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Azo polymers for colon-specific drug delivery

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Summary

In order to develop a colon-specific drug delivery system, copolymers of 2-hydroxyethyl methacrylate and methyl methacrylate were prepared in the presence of bis(methacryloylamino)azobenzene. The conditions of polymerization were optimized to prevent crosslinking. Film coatings were prepared with the azo polymers. The films were insoluble in simulated gastric and intestinal juice. Dependent on the content of the hydrophilic groups in the polymers, the films were more or less permeable to water. In vitro and in vivo tests prove that it is possible to use the polymers to deliver drugs to the large intestine. Due to the presence of the azo compound, the azo polymer coatings can be degraded by intestinal bacteria.

Introduction

Colon-selective drug delivery systems have been the focus of increasing interest during the last decade. Targeting of drugs to the large intestine can be achieved in several ways.

The drug can be formulated in a solid dosage form, coated with a polymer able to withstand the lower pH values, but desintegrating at the slightly alkaline pH values of the ileo-caecal junction and the large intestine. For this purpose, Lehmann (1975) developed a copolymer of methacrylic acid and methyl methacrylate.

Several factors, however, such as the presence of short-chain fatty acids, residues of bile acids, carbon dioxide or other fermentation products, can reduce the colonic pH to approx. 6, and call the pH of the colon as a trigger into question (Rubinstein, 1990).

Drugs can also be administered as prodrugs to reach the large intestine where the active compound is formed enzymatically by the intestinal microorganisms (Friend and Chang, 1984). The metabolic capacity of gastrointestinal bacteria can be very large and foreign compounds may be exposed quite easily to these effects (Scheline, 1973). The use of salicylazosulfapyridine (SAS) is well established in the treatment of inflammatory bowel disease. In the colon, the azo bond of SAS is split with the liberation of 5-aminosalicylic acid (5-ASA) and sulfapyridine (Peppercorn and Goldman, 1972; Khan et al., 1983; Klotz, 1985).

It appears that reduction of azo bonds is a general reaction rather than a reaction carried out by only one or a few intestinal bacteria (Scheline, 1973).

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Saffran and co-workers (1986, 1988) succeeded in delivering peptide drugs to rats and dogs via oral administration. They developed a copolymer of styrene and 2-hydroxyethyl methacrylate crosslinked with divinylazobenzene to coat oral dosage forms. On arrival at the colon, the coating will be degraded by bacterial azo reductase. However, problems due to variability in absorption rates are encountered when administering coated drugs. The variations observed are probably due to intra- and intersubject differences in microbial digestion of the coatings, which might be caused by the polymer applied being insufficiently hydrophilic.

Therefore, we present in this paper the synthesis of new azo polymers in order to prepare colon-selective coatings. Copolymers of 2-hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA) were prepared in the presence of a bifunctional azo compound, bis(methacryloylamino)azobenzene (BMAAB). To investigate the influence of the hydrophilicity of the polymers on the degradation by intestinal bacteria, copolymerizations were achieved over a wide range of monomer feed compositions. In vitro and in vivo tests show that only coatings with a high degree of hydrophilicity are susceptible to bacterial degradation.

Materials and Methods

Materials

HEMA and MMA (both Janssen Chimica, Beerse, Belgium) were purified by vacuum distillation. Azobisisobutyronitrile (AIBN) (Janssen Chimica, Beerse, Belgium) was recrystallized from methanol. Methacryloyl chloride (Janssen Chimica, Beerse, Belgium) was used without purification. Ethanol and methylene chloride were purified by distillation. 4-Nitroaniline (Janssen Chimica, Beerse, Belgium) was used without further purification.

Instrumentation

UV spectra were recorded with an HP8452A diode array spectrophotometer (Hewlett Packard Co., Santa Clara, CA, U.S.A.). In vitro degradation tests were carried out in a Compact Anaerobic Workstation (DW Scientific, West Yorks, U.K.).

Synthesis of BMAAB

4,4′-Diaminoazobenzene (4,4′-DAAB) was synthesized according to the method of Witt and Kopetschini (1912); m.p., 250°C; λ_{max}, 400 nm.

