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PROCEEDINGS OF THE TAKASAKI SYMPOSIUM ON RADIATION PROCESSING OF NATURAL POLYMERS

NOVEMBER 23 AND 24, 2000, JAERI, TAKASAKI, JAPAN

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Functional Materials Laboratory I

日本原子力研究所
Japan Atomic Energy Research Institute

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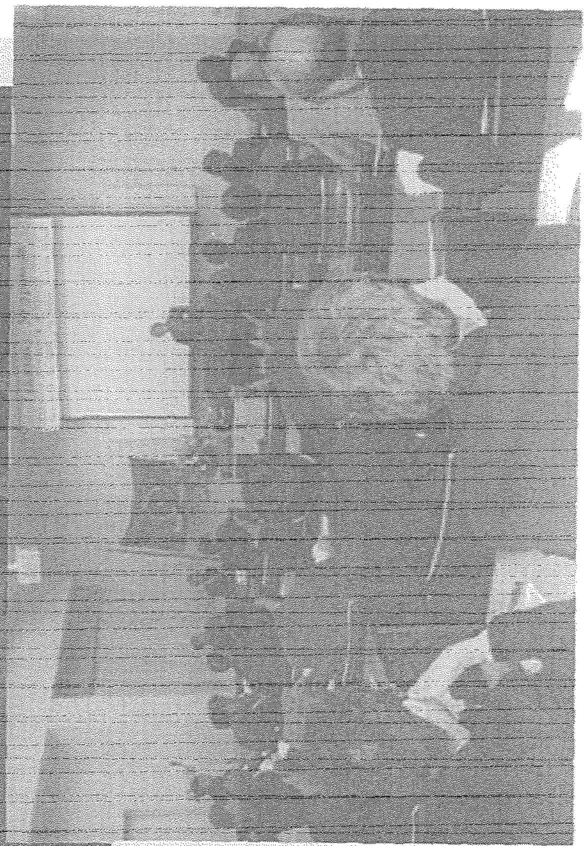

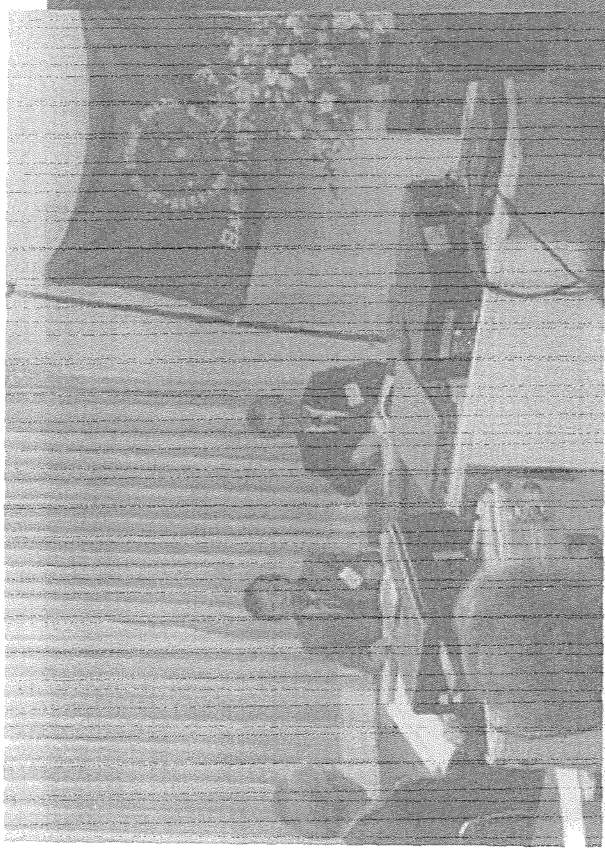

Proceedings of the Takasaki Symposium on Radiation Processing of Natural Polymers

November 23 and 24, 2000, JAERI, Takasaki, Japan

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

(Received January 30, 2001)

This Takasaki symposium was held as enlargement of the Takasaki workshop on bilateral cooperations for radiation processing of natural polymers in 1999. The attendants were increased from 61 to 80 due to the expansion from five bilateral cooperation countries last year to further 3 Asian countries. At the symposium, the enhancement effects of plant growth and antifungal activity by radiation-degraded carbohydrates, hydrogels as a wound dressing material, and production of an protein free natural rubber grove were of major concern. Many domestic and foreign attendants were especially interested in novel functionality of radiation-treated natural polymers and exchanged their opinions actively. Furthermore, since outstanding researchers in this field were invited from the Asian countries, we could also exchange the opinions about the present situation of the research activities and interregional cooperation of the radiation processing in Asia.

In the symposium we had presentations and discussions on the outcomes of the bilateral cooperations. The 80 participants attended to the symposium including 20 from abroad, 28 from domestic, and 32 from JAERI. The symposium program consisted of 17 presentations from 8 Asian countries, 10 from JAERI, and 2 invited talks. This proceeding compiles the invited and contributed papers.

Keywords: Radiation Processing, Natural Polymers, Rubber Latex, Silk Proteins, Starch, Growth Enhancement, Antifungal Activity, Hydrogel, Wound Dressing

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※ Eds. Tamikazu Kume and Yasunari Maekawa

天然高分子の放射線加工に関する高崎シンポジウム論文集
2000年10月23日～24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
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(2001年1月30日受理)

本シンポジウムは、昨年の2国間研究協力相手国5ヵ国のみを対象とした天然高分子の放射線加工ワークショップに引き続き、範囲をその他のアジア地域関連国まで拡大して開催したものである。会議では、放射線分解した多糖類の植物成長促進効果や抗菌作用、創傷被覆剤としてのハイドロゲル、タンパクアレルギーを起こさないゴム手袋の製造などに関する成果が報告され、それぞれの天然高分子の放射線処理による新機能発現に関して、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。また、今回のシンポジウムでは、アジア各国の要職にある研究者も招へいしたため、天然高分子の放射線処理による新機能発現に関する成果報告のみならず、アジア各国の放射線処理に関する研究の現状や国際協力のあり方などについての意見交換を行うことができた。

本シンポジウムには国内外の研究者80名（外国人20名、国内28名、原研32名）が参加し、アジア諸国8カ国から17件、原研から10件、外部機関からの招待講演2件の講演を行った。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。

高崎研究所：〒370-1292 群馬県高崎市綿貫町1233
※ 編集者：久米民和、前川康成

Contents

目　　次

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

Hiroshi Watanabe
Director General, Takasaki Radiation Chemistry Research Establishment,
Japan Atomic Energy Research Institute
1233 Watanuki, Takasaki, Gunma 370-1292, Japan

Good morning, Ladies and gentlemen. It is a great pleasure for me to give an opening address for the "Takasaki Symposium on Radiation Processing 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 been playing a leading role in the R & D of radiation processing in Japan and also in IAEA/RCA project as an international cooperation.

The application of radiation technology is now adopted widely in industry, medicine, and agriculture. On the review of economic scale of radiation applications in Japan, it was revealed that the total scale was about 70 billion US dollars and 60 billion dollars was for the industrial application. However, in the future, we have to aim to establish the harmonizing society with nature. The radiation technology would be well applicable for the conservation of environment and the recovery of resources.

From these viewpoints, we have been conducting the R & D of natural polymers by radiation modification mainly in the bilateral collaborations that are carried out as the four research programs with Asian countries. These R & D are closely related in the fundamental analysis and mechanism though the materials are different in the programs. For the further development and acceleration of these programs, therefore, it will be very useful to exchange the technical information and to make enough discussion between researchers joining the collaboration and the specialists in this field. I believe that this Symposium will give such a good occasion and bring to fruitful results.

Today we invited the special guests for keynote lecture, Prof. M.Suzuki, dean of School of Medicine, Gunma University, and Dr. S.Machi, executive director of JAIF. We have also received about 80 participants here from 8 countries of Asia and several Japanese organizations. I hope you all participate the discussion and comments, which contribute to the progress of these research and development.

Thank you very much.

2 Opening Remarks
"Symposium on Radiation Processing of Natural Polymers"

S. Machi, S. M. D., JAIF and C. R.D., FNCA

Distinguished Participants, ladies and gentlemen:

On behalf of JAIF and FNCA, and as a former Director General of Takasaki JAERI, and former DDG of IAEA I, first of all, like to welcome all distinguished participants from Asian countries to this important and interesting meeting.

It seems to me that this Symposium shows a remarkable success of international cooperation among Asian countries in R and D of radiation processing organized by JAERI, Takasaki. I, therefore, would like to congratulate all participants and JAERI management for the opening of this symposium.

Twenty years ago I was IAEA/RCA coordinator as sell as Head of Section for Industrial Application and Chemistry and initiated the important UNDP project including radiation processing. Past 20 years commercial application of radiation processing has largely expanded in Asia. It should be also recognized that research capacity for radiation chemistry and application has greatly grown in Asian countries by their own efforts with IAEA support and the regional cooperation.

Several R/D activities in Asian countries have achieved significant results, such as vulcanization of natural rubber latex, utilization of agricultural wastes, hydrogel for medical uses and more recently utilization of marine natural polymers like seaweeds.

I strongly wish that those R/D works will bear the fruits of commercial application with socio-economic benefits in years to come.

The FNCA, Forum for Nuclear Cooperation of Asia, participated by 8 governments, has been established in 1990 to enhance regional cooperation aiming promotion of nuclear applications for benefits of humankind. FNCA in Japan is operated by AEC, STA and JAIF with collaboration of relevant ministries and institutes. Currently, FNCA has seven areas of cooperation including agricultural application, medical uses, research reactor, nuclear safety culture, waste management, public acceptance and human resource management. I am confident that radiation processing application should be a priority activity in near future in FNCA.

There are, in fact, several Asian nuclear cooperation programs where Japan plays significant roles, such as RCA/IAEA, scientist exchange program by STA, nuclear training program by STA, and bilateral research cooperation with research centers in Asia, and FNCA. These programs should be coordinated to be complementary and synergistic.

Finally I hope you have excellent presentation and information exchange which should be useful for your future R/D work and further enhance collaboration among participating countries and scientists.

Thank you for your attention.

3 Collaboration of science, industry and business in the Asian area in the 21st century through the application of biotechnology

Mamoru Suzuki, M.D., Ph.D.

Dean of Gunma University School of Medicine

The science in the 21st century will develop on the continuous line of tremendous piles of achievements of fascinating findings, discoveries, applications and development of new technologies. Today, I would like to present a brief note on the development of research in the life sciences and the resulted new concept of biotechnology and bioindustry

In 1858, Charles Darwin published his revolutional views on natural selection in the evolution of living creatures. The book titled "The Origin of Species" came out in the following year. At that time, of course, nobody presumed that Watson and Crick's model of the double stranded DNA helix would come out 100 years later. The DNA theory substantially support Darwin's concept. The mechanism of genetics can be explained at molecular level, and modern genetics promoted DNA central dogma, which has overwhelmed in every field of life sciences. Any structure of animals, plants, uni-cellular organisms and viruses is the result from the informations described in the DNA strands. Every cell activity is eventually under the control of DNA. The reading of whole DNA structure of humans has been promoted as the human genome project. Nearly complete mapping of the human genome has been endevoured by the year 2000, and in consequence, studies on the functions of the read sequences are now on-going works. A new research concept has prevailed in many fields of bio-science. If any specified gene structure can be determined, the gene molecule can be amplified by PCR technique. Thus, the technical methods to industrialize DNA informations has been established. The biotechnology is not the exclusive property of biologists or medical scientists anymore, instead, this new technology has attracted attention of scientists working in the other areas, engineers, businessmen so far as sociologists with various backgrounds and disciplines, and thus a new complex of technology, industry and business has altogether set in action.

In the United States, investment in life science was $5 billion in 1970, however the budget was boosted to nearly $15 billion in 1999, while investments in the other studies such as engineering, physical sciences, environmental sciences, mathematics and computer sciences, social sciences, psychology have been remained at the same level for the nearly 30 years. Similar extension of the budget in bio-science and technology is shown in Japan and the market size of bio-industries in

1999 reached 1200 billion yen, which is equivalent to 11 billion US dollars. With such statistics, it is estimated that by the year 2010, total budget in the bio-market will reach 240 billion US dollars (25 trillion yen) per year if some boosting action is taken place by the government in the priority research areas.

At present, application of biotechnology is to be the leading program in medical and health industries, agriculture and fishery, environmental monitoring, chemical industries, information service business, and electronics engineering. Biotechnology is now being converted into bioindustry covering various kind of production activities followed by the economic development. With such background, in July 2000, Japan Biological Informatics Consortium was organized by the link leadership of government representatives, a group of the eminent scientists and the top managers of the industries and trades & commerce. Seventy five Japanese big leading companies for instance, Mitsubishi Chemical Co., Ajinomoto Co., Hitachi, Shimadzu, Fujitu, Suntory, Takeda Pharmaceutical Co., etc have joined the Consortium.

Let me present some examples of a collaboration networking group within our School of Medicine working in close contact with bioindustry.

An interdisciplinary studies on the neurophysiology, brain science, psychiatry, psychology, behavior science, are being enrolled in an order to investigate into the personality building by new methods. One research group are working to elucidate the brain development and the environmental factors which effect the formation of mentality, behavior of an individual during the steps of the growth. Thus behavior science may be studied and analyzed at a molecular or gene level. The other group are studying the cross talk between the grown brain and the diseased body of an adult person. The brain metabolism resulted from psychiatric response can be imaged by the administration of short term isotope by means of positron emission tomography. A patient effected by a malignant tumor and in consequence falls into psychiatric depression can be diagnosed by lowered glucose metabolism in the brain. Other patients who are simply get psychological adverse reaction does not show lowed glucose metabolism. A psychiatrist can differentiate the depression state from the mental reaction and prepare an appropriate therapy for each type of patient. The gene technology makes the common basis in these studies and each study component can be closely linked with industries outside the University.

The Clinical investigation and Research Unit (CIRU) at Gunma University Hospital is officially open its activities from April 2001. The research unit has made typical collaboration with the bioindustry, in particular, with pharmaceutical companies. A new drug is tested at this unit before commercialized. The volunteer patients are invited to test a new drug. Before the test, poor metabolizers among the volunteer are examined by a DNA technology. The person who are deficient of the enzymes which catabolize the given drug is detected by the preliminary study and will be excluded from the test to avoid adverse side reaction which might be caused by the uncatabolized test drug. Also, the polymorphism of the drug receptors of the volunteers will be studied in order to design a scientific drug administration. The CIRU thus keeps its activities in close link with pharmaceutical industries in this new area of academic-industry collaborations.

The third example makes a potential future application in the bio-industry after making the basic studies at the laboratory level. Department of Parasitology Gunma University School of Medicine has worked on malaria for more than 20 years. For these several years, intensive collaboration studies have been conducted with Faculty of Engineering of Gunma University and partly with Japan Atomic Energy Research Institute to analyze and synthesize a parasite enzyme molecule which might be high-lighted as the malaria vaccine candidates. By the use of a computer system, Professor Katakai and his associates prepared a molecule model and consequently, active sites of the molecule were determined. The active sites were produced by the excellent synthesis technique of the research staff in his laboratory and the reactivity of malaria patients to the product was assessed using the sera from severe malignant falciparum malaria patients and the sera from patients with mild manifestation and vivax malaria patients. Obviously, the synthesized product showed high reactivity with the sera from severe falciparum patients. The results looked promising to prepare a product which can be a candidate for malaria vaccine if safety tests are cleared.

At the end of this presentation, I would like to stress that the application of atomic science in this new trend of bio-science and technology will promote an unique and important contributions in the bioindustry in the 21st century. In particular, harmonized collaborations amongst scientists, engineers and industry people in Asian area will bring out a new view in the bio-industry, which will never come out from a single research group. Such results will support industries and economics of respective country and eventually bring welfare and prosperity of the people.

Session 1

Radiation Processing of Marine Carbohydrates

JP0150453

4 Bilateral Cooperation Between VAEC and JAERI

Tamikazu Kume

Takasaki Radiation Chemistry Research Establishment,
Japan Atomic Energy Research Institute
1233 Watanuki, Takasaki, Gunma 370-1292, Japan

"**Cooperative Research Program on Utilization of Marine Carbohydrates by Radiation Processing**" was sighed by the Vietnam Atomic Energy Commission (VAEC) and the Japan Atomic Energy Research Institute (JAERI) on 29 September 2000.

1. Background

A huge amount of bio-resources are underutilized or discarded. Radiation treatment has being applied to improve or pasteurize these bio-resources because it has advantages not only to recycle the bio-resources but also to reduce the pollution of environment.

Vietnam and Japan are the maritime countries having a long coast and the utilization of marine resources is a mutual subject for both countries. Radiation processing of the marine carbohydrates such as sodium alginate, carrageenan, chitin/chitosan, etc. has a potential for the development of new radiation application. Vietnam and Japan have preliminary investigated the radiation processing of agro-resources through the STA scientist exchange program and obtained the interesting results, i.e. growth promotion of plants by irradiated sodium alginate, reduction of heavy metal toxicity by irradiated chitosan.

Therefore, VAEC and JAERI agreed to carry out the cooperative research activities under the Arrangement on the Research Cooperation in the Field of Radiation Processing to develop the research work on upgrading of marine carbohydrates further. The cooperative program is important for both parties in the following points:

- Strengthening the cooperative relation between Japan and Vietnam on Science and Technology

- Reduction of environmental pollution
- Recycling of resources
- Development of new radiation processing
- Development of knowledge and expertise in radiation processing
- Development of new materials and new field of marine carbohydrates

2. Scope of Research

The purpose of the cooperative research program (hereinafter referred to as Program) is to provide the basic technology for the new functional materials from natural resources. The program is aimed to investigate the radiation treatment of marine carbohydrates for the effective utilization of bio-resources.

The following marine carbohydrates obtained from sea products will be mainly used in the Program:

- Alginate
- Carrageenan
- Chitin / Chitosan
- Others

By radiation degradation and crosslinking, the capability to improve the quality of the products will be studied for agricultural and medical use.

3. Major Items of the Work and Time Schedule

Contents	2000	2001	2002
1) Study on materials preparation			
• Extraction of carbohydrates from various marine products	→		
• Enhancement of extraction yields by radiation	→		
2) Study on induction of physiological functions			
• Analysis of radiation degraded carbohydrates	→	→	
• Test for functional activities		→	→
• Optimum radiation condition for induction of functions		→	→
3) Study on plant cultivation			
• Tissue culture (*in vitro*) test		→	→
• Field test in Vietnam			→
4) Other application			
• Application for feeds			→
• Application for medical field			→

5 SOME FEATURES OF IRRADIATED CHITOSAN AND ITS BIOLOGICAL EFFECT

Le HAI[1], Nguyen Quoc HIEN[1], Le Quang LUAN[1], Truong Thi HANH
Nguyen Tan MAN[1], Pham Thi Le HA[1], Tran Thi THUY[1]
Fumio YOSHII[2] and Tamikazu KUME[2]

1. Nuclear Research Insitute, VAEC, Dalat, Vietnam
2. Takasaki Radiation Chemistry Research Establishment, JAERI
1233 Watanuki, Takasaki, Gunma, 370-12 Japan

Abstract

Preparation of chitosan oligomer by radiation degradation was carried out on the gamma Co-60 source. The radiation degradation yield (G_d) of the chitosan was found to be of 1.03. The oligochitosan with 50% of dp>8 fraction was obtained by irradiating the 10% (w/v) chitosan solution in 5% acetic acid at 45 kGy for the chitosan having the initial viscometric average molecular weight, Mv= 60,000. Irradiated chitosan showed higher antifungal effect than that of unirradiated one. Furthermore, the irradiated chitosan also showed the growth-promotion effect for plants.

Keywords: Chitosan, Irradiation, Antifungal Effect, Plant Growth-Promotion

1. Introduction

Chitosan is one of the natural polymers, poly-β- (1-4) -N- acetyl-D-glucosamine, derived from chitin by alkali deacetylation. This natural polymer has been produced mainly from the shell of crustaceans, such as shrimp, lobster, crab, squid, etc.. [1]. The annual production of chitosan in the world is of about 2,000 tons [2].

Chitosan and its derivatives have been extensively studied and widely utilized in medicine and cosmetics as carriers for drug, enzyme, tissue, wound dressing, hair spray, skin care cream [1-5]. In agriculture, chitosan has been also used as plant regulator, agro-products preservative [6]. In addition it has been used for metal adsorption in waste water treatment and for purification in fruit juice production,.. Matshuhashi and Kume (1997) reported the higher antimicrobial activity of chitosan induced by radiation and their results indicated that chitosan with molecular weight (MW) from 10^5 to 3.10^5 exhibits fairly high antimicrobial activity *in vitro* [7]. Recently Ha et al (1999) reported the higher antifungal activity of irradiated chitosan [8].

Oligochitosan with degree of polymerization (DP) from 7 to 14 has been recognized as phytoalexin inducer to prevent infection of many fungi for plants [9,10]. Thus oligochitosan is very promising to utilize in agriculture and medicine for enhancement of immune system against infection of diseases [4, 6].

Several degradation methods to regulate MW of polysaccharides including chitosan have been studied such as chemical method used oxidative agents, biological method used enzymes [11-13], radiation method used γ-ray from a Co-60 source, electron beam and ultraviolet ray [7,14-17]. Radiation method for degradation and/or modification of natural polysaccharides is considered as a useful tool with the advantages of *i*) process carrying out at ambient temperature *ii*) products with highly purity grades and *iii*) reliability for regulating MW of polysaccharides through adjustment of dose [7,16].

This report presents some features and the antifungal effect of irradiated chitosan.

2. Experimental

2.1. Irradiation of chitosan

Chitosan 8B in powder form with deactylation degree of 80% and viscosity average molecular weight M_{v0} =710,000, a product of Katokichi Co. Ltd., Japan was used without further purification. Solution chitosan 8B of 10% (w/v) was prepared in 5% (w/v) acetic acid and irradiated on a gamma Co-60 source, at room temperature, and dose rate of 2kGy/h. The viscosity average MW (M_v) of chitosan samples irradiated in powder form was measured by Ubbelohde viscometer using 0.1M CH_3COOH/ 0.2M NaCl as solvent system and calculated by equation $[\eta] = k.\ M_v^{\alpha}$ where $k = 1.81.10^{-3}$ cm^3/g and $\alpha = 0.93$ at 25°C [18]. Oligochitosan from irradiated chitosan solutions was determined by fraction coagulation method using CH_3OH/H_2O = 9/1 (v/v) as solvent [13].

Chitosan in flake form was prepared from shrimp shell chitin by deacetylation in NaOH solution of 40%w/w, at 125°C, for 3 hours. The chitosan samples were dissolved in 2.5% CH_3COOH and their viscosity were measured by Brookfield viscometer, at 30°C.

2.2. Biological effect test

2.2.1. Fungicidal effect of irradiated chitosan

a) In vitro

Chitosan in powder form was irradiated by gamma radiation of Co-60 source at 75 kGy then dissolved in 2.5% CH_3COOH and adjusted to pH 6.0 with 1N NaOH. Chitosan solution was filtered with Millipore MILLEX-GS (pore size: 0.22μm, Millipore MA, USA) and diluted with concentration range from 50 to 2800μg/ml in the medium (Czapeck-Dox). Spores or micellial fragments of fungi were dispersed in the medium, then incubated at 25°C. The fungicidal effect of chitosan and irradiated chitosan was tested by recording the fungal growth when the fungus in the control dishes had attained maximum growth.

b) In vivo

The antifungal activity *in vivo* of irradiated chitosan (the product received so-called as plant protector) was tested as follows: 9 month old tea plants were divided into 3 beds, 50 plants for each. Bed 1 was the reference control and bed 2 and 3 were artificially inoculated with *Exobasidium vexans* (foliar pathogen) by spraying 3 times on the leaf surface and pouring over the soil surface with suspension of spores or micellial fragments of fungi (10^6/ml). Two days after infected with fungi, bed 2 was treated with 0.1% irradiated chitosan, bed 3 was treated with 0.05% acetic acid. The number of the infected leaves frequency (number of infected leaves per total of leaves) were recorded within 50 days.

2.2.2. Effect of growth-promotion for plant

In the above test of antifungal effect *in vivo*, the number of leaves and the height of plant were also recorded.

Irradiated chitosan solution was added into MS medium of tissue culture with different concentrations: 0, 20, 50 and 100ppm and *Chrysanthemum revert* plants was used for study.

The length of stem, root and the fresh biomass (including all of root, stem and leaves) of the tissue culture plants were recorded after 20 days incubating at 25°C.

3. Results and discussion

3.1. The features of irradiated chitosan

Results in Fig. 1 clearly indicated that M_v of chitosan decreases with the increase of dose. The decrease of MW can be evaluated on the basis of the G_d (sissions/100eV). $G_d = 1.03$ was derived from the results in Fig. 1.

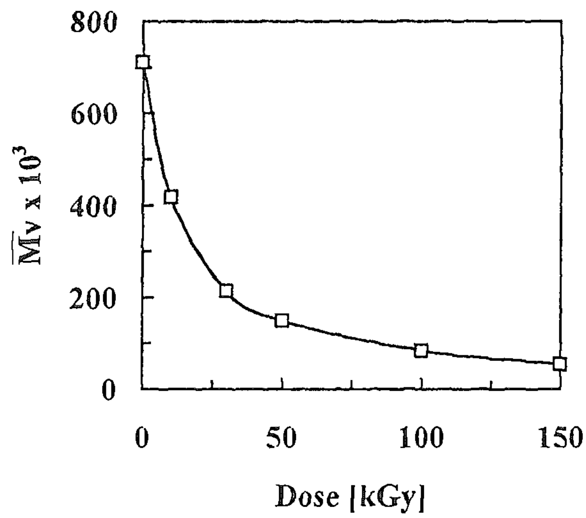

Fig. 1. The relationship between M_v of chitosan and absorbed dose

The obtained G_d value for chitosan was fairly good in agreement with G_d value reported by Ulanski and Rosiak, $G_d = 1.10$ using HPLC technique to determine the number average MW [15].

Accordingly, radiation technique can be usefully utilized to regulate chitosan M_v to a certain low level. On the other hand, for preparation of oligochitosan, the iradiation of chitosan in form of dry powder at high dose may cause the destroy of glucosidic ring and breaking of $-NH_2$ linkage. Radiation degradation reaction for chitosan in solution may proceed in mild condition due to the contributions of indirect effect mainly from radiolysis products of water.

The relationship of oligochitosan fraction and dose in Fig. 2 showed that the oligochitosan DP>8 fraction with 50% of mass product can be obtained at the dose of 45kGy for chitosan having initial Mv = 60,000. At this given dose a half of irradiated chitosan was not coagulated by solvent mixture methanol/water (9/1 v/v) will have DP<8, assuming that opening of glucosamine ring of oligochitosan DP dp>8 to form strongly electrolytic products which are soluble in above mentioned solvent mixture system does not occur. Further study using fractionation LC and HPLC techniques to determine the degree of MW distribution of oligochitosan is still in progress.

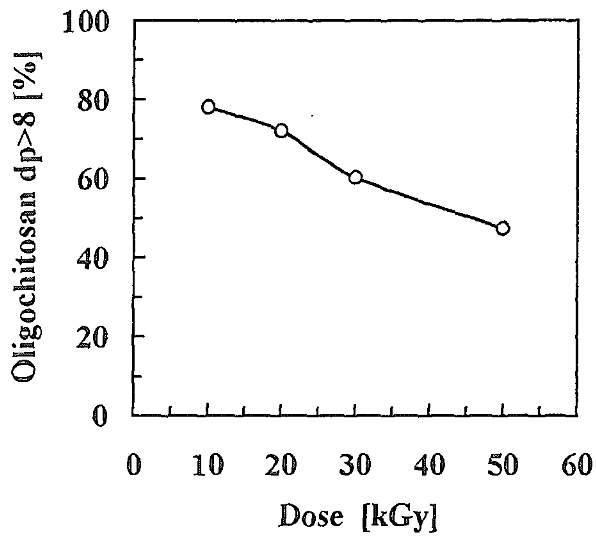

Fig. 2. The fraction of oligochitosan with DP>8 with dose

To prepare oligochitosan by the chemical degradation method, the rather low chitosan concentration solution (1%) was used and after degradation process the oxidative agent has to be washed off as well as further purification of oligochitosan has to be done. For those reasons the radiation degradation method could be more advantageous due to higher concentration of chitosan in solution (>10%) may be used and the products without contaminated catalyst agents can be obtained.

The relationship between viscosity of chitosan solution with different concentrations was presented in Fig. 3. The result showed that the viscosity of chitosan solution decreased sharply with increasing absorbed dose.

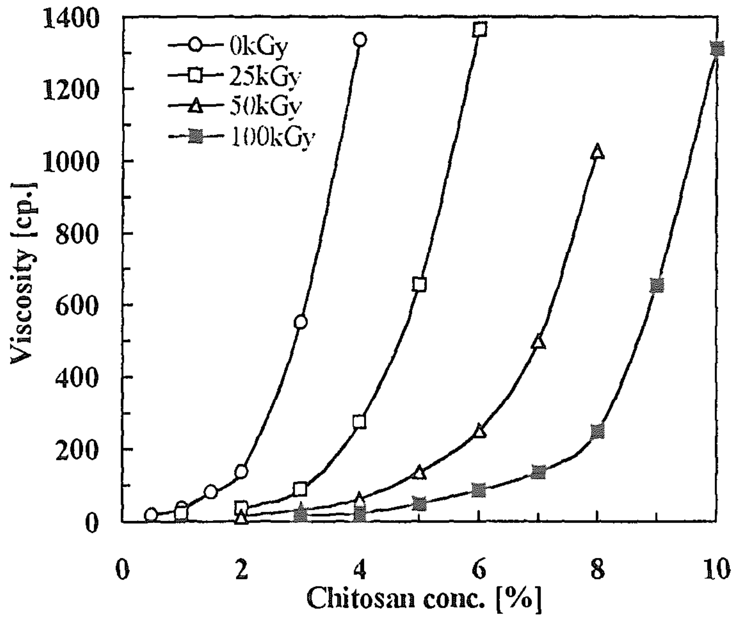

Fig. 3. Viscosity of chitosan solution vs. concentration and dose
Chitosan (M_v ~ 300,000) prepared from shrimp shell and irradiated in flake form

3.2. Biological effect of irradiated chitosan

3.2.1. Antifungal effect

The results in Table I indicated that both unirradiated and irradiated chitosan inhibited the growth of all fungi strains studied, but irradiated chitosan showed higher fungicidal activity than that of unirradiated one. The change in surface charge of chitosan by irradiation in dry state was small [17], so it was presumed that when chitosan was degraded by irradiation, more effective fragments were produced. The results in Table I also revealed that the concentration of chitosan which suppressed the growth was different for different strains of fungi, *Colletotrichum sp.* was the most resistant.

Table I. Effects of chitosan and irradiated chitosan on the growth of fungi

Minimum concentration (μg/ml) at which no growth was detected		
Fungi	Chitosan	Irradiated chitosan
Phytophthora cactorum	300	250
Fusarium oxysporum	1150	800
Aspergillus awamori	400	250
Exobasidium vexans	1000	550
Septoria chrysanthemum	700	350
Gibberella fujikuroi	400	250
Septobasidium theae	1450	1000
Colletotrichum sp.	1500	1050

Table II showed the frequency of infected leaves that was similar in bed 1, 2, 3 at the beginning of the test. After 1 week, the frequency in bed 2, 3 became higher than that in the control (bed 1) because the tea leaves were infected not only by pathogenic fungi naturally but also by artificial inoculation. In comparison with bed 1 and 3, the infected leave frequency of bed 2 was lower after 1 month. It proved that irradiated chitosan can protect tea leaves against infection by *Exobasidium vexans.*

Table II. Effect of irradiated chitosan on infection of *Exobasidium vexans* to tea leaves

	Infected leave frequency				
	Recording date				
Bed	June, 30	July, 8	July, 15	July, 22	July, 30
1	0.078	0.132	0.345	0.394	0.421
2	0.078	0.153	0.180	0.140	0.173
3	0.077	0.420	0.431	0.442	0.471

3.2.2. Growth-promotion effect for plants

Table III showed that foliar spraying with chitosan not only protected tea leaves against *Exobasidium vexans* but also promoted the growth of tea according to the height of plants and the number of leaves.

Table III. Effect of irradiated chitosan on the number of leaves and the height of tea plant

Bed	June, 30				July, 30			
	Height (cm)	%	No. of leaves/tree	%	Height (cm)	%	No. of leaves/tree	%
1	38.1±0.2	100	11.4±0.1	100	41.0±0.1	100	13.1±0.1	100
2	38.0±0.4	99.7	11.3±0.1	99.1	41.8±0.2	102.0	14.2±0.2	108.4
3	38.2±0.1	100.3	11.0±0.2	96.5	40.6±0.3	99.0	12.8±0.1	97.7

(Recording date: June, 30 and July, 30)

The effect of oligochitosan on plant growth-promotion of *Chrysanthemum revert in vitro* was presented in Table IV. The result showed that the culture medium contained oligochitosan from 20 to 50ppm made the length of stem, the length of root and the fresh of biomass increase of 2.9, 68.4 and 60.6%, respectively.

Table IV. Effects of oligochitosan on plant growth-promotion *in vitro*

Oligochitosan conc. (ppm)	Growth-promotion effect					
	The length of stem		The length of root		Fresh biomass	
	mm	%	mm	%	g/22plants	%
0	30.7	100	34.3	100	4.57	100
20	31.6	102.9	48.6	141.7	7.63	160.6
50	31.6	102.9	57.8	168.5	7.40	155.8
100	29.1^{NS}	94.8	48.6	141.7	6.43	135.4
LSD	0.8		6.1		1.23	

LSD: The least significant difference, NS: None significant difference

4. Conclusions

The radiation degradation yield for chitosan was found to be of G_d = 1.03. The oligochitosan DP>8 with 50% fraction in the final product can be obtained by irradiating 10% (w/v) chitosan solution in acetic acid at dose of 45kGy for chitosan having initial Mv=60,000.

Irradiation could improve the fungicidal activity of chitosan. Of 8 strains of fungi tested, the concentration of irradiated chitosan for suppression the growth of fungi was smaller than that of unirradiated chitosan. In *in vivo* test, the frequency of diseased leaves was 0.173 for tea bed sprayed with irradiated chitosan compared to 0.421 for the control.

Oligochitosan produced by radiation technique has growth-promotion effect for plants.

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6 Radiation-Induced Enhancement of Antifungal Activity of Chitosan on Fruit-Spoiling Fungi during Postharvest Storage

Tran Bang DIEP[1], Nguyen Duy LAM[1], Tran Minh QUYNH[1] and Tamikazu KUME[2]

[1]Institute for Nuclear Science and Technique - VAEC

[2]Takasaki Radiation Chemistry Research Establishment - JAERI

Abstract

Experiment conducted four fruit-spoiling fungal strains that were isolated from spoilt fruits (mango and dragon fruit) and were identified as follows: *Fusarium dimerum* Penzig, *Aspergillus nidulans* Wint, *Aspergillus fumigatus* Fresenius and *Aspergillus japonicus* Saito. Chitosan samples with various deacetylation degree (70-99%) were irradiated at doses ranging from 20 to 200kGy, then were supplemented to liquid medium for growth of fungi. We have found that chitosan possesses not only well known antibacterial activity but also the antifungal one on fruit-spoiling fungi. Method of fungal cultivation using liquid medium showed that it has higher sensitivity compared with the cultivation on agar plate, so we recommend this method should be used for evaluation of antimicrobial activity of chitosan. Our study also indicated that deacetylation degree of chitosan clearly affects its antifungal activity, the higher the deacetylation of chitosan, stronger antifungal activity can be observed. This finding recommends the use of chitosan with higher deacetylation for fruit coating and other pharmacology utilization. Results from the minimal inhibitory concentrations (MIC) on fungal growth showed that radiation treatment increased antifungal activity of chitosan and dose of 60kGy gave highest activity.

Key words: chitosan, antifugal activity, radiation treatment, fruit-spoiling fungi

1. Introduction

Coating of fresh fruits and their cut products by biopolymer for preservation have been being a popular processing and strongly developed in number of countries. Coating membrane can create changeable micro-atmosphere covering fruit and form a layer, which hinders water loss and exchange of gases. Coating is also useful when they are used as carrier for prosthetic substances with the aims of pasteurization, preservation, anti-oxidization or flavoring. To form an edible coating that is eatable or harmless to consumers, one kind of biopolymer or its mixture with others can be used. Polysaccharides and proteins are most popular biopolymers for this purpose. Principle of most of completed studies on application of coating with chitosan based on the modification of internal atmosphere of fruit and the semi-permeability of chitosan membranes *[2,8]*. Antimicrobial activity of chitosan on fruit-spoiling microorganisms has not been studied intensively.

Enhancement of antimicrobial activity of chitosan plays a certain important role in practical application of pharmacy and preservation of fresh fruits. With increased activity, chitosan can be used for fruit preservation instead of series of toxic synthetic fungicides, which are limited or banned in use. As action of hydrolytic enzymes, radiation treatment can cause changes in physico-chemical properties of chitosan and can increase its antibacterial activity *[3,7]*. However, there is a lack of references related to the enhancement by irradiation. Therefore, in the study we investigated the enhancement of antifungal activity of chitosan by irradiation in order to improve the chitosan coating utilization.

2. Material and method

2.1. Materials

Mature-green grade mango fruits were collected directly from mango tree growing in suburb of Hanoi City, while dragon fruits were purchased at markets around of Hanoi. Chitosan samples with various deacetylation degrees (7B-10B) were provided by KATOKICHI Company (Japan). Domestic chitosan sample with deacetylation about 80% was supplied by Chemical Institute.

2.2. Isolation and identification of fungi contaminated on spoilt fruits

Three mediums were used for fungal growth study: Czapek-Dox, Peptone-glucose and Sauboraud-glucose. Mangoes and dragon fruits after buying left to become spoilage naturally. Fungi on spoilt fruits were isolated by two methods: shake-washing polyspores of fungi from samples or directly cultivating pieces of spoiled fruits on agar plates. To identify fungal strains, we observed general characteristic appearance of colony by microscope with small objective. After that, we prepared microscopic slides to observe the reproductive organs such as phialospore, gloespore, and appearance of polyspores. At last, fungal strains were identified according to Bui Xuan Dong's classification key *[1]*.

2.3. Fungal cultivation in liquid medium

Two fungal strains of *Fusarium dimerum* Penzig and *Aspergillus nidulans* Wint were cultivated separately in 100 ml PDB medium (Difco, USA) for preparing primary seed. In the next shaking experiment, 1 ml of suspension of primary seed was transferred into Erlenmeyer flasks containing liquid medium. The incubation condition was conducted with 120 rpm of shaking rate at 28-30^{o}C for several days. This cultivation method was used to investigate inhibitory activity of chitosan on growth of fungi, and from that, the minimal inhibitory concentration (MIC) was determined. Through the latter value, influence of original and irradiated chitosan on fungi, effect of deacetylation degree, and sensitivity of fungal strains to chitosan was defined. The inhibition (%) of chitosan was calculated by weighting dried biomass of fungal strains by formula: {[biomass of control sample (g/l)] – [biomasss of treated sample (g/l)]}/ [biomass of control sample (g/l)].

3. Result and discussion

3.1. Typical fruit-spoiling fungi on mango and dragon fruits

Based on the characteristics of colony's morphology, the form of polyspore spreading, reproductive organ and according to the classification key of Bui Xuan Dong *[1]*, we have found three fungal species with eight strains from different mediums as shown in *Table 1*. Among these strains, only one strain that belong to *Penicillium* was found in spoiled dragon fruits but not in mango. Four strains with highest frequency of occurrence on both of mango and dragon fruits were identified.

Data from the results showed that amount of fungal strains growing on pepton-glucose and Sauboraud-glucose mediums were higher than that on Czapek-Dox medium. Among eight strains that belong to three spices, *Aspergillus* and *Penicillium* sp. always appeared with higher frequency on all of three mediums. The finding agreed with many previous results due to the fact that *Aspergillus* and *Penicillium* sp. are always dominated in plant-originated materials. Only one strain of *Fusarium* sp. was found (*Fusarium dimerum* Penzig) but it was typical fruit-spoiling strain on both of mango and dragon fruits. From many documents, *Fusarium* was considered as the main fungi that cause spoilage on fruit during postharvest storage. Four strains with highest frequency of occurrence on both of mango and dragon fruits were: *Fusarium dimerum* Penzig, *Aspergillus nidulans* Wint, *Aspergillus fumigates* Freeness and *Aspergillus japonicas* Saito.

Table 1: Fungi contaminated on spoiled mango and dragon fruits

No.	Species	Occurrence frequency of fungus on various mediums		
		Czapek - Dox	Pepton - glucoza	Sauboraud – glucoza
1	*Fusarium*	1	1	1
2	*Aspergillus*	3	5	4
3	*Penicillium*	2	2	3

3.2. Effect of chitosan on the growth of fruit-spoiling fungi

Two fungal strains namely *Fusarium dimerum* Penzig and *Aspergillus nidulans* Wint were used in this experiment. Fungi were inoculated into PDB liquid medium that has been added with chitosan at different concentration (from 50 to 200ppm) for liquid culture. Effect of chitosan at different concentration on the growth of *Fusarium dimerum* Penzig and *Aspergillus nidulans* Wint were indicated in *Table 2*. The results showed that biomass of two fungal strains obtained after 84 hours of incubation decreased with increase in concentration of chitosan. The growth of two strains was inhibited clearly even at very small concentration of chitosan: 150-200ppm for *Fusarium dimerum* Penzig and 50 -100 ppm for *Aspergillus nidulans* Wint. By comparison on sensitivity of fungal strains to chitosan, it was realized that *Fusarium dimerum* Penzig was less sensitive than *Aspergillus nidulans* Wint. The MIC of chitosan on *Fusarium dimerum* Penzig Wint was two times higher than that of *Aspergillus nidulans.*

Experimental evidences of chitosan effect on the growth of fungi are very limited. Tsay et al. *[5]* indicated that high concentration (1000 -5000 ppm) in plate culture could inhibit growth of fungi. In our experiment, chitosan showed the inhibition on *Fusarium dimerum* Penzig and *Aspergillus nidulans* Wint at ten times smaller concentration. Beside of difference in using fungal strains for experiment, the main reason of quite different effectiveness on fungal growth was from culture techniques. First time we used liquid culture to study microbial effect of chitosan instead of the cultivation on agar plates that have been used regularly in previous investigations *[5]*. 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 *[4]*. Other mechanism proposed that chitosan is able to block description to RNA from DNA *[6]*. The way of action of two mechanisms is not the same depending on the molecular weight of chitosan fractions. Fractions with average molecular weight acts by the first mechanism. In the second mechanism, chitosan must be hydrolyzed to the smaller fractions in order to permeate easily in to the cell. Cultivation in shaking liquid medium maintained a homogenic solution of chitosan in medium leading to the effect of chitosan on fungi was kept by both above-mentioned mechanisms. This way gave the higher sensitivity of method when we evaluated the inhibition of chitosan on the growth of microorganism. Stimulation of chitosan on the growth of fungi was not observed in our experiment, although it was showed by Tsay et al *[5]* when they used 500-2000 ppm of chitosan in agar plates.

3.3. Influence of deacetylation degree of chitosan on its antifungal activity

Fusarium dimerum Penzig was selected to investigate the effect of chitosan with different deacetylation degree (from 70% to 99%) on the growth of fungi. Different concentrations of chitosan (from 100 to 350 ppm) were supplemented to PDB medium for fungal cultivation. Results in *Table 3* indicated that the deacetylation of chitosan obviously affects its antifungal activity. The MIC of chitosan on *Fusarium dimerum* Penzig decreased with deacetylation degree increased.

The influence of deacetylation degree of chitosan on the growth of fungi was observed not only at minimal inhibitory concentration (MIC) as indicated by data from *Table 3*, but also at every lower concentrations of chitosan (data were not given here). This fact demonstrates that chitosan is possessed antifungal property depending on deacetylation degree. Our finding on deacetylation-depended antifungal activity is in good agreement with other study on antibacterial activity, in which antibacterial activity of chitosan was shown to increase with increase of deacetylation degree *[5]*. Thus, the deacetylation of chitosan affects its anti-microbial activity including the antibacterial and antifungal activity.

Table 2: Influence of chitosan at various concentrations on the growth of *Fusarium dimerum* Penzig and *Aspergillus nidulans* Wint

Chitosan (ppm)	Biomass (g/l)	Inhibition (%)
	Fusarium dimerum Penzig	
Control (0)	3,67 ± 0,29	-
150	2,72 ± 0,06	25,8
160	2,01 ± 0,001	45,2
170	1,50 ± 0,04	59,1
180	1,12 ± 0,05	69,4
190	0,96 ± 0,04	73,8
200	0	100
	Aspergillus nidulans Wint	
Control (0)	7,91 ± 0,09	–
50	6,11 ± 0,01	22,7
60	6,04 ± 0,06	23,6
70	4,33 ± 0,04	45,2
80	0,76 ± 0,02	90,3
90	0,76 ± 0,02	90.3
100	0	100

Table 3: Influence of chitosan with different deacetylation degree on the growth of *Fusarium dimerum* Penzig

Deacetylation degree (%)	MIC (ppm)
70	350
80	230
90	180
100	160

3.4. Effect of gamma radiation on antifungal activity of chitosan

Fusarium dimerum Penzig and *Aspergillus nidulans* Wint fungi were also utilized in this experiment. Chitosan in dry state were exposed to gamma radiation at doses: 20, 60, 100, and 200kGy. The fungi were cultivated in PDB medium supplemented with irradiated chitosan at various concentrations for 84 hours in order to define MIC. The results from the experiment were shown in *Figure 1*. 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 counted at dose of 60 kGy. At this dose, the MIC values of chitosan on *Fusarium dimerum* Penzig and *Aspergillus nidulans* Wint were 150ppm and 70ppm, respectively.

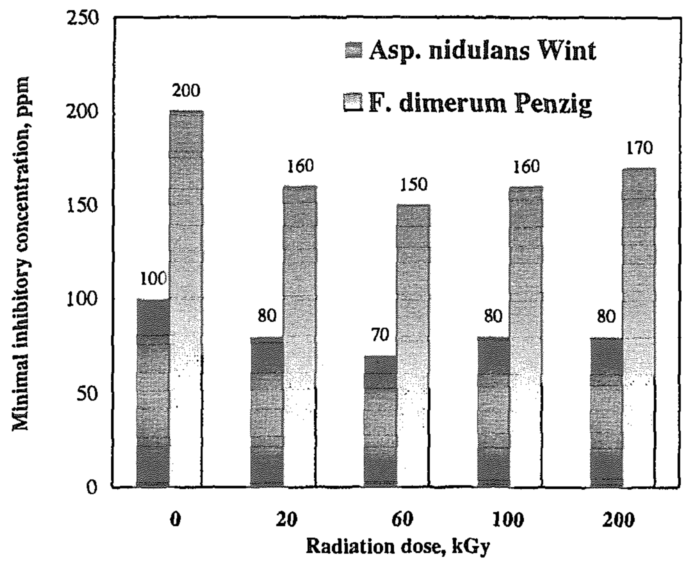

Fig 1: Changes in MIC on *Fusarium dimerum* Penzig and *Aspergillus nidulans* Wint by radiation treatment of chitosan

Masuhashi and Kume *[3]* observed firstly the enhancement of antibacterial effect of irradiated chitosan on *E.coli*. They indicated that chitosan, which have been irradiated at dose of 100kGy, could inhibit the growth of *E.coli* even at low concentration of 3mg/l. The 500 kGy-irradiated chitosan also possessed antibacterial activity, but its effectiveness was lower than that of 100kGy-irradiated chitosan. Antibacterial activity of chitosan irradiated at dose higher than 500kGy were to be weak gradually and the activity disappeared at dose of 2000kGy and higher. Our study gained the same results and a maximal effect was observed even at dose of 60kGy, and the antifungal activity still maintained at other doses treated.

For partly explaining the radiation-induced enhancement of antimicrobial activity of chitosan, it is necessary to repeat the action mechanism of chitosan. The proposed mechanism is action causing the reduction of metabolism by stacking chitosan macromolecules to bacterial cell wall, and other mechanism by inhibiting the description to RNA from DNA *[4,6]*. Effect of radiation on chitosan has been reported earlier with the break of glycosidic link to produce different lower molecular-weight fragments *[3,7]*. Microbial inhibition caused by radiation-formed chitosan fragments is stronger than that by original chitosan molecules due to the contribution of both mechanisms occurred simultaneously in case of irradiated chitosan. Action mechanism of original chitosan abides mainly by only one mechanism, in which chitosan macromolecules stack to cell wall. Because radiation-formed fragments are varied in size including a wide range of molecular weights, so the high molecular-weigh fragments act by mechanism as that of the original chitosan macromolecules, while mechanism of lower molecular-weigh fragments base on inhibiting genetic materials of cell. It should be note that chitosan macromolecules consist of glucosamin and *N*- acetyl glucosamin units, both of which are linked by 1 – 4 glycosidic bonds. So the radiation treatment with very high dose as did by Masuhasi and Kume *[3]* could break most of bonds leading to completed degradation with monomer and short-chain oligomers as the irradiated products. These fragments could not create inhibition effects on the growth of microorganism, even they might stimulate the growth. as observed in some plate-culture conditions *[5]*.

3.5. Sensitivity of fruit-spoiling fungi to original and irradiated chitosan

The experiment conducted four fungal strains: *Fusarium dimerum* Penzig, *Aspergillus nidulans* Wint, *Aspergillus fumigatus* Fresenius and *Aspergillus japonicus* Saito. The sensitivity to chitosan of these fungi was measured and compared between non-irradiated and irradiated chitosan. Results indicated that *Aspergillus nidulans* Wint was the most sensitive to chitosan (the lowest MIC value recorded), while *Fusarium dimerum* Penzig was the last one. All of strains that belong to *Aspergillus* species were more sensitive than *Fusarium dimerum* Penzig, and within these species, the sensitivity increased with following order: *Aspergillus fumigatus* Fresenius, *Aspergillus japonicus* Saito and *Aspergillus nidulans* Wint. Similar to that of non-irradiated chitosan, the sensitivity of the fungi to irradiated chitosan also was expressed with order: *Fusarium dimerum* Penzig, *Aspergillus fumigatus* Fresenius, *Aspergillus japonicus* Saito and *Aspergillus nidulans* Wint. According to Tsay et al. *[5]*, sensitivity of negative-gram bacteria to chitosan is always higher than positive-gram bacteria. Sufficient data on the sensitivity of fungi to chitosan have not been published.

Table 4: Sensitivity of fungi to chitosan

Fungal strains	MIC of chitosan (ppm)	
	Non-irradiated	Irradiated at 60kGy
Fusarium dimerum Penzig	200	150
Aspergillus fumigatus Fresenius	150	120
Aspergillus japonicus Saito	120	80
Aspergillus nidulans Wint	100	70

4. CONCLUSION

Three species of fungi, include eight of typical fruit spoiling strains were isolated from mango and dragon fruits. Among them, four strains: *Fusarium dimerum* Penzig, *Aspergillus nidulans* Wint, *Aspergillus fumigatus* Fresenius and *Aspergillus japonicus* Saito were found with highest frequency of occurrence and were the most typical on both of mango and dragon fruits. Chitosan possesses not only the well-known antibacterial activity but also high antifungal activity tested on typical fruit-spoiling fungi. The fruit- spoiling fungi showed to have different sensitivity with chitosan, *Aspergillus sp.*, for example, have higher sensitivity compared to *Fusarium sp.*

Radiation treatment increased antifungal activity of chitosan. Irradiated chitosan at 60kGy in dry status is enough to induce the highest antifungal activity. Deacetylation degree of chitosan is directly influences on antifungal activity of chitosan. The higher deacetylation of chitosan is, the stronger antifungal activity of chitosan is.

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7 Suppression of Zn Stress on Barley by Irradiated Chitosan

N. Nagasawa[1], P. T. L. Ha[2], S. Watanabe[3], T. Ito[3],
H. Mitomo[1], H. Takeshita[3], F. Yoshii[3] and T. Kume[3]

[1]Department of Biological and Chemical Engineering, Faculty of Engineering, Gunma University, 1-5-1 Tenjin, Kiryu, Gunma 376-8515, Japan
[2]Nuclear Research Institute, Dalat, Vietnam
[3]Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute, Takasaki, Gunma 370-1292, Japan

Abstract

Chitosan was irradiated up to 1000 kGy in solid state. Irradiation of chitosan caused the reduction of molecular weight. The molecular weight of the chitosan reduced from *ca.* 4×10^5 to *ca.* 6×10^3 by irradiation at 1000 kGy. For the barley growth promotion, irradiated chitosan showed the significant effect and 1000 kGy irradiated chitosan improved 20 % of growth. Using the positron emitting tracer imaging system (PETIS), the effect of chitosan on uptake and transportation of ^{62}Zn in barley were investigated. It was found that the transportation of Zn from root to shoot and the damage of plant by Zn were suppressed with irradiated chitosan.

Keywords: Radiation degradation, Chitosan, Growth promotion, Suppression of Zn stress, Positron emitting tracer imaging system (PETIS)

1.Introduction

Polysaccharides such as chitin / chitosan, alginate and carrageenan are widely used in food processing to increase viscosity, and as emulsifiers in medicine and cosmetic fields [1]. Recently, oligosaccharide derived from depolymerization of alginates by enzyme was reported to have novel features such as stimulation of growth of *Bifidiobacteria*, promotion of germination and shoot elongation of plants [2, 3, 4, 5]. Oligochitosan degraded by chitosanase, has been also reported to enhance for antimicrobial activity [6]. These oligosaccharides can be prepared by acid hydrolysis or enzymatic degradation of polysaccharides [7, 8, 9, 10]. On the other hand, radiation technique can provide a useful tool for degradation of polysaccharides. It has been well known that polysaccharides such as cellulose can be degraded due to scission of glycosidic bond by radiation [11, 12]. We have been studied on radiation effect of these polysaccharides and its biological activities for plants. They were easily degraded by irradiation and induced various kinds of biological activities such as promotion of plant growth, anti-bacterial activity, and so on. Irradiated alginate has been proved to promote growth of rice and barley through our cooperative study with Vietnam Atomic Energy Commission. Furthermore, we found that chitosan irradiated in 1% solution (0.5M acetic acid) at 100 kGy not only recovered seedlings growth damaged by heavy metals but also reduced vanadium levels in seedlings [13]. In this study, suppression of zinc (Zn) stress on barley by irradiated chitosan was investigated.

2. Experimental

2.1. Materials and irradiation

Plant of barley (*Hordeum vulgare*) was used for experiment. Chitosan (degree of deacetylation = 80%) used in this work were purchased from Katokichi Bio Co. Ltd., Japan. This material in solid (less than 200-mesh powder) was sealed in a glass tube with the air atmosphere, and then irradiated. The irradiation was carried out using gamma rays from Co-60 sources with a dose rate of 10 kGy/hr.

2.2. Analytical procedures

Molecular weight of irradiated chitosan was measured by gel permeation chromatography (SEC-MALLS; Shodex Co., Ltd., Japan). The instrument was equipped with Shodex Asahipak GF-7MHQ and elution at room temperature (27°C) with 1/3M acetic acid + 0.1 M sodium acetate aqueous solution with a flow rate of 0.6 mL/min. The eluent was monitored by a Shodex RI-71 differential refractometer and a Wyatt DAWN DSP and polymer concentration was *ca.* 0.1%(w/v).

2.3. Toxicity test of Zn on plant growth

Germinated seeds were sown on plastic nets floating on nutrient solution of Hyponex® at 1/1000 in controlled room (25 °C). Zinc (Zn) stresses were applied at 1.0 – 2.0 mM (Zn as $ZnSO_4$) on 3 day old seedlings with 30 plants per each treatment. After 14 days growing, each 20 seedlings were collected, washed gently, air dried and finally dried in oven at 105 °C for 24 hr, and weighed

2.4. Production of ^{62}Zn

^{62}Zn was produced from the reaction ^{63}Cu (p, 2n) ^{62}Zn by bombarding Cu plate with 0.3 mA of 30 MeV H^+ particles from TIARA AVF cyclotron for 90 min. This Cu plate was dissolvied in nitric acid, and then added hydrochloric acid and pure water. The solution was purified through anion exchange column, and ^{62}Zn solution produced *ca.* 5.55 MBq in 7mL of 5mM HCl solution.

2.5. Analysis of ^{62}Zn transport in plants

The solution containing *ca.* 2.7 MBq/mL of ^{62}Zn was fed to the root of plants and then the plants were dipped in water for 18 hr during the image analysis of tracer activity by using positron emitting tracer imaging system (PETIS) [14].

3. Result and Discussion

3.1. Irradiation effect of chitosan

Chitosan, polymer of glucosamine, irradiation effect of chitosan has been investigated. Figure 1 shows the relationship between number-average molecular weight ($\overline{Mn}$) and the irradiation dose. It can be seen that $\overline{Mn}$ of chitosan in solid state decreased remarkably by irradiation. The molecular weight of the chitosan reduced from *ca.* 4×10^5 to *ca.* 6×10^3 by irradiation at 1000 kGy. This degradation behavior of chitosan is almost the same as that of alginate [15].

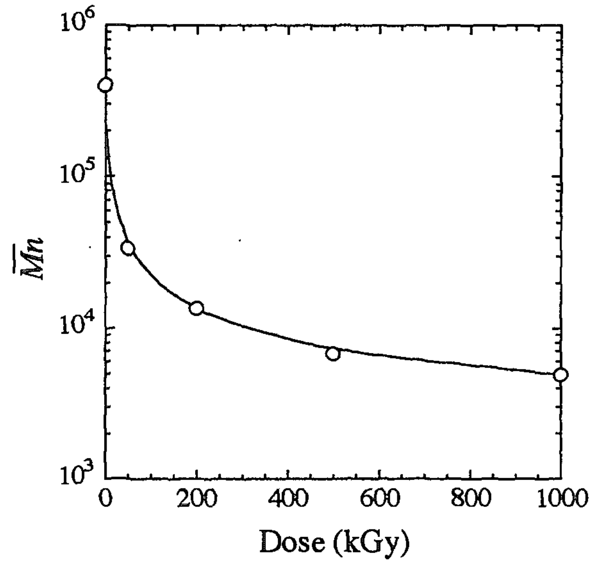


Fig. 1 Change in molecular weight of chitosan in solid state by irradiation. Molecular weight was measured by GPC.

3.2 Effect of irradiated chitosan on growth of barley

Chitosan affects the growth of barely plant. Figure 2 shows the effect of irradiation dose of chitosan on barely growth. Degraded chitosan obtained from higher dose (up to 1000 kGy) shows a stronger effect on the increase of dry matter of barely seedlings. It is expected that a certain molecular weight of degraded chitosan ($\overline{Mn}$ *ca.* 6×10^3 at 1000 kGy) is effective for plants growth.

The growth of barely at various concentrations of irradiated chitosan is shown in Fig. 3. The results show that the optimium concentration of degraded chitosan is 20 ppm.

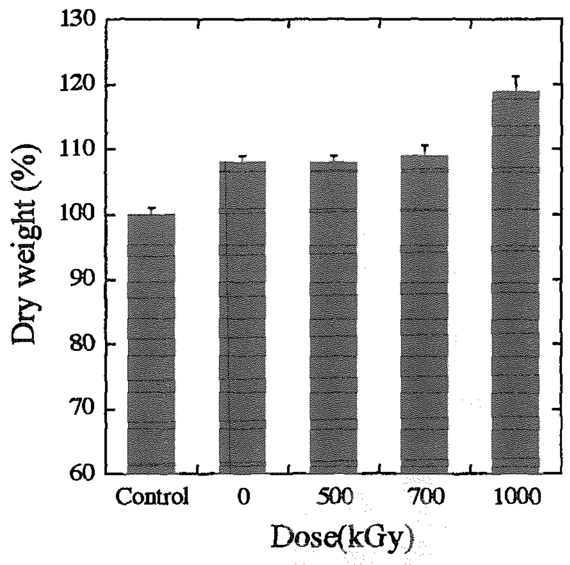


Fig. 2 Effect of irradiation dose for chitosan on barley growth. Barley was cultivated for 14 days in hydroponic solution with 20 ppm chitosan irradiated in solid state.

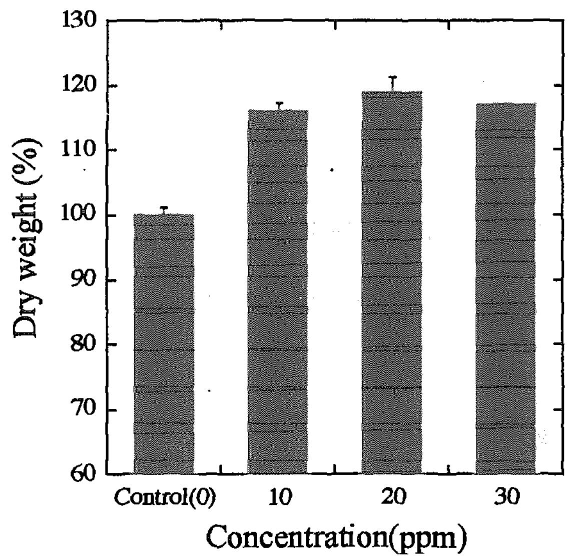


Fig. 3 Concentration effect of irradiated chitosan on barley growth. Barley was cultivated for 14 days in hydroponic solution with chitosan irradiated at 1000kGy in solid state.

Using the chitosan irradiated at 1000 kGy with the concentration of 20 ppm, effect of irradiated chitosan on barley growth under Zn stress was tested. Figure. 4 shows the damage of barley by Zn (left) and the suppression of Zn damage on barley with irradiated chitosan. The growth of barley decreased with increasing concentration of $ZnSO_4$. With 2.0 mM $ZnSO_4$, significant damage (45%) was observed but it was slightly smaller recoverd (65%) with 20 ppm of irradiated chitosan.

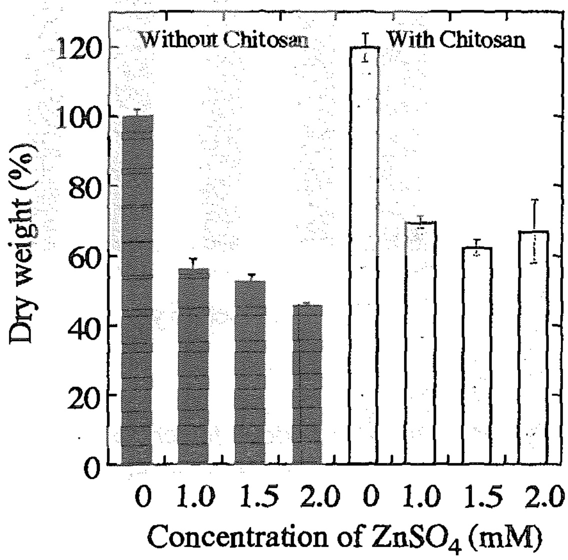
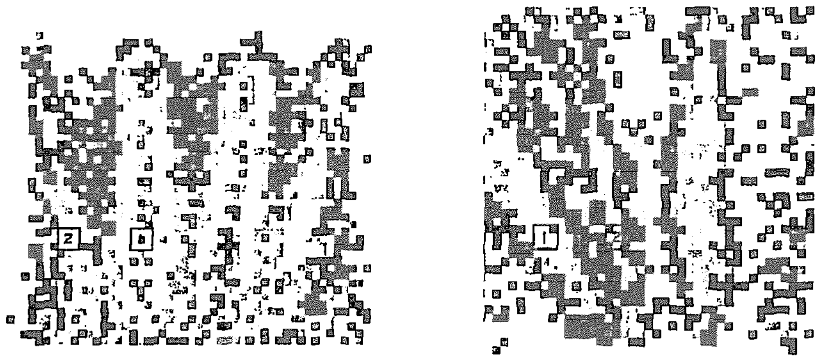


Fig. 4 Effect of irradiated chitosan (1000kGy, 20ppm) on barley growth under zinc stress at different concentrations.

3.3 Uptake and translocation of Zn in Plants

The recovery of metal damage when treated with irradiated chitosan at 1000kGy suggest that chitosan under irradiation can regulate transportation of Zn. Figure. 5 shows the ^{62}Zn imaging of barley leaves using PETIS. The results revealed that the plant treated with 20 ppm irradiated chitosan transported Zn very weakly into the shoot compared to without chitosan. The results show that the radiation degraded chitosan are effective to suppress the uptake and translocation of Zn into the shoots and suppress the heavy metal damage on the growth of plants.



Without chitosan　　　　With chitosan

Fig. 5 PETIS images of ^{62}Zn in barley leaves with or without irradiated chitosan(1000kGy, 20ppm).

Acknowledgment

The authors would like to thank Mr. A. Tsuji (Hamamatsu Photonics Co. Japan) for his helpful suggestion.

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8 Chitosan-Crosslinked Gels Prepared by a Simultaneously Occurring Reaction of Radiation-Induced Polymerization and Self-Bridging of Acrylic Acid in Aqueous solutions

Amr ELHAG ALI[a], Elsayed Ahmed HEGAZY[a], John HENDRI[b], Ryoichi KATAKAI[b], Yasunari MAEKAWA[c], Tamikazu KUME[c], Masaru YOSHIDA[c]

[a] National Center for radiation research and technology, Cairo, Egypt

[b]Faculty of Engeneering, Gunma University, Kiryu, Gunma 376, Japan.

[c]Japan Atomic Energy Research Institute, Takasaki Radiation Chemistry research establishment, Takasaki, Gunma 370-12, Japan

(E-mail: Elhagali_a@hotmail.com)

Abstrasct

Chitosan is one of the most interesting natural polymers, in addition to its biodegradability it shows wide biological properties such as antifibrolastic and antimicrobial activities, which verify its biomedical application. Novel Acrylic acid/Chitosan hydrogel was prepared by means of γ-irradiation as a clean source for initiation, and crosslinking. The nature of the AAc/CS gel and the effect of the presence of chitosan on the behavior of AAc were characterized. The effect of pH on the degree of swelling of different gels and time course swelling studies show the effect of presence of chitosan and its molecular weight on the swelling of the gels. DSC and TGA were used to study the effect of the presence of chitosan on the thermal behavior of PAAc. It was found that chitosan change thermal behavior of AAc. These results support our assumption for the formation of crosslinking between PAAc and CS chains via polyelctrolyte complex formation, attributed to the high affinity between CS and AAc, accompanied by homopolymerization and self-bridging. This crosslinking increase with CS molecular weight increasing and affect the thermal behavior of PAAc.

