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Preparation and Properties of Hydrogels of PVA/PVP/Chitosan by Radiation

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Abstract

The radiation can induce chemical reaction to modify polymer under even the solid condition or in the low temperature. The radiation crosslinking can be easily adjusted and is easily reproducible by controlling the radiation dose. The finished product contains no residuals of substances required to initiate the chemical crosslinking which can restrict the application possibilities. In these studies, hydrogels from a mixture of chitosan and polyvinyl alcohol(PVA)/Poly-N-vinylpyrrolidone(PVP) were made by "freezing and thawing", or gamma-ray irradiation or two steps of "freezing and thawing" and gamma-ray irradiation for wound dressing. The mechanical properties such as gelation, water absorptivity, and gel strength were examined to evaluate the hydrogels for wound dressing. The composition of PVA:PVP was 60:40, PVA/PVP: chitosan ratio was in the range of 9:1 - 7:3, and the solid concentration of PVA/PVP/chitosan solution was 15wt%. Gamma irradiation doses of 25, 35, 50, 60 and 70kGy, respectively were exposed to a mixture of PVA/PVP/chitosan to evaluate the effect of irradiation dose on the mechanical properties of hydrogels. Water-soluble chitosan was used to in this experiment. The mechanical properties of hydrogels such as gelation and gel strength was higher when two steps of "freezing and thawing" and irradiation were used than only "freezing and thawing" was utilized. Gel content was influenced slightly by PVA/PVP:chitosan composition and irradiation dose, but swelling was done greatly by them. Swelling percent was much increased as the composition of chitosan in PVA/PVP/chitosan increased.

1. Introduction

The principal function of a wound dressing is to provide an optimum healing environment. For example, a wound must be isolated from the external environment before healing begins. A wound dressing covers the wound mimicking the natural barrier function of the epithelium. To provide an optimum healing environment, a wound dressing should control bleeding, protect the wound from the external environment, prevent further contamination or infection and maintain a moist micro-environment next to the wound surface. Hydrogels consist of the hydrophilic polymer which forms a three dimensional network and much water in its network. They are one of the most promising material for biomedical applications, which has several advantages and characteristics for wound dressing, contact lenses, drug delivery system and so on. due to their biocompativility with blood, body fluids and tissue[1,2]. They must sustain their mechanical strength and contain over 75% water. Their water adsorption is due to the hydration which is related to the chemical groups such as -COOH, $CONH_2$, -CONH-, SO_3H, capillary effect, and osmotic pressure[3]. The hydrogels have been prepared by using chemical method for a long period, however, in recent years, irradiation technique to produce the hydrogels is being used increasingly around the world. This simple and compact technology is so convenient. The physical and mechanical properties can manipulate easily by irradiation dose[4]. Ideal hydrogels wound dressing must have the basic features such as absorbtion of the exudates, prevention of excessive loss of body fluids, a good adhesion to the wound, non-toxicity and prevention of infection[5].

Chitosan, the partially deacetylated form of chitin, is another material known in the wound-management art for its hemostatic properties. Additionally, it possesses many other biological properties including bacteriostatic and fungistatic properties particularly useful for wound treatment. Chitosan has heretofore been employed in various physical forms for wound treatment, e.g. as a solution/gel; film/membrane; sponge; powder or fiber. In general, chitosan with a high molecular weight is not soluble in water. but dissolved in acid water solution. To make the hydrogels using chitosan in aqueous acid solution, the repeated cleaning process was necessary to neutralize the acid. The water soluble chitosan was used to simplify the process of making the hydrogels in this study.

In this work, attempts were made to obtain the hydrogels for wound dressing which consisted of PVA, PVP and chitosan. Hydrogels from a mixture of chitosan and PVA/PVP were made by "freezing and thawing", or exposing ^{60}Co gamma-ray or two steps of "freezing and thawing" and ^{60}Co gamma-ray irradiation. The mechanical properties such as gelation, swelling, and gel strength were examined to evaluate the hydrogels for wound dressing.

