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Synthesis and characterisation of inulin-azo hydrogels designed for colon targeting

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Abstract

The present paper describes the synthesis and characterisation of new hydrogel systems designed for colon targeting. The gels were composed of methacrylated inulin (MA-IN), copolymerized with the aromatic azo agent bis(methacryloylamino)azobenzene (BMAAB) and 2-hydroxyethyl methacrylate (HEMA) or methacrylic acid (MA). The gels were assessed by studying the influence of various parameters on the dynamic and equilibrium degree of swelling. It was shown that the uptake of water in the gels was inversely proportional to the MA-IN feed concentration, the degree of substitution of the inulin backbone, and the concentration of BMAAB. The latter can probably be explained by the hydrophobic nature and rigidity of the aromatic azo agent. Incorporation of the hydrophilic monomers HEMA or MA also reduced the equilibrium degree of swelling. An increasing network density and hydrogen bonding propensity, can suggested to be responsible for this observation. It was shown that water uptake in the hydrogels was controlled by both relaxation and diffusion mechanisms (anomalous behaviour). When the release of the model drug prednisolone was studied in phosphate buffer, it was shown that $>80\%$ of the drug was released during the first 3 h from hydrogels of MA-IN:HEMA. Although drug release decreased significantly from MA-IN:HEMA:BMAAB hydrogels, it remained too high: $\sim 50\%$ of the drug was released after 5 h. The same observation was made for hydrogels containing MA instead of HEMA. These results clearly point out the difficulty in finding the optimal balance between swelling to allow degradation in the colon (high swelling of the gels) and low premature drug release before the colonic environment is reached (low swelling properties).

Keywords: Colon targeting; Azo polymers; Hydrogels; Inulin

1. Introduction

Colon targeting still remains one of the most challenging areas in the field of controlled drug

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delivery, mainly because after more than 15 years of research, systems that deliver drugs specifically to the colon have not yet entered the market, although during the past 15 years many inventive systems have been proposed. The reader is referred to some representative review articles (Friend, 1991; Van den Mooter and Kinget, 1995; Van den Mooter et al., 1997; Kinget et al., 1998). Among the systems developed, polymeric systems that deliver the drug into the colon after polymer biodegradation triggered by colonic bacteria are truly site specific. Biodegradable polymers were either designed to act as coating material for solid dosage forms or as drug loaded hydrogels. The latter can be defined as three dimensional insoluble polymer networks which can take up a significant amount of liquid, without loss of structural integrity.

In a previous paper we reported on the development of hydrogels based on derivatised inulin, a naturally occurring polysaccharide, which belongs to the group of the glucofructans (Vervoort et al., 1997). It was found that the inulin based hydrogels were susceptible to bulk degradation as a consequence of bacterial mediated hydrolysis after incubation in a rat caecal and human faecal degradation medium (Vervoort, 1998; Vervoort et al., 1998). However the rate of degradation was too slow, which made them less suitable for colon targeting purposes. Since we previously developed and characterized different types of azo polymers for colon specific drug delivery (Van den Mooter et al., 1992, 1993), we decided to combine both systems in order to increase the susceptibility of the hydrogels to bacterial degradation. The present paper reports on the synthesis and characterisation of a new generation of hydrogels based on methacrylated inulin which is copolymerized with bis(methacryloylamino) azobenzene (BMAAB) and other monomers such as 2-hydroxyethyl methacrylate (HEMA) and methacrylic acid (MA). The characterisation was performed by studying both the swelling kinetics and the equilibrium degree of swelling of the hydrogels. The effect of both synthesis parameters and composition of the swelling medium on the swelling properties was investigated. The synthesis parameters investigated were the degree of substitution (DS) of MA-IN as well as its feed concentration, the feed concentration of BMAAB and hydrophilic monomers (HEMA, MA), the polymerisation time, and the azobisisobutyronitrile (AIBN) concentration (free radical generating system). In the swelling medium, temperature, ionic strength and pH were varied. The hydrogels were further characterized by studying the release of prednisolone from selected hydrogels in phosphate buffer.

