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IAEA-TECDOC-1745

## **Radiation Processed Materials in Products from Polymers for Agricultural Applications**



**IAEA**

International Atomic Energy Agency

RADIATION PROCESSED MATERIALS  
IN PRODUCTS FROM POLYMERS FOR  
AGRICULTURAL APPLICATIONS

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# RADIATION PROCESSED MATERIALS IN PRODUCTS FROM POLYMERS FOR AGRICULTURAL APPLICATIONS

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© IAEA, 2014  
Printed by the IAEA in Austria  
July 2014

IAEA Library Cataloguing in Publication Data

Radiation processed materials in products from polymers for  
agricultural applications. — Vienna : International Atomic  
Energy Agency, 2014.  
p. ; 30 cm. — (IAEA-TECDOC series, ISSN 1011-4289  
; no. 1745)  
ISBN 978-92-0-106414-1  
Includes bibliographical references.

1. Polymers — Effect of radiation on. 2. Irradiation —  
Industrial applications. 3. Radiation chemistry — Industrial  
applications. I. International Atomic Energy Agency. II. Series.

## FOREWORD

In recent years, the steady decline of global agricultural production and crop yields has raised fears that one day the world may be unable to grow enough food to feed the population adequately. The effects of climate change and population growth have contributed to this grim scenario. Serious problems already exist in some States at both national and local levels, and the current situation may even worsen unless States undertake concerted efforts to address the problem. There is an urgent need to examine technologies that can greatly improve and increase crop production and ensure the preservation of the products until they reach consumers.

Radiation technology has long been recognized as a process that can degrade, graft and crosslink polymers to fabricate novel value added products. Considering the crucial role that radiation technology plays in the production of commodities aimed at increasing crop yields and consequently preserving these crops, the IAEA and its Member States have initiated and supported projects related to the radiation processing of polymers for agricultural applications. Completed projects include the coordinating research project (CRP) Development of Radiation Processed Products of Natural Polymers for Application in Agriculture, Healthcare, Industry and Environment (2008–2012) and RCA RAS/8/109 Supporting Radiation Processing of Polymeric Materials for Agricultural Applications and Environmental Remediation (2009–2012). Currently active projects include the CRP Application of Radiation Technology in the Development of Advanced Packaging Materials for Food Products.

The technical meeting on Radiation Processed Materials in Products from Polymers for Agricultural Applications was held from 8 to 12 July 2013 at the IAEA. The meeting provided a forum for the sharing of practical experiences and lessons learned. This publication is a result of this meeting, and it reviews the recent developments in the use of radiation technologies for the preparation of green products based on polymers for agricultural applications.

The IAEA acknowledges the valuable contributions of all the participants at this technical meeting and the assistance of M.H.O. Sampa (Brazil) and L.V. Abad (Philippines) in the preparation of this publication. The IAEA officer responsible for this publication was A. Safrany of the Division of Physical and Chemical Sciences.

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## CONTENTS

CHAPTER 1. INTRODUCTION .....	1
1.1 Background.....	1
1.2 Objectives of the Technical Meeting .....	2
1.3 Topics discussed .....	2
1.4 Conclusions .....	10
1.5 Recommendations.....	12
CHAPTER 2. STATUS OF R&D ON RADIATION PROCESSING OF NATURAL POLYMERS AT THE NUCLEAR RESEARCH CENTER OF ALGIERS ..	13
<i>M. Mahlous</i>	
2.1 Introduction .....	13
2.2 Radiation chemistry .....	13
2.3 Environmental applications.....	14
2.4 Healthcare applications .....	15
2.5 Agriculture applications .....	17
References .....	17
CHAPTER 3. RADIATION PROCESSING OF MARINE ALGAL POLYSACCHARIDES AS PLANT GROWTH PROMOTERS .....	19
<i>E. Smolko, L. Cerchietti, M. Clozza, E.B. Giardina, F. Villela, M.D. Divo</i>	
3.1 Introduction .....	19
3.2 Material and methods.....	20
3.3 Results and discussion.....	21
3.4 Conclusions .....	24
References .....	24
CHAPTER 4. OVERVIEW OF THE ACTIVITIES ON RADIATION PROCESSING OF POLYMERIC MATERIALS IN BANGLADESH.....	26
<i>M. Haque, N.C. Dafader, S. Sultana, M.Z.I. Mollah, M.M.A. Mandal</i>	
4.1 Introduction .....	26
4.2 Extraction of chitosan from prawn shrimp shell.....	27
4.3 Effect of radiation dose on the molecular weight of chitosan .....	27
4.4 Preparation of oligochitosan.....	28



4.5	Application of oligochitosan as plant growth promoter.....	28
4.6	Works on super water absorbent.....	33
4.7	Achievement through TC project .....	33
4.8	Government contribution .....	33
References	.....	34

CHAPTER 5.	INDUSTRIAL PRODUCTION OF FOOD PLASTIC PACKAGING ANDTHE USE OF IRRADIATION FOR MODIFYING SOME FILM PROPERTIES .....	35
	<i>A.V. Ortiz, E.A.B. Moura</i>	

5.1	Introduction .....	35
5.2	Industrial processes .....	35
5.3	Industrial use of irradiation for plastic packaging .....	39
5.4	An important issue for plastic packaging in direct contact with food products .....	40
5.5	Conclusions .....	41
References	.....	41

CHAPTER 6.	DEVELOPMENT OF NEW ADVANCED COATING AND PACKAGING WITH IRRADIATION TREATMENT .....	43
	<i>AM. Lacroix, S. Salmieri, K. Vu, T. Huq, A. Khanh, P. Takala, N. Sharmin, R. Khan, M.M. Senna, H.M.M. Ibrahim, A. Safrany</i>	

6.1	Introduction .....	43
6.2	Material and methods.....	44
6.3	Results .....	48
6.4	Conclusion.....	50
References	.....	51

CHAPTER 7.	RADIATION PROCESSED MATERIALS IN PRODUCTS FROM POLYMERS FOR AGRICULTURAL APPLICATIONS IN CHINA .....	53
	<i>M. Zhai, G. Wu, L. Xu,</i>	

7.1	Introduction .....	53
7.2	Radiation degradation of chitosan .....	54
7.3	Commercial products of chitosan in China .....	56
7.4	Applications of radiation degraded chitosan .....	56
7.5	Challenges of commercialization.....	58
References	.....	58

CHAPTER 8.	EVALUATION OF RADIATION PROCESSED POLYMERS AS MATERIALS FOR AGRICULTURAL PURPOSES .....	59
	<i>H. Abd-El-Rehim</i>	
8.1	Introduction .....	59
8.2	Experimental.....	60
8.3	Results and discussion.....	61
8.4	Applications of copolymer hydrogels in agricultural purposes.....	68
References	.....	72
CHAPTER 9.	PRODUCTS BASED ON BIO-RESOURCED MATERIALS FOR AGRICULTURE. RADIATION PROCESSED BIODEGRADABLE POLYMERS, PLANT GROWTH PROMOTERS AND SUPERABSORBENT POLYMERS .....	75
	<i>K.A. Dubey, Y.K. Bhardwaj, C.V. Chaudhari, L. Varshney</i>	
9.1	Introduction .....	75
9.2	Material and methods.....	76
9.3	Results and discussion.....	78
9.4	Conclusion.....	83
References	.....	84
CHAPTER 10.	PREPARATION OF LOW MOLECULAR WEIGHT CHITOSAN BY RADIATION AND ITS APPLICATION FOR PLANT GROWTH PROMOTER .....	86
	<i>D. Darwis, T. Puspitasari, D. Iramani, Sri Susilowati, D.D. Pangerteni</i>	
10.1	Introduction .....	86
10.2	Preparation of low molecular weight chitosan .....	87
10.3	Results and discussion.....	90
10.4	Conclusion.....	96
References	.....	97
CHAPTER 11.	EXPLOITING NANOTECHNOLOGY AND RADIATION TECHNOLOGIES TO DEVELOP NEW ECO-SUSTAINABLE NANOMATERIALS FOR FOOD PACKAGING SUITABLE FOR STERILIZATION BY IRRADIATION .....	99
	<i>C. Silvestre, M. Pezzuto, D. Duraccio, A. Marra, S. Cimmino</i>	
11.1	Introduction .....	99
11.2	Experimental.....	100
11.3	Results and discussion.....	101

11.4	Conclusions .....	103
References	.....	104
CHAPTER 12.	APPLICATIONS OF BIOPOLYMERS MODIFIED BY RADIATION PROCESSING..... <i>M. Tamada</i>	105
12.1	Introduction .....	105
12.2	Radiation processing of biopolymer .....	106
12.3	Conclusions .....	113
References	.....	113
CHAPTER 13.	UP-SCALING RADIATION PROCESSED OLIGOCHITOSAN AND ITS APPLICATION IN THE PLANTATION OF RICE..... <i>K. Zaman, K. Hashim, M. Mahmud, N. Yaacob, N. Talip, Abd Rahim Harun</i>	115
13.1	Introduction .....	115
13.2	Experiments.....	116
13.3	Results and discussion.....	116
13.4	Conclusion.....	124
References	.....	125
CHAPTER 14.	INFLUENCE OF IRRADIATED CHITOSAN ON GROWTH AND FLOWER QUALITY OF GLADIOLUS AT DIFFERENT SOWING DATES AND SYNTHESIS OF RADIATION CROSS-LINKED POLY(ACRYLIC ACID) HYDROGEL FOR AGRICULTURE APPLICATIONS .....	127
	<i>U. Habib, N. Ahmed, S. Zahid, T. Yasin</i>	
14.1	Introduction .....	127
14.2	Materials and methods .....	127
14.3	Results .....	128
14.4	Synthesis of radiation cross-linked poly(acrylic acid) hydrogel for agriculture applications .....	130
14.5	Conclusion.....	134
References	.....	134
CHAPTER 15.	ROADMAP TOWARDS REGISTRATION AND TECHNOLOGY TRANSFER OF RADIATION PROCESSED PLANT GROWTH PROMOTERS/ELICITORS: THE PHILIPPINE EXPERIENCE..... <i>L.V. Abad, C.T. Aranilla, G.L. Magsino, C.A. Asis</i>	136

15.1	Introduction .....	136
15.2	Research and development on radiation processed oligochitosan and oligocarrageenan as plant growth promoter/elicitor .....	137
15.3	Research and development gaps on radiation processed oligomers as plant growth promoter/elicitor .....	141
15.4	Philippine regulation for the registration of plant growth promoters/elicitors .....	141
15.5	Philippine roadmap towards commercialization of PGPS .....	142
References .....		143
CHAPTER 16.	STUDIES ON THE RADIATION-CHEMICAL BASIS OF SYNTHESIZING OLIGOSACCHARIDES AND POLYMER MICROGELS, AND ANALYTICAL METHODS FOR THEIR CHARACTERIZATION .....	145
	<i>R. Czechowska-Biskup, S. Kadlubowski, B. Rokita, J.M. Rosiak, P. Ulanski, R. Wach</i>	
16.1	Contributions to the radiation chemistry of polysaccharides .....	145
16.2	Determination of basic physicochemical properties of polysaccharides .....	148
16.3	Radiation synthesized microgels for soil moisture retention .....	150
16.4	Concluding remarks .....	151
References .....		152
CHAPTER 17.	SEALED, COMPACT, ECONOMICAL, LOW ENERGY EBEAM ENIGLES – WELCOME TO THE EBEAM RENAISSANCE.....	156
	<i>I. Bland</i>	
17.1	Introduction .....	156
17.2	Results and discussion.....	160
17.3	Conclusion.....	161
References .....		162
CHAPTER 18.	SUPERABSORBENT PREPARED BY RADIATION INDUCED GRAFT COPOLYMERIZATION OF ACRYLIC ACID ONTO CASSAVA STARCH .....	163
	<i>P. Suwanmala, T. Tangthong, K. Hemvichian</i>	
18.1	Introduction .....	163
18.2	Experimental.....	164
18.3	Results and discussion.....	165
18.4	Conclusion.....	167
References .....		168

CHAPTER 19.	RADIATION SYNTHESIS AND CHARACTERIZATION OF NATURAL AND NATURAL-SYNTHETIC HYBRID SUPER ABSORBENT POLYMERS FOR AGRICULTURAL APPLICATIONS .....	169
	<i>M. Şen, H. Hayrabolu, O. Güven</i>	
19.1	Introduction .....	169
19.2	Synthesis and characterization of the swelling properties of acrylic acid sodium salt/locust bean gum hydrogels .....	170
19.3	Synthesis and characterization of carboxylated locust bean gum hydrogels .....	178
19.4	Conclusions .....	183
References	.....	184
CHAPTER 20.	NEW OPPORTUNITIES FOR EBEAM TECHNOLOGIES IN ONE HEALTH.....	187
	<i>S.D. Pillai</i>	
20.1	Introduction .....	187
20.2	Global distribution and consumption of foods .....	188
20.3	Ionizing radiation technology.....	188
References	.....	194
CHAPTER 21.	RADIATION PROCESSING OF BIO-POLYMERS FOR AGRICULTURE APPLICATIONS.....	196
	<i>Tran-Minh Quynh</i>	
21.1	Introduction .....	196
21.2	Experimental.....	197
21.3	Improving antimicrobial activity of chitosan by gamma radiation for food preservation .....	198
21.4	Preparation of superabsorbent hydrogels based on starch .....	201
21.5	Conclusion.....	203
References	.....	203
LIST OF PARTICIPANTS		205

## Chapter 1

### INTRODUCTION

#### 1.1. BACKGROUND

Agriculture represents the single largest human use of land and water, as people around the world depend on it for their most basic needs. Efforts are being made to increase food productivity through the development of improved crop varieties, improved plant nutrition, integrated pest management, and livestock improvement strategies. While it is important to find ways to make the process of growing food more efficient, it is also imperative to preserve what has already been produced, as it is estimated that about 30–40 % of the total amount of food produced are lost between its points of production and consumption. The need to preserve and protect our environment by providing opportunities to farmers to use less water and land, and to efficiently and effectively utilize fertilizers, herbicides, and pesticides, pose an additional challenge.

The IAEA, through its technical cooperation programmes, coordinated research projects, its pool of consultants, and through its several technical meetings and conferences aims to promote the peaceful use of radiation technologies.

Because of the IAEA's support, green technologies that harness the use of radiation processing of non-toxic and renewable bio-resourced materials have been successfully applied in both developed and developing countries. Likewise, substantial industries based on several mature radiation processing applications are now in place. In the last few years, there has been considerable success in the modification of readily available natural polymers through radiation processing for various agricultural applications. Radiation-degraded polysaccharides, such as alginate, carrageenan and chitosan could be used as a plant-growth promoter, a plant-protector from diseases, and as a natural antioxidant for the preservation of food and allied products. It has been proven that these oligosaccharides inhibit some common plant viruses, fungal diseases and bacterial infection. Numerous field tests have demonstrated an increase in yield of various crops and the reduction of the disease index by more than 50% when compared to control, hence increasing the productivity of crops by more than 10%. Utilizing such natural, non-toxic, non-polluting and biodegradable products instead of the large scale use of chemical fertilizers can significantly benefit the environment.

Radiation-crosslinked water-soluble polymers that absorb huge amounts of water (ten or hundred times their dry weight) can be used as soil conditioners and, in the process, reduce the watering frequency, increase the soil porosity, decrease leaching, and cut the usage of fertilizers. By using such superabsorbents, crop production is not only improved, but the environment is also protected against drought and groundwater pollution.

Radiation techniques are also being successfully utilized in the preparation and production of advanced coating and packaging materials for various food products, which have contributed to a longer shelf life and a decreased incidence of contamination.

While there are numerous success stories, there is still a limited use of radiation technologies in the large scale industrial production of advanced materials and products for agricultural applications. Existing gaps in both research and development, as well as challenges in the up-scaling of the production, have to be addressed.

The IAEA has been continuously supporting Member States in their efforts to address these challenges, and has recently concluded a highly successful coordinated research project (CRP) on the Development of Radiation-Processed Products of Natural Polymers for Application in Agriculture, Healthcare, Industry and Environment. During this particular CRP, the participants were able to develop guidelines for the production and the detailed characterization of radiation-degraded chitosan, and had first-hand experience in the field-scale application of oligosaccharides on various crops. These efforts were complemented by a regional cooperative project on Supporting Radiation Processing of Polymeric Materials for Agricultural Applications and Environmental Remediation (RAS/8/109).

Recently, the IAEA initiated a CRP that focused on the development of new packaging materials based on natural and synthetic polymers by radiation techniques. It also enabled its participants to assess the effects of ionizing radiations (gamma, electrons, and X rays) on commercial and emerging food packaging materials, especially in their use for pre-packaged foods intended for radiation processing. This project also included the development of recyclable, biodegradable, bioactive, and smart packaging and coatings.

The technical meeting on Radiation Processed Materials in Products from Polymers for Agricultural Applications was held 8 - 12 July 2013 at the IAEA in Vienna. This meeting provided a forum for review and discussion of the progress in radiation technologies for the preparation of new materials and products, and the characterization and performance testing of these products for various agricultural applications.

## 1.2. OBJECTIVES OF THE TECHNICAL MEETING

One of the major objectives of the 20 country participants who took part in this Technical Meeting was to review the recent developments as well as revisit global, regional, and national level initiatives for the use of radiation technologies in the preparation of green products based on polymers for agricultural applications.

Focus was given to presenting new concepts and the practical utilization of radiation techniques with the end result of evolving a strategy for the deployment of these technologies. The need for further scientific and technological development in this area has also been recognized. The meeting provided a forum for the sharing of practical experiences and lessons learned in the use of radiation technologies in the preparation of products for various agricultural uses. It also served to strengthen contacts and encourage cooperation between technology providers and end-users.

## 1.3. TOPICS DISCUSSED

The meeting focused on the following topics:

### **1.3.1. Radiation technologies for the preparation of advanced materials and characterization of products based on bio-resourced materials**

In the past 60 years, extensive research and development for the radiation processing of polymers have been conducted, and there have been numerous successful commercialized applications in various industries. During the technical meeting, the participants discussed the results obtained from these recent studies, together with the practical environmental applications of advanced materials prepared by radiation technology. Protocols for the preparation of new polymeric materials for agricultural applications were presented, with the particular emphasis on the emerging applications of new materials prepared through radiation

techniques (oligosaccharides, hydrogels and packaging materials) in plant protection and growth promotion, soil conditioning and food preservation.

Characterization of products based on bio-resourced materials is one of the critical issues in the identification of application areas of these materials. These areas include the determination of: (a) the network structure and effective crosslink density of the hydrogels prepared, (b) their swelling and deswelling properties in water and in the presence of nutrients for soil conditioner applications, and (c) the molecular weight and DD of oligochitosan when used as plant growth promoter. During the recently completed CRP, detailed protocols for the determination of the average molecular weight and the deacetylation degree of chitosan have been enhanced and tested in interlaboratory studies and were made available to the IAEA Member States. Harmonization of MW, DD, dose and dose rate measurement have already been established and now need to be documented and circulated to member countries. Basic and advanced techniques for the characterization of the network structure of super water absorbents and the physical and chemical characterization of oligosaccharides were described in the reports.