2. Experimental

2.1. Materials

PVA(Mw: 8.5×10^4 - 1.46×10^3) was supplied by Aldrich Chemical Co.. PVP of average molecular weight of 1.3×10^6 were purchased from Sigma & Aldrich Chemical Co., MO, USA. These Polymers were used without further purification. The water used as a solvent in all experiments was distilled water.

The water-soluble chitosan was supplied by Zakwang Co., Korea, which is manufactured by the processes of acid/enzyme, ion exchange column treatment and membrane treatment. The average molecular weight of chitosan is 300,000.

2.2. Preparation of hydrogels

PVA/PVP(60/40 composition) was dissolved in distilled water of 95℃, and then mixed with chitosan by mechanical stirrer at room temperature to give PVA/PVP/chitosan solution. The solutions were poured into petri dish at room temperature. The solution was kept at room temp for 24 h in order to remove air bubble. Hydrogels from a mixture of chitosan and PVA/PVP were made by "freezing and thawing", or exposing ^{60}Co gamma-ray or two steps of "freezing and thawing" and ^{60}Co gamma-ray irradiation. PVA/PVP: chitosan ratio was in the range of 9:1 - 7:3, and the solid concentration of the total PVA/PVP/chitosan solution was 15wt%, and the composition of PVA:PVP was 60:40. Water-soluble chitosan was used in this experiment. Gamma irradiation doses of 25, 35, 50, 60 and 70 kGy, respectively were exposed to a mixture of PVA/PVP/chitosan to evaluate the effect of irradiation dose on the mechanical properties of hydrogels. "Freezing and thawing" was repeated up to 5 times to crosslink the PVA/PVP/chitosan solution physically. Each cycle of "freezing and thawing" involved lowering the temperature to -30℃, standing at this temperature for 1 h, then raising the temperature to room temperature.

2.3. Gel content

The gel content of the hydrogels was measured by extraction in a hot distilled water of 50 ℃ for 72 h and vacuum dried at 70℃ about 48h until they reached constant weight. The gel content was defined as the eq.(1). W_d is the dried gel weight after extraction, and W_i the initial weight of the polymer in polymer solution.

$$\text{Gel}\ (\%) = (W_d / W_i) \times 100 \qquad (1)$$

2.4. Degree of swelling

The degree of swelling could be described as water absorptivity (eq.2) of the hydrogels. The gel samples were immersed in distilled water for 48h at the room temperature until the gel reached the equilibrium state of swelling. After the water on the surface of the swollen gels was removed by using cellulose paper, the mass was determined. The dried gels were obtained by drying at 70℃ until they reached constant weight. W_s is the weight of the swollen gels and W_d is the dried gel weight

$$\text{Water absorptivity} = \{(W_s - W_d) / W_d\} \times 100 \qquad (2)$$

2.5. Gel strength (Rupture force and elongation at break)

The mechanical properties of the hydrogels was obtained by determining gel strength (eq. 3) which is the peak force (F_B) in gram multiplied by the distancerm (☐D) to the rupture measured in centimeter. Tests were conducted by using "TA-XT2" texture analyzer at room temperature.

$$\text{Gel Strength} = F_B \times ☐D \qquad (3)$$

2.6. Healing test of the hydrogels for wound dressing

Rats (200g) were anesthetized with diethyl ether and Kentamine purchased from Yuhan Co. Seoul, Korea, and then the dorsal fur was removed with electric clippers. The skin was cleansed with H_2O_2. After two wounds of 1cm diameter in the dorsum was prepared, the skin of rats was disinfected with Povidone Iodine Topical Solution purchased from Sung Kwang, Buchon, Korea. And, one of both wound was covered with one of the prepared hydrogels and the other was covered with a commercially used vaseline gauze as a control. And then Tegaderm (3M) was covered over the hydrogels and gauze, respectively. Chitosan/PVA/PVP hydrogels made by two steps of "freezing and thawing" and ^{60}Co gamma-ray irradiation were used for the healing test of rats. PVA/PVP: chitosan ratio was in the range of 9:1, and the solid concentration of the total PVA/PVP/chitosan solution was 15wt%, and the composition of PVA:PVP was 60:40. Wounds of 1cm diameter formed in back skin of rats were covered with the hydrogel samples of 1.5×1.5×0.3 cm and vaseline gauge.

