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Synthesis of starch-*g*-poly(acrylic acid-*co*-2-hydroxy ethyl methacrylate) as a potential pH-sensitive hydrogel-based drug delivery system

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The present work focused on the design of a drug delivery system (DDS) based on pH-sensitive hydrogel. The hydrogels were prepared via graft copolymerization of mixtures of acrylic acid (AA) and 2-hydroxy ethyl methacrylate (HEMA) onto starch backbones by a free radical polymerization technique. Sodium bicarbonate ($NaHCO_3$) was added to function as a foaming agent under acidic conditions, rendering the hydrogels to be porous. The porous structure of the hydrogel was essential in this system to yield a large surface area so that 5-fluorouracil (5-FU) release could be facilitated. The hydrogel, thus prepared, possessed a porous structure as determined by scanning electron microscopy.

The water absorbency of the hydrogels was measured in solutions with pH levels ranging from 1 to 13. The starch-based hydrogel exhibited a pH-responsiveness character such that a swelling-deswelling pulsatile behavior was recorded at pH levels of 2 and 7. Using the drug 5-FU as a model molecule, the in vitro controlled drug-release behaviors of these hydrogels were investigated. The results indicate that the main parameter affecting the drug-release behavior of hydrogels is the pH of the solution. The release rate of 5-FU from hydrogel at pH 7.4 was faster than that at pH 1.2 due to the shrinkage of the hydrogel at pH 1.2.

These results suggest that a porous hydrogel could potentially be a useful local delivery system to release drugs, primarily at a specific site of body.

Key Words: Starch, acrylic acid, 2-hydroxy ethyl methacrylate, hydrogel, drug delivery

Introduction

Drug release from solid matrix systems, made of polymer(s) and drug(s), is a basic concept for studies on controlled drug delivery. The most interesting class of polymers in this application is the hydrogel class.

Hydrogels are special soft and pliable polymeric materials that can absorb large quantities of water, saline, or physiological solutions while the absorbed solutions are not removable even under pressure.[1] Stimuli-responsive smart hydrogels that can respond to environmental physical and chemical stimuli, such as temperature,[2] pH,[3] light,[4] magnetic field,[5] and substance species,[6] have attracted great interest in recent years due to their versatile applications such as controlled drug and gene delivery systems,[7–11] chemical-/bio-separations,[12] and sensors and/or actuators.[13] Among those smart hydrogels, pH-responsive hydrogels have been extensively investigated for potential use in site-specific delivery of drugs to specific regions of the gastrointestinal tract and have been prepared for delivery of low-molecular-weight drugs.

In the swollen state, these become soft and rubbery, resembling a living tissue, and some possess excellent biocompatibility.[1] Interest in biodegradable polymers, and specifically in a DDS matrix, has been growing. The main reason for this is that delivery systems based on biodegradable polymers do not require removal of the polymers from the body at the end of the treatment period, as they degrade into physiologically occurring compounds that can be readily excreted from the body.[14] Thus, polymeric hydrogels are of considerable interest as biomaterials in drug delivery research.[15–19]

Hydrogels are formed from hydrophilic synthetic polymers and many natural polymers such as proteins and polysaccharides. Natural polymer gels are useful for pharmaceutical fields such as controlled delivery devices because of their nontoxic nature, low cost, ready availability, biocompatibility, and biodegradability.

Recently, drug delivery systems based on natural hydrogels have been extensively explored to achieve higher concentrations of drugs in a specific region or tissue and a controlled release profile for extended time periods.[20–23]

In the current study, we investigated the synthesis and utility of an anionic hydrogel from the graft copolymerization of acrylic acid (AA) and 2-hydroxy ethyl methacrylate (HEMA) onto starch backbones for the controlled release of a model drug, 5-fluorouracil (5-FU). HEMA is as a comonomer used mainly for increasing the hydrophilicity of the resultant network. The drug absorption and release capacities of the hydrogel systems and the influence of the pH of the medium, porosity, and crosslinker content on the release properties were also examined.

Materials and methods

Materials

Starch, methylenebisacrylamide (MBA), and ammonium persulfate (APS) (all Fluka, Buchs, Switzerland) were used without further purification. AA and HEMA (Merck, Darmstadt, Germany) were used after vacuum distillation. All other chemicals were also of analytical grade. Bidistilled water was used for the hydrogel preparation and swelling measurements. The drug 5-FU was obtained from Jaberebne Hayan Pharmaceutical Co. (Tehran, Iran). The chemical structure of 5-FU is shown in Figure 1. The simulated gastric fluid (SGF, pH 1.2), composed of 21.25 mL of HCl, 11.18 g of KCl, and 1000 mL of distilled water, and the simulated intestinal fluid (SIF, phosphate buffer solutions, PBS, pH 7.4), composed of 3.6 g of KH_2PO_4, 4.8 g of Na_2HPO_4, and 1000 mL of distilled water, were prepared as described in *US Pharmacopoeia 30.*[24]

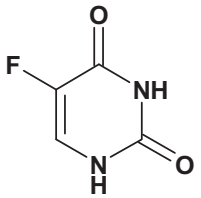


Figure 1. Chemical structure of 5-FU.

