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Azopolymers: a means of colon specific drug delivery?

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Improved drug delivery systems are required for drugs currently used to treat localised diseases of the lower intestinal tract such as ulcerative colitis and irritable bowel syndrome. Such diseases are most effectively treated by the local delivery of anti-inflammatory agents to the large intestine. However, these agents are either delivered inefficiently and unpleasantly by large-volume rectal enema or exhibit unacceptable side effects after oral administration because of absorption in the upper regions of the gastrointestinal tract (Friend, 1991).

It has also been suggested that the delivery and release of therapeutic peptides reliably and specifically within the colon represents the best opportunity for the absorption of such drugs after oral administration since the colon has the advantages of longer drug residence time, a more permeable epithelium and purportedly fewer proteolytic enzymes than elsewhere in the intestine (Mackay and Tomlinson, 1991; Ritschel, 1991). Consequently drugs, particularly peptides, which are susceptible to proteolytic degradation and deactivation in the upper intestine, may be more effectively absorbed from the colon. For example, the injection of insulin into the ascending colon has been shown to cause a 50% decrease in blood glucose levels whilst its injection into the ileum had no effect unless it was coadministered with a trypsin inhibitor (Kidron et al., 1982). The colonic absorption of calcitonin has also been demonstrated in both rats and man (Antonin, 1992; Hastewell et al., 1992). Furthermore, evidence also exists which shows that paracellular and lymphatic uptake of peptides, avoiding first pass metabolism, occurs in the colon (Rubas and Grass, 1991).

A number of different physiological triggers have been proposed for targeted release of drugs in the colon. One approach has been to utilise the plethora of microorganisms which pervade the lower gastrointestinal tract. These organisms are capable of mediating a wide variety of metabolic processes including the reduction of water soluble azo dyes such as Prontosil (Scheline, 1973). Microbial azo reduction has also been shown to be responsible for the reductive cleavage of the prodrug sulphasalazine, used in the

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treatment of ulcerative colitis, to 5-aminosalicylic acid and sulphapyridine in the colon (Peppercorn and Goldman, 1972) and has been exploited in the design of water soluble polymeric prodrugs (Brown et al., 1983).

Such studies on water soluble azo compounds and polymers led Saffran et al. (1986) to investigate the use of insoluble polymers containing azoaromatic crosslinks as capsule and tablet coatings for the delivery of medicament to the lower bowel. These workers believed that such coatings would be impervious to water in the stomach and small intestine but that reduction of the azo bonds in the lower intestine would facilitate aqueous permeation and subsequent drug release. Although Saffran et al. (1986, 1990, 1991) and Saffran and Neckers (1987) have shown that peptides administered to pancreatectomized dogs and rats using azopolymer-coated devices elicit secondary pharmacological responses, such as antidiuresis and hypoglycaemia on administration of vasopressin and insulin, respectively, their studies provide no evidence to demonstrate that the devices reached the large intestine or that the peptides were released as a consequence of microbial degradation of the azo polymer. The studies are further confounded by the use of a stoichiometric mixture of 5-methoxysalicylic acid and sodium bicarbonate in some studies. The salicylic acid acts as a penetration enhancer and also serves, in conjunction with the sodium bicarbonate, to generate carbon dioxide pressure to burst the capsule and facilitate the release of the drug. There was no consideration given by these workers to either the ease or extent of reduction of the chosen azo crosslinkers nor to the desirability or importance of the swellability of the polymer coating. As no control experiments were reported in any of their studies, using a similar polymer coating without the azo crosslinkers, the possibility of degradation and chemical breakdown of the polymer backbone remained uninvestigated. In addition, there was no discussion of the fact that the polymers used for these studies were insoluble whereas all those studied previously had been water soluble.

