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Synthesis and Characterization of Methacrylic Derivatives of 5-Amino Salicylic Acid with pH-Sensitive Swelling Properties

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ABSTRACT The purpose of this study is to develop novel colon-specific drug delivery systems with pH-sensitive swelling and drug release properties. Methacrylic-type polymeric prodrugs with different content levels of 5-amino salicylic acid (5-ASA) were synthesized by free radical copolymerization of metacrylic acid (MAA), polyethylene glycol monomethacrylate (PEGMA), and a methacrylic derivative of 5-ASA (methacryloyloxyethyl 5-amino salicylate [MOES]). The copolymers were characterized, and the drug content of the copolymers was determined. The effect of copolymer composition on the swelling behavior and hydrolytic degradation was studied in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.2). The swelling and hydrolytic behavior of the copolymers was dependent on the content of MAA groups and caused a decrease in gel swelling in SGF or an increase in gel swelling in SIF. Drug release studies showed that increasing content of MAA in the copolymer enhances the hydrolysis in SIF but has no effect in SGF. The results suggest that hydrogen-bonded complexes are formed between MAA and PEG pendant groups and that these pH-sensitive systems could be useful for preparation of a controlled-release formulation of 5-ASA.

KEYWORDS: 5-ASA, Hydrogel, Hydrolysis, Methacrylic copolymers, Colon targeting.

INTRODUCTION

Orally administered colonic delivery systems are difficult to design because of the anatomic location of the colon at the end of the alimentary canal. This presents a formidable challenge in targeting drugs specifically to this site for local absorption so that systemic absorption is reduced and painful treatments, such as enemas, can be avoided. Local treatment of inflammatory bowel disease, for example, would increase the efficiency of drugs used in its treatment, such as salicylic acid derivatives (eg, 5-amino salicylic acid [5-ASA] and mesalazine).

Colonic azoreductase is used in a number of prodrugs, polymeric coatings, and cross-linked hydrogels [1] . These systems are aimed primarily at the specific release of 5-ASA in the colon. The interest in 5-ASA delivery systems stems from the successful use of sulfasalazine in the treatment of inflammatory bowel disease. Sulfasalazine is a combination of a salicylate and a sulfonamide linked by azo bonds. The recognition that 5-ASA is the active moiety in mesalazine has led to the replacement of mesalazine by sulfapyridine as a carrier entity in 5-ASA therapy.

Another approach that can be used in colon-specific delivery is to attach 5-ASA via an azo bond to a polymeric carrier [2-4]. Advantages such as nonabsorption from the small intestine, elimination of sulfapyridine-produced side effects, and nonabsorption of the polymeric cleavage products were claimed for some of the water-soluble polymeric prodrugs containing 5-ASA residue with azo linkage to the polymer backbone. Publications show that azo polymer-based colonic drug targeting presents some problems [5,6] ; it seems that microbial degradation of the investigated azo polymers is slow. On the other hand, azo aromatic compounds include several substances that are known to be carcinogens [6] . A system that would protect the drugs from the gastric environment yet allow release and absorption in the colon would provide a significant advantage in colon-specific drug delivery. These requirements have prompted the development of polymeric systems that swell minimally under acidic conditions but extensively in basic intestinal medium [7,8] .

Responsive hydrogel networks consisting of polymethacrylic acid (PMAA) and polyethylene glycol (PEG) are classic examples of pH-sensitive carriers that exhibit swelling transitions in response to changes in pH [9,10] . PEG and PMAA may be associated to form

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hydrogen-bonded complexes under acidic conditions. A hydrogel containing a backbone of PMAA and grafts of PEG exhibits a relatively low degree of swelling under complex-promoting conditions (low pH when acid is protonated) and a high degree of swelling when the complex is broken (high pH when the acid is neutralized) [11]. Several hydrogel networks, including a PMAA backbone and PEG grafts, were synthesized and used to protect sensitive drugs from proteolytic degradation in the stomach and upper portion of the small intestine. In all cases, the drug was physically trapped in a polymer matrix, and drug release occurred via diffusion from the interconnected hydrogel matrix [12,13] .