Preparation of hydrogel

A general procedure for chemically crosslinking the graft copolymerization of AA and HEMA onto starch backbones was conducted as follows. Starch (2.0 g) was added to a 3-neck reactor equipped with a mechanical stirrer (Heidolph RZR 2021, 3-blade propeller type, 300 rpm), including 35 mL of doubly distilled water. In order to homogenize the starch solution and to decompose the thermally dissociating initiator, i.e. APS, the reactor was immersed in a thermostated water bath preset to 80 °C. A definite amount of APS solution (0.1 g in 5 mL of H_2O) was then added to the starch solution and was allowed to stir for 10 min. After adding the APS, certain amounts of AA and HEMA (AA: 1.50 g, HEMA: 1.50 g) were added simultaneously to the starch solution. MBA solution (0.05 g in 5 mL of H_2O) was added to the reaction mixture after the addition of monomers and the mixture was continuously stirred. After 60 min, the reaction product was allowed to cool to ambient temperature and neutralized to pH 8 by the addition of 1 N sodium hydroxide solution. After 50 mg of $NaHCO_3$ was added, the mixture was stirred to yield an evenly bubbling formation and to accelerate the hydrogel formation. The hydrogel, starch-*g*-poly(NaAA-*co*-HEMA), was poured to excess in nonsolvent ethanol (200 mL) and kept for 3 h to dewater. The ethanol was then decanted and the product was cut to small pieces. Again, 100 mL of fresh ethanol was added and the hydrogel was kept for 24 h. Finally, the filtered hydrogel was dried in an oven at 60 °C for 10 h. After grinding using a mortar, the powdered superabsorbent was stored away from moisture, heat, and light.

Swelling measurements

An accurately weighed sample of the powdered superabsorbent (0.2 ± 0.001 g), with average particle sizes between 40 and 60 mesh (250-350 μm), was immersed in distilled water (200 mL) and allowed to soak for 3 h at room temperature. The equilibrium swelling (ES) capacity was measured twice at room temperature according to a conventional teabag method and using the following formula.

$$ES(g/g) = \frac{\text{Weight of swollen gel-Weight of dried gel}}{\text{Weight of drieds gel}} \tag{1}$$

Absorbency at various pHs

Individual solutions (50 mL) with acidic and basic pHs were prepared by dilution of NaOH (pH 10.0) and HCl (pH 1.0) solutions (0.1 M) to achieve pH $\geq$ 6.0 and pH $<$ 6.0, respectively. The pH values were precisely checked with a pH-meter (Metrohm/620, accuracy $\pm$0.1), and then 0.5 ± 0.001 g of the dried hydrogel was used for the swelling measurements according to Eq. (1). The sensitivity of the hydrogel to pH was investigated

in terms of the swelling and deswelling of the final product at basic (pH 7.0) and acidic (pH 2.0) solutions, respectively. The swelling capacity of the hydrogels at each pH was measured according to Eq. (1).

Determination of drug loading

Hydrogel (0.10 g) was immersed in 10 mL of the phosphate buffer solution (pH 7.4) of 5-FU, with 0.54 g of the drug dissolved in 50 mL of solution in a 50-mL beaker for complete swelling at 37 °C. The loaded swollen hydrogels were crushed in an agate mortar with a pestle and transferred to a conical flask; then about 20 mL of the fresh phosphate buffer solution was added to the conical flask and the homogeneous mixture was sonicated for 20 min. The 5-FU solution was separated from the mixture after being centrifuged for 20 min at 5000 rpm. The amount of 5-FU was determined using a UV spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan). More precisely, the amount of 5-FU entrapped was estimated by the difference between the initial and the final amount of drug in the gelling media. The drug loading (%) was calculated using the following equation.

$$\text{Drug loading (\%)} = \frac{\text{Weight of drug in hydrogel}}{\text{Weight of hydrogel}} \times 100 \tag{2}$$

In vitro drug release

The dry samples (0.1 ± 0.0001 g) were immersed in 50 mL of release media (SGF and SIF) with different pH values (pH 1.2 or 7.4) at 37 °C with agitation. At given time intervals, 1 mL of release medium was removed using a syringe attached to a 0.45-μm Millipore filter, and, after suitable dilution, the concentration of the released drug was measured by UV spectrophotometer at 266 nm. The drug release percentage was calculated twice using the following equation.

$$\text{Released drug (\%)} = \frac{R_t}{L} \times 100 \tag{3}$$

Here, L and R_t represent the initial amount of drug loaded and the final amount of drug released at time t, respectively.