At the certain postoperative day, macroscopic observation of wound status was made. The observation was proceeded totally for 14 days. After all experiments, all the rats were sacrificed with an overuse of Kentamine.

3. Results and discussion

Up to and including the late 1950's, it was generally accepted that, in order to prevent bacterial infection, a wound should be kept as dry as possible. However, a variety of studies have questioned this philosophy and found that wounds that were kept moist actually healed more rapidly than those that were left exposed to the air or covered with traditional dried dressings. In a review of the properties of occlusive dressings, W. H. Eaglestein concluded that occlusive dressings that keep wounds moist could increase the rate of epidermal resurfacing by some 40%.

Hydrogels are complex lattices in which the dispersion medium is trapped rather like water in a molecular sponge. Available hydrogels are typically insoluble polymers with hydrophilic sites, which interact with aqueous solutions, absorbing and retaining significant volumes of fluid.

A wider range of polymers can be crosslinked by radiation than by any chemical method. The radiation crosslinking can be easily adjusted and is easily reproducible by controlling the radiation dose. The finished product contains no residuals of substances required to initiate the chemical crosslinking which can restrict the application possibilities, or can increase the failure rate. In these studies, hydrogels from a mixture of chitosan and PVA/PVP were made by "freezing and thawing", or exposing ^{60}Co gamma-ray or two steps of "freezing and thawing" and ^{60}Co gamma-ray irradiation.

Figure 1 shows gelation behavior of the hydrogels which were synthesized by the repeated "freezing and thawing". PVA/PVP: chitosan ratio was in the range of 9:1 - 7:3, and the solid concentration of the total PVA/PVP/chitosan solution was 15wt%. The composition of PVA:PVP was fixed as 60:40. Gel content increased as chitosan concentration in PVA/PVP/chitosan decreased, and "freezing and thawing" was repeated. "Freezing and thawing" caused gel content to enhance greatly up to 4 cycles of "freezing and thawing", after that, its increase levelled off. It is well known that this procedure of PVA results in the formation of crystallites that serve as physical crosslinks to render the material insoluble in water. Gel content(%) in this experiment was continuously decreased as the composition of chitosan in PVA/PVP/chitosan increased because chitosan is not crosslinked by "Freezing and thawing". Figure 2 shows the swelling behavior of the same hydrogels as shown in Figure 1. The swelling percent was inversely proportional to the gel percent because crosslinking density increases with increasing gelation.

Gamma irradiation doses of 25, 35, 50, 60 and 70kGy, respectively were exposed to an aqueous mixture of PVA/PVP/chitosan to evaluate the effect of irradiation dose on the gel content(%) and swelling behavior of hydrogels(Figure 3, 4). Gel content was influenced slightly by PVA/PVP:chitosan composition and irradiation dose, but swelling was done greatly by them. When radiation process or "freezing and thawing" process was used to obtain hydrogels, the difference between two processes is that gel content is influenced by PVA/PVP:chitosan composition in the case of "freezing and thawing" process, not much influenced by PVA/PVP:chitosan composition in the case of irradiation. It can be explained by the fact that the crosslinking network can be formed between PVA/PVP and chitosan molecules when irradiation process is used. The gels result from the coupling of the polymer radicals which were directly and indirectly produced from PVA, PVP or chitosan by gamma rays. The indirect formation of polymer radicals is mainly due to the $H^{\cdot}$ and $^{\cdot}OH$ radicals arising from water molecules, both of which abstract the hydrogen atoms to form the polymer radicals. There are two types of crosslinking processes: intermolecular and intramolecular crosslinking. The former allows the increase of the Mw of crosslinked polymer via the coupling of two or more polymer radicals. The latter does not alter Mw, but affect the quantities relating to a polymer chain dimension because the radical coupling reaction should take place within the same polymer chain[6].

