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# Preparation and Characterization of pH-Sensitive Poly(ethylene oxide) Grafted Methacrylic Acid and Acrylic Acid Hydrogels by $\gamma$-ray Irradiation

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**Abstract:** pH-sensitive hydrogels were studied as a drug carrier for the protection of insulin from the acidic environment of the stomach before releasing it in the small intestine. In this study, hydrogels based on poly(ethylene oxide) (PEO) networks grafted with methacrylic acid (MAA) or acrylic acid (AAc) were prepared via a two-step process. PEO hydrogels were prepared by $\gamma$-ray irradiation (radiation dose: 50 kGy, dose rate: 7.66 kGy/h), grafted by either MAA or AAc monomers onto the PEO hydrogels and finally underwent irradiation (radiation dose: 5-20 kGy, dose rate: 2.15 kGy/h). These grafted hydrogels showed a pH-sensitive swelling behavior. The grafted hydrogels were used as a carrier for the drug delivery systems for the controlled release of insulin. Drug-loaded hydrogels were placed in simulated gastric fluid (SGF, pH 1.2) for 2 hr and then in simulated intestinal fluid (SIF, pH 6.8). The *in vitro* drug release behaviors of these hydrogels were examined by quantification analysis with a UV-Vis spectrophotometer.

*Keywords*: radiation, crosslinking, radiation grafting, poly(ethylene oxide), pH-sensitive hydrogels, drug delivery, insulin.

## Introduction

Hydrogels are three-dimensional networks of hydrophilic polymers held together by crosslinks of covalent bonds or ionic bonds and secondary forces in the form of hydrogen bonds or hydrophobic interactions.[1,2] Environmentally sensitive hydrogels have an enormous potential for various applications. Some environmental variables, such as pH and elevated temperatures, are found in the body. For this reason, either pH-sensitive and/or temperature-sensitive hydrogels can be used for a site-specific controlled drug delivery.[3] Especially, pH-sensitive hydrogels have been most frequently used to develop controlled release formulations for oral administration.[3,4] All the pH-sensitive hydrogels contain pendent acidic, for example carboxylic and sulfonic acids, or basic, for example ammonium salts, groups that either accept or release protons in response to changes in environmental pH.[3-8] These ionic hydrogels are the swollen polymer networks which show sudden or gradual changes in their dynamic and equilibrium swelling behavior as a result of changing the external pH. In these gels, ionization occurs when the pH of the environment is above the $pK_a$ of the ionizable group.[5-9] As the degree of ionization increases (pH increase in the system), the number of fixed charges increases, resulting in increased electrostatic repulsions between the chains.[4]

There are many advantages in using ionic over neutral networks in drug delivery. Their properties can be applied in a wide variety of biomedical applications, such as dental adhesives and restorations, controlled release devices, prodrugs and adjuvants, and biocompatible materials.[1,4,9-11]

Many researchers have studied the dynamic swelling of pH-sensitive networks. One of these, studies by Khare and Peppas[9] examined the swelling kinetics of poly(methacrylic acid) or poly(acrylic acid) (PAAc) with poly(hydroxy ethyl methacrylate). They observed the pH- and ionic strength-dependent swelling kinetics in these gels. It is known that PAAc has been considered as a pH and electrical sensitive material due to the ionic repulsion between the anionic charged groups, and thus forms polymer complexes with polybases such as poly(ethylene oxide), polyvinylpyrroidone, or polyacrylamide.[12-15]

Irradiation, especially if combined with simultaneous sterilization of the product, is a very convenient tool for the

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synthesis of hydrogels. Radiation processing has many advantages over other conventional methods.[16] For initiation processes, radiation differs from chemical initiation. In radiation processing, no catalysts or additives are needed to initiate the reaction. The advantages of the radiation methods are that they are relatively simple, and moreover, the degree of crosslinking, which strongly determines the extent of swelling in hydrogels, can be controlled easily by varying the absorbed dose.[17,18] Therefore, these methods are found to be very useful in preparing hydrogels for medical applications, where even a small contamination is undesirable.

Oral delivery of peptides, proteins and other drugs to the gastrointestinal (GI) tract is one of the most challenging issues, and thus, under much investigation.[19-23] There are many hurdles, including protein inactivation by digestive enzymes in the GI tract, and the poor epithelial permeability of these drugs. Certain hydrogels may overcome some of these problems by appropriate molecular design or formulation approaches.

In this work, we prepared the pH-sensitive hydrogels by the $\gamma$-ray radiation grafting technique, as an oral delivery carrier for a drug. The effects of radiation dose and monomer composition in the gel formation were investigated as well as the stimuli-sensitive property such as the specific pH for applications for the controlled drug release systems *in vitro*.