To a solution of 4.25 g (0.02 mol) of 4,4′-DAAB in 40 ml of dry pyridine, 5.9 ml (0.06 mol) of methacryloyl chloride was added dropwise with stirring at room temperature. After complete addition, the reaction mixture was heated at 60°C for 1 h. After cooling it was poured into ice water, acidified to pH 4 and filtered to isolate the precipitate. The precipitate was washed with a saturated solution of sodium bicarbonate and then with water. The crude reaction product was recrystallized four times from ethanol; m.p., 278°C; yield, 83%; λ_{max}, 370 nm.

Synthesis of azo polymers

All copolymerizations were carried out with HEMA and MMA in different ratios. The amount of BMAAB, added to the reaction mixture to prepare the different azo polymers listed in Table 1, was 2% w/w. The polymers were formed via a free radical mechanism using AIBN as initiator at a concentration of 1% w/w. All polymerizations were carried out at 60°C. Dependent on the HEMA/MMA ratio in the copolymers, the polymerization reactions were carried out in ethanol or ethanol-methylene chloride (50:50). The over-

TABLE 1

Influence of HEMA content on bacterial degradation of the azo polymers

Polymer	MMA/HEMA ratio	Degradation
A	1:1	−
B	1:2	−
C	1:3	−
D	1:4	−
E	1:5	− − /+
F	1:6	+ + +
G	no MMA added	+ + +

The amount of BMAAB added to the reaction mixture was 2% w/w.

TABLE 2

Composition of V.P.I. diluent

$CaCl_2$ (anhydrous)	200 mg
$MgSO_4 \cdot 7H_2O$	480 mg
$K_2HPO_4 \cdot 3H_2O$	1.3 g
KH_2PO_4	1.0 g
NaCl	2.0 g
$NaHCO_3$	10.0 g
H_2O to	1 000 ml

all concentration of monomers, in order to obtain soluble polymers, was never higher than 7% w/w. The polymers were isolated and purified by repeated precipitation in diethyl ether. The yield after 24 h of reaction was 80%. The inherent viscosity in dimethylformamide at 24°C was 0.8.

Preparation of isolated films

Polymer solutions containing 10% w/w of the azo polymers in ethanol or in ethanol-methylene chloride (50:50) were cast on a teflon-coated glass plate, using a film casting knife (Gardner Multicator type 411). To slow down solvent evaporation, the glass plate was covered with a funnel. After complete evaporation of the solvent, the films were removed from the glass plate, dried to constant weight at 35°C and stored in a desiccator for 2 weeks. The thickness of the films was measured with a micrometer (Lorentzen & Wetters, Van der Heyden, Brussels, Belgium).

Bacterial degradation tests

In vitro Isolated films were prepared with the azo polymers. The films were incubated anaerobically in V.P.I. diluent (Table 2), inoculated with freshly voided, human feces. Subsequently, the films were rinsed with distilled water, examined for degradation and compared with films which were incubated with V.P.I. diluent, but not inoculated with human feces.

In vivo Capsules (no. 9, Elanco Lilly) were coated with polymers F and G and filled with fluorescein as a tracer substance. The coating solution had a polymer concentration of 10% w/w in ethanol. Male Wistar rats (300 g) were anesthetized intraperitoneally with sodium pentobarbital (60 mg/kg). The coated capsules were put in the caecum. Ligatures were made before and after the caecum in order to prevent the capsule moving out of the caecum. After 6, 12, 18 and 24 h, the rats were killed with diethyl ether and the caecum removed.

Permeability of isolated films

The permeability of isolated films was studied by determination of the diffusion of caffeine, used as a tracer substance, through the films. The films were pinched between the donor and acceptor compartments of a diffusion cell. The amount of caffeine diffusing from the donor to the acceptor compartment was spectrophotometrically determined at 272 nm.

