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PROCEEDINGS OF THE TAKASAKI SYMPOSIUM ON RADIATION APPLICATION OF NATURAL POLYMERS IN ASIA OCTOBER 1 AND 2, 2001, JAERI, TAKASAKI, JAPAN

March 2002

Eds. Tamikazu KUME, Yasunari MAEKAWA,
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Takasaki Symposium on Radiation Application of Natural Polymers in Asia

October 1 and 2, 2001,

JAERI, Takasaki, Japan

(Eds. T. Kume, Y. Maekawa, Functional Materials Laboratory I)

Department of Material Development

Takasaki Radiation Chemistry Research Establishment

Japan Atomic Energy Research Institute

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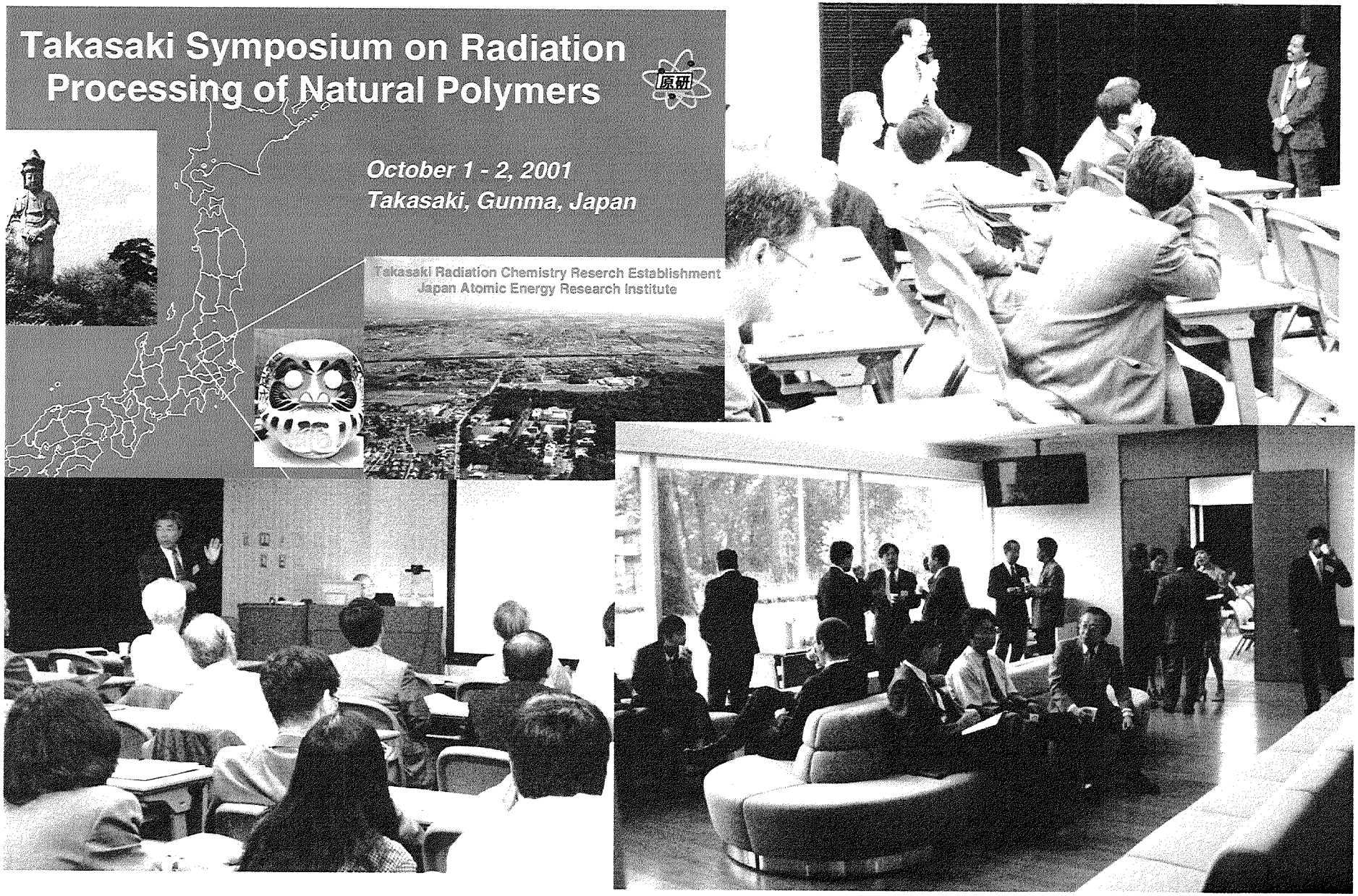

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Proceedings of the Takasaki Symposium on Radiation Application of Natural Polymers in Asia

October 1 and 2, 2001, JAERI, Takasaki, Japan

Eds. Tamikazu Kume, Yasunari Maekawa, Functional Materials Laboratory I

Department of Material Development
Takasaki Radiation Chemistry Research Establishment
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Watanuki-cho, Takasaki-shi, Gunma-ken

(Received January 30, 2002)

This Takasaki symposium was annually held as the 3rd meeting for radiation processing of natural polymers through research cooperation among Asian countries. The symposium includes the presentations of research outcomes on radiation processing of starches, silk proteins and marine carbohydrates. In the researches of starch and cellulose, radiation crosslinking of biodegradable polysaccharides was achieved by modifying it to be water-soluble paste, showing the wide range of its application to wound dressing and biodegradable plastics. In silk protein researches, pulverization and water-solubilization of the irradiated silk proteins and its antibacterial properties were reported. In the researches of marine carbohydrates, it was reported that radiation-degraded chitosan and alginate showed promotion effects for plant growth, enhancement of antibacterial properties, and capability to be applied for bioadhesive materials. In addition, in estimation of economic scale of radiation application, comparisons between Japan and U.S. in medical, agricultural and industrial fields were introduced. Many domestic and foreign attendants were very interested in reports and exchanged their opinions actively. Radiation application of natural polymers are the most important subjects from the view points of resource recycling and environment protection and are good agreement with the needs of Asian countries. Outcomes of this symposium are expected to contribute the progress in radiation applications in south Asian countries as well as Japan.

In the symposium we had the 63 participants consisted of 16 foreign researchers from not only bilaterally cooperated Malaysia, Thailand and Vietnam, but also Indonesia and China, 28 from domestic universities, governmental institutes and companies, and 32 from JAERI. This proceeding compiles the invited and contributed papers.

Keywords: Radiation Processing, Natural Polymers, Starch, Silk Proteins, Growth Enhancement, Antibacterial Property, Marine Carbohydrates, Wound Dressing

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天然高分子に関するアジア地域放射線利用高崎シンポジウム論文集

2001年10月1日～2日、高崎研究所、高崎市

日本原子力研究所高崎研究所材料開発部
高機能材料第1研究室※

(2002年1月30日受理)

本シンポジウムは、アジア諸国との研究協力のもとに進めている天然高分子の放射線加工処理に関する第3回目の会合として、昨年に引き続き開催された。会議では、澱粉、絹タンパク質、海産多糖類の放射線加工などについて、研究成果を中心に報告及び討議が行われた。澱粉及びセルロースに関しては、放射線分解型の多糖類を水溶化してペースト状で照射することにより橋かけし、創傷被覆材や生分解性プラスチックとして幅広い利用の可能性があることが示された。絹タンパク質に関しては、放射線分解した絹繊維の微粉化、水溶化、抗菌性などの成果が報告された。海産多糖類に関しては、キトサンやアルギン酸の放射線分解物は、植物の生長に極めて有効であること、抗菌性が増すこと、飼料の結着材として利用可能であることが報告された。また、放射線利用の経済規模に関して、医学、農業、工業の各分野における日米比較の結果が報告された。これらの報告に対し、国内外参加者から強い興味が示されると共に活発な意見交換が行われた。天然高分子の放射線利用は、資源のリサイクル、地球環境保全の上で重要な課題であり、アジア諸国のニーズに合致したテーマでもある。本シンポジウムの成果は、日本及び東南アジア諸国の放射線利用分野の発展に寄与できるものと期待される。

本シンポジウムには、二国間研究協力を進めているマレーシア、タイ、ベトナムに加えて、インドネシア、中国などからの外国人16名、国内の大学・国公立機関及び民間企業から23名、原研24名の計63名が参加した。本論文集は、シンポジウムで発表された論文等を収録したものである。

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※ 編集者：久米民和、前川康成

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1 Opening Address for Takasaki Symposium on Radiation Application of Natural Polymers

Hiroshi Watanabe

Director General, Takasaki Radiation Chemistry Research Establishment,
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1233 Watanuki, Takasaki, Gunma 370-1292, Japan

Good morning, Ladies and gentlemen.

On behalf of JAERI-Takasaki, It is a great pleasure for me to give an opening address for the "Takasaki Symposium on Radiation Application of Natural Polymers". First of all, I wish to extend my hearty welcome to you all here today.

Regarding the radiation applications, JAERI-Takasaki has so far carried out R&D of radiation processing as a research center of Japan. A lot of radiation technologies developed in JAERI have been already transferred to the industry in Japan, and also spreading to Asian countries through the cooperation with RCA and IAEA, and the bilateral collaborations. Nowadays the application of radiation technology is highly appreciated in many fields of industry, medicine and agriculture. It is our pleasure to spread radiation applications to the world, because radiation is a valuable tool for the increased quality of our life.

Recently it is said that the 20-century was an era of development of economy and science and technology. The remarkable progress of technology really promoted economic activities, and also increased the quality of life. However, we are now faced with sever environmental pollution on the earth. Therefore, I believe that the 21-century should be an era of environment, and we have to aim the construction of society harmonizing with nature.

From the viewpoint, the radiation technology will be highly expected in the field of environment. Indeed in JAERI-Takasaki, 7 laboratories out of 10 are doing research on environmental protection and the related subjects such as biodegradable polymers, heavy metal absorbents, electron beam treatment of gases including dioxins from incinerator, bioremediation and so on. In this way, the utilization of natural resources is one of the most important research items.

For the further development of radiation processing of natural polymers, it will be very useful to exchange the technical information and to make enough discussion between researchers joining the collaboration.

I expect that this Symposium will give such a good occasion and bring to fruitful conclusion through the stimulating discussion, and all the participants will contribute to the progress of these research and development.

Thank you very much for your attention.

2 Opening Remarks for Takasaki Symposium on Radiation Application of Natural Polymers

Sueo Machi

Japan Coordinator of FNCA

Dr. Watanabe, Dr. Kume, Distinguish Participants from Malaysia, Thailand, Viet Nam and Japan. I am very pleased to be invited to attend this regional meeting.

I assume that Dr. Watanabe invites me because of my past role in formulation and initiation of the bilateral cooperation with Malaysia, Thailand, and Indonesia more than 10 years ago when I was the Director General of Takasaki Establishment. Therefore, I am happy to learn that these bilateral research cooperation has been promoted successfully including newly started cooperation with Viet Nam.

In my view radiation processing can provide unique technique useful for production of new value added products. In addition radiation technology is environmentally friendly, simple and saving energy. In fact as you know, its industrial application has grown to large extent in the past 30 years in particular upgrading polymer materials, sterilization of medical products, food irradiation and more recently flue gas cleaning.

The IAEA where I had been working is assisting Member States to use radiation technology to meet their need improving everyday life of people with good success. Japanese Government has been cooperating with Asian Countries through bilateral arrangements and multilateral mechanism such as FNCA (Forum for Nuclear Cooperation in Asia) and RCA.

This meeting is to review the outcome of bilateral cooperation with MINT of Malaysia, OAEP of Thailand, and VAEC of Viet Nam. The Major focus of cooperative research is radiation technology for processing natural polymers which is a new promising field. Natural polymers such as marine carbohydrates, starch, silk and cellulose are converted efficiently into value added products by radiation processing. Interesting results have been achieved by the cooperation in laboratory scale. Some of them should be developed to industrial scale applications in the near future after careful assessment.

As for radiation facilities, electron accelerator can offer economical radiation

services if you have large volume of products to be irradiated. In particular self-shielded low energy accelerator is low cost and easy in its operation though the penetration range is short. Development of industrial scale irradiation system using low energy accelerator will be developed by Takasaki Establishment in cooperation with the FNCA in coming years.

In November this year, Ministers of 9 FNCA countries in charge of nuclear science and technology will meet in Tokyo to exchange their views on strategy of development of nuclear project under FNCA such as mutation breeding, cancer therapy, research reactor application, radioactive wastes management, safety culture, human resource development, and public information. The bilateral cooperation should be synergetic with FNCA since all 4 countries participating this meeting are members of FNCA.

In concluding my remarks I would hope that the meeting is fruitful and successful to review the results and formulate future work plan, and also wish you to have nice stay in Takasaki.

Thank you.

3 Biodegradable Water-absorbent Synthesized from Bacterial Poly(amino acid)s

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Abstract

Biodegradable hydrogels prepared by γ-irradiation from microbial poly(amino acid)s have been studied. pH-Sensitive hydrogels were prepared by means of γ-irradiation of poly(γ-glutamic acid) (PGA) produced by *Bacillus subtilis* and poly(ε-lysine) (PL) produced by *Streptomyces albulus* in aqueous solutions. When a dosage of γ–irradiation was 19 kGy or more and a concentration of PGA in water was 2 wt% or more, transparent hydrogels could be produced. In the case of 19 kGy, the produced hydrogel was very weak, however, the specific water content (wt. of absorbed water/wt. of dry hydrogel) of this PGA hydrogel was approximately 3,500. The specific water content was decreased to 200 increasing in the γ-irradiation dose over 100 kGy. Under acid conditions or on addition of electrolytes, PGA hydrogels shrinked. PGA hydrogel was pH-sensitive and changed the volume of the hydrogel depend on the value of pH outside hydrogel in the swelling medium. This PGA hydrogel was hydrodegradable and biodegradable. New novel purfier reagent (coagulant), made from PGA hydrogels, for contaminated turbid water was found and developed by Japanese venture company. Very small amount of this coagulant (only 1 ppm in turbid water) can work for purification of turbid water.

PL aqueous solution also can change to hydrogel by γ-irradiation. The specific water content of PL hyrdogel was range from 20 to 160 depend on the preparation conditions. Under acid conditions, the PL hydrogel swelled due to the ionic repulsion of the protonated amino groups in the PL molecules. The rate of enzymatic degradation of the respective PL hydrogels by a neutral protease was much faster than

the rate of simple hydrolytic degradation.

Keywords: Hydrogel, Biodegradation, γ–Irradiation, Poly(γ-glutamic acid), Poly(ε-lysine), Coagulant

Introduction

Biodegradable polymer is very useful for plastic products which are impossible to recycle such as films protecting seeds or young plants, soil development reagent for greening of deserts, water cleaning treatment reagent, paper diapers and plastic trays highly contaminated by foods. For these purpose, many kinds of biodegradable polymers have been studied. The amount of water absorbent polymers including in paper diapers are growing more and more in comfortable baby-care and old-care life. Water absorbent polymers are not suitable for material recycling after using. However, usage of compost made from used biodegradable absorbent polymer in the vegetable fields is very important in the point of view of resources saving. If these biodegradable absorbent is natural origin, these polymers incorporate to natural carbon cycle that is natural recycling.

Two kinds of poly(amino acid)s are known as produced by microorganisms. One is poly(γ-glutamic acid)[1] (PGA (1) in **Fig.1**) included in traditional Japanese food, *natto*, made from soy beans fermented by *Bacillus* strains. PGA is water-soluble and biodegradable with a high molecular weight (100,000-1,000,000). Another is poly(ε-lysine)[2] (PL (2) in **Fig.3**) which was found in Japanese soil sample and synthesized by *Streptomyces albulus*, an actinomycete. PL (Mn; ca 4,000) showed antimicrobial activity against Gram-posittive and –negative bacteria.

In this paper, a pH-sensitive, biodegradable bydrogel based on crosslinked PGA[3-5] and PL[6,7] by γ–irradiation was developed. Biodegradability and swelling properties of these hydrogels were studied. In addition, novel coagulant[8] using PGA hydrogels developed by Japanese venture company was introduced.

Exprimental

Materials; PGA fermented by *B. subtilis* F-02-1 was obtained from Meiji Seika Kaisha, Ltd. (Tokyo, Japan). The number-average molecular weight, Mn, of PGA was

5.5 x 10^5 as analyzed by gel permeation chromatography. PL fermented by *Streptomyces albulus* was obtained from Chisso Corp. (Tokyo, Japan). The Mn of PL used here was approximately 4000.

Preparation of hydrogels; Hydrogels were prepared by γ irradiation (1.6 kGy/h) of PGA or PL aqueous solutions (1-10 wt%) using an irradiation system with a ^{60}Co (110 TBq) source. The solution (2 mL) was contained in a 10-mL glass vial with a cap under nitrogen. The resultant hydrogels were swollen to equilibrium for 1 week. During this time, the uncrosslinked PGA or PL was removed by changing the swelling media daily.

Results and discussion

PGA hydrogels have been prepared by means of γ–irradiation of PGA solutions. When a dosage of γ–irradiation was 19 kGy or more and a concentration of PGA in water was 2 wt% or more, transparent hydrogels could be produced . In the case of 19 kGy, the produced hydrogel was very weak, however, the specific water content (wt. of absorbed water/wt. of dry hydrogel) of this PGA hydrogel was approximately 3,500 (**Fig.1**). It was found that the water sorption capability of PGA hydrogels was very high.

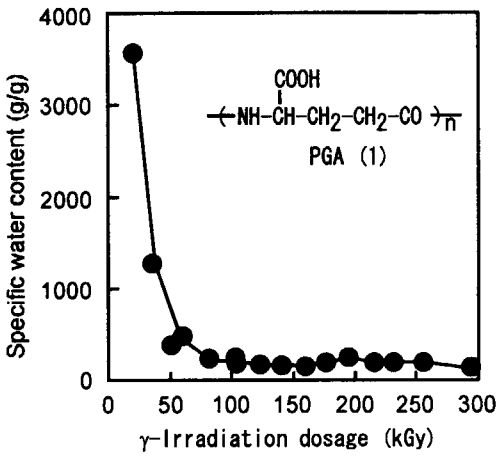


Fig.1 Specific water content of PGA hydrogels prepared by γ–irradiation of 5 wt% PGA solution.

The influence of various pH values or the presence of salts in the swelling medium of a hydrogel is of importance in agricultural and biomedical applications such as diapers, water reservoirs in agriculture, and hydrogels as implants for drug-release applications. Swelling equilibria of PGA hydrogels were measured in aqueous solutions of various pH values. **Fig. 2** (●) shows the relative weights of PGA hydrogels in aqueous solutions at different pH values, and it can be seen that the specific water content was strongly dependent on pH. Under acid conditions, PGA hydrogel was shrunk.

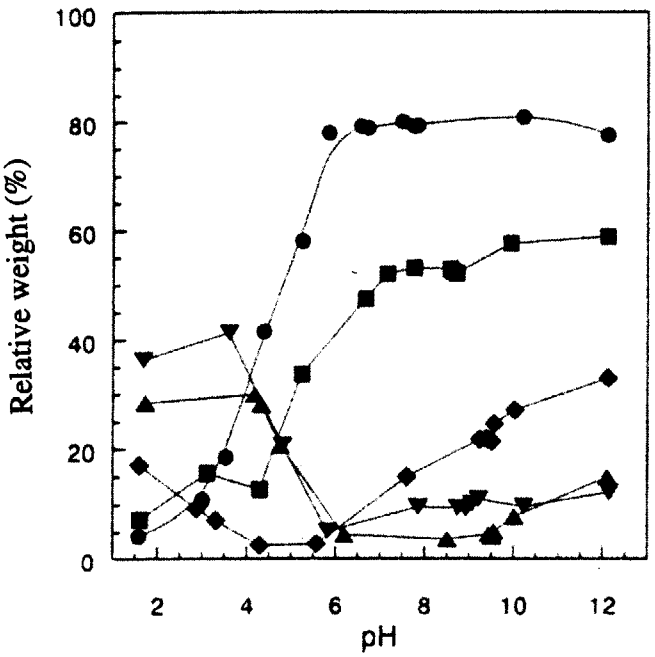
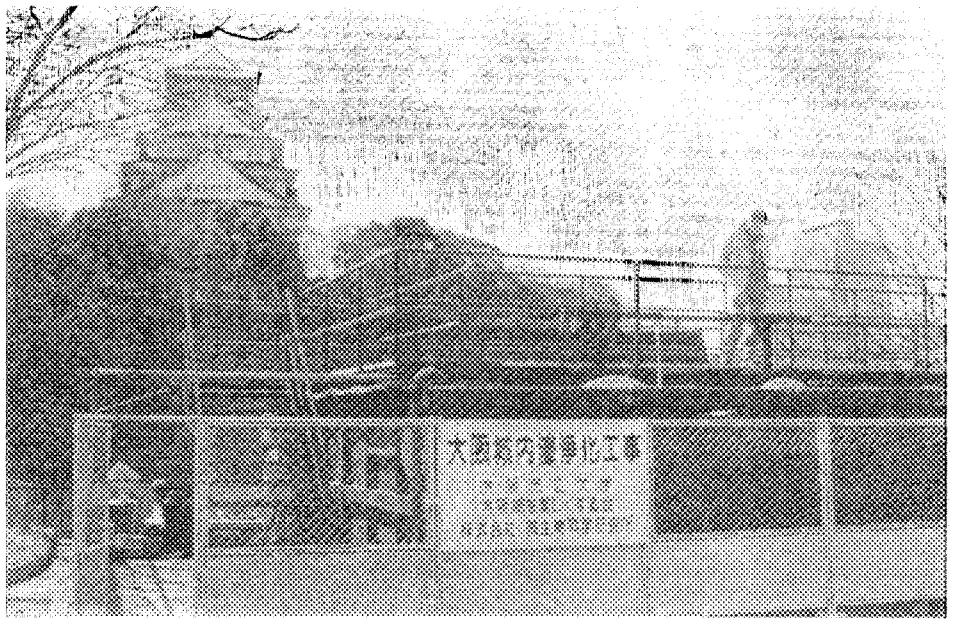


Fig.2 Swelling of PGA/PL mixed hydrogels (90 kGy) in the aqueous solutions of various pH's. PGA/PL wt%: (●) 100/0; (■) 80/20; (◆) 50/50; (▲) 20/80; (▼) 0/100. McIlvaine buffers 25 mM were used.

Recently, new novel purifier reagent (coagulant), made from PGA hydrogels, for contaminated turbid water, was found and developed by Japanese venture company (Biseibutsukagakukenkyusho (microorganism science laboratory) Co., Ltd., Osaka, biken@pk.highway.ne.jp). If this dried and improved PGA hydrogel is added to contaminated turbid water under only 1 ppm concentration, clear purified water is obtained by precipitation of waste-corruption for only 1 hour. These sludge (if no toxic materials) can be used for compost in the vegetable fields. Using this reagent,

the cleaning up project for contaminated water in the inner moat of the Osaka Castle is going on.



Picture Water purification tanks for the inner moat of the Osaka Castle[8].

PL hydrogels can be prepared by means of γ–irradiation of PL solutions as well as PGA. Under acid conditions, the PL hydrogel swelled due to the ionic repulsion (▼ in Fig. 2). The swelling-deswelling pattern of PL hydrogel was opposite to that of PGA hydrogel. Aqueous PGA/PL polymer mixed solutions were crosslinked when exposed to γ irradiation under a N_2 atmosphere. It was found that these hydrogels were amphoteric (acidic and basic) hydrogels (◆ in Fig.2). These PL hydrogels could be degraded by protease (*Protease A* (*Amano*)) produced from *Aspergillus oryzae* at 40 °C and pH 7.0. The rate of enzymatic degradation of the respective PL hydrogels was much faster than the rate of simple hydrolytic degradation and decreased with the increase in γ–irradiation dose during preparation of the PL hydrogel (**Fig.3**).

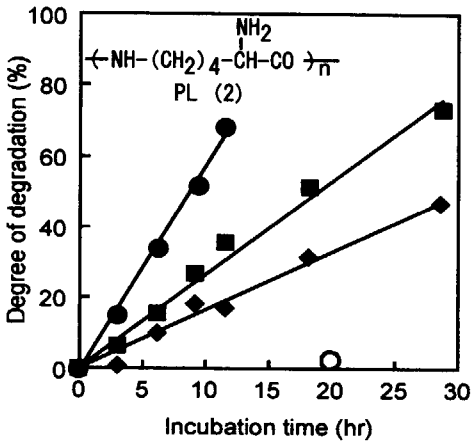


Fig. 3 Enzymatic degradation profiles on PL hydrogels in the aqueous solution of protease A at 40 °C and pH 7.0. (●) prepared at 101 (■) 147 (◆) 203 kGy in enzyme solution.

(○) 101 kGy in solution without enzyme.

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4 Hydrogel Wound Dressing by Radiation

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Abstract

Water soluble polymers such as polyethyleneoxide (PEO), polyvinyl alcohol (PVA) were irradiated in solid and molten states as well as in aqueous solution in order to synthesize a hydrogel. PEO undergoes crosslinking at all phases by radiation initiation. Among these phases, the radiation in the aqueous solution requires the lowest dose for crosslinking due to the contribution of OH radical created in radiolysis of water.

The hydrogel prepared by irradiation in aqueous solution was applied to a dressing for healing of wound. In order to evaluate the healing effect of the PEO hydrogel dressing, wounds formed on the back of marmots were covered by the hydrogel. The healing under the wet environment of the hydrogel dressing had three advantages, compared with that of gauze dressing, which gives a dry environment: (1) enhancement of healing rate, (2) facilitation for changing the dressing, i.e. the hydrogel can be peeled off without any damage to the regenerated skin surface, and (3) hydrogel dressing material does not remain stuck on the wound.

Key words: Hydrogel, Wound dressing, Healing, PEO, Aqueous solution

Introduction

Hydrogel occluded much water in the three dimensional structure formed by crosslinking of polymer. Water in the hydrogel cannot be released by pressure. Hydrogels usually have –OH, -O-, -COOH, -COONa, -SO_3Na functions in main chain or branch chain. There are two methods to form hydrogel by irradiation. One is due to radiation polymerization/crosslinking of monomer such as acrylic acid, acrylamide and 2-hydroxyethyl methacrylate. Another one is due to crosslinking by recombination of radicals formed by irradiation on the polymer chains. In polymerization/crosslinking, since it is difficult to do polymerize 100% of monomer,

un-polymerized monomer remains after radiation polymerization. This technique is not favorable for application on medical field, because the monomers are usually toxic. On the contrary, hydrogel formed by radiation crosslinking of polymer is preferable for medical application because of its purity after irradiation and lack of low-molecular weight toxic substances.

In 1960, Winter found that moist environment of wound covered by wet material is effective for the healing process[1]. After that, hydrocolloid type dressing formed by blending of natural rubber latex and carboxymethylcellulose was developed. Hydrogel gives wet environment for wound, hence it was considered as useful for applications on wound dressing. In this article the results of crosslinking of water-soluble polymer in various phases by irradiation and evaluation test for application on wound dressing of obtained hydrogel are reported.

Experimental

Poly (vinyl alcohol)(PVA), poly (ethylene oxide) (PEO) and poly (vinylpnroridone) (PVP), the typical water-soluble polymers, were used for hydrogel preparation. PVA117 obtained from Kuraray Co. Ltd and PVP K-90 obtained from Kishida Chemical Industry Co Ltd have molecular weights of 7.3×10^4 and 3.6×10^5, respectively. Molecular weights of PEO obtained from Meisei Chemical Industry Co. Ltd are 1.4×10^5, 4×10^5 and 3.8×10^6. These materials were irradiated at various phases such as solid, molten and aqueous solution in order to introduce crosslinks at a lower irradiation doses. Clinical test for application to wound dressing was carried out on marmots.

RESULTS AND DISCUSSION

Crosslinking behavior of water soluble polymers

PVA, PEO and PVP were irradiated at various phases. Irradiation was carried out at vacuum conditions except for aqueous solution. The PVA undergo crosslinking at molten state and in aqueous solutions, but crosslinking in solid phase at room temperature is not observed. PVP undergoes crosslinking at solid phase and aqueous solution by irradiation, however irradiation at molten state results in discoloration. Thus, this irradiation is not preferable for crosslinking of PVP. Three types of PEO with different molecular weight were irradiated to form hydrogel.

Irradiation induces crosslinking of PEO at every examined phase. The crosslinking was remarkably affected by molecular weight and irradiation temperature. PEO gives the highest gel fraction at a temperature higher than its melting point, e.g. at 70℃. High molecular weight samples of PEO are better for crosslinking at every phase. The high molecular weight PEO (3.8 x 10^6) has gel fraction of 40% even 0 kGy, due to entanglements of molecules chains. Hence, gel fraction of 80% is obtained at low dose, 30 kGy. Figure 1 shows the gel fraction of middle molecular weight PEO (4 x 10^5) irradiated at three phases. Among them, irradiation in an aqueous solution gives the highest gel fraction and a required dose for crosslinking is the smallest. It is considered that acceleration of crosslinking is due to a contribution of OH radical formed by radiolysis of water. To elucidate swelling properties, PEO irradiated at various phases were immersed in water for 48 hours. Swelling reflects crosslinking density and low swelling gels have high crosslinking density. According to Figure 2, PEO irradiated in aqueous solutions give the highest crosslinking density and 1g dry gel absorb water of about 20 g at 40 ~ 60 kGy.

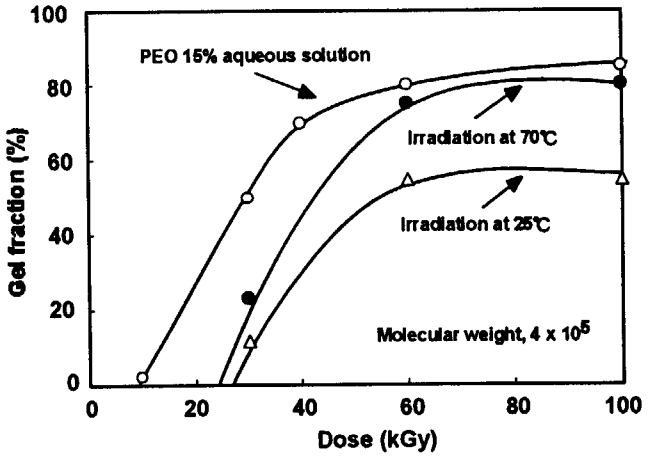

Fig.1 Crosslinking of PEO at various phases

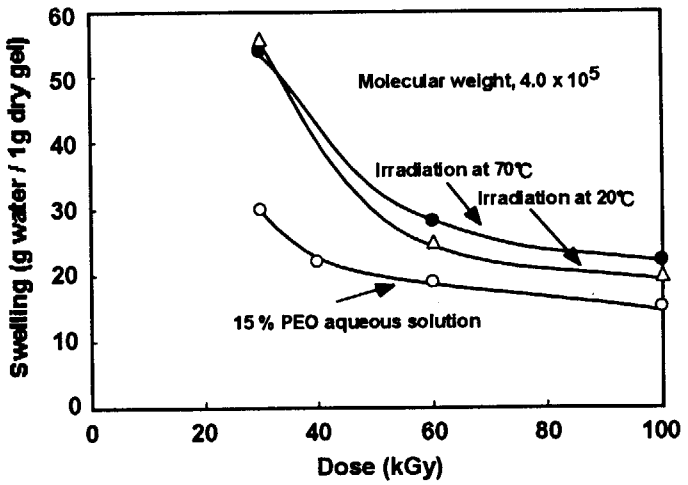

Fig.2 Swelling behavior of crosslinked PEO.

Evaluation of PEO hydrogel for wound dressing

The PEO hydrogel was prepared for healing test of wound and applied as a dressing to five marmots. The γ -sterilized hydrogel was applied to the wound formed on the marmots back. The dressings usefulness was evaluated by taking weights of the marmots during healing and the weight of the hydrogel before and after use. Simultaneously the healing ratio was determined as well. The regular gauze dressings were applied as a reference. In both cases of the hydrogel and gauze dressing, the weight of marmots decreased 5 ~ 10 % in the initial period (up to 7 days) owing to the wound and after that time the weight increased gradually with healing. The dressing was changed three times, after 4, 7, and 11 days during the healing period. Figure 3 shows the area of wound, which size decreases with time. The lower value for the shorter period reflects faster healing. An area of the wound covered by hydrogel decreases obviously with increasing healing period. Almost all wounds were healed after 14 days. On the contrary, the wound covered by gauze dressing reduced its size by only half a percent even after 14 days. According to these findings, healing is faster

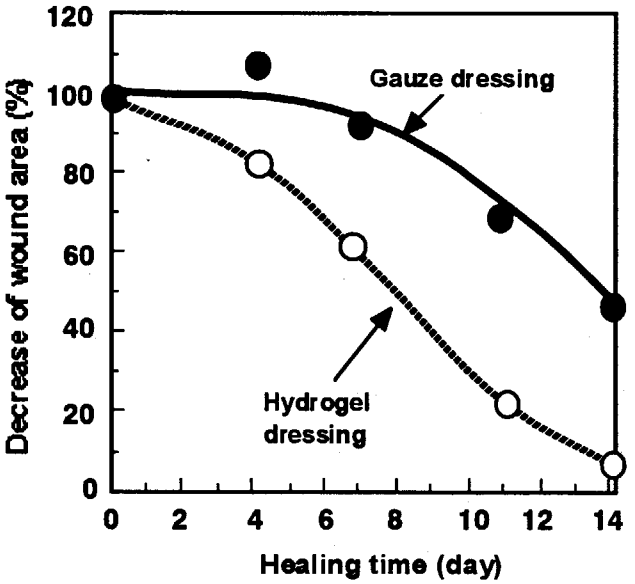

Fig.3 Healing of wound by gauze and hydrogel dressing

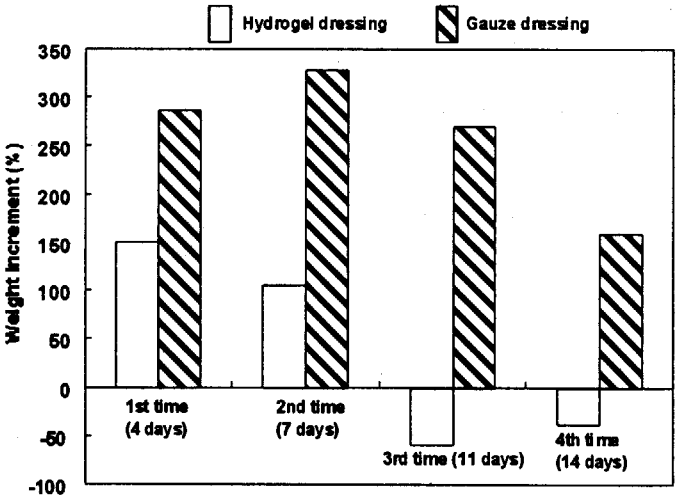

Fig.4 Absorption of effusion from wound of dressing during healing

with the hydrogel dressing than with the gauze dressing.

We have reported that PVA hydrogel obtained by combination of acetalization and irradiation is durable after autoclave sterilization (121℃), and the hydrogel accelerates healing in comparison to gauze dressing[2]. The same tendency was also obtained in the case of PEO hydrogel. As shown in Figure 4, the weight of the hydrogel increases quickly at the earlier stages, up to 4 days, due to absorption of effusion produced on the wound. After that, the production of effusion ceases and weight of the hydrogel decreases due to evaporation of the water from the hydrogel. This means that the healing of wound proceeds smoothly with time. The hydrogel can be peeled off easily from the wound when the dressing needs changing. According to Figure 4, the weight of gauze dressing increases even 14 days, indicating that effusion from the wound continues due to the slower healing rate. Moreover, since the gauze dressing adheres closely to the wound, it gives additional damage for the wound during dressing change. It is concluded that wet environment formed by PEO hydrogel is effective for fast healing of wounds.

CONCLUSION

Radiation crosslinking of water-soluble polymers was carried out at solid and molten state, as well as in an aqueous solution. Among these phases, water-soluble polymer irradiated in aqueous solution induced crosslinking and formed hydrogel at the lowest doses. It was confirmed that PEO hydrogel crosslinked in an aqueous solution by irradiation is effective as a wound dressing.

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Session 1

Radiation Processing of Starch and Cellulose

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5 PVA-Sago Starch Hydrogel and the Preliminary Clinical Animal Study of the Hydrogel

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Abstract

Sago starch granule dissolves in hot water to form physically crosslink semi-gel structure. Polyvinyl alcohol (PVA) in aqueous solution is chemically crosslink and form hydrogel after expose to gamma or electron beam irradiation. Combination of sago starch and PVA give tremendous improvement on strength and elasticity of the gel. Adding additive such as carboxymethyl cellulose enhance the swelling or absorption property of the gel. These properties of hydrogel are important for wound dressing application.

The preliminary clinical animal study on the PVA Sago hydrogel dressing shows promising results of healing process in comparison with the conventional dressing using vaseline impregnated gauze acting as control dressing. This re-confirmed by biopsy tests on the wound tissue taking during the healing process. The tests show the increasing amount of fibroblast and endothelial cells on both wounds using hydrogel and jalonet during the healing process. Also, the rate of epitheliazation is almost completed for both wounds after 10 days of dressing and the lymphocytes cell increase tremendously for the first 14 days with hydrogel dressing.

Keywords: Hydrogel, Sago Starch, Clinical test, Wound dressing, Radiation

1. Introduction

Hydrogels have received significant attention because of their physical properties make them attractive for a variety of biomedical and pharmaceutical applications (1). An integral part of the physical behavior of hydrogels is their swelling behavior in water, since upon the preparation they must be brought in contact with water to yield the final, solvated network structure. Generally, highly swollen hydrogels are those of cellulose derivatives, polyvinyl alcohol, polyvinyl pyrrolidone, polyacrylamide and polyethylene glycol. Their biocompatibility allows them to be considered for medical applications (2), whereas their hydrophilicity can impart desirable release characteristics to controlled and sustained release formulation (3).

The ability of hydrogel to absorb exudates and keep moist environment on the wound, impermeable to bacteria but permeable to oxygen and water, follow the contour of the wound surface and easy removal, make it most suitable to be use as wound dressing (4-6). Moist environment is found to accelerate wound healing where new epidermis formation is twice as much as in dry environment. In moist condition, epidermis migrates through the serous exudate on the wound surface above the fibrous tissue of the dermis. Normal scab including fibrous tissue is not formed, and therefore the leukocytes migrate out from the dermis into the exudate. The increasing amount of fibroblast cells in the proliferative phase of the dermal repair, in moist compare to dry condition indicate the accelerating healing process. The development of hydrogel for wound dressing has to proceed with clinical test to justify the beneficial of this dressing compare to conventional dressing. In this study, the clinical test using PVA sago hydrogel and conventional jalonet dressing as a control have been carried out on the rabbit. The biopsy test on the wound is use to monitor the formation of various cells such as leukocyte, polymorphonuclear, fibroblast and endothelial, during the healing process.

2. Experimental and Method

2.1 Materials

Food grade sago powder was secured from Nitsei Sago Industries Sdn. Bhd., Sarawak, Malaysia. PVA 117 with 1700 polymerisation and 98-99% specification was purchased from Kuraray Co. Ltd., Japan. Low viscosity carboxymethyl cellulose (CMC) sodium salt with viscosity 10 –20 cps (2% aqueous solution at 25°C) was purchased from Sigma Chemical Co. All the materials were used as-receive. The distilled water was used through out this experiment.

2.2 Sample Preparation

Preparation of semi-gel sample as describe in the previous work (7) is a mixture blend of sago starch solution (w/v) with water-soluble polymers PVA (w/v). In some case, the blend is added with additive such as CMC aqueous solution (w/v). Then, the blend is heated in water bath at 90°C for 30 minutes and let it cooled in the PET plastic mould with thickness less than 3mm, at room temperature to form semi-gel sample.

2.3 Irradiation Process

Semi-gel samples were irradiated at 25kGy i.e. sterilization dose, using Nissin High Voltage electron beam accelerator at 2MeV voltage and 6mA beams current. The samples were kept in cool environment prior for analysis.

2.4 Gel Fraction Measurements

Gel fraction is performed by placing samples in stainless steel pouches and put into a bottle, filled with distilled water. The bottle was heated in an autoclave at 121°C for 1 hour. Then, the samples were dried in vacuum oven at 60°C until constant weight. Gel

fraction was calculated from the ratio of dry extracted sample to the initial mass of dry gel sample.

2.5 Tensile Strength and Elongation Measurements

Tensile strength (gel strength) and elongation at break is determined on rectangle gel sample (1cm x 10cm) using Toyoseiki Strograph-RI Universal Testing machine at crosshead speed of 50mm/min and samples thickness is between 0.3 to 0.5 mm.

2.6 Swelling Measurements

Swelling test is performed by immerse the gel samples in distilled water for 72 hours at room temperature. The gel samples were weighted after carefully wiping out the excess water from the surface of the sample. The degree of swelling was calculated from the ratio of the swollen gel mass to that of the initial dry gel mass.

2.7 Clinical Tests

For this test, 12 healthy rabbit with weight range from 2 to 3.5 kg were selected. The rabbits were shaved on the back and both flank, and anaesthetized with mixture solutions of Ketamine and Xylozine. The area was cleaned with povidone iodine and marked with sterile ink. The partial thickness wound with approximately 5.0 x 2.5 cm in size and 0.6 mm in depth was created using Humby knife. The bleeding was stop with saline sterile gauze.

The biopsy test was carried out by cutting tissue of size approximately 0.5 x 0.5 x 0.5 cm at area of unhealed wounds or 1cm from the wound margin using sterilize scalpel blade. The specimen was kept in the 10% formalin solution before transfer to pathology laboratory for analysis of cells such as lymphocytes, polymorphonuclear, fibroblast and endothelial. The biopsy site was closed with plain 3/0 catgut sutures.

The wounds were covered with 3 layers of vaseline impregnated gauze (jalonet dressing) as control dressing or one layer of PVA sago hydrogel as test dressing. The dressings were covered with 2 pieces of normal saline wet gauze follow with dry dressing and anchored with silk net. TG-grip was applied in order to keep the dressing in place.

The wounds were inspected on day 4, 10, 14 and 21 of operative day. Each time of inspection, the biopsy test was performed on un-healing wound area. After that, the new dressing will be applies on the wound. This procedure will be continuously repeated on days 4, 10, 14 and 21 of operative days.

3. Results and Discussion

3.1 Properties of PVA sago hydrogel

PVA in aqueous solution can easily crosslink by gamma or electron beam to yield gel that can absorb large amount of water. However, the low gel strength and elasticity properties of this gel need to be improved so that it can be use for wound dressing application. Carrageenan (8) and agar (4) have been used to enhance the gel strength and elasticity properties of PVP and PVA hydrogel. In this study, we found that sago starch increase the gel strength and elongation at break of PVA hydrogel, as shown in the Figures 1 and 2, respectively. Its also show that the properties are proportionally depend on the amount of sago starch, where increasing amount of sago starch will increase the properties of the gel. It indicate that there is some interaction either crosslink or grafting taking place between the starch and PVA molecules in the network. Even though starch in solid form degrade when expose to irradiation, but in aqueous condition, there is a possibility of crosslink or grafting with the help of OH radicals from radiolysis of water molecule during irradiation processing.

Adding starch into the system will reduce the capability of PVA hydrogel to absorb water and at the same time also reduce the gel fraction of PVA hydrogel, as shown in Figures 3 and 4, respectively. The swelling property of the hydrogel is inversely proportional to the

amount of starch where increasing the starch content will decrease the water uptake. It indicates that the PVA gel networks occupied by the sago starch either crosslink/grafting with PVA or trap in the network during the irradiation process.

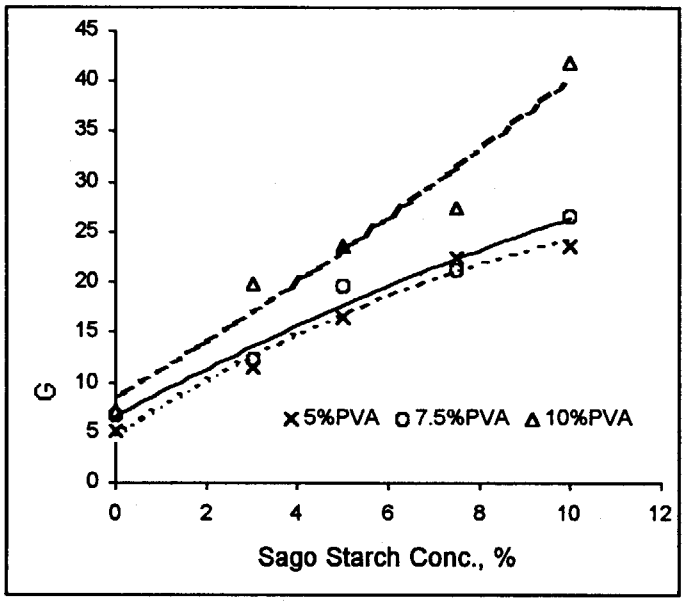

Figure 1: Gel strength of PVA/sago hydrogel at specifies concentration of PVA with increasing amount of sago starch

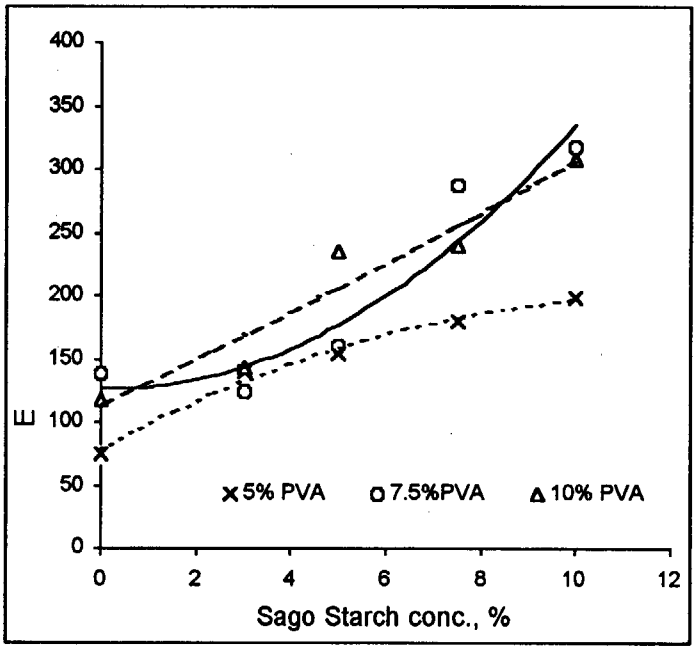

Figure 2: Elongation of PVA Sago hydrogel at specifies PVA concentration with increasing amount of sago starch

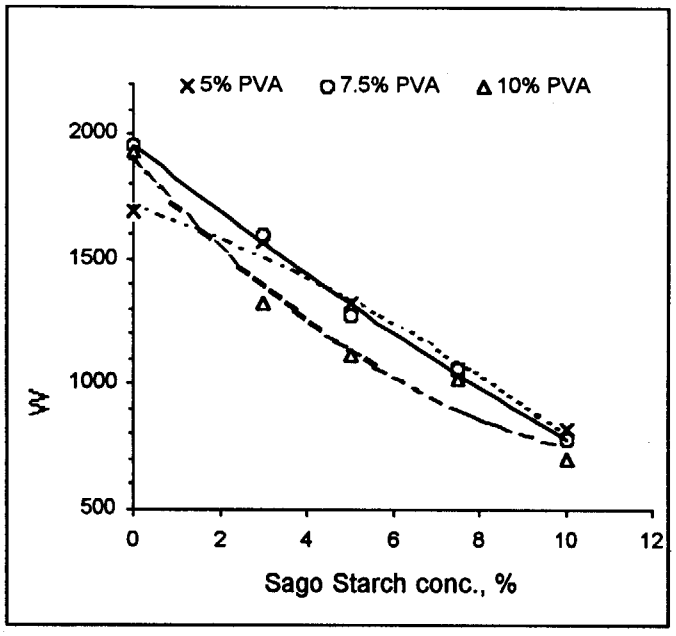

Figure 3: Swelling of PVA Sago hydrogel at specify PVA concentration with increasing amount of sago starch.

Theoretically, swelling depends on the gel fraction of the crosslink material where increase gel fraction will decrease the swelling ability and vise-versa. The dense network will reduce the entrapment of water molecule in the network. In the case of PVA sago hydrogel, the both properties, gel fraction and swelling reduces with increasing amount of sago starch in the system, as shown in Figures 4 and 3, respectively. It reveals that more sago starch trap in the PVA network than the crosslink/grafting with PVA. The 7.5% PVA shows low swelling ability than the 5% and 10% PVA and also the gel fraction much more reduce at higher concentration of sago starch. It indicates that more trap sago starch than crosslink taking place for the system having 7.5% PVA. At much higher PVA concentration (10% PVA), the gel fraction increases compare to 7.5% PVA at specific starch concentration. It indicates that there is some increasing on the crosslink/grafting of starch in the network beside increase the PVA crosslink. The gel fraction of 5% PVA at higher sago starch concentration also increase which indicate some crosslink of sago starch in the system.

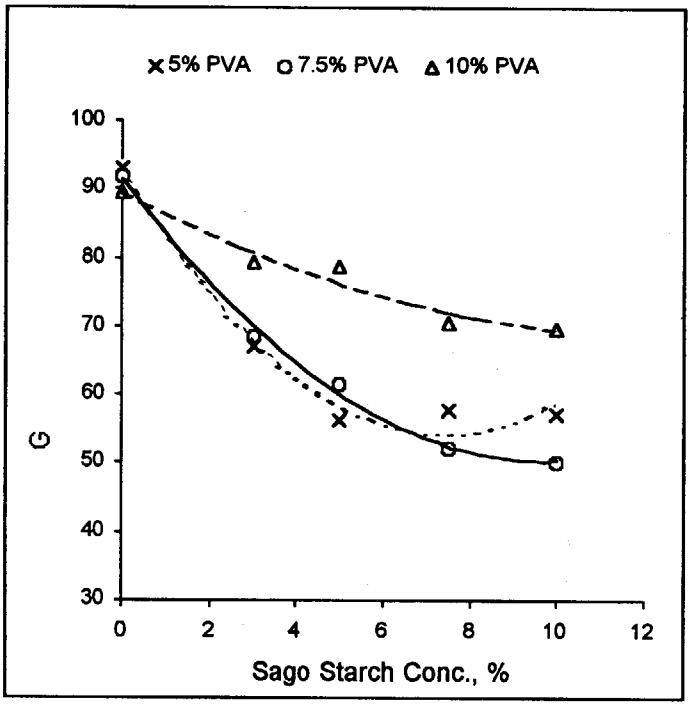

Figure 4: Gel Fraction of PVA Sago hydrogel at specifies PVA concentration with increasing amount of sago starch.

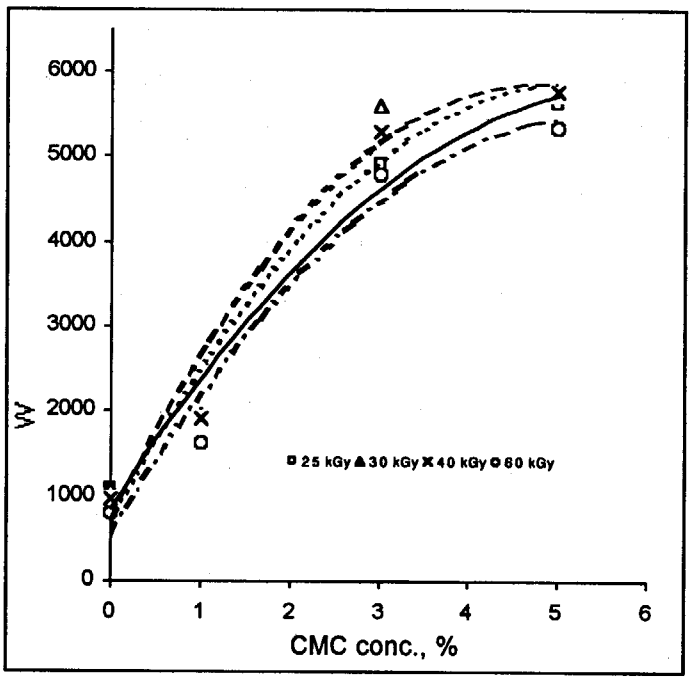

Figure 5: Effect of CMC on the swelling property of PVA Sago hydrogel

Since the absorption property of hydrogel is one of the important criteria for wound dressing application, so improvement on the swelling property of the PVA sago hydrogel has to be considered. In this case, carboxymethyl cellulose (CMC) is introduced into the system to enhance the swelling property of PVA sago hydrogel. The swelling property of the PVA sago hydrogel increases with increasing amount of CMC especially at low concentration of CMC, as shown in the Figure 5. For example, CMC 5% in the hydrogel will uptake about 55g of water for 1g of PVA sago hydrogel. The carboxy group in the CMC is the key factor of retaining water in the hydrogel by forming hydrogen bond with water molecules. Figure 5 also indicates that effect of irradiation dose are not so much compare to the effect of CMC on swelling property of PVA sago hydrogel. It shows that CMC does not involved in the crosslink process to form hydrogel because it is only crosslink in the paste like condition (9).

3.2 Clinical tests on PVA sago hydrogel

In the wound dressing management, there are three overlapping phases taking place during the healing process. The early phase is called inflammation phase follow with granulation tissue formation or proliferative phase and the final phase is matrix formation and remodeling. Inflammatory phase involved the formation of a group of white cell such as polymorphonuclear and lymphocytes. The function of polymorphonuclear cell is to digest microorganism and debris by infiltrate to the wound while the lymphocytes cell is beneficial for body protection, in this study to the rabbit. These cells produce chemo-attractant material and accumulate at the wound area, in order to functionally remove or digest nectrotic tissue, debris and phatogenic organisms.

In this clinical study, Figure 6 and 7 show the formation of polymorphonuclear and lymphocytes cells, respectively, during the healing process of the wound using PVA sago hydrogel and jalonet dressings. For the polymorphonuclear cell, hydrogel dressing show slightly decline in cell formation during healing process while the jalonet dressing sharply reduce to zero but increase again on day 21 of operative days. In the case of lymphocyte cell, both dressing reveals the increasing in cell formation through out the healing process, as shown in Figure 7. The hydrogel dressing shows optimum cell

formation at day 14 of operative days while the jalonet dressing continuously increase with healing process. Toward the end of healing process, that is day 21, both dressing shows nearly same amount of cell development on the wound. This indicates that hydrogel dressing is as good as jalonet dressing for wound healing application.

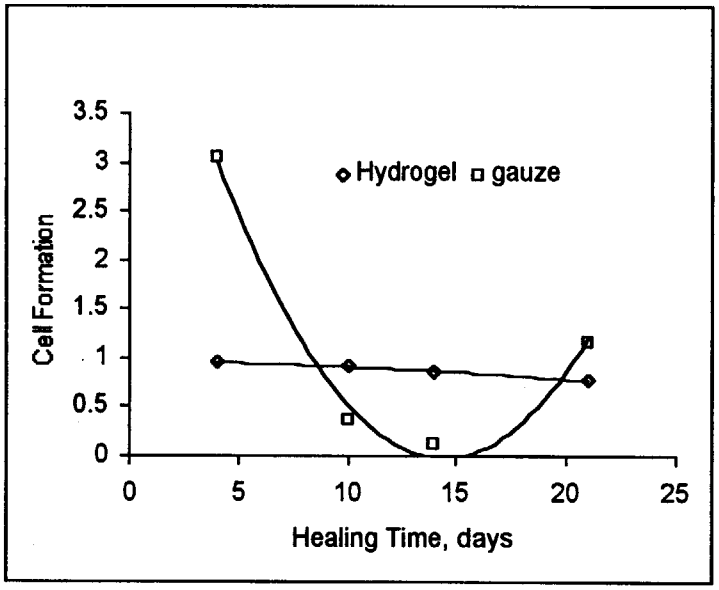

Figure 6: Polymorphonuclear test on the wound tissue during the healing process by PVA Sago Hydrogel and gauze dressing

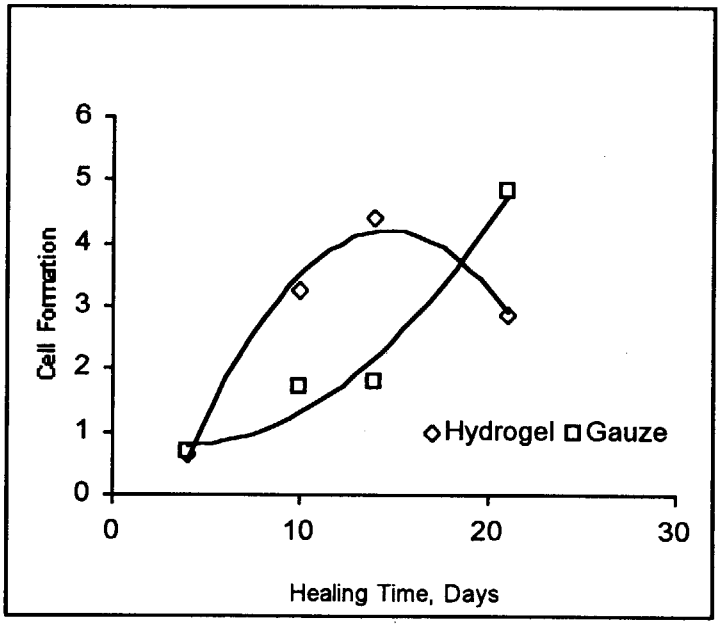

Figure 7: Lymphocyte test on the wound tissue during the healing process by PVA Sago Hydrogel and gauze dressings.

