Radiation processing of polysaccharides
Radiation processing of polysaccharides
FOREWORD

Radiation processing has been applied in many fields of national economy. Sterilization, polymer cross-linking (tapes, tubes, cables, etc.), tyre belt vulcanization, conservation of art objects, and irradiation of selected food items are well established technologies. Two types of irradiators, gamma sources and electron accelerators, are being applied for the process.

The International Atomic Energy Agency — through its technical cooperation programmes, coordinated research projects, consultants and technical meetings and conferences — is promoting the peaceful use of nuclear science and radiation technologies. Due to the IAEA’s support, some new radiation technologies (e.g. hydrogel wound dressings, sulphur-free radiation vulcanized latex) were developed and transferred to Member States during the past ten years.

Special attention was given to the radiation processing of natural polymers under the framework of the Regional Cooperative Agreement (RCA) for Research, Development and Training Related to Nuclear Science and Technology for East Asia and the Pacific. This region is especially rich in respect to wide variety of indigenous natural polymers, such as chitin/chitosan, carrageenans and alginates. Therefore, the IAEA has been organizing research and technical cooperation projects in this area. A Coordinated Research Project on Radiation Processing of Indigenous Natural Polymers was conducted from 1997 to 2000; and Workshops on Radiation Processing of Chitin/Chitosan, Radiation Processing of Polysaccharides, Radiation Processing of Natural Polymers for Agro- and Agro-chemical Industry, Technical, Economic and Environmental Advantages of Radiation Processing of Cellulose and Radiation Processing for Upgrading of Cellulosic Agro-Waste into Animal Feed were organized in the region. The research groups in the nuclear institutes and universities have already developed new products from these natural indigenous polysaccharides, such as plant growth promoters, novel wound dressing for burn patients, fruit preservatives, absorbents, etc.

A Technical Meeting on Emerging Applications of Radiation Processing for the 21st Century held in Vienna, 28–30 April 2003, reviewed the present situation and possible developments of radiation technology to contribute to sustainable development. Subsequently a series of meetings to discuss particular subjects has been organized. Consultants Meetings on Advances of Radiation Chemistry of Polymers and on Status of Industrial Scale Radiation Treatment of Wastewater held in the USA, September 2003 and in the Republic of Korea, October 2003, respectively, discussed the status and trends of radiation technology in these areas.

The Consultants Meeting on Radiation Processing of Polysaccharides held at the Japan Atomic Energy Research Institute (JAERI), Japan, in November 2003 reviewed the recent developments concerning radiation processing of polysaccharides.

This TECDOC provides basic information on radiation processing of polysaccharides and promotes experience exchange for further development of radiation processing. Public awareness and technology acceptance are other factors to be considered for further dissemination.

The IAEA acknowledges the valuable contributions of all the participants in the consultants meeting. The IAEA officer responsible for this publication was A.G. Chmielewski of the Division of Physical and Chemical Sciences.
CONTENTS

SUMMARY ........................................................................................................................................... 1

STATUS REPORTS

Radiation processing in India: Current status and future programme ........................................... 9
Sunil Sabharwal

Radiation processing in Japan: R&D for technology transfer ...................................................... 17
M. Tamada

Status of radiation processing in the Republic of Korea ............................................................... 21
Young-Chang Nho

REPORTS OF PARTICIPANTS

Radiation processing of natural polymers: Achievements & trends ........................................... 29
S. Sabharwal, L. Varshney, A.D. Chaudhari, S.P. Ramnani

Radiation degradation and crosslinking of polysaccharides and its application ......................... 39
F. Yoshii

Prevention of surgical adhesions using barriers of carboxymethyl cellulose and
polyethylene glycol hydrogels synthesized by irradiation ....................................................... 45
Joon-Ho Lee, Young-Chang Nho

Review of alternative methods applying to cellulose and
chitosan structure modification .................................................................................................. 55
H. Struszczyk, Dciechańska, D. Wawro, A. Niekraszewicz, G. Strobin

Radiation degradation of chitosan and some biological effects ............................................... 67
Nguyen Quoc Hien

LIST OF PARTICIPANTS .................................................................................................................... 75
1. BACKGROUND

The consultants meeting on “Radiation Processing of Polysaccharides” was held at the Takasaki Radiation Chemistry Research Establishment (TRCRE), of the Japan Atomic Energy Research Institute (JAERI), Japan on 24-27 November 2003. TRCRE has been at the forefront of developing a large number of radiation based technologies, especially in the area of processing of polymeric materials. Five experts from India, Japan, Korea, Poland and Vietnam and two observers from Japan attended the Meeting. They contributed to the preparation of this technical report.

The discussions at the Meeting were focused towards the following two objectives:

- Review of the radiation processing activities in the participants’ countries and the future trends in the field
- Radiation processing of natural polymers with special emphasis on the commercial successes achieved and the emerging applications of radiation processed natural polymers.

Radiation processing is a very convenient tool for imparting desirable effects in polymeric materials and it has been an area of enormous interest in the last few decades. Commercial success has been achieved in radiation processing of synthetic polymers, which is now a multi billion dollar industry.

The success of radiation technology for processing of synthetic polymers can be attributed to two reasons, namely, their ease of processability in various shapes and sizes, and secondly, the fact that most of these polymers undergo crosslinking reaction upon exposure to radiation. On the other hand, naturally occurring polymers were difficult to process and degraded when exposed to high energy radiation. Thus, the area of radiation processing of natural polymers largely remained unexplored and industrial applications have been difficult to achieve.

A lot of research work specially related to the use of radiation technology for minimizing environmental pollution associated with the processing of natural polymers such as dissolution of cellulose in the viscose-rayon process was carried in countries like Canada and Russia. However, the process could not be commercialized as the viscose producing plants were relocated to countries in South-East Asia where the environmental issues were less demanding. In recent years, natural polymers are being looked again with renewed interest because of their unique characteristics like inherent biocompatibility, biodegradability and easy availability.

Traditionally, the commercial exploitation of natural polymers like carrageenans, alginites or starch etc. has been based, to a large extent, on the empirical knowledge. But now, the applications of natural polymers are being sought in knowledge-demanding areas such as pharmacy and biotechnology, which is acting as a locomotive for further scientific research in their structure-function relationship.

This opportunity was realized a few years ago by the Member States of the RCA region and a project on utilization of naturally occurring polymers for value addition through radiation processing was initiated in the RCA region in 1998. The project was especially of relevance to the region keeping in view the available natural resources and the prevailing socio-economic conditions. A variety of natural polymers occur in the region such as carrageenans, alginites, starch etc. Besides this, it was also envisaged to utilize the large amount of agricultural waste (byproducts) generated in the region for value addition.
Under the project, work was focused to achieve the following:

- Development of applications of low molecular weight polysaccharides in the area of agriculture as plant growth promoter
- Utilize the specific properties of natural polymers as an additive to produce radiation processed hydrogels
- Development of methods for producing radiation crosslinked completely bio-degradable hydrogels
- Explore the possibility of using radiation modified materials or methods for environmental remediation.

During the course of the implementation of the project, a number of regional training courses and workshops were organized to provide necessary training to technologists as well as to assess achievements and explore areas wherein commercial prospects of the technology can be exploited.

The final Meeting of the IAEA/RCA Project Coordinators was held in Bangkok, Thailand on 18-20 March 2002, where various aspects of progress made in the project were evaluated for achievements. The discussions at this meeting indicated that the R&D efforts put in by many Member States have started yielding good results in many areas and it was recommended that the applied work carried out must be converted into the technologies. Since then, considerable progress has been made and some of the radiation processed polymers based products have been commercialized in some countries. It was therefore felt necessary to organize this Consultants Meeting to achieve the following objectives:

- to assess the progress achieved in this direction so far
- to identify methodologies and analytical methods for investigating their structure-property relationships with respect to radiation produced changes
- to identify the testing protocols required for implementing the programme on a large scale and integrate the programme with specialists from the other fields such as biotechnology, agriculture and medicine
- identify new areas of application, to chart out future programme of the project
- to explore the possibilities of implementing the developed technologies in the other regions such as Africa and South America which have similar socio-economic conditions.

2. ACHIEVEMENTS IN RADIATION PROCESSING OF NATURAL POLYMERS

Synthesis of Radiation Crosslinked Natural Polymers or their Derivatives

As mentioned earlier, naturally occurring polymers or their derivatives under normal conditions of irradiation undergo chain scission reaction. The work done under the project has shown that under suitable conditions, polymers such as carboxymethyl cellulose (CM-cellulose) or carboxymethyl starch can be crosslinked to form a hydrogel material. This offers an opportunity to obtain non-toxic, additive free, totally biodegradable and biocompatible crosslinked hydrogels for many applications.

The work done at JAERI has resulted in the development of a non-bed sore forming hydrogel mat based on radiation crosslinked CM-Cellulose hydrogel. The product was extensively tested in collaboration with a local hospital and found to be extremely useful. The technology for the product has been transferred to a private manufacturer and the product has been successfully commercialized. Similarly, radiation crosslinking of chitosan in aqueous solutions has been achieved by irradiating it in presence of a sensitizer, carbon tetrachloride.
The crosslinked material is now being evaluated as an adsorbent for removing toxic materials from the waste waters. Formation of crosslinked polymer hydrogels from natural polymers and their derivatives offers new avenues for applications of such materials in many areas.

Applications of Radiation Processed Natural Polymers for Health Care

Radiation Processed Burn/Wound Dressings

The healing of wounds, especially the burn wounds has been a challenging medical problem as such wounds take a long time to heal and need to be protected to prevent infection of the wound. A radiation processed wound dressing based on PVP, agar and polyethylene glycol has been well established in the market. Naturally occurring polymers like alginates and carrageenans are known to possess excellent wound healing characteristics. In order to utilize the functional properties of these polymers for wound healing, a PVA based hydrogel containing naturally occurring polymers like agar and carrageenan has been developed and commercialized in India. Similarly, a PVP based hydrogel containing carrageenan has been developed and extensively tested on patients in Philippines with very encouraging results.

An additional feature of these hydrogels is that they have been found to be useful in curing wounds in patients having diabetic ulcers which are otherwise difficult to heal indicating that such gels could be extremely useful in managing difficult-to-heal wounds. The PVP-carrageenan gel has the additional advantage of being a haemostatic agent, which can be extremely helpful in many medical emergencies. The successful development of such materials provides new opportunities for use of natural polymers in knowledge based technologies.

Radiation Processed Hydrogel for Prevention of Surgical Adhesions

Post surgical adhesions are the undesired tissue attachments that are formed in the cuts or abrasions in the tissues especially after abdominal surgery and result in a number of complications such as pain, infertility and obstruction of intestines. 60-95% of the patients develop adhesions following major abdominal surgery. Radiation crosslinked CM-cellulose and polyethylene glycol (PEG), which are biocompatible and biodegradable, have been tested as physical barriers for preventing surgical adhesions in rats. The results have shown that animals tested with CM-cellulose/PEG hydrogel or its solution form had a significantly lower average adhesion score as compared to the control animals indicating that such materials could be of potential use for preventing surgical adhesions and reduce the cost associated with adhesiolysis and subsequent hospitalization.

Radiation Processed Low Molecular-weight Polysaccharides for Agricultural Applications

Low molecular weight naturally occurring polysaccharides like chitosan and alginates, prepared by conventional methods, have been reported to possess novel features such as promotion of germination and shoot elongation and stimulation of growth of Bifidobacteria. As compared to the conventional techniques, like acid or base hydrolysis or enzymatic methods, radiation processing offers a clean one step method for the formation of low molecular weight polysaccharides in aqueous solutions even at high concentrations.

A lot of studies have been carried in countries like Japan, Vietnam, China, India and Philippines to investigate the plant growth promotion and plant protection effect of radiation processed polysaccharides in a variety of crops under different environmental conditions.

The results of these studies have clearly shown that radiation processed polysaccharides even at very low concentrations of a few tens of ppm are very effective for use as plant growth promoter. This application offers tremendous opportunity to use them as highly effective organic fertilizers. Their
biodegradability will be an additional advantage of using such materials as plant growth promoters. In Vietnam, three such formulations have already been commercialized namely, Olicide and Gold Rice (chitosan based) and T&D (alginate based).

The antibacterial and antifungal effect of low molecular weight chitosan has been demonstrated for reducing the post harvest losses by prolonging the shelf life of many fruits and vegetables by coating them with these polysaccharides. More importantly, low molecular weight chitosan has been demonstrated to be an effective plant protector against infectious diseases and environmental stress.

**Combined Radiation and Conventional Processes for Efficient Processing of Natural Polymers**

Many processes of radiation treatment of natural polymers, though known for a long time, have not yet been commercialized either because of the high cost of irradiation (high dose) or because of the reluctance on part of the industry to adapt the radiation technology. It is therefore of importance to consider combining the beneficial effects of conventional technology along with radiation technology to overcome such problems.

The use of electron beam processing of cellulose pulp for reducing its degree of polymerization (DP) has been studied very extensively as it offers advantage in terms of reducing the concentration of carbon disulphide in the viscose process. The results presented at the Meeting showed that by suitably combining radiation technology along with the enzymatic treatment it can be used to dissolve cellulose pulp even without addition of the toxic carbon disulphide solvent used in the conventional viscose-rayon process. The developed process utilizes the electron beam treatment for initially reducing the DP to a desired level and enzymatic modification to produce alkali soluble cellulose. The combined process offers many unique advantages over the existing viscose-rayon process, which is now facing stiff environmental regulations due to large pollution associated with the process.

Similarly, the cost of production of radiation processed low molecular weight chitosan is still high because the dose required to produce oligo-chitosan is rather high. The work reported at the Meeting has shown that chemical treatment combined with radiation treatment can be useful in producing low molecular weight chitosan in a more cost effective manner.

Another concept of combining radiation technology with the conventional technology that has been successfully demonstrated is in the area of converting cellulosic agro byproducts (agrowastes) material into substrate for growing mushroom and subsequently using it as an animal feed. The process involves radiation pasteurization of cellulosic waste followed by inoculation with desirable fungi to grow mushrooms. The process has been demonstrated to be technically and economically feasible on a large scale using electron beam accelerator to achieve the desired output.

### 3. IDENTIFICATION OF NEW EMERGING AREAS

**Waste water engineering**

The complex-forming ability as well as interaction with the negatively charged surfaces of polymers like chitosan makes them ideal candidates for use as adsorbents and flocculants. Many of these applications are currently being explored. However, the limited solubility of chitosan in near neutral and basic solutions has been a limiting factor in flocculation applications. Radiation processing offers unique advantages in terms of providing low molecular weight chitosan having nearly the same charge and higher solubility.

On the contrary, the solubility of chitosan films or fibre in acidic solutions is a hindrance in its application for use as an adsorbent for removing toxic metals and dyes from industrial effluents. In this case, radiation technology can provide crosslinked or grafted chitosan for use as adsorbents.
Organoleptic gels for absorption of organic solvents

Recently, novel crosslinked cellulose ether hydrogels have been synthesized by radiation processing techniques. Such gels have been found to uptake large amount of organic solvents such as pyridine, dimethyl formamide and dimethyl sulphoxide. These gels have the potential to be used as selective adsorbents for removing toxic organic chemicals from the waste streams. Further work should be directed to develop such applications.

Bio-medical Applications

Natural polysaccharides like chitosan have been demonstrated to be useful materials for biomedical applications such as culture material for skin graft and bone cells. Radiation processed polysaccharides should be tested in such applications.

Development of Biodegradable Plastics

The development of biodegradable plastic for environmental conservation needs to be addressed on an urgent basis as a large amount of non-degradable waste is generated throughout the world especially in the advanced countries. On the other hand, enormous amounts of agro-waste materials are produced in the developing countries, which in various forms can be suitably used as fillers or additives in the plastics to increase the environmental degradability of the composite materials. Besides taking care of the waste generated, this step will impart value addition to the waste and enhance the economic condition of the people engaged in agro-related industries in such countries. Radiation processing can be effectively utilized to develop synthetic-natural polymer based composites that can be crosslinked to impart the desired physico-chemical properties to these materials.

Development of hybrid technologies for processing of cellulose based materials

The tightly bound network structure of cellulose necessitates use of very harsh and toxic conditions for solubilizing it. New biotechnological methods have been developed in recent years, which allow dissolution of cellulose in alkali solutions. Radiation technology methods along with such biotechnical methods should be investigated for bringing economical benefits to the industries where cellulose is used as a raw material for manufacture of products like fibres, films, fibroids and in emerging area of nano structured products. The hybrid technology can also be beneficially utilized to convert cellulosic waste into useful products.

4. FINDINGS

After extensive discussion and deliberations at the Consultants Meeting, the following findings are being put forward.

- Naturally occurring polymers or their derivatives are diverse, abundant, possess unique properties and are now being explored for various knowledge-based applications. In a short span of about five years, considerable success has been achieved in some of areas related to health care and agriculture practices by the use of radiation processed polymeric materials. Further efforts are necessary for demonstrating applications on a large scale.
- The scope of the programme should now be expanded by implementing radiation processing in other Member States where socio-economic conditions necessitate use of similar technology.

- Radiation processing of natural polymers for value addition has taken roots in the RCA region. The achievements of the programme need to be publicized for its further propagation in other countries.

- A new international cooperation programme could be initiated to develop further applications of radiation processed natural polymers in the emerging areas of biotechnology, pharmaceutical and medical industry. This can serve the twin purpose of integrating professionals in the other fields to utilize the benefits of radiation technology as well as opening new avenues for use of radiation processed natural polymers.
STATUS REPORTS
RADIATION PROCESSING IN INDIA: CURRENT STATUS AND FUTURE PROGRAMME

SUNIL SABHARWAL
Bhabha Atomic Research Centre (BARC), Trombay, Mumbai, India

Abstract

Radiation processing is an alternative to conventional methods such as thermal and chemical processing in many industrial applications. In India, this technology has found extensive applications in areas of healthcare, agriculture, food preservation, industry and environment. Both gamma radiation and electron beam accelerators are being utilized for this purpose. Presently, six commercial Co-60 gamma irradiators and three commercial electron beam (EB) accelerators with installed capacity of 185 kW are commercially operating in India. Five demonstration plants housing a total of 18.5x10^7 GBq Co-60 have been set up to assess the techno-commercial viability of the processes such as radiation vulcanization of natural rubber latex, decontamination of spices, hygienization of sewage sludge, shelf-life extension of onions. The new areas being explored include use of electron beam treated pulp for viscose-rayon process, radiation processed “hydrogel” burn/wound dressings and radiation processing of natural polymers. In the present paper, the current status of this program, especially the recent developments and future direction of radiation processing technology in India is reviewed.