Our group has previously investigated the use of azopolymer coated capsules for colon specific drug delivery in rats (Hastewell et al., 1991). Using a lipophilic marker, it was possible to identify the site of capsule release in the gastrointestinal tract. These results suggested that the release from such devices was consistent with the activation of azopolymer drug delivery systems by azoreductase in the lower gastrointestinal tract. However, subsequent studies using the same polymer demonstrated that the release from these devices was in fact time dependent (Lloyd et al., 1992). Furthermore, these polymers were shown to be resistant to chemical reduction by sodium dithionite or zinc and hydrochloric acid but nevertheless susceptible to degradation by sodium hydroxide solution. These observations suggested that the time dependent release for these systems was at best partially a consequence of hydrolysis of the polymer backbone to give an inherently more swellable and soluble polymer. A number of further studies have also been reported over the intervening years using a variety of azocrosslinkers and polymers (Pradny and Kopecek, 1990; Schacht and Wilding, 1991; Brondsted and Kopecek, 1992a,b; Kimura et al., 1992; Kopecek et al., 1991, 1992; Van den Mooter et al., 1992, 1993). Some of these studies have involved using colorimetric changes as a means of monitoring the cleavage of the azo bond by reduction to the free amines and hence have failed to consider the opacity caused by the swelling of the azopolymer gels alone. Hydrogels have a tendency to become opaque as the gel swells as a consequence of changes in refractive index of the gel. Such a change in opacity may mask any colour change attributable to the simultaneous reduction of the azo bonds within a hydrogel. Colorimetric methods are also unable to distinguish between reduction of an azo bond to the intermediate hydrazo moeity, a process which is often reversible, from the complete reduction of this species to the free amines. Other workers have investigated the swellability, permeability or the viscosity of the polymers before and after in vitro or in vivo incubation with caecal contents. These experiments are unable to distinguish the changes in macromolecular structure which occur to the polymer as a result of azo reduction from that which would occur upon degradation of the poly-

mer by some other mechanism, such as the hydrolysis of hydroxyethyl esters. Although these studies provide useful information relating to the caecal degradation of such polymers it has not been conclusively demonstrated that degradation occurs as a consequence of azo reduction.

It would also seem preferable to achieve complete reduction of the azo bond to the corresponding amines despite the fact that Kimura et al. (1992) have suggested that the conversion of azo to hydrazo bonds in polyurethane based azopolymers may be sufficient to trigger drug release. If reduction to the free amines is to be achieved then more consideration must be given to the nature of the azoaromatic crosslinkers. To date there has been no rationale selection of azo crosslinker molecules taking into account the electronic parameters affecting the ease of reduction of the azo bonds within the colonic environment, where the redox potential has been shown to vary between −200 and −250 mV (Friend, 1991). It is well documented, for example, that whereas certain azo compounds are readily reduced electrochemically to the corresponding amines, the others are only reduced as far as the corresponding hydrazo compounds (Florence, 1974). Dubin and Wright (1975) have suggested previously that the electrochemical reduction of certain azo dyes can be directly correlated with the bacterial reduction and this has been recently confirmed experimentally by comparing the halfwave potentials for a series of water soluble azo dyes with their rate of reduction by *Bacteroides fragilis* under anaerobic conditions (Bragger et al., 1993a). As the biological reduction of the azoaromatic compounds appears to correlate with the electrochemical reduction of these species it is reasonable to conclude that the extent of biological reduction will be dependent on the electronic structure of the azo compound. Table 1 shows the results of a recent study of the polarographic reduction of various azo compounds. Solutions of the azo compounds (0.01 mM) were prepared in 0.02 M phosphate buffer (pH 7.0) containing 20% v/v ethanol. The DC current-voltage curves were obtained at 21 ± 1°C using a recording polarograph with a polarizing range of −420 to −920 mV vs Ag/AgCl reference electrode, a current range of 0.02 mA and a voltage sweep rate of 10 mV s^{-1}.

Table 1
The half-wave potential ($E_{1/2}$ (Ag/AgCl)) and the relative number of electrons (n) involved in the polarographic reduction calculated from the corresponding DC polarographs of a number of different azo compounds

Azo compound	Half-wave potential (mV)	n[a]
Azobenzene	−587	2.0
Azotoluene	−656	2.0
4,4'-Dihydroxyazobenzene	−736	4.3
Amaranth	−677	4.0
Orange II	−784	3.2

[a] Based on studies of Florence (1974) and assuming that the diffusion coefficient for the different azo compounds is the same.

In analysing the results of this study it is important to compare only structurally similar molecules as in calculating the apparent number of electrons involved in the polarographic reduction it is assumed that the diffusion coefficients of the compounds are the same. Azo compounds always reduce by means of a two-electron process to the corresponding hydrazo compound which then disproportionate to the free amines, if possible, at different rates (Florence, 1974; Madajová and Zelensky, 1981). As a consequence, the complete reduction to the corresponding free amines employs a total of four electrons. This study confirms that amaranth undergoes a four-electron reduction in 20% v/v buffered ethanol. The apparent number of electrons involved in the polarographic reduction will be dependent on whether the disproportionation occurs during the lifetime of the polarographic mercury drop. The results therefore suggest that the disproportionation of the reduced hydrazo derivative of Orange II may occur less rapidly than that of amaranth. The apparent number of electrons involved in the reduction of the 4,4'-dihydroxyazobenzene may reflect the increase in the diffusion coefficient of this molecule given that the molecule is much smaller than amaranth and Orange II. As azobenzene and azotoluene are structurally similar, it is acceptable to compare the polarographic data for these two compounds. Florence (1974)