The second phase of healing process occur immediately or overlapping with the first phase. The granulation stage or sometime call proliferative phase is taken place from day 3 to 2 weeks. At this stage, the fibroblast and endothelial cells are the important components during the healing process. The fibroblast cell will produce collagen and lay down extra-cellular matrix or ground substance to strengthen the wound. At the same time, the endothelial cell will start to develop the lining cell of various body cavities and blood vessel.

During the healing process by PVA sago hydrogel and jelonet dressings, both dressing has similar trend in the development of fibroblast and endothelial cells at wound area. Fibroblast cell formation gradually increases with healing process for both type of dressing, as shown in Figure 8. In the case of endothelial cell, it shows optimum formation of cell at the middle stage of healing process for both type of dressing. Both dressing gives good healing of the wound. Based on the biopsy test, the PVA sago hydrogel can be use as wound dressing that has the same quality as the conventional dressing.

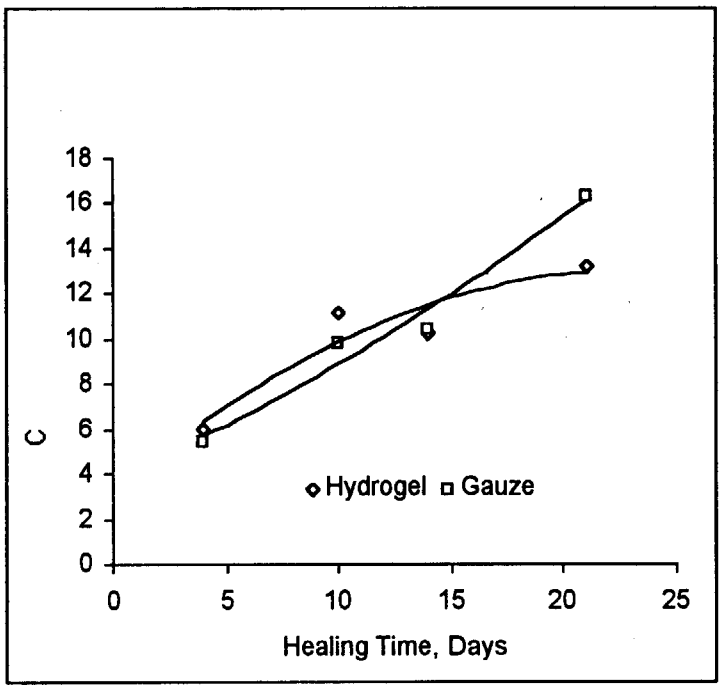

Figure 8: Fibroblast test on the wound tissue during the healing process by PVA Sago hydrogel and gauze dressings.

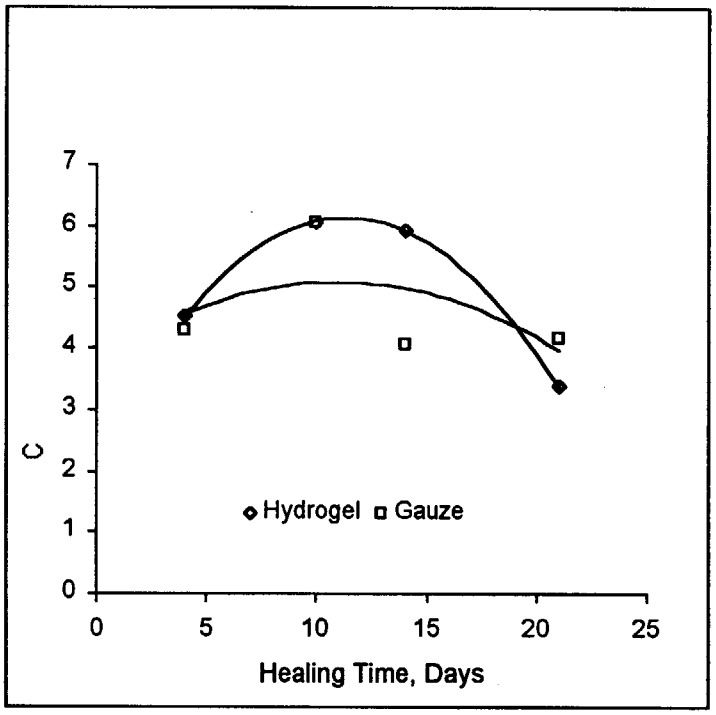

Figure 9: Endothelial test on the wound tissue during the healing process by PVA Sago hydrogel and gauze dressings.

4. Conclusion

Sago starch increases the gel strength and elasticity properties of PVA hydrogel. On the other hand, it reduces the swelling and gel fraction of PVA hydrogel. Based on these properties, it indicates that sago starch is either crosslink/grafting with PVA or trap in the PVA network, depend on the concentration of sago starch and PVA in the system. Addition of CMC into the system gives tremendous increase on the absorption ability of the PVA sago hydrogel. From the clinical study, based on the biopsy test, the PVA sago hydrogel has the same performance as the conventional dressing. It indicates that the hydrogel can be use for wound healing in biomedical application.

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6 Radiation Modified Sago-Blends and Its Potential for Biodegradable Packaging Materials

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Abstract

This paper describes work at MINT on the characterisation and development of sago blends as alternative biodegradable packaging materials. A study was undertaken to investigate the effect of formulation, mixing temperature and irradiation dosage on expansion of sago starch-polyvinyl alcohol (PVA) and sago-polyvinyl pyrrolidone (PVP) blends based foam. The foams were produced by microwaving irradiated hydrogels prepared by mixing sago starch with aqueous PVA or PVP.

In the development of starch-based plastic, the effect of different composition and different irradiation dosage were studied to evaluate films with good tensile properties, elongation, gas permeability and water vapor transmission rate and also the biodegradability of the film using soil burial test.

In another development, irradiation i.e. microwave, electron beam and gamma, has been investigated as a means of degrading the starch granules, which leads to an increase in the amount soluble materials leached. Results showed that irradiation caused an increase in leaching, and a concomitant drastic reduction in swelling volumes of starch granules. It is also showed that the strength of starch gels and viscosity decreased as the levels of irradiation was increased. The degraded starches will be incorporated as an ingredient in the fish cracker and characterized its properties.

Key words:

Sago-starch blends, polyvinyl alcohol, polyvinyl pyrrolidone, irradiation (gamma and electron beam), biodegradable foam and film and packaging materials.

1.0 INTRODUCTION

Owing to the rapid population and economic growth, many countries are facing environmental problems such as that created from plastic consumption and those related to garbage disposal. One of the items that is contributing further to this problem would be foams and plastic wrappers used in packaging.

Several serious problems are being faced by the foam industries. These include flammability (combustibility), waste disposal (biodegradability) or recycling wherever possible or economically feasible, and the problem of ozone depletion by fluorocarbon (CFC) blowing agents [Frisch, 19991]. The development of biodegradable foam would thus be a step forward in the right direction for the aforementioned industry. Utilization of annually renewable agriculturally derived products such as starch as extenders and replacements for synthetic, petroleum-based polymer is currently an active area of research [Tsiapouris, 2000 and Onteniente, 200].

Starch and its derivatives are currently used as plastic materials for disposable items such as packaging loose-fills and picnic tableware [Lim, 1996]. Use of polysaccharides in plastics not only reduces our dependence on petrochemical-derived monomers, but the polysaccharide portion will also biodegrade, causing the finished plastic article to lose its integrity and be reduced to the small particles [Patil, 1994]. Sago starch, plentiful in south Asia, is produced from pith of sago palm. With it's abundant and readily available, it is an attractive indigenous polymer to be use in the development of biodegradable raw materials and or as composites.

Electron beam irradiation of polymer has been employed in the production of foam. High-energy irradiation typically produces free radicals can readily interact with each other forming crosslinking and strengthening the polymer structure. Crosslinking will also enhance the resistance of the cellular product to thermal collapse [Ghazali, 1999].

2.0 Materials and Methods

2.1 Materials

Industrial grade sago starch was obtained from Nitsei Sago, Mukah in Sarawak Malaysia. Sago starch sample was used as provided without any further treatment. In some cases, sago and tapioca starch samples were also obtained from Ajinomoto (M) Sdn. Bhd. It has moisture content about 14 and 12 %, respectively. Carboxyl methyl cellulose was supplied by Aldrich (DS=0.9 and Average MW ca. 700,00). Poly vinyl alcohol was obtained from Kuraray Co. Ltd, Japan which has a degree of hydrolysis of 98 – 99%.

2.2 Preparation of Sago- blends foams

A 10/30/100, 15/25/100, 20/20/100, 25/15/00 and 30/10/100 of sago starch/PVA/water mixtures were prepared by solubilised PVA in distilled water at 121°C for 10 min. They were then left to cool. Sago starch was then mixed with aqueous PVA before the irradiation. 30 ml of blend was poured into a square petri dish (10 cm x 10 cm), irradiated at 10, 15, 20, 25 and 30 kGy, and foam by microwave heating for 5-8 min. In this study, the distilled water acted as the blowing agent.

2.3 Preparation of Sago- blends film

Sago starch was first heated above gelatinization temperature at 80 °C using double boiler. It was then cooled to room temperature. Polyvinyl alcohol (PVA) was mixed with distilled water and heated in autoclave for complete dissolution. Carboxyl methyl cellulose was dissolved in distilled water by vigorous stirring. Film formation was conducted by using solvent casting method. Solvent casting method is a relatively easy way to make films. Approximately 20 ml of sago starch solution were poured onto a petri dish. The film was then subjected to EB irradiation at 2 MeV, 10 mA, after 24 hours dry to half gel. The film was allowed to dry for several hours at ambient temperature. The resultant film was removed from the petri dish. The carboxyl methyl starch and PVA films were also synthesized using solvent casting.

2.4 Irradiation

Irradiation was carried out at Alurtron (MINT) using EPS-3000 Nissin High Voltage Accelerator at 0, 25, 50, 75 and 100 kGy.

2.5 Clarity

The clarity (% T_{650}) of 1 % starch paste, which had been heated in a boiling water bath for 30 minutes and cooled to 25°C for 1 hour, was evaluate by method as described by Craig et. al., (1989). It was evaluate using percent transmittance at 650 nm against water blank in a Ultrospec 2000 UV-VIS Spectrophotometer.

2.6 Gel strength

The starch pastes from pasting property determination were used. The sample was poured into a height of 2.7 cm in a cylindrical plastic container (D=4.0 cm, H=5.5 cm). The pastes were measured after aging at 25°C for 24 hours. The gel strength was measured by using a Stable Micro System (TA-XT2/ Texture Analyzer). The gel was compressed at a speed of 2.0 mm/s to a distance of 15 mm using a cylindrical probe (10 mm in diameter) and the refracted of the speed of 0.1 mm/s to obtain the curve. The maximum force was termed as the gel strength.

The gel strength of the blend was determined based on the force required to penetrate it and measured using a Texture Analyser (TAXT2). The testing condition were as follows: Pre-test speed of 10mm/sec, test speed of 1mm/sec, post test speed of 10 mm/sec, penetration distance 2 mm using a cylindrical probe (15 mm diameter).

2.7 Gel content determination

The sample obtained after moisture content of the irradiated sago starch-PVA blends (in the form of gels) was determined. They were then weighed, placed in between steel nets, and then autoclaved for 15 min. the autoclaved gel were later washed with distilled water and dried in an oven at 60°C overnight. The gel content was calculated using the equation below:

% Gel = (weight of dry gel after extraction/weight of initial polymer) x 100

2.8 Morphology

A Joel JSM-35 Scanning Electron Microscope (Joel Ltd. Tokyo) was used to evaluate the morphology of blends and foams samples. Samples were mounted onto circular aluminium stubs with double-sided sticky tape. The stubs were then sputter coated with gold before viewing.

2.9 Linear expansion of foams

The percentage linear expansion was obtained on foaming the sago starch-PVA blends in a microwave. The unfoamed blends were ruled with a line across using a fine oil pen. Each line was measured before and after forming. The percentage linear expansion was calculated as follows:

$$\text{Linear expansion (\%)} = \frac{(\text{Length after foaming} - \text{Length before foaming}) \times 100}{(\text{Length before foaming})}$$

2.10 Swelling properties of sago film

Triplicate irradiated film were cut into small pieces of known weight (0.2 g) and placed in tea bag. The tea bags were then placed in a 1 L glass beaker containing distilled water overnight. The final weight of the sample was measured and percent of swelling was calculated from the ratio of final weight over the original weight.

2.11 Tensile and Elongation

Measurement of the tensile strength and elongation of the composite films were performed on a tensile testing machine (Shimadzu Autograph Model: AGS-10kNG).

The tensile strength of films were measured under the following conditions : a gauge length of 20 mm; a test speed of 1 mm/min ; and at 50 % humidity using 500N load cell.

3.0 RESULTS AND DISCUSSION

3.1 Starch Pastes Clarity

Fig.1 shows the starch pastes clarity from the % Transmittance determination. Irradiation treatment had a pronounced effect on starch pastes clarity. Non-irradiated sago and tapioca starch gave a translucent pastes and showed 35.4 ± 0.39 and 23.4 ± 0.05, respectively. As we can see, the percent transmittance of irradiated samples was increased with the increased in irradiation dosage. These may be due to the breakdown of the glucosidic bonds that produce more soluble matters. The light can pass through the granule, which result in more clear solution.

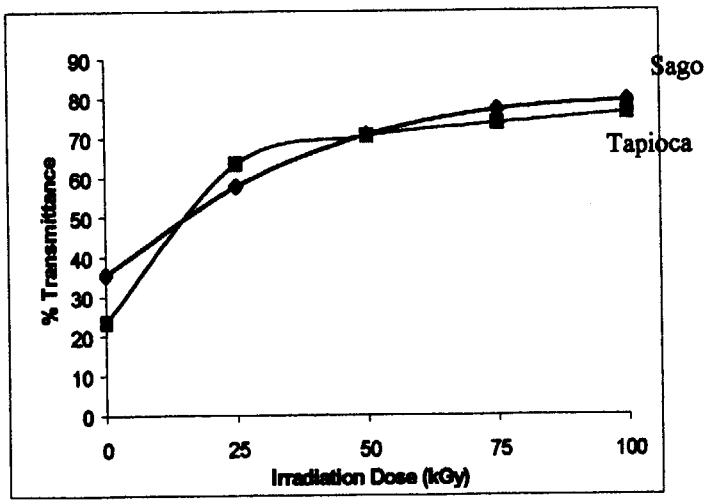

Fig. 1: Effect of Irradiation on the Clarity of Starch Pastes

3.2 Gel Strength

The gel strength found to decrease rapidly with increase in radiation dose. [Rosenthal, 1992] reported that on irradiation, hydrolysis and oxidative degradation will take place. Large carbohydrate molecules are split to smaller fragment by cleavage link, resulting in scissioning and depolymerization. Some reports shows that irradiation by using UHF treatment may result in breaking some branches at α 1-6 linkages (Marquette, 1982), the side chain bonded to the main chain may be separated. Thus, chains of starch may be shortened with a loss of ability to form gel.

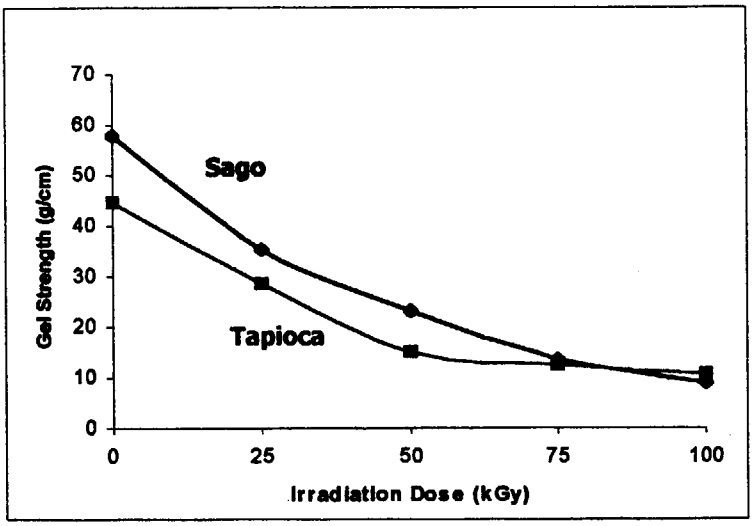

Fig. 2: Gel Strength for Sago and Tapioca Starch

Results also showed that irradiation caused an increase in leaching and as a result drastic reduction in swelling volumes of starch granules (Fig. 3), and rate of gelation (Table 1). These changes were due to cleavage of the amylose and amylopectin fractions by radiation energy and thus a decrease in the molecular weight (Table 2) of starch polymers. .

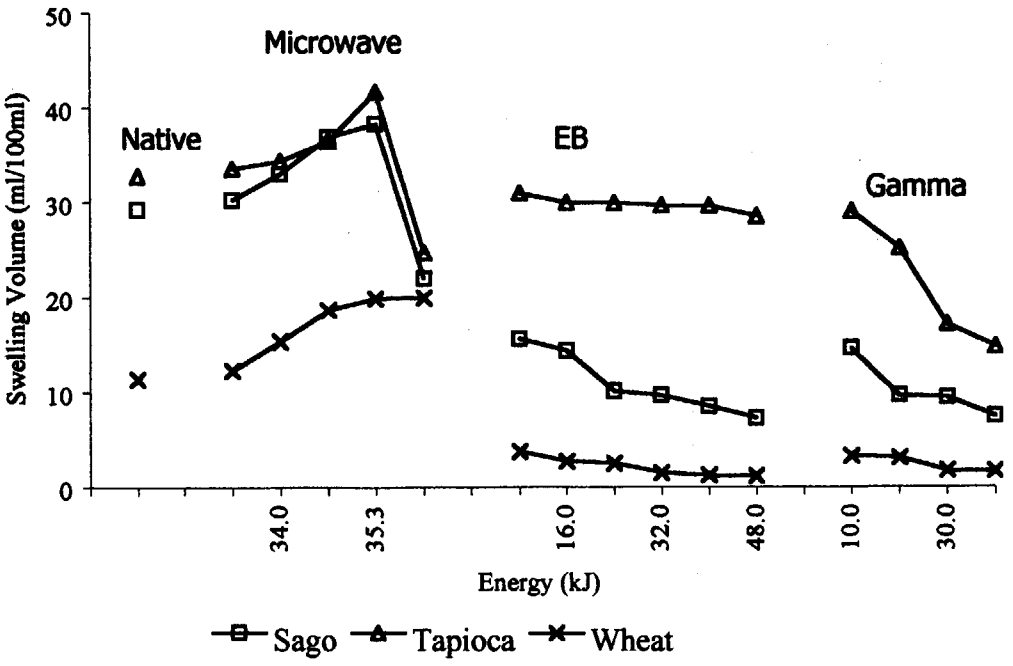

Fig.3 : Swelling behaviour of starches treated by different irradiation type.

Table 1: Rate of retrogradation for native and irradiated starches

Treatment	Rate of Retrogradation*(h^{-1})		
	Sago	Tapioca	Wheat
N	0.3314^{a}	0.2132^{a}	0.2310^{a}
M10	0.3924^{a}	0.2426^{a}	0.2677^{a}
E5	0.6504^{b}	0.2876^{b}	0.3510^{b}
E30	0.7209^{d}	0.5604^{e}	0.5800^{d}
G5	0.6531^{b}	0.4236^{c}	0.5151^{c}
G20	0.6702^{c}	0.5395^{d}	0.6212^{e}

* Means of duplicate measurements
a-e: Means within a column with different letters are significantly different ($p < 0.05$)

Table 2: Effect of Irradiation on the Intrinsic Viscosity and Molecular Weight of Starches

SAMPLE	SAGO				TAPIOCA				WHEAT			
	SLOPE	R^2	[η]	$M_W(10^5)$	SLOPE	R^2	[η]	$M_W(10^5)$	SLOPE	R^2	[η]	$M_W(10^5)$
N	41.18	0.9983	191.00	7.13	14.50	0.9961	179.58	6.65	19.24	0.9965	138.30	4.96
M2	38.55	0.9782	184.57	6.86	13.34	0.9978	177.02	6.54	19.65	0.9870	134.57	4.81
M4	36.59	0.9987	178.59	6.61	12.55	0.9936	174.98	6.46	19.21	0.9567	133.01	4.75
M6	35.65	0.9666	176.68	6.53	13.65	0.9845	172.00	6.34	18.25	0.8759	131.02	4.67
M8	33.25	0.9680	173.65	6.40	12.22	0.9870	170.24	6.26	18.11	0.7968	129.61	4.61
M10	32.60	0.9867	169.66	6.24	12.36	0.9920	166.67	6.12	16.32	0.8576	127.56	4.53
E5	32.28	0.7792	156.00	5.68	12.45	0.9967	142.31	5.12	16.48	0.8999	121.45	4.29
E10	30.60	0.8128	142.23	5.12	13.66	0.9945	128.23	4.56	16.22	0.9245	108.40	3.77
E15	28.24	0.8266	132.83	4.74	13.31	0.9985	118.83	4.18	15.65	0.9687	92.58	3.16
E20	27.49	0.8738	117.57	4.13	15.81	0.9396	103.57	3.58	14.65	0.9652	83.22	2.80
E25	25.33	0.9342	95.27	3.26	17.83	0.9776	81.27	2.73	13.35	0.9754	80.12	2.69
E30	21.57	0.9349	87.79	2.98	15.53	0.9534	73.79	2.45	13.37	0.9857	75.06	2.50
G5	12.85	0.9878	121.56	4.29	12.70	0.9878	115.98	4.07	13.00	0.9457	117.90	4.15
G10	14.64	0.9840	102.20	3.53	14.57	0.9840	87.33	2.96	12.69	0.9358	93.76	3.20
G15	13.42	0.9607	88.57	3.01	14.46	0.9607	76.32	2.54	12.70	0.9645	80.90	2.70
G20	16.33	0.9870	74.31	2.47	15.36	0.9870	67.57	2.22	12.99	0.9974	72.01	2.38

3.3 Gel content

A major practical use of high-energy radiation to modify materials has been in the crosslinking of polymers. Generally, the extent of radiation induced crosslinking of polymers can be estimated from gel content determination [Ratnam, 1999]. The gel content analysis of irradiated polymers allows to estimate

important radiation parameters as yield of crosslinking and degradation, gelation dose, etc and to correlate these with some physico-chemical properties [Charlesby, 1960]. Thus, in order to elucidate radiation-induced crosslinking, the gel content was determined and the results were plotted in Fig. 4. Apparently, it was observed that the gel content was increased when the irradiation dose was increased. This phenomenon shows that PVA is a crosslinkable type polymer as reported by other researchers [Charlesby, 1960 and Chapiro, 1962].

The dramatically increase was observed at 10-20 kGy. Thereafter, it reached a plateau indicating crosslinking reach saturation. It is interesting to note that when the PVA content in the blend was increased from 10-20% (blends 10% SS: 30% PVA, 15% SS: 25% PVA and 20% SS: 20% PVA), the saturated point was at 15 kGy while it was at 20 kGy when the PVA content in the blend was 25-30% (blends 25% SS: 15% PVA and 30% SS: 10% PVA). The difference might be due to higher PVA content in the blend. Therefore, higher dose was required.

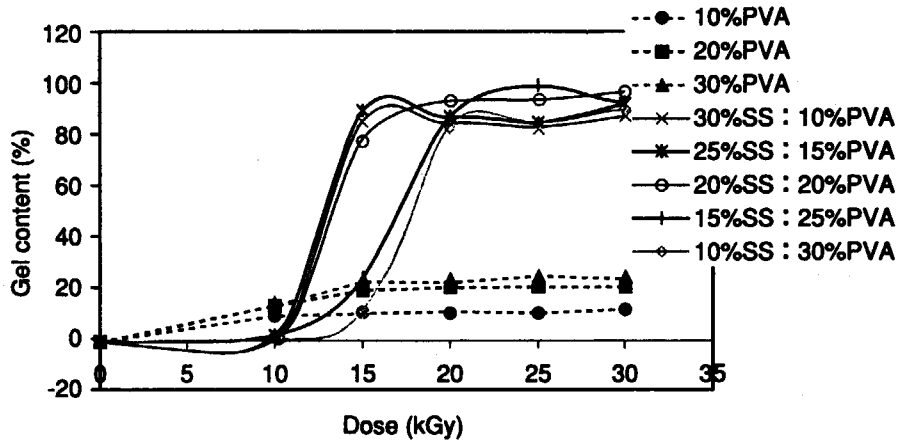

Fig. 4. Effect of irradiation doses on the gel content of sago starch (ss)-polyvinyl alcohol blends (PVA)

The gel content of blends containing 25-30% PVA were found to be lower than that of blends containing 10-20% PVA. This was probably due to efficiency of PVA to form crosslinking. As it can be seen that the gel content of 10, 20 and 30% aqueous PVA were far below that of the blends. This suggest that sago starch enhanced the crosslinking ability of blends. Surface grafting of sago starch onto

PVA may have taken place. The free radical in the starch was suspected to occur more in the amylose part since in the crystalline regions (amylopectin), molecular chains are more tightly packed and less mobile than the amorphous regions, which can be considered as semi solid. Crosslinks (which require a considerable local rearrangement of molecular chains) may therefore be expected to occur preferentially in amorphous region [Charlesby, 1960]. Optimum crosslinking is important for the foam production. Crosslinking not only stabilizes bubbles during expansion but also enhances the resistance of the cellular products to thermal collapse. This enhanced resistance to collapse is necessary for the applications [Ghazali, 1999].

3.4 Texture of blends

Fig. 5 shows the variation in gel strength of sago starch-PVA blends with respect to the irradiation dose. It is apparent that blends showed a gradual increment in gel strength until dose of 25 kGy. A gradual increment in gel strength with dose was also observed for irradiated aqueous PVA, indicating that sago starch-PVA blends were rather harder than irradiated PVA. The surface of irradiated blends was also observed to be less sticky. This implied that the sago enhanced the gel strength of the blends presumably due to the effect of crosslinking. The overall effect of crosslinking is that the molecular weight of the polymer steadily increases with dose, leading to branch chains, until ultimately a tri-dimensional network is formed when on the average each polymer chain is linked to another chain.

Polymer crosslinking may be defined as any process whereby a weak, plastic material is transformed into a stronger, extensible, more rubber like material [Park, 1991]. As mentioned earlier, PVA is categorised as the crosslinking polymer, therefore, the gel content was observed to increase when the irradiation dose was increased. The relationship of gel strength and gel content of blends seemed to be directly proportional up to 25 kGy for all blends. Thereafter, the gel strength in blends dropped rapidly. This indicate that at higher irradiation dose rate, chain

scission is predominant over crosslinking and hence the polymer degrades and lost its strength.

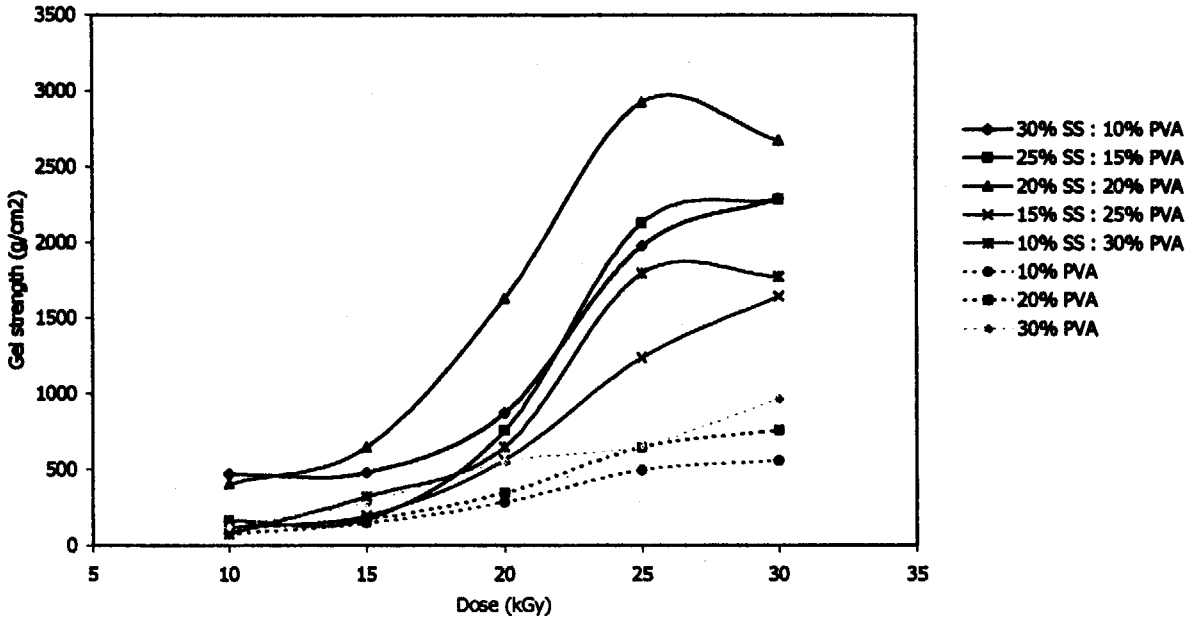

Fig. 5. Effect of irradiation doses on the gel strength of sago starch (SS)-polyvinyl alcohol blends (PVA)

3.5 Expansion ratio of foams

Fig. 6 depicts the expansion ratio of the sago starch-PVA foams. The maximum expansion ratio for all blends was obtained when they were irradiated at 15 kGy. This value coincided with when the crosslinking reached the saturated point. Crosslinking will also enhance the resistance of the cellular product to thermal collapse. When the irradiation dose was further increased to 30 kGy, the linear expansion of the foams tended to be decreased. This is due to the fact that more crosslinking occurred with increment in the irradiation dose resulting in less expansion of the foam. The crosslinked polymer inhibited the mobility of the chain. Furthermore, it might be due to the degradation of the sago granule and the scission of the PVA chains in the system. The expansion of foam seemed to be very much dependent on the level of crosslinking density in the blends. The ability of the foams to expand was restricted by an increase in crosslinking density. The gel strength of the blends increases as the crosslinking density increased.

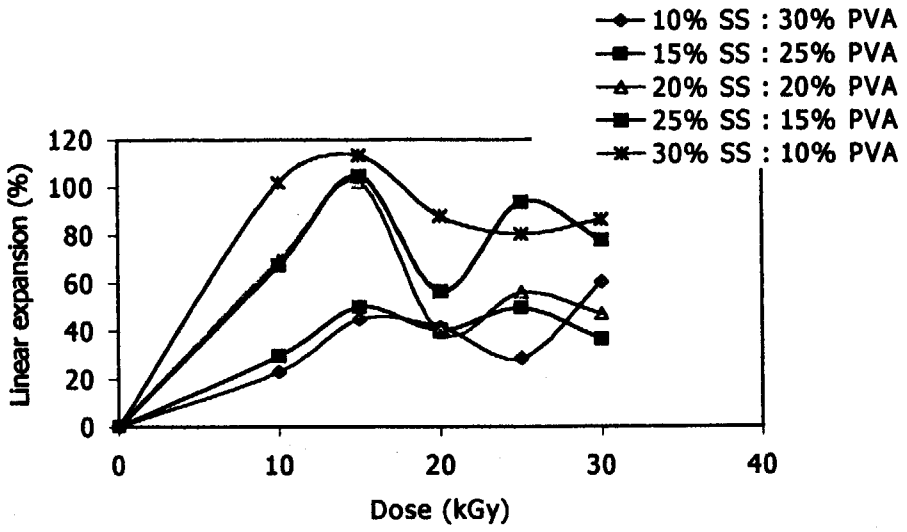

Fig. 6. Effect of irradiation doses on the linear expansion of sago starch (SS) : polyvinyl alcohol (PVA) blends

As mentioned earlier, the more developed the network linking the molecular chains the more limited the mobility of the molecular chains thus producing a restraining effect of foam development. With more dense networks the less is the ability of the gases to expand and form the foam cells. Hence, the expansion value reduced. At 15 kGy, the comparison between the blends was made. It was found that the maximum expansion was blends containing 30% sago starch followed by 25%, 20%, 15% and 10%, respectively. This showed that the increment of the sago starch in the blends enhanced the foam expansion.

3.6 Sago Film

Starch is thermally processable when a plasticizer such as water is added to lower its melting temperature below the decomposition temperature. Addition of polyethylene and polypropylene are known to add water stability, elasticity and toughness to processed starch-filled films. Unfortunately, polyethylene and polypropylene are compounds which have been shown not to be biodegradable. As a result, only the starch portion of the composite film biodegrades while the remaining copolymers remain intact. Graft copolymers are not biodegradable and thus the final product is actually only partially biodegradable due to decomposition of the starch component.

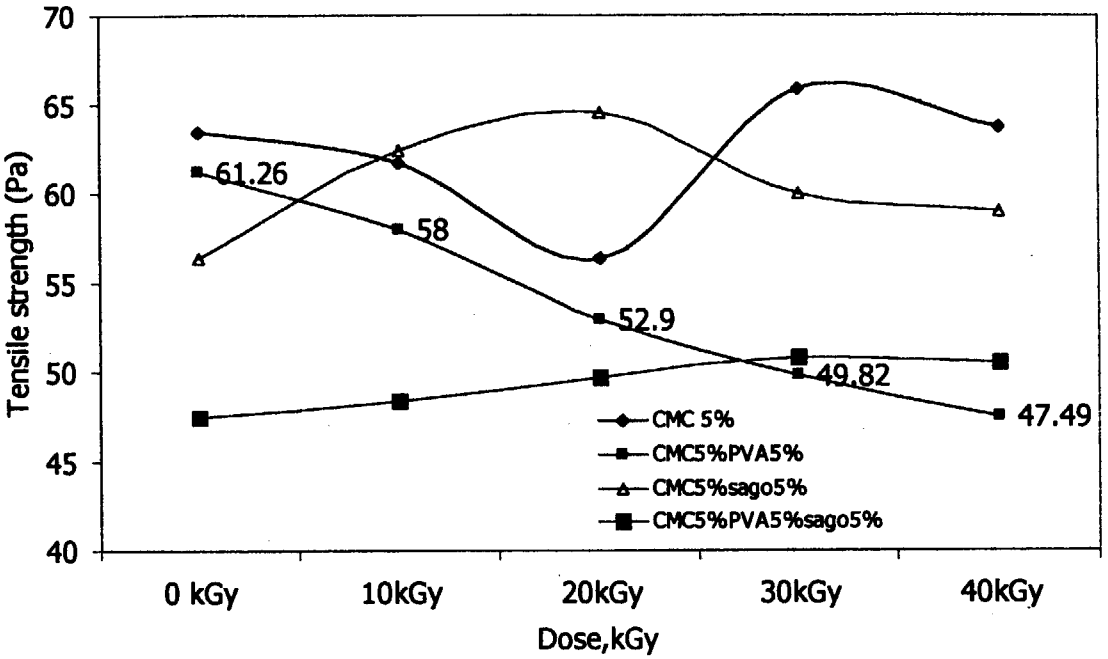

Fig. 7: Tensile properties of sago-blend films

Polymer films have been prepared using sago starch as a component and other additives like carboxyl methylcellulose and polyvinyl alcohol to improve the properties. Starch must be combined with other materials in order to produce a satisfactory film because starch alone produces a brittle and water sensitive film.

Fig. 7 shows the tensile properties of sago blend films. The tensile of CMC-PVA film decreases with increasing irradiation dose. This shows that CMC-PVA blend degraded if subjected to electron beam irradiation. However, it is interesting to note that adding sago in the blends reverse the trend of the tensile of CMC-sago and CMC-PVA-Sago blends i.e. an increase in tensile properties with increasing doses. However, the property start to deteriorate at doses of higher than 25 kGy. The tensile property of CMC-sago blend is highest at 20 kGy dose that is about 65 Pa.

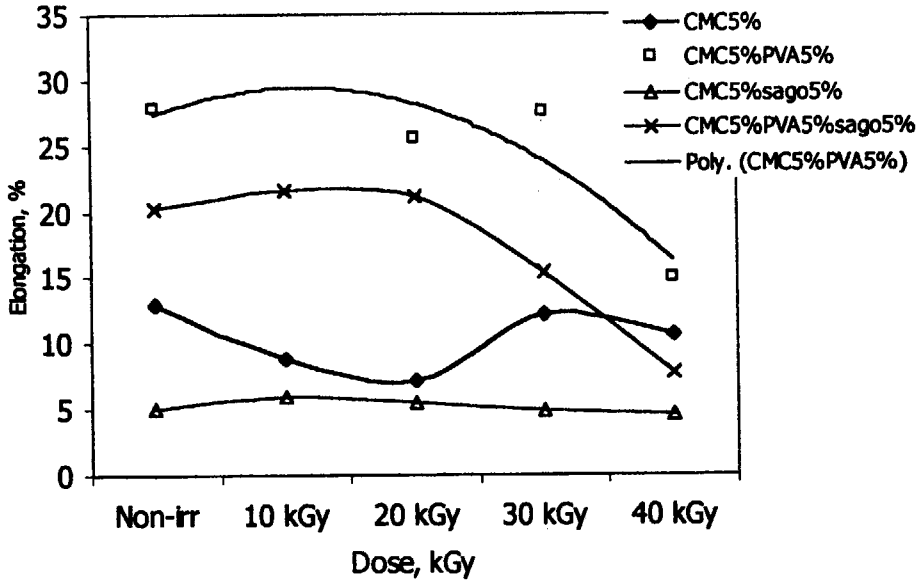

Fig 11. Elongation properties of sago films

All blends show a decreasing trend of elongation property against increasing irradiation dose. This is show in Fig. 11. This indicates that the film property is less resilience or lack of elasticity at higher irradiation dose. Fig. 12 displays the swelling property of the sago-blends films. CMC-sago has less swelling % and relatively constant at around 650% with increasing doses. High irradiation dose affected much of swelling in blends CMC-PVA-sago and CMC-PVA especially at 20-30 kGy doses. Higher swelling % is less desirable for film since it tend to absorb more water present in the atmosphere.

More properties such as tear resistance, permeability and others will be carried out to further evaluate the film properties and its potential as wrapping material in packaging applications. At the moment, the tear property is relatively low as reflected in elongation property. Adding more elastomeric type polymer in the blend may help improve the property.

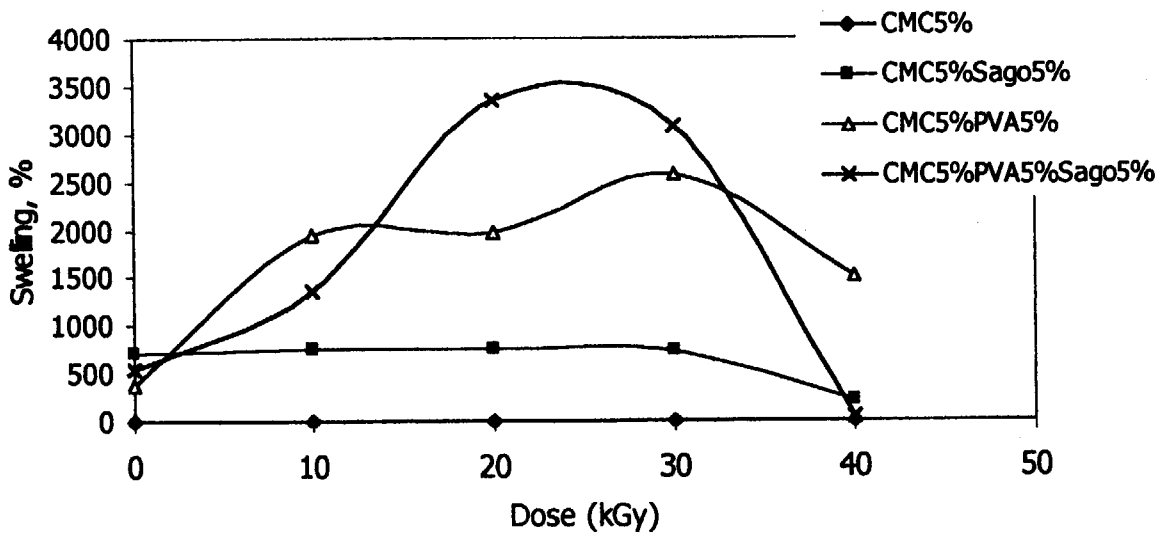

Fig.12 : Swelling properties of sago films

SUMMARY

Crosslinking of sago can also be achieved by blending sago with water-soluble polymers such as PVA. The present study indicates that it is possible to produce biodegradable crosslinked sago foams. Potential biodegradable sago foams can be obtained by blending sago with PVA and other water soluble polymers. Foams produced from sago-PVA blends seemed to be softer and has good resilience properties and would be suitable for packaging application such as replacing polystyrene loose fill foam.

Promising film from sago blends have been produced. However, more experimental work is required to evaluate further these foam properties. This is especially so in tear resistance property of the film. Further work is being carried out to also blend sago with glycerin and grafting palm oil resin into sago to improve the property.

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7 Crosslinking of Starch Derivatives by Radiation

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Abstract

The novel starch derivative hydrogel, carboxymethyl starch (CMS) hydrogel, was synthesized by irradiation in high concentrated solution (in called so paste-like condition). The effect of the solution concentration on the crosslinking of CMS, the properties of formed hydrogel and the biodegradability estimated from CO_2 formation in composting test were investigated. Furthermore, to elucidate the mechanism of CMS crosslinking by radiation, carboxymethyl amylopectin (CMAP) and carboxymethyl amylose (CMA) were also irradiated at paste-like condition, and the properties such as gel fraction and swelling ratio were measured.

The crosslinking of CMS was induced by irradiation at concentration from 20 to 60 %. Among them, higher concentration (paste-like condition) was very effective for crosslinking of CMS by irradiation. 1 g of the dry gel (formed from the solution at concentration of 15 %) crosslinked at low dose, 3 kGy was able to absorb about 500 g of distilled water. Crosslinked CMAP had higher gel fraction and water-uptake at high concentration than that of CMA. Hence, CMAP is the predominant component in crosslinking of CMS. Biodegradation of crosslinked CMS by controlled compost was about 24.0 % after 1 week. The biodegradation is faster than cellulose powder.

Keywords; Crosslinking, Starch derivatives, Radiation, Carboxymethyl starch, Hydrogel, Carboxymethyl amylopectin, Carboxymethyl amylose

1. Introduction

Research and development of biodegradable polymers have been very active owing to concerns related to the environmental pollution by nondegradable plastics wastes. However, many of the candidates for biodegradable polymers have same limitations in their properties or costs.

Starch is a potentially useful material for biodegradable plastics because of its natural

abundant polysaccharide produced as a storage polymer from many plants. It usually has two major components and appears as a mixture of two glucosidic macromolecules very different in structure and properties: linear amylose consisting of α-(1→4)-linked D-glucose, and amylopectin, having the same backbone as amylose but with a myriads of α-(1→6)-linked branch points. It and the starch derivatives such as carboxymethyl starch (CMS), hydroxyethyl starch, and hydroxypropyl starch that are soluble in water have been widely used in many industrial applications such as food, medicine and cosmetic fields [1].

Radiation is a very convenient tool for modification of polymer materials through crosslinking, grafting and degradation. Polysaccharide materials such as cellulose, starch and their derivatives, exposed to ionizing radiation, had been recognized as degraded type polymers since long [2]. On the contrary, CMS has been found to lead crosslinking in paste-like condition under irradiation. In this article, the effect of the solution concentration on the crosslinking of CMS, the properties of formed hydrogel and the biodegradability estimated from CO_2 formation in composting test are reported. Furthermore, to elucidate the contribution of crosslinking, carboxymethyl amylopectin (CMAP) and carboxymethyl amylose (CMA) were irradiated at paste-like condition.

2.Experimental

2.1. Materials

All starch derivatives were received from Gun-Ei Chemical Industry Co. Ltd., Japan. The degrees of substitution (DS) were 0.15, 0.22 for CMS, 0.25 for CMAP and 0.24 for CMA, respectively. All chemicals were used analytical grade without more purification.

2.2. Sample Preparation and Irradiation

CMS was added to deionized water, which was then mixed until homogeneous using hybrid mixer HM-500 (KEYENCE Co. Japan). This sample was irradiated at dose range of 1 to 50 kGy in diluted solution, viscous solution, paste-like condition (high concentrated solution) well mixed with deionized water, and solid state. Furthermore, CMAP and CMA were also irradiated at same dose range in paste-like condition. The irradiation was carried out using beam current of 1 mA and acceleration energy of 2 MeV generated by a Dynamitron electron beam accelerator (3 MeV, 25 mA).

2.3. Gel content and swelling of hydrogel

After irradiation, the CMS, CMAP and CMA mixed with water were freeze-dried using

freeze dryer FD-550 (purchased from Tokyo Rikakikai Co., Ltd., Japan). Then the gel content in the dried samples was estimated by measuring its dried insoluble part after immersion in deionized water for 48 h at room temperature. The gel fraction was calculated as follows;

$$Gel\ Fraction\,(\%) = \left(\frac{Wd}{Wi}\right)\times 100 \qquad (1)$$

where Wi is the initial weight of dried sample after irradiation and Wd is the weight of the insoluble part after extraction with water.

The swelling of crosslinked sample was estimated by Japan Industrial Standard (JIS) K8150. The dry gel was immersed in deionized water for 48 h at room temperature. After swelling, the hydrogel was filtered by a stainless steel net of 30 meshes. The swelling was calculated as follows;

$$Swelling = \frac{(Ws - Wi)}{Wi} \qquad (2)$$

where Ws is the weight of hydrogel in swollen state.

2.4. Biodegradation method

Biodegradation of sample was carried out using controlled activated compost in Microbial Oxidative Degradation Analyzer (MODA) from Saida Ironworks Co., Ltd., Japan. The biodegradability of crosslinked CMS was evaluated by measuring formed CO_2 in the composting test.

3. Results and discussion

3.1. Effect of concentration of CMS on crosslinking by radiation

CMS was irradiated with electron beam in the presence of water to induce crosslinking structure. The irradiation was carried out at diluted solution, viscous solution, paste-like condition well-mixed CMS with deionized water, and solid CMS. The result is shown in Figure 1. Crosslinking of CMS was led due to irradiation with a dose of 50 kGy at high concentration from 20 to 60 %. Such CMS of high concentration before irradiation were strongly kneaded with water in glass vessel by using glass bar to achieve a homogeneous

mixture. It is so-called paste-like condition. It is found that paste-like condition is effective for crosslinking of CMS by irradiation. At 70 % of polymer, water is insufficient for mixing homogeneously with CMS powder, consequently such material gives lower gel fraction than that of 20 ~ 60 %. The irradiation causes degradation for diluted aqueous solution, below 10 % and solid state of CMS. Crosslinkers such as formaldehyde and epichlorohydrin are frequently used for introducing intermolecular bonding of CMS. It is predicted that unreacted chemical remain in crosslinked CMS. Crosslinking of paste-like samples is induced only by irradiation. This advantage makes simple technique for crosslinking of CMS and crosslinked CMS is very pure.

3.2. Effect of irradiation dose on crosslinking of CMS.

Relationship between gel fraction and dose in crosslinking of CMS of paste-like condition is shown in Figure 2. At 10 %, small gel fractions arise at 10 kGy, but gel fraction disintegrate by degradation at high dose, 20 to 40 kGy. Even for concentration of 15 and 20 %, decrease of gel fraction by degradation is observed at higher dose such as 40 kGy. Maximum gel fraction is remarkably affected by high concentration. At high concentration (40, 50 %), gel fraction reach 50 % at low dose, 3 kGy and saturate at 5 kGy. Required dose for crosslinking of CMS is relatively smaller. From this finding, it is obviously that required dose for crosslinking at paste-like condition is very small compared with crosslinking of solid polymer such as polyethylene or rubber [3,4]. Also irradiation of carboxymethyl cellulose (CMC) at paste-like condition led efficiently crosslinking as described in previous paper [5].

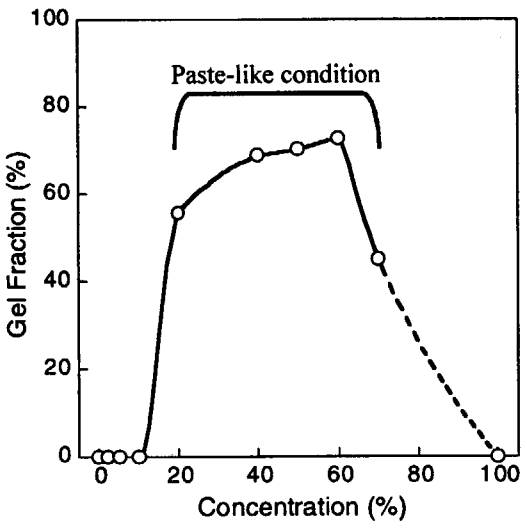

Fig. 1. Gel fraction of CMS irradiated at various concentrations.

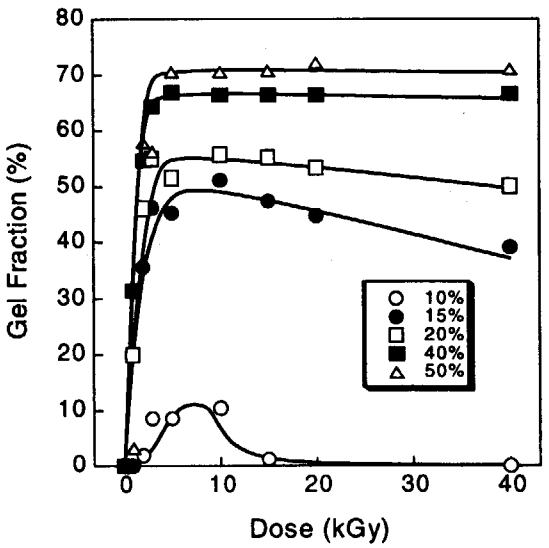

Fig. 2. Effect of polymer concentration on crosslinking of CMS

3.3. Water uptake of crosslinked CMS.

Relationship between swelling and dose of crosslinked in paste-like condition CMS is shown in Figure 3. CMS after irradiation was immersed in pure water for 48 hours to estimate swelling ratio (SR). The SR decreases with increasing dose, furthermore it decreases with increasing concentration of CMS. It might be due to depend on density of crosslinks in CMS gel. 1 g of the dry gel (concentration of 15 %) crosslinked at low dose, 3kGy formed hydrogel, which is able to absorb about 500 g of distilled water. This is very important factor for its future application.

3.4. Biodegradation of crosslinked CMS.

Relationship between the biodegradability of crosslinked CMS and the incubation time in the controlled compost is shown in Figure 4. The biodegradation of unirradiated and crosslinked CMS (5 kGy irradiated 50 % solution) by controlled compost was about 28.5 and 24.0 % after 1 week, respectively. The biodegradation of unirradiated and crosslinked CMS were faster than that of cellulose powder standard.

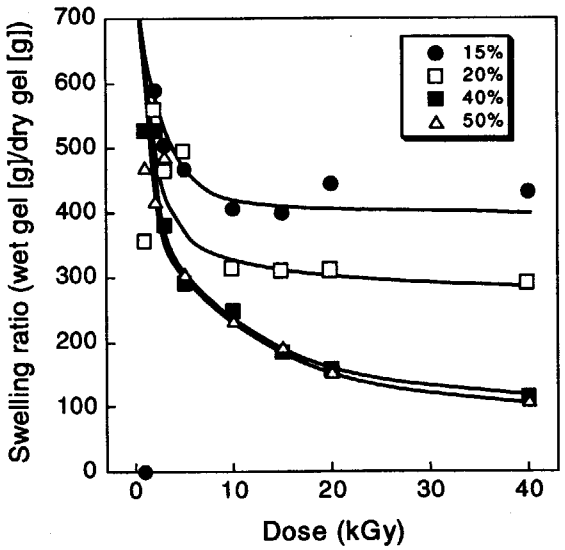

Fig. 3. Swelling of crosslinked CMS.

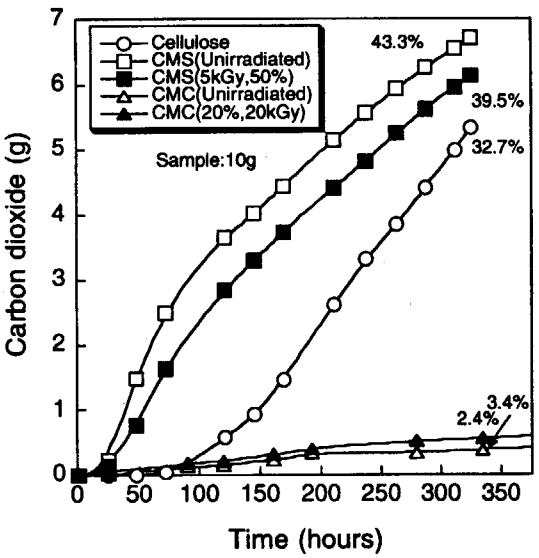

Fig. 4. Biodegradability of crosslinked CMS.

3.5. Contribution of CMAP and CMA on crosslinking of CMS

Starch consists of amylopectin and amylose. To elucidate the mechanism of crosslinking, carboxymethyl amylopectin (CMAP) and carboxymethyl amylose (CMA) were irradiated at paste-like condition. After irradiation, gel fraction and SR were measured. The gel fraction and the SR of crosslinked CMAP and CMA of paste-like condition are shown

in Figure 5 (a) and (b), respectively. The CMAP has higher gel fraction and water-uptake at high concentration (10-50%) than that of CMA. Unirradiated CMAP, however, has also a high gel fraction. The high concentrated CMAP dissolved completely in water when they were not freeze-drying. In unirradiated CMAP, gelation occurs. This is because it possesses a myriads of α-(1→6)-linked branches onto the same backbone as amylose. It is easy to recystalize by freeze-drying. Hence, amylopectin is the predominant component in crosslinking of CMS.

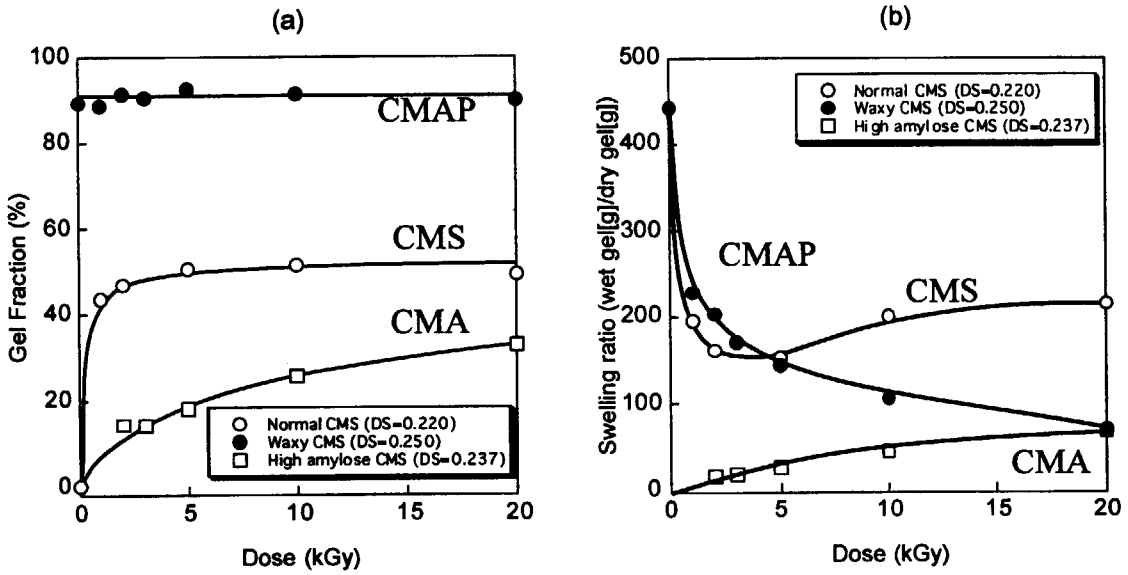

Fig. 5. Gel fraction and swelling of crosslinked CMS, CMAP and CMA.
(a); Gel fraction, (b); Swelling ratio

4. Conclusion

Hydrogel from starch derivatives were synthesized by ionizing radiation without any additives. It was found that high concentration in aqueous solution, so-called paste-like condition of polymer was favorable for crosslinking. The formed hydrogel was able to swell significantly by water absorption, and the swelling varied due to the concentration in which the polymer was irradiated. The hydrogel was a biodegradable material. It was able to degrade in controlled compost. Amylopectin in starch might be the predominant component in crosslinking of CMS.

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8 Syntheses of PVA/starch Blend Hydrogels by Irradiation

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Abstract

A series of excellent PVA/starch blend hydrogels were prepared by gamma and electric beam (EB) radiation at room temperature. The influence of dose, the content of starch in blend system on the properties of the prepared hydrogels were investigated. The gel strength was improved obviously after adding starch into PVA hydrogels, but the swelling properties decreased slightly due to low swelling capacity of starch. The effect of component of starch on the properties of PVA/starch blend hydrogels as well as the reaction mechanism between PVA and starch under irradiation were investigated further. Comparing with PVA/starch blend hydrogels, PVA/amylose blend hydrogels had higher gel fraction, mechanical strength, and lower swelling capacity. PVA/amylopectin blend hydrogels were over the left. It indicated that the amylose of starch was a key component that influenced the properties of PVA/starch blend hydrogels. The analyses of FTIR and DSC spectra of the prepared gel samples after extracting sol indicated that there was a grafting reaction between PVA and starch molecules except for the crosslinking of PVA molecules under irradiation, and the amylose of starch was a key reactive component.

Keywords: Starch, Amylose, Amylopectin, PVA, Radiation, Crosslinking, Grafting

1. Introduction

Hydrogels are three-dimensional hydrophilic polymer networks capable of imbibing large amounts of water, which have been used widely in the field of biomedicine and pharmacy [1]. Synthetic polymers, such as PVA, PVP, PEO hydrogels have been studied widespread but their properties need to be improved further for special applications [2]. Hydrogels of natural polymers, especially polysaccharides also have been used recently because of their unique advantages. Polysaccharides are, in general, non-toxic, biocompatible, biodegradable, and abundant[3]. However, as polysaccharides dissolve easily in water, cannot form stable hydrogel, a effective method is make them into a synthesized polymer gel networks to form natural and synthesized polymer blend hydrogels. kappa-carrageenan (KC) has been found to enhance the properties of the

hydrogels by incorporation KC into water-soluble polymer system [4].

Starch is one of the most abundant and cheap polysaccharides. Usually starch includes about 30%amylose and 70%amylopectin.Chemical modification of starch via graft copolymerization of vinyl monomers onto it has been studied widely in recent years [5]. But few studies on starch/synthesized polymer blend hydrogels have been reported [6]. In this work, a series of excellent PVA/starch blend hydrogels will be prepared by irradiation technique, and in the meantime the formation mechanism and characteristics of the prepared hydrogels will be studied in detail.

2. Experimental

PVA, starch, amylose, amylopectin were used for hydrogel preparation. PVA-117 (Mw, 7.3×10^4) was supplied by Kuraray Co. LTD, Japan. Starch (corn), Amylose and Amylopectin were provided by Gunei Chemical Industrial Co. LTD, Japan.

The hydrogels were prepared by gamma (^{60}Co gamma source) and electric beam (Cockroft Walton Electron Beam Accelerator, 1mA and 2MeV) radiation at room temperature.

The properties of the prepared hydrogels, such as gel strength, elongation at break, gel fraction and swelling behavior were measured by using conventional methods. The FTIR, DSC of gel portion were determined using Shimadzu FTIR-8100A spectrometer, Perkin-Elmer Model DSC-7, respectively.

3. Results and discussion

3.1 The characteristics of the PVA/starch blend hydrogels

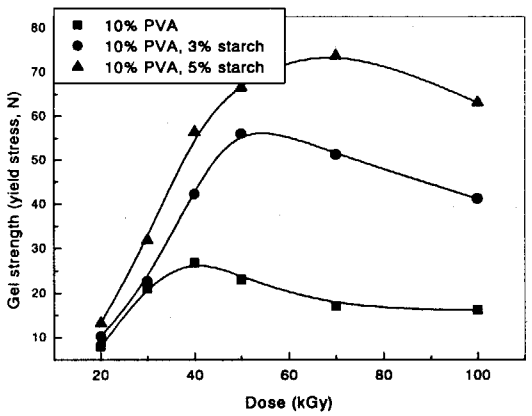

Fig.1 Gel strength of PVA/starch blend hydrogels prepared by gamma irradiation

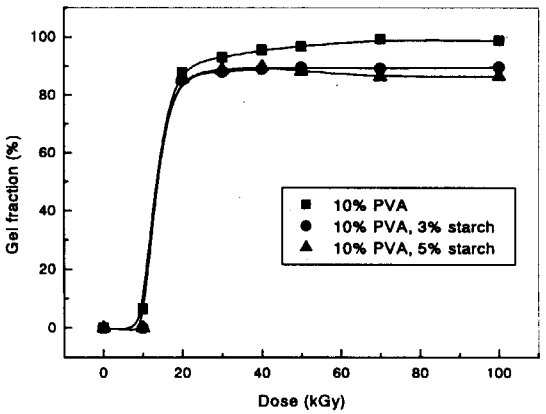

Fig.2 Gel fraction of PVA/starch blend hydrogels prepared by gamma irradiation

The influence of absorbed dose, the content of stach blend system on the properties of the prepared hydrogels, such as the gel fraction, gel strength (compressed and tensile strength), elongation at break and swelling behavior were investigated. The gel strength was improved obviously after adding starch into PVA hydrogel (Fig.1), but the swelling

properties decreased slightly due to low swelling capacity of starch. The gel fraction determined gravimetrically showed that a part of starch could not be removed by extraction (Fig.2). It seems that there is a chemical reaction, i.e. grafting reaction of cornstarch to PVA gels except for crosslinking of PVA molecules under irradiation.
Polysaccharide and synthesized polymer blend hydrogels have been prepared and studied widely, and most researchers thought maybe that there was chemical reaction between polysaccharide and synthesized polymer molecules due to saccharide groups contains two reactive groups at C-2 and C-6 positions, but up to now no relative research was reported. Starch is a kind of polysaccharide. Is there the grafted starch in PVA/starch blend hydrogels prepared by irradiation? In addition, because starch consists of amylose and amylopectin, before discussion on grafting reaction between PVA and starch molecules, it is essential to elucidate the influence of component of starch on the properties of PVA/starch blend hydrogels.

3.2Effect of component of starch on the properties of PVA/starch blend hydrogels

In order to elucidate the effect of component of starch on the properties of PVA/starch blend hydrogels, amylose and amylospectin were chosen to blend with PVA to prepare

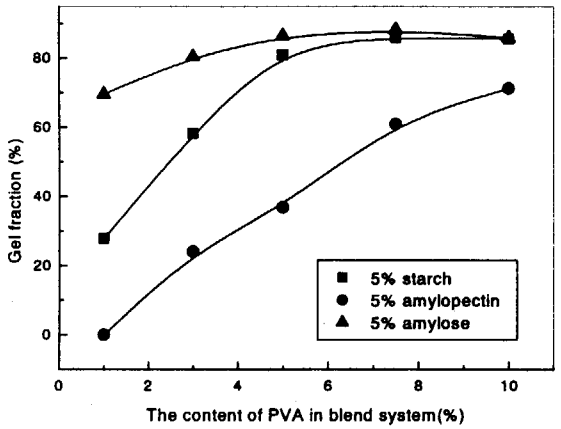

Fig.3 Gel fraction of the blend hydrogels prepared by gamma irradiation (20kGy)

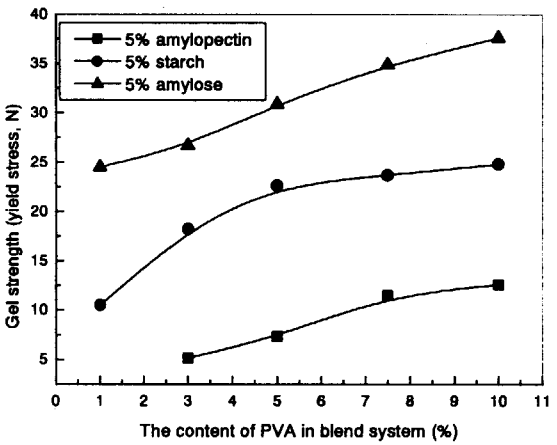

Fig.4 Gel strength of the blend hydrogels prepared by gamma irradiation (20kGy)

the hydrogels, respectively. Comparing with PVA/starch blend hydrogels, PVA/amylose blend hydrogels had higher gel fraction (Fig.3), mechanical strength(Fig.4), and lower swelling capacity. PVA/amylopectin blend hydrogels was over the left. It was very difficult to form homogeneous PVA/amylopectin mixed solution before irradiation due to bad intermiscibility of PVA and amylopectin molecules. After irradiation, the prepared PVA/amylopectin blend hydrogels was very weak, and the gel fraction was very low. When the content of PVA was 1% in the blend system, an excellent PVA/amylose or PVA/starch blend hydrogel could be gained, and the gel strength of PVA/amylose blend hydrogel was more than that of PVA/starch blend hydrogel, but the PVA/amylopectin blend hydrogel could not. It indicated that the possibility of reaction between PVA and

amylopectin was very low, so the amylose of starch was a key component that influenced the properties of PVA/starch blend hydrogels. If there is a chemical reaction, i.e. grafting reaction between PVA and starch molecules except for crosslinking of PVA molecules under irradiation, the main reactive component of starch will be amylose.

3.3 Evidence for grafting reaction of starch to PVA gel

When polymer aqueous solution was subjected to ionizing irradiation, hydroxyl radicals were the main reactive species responsible for reactivity transfer from water to polymer chains [4]. After PVA/starch/water blend system was irradiated, hydroxyl radicals could initiate PVA and starch radials. PVA free radicals reacted easily with other PVA molecules to form crosslinked PVA networks [2]. Reaction probability between starch radials and other starch was very low, after irradiation, starch degraded obviously [6]. But starch radicals reacted with PVA to initiate graft reaction. Graft reaction between PVA and starch molecules depended on the probability of the combination of PVA and starch macroradicals. The following experiments will demonstrate further it.

3.3.1 Infrared spectral analyses of gel portion

The FTIR spectra of the prepared gels after extracting sol were shown in Fig.5. To comparison, a FTIR spectrum of irradiated starch (5% starch aqueous solution was irradiated at 20kGy) was also appeared. The FTIR spectrum of PVA/starch or PVA/amylose gel after extracting sol showed obviously characteristic absorption bands at 1646 and 1024 $cm^{-1}$for O-H and C-H bending of starch or amylose in addition to the absorption bands of PVA, but The FTIR spectrum of PVA/amylopectin gel had not obvious absorption bands for amylopectin molecules. The results suggested that there was obvious grafting reaction in amylose /PVA and PVA/starch blend systems, and the amylose of starch was main reactive component.

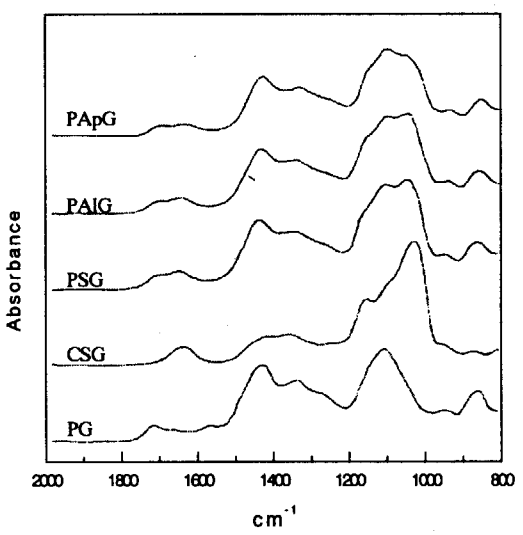

Fig.5 FTIR spectra of the gels prepared by gamma irradiation at 20kGy
PG: 10%PVA gel, CSG: 5%starch solution, PSG: 10%PVA, 5%starch,
PAlG: 10%PVA, 5%amylose, PApG: 10%PVA, 5%amylopectin

3.3.2 Thermal analyses of gel portion

DSC curves of gel samples after extracting sol and mechanical mixture of irradiated PVA and irradiated starch at the same dose were shown in Fig.6. DSC curves of the second run

of the prepared gel samples (dotted line) displayed that there still was a melting peak at pure PVA gel, mechanical mixture of PVA and starch or PVA/ amylopectin blend gel, but in PVA/starch or PVA/amylose blend gels melting peak almost disappeared with the addition of starch or amylose into PVA gel, i.e. the incorporation starch or amylose into PVA by irradiation prevented the recrystallization of PVA. It also showed that the reactivity between PVA and amylose under irradiation was higher than that of PVA and amylopectin, and the amylose of starch was main reactive component.

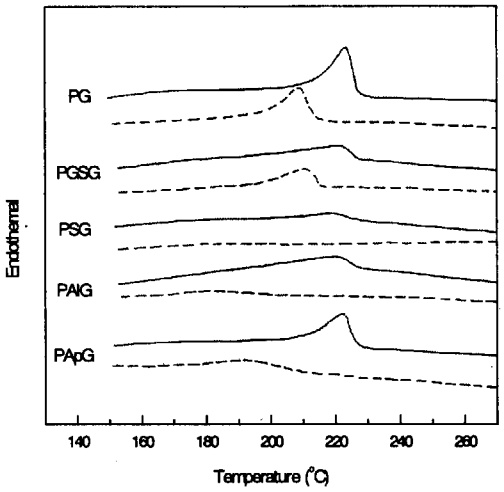

Fig.6 DSC heating curves of the gels prepared by gamma irradiation at 20kGy. PGSG: PVA, starch mechanical mixture(70%PVA, 30%starch, others are the same as that in Fig.5

4. Conclusions

A series of excellent PVA/starch blend hydrogels used for wound dressing could be prepared by changing the composition and the dose under gamma or EB irradiation.

There was a grafting reaction between PVA and starch molecules except for the crosslinking of PVA molecules under irradiation, and the amylose of starch was a key reactive component.

The amylose of starch was a key component to influence on the properties of PVA/starch blend hydrogel, too.

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9 Electron Beam Processing of Oil Palm Empty Fruit Bunch Fibers - Polypropylene Composites

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Abstract

Researches on oil palm empty fruit bunch (EFB) fibers and thermoplastic composites have been carried out by many workers in the last decade. The main focus was to enhance the properties of the resultant composites in view of the incompatibility of the two components. Thus, efforts have been made to enhance their properties by using coupling agents, treating the fibers and modifying the matrices. In this study, the effects of electron beam (EB) irradiation and some reactive additives (RAs) on the mechanical properties of EFB-PP (polypropylene) composites were evaluated. Different modes of irradiation were investigated. Mono, di and tri functional of monomers of RAs were used.

Irradiating PP alone, compared to irradiating the EFB fibers or irradiating both components, gave optimum properties for EFB-PP composites. Further improvements of the properties of the composites were achieved with the addition of RAs with TMPTA (trimethylol propane triacrylate) giving the optimum results.

Keywords: EFB fibers, Composites, Polypropylene, Reactive Additives, Radiation

1. Introduction

One of the natural fibers that are drawing much attention in Malaysia is the fiber from trunks; fronds and empty fruit bunches of oil palm (*Elaeis guineensis*, Jacq.). The palm oil industry generates between 25 and 30 million tons of fibers annually [1]. With the

pressing need to utilize these by-products, the search for their effective conversion into more value-added products continues. Considerable research and development has proven that oil palm fibers is a potential source of lignocellulosic materials for the manufacture of composite products. To date, studies had been conducted on the manufacture of paper [15, 16, 22, 23], medium-density fiberboard [10, 24], particleboard [9, 17] and natural rubber composites [7, 8].

Among the oil palm fiber residues, EFB offers the best prospect for commercial exploitation because it is readily available at the palm oil mill minimizing transportation and procurement costs. The amount of EFB generated by the palm oil industry in Malaysia is very high estimated to be 15 million tons per year [19]. Despite the tremendous and sustainable supply of EFB, however, its commercial use is limited only to the manufacture of lower value-added products such as mattresses, car seats cushions, composts, mulch, etc.

Thermoplastic-natural fiber composite is one of the potential products that can be manufactured from oil palm residues. Studies on the use of oil palm fibers and thermoplastic residues were conducted [11, 12, 14, 20, 25] showing that oil palm fibers, like other lignocellulosic fibers, resulted in poor interfacial adhesion with thermoplastics when used without any treatment. This is mainly due to the incompatibility of the hydrophilic cellulosic fibers and the hydrophobic thermoplastic matrices. Improving the compatibility between these two types of polymers would greatly improve the thermoplastic-natural fiber composite properties, thus, facilitating their use in a number of plastic composite applications.

Maleated polypropylene (MAPP) as a compatibilizer for the natural fibers and thermoplastics, specifically PP, improved most of the properties of the plastic composites [5, 13, 18, 24]. Radiation treatment was also found to enhance the properties of wood fiber-thermoplastic composites [2, 3, 13]. This study evaluated the effects of different irradiation techniques and some reactive additives on some properties of EFB-PP composites.

2. Material and Methods

Thermomechanically pulped EFB fibers, 0.5- 2 mm in size with about 5% moisture content were used. The PP used is a product of Titan Polymer Malaysia Sdn. Bhd. It has specific gravity of 0.9 and melt-flow index of 14.0 g/10 min. The RAs used are 2-ethylhexyle acrylate (EHA), 1,6-hexadiol diacrylate (HDDA), tripropylene glycol diacrylate (TPGDA) and trimethylol propane triacrylate (TMPTA) which are products of UCB Asia Pacific Ltd.

The modes of irradiation applied are as shown in Table 1 using an EB machine EPS Model-3000. All samples were irradiated at room temperature with an accelerator voltage of 3 *Me*V, beam current of 1*mA* and a dose of 10kGy.

All composites were prepared with equal amounts of PP and EFB fibers (50:50 %w/w). Melt blending was done using a Brabender Plastic Corder PL2000-6. The kneaded samples were molded in hot-and cold press machine into 15-cm x 15-cm boards.

All mechanical tests were carried out using ASTM standards. All samples were conditioned for at least 24 hours in a room at 27±2°C and RH of 65±5% before testing.

3. Results and Discussions

3.1 Preparation of composites

Table 1 shows the modes of irradiation carried out for making the EFB-PP composites. The purpose of using several modes of irradiation is to determine the suitable method of irradiation that can produce optimum mechanical properties of the composites. Polypropylene is well known to degrade easily upon irradiation, which is due to its high crystallinity. Upon irradiation at 10 kGy, melt flow PP increases tremendously from 7.0 to ~ 38.0 g/10 min. Similarly, cellulose has been shown to produce radicals upon irradiation [4]. This is further demonstrated when the irradiated materials were blended in

the Brabendar mixer as shown from the mixing torque in Figure 1. The torque decreases 31%, 25% and 34% when irradiated PP, irradiated EFB and both irradiated PP/EFB were introduced in the brabender mixer respectively in comparison with the blend without radiation treatment. PP and cellulosic degradation due to radical induced oxidation chain scission results in molecular weight reduction, cause increase in flow of the materials and thus reduce torque. Lower torque during compounding can lower the energy consumption. In addition, higher material flow will allow higher fiber loading in composites and facilitate the continuos processing of the composite using injection and extrusion molding techniques.

Table 1. Modes of Irradiation Evaluated in this Study

Composite	Description	Remarks
1	No Irradiation	Neither PP nor EFB fibers was irradiated
2	Both EFB&PP	The two main components, PP and EFB fibers, were irradiated
3	PP only	Only PP were irradiated
4	EFB only	Only the EFB fibers were irradiated

The addition of reactive additives in the form of liquid further reduced the torque of the mixing of the EFB and irradiated PP composites as shown in Figure 2. In this case, reactive additives act as lubricating agents during mixing [4]. Further reduction in torque will facilitate the processing of the blend of the composite materials. The reduction in mixing torque of the blends with RAs are attributed to the following order EHA < HDDA < TMPTA.

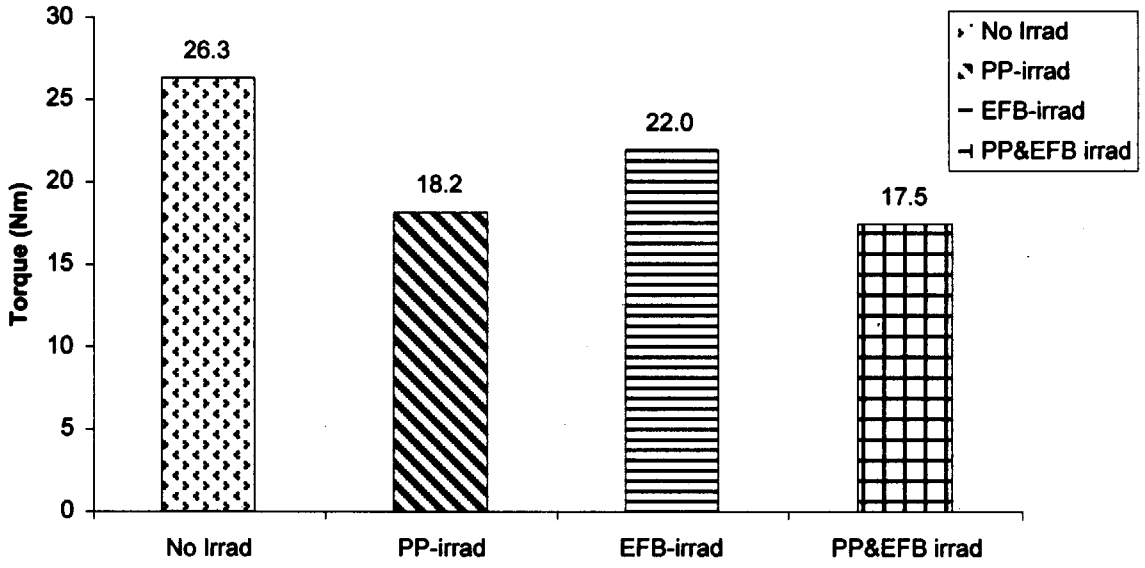


Figure 1. Average torque values for EFB-PP composite using different modes of irradiation

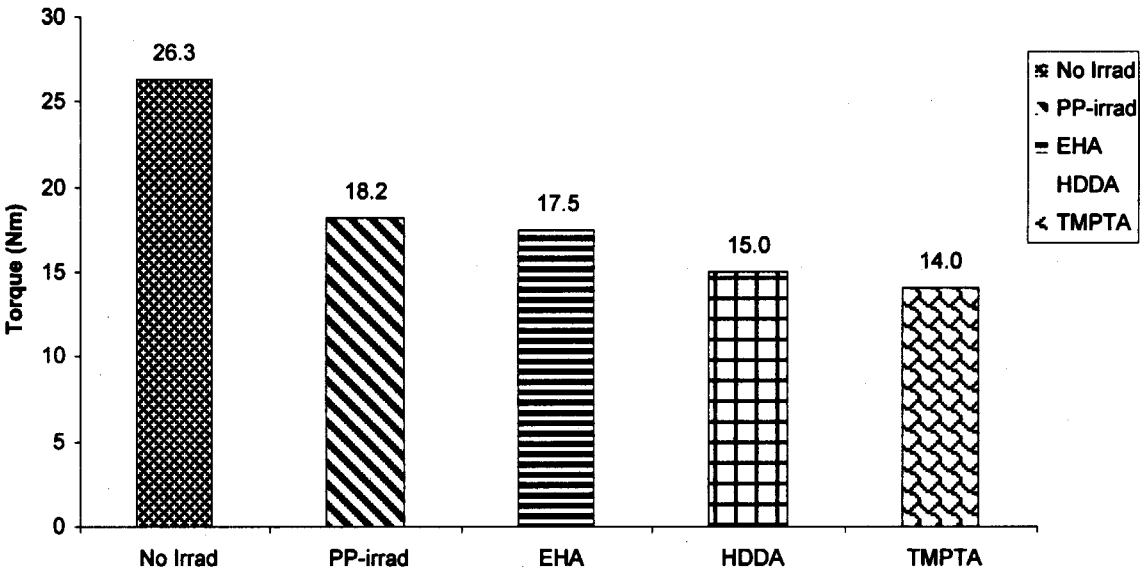


Figure 2. Effect of Reactive Additives on the Torque of EFB-PP Composites

3.2 Properties of composites

Following the brabendar mixing, the resultant composites were subjected to the melt flow rate (MFR) measurement. MFR will determine whether the composites can be used for extrusion or injection process in the manufacturing of products. The changes in MFR will also indicate the changes at the molecular levels that affect the mobility of the molecules. Figure 3 shows the MFR of EFB-PP composites treated with EB irradiation using different modes. The addition of 50% EFB fibers to PP has resulted the drop in MFR of the composite to 1.03 from the initial value of 14.0 of PP. However, the reduction of molecular weight of PP by radiation increased the melt flow of the composite from 1.03 to 4.22. At this stage, the changes in MFR can only be attributed to the physical changes of the PP matrixes. However, similar trend was observed for irradiated PP and irradiated EFB composites. For composite comprises of PP and irradiated EFB, MFR decrease to 2.15 from the initial value of 14.0 of PP. However, it is higher than MFR of unirradiated PP/EFB of 1.03. This shows that irradiated EFB has undergone degradation that cause the breakdown of the fibers during melt blending which make the resultant composite rather easy to flow. This can be the indication of radical chain scission of the cellulosic components of the fibers.

The introduction of RAs in the blends has caused the MFR of the composite to decrease to almost 50% of the initial value as shown in Figure 4. At this stage, it is indicated that crosslinking or interfacial bonding has occurred which make the composite difficult to flow when melted. The amounts of RAs have not significantly influence the decrease in MFR of the composites. However, the functionality of RAs affect the MFR of the composite in the following order; TMPTA> HDDA>EHA. It is envisaged that RAs act as crosslinking agent to facilitate the interaction between radicals of long chain PP and radicals of cellulosic fibers. However, it is still not clear whether the interfacial reaction between PP and cellulosic fibers have taken place. The possible reactions are as follow [4]:

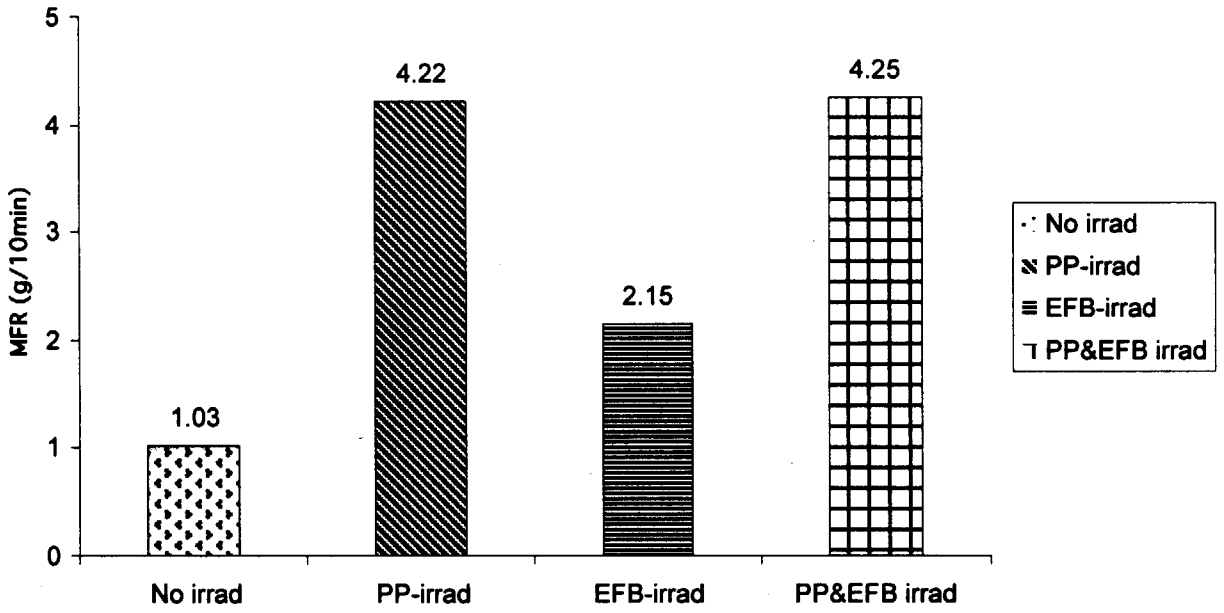


Figure 3. Melt Flow Rate of EFB-PP Composites using different modes of irradiation

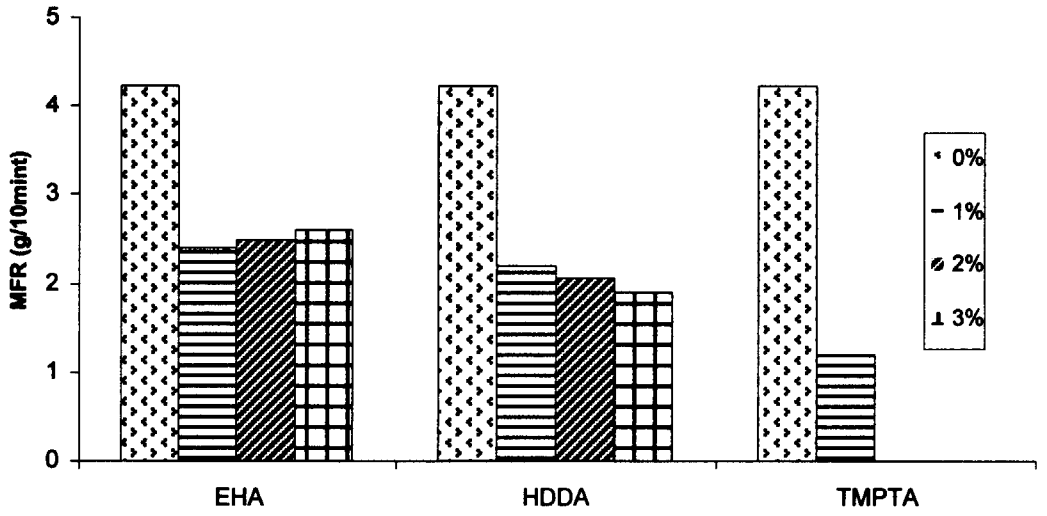
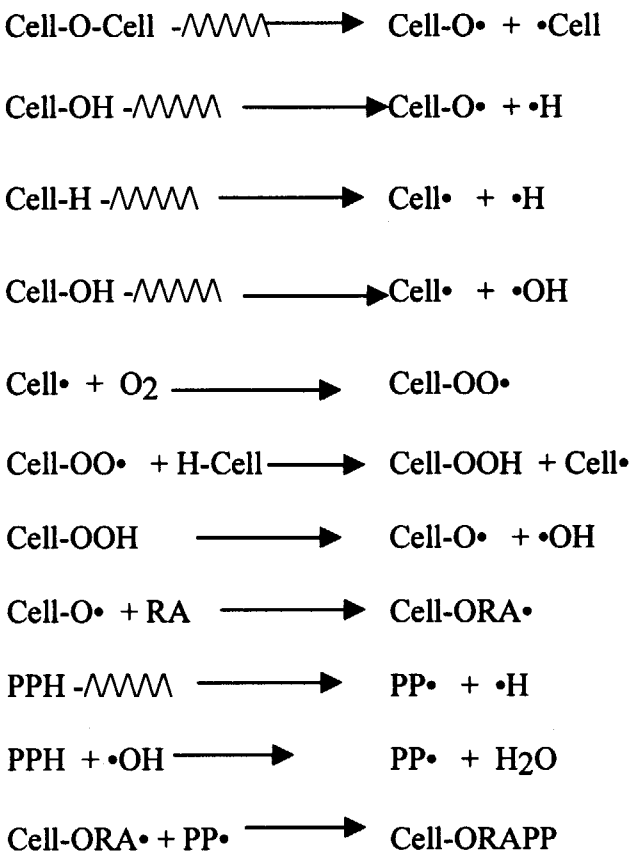


Figure 4. Effect of Reactive Additives on the Melt Flow Rate of EFB-PP Composites



The above reactions are the most common mechanisms for radiation crosslinking of polymeric materials in the presence of air or oxygen. However, there is also possibility that short chain PP radicals may interacts between themselves (polymerization), to form grafting with the long chain PP and crosslink with other PP molecule and with cellulosic fibers. All these reactions occur during melt blending of PP and EFB. In the presence of RAs, the above reactions are facilitated and enhanced.

Table 2 shows the properties of the EFB-PP composites prepared using different modes of irradiation. The addition of EFB fibers onto PP resulted in the drop of strength of the resultant composite. However, the presence of fibers in the PP matrix help to increase the modulus of the resultant composite and significantly increase in the flexural properties of the composite compare to the initial properties of PP. EFB fibers provide some flexibility to the PP matrix and help to absorb bending force applied on the composites. This

property is very important in certain applications that require higher bending property such as for door panel, parcel tray and booth cover of the automotive components. The impact properties of PP composites are maintained or slightly increase in the presence of fibers.

For general improvement of the PP composite, various methods of preparing the composite have been carried out. Table 2 shows that composite comprise of irradiated PP and non- irradiated EFB (PP-irrad/EFB) has higher mechanical properties than others. Subjecting both PP&EFB fibers to EB radiation also improved the strength of the resultant composite causing an increment of 71% over the unirradiated composite. The improvement in the strength when PP was irradiated can be due to the increasing network of PP matrix as suggested earlier. The properties of unirradiated PP/EFB composites are not much different from the composite of unirradiated PP/irradiated EFB. This further explained that EFB radicals alone can not induce radical reaction with PP.

Table 2. Properties of the EFB-PP Composites Prepared Using Different Modes of Irradiation

Composite	Description	Tensile Strength (MPa)	Tensile Modulus (MPa)	Flexural Strength (MPa)	Flexural Modulus (MPa)	Izod Impact Strength (J/m)
1	Without Irradiated Component	17	631	47	2910	26
2	With Irradiated EFB Fibers	20	610	45	2800	27
3	With Irradiated PP	**36**	**778**	**60**	**3018**	**31**
4	With both EFB and PP Irradiated	29	692	49	2725	27
Control	PP sheet (Unfilled)	36	411	29.8	1750	22

A better transfer of stress from the matrix to the fiber through the improved interphase can give higher strength [6, 23]. Increase in strength when PP was irradiated, is due to the several possible reactions of PP radicals as described earlier that strengthen the composite.

Tensile modulus of the composites where PP was irradiated was also improved showing a 23% and 10% increase when irradiating PP only and when irradiating both PP&EFB fibers, respectively, compared to the unirradiated sample. The increase, however, is not so dramatic as with the improvement in strength. The mere presence of fibers in the composites tremendously increased their tensile and flexural modulus when compared with the unfilled PP. The improved adhesion/packing between the two components brought about by the irradiation of PP might impart greater stiffness to the composite with irradiated PP, thus, higher tensile modulus was observed.

Generally, the addition of fibers to the PP matrix improved impact strength. This enhancement in impact strength can be attributed to the ability of the material to resist fracture under stress applied at high speed due to better interaction of the composite components. The irradiated PP/EFB composite shows greater resistant towards propagation of micro cracks (craze) during the moment of impact compared to the unirradiated composite.

Table 3 presents the properties evaluated when EHA, HDDA, TPGDA and TMPTA were incorporated into the EFB-PP composites as reactive additives where irradiated PP was used. Generally, the addition of RAs enhanced the strength of the composites comparable to the strength of PP. They also improved impact properties. However, flexural properties have not changed significantly. There is no clear indication of trends on the functionality of RAs that influence the properties of the composites. However, TMPTA is still the best RAs that can provide the overall improved mechanical properties of the PP/EFB composites. This could be due to the tri-functionality of TMPTA that facilitate and enhance the formation of crosslinking networks of PP that improved the cohesion between fibers and PP.

Table 3. Properties of the EFB-PP Composites that comprised of irradiated PP, EFB fibers and different Reactive Additives

Composite	Description	Tensile Strength (MPa)	Tensile Modulus (MPa)	Flexural Strength (MPa)	Flexural Modulus (MPa)	Izod Impact Strength (J/m)
	Unfilled PP	36	411	29.8	1750	22
A	No RA	36	778	60	3018	31
B	With EHA	32	737	57	3019	34
C	With HDDA	41	720	59	2900	38
D	With TPGDA	37	639	53	2621	37
E	With TMPTA	**42**	**775**	**65**	**3071**	**34**

4. Conclusions

From this study it shows that radiation can upgrade the mechanical properties of EFB fibers - PP composites higher than the properties of PP plastic. By irradiating PP alone, the composites can be easily processed using the commonly used polymer mixer. The addition of reactive additives such as acrylate monomers acts as processing or lubricating agents. The addition of reactive additives also further enhanced the properties of the irradiated EFB-PP composites with TMPTA giving the best results.

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10 Hydrogel of Biodegradable Cellulose Derivatives Radiation-Induced Crosslinking of HPC

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Summary

Hydroxypropylcellulose (HPC) hydrogel combines properties of the polymer, which made up the network and as a material of natural origin is still susceptible to biodegradation. In this report the effects of high-energy radiation on the ether of cellulose - HPC are presented. The polymer irradiated in solid state or in diluted aqueous solution underwent mainly degradation by the cleavage of glycosidic bonds in its main chain. Irradiation of HPC in aqueous solutions at moderate concentrations resulted in the formation of hydrogels. Chemical crosslinks bond the chains of polymer turning it to an insoluble macroscopic gel. We have found that beside the concentration, dose and dose rate can affect the results of irradiation. Electron beam irradiation gave higher gel fraction, up to 90%, than gamma irradiation, which has a maximum of 65%. Swelling of the crosslinked hydrogels was related to the density of crosslinks and was the highest at low irradiation doses. HPC hydrogels displayed thermally reversible character in their swelling. The volume of gel underwent continuous deswelling due to an increase of the solution temperature with its emphasis over 40°C. At elevated temperature the hydrogel collapsed, loses its transparency and turned into translucent white. This transition was fully reversible when the gel was placed in the medium of low temperature. The hydrogel demonstrated superior mechanical properties. Despite of the stable three-dimensional crosslinked network, the gels underwent biodegradation under controlled conditions when enzyme was used.

Keywords: Hydrogel, Hydroxypropylcellulose, Crosslinking, Irradiation, Swelling

1 Introduction

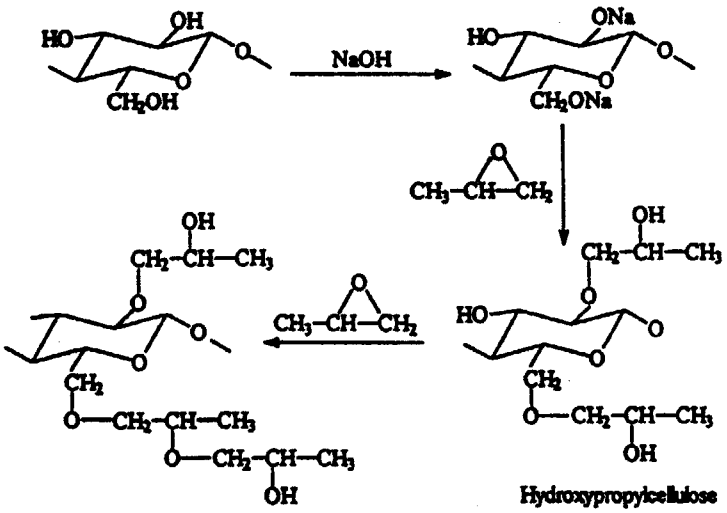
Polysaccharides, such us cellulose and its derivatives, exposed to ionizing radiation, had long been recognized as degradable type of polymers [1, 2]. The ethers of cellulose suffer reduction of molecular weight when exposed to gamma rays or electron beam irradiation. Random cleavage of glycoside bonds in the main chain, initialized by radicals placed on macromolecules is the leading reaction of natural polymers. Irradiation of some cellulose derivatives in an aqueous solution under certain conditions resulted in the formation of three-dimensional network of crosslinked gel. The first investigations on cellulose ethers have been performed by Leavitt, who suggested that the crosslinking reactions involved macroradicals created through an indirect effect of radiation by water radiolysis products [3, 4].

Hydrogels are polymeric networks, which may absorb and retain a large quantity of solvent increasing their volume but still maintain the initial shape and poses some mechanical resistant. Physical gels are held together by secondary forces including ionic, H-bonding or hydrophobic interaction and/or molecular entanglements. All of these interactions are reversible and can be disrupted by changes in physical conditions or stress. On the contrary, chemical gels are insoluble in water and their crosslinked network can be damaged irreversibly only by a rupture of covalent bonds [5]. Usually the network of hydrogel consist of hydrophilic polymer or copolymers, bonded together by chemical crosslinkers or by direct reactions between macromolecules initiated by ionizing radiation, which is in some aspects more convenient method. The presence of several functional groups along polymer chain often makes the hydrogel sensitive to the ambient conditions and is commonly referred to as environmentally reversible materials [6].

Hydrogels possessing abilities of absorption of water or solvents of various ionic strength or pH have found applications in a wide range of industries as super-absorbents, water reservoirs (i.e. agriculture, forestry) [7]. Also biomaterials in medicine and pharmacy are among one of the basic utilization of hydrogels, like wound care coverings, controlled drug delivery systems, dental materials, implants, ophthalmic applications and others [8, 9]. Particularly, natural polymers or hydrogels, which consists of natural macromolecules or their composites with synthetic polymers, formed by irradiation technique have received intense attention by researchers due to their biodegradability and availability at low costs [10, 11]. Ethers of cellulose, because of their novel feature of the gel formation ability and easy biodegradation seem to be an excellent material.

Hydroxypropylcellulose (HPC) is a water-soluble derivative of cellulose with *iso*-hydroxypropyl side chains. Degree of molecular substitution (MS) expresses the number of

hydrogen in OH groups of cellulose or a side chain itself, which are replaced by a side chain. Thus, in the case of HPC, MS can outnumber 3. In Scheme 1 a simplified route of HPC production is presented. One of the important properties of HPC is its reversible temperature-dependent phase behavior.



Scheme 1 Simplified reaction route in production of HPC

Water solution of HPC forms one-phase system at room temperature, but when it is heated above a critical temperature, so called the cloud point, it separates into two phases [12]. The hydration shell vanishes at temperatures exceeding ~40°C, depending on the degree of hydroxypropyl substitution [13-15]. In this report results of hydrogel formation by irradiation of HPC are presented. Investigations on basic properties of obtained hydrogels were also in the interest of the research.

2 Experimental

2.1 Materials

HPC of two molecular-weight grades obtained from Nippon Soda Co. Ltd. Japan was used in our experiments. The basic characteristic of the polymer is summarized in Table 1. The average molecular weights were determined by measuring the intrinsic viscosity.

Table 1. Characteristic of HPC samples.

Sample	MS[a]	Intrinsic viscosity dL. • g^{-1}	Weight-average molecular weight[b]
HPC1	3.0	4.49	$6.60 \cdot 10^5$
HPC2	3.0	7.98	$12.5 \cdot 10^5$

[a] Degree of molecular substitution, MS defines the average number of hydroxypropyl groups per anhydroglucose unit and includes these attached directly to cellulose unit and those attached to a substituent. The value provided by the manufacturer.

[b] see Experimental part for details

2.2 Sample preparation and irradiation

Deionised water was added to the polymer and mixed using a blending machine. The material was kept for few days at room temperature to ensure complete dissolution of polymer chains. Irradiation of the mixture in air was carried out in polyethylene bag; for air-free irradiation, the mixture was heat-sealed in poly(vinylidene chloride) bag to avoid the penetration of oxygen during irradiation, after removal of the air by vacuum machine. Irradiation of samples was conducted with gamma rays generated from a ^{60}Co source at a dose rate of 10 kGy/h at ambient temperature. For irradiation by electron beam (EB), the 2MeV accelerator was used at the irradiation parameters: current 1 mA, voltage 1 MeV and the dose per pass 10 kGy.

2.3 Viscosity and molecular weight of the polymer

Weight-average molecular weight of the polymer was determined from intrinsic viscosity on the basis of the Mark-Houwink equation $[\eta] = K \bullet DP_w^a$, where K and a are empirical constants, DP_w is the weight-average degree of polymerization. To calculate the molecular weight, DP_w was multiplied by the average mass of the substituted anhydroglucose unit. The intrinsic viscosity was measured by an Ubbelohde viscometer in water as a solvent at 25°C. The time of flow of the solvent was 91.0 sec. The intrinsic viscosity was found by plotting the obtained reduced viscosity η_{sp}/c and $\ln(\eta/\eta_{sp})/c$ against concentration ($g \bullet dl^{-1}$) and extrapolating to zero concentration. For the above conditions, to yield weight-average molecular weight the constants K equals $7.2 \bullet 10^{-3}$ [$dl^{-1} \bullet g$] and a 0.915 [16].

2.4 Gel content and swelling of hydrogel

After irradiation, the crosslinked hydrogel was dried for 24 h at 30°C under atmospheric pressure following by drying at the same temperature under vacuum for another 24 h. The gel content was estimated gravimetrically by measuring its insoluble part after extraction of sol. Thus, hydrogel was kept in deionized water for 7 days at room temperature and was occasionally shaken. The residue was made up of only crosslinked gel. The gel fraction was calculated as follows

Gel fraction (%) = (Gd / Gi) × 100

where G_i is the initial weight of dried hydrogel after irradiation and G_d is the weight of insoluble part after extraction with water.

Swelling was conducted by immersing a dried gel sample in deionised water at room temperature. After the equilibrium water uptake was reached, the hydrogel was filtered by stainless steel net of 30 mesh and lightly blotted out by filter paper to remove surface water

prior to weighing. Swelling, in grams of absorbed solvent per gram of dried gel, was calculated as follows

Swelling = (Gs - Gd) / Gd

where G_s is the weight of hydrogel in a swollen state. Swelling in NaCl solution and solutions of different pH value was performed after removal of soluble part from the gel and drying.

2.5 Mechanical properties

In order to estimate the mechanical properties of crosslinked material, hydrogels were examined in relaxed state (immediately after irradiation) and after drying. The dumbbell shaped samples with a central cross section of 5 x 0.5 mm (relaxed state) and 5 x 0.1 mm (dried state) were tested (Japan Industrial Standard K-6301) at 25°C, at a strain rate of 50 mm/min by using Strograph R1. At least 5 measurements of the tensile strength and elongation for each sample were recorded and a mean value was calculated.

2.6 Biodegradation

Enzymatic degradation was carried out in an acetic acid - NaOH buffer, at pH 5.0, by cellulase enzyme, from *penicillium funiculosum*. About 10 mg of dried film of the gel, with a thickness of about 0.3 mm, was immersed in the enzyme solution (2.5 ml) at 37°C. The concentration of the cellulase enzyme in buffer solution was 0.1 mg mL^{-1}. After incubation, the samples were washed and kept in an excess of distilled water to wash away the degraded polymer and dried at 35°C under vacuum. Results of the degradation are expressed as:

Weight loss (%) = Ge/Gd × 100%

where Ge and Gd denote the weights of films after and before enzymatic tests, respectively.

3. Results and discussion

3.2 Radiation crosslinking of HPC polymer

HPC exposed to γ rays either in solid state or in diluted aqueous solution, in the atmosphere of air as well as in the absence of oxygen, undergo degradation. It corresponds well with the results reported earlier for another cellulose derivative, carboxymethylcellulose, CMC [17]. When HPC is irradiated at moderate or high concentration, reactions leading to intermolecular crosslinking prevail and insoluble gel is formed. The crosslinking process of HPC in aqueous solution initiated by electron beam from an accelerator is presented in Figure 1. For all of the samples the gel fraction increases with absorbed dose, steeply at the beginning of gelation, and levels off asymptotically to the maximum value, which for HPC2 is at dose around 40 kGy. At the investigated region of concentrations there is no meaningful

diversification in the gelation of polymer of both examined molecular-weight grades. All experimental points lie along the average curves. The maximum gel fraction is independent of the initial molecular weight of the polymer and reaches ca. 90% at 100 kGy of the absorbed energy. The only distinction is perceptible in the gelation point; the gel starts to arise earlier in the case of HPC2, the samples of higher molecular-weight grade.

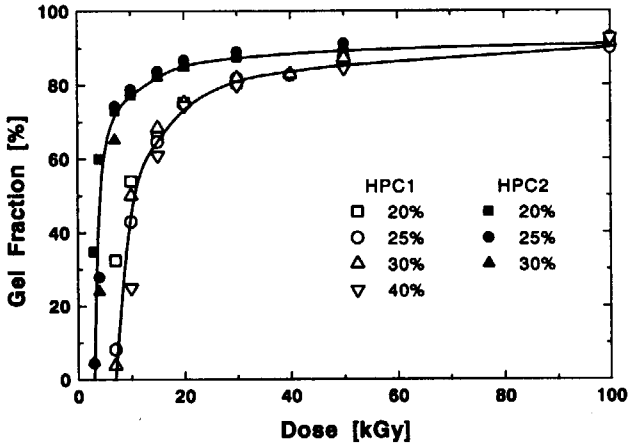


Figure 1. Effect of the initial molecular weight of HPC and the concentration of polymer in aqueous solution during irradiation on the gel fraction. Irradiation performed by electron beam in air-free atmosphere.

Results obtained for the HPC1 irradiated in the absence of oxygen by gamma rays with the dose rate of 10 kGy/h are presented in Figure 2. Gel fraction of HPC1 approaches the maximum over 40 kGy, and reaches 60% (concentration of 30 - 40%). Polymer processed at lower concentrations, 20% degrades with subsequent irradiation. The gel fraction drops, apparently scission prevails over intermolecular crosslinking. Gel fraction of polymers irradiated at higher concentrations does not decrease, up to 200 kGy; obviously intermolecular crosslinking overweighs cleavage reaction.

Comparing the obtained results of irradiation by EB and γ-rays, one can notice that the significant impact on the gelation have the dose rate and the polymer concentration during irradiation. Energy of 10 kGy is delivered to the sample within 1 hour from the ^{60}Co source, whereas within a few seconds for EB irradiation. Thus, the higher concentration of radicals

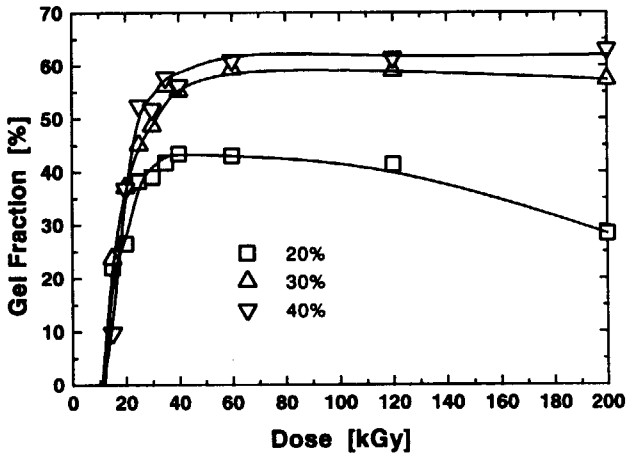


Figure 2. Gel fraction of HPC1 hydrogel formed by irradiation of the polymer by γ rays in aqueous solutions in air-free atmosphere.

is conducive to hydrogel formation. For the creation of a crosslinking bond, the presence of two radicals on adjacent chains is required. Their subsequent recombination results in the formation of a chemical bond between two polymeric chains. The best conditions for crosslinking of HPC leading to gelation, according to our results, seems to be moderate and high concentration of the polymer in water solution, namely 30 - 40% and about 25 - 30% for HPC1 and HPC2, respectively. Water acts like a plasticizer; it allows the freedom of mobility of the polymer and is not a barrier to the coupling of macroradicals.

3.2 Swelling of HPC hydrogel

The main feature of hydrogel is its ability to absorb and hold in its structure an amount of solvent. Swelling is defined as the mass of solvent absorbed per 1 gram of dried gel and is dependent on the hydrophilicity of the polymer, the density of intermolecular links, etc.

The maximum water uptake of a hydrogel produced by radiation crosslinking from neat HPC is easy to control and depends on a delivered dose. Figure 3 shows the swelling of HPC hydrogel, samples made in aqueous solution by EB, versus absorbed dose. Obtained shape of swelling curves is common for gels formed by ionizing radiation [18]. Swelling is the highest at the beginning stages of irradiation, just after the dose oversteps gelation point. To form a gel, statistically one crosslink per chain is necessary to form an insoluble macroscopic gel [19]. Then, the network is weak and susceptible to break but, because of a relatively low number of intermolecular bonds, more water molecules can easily penetrate and retain inside the crosslinked matrix of a polysaccharide. With subsequent increase of the density of crosslinks, due to the further irradiation, the absorption ability of the gel decreases and voids accessible for water shrink. The hydrogel structure becomes more firmly connected and rigid. The swelling drops sharply with dose and finally, over a dose of 30 kGy, remains at the level of ca. 10 - 20.

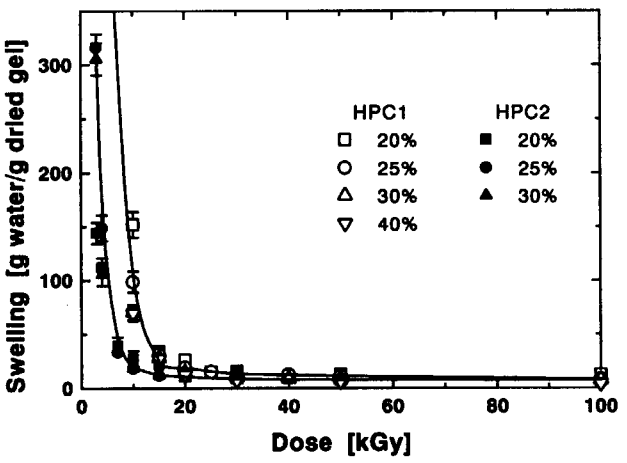


Figure 3. Swelling of HPC hydrogels in deionised water at 25°C. Gels were formed by EB irradiation of aqueous solutions of the polymer in air-free atmosphere.

HPC is an example of a thermally responsive polymer. At temperatures between 40 - 45°C diluted aqueous solutions of the polymer become turbid, then the macromolecules coagulate and precipitate. The degree of substitution of cellulose repeating units, the solution components and the concentration of polymer itself can influence this cloud point [20, 21]. The temperature-sensitive swelling of neat HPC hydrogel is shown in Figure 4. Hydrogels display similar tendency in their thermo-responsive character regardless of the type of water based solvent used. The continuous decrease in gel capacity was observed due to an increase of the temperature, with its intensification above 40°C. Samples kept in deionised water diminish their masses continuously, while those kept in buffer solutions shrinks faster. It is most pronounced for samples bathed in solution at pH 11.5 - with temperature above 40°C the samples collapse. Probably, the components of the buffer – salts interact by weak secondary forces with hydroxyl groups of the polymer. It is believed that the effect of salting out occurs. The solubility of the polymer is reduced by an addition of another solute. The ionic salt removes the water associated with the polymer and takes it place. This is in accordance with the fact that the presence of salts lowers the cloud point of aqueous solutions of HPC and other ethers of cellulose [13].

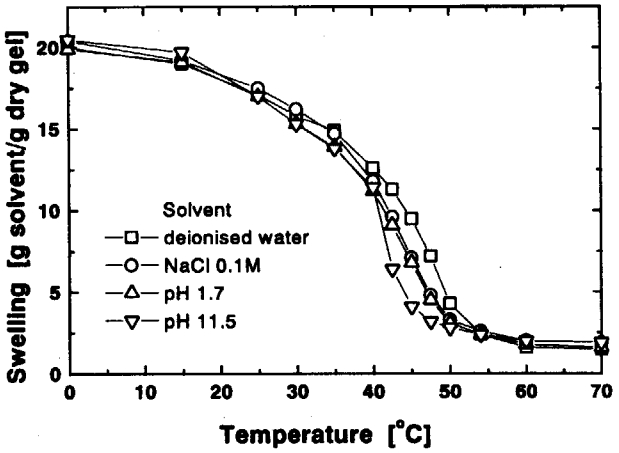


Figure 4. Temperature-sensitive equilibrium swelling of HPC2 gel samples in various media. Gels were produced by EB irradiation at 80 kGy of the film of HPC2 in 20% aqueous solutions. Sol fraction was extracted and the material was dried before the experiment.

The ratio of swollen to collapsed gel is about 13 between temperatures 0 and 60°C. A change in the temperature of bathing solution has a significant effect on the relative segment-solvent and segment-segment interactions of HPC. Thus, with increasing temperature, a rise of mutual attraction of chain parts occurs. Hydrogel shrinks abruptly, changing its transparency to translucent white. The transition of hydrogels is fully reversible.

3.3 Mechanical properties of gel

The effect of irradiation dose on mechanical properties of HPC1 and HPC2 irradiated by EB in 20% concentration was measured. Tensile strength and elongation at break of hydrogels samples in relaxed and dried state are presented in Figure 5.

The tensile strength of both fractions of HPC increases with an absorbed dose. This effect is due to the increasing crosslink density. For samples in relaxed state it alters from 0.12 to 0.28 MPa at doses of 10 and 50 kGy, respectively for HPC2 and from 0.074 to 0.106 MPa at the corresponding doses for HPC1. The faster increase of mechanical strength in the case of higher molecular-weight grade HPC is probably due to the preservation of molecular entanglements by fixing them by junction points. In the uncrosslinked state or at low dose the tangles of the chains can be easily disentangled by application of stress. The number of repeating units in an average chain of HPC2 is nearly twice than that in HPC1 chain. Thus, entanglements occur more frequently and can be easily fixed by crosslinking points in the case of longer chains. This seems to be the main reason for faster increase of tensile strength of HPC2 samples. Tensile strength recorded in dry state for HPC2 after irradiation at 40 kGy almost double the initial value. It increased from 32 to 59 MPa, while for HPC1 this increased from 28.6 (unirradiated film [22]) to 39.2 MPa at 50 kGy. Elongation at break of HPC of both molecular-weight grades decreases with absorbed dose. The samples after irradiation and drying still maintain their initial elasticity, without evidence of brittleness. According to these results, irradiated material even in dried state has favorable properties, which allow for applications not only as a hydrogel but also as covering material of dried film.

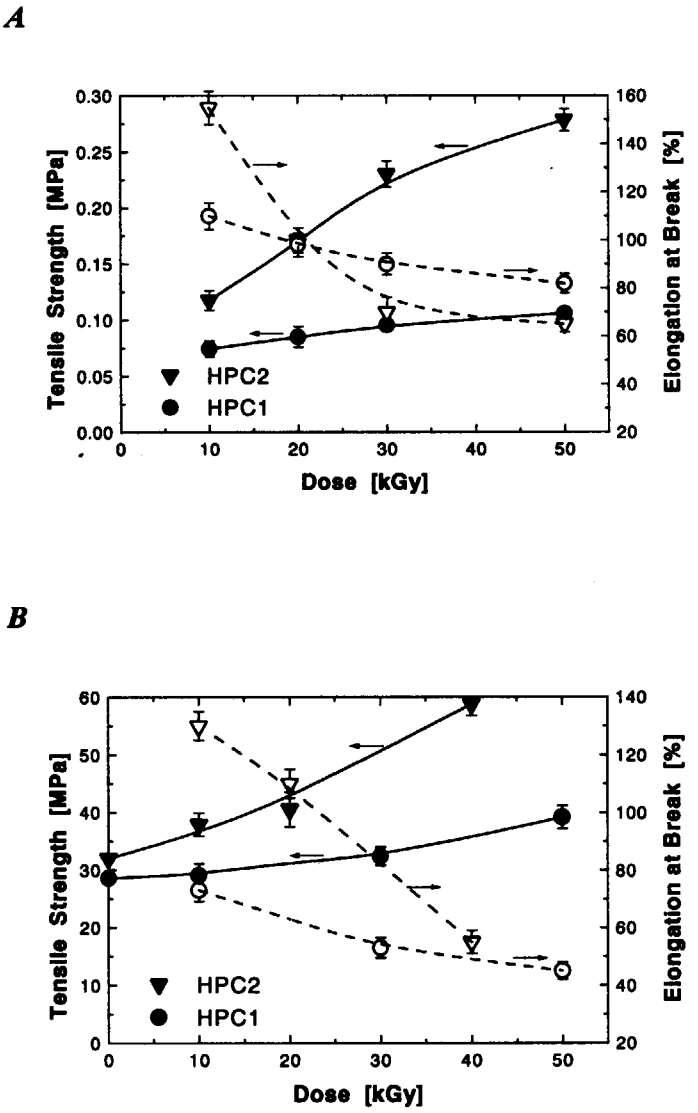


Figure 5. Tensile strength and elongation at break of hydrogel film in relaxed conditions, A) and in dried state, B). Material was prepared from polymer in 20% aqueous solutions by EB irradiation in air-free atmosphere.

3.4 Enzymatic degradation

The crosslinked HPC hydrogel (gel fraction only, after washing out sol part) undergo gradual degradation by cellulase enzyme as depicted in Figure 6. Samples were irradiated by EB at the concentration of 20% in air-free atmosphere. The rate of degradation is faster for the gel prepared by irradiation at lower dose, 20 kGy than that crosslinked by 50 kGy. The weight of the samples after 72 hours of incubation decreases to 96 and 92% of the initial values for samples irradiated at 50 and 20 kGy, respectively. It is in accordance with an increase in crosslinking density due to increasing dose. The moderate rate of degradation is the consequence of a high degree of substitution of anhydroglucose rings and the size of hydroxypropyl group [23-25]. HPC used in our investigations is characterized by high degree of substitution, which facilitates the crosslinking but, on the contrary, hampers the biodegradation.

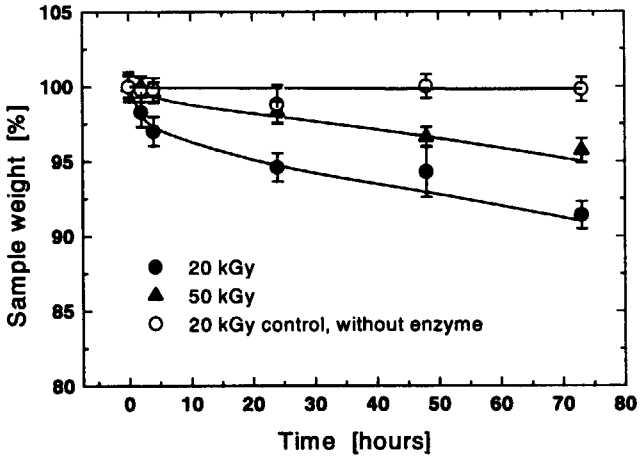


Figure 6. Biodegradation of crosslinked HPC2 by the cellulase enzyme C-0901 from P. funiculosum in an acetic acid – NaOH buffer (pH 5.0). Hydrogels were prepared from 20% HPC2 aqueous solution by EB irradiation under air-free conditions.

Conclusion

A novel type of hydrogel has been formed from HPC. Polymer chains were chemically crosslinked in aqueous solutions at medium concentrations by the ionizing radiation without any bifunctional crosslinking compounds. Hydrogels of HPC swelled and collapsed due to temperature alternation, maintaining their shape of circular films, thereby entering the class of stimuli-response hydrogel. The crosslinked material demonstrated superior mechanical properties and even after crosslinking maintained its ability to biodegradation.

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Session 2

Radiation Processing of Silk Protein

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11 Change in Silk Protein by Radiation

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Abstract

Silk fibroin fiber irradiated with an accelerated electron beam in the dose range of 250 - 1,000 kGy was pulverized by using a ball mill. As irradiation dose increased, the conversion efficiency from fiber to powder increased, which reached 94% at 1,000 kGy. Silk fibroin powder obtained by this method dissolved 57% into water of ambient temperature. It is a very interesting phenomenon that silk fibroin obtains solubility without chemical treatment. In order to study mechanism of solubilization of silk fibroin powder, amino acid component of soluble part of silk powder was performed. The more irradiation dose up, the more recovery fraction of glycine or alanine decreased, which is, however, reached the minimum about 50%. To consider this result with crystal structure of silk fibroin, it is suggested that irradiation on silk fibroin fiber selectively degrades glycine and alanine in amorphous region, which makes it possible to pulverize and to dissolve for silk fibroin powder. Molecular weight of soluble part was also measured, but it had no serious concern with irradiation dose. Particle size distribution of silk fibroin powder was measured in order to study reduction of irradiation dose needed for pulverization. This measurement exhibited the possibility that lengthening of pulverization time reduces of irradiation dose. In addition, structure of particle was inferred from result of this measurement.

Key words : Silk Fibroin Powder, Radiation, High Solubility, Amino Acid Analysis, Particle Size Distribution Measurement

1. Introduction

Recently silk fibroin is considered natural protein with interesting characters, and then applications to new fields, particularly medical or cosmetic materials, are in progress. It is, however, often necessary to transform silk fiber into other forms on practical application. For example, film and block are studied for artificial skin and contact lens respectively [1]. Powder has been already used as additions for food or cosmetics or as finishing chemicals for textile.

These facts show that silk fibroin powder is a useful material from the point of width and progress in practical use. But silk fibroin fiber has mechanical strength because of its crystal structure, so it is difficult to pulverize silk fibroin with only physical method. Then, it is necessary for preparing silk fibroin powder to treat before pulverization, for instance, to weaken by alkali treatment [2], to dissolve in concentrated neutral salt solution, such as $CaCl_2$ or LiBr, followed by desalting and desiccating [3]. These are, however, wet methods using water system, so the drainage and many processes are large burdens.

We studied the radiation method, as a new dry one, in order to pulverize silk fibroin [4]. We have reported pulverization of silk fibroin by the radiation method and marvelous solubility of the powder. In this report, molecular weight and particle size distribution of silk fibroin powder will be reported, in addition to the last report.

2. Experimental

2.1 Materials

Silk fibroin fiber is obtained from raw silk of *Bombyx mori*. The raw silk has the double-layer structure, the inside is fibroin and the outside is sericin. In order to eliminate sericin, raw silk was degummed. 200 Grams of raw silk were soaked in water at 40(±5)°C for 1 hour. Lightly wrung raw silk fibers were soaked in 5 l of water containing 10 cm^3 of enzyme solution (Alkalase2.5L, NovoNordisc, Denmark), 15 g of $NaHCO_3$ (99.6%, Kanto Chemical Co., Inc., Japan) and 5 g of nonionic surfactant (Shunel SB#14, Morin Chemical Industries Co., Ltd., Japan) at 60(±5)°C for 2 hours. Then degummed silk fibroin fibers were rinsed with running water, and were dried. The degumming loss on this scheme was 23.2%.

2.2 Irradiation

About 2 g of silk fibroin fiber were packed into a plastic bag with O_2 gas, which were irradiation sample. Irradiation was carried out at room temperature with 1 MeV electron beam. Sample were transferred under scanned electron beam of 1 mA at a speed of 1.17 m/min to yield a dose of 50 kGy/pass. Total doses ranged from 250 to 1,000 kGy.

2.3 Pulverization

Irradiated silk fibroin fiber was cut into about 1 cm in length. About 0.8 g of cut fiber were pulverized for defined time using a ball mill (Frisch Pulverisette type 6, Frisch Japan, Japan). After pulverizing, silk fibroin was filtered with 90 μm sieve. In order to compare degree of pulverization, the conversion fraction C (%) from fiber to powder was defined as below.

$$C = 100\ (1 - R / A)$$

R is weight of residue on sieve, and A is one of silk fibroin fiber before pulverization.

2.4 Solubility measurement

Soluble part was extracted from silk fibroin powder or fiber with distilled water and then supernatant was transferred to another vessel. The extraction process was repeated again and the second supernatant was added to the first one. Water was evaporated from the extract solution and then soluble part was obtained as residue.

2.5 Molecular weight measurement

Soluble part of silk fibroin powder in each dose was extracted and then dissolved into water at 0.2 mg/cm^3 in concentration. Gel permeation chromatography (GPC) was used for measurement. Molecular weight markers (range 2512-16949, Pharmacia Biotech Inc. NJ) were used for standard.

2.6 Amino acid analysis

Soluble part of silk fibroin powder in each dose was extracted from 10 mg of powder with 1 x 2 cm^3 of distilled water. After elimination of water, extracts were treated with 6 mol/l of HCl at 110 ℃ for 24 hours, which is usual preparation. This was analyzed by high performance liquid chromatography (HPLC).

2.7 Particle size distribution

Measurement of particle size distribution was performed by laser diffraction scattering

method using SK laser micronsizer LMS-24 (Seishin Enterprise Co., Ltd. Japan). Solvent of total powder (including both soluble and insoluble part) was hexane, and that of insoluble part was water.

3. Results and Discussion

3.1 Effect of irradiation on pulverization of silk fibroin fiber and solubility of obtained powder

Fig.1 shows the influence of irradiation on the conversion fraction C. Unirradiated silk fibroin fiber was not pulverized at all. But the more irradiated silk fibroin fiber is, the larger the conversion fraction C is. Finally the conversion fraction of silk fibroin fiber irradiated 1,000 kGy reached to 94%.

Fig.2 shows the electron microscope photograph of silk fibroin powder obtained from fiber irradiated 1,000 kGy. It shows fine particles under 10 μm in diameter and its aggregates. There is a fragment of silk fibroin fiber at the upper side. Diameter of silk fibroin fiber is about 15 μm, therefore it is apparent that silk fibroin fiber is severed not only the direction of length but also the direction of width.

Fig.3 shows the solubility of silk fibroin fiber and powder into water. Properly unirradiated silk fibroin fiber is not soluble in water. Irradiated silk fibroin fiber, however, obtained slight solubility, 8% of silk fibroin fiber dissolved in case of 1,000 kGy dose. Pulverization made silk fibroin more soluble, 57% of powder dissolved into water in the same dose. It is a very interesting phenomenon that silk fibroin obtains solubility without chemical treatment.

3.2 The mechanism of solubilization of silk fibroin powder

To study solubility of silk fibroin powder, molecular weight measurement and amino acid

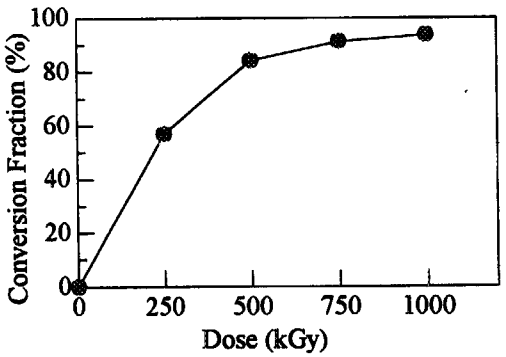

Fig.1 The Influence of Irradiation on the Conversion Fraction.

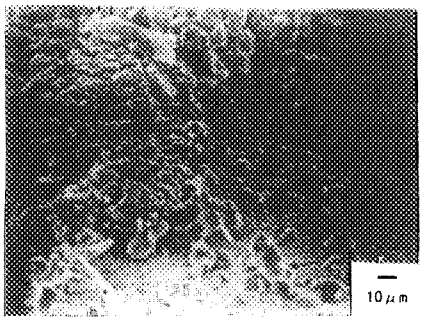

Fig.2 Photograph of silk poder (1000 kGy)

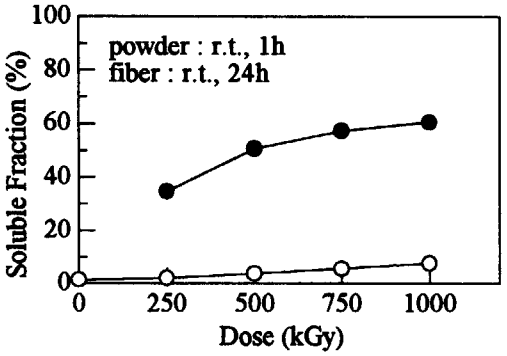

Fig.3 Solubility of Irradiated Silk Fibroin.

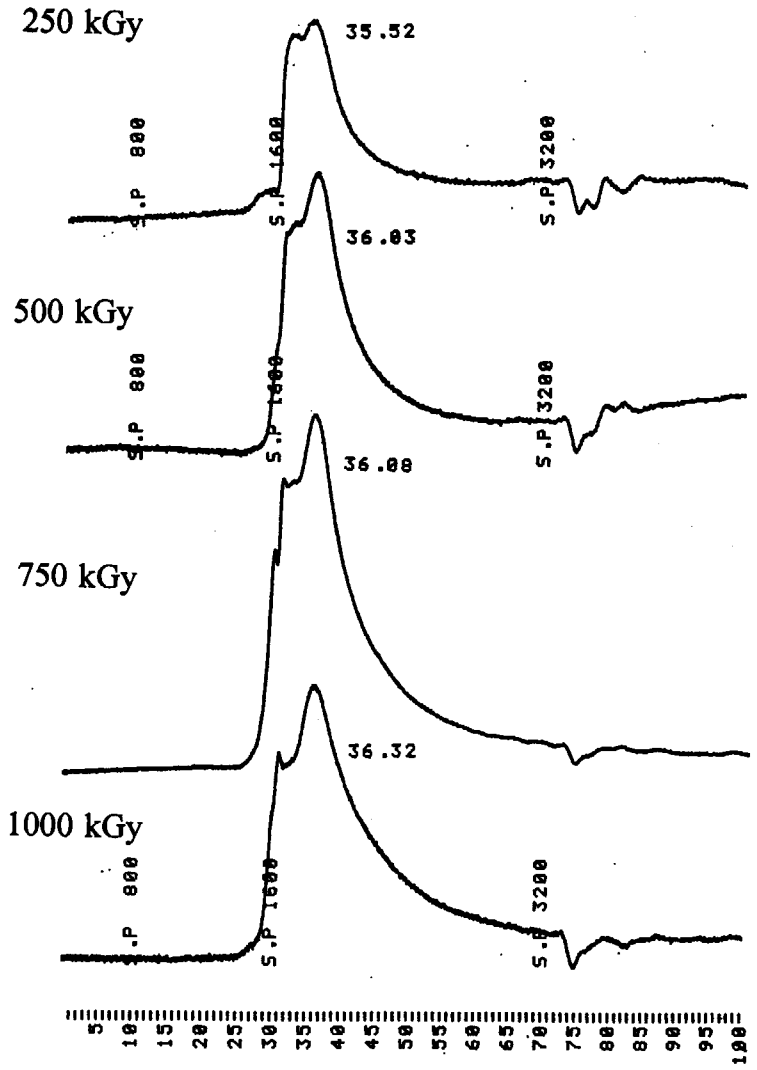

Fig.4 Gel Chromatography
of Soluble Part of Silk Fibroin Powder.

Tab.1 Recovery Fraction of Amino Acid from Soluble Powder.

literatural amino acid construction (Lc(%) [5])	symbol	head	unit	250 kGy soluble	500 kGy soluble	750 kGy soluble	1000 kGy soluble
	a	ratio of soluble part	%	34.56	48.64	53.24	56.64
Gly 44.46	b1	Theoretical Weight	μ mol	21.10	29.70	32.51	34.58
	b2	Detection Weight	μ mol	13.30	14.86	15.36	15.60
	b3	Recovery Fraction	%	63.01	50.04	47.26	45.11
Ala 30.24	c1	Theoretical Weight	μ mol	14.35	20.20	22.11	23.52
	c2	Detection Weight	μ mol	12.93	11.00	11.60	11.73
	c3	Recovery Fraction	%	90.07	54.48	52.46	49.86
Ser 11.91	d1	Theoretical Weight	μ mol	5.65	7.96	8.71	9.26
	d2	Detection Weight	μ mol	5.16	7.32	7.93	7.95
	d3	Recovery Fraction	%	91.36	92.01	91.11	85.86
Tyr 4.88	e1	Theoretical Weight	μ mol	2.32	3.26	3.57	3.80
	e2	Detection Weight	μ mol	2.05	2.76	3.05	3.05
	e3	Recovery Fraction	%	88.38	84.81	85.39	80.38
Var 2.09	f1	Theoretical Weight	μ mol	0.99	1.40	1.53	1.63
	f2	Detection Weight	μ mol	0.88	1.26	1.45	1.51
	f3	Recovery Fraction	%	88.62	90.23	94.56	92.77
Asp 1.38	g1	Theoretical Weight	μ mol	0.65	0.92	1.01	1.07
	g2	Detection Weight	μ mol	0.59	0.88	1.00	0.97
	g3	Recovery Fraction	%	90.29	95.17	98.68	90.82
Thr 0.98	h1	Theoretical Weight	μ mol	0.47	0.65	0.72	0.76
	h2	Detection Weight	μ mol	0.37	0.53	0.59	0.58
	h3	Recovery Fraction	%	78.51	80.39	82.58	76.70
Glu 0.93	i1	Theoretical Weight	μ mol	0.44	0.62	0.68	0.72
	i2	Detection Weight	μ mol	0.43	0.63	0.73	0.74
	i3	Recovery Fraction	%	97.42	102.03	107.75	101.92

average molecular weight of amino acid residue : 75 (calculated on reference [5].)
sample weight of amino acid analysis : 10.3 mg

Theoretical Weight = (sample weight (mg) × soluble part fractuon (w%)
/ average molecular weight of amino acid residue)
× literatural amino acid construction
= (10.3 × (a/100) / 75) × Lc/100
= 1.03aLc/75000 (mmol)
= 1.03aLc/75 (μ mol)

Recovery Fraction = 100 (detection molar (μ mol)) / (theoretical molar (μ mol))
= 100(n2)/(1.03aLc/75)
= 7500n2/1.03aLc (%) (n2 means b2, c2, ..., i2)

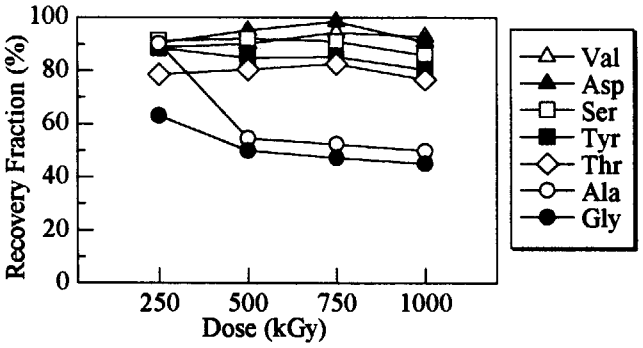

Fig.5 Recovery of Amino Acid from Soluble powder. These plots are founded on Tab.1.

analysis of soluble part of powder were performed.

The measurement of molecular weight was performed using gel permeation chromatography (GPC). Results of GPC analysis showed that molecular weight of soluble part of silk powder were invariable around 18,500 and 16,500 in spite of dose range from 250 to 1,000 kGy (Fig.4). By this fact, it is concluded that molecular weight has no serious concern with solubility. The peak of 18,500, however, became relatively smaller and the peak 16,500 shifted to more low-molecular side with irradiation dose up. These facts say that the more irradiation dose up, the smaller the molecular weight became even though slightly.

Amino acid component of soluble part of silk fibroin powder was analyzed. Tab.1 and Fig.5 show the recovery fraction of each amino acid. The recovery fractions were calculated on results of amino acid analysis and known amino acid contents of silk fibroin in literature [5]. As irradiation dose up, the recovery fraction of glycine decreased, but it reached a minimum, about 45%. The recovery fraction of alanine is similar to that of glycine, and a minimum was about 50%. Other amino acids were recovered 80% even at the minimum.

By the way, silk fibroin fiber consists of crystal region and amorphous region, which closely relates with amino acid composition. The crystal region consists of the distinctive primary structure, repeats of 6 amino acids, Gly-Ala-Gly-Ala-Gly-Ser. In amorphous region, amino acid sequence is not so definitive and then many kinds of amino acids are contained, different from in crystal region. The ratio of glycine and alanine in crystal region is 45% and 44% respectively (Tab.2). These ratios are very close to the those of recovery fractions ofglycine and alanine. And it is known that degradation occurs on the amorphous region in many cases. Synthetic considering these things suggests following things. (1) Irradiation of silk

Tab.2 The Ratio of Glycine and Alanine
in Crystal Region and Amorphous Region

amino acid	all fibroin	crystal region	amorphous region	recovered fraction
glycine	44.5 (100)	20.0 (45)	24.5 (55)	45
alanine	30.2 (100)	13.3 (44)	16.9 (56)	50

Upper lines show the rates for total amino acid.
Lower lines show the rates in each amino acid.

fibroin selectively degrades glycine and alanine in amorphous region. (2) Degradation of glycine and alanine weakens mechanical strength of silk fibroin and makes it possible to pulverize silk fibroin fiber. (3) The degradation by irradiation and the pulverization by physical method changed silk fibroin fiber into small fragments which are soluble in water.

3.3 Particle size distribution

In order to study reduction of irradiation dose by lengthening of pulverization time, particle size distribution is measured. Fig.6 makes it clear that particle size distribution of 500 kGy 2 hours and 3 hours are very similar to that of 1,000 kGy 1 hour. From this result, the possibility that lengthening of pulverization time reduces irradiation dose is exhibit.

Comparison of distribution shows that particle size of insoluble part of silk fibroin powderis as half as that of total powder ("Total powder" has not been extracted, so include both soluble

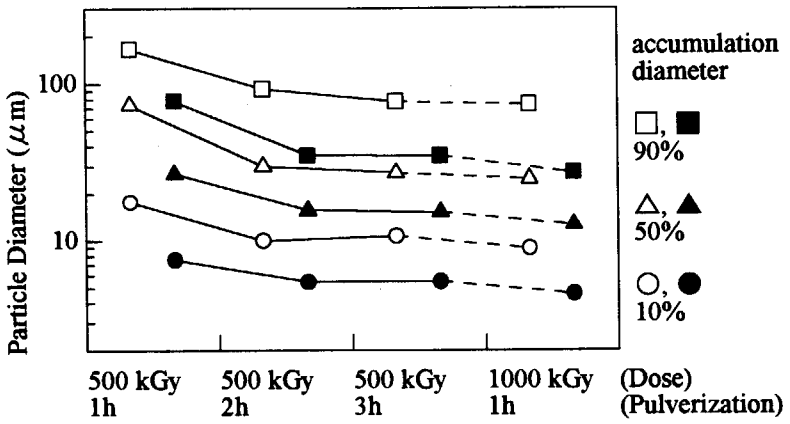

Fig.6 Particle Size Distribution of Total Silk Powder and Insoluble Powder
kinds of particle
□, △, ○ : total silk powder in hexane
■, ▲, ● : only insoluble powder in water

part and insoluble part). An inferable cause of the difference of particle size is described below. Measurement of total powder was performed in hexane solvent, so aggregates, which is formed insoluble particles attached by soluble part, do not separate because of strong affinity between insoluble particle and soluble part. Insoluble part, which had obtained by dispersing of silk fibroin powder in water, does not include soluble part. So, aggregate has been in pieces and measured particle size is smaller than that of soluble part.

4. Conclusions

(1) Irradiation of an electron beam to silk fibroin fiber made it possible to obtain high-solubility silk powder.

(2) Amino acid analysis made it clear that glycine and alanine of silk fibroin powder were remarkably degraded. From this fact, an inferable mechanism of degradation of silk fibroin was presented.

(3) The measurement of particle size distribution showed the possibility of reduction of irradiation dose in order to obtain silk fibroin powder. And from the result of this measurement, aggregate structure of silk fibroin powder was inferred.

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12 Status of Silk Industry in Thailand

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Abstract

At the present, there are 193,500 sericulture households in Thailand while the area of mulberry fields is 218,900 rai (6.25 rai = 1 hectare). Approximately of 80% of the total sericulturists are in the northeast, the remaining 20 % in the north, central area, west area and in the far south. In total, they can produce raw silk of about 1,357 tons a year, which can be divided into 2 types of raw silk as following:

Weft silk. There are 177,947 small-scale farmers producing yellow cocoons (Local polyvoltine silkworm race and polyvotine-bivoltine silkworm race) of approximately 900 tons of raw silk.

Warp Silk. There are 4,841 farmers producing white cocoons to be entirely reeled by reeling factories. Though this type of raw silk is a smallest group but they are able to produce a total of warp silk of 346 tons.

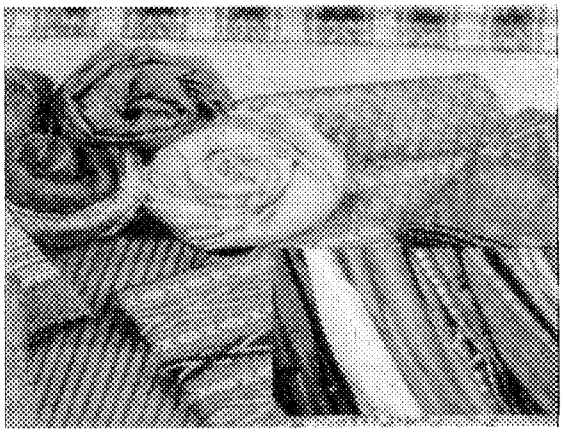
However, the production in the country can not reach the demand of weaving factories, so Thailand still has to import about 300 tons of raw silk. Therefore, it is planed in the 8th National Social and Economic Development Plan (1997–2001) that in 2001, the target raw silk production is expected 1,794 tons.

In the year 2000, there were 317 tons of silk waste caused by the processing of Thai Silk production. Mainly 178 ton of silk waste caused by the small-scale farmers who continuously to lay the silkworms egg by themselves, while another 26 ton caused by 8 reeling factories and 13 ton caused by egg production centers belonged to the government. Today, some of silk waste left from pulling and garnetting has been started to be exported. However, utilization of silk waste in Thailand should still be discussed.

Keywords: Thai silk, industry, silk waste, weft silk, warp silk, raw silk, sericulturist

Introduction

Thai silk has developed into one of the most important exporting products. It has gradually become very popular for its high quality textile fabrics owing to its attributed and unparalleled characteristics includind silky and luster properties, elasticity, good tenacity, draping quality and hygienic properties. The type of silk which is now widely acclaimed, is called Mud-mee silk. The technique for producing Mud-mee silk is tie-dyeing prior to weaving i.e. the silk threads will be wound on a frame according to the pattern designed by hearts of weavers who learns this local know-how fromtheir mothers. All production created by hand made under local know how of the farmers.



In Thailand, there are 193,500 sericultural households while the area of mulberry fields is 218,900 rai (6.25 rai = 1 hectare)(table 2). Over 80% of sericulture farms is located in the northeastern region about 14 to 18 N latitude and 100 to 106 E longitude with average elevation of 200-230 meters above sea level. The remaining of about 20% is in the northern, central and western area and far southern areas. Raw-silk production is approximately 1,200–1,350 tons per year. But, this amount of production cannot response the demand of weaving factories in the country yet. Thus, Thailand still needs to import raw silk about 300 tons/year.

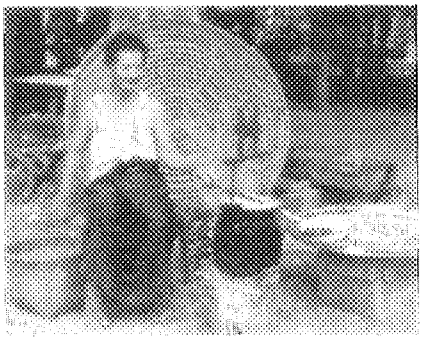
Raw silk production

Raw silk that the Thai sericulturists produce may be summarized in two following categories:

1. **Weft silk**:

Silk is considered as an agro-industrial activity because this occupation is popular practiced among small and traditional sericulturists in the Northestern part of Thailand as part time job after growing rice. It is found to be rather hard to gather and process information concerning this type of activity. There are about 188,656 households producing 1,011 ton of raw silk from yellow cocoons. Sericulturists who produce yellow weft silk may be classified into 2 groups as followed:

1.1 Traditional sericulture.



Sericulturists at present of 164,249 households, are small landholders with relative low income, no resources for investment and are not able to expand the silkworm varieties to be used are improved native varieties. Earning of these sericulturists on their type of farming is within the range of 4,000-6,000 Bath/year. Their cycle to raise rearing is around 4–6 crops, and each crop will be 10–15 trays only.

1.2 Improved sericulture

In Order to improve cultivars of mulberry, the 24,410 households have grown mulberry plants in larger area to obtain better quality of silkworm eggs multi x bivoltine hybrid from governmental agencies. Silkworm rearing can be raised to 4–6 crops/year. Farmers mostly sell yellow cocoon to reeling factories and some reel them for weaving. These sericulturists who conduct such farming earn income within the range of 8,000– 10,000 baht/year.

2. Warp silk:

Sericulturists of 6,792 households who are classed as industrial sericulturists and produce white cocoons. This group of sericulturists own larger area of land, and cultivate mulberry plants ranging from 5-20 rai which is assumed that least mulberry leaves should be avilable for rearing bivoltine hybrid of 6-10 crops/year and 4-6 sheets per crop. For this group, all cocoon are sold out to the reeling factories. In addition,

Reeling factory to produce bivoltine raw silk.

sericulturists under this group normally earn their income in the range of 72,000-210,000 baht/year. Though this group is small, but sericulturists under this group can produce white raw silk of 346 tons from bivoltine races in total. In the year 2000, Chul Thai Silk Company was the major producer who are able to produced 256 tons or 74% of total production while the rest or 26% of total production was produced by other 8 reeling factories.

Silk waste in Thailand

Thereare approximately 317 tons of silk waste in Thailand. They are obtained from three sources, mainly, 178 tons obtained from the small-scale farmers who continuously lay the silkworm egg for next rearing crop by themselves, another silk waste of 126 tons are obtained from 9 reeling factories and 13 tons from egg production centers of the government. (table 3)

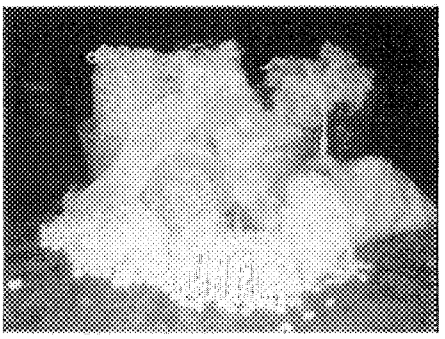
Utilization of silk waste

There are two kinds of silk waste as followed:

1) Cocoon shell waste and soiled cocoon, which are derived from the processing of silkworm egg production both in the center of government and small-scale farmer. Some of them like the farmers, house keepers, and students used them for handicraft and outer cocoons for invention, etc. Otherwise, small group of farmers tried to reel for weft silk, but then a little bit waste shell be used.

2) Waste derived from raw silk production process at reeling factories include

- silk waste in groping end
- silk yarn waste
- brushing waste of cocoon



Silk wastes from reeling factories

These raw silk wastes are normally sold to dupion silk factories in Thailand and some of them will be exported to other countries in Asia but the price is quite low. The value added in silk waste production should be discussed.

Raw Silk Production Plan

In the 8th National Social and Economic Development Plan (1997-2001). Thailand has determined the target for raw silk production as followed:

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- Dr. Suwanee Khunwong, The President of Chul Thai Silk Company, Thailand.
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- Pojana Weerasophon, Chief of Monitoring & Evaluation, Sericultural Research Institute, Department of Agriculture (DOA), Ministry of Agriculture and Cooperative, Thailand.
- Sericulture Extension Section, Department of Agriculture Extension (DOAE), Ministry of Agriculture and Cooperative, Thailand.

For data source supplied.

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For suggestion and helping to prepared of full paper.

Table 1 Target determined for raw silk production during 1997-2001.

Unit: Tons

Year	Hand reeled raw silk	Machine reeled Raw silk	Total
1997	1,249	410	1,659
1998	1,273	418	1,691
1999	1,298	426	1,724
2000	1,324	435	1,759
2001	1,350	444	1,794

Source: Ministry of Agriculture and Cooperatives.

Table 2 The sericulture household, mulberry field area and raw silk production in Thailand in 1997– 2000.

Year	Farmer households	Area of mulberry field (rai)*	Raw silk production		Total (tons)
			Hand reeled raw silk (tons)	Machine reeled raw silk (tons)	
1997	193,445	218,920	715	330	1,045
1998	177,947	208,485	733	340	1,073
1999	168,418	190,734	946	330	1,276
2000	193,500**	218,900	1,011	346	1,357

Source: Dept. of Agricultural Extension

Note: * 6.25 rai = 1 hectare

** Number of farmers producing cocoon = 6,792 households
Number of farmers producing raw silk = 186,708 households

Table 3 Waste of silk obtaine from various sources in Thailand in the year2000.

Sources		Silk waste (tons)
1. Small-scale farmers		178
2. Reeling factory (9 factories)		126
3. Egg production center		13
3.1 Under DOA center (17 stations/centers)	(5.8)	
3.2 Under DOAE center (9 centers)	(7.2)	
Total		316

Note:
DOA = Dept. of Agriculture
DOAE = Dept. of Agricultural Extension

Table 4 Cocoon production and silk waste obtained from 9 reeling factories in a year.

Reeling factories	Cocoon production (ton)	Silk Waste (ton)
1.Chul Thai Silk	1,564.00	88.00
2. Thai Silk Product	8.82	0.44
3. Udon Saenrung Thai Silk	6.90	0.34
4.Thip May Thai	175.00	8.75
5. Thai Silk Industry	381.00	15.91
6. Silk City	5.40	0.27
7. Kong Fuji	5.40	0.27
8. Thai Nan Silk	73.50	3.67
9. General Farm Supply	180.00	9.00
Total	2400.02	126.65

Table 5 Raw silk and silk waste imports and exports in 1998-2000

Unit of Quantity: tons,
Unit of Values: million bath,
1 USD= 47 Baht)

Items	1998		1999		2000	
	Quantity (ton)	Values (million baht)	Quantity (ton)	Values (million baht)	Quantity (ton)	Values (million baht)
Raw silk						
Import	51.27	51.59	75.27	57.98	138.56	125.25
Export	3.68	3.64	7.99	13.70	0.36	0.67
Silk thread						
Import	82.98	92.83	148.17	138.79	189.77	184.18
Export	4.54	1.65	9.70	2.28	44.45	4.30
Dupion						
Import	7.39	5.66	6.08	3.84	34.21	4.77
Export	229.51	229.92	353.99	324.45	456.49	415.07
Silk waste						
Import	1,916.99	471.63	2,154.97	352.97	1,691.99	234.18
Export	262.07	142.09	280.95	159.24	699.61	166.98

Sources: Dept. of custom and Dept. of Economic and Commercial

13 SOLUBILIZATION OF SILK PROTEIN BY RADIATION

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Abstract

Gamma irradiated silk fibroin at doses of 0, 5, 10, 20, 40, 60, 80, 100, 125, 250, 500, 750 and 1000 kGy were soaked in water for 1 hr. Silk fibroin solubilized percentage was investigated from lost weight of sample (dried at 105^0C), they were 0, 0, 0.7, 0, 0.11, 0.11, 0, 0.73, 0.77, 4.38, 8.32, 10.22 and 18.52 respectively. It showed that at the higher dose up to 250 kGy had direct effect to solubility, and increased with increasing dose. In addition, silk sericin dissolved 77.76, 82.22, 83.55, 84.31, 86.04, 86.67 and 87.37% after gamma irradiation at the doses of 0, 50, 100, 200, 500, 750 and 1000 kGy respectively.

It presents that radiation can cause silk protein, fibroin and sericin dissolve because of their degradation.

Key words : Silk protein, Gamma radiation, Solubilization.

1. Introduction

Silk is one of the important resources of Thailand which ranks the seventh largest silk producing country in the world with the production of 1,000 tons per year. More than 10% of silk are discarded as waste. Raw silk consists of two kinds of protein, produced by silkworm, namely, fibroin coated with 20-30% sericin (1). Over the centuries silk fibroin has been used as the highly valuable textile fibers due to its qualities of strength, elasticity, softness, absorbency and affinity for dyes. Recently much attention has been focused on the non textile use of silk proteins. They display various properties. Engel *et al.* (2) reported that sericin has a skin moisturizing and antiwrinkle action. Kato *et al.* (3) revealed that study provided the first evidence for antioxidant action of sericin by showing that sericin suppressed in vivo lipid oxidation. Furthermore. They found that sericin can inhibit tyrosinase activity.

At the present, several researchers investigated the properties of silk fibroin including water vaper permeability. It is one of possible biomedical materials because of its biocompatibility, therefore it can be used as a surgical suture (4). The application in membrane form is expanding. Asakura *et al.* (5) immobilized enzyme in fibroin membrane and developed an enzyme electrode equipped with the membrane (6). Hirostu *et al.* (7) reported that silk fibroin membrane can separate water from an ethanol-water mixture. It is also expected that silk fibroin membranes will be used widely in biomaterials such as contact lens , artifical skin, burn wound dressing, drug delivery system, artificial lungs and pharmacological agents because silk fibroin lowers the blood glucose level and enhances the alcohol metabolism of the liver (4).

Usually silk fibroin is water insoluble without chemical treatment but it has become possible to dissolve with silk powder technique (8). Some experts have classified sericin as alpha sericin and beta sericin. Alpha sericin is in the inner layer of cocoon and does not easily dissolve in water whereas beta sericin which is in the outer layer of cocoon does. In general, sericin considered as waste in texile industry is removed through a degumming process. However, sericin has cosmetic activity including a good retention ability of water on the cutaneous surface due to the presence of several hydroxyl groups. (4)

In this study presents that radiation can cause fibroin and sericin dissolve because of their degradation.

2. Methods

Two kinds of silk waste sample, silk waste fiber (obtained from Shinano Kenchi (Thailand) Co. Ltd.) and silk waste cocoons (obtained from Sericultural Research Institute) were used.

Silk waste fiber was prepared for fibroin and gamma irradiation was carried out at room temperature in air equilibrium atmosphere at the doses of 5, 10, 20, 40, 60, 80, 100, 250, 500, 750 and 1,000 kGy. Non-irradiated and irradiated fibroin were weighed and soaked in water for 1 hr. Centrifugal was done for 15 min at 13,000 rpm. The supernatant was left and wet silk fibroin was dried at 105^0C. Re-weigh of completely dried silk fibroin was done. Silk fibroin solubility was calculated from lost weight.

Silk waste cocoons were soaked in distilled water and autoclaved for 2 hours. Filtrate was obtained from gum solution filtration and then freeze-dried, silk sericin powder was already to irradiate. Gamma irradiation was applied to silk sericin powder at the doses of 50, 100, 200, 500, 750 and 1,000 kGy at room temperature in air equilibrium atmosphose. Non irradiated and irradiated sericin solubility were measured in the some methods as silk fibroin.

3. Results and Discussion

3.1 Fibroin solubility

It is known that fibroin is a kind of protein in silk fiber and not able to dissolve in water. From this experiment silk fibroin can be possible to dissolve by gamma radiation.

Effect of gamma irradiation on silk fibroin solubility was shown in Table 1 and Fig.1. It indicated that at the dose range of 5-125 kGy there was no difference in solubility. At the dose higher than 250 kGy the silk fibroin solubilized percentage were 4.38, 8.32 10.22 and 18.52 for 250, 500, 750 and 1,000 kGy respectively. It means radiation causes silk fibroin and increase with increasing dose.

3.2 Sericin Solubility

In general, some type of silk sericin can be able to dissolve in water especially beta sericin which is in the outer layer of cocoon. This experiment, sericin powder was obtained from cocoons not fiber, therefore its solubility is rather high even non-irradiated one. However radation can be effect on it and shown in Table 2 and Fig 2. There was a little difference in sericin solubility percentage between them, at a dose of 1000 kGy can increase 10% sericin solubility whereas 5% at a dose of 50 kGy approximately when compared to non-irradiated one.

4. Conclusion

Silk fibroin is water-insoluble without chemical treatment, but radiation has direct effect on solubility of fibroin as well as sericin. It shows that silk proteins can be easily use for many applications by radiation.

5. Acknowledgement

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Table 1 Comparison of effect of radiation on fibroin solubility

Doses/kGy	% Solubility
0	0
5	0
10	0.70
20	0
40	0.11
60	0.11
80	0
100	0.73
125	0.77
250	4.38
500	8.32
750	10.22
1000	18.52

Table 2 Radiation effect on sericin solubility

Doses/kGy	% Solubility
0	77.76
50	82.22
100	83.55
200	84.31
500	86.04
750	86.67
1000	87.37

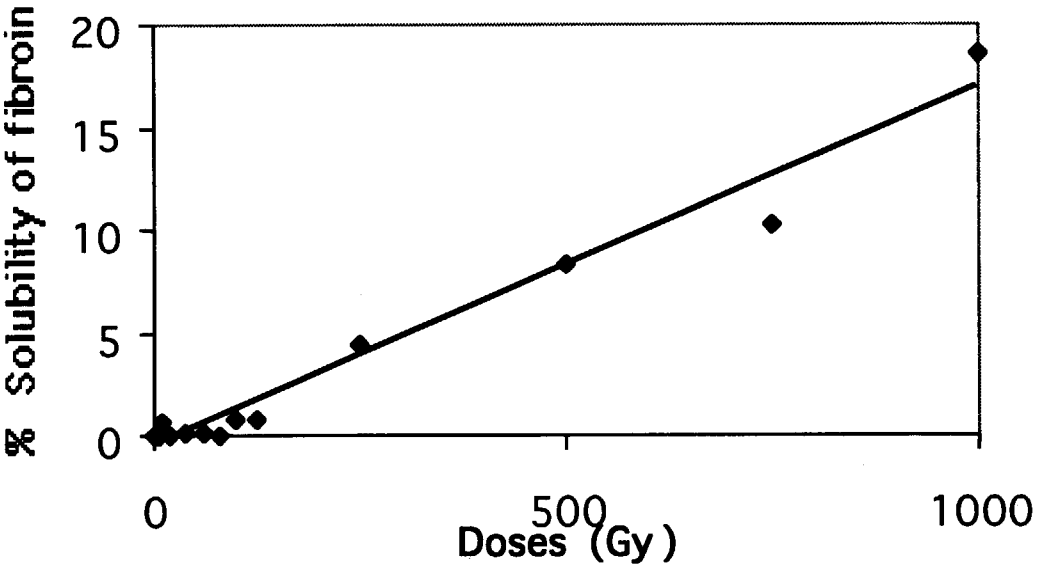

Fig. 1 Effect of gamma radiation on solubility of silk fibroin

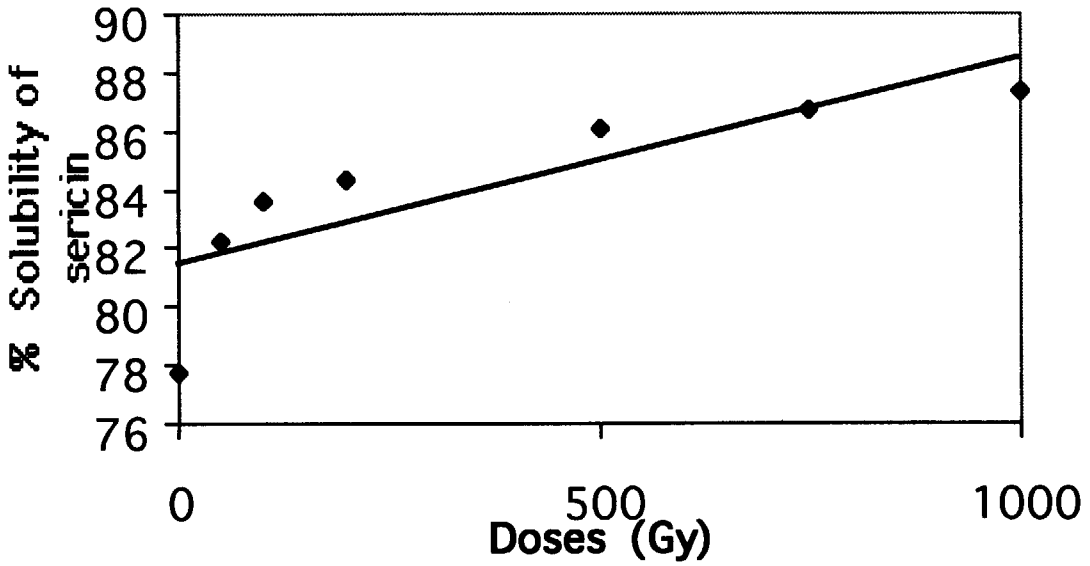

Fig. 2 Effect of gamma radiation on solubility of silk sericin

14 Minimum Inhibitory Concentration of Irradiated Silk Protein Powder for Bacterial Activity

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Abstract

The objective of this research was to study a minimum concentration level of irradiated silk protein powder, which inhibited bacterial activity. The concentration of 100 kGy irradiated silk protein powder (ISP) solution was ranged from 5 to 15 % in distilled water. The activities of three types of bacteria, *Escherichia coli* B/r, *Bacillus subtilis* M3-1 and *Staphylococcus aureus* K, were tested by using minimum inhibition concentration method (MIC). The results indicated that the minimum concentration level that inhibited growth of *E. coli* B/r and *S. aureus K* was 5 % ISP and all concentration levels studied could not inhibit the *Bacilus subtilis* M3-1 activity.

Keywords: Irradiated Silk Protein Powder, Bacterial Activity Inhibition, Minimum Inhibitory Concentration

1. Introduction

One of the waste products from silk industry is remainder silk fiber, which contains proteins. The silk proteins consist mainly of fibroin and sericin [1].

The silk protein have been investigated widely. Particularly, the project of "Cooperation Research Program on Radiation Processing of Silk Protein", has the priority to improve of silk protein processing by radiation, especially on radiation-degradation of silk protein and physiological properties of irradiated silk protein [2].

For the physiological properties, Bunnak and Chaisupakitsin [3] reported that a 1:3 volume ratio of 3 % silk proteins at 50 kGy gamma irradiation under N_2 atmosphere and 3 % polyvinyl alcohol (PVA) could inhibit growth of *E. coli*, *B. subtilis* and *S. aureus,* but could not inhibit that of *S. epidermidis*. It means the irradiated silk protein powder has a property of antibacterial activity. However, the silk protein preparation by using PVA, which is a chemical treatment, might not be suitable for medicine. For the degradation property, many methods, particularly, chemical treatment, are used to study silk protein solubility [4]. Ishida et al. [4], however, found that irradiated silk fibroin in a form of powder was soluble in water without being treated by chemical. They also reported that 57 % in weight of powder at 1,000 kGy dissolves in water and soluble part of silk protein dissolves in water at room temperature rapidly. Meanwhile, Sudatis and Pongpat [5] found that irradiated silk fibroin at 1,000 kGy could be also dissolved 18.52 % in water.

Because of the properties of the irradiated silk protein powder mentioned above, an application for medical products could be advantageous. Therefore, it was very interesting to know, at irradiation of 100 kGy how minimum concentration of the silk protein powder could inhibit growth of bacteria. This research was conducted in order to study the minimum concentration of the powder for inhibition bacterial activity.

2. Experiment

2.1 Material

Silk protein fiber was irradiated with ^{60}Co γ-ray up to 100 kGy with a does-rate of 10 $kGyhr^{-1}$. The fiber was soaked in 1N HCl for 72 hrs. Neutralization of the fiber with distilled water was done, and it was dried in hot-air oven at 50 °C. Then the fibers were pulverized by using Ball-Mill Cashing Machine (Fritsch Pulveriette type 6, Fritsch Germany) [4].

2.2 Preparation of irradiated silk protein powder solution

The 0.05, 0.10 and 0.15 g of powder were measured in order to make solution in 3 concentration levels, 5, 10 and 15 %, respectively. Then 1 ml distilled water was added to each of weighed of powder. All of the solutions were warmed in water-bath at 50 ° C for 30 min. Then they were centrifuged at 10,000 rpm for 30 min. After that their suspended particles were filtered with Millipore MILLEX-GS (pore size 0.22 μm, Millipore Corporation, Bedford, U.S.A.) to remove microbial contamination.

2.3 Preparation of bacteria and media

E. coli B/r, *B. subtilis* M3-1 and *S. aureus* K. were incubated at 37 °C for 24 hrs using Nutrient broth (Difico, Michigan, USA). The optical density of each kind of bacteria was measured at 660 nm by Spectrophotometer (Shimadzu, Japan). The acceptance optical density of each kind of bacteria, which contained $1.6x10^6$ cells in 1 ml, was ranged from 0.014 to 0.020, but not over than 0.030.

4 ml of the medium was transferred to a L-shaped tube and capped with a silicon plug. All of the tubes were sterilized.

2.4 Antibacterial activity testing

400 μl of filtered silk solution at each concentration level (2.2) was mixed with nutrient broth and 100 μl of each kind of bacteria (2.3) was added to the solution. Controls of the test were the medium plus each kind of bacteria without silk solution. All of the solutions were incubated at 37 ° C for 78 hrs and the optical density (OD) at 650 nm was measured by using a Bioscanner (Ohtake Seisakusho, Tokyo, Japan).

3. Result and discussion

3.1 Growth suppression of *E. coli* B/r

The growth suppression of *E. coli* B/r of different concentrations of irradiated silk powder (ISP) solution is showed in Fig. 1. The result indicated that the growth curves of *E. coli* of each concentration were slightly decreased. The optical density (OD) of all of concentrations with ISP was lower than that of the control (0 % ISP). Especially, the OD of 5 % concentration of ISP was the lowest. It meant that the 100 kGy irradiated silk

protein powder could inhibit the activity of *E. coli* B/r. and 5% of the powder is the minimal concentration.

3.2 Growth suppression of B. *subtilis* M3-1

Fig.2 showed the growth of *B. subtilis* M 3-1. The optical density of all concentration solution with ISP was higher than that of the control (0 % IPS). It is clear that the studied concentration levels of irradiated silk protein powder could not suppress the growth of such kind of bacterial.

3.3 Growth suppression of *S. aureus* K

The suppression of the growth of *S. aureus* K of the different concentration levels of ISP solution is showed in Fig. 3. It could be seen that at 5 % and 15 % of ISP, each OD was decreased. Meanwhile, at 10 % of ISP, the OD was also decreased until 32 hr. of incubation time, then it was slightly increased. Comparing the OD of the control with all of concentrations with ISP, it is found that 5 % of the ISP is the minimum concentration level, which could inhibit the bacterial growth at least for 32 hrs.

The results of this study indicated that the 5 % of the 100 kGy irradiated silk protein powder in distilled water was the minimum concentration level for inhibiting the activities of both *E. coli* B/r and *S. aureus* K, but no studied concentration levels could inhibit the growth of *B. subtilis* M3-1. These results are similar to Tungsupap [6], who reported that either 5 % of 500 kGy and that of 1,000 kGy irradiated silk protein powder was also the minimum concentrations for inhibitory activities of *E. coli*, *S. aureus* and *S. epidermidis*, but could not inhibit that of *B. subtilis*. However, the result of this study indicated that instead of higher doses, 500 or 1,000 kGy of the irradiation of silk protein powder and amount of 800 µl of those solution [6], the 100 kGy and that of 400 µl could also inhibit both the activities of *E. coli* B/r and *S. aureus* K.

Acknowledgement

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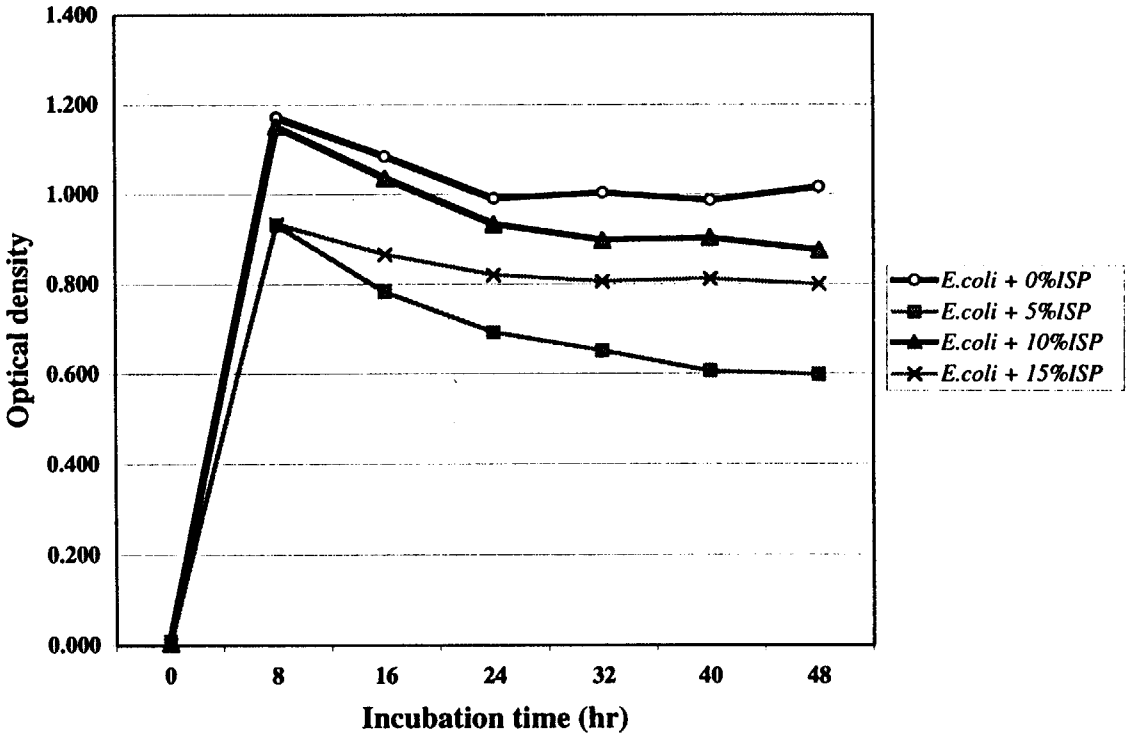

Fig. 1 Suppression of the growth of *E. coli* B/r in different concentrations of 100 kGy irradiated silk protein powder.

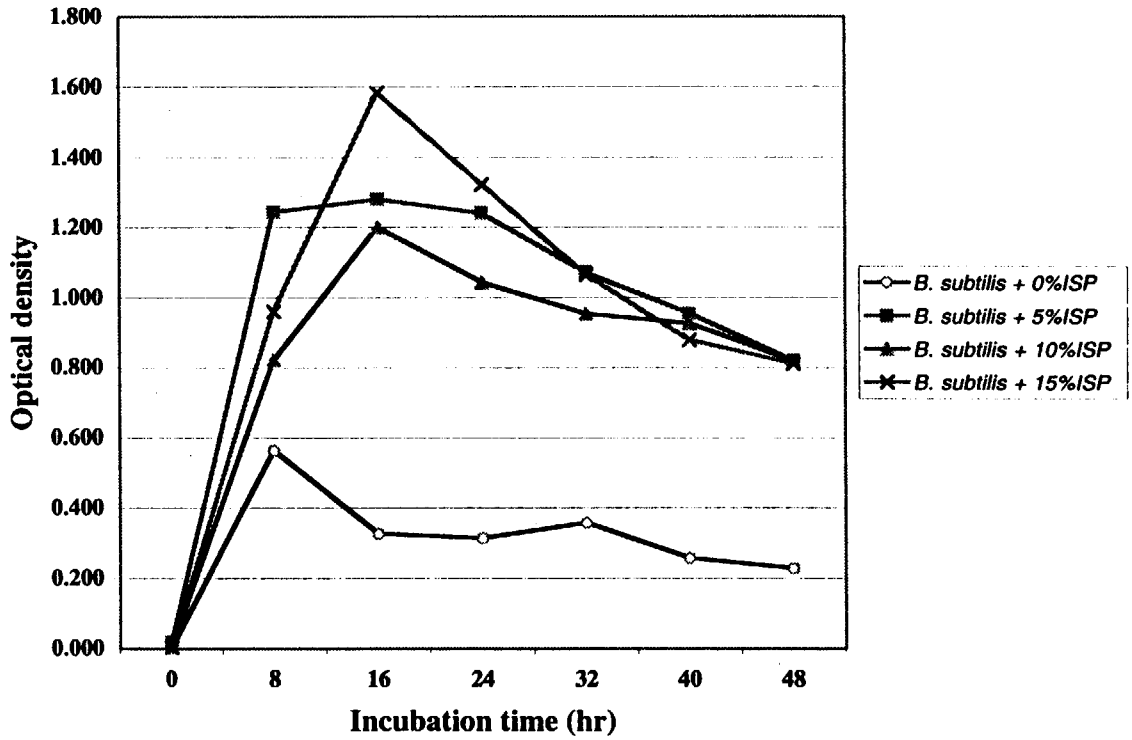

Fig. 2 Suppression of the growth of *B. subtilis* M3-1 in different concentrations of 100 kGy irradiated silk protein powder.

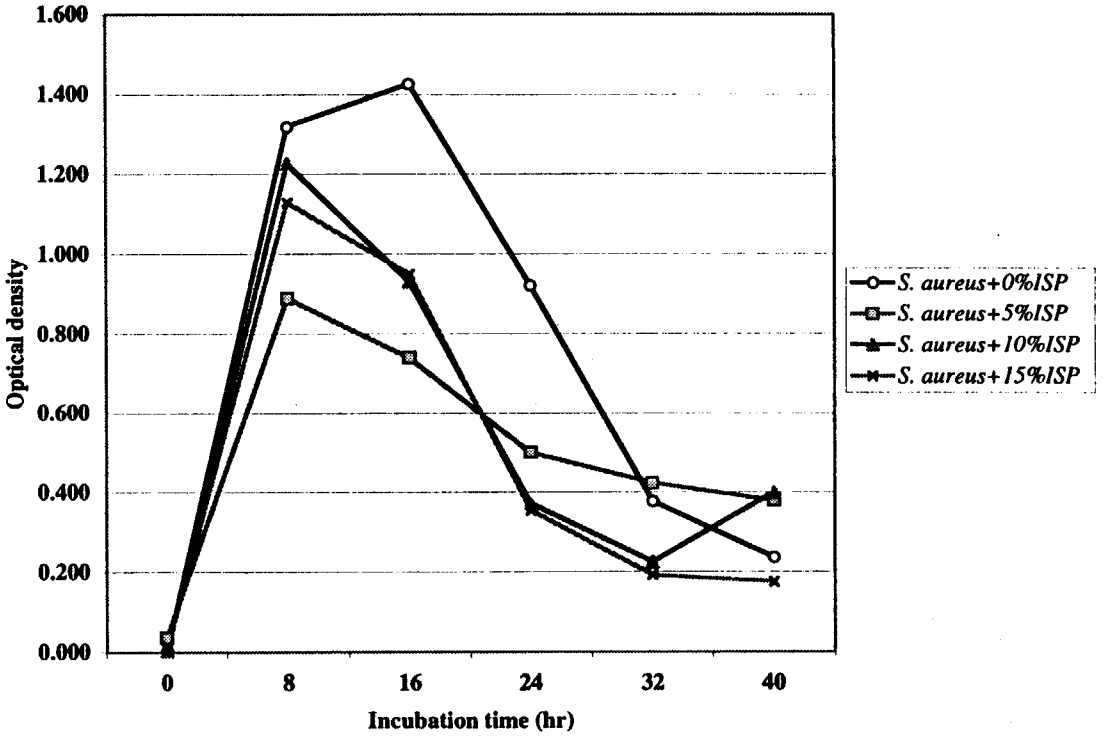

Fig. 3 Suppression of the growth of *S. aureus* K in different concentrations of 100 kGy irradiated silk protein powder.

15 Nutritional Value of Silk Powder from Irradiated Silk Waste

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Abstract

Silk waste from Thai reeling factory was developed into purified silk protein. Cleanliness of silk filaments by boiling in water was firstly needed. After air drying, they were irradiated with Gamma ray using Cobalt–60 at doses of 750 and 1,000 kGy, then the irradiated silk filaments were dissolved in calcium chloride–ethanol–water solution. The next steps would be dialysis followed by freezed dried process to obtain irradiated silk powder.

Two samples of 750 and 1,000 kGy irradiated silk powder were analyzed for nutritional components compared with non irradiated silk filaments. It was found that, the fat content in two irradiated samples was distinctive lower than the non irradiated one, however the protein content was nearly the same in three samples. In addition the moisture content in two irradiated samples was distinctive higher than the non irradiated one. These results show that irradiation technique is useful for development of valuable silk protein as biomaterial.

Keywords : Silk Powder, Radiation Degradation

1. Introduction

Silk fiber has been used as the valuable textile fiber in Thailand for a long time. Many tons of them are discarded as wastes each year. Since raw silk consists of silk fibroin and sericin [1] which have good physicochemical properties [2,3,4], various applications of silk fiber as non-clothing purpose are investigated. The products developed from silk protein are useful in many fields. Silk powder is used as moisturizer in cosmetics and as dietary supplement in foods [5]. In addition silk fibroin membrane which has oxygen-permeable property is used for medical application [2], and hydrogel containing silk protein is used for wound dressing [6]. In this study, we analyzed nutritional components of silk powder obtained from Thai silk wastes with the aim for utilization of silk protein as foods in order to recycle these silk wastes.

2. Materials

Silk fiber was obtained from a reeling factory in Thailand. All chemicals were analytical grade from chemical companies.

3. Method

3.1. Preparation of silk powder

Silk fiber was firstly cleansed by boiling in water at 95-98 °C for 30-45 minutes. Further cleaning 2-3 times in addition water were needed, then dried it in ambient temperature. Dried silk fiber was divided into 3 groups and irradiated them with gamma ray using Cobolt-60 at doses of 0, 750 and 1,000 kGy. Afterwards two groups of the irradiated silk fiber were dissolved in calcium chloride – ethanol – water solution, in the mole ratio of 1:2:8, at 95-98 °C . Completely dissolving time was about 4-5 minutes. Silk fibroin solution was then dialyzed by seamless cellulose tube against deionized water to remove calcium chloride. Finally, silk powder was obtained from silk fibroin solution by freezed dried process.

3.2 Analysis of nutritional components of silk powder

3.2.1 Nitrogen

Method : Kjeldahl Method[7].

Sample is digested in concentrated sulfuric acid and potassium sulfate, organic nitrogen in food is converted to ammonium sulfate. After adding excess sodium hydroxide solution and heating the solution, ammonia is liberated and can be trapped in a boric acid solution. The concentration of total nitrogen content is determined by titration with standard hydrochloric acid solution.

3.2.2 Fat (Ether extract)

Method : Acid hydrolysis method[8].

The sample is hydrolyzed by concentrated hydrochloric acid at 70-80 °C . As the protein dissolved in the acid, the fat will separate and can be extract by diethyl and petroleum ether. The solvent is then removed by evaporation and the residue of fat is dried and weighed.

3.2.3 Iron , Calcium

Method : Atomic Absorbtion Method[9].

After removal of organic material by dry ashing. The residue is dissolved in 20% HNO_3. The lanthanum chloride is added to the solution for calcium analysis. The solution is sprayed into the flame of the atomic absorption apparatus, and the absorption of Calcium and Iron is measured at 422.7 nm and 248.3 nm respectively.

3.2.4 Moisture(Loss on drying)

Method : Vacuum oven method[10].

The sample is weighed into a dish and dried to constant weight (about 6 hrs.) in a vacuum oven at 70 °C, 25 mmHg, then cooled in a desiccator and weighed. The moisture is the difference of the weight measured before and after drying.

3.2.5 Ash (Residue on ignition)

Method : dry ashing method[11].

The sample is weighed into a porcelain dish. The dish and sample are ignited gently over a low flame and then ignite in furnace at 550 °C until the residue free from carbon (about 6 hrs.). The dish containing the residue is cooled in a desiccator and amount of residue is determined by weighing.

4. Results and discussion

Three samples of silk products were analyzed for nutritional components. Results of total nitrogen, fat, iron, calcium, moisture and ash contents were shown in the table.
Total nitrogen content which represents protein content was higher than total fat content in three samples. They were 16.56, 15.90 and 16.26 % in 0, 750 and 1,000 kGy samples respectively. The increase of irradiation dose from 750 to 1,000 kGy had no effect on total nitrogen content. Total fat content in non irradiated sample was only 1.50 %. After irradiation, it was decreased to 0.26 and 0.33% in 750 and 1,000 kGy samples respectively. It was distinctive lower than non irradiated one. It implied that radiation had some effect on total fat content in irradiated samples. However the type of fatty acid could not be illucidated in this study, therefore more study is needed to be done.
Regarding total mineral content, the results showed that total iron contents were 3.1, 4.6 and 2.0 % in 0, 750 and 1,000 kGy samples respectively; total calcium contents were 381.6, 382.7 and 157.0 % in 0,750 and 1,000 kGy samples respectively. In general food such as milk and products, the total contents of iron and calcium are not high as these silk products [12]. It was remarkable that increasing dose of 1,000 kGy decreased total iron and calcium contents distinctively. In this case, some degraded compounds of iron and calcium might be occurred which could not be detected.
In addition total moisture content was 0.05, 5.67 and 4.43 % in 0, 750 and 1,000 kGy samples respectively. It showed that total moisture content was increased after irradiation. Molecular structure of irradiated samples might be different from non irradiated one. Therefore they changed their physiological characteristic. Irradiated silk powder had moisturizing or humectant effect which could be utilized in cosmetic preparation.

5. Conclusion

Our result is one of the greatest challenge, not only the silk fiber can be used as raw material in textile industry, their wastes might be an important source of protein and calcium for elderly, the requirement of dietary fat is lower in aging person. The collection of data on nutritional value of silk wastes was needed to be done in order to make recommendation that it is useful as human food. However in cosmetics, the properties of silk powder as moisturizer or humectant were published. There were many commercial products that were available in the market.

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Table : Nutritional Components of Silk Powder from Irradiated Silk Wastes.

Component	Irradiated Sample		
	0 kGy	750 kGy	1,000 kGy
Total nitrogen (g%)	16.56	15.90	16.26
Fat (g%)	1.50	0.26	0.33
Iron (mg%)	3.1	4.6	2.0
Calcium (mg%)	381.6	382.7	157.0
Moisture (g%)	0.05	5.67	4.43
Ash (g%)	1.17	1.38	0.53

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Session 3

Radiation Processing of Marine Carbohydrates

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16 Biopolymer Molecular Weight Control by Radiation Treatment for Functional Property Improvement

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Abstract

Solid-state radiation treatment of chitosan at dose range of 30-100 kGy and of gelatin at dose range of 30-40 kGy significantly improved the water-stability of shrimp feed. In these dose ranges, the viscosity-average molecular weight (Mv) of bioadhesive was reduced from 552,000 to 250,000-130,000 and from 57,000 to 38,000-32,000 for chitosan and gelatin, respectively. Gelatin, which has been irradiated in liquid state, could not be used as bioadhesive due to the forming of higher macromolecules based on chain-crosslinks. Liquid-state radiation treatment, in comparison with solid-state irradiation at 100 kGy, required only dose of 4-6 kGy for similar MW and for the same optimal improvement of adhesive property.

Radiation treatment with 60-100 kGy maximally enhanced the anti-microbial activity of chitosan. In addition, the optimal dose required for activity is depended on chitosan origin. Chitosan with initial MW of 830,000 required dose of 100 kGy to reduce to 120,000, while other kind of chitosan with initial MW of 550,000 required dose of 60-75 kGy to reduce to 170,000-150,000 for optimal enhancement.

The antimicrobial activity is regulated by not only MW but also by its distribution. Irradiated chitosan that has largest width of MW distribution (Mw/Mn) may possesses highest antimicrobial activity. Result from fractionation by using centrifugal filter devices showed that fraction of 3-5 x 10^4 has mainly contributed to the suppression of microbial growth.

Key words: Chitosan, Gelatin, Radiation Treatment, Molecular Weight, Molecular Weight Distribution, Adhesive, Antimicrobial activity.

1. Introduction

The functional and biological application of biopolymers has greatly increased recently. Chitosan and gelatin, being high-molecular-weight biopolymer whose functional reactive groups are readily available for chemical reactions to alter their typical mechanical, physical, and solution properties. Functional property of biopolymer also depends on their polyelectrolyte property. Gelatin carries negative charge, while chitosan has high positive charge density, one charge per glucosamin unit.

Radiation treatment has potential to degrade biopolymer by breaking them to low molecular fractions. Dose-effect investigation has recently been utilized for sodium alginate [1], carrageenan [2] and chitosan [3, 4] in developing of novel plant growth promoters. For sodium alginate and chitosan, molecular weight must being less than 10,000 for achieving of maximal effectiveness. Carrageenan also needs to be irradiated at dose of 100 kGy for Kappa- and 500 kGy for Iota-carrageenan in order to reduce molecular weight to *ca.* 10,000- 20,000. To degrade biopolymer in order to get effective size; each of biopolymer, even each one from the same kind but varied in origin, requires different radiation dose. Irradiated biopolymer is comprised of mixtures of molecules of various sizes. This distribution is affected by radiation dose. However, the influence of molecular weight distribution of the mentioned biopolymer on plant promotional activity has not been investigated intensively.

Researchers in number of countries have recently showed the enhanced antimicrobial activity of irradiated chitosan in comparison with the original one tested against *E. coli* [5, 6], fruit-spoiling fungi [7], and disease-causing fungi on plant [8]. Results from those studies showed 100 kGy as the optimal dose for activity enhancement. The results also indicated the difference in molecular weight of various chitosans irradiated at the same dose of 100 kGy. In recent study, we received the maximal antifungal activity of chitosan with molecular weight (Mv) 170,000 when irradiated at 60 kGy [9]. Thus, the enhancement of antimicrobial activity of chitosan by radiation treatment depend not only on radiation dose, but also on size of molecules including size before treatment, and probably on distribution of molecular weight.

In present article, by connecting experimental data, we tried to show the relation of some functional properties of biopolymer to its molecular weight, to molecular weight distribution. Adherent property of chitosan and gelatin, and antimicrobial activity of chitosan were described as examples.

2. Experimental

2.1. Materials

The first type of chitosan was provided by Institute of Chemistry (National Center of Natural Science and Technology, Vietnam). This chitosan sample has viscosity-average molecular weight Mv 550,000, and degree of deacetylation (DDA) of 85%. The second type of chitosan namely 10B was purchased from Katokichi Company, Japan. Chitosan 10B has Mv of 830,000, and DDA of 99%. Gelatin was a product from Shanghai Chemical Company with Mv of 57,500.

Microorganisms used in the study included two bacterial strains (*Escherichia coli B/r, Pseudomonas fruorescens*), two fungal strains (*Fusarium dimerum* Penzig and *Aspergillus nidulans)* and three kind of yeast (*Aureobasidium sp.* B12, *Candida lypolitic, and Saccharomyces cerevisiae* 52a). Two fungi were isolated from spoilt mango and dragon fruits as described in previous study [9]. Strains of bacteria and yeast were obtained from Department of Radiation Research for Environmental Resources, Takasaki Radiation Chemistry Research Establishment (TRCRE, JAERI).

2.2. Radiation treatment

Chitosan in powder (10B chitosan) or in flake form (domestic chitosan) was irradiated on gamma Co-60 source (TRCRE), at room temperature, and dose rate of 10 kGy/h. Radiation dose was arranging from 25 kGy to 1000 kGy. Paste-like chitosan solution of 10% (w/v) was prepared in 5% acetic acid and was packaged in two layers of PE bag. Irradiation was carrying on the gamma Co-60 source of Hanoi Irradiation Center with dose arranging from 2 to 45 kGy and dose rate of 2 kGy/h. Gelatin also was gamma-irradiated in solid and liquid state. Treatment in solid state was undertaken in air with dose arranging from 10 to 40 kGy. Liquid state treatment was under anoxia condition with 2% gelatin solution, and dose range of 2-15 kGy.

2.3. Measurement of antimicrobial activity

- The minimal inhibitory concentration (MIC) was determined by using method as described in previous study [9].

- Measurement of antimicrobial activities using optical density method: A loopful of each culture was spread to give the single colonies on the Nutrient Agar and incubated at 37^0C for 24 h (for bacteria) or on the YM Agar and incubated at 25^0C for 24 h (for yeast). A representative colony of each strain was picked off with wire loop and placed in Nutrient

Broth or YM Broth, which were then incubated overnight at 37^{0}C or 25^{0}C for two times to adapt to culture medium. A culture where bacteria or yeast grew in a logarithmic growth phase was prepared for antimicrobial tests. One hundred micrometer of an overnight-culture of target organisms was transferred to L-shaped tubes containing medium with different of chitosan concentrations. All of samples were inoculated under shaken cultivation at 37^{0}C (for bacteria) and 25^{0}C (for yeast) for 78 h - 96 h. The turbidity of medium was measured at 650 nm by using bioscanner (Ohtake Seisakusho, Tokyo, Japan) during whole incubation.

2.4. Measurement of molecular weight and its distribution

Weight-average molecular weight (Mw), number-average molecular weight (Mn), and molecular weight distribution (Mw/Mn) were determined using gel permeation chromatography (GPC). The measurement was performed on HLC-802A (Tosoh Co. Ltd., Japan) equipped with three TSK gel PW_{XL} columns in series ($G6000PW_{XL}$, $G3000PW_{XL}$ and $G2500PW_{XL}$; Tosoh Co. Ltd., Japan) in combination with an TSK guard column PW_{XL}. Aqueous solution containing 0.5M CH_3COOH and 0.5M CH_3COONa was used as eluent. Viscosity-average molecular weight (Mv) of chitosan and gelatin was calculated by the Mark-Houwink equation $[\eta] = K_m M^{\alpha}$ using the Ubbelohde, where $K_m = 1.81 \times 10^{-3}$ cm^3/g, $\alpha = 0.93$ at 25°C for chitosan [10], and $K_m = 1.66 \times 10^{-3}$, $\alpha = 0.885$ for gelatin [11].

2.5. Fractionation of irradiated chitosan

Irradiated chitosan solutions were filtered with Centrifugal Filter Devices (CENTRIPREP, Millipore-USA) with molecular weight exclusion limits of 3×10^3, 1×10^4, 3×10^4, 5×10^4 and 1×10^5. Portion of each fraction of irradiated chitosan was freeze-dried and weighed for percentage calculation.

3. Results and discussion

3.1. Adherent property of biopolymer in the molecular weight dependence

3.1.1. Chitosan

Feed pellets for shrimp aquaculture were prepared as described in other article of this proceeding [12]. The water-stability of feed pellets using chitosan that has been irradiated at different doses is indicated in Table 1. Chitosan samples were irradiated in solid and in paste-like state. All of chitosan solutions were prepared with the same concentration of

0.75% in 0.0635M acetic acid. Each solution then was used to moisturize feed material to get ratio of chitosan 0.48% to feed. Three other samples were put into experiment as the control ones; they were the unirradiated chitosan, carboxymethylcellulose (CMC), and sample without adhesive addition. The result showed that solid-state radiation treatment clearly increased the water-stability of feed pellets. In addition, the activity was increasing as observed with increase of radiation dose, even from dose of 10 kGy. Dose of 20-40 kGy could modify chitosan to make it reach six hours of water-stability, which equal to that of imported feed. Thus, 20-30 kGy as known as sterilization dose, can be recommended to degrade chitosan for adhesive property enhancement. Treatment of chitosan in paste-like state could also improve the adherent property. In this case, the required dose was small compared to dose used in solid state. Dose of 4-6 kGy was optimal for inducing of maximal enhancement because the disappearance of effect started at 10 kGy.

TABLE 1. Influence of irradiated chitosan on the water-stability of shrimp feed pellet

No.	Treatment	Water-stability, hrs	Level of standard
1	Unirradiated chitosan	4	> SVNMF
2	10 kGy (SS)	5	> SVNMF
3	20 kGy (SS)	6	ERS
4	40 kGy (SS)	7	ERS
5	60 kGy (SS)	8	ERS
6	100 kGy (SS)	> 8	Hard
7	4 kGy (LS)	6	ERS
8	6 kGy (LS)	6	ERS
9	10 kGy (LS)	4	> SVNMF
10	No adhesive added	0.5	< SVNMF
11	CMC 2%	1	< SVNMF

Chitosan / feed = 4.8/1000; SS, LS: treatment in solid and liquid stare; ERS: Equal to Regional Standard; SVNMF: Standard of Vietnam Ministry of Fisheries [13];

Since chitosan is one of the few cationic polyelectrolites, while most of feed ingredients (proteins, anionic polysaccharides) carry negative charges, the positive charge of chitosan interacts strongly with negative surfaces to give electric neutrality [14].

Effect of radiation treatment, which was implemented in solid and paste-like state on, the average-viscosity molecular weight (Mv) of chitosan was shown in Fig1 and Fig 2, respectively. Data from Fig 1 showed that Mv sharply decreased in a dose range to 150

kGy, then slowly decreased from 150 to 500 kGy. The polysaccharides including chitosan are typical degradable materials due to ionizing radiation [15, 16]. In this study, the original chitosan has molecular weight (MW) of 552,000; it reduced to ca. 250,000 when irradiated at 30-40 kGy. Irradiated chitosan solution with reduced molecular weight has low viscosity, and therefore easy to flow into the crevices and asperities found in solid surfaces of material like feed particles.

Liquid-state radiation treatment (Fig 2), in comparison with solid-state irradiation, required only dose of 4-6 kGy for the same optimal improvement of adhesive property. At that dose, MW of chitosan (domestic) was reduced to *ca.* 140,000. To get similar MW by using solid-state irradiation, the absorbed dose must require 100 kGy as indicated in Fig 1. The improvement of adherent property was not observed with chitosan irradiated at 10 kGy in liquid state, so molecular weight 90,000 of 10 kGy-irradiated chitosan probably is lowest limit of MW for improvement. Results from Fig 1 also showed that although there was difference in MW of two chitosan samples before treatment (MW 552,000 and MW 829,000), no significant difference in reduction and size of MW was observed when irradiated at doses higher than 50 kGy.

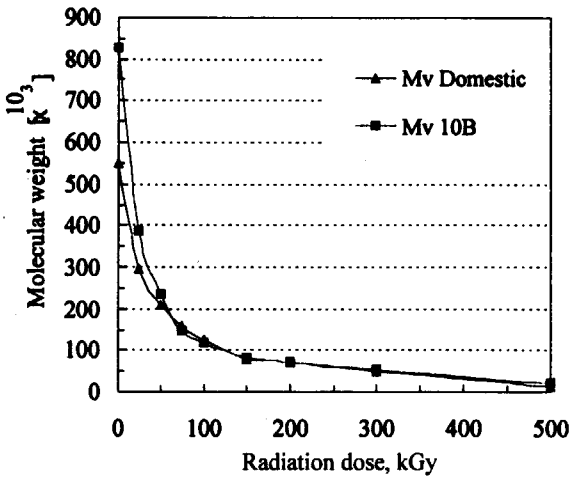

FIG 1. Change in viscosity-average MW of chitosan as a function of dose by solid-state radiation treatment. Chitosan with M_v 552,000 (domestic) and 829,000 (10B) in origin.

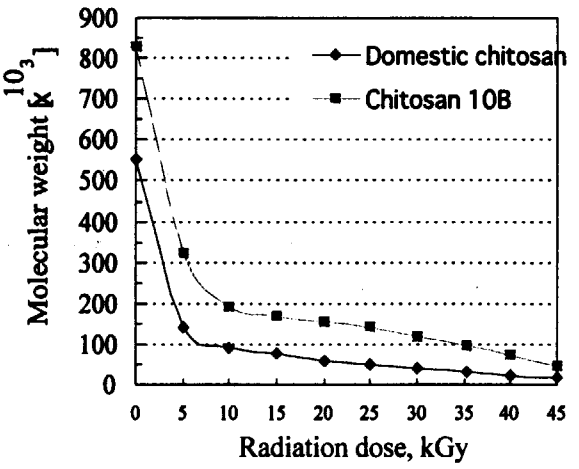

FIG 2. Change in viscosity-average MW of chitosan as a function of dose by radiation treatment in paste-like state. Chitosan with M_v 552,000 (domestic) and 829,000 (10B) in origin.

3.1.2. Gelatin

Influence of irradiated gelatin and its content on the water-stability of shrimp feed was shown in Table 2. Similar to chitosan, radiation treatment in solid state had raised the

adherent property of gelatin applying in feed as bioadhesive. Radiation dose required for treatment was also around of 30 kGy. Ratio of gelatin to feed material could be reduced by about 50% (from 5 to 2-3% in feed). Liquid-state treatment of gelatin, contrary to chitosan, reduced the adherent potential (data not shown in Table 2).

Change in viscosity of gelatin solution that has been irradiated in liquid state is indicated in Fig 3. The data showed that radiation treatment clearly increased the viscosity of gelatin solution. Thus, irradiation could be a factor caused the change in protein property like the act of thermal treatment. These thermally induced changes are caused by segments of gelatin chains organizing intramolecularly into the collagen-fold structure [17]. On the contrary with liquid-state irradiation, the viscosity of solid-state irradiated gelatin has been reduced gradually by increasing of absorbed dose (Fig 4). The reduction was linear function of dose arranging from 10 to 40 kGy. Solid-state radiation treatment of gelatin at dose range of 30-40 kGy significantly improved the water-stability of shrimp feed. In these dose ranges, the Mv was reduced from 57,000 to 38,000-32,000 (Fig 5).

TABLE 2. Influence of irradiated gelatin and its content on the water-stability (in hrs) of shrimp feed pellet (treatment in solid state)

No.	Ratio of gelatin to feed, %	Radiation dose, kGy			
		0	20	30	40
1	2	< 2	3.5	4	4.5
2	2.5	2.5	5	5	6
3	3	3.5	6.5	6.5	-
4	4	4.5	-	-	-
5	5	-	-	-	-

Irradiation of gelatin in solid state is the direct effect of gamma-radiation, which breaks covalent cross-linkages or peptide bonds. The higher radiation dose, the greater effectiveness caused by chains cleavage. Gelatin peptide bonds are believed to be less stable to acid- and base-catalyzed hydrolysis than those of most protein [14]. This concept may be considerable for radiation degradation. An adhesive must be applied as a liquid, preferably of a low viscosity, both to wet the adherent surfaces and to flow into the crevices and asperities universally found in solid surfaces. Eventually, the adhesive must undergo a phase change, i.e., by solvent evaporation, or reaction, to a solid in order for the joint to acquire the necessary strength [14]. Radiation treatment reduced chitosan and

gelatin macromolecules to lower molecular-weight fragments. Their solution has lower viscosity, and therefore easy to flow into the crevices and asperities found in solid surfaces of feed particles. Gelatin solution that has been irradiated in liquid state, showed decreased adherent property due to its viscosity higher than unirradiated one.

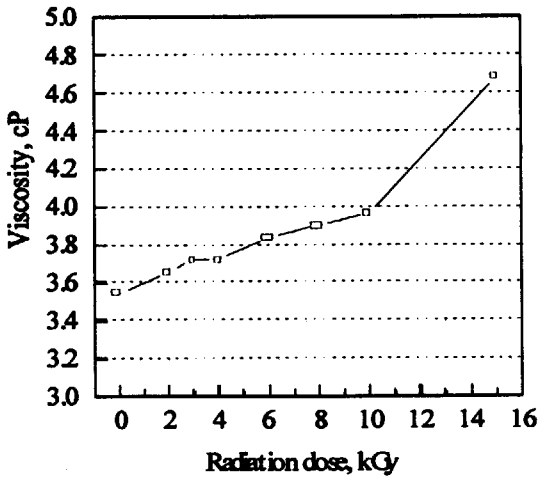

FIG 3. Change in viscosity of gelatin solution by liquid-state radiation treatment (2% gelatin solution under anoxia condition).

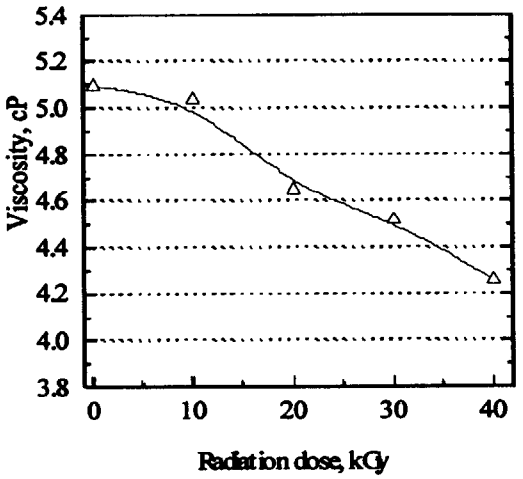

FIG 4. Change in viscosity of gelatin solution by solid-state radiation treatment (4% gelatin solution, Brookfied viscometer).

3.2. *Antimicrobial activity of chitosan in molecular weight dependence*

Fusarium dimerum Penzig and *Aspergillus nidulans* Wint fungi was cultivated in PDB medium with supplementing of irradiated chitosan (MW 552,000) at various concentrations for 84 hours in order to define MIC. The results from the experiment were shown in Fig 6. The data proved that antifungal activity of chitosan on two fungal strains increased in all of chitosan samples irradiated at dose of 20-200kGy. However, the highest enhancement effect on antifungal activity of chitosan was observed at dose of 60 and 75 kGy, where MW was 150,000-170,000. At this dose range, the MIC values of chitosan on *F. dimerum* Penzig and *A. nidulans* Wint were 150 ppm and 70 ppm, respectively. Dose of 60 kGy also was proved as optimal dose for antibacterial activity tested against *E. coli* and other fungi (data not shown).

Several mechanisms were proposed for the antimicrobial activity of chitosan. One of major proposals is relied on the reduction of bacterial metabolism by stacking of chitosan molecules to bacterial cell wall [19]. Other mechanism proposed that chitosan is able to

block description to RNA from DNA [20]. The way and intensity of action of two mechanisms is not similar depending on the molecular weight of chitosan. Fractions with average molecular weight acts with the first mechanism. In the second mechanism, chitosan must be hydrolyzed to the smaller fractions in order to permeate easily in to the cell. Effect of radiation on chitosan as reported with the break of glycosidic link to produce different lower molecular-weight fragments. Microbial inhibition caused by irradiated chitosan fragments is stronger than original chitosan molecules due to the contribution of both mechanisms that simultaneously occurred.

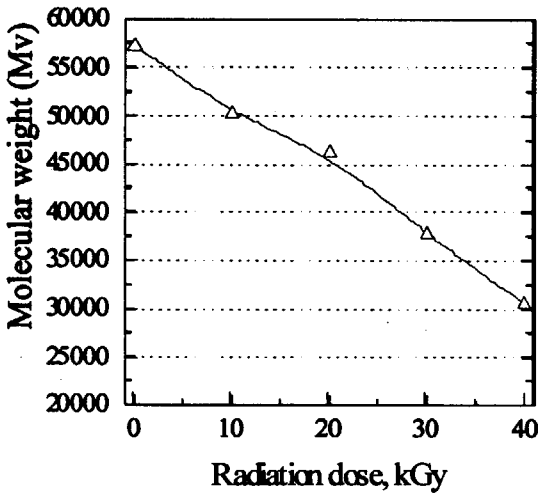

FIG 5. Change in viscosity-average MW of gelatin by solid-state radiation treatment.

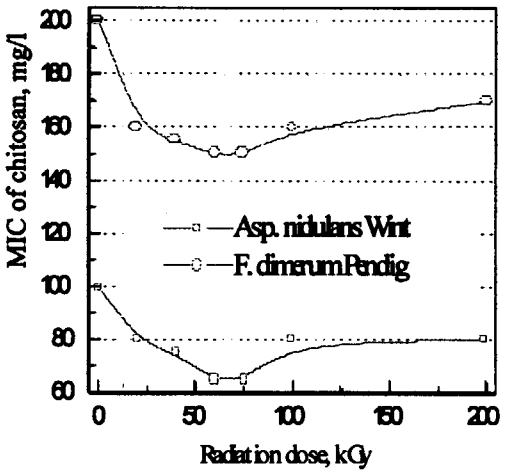

FIG 6. Change in minimal inhibition concentration of chitosan irradiated at different doses testing on fungi.

Experiment investigating antimicrobial activity of irradiated chitosan (MW 830,000) were carried out by using two bacteria strains (*Ps. fruorescens, E. coli* B/r) and three kinds of yeast (*C. lypolitic, S. cerevisiae* 52a, *Aureobasidium.sp* B12). The antimicrobial activity was determined by measuring time of growth in logarithmic phase of microorganism. The longer the logarithmic phase is, the higher is the antimicrobial activity of the correspondent chitosan. Results were shown in Fig 7 and Fig 8 for bacteria and yeast, respectively. According to the data, the activity was increased with radiation dose varying from 50 to 100 kGy, then was decreased with doses higher than 100 kGy. Chitosan 10B with initial MW of 830,000 required dose of 100 kGy to reduce to 120,000, and the antimicrobial activity appeared as maximum for chitosan with this molecular weight.

Summing up the results, it was seen that radiation treatment with 60-100 kGy maximally enhanced the anti-microbial activity of chitosan. In addition, the optimal dose required for activity is depended on chitosan origin. Thus, the optimal MW required for the maximal enhancement is different depending on radiation dose and initial MW.

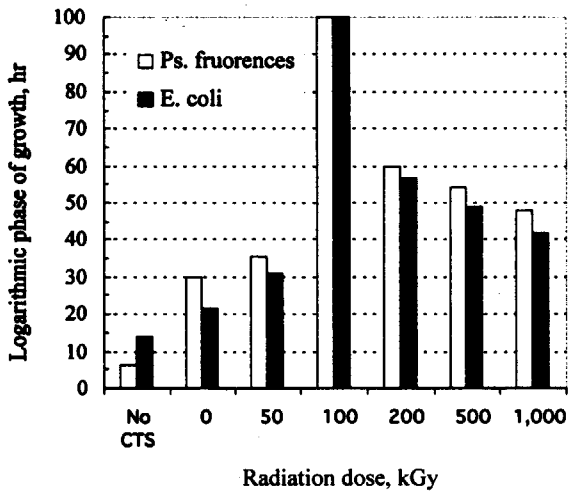

FIG 7. Effect of chitosan irradiated at different dose on the growth of bacteria (*Ps. fruorences* and *E. coli* B/r in medium with chitosan concentration 166 mg/l and 20 mg/l, respectively).

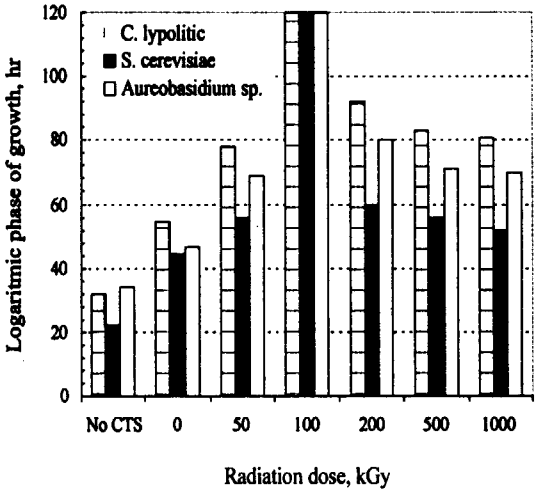

FIG 8. Effect of chitosan irradiated at *lypolitic*, *S. cerevisie* 52a, *Aureobasidium sp.* in medium with chitosan concentration 50, 64 and 55.5 mg/l, respectively)

The weight-average molecular weights (Mw) and number-average molecular weights (Mn) of irradiated chitosan samples were calculated with pollutan standards and their ratio (Mw/Mn) are presented in Fig 9. In comparison with viscosity-average molecular weight (Mv) as indicated in Fig 1, the Mn and Mw values were lower than Mv observed at the original and 50 kGy-irradiated chitosan, where molecular weight was high. The width of molecular weight distribution, defined as Mw/Mn, was equal to 3.2 for unirradiated chitosan. This ratio was increasing rapidly towards 5.6 at dose of 50 kGy, then decreasing gradually to 1.9 at 1,000 kGy equal to that of the original chitosan. It is worthy of remark according to Ulanski and Rosiak et al [16] that the polydispersity (Mw/Mn) values reported for chitosan irradiated at doses up to 50 kGy were increased from 1.5 to 2.

3.3 *Antimicrobial activity of chitosan in molecular-weight-distribution dependence*

As above mentioned, two chitosan samples with initial MW 532,000 and 829,000 exhibited the maximal microbial activity when they were irradiated at 60 and 100 kGy for reducing MW to 170,000 and 120,000, respectively. Thus, low molecular weight was

needed to enhance the activity, but its size was not similar. Investigation of effect of molecular weight distribution on antimicrobial activity by using GPC to measure Mw/Mn and using filter devices to fractionate different weight portions was carried out.

The GPC elution patterns of chitosan irradiated at different doses showed peak shifts to longer retention time as the dose is increased (data not shown). The maximum retention times of those different chitosans were ≈ 31 min; however, higher molecular weight ones had shorter retention time. In addition, the distribution became wide as the partial degradation proceeded by increasing of radiation dose up to 100 kGy. But, the distribution became narrow again when the molecular weight was decreased by increasing of radiation dose from 500 to 2000 kGy. Thus, the change of polydispersity (Mw/Mn) in Fig 9 supports the retention time of the molecular weight distribution implied from the GPC profiles.

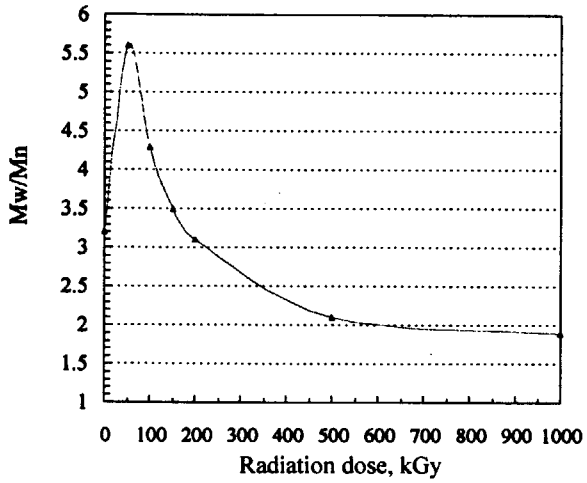

FIG 9. Change of molecular weight distribution (Mw/Mn) of chitosan by radiation dose.

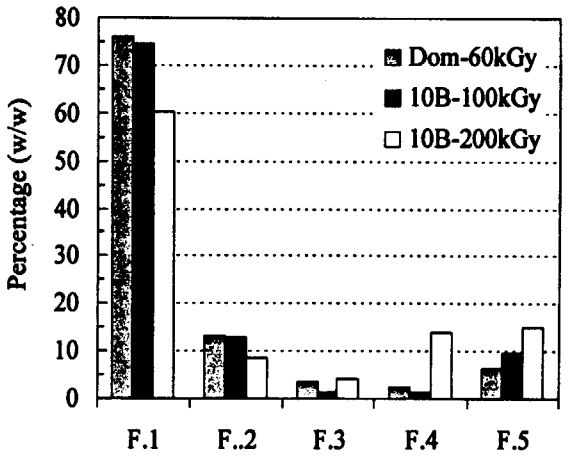

FIG 10. Change of fractionated chitosan portions as function of dose and of initial MW. F.1: > 5 x 10^4 ; F.2: (3-5) x 10^4 ; F.3: (1-3 x 10^4; F.4: (3-10) x10^3; F.5: <3 x 10^3.

Based on the results, it is considered that the maximal antimicrobial activity of irradiated chitosan is exhibited at radiation dose of 100 kGy, which can break chitosan molecules to make the molecular weight distribution get the larger width. In this study, chitosan 10B required 100 kGy in order reduce Mv to 120,000 with maximal value of Mw/Mn = 4.3. However, it is needed to determine MW distribution of chitosan with initial Mv 530,000 (domestic) irradiating at 60 kGy and of chitosan 10B at 75 kGy to confirm the proposal that the distribution must be highest width for the maximal antimicrobial activity.

The distribution of weight portions of two irradiated chitosan samples is illustrated in Fig 10. Interestingly, radiation treatment at dose of 100 kGy for chitosan 10 B and at 60

kGy for "domestic" one has formed a distribution with substantially similar manner. The (3-5) x10^4 fraction from two chitosans was *ca.* 12%. Portions of higher molecular weight had higher percentage compared to low molecular weight ones. The latter was going to increase with dose increasing, while the former decreased. The (3-5) x10^4 fraction, for example, was reduced from 12% at 100 kGy to 8% at 200 kGy. Since the maximal antimicrobial property was observed at dose of 100 kGy for chitosan 10B and 60-75 kGy for chitosan "domestic", the distribution of weight portions may have principle role for enhancing the activity.

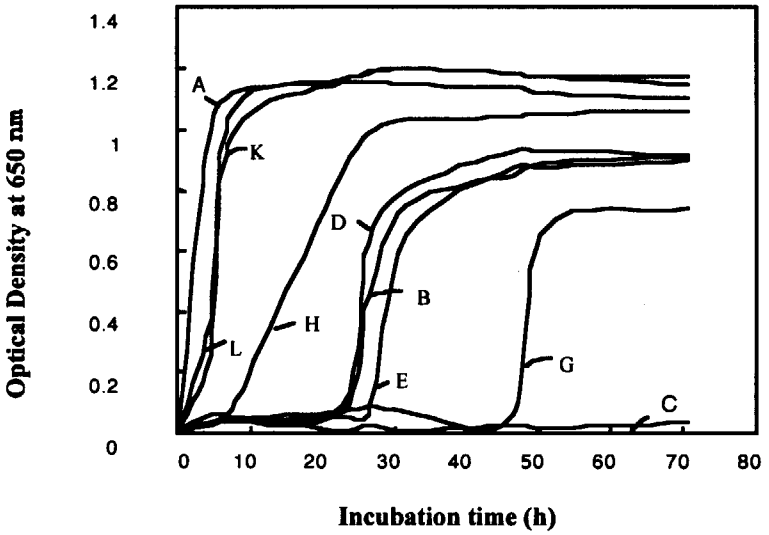

Fig 11. Antibacterial activity of fractionated chitosan fragments against *Ps. fuorescens.*
A : No chitosan added; B: 166 mg/l original chitosan;
C : 100 kGy-irradiated chitosan; D : >1 x 10^5; E : 5-10 x 10^4;
G : 3-5 x 10^4; H : 1-3 x 10^4; K : 3-10 x 10^3; L : <3 x 10^3.

All of fractionated potions were separately used for antimicrobial test to clarify the individual activity of each portion. The result from Fig 11 testing on *Ps. Fruorescens* showed that the 3-5 x 10^4 fraction (line G) has highest antimicrobial activity, while 3-10 x10^3 (line K) and < 3 x10^3 (line L) fractions lost the enhanced activity. By comparison at the same concentration, however, the antimicrobial activity of the 3-5 x 10^4 fraction was lower than that of 100 kGy-irradiated chitosan (line C). This confirmed that high antimicrobial property of irradiated chitosan is obtained by contribution of different fractions, especially from those whose size larger than 3-5 x 10^4. During the growth of microorganisms in medium containing chitosan, several chitosan breaking enzymes such as chitosanase were triggered to form and released to the medium resulting to additional degradation of chitosan. This degradation may lead to the decrease in antimicrobial activity of 3-5 x 10^4 fraction when it alone was taken to supplement medium for microbiological culture. The

activity tested on yeast (*S. cerevisiae* 52a) also received with the same manner; among fractionated portions, the 3-5 x 10^4 fraction also had the highest activity (data not shown).

4. Conclusion

Solid-state radiation treatment of chitosan at dose range of 30-100 kGy and of gelatin at dose range of 30-40 kGy principally improved the water-stability of shrimp feed. In correspondence with these doses, the viscosity-average molecular weight (Mv) of the bioadhesives was reduced from 552,000 to 250,000-130,000 and from 57,000 to 38,000-32,000 for chitosan and gelatin, respectively. Gelatin, which has been irradiated in liquid state, could not be used as bioadhesive due to the forming of higher macromolecules based on chain-crosslinks. Liquid-state radiation treatment of chitosan, in comparison with solid-state irradiation at 100 kGy, required only dose of 4-6 kGy for similar size of MW and for the same optimal improvement of adhesive property.

Radiation treatment with doses arranging from 20 to 1000 kGy enhanced the anti-microbial activity of chitosan. However, treatment at dose of 60-100 kGy showed the highest effectiveness. The antimicrobial activity of chitosan was regulated by not only MW but also by its distribution. Irradiated chitosan that has larger Mw/Mn may possess higher antimicrobiological activity. Fraction of 3-5 x10^4 has highest contributed to the suppression of microbial growth. However, weight portion of this fraction must higher than 12%.

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17 The Use of Chitosan as Bioadhesive and Its Property Improvement by Radiation Treatment for Water-Stable Shrimp Feed Production

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Abstract

Among marine polysaccharides, only chitosan with small content in feed (0.48-0.75%) could be selected to prepare shrimp feed-pellet having so high water-stability that meet the Standard of Vietnam Ministry of Fisheries 28-TCN 102/1997. Solid-state radiation treatment of chitosan with dose ranging from 10 to 200 kGy not only increased its solubility in solvents of dilute acid, but also improved the water-stability of feed-pellet product. Radiation treatment at sterilization doses (20-40 kGy) was evaluated as the most practical technology because irradiated chitosan with reduced content of 0.34% has capacity to be prepared feed-pellets stable as comparable to imported products. Results from feeding trials shown that chitosan-containing feed did not affect the growth response and feed utilization efficiency such as weight gain (WG), feed conversion ratio (FCR) and productivity at harvest.

Introduction

For the past few years, the shrimp culture of Vietnam has been rapidly developed and its product gave the increasingly economical benefit through export. For sustainable development

*) Mr. Hung presented content of this article at the Takasaki symposium.

of shrimp culture, one of the most important strategies is exchanging of extensive farms, which is popular mode at present to semi-intensive and intensive ones. These new modes required good support from industries such as locally developed farm equipment, commercial feeds, farm supplies, seafood processing and international trade industries [1-3]. High quality feed is a very important factor in shrimp culture because it is required to meet the increased demand from farmers on quantity and nutritive quality for minimizing feed losses and for avoiding the over-feeding problem. In other side, the industrially produced feed with high water-stability also greatly contributes in reduction of pond pollution, which is one main reason related to shrimp diseases [1, 2]. For industrial feed-pellet production, most of small and medium-scale enterprises have to import not only macroquantitative feed compositions (fish meal, wheat flour) but also microquantitative ones such as vitamins, minerals, and other additives including adhesive [1]. The latter plays a role of binding material particles, making the formulated pellets stable in pond water.

Because imported feeds maintain the water-stable structure in 6-8 hrs, while most of domestic shrimp-feed from small-scale enterprises has only to be required 2 hrs for water stability as based on the Standard of Ministry of Fisheries 28-TCN 102/1997 [1, 4], the aim of the present study was to investigate for selecting of the locally available adhesives from marine polysaccharides (alginate sodium, carrageenan, agar, and chitosan) for shrimp feed production. Quality of adhesives was evaluated in terms of their ability for making of feed pellet stable in water to meet the national and regional standards. Gamma irradiation has been utilized to control the molecular weight of adhesive in order to get higher efficiency. The study will provide a basis for using local bioadhesives instead of gluten, which is a product imported from wheat-producing countries.

2. Material and method

2.1. Marine polysaccharides

Alginate sodium (medium viscosity) was obtained from Sigma Chemical Company. Carrageenan (type WG-115) was a product of Genugel Carrageenan, Denmark. Chitosan was provided from Chemistry Institute, Vietnam. To use biopolymer as adhesive for feed

preparation, each of selected polymers was mixed directly with feed ingredients or it was dissolved in suitable solvent before mixing.

2.2. Radiation treatment and chitosan analysis

Domestic chitosan with deacetylation degree (DD) of about 90% and viscosity-average molecular weight (M_v) of 550,000 were used without further purification and were gamma-irradiated in solid state. The radiation treatment was undertaken at dose of 20, 40, 60, 75, 100, 150, and 200 kGy with dose rate of 10 kGy/h in Takasaki Radiation Chemistry Research Establishment. The viscosity-average molecular weight (M_v) was calculated using Mark-Houwink equation relating to intrinsic viscosity: $[\eta] = K_m M^a_v$ where $K_m = 1.81 \times 10^{-3}$ and $a = 0.93$ are the empirical viscometric constants that are specific for a given polymer, solvent and temperature [5].

2.3. Shrimp feed-pellet preparation

The preparation of experimental diet was done as those reported in detail by Thoa et al. [6]. The evaluated foodstuffs were provided as indicated: fish meal 53.5%, soybean concentrate 9.0%, rice bran 12.0%, cassava flour 13.5%, fish premix 1.0%, and adhesive . The diets were prepared by thoroughly mixing the dry ingredients with adhesive then adding water until the whole mixture reached moisture 40-45%. The dough was pelleted through a 2-mm die, and then dried in an oven overnight at 60^0C. Other procedure was also utilized for feed preparation using different adhesives. The adhesive in first step has been dissolved in water or suitable solvent, and then the received solution was taken to moisturize the diet mixture. The pellet water-stability was evaluated according to the 28-TCN 102/1997 [4].

Diets for feeding trials using indoor tanks were prepared as follows: Two commercial feeds from CP Company (Thailand) and KP-90 (Vietnam) were used without additional treatment for feeding control shrimps. Test diets were prepared by using two mentioned commercial feeds as the initial material for nutritive evaluation of chitosan adhesive. Commercial pellets were finely ground and passed through a 0.5-mm sieve, then well mixed with irradiated chitosan solution to get suitable moisture. After pelletizing, feed was dried overnight at 75^0C. By this preparation, the test feeds were available from CP and KP-90, respectively. Thus, there were four lots of experimental feed.

Diets for feeding trials using pond shrimp-culture were ordered for Halong Can Food Company Ltd. (170 Le Lai, Haiphong, Vietnam). The company's commercial shrimp feed (HCFC) was used as control feed and chitosan-added HCFC was the test feed.

2.4. Feeding trial for nutritive evaluation

a) *Feeding trials using indoor tanks* were carried out as follows: Twenty *Penaeus monodon* fabrius shrimps per tank (two tanks per treatment) were randomly distributed in 2 m^3 circular plastic composite tanks (water depth of 1 m) equipped with a system supplying air and brackish water. The animals were then acclimatized for a further 7 days to adapt to the medium conditions and experimental diets. Another shrimp from the reserve tank replaced any shrimp, which died during the experiment. The water temperature, pH and dissolved oxygen during 60-day culture period varied between 27 and 29^0C, 7.2 and 7.8, and 5.8 and 7.7 mg/l, respectively. All shrimps in each tank were initially fed 8% of total body weight daily. Shrimp with mean initial weight 5.54 ± 0.42 g were fed the experimental feed for 60 days three times per day. During the experiment, the feed amount was progressively changed and adjusted according to the shrimp acceptance. Every morning and afternoon before each feeding time, uneaten feed and faeces were removed from the tanks. Every ten days shrimp from each tank were weighed and measured to evaluate the growth.

b) Feeding trials using pond shrimp-culture were prepared as follows: The experiment for evaluation of feed-pellet and adhesive (chitosan) quality was implemented using 4 earthen ponds with 1000 m^2 per each (2 ponds per treatment). Density at the release was 8 shrimps/m^2 with shrimp size P45 (5.54 ± 0.42 g). The control shrimps fed commercial feed obtained from Halong Can Food Company Ltd. (HCFC). The test shrimps fed feed that produced by HCFC with the same materials supplemented with 0.75% (w/w) irradiated chitosan (60 kGy). The animal growth and biological parameters were measured and calculated as similar to that of the indoor experiment.

3. Results and discussions

3.1. Selection of suitable polysaccharide as bioadhesive for water-stable feed production

3.1.1. Alginate and carrageenan

Sodium alginate and carrageenan in powder form were used as bioadhesive at content of 2-5% to prepare feed pellets. These polysaccharides were added to feed material in two ways, e.g. in powder form by well mixing with feed ingredients before water added, and in liquid state by polymer solution. All of received feed dissolved quickly in water after several minutes only. Thus, alginate and carrageenan cannot be used as adhesive for producing the water-soluble feed.

3.1.2. Chitosan

In contrast to alginate and carrageenan, chitosan provided high water-stability when it was added to feed in solution form, even at low chitosan content of 0.5% (see Table 1). Content of 0.5% made feed pellets water-stable exceeding the Standard 28-TCN 102/1997, and content of 0.75% chitosan provided feed meets the parameter equal to the regional standard. Acetic acid is commonly used to dissolve chitosan, however the acid taste may influence the palatability of animal.

For conclusion of selection of suitable bioadhesive from marine polysaccharides, only chitosan was selected due to its suitable adhesive properties such as its low content in feed is required and its raw main resource for extraction is available from shrimp shell.

3.2. Effect of radiation treatment on chitosan

3.2.1. Improvement of solubility of chitosan in acid solvents by irradiation treatment

To dissolve chitosan for food/feed preparation the organic acids, especially acetic acid was commonly used. However, the content and taste of the selected acid can affect the palatability of the animals, so increasing of chitosan solubility with reduction of acid content could be useful in some chitosan utilization, including the supplement to shrimp feed. In this part the potential of radiation treatment was investigated to clarify how much its effectiveness to increase chitosan solubility. Results of the solubility in radiation dose dependence, when treated in solid state, are shown in Fig 1. The solubility time of 1% (w/v) chitosan in acetic and oxalic acid reduced quickly in a range from 10-75 kGy. Solubility time of original chitosan in acetic acid, for example, from 80 min was reduced to 39 min by 60 kGy irradiation. The reduction expressed at lower rate with increasing radiation doses from 75 kGy to 200 kGy. Chitosan regularly takes long time to dissolve in diluted acid; the time can be reduced clearly as based on the received result.

Table 1. The water-stability of feed pellet formulated with chitosan as bioadhesive

Chitosan in feed, % (w/w)	Water-stability of feed pellet, hrs
0.5	4-5, > VNS
0.75 – 1.0	6-8, = RS
1.5	>10, hard

VNS = Standard of VN Ministry of Fisheries,
RS = "Regional standard"

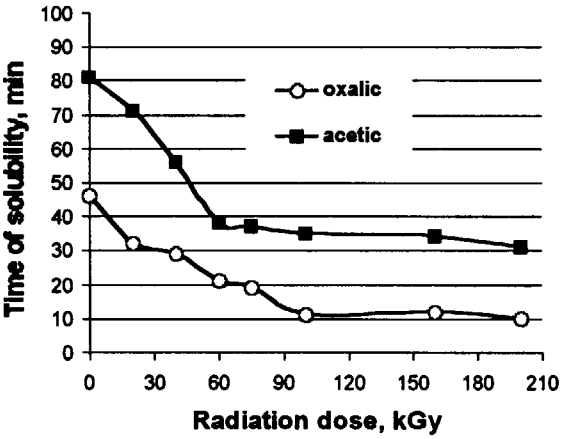

Fig 1. Effect of radiation treatment on the solubility of chitosan in acetic and oxalic acid

In laboratory practice, some technicians can easily prepare chitosan solution even at high concentration by using very high acid content. However, 10% chitosan in 5% acetic acid probably is the most optimal way according to our experience. From this stock chitosan paste, 1% chitosan solution in 0.5% acetic acid can easily be received by water dilution. This technique cannot be used to prepare solution of 1% chitosan in acetic acid with concentration less than 0.5%. For this reason, we used 0.125 M (0.65%) acetic acid to prepare 1, 2, 3 and 4% chitosan solution. Two chitosan samples of unirradiated and irradiated at 60 kGy were taken to experiment to compare the effectiveness of radiation treatment on preparation of 1% chitosan in diluted acetic acid (concentration lower than 0.5%). Table 2 shows that it takes 158 min to complete 3% chitosan solution in 0.65% acetic acid, while in practice can not prepare 4% chitosan with the same condition because it takes very long time. It was required shorter time (85 min) for completing 3% 60 kGy-irradiated chitosan and not so difficult to prepare 4% (153 min). From the latter solution, the 1% chitosan can be received by water dilution and its acid concentration was 0.16% only. Thus, radiation treatment can be used to degrade chitosan making it easy to dissolve in diluted acid, by which no side effect can be caused from acid content and taste.

Table 2. Effect of radiation treatment on solubility of chitosan at different concentrations using 0.125 M (0.65% w/v) acetic acid as solvent

Chitosan, % (w/v)	Unirradiated		Irradiated at 60 kGy	
	Solubility time, min	pH	Solubility time, min	pH
1	80	4,45	39	4,43
2	111	5,32	64	5,22
3	158	5,81	85	5,66
4	-	-	153	6,06

3.2.2. Change in viscosity and molecular weight of chitosan by radiation treatment

Domestic chitosan samples with deacetylation degree (DD) of about 90% and viscosity-average molecular weight (M_v) of 550,000 were gamma-irradiated in solid state. The radiation treatment was undertaken at 20-200 kGy dose range and dose rate of 10 kGy/h. The result from investigating of viscosity change of 0.75% chitosan solutions (in 0.0625 M acetic acid) was shown in Fig 2. For viscosity measurement, the Brookfield viscometer was used. The change tendency has a correspondence with the reduction of solubility time with increasing radiation dose. The viscosity decreased quickly at doses lower than 100 kGy, then slowly decreased.

The viscosity-average molecular weight (M_v) was calculated using Mark-Houwink equation relating to intrinsic viscosity: $[\eta] = K_m M^a_v$, where $K_m = 1.81 .10^{-3}$ and a = 0.93. Fig 3 showed the radiation dose dependent average-viscosity molecular weight (Mv) of chitosan. The Mv sharply decreased in a dose range to 100 kGy, then slowly decreased from 100 to 200 kGy. The polysaccharides including chitosan are typical degradable materials due to ionizing radiation. Effect of irradiation on chitosan has been reported earlier with the break of glycosidic link to produce low molecular-weight fragments [10, 11]. Our result was very well agreed with the referential data. The original chitosan has molecular weight (MW) of 550,000;

it was reduced to *ca.* 250,000 when irradiated at 40 kGy. Dose of 100 kGy degraded chitosan to MW 120,000, while the radiation treatment of 200 kGy reduced it to MW 70,000.

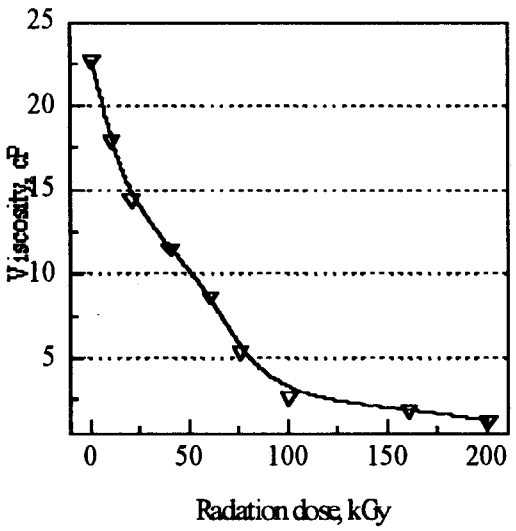

Fig 2. Change in viscosity of chitosan solution by solid-state radiation treatment

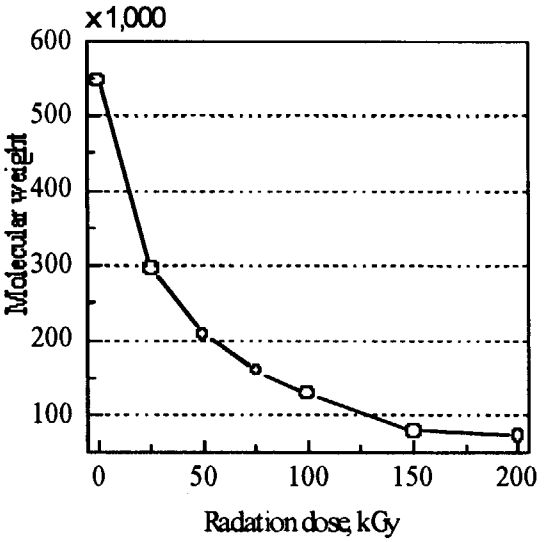

Fig 3. Change in viscosity-average molecular weight of chitosan by solid-state radiation treatment

3.2.3. Improvement of water-stability of feed by using irradiated chitosan as bioadhesive

The water-stability of feed pellets using chitosan that has been irradiated in solid state at different doses is shown in Table 3. All of chitosan solutions were prepared with the same concentration of 0.75% in 0.0635 M acetic acid. Each solution then was used to moisturize feed material to get chitosan content 0.48% of feed. To make the comparison with chitosan samples those have been irradiated at dose: 10, 20, 40, 60, 75, 100, 160, and 200 kGy three other samples were put into experiment as the control ones, they were the unirradiated chitosan, carboxymethylcellulose (CMC), and sample without adhesive addition. The result showed that radiation treatment clearly increased the water-stability of feed pellets. In addition, the activity was increasing as observed with increase of radiation dose, even from dose of 10 kGy. Dose of 20 kGy could modify chitosan to make it reach six hrs of water-stability equal to that of imported feed. Dose of 60 kGy and higher showed a very high water-stability which

may cause hard feed, so it is no need to irradiate chitosan at dose higher than 40 kGy. On other hand, higher dose requires high cost and time. For these reasons, 20-30 kGy as known as sterilization dose, can be recommended to degrade chitosan for adhesive property enhancement.

3.2.4. Lowest optimal ratio of chitosan to feed

In the above part, the irradiated chitosan with content 4.8% of feed was proved to have the required criteria in water-stability. In this part, we investigate to select the lowest optimal content of chitosan. Feed containing irradiated chitosan at various content (0.25 - 0.48%) was prepared. The received results showed that to reach 5 hr- water-stability, 0.45% of unirradiated chitosan content is lowest content required, while only 0.34-0.38% of 20 kGy-irradiated chitosan can be used for 6-7 hr- water-stability. Thus, radiation treatment of chitosan not only increases the water-stability of feed pellets, but also reduces the ratio of chitosan to feed. When chitosan is irradiated at sterilization dose, its content of 0.34% is evaluated as optimal lowest content giving the feed water-stability reaching the regional criteria. In addition, content of 0.75% unirradiated chitosan must to be required to get water-stability similar to that of content of 0.34% irradiated chitosan.

Table 3. Influence of irradiated chitosan on the water-stability of shrimp feed pellets

No.	Treatment	Water-stability, hrs	Level of standard
1	Unirrad. chitosan	4	> VNS
2	10 kGy	5	> VNS
3	20 kGy	6	RS
4	40 kGy	7	RS
5	60 kGy	8	RS
6	100 kGy	> 8	RS
7	No adhesive added	0.5	< VNS
8	CMC 2%	1	< VNS

Radiation treatment in solid state, chitosan content in feed = 4.8/1000

3.3. Mechanism of radiation action for improvement of adhesive property

The way to stabilize feed by using adhesive, in our opinion, can be consisted of three forms as entrapping, croslinking and binding. In the entrapping form, the intermolecular forces stabilizing the adhesive structure are mainly non-valent such as hydrogen bonds, hydrophobic interactions, electrostatic-'salt links' and electrostatic-'metal ion bridges'. These bonds are weak and often appeared in natural polymer like carrageenan, alginate or agar [7]. This concept can explain for low stable structure of feed containing alginate and carrageenan as above-mentioned. The second linkage form is covalent croslinking. These links are very popular with proteinous adhesive. The covalent linkages between proteins are the disulfide bonds of cystine residues. The Maillard reactions also are introduced as covalent linkages that are a complex series of reactions between amino and carbonyl compounds [8]. The third form is linkage between adhesive and feed structures, by which the adhesive is attached to a surface of the feed ingredient structures, either by a non-covalent or covalent bonds. The linkages through non-covalent adsorption rely on electrostatic, hydrophobic and dipole-dipole interactions, and hydrogen bonds [7]. The typical chemical bonds of covalent linkage are much stronger. The type of binding depends very much on the feed ingredients.

Chitosan, being a high-molecular-weight biopolymer, is a linear polyelectrolyte whose reactive amino groups and primary and secondary hydroxyl groups are readily available for chemical reactions to alter its mechanical, physical, and solution property. At pH below 6.5, chitosan in solution carries a positive charge. As chitosan is a polymer, it has a high positive charge density, one charge per glucosamine unit. Since chitosan is one of the few cationic polyelectrolites, it is an exception to the current industrial high molecular weight hydrocolloids or polysaccharides, which are mostly neutral or polyanionic, and provides a great variety of potential applications [9]. Since most of feed ingredients (proteins, anionic polysaccharides) carry negative charges, the positive charge of chitosan interacts strongly with negative surfaces to give electric neutrality. Therefore, chitosan as bioadhesive has higher potential than gelatin at the same content.

The molecular weight of chitosan is a very important property because a minimum molecular weight is most often needed to achieve desired properties. Hence, the polymeric behavior as well the mechanical properties of chitosan depends critically on the average size and the distribution of sizes of macromolecules in the product. Radiation treatment in dose

dependence has potential to degrade chitosan polymers by breaking them to low molecular fraction [10]. Their solution also become low viscosity, and therefore easy to flow into the crevices and asperities found in solid surfaces of material like feed particles [12].

3.4. Feeding trial for evaluating chitosan nutritive quality by shrimp-culture experiment

The growth response and feed utilization efficiency data of feeding trial using in-house tanks are presented in Table 4. The CP feed produced greater weight-gain than domestic diet KP90. The CP diets also produced better feed conversion ratios (FCR) compared to the KP90 ones. Shrimp survival remained 100% at both of diet throughout the 40- day period. Total feed intake data reversally reflected the weight gain data. Feed intake for shrimp fed CP diets were lower than those of shrimp fed KP90 diets. The growth, FCR and total feed intake were not significantly different among shrimp fed diet with no chitosan added and diets containing chitosan.

Table 4. Results of the 40-day feeding trial on tank-scale for *P. monodon* fed CP and KP90 diets containing 0.5% irradiated chitosan

Index	Diet			
	CP	CP + CTS	KP90	KP90+CTS
Initial weight (g)	5.4	5.7	5.6	5.6
Final weight (g)	16.5	16.7	15.8	15.7
Final weight gain (%)	205.6 [b]	193.0 [b]	182.1 [a]	180.4 [a]
Feed conversion rate	2.1 [b]	2.2 [b]	2.6 [a]	2.5 [a]
Survival (%)	100	100	100	100
Total feed intake (g/shrimp)	23.3 [b]	22.1 [b]	26.8 [a]	25.1 [a]

Mean within the same row having different superscript is significantly different ($P < 0.05$).
Weight gain (%) = (final weight - initial weight) / initial weight x 100; Survival (%) = Number of final shrimp/number of initial shrimp x 100; Feed conversion ratio (FCR) = Dry feed intake/wet weight gain.

Table 5. Results of the 50-day feeding trial on pond-scale for *P. monodon* fed HCFC diets containing 0.5% irradiated chitosan

Index	Diet	
	HCFC without chitosan	HCFC with chitosan
Initial weight (g)	5.7	5.6
Final weight (g)	25.7	27.7
Final weight gain (%)	350.9 [b]	394.6 [a]
Feed conversion rate	2.5 [a]	2.4 [a]
Dry matter feed intake (g/shrimp)	50.3 [a]	52.2 [a]
Productivity (kg/ha)	1450 [b]	1520 [a]

Mean within the same row having different superscript is significantly different ($P < 0.05$)

Table 5 shows result of the pilot-scale shrimp-culture experiment that was carried out using four 800 m^2 earthen ponds (two ponds per treatment) to evaluate two kinds of feed: HCFC (without chitosan) and HCFC+CTS (contained 0.5% chitosan). Difference in the average weight at harvest between shrimp fed diet containing chitosan (HCFC+CTS) and shrimp fed HCFC was observed (28.7 and 25.5 g/shrimp, respectively). The productivity at harvest of shrimp also was different that reflecting the higher weigh gain of shrimp fed diet which containing chitosan. The FCR was 2.5 and 2.4 respectively. This proved that chitosan as bioadhesive neither causing side effect nor making influence on normal growth of shrimp-culture. The chitosan composition also did not affect the feed palatability of animals.

4. Conclusion

By experiment on various marine polysaccharides, we have selected chitosan to use as bioadhesive for recycling in production of water-stable shrimp feed-pellets. Shrimp feed containing *ca.* 0.5% can meet the requirement of Standard of Ministry of Fisheries in water-stability, while feed containing 0.75% provides the stability equal to that of imported feed. Solid-state radiation treatment at sterilization dose (20-30 kGy) markedly increases their

adhesive property. Radiation treatment of chitosan not only increases the water-stability of feed pellets, but also reduces the content of feed chitosan. When chitosan is irradiated at the sterilization dose, its content of 0.34% is evaluated as optimal lowest content giving the feed water-stability reaching the regional criteria.

The price of adhesive in feed reduced considerably leading to a practical possibility of application of radiation technology for shrimp feed production with economical benefit. The use of liquid adhesive to moisturize feed materials is evaluated as an improved procedure since feed becomes higher water-stability at lower content of added adhesive. Chitosan adhesive does not influence the growth and palatability during shrimp culture.

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18 Effect of Radiation-Degraded Chitosan on Growth Promotion of Flower Plant in Tissue Culture

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Abstract

Radiation is a useful tool for degradation of polysaccharides, such as starch, carrageenan, alginate and chitin/chitosan. The viscosity molecular weight (Mw) of chitosan with 80% degree of deacetylation was reduced to $1.5x10^5$ by irradiation of 50kGy in solid phase. The solution of 10% of chitosan with Mw *ca.* $15x10^5$ was then irradiated at doses ranging 10-250kGy for further degradation and the products were supplemented into cultural media for testing of plant growth promotion effect. The results indicated that irradiated chitosan showed a strong growth-promotion effect on the increase of the length of shoot, the length of root and fresh biomass for flower plants namely *Limonium latifolium*, *Eustoma grandiflorum* and *Chrysanthemum morifolium* in tissue culture. The growth-promotion effect was obtained by the treatments with 50ppm of chitosan irradiated at the doses of 75-100kGy in 10% solution. The suitable concentrations of chitosan irradiated at 100kGy are ca. 100ppm for *C. morifolium*, 30ppm for *E. grandiflorum* and 40ppm for *L. latifolium* . In addition, our study also indicated that the survival ratio of transferred flower plantlets treated with irradiated chitosan was improved after acclimatizing for 30 days in the greenhouse.

Accodingly, it is concluded that degraded chitosan obtained by radiation degradation technique is effective as a plant growth promoter as well as irradiated alginate.

Keywords: Chitosan, irradiation, flower plant, plant growth promotion, tissue culture, acclimatization, root, shoot, fresh biomass.

1. Introduction

Chitosan, a poly[β-(1-4)2 amino-2-deoxy-D-glucose] is a functional and basic linear polysaccharide and prepared by alkali deacetylation of chitin. Chitosan, the most abundant natural polysaccharide on the earth after cellulose [1], has been produced mainly from the shell of crutanceans such as shrimp, crab, lobster, squid, etc.[2]. The manual production of this natural polymer in the world is about 2,000 tons [3].

Chitosan and its derivatives have been extensively studied and widely utilized in medicine as a biodegradable for drug derivative system [4], wound dressing, contact lens and in cosmetic as skin care cream and hair sprayer. Chitosan was not only used in waste water treatment for metal ions absorption [2,5] and flocculant of protein, amino acid, etc, but also in biotechnology for enzyme and cell immobilization, protein separation and chromatography.

Chitosan with different molecular weight (Mw) and degree of deacetylation has not only antimicrobial [6] but also plant growth stimulation effect, therefor it has been used in agriculture as agro-products preservative, seed coating and fertilizer. Recently, degraded chitosan was reported to have more applications [7]. Chitosan with Mw from 10^5 - $3x10^5$ induced by irradiation method exhibits fairly high antimicrobial activity in vitro [6]. Irradiated chitosan has been found to reduce the damages of Vanadium and promote the growth and development of rice, wheat and barley [8].

Oligosaccharides including oligochitosan have been recognized to have ability of regulation process of morphogenis [9] and phytoalexin induction to prevent infection of fungal diseases for plant [10]. Fractions of chitosan display elicitor activity inducing a defense reaction tissue of whole plant and suspensions of cultural cells [11]. Oligochitosan can cause an accumulation of antibiotic phytoalexins [12-16], stimulate plant growth and format the root system and strengthen stem. Thus, Oligochitosan induced by enzymatic hydrolysis degradation or by extracttion from cell wall has various effects on plant. In this study, the growth promotion effect of oligochitosan prepared by irradiation degradation method was investigated on plant tissue culture in order to develop the application of irradiated chitosan for plant *in vitro* propagation, because plant tissue culture has been recognized as an efficient tool for plant breeding, plant propagation and biotechnology.

2. Material and method

2.1. Preparation of irradiated chitosan

Chitosan 8B with deacetylation degree of 80% was supplied by Katokichi Chemical Co., Japan. To prepare chitosan with Mw *ca.* 1.5x 10^5, chitosan 8B was irradiated at the dose of 50kGy in powder form [17]. To obtain chitosan solution of 10% (w/v), the chitosan was kept in solution of acetic acid 2.5% overnight at room temperature for swelling and then stirred for 5 hours to completely dissolve. For further degradattion, the chitosan solution was then irradiated by gamma rays from a Co-60 source at the dose up to 250 kGy with the dose rate of 2 kGy/h.

2.2. In vitro growth test

The flower plants used in the present experiment are *Limonium latifolium*, *Eustoma grandiflorum* and *Chrysanthemum morifolium*. To investigate plant growth promotion effect of chitosan irradiated at different doses, 20 shoots of flower plants were cultured in vessel containing rooting medium including half strength of Murashige and Skood's medium [18], 3% sucrose, 0.8% agar for *C. morifolium* and supplemented with 1mg/l 3-indol butyric acid (IBA) for *L. latifolium* and 1mg/l IBA and 0.01mg/l 1-Napthylacetic acid (NAA) for *E. grandiflorum*. Chitosan irradiated at different doses were supplemented into cultural medium with the concentration of 50ppm. The optimum concentration of irradiated chitosan was observed by culturing the flower plant shoots in one-half MS medium as described above and supplemented with 5-100ppm chitosan irradiated at 100kGy. Media was adjusted to pH 5.8 by KOH and HCl before autoclaving at 121°C and 105 kPa for 15 min. All cultures were incubated in the light culture room at 25 ± 1°C with photoperiod 12h per day.

2.3. Transfer to soil

100 plantlets cultured on the medium supplemented with chitosan irradiated at the dose ranging from 50 to 250 kGy with well developed roots were removed and washed handle with care in water. Then they were transferred to pots containing a mixture of soil, ashes and fertilizer and cultivated for 30 days in the greenhouse.

2.4. Data collection and statistical analysis

For each experiment, at least 100 shoots were used and experiments were repeated three times. Data was recorded after incubating for 15days with *C. morifolium.* and 30 days with *L. latifolium* and *E. grandiflorum* and statistically analyzed by variance analysis (ANOVA) with $LSD_{0.05}$.

3. Results and discussion

3.1. Plant growth promotion effect of chitosan irradiated at various doses

The growth promotion effects of chitosan with initial Mw of $1.5x10^5$ irradiated at various doses in 10% solution was investigated on flower plants namely *L. latifolium*, *E. grandiflorum* and *C. morifolium* in tissue culture.

The results in Table 1 indicate that the irradiated chitosan obtained by the doses from 50 to 200 kGy shows stronger effect on the increase of the shoot length 4.0 - 7.1% and fresh biomass 7.8 - 22.3% of *C. morifolium* compared to the untreated one, but the growth effect on the increase of root length is only observed at the doses of 75 and 100 kGy (21.6 - 23.0%). On the other hand, these effects are not observed in the treatments of chitosan unirradiated or irradiated at low dose of 30kGy, while the treatments of chitosan irradiated at the doses ranging from 50 - 100kGy show the higher effect with the higher dose. The results are in agreement with the growth promotion effect of irradiated chitosan on shoot and root of rice and wheat seedling reported by Tham et al. [8]. Furthermore Nagasawa et al. [19] also informed that chitosan with Mn *ca.* 6.10^3 obtained by irradiation of 500 - 1000kGy in solid state showed a strong effect on barley seedling.

Table1. Effect of irradiation dose for chitosan on the growth of *C. morifolium*

Dose kGy	Growth promotion effect					
	The length of shoot		The length of root		Fresh biomass	
	mm	%	mm	%	g/20plants	%
Control	30.6	100.0	22.6	100.0	3.07	100.0
0	30.5	99.8^{NS}	23.1	102.2^{NS}	2.84	92.5^{NS}
10	31.1	101.6^{NS}	21.6	95.4^{NS}	3.23	105.2^{NS}
30	31.1	101.7^{NS}	23.8	105.0^{NS}	3.24	105.7^{NS}
50	33.2	108.7	20.9	92.2^{NS}	3.51	114.6
75	32.6	106.7	27.5	121.6	3.75	122.3
100	32.7	107.1	27.8	123.0	3.74	121.8
150	32.1	105.0	24.3	107.4^{NS}	3.71	121.1
200	31.8	104.0	23.7	104.9^{NS}	3.35	109.3^{NS}
$LSD^*_{0.05}$	1.2	3.6	3.1	13.1	0.39	11.4

** $LSD_{0.05}$: The least significant difference with p=0.05, ** NS: None significance*

Effect of irradiated chitosan on the growth of *E. grandiflorum* is given in Table 2. It is clear that the treatment of chitosan irradiated at dose of 50 - 100 kGy also reaches remarkable growth promotion effect on *E. grandiflorum*. The presentation of the irradiated chitosan into cultural medium makes the root and shoot length and fresh biomass of *E. grandiflorum* increase in 10.4 - 12.7%, 18.6 - 43.6% and 36.1 - 55.2%, respectively. Effect of chitosan irradiated on the growth of *L. latifolium* is also invetigated and the results are presented in Table 3. The treatments of chitosan irradiated at the doses ranging from 50 to 150 kGy also show a strong effect on elongation of shoot length 4.7 - 11.4%, root length 98.7 - 167.9% and increase of fresh biomass 7.8 - 22.3% compared to the untreated one.

Table 2. Effect of irradiation dose for chitosan on the growth of *E. grandiflorum*

Dose kGy	Growth promotion effect					
	The length of shoot		The length of root		Fresh biomass	
	mm	%	mm	%	g/20plants	%
Control	22.5	100.0	12.5	100.0	3.81	100.0
0	22.9	101.8 ^{NS}	12.7	101.6 ^{NS}	4.37	114.6 ^{NS}
10	23.7	105.2 ^{NS}	12.2	105.6 ^{NS}	4.33	113.6 ^{NS}
30	24.3	107.7 ^{NS}	12.5	108.0 ^{NS}	5.29	138.6
50	24.9	110.4	14.9	118.6	5.19	136.1
75	26.5	117.5	15.0	119.7	5.79	151.9
100	25.4	112.7	17.9	142.6	5.92	155.2
150	23.1	102.4 ^{NS}	18.0	143.6	5.01	131.4
200	22.7	100.9 ^{NS}	13.4	106.9 ^{NS}	4.88	128.0
$LSD_{0.05}$	2.0	8.2	1.7	11.8	1.09	22.1

Table 3. Effect of irradiation dose for chitosan on the growth of *L. latifolium*

Dose kGy	Growth promotion effect					
	The length of shoot		The length of root		Fresh biomass	
	mm	%	mm	%	g/20plants	%
Control	35.0	100.0	2.8	100.0	1.82	100.0
50	36.5	104.7	5.6	198.7	2.50	137.6
100	38.2	109.2	5.8	204.6	2.41	132.3
150	39.0	111.4	7.6	267.9	2.59	142.3
250	36.9	105.4	4.7	165.2	2.10	115.6
$LSD_{0.05}$	1.1	3.0	2.3	43.3	0.34	14.8

Farmer et al. (1991) [20] and Darvill et al. (1984) [13] reported that Oligosaccharides including oligochitosan play a role of cell signaling in plants for induction of phytoalexin that protects the plant from fungal infection. Recently, irradiated chitosan has been found to reduce the stress of heavy metal (zinc, vanadium, etc...) on plant. On the other hand, according to Hien et al. [21], irradiated alginate not only showed a remarkable growth promotion effect but also could prevent the attack of green agae on root of rice.

The results in Table 4 show that the presentation of irradiated chitosan not only promotes the growth of shoot, root and fresh biomass of flower plant but also improves the survival ratio of transferred flower plantlets after acclimatizing in the greenhouse. The result is clearer after acclimatizing for 30 days.

Based on the results it can be seen that irradiated chitosan obtained by irradiation of chitosan 8B (Mv~$1.5x10^5$) at the dose of 75-100 kGy in 10% solution shows a stronger growth promotion effect on root and shoot length and fresh biomass of *C. morifolium*,*E. grandiflorum* and*L. latifolium*. But the product obtained by the dose of 100kGy shows the strongest effect.

Table 4. Effect of irradiated chitosan on the survival ratio of transferred flower plantlets after acclimatizing in the greenhouse

	Survival ratio, %								
Dose,	*C. morifolium*			*E. grandiflorum*			*L. latifolium*		
kGy	10days	20days	30days	10days	20days	30days	10days	20days	30days
Control	86.0	84.0	78.0	84.7	64.4	55.0	88.0	80.3	77.0
50	94.7	94.0	92.0	85.6	67.8	57.6	97.7	89.0	86.0
100	95.0	92.7	90.3	90.9	80.3	76.5	97.7	88.0	87.0
150	94.3	94.0	92.0	89.1	71.0	61.9	98.0	85.3	83.1
250	91.7	89.0	89.0	89.4	71.1	61.4	95.3	85.3	82.8
$LSD_{0.05}$	4.7	4.8	7.3	4.1	3.7	2.6	3.0	4.0	4.4

3.2. Optimum concentration of irradiated chitosan on plant growth promotion

Chitosan irradiated at 100kGy was chosen to investigate the optimum concentration for flower plant in tissue culture. The effect of irradiated chitosan concentration on *C. morifolium* is presented in Table 5. The results indicate that the supplements of irradiated chitosan show strong effect on *C. morifolium* . The significant effect on the increase of shoot and root length and fresh biomass is observed at the concentrations of 50-150ppm, but this effect is decreased at the concentration higher than 200ppm. The optimum concentration of irradiated chitosan is found at 100ppm. The result is good in agreement with the results were reported by Tham et al. [8] that irradiated chitosan (obtained by irradiation of 100kGy in 1% solution) showed strong effects on wheat and rice seedlings at the concentration of 100ppm.

Table 5. Effect of irradiated chitosan concentration on the growth of *C. morifolium*

Irradiated chitosan conc., ppm	Growth promotion effect					
	The length of shoot		The length of root		Fresh biomass	
	mm	%	mm	%	g/20plants	%
0	29.6	100	38.6	100	2.70	100
5	31.5	106.3 ^{NS}	40.1	104.1^{NS}	3.36	124.7
10	30.5	102.9 ^{NS}	40.2	104.2^{NS}	3.21	118.9
20	30.3	102.5 ^{NS}	42.3	109.6	3.13	116.2
30	32.2	108.8^{NS}	40.7	105.6^{NS}	3.19	118.2
50	33.2	112.2	44.1	114.4	3.33	123.4
70	32.7	110.6	43.5	112.8	3.34	123.9
100	35.3	119.4	54.2	140.6	4.53	168.1
150	34.7	117.3	45.4	117.6	3.59	133.0
200	30.8	104.2 ^{NS}	32.3	83.8 ^{NS}	2.44	90.4 ^{NS}
$LSD_{0.05}$	3.0	9.3	3.5	8.3	0.35	10.8

Table 6. Effect of irradiated chitosan concentration on the growth of *E. grandiflorum*

Irradiated chitosan conc., ppm	Growth promotion effect					
	The length of shoot		The length of root		Fresh biomass	
	mm	%	mm	%	g/20plants	%
0	21.5	100.0	14.7	100.0	2.98	100.0
5	23.3	108.5	15.8	107.7 ^{NS}	3.04	101.9 ^{NS}
10	24.4	113.7	17.2	116.8	3.20	107.3
20	24.7	115.2	19.4	131.8	3.66	122.8
30	25.0	116.5	24.5	166.9	4.44	148.5
50	24.6	114.6	17.4	118.6	3.16	106.2
100	23.1	107.5	14.5	98.9 ^{NS}	3.12	104.9 ^{NS}
$LSD_{0.05}$	1.8	7.37	2.0	11.6	0.16	5.1

As given in Table 6 that irradiated chitosan supplemented into cultural medium shows strong effect of growth promotion on *E. grandiflorum*. The shoot length, root length and fresh biomass increase with the irradiated chitosan concentration ranging from 20-50 ppm

and the concentration of 30 ppm shows the strongest effect. The result from Nagasawa et al. [18] indicated that irradiated chitosan (obtained by irradiated at 1000kGy in powder state) showed a hight effect on the increase of growth and development of barley seedling at the concentration of 30ppm.

The results in Table 7 indicate that irradiated chitosan supplemented with the concentrations of 20-60ppm show remarkable effect on growth of *L. latifolium*. The optimum concentration is 40ppm.

Table 7. Effect of irradiated chitosan concentration on the growth of *L. latifolium*

Irradiated chitosan conc., ppm	Growth promotion effect					
	The length of shoot		The length of root		Fresh biomass	
	mm	%	mm	%	g/20plants	%
0	26.2	100.0	4.9	100.0	1.39	100.0
20	33.9	129.5	5.4	109.6 ^{NS}	1.86	133.4
40	35.1	133.9	6.1	123.4	2.14	153.6
50	33.1	126.5	5.4	109.6	1.75	126.1
60	34.5	131.7	5.8	118.3	1.78	127.7
80	29.1	111.2	5.2	105.3	1.55	111.7
100	28.9	110.2 ^{NS}	4.6	92.0 ^{NS}	1.24	89.4 ^{NS}
$LSD_{0.05}$	3.3	10.5	0.8	15.49	0.16	9.7

4. Conclusions

According to the results, it can be concluded that Chitosan with initial Mv *ca.* $1.5x10^5$ irradiated at 100kGy in 10% solution shows a strong growth promotion effect on flower plants namely *Limonium latifolium*, *Eustoma grandiflorum* and *Chrysanthemum morifolium* in tissue culture. The suitable concentrations of chitosan irradiated at 100kGy are found to be 100ppm for *Chrysanthemum morifolium,* 30ppm for *E. grandiflorum* and 40ppm for *L. latifolium*. In addition, the supplementation of irradiated chitosan makes the survival ratio of transferred flower plantlets improve after acclimatizing for 30 days in the greenhouse.

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19 Preparation and Characteristics of Chitosan-ContainingGels by γ-Irradiation

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INTRODUCTION

The radiation-chemistry has become one of the effective methods for preparation of synthetic biopolymers. The principal advantage of this method is free from toxic impurities such as polymerization catalyst and cross-linking agent and also involves a radiation-induced sterilization effect.

Chitosan (CS), which is produced by alkaline deacetylation of chitin, is soluble in water only at low pH. It is known that CS forms polyelectrolyte complexes with a weak acid such as poly(acrylic acid)(AAc) through electrostatic attraction. Such a polyelectrolyte complex is applicable to responsible biopolymer materials as interpenetrating polymer networks because of its unique feature of relative independence [1].

We studied the synthesis and its characteristics of a series of multifunctional biomedical materials by the simultaneously occurring reactions of radiation-induced polymerization and self-bridging in aqueous solutions without any initiator or cross-linking agent [2]. In this study, we synthesized a novel CS crosslinked AAc gel by radiation processing in combination with complexation of the polymers, and will report the preliminary.

EXPERIMENTAL

CS10, CS 100, and CS1000 with different molecular weights were purchased from Wako Pure Chemical Industries, Ltd. AAc and acetic acid were obtained from Kanto Chemical Inc. The swelling behavior of the CS-crosslinked AAc gels was measured in buffer solutions with pH 3.0 – pH 8.0. Other chemicals were of reagent grade.

To synthesize the CS-crosslinked AAc gel, 2 ml of aqueous solutions containing 0.4 ml and 0.8 ml of AAc was homogeneously mixed with 0.02 g of CS and then this solution was irradiated for 3 hours at a dose rate of 10 kGy/h and 0°C (ice water temperature) with γ-rays from a ^{60}Co source. After irradiation, the samples obtained were washed with a 2 % aqueous solution of acetic acid to remove unreacted products and the resulting insoluble fraction was defined as the gel involving the crosslinked network structure. The modified CS gels were lyophilized after replacing with distilled-deionized water, immersed in a certain pH buffer solution at 37°C and kept until reaching the equilibrium.

Perkin-Elmer DSC-7, was used to investigate the physico-chemical character of the three-dimensional polymer networks generated by a radiation crosslinking reaction

between the CS chains and the poly(AAc) chains. DSC was run at a constant rate of 5°C / min in the range of 25 to 300°C.

RESULTS AND DISCUSSION

It is well known that CS dissolved in an aqueous acetic acid solution is subject to main chain scission during γ-irradiation [3]. On the other hand, CS possessing basic amino groups can interact with the carboxyl (-COOH) groups of AAc as the anionic electrolyte through electrostatic interaction. Since the gel fraction of the CS modified samples obtained with 30 kGy at 0°C reached 100 %, all the chitosan molecules in the mixture system were incorporated into the obtained AAc networks.

The pH dependence of equilibrium swelling (Sw) as a function of AAc concentration at 37°C for CS10 modified gels is shown in Figure 1. A polymer of AAc has a pKa of 4.7, in contrast to a pKa of 6.1 for CS. The gels were in shrunken state at < pH 3.0 in all cases as seen clearly in Fig. 1. At > pH 3.0, the swelling behavior exhibited a big difference by introducing CS10 into poly(AAc) chains; the Sw values of the two gels, 40 % AAc without CS10 and 40 % AAc with CS10 were observed to be approximately 80 and 25, respectively, at pH 5.0. This gap is strongly dependent on the formation of networks crosslinked between the poly(AAc) chains and the CS chains.

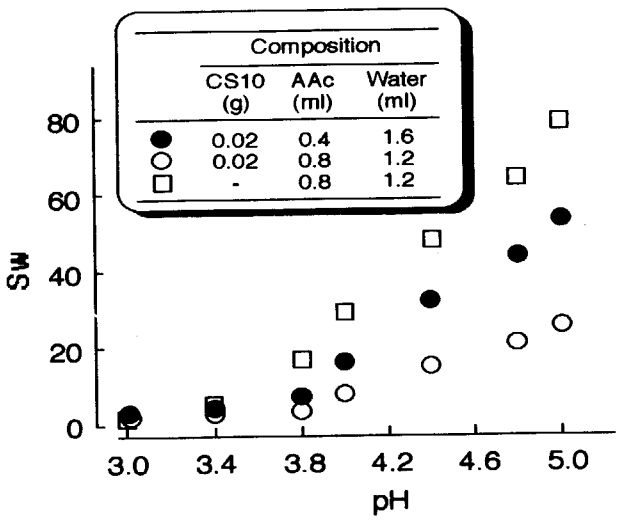

Figure 1. The pH dependence of equilibrium swelling as a function of AAc concentration for CS10 modified gels obtained with a dose of 30 kGy at 0°C.

The time-dependence of swelling ratio swollen in pH 7.0 at 37°C for AAc gels crosslinked with different molecular weight CS chains was investigated and it was found that the rate of swelling of CS10 modified gel was much faster than those of CS100 and CS1000. This slower swelling of higher molecular weight CS may be attributed to the formation of much more efficiently crosslinked networks between the higher molecular weight CS chains and poly(AAc) chains, in other words, the formation of a more tightly crosslinked networks.

The DSC profiles of CS10 modified gels which were lyophilized after treated in buffer solution at a certain pH are shown in Figure 2. A lyophilized gel of AAc swollen at pH 7.0 exhibited two endothermic peaks at temperatures of 145°C and 220

- 240°C; the former is due to the moisture in the gel, while the latter is attributable to the intramolecular dehydration reaction which led to the formation of anhydride structures in the macromolecular chain, followed by a decarboxylation of AAc chains (Figure 2a). In the case of CS10 modified gel swollen at pH 7.0 and then lyophilized (Figure 2b), a broad peak in the range of 220 to 240°C completely disappeared, compared with a pure poly(AAc). This peak disappearance is due to the decomposition without intermolecular dehydration reaction according to the structural change in the gel network induced by the existence of CS chains. Such a structural change is the so-called scrambled-egg structure that sterically inhibits the reaction between the COO^- groups in AAc chains and $^+NH_3$ groups in CS chains. Figure 2b also showed a new endothermic peak at 200°C, assuming that the intermolecular esterification reaction takes place between the OH groups in CS chains and the COOH groups in AAc chains. Contrary to the results of CS10 modified gel at pH 7.0, at pH 3.0 the gel used in this study is in collapsed state. The DSC profile indicated in Figure 2c is characterized by an appearance of stronger peak at approximately 250°C owing to the amidation reaction between the NH_2 groups in CS chains and the COOH groups in AAc chains.

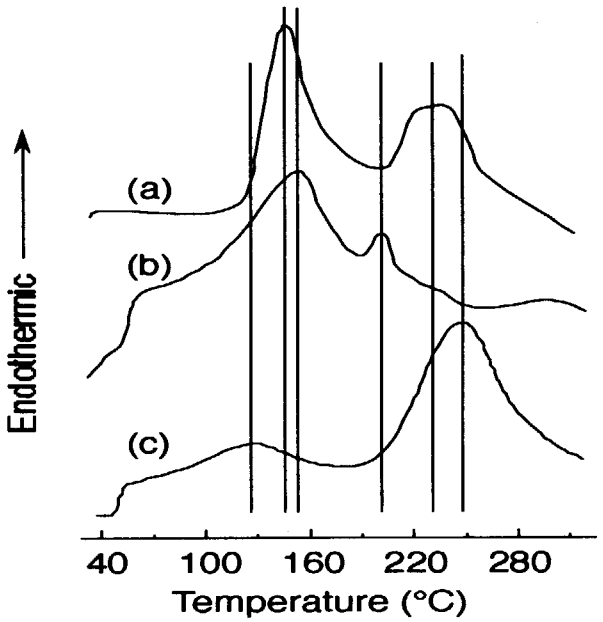

Figure 2. DSC profiles of (a) pure poly(AAc) gel lyophilized after swollen at pH 7.0 and CS10 modified gels lyophilized after swollen at (b) pH 7.0 and (c) pH 3.0.

On the basis of the results of swelling kinetics and DSC data, the proposed model for illustrating the formation of CS cross-linked AAc gels by radiation processing is shown in Figure 3. This reaction process consists of three steps, (a) formation of polyelectrolyte complex, (b) polymerization and self-bridging of AAc, and (3) cross-linking between the CS chains and the AAc chains. When CS chains were dissolved in an aqueous solution of AAc monomer with a pKa value of 4.26, this biopolymer partially forms a linear polyelectrolyte complex although most of AAc monomer is set free in its solution. During γ-irradiation, either free AAc or AAc contained in the polyelectrolyte complex is first polymerized to give linear polymers. It should be

pointed out here that CS chains in polyelectrolyte complex partially undergo degradation during the course of polymerization of AAc. The subsequent reaction steps are the formation of cross-linked networks between the two chains, AAc • AAc and AAc • GS.

In conclusion, we synthesized a novel CS cross-linked AAc gel by means of simultaneously occurring radiation reactions of polymerization and self-bridging in aqueous solutions without any cross-linker. In this radiation process, the mutual

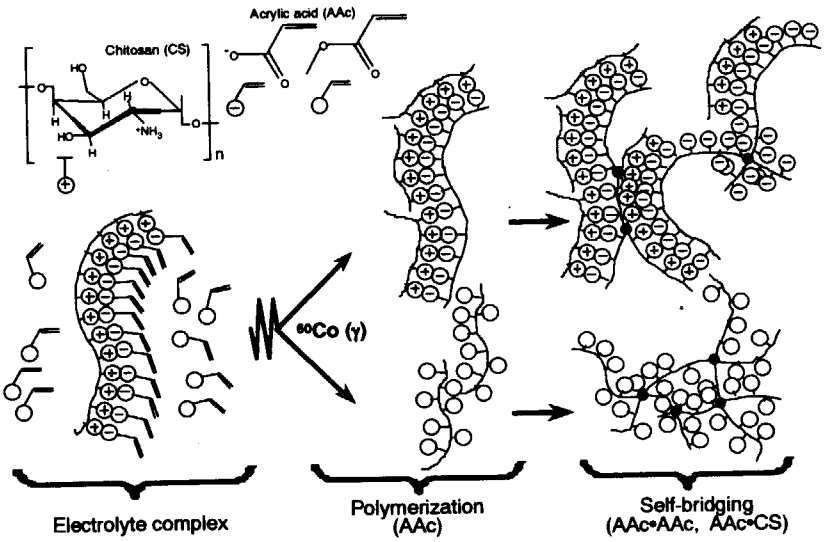

Figure 3. Proposed model for illustrating the simultaneously occurring process of radiation-induced polymerization and self-bridging of AAc in aqueous solutions, followed by a formation of crosslinked networks between the poly(AAc) chains and the CS chains according to a strong chemical affinity between the NH_2 groups (CS) and the COOH groups (AAc).

interaction among the formation of electrostatic polyelectrolyte complex between the CS chains and the AAc chains, main chain scission of CS, polymerization and self-bridging of AAc, and cross-linking between the CS chains and the AAc chains. This radiation technique, which is free from toxic ingredients (polymerization initiators, cross-linkers, etc.), is a useful tool for the design of novel biomedical polymers by employing biological substances such as glucosamine, amino acids, proteins, carrageenans, and hyaluronic acid.

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Session 4

Radiation Processing of other Polymers

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20 Radiation Vulcanization of Natural Rubber Latex with Low Energy Accelerator-II

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Abstract

The natural rubber latex (NRL) was radiation vulcanized under a low energy electron accelerator. Accelerating voltage and maximum beam current of this accelerator are 250 kV and 10 mA respectively. Irradiation was carried out in a reaction vessel with constant stirring. The capacity of the vessel is 18 liters. Radiation vulcanization accelerators (RVA) were normal butyl acrylate (*n*-BA) and nonane-diol-diacrylate (NDDA). NDDA has no bad smell like that of *n*-BA. 20 minutes irradiation time is enough to vulcanize 14 liters of latex when 5 phr RVA (both types) are used. Maximum of ~30 MPa tensile strength was obtained with 5 phr NDD-A. However the remained NDDA is difficult to remove due to high molecular weight.

Water-extractable proteins content was determined in dipped films for various leaching conditions without and with additive (polyvinyl alcohol, PVA). Water-extractable proteins content is reduced to ≤ 41 by adding 5 phr PVA and leaching for 8 hours. The tackiness of the dipped films is reduced to 0.1 from 9 gf by mixing 6 phr PVA with the irradiated latex.

Hand gloves (surgical and examination) were successfully produced from the irradiated latex.

Keywords: Natural rubber latex, Radiation vulcanization, Electron beam, RVA, NDDA, *n*-BA, Tensile strength, Aging, Protein content, Tackiness.

1. Introduction

Radiation method for the vulcanization of natural rubber latex (NRL) emerged in late fifties and got popularity for some distinct advantages over the conventional one especially for its approach to cleaner environment. But it was not proved to be suitable for industrial application due to some drawbacks such as high cost in establishing a radiation source (Co-60 for gamma rays) and of course low quality of products. Irradiations of NRL were carried out by electron beam of various types and variable energies [1-6]. It is expected that radiation vulcanization of natural rubber latex (RVNRL) with low energy electron accelerator would reduce the initial investment and irradiation cost. A pilot plant with 250 keV electron beam (EB) for RVNRL was installed recently at Takasaki Radiation Chemistry Research Establishment, Takasaki, Japan as an alternative source.

NRL can be vulcanized by ionizing radiation without any accelerator or additive [7-8]. But the required dose is very high. So a radiation vulcanization accelerator (RVA) is used to reduce the vulcanization dose for economic reason. Till now normal butyl acrylate (*n*-BA) is being used as an effective RVA. But it has bad smell and enhances the coagulation of latex [9-10]. In this investigation a new RVA with no smell was used. *n*-BA was also used for comparison.

Latex proteins allergy is of concern worldwide. The NRL gloves cause the problem for the latex allergy sensitive people. The water-extractable (EP) proteins contained in the latex are responsible for the allergy. In this investigation efforts were made to minimize the protein content in the RVNRL films.

The aging property of RVNRL products is poor [11-12]. In the present study antioxidants were added to improve the aging property of RVNRL products.

The tack of rubber causes serious problem in the manufacturing process during stripping of gloves from the formers. For dipped products this tack should be removed. Various aspects for the reduction of tackiness have been investigated.

In order to produce RVNRL with suitable strength and quality for the production of dipped goods, especially hand gloves, various schemes were adopted in irradiating latex with electron beam pilot plant. These are,

- Optimization of various parameters of EB pilot plant for the production of good quality RVNRL.
- Improvement of aging property of the RVNRL products (rubber films, hand gloves etc.).

- Reduction of proteins in RVNRL.
- Reduction of tackiness in the RVNRL products.
- Test production of hand gloves (surgical and examination).

The aim of these research works was to produce dipped goods from EB irradiated latex suitable for commercialization.

2. Experimental

2.1. Irradiation of latex under EB

The latex was irradiated in a reaction vessel under EB after mixing with RVA (NDDA and / *n*-BA) with constant stirring [13]. An antifoam, BYK022, from BYK-Chemie GmbH Co. Ltd., Germany was mixed with latex to suppress the foam formation. The mixing of RVA was done for 30 minutes in the reaction vessel with 150 rpm.

2.2. Preparation, leaching and drying of RVNRL films

Spreading 25-35 mL latex over each of several raised rimmed glass plates made cast films of latex. They were allowed to air dry till transparent. The films were heated in an oven at 80°C for 1 hour after leaching with 1% ammonia and air drying. Dipped films were made by dipping cleaned glass plates into the latex for a definite length of time. The films were air dried up to gel formation and leached in wet-gel condition with hot water at 56°C for various lengths of time. They were heated in an oven at 80°C for 40 minutes.

2.3. Measurement of tensile properties

Dumbbell shaped test pieces were cut using dumbbell cutter of precised size for natural rubber film. Tensile and related properties were measured with a tensile machine, Strograph-R1, Toyoseiki, Japan.

2.4. Measurement of swelling ratio and cross-link density

A weighed amount of rubber film was immerged into toluene for 24 hours. The swelling ratio was calculated from the difference of the weight before and after swelling. The cross-link density was calculated using Flory and Rehner equation [14].

2.5. Use of antioxidants for the improvement of aging property

The antioxidants used are Nonflex- arba, Nonflex CBP, Nonflex BB, Nonflex EBP, Nonflex LAS, Nonflex WS, Nonflex TNP, Nonflex DCD, BHT suwanox, Ozonone-35, Ozonone-6C, Ozonone EX-SX supplied by Seiko Chemical Co. Ltd., Japan,

Nocrac DAH, Nocrac CD, Nocrac SP, Nocrac white supplied by Ouchishinko Chemical Industrial Co. Ltd., Japan and Sumilizer GA-80, Sumilizer P16 supplied by Sumitomo Chemicals, Japan. In order to find out the retention of weight fraction, RVNRL films were extracted in xylene in presence of antioxidants. Emulgen-147 and Emulgen-420 from Kao Corporation, Japan were used for the preparation of antioxidant emulsion.

The dipped films from the antioxidant mixed RVNRL were prepared and accelerated aging test was done at 100°C for 22 hours in an air-circulated oven, Geer oven 45-P, Toyo Seiki, Japan.

2.6. Determination of EP in RVNRL films

The RVNRL films were cut into small pieces. 1 g of the pieces for each sample was weighed and extracted in water for 2 hours at 37°C. The concentration of proteins in the extract was determined following Pierce Micro BCA™ Protein Assay. Test tube protocol with a linear working range of 0.5-20 μg/mL was followed [15].

2.7. Measurement of tackiness

The tackiness of the RVNRL films were measured between the rubber films and stainless steel by tack tester, TACII, Rhesca Co., Ltd., Japan.

3. Results and discussion

3.1. Optimization of various parameters of EB pilot plant for the production of good quality RVNRL

For a suitable setting of experimental condition for RVNRL under EB various parameters were optimized.

3.1.1. Irradiation with varying EB current (10 mA and 5 mA): The maximum value of tensile strength Tb (26 MPa) is obtained for 30 minutes irradiated latex film using NDDA but 40 minutes is needed if the latex is irradiated with low EB current (5 mA) [1].

3.1.2. Use of n-BA as RVA: NRL was irradiated under EB with 10 mA current using *n*-BA as RVA instead of NDDA. Fig. 1 shows the Tb and Eb of RVNRL films for various lengths of irradiation times. The maximum Tb (26 MPa) is obtained from 30 minutes irradiated latex. The time required to obtain maximum Tb is same as that of the film obtained by using NDDA. Similar accelerating efficiency is obtained from both *n*-BA and NDDA. Fig. 2 shows the plot of swelling ratios and cross-link density

of those films against lengths of irradiation time. Little lower cross-link density is obtained from RVNRL films with *n*-BA especially at longer lengths of irradiation time and thus Eb is higher than that of with NDDA [1].

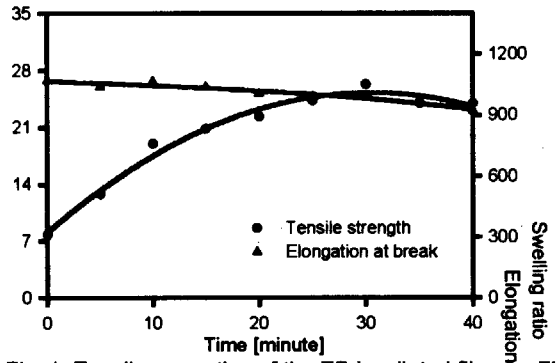

Fig. 1. Tensile properties of the EB irradiated films versus irradiation time.

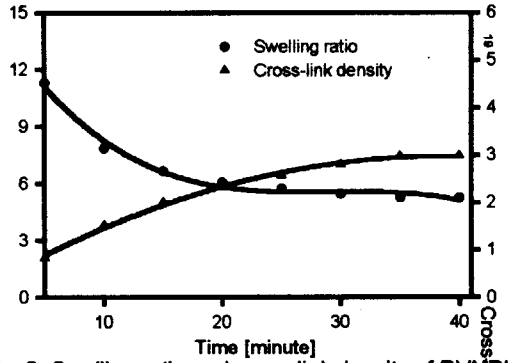

Fig. 2. Swelling ratio and cross-link density of RVNRL film versus irradiation time.

3.1.3. Effect of mode of RVA addition: 50% emulsion of RVA was prepared using 4 phr *n*-BA and 1 phr NDDA with water and emulgen-420. For the addition of RVA two scheme were adopted (1) all together pouring slowly by stirring before irradiation and (2) fractional addition during irradiation (1/4th each at 10 minutes interval). Table 1 shows the Tb and Eb of the RVNRL films for various lengths of irradiation time.

Table 1. Tensile properties at various lengths of irradiation time for two different modes of RVA addition.

Time (min.)	At a time addition		Fractional addition	
	Tb (MPa)	Eb (%)	Tb (MPa)	Eb (%)
0	3.89	960	4.67	1095
5	14.47	1075	11.24	1090
10	20.81	950	16.08	1145
15	23.14	950	19.17	1090
20	21.83	875	18.37	1060
25	18.93	825	23.31	1025
30	17.31	775	21.91	960
35	15.06	740	20.42	910
40	13.46	720	20.98	835

It is found that maximum 23 MPa was obtained from the RVNRL film obtained by irradiating 15 minutes if the RVA is added at a time. In case of fractional addition of RVA it is seen that the length of time required to obtain maximum value of Tb is increased (25 minutes). This increment seems to be obvious because the RVA molecules have less scope to come in contact and to form free radical. So to have sufficient cross-linking it is to wait for new RVA for reacting sites. However the value for maximum Tb in both the cases is same.

3.1.4. Effect of volume of latex: The capacity of the reaction vessel is 18 liters. But due to stirring and heating effect the volume of latex used for irradiation is considered important. 16 liters latex was irradiated in the reaction vessel maintaining other parameters same. It is seen that the Tb obtained by using higher volume (16 L) of latex is lower. So 14 liter of latex was selected as the ideal volume. Further lowering of volume could not work well because the distance of the latex surface and beam window would become large that would cause delayed vulcanization.

3.1.5. Effect of dilution and centrifugation: For this purpose the Iotex brand latex was diluted to 25% by 1% ammonia, irradiated for various lengths of time (20, 30 and 60 minutes, separately) using 5 phr NDDA. After irradiation 0.2 phr of PVA-105 as 10% solution in water was added to the latex and centrifuged. The centrifuged latex was again diluted to 50% TSC. Table 2 shows the tensile properties and swelling ratio for RVNRL films obtained by each length of irradiation time. It is seen that the Tb for 20 minutes irradiated NRL film is maximum. The Tb decreases at higher lengths of irradiation time. The low TSC latex gives better Tb after irradiation. In this case the value of Tb obtained by 20 minutes irradiation is also high enough. The low TSC latex contains much water. So the formation of free radical is higher and the mobility of the rubber molecules also high. So the frequency of moving the rubber particles to expose under the beam becomes high. Ultimately cross-linking between the rubber chains becomes larger.

Table 2. Tensile properties and swelling ratio

Irradiation time (min.)	Tensile strength (MPa)	Eb (%)	S. R.
0	6.43	1300	-
20	29.82	995	4.9567
30	27.35	900	4.3295
60	15.39	635	3.4665

This is fact is evident from swelling ratio data in table 2. The swelling ratio obtained under this condition is similar to that obtained with latex of 50% TSC irradiated for 30 minutes. At higher doses cross-link increases too much and the films becomes hard. So cracking was found in the cast film from 30 and 60 minutes irradiated latex. Moreover by centrifugation the non-rubber and some particles that may have been produced by heating during irradiation are removed that helps the rubber molecules come closer to have better entanglement. The films obtained after centrifugation was

also very transparent. This irradiation process of latex could be helpful in practical application if field latex is irradiated first and then centrifuged.

3.1.6. Cover of the vessel: The reaction vessel has a cooling cover with titanium film window. Titanium film is very expensive. So aluminum foil was used as the cover of the vessel. It is simple and easy to use and also very cheap in comparison to titanium film. There was no difference in quality and strength of the RVNRL films obtained by covering with aluminum foil to that obtained with titanium film.

3.2. Improvement of aging property

3.2.1. Extraction of rubber films in presence of antioxidants: To find out a suitable antioxidant for preventing degradation, RVNRL films were extracted in xylene in presence of 1% antioxidants and with air bubbling. It was found that after 8 hours extraction the film extracted without any antioxidant has ~63% retention of weight fraction. Nonflex TNP has 95% retention of weight fraction that is maximum value among all antioxidants. But it could not be used due to its harmful effect to the endocrine system. Nonflex arba, Nonflex CBP, BHT Suwanox, Nonflex WS, Sumilizer P16, Ozonone-35, Nocrac DAH, Ozonone 6C possess retention value >91%. Considering color and retention value three antioxidants, Nonflex CBP, Sumilizer P16 and Nocrac DAH were selected. As DAH produces color during extraction it was used with other antioxidants to minimize coloration.

3.2.2. Aging test in presence of selected antioxidants: The best combination was used for aging test at 100°C for 22 hours as specified in the ASTM standard for aging test. 100% retention of Tb was obtained by using DAH, CBP and P16. 1 phr PVA was added to find out its effect on aging. 100% retention was achieved for both the films with added PVA and without added PVA. But the Tb reduces by the addition of PVA to some extent. The values of elongation of the films are within a good range.

3.3. Reduction of protein content in RVNRL films

3.3.1. Protein content in dipped film from EB irradiated latex: Dipped films were prepared from RVNRL containing 5 phr PVA using $Ca(NO_3)_2$ as coagulant. Table 3 shows the protein content after various lengths of leaching time.

Table 3. Residual protein content after various lengths of leaching time

Length of leaching time	Protein content (μg/g) 1% aqueous ammonia	Water at 56°C	Water at 72°C
0	391	391	391
10 m	152	79	115
20 m	105	83	100
30 m	71	78	88
1 h	54	63	73
2 h	58	53	68
4 h	47	50	27
8 h	20	41	23*
24 h	-	19*	-

Note: Film thickness = 0.1-0.13 mm, *films became brown

It is found that the protein removal extent varies with the type of agent. After 8 hours leaching it reduces to 20, 41 and 23 μg/g with 1% ammonia, water at 56°, and 72°C respectively. But with 1 hour leaching the concentration of proteins reduces to fairly low value.

3.4. Reduction of tackiness in RVNRL films

3.4.1. Effect of PVA concentration: Dipped films were made using $Ca(NO_3)_2$ as coagulant. PVA of various concentrations was added to the RVNRL before casting.

Table 4. Tackiness of dipped film at various concentrations of PVA

Concentration of PVA (phr)	Tackiness (gf) Outer surface	Inner surface
0.5	9.28	12.04
1.0	6.82	9.58
1.5	5.82	9.18
2.0	4.40	6.24
3.0	2.80	3.23

It is found that the tackiness is reduced with the increased concentration of PVA. It is about 3 gf at the highest concentration of 3 phr. The tackiness of the outer surface is less than that of the inner surface. As PVA is water- soluble it might be migrated to the outer surface during wet-gel leaching.

3.4.2. Effect of length of leaching time with higher concentration of PVA: The concentration of PVA addition to RVNRL was increased further (6 phr) and the films were leached for various lengths of time with 1% ammonia and water at 56°C. Table 5 shows the effect of length of leaching time with two different leaching agents. By

leaching for 5 to 10 minutes the tackiness becomes very low. It is less for the outer surface than for inner surface with both the agents. By further increasing the leaching time the tackiness does not decrease further. It seems that the outer surface tack of 0.1 gf is due to PVA itself. The PVA film possesses same level of tackiness.

Table 5. Tackiness at various lengths of leaching time

Length of leaching Time (min.)	Tackiness of water (56°C) leached film (gf)		Tackiness of ammonia leached film (gf)	
	Outer surface	Inner surface	Outer surface	Inner surface
0	0.22	2.18	0.22	2.18
5	0.06	1.40	0.10	2.22
10	0.10	0.43	0.10	1.84
20	0.08	1.50	0.10	1.68
30	0.06	1.66	0.10	1.68
60	0.18	1.12	0.10	0.92
120	0.36	0.86	0.10	1.26

3.5. Production of hand gloves

Hand gloves were produced successfully from EB irradiated latex.

4. Conclusion

1. For the vulcanization of NRL higher EB current needs shorter vulcanization time. NDDA and *n*-BA show similar accelerating efficiency with EB irradiation.
2. At a time addition of RVA is better than fractional addition. Better Tb is obtained by irradiation of low TSC latex followed by centrifugation.
3. Aluminum foil can be used as a cover of the reaction vessel of EB.
4. Using antioxidant 100% retention of Tb after aging can be obtained.
5. Water extractable proteins can be reduced to the acceptable level by PVA addition and leaching.
6. PVA can reduce tackiness to a very low level but can not removed completely.
7. Hand gloves can be produced successfully from EB irradiated latex.

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21 Radiation Cosslinking of Bionolle and its Biodegradation

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Abstract

Biodegradable aliphatic polymer such as poly(butylene succinate) (Bionolle#1001), poly(butylene succinate adipate copolymer) (Bionolle #3001 and #3020) were irradiated to give crosslinking structure in the presence of inorganic materials. Pure Bionolle#1001, Bionolle#3001 and #3020 have low gel fraction in radiation. These samples have high gel fraction by irradiation in the presence of silicon dioxide and carbon black, especially for Bionolle#3001. Biodegradability of crosslinked Bionolle#3001 evaluated by enzymatic and soil burial tests were accelerated by addition of inorganic materials.

Keywords: Bionolle, Inorganic Materials, Radiation Crosslinking, Biodegradation.

1. Introduction

Radiation crosslinking is major technology of radiation processing in the industry. Radiation is effective for improvement of heat resistance and processability of polymer materials. These properties are given by radiation crosslinking technique. Cable and mobile tire are often irradiated to introduce crosslinking structure in industry. Hence, radiation is useful tool for modification of polymer materials. Biodegradable polymer degrades by bacteria in the soil, it is so-called friendly acceptable polymer. It is reported that electron beam irradiation is effective to produce biodegradable Bionolle foam [1]. Heat resistance of Poly(ε-caprolactone) (PCL) was improved by irradiation at super cooled state[2]. Bionolle have melting point of 90~120°C, this thermal properties is similar to that of polyethylene. Bionolle products cause deformation by contacting of hot water. Thus, improvement of heat resistance is

essential for expansion of application field. We have attempted to enhance radiation crosslinking efficiency of Bionolle in the presence of inorganic material. In this article, radiation crosslinking behavior in the presence of silicone oxide or carbon black and its biodegradability was investigated.

2. Experimental

2.1. Materials

The properties of Bionolle samples are shown in Table 1. These samples were produced at Showa High Polymer Co. Ltd., Japan.

Table 1. Properties of Bionolle used in this work

Commercial Sample name	Mw (x 10^5)	Melting point (°C)	Density (g/cm^3)	△H (J/g)
Bionolle#3001 (PBSU-co-AD)	2.96	92	1.23	45
Bionolle#3020 (PBSU-co-AD)	1.56	93	1.23	44
Bionolle#1001 PBSU	1.75	114	1.26	72

Carbon black (Charcoal, activated, Granural) and Silicon Dioxide (Wako Pure Chemical Industries.Ltd) are used without purification.

2.2. Preparation of samples and irradiation

Biodegradable polymer and inorganic material was mixed in a labo plastomill model 50C150 (Toyo Seiki), and the speed of mixing was 20 rpm, at 150°C. After mixing the sample was press to form 0.5 mm thickness sheet in a Ikeda hotpress at150°C for 3 min preheating, then were heated at the same temperature for another 3 min at pressure of 120 kgf/cm^2, after that the sample was cooled to room temperature by cold press using water as a coolant for 3 min. Irradiation of samples was carried out in the atmosphere of air and vacuum using an accelerator with energy 1 Mev and current 1 mA, at various doses from 30 to 200 kGy at a dose rate of 10 kGy/pass.

2.3. Gel content

Gel content of irradiated samples were estimated by measuring the insoluble part in boiling chloroform for 48 h. The gel fractions were calculated as a ratio of dried gel to the initial weight of the polymer.

$$\text{Gel fraction (\%)} = [(W_g/W_i)] \times 100\%$$

Where W_g is the weight of gel and W_i is the weight of original sample.

2.4. Enzymatic degradation test

The evaluations of enzymatic degradation of Bionolle samples were carried out by determining the rate of their solubilization. The samples were immersed in phosphate buffer solution (pH 7.4) with composition of reaction mixtures as follows :

0.2 M phosphate buffer (pH 7.0) 4.0 ml

enzym, Lipase AK (10 mg/ml) 1.0 ml

0.1 % surfactant ($MgCl_2$) 1.0 ml

The thin films of Bionolle samples with initial weight about 9 mg with a dimension of 10 mm x 10 mm x 0.1 mm were oven dried at 40°C for 24 h. The films were put into test tubes and 5.0 ml of reaction mixture was added and incubated at 55°C with shaking for various times. Then the films were taken out from the test tubes and washed with distilled water, methanol and dried in oven to constant weight at 40°C. The enzymatic degradation is expressed as percentage of weight loss.

2.5. Soil burial test

The soil burial test was performed in plastic troughs, 57 cm long by 17 cm wide by 14 cm high. The soil consisted of composted top soil, black garden soil, pond sludge in equivalent ratios maintained at a pH of 7 and about 40% moisture content. The dumbell cut irradiated Bionolle samples (dosage 0 and 160 kGy) with thickness of 0.5 mm were buried about 3 cm from the soil surface, for various burial times from 2 weeks to 4 months. After each interval, the samples were removed from the soil and wiped by tissue paper, clean with running water and dried in oven to constant weight in vacuum oven at 40°C.

3. Result and Discussion

3.1. Crosslinking behaviors of Bionolle in presence of inorganic material.

Bionolle undergo crosslinking in irradiation, but gel fraction formed by crosslinking is not so high. Hence, Irradiation at high temperature to enhance crosslinking has been carried out [1]. Figure 1 shows the gel content of three kinds of unirradiated and irradiated Bionolle samples mixed with 2% silicon dioxide (irradiation in the atmosphere of air). As expected the gel content of mixed samples increase significantly with increasing irradiation dose in the presence of silicon dioxide. The gel content reaches to 29% for Bionolle#3001, 17% for Bionolle#3020 and 7% for Bionolle#1001 at dose of 160 KGy.

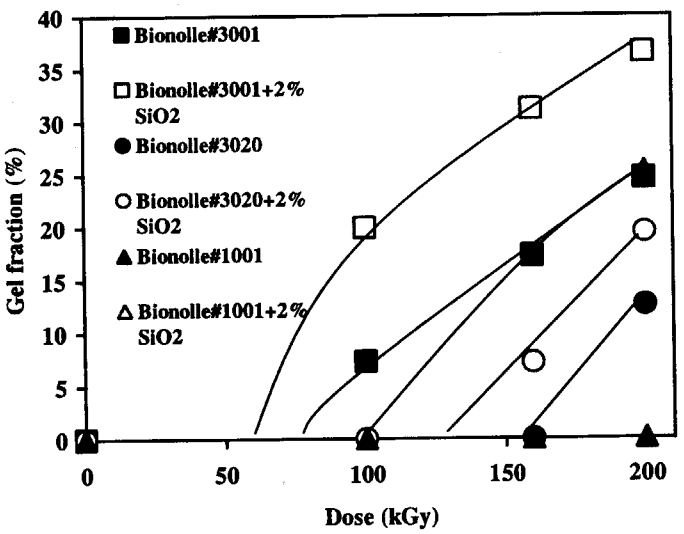

Fig. 1. Gel fraction of irradiated Bionolle, Irradiated in air-free atmosphere.

Effect of atmosphere on crosslinking of Bionolle in the present of silicon dioxide is shown in Figure 2. It can be seen that Bionolle#3001 irradiated in atmosphere of vacuum gave higher gel fraction than that of irradiated in air. Air condition is not preferable for crosslinking of Bionolle#3001. Crosslinking behaviors of irradiated Bionolle #3001 in presence of carbon black gave the same result as shown in Figure 3. From these findings, it was found that silicon dioxide and carbon black are favorable for crosslinking of Bionolle#3001.

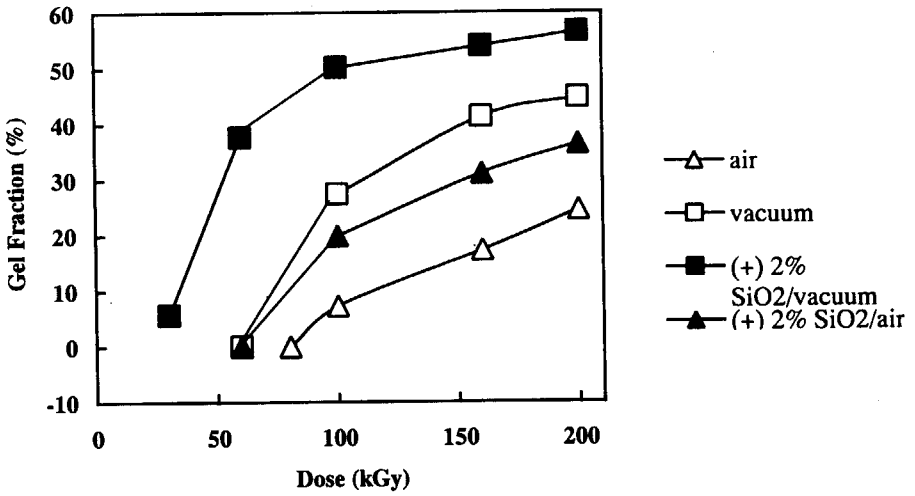

Fig.2. Effect of atmosphere on crosslinking of Bionolle#3001 in the present of silicon dioxide.

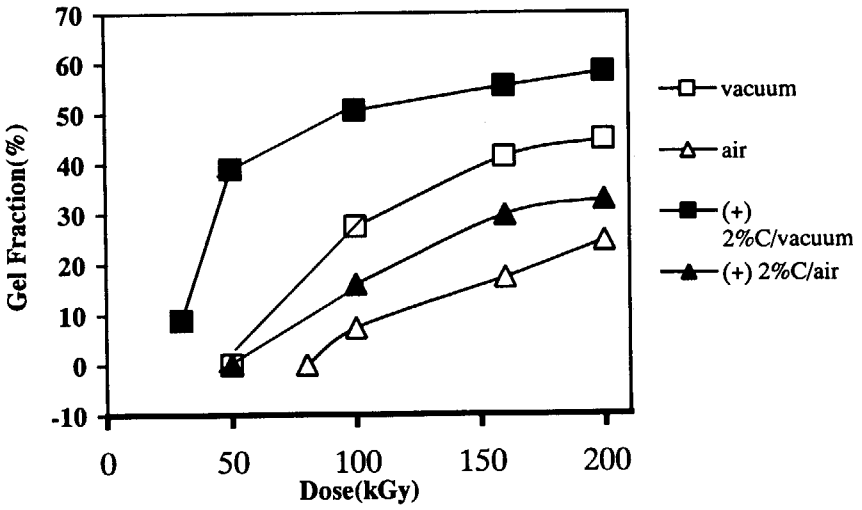

Fig.3. Effect of atmosphere on crosslinking of Bionolle#3001 in the present of carbon black.

3.2. Biodegradation Test

Biodegradable polymer converts to H_2O and CO_2 by biodegradation of bacteria in the soil and the weight decrease with increasing time.

The enzymatic degradation test of unirradiated and irradiated Bionolle#3001 in the presence of silicon dioxide was carried out. The results are shown in Figure 4. Silicon dioxide accelerating enzymatic degradation for irradiated and unirradiated Bionolle#3001, especially silicon dioxide is effective for irradiated Bionolle#3001. This is due to the degradation first on the polymer surface by contacting with enzyme, then gradually goes to the inside. The Enzymatic degradation of Bionolle#3001 in the presence of 2% silicon dioxide easily occurs, even crosslinking structure is introduced by irradiation. This is due to change of morphology of aliphatic polyester by silicon dioxide.

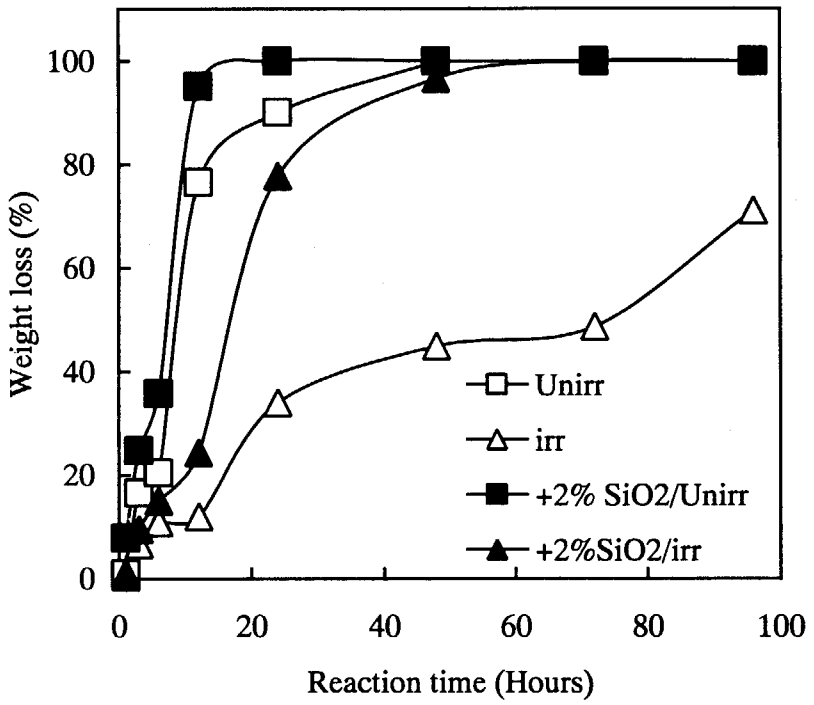

Fig. 4. Enzymatic degradation of irradiated Bionolle#3001 in the presence of 2% silicon dioxide (Irradiation dose 160 kGy).

Effect of carbon black on degradability in soil burial test of unirradiated and irradiated Bionolle is shown in Figure 5. The weight loss of the Bionolle#3001 mixed with 2% carbon black is larger than that of Bionolle#3001 without carbon black. After 4 months, Biodegradability reaches at 23% for irradiated Bionolle#3001 with 2% carbon black and 20% for Bionolle#3001 without one. Hence, it was conformed that inorganic materials is effective for soil burial degradation of Bionolle#3001.

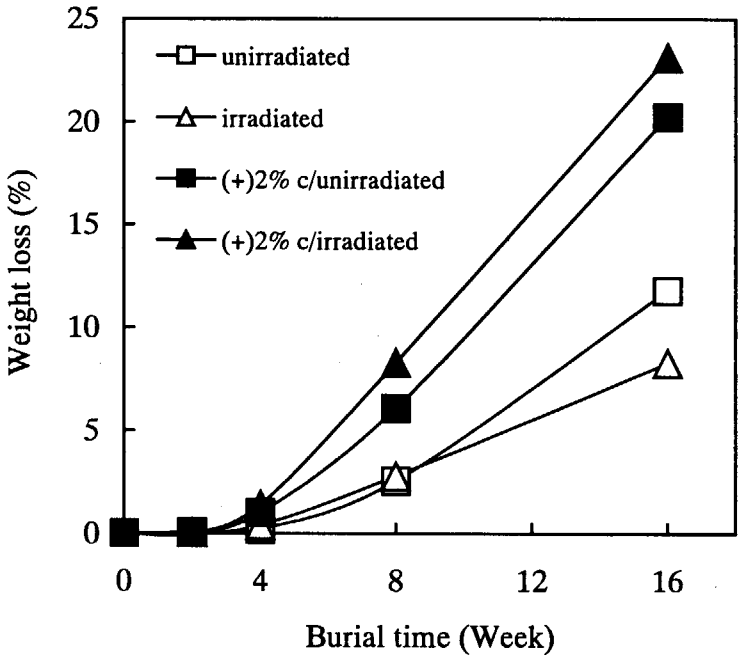

Fig. 5. Soil burial degradation of irradiated Bionolle#3001 in the presence of 2% carbon black (Irradiation dose 160 kGy).

Conclusion

Inorganic materials were effective to enhance gel fraction of Bionolle#3001, especially, silicon dioxide and carbon black. Such material was useful to accelerate biodegradation of crosslinked samples.

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Session 5

Economic Scale of Radiation Application

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22 Economic Scale of Utilization of Radiation In Japan-Overview

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Abstract

Utilization of radiation in Japan is progressing in the fields of industry, agriculture and medicine. Main tools used were gamma rays and electron beams. An increased usage of radiation is aiming at increase of welfare as well as quality of life among Japanese peoples.

To understand the actual state of progression for utilization of radiation, economic scale as a measure was studied. Economic scale revealed in 1997 in Japan was 71 billion dollars (b$, 1$=121¥), consisting of 60 b$ for industry, 1 b$ for agriculture and 10 b$ for medicine. The value 71b$ corresponds to 1.7% of gross domestic products (GDP). Economic scale of " nuclear energy " at that time was 61b$. Hence, the sum of radiation (71b$) and nuclear energy (61b$) becomes 132 b$. This value corresponds to 3.2% of GDP. An occupational ratio between the two was 54 % *vs.* 46 %. It is worthy of mentioning that as to the case of study made by U.S.A., it becomes 79 % *vs.* 21 % in 1995.

Keywords: Utilization of Radiation, Economic Scale, Industry, Agriculture, Medicine

1. Introduction

Last year, the Science and Technology Agency (STA, now the Ministry of Education, Culture, Sports, Science and Technology, abbreviated as MEXT) of Japan offered to the Japan Atomic Energy Research Institute (JAERI) to conduct a study of an **economic scale of utilization of radiation** (gamma ray, electron beam, etc.) by Japanese

industries from the viewpoint of public acceptance (PA). The study was undertaken to enhance the understanding of benefit from the utilization of radiation. This research was not for profit in nature. Confidential data provided by private companies was omitted from the source material. Now the study was completed and the results were disclosed partially. On this occasion, JAERI organized the committee called " The Special Committee for Evaluation of Economy on Utilization of Radiation ". It consisted of about 20 Japanese specialists from universities, research institutes and companies working at fields of industry, agriculture and medicine.

As far as we know, utilization of radiation in Japan has been actively promoted by various agencies including the Takasaki Radiation Chemistry Research Establishment (Hereinafter abbreviated as the Takasaki) of JAERI. The Takasaki was established in April 1963 for the purpose of promoting research and development (R&D) activities with gamma rays and electron beams. Fields addressed to were industry and agriculture mostly and a small part of medicine. Results obtained from R&D works are aiming at contributing to welfare as well as quality of life of Japanese peoples.

In U.S.A., an economic scale of utilization of radiation in the field of industry, agriculture and medicine was studied in year 1991[1]. One of prominent result from the study is that the ratio of economic scale between utilization of radiation and that of nuclear energy was 79 % *vs.* 21 %. The radiation is rather advanced in U. S. A. This was interesting indication for us.

2. Objectives

Outline of study is shown in **Table 1**. Principally, economic scale is defined as the sale of products using radiation. Here, efficiency of radiation in the course of manufacturing process is abbreviated because of its difficulty. Data sources used for this study were addressed to those from opened to public. If one met difficulties for data procuring, counterplan such as questionnaire, interview, and hearing by telephone were taken into consideration. A private research institute assisted us. After completion of data procurement, discussions were made in three working groups (WG), that is, industry (Chairman, Dr. Tagawa, Osaka University), agriculture (Dr. Chino, Akita Prefecture University) and medicine (Dr. Inoue, Gunma University). More details on methodology and concept are described elsewhere [2].

Table 1 Outline of study

Objectives:	Systematic study to know a wide use of radiation by means of a economic scale as a measure.
Method:	Commissioned to JAERI from STA
Field:	Applications in Industry, Agricult[illegible] and Medicine, Health & Therapy at a year of 1997.
Economic sale is to be:	
●Industry:	Sale of Industrial products recognized as radiation source, measuring apparatus and manufactured goods by radiation.
●Agriculture:	Goods produced by mutation breeding, food irradiation and SIT (sterile insect technology)
●Medicine:	A expenses for medical diagnosis and treatment carried out by using radiation.

3. Results

3.1 Applications in Industry.

In WG, the economic scale of utilization of radiation in fiscal year (FY) 1997 was studied by using published documents. The study revealed that the scale was about **60 b$**, of which semiconductor related manufacturing totaled 44 b$ while others such as radial tires were 16 b$.

The share of individual items is shown in **Fig. 1**. In industry, the largest is production of semiconductor (about 75%). The second is radiation processing (15%) including radial tire production (8,385M$) fabricated by 5 big Japanese tire companies. At irradiation facility, medical instruments were included. Electron beam accelerators as large as 308 units were run at that time. Summary is shown in **Table 2.**

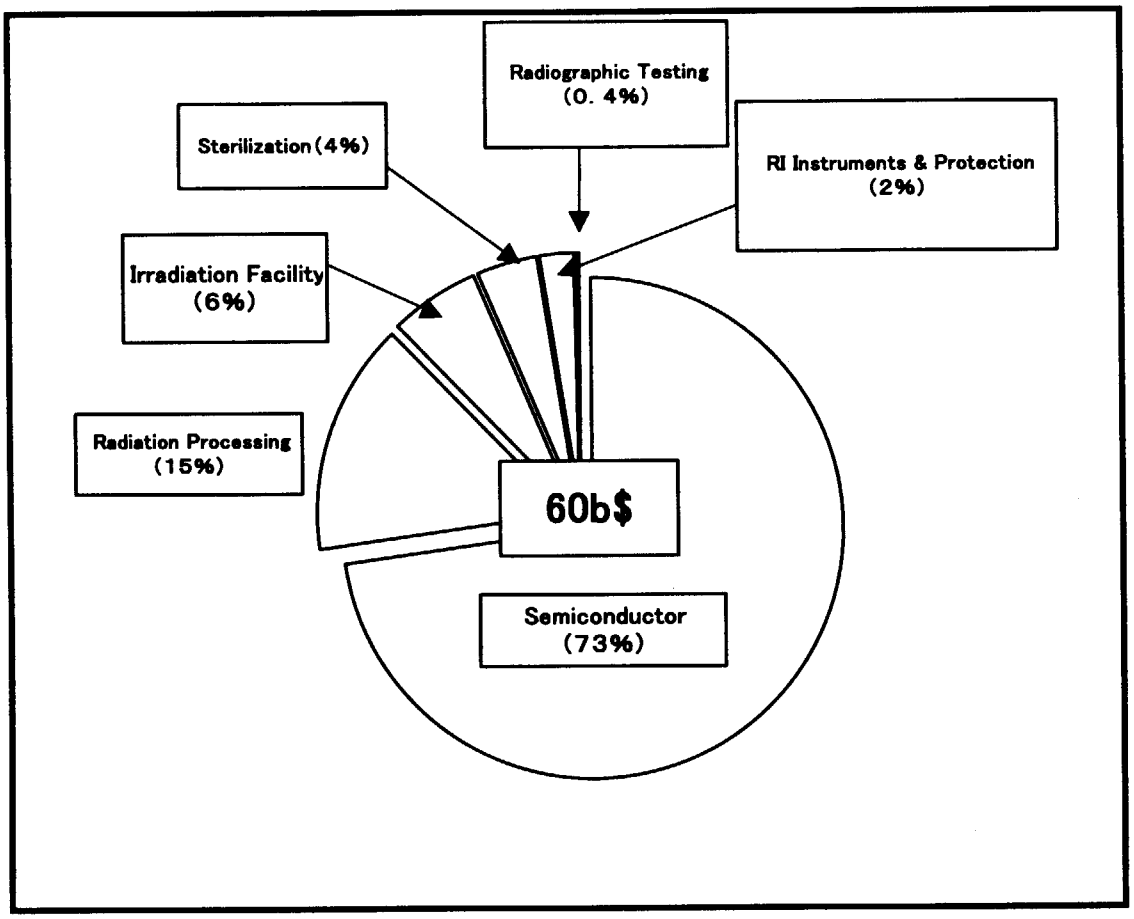

Fig. 1 Economic share at industry

Table 2 Economic scales of applications in industry

Field			Products (M$)	Sum (M$)
(1) Irradiation facility				3,531
	Industry	Cobalt Facility	7	
		EB	226	
		Ion Accelerator	247	
	Medicine	X ray apparatus for diagnosis	1,695	
		Medical apparatus for imaging	84	
		Medical apparatus for nuclear medicine	2	
		Apparatus for RI treatment	15	
		Sealed RI for medicine	3	
		Particle accelerator for diagnosis	88	
		Apparatus and goods related to medicine	1,164	
(2) Measuring instruments fro radiation				540
		Instruments		
		Protection		
(3) Radiographic testing(RT)				258
(4) Sterilization of disposable medical instruments				2,348
	Gamma		2,188	
	Electron		160	
(5) Fabrication process				9,080
	Cross-Linking	Tires	8,385	
		Wire & Cable	372	
		P. E. Foam	148	
		Heat shrink tube /Film	136	
		Others	2	
	Degrad.	PTFE	4	
	Curing	Coating, Converting, Magnetic materials	25	
	Graft	Cell separator, Absorbent	8	
Sum:(1)～(5)				15,757
(6) Semiconductor				44,263
Total Sum :(1)～(6)				60,020

3.2 Agricultural Use

In WG, agricultural use including radioisotopes was studied. Results are shown in **Table 3.** The scale of agricultural use is about **0.97 b$.**

Table 3 Applications in agriculture and radioisotopes

Field	Items	Products (M$)	
1. Irradiation & Insect Control		136	
(1) Food irradiation	Potatoes		
(2) SIT	Fruit flies		
(3) Sterilization	Bag-in-box		
	Feed for laboratory animals		
2. Mutation breeding		804	
	Rice (17 species)		
	Pear (Gold 20century, others)		
	Beans (4 species)		
	Others (Peach, Chrysanthemum etc.)		
3. Research & Development by RI Utilization		24	
(1) Laboratory work	RI provision in Agri/biological studies		
	RI depositions (solid, liquid etc.)		
(2) Environmental protection	Air pollution analysis		
(3) Chronology	Geology & Archeology by C-14 isotopes etc.		
Sum		964	

With respect to food irradiation, potatoes of 15,000t/y were irradiated at Hokkaido with price by 1.1$/kg. Extermination of melon fly around Okinawa area and so on brought large benefits as high as 69M$. Production of rice born by mutation breeding is

about 3% of overall rice production in Japan and economic scale totaled 774M$. Utilization of radioisotope (RI) is principally used at laboratory work and for environmental analysis and chronological purpose at private university. The scale is as high as 24M$.

The share of agriculture is shown in **Fig. 2** as about **1b$**, consisting of irradiation 14b$ (14%), mutation breeding 0.80b$ (83%) and radioisotope utilization 0.02 b$ (3%), respectively.

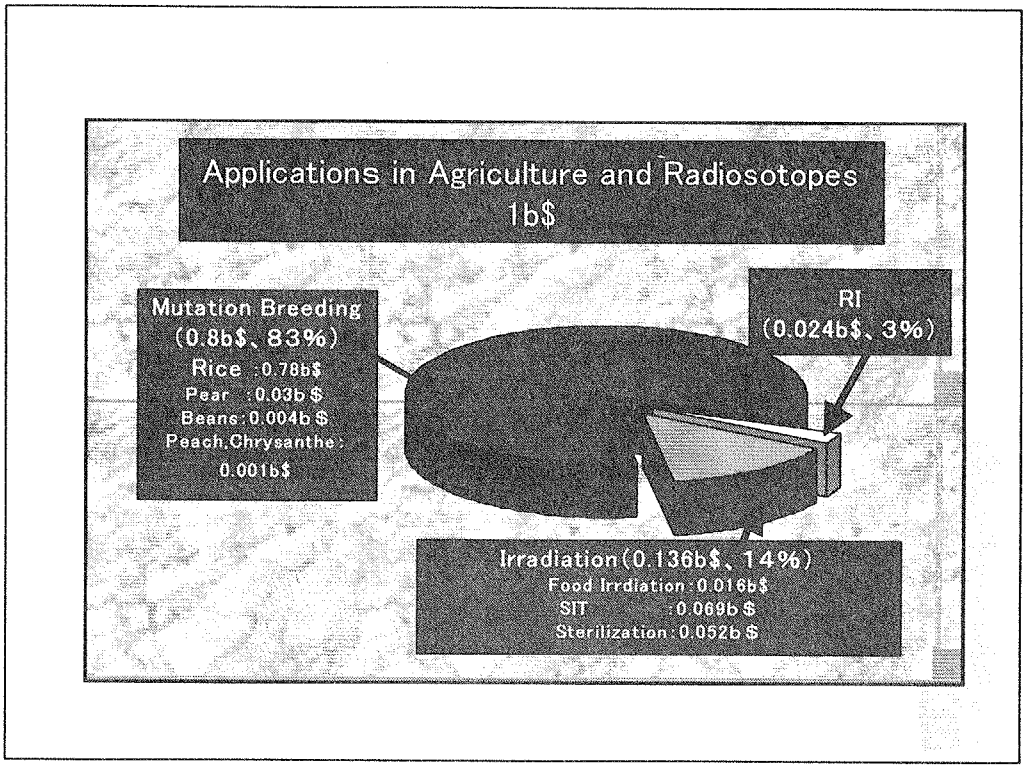

Fig. 2 Economic share at agriculture

3.3 Applications in Medicine.

For medicine, an economic scale was studied by using reimbursed receipts. Statistical data were released from Ministry of Health/Welfare and Labor. Results are summarized in **Table 4**. In medicine, an economic scale was **9.8 b$**. About 99% was reimbursed items and 0.3% was not. An occupational ratio between medical care and dental care becomes 91%vs.. 9%, where MRI (magnetic resonance inspection) was omitting due to non-radioactive item. The content of medical care is shown in **Fig. 3**. X-ray diagnosis, computed tomography (CT) is large in magnitude of 84%.

Table 4 Summary of Medicine

Applications in Medicine, Health & Therapy		
1. Insurance Coverage		Cost(M$)
(1) Medicine		
	1.1 Diagnosis(Exc.MRI)	
	1) X-ray Inspection	4,192
	2) Nuclear medicine	1,035
	3) Computed Tomograph	3,287
	1.2 Radiation Treatment	466
	1.3 Inspection	2
	Sum(1)	8,982
(2) Dentistry		
	2.1 Diagnosis	849
	2.2 Radiation Treatment	4
	Sum(2)	853
Sum(1+2)		9,835
2. No Insurance Coverage		Cost(M$)
	1) PET	Not decided
	2) Proton Treatment	3
	3) BNCT	0.4
Sum.		3
Total Sum = 9,835+3=9,838 M$		

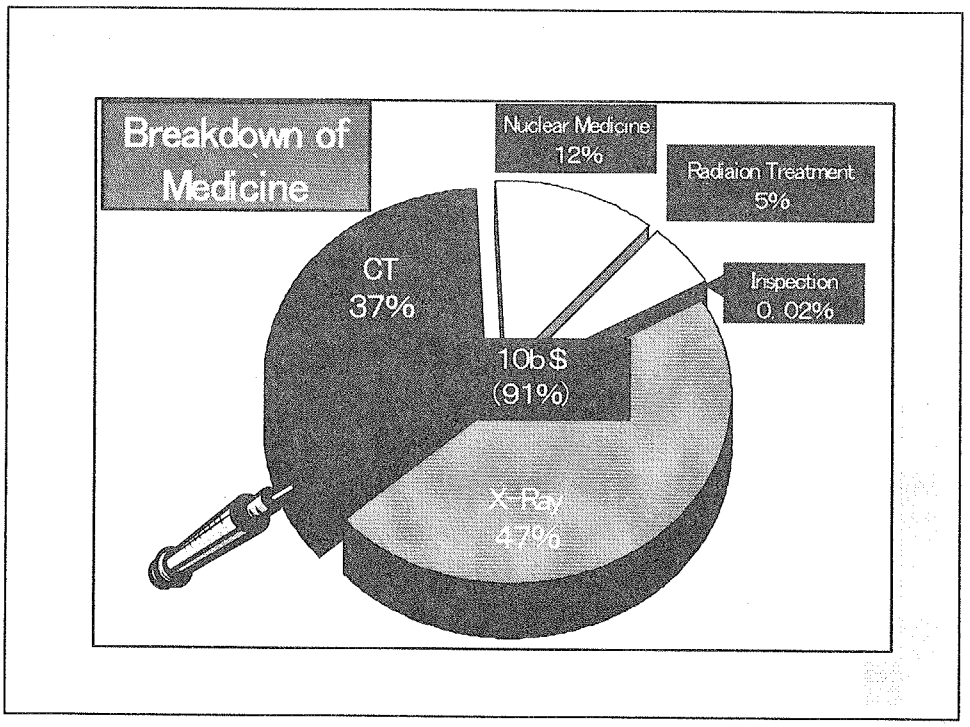

Fig. 3 Share of Medical Care consisted of Diagnosis, Radiation treatment (Radiotherapy) and Inspection

3.4 Economic scale of industry, agriculture and medicine

Results of study were summarized in **Fig. 4.** It revealed that the scale of utilization of radiation in that fiscal year stood at 71 b$. It represents about 1.7% of the gross domestic products (GDP). The value of 71 b$ consisted of 60 b$ for industry, 1 b$ for agricultural and 10 b$ for medicine, respectively.

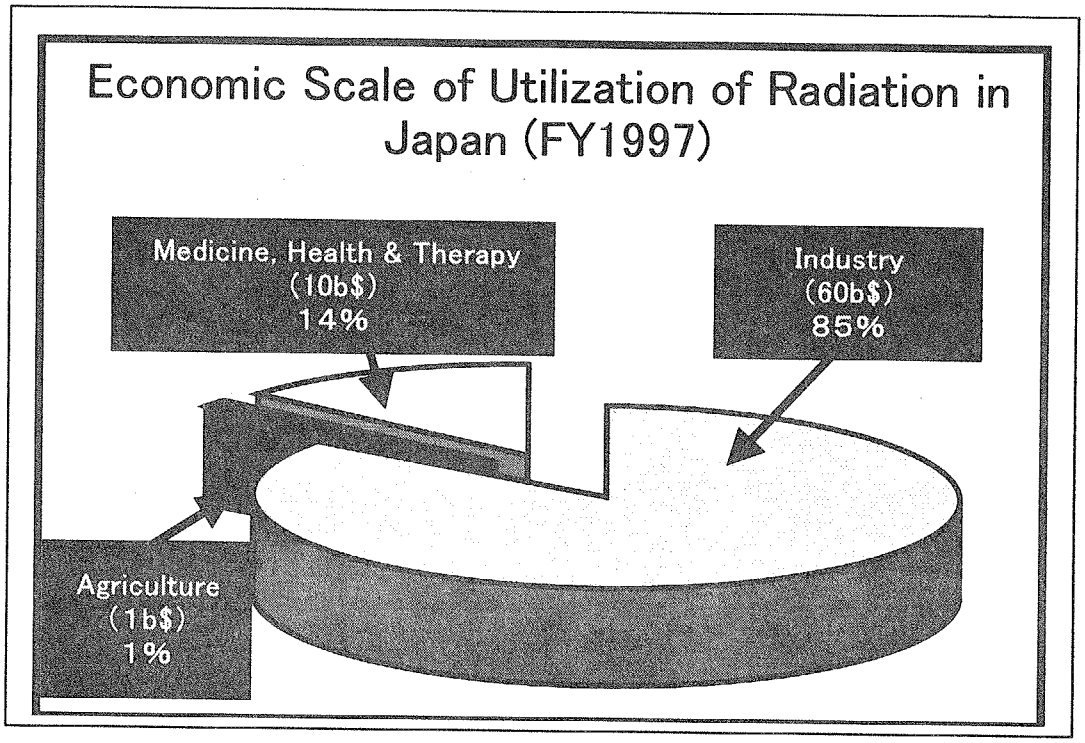

Fig. 4 Economic scale of utilization of radiation in Japan

3.5 Comparison with nuclear energy

Economic scale of radiation was simply compared with that of nuclear energy. As preliminary study, as shown in the **Table 5,** sales of electricity from 52 nuclear power plants (NPP) were found to be **47b$** as maximum case. This includes distributive price of electricity at transmittal. Adding to this, sales of reactor components and fuels and so on were found to be **13b$.** Therefore, as shown in **Fig. 5,** economic scale of nuclear energy stood at **60b$.**

Table 5 Sales of electricity generated from 52 nuclear power plants at the end of transmittal

No	Electric Power Co.	Gross Sales (billion $)	Share of Nuclear %	Sales by Nuclear (billion $)
1	Hokkaido	5	27	1
2	Tohoku	13	13	2
3	Tokyo	44	43	19
4	Chubu	18	23	4
5	Hokuriku	4	22	1
6	Kansai	22	52	11
7	Chugoku	9	15	1
8	Shikoku	5	42	2
9	Kyushu	12	48	6
	Sum	130		47
	Ave.		36	

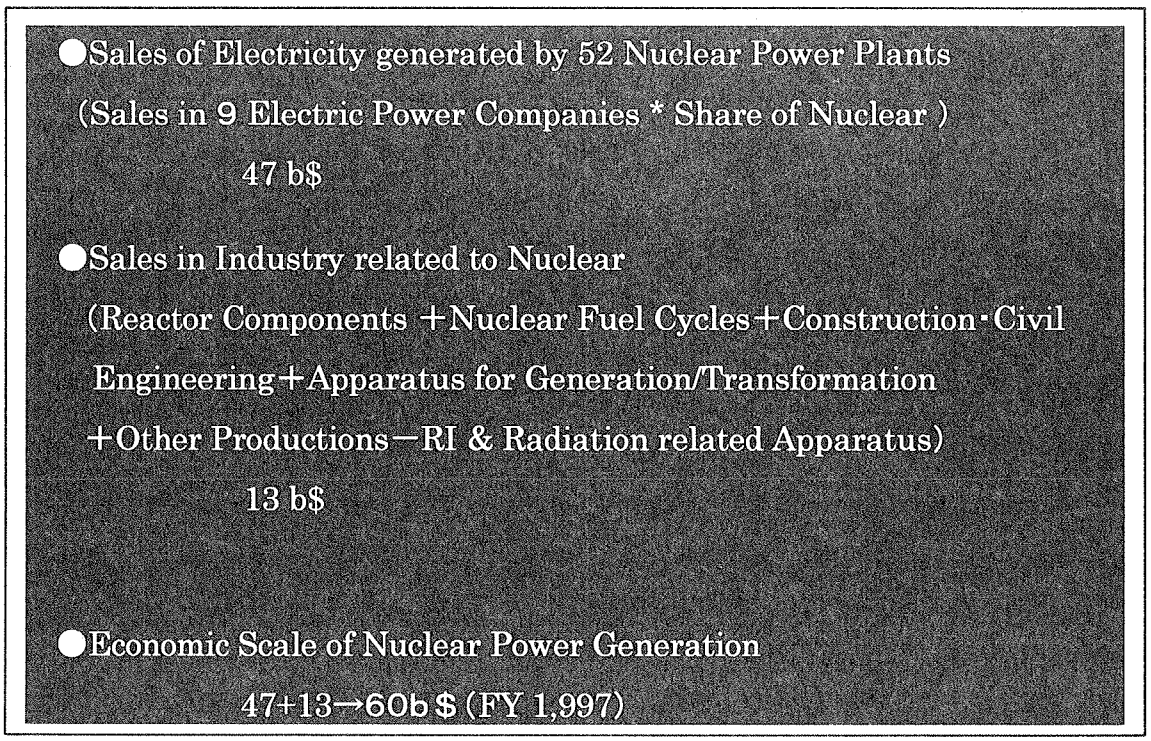

Fig. 5 Economic scale of nuclear energy in 1997

4. Conclusion

(1) Economic scale of utilization of radiation revealed in 1997 was 71 billion dollars (b$, 1$=121¥), consisting of 60 b$ for industry, 1 b$ for agriculture and 10 b$ for medicine. It corresponds to 1.7% of GDP

(2) Economic scale of nuclear energy in 1997 was 61b$.

(3) Sum of radiation and nuclear energy is 132b$ as shown in **Fig. 6**. It corresponds to 3.2% of GDP. An occupational ratio between the two becomes 54 % vs.. 46 %. It is worthy of mentioning that as to the case of U.S.A., the ratio was 79 % vs.. 21 % in 1995.

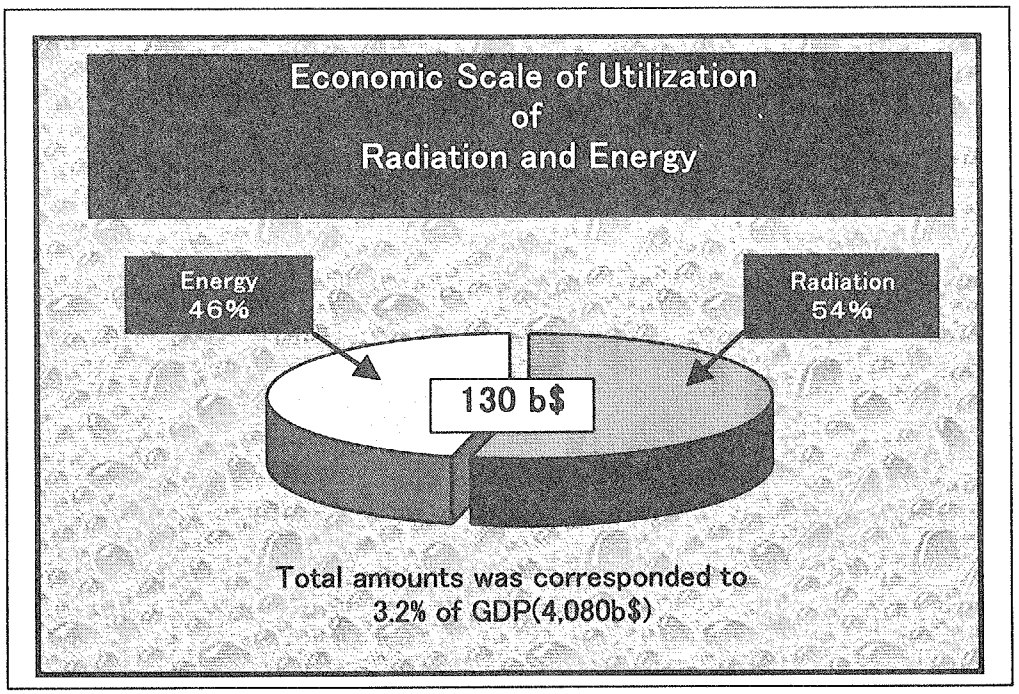

Fig. 6 Economic scale of utilization of radiation and nuclear energy in Japan as of 1997

Acknowledgment

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Economy on Utilization of Radiation for their excellent contribution to this work.

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23 Economic Scale of Utilization of Radiation in Japan

- Medical Field -

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5. National Institute of Radiological Sciences,
6. GE Yokogawa Medical Systems,
7. Japan Radioisotope Association

Abstract

In working group for medicine, a direct economic scale of radiation medicine in Japan in 1997 was studied by data obtained from the Ministry of Health, Labor and Welfare. The revealed amounts were 9.8 b$. Majorities of those (>99.7%) were from medical and dental fields, and fully reimbursed national health security. A ratio between medical and dental service was 91% *vs* 9%. Obtained results were then compared directly to those of USA taking the different medical systems into consideration. For example, national medical expenditures in Japan was about 240b$ while personal medical expenditures in USA was 967b$. The latter is 4 times greater than that of the former. The result of term-to-term comparison will be described in detail in the text.

Keywords: Medicine, Economic Scale of Japan and USA

1. Introduction

In Japan, national health expenditures were increased with year and in 1997 it reached to 240b$ [1]. The figure is corresponding to 0.24% of Gross Domestic Products (GDP)[2] and 1,903$ for personal expenditures of Japanese people. Under this situation, we tried to find the economic scale of utilization of radiation in the medical field because of no

existence of authorized data up to that time. This trial was carried out in the year of 1999. Then, a comparison of economic scale between Japan and United States of America (USA) was made. This report is described the results obtained from two previous studies [31].

2. Method

For study, a total of seven Japanese specialists involving medical doctors and researchers working in university, institute and private pharmaceutical company were participated and formed the medical working group (MWG). The MWG was a branch of special committee for studying economic effect on utilization of radiation (Chairman, Dr. M. Shimizu, Professor Dean, Economic Division of Keio University). In the committee, industrial and agricultural working groups were also formed. Hereinafter we called the special committee as the parents committee. The parents committee was supported by the Takasaki Radiation Chemistry Research Establishment belonged to the Japan Atomic Energy Research Institute (JAERI).

In MWG, domestic data was studied in a year of 1999 and USA data was did in a year of 2000. In the former, main data source was " Research for socio/medical diagnostic acts in 1997- vol.1, outline and statistics", published by the Ministry of Labor, Health and Welfare [3] .On the other hand, Data source in USA was large and dispersed. From this point of view, a recommendation from the parents committee to the MWG was given that the comparison should not be addressed to overall matters due to a great many systematic differences laid between two countries. According to this, a simple with reasonable background is focused to obtain. In this procedure, the MWG was assisted by private think-tank. The assumptions set to this study are as follows:

1) A target year is 1997.
2) A data should be from open to public such as Internet and publications.
3) At the last stage, a double check should be performed by MWG and corresponding person in USA.

3. Results and Discussion

3.1 Economic Scale of Medicine in Japan in 1997

As shown in **Fig.1,** a radiological use in medicine at the national expenditures of 240b$ in Japan was about 4%, that is, 9.9 b$ (billion dollars)[4]. The breakdown of the amount is shown in **Fig. 2.** It consists of 9.1b$ for medical care and 0.8b$ for dental care and very small amounts for charged particle therapy and boron neutron capture

therapy (BNCT). Former big two were reimbursed and latter small were not. In medical care, a main radiological use is diagnosis consisting of X-ray diagnosis, nuclear medicine and computed tomography (CT). This is also observed in the dental care.

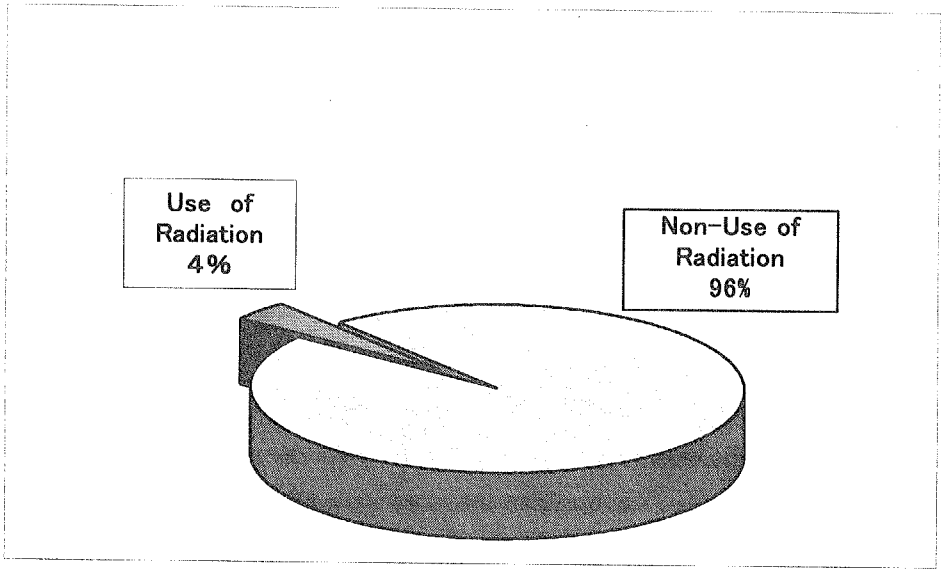

Fig. 1 Radiological Usage in Medicine in 1997 against the national Healthcare Expenditures of 240b$

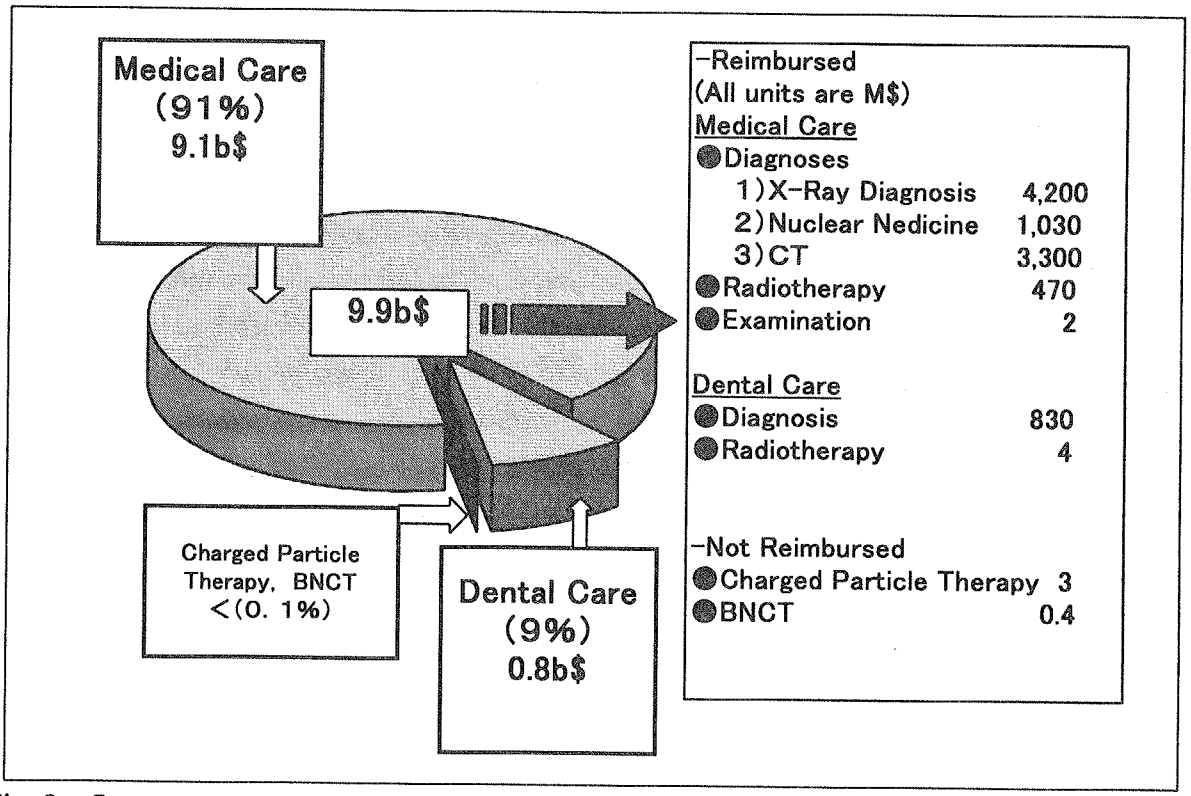

Fig. 2 Summary: Economic Scale of Medicine in 1997 in Japan

3.2 Economic Scale of Medicine in Japan and USA in 1997

Through discussion, MWG decided items of study as shown in **Table 1.** At classification " general ", ①total expenditures and ②radiation imaging were chosen as studying target. While, at classification " specified " ,③Prostate cancer and charged particle therapy, ④ Fluorodeoxyglucose (FDG) Positron Emission Tomography (FDG-PET), ⑤ Medical equipments, ⑥ Radiopharmacy, ⑦ Contrast media, were chosen as studying purposes.

It should be noted that the ratio of population between Japan and USA in 1997 was 120million *vs.* 270million, that is about 1*vs* 2. While, the ratio of GDP was 4,800b$ *vs.* 7800b$, that is about 1*vs* 1.6.

Table 1 Topics for Comparison

General	①	Total Expenditures
	②	Radiation Imaging
Specified	③	Prostate Cancer and Charged Particle Therapy
	④	FDG-PET
	⑤	Medical Equipments
	⑥	Radioisotopes
	⑦	Contrast Media

3.2.1 General

(1) Total Expenditure

With respect to total expenditures, a concept of Total Domestic Health Expenditures (TDHE) [5,6] was used. It consisted of 1) National medical expenditures, 2) Indirect part of the former 1) including administrative cost like salaries necessary for operating medical insurance systems, 3) Public aid from the government to 1) subsidy to running medical agency and 4) Medical services not covered by insurance. Adding to TDHE, national health care in Japan was contrasted to personal health care in USA though standing base was not equal but only as indication.

Results are shown in **Table 2.** TDHE in Japan in 1997 was 279b$ while that in USA was 987b$, respectively. TDHE in USA is greater than that in Japan to the magnitude of 3.5, which is not proportional to population. As one indication, national health care in Japan was 240b$ and personal health care in USA was 969b$. USA was greater to the magnitude of 4. Personal health care system was advanced in USA much.

Table 2 Total Expenditures

Japan	TDHE	279
	National Health Care	240
USA	TDHE	987
	Personal Health Care	969

Note: 1$=121¥, TDHE=Total Domestic Health Expenditures

(2) Radiation Imaging

When one goes to a hospital by disease, first encounter to him may be the radiation imaging. Despite of popularity, a magnetic resonance imaging (MRI) and an ultrasonography were omitted in this study because they were non-radiological modalities for imaging. Radiation imaging consisted of X-ray, CT and nuclear medicine, respectively. Economic scale of those is summed up in **Table 3**. In two countries, X-ray is used widely, that is, 49% in Japan and 74% in USA [7] and economic scale was 4,189M$ for Japan and 31,983M$ in USA. This implies that USA was about 8 times greater in magnitude than that in Japan. In general sense it seems to be clear that USA uses radiation imaging positively. In total, USA is 5 times greater in economic scale for radiation imaging than that of Japan.

Table 3. Radiation Imaging

Medicine	Japan, Ref.[4]	%	U.S.A., Ref.[7]	%
X-ray	4,189	49	31,983	74
Computed Tomography (CT)	3,286	39	8,995	21
Nuclear Medicine	1,034	12	2,099	5
Total (M$)	8,509	100	43,077	100

1 $＝121¥

3.2.2 Specified

(3) Prostate Cancer and Charged Particle Therapy

(3-1) Cancer and Radiotherapy (RT)

RT is one of the most useful treatments for various types of cancer, and it is well known from old days that American patient is more aggressive for use of RT than Japanese to cure his disease. However, one shows no clear evidence regarding this matter to date. So, MWG revealed this as shown in **Table 4.** Once American had a cancer (1.15million

patients in 1994), 49% (560,262) of those received RT [8-10]. How about the Japanese? Of new patients of 440,001 in 1995, only 15% (71,696) were given RT [11-12]. Whether we do not know it is fact or not that Japanese patients are willing to have surgical operations than RT. It may exist many reasons behind the difference in two figures.

Table 4. Application Rate of Radiotherapy (RT) for Patients with Cancer

For U. S. A.					Rate of
Year	New Patients	Ref.	Patients treated by RT	Ref.	RT(%)
1994	**1,150,000**	[8]	**560,262**	[10]	**49**
1995	**1,252,000**	[9]			
For Japan					Rate of
Year	New Patients	Ref.	Patients treated by RT	Ref.	RT(%)
1994					**15**
1995	**440,001**	[11]	**71,696**	[12]	

(3-2) Prostate Cancer

Prostate cancer [14-18] is a typical disease for male. This disease is top of order in malignant neoplasms of Americans in 1997. Very detail comparison about this cancer was made and result is shown in **Table 5.** Rate of prostate cancer is higher in USA to the magnitude of 7, implying that American has a constitutional predisposition to prostate cancer. Application rate of RT in USA is 30% but that of RT in Japan is only 5%. In the latter case, a hormone therapy is adopted frequently. Cost per RT course is, however, cheaper in Japan (3,306$) than that in USA (15,000$) to the factor of 4.5. Finally, economic scale of prostate cancer in USA is 576M$, while that in Japan is 0.72M$, respectively. About two orders are different between two countries.

Table 5 Prostate Cancer

For U. S. A.	
(1) Population, male (Average from 1993 to 1997)	128,261,888
(2) Rate	139.1/100,000
(3) No. of Patients, (1)*(2)	178,412
(4) Rate of local disease (%)	71.7
(5) Applicator of RT (%)	30
(6) No. of Pts, (3)*(4)*(5)	178,412 * 0.717 * 0.30
	38,376
(7) Cost per RT course ($)	15,000
(8) Total Cost	38,376 * 15,000
(M$)	576
For Japan	
(1) Population, male (1995 National census)	61,574,000
(2) Rate	19.5/100,000
(3) No. of Patients,(1)*(2)	12,000
(4) Rate of local disease (%)	30
(5) Applicator of RT (%)	5
(6) No. of Pts, (3)*(4)*(5)	12,000*0.3*0.05
	180
(7) Cost per RT course ($)	3,306
(8) Total Cost	180*3,306
(M$)	0.72

(3-3) Charged Particle Therapy for Prostate Cancer

With respect to prostate cancer, charged particle RT, especially proton therapy, is recently adopted to treat the cancer and some being disorders more effectively with radiation under controlled beam delivery. In USA, proton therapy is carried out at Loma Linda University's Proton Treatment Center in Southern California [19]. In Japan, charged particle therapy has been carried out at the National Institute of Radiological Sciences (NIRS) [20] by means of carbon ion and Tsukuba University by means of proton [21]. Payment in USA is covered by insurance but that in Japan is not to date. Economic scale of charged particle therapy for prostate cancer is shown in **Fig. 2**. Apparently, USA is more advance in this field because economic scale is 15.2M$ having 507 therapies in a year. In Japan, charged particle therapy is just started and economic scale is 0.22M$ having 13 (10 by NIRS and 3by Tsukuba) therapies.

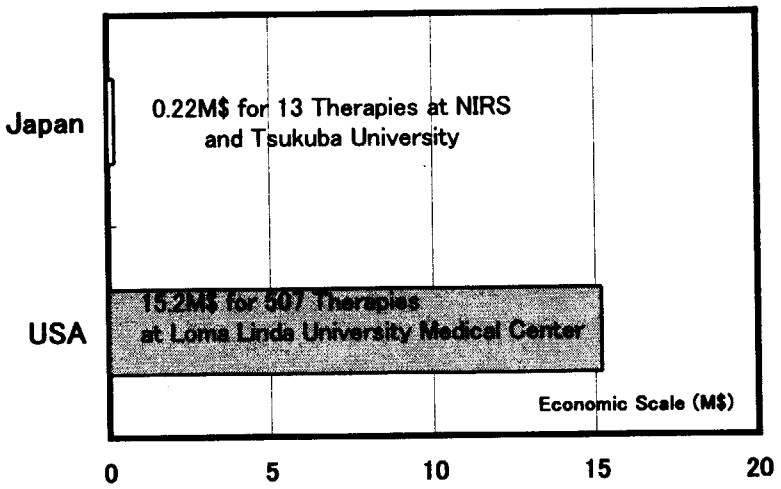

Fig. 2 Charged Particle Therapy for Prostate Cancer (1997)

(4) FDG-PET

In Japan [22], FDG-PET under no insurance performed in 12 institutes up to a year of 2000. By using owned cyclotrons, clinical researches on FDG-PET at about 20 institutes are carried out. A total number of examinations were 5,740 at 24 institutes in 1997. While, in USA [23], reimbursement of FDG-PET for lung cancer started from 1998 and now the costs of FDG-PET for 4 kinds of cancers including lung cancer are reimbursed. A total number of examinations were 100,000 at 168 institutes in a year of 2000. This increase was markedly influenced by inception of reimbursement. Delivery of FDG from PET center to neighbor clinics is possible within a states. **Fig. 3** shows an economic scale of FDG-PET in both countries. In 1997, FDG-PET in USA is 50M$, while that in Japan is about 2M$. After reimbursement occurred in 1998, FDG-PET in USA increased its scale rapidly from 50 M$ (1997) to 210 M$ (1999). Unit cost for FDG-PET in USA is about 1,980$ while that in Japan is about 397$. This big difference (5 times difference) may be due to inclusion of depreciation cost for the case of USA. As a general trend, USA accepted quickly the well-developed tools like FGD-PET for the best treatment.

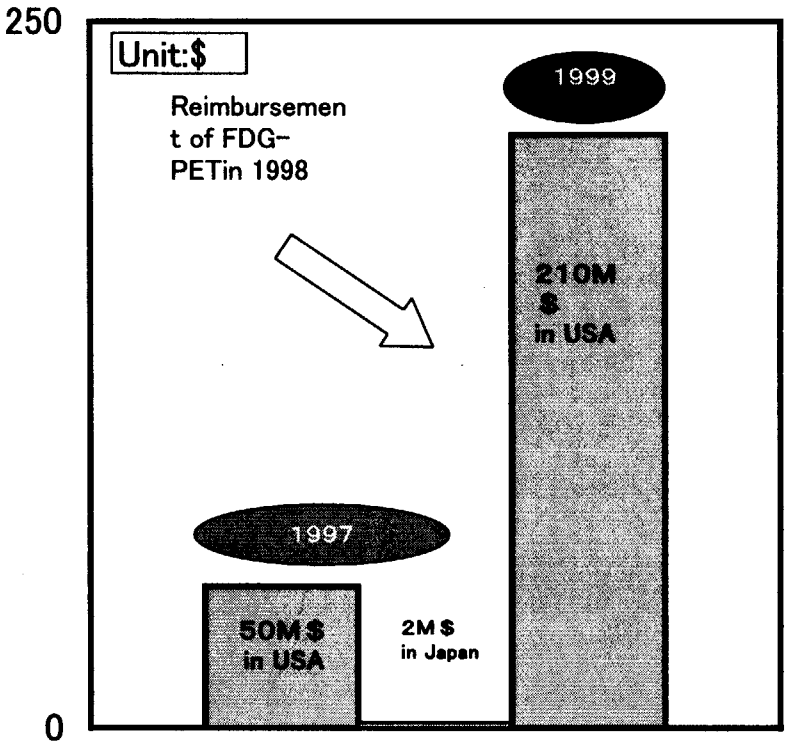

Fig. 3 Economic Scale of FDG-PET in 1997 (USA and Japan) and 1999 (USA)

(5) Medical Equipments

Economic scale of medical equipments used for diagnostic imaging such as X-ray, RT, nuclear medicine and CT was studied. Data sources in USA included MRI and ultrasound as tool for diagnostic imaging. Their shares were 12% and 23%, respectively. For comparison with Japanese data, however, the two were omitted. Here, production costs of equipments at factory are utilized. Obtained results are shown in **Fig. 4.** Economic scale of medical equipments in USA [24,25] is 3,200M$ and that in Japan [26,27] is 1,820M$, respectively. USA is larger to the magnitude of 1.8. Almost half of the total was occupied by production costs of X-ray and percentage in two countries showed little difference. Regarding RT, there existed poor database in Japan, therefore, it showed as zero in the figure. It is worthy of mentioning that production costs of CT in Japan (43%) are greater than that of USA (17%). This difference is attributed to numbers of hospitals owning to CT and numbers of CT per hospital. Hence, CT in Japan (11,000 units, majority is normal type) was popularized than that in USA (6000 units, partly high grade helical type). However, as general, equipments for nuclear medicine are overwhelming by USA.

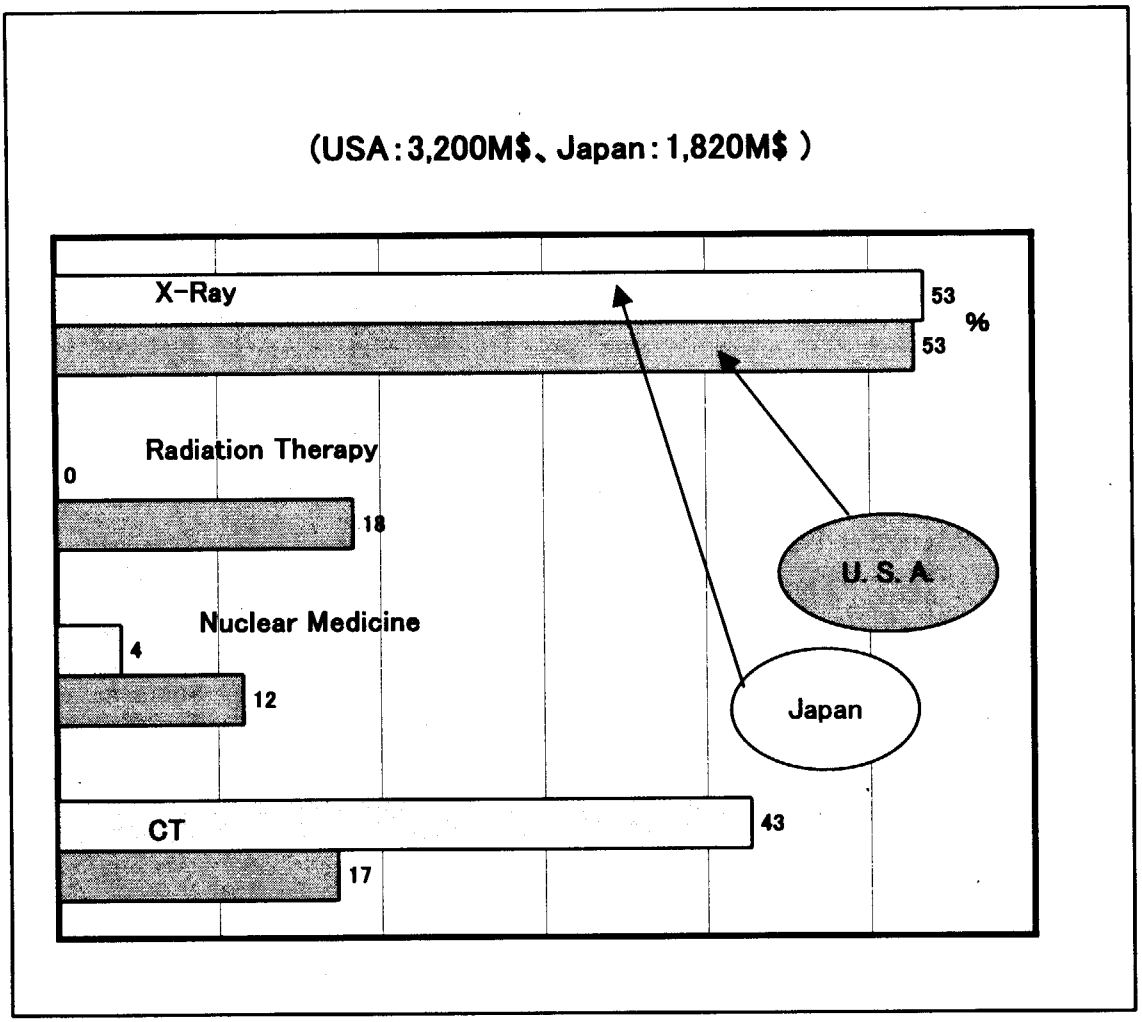

Fig. 4 Economic Scale of Equipments for Diagnostic Imaging in 1997

(6) Radiopharmacy

In USA [28], radiopharmacy, that is, nuclear medicine for imaging was sold not only by the makers but also by the pharmacy. Here, sales of radiopharmacy from makers are compared. Resultant table is shown in **Table 6.** Radiopharmacy in USA is 736M$ in 1998 and that in Japan is 366M$, respectively. USA is two times greater. Shipment of radipharmacy was almost Tc-99m (>80%) without including fee for transportation. In case of Japan, transportation and packing costs were included.

Table 6 Radiopharmacy (Nuclear medicine for imaging)

Radiopharmacy			(Unit:M$)
Year	1997	1998	1999
U. S. A.	-	736	832
Japan	-	366	374

Note: Utilization of Tc-99m in USA >80%

(7) Contrast Media

Contrast media used by MRI and X-ray/CT examinations, however, the former was omitted and iodine contrast media for X-ray/CT was studied here [28-30]. Comparison was addressed to sales cost at pharmaceutical suppliers. Result is shown in **Table 7**. Difference in sales between two countries seems to be not so much. Use of contrast media of iodine in 1997 was 37.76×10^6 in USA and percent (%) usage of CT was about 45. While, use of contrast media of iodine in 1997 was 22.59×10^6 for CT in Japan and percent usage of CT was about 24. Despite of different counting system on CT lied on two countries, price of CT in USA is slightly cheaper than that in Japan.

Table 7 Contrast media

	Year	1996	1997	1998	1999
U. S. A.	Sales (b$)	1.11	1.12	1.12	1.15
	Total usage numbers (x1E06)	35.78	37.76	39.47	41.35
	% Usage for CT	44.0	44.7	45.5	46.7
	% Usage for X-Ray	47.8	46.6	45.9	44.1
Japan	Sales (b$)		0.93	0.89	
	% Usage for CT		23.6		

Discussion about radioisotopes was omitted here. Only resultant figure will be shown in the summary table shown in the below.

4. Conclusion

4.1

The radiological use in medicine against national expenditures of 240b$ in Japan was about 4%, that is, 9.9 b$ (billion dollars). The value consists of 9.1b$ for medical care and 0.8b$ for dental care.

4.2

Summary for comparison between Japan and USA is shown in **Table 8.**

(1) TDHE is 983 b$ for USA and 281 b$ for Japan. The former is greater to the magnitude of 3.5.
(2) Occupational ratio of TDHE against GDP is 12.6% in USA and 6.9% in Japan. The former is roughly 2 times larger; it is just a population ratio.
(3) Sum of specified items such as radiation imaging, prostate cancer, FDG-PET, medical equipments, radiopharmacy (including radioisotopes), and contrast media is 48,942 M$ for USA and 11,666 M$ for Japan, respectively. Regarding specified items, the value is roughly about 4 times greater in USA than in Japan.
(4) Occupational ratio of specified items against GDP is 0.6% for USA and 0.2% for Japan. The former is roughly 3 times larger than the latter.

Table 8 Summary: Comparison between Japan and USA
(1$=121¥, b$=billion dollars, M$=million dollars)

General	Unit	U. S. A.	Japan	Ratio
Personal and National Health Care	b$	967	240	4
Total Domestic Health Expenditure	b$	983	281	3.5

Specified	Unit	U. S. A.	Japan	Ratio
Radiation Imaging	M$	43,077	8,509	5.1
Prostate Cancer	M$	576	0.6	996
Charged Particle Therapy	M$	15	0.2	71
FDG-PET	M$	50	2	21
Cyclotron :RI	Unit	19	6	3.2
:PET	Unit	47	25	2
Medical Equipment	M$	3,133	1,844	1.7
Radioisotopes	M$	140	9	16.2
Radiopharmacy	M$	832	374	2.2
Contrast Media	M$	1,119	926	1.2
Sum (M$)	M$	48,942	11,666	4.2

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24 Economic Scale of Utilization of Radiation in Japan - Agricultural Field -

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Abstract

The economic scale of radiation application in the field of agriculture in Japan, which was estimated from public documents, is about 970 M$. In food irradiation, the irradiated amount of 15,000 t/y potatoes at Hokkaido is calculated to be 16 M$. Sterile Insect Technique (SIT) for melon fly mainly in Okinawa area brought much benefits as high as 60 M$. Production of rice born by mutation breeding is about 3% of overall production in Japan and the economic scale was 774 M$. Radioisotope (RI) utilized in a laboratory work, environmental analysis and chronology is counted to be as high as 24 M$. The relative ratios of radiation processing (136 M$), mutation breeding (804 M$) and RI utilization (24 M$) are 14, 83, and 3 %, respectively.

In USA, the economic scale in food irradiation and mutation breeding was studied for the comparison with that in Japan. The food irradiation and mutation breeding were scaled to be 206 – 3,903 M$ and 3,851 – 13,593 M$, respectively. The economic scale in US is 4 – 18 times higher than that in Japan.

Key words: Economic scale, Radiation processing, Agriculture, Food irradiation, Mutation breeding, Sterile insect technique

1. Introduction

The economic scales of radiation application in Japan and USA were studied by Japan Atomic Energy Research Institute at the instance of the Science and Technology Agency (STA, now the Ministry of Education, Culture, Sports, Science and Technology) in 1999 [1] and 2000 [2], respectively. The study included three fields, Industry, Medicine and Agriculture. The economic scale of radiation application in Japan in

1997 was amounted to about 86 b$. It consisted of 60 b$ (85%) for industry, 10 b$ (14%) for medicine and 1 b$ (1%) for agriculture.

The economic scale of agriculture is only 1% but it is important and essential for human life because we cannot survive without foods. This paper describes the results of economic scale evaluation of Agricultural field in Japan investigated in 1999 and the comparison of food irradiation and mutation breeding in USA and Japan investigated in 2000.

2. Economic scale of radiation application for agriculture in Japan

The economic scale of radiation application in 1997 was studied using published documents. The field of agriculture was divided as 1) radiation processing including food irradiation, sterile insect technique (SIT) and sterilization, 2) mutation breeding and 3) radioisotope (RI) utilization. Table 1 summarized the economic scale in the field of agriculture in 1997. Total cost was 964 M$ consisted of 136 M$ for radiation processing, 804 M$ for mutation breeding and 24 M$ for RI utilization.

2.1 Radiation processing

Radiation processing was estimated as 16 M$ (12%) for food irradiation, 69 M$ (50%) for SIT and 50 M$ (38%) for sterilization (Fig. 1).

For food irradiation, only sprout inhibition of potato by radiation is approved and commercialized in Japan since 1974. The economic scale of potato irradiation was calculated as 16 M$ using the amount of 15,000 ton/y with the cost of 125 yen/kg. The share of irradiated potato is only 0.6 % (15,000t/2,440,000t). The irradiation of potato in Shihoro, Hokkaido is continued more than 25 years but other food item is not approved in Japan. Recently it is expected to commercialize the radiation processing of 1) sterilization of spices, 2) sterilization of herbs and medical plants and 3) quarantine control of cut flower, and the expected economic scale of radiation processing in these fields are 218 M$, 440 M$ and 141 M$, respectively (Table 2).

SIT is very useful and important technique to eradicate the fruit fly without the environmental hazard. The project of SIT in Okinawa Island was conducted in 1977 and succeeded to eradicate the melon fly from whole Okinawa area in 1993. The economic scale in Okinawa Prefecture benefited by SIT was 61 M$ (Table 3). It is the result of increase the export from Okinawa to main land (32 M$) and consumption in Okinawa (29 M$).

The economic scale of sterilization of medical supplies was studied in the field of industry. Here, we studied the sterilization field relating to the food, i.e. laboratory

animal feeds and food packaging materials (Table 4). The total cost of radiation sterilization was calculated as 49 MS consisted of 1.2 M$ for laboratory animal feeds and 48 M$ for bag in box (BIB).

2.2 Mutation breeding

Plant mutation breeding by radiation has been investigating for long time in many countries. Using radiation technique 128 varieties were developed in Japan. Many new species were developed for decease resistant crops, i.e. 55 species of rice, 10 of barley and 2 of wheat. Other species of beans, fruits including pears resistant for black spot decease, grass, vegetables, etc, were also developed (Table 5). Figure 2 shows the economic scale of mutation breeding. Rice with 17 species is the biggest as 774 M$ (96.3 %) following 25 M$ (3.1%) of pear, 4 M$ (0.5%) of beans and 1 M$ (0.1%) of others including peach and chrysanthemum.

2.3 Radioisotopes

Radioisotopes (RI) utilization include 1) laboratory work for RI provision in agro/biological studies and RI deposition, 2) environmental protection of air pollution analysis, 3) chronology for geology and archeology by ^{14}C, and the total economic scale was estimated as 964 M$. The details are 6 M$ (24%) for laboratory research work, 17 M$ 72 (%) for environmental analysis and 1 M$ (1%) for chronology (Fig. 3).

3. Comparison of Economic scale in USA and Japan

The economic scale of radiation application in USA was studied in 2000. It was planned to evaluate by using the data of 1997 as same as the data in Japan. However, the published data in 1997 was difficult to obtain and we used the data in 1999 for the study of agricultural field. In this study, only food irradiation and mutation breeding were selected and compared the economic scale in USA and Japan.

3.1 Food irradiation

USA is the most developed country for food irradiation. The report of United States General Accounting Office (GAO) estimated the annual quantities and percentage of consumption for irradiated food in US as of January 2000 [3]. Using this data, we estimated the economic scale of food irradiation in1999 (Table 6). Amount of irradiated spices including dry or dehydrated aromatic vegetable substances was 43,092 lb and the cost was estimated as min. 146 M$ by raw material cost of black pepper and max. 3,202 M$ by commercial cost in local market. Fruits & vegetables and meets of

fresh & frozen uncooked poultry were estimated as 5 M$ and 19 M$, respectively. In this data, the irradiation of ground beef to kill the food borne pathogen such as *E. coli* O157:H7 is not including but the commercial irradiation is increasing in 2000. Radiation facilities for food irradiation in USA are listed in Table 7. Most of the facilities for food irradiation are used with other purpose such as sterilization of medical devices. In June 2000, the X-ray irradiation facility using electron beam in Hawaii opened for the quarantine control of fruit. Some new facilities are also planned to start the meet irradiation in 2001. Under these conditions, it is expected that the irradiation of spices will increase 10% annually and the amount of irradiated beef will reach to 3 M lb within 3 years.

3.2 Mutation breeding

Recent IAEA data shows that the number of mutant by mutation breeding in USA is 128 (Table 8). The number of mutant in Japan described in the IAEA report is 120 and it was slightly lower than 128 species reported in Japan (Table 2). The economic scale of mutation breeding in USA is estimated as 11,234 M$ with wheat 7,864 M$, rice 1,756, beans 577, etc (Table 9). The amount of 11,234 M$ was calculated as the total cost of irradiated spices. The scale of rice (1,756 M$) in USA is higher than 750 M$ in Japan even though these species are the main products in Japan. For the fruits, grapefruits (156 M$) and peach (24 M$) is the main species in USA and Japan. Other plants such as wheat, barley, oats, beans, peanuts, and peppermint were much higher in USA.

4. Conclusion

The total cost of food irradiation and mutation breeding in USA was 3,246 – 13,997 M$ (Table 10). The scale of food irradiation in USA was 165 – 3122 M$ and that of mutation breeding was 3,081 – 11,234 MS. As it was difficult to find the production cost of irradiated items, min. and max. values were shown in the result. For mutation breeding, the minimum value was estimated by assuming the 3% share of irradiated species. From these results, it was concluded that economic scale in USA is 4 - 18 bigger than that in Japan. The ratio in USA and Japan is 11 – 208 for food irradiation and 4 – 14 for mutation breeding.

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Table 1. Applications in Agriculture

Field		Products (M$)	Break Down (M$)
1. Radiation Processing		136	
(1) Food irradiation	Potatoes		16
(2) SIT	Fruit flies		69
(3) Sterilization	Bag-in-box		50
	Feed for laboratory animals		2
2. Mutation Breeding		804	
	Rice (17 species)		774
	Pear (Gold 20 century, others)		25
	Beans (4 species)		4
	Others (Peach, Chrysanthemum etc.)		1.0
3. RI Utilization		24	
(1) Laboratory work	RI provision in Agri/biological studies		4
	RI depositions (solid, liquid etc.)		2
(2) Environmental protect	Air pollution analysis		17
(3) Chronology	Geology & Archeology by C-14 isotopes etc.		1
Sum		964	

Table 2. Economic Scale in Expected Fields

	Production Volume	Production Price
Sterilization of Spices	Imported 113,274 ton	218 M$
Sterilization of Herbs and Medicinal Plants	Imported 1,050 ton	440 M$
Quarantine Control of Cut Flower	15,001 ton	141 M$

Table 3. Economic Scale of SIT for Melon Fly in Okinawa Island

Export from Okinawa	
Production cost of host plant	32 M$
Cost saving of analysis and fumigation	0.1 M$
Consumption in Okinawa Prefecture	
Total production cost of host plant	(95 M$)
Cost for increasing the production yield	29 M$
Total	61 M$

Table 4. Economic Scale of Sterilization for Food Package and Feed

	Whole scale		Radiation	
Laboratory Animal Feeds	10,000 ton	8 M$	15% (10-20%)	1.2 M$
BIB (Bag in Box)	86.1 M (bags)	304 M$	16% (14-18%)	48 M$

Table 5. Mutation Breeding in Japan

	Common Name	Direct	Indirect
Crops	Rice (Kinuhikari, etc)	8	47
	Barley	6	4
	Wheat	2	
Beans	Soybean	9	1
	Others	2	
Fruits	Apple	1	
	Pear	3	
	Peach	1	
Grass	Lawn	4	
Flower	Chrysanthemum, etc	39	
Vegetables	Tomato, etc	6	
Sub total		88	56
Total		128	

Table 6. Economic Scale of Food Irradiation in USA

	Item	Production (M lb)	Unit ($/kg)	Economic scale (M$)	Annual consumption (%)
USA	Spices	43,092	min. 3.39 max. 74.2	min. 146 max. 3,202	9.5
	Fruits, Vegetables	680	7.7	5	0.002
	Meats	227	83.0	19	0.002
Japan	Potato	15,000	1.1	16	0.6

Table 7. Irradiatiion Facilities in USA

	Facilty	Place	Spices	Fruits	Meet	Others
γ-ray (Co-60)	Applied Radiant Energy Corporation	Lynchburg, VA	·	·	·	Wood Flooring
	Food Technology Service, Inc.	Mulberry, FL	○	○	○	
	STERIS Isomedix, Columbus	Columbus, Mississippi	·	·	·	
	STERIS Isomedix, Dover	Dover, New Jersey	·	·	·	
	STERIS Isomedix, Liberty	Liberty,Illinois	·	○	·	
	STERIS Isomedix, Morton Grove	Morton Grov, Illinois	·	○	·	
	STERIS Isomedix, Northborough	Northborough, Massachusetts	·	·	·	
	STERIS Isomedix, Sandy	Sandy, Utah	·	·	·	
	STERIS Isomedix, Spartanburg	Spartanburg, ,South Carolina	·	·	·	
	STERIS Isomedix, Texas		○	·	·	
	STERIS Isomedix, Whippany	Whippany, New Jersey	○	○	·	
	IBA (North Carolina)	Haw River, NC	○	·	·	Medical, Food packaging
	IBA (North Jersey)	Rockaway, NJ	○	·	·	Cosmetics, Food packaging
	IBA	West Memphis, Arkansas	○	·	·	
	Radiation Sterilizers, Decatur	Decatur, Georgia	Closed			
	IBA, Shaumburg	Shaumburg, Illinois	○	·	△	Food packaging
	IBA, Tustin	Tustin, California	○	○	·	Food packaging
	IBA	Forth Worth, Texas	○	·	·	Food packaging
	IBA	RTI/Salem, NJ	○	·	·	Medical, Food packaging
	IBA Gilroy	Gilroy, CA	○	·	·	Animal treats
EB (X-ray)	AMES, IA	Ames, IA	·	·	○	(For research in Iowa Univ.)
	Titan SureBeam	Sioux, Iowa	·	·	○	(Open in 2000)
	IBA, Bridgeport	Bridgeport, NJ	△	△	△	Polymers
	Hawaii Pride	*Hilo*, HI	·	○	·	

○; Operating, △; Open in 2001

Table 8. Mutation Breeding in USA: IAEA data

	Scientific name	Common Name	Numbers
Crops	*Avena*	Oats	12
	Hordeum	Barley	13
	Oryza	Rice	23
	Triticum	Wheat	3
Beans	*Phaseolus*	Kedny	26
	Arachis	Peanuts	1
Fruits	*Citrus*	Grapefruit	2
Raw materials	*Mentha*	Peppermint oil	2
	Nicotiana	Tobacco	1
	Humulus	Hop	3
Grass		Lawn, Grass	11
Flower	*Begonia*	Begonia, etc	16
Flower trees	*Rosa*	Rose, etc	12
Vegetables	*Lactuca*	Lettuce, etc	3
Total		128	

Table 9. Economic Scale of Mutation Breeding in USA and Japan

	USA (M$)	Japan (M$)
Oats	273	
Barley	379	
Wheat	7,864	
Rice	1,756	750
Peanuts	100	
Dry edible beans	577	4
Peppermint oil	129	
Fruits	156 (grapefruit)	24 (pear)
Others (peach, chrysanthemum)		1
Total	11,234	778

Table 10. Comparison of Economic Scale in Agriculture

		Food Irradiation (M$)	(Ratio)	Mutation Breeding (M$)	(Ratio)	Total (M$)	(Ratio)
USA	Min.	165	(11)	3,081	(4)	3,246	(4)
	Max.	3,122	(208)	11,234	(14)	13,997	(18)
Japan		15	(1.0)	778	(1.0)	794	(1.0)

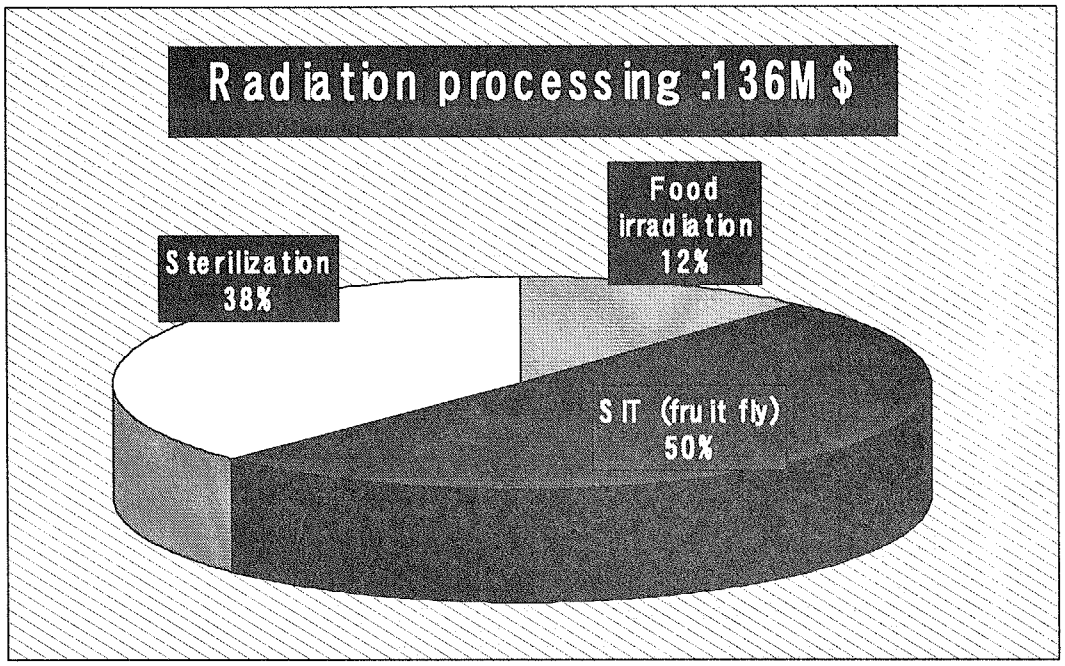

Fig.1. Economic Scale of Radiation Processing

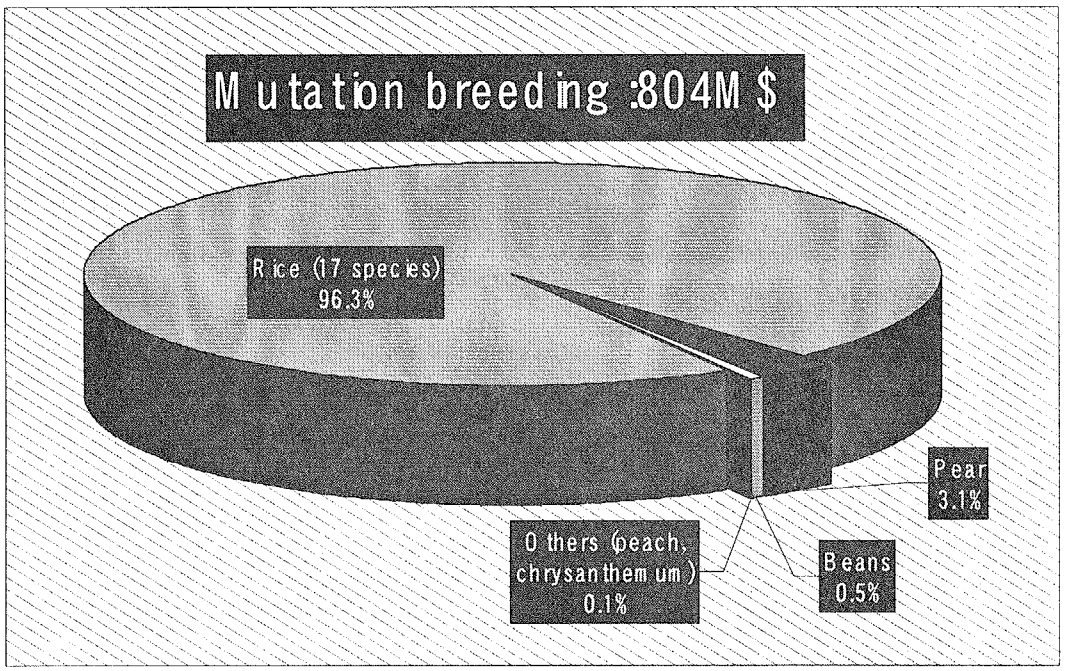

Fig.2. Economic Scale of Mutation Breeding

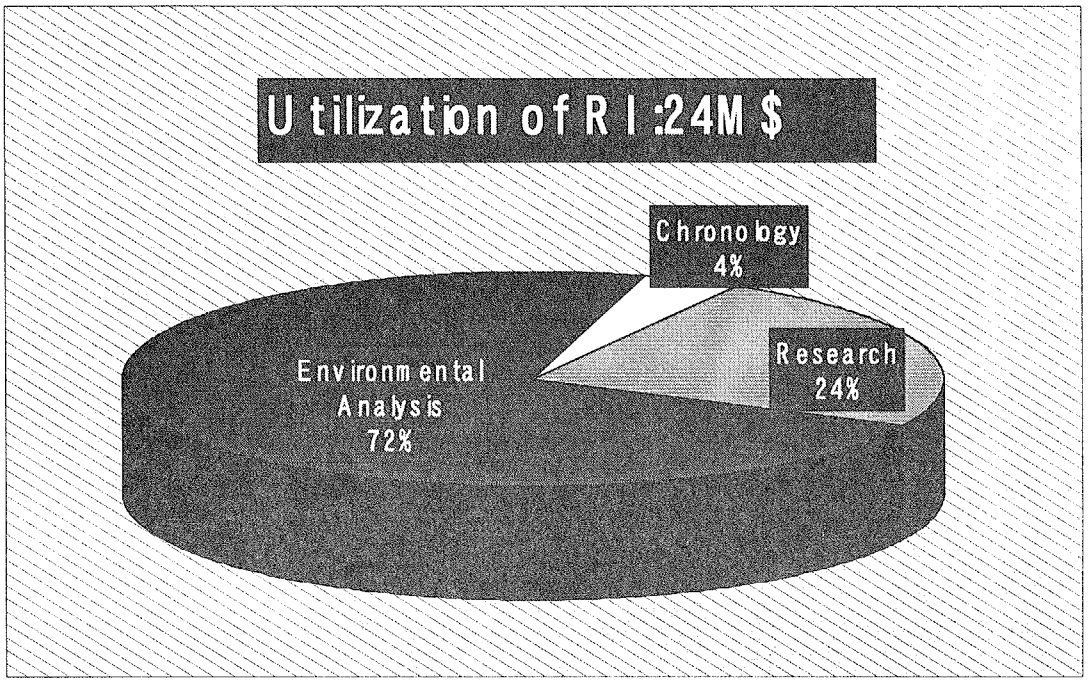

Fig. 3. Economic Scale of RI Utilization

25 Economic Scale of Utilization of Radiation in Japan - Industrial Field -

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Abstract

Radiation is contributing to the growth of Japanese industry directly and indirectly. The direct contribution is the manufacturing of industrial products such as tires and IC by processing with electron beams, γ-rays and ion beams. Construction of radiation facilities and production of radiation instruments/equipments also play an important role in nuclear industry. The total shipments of radiation related products were ¥ 7,236 billion in FY 1997. Industrial application of nucleonic control system (NCS) and nondestructive testing (NDT) based on radiography are indirect contribution of radiation to the industry. The indirect economic contribution of NCS and NDT will be more than several thousands billion yen.

Keywords: Radiation, Industrial Application, Economic Scale, NDT, NCS

1. Introduction

Total value created by industry in Japan was ¥ 130 trillion in 1997. Radiation is used in many industrial fields and contributing to the growth of Japanese industry. Most typical contribution of radiation in industry is the manufacturing of products such as tires and IC by processing with electron beams, γ-rays and ion beams. This can be referred to as direct effect of radiation to industry. The economic scale of the direct effect in 1997 was estimated by summing up the shipment values of these products. In addition to the direct effect, radiation techniques such as nucleonic control system (NCS) and nondestructive testing (NDT) based on radiography are also contributing to the Japanese industry vitally by improvements of productivity and quality of products, saving raw materials and energy. This can be referred to as indirect effect of radiation to industry. The direct and indirect effect of radiation to the Japanese industry will be discussed in this paper. This study was carried out by a Working Group founded in JAERI in 1999. The members of the Group were S. Tagawa (Group Leader), M. Kashiwagi, T. Kamata, H. Tominaga, K. Masubuchi, K. Oka and K. Makuuchi. Data shown in this paper without reference were estimated by the Working Group.

2. Economic Scale of Radiation Processing

Direct contribution of radiation to Japanese economy can be divided into two groups, (1) production of radiation related hardware and (2) manufacturing of industrial products by radiation processing. Shipment value of hardware is illustrated in Table 1. Total value of

hardware was ¥493 billion in 1997. Main part of radiation related hardware is medical radiation facilities such as apparatus of X-ray diagnosis and X-ray CT. Industrial radiation facilities includes construction of 3 Gamma facilities, 21 electron accelerators and 105 ion accelerators. Another big area was the nucleonic instrument for NDT and NCS.

Table 1 Shipment value of radiation related hardware

Hardware	Shipment value
Medical radiation facility	¥ 369 billion
Industrial radiation facility	¥ 58 billion
Nucleonic instrument	¥ 62 billion
Radiation protection equipment	¥ 4 billion
Total	¥ 493 billion

Ion beams, electron beams and γ-rays are used for radiation processing. Table 2 shows shipment value of radiation-processed products by type of radiation sources in 1997. Approximately 74 % of the total production value was brought about by ion beam processing for the fabrication of IC. Semiconductors and integrated circuits are produced by 11 companies in Japan. All companies use three unit operations of ion beam processing, namely ion implantation, sputtering by ion beams and deposition of ions. The total shipment value of these products was ¥ 5,356 billion.

Table 2 Shipment value of radiation processed products

Radiation source	Main products	Shipmen value
Ion beams	Semiconductor, ICs	¥ 5,356 billion
Electron beams	Tires, wire/cable	¥ 1,126 billion
γ-rays	Sterilized medical devices	¥ 265 billion
	Total	¥ 6,747 billion

Electron beams are used for crosslinking of polymer, curing, graft polymerization and sterilization. Table 3 shows the number of electron accelerators installed in industry by the end of 1997. More than 300 electron accelerators were installed.

Table 3 Number of installed EB machine

Process/product	Total	
Crosslinking	116	
Wire & cable		51
Plastic foams		14
Heat shrinkable tube & film		28
Tires		23
Curing	46	
Sterilization	11	
Flue gas treatment	4	
Others	16	
R&D	115	
Total		308

Crosslinking of polymer is the commercially most important area of radiation chemistry of polymers and holds the largest shipment values as shown in Table 4. Tires are superior among the radiation crosslinked products, followed by wire/cable, foams and heat shrinkable tube/film. Other crosslinked products include small rubber parts, SiC fiber and latex gloves. Five tire manufacturers are using electron accelerators for radiation crosslinking of tire components.

Table 4 Shipment value of electron beam processed products

Process/product	Production Value
Crosslinking	¥ 1,098 billion
Tires	¥ 1,018.5 billion
Wire & Cable	¥ 45.0 billion
Foams	¥ 17.9 billion
Heat Shrinkable Tube & Film	¥ 16.5 billion
Other crosslinked products	¥ 0.2 billion
EB Curing	¥ 3 billion
Graft Polymerization	¥ 1 billion
Sterilization	¥ 19 billion
Total	¥ 1,121 billion

Table 5 lists the main products of γ-ray processing. Gamma rays are used for sterilization of medical devices, packaging materials and animal foods, and degradation of polytetrafluoroethylene.

Table 5 Shipment value of γ-ray processed products

Field	Shipment value
Sterilized Medical Devices	¥ 265 billion
Sterilized Packaging Materials	¥ 6 billion
Polymer Degradation	¥ 0.3 billion
Sterilized Animal Food	¥ 0.2 billion
Total	¥ 271.5 billion

2. Indirect Effect of Radiation to Japanese Industry

Non-destructive testing (NDT) and nucleonic control system (NCS) are adopted universally in industry for product reliability and process control. Contribution of NDT and NCS to the industry is immeasurable.

2.1 NDT

Table 6 shows the distribution of γ-ray NDT radiography in industry. The total numbers of γ-ray NDT radiography was 972. Besides inspection services companies, many other enterprises use γ-ray NDT radiography. Main radioisotopes for γ-ray radiography apparatus for NDT are

^{60}Co and ^{192}Ir. The ratio of Co:Ir was 194:750 in 1997.

Table 6 Distribution of γ-ray NDT radiography

Industrial Firms	Number of Radiography	
	1997	1999
Iron & Steel	61	53
Fabricated Metals	15	15
Electric Machines	29	29
Precising Machinery	31	34
Transportation Equipment	33	16
Machinery	50	45
Other Manufacturing	18	31
NDT Inspection Services	633	771
Other Inspection Services	73	59

In addition, x-ray NDT radiography is also used in man industrial firms. Total number of x-ray NDT radiography is estimated to 1,000. X-ray inspection apparatus are installed in airports and semiconductor factories. Table 7 shows number and shipment value of x-ray inspection apparatus from 1996 to 1998.

Table 7 Number and shipment value of x-ray inspection apparatus installed in Japan

Year	Installed	Shipment value
1996	175	¥ 3.92 billion
1997	195	¥ 4.36 billion
1998	225	¥ 4.95 billion

There are 132 NDT inspection companies in Japan in 1997. Total employees in these companies were 9,210. Total service charge: was ¥106.7billion in 1997. These companies use various NDT methods such as Visual Testing (VT), Magnetic Particle Testing (MT), Liquid Penetration Testing (PT), Eddy Current Testing (ET), Ultrasonic Testing (UT), Acoustic Emission Testing (AE) and Radiographic Testing (RT). The percentage of radiography method in the total of nondestructive testing was 25.5%. Figure 1 shows distribution of clients.

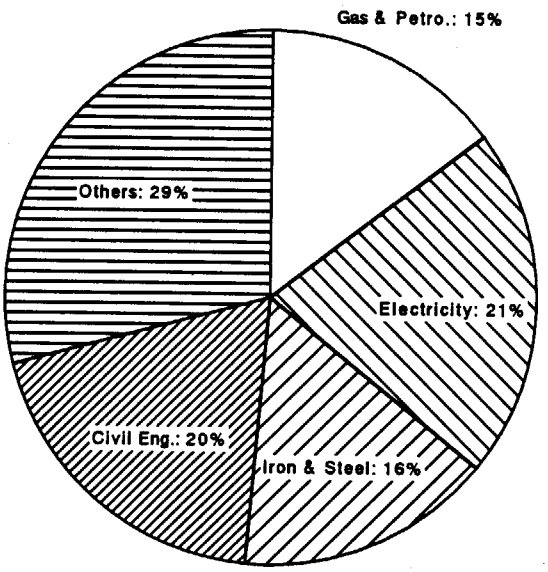

Figure 1 Main clients of RT-NDT services

3. NCS

Various RI gauges are used for NCS in industry as shown in Table 8[1]. Thickness gauge is most widely accepted in many industries.

Table 8 Number of RI gauges

RI gauge	Year			
	1996	1997	1998	1999
Thickness gauge	2,751	2,678	2,757	2,726
Level gauge	1,430	1,406	1,387	1,218
Density gauge	857	957	856	814
Moisture gauge	133	133	129	127

Table 9 shows typical thickness gauges used in steel industry and paper industry[1].

Table 9 RI for thickness gauge

Objective	RI	Activity	Sensitivity
Iron	^{137}Cs (g)	1.11 TBq (30Ci)	4 ~ 100 mm
Iron	^{241}Am (g)	18.5 GBq (500mCi)	0.1 ~ 8 mm
Paper	^{90}Sr (b)	740 MBq (20mCi)	100 ~ 5,000 g/m^2
Paper	^{85}Kr (b)	37 GBq (1Ci)	10 ~ 1,200 g/m^2
Paper	^{147}Pm (b)	18.5 GBq (500mCi)	5 ~ 100 g/m^2

Table 10 shows the number of γ-ray thickness gauges in industries and shipment value of each industry[1]. For example, γ-ray thickness gauges are installed in 73 % of papermaking machine. Since the total shipment of papers was ¥ 2.67 trillion, the indirect contribution of NCS can be estimated at ¥ 1.95 trillion.

Table 10 Number of γ-ray thickness gauges in industries

Industry	Number of gauges	Value of Shipment
Paper	930	¥ 2.67 trillion
Iron & steel	610	¥ 4.60 trillion
Plastic films	349	¥ 1.18 trillion
Nonferrous metals	63	¥ 1.36 trillion
Total	1,952	¥ 9.81 trillion

4. Trend and Prospect

Fig 2 shows changes with the year in the number of users in industrial firms[1]. There two types of users in Japan according to the regulation. The reported users are allowed to use only sealed RI of less than 3.7 GBq. The permitted uses allowed to use sealed RI of more than 3.7 GBq or unsealed RI. Clearly, the number of permitted users is decreasing. While the number of reported users is increasing to avoid troublesome procedures to obtain approval from a

government office.

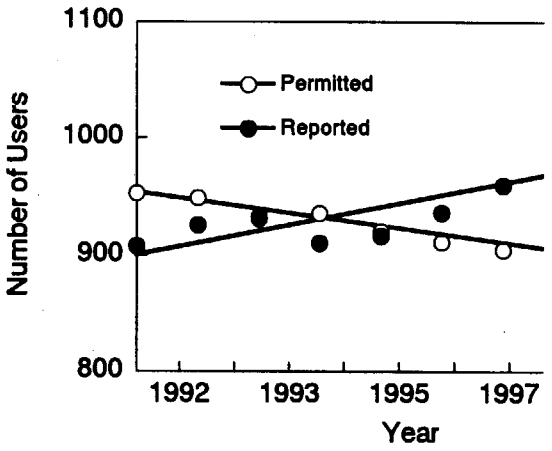

Fig. 2 Changes with the year in the number of users in industrial firms

Further, non-radiation process and method are developed to take place the radiation process and method. Typical example is observed in smoke detector. Ionization detector with RI was mostly used in Japan. However, it has being replaced by photoelectric detector. Percentage ionization detector in the smoke detector is decreased to less than 1%. Deregulation of radiation is needed for the further contribution of radiation to Japanese industry.

Reference

[1] Japan Radioisotope Association, Statistics on the Use of Radiation in Japan, 1997, 1998, 1999 and 2000.

26 Conclusion Remarks

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In this Symposium various researches and developments based on Bilateral Cooperation were introduced using the excellent effects of radiation. The presentations and discussions are summarized below.

Invited Presentations

(1. Biodegradable Water-absorbent Synthesized from Bacterial Poly(amino acid)s,
2. Hydrogel Wound Dressing by Radiation)

1) Two presentations were very helpful to understand the background of the research, principle of the technologies, reaction mechanisms and practical applications.
2) These technologies are environmentally friendly as well as wide practical applications.

Session 1 (Radiation Processing of Starch and Cellulose)

1) Radiation crosslinking was successfully performed under the paste condition.
2) Irradiated products are useful for wound dressings and biodegradable plastics.
3) Irradiation of PP or PP/EFB gave optimum properties for EFB-PP composites.

Session 2 (Radiation Processing of Silk Protein)

1) Radiation can make fine powder.
2) Radiation can give high water-solubility.
3) Radiation can be expected to give special functions such as resistance to microorganisms.

4) Further research is required to clarify the functions of irradiated products & to reduce total dose.

Session 3 (Radiation Processing of Marine Carbohydrates)

1) Radiation treatment of chitosan significantly improve anti-microbial activity & the water-stability of shrimp feed by its bio-adhesive effects.
2) Low molecular compounds produced from chitosan by irradiation can promote growth of plants significantly.
3) Hydrogel occluding chitosan in the networks of acryloyl L-proline methyl ester and acrylic acid was successfully obtained.

Session 4 (Radiation Processing of Other Polymers)

1) Vulcanization of natural rubber latex was performed using a low energy electron accelerator with high productivity and cost effectiveness.
2) Addition of inorganic compounds is effective for radiation crosslinking of PBS as well as improvement of biodegradability.

Session 5 (Economic Scale of Radiation Application)

1) The economic scale in Japan at a fiscal year 1997 was 71 B$. It corresponds to a ratio of about 1.7 % gross domestic production (GDP).
2) The value of 71 B$ consisted of 60 B$ for industrial applications, 10 B$ for medical/health uses and 1 B$ for agricultural uses, respectively.

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国際単位系（SI）と換算表

表1　SI基本単位および補助単位

量	名称	記号
長さ	メートル	m
質量	キログラム	kg
時間	秒	s
電流	アンペア	A
熱力学温度	ケルビン	K
物質量	モル	mol
光度	カンデラ	cd
平面角	ラジアン	rad
立体角	ステラジアン	sr

表2　SIと併用される単位

名称	記号
分，時，日	min, h, d
度，分，秒	°, ′, ″
リットル	l, L
トン	t
電子ボルト	eV
原子質量単位	u

1 eV=1.60218×10^{-19}J

1 u=1.66054×10^{-27}kg

表3　固有の名称をもつSI組立単位

量	名称	記号	他のSI単位による表現
周波数	ヘルツ	Hz	s^{-1}
力	ニュートン	N	m·kg/s^2
圧力，応力	パスカル	Pa	N/m^2
エネルギー,仕事,熱量	ジュール	J	N·m
工率，放射束	ワット	W	J/s
電気量，電荷	クーロン	C	A·s
電位，電圧，起電力	ボルト	V	W/A
静電容量	ファラド	F	C/V
電気抵抗	オーム	Ω	V/A
コンダクタンス	ジーメンス	S	A/V
磁束	ウェーバ	Wb	V·s
磁束密度	テスラ	T	Wb/m^2
インダクタンス	ヘンリー	H	Wb/A
セルシウス温度	セルシウス度	℃	
光束	ルーメン	lm	cd·sr
照度	ルクス	lx	lm/m^2
放射能	ベクレル	Bq	s^{-1}
吸収線量	グレイ	Gy	J/kg
線量等量	シーベルト	Sv	J/kg

表4　SIと共に暫定的に維持される単位

名称	記号
オングストローム	Å
バーン	b
バール	bar
ガル	Gal
キュリー	Ci
レントゲン	R
ラド	rad
レム	rem

1 Å=0.1nm=10^{-10}m

1 b=100fm^2=10^{-28}m^2

1 bar=0.1MPa=10^{5}Pa

1 Gal=1cm/s^2=10^{-2}m/s^2

1 Ci=3.7×10^{10}Bq

1 R=2.58×10^{-4}C/kg

1 rad=1cGy=10^{-2}Gy

1 rem=1cSv=10^{-2}Sv

表5　SI接頭語

倍数	接頭語	記号
10^{18}	エクサ	E
10^{15}	ペタ	P
10^{12}	テラ	T
10^{9}	ギガ	G
10^{6}	メガ	M
10^{3}	キロ	k
10^{2}	ヘクト	h
10^{1}	デカ	da
10^{-1}	デシ	d
10^{-2}	センチ	c
10^{-3}	ミリ	m
10^{-6}	マイクロ	μ
10^{-9}	ナノ	n
10^{-12}	ピコ	p
10^{-15}	フェムト	f
10^{-18}	アト	a

（注）

1．表1－5は「国際単位系」第5版，国際度量衡局 1985年刊行による。ただし,1 eVおよび1 uの値はCODATAの1986年推奨値によった。

2．表4には海里，ノット，アール，ヘクタールも含まれているが日常の単位なのでここでは省略した。

3．barは，JISでは流体の圧力を表わす場合に限り表2のカテゴリーに分類されている。

4．EC閣僚理事会指令ではbar，barnおよび「血圧の単位」mmHgを表2のカテゴリーに入れている。

換算表

力	N(=10^{5}dyn)	kgf	lbf
	1	0.101972	0.224809
	9.80665	1	2.20462
	4.44822	0.453592	1

粘度　1Pa·s(N·s/m^2)=10 P(ポアズ)(g/(cm·s))

動粘度　1m^2/s=10^{4}St(ストークス)(cm^2/s)

圧力	MPa(=10bar)	kgf/cm^2	atm	mmHg(Torr)	lbf/in^2(psi)
	1	10.1972	9.86923	7.50062×10^3	145.038
	0.0980665	1	0.967841	735.559	14.2233
	0.101325	1.03323	1	760	14.6959
	1.33322×10^{-4}	1.35951×10^{-3}	1.31579×10^{-3}	1	1.93368×10^{-2}
	6.89476×10^{-3}	7.03070×10^{-2}	6.80460×10^{-2}	51.7149	1

エネルギー・仕事・熱量	J(=10^7 erg)	kgf·m	kW·h	cal(計量法)	Btu	ft·lbf	eV
	1	0.101972	2.77778×10^{-7}	0.238889	9.47813×10^{-4}	0.737562	6.24150×10^{18}
	9.80665	1	2.72407×10^{-6}	2.34270	9.29487×10^{-3}	7.23301	6.12082×10^{19}
	3.6×10^6	3.67098×10^5	1	8.59999×10^5	3412.13	2.65522×10^6	2.24694×10^{25}
	4.18605	0.426858	1.16279×10^{-6}	1	3.96759×10^{-3}	3.08747	2.61272×10^{19}
	1055.06	107.586	2.93072×10^{-4}	252.042	1	778.172	6.58515×10^{21}
	1.35582	0.138255	3.76616×10^{-7}	0.323890	1.28506×10^{-3}	1	8.46233×10^{18}
	1.60218×10^{-19}	1.63377×10^{-20}	4.45050×10^{-26}	3.82743×10^{-20}	1.51857×10^{-22}	1.18171×10^{-19}	1

1 cal= 4.18605J（計量法）

= 4.184J（熱化学）

= 4.1855J（15℃）

= 4.1868J（国際蒸気表）

仕事率　1 PS(仏馬力)

= 75 kgf·m/s

= 735.499W

放射能	Bq	Ci
	1	2.70270×10^{-11}
	3.7×10^{10}	1

吸収線量	Gy	rad
	1	100
	0.01	1

照射線量	C/kg	R
	1	3876
	2.58×10^{-4}	1

線量当量	Sv	rem
	1	100
	0.01	1

（86年12月26日現在）

Proceedings of the Takasaki Symposium on Radiation Application of Natural Polymers in Asia October 1 and 2, 2001, JAERI, Takasaki, Japan