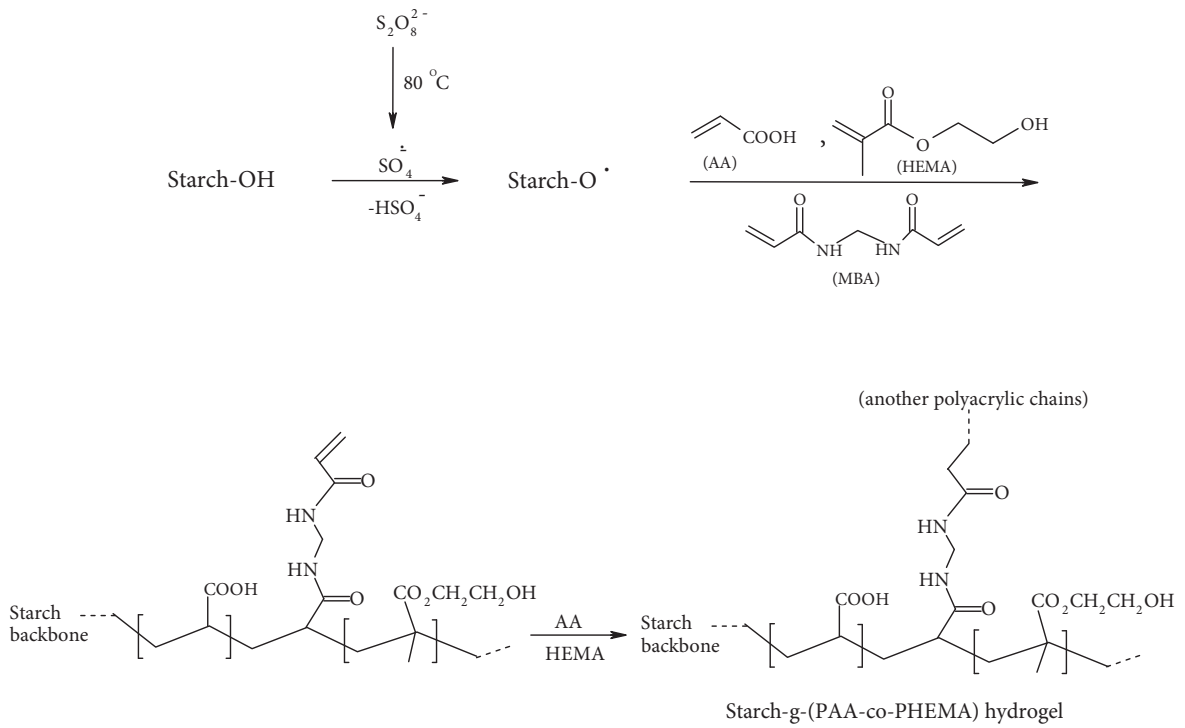
Instrumental analysis

Fourier transform infrared (FTIR) spectra of samples were taken in KBr pellets using an ABB Bomem MB-100 FTIR spectrophotometer (Quebec, Canada) at room temperature. The surface morphology of the gel was examined using scanning electron microscopy (SEM). After Soxhlet extraction with methanol for 24 h and drying in an oven at 70 °C, superabsorbent powder was coated with a thin layer of gold and imaged in a SEM instrument (Leo, 1455 VP). The Soxhlet extraction was performed in order to remove the linear polymer from the reaction mixture. Brunauer-Emmett-Teller (BET) analysis was used to determine the pore size of the hydrogels.

Results and discussion

Synthesis and spectral characterization

The mixture of the monomers, AA and HEMA, was simultaneously grafted onto starch backbones in a homogeneous medium using APS as a radical initiator and MBA as a crosslinking agent. A general reaction mechanism for starch-*g*-poly(AA-*co*-HEMA) hydrogel formation is shown in the Scheme. In the first step, the thermally dissociating initiator, i.e. APS, is decomposed under heating to produce a sulfate anion radical. The anion radical then abstracts hydrogen from the –OH groups of the starch backbones to form the corresponding radical. Thus, these macroradicals initiate monomer grafting onto starch backbones, leading to a graft copolymer. In addition, a crosslinking reaction was carried out in the presence of a crosslinker, i.e. MBA, so that a 3-dimensional network was obtained. It should be pointed out the grafting reaction was performed in the presence of a crosslinker (MBA) because of the synthesis of an insoluble polymeric hydrogel.