Hydrogels from a mixture of chitosan and PVA/PVP were made by two steps of "freezing and thawing" and ^{60}Co gamma-ray irradiation. The gelation content and the degree of swelling were examined to evaluate the hydrogels for wound dressing(Figure 5,6). Gel percent of hydrogels was higher when two steps of "freezing and thawing" and irradiation were used than only "freezing and thawing". Irradiation of more than 25kGy did not have a great effect on gel percent of hydrogels. The swelling degree was greatly influenced by irradiation dose(Figure 6).

Hydrogels from a mixture of chitosan and PVA/PVP were made by "freezing and thawing", or ^{60}Co gamma-ray irradiation or two steps of "freezing and thawing" and ^{60}Co gamma-ray irradiation. Figure 7 shows their respective gel percent of hydrogels. Gel content increased as chitosan concentration in PVA/PVP/chitosan decreased, and two step of "freezing and thawing" and irradiation was used. Figure 8 shows the swelling percent of hydrogels from Figure 7. Swelling degree of hydrogels obtained from only irradiation process was much higher than those obtained from "freezing and thawing" or two step of "freezing and thawing". Figure 9 shows swelling degree vs.

immersing time on the same samples as Figure 7. The swelling percent increased rapidly up to 1 hr., and continued to rise steadily up to 20 hr., and then levelled off.

Chitosan/PVA/PVP hydrogels made by two steps of "freezing and thawing" and ^{60}Co gamma-ray irradiation were used for the healing test of rats. PVA/PVP: chitosan ratio was in the range of 9:1, and the solid concentration of the total PVA/PVP/chitosan solution was 15wt%, and the composition of PVA:PVP was 60:40. Wounds of 1cm diameter formed in back skin of rat were covered with the hydrogel samples of 1.5×1.5×0.3 cm and vaseline gauge, or vaseline gauge and non dressing. At the certain postoperative day, macroscopic observation of wound status was made. Vaseline gauge was so fast in drying rate and sticky to the wound of rat. PVA/PVP/chitosan hydrogel dressing stopped the blooding from wound, and had the best curing effect. The observation was proceded totally for 14 days.

4. Conclusions

Hydrogels from a mixture of chitosan and PVA/PVP were made by "freezing and thawing", or gamma-ray irradiation or two steps of "freezing and thawing" and gamma-ray irradiation for wound dressing. The mechanical properties of hydrogels such as gelation and gel strength was higher when two steps of "freezing and thawing" and irradiation were used than only "freezing and thawing" was utilized. Gel content was influenced slightly by PVA/PVP:chitosan composition and irradiation dose, but swelling was done greatly by them. Swelling percent was much increased as the composition of chitosan in PVA/PVP/chitosan increased.

Wounds of 1cm diameter formed in back skin of rat were covered with the hydrogel samples of 1.5×1.5×0.3 cm and vaseline gauge, or vaseline gauge and non dressing. At the certain postoperative day, macroscopic observation of wound status was made. Vaseline gauge was so fast in drying rate and sticky to the wound of rat. PVA/PVP/chitosan hydrogel dressing stopped the blooding from wound, and had the best curing effect.

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REFERENCES

1. J. M.iak, P. Ulanski, and A. Rzeznicki, Hydrogels for biomedical purposes, *Nucl. Instr. and Meth. in Phy. Res.*, **B105**, p.335(1995).
2. F. Yoshii, Y. Zhnshan, K. Isobe, K. Shinozaki, K. Makuuchi, Electron beam crosslinked PEO and PEO/PVA hydrogels for wound dressing, *Radiat. phys. Chem.*, **55**, p.133(1999).
3. J. M. Rosiak, Radiation formation of hydrogels for drug delivery, *J. controlled Release*, **31**, p.9(1994).
4. J. M Rosiak, P. Ulanski, L. A. Pajewski, F. Yoshii, and K. Makuuchi, Radiation formation of hydrogels for biomedical purpose. Some remarks and comments, *Radiat. Phys. Chem.,* **46**, p.161(1995).
5. J. M. Rosiak, Hydrogel dressings, *Radiation Effects on Polymer*, Chap. 17, ACS Series 475, ACS Washington, DC, p.271(1991).
6. B. Wang, M. Kodama, S. Mukatake, E. Kokufuta, On the intermolecular crosslinking of PVA chains in an aqueous solution by ã-ray irradiation, *Polym. Gels and Networks*, **6**, p. 71(1998).