In the present study, the synthesis and swelling properties of poly (methacrylic acid-co-ethyleneglycol monomethoacrylate-co-methacryloyloxyethyl 5-amino salicylate) (poly [MAA-co-EGMA-co-MOES]) are reported. A previous study reported on the synthesis and copolymerization of methacryloyloxyethyl 5-amino salicylate (MOES) with methacrylic acid (MAA) (poly [MOES-co-MAA]) or hydroxyethyl methacrylate (HEMA) (poly [MOES-co-HEMA]) [14] . In this research, the synthesis of a pH-sensitive hydrogel network consisting of MOES, MAA, and polyethylene glycol monomethacrylate (PEGMA) is described. Complex-forming constituents of the hydrogel were covalently linked to each other and to the drug-linked monomer, and the swelling characteristics and drug release properties of the hydrogel were studied in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.2).

MATERIALS AND METHODS

Chemicals

HEMA, 5-ASA, MAA, and N,N-dicyclohexyl carbodiimide (DCC) were from Merck (Whitehouse Station, NJ). PEGMA (with molecular weight of 400) and benzoyl peroxide were purchased from Sigma Chemical Co. (St Louis, MO).

Synthesis of methacryloyloxyethyl 5-amino salicylate (MOES)

A solution of 0.03 M of DCC in 40 mL of N,N-dimethylformamide (DMF) was added dropwise at -20°C to a solution of 0.03 M of 5-ASA and 0.003 M of dimethylaminopyridine (DMAP) dissolved in 50 mL of DMF. A solution of 0.03 M HEM in 20 mL DMF was added to the mixture at -20°C. The reaction mixture was slowly returned to room temperature and stirred for 3 hours. The white precipitation of dicyclohexylurea was filtered, and DMF solution was extracted by 10 wt% of $NaHCO_3$ 3 times. The extracted solution was dried over $MgSO_4$ and the solvent evaporated in vacuum. The oily residue was solidified by cold methanol and recrystallized from methanol to yield 61% MOES. Fourier transform infrared spectroscopy (KBr , cm^{-1}); 1725 , 1730 (C = O), 1635 (C = C), 1160 (C-O), ^{1} H - NMR (DMSO - d6 , T) 1.85 (s,3H, C = C - CH3), 4. 25 (s, 4H, OCH_2 CH_2 O), 6.6-7.1 (m, 3H, Ar-H). Analysis: Calculated for C_{13} H_{15} NO_5 , C 58.8, H 5.66, N 5.28. Found C 57.92, H 5.21, N 5.98.

Preparation and Properties of Copolymers

Drug-linked copolymers were synthesized by copolymerization of MAA, PEGMA, and MOES in a solution of methanol with a variable feed ratio as shown in **Table 1**. Copolymerization was carried out in the presence of benzoyl peroxide as an initiator. Each mixture was stirred for 6 hours at 60°C under nitrogen. The copolymers obtained were precipitated with acetone, centrifuged at 3000 rpm for 2 hours, and washed twice with a mixture of ethanol-acetone (1/1 vol/vol).

Table 1. Characterization of Polymers

Sample	Feed Composition (mmol/L)[a]			MOES[b]	MAA	5-ASA	Molecular
MOES	MAA	PEGMA	(mmol/g)	(mmol/g)	(g/100)	Weight	
1P	1	18	1	0.05	7.83	12.08	52,000
2P	2	14	4	0.083	6.01	20.04	48,000
3P	3	16	1	0.098	7.48	21.72	51,000
4P	5	11	4	0.195	4.45	22.79	49,000

MOES = methacryloyloxyethyl 5-amino salicylate; MAA = metacrylic acid; PEGMA = polyethylene glycol monomethacrylate; 5-ASA = 5-amino salicylic acid.

[a]The weight ratio of monomers to initiator and solvent was 12.5 : 0.5 : 8.7. Copolymerization was performed in methanol at 60°C for 6 hours under nitrogen atmosphere using benzoyl peroxide.

[b]Results obtained by UV are the mean of 3 measurements. Standard deviation of the measurement was ±4%.

