# Preparation of pH-Sensitive Poly(vinyl alcohol-*g*-methacrylic acid) and Poly(vinyl alcohol-*g*-acrylic acid) Hydrogels by Gamma Ray Irradiation and Their Insulin Release Behavior

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**ABSTRACT:** A series of pH-responsive hydrogels were studied as potential drug carriers for the protection of insulin from the acidic environment of the stomach before releasing in the small intestine. Hydrogels based on poly(vinyl alcohol) networks grafted with acrylic acid or methacrylic acid were prepared by a two-step process. Poly(vinyl alcohol) hydrogels were prepared by gamma ray irradiation (50 kGy) and then followed by grafting either acrylic acid or methacrylic acid onto these poly(vinyl alcohol) hydrogels with subsequent irradiation (5–20 kGy). These graft hydrogels showed pH-sensitive swelling behavior and were used as carriers for the controlled release of insulin. The *in vitro* release of insulin was observed for the insulin-loaded hydrogels in a simulated intestinal fluid (pH 6.8) but not in a simulated gastric fluid (pH 1.2). The release behavior of insulin *in vivo* in a rat model confirmed the effectiveness of the oral delivery of insulin to control the level of glucose.
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**Key words:** irradiation; drug delivery systems; hydrogels; pH-responsive; insulin

## INTRODUCTION

Hydrogels are hydrophilic polymer networks that are capable of imbibing large amounts of water, yet are insoluble because of the presence of physical or chemical crosslinks, entanglements, or crystalline regions. Because of the presence of certain functional groups along the polymer chains, hydrogels are often sensitive to the conditions of the surrounding environment. This environmentally sensitive behavior has led to the extensive use of hydrogels in controlled drug delivery systems[1–6] and in membrane separations,[7] where they can respond to changes in the environment and thus regulate drug release or solute diffusion.

Irradiation, especially if combined with simultaneous sterilization of the product, is a very convenient tool for the synthesis of hydrogels. Radiation processing has many advantages over other conventional methods.[8] In radiation processing, no catalysts or additives are needed to initiate the reaction. The advantages of radiation methods are that they are relatively simple, and the degree of crosslinking, which strongly determines the extent of swelling of hydrogels, can be controlled easily by varying the radiation dose.[9,10] Therefore, these methods are found to be very useful in preparing hydrogels for medical applications, where even the slightest contamination is undesirable.

Oral delivery of peptides and proteins to the gastrointestinal (GI) tract is one of the most challenging issues. There are many hurdles, including protein inactivation by digestive enzymes in the GI tract, mainly in the stomach, and poor epithelial permeability of these drugs. However, certain hydrogels may overcome some of these problems by appropriate molecular design or formulation approaches. In this work, we used pH-responsive hydrogels, prepared by gamma radiation grafting, as oral delivery carriers for insulin to protect the insulin from the acidic environment of the stomach. We investigated the insulin release profile of these pH-responsive hydrogels both *in vitro* and *in vivo*.

## EXPERIMENTAL

### Materials

Poly(vinyl alcohol) (PVA; $M_w$ 85,000–146,000, degree of hydrolysis: 99+%) was purchased from Aldrich Co. (Milwaukee, WI). Methacrylic acid (MAA) and acrylic acid (AAc), obtained from Junsei Chemical Co. (To-

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kyo, Japan), were purified by an inhibitor removal column (manufactured by Korea Atomic Energy Research Institute, diameter 5 cm, Pyrex) packed with alumina (Junsei, Tokyo, Japan). Insulin (from bovine pancreas, 28.2 IU/mg) was purchased from Sigma Chemical Co. (St. Louis, MO). Ferrous ammonium salt was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

### Preparation of hydrogels

A 20% (w/v) aqueous solution of PVA was prepared by dissolving PVA in deionized water at 90°C for 24 h. To crosslink the PVA solution, irradiation was carried out by Co-60 source at room temperature. The dose rate was $8.74 \times 10^5$ rad/h. After irradiation, the PVA hydrogel was washed in deionized water for 48 h to remove uncrosslinked polymer and vacuum-dried for 48 h at room temperature. Solutions of MAA and AAc [10, 20, and 30% (v/v)], containing 0, 0.005, 0.01, 0.015, and 0.02*M* of ferrous ammonium salt (Mohr's salt) , respectively, were prepared. The grafting reactions were carried out by subsequent irradiation to PVA hydrogels swollen with these monomer solutions in a capped vial. The PVA-*g*-MAA and PVA-*g*-AAc hydrogels were washed in deionized water for 48 h and vacuum-dried for 48 h at room temperature. The degree of grafting is defined as

$$\text{Degree of grafting (\%)} = \frac{W_g - W_0}{W_0} \times 100 \quad (1)$$

where $W_g$ and $W_0$ denote the dry weights of the grafted and the nongrafted PVA hydrogel, respectively.

### Measurement of swelling characteristics

After irradiation, the crosslinked PVA was vacuum-dried for 48 h at room temperature and weighed. Then, hydrogel was kept in deionized water for 48 h at room temperature and was occasionally shaken. The insoluble part of hydrogel, which consisted of only the crosslinked hydrogel, was vacuum-dried for 48 h at room temperature and weighed. Gel content is defined as

$$\text{Gel content (\%)} = \frac{W_d}{W_i} \times 100 \quad (2)$$

where $W_i$ is the initial weight of dried hydrogel after irradiation and $W_d$ is the weight of the dried insoluble part after extraction with water.

Swelling studies were performed on both PVA-*g*-MAA and PVA-*g*-AAc hydrogels as a function of pH of swelling medium. The weight swelling ratio ($Q$) is defined as

$$Q = \frac{W_s - W_d}{W_d} \quad (3)$$

where $W_s$ and $W_d$ denote the weight of the hydrogels at the swelling state and dry state, respectively. To measure the weight swelling ratio, preweighed dry samples were immersed in swelling medium for a certain period of time. After the removal of surface water with filter paper, the weight of the swollen samples was measured.

### Insulin loading and releasing *in vitro*

Insulin was dissolved in 0.1 mL of 0.1*N* HCl. The insulin solution was diluted with 19.8 mL of deionized water, 50 (v/v%) ethanol solution, or 100% ethanol and then normalized with 0.1 mL of 0.1*N* $NH_4OH$, respectively. Loading was accomplished by soaking the dried PVA-*g*-MAA and PVA-*g*-AAc hydrogels in the insulin solution for 48 h at room temperature. Then the hydrogels were washed with 100 mL of 0.1*N* HCl solution to remove any insulin that remained on their surface. The insulin-loaded hydrogels were dried at room temperature in a vacuum oven of the lowest possible gauge pressure for 24 h and stored at 4°C. Insulin-loaded hydrogels were placed in 30 mL simulated gastric fluid (SGF, pH 1.2, prepared by dissolving 2 g of sodium chloride and 7 mL of concentrated HCl in 1 L of distilled water) at 37°C for 2 h and then in 30 mL simulated intestinal fluid (SIF, pH 6.8, prepared by mixing 250 mL of 0.2*M* $KH_2PO_4$ and 118 mL of 0.2*N* NaOH) at 37°C for 6 h to mimic *in vivo* conditions in the GI tract. At several different time intervals, 3 mL of the release medium was collected and replaced by the same volume of buffer (SGF or SIF). The released insulin concentration was analyzed at 274 nm by using a UV-spectrophotometer. A series of insulin solutions ranging from 0 to 0.5 mg/mL in concentrations were used to prepare a standard calibration curve.

### Insulin loading and releasing *in vivo*

Male Wistar rats (200g) were used. Diabetes was induced in the rats by intraperitoneal injection of streptozotocin (Sigma, 40 mg/kg body weight, once daily for 3 consecutive days) dissolved in citrate buffer at pH 4.6. The rats were considered diabetic when the glucose level exceeded 250 mg/dL after the streptozotocin treatment. Insulin was administered to the rats for *in vivo* study by either oral delivery of the insulin-loaded PVA-*g*-AAc hydrogel or injection of insulin solution. The insulin-loaded and dried hydrogels were

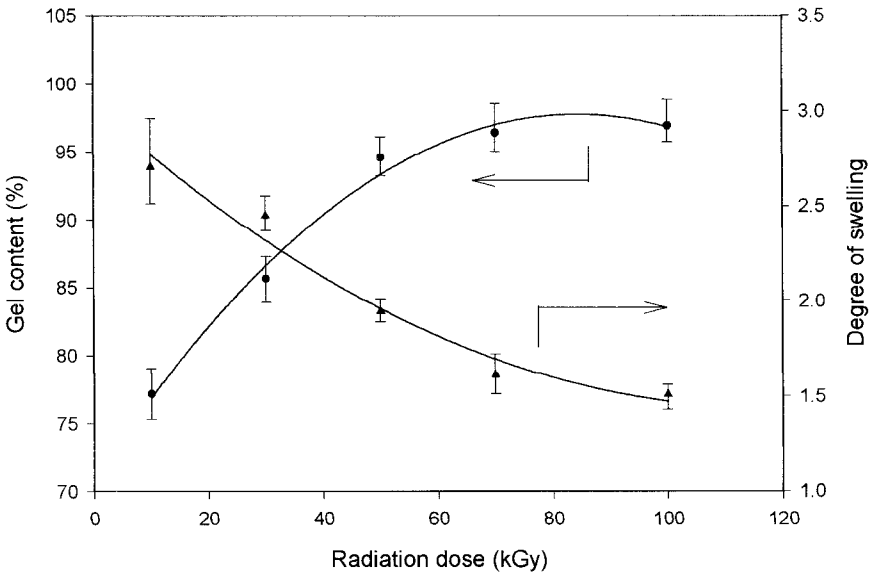


**Figure 1** Gel content and weight swelling ratio of PVA hydrogel as a function of irradiation dose.

administered orally through the tube inserted to the throat in the form of granules. Serum glucose levels of rats were measured by using a blood glucose meter (Surestep, Lifescan Inc.). The data represent mean values from five independent experiments.

## RESULTS AND DISCUSSION

Figure 1 shows the gel content of PVA hydrogel as a function of the irradiation dose. As irradiation dose increased, the gel content increased slightly because of the enhancement in degree of crosslinking. The degree of swelling of PVA hydrogel as a function of irradiation dose is also shown in Figure 1. The higher the irradiation dose, the less the degree of swelling, attributed to the formation of a more tightly crosslinked structure.

Figure 2 shows the effect of irradiation dose on the grafting of MAA onto PVA hydrogels. All of the samples tend to exhibit a similar trend in grafting as a function of radiation dose. As the concentration of MAA solution for grafting increased, the grafting yield increased. The rate of increase in grafting was higher in the lower dose range. No further increase in

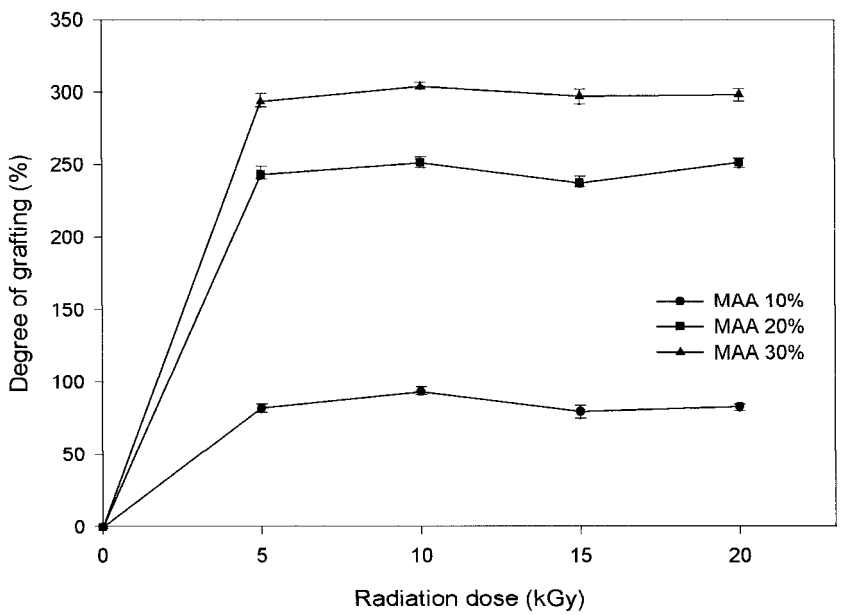


**Figure 2** Effect of irradiation dose on the grafting of methacrylic acid onto PVA hydrogel.

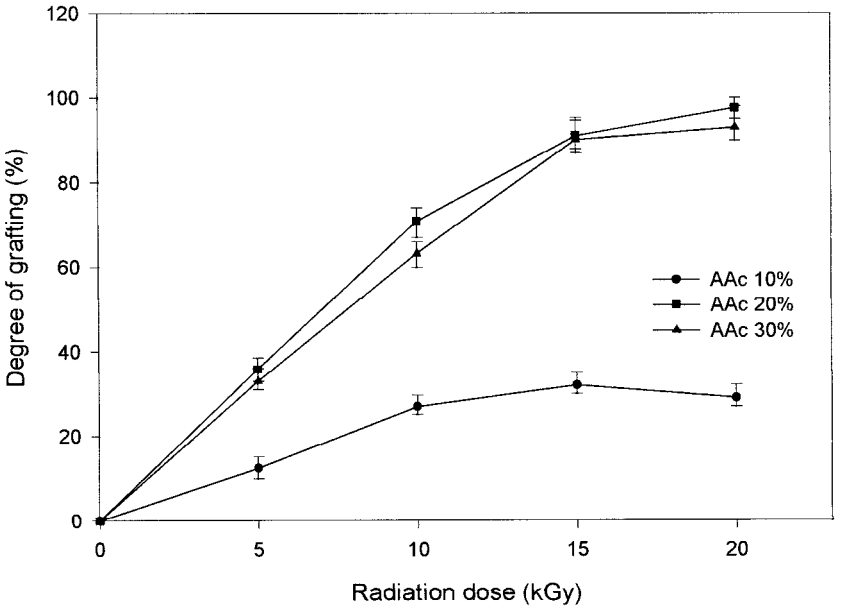


**Figure 3** Effect of irradiation dose on the grafting of acrylic acid onto PVA hydrogel.

grafting was observed above the radiation dose of 5 kGy.

Figure 3 exhibits the effect of irradiation dose on the grafting of AAc onto PVA hydrogels. Contrary to MAA-*grafted*-PVA, grafting yield increased in proportion to the radiation dose up to 15 kGy. When the irradiation dose exceeded 15 kGy, both homopolymerization and grafting of AAc took place competitively. Thus grafting yield was not further increased above an irradiation dose of 15 kGy.

The grafting yield of MAA was much higher than that of AAc, as shown in Figures 2 and 3. The reactivity of monomers and radicals on copolymerization is determined by the nature of the substituents on the double bond of the monomer. The methyl group of methacrylic acid may activate the double bond, making the monomer more reactive than acrylic acid. It was known that activation energies for polymerization of acrylic and methacrylic acid in salt-free solutions were 16.7 and 15.6 kcal/mol, respectively.[11]

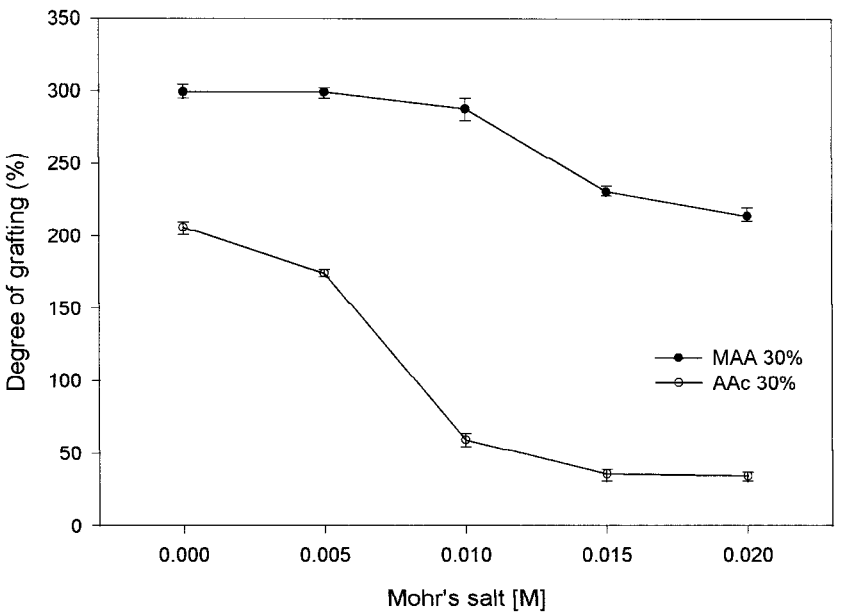


**Figure 4** Effect of Mohr's salt on the grafting of methacrylic acid and acrylic acid to PVA hydrogel.

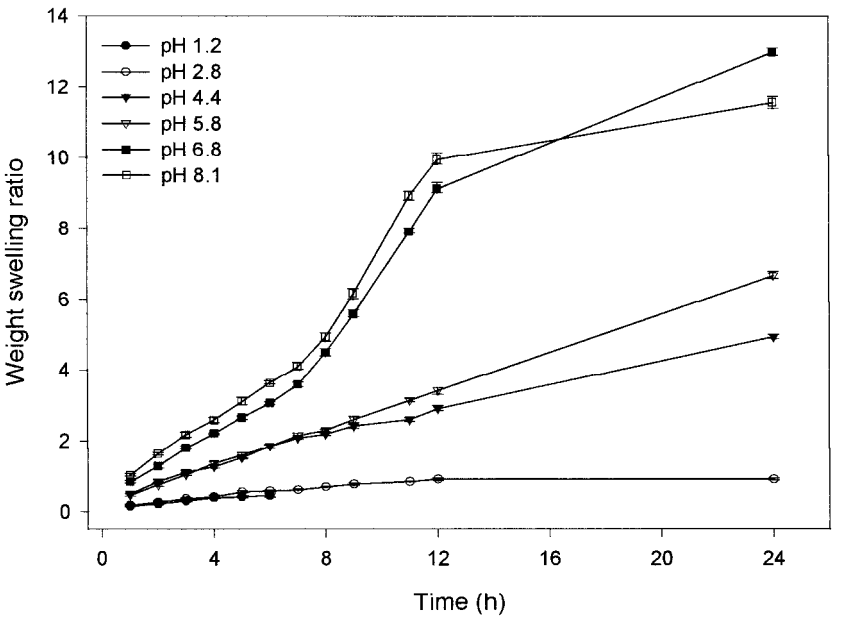


**Figure 5** Weight swelling ratio of PVA-*g*-MAA hydrogels swollen in several different buffer solutions as a function of time at 25°C.

Figure 4 shows the effect of Mohr's salt concentration on the grafting of MAA and AAc to PVA hydrogels. The addition of Mohr's salt in the graft polymerization system led to a decrease in grafting yield. Mohr's salt was incorporated to suppress the side reactions such as homopolymerization. Metallic salts such as $Fe^{2+}$ and $Cu^{2+}$ play a role in inhibiting the grafting reaction as well as homopolymerization above a certain concentration. The degree of grafting decreased with the addition of more than $5 \times 10^{-3}$ *M* Mohr's salt.

Figures 5 and 6 show the typical swelling behavior of the graft hydrogels at several different pH values. The swelling rate was significantly faster for the hydrogel network in solutions of pH above 5.5 than for that in lower pH media. At lower pH, where complexation occurred, both the swelling rate and swelling ratio were low. Complex formation resulted from the

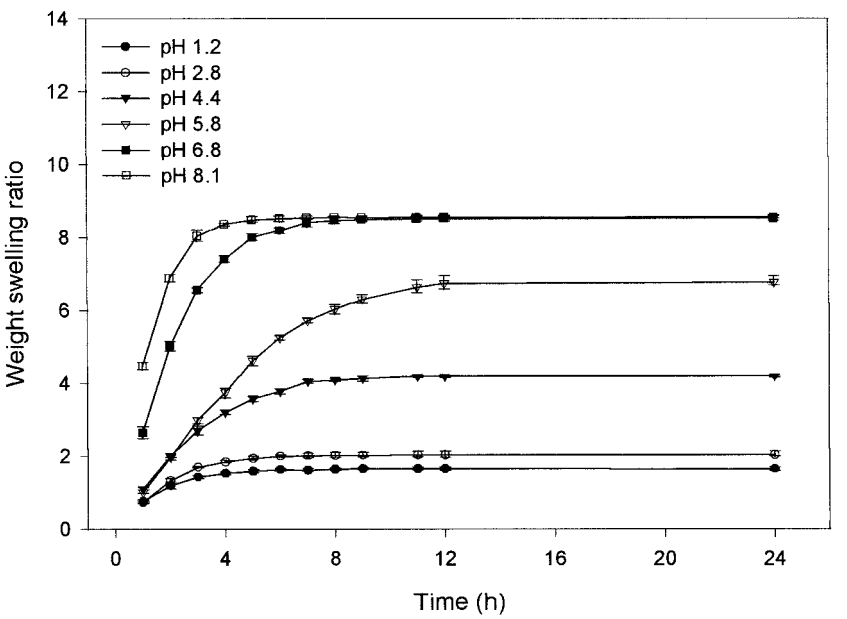


**Figure 6** Weight swelling ratio of PVA-*g*-AAc hydrogels swollen in several different buffer solutions as a function of time at 25°C.

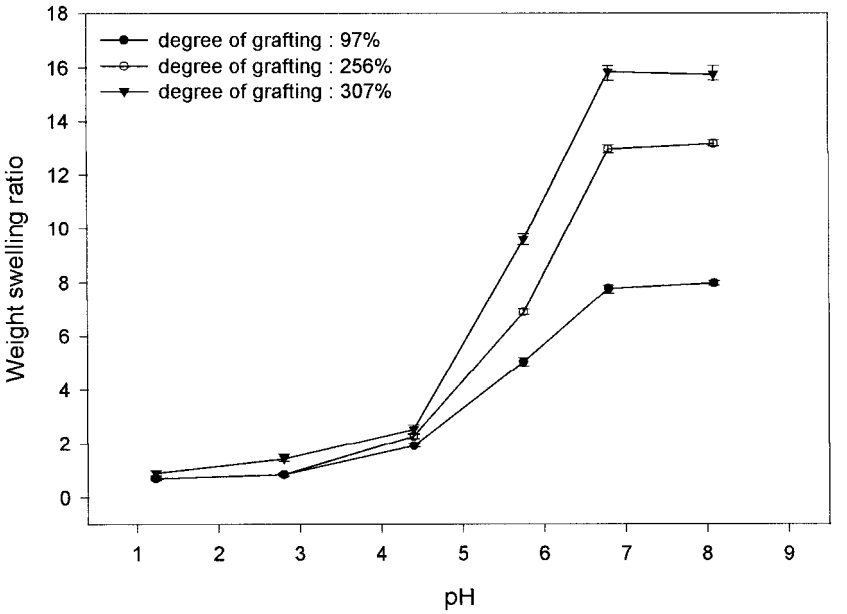


**Figure 7** Swelling behaviors of PVA-*g*-MAA hydrogels depending on pH at 25°C.

formation of temporary physical crosslinks attributed to hydrogen bonding between PVA segments and carboxyl groups of MAA or AAc graft segments. This hydrogen-bonded complex caused the polymer network to be less hydrophilic because the carboxyl groups on the MAA and AAc graft chains participated in the complex formation. As the pH increased, the complexation did not occur, resulting in both faster swelling rate and higher swelling ratio. In higher pH media, the complexes were broken and the carboxylic acid groups on the MAA and AAc graft became progressively more ionized. In these cases, the hydrogels swelled more rapidly as a result of a large swelling driving force created by the electrostatic repulsion between the ionized carboxylate groups. In the transition region of pH between 4.4 and 5.8, the swelling was governed by ionic interactions as well as interpolymer complexation. Because AAc graft is more hydrophilic than MAA graft, AAc grafted hydrogel reached the equilibrium swelling earlier.

Figures 7 and 8 exhibit typical equilibrium swelling as a function of pH. Both PVA-*g*-MAA and PVA-*g*-

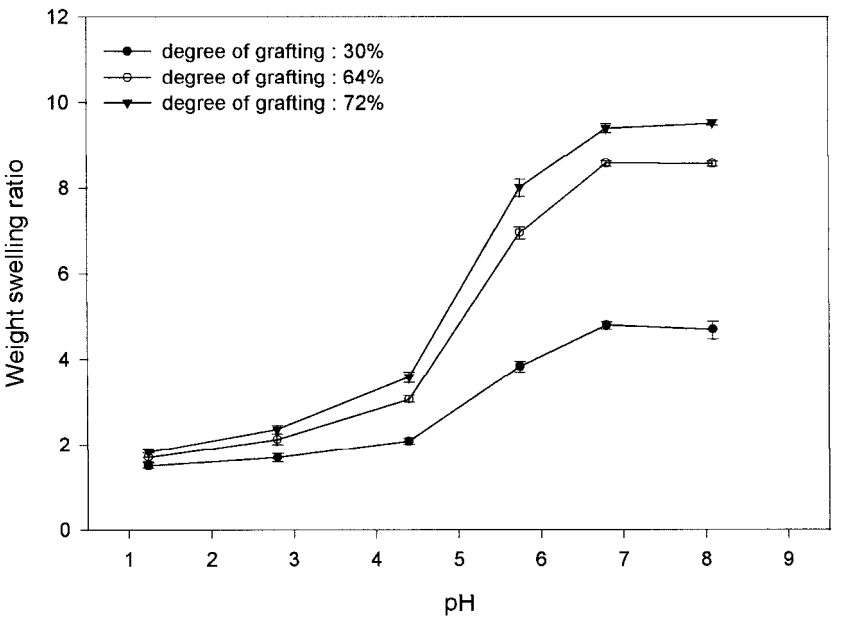


**Figure 8** Swelling behaviors of PVA-*g*-AAc hydrogels depending on pH at 25°C.

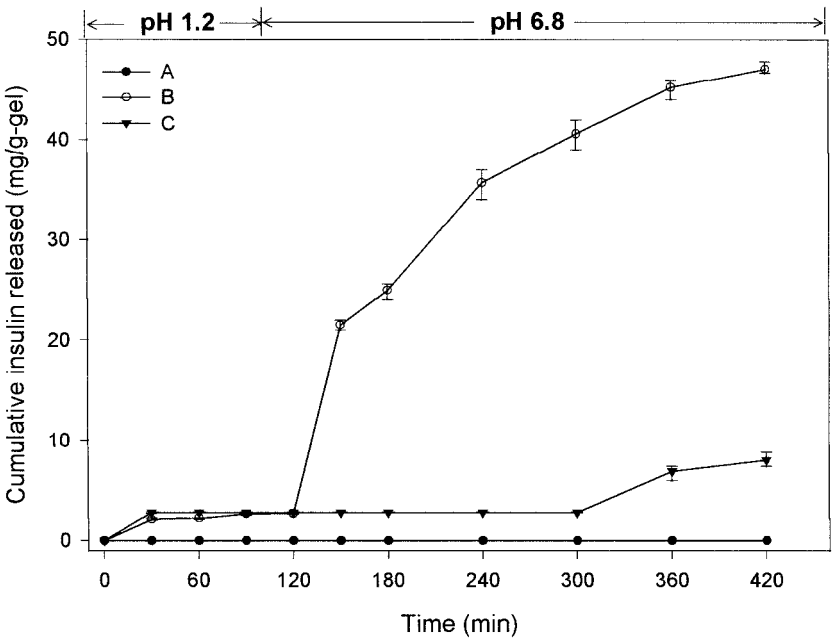


**Figure 9** Insulin release profile from PVA-*g*-AAc hydrogel at two different pH conditions depending on time.

AAc hydrogels showed similar swelling behaviors as a function of pH. It was found that the graft copolymers containing the highest amount of MAA and AAc gave rise to higher swelling ratio. This swelling behavior was explained by the fact that higher MAA and AAc content resulted in a larger electrostatic repulsion attributed to the higher content of ionized carboxylate groups and thus a higher swelling ratio.

Figure 9 shows *in vitro* insulin release profiles from PVA-*g*-AAc hydrogel at two different pH environments depending on time. An insulin-loading solution was prepared with either 50 (v/v%) ethanol solution, 100% ethanol, or deionized water. When 50% ethanol solution was used, the optimum release profile could be obtained. Because of the slight hydrophobic nature of insulin, the solubility of insulin was higher in ethanol solution than in deionized water. When deionized water was used, the precipitation of insulin oc-

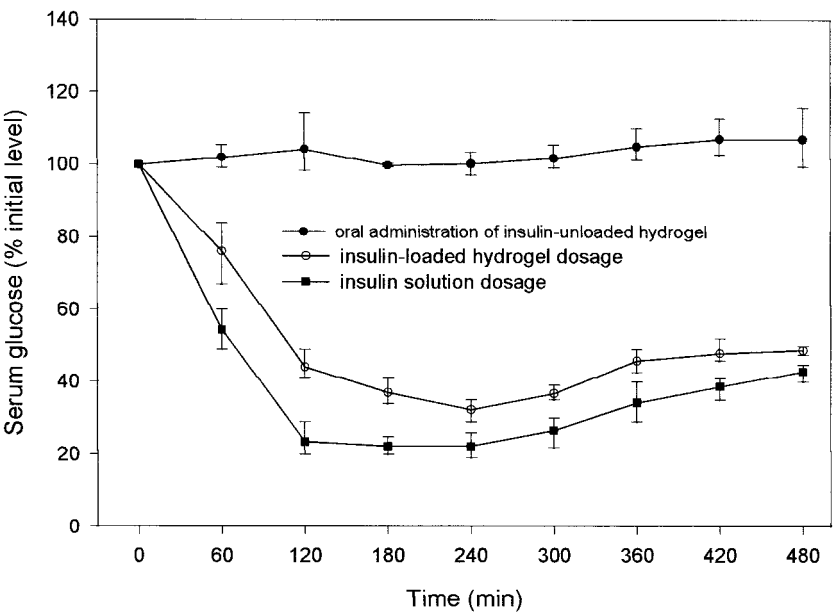


**Figure 10** Blood glucose response in rats after the oral administration of insulin-loaded PVA-*g*-AAc hydrogel and injection of insulin solution.

curred in several hours. Therefore, ethanol was used to prevent precipitation of insulin. Figure 10 shows a variation of blood glucose level according to both the oral administration of insulin-loaded PVA-*g*-AAc hydrogel and injection of insulin solution. After 2 h of receiving both the polymeric dosage form and direct injection of insulin, the blood glucose level reached the lowest level in a similar manner. This result confirmed that the insulin could be delivered effectively to the small intestine in the biologically active form.

## CONCLUSIONS

A series of pH-responsive hydrogels based on PVA-*g*-MAA and PVA-*g*-AAc were developed as drug carriers for oral drug delivery. The preparation of these graft copolymeric hydrogels was carried out using gamma radiation–initiated polymerization. The degree of grafting of these hydrogels increased as the concentration of MAA and AAc monomers increased. The equilibrium swelling measurements of these hydrogels, which were carried out in simulated gastrointestinal fluids, showed their pH-responsive nature. The *in vitro* release profile of insulin was obtained in both simulated gastric fluid and simulated intestinal fluid. The release behavior of PVA-*g*-MAA– and PVA-*g*-AAc–based pH-responsive hydrogels indicated that these hydrogels could be applied successfully for oral drug delivery to the gastrointestinal tract. The oral administration of insulin-loaded hydrogels to rats obviously decreased the blood glucose levels for at least 4 h because of the absorption of insulin in the gastrointestinal tract.

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## References

1. Hoffman, A. S. Adv Drug Deliv Rev 2001, 43, 3.
2. Lowman, A. M.; Peppas, N. A. Macromolecules 1997, 30, 4959.
3. Lowman, A. M.; Morishita, M.; Kajita, M.; Nagai, T.; Peppas, N. A. Am Chem Soc Am Pharm Assoc 1999, 88, 933.
4. Siegel, R. A. J. Controlled Release 1988, 8, 179.
5. Qiu, Y.; Park, K. N. Adv Drug Deliv Rev 2001, 53, 321.
6. Ravichandran, P.; Shantha, K. L.; Rao, P. K. Int J Pharm 1997, 154, 89.
7. Peppas, N. A. J Bioact Compat Polym 1991, 6, 241.
8. Bhattacharya, A. Prog Polym Sci 2000, 25, 371.
9. Jabbari, E.; Nozari, S. Eur Polym J 2000, 36, 2685.
10. Rosiak, J. M.; Ulanski, P. Radiat Phys Chem 1999, 55, 139.
11. Nho, Y. C.; Jin, J. H. J Appl Polym Sci 1997, 63, 1101.