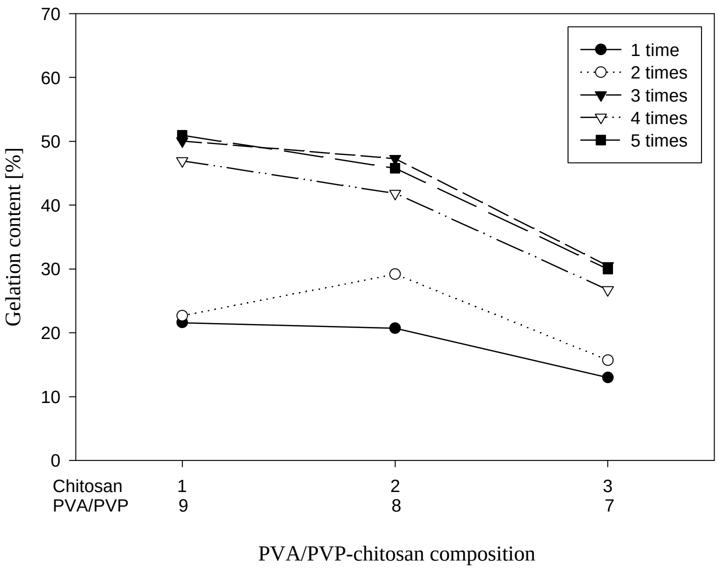

Figure 1. Gelation content of PVA/PVP-chitosan hydrogels vs. PVA/PVP-chitosan composition after repeated freezing and thawing without irradiation doses.
(PVA : PVP = 6 : 4, solid concentration 15wt%.)

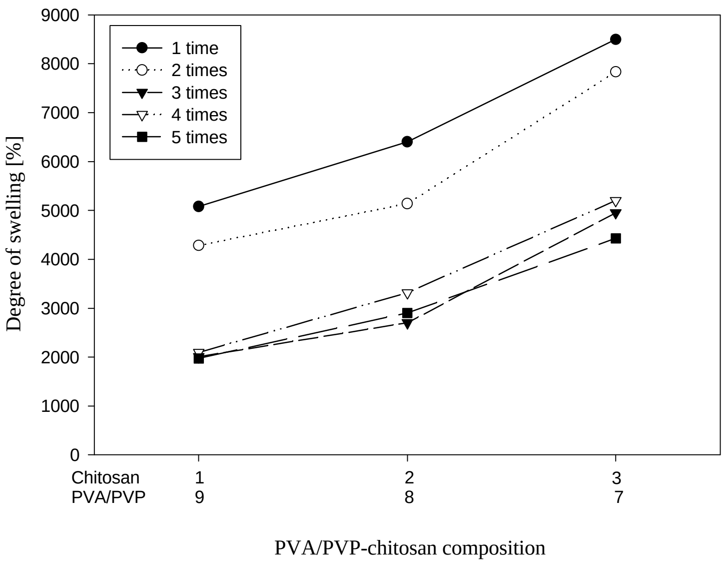

Figure 2. Degree of swelling of PVA/PVP-chitosan hydrogels vs. PVA/PVP-chitosan composition after repeated freezing and thawing without irradiation doses.
(PVA : PVP = 6 : 4, solid concentration 15wt%.)