Results and Discussion

In order to obtain soluble copolymers, several copolymerizations at different monomer/solvent ratios were carried out. The results are given in Table 3. These data show that soluble copolymers were obtained when the overall concentration of monomer was not higher than 7% w/w. Crosslinked polymers are formed when the total monomer concentration equals or exceeds 10% w/w.

In another experiment, the yield was determined as a function of polymerization time. The results are depicted in Fig. 1. When the total monomer concentration was 7% w/w, a yield of more than 80% was achieved after 24 h. When the overall monomer concentration was 15%

TABLE 3

Influence of total monomer concentration on solubility of the azo polymers

Monomer concentration (% w/w)	Solubility
25.4	– [a]
16.6	– [a]
12.5	– [a]
10.0	– [a]
7.0	+
5.0	+

[a] Crosslinked polymers were formed.
Polymerization time was 24 h. HEMA/MMA ratio, 6:1.

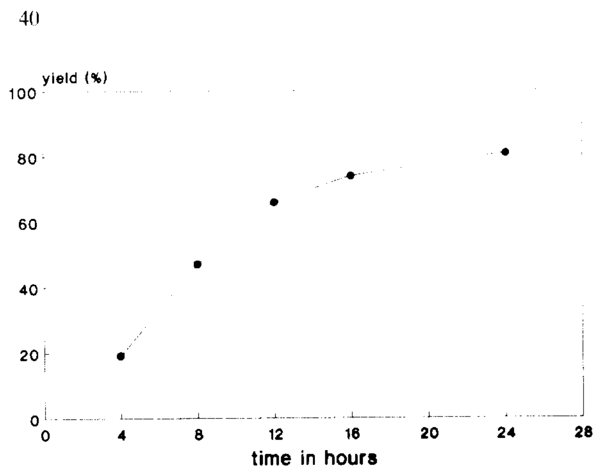

Fig. 1. Yield of polymerization as a function of time for polymer F.

w/w, only 54% of soluble azo polymer could be isolated, since crosslinking began to occur after 4 h of reaction.

TABLE 4

Amount of BMAAB incorporated into polymer F as a function of amount added to the reaction mixture

% (w/w) added	% (w/w) incorporated [a]
1.00	0.48
2.00	1.12
3.00	1.48

[a] After 24 h of polymerization.
The overall monomer concentration was 7% w/w.

The concentration of BMAAB incorporated into the copolymers can be calculated from UV spectrophotometry at 370 nm. The results are given in Table 4. These data show that almost 50% of the BMAAB concentration in the monomer mixture was incorporated into the polymer chain.