Scheme. Proposed mechanistic pathway for synthesis of the starch-based hydrogels.

The grafting was confirmed by comparing the FTIR spectra of the starch substrate with that of the grafted products (Figure 2). In the spectra of the hydrogel, the characteristic band at 1578 cm^{-1} was attributed to C=O asymmetric stretching in the carboxylate anion. This was confirmed by another peak at 1411 cm^{-1}, which is related to the symmetric stretching mode of the C=O of carboxylate groups. The main contribution to the absorption band at 1735 cm^{-1} is due to both the carboxylic acid (AA) and ester (HEMA).

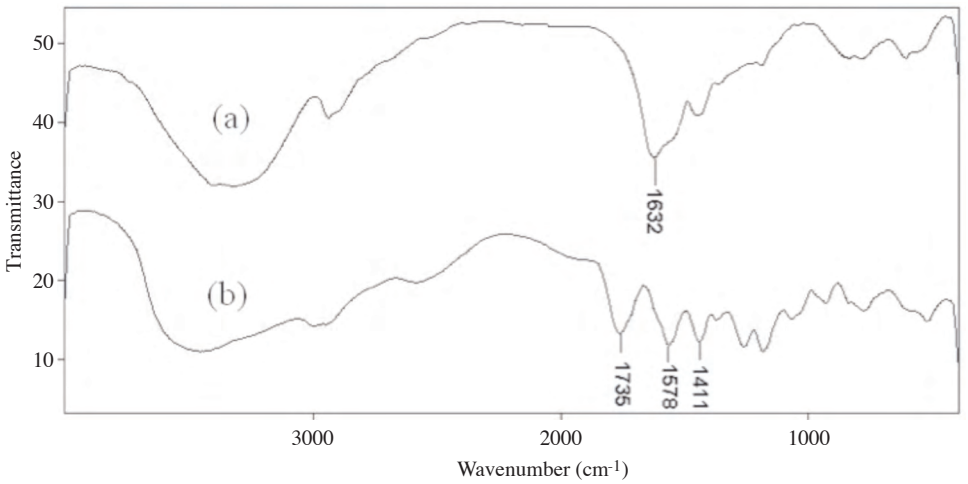


Figure 2. FTIR spectra of starch (a) and starch-*g*-poly(AA-*co*-HEMA) hydrogel (b).

One of the most important properties that must be considered is the hydrogel microstructure morphology. Figure 3 shows the SEM photographs of the surface (Figure 3A) and the cross-sectional area (Figure 3B) of the hydrogel with interconnected pores. The hydrogel has a porous structure. It is supposed that these pores are the regions of water permeation and interaction sites of external stimuli with the hydrophilic groups of the graft copolymers. The cross-sectional view of the hydrogels (Figure 3B) also exhibits a large, open, channel-like structure.

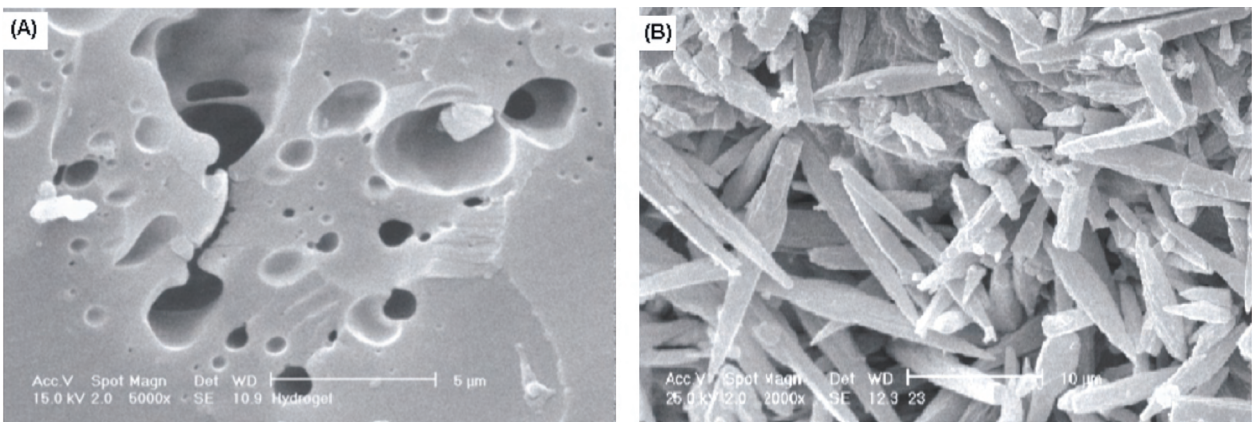


Figure 3. SEM photograph of the hydrogel: A) surface of porous hydrogel, B) cross-sectional area of porous hydrogel. The average pore diameter of the synthesized hydrogel was 12.4 nm.