Key words: Chitosan; Acrylic acid; Irradiation; pH-sensitivity; Thermal analysis

Introduction

Chitosan (CS), which is produced by alkaline deacetylation of chitin, is soluble in water only at low pH owing to the protonation of amino groups. It is known that CS forms polyelectrolyte complexes with a weak acid such as poly(acrylic acid) through electrostatic attraction. As one example, such a polyelectrolyte complex is applicable to responsible biopolymer materials as interpenetrating polymer networks because of its unique feature of relative independence [(1)]. In pervious attempts, copolymerization of AAc and CS takes place by chemical method or by blending through different mechanisms; the amidation between the free carboxylic groups and the amino groups in CS, the intercomplexation of AAc and CS or polymerization of AAc in the presence of CS obtaining interpenetrating network of PAAc and CS [(2 - 4)]. Radiation-chemistry has become one of the effective methods for preparation of synthetic biopolymers [(5)]. Recent studies also showed the importance of water as a solvent in the radiation processes. The radiolysis of water produces active species such as $H^{\bullet}$, $OH^{\bullet}$ and e , these active species assist chain polymers to be crosslinked and/or self-bridged. 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.

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 and cross-linking agent [(6)]. In this study, we synthesized a novel CS crosslinked AAc gel by radiation processing in combination with complexization of the polymers, and will report the preliminary.

EXPRERIMENTAL

Materials

Chitosan (CS) of molecular weights, 10, 100, and 1000 from Wako (Japan), Acrylic acid, (AAc), and Acetic acid, 99.7, Cica-reagent, Kanto chemical co. (Japan), buffer solutions of pH range 3-8. All chemicals were used as received without further purification.

Preparation of Chitosan/Acrylic acid Gels

CS/AAc gels were obtained by radiation induced copolymerization of mixture of CS (0.02 g) and AAc (0.8 and 0.4 ml) in 2ml of distilled water using γ-rays from ^{60}Co source at dose rate 10 k Gy/h at 0°C for 3 hours. All samples were washed in excess 2% acetic acid aqueous solution to remove the unreacted component then freeze-dried.

Differential Scanning Calorimetry (DSC)

A Perkin-Elmer DSC-7 was used to measure the phase transition of water in CS/AAc gels. DSC curves were obtained in the temperature range from 30 to 300 °C and scanning rate was 5°C min^{-1}. Sample weights were 6-10 mg.

Results and Discussion

It is well known that during the polymeriztion of CS as polysaccharide and AAc as vinyl acid by chemical methods or by blending. Quantities of copolymers can be formed by two different mechanisms; the amidation between the free carboxylic groups of AAc and the amine groups of CS which provide the grafting of PAAc chains onto CS macromolecules, copolycondisation. The intercomplexation of CS and AAc or polymerization of AAc in the presence of CS obtaining interpenetrating network of PAAc and CS (7). But on using radiation, the case is completely different. Gel/sol fraction test shows that the degree of conversion is almost 100% which means that CS is perfectly included in the gel and that was confirmed by determining the N_2 content in the gel which is corresponding to the amount of CS in the gel.

Fig. (1) shows the effect of pH on the swelling degree of AAc/Cs gel having CS of the same molecular weight, 10, and different content of AAc compared with that of pure poly (AAc). Figure shows that the presence of Cs in the same AAc concentration dramatically decrease its swelling degree from 80 to 30. In the same time reducing the AAc concentration increase the swelling degree once more. The motive force in both cases might be the degree of crosslinking, which increased by the existence of CS leading to that dramatic decrease in the swelling. Degree of crosslinking also decreases by reducing the AAc concentration, which allow the swelling of the gel but lower than that of pure AAc.

The effect of CS molecular on the swelling of AAc/CS gel was also investigated. Fig. (2) shows that the increasing in CS molecular weight reducing the swelling degree of the gel. This also may be attributed to the degree of crosslinking achieved by increasing the CS molecular weight.

DSC is used to get some information about the structure by attributing the observed peak to specific physical or chemical processes. Figure (3) shows the DSC thermograms of freeze dried CS/AAc after swelling in buffer solutions of pH 3 and 7 compared with that of PAAc swollen in pH 7. As shown, the three thermograms show peaks in the range 125-145°C, both PAAc at pH 7 and AAc/CS gel at pH 3 show peaks in the range 240-250°C and this peak is completely absent in case of the gel at pH 7.

The endothermic peak observed in the range 125-145°C attributed to the liberation of moisture whereas that peaks around in the range 240-250°C may due to intermolecular dehydration reaction forming cyclic anhydride structure. This peak, in the range 240-250°C, is completely absent in case of the gel swollen in pH 7[8]. These results show that both gel in the collapsed state, at pH 3, and PAAc even in swollen state, at pH 7, show almost the same thermal behavior. This behavior changed as soon as the gel swollen in pH 7 giving the chance to chitosan to participate freely in network structure. This change, decomposition without intermolecular dehydration reaction, may due to structural change in the network due to the presence of CS, this structural change is so-called "scrambled egg" structure which sterically hinders the reaction between adjacent COO [9]. These results were also confirmed by studying the TGA traces of the AAc/CS gel swollen in pH 3 and 7 in compared with that of PAAc in pH 3 and 7 and pure chitosan. AAc/CS gel and PAAc swollen in pH 3 almost have the same TGA trace showing weight loss stage in the range less than 180°C results from evolution of water associated with the gel. High weight loss at 200°C corresponding to intramolecular and intermolecular dehydration forming anhydride ring structure then decarboxylation at temperature range over 250°C and finally back bone degradation at temperature above 350°C. On the other hand, when on comparing the TGA trace of AAc/CS gel swollen in pH 7 and that of PAAc in the same conditions. It was observed that there is a clear change between these two traces. The PAAc trace looks similar to that of PAAc in pH 3 and AAc/CS gel in pH 3. The high weight loss in the first stage may due to higher humid absorbed by the gel whereas that of the gel show no sharp weight loss which confirm the results obtained from DSC analysis.

Conclusion

According to these results we can assume that there a high affinity between AAc and CS as a direct result of polyelectrolyte complex between COOH of AAc and NH_2 of CS. On radiation, homopolymerization of AAc takes place, parallely, the active species produced from the radiolysis of water assist PAAc self-bridging and crosslinking with CS chain or sigment might be produced due to the chain scission. The following schematic diagram, Fig (4), shows the estimated mechanism of AAc/CS gelation.

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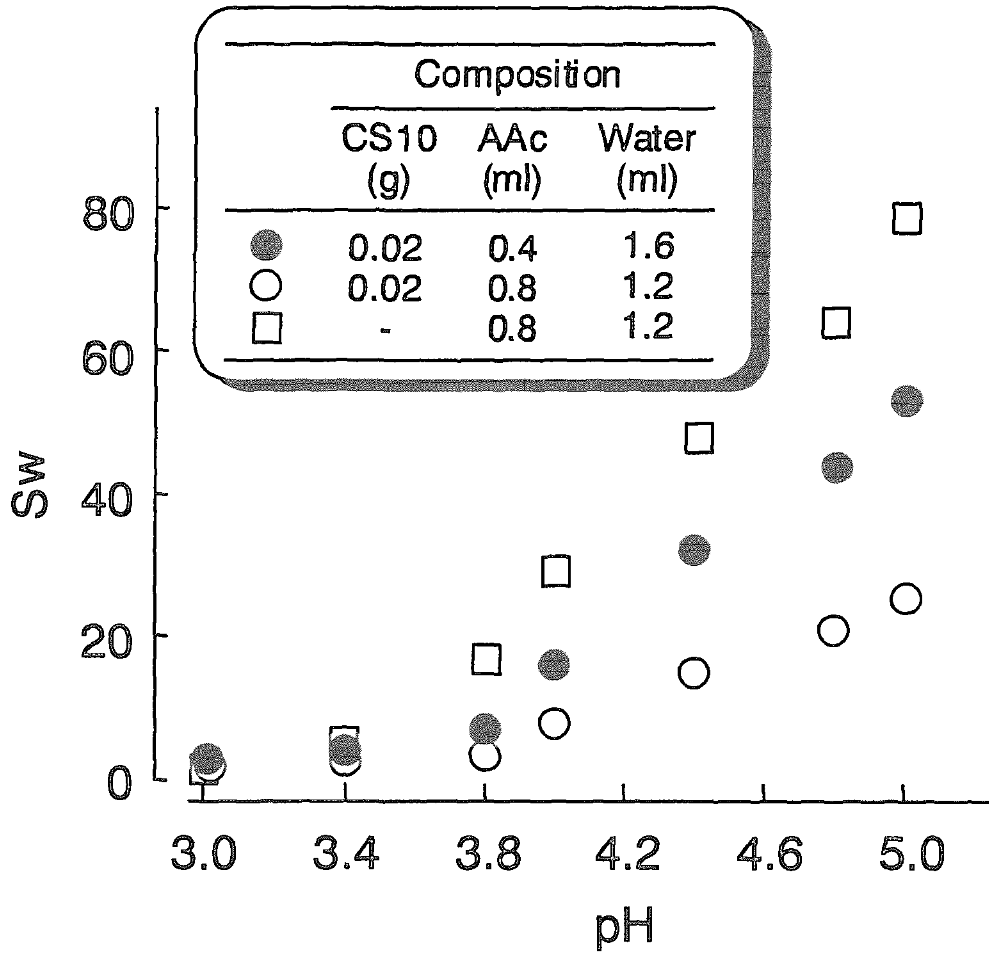


Figure 1. The pH dependence of equilibrium swelling as a function of AAc concentration for CS10 modified gels obtained with a dose of 30 kGy at 0°C.

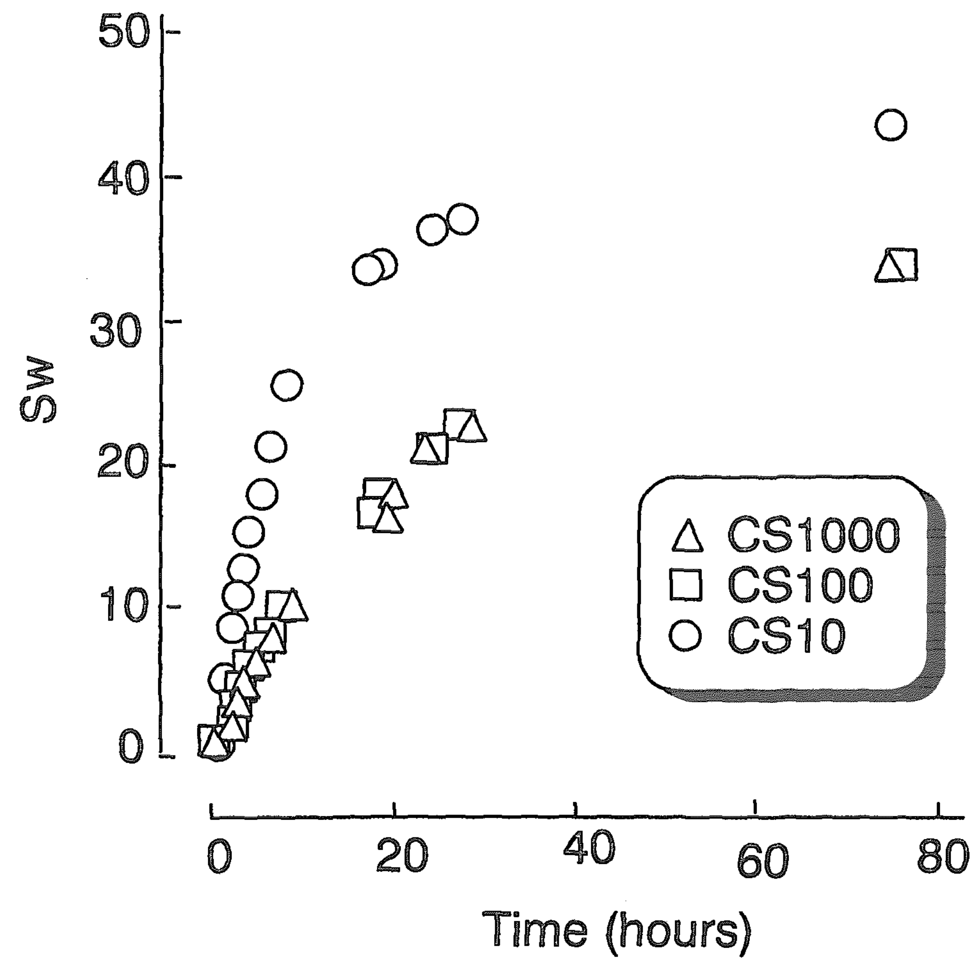


Figure 2. Time-course of swelling ratio swollen at pH 7.0 and 37°C for AAc gels crosslinked with different molecular weight CS chains. The CS modified gels were obtained by irradiating up to 30 kGy at 0°C using a mixture of 0.02 g of CS, 0.8 ml of AAc, and 1.2 ml of water.

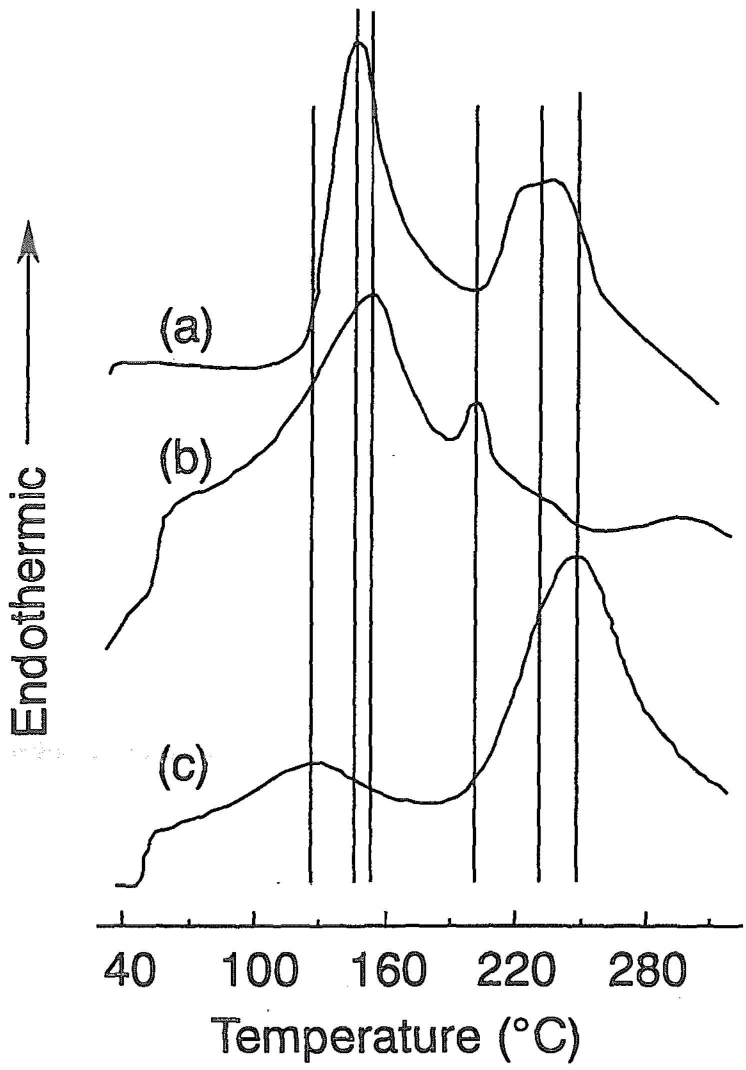


Figure 3. DSC profiles of (a) pure poly(AAc) gel lyophilized after swollen at pH 7.0 and CS10 modified gels lyophilized after swollen at (b) pH 7.0 and (c) pH 3.0.

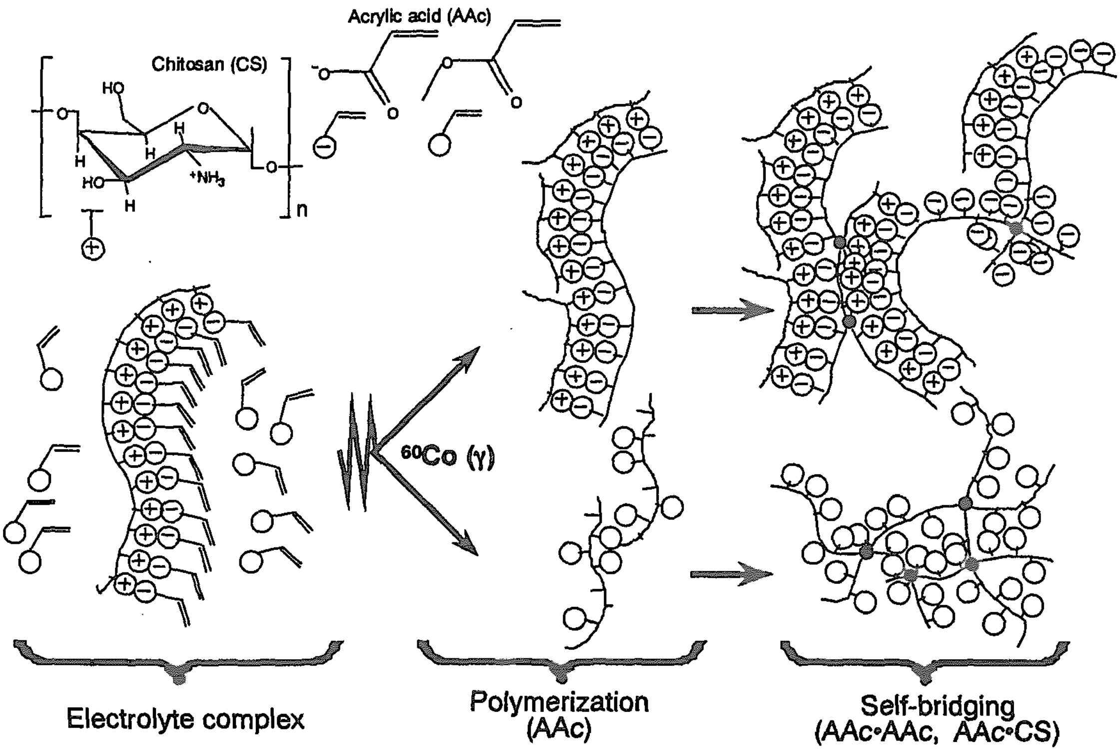


Figure 4. Proposed model for illustrating the simultaneously occurring process of radiation-induced polymerization and self-bridging of AAc in aqueous solutions, followed by a formation of crosslinked networks between the poly(AAc) chains and the CS chains according to a strong chemical affinity between the NH_2 groups (CS) and the COOH groups (AAc).

9 Biological Activities of Radiation-Degraded Carrageenan

Lorna RELLEVE, Alumanda DELA ROSA, Lucille ABAD, Charito ARANILLA and Anne Kathrina ALIGANGA[1]

Fumio YOSHII, Tamikazu KUME and Naotsugu NAGASAWA[2]

1. Philippine Nuclear Research Institute, Commonwealth Ave. Diliman, Quezon City 1101, Philippines
2. Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute, 1233 Watanuki, Takasaki Gunma 370-1292, Japan

Abstract

Carrageenans were irradiated in solid state to doses 50-1000 kGy in air at ambient temperature. Changes in their molecular weight and functional properties with respect to their FT-IR and UV spectra were evaluated. Irradiation of carrageenans resulted in a rapid decrease of molecular weight indicating main chain scission in their polymeric structures. Formations of some compounds were evident by new absorption peaks in their UV and FT-IR spectra and quantitative analyses of the FT-IR spectra which, in addition, support that there is a breakdown in the carrageenan structure.

Irradiated carrageenans were investigated for their plant growth-promoting activity. Carrageenans were added to the nutrient solutions for rice seedlings under non-circulating hydroponics cultivation. Irradiated carrageenan induced weight gain in treated rice seedlings. Maximum weight gain was obtained with KC irradiated at 100 kGy while treatment with IC at 500 kGy. IC exhibited less growth promoting properties than KC. The growth of fungi on the roots disappeared with treatment of IC and KC irradiated at 500 kGy. Growth promotion of some leafy vegetables was also observed with application of degraded KC.

The carrageenan molecule has been broken down to smaller molecule(s) or compound(s) that can be absorbed effectively as nourishment factors and anti-microbial agents by plants.

Keywords: carrageenan, radiation-degraded, growth-promoting properties

1. Introduction

Carrageenans are sulfated anionic polymers that comprise the main structural polysaccharides of red seaweed (*Rhodophyceae*) [1]. They are composed of D-galactose units linked alternately with α 1,3 and β 1,4 linkages. These sulfated galactans are classified according to the presence of the 3,6 anhydrogalactose on the 4-linked residue and the number and position of the sulfate group [2]. The repeating units of the principal gel formers, kappa-, iota- and lambda carrageenans are shown in Fig. 1. The Philippines is one of the major producers of carrageenan , primarily of the kappa- and iota types. Carrageenans are used commercially as thickeners and gelling agents in the food industry and binders in some consumer products.

Natural polymers like polysaccharides have been receiving a great deal of attention by many researchers because of their biodegradability and availability at low cost. The native structures and properties of the carrageenans may be exploited for non-food applications, i. e., hydrogel for microencapsulation, controlled-released of chemicals and biological products, and wound dressings. It has been known that gamma irradiation can modify the structures and chemical and biological properties of materials via either degradation, crosslinking or grafting. Enzyme- or radiation-degraded polysaccharides has been found to induce various kinds of biological activities such as anti-microbial, promotion of plant growth, suppression of heavy metal stress etc [3]. This study aims to modify carrageenan by radiation degradation and to investigate their biological activities.

This paper presents the results of the various studies undertaken to investigate the effect of gamma radiation on the molecular weights and structures of kappa-, iota and lambda-carrageenan and the effect of radiation-degraded carrageenan on the growth of plants. The relationship of the growth-promotion activity and molecular weight of irradiated carrageenans and the influence of the sulfate group in the radiolysis of the three types of carrageenan are discussed.

2. Materials and Methods

Materials/Irradiation

Refined kappa-carrageenan (KC) and iota-carrageenan (IC) were obtained from Copenhagen Pectin A/S, Denmark and Shemberg Corporation, Philippines. The samples were used without further purification and gamma irradiated to doses 50-1000 kGy in air at ambient temperature. Carrageenans used for GPC and UV experiments were purchased from Marine Resources and Development Corporation, Philippines.

Methods

Analytical Measurements

Molecular weights of irradiated carrageenan were determined by gel permeation chromatography using Waters HPLC instrument equipped with Waters Ultrahydrogel column and elution was monitored by an RI detector using 0.1 M $NaNO_3$ as the mobile phase at a flow rate of 1 ml/min. The carrageenan concentration used was 0.1%. A calibration curve was constructed using PEO/PEG standards. FT-IR spectra were recorded using diffuse reflectance spectroscopy (DRS) with a Shimadzu FT-IR spectrometer. Samples for FT-IR were prepared by pipetting 20 μl of 1% carrageenan solution on an aluminum pan and dried in a vacuum oven at 40^0C. UV spectra were measured in water with a Perkin Elmer Lambda 20 UV-Vis spectrometer in the range 200-400 nm at 0.25% carrageenan concentration.

Plant Growth Studies

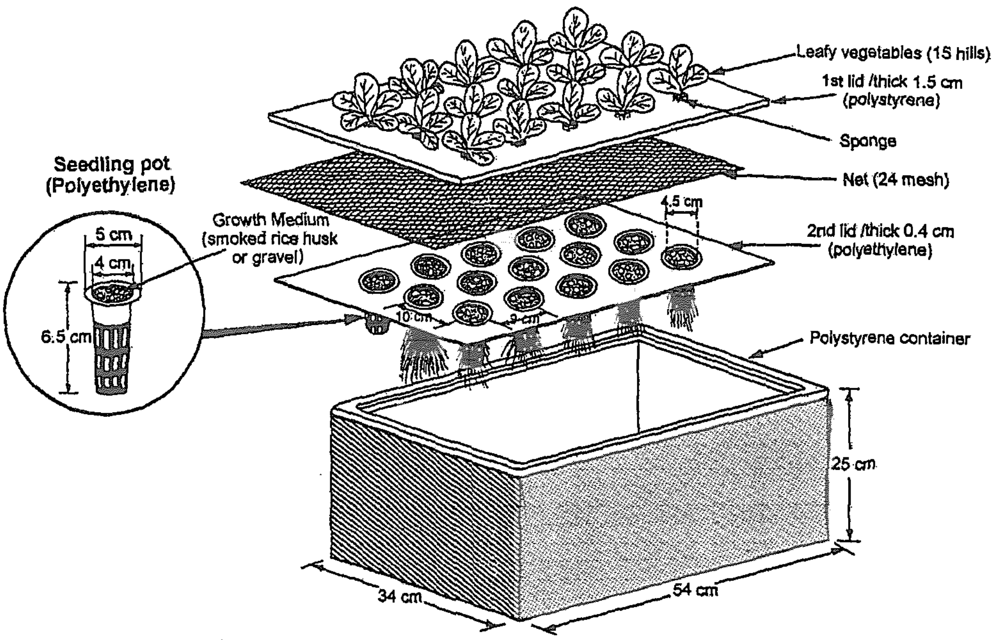
Cultivation of rice and vegetable

Rice seeds (*Japonica*) were washed in running water for 24 hrs and germinated by immersion in water at 70^0C in the darkroom. Twenty-five germinated seeds were then sowed onto plastic net floating in plastic pots containing 80 ppm of carrageenan in diluted hyponex solution (1/2000).

Bok choi seeds were sowed directly in cup containing perlite. The cup was then immersed in nutrient solutions contained in polystyrene box with holes (see diagram below). This set-up was adapted from Asian Vegetable Research and Development Center, Taiwan. Nutrient solutions for this experiment were prepared as follows:

CaNO3	635	Fe-EDTA	22.6
$NH_4H_2PO_4$	53	$MnSO_4$	1.54
K_2HPO_4	16	$CuSO_4$	0.08
KH_2PO_4	28	$ZnSO_4$	0.22
KNO_3	217	H_3BO_3	2.86
$MgSO_4$	504	$NaMoO_4$	0.03

*concentration in ppm



After 1 month of growth in a greenhouse, rice seedlings and Bok choi were collected, gently washed and dried at 105^{0}C for 12 hours. % weight gain of dried rice seedling and Bok choi were determined for evaluation of growth promotion activity of carrageenan and calculated as:

$$\%\,Weight\ gain = \frac{\text{dry wt. of plant}_{\text{treated with carrageenan}} - \text{dry wt. of plant}_{\text{untreated}}}{\text{dry wt. of plant}_{\text{untreated}}}$$

Artemia salina L. Lethality Test

The assay was performed as described by Solis et al [4] with minor modifications. Brine shrimp (*Artemia salina* Leach) cysts were hatched in artificial seawater supplemented with 6 mg/l dried yeast and oxygenated with an aquarium pump in an improvised double-chamber brine tank. After 48 hours, nauplii (baby shrimp or larvae) were gathered and placed in a multichannel micropipette reservoir. Approximately 15-

20 larvae (in a total volume of 100 μl) were pipetted in 96-well microtiter plates (Nunc.). 100 μl of irradiated iota, kappa, and lambda carrageenan (0, 50, 100, 500 and 1000 kGy) diluted in salt water (with phosphate buffer, pH 7.0) at concentrations of 25, 250, 1000 and 5000 μg/ml were added. After 24 hours, non-viable shrimps were counted with the aid of a binocular microscope (Nikon). The plates were then incubated to 50 °C for 10 minutes to kill the remaining live shrimps and their total number was recorded. Percent survival was plotted against concentration of the test substance to obtain a survival curve

3. Results and discussion

Effect of radiation on the molecular weight and structure of carrageenan

The properties of polymeric materials are affected upon irradiation as a result of chemical changes in the polymer molecules[5]. These changes include molecular weight and structure. GPC elution profiles or kappa-carrageenan at different irradiation doses is shown in Fig. 2. As expected, peak shifts to longer retention as the dose is increased. Irradiation of carrageenan led to the reduction in molecular weight. Carrageenan degraded at higher doses showed bimodal molecular weight distribution. Molecular weight of carrageenan decreases remarkably with dose as demonstrated in Fig. 3. KC and IC exhibited different degradation behavior. Degradation of KC at 100 kGy corresponds to dose of about 200 kGy for IC. The susceptibility of the compound to degradation can be compared by calculating their G-values. Radiation-chemical yields of degradation, G_d were determined from the Charlesby-Pinner equation:

$$\left(\frac{1}{Mn}-\frac{1}{Mn_0}\right)=G_d\times 1.04\times 10^{-7}\times D$$

where Mn; number-average molecular weight of carrageenan at absorption dose, Mn_0; initial number-average molecular weight, D absorbed dose (kGy) [6]. From Fig. 4., the yields of degradation (G_d) of carrageenans are calculated as follows: kappa; 2.13 and iota:1.51. Comparing the G_d values, kappa was found to be more susceptible to radiolysis than iota. This observation has also been reported in the literature [7]. Thus, the sulfate groups may have played a role in the radiolysis of the carrageenans. Analysis of lambda carrageenan will verify the hypothesis.

The UV spectra (Fig. 5) of irradiated carrageenans show an absorbance peak at around 265 nm and the intensity of which increases with increasing dose. This peak is probably due to the formation of double bond. Double bond formation has been proven by bubbling solution of irradiated alginate with ozone [8]. Fig. 6 shows the plot of ratio of the absorbance at 265 nm of irradiated and unirradiated carrageenan with dose. This plot gives the rate of formation of the double bond. It can be seen that greater formation of double bond was obtained with KC. These results were in good agreement with the MW measurement and support the assumption that sulfate groups influence the radiolysis of carrageenans. In Fig. 7, the relationship of MW and the absorbance at 265 nm is presented. Reduction of MW resulted in a rapid increase of absorbance up to 100 kGy and increases gradually on continued exposure to radiation. Thus, measurement of UV absorbance at 265 at a given concentration can give a good estimate of the molecular weight.

Infrared spectral absorption of carrageenans, notably the glycosidic, 3,6 anhydrogalactose and the covalent sulfate, are listed in Table 1 and sample spectra of iota-carrageenan are shown in Fig 7. IC and KC differ chemically in the presence of another 2-sulfate in the disaccharide unit of iota and this is shown in their FT-IR spectra. KC does not have an absorption peak at 805 cm^{-1} (Table I). As illustrated in Fig. 8, important functional groups are retained even after irradiation at 500 kGy while there is the appearance of a new peak at 1728 cm^{-1}. This peak could be attributed to the formation of a carbonyl group in the structure. The same is observed for kappa carrageenan. Quantitative FT-IR analysis was undertaken. Table II shows that for both IC and KC, there is a considerable decrease in the peak heights corresponding to the glycosidic, 3,6 anhydrogalactose and methylene groups with increase in radiation dose. Peaks due to covalent sulfates and sulfates of 3,6 anhydrogalactose were likewise decreased with radiation.

Growth-promotion effect of irradiated carrageenan

Upon irradiation, carrageenan is depolymerized with the formation of shorter fragments the molecular size of which depends on the radiation dose used. When solutions of the irradiated KC and IC were mixed with the growth medium for rice seedlings under hydroponic conditions, stimulation of growth was observed. As shown in Fig. 9a, the maximum % weight gain of rice seedlings was obtained with KC irradiated at 100 kGy.

It decreased to the level of the control at doses of 500 and 1000 kGy, indicating that no toxic compounds are formed at high radiation dose. This observation is supported by the brine shrimp toxicity assay. Unirradiated IC and KC has also stimulated growth of rice to some extent. This strongly suggests that a certain range of molecular weight is suitable for growth-promotion of plants. Depending on the molecular weight, carrageenans exhibited various level of growth-promotion effect on rice. Table III summarized the results of MW by GPC experiment. In this study, KC at 100 kGy showed the highest effect of growth promotion, thus MW of about 24,000 is suitable for growth promoters of plants. IC exhibited less growth promoting properties than KC. Maximum weight gain was attained with the application of IC irradiated at 500 kGy. Like KC no toxic compounds are formed at higher radiation doses. Optimum growth-promotion of IC was not achieved since to get MW of 24,000, IC has to be degraded at about 255 kGy. This value was calculated from the Charlesby-Pinner equation using the calculated G_d. From these results, it is clearly suggested that growth-promotion effect of radiation-degraded carrageenan depends mainly on its molecular weight. Fig. 9b shows the photograph of rice seedling treated with irradiated carrageenan. It is evident that chlorosis or yellowing of leaves was inhibited with the application of both KC and IC as compared to the control. Furthermore, growth of fungi was inhibited with treament of KC at 500 kGy (Fig 10). It can be noticed from the photograph that IC can also inhibit growth of fungi on roots even at 0 kGy and inhibition increases with increasing dose. This noticeable anti-microbial activity will be further studied against *E. choli* and other microorganisms. Several studies have been reported on the anti-microbial activities of ethanolic extract of species of red algae against *Escherechia choli*, *Staphylococus areus, Candida albicans* etc [9,10].

Fig. 11 illustrates the growth-promotion activity of carrageenan on vegetables like mustard and bok-choi.

Toxicity of Irradiated Carrageenan

The irradiated carrageenans were subjected to a microwell cytotoxicity assay based on a 24-hour lethality of *Artemia salina* larvae. Brine shrimp larvae have been used as a convenient and reliable bioassay system to screen for a wide spectrum of potentially toxic substances such as mycotoxins, co-carcinogens, anti-tumor compounds, dinoflagellate toxins, pesticide residues and other pollutants [11,12]. LC_{50} values of less than 200 μg/ml are considered cytotoxic. At physiologically neutral pH, irradiated carrageenan is unlikely to exhibit cytotoxicity. LC_{50} values of irradiated carrageenan are summarized in Table IV. We predict two possible bioactive compounds of potential pharmacologic value: one has an LC_{50} of around 1300 ug/ml and another at 400 ug/ml. Future work will need a preparative HPLC coupled to a bioassay-driven purification scheme.

4. Conclusion

Carrageenans were highly sensitive to gamma radiation with significant changes in their molecular weight and structure. Kappa-carrageenan was found to be more susceptible to radiolytic breakdown. Carrageenan with specific molecular weight range can be readily produced by radiation degradation. Radiation degraded carrageenans promoted growth of rice and leafy vegetables. Carrageenan with molecular weight of about 24,000 can be used as growth-promoter of plants.

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Table I. Major peaks from the FTIR spectra of carrageenan

Absorption cm^{-1}	Functional groups	Peak intensity	
		kappa	iota
1640-1645	polymer bound water	s	s
1370-1375	methylene group	m	m
1210-1260	covalent sulfate	vs	vs
1010-1080	glycosidic linkage	vs	vs
928-933	3,6 anhydro-D-galactose	s	s
840-850	D-galactose-4-sulfate	m	m
800-806	3,6 anhydro-D-galactose-2 sulfate	a	m

vs-very strong, s-strong, m-medium, a-absent

Table II. Effect of radiation dose on the major functional groups in carrageenan

(A) iota-carrageenan

Absorption (cm^{-1})	Peak Height			
	IC-0	IC-100	IC-500	IC-1000
1640-1641	13.16	1.01	21.16	9.43
1371-1375	5.29	5.62	6.14	3.43
1230-1250	7.59	40.30	33.16	2.32
1068-1072	37.89	12.52	10.38	5.06
929-931	26.16	15.63	14.39	1.85
846-847	18.17	8.79	9.65	8.10
804-806	12.80	5.79	7.61	---

(B) kappa-carrageenan

Absorption (cm^{-1})	Peak Height			
	IC-0	IC-100	IC-500	IC-1000
1640-1641	1.4185	0.2477	0.1160	0.0906
1371-1375	0.8425	0.1325	0.0672	----
1230-1250	6.2183	3.5427	0.9331	0.7107
1068-1072	1.0690	6.3040	1.1402	0.8832
929-931	5.2440	2.0705	0.4280	0.3412
846-847	2.2053	1.2121	0.2589	0.2059

Table III. Effect of radiation on the molecular weight of carrageenan

Dose (kGy)	Molecular Weight (Da)	
	kappa	iota
0	1,032,905	3,545,093
50	65,550	209,425
100	26,625	114,029
500	5,457	13,005
1000	4,738	7,200

Table IV. LC_{50} values of irradiated carrageenan

Dose (kGy)	LC_{50} (μ/ml)		
	lambda	iota	kappa
0	>2500	>2500	>2500
50	1350	1500	>2500
100	1350	1500	>2500
500	400	1500	1250
1000	400	1500	1250

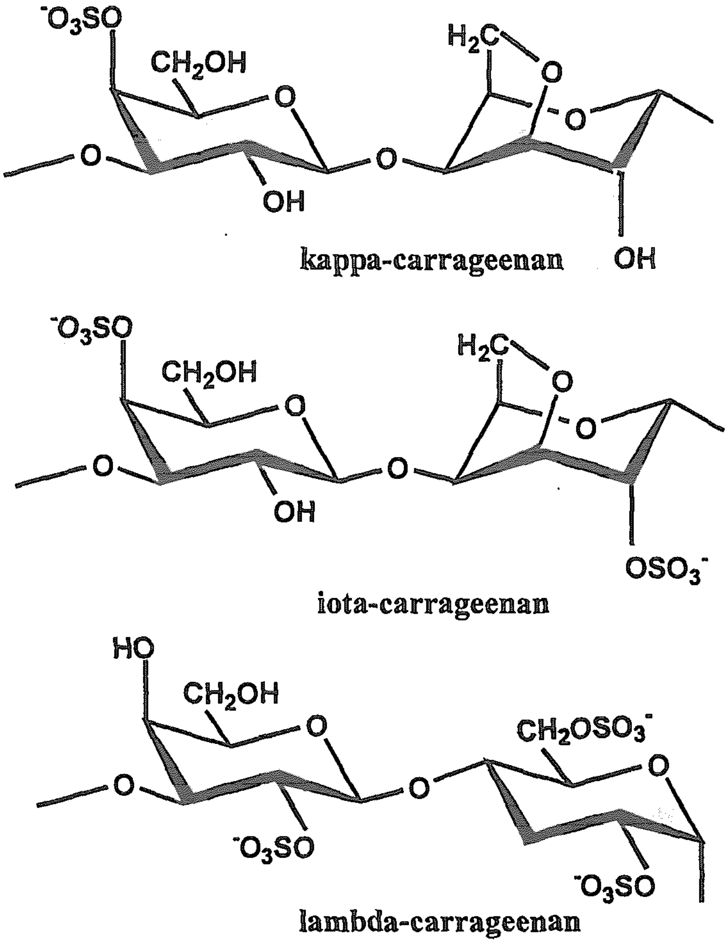

Fig. 1. Idealized structures of different types of carrageenan

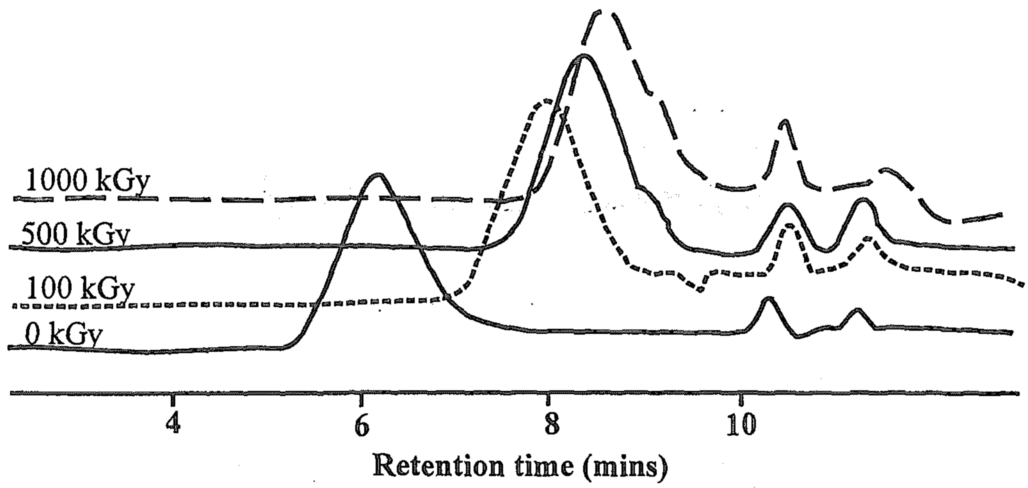

Fig. 2. GPC elution profile of kappa-carrageenan irradiated in solid state

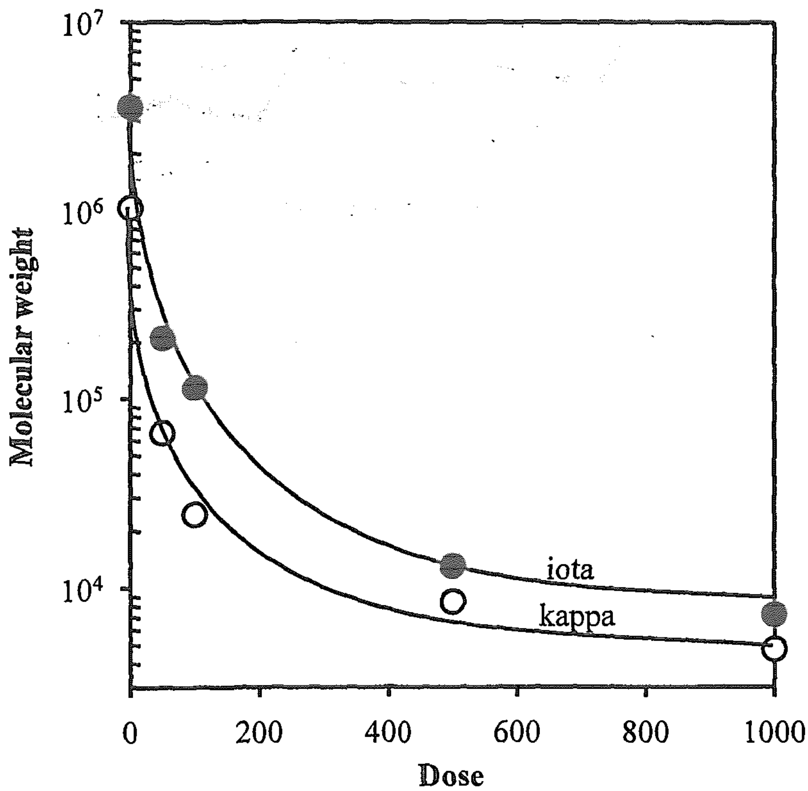

Fig. 3. Effect of radiation on the molecular weight of carrageenan

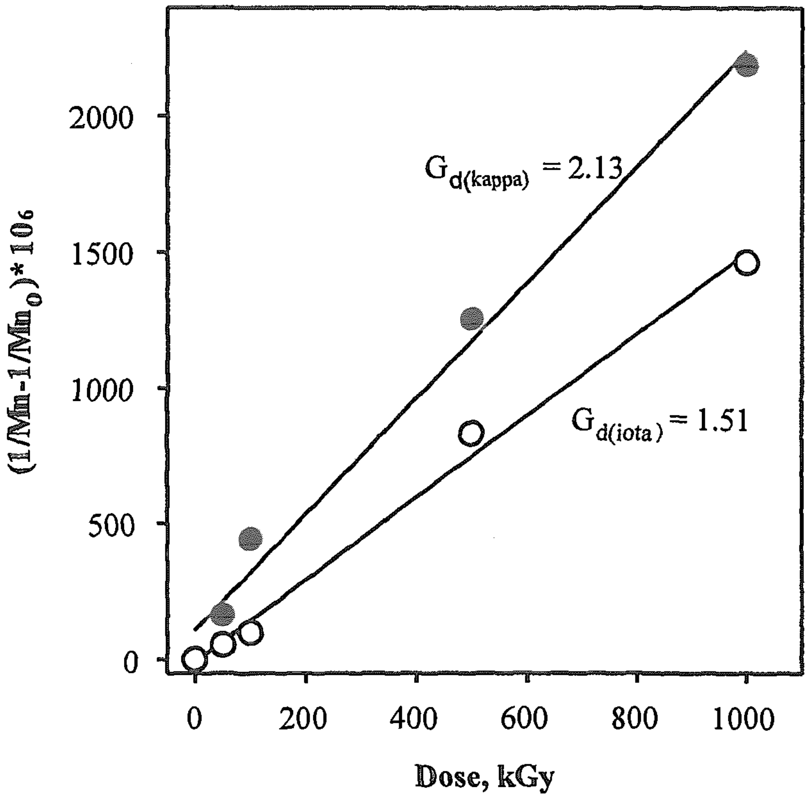

Fig. 4. Degradation yields of carrageenans

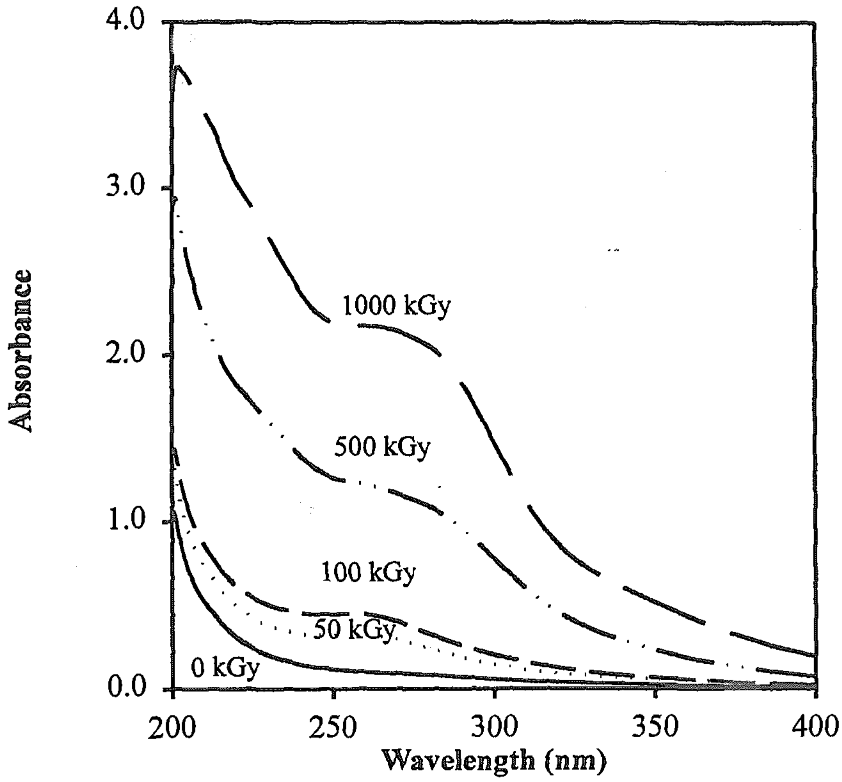

Fig. 5. UV spectra of kappa-carrageenan at different doses

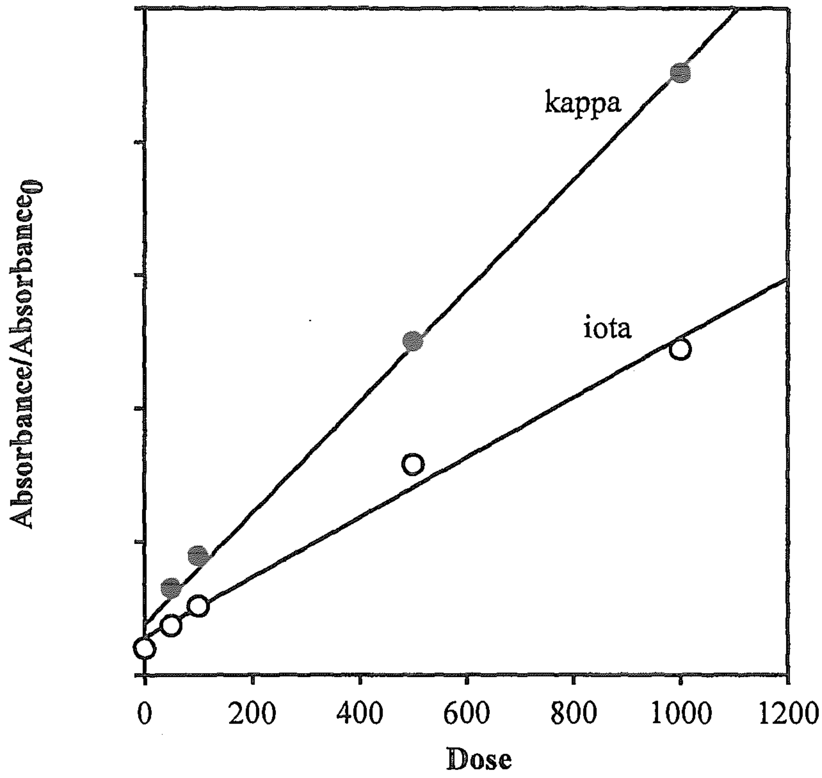

Fig. 6. Absorbance at 265 nm as a function of dose

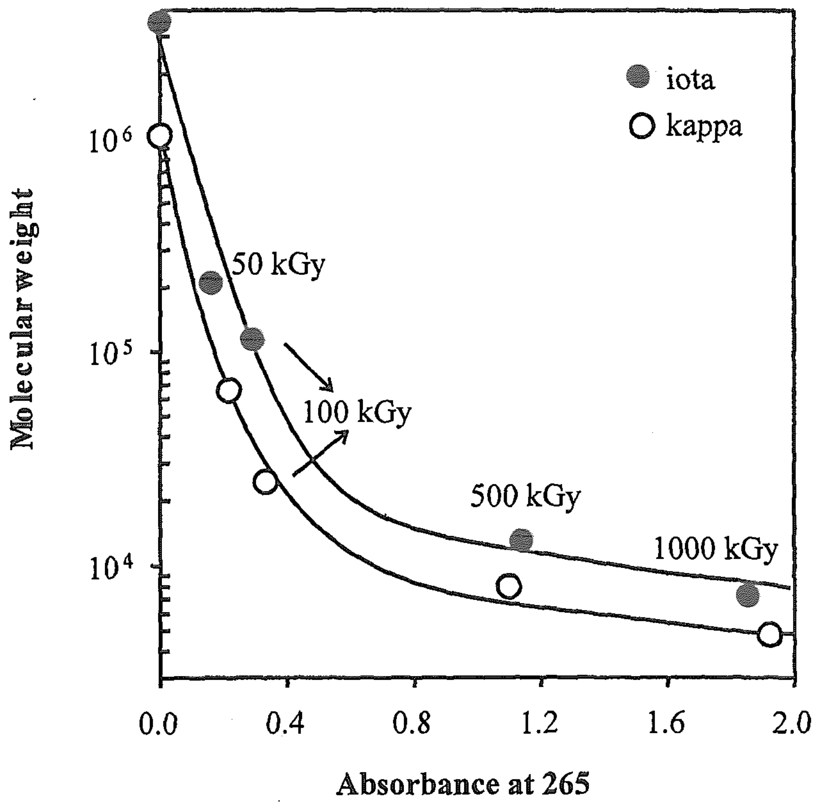

Fig. 7. Relationship of molecular weight and absorbance at 265 nm of carrageeenan at differnet doses

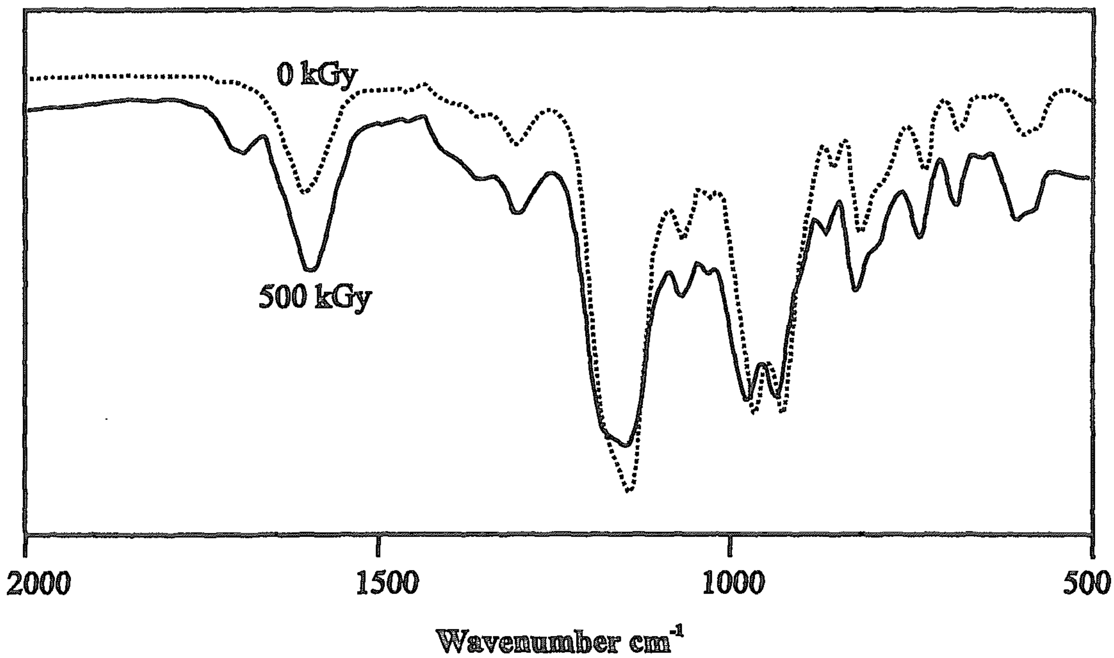

Fig. 8. FT-IR spectra of iota carrageenan at 0 kGy and 500 kGy

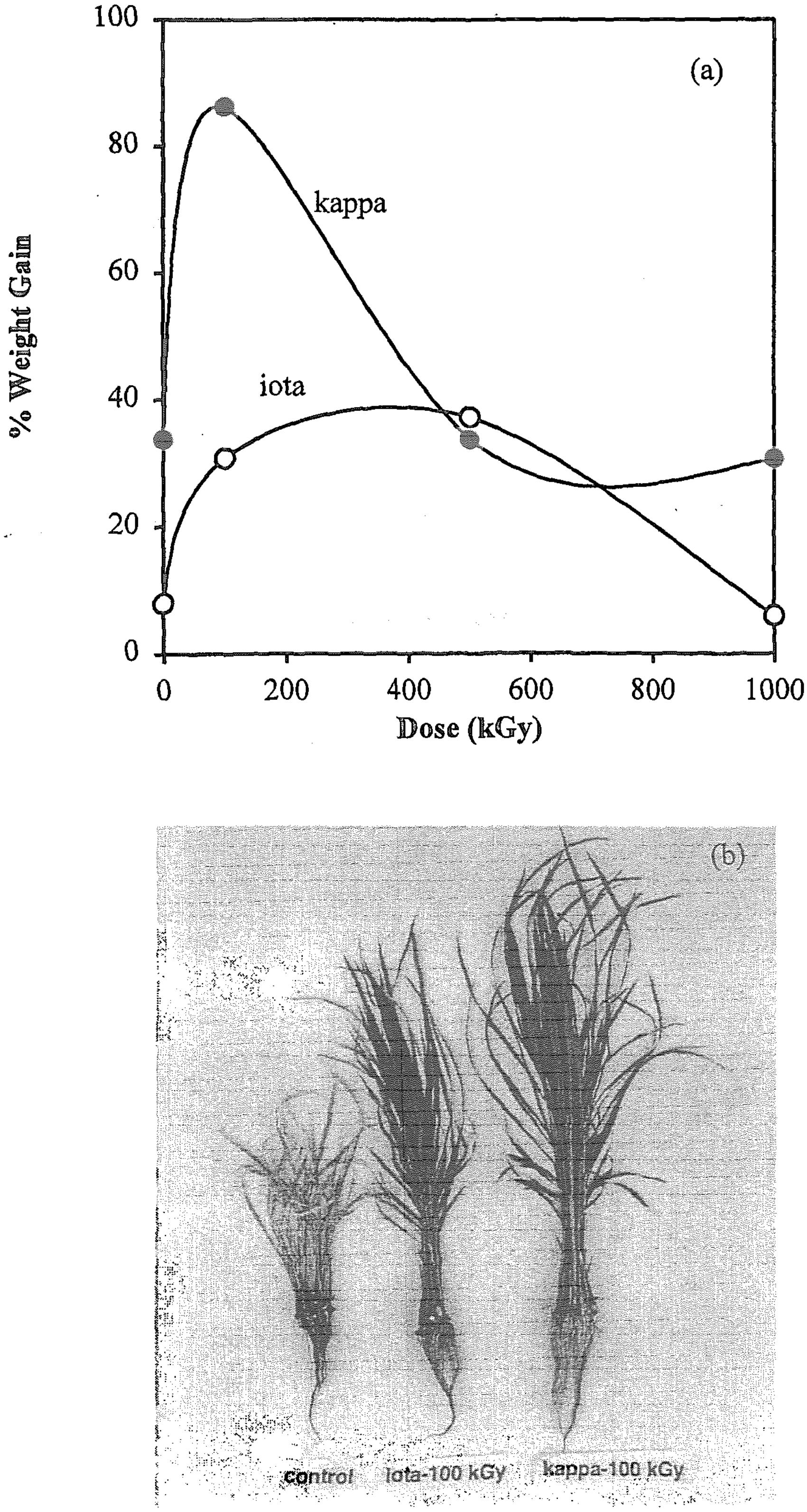

Fig. 9. Growth promotion of rice by irradiated carrageenan
(a) as a function of dose(b) at 100 kGy

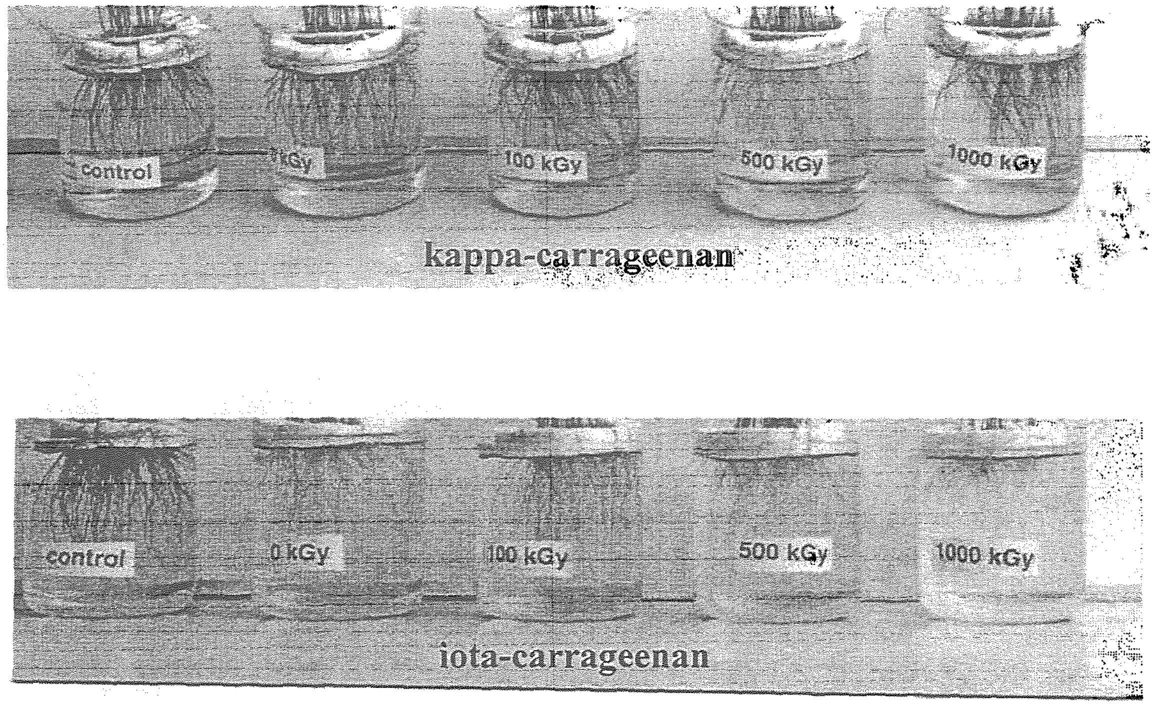

Fig. 10. Growth of fungi on roots in hydroponics solution with carrageenan

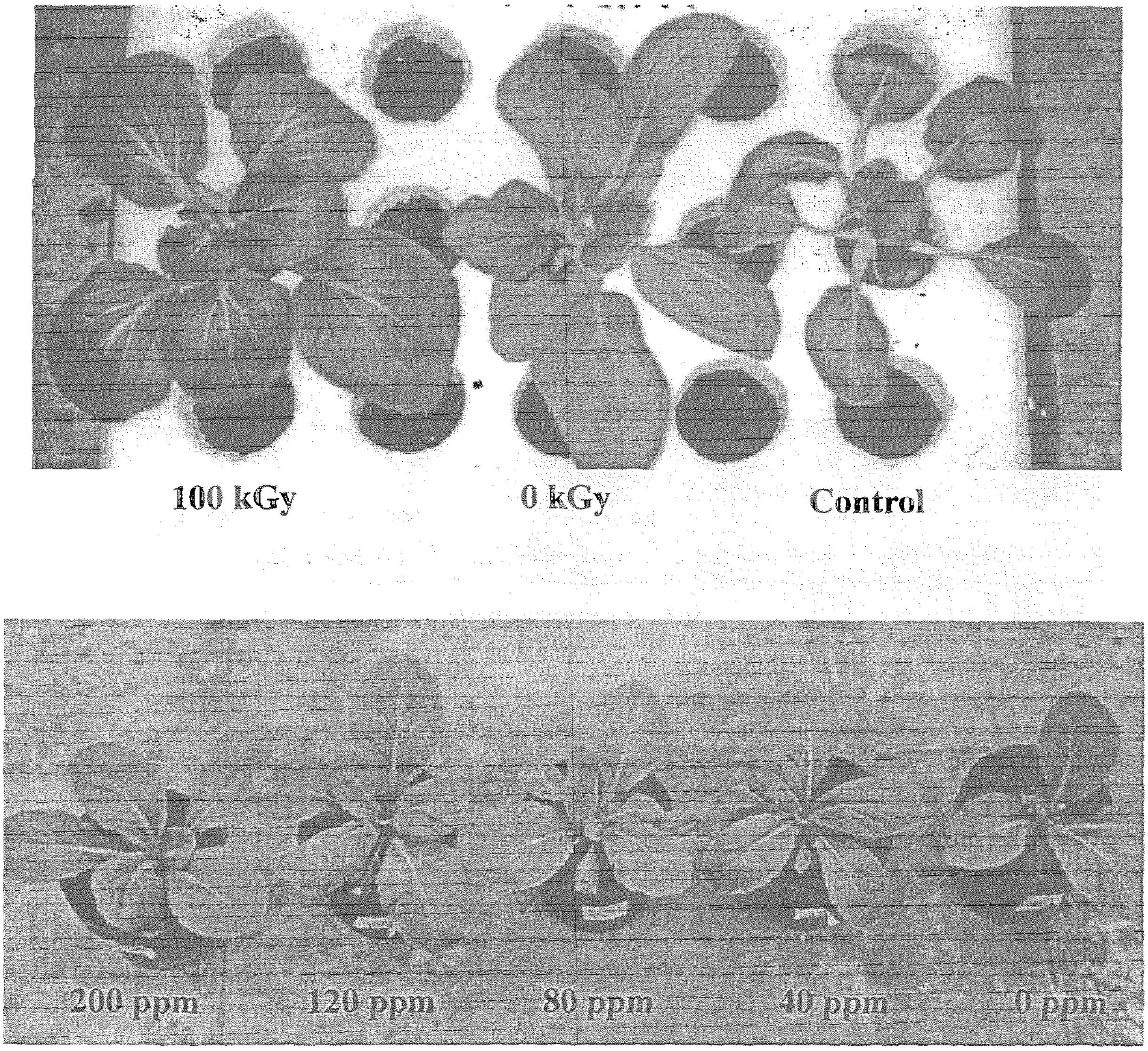

Fig. 11. Growth-promotion effect of carrageenan on bok-choi and mustard

Session 2

Radiation Crosslinking of Starch and Other Polysaccharides

10 Bilateral Cooperation between MINT and JAERI

Fumio Yoshii

Takasaki Radiation Chemistry Research Establishment,
Japan Atomic Energy Research Institute
1233 Watanuki, Takasaki, Gunma 370-1292, Japan

Implementing Arrangement between the Malaysian Institute for Nuclear Technology Research, (MINT) and the Japan Atomic Energy Research Institute (JAERI) in the field of radiation processing were signed on 9 December 1987. Under the Implementing Agreement, the "Cooperative Research Program on Upgrading Oil Palm Wastes to Animal Feeds by Radiation and Fermentation Processing" was implemented on 1987 - 1997.

Then, the "**Cooperative Research Program on Radiation Crosslinking of Starch**" was started on 9 December 1997.

1. Scope of Research

Starches are renewable natural polymers that are easy to decompose in the environment and can be modified to various applications such as biomedical, agricultural, pharmaceuticals and industrial materials that are friendly to the environment and human body. In this cooperative research program the modification of starch can be carried out by radiation crosslinking technique and this development will produce value-added starches. However, special radiation crosslinking technique should be developed because starches tend to decompose by radiation. Starches such as sago and tapioca are produced abundantly in Malaysia. Currently cassava for tapioca and sago palm are planted over 2,124 and 28,000 hectares, respectively, in Malaysia. The cooperative research will benefit both parties in terms of:

- Development of new technique of radiation crosslinking
- Development of new materials from starch
- Diversified application of starch

- Development of knowledge and expertise in radiation processing
- Enhancement of collaboration between Malaysia and Japan in science and technology

2. Major Items of the Work

(1) Research on radiation crosslinking of starch

1) Selection of radiation crosslinking method of starch

Effect of the addition of polyfunctional monomers and water-soluble crosslinking –type polymers on radiation crosslinking of starch will be studies. Powder paste and aqueous solution of starch will be used. Optimum crosslinking method will determine by measuring the gel fraction of the irradiated starches.

2) Selection of suitable starch for radiation crosslinking

Effect of chemical composite and molecular weight of starch on the radiation crosslinking will be studied. The optimum crosslinking method that was determined in 1) should be used

(2) Research on physical properties of the radiation crosslinked starch

The following physical properties of the radiation crosslinked starch will be investigated.

1) Microstructure of crosslinked starch
2) Mechanical properties
3) Swelling properties
4) Thermal stability
5) Biodegradability

(3) Final report

3. Time schedule

	1998	1999	2000	2001
(1) Research on radiation crosslinking of starch				
1) Selection of radiation crosslinking method of starch	→	→		
2) Selection of suitable starch for radiation crosslinking		→		
(2) Research on physical properties of the radiation crosslinked starch				
1) Microstructure of crosslinked starch			→	
2) Mechanical properties, Swelling ratio, Thermal stability, Biodegradability			→	

11 Development of Sago Starch Hydrogel for Wound Dressing

Kamaruddin HASHIM[1], Khairul Zaman HJ. MOHD DAHLAN[1], Kamarudin BAHARI[1]. Fumio YOSHII[2] and Tamikazu KUME[2]

1. Malaysian Institute for Nuclear Technology Research (MINT), Bangi, Malaysia.
2. Takasaki Radiation Chemistry Research Establishment, JAERI, Takasaki, Japan.

Abstract

Sago starch is utilized in Malaysia mainly for food production. The purpose of the research is to diversify the use of sago starch for medical application particularly in development of hydrogel burn wound dressing. The sago starch is blending with mixture of PVP and PVA to improve the degree of crosslink, mechanical properties, swelling ability and tackiness of the blend hydrogel (sago/PVA and sago PVP). Additives have been introduced into the system such as, polypropylene glycol or carboxymethyl cellulose to improved further the swelling ability and tackiness properties of the blend hydrogel as well as other properties. Effect of irradiation dose on the blend hydrogel has also been studied to optimize the effective dose for blend hydrogel and simultaneously for sterilization purpose.