## Experimental

**Materials.** Poly(ethylene oxide) (PEO), $M_w$ $2.0 \times 10^5$, was purchased from the Aldrich Chemical Company Inc. Milwaukee in the USA. Methacrylic acid (MAA) and acrylic acid (AAc) monomer obtained from the Junsei Chemical Co. Tokyo in Japan, were purified by an inhibitor removal column packed with aluminum oxide (Junsei Chemical Co. Tokyo in Japan). Ferrous ammonium sulfate ($FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$, Mohr's salt) was purchased from the Wako Pure Chemical Industries Ltd. Osaka in Japan. Insulin (from bovine serum, 28.2 IU/mg) was purchased from the Sigma Chemical Co. St. Louis in USA.

**Preparation of Hydrogles.** An aqueous solution of PEO was prepared by dissolving PEO in distilled water at room temperature with a stirrer. In order to crosslink the PEO solution, irradiation was carried out by a $^{60}Co$ source. The dose rate was 7.66 kGy/hr. After irradiation, the crosslinked PEO hydrogel was dried and weighted. Then, the hydrogel was kept in deionized water for 48 hr at room temperature and was occasionally shaken. The insoluble part of the hydrogel made up of only the crosslinked hydrogel, was dried and weighed. The gel content is defined as

$$\text{Gel content}(\%) = \frac{W_d}{W_i} \times 100 \quad (1)$$

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

10, 20 and 30% (v/v) solutions of MAA and AAc which contain 0, 0.005, 0.01, 0.015 and 0.02 M of $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ (Mohr's salt) were prepared, respectively. The grafting experiments were performed by the mutual irradiation method for the PEO hydrogel swollen with these monomer solutions in a capped vial. The PEO-*g*-MAA and PEO-*g*-AAc hydrogels were washed in deionized water and dried in air. The degree of grafting is defined as

$$\text{Degree of grafting}(\%) = \frac{(W_g - W_o)}{W_o} \times 100 \quad (2)$$

Where $W_g$ and $W_o$ denote the weights of the grafted and the ungrafted PEO hydrogel, respectively.

**Measurement of Swelling Characteristics.** Swelling behavior was examined for both the PEO-*g*-MAA and PEO-*g*-AAc hydrogels as a function of the pH of the swelling medium. The weight swelling ratio ($Q$) is define 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 a swelling medium for a certain period of time. After the excessive surface water on the swollen hydrogel was removed with filter paper, the weight of the swollen sample was measured.

**Instrumental Analysis.** The ATR-FTIR spectroscopic investigations were carried out with a Bruker TENSOR 37 spectrophotometer equipped with a DLaTGS detector. Transmission and ATR spectra were recorded at 16 scans with a resolution of 4 $cm^{-1}$. DSC thermogram was recorded using a TA instruments Q-1000, which were scanned up to 300 °C at a heating rate of 10 °C/min under a $N_2$ flow. The surface morphologies of hydrogels were observed by a XL Series 30 s scanning electron microscope (SEM) (Philips Co., Netherlands). UV-vis analysis was performed with a SCINCO Co. S-1100.

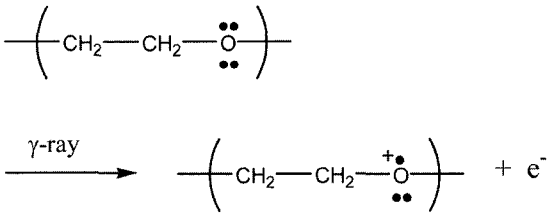
**Drug Loading.** In this experimental, insulin (100 mg) was dissolved in 1 mL of 0.1 N HCl. The insulin solution was diluted with a 198 mL 25 vol% ethanol aqueous solution and normalized 1 mL of 1 N NaOH. Loading was accomplished by soaking the freeze dried PEO-*g*-MAA and PEO-*g*-AAc hydrogels for 48 hr in the insulin solution. Then the hydrogels were filtered using filter paper with Whatman No. 1 and washed with 100 mL 0.1 N HCl solution to remove the remaining insulin from their surface. The insulin-loaded hydrogels were dried under vacuum and stored at 4 °C.

**Drug Releasing *in vitro*.** Drug-loaded hydrogels (100 mg) were placed in a 30 mL enzyme-free simulated gastric fluid (SGF, pH 1.2, prepared by dissolving 2 g of NaCl and 7 mL of concentrated HCl in 1 L of distilled water) at 37 °C for

2 hr and then in a 30 mL enzyme-free 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 8 hr in order 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 a buffer solution (SGF or SIF). The released insulin concentration was analyzed by UV-Vis spectrophotometer.