2. Materials and methods

2.1. Materials

Inulin (Raftiline HP, with an average degree of polymerization between 22 and 25) was kindly provided by Orafti (Tienen, Belgium). *N*,*N*-dimethylformamide (DMF) (BDN, UK), glycidyl methacrylate (Aldrich, Steinheim, Germany), dimethylaminopyridine (DMAP) (Sigma, St. Louis, MO), p-nitroaniline, sodium hydrogen sulfide, and ammonium persulfate (all Acros Organics, Geel, Belgium) were used without any further purification. Methacrylic acid (UCB, Leuven, Belgium), methyl methacrylate (Merck, Schuchardt, Germany), methacryloylchloride (Acros Organics, Geel, Belgium) and 2-hydroxyethyl methacrylate (Acros Organics, Geel, Belgium) were purified by vacuum distillation. Azobisisobutyronitrile (AIBN) (Acros Organics, Geel, Belgium) was recrystalized from methanol. All other materials were of analytical or HPLC grade and were used without purification.

2.2. Synthesis of *N*,*N*′-bis(methacryloylamino)azobenzene (BMAAB)

BMAAB was synthesized as described by Van den Mooter et al. (1992). In brief, 50 g (0.36 mol) of p-nitroaniline was dissolved in a mixture of 125 ml of concentrated sulfuric acid and 550 ml of water; 175 g of ammoniumpersulfate was added while stirring. After 24 h of reaction, the brown–yellow precipitate was collected on a Büchner funnel, washed with water, and dried at 60°C. A

total of 30 g of the dried precipitate was suspended in a mixture of 50 ml of ethanol and 100 ml of water. To this mixture, 70 ml of a 44% w/v solution of sodium hydrogen sulfide was added slowly. The reaction mixture was placed on a water bath (60°C) for ~3 h, after which time 4,4′-diaminoazobenzene was collected, dried and subsequently dissolved in a solution of HCl (1N). From this solution, the hydrochloride of 4,4′-diaminoazobenzene was precipitated by addition of concentrated HCl. From a solution of the hydrochloride, the free base was precipitated by addition of NaOH; 4,4′-diaminoazobenzene was then recrystallised from ethanol.

To a solution of 4.25 g (0.02 mol) of 4,4′-diaminoazobenzene in 40 ml of dry pyridine, 5.9 ml (0.06 mol) of methacryoylchloride was added dropwise, while stirring at room temperature. After complete addition, the reaction mixture was heated at 60°C for 1 h. After cooling, the mixture was poured in ice, acidified to pH4, filtered and washed with a saturated solution of sodium bicarbonate, and consequently with water. The crude reaction product was recrystallised from ethanol.

Overall yield: 36.52%

Melting point: 278°C

2.3. Synthesis of methacrylated inulin

Methacrylated inulin (MA-IN) was synthesized and isolated according to the method of Vervoort et al. (1997). In brief, 50 g of dried inulin was dissolved in 200ml of DMF. After dissolution of DMAP (10 mol.% versus fructose units), a calculated amount of glycidylmethacrylate was added, depending on the desired degree of substitution of inulin (DS; defined as the amount of methacryloyl groups per 100 fructose units). The reaction mixture was stirred for 72 h at room temperature after which the reaction product MA-IN was precipitated in isopropanol, followed by a washing cycle with the same solvent. The precipitate was subsequently dissolved in water and dialyzed for 10 days at 4°C against water (MWCO 500; Spectrum Medical Industries, Houston, TX). Prior to the isolation/purification, DMAP was neutralized by HCl in order to prevent alkaline hydrolysis of the methacrylic ester during dialysis. After dialysis, MA-IN was recovered by lyophilisation.

DS was determined with ^{1}H-NMR. The proton signals of the sugar units are situated at a shift ranging from 3.73 to 4.45 ppm. The anomeric proton of the glycosilic unit shows a signal at $\delta = 5.44$ ppm; the methyl group of the methacrylated product gives a signal at $\delta = 1.96$ ppm, and sp2-protons from the double bond give signals at 5.78 and 6.21 ppm. Using the following equation, DS can be determined:

$$DS = 100\left(\frac{7X}{Y+Z}\right)$$

where X represents the mean integral of the signals from sp2-protons of the methacrylated product; Y is the integral of the glucose and fructose protons, and Z is the integral of the anomeric proton. MA-IN with DS of 6, 14, and 20 were synthesized.