Keywords: Sago starch, Hydrogel, Wound dressing, Electron beam, Radiation

1. Introduction

Wound dressing hydrogel has been developing successfully for commercial medical application [1,2,3]. One of the components use in developing wound dressing hydrogel consist of polysaccharide such as agar, alginate and carrageenan [4], since most of the polysaccharides are non-toxic, biocomaptible and biodegradable. Requirement needed for wound dressing application [5,6], the product must be non-toxic, good adhesion to skin and wound, readily remove without tendency to stick to the wound, exhibit high elasticity and also mechanical strength, ability to absorb fluid or exudates come out from the wound, permeable for oxygen to get to the wound, prevent bacteria from environment enter into the wound and good transparency for easy monitoring the healing process of the wound.

Sago starch alone cannot be crosslink to form hydrogel since it is degradable upon irradiation. It need medium or material that can be crosslink and the formation of semi-gel form from sago starch will assist in handling prior for irradiation. Since sago starch dissolved in hot aqueous solution, the suitable material is the water-soluble polymer such as polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA), polyethylene oxide (PEO), polyacrylic acid (PAA) and polyacrylamide (PAAm). These polymers either in solid or solution condition can easily form hydrogel upon irradiation with gamma or electron beam. So the aim of this research work is to utilize sago starch in the development of wound dressing hydrogel by blending with PVP and/or PVA and also with some additive such as polypropylene glycol and carboxymethyl cellulose, follow with study on gel strength, elasticity, swelling ability and tackiness properties of the blend hydrogel.

2. Materials and Methodology

2.1 Materials

Food grade sago powder was secured from Nitsei Sago Industries Sdn. Bhd., Mukah, Sarawak, Malaysia. PVA 117 with 1700 polymerisation and 98-99% specification, and PEO E30 with molecular weight 30,000 to 50,000 were supplied by Takasaki Radiation Chemistry Research Establishment, JAERI, Japan. Medical grade PVP, Colliding 90F was purchased from BASF of Germany. Low viscosity carboxymethyl cellulose (CMC) sodium salt with viscosity 10 –20 cps (2% aqueous solution at 25°C) was purchased from Sigma Chemical Co. PPG with average M_n ca. 425 was purchased from Aldrich Chemical Company Inc. The water used in this experiment is distilled water and all chemicals were analytical grade reagents and used as-receive.

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 various concentration (w/v) of water-soluble polymers PVA or/with PVP. In some case, the blend is added with additive such as CMC aqueous solution (w/v) or PPG aqueous solution (w/v). Then, the blend is heated in water bath at 90°C for 30 minutes and let it cooled in the mould, at room temperature to form semi-gel sample.

2.3 Irradiation Process

Semi-gel samples were irradiated at various doses from 20 to 60kGy 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 Analysis Methods

2.4.1 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.

2.4.2 Tensile Strength and Elongation Measurements

Tensile strength (gel strength) and elongation 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.

2.4.3 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.

2.4.4 Tackiness Measurements

Tackiness of gel samples are obtained by using probe type Rhesca Tackiness Tester apparatus, model TAC-II at 10gf load, 1 second pressing time and 120 mm/min speed.

3. Results and Discussion

3.1 Effect of PVP or PVA on the blend hydrogels

In the previous work [7,8], we have found that sago/PVA blend hydrogel has better mechanical properties (gel strength and elasticity) but less swelling ability compare to sago/PVP and sago/PEO blend hydrogels. Figures 1 and 2 will reveal the effectiveness of each polymer on the mechanical properties of the blend hydrogel. Figure 1 shows that the gel strength of sago/PVA hydrogel is enhanced by adding 5% of PVP on blend hydrogel, especially at low irradiation dose from 20 to 30kGy but decrease with increasing amount of PVP in the blend hydrogel. At higher irradiation dose, it only gives very significant improvement on gel strength. Even though adding PVA on sago/PVP hydrogel give tremendous increase on gel strength but the strength gain from this effect is more or less the same as sago/PVA blend hydrogel. It seems like PVP is considered as additive in the system of sago/PVA blend rather than PVA to the sago/PVP blend that has less gel strength than the former one.

In the case of elasticity (elongation) property, Figure 2 shows that sago/PVA blend hydrogel has better elasticity than the sago/PVP blend hydrogel and the elasticity reduce with increasing irradiation dose. Even though adding 5% PVP to sago/PVA blend hydrogel enhanced the gel strength but the present of PVP decrease the elasticity of the sago/PVA blend hydrogel. Adding PVA into the sago/PVP blend improve the elasticity of the blend hydrogel because of the existence of PVA that has good elasticity property.

Although, the sago/PVA blend hydrogel has better mechanical property than the sago/PVP blend hydrogel, the sago/PVP blend hydrogels has good swelling ability to absorb water compare to sago/PVA blend hydrogel as shown in the Figure 3. So, adding PVA into the sago/PVP blend hydrogel will definitely reduce the swelling performance of the blend hydrogel and it reduces with increasing irradiation dose. So it is important to have PVP in the sago/PVA blend hydrogel in order to improve the swelling ability of sago/PVA blend hydrogel, since the swelling ability of hydrogel is one of the factors that determine the performance of wound dressing hydrogel as mention earlier.

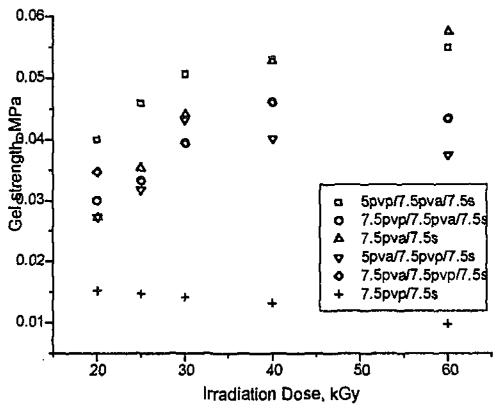

Figure 1: Effect PVP or PVA and irradiation dose on gel strength of blend hydrogels

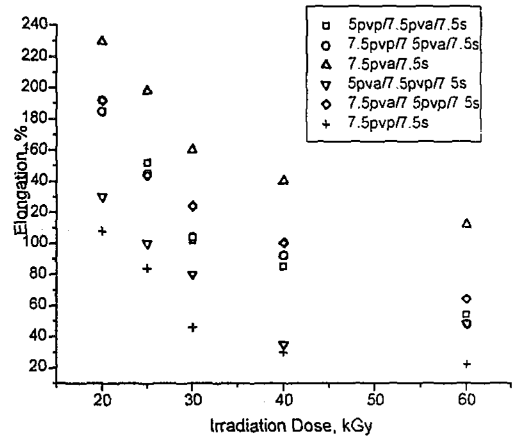

Figure 2: Effect PVP or PVA and irradiation dose on elongation of blend hydrogels

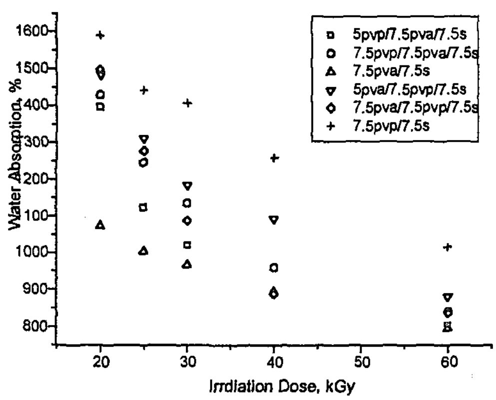

Figure 3: Effect PVP or PVA and irradiation dose on swelling ability of blend hydrogels.

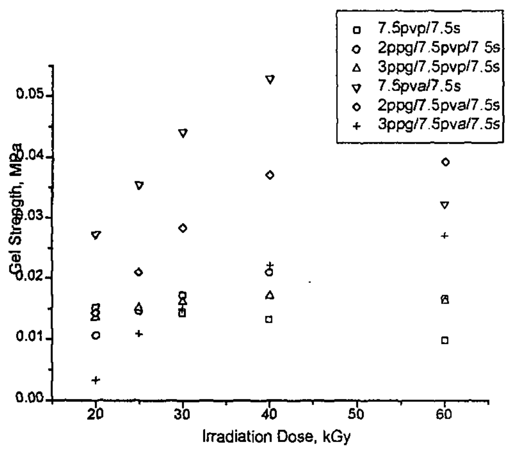

Figure 4: Effect PPG and irradiation dose on gel strength of blend hydrogels

3.1 Effect of PPG on the blend hydrogels

Polypropylene glycol (PPG) is a moisturizer (humectant) that has been frequently used as food additive in preventing cake or shredded coconut form drying out. Polyethylene glycol and PPG have been used in wound dressing [9] application in order to have a biocompatible humectant wound dressing and to avoid such a situation that the hydrogel dressing might form a rigid, fragile plate after drying or preventing from complete loss of water.

Figures 4, 5 and 6 illustrate the effect of PPG on the blend hydrogels. Adding PPG onto the sago/PVA blend hydrogel reduce the gel strength but increase significantly the gel strength of sago/PVP blend hydrogel only at higher irradiation dose. On the other hand, it improved the swelling ability of the sago/PVA hydrogel, as shown in Figure 5, but reduce the swelling property of sago/PVP blend hydrogel. So the present of PPG in the sago/PVA blend hydrogel reduce the gel strength but improved the swelling properties of the blend hydrogel, differ from the case of adding PVP into the blend hydrogel that improve both property of blend hydrogel.

Another parameter have to be consider is the tackiness property of hydrogel dressing, that reflect the behavior of dressing, adhere to the wound and healthy part of the skin as describe earlier. Figure 6 show that sago/PVP blend hydrogel has higher tackiness than the sago/PVA blend hydrogel. Adding PPG to the sago/PVA blend hydrogel will increase the tackiness property of blend hydrogel especially at low irradiation dose less than 25 kGy but at much higher irradiation dose, the tackiness drop to the level lower than the tackiness of blend hydrogel. On the other hand, PPG does not improve the tackiness property of sago/PVP blend hydrogel.

3.2 Effect of CMC on the blend hydrogels

Carboxymethyl cellulose is the derivative of the cellulose that has same molecular structure as starch molecule. The existence of functional group i.e. carboxymethyl on the cellulose and the advantage of same structure as starch, will influence the compatibility of starch molecule with other components, in this case PVA and PVP, in the blend. The CMC also has been use as binder, stabilizer and thickener in food, pharmaceutical and industrial applications.

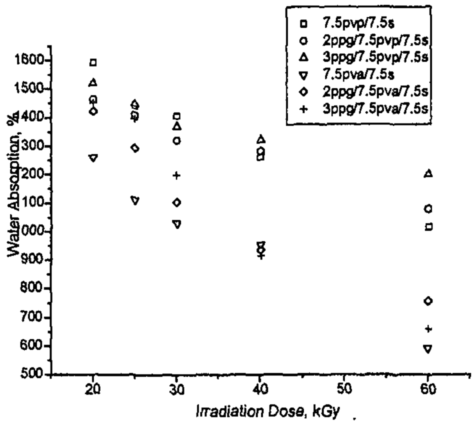

Figure 5: Effect PPG and irradiation dose on swelling ability of blend hydrogels.

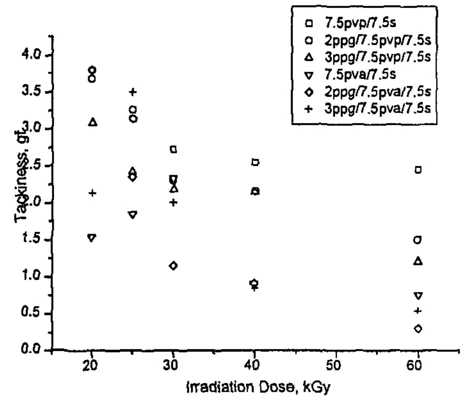

Figure 6: Effect PPG and irradiation dose on tackiness of blend hydrogels.

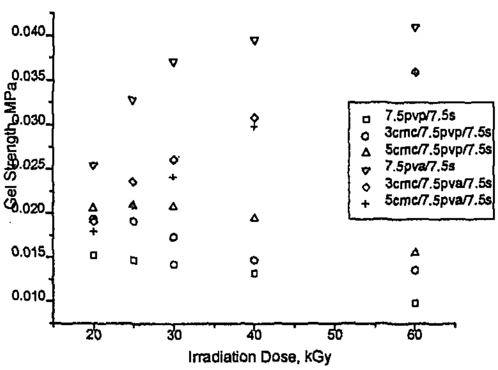

Figure 7: Effect of CMC and irradiation dose on gel strength of blend hydrogels

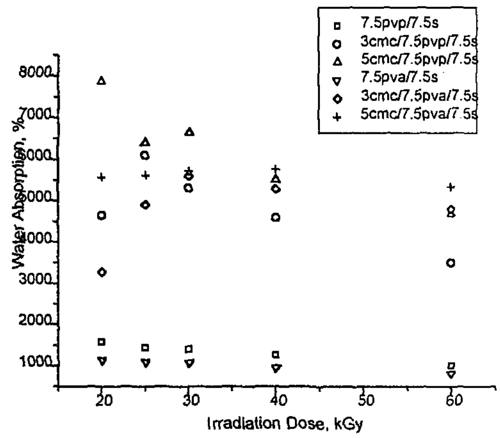

Figure 8: Effect of CMC and irradiation dose on swelling ability of blend hydrogels.

The effect of CMC on the gel strength of blend hydrogel is almost the same as the effect of PPG where it reduce the gel strength of sago/PVA blend hydrogel but the gel strength increase with increasing irradiation dose, as shown in Figure 7. From Figures 8 and 9, adding CMC into the sago/PVA and sago/PVP blend hydrogel, increase tremendously the swelling ability and tackiness property of the blend hydrogel. However, Its also show that the swelling and tackiness properties is better than using PPG. Even though CMC reduce the gel strength of sago/PVA hydrogel, the swelling ability is 4 times better than blend hydrogel or blend hydrogel with PPG. The rate of tackiness enhancement by CMC on tackiness property of sago/PVA and sago/PVP blend hydrogels is more or less the same and the tackiness decrease with increasing irradiation dose.

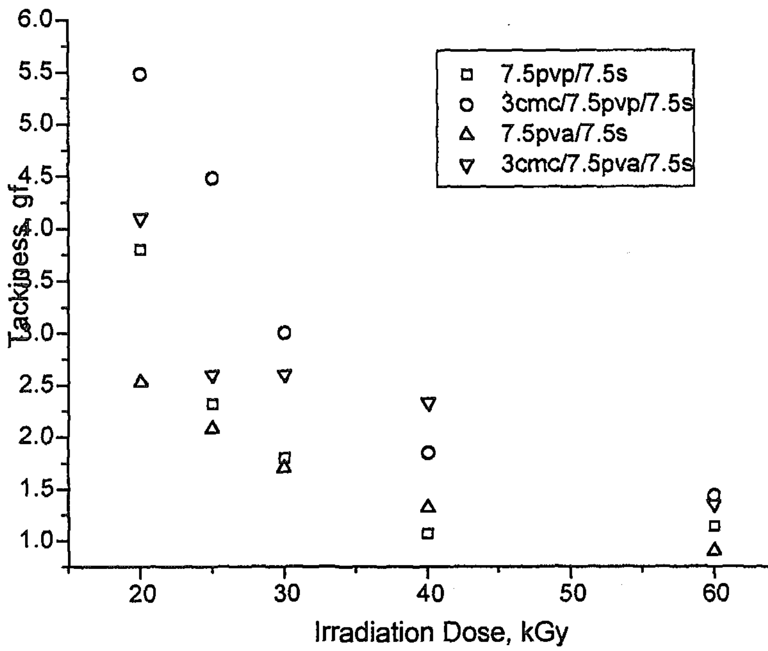

Figure 9: Effect of CMC and irradiation dose on tackiness of blend hydrogels

4. Conclusion

Based on the studies, it can be conclude that sago/PVA is one of the major components beside water in the development of hydrogel dressing and PVP is consider as additive in the blend, in order to improve especially the gel strength of the hydrogel. Other additive such as PPG and CMC is applied to enhance the swelling and tackiness properties of blend hydrogel. From the observation, we found that CMC is the right choices of additive to be use for future development in formulate the ingredient for wound dressing hydrogel.

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12 RADIATION PROCESSING OF INDIGENOUS NATURAL POLYMERS

- Properties of radiation modified blends from sago-starch for biodegradable composite

Ghazali[1] Z., Dahlan[1] K. Z., Wongsuban, B.[2], Idris S.,and Muhammad[2] K.
[1]Malaysian Institute for Nuclear and Technology Research (MINT), Bangi, 43000 Kajang Malaysia
[2]Department of Food Science, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia, Serdang, Malaysia

Abstract

Research and development on biodegradable polymer blends and composites have gained wider interest to offer alternative eco-friendly products. Natural polysaccharide such as sago-starch offers the most promising raw material for the production of biodegradable composites. The potential of sago, which is so abundant in Malaysia, to produce blends for subsequent applications in composite material, was evaluated and explored. Blends with various formulations of sago starch and polyvinyl alcohol (PVA), and polyvinyl pyrrolidone (PVP) polymers were prepared and subjected to radiation modification using electron beam irradiation. The effect of irradiation on the sago and its blends was evaluated and their properties were characterized. The potential of producing composite from sago blends was explored. Foams from these blends were produced using microwave oven while films were produced through casting method. The properties such as mechanical, water absorption, expansion ratio, and biodegradability were characterized and reported in this paper.

Key words:

Biodegradable, foams, films, sago-starch, polyvinyl alcohol, polyvinyl pyrrolidone, irradiation, crosslinking and electron beam.

1.0 INTRODUCTION

Starch, produced in abundance, biodegradable is cheap and can be obtained from many renewable sources). In South Asia, sago starch, produced from pith of sago palm, is a useful resource and an important product for commercial raw materials and foodstuff. The sago starch is also abundant especially in East Malaysia. Malaysia is the principle exporter of sago starch to the world market. With its abundant availability, sago is offering an attractive candidate for use in development of biodegradable starch-based composites. Interest in biodegradable packaging has increased significantly in recent years due to many factors, including rapidly diminishing landfill space, concern over future oil prices for the manufacture of synthetic plastic packaging, public awareness of environmental issues, damage to marine life due to discarded plastics and the development of new technologies for alternative degradable packaging materials. (D.L. Kaplan, 1994). Natural polysaccharides are among the most promising materials for the production of biodegradable foams. One of them is sago starch, which is abundant in Malaysia. Starch is totally biodegradable in a wide variety of environments and could assist in developing totally degradable materials to meet niche market demands for biodegradable plastics. Degradation or incineration of starch in plastics would recycle atmospheric CO_2 trapped by the plant and would not increase potential global warming.

2.0 EXPERIMENTAL

2.1 Viscosity profile

The pasting of starch sample was examined in a Brabender Viscograph using 75 rpm and a toque of 700 cmg equivalent to 1,000 BU. The starch slurry (400 ml at 6.0% starch solids) was adjusted to pH 6.5 with a few drops of 5%HCl or 5% NaOH solution, paste at a heating rate of 1.5 °C/min from 50 to 95°C, held at 95°C for 30 min, cooled from 95°C to 50°C, and finally held at 50°C for 30 min. The following measurements were taken from the Viscograph curve: temperature at begins of gelatinisation, Torque at maximum viscosity, temperature at maximum viscosity, torque at start of holding period. Torque at start of cooling period, torque at the end of cooling period, torque at the end of final cooling period.

2.2 Viscosity

The 2% starch solution was heated in a boiling water bath for 30 min and cooled to room temperature for 1 hour. An 8 ml of each sample was measured in triplicates using a Brookfield DV-III Rheometer (V1.2 RV) with an appropriate spindle no. SC4-18. The viscosity (cPs) was recorded within 30 seconds at every 10 rpm interval with a speed from 10 to 100 rpm.

2.3 Gel Strength

The 8% starch paste was prepared. The sample was poured into a height of 2.7 cm in a cylindrical plastic container (diam. =4.0 cm, height 5.5 cm) as a mold. The pastes were measured after aging at 4 and 25°C for 24 hours. The gel strength of the texture was determined using a Stable Micro System (TAXT-2 Texture Analyser). The paste in the mold was compressed at a speed of 2.0 mm/sec to a distance of 15 mm using a cylindrical probe (10 mm in diameter) and then retracted at the speed of 0.1 mm/sec to obtain the curves. The maximum force was termed gel strength.

2.4 Swelling power and solubility

Swelling and solubility was determined as described by Schoch (1964). Starch was accurately weighed (2g, dry basis) into a dry tarred pre-weighed 250 ml centrifugal bottle. Distilled was added to give a total volume of water equivalent to 180g. The starch was completely suspended by stirring at 200 rpm using magnetic stirrer. After taking out the stirrer, the bottle was immediately placed in constant temperature shaking water bath at 85°C±0.2 with continuous shaking at 200 rpm for 30 minutes. The centrifugal bottle was then dried and placed on a balance followed by the addition of distilled water to bring a total weight of 200 g. After capping, the bottle was centrifuged for 15 minutes at 1000xg. To measure solubility, 50 ml of the supernatant was then pipetted and transferred into an evaporating petri dish and dried overnight in a hot air oven at 105°C. The dried residue was then cooled in a dessicator and weighed for soluble starch. To measure the swelling power, the supernatant was carefully removed and discarded. The bottle with the sediment paste was then weighed to give the weight of swollen starch granules. The result was expressed by the calculation below:

$$\% \text{ solubility (on dry basis)} = \frac{\text{Weight of soluble starch}}{\text{Weight of sample on dry basis}} \times 400$$

$$\text{Swelling power} = \frac{\text{Weight of sediment paste} \times 100}{\text{Weight of sample on dry basis x(100\%solubility)}}$$

2.5 Preparation of Sago-water Soluble Polymer Blends

PVA solution (100 ml) of known concentration was heated continuously in a water bath at 90°C. Sago powder of known weight was added slowly and stirred for 2 minutes or until a gelatinized solution was about to achieved. The sago gel was then poured into a petri dish (9 x 9 mm) and cooled to a room temperature to form solid sample of about 4 mm. These samples were then subjected to irradiation and foaming processes.

PVP-sago, HEMA-sago and HEA-sago blends were prepared in the same manner as PVA-Sago gel. To some of the blends blowing agents, $NaHCO_3$ or nitrogen gas (bubbled N_2 gas) were added. This is done to evaluate the possibility of foaming sago-polymer blends with blowing agent.

2.6 Irradiation crosslinking

Petri dishes containing sago-gel were irradiated at 10, 15, 20, and 30 kGy. The irradiation parameters used were 3 MeV acceleration voltage and 10 mA current. The accelerator used was a Cockcroft Walton type having maximum acceleration voltage of 3 MeV and 30 mA beam current. The irradiation dose rate was set at 20 kGy per pass. The sample distance from beam window was 200 mm. Irradiation was carried out at an irradiation chamber temperature of 35°C approximately and at atmospheric pressure (760 mm Hg, approximately).

2.7 Gel Content Measurement

Triplicate irradiated sago-gel samples were cut into small pieces of known weight (about 0.2 g) and placed in stainless steel pouches. The pouches were placed in glass bottle containing 0.5 M NaOH overnight. Samples were rinsed thoroughly to neutral state and dried in a vacuum oven at 60 °C overnight. The final weight of the sample was measured and the gel content was calculated from the ratio of final weight over the original weight (taking into account the initial water content in gel samples).

2.8 Foaming

Irradiated samples of 30 x 45 mm sizes were foamed in a microwave oven for 3 minutes at 150 °C (high heating mode). The foams were evaluated of its foamability, resilience, water absorption, expansion ratio and biodegradability.

2.9 Expansion Ratio

Expansion capability of sago to foam was also measured. Irradiated sago-PVA blends were cut into 30 x 45 mm size and foam in microwave oven for 3 minutes. The difference of weight over initial weight was calculated as expansion percentage.

2.10 Degradability/Soil Burial Test

Biodegradability of sago foams was evaluated using soil burial method. The soil burial test was performed by burying the samples in the clay 20 cm depth with a pH from 6.5 to 7.5. The soil was not supplemented with any additional nutrients or microorganisms. Sample foams (4cm X 5cm) of known weight were cut from prepared foams prior to exposure in the soil testing systems. Sago foams were labeled and placed in a quadrant of 1 feet deep dug-up soil. The area was then carefully filled with soil. The foams appearance and weight was monitored at 2, 3, 4 and 5 days

In another experiment, foam sample was also held in 10% (w/v) soil water and incubated in shaker at 27°C and 90 rpm. After each interval, the samples were removed from the soil and clean with running water and dried in an oven to constant weight at 60°C.

3.0 RESULTS AND DISCUSSION

3.1 Viscosity

Starch displays unique properties on heating with water. It exhibits unique viscosity behavior with change of temperature, concentration and shear rate. Flow behavior characteristics can be estimated from the viscosity and shear rate relationship. The power law model is also widely used to describe the flow behavior characteristics of many food systems such as tomato products, citrus juices, soup and sauces (Nurul et al., 1998). In this experiment, effect of irradiation dose on the rheological behavior of gelatinized sago starch was studied over the shear range of 13.2-132.0 at 2% starch concentration. Figure 1 and 2 show the relationship between the viscosity and the irradiation dose of sago and tapioca starch. The viscosity was decreased when the shear rate was increased. The decrease of viscosity with the increase of shear rate suggested that the increasing shear rate progressively disentangles the arrangements of long chain molecules and helps to overcome the intermolecular resistance to flow. On the other hand, the highly solvated molecules or particles present in the dispersion medium may be progressively sheared away with increasing shear rate causing a reduction in the effective size of the particles and hence a reduction in apparent viscosity (Holdsworth, 1977)

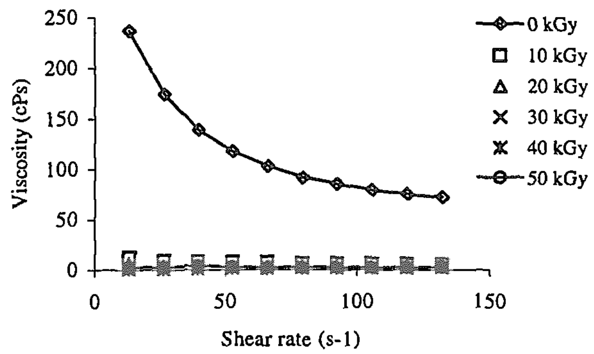

Figure 1: Effect of Irradiation Dose on Viscosity of Sago Starch

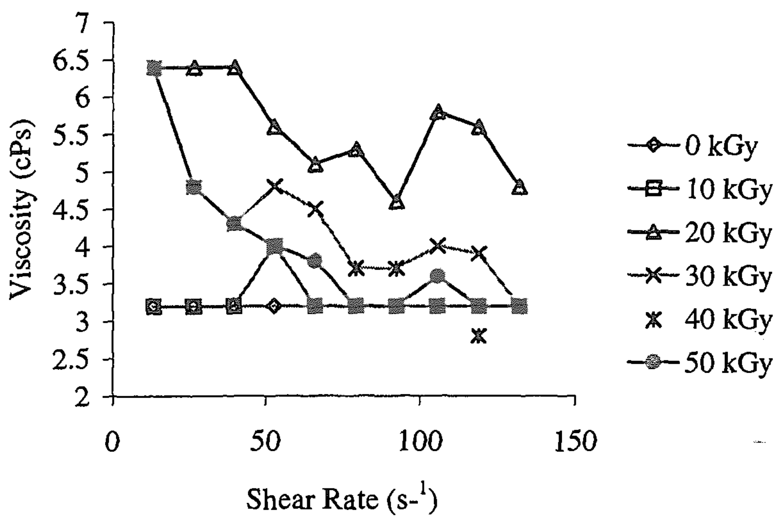

Figure 2: Effect of Irradiation Dose on the Viscosity of Tapioca Starch

Flow behavior characteristics can be estimated from the viscosity and shear rate relationship (Nurul et al., 1998). The flow behavior characteristic is influenced by the irradiation dose as the analysis of the results are shown in Table 1.

Table 1: Effect of Irradiation Dose on the Flow Behavior and Consistency of Sago and Tapioca Starch at 2% Starch Concentration

Irradiation dose (kGy)	Flow behavior (n)		Consistency (K)	
	Sago	Tapioca	Sago	Tapioca
0	0.5344	0.2985	2.45	0.2924
10	0.3305	0.1196	1.15	0.2840
20	0.2603	0.0057	0.8	0.0809
30	0.1133	0.2377	0.60	0.0969
40	0.2570	0.3134	0.05	0.0915
50	0.1276	0.2985	0.59	0.0915

Fig. 1 and 2 show that the viscosity of irradiated sago and tapioca starch in a decreasing trend. This might be due to the reduction of the amylose content in the starch. However, the results have not clearly seen due to the less efficient of the viscometer. The intrinsic viscosity measurement would be the better method. The flow behavior of the native sago starch (at 0 kGy) indicated that the starch is pseudoplastic and its pseudoplasity decreased when the irradiation dose was increased.

3.2 Gel strength

Fig.3 shows the effect of EB irradiation on gel strength of sago. Starch paste after gelatinisation consists of solubilised carbohydrates almost all of which is amylose and of swollen starch granules or their fragments, and it exhibits viscoelastic properties. On cooling of concentrated starch paste, starch gel quickly develops and solubilised carbohydrates form a continuous network which links swollen starch granules (Ott and Hester, 1965; Hikone, 1998). In this experiment, the gel strength of sago and tapioca starch decreased rapidly when irradiation dose was increased. Rosental (1992) reported that upon irradiation, hydrolysis and oxidative degradation of carbohydrate molecules may occur. Lower saccharides may be oxidized at the end of the molecule to form acid, and as a result of ring scission aldehydes may be formed. Large carbohydrate molecules are split into smaller units by cleavage link, resulting in depolymerization. This, chains of carbohydrtaes may be

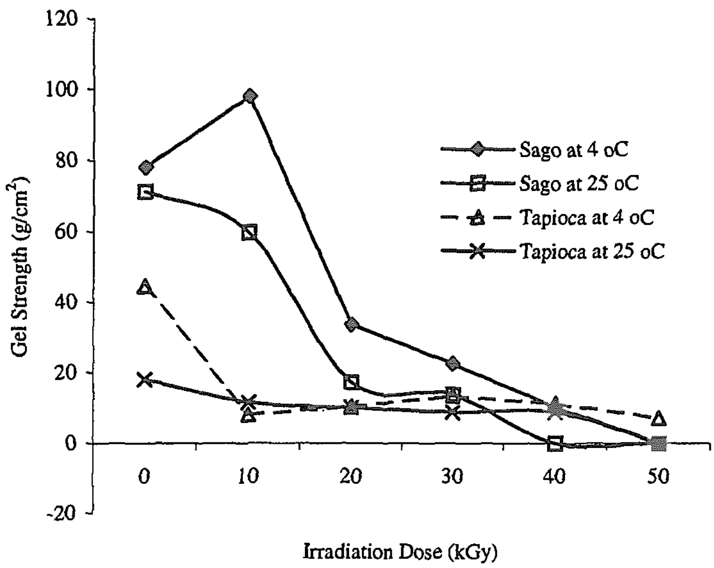

Figure 3: Effect of Irradiation Level on Gel Strength of Sago and Tapioca Starch at 4 and 25° C Storage Temperature

shorten with a loss of gelling power. Zanoni et al. (1991) reported that gelatinisation can be referred to as desegregation of starch granules within an aqueous environment at a suitable temperature. In the first stages of gelatinisation the shorter micelles dissociate. The longer micelles will persist to higher temperature. When starch granules are gelatinised in excess water, there is a phase change from order to disorder configuration (Donovan, 1979).

3.3 Swelling power and solubility

Figure 4 shows the swelling power of irradiated sago and tapioca. It was observed that the swelling decreases as the irradiation dose increases. The swelling power determined as the weight of hydrated granules was reduced in all irradiated samples. This was due the scission of the amylose and amylopectin chain the starch molecule (Rosenthal, 1992). The result of % solubility is shown in Fig 5. It solubility increases when the irradiation dose is increased. Tester and Merrison (1994) found that the increment in damaged starch level led to and increment in swelling power of wheat starch. That means sago and tapioca starch was damaged by irradiation as the swelling power was decreased. Several researchers reported the increment in material soluble in water as evidence of molecular degradation of starch by irradiation (Radley, 1960; Whistler and Ingle, 1965; Tollier and Guilbot, 1972; Duarte and Rupnow, 1994).

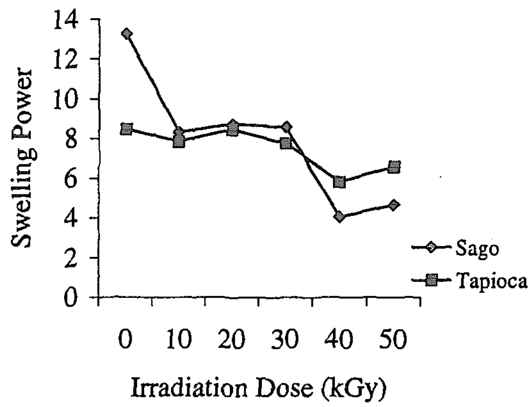

Figure 4: Effect of Irradiation Dose on Swelling Power of Sago and Tapioca Starch

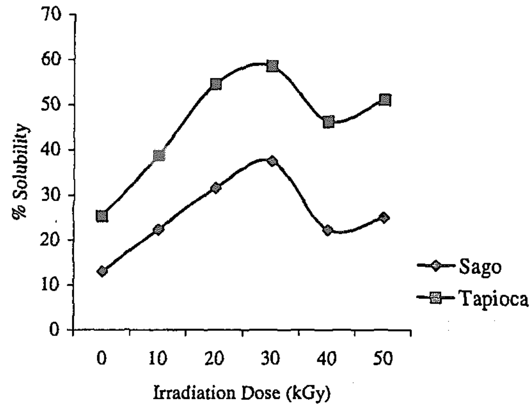

Figure 5: Effect of Irradiation Dose on % Solubility of Sago and Tapioca Starch

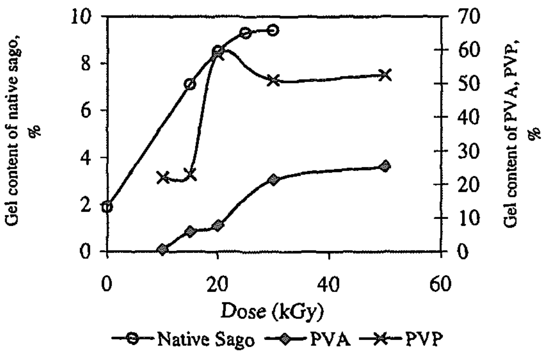

Fig.6: Crosslinking behavior of sago, PVA & PVP

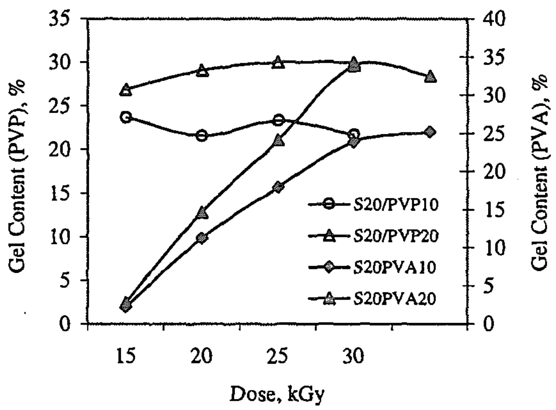

Fig. 7: Crosslinking properties of sago blends

3.4 Crosslinking of Sago and Sago Blends

Although sago on its own do not readily crosslink as shown in Fig 6, it is interesting to note that blending sago with PVA shows a significant increase in gel content value indicating greater crosslinking ability. This is observed in Fig. 7. As much as 35% gel was observed in blends of 20:20 ratio (Sago:PVA) at 30 kGy dose. The PVA content added in the blend does affect to certain extends the crosslinking ability of sago blends especially at higher irradiation dose of more than 20 kGy.

The Sago-PVP blends however, showed a contrast crosslinking properties compared to Sago-PVA. The presence of sago reduced the crosslinking ability of PVP as seen in Fig. 7. In general, the gel content values of these blends are 23% for the one containing 10% PVA and about 30 % for blends containing 20 % PVP.

3.5 Sago Foams

Sago on its own could not be expanded to produce foam. It is hoped that introducing crosslinking in sago or rather its blends would assist foaming. Thus, electron beam irradiation was used to induce crosslinking. The introduction of crosslinking hopefully helps prevent foam from collapsing once it cooled down. At present foam expansion was achieved using microwave oven. In subsequent work, compression mould and extrusion will be use for expansion process.

The sago-PVP formulations foamed very well but produced hard foams. Formulations having more than 20% sago and 15% produced better foams than others. It was observed also that irradiation dose of more than 25 kGy gave rigid foams.

In general, it was observed that Sago-PVA formulations gave better foams than the others. The foams produced are also softer and more resilience. For sago-PVA blends, the optimum irradiation dose was between 15-20 kGy. It was observed that best foams could be obtained with more than 15% PVA content. As much as 80% sago can be added. Evaluation on water absorption properties, expansion and biodegradability were evaluated using these Sago-PVA blends.

Blend should have sufficient mechanical properties to sustain foam cell from collapsing. In some foam made from polyolefin, crosslinking was introduced to achieve required mechanical properties to avoid this phenomenon. Having too much crosslinking also restricts formation of foam cells during expansion process. This dynamic properties requirement is clearly show in Fig. 8. The highest expansion for sago-PVA blends (S40PVA20) was achieved at 15 kGy irradiation dose i. e. indicating a balance properties of expansion and

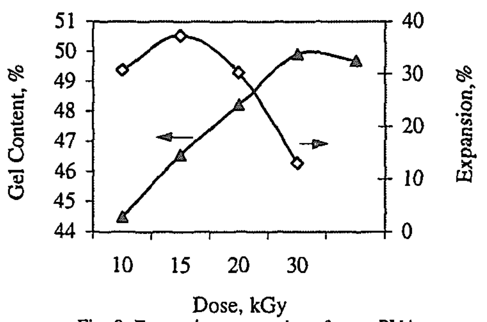

Fig. 8: Expansion properties of sago-PVA foams

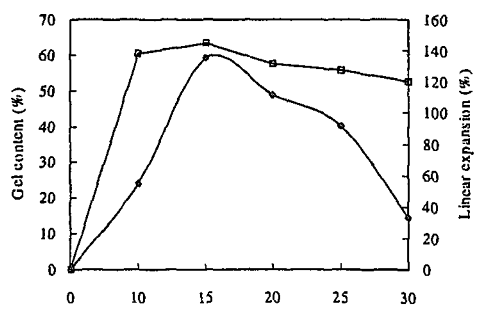

Fig.9: Expansion properties of Sago-PVP blends

mechanical strength. At irradiation dose exceeding higher than 15 kGy, the blend ability to foams decrease tremendously. Similar properties was observed for sago-PVP blends as shown in Fig. 9.

3.6 Biodegradibility of Sago Foams

Exposures in natural environments provide the best true measure of the environmental fate of a polymer because these tests include a diversify of organisms and achieve a desirable natural closeness of fit between the substrate, microbial agent and the environment (Ching., C. et. al. 1993). Measuring the degradability of materials after exposure testing can be relatively straightforward. In this work, the soil burial test was performed by burying the samples in the clay 20 cm depth with a pH from 6.5 to 7.5. Sample foams (4cm X 5cm) of known weight were cut from prepared foams prior to exposure in the soil testing systems. The soil was not supplemented with any additional nutrients or microorganisms. Initial soil burial test indicated that the sago-PVP and sago-PVA foams are biodegradable. This was observed that after 1 week, all samples tested disappeared. The photographs taken for samples after 2 and 5 days clearly indicated biodegradability of sago foams. It was presumed that the foams were digested by termites.

In another experiment samples were dipped in 10% (w/v) soil water and incubated in shaker at 27°C and 90 rpm. After each interval, the samples were removed from from the soil and clean with running water and dried in an oven to constant weight at 60°C. In Fig. 10 the weight loss of foamed sample increases to burial time. Living organisms are capable of producing enzymes that can attack this foam more easily due the presence of a large number of cells. The lower percentage in weight loss is also because to the burial time is too short and there were insufficient number of active microorganisms to attack these foam. There are several factors such as temperature, pH, oxygen concentration, humidity and availability of mineral nutrients that may effect this result.

Figure 11 shows the weight loss of the foamed sample after they were incubated in the soil water after 6 months. There is no significant change in weight loss for the foam that was stored in the water soil.

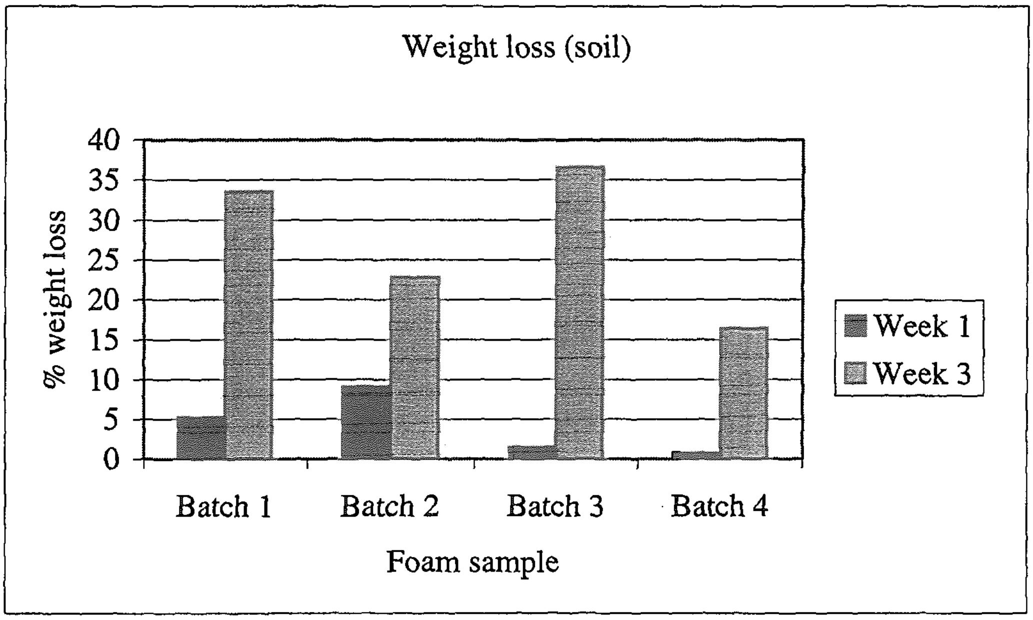

Figure 10: Biodegradibility of Sago Foams (Soil Burial Test)

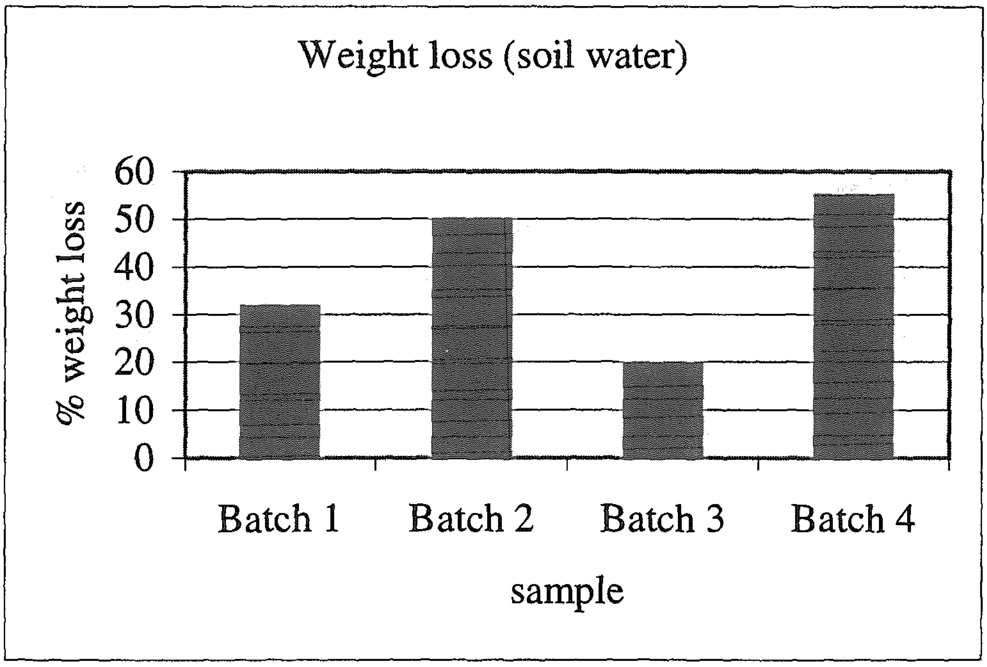

Figure 11: Biodegradibility of Sago Foams (Soil-water Burial Test)

4.0 CONCLUSION

Crosslinking of sago can also be achieved by blending sago with water-soluble polymers such as PVA and PVP. Addition of crosslinking accelerator does promote crosslinking of sago very much. However, blending these monomers and crosslinking accelerator does not render sago foamable.

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 PVP polymers. Foams produced from sago-PVA blends seemed to be softer and more resilience than that of sago-PVP blends. However, both types of foams could finds different end uses.

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13 Radiation Processing of Biodegradable Polymer Hydrogel from Cellulose Derivatives

Radoslaw A. WACH[1], Hiroshi MITOMO[1], Fumio YOSHII[2], Tamikazu KUME[2]

1. Department of Biological and Chemical Engineering, Faculty of Engineering, Gunma University, Kiryu, Japan
2. Takasaki Radiation Chemistry Research Establishment, JAERI

Abstract

The effects of high-energy radiation on ethers of cellulose: carboxymethyl-, hydroxypropyl- and hydroxyethylcellulose have been investigated. Polymers were irradiated in solid state and aqueous solution at various concentrations. Degree of substitution (DS), the concentration in the solution and irradiation conditions had a significant impact on the obtained products. Irradiation of polymers in solid and in diluted solution resulted in their degradation. A novel hydrogels of such natural polymers were synthesized, without using any additives, by irradiation at high concentration. It was found that high DS of CMC promoted crosslinking and, for all of the ethers, the gel formation occurred easier for more concentrated solutions. Paste-like form of the initial material, when water plasticized the bulk of polymer mass, along with the high dose rate and preventing oxygen accessibility to the sample during irradiation were favorable for hydrogel preparation. Up to 95% of gel fraction was obtained from 50 and 60% CMC solutions irradiated by gamma rays or by a beam of accelerated electrons (EB). The other polymers were more sensitive to the dose rate and formed gels with higher gel fraction while processed by EB. Moreover, polymers (except CMC) treated by gamma rays were susceptible to degradation after application of a dose over 50 – 100 kGy. The presence of oxygen in the system during irradiation limited a gel content and was prone to easier degradation of already formed gel. Produced hydrogels swelled markedly by absorption when paced in the solvent. Crosslinked polymers showed susceptibility to degradation by cellulase enzyme and by the action of microorganisms in compost or under natural conditions in soil thus could be included into the group of biodegradable materials.

Keywords: Hydrogel, Radiation Crosslinking, Biodegradation, CMC, Cellulose Ethers

1. Introduction

Polysaccharides such us cellulose and its derivatives, exposed to ionizing radiation, had been recognized as degraded type polymers since long [1]. The ethers of cellulose suffer the reduction of molecular weight while exposed on the influence of gamma rays or high-energy electrons from accelerators. For such modified natural polymers the main occurring reactions, initialized by radicals placed on macromolecules, lead to random cleavage of glycoside bonds in the main chain. Furthermore, irradiation of polymers of this type, like i.e. carboxymethylcellulose [2] resulted in their faster degradation when processed in diluted aqueous solution. Presence of water in a system causes an acceleration of radio-chemical reactions by the action of intermediate products of water radiolysis. The decomposition of water molecules results in creation of unstable species, like hydroxyl radicals, hydrogen atoms and hydrated electrons. Out of them hydroxyl radicals are the mains responsible for the transfer of reactivity from water to the polymer chains. They abstract hydrogen atoms from macromolecules and consequently macroradicals are formed. But an irradiation of some cellulose derivatives in an aqueous solution upon certain conditions resulted in formation of three-dimensional network of crosslinked gel. The first successful attempts have been done by Leavitt [3, 4]. The author suggested that the crosslinking reaction involved macroradicals created through an indirect effect of radiation by water radiolysis products.

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. Chemical gels, on the contrary, are insoluble in water and their crosslinked network can be damaged only irreversibly by rupture of covalent bonds [5]. Usually the network of hydrogel consist of hydrophilic polymers, bond together by chemical crosslinkers or, which is more convenient, by reactions between these polymers initiated by an ionizing radiation. The hydrophilicity of these materials is due to the presence of hydrophilic groups along the polymer chains, such as –OH, -COOH, -CONH, $-CONH_2$ and others [6, 7].

Hydrogels possessing abilities to absorption of 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). Also biomaterials in medicine and pharmacy are among one of the basic utilization of hydrogels, i.e. wound care coverings, controlled drug delivery systems, dental materials, implants, ophthalmic applications and others [8]. Particularly, natural polymers or hydrogels, which consists of natural macromolecules or their composites with synthetic polymers, formed by irradiation technique have been under an attention of researchers due to their biodegradability and availability at low costs [9, 10]. Ethers of cellulose, due to their novel feature of gel formation ability and easy biodegradation seem to be an excellent material. In the present research selected cellulose ethers were examined under ionizing radiation. It was found that carboxymethylcellulose (CMC), hydroxypropylcellulose (HPC) and hydroxyethylcellulose (HEC) form biodegradable hydrogels when irradiated in moderately concentrated solutions.

2. Experimental

2.1. Materials

Four ethers of cellulose were used in our investigations: carboxymethylcellulose (CMC) obtained from Daicel Co. Ltd., Japan, hydroxypropylcellulose (HPC) from Nippon Soda Co. Ltd. Japan and hydroxyethylcellulose (HEC) from Sumitomo Seika Co. Ltd. Japan. Characteristic of these polymers is summarized in Table 1. Average molecular weights were determined by measuring the intrinsic viscosity. Cellulase C-0901 enzyme, from *penicillium funiculosum* was obtained from Sigma Chemical Co., USA. All chemicals were analytical grade.

2.2. Sample preparation and irradiation

Deionized water was added to the polymer and mixed. For higher concentrations, above 5%, due to its high viscosity, the polymer was kneaded well with water. Then, material was kept for few days at room temperature to ensure complete dissolution and uniform distribution of polymer chains. Higher concentrated solutions had a form of very thick, thixotropic, paste-like gels. In the presence of air, irradiation of the mixture 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, after the removal of air by a vacuum machine.

Table 1. Characterization of cellulose ethers samples.

Sample	Degree of substitution (DS)	Intrinsic viscosity dL./g	Weight-average molecular weight
CMC 2.2	2.2	5.59	5.22×10^5
CMC 1.29	1.29	7.38	5.39×10^5
CMC 0.86	0.86	6.79	4.22×10^5
HPC1	3.0	4.49	6.60×10^5
HPC2	3.0	7.98	12.5×10^5
HEC7	2.0	3.72	2.02×10^5
HEC8	2.0	5.39	3.09×10^5
HEC9	2.0	9.35	5.82×10^5

Irradiation of samples was conducted with gamma rays generated from a ^{60}Co source at a dose rate of 10 or 1 kGy/h at inert temperature. For irradiation by high-energy electrons, 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 polymers

Initial weight-average molecular weights of the polymers were determined from an intrinsic viscosity on the basis of the Mark-Houvink equation $[\eta] = K \times DP^a$. K and a are constants, DP is the weight-average degree of polymerization and M_w is the weight-average molecular weight. To calculate a molecular weight obtained DP was multiplied by the average mass of the substituted anhydroglucose unit. The intrinsic viscosity was measured by an Ubbelhode viscometer in water or 0.1M NaCl solution, in the case of CMC at 25°C. The intrinsic viscosity were found by plotting obtained reduced viscosity η_{sp}/c and $\ln(\eta/\eta_{sp})/c$ against concentration [in g dl^{-1}] and extrapolating to zero concentration. For these polymers constant K is equal to 1.8×10^{-2}, 7.2×10^{-3}, 3.16×10^{-3} and a to 0.97, 0.915, 0.55 for CMC [11], HPC [12] and HEC [13], respectively.

Viscosity of degraded samples was examined with a rotary viscometer made by Tokyo Keiki Co. Ltd., Japan, after dilution to the concentration of 1%.

2.4. Gel content and swelling of hydrogel

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, occasionally shaken. The residue was made up of the crosslinked gel only. The gel fraction was calculated as follows

$$\text{Gel fraction (\%)} = (Gd / Gi) \times 100 \qquad (1)$$

where Gi is the initial weight of dried hydrogel after irradiation, Gd is the weight of insoluble part after extraction with water. The swelling of crosslinked hydrogel was estimated according to Japan Industrial Standard (JIS) K8150. The dry gel was immersed in deionized water for 24h at room temperature. Swelling, in grams of absorbed solvent per gram of dried gel, was calculated as follows

$$\text{Swelling} = (Gs - Gi) / Gi \qquad (2)$$

were Gs is the weight of hydrogel in a swollen state.

2.5. Biodegradation methods

Enzymatic degradation was carried out using a Cellulase C-0901 enzyme, from *penicillium funiculosum* in an acetic acid - NaOH buffer of pH 5.0 at 37°C with shaking. After an extraction of the soluble part, samples of gel of about 10mg were incubated in an enzyme solution at different times. Concentration of the enzyme in buffer was 0.1 mg mL^{-1}. Washing with an excess of distilled water and drying in vacuum at 35°C to a constant weight followed the incubation. The result of degradation is expressed as a percentage of the weight loss

$$\text{Weight loss \%} = (W_0 - W_1)/W_0 \times 100\% \qquad (3)$$

where W_0 and W_1 are, respectively, the weights of gel before and after enzymatic treatment.

The microbial degradability of polymer under the controlled soil was evaluated by the production of CO_2. Specially designed apparatus – MODA: Microbial Oxidative Degradation Analyzer [14], comprised of 4 independent lines of columns, was used. 10 grams of the sample along with rinsed sea sand - 450 g, and compost - 130 g, after mixing, was placed in a heated reaction column. Inside the column monitored temperature of the test was 35°C and the flow of the carbon dioxide-free but moisturized air was 30 ml min^{-1}. After flowing by the sample, the air, caring formed due to polymer decay CO_2 was passing through a series of columns filled in turn with silica gel, calcium chloride, soda lime and calcium chloride. Ammonia, also formed from the sample, was trapped in sulfuric acid solution, and water vapor was absorbed into first two columns (silica gel and calcium chloride). The CO_2 was collected quantitatively by soda lime and producing during the reaction water was caught in the last $CaCl_2$ column. Thus mass of produced carbon dioxide was calculated as a difference in the weight of two last columns (containing soda lime and calcium chloride) at the beginning and the end of the test. Pure compost mixed with sea sand was used as a blank and cellulose as a reference sample.

3. Results and discussion

3.1. Degradation

The all examined polymers exposed on γ-rays in solid state and in diluted aqueous solution in the atmosphere of air as well as under vacuum undergo mainly degradation. At Figure 1 there is presented the degradation of HPC2 as the ratio of the viscosity: V_{irr}/V_0 (viscosity after irradiation / initial viscosity of the polymer). Solutions were examined after dilution to the concentration of 1%. Samples lessen their viscosity the most significant at the early stages of irradiation, at low doses. Than, the rate of decreasing becomes not so high, reaching the level of pure solvent at high doses. The rate of viscosity reduction is lower for polymer irradiated in solid than irradiated in solution. It proves that presence of water accelerates radiation-induced reactions by indirect effect, through intermediate products of water radiolysis, here – breaking of the main chain. The reduction of the solution viscosity is directly related to molecular weight with emphasis

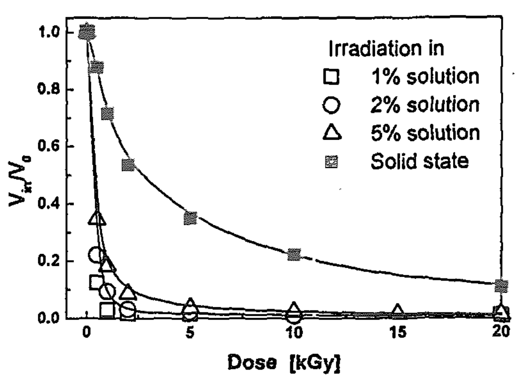

Fig .1. Degradation of HPC2 irradiated in solid and diluted aqueous solution. Viscosity after dilution to 1% concentration: V_{irr} – after irradiation, V_0 – initial.

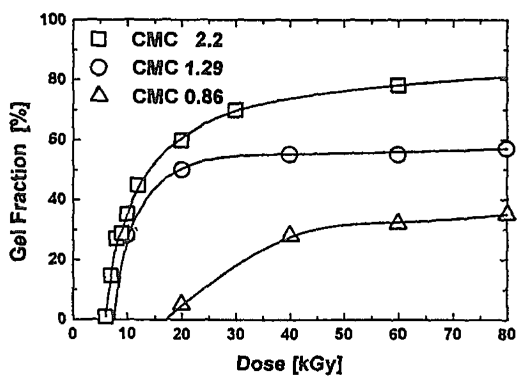

Fig .2. Crosslinking of CMC of different degree of substitution.

on the presence of longer chains. Polymers decompose to the form of low molecular compounds, like oligo- or mono- saccharide. The cleavage of bonds in normal chain occurs randomly; thus the longest chains suffer from the most frequent breaks. It was postulated that cellulose and its derivatives degrade by the rupture of glycosidic bonds, which are the weakest points in such macromolecules. [15, 16]. HPC1, the lower molecular fraction of the same polymer, CMC and HEC, when irradiated in solid state or in dilution, degrade at the same manner under gamma rays.

3.2. Hydrogel of carboxymethylcellulose

3.2.1. *Crosslinking of CMC and swelling*

Crosslinking successfully competes with glycoside bone cleavage as the concentration of polymer in a solution oversteps the critical value, then insoluble gel appears. Gelation occurrence depends on the molecular weight, the degree of substitution (DS) of the cellulose unit and irradiation conditions. CMC with higher degree of substitution build gel easier and more efficiently than that of lower DS as presented at Figure 2 for irradiation of 20% solutions. CMC of DS 2.2 gives the highest gel content among the three samples, about 80%, moreover, CMC of DS 1.29 and 0.86 produce ca. 60 and 35% of gel, respectively. Hence, it can be concluded that intermolecular linkages are formed as a result of the ether function. At Figure 3 the gel fraction against delivered dose is shoved for CMC 2.2 irradiated at high concentrations after degassing by EB, which does not differ from the results got for EB processed samples. Irradiation of polymer in concentration of 50 to 60% by gamma rays and by a beam of accelerated electrons resulted in obtaining high gel fraction ca. 90 and 95%, respectively.

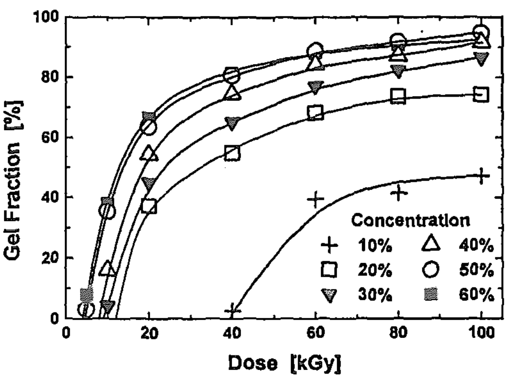

Fig. 3. Crosslinking of CMC 2.2 irradiated in aqueous solution by EB without an access of air.

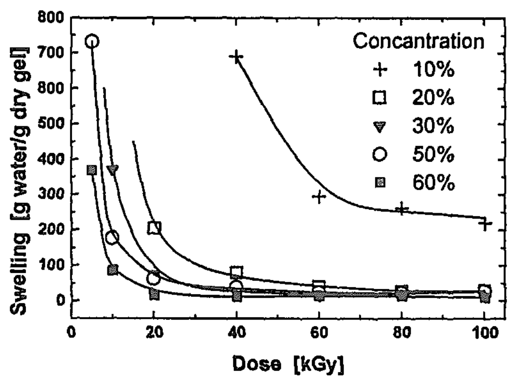

Fig. 4. Swelling of CMC 2.2 hydrogels formed by EB in water.

Medium concentrated solutions form not as high gel fraction but the maximum water uptake greatly enhances. In Figure 4 swelling of hydrogels is presented. CMC 2.2 formed by EB exhibit various swelling properties related to initial concentration of polymer and applied dose. Swelling has the maximum value for hydrogels at low doses of irradiation and drastically reduces with increasing the dose. After the dose oversteps so called 'gelation dose' the gel is formed, statistically one crosslink per chain is necessary [17]. The network is weak and susceptible for breaking but, because of a relatively low number of intermolecular bonds, is able to expand by holding a solvent into its voids. With subsequent increasing the density of crosslinks due to the further irradiation the absorption ability of the gel decreases. The hydrogel structure becomes more tightly connected and rigid. The swelling remains at the level of ca. 20, even for hydrogels irradiated at the dose as high as 100 kGy. An exception is the hydrogel formed from 10% CMC solution at 100 kGy, which can absorb over 220 grams of water per one gram of dried gel.

3.2.2. Enzymatic degradation of CMC

Crosslinked hydrogels were degraded by cellulaze enzyme in acetic acid – NaOH buffer pH 5.0, The results shown in Figure 5, for an example of CMC 2.2, demonstrates high degradability of the investigated material. Hydrogels were prepared from 50% CMC aqueous solutions by gamma irradiation at various doses. Hydrogels of less applied dose degrade faster than those crosslinked more tightly at higher dose due to the increasing

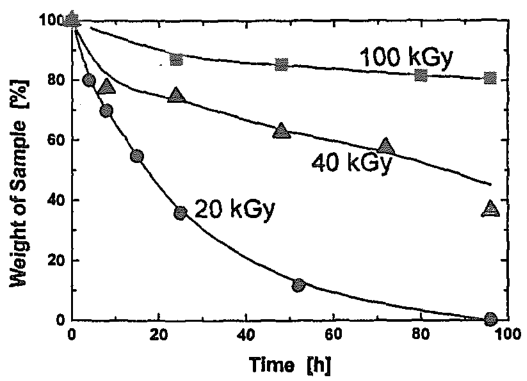

Fig. 5. Enzymatic degradation of CMC 2.2 hydrogel formed from 50% aqueous solution by γ-rays in with no access of air, cellulase enzyme in acetic acid – NaOH buffer, pH 5.0 at 37°C.

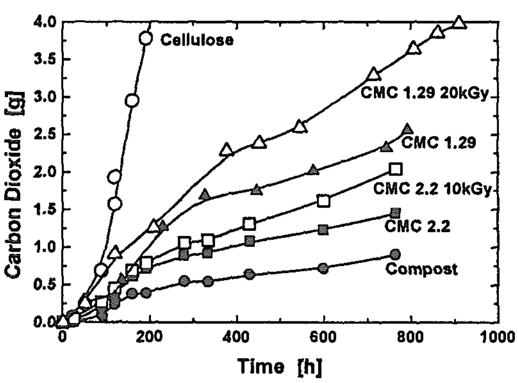

Fig. 6. Microbial oxidative degradation of CMC 2.2 and 1.29 unirradiated and irradiated in the compost soil at 35°C.

number of intermolecular bonds. Samples formed by the energy of 20 kGy disintegrate completely after 95 hours, while those formed by 10 kGy disappear before 8 hours of enzymatic treatment – not showed at the picture – despite, they posses relatively high gel fraction, over 40%. For gels irradiated at dose 40 kGy and above the degree of degradation do not exceed 60% after 96 hours of incubation. The material characterized by a huge swelling ability – the most desired from the point of view of further applications – irradiated at low doses, undergo the fastest biodegradation by the action of the enzyme.

3.2.3. Microbial degradation of CMC

Microbial degradability was evaluated by measuring of carbon dioxide produced from decomposed polymeric material. Samples of carboxymethylcellulose of DS 1.29 and 2.2, also cellulose as a reference material, were used in the experiment. The results are presented in Figure 6. Cellulose undergoes the fastest degradation, which reaches c.a. 80% after 1 month (after subtraction of the carbon dioxide produced in blank test – only compost). CMC decompose much slower but the rate of producing of CO_2 is still significant. After 1 month CMC 2.2 degrades in 3.8% - unirradiated and 8.4% - irradiated and CMC 1.29 degrades in 12.3% and 18.7%, respectively. The higher substituted samples disintegrate slower. Side chains prevents form cleavage of backbone by making the access of microorganisms to the vicinity of glycosidic bind difficult due to a spatial atomic configuration. Radiation processing of the polymer improves its susceptibility for bacterial digestion. Polymers irradiated in 20% aqueous solution at low dose, 10 and 20 kGy for DS 2.2 and 1.29 respectively, yield faster decomposition, in spite of some crosslinks were introduced. The gel fraction of examined hydrogels was 15% for CMC 2.2 and 47% for CMC 1.29. It can be explained in that way, that even in crosslinked hydrogels, polymer chains are shorter than in initial material. During irradiation of polysaccharides scission is the predominant reaction, but with increasing the degree of etherification crosslinking enhances its share. Random scission causes in diminishing average molecular weight and consequently, degraded as well as not degraded chains can be crosslinked. Hence, after irradiation unbonded macromolecules exist beside the network of gel and it is apparent that they undergo the fastest biodegradation. Crosslinked material also experience decomposition process. Hydrogel consist of chains, which are mainly cut for smaller fractions but bonded each to another. The existence of a few intermolecular crosslinks per initial chain should not inhibit greatly the total degradation. Thus, it is postulated that on the increasing of biodegradation rate of irradiated hydrogel, apart the radiation degraded chains, has also crosslinked fraction.

3.3. Hydrogel of hydroxypropyl and hydroxyethylcellulose

Opposite to CMC 2.2, crosslinking of HPC and HEC differs considerably due to the type of irradiation. The gel fraction is significantly higher when polymer is irradiated with high dose rate as presented in Figure 7 for an example of HPC2 in 20% aqueous solution. EB irradiation results in the content of gel as high as 85%. Gamma processing with the dose rate 10 and 1 kGy/h gives maximum part of gel less than 60 and 30, respectively. The concentration of free radicals in the system, determined by the dose rate, is the important factor influencing the gel formation. To the intermolecular recombination it is required the existence of two such species on neighboring chains at the same time. The probability of such a coupling is higher as higher is a dose rate. Under the present conditions of irradiation the concentration of radicals in the system should have differed 500 (or 5000 for 1 kGy/h) times. It is due to the fact that EB gives more than 500 times higher dose rate comparing to 10 kGy/h gamma irradiation.