### **1.3.2. Plant growth promoters and elicitors**

Agriculture remains to be one of the most important sectors in our global economy. However, the combined adverse effects of climate change and other evolving factors such as population growth have consequently affected much of the world's agricultural production leading to the decline of crop yields in many producing countries. Inevitably, efforts are now focused towards developing technologies that can increase and sustain crop productivity. Plant growth promoters (PGP) have been considered to be one among these technologies. PGPs are organic compounds, either natural or synthetic, that can modify or control one or more specific physiological processes within a plant to accelerate its rate of growth or maturation. These are often referred to as plant hormones. Lately, oligosaccharides have been recognized to possess important functionalities such as their ability to signal molecules in various processes of plant growth and development, and the significant role they play in plant adaptive responses to abiotic and biotic stress.

Oligosaccharides from naturally occurring polysaccharides like chitosan and prepared through conventional methods either by enzymatic or acid/base hydrolysis, are reported to possess novel features such as the promotion of germination, shoot elongation, stimulation of growth, and disease control in plants.

Degradation by radiation processing of polysaccharides has recently gained much attention owing to its technological effectiveness in producing low molecular weight oligomers. Upon irradiation, polysaccharides (e.g. carrageenan, alginate and chitosan) can be degraded in a controlled way to form shorter fragments. As compared to the conventional techniques such as 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.

Through the CRP and RAS projects, Participating Member States carried out several studies that have demonstrated the effectiveness of radiation-modified oligomers from chitosan, alginate and carrageenan as growth promoters and protectors of a variety of crops. These oligomers, when applied to plants in the form of foliar sprays, elicit various kinds of biological and physiological activities, including promotion of plant growth, seed germination, shoot elongation, root growth, flower production, suppression of heavy metal



stress, increase in essential oils, and control of plant diseases. The responses vary, depending on the kind of crops and environmental factors such as location, climate, season, type of soil, etc. A summary of these results are shown in Tables 1–3.

TABLE 1.1 INCREASE IN YIELD OF VARIOUS CROPS TREATED WITH RADIATION MODIFIED OLIGOMERS

Plants	Country	Oligosaccharide used	Experimental Test	Increase in yield (%)
Rice	Bangladesh, Indonesia, Malaysia, Vietnam	Chitosan	Field	3-20
Mungbean	Bangladesh, Philippines	Chitosan, Carrageenan	Pot	30-100
Soybean	Indonesia	Chitosan	Field	38
Bitter Gourd	Indonesia	Chitosan	Field	40
Chili	Thailand, Indonesia	Chitosan	Pot & Field	34-50
Potato	Indonesia	Chitosan	Field	25
Carrot	Indonesia	Chitosan	Field	55
Tomato	Bangladesh, Sri-lanka	Chitosan	Pot	25-56
Strawberry	Bangladesh	Chitosan	Pot	71
Cucumber	China	Chitosan	Field	23
Sugarcane	Vietnam	Chitosan	Field	13
Faba bean	Egypt	Chitosan, Alginate	Field	25-27
<i>Zea maize</i>	Bangladesh, Philippines, Egypt	Chitosan, Alginate Carrageenan	Field	24-40
Wheat	Egypt, India	Alginate, Chitosan	Field	30-38

going: kale, lettuce, spinach, sugar cane, tea, coffee, green pepper, cabbage, cauliflower, barley, Marian plum

TABLE 1.2. INCREASE IN YIELD OF OIL IN PLANTS TREATED WITH RADIATION MODIFIED OLIGOMERS

Plants	Country	Oligosaccharide used	Experimental Test	Increase in yield (%)
Menthol in <i>Mentha arvensis L.</i>	India	Carrageenan	Pot	37
Essential oil in <i>Foeniculum vulgare</i> Mill	India	Carrageenan	Pot	66
Alkaloid from <i>Catharanthus roseus L.</i>	India	Alginate	Pot	15
Essential oil and citral content in Lemon Grass	India	Alginate	Pot	>100
Artemisinin in <i>Artemisia annua L.</i>	India	Alginate	Pot	

TABLE 1. 3. POTENTIAL OF RADIATION MODIFIED OLIGOMERS IN CONTROLLING PLANT DISEASES

Plants	Country	Oligosaccharide used	Experimental Test	Plant diseases
agar wood	Malaysia	chitosan	Field	fungus

rice	Vietnam Malaysia	chitosan	Field	<i>Pyricularia oryzae</i> , <i>Pyricularia grisea</i> and other blast diseases
tomato	Sri-Lanka Egypt	chitosan alginate	Field	leaf blight, bacterial wilt, tobacco mosaic virus, and Grady mold
chili	Indonesia, Thailand	chitosan	Field	anthracnose, aphids
soybean	Indonesia	chitosan	Field	<i>Phakopsora pachyrhizi</i>
rambutan	Sri-lanka	chitosan	Field	powdery mildew
dragon fruit	Sri-lanka	chitosan	Field	<i>Botryosphaeria dothidea</i>
banana	Egypt	alginate	Field	tobacco mosaic virus

The extensive research conducted by the participating countries in demonstrating the efficacy of radiation modified oligomers as plant growth promoter and elicitors led to some success stories. Results of these studies were openly shared to the public through seminars, workshops, demonstrations and exhibits, and this has attracted the interest of several groups including government institutions, farmers and private companies. In some countries like Vietnam, radiation modified oligomers have been commercialized under the trade names Olicide, Gold Rice (chitosan based) and T&D (alginate based). Japan has also commercialized azubio, an oligo-glucosamine-L. Other countries like Indonesia, Malaysia and Thailand are now heading in the direction towards the technology transfer of their products. Meanwhile, it is also quite encouraging to note that in most countries where field trials have been conducted, the local farmers are very eager to obtain these products to increase their crop yield.

The future outlook for the utilization of radiation processed oligomers such as plant bio-stimulants is quite positive, considering the innumerable successful field trials. The readily available raw materials and the ease by which radiation technology can degrade the polysaccharides in a controlled way into oligomers, together with the combination of green materials and green technology can further propel this technology towards the market. From the economic standpoint, cost of technology is seen to be minimal as compared to the expected gains from yield. However, despite this optimistic viewpoint, further concerns still need to be addressed and resolved to effectively guarantee the successful commercialization of these products. These include:

(a) Technical issues on:

- Standardization of the range of molecular weight of oligomers;
- Stability of the oligomers with storage;
- Consistency of the quality of raw materials;
- Long term effect of PGPs on level of micronutrients;
- Systematic R&D to determine the mechanism of action for plant growth / elicitor.

(b) Product registration needs

- Field trials comprising of at least two plant cycles;
- Testing of PGPs for each particular crop;
- Complete characterization of the product.

### (c) Commercialization

- Company dependence on the irradiation centers;
- Differentiation compared to competitor products;
- Features and expected benefits;
- Cost-benefit efficiency.

#### 1.3.3. Super water absorbents (SWA)

Super water absorbents are considered artificial humus. These are hydrogels that can absorb a hundred times the amount of water and are mainly applicable in increasing the available water supply in the soil, especially in arid areas. An SWA amendment to soils reduces the evapotranspiration rate of the plants which, in turn, induces a significantly higher growth rate in plants. SWA can be synthesized by radiation processing of polysaccharides and their derivatives. Some concrete examples are hydrogels from radiation grafted hydrophilic monomers (e.g. acrylic acid) onto cellulose and starch and crosslinked carboxymethylcellulose (CMC), a water soluble polysaccharide derivative. CMCs are crosslinked in paste-like conditions by ionizing radiation. These radiation-processed SWAs lose their original solubility and largely swell in water, imparting their specific functionality as SWA. The resulting SWA does not contain any impurities like catalysis residues which gives a definite advantage over chemically processed SWA. The compositions of radiation processed SWA developed by Member States are listed in Table 1.4.

TABLE 1.4. CURRENT R&D STATUS OF SWA

Country	Composition	Stage
Poland	PVP	R
Turkey	LBG, GG, TG /AacNa, CLBG	R
Japan	CMC	Green house
Malaysia	Sago waste/AAc	R
Indonesia	Starch/AAc	R
Argentina	Alginate/AAc	R
Vietnam	Cassava starch/PVA, PAAm	P
Philippines	CM-carrageenan	R
India	AAc + CMC, carrageenan+AAc	R
Egypt	PAAm/PAAc	R
Pakistan	PAAc	R
Bangladesh	AAM/CMC, AAc/CMC, AAM/AAC	R, P
Thailand	Cassava starch/AAc	P

*R: Research, P: Pot test*

Several R&D procedures to improve the properties of SWA have been undertaken. Network structures such as swelling behavior, mechanical properties, and rheological properties are important parameters for the characterization of SWA. Studies show that the swelling behaviour of SWA is highly affected by pH, ionic strength and temperature. The presence of a

crosslinker (e.g. phenyltriethoxysilane) (PTES) can enhance the swelling properties of acrylic acid-based superabsorbent hydrogel. The rate of water absorption may also be improved by the preparation of microgels and nanogels using reverse emulsion and intramolecular crosslinking by pulse EB. Higher water uptake is also achieved by adding a small fraction (1%) of carrageenan in neutralized acrylic acid (AA). This SWA has a degree of swelling of 800 g/g.

The effectiveness of using radiation-processed SWA on plants has been studied on certain crops. Pot experiments of SWA showed an improvement of water retention and percent germination. Results indicate that the sand mixed with 0.1% wt SWA can maintain the water content two times longer than that of sand without SWA. Percent germination of corn seeds with 0.5% SWA is two times higher than those without SWA. These experimental results indicate that SWA has considerable effects on seed germination and the growth of young plants. Semi field trials on tomato plants using SWA prepared from 5% acrylamide blended with 3% CMC reveal twice the fresh yield of 31 Mg/ha. Field trial of acrylic acid/ CMC based SWA conducted on sorghum indicates that, with the addition of 20 kg/ha of SWA, the crop yield can be increased by almost 18.5% together with an increase in plant height of 8.5%. Equilibrium degree of swelling (EDS) of the acrylic acid/CMC is found to be 460 g/g.

The Thailand Institute of Nuclear Technology (TINT) has shown success in their prepared SWA from radiation-induced grafting copolymerization of acrylic acid onto cassava starch. Application of this SWA increases the survival rate of rubber tree transplants from 20% to 85%. It is now cooperating with a GO and an NGO for the production of these hydrogels for use in young rubber trees that will eventually be transplanted. This move could certainly pave the way for a subsequent technology transfer.

Aside from the utilization of SWA as an effective water retainer, new directions for R & D are now heading towards the production of value-added SWA. Nutrient solutions containing N, P, and K ions may be incorporated in the hydrogels and may act as slow release fertilizers. The use of gum/acrylic acid which has the same swelling range as the commercial sodium acrylic acid for urines is also being explored. This gel has a high possibility for the application of SWA in salty water irrigation system.

The use of radiation modified biodegradable polymers such as SWA presents promising results because it has an advantage over chemically crosslinked SWAs as far as environmental hazards are concerned. Radiation processed SWA can cut carbon emissions in the atmosphere and reduce the environmental burden. In addition, its cost can be much lower. The SWA produced by TINT is priced at about 5–8 USD/kg as compared to the chemically produced imported SWA which is around 15–20 USD/kg. SAP (sodium acrylic-acid polymer) is the most popular commercial SWA used in diapers. This is produced by the polymerization of acrylic-acid with a chemical crosslinker. In Japan, SAP costs 4 000 yen /500g from the book of chemical catalogs. In bulk, a Japanese company sells it only at 150 yen /kg. Biodegradable radiation crosslinked CMC is estimated to cost around 1 000–2 000 yen /kg. The price is expected to be much lower if produced commercially in large amount.

Despite the above mentioned advancements on the development of radiation processed SWA, some gaps still need to be addressed. Among these are the following:

- The need to coordinate and harmonize protocols on the properties and application conditions of SWA (i.e. dosage of SWA, the irrigation period for different agricultural and

horticultural applications, the effect on climate and soil conditions, and the swelling ratio and biodegradation properties of SWA).

- Cost estimation for the production of SWA is needed for technology transfer.

#### **1.3.4. Packaging materials**

It is imperative to increase and protect the world's food supply to meet the growing demands of the increasing global population. However, crop yield is just one aspect of meeting this increased need for food supply. It is equally important to protect what is produced. A significant quantity of harvested crops around the world fails to make it to the consumer. It is estimated that the loss between the points of production and consumption could reach as high as 30–40% of the total amount of food produced. Considerable research and development are being conducted in different Member States to develop and improve new packaging materials and coatings to protect our food.

Ionizing irradiation plays a major role in the development and improvement of packaging polymers as well as in sterilizing packaging materials used in aseptic packaging. Innovative packaging based mainly on natural polymers in conjunction with other technological advanced material modifications (e.g. in nanotechnology) holds a promising future in this field.

#### **Post harvest edible coating for fruits and vegetables**

Irradiated cellulose derivatives, alginate, chitosan are promising materials for the development of natural edible coatings. The use of irradiation to crosslink cellulose derivatives and alginate polymers has several beneficial effects such as improving the shelf life, eliminating pathogens, ensuring the overall quality of ready-to-eat coated vegetables and fruits (i.e. coated pre-cut broccoli and strawberries), and preserving the bioactivity of active compounds immobilized in the polymers during storage. The addition of nanocrystalline cellulose (NCC) in the polymer formulations has a synergistic effect with irradiation and improves the physico-chemical properties of the polymers, which controls the release of active compounds and protects the bioactivity of natural compounds during storage.

The effect of irradiated chitosan as a postharvest preservative edible coating on oranges, mango and bell pepper was studied. Irradiated chitosan coating on these fruits reduces the weight loss and respiration rate during storage, delays and minimizes the changes in ascorbic acid contents, titratable acidity, and preserving the taste and quality of fruits, as compared to untreated fruits.

#### **Heat shrinkable barrier films for food packaging**

Ionizing radiation also plays an important role in the packaging industry, especially in the heat shrinkable barrier film production process. In this case, irradiating the film structure is aimed mostly at the crosslinking of the polyolefin. Generally, the main formulations are polyolefin /EVOH/polyolefin or polyolefin /PVdC /polyolefin, for high oxygen barrier applications like fresh meat and fresh poultry; and polyolefin /PA/polyolefin, for medium-high oxygen barrier applications like cheese. Cross-linked polyolefin can withstand higher stretching rates. The stretched film has a memory effect which goes back to its original dimensions when exposed to a much higher temperature. Non-irradiated crosslinked films may start shrinking slowly if submitted to temperatures over 35 °C. When submitted to temperatures higher than 85 °C, both the irradiated and non-irradiated films will shrink in a few seconds. However, the

shrinking factor in irradiated film is much higher, and this results to a better final packaging presentation especially in odd-shaped products. The use of ionizing radiation for cross-linking polymers is one of the most successful applications used by the packaging industry.

### **Pre-packaged food packaging materials suitable for sterilization by irradiation**

Ionizing radiation using gamma or e-beam rays has been used in the treatment of packaged food. The irradiation process can reduce microbial contamination of food, resulting in its extended shelf life. From a commercial standpoint, food is generally pre-packaged in its final form before irradiation to avoid recontamination.

Radiation can cause physical, mechanical and chemical changes in the packaging materials which could alter some of its important functionalities (e.g. oxygen barrier). Thus, the materials utilized for packaging must present resistance to radiation and must not transfer any radiolytic substances to the stored product.

The use of irradiation technologies for pre-packaged foods requires a clear understanding of some federal regulations governing the doses and types of polymers that are acceptable for use with pre-packaged food. Quite a variety of polymers used for pre-packaged irradiated foods in specific doses have already been approved by the FDA. Developing new food packaging materials and evaluating their ionizing radiation compatibility for use in the market distribution channel has significant commercial potential.

Recent developments in the preparation of radiation-resistant packaging materials include the incorporation of nanomaterials, the use of multilayer food packaging, the exploitation of biobased and compostable materials, and the combination of modified atmosphere packaging (MAP) and food irradiation, among others. Table 1.5 shows on-going researches on the use of these technologies.

TABLE 1.5. R&D OF PRE-PACKAGED FOOD PACKAGING MATERIALS

Technology used	Base materials / ionizing radiation used	Effects
Nanomaterials	Synthetic polymer (iPP) and biopolymer (PLA).	High barrier against gas and good mechanical and processing properties
Multilayer food packaging	EVA/EVOH/EVA, with natural clay/graphene addition into EVOH phase	Reduction of EVOH layer without reduction of oxygen barrier;
Biobased and compostable packaging	Aliphatic-Aromatic Copolyester/Starch Blend Aliphatic-Aromatic Copolyester/Polylactic Acid Blend reinforced with filler from natural resources (vegetal fibres, nut shell particles, egg shell particles, bio-CaCO <sub>3</sub> nanoparticles, green silica nanoparticles, natural clay, metal nanoparticles, graphene)	Improved biodegradability of packaging
Modified atmosphere packaging (MAP) and food irradiation	eBeam processing at very low doses (< 1 kGy)	Reduction of bioburden as compared to control (non-irradiated MAP) on fresh produce e.g. grapes, watermelon, cherry tomatoes, avocado and strawberries.

The technology for the modified atmosphere packaging (MAP) in combination with food irradiation is one of the more promising researches which is already in its pilot stage of production. Overall, objective sensory quality tests such as moisture content, soluble solids or titratable acidity levels on the eBeam MAP treated product samples showed that these quality attributes were unaffected by the eBeam treatment. The significance of combining eBeam technology with MAP is that the eBeam treatment will reduce the bioburden and the MAP conditions will retard the spoilage process. The bioburden reduction by eBeam has the key additional benefit that the 1 kGy eBeam dose will inactivate bacterial and viral pathogens in varying levels and thereby reduce potential infection risks. The ability to extend the shelf life and ensure the microbiological safety of high value fresh produce can open up new large markets especially in the vending machine distribution channels. Vending machine distribution channels are currently valued at approximately \$20 billion in the USA. In addition, the federal and local regulations to remove high calorie vending machine items in schools open up a completely new market for eBeam treated foods. The commercial availability of low energy eBeam and X ray technology opens up exciting opportunities for additional research on polymer grafting and/or modifications.

Novel packaging materials for high-value foods such as fresh produce opens up new challenges for more research in areas such as the incorporation of vaccines and sensor systems for monitoring spoilage in the packaging materials. Packaging materials imprinted with branched 3D networks may be embedded with slow delivery solute systems and food spoilage sensors. Collaborative research among chemical engineers, polymer scientists, eBeam technologists and microbiologists is currently exploring the possibility of incorporating eBeam derived immunomodulators (vaccines) directly into edible food packaging material. The ability to deliver immunomodulators (vaccines) via edible food packaging material allows for the delivery of vaccines to target populations by piggybacking on the supply chain efficiencies of fresh produce. Utilizing the same supply chain could significantly reduce transportation costs. It is also envisioned that membrane bound immunomodulators can lead to novel vaccine delivery systems.