1. INTRODUCTION

The unique advantages of high energy ionizing coupled with the availability of reliable high intensity radiation sources have resulted in ushering the era of radiation processing for a variety of applications. These include the areas of health care, food, polymer processing industry and environment. The value of radiation processed products currently runs into several billion dollars, the actual industrial deployment, on commercial basis, depends on the need of the individual society and varies from country to country.

In India, the program of radiation processing made a beginning with the establishment of ^60^Co gamma radiation source based radiation sterilization plant (ISOMED) in 1974. Since then, the applications of radiation technology in a variety of areas have been explored with emphasis on developing applications that are required to meet specific needs of Indian industry. The major emphasis of radiation processing applications in the initial years was on the use of ^60^Co as radiation source mainly because it could be easily produced in nuclear reactors indigenously and gamma radiation having large penetration offered an easy means for irradiation of thicker materials.

A 2-Mev, 20 kW electron beam accelerator, installed at Isotope Group, BARC in 1988, ushered in the era of electron beam processing in India. Presently, both gamma radiation sources as well as electron beam accelerator technology are being applied for developing such applications. The current status of this technology, with the recent developments, especially over the last 2-3 years, and the future prospects are presented in this paper.

2. LARGE SCALE RADIATION PROCESSING FACILITIES IN INDIA

The major large scale radiation processing facilities, their designed capacity and indented use are listed in Table I. It is clear from the table that two new facilities based on gamma irradiation, namely Spice irradiator and Onion irradiator have been commissioned during the last 2-3 years.
Two new electron beam accelerators have been commissioned in the private sector by the industry, while the 2-MeV electron beam accelerator, earlier located at BARC, has been relocated outside for better interaction and offering irradiation services to the industry. The Table I also shows that two new accelerators, one a 3 MeV and other 10 MeV, both indigenously developed, are being commissioned to augment the existing 2 MeV and 500 keV electron beam accelerators operating at BARC.

TABLE I. STATUS OF LARGE SCALE RADIATION PROCESSING FACILITIES CURRENTLY UNDER OPERATION/CONSTRUCTION IN INDIA

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Facility</th>
<th>Source</th>
<th>Designed Capacity</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ISOMED, Mumbai</td>
<td>$^{60}$Co</td>
<td>37 PBq</td>
<td>Radiation sterilization</td>
</tr>
<tr>
<td>2</td>
<td>RASHMI, Bangalore</td>
<td>$^{60}$Co</td>
<td>18.5 PBq</td>
<td>Radiation sterilization</td>
</tr>
<tr>
<td>3</td>
<td>SARC, Delhi</td>
<td>$^{60}$Co</td>
<td>18.5 PBq</td>
<td>Radiation sterilization</td>
</tr>
<tr>
<td>4</td>
<td>RAVI, Jodhpur</td>
<td>$^{60}$Co</td>
<td>18.5 PBq</td>
<td>Multi purpose</td>
</tr>
<tr>
<td>5</td>
<td>SPICE irradiator, Navi Mumbai</td>
<td>$^{60}$Co</td>
<td>37 PBq</td>
<td>Decontamination of spices**</td>
</tr>
<tr>
<td>6</td>
<td>SHRI, Vadodara</td>
<td>$^{60}$Co</td>
<td>18.5 PBq</td>
<td>Hygienization of sewage sludge</td>
</tr>
<tr>
<td>7</td>
<td>Krushak, Nashik</td>
<td>$^{60}$Co</td>
<td>11.1 PBq</td>
<td>Onion irradiation*</td>
</tr>
<tr>
<td>8</td>
<td>RVNRL, Kottayam</td>
<td>$^{60}$Co</td>
<td>3.7 PBq</td>
<td>Radiation vulcanisation of natural rubber latex</td>
</tr>
<tr>
<td>9</td>
<td>ILU-6, Navi Mumbai</td>
<td>EB</td>
<td>2 MeV, 20 kW</td>
<td>Multi purpose***</td>
</tr>
<tr>
<td>10</td>
<td>NICCO Cables, Kolkata</td>
<td>EB</td>
<td>3 MeV, 150 kW</td>
<td>Wire and cable crosslinking, contract irradiation service*</td>
</tr>
<tr>
<td>11</td>
<td>Radian Cables, Hyderabad</td>
<td>EB</td>
<td>1.5 MeV, 15 kW</td>
<td>Wire and Cable crosslinking*</td>
</tr>
<tr>
<td>12</td>
<td>500-keV, Mumbai</td>
<td>EB</td>
<td>500 keV, 10 kW</td>
<td>Surface curing, R&amp;D</td>
</tr>
<tr>
<td>13</td>
<td>3-MeV, Kharghar</td>
<td>EB</td>
<td>3 MeV, 30 kW</td>
<td>Multi purpose irradiation**</td>
</tr>
<tr>
<td>14</td>
<td>10-MeV, Kharghar</td>
<td>EB</td>
<td>10 MeV, 10 kW</td>
<td>Multi purpose irradiation**</td>
</tr>
</tbody>
</table>

*Commissioned in last 2-3 years, ** Under Construction, *** Relocated to new place

3. APPLICATIONS USING ELECTRON BEAM ACCELERATOR

**Crosslinking of wire and cables**

The use of high-energy radiation using EB accelerators has been well established for crosslinking of wire & cables as it results in a product that offers distinct advantages over chemically crosslinked cables viz. higher operating temperature, much lesser thickness of the insulating material and high throughput. Extensive work has been carried out at BARC in collaboration with Indian cable manufacturers and other research institutions to develop the formulations for various types of insulation’s such as polyethylene, PVC and EPDM based materials using both these methodologies [1, 2]. Suitable under beam conveyor systems for uniform irradiation of the products have been designed and installed at the 2 MeV ILU-6 accelerator and over 100 km of crosslinked wires and cables of varying insulation thickness have been processed this year.
The hand on experience gained in this technology has helped the Indian cable industries to establish in-house EB facilities. Two of the major cable industries have installed their own EB machines which are currently being operated on commercial basis for meeting the demands of Indian railways, mining industry, automobile and home appliances industry.

**Radiation crosslinking of polyethylene “O” rings**

Radiation induced crosslinking of polymer can enhance the useful working range of the polymer as well as its resistance to aggressive solvents, thereby imparting high performance characteristics in the materials. Thus, simple thermoplastic polymers after crosslinking can replace costlier special heat resistant polymeric materials for many applications. For example, uncrosslinked low density polyethylene (LDPE) has a melting temperature of 110°C and softening temperature of about 60°C. However, crosslinked LDPE can retain its dimensional stability at higher temperature. Process optimization studies have been carried out at BARC for radiation crosslinking of polyethylene “O” rings to impart dimensional stability at 200°C [3]. These “O” rings are used as gaskets for drum closures especially for edible oils. In order to induce uniform crosslinking of the product on a commercial scale, a rotating multi – spindle conveyor system has been designed to meet the desired objective. The process has been commercialized and presently, 80,000 rings can be irradiated per day using a 16-spindle under beam geometry. Radiation crosslinking has the additional advantage that since no chemical additives is added to the product, the “O” rings can be used as gasket even when these have to come in contact with edible oils thus meeting the FDA specifications. Three major companies using radiation technology for this purpose and more than 6 million rings have been processed till now.

**Radiation Crosslinked Heat-Shrinkable Tubes**

An important feature of radiation crosslinked semicrystalline polymers is its viscoelastic memory. The second most important application of radiation crosslinking is the production of heat-shrinkable products. Development work in collaboration with an industrial partner to develop heat-shrinkable tubes for joining electric and electronic cables has been completed on a laboratory scale. The process is being up-scaled to meet the quality assurance requirements of a large scale irradiation.

**Colouration of Gem Stones**

Gem and jewelry export constitutes one of major market in the Indian exports. Coloured diamonds and precious stones command a better price in the market. Use of high energy electron beam accelerators for enhancing the colour of natural diamonds has emerged as a novel technique that has been well accepted in the diamond industry as the radiation treated colour diamonds are much cheaper than the naturally occurring coloured diamonds [4]. The process is now regularly being carried out in India on a commercial basis.

**Electron Beam Processing for Viscose Rayon Process**

Viscose rayon industry is presently facing stiff regulations from environment protection agencies due to emission of hazardous chemicals like H₂S, CS₂ etc. to the atmosphere. Several manufacturers in the developed countries have closed the viscose rayon units in their countries or have severely curtailed the production. Therefore, several research centers and private companies are exploring the possibilities of modifying the overall viscose process. This search is very important for the developing countries like India, which have a large capacity of viscose production units and may benefit from processes that shall allow extension of the plant operation under suitable condition rather than closing such units.
BARC has been working in close collaboration with one of the leading rayon producer of the country to evaluate and assimilate this technology. The work carried out so far has demonstrated the degree of polymerization of the cellulose pulp can be accurately controlled by electron-beam irradiation; substantial carbon disulphide reduction (up to 25%) can be achieved while yielding the same high quality viscose. The pilot scale studies also show that $K_w$ value of the viscose show a increase and the wet tensile strength of the end fiber product shows a slight decrease when conventional viscose process conditions are used indicating that extra hemicelluloses is generated that adds to the cost of managing removal. This show that the process parameters of the conventional viscose process such as spin bath conditions and alkali requirements needs to be re-optimized for using the electron beam treated pulp.

4. APPLICATIONS USING GAMMA RADIATION SOURCES

Radiation Processed Hydrogels for use as Burn Dressings

In recent years, hydrogels have emerged as an important class of biomaterials as they possess excellent biocompatibility. The conventional methods involved for producing hydrogels use toxic additives to bring about polymerization/crosslinking and thus are not suitable for production of biocompatible hydrogels. Ionizing radiation possesses the unique ability to initiate polymerization and/or crosslinking reactions without the need to add toxic chemicals. Therefore radiation processing is emerging as an excellent tool to produce hydrogels for a variety of medical applications. Healing of wide and serious burn wounds is one such difficult medical problem as the healing takes a lot of time and the dressing used for protection have to be changed regularly till the healing is complete. A PVP based radiation processed hydrogel wound dressing was developed by Rosiak (1994) and has been successfully commercialized [5].

In India also, about three years ago, BARC initiated the program of developing poly vinyl alcohol (PVA) based synthetic additive free hydrogel for use as wound and burn dressings. These efforts have resulted in development of PVA based hydrogel dressing that possesses excellent characteristics for the desired use. The product has been extensively tested in the local hospitals; more than 10,000 pieces have been supplied to various users [6]. The technology of production has been transferred to a private entrepreneur for production on a commercial basis and the product is now available in the Indian market.

Radiation Processing of food products

Spice irradiation

India is one of the major producers of spices with a production of over 3 million tons per year, of which about 0.2 million tons are exported. During storage, spices are vulnerable to microbial attack and insect infestation and common spices are reported to have contamination levels as high as $10^8$ CFU/g. The ban on use of ethylene oxide (EtO) by European Union, Japan and many other countries has compelled the spice industry to employ alternative processes.

In order to offer a reliable, cost effective process that can be commercially utilized to reduce microbial contamination in spices, Board of Radiation and Isotope Technology (BRIT), a constituent of Department of atomic Energy, commissioned an indigenously built radiation plant at Navi Mumbai. The facility, ideally located near a spice whole sale market, is designed for housing a maximum of one million curie cobalt-60 source with a throughput of 12,000 tonnes per annum at an average dose of 10 kGy [7]. Since its commissioning, the plant has been working on commercial basis and has processed more than tones of various spices such as black pepper, turmeric, chili, ginger, cardamom and onion powder. Availability of this facility will ensure supply of quality spices from India for local use as well as export.
**Onion Irradiation Facility**

India has an annual production of over 5 million tones with post-production losses of about 25% due to tropical region climate of high temperature and high humidity. In order to establish the assess the advantages of using radiation processing technology to overcome these losses on a techno-commercial basis, and demonstrate it to potential users, an onion irradiator “Krushk” has been established by BARC in October 2002 near Nasik, Maharashtra where about 25% of the country’s onion are produced. The facility designed for a throughput of 10 tonnes/hr of onion with an initial loading of 3.7 PBq $^{60}$Co source. The facility is expected to be a forerunner for a number of such facilities expected to be commissioned in the coming few years.

**Radiation sterilization of medical products**

The technology of radiation sterilization of medical products is well established in India. The total market of sterilized single use medical products in India presently is about 18.5 million cubic feet, of which, gamma sterilization constitutes about 2 million cubic feet. This constitutes a market share of about 11%. At present, four Cobalt-60 based gamma irradiators are operating in the country to meet this requirement. In an effort to further demonstrate the effectiveness of radiation sterilized product, particularly for the rural segment where infrastructure facilities are inadequate, a number of specific kits have been developed. One such product is gamma sterilized “Dai Kits” which contains radiation sterilized materials such as absorbent gauze, cotton, blades, umbilical tapes, soaps and drape sheets etc. normally required for delivery procedure in rural homes. The use of these kits is reported to have significantly reduced the infant morbidity and mortality rates in rural areas [8].

**5. ENVIRONMENTAL APPLICATIONS**

**Radiation Treatment of Sewage Sludge**

Use of ionizing radiation for controlling environmental pollution is an important application in the area of public health. The rapid growth of large population centers especially in the developing countries, has led to the problem in management of municipal wastes. Municipal sludge, if released untreated, can endanger human health as it contains a high load of pathogenic bacteria. Conventional methods of sludge treatment such as aerobic and anaerobic digestion at best may reduce the decaying organic matter but do not mitigate the problem of pathogens. It has been demonstrated by many researchers that ionizing radiation can effectively disinfect the sewage sludge and the disinfected sludge can be used as soil conditioner and as an additive to animal fodder. This field has been investigated mainly on a laboratory scale except one large plant near Munich, Germany.

In order to gain hands on experience in this important area, the Department of Atomic Energy (DAE) has designed, built and since 1992 operating a Sludge Hygienization Research Irradiator (SHRI), adjacent to the municipal sewage sludge treatment plant in Vadodara. The SHRI plant is a batch flow irradiator designed to house 18.5 PBq of $^{60}$Co and has the capacity to process 110 m$^3$/day of sewage sludge by irradiating it to a dose of 3-4 kGy that can achieve a pathogen reduction factor of $10^6 – 10^7$. The plant has provided valuable experience regarding the design, operational parameters and economics of the process and it has been established that physico-chemical properties of the effluents remain unaltered by radiation treatment [9, 10]. Thus, irradiated sludge can be gainfully used in agricultural practice. It is expected that in future radiation processing will become an important technology for sewage sludge hygienization.
6. RESEARCH AND DEVELOPMENT ACTIVITIES

Development of Radiation Grafted Membranes for Selective Adsorption of Metal Ions

Radiation induced grafting is a potentially very important application of the ionizing radiation since it offers a unique method to modify the bulk or surface properties of a substrate, although the industrial success has been small so far. Many of the desired characteristics can easily be incorporated in a much inexpensive material to meet the demands of a particular application. Radiation grafting of anionic and cationic monomers to impart ion exchange characteristics is a very promising area and is being actively investigated. Amidoxime group containing fibrous adsorbents for the recovery of uranium from sea water or useful metal ions from waste waters has been synthesized by radiation grafting of acrylonitrile followed by amidoximation of cyano groups with hydroxylamine. Experimental conditions have been optimized for achieving over 110% grafting of acrylonitrile on non-woven polypropylene using post-irradiation grating method using EB accelerator and subsequently converting acrylonitrile into an amidoxime. The membranes prepared have been tested under actual test conditions in sea water and have been found to selectively take up uranium from sea water. Further studies to upgrade the process are being planned.

Radiation Processing of Natural Polymers

The area of radiation processing of natural polymeric material has largely remained unexplored as most of them degraded when exposed to radiation. These materials possess complex chemical structures, perform different physiological functions and above all are useful for a wide range of applications, many of which are still emerging. All of these molecules possess the following important characteristics namely, non toxicity, bio degradability, harvestable at low cost and have been used by human beings in some form or the other for centuries. Over last few years, there has been a rapidly expanding interest in such natural polymers for finding applications which can do value addition to such products. It is now being realized that radiation processing can also be beneficially utilized either to improve the existing methodologies used for processing natural polymers or to impart value addition to such products by converting them into more useful form. Experimental conditions so as to achieve radiation crosslinking of Chitosan in aqueous solutions in presence of a suitable sensitizer are established [11]. Studies to assess the effectiveness of low molecular weight polymers produced by radiation degradation for use as plant growth promoters are in progress.

7. CONCLUSION

Radiation processing has established itself as a commercially viable technology in India that can be beneficially used to enhance characteristics of many materials or can be used as an energy efficient process for a variety of applications. The technology of radiation sterilization is well established while that of food irradiation is on the way to achieve commercial success. Crosslinking of poly olefins such as polyethylene imparts high temperature dimensional stability and improves the solvent resistance of the materials. These benefits have been utilized for radiation crosslinking of wire & cables and producing LDLE “O” rings on an industrial scale using the electron beam accelerator. Crosslinking induces "memory effect" to the polymer, which makes it useful for producing heat shrinkable materials useful for cable industry. Radiation processing of natural polymers is an area that is expected to be of special interest to country like India that is so rich in natural polymers. New avenues are also being explored to use electron beam treated pulp in the Indian viscose rayon industry, which is one of the largest in the world. Formation of trapped radicals/ ions in polymers has been used for initiating grafting reactions, which can help to design and synthesize polymers having specific metal ion uptake properties. The availability of 3 MeV and 10 MeV accelerators in the near future is expected to further enhance the scope of applications in areas such as food irradiation, radiation sterilization and crosslinking thicker sections of polymer materials for many applications.
REFERENCES


RADIATION PROCESSING IN JAPAN: R&D FOR TECHNOLOGY TRANSFER

M. TAMADA
Takasaki Radiation Chemistry Research Establishment,
Japan Atomic Energy Research Institute, Takasaki, Japan

Abstract

Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute (TRCRE, JAERI) has led the radiation processing in Japan. A number of achievements in TRCRE have been transferred to the private sector and commercialized. To promote the industrialization by using 240 patents belonging to TRCRE, an open seminar has been monthly held to elucidate the interesting results to the private companies. In one year, 70 companies gave us the technical consultation. In the radiation processing, graft polymerization can synthesize a metal adsorbent which is a promising material for industrialization. Recovery of uranium from seawater and removal of cadmium from scallop processing were shown as examples for ongoing R &D.