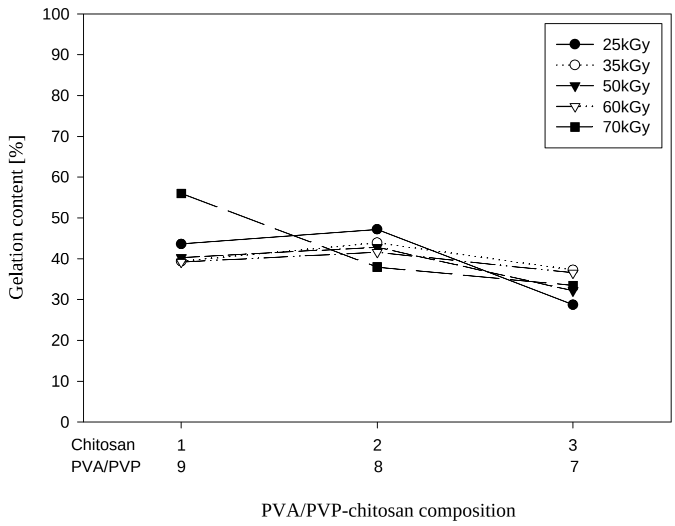

Figure 3. Gelation content of PVA/PVP-chitosan hydrogels vs. PVA/PVP-chitosan composition at different irradiation doses. (PVA : PVP = 6 : 4, solid concentration 15wt%.)

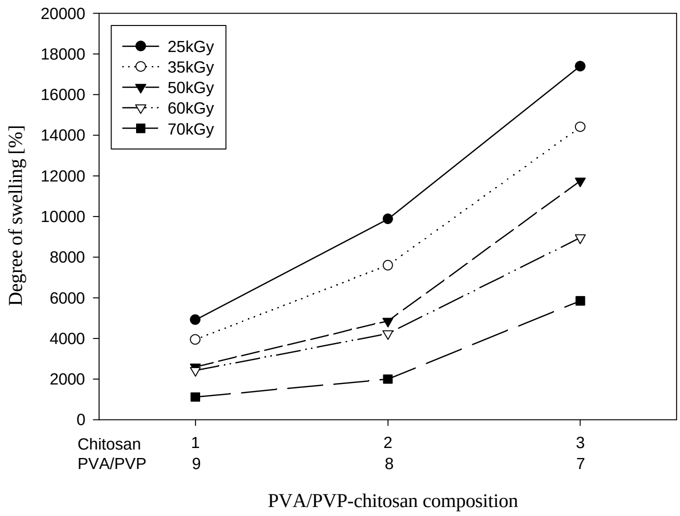

Figure 4. Degree of swelling of PVA/PVP-chitosan hydrogels vs. PVA/PVP-chitosan composition at different irradiation doses. (PVA : PVP = 6 : 4, solid concentration 15wt%.)

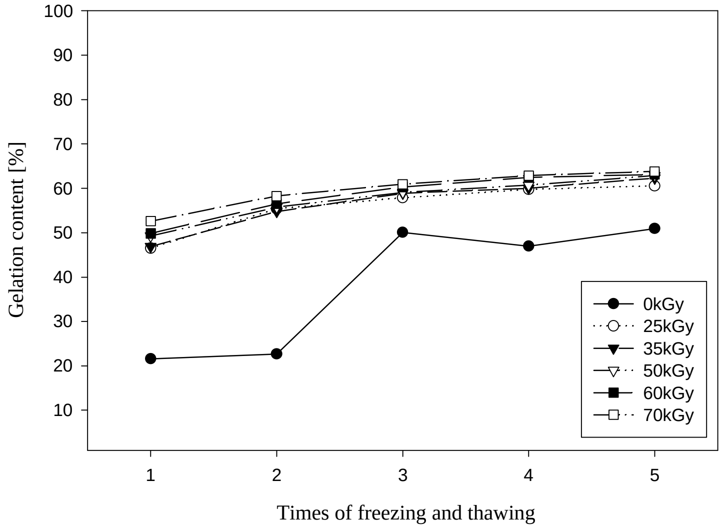

Figure 5. Gelation content of PVA/PVP-chitosan hydrogels vs. freezing and thawing at different irradiation doses.
(chitosan : PVA/PVP = 1 : 9, PVA : PVP = 6 : 4, solid concentration 15wt%.)