The successful technology transfer of packaging materials as coating, as heat shrinkable barrier films, or as pre-packaged food packaging materials would be given a push while considering the following factors:

- The development of active packaging and coating using bio-based polymers and technologies involving irradiation as sanitary treatment have to be supported as an innovative approach to ensure food safety;
- The need for a data bank under the current CRP on packaging which contains a list of all the materials used, their sources, their molecular characteristics, processing/radiation conditions, and testing conditions for properties evaluation;
- The need for further R&D on new technologies involving nanocomposites to improve the mechanical and barrier properties of biopolymers, and reproducible procedures for preparing homogeneous dispersion of nanoparticles into the polymer matrix.

#### 1.4. CONCLUSIONS

Longstanding efforts by research establishments of Member States which have participated in various IAEA activities related to radiation chemistry and technology have led to significant developments in the field of radiation processed materials in products from polymers for agricultural applications.

Some of these elaborated products have successfully passed large-area field tests. It has been demonstrated beyond any doubt that applying low amounts of some oligosaccharides in the form of foliar sprays to selected industrially and economically important crops significantly increases the crop yields and, in many cases, can elicit plant resistance against pathogens. The balance between profits from higher crop yields and costs of the treatment is definitely positive. Some of these products are in the market and, in some Member States, the capability to manufacture these products on demand already exists.

Super water absorbents for soil conditioners using indigenous biodegradable polysaccharide have been produced, thus reducing the environmental burden and carbon emissions in the atmosphere. SWA enhanced the germination rates and production yield in pot and green house tests in arid conditions. It can assist in the irrigation system to maintain the water content in soil. Cultivation yield can increase in areas in arid condition. The transplantation of rubber trees is a new and promising application field for SWA. Collaboration of agricultural institutes will accelerate the progress in the field test for commercialization. Value-added SWAs that have the function of slow release of N, P, K, essential nutrient metals, and urea have been developed. The supply of SWA samples and mass media such as TV give us the opportunity to meet new customers.

Ionizing radiation has also been shown to be a very useful tool in the manufacturing of modern food packaging materials, including nanocomposites, combining excellent barrier properties and biodegradability. The eBeam treatment of MAP packaging opens up completely new markets for foods and food ingredients and for delivering microbial cells to a variety of industrial and therapeutic applications. Packaging materials imprinted with branched 3D networks provide new opportunities for embedding slow delivery solute systems as well as incorporating next generation food spoilage sensors, etc. Heat shrinkable packaging is considered to be one of the most successful breakthroughs in the plastic packaging field. Cross-linked films based on milk proteins using irradiation have been developed. Grafting and some characterization of grafted natural polymers were successfully obtained. The development of active coating and packaging and in situ tests on fruits, vegetables and meat have permitted the commercialization of some active coatings. Improved and tailored properties of polymer nanocomposites can be achieved only if a good dispersion and distribution scheme is realized in the polymer nanomaterials. Irradiation is a viable industrial process, but its effects and the modifications it can cause in plastic films for food packaging must be carefully assessed, especially because these irradiated materials are supposed to get in direct contact with food products.

The results of field tests for plant growth promoters and superwater absorbents as well as the new packaging materials developed indicate a tremendous potential for the utilization of these products. Nevertheless, great efforts have to be exerted to position these products in the market. This can be effectively done by stressing their advantages over competitor products, their features and expected benefits and cost-benefit efficiency.

TM participants have stressed that the support provided by the IAEA has been vital in achieving these outcomes. Regional courses and CRPs have provided tools for knowledge transfer, the creation of new concepts and the formulation of practical solutions. They also provided tools, guidance and protocols for the determination of basic physicochemical properties of chitosan, and opportunities to test relevant analytical competence by interlaboratory studies within the CRP.



## 1.5. RECOMMENDATIONS

The technical meeting recognized that the ultimate goal for all the R&D undertaken on radiation processed materials in products from polymers for agricultural applications should be to put all these products in the market. Notwithstanding the limitations and difficulties of pushing all these to commercialization stage, the participants recommend the following:

- Before initiating any R&D in the planning and development of new products, the following factors should be seriously considered: the need for a selected product, its possible end-users, desired end-product parameters and the prevailing competitive environment, added value/clear advantages/benefits of the planned product, estimated costs and benefits in comparison with similar existing products and solutions.
- There must be consistency of all chain processes in product development as an important key towards technology transfer. To achieve this, there is a need to establish methods and protocols for analyzing basic physicochemical parameters of the substrates and products, and consistency of quality and supply of materials.
- There must be cooperation between the industry and end-users, starting from the early stages of product development to ensure a smooth transfer of technology. The end-users and general public should be made aware of the developments and potential benefits of the product.
- The need to identify the formal and regulatory requirements regarding the product. Institutions should cooperate with relevant national regulatory bodies.
- Information dissemination on radiation technology is a necessity. The target industry can be provided with relevant background information about radiation technology and include it as part of the option for their technological process. It can be emphasized that there is no need for the company to invest in their own irradiation facility.
- The nuclear research organizations are advised and encouraged to cooperate and work closely with relevant agencies/authorities such as agriculture authorities in the development and commercialization of new products and technologies for the agriculture applications, and to facilitate the acceptance and usage of radiation processed products.
- Pilot scale production and field trials are expensive but necessary in order to demonstrate and prove the technical and economic viability of the projects. Nuclear Research institutes should take the initiative to develop pilot scale plants using their existing facilities and MoU with private entrepreneurs.

Collaboration and cooperation among Member States should continuously be encouraged to facilitate technical assistance in performing characterization of the starting materials and products in laboratories equipped with appropriate equipment.

## Chapter 2

### STATUS OF R&D ON RADIATION PROCESSING OF NATURAL POLYMERS AT THE NUCLEAR RESEARCH CENTRE OF ALGIERS

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#### Abstract

In recent years, considerable success in the modification of natural polymers by irradiation with the purpose of producing properties for specific applications has been recorded. Research groups in many countries have developed new products based on radiation-processed polysaccharides. Some developed products have already been commercialized. These include plant growth promoters and protectors for application in agriculture, wound dressing hydrogels containing radiation-processed polysaccharides for the health sector, and adsorbents of metal ions and organic pollutants for the protection of the environment. In Algeria, although the commercialization stage of radiation-processed polysaccharides has not yet been reached, special attention is being given to research in this area. Several studies related to the effects of ionizing radiation on polysaccharides such as chitosan and sodium alginate have been conducted. Work activities aimed at the development of materials based on natural polymers for medical, environmental and agricultural applications have either been completed or are currently being done. The results of some of these studies are presented below.

#### 2.1. INTRODUCTION

Natural polymers, such as polysaccharides, manifest properties that make them highly sought for, especially in the development of new materials for applications in various areas, like health care, agriculture and environmental protection. The most important among these properties are their non-toxicity, biocompatibility and biodegradability. Along with these unique and highly sought for properties, polysaccharides possess high molecular weights that greatly reduce their solubility and, therefore, limit their applications [2.1]. In addition, each application requires a specific range of molecular weights. On the other hand, other applications such as the adsorption of metal ions in liquid effluents require cross-linked materials. Several methods of reducing the molecular weight have been developed, among which are enzymatic degradation and acid hydrolysis. But radiation processing is still the most appropriate one for both degradation and cross-linking, since it is free of initiators and side products that result in a chemically pure final product [2.2]. It offers a clean and additive-free method for preparation of value-added novel materials [2.3] based on natural polymers. Like the other nuclear research centers involved in the valuation of natural polymers through radiation technology, the Nuclear Research Centre of Algiers has initiated a number of research activities in this area. Research works that have either been completed or are currently in progress are presented in brief in this paper.

#### 2.2. RADIATION CHEMISTRY

##### 2.2.1. Gamma irradiation-aided chitin/chitosan extraction from prawn shells

In this development [2.4], chitin and chitosan were extracted from prawn shells. The influence of a 25 kGy irradiation dose on the deproteinization process was investigated. The deproteinization degree was followed by the Lowry–Folin method. The demineralization degree versus reaction time with 1 N hydrochloric acid solution was followed by atomic absorption spectrometry. Chitin and chitosan obtained were characterized by FTIR spectrometry. The influence of some parameters, such as reaction time, alkaline concentration and temperature, on the deacetylation degree was also investigated. The deacetylation degree was evaluated by

FTIR spectrometry using the bands at  $1320\text{ cm}^{-1}$  and  $1420\text{ cm}^{-1}$ . It was found that the irradiation of the shells at a dose of 25 kGy reduces the time of the deproteination reaction by a factor of three, as compared to the non-irradiated samples.

### **2.2.2. Beneficial effect of gamma irradiation on the deacetylation of chitin to form chitosan**

The main aim of this study was to investigate the effect of gamma irradiation on the N-deacetylation of chitin, and the determination of susceptibility of gamma irradiated chitin to achieve milder deacetylation conditions [2.5].

Chitin from crab shells was irradiated up to 20 kGy, then N-deacetylated in aqueous NaOH solution (40% and 60% w/w) at  $60^{\circ}\text{C}$  and  $100^{\circ}\text{C}$  for 60 min. The degree of N-deacetylation (DD) of non-irradiated and irradiated samples was determined by IR-band ratio method. It was found that the DD of chitosan samples prepared from irradiated chitin samples was higher than the DD of chitosan samples prepared from non-irradiated chitin under similar deacetylation conditions. Even low dose irradiation has been found to make chitin more susceptible to N-deacetylation. The reason for radiation-induced enhancement of N-deacetylation was found to be mostly due to the reduction in the molecular weight of irradiated chitin. Milder N-deacetylation conditions were achieved by low dose gamma irradiation of chitin.

### **2.2.3. Effect of molecular weight on radiation chemical yield of chain scission of gamma-irradiated chitosan in solid state and in aqueous solution**

The purpose of this development [2.6] was to study the effect of initial molecular weight on radiation chemical yield  $G(s)$  of chain scission of  $\gamma$ -irradiated chitosan in solid state and in aqueous solution. Chitosan samples A1, A2 and A3 with molecular weight of 471, 207 and 100 kDa, respectively, produced from squid pen chitin were degraded by gamma rays in the solid state and in aqueous solution with various doses. The molecular weight (MW) changes were monitored by capillary viscometry method and the chemical structure changes were followed by UV analysis. The radiation chemical degradation yield  $G(s)$  and degradation rate values were calculated. The results showed that the degradation of chitosan was faster in solution than in solid state. The chitosan sample A3 with the lowest MW was more sensitive to radiolysis than A1 and A2 samples. Structural changes in irradiated chitosan were revealed by the appearance of absorption peaks of carbonyl groups. The peak intensity was higher in the chitosan A3 sample than in A1 and A2 samples; the oxidative products decreased with the increasing molecular weight of chitosan.

## **2.3. ENVIRONMENTAL APPLICATIONS**

### **2.3.1. Radiation synthesis of chitosan beads grafted with acrylic acid for metal ions sorption**

Chitosan is an effective ion-exchanger, as it contains a large number of amino groups that forms chelates with almost all the metal ions. However, raw chitosan presents some major drawbacks in sorption processes, such as low mechanical properties, low heat resistance, solubility in acidic media, high swelling ratios that limit its use in columns, and limited sorption capacities for some metal ions. To overcome the first four limitations, cross-linking could improve the stability of chitosan, while for the last one, grafting of polymer with functional groups to the chitosan would increase its sorption capacity. Radiation-induced graft polymerization is a convenient technique to introduce desirable properties in polymers.

It has many advantages over other conventional methods, such as chemical and photochemical grafting. For instance, the method is relatively simple and no catalyst or additives are needed to initiate the reaction. In this study [2.7], raw chitosan beads have been suitably modified by cross-linking with glutaraldehyde to increase their resistance to chemical and biological degradation, and then modified by radiation grafting reaction introducing carboxyl groups to increase their sorption capacity. The efficiency of modified and unmodified chitosan beads to adsorb cadmium and lead ions, considered highly toxic, was investigated. Radiation-induced grafting of acrylic acid onto chitosan beads was performed in solution at a dose rate of 20.6 Gy/min by  $^{60}\text{Co}$  gamma rays. The effect of the absorbed dose on grafting yield was investigated. Characterization of the grafted material was performed by FTIR spectroscopy and swelling measurements at different pHs. The grafting yield increased with the dose; it reached 80% at 40 kGy irradiation dose. The removal of Pb and Cd ions from aqueous solutions was investigated with both ungrafted and grafted chitosan beads. The sorption behavior of the sorbents was examined through pH, kinetics and equilibrium measurements. Grafted chitosan beads presented higher sorption capacity for both metal ions than unmodified chitosan beads.

### **2.3.2. Adsorption of dyes and metal ions by acrylamide - co - acrylic acid hydrogels synthesized by gamma radiation**

Among the processes applied in the treatment of wastewater, adsorption has the advantages of being efficient and economically feasible. Great attention has been focused on hydrogels as an adsorbent for the removal of dyes and metal ions from wastewater. The most efficient method for synthesizing hydrogels is the simultaneous free radical copolymerization and cross-linking of monomers in solution. The objectives of the present work [2.8] were to synthesize a hydrogel based on acrylamide and acrylic acid, and to determine its efficiency in removing dyes and metal ions, such as methyl violet (MV), Congo red (CR), and cupric ions, respectively, from aqueous solutions. The monomer solutions prepared at 15/85 mole ratio (AAM/AAC) were irradiated at 8 kGy at a dose rate of 24.5 Gy/min. The swelling kinetics was followed gravimetrically at 25°C and pH ranging from 3–9. Equilibrium adsorption measurements were conducted in solutions containing 50 mg/l of MV and 200 mg/l of RC at initial pH ranging from 3–8. Concentration in the solution was followed by spectrophotometric analyses at 598 nm for MV and 464 nm for RC. Adsorption of  $\text{Cu}^{2+}$  ions by the hydrogel was determined by using aqueous solutions of  $\text{CuSO}_4$  (2000 ppm) at pH ranging from 1–5. The concentrations of the solutions were measured at 810 nm. The results showed that the swelling rate of the hydrogel depended on the pH and the ionic nature of the dye. Diffusion of water and dye solutions within the hydrogel was found to be of non-Fickian character. Contrary to the swelling behavior, the capacity of the hydrogel to adsorb dyes seemed to be higher at weak pHs. The results also showed that adsorption of  $\text{Cu}^{2+}$  increased with pH increasing from 1–4.

## **2.4. HEALTHCARE APPLICATIONS**

### **2.4.1. Synthesis and characterization of hydrogels based on poly(vinyl pyrrolidone)**

Hydrogels have a number of biomedical applications, such as wound care products, dental and ophthalmic materials, drug delivery systems, elements of implants, constituents of hybrid-type organs, as well as stimuli-sensitive systems. This work [2.9] is devoted to the synthesis of hydrogels based on polyvinyl pyrrolidone (PVP) for wound dressings. Among the various methods used in the production of hydrogels, the radiation technique proved to have many advantages, as it is a simple, efficient, clean and environment-friendly process. PVP

hydrogels were cross-linked by using gamma irradiation technique. The effects of the PVP concentration and the absorbed dose on gel content were investigated. An equilibrium swelling study was performed to calculate molecular weight between cross-links of swollen gel using the Flory Rhenner theory. The oxygen effect was studied through the yield of cross-linking  $G_x$  that was deducted from the gel-sol analysis performed with the GelSol 95 program. Systematic analysis of the influence and the concentration of the dose showed that the gel fraction increased together with the increasing dose and PVP concentration. The highest degree of swelling of PVP hydrogels was obtained at doses near the gelation point. The polymer concentration of 7% seemed to be the most adequate to obtain an appreciable gel content, even at low irradiation doses. The cross-linking process is affected by the presence of oxygen in the solution. The microbe penetration test results showed that this hydrogel constitutes a good barrier against bacteria.

#### **2.4.2. Influence of some factors affecting antibacterial activity of pva/chitosan-based hydrogels synthesized by gamma irradiation**

The continuous search for hydrogels with improved specific properties was oriented towards the development of composites from synthetic and natural materials. Polysaccharides are interesting materials that can be explored in combination with synthetic polymers for developing hydrogel systems. The aim of this study [2.10] was to synthesize wound dressing by gamma irradiation from Polyvinyl alcohol (PVA) based hydrogels containing different concentrations of chitosan of two different molecular weights, and to investigate their antibacterial activities. PVA-based hydrogels containing different concentrations of chitosan with molecular weight of 471 and 101 kDa were cross-linked by gamma irradiation at a dose of 25 kGy. The swelling behavior, gel content and morphological structure of the blend were investigated. The antibacterial effect as a function of chitosan content and molecular weight in the hydrogel was investigated against *Escherichia coli* and *Bacillus subtilis*. The results of antibacterial activity tests revealed that chitosan was more effective in inhibiting growth of gram positive bacteria than the gram negative ones. It was also observed that the chitosan content, as well as its molecular weight, had a direct influence on bacterial growth inhibition: the higher the chitosan content in the blend and the higher its initial molecular weight, the larger the inhibition zone diameter. The bacteria growth inhibition was attributed to the diffusion of entrapped chitosan from the hydrogel blend to the culture medium. The PVA/chitosan-based hydrogel could be used as wound dressing material.

#### **2.4.3. Evaluation of healing activity of pva/chitosan hydrogels on deep second degree burn: pharmacological and toxicological tests**

Chitosan possesses many properties that are advantageous for wound healing such as biocompatibility, biodegradability, hemostatic activity, healing acceleration, non-toxicity, adsorption and anti-infection. The most important characteristics of chitosan are the degree of deacetylation (DD) and molecular weight that influence its physico-chemical behavior. The main aim of this study [2.11] was to synthesize PVA hydrogels containing chitosan using gamma irradiation, and to investigate in vivo the influence of chitosan present in their composition on the wound healing process. Hydrogel based on PVA containing 0.25% of chitosan was evaluated as wound dressing material on a rat burn model. Histological analyses, primary irritation index (P.I.I.), and ocular irritation index (O.I.I.) were investigated. The comparative study showed that the wounds treated with PVA/chitosan hydrogel healed on the 9<sup>th</sup> day, while those treated with paraffin gauze dressing and cotton gauze healed on the 16<sup>th</sup> day. Histological analysis showed that new granulation tissue and epithelialization progressed better in wound treated with hydrogel PVA/chitosan. The determined values of

P.I.I. and O.I.I. of the PVA/chitosan hydrogel indicated that it can be considered as non-irritating to the skin. PVA/chitosan hydrogels showed a good healing efficiency that can be exploited in therapeutics and has a beneficial influence on the various wound healing phases.

## 2.5. AGRICULTURE APPLICATIONS

### 2.5.1. Effect of radiation processed sodium alginate on germination rate of barley (*hordeum vulgare*)

Sodium alginate was irradiated up to 150 kGy. Solutions of sodium alginate oligomers obtained were prepared with 20, 50 and 100 ppm concentrations. Seeds of barley (*Hordeum vulgare*) containing 12% of humidity were first disinfected with ethanol (70%) and with sodium hypochlorite solution (2%), then impregnated in different solutions of sodium alginate. Seeds were then wrapped in absorbent paper and put in Petri dishes (25 grains per dish, 4 replicates for each dose and each concentration). After 3 days, the germination rates were calculated. The results are summarized in Table 2.1.