1. R &D IN RADIATION PROCESSING

Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Institute (TRCRE, JAERI) was founded in April 1963 as a center for R &D of radiation chemistry in Japan. Its research focuses on two principal areas: radiation processing using gamma rays and electron beams (EB), and advanced radiation technology using ion beams. Since then, the radiation processing fields in materials, environment, evaluation, and biology have been activated toward the industrialization. A number of achievements in the radiation processing have been transferred to the private sector and commercialized as shown in Figure 1.

![Diagram](image_url)

FIG. 1. R&D in TRCRE, JAERI. Solid line: industrialization; triple lines: under development

The commercial products of radiation processing such as radial tire and button-shaped battery were widely used by consumers in Japan. The radiation processing is considered inevitable technique in industrial manufacture. The number of radiation facilities proves this result.
Eight facilities of gamma irradiation and 336 EB accelerators were already installed in the research institute and the production companies in Japan. Especially, the electron beam accelerators have been widely used since the EB processing had merits in easy control of beam energy and current, simple maintenance of the machine, and high power.

TRCRE has 240 valid patents for R&D of material for gamma rays, EB, and ion beams at present. To promote the industrialization by using these patents, TRCRE holds the open seminar every month to elucidate the interesting results to the private companies. Simultaneously, technical consultation was opened. In one year, 70 companies have given us the technical consultations.

2. RADIATION PROCESSING OF POLYMERS

The half of TRCE patents have been applied in the research field of organic and polymer materials. Therefore, the radiation processing of polymer is considered a very attractive field for industrialization. Three major processes are graft polymerization, crosslinking, and degradation. The graft polymerization is a sophisticated technique to synthesize new functional materials. The crosslinking is an excellent technique to improve the properties of polymers. The degradation of polymer leads the new functional chemicals.

Metal adsorbents are interest from the point of view of metal resources and environmental preservation. If a novel adsorbent has a high selectivity to a certain metal, the adsorbent will collect this selected metal from seawater and streaming water. Such an excellent metal adsorbent can be synthesized by graft polymerization as shown in Figure 2. First, the trunk polymer is irradiated with EB or gamma rays. To avoid the contact with oxygen, the trunk polymer is packed into the sealed polyethylene bag and inside air is substituted for nitrogen gas. After irradiation, the irradiated trunk polymer is put into monomer solution. The graft chain propagates during the reaction time. The obtained material is available for the recovery of uranium from seawater and the removal of cadmium from scallop processing.

![FIG. 2. Synthesis of metal adsorbent by graft polymerization](image-url)
**Recovery of uranium from seawater**

The graftpolymerization enables us to synthesize the adsorbent having selectivity to uranium in seawater. One ton of seawater contains 3.3 mg of uranium. This concentration is extremely low. However, the total amount of uranium in seawater is 4.5 billion tons. This amount is 1000 times as much as that of uranium mines. A quantity of $5.2 \times 10^6$ t of uranium is estimation for Japan in a year. If 0.2% of this amount of uranium resource is collected, the demand for electric power plants will be covered by uranium collected from seawater (Figure 3).

The adsorbent of amidoxime type is suitable for recovery of uranium from seawater. This adsorbent was synthesized by co-grafting acrylonitrile and methacrylic acid onto polyethylene nonwoven material. After grafting, the introduced CN functions were converted to amidoxime by the chemical treatment of hydroxylamine. The obtained adsorbent can maintain its strength in severe marine conditions.

The ability of the adsorbent was evaluated in marine experiment. The adsorbent stacks of 350 kg were soaked in sea. The twelve soaking experiments of adsorbent stacks achieved the collection of 1 kg uranium as yellow cake [1].

**Removal of cadmium from scallop processing**

Scallop is edible shell and is raised in the northern part of sea in Japan. Annually, the scallop processing produces some thousand tons of its waste. This waste contains much protein and fat. However, the waste including mid-gut gland of the scallop is incinerated since it contains 10-40 ppm of cadmium. The incineration produces flying ash of cadmium. The removal of cadmium by means of adsorption can help to avoid the spread of cadmium in the flying ash (Figure 4). Collected cadmium on the adsorbent is eluted by acid solution and can be used as a metal resource. The mid-gut gland without cadmium is utilized for livestock's feed.
The two kinds of adsorbent (amidoxime type and iminodiacetic acid type) were synthesized by radiation-induced grafting. To extract cadmium from the mid-gut gland, malic acid was used. The pH of upper layer became 3 after malic acid treatment. In this region of pH, the adsorbent of iminodiacetic acid type had higher selectivity to cadmium than that of amidoxime type. The column experiment revealed that 0.1 g adsorbent can remove 50 bed volumes of 2 ppm cadmium.

The removal of cadmium from scallop processing with graft adsorbent has been financially supported for three years by the Ministry of Economy, Trade and Industry in Japan.

3. CONCLUSIONS
- Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute (TRCRE, JAERI) has led the radiation processing in Japan.
- TRCRE has monthly held the seminar and the service of technical consultation to promote the technical transfer of radiation processing to the companies.
- Metal adsorbent synthesized by the graft-polymerization is a promising material for industrialization.
  - Recovery of uranium from seawater has been technically established.
  - Removal of cadmium from scallop processing has been adopted in technology transfer program in METI.

REFERENCES
STATUS OF RADIATION PROCESSING IN THE REPUBLIC OF KOREA

YOUNG-CHANG NHO
Korea Atomic Energy Research Institute,
Yusong, Daejon, Republic of Korea

Abstract
Radiation processing is a relatively sophisticated industrial technique to make products of good quality, which can not be manufactured by the conventional thermal and chemical processes. The radiation-induced changes in polymer materials such as plastics or elastomer provide some desirable combinations of physical and chemical properties in the end product. Radiation can be applied to various industrial process involving crosslinking, curing and sterilization. Current commercial usage for radiation processing in Korea is dominated by several major applications: crosslinked wire, shrinkable tube, procuring of some automobile tire components, curing of surface coating, polyolefin foam, PTC conducting polymer, sterilization of disposable medical products. A New National Core Research Center for R&D of Radiation Technology in Jeongup, Chollabukdo Korea will be constructed until the year of 2005 in order to meet the rapidly growing market in the areas of radiation technology. This report describes an overview of commercial radiation processing and research works carried out in Korea.

1. INDUSTRIAL APPLICATION OF RADIATION TECHNOLOGY

Crosslinking

Since radiation-crosslinked wire is usually more expensive than the conventional product, it is used only when special products such as high temperature resistance are necessary and cheaper chemical crosslinking methods are not satisfactory. Major markets included wiring for automobile, process control instruments in which superior environmental resistance is important. Practical commercial radiation application in wire/cable has been limited to small-size wire because of limitation in EB penetration. The principle unique advantage of irradiation is that it provides a means of crosslinked structure to thermoplastics without peroxide and heat, which improves mechanical properties such as abrasion resistance and resistance to flow at elevated temperatures. The use of the electron beam processing system, since its application to crosslinking polymers for wire insulation at LG Cable Co. in 1985, has undergone steady growth. This suggests a growing demand for electron beam-irradiated wire in such industries as electronic equipment and automobile and related industries.

The production of crosslinked wire in LG Cable Co. was the beginning of commercial radiation application of electron beam accelerator in Korea. Thus far, the company has five electron beam accelerators, which have been operated to produce crosslinked wire and shrinkable tube. Other cable companies such as Kyungshin Industrial Co. Daewon Cable Co. and Yeonhab Electric Cable Co. installed a total of 3 EB machines to produce crosslinked wire and shrinkable tube.

Crosslinked polyolefin foams are manufactured by EB process at Youngbo Chemical Co. and Tongil Industries Co. The reason for irradiation for the polyolefin is to increase the viscosity of the molten polymer material by crosslinking. The higher viscosity makes it more difficult for gaseous products from the foaming agent to escape from the melt, and these results in a large volume expansion and uniform foam size.
It is known that the use of radiation to precure tire components can simplify manufacturing and save materials costs. In tire manufacture, the advantages of radiation processing are mainly interdependent with each other elements in overall production process. Radiation does not impart a significant improvement in physical properties of the final tire. Consequently, it is known that its adoption is based on pure economics, which can vary for each manufacturer. Hankook Tire Co. applied EB accelerators (500 keV and 65 mA, 1000 keV and 65 mA) for the precuring of tire rubber sheet.

**UV/EB curing of coating**

The main difference between UV and electron beam is that the UV energy is too low to break chemical bonds in a short time. Therefore, photoinitiators are added to the materials that are to be irradiated. The main advantage of UV, compared EB is that the cost of equipment is much lower. The major disadvantages are the limited penetration of thick or heavily pigmented materials, a higher material cost because photoinitiators are required, and toxicity of monomer and solvent. Curing process in Korea depends on largely on thermal and UV process. Coating technology in Korea has been greatly used for printing, treating the surface of wood, plastics, furniture, metal and paper to give gloss, water proofing and prevention of corrosion.

EB curing was introduced in 1989 as an industrial process by Hankook Tetrapack Ltd., which installed an accelerator of 175 keV, 300 mA. The private company operated to cure coating ink on paper used for tetra-pack.

**Radiation Sterilization**

A Co-60 sterilization facility installed at Seoul site of KAERI under the UNDP financial support had been operated not only for training /demonstration but also for commercial sterilization of medical products until 1987, when a private company, Green Pia Tech installed a new commercial irradiator of 3x10^7 GBq with maximum capacity of 18,5x10^7 GBq Co-60. As Green Pia Tech has enough capacity to sterilize all the amounts of irradiation materials in Korea, the Co-60 irradiator (0.75 x10^7 GBq) which was reinstalled in 1992 at Daejon site from Seoul site, has been used for conducting research/development and qualification of equipment in nuclear power plants.

Green Pia Tech sterilizes medical products such as surgical gloves, needles, bandages, blood bags, vials, mud and Chinese hubs. Another private irradiation facility (Soya Co.) was built to meet the growing demand of medical products and foods.

2. RECENT RESEARCH AND INDUSTRIAL APPLICATIONS

**Conductive polymer with PTC**

A radiation process for conductive polymer composition exhibiting positive temperature coefficient (PTC) behavior was developed. PTC polymer conductive compositions are suitable for use in electrical devices such as circuit over current protection devices.

The main feature of the PTC material is that its electrical resistivity increases with increase of temperature when the filler concentration is sufficient. The electrical resistivity effect is a result of the thermal expansion of the polymer matrix or breaking of conducting pathways in the filler. Several ways to decrease the room temperature volume resistivity in carbon black/polymer blends and to increase PTC behavior were tried based on the use of additives, optimization of processing conditions, and selection of carbon blacks having an appropriate size distribution, and porosity of carbon black and thermal treatment. These days, a Korean company is producing the conductive polymer suitable for use in electrical devices such as circuit over-current protection devices (Figure 1).
Wound dressing

To provide an optimum healing environment, a wound dressing should control bleeding, protect the wound from the external environment, and prevent further contamination or infection. Hydrogels from a mixture of chitosan and polyvinyl alcohol (PVA)/Poly-N-vinylpyrrolidone (PVP) were developed by two steps of "freezing and thawing" and gamma-ray irradiation for wound dressing (Figure 2). Clinical testing for burn wound using the synthesized hydrogels was conducted at the Burn center of the Kuro Sungsim Hospital. The hydrogel was transparent, which allows the doctors to observe the healing process without disturbing the patient, thus decreasing the pain considerably. Recently, we are taking the steps necessary to get a government approval for making a commercial product.

Wear-resistant ultra high molecular weight polyethylene for hip joint

Ultra high molecular weight polyethylene (UHMWPE) has mechanical and chemical properties highly suitable for orthopaedic implants. Radiation crosslinking process was used to increase wear resistance of acetabular cup in artificial hip joint (Figure 3). The effect of e-beam irradiation on the structure and mechanical properties of UHMWPE specimen was irradiated at 140 and room temperature with different dosages of electron beam. Following irradiation at room temperature, the UHMWPE were thermally treated at 110, 130, 145 to eliminate all the remaining free radicals. The crystalline structure and mechanical properties were compared after crosslinking of electron beam. The crystalline structure and degree of crystallinity of irradiated UHMWPE were investigated using DSC, SAXS and WAXD. Mechanical properties such as tensile strength were measured. The polymer pin on a metal plate type-testing apparatus was used to test the wear property.
The irradiation of electron-beam in molten state resulted in the best wear resistant property of UHMWPE. We expect that UHMWPE having the enhanced crosslinking after irradiation will be used to extend the lifetime of artificial joints.

Drug delivery systems

- pH-sensitive hydrogels were studied as a drug carrier for the protection of insulin from the acidic environment of the stomach before releasing it in the small intestine. In this study, hydrogels based on poly (ethylene oxide) (PEO) networks grafted with methacrylic acid (MAA) or acrylic acid (AAc) was prepared via a two-step process. PEO hydrogels were prepared by γ-ray irradiation (radiation dose: 50 kGy, dose rate: 7.66 kGy/h), and then grafting by either MAA or AAc monomers onto the PEO hydrogels with subsequent irradiation (radiation dose: 5-20 kGy, dose rate: 2.15 kGy/h). These grafted hydrogels showed a pH-sensitive swelling behavior. The grafted hydrogels were used as a carrier for the drug delivery systems for the controlled release of insulin. The in vitro drug release behaviors of these hydrogels were examined by quantification analysis with a UV/vis spectrophotometer. Insulin was loaded into freeze-dried hydrogels (7×3×2.5 mm) and administrated orally to healthy and diabetic Wistar rats. The oral administration of insulin-loaded hydrogels to Wistar rats decreased the blood glucose levels obviously for at least 4 h due to the absorption of insulin in the gastrointestinal tract.

- Biocompatible and biodegradable pH-responsive hydrogels based on poly acrylic acid (AAc) and chitosan were prepared for controlled drug delivery. These interpolymeric hydrogels were synthesized by a gamma irradiation polymerization technique. The degree of gelation was over 96% and increased as the chitosan or acrylic acid content increased. The equilibrium swelling studies of hydrogels prepared in various conditions were conducted in an aqueous solution, and the pH sensitivity in the range of pH 1-12 was investigated. The AAc/chitosan hydrogels showed the highest water content when the 30 vol.% AAc and 0.1 wt.% chitosan were irradiated with a 30 kGy radiation dose. Also, an increase of swelling degree, with an increase in the pH was noticed and showed the highest value at pH 12. The drug, 5-fluorouracil (5-FU), was loaded into these hydrogels and the release studies were carried out in simulated gastric and intestinal fluids (SGF and SIF).

The in-vitro release profiles of the drugs showed that more than 90% of the loaded drugs were released in the first 1h at the intestinal pH and the rest of the drug had been released slowly.

Radiation curing of epoxy

Epoxy resins are widely used as high performance thermosetting resins for many industrial applications. The effect of an electron beam (E-beam) and gamma-ray irradiation on the curing of epoxy resins was investigated. Diglycidyl ether of bisphenol-A (DGEBA), diglycidyl ether of bisphenol-F (DGEBF) as epoxy resins and triarylsulfonium hexafluoroantimonate (TASHFA),
triaryl sulphonium hexafluorophosphate (TASHFP) as initiators were used. The chemical and mechanical characteristics of irradiated epoxy resins were compared after curing of E-beam and γ-ray irradiation up to 50 kGy in N\textsubscript{2} and air atmosphere. We ascertained the effect of oxygen on the radiation curing of epoxy resin. The thermal properties of cured epoxy were investigated using DMA and TGA. Mechanical properties such as flexural strength were measured. The chemical structures of cured epoxy were characterized by FT-NIR. The gel fraction and the strength at yield of epoxy resins irradiated by E-beam and γ-ray in N\textsubscript{2} atmosphere were also compared with those of epoxy resins irradiated by E-beam and γ-ray in air.

**Preparation of nano-particles**

Nickel nanoparticles have been prepared by the reduction of nickel chloride induced by γ-ray irradiation with the dose of 5~30 kGy and dose rate of 0.65~3.9 kGy/hr at room temperature under atmospheric pressure. It was revealed that the prepared particles were the cubic phase of nickel by X-ray diffraction (XRD) analysis. Transmission electron microscope (TEM), scanning electron microscope (SEM), and particle size analyzer (PSA) characterizations indicated that these nickel nanoparticles had diameters of 10~15 nm with a narrow size distribution. Also, it was shown that parameters such as the concentration of nickel ion and isopropyl alcohol, irradiation dose, and types of surfactants could be used to control the size of the nickel nanoparticles.

3. NEW NATIONAL CORE RESEARCH CENTER FOR R&D OF RADIATION TECHNOLOGY

The Korean government has decided to establish a New National Core Research Center for R&D of Radiation Technology in Jeongup, Chollabukdo Korea until the year of 2005 in order to meet the rapidly growing market in the areas of radiation technology. The laboratory will develop the technologies associated with the application of radiation in the areas of, material engineering, plant engineering, nondestructive tests, radioisotope tracer application, environment engineering, medical science, food, biotechnology, agriculture, sterilization, evaluation of aging of nuclear power plant and aerospace.

Most of the administrative and legal procedures, procurement of the land and civil engineering works have been accomplished well. The laboratory is to be constructed in the next year. The major facilities to be built are two Co-60 irradiators of $3.7\times10^7$ GBq, one electron accelerator of 10 MeV, one ion beam accelerator of 300 KeV, one cyclotron of 30 MeV and one gamma irradiation farm. The relevant industries shall be accommodated around the center. We believe that the Center will become an efficient tool to make a significant role for the cooperation between Korea and RCA member countries in the field of radiation technology.

