

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

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Abstract

The present work focused on the design of drug delivery system (DDS) based on a 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 (NaHCO₃) was added to function as a foaming agent under acidic conditions, rendering the hydrogels to be porous. Porous structure of 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. Due to the reversible swelling behavior of the hydrogels, the synthesized networks can sense the environmental pH change and achieve an oscillatory release pattern. Using 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.

Keywords: Hydrogel, starch, drug delivery, acrylic acid, 2-hydroxy ethyl methacrylate.

Introduction

Drug release from solid matrices 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 given by hydrogels, also pH-sensitive ones. 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. In the swollen state, these become soft and rubbery, resembling a living tissue and some possess excellent biocompatibility (Buchholz & Graham, 1997). Thus, polymeric hydrogels are of considerable interest as biomaterials in drug delivery research (Yoshida *et al.*, 1995; Ju *et al.*, 2001; Hoffman, 2002; Kikuchi & Okano, 2002;). 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 non-toxic, low cost, free availability, biocompatibility and biodegradability.

Recently, drug delivery systems based on natural hydrogels have been extensively explored to achieve the higher concentration of drugs in the specific region or tissue and the controlled release profile for extended time periods (Zhang J, Peppas NA, 2000); hang XZ, Wu DQ, Chu CC, 2004); Liu YY, Fan XD, Kang T, Sun L, 2004).

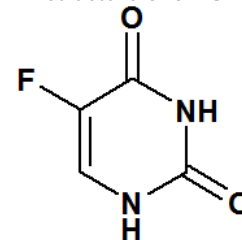
In the current study we investigated the synthesis and utility of an anionic hydrogel from graft copolymerization of acrylic acid and 2-hydroxy ethyl methacrylate onto starch backbones, for the controlled release of a model

drug, 5-fluorouracil (5-FU). Drug absorption and release capacities of hydrogel systems and influence of pH of the medium, porosity and the crosslinker content on the release properties were also examined.

Materials and methods

Starch, methylenebisacrylamide, and ammonium persulfate (all Fluka, Buchs, Switzerland), were used without further purification. Acrylic acid (AA) and 2-hydroxyethyl methacrylate (HEMA) (Merck, Darmstadt, Germany) was used after vacuum distillation. All other chemicals were also of analytical grade. Double distilled water was used for the hydrogel preparation and swelling measurements. The drug, 5-Fluorouracil, was obtained from Jaberebne Hayan Pharmaceutical Co. (Tehran, Iran). The chemical structure of 5-Fluorouracil is shown in Fig.1. The simulated gastric fluid (SGF, pH 1.2) composed of 21.25 mL HCl, 11.18 g KCl and 1000 mL distilled water and the simulated intestinal fluid (SIF, phosphate buffer solutions, PBS, pH 7.4) composed of 3.6 g KH₂PO₄, 4.8 g Na₂HPO₄ and 1000 mL distilled water were prepared as described in *US Pharmacopoeia 30*.

Fig.1. Chemical structure of 5-FU



Preparation of hydrogel

A general procedure for chemically crosslinking graft copolymerization of AA and HEMA onto starch backbones was conducted. Starch (2.0 g) was added to

a three-neck reactor equipped with a mechanical stirrer (Heidolph RZR 2021, three blade propeller type, 300 rpm), including 35 mL doubly distilled water. The reactor was immersed in a thermostated water bath preset at 80 °C. Then a definite amount of APS solution (0.1 g in 5 mL H₂O) was added to starch solution and was allowed to stir for 10 min. After adding 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 H₂O) 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 addition of 1N sodium hydroxide solution. The hydrogel, starch-*g*-poly(NaAA-*co*-HEMA), was poured to excess non solvent ethanol (200 mL) and kept for 3 h to dewater. Then ethanol was decanted and the product cut to small pieces. Again, 100 mL fresh ethanol was added and the hydrogel was kept for 24 h. Finally, the filtered hydrogel is dried in oven at 60°C for 10 h. After grinding using 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 40-60 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 tea bag method and using the following formula:

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

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 ≥ 6.0 and pH < 6.0, respectively. The pH values were precisely checked by a pH-meter (Metrohm/620, accuracy ±0.1). Then, 0.5 (± 0.001) g of the dried hydrogel was used for the swelling measurements according to Eq. 1. Sensitivity of the hydrogel to pH was investigated in terms of swelling and deswelling of the final product at two basic (pH 7.0) and acidic (pH 2.0) solutions, respectively. 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) in a 50 mL beaker for completely swelling. The swollen hydrogels were crushed in an agate mortar with a pestle and transferred into a conical flask, and 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 UV spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan). The drug loading (%) was calculated using the following equation:

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

In vitro drug release

The samples (0.1 ± 0.0001 g) were immersed into 50 mL of the release medium (simulated gastric and intestinal fluids, 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 the release medium was removed using a syringe attached with a 0.45 μm Millipore filter and after suitable dilution the concentration of released drug was measured by UV spectrophotometer at 266 nm. The drug release percent was calculated twice using the following equation:

$$\text{Re leased drug (\%)} = \frac{R_t}{L} \times 100 \quad (3)$$

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

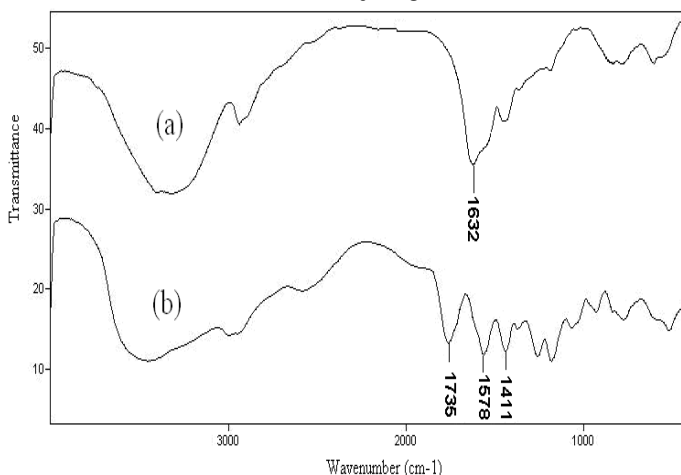
Instrumental analysis

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, superabsorbent powder was coated with a thin layer of gold and imaged in a SEM instrument (Leo, 1455 VP).