The copolymers were characterized by the content of aromatic ester (MOES) and carboxylic groups (MAA). The content of aromatic ester was determined by measuring UV absorbance at 240 nm. The content of 5-ASA pendant groups was determined by nitrogen analysis and an exhaustive hydrolysis method. The hydrolysis was performed in a dialysis tube at pH 10 and 70°C for 24 hours. The quantity of released drug was measured by means of a UV spectrophotometer (2100 Shimadzu, Kyoto, Japan). The results were compatible with each other. The carboxylic acid content of copolymers was determined according to the method described in the literature [10] . The molecular weight of polymers was measured by gel permeation chromatography (GPC) using a Waters model 150-C (Milford, MA) containing 4 Shodex GPC KF-800 series columns (SDK, Tokyo, Japan). DMF was used as the eluent at a flow rate of 1 mL/min. The column setting was calibrated by using well-characterized polyethylene oxide within the range of 2600 to 885 000 MW.

Measurement of Swelling Ratio

The hydrogels were characterized by the equilibrium swelling ratio. Dried copolymers were allowed to swell in 50 mL of enzyme-free SGF (pH 1.2) or SIF (pH 7.2) at 37°C. SIF and SGF were prepared according to the method described in the *US Pharmacopeia* [15] . At a specific time, the hydrogel was removed from the swelling medium and the excess solution on the swollen sample was absorbed by gentle tamping between filter paper. The sample was weighed, and the procedure was repeated until a constant weight was achieved. The swelling ratio (Q) of hydrogels was calculated according to the expression

$$Q = Ws / Wd, \quad (1)$$

where Ws is the weight of the swollen hydrogel and Wd is the weight of dry hydrogel. The weights were calculated as the +SD from 3 independent experiments.

Method of Hydrolysis

The copolymers (200 mg) were poured into 5mL of SGF (pH 1.2) or SIF (pH 7.2). The mixture was introduced into a cellophane membrane dialysis bag. The bag was closed and transferred to a flask containing 25 mL of the same solution maintained at 37oC. The external solution was continuously stirred, and 3 mL samples were removed at selected intervals. The volume removed was replaced with SGF or SIF. Triplicate samples were used. Concentrations of 5-ASA were determined at 300 nm for pH 1.2 and 328 nm for pH 7.2 using a 2100 Shimadzu UV-VIS spectrophotometer.