Main cooperation can involve the followings: 1) the mutual exchange of technical information, 2) collaborative researches, 3) mutual exchange of related scientists 4) use of the Center's radiation and experimental facilities, etc.
REPORTS OF PARTICIPANTS
RADIATION PROCESSING OF NATURAL POLYMERS: ACHIEVEMENTS & TRENDS

S. SABHARWAL, L. VARSHNEY, A.D. CHAUDHARI*, S.P. RAMNANI*
Bhabha Atomic Research Centre, Trombay, Mumbai India
*Department of Botany, University of Nagpur, Nagpur, Maharashtra, India

Abstract

Radiation processing of natural polymers has been an area of active research during the last few years for developing new applications for value addition to these materials. The areas being explored include health care, agriculture and environment. To fulfill the demands of specific applications, the natural polymers need to possess different characteristics, for example, while for agricultural applications radiation processing should lead to formation of lower molecular products, the utilization for environmental applications demands formation of crosslinked network structures. This requires understanding the radiation effect on these materials as well as optimizing the experimental conditions for a particular application. In India, various aspects of radiation processing are being investigated. These include (i) radiation crosslinking of chitosan in presence of a sensitizer carbon tetrachloride, (ii) development of a radiation processed PVA based hydrogel containing only natural polymers such as agar and carrageenan as additives for use as burn or wound dressing and (iii) to test the efficacy of using radiation processed oligo-saccharides as plant growth promoter for two crops, namely, wheat (Triticum aestivum) and chickpea (Cicer arietinum). The results of these studies along with some of the emerging applications being pursued in the RCA region are presented in this paper.

1. INTRODUCTION

In recent years, natural polymers are being explored for many new applications because of their unique characteristics such as easy availability, biocompatibility and biodegradability. A considerable amount of research on natural polymers has emanated from Asian countries and the studies have covered the whole spectrum of research, from better production and purification methods, the derivatization chemistry and applications [1, 2].

The areas of applications of natural polymers being explored include health care applications [3, 4], in agricultural applications wherein it has been observed by a number of researchers that some low molecular natural polymers, particularly polysaccharides such as chitin/chitosan or alginates show very interesting properties [5-8] and in environmental conservation due to their molecular structure, many natural polymers has an extremely high affinity for many toxic metal ions and dyes [9-12].

Radiation processing has established itself as a commercially successful technology for modification of a variety of synthetic polymeric materials for a variety of applications such as crosslinking of wire & cable, production of heat shrinkable materials, modification of rubber tires and production of foamed materials [13, 14].

Radiation processing of natural polymers, however, has received much less attention over the years because of two reasons namely:

(i). most of the natural polymers undergo chain scission reaction when exposed to high energy radiation and
(ii). difficulty in processing natural polymers in various forms and sizes.

High energy radiation technique can be effectively used for reducing molecular weight of different polymers including natural polysaccharide such as alginate and Chitosan [15, 16].
As mentioned earlier, conventionally synthesized low molecular weight oligosaccharides are being explored as plant growth promoters, however, until recently, the effect of the radiation degraded alginate and chitosan products on the growth of plants was not studied much in detail.

In past few years, radiation depolymerized alginates; chitosan and carrageenans have been tested on a number of crops such as Rice (Oryza sativa L. var. japonica), peanut seeds (Arachis hypogea), [17, 18]. Hien et al. have reported the effect of alginates on the growth of rice at various concentrations. The results showed that a suitable concentration range of alginates is 20-50 ppm [19] suggesting that the higher activity of products obtained from radiation degraded alginate. Most of these studies have been carried out in hydroponic systems and there is a need to extend these studies to other crops to optimize the experimental conditions for obtaining maximum yields.

Polymeric biomaterials are widely used in medical field in a variety of ways, namely, as therapeutic devices, drug delivery systems and clinical diagnostic devices. In recent years, hydrogels have emerged as an important class of biomaterials as they possess excellent biocompatibility. These hydrogels are three dimensional crosslinked network structures that are produced by simultaneous polymerization and crosslinking of suitable monomers or by crosslinking of linear polymers.

The conventional crosslinking methods involve use of toxic additives to bring about polymerization/crosslinking methods and thus are not suitable for production of biocompatible hydrogels. Ionizing radiation possesses the unique ability to initiate polymerization and/or crosslinking reactions without the need to add toxic chemicals. Therefore radiation processing is emerging as an excellent tool to produce hydrogels for a variety of medical applications. Healing of wide and serious burn wounds is one such difficult medical problem as the healing takes a lot of time and the dressing used for protection have to be changed regularly till the healing is complete. A PVP based radiation processed hydrogel wound dressing containing synthetic polymers as plasticizer has been developed by Rosiak (1994) and has been successfully commercialized.

Natural polymers like alginates, carrageenans or derivatives of natural polymers like chitosan are known to possess wound healing characteristics, besides having good biocompatibility. Incorporation of such natural polymers in synthetic polymers can produce biomaterials that combine the features of synthetic polymers with specific biocompatible and wound healing characteristics of natural polymers.

The applications of natural polymers in health care sector or in environmental conservation necessitate use of these polymers in the crosslinked form as hydrogels, however as mentioned earlier, these polymers typically undergo chain scission reaction when exposed to high energy radiation [20]. Studies have been carried out in recent years on polysaccharides and their derivatives to attempt radiation induce crosslinking in cellulose, starch and chitin/chitosan water-soluble derivatives under various experimental conditions. It was found that polysaccharides water-soluble derivatives such as carboxymethylcellulose (CMC), carboxymethylstarch (CMS) and carboxymethylethelitin (CMCT), carboxymethylchitosan (CMCTS) lead to radiation crosslinking when irradiated as highly concentrated aqueous solution in paste-like state [20]. However, natural polymers themselves undergo predominantly degradation reaction. Thus, more detailed studies or different approaches are required to attempt radiation induced crosslinking of these materials.

2. OBJECTIVE OF THE PRESENT RESEARCH

The objectives of the present research presented in this work were the following:

- Establish conditions for radiation induced crosslinking of chitosan in aqueous solutions in presence of a suitable sensitizer
- Development of a PVA hydrogel containing only natural polysaccharides as additives for use as burn or wound dressing
- Explore the use of radiation processed polysaccharides as plant growth promoter in crops of wheat (Triticum aestivum) and chickpea (Cicer arietinum)

3. RADIATION INDUCED CROSSLINKING OF CHITOSAN IN AQUEOUS SOLUTIONS

Chitin is the second most abundant natural polymer polysaccharide found in the outer skeleton of insects, crabs, shrimps and other marine animals [21]. The limited solubility of chitin in the common solvents limits its commercial applications. However chitin can be easily converted into chitosan, by alkali hydrolysis using 50% sodium hydroxide solution under boiling condition [22].

Chitosan has many applications due to the presence of reactive –NH\(_2\) group at position 2 and two hydroxyl group at position 3 and 6 respectively of 2- deoxy-D-Glucose residue. Due to the presence of amino group at position 2 and hydroxyl group at position 3, chitosan forms chelates with a large numbers of meta ions [23]. Presence of amino group in chitosan renders it soluble in weak acids such as dilute acetic acid, lactic acid, citric acid etc. Solubility of chitosan in acid media has several advantages in it’s processing for various commercial applications. However for applications such as chromatographic separation, effluent treatment it is necessary to crosslink chitosan to make it insoluble in the entire pH ranges. Chitosan may be crosslinked chemically by aqueous solution of gluteraldehyde, chlorohydrin etc. [24]. The resultant crosslinked chitosan is insoluble in acid media unlike normal chitosan, which is soluble in acidic medium. Chemical modification of chitin/chitosan has been studied widely, but up to recent years, only a little work is reported on the radiation-induced modification. Dutta et al. have reviewed the chemical modification of chitosan and its applications [25].

Chitosan and other natural polymers such as cellulose, carrageenan and alginates predominantly undergo chain scission when exposed to ionizing radiation [26-29]. Although methods for crosslinking of chitosan by chemical means are reported in the literature, no method is reported where chitosan is cross-linked using ionizing radiation. We have developed a new method for Co\(^{60}\) gamma ray induced crosslinking of chitosan in the presence of carbon tetrachloride.

**Effect of gamma irradiation on chitosan**

Figure 1 shows the change in the molecular weight of chitosan irradiated in air, as determined by intrinsic viscosity measurement as a function of radiation dose. The changes in molecular weight were evaluated from intrinsic viscosity measurement using the following equation.

\[
\eta = K \times M_v^\alpha \quad (1)
\]

where \(M_v\) is the viscosity average molecular weight and K and \(\alpha\) are constants having values 1.81x10\(^{-3}\) and 0.93 respectively [28].

As can be seen from the Fig. 1, the chitosan molecule undergoes depolymerization resulting in the reduction of its molecular weight. However, with increasing radiation dose the decrease in the molecular is very fast initially followed by leveling off of molecular weight beyond 50 kGy. These results are in agreement with results reported earlier by many workers [29-31].
FIG. 1. Changes in the molecular weight of chitosan on exposure to gamma Radiation

Synthesis and Characterization of Crosslinked chitosan

Since carbon tetrachloride is insoluble in water, a mixture of water and methanol was used to dissolve CCl₄. A solution containing 30:65:5 parts by weight of water, methanol and carbon tetrachloride was prepared and to which chitosan was added till it is completely soaked by the solution. A 5 gm of chitosan requires 18 gm of solvent for complete soaking. This mixture was kept overnight to allow the solvent to get diffuse into microstructure of chitosan. The mixture was then irradiated in Co⁶₀ gamma chamber for a required period of time. The irradiated mixture was washed first with methanol to remove any traces of carbon tetrachloride and other radiolytic products formed during the irradiation followed by washing with water. Washing was repeated twice and the resultant product, which is yellow in color, was again washed with acetone followed by vacuum drying at 50⁰ C.

The product formed (referred to as CR-Chitosan) after the irradiation showed the following characteristics:

- CR-Chitosan is insoluble in 1% aqueous acetic acid solution in which chitosan otherwise dissolves.
- CR-Chitosan swells in 1% acetic acid solution
- CR-Chitosan is yellowier than the product formed when chitosan is irradiated in water-methanol mixture in the absence of CCl₄.

X ray diffraction (XRD) pattern of chitosan exhibits a characteristics peak at ~ 2θ = 20 [32]. The XRD pattern of chitosan, irradiated chitosan and CR-chitosan showed that chitosan and irradiated chitosan yield similar XRD pattern with characteristic peak at 2θ = 20.6. However, CR-chitosan showed a very broad pattern with substantial decrease in the intensity. Thus it appearrs that when chitosan is irradiated with gamma rays there are no changes in the crystalline structure upon irradiation. However, when chitosan is irradiated in a solvent containing 60:35:5 parts by weight of methanol, water and carbon tetrachloride respectively, there is a change in the crystalline structure as depicted by the XRD pattern of CR-chitosan which is very broad with low intensity. This is further supported by Scanning Electron Microscopy (SEM) results on chitosan samples. The SEM pictures of chitosan, irradiated chitosan and CR-chitosan are shown in Figure 2. The results show that irradiated chitosan samples (A) showed small structures with large voids. This is because in chitosan polymer chains are tightly bound to each other by strong hydrogen bonding between the chains [33].
As discussed earlier, when chitosan is irradiated with gamma rays it undergoes degradation resulting in the breaking up of polymer chains therefore irradiated chitosan shows smaller structures as depicted in SEM picture. However, SEM picture of CR-chitosan (Figure 2B) showed very big structures interlinked with each other confirming that when chitosan is irradiated in a solvent (65:30:5 methanol, water and carbon tetrachloride respectively), the polymer chains gets crosslinked with each other resulting in the changes at the microstructure level.

![SEM pictures of (A) Chitosan irradiated with gamma radiation, dose = 75 kGy and (B) CR-chitosan (radiation dose = 75 kGy).](image)

Most of the applications of chitosan are due to the presence of -NH$_2$ group in its structure, which makes it cationic polymer. Also due to the presence of –NH$_2$ group and OH group at position 2 and 3 respectively, it shows chelating properties. Therefore, for applications of crosslinked chitosan in chromatographic separation and effluent treatment it is essential that –NH$_2$ group should be retained after modification. To establish this, ionic capacity of chitosan, irradiated chitosan, CR-chitosan and CRH-chitosan was determined. A known weight of chitosan or crosslinked chitosan was added to known volume of 0.1 mol dm$^{-3}$ H$_2$SO$_4$ solution and the mixture was kept aside for 2 hrs. The mixture was filtered and an aliquot was titrated against standard solution of sodium hydroxide. Similarly blank titration without the addition of chitosan was also run. From the difference in the volume of NaOH required for neutralization, ionic capacity of chitosan samples were calculated using following equation:

$$\text{Ionic capacity} = (V_2 - V_1) \times \frac{a}{w} \text{ (meq g$^{-1}$)}$$  \hspace{1cm} (2)

Where $V_2$ and $V_1$ are the volumes of NaOH require for complete neutralization of H$_2$SO$_4$ in the absence and presence of chitosan respectively, $a$ is the normality of NaOH and $w$ is the weight of sample taken for analysis. The results of preliminary studies on ionic capacities of different chitosan samples are only marginally lower than chitosan and irradiated chitosan, indicating that irradiation of chitosan in the presence of CCl$_4$ does not affect the –NH$_2$ group.

Therefore, chitosan, which normally undergoes depolymerization when exposed to ionizing radiation, can be crosslinked when irradiated in the presence of sensitizer such as CCl$_4$. The physical characterization of crosslinked Chitosan by XRD and SEM confirmed that Chitosan when irradiated in the presence of CCl$_4$ undergoes crosslinking and substantial change in its microstructure. The resultant crosslinked product has –NH$_2$ group intact as evident from the results from ionic capacity determination which makes the crosslinked product useful for environmental applications which are presently under investigation.

33
4. DEVELOPMENT OF RADIATION PROCESSED PVA-POLYSACCHARIDES HYDROGEL BURN OR WOUND DRESSINGS

Radiation processing is emerging as an excellent tool to produce hydrogels for a variety of medical applications. Healing of wide and serious burn wounds is one such difficult medical problem as the healing takes a lot of time and the dressing used for protection have to be changed regularly till the healing is complete. A PVP based radiation processed hydrogel wound dressing containing synthetic plasticizers has been successfully commercialized in Poland [34].

In India also, about three years ago, BARC initiated the program of utilizing radiation processing for developing suitable PVA based hydrogel containing only natural polymers as additives for use as wound and burn dressings. Pure PVA aqueous solution, when exposed to gamma radiation at normal sterilization dose of 25 kGy, forms brittle hydrogel, which are impractical for use as dressing. Moreover, the unreacted PVA has to be washed out and to be sterilized before use. Natural polysaccharides have been observed to bring desirable characteristics to the hydrogel. Presence of polysaccharides like agar and carrageenan in formulations significantly improve mechanical properties and water absorption properties of the hydrogel (Majumdar and Varshney, 1999) [35]. These changes have been attributed to variation in the degree of crosslinking and network structure during irradiation brought about by the use of the polysaccharides. PVA mainly cross links and polysaccharides form interpenetrating network structure with PVA. The extent of gelling power of the mixture of polysaccharides and their high affinity for water in the formulation brings desirable characteristics to the dressing and eliminates use of synthetic plasticizers and humectants. Besides burn, these dressings have been observed to heal difficult wounds like leprosy & diabetic ulcers, bed sores, post surgical wounds, etc. These efforts have resulted in development of PVA based hydrogel dressing that possesses excellent characteristics for the desired use. The radiation processed PVA hydrogels containing natural polymers have been successfully commercialized in India.

5. USE OF RADIATION PROCESSED POLYSACCHARIDES AS PLANT GROWTH PROMOTER

As mentioned earlier, radiation processed polysaccharides are now being investigated as plant growth promoters by a number of researchers. These studies have mainly been carried out mainly in hydroponic systems for some specific type of crops only; hence, more studies are desired to establish the optimum conditions for use of such materials for a large number of crops. With this in view, the growth promotion activity of radiation depolymerized Chitosan and alginate has been investigated in wheat (Triticum castivum) and chickpea (Cicer arietinum).

The growth promotion effect on the seedling growth of two crops was studied as seedling growth phase is the best to analyze such effects. The aqueous irradiated solutions of oligosaccharides were applied in the following 2 modes.

(i). in the first mode, the dry and 4 hours pre-soaked seeds were treated with different concentrations (20 -100 ppm) of oligosaccharides for 18 hours. The unirradiated polysaccharides were used as a control. The water control was also maintained and handled in the same way. The treated and control seeds were then sown in a tray containing soil. The trays were kept at room temperature under 16/8 illumination and irrigated with water every alternate day.

(ii). in the second mode, the wheat and chickpea seeds were soaked in water for 4 hours and sown in paper cups containing soil. The cups were irrigated with 20 ml solution of different concentrations of oligosaccharides or water every alternate day till 8th or 10th day. After the emergence of the first leaf, (8th day in chickpea and 10th day in wheat) the seedling height was measured from transition zone to the tip of the shoot.

The irradiations were carried out in aqueous solutions using a Gamma Chamber at a dose rate of 5 kGy hr⁻¹.
All experiments were repeated 5 times and every time experimental sets were run in triplicate. The data of all the experiments was pooled averaged and analyzed statistically to determine the significance level. The results of the study showed the following:

- Unirradiated and irradiated alginate as well as Chitosan did not show any effect on the germination. The percentage of germination and the emergence of radical were not affected at all.

- The subsequent growth of the seedling was found to be influenced by the presence of oligosaccharides. In both the crop species, the growth promotion activity was statistically significant at 1 to 5% level with irradiated polysaccharides whereas unirradiated polysaccharides did not induce growth promotion. The oligosaccharides also promoted the root growth and proliferation in both crop species.