TABLE 2.1. EFFECT OF RADIATION PROCESSED SODIUM ALGINATE ON GERMINATION RATE OF BARLEY (*HORDEUM VULGARE*) AFTER 3 DAYS

Concentration (ppm)	Doses (kGy)						
	0	5	10	25	50	70	150
20	72	72	74	85	100	100	100
50	72	71	73	81	100	100	100
100	72	74	76	88	100	100	100
Control	70						

The control samples (non-treated with sodium alginate) reached the germination rate of 70% after 3 days, while 100% rates were obtained in all samples treated with sodium alginate irradiated from 50 kGy to 150 kGy. In the range of 0 to 25 kGy, the germination rates were from 72% to 88%, depending on the dose and the concentration. But the effect of concentration seems to be less important than that of the dose.

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## Chapter 3

### RADIATION PROCESSING OF MARINE ALGAL POLYSACCHARIDES AS PLANT GROWTH PROMOTERS

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#### Abstract

After having participated in the IAEA Coordinated Research Project (CRP) on the “Development of radiation-processed products of natural polymers for application in agriculture, healthcare, industry and environment” with a project entitled “Assay oligosaccharides as plant growth promoters”, Argentina started mobilizing technical experts from diverse fields to introduce the technology in the country. Agronomers, radiation research scientists, and resource persons from seaweed industries were invited to participate in the project. The project team members consisted of Dr. Mario Clozza and his group from the Faculty of Agronomy of the Buenos Aires University, members of the radiation processing division of the National Atomic Energy Commission of Ezeiza Atomic Centre, and resource persons from the Soriano S.A., a seaweed industry. Under the scope of the CRP, degraded alginic acid from *Macrocystis periferia* and other algae from the coastal areas were used for the study. Despite the fact that Argentina has huge brown algae farms in the Patagonia region, these are not commercially available due to the lack of specialized vessels for harvesting. Soriano S.A. produces good quality carrageenan from red algae, sourced from the northern part of the extended national maritime littoral of more than 2000 km long. The project involved the utilization of radiation-processed polysaccharides such as carrageenan, alginic acid, and chitosan of commercial origins on tomato, lettuce, spinach, and cabbage plants.

#### 3.1. INTRODUCTION

Polysaccharide-based natural polymers and waste agricultural products treated with high radiation doses can play a significant role in agriculture as plant growth promoters and plant stress suppressors. Seaweeds have long been used as additives to soils, mainly in coastal areas where wet or partly dried seaweed are used as fertilizers. The high fibre content of the seaweed acts as a conditioner and the mineral content as fertilizer. In recent times, liquid seaweed extracts have been marketed for use in expensive crops such as vegetables and berry fruits. The presence of auxin-type plant hormones in the extracts have been linked to faster growth and better products.

Various red and brown seaweeds were used to produce three hydrocolloids: agar, alginate and carrageenan. The use of seaweeds as a source of these hydrocolloids dates back to 1658 in Japan when agar was first extracted from red seaweed using hot water. Today, research into their life cycles has led to the development of cultivation industries that supply a major portion of the raw material for hydrocolloid production.

It is reported that the oligosaccharides from a higher plant cell wall induce various kinds of biological activities such as the anti-microbial promotion of plant growth, the suppression of environmental stress, and the synthesis of phytoalexins. Low molecular weight carbohydrates and oligosaccharides are usually prepared either through acid hydrolysis or enzymatic degradation of polysaccharides. Radiation is one of the more effective procedures used in the fragmentation of polysaccharides generated by random scission [3.1].



Carrageenans and alginates easily degrade when subjected to ionizing radiation. Molecular weight obtained is as low as  $8 \times 10^3$  with doses of up to 1000 kGy. When solutions of the irradiated  $\kappa$ - and  $\iota$ -carrageenans are mixed with the growth medium for rice seedling under hydroponics conditions, stimulation of growth is observed. Similarly, growth promotion activity of  $\kappa$ -carrageenan is observed on bok-choi vegetable applied also under hydroponics condition. This oligosaccharide also shows a high growth promoting effect on potato in tissue culture [3.2, 3.3]. Degraded alginate (MW ca.7000) as a growth promoter has a remarkable effect on rice. In peanut plants, dry matter of shoots significantly increases under hydroponic conditions. Foliar spraying of degraded alginate on tea, carrot, cabbage, tomato, grape, and onion plants leads to the increase in productivity of ca.15-60%. [3.4, 3.5].

The radiation-induced degradation technology of polysaccharides is a new and promising application of ionizing radiation. This technology has a drawback, though, since it may not be economical. One way to reduce cost is to degrade polysaccharides at low irradiation doses. Kume [3.1] observes that the irradiation of alginate in liquid state (4% solution) increases degradation by about ten times as compared to that in solid state. However, if the practical utilization of radiation technology to produce degraded alginate is considered, then irradiation in solid state is better than in liquid state.

Another way to lower the cost is to irradiate the polysaccharide in the presence of some additives such as potassium per-sulphate, ammonium per-sulphate, or  $H_2O_2$ . Abel-Rehim et al, [3.6] shows that the irradiation of alginate in the presence of ammonium per-sulphate at moderate doses results in an increasing bean plant growth when sprayed with 100 ppm of sodium alginate.

In horticultural crops, the production of good quality seedlings is the most important stage. In the preliminary phase of this study, different polysaccharide treatments, radiation doses, and concentrations were studied to determine their effects on the quality of the horticultural seedlings. These were then evaluated through leaf and root development, shoot-root ratio, and photosynthetic activity in lettuce, spinach, cabbage, and tomato plants. Experiments were conducted under greenhouse conditions and soilless culture. Effects on the application of radiation-degraded polysaccharides have been analyzed through the growth and development of the plants. The main expected output from this study is an increase in dry matter through increased biochemical and physiological functions in plants [3.7].

## 3.2. MATERIAL AND METHODS

### 3.2.1. Experiments

Experiments were carried out at the Faculty of Agriculture, (34°45'S, 60°31'W), under a greenhouse N-S oriented that has been improved for seedlings production. The growth and development of seedlings of lettuce, tomato, and cabbage which were foliar sprayed with different irradiated polysaccharides were compared with untreated control plots. Plants were obtained in plugs and grown in soilless culture in order to control the presence of all essential nutrients, using a "perlita" and Sphagnum peat (50% v/v) substrate and a nutrient solution (Hoagland). The spray solutions which were prepared from solid polysaccharides just before application were applied twice a week. At the end of the experiments, seedlings and plants were harvested. The dry weight (ventilated oven, 60°C for a week) of leaves (including petioles), stems, and roots were measured and the shoot/root ratio was calculated. Data were analyzed by using the statistical software InfoStat and Duncan test.

#### 3.2.1.1. High dose experiment

Alginate degraded through ionizing radiation was utilized as a plant growth promoter and for biocide property studies. Non-irradiated, irradiated alginate solutions (irradiated in solid at 500, 750 and 1000 kGy), and a control (water sprayed) were applied on spinach (*Spinacia oleracea* L.), cabbage (*Brassica oleracea* L.), and tomato (*Solanum lycopersicum* L.). Polysaccharide solutions for foliar spraying were prepared in 20 and 100 ppm concentrations. Aerial biomass, shoot/root ratio, and Chlorophyll (SPAD-502 meter), Polyphenol (Folin-Ciocalteu reactive) and Anthocyanin (differential pH method) contents were measured [3.8].

#### 3.2.1.2. Low dose experiment

Radiation-processed alginate, carrageenan, and chitosan were used as plant growth promoters in cabbage, tomato and lettuce (*Lactuca sativa* L.). Polysaccharides were gamma irradiated in solid form at doses of 50, 100 and 300 kGy. Aqueous solutions of 100 ppm concentration were applied to plants through foliar spraying. To study the possible vegetal growing compensation effect under nutrient stress conditions, plants were provided with different nutrients containing nitrogen (N200: 200 ppm N; N50: 50 ppm N). Chlorophyll leaf content was estimated using a SPAD-502 meter [3.7].

#### 3.2.1.3. Polysaccharides irradiation

Sodium alginate (Bernal and Sigma),  $\kappa$ -carrageenan 200 (Soriano S.A. Argentina), and chitosan from shrimp shell CH-091504, DDA 83.2% (Federal Laboratories Corp., USA) were used in the experiments. These polysaccharides were irradiated in air in solid state (powder form) with gamma rays using the  $^{60}\text{Co}$  facility of the semi-industrial irradiation plant of CNEA (Ezeiza Atomic Centre) and the private Industrial Irradiation Plant Ionics S.A. (Don Torcuato, Buenos Aires Province). Doses were measured using potassium dichromate dosimeters at CNEA and with Harwell Red Perspex dosimeters films at Ionics S.A. Irradiation took place in air at normal temperatures and at a dose rate of 10 kGy/h. Viscosity molecular weight determination of alginate and carrageenan were done following the protocol obtained in the interlaboratory study organized within the framework of the IAEA Coordinated Research Project (CRP) on “Developments of radiation-processed products of natural polymers for application in agriculture, healthcare, industry and environment”.

### 3.3. RESULTS AND DISCUSSION

#### 3.3.1. Biomass partitioning and physiological response

Tables 3.1–3.3 show the effect of the application of different concentrations of alginate on the aerial biomass and shoot-root ratio, and the chlorophyll, polyphenol and anthocyanin contents in spinach, cabbage and tomato. Results indicate varying effects depending on the radiation dose and spray concentration.

TABLE 3.1. AERIAL BIOMASS, SHOOT-ROOT RATIO AND CHLOROPHYLL, POLYPHENOL AND ANTHOCYANIN CONTENTS IN SPINACH WITH DIFFERENT CONCENTRATIONS OF ALGINATE

SPINACH	Aerial biomass (mg)		Shoot-root ratio		Chlorophyll content ( $\mu\text{g}/\text{cm}^2$ )		Polyphenol (mg/100g Fresh weight)		Anthocyanin* (mg/100mg Fresh weight)	
Control	36 c		2.60 c		10.67 b		0.34 a		0.30 a	
kGy	20 ppm	100 ppm	20 ppm	100 ppm	20 ppm	100 ppm	20 ppm	100 ppm	20 ppm	100 ppm
0	127 a <sup>z</sup>	86 b	3.93 ab	4.32 a	11.20 a	10.32 b	0.20 b	0.17 b	0.20 a	0.43 a
500	136 a	88 b	5.02 a	3.28 b	11.49 a	10.51 b	0.16 b	0.11 b	0.23 a	2.53 a
750	120 a	80 b	5.11 a	4.70 a	11.45 a	10.79 ab	0.14 b	0.12 b	0.13 a	1.23 a
1000	124 a	90 b	1.68 d	4.97 a	10.68 b	9.66 c	0.16 b	0.20 b	0.00 a	2.77 a

\* Anthocyanin (mg/100 mg)  $\times 10^{-2}$  fresh weight.

<sup>z</sup> Values within each column with different letters differ significantly at P=0,05 by Duncan's test

TABLE 3.2. AERIAL BIOMASS, SHOOT-ROOT RATIO AND CHLOROPHYLL, POLYPHENOL AND ANTHOCYANIN CONTENTS IN CABBAGE WITH DIFFERENT CONCENTRATIONS OF ALGINATE SPRAY

CABBAGE	Aerial biomass (mg)		Shoot-root ratio		Chlorophyll content ( $\mu\text{g}/\text{cm}^2$ )		Polyphenol (mg/100g Fresh weight)		Anthocyanin* (mg/100mg Fresh weight)	
Control	448 b		2.34 d		16.37 b		0.20 a		0.40 ab	
kGy	20 ppm	100 ppm	20 ppm	100 ppm	20 ppm	100 ppm	20 ppm	100 ppm	20 ppm	100 ppm
0	536 a <sup>z</sup>	447 b	2.80 d	4.89 b	17.74 a	16.67 b	0.21 a	0.26 a	0.00 b	0.03 b
500	512 a	456 b	3.42 c	6.12 a	17.48 a	16.99 b	0.21 a	0.18 a	0.07 b	0.63 ab
750	534 a	452 b	2.35 d	4.34 b	17.87 a	16.49 b	0.22 a	0.17 a	0.27 ab	0.73 ab
1000	562 a	434 b	2.63 d	4.21 b	17.86 a	16.53 b	0.22 a	0.27 a	0.57 a	1.93 a

\* Anthocyanin (mg/100 mg)  $\times 10^{-2}$  fresh weight

<sup>z</sup> Values within each column with different letters differ significantly at P=0,05 by Duncan's test

TABLE 3.3. AERIAL BIOMASS, SHOOT-ROOT RATIO AND CHLOROPHYLL, POLYPHENOL AND ANTHOCYANIN CONTENTS IN TOMATO WITH DIFFERENT CONCENTRATIONS OF ALGINATE SPRAY

TOMATO	Aerial biomass (mg)		Shoot-root ratio		Chlorophyll content ( $\mu\text{g}/\text{cm}^2$ )		Polyphenol (mg/100g Fresh weight)		Anthocyanin* (mg/100mg Fresh weight)	
Control	290 c		3.83 a		8.81 a		0.34 a		0.17 a	
kGy	20 ppm	100 ppm	20 ppm	100 ppm	20 ppm	100 ppm	20 ppm	100 ppm	20 ppm	100 ppm
0	397 a <sup>z</sup>	341 b	2.28 c	3.51 a	8.71 a	8.74 a	0.34 a	0.39 a	0.27 a	0.13 a
500	412 a	278 c	2.45 c	3.80 a	8.79 a	8.71 a	0.39 a	0.39 a	0.17 a	0.33 a
750	392 a	378 a	2.30 c	3.55 a	8.69 a	8.89 a	0.34 a	0.30 a	0.00 a	0.43 a
1000	388 a	368 ab	2.09 c	3.19 b	8.63 a	8.63 a	0.37 a	0.46 a	0.23 a	0.10 a

\* Anthocyanin (mg/100 mg)  $\times 10^{-2}$  fresh weight

<sup>z</sup> Values within each column with different letters differ significantly at P=0,05 by Duncan's test

For aerial biomass, shoot-root ratio and chlorophyll content, treatments are considered effective when statistical values are higher than the control, as these parameters indicate better growth and yield performance. When the polyphenol and anthocyanin values are lower than controls, treatments are considered positive as these help in improving plant protection under environmental stress and, in the process, limiting carbohydrates production which results in plant growth.

Application of alginate in any dose and concentration on spinach increased the aerial biomass and shoot-root ratio. The chlorophyll content was higher at a lower concentration of 20 ppm. The polyphenol content decreased with the application of alginate as compared to the control. No effect was observed in anthocyanin content.

For cabbage, both the aerial biomass and chlorophyll content increased at lower concentration of 20 ppm but shoot-root ratio increased at 100 ppm, independent of the radiation doses. No effects were observed in its polyphenol and anthocyanin contents.

Tomato presented a higher aerial biomass at 20 ppm concentration only but had a lower shoot-root ratio. This indicates a proportionally greater radicular biomass than the other treatments. No differences were observed in its chlorophyll, polyphenols, and anthocyanins contents as compared to the control.

### **3.3.2. Physiological response**

For low dose experiments, chlorophyll contents varied among lettuce, cabbage and tomato plants. This variation depended on the type of polysaccharide added, radiation dose, and nitrogen availability (Table 3.4).

For high dose experiments (40 plants/crop), the percentages of the number of plants with an aerial biomass higher than that of the control were 80, 70 and 35% for spinach, cabbage and tomato, respectively. Results were higher when 20 ppm alginate solutions with an over-all percentage of 66.6%, and 95 %, 75 % and 35% were applied to spinach, tomato, and cabbage plants. At radiation doses of 0, 500, 750 and 1000 kGy, number percentages were 63.3, 60, 70 and 50%, respectively.

At low dose experiments, the application of polysaccharides on plants showed varying chlorophyll contents which depended on the crop and N availability (Table 3.5). All plants showed higher chlorophyll content at 200 ppm N than at 50 ppm N. The addition of 200 ppm nitrogen increased the chlorophyll content by around 10% for cabbage and tomato plants for all treatments. Increase in chlorophyll content for lettuce plants was observed only when it was applied with chitosan.

In general, a radiation dose of 100 kGy showed the best results, with 77.7% of the plants having higher chlorophyll content than the control, and specifically 83, 66.6 and 54.1% for tomato, lettuce and cabbage plants, respectively. The addition of chitosan, alginate, and carrageenan increased the number percentages by 83.3 %, 66.6 % and 58.3%, respectively.

TABLE 3.4. CHLOROPHYLL CONTENT IN LETTUCE, CABBAGE AND TOMATO

CHLOROPHYLL CONTENT ( $\mu\text{g cm}^{-2}$ )						
Nitrogen Availability	Dose	LETTUCE		CABBAGE		TOMATO
	(kGy)	N200	N50	N200	N50	N200 N50
Control		4.90 b	3.67 a	17.35 c	15.34 b	8.42 b 7.50 a
Alginate	0	4.67 bc	3.42 b	19.15 a	15.56 b	9.34 a 7.84 a
	50	4.80 b	3.69 a	18.75 b	14.76 c	9.01 a 7.49 a
	100	5.05 ab	3.73 a	19.46 a	15.64 b	9.40 a 7.64 a
	300	4.80 b	3.48 b	19.51 a	17.21 a	9.03 a 7.42 a
Carrageenan	0	4.40 c	3.80 a	18.79 b	17.04 a	9.44 a 7.16 a
	50	4.94 b	3.54 ab	19.87 a	15.79 b	9.18 a 7.39 a
	100	4.94 b	3.58 ab	18.98 b	14.81 c	9.37 a 7.22 a
	300	5.26 a	3.42 b	18.93 b	15.19 b	9.13 a 7.51 a
Chitosan	0	5.05 ab	3.64 a	19.77 a	15.16 b	8.98 a 7.58 a
	50	5.50 a	3.79 a	18.43 b	14.73 c	9.38 a 7.45 a
	100	5.80 a	3.73 a	19.06 ab	15.28 b	9.32 a 7.63 a
	300	5.57 a	3.88 a	19.32 a	15.63 b	9.11 a 7.59 a

<sup>z</sup> Values within each column with different letters differ significantly at  $P=0.05$  by Duncan's test

TABLE 3.5. INCREASE IN CHLOROPHYLL IN PLANTS AT VARYING NITROGEN AVAILABILITY

Nitrogen availability	% Chlorophyll Increase		
mg/L	Cabbage	Tomato	Lettuce
200	10.48	9.50	3.46
50	1.50	-0.13	-0.81

### 3.4. CONCLUSIONS

Irradiated alginates applied through foliar spray at low nitrogen availability (N50) did not improve the chlorophyll content in lettuce, cabbage, and tomato. Under N200 treatments, alginate application increased the chlorophyll content in cabbage and tomato, regardless of the radiation dose. Carrageenan applications also showed the same response. Chitosan exhibited the best results with an increased chlorophyll content under low or high availability of nitrogen.