The results of BET analysis showed that the average pore diameter of the synthesized hydrogel was 12.4 nm. In general, the size of the pores can be controlled by adjusting various factors such as the type and amount of surfactant, porosigens and gas-forming agents during crosslinking polymerization, and the amount of diluent in the monomer mixture (i.e. the monomer-to-diluent ratio). For example, as the amount of diluent (usually water) in the monomer mixture increases, the pore size also increases up to the micrometer range.[25]

The porosity plays the multiple role of enhancing the total water sorption capability and the rate of response by reducing the transport resistance.[26,27] Therefore, creation of porosity in hydrogels has been considered to be an important process in many ways. The phase-separation technique,[28] the water-soluble porogens,[29] and the foaming technique[30,31] are 3 different methods for preparing porous hydrogel structures.

pH-Sensitivity and pulsatile behavior

The equilibrium swelling studies indicated that the ionic hydrogels were sensitive to environmental pH. Therefore, in this series of experiments, the swelling rate of the synthesized hydrogels was measured in different pH solutions ranging from 1.0 to 13.0 (Figure 4). Since the swelling capacity of all "anionic" hydrogels is appreciably decreased by the addition of counterions (cations) to the swelling medium, no buffer solutions were used. Therefore, stock NaOH (pH 10.0) and HCl (1.0) solutions were diluted with distilled water to reach desired basic and acidic pHs, respectively. Maximum swelling (75 g/g) was obtained at pH 8. In acidic media, most of the carboxylate groups are protonated, so the decreased repulsion of anionic groups leads to a decreased swelling rate. With the increase in the pH of the medium after the pKa of AA, the carboxyl groups of AA convert to COONa groups with neutralization by NaOH in solution; then they can ionize depending on the pH of the medium. The reason for the swelling-loss in the highly basic solutions is the "charge screening effect" of excess Na^+ in the swelling media, which shields the carboxylate anions and prevents effective anion-anion repulsion.[32]

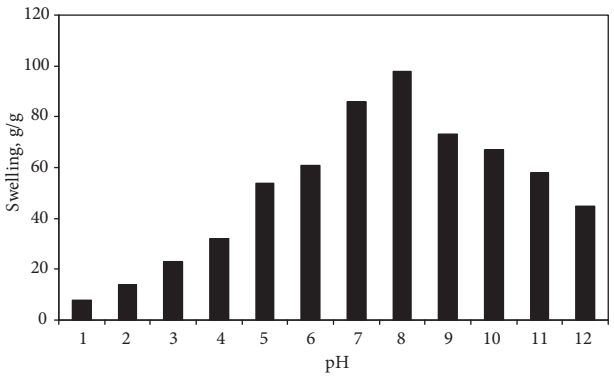


Figure 4. Effect of pH of solution on swelling of starch-*g*-poly(AA-*co*-HEMA) hydrogel.

The starch-g-(PAA-co-PHEMA) hydrogels also showed reproducible swelling-deswelling cycles at pH levels of 2.0 and 7.0, as demonstrated in Figure 5. At pH 7.0, the hydrogel swelled up to 75 g/g due to anion-anion repulsive electrostatic forces, while, at pH 2.0, it shrank within a few minutes due to the protonation of the carboxylate groups. This sharp swelling-deswelling behavior of the hydrogels makes them suitable candidates for controlled drug delivery systems.

Drug loading efficiency

The drug loading of hydrogels with different crosslinker contents is shown in Figure 6. As can be seen, the amount of drug loaded in the hydrogel beads decreased with increased content of the crosslinker, MBA. The

increase in crosslink density decreased the swelling of the hydrogel, and, for that reason, the amount of drug loaded into the hydrogel decreased.[33]

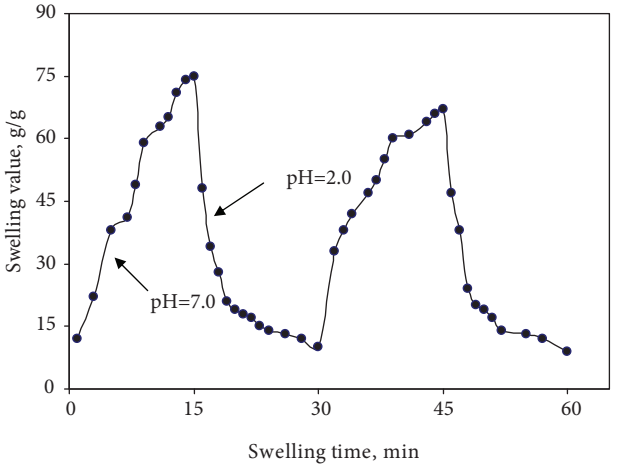


Figure 5. On-off switching behavior as reversible pulsatile swelling (pH 7.0) and deswelling (pH 2.0) of the hydrogel. The time interval between the pH changes was 15 min.

