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Research article

PROPERTIES OF A RESPONSIVE HYDROGELS BASED ON PROTEIN FOR ORAL DELIVERY OF DRUGS

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ABSTRACT: In this study, to following synthesis of the hydrogels via graft copolymerization of acrylic acid (AA) onto animal's protein backbones, loading and releasing drug was investigated. In vitro drug release studies in different buffer solutions showed that the most important parameter affecting the drug-release behavior of hydrogels is the pH of the solution. The release rate of acyclovir 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.
Keywords: Protein, Hydrogel, Monomer, Acyclovir, Release drug.

INTRODUCTION

The development of various advanced drugs over the past decade has created a need for new methods of controlled delivery for these compounds, including peptides, proteins, plasmid DNA, antisense oligodeoxynucleotides, and immunotoxins. Activity of such molecules depends on their ability to reach the targeted sites; however, they are easily degraded by proteases or DNA-degrading enzymes in vivo once they enter into the body system [1]. Besides cross linking hydrogels, externally-smart sensitive drug delivery systems have been investigated as novel controlled delivery approaches to control the release of drugs in response to changes in the surrounding environment. To develop such drug delivery systems, studies have been performed to manufacture stimuli-sensitive materials undergoing phase transitions in response to changes in ionic strength, pH or temperature. In particular, among the pH-sensitive hydrogels reported to date, poly(*acrylic acid*) (PAA) and its copolymers have been widely used for cell separation as well as for pharmaceutical and tissue engineering applications because of their unique pH properties[2-3]. These hydrogels responding to external stimuli such as heat, pH, electric field, chemical environments, etc, are often referred to as "intelligent" or "smart" hydrogels. In this work, we attempted to investigate the pH-reversibility properties and influence of pH of the medium on the drug release of hydrogel systems.

MATERIALS AND METHODS

Materials

The gelatin (Merck) was used as received. Acrylic acid (AA, Merck) was used after vacuum distillation. Ammonium persulfate (APS, Merck) was used without purification. All other chemicals were of analytical grade. Acyclovir was purchased from Hakim Pharmaceutical Co. Tehran, Iran. Structure of Acyclovir shows in figure 1. All other chemicals were of analytical grade.

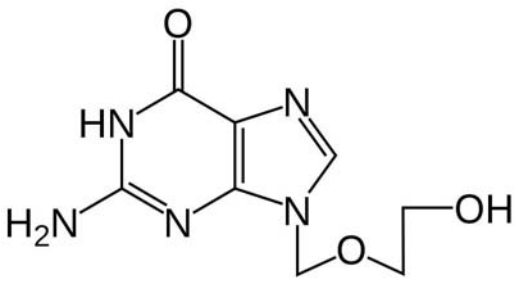

Figure-1. The Chemical structure of Acyclovir.

All the tests including determination of drug content, swelling and *in vitro* drug cumulative release were carried out in triplicate and the averages were reported. Statistical data analysis was performed using the Student's *t*-test with $p < 0.05$ as the minimal level of significance. The reproducibility of the experiments was n=3. The average of three independent determinations was considered and the accuracy of the measurements was ±3%.

Preparation of hydrogel

A general procedure was conducted as follows. In order to remove of oxygen, deionized doubly-distilled water was boiled for 30 min., and after cooling to room temperature, pure argon gas was bobbled in water for 15 min. animal protein (0.0-2.0 g) was dissolved in 35 mL distilled water in a three-neck reactor equipped with mechanical stirrer (three blade propeller type, 600 rpm) and a reflux condenser. The reactor was immersed in a thermostated water bath preset at desired temperature (35-70 ^{o}C). Then, a definite amount of APS solution (0.1 g in 5 mL H_2O) was added to protein solution and was allowed to stir for 10 min. After adding APS, variable amounts of AA(0.40–1.60 g,) were added simultaneously to the gelatin solution. Then, in order to investigate the effect of crosslinker concentration on swelling capacity, methylene bisacrylamide solution (0.03–0.08 g in 5 ml H_2O) was added to the reaction mixture after the addition of monomers and the mixture was continuously stirred. Finally, the filtered hydrogel was dried in oven at 60^{o}C for 10 h. After grinding by mortar, the powdered superabsorbent was stored by protecting from moisture, heat and light.

Swelling at various pHs

Individual solutions (50 mL) with acidic and basic pHs were prepared by the 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[3]. Sensitivity of the hydrogel to pH was investigated in terms of swelling and deswelling of the final product at two basic (pH 7.4) and acidic (pH 1.2) solutions, respectively. Swelling capacity of the hydrogels at each pH was measured according to Eq. 1 at consecutive time intervals (15 min).

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

Effect of pH on equilibrium swelling

In this series of experiments, swelling ratio for the synthesized hydrogels was measured in different pH solutions ranged from 1.0 to 13.0 (Figure 2). Since the swelling capacity of all "anionic" hydrogels is appreciably decreased by the addition of counter ions (cations) to the swelling medium, no buffer solutions were used. Therefore, stock NaOH (pH 10.0) and HCl (pH 1.0) solutions were diluted with distilled water to reach desired basic and acidic pHs, respectively. Maximum swelling (87 g.g^{-1}) was obtained at pH 8. In acidic media, most carboxylate groups are protonated, so decreased repulsion of anionic group's leads to a decreased swelling ratio. At higher pHs (3– 8), some carboxylate groups are ionized and the electrostatic repulsion between carboxylate groups causes an enhancement of the swelling capacity. The reason of the swelling loss for the highly basic solutions is the charge screening effect of excess Na^+ in the swelling media, which shield the carboxylate anions and prevent effective anion–anion repulsion [4-6].

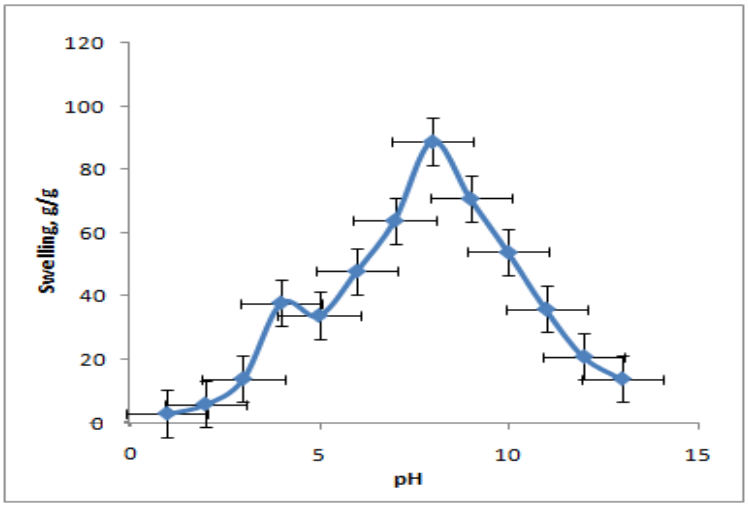

Figure 2. Effect of pH of solution on swelling of protein-*g*-polyacrylic acid hydrogel.

pH-responsiveness behavior of the hydrogel

Since the hydrogels show different swelling behaviors at various pHs, their pH-reversibility was investigated in solutions buffered at pHs 2.0 and 7.0 (Figure 3). The figure shows a stepwise reproducible swelling change of the hydrogel at 25°C with alternating pH between 1.2 and 7.4. At pH 7.4, the hydrogel swells up to 63 $g.g^{-1}$ due to anion–anion repulsive electrostatic forces, while at pH 1.2, it shrinks within few minutes due to protonation of carboxylate groups. This sharp swelling-deswelling behavior of the hydrogels makes them suitable candidates for controlled drug delivery systems [7-8].

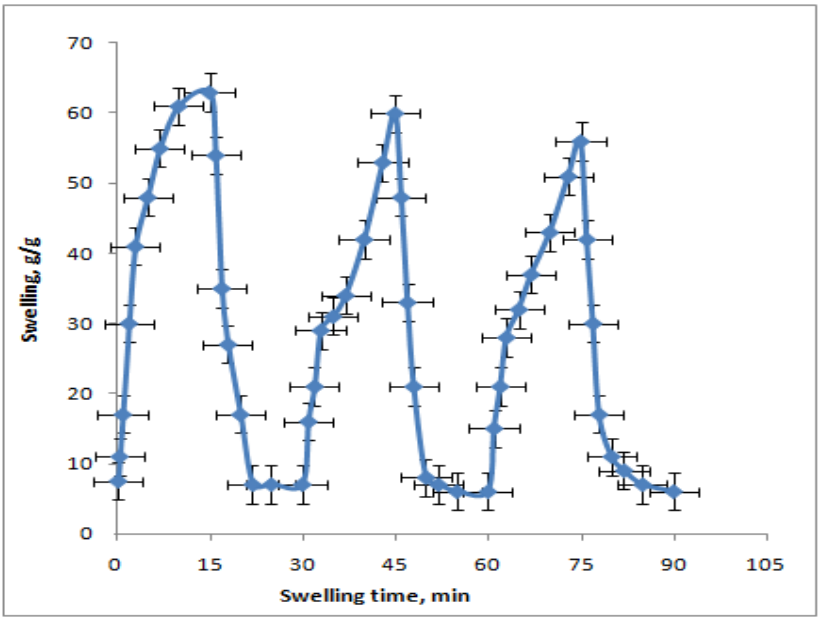

Figure 3. On-off switching behavior as reversible pulsatile swelling (pH 7.4) and deswelling (pH 1.2) of the hydrogel.

In vitro acyclovir Release in the Simulated Human Gastrointestinal System

To determine the potential application of protein-based superabsorbent containing a pharmaceutically active compound, we have investigated the drug release behavior acyclovir form this system under physiological conditions. The percent of released drug from the polymeric carriers as a function of pH is shown in table 1. The concentration of acyclovir released at selected pH intervals was determined by UV spectrophotometer. The drug-loaded hydrogels with high degrees of drug loading (>69%) were prepared by the swelling-diffusion method. The amount of drug released in a specified time from the protein-based hydrogel decreased as the pH of the dissolution medium was lowered, indicating better release in a medium with a pH much higher than that of the stomach [9,10].

Table 1. The percent of released acyclovir from the polymeric carriers as a function of pH.

pH	1.2	2	3	4	5	6	7	7.4	9	10	11	12
Concentration (10^{-4} mol/L)	1.30	1.78	2.10	2.37	2.5	2.47	2.61	3.26	2.49	2.31	1.35	1.08
Percent released	12%	17%	33%	41%	53%	51%	56%	69%	50%	37%	14%	9%

At low pH values, electrostatic repulsion between the carboxylic acid groups of backbone is low, thus decreases gel swelling and minimizes release of drug 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 drug was increased [11,12].

The release rate experiments were also performed in SFG (pH 1.2) and SIF (pH 7.4) solutions at 37°C (Figure 4). As can be seen from Table 1, when pH of the medium is 1.2, the cumulative release ratio of drug from the test hydrogels is below 12% at the end of the experiment (40 h), whereas almost 51% of the loaded drug is released within 10 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 polyacrylate on the hydrogel accelerates the release of drug from the hydrogel [13].

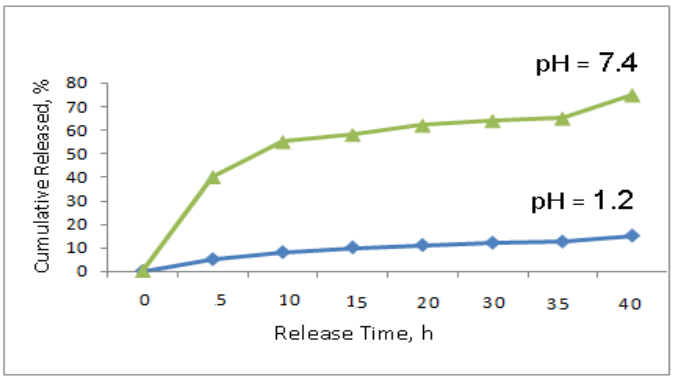

Figure 4. Release of drug from hydrogel carrier as a function of time and pH at 37ºC.

CONCLUSION

A novel biopolymer-based superabsorbent hydrogel, protein-*g*-polyacrylic acid, was synthesized through cross linking and graft polymerization of acrylic acid onto animal's protein. 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.The loading and release of the drug from the pH-sensitive hydrogels was effective. The release value of acyclovir from hydrogels at pH 7.4 was higher than that at pH 1.2 due to the electrostatic repulsion between carboxylate groups.

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