## Results and Discussion

Figure 1 shows the gel content of the PEO hydrogel as a function of the irradiation dose. As the irradiation dose and the concentration of the PEO increased, the gel content of the PEO increased. Highly crosslinked hydrogels have a tighter structure, and swell less compared to the hydrogels with lower crosslinking ratios. Crosslinking hinders the mobility of the polymer chain, hence it lowers the swelling ratio. When aqueous solutions of PEO are subjected to γ-ray irradiation, the radicals are formed in the polymer chain either by a direct action of the high energy radiation, or by an indirect attack of the solvent derived radicals.[24] Based on an ESR study, Ferloni *et al.*[25] have found that γ-ray radiation of the PEO chains leads to the localization of positive charges in the PEO oxygen atoms as shown below



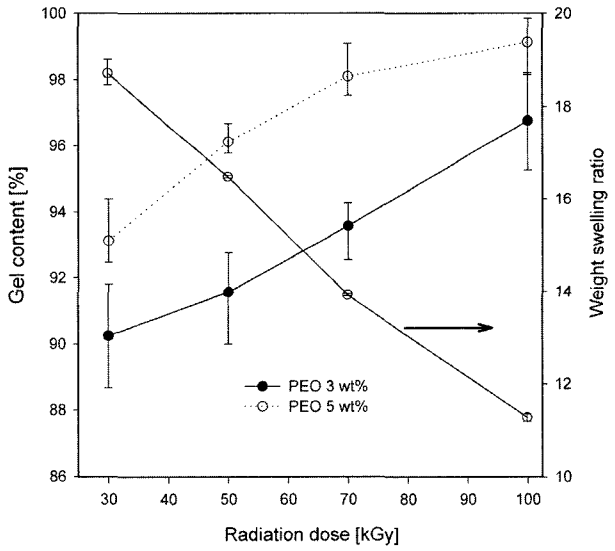
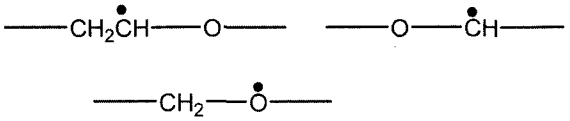




**Figure 1.** Gel content of PEO hydrogels as a function of irradiation dose (PEO $M_w=2\times10^5$).

The positive polymer radicals then undergo α– and β–scissions resulting in three types of radicals



Finally, the combination of these radicals can lead to crosslinking in the PEO structure.

The hydrogels synthesized by irradiating (50 kGy) PEO 5 wt% aqueous solution were used for grafting because the hydrogel had the suitable physical properties for pH-sensitive hydrogels. Figure 2 shows the effect of irradiation dose on the grafting of MAA onto PEO hydrogels. As the concentration of MAA solution for grafting increased, the grafting yield increased. In the case of 30% MAA, the high grafting yield was obtained even at a low dose of 5 kGy. On the other hand, the degree of grafting of 10% MAA monomer increased slightly with an increase of radiation dose.

Figure 3 exhibits the effect of irradiation does on the grafting of AAc onto PEO hydrogels. When the irradiation dose was more than 15 kGy, the rate of the grafting was low at 10, 20% AAc concentration. The grafting yield of 30 wt% of the MAA was much higher than that of the AAc as shown in Figures 2 and 3. The reactivity of the monomers and radicals in copolymerization is determined by the nature of substituents in the double bond of the monomer. The methyl group of methacrylic acid may activate the double bond, making the monomer more reactive than the acrylic acid. It is known that activation energies for polymerization

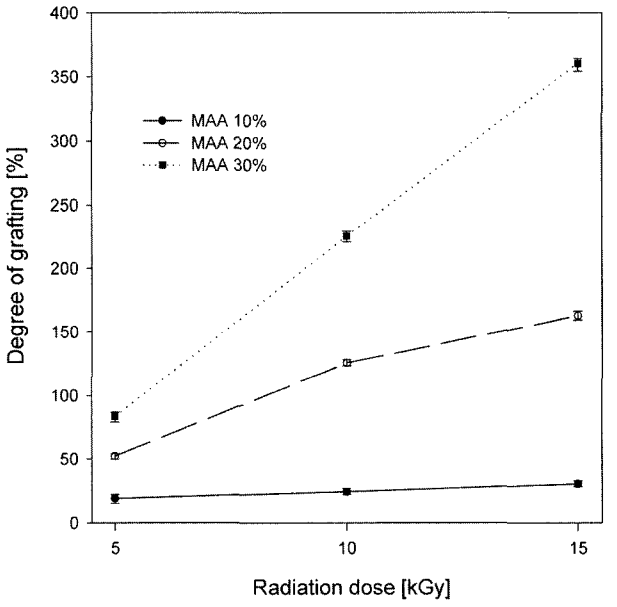



**Figure 2.** Effect of irradiation dose on the grafting of MAA onto PEO hydrogel. 0.01 M Mohr's salt was added in the monomer solution.