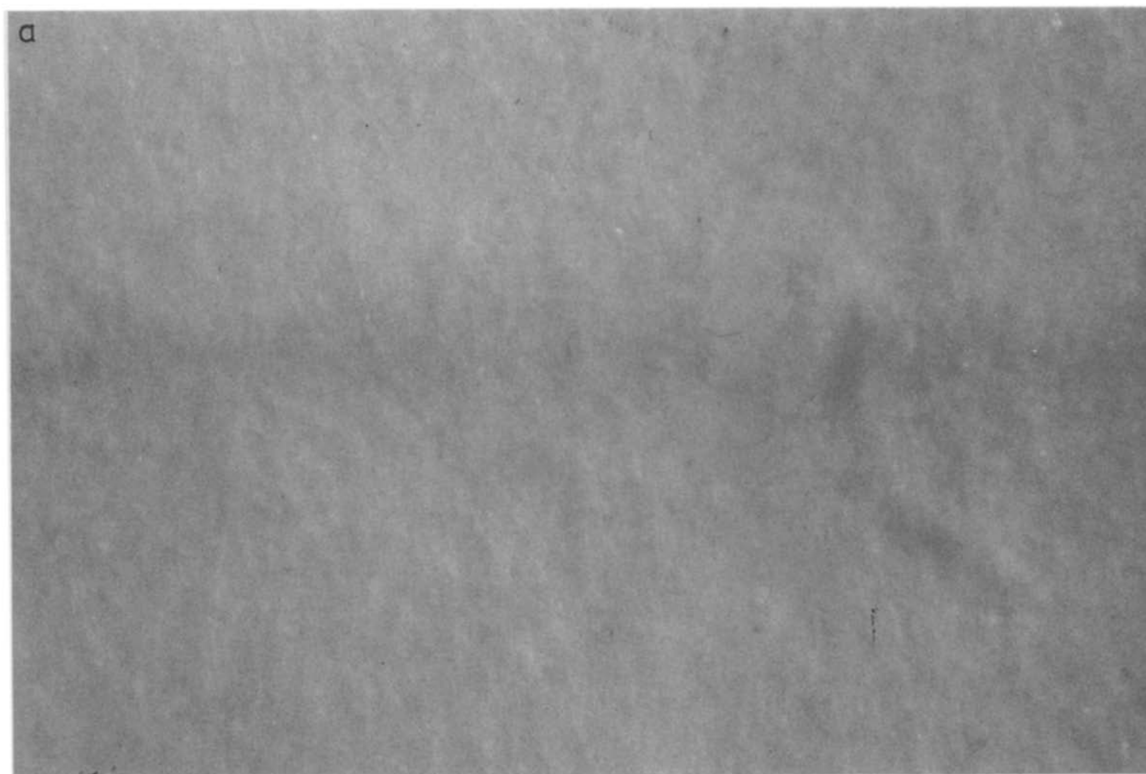

Fig. 2. (a) Isolated film of polymer G after 24 h incubation in V.P.I. diluent. Magnification, 30 ×. (b) Isolated film of polymer G after 24 h incubation in V.P.I. diluent inoculated with human feces. Magnification, 30 ×.

Fig. 2b.

Bacterial degradation of the azo polymer coatings

In vitro

Isolated films were prepared with the different azo polymers listed in Table 1. The coating solutions contained 10% w/w of polymer in ethanol or ethanol-methylene chloride (50 : 50). The thickness of the films was between 32 and 46 μm.

The polymer films were incubated anaerobically for 24 h in V.P.I. diluent and inoculated with freshly voided, human feces. After 24 h, the films were rinsed with distilled water and then compared macro- and microscopically with azo polymer films which were incubated anaerobically with V.P.I. diluent but not inoculated with human feces. The results of bacterial degradation as a function of HEMA content in the azo polymers are given in Table 1. No degradation by intestinal bacteria was observed with films A–D. As is evident from Table 1, film E is only very slightly affected, whereas films F and G are strongly influenced. Film G shows a large number of irregularly shaped holes (Fig. 2a and b). Compared with the blank films, the surface of films F and G has become rough and in some places film G has decomposed into a gelatinous substance.

The bacterial degradation of polymer F films is visually more difficult to detect as compared to polymer G films. To quantify the degradation of polymer F films, we determined the permeability of isolated films before and after incubation in V.P.I. diluent inoculated with human feces. This permeability was studied by determination of the diffusion of caffeine through the films.

Supposing that the diffusion takes place under steady-state conditions, we can write:

$$C_2 = \frac{PSC_1t}{xV_2} \quad \text{(Peeters, 1990)}$$

where C_2 and C_1 represent the concentration of caffeine in the acceptor and donor compartment,

respectively, x is the thickness of the film, V_2 denotes the volume of acceptor compartment, P is the permeability constant and t denotes the time.

P can be calculated from the graph of C_2 as a function of time. The results are shown in Fig. 3. After 16 h incubation, P was determined to be 1.95×10^{-9} cm^2/s and after 48 h the value of P was 3.33×10^{-9} cm^2/s, corresponding to an increase by a factor of 5.5. No significant change in P was observed for the control films, which were only incubated in V.P.I. diluent. The above results indicate that the increase in P is caused by bacterial degradation of the azo polymers.

All coatings had a vivid yellow color before incubation in human feces, while after incubation, only films F and G showed a pale yellow color. The change in color is probably due to the reduction of the azo bonds.