- All samples of irradiated alginate and Chitosan promoted the seedling growth in wheat and chickpea. The biological activity in terms of seedling growth promotion remarkably increased with samples irradiated in aqueous solution to a dose of 5 kGy, the maximum height was obtained using alginate samples irradiated to 10 kGy or using Chitosan samples irradiated to 15 kGy. Further increase in dose did not affect the biological activity of oligosaccharides.

- Various concentrations ranging from 20 ppm to 100 ppm were used to study the effective concentration. In both the crop species, all concentrations were nearly equally effective in stimulating the seedling growth. The concentration of oligosaccharides in excess of 100 ppm upto 500 ppm did not show any additional beneficial or adverse effect.

- Both seed treatment and irrigation with oligosaccharides were effective in chickpea whereas only seed treatment was effective in wheat. It is interesting to note that in physiologically active (pre-soaked seeds) and inactive (dry seeds), the irradiated oligosaccharides produced phytophermone like activity indicating that readily taken up by the imbibing seeds and the oligosaccharides might be producing hormones like effect or metabolic burst resulting in increased growth.

- While oligo alginate was very effective mainly in chickpea, oligo-chitosan was effective in chickpea as well as in wheat crop.

These results clearly show that irradiated alginate and chitosan are effective in promoting plant growth. The seed treatments as well as soil application are both effective ways for stimulating plant growth and radiation processed chitosan exhibited relatively better plant growth activity.

6. SOME EMERGING APPLICATIONS OF RADIATION PROCESSED NATURAL POLYMERS IN THE RCA COUNTRIES

As mentioned earlier, the use of natural polymers is now being investigated for many new applications based on their special characteristics. Carrageenan films are known to possess haemostatic properties. Surgical situations may arise wherein bleeding occurs over an area where there is no identifiable blood vessel amenable to the clamp and tie method, the electrocautery method, or attempting to do so would do more harm. In such cases, the use of haemostatic materials may be indicated. In Philippines, radiation processed KC/PVP hydrogel has been tested for haemostatic effectiveness and biodegradability and tissue reactivity in surgically created muscular flaps in the thigh muscles of New Zealand rabbits (Orytolagus cuniculus) [36]. This was compared to negative controls and to commercially available Gelfoam™. Hemeostasis was achieved by application of one of the topical agents plus moderate digital pressure.
The mean Bleeding time was significantly lower in the KC/PVP hydrogel group (16.9 ± 6.9 seconds) compared to 38.0 ± 27.6 seconds for the Control group but was not significantly different from the Gelfoam group (26.0 ± 24.6 seconds) at p=.05. Microscopic studies showed comparable healing and biodegradability with varying degrees of residual material and inflammation. Carrageenan hydrogel was found to be effective as a topical haemostatic agent comparable, if not superior to the more expensive, commercially available agents. Likewise, its biodegradability and tissue reactivity are similar to the presently available and approved haemostatic agents. This can be used in several clinical applications such as in thyroid and mastoid surgeries and sinus pack sponges used in sinus surgeries.

Many natural polymers possess biocompatible characteristics along with excellent film forming ability that makes them useful for cosmetic applications. Natural polymers like Chitosan are essential ingredients of many a cosmetic preparations as it provides a moisturizing effect on the skin. In Malaysia, sago starch is obtained from sago palm that is grown abundantly in the region. Researchers working at Malaysia Institute of Nuclear Technology (MINT) have synthesized a radiation processed PVA/PVP based hydrogel containing sago starch as an additive to develop a beauty mask for cosmetic applications [37]. The mask has been tested for its commercial utility and Technology transfer agreement has been signed with a private company for producing it on a commercial basis.

7. CONCLUSION

In conclusion, radiation processing of natural polymers is an exciting and emerging area wherein the unique characteristics of these polymeric materials are being explored for knowledge-based applications in areas related to health care, agriculture and environmental remediation. Already success has been achieved in some of these areas, such as in natural polymer based hydrogels, as plant growth promoters and for cosmetic applications. However, lot more research work needs to be done to utilize their full potential.

REFERENCES


JEUNIAUX, C., VOSS-FOUCART, M.F., POULICEK, M., BUSSERS, J.C. “Sources of chitin, estimated from new data on chitin biomass and production”. In G. Sjåk-Break T. Anthonson and P. Sandford, Chitin and chitosan London /New York: Elsevier Applied Science. 1989 (pp. 3-11)


Charlesby, A. Atomic Radiation and polymers, Pergamon Press, 1960

YOSHII,F., “Radiation induced degradation in alginate”, Presented at Second Regional Coordinated Research Meeting on Natural Polymers, Mumbai, India (1999)


CHIRACHANCHAI, S., RCA/IAEA Regional Workshop on “Radiation Processing of Natural Polymers for Agro and agro-Chemical Industry” 25-28 September, 2001, held at Takasaki, Japan


RADIATION DEGRADATION AND CROSSLINKING OF POLYSACCHARIDES AND ITS APPLICATION

F. YOSHII
Takasaki Radiation Chemistry Research Establishment, JAERI, Japan

Abstract

Polysaccharides such as alginate and chitin/chitosan undergo degradation by irradiation. Degradation behavior was different under irradiation conditions. It was found that required dose to get small molecular weight sample was lower for aqueous solution state than solid phase. Degraded alginate and chitosan were effective as a plant growth promoter and the favorable molecular weight was less than 10,000. Carboxymethylcellulose (CMC), Carboxymethylchitin (CM-chitin) and carboxymethylchitosan (CM-chitosan) form gels when irradiated at paste-like condition. Bedsore prevention mat filled up CMC hydrogel crosslinked by irradiation at paste-like condition was practical applied as a health care products. It was found that CM-chitosan hydrogels have anti-microbial activity and effective as absorbents to remove metal ions. Cellulose derivative having phthalate function (hydroxypropyl methylcellulose phthalate, HPMCP) crosslinked with EB-irradiation at paste-like condition absorbed organic solvents such as chloroform and pyridine

1. INTRODUCTION

Natural polymer such as polysaccharides, cellulose, starch, chitin/chitosan, alginate and carrageenan are widely used in food, medical and cosmetic fields owing to their unique structure, distinctive properties, safety and biodegradability. It is well known that polysaccharides can be degraded due to scission of glycoside bonds by ionizing irradiation. We found that low molecular weight component obtained by radiation degradation was useful for acceleration of plant growth. Here, the degradation behavior of alginate and its application for agriculture is presented.

The hydrogels based on polysaccharides and their derivatives have been studied widespread but no radiation crosslinking of polysaccharides and their derivatives have been reported so far. Polysaccharides and their derivatives are typical degradation polymers in radiation processing [1]. But in several studies conducted, it was found that polysaccharide derivatives such as carboxymethylcellulose (CMC), methylcellulose (MC), carboxymethylstarch (CMS) and carboxymethylchitosan (CM-chitosan) undergo crosslinking when irradiated at paste-like condition [2-5].

In this report, application of radiation degraded alginate for agriculture and crosslinking behavior of polysaccharide derivatives at paste-like condition were reported.

2. DEGRADATION BEHAVIOR OF ALGINATE AND CHITIN/CHITOSAN

Alginate was irradiated at solid phase and aqueous solution. Indirect effect of water on degradation of alginate was investigated. Figure 1 shows number-average molecular weight (Mn) measured by GPC against irradiation dose [6]. It can be seen that Mn of alginate in aqueous solution decrease remarkably in irradiation. The Mn for alginate aqueous solution degraded at 20 kGy for 1% (w/v) or at 50 kGy for 4% (w/v) was equivalent to that of alginate irradiated at 500 kGy. Hence, required irradiation dose to get the same molecular weight of alginate in 1% (w/v) aqueous solution was equivalent to ca. one 20th of the dose needed for soil phase irradiation. This may be mainly due to the suppression of recombination of main chain because of high mobility of alginate chain in aqueous solution. The H and OH radicals formed by radiolysis during irradiation of water accelerate the molecular chain scission of alginate. Reaction between the above free radical and alginate molecules lead to rapid degradation of alginate in aqueous solution.
Chitosan dissolved in 1% acetic acid and the aqueous solution is γ-irradiated. Degradation is larger in aqueous solution than solid phase as well as alginate. Lower molecular weight chitosan is obtained in aqueous solution at smaller dose.

**FIG. 1.** Change in molecular weight of alginate by radiation

**FIG. 2.** Effect of irradiated alginate on rice growth promotion. Rice was cultivated for 9 days in Hyponex solution (1/1000-fold). I- increase of dry matter (%).
3. GROWTH PROMOTION EFFECT OF RADIATION DEGRADED ALGINATE ON PLANT

To confirm the effect of growth promotion of plant, irradiated alginate was supplied to rice through hydroponic solution. Degraded alginate obtained from higher dose (up to 200kGy irradiated in liquid state) shows a stronger effect on the increase of dry matter of rice seeding (Figure 2).

It is expected that a certain molecular weight of degraded alginate maybe suitable for plant growth. For rice, degraded alginate from 4% alginate solution irradiated at 100 kGy (Mn ca 7,000) impacts remarkably on growth promotion. A similar effect is also observed for alginate powder irradiated at 500 kGy. It can be seen that addition of irradiated alginate is the most effective for formation of bigger root. From these findings, formation of stronger plant for various environments is expected. The growth of rice at various concentrations of irradiated alginate is investigated. The results indicate a suitable range of degraded alginate concentration, 20 to 50ppm. Furthermore, degraded alginate by irradiation is preferable for growth promotion of carrot, cabbage and peanut.

4. CROSSLINKING OF CMC AT PASTE-LIKE CONDITION AND ITS APPLICATION ON BEDSORE PREVENTION

CMC aqueous solution was irradiated at a high concentration (10-50%) to lead crosslinkings [2]. A high concentration in aqueous is favorable for crosslinking. The presence of water enhances the yields of macroradicals. So from the practical point of view, the polymers should be mixed with water well to prepare homogeneous samples. A high degree of substitution (DS) also was found favorable crosslinking. The CMC with a DS of 2.2 gives the highest gel fraction in high concentration among these samples. It can be explained that intermolecular linkages are a result of ether function.

Carboxymethylcellulose (CMC) soft hydrogel is applied as a healthcare product in hospitals and is used as bed mats for operation procedures. Before operation is conducted, the mat is pre-heated to body temperature (37 °C) using an oven heater. Temperature could be maintained for a long time during operation. The hydrogel mat is shown to disperse body pressure and improve circulation of blood during operation, thus, it could prevent bedsores in patients. This hydrogel converts into fertilizer by degradation of microorganism in soil. In addition, biodegradability is a big advantage of this hydrogel.

5. CROSSLINKING OF CM-CHITIN/CM-CHITOSAN AND THEIR APPLICATION FOR METAL ION ADSORPTION

Crosslinking of CM-chitin and CM-chitosan was also observed at paste-like state (above 10 %) of high concentration. The crosslinking behavior is similar like CMC. High concentrated paste-like condition was favorable for crosslinking [4, 5]. In the case of CM-chitosan, high degree of deacetylation was found to negatively correlates to crosslinking even if it has a high DS. The hydrogels created from carboxymethylated chitin derivatives, exhibited excellent mechanical properties and good swelling in water. Irradiation of 30% CM-chitin and CM-chitosan sheets gave maximum gel strength of 0.45 MPa at 70 kGy and 0.75 MPa at 50 kGy, respectively. Swelling of these gels showed dependence on concentration with irradiation and swelling range is from 20 to 150 g water / 1 g dry gel. These values are less than that of CMC gel.

It was found that CM-chitosan hydrogels have anti-microbial activity and effective as absorbents to remove metal ions. In our research, a new type adsorbed based on chitin derivatives (CM-chitin, CM-chitosan) was prepared by irradiation [6]. The adsorption of Cu (II) ions onto these crosslinked chitin derivatives was investigated. Adsorption kinetic studies indicated a rapid removal of copper (II) ions from aqueous solutions.
Also, isothermal adsorption data revealed that Cu (II) could be removed by these cross-linked carboxymethylated chitin derivatives with high efficiency. Adsorption isothermal data could be well interpreted by the Langmuir equation.

The uptakes of Cu (II) ions on cross-linked CM-chitin were 161 mg/g, and on cross-linked CM-chitosan was 172 mg/g at pH 5.5. Low pH is favorable for Cu (II) adsorption. The Cu (II) ions can be desorbed from the crosslinked matrix rapidly and completely by treatment in diluted HCl solution and at the same time the adsorbents can be regenerated to be used again to adsorb heavy metal ions.

6. CROSSLINKING OF HPMC FOR ORGANIC SOLVENT ADSORPTION

Hydroxypropyl methylcellulose phthalate (HPMCP), cellulose ether with a phthalate functional group, is widely used in the pharmaceutical industry because it is less susceptible to hydrolysis than cellulose acetate phthalate. Radiation crosslinking of cellulose derivative having phthalate function (Hydroxypropyl methylcellulose phthalate, HPMCP) kneaded with aqueous alkali solution was achieved with EB-irradiation at paste-like condition (Fig. 3).

Low alkyl chain alcohol, ethers and ketone can be used as the media for radiation crosslinking of HPMCP [7]. The gels have good swelling ability in chloroform and acetone. The HPMCP gel absorbed organic solvents such as chloroform and pyridine. Accordingly, applications for absorbent to adsorption of organic solvent are expected.

![Graph showing gel fraction (%) vs. HPMCP conc. (%)](image)

**FIG. 3. Crosslinking of HPMCVP at past-like condition**
REFERENCE


PREVENTION OF SURGICAL ADHESIONS USING BARRIERS OF CARBOXYMETHYL CELLULOSE AND POLYETHYLENE GLYCOL HYDROGELS SYNTHESIZED BY IRRADIATION

JOON-HO LEE, YOUNG-CHANG NHO

Korea Atomic Energy Research Institute, Yusong, Daejon, Republic of Korea

Abstract

Biocompatible and biodegradable hydrogels based on carboxymethyl cellulose (CMC) and polyethylene glycol (PEG) were prepared for physical barriers for preventing surgical adhesions. These interpolymeric hydrogels were synthesized by a gamma irradiation crosslinking technique. The 1.5cm×1.5cm of cecal serosa and adjacent abdominal wall were abraded with bone burr until the serosal surface was disrupted and hemorrhagic but not perforated, and the serosa of the cecum was sutured to the abdominal wall in 5 mm apart from the injured site. The denuded cecum was covered with either CMC/PEG hydrogels or solution from CMC/PEG hydrogel. Control rat serosa was not covered. Two weeks later, the rats were sacrificed and adhesion was scored on a 0-5 scale. No treatment showed the significantly higher incidence of adhesions than either CMC/PEG hydrogels or solution from CMC/PEG hydrogel. In conclusion, these studies demonstrate that CMC/PEG hydrogels have a function of prevention of intra abdominal adhesion in a rat model.

1. INTRODUCTION

Postsurgical adhesions are abnormal tissue attachments that result from cuts or abrasions to tissues during surgery. These adhesions develop as part of the normal wound healing response of the tissues to the trauma and occur in over two thirds of all abdominal surgical patients. The consequences of these adhesions are varied and depend upon the surgical site involved. Problems may include pain, infertility, obstruction of the intestines, and even an increased risk of death after cardiac surgery. The process of adhesion formation initially involves the establishment of a fibrin framework and normal tissue repair.

The normal repair process allows for fibrinolysis alongside mesothelial repair. However, in surgical adhesion formation the fibrin matrix matures as fibroblasts proliferate into the network and angiogenesis occurs resulting in the establishment of an organized adhesion within 3 to 5 days. 60-95% of patients develop adhesions following major abdominal surgery [1]. Adhesions are responsible for about 60% of bowel obstructions and 20% of all infertility with substantial costs associated with adhesiolysis and hospitalizations [2, 3, 4].

Interventional attempts to prevent the formation of postsurgical adhesions have included the use of hydroflotation techniques or barrier devices. Hydroflotation involves the instillation of large volumes of polymer solutions such as dextran or carboxymethyl cellulose [5, 6] into the surgical space in an attempt to keep the organs apart. However, this technique has produced marginally beneficial effects in animals or humans. Synthetic barrier membranes made from oxidized regenerated cellulose (Interceed) or polytetrafluoroethylene (Gore-tex surgical membrane) have demonstrated some limited inhibition of adhesion formation in humans.

Resorbable barrier materials that have received research attention include Dextran-70, hyaluronic acid, hyaluronic acid [7], Poloxamer 407 [8], Interceed (Johnson & Johnson Medical, Inc., Arlington, TX), carboxymethylcellulose, fibrin glue, sodium hyaluronate/carboxymethyl cellulose (SH/CMC), and amnion. Only two products have been approved for clinical use: Interceed [9, 10] (Johnson & Johnson Medical, Inc., Arlington, TX) and Seprafilm (Genzyme Corp., Cambridge, MA).
Hydrogels are most often defined as two component systems where one of the components is a hydrophilic polymer, insoluble in water because of three dimensional networks joining as chains, and the second one is water. These systems may swell in water up to an equilibrium state and retain their original shape. The interactions responsible for water sorption by hydrogels include the processes of hydration, which is connected with the presence of such chemical groups as -OH, -COOH, -CONH₂, -CONH-, -SO₃H, and the existence of capillary areas and differences in osmotic pressure.

The forces, which make hydrogel dissolution impossible, are connected with the existence of covalent bonds between individual polymer chains although they may also have a character of electrostatic or hydrophobic interactions. The polymer gels have a very low modulus of elasticity, and therefore cause minimal mechanical irritation. They usually show good biocompatibility in contact with blood, body fluids and tissues. In recent years, much attention has been focused on the research and development of polymer hydrogels for biomaterials, such as contact lenses, wound dressing, enzyme immunoassay, catheters and drug delivery systems.

Irradiation has been recognized as a very suitable tool for the formation of hydrogels. The radiation process has various advantages, such as easy process control, possibility of joining hydrogel formation and sterilization in one technological step, no necessity to add any initiators and crosslinkers possibly harmful and difficult to remove. They make irradiation the method of choice in the synthesis of hydrogels.

It is known that water-soluble polysaccharides such as carboxymethylcellulose (CMC), carboxymethylstarch (CMS) and carboxymethylchitin (CMCT), carboxymethylchitosan (CMCTS) are crosslinked by radiation at more than 10% aqueous solution [11].