Increase in aerial biomass (377%) and decrease in anthocyanin (212%) and polyphenol (130%) contents were obtained in spinach treated with a solution of irradiated alginate (500 kGy) containing 20 ppm N. Likewise, aerial biomass increased in cabbage (125%) and in tomato (142%) but with no significant changes in their physiological parameters.

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## Chapter 4

### OVERVIEW OF THE ACTIVITIES ON RADIATION PROCESSING OF POLYMERIC MATERIALS IN BANGLADESH

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Bangladesh

#### Abstract

Natural polymers processed through radiation are used in agriculture, health care, and environmental protection. The process of chitosan extraction from prawn/shrimp shells has been established. The method used in determining the molecular weight of chitosan had previously been standardized during the harmonization program of protocol for molecular weight determination organized by IAEA in cooperation with Poland and UK during which Bangladesh was a participant. Increased yield of several crops over the control was achieved using chitosan as plant growth promoter: up to 13% for rice when chitosan with 50 ppm concentration was applied two times, up to 56% for summer tomato when chitosan with a concentration of 75 ppm was sprayed one time, up to 30% for mungbean when 50 ppm chitosan was applied two times, and up to 24% for maize when 100 ppm chitosan was applied three times. Field tests covering three seasons to confirm any increase in the yield of tomato and maize are still being conducted. Funding from the CRP helped carry out these activities to a great extent. Through the IAEA TC project, radiation processing laboratories were enhanced with new equipment, chemicals/monomers/polymers, and the capability of staff members was improved through additional training, expert assistance and scientific visits. One PhD student and several MSc students were able to complete their theses and had them published. Super Water Absorbent (SWA) hydrogel was prepared using 5% Acrylamide blended with 3% carboxymethyl cellulose (CMC) and irradiating at 25 kGy. It was applied on tomato plants as soil conditioner and positive results were obtained. Government funding aided in the establishment of a pilot plant that eventually became a full-fledged institute for radiation processing.

#### 4.1. INTRODUCTION

Bangladesh is crisscrossed with many rivers where a lot of shrimps and prawns abound. Moreover, Bangladesh has extensive coastal lines that stretch to about 750 kilometers. There is an increase in shrimp hatcheries all over the country and shrimps/prawns are now being exported to various parts of the world, notably Europe and North America. But shrimp/prawn shells remain unutilized and are causing a threat to the environment as unprocessed garbage. Chitin (Fig. 4.1) is extracted from waste shells and is converted to chitosan (Fig. 4.2) through further treatment [4.1–4.2]. There is a great demand for chitosan in the pharmaceutical and agricultural fields for its excellent biodegradability [4.3], biocompatibility [4.4–4.5] and bioactive properties. Chitosan films and membranes are used in dialysis, contact lenses, dressings [4.6–4.7] and in the encapsulation of mammal cells, including cell cultures. Chitosan sponges, when used as dressings, stop the bleeding of mucous membranes.

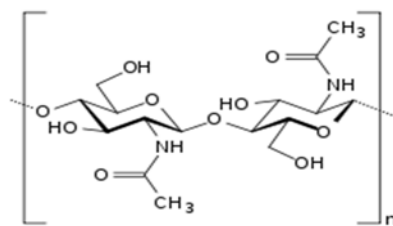


FIG. 4.1. Structure of Chitin.

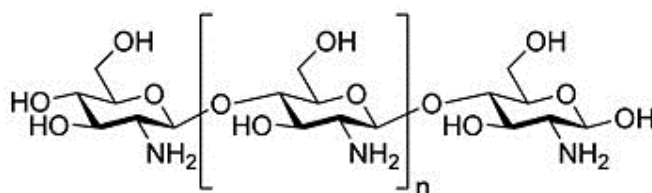


FIG. 4.2. Structure of Chitosan.

Bangladesh was one of the participants in a Coordinated Research Project (CRP) entitled, “Modification of Natural Polymers by Radiation Processing for Value Added Products”, contract No. 14769 as part of the CRP on “Development of Radiation-processed Products of Natural Polymers for Application in Agriculture, Healthcare, Industry and Environment”, during the period 2009-2011. Under this CRP, extraction of chitosan from prawn/shrimp shell, its characterization by measuring molecular weight, production of oligochitosan by applying radiation, utilization of oligochitosan as plant growth promoter were explored.

#### 4.2. EXTRACTION OF CHITOSAN FROM PRAWN/SHRIMP SHELL

Prawn shells were collected from the local market and cleaned by washing with water. These were then dried in a vacuum oven at 50°C. Five hundred g of the dry raw shells were treated with 4% sodium hydroxide for deproteinization. The deproteinized prawn shells were washed with water to remove the alkali. It was then treated with 3.5% hydrochloric acid to produce chitin. The chitin was treated with 20M sodium hydroxide to produce chitosan.

#### 4.3. EFFECT OF RADIATION DOSE ON THE MOLECULAR WEIGHT OF CHITOSAN

Chitosan was heated in an oven at 60°C and weighed to constant weight. Dry chitosan weighing 0.01–0.3g was dissolved in 100 ml 0.2 M acetic acid/0.15 M ammonium acetate (pH ~4.8). The viscosity of the chitosan solution was measured using the Ostwald Viscometer in a thermostatic water bath at ~30°C. A 5 ml chitosan solution was charged in viscometer and flow time was measured with a stop- watch; viscosity was determined using the flow time. The molecular weight of chitosan was measured using the equation:

$$M = (\eta / K)^{1/\alpha} \quad (1)$$

where  $\eta$  is intrinsic viscosity,  $K=9.66 \times 10^{-5}$  L/g and  $\alpha= 0.742$

Chitosan in solid and solution forms were irradiated at different radiation doses at room temperature. The molecular weight and viscosity of irradiated chitosan with different radiation doses are shown in Table 4.1 and Table 4.2. Both its molecular weight and viscosity



decreased with increased radiation dose. The decreasing trend of molecular weight and viscosity of irradiated chitosan in solution form were faster than those of irradiated chitosan in solid state. In the case of irradiation of chitosan in dry form, the degradation is achieved from a direct effect of irradiation.

TABLE 4.1. EFFECT OF RADIATION DOSE ON VISCOSITY AND MOLECULAR WEIGHT OF CHITOSAN IN SOLUTION FORM

Radiation dose [Gy]	Intrinsic viscosity [dm <sup>3</sup> /L]	Molecular weight
0	0.779	1.84 x 10 <sup>5</sup>
50	0.615	1.35 x 10 <sup>5</sup>
100	0.521	1.06 x 10 <sup>5</sup>
200	0.385	0.72 x 10 <sup>5</sup>
300	0.272	0.45 x 10 <sup>5</sup>

TABLE 4.2. EFFECT OF RADIATION DOSE ON VISCOSITY AND MOLECULAR WEIGHT OF CHITOSAN IN SOLID FORM

Radiation dose [kGy]	Intrinsic viscosity [dm <sup>3</sup> /L]	Molecular weight
0	0.779	1.84 x 10 <sup>5</sup>
1	0.767	1.80 x 10 <sup>5</sup>
5	0.578	1.24 x 10 <sup>5</sup>
10	0.412	0.78 x 10 <sup>5</sup>
20	0.297	0.502 x 10 <sup>5</sup>
25	0.297	0.502 x 10 <sup>5</sup>
30	0.273	0.448 x 10 <sup>5</sup>

#### 4.4. PREPARATION OF OLIGOCHITOSAN

Chitosan was heated in an oven at 60°C and weighed up to constant weight. It was then irradiated with a dose of 25 kGy at room temperature to reduce the molecular weight of chitosan. The irradiated solid chitosan was dissolved in lactic acid, and hydrogen peroxide (0.3%) was added. The chitosan solution was again irradiated at 10 kGy radiation dose. The pH of the chitosan was controlled at ~5 by adding sodium hydroxide solution. The molecular weight of the obtained oligochitosan was ~7 000.

#### 4.5. APPLICATION OF OLIGOCHITOSAN AS PLANT GROWTH PROMOTER

##### 4.5.1. Effect of different levels of chitosan application on yield of rice

Chitosan was applied on two rice varieties, BRRI dhan-34 and Kalozirain in Aman season (harvesting period: November - December) and on one variety, Iratom-24 in Boro season (harvesting period: April - May). In this report, the field test for Iratom-24 in Boro season is highlighted.

The experiment was conducted from December 2010 to May 2011 in the fields of the Bangladesh Institute of Nuclear Agriculture, but in a different place called Jamalpur, about 66 km away from where the first study was conducted. Its aim was to investigate the response of grain yield to different concentrations of chitosan under three growth stages. Iratom-24 was used as planting material. The experiment comprised of (a) the spraying of chitosan during the three growth stages of the vegetative, booting, and heading phases: (i) once at the tillering stage only (T<sub>1</sub>) 30 days after transplanting (DAT); (ii) twice both at tillering and booting stages (T<sub>2</sub>) 30 and 60 DAT; and (iii) three times at tillering, booting and heading stages (T<sub>3</sub>) 30, 60 and 90 DAT; and (b) four levels of Chitosan concentrations that are 0, 25, 50 and 75 ppm. The experiment was laid out in two factors randomized complete block design with three replications. The unit plot size was 4m×3m. Plant spacing was 20cm×20cm. Fertilizers

such as urea, triple super phosphate (TSP), muriate of potash (MP), and gypsum were applied at 185, 150, 80 and 40 kg ha<sup>-1</sup>, respectively. Urea was applied in three equal portions at 10, 30 and 50 DAT, while other fertilizers were applied as basal dose during the final land preparation. Other cultural practices such as weeding and pest control were done as needed for normal plant growth and development.

Results in Table 4.3 showed that grain yield is highest (7.0 t ha<sup>-1</sup>) when chitosan was sprayed at 50 ppm at the two growth stages of tillering and booting, and also showed good dry matter partitioning to economic yield (51.5%). The highest yield increment over control is observed in the treatment combination of T<sub>2</sub>×50 ppm chitosan (13.3%). Thus, chitosan may be applied at 50 ppm at two growth stages of tillering and booting for increased grain yield of boro rice.

TABLE 4.3. INTERACTION OF APPLICATION FREQUENCY AND CONCENTRATION OF CHITOSAN APPLICATION ON YIELD ATTRIBUTES AND GRAIN YIELD OF BORO RICE CV. IRATOM-24

Treatments	Plant height (cm)	Effective tiller hill <sup>-1</sup> (no.)	Filled grains panicle <sup>-1</sup> (no.)	1000-grain weight (g)	Grain yield (t ha <sup>-1</sup> )	Straw yield (t ha <sup>-1</sup> )	Harvest index (%)	Yield increase/decrease over control (%)
T <sub>1</sub> × 0 ppm	71.6	11.20 b	95.0 cd	27.3 c	5.95 bc	6.83 ab	46.5 bc	---
25 ppm	72.5	10.40 b	105.0 ab	29.0 bc	6.42 ab	6.65 abc	49.1 abc	+ 7.90
50 ppm	73.5	10.70 b	103.0 ab	28.6 bc	6.42 ab	6.60 abc	49.3 abc	+ 7.90
75 ppm	72.4	11.20 b	90.0 d	29.9 ab	5.54 c	6.30 c	46.8 bc	- 6.89
T <sub>2</sub> × 0 ppm	73.9	10.53 b	91.0 cd	27.1 c	6.18 b	6.13 c	50.2 ab	---
25 ppm	74.7	10.40 b	94.0 cd	30.9 a	6.43 ab	6.15 c	51.1 a	+ 4.00
50 ppm	74.4	11.07 b	106.6 a	28.4 bc	7.00 a	6.60 abc	51.5 a	+ 13.3
75 ppm	74.9	10.93 b	98.0 bc	30.0 ab	6.53 ab	6.29 c	50.9 a	+5.66
T <sub>3</sub> × 0 ppm	73.5	12.30 a	90.0 d	28.2 bc	5.74 c	6.52 bc	45.9 c	---
25 ppm	73.7	12.37 a	108.0 a	27.7 c	6.42 ab	6.52 bc	49.6 abc	+ 11.8
50 ppm	73.4	12.27 a	95.0 cd	27.5 c	6.08 bc	7.05 a	46.3 bc	+ 5.92
75 ppm	73.2	10.73 b	103.0 ab	28.2 bc	6.30 b	6.60 abc	48.8 abc	+ 9.75
F-test	NS	**	**	*	*	*	*	
CV (%)	2.91	4.76	5.40	3.57	5.44	4.16	4.49	

In a column, figures bearing same letter (s) do not differ significantly at  $P \leq 0.05$  by DMRT; DAT = Days after transplanting; Ns = Not significant; \* and \*\* indicate significance at 5% and 1% level of probability, respectively.

#### 4.5.2. Effect of different levels of chitosan application on yield of tomato grown in summer season

The experiment was conducted from February to May 2011 at the pot yard of the Bangladesh Institute of Nuclear Agriculture in Mymensingh to investigate the response of different concentrations of chitosan under three growth stages of tomato grown during the summer season. BINA tomato-6 was used as planting material. Five different concentrations of chitosan at 0, 25, 50, 75 and 100 ppm were sprayed at three growth stages of tomato, i.e. chitosan sprayed 0, 25, 50, 75 and 100 ppm; (i) once at its vegetative stage 25 days after transplanting (DAT); (ii) chitosan sprayed twice both at its vegetative and flowering start stages, 25 and 40 DAT, and (iii) chitosan sprayed three times at its vegetative, flowering start and peak flowering stages, 25, 40 and 55 DAT. In control, water was sprayed as per treatment. The pots were filled with 12 kg sandy loam soil. Fertilizers using the fertilizer recommendation guide of the Bangladesh Agriculture Research Council (BARC) [4.8] were applied. The experiment was laid out in a two factor completely randomized design with four replications. Each pot contained one plant and denoted a replication. Twenty five day-old seedlings were transplanted on 24 February, 2011. Other cultural operations like irrigation, weeding, mulching, and pest control were followed as necessary for normal plant growth and development.

The effect of chitosan application on yield attributes and fruit yield at different growth stages was significant. Results revealed that flower and fruit number, reproductive efficiency (RE), and fruit yield decreased with the increasing frequency of chitosan application. This result indicates that one spray at vegetative stage of tomato has a positive effect on fruit yield in tomato. The flower and fruit number, RE, and fruit yield are greater in chitosan-applied plants than control plants. It indicates that flower and fruit number and fruit yield increased with increasing concentration of chitosan of up to 75 ppm. Application of 100 ppm chitosan may already have a negative effect on plant growth and development of fruit yield. The highest fruit yield recorded was at 75 ppm chitosan (614 g/plant). This gave a higher number of fruits/plant with superior RE. In contrast, the lowest fruit yield was obtained in the control (393 g/plant). Yield attributes as well as fruit yield are maximum when chitosan was sprayed at 75 ppm at vegetative stage of tomato during the summer season (Table 4.4).

TABLE 4. 4. INTERACTION OF CHITOSAN APPLICATION STAGES AND CONCENTRATIONS ON YIELD ATTRIBUTES AND FRUIT YIELD OF SUMMER TOMATO CV. BINA TOMATO-6

Frequency concentration (ppm)	and	Flowers plant <sup>-1</sup> (no.)	Fruits plant <sup>-1</sup> (no.)	Single fruit weight (g)	Reproduc-tive efficiency (%)	Fruit yield plant <sup>-1</sup> (g)
Control × T <sub>1</sub>		28.7 bcd	9.00 cd	50.1 ab	31.4 g	451 ef
Control × T <sub>2</sub>		20.0 f	7.00 e	43.3 cde	35.0 efg	303 h
Control × T <sub>3</sub>		25.7 cde	8.50 d	50.0 ab	33.0 fg	425 fg
25 × T <sub>1</sub>		25.2 c-f	11.5b	46.8 a-d	45.5 ab	538 cd
25 × T <sub>2</sub>		28.0 bcd	11.2b	44.2 b-e	40.2 cde	497 de
25 × T <sub>3</sub>		24.5 def	9.50cd	40.9 de	38.8 de	388 g
50 × T <sub>1</sub>		27.3 b-e	13.3a	45.6 a-d	48.7 a	606 ab
50 × T <sub>2</sub>		28.3 bcd	11.3b	48.6 abc	40.0 cde	550 bcd
50 × T <sub>3</sub>		26.7 b-e	8.67 d	51.4 a	32.5 fg	446 efg
75 × T <sub>1</sub>		32.0 ab	14.3 a	44.3 b-e	44.8 abc	636 a
75 × T <sub>2</sub>		34.7 a	14.3 a	44.7 b-e	41.3 bcd	640 a
75 × T <sub>3</sub>		28.7 bcd	11.3 b	50.1 ab	39.5 de	567 bc
100 × T <sub>1</sub>		30.3 abc	11.7 b	43.8 cde	38.5 de	511 cd
100 × T <sub>2</sub>		22.5 ef	8.33 de	39.1 e	37.0 def	325 h
100 × T <sub>3</sub>		25.0 c-f	10.3 bc	42.8 cde	41.2 bcd	440 efg
F-test		**	**	*	**	**
CV (%)		10.49	7.69	6.78	6.98	7.94

In a column, figures bearing same letter (s) do not differ significantly at  $P \leq 0.05$  by DMRT; \* and \*\* indicate significance at 5% and 1% level of probability, respectively.

#### 4.5.3. Effect of different levels of chitosan application on yield of mungbean

The experiment was conducted from February to May 2011 at the pot yard of the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh to investigate the response of different concentrations of chitosan in 2 summer mungbean varieties viz., BINAmung-7 and BINAmung-8. Five different concentrations of Chitosan at 0, 25, 50, 75 and 100 ppm were sprayed twice at two growth stages of mungbean i.e., chitosan with concentrations of 0, 25, 50, 75 and 100 ppm was sprayed at both the vegetative (25 days after sowing) and flowering and fruiting stages (40 days after sowing). The control was only sprayed with water. The pots were filled with 12 kg sandy loam soil. Fertilizers were used as stated in reference [4.8]. The experiment was laid out in two factor completely randomized design with four replications. Each pot contained two plants and denoted a replication. Intercultural operations like irrigation, weeding, mulching, and pest control were followed as necessary for normal plant growth and development.

The effect of chitosan application on yield attributes and yield of mungbean at different concentrations was significant (Table 4.5). Results revealed that plant height, number of pods plant<sup>-1</sup>, seeds pod<sup>-1</sup>, 1000-seed weight as well as seed yield plant<sup>-1</sup> were greater in chitosan-

applied plants than in control plants. Results further revealed that seed yield increased with increasing chitosan concentration till 50 ppm followed by a decline. This result indicates that application of chitosan with concentrations of 75 and 100 ppm may be toxic for plant growth, thereby decreasing seed yield. The highest seed yield (9.27 g/plant) was recorded at 50 ppm chitosan with higher number of pods plant. In contrast, the lowest seed yield was observed in control plants (7.15 g/plant) with lower yield attributes. Yield attributes and seed yield were highest in BINAmung-7 when sprayed with 50 ppm chitosan. The seed yield of BINAmung-8 was highest at concentrations of 50 ppm and 75 ppm chitosan with about 30% higher seed yield over control. Between the two varieties, the seed yield and yield related traits were superior in BINAmung-7 than BINAmung-8.