The absorption peak of the azo polymers at 370 nm due to the presence of azo bonds offers the possibility of performing the quantitative determination of the reduction of the azo groups. We measured the absorption of polymer G films at 370 nm in ethanol. Data were collected after 24, 48 and 120 h incubation in human feces and compared with a non-incubated polymer G film. We determined the absorption per unit weight (abs/w) of the coatings. The results are depicted in Fig. 4. After 48 h the absorption per unit weight at 370 nm decreased by about 35% and after 120 h by about 64% as a result of the reduction of azo groups.

In another experiment, the length of different azo polymer films was measured. One part of the isolated polymer films was incubated anaerobically for 48 h in V.P.I. diluent inoculated with human feces, another part being incubated anaerobically in V.P.I. diluent. After 48 h, the films were rinsed with distilled water, the length was measured again and the elongation factor L was determined. L was calculated as the ratio of

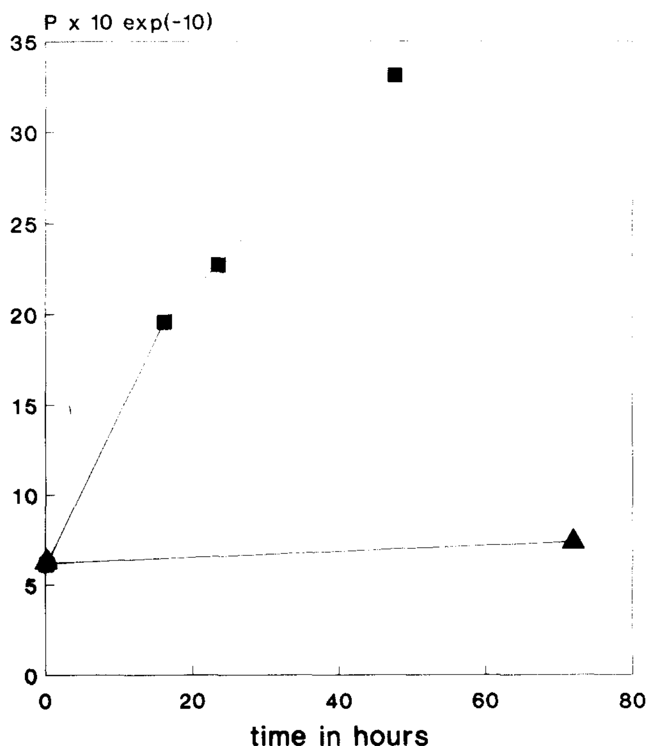

Fig. 3. Influence of incubation time in human feces on the permeability constant of isolated films of polymer F. Incubated in human feces (■); incubated in V.P.I. diluent only (control) (▲).

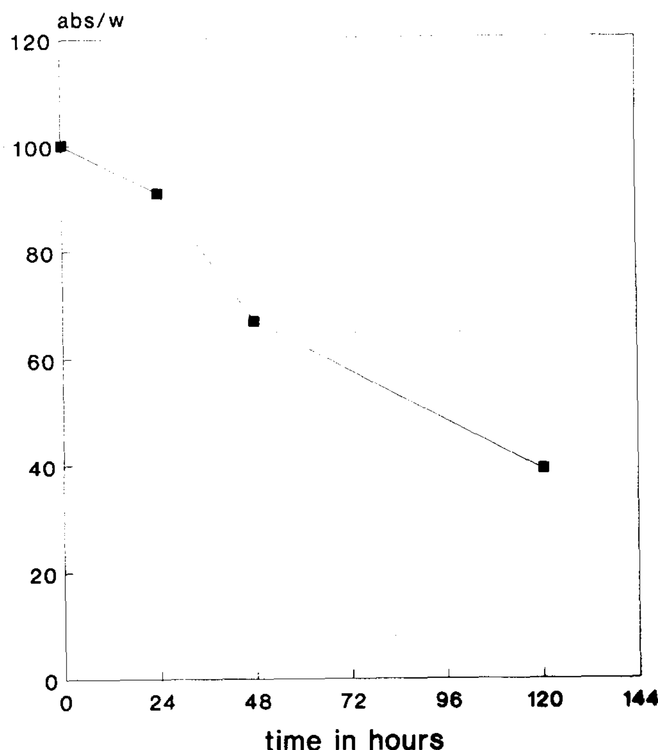

Fig. 4. Influence of incubation time in human feces on reduction of azo bonds in polymer G.

the length of the films after incubation to that before incubation. The results are listed in Table 5.