2.4. Synthesis of hydrogels

Solutions made up of MA-IN, and various amounts of AIBN, BMAAB, 2-hydroxyethyl methacrylate (HEMA), methacrylic acid (MA) or methyl methacrylate (MMA) in DMF were purged for 15 min with nitrogen in an oxygen free workstation (DW Scientific, West Yorkshire, UK). A total of 150 μl of the solution was transferred in a closed cylindrical polypropylene mould and placed at 60°C during 8 h. After synthesis, the gels were isolated and washed several times with DMF and subsequently with demineralized water to remove DMF. The time interval between two consecutive solvent changes was 12 h. After the washing procedure, the gels were dried in air to constant weight and stored until further use.

2.5. Determination of the azo content in the hydrogels by the Kjeldahl method

A total of 1 g of hydrogel (or an equivalent quantity) was suspended in a Kjeldahl destruction tube in 20 ml of a mixture of sulfuric acid and phosphoric acid (95:5; v/v), subsequently 15 ml of hydrogen peroxide was added, and finally 15 g of

a mixture of potassium sulfate and copper sulfate (15:35). The total mixture was then heated at 420°C for 30 min. Subsequently, the mixture was cooled to 100°C, NaOH (40% w/v) was added and the produced ammonia was steam distilled, captured in 25 ml of a 4% solution of boric acid, and titrated with sulfuric acid (0.02 M).

2.6. Characterisation of the hydrogels by determination of the dynamic and equilibrium degree of swelling

After synthesis, the gels were washed as described above, and dried to constant weight at room temperature. After recording of the mass of the dried gels (W_d), they were immersed in the swelling medium. At regular time intervals the gels were removed from the swelling medium, blotted with tissue paper to remove free water from the gel surface, and the weight of the swollen hydrogels (W_s) was determined. The swelling ratio (q) was calculated according to:

$$q = \frac{W_s}{W_d}.$$

When the hydrogels reached a constant mass, i.e. when no sorption of the swelling medium did occur anymore, the swelling ratio was considered to be the equilibrium swelling ratio.

In order to study the influence of the synthesis parameters on the degree of swelling, gels were prepared in which the following parameters were varied: MA-IN feed concentration (15; 22.5; 30% w/w), reaction time at 60°C (4, 8, 16 h), concentration of AIBN (0.5, 1.0, 2.0% w/w), concentration of BMAAB in the reaction mixture (0, 0.5, 1.0 equivalents, calculated towards MA-IN).

The effect of salt concentration on hydrogel swelling behaviour was studied by soaking the hydrogels in NaCl solutions in a concentration range between 0.01 and 0.4 M.

The influence of pH on the swelling behaviour of gels containing MA was studied by immersing the gels in a 0.2 M NaCl solution, the pH of which was adjusted with NaOH or HCl. In order to study the swelling behaviour at pH 8, gels were soaked in 0.2 M phosphate buffer.

The influence of temperature on the swelling behaviour of the hydrogels was studied by immersing the gels at 25, 35, and 45°C.

2.7. Release of prednisolone from the hydrogels

Hydrogels were loaded with prednisolone (Bufa, Belgium) by soaking the gels in a saturated solution of prednisolone in water-ethanol (70:30, v/v) for 72 h while gently shaking. Subsequently, the gels were removed from the solution, blotted with tissue paper to remove the solution from the surface of the gels, dried to constant weight and stored in air till further use.

The release study of the drug from the hydrogels was carried out in 200 ml of phosphate buffer (0.05 M, pH 6.8). At regular time intervals, 2 ml samples were withdrawn, immediately followed by replacing the release medium with 2 ml of fresh buffer solution. The concentration of prednisolone in the release medium was determined using an HPLC system equipped with a L-7000 LaChrom pump, a L-7400 LaChrom UV detector set at 242 nm, a D-7000 interface, and a model L-7200 injecting system (all from Merck-Hitachi, Darmstadt, Germany). UV signals were monitored and peaks were integrated using the D-7000 HSM software. The column used was a LiChrospher 60 RP Select B (5 μm, 12.5 × 0.4 cm) (Merck, Darmstadt, Germany). The flow rate was 1 ml/min, and the injected volume 20 μl. The mobile phase consisted of acetonitrile–water (25:75; v/v). Concentrations were calculated using calibration curves made up of standard solutions of known concentrations.