RESULTS AND DISCUSSION

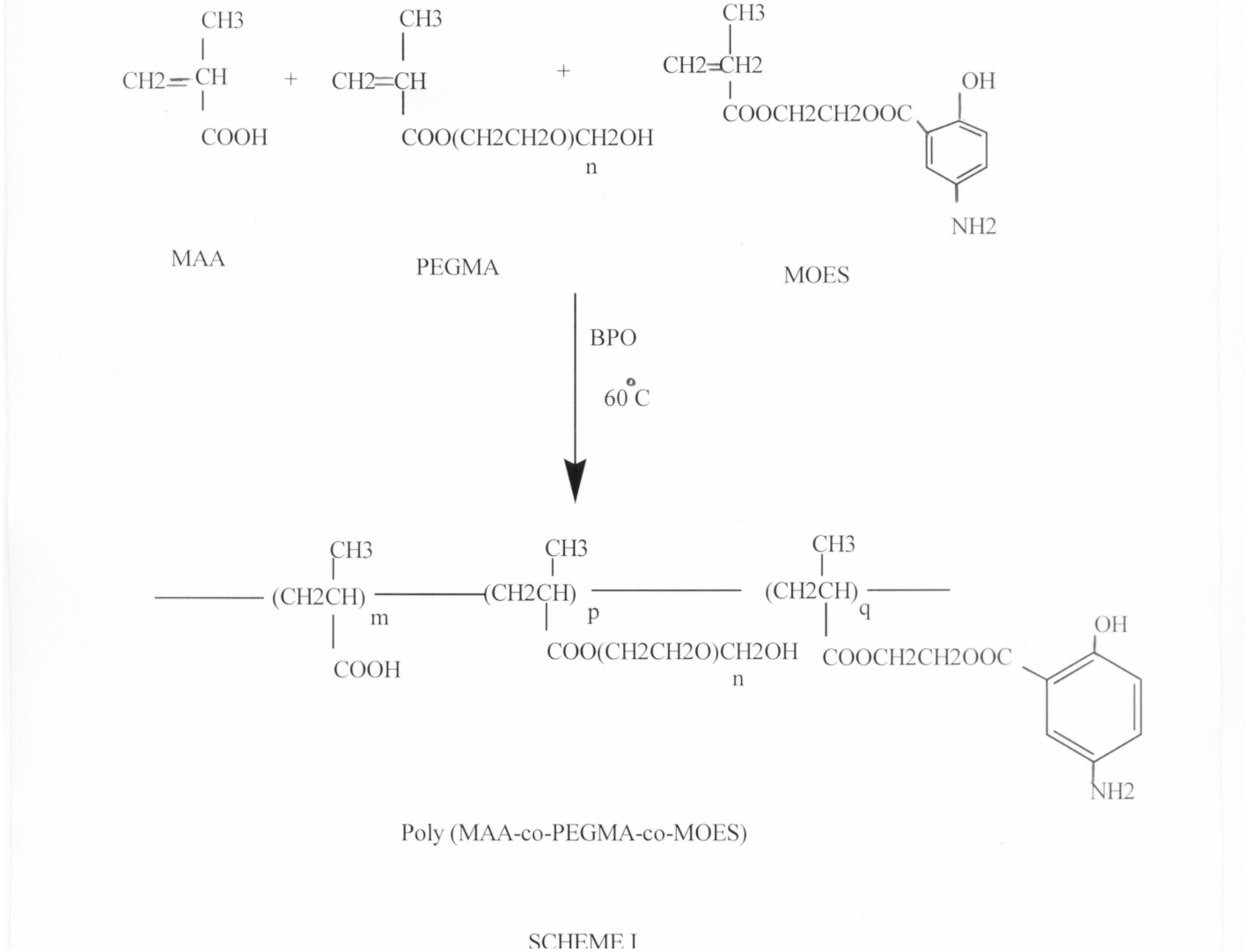
Synthesis of Copolymers Having 5-ASA Pendant Groups

MOES was prepared by direct esterification of 5-ASA with HEMA in the presence of DCC-DMAP in DMF solution. When hydrophilic polymers containing methacryloyloxyethyl-type monomers are to be prepared, copolymerization with water-soluble monomers is one possible approach. In the present case, MOES and PEGMA were copolymerized with MAA in the presence of benzoyl peroxide as an initiator at 60°C for 6 hours in methanol after degassing in a sealed tube (**Scheme 1**). When the water-soluble monomers (PEGMA and MAA) were rich in the feed, a water-swellable graft copolymer resulted. When the hydrophobic monomer (MOES) was rich in the feed, the resulting copolymers were well dispersed in water and methanol.

An increase in the amount of MAA in the feed ratio produces water-soluble copolymers that were, however, not collected due to their solubility under the present conditions. **Table 1** shows that samples 2, 3, and 4 produced water-swellable hydrogels with a varying amount of drug-linked monomer in the copolymer composition and that sample 1 was soluble in SIF.

pH-Sensitive Swelling Studies

The equilibrium swelling ratio, Q, defined as the volume of the equilibrium swollen gel divided by the volume of the same gel before swelling, was found to be influenced by pH (**Figure 1**). At the low pH of SGF (pH 1.2), the hydrogels were un-ionized and the swelling ratio was low (relevant values of Q for samples 1P-4P were 1.65, 1.22, 1.41, and 1.37 in SGF after 4 hours-presumably near-equilibrium state). At a higher pH (7.2 in SIF), the swelling reached equilibrium rapidly, which could be attributed to the higher degree of ionization (values of Q for samples 1P-4P were 3.01, 1.63, 2.22, and 1.46 in SIF after 4 hours). The equilibrium swelling was dependent on the content of carboxylic acid groups and the content of hydrophobic monomer (MOES). As shown in **Figure 1** , an increase in the content of MAA groups resulted in less swelling in SGF but greater swelling in SIF.