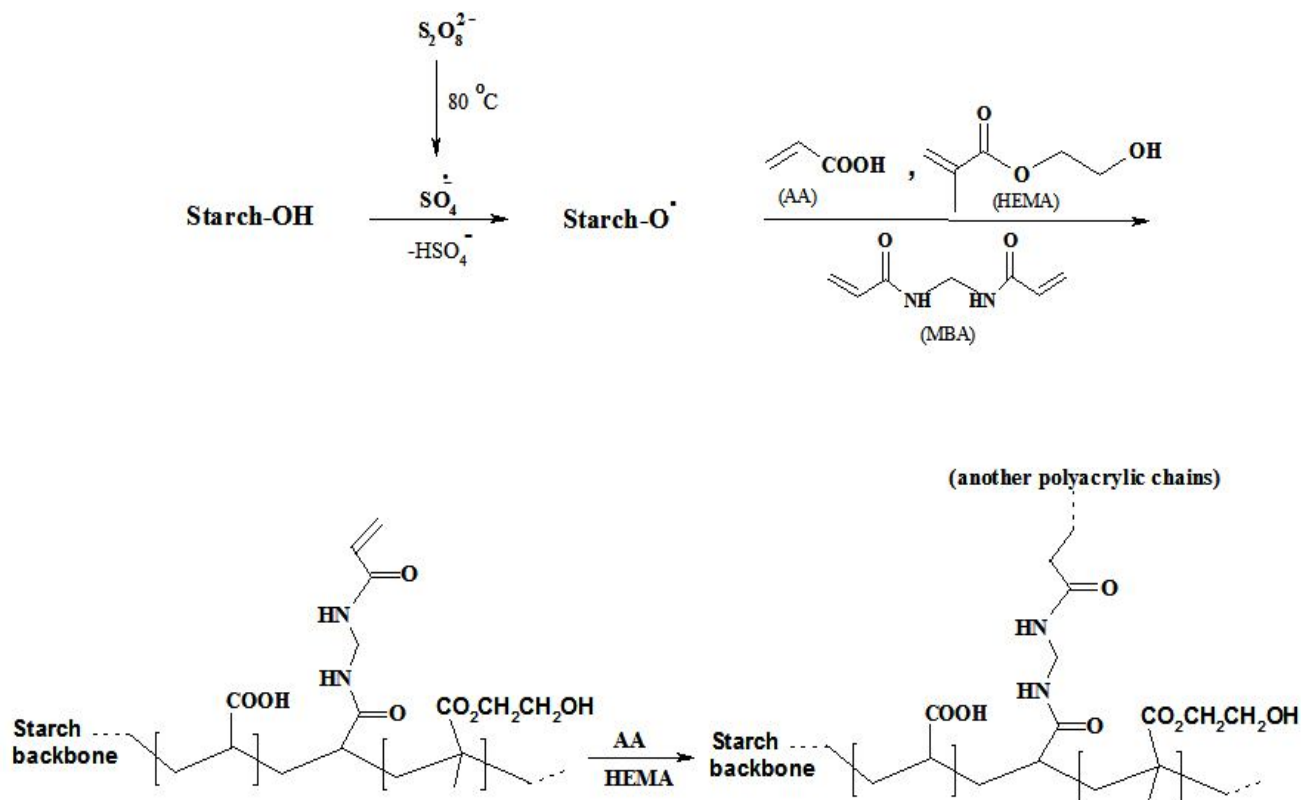
Result and discussion

Synthesis and spectral characterization

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



Scheme 1. Proposed mechanistic pathway for synthesis of the starch-based hydrogels.



Starch-g-(PAA-co-PHEMA) hydrogel

The mixture of monomers, AA and HEMA, 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 Scheme 1. At the first step, the thermally dissociating initiator, i.e. APS, is decomposed under heating to produce sulfate anion-radical. Then, the

anion-radical abstracts hydrogen from -OH groups of starch backbones to form corresponding radical. So, these macroradicals initiated monomer grafting onto starch backbones led to a graft copolymer. In addition, crosslinking reaction was carried out in the presence of a crosslinker, i.e., MBA, so that a three dimensional network was obtained.

The grafting was confirmed by comparing the FTIR

Fig. 3. SEM photograph of the hydrogel. Surfaces were taken at a magnification of 5000, and the scale bar is 5 μm.

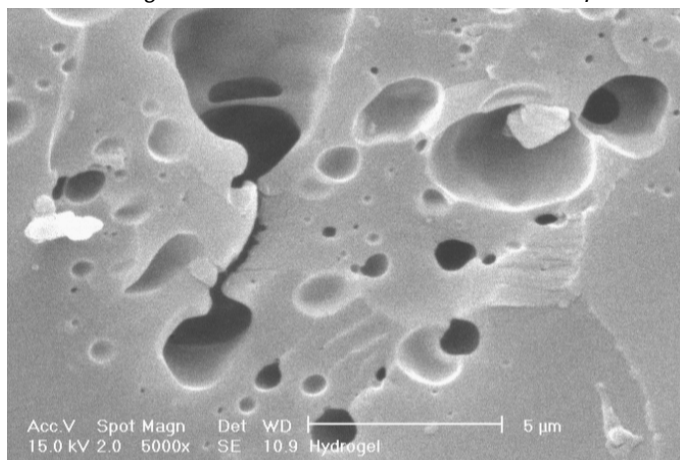


Fig. 4. Effect of pH of solution on swelling of starch-g-poly (AA-co-HEMA) hydrogel.