The presence of air causes degradation, by increasing the yield of scission. For γ-irradiated HPC2 glycosidic bond cleavage finally prevails over crosslinking and the created network of the gel once more becomes soluble. During irradiation the oxygen present in the system diminishes the maximum of gel fraction for the all investigated polymers. It is due to the reaction of oxygen with macroradicals, which transform into

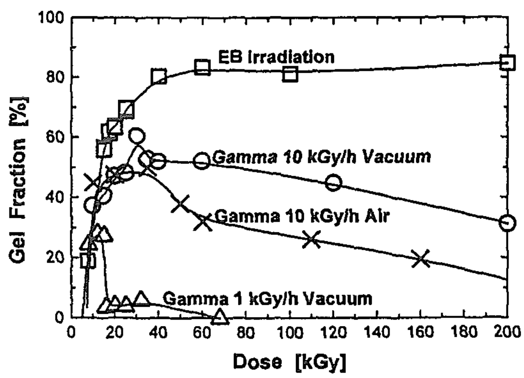

Fig. 7. Crosslinking of HPC2 irradiated at 20% aqueous solution at different dose rate.

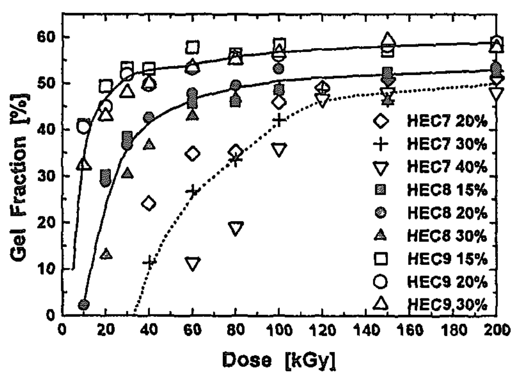

Fig. 8. EB irradiation of various molecular weight samples of HEC in aqueous solution without an access of oxygen.

peroxy-macroradicals, and subsequently decompose by rupture of main chain bond of the macromolecule [18]. Also HEC and CMC undergo degradation while irradiated in air. The length of a polymer chain affects the results of irradiation as depicted in Figure 8 for an example of HEC; the degree of molecular substitution was 2.0 for all fractions. Higher initial average molecular weight of the polymer enhances a share of gel. The maximum gel fraction reaches the level of 50, 53 and nearly 60% for increasing molecular weight of samples. Moreover, growing of the insoluble part at initial stages of gelation also occurs earlier, lower gelation dose, and faster for larger macromolecules. Thus, the energy utilized to produce 50% of gel of HEC9 corresponds to 25 kGy, while for HEC8 and HEC7 is as much as 80 and 200 kGy, respectively. Comparable results, with a strong dependence on the initial molecular weight and irradiation conditions were obtained for the other investigated polymers, except the effect of dose rate on CMC.

Conclusion

Hydrogels from four kinds of cellulose ethers were synthesized by ionizing radiation without any additives. It was found that high concentration in aqueous solution and more complete substitution by a side chains in glucopyranose ring of a polymer were favorable for crosslinking. Considerable higher gel fraction was obtained for solutions irradiated by EB than that processed by gamma rays, except for CMC for which gel formation was only slightly dependent on the dose rate. Access of oxygen to the sample during irradiation causes diminishing of the maximum gel content. Formed hydrogels was able to swell significantly by water absorption, and the swelling varied due to the concentration in which the polymer was irradiated and to applied dose. Test carried out by using a cellulase enzyme and in activated compost, confirmed that obtained hydrogels are biodegradable.

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14 Industrial Wastewater Treatment with Electron Beam

Bumsoo HAN, JaeIn Ko, JinKyu KIM, Yuri KIM, WooHo Chung

Central Research Institute of Samsung Heavy Industries Co.
103-6 Munji-dong Yusung-ku, Taejon 305-380, Korea (rep. of)

Abstract

Global withdrawals of water to satisfy human demands have grown dramatically in this century. Between 1900 and 1995, water consumption increased by over six times, more than double the rate of population growth. This rapid growth in water demand is due to the increasing reliance on irrigation to achieve food security, the growth of industrial uses, and the increasing use per capita for domestic purposes. Given the seriousness of the situation and future risk of crises, there is an urgent need to develop the water-efficient technologies including economical treatment methods of wastewater and polluted water.

In the Central Research Institute of Samsung Heavy Industries (SHI), many industrial wastewater including leachate from landfill area, wastewater from papermill, dyeing complex, petrochemical processes, etc. are under investigation with electron beam irradiation. For the study of treating dyeing wastewater combined with conventional facilities, an electron beam pilot plant for treating 1,000m^3/day of wastewater from 80,000m^3/day of total dyeing wastewater has constructed and operated in Taegu Dyeing Industrial Complex. A commercial plant for re-circulation of wastewater from Papermill Company is also designed for S-paper Co. in Cheongwon City, and after the successful installation, up to 80% of wastewater could be re-used in paper producing process.

1. Introduction

Samsung Heavy Industries (SHI) was established in 1974 and has managed to enter a wide range of successful business areas. These include plant engineering, Industrial machinery, shipbuilding, and constructions. The volume of annual sales is over 3 billion dollars with the 10,000 employees. The Central Research Institute of SHI is located in Daeduk Science Town, which is at the central part of Korea. The Accelerator Laboratory in this institute is devoted for the development of accelerator technologies and applications.

The research activities for the applications of accelerator include wastewater treatment, combustion flue gas purification, semi-conductor treatment, and other radio-chemical processing. The treatment of industrial wastewater with electron beam is one of the actively studied subjects for the environmental application in Central Research Institute. The method for the removal of heavy metals from wastewater and other technologies [1,2] are developed with the joint works of Central Research Institute of SHI and Institute of Physical Chemistry (IPC) of Russian Academy of Sciences.

2. Increase Of World Water Consumption

Actually 70% of the world is covered with water, however, the reality is that 97.5% of all water on earth is salt water, leaving only 2.5% as fresh water. Nearly 70% of that fresh water is frozen in the icecaps of Antarctica and Greenland, and most of the remainder is present as soil moisture, or lies in deep underground aquifers as groundwater not accessible for human use. As a result, less than one per cent of the world's fresh water, or about 0.007 per cent of all water on earth, is readily accessible for direct human uses [3]. This is the water found in lakes, rivers, reservoirs and those underground sources that are shallow enough to be tapped at an affordable cost. Only this amount is regularly renewed by rain and snowfall, and is therefore available on a sustainable basis.

It has been estimated that the amount of the fresh water that is readily accessible for human use at about 9,000 cubic kilometers a year, and another 3,500 cubic kilometers of water that is captured and stored by dams and reservoirs could be added. Harnessing the remaining water resources for human needs becomes increasingly costly, because of topography, distance and environmental impacts. Currently, humans are using about half the 12,500 cubic kilometers of water that is readily available. Given an expected population increase of about 50% in the next 25 years, coupled with expected increases in demand as a result of economic growth and life-style changes, this does not leave a great room for increased consumption (Fig.1). Water needs to be left in rivers to maintain healthy ecosystems, including fisheries. When global water picture is examined at a country level, some countries still have large amounts of water per capita, but others, however, are already facing serious difficulties. Future increases in demand due to population growth and increased economic activities will inevitably impinge further on the available water resources.

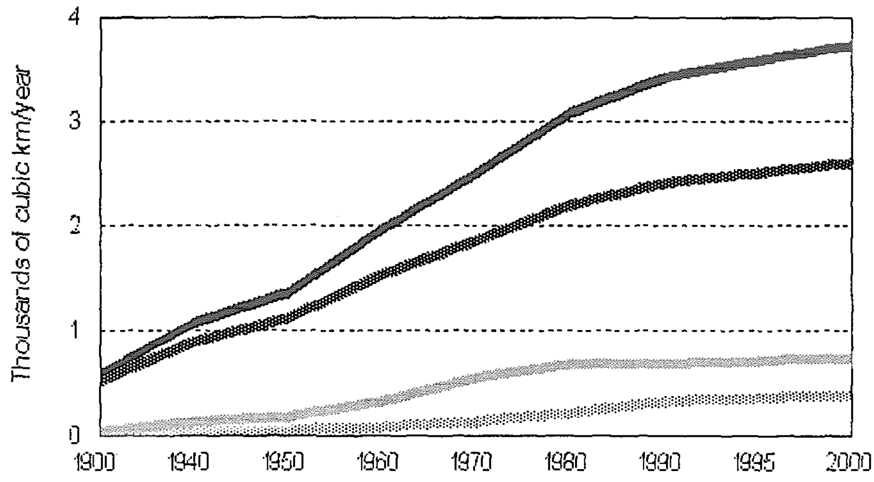

FIG. 1 Increase in world water consumption [3]

Despite improvement in the efficiency of water use in many developed countries, the demand for fresh water has continued to climb as the world's population and economic activities have expanded. From 1900 to 1995, withdrawals of fresh water from rivers, lakes, reservoirs, underground aquifers, and other sources increased by more than a factor of six. Increases in irrigation and, to a lesser extent, industrial uses of water have been the largest sources of this growing demand. At the same time, contamination by pollutants has seriously degraded water quality, effectively decreasing the supply of fresh water.

The results have been increased pressure on fresh water resources in most regions of the world and a lack of adequate supplies in some localities. Water experts and international institutions warn that water shortages could become critical in some regions.

In Korea, The annual average rainfall is 1.3 times greater than that of world, but it varies too much with season and area to control for withdrawal. Only 24% of rainfall could be accessible for human uses. If in the absence of far more effective management of water resources, this could be a pose serious long-term obstacle to sustainable development of Korea.

3. Electron Beam Treatment of Wastewater

People have used water as a convenient sink into which to dump wastes. The pollution comes from many sources, including untreated sewage, chemical discharges, petroleum leaks and spills, agricultural chemicals, etc.. The wastewater discharged has outstripped nature's ability to break them into less harmful elements. Pollution spoils large quantities of water, which then cannot be used. Virtually all pollutants can be removed from water, especially in the case of toxic substances, is very expensive, and requires sophisticated techniques.

The treatment of municipal and industrial wastewater becomes a more important subject in the field of environment engineering. The treatment of the industrial wastewater containing refractory pollutant with electron beam is actively studied in Samsung Heavy Industries. Electron beam treatment of wastewater often leads to their purification from various pollutants. It is caused by the decomposition of pollutants as a result of their reactions with highly reactive species formed from water radiolysis (hydrated electron, OH free radical and H atom).

TABLE I. Supply and Demand of Fresh Water in Korea (in billion tons)

Year	1994	2001	2006	2011
Demand	2.990	3,364	3,499	3,665
Supply	3,222	3,429	3,454	3,465
Shortage	-	-	45	200

TABLE II. Amount of Wastewater Generated/Discharged in Korea [4] (in 1,000m^3/day)

	Number of Companies (%)	Amount of waste-water generated (%)	Amount of waste-water discharged (%)
Textile co.	1,423 (5.6)	473 (5.4)	457 (19.2)
Papermill	268 (1.1)	711 (8.1)	364 (15.3)
Light Ind.	511 (2.0)	390 (4.5)	243 (10.2)
Processing Ind.	3,376 (13.3)	439 (5.0)	200 (8.4)
Metal fabrication	437 (1.7)	5,346 (61.1)	169 (7.1)
Others	19,284 (76.2)	1,382 (15.8)	942 (39.7)
Total	25,299 (100)	8,741 (100)	2,375 (100)

Sometimes such reactions are accompanied by the other processes, and the synergistic effect upon the use of combined methods such as electron beam treatment with ozonation, electron beam and adsorption and others improves the effect of electron beam treatment of the wastewater purification.

In Korea, where the industries are concentrated in near urban areas, resulting in severe water pollution problems in most large cities. Major sources of water pollution include chemical-intensive industries such as textiles, metal plating, electronics, papermill and refineries. Typical contaminants include non-biodegradable substances, grease and oils, acids and caustics, heavy metals such as cadmium and lead, sludge and a long list of synthetic organic compounds. The amounts of wastewater generated and discharged in Korea are summarized in TABLE II.

TABLE III. Wastewater under study at SHI

Wastewater (from)	Purpose of investigation	Results
Dyeing company	Removal of color and organic Impurities	Pilot plant constructed. Improve removal efficiencies
Papermill	Decrease COD, color Increase re-use rate	Reduction in impurities Commercial plant designed
Petrochemical co.	Removal of organic residues after processing	Removal of TCE,PCE,PVA, HEC and other substances
Leachate from landfill area	Removal of organic impurities Improvement of Bio-treatment	Bio-treatment efficiency improved
Heavy metals	Decrease the content of heavy metal ions in water	Removal of Cd,Cr^{+6},Hg up to 98% (95% in Pb)
Power plant Clean-up	Decrease the content of organic acid and detergent	Decrease the content of organic substance

The wastewater under current investigation at SHI are from dyeing companies, petrochemical processes, papermill and leachate from sanitary landfill area etc.. Those are also summarized in TABLE III. Two electron accelerators of energy 1 MeV each in continuous mode at Daeduk (SHI) and one accelerator of 5 MeV in pulsed mode at Moscow (IPC) are used in experiments. Applied sets of dose for the experiments were measured with an ordinary or modified Fricke dosimeter and dichromatic dosimeter.

4. Pilot Plant for Wastewater from Dyeing Process

An electron beam pilot plant for treating 1,000m^3/day of dyeing wastewater from 80,000m^3/day of total wastewater has constructed in Taegu Dyeing Industrial Complex (TDIC). TDIC includes now more than hundred factories occupying the area of 600,000m^2 with 13,000 employees in total. A majority of the factories has equipment used for dip dyeing, printing, and yarn dyeing. The production requires high consumption of water (90,000m^3/day), steam, and electric power, being characterized by large amount of highly colored industrial wastewater. Therefore, intensive and effective purification of the wastewater is one of the most complicated and actual problems of TDIC's current activities.

Purification of the wastewater is performed by Union wastewater treatment facilities in Fig.2 using conventional methods schematically described in Fig.3. Current facility treats up to 78,000m^3 of wastewater per day, extracting thereby up to 730 m^3 of sludge.

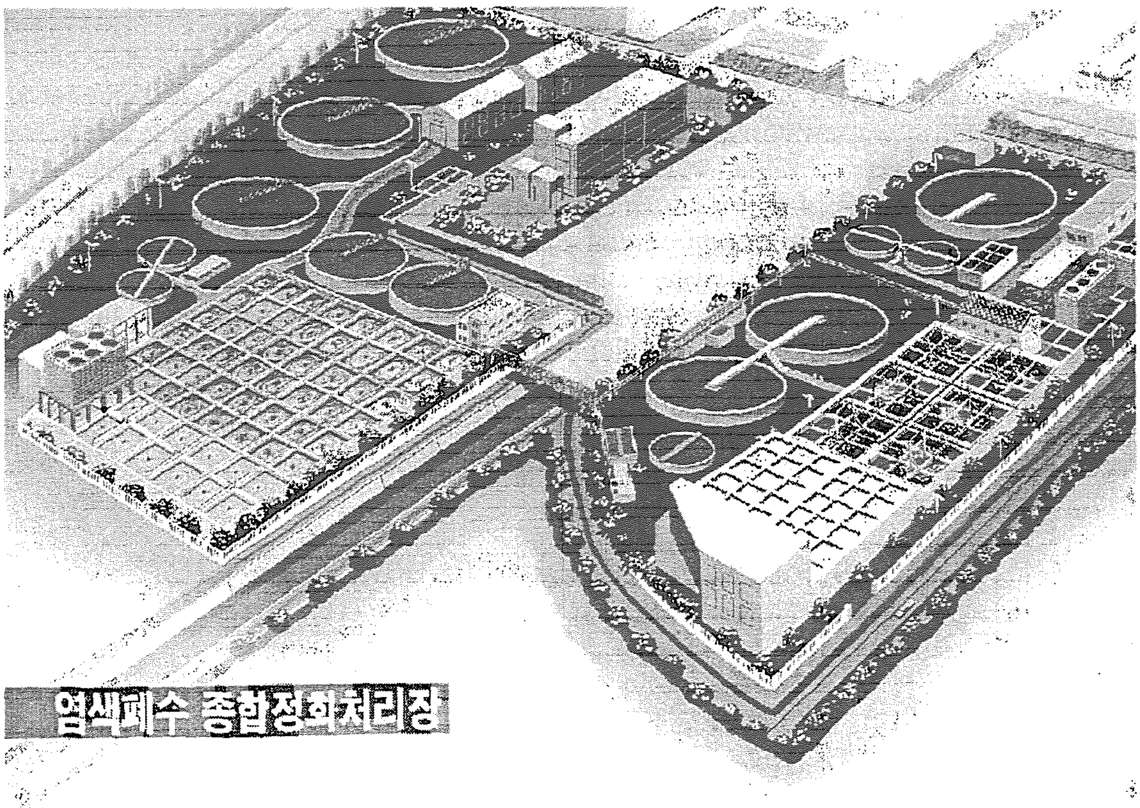

FIG. 2 Wastewater treatment Facility in TDIC

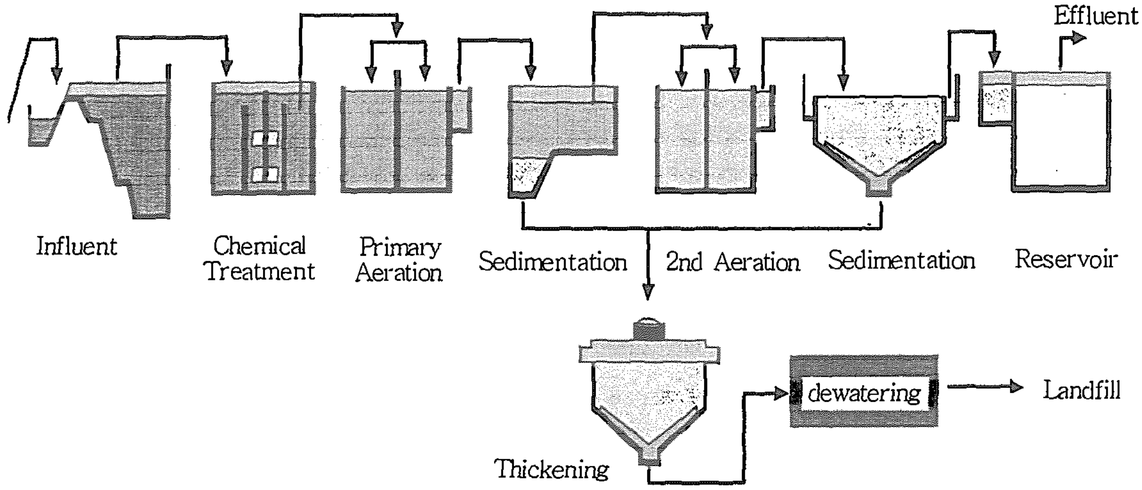

FIG.3 Process flow in dyeing wastewater

Rather high cost of purification results from high contamination of water with various dyes and ultra-dispersed solids. Because of increase in productivity of factories and increased assortment of dyes and other chemicals, substantial necessity appears in re-equipment of purification facilities by application of efficient methods of wastewater treatment. The existing purification system is close to its limit ability in treatment of incoming wastewater. Some characteristics of raw wastewater as well as characteristics of treated water after several treatment stages and of effluent are listed in the TABLE IV.

The studies have been carried out regarding the possibility of electron beam application for purification of wastewater. With the co-works of SHI Central research Institute and IPC, the experiments on irradiation of model dye solutions and real wastewater samples (from various stages of current treatment process) have been performed. [4] The results of laboratory investigations of representative sets of samples showed the application of electron beam treatment of wastewater to be perspective for its purification (Fig. 4). The most significant improvements result in decolorizing and destructive oxidation of organic impurities in wastewater. Installation of the radiation treatment on the stage of chemical treatment or immediately before biological treatment may results in appreciable reduction of chemical reagent consumption, in reduction of the treatment time, and in increase in flow rate limit of existing facilities by 30-40%.

TABLE IV. Typical Characteristics of Wastewater

Parameter	pH	BOD_5 (mg/l)	COD_{Mn} (mg/l)	Suspended solids (mg/l)	Color units
Raw wastewater	12	2,000	900	100	1,000
After chemical treat	6.8-7.5	1,700	450	50	500
After 1st Bio-treat	7.0-8.0	1,300	250	50	400
After 2nd Bio-treat	7.0-8.0	30	60	50	250

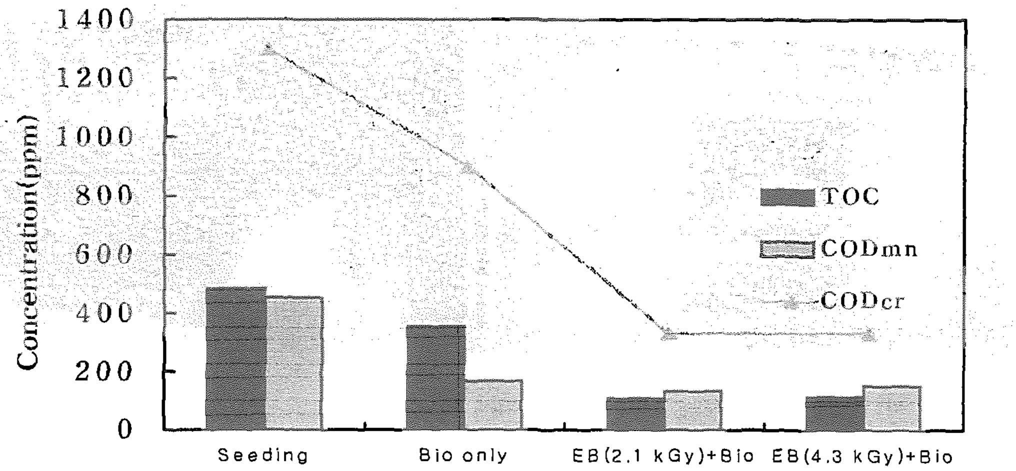

FIG. 4 Combined effect of e-beam and biological treatment.

A pilot plant for a large-scale test (flow rate of 1,000m^3 per day) of wastewater starts operation with the electron accelerator of 1MeV, 40kW (Fig. 5). The accelerator was installed in Feb. 1998 and the technical lines are finished in May. The Tower Style Biological treatment facility (TSB) which could treat up to 1,000m^3 per day has also installed in October. The wastewater from various stages of the existing purification process can be treated with electron beam in this plant to investigate the experimental possibilities of the pilot plant, and it will give rise to elaborate the optimal technology of the electron beam treatment of wastewater with increased reliability at instant changes in the composition of wastewater. The wastewater is injected under the e-beam irradiation area through the nozzle type injector to obtain the adequate penetration depth. The speed of injection could be varied upon the dose and dose rate.(Fig.6)

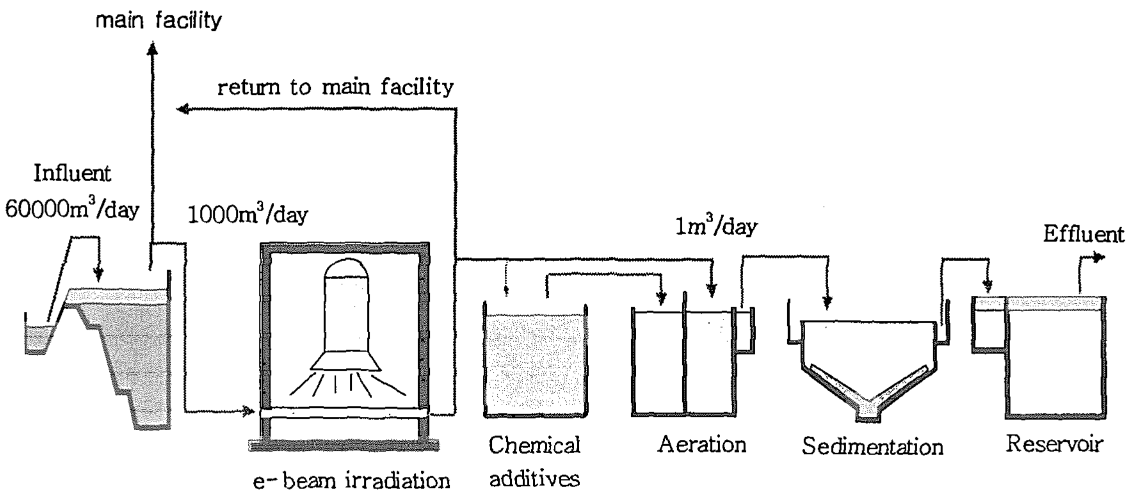

FIG.5 Schematic diagram of pilot plant at Taegu dyeing complex

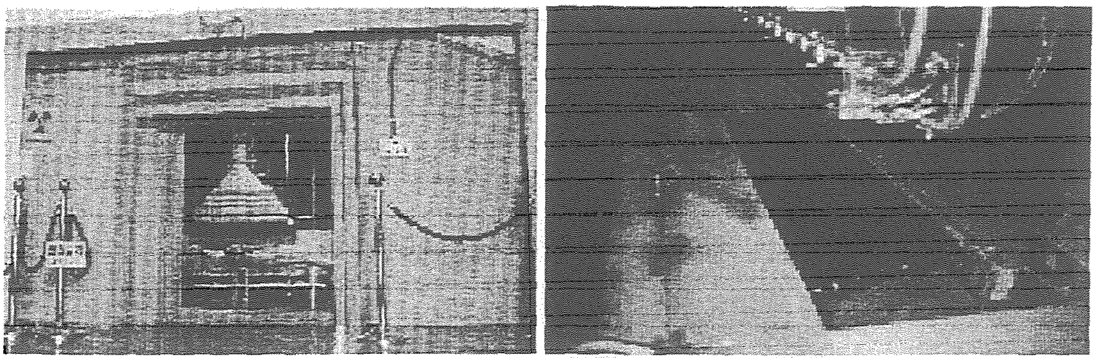

FIG.6 Electron Accelerator and Wastewater under Injection

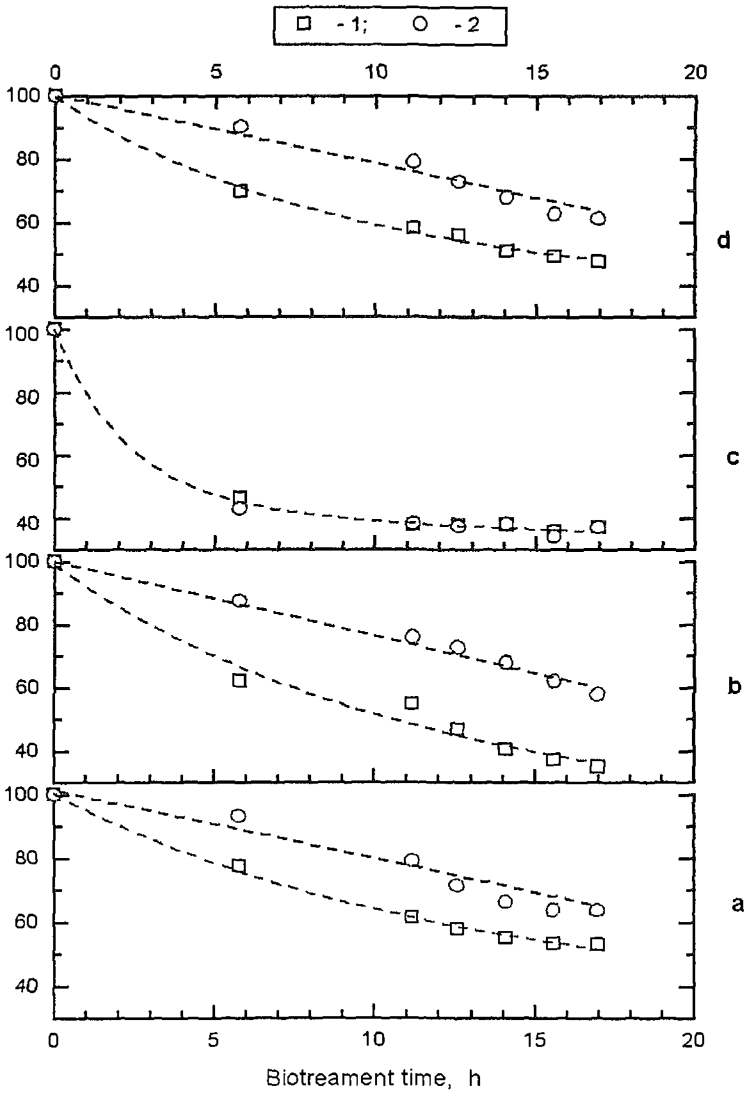

FIG.7 Effect of irradiation and biological treatment on wastewater parameters: a-TOC; b-COD_{Cr}; c-COD_{Mn}; and d-BOD. 1- after EB treatment; 2-without EB treatment

5. Commercial Plant Design for Wastewater from Papermill

A commercial plant for re-circulation of wastewater with electron beam from Papermill Company is also under planning in S-paper Co. and SHI. S-paper co. is located in Cheongwon City, 120km south of Seoul, and consumes 18,000 m^3 of water per day. The major products of this company are papers for newsprint (450t/day) and are mainly made of recycled paper (91%) and pulps. Purification of wastewater is now performed by 2-stages of chemical and biological treatment facilities.

For the economical point of view, this company tried to recycle the treated water to production lines, but used only 20-30% at total water since the amount of organic impurities after treatment are high and some of them are accumulated during re-circulation. In order to develop the most efficient method for re-circulation of wastewater, the experiments were conducted with samples in various stages of treatment. The best result obtained is irradiation of water after biological treatment combined with coagulation and filtration (Fig. 8). Irradiation in this stage, the additional removal of impurities is up to 80% in TOC (Total Organic Carbon) values.

On the base of data obtained by SHI and IPC the suitable doses in this case are determined as around 1 kGy for the flow rate of 15,000 m^3 wastewater per day (since the 3,000m^3 of wastewater is returned to initial stage with sludge). Therefore, three accelerators with the total power of 300kW and treating systems are designed. After the successful installation of electron beam treatment facilities, up to 80% of wastewater could be re-used in paper producing process (Fig. 9).

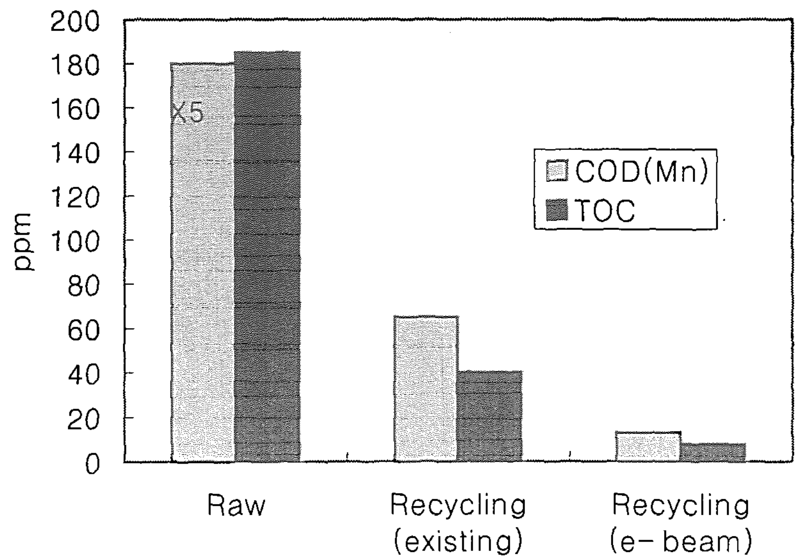

FIG.8 Treatment of wastewater from papermill.

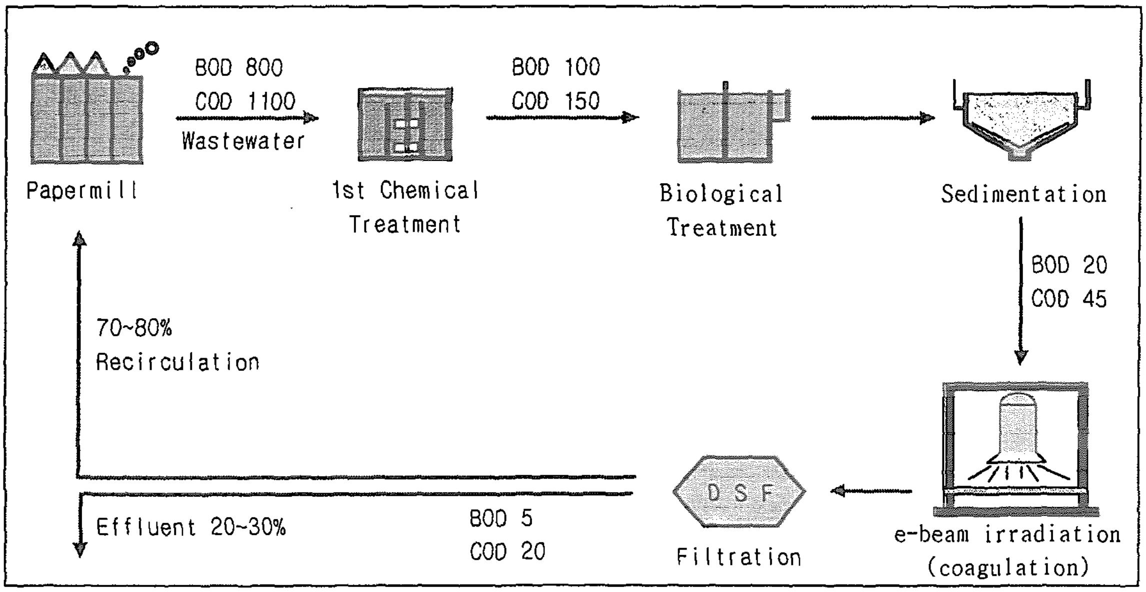

FIG. 9 Process flow of e-beam facility for wastewater from papermill.

6. Summaries and Conclusion

6.1 General

6.1.1 Electron beam treatment of industrial wastewater is under development in Samsung Heavy Industries for the removal of non-degradable wastes, and also for the re-use of wastewater to production process.
6.1.2 For industrial wastewater with low impurity levels such as contaminated ground water, cleaning water and etc., purification only with electron beam is possible, but requires high amount of irradiation doses.
6.1.3 For industrial wastewater with high impurity levels such as dyeing wastewater, leachate and etc., purification only with electron beam requires high amount of doses and far beyond economies.
6.1.4 Electron beam treatment combined with conventional purification methods such as coagulation, biological treatment etc. is suitable for reduction of non-biodegradable impurities in wastewater and will extend the application area of electron beam.

6.2 Experimental

6.2.1 A pilot plant with electron beam for treating 1,000m^3/day of wastewater from dyeing industries has constructed and operated continuously since Oct 1998.
Electron beam irradiation instead of chemical treatment shows much improvement in removing impurities and increases the efficiency of biological treatment.
Actual plant is under consideration based upon the experimental results.
6.2.2 Commercial plant for re-circulation of 15,000m^3/day of wastewater from papermill is also investigated, and after the successful installation, up to 80% of wastewater could be re-used in paper producing process.

Acknowledgement

The author wishes to acknowledge the support of the International Atomic Energy Agency and the Ministry of Foreign Affairs and Trade of Korean Government.

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Session 3

Radiation Processing of Silk Protein

15 Bilateral Cooperation Between OAEP and JAERI

Tamikazu Kume

Takasaki Radiation Chemistry Research Establishment,
Japan Atomic Energy Research Institute
1233 Watanuki, Takasaki, Gunma 370-1292, Japan

Implementing Arrangement between the Office of Atomic Energy for Peace, Thailand (OAEP) and the Japan Atomic Energy Research Institute (JAERI) in the field of radiation processing was signed on 20 March 1990. Under the Implementing Agreement, the "Cooperative Research Program on Sludge Pasteurization and Upgrading by Radiation" and the "Cooperative Research Program on Upgrading of Sludge and Agricultural Wastes by Radiation" were implemented on 1990 - 1994 and 1994 - 1998, respectively.

Then, the "**Cooperative Research Program on Radiation Processing of Silk Protein**" was started on 11 December 1998.

1. Scope of Research

Application of Radiation Processing has been developed in various fields such as sterilization of medical supplies, improvement of polymer materials, etc. However, the radiation processing using the petrochemical products is not suitable for developing countries because of difficulties in production and high cost. It is, therefore, important to develop the technique using natural resources to avoid environmental pollution.

Most of Asian countries are agricultural countries and produce the huge amount of renewable and environmentally friendly resources. Silk is one of the important resources and Thailand is the fifth country producing silk in the world with the production of 13,000 tons per year. Ten percent of silk is discarded each year or under utilized. As silk protein has excellent physiological characters, it is expected to develop the utilization of silk as new functional materials. Therefore, in this cooperative research program, the radiation processing on silk protein is investigated to decrease the environmental pollution and recycle resources.

2. Major Items of the Work

(1) Research on improvement of silk protein by radiation

1) Radiation degradation of silk protein

Change of silk protein under various radiation conditions such as temperature, oxygen, irradiation dose will be studied to solubilize the silk wastes.

2) Physiological properties of irradiated silk protein

Physiological properties of degraded polypeptides by radiation from fibroin and sericin which are the main components of silk protein will be studied to clarify the anti-bacterial and anti-oxidative activities to decrease blood cholesterol, etc.

(2) Research on radiation crosslinking of silk protein

1) Selection of radiation closslinking method of silk protein

Radiation effect on silk protein in solid or liquid state, with or without oxygen, etc. will be studied to find out the suitable condition for crosslinking. Also the effect of the addition of polyfunctional monomers and water soluble crosslinking-type polymers on radiation crosslinking of silk protein will be studied.

2) Characteristics of crosslinked products

Physical properties of closslinked silk such as microstructure, mechanical properties, swelling properties, thermal stability, biodegradability, bio-affinity, etc. will be investigated.

3. Collaborative Party

The cooperative program will be implemented by the staff of OAEP, JAERI and Cooperative Parties. The Cooperative Parties of OAEP are as follows:

1) National Metal and Materials Technology Center (MTEC)
2) Department of Agriculture
3) Department of Medical Sciences
4) Chiang Mai University

5) Chulalongkorn University

6) Prince of Songkla University

7) King Mongkut's Institute of Technology Ladkrabang

3. Time Schedule

	1999	2000	2001	2002
(1) Research on improvement of silk protein by radiation	────	───→		
1) Radiation degradation of silk protein		────	────	───→
2) Physiological properties of irradiated silk protein				
(2) Research on radiation crosslinking of silk protein			────	───→
1) Selection of radiation closslinking method of silk protein			────	───→
2) Characteristics of crosslinked products				

16 Culture of Human Skin Sheet for Wound Therapy

Nares Damrongchai

National Center for Genetic Engineering and Biotechnology (BIOTEC),
National Science and Development Agency,
Ministry of Science, Technology and Environment
73/1 Rama 6 Road, Rajadhevee, Bangkok 10400, Thailand

Thailand has annually several thousand cases of burn including serious burn patients that is life-threatening. Among these, roughly 40% of the patients lost their lives due to lack of appropriate skin substitute or skin grafting. However, recent development in cell culture technique has enable one alternative solution to the problem of this serious shortage of skin grafts by mean of cultured skin system. This involves the primary culture of epidermal human keratinocytes for epidermal reconstruction and grafting of the cultured skin sheet to the wound site as replacement of the lost skin. The material used for cell culture was normal cell culture flasks and petri dishes, with special treatment for culture of anchorage-dependent cells. Silk protein has been considered suitable as coating material for skin cell culture but has not been tried out in this experiment.

Our group has been performing keratinocyte cultures in the laboratory with sample biopsy from children's foreskin as well as normal adult skin (1^{st} phase). In this phase, emphasis was given on the qualitative and quantitative evaluation of the laboratory's ability to produce cultured skin sheet from small sample biopsy of normal human skin within the given period of time and with minimal contamination rate. We were successful in preparing cultured keratinocyte sheets from these skin sample biopsies in 14-21 days with relatively low contamination rate. These data will provide fundamental support for the proceeding into the clinical trial (2^{nd} phase) with long term assessment on patients.

Reference:

Falanga, V. *et al.* (1998) *Arch Dermatol* 134.

17 Study on Antibacterial Activity of Hydrogel from Irradiated Silk Protein

J. BUNNAK[1] and M. CHAISUPAKITSIN[2]

1. Department of Industrial Education, King Mongkut's Institute of Technology Lardkrabang, Bangkok, Thailand, 10520
2. Department of Chemistry, King Mongkut's Institute of Technology Lardkrabang, Bangkok, Thailand, 10520

ABSTRACT

Hydrogels for biomedical application were prepared from solution blends of 3% silk protein and 3%, 10% poly(vinyl alcohol) (PVA) and followed with irradiation. Mixture of hydrogels were gamma irradiated at 10, 20, 30, 40 and 50 kGy under N_2 atmosphere. To clarify anti-bacterial activity of hydrogels, modified of the Agar disk diffusion method and American Association of Textile Chemists and Colorists, AATCC Test Method 90-1977, were carried out. The four kinds of bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, were used. It was found that a 1:3 volume ratio of 3% silk protein and 3% PVA respectively, at 50 kGy irradiation, is suitable conditions for preparation hydrogels and trend to indicate the highest of an antibacterial activity against *E. coli*, *B. subtilis and S. aureus*. However the antibacterial activity of hydrogels against *S. epidermidis* was not clearly. These results are very useful to expand the application of hydrogel from irradiated silk protein to the medical products.

Keywords : Silk protein, Antibacterial activity, Hydrogel, Poly (vinyl alcohol)

1. Introduction

Silk fiber consists of two different types of protein, Fibroin and Sericin. Fibroin is a structural material and sericin is a covering material. A large amount of silk fibers have been wasted from hand and machine reeling in Thailand. It is reported about 200 tons per year as silk waste. The utilization of silk waste is interested to develop in the field of bio-materials. Products from silk have excellent physiological characters, it is expected to consider and develop the utilization as a new functional bio-materials [1].

Recently published papers reported gamma irradiation is the possible method for preparing hydrogel from poly (amino acid) such as poly (ε-lysine) [2] and poly (γ - glutamic acids) [3].

Hydrogels have been used widely for cosmetic and biomedical applications such a burns wound dressing, contacts lens, artificial skin and also in drug delivery system. Hydrogels for wound dressing have been produced by electron beam crosslinking on a mixture of natural and synthetic polymers, poly(vinyl pyrrolidone), polyethylene glycol and agar [4] and poly(vinyl alcohol) [5][6].

Poly(vinyl alcohol) is a nontoxic water soluble synthetic polymer, which is widely use in biochemical and biomedical applications [7]. PVA gels can be used in the medical field because they possess good biosuitability [8]. Hydrogels from solution blends of silk fibroin and PVA have been prepared by gamma irradiation under nitrogen [9]. However hydrogels were prepared for biomedical application, have to sterilize or prevent from many of microorganisms. These properties will be the most valuable hydrogel in the field of biomedical application. No reports have been found in the literature on an antibacterial activity of hydrogel preparing from irradiated silk protein. Therefore the aim of this research is to clarify an antibacterial activity of irradiated silk protein /PVA hydrogel.

2. Experimental

2.1 Materials

Raw silk waste fibers (STBII) was supplied from the SHINANO KENSHI (THAILAND) Co., LTD., Saraburi province, Thailand. The media to cultivate of bacteria were purchase from Difco Laboratories. Blank Paper disks (6 mm diameter) were purchase from Becton Dickinson and Company, USA (BBL^{TM}).

2.2 Preparation of silk protein solution

10 g of cleaned and crushed of silk waste fibers were dissolved in 90 ml $CaCl_2$ solution (73 g $CaCl_2$. $2H_2O$: 54ml H_2O : 47 g C_2H_5OH) at 90 °C for 30 min. The silk protein solution was dialyzed using seamless cellulose tube (molecular weight cut off 12,000-14,000 Da.) with de-ionized water at least for 3 days. Concentration of silk protein solution obtained after dialysis was 3w/v%. Dialysis process should be done just before mixing with PVA solution, to avoid silk protein crystallization.

2.3 Preparation of silk protein/PVA hydrogel

Two kinds of different concentrations of PVA, 3% and 10 w/v% were prepared by dissolving PVA with sterilize deionised water. Then 3% silk proteins were blended with 3% and 10% PVA (silk proteins : PVA, 1: 3). Subsequently the mixtures in sealed each glass tube, was filled with nitrogen gas and irradiation.

2.4 Irradiation

Irradiation was carried out at room temperature using Co-60 Gamma cell-220 irradiator with a dose rate of 100Gy/min. The doses of irradiation were conducted 10, 20, 30, 40 and 50 kGy.

2.5 Culture Medium

AATCC Broth contains of Peptone (Bacto-peptone) 10g, Beef extract 5g, sodium chloride 5g, and distilled water 1000ml. And AATCC agar, add 1.5% agar. Boil to dissolve ingredients. Adjust to pH 6.8-7.0, Dispense in 150ml amounts in Duran bottles and sterilized at 121 ° C (15 psi) , 15 min.

2.6 Test Bacteria

Pure cultures of bacteria were cultivated and obtained from Bamrajnaradul Hospital, Nonthaburi province, Thailand. Four kinds of organisms such *as Escherichia coli* (gram-negative), *Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis* (gram-positive) were carried out.

2.7 Hydrogels preparation to determine the antibacterial activity

The specimen of hydrogels in each glass tubes was removed by breaking tubes. Then hydrogels was cut into a circular piece by a sharp cutter using aseptic technique. Each pieces of hydrogel was placed on and pressed down lightly to a heavily seeded bacteria.

2.8 Antibacterial activity testing

The procedure was performed as the modified methods of Agar disk diffusion method [10] and AATCC Test method 90-1977 [11]. The procedures are as following :

1. Twenty-four-hour AATCC broth cultures of four test bacteria, were conducted.

2. Count a number of each test bacteria (direct count with counting chamber and measure turbidity of OD 660 nm) and dilute become to 1.0×10^6 cells/ml.
3. Melt 150 ml of AATCC Agar in a Duran bottle, cool at 45 °C, inoculate with 1.0×10^6 cells/ml of a tested bacterium.
4. Pour the test organisms with medium into a 100 mm diameter Petri-dish. Allow 25 minutes lapse of time before placed on the tested specimen
5. Gently press down the tested hydrogel (d) and an untreated control (PVA solution (a), non-irradiated silk/PVA mixture (b) and silk protein solution (c) using a blank paper disk) into intimate contact with the seeded agar Petri-dish, using sterile forceps. Set tests in duplicate.
6. Incubated the Petri-dish for 24-48 hours at 37 °C
7. Observed and measured clear zone around the hydrogel specimens through the bottom of the plate. Calculated the width of the clear zone as follows:

$$W = \frac{T - D}{2}$$

   Where:

   W : width of clear zone in millimeter (mm.)

   T : total diameter of test specimens and clear zone

   D : diameter of test specimens
8. A clear zone of no growth surrounding the specimen, indicates an antibacterial activity of hydrogel specimens.

3. Results and Discussion

3.1 To select a suitable concentration of silk protein and PVA for preparation hydrogels on antibacterial activity testing

The 3% silk protein blended with 3% PVA and 10% PVA, and followed with γ – irradiation, were carried out. The appearances of hydrogels which contained 3% silk protein /10% PVA at a ratio of 1:3 respectively, were brittle and easy to break. Furthermore the resulting hydrogel contained many bubbles which come from gases evolved during irradiation. Thus it is very difficult to cut into a circular shape for an antibacterial activity testing. And it was reported that hydrogel derived from 3% silk protein /3% PVA concentration , showed higher water absorption than 10% PVA (Fig. 1)[9]. Absorbs fluid is one required properties of hydrogel for wound dressing [12]. Based on these reasons, 3% PVA concentration was selected to prepare hydrogel on antibacterial activity testing.

3.2 To clarify an antibacterial activity of irradiated silk protein/ PVA hydrogels

Four kinds of bacteria, such as *E .coli, B. subtilis, S. aureus* and *S. epidermidis*, were examined. Clear zone of growth inhibition adjacent to hydrogel indicates antibacterial activity which was compared with silk protein/PVA without irradiation (b) as a control. An antibacterial activity of hydrogels at 20 (Fig.2) and 50 kGy (Fig. 3) against *E. coli*, were clearly observed, whereas low activity was observed in PVA (a), non-irradiated silk protein/PVA solution (b) and no activity on silk protein solution (c). Similar phenomenon of hydrogels (d) were also observed against *B. subtilis* at 20 kGy (Fig. 4) and at 50 kGy (Fig. 5) and *S. aureus* at 20 kGy (Fig. 6) and at 50 kGy (Fig. 7). In addition, no activity of untreated control (a, b, c) against *B. subtilis* and *S. aureus* were found. These results suggest that *E. coli* is highly sensitive to PVA and silk protein solution. On the other hand a clear zone of growth inhibition of hydrogels against *S. epidermidis* at 20 kGy (Fig. 8) and at 50 kGy (Fig. 9), was not clearly observed. This indicated that prepared hydrogel trend to lack of that activity against *S. epidermidis*.

3.3 To study the effect of irradiation doses on an antibacterial activity of hydrogel

The appearance of silk protein/PVA at 10 kGy, hasn't been a hydrogel, it is still liquid solution. From this experience, we used the samples which were irradiated at 20, 30, 40 and 50 kGy for an antibacterial activity testing. An antibacterial activity was determined by the presence of growth inhibition (clear) zones surrounding the various disks and hydrogel used. Width of the diameters of such zones can be measured with a fine metric ruler. An antibacterial activity of hydrogel against four bacteria at various doses was shown in Fig. 10. It can be seen that an antibacterial activity of hydrogel against *E. coli* (gram negative) was increased gradually with increasing doses of irradiation especially at 50 kGy, can be clearly observed. Similar trend of this activity of hydrogel against *B. subtilis* and *S. aureus* (gram positive) (Fig. 10) were found whereas that of activity against *S. epidermidis* was not clearly observed. It can be seen that the maximum of such activity of hydrogel was found at 50 kGy [Fig. 10]. It mentioned that irradiation degraded silk protein to produce lower molecular weight products [9][13]. Base on these reports, silk fiber changed to low molecular weight fragments by irradiation. Therefore it assumed that increased inhibitory effect of hydrogel on each bacteria was higher than that of non-irradiated one. This similar behavior was found in irradiated chitosan has an antibacterial activity than that of non-irradiated chitosan [14]. Similar effect of molecular weight dependent antimicrobial activity by chitosan, was found [15][16]. The results can be explained on the higher irradiation doses degraded higher silk proteins. No significant different in the width of clear zone of growth inhibition for three kinds of bacteria (*E. coli, B. subtilis* and *S. aureus*) were observed. On the contrary, it is a difference of *S. epidermidis*. It was supposed that an antibacterial susceptibility of hydrogel depended upon species of microorganisms. However the hydrogel seem to be the highest antibacterial susceptibility to *E. coli.* As the previous investigators mentioned that the size of growth inhibition zones can be affected by several factors, including (1) the culture medium used; (2) incubation conditions; (3) the rate of diffusion of the solution or antibiotic; (4) the concentrations of the antibiotics used; and the antibiotic sensitivity of the organism being tested[10][17]. These conditions are very important role to determine an antibacterial activity. However, irradiation doses are trend to indicate the effective to hydrogel on an antibacterial activity.

Acknowledgment

This research was supported by the bilateral cooperative project between Office of Atomic Energy for Peace of Thailand (OAEP) and Japan Atomic Energy Research Institute (JAERI) (Takasaki). The author would like to thank Mr. Arak from OAEP for their kind assistance of irradiation. And I would like to thank Bamrajnaradul Hospital for cultivation four pure cultures of bacteria to test in this research.

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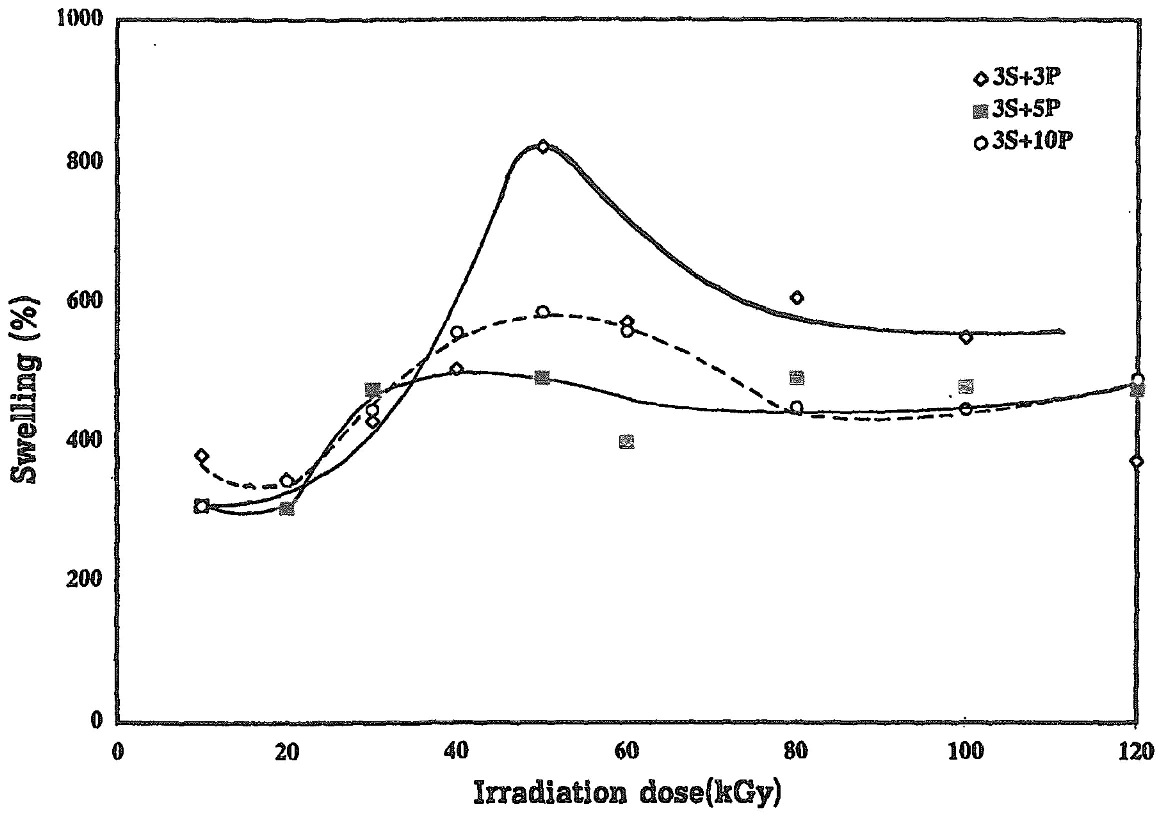

Fig. 1 Change in the swelling of silk fibroin/PVA hydrogels as a function of irradiation dose

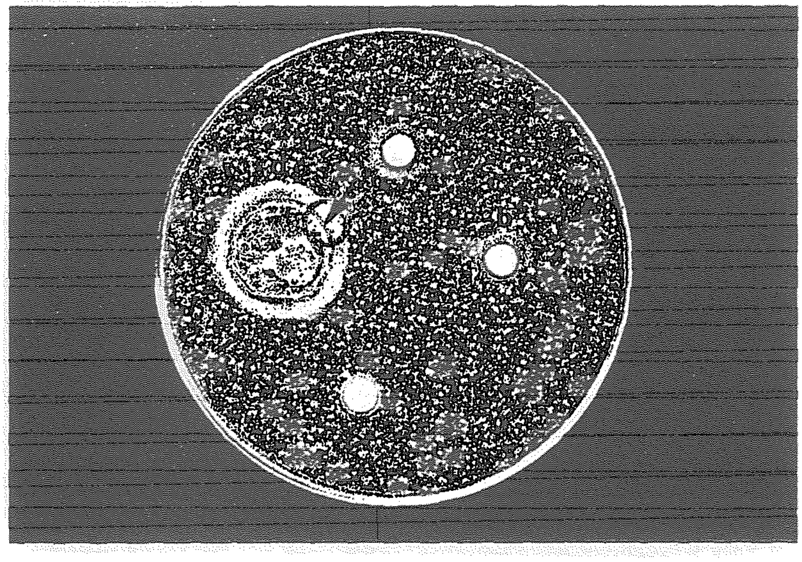

Fig. 2 Clear zone of growth inhibition of *E.coli* at 20 kGy

a : PVA
b : silk protein / PVA
c : silk protein solution after dialysis
d : hydrogel (irradiated silk protein / PVA)

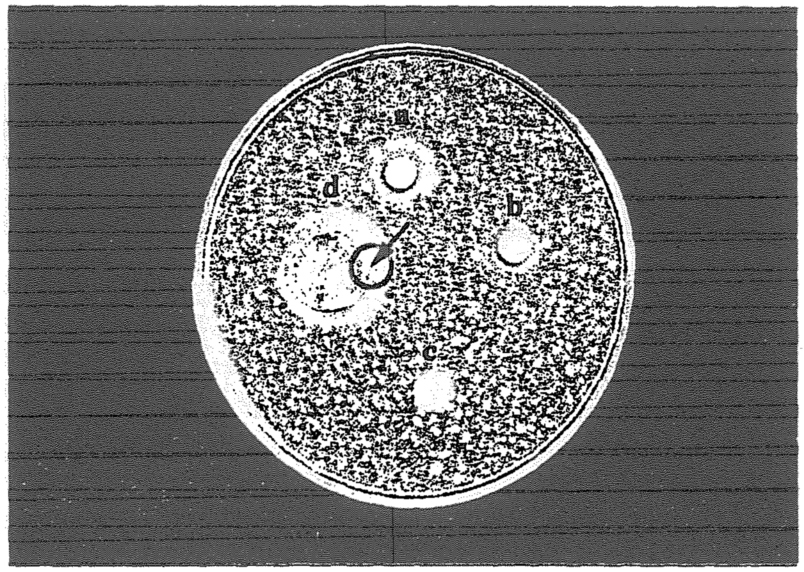

Fig. 3 Clear zone of growth inhibition of *E.coli* at 50 kGy

a : PVA
b : silk protein / PVA
c : silk protein solution after dialysis
d : hydrogel (irradiated silk protein / PVA)

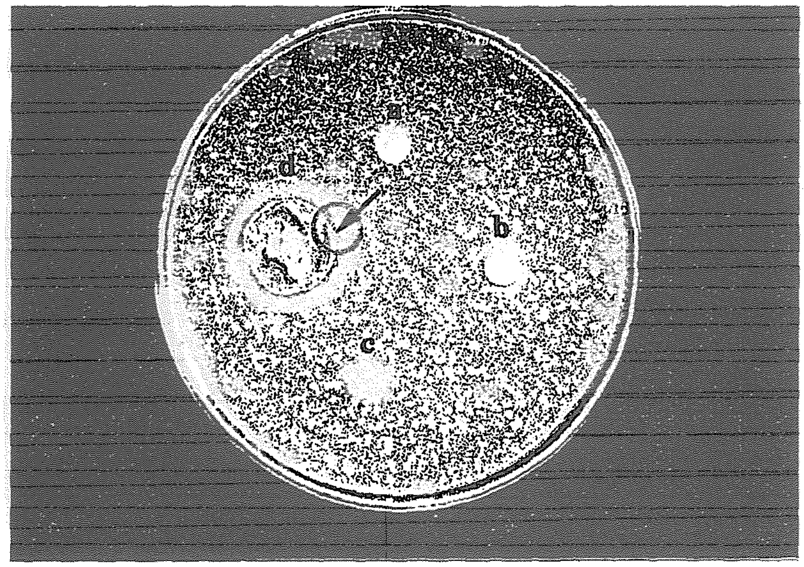

Fig. 4 Clear zone of growth inhibition of *Bacillus subtilis* at 20 kGy

a : PVA

b : silk protein / PVA

c : silk protein solution after dialysis

d : hydrogel (irradiated silk protein / PVA)

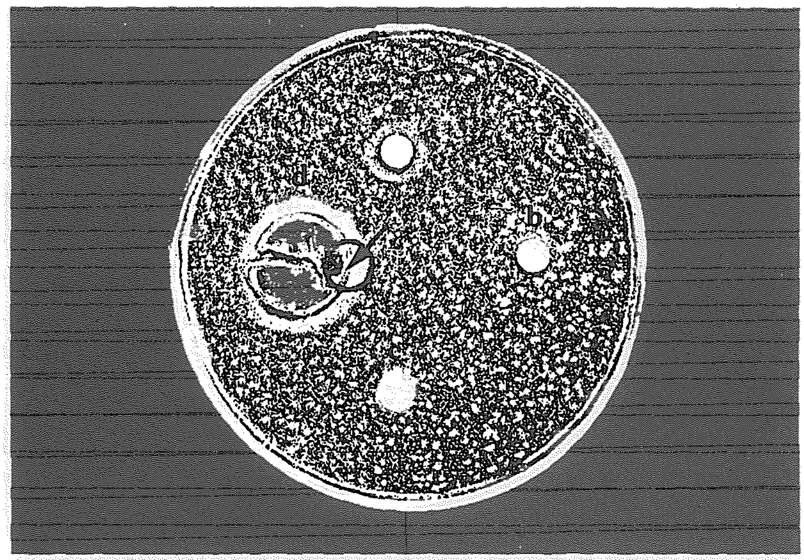

Fig. 5 Clear zone of growth inhibition of *Bacillus subtilis* at 50 kGy

a : PVA

b : silk protein / PVA

c : silk protein solution after dialysis

d : hydrogel (irradiated silk protein / PVA)

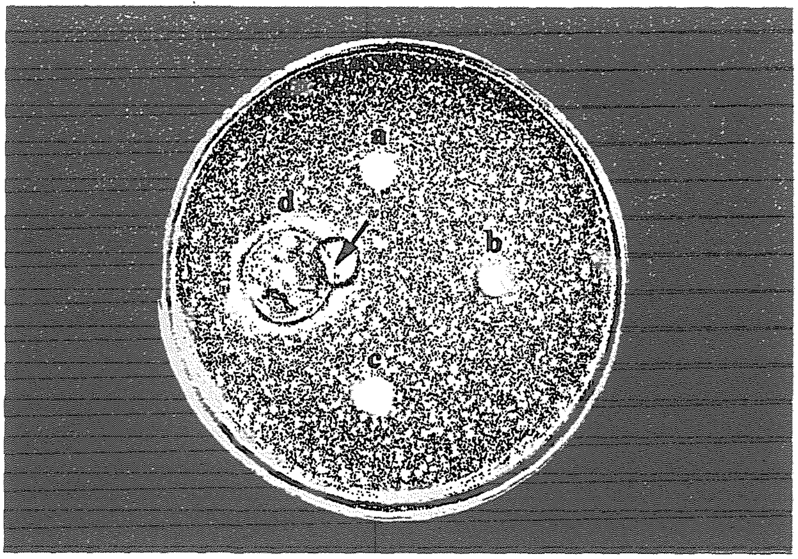

Fig. 6 Clear zone of growth inhibition of *S. aureus* at 20 kGy

a : PVA
b : silk protein / PVA
c : silk protein solution after dialysis
d : hydrogel (irradiated silk protein / PVA)

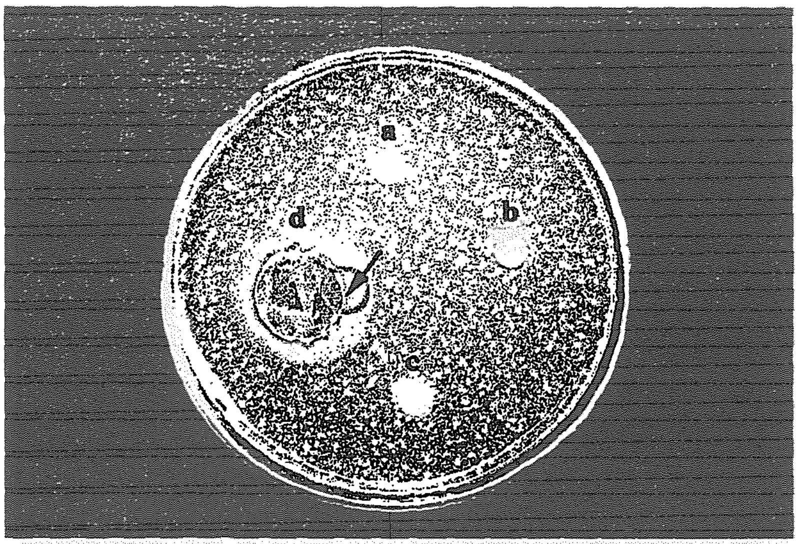

Fig. 7 Clear zone of growth inhibition of *S. aureus* at 50 kGy

a : PVA
b : silk protein / PVA
c : silk protein solution after dialysis
d : hydrogel (irradiated silk protein / PVA)

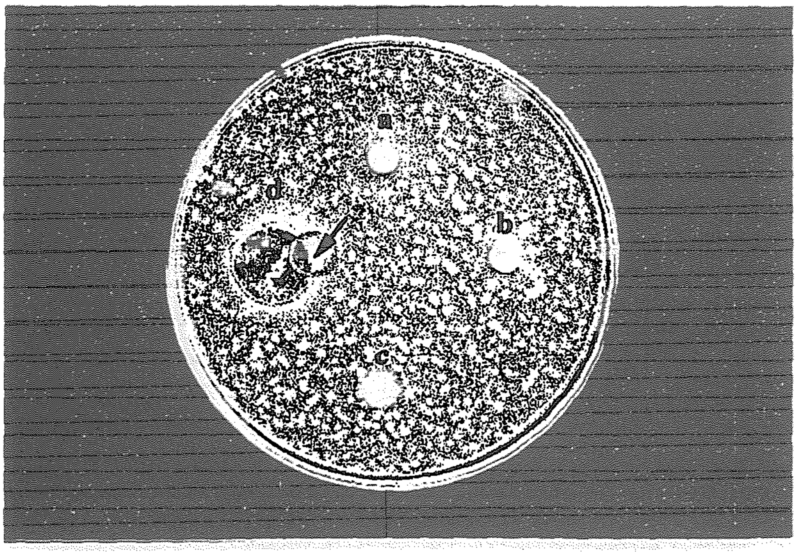

Fig. 8 Clear zone of growth inhibition of *S.epidermidis* at 20 kGy

a : PVA
b : silk protein / PVA
c : silk protein solution after dialysis
d : hydrogel (irradiated silk protein / PVA)

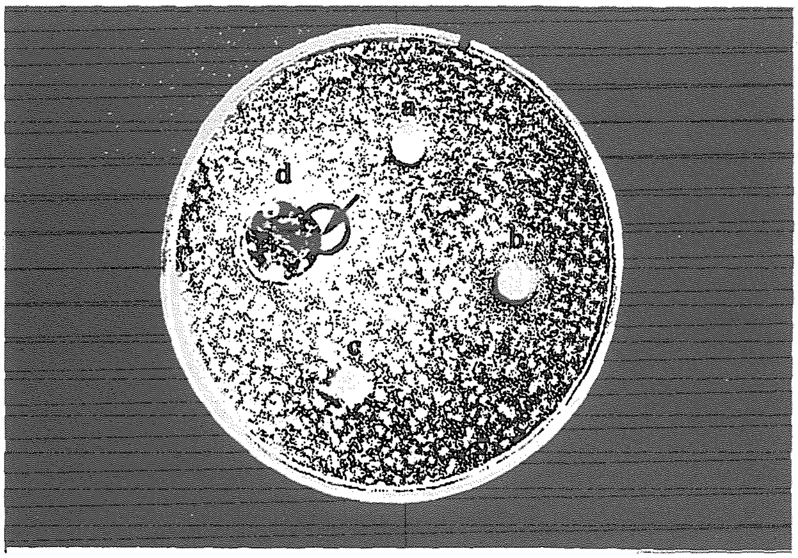

Fig. 9 Clear zone of growth inhibition of *S.epidermidis* at 50 kGy

a : PVA
b : silk protein / PVA
c : silk protein solution after dialysis
d : hydrogel (irradiated silk protein / PVA)

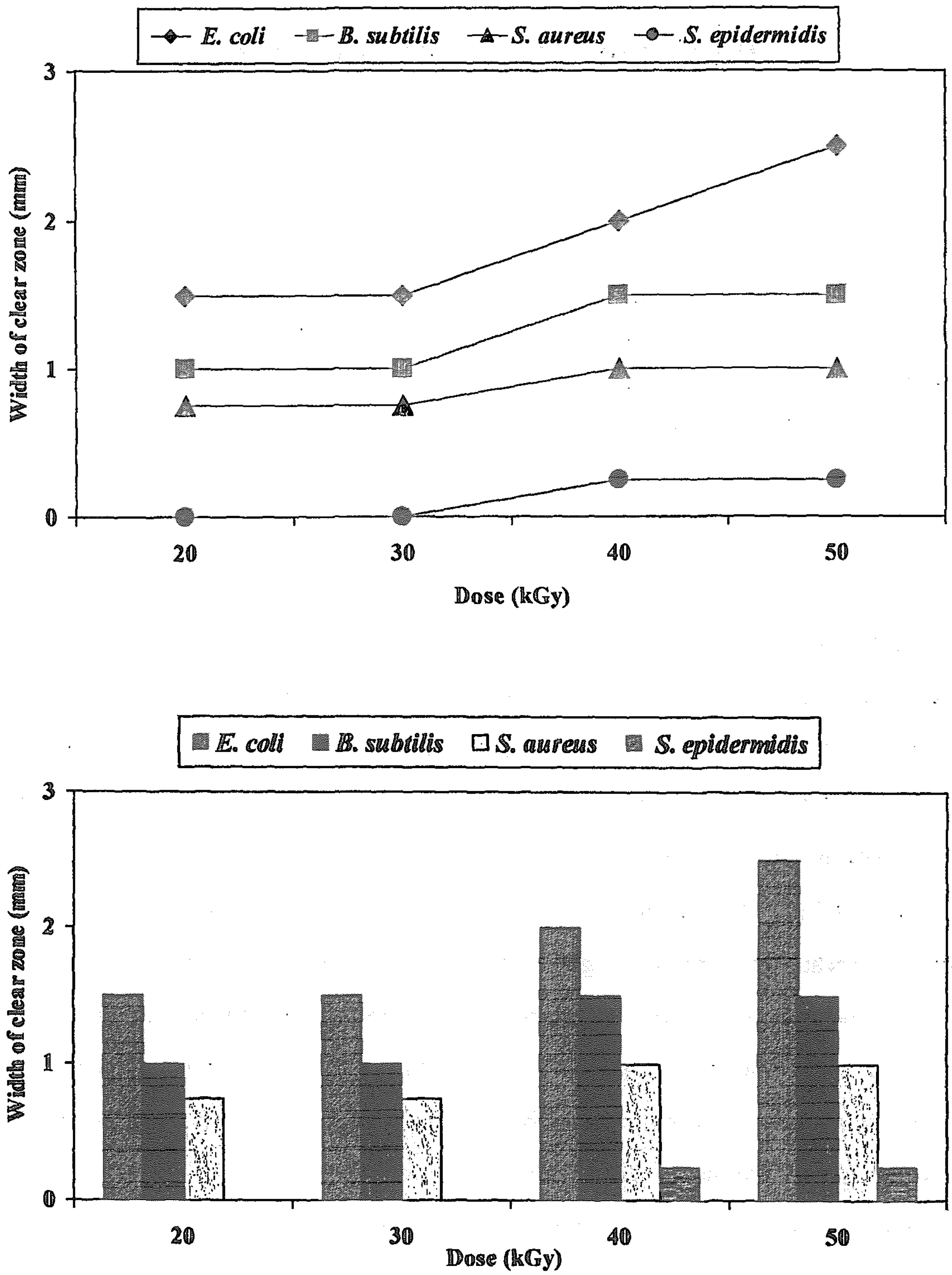

Fig. 10 Width of clear zone of growth inhibition of four bacteria by hydrogel at various doses

18 Radiation Degradation of Silk

Kazushige ISHIDA[1], Hidefumi TAKESHITA[2], Youichi KAMIISHI[1], Fumio YOSHII[2] and Tamikazu KUME[2]

1. Textile Research Institute of Gunma, 5-46-1 Aioi-cho, Kiryu, Gunma 376-0011, Japan
2. Takasaki Radiation Chemistry Research Establishment, Japan Atomic Research Institute, 1233 Watanuki-machi, Takasaki, Gunma 370-1292 Japan

Abstract

Silk fibroin powder was prepared from irradiated silk fibroin fiber by means of only physical treatment. Silk fibroin fiber irradiated with an accelerated electron beam in the dose range of 250 - 1000 kGy was pulverized by using a ball mill. Unirradiated silk fibroin fiber was not pulverized at all. But the more irradiation was increased, the more the conversion efficiency from fiber to powder was increased. The conversion efficiency of silk fibroin fiber irradiated 1000 kGy in oxygen was 94%. Silk fibroin powder shows remarkable solubility, which dissolved 57% into water of ambient temperature. It is a very interesting phenomenon that silk fibroin which did not treat with chemicals gets solubility only being pulverized. In order to study mechanism of solubilization of silk fibroin powder, amino acid component of soluble part of silk fibroin powder was analyzed. The more irradiation dose up, the more glycine or alanine degraded, but degradation fraction reached bounds about 50%. Other amino acids were degraded only 20% even at the maximum. To consider crystal construction 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 silk fibroin powder.