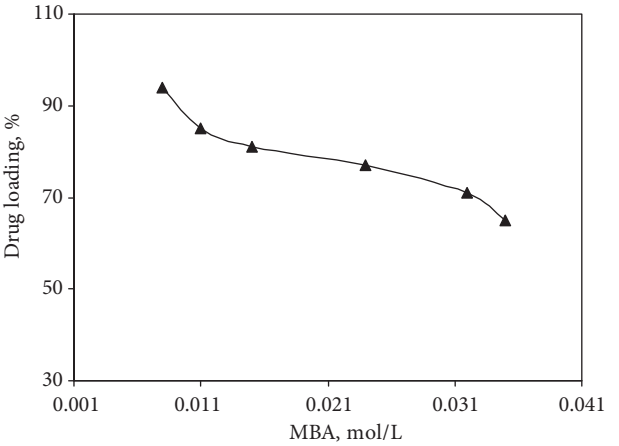


Figure 6. Effect of crosslinker content on 5-FU release.

In vitro release behavior of hydrogels

To determine the potential application of a starch-based superabsorbent containing a pharmaceutically active compound, we investigated the drug release behavior of this system under physiological conditions. The percentage of released drug from the polymeric carriers as a function of pH is shown in Figure 7. The concentration of 5-FU released at selected time intervals was determined with a UV spectrophotometer. The amount of 5-FU released in a specified time from the starch-based hydrogels decreased at pH levels lower and higher than 8. At acidic pH values, electrostatic repulsion between the carboxylic acid groups of the backbone was low, thus decreasing gel swelling and minimizing release of 5-FU via diffusion. However, in alkaline media (pH $>$ 8), -COONa groups of PAA cannot ionize due to the screening effect of counterions (Na^+) in the swelling medium, and, for that reason, the swelling of the hydrogel decreases. As a consequence of the decrease in the swelling of the gel, drug release from the hydrogel decreases.

The release rate experiments were also performed in SFG (pH 1.2) and SIF (pH 7.4) solutions at 37 °C (Figure 8). As can be seen from Figure 8, when the pH of the medium is 1.2, the cumulative release rate of 5-FU from the test hydrogels is below 35% at the end of the experiment (24 h), whereas almost 90% of the loaded drug is released within 15 h in medium with a pH of 7.4. Again, these results indicate that the higher swelling rates of the hydrogel create larger surface areas to diffuse the drug. In basic solutions (pH 7.4), the electrostatic repulsion between COO^- anions of grafted poly(sodium acrylate) on the hydrogel accelerates the release of 5-FU from the hydrogel.

Figure 9 shows the effect of the porosity of the hydrogel on 5-FU release. When compared to the dense hydrogel, the porous hydrogel provided a much faster 5-FU release. An initial burst release of 55 μg of 5-FU from the porous hydrogel was observed during the first 12 h of experiments, followed by a continuous release

of 5-10 μg of 5-FU for up to 20 days. On the other hand, the hydrogel with a dense structure showed a much lower initial burst of 5-FU release, followed by a slower 5-FU release for up to 10 h. It should be pointed out that a dense structure would allow 5-FU release to occur primarily at the surface. In this regard, drug release is more dependent on the swelling kinetics of the hydrogel.[34–36]

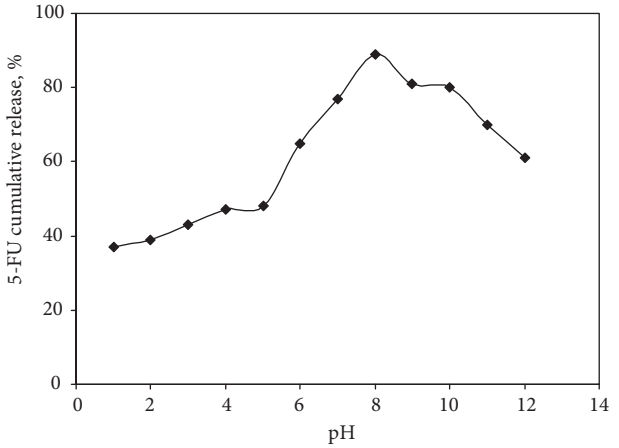


Figure 7. Release of 5-FU from hydrogel carrier as a function of pH at 37 °C.