TABLE 4.5. EFFECT OF FOLIAR APPLICATION OF CHITOSAN ON YIELD ATTRIBUTES AND SEED YIELD OF MUNGBEAN CULTIVARS

Treatments	Plant height (cm)	Pods plant <sup>-1</sup> (no.)	Seeds pod <sup>-1</sup> (no.)	1000-seed weight (g)	Seed yield plant <sup>-1</sup> (g)	Yield increased over control (%)
<b>Variety</b>						
BINAmung-7 (V <sub>1</sub> )	29.8 b	30.5 a	10.7 b	29.71 b	8.74 a	
BINAmung-8 (V <sub>2</sub> )	39.9 a	16.6 b	11.6 a	45.08 a	7.81 b	
F-test	**	**	**	**	**	
<b>Concentration (ppm)</b>						
0	32.1 c	21.1 c	10.8	37.32 ab	7.15 c	--
25	36.6 a	23.9 b	11.2	37.62 a	8.37 b	17.1
50	36.2 a	26.4 a	11.2	37.60 a	9.27 a	29.6
75	35.2 ab	23.3 b	11.2	37.65 a	8.45 b	18.2
100	34.4 b	23.1 b	11.4	36.80 b	8.16 b	14.1
F-test	**	**	NS	*	**	
<b>Interaction of frequency (V) and concentration (ppm)</b>						
V <sub>1</sub> × 0	27.7 e	27.5 c	10.6 cd	29.43 cd	7.74de	---
V <sub>1</sub> × 25	32.2 c	31.8 b	10.8 bcd	30.14 bc	9.31b	20.3
V <sub>1</sub> × 50	31.1 c	35.3 a	10.4 d	30.50 b	10.1a	30.4
V <sub>1</sub> × 75	30.0 cd	28.3 c	11.0 bcd	29.90 bc	8.38c	8.30
V <sub>1</sub> × 100	28.3 de	29.5 c	10.8 bcd	28.60 d	8.20c	5.90
V <sub>2</sub> × 0	36.5 b	14.6 f	11.0 bcd	45.20 a	6.56f	---
V <sub>2</sub> × 25	41.0 a	16.0 ef	11.6 ab	45.10 a	7.44e	13.4
V <sub>2</sub> × 50	41.3 a	17.5 de	12.0 a	44.70 a	8.44c	28.6
V <sub>2</sub> × 75	40.5 a	18.3 d	11.4 abc	45.40 a	8.52c	29.9
V <sub>2</sub> × 100	40.5 a	16.7 def	12.0 a	45.00 a	8.12 cd	23.7
F-test	*	**	*	*	**	
CV (%)	4.25	6.09	4.75	1.59	4.45	

In a column, figures bearing same letter (s) do not differ significantly at  $P \leq 0.05$  by DMRT; NS = Not significant; \* and \*\* indicate significance at 5% and 1% level of probability, respectively.

#### 4.5.4. Effect of different levels of chitosan application on yield of maize

The experiment was conducted from December 2011 to April 2012 at the farmer field in Mymensingh, Bangladesh to investigate the response of grain yield to different concentrations of Chitosan as growth promoter. Quality protein maize-1 (QPM-1) was used as planting material. Five concentrations of Chitosan such as 0, 50, 75, 100 and 125 ppm were sprayed three times at 40, 55 and 70 days after sowing (DAS). The control was sprayed with water only. The experiment was laid out in a randomized complete block design with three replications. The unit plot size was 4m × 5m. Plant spacing was 70cm × 30cm. Fertilizers such as urea, TSP, MP, and gypsum were applied at the rate of 285, 250, 180 and 40 kg ha<sup>-1</sup>, respectively. Urea was applied in three equal portions at 30, 50 and 70 DAS, and other fertilizers were applied as basal dose during the final land preparation. Other cultural

practices such as weeding and pest control were done as needed for normal plant growth and development.

Chitosan concentration had a significant effect on plant height, biological yield, harvest index, yield components, and seed yield in maize except for the number of cobs plant<sup>-1</sup> (Table 4.6). Results revealed that all the plant parameters were greater in Chitosan applied plants as compared to the control except for 50 ppm Chitosan. The highest plant height (218 cm), biological yield (278.0 g plant<sup>-1</sup>), yield attributes (except 100-seed weight) and seed yield (132.7 g plant<sup>-1</sup> and 6.32 t ha<sup>-1</sup>) were recorded at 100 ppm followed by 125 ppm and 75 ppm concentrations. The seed yield was higher at 100 ppm. Chitosan may increase the number of seeds cob<sup>-1</sup>. In contrast, the lowest mentioned parameters were recorded in control plants where no Chitosan was sprayed. Furthermore, the highest 100-grain weight and harvest index were recorded in 125 ppm Chitosan concentration indicating that dry matter partition to economic yield was better at 125 ppm concentration than the other concentrations. However, the grain and biological yield were lower in 125 ppm than 100 ppm Chitosan concentration indicating application of 125 ppm of Chitosan may not be good for maize production. Thus, Chitosan may be applied thrice at 100 ppm concentration for increased grain yield of maize.

TABLE 4.6. EFFECT OF DIFFERENT LEVELS OF CHITOSAN ON SOME MORPHOLOGICAL CHARACTERS, YIELD ATTRIBUTES AND SEED YIELD IN MAIZE CV. QPM-1

Concentration	Plant height (cm)	Biological yield plant <sup>-1</sup> (g)	Cobs plant <sup>-1</sup> (no.)	Cob length (cm)	Seeds cob <sup>-1</sup> (no.)	100-seed weight (g)	Seed weight plant <sup>-1</sup> (g)	Seed yield (t ha <sup>-1</sup> )	Harvest index (%)
0	190.0 c	235.5 c	1.00	15.8 b	456.2 b	22.94 d	107.6bc	5.10 c	45.69ab
50	188.0 c	229.3 c	1.00	15.6 b	436.2 c	23.31cd	95.52 c	4.64 d	41.66 b
75	211.0 b	258.8b	1.14	16.8 a	450.0 b	24.08bc	117.3ab	5.59 bc	45.48ab
100	218.0 a	278.0 a	1.14	17.4 a	511.7 a	23.95 b	132.7 a	6.32 a	47.74 a
125	212.0ab	255.6b	1.14	17.0 a	460.7 b	25.08 a	125.0 a	5.95 ab	48.91 a
F-test	**	**	NS	*	*	*	**	**	**
CV (%)	3.43	7.81	7.73	4.67	5.94	4.07	9.77	5.55	5.76

In a column figures having same letter (s) do not differ significantly at  $P \leq 0.05$ ; \* and \*\* indicate significant at 5% and 1% level of probability; NS = Not significant.

#### 4.5.5. Summary of chitosan application

For rice, yield increased up to 13% by applying Chitosan twice with a concentration of 50 ppm at tillering (25 DAT) and booting stages (50 DAT). For summer tomato, fruit yield increased up to 56% when Chitosan was sprayed once with 75 ppm concentration during vegetative stage (25 DAT). For mungbean, yield increased up to 30% by applying Chitosan twice with 50 ppm concentration at vegetative (25 DAS) and flower initiation stages (40 DAS). For maize, yield increased up to 24% by applying 100 ppm chitosan three times (40, 55 and 70 days after sowing).

#### 4.5.6. Planned activities towards the sustainability of the project

- Field test for three seasons for additional confirmation of increase in the yield over the control for tomato, mungbean and maize;
- Investigation of effect of chitosan on those crops as elicitor;
- Registration of product in the appropriate agency; and
- Transfer of technology.

#### 4.5.7. Follow up field tests

- Field test has been carried out for tomato in winter season: harvested, data not available

(b) Field test for maize at different area has been carried out, waiting for harvesting.

#### 4.6. WORKS ON SUPER WATER ABSORBENT

Super water absorbent was prepared using acrylamide and carboxymethyl cellulose (CMC) and irradiating it with gamma rays. Three percent (3%) CMC mixed with acrylamide and irradiated at 25 kGy radiation dose gave maximum swelling value of 28000% when kept in water for 48 h (Fig.4.3). The prepared hydrogel was tested for water retention capacity and applied on crops for germination and growth promotion. Promising results were obtained in the preliminary laboratory scale tests.

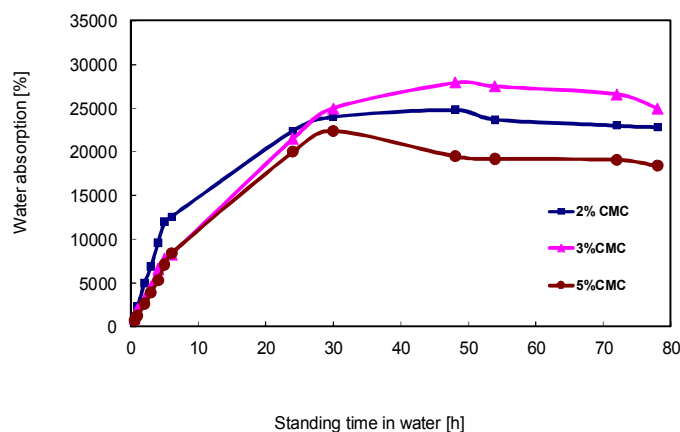


FIG. 4.3. Effect of CMC on water absorption of AAm/CMC blend hydrogel prepared at 25kGy.

#### 4.7. ACHIEVEMENT THROUGH TC PROJECT

The following results were obtained through the Technical Cooperation Project BGD8021: Promoting Radiation Processed Biodegradable Materials for Healthcare and Environmental Protection:

- The Nuclear and Radiation Chemistry (NRC) Laboratory acquired new equipment and chemicals/monomers/polymers.
- Capacity building of NRC staff members increased by:
  - (a) Training through fellowships: two in biodegradable polymers and one in super water absorbent,
  - (b) Expert assistance: one on biodegradable polymers and one on super water absorbent,
  - (c) Scientific visit: Malaysia and Republic of Korea,
  - (d) Considerable progress in the preparation of biodegradable film and super water absorbent has been achieved,
  - (e) Several MSc students and one of the team members worked for PhD degree during the period of TC and CRP projects (Completed and thesis submitted).
  - (f) Several papers were published.

#### 4.8. GOVERNMENT CONTRIBUTION

Government funding helped to establish a pilot plant making it a full-fledged radiation processing institute housed with a 350 kCi<sup>60</sup>Co irradiator.

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## Chapter 5

### INDUSTRIAL PRODUCTION OF FOOD PLASTIC PACKAGING AND THE USE OF IRRADIATION FOR MODIFYING SOME FILM PROPERTIES

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#### Abstract

The four main industrial processes needed to produce a plastic packaging structure are: cast extrusion, blown extrusion, injection moulding, and blown moulding. Since one polymer may not offer all the protection and marketing properties required for a specific food product, multilayer films can be produced. Each layer will be composed of a different polymer and additives to meet all the requirements. Ionizing radiation plays an important role in the packaging industry, especially in the heat shrinkable barrier film production process. In this process, irradiating the film structure is aimed mostly at the cross-linking of the polyolefin. Cross-linked polyolefin-based films can withstand higher stretching rates, be better stabilized, and will both have a high degree of shrinkage and higher shrinking forces. This leads to very thin structures with very well balanced cost-benefit ratios and better final packaging presentation. The use of ionizing radiation for cross-linking polymers is one of the most successful cases of irradiation used by the industry. Besides cross-linking, scission may also occur in the polymeric structure, and it may liberate toxic or unwanted substances that can be transferred to the food. Therefore, irradiated food packaging materials should be thoroughly assessed according to active legislation to guarantee that it will not harm the consumer's health either in the short or the long term.

#### 5.1. INTRODUCTION

Ionizing radiation can cause structural changes in packaging materials, altering their physical and chemical properties such as mechanical resistance and barrier. Materials utilized for this kind of processing must have physical and chemical resistance to radiation, and must not suffer either a significant reduction or enhancement of some key characteristics. They should neither transfer toxic substances nor cause a strange odour and flavour to the stored product [5.1, 5.2].

The objective of this paper is to present the common industrial processes for producing mono- and multi-layer plastic structures intended to get in contact with food, including the ionizing radiation process. A well-established industrial process is the use of ionizing radiation to enhance the mechanical properties of the polyolefin in a heat shrinkable structure. This paper also gives an example that not all commercially available structures can be irradiated in order to become more suitable for agricultural products or similar applications. If ionizing radiation is to be applied, it is important to design a structure that will benefit mostly from cross-linking, and avoid structures with polymeric materials that will mostly degrade as a result of the irradiation treatment.

#### 5.2. INDUSTRIAL PROCESSES

##### 5.2.1. Initial processes

Initially, the process of producing plastic packaging is based on a similar principle: a polymeric material in the pellet or powder form has to be transformed into a melt that will acquire a specific form by the use of heat and pressure. This initial process for extrusion, injection moulding, and blown injection moulding normally starts in a screw and barrel heated assembly. This assembly generally has three specific regions: screw feeding, screw compression, and metering. In the first region, the material that is kept in a hopper is fed to



the assembly and is heated. Upon reaching the second region, it is compressed and the melt begins to form. Finally, the last region homogenizes and leads the melt to an opening with a specific design depending on the process. The pressure of the melt that flows out of the assembly needs to be high enough to force it into the opening, which is commonly called a die, and to quickly fill the channels of a moulding chamber [5.3]. Figure 5.1 shows the main components and regions of the screw and barrel heated assembly.

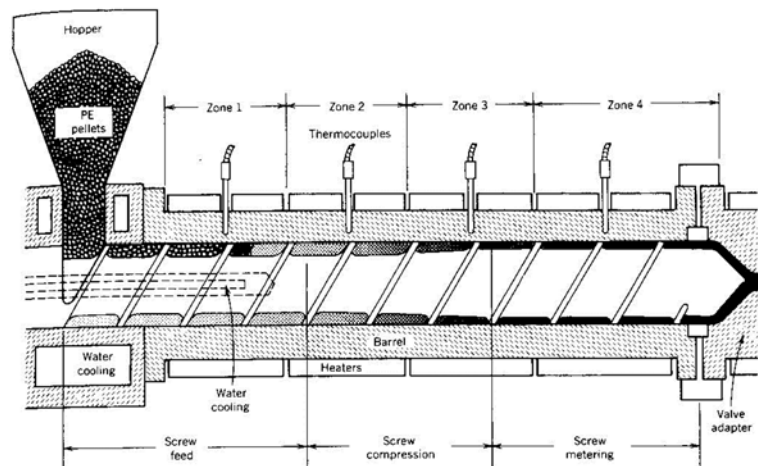


FIG. 5.1. The screw and barrel heated assembly [5.4].

#### 5.2.1.1. Cast and Blown Extrusion

For the production of a cast film or sheet, the die opening through which the melt leaves the screw and barrel assembly is a slit so that a rectangular profile is produced. The melt leaving the die usually gets into immediate contact with chilling rolls that will bring the material temperature down so that it can be trimmed and wound (Fig.5.2).

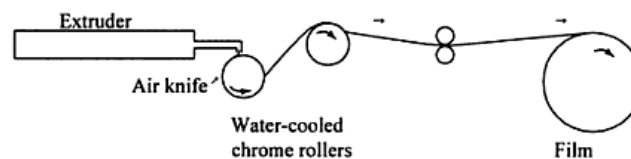


FIG. 5.2. Cast extrusion [5.3].

In the blown extrusion process, the melt must go through an annular opening. As soon as the melt leaves the die, it is cooled down with a high flux of cold air. In this case, the quick temperature decrease is necessary to start hardening the melt, and then air is blown into the plastic cylinder formed at the end of the die. The cylinder diameter will increase and form a bubble that will be expanded until the desired thickness and diameter are reached. With the help of guiding rolls, the bubble is conducted into a tower while it keeps cooling down (Fig.5.3). It is collapsed by frames and pinching rolls and then it follows to a winder where it can be slit and wound [5.4].

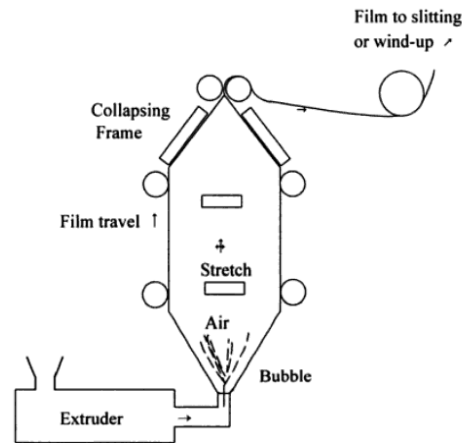


FIG. 5.3. The blown extrusion [5.3].

### 5.2.1.2. Injection Moulding

Injection moulding is a manufacturing process for producing parts by which a material is injected into a mould. Trays, cups, and lids are examples of food packaging usually produced through injection moulding. It is different from the extrusion process wherein the screw keeps turning the whole time the machine is producing. In this case, the motor is activated for a few seconds, enough time for the melt to be forced into the mould and fill all its channels and cavities. Then, the screw stops and the material in the mould is allowed to cool and harden. The mould opens, the piece is removed, and the injection process begins all over again. Figure 4 shows the clamping unit of an injection-moulding machine.

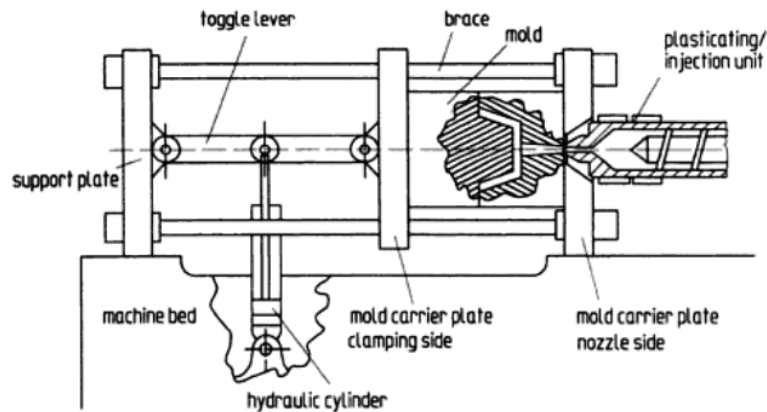


FIG. 5.4. Injection mould machine [5.5].

### 5.2.1.3. Injection blown moulding

In the injection blown moulding, the motor turns the screw at certain points, just like in the injection moulding process. But, in this case, the melt is forced into an annular opening and a hot tube called a parison or pre-form is formed. Next, the parison is placed inside a split mould with a hollow cavity. The mould sides are clamped together, pinching and sealing the parison bottom. Air is blown into the hot tube and the parison walls are expanded until they reach the mould wall and acquire the shape of the cavity. Then the mould is cooled and the melt hardens. The mould is opened and the piece can be removed, and the process begins all over again [5.6]. Figure 5 shows the basic blown moulding process.