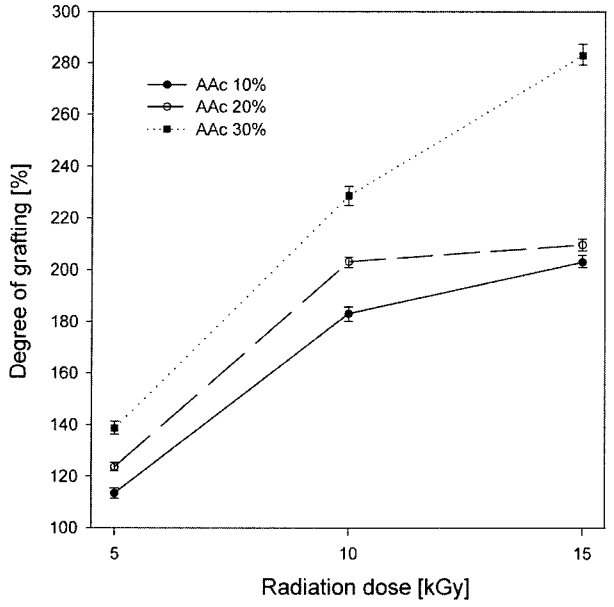



**Figure 3.** Effect of irradiation dose on the grafting of AAc onto PEO hydrogel. 0.01 M Mohr's salt was added in the monomer solution.

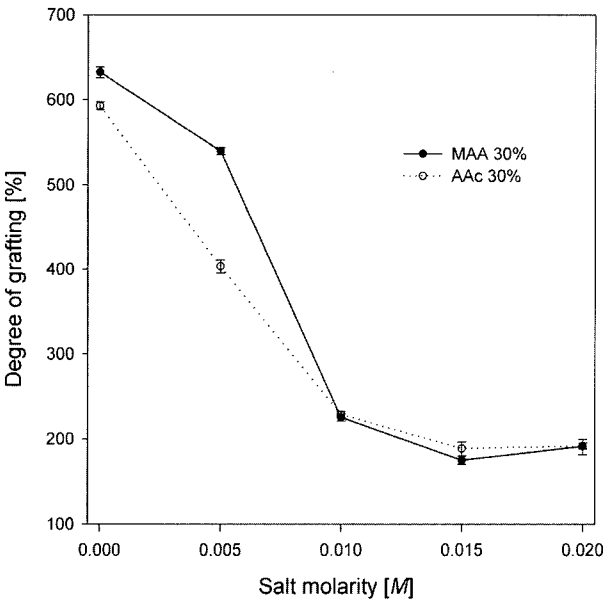



**Figure 4.** Effect of Mohr's salt on the grafting of MAA and AAc to PEO hydrogel (10 kGy irradiation).

of AAc and MAA in salts-free solutions are 16.7 and 15.6 kcal/mol, respectively.[26]

Figure 4 shows the effect of Mohr's salt ($FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$) concentration on the grafting of the MAA and AAc onto the PEO hydrogels. The addition of the salt in the graft polymerization system led to a decrease in grafting yield. Mohr's salt was incorporated in order to suppress the side

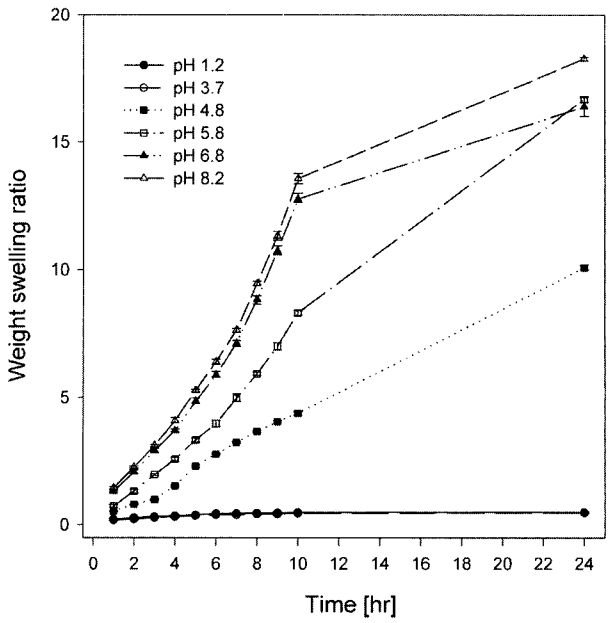



**Figure 5.** Degree of swelling of PEO-*g*-MAA hydrogels swollen in several different buffer solutions as a function of time at 25 °C (10 kGy irradiated, degree of grafting of hydrogel : 225%).

reactions such as homopolymerization and extra gelation of the monomer solution. Metallic salts such as $Fe^{2+}$ and $Cu^{2+}$ play a role in inhibiting the grafting reaction as well as homopolymerization above a certain content. The degree of grafting of the AAc monomer was drastically decreased with the addition of $1 \times 10^{-2}$ M Mohr's salt.

Figures 5 and 6 show the typical swelling behavior of the graft hydrogels at several different pH values. It is obvious that the swelling ratios of the hydrogels are significantly higher at a pH above 5.8 compared to the lower pH media. At a low pH, when the complexation occurred, both the swelling rate and ratio were low. Complex formation results from the formation of temporary physical crosslinks due to hydrogen bonding between the PEO and PMAA or PAAc pendent groups. This hydrogen-bonded complex causes the polymer network to be less hydrophilic because the carboxyl groups in the PMAA and PAAc graft chain participate in the complex formation. As the pH increases, complexation does not occur resulting in both a faster swelling rate and higher swelling ratio. In a higher pH media, the complexes are broken and the carboxylic acid groups in the PMAA and PAAc become progressively more ionized. In these cases, the hydrogels swell more rapidly due to a large swelling force created by electrostatic repulsion between the ionized carboxylate groups. In the transition region of a pH between 4.8 and 5.8, the swelling is governed by the ionic interactions as well as interpolymer complexation.