3. Results and discussion

Inulin was derivatized with glycidylmethacrylate in order to incorporate polymerizable vinyl groups in the polysaccharide backbone. The methacrylation of inulin was based upon a method which was originally developed by Van Dijk-Wolthuis et al. (1995) for derivatization of dextran, and subsequently modified by Vervoort et al. (1997) for inulin. Methacrylic groups are necessary to prepare inulin based hydrogels by

free radical solution polymerization. Using ^{1}H-NMR, the degree of substitution was determined. The efficiency of the coupling reaction, calculated as the ratio of the obtained DS to the theoretical value ranged between 60 and 70%. DS obtained was 6 (theoretical value 10); 14 (theoretical value 20), and 20 (theoretical value 30).

Hydrogels with a MA-IN backbone and different groups (HEMA, MA, BMAAB, MMA) were obtained by free radical polymerisation of a solution of MA-IN and the other monomers in DMF. In contrast to the method developed by Vervoort et al. (1997), DMF was used as the polymerisation solvent instead of water, since BMAAB and MMA are water insoluble monomers. The resulting hydrogels were orange coloured and transparent. Hydrogels based on MA-IN with a DS of 14 were fragile and difficult to manipulate in order to avoid fragmentation, while no gelification could be observed using MA-IN with DS of 6. As a matter of fact, only gels based on MA-IN with a DS 20 could be handled without danger of rupturing or fragmentation.

The efficiency of the incorporation of BMAAB in the hydrogels was analysed using the Kjeldahl method and ranged between 75 and 94% of the theoretical value, calculated as the theoretical fraction of BMAAB in the dried hydrogel.

In order to optimize hydrogel synthesis and to pinpoint the factors in the synthesis procedure that determine the swelling properties of the gels, the influence of the following parameters on the water uptake was investigated: feed concentration of MA-IN in the DMF solution, polymerisation time and concentration of AIBN and BMAAB. The results are depicted in Fig. 1, and it is clear that in general AIBN concentration and polymerisation time do not significantly affect the equilibrium degree of swelling. However, a higher concentration of initiator affects the kinetics of