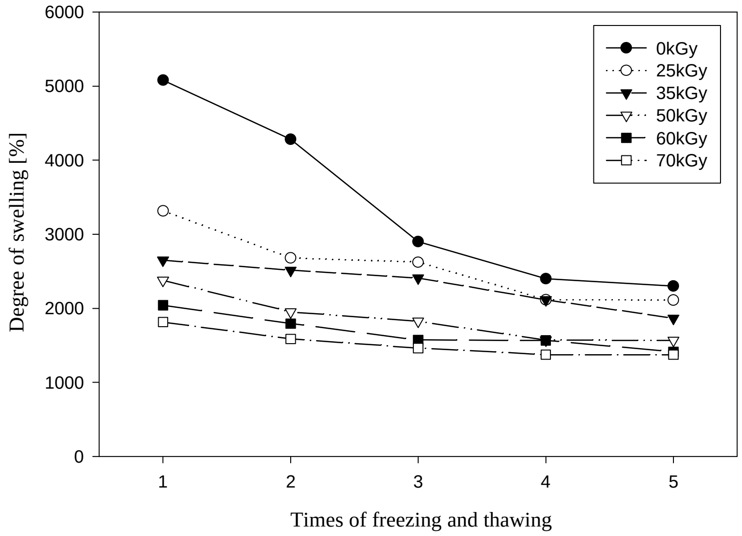

Figure 6. Degree of swelling of PVA/PVP-chitosan hydrogels vs. irradiation dose after repeated of freezing and thawing.
(chitosan : (PVA/PVP) = 1 : 9, PVA : PVP = 6 : 4, solid concentration 15wt%.)

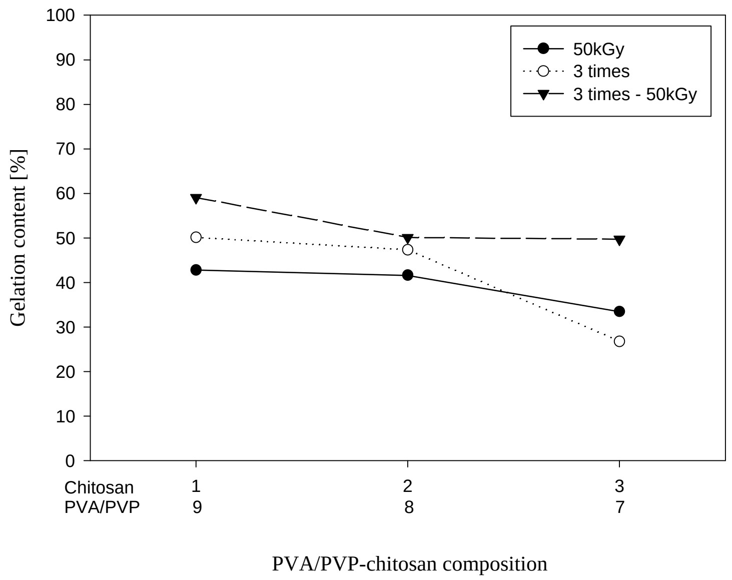

Figure 7. Gelation content of PVA/PVP-chitosan hydrogels vs. PVA/PVP-chitosan composition. (PVA : PVP = 6 : 4, solid concentration 15wt%.)

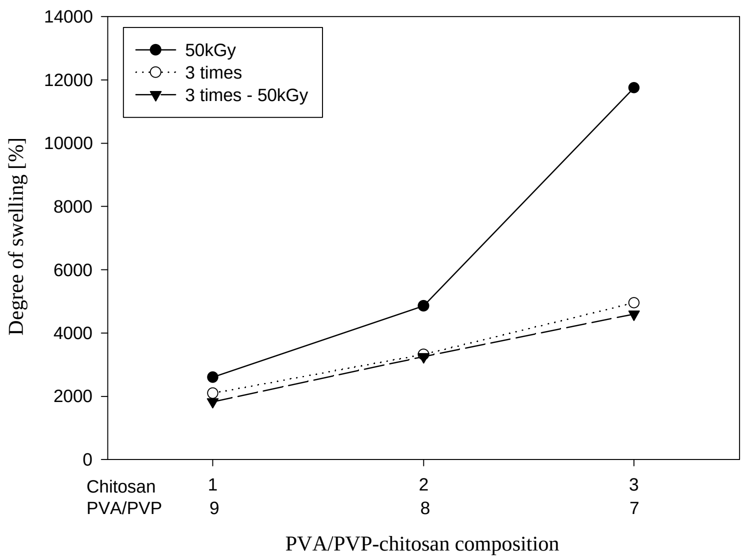

Figure 8. Degree of swelling of PVA/PVP-chitosan hydrogels vs. PVA/PVP-chitosan composition. (PVA : PVP = 6 : 4, solid concentration 15wt%.)