The films incubated with human feces demonstrate elongation, reaching about twice the original length in the case of film G. Films incubated in the V.P.I. diluent only show an elongation factor of 1.11 at the most, due to water uptake.

TABLE 5

Elongation factor of azo polymer coatings after incubation for 48 h in human feces

Polymer	L	
	After incubation	Blank
A	1.00	1.00
B	1.00	1.00
C	1.00	1.01
D	1.01	1.01
E	1.04	1.03
F	1.36	1.09
G	1.86	1.11

Elongation of the polymer films is a consequence of the reduction of azo bonds, by which a change in structure of the films appears.

The results described above show that among the investigated azo polymers only those polymers containing a large amount of HEMA are susceptible to bacterial degradation.

In vivo

Although the gastrointestinal microflora of rats and humans differ, in vivo experiments with rats can give a good indication of the biodegradation of the azo polymer coatings.

For the in vivo experiments, we only used polymers F and G, since we knew from the in vitro experiments that only these polymers were susceptible to bacterial degradation.

Capsules (no. 9, Elanco Lilly), filled with fluorescein as a tracer molecule, were dipped four or five times in the coating solutions. The amount of polymer coating on the capsules was between 9.2 and 9.8% w/w and between 11.7 and 12.4%

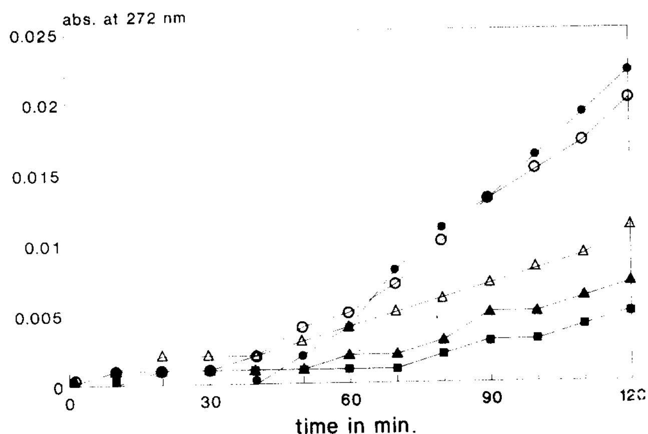

Fig. 5. Release of caffeine in simulated gastric fluid from capsules coated with polymers F and G. Polymer F: 4.7% (●), 7.4% (△), 9.4% (■); polymer G: 12.3% (○), 14.4% (▲).

w/w, calculated as the weight gain on the capsule on dipping the capsules four or five times, respectively, in the polymer solution.

After 6 h residence in the caecum of the rat, both polymer coatings were only slightly affected and the caecum was not colored with fluorescein. Coatings of polymer G showed a more advanced degree of decomposition after 12 h. After 18 h the coating had become completely pale yellow and after 24 h it had a gelatinous appearance. Polymer F was more resistant to bacterial degradation. The caecum was partially colored after 18 h and the surface of the coating was rough. After 24 h the caecum was completely colored and the surface of the coating was covered with irregularly shaped holes and showed a pale yellow color due to azo reduction. Unlike polymer G coatings, polymer F coatings did not have a gelatinous appearance.

No large differences in the rate of degradation were observed when the capsules were dipped four or five times in the coating solution.