Key words : Silk fibroin, powder, Radiation degradation, Dissolution, Amino acid analysis

1. Introduction

Silk fibroin has been used as textile material for thousands years because it has excellent natures, for example lightness and warmth on wearing, beautiful gloss, and so on. Recently silk fibroin is also considered to be natural protein with interesting characters and application to new fields, particularly medical or cosmetic materials, are extensively studied, because silk fibroin has affinity to human skin proved by the long history of using as textile material. On practical application, it is necessary to change form of silk fiber in many cases. For example, film skin and block are studied for artificial skin and for 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], which is the new method recently spread. But these are wet methods using water, so it is necessary to many processes and the drainage disposal is an indispensable problem.

We studied the radiation method, as dry method, in order to pulverize silk fibroin. Now, we report the influence of irradiation on pulverizing of silk fibroin fiber, marvelous solubility of silk fibroin powder and the mechanism of solubilization of silk fibroin powder.

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 by enzyme (Alkalase2.5L, NovoNordisc, Denmark). About 2 g of silk fibroin fiber for irradiation was packed into a plastic bag with O_2 or N_2 gas.

2.2 Irradiation

Irradiation was carried out at room temperature with 1 MeV electron beam. Sample to be irradiated 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 1000 kGy.

2.3 Pulverization

Irradiated silk fibroin fiber was cut into about 1 cm in length. About 0.8 g of fiber was put into a ball mill pot (Frisch Pulverisette type 6, Frisch Japan), and was pulverized for 1 h. 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 with weight of residue R and weight of silk fibroin fiber put into pot A.

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

2.4 Solubility measurement

Soluble component 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 the precipitation was weighed, which is the weight of soluble component.

2.5 Amino acid analysis

Soluble part of silk fibroin powder in each dose was extracted from 10 mg of powder with 1x2 ml of distilled water. After elimination of water, extract was treated with 6 mol/l of HCl at 110 ℃ for 24 has usual preparation. This was analyzed by liquid chromatography.

3. Results and Discussion

3.1 Influence of irradiation on pulverization of silk fibroin fiber

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 1000 kGy in oxygen atmosphere reached to 94%. The conversion fraction of silk fibroin irradiated in the oxygen atmosphere is larger than that in the nitrogen atmosphere on all doses. This tendency is more obvious in low irradiation dose than in high one.

Fig.2 shows the electron microscope photograph of silk fibroin powder obtained from fiber irradiated 1000 kGy in the oxygen atmosphere. It shows fine particles under 10 μm in diameter and its aggregates. And 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.

3.2 Solubility of silk fibroin powder

Fig.3 shows the solubility of silk fibroin fiber and powder into water. Properly unirradiated silk fibroin fiber is not soluble in water. But irradiated silk fibroin fiber obtained solubility slightly, soluble part of silk fibroin fiber was 8% at 1000 kGy. Pulverization made silk fibroin more soluble, 57% in weight of powder at 1000 kGy dissolved into water. It is a very interesting phenomenon that silk fibroin which was not treated with chemicals but pulverized gets solubility.

Fig.4 shows the influence of water temperature on solubility of silk fibroin powder. Silk fibroin powder dissolved in water at room temperature as well as at 121 degrees. In other words, the soluble part of silk fibroin powder dissolves in water at room temperature rapidly and the insoluble part of silk fibroin powder does not dissolve in water even at 121 degrees. This fact suggests that the soluble part and the insoluble part of silk fibroin powder have entirely different nature respectively.

3.3 The mechanism of solubilization of silk fibroin powder

Amino acid component of soluble part of silk fibroin powder was analyzed. Fig.5 shows the degraded fraction D of each amino acid in soluble part of silk fibroin fiber. The degraded fractions were calculated on results of amino acid analysis and known amino acid contents of silk fibroin in literature [4]. The more irradiation dose up, the more glycine degraded, but the degraded fraction reached to bounds, about 55%. Similar to glycine, the more irradiation dose up, the more the degraded fraction of alanine reached to bounds, about 50%. Other amino acids were degraded only 20% even at the maximum.

By the way, silk fibroin fiber consists of crystal region and amorphous region, and amino acid composition closely relates with this crystal construction. 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 as in crystal region. And many kinds of amino acids are contained in amorphous region different from in crystal region. The ratio of glycine and alanine in amorphous region is 55% and 56% respectively (Table 1). These ratios are very close to the those of degraded fractions of glycine and alanine. And it is known that degradation occurs on the amorphous region in many cases. Synthetic considering, these things suggests following things. First, irradiation of silk fibroin selectively degrades glycine and alanine in amorphous region. Second, degradation of glycine and alanine weakens mechanical strength of silk fibroin and makes it possible to pulverize silk fibroin fiber. Third, the degradation by irradiation and pulverization by physical method make the change of silk fibroin fiber to small fragments which are soluble in water.

4. Conclusion

(1) Irradiation electron beam makes it possible to pulverize silk fibroin fiber without chemicals.

(2) Silk fibroin powder by irradiation method shows marvelous solubility into water.

(3) Irradiation of silk fibroin degrades glycine and alanine in amorphous region selectively. And it is suggested that degradation of glycine and alanine in amorphous region allow of pulverization only physical method and that irradiation and pulverization make the change of silk fibroin fiber to small fragments which is soluble water.

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Fig.1 Pulverization of irradiated silk fibroin fiber

Fig.2 Photograph of silk fibroin powder taken with electron microscope (1000 kGy, Oxygen)

Fig.3 Solubility of silk fibroin fiber or powder

Fig.4 Comparison of solubility between for room temperature water and for 121 ℃ water

Fig.5 Degradation of amino acids by irradiation. ○ : glycine, ● : alanine, △ : serine, ▲ : tyrosine, □ : valine, ■ : asparagine, ▽ : threonine.

Table 1 Degradation of amino acids and the crystal structure

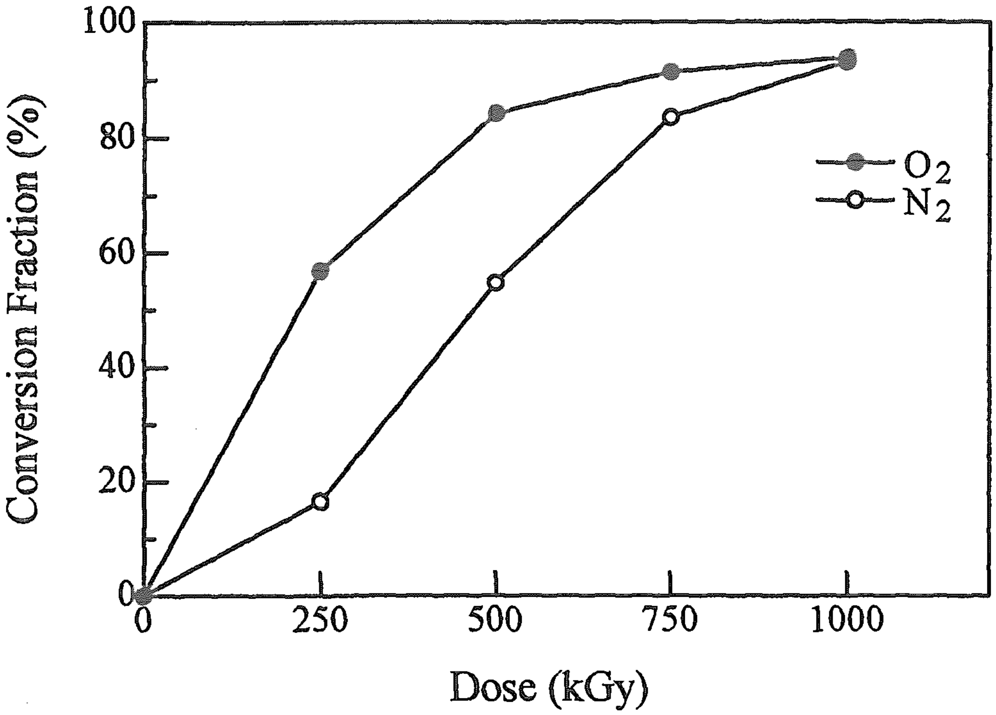

Fig. 1 Pulverization of irradiated silk fibroin fiber

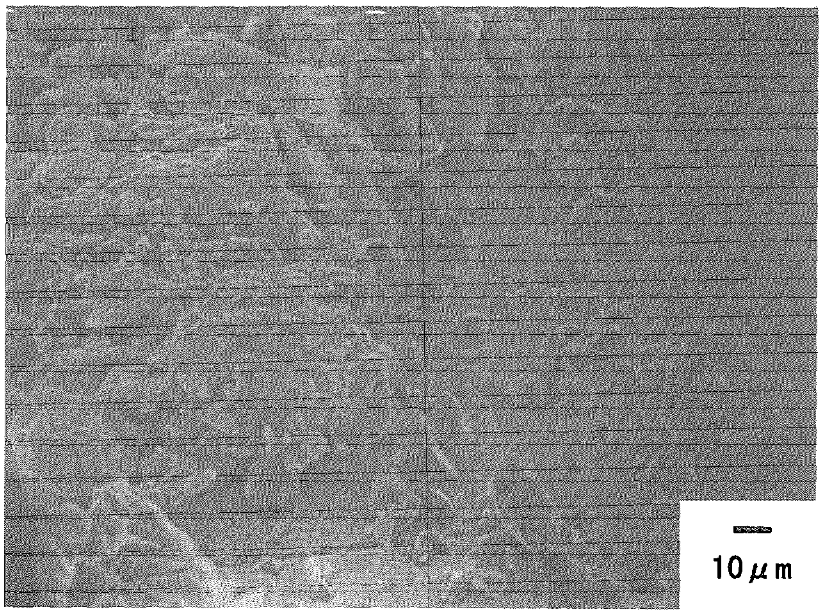

Fig.2 Photograph of silk fibroin powder taken with electron microscope (1000 kGy, Oxygen)

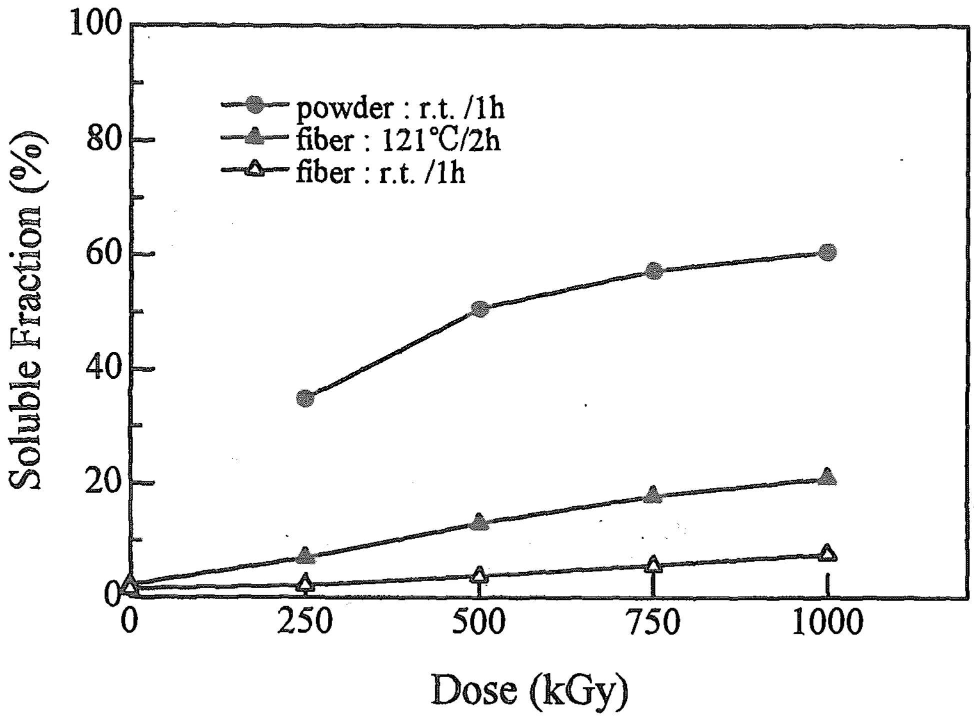

Fig.3 Solubility of silk fibroin fiber or powder

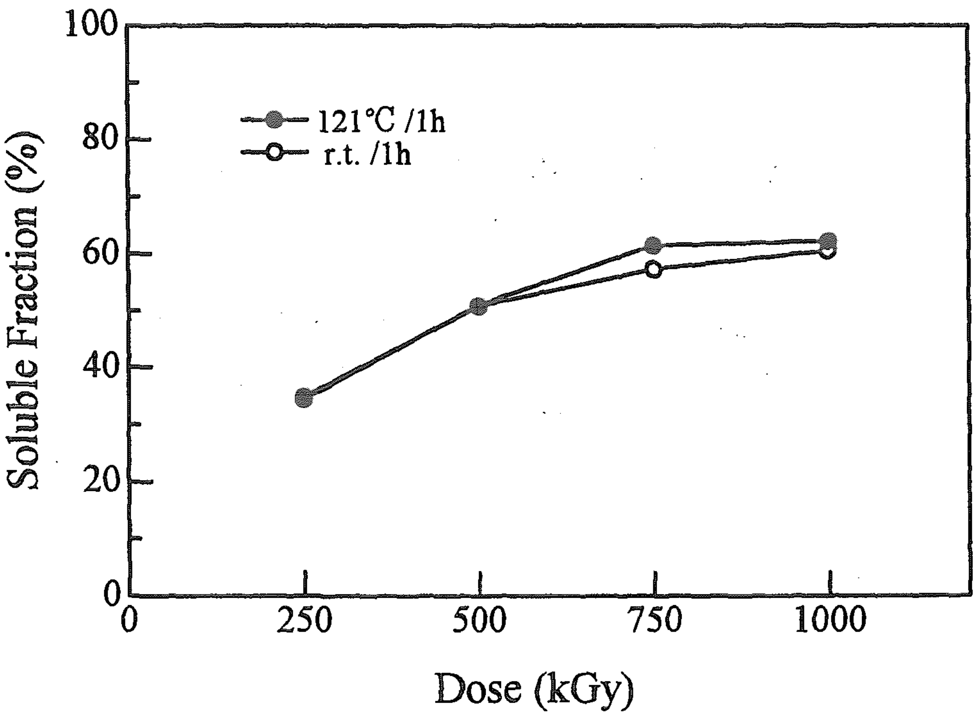

Fig.4 Comparison of solubility between for room temperture water and for 121℃ water

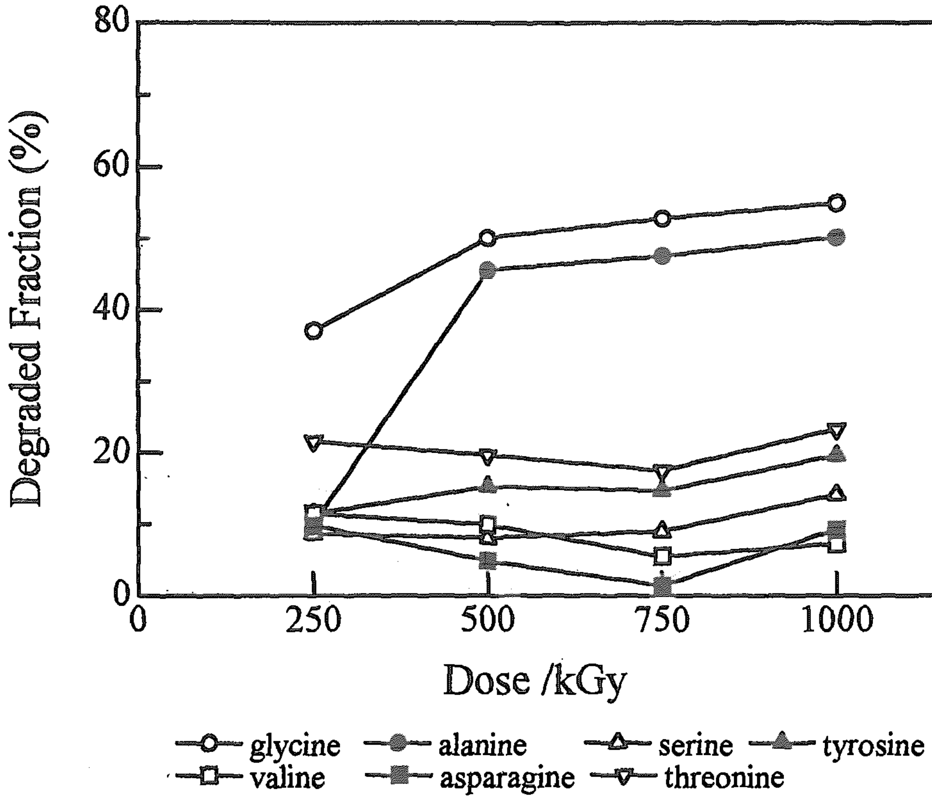

Fig.5 Degradation of amino acids by irradiation

Table 1 Degradation of amino acids and the crystal structure

	Amino acid content [4]	Crystal region (40%)	Amorphous region (60%)	Degraded fraction (1000 kGy)
Glycine	44.5 (100)	20.0 (45)	24.5 (55)	55
Alanine	30.2 (100)	13.3 (44)	16.9 (56)	50

19 Preparation and Water Absorption of Cross-Linked Chitosan/Silk Fibroin Blend Films

Jantip SUESAT*, Ratana RUJIRAVANIT*, Alexander M. JAMIESON**, Seiichi TOKURA***

*The Petroleum and Petrochemical College, Chulalongkorn University, Bangkok, Thailand

**Department of Macromolecular Science, Case Western Reserve University, Cleveland, U.S.A.

***Faculty of Engineering, Kansai University, Osaka, Japan

Abstract

Natural polymer blend films composed of chitosan and silk fibroin were prepared by varying the ratio of chitosan to silk fibroin, with and without glutaraldehyde as a crosslinking agent. The effects of the ratio of chitosan to silk fibroin and crosslinking agent on swelling behavior of the blend films were studied. For the swelling behavior, the blend films exhibited a dramatic change in the degree of swelling when immersed in acidic solutions. The degree of swelling of the films increased as the chitosan content increased; the blend film with 80 % chitosan content had the maximum degree of swelling. It appeared that crosslinking had occurred in the blend films which helped the films to retain their three dimensional structure. In addition, FTIR spectra of the films showed evidence of hydrogen bonding interaction between chitosan and silk fibroin. For the effect of salt type, the films were immersed in various types of aqueous salt solutions, viz NaCl, LiCl, $CaCl_2$, $AlCl_3$, and $FeCl_3$. The films immersed in $AlCl_3$ and $FeCl_3$ aqueous solutions gave the maximum degree of swelling. The effects of $AlCl_3$ and $FeCl_3$ concentrations on swelling behavior were also investigated. It was found that the maximum degree of swelling of the films occurred at 1.0×10^{-2} M of $AlCl_3$ and $FeCl_3$ aqueous solutions.

Keywords: Chitosan, Silk fibroin, Blend film, Swelling property, Degree of swelling, Crosslinking agent

1. Introduction

Increasingly, natural polymers are becoming more important because they are renewable resources and have low cost. Due to their interesting properties, including nontoxicity, biocompatibility, and biodegradability, natural polymers have been investigated with a view to expanding their utilization. Chitosan is an aminopolysaccharide derived from chitin via deacetylation by alkali hydrolysis. It is a copolymer consisting of β-(1-4)-2-acetamido-D-glucose unit and β-(1-4)-2-amino-D-glucose unit with the latter usually greater than 75% [1]. Chitosan can be blended with synthetic polymers to yield products whose physical and chemical properties have some potential applications. In addition, chitosan can be blended with biomaterials, like cellulose [2], silk fibroin [3], pectin [4], collagen [5], etc. Chen *et al.* [3] investigated a conformational transition of silk fibroin induced by blending with chitosan. The rigid chain of chitosan can induce transformation of the random coil conformation of silk fibroin to the β-sheet conformation because of the occurrence of hydrogen bonding between chitosan and silk fibroin.These two biopolymers can also form a hydrogel having a semi-interpenetrating polymer network morphology that is sensitive to pH and ion concentration changes. Chen, *et al.* [6] studied the swelling behavior of chitosan/silk fibroin blend film in pH buffer solutions and reported that swelling-shrinking behavior of chitosan/silk fibroin blend films exhibited a fine reversibility.

In this study, chitosan/silk fibroin blend films were prepared with varying chitosan content. The effect of blend composition on the swelling behavior of films were studied. The swelling behavior of the blend films was determined in pH buffer solutions and various types of salt solutions. Furthermore, the effect of varying concentration of various salt types on the swelling property of the blend films was also determined.

2. Experiment

Chitin was prepared by the method of Shimahara *et al.* [7]. The decalcification of shrimp shell was performed by immersing in 1 N HCl solution for 2 days. Protein removal of the decalcified product was performed in 4 % w/w of NaOH solution by boiling further at 80-90 °C for 4 h. The deproteinized portion was washed with deionized water until neutral. For chitosan preparation, chitin flakes were deacetylated by heating in 50 % by weight of NaOH solution containing 0.5 % by weight of $NaBH_4$ added based on the weight of chitin to prevent depolymerization. The deacetylation was performed in an autoclave at 110 °C for 1 h. The degree of deacetylation of chitosan was 80% based on the infrared spectroscopic method of Sannan *et al.* The viscosity-average molecular weight of chitosan was 7.12×10^5 acccording to the method of Lee [8]. Chitosan solution was prepared by dissolution of chitosan in 1% by weight acetic acid solution. The chitosan solution was allowed to stand overnight at 4 °C in a refrigerator to get rid of air bubbles before use.

To obtain silk fibroin solution, silk fibroin 6 g was dissolved in 94 g of 2:2:8 by mole of $CaCl_2$: EtOH: H_2O solvent system at 100 °C for 15 minutes [9]. The resulting silk fibroin solution was filtered through the sintered glass filter and subsequently dialyzed against distilled water for 7 days. The dialyzed silk fibroin solution was filtered and diluted to achieve a concentration of 1% w/w.

The blend films of chitosan and silk fibroin were prepared by mixing various ratios of 1% by weight of silk fibroin solution and 1% by weight of chitosan solution. The blend solution was stirred slowly for 12 h and left overnight to get rid of air bubbles before casting onto the clean dry petri dishes in a dust-free atmosphere at room temperature. When the films were dried completely, they were soaked in 0.5% by weight of NaOH in MeOH for 24 h to neutralize the acid and then washed exhaustedly with MeOH. The blend films were dried at ambient temperature and stored over silica in a desiccator before use.

For the swelling behavior determination, the samples were cut into the disk form with diameter of 16 mm and 25-30 μm in thickness. The samples were immersed in pH buffer solutions at various pH values and in various types of salt solutions. The degree of swelling was calculated from the following equation:

$$Degree\ of\ swelling\ (\%) = \frac{W - Wo}{Wo}$$

where W_0 is the weight of the films at dry state and W is the weight of swollen films.

3. Results and discussion

Swelling study

Effect of pH

The effect of pH on the degree of swelling of pure and the blend films with crosslinking is shown in Figure 1.

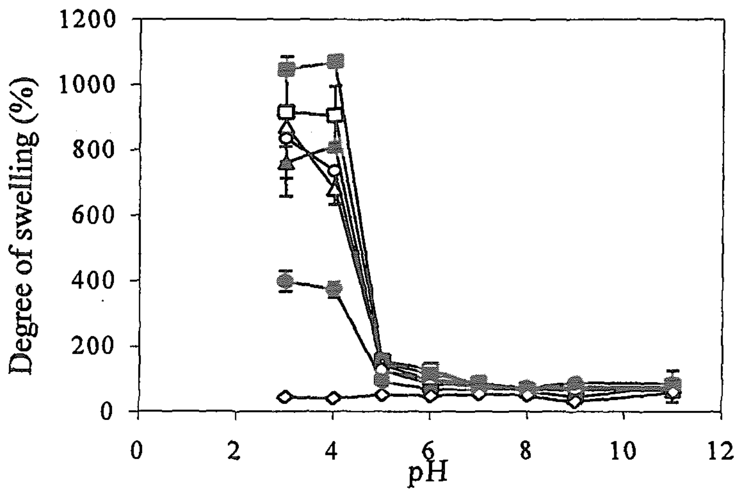

Figure 1 Degree of swelling of pure and blend films of chitosan and silk fibroin with crosslinking in pH buffer solution. The amount of added glutaraldehyde was 0.01 mole/glucosanime unit of chitosan. The ratio of chitosan to silk fibroin is as follow:
□ 100/0; ■ 80/20; ▲ 60/40; △ 50/50; ○ 40/60; ● 20/80; ◇ silk fibroin

When pHs were less than 5, the degree of swelling of the crosslinked blend films increased with increasing chitosan content. The maximum degree of swelling was observed for the blend film with 80 % chitosan content. This may be due to the dissociation of hydrogen bond between chitosan and silk fibroin chains caused by the protonation of amino groups of chitosan. Unlike that of the blend films without crosslinking, the crosslinked blend films with higher than 50% chitosan content can maintain their structures in acidic pH range. However, the degree of swelling of the crosslinked blend films was lower than the blend films without crosslinking. Wang *et al.* [10] reported that the crosslinked chitosan network can prevent the semi-interpenetrating polymer network of chitosan and poly(acrylic acid) from collapse at low pH but crosslinking could limit swelling of the films. It can be explained that the crosslinking makes chitosan chains covalently link together and form a network that can prevent the dissolution of the films even though it restricts the swelling of the films. In contrast, the degree of swelling of the blend films did not exhibit any difference when the pH is higher than 5. This can be explained by the fact that, at alkaline pH range, the number of protonated amino groups of chitosan is very low. The pKa of chitosan is about 6.3-6.5 [11], which indicates that chitosan tends to be protonated in acidic solution. Thus the degree of swelling of the blend films in alkaline solution is very low as compared to that of the blend films in acidic solution.

From the above, it is evident that the blend films of chitosan and silk fibroin are pH-responsive.

Effect of salt type

The degree of swelling of pure and the blend films with crosslinking in various types of salt solutions is shown in Figure 2.

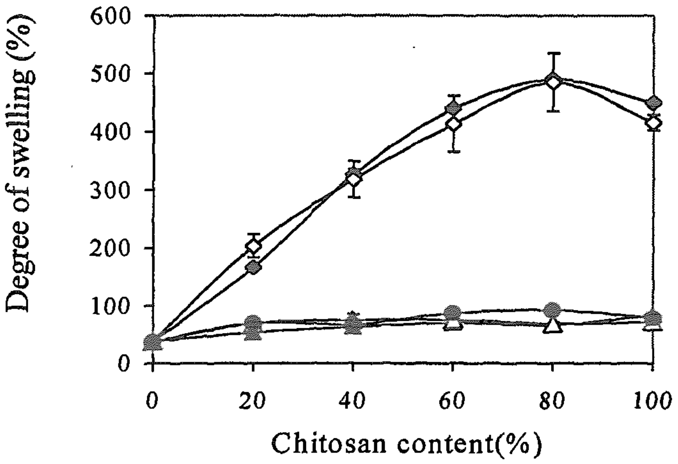

Figure 2 Degree of swelling of pure and blend films of chitosan and silk fibroin with crosslinking in various types of salt solutions. The amount of added glutaraldehyde was 0.01 mole/glucosamine unit of chitosan.
◆ 0.25 M $FeCl_3$; ◇ 0.25 M $AlCl_3$
▲ 0.25 M $CaCl_2$ solution; △ 0.25 M NaCl solution; ● 0.25 M LiCl solution

As chitosan content increases, the degree of swelling of pure and the blend films with crosslinking increases significantly in the case of immersing the films in $AlCl_3$ and $FeCl_3$ solutions. This is believed to be due to the formation of a coordinate covalent bond between nitrogen at amino group of chitosan and Al^{3+} or Fe^{3+}. In solution, Al^{3+} is surrounded by six molecules of water while Cl^- acts as a counter ion [12]. This Al^{3+} hydrated still has empty orbital that is electron deficient. The nitrogen atom of the amino group of chitosan has a lone pair electron that can coordinate to Al^{3+}. So Al^{3+}can form a coordinate covalent bond with the amino group of chitosan resulting in greater degree of swelling at higher chitosan content. The transition metal ion, like Fe^{3+}, can also form a coordinate covalent bond with the amino group of chitosan. The covalent binding of Al^{3+} or Fe^{3+} has the effect of increasing the net positive charge on the chitosan chains. Therefore, increase of chitosan content can enhance the degree of swelling of the blend films in both $AlCl_3$ and $FeCl_3$ solutions. At chitosan content greater than 50%, the degree of swelling can not be reported because overswelling led to the disintegration of the films. The degree of swelling of the crosslinked blend films with greater than 50 % chitosan content could be observed and the highest degree of swelling was observed at 80 % chitosan content. This indicated that the presence of crosslinks can prevent the disintegration of the films by the formation of network between the chitosan chains. However, the crosslinks limited the swelling ability of the films. The degree of swelling of the crosslinked blend films was lower than that of the non-crosslinking blend films at the corresponding blend compositions. The degree of swelling of the crosslinked blend films was very low in 0.25 NaCl, 0.25 M LiCl, and 0.25 M $CaCl_2$ solutions for the whole range of chitosan content. The reason is that the amino group of chitosan could not bind or bind weakly to alkali and alkali earth metal ions such as Na^+, Li^+, and Ca^{2+} resulting in a lower degree of swelling [10].

Effect of $AlCl_3$ and $FeCl_3$ concentrations

From the previous results, the chitosan and the blend films with crosslinking showed strong swelling in $AlCl_3$ and $FeCl_3$ solutions. Therefore, we further investigated the effect of $AlCl_3$ and $FeCl_3$ concentrations on the swelling behavior of pure and the blend films with crosslinking.

The effect of $AlCl_3$ concentration on the degree of swelling of pure and the blend films with crosslinking is shown in Figure 3.

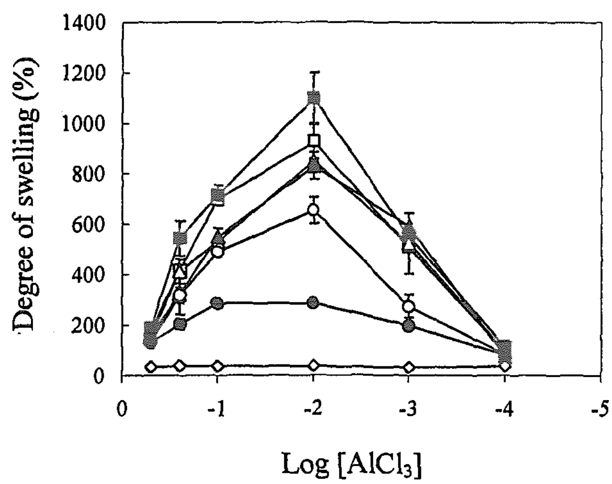

Figure 3 Degree of swelling of pure and blend films with crosslinking immersed in various concentrations of $AlCl_3$ salt solutions. The amount of added glutaraldehyde was 0.01 mole/glucosamine unit of chitosan. The ratio of chitosan to silk fibroin is as follow: □ 100/0; ■ 80/20; △ 60/40; ▲ 50/50; ○ 40/60; ● 20/80; ◇ silk fibroin.

The $AlCl_3$ concentration did not affect the degree of swelling of pure silk fibroin film at all salt concentrations because of the very low interaction between Al^{3+} and silk fibroin. However the chitosan and the blend films showed a strong variation in the degree of swelling related to the changes in salt concentration. The degree of swelling increased significantly as $AlCl_3$ concentration increased. When $AlCl_3$ concentration was $1.0x10^{-2}$ M, the degree of swelling of pure chitosan and the blend film showed a maximum value. The maximum degree of swelling belonged to the blend film with 80 % chitosan content. The occurrence of the highest degree of swelling at $AlCl_3$ concentration of $1.0x10^{-2}$ M can be explained by the Donnan equilibrium effect [13]. The Donnan equilibrium effect arises from the ionic osmotic pressure generated from the mobile counterions, which accompany the bound ions on the network strands [14]. The high counterion concentration causes a large swelling pressure in the absence of excess mobile salt. At the $AlCl_3$ concentration of $1.0x10^{-2}$ M, the difference of ion concentration between the interior and exterior of the films is maximal, leading to a large imbalance in osmotic pressure, which causes the maximum degree of swelling of the films. The decrease in the degree of swelling when the $AlCl_3$ concentration exceeds $1.0x10^{-2}$ M occurs because of there is now an excess mobile ion concentration in the external solution. The concentration of amino groups of chitosan in the film available to bind Al^{3+} is depleted, no additional Al^{3+} bound to the network strands, the excess mobile ions can penetrate the film and screen the bound charges, and the degree of swelling decreases.

The degree of swelling of pure and the blend films with crosslinking in various concentrations of $FeCl_3$ solution is shown in Figure 4.

The effect of $FeCl_3$ concentration on the degree of swelling of pure and the blend films with crosslinking is similar to that of the films immersed in $AlCl_3$ solutions. The $FeCl_3$ concentration that causes the maximum degree of swelling is $1.0x10^{-2}$ M. The blend film with 80% chitosan content has the highest degree of swelling. The difference in the degree of swelling of the films immersed in different concentrations of $FeCl_3$ solution is also caused by the Donnan equilibrium effect, as mentioned in the case of $AlCl_3$ solution. The degree of swelling of pure silk fibroin films is not affected by the $FeCl_3$ concentrations.

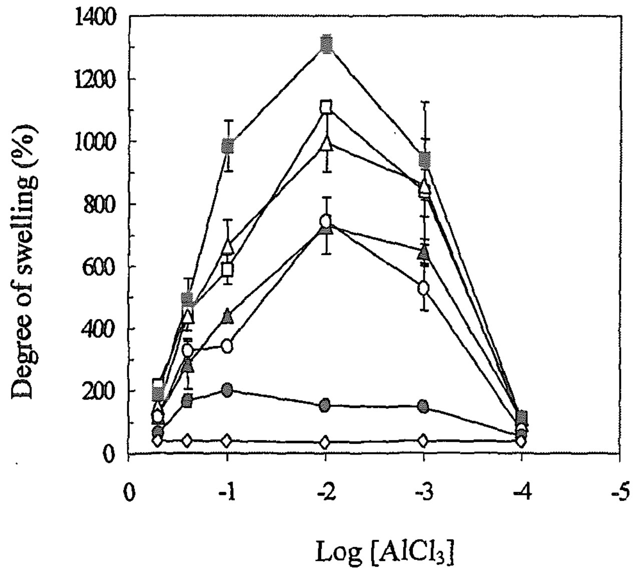

Figure 4 Degree of swelling of pure and blend films with crosslinking immersed in various concentrations of $FeCl_3$ salt solutions. The amount of added glutaraldehyde was 0.01 mole/glucosamine unit of chitosan. The ratio of chitosan to silk fibroin is as follow: □ 100/0; ■ 80/20; △ 60/40; ▲ 50/50; ○ 40/60; ● 20/80; ◇ silk fibroin.

Conclusion

The composition of chitosan/silk fibroin blend films had a large effect on the mechanical properties, physical properties, and swelling behavior of the blend films. Blending silk fibroin with chitosan resulted in an improvement in tensile strength and elongation at break, and an increase in crystallinity. On the other hand, silk fibroin enhanced the thermal stability of chitosan. The addition of crosslinking agent to the blend films enhanced the mechanical properties. Furthermore, crosslinking was very important for the swelling behavior since it enabled retention of structural integrity of the films in the acidic pH buffer solution, even though it reduced the degree of swelling of the films. The properties of chitosan/silk fibroin blend films varied strongly with respect to changes in pH, salt type, and salt concentration. Therefore, these chitosan/silk fibroin blend films had pH and salt-responsive properties.

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20 SOME THOUGHTS ON NUCLEAR COOPERATION IN THE ASIA PACIFIC REGION

Azhar Djaloeis,
Deputy Chairman,
National Nuclear Energy Agency, Indonesia

Abstract:

This paper describes some thoughts of the author on the importance of the role of nuclear cooperation in the Asia-Pacific region in giving significant contributions to the development of the individual countries and to the region as a whole. The paper focuses on the role of nuclear science and technology and the importance of nuclear cooperation in the Asia-Pacific region, in particular to meet the challenges and opportunities in the new millennium. The paper starts off with a brief discussion on some important aspects of the Asia-Pacific region in view of its strategic location and its vast potential in terms of human and natural resources. Some of the major needs, challenges and opportunities in association with a future vision are briefly presented in terms of areas of activities that can be supported by the application of nuclear science and technology. The paper then discusses some ideas on how to further strengthen the nuclear cooperation in the Asia-Pacific region, followed by some concluding remarks and suggestions. <end>.

1. INTRODUCTION

At the very outset, I wish to express my sincere appreciation and thanks to the Organizing Committee for having invited me to attend this "**Second Takasaki Symposium on Radiation Processing**". It is indeed an honour for me, at the request of the organizing committee, to present a paper entitled "**Some Thoughts on Nuclear Cooperation in the Asia-Pacific Region**". I shall treat this subject mainly from the point of view of a policy maker, and thus regard the nuclear techniques as a tool in solving various scientific-technical problems in the process of achieving sustainable national and regional development.

As a development tool, nuclear techniques possess basically three agents of benefits. The first of these is **radiation**, namely the radiation of photons, electrons, neutrons or any species of ions. The interaction of radiation with materials/matter may be used to give information or to induce certain desired effects on the materials. The second agent of benefits, widely known as "**tracers**", involves the use of radioisotopes. Detection and measurement of the spatial distribution as a function of time of injected appropriate tracers into a system of interest can give accurate information on the system performance. Such systems may be plants, animals, human beings, industrial plants, or natural systems such as rivers. Finally, the third agent of benefits involves the **heat** produced by nuclear processes, such as nuclear fission or nuclear fusion.

In view of the aforementioned, it is evident that nuclear techniques can bring a wide spectrum of benefits, such as in the field of agriculture, health, industry, energy, hydrology and environmental care. The question then is how to develop and utilize nuclear sciences and technology, especially in a developing country like Indonesia. The next relevant question is how to develop or intensify nuclear cooperation in the Asia-Pacific region, in order to promote safe and peaceful and at the same time prevent military applications of nuclear techniques for the benefits of the individual countries and the region as a whole.

2. ASIA-PACIFIC REGION IN THE 21ST CENTURY

The Asia-Pacific region is in my view characterized by three major features. The first is the large population; with the present population of over 2 billion people with an increasing trend, the region has a vast resource of brain power and market potential. The second feature is the strategic location and the availability of natural resources, on or in the ground and in the sea. Given the backbone of Science and Technology, the region has in my view a good chance of becoming the most prosperous and powerful region of the world in the 21st century. The third feature of the region, however, is the large spectrum of economic-technological of the countries in the region, ranging from highly developed countries such as Japan and Australia to developing and less developing countries.

In order to capture the above stated chance, individual national efforts should be intensified, bilateral and multilateral links and collaborations among the Asia-Pacific countries should be further strengthened and focused, in particular to provide optimal solutions to the following questions:

- How to maintain **peace and socio-political stability** in the region, thus giving to the peoples of the region the feeling of **security and safety**, which is a basic condition for economic activities and progress.
- How to accelerate **economic growth**, in particular in the developing and less developing nations.
- How to accelerate the **development and application of Science and Technology**, in particular nuclear science and technology, in the developing member states in the region.

In this context, the regional cooperation schemes may be expected in the future to play a stronger role in stimulating the growth of nuclear and related sciences and technologies in the developing member states through systematic and well managed programs of training and R&D activities (capacity building).

With respect to the needs of the region, of high priority in my view are the following:

- **Food & Nutrient Security and Food Safety**

  With a total population of already over 2 billion people, food and nutrient security as well as food safety constitute a tremendous fundamental task to solve in the Asia-Pacific region, and indeed for the whole world! How to produce cheap and nutritious food for the mass of population constitutes a challenge that has to be overcome. The development and application of science and technology here spans all the way from food **production** (plant based, animal based and artificially/chemically based), food **processing** and food **preservation** to food **trade**.

- **Health Care**

  Provision of good health care at affordable price for the mass of population, such as facilities for general health check-ups, specific diagnostic measures, therapeutic treatments, preventive services and general consultations is expected to become another major challenge for the future, in particular considering the continuous rise in the living standard of the population.

- **Industrial Development**

  Industrial development in its broadest sense, namely the development of small, medium and large scale business enterprises to produce market competitive (preferably high) added value products, would give positive impacts to the economy and provide employment for the people. Included in this category are agrobased industries which can play a major role in many of the Asia-Pacific countries.

- **Energy**

  Energy is the fuel of the economy! With increasing size and standard of living of the population and the growth of industries, the demand for energy in the Asia-Pacific region is expected to grow at a tremendous rate in the future.

  With the rapid depletion of the finite and limited hydrocarbon resources, their potentials to be utilized for the production of higher added value products, and the environmentally detrimental effects of their burning, humanity would be forced to seek or develop other sources of energy.

In the Asia-Pacific region, Japan and Korea have adopted and implemented a consistent policy, strategy and programs for the utilization of nuclear power as one of the supporting pillars in their efforts to meet their increasing energy demand. In this context, it would be of vital importance for the Asia-Pacific nations to think strategically on the role and impacts of nuclear power plants, of which the number is expected to increase in the region.

Alongside the economics and technology of nuclear power generation, the questions related to safeguards and to the safety of nuclear power plants and radiation protection become quite relevant to the whole region.

- **Environmental Care and Provision of Clean Air and Potable Water**

Care for the environment and provision of clean air and potable drinking water are crucial issues that demand increasing attention in the future. Environmental care, however, should not considered in isolation, but rather be seen as an integral part of national development, which includes the economic and industrial aspects, especially in developing countries.

Nuclear techniques can give significant contributions and in some cases unique, in meeting the aforementioned needs and challenges. Planners should therefore be well informed on the potential benefits of these techniques, and incorporate them into the overall national and regional development planning activities.

3. NUCLEAR COOPERATION IN THE FUTURE

In my view, nuclear science and technology will play a greater role in the Asia-Pacific region in the future, not only for power, but also for non-power applications, such as agriculture, human health/medicine, industry, energy and environment. Given the situation and conditions in various countries of the Asia-Pacific region, one basic challenge is how to intensify mutually beneficial nuclear cooperation to promote safe and peaceful and at the same time to prevent military applications of nuclear techniques. From the point of view of a developing country, the interests seem clear. Nuclear techniques should finally be used as a cost-competitive tool in solving various technical problems associated with the national development, such as in agriculture, health, industry, energy and environment. Nuclear cooperation is seen therefore as a network to facilitate and assist in capacity building, specific R&D activities and public awareness campaigns. The technologically developed countries on the other hand, in my view, derive primarily political, economic and scientific benefits through provision of high technology goods and services to customers and advancement of scientific-technical knowledge through joint research activities. The availability of such large market needs/interests will serve as fertile ground for the growth of regional nuclear cooperation in the future. Nevertheless, this has to be supplemented with conscious and systematic effort to develop the proper mind set, to strengthen of necessary regional resources and to intensify regional awareness and support. Such efforts include:

- Creation of **genuine mental shift** among the decision makers in the member states in the region to put more emphasis on the concept "**we and ours**" rather than "**you/I and yours/mine**", thus creating an atmosphere towards developing a common prosperity in the region.

- Development of distributed regional competence, intelligence, or strength in nuclear science and technology by developing Regional Resource Centers/Units (**RRC's or RRU's**) in various different member states.

- Polishing of **public image** of nuclear science and technology, primarily through

☑ Better safety of installations
☑ Better public awareness campaign
☑ Better output, outcome and socio-economic impact of nuclear techniques

In practice, the existing multilateral cooperation schemes (Regional Cooperative Agreement - RCA - under the auspices of the International Atomic Energy Agency – IAEA, and the Forum for Nuclear Cooperation in Asia under the sponsorship of Japan) should be supplemented with **TCDC** (Technical Cooperation among Developing Countries). The participation of end users should encouraged in the future. The end users, who so far have not played a dominant role in the cooperation programs, should as far as possible be involved in the program activities from the beginning. Furthermore, national governments should demonstrate a stronger commitment, be made more aware of the benefits of nuclear science and technology.

With respect to the cooperation programs, the activities should be more oriented towards problem solving with end-user focus, designed to address the real needs of the region and the individual member states and to capture opportunities. The problem solving activities should be supported by capacity building in terms of the development of scientific facilities, human resources and legal infrastructure. In addition, awareness campaign should be intensified, in particular to get

☑ Commitment and support on the government
☑ Participation of end-users
☑ Public appreciation and support

☑ Financial support from potential funding/donor agencies/bodies.

Last but not least, special treatment for developing countries should be provided in order to accelerate the pace of progress/development. In general capacity building activities should be strengthened, with a special focus on the development of regional resource centers or units in those developing countries wherever appropriate. In addition, technical cooperation among developing countries should be promoted at bilateral or multilateral levels.

4. CONCLUDING REMARKS

From the foregoing discussion, several points may be pointing out as concluding remarks:

- Asia-Pacific region, with its strategic location and vast amount of natural and human resources, has a great potential to become the most prosperous and powerful region of the world in the 21st century.
- The challenges and opportunities for the region include the adequate supply of food, potable water and clean air, the provision of health care and the care of the environment. These are strongly coupled to the industrial development and the provision of adequate energy. Nuclear science and technology has a wide spectrum of application, and can offer significant benefits for the individual countries and the region as whole.
- Optimal development and application of nuclear science and technology in the member states can be strengthened through regional cooperation schemes, such as the existing RCA and FNCA as well as other forms regional cooperation (TCDC, etc.). Key to the success is the government commitment; at the implementation level, the Regional Resource Center's or Units constitute a strong backbone in providing various services for the benefits of the region as a whole.

Session 4

Radiation Vulcanization of Natural Rubber Latex

21 Bilateral Cooperation between BATAN and JAERI

Fumio Yoshii

Takasaki Radiation Chemistry Research Establishment,
Japan Atomic Energy Research Institute
1233 Watanuki, Takasaki, Gunma 370-1292, Japan

Implementing Arrangement between the National Atomic Energy Agency, Indonesia (BATAN) and the Japan Atomic Energy Research Institute (JAERI) in the field of radiation processing were signed on 9 May 1984. Under the Implementing Agreement, the "Cooperative Research Program on Modification of Natural Rubber Latex by Radiation Graft Polymerization" "Development of Biomedical Polymer by Radiation Technique" and "Development of Environmental Adaptable Polymer by Radiation Processing" were implemented on 1984 – 1990, 1990 - 1994, and 1994 – 1997, respectively.

Then, the "**Cooperative Research Program on Development of Protein Free Radiation Vulcanized Natural Rubber Latex**" was started on 1 May 1998.

1. Scope of Research

The cooperative research program is aimed to provide the basic technology of the new industrial process for production of pre-vulcanized protein free NR latex. I addition, fundamental research on the radiation chemistry of rubber proteins should be performed to contribute the further development of RVNRL technology.

2. Major Items of the Work

A) Optimum process development

1) Radiation vulcanization process of field latex
2) Dilution of vulcanized process field latex
3) Centrifugation process of diluted vulcanized field latex
4) Determination of optimum processes

B) Test and evaluation of centrifugalized vulcanized latex prepared by the optimum process

1) Characterization of centrifugalized vulcanized latex

(1) extractable proteins

(2) mechanical properties

(3) aging properties

2) Stability of centrifugalized vulcanized latex during storage

C) Fundamental research on chemistry of rubber proteins

1) Effect of irradiation on rubber proteins

2) Change in rubber proteins in cenrtifugalized vulcanized latex during storage

D) Final report

3. Time schedule

	1998	1999	2000	2001
A) Optimum process development 1) Radiation vulcanization process of field latex 2) Centrifugation process of diluted vulcanized field latex	→	→		
B) Test and evaluation of centrifugalized vulcanized latex 1) Characterization of centrifugalized vulcanized latex 2) Stability of centrifugalized vulcanized latex during storage		→	→	
C) Fundamental research on chemistry of rubber proteins 1) Effect of irradiation on rubber proteins in field latex 2) Change in rubber proteins in centrifugalized vulcanized latex during storage	→	→	→	→

22 Radiation Vulcanization of Natural Rubber Latex with Low Energy Accelerator

Md. Emdadul HAQUE[1], Keizo MAKUUCHI[1], Hiroshi MITOMO[2], Kenichi IKEDA[1], Fumio YOSHII[1] and Tamikazu KUME[1]

[1]Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute, 1233 Watanuki, Takasaki, Gunma 370-1292, Japan

[2]Department of Biological and Chemical Engineering, Faculty of Engineering, Gunma University, Kiryu, Gunma 376-8515, Japan

Abstract

The radiation vulcanization of natural rubber latex (RVNRL) with the recently installed electron beam (EB) pilot plant at Takasaki Radiation Chemistry Research Establishment, Takasaki, Japan has been discussed. The accelerating voltage and beam current of the plant are 250 kV and 10 mA respectively. The plant has a reaction vessel with the capacity of 18 liters latex to irradiate at a time. In order to obtain a suitable setting of experimental for RVNRL under EB of the plant the parameters such as irradiation time, defoamer concentration, volume of latex, beam current etc. are being optimized by varying the individual parameter at a constant set of the other variables.

Keywords: Natural Rubber Latex, Radiation Vulcanization, Electron Beam, Pilot Plant, Tensile Strength, Cross-link Density, Swelling Ratio

1. Introduction

Radiation vulcanization of natural rubber latex (RVNRL) is carried out with electron beam (EB) from accelerator machine side by side with gamma rays from Co-60. But vulcanization with EB did not become so popular because of its low penetration into the natural rubber latex (NRL). There are also possibilities of over dosing and rise of temperature of latex during irradiation. Efforts were made to overcome these critical drawbacks. The RVNRL without radiation vulcanization accelerator (RVA) have been studied in China by using a van de Graaff electron accelerator (2 MeV, 0.15 mA) with constant stirring to ensure homogeneity [1]. A pilot plant having the energy of 6 MeV was installed in France to irradiate latex with linear electron accelerator continuously in a specially designed vessel connected to a circulating pump [2]. It was reported that the resulting RVNRL and its film were of good properties. In Germany NRL was irradiated with a Dynamitron accelerator (1.5 MeV, 25 mA) by flow of latex on metal slope using a polyfunctional monomer as RVA [3]. At Takasaki Radiation Chemistry Research Establishment RVNRL was carried out with 3 MeV electron accelerator (Dynamitron) using a cylindrical stainless steel reaction vessel of 3.2 liters capacity having outer jacket and propeller type stirrer [4]. For homogeneity of vulcanization higher stirring speed was suggested.

Attention was drawn to the low energy electron accelerator because it does not require any special building with thick bio-shielding. NRL was irradiated with the accelerating voltage of 300 kV varying the beam current, stirring speed etc. [5]. The effect of beam current on tensile strength of RVNRL film was not found but with higher stirring speed shorter vulcanization time could be achieved. The RVNRL also carried out with electron accelerator CB 250/15/180L having drum type reaction vessel and was reported to be good enough [6]. In this paper various aspects of the EB pilot plant for RVNRL recently installed at Takasaki Radiation Chemistry Research Establishment (TRCRE), Takasaki, Japan have been discussed.

2. Experimental

2.1. Main features of EB pilot pant for RVNRL at TRCRE

The pilot plant for RVNRL at TRCRE has a reaction vessel (Fig. 1) with the capacity of 18 liters latex to irradiate at a time. The accelerating voltage and beam current of the plant are 250 kV and 10 mA respectively. The length and width of the beam window are 20 and 6 cm respectively. The latex irradiation vessel under EB is a cylindrical stainless steel vessel (29 cm Φ and 30.5 cm height) containing four baffle plates onto the inner wall at 90° intervals and fitted with a propeller type stirrer and outer jackets. The vessel is covered with a cooling plate having titan film (thickness = 0.0015 cm) window (20 cm x 8 cm).

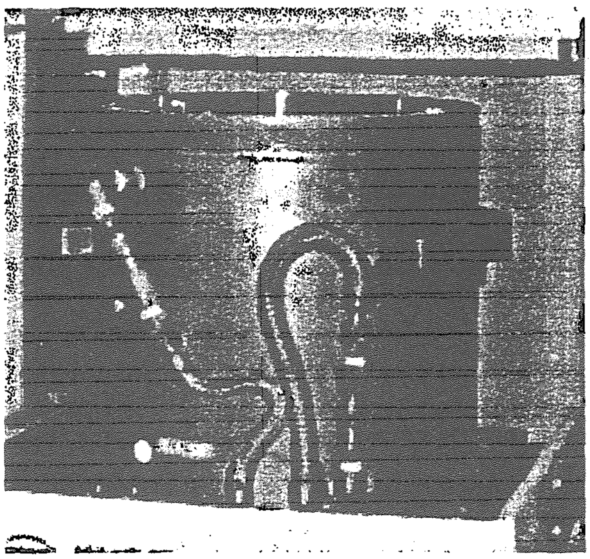

Fig. 1. Photograph of reaction vessel

The reaction vessel is installed on a basement under the beam window. Backward and forward movements of the vessel together with the basement are done manually. The vessel is pulled forward for feeding the latex and after completion of feeding it is again replaced to the position by pulling backward. The upward and downward movements are done mechanically with the help of the right and left turns of a key. The vessel has an outlet at the bottom to channel out the irradiated latex and to remove the waste- water after washing the vessel. The irradiation is carried out after setting the vessel at the inbuilt set top up position and shutting up the plant door.

2.2. Irradiation of NRL with radiation vulcanization accelerator (RVA) under EB

A high ammonia latex concentrate (Microtex, Malaysia) was used after diluting with 1% aqueous ammonia to 50% total solids. 1,9-nonanediol diacrylate (ND-A) obtained from Kyoeisha, Japan, was mixed with latex without further purification by stirring for ~ 30 minutes. A defoamer, BYK022 from BYK-Chemie GmbH Co. Ltd., Germany was mixed with latex to suppress the foam formation. The latex was irradiated under EB after mixing with ND-A with constant stirring at a fixed rpm.

2.3. Preparation of rubber film and measurement of properties

The irradiated latex was cast on glass plates for making films. The films were dried, leached in 1% aqueous ammonia and heated at 80°C for an hour. The tensile strength at break (Tb) and elongation at break (Eb) were measured with a tensile machine, Strograph-R1, Toyoseiki, Japan. The swelling ratio (wt.) of the film was determined from the weight change of the film immersed in toluene for 24 hours.

3. Results and Discussion

3.1. Favorable characteristics of the new RVA

The accelerating efficiency of a di-functional monomer, ND-A for RVNRL has been investigated to replace *n*-BA [7]. ND-A has no smell. It possesses low primary irritation index (PII = 2). The ND-A mixed latex remains in a very good physical state (in terms of color and viscosity) up to several days (does not coagulate for at least 7 days storage).

3.2.Operation of EB pilot plant for RVNRL

A view of the recently installed EB pilot plant is shown in Fig. 2.

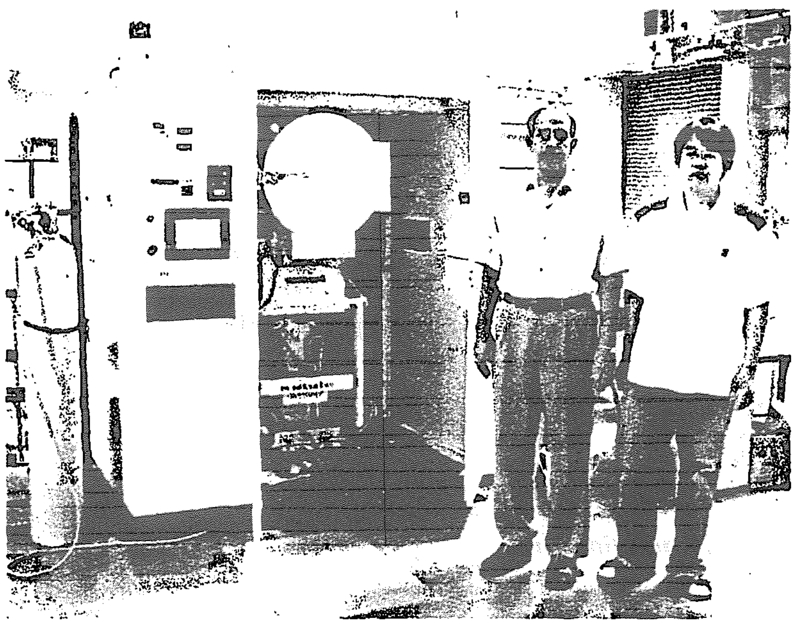

Fig. 2. A view of EB pilot plant

The advantageous features of this plant are:

- Small size
- No special building with thick bio-shielding is required
- Self- shielded

- High output
- The electron beam is stopped with the shut down of the power source
- Low irradiation cost (expected)

The pilot plant is being optimized in order to obtain a suitable setting of experimental for RVNRL under EB.

3.3. Optimization of defoamer concentration

If the latex is stirred bubbles are formed and create problem during irradiation. So a defoamer is used to suppress the bubble formation [8]. To optimize the defoamer concentration varying quantities of defoamer were added to the 5 phr ND-A impregnated latex separately and stirred with low to high speed for 20 minutes. It was found that 0.2 phr concentration of defoamer is sufficient to suppress the bubble formation when latex is stirred at $\leq$ 360 rpm. The maximum rotational speed of the stirrer in the EB reactor vessel is 360 rpm. So 0.2 phr of the defoamer concentration could be used at any stirring speed of the stirrer. The foam formation was examined by taking 16 liters of latex at various stirring speed. No bubble was formed.

3.4. Optimization of irradiation time

For this purpose NRL was irradiated under EB for various lengths of time using fixed volume, RVA concentration, defoamer concentration and stirring speed at the beam current of 10 mA and accelerating voltage of 250 kV. Fig. 3 shows the Tb and Eb plotted against the irradiation time. It is found that Tb increases with the increased length of irradiation time attaining a maximum value at around 30 minutes irradiation time beyond which it again shows downward trend. The maximum Tb obtained from the RVNRL film prepared with the latex irradiated for 30 minutes is ~ 26 MPa. Elongation at break however decreases gradually with increased irradiation time.

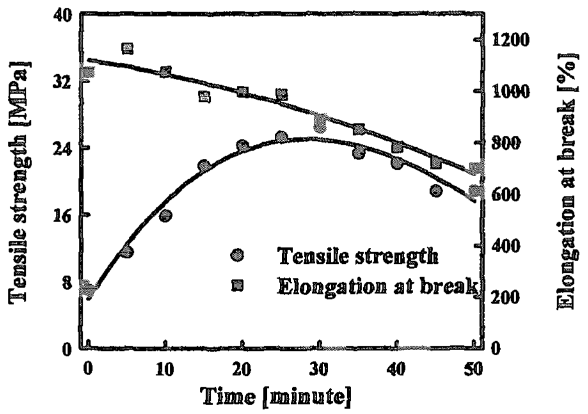

Fig. 3. Tensile properties of EB irradiated latex film versus length of irradiation time.

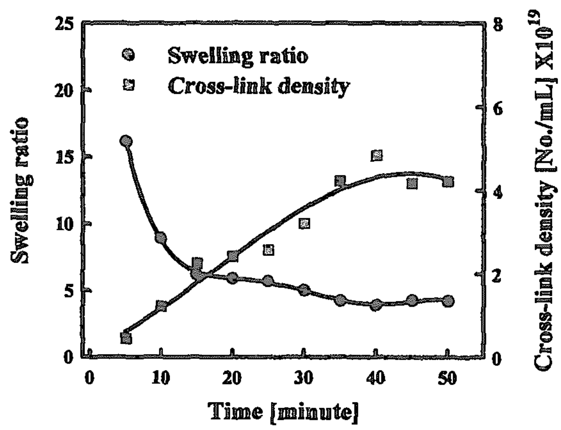

Fig. 4. Swelling ratio and cross-link density of EB irradiated latex film versus length of irradiation time.

Conditions of irradiation :
Latex used = 14 L (50% TSC)
Accelerator = 5 phr ND-A
Defoamer = 0.2 phr BYK022
Current and voltage = 10 mA and 250 kV
Stirring speed = 210 rpm

Fig. 4 shows the swelling ratio and corresponding cross-link density of the RVNRL films plotted against the length of irradiation time. The swelling ratio goes down to a minimum value (which corresponds to a maximum value of cross-link) for the film obtained from the latex irradiated for 40 minutes. As discussed earlier the maximum value of Tb is obtained for 30 minutes irradiated sample. From this observation it can be inferred that to obtain a maximum value of Tb a certain number of cross-link is required that can be termed as optimum cross-link and this value spread over a range. In this case optimum value of cross-link is obtained by irradiating the latex for 30 minutes.

Fig. 5 shows the Tb and Eb of the RVNRL film prepared by irradiating latex at lower EB current (5 mA) maintaining the other conditions as stated earlier. At this EB current similar trends in the Tb and Eb values are observed. But the maximum Tb (~ 26 MPa) is obtained for the RVNRL film from the latex irradiated for 40 minutes. Thus the length of irradiation time required to obtain maximum Tb is less at higher EB current. The swelling ratio and corresponding cross-link density of these films are shown in Fig. 6. At 40 minutes irradiation time for 5 mA beam current the value of cross-link is within the similar range to that of the 30 minutes irradiation time for 10 mA beam current.