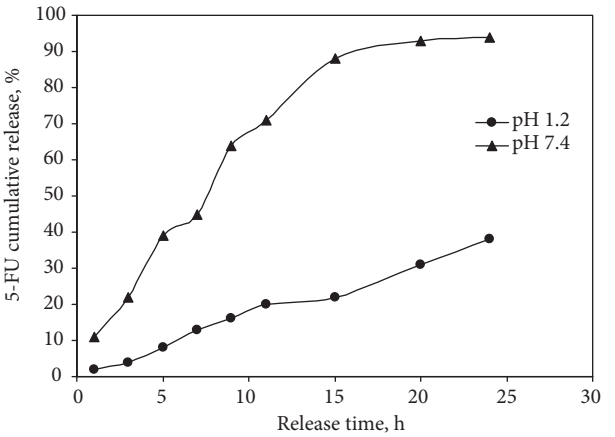


Figure 8. Release of 5-FU from hydrogel carrier as a function of time and pH at 37 °C. Reaction conditions: kC, 2 wt%; CAN, 0.004 mol/L; temperature, 60 °C; time, 80 min.

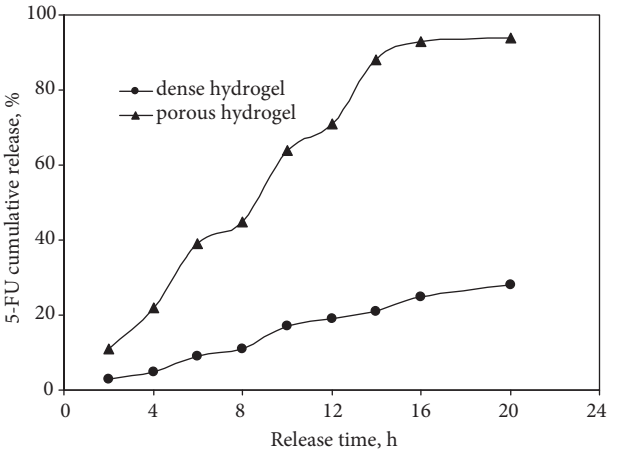


Figure 9. Effects of porosity of the starch-based hydrogel on 5-FU release at pH 7.4 and 37 °C.

Conclusion

A novel biopolymer-based superabsorbent hydrogel, starch-*g*-poly(AA-*co*-HEMA), was synthesized through simultaneous crosslinking and graft polymerization of acrylic acid and 2-hydroxy ethyl methacrylate mixtures onto starch. The superabsorbent hydrogels exhibited a high sensitivity to pH, such that several swelling

changes of the hydrogel were observed in pH variations of a wide range (1-13). Ionic repulsion between charged groups incorporated in the gel matrix by an external pH modulation could be assumed as the main driving force responsible for such abrupt swelling changes. Furthermore, the reversible swelling-deswelling behavior in solutions with acidic and basic pH makes the hydrogels suitable candidates for controlled drug delivery systems.

Our results indicated that the porous hydrogel, prepared by the gas-foaming technique, could potentially be used as a carrier for local and controlled delivery of drugs.

The release value of 5-fluorouracil from hydrogels at pH 7.4 was higher than that at pH 1.2 due to increased electrostatic repulsion between negatively charged polymer chains. Overall, the hydrogels presented in this study may serve as a platform for a wide range of pharmaceutical uses to improve the bioavailability of nonsteroidal antiinflammatory drugs.