Gastro-intestinal resistance of the coatings

To investigate whether the azo polymer coatings could withstand gastric and intestinal fluid, we determined the permeability of isolated films

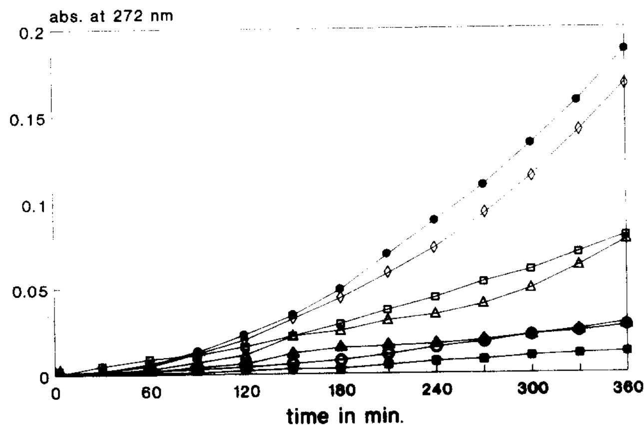

Fig. 6. Release of caffeine in simulated intestinal fluid from capsules coated with polymers F and G. Polymer F: 4.8% (●), 7.2% (□), 9.2% (○), 12.1% (■); polymer G: 9.7% (◇), 11.9% (△), 14.9% (▲).

TABLE 6

Permeability constants of different azo polymer coatings in simulated gastric and intestinal fluid

Coating	P ($\times 10^{-11}$)	
	Intestinal fluid	Gastric fluid
Polymer B	4.1 (14.0%)	– [a]
Polymer C	16.0 (16.0%)	16.4 (15.3%)
Polymer D	33.0 (8.3%)	28.1 (9.1%)
Polymer E	44.1 (16.2%)	44.3 (1.3%)
Polymer F	61.3 (8.1%)	51.3 (14.8%)

[a] The lag time was too long to determine P.
Relative standard deviations are indicated in parentheses.

and the amount of drug released from a coated capsule in simulated gastric and intestinal fluid (USP XXII). The residence time was 2 h in simulated gastric fluid and 6 h in simulated intestinal fluid. The results have been combined in Table 6. They show that there is only a negligible difference in permeability of the coatings investigated in gastric or intestinal fluid. This was expected, since all copolymers are only very slightly pH-sensitive if at all. However, there is a large difference in permeability among the different polymers investigated. The permeability constants increase with decreasing MMA content which corresponds with increasing hydrophilicity.

The release of caffeine in gastric and intestinal fluid from a coated capsule was also determined at 272 nm. As a consequence of the results of the in vitro degradation tests, only polymers F and G were investigated. The results are demonstrated in Figs 5 and 6 and Table 7. They show that for about the same amount of polymer coating on the capsule, the percentage of drug released through a polymer G coating, compared with a polymer F coating, is about 5-times higher in intestinal juice and more than 20-times greater in gastric juice.

The permeability and release experiments indicate that both coatings are indeed able to withstand gastric acid and intestinal fluid. Neither of the coatings was dissolved, however, only coatings made of polymer F are able to provide sufficient protection for drugs in the gastrointestinal tract.

Conclusion

The experiments and results reported here indicate that azo polymers can be used in colon-selective release formulations. In vitro and in vivo experiments show that the film coatings made of these polymers are degraded by intestinal bacteria. However, a balance must be found in the ratio between the amount of hydrophilic component which ensures a good availability of the azo group for bacterial reduction and the more hydrophobic component which provides resistance

TABLE 7

Percentage of caffeine released from capsules coated with polymers F and G in simulated gastric fluid and simulated intestinal fluid

	Polymer F		Polymer G	
	% of polymer [a] on the capsule	% of caffeine released	% of polymer [a] on the capsule	% of caffeine released
Simulated gastric fluid	4.7	2.2	4.6	–
	7.4	1.1	7.2	27.1
	9.4	0.5	9.4	10.0
	11.8	0.1	12.3	2.1
	14.7	0.0	14.4	0.7
Simulated intestinal fluid	4.8	20.2	4.6	–
	7.2	8.6	7.0	43.2
	9.2	3.1	9.7	18.2
	12.1	1.4	11.9	8.3
	14.4	0.5	14.9	2.9

[a] Calculated as weight gain on the capsule.

to the gastric juice and small intestinal fluid. These are the aims of further investigations.

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