has previously shown that azobenzene will only undergo a two-electron polarographic reduction to the corresponding hydrazo compound, since it lacks the functionality required for disproportionation to the free amines. Furthermore, the results above suggest that azotoluene, which can be considered as a model compound for the azostyrene crosslinker within previously synthesised polymers (Saffran et al., 1986), is less readily reduced than azobenzene since it has the more negative half-wave potential (Fig. 1). In addition, the relative number of electrons involved in the polarographic reduction suggests that this compound undergoes, at best, a two-electron polarographic reduction. If the lack of functionality required for the subsequent disproportionation of the hydrazo intermediate is also considered, it seems very unlikely that azotoluene will be reduced to the free amines under the redox conditions extant in the colon. It is therefore our opinion that some of the azo crosslinkers used in previously published studies are not reduced within the gastrointestinal tract. Moreover, even azo crosslinkers which are reduced may not be once incorporated into a polymeric matrix through which the biological reducing species must diffuse in order to effect azo reduction.

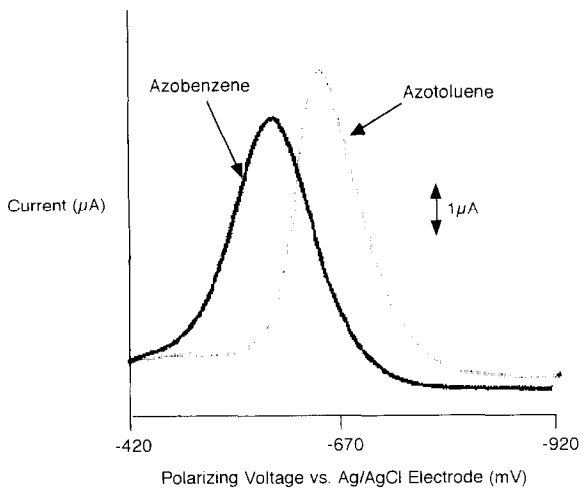

Fig. 1. A differential pulse polarograph showing the polarographic waves for azobenzene and azotoluene in 0.02 M phosphate buffer (pH 7.0) containing 20% ethanol at 21 ± 1°C.

The selection of more suitable crosslinking agents will also be facilitated by an improved understanding of the mechanism and factors affecting the reduction of azo compounds. Computer-aided modelling may offer a possible means of selecting suitable azo compounds, since it has recently been demonstrated that this technique may be used to predict effectively the ease of reduction of azo compounds (Bragger et al., 1993b). Rafii and Cerniglia (1993), using immunoelectron microscopy, have suggested that azo reductase is synthesised throughout the cytoplasm and secreted extracellularly directly, without cellular accumulation. However, recent evidence has suggested that azo reduction is in part mediated by low molecular weight electron carriers such as NADPH rather than specific azo reductase enzymes (Lloyd et al., 1993). On this basis, we believe that the azo compounds may act as an electron receptor as part of the electron transport process. Furthermore, this would suggest that successful degradation of an azo polymer is not dependent upon the presence of a particular organism possessing a specific azo reductase in the colon and hence the principle of using azo polymers for colon specific drug delivery is a valid approach, since low molecular weight electron mediators, such as NADPH, should be able to penetrate a swollen polymeric matrix. However, further studies are required to prove conclusively that suitable low molecular weight redox carriers mediate azo reduction in the colon.

Finally, initial studies of any polymeric system must also include, in our opinion, evidence to demonstrate that the incorporated azo crosslinker is susceptible to reduction in the absence of the supporting polymer. Although Brondsted and Kopecek (1992b) have attempted to show that their crosslinkers are enzymatically reduced, the studies are less than satisfactory as the degradation was carried out using an enzymatic system which included ethanol as a co-solvent to solubilise the crosslinkers. Ideally, such studies should be carried out using anaerobic cultures which are representative of the colonic microflora and should involve an assessment of both the degradation of the azo compound and the formation the corresponding amines by HPLC using ap-

proaches similar to those described by Bragger et al. (1993c).

In conclusion, our work suggests that the majority of previous studies in this field do not withstand critical scientific analysis and the conclusions of such studies require re-evaluation. However, we believe azo polymers will offer substantial opportunities for colon specific drug delivery if a more systematic approach is taken towards the design of such systems.

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