The goal of this study was to evaluate the efficacy of CMC/PEG hydrogels as barrier for reducing postsurgical adhesions in a rat cecal abrasion model.

2. EXPERIMENTAL

Materials

Na-carboxymethylcellulose (CMC, average Mw. 7.0×10⁵, and DS 0.9) and polyethylene glycol (PEG, average Mw. 4×10³) were supplied by Aldrich Chemical Company. Distilled water was used as a solvent in all experiments.

Preparation of Hydrogels

CMC/PEG (various weight compositions, Table I) was dissolved in distilled water and then mixed by a physical blender at room temperature to give a CMC/PEG solution. The dried content of CMC/PEG was in the range 11.2 - 12.3 wt%, and the weight composition percent of CMC/PEG was 88/12, 85/15, 82/18, 79/21. The homogeneous paste was then put into the cavity between plastic plates with 2mm spacer. The paste was exposed to 25 kGy of gamma ray to make hydrogels.

Preparation of Solution from Hydrogels

A sample of CP88/12 in Table I was diluted with distilled water and agitated mildly to make 2wt% concentration of CMC/PEG. This solution which consisted of the small particles of hydrogels distributed in water was used for coating for the purpose of the prevention of intra abdominal adhesion in a rat model.
TABLE I. FORMULATION FOR PREPARING HYDROGELS

<table>
<thead>
<tr>
<th>Hydrogel Composition</th>
<th>CMC/PEG Concentration (%)</th>
<th>Composition of CMC/PEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cp88/12</td>
<td>11.2%</td>
<td>88% 12%</td>
</tr>
<tr>
<td>Cp85/15</td>
<td>11.6%</td>
<td>85% 15%</td>
</tr>
<tr>
<td>Cp82/18</td>
<td>11.9%</td>
<td>82% 18%</td>
</tr>
<tr>
<td>Cp79/21</td>
<td>12.3%</td>
<td>79% 21%</td>
</tr>
</tbody>
</table>

CMC Mw=7.0×10^5, DS = 0.9, Total Dose = 2.5Mrad, Dose rate = 5kGy/hr
PEG Mw=4.0×10^3

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 at room temperature until the gel collapsed. After the water on the surface of the swollen gels was removed with cellulose paper, the mass was determined. The degree of swelling was defined as the eq. 2; where \( W_s \) is the weight of the swollen gels and \( W_d \) is the initial CMC/PEG weight.

\[
Water absorptivity = \frac{W_s - W_d}{W_d} \quad (2)
\]

Gel Strength

A cylindrical hydrogel specimen, with a height of 4.8 mm and a diameter of 12 mm, was used for the compressive strength tests. The compressive strength tests were conducted by Instron model 4400 Universal testing machine at room temperature. A cylindrical hydrogel specimen was placed on the base and the probe was lowered until contact was made. The probe was then lowered at 10 mm/min until 70 % relative deformation, and then raised. The compressive strength used in this experiment is the value measured at 70% relative deformation. The mechanical properties of the hydrogels were obtained by determining the compressive strength.

Adhesive Force

The adhesive force was obtained by measuring the force required to break the contact between the adhesive force between CMC/PEG hydrogel and mucosa layer of intestine.

Animal Test

Female Wistar rats (250-300g) were purchased from Kyeryong Science Co. (Daejon, Korea). Rats were anesthetized by intramuscular injection of ketamine (200mg/kg) and their ventral hair was removed with electric clippers. Using the aseptic technique, a 7 cm incision was made on the midline of the abdominal wall, and 1.5 cm x 1.5 cm of cecal serosa and adjacent abdominal wall were abraded with bone burr until the serosal surface was disrupted and hemorrhagic but not perforated, and the serosa of the cecum was sutured to the abdominal wall in 5 mm apart from the injured site (Figure 1).

To evaluate the effects of CMC/PEG hydrogel (Cp8812) or 2% solution from CMC/PEG hydrogel (Cp8812) as physical barrier for the prevention of intra abdominal adhesion in a rat model, we divided 30 female rats into three equal groups [12].
FIG. 1. Treatment procedure of cecal serosa and adjacent abdominal wall for adhesion evaluation. (A) Abrasion injury on cecal serosa. (B) Abrasion injury of adjacent abdominal. (C) Application with solution prepared from hydrogel(cp88/12) on injury.

In Group I (control) the defect was not repaired; in Group II the defect was repaired after a layer of CMC/PEG hydrogel was laid over the viscera; in Group III the defect was repaired after solution from CMC/PEG hydrogel was coated over the viscera. Ten of the animals from each group were sacrificed at postoperative day 14, and the adhesion severity and strength were scored according to Vlahos’ experiment [13].

The adhesion severity was classified as follows (Table II):

0: No adhesion, 1: one thin filmy adhesion, 2: two or more thin filmy adhesion, 3: thick adhesion with focal point, 4: thick adhesion with planar attachment, 5: very thick vascularized adhesion. The adhesion strength was classified as follows: 1: adhesion was filmy and easily torn with very light pressure, 2: adhesion was substantial and needed the moderated pressure to tear, 3: adhesion was heavy and required significant pressure to rupture, 4: adhesion was very heavy and difficult to rupture.

Some of the model rats were sacrificed at 3, 7 and 14 days after surgery to observe the absorption of CMC/PEG hydrogel (Cp8812) in the injured site.

At death, the abdominal wall of the injured site and opposed cecum was removed and fixed in 10 w% formalin solution. Specimens were dehydrated in a graded series of ethanol and in toluene, and then embedded in paraffin. Sections were cut at 4 to 5 microns. The tissues were processed by the standard procedure for histological examinations and their thin sections were examined after staining with hematoxylin-eosin (H-E).

TABLE II. CLASSIFICATION OF ADHESION SEVERITY AND STRENGTH

<table>
<thead>
<tr>
<th>Adhesion severity</th>
<th>Adhesion strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>0  No adhesion</td>
<td>1  Adhesion was filmy and easily torn with very light pressure</td>
</tr>
<tr>
<td>1  One thin filmy adhesion</td>
<td>2  Adhesion was substantial and needed moderate pressure to tear</td>
</tr>
<tr>
<td>2  Two or more thin filmy adhesion</td>
<td>3  Adhesion was heavy and required significant pressure to rupture</td>
</tr>
<tr>
<td>3  Thick adhesion with focal point</td>
<td>4  Adhesion was very heavy and difficult to rupture</td>
</tr>
<tr>
<td>4  Thick adhesion with planar attachment</td>
<td></td>
</tr>
<tr>
<td>5  Very thick vascularized adhesion</td>
<td></td>
</tr>
</tbody>
</table>
3. RESULTS AND DISCUSSION

Physical properties

There are several methods of preparing crosslinked hydrogels, such as radiation and chemical crosslinking. Radiation reactions utilize electron beams, gamma ray, X rays or ultraviolet light to excite a polymer and produce a crosslinked structure. Chemical crosslinking needs the use of at least one difunctional, small molecular weight crosslinking agent. This agent usually links two longer molecular weight chains through its di- or multifunctional groups. The radiation crosslinking can be easily adjusted by controlling the radiation dose and is reproducible. All the samples prepared by the formulation in Table I had very soft and flexible properties. When the prepared hydrogels were in kept in water for more than 48 hrs, it was unable to evaluate the gel percent because the forms of hydrogels were disintegrated. Figure 2 shows the degree of swelling behavior of the hydrogels that were synthesized by gamma irradiation. The swelling percent increased as PEG amount in CMC/PEG increased.

Crosslinking transforms a linear polymer into a three-dimensional molecule, resulting in a significant increase in molecular mass, lower solubility in organic solvents, and improve mechanical properties. Degradation results in a decrease in molecular mass, and has the opposite effect on the physical properties of the polymer. Crosslinking and degradation occur simultaneously. However, the ratio of their rates depends on the chemical structure of the polymer, its physical state, and the irradiation state. Polymers are generally divided into those that predominantly crosslink and those that predominantly degrade. CMC and PEG are crosslinked in their homogeneous mixture with water. As the molecular weight of PEG in these experiments was low, the increase of PEG in CMC/PEG solution resulted in the decrease in gelation of hydrogels.

FIG. 2. Swelling behaviour for hydrogels prepared by the formulation of Table I. Hydrogels are disintegrated cp88/12(48hr), cp85/15(24hr), cp82/12(13hr), cp79/21(7hr) in water.
The compressive strength used in this experiment is the value measured at 70 \% relative deformation. The gel strength of the hydrogels was obtained by determining their compressive strength (Figure 3). The compressive strength and modulus decreased as concentration of PEG in CMC/PEG increased. Because PEG of low molecular weight does not contribute to the crosslinking, the increase in PEG in /CMC/PEG resulted in the decrease in the compressive strength and modulus of hydrogels.

FIG. 3. Compressive strength and modulus of hydrogels prepared by the formulation of Table I.

Figure 4 shows the adhesive force between CMC/PEG hydrogel and the mucosa layer. Mucoadhesion, which adheres to the mucosa layer, may be useful to fix the hydrogels on the viscera. The higher the content of PEG in the CMC/PEG hydrogels, the lower the adhesive force. It was indicated that the density of carboxylic groups on the polymer chain was important for a mucoadhesion.

FIG. 4. Adhesive force of CMC/PEG hydrogel and the mucosa layer.
Figure 5 shows the FT-IR spectra of CMC, PEG and CMC/PEG film prepared from CMC/PEG hydrogel. PEG exhibits the absorption features at 2938-2976 cm\(^{-1}\) (C-H) and around 1110 cm\(^{-1}\) (C-O-C). The intensities of carboxyl band at 1589 cm\(^{-1}\), which is not observed in pure PEG, was shown for the CMC/PEG film prepared from the CMC/PEG hydrogels.

![FTIR spectra](image)

**FIG. 5.** FTIR spectra of CMC, PEG and CMC/PEG (CMC/PEG film prepared from hydrogel involving CMC/PEG (88/12 wt%).

### Animal studies

Adhesions are unwanted tissue growths occurring between layers of adjacent bodily tissue of between tissues and internal organs. Adhesions commonly form during the healing which follows surgical procedures, and when present, adhesions can prevent the normal motions of those tissues and organs with respect to their neighboring structures.

The results of adhesion scoring are summarized in Table III. The control animals formed the dense adhesions in the area where the cecal serosa was approximately directly to the abdominal wall. Animals treated with CMC/PEG hydrogel (Cp8812) or 2 wt% solution from CMC/PEG hydrogel (Cp8812) had a significantly lower average adhesion score than the controls. There was not much difference in adhesion scores between groups II and group III. At day 14, no residual HA/CMC hydrogels were visible in the treated animals.

**TABLE III. EVALUATION OF ADHESION FOR RATS TREATED WITH CMC/PEG HYDROGEL (CP8812) OR SOLUTION FROM CMC/PEG HYDROGEL (CP8812)**

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Adhesion degree</th>
<th>Adhesion area (cm(^2))</th>
<th>Adhesion strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>4.6 ± 0.5</td>
<td>3.61 ± 0.24</td>
<td>3.6 ± 0.51</td>
</tr>
<tr>
<td>2% solution CMP/PEG</td>
<td>0.8 ± 0.78</td>
<td>0.105 ± 0.10</td>
<td>1.3 ± 1.25</td>
</tr>
<tr>
<td>Hydrogel Cp88/12</td>
<td>1.0 ± 0.81</td>
<td>0.162 ± 0.14</td>
<td>1.5 ± 1.17</td>
</tr>
</tbody>
</table>

* P value < 0.05 versus controls.
The mechanism of action by which CMC reduces adhesion formation is not clear. It has been found that CMC when implanted intraperitoneally attracts fluid in its surrounding and thereby prevents serosa to peritoneal contact - the so-called “hydroflotation effect”. In addition, there is evidence to suggest that CMC coats intraperitoneal surfaces and reduces the direct apposition of traumatized structures- the so-called “siliconizing effect” [14]. The other proposed mechanism of action of SCMC is through its effect on fibroblastic and cellular activities [15].

At time of application, the hydrogels adhered readily to serosal surfaces and did not have to be sutured in place. In both control and experimental groups, the celiotomy incisions healed normally. The sutures holding the bowel to the abdominal wall were still in place (Figure 6).

FIG. 6. Necropsy taken 14 days after operation of rat to find the difference of adhesion between control (A) and solution prepared from hydrogel (cp88/12) (B).

The bioresorbability of CMC/PEG hydrogel (Cp8812) between skin and abdominal layer of rat was evaluated by observing the forms of hydrogels on cecal serosa on the 3rd, 7th, 14th day after surgery. The hydrogel was found to be the loose gel on the 3rd postoperative day, and was slightly detectable at 7th, almost not detectable at the 14th postoperative day (Figure 7). Until now, the mechanism of the bioresorbability of CMC or PEG was not well known.

FIG. 7. Procedure of bioresorbability of CMC/PEG hydrogels. (A) immediately after application of hydrogel. (B) 3 days after application of hydrogel. (C) 7 days after application of hydrogel. (D) 14 days after application of hydrogel.
Figure 8 shows the histological appearance of tissue in the part of cecal serosa and abdominal wall. In control, the dense fibrous adhesion between the intestine (cecal serosa) and abdominal wall was found, and variable inflammatory cells and neovascular structure were seen in granulation tissue. Serosal area and surrounding fat tissue show mild inflammatory cell infiltrations. On the other hand, rat tissue treated with hydrogel show no granulation tissue formation.

![Figure 8](image)

**FIG. 8.** Histological appearance of tissue in the part of cecal serosa and abdominal. (A) control. (B) Application of hydrogel (cp88/12).

Figure 9 shows the histological appearance of tissue in the part of cecal serosa and abdominal wall 12 hrs (A), 3days (B) and 7days(C) after application of hydrogels on the injuries. We can detect a focal abdominal wall and mucosal erosion (A) and the completely formed muscular tissue and mesothelial area by wound healing(C).

![Figure 9](image)

**FIG. 9.** Histological appearance of tissue in the part of cecal serosa and abdominal. (A) 12 hrs after application of 2w% solution from hydrogel, (B) 3days after application of 2w% solution from hydrogel, (C) 7days after application of 2w% solution from hydrogel.

4. CONCLUSION

Hydrogels based on CMC/PEG were prepared for physical barriers for preventing surgical adhesions using radiation. These interpolymeric hydrogels were synthesized by a gamma irradiation crosslinking technique.
Animals treated with CMC/PEG hydrogel (Cp8812) or solution from CMC/PEG hydrogel (Cp8812) had a significantly lower average adhesion score than the controls. At day 14, no residual HA/CMC hydrogels were visible in the treated animals.

Hydrogels prepared by radiation was found significantly to reduce postsurgical adhesions in a rat cecal abrasion model.

REFERENCES

Review of Alternative Methods Applying to Cellulose and Chitosan Structure Modification

H. Struszczyk, D. Ciechańska, D. Wawro, A. Niekraszewicz, G. Strobin
Institute of Chemical Fibres, Lodz, Poland

Abstract

The Institute of Chemical Fibres specializes in modification of natural polymers, specially cellulose and chitosan. Within research work the effects of chemical treatment (18 wt% aq. solution of NaOH), γ radiation and action of cellulolytic enzymes on changes of cellulose molecular structure and its solubility in 9 wt% NaOH solution have been investigated. The studies on the estimation of changes in chitosan molecular structure caused by its degradation by chemical, enzymatic and radiation methods have been conducted.

1. INTRODUCTION

Cellulose

Cellulose is a widely occurring polymer in nature. The main sources of cellulose used in the chemical fibres industry are wood pulps, cotton and other plant materials.

The practical application of cellulose pulps for different processes is connected with selection of methods for modification of this raw material in order to improve its reactivity and solubility in inorganic and organic solvents. The cellulose modification process can be conducted by chemical, physical or biochemical methods. Alkalization of cellulose is the oldest and most frequently used method of its modification. The process includes treating cellulose with 18-19 wt% aqueous sodium hydroxide. Many chemical reactions and physical processes take place and as results intermolecular bond become weaker and polymerization degree decreases [1-2].

Modification of cellulose by radiation has been subject of examination for many years. Under the influence of an electron beam or γ-irradiation the cellulose reactivity, processability into esters and ethers as well as its solubility in alkaline solution increases [3-6].

Steam-explosion method of cellulose modification was developed mainly for wood and biomas recycling and consists in subjecting wet cellulose to high pressure (1-5 MPa) and temperature (180 - 250°C) in very short time (15-300 sec). Kamide and Yamashiki applied that method to cellulose pulp with α-cellulose content above 90% and to cotton linters. Cellulose modified in that way was characterized by 99% solubility degree in aqueous NaOH. They explained that phenomenon as a result of breaking of intermolecular hydrogen bonds in cellulose [7-9].

The latest meaningful method of cellulose modification is based on the action of cellulase and xylanase enzymes. The process of biotransformation of cellulose creates new options to change physico-chemical characteristics of cellulose in order to obtain a polymer directly soluble in 7 - 9 wt% aqueous solutions of sodium hydroxide [10-13].

Chitosan

Chitin, poly [β-(1, 4)-2-acetamid-2-deoxy-D-glucopiranose] is most abundant, next after cellulose, renewable natural polymer [14]. It occurs in the shells of marine crust fish, insects as well as some fungi wall cells. The chitosan, poly [β-(1, 4)-2-amino-2-deoxy-D-glucopiranose] is a product of chitin deacetylation. Low solubility of chitin affects on its narrow range of practical application.

Chitosan solubilized in the aqueous acid solutions, contrary to chitin, has been utilized in several practical applications, especially in medicine. Chitosan is non-toxic, biodegradable polymer distinguished by LD50>16g per 1kg of body weight. The bioactivity is specific property of this...
polymer. This phenomenon results from several processes such as biodegradability, membrane effect, polycationic character or stimulation of natural organism resistance.

The most essential medical applications of chitosan are as follows [15]:

- wound healing promoting dressings
- dermatological agents
- biodegradable carriers for slow release drugs
- anticoagulant agents
- optimological, dentistical and orthopedic agents
- tumor cell metabolism reducing agents

Microcrystalline chitosan (MCCh) elaborated at the Institute of Chemical Fibres is a most useful form in medical applications [16-17]. This form is distinguished by improved bioactivity and susceptibility to a biodegradation, in comparison to the initial chitosan.