Poly (MAA-co-PEGMA-co-MOES)

SCHEME I

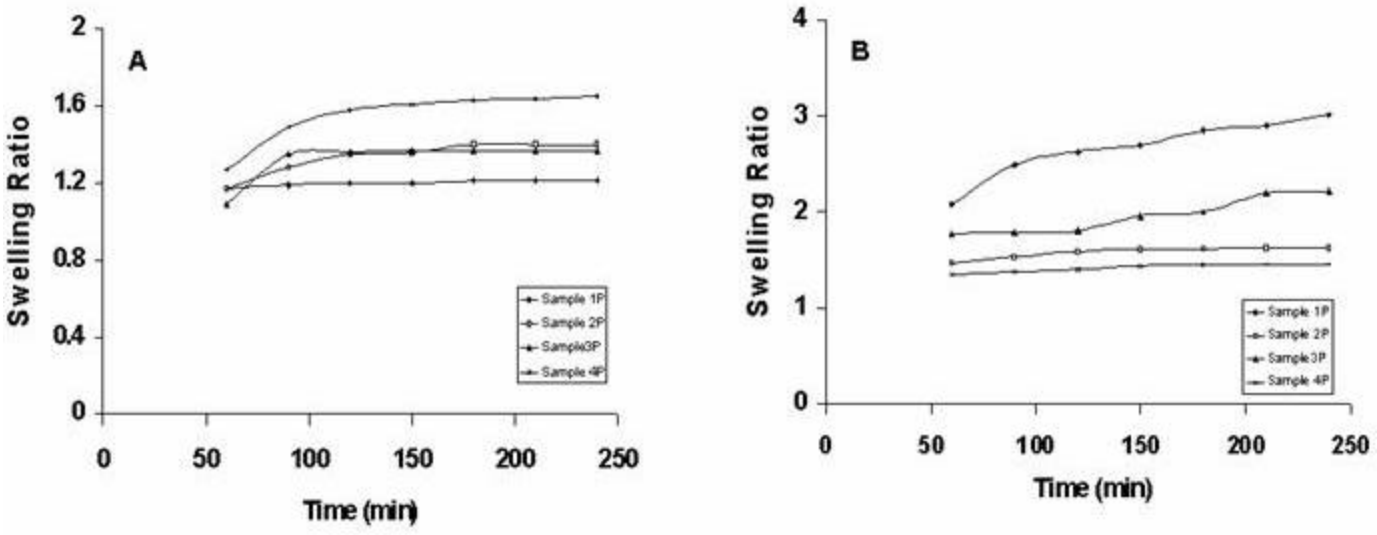


Figure 1. The change in the swelling index (Q) of copolymers as a function of time in (A) simulated gastric fluid (SGF, pH 1.0) and (B) simulated intestinal fluid (SIF, pH 7.2) at 37ºC (mean ± SD, n = 3).