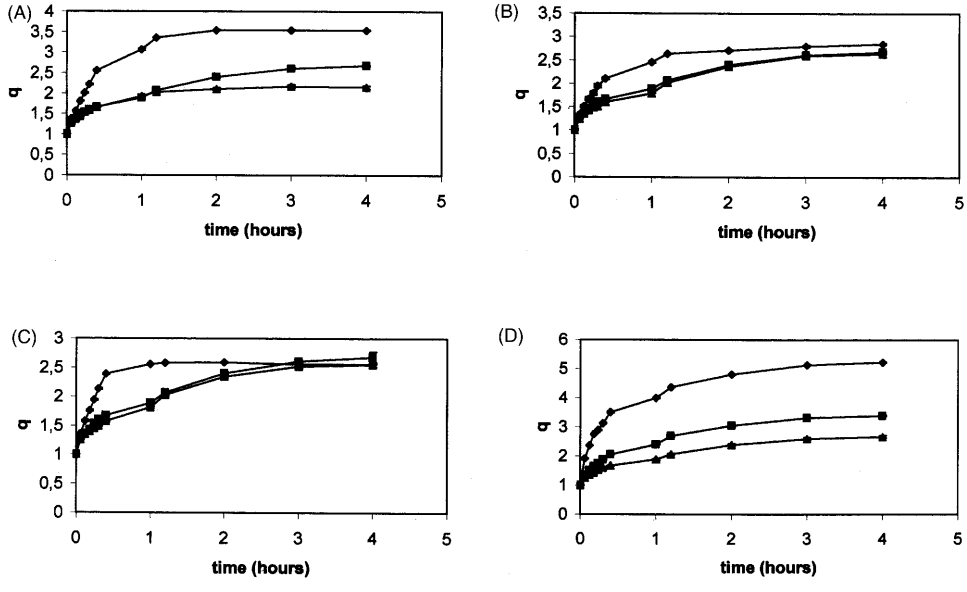

Fig. 1. Degree of swelling of hydrogels as a function of the residence time in demineralized water ($n = 3$). (A) Influence of MA-IN feed concentration (◆, 15% w/w; ■, 22.5% w/w; ▲, 30% w/w), polymerisation time = 8 h, polymerisation temperature = 60°C; DS = 14; AIBN concentration = 1% w/w; BMAAB concentration = 1 equivalent. (B) Influence of polymerisation time (◆, 4 h; ■, 8 h; ▲, 16 h), MA-IN feed concentration = 22.5% w/w, polymerisation temperature = 60°C; DS = 14; AIBN concentration = 1% w/w; BMAAB concentration = 1 equivalent. (C) Influence of AIBN concentration (◆ 0.5% w/w; ■, 1% w/w; ▲ = 2% w/w), polymerisation time = 8 h, polymerisation temperature = 60°C; DS = 14; BMAAB concentration = 1 equivalent, MA-IN feed concentration = 22.5% w/w. (D) Influence of BMAAB concentration (◆, 0 equivalents; ■, 0.5 equivalents; ▲, 1 equivalent), polymerisation time = 8 h, polymerisation temperature = 60°C; DS = 14; AIBN concentration = 1% w/w; BMAAB concentration = 1 equivalent, MA-IN feed concentration = 22.5% w/w.

Table 1
Influence of NaCl concentration and temperature on hydrogel diffusional exponent[a]

Hydrogel	*n*					
	0.02 M/25°C	0.1 M/25°C	0.2 M/25°C	0.4 M/25°C	0.2 M/35°C	0.2 M/45°C
A1	0.65 (0.01)	0.69 (0.10)	0.64 (0.01)	0.66 (0.01)	0.61 (0.01)	0.57 (0.04)
A1H0.1	0.66 (0.00)	0.69 (0.02)	0.63 (0.02)	0.62 (0.03)	0.67 (0.02)	0.59 (0.03)
A1H1	0.59 (0.02)	0.62 (0.01)	0.64 (0.02)	0.62 (0.01)	0.64 (0.02)	0.56 (0.01)
A1MA0.05	0.62 (0.01)	0.64 (0.04)	0.62 (0.06)	0.66 (0.05)	0.59 (0.02)	0.55 (0.01)
A1MA0.5	0.64 (0.01)	0.60 (0.04)	0.62 (0.01)	0.63 (0.02)	0.63 (0.01)	0.60 (0.00)

[a] A1, 1 equivalent of BMAAB; H1, 1% w/w of HEMA; H0.1, 0.1% w/w of HEMA; MA0.05, 0.05% w/w of MA; MA0.5, 0.5% w/w of MA. Standard deviations are indicated in parenthesis.

the solvent uptake process. Indeed, a higher contribution of the initiator in the beginning of the polymerization decreases the relative contribution of the propagation step, leading to slightly different network structure. Hydrogels which were isolated after a reaction time of 4 h showed a marginally higher value of q indicating that the reaction was not completely finished after 4 h, and a less dense network results. On the other hand, the MA-IN feed concentration (30, 22.5, 15% w/w) significantly influences the hydrogel network. A low polymer feed concentration promotes intramolecular crosslinking, since the probability of bond formation between different chains is proportional to the probability that these bonds lie in the same small volume element (James and Guth, 1947; Yeh et al., 1995). This implicates that less chains can be incorporated in the hydrogel network of low feed concentration (increased sol fraction). The resulting network will exhibit restricted rigidity because of the limited amount of intermolecular crosslinking. As a consequence, hydrogels of low feed concentration exhibit less restriction to water penetration, and are therefore characterized by a higher equilibrium degree of swelling.