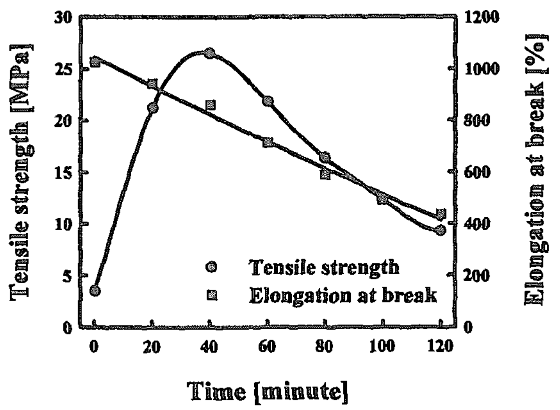

Fig. 5. Tensile properties of EB irradiated latex film versus length of irradiation time (5 mA and 250 kV).

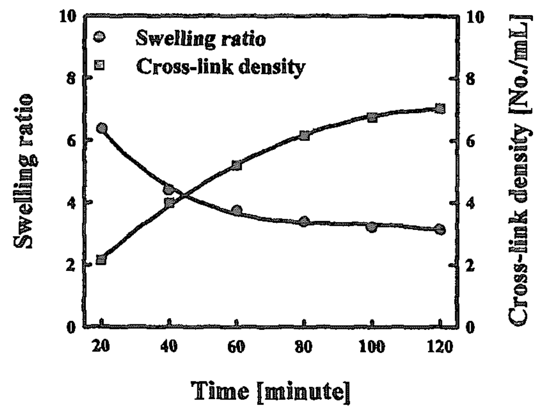

Fig. 6. Swelling ratio and cross-link density of EB irradiated latex film versus length of irradiation time (5 mA and 250 kV).

The time required in this condition of latex irradiation under the EB of this plant seems to be long and the maximum value of tensile strength is also not up to the level of maximum tensile strength (~ 30 MPa) obtained from γ-rays irradiated latex film. Further investigation is being continuing by varying the individual parameter at constant sets of the other variables to determine the ideal setting for maximum efficiency of the machine.

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23 Production of Surgical Gloves from Low Extractable Protein RVNRL

Marga Utama[1], Yanti S.[1], Made Sumarti [1], Marsongko[1], Tita Puspitasari,[1], Dian Iramani[1], K.Makuuchi[2], F.Yoshii[3], Siswanto [4]

[1].Center for Research and Development of Isotopes and Radiation Technology, National Nuclear Energy Agency, Jakarta, Indonesia.
Jl.Cinere Ps. Jumat P.O.Box 7002 JKSKL, Jakarta 12070, Indonesia.

[2].EB System Cooperation
1233 Watanuki, Takasaki, Gunma, 370-1207 Japan

[3]Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute,
123 Watanuki –Machi, Takasaki-Shi, Gunma 370-1292, Japan.

[4] Research Unit for Biotechnology of Estate Crop
Jl. Taman Kencana No.1 Bogor

Abstract
Study on the production of surgical gloves from low extractable protein RVNRL (Radiation Vulcanization of Natural Rubber Latex) in home industry scale with normal butyl acrylate as sensitizer has been carried out. The variation of dipping speed, concentration of coagulant agent and selection of antioxidant for producing good quality of surgical gloves were evaluated. The water-extractable protein and PBS (Phosphate Buffer Saline) - extractable protein content, the physical and mechanical properties of gloves were measured. The results show that for producing a good quality of surgical gloves from low extractable protein RVNRL , the concentration of latex is 50% with calcium nitrate as coagulant agent between 15-20%. By using this condition the physical and mechanical properties of surgical gloves is required to ASTM standard such as tensile strength more than 24 MPa, PBS-extractable protein is around 41-68 ug/g and water-extractable protein contents is around 23-35 ug/g.

Keywords : Surgical gloves, low extractable protein, dipping technique.

Introduction

In western countries there are very strong social/political pressure to ensure that all employers/consumers/user are given maximum protection against all the hazards, they are likely to encounter [1]. As part of this general trend it can be expected that latex products which directly contact with human body should give "protection" and "safer" in use.

As a generally known dithiocarbate used as accelerators in curing process for producing latex products cause the formation of nitrosamines [2,3]. It has already been observed that various N-nitrosamines have been shown to be carcinogens in animals, and IARC classification as probable human carcinogens has emerged [4].

In 1982 the German BGA introduced a limit of 10 ppb (u/kg) for total N-nitrosamines in rubber teats and soothers to gether a 200 ppb limit nitrosable amines [5].

It has been reported that radiation vulcanization technique more advantages over sulfur vulcanization such as absence of N-nitrosamines, very low toxicity, easy degradation in the environment, transparency, softness and less formation of sulfur dioxide when burning, because the fact that radiation vulcanization technique does not contain dithiocarbamats, sulfur, and zinc oxide [6-10].

Between 1989-1992, the US Food and Drug Administration (FDA) reported a number of cases of hypersensitive reaction have attributed to protein allergens presents in natural rubber latex [11].

Great amount of work has been carried out on reduction of protein content of RVNRL. They reported RVNRL film are influenced by many factors such as dose of irradiation, storage periods, and leaching time of film [12-13].

This paper describes the production of surgical gloves made from low extractable protein RVNRL prepared by home industry at Serpong, Tanggerang, West Jawa, Indonesia. The optimum condition processing and characteristic of surgical gloves will be reported in this paper. The focus of the study is to prove that gloves from RVNRL have lowest extractable protein content.

Material and method.

Material. Low protein RWNRL produced by P3TIR-BATAN on February 1999, with the technical specification on Table 1 were used on this experiment. Calcium nitrate as coagulant agent, and some chemical such as BSA (Bovin Serum Albumin) Standard, sodium carbonate, sodium bicarbonate, bicinchonimic acid etc., were used for analysis the extractable protein content of surgical gloves.

Table 1. Typical properties of low extractable protein content of RVNRL produced by P3TIR-BATAN on February, 1999. (Jalupang Plantation).

Properties	Minimum	Maximum	Average	ASTM
- Total Solid Content, %.(TSC).	64.70	64.84	64.77	61.5*
- Dry Rubber Content, %.(DRC)	64.56	64.56	64.43	60.0*
- Viscosity, cP.	90	98	94	-
- pH	10.2	10.4	10.3	-
- VFA number	0.0029	0.0031	0.0030	0.2**
- Mg Content, %TSC.	0.00049	0.00051	0.00050	0.005**

- Minimum, ** Maximum.

Apparatus. Surgical gloves former from fiber glass, dipping tank, Toyoseiki Strograph-RI Tensile Tester, and UV Spectrometer

Production of surgical gloves.. Figure 1 shows a flow diagram of a surgical gloves production line. The coagulant bath contain calcium nitrate at concentration 15-30%, dependent on the final thickness gloves required. After cleaning the former dipped into coagulant, draw up, dipped into latex, draw up, dried at room temperature, over night (17-20 hours). The next stage was beading and stripping, then leaching in ht water for 1 hour and drying at room temperature.

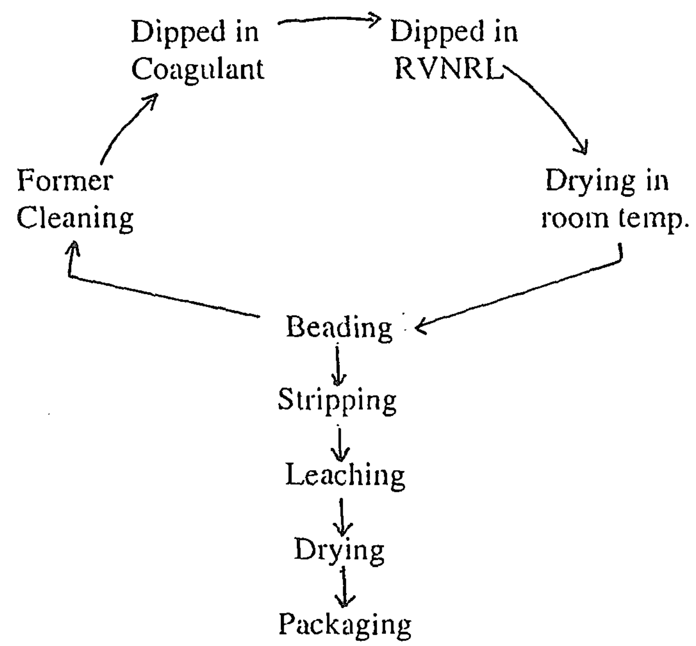

Figure 1. Schematic diagram of surgical gloves production

Evaluation of quality latex and surgical gloves . The procedure for measuring the RVNRL and surgical gloves properties such as TSC, DRC, viscosity, pH, tensile strength, etc., were measured according to ASTM standard. [14-15]

Determination of Extractable protein. The procedure for determination of water and PBS extractable protein content of surgical gloves, was carried out according to ASTM and LOWRY procedure [16-18].

Results and discussion.

The gloves production were carried out on small (home) industry scale (20 pair/hour) with calcium nitrate as coagulant agent. The importance factor for producing surgical gloves from RVNRL by dipping procedure are selection of antioxidant, concentration of calcium nitrate, and dipping speed.

Selection of antioxidant . The solid content of field natural rubber latex is around 20-40%. The fraction content of rubber content (poly-isoprene) which normally is about 95%, and the rest is non rubber constituent such as protein, fatty acid, antioxidant which normally present in latex. It is clear that the addition of antioxidant is necessary to replace the natural antioxidant already destroyed by irradiation [22]. Phenolic antioxidant were used in this experiment (Figure 2), because they are less discoloring then amines antioxidants. They are used in the application where the properties are necessary such as surgical gloves. Table 2 shows the physical and mechanical properties of gloves with 3 kind of antioxidants before and after aging 70 C for 168 hours. It can be selected that Lowinox 44B25 is a better antioxidant, because the decreasing of physical and mechanical properties after aging is lowest compared with the others, and the color of glove are not change.

Selection of calcium nitrate concentration . Some factor must be considered when choosing a chemical coagulant system i.e: as rapid de-stability of the colloid system, it must be non foaming, good shelf stability, cost, toxicity, and environment factors must be considered. According to these reasons, calcium nitrate is a chosen system for this trial production. Figure 3 shows the relation between concentration of calcium nitrate with the thickness of gloves at one time dip. It can be assumed that for producing gloves with

Table 2. Physical and mechanical of gloves with three kind of lowinox antioxidant before (A) and after (B) aging 30 C for 168 hours.

Properties		Lowinix 22B46	Lowinox 44B25	Lowinox P24S
-Modulus 600%,MPa.	(A)	1.9	2.0	2.0
	(B)	2.1	2.5	2.4
-Tensile strength,MPa.	(A)	26.1	26.8	26.5
	(B)	20.5	23.1	21.6
-Elongation at break,%.	(A)	1000	1000	1000
	(B)	1000	1000	1000
-Permanent set,%.	(A)	10	10	10
	(B)	7	7	7
-color after aging		change	not change	change

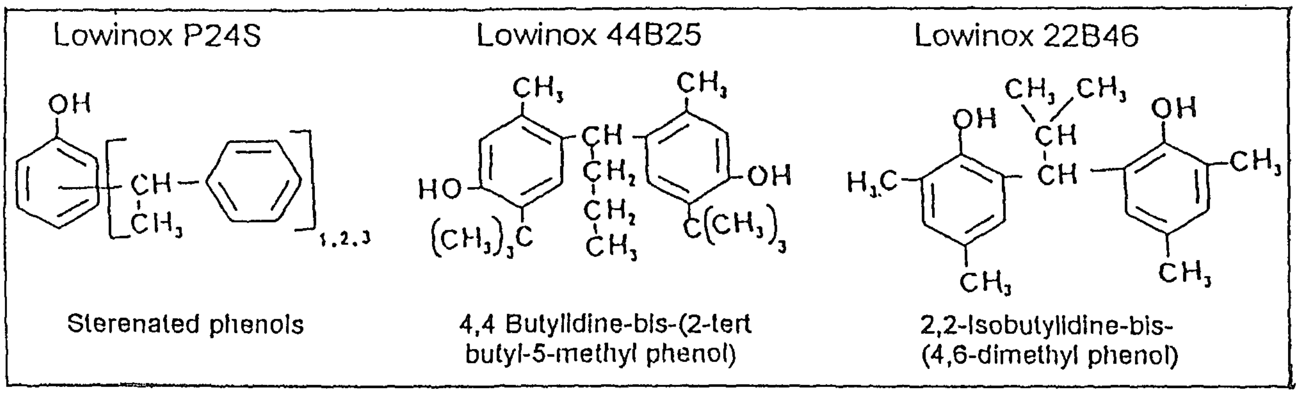

Figure 2. Chemical structure of phenolic antioxidant to be used in this experiment

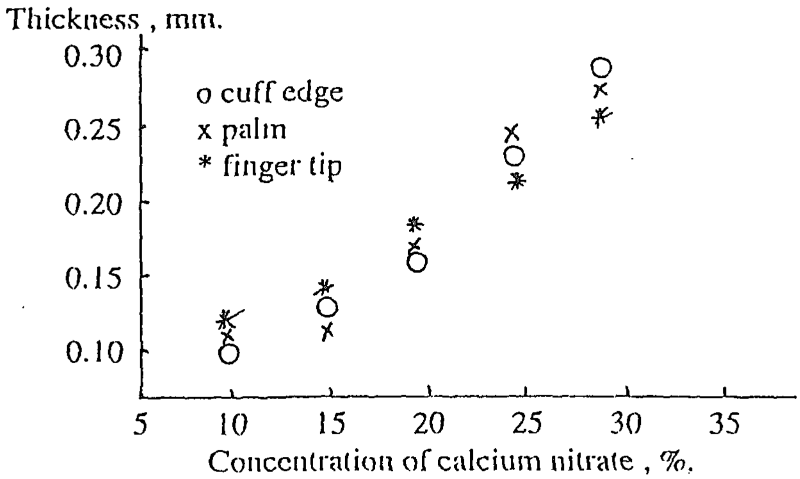

Figure 3. The relation between concentration of calcium nitrate with thickness of gloves.

thickness 0.2 mm the total solid content of RVNRL is around 50%, and the concentration of calcium nitrate is around 15-20%.

Selection of dipping speed. In the straight-dipping process, the latex compound adhering to the form is simply air-dried. In the coagulant-dipping process, the latex compound comes into direct contact with latex stability, consign almost instant gelatin

Table 3.The thickness of gloves (mm), with different speed of dipping and drawing.

Position	Speed of drawing, m/sec.		
	0.1	5	5 to 0.1
- Finger tip	0.31	0.31	0.23
- Palm	0.21	0.28	0.24
- Cuff edge	0.21	0.23	0.24

of. latex compound in the area where contact is made, and the speed of dipping produced thicker film per dip. Table 3 shows the effect of dipping speed on the uniformity thickness of gloves. It indicates, that for getting uniformity thickness of gloves the speed of dipping are as follow : the former is dipped quickly then be pulled at the speed of 50 m/sec., after half position of gloves former position, the speed to be decreased up to 0.1 m/sec.

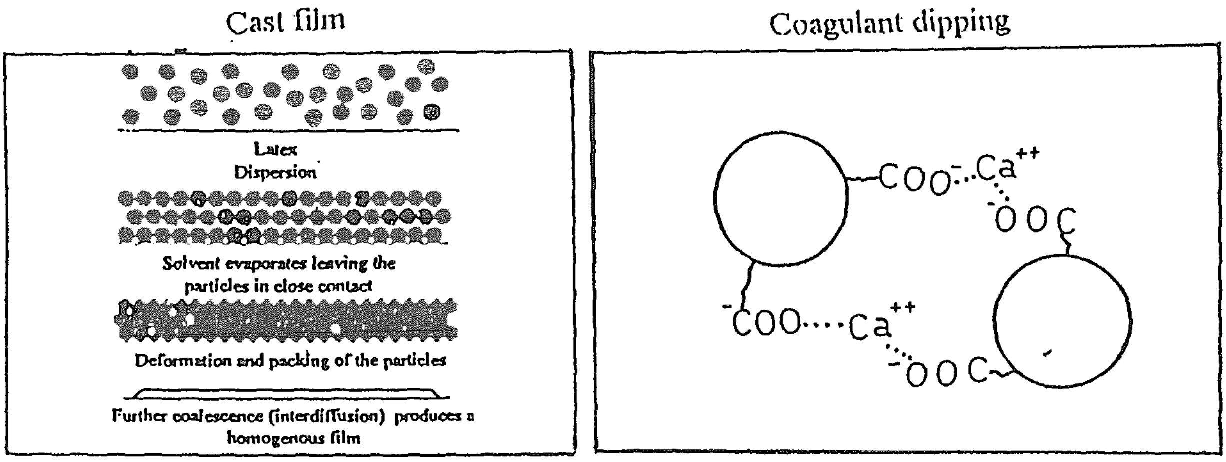

Figure 4. Schematic of film forming [19-20].

Physical and mechanical properties. Figure 4 shows the film formation process prepared by casting and dipping film. Film formation in latex is complex process depend on drying or coagulation process which bright latex particles together. In casting film, film formation is controlled by some factors such as concentration of latex, Tg (glass transition), temperature of drying, particles cross link density, and temperature of which film formation is taking place. In coagulant film is controlled not only those factors, but also controlled by coagulant agent, where latex destabilized. Film made by this process, therefore, here a higher degree of disorder than film made by casting process. Consequently tensile strength more higher (Figure 5), because ionic cross linking among rubber particles are form by the metal oxide, usually calcium from coagulant.

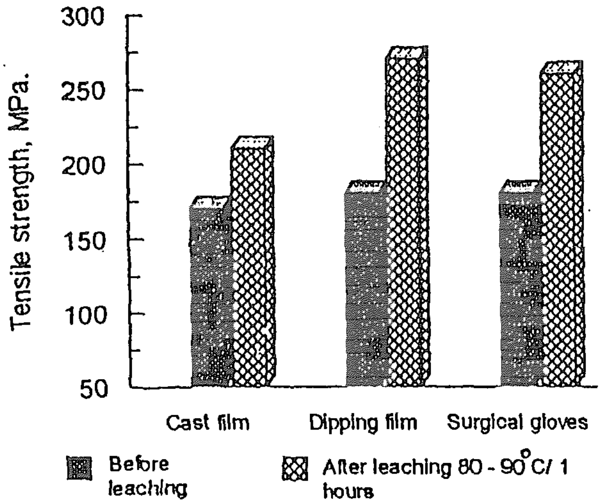

Figure 5. Tensile strength of cast film and coagulant dipping film.

Ionic cross link begin forming at high temperature (70-80 C) immediately after the film is leached in hot water. The cross link contribute to both of high modulus and high tensile strength.

Protein content. Latex protein is reported as a cause of Type I sensitivity in some individuals who have been exposed to latex containing devices. Since May 1991, the FDA (Food and Drug Administration) has recommended that manufacturers of latex devices reduce the water-extractable protein in their natural rubber latex devices. The FDA recommended limit for water-extractable protein is 1200 ug per glove or 120 ug/g [2,3].

Leaching is the process removal hydrophilic materials from latex dipped produce by washing them in hot water. The removal excess calcium nitrate in water soluble compounding greatly improves the clearly physical properties of film, particularly film obtained pre-vulcanized latex. Leaching can be carried out through wet gel leaching and dry film leaching.

A practical and most effective means of reducing the extractable protein in latex gloves will be the use of pre-vulcanized natural latex with combination of wet leaching and dry-film leaching. From the production line of surgical gloves prepared in small (home) industry scale (20 pairs/hour) shows that the water-extractable protein content of gloves from RVNRL were around 23-35 ug/g and PBS-extractable protein were around 41-68 u/g, while the water-extractable protein content of gloves with sulfur vulcanization technique was around 498-2500 ug/g, and PBS-extractable protein content was around 1812 – 3840 ug/g, and PBS-extractable protein content of gloves from sulfur and clorination technique was 625 ug/g (Table 4). From the results it can be indicated

Table 4. The water-extractable and PBS extractable protein content (ug/g) of gloves prepared from RVNRL, sulfur vulcanization, and clorination technique.

Method	water-extractable	PBS-extractable
- RVNRL	23 – 35	41- 68
- Sulfur vulcanization	496-2500	1812-3840
- Sulfur and clorination	400	625

Table 5. The quality of gloves from RVNRL prepared by home industry in Tanggerang, Indonesia on March – December 1999.

Properties		Bath I	Bath II	Bath III	ASTM Type I	ASTM Type II
- Modulus 500%, MPa.		2.2	2.1	2.0	5.5**	5.5**
- Tensile strength,MPa.	(A)	26.9	25.1	26.1	24 *	20*
	(B)	20.7	21.2	20.2	18*	-
- Elongation at break,%.	(A)	1000	1100	1000	750*	750*
	(B)	1000	1000	1000	650*	-

A = before aging, B = after aging 70 C/168 hour.

the removal of extractable protein during drying and leaching become easier since the protein macromolecules have been degraded by irradiation. This values prove that gloves from RVNRL prepared by home industry scale have a lowest water-extractable protein content.

Quality. According to ASTM, the importance physical and mechanical properties of surgical gloves are: modulus, tensile strength, elongation, thickness, integrity, pinhole etc. Table 5 shows the quality of surgical gloves from RVNRL prepared by home industry which indicates that the physical and mechanical properties is require to ASTM standard, but for commercial purpose the physical and mechanical properties should be improved.

Conclusion

From this study it can be concluded that the optimum condition processing for production surgical gloves from low protein RVNRL in home industry scale are as follow:

- The optimum concentration of RVNRL for producing surgical gloves was around 50%, with Lowinox 44B25 (4,4-Butylidine-bis-(-2-tert.butyl-5-methyl-phenol) as antioxidant.
- The optimum calcium nitrate as coagulant agent is around 15-20%, with speed of drawing was 50 m/min. than after half position of the gloves former the drawing speed should be decreased to 0.5 m/min.
- By using this optimum condition the quality of surgical gloves is required to ASTM standard, and the water-extractable protein content is around 23-35 u/g, and the PBS-extractable protein content is around 41-68 ug/g.

Acknowledgement

The authors thanks to Mr.Tjutju Rahayu Liusman and his staffs of Surgical Glove Home Industry Serpong Tanggerang, for the participation trial production of surgical gloves in home industry scale.

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Session 5

Application of Radiation in Asian Countries

24 Application Of Radiation In Bangladesh

Naiyyum CHOUDHURY, Najmul Alam CHOWDHURY and Feroza AKHTAR
Bangladesh Atomic Energy Commission
4, Kazi Nazrul Islam Avenue, G.P.O. Box No. 158
Dhaka-1000, Bangladesh

Abstract

Radiation technology offers a very wide scope for utilisation and commercial exploitation in various field. All over the world, this technology is being favourably considered for different applications like radiation sterilisation of medical products, preservation of food by controlling the physiological processes for extending shelf-life and eradication of microbial and insect pests, radiation processing of polymeric materials and treatment of sewage sludge.

Bangladesh Atomic Energy Commission has taken radiation processing programmes in a big way right from its inception. This paper describes the studies carried out by various research groups in Bangladesh Atomic Energy Commission mainly using Cobalt-60 gamma radiation. The investigation covers medical sterilisation, food preservation and development and modification of polymeric materials by gamma radiation. Both food preservation and radiation sterilisation of medical products are now being commercially carried out in the Gammatech facility as a joint venture company of BAEC and a private entrepreneur. Bangladesh is soon going to establish a full-fledged Tissue Bank to cater the needs of various tissue allografts for surgical replacement. Recently Government of Bangladesh has allocated US$ 1.00 million for strengthening of the Tissue Banking Laboratory. BAEC has made quite a good research contribution on vulcanization of natural rubber latex, wood plastic composites, surface coating curing, polymer modification etc. As a result of successful achievement of R & D activities in all these projects, a pilot plant project involving about US$ 4.00 million is under implementation at the Atomic energy Research Establishment campus of BAEC. In addition a project on 'National Polymer Centre' at a cost of US$ 2.00 million has already been approved. It is expected that work on radiation processing including commercialization will be accelerated with the implementation of these projects. The impact of radiation processing in selected areas will no doubt be significant in coming years.

Keywords: Radiation, applications, sterilization, processing, vulcanization, composites, hydrogel.

1. Introduction

Radiation technology namely the technology of utilization of intense radiation is a veritable source with immense potential for application in diverse fields. The knowledge about the effects of radiation on materials, components and systems opened up a new vista for large scale industrial exploitation. With the emergence of this new technology, the use of ionising radiation has to a very large extent revolutionised industrial scene all over the world during the last three decades.

The manifold advantages that radiation technology offers are of immense consequence to industry and potential benefits to public health and environment. The major applications of intense radiation include radiation sterilization of medical products, radiation preservations of food grains, radiation chemical processing, radiation polymerization, production of wood-plastic composites, radiation curing of paints and coatings and so forth.

It is worth while to indicate that the cost of conventional energy has multiplied manifold and is expected to go higher whereas the cost of radiation power has come down considerably. All over the world, there is a general tendency to re-evaluate the radiation route for industrial process techniques, so far discarded merely on the basis of poor economics.

With the availability of multi-kilo curie quantities of various radioisotopes as a consequence of the operation of nuclear power stations world-wide, utilization of this untapped potential of energy is of great technological significance to industry and public health in the country. Electron beam irradiation is fast catching up with gamma irradiation techniques in various parts of the world. Major breakthrough has been achieved in the areas of sterilization of sea foods, poultry products, cured coatings, cracking of cured petroleum and other allied fields.

Some of the major areas where research and development activities have been undertaken in this particular field in Bangladesh, and the current status of this programme with a glimpse into the future prospects are highlighted in this paper.

2. R & D activities at BAEC

During the last three decades, Bangladesh Atomic Energy Commission (BAEC) has undertaken an elaborate R & D programme covering a wide spectrum of activities in different fields of radiation processing research and its technological applications. This presentation is confined to the activities categorised as follows :

Radiation sterilization of medical products and pharmaceutical raw materials,
Radiation sterilization of tissue grafts for use in rehabilitative surgery,
Radiation preservation of foods,
Radiation biotechnology,
Radiation Vulcanization of Natural Rubber Latex,
Wood-plastic composites using gamma irradiation,
Curing of surface coatings by UV radiation, and
Preparation of hydrogel for burn dressing.

2.1. Radiation sterilization of medical products

Detailed investigations have been made on locally produced absorbent cotton, surgical bandages/dresings, family planing kits, empty ophthalmic bottles etc. in order to evaluate its bioburden and microbial radiosensitivity [1,2]. The contaminating bacteria and fungi have been isolated, purified, identified and the D_{10} value of some comparatively radioresistant isolates were determined. The cotton based products showed quite a high level of contamination sometimes showing values as high as 10^3 or 10^4 [1]. But now-a-days with stricter adherence to GMP the cotton based products have shown a remarkable increase in their microbiological quality. The radiation dose level for sterilization of different medical products ranged between 25 kGy to 30 kGy with a sterility assurance level (SAL) of 10^6 (Table 1).

2.2. Radiation sterilization of pharmaceutical raw materials

Extensive R & D was also carried out to explore possibilities of extending radiosterililization technique to sterilize certain pharmaceutical products which included antibiotics, steriods, vitamins etc. The following pharmaceutical raw materials were studied in detail to find their suitability for radiation sterilization.

Antibiotics : Oxytetracycline-HCl, Amoxicillin tri-hydrate, Chloramphenicol and Procaine penicillin G.

Steriods : Dexamethasone sodium phosphate.

Vitamins : Riboflavin 5'-phosphate and Thiamine HCl.

Each of the above mentioned raw materials were irradiated at doses of 5, 10, 25, 50 and 100 kGy and the consequent microbiological, physical and chemical changes were observed. In general, the products were examined for microbial load, colour, pH, potency, absorption spectra and HPLC to check if there was any degradation upon irradiation. Analytical studies failed to prove any physico-chemical, biological potency and safety alterations. It was evident from the above studies that all the samples tested are suitable for radiation sterilization even at a high dose of 50 to 100 kGy (Table 2 and3, Figure 1 and 2) [3,4]. Re-examination of radiosterilized antibiotics stored under room temperature for 12 months after radiation treatment showed compliance with BP requirements as regards physico-chemical and microbiological specifications.

Radiation sterilization is particularly well-suited for Bangladesh. The country lacks sterilization facilities in most areas. Regular supplies of radiation sterilized medical products can greatly improve the country's health care programmes. Bangladesh has made a modest beginning in this respect. Institute of Food and Radiation Biology of the Bangladesh Atomic Energy Commission offered radiation sterilization services to many governmental and autonomous organization and private companies since 1981. With the 50,000 Curie Co60 source , it became impossible to serve all the organizations and to meet the increasing demand of radiosterilized medical and pharmaceutical products.

The sterilization demand of the country was 68,000 cubic feet/year in 1985 which stood at 225,000 cubic feet/year in 1995 and it is increasing with the extension and improvement of the health care programmes. The country badly needs upgrading of the medical products, particularly those manufactured locally which will be sufficient for running a commercial irradiator year round. With these view in mind a commercial irradiator (100,000 Ci Co60) was installed at Chittagong, in 1993 under the joint venture of BAEC and BEXIMCO, known as GAMMATECH.

2.3. Tissue Banking

Tissue banking represents a well established service in many countries in the health sector where many different kinds of tissues are processed, sterilized and supplied. Tissue bank involves selection, collection, processing, storage and distribution of tissues for their subsequent clinical use. Skin, amnion membrane, bone, heart valve, tendon, dura mater, middle ear etc. are typical example of tissues that are converted to grafts. Radiation preservation has opened a new dimension in these areas. Properly processed grafts can now be effectively sterilized by radiation and preserved for long time without any loss of efficacy. In collaboration with the International Atomic Energy Agency (IAEA) a project on "Tissue Banking" was initiated at the Institute of Food and Radiation Biology (IFRB), Bangladesh Atomic Energy Commission at the end (December) of the year 1985.

Techniques for the preparation of radiation sterilized amnion membrane and bone grafts have been established. Fresh amniotic membranes and bones are collected from the labour unit of Azimpur Maternity Hospital, Gonoshastho health complex, from the operation theater of Dhaka Orthopaedic Hospital (RIHD) and Dhaka Medical College Hospital respectively. The prepared amnion membrane and bone allograft are sterilized by gamma irradiation (20/25 kGy)[5,6,7]. Radiation sterilized amnion and bone grafts are now being prepared and supplied regularly to 12 different hospitals of the country. Since the out-set of the research work on tissue banking in Bangladesh more than 13,500 amniom and bone allografts were prepared and 700 different types of patients (burn, leprotic ulcer, bedsore, traumatic open wound, diabetic wound, orthopaedic and maxillo-facial defective) were successfully treated with these radiation sterilized tissue allografts (Figure 3 and 4) [8].

The ultimate objective of the present research and development activities on Tissue Banking in Bangladesh is to establish a full-fledged TISSUE BANK to cater the needs of various tissue allografts for surgical replacement. Recently Government of Bangladesh has allocated 1.00 million US$ for strengthening of the Tissue Banking Laboratory.

2.4. Food Irradiation

Radiation provide effective means for preservation of foods by controlling the physiological processes that cause spoilage and through eradication of microbial and insect pests. Research and development work at BAEC has involved obtaining substantial basic information on spoilage factors - enzymatic, microbial and chemical - along with evaluation of nutritional and organoleptic quality aspects and toxicological safety of irradiated foods. This approach was necessary since the main objective has been on the extension of post-harvest storage of various items in natural form.

The studies carried out at BAEC have been aimed at the following applications :

(i) Disinfestation of pulses
(ii) Sprout inhibition in potatoes and onions
(iii) Delayed ripening of fruits
(iv) Extension of shelf life of fish and meat
(v) Elimination of food borne pathogens
(vi) Microbial decontamination of pathogens
(vii) Promotion and commercialization of food irradiation technology

Research has been carried out on preservation and shelflife extension of a number of food items such as potato, onion, sweet potato, dried fish, fish, poultry, fruits and vegetables[9,10,11,12,13]. Pilot and semi-commercial scale irradiation and storage studies of promising food items such as potato, onion, dried fish and pulses were carried out in collaboration with the wholesalers and traders (Table 4). Process parameters for irradiating different food items were established (Good Irradiation Practice). Nutritional and toxicological studies have been conducted on irradiated food items. Consumer acceptability trials and test marketing of irradiated products were carried out. It has been found from the test marketing studies that the consumers have accepted irradiated products very favourably, especially when the superior quality and hygiene of the irradiated products are immediately apparent [14].

To provide the legal framework for the commercialization of the food irradiation technology, clearance for irradiation of 13 food items was issued by the Bangladesh Government in 1983 (Table 5).

Based on these developments a demonstration-cum-commercial Cobalt-60 irradiation plant was set up in March 1993 in the port city of Chittagong as a joint venture project (GAMMATECH Limited) of Bangladesh Atomic Energy Commission and Beximco- a leading private company in the country. By May 1997, GAMMATECH Ltd. irradiated 909,943 kg of different food items brought by clients and these items have been marketed successfully both at home and abroad (Table 6). Every year new items are being added to the list of food items being irradiated and marketed (Table 7). GAMMATECH now earns about Tk. 22,00,000 to Tk. 24,00,000 annually as service charge for irradiation and can meet about 95 - 100% of its operational expenses.

Later on, for harmonization of regulation, Bangladesh adopted in 1995 a 'Specification for Authorization of Irradiation by Groups/Classes of Foods' in line with the guidelines framed by ICGFI which has been formed under the aegis of IAEA, FAO and WHO. This will replace the clearance given earlier to irradiation of individual food items.

2.5. Sterile Insect Technique

Large scale laboratory rearing and radiosensitivity of some economically important pests such as, blow flies, fruit flies (e.g. melon fly and oriental fruit fly), jute hairy caterpillar have already been established as a prerequisite for the eradication / suppression of the concerned pests through SIT. A preliminary survey and trapping of blow fly for species identification and density of target pest have been conducted in the off shore island Sonadia[15].

2.6. Biotechnology

Biotechnology work in this field may be broadly classified under two broad heading, (i) upgrading of agro-wastes by radiation and fermentation processing. (ii) development of useful mutants through low dose of gamma irradiation.

Currently work on the following programmes are being conducted at BAEC:

(a) Isolation of good fungal and bacterial strains and to develop good enzyme system for obtaining fermentable sugars from residues like sugarcane, bagasse, jute stick, rice straw, saw dust etc[16, 17, 18].

(b) Effects of radiation and other physical and chemical methods on enzymatic digestion of lignocellulosic wastes[19].

(c) Bioconversion of cellulosic and other residues to different useful products through saccharification and fermentation.

(d) Development of useful mutants through low dose of gamma irradiation of certain microorganisms for citric acid fermentation[20, 21].

2.7. Radiation Vulcanized Natural Rubber Latex

Bangladesh produces about 15,000 metric tons natural rubber field latex. In 1990 the project was taken under the financial assistance of IAEA. Laboratory scale production of concentrated latex production was started with help of the separator machine obtained from IAEA. Two experts from Japan (Dr. K. Makuuchi and Dr. F. Yoshii of JAERI) and one expert from Indonesia Dr. Marga Utama helped to start the project. The characteristic properties of the latex concentrate thus obtained were evaluated and compared to those as required by the ISO. Bangladeshi latexes posses very good quality. The process of vulcanization of natural rubber latex by radiation and sesitizer(chemical used to reduce the vulcanization dose) has been optimized and the optimum radiation dose is around 12 kGy [22-26] shown in figures 5-6. Rubber products like hand gloves, baby teats, balloons, etc. have been prepared with radiation vulcanized latex and these posses good qualities. The storage stability of rubber films has been observed in open air in the laboratory and found that the properties remain good for a long period shown in figures 7-8. By coating the radiation vulcanized latexes on jute mats/ hessian cloths, jute-rubber combination has been prepared and this is expected to enhance the field of application of jute in the market of home and abroad. Some individual small companies have shown interest to adopt the technology. Bangladesh does not produce concentrated latex, so individual manufacturers of dipped products import latex concentrate from abroad. Some private rubber gardens are interested to procure industrial scale separator.

2.8. Hydrogel for medical Application

The polymer based hydrogel material would be prepared using various monomers/polymers and additives by radiation technique for treatment of patients with burn injuries. IAEA would provide financial/technical support to implement the project through BGD/2001/005PL for 2001-2002. Experts from IAEA (Dr. Yoshii F., Japan; Dr. Rosiac J., Poland; Dr. Guven O., IAEA) would help to initiate the project. After completion of the project the product would be produced on large scale in the Pilot Plant

2.9. Surface Coating Curing

A formulated solution is coated on a substrate (wood, leather, hessian cloths etc) and then cured by radiation to make film on the surface of the substrate, selection of coating formulation depends on the nature of the substrate on which coating is made. Flexible coated film is made on the flexible surface like leather hessian cloths, etc. and is prepared with urethane type oligomer in combination with other monomers and additives. Similarly hard coating is obtained using formulation polyester oligomer for the surface of hard substrate like wood and wood products.[27]. Figure 9 shows the properties of cured wood surface containing various percentage of additives (sand in base coat). Bangladesh exports leather worth about Tk. 500 crore each year. Tanning on the raw leather essential to improve properties of raw leather and it is quite expensive. Coating on raw leather improves surface properties and reduces the coating of tanning. The strength of the coated leather is slightly improved and the treated leather can be stretched by 75% more than the untreated leather. Modification of natural rubber, wood, silk by radiation to yield better quality products.

2.10. Wood Plastic Composite

Wood is a versatile engineering material used in almost every sphere of life starting as propeller shaft in nuclear submarine to kitchen as spoon. The use of wood is increased with the growth of industrialization. But production of wood depleting very fast with modernization and high quality wood has almost disappeared while low quality wood faces qualitative problems. WPC project was taken under the financial assistance of IAEA. Low quality woods (simul, korai, kadam, mango, debdaru etc.) after impregnation with monomers (MMA, AN, etc.) and additives (urea salts etc.) are exposed to gamma radiation to prepare wood plastic composites. The wood composite thus prepared has acquired enhanced mechanical strength by more than 90% decreased moisture/water absorption by 50% and its resistance to chemical salt corrosion and attacks by insets microbes etc. It has very low abrasion (wear and adhesion) loss [28-32]. Figure 10 shows the results of WPC prepared by impregnating with monomer MMA containing urea as additive.

2.11. Jute Rubber, Jute Plastic Composites

Jute is a type of natural fiber which grows abundantly in Bangladesh. With the advent of synthetic fibers, use of jute has enormously depleted. With a view to improve properties like, strength, water resistance etc. BAEC has taken works to coat hessian cloths as well as to graft jute fibers/string/treads with RVNRL or monomers and additives. Rubberized jute material has improved water resistance and very good as a packaging material. Polymer coated jute material is durable in dry soil and water but quite degradable in mud and soft day.

2.12. National Polymer Composite Centre

The objective of the project is to achieve design and development of durable and sustainable polymer composites reinforced with polymer resins and indigenous natural fiber and synthetic fibers of glass and carbon origins which can be used to fabricate unbreakable civil structural materials such as sewerage pipes, water pipes, parts of electronic appliances, cables, door and window frames, staircases, tables etc. The project has been approved by the Government of Bangladesh and the cost of the project is approximately US$ 2 million.

3. Regulatory aspect

Bangladesh promulgated in 1993 a "Nuclear Safety and Radiation Control" Act covering all activities related to nuclear sources and devices and use of radioactive materials. Subsequently NSRC rules were gazetted in 1997. Establishment and operations of all radiation processing facilities are governed by the provisions of the National Nuclear Safety and Radiation Control Rules.

4. Conclusion

The application of gamma irradiation for radiation sterilisation of medical products and food irradiation has been fully demonstrated in the country. The areas of production of wood-plastic composites, RVNRL and curing of surface coating are in the process of exploitation on a semi-commercial scale.

Electron beam irradiation techniques for other applications like radiation curing of paints, wire and cable insulation, production of heat shrinkable plastics, vulcanisation of rubber open up new areas which need to be set up and demonstrated in the country.

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Table 1: Estimated sterilisation dose for absorbent cotton wool and surgical gauze and a single recommended dose for cotton based medical products.

Medical Products	Radiation resistant isolate	Maximum initial microbial count (CFU/g)	D_{10} values of the isolates (Mrad)	Estimated safety level sterilisation dose (Mrad)
Absorbent cotton wool	ACR-OR	3.3×10^2	0.213	1.70
	ACR-LU	4.1×10^3	0.298	2.68
	ACR-MI	3.5×10^3	0.220	1.98
	ACR-ME	2.8×10^2	0.215	1.93
	ACR-MU	1.1×10^2	0.187	1.50
	ACR-PE	1.9×10^3	0.201	1.81
Surgical Gauze	SGR-OR	5.1×10^2	0.246	1.97
	SGR-LU	4.9×10^3	0.180	1.62
	SGR-MI	3.7×10^3	0.240	2.16
	SGR-ME	3.4×10^2	0.270	2.16
	SGR-MU	4.6×10^3	0.158	2.42
	SGR-PR	5.9×10^3	0.294	2.42
Recommended*		5.9×10^3	0.298	2.911

*One single dose recommended for cotton based medical products (absorbent cotton wool and surgical gauze), based on maximum contamination level (5.9×10^3 CFU/g) and the highest radioresistance (D_{10} value 0.298 Mrad) of the isolate.

Sterilisation Dose (S.D) recommended = $0.298\ (\text{Log } 5.9\times10^3 - \text{Log } 10^{-6}) = 2.911$ Mrad

Table 2: Determination of biopotency of control and irradiated oxytetracycline HCl powder as done by disk diffusion and turbidimetric method.

Radiation dose (kGy)	Diameter of zone of inhibition (mm) by disk diffusion method (Kirby-Bauer)	Potency (mg/g) turbidimetric method
Control (unirradiated)	8.30	912.00
10	8.25	916.76
25	8.20	921.36
50	8.15	924.74
100	8.30	900.65

Table 3: HPLC retention time and area of control and irradiated samples of Oxytetracycline HCl powder

Radiation dose (kGy)	Retention time (minute)	Area
Control (unirradiated)	15.30	2,06,369
10	15.33	1,95,986
25	15.37	1,98,481
50	15.38	2,02,304
100	15.40	2,02,153

Table 4: Test marketing of irradiated potatoes, onions and dried fish in 1984 – 1988 seasons.

Months	Sales in kg in different seasons			
	1984	1985	1986	1988
Irradiated potatoes				
August	1000	1000	1200	500
September	1600	1500	2000	2000
October	2500	3000	2500	4000
November	1100	800	800	-
Total	6200	6300	6500	6500
Irradiated onions				
September	1000	500	1500	300
October	1800	2000	5500	1000
November	2000	2000	4000	3000
December	700	500	2000	3000
January	500	500	1000	1000
Total	6000	5500	1400	8300
Irradiated dried fish				
July	250	250	500	500
August	250	250	500	500
September	250	250	500	500
October	250	250	500	500
Total	1000	1000	2000	2000

Table 5: Clearance for irradiation of 13 food items by the Bangladesh Government in 1983.

ITEM NAME	CODE & TYPE OF CLEARANCE		DATE	DOSE MAX (kGY)
CHICKEN	3	UNCONDITIONAL	83/12/29	7
CONDIMENTS	2	UNCONDITIONAL	83/12/29	1
CONDIMENTS	3	UNCONDITIONAL	83/12/29	10
FISH	3	UNCONDITIONAL	83/12/29	2.2
FISH (DRIED)	2	UNCONDITIONAL	83/12/29	1
FISH PRODUCTS	3	UNCONDITIONAL	83/12/29	2.2
FROG LEGS	3,5	CONDITIONAL	83/12/29	7
MANGO	1,2	UNCONDITIONAL	83/12/29	1
ONIONS	6	UNCONDITIONAL	83/12/29	0.15
PAPAYA	1,2	UNCONDITIONAL	83/12/29	1
POTATO	6	UNCONDITIONAL	83/12/29	0.15
PULSES	2	UNCONDITIONAL	83/12/29	1
RICE	2	UNCONDITIONAL	83/12/29	1
SHRIMP	3,5	CONDITIONAL	83/12/29	5
SPICES	2	UNCONDITIONAL	83/12/29	1
SPICES	3	UNCONDITIONAL	83/12/29	10
WHEAT	2	UNCONDITIONAL	83/12/29	1
WHEAT PRODUCTS	2	UNCONDITIONAL	83/12/29	1

Explanation for codes: 1. Delay ripening/physiological growth, 2. Disinfestation, 3. Microbial control, 5. Shelf-life extension, 6. Spouting inhibition.

Table 6: Item wise quantity of products irradiated and revenue earned by Gammatech Ltd.

Year / Products	1993 (July-Dec)	1994 (Jan-Dec)	1995 (Jan-Dec)	1996 (Jan-Dec)	1997 (Jan-May)	Total
Food items						
Dried fish (Kg)		129,959	494	154	1,815	132,422 Kg
Frozen foods (Kg)		8,842	319,453	276,193	58,642	663,130 Kg
Beef casing (Kg)		8,842	8,980	10,475	---	28,297 Kg
Bean/pulses (Kg)		---	2,000	---	4,000	6,000 Kg
Flour (Kg)		---	11,940	4,620	---	16,560 Kg
Turtle meat (Kg)		---	---	200	---	200 Kg
Macaroni (Kg)		---	---	33,176	30,158	63,334 Kg
Total (Kg)		**147,643**	**342,867**	**323,818**	**94,615**	**909,943 Kg**
Medical sterilisation						
Shell dressing (pcs)	9,542	---	10,260	---	---	19,802 Pcs
plastic bottle, caps, droppers etc (cft)	886	1,559	1,253	1,992	272	5,962 cft
Magnesium trisilicate (Kg)	7,650	---	5,120	---	---	12,770 Kg
Magnesium Silicate (Kg)	---	---	52,850	51,326	40,065	144,241 Kg
Revenue earned (Tk)	**379,504**	**812,930**	**2,400,000**	**2,300,000**	**680,000**	**6,572,434**

Table 7: Charges for Irradiation (The present charges for irradiating different items by Gammatech are as follows)

1. Potato and onion:	Tk. 0.50/kg	(1.25 US Cent/kg)
2. Dry fish and pulses:	Tk. 3.00/kg	(7.50 US Cent/kg)
3. Frozen fish and shrimp:	Tk. 5.00/kg	(12.50 US Cent/kg)
4. Medical products:	Tk. 250.00/cft	(6.25 US $/cft)

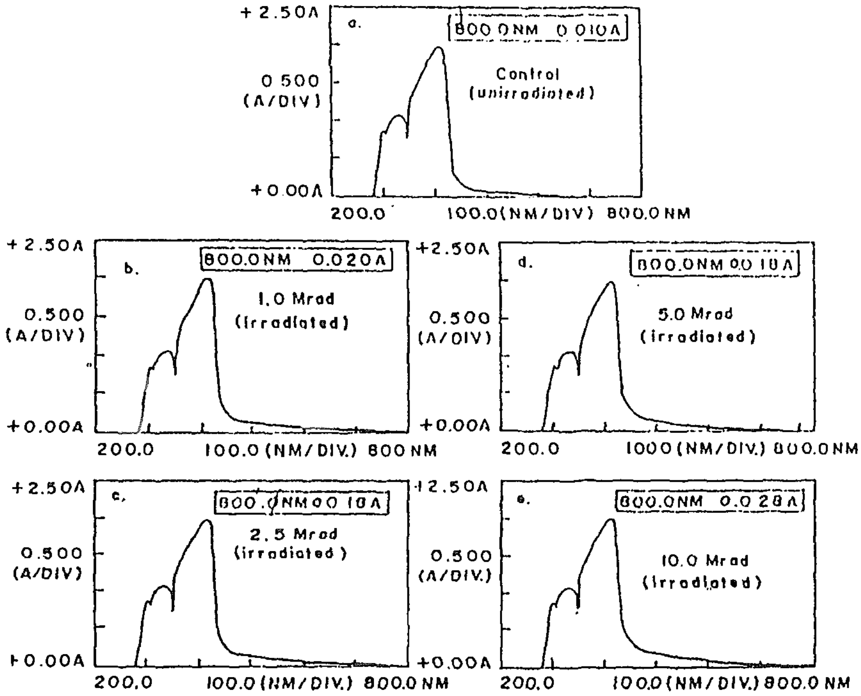

Figure 1. Absorption spectra of control and irradiated oxytetracycline-HCl powder

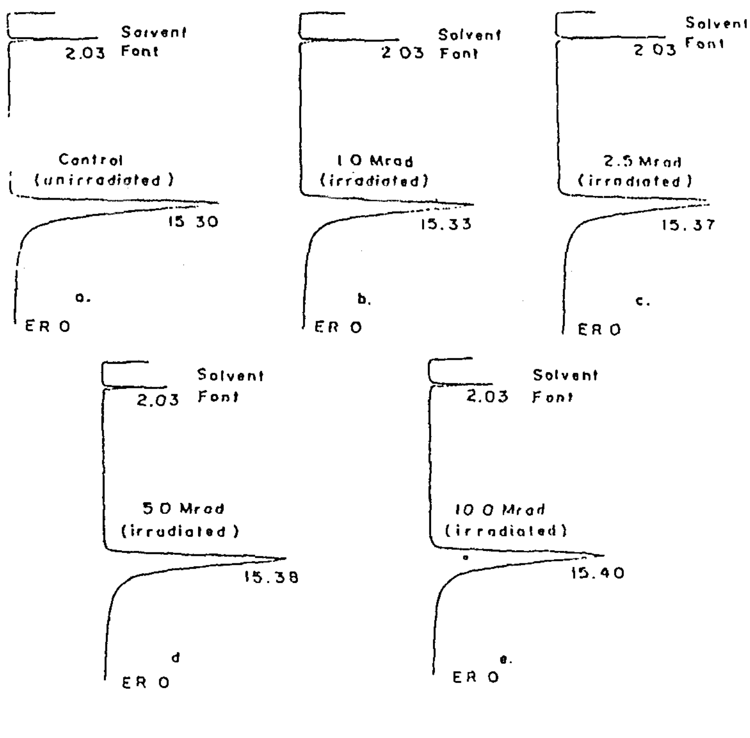

Figure 2. HPLC chromatogram of control and irradiated oxytetracycline-HCl powder

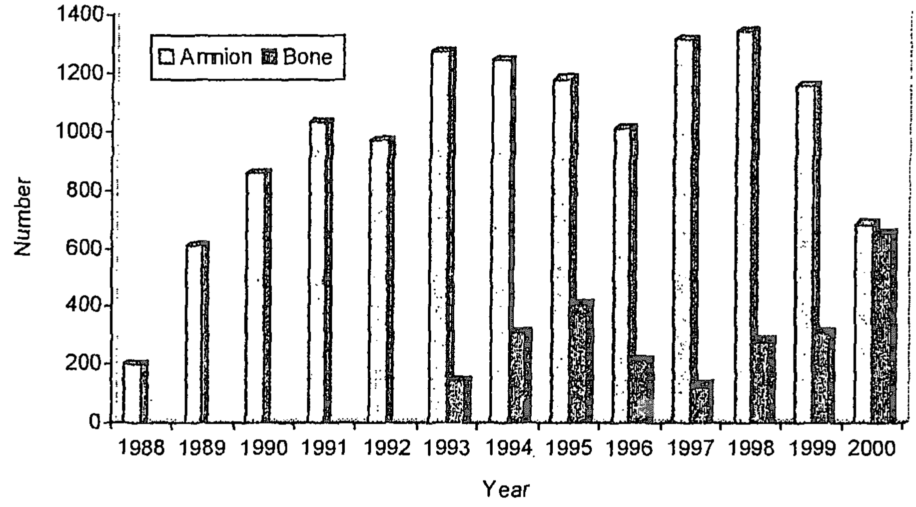

Figure 3 : Bar diagram showing the number of grafts prepeared and sterilization (1988-Sept. 2000).

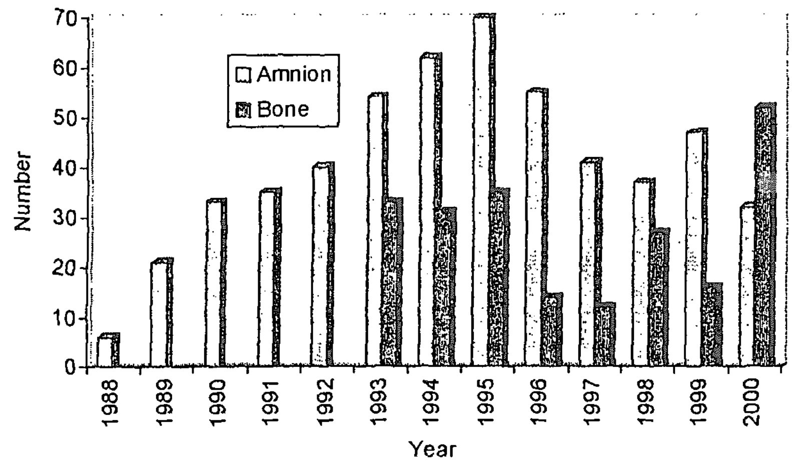

Figure 4 : Bar diagram showing the number of patient treated with radiation sterilized tissue allografts (Amnion & Bone)

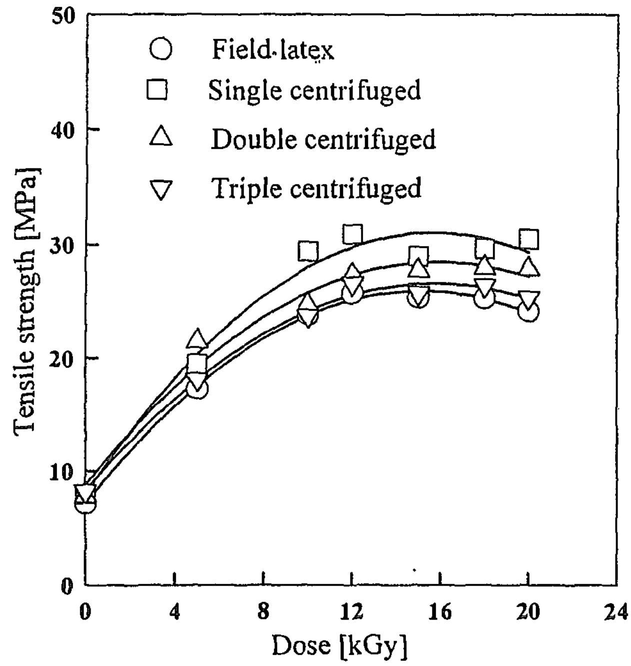

Fig. 5 Tensile strength of RVNRL film at various radiation doses.

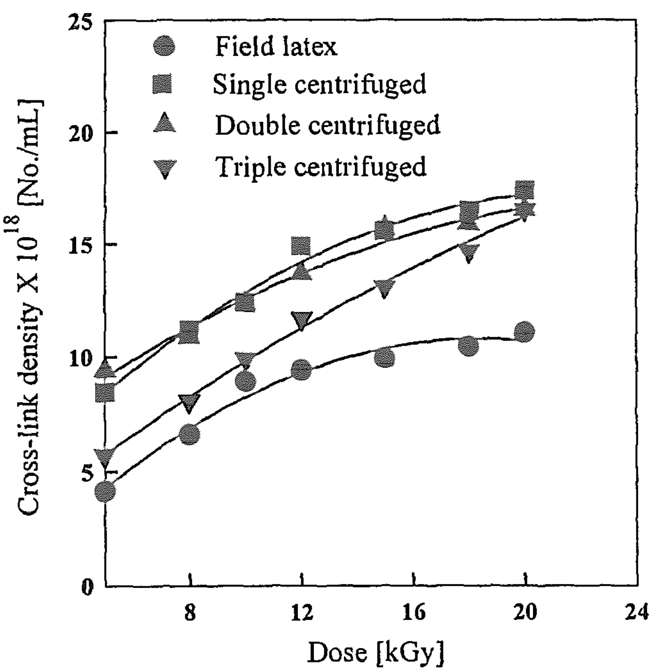

Fig. 6 Cross-link density of RVNRL film at various radiation doses.

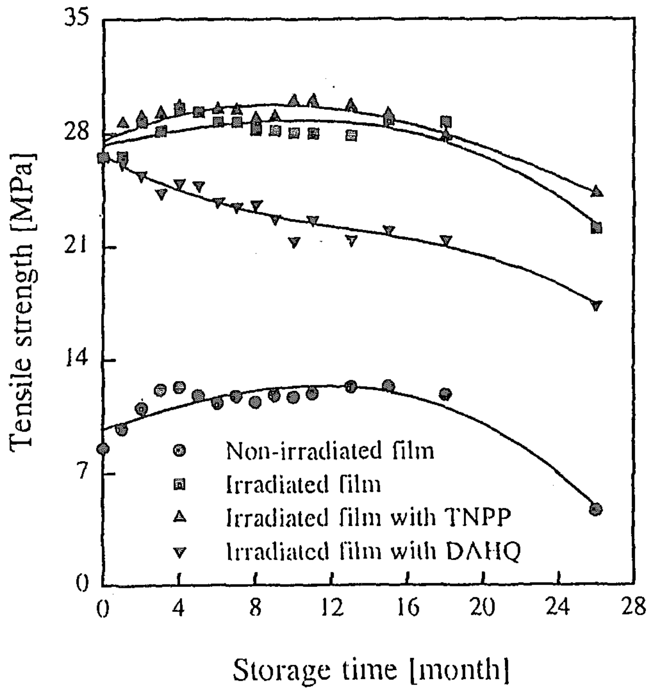

Fig. 7 Effect of storage time in air on the tensile strength of various types of rubber films.

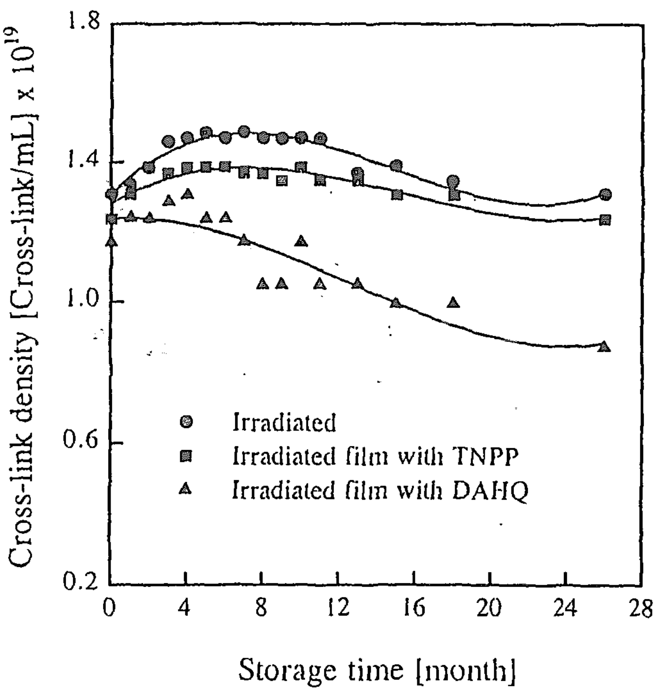

Fig.8 Effect of storage time in air on cross-link density of various types of rubber films.

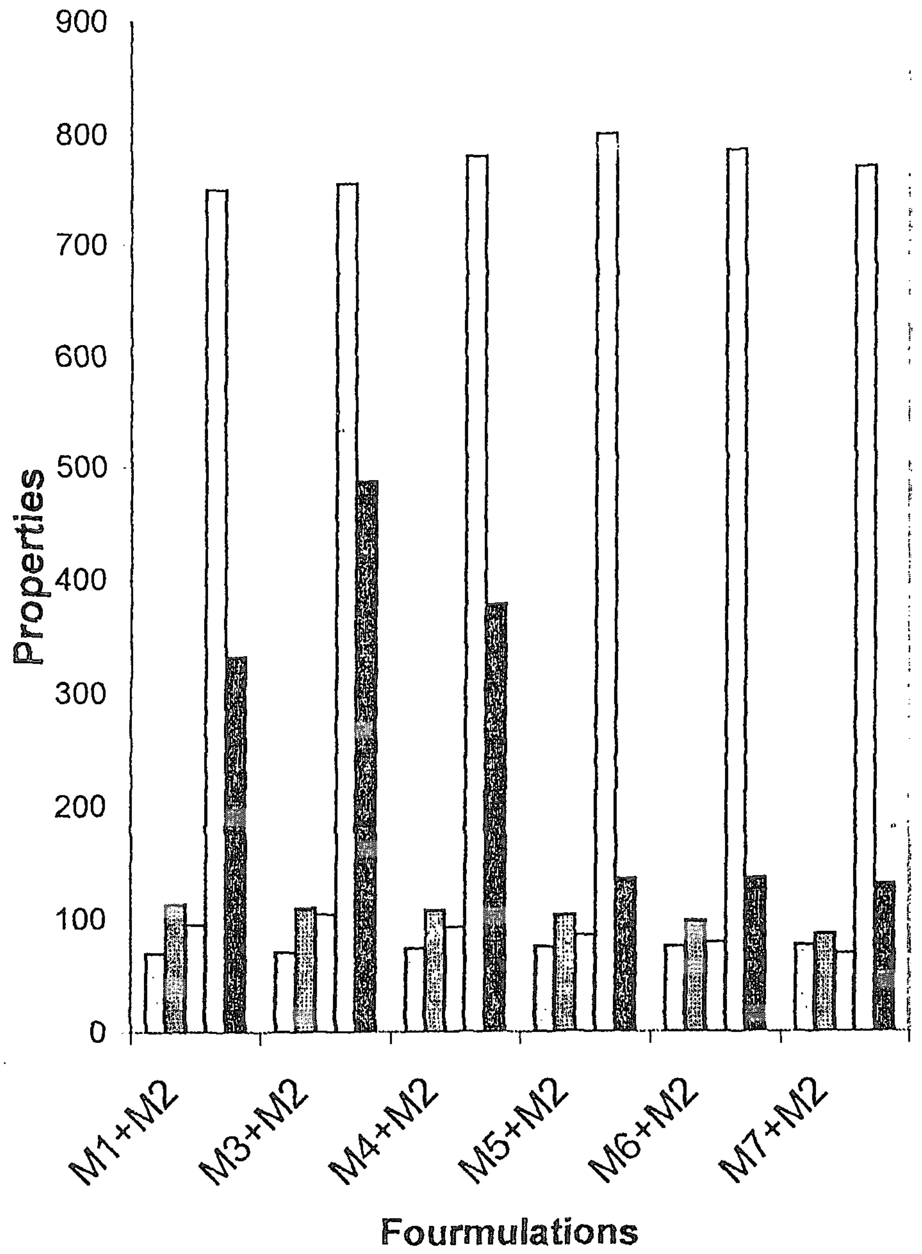

Fig.9 Curing of Surface Coating by UV radiation.

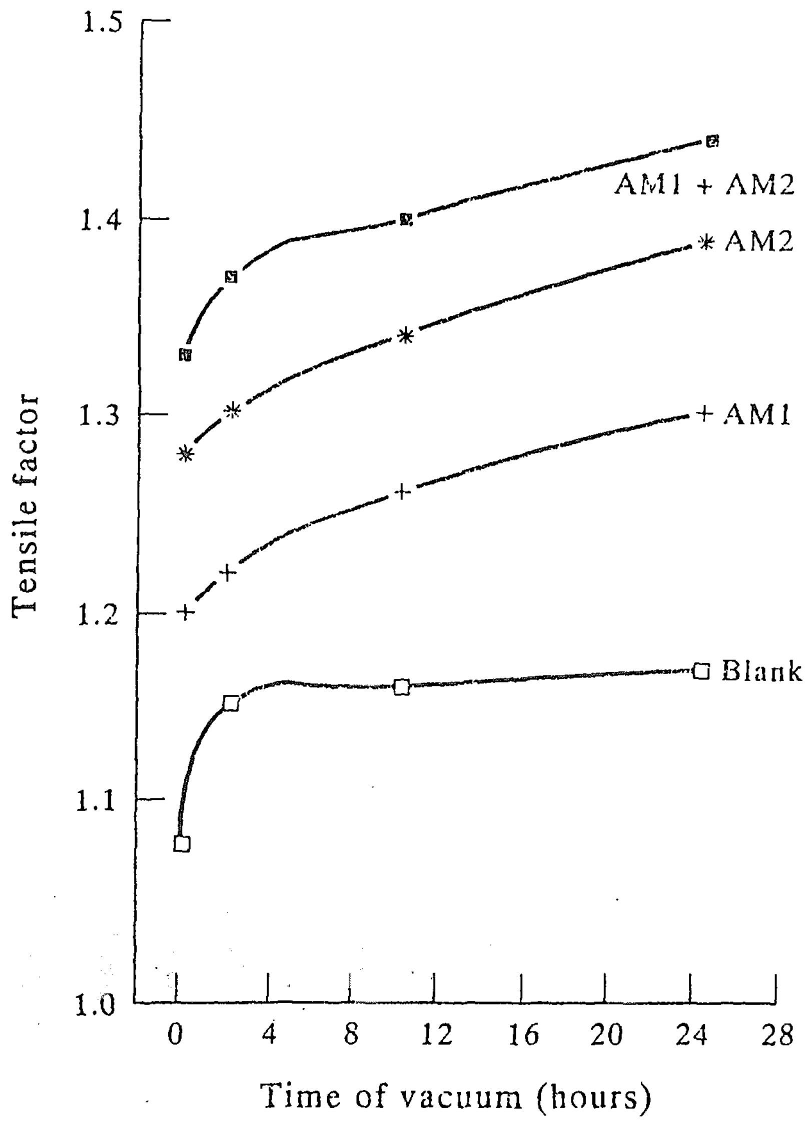

Fig.10 Tensile strength factor of the composites prepared with different impregnating solutions against vacuum time.(TS of simul = 3770 kg/m^2).

25 Status of Radiation Processing in the Philippines

ALUMANDA M. DELA ROSA

Philippine Nuclear Research Institute

Commonwealth Avenue, Diliman

Quezon City 1101 Philippines

Abstract

Radiation processing, one of the well established applications of radiation and radioisotopes, has been successfully integrated into the industrial development of many countries worldwide. Environmental considerations embodied in the Montreal Protocol, the need for energy conservation brought about by escalating costs of fossil fuels, and the bright prospect of utilizing indigenous materials for value-added products have catalyzed the rapid development of radiation processing in the Asian region.

This paper presents an overview of current developments in research activities and commercial applications of radiation processing in the Philippines. The areas of application include radiation sterilization of medical products, food irradiation, radiation vulcanization of natural rubber latex, radiation modification of the marine polysaccharide, carrageenan, and upgrading of cellulosic agriwaste by irradiation.

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[1]Presented at the Takasaki Symposium on Radiation Processing of Natural Polymers, Takasaki, Japan, 23-24 October 2000.