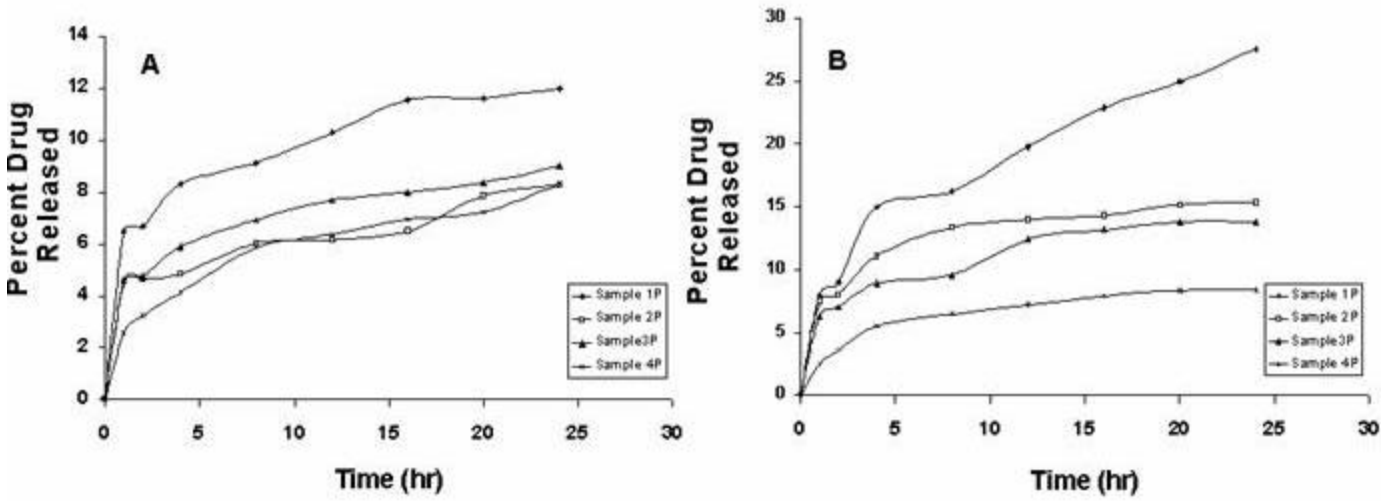


Figure 2. Release of 5-ASA from hydrogels in (A) simulated gastric fluid (SGF, pH 1.0) and (B) simulated intestinal fluid (SIF, pH 7.2) at 37°C.

In a previous study, we reported the synthesis and swelling properties of poly (MOES-co-MAA) units [14] in which the weight percent of MAA units in the copolymer was similar to that of sample 1P.

The value of Q was about 1.34 for poly (MOES-co-MAA) and about 1.4 for sample 1P at pH 1.2. In SIF (pH 7.2), the equilibrium swelling ratio of poly (MOES-co-MAA) was 1.4 compared with the swelling ratio of 3.01 for sample 1P.

These results indicate that the incorporation of hydrophilic PEG units increases the swelling ratio in SIF (pH 7.2) but has an inverse effect in SGF (pH 1.2). It seems that hydrogen-bonded complexes may form between the PEG and MAA units of the hydrogel under acidic conditions.

Consequently, the hydrogels exhibit a relatively low degree of swelling in SGF. Furthermore, the insoluble hydrogel formed in SGF dissolves in the presence of ethanol or methanol because the complexation bonds are weakened. The hydrogel exhibits a high degree of swelling in SIF (**Figure 1B**), which could be related to the break of a hydrogen-bonded complex between MAA and PEG units.

As shown in **Figure 1B**, the swelling ratio was higher for the MAA composition (1P) in SIF. It is of interest to note that the equilibrium swelling ratio of 1P was reached after about 4 hours and that beyond this time the copolymer dissolved. After about 4 hours, it was observed that the size of hydrogel was reduced over time, indicating that dissolution was occurring.

Drug Release Studies

Figure 2 shows the release profiles of hydrogels at 37°C in SGF (**Figure 2A**) and SIF (**Figure 2B**) as a function of time. As shown in this figure, the drug release proceeds more efficiently at a higher pH (SIF). Furthermore, increasing content of the MAA units in copolymer enhances the rate of drug release in SIF but has essentially no effect on SGF. The percentage of 5-ASA released from sample 1P was about 27.5%, and it was only 8% from sample 4P after 24 hours in SIF (**Figure 2B**). In comparison, only 12% and 8% of the drug was released after 24 hours in SGF from samples 1P and 4P, respectively.

As shown in **Figure 2B** , sample 1P showed about 15% release after 4 hours in SIF. This initial quick release could be related to the solubility of the hydrogel after 4 hours in SIF. Sample 1P contains the higher composition of ionizable MAA groups, and no cross-linking agents are present. Consequently, during hydrolysis at pH 7.2, the sample dissolves gradually. The hydrolysis of ester bonds is catalyzed by acids and bases, and the ionization of MAA groups increases with the basic conditions. Both factors increase the swelling of hydrogel and thus enhance the rate and extent of drug release in SIF.