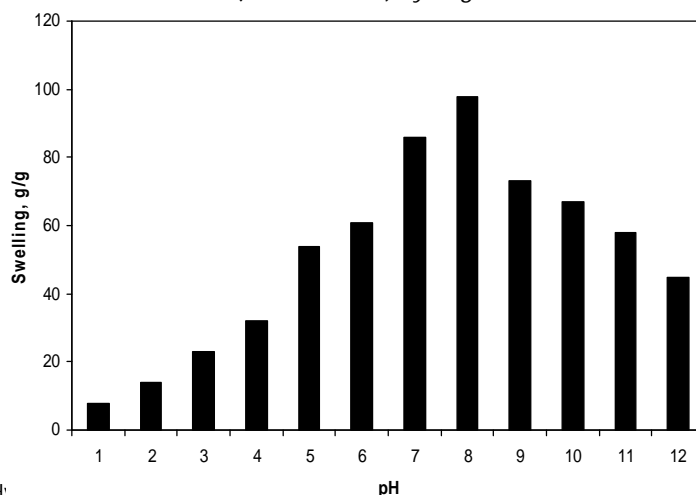
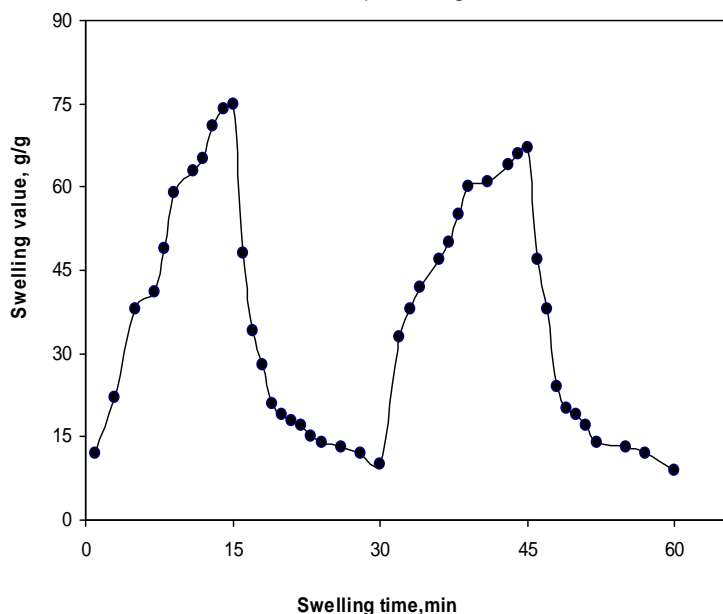


Fig.5. On-off switching behavior as reversible pulsatile swelling (pH 7.0) and deswelling (pH 2.0) of the hydrogel. The time interval between the pH changes was 15 min.



spectra of the starch substrate with that of the grafted products (Fig.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 carboxylate groups. The main contribution to the absorption band in the 1735 cm^{-1} is due to the ester group from the poly (2-hydroxyethyl methacrylate).

One of the most important properties that must be considered is hydrogel microstructure morphologies. The surface morphology of the samples was investigated by scanning electron microscopy (SEM). Fig.3 shows an SEM micrograph of the polymeric hydrogels obtained from the fracture surface. 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.

pH-Sensitivity and pulsatile behavior

Equilibrium swelling studies indicated that the ionic hydrogels were sensitive to environmental pH. Therefore, in this series of experiments, swelling ratio for the synthesized hydrogels was measured in different pH solutions ranged from 1.0 to 13.0 (Fig.4). Since the swelling capacity of all "anionic" hydrogels is appreciably decreased by addition of counter ions (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, the most of carboxylate groups are protonated, so decreased repulsion of anionic groups leads to a decreased swelling ratio. At higher pHs (5-8), some of carboxylate groups are ionized and the electrostatic repulsion between COO^- groups causes an enhancement of the swelling capacity. The reason of the swelling-loss for the highly basic solutions is "charge screening effect" of excess Na^+ in the swelling media which shield the carboxylate anions and prevent effective anion-anion repulsion (Jenkins & Hudson, 2001).