Microcrystalline chitosan is a new useful form prepared by macromolecule aggregation process suitable especially for several special applications. An aqueous gel-like dispersion is a basic useful form of MCCh. Microcrystalline chitosan can be used instead of initial chitosan with practical and economical advantages as well as in the new applications, where an initial chitosan can not be used. MCCh is distinguished by all valuable properties of initial chitosan with simultaneously higher bioactivity, biodegradability and other unique properties such as ability to film preparation directly from the aqueous dispersion.

2. EXPERIMENTAL

Materials

The viscose – grade pulps such as Ketchikan pulp (Ketchikan Pulp Co., Brownfield, USA), Fibrenier pulp (ITT, Rayonier, USA), beech pulp (Swiecie Co., Poland) have been applied to investigations relevant related to cellulose structure modification.

The initial chitosan in form of flakes produced by Chempol (India) has been used for studies concerned the chitosan structure modification.

Methods

Chemical degradation of cellulose

Dry cellulose pulp was immersed into NaOH solution (18-wt.%) at 40°C. After 2 hrs of soaking the pulp was compressed, disintegrated and then stored at 40°C under stationary conditions. The process of cellulose degradation was stopped by introduction of sample into distilled water and neutralization by means of 10 % acetic acid.

Radiation degradation of cellulose

Dry cellulose samples were subjected to γ-radiation from $^{60}$Co source, at the Institute of Applied Radiation Chemistry of Lodz, Poland. The activity of the radiation chamber was $7.4 \times 10^5$ GB2 (20 kCi) and dose rates were 3, 6, 9, 12 and 15 kGy, respectively.

Enzymatic modification of cellulose
Cellulose samples were treated with cellulase solution in acetate buffer of pH 4.8. The enzymatic reaction was carried out at 50°C for time up to 48 hrs. and continuous shaking. Next the enzyme solution was separated by filtration on the Buchner funnel. The sample of modified cellulose was washed with hot water (90°C) and then twice with cold distilled water and dried in air.

*Preparation of chitosan forms (MCCh)*

Microcrystalline chitosan (MCCh) was prepared according to original continuous method elaborated at Institute of Chemical Fibres.

*Ionizing radiation of chitosan*

The chitosan samples were subjected to γ-radiation from 60Co source at the Institute of Applied Radiation Chemistry of Lodz, Poland. The activity of the radiation chamber was 20 kCi and the dose rate was 5 kGy/h. The applied doses were 5, 10, 20, 50, 100 and 150 kGy.

*Chemical degradation of chitosan*

The degradation of chitosan was carried out in a presence of sodium perborate with concentration ranged from 0.1 - 2.0 wt% at temperature of 2 - 100°C for time up to 2 hrs.

*Enzymatic degradation of chitosan*

Enzymatic degradation was carried out in lysozyme (mucoprotein N-acetyl-muramyl-hydrolase) solution in phosphate-citric buffer, pH 7.23. Lysozyme was a product of Merck, Germany, and its activity was 100,000 U/mg. The test was performed in an incubator at 37°C under static conditions. All the tested samples in the buffer solution were sterilized, prior to addition the lysozyme, in autoclave, at 121°C for 15 min. The samples were removed from baths by centrifugation at predetermined time intervals of 3, 7, 14 and 28 days. After removal each samples was washed first with distilled water at 50°C and then with 70% ethyl alcohol. The washed samples were then dried to a constant weight at 45°C.

*Analytical methods*

*Gel Permeation Chromatography (GPC)*

The GPC system used for measurement of cellulose and chitosan samples consist of an isocratic pump HP 1050 (Hewlett-Packard Co, Germany), manual injector (Rheodyne, USA) and refractive index detector 1047A (Hewlett–Packard Co, Germany).

Parameters for cellulose measurement:

- The mobile phase i.e. DMAC/0.5% LiCl was pumped with constant flow rate 0.8 ml/min
- The set of two columns Plgel Mixed B (5 μm, 300 mm) Polymer Lab. Shropshire U.K., operated at 80°C
- The volume of injections was 70 μl
- Calibration of GPC system using a set of ten polystyrene standards (M from 3,250 to 7,100,000) Polymer Lab. Ltd Shropshire U.K.
- Parameters for chitosan measurements
- The mobile phase i.e. 0.33 M acetic acid / 0.2 M sodium acetate was pumped with constant flow rate 1 mL / min
- The volume of injections was 70 μl
- Calibration using the standards poly (ethylene oxide) and poly (ethylene glycol) of $M_w$ from 1,400 – 930,000.
The date of measurements were collected and processed by PL Caliber GPC/SEC Software (Polymer Lab. Ltd. Shropshire U.K.)

*Solubility degree of cellulose in alkaline solutions*

The cellulose samples after structure modification were dissolved in 9 wt% NaOH aqueous solution at –5°C with intensive homogenisation according to procedure described in [18].

3. RESULTS AND DISCUSSION

**Studies on cellulose modification in aspect of its processability**

Works on new cellulose fibres technologies have been carried out for many years. Their main objective is to develop new methods for manufacturing cellulose fibres, competitive to the technology based on a viscose process. Studies on new methods based on preparation of cellulose directly soluble in alkaline aqueous solutions (Celsol) are still in progress. Above mentioned method which is based on the wet spinning technique, need preliminary modification of cellulose pulp for improving its reactivity and solubility.

Within the studies conducted at the Institute of Chemical Fibres different methods for cellulose structure modification were tested regarding their use ability for Celsol process. In continuation of our previous investigations changes on molecular characteristics exclusively have been examined.

Using GPC, changes in molecular weight distribution (MWD) and average molecular weight \( \overline{M}_n \) and \( \overline{M}_w \) caused by \( \gamma \)-radiation, chemical action and enzymatic treatment have been presented.

The molecular parameters of cellulose pulps treated by alkalization and \( \gamma \)-irradiation are shown in Tables I and II respectively. The changes in MWD curves of cellulose chemically treated and \( \gamma \)-irradiated are illustrated in Fig. 1 and 2.

**TABLE I. MOLECULAR PARAMETERS OF CHEMICALLY TREATED KETCHIKAN PULP**

<table>
<thead>
<tr>
<th>Time of treatment [h]</th>
<th>( \overline{M}_n ) [kDa]</th>
<th>( \overline{M}_w ) [kDa]</th>
<th>( \overline{M}_w/ \overline{M}_n )</th>
<th>DPw</th>
<th>Content of fraction, DP [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;200</td>
</tr>
<tr>
<td>0</td>
<td>34</td>
<td>133</td>
<td>3.9</td>
<td>821</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>73</td>
<td>2.3</td>
<td>451</td>
<td>33</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>46</td>
<td>1.9</td>
<td>284</td>
<td>46</td>
</tr>
<tr>
<td>24</td>
<td>15</td>
<td>23</td>
<td>1.5</td>
<td>142</td>
<td>80</td>
</tr>
<tr>
<td>48</td>
<td>12</td>
<td>17</td>
<td>1.4</td>
<td>105</td>
<td>91</td>
</tr>
</tbody>
</table>
FIG. 1. MWD curves of chemically treated Ketchikan pulp

Based on the results (Table I) it was found out that in a case of cellulose alkalization, a rapid decrease of Mn, Mw and polydispersity is observed. The highest polymer fraction DP > 550 disappears and amount of lower fraction DP < 200 increases.

TABLE II. MOLECULAR PARAMETERS OF $\gamma$ IRRADIATED FIBRENIER PULP.

<table>
<thead>
<tr>
<th>Radiation dose [kGy]</th>
<th>Mn [kDa]</th>
<th>Mw [kDa]</th>
<th>$\tilde{M}_w$/ Mn</th>
<th>DPw</th>
<th>Content of fractions, DP [%]</th>
<th>&lt;200</th>
<th>200-550</th>
<th>&gt;550</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>35</td>
<td>106</td>
<td>3.0</td>
<td>654</td>
<td>23</td>
<td>36</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>86</td>
<td>2.6</td>
<td>530</td>
<td>26</td>
<td>40</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>75</td>
<td>2.6</td>
<td>463</td>
<td>31</td>
<td>40</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>27</td>
<td>66</td>
<td>2.4</td>
<td>405</td>
<td>34</td>
<td>43</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>26</td>
<td>60</td>
<td>2.3</td>
<td>370</td>
<td>37</td>
<td>43</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>24</td>
<td>55</td>
<td>2.3</td>
<td>339</td>
<td>41</td>
<td>42</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

FIG. 2. MWD curves of $\gamma$ irradiated Fibrenier pulp; 1 – initial sample; Sample irradiated with: 2 – 3kGy; 3 – 6 kGy; 4 – 9 kGy; 5 – 12 kGy; 6 – 15 kGy
Based on the results (Table II) it was found out that in case of γ-irradiation reduction of average molecular weight \( \bar{M}_n \) is accompanied by low decrease of polydispersity \( \bar{M}_w/\bar{M}_n \). Shapes of MWD curves remains similar each other.

The application of enzymes for cellulose modification makes it possible to join partial depolymerization and degradation of its structure leading to obtention of direct soluble polymer in aq. sodium hydroxide.

The molecular parameters of enzymatically treated cellulose are presented in Table III and Figure 3.

**TABLE III. MOLECULAR PARAMETERS OF ENZYMATICALLY TREATED BEECH PULP (CELLULASE).**

<table>
<thead>
<tr>
<th>Time of treatment [h]</th>
<th>( \bar{M}_n ) [kDa]</th>
<th>( \bar{M}_w ) [kDa]</th>
<th>( \bar{M}_w/\bar{M}_n )</th>
<th>DPw</th>
<th>Content of fractions, DP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;200</td>
</tr>
<tr>
<td>0</td>
<td>28</td>
<td>90</td>
<td>3.2</td>
<td>555</td>
<td>30</td>
</tr>
<tr>
<td>0.5</td>
<td>26</td>
<td>82</td>
<td>3.2</td>
<td>506</td>
<td>34</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>75</td>
<td>3.1</td>
<td>462</td>
<td>39</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>69</td>
<td>3.1</td>
<td>426</td>
<td>43</td>
</tr>
<tr>
<td>24</td>
<td>21</td>
<td>64</td>
<td>3.1</td>
<td>395</td>
<td>46</td>
</tr>
<tr>
<td>48</td>
<td>20</td>
<td>62</td>
<td>3.1</td>
<td>383</td>
<td>47</td>
</tr>
</tbody>
</table>

*FIG. 3. MWD curves of enzymatically modified Beach pulp.*

The enzymatic degradation of cellulose differs significantly from previously considered methods. Based on the results (Table III) it was found out that:

- reduction of molecular weight runs very slowly,
- \( \bar{M}_w/\bar{M}_n \) values remain at the same level despite of considerable changes of \( \bar{M}_n \) and \( \bar{M}_w \)
The selected cellulose samples characterized by similar values of average molecular weight (\( \text{Mw} \approx 75,000 \)) were tested with regard to their suitability in Celsol process. Determination of degree of cellulose solubility was carried out in 9 wt\% aqueous sodium hydroxide solution. The data on cellulose solubility are presented in Table IV.

TABLE IV. SOLUBILITY OF SELECTED PULPS AFTER VARIOUS KINDS OF TREATMENT

<table>
<thead>
<tr>
<th>Method of treatment</th>
<th>Mw [kDa]</th>
<th>Solubility degree [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>chemical</td>
<td>73</td>
<td>20</td>
</tr>
<tr>
<td>( \gamma ) radiation</td>
<td>75</td>
<td>28</td>
</tr>
<tr>
<td>enzymatic</td>
<td>75</td>
<td>&gt;95</td>
</tr>
</tbody>
</table>

Based on the results (Table V) it was found out that the changes in cellulose molecular structure which took place during degradation do not guarantee total solubility of cellulose in 9 wt\% NaOH solution. The highest solubility was attained in case of applying the enzymes for cellulose modification.

Enzymatic treatment was selected as the best method for improving cellulose solubility in aqueous alkaline solution and for applying in Celsol process.

Studies on different methods for chitosan structure modification

Some essential properties of chitosan amongst other solubility, processability and bioactivity, as well as applications ability in medicine or agriculture, are closely connected with its molecular structure. Commercial chitosan types are generally characterised by high viscometric average molecular weight than 400 kDa, as well as deacetylation degree higher than 70\%. Different methods permitting reduction of \( \text{Mv} \) such as physical (\( \gamma \)-radiation), chemical (sodium perborate) and enzymatic (lysozyme) degradation are stated.

Radiation degradation

Studies on the modification of molecular structure of chitosan forms by using different doses of \( \gamma \) radiation (5 – 150 kGy) issued from \(^{60}\text{Co}\) isotope were conducted.

Molecular characteristic of initial chitosan after \( \gamma \)-radiation is presented in Table V and Figure 5.

TABLE V. MOLECULAR CHARACTERISTICS OF STANDARD CHITOSAN AFTER \( \gamma \) RADIATION DEGRADATION.

<table>
<thead>
<tr>
<th>No. of sample</th>
<th>Dose [kGy]</th>
<th>Mn [kDa]</th>
<th>Mw [kDa]</th>
<th>Mw / Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>38</td>
<td>270</td>
<td>7.1</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>35</td>
<td>198</td>
<td>5.7</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>27</td>
<td>138</td>
<td>5.1</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>27</td>
<td>102</td>
<td>3.8</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>16</td>
<td>55</td>
<td>3.4</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>9</td>
<td>32</td>
<td>3.6</td>
</tr>
<tr>
<td>7</td>
<td>150</td>
<td>6</td>
<td>19</td>
<td>3.2</td>
</tr>
</tbody>
</table>
FIG. 4. Molecular weight distribution curves of chitosan degraded by ionizing radiation

The shape of MWD and also changes in $\overline{M}_w / \overline{M}_n$ suggest that degradation by radiation is associated with a random statistical breaking up of the chains with reduction of $\overline{M}_n$ and $\overline{M}_v$ values and polydispersity.

The influence of $\gamma$-radiation dose on average molecular weight of both initial and microcrystalline chitosan has been investigated. The results of studies are presented in Table VI.

TABLE VI. INFLUENCE OF $\gamma$ RADIATION DOSE ON AVERAGE MOLECULAR WEIGHT OF DIFFERENT CHITOSAN FORMS

<table>
<thead>
<tr>
<th>Chitosan sample</th>
<th>Dose [kGy]</th>
<th>$M_v$ [kDa]</th>
<th>Reduction of $M_v$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard chitosan</td>
<td>0</td>
<td>473</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>371</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>286</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>201</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>105</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>53</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>32</td>
<td>93</td>
</tr>
<tr>
<td>Microcrystalline chitosan (MCCh)</td>
<td>0</td>
<td>280</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>43</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>40</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>26</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>11.5</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>290</td>
<td>3.3</td>
<td>99</td>
</tr>
</tbody>
</table>
Based on results it was found out that:

- γ-radiation treatment is very effective method for reduction of average molecular weight both for initial as well as microcrystalline chitosan,
- MCCh indicates higher susceptibility on degradation with similar radiation dose such as an initial chitosan,
- use of dose of 290 kGy caused nearly completely degradation of MCCh to the oligomeric forms

**Enzymatic degradation**

Biodegradation of chitosan seems to be one of the most important properties of this polymer. The low molecular weight oligoaminosaccharides obtained during biodegradation process affects significantly on several biological process occurring ion the living organism.

The process of initial chitosan degradation by selected enzymes such as lysozyme present in the body fluids of mammals is illustrated by Figure 7. The changes in molecular weight distribution taking place in the process are presented in Table VII.

**TABLE VII. MOLECULAR CHARACTERISTICS OF STANDARD CHITOSAN AFTER ENZYMATIC DEGRADATION BY LYSOZYME**

<table>
<thead>
<tr>
<th>No. of sample</th>
<th>Time of degradation [day]</th>
<th>Mn [kDa]</th>
<th>Mw [kDa]</th>
<th>Mw / Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>38</td>
<td>270</td>
<td>7.1</td>
</tr>
<tr>
<td>2</td>
<td>sterilized</td>
<td>32</td>
<td>265</td>
<td>8.3</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>22</td>
<td>156</td>
<td>7.1</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>20</td>
<td>131</td>
<td>6.6</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>17</td>
<td>115</td>
<td>6.8</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
<td>11</td>
<td>76</td>
<td>6.9</td>
</tr>
</tbody>
</table>

**FIG. 5. Molecular weight distribution curves of enzymatically degraded chitosan**
On the base of results (Table VII, Figure 7) it was found out that:

- the enzyme readily affects the lower fraction while the high fractions are less susceptible to changes,
- polydispersity $\frac{M_w}{M_n}$ remains practically at a constant level.

Chemical degradation

The degradation of chitosan in a presence of sodium perborate with concentration ranged from 0.1 to 2.0 wt% at temperature of 100°C for 2 hrs was carried out. The results of studies are presented in Table VIII.

**TABLE VIII. INFLUENCE OF SODIUM PERBORATE CONCENTRATION ON AVERAGE MOLECULAR WEIGHT OF CHITOSAN.**

<table>
<thead>
<tr>
<th>Sodium perborate concentration [wt%]</th>
<th>Molar ratio of sodium perborate to chitosan</th>
<th>$M_v$ [kD]</th>
<th>Reduction of $M_v$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>433</td>
<td>0</td>
</tr>
<tr>
<td>0.10</td>
<td>0.04</td>
<td>126</td>
<td>70.9</td>
</tr>
<tr>
<td>0.25</td>
<td>0.10</td>
<td>75</td>
<td>82.7</td>
</tr>
<tr>
<td>0.50</td>
<td>0.20</td>
<td>49</td>
<td>88.7</td>
</tr>
<tr>
<td>1.00</td>
<td>0.40</td>
<td>34</td>
<td>92.1</td>
</tr>
<tr>
<td>2.00</td>
<td>0.80</td>
<td>12</td>
<td>97.2</td>
</tr>
</tbody>
</table>

A concentration of sodium perborate of 0.1 wt% in degradation bath was resulted in reduction of average molecular weight from $M_v = 433 \text{ kDa}$ to 126 kDa (approx. 70 %). The application of sodium perborate with concentration of 20 wt% was resulted in reduction of $M_v$ of 97% in comparison to the initial chitosan.