1. Introduction

R & D activities in radiation processing commenced in the mid 1960's with experiments on radiation sterilization of medical products, wood plastic combination, and food irradiation. Some notable results of the early experiments included wood plastic combinates of lower quality wood with monomers, the requisite doses for the radiation sterilization of medical products, pharmaceuticals, and tissue grafts, and the radiation doses for the preservation of food products.

To introduce and demonstrate radiation technology to the local industry, the Philippine Nuclear Research Institute (PNRI), with the technical assistance of the International Atomic Energy Agency (IAEA), established a multipurpose gamma irradiation facility (Fig.1). It is a batch type irradiator, a Gammabeam 651T, from Nordion International Co., Ltd. and it was commissioned in 1989 with an initial loading of 1 PBq 60Co. Presently, the facility has a total 60Co loading of 4.4 PBq [1]. Results of dose intercomparison of PNRI with the International Dose Assurance Service (IDAS) of the IAEA for the past 10 years show that the dose measured by the PNRI Fricke dosimeters agrees to within ± 1.7% of the IDAS estimated dose, while the dose measured by the PNRI ethanol chlorobenzene (ECB) dosimeters is in agreement within ± 4.4% with that of alanine of IDAS (Table I).These intercomparison studies have shown the reliability of the dosimetry system of PNRI using the Fricke and ECB dosimeters [2].

The multipurpose irradiation facility is designed for research and pilot scale studies. Though in a limited scale, some local industries are using it for the sterilization and decontamination of their products. Indeed, the availability of the irradiator has provided the impetus for the development of radiation processing in the country and for the transfer of the technology to the local industry. The areas of application presently being pursued using the irradiation facility are radiation sterilization of medical products, food irradiation, radiation sterilization of tissue grafts, radiation vulcanization of natural rubber latex, radiation modification of carrageenan, and agrowaste irradiation.

Food Irradiation

Current activities on food irradiation are focused on rice and corn, meat and seafood products, mangoes and spices. Table II presents the level of disinfestation of stored rice as a function of dose. A radiation dose of 0.5kGy was found effective for the disinfestation of local varieties of rice and corn [3]. A shelf-life extension of three months for rice and five months for corn was obtained. As shown in Fig. 2, the overall sensory acceptability of milled rice was not affected significantly by a dose of 0.5 kGy. Radiation treatment can prevent about 20% - 30% loss by infestation in these important food products. The National Food Authority (NFA) which operates stored grain warehouses in various regions of the country, and which collaborated with PNRI in this study, is keen in applying radiation disinfestation in its operations.

The increasing incidence of food-borne diseases has become a serious problem of the food industry as it impacts on food safety and public health. The Filipino consumers have been steadily acclimatized to consuming processed foods, hence increasing the risk of contracting food-borne diseases. The effect of irradiation on processed foods such as hotdogs and squid balls was investigated [4]. Results showed that, in the case of hotdogs, a radiation of 2.0 kGy reduced the total microbial load by 2-3 log cycles, and the organoleptic qualities of the product did not differ significantly from the non-irradiated product. A radiation dose of 4-6 kGy reduced the microbial load of squid balls by 2-3 log cycles. The organoleptic qualities of the irradiated product did not differ significantly from the control. Thus, irradiation offers an alternative cold decontamination treatment for foods that are to be marketed raw or are minimally processed.

The recurrent outbreaks of toxic red tide *Pyrodinium* blooms in the Philippines have caused not only a number of mortalities but also economic losses to the coastal fishing industry particularly in the cultivation of green mussels. Post harvest treatments such as thermal processing, depuration did not eliminate or reduce the toxicity of contaminated mussels [5],[6]. Radiation treatment was employed in detoxifying the red tide toxin. Radiation resistance of the paralytic shellfish poisoning (PSP) toxins was determined by subjecting the semi-purified toxin extract as well as the shellstocks of green mussels to various doses of irradiation. The PSP toxin concentration was determined by the standard mouse bioassay. Results showed that D_{10} values depend on the initial PSP toxin concentration. The D_{10} of samples with initially high levels of PSP toxin are lower than those of samples with initially low values of PSP toxin. Thus, the former have D_{10} values of 28 kGy in shellstocks and 18 kGy for the semi-purified toxin. While the latter have D_{10} values of 55 kGy in shellstocks and 43 kGy in semi-purified toxin extract. These data indicate that the redtide toxin can be detoxified by irradiation.

The Philippines has previously established the quarantine dose for the oriental fruit fly. In view of recent claims that there are two species of fruit flies in the Philippines, namely, *Bactrocera philippinensis* and *Bactrocera occipitalis,* the probit 9 experiment is being repeated for each species.

Spices are routinely irradiated on a semi-commercial scale at the multipurpose irradiation facility. Test marketing of irradiated onions have been conducted with positive results.

The Philippine Bureau of Food and Drugs, in cooperation with the PNRI has drafted an administrative order prescribing the regulations for food irradiation. These regulations are based on Codex Alimentarues and patterned after the model harmonized regulations of food irradiation for Asia and the Pacific which was adapted by the ASEAN Ministers of Agriculture and Food through the ASEAN *ad hoc* Working Group on Food Irradiation.

Radiation Sterilization of Tissue Grafts

The Philippines currently maintains two tissue banks, one for bone graft at the University of the Philippines, Philippine General Hospital (UP-PGH), and one for human amnions at the PNRI. Both tissue banks use radiation to sterilize the tissue grafts. One private hospital in Metro Manila is seriously planning to set up its own tissue bank initially for bone grafts.

The three-year experience of the PNRI in the production of radiation-sterilized human amniotic membranes for clinical applications have shown encouraging results [7]. The amnions are collected from the cooperating hospitals, processed, and sterilized with 25 kGy. The blood samples taken from the donors are tested for hepatitis and HIV viruses. From a total of 950 blood samples, about 2% were positive for hepatitis virus. There was no incidence of HIV virus in the total samples analyzed. Samples positive for hepatitis virus were not included in the routine processing of the amnion membranes.

Clinical trials conducted by various surgeons have shown the effectiveness of the radiation-sterile amnion dressing in terms of its ability to reduce pain and increase the rate of healing. For 2^{nd} degree burns, good appearance of the wound was observed after seven days of dressing. In club-foot surgery patients, the wounds have shown complete healing four weeks after surgery. Moreover, epithelialization was achieved within two weeks, and the new skin formed had a smooth texture. In patients with 3^{rd} degree sacral pressure bedsores, the reported success rate of healing for deep wounds was from 15 to 80% after one month of application of the amnion dressing. Fig. 3 and Table II present the clinical applications of the amnion dressing for burns, 3rd degree sacral pressure sores, bedsores, and open wounds of club-foot surgery patients.

Radiation Vulcanization of Natural Rubber Latex

Earlier studies have shown the suitability of the natural rubber latex from Mindanao to radiation vulcanization [8]. The Philippine latex is compatible with RVNRL. The cast films from irradiated latex have good mechanical properties which remain within acceptable limits even after storage of 12 months. The current investigation has been focused on natural antioxidants for RVNRL. Several natural products were tested for their ability to prevent or reduce the oxidative degradation of radiation-vulcanized natural rubber latex [9]. Non-water soluble amino acids such as cystine, asparagine and alanine have good anti-aging effect on RVNRL. Keratin from chicken feathers has a good potential as a natural antioxidant for RVNRL (Table III). Tocopherol, lignin, peroxidase, and polymer-grafted RVNRL proteins did not exhibit any anti-aging properties on RVNRL.

Radiation Modification of Carrageenan

Carrageenans are sulfated anionic polymers which comprise the main structural polysaccharides of red seaweed (*Rhodophyceae*). They are composed of D-galactose units linked alternately with α-1,3 and β-1,4 linkages.

The Philippines is one of the major producers of carrageenan, primarily of the kappa- and iota-types. Several studies have been undertaken with the objective of preparing new products from the degradation of carrageenan by radiation ,and the synthesis of new products from the interaction of carrageenan with monomers and polymers via radiation crosslinking/grafting.

Hydrogels with varying compositions and properties were prepared from carrageenans and the monomers, N-vinyl pyrrolidone and N-isopropylacrylamide [10]. Another set of hydrogels were prepared by irradiating gels of carrageenans with the polymers polyvinylpyrrolidone (PVP) [11] and polyethyleneoxide (PEO) [12]. These hydrogels were characterized with respect to their gel fraction, tensile strength, and swelling behaviors. Effects of temperature, ionic strength, and pH on the swelling properties of the hydrogels were studied. Results showed that the kappa-carrageenan (KC) and the iota-carrageenan (IC) increased the physical and mechanical properties of the hydrogels.

The PVP and NiPAAm-based hydrogels demonstrated strong volume phase transition changes with temperature and pH while the PEO-based hydrogels did not. The carrageenan/PVP hydrogel has been successfully tested clinically as wound dressing . The hydrogels were applied to 20 donor sites of a split-thickness graft (Fig.4). The hydrogel dressings were found to be comparable to the commercially available hydrocolloids that were applied also to the grafts. Healing took place within 7-10 days. The hydrogels were transparent which enabled the doctors to observe the healing process without disturbing the wound. According to the patients, the carrageenan hydrogel dressing had a cooling effect, and decreased the pain significantly.

The KC and IC irradiated at 100 kGy induced weight gain in rice seedlings and bok-choi vegetable. This effect may be attributed to the absorption of the degraded carrageenan by the plant [13].

Other applications of the radiation-modified carrageenan are presently being pursued.

Upgrading of Sugarcane Wastes by Radiation

The sugar industry generates an estimated six tons of cellulosic sugarcane wastes per annum. These are being utilized as fuel for the sugar mills and as organic fertilizers. However, a great quantity of these wastes are either burned or dumped in the environment causing environmental pollution. In order to address this problem, the PNRI, the Sugar Regulation Administration (SRA), and the Bureau of Animal Industry (BAI) have collaborated to undertake this project on upgrading sugarcane wastes to useful products. The project aims to convert sugarcane wastes to value added products such as substrates for the growing of mushrooms and as animal feeds. The potential use of sugarcane wastes as substrate for the growing of mushrooms is rather high in view of the fact that the current demand for mushrooms in urban areas is more than the current supply at four tons/day. On the other hand, the total imported feed ingredients grow annually at 17-25%.

Initial work [14] has been conducted by irradiating one batch of ground fresh sugarcane bagasse as well as mudpress, and autoclaving another batch at 121°C, 15 psi for 40 minutes. These were inoculated with the mushroom spawn seeds (*Pleurotus* sajur caju and *P. florida*) obtained from commercial mushroom growers. Microbiological evaluation indicated that 15 kGy reduced the bacterial load by 3 log cycles (108 cfu/g to 105 cfu/g). The physico-chemical evaluation showed that the crude protein content was higher in the irradiated batch, but the crude fiber content was decreased compared to the autoclaved batch. The average total number of flushes and the total number of umbrella produced were the same in both irradiated and autoclaved batches. Further experiments are presently being conducted to verify the earlier results.

The PNRI has included this project under the Department's flagship project on clean technologies and on assistance to the poor . The success of this project will redound to greater benefit for the marginal laborers in the sugar industry as the products from the sugarcane wastes can extend the income of the laborers.

Radiation Sterilization Of Medical Products

The local medical and pharmaceutical industry has adopted the technology albeit in a limited scale due to the limitation of the existing irradiation facility. The medical products that have been irradiated in the multi-purpose irradiation facility include rubber gloves, empty aluminum tubes, empty gelatin capsules, empty plastic bottles, orthopedic implants and cosmetic raw materials, carrageenan powder.

In a technology park south of Metro Manila, Terumo Philippines has recently established a syringe plant with 10 MeV electron beam facility for in-house radiation sterilization, construction of the plant started in October 1998 and operation commenced in the early part of 2000. It is expected that their products will be mostly for export.

Conclusion

Results of over 30 years of R&D clearly show the relevance and adaptability of radiation processing as a tool for technological and socio-economic development of the Asian region. The Philippine experience further shows the importance of the requisite facility in nuclear technology. A commercial facility is now imperative to bring about full transfer of technology to the local industry.

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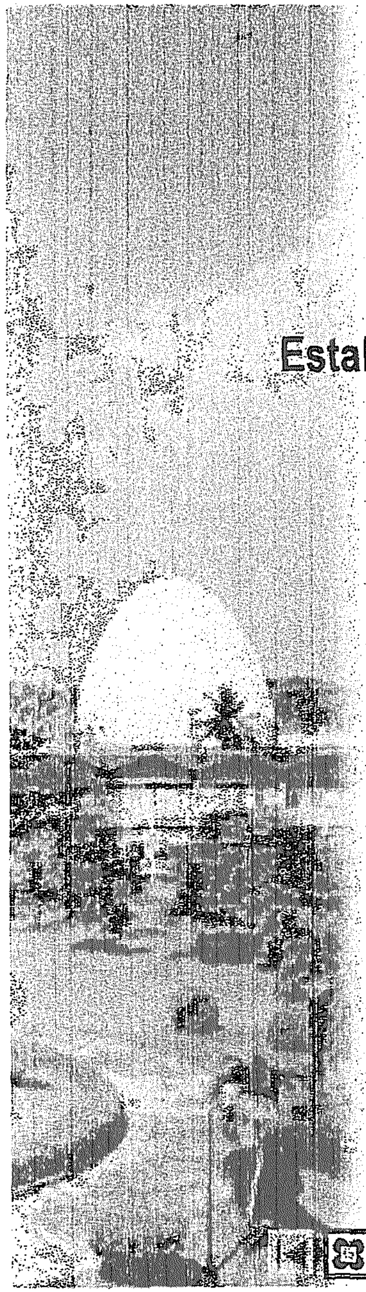
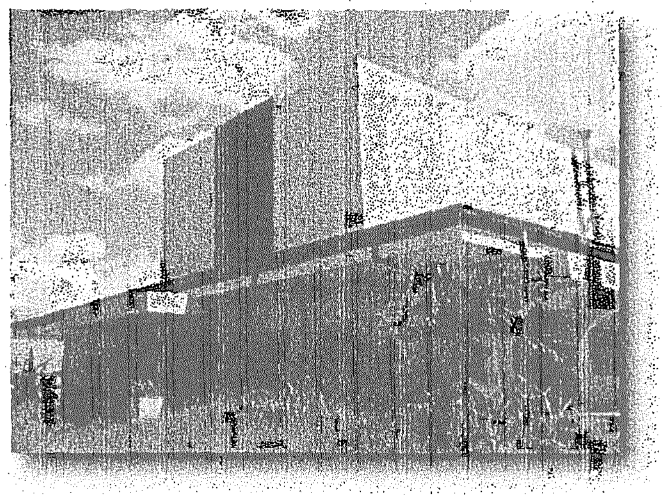


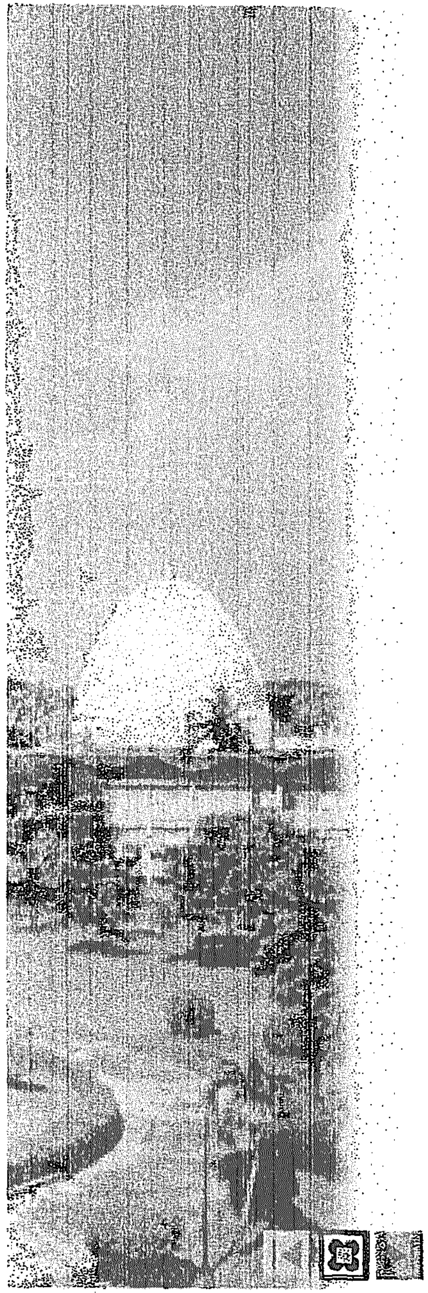
High Priority Flagship Programs

Integrated R&D Program on Clean Technologies

Establishment of Commercial Gamma Irradiation Facility

- To meet the present and future demand of industry and to promote wider application of clean technologies with the use of radiation
- At present, the PNRI, the National Development Company and the private sector are keenly working together to pursue the construction of a commercial-scale irradiation facility.



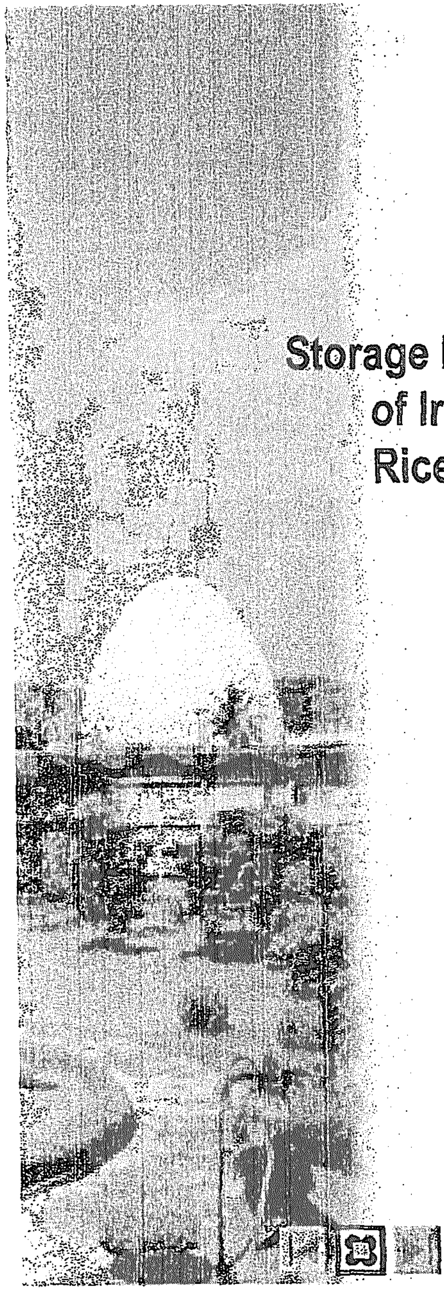


High Priority Flagship Programs

Integrated R&D Program on Clean Technologies

Results of Intercomparison with IDAS

	Dosimeter	PNRI Nominal Dose (kGy)	IDAS Estimated Dose (kGy)	Deviation (%)
1989	ECB	14.50	13.80	5.07
	Fricke	0.2147	0.2095	2.48
1990	ECB	12.3	11.8	4.24
	Fricke	0.16311	0.161	1.31
1991	ECB	20.45	19.4	5.41
	Fricke	0.214	0.216	-0.93
1993	ECB	6.00	5.701	5.24
	ECB	26.32	24.958	5.46
1994	ECB	26.9	25.83	4.14
	Fricke	0.2	0.2047	-2.3
1995	ECB	24.47	25.13	-2.63
	Fricke	0.203	0.207	-1.93
1996	Fricke	0.2004	0.202	-0.79
1997	ECB	5.4	5.58	-3.4
	ECB	25.4	26.94	-5.7
1998	ECB	6.006	6.069	-1.04
	ECB	24.00	25.68	-6.54
	Fricke	0.1997	0.2043	-2.25



High Priority Flagship Programs

Integrated R&D Program on Clean Technologies

Storage Evaluation of Irradiated Rice and Corn

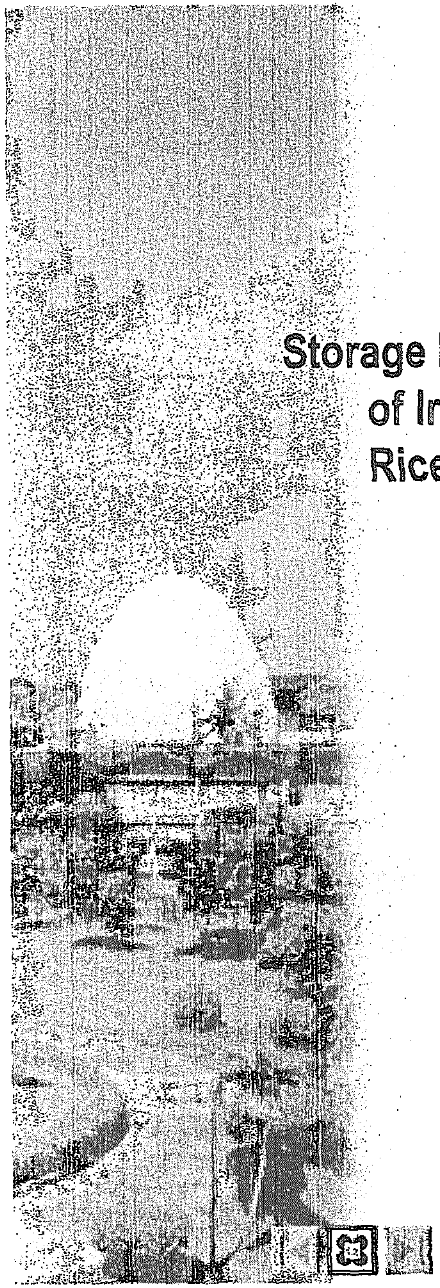
Comparison of the No. of Adult Insects on Irradiated and Non-irradiated **Rice** during Storage at Ambient Temperature

DOSE (kGy)	PACKAGING	STORAGE PERIOD (months) 0	1	2	3	4	5	6	7	9
0	1**	0.33	0	0	0	2.33	2.33	20.0	15.7	9.7
	2	0.66	2.0	21.0	56.0	0.67	0	0.6	13.4	0
	3	0	6.0	6.34	8.67	0	0.6	2.6	0	8.3
0.5	1	0	0.33	0.33	0	0	0	2.33	8.0	14.7
	2	0	0	0	0	0	0	0	0	0
	3	0	0	0.33*	0	0	0	0	0	0
1.0	1	0	0.330	0	0	0	0	1.0	2.3	3.0
	2	0	0.33*	1.33*	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0

Comparison of the No. of Adult Insects on Irradiated and Non-irradiated **Corn** during Storage at Ambient Temperature

DOSE (kGv)	PACKAGING	STORAGE PERIOD (months) 0	1	2	3	4	5	6	7	9
0	1**	11.0	1.5	15.0	27.0	17.5	34.0	25.0	29.0	29.0
	2	0.5*	0	3.0	145.0	0	1.5*	17.5	55.0	41.5
	3	6.5	2.95	4.0	86.1	0	12.0*	84.5	12.0	0
0.5	1	0	10.5	0	0	1.0	1.0	2.0*	4.0	21.0
	2	0	0	0	2.0*	0	0	2.0*	0	0
	3	0	1.0*	0	6.0*	0	0	5.5*	0	0
1.0	1	0.5*	1.0*	0	0	0	0	0	4.0	27.5
	2	0	1.5*	0	0	0	0	0	0	0
	3	0	0	0	2.5*	0	0	2.5*	0	0

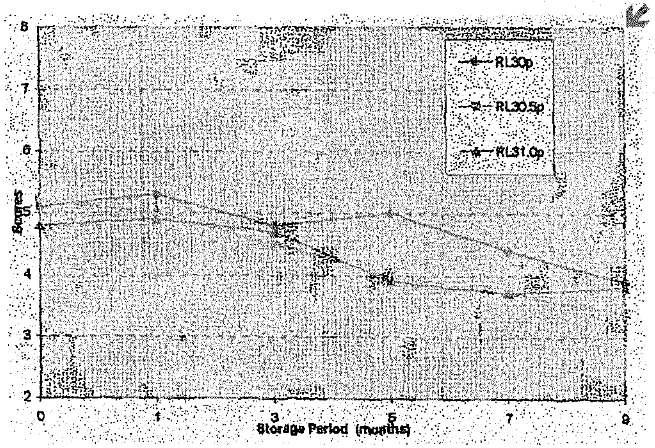
* Dead insects
** 1- in polypropylene sack, 2- in polyethylene bag, 3- in polypropylene sack line with polyethylene bag



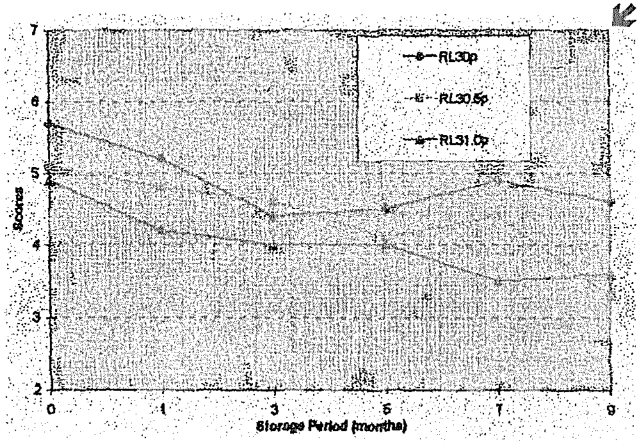
High Priority Flagship Programs

Integrated R&D Program on Clean Technologies

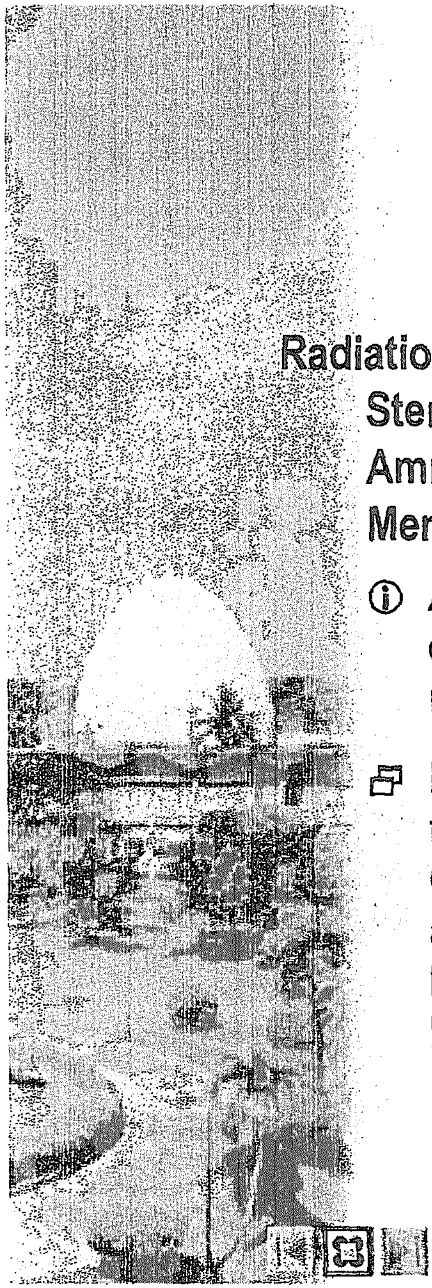
Storage Evaluation of Irradiated Rice and Corn



Sensory Quality (Acceptability) of Irradiated **Raw Rice**



Sensory Quality (Acceptability) of Irradiated **Cooked Rice**

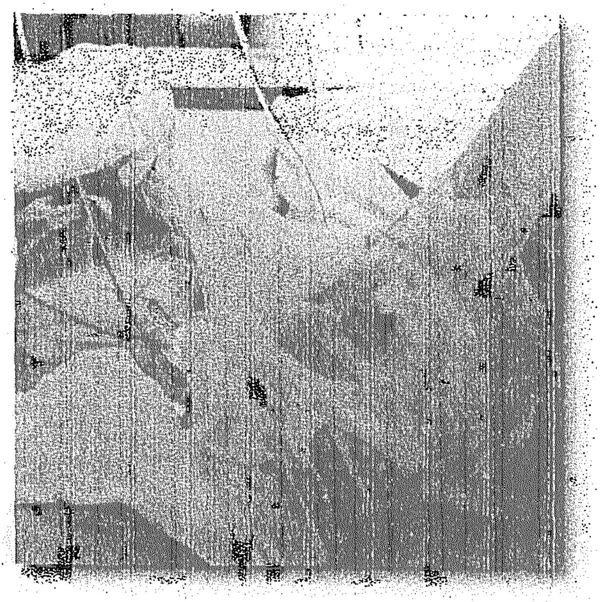


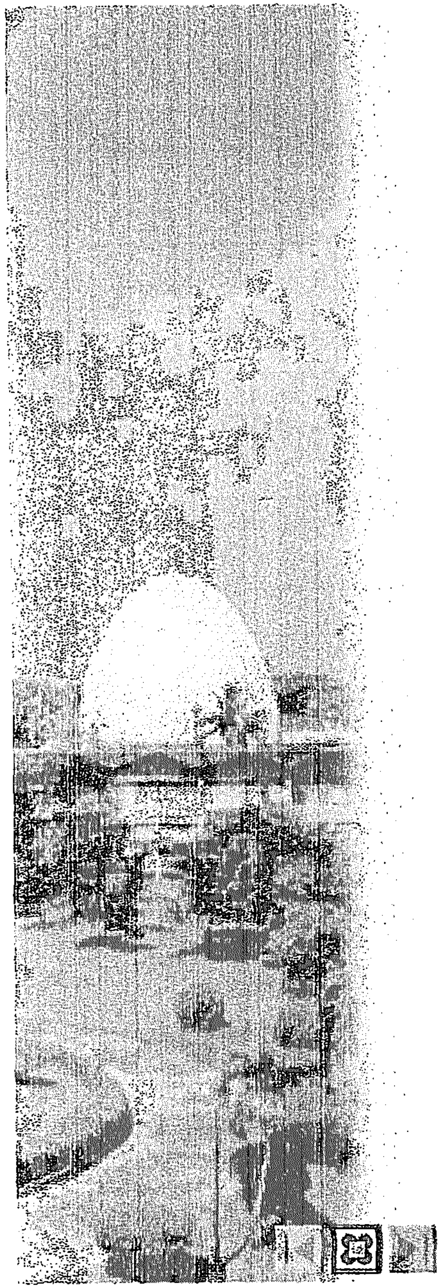
High Priority Flagship Programs

S&T Intervention Program for the Poor, Vulnerable and Disabled

Radiation Sterilization of Amnion Membranes

- A study to provide continuous supply of radiation-sterilized amnion membranes for clinical applications
- Burn victims of the recent Meralco transformer explosion in Caloocan City have benefited from the application of amnion membrane biologic dressing processed by PNRI.





High Priority Flagship Programs

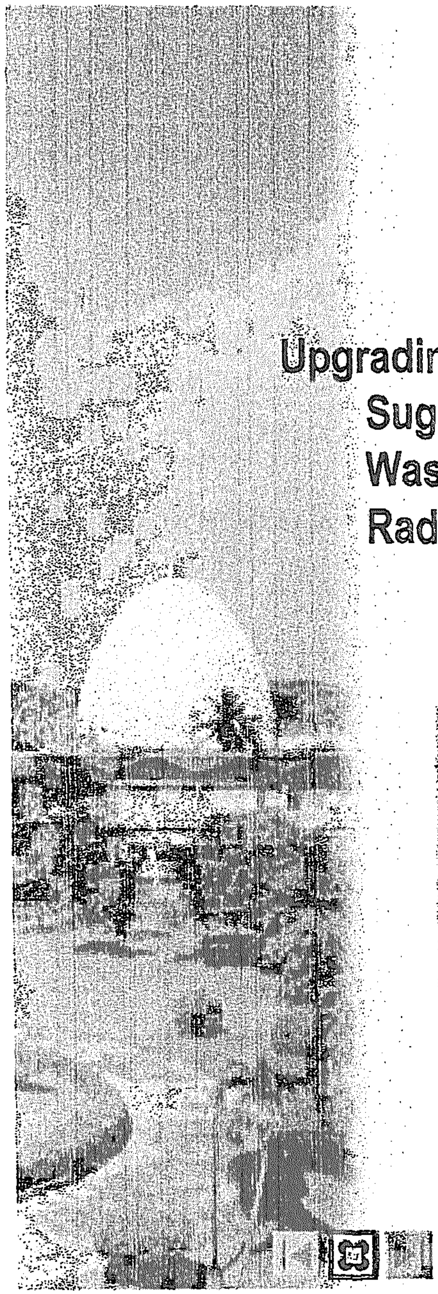
S&T Intervention Program for the Poor, Vulnerable and Disabled

Clinical Applications of Radiation-Sterilized Human Amnions Dressing (1999 – 2000)

Hospitals	No. of Amnions	No. of patients served		Purpose
		1999	2000	
Philippine Orthopedic Center	883	35	31	Club foot surgery, bedsore, open wounds
Veterans Hospital	165	-	11	Bedsore
Chinese General Hospital	121	5	3	Burns
Rizal Medical Center	109	8	8	Burns
Jose Reyes Medical Center	158	-	7	Burns
New Era Hospital	20	2	-	Bedsore
Capitol Medical Center	68	4	-	Burns
Fairview Hospital	50	2	4	Burns
TOTAL	1574	56	64	

High Priority Flagship Programs

Integrated R&D Program on Clean Technologies



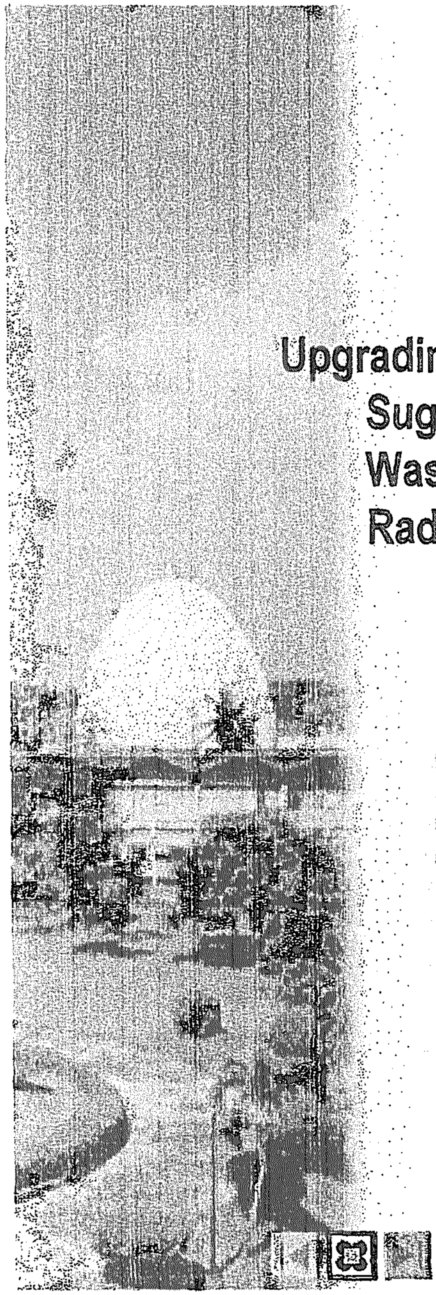
Upgrading of Sugarcane Wastes Using Radiation

Physico-chemical components	Components (%)
moisture content	40 - 55
crude protein	1.97 - 2.10
pH	3.4 - 4.2
crude fiber	40 - 45
water holding capacity	8 - 11

Physico-chemical component of fresh bagasse collected from sugar mills

Storage time (week)	No. of bag	pH	Moisture (%)
0	1	6.5	65.0
	2	6.2	70.0
	3	6.0	65.0
1	1	6.8	68.0
	2	6.5	65.0
	3	6.5	65.0
2	1	7.0	68.0
	2	7.0	64.0
	3	7.0	64.0

Effect of pH and moisture content on stored bagasse substrate at room temperature



High Priority Flagship Programs

Integrated R&D Program on Clean Technologies

Upgrading of Sugarcane Wastes Using Radiation

Production/Yield	Irradiated	Autoclaved
%Fruited bags	52.8 (80 bags)	45.3 (80 bags)
Total yield (gm)	404.1	543.7
Ave. yield/bag (kg)	4.94 (5.0)	4.37 (4.4)
Total no. of umbrellas	231	223
Ave. no. of umbrellas	8.6	10.5
Days of 1st flush	34-36	30
Ave. no. of flushes	1.8 (2.0)	2.048 (2.1)

Yield of mushroom on irradiated and autoclave sugarcane bagasse substrate

	Crude Protein (%)		Crude Fiber (%)	
	Autoclaved	Irradiated	Autoclave	Irradiated
Unfermented	2.2	6.6	13.1	11.9
Fermented				
(P. sajur)	2.4	6.8	7.1	4.3
(P. florida)	4.0	5.4	8.5	4.7

Crude fiber and protein content of fermented and unfermented sugarcane bagasse

26 Utilization of Natural Polysaccharides by Radiation in Korea

Lee, Young-Keun and Young-Chang Nho
Korea Atomic Energy Research Institute, Yusong-ku DuckJin-dong 150, Taejon 305-606, Korea

Abstract

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

Introduction

The principal function of a wound dressing is to provide an optimum healing environment. For example, a wound must be isolated from the external environment before healing can begin. A wound dressing covers the wound mimicking the natural barrier function of epithelium. To provide an optimum healing environment, a wound dressing should control bleeding, protect the wound from the external environment, prevent further contamination or infection and maintain a moist micro-environment next to the wound surface.

Hydrogels consist of the hydrophilic polymer which forms a three dimensional network with much water in this network. They are one of the most promising materials for biomedical applications, and have several advantages and characteristics for wound dressing, contact lenses, drug delivery systems and so on. Due to their biocompativility with blood, body fluids and tissue[1,2,3]. They sustain their mechanical strength and contain over 75% water. Their water adsorption is due to hydration which is related to chemical groups such as -COOH, CONH2, -CONH-, SO3H, capillary effect, and osmotic pressure[4]. These hydrogels also undergo volume change, do not dissolve, and have stable strength, which is caused by ionic, hydrophobic and van der waals forces, but their effects are small and covalent bonding is prevalent[5]. In fact, hydrogels have been prepared by using chemical methods for a long period. However, in recent years, irradiation techniques to produce hydrogels has been used increasingly around the world. This simple and compact technology is so convenient. Physical and mechanical properties can be manipulated easily by irradiation dose[6,7]. An ideal hydrogel wound dressing must have the basic features of absorbtion of the exudates, prevention of excessive loss of body fluids, good adhesion to the wound, non-toxicity and prevention of infection[8].

In this work, attempts were made to obtain hydrogels for wound dressing which consisted of PVA, PVP and chitosan. In these studies, radiation crosslinking was used to develop hydrogels for wound dressing. Hydrogels from a mixture of chitosan and PVA/PVP were made by "freezing and thawing", or exposing to 60Co gamma-ray or two steps of "freezing and thawing" and 60Co gamma-ray irradiation. Mechanical properties such as gelation, water absorptivity, and gel strength were examined to evaluate the hydrogels for wound dressing.

Experimentals

1. Materials

PVA(Mw: 8.5× 104 - 1.46× 103) was supplied by Aldrich Chemical Co.. PVP [8] of average molecular weight was purchased from Sigma & Aldrich Chemical Co., MO, USA. These polymers were used without further purification. The water used as a solvent in all experiments was distilled water. Chitosan was obtained from Jakwang Co. Ltd. Seoul, Korea.

2. Preparation of Hydrogels

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

3. Gel content

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

Gel (%) =rm W _d over Wi TIMES 100

(1)

4. Degree of swelling

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

Water absorptivity = rm W_s - W_d over W_d TIMES 100

(2)

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

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

Gel Strength = rm F_B times TRIANGLE D

(3)

Results and discussion

Up to and including the late 1950's, it was generally accepted that. In order to prevent bacterial infection, a wound should be kept as dry as possible. However, a variety of studies have questioned this philosophy and found that wounds that were kept moist actually healed more rapidly than those that were left exposed to the air or covered with traditional dried dressings. In a review of the properties of occlusive dressings, W. H. Eaglestein[9] concluded that occlusive dressings that keep wounds moist could increase the rate of epidermal resurfacing by some 40%.

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

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

Figure 1 shows gelation behavior of the hydrogels which were synthesized by repeated "freezing and thawing". The PVA/PVP: chitosan ratio was in the range 9:1 - 7:3, and the solid concentration of the total PVA/PVP/chitosan solution was 15wt%. The composition of PVA:PVP was fixed as 60:40. Gel content increased as chitosan concentration in PVA/PVP/chitosan increased, and "freezing and thawing" was repeated. "Freezing and thawing" caused gel content to be enhanced greatly for up to 4 cycles of "freezing and thawing", after that, its increase levelled off. It is well known that this procedure of PVA[10] results in the formation of crystallites that serve as physical crosslinks to render the material insoluble in water. Gel content(%) in this experiment was continuously decreased as the composition of chitosan in PVA/PVP/chitosan increased because chitosan is not crosslinked by "Freezing and thawing". Figure 2 shows the swelling behavior of the same hydrogels as shown in Figure 1. The swelling percentage was inversely proportional to the gel percentage because crosslinking density increases with increasing gelation.

Gamma irradiation doses of 25, 35, 50, 60 and 70kGy were exposed to an aqueous mixture of PVA/PVP/chitosan to evaluate the effect of irradiation dose on the gel content(%) and swelling behavior of hydrogels(Figure 3, 4). Gel content was influenced slightly by PVA/PVP:chitosan composition and irradiation dose, but swelling was influenced greatly by them. When the radiation process or "freezing and thawing" process was used to obtain hydrogels, the difference between the two processes is that gel content is influenced by PVA/PVP:chitosan composition in the case of the "freezing and thawing" process, but is not influenced by PVA/PVP:chitosan composition in the case of irradiation. This can be explained by the fact that the crosslinking network can be formed between PVA/PVP molecules and chitosan molecules when the irradiation process is used. The gels result from the coupling of the polymer radicals which were directly and indirectly produced from PVA, PVP or chitosan by gamma rays. The indirect formation of polymer radicals is mainly due to the H. and .OH radicals arising from water molecules, both of which abstract the hydrogen atoms to form polymer radicals. There are two types of crosslinking processes: intermolecular and intramolecular crosslinking. The former allows the increase of the Mw of crosslinked polymer via the coupling of two or more polymer radicals. The latter does not alter the Mw, but affects the quantities relating to a polymer chain dimension because the radical coupling reaction should take place within the same polymer chain[11].

Gelation of hydrogels was higher when two steps of "freezing and thawing" and irradiation were used, than when only "freezing and thawing" or irradiation was utilized(Figure 5,6).

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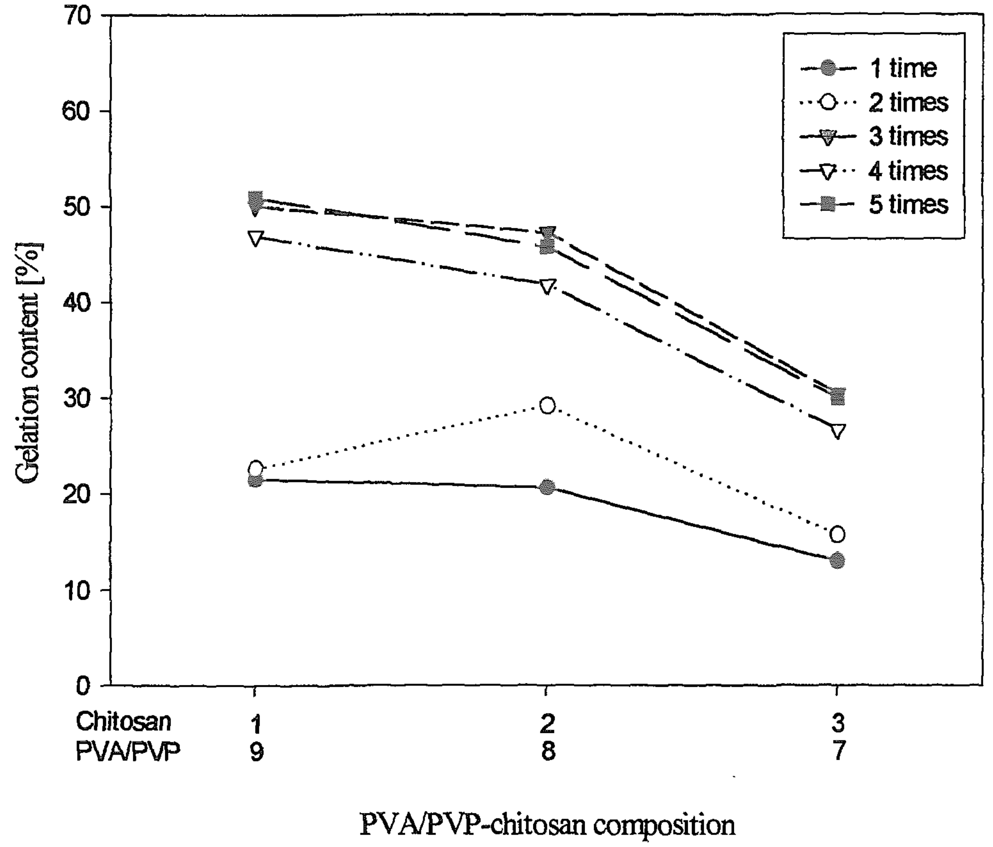


Figure 1. Gelation content of PVA/PVP-chitosan hydrogels vs. PVA/PVP-chitosan composition after repeated freezing and thawing without irradiation doses.
(PVA : PVP = 6 : 4, solid concentration 15wt%.)

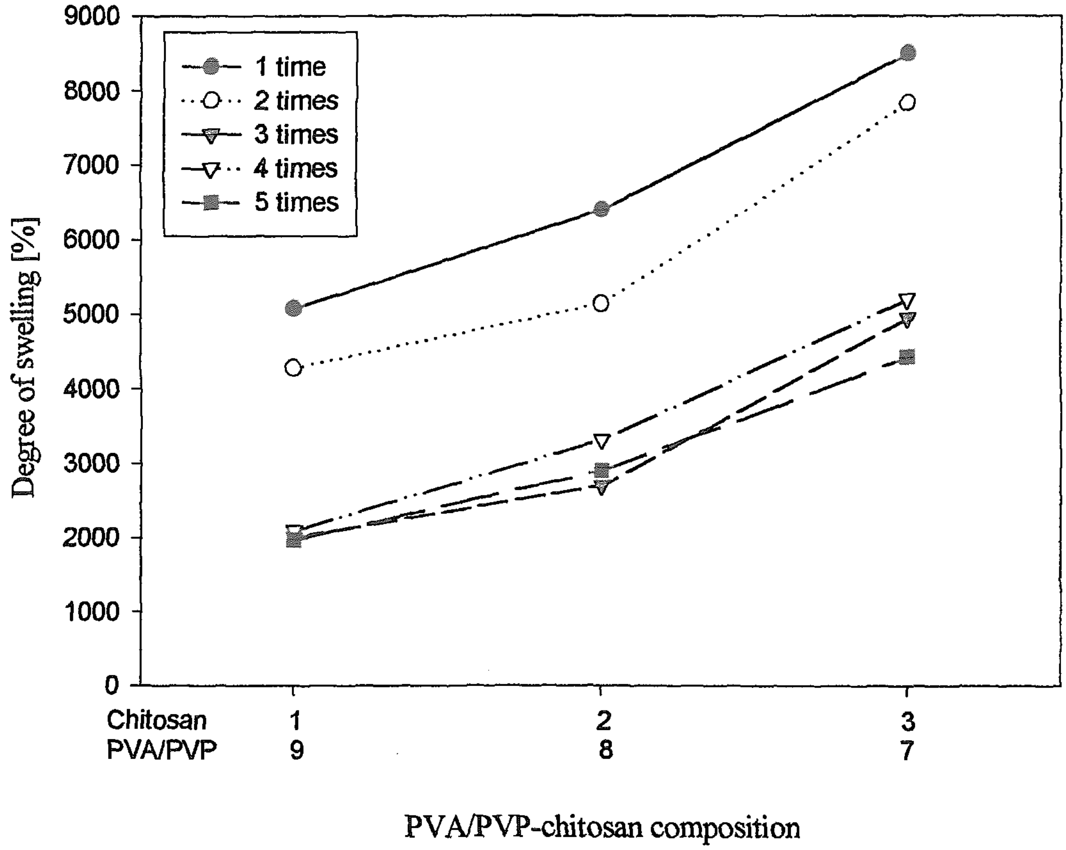


Figure 2. Degree of swelling of PVA/PVP-chitosan hydrogels vs. PVA/PVP-chitosan composition after repeated freezing and thawing without irradiation doses.
(PVA : PVP = 6 : 4, solid concentration 15wt%.)

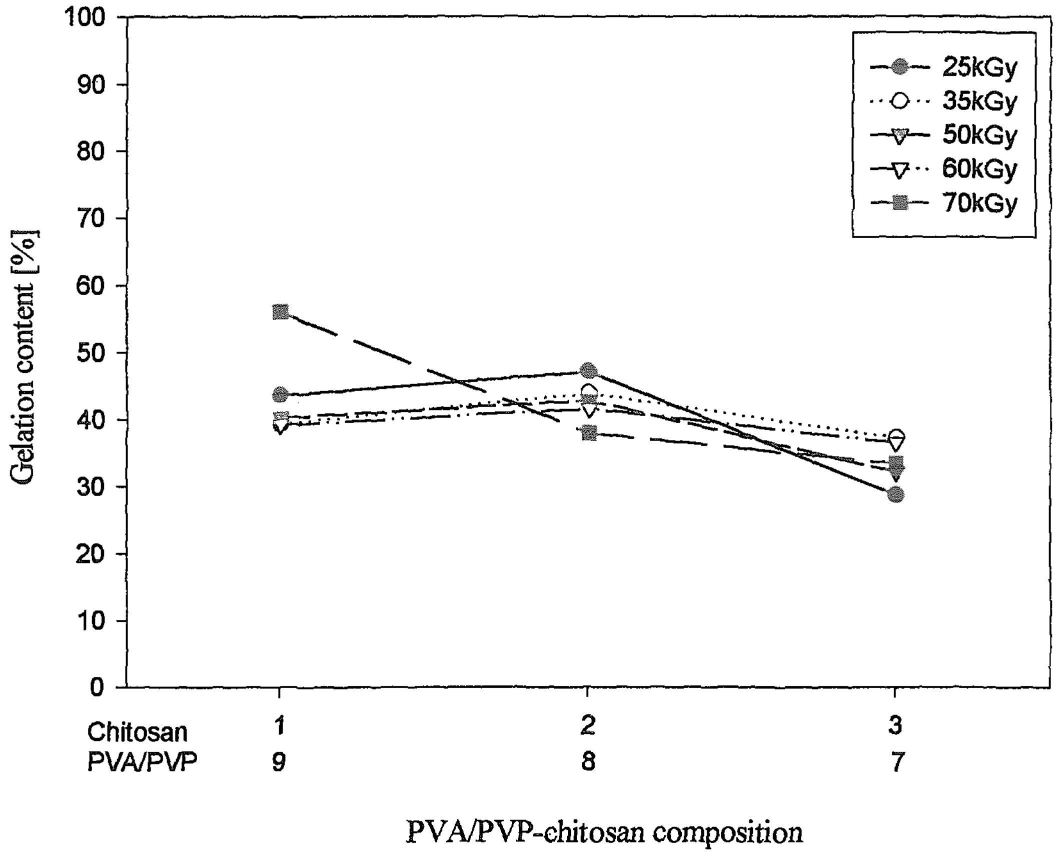


Figure 3. Gelation content of PVA/PVP-chitosan hydrogels vs. PVA/PVP-chitosan composition at different irradiation doses. (PVA : PVP = 6 : 4, solid concentration 15wt%.)

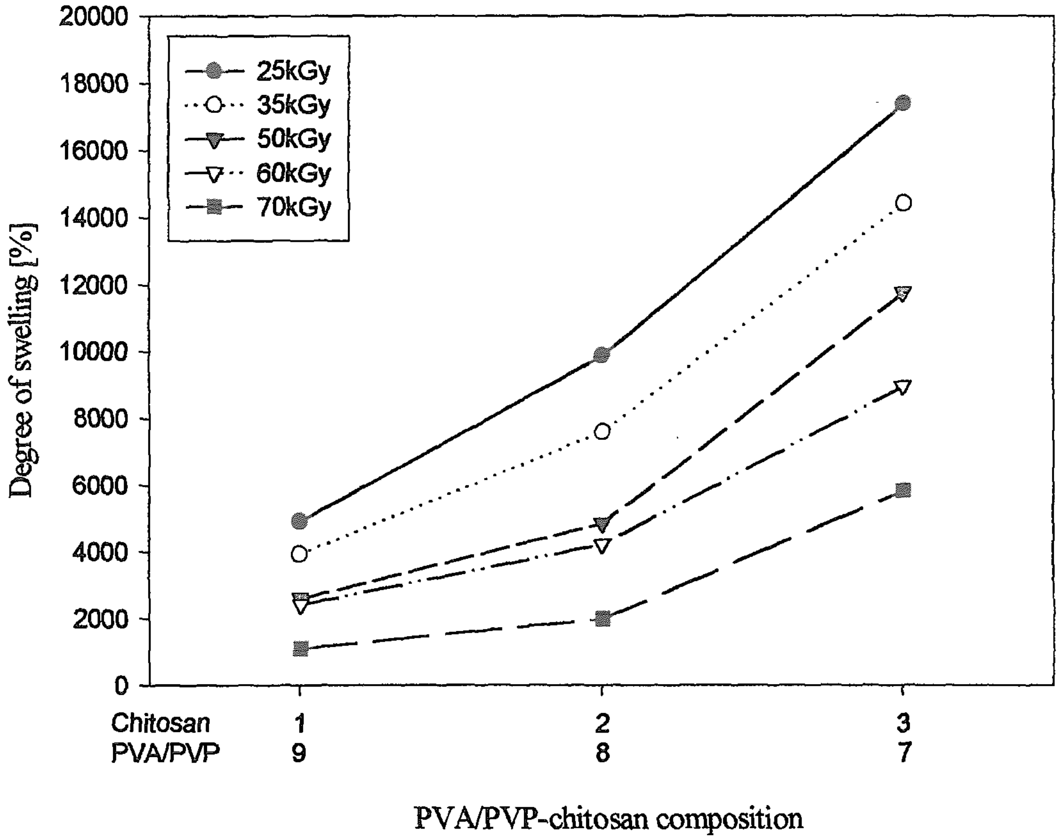


Figure 4. Degree of swelling of PVA/PVP-chitosan hydrogels vs. PVA/PVP-chitosan composition at different irradiation doses. (PVA : PVP = 6 : 4, solid concentration 15wt%.)

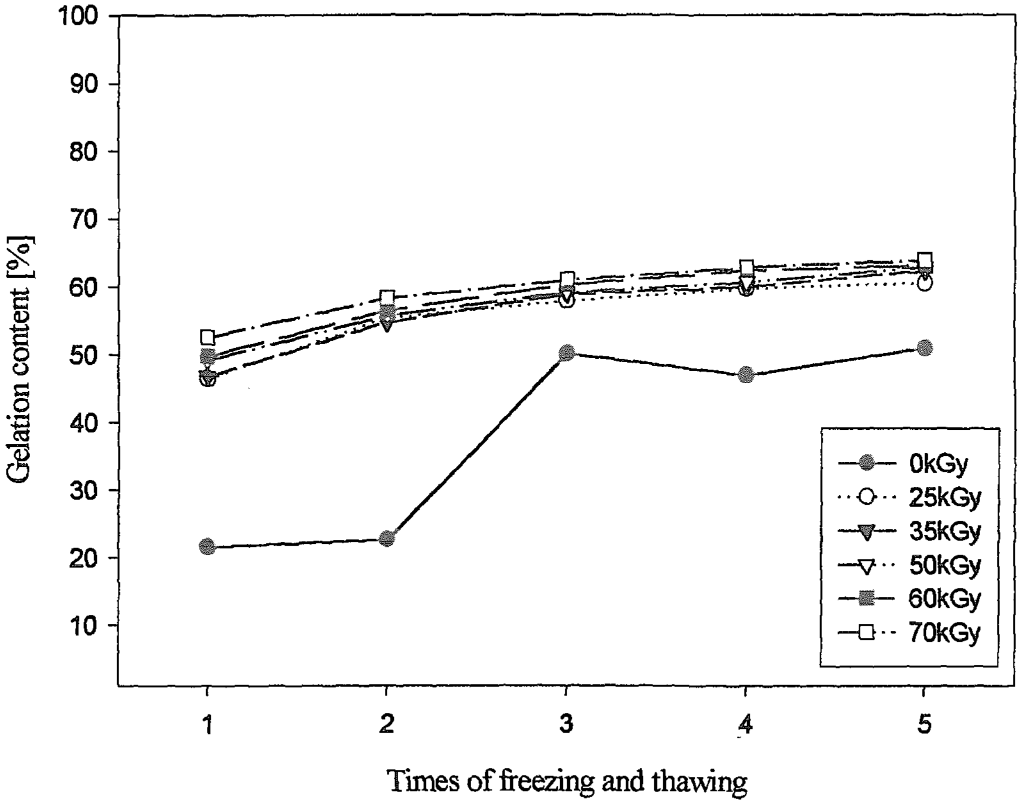


Figure 5. Gelation content of PVA/PVP-chitosan hydrogels vs. freezing and thawing at different irradiation doses.
(chitosan : PVA/PVP = 1 : 9, PVA : PVP = 6 : 4, solid concentration 15wt%.)

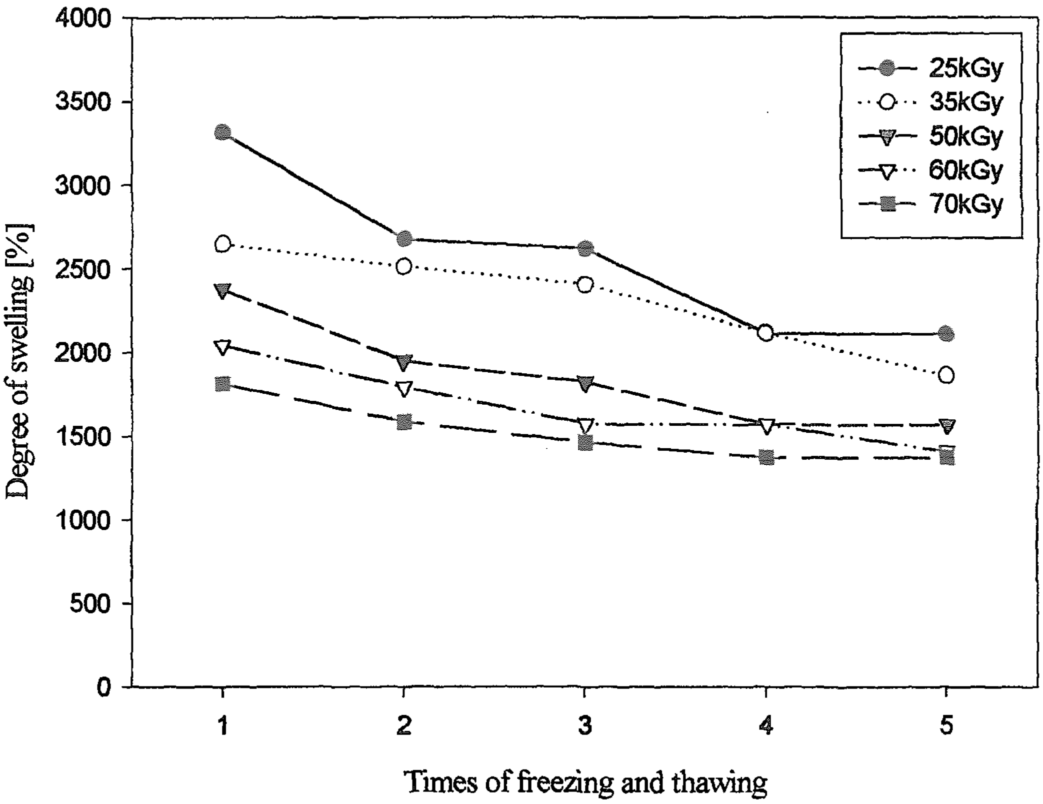


Figure 6. Degree of swelling of PVA/PVP-chitosan hydrogels vs. freezing and thawing at different irradiation doses.
(chitosan : (PVA/PVP) = 1 : 9, PVA : PVP = 6 : 4, solid concentration 15wt%.)

27 Status and Development of Nuclear Techniques for Commercial Application in Sichuan

Hao CHEN Pingchuan PAN Zhuping CHEN Yun ZHU Xiaochuan CHAO

Sichuan Province Institute of Nuclear Technology Application,

124 Sha He Bao, Chengdu, Sichuan 610066, P.R.China

Abstract

This article presents information on fields of radiation techniques in Sichuan and their potential development in future.

Keywords: Radiation, Processing, Sichuan

Introduction

Sichuan is one of biggest provinces and is a center of economy and art in the southwest of China. Research of nuclear technology began from 1960.The studies at the beginning had focused on agriculture, radiation chemistry industry and radiation medicine. Over the past 30 years, nuclear techniques are used in a wide range of applications, from food preservation to crosslinking. They play an increasingly valuable and often unique role in Sichuan economy.

Distribution of radiation facilities

There are 5 Co^{60} facilities in Sichuan. Two facilities are in Sichuan Province Institute of Nuclear Technique Application at Chengdu, a facility's designed capacity is 500kci and its activity is 350kci, another designed capacity is 150kci and its activity is 50kci , only for research. The 9th academy of China Nuclear Industry Co. has a Co^{60} with 1,000kci of designed capacity, its activity is only 200kci at Mianyang. Sichuan Province Academy of Agriculture Science has a Co^{60} with designed capacity of 200kci and its activity is 150kci. Chengdu

Chuanshuang Crosslinking Products Factory has a Co^{60} facility with designed capacity of 500kci and its activity is 100kci.

Besides Co^{60}, there are 5 electron beam accelerators in Sichuan. Sichuan Province Institute of Nuclear Technology Application, Chengdu Cable Factory and Dayi Co.Ltd each has a EB accelerator with 2.0Mev (15mA). The 9th Academy of China Nuclear Industry Co. has two, one is 3.0mev (10mA) made in Japan and another is 10mev(5mA) made in Russia.

Mutation plant breeding

Research on mutation plant breeding has been carried out by radiation in Sichuan since 1970s'. Under studies, plant breeders improved their classical procedures and established a series of systems including tissue culture and gene techniques to cross and select newer mutant cultivars. Great progress had been made in 1975 that mutant cultivar Fuhui 06 treated as recovery series has been found. With Fuhui 06 plant breeders established a newer system to select mutant cultivars. Up to date, about 10 cultivars(rice) are released, and have been cultured in 5 million hectares in South of China. The ? Y 838 is very excellent in all mutant cultivars. It produces more than 1.8 million tons of rice than before. ? Y 838 is exported to some countries of south Asia and Africa, and culture results are satisfied. Now, a new mutant rice named Fu Y 718 is expected to work better than its sisters.

Animal Production

Livestock are an important component in the Northwest of Sichuan. Researchers carried out studies on radioimmunoassay (RIA) with iodine 125 as a label to measure the level of the hormone progesterone in blood or milk of female animals to determine their reproductive status. The technique is tested helpful and effective in breeding of animals in Sichuan.

Food preservation by irradiation

About one-third of harvest in Sichuan every year may be lost due to spoilage and infestation on its way to the consumers. Sichuan Government pays attention to new techniques to preserve foods. As soon as irradiation is realized to be potential technique for food preservation , studies on food irradiation have been carried out since 1967. Research focused on disinfection of grain, spices, dried fruit and vegetables, on sprout inhibition of garlic, ginger, onions and potatoes, on elimination of food borne diseases in pork and poultry, and on extention of the shelf like of meat products. In 1983, the Codex Alimentarius Commission published an international standard for food irradiation. It's a landmark that commercialization of food irradiation is beginning in Sichuan. Under the aegis of the FAO, IAEA and WHO, co-ordinated research programs on food process control and quality control during irradiation as well as market testing had been carried out. Since then, commercial applications of food irradiation are accelerated. Despite economic handicaps and the resistance of some consumer groups, the number of facilities for food irradiation has steadily grown, and national hygienic standards of rice, meat, poultry, spices, dried fruit and vegetable have been ratified in Sichuan. Total amount of foods to be irradiated is more than 0.2 million tons per year.

Medicine fields

Radiation is mainly used to sterilize medical products such as gloves and scalpels for operation, injection and infusion products. Isotope labels for diagnosis and curing are produced too, such as ^{199}Ti, ^{153}Sm-EDTMP, ^{99m}.TC-D extran and ^{99m} TC-HMPAO, etc. Many of the products are produced by companies of Sichuan University.

In industry

Radiation application in the aspect of industry in Sichuan is concentrating on crosslinking and radiated cable. Research on crosslinking began from 1984. Up to 1990, about 10 factories to produce crosslinking products in Sichuan, 9 of which at Chengdu. The output is one-second in China, and is about 80 million US$ every year. But radiated cable is very small amount. Because car and ship producers are willing to buy cheaper normal cables instead of radiated ones despite of its good quality. Compared to North of China, the radiated cables are very welcomed and their products are more than 0.2 billion US$ per year. In addition, A few factories in Sichuan produce improved paint by radiation fixing for house and cars painting, but the amount is small.

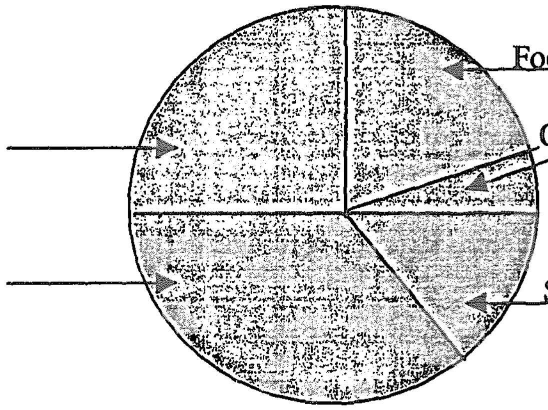


Fig.1 Contribution of radiation process to Sichuan Economy (0.1 billion US$) in 1999.

Trends

Sichuan is base of research on atomic energy in China. There are about 10 National or Provincial institutes or academies for nuclear techniques. After clod war, most of those institutes are changing into peaceful use of atomic energy, and transfer their techniques to commercial markets. Sichuan Government encourages these institutes to carry out researches on environment protection, agricultural production and medical products by atomic energy. Meanwhile, Center Government in Beijing is organizing exploitation of the west of China, including Sichuan. So policy and finance support will be very helpful to Sichuan development and it will accelerate opening and exploitation of Sichuan. We believe that the peaceful use of atomic energy will developed very fast and contribute more to Sichuan economy and natural environment. It's no doubt that it is good time for institutes on nuclear techniques to carry out cooperation with their colleagues in the west and neighbor countries and shake hands on peaceful use of atomic energy and contribute great to development of world.