Figures 7 and 8 show the swelling ratio in SIF/SGF of the grafted hydrogels. With increasing the SIF/SGF values, it can expect that drug release effect will be increased in

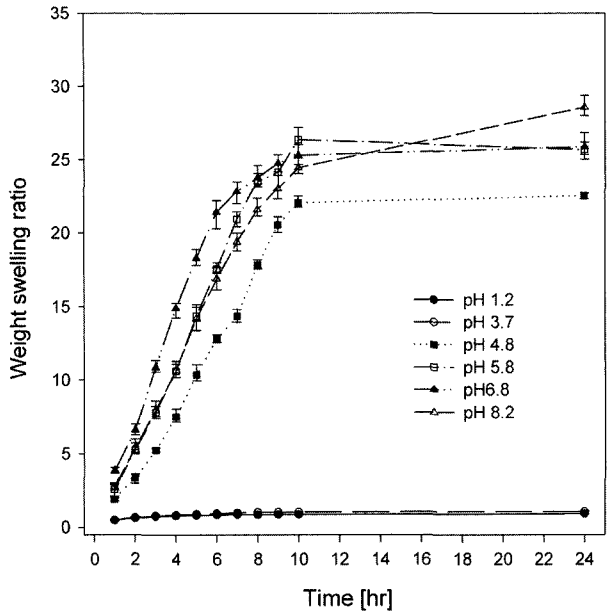



**Figure 6.** Degree of swelling of PEO-*g*-AAc hydrogels swollen in several different buffer solutions as a function of time at 25 °C (10 kGy irradiated, degree of grafting of hydrogel : 203%).

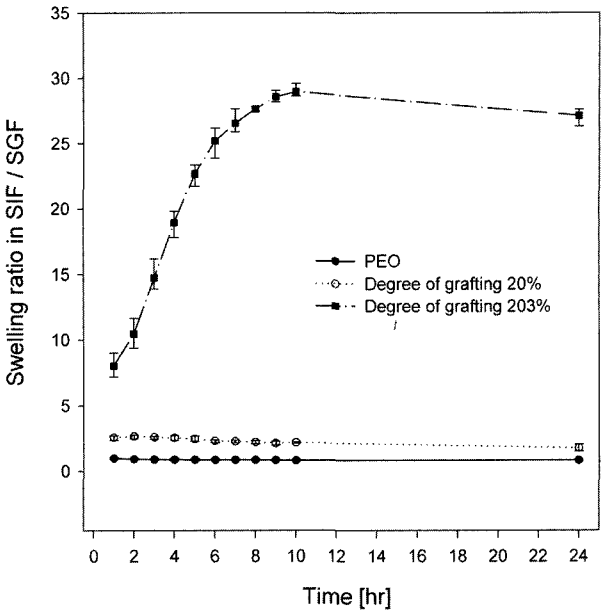



**Figure 8.** Swelling ratio in SIF/SGF of PEO-*g*-AAc hydrogels swollen in SIF and SGF as a function of time at 25 °C (10 kGy irradiation).

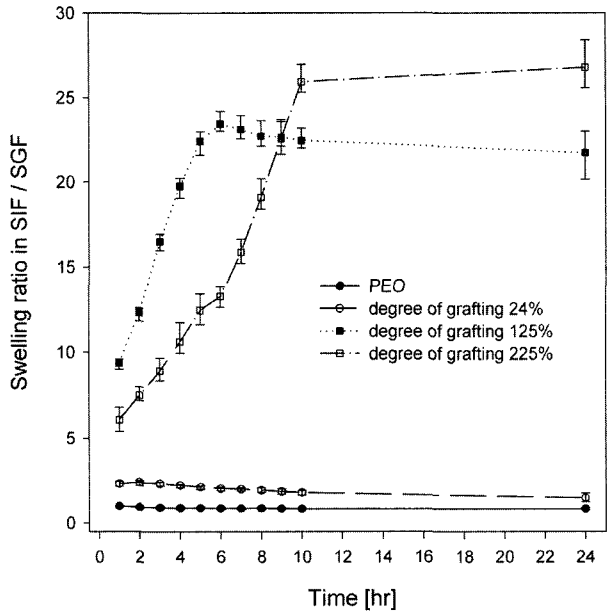



**Figure 7.** Swelling ratio in SIF/SGF of PEO-*g*-MAA hydrogels swollen in SIF and SGF as a function of time at 25 °C (10 kGy irradiation).