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

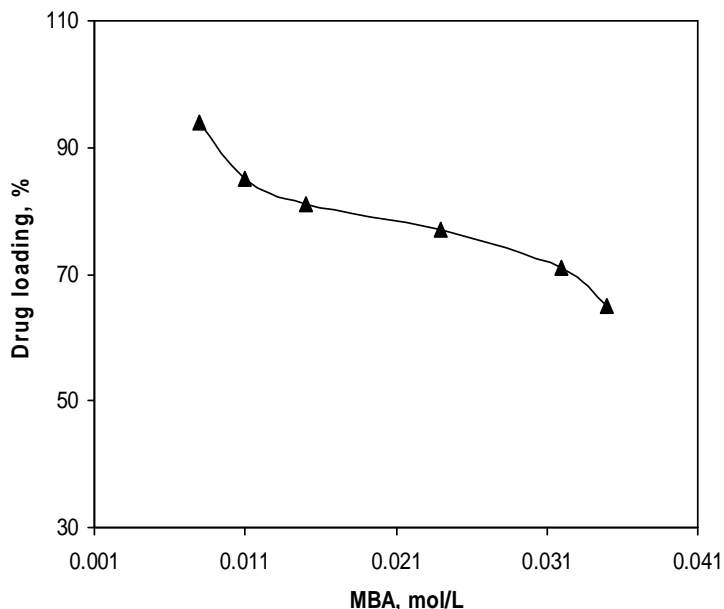


Fig.7. Release of 5-FU from hydrogel carrier as a function of pH at 37°C .

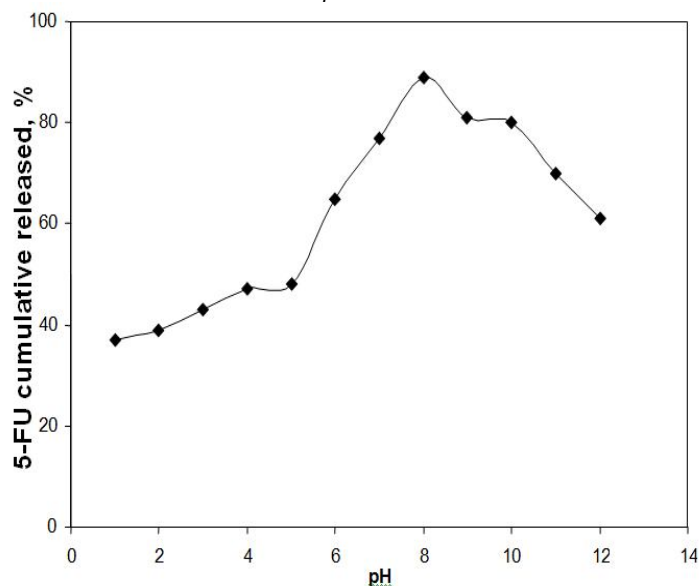
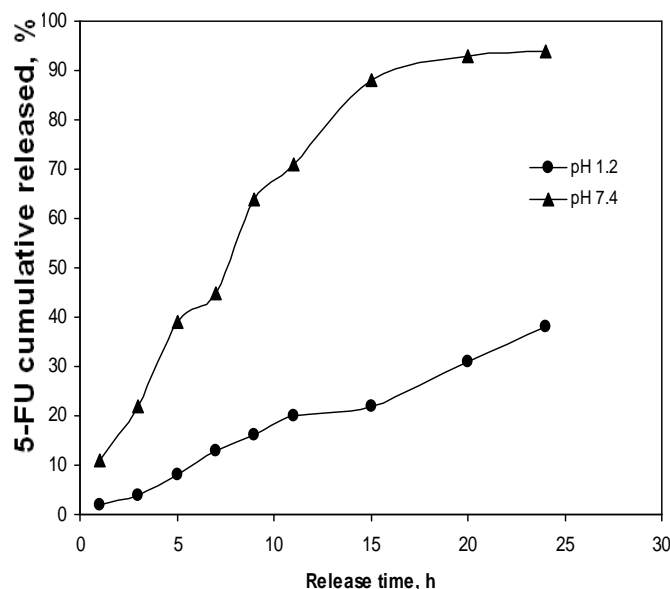


Fig. 8. Release of 5-FU from hydrogel carrier as a function of time and pH at 37 °C.