References

1. Buchholz, F. L.; Graham, A. T. *Modern Superabsorbent Polymer Technology*, Elsevier, Amsterdam, 1997.
2. Chu, L. Y.; Kim, J. W.; Shah, R. K.; Weitz, D. A. *Adv. Funct. Mater.* **2007**, *17*, 3499-3505.
3. Kim, S. J.; Spinks, G. M.; Prosser, S.; Whitten, P. G.; Wallace, G. G.; Kim, S. I. *Nat. Mater.* **2006**, *5*, 48-56.
4. Tatsuma, T.; Takada, K.; Miyazaki, T. *Adv. Mater.* **2007**, *19*, 1249-1257.
5. Wang, W.; Liu, L.; Ju, X. J.; Zerrouki, D.; Xie, R.; Yang, L. *Chem. Phys. Chem.* **2009**, *10*, 2405-2052.
6. Zhang, S. B.; Chu, L. Y.; Xu, D.; Zhang, J.; Ju, X. J.; Xie, R. *Polym. Advan. Technol.* **2008**, *19*, 937-943.
7. Soppimath, K. S.; Aminabhavi, T. M.; Dave, A. M.; Kumbar, S. G.; Rudzinski, W. E. *Drug Dev. Ind. Pharm.* **2002**, *28*, 957-964.
8. Chu, L. Y.; Yamaguchi, T.; Nakao, S. *Adv. Mater.* **2002**, *14*, 386-391.
9. Thornton, P. D.; Mart, R. J.; Ulijn, R. V. *Adv. Mater.* **2007**, *19*, 1252-1258.
10. Hamidi, M.; Azadi, A.; Ra?ei, P. *Adv. Drug Deliver. Rev.* **2008**, *60*, 1638-1644.
11. Cheng, H.; Zhu, J. L.; Sun, Y. X.; Cheng, S. X.; Zhang, X. Z.; Zhuo, R. X. *Bioconjugate. Chem.* **2008**, *19*, 1368-1372.
12. Yang, M.; Chu, L. Y.; Wang, H. D.; Xie, R.; Song, H.; Niu, C. H. *Adv. Funct. Mater.* **2008**, *18*, 652-658.
13. Eddington, D. T.; Beebe, D. J. *Adv. Drug Deliver. Rev.* **2004**, *56*, 199-204.
14. Kakinoki, S; Taguchi, T; Saito, H; Tanaka, J; Tateishi, T. *Eur. J. Pharm. Bio.* **2007**, *66*, 383-390.
15. Qu, A.; Wirsen, A.; Albertsson, A. C. *Polymer* **2000**, *41*, 4589-4598.
16. Kim, H. Y.; Bae, Y. H.; Kim, S. W. *J. Control. Rel.* **1994**, *28*, 143-152.
17. Vazquez, B.; Gurruchaga, M.; Goni, I. *Polymer* **1995**, *36*, 2311-2314.
18. Podual, K.; Doyle, F. G.; Peppas, N. A. *J. Control. Rel.* **2000**, *67*, 9-17.
19. Chatterji, P. R. *J. Appl. Polym. Sci.* **1989**, *37*, 2203-2222.
20. Raghavendra, V.; Kulkarni, V.; Setty, S. M.; Sa, B. *Macromolecules* **2010**, *47*, 520-527.
21. Zhou, H. Y.; Zhang, Y. P.; Zhang, W. F.; Guang, X. *Carbohyd. Polym.* **2011**, *83*, 1643-1651.
22. Hua, S.; Yang, H.; Wang, W.; Wang, A. *Appl. Clay Sci.* **2010**, *50*, 112-117.

23. Hori, K.; Sotozono, C.; Hamuro, J.; Yamasaki, K.; Kimura, Y.; Ozeki, M.; Tabata, Y.; Kinoshita, S. *J. Control. Rel.* **2007**, *118*, 169-176.
24. US Pharmacopeia 30 NF 25. US Pharmacopeial convention, Rockville, MD, 2007.
25. Chirila, T. V.; Constable, I. J.; Crawford, G. J.; Vijayasekaran, S.; Thompson, D. E.; Chen, Y. C.; Fletcher, W. A.; Griffin, B. J. *Biomaterials* **1993**, *14*, 26-38.
26. Chen, J.; Park, K. *J. Control. Release* **2000**, *65*, 73-82.
27. Chen, J.; Park, K. *Carbohyd. Polym.* **2000**, *41*, 259-268.
28. Gotoh, T.; Nakatani, Y.; Sakohara, S. *J. Appl. Polym. Sci.* **1998**, *69*, 895-906.
29. Badiger, M. V.; McNeil, M. E.; Graham, N. B. *Biomaterials* **1993**, *14*, 1059-1063.
30. Smith, S. J.; Lind, E. J. *US Patent* **1995**, 5399591.
31. Smith, S. J.; Lind, E. J. *US Patent* **1993**, 5314420.
32. Flory, P. J. *Principles of Polymer Chemistry*, Cornell University Press, Ithaca, New York, 1953.
33. Zhang, J. P.; Wang, Q.; Wang, A. Q. *Carbohyd. Polym.* **2007**, *68*, 367-374.
34. Markland, P.; Zhang, Y.; Amidon, G. L.; Yang, V. C. *J. Biomed. Mater. Res.* **1999**, *47*, 595-602.
35. Brazel, C. S.; Peppas, N. A. *J. Control. Release* **1996**, *39*, 57-64.
36. Obaidat, A. A.; Park, K. *Biomaterials* **1997**, *18,* 801-806.