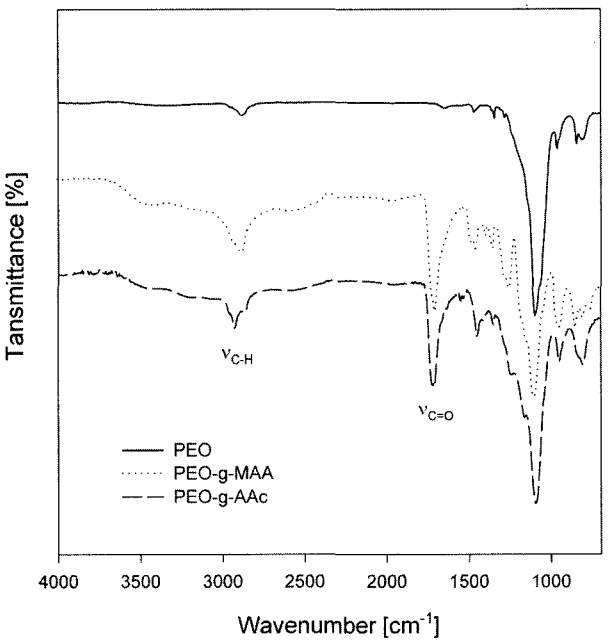



**Figure 9.** ATR-FTIR spectra of PEO, PEO-*g*-MAA and PEO-*g*-AAc hydrogels (PEO; 50 kGy irradiated, PEO-*g*-MAA and PEO-*g*-AAc; 10 kGy irradiated, degree of grafting of hydrogel : 24%, 183% respectively).

intestine. Virgin crosslinked PEO showed a similar swelling behavior in SIF and SGF. Both PEO-*g*-MAA and PEO-*g*-AAc hydrogels showed a similar swelling behavior as a function of time. It was found that the graft copolymers containing a high amount of MAA and AAc gave rise to a high swelling ratio in SIF/SGF. This swelling behavior was explained by the fact that a higher MAA and AAc content resulted in a larger electrostatic repulsion due to the higher content of the ionized carboxylate groups and thus a higher swelling ratio.

Radiation crosslinked hydrogels were characterized by an ATR-FTIR spectroscopy. In Figure 9, ATR-FTIR spectra of the PEO, PEO-*g*-MAA and PEO-*g*-AAc are shown. PEO exhibits the absorption features at 2938-2976 $cm^{-1}$ ($\nu_{C-H}$) and around 1110 $cm^{-1}$($\nu_{C-O-C}$). PEO-*g*-MAA and PEO-*g*-AAc show an intense band (1734 $cm^{-1}$) corresponding to the carbonyl group shifted by the hydrogen bonding between the ether group of the PEO and the hydroxyl group of the carboxyl group of the AAc or MAA.

Figure 10 shows the ATR-FTIR spectra of the PEO-*g*-AAc hydrogel depending on the swelling time. A peak at 2938-2976 $cm^{-1}$($\nu_{C-H}$) and 1734 $cm^{-1}$($\nu_{C=O}$) in the swelling condition disappeared partially. On the other hand, the peak at 1558 $cm^{-1}$($\nu_{COO^-}$) appeared clearly. As the swelling time is increased, the $\nu_{COO^-}$ peak intensity increases because the -COOH group is dissociated into $-COO^-$ and $H^+$.

Figure 11 shows the DSC melting endotherms of the PEO and PEO-*g*-MAA hydrogels. PEO hydrogel has $T_m$ (49.8 °C). We can detect a small melting peak of PEO in the DSC thermogram of the PEO-*g*-MAA hydrogel. This phenomenon can be attributable to the decrease in a crystalline structure of the PEO after the PEO was grafted with MAA.

Figure 12 shows the scanning electron microscope of the PEO-*g*-MAA hydrogels which were freeze-dried after swelling for 10 hr in SGF and SIF. Very few pores were found when hydrogels were freeze-dried after swelling in SGF. On the other hand, hydrogels which were freeze-dried after swelling in SIF had porous structure on the surface and the inside.

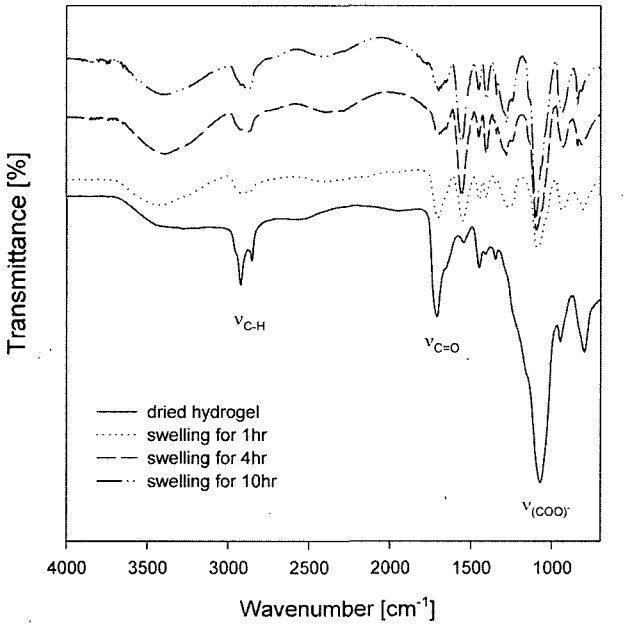



**Figure 10.** ATR-FTIR spectra of PEO-*g*-AAc hydrogel depending on swelling time (10 kGy irradiated, degree of grafting of hydrogel : 203%).