The rationale for the development of the inulin-azo hydrogels was to combine the systems developed by Van den Mooter et al. (1992, 1993) with those developed by Vervoort et al. (1997). The implication of the incorporation of the azo agent is shown in Fig. 1(d). Both the rate of water uptake as well as the equilibrium degree of swelling decreased with increasing azo concentration in the hydrogels, which can be explained as the consequence of the hydrophobic nature of BMAAB in combination with the increased crosslinking density. Compared to the pure MA-IN hydrogels, q decreased with a factor two if one equivalent of BMAAB was incorporated. This will obviously influence both the rate of azoreduction as well as the rate of hydrolysis as already described by Van den Mooter et al. (1993, 1994), Vervoort (1998).

In order to compensate for the decreased water uptake, other monomers such as HEMA and MA were incorporated. The kinetics of the uptake of sorption medium in these hydrogels was analyzed using the power law expression of Ritger and Peppas (1987):

$$\frac{M_t}{M_\infty} = kt^n$$

where n is the diffusional exponent which is indicative of the mechanism of water transport in the hydrogel, k is a kinetic constant characteristic for the polymer system, M_t is the mass of the solvent sorbed at time t and M_∞ is the mass of solvent sorbed at equilibrium. For cylindrical samples, solvent sorption is considered to be Fickian, i.e. diffusion controlled if $n = 0.45$, relaxation controlled if $n = 0.89$, and anomalous, i.e. diffusion and relaxation controlled if $0.45 < n < 0.89$. Using the initial stage of swelling (up to 60% of the sorbed medium necessary to reach equilibrium), the diffusional exponent was calculated from the slope of a plot of $\ln M_t/M_\infty$ versus $\ln t$; the results of which are given in Table 1. Since

$0.45 < n < 0.89$ for all hydrogels tested, irrespective of the salt concentration or the temperature of the sorption medium, solvent uptake is considered to be anomalous, which is common for glassy hydrogels which become rubbery upon water uptake (Gehrke and Lee, 1990). In order to further characterize the swelling rate, the mean swelling time (MST) was calculated (Möckel and Lippold, 1993):

$$MST = \frac{n}{n+1} k^{-\frac{1}{n}}$$

A significant influence of the temperature of the sorption medium could be observed (Fig. 2); no clear influence of the ionic strength could be observed (data not shown). This prompted us to synthesize hydrogels in which the ratio of HEMA and MA was varied in a wider range. These hydrogels were characterized by their equilibrium degree of swelling. The results of swelling experiments are reported in Table 2. Surprisingly, the incorporation of HEMA did not increase q, but led to a decrease which was proportional to the amount of HEMA incorporated in the hydrogels. Although HEMA is clearly a hydrophilic monomer, the increased crosslinking density as a consequence of HEMA incorporation might at least partially explain this behaviour. The same observation was noticed for hydrogels containing MA. The incorporation of this monomer should enable us to regulate swelling in the gastro-intestinal tract since the pKa of polyMA is 5.65 (Ranjha and Doelker, 1999), hence deprotonation in the distal part of the small intestine and colonic environment will lead to higher swelling ratio and hence higher accessibility of degradable bonds, whereas premature swelling (hence drug release) in the stomach and proximal part of the small intestine can be avoided. From Table 2, it is clear that incorporation of MA indeed leads to a pH dependent increase of q, but the influence of pH was less than expected. Again, the increased network density, can be responsible, while on the other hand increased hydrogen bonding possibilities may also contribute. Indeed, increased

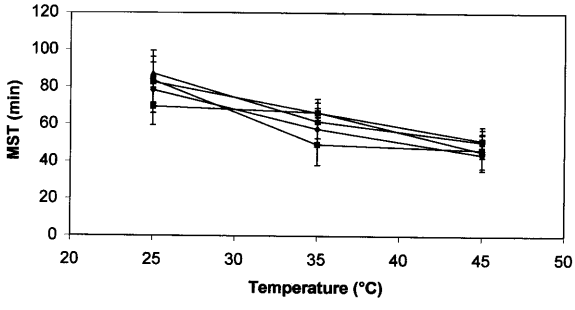

Fig. 2. Influence of temperature on MST in 0.2 M NaCl ($n = 3$). ■, A1; ◆, A1H0.1; ●, A1H1; –, A1MA0.05; ▲, A1MA0.5 (A1 = 1 equivalent of BMAAB; H1 = 1% w/w of HEMA; H0.1 = 0.1% w/w of HEMA; MA0.05 = 0.05% w/w of MA; MA0.5 = 0.5% w/w of MA).