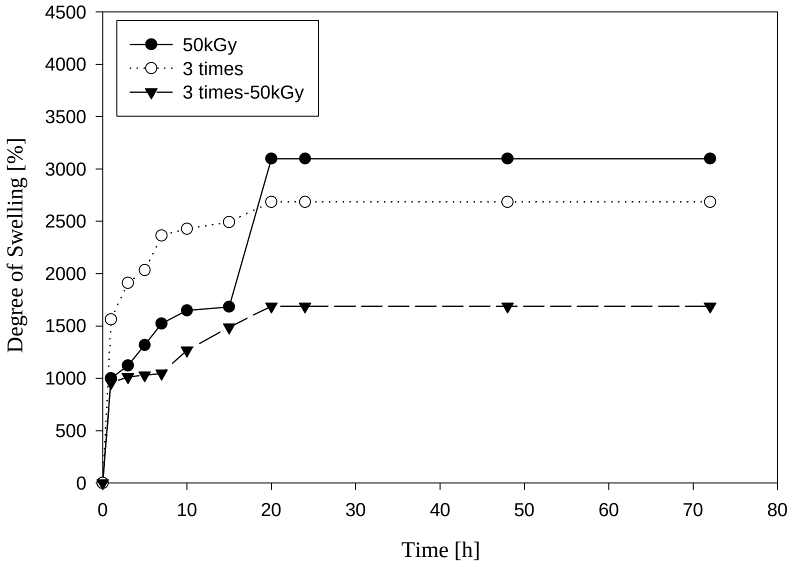

Figure 9. Degree of swelling of PVA/PVP-chitosan hydrogels vs. time.
(chitosan : (PVA/PVP) = 1 : 9, PVA : PVP = 6 : 4, solid concentration 15wt%.)

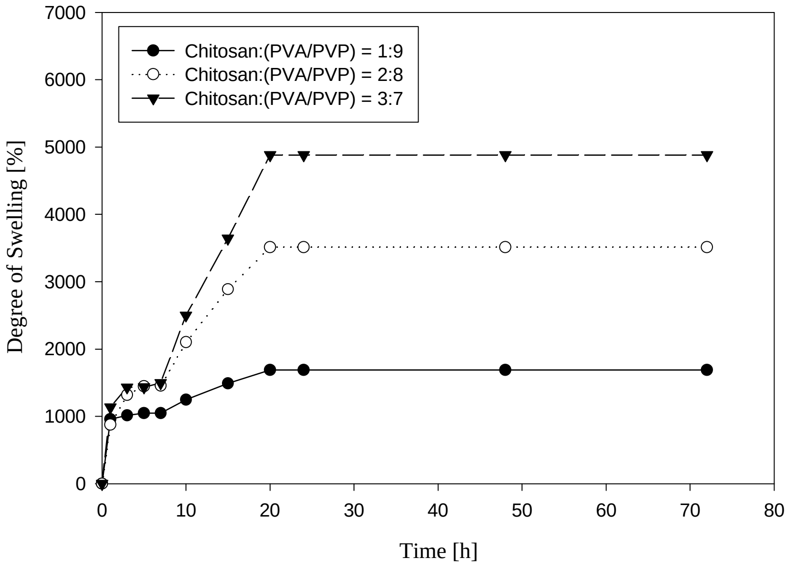

Figure 10. Degree of swelling of PVA/PVP-chitosan hydrogels vs. time
at 50kGy after 3 times of freezing and thawing.
(PVA : PVP = 6 : 4, solid concentration 15wt%.)