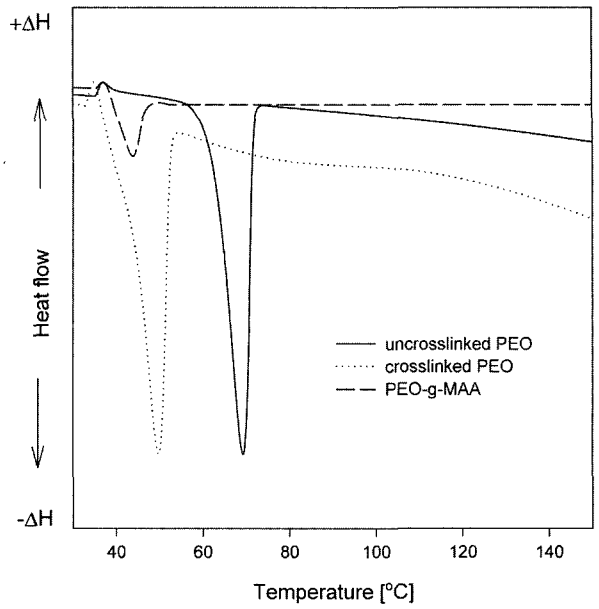



**Figure 11.** DSC thermogram of PEO-*g*-MAA (grafting ratio : 24%) and PEO hydrogels.

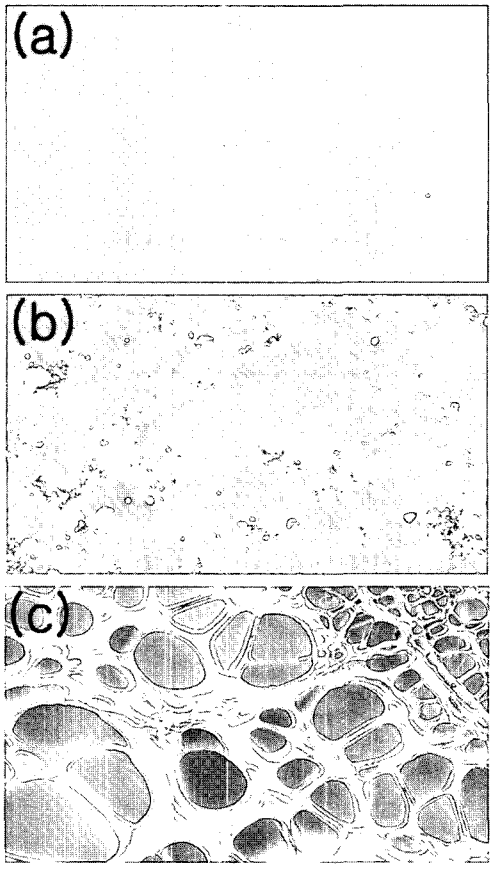



**Figure 12.** Surface SEM analysis of MAA-grafted PEO(grafting ratio : 225%). (a) dried hydrogel, (b) freeze drying of hydrogel swollen in SGF for 10 hr, and (c) freeze drying of hydrogel swollen in SIF for 10 hr.

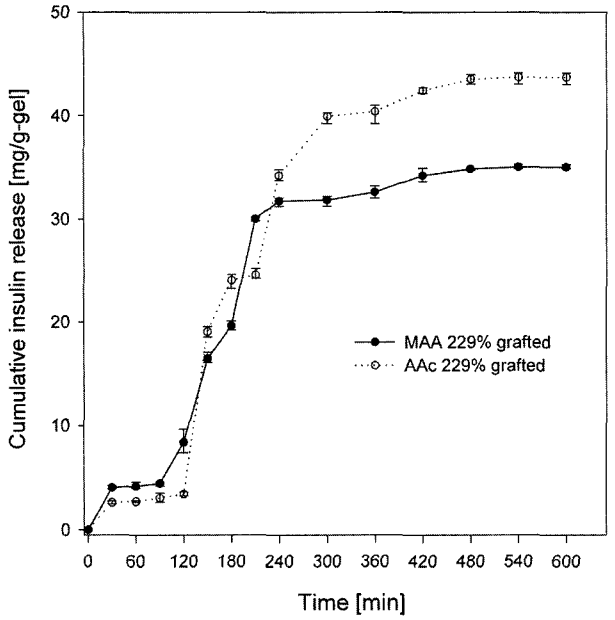



**Figure 13.** Insulin release profile from MAA and AAc grafted onto PEO at 37°C (0 min to 120 min in SGF, 120 min to 600 min in SIF).