Within the studies on the influence of degradation treatment time on average molecular weight of chitosan. The degradation of initial chitosan in a presence of sodium perborate with concentration of 1.0 wt% at temperature of 100°C with time ranged from 0.25 to 2 hrs. The results of studies are presented in Table IX.

**TABLE IX. INFLUENCE OF TREATMENT TIME ON AVERAGE MOLECULAR WEIGHT OF CHITOSAN.**

<table>
<thead>
<tr>
<th>Treatment time [h]</th>
<th>$\bar{M}_v$ [kD]</th>
<th>Reduction of $\bar{M}_v$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>433</td>
<td>0</td>
</tr>
<tr>
<td>0.25</td>
<td>46</td>
<td>89.4</td>
</tr>
<tr>
<td>0.50</td>
<td>37</td>
<td>91.5</td>
</tr>
<tr>
<td>1.00</td>
<td>36</td>
<td>91.7</td>
</tr>
<tr>
<td>2.00</td>
<td>33</td>
<td>92.4</td>
</tr>
</tbody>
</table>
The application of chitosan treatment time equal 0.25 h was resulted in reduction of $\tilde{M}_v$ of 90% in comparison to the initial chitosan. Prolongation of treatment time up to 2 hrs was not caused further reduction of $M_v$. The degradation process of chitosan in presence of sodium perborate seems to be simple, not expensive and fast method for $M_v$ reduction.

REFERENCES

Abstract

Chitosan was irradiated by the gamma ray from a Co-60 source for degrading and preparing the oligochitosan. The radiation degradation yield \( (G_s) \) of the chitosan was found out to be of 1.03. The oligochitosan DP<8 with 50% mass fraction was obtained by irradiating the 10% (w/v) chitosan solution at 45kGy for the chitosan having the initial viscosity average molecular weight, \( M_v = 60,000 \). Biological effects induced by irradiation of chitosan particularly anti-fungal, phytoalexin induction, growth-promotion effect for plants have been also investigated.

1. INTRODUCTION

The objective of the research is to study the degradation process of chitosan by gamma Co-60 radiation and to investigate some biological effects of irradiated chitosan induced by radiation degradation process.

Chitosan is a linear aminopolysaccharide (polyglucosamine) derived from chitin, a naturally abundant mucopolysaccharide, poly \( \beta - (1 \rightarrow 4) \) - linked N-acetyl-D-glucosamine by alkali deacetylation. Chitosan has a wide range of applications, such as in waste water treatment, in medicine and cosmetics, in food and functional food, in agriculture, [1-4]. The production of chitin/chitosan is currently based on crab and shrimp shells discarded as a food industry waste. The global annual estimate of shellfish processing discards is more than one million metric tons [1]. Thus, disposal of shellfish wastes has been a challenge for most of the shellfish-processing countries. Therefore, production of value-added products such as chitin/chitosan, oligomers and their derivatives for utilization in different fields is of utmost interest.

Low molecular weight polysaccharides and/or oligosaccharides can be produced by degradation of corresponding polysaccharides including marine polysaccharides such as alginate, chitin/chitosan, and carrageenan. Chemical, enzymatic and radiation processing technologies can be applied for degradation process.

Recently much attention has been paid to the application of radiation processing technology for degradation of natural polysaccharides [5] due to several reasons, for instances:

i) carrying out at room temperature
ii) reliability of process control and large scale application
iii) economic competitiveness to alternatives.

Both gamma Co-60 and electron beam can be applied for degradation process. Recently a unique design of low energy electron beam machine can be considered as a feasible economical facility for the purpose of degradation of polysaccharides by irradiation in solution state (liquid) [6].

Oligochitosan has been recognized as potent phytoalexin inducer (elicitor) to resist infection of diseases for plants [7-10]. Thus it is promising to utilize oligosaccharides particularly oligochitosan in agriculture as biotic elicitor to enhance defense responses against diseases and as growth promoter for plants [10-11]. Earlier work on irradiation of chitosan was carried out by Kume and Takehisa in 1982 [12]. The authors reported that viscosity of irradiated chitosan decreased significantly at low dose (10 kGy). In 1992, Ulanski and Rosiak studied on radiation-induced changes in chitosan and reported radiation yields of scission of chitosan in solid state, \( G_s = 0.9 \) in vacuum, \( G_s = 1.1 \) in air and \( G_s = 1.3 \) in oxygen [13]. A post effect of degradation slightly occurred.
The enhancement of antimicrobial activity of irradiated chitosan was investigated by Matsuhashi and Kume in 1997 [14]. It was concluded that irradiated chitosan having molecular weight of 105 to 3 x 105 exhibited highly antimicrobial activity. Following this direction of research, in 1999, Ha et al. also recognized the enhancement of antifungal activity of irradiated chitosan for different fungi strains [15]. More recently two papers reported the preparation of oligochitosan by radiation degradation of chitosan in solid state [16] and in solution [17]. Results indicated that in order to obtain oligochitosan in case of irradiation of chitosan solution, the dose should be higher than 10 kGy while very high dose and followed by fractionation should be needed for irradiating chitosan in solid state.

Thus for the purpose to prepare oligosaccharide, irradiation for degradation in solution state may be favorable. In this report the irradiation effect on chitosan in solid state to regulate chitosan molecular weight, the yields of oligochitosan by irradiating chitosan solution and some biological activities of resultant products such as anti-fungi, pisatin induction and plant growth-promotion have been investigated.

2. MATERIALS AND METHODS

Chitosan and irradiation

Chitosan with degree of deacetylation of 80% [18,19] and viscosity average molecular weight (MW), \( Mv_o = 710,000 \) was used for study. Chitosan solution of 10% (w/v) was prepared in 5% (w/v) acetic acid. Irradiation was carried out on a gamma Co-60 source at room temperature, dose rate of 2 kGy/h. The viscosity average MW (Mv) of chitosan samples irradiated in powder form was measured by Ubbelohde viscometer using 0.1 M CH\(_3\)COOH/ 0.2 M NaCl as solvent system and calculated by equation \( [\eta] = k \times Mv^\alpha \), where \( k = 1.18 \times 10^{-3} \) cm\(^3\)/g and \( \alpha = 0.93 \) at 25°C [20]. Oligochitosans from irradiated chitosan solutions were determined by fraction coagulation method using CH\(_3\)OH/H\(_2\)O = 9/1 (v/v) as solvent [21].

Biological effect test

Fungicidal effect of irradiated of chitosan

a) In vitro

Chitosan powder was dissolved in 2.5% CH\(_3\)COOH, adjusted to pH ~ 6.0 by 1N NaOH. Chitosan solution was filtered with Milipore MILLEX-GS (pore size: 0.22\( \mu \)m, Millipore MA, USA) and diluted from 1200- 50\( \mu \)g/ml in the medium (Czapeck-Dox). Spore or mycelia fragments of fungi were dispersed in the medium, and 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 of irradiated chitosan in vivo was tested as follows: tea plants of 9 month old were divided into 3 beds, 50 plants for each. Bed 1 was the reference control and bed 2 and bed 3 were artificially inoculated with E. vexans (foliar pathogen) by spraying 3 times on the leaf surface and pouring over the soil surface with suspension of spores or mycelia 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 infected leaves frequency (number of infected leaves per total of leaves) was recorded within 30 days. Phytoalexin particularly pisatin induced in immature pea pod by treating with oligochitosan was determined according to method described by Hadwiger and Beckman [22].

68
**Effect of growth-promotion for plant in tissue culture**

Irradiated chitosan solution was added into medium of tissue culture with different concentration: 0, 10, 30, 50, 100 and 150 ppm and *Chrysanthemum* plant was used for study. The shoot height, root length and fresh biomass (including root, shoot and leaves) of the tissue culture plants were recorded after 20 days incubating at 25°C.

3. RESULTS AND DISCUSSION

**Radiation degradation of chitosan**

Results in figure 1 clearly indicated that Mv of chitosan decreased as the increase of dose. The degree of MW decrease can be evaluated by the radiation degradation yield, Gs (sissions/100eV). Gs = 1.03 was derived from the results in the figure 1. The obtained Gs value for chitosan was fairly good in agreement with Gs value reported by Ulanski and Rosiak, Gs = 1.1 using HPLC technique to determine the number average MW (Mn). Accordingly, radiation technique can be usefully utilized to regulate chitosan MW to a certain low level. On the other hand, for preparation of oligochitosan, the irradiation of chitosan in form of dry powder at high dose may cause the destroy of glucosidic ring and breaking of –NH₂ linkage.

![Graph showing the relationship between Mv and dose](image)

**FIG. 1. The relationship between Mv of chitosan and dose**

Radiation degradation reaction for chitosan in solution may proceed in mild condition due to the contribution of indirect effect from solvent (mainly water). The relationship of oligochitosan fraction and dose in figure 2 showed that oligochitosan DP>8 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 degraded 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 with DP<8 to form strongly electrolytic products which are soluble in above mentioned solvent mixture system doesn’t occur. Further study to fractionate by liquid chromatograph technique to determine the index of MW distribution of oligochitosan should be still needed.
Recently Qin et al. studied the effect of hydrogen peroxide treatment on the MW and structure of chitosan [23]. Results revealed that H$_2$O$_2$ also caused the scission of chitosan chain and the polydispersity index (Mw/Mn) became narrower with the decrease of chitosan MW. Chitosan with MW more than 50x10$^3$ retained almost the original chemical structure. However further degradation of chitosan with H$_2$O$_2$ to prepare lower MW chitosan or oligochitosan could not be done due to ring-opening oxidation that modified the chemical structure of chitosan [23]. The enzymatic hydrolysis of chitosan for preparation of oligochitosan has been recognized as a promising method [21]. However, in this method the rather low chitosan concentration solution (~3%) was used and further purification of oligochitosan has to be done after degradation process. For those reasons, 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.

**Biological effect**

*Fungicidal effect of irradiated of chitosan*

Effect of irradiated chitosan on growth of various fungi is given in Table I.

| TABLE I. EFFECT OF IRRADIATED CHITOSAN ON GROWTH OF VARIOUS FUNGI |
|--------------------------|--------------------------|--------------------------|--------------------------|
| Fungi                    | Minimum concentration of chitosan \( \mu g/ml \) for suppression of fungi | Non-irradiated | Irradiated* | Enhancement ratio (%) |
|--------------------------|--------------------------|--------------------------|--------------------------|
| Phytophthora cactorum     | 300                      | 250                      | 16.7                     |
| Fusarium oxysporum       | 1150                     | 800                      | 30.4                     |
| Aspergillus awamori      | 400                      | 250                      | 37.5                     |
| Exobasidium vexans       | 1000                     | 550                      | 45.0                     |
| Septoria chrysanthemun   | 700                      | 350                      | 50.0                     |
| Gibberella fujikuroi     | 400                      | 250                      | 37.5                     |
| Setobasidium theae       | 1450                     | 1000                     | 31.0                     |
| Collectotrichum sp.      | 1500                     | 1050                     | 30.0                     |

*chitosan irradiated 75 kGy in solid state
The results in table I indicated that both non-irradiated and irradiated chitosan inhibited the growth of all fungi strains studied, but irradiated chitosan showed higher fungicidal effect than that of non-irradiated one. The change in surface charge of chitosan by irradiation in solid state was small [12], so it was presumed that when chitosan was degraded by irradiation, more effective fragments for anti-fungal activity were produced. Results in table I also revealed that the concentration of chitosan which suppressed the fungi growth was different for various fungi strains. Of the 8 fungi strains studied, *Collectotrichum sp.* showed the highest resistance and *Phytophthora catorum* was the lowest. This phenomenon already observed in the study carried out by Allan and Hagwiger on the fungicidal effect of chitosan on fungi of varying cell wall composition [24].

**TABLE II. EFFECT OF IRRADIATED CHITOSAN** **AGAINST INFECTION OF *E. VESANS* FOR TEA LEAVES**

<table>
<thead>
<tr>
<th>Bed</th>
<th>Infected leave frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
</tr>
<tr>
<td>1</td>
<td>7.8</td>
</tr>
<tr>
<td>2</td>
<td>7.8</td>
</tr>
<tr>
<td>3</td>
<td>7.7</td>
</tr>
</tbody>
</table>

Chitosan solution of 10% (w/v) irradiated 50kGy

The fungus *E. vexans* causes a disease of tea leaves resulting low quality and yield of tea products. Chitosan can be used as safe agent not only against fungal infection but also to increase the quality of tea. Table II showed that the frequency of infected leaves of bed 2 treated with irradiated chitosan was lower compared to that of bed 1 and 3. Thus it is obviously that irradiated chitosan can protect tea leaves against infection of *E. vexans*.

**Phytoalexin (pisatin) induction activity**

Phytoalexins are antimicrobial compounds that are both synthesized by and accumulated in plants after exposure to pathogens [7]. Phytoalexins are absent in healthy plants. The molecules that signal plants to synthesize phytoalexin are called elicitors. Thus radiation degraded polysaccharides such as pectic, chitosan exhibited phytoalexin induction effect that is different due to molecular size (or absorbed dose) [5].

**TABLE III. EFFECT OF PISATIN INDUCTION BY OLIGOCITOSAN**

<table>
<thead>
<tr>
<th>Oligochitosan Conc., ppm</th>
<th>A (absorbance at 309nm)</th>
<th>Pisatin eq., %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.24</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>0.28</td>
<td>117</td>
</tr>
<tr>
<td>100</td>
<td>0.34</td>
<td>142</td>
</tr>
<tr>
<td>500</td>
<td>0.31</td>
<td>129</td>
</tr>
</tbody>
</table>

Table III showed the effect of pisatin induction in peas by treatment of oligochitosan. Results in table III clearly indicated that pisatin; a plant phytoalexin was effectively induced by treatment of oligochitosan. However molecular weight or degree of polymerization (DP) of oligochitosan is most important factor for elicitation effect [9, 25, and 26]. Further study to find out DP that induces phytoalexin most effectively should be carried out.

**Growth-promotion effect for plant**

The results in table IV indicated that oligochitosan with the concentration from 10 to 150ppm in culture medium made the shoot height and root length of *Chrysanthemum* plants increase up to about 20% and 40% respectively compared to the control one.
Finally, the fresh biomass of *Chrysanthemum* plants increased up to 68% at the treatment of 100ppm of oligochitosan. It was clearly an evidence that oligochitosan produced by radiation degradation method showed strongly the growth-promotion effect for plants.

**TABLE IV. EFFECT OF OLIGOCHITOSAN ON THE GROWTH OF *CHRYSANTHEMUM* PLANT IN TISSUE CULTURE**

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Shoot height</th>
<th>Root length</th>
<th>Fresh biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm</td>
<td>%</td>
<td>mm</td>
</tr>
<tr>
<td>0</td>
<td>29.6</td>
<td>100.0</td>
<td>38.6</td>
</tr>
<tr>
<td>10</td>
<td>30.5</td>
<td>102.9</td>
<td>40.2</td>
</tr>
<tr>
<td>30</td>
<td>30.2</td>
<td>108.8</td>
<td>40.7</td>
</tr>
<tr>
<td>50</td>
<td>33.2</td>
<td>112.2</td>
<td>44.1</td>
</tr>
<tr>
<td>100</td>
<td>35.3</td>
<td>119.4</td>
<td>54.2</td>
</tr>
<tr>
<td>150</td>
<td>34.7</td>
<td>117.3</td>
<td>45.4</td>
</tr>
<tr>
<td>200</td>
<td>30.8</td>
<td>104.2</td>
<td>32.3</td>
</tr>
</tbody>
</table>

Chitosan solution of 10% (w/v) irradiated 100kGy

**4. CONCLUSIONS**

Chitosan is degraded by irradiation with radiation degradation yield of about 1.03 (sissions/100eV) in solid state. Oligochitosan DP<8 with 50% mass fraction can be obtained by irradiating 10% (w/v) chitosan solution with gamma Co-60 radiation at dose of 45 kGy for chitosan having initial Mw=60,000. Irradiated chitosan exhibits higher fungicidal effect than that of non-irradiated one. Furthermore, oligochitosan possesses not only protection against infection of diseases but also growth-promotion effect for plants. Based on results obtained it can be considered that radiation technology is promising to produce oligosaccharides particularly oligochitosan used as biotic elicitor to enhance defense responses against diseases and as growth-promoter for plants.

**REFERENCES**


BIBLIOGRAPHY

LIST OF PARTICIPANTS

Ciechanska, D. Institute of Chemical Fibres, Ul. M. Sklodowskiej-Curie 19/27, 90-570 Lodz, Poland

Chmielewski, A.G. International Atomic Energy Agency, Wagramer Strasse 5, P.O. Box 100, 1400 Vienna, Austria

Hien, N.Q. Research and Development Center for Radiation Technology, Truong tre Str., Linh xuan Ward, Thu duc District, Ho Chi Minh City, Vietnam

Machi, S. Japan Atomic Industrial Forum, Inc. (JAIF), Dai-ichi Chojiya Building, 2-13, Shiba-daimon 1-chome, Minato-ku, Tokyo 105-8605, Japan

Nho, Y.Ch. Korea Atomic Energy Research Institute (KAERI), POB 105, Yusong, Daejon 305-600, Republic of Korea

Kume T. Japan Atomic Energy Research Institute (JAERI), Takasaki Radiation Chemistry Research Establishment, 1233 Watanuki, Takasaki, Gunma 370-12, Japan

Sabharwal S. Radiation Technology Development Section, Bhabha Atomic Research Center (BARC), Trombay, Mumbai 400-085, India

Tamada, M. Japan Atomic Energy Research Institute (JAERI), Takasaki Radiation Chemistry Research Establishment, Dpt. of Material Development, 1233 Watanuki, Takasaki, Gunma 370-12, Japan

Yoshii, F. Japan Atomic Energy Research Institute (JAERI), Takasaki Radiation Chemistry Research Establishment (TRCRE), Dpt. of Material Development, 1233 Watanuki, Takasaki, Gunma 370-12, Japan