Table 2
Equilibrium degree of swelling of different MA-IN hydrogels[a]

Hydrogel	DS	pH	q
A0	20	7.0	5.76 (0.18)
A1	20	7.0	3.03 (0.01)
A0H1	20	7.0	5.61 (0.13)
A0H2.5	20	7.0	5.26 (0.09)
A0H5	20	7.0	4.74 (0.28)
A0H10	20	7.0	4.16 (0.08)
A0MA0.5	20	3.0	5.47 (0.09)
		5.0	5.47 (0.18)
		8.0	5.97 (0.01)
A0MA2.5	20	3.0	4.79 (0.12)
		5.0	4.63 (0.14)
		8.0	4.88 (0.08)
A0MA5	20	3.0	4.27 (0.11)
		5.0	4.29 (0.04)
		8.0	4.65 (0.02)
A0MA10	20	3.0	3.78 (0.02)
		5.0	3.88 (0.05)
		8.0	4.70 (0.01)
A0MA20	20	3.0	3.07 (0.02)
		5.0	3.13 (0.01)
		8.0	4.50 (0.02)
A0MA2.5	14	3.0	9.00 (0.19)
		5.0	9.12 (0.19)
		8.0	9.64 (0.37)
A0MA2.5MMA0.5	20	3.0	4.90 (0.06)
		5.0	4.75 (0.02)
		8.0	5.12 (0.03)

[a] A0, no BMAAB; A1, 1 equivalent of BMAAB; H1, 1% w/w of HEMA; H2.5, 2.5% w/w of HEMA; H5, 5% w/w of HEMA; H10, 10% w/w of HEMA; MA0.05, 0.05% w/w of MA; MA0.5, 0.5% w/w of MA; MA2.5, 2.5% w/w of MA; MA5, 5% w/w of MA; MA10, 10% w/w of MA; MA20, 20% w/w of MA; MMA0.5, 0.5% w/w of MMA. Standard deviations are indicated in parenthesis.

crosslinking density will reduce the network elasticity, and ionisation of carboxylic groups of MA, clearly is not sufficient to induce a significant pH dependent swelling of the hydrogels. If increased hydrogen bonding impedes network swelling, then incorporation of monomers that disturb hydrogen bonding should lead to a higher equilibrium degree of swelling. In a next set of experiments, hydrogels were prepared with the following composition: MA-IN:MA:MMA (100:2.5:0.5; w/w/w). As shown in Table 2, q increased for these hydrogels which confirms the influence of hydrogen bonding on q. Another option to increase water uptake of the hydrogel is to use MA-IN with a lower DS. As shown in Table 2, q did indeed increase significantly when DS 14 was used. Unfortunately, as already mentioned, these gels were difficult to manipulate and of little practical use in the development of a drug delivery system.

The dependency of the equilibrium degree of swelling of the hydrogels on the concentration of the incorporated monomers prompted us to investigate whether incorporation of these hydrophilic monomers would influence drug release. The release of prednisolone was monitored from MA-IN hydrogels with BMAAB, and with two different concentrations of MA. The obtained release profiles were then compared to that of a hydrogel containing HEMA instead of MA, in order to point out the influence of the type of monomer on the drug release. The influence of incorporation of BMAAB was investigated by comparing the release profile of the HEMA hydrogel with the release profile of an analogous hydrogel, but without BMAAB. Since it was shown that too high an amount of HEMA or MA negatively influences the swelling properties of the gels, in this experiment gels were selected in which the concentration of HEMA or MA did not exceed 1 or 0.5% w/w, respectively.