The release profile of sample 1P was compared with that of poly (MOES-co-MAA), which had been prepared earlier [14] . The amount of the drug that was released from the poly (MOES-co-MAA) was about 15.5% at pH 1.2 and about 23% at pH 7.2 after 24

hours of hydrolysis in phosphate buffer solution. It was expected that the incorporation of a water-soluble macromonomer such as PEGMA could enhance the extent and rate of drug release, but reverse effects were observed (12% release from sample 1P after 24 hours in SGF) at pH 1.2, compared with those of poly (MOES-co-MAA) with the same content of MAA units. This observation could confirm the formation of a hydrogen-bonded complex between MAA and PEG in blocked-type copolymers, which leads to the relative protection of the drug from hydrolytic degradation in acidic environments (such as SGF).

CONCLUSION

The experiments reported here indicate that these hydrogels can be used in controlled release formulations of 5-ASA. However, a balance must be found in the ratio of drug-containing hydrophobic component (such as MOES), which leads to an appropriate amount of drug loading, and the more hydrophilic components, which ensure good swellability and availability of the ester bond hydrolysis. Results published so far confirm that the PEG-PMAA gels adhere more strongly to the mucosa of the intestine than to the mucosa of the stomach, and this can localize the delivery system in a site-specific manner[9] . However, it is important to design a ratio that provides resistance to the gastric juice and small intestinal fluid for use in colon-selective formulations, and this should be the aim of future investigations.

REFERENCES

1. Van den Mooter G, Maris B, Samyn C, Augustijns P, Kinget R. Use of azo polymers for colon-specific drug delivery. J Pharm Sci. 1997;86(12):1321-1327.

2. Schacht E, Gevaert A, Kenawy ER, Molly K, Verstraete W, Adriaensens P, Carleer R, Gelan J. Polymers for colon specific drug delivery. J Control Release. 1996;39:327-338.

3. Kopeckova P, Kopecek J. Release of 5-amino salicylic acid from bioadhesive N-(2-hydroxypropyl) methacrylamide copolymers by azoreductases in vitro. Makromol Chem. 1990;191:2037-2045.

4. Kopeckova P, Rathi R, Takada S, Rihova B, Berenson M, Kopeck J. Bioadhesive N-(2-hydroxypropyl) methacrylamide copolymers for colon specific drug delivery. J Control Release. 1994;28:211-222.

5. Chung KT, Stevens SE Jr, Gerniglia CE. The reduction of azo dyes by the intestinal microflora. Crit Rev Microbiol. 1992;18:175-190.

6. Chung KT, Gerniglia CE. Mutagenicity of azo dyes: Structure-activity relationship. Mut Res. 1992;277:201-220.

7. Van den Mooter G, Samyn C, Kinget R. The relation between swelling properties and enzymatic degradation of azo-polymers designed for colon-specific drug delivery. Pharm Res. 1994;11(12):1737-1741.

8. McLeod AD, Friend DR, Tozer TN. Glucocorticoid-dextran conjugates as pol-entical prodrugs for colon-specific delivery: Hydrolysis in rat gastrointestinal tract content. J Pharm Sci. 1994:83(9):1284-1288.

9. Lowman AM, Morishita M, Kajita M, Nagai T, Peppas NA. Oral delivery of insulin using pH-responsive complexation gels. J Pharm Sci. 1999;88(9):933-937.

10. Haglund BO, Kenneth RJ, Himmelstein J. An in situ gelling system for parenteral delivery. J Control Release. 1996;41:229-235.

11. Mathur AM, Hammonds KF, Klier J, Scranton AB. Equilibrium swelling of poly (methacrylic acid-g-ethylene glycol) hydrogels: Effect of swelling medium and synthesis conditions. J Control Release. 1998;54:177-184.

12. Peppas NA, Klier J. Controlled release by using poly (methacrylic acid-g-ethylene glycol) hydrogels. J Control Release. 1991;16(1-2):203-214.

13. Peppas NA, Khare AR. Preparation, structure and diffusional behaviour of hydrogels in controlled release. Adv Drug Del Rev. 1993;11(1-2):1-35.

14. Davaran S, Hanaee J, Khosravi A. Release of 5-amino salicylic acid from acrylic type polymeric prodrugs designed for colon-specific drug delivery. J Control Release. 1998;58:279-287.

15. US Pharmacopeial Convention, Inc. The United States Pharmacopeia, 24th ed. Rockville, MD: US Pharmacopeial Convention, Inc.; 1999.