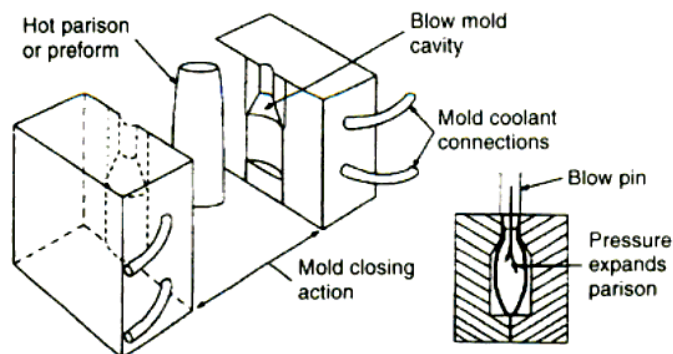


FIG. 5.5. Blown moulding process [5.6].

### 5.2.2. Production of multilayer structures

The above processes are used to produce monolayer structures of either one component or a blend of polymers and/or additives that can be used to form a single layer. However, in the desire to protect food, it is impossible to find only one kind of polymer or blend which will provide all the necessary properties (mechanical, optical, barrier) required for extending the food's shelf-life and its marketing purposes.

One example is sausage which is a form of industrialized meat. It is a food product made up of meat, fat, water and spices. The meat component will require that the sausage packaging be a good oxygen barrier, while the water component will require a water vapour barrier. Still, this packaging will require the sausage to be vacuum-sealed. One common packaging alternative is to have polyamide (PA) and polyolefin in the composition of this packaging. PA is a good oxygen barrier and has good mechanical resistance. The low density polyethylene (LDPE) is a polyolefin that can be sealed and is also a good water vapour barrier. It is not possible to mix them as their polarities and other characteristics make them chemically incompatible. In this case, the solution would be to co-extrude them. There are special dies with channels that will simultaneously direct different melts inside and organize these melts into layers, that is, depositing them one over the other. In this process, the sausage packaging structure will have an internal layer of LDPE and an external layer of PA. The layer between the PA and the LDPE, called a tie layer, will carry a grafted copolymer. Materials used in tie layers are usually polyolefins grafted with maleic anhydride. The polarity of the grafted molecules can attract the polar PA layer while the polyolefin base will be able to attract the LDPE layer, and together they will form a 3-layer single structure.

### 5.2.3. Converting already extruded films

Lamination is the process that involves the production of a new structure by joining at least two substrates. These substrates can be previously extruded plastic films or sheets, paper, or aluminium foil. The heart of this process is the group of laminating rolls, responsible for joining the substrates and distributing homogeneously the adhesive that is already coated over one of the substrates. A basic laminating system can be seen in Figure 5.6.

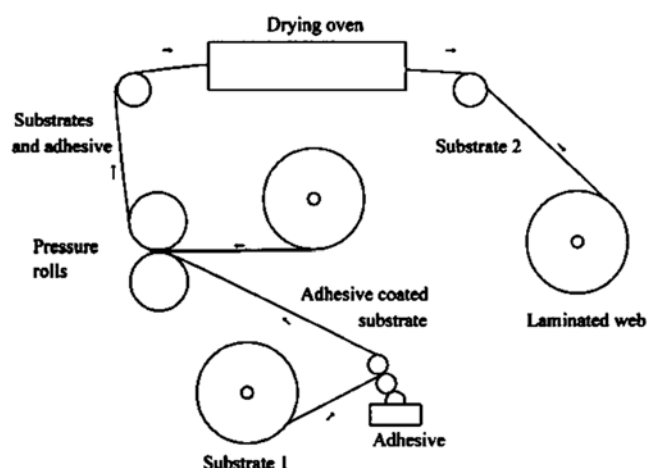


FIG. 5.6. The laminating process [5.3].

### 5.3. INDUSTRIAL USE OF IRRADIATION FOR PLASTIC PACKAGING

In the plastic packaging industry, it is possible to find the use of two different irradiation technologies. The first technology is ultraviolet (UV) radiation. The UV energy is widely used for curing inks and adhesives. This is a growing technology, so long as it avoids the use of solvent-based inks and adhesives. The use of solvent in these processes becomes an environmental issue once it is evaporated to allow the ink or adhesive to cure and, in most cases, the organic solvent is liberated directly into the atmosphere. The UV-cured inks and solvents are of great interest to many producers only if the cure is based on a cross-linking curing chemical reaction that will start once the inks and adhesives go through a high exposition to UV light. This is because the curing process is totally chemical and no by-products are generated [5.7].

Besides the UV technology, ionizing radiation plays an important role in the industry. The main industrial uses of ionizing radiation can be summarized in five categories: crosslinking wire insulation, heat shrinkable tubing, heat shrinkable food packaging, surface curing, and tire manufacturing. Industrial electron-beam equipment can be found all over the world. Cryovac, a global leader in heat shrinkable food packaging, owns over 130 electron beam units specifically for producing heat shrinkable food packaging [5.8, 5.9].

Industrial electron accelerators usually operate between 70 keV and 10 MeV. This accelerating potential determines the depth of electron penetration into matter [5.8]. Table 5.1 shows a typical penetration of electrons according to the voltage applied and end-use application.

TABLE 5.1. Electron beam penetration according to electron energy and application [5.8]

Market Segment	Electron Energy	Typical Penetration
Surface Curing	80 – 300 keV	0.4 mm
Shrink Film	300 – 800 keV	2 mm
Wire & Cable	0.4 – 3 MeV	5 mm
Sterilization	3 –10 MeV	38 mm

In the production of heat shrinkable films, e-beam irradiation energy varies from 300–800 keV with typical penetration of up to 2 mm, and its irradiation aims mostly at the cross-

linking of the polyolefin. Radiation cross-linked polyolefins are widely used as heat-shrinkable material because they can store elastic memory that can be recovered on application of heat. The phenomenon is referred to as memory effect or elastic memory [5.10]. Cross-linked polyolefins withstand higher stretching rates, and the stretched film can be totally heat stabilized. When it is submitted to a much higher temperature, the stretched film will shrink as it tries to get back to its original dimensions. A film that was stretched and heat stabilized, but not irradiated, will probably start shrinking slowly if submitted to temperatures over 35°C. However, when submitted to higher temperatures over 85°C, both the irradiated and non-irradiated films will shrink in a few seconds, although shrinking forces are higher for the irradiated film. This shrink technology leads to a much better final packaging presentation especially in odd shaped products because of its high flexibility to product dimension and its instantaneous adjustment to product shapes. The use of ionizing radiation for cross-linking polymers is one of the most successful cases of irradiation use by the industry.

In 2011, a study of polyethylene terephthalate/polypropylene, bi-oriented polypropylene/polypropylene, bi-oriented polypropylene/bi-oriented polypropylene and bi-oriented polypropylene/polyethylene terephthalate non-shrinkable structures that are currently used for packing dry pasta, cereals, cereal bars and granola was the subject of a PhD thesis developed at IPEN – Nuclear Energy Research Institute and the Biology Institute in São Paulo, Brazil [5.11]. In this study, samples of the above structures, both irradiated and non-irradiated, were tested against insect perforation. The insects used for the experiment were *Lasioderma serricorne*, *Plodia interpunctella* and *Sitophilus zeamais*. These three insects are common in Brazil and usually attack grains and cereals, even when these are packed in plastic film structures. The non-irradiated samples and irradiated samples (10, 20 and 60 kGy) containing granola were kept in a box infested with these insects for 30 days. Both the non-irradiated and irradiated structures were not resistant to the insect attack. In this case, radiation was not able to change the film puncture resistance properties. Tensile strength test according to ASTM D 882-91, puncture resistance test according to ASTM F 1306-90, and sealing resistance test according to ASTM F 88-00 were also performed on the structures 8 days, 2 months and 6 months after irradiation. Results showed that, as time went by, the materials started to present significant loss of mechanical properties, including major losses in sealing resistance in the irradiated materials that were sealed and tested 6 months after irradiation [5.11]. This study showed that not only was irradiation unable to increase the material resistance to prevent insect puncture, but it also accelerated the degradation of its components, making it more fragile than the original material. Aside from this study, there is no consistent scientific work involving the study of current polymeric packaging available in the market treated with ionizing radiation to better meet the requirements of agricultural products in Brazil.

#### 5.4. AN IMPORTANT ISSUE FOR PLASTIC PACKAGING IN DIRECT CONTACT WITH FOOD PRODUCTS

A plastic material used for food contact must comply with the specific health legislations of the country where the product is being commercialized. These regulations, formulated by either government agencies or public health institutions, aim to protect the consumer's health by disallowing the use of plastic materials and additives in food packaging that may liberate contaminants which can either intoxicate or harm the consumer upon ingestion. Furthermore, the prolonged and constant intake of such substances may even increase the consumer's chances of developing specific diseases such as cancer.

Irradiating film will lead to two results in the plastic material: scission and cross-linking. These two reactions will happen simultaneously and, depending on the dose, one will overcome the other. Scission of polymeric chains may liberate monomers and oligomers. Toxic additives and grafts which are inert when properly incorporated in the polymer matrix may also be released. Due to these possible changes in the packaging chemical structure, countries where specific food contact legislation is implemented foresee that a non-irradiated film which is originally approved for food contact must be reassessed if, at a certain point, its production process starts involving irradiation or any process that may bring about the same effects. Such assessment is carried out in order to guarantee that no unexpected substances will affect the consumer's health in the short or long term.

## 5.5. CONCLUSION

Researchers all over the world are constantly studying and developing new polymers and processes in order to obtain the best results in food packaging and consequently improve food preservation. It is important to keep in mind that all polymeric packaging materials have to comply with specific physical and chemical properties which allow them to be processed by standard cost-competitive industrial equipment. Irradiation is an important industrial process to achieve materials with superior mechanical properties. Heat shrinkable packaging is considered one of the most successful breakthroughs in the plastic packaging field.

Limited study has been developed in Brazil with commercial traditional packaging treated with irradiation for agricultural packaging products. A PhD study [5.11] demonstrated that irradiation of present commercial multilayer flexible plastic packaging used for grains, cereals and minimum processed agricultural products will not necessarily lead to improvement of material properties. Polypropylene was one of the components in all the studied packaging structures. Studies show that polypropylene without the proper stabilizers for irradiation mostly tends to scission than to cross-link [5.12].

Finally, it is important to point out that irradiation is a viable industrial process, but the effects of the irradiation and the modifications it can cause in plastic films for food products must be carefully assessed, especially because these irradiated materials are supposed to get in direct contact with food products.

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## Chapter 6

### DEVELOPMENT OF NEW ADVANCED COATING AND PACKAGING WITH IRRADIATION TREATMENT

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#### Abstract

This research has demonstrated that cellulose derivatives and alginate are promising polymers for the development of natural edible coatings. The use of irradiation to crosslink these polymers have beneficial effects to improve the shelf life and to preserve the overall quality of ready to eat coated vegetables and fruits i.e. coated pre-cut broccoli and strawberries, and to preserve the bioactivity of active compounds immobilized in the polymers during storage. The addition of nanocrystalline cellulose (NCC) in the polymer formulations has a synergistic effect with irradiation in order to improve the physico-chemical properties of the polymers and gave promising results with regards to the control release of active compounds but also on the protection of the bioactivity of natural compounds during storage. A synergistic effect between the presence of natural antimicrobial compounds and irradiation was also observed in order to eliminate pathogens and on the shelf life extension of ready to eat food. Mechanism of action of both treatments on bacterial radiosensitization is undergoing and important information has already been discovered. Similar results have been obtained with biodegradable packaging. Chitosan, methylcellulose, polycaprolactone, zein, caseinate and alginate are promising natural polymers witch could apply as food packaging. The incorporation of NCC and the microfluidization treatment of film formulations improve significantly the mechanical and barrier properties of the bio-based films. The irradiation treatment for crosslinking or grafting of monomers on natural films induced a significant improvement of the physico-chemical properties of films, can improve the compatibility of the polymer network and improve the water resistance.

#### 6.1. INTRODUCTION

Food packaging and coating have the function to protect the food against external contamination and to extend the shelf life of food. The global market of packaging is estimated at more than \$ 417 billion and food packaging represents 65 % of the market. Until now, petrochemical based plastics have been increasingly used because of their large availability at relatively low cost and because of their good mechanical properties. The current plastics consumption is estimated at 200 million tonnes/year. However, plastic represent a treat to the environment. To reduce the pollution problems caused by synthetic and



non-biodegradable packaging films, edible or biodegradable packaging materials are encouraged to be developed especially in case of antimicrobial packaging.

Post-processing protection by the development of active packaging and coating has been proposed as an innovative approach. Active packaging allows interaction with food products and the environment and plays a dynamic role in food protection [6.1].

Bio-based packaging is defined as packaging containing raw materials originating from agricultural sources. However, these compounds have high moisture sensitivity and variable gas and water permeability. Edible coatings or biodegradable films should possess the appropriate barrier and mechanical properties and should retain its properties during commercial marketing of foods. Proteins and polysaccharides have been used to develop edible coatings and biodegradable films. However, these polymers are soluble in water and their functional properties should be improved. Ionizing irradiation can play a major role in the development and improvement of packaging polymers properties as well as sterilizing material used in aseptic packaging. Irradiation can produce crosslinking, can permit grafting and improve the compatibilization of polymer blend. The use of natural nanopolymers can also act as reinforcing material, modulate the barrier properties and potentially assure a control release of active compounds.

This report will focus on the use of different methods of crosslinking and functionalization of polymers by gamma irradiation for the development of active edible coating, beads and packaging for food applications. The use of gamma irradiation in combination with active packaging, active coating and active beads to assure food safety is also demonstrated.

## 6.2. MATERIAL AND METHODS

### 6.2.1. Films preparation and functionalization

#### 6.2.1.1. Methylcellulose (MC) based films grafted with trimethylolpropane trimethacrylate (TMPTMA) or with 2-hydroxyethyl methacrylate (HEMA)

Methylcellulose (MC) solution (1% W/W) was prepared and TMPTMA monomer (0.1% w/w) and glycerol (0.25%) were added to the MC solution. The solution was homogenized and applied to the petri dish and allowed to dry for 48 hours at room temperature. Crystalline nanocellulose (NCC) solution was also added to the MC-based formulation containing MC, TMPTMA and glycerol at concentrations from 0.025–1% (w/w). Dried films were then casted, irradiated and stored in plastic bags for characterization [6.2].

MC was also grafted with 2-hydroxyethyl methacrylate (HEMA) (0.1–1%, w/w). HEMA was incorporated into the MC-based formulation and films were prepared by casting. Films were then exposed to gamma radiation. Dried water soluble films were peeled off manually using spatula and stored in polyethylene bags prior to characterization [6.3].

#### 6.2.1.2. Chitosan based films

Chitosan (0.1–1% w/w) solution was prepared at room temperature (25°C) in 2% acetic acid solution. MC solution (1%, w/w) was prepared in an ice bath using deionized water with continuous stirring. Then 0.5% vegetal oil (hydrophobic agent), 0.25% glycerol (plasticizer) and 0.025% Tween<sup>®</sup> 80 (emulsifier) along with chitosan solution (as filler) were directly poured into the MC solution. The MC-based formulation was then homogenized using an IKA<sup>®</sup> T25 digital Ultra-Turrax disperser (IKA<sup>®</sup> Works Inc., Wilmington, NC, USA) at 45°C

and 24 000 rpm for 1 min. Films were then cast by applying 12 mL of the film-forming solution onto Petri dishes and allowed to dry for 24 h, at room temperature and 35% relative humidity. Dried water soluble films were peeled off manually using spatula and stored in polyethylene bags prior to characterization.

Crystalline nanocellulose (NCC) reinforced chitosan based films were prepared by dispersing spray dried NCC powder in distilled water with magnetic stirring, followed by sonication. Then, 0.5% ethylene glycol and 2% high mol. wt. chitosan (DD: 85–90%, 85/2500 Heppemedical, GmbH) were incorporated in the suspension. The suspension was magnetically stirred overnight followed by homogenization with an IKA RW-20 mechanical homogenizer at 1500 rpm (revolutions per minute) for 5 hours. The films are made by casting a 15 mL of the nanocomposite suspension on Petri dishes and were allowed to dry at room temperature with 30–35% relative humidity (RH) for 2 days. After that films are treated with 1M NaOH for 2 min and washed several times with deionized water to render the films insoluble [6.4].

Microfluidization at a fixed pressure of 20 000 psi (for 1, 3 and 5 cycles) was done on chitosan based-formulation. With the optimized microfluidization cycle the suspension was passed through two different microfluidization pressures: 5000 psi and 10 000 psi.

Grafting of chitosan with 3-aminopropyl triethoxy silane or 2-hydroxyethyl methacrylate (HEMA) was done following the present procedure. A 1% (w/v) chitosan was dissolved in 2% aqueous acetic acid solution. The chitosan solution was gamma irradiated with 0.1 to 5 kGy doses. Then the irradiated solution was casted onto Petri dishes and was allowed to dry at room temperature (RH was 40–50%). The HEMA or silane grafted chitosan films were prepared by slowly incorporating HEMA or silane (0.1–1%, w/v) into the chitosan solution under constant stirring. The solution was stirred for 1 hour and then subjected to gamma irradiation at different doses under air. After irradiation treatment, the solution was again stirred for 1 hour and the films were prepared by casting [6.5].

#### 6.2.1.3. Preparation of polycaprolactone (PCL) – based composite films by compression molding

PCL films were prepared from PCL granules by compression molding machine (Carvar, Inc., Indiana, USA, Model 3912) at 110°C under a consolidation pressure of 2 tons for 1 min. The mould containing the PCL film was rapidly cooled in an ice bath for 2 min. The PCL film was then taken out from the mould and kept in a desiccator prior to characterization. NCC reinforced PCL composites were prepared by melt blending. PCL granules were melted at 110°C and mixed with NCC powder under continuous stirring for 10 minutes. The mixture was hot pressed in the same compression molding machine using the same parameters [6.6].

An aqueous solution containing 3% alginate (ALG) and 2% glycerol was prepared at room temperature under stirring. The NCC/bioactive mixture emulsion was incorporated in the ALG/Glycerol solution in order to obtain final concentrations of 0.25% NCC. Then, 1% PCL was added under melted state to the mixture under vigorous stirring for 5 min at 45°C. The solution was then homogenized using an IKA® T25 digital Ultra-Turrax disperser (IKA® Works Inc.) at 45°C and 24 000 rpm for 1 min, maintaining the temperature at 45°C. Films were then cast by applying 20 mL of the film-forming solution onto Petri dishes and allowed to dry for 24 h, at 20°C and 35% RH [6.7].