A typical release profile of a MA-IN hydrogel with 1 equivalent if BMAAB and 1% HEMA is given in Fig. 3(A). In order to demonstrate the influence of the presence of BMAAB in the gels, the release profile of prednisolone from a hydrogel without BMAAB is given in the same figure. Approximately 19% of prednisolone was released after 30 min from the azo containing hydrogel; the amount increased up to 56% after 4 h and 80% after 8 h. From the hydrogel without azo the release occurs even faster. Considering the transit in the gastro-intestinal tract (Rubinstein et al., 1988), this indicates that a major part of the drug will be released before the colon is reached. The release of prednisolone from BMAAB-MA-IN hydrogels containing MA is shown in Fig. 3(B). The rate of prednisolone release was slightly higher than that obtained with gels containing HEMA, and the difference between gels containing 0.5 and 0.05% w/w of MA was very small. The release rates from these hydrogels are inversely proportional to their equilibrium degree of swelling in 0.2 M NaCl, which have been reported by Maris et al. (2000). If the main concern is the high release rate during the first part of the experiment, using hydrogels that have a lower equi-

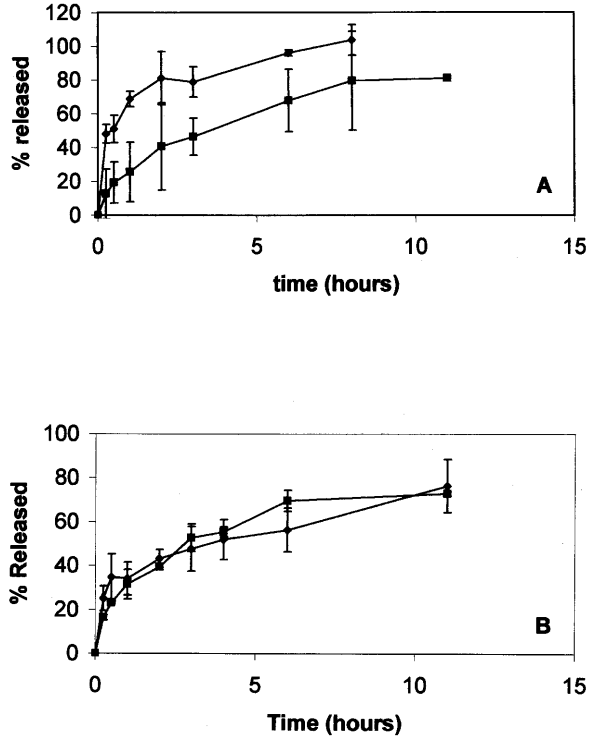

Fig. 3. Release of prednisolone in phosphate buffer pH 6.8 ($n = 3$) (A) ◆, A0H1; ■, A1H1; B: ◆, A1MA 0.05; ■, A1MA0.5 (A0 = no BMAAB; A1 = 1 equivalent of BMAAB; H1 = 1% w/w of HEMA; MA0.05 = 0.05% w/w of MA; MA0.5 = 0.5% w/w of MA).

librium degree of swelling would probably solve this problem, and based upon the results of the swelling experiments, it should be possible to tune the release by the HEMA or MA concentration in the gels. However, the other major concern is that degradable bonds in the hydrogels are accessible to the enzymatic system responsible for hydrogel degradation in the colonic environment, and this clearly implicates that the hydrogels need a sufficient degree of swelling. Preliminary experiments showed that hydrogels made up of MA-IN and BMAAB were not significantly degraded in the colonic environment, probably due to a lack of hydrophilicity. It is of utmost importance to find a balance between degradability and avoidance of premature drug release. The incorporation of ionisable groups such as MA was considered for that purpose. Unfortunately, the increased crosslinking density as a consequence of its incorporation clearly reduces the elasticity of the network and ionisation of the carboxylic groups is insufficient to induce a significant pH dependent swelling of the hydrogels.

In order to overcome this problem, new hydrogels are currently under investigation in which the ionisable groups are a part of the backbone without leading to increased network density.

4. Conclusion

The incorporation of BMAAB in MA-IN hydrogels, a strategy to increase hydrogel degradability in the colonic environment, led to decreased hydrophilicity and increased crosslinking density as shown by its equilibrium degree of swelling. Copolymerisation with hydrophilic monomers such as HEMA and MA did not increase the equilibrium degree of swelling, which is probably caused by increased network density. This could also explain, together with increased hydrogen bonding, the negligible pH dependency of gels containing MA.

Release studies using prednisolone as a model drug clearly showed the necessity to find a balance between avoidance of premature drug release caused by too high a degree of swelling, and on the other hand sufficient swelling necessary for accessibility of degradable bonds.

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