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

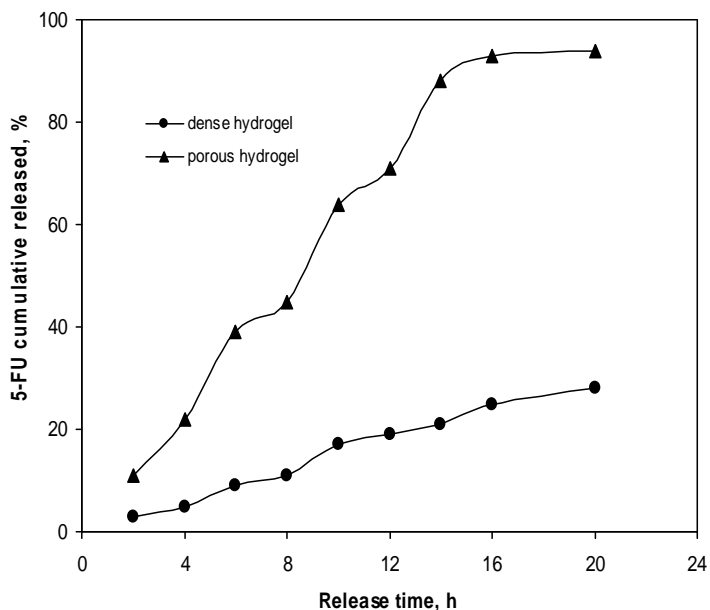
Drug loading efficiency

In this series of experiments, the drug loading of the hydrogels with different crosslinker content were shown in Fig.6. As can be seen, the amount of drug loaded in the hydrogel beads decrease with increasing the content of crosslinker, MBA. The greater the crosslinking density, the worse the elasticity of the polymer chains, which could restrict the penetration of 5-FU into hydrogel, and then leads to the decrease of the loading amount for drug (Zhang & Wang 2007).

In vitro release behavior of hydrogels

To determine the potential application of starch-based superabsorbent containing a pharmaceutically active compound, we have investigated the drug release behavior from this system under physiological conditions. The percent of released drug from the polymeric carriers as a function of pH is shown in Fig.7. The concentration of 5-FU released at selected time intervals was determined by UV spectrophotometer. The amount of 5-FU released in a specified time from the starch-based hydrogels decreased at pHs lower and higher than pH 8. At acidic pH values, electrostatic repulsion between the

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



carboxylic acid groups of backbone is low, thus decreases gel swelling and minimizes release of 5-FU via diffusion. However, in alkaline media the presence of OH⁻ increases the electrostatic repulsion between carboxylate groups, thus increases the gels swelling degree and so the release of 5-FU increased.

The release rate experiments were also performed in SFG (pH 1.2) and SIF (pH 7.4) solutions at 37 °C (Fig.8). As can be seen from Figure 8, when pH of the medium is 1.2, the cumulative release ratio 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 pH 7.4 medium. Again, these results indicate that the higher swelling ratios 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.

Fig.9 shows the effect of 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 dense structure showed a much lower initial burst 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 mediated by the environment pH changes (Saraydın *et al.*, 1994; Akkas *et*

al., 1999; Sen & Yakar, 2001; Mahkam *et al.*, 2004; Mahkam *et al.*, 2006).

Conclusion

A novel biopolymer-based superabsorbent hydrogel, starch-*g*-poly(AA-*co*-HEMA), was synthesized through simultaneous crosslinking and graft polymerization of acrylic acid/2-hydroxy ethyl methacrylate mixtures on to starch. The superabsorbent hydrogels exhibited high sensitivity to pH, so 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 a suitable candidate 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 (5-FU) from hydrogels at pH 7.4 was higher than that at pH 1.2 due to the electrostatic repulsion between carboxylate groups. Overall, the hydrogels presented in this study may serve as a platform for a wide range of pharmaceutical uses to improve the bioavailability of non-steroidal anti-inflammatory drugs.

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