#### 6.2.1.4. Preparation of Zein/PVA films

At first, a 5% w/w stock solutions of Zein dissolved in ethanol/water mixture (80/20%) and PVA dissolved in hot water at different ratios were prepared. The solutions were continuously stirred and the required ratio of glycerol (20%) was added to the solutions, and then a vacuum was applied to remove dissolved air. The solutions were then poured in bottles under a flow of nitrogen atmosphere. The bottles were exposed to gamma rays with a  $^{60}\text{Co}$  source at a mean dose rate of 19 965 kGy/h. Films were then cast by pipetting 5 ml of the solution onto polymethacrylate plates, and sitting on a level surface. Solutions were allowed to dry overnight in ventilated oven at about temperature ( $60 \pm 2^\circ\text{C}$ ). Dried films could be peeled intact from the casting surface [6.8].

#### 6.2.1.5. Mechanical properties of films

Puncture Strength (PS), Puncture Deformation (PD) and Elongation (Eb). Puncture strength (PS) and puncture deformation (PD) were measured using a Stevens-LFRA texture analyzer (model TA-1000; Texture Technologies Corp., Scarsdale, NY, USA). Films were fixed between two perforated Plexiglass<sup>®</sup> plates (3.2 cm diameter), and the holder was held tightly with two screws. A cylindrical probe (2 mm diameter; scale: 0–900 g; sensitivity: 2 V) was moved perpendicularly to the film surface at a constant speed (1 mm/s) until it passed through the film. Strength values at the puncture point were used to calculate the hardness of the film. The PS values were divided by the thickness of the films in order to avoid any variation related to this parameter. PS was calculated using the equation:  $\text{PS (N/mm)} = (9.81 F)/x$ , where  $F$  is the recorded force value (g),  $x$  is the film thickness ( $\mu\text{m}$ ), and  $9.81 \text{ m.s}^{-2}$  is the gravitational acceleration. PD of the films was calculated from the PS curve, using the distance (mm) recorded between the time of first probe/film contact and the time of puncture point. Elongation (Eb) (%) values were automatically collected after film break due to elongation, using the Test Navigator<sup>®</sup> software.

#### 6.2.1.6. Water vapor permeability of films (WVP)

The WVP tests were conducted gravimetrically using ASTM procedure. Films were mechanically sealed onto Vapometer cells (No. 68-1, Thwing-Albert Instrument Company, West Berlin, NJ, USA) containing 30 g of anhydrous calcium chloride (0% RH). The cells were initially weighed and placed in a Shellab 9010 L controlled humidity chamber (Sheldon Manufacturing Inc., Cornelius, OR) maintained at  $25^\circ\text{C}$  and 60% relative humidity (RH) for 24 h. The amount of water vapor transferred through the film and absorbed by the desiccant was determined from the weight gain of the cell. The assemblies were weighed initially and after 24 h for all samples and up to a maximum of 10% mass gain. Changes in the weight of the cell were recorded to the nearest  $10^{-4}$  g. WVP was calculated according to the combined Fick and Henry laws for gas diffusion through coatings and films, according to the equation:  $\text{WVP (g}\cdot\text{mm}\cdot\text{m}^{-2}\cdot\text{day}^{-1}\cdot\text{kPa}^{-1}) = \Delta w x / A \Delta P$ , where  $\Delta w$  is the weight gain of the cell (g) after 24 h,  $x$  is the film thickness (mm),  $A$  is the area of exposed film ( $31.67 \times 10^{-4} \text{ m}^2$ ), and  $\Delta P$  is the differential vapor pressure of water through the film (3.282 kPa at  $25^\circ\text{C}$ ).

#### 6.2.1.7. Swelling properties

A water uptake apparatus is designed to study the water absorption properties of films or beads and consequently to determine the rate of gel swelling. Films or beads were dried at  $40^\circ\text{C}$  for 24 h in a drying oven and placed in a 5 mL graduated cylinder (0.2 mL subdivision).

Water penetration into films or beads was measured as a function of time [6.9, 6.10]. The water uptake is expressed in the rate of film swelling (percent weight increase).

#### 6.2.1.8. Coating of broccoli

Methylcellulose was poured into a beaker containing warm water (60°C) to obtain a final concentration of 2% (wt/vol) and subjected to constant stirring for MC pre-gelatinization. The suspension was first placed in an ice bath, under strong stirring, to insure complete solubilization. The addition of 1% vegetable oil, 0.5% glycerol and 0.025% Tween<sup>®</sup>80 was performed at room temperature, under strong stirring for 5 min. The bioactive agents were then incorporated into the coating formulation and stored at 4°C prior to application. Inoculated broccoli florets were dipped in baths containing different antimicrobial coating formulations. The antimicrobial formulations were composed of organic acids mixture, lactic acid bacteria metabolites, spice mixture extracts, spice extracts and fruit extract. After dipping, broccoli were drained and dried on aluminum paper for 90 min, a portion of 25 g of vegetables were packaged in 0.5-mil metallized polyester-2-mil ethylene vinyl acetate copolymer bags (205 × 355 mm, Wipak Division Ltd) and all samples were left overnight at 4°C before irradiation treatment [6.11].

#### 6.2.1.9. Packaging of broccoli

First, antimicrobial formulations were prepared in order to obtain a concentration of 60 g/L organic acids mixture, 13.5 g/L spice extract, and 6 g/L spice mixture or 6 g/L spice extract in the final film-forming suspension prior to casting. The mixtures were homogenized at room temperature using an Ultra-Turrax disperser (T25 model; IKA<sup>®</sup> Works Inc., Wilmington, NC, USA) at 12 000 rpm for 1 min immediately followed by 24 000 rpm for 1 min.

An aqueous solution containing 30 g/L alginate and 20 g/L glycerol was prepared at room temperature under stirring. The antimicrobial formulation was incorporated into the alginate/glycerol suspension, under stirring at room temperature. 10 g/L polycaprolactone was added under molten state to the mixture under vigorous stirring for 5 min at 45°C. The polymer suspension was homogenized using an IKA<sup>®</sup> T25 digital Ultra-Turrax disperser (IKA<sup>®</sup> Works Inc.) at 24 000 rpm for 1 min, by maintaining the temperature at 45°C. Films were then cast by applying 20 mL of the film-forming suspension onto Petri dishes (50 × 9 mm; VWR International, Ville Mont-Royal, QC, Canada) and allowed to dry for 24 h, at 20°C and 35% relative humidity (RH) before peeling [6.12].

An antimicrobial film based on methylcellulose was prepared. The methylcellulose powder was poured into a beaker containing warm water (60°C) to obtain a final concentration of 10 g/L and subjected to constant stirring for MC pre-gelatinization. The suspension was then placed in an ice bath, under strong stirring, to ensure a complete solubilization. The addition of 5 g/L VO, 2.5 g/L glycerol and 0.25 g/L Tween<sup>®</sup>80 was performed at room temperature, under strong stirring for 5 min. The antimicrobial formulation was then incorporated into the methylcellulose suspension and stored at 4°C prior to application. Films were then cast by applying 20 mL of the film-forming suspension onto Petri dishes (50 × 9 mm; VWR International) and allowed to dry for 24 h, at 20°C and 35% RH before peeling. This film was fixed to the polycaprolactone based film to form a bilayer film.

#### 6.2.1.10. Preparation of meat sample

Boneless pork loin meat was purchased from a local grocery store. Approximately 10 g of meat samples were cut and sandwiched between two films. The samples were put in a sterile bag, vacuum sealed and kept at 4°C until the day of the analysis. Six different formulations of meat samples were prepared.

#### 6.2.1.11. Active packaging preparation and irradiation of packaged meat

Chitosan, a natural linear polysaccharide consisting of 1,4-linked 2- amino-deoxy-β-d-glucan was used to develop a crosslinked films via enzymatic treatments. The antimicrobial formulation (lactic acids metabolites/fruit extract/spice extract) was mixed with the polymer and microfluidized five times under 23 000 psi. The solution was left to dry and the films were peeled and used to packaged ground meat. The meat samples were irradiated and microbial analysis was done to evaluate bacteria count during storage.

#### 6.2.1.12. Active beads preparation and irradiation of meat

An aqueous suspension containing 1.5% (w/v) alginate and NCC were homogenized using an Ultra-Turrax TP18/1059 homogenizer (Janke & Kunkel, Staufen, Germany) at 23°C and 25 000 rpm for 1 min. A 0.5% w/v lecithin was slowly added to the alginate suspension and heated to 60°C for 30 min. The pH of alginate-lipid suspension was adjusted from 7.5 to 8.0 by using 0.1M NaOH for the proper interaction of alginate and lecithin [6.13]. Essential oil such as *Origanum compactum*, 0.025% w/v was added to the alginate-lipid suspension and formed the emulsion. The pH of the emulsion was lowered to 5.5–6.0 by using 0.05 M lactic acid. A 0.25 % w/v of Nisin was mixed in 0.01 M CaCl<sub>2</sub> solution. The antimicrobial gel beads were obtained by addition of 0.01 M CaCl<sub>2</sub> containing Nisin to the emulsion under vigorous stirring at room temperature. Irradiation of meat was performed in an underwater calibrator <sup>60</sup>Co irradiator at the Canadian Irradiation Centre. Vegetable products were irradiated under air and ready to eat meat under vacuum.

### 6.3. RESULTS

#### 6.3.1. Effect of tmptma and silane grafting on mc-based films

The incorporation of NCC as reinforcing agent in films led to significant improvement of some films properties. NCC addition (0.025%), grafting with TMPTMA and irradiation treatment (1 kGy) improved significantly the mechanical properties of the films. The use of irradiation (0.5 kGy) and NCC addition was also able to show the lowest WVP [6.5]. Silane monomer (3-aminopropyl tri-ethoxy silane) (0.1–1%, w/w) was also incorporated into the MC-based films and was found to improve the strength of the films significantly. Surface morphology of the grafted films was examined by scanning electron microscopy and suggested better appearance [6.3].

##### 6.3.1.1. Chitosan-based films properties

The addition of NCC (5%) has permitted an increase of 46% of the tensile modulus, a decrease by 19% of the elongation at break, a decrease by 33% of the WVP and a decrease of 10% of the swelling of chitosan based films [6.4]. Microfluidisation has permitted a 23% increase of the tensile strength and an 86% decrease of the elongation at break of chitosan based films. Irradiation of PCL/NCC films showed an increase of 51% of the tensile strength as compared to 43% without NCC at the same dose of irradiation (10 kGy). A 44% and 53%

increase of the elongation at break was also observed for PCL and PCL/NCC based films when irradiated at 10 kGy.

The results of grafting of chitosan based films with silane and HEMA showed that irradiation at a dose of 0.3 kGy has permitted an increase of 5.7% the puncture strength, an increase by 43% the viscoelasticity and a decrease of 7% of the WVP of films after grafting with HEMA. When silane was used, an irradiation dose of 10 kGy has permitted an increase of 25% of the tensile strength. Grafted chitosan films with silane was also prepared as a composite films with PCL-based films and results showed that irradiation at 20 kGy, has permitted a 32% increase of the tensile strength. The grafted films with silane showed also a smoother appearance by visualization by scan electronic microscopy [6.13, 6.5].

#### 6.3.1.2. Zein based films

Mechanical properties of zein-based films indicated that the addition of PVA in zein-based films has permitted an increase of the toughness of the blends. The addition of 30% of PVA in the blend gave the minimum ratio to obtain a compatible phase with zein. Grafting of acrylic acid by irradiation to the blend improved significantly ( $P \leq 0.05$ ) the puncture deformation suggesting that grafting improved the compatibility of the zein/PVA network [6.8].

#### 6.3.1.3. Coating and packaging of broccoli

This study showed that the radiosensitization of bacteria varied according to the bioactive coating that was applied onto broccolis. Indeed, the organic acids mixture/fruit extract coating was found to be the most effective coating in increasing the radiosensitivity of *E. coli* and aerobic microflora whereas the organic acids/lactic acid bacteria metabolites coating was the most efficient for *S. Typhimurium*. The organic acids mixture/spice extract coating was also found to be as efficient as organic acids/fruit extract coating against aerobic microflora. On the other hand, all coatings (organic acids/lactic acid bacteria metabolites, organic acids/fruit extract, organic acids/fruit extract/spice mixture and organic acids/spice extract) had similar radiosensitizing effects ( $p > 0.05$ ) to *L. monocytogenes* but generated a lower relative radiosensitivity ( $< 1.5$ ) as compared to other bacteria (2.40 for organic acids/lactic acid bacteria metabolites against *S. Typhimurium*) [6.11]. The use of one of the best active formulation combined with irradiation has permitted to reduce the dose necessary to eliminate pathogens *i.e.* *Listeria monocytogenes*, *Escherichia coli*, *Salmonella Typhimurium* and the total aerobic count. A respective relative radiosensitization of 1.36, 1.95, 2.40 and 1.60 was observed [6.11].

Immobilization of the same antimicrobial formulation in PCL-based films showed a good efficiency to control *S. Typhimurium* on broccoli, with a significant reduction of bacterial concentration until total inhibition after 12 days of storage. In general, bioactive films showed a significant reduction and a good capacity to control the growth of *L. monocytogenes* and *E. coli* at short-term storage (4 days). Therefore, these results demonstrated the high antimicrobial potential of both types of films via the diffusion of antimicrobial volatiles on pathogenous bacteria in pre-cut vegetables [6.12].

#### 6.3.1.4. Chitosan based-film and microbial quality of meat

Gamma irradiation of meat at doses of 0.5 and 1.5 kGy reduced bacterial count of meat and a lower level of bacteria was maintained throughout the storage compared to the control (without irradiation). At day 13, samples irradiated at 1.5 kGy had a reduced psychrophilic bacteria count by 2.98 log (CFU/g) compared to the control. Combination of antimicrobial

films with 1.5 kGy irradiation (Active Film (AF) +1.5 kGy) further reduced the bacterial count showing. The shelf life of the control was 6 days as compared to 8 and 16 days for meat packaged with active films and treated at 0.5 and 1.5 kGy respectively. Results for mesophilic bacteria demonstrated that AF and AF + 0.5 kGy were not able to reduce the bacterial population during storage. Irradiation dose of 1.5 kGy was, however, found to be very effective on the reduction of mesophilic bacteria. Bacterial count was beyond detection limit at day 1 for the control +1.5 kGy and AF +1.5 kGy formulations. Combination of antimicrobial films and gamma irradiation increased the shelf life of fresh pork sausages from 6 days to the control to 14 days for samples treated with AF and 1.5 kGy.

#### 6.3.1.5. Alginate-based films

The addition of 5% NCC (w/w) has permitted to improve by 37% the tensile strength and decrease by 44% and by 31% the elongation at break and the WVP of alginate-based films [6.14]. Irradiation at 0.1 kGy of beads prepared with the same formulation has permitted to reduce by 17% the rate of gel swelling [6.14]. These beads were used to prepare active beads and these beads were sprayed on ham. The combined effect of irradiation and microencapsulated antimicrobials microbeads was very effective in reducing *L. monocytogenes* in ready to eat (RTE) ham during subsequent storage. Antimicrobial ingredient combinations (bacteriocins + essential oils) could be used in addition with irradiation dose at 1.5 kGy of RTE ham to inhibit the growth of surviving pathogens during prolonged storage (35 days) at 4°C. The microencapsulated essential oils showed an effective ability to prolong the efficacy of the active compounds against the food pathogen *L. monocytogenes*. The principal finding of this work is that alginate-NCC microbeads can be used to deliver bioactive compounds in food system. The combination of antimicrobial and gamma irradiation could protect the food system from food pathogen *L. monocytogenes*. This study was conducted from the perspective of food applications; however, the methodology established can be broadly used in various biological and physiological systems.

#### 6.4. CONCLUSION

MC, chitosan, PCL, alginate and zein are promising natural polymers which could apply to various potential uses as packaging and coating materials. The incorporation of NCC and the microfluidization treatment of polymer formulations improved significantly the mechanical and barrier properties of the bio-based films. The addition of NCC, the irradiation treatment or grafting of monomers on natural polymers by irradiation induced a significant improvement of the physico-chemical properties of films and can improve the compatibility of the polymer network as well as producing crosslinking reactions between polymers and improve the water resistance. Results on active coating and irradiation of broccoli showed that the combined treatments (coating or packaging with active polymers and irradiation) has permitted a bacterial radiosensitization and reduced the dose necessary to eliminate pathogens. The combined effect of irradiation and microencapsulated antimicrobials in microbeads or in films is also very effective in reducing *L. monocytogenes* in ready to eat ham during subsequent storage. Antimicrobial ingredient combinations (bacteriocins + essential oils) could be used in addition with irradiation to inhibit the growth of surviving pathogens during prolonged storage at 4° C. A synergy between antimicrobial compounds and also between antimicrobial formulations and irradiation has been observed in order to eliminate pathogens and on the shelf life extension.

## Acknowledgements

This research was supported by the Natural Science Engineering Research Council of Canada, Ministry of Agricultural Fisheries and Food of Québec, Canada, FP Innovation and International Atomic Energy Agency (IAEA). Nordion is acknowledged for irradiation treatments.

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## Chapter 7

### RADIATION PROCESSED MATERIALS IN PRODUCTS FROM POLYMERS FOR AGRICULTURAL APPLICATIONS IN CHINA

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#### Abstract

Oligochitosan has been found to be water-soluble and effective to elicit multiple plant defense responses. In China, Peking University and Shanghai Institute of Applied Physics, Chinese Academy of Science investigated on the radiation degradation of chitosan. The results showed that the molecular weight (Mw) of more than 95% of chitosan was lower than 3000 with narrower Mw distribution when compared with enzyme radiolysis products. The mechanism of radiation degradation was well elucidated. The technology was transfer to Xiamen Blue Bay Co. Ltd and Jiaying Kerui Biology Technology Co. Ltd by Peking University and Shanghai Applied Physics Institute, China Academic, respectively. The oligochitosan was used to produce animal feeds for health care drug, aquaculture feed for shrimp and fish, animal feed for bird species, animal feed for piglet and so on. Data for the mentioned applications as well as the promotion of cucumber growth was shown. In summary, oligochitosan prepared by radiation degradation has marvelous prospects on enhancing animal breed and plant growth at low cost.

#### 7.1. INTRODUCTION

Chitosan is a linear polysaccharide composed of randomly distributed  $\beta$ -(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). Chitosan is biodegradable, non-toxic and biocompatible and has shown to be particularly useful in plant protection due to its dual function: antifungal effects and elicitation of defense mechanisms in plant [7.1,7.2]. However, its high viscosity and insolubility in neutral aqueous solution restrict its uses in many purposes. Recent studies on chitosan have attracted interest for converting chitosan to oligosaccharides [7.3]. Oligochitosan, obtained by hydrolysis or degradation of chitosan, is not only water-soluble but also has shown to be more effective than chitosan to elicit multiple plant defence responses.

## 7.2. RADIATION DEGRADATION OF CHITOSAN

### 7.2.1. Molecular weight of radiation degraded chitosan

Compared with chemical radiolysis, enzyme radiolysis as well as other degradation methods, radiation degradation of chitosan has been found to be more environmental friendly. Radiation degradation of chitosan has been carried out in China by Peking University and Shanghai Institute of Applied Physics, Chinese Academy of Science.

Degradation of chitosan in solid state needs high dose, and in