28 Application of Radiation in Agriculture in Indonesia

MASRIZAL, Riyanti SUMANGGONO, Ahmad Nasroh KUSWADI, Muchson ARIFIN and Soertini GANDANEGARA.
Research and Development Center for Isotopes and Radiation Technology
National Nuclear Energy Agency of Indonesia (BATAN)
PO Box 7002 JKSKL Jakarta 12070
Telephone +62-21-7690709, Fax+62-21-7691671

Abstract

Radiation has been used in agriculture research and development in Indonesia since several decades ago, especially in fields of mutation breeding, pest control, plant nutrition, and animal health. Mutation breeding using gamma radiation has contributed several crop varieties, namely seven varieties of rice, three of soybean, and two of mungbean to national agriculture. Besides, hundreds of promising mutant lines of rice, soybean, mungbean, peanut, sorghum, horticultural crops, and industrial plants which are ready for multi-location trials. Radiation is also used for the control of insect pests, either field or storage pests. Storage pests are eradicated by lethal dosage for direct killing, while the population of field pests (especially fruit flies) is eliminated by the release of radiosterilized insects in the program of sterile insects technique (SIT). A pilot scale trial of SIT to control fruit fly is being conducted in East Java province. Lethal dosage of gamma irradiation is used as post-harvest treatment for food of such as dried spices, fresh fruits, and packed foods. Gamma radiation has been also used to sterilize culture media of *Bradyrhizobium*, a nitrogen fixative bacteria that is the symbiont of soybean to be used in yield improvement. In animal health, gamma radiation can be used directly or indirectly to eliminate, decrease diseases. Disease control can use irradiation to develop vaccines, and to produce the diagnostic reagent kits. Vaccines for *Coccidiosis* (chick) diseases have been produced and disseminated in Indonesia.

Key Words: Mutation Breeding, Sterile Insect Technique, Animal Health, Radio-vaccine.

Introduction

Agriculture, which is assumed to be the prime mover of the national economy in the present development program of Indonesia, has the ultimate goal to improve the welfare of the Indonesian people through the provision of adequate and safe food, animal feed and industrial raw materials at the same time as conserving land use resources to sustainability. The main problems encountered in the crop sub-sector are lack of varieties with desired characteristics, various pest and diseases, and limiting environmental conditions such as soil fertility problems, and drought. For the livestock sub-sector, the main problems are: low productive rate; poor nutrition condition; inadequate disease control and prevention; attitude, knowledge and skill of farmers; low entrepreneurial spirit; inadequate technical knowledge and skills; and inadequate farm management capacity.

Rice is the staple food for almost all of Indonesian people, whereas legume i.e. soybean and mungbean are important secondary crops for supplement food, especially for food diversity. In Indonesia rice is grown in areas up to 1,800 meters above sea level, during the wet and the rainy season. Up to now, more than 11,871 local and 103 improved varieties have been listed (Table 1). Due to population increase, increasing food crop production is the main program of National Agriculture Development. Rice consumption rate per person since 1990 up to 1996 decreased from 118.00 to 111.48 kg, it was showing that food diversification occurred from rice to non-rice [1].

The national rice demand, in fact, is more than national rice production, while the harvested area is mostly stable from year to year; causing the imported rice decreased (Table 2) [2]. Java Island (56.46 %) is the main region for rice production, followed South Sulawesi (7%) and North Sumatra (6.51 %).

Soybean and mungbean are secondary crops, important for their high nutrition value. The importance of these crops is especially for food diversification and food supplement when climatic stress (drought) takes place.

Soybean have been included in the National Food Program since 1984 due to high protein content, fat, vitamin and minerals for increasing nutritious food in people diet. The harvested area is about 1.3 million hectares, and the productivity is about 1.1 ton per hectare. Most soybean production is used for direct consumption, processed in forms, such as tempe (fermented bean), tofu, and soy sauce, feed stock and also for industrial raw material. Tempe and tofu are very popular in Indonesia.

Mungbean is usually used as vegetables (bean sprout/30 %), baby's food, porridge, cakes, feed stock and industrial raw material (cosmetics). Mungbean porridge is popular dish for breakfast. Although mungbean is rich in vitamins (especially B1 vitamin), the farmers are reluctant to grow it due to having low yield.

The importance of these crops is indicated by their increasing demand over the last decade. In 1987 a total of 1.4 million tons of soybean, 0.8 million tons of groundnut, 0.3 million tons of mungbean and 0.2 other grain legumes were consumed as food and feed stock, and also used as row material for local industry. Demand by the year 2000, both as food and feed stocks, are even greater, is at 3.1 million tons of soybean, 1.9 million tons of groundnut and 0.6 million tons of mungbean [3].

The national soybean and mungbean demand, in fact, in more than their production. To solve this problem, imported legumes are the only way. Soybean and mungbean import was increased every year (Table 3, 4) [2]. Soybean import in 1996 up to 1998 was reach about 700,000 tons a year, or more than 49%, whereas mungbean import in 1998 was more than 50% of the total production. Although improving variety is not the only way to increase the productivity, it gives an important contribution in increasing the production.

To help these problems, the Research and Development Center for Isotope and Radiation Technology (RDCIRT) - BATAN have used gamma radiation in the researches on mutation breeding, plant nutrition (biological nitrogen fixation), insect pest control (sterile insect technique), post- harvesting, and animal health.

Mutation Breeding

Plant breeding activities at BATAN had been carried out on food crops, horticultural crops and industrial crops by induced mutation, using gamma rays from Cobalt 60. Rice is the staple food for almost all of Indonesian people, whereas legumes i.e. soybean, mungbean and peanut are important supplement food. Food demand has been increasing year by year, due to population increment. Some problems facing in increasing food production are mostly caused by biotic and abiotic constrains.

Mutation breeding on rice, soybean, mungbean, sorghum and peanut was started in 1972, 1980, 1983, 1995 and 1997 respectively. The main obstacle in rice production is damage by Brown Plant Hopper pest, soybean is by rust (*Phakopsora pachirizi* SYD), and mungbean is by *Cercospora* leaf spot. Objective of sorghum breeding is to increase starch quality. Some promising mutant lines have been obtained; moreover, ten new mutant varieties of food crops have been officially released from these activities (Table 1).

Mutation breeding on horticultural crops such as banana, chilly, chrysanthemum, jasmine, onion and garlic was started in around 1995 - 1999, by exposing buds and gall to gamma rays. Fusarium disease is the main problem in banana plantation, whereas anthracknose disease damages chilly seriously in the wet season. Some promising banana and chilly mutant lines have been obtained.

Mutation breeding activities have also been carried out in industrial plants, such as ginger, patchouli, and cotton. These activities were commenced around 1996-1999. The objective of these activities was to increase oil content and quality of patchouli, tolerance to *Pseudomonas solanacearum* and *Fusarium oxysporum f. zingiberi* in ginger, tolerance to some diseases in cotton.

Sterile Insect Technique to Control Insect Pests

In Indonesia, researches on controlling insect pests by using sterile insect techniques (SIT) have been conducted since 1968. Irradiation of gamma from ^{60}Co was used to sterilized the insects. Untill 1995, the research were concentrated on lepidopterous cabbage pests i.e. *Plutella xylostella* and *Croccidolomia binotalis*. The results showed that releases of sterile insests into infested cabbage plants in a semifield plots were able to decrease pest population [3]. The implementation of SIT to control these cabbage pests in the filed however, is hindered by the cost of the insects mass rearing.

In the last decade, the Indonesian government has extensively developed fruit production centers in many provinces in order to accomplish domestic fruit demand and to increase fruit export. Mango production centers were developed in West Java, East Java and South Sulawesi, which are consisted of thousand hectares of mango orchards aggregation. Fruit fly *Bactrocera carambolae* (Drew & Hancock), a key pest of main fruits in Indonesia, become a serious thread for the fruit production centers. Sterile insect techniques by using radio-sterilized fruit fly will be utilized to cope with the fruit flies problem in the production centers.

The *B. carambolae* has been successfully mass reared in our laboratory on inexpensive artificial diet of *B. dorsalis*, consisted of wheat bran (26.0 %), brewer yeast (3.6 %), sucrose (12.0 %), methyl-P-hydrobenzoate (0.1 %), Na-benzoate (0.1 %), HCl (0.2 %) and water (58.0 %) (Kuswadi, et.al. 1999a). Pupae of 6 days old (2 days before emergence) were irradiated with gamma to obtain radiosterilized flies. Data on sterility of the flies, observed by pairing twenty male flies emerged from the irradiated pupae with the same number of unirradiated females in a small cage, and observing the fertility of eggs produced, showed that irradiation of > 90 Grey caused (almost) full sterility to the male flies (Figure 1).

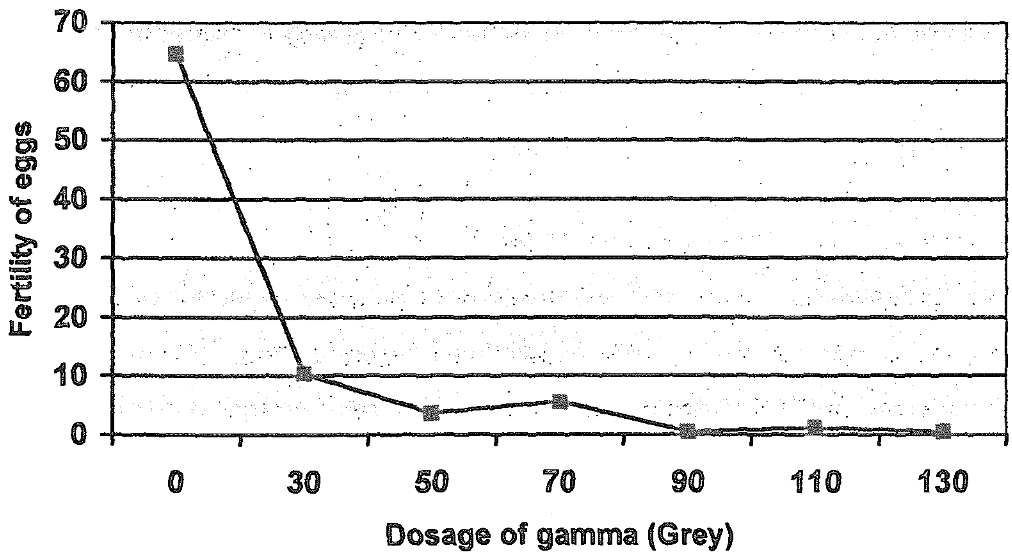
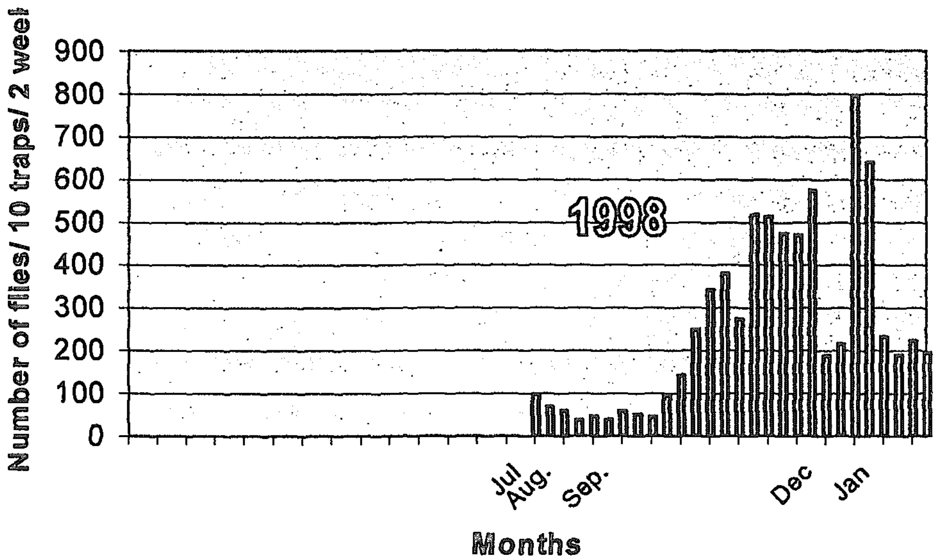


Figure 1. Fertility of eggs produced by normal flies of *Bactrocera carambolae* when mated with irradiated males.

Data on population dynamic the fruit fly in mango orchard in East Java have been collected by observing the number of flies caught every other week by metyl eugenol traps set in the orchards [4, 5] The fruit fly population remained low before the harvesting time, and started to increase significantly in October and then decreased again after January (Figure 2), meaning that to maximize the effectiveness SIT, the sterile flies should be released before October. Mango in Java is harvested from September to December.



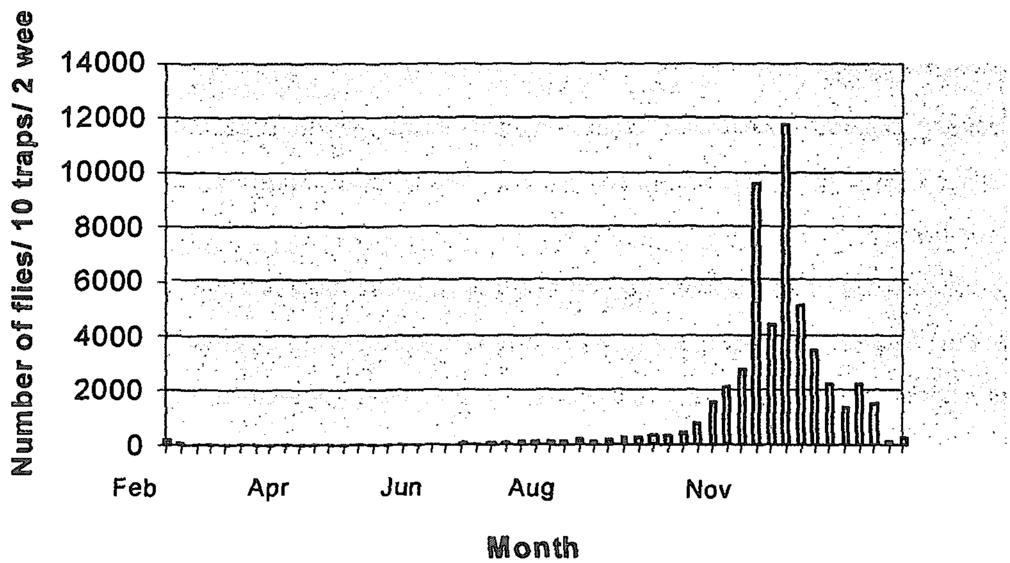


Figure 2. Population dynamic of *Bactrocera carambolae* in mango orchard in East Java as recorded by methyl eugenol traps in 1998 and 1999.

A pilot scale program for controlling *B. carambolae* by SIT (in 250 ha of mango orchard which is relatively isolated from other crops by forest) is beeing conducted in a mango orchard in East Java. Absolute population of the fruit fly in the orchard was observed by using *release and recapture method* in August 1999, in order to estimate the number of sterile flies needed in each release. A density of about 300 male flies, or 600 flies of both male and female was found in every hectare of orchard. The releases of sterile flies have been started in August 2000. About 44 liters of irradiated pupae, which are equal to about two million pupae or one and a half million sterile flies, were released every other week into the orchard.

Biological Nitrogen Fixation

In attempt to gain benefit of symbiotically nitrogen fixing soil microorganism Bradyrhizobium with soybean, a program was developed to obtain Bradyrhizobium inoculants to enhance N fixation and increase soybean production in acid soil. In these activities Bradyrhizobium strain and soybean mutant lines developed for acid soil from Mutation Breeding Section were used. Some of research activities were:

- Isolation and identification of Bradyrhizobium strains from root nodules of some soybean varieties.
- Screening of Bradyrhizobium isolates toward acidity on acid culture media in the laboratory, then followed by further testing in the green house with some soybean mutant lines.
- In the field, some experiments had been done to study the effectiveness of selected strains of soybean mutant line on N fixation and grain yield. Compatibility between Bradyrhizobium strains with some mutant lines, and inoculation technology were also studied. These activities were carried out in ultisol soil at Sembawa and tidal swamps soil at Karang Agung Ulu, South Sumatra.
- Good strains in enhancing N fixation and grain yield of soybean mutant lines were obtained

- To face problems on the wide spectrum of varieties use in different areas mixed inoculants was prepared. These Bradyrhizobium inoculants named Rhisora, which could substitute 60 kg of N fertilizer (urea).
- In preparing inoculants, gamma irradiation from ^{60}Co was used to sterilize the peat. At the moment we are studying the use of agro-waste of sugar refinery for *Azosprillum* inoculants carrier. These materials are abundant in the area, for instance from one sugarcane plantation in Lampung area around 120 tons bagasse and 100 tons filtermud were produced per day during harvesting season. Besides using the ^{60}Co, to sterilize the carrier Electron Beam Machine was also used.

Animal Health

Application of nuclear technique in animal husbandry was initially introduce by RDCIRT – BATAN of Indonesia, especially in the development of radio-vaccine, for the improvement of poultry production, to meet animal protein demand with low cost. The development of radio-vaccine in Indonesia is considered important due to its tropical and humid climate, where various poultry diseases are potential and hampers the development of poultry farms. For example, *Coccidiosis* may causes mortality rate increased by up to 20%, and some time may be higher.

In the development of radio-vaccine in Indonesia, joint work collaboration, between RDCIRT and other Research Institutes (*i.e.* Research of Veterinary Science – Bogor and Center for Pharmaceutical of Veterinary Science – Surabaya), has been developed. This joint collaboration then grown into mutual collaborating since RDCIRT – NNEA initiated the development of radio-vaccine for *coccidiosis* (*E. tennela*) using ^{60}Co gamma irradiation. Sporulated oocysts – in potassium bichromate 2.5% media for 48–74 hours in 27–29^{0}C – was irradiated by 125 Gy dose of gamma rays with 10% shielding. Intensive and extensive field trials were carried out at several locations under the coordination of Directorate General Livestock Services (DGLS), Department of Agriculture prior officially approval for commercially product of the **Coccivet Vaccine** (Surabaya, September 1990 – Registration No. D. 90081333 VKC).

Following the introductory of Coccivet Vaccine, this special effort was then turned into a greater opportunity for CRDIRT to develop a broad spectrum of *coccidiosis* radio-vaccine. Using 6 oocysts (*i.e. E. tennela; E. necatrix; E. maxima; E. mitis; E. acervulina; and E. praecox*), **Coccivet Polyvalent Supra 95 Vaccine** (Jakarta, August 1995 – Registration No. 9508115) was approved and released for mass production, following extensive field trials using 10 days old SPF chicken.

Conclusion

The program of RDCIRT-BATAN in agriculture research by using radiation and isotope technology has been conducted and will be continued. Mutation breeding program uses ionizing radiation as well as chemical. Fruit fly is a key pest of mango orchards, and sterile insect technique has been used to solve the problem. Radio-vaccine has been developed by gamma radiation, thence other radio-vaccine will be developed.

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Table 1: Number of released rice varieties in Indonesia from 1969 to 1996.

	NUMBER OF RELEASED RICE VARIETY					
	69-73	74-78	79-83	84-88	89-93	94-96
SAWAH L.L	3	4	14	11	9	6
SAWAH H.L	0	3	1	1	1	0
SAWAH T.W.	0	0	2	4	2	0
DRY LAND	0	2	3	6	4	0
INTRODUCTION	1	8	3	6	6	0
	TOTAL RELEASED RICE VARIETY = 103					
	TOTAL LOCAL RICE VARIETY = 11.871					

Table 2. Production of rice variety in Indonesia from 1991 to 1998.

Year	Harvested Area (ha)	Production (ton)	Import (ton/%)
1991	10281597	44688247	190572 / 0.43%
1992	11103317	48240009	621070 / 1.29%
1993	11012800	48181087	32437 / 0.07%
1994	10733830	46641524	636856 / 1.37%
1995	11438764	49744140	1819226 / 3.66%
1996	11569729	51101206	2149758 / 4.21%
1997	11365523	49568170	2190511 / 4.42%
1998	11889619	52914744	2170619 / 4.10%

Table 3. Production of soybean in Indonesia from 1991 to 1998.

Year	Harvested Area (ha)	Production (ton)	Import (ton/%)
1991	1368199	1555453	631038 / 40.57%
1992	1665706	1869713	687550 / 36.77%
1993	1470206	1708528	700156 / 40.98%
1994	1406918	1564847	628159 / 40.14%
1995	1477432	1680007	496888 / 29.58%
1996	1279286	1517181	743532 / 49.01%
1997	1265853	1373049	732745 / 53.37%
1998	1300501	1547263	723501 / 46.76%

Table 4. Production of mungbean in Indonesia from 1991 to 1998.

Year	Harvested area (ha)	Production (ton)	Import (ton/%)
1991	301267	237447	21094 / 8.88%
1992	393073	326750	15593 / 4.77%
1993	404673	357991	31687 / 8.85%
1994	292095	283886	73191 / 25.78%
1995	361115	325342	68235 / 20.97%
1996	338404	308330	109738 / 35.59%
1997	395538	359644	157803 / 43.88%
1998	400984	387312	208631 / 53.87%

Table 5. Planted area of 6 rice mutant varieties in 1997 and 1998 in Indonesia.

No	VARIETY	1997		1998	
		DRY (Ha/%)	WET (Ha/%)	DRY (Ha/%)	WET (Ha/%)
1	ATOMITA1	211 / 0.011	0	35	0
2	ATOMITA2	157 / 0.008	0	0	0
3	ATOMITA3	0	61	0	0
4	ATOMITA4	125 / 0.007	7723 / 1.017	12432 / 0.53	968 / 0.14
5	CILOSARI	2	2614 / 0.35	9940 / 0.43	1073 / 0.15
6	SITUGINTUNG	0	0	0	0

Source: Central Bureau of Statistic, Statistic Division of Food Crops, 1998

Table 6. Some mutant varieties developed in the Center for Research and Development of Isotope and Radiation Technology, BATAN, 1982 to 1998.

No	Name of crop	Name of variety	Year of release	Original variety	Improved characters
1	Lowland rice	Atomita 1	1982	Pelita I/1	Yield, early maturity, resistance to Bph-1, Glh and Blb.
2	-"-	Atomita 2	1983	-"-	Yield, early maturity, tolerance to salinity, resistance to Bph-1, Glh and to Blb.
3	-"-	Atomita 3	1990	-"-	Yield, resistance to Bph-1 and 2, and to Blb.
4	-"-	Atomita 4	1991	Cisadane	Yield, resistance to Bph-2 and early maturity
5	-"-	Cilosari	1996	SM 268 x IR 36	Yield, resistance to Bph-2 and *Xanthomonas oryzae* strain 3, and good cooking quality
6	Upland rice	Danau Atas	1988	Seratus Malam	Yield and resistance to blast
7	-"-	Situgintun g	1992	-"-	Yield, resistance to blast and Bph-1
8	Soybean	Muria	1987	Orba	Yield and mature in 80 days
9	-"-	Tengger	1991	-"-	Yield, early maturity and MR to *Phakopsora pachyrhizi*
10	-"-	Meratus	1998	-"-	-"-
11	Mungbean	Camar	1991	Manyar	Yield, synchronize in maturity and tolerance to *Cercospora* leaf spot

29 Radiation Technology in Vietnam

Vo Van THUAN
Institute for Nuclear Science and Technique, VAEC,
B.O.Box 5T-160, Hoang Quoc Viet Road, Nghiado, Caugiay, Hanoi, Vietnam

Abstract
Most of researches and developments in the field of radiation technology that have completed in a decade before 1995 were concentrated to sterilization and food irradiation. A series of medical devices and products were the main commodities for research and application trials. Also, many kind of food have attracted the scientists and technologists to investigate the application and commercialization of irradiated food. In addition, the radiation technology also was utilized for processing of non-food items including herbs, medicinal produces, and tobacco material. Since 1996 VAEC and INST has realized the important role of radiation processing on natural polymers. Hence, along with the commercialization of radiation technology, three research teams were established for the target. This report reviews the recent activities and achievements on radiation technology in the country emphasizing on the radiation processing of polysaccharides. A number of polysaccharides, which originated from bio-/agro-wastes such as seaweed, shrimp shells, lignocelluloses, was modified or degraded by irradiation to prepare hydrogel and bio-active material using for health-care and crop production.

Keywords: radiation technology, Vietnam, sterilization, food irradiation, natural polymer, polysaccharides, lignocellulose.

1. VAEC and the development of radiation technology

Most of research items, which related to application of isotopic and radiation techniques, were conducted in three institutes of VAEC, namely Dalat Nuclear Research institute (DNRI), Center for Research and Application of Radiation Technology

(CRART) in Ho Chi Minh city, and Institute for Nuclear Science and Technique (INST) in Hanoi. Within the country VAEC is the only institution, whose manpower is crowded with scientists specified in nuclear science and technology. For its effective operation VAEC has a important policy to establish cooperation with other institutes and end-user institutions. For example, VAEC has good link to many universities in the field of education and training, to specified institutes for research and to factories and private sector for technology application and transfer.

Radiation technology in Vietnam was taken off from 1983 when a research cobalt irradiator of 16.5kCi was established in DNRI. There, Radiation Technology Department and Radiation Biology Department are the key units that exploited the radiation source, have carried out a series of studies, such as: radiation sterilization, wood – polymer composite manufacture, food preservation, radiation mutation breeding. The advanced technology of radiation vulcanization of natural rubber latex was established and implemented firstly in DNRI. The achievements of this stage were the convincing consumers in radiation sterilization and insect disinfestation, and preparation of the background to transfer the technology in to industry.

2. The Hanoi Irradiation Center – A demonstration of radiation technology in Vietnam

Under IAEA TC Project VIE/8/004, a semi-commercial Cobalt facility was set up in the Hanoi Irradiation Center (HIC) in 1991. The facility with activity 110 kCi at the first loading has played an important role to implement the R-D programs during 1991-1995 for development and then for commercialization of radiation technology. Food irradiation as the main purpose at beginning, so this subject was under extensive operation. At present, the application and development of food irradiation, however, is evaluated to require further enhancement of the regional and international regulatory harmonization. A remarkable amount of medicinal herbs have been irradiated for microbial and insect control. Sterilization of different surgical plastic/rubber items has been successfully tested. Achievements of this program have been overviewed in Ref. [1].

The following data of health-care product sterilization as illustrative example shows the commercial operation in Hanoi Irradiation Center last year: surgical gloves (120 m^3), surgical catgut (80,000 units), tissue graft, medicinal herbs (100 m^3), bandage set (50 m^3). Sterilization of medical products has turned to an industrial opportunity.

Under the IAEA TC project VIE/7/004 and the Regional cooperation project RAS/7/008, a bank of tissue grafts for health care has been established and being operated effectively. For sterilization of tissue grafts of the bank, only gamma irradiation has been being used. For 1991 - 1999, 36,000 bio-membranes (amniotic, from frog and pig skin) and 4,800 allograft bones have distributed to 20 hospitals and contributed to rescue 1,350 burn patients, and grafted more than 1,000 patients. Recently, radiation sterilization of deep frozen massive bones also are available in the bank.

A part of time is delivered to research such as: glass coloring for decoration, pasteurization of selected agro-wastes for cultivation of high quality mushroom, formulation of radiation resistant polymers, etc. An advanced research undergoing is radiation degradation of natural polymers, being detailed below in the point 4.

The average operating time of the Hanoi Irradiation Center achieving 3,500 hours/year in the period 1996-1998 without any trouble, and it reached 4,600 hours in 1999. Experience from extensive practical activity convinces the skill of operators and operating staff, who enable for training and transferring the technology to industrial scale. In fact, specialists from Hanoi Irradiation Center have taken an active part in the project designing and construction of the commercial irradiation facility located in HCM city.

3. The Center for Research and Application of Radiation Technology and commercialization of radiation technology in Vietnam

To meet the rapid growth of domestic production of medical products, a second irradiation center with a commercial scale (400 kCi of cobalt-60 at first loading) was established in HCM city in 1998 serving for industrial sterilization of the medical products and commercial test of the food preservation. For illustration we list some significant items tested in the Center for Research and Application of Radiation Technology (CRART) in recent months from March 1999 to June 2000: 560m^3 of medical products (surgical gloves, bandages, clinical tools, microbiological tools), 320m^3

of pharmaceuticals (powder and pellets, products and semi-products of medicinal herbs), hundreds tones of different foodstuff (frozen and dried seafood, dried meat, tea, spices).

There is a significant demand of the cross-linked polymers in the Country, including wire and cable, and shrinkable packages used in food industry and in medicine which calls for the next application of electron beam technology.

Along with the expanding the capacity of CRART, the Hanoi Irradiation Center is also expected to be upgraded by the Government with an IAEA support under the newly approved TC project VIE/8/014 for 2001-2002 . Beside of sterilization of non-food products, food irradiation would be promising in near future. Preservation of food and medical herbs are very specific for tropical countries including Vietnam. The radiation quarantine of fresh vegetable and fruits is an important tool for supporting import/export when Vietnam starts joining to the regional free market.

4. A new trend - research on radiation processing of natural polymers

The need of environment-friendly products for sustainable development calls the regional countries to cooperation in a new R-D program for application of natural polymers. Rubber latex is a classical example of natural polymers with high capability in the Region. Following the pioneer research of Japanese scientists, since 1987, Nguyen Quoc Hien et al. [2] has been carried out research on radiation vulcanization of natural rubber latex (RVNRL) in a continuous support of IAEA and the Regional cooperation. Recently, supported by a Regional research cooperation project this group has improved the aging properties of RVNRL by using natural antioxidant which made from hair keratin by hydrolysis in alkaline [3]. Tran Tich Canh et al. [4] are pioneers in Vietnam to study radiation modification of natural polysaccharides for immobilization of bioactive substances. Pre-irradiation grafting of acrylamide onto starch has been studied, for example, at low temperature by a dose of 5 kGy, that 10% concentration of acrylamide grafted onto solid state of starch achieves 30-40% grafting efficiency. In the recent bilateral and regional cooperation, Vietnam has a commitment to enhance the research on radiation processing of marine polysaccharides which are suitable for Vietnam, as a tropical country with more than 3,000 km sea coast.

5. Radiation modification of chitosan/chitin

Radiation grafting hydrophilic monomers onto chitin has been carried out by Nguyen Anh Dzung et al. [5] to modify its hydrophobia. The grafted products, based on chitosan as well as starch have been used for immobilization of enzyme, for example, trypsin. The bio-activity is proved stable with increase of the grafting efficiency.

Radiation cross-linking has been studied [5] to preparing chitosan-HEMA copolymer, following the procedure for formulation of interpenetrating network hydrogel. The radiation crosslinked chitosan-HEMA copolymer gels have a smooth and stable surface improving its mechanical property. The product is suitable for bio-medical applications, such as for wound dressing which now widely succeeds in the burn treatment.

6. Degradation of chitin/chitosan and alginates

Le Xuan Tham et al. [6] and Nguyen Duy Lam et al. [7] studied the radiation degradation of chitin/chitosan, which extracted from shrimp and crab shell. The following experiments with degraded chitosan in cultivation of plant have shown a positive effect of growing rate of rice plant in soil polluted with salt and heavy metal. This seems to prove the enhanced bio-activity of irradiated chitosan, which prevents the absorption of salt and harmful metals such as vanadium and zinc.

Kieu Ngoc Lan et al. [8] is carrying out the study on application of degraded chitin/chitosan for coating fresh fruits to extend the self-life of mango. The enhancement of anti-fungal activity of irradiated chitosan using for fruit coating is to be reported in this symposium by Tran Bang Diep [9].

Degradation of alginate extracted from brown sea-weed *Sagassum* has been studied by Nguyen Quoc Hien et al. [10] applying to learn effect on growth-promotion of plants in green house condition. Preliminary result shows a clear correlation of the growth-rate and the concentration of applied alginate in a range from 20 to 200 ppm. Next steps of progress will be reported in the present symposium by Le Hai [11].

7. Agro-waste treatment

The regional cooperation encourages a research program on radiation processing of agro-wastes, which would contribute, to the environmental conservation and improving the rural living standard. In this trend, it is important to select a right agro-waste item and the goal of technology to get economic benefit. Nguyen Manh Hung et al., in cooperation with the Institute for Agricultural Genetics [12] carried out research with the aim of using radiation treatment instead of autoclaving for sterilization of substratum in spawn seed production encouraging a National project for commercialization of mushroom cultivation. Nguyen Duy Lam et al. [13] have developed an advanced procedure in radiation pasteurization of sugarcane bagasse used for fungal fermentation. Final products, which are to be expected, are feed for ruminant and edible mushroom. By *in sacco* test for digestibility, the nutritional value of fermented bagasse is clearly increased.

Application of radiation technology for treatment of agro-wate waste have been widely studied. However, if the radiation is to have a role as practical method of treatment for agro-waste, lower doses than those currently necessary must be achieved for the process to have commercial feasibility. In fact, limit of practical application of radiation in this field still remains on substratum sterilization for fermentation with radiation dose more than 10 kGy. The radiation treatment for lignocellulosic materials will also be more prospective if it can produce certain bio-active substances that may have some physiological impacts on plant life. Extracts from lignocelluloses including bagasse and oil palm fiber after radiation treatment posses a number of biological activities. Some certain substances may induce higher defensive response in plant. Other substances can make seeds dormant [14]. The extracts applied at lower content may promote plant's growth rate and obviously support them to tolerate environmental stresses [15].

8. Regional and bilateral cooperation

Vietnam being a member of the Regional Cooperation in the field of radiation technology for the last decade (RAS/92/073) was taking active part in harmonization of food irradiation and carrying out cooperative research program on radiation processing of natural polymers. Vietnam and Japan have just enhanced the cooperation by signing the bilateral cooperative agreement between VAEC and JAERI aiming for the first 3 years (2000-2002) at the research on radiation processing of marine polysaccharides. The achievements in these new trends should be developed more extensively to get the commercial application. The Radiation Technology can contribute its potentials as another new possibility opening for agriculture and for agro-waste management towards the environment-friendly sustainable development.

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30 Status and Prospect of Radiation Processing Technology in Malaysia

KHAIRUL ZAMAN Hj. Mohd Dahlan and NAHRUL KHAIR Alang Md Rashid

Malaysian Institute for Nuclear Technology Research (MINT),
Bangi, 43000 Kajang, Selangor Darul Ehsan, Malaysia

Abstract

Radiation processing technology in Malaysia is gaining acceptance by the local industry. The technology has proven to enhance the industrial efficiency, productivity and improve product quality and competitiveness. For many years, variety of radiation crosslinkable materials based on synthetic polymers have been produced either in the form of thermoplastic resins, polymer blends or composites. Today, effort is being focused towards producing environmentally friendly and biodegradable materials using natural polymers.

The government of Malaysia through the Malaysian Institute for Nuclear Technology Research (MINT) has developed research program to utilize indigenous materials such as natural rubber, palm oil and polysaccharide. Radiation processing technology is used to process (crosslink/grafting/curing) the materials at a competitive cost. This technology can be applied in several industrial sectors such as automobile, aerospace, construction and healthcare.

Keywords: radiation processing, indigenous materials, natural polymer, crosslinking, curing.

INTRODUCTION

Radiation processing is one of the core research programs of the Malaysian Institute for Nuclear Technology Research (MINT). As a government research institute, research activity of MINT is designed to meet the government policies and aspiration of developing knowledge driven economy (k-economy). It is recognized that knowledge is the main driving force for economic growth of a country. In this connection, R & D program in Malaysia is designed to generate knowledge that can meet market demands and needs.

The Second Malaysian Industrial Master Plan (IMP 2) (1996 - 2005) focuses on the manufacturing strategy and cluster-based development emphasis on the full integration of operations along the value chain R & D and product designs to marketing and distribution. IMP 2 has classified the industries into three groups namely, internationally linked, policy-driven and resource-based groups as shown in Annex 1. Participation of both the public and private sectors in the implementation and monitoring of the plan are necessary. Both parties, government and industry are encouraged to work together as early as in the design and approval of the project budget and to follow through the development until it reaches the market place.

National Priorities

The government supports R & D and technologies that promote growth (increase export & reduce import); enhanced industrial efficiency, productivity and competitiveness; generate home-grown technology with own brands of goods and services; reduce labor with increasing automation and improve quality of life.

Within the manufacturing industry, advanced materials such as composites, either polymer-based, metal-based or ceramic-based are given priority. It is much so, if resource based material can be integrated into the development of advanced materials. In this case, natural rubber and oil palm are the main sources. The by-products of the two resources such as rubber wood, rubber wood fibers, oil palm fronds and empty fruit bunches are the primary materials for further utilization - value added and meeting the zero waste concept. Medium density fiberboard (MDF) made of rubber wood fibers and oil palm fibers is the example of new product emerged in the market recently. The rubber wood MDF increases its popularity rapidly in the furniture industry both locally and internationally.

In addition to composite materials, the modification of resource base materials that have commercial value is also given high priority. Epoxidised natural rubber and thermoplastic natural rubber elastomer are amongst the products that have been developed and commercialized in Malaysia. Modified palm oils such as polyol and epoxidised palm oil are developed and used as starting materials for polyurethane and polyester based resins for various applications.

In the light of IMP 2 and the above strategy, the current industrial application of nuclear technology such as radiation processing fits in well into the country's development program. Radiation processing is one of the industrial processes that can be used for cross-linking, grafting, elimination of microorganisms, modification of organic compounds, etc. The radiation processing technology can be an integral part of the manufacturing line for the production of flame/fire resistant wire and cable, heat shrink tube, hot water tube, heat shrink film for packaging, sleeve, composite materials, viscose rayon and many other profile and molded products. It has been proven as unique and commercially viable process. On the other hand, the materials used for radiation processing are specifically compounded and are not easily available. Therefore, the introduction of radiation processing technology in local industry requires mix strategies as follows;

- Established technology/material/product - development of radiation crosslinkable materials for specific.
- New technology/material/product – development of advanced and modified materials based on indigenous and locally available raw materials

In addition, radiation processing has also been proven as a viable process for cleaning up flue gasses from power station and incinerator. It can also be used for cleaning of volatile organic compounds and drinking water. Therefore, it has great potential to be used for protection of environment.

RADIATION PROCESSING PROGRAMME

Radiation processing projects are formulated by taking into account the national priorities such as manufacturing strategy, resource based clusters and the expected output of the project. The following are some of the possible materials, processes and out put of the research projects undertaken by the Malaysian Institute for Nuclear Technology Research (MINT);

Materials	Process	Output Product/Application
Natural rubber - SMR (Std.Malaysian Rubber) - Epoxidized natural rubber - Latex - Rubber wood fibers **Oil palm** - crude and refined oil - Expoxidized palm oil - Palm oil fibers **Polysaccharide** - Starch from Sago & Tapioca - Chitosan **Thermoplastic** - Low Linear Density Polyethylene (LLDPE) - Linear Density Polyethylene (LDPE) - Ethylene Vinyl Acetate (EVA) - Polypropylene - Polyvinyl chloride - Polystyrene **Thermoset Composite** - polyester - epoxy - carbon fiber	• Blending/Composite • Modification/synthesis • Compounding • Extrusion • Injection molding • Coating/lamination • Casting • Gamma vulcanization, • Electron beam crosslinking, grafting and curing	• Composite profiles such as panels, frame, flooring for construction industry and aerospace industry. • Continues extrusion tube/hoses, sheet/film, pipe, foam, membrane as industrial products • Continues composite sheet, foam for automotive parts such as window trim, rear shaft and front panel etc. • Injection molding of modified and composite materials for automotive parts • Hydrogel for bio-medical application
Environmental pollution - SOx and NOx from power stations and incinerator.	• Cleaning the gases	• Clean air release to the atmosphere. Fertilizers as by product

The list of projects that are being supported by the government and other related agencies and institutions locally and internationally are shown in Annex 2. As can be seen in Annex 2.0, the established technology/material/product type project will only be carried out if there is an end user. This type of project involves the development of specific compound to meet the product specifications as required by the company or the development of technology that will be immediately transferred to industry.

For a new technology/material/product type project, it is more for applied science and engineering. Although partnership with industry is not prerequisite, nevertheless, the project must indicate the future application and meeting the government requirement on R & D such as increase export, increase quality, productivity, using local materials etc. The output of the project must be clear and the target application should also be identified. Generally, this type of project is related to material development using indigenous polymer and radiation processing technology for industrial or medical applications.

In addition to R & D on new materials and applications using radiation processing, MINT continues to promote radiation technology as safe and reliable technique for sterilization. Gamma sterilization is well accepted and established technology in this region. The effects of radiation on packaging materials, medical products and others such as herbs, pharmaceutical raw materials, species are well documented and published. There is enough industrial experience in radiation sterilization that it is now become routine industrial services and also highly competitive.

Institutional Linkages

The linkages and networking with other institutions and universities are encouraged to optimize the utilization of manpower-expertise and facilities.

In the case of radiation processing, MINT is the leading institution. Other research institutions and universities in Malaysia have no radiation sources to carry out R & D on radiation processing. However, certain institutions have specific expertise and facilities which can be tapped by MINT such as Malaysian Rubber Board (the source of various type of natural rubber and expertise in natural rubber), Universiti Putra Malaysia (the source of research students and fiber characterization), Forest Research Institute of Malaysia (the source for fibers and preparation of fibers), Palm Oil Research Institute of Malaysia (the source of polyol and epoxidized palm oil) etc.

Regional and bilateral linkages are another means of forming strategic alliances whereby each participating country contributes to the development of science and technology on a wider perspective. Utilization of natural and indigenous polymer is one of a good example for this region to collaborate since this region is rich of under utilized natural resources.

Commercialization

In Malaysia, the commercialization of R & D finding can take place in several ways. The usual approach is through direct negotiation between the inventor and the interested company, who has been identified earlier during market survey. However, there are several venture capital agencies that specialize in providing funds for commercialization and for getting industrial partner. In Malaysia, government has developed several agencies to look into the commercialization aspect of research finding. Malaysian Technology Development Corporation (MTDC) has been established for this purpose. MTDC is also acting as a venture capital agency. It is authorized by the government to manage Commercialization of R & D Fund (CRDF) and Technology Acquisition Fund (TAF) to assist the entrepreneur and the private company in the respective areas. In addition, the Ministry of Science is also providing similar fund in the form of grant called 'Industrial Grant Scheme (IGS)' to assist the company to commercialize the R & D finding. The mechanisms for dissemination of R & D finding to the industry are well in place.

On-Going and Futures Projects

As shown in Table 2, most of the on-going projects, which are categorized under the new technology/material/product, are based on the indigenous materials such as natural rubber, palm oil and polysaccharide. The aim of the research is to develop new materials that can be processed (crosslink/grafting) by electron beam irradiation at a competitive cost and to be used either in automobile, construction, or healthcare industry. Other applications of the new materials are for curing of coatings, adhesive, lamination and printing ink.

For future projects, radiation processing of composite materials for automobile, aerospace and construction industry will be given more attention. Local universities and MINT have initiated research on radiation grafting of membranes for fuel cell (battery) and cellulose fibers for plastic composite and the work will continue. Radiation crosslinking of conductive and magnetic polymer for electronic and electrical industry is also being studied at the local university in collaboration with MINT.

At the same time, MINT continues to promote and assist local industry in adopting the established technology such as heat and fire resistant wire, hot water pipe, heat shrink products and others whenever the need arises. On the other hand, purification of flue gases has received great interest from utility company. Joint project between MINT and utility company is initiated and the EB flue gas purification system is operational.

To facilitate the commercialization of the above research work, a facility for polymer processing is being constructed which is expected to be ready by end of 2000. The facility comprises of offices, testing laboratory, workshop and several testing equipment including pilot cum industrial polymer processing machines such as compounder, extruder and injection molding machine.

As for radiation services such as gamma sterilization, the effort is more on product diversification. Currently there are 3 gamma irradiation service facilities in Malaysia. The number may increase in future. This has created a tough competition on the traditional medical disposable products such as rubber gloves, catheters etc. Therefore, efforts are now focus on non-traditional products such as pharmaceuticals, cosmetics, herbs, medical herbs, species and animal feeds. SINAGAMA-MINT gamma sterilization facility has also been awarded ISO9002 and EN46002 which make it attractive for products to be marketed in USA and Europe.

CONCLUSION

In the past several years, there is a significant progress and development on the application of radiation processing in Malaysia. Government continues to support R &

D on this field by providing the necessary infrastructure, facility, trained manpower and research funds. Various mechanisms for commercialization are also in placed to facilitate the transfer of technology from laboratory to industry.

In the private sector, several units of electron bream machines are in operation such as 3 units for heat shrink films and 1 for crosslinking of wire. A few more are in the planning stage for crosslinking of wire and heat shrink sheet. For gamma sterilization facility, three industrial plants are in operation including one at MINT, and two more are at final stage of implementation. It is envisaged that radiation processing will continue to contribute to the progress and development of industry in Malaysia.

ANNEX 1

CLUSTER-BASED INDUSTRIAL DEVELOPMENT

Internationally –Linked Cluster

Industry Groups	Potential Cluster
Electrical and Electronic	Electrical and Electronic
Chemical	Petrochemical / Pharmaceuticals
Textile and Apparel	Textiles and Apparel

Policy – Driven Cluster

Industry Groups	Potential Clusters	
Transportation	Automotive	Marine
	Motocycles	Aerospace
Chemical	Polymer	Composites
	Metals	Ceramics
Machinery and Equipment	Machinery and Equipment	

Resource-Based Cluster

Industry Groups	Potential Clusters
Resource-Based	Wood-Based Products
	Rubber-Based Products
	Palm Oil-Based Products (Food)
	Oil Palm-Based Products (non-food)
	Cocoa-Based Products
Agro-Based and Food Products	Fish & Fish Products
	Livestock & Livestock Products
	Fruits and Vegetable
	Floriculture

ANNEX 2

1999/2000 LIST OF PROJECTS

Division of Radiation Processing Technology, MINT

Established technology/material/product

- Development of heat shrinkable compounds (flame retardant) – funded by IRPA for 1997 – 1999 and supported by local company
- Development of electron beam facility for processing of heat shrink sleeve and heat shrink tubes – funded by local company in 1999/2000
- Electron beam sterilization of medical items. – trial run on industrial samples.
- Utilization of electron beam technology for purification of flue gases in the applications of electricity supply industry - Research cooperation between MINT and Tenaga Nasional Research Development Sdn. Bhd. from 1998 - 2000. The research fund is partially provided by company.

New technology/material/product

- Enhancement of the properties of thermoplastic natural rubber – funded by IRPA for 1997 – 1999. R & D in cooperation with Malaysian Rubber Board.
- Blending of natural rubber with thermoplastic using liquid natural rubber and other additives as compatibilizers – Collaboration with university and supported by IRPA, 2000 - 2002
- Property enhancement in radiation vulcanized natural rubber latex – funded by IRPA for 1997 – 1999. R & D in cooperation with several natural rubbers dipped product manufacturers.
- Radiation processing of water-soluble polysaccharide – funded by IRPA for 1999 and 2000. This project is part of the bilateral project between MINT and Takasaki Radiation Chemistry Research Establishment, JAERI, 1998 – 2000 entitled Radiation Crosslinking of Starch.
- Radiation processing of indigenous polymer (modification of starch from SAGO for biodegradable foam) – RCA/IAEA funded CRP project, 1998 – 2000.
- Radiation processing of chitin/chitosan – funded by IRPA for 1999 and 2000.
- Development of radiation curable materials from palm oil for pressure sensitive adhesive and other application – in house funding and partly funded by company (1996 –1998)

- Basic study on radiation processing of bio-fibers polymer composite materials – funded by IRPA (UPM). Research cooperation between Universiti Putra Malaysia (UPM) and MINT, 1997 - 2000

Note: IRPA (Intensified Research in Priority Area) is a funding scheme for R & D managed by the Ministry of Science, Technology and the Environment.

31 Radiation Processing in Japan

Keizo MAKUUCHI
Takasaki Radiation Chemistry Research Establishment, JAERI

1. Introduction

Economic scale of nuclear industry in Japan in 1997 was investigated by a study group organized by TRCRE JAERI in 1999. Table 1 shows summary of the investigation. It was ¥15.85 trillion ($ 13,108 million), including nuclear energy industry and radiation applications in industry, agriculture and medicine. This value is higher than GDP (Gross Domestic Product) of Malaysia ($ 9,788 million) and lower than that of Thailand ($ 15.391 million). Total production value of radiation application accounted for 54% of nuclear industry. Clearly, radiation application has larger economic impact than that of nuclear energy industry.

Table 1 - Economic Scale of Nuclear Industry in 1997 (Unit: ¥billion)

	Field	Production Value		Share
Radiation Application		8,573.8		54.1%
	Industry		7,266.6	
	Agriculture		116.7	
	Medicine		1,190.5	
Nuclear Energy		7,274.2		45.9%
	Nuclear Power Generation		5,661.3	
	Nuclear Industries		1,612.9	
	Total	15,848.0		

Industrial application of radiation is divided into five groups, namely non destructive test (NDT), RI instruments, radiation facilities, radiation processing and ion beam processing. Table 2 shows actual production value by these groups. More than 70% of the total production value was brought about by ion beam processing. Ion beams are used for the production of IC and semiconductors. There were about 310 NDT inspection companies. Inspection service charges were evaluated as production value, instead of production value of radiography apparatus. NDT using radiation was estimated to be 24.6% of service charges of ¥126.7 billion. RI instruments used were thickness gauges, level gauges, density gauges, moisture gauges and gas chromatograph. Total production value was estimated to be ¥65.4 billion. Total installation costs of radiation facilities were estimated to be ¥369.3 billion in medical field and ¥29.9 billion in industrial field. Economic scale of radiation processing is evaluated by production value of radiation processed products. It was estimated to be ¥1.39 trillion.

Table 2 - Production Value of Industrial Application of Radiation (Unit: ¥100 million)

Field	Production Value
Non Destructive Test	312
RI instruments	654
Radiation Facilities	4,274
Radiation Processing	13,867
Ion Beam Processing	53,559
Total	72,666

In this paper present and future economic prospect of radiation processing of polymers will be reviewed.

2. Radiation Processing

Radiation processing is defined as an industrial application of chemical reactions induced by radiation. Radiation reactions are utilized in polymer processing, radiation sterilization and food irradiation. Main radiation reactions are chain scission, recombination of radicals, addition of radicals to unsaturated double bond and oxidation. Practically important radiation reactions are crosslinking of polymers by recombination of polymer radicals, initiation of radical polymerization and degradation of polymers. Table 3 shows production value of radiation processed products. Polymer processing accounts for 80% of the total value of radiation products.

Table 3 - Production Value of Radiation Processing (Unit: ¥100 million)

Field	Production Value
Polymer Processing	11,026
Sterilization	2,903
Food Irradiation	19

2.1 Polymer processing

Crosslinking, graft polymerization, EB curing, degradation and polymerization are the main fields in polymer processing by radiation. No plant is operated for radiation polymerization in Japan. Table 4 shows the production value of radiation processed polymer products. Contribution of the crosslinking technique to the economy is outstanding among them due to wide applications to improve thermal and mechanical properties of polymers.

Table 4 - Production value of Radiation Processed Products (Unit: ¥100 million)

Field	Production Value
Crosslinking	10,981
EB Curing	30
Graft Polymerization	10
Degradation	5

2.1.1 Radiation crosslinking

The first success in radiation processing in Japan was the production of heat resistant wires by crosslinking in 1964. Subsequently, large numbers of radiation crosslinked products are supplied to the car industry and electronic industry. Table 5 shows the production value of radiation crosslinked products. Tires are outstanding 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.

Table 5 – Production Value of Radiation Crosslinked Products (Unit: ¥100 million)

Field	Production Value
Tires	10185
Wire & Cable	450
Foams	179
Heat Shrinkable Tube & Film	165
Other crosslinked products	2

A. Tires

Six companies (Bridgestone, Yokohama Tires, Sumitomo, Toyo Tires, Ohtsu Tires, and Michelin-Okamoto) produce about 170 million tires in Japan. Among them five companies installed electron accelerators. Fig. 1 shows increases in production of radial tires and total energy of medium energy electron accelerators (500 - 800 keV) installed in tire manufacturers. These accelerators are used for pre-vulcanization of carcass ply of radial tires to increase green strength. Pre-vulcanization of inner liner of radial tires is also applied by few companies by using low energy electron accelerators. Radial tires accounted for 91.3 % of tires in 1997. Total production value of tires by five companies was ¥1.110 trillion. The production value of radiation processed tires was estimated to be ¥1,018.5 trillion.

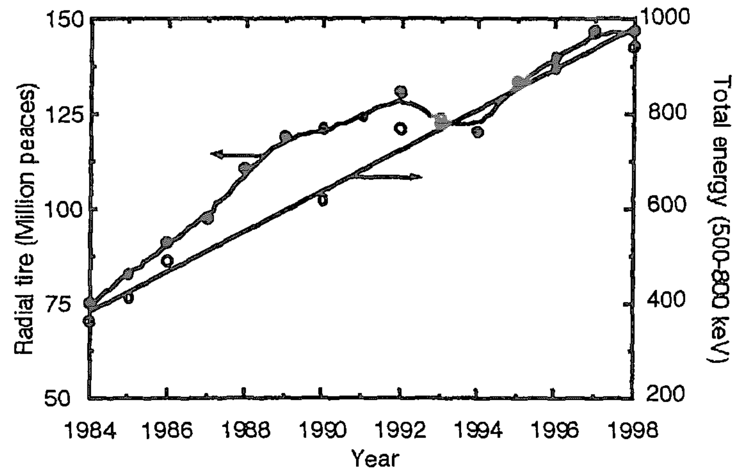

Fig. 1 - Production of radial tires and total energy of medium energy electron accelerators in tire industry in Japan

B. Wires and cables

Total production value of wires and cables was ¥1.2335 trillion in 1997. Radiation crosslinking of wires and cables was applied by 16 companies. Production value of radiation crosslinked wires and cables were estimated to be ¥45 billion. The radiation crosslinked wires and cables show excellent heat resistant and abrasion resistance. Main markets of radiation crosslinked wires and cables are car industry and electrical industries. Huge amounts of radiation crosslinked wires and cables are incorporated in a car to reduce the total weight and to increase processability of the car.

C. Polyolefin foams

Radiation crosslinked, closed-cell type polyolefin foams were developed by Sekisui Chemical and Toray. Polyolefin foams are lightweight, heat insulating, shock absorbing, highly moldable, and non-water-absorbency. Due to these properties polyolefin foams have a wide range of applications such as molded interior car components, insulation materials for construction uses, jointing materials, pipe covers, miscellaneous industrial materials, consumer goods, and healthcare and sports products. Polyethylene and polypropylene are representative polyolefins used for production of plastic foams. Production values of polyethylene foams and polypropylene foams were ¥9.85 billion and ¥8.0 billion, respectively.

D. Heat shrinkable tubes

Heat shrinkable tubes were commercialized by four companies and total production value was estimated to be ¥15 billion. Crystalline polymers such as polyolefin and fluoropolymers are used for these products. Heat shrinkable tubes are used in many areas besides cars and airplanes. Protection of connecting parts of wedded wires and cables, optical fibers and pipelines. Heat shrinkable films produced by radiation are used for food packaging. Domestic production and import of the heat shrinkable films were 500 tons and 1,000 tons, respectively. Production value of heat shrinkable films was estimated to be ¥1.5 billion.

E. Other crosslinked products

Rubber parts built in cars such as O rings and oil hose are crosslinked by radiation. A plant for the production of SiC fibers by radiation crosslinking technique is operating by Japan Carbon Co. Ltd. Radiation vulcanized natural rubber latex gloves are used in nuclear power plants. Total production value of these products in 1997 was estimated to be about ¥ 200 million.

2.1.2 EB curing

Radiation curing of resins with low energy electron accelerator is the environmentally friendly process because it is solvent free and energy saving process. Examples of commercial application of electron curing in Japan are precoated steel panels, precoated steel coils, tunnel interior panels, floppy discs, anti-fogging films, thermo-sensitive paper, and pressure-sensitive adhesive films. However, this technique has not been utilized as expected due to emerging of UV curing technique. Total production value of EB cured products was estimated to be about ¥3 billion.

2.1.3 Radiation graft polymerization

Radiation graft polymerization is known as one of the best methods to modify solid polymeric materials such as film, membrane, fiber, and cloth. Battery separators and super-clean-air-filters used in LSI factories are commercially produced by this technique. Total production value of radiation grafted products was estimated to be about ¥1 billion.

2.1.4 Radiation degradation

Fine powders of PTFE are produced by radiation degradation technique in Japan. Total production value of radiation degraded products was estimated to be about ¥500 million.

2.2 Radiation sterilization

The market for industrial sterilization of medical devices is around ¥472.8 billion. As shown in Fig. 2, 56% of sterilized medical articles are sterilized by Co-60 and 4 % by electron beams. Sterilization with high-energy electron accelerator of 5-10 MeV is increasing. Three 10 MeV electron linear accelerators are installed in Hogi Medical Co. Ltd. to sterilize medical clothes. Four 5 MeV electron accelerators are also operating as contract irradiators.

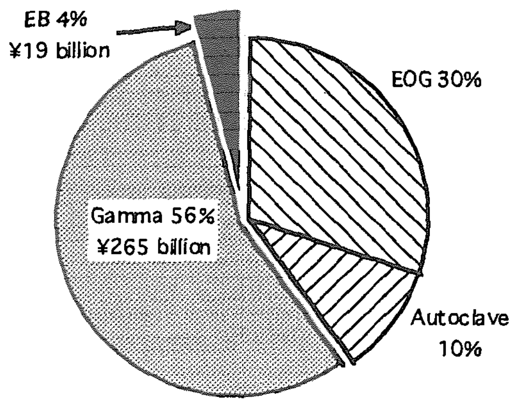

Fig. 2 - Share of sterilization methods

Packaging material and animal foods are sterilized by radiation, thought the amounts are not large as shown in Table 6.

Table 6 - Production Value of Radiation Sterilized Products 1997 (Unit: ¥100 million)

Field	Production Value
Medical Devices	2841
Packaging Materials	60
Animal Food	2

2.4 Food irradiation

Food irradiation was limited for the sprout inhibition of potatoes. The production value of the potatoes was ¥1.9 billion.

3. Future prospects of Radiation Processing of Polymers

Table 7 shows estimated relative productivity of EB machine (production value/number of operating EB machine). These data indicate that steady growth can be expected in crosslinking, but hard to growth in other processing.

Table 7 - Relative productivity of EB machine for Radiation Processing

Radiation Processing	Main Products	Number of EB		Production Value (¥billion)	Relative Productivity
		Installed	Operating		
Crosslinking	Radial Tires	28	17	1,018	59.9
	Wire/Cable	60	43	45	1.05
	Foams	16	8	18	2.25
	Tube/Film	14	12	17	1.42
Graft Polym.	Membrane	<5	2	1	0.5
EB Curing	Coating etc	48	<20	3	<0.15

3.1 Crosslinking

Most of the radiation crosslinked products are used in car industry. Cost reduction requirement from car companies to the parts suppliers is severe. There are competitive technologies for crosslinking. For example, the percentage of radiation crosslinked plastic foams in the total of plastic foams is only 2.5%. Radiation crosslinking technology has several advantages over the other technologies. However, cost of crosslinking by radiation is higher than the other crosslinking techniques. Reductions of the initial investment (price of EB machine) are desirable to advance the traditional radiation crosslinking technique.

Radiation crosslinking technique has been applied to solid products so far. Crosslinking of polymer in aqueous solution or dispersion will expand the radiation processing. Hydrogel prepared by irradiation of aqueous solution of synthetic polymers such as PVA and PVP are applied for wound dressing. The mechanical properties of the PVA hydrogel is improved by in incorporation of carrageenan. Though carrageenan degrades by radiation, carrageenan incorporated hydrogel by irradiation has an excellent mechanical properties. This fact is interesting from the standpoint of application of the hydrogel and radiation crosslinking of degradable polymers.

Example of radiation crosslinking of dispersed polymer in water is vulcanization of natural rubber latex. Natural rubbers (cis-l, 4-polyisoprene) dispersed in an aqueous medium as fine particles are crosslinked easily with low dose. Fig. 3 shows the recently developed RVNRL process. The process consists of five steps, (1) mixing latex with n-butylacrylate (vulcanization accelerator), (2) irradiating the mixture, (3) dilution, (4) addition of PVA, and (5) centrifugation.

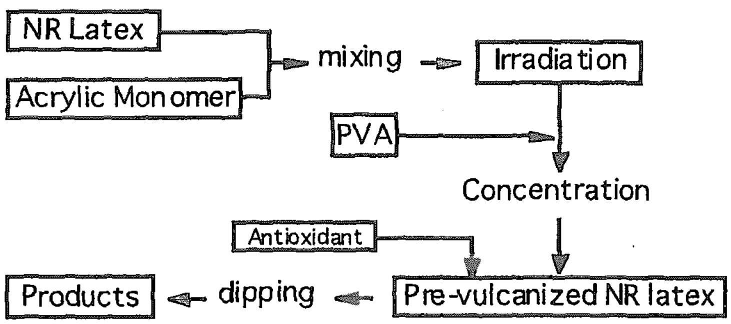

Fig. 3 - Process of radiation vulcanization of natural rubber latex

The resulting radiation vulcanized latex is used for the manufacture of latex products by a conventional coagulant dipping process. The radiation vulcanized latex does not contain dithiocarbamates, sulfur and zinc oxide that are used in the conventional vulcanization. In addition, radiation decomposes natural rubber proteins that cause serious allergy. Combination of dilution, PVA addition, and centrifugation of the radiation vulcanized latex reduces the amount of proteins of the rubber films to very low level by short term leaching. The radiation vulcanized latex has the following advantages over conventionally vulcanized NR latex with sulfur:

No fear of protein allegy
Absence of N-nitrosamines
Very low cytotoxicity

Easy degradation in the environment
Transparency and softness
Less formation of SO_2 when burned

Another promising crosslinking techniques that have not yet utilized are crosslinking of polymer blends at high temperature. Polymer material that has gradient distribution of crosslinking can be produced by irradiation of electron beams with adequate energy.

3.2 Graft polymerization

Besides radiation method, there are several graft polymerization methods. Most popular method is chemical initiator method. 540,000 tons of ABS resins are products by the chemical initiator method. Advantage of radiation graft polymerization is the modification of deep inside of arbitrary shape of product. Radiation method has limited uses to produce value added functional materials. Extreme reductions of the initial investment (price of EB machine) and to find proper products are necessary factors for the further promotion of radiation graft polymerization technique.

3.3 EB curing

EB curing has been facing with severe competition with UV curing. Extreme cost reductions of EB machine and EB curable resins are essential for the further encouragement of EB curing.

3.4 Degradation

Emerging application of radiation degradation technique is focused on natural polymers. Radiation degraded marine carbohydrates such as alginate, carrageenan, and chitin/chitosan have special biological activities. Plant growth promoter, antimicrobias, heavy metal suppressor will be produced from radiation degraded marine carbohydrates. Radiation degradation of pulp cellulose can significantly lower production costs and can provide critical environmental benefits in viscose industry.

3.4 Polymerization

Radiation-induced radical polymerization is hard to compete with conventional polymerization in terms of initial investment and quality of polymers. Undeveloped area of radiation-induced polymerization is high dose rate polymerization with electron beams. New polymer can be produced at high dose rate irradiation because cationic polymerization processs becomes predominant.

4. Conclusion

Present situation of radiation processing was reviewed. Very slow expansion is anticipated in the crosslinking of solid products, graft polymerization and EB curing. New generation of radiation processing will be crosslinking of polymers in solution and dispersion. Radiation degradation of natural polymers is expected to develop new area of radiation processing. Furthermore, remarkable progress in electron accelerator is necessary to support the radiation processing.

32 Conclusion Remarks

Tadao SEGUCHI
Director, Department of Material Development
Takasaki Radiation Chemistry Research Establishment,
Japan Atomic Energy Research Institute
1233 Watanuki, Takasaki, Gunma 370-1292, Japan

In this symposium during two days we have many interesting presentations and valuable discussions for the research and development of radiation processing of natural polymers. Especially, the talks by the special guests, Dr. S. Machi, Prof. M. Suzuki, and Dr. A. Djaloeis, are the useful suggestion to guide this subject in future.

The subjects carried out by bilateral cooperation could be summarized as follows.

(1) Marine carbohydrates;

The products induced by radiation decomposition are very effective to growth of plants or to extension of fruits storage, and the dependencies on irradiation dose have been cleared. The application seems to be highly expected. However, the products analysis and profitable irradiation techniques are not enough, which are the future works.

(2) Starch and polysaccharides;

The radiation crosslinking was confirmed by the modification of law materials or by the mixing of other polymers. As the materials by crosslinking behave as hydro-gel, the application will be very wide when the crosslinking technology is developed.

(3) Silk protein;

The radiation degradation behavior of silk is progressed, and the decomposed products of silk proteins are found to have antibacterial activity in hydro-gel. Silk has intrinsically good compatibility with human body, so the degradation products may have high potentiality to apply for cosmetics and medicines.

(4) Natural rubber latex;

The fundamental research for radiation crosslinking has been completed, and now it is final stage of R & D for the processing to commercial products. Low energy electron beam accelerator is presented to be economical radiation source.

The above research works except natural rubber latex are rather less in the mechanisms. As the considerable data are accumulated, the analysis is expected to image a model of chemical reaction mechanisms. When the mechanism was composed, then the next experimental could be foreseen to develop extensively.

The radiation applications at present in 8 Asian countries, which are presented by the representative from each country, are very valuable in the various aspects for all participants. The information exchange at this symposium should contribute to develop R & D of radiation science and application in Asian countries.

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 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	$m \cdot kg/s^2$
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
線量当量	シーベルト	Sv	J/kg

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

$1\ eV = 1.60218 \times 10^{-19}\ J$
$1\ u = 1.66054 \times 10^{-27}\ kg$

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

$1\ Å = 0.1\ nm = 10^{-10}\ m$
$1\ b = 100\ fm^2 = 10^{-28}\ m^2$
$1\ bar = 0.1\ MPa = 10^5\ Pa$
$1\ Gal = 1\ cm/s^2 = 10^{-2}\ m/s^2$
$1\ Ci = 3.7 \times 10^{10}\ Bq$
$1\ R = 2.58 \times 10^{-4}\ C/kg$
$1\ rad = 1\ cGy = 10^{-2}\ Gy$
$1\ rem = 1\ cSv = 10^{-2}\ Sv$

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(=10^5dyn)	kgf	lbf
1	0.101972	0.224809
9.80665	1	2.20462
4.44822	0.453592	1

粘　度　$1\ Pa \cdot s(N \cdot s/m^2) = 10\ P$(ポアズ)$(g/(cm \cdot s))$

動粘度　$1\ m^2/s = 10^4 St$(ストークス)(cm^2/s)

圧力

MPa(=10 bar)	kgf/cm²	atm	mmHg(Torr)	lbf/in²(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 = 4.18605 J (計量法)
　= 4.184 J (熱化学)
　= 4.1855 J (15 °C)
　= 4.1868 J (国際蒸気表)

仕事率　1 PS (仏馬力)
　= 75 kgf·m/s
　= 735.499 W

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）

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