Figure 13 exhibits the *in vitro* drug release profile from the hydrogels in two different pH environments as a function of drug-releasing time. The drug release was carried out for the first 2 hr in simulated gastric fluid and for the next 6 hr in simulated intestine fluid in order to create a similar condition with the human body. Both PEO-*g*-MAA and PEO-*g*-AAc hydrogels released only a small amount of insulin for the first 2 hr in the simulated gastric fluid, and then a lot of insulin rapidly for the next 6 hr in the simulated intestine fluid.

## Conclusions

pH-sensitive hydrogels based on PEO-*g*-MAA and PEO-*g*-AAc were synthesized as a drug carrier for oral drug delivery. The hydrogels based on poly(ethylene oxide) (PEO) networks grafted with methacrylic acid (MAA) or acrylic acid (AAc) were prepared via a two-step process. PEO hydrogels were prepared by γ-ray irradiation, and then grafting by either MAA or AAc monomers onto the PEO hydrogels with subsequent irradiation. 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 a pH-sensitive nature. The *in vitro* release profiles of the drugs were obtained in both a simulated gastric fluid and simulated intestinal fluid. The release behavior of the pH-sensitive PEO-*g*-MAA and PEO-*g*-AAc hydrogels indicated that these gels could be applied successfully for oral drug delivery to the gastrointestinal tract.

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## References

(1) E. J. Mack, T. Okano, S. W. Kim, and N. A. Peppas, *Hydrogels in Medicine and Pharmacy Polymers*, CRC Press, Boca Raton, USA, 1988, Vol. II, p. 65.
(2) J. M. Rosiak and P. Ulanski, *Radiat. Phys. Chem.*, **55**, 139 (1999).
(3) Y. Qiu and K. N. Park, *Adv. Drug Delivery Reviews*, **53**, 321(2001).
(4) N. A. Peppas, P. Bures, W. Leobandung, and H. Ichikawa, *Eur. J. Pharm. Biopharm.*, **50**, 27 (2000).
(5) L. Brannon and N. A. Peppas, *Chem. Eng. Sci.*, **46**, 715 (1991).
(6) A. B. Scranton, B. Rangarajan, and J. Klier, *Adv. Polym. Sci.*, **120**, 1 (1995).
(7) R. Skouri, F. Schoesseler, J. P. Munch, and S. J. Candau, *Macromolecules*, **28**, 197 (1995).
(8) E. Yu, A. Kramarenko, and A. R. Khoklov, *Macromolecules*, **30**, 3383 (1997).
(9) A. R. Khare and N. A. Peppas, *Biomaterials*, **16**, 559 (1995).
(10) C. Donini, D. N. Robinson, P. Colombo, F. Giordano, and N. A. Peppas, *Int. J. Pharm.*, **245**, 83 (2002).
(11) H. C. Chiu, Y. F. Lin, and Y. H. Hsu, *Biomaterials*, **23**, 1103 (2002).
(12) O. E. Philippova, D. Hourdet, R. Audebert, and A. R. Khokghlov, *Macromolecules*, **29**, 2822 (1996).
(13) G. Staikos, G. Bokias, and K. Karayanni, *Polym. Int.*, **41**, 345 (1996).
(14) P. M. Torre and S. Torrado, *Biomaterials*, **24**, 1459 (2003).
(15) J. W. Lee, S. Y. Kim, S. S. Kim, Y. M. Lee, K. H. Lee, and S. J. Kim, *J. Appl. Polym. Sci.*, **73**, 113 (1999).
(16) A. Bhattacharya, *Prog. Polym. Sci.*, **25**, 371 (2000).
(17) E. Jabbari and S. Nozari, *Eur. Polym. J.*, **36**, 685 (2000).
(18) J. M. Rosiak and P. Ulanski, *Radiat. Phys. Chem.*, **55**, 139 (1999).
(19) P. Markland, Y. Zhang, G. L. Amidon, and V. C. Yang, *J. Biomed. Mat. Res.*, **47**, 595 (1999).
(20) W. Leobandung, H. Ichikawa, Y. Fukumori, and N. A. Peppas, *J. Control. Release*, **80**, 357 (2002).
(21) T. Traitel, Y. Cohen, and J. Kost, *Biomaterials*, **21**, 1679 (2001).
(22) M. K. Chun, C. S. Cho, and H. K. Choi, *J. Control. Release*, **81**, 327 (2002).
(23) F. A. Dorkoosh, J. C. Verhoef, M. H. C. Ambagts, M. Rafiee-Tehrani, G. Borchard, and H. E. Junginger, *Eur. J. Pharm. Sci.*, **15**, 433 (2002).
(24) V. S. Bhalerao, S. Varghese, A. K. Lele, and M. V. Badiger, *Polymer*, **39**, 2255 (1998).
(25) P. Ferloni, A. Magistris, G. Choidelli, A. Faucitano, and A. Buttafava, *Radiat. Phys. Chem.*, **37**, 615 (1991).
(26) Y. C. Nho and J. H. Jin, *J. Appl. Plym. Sci.*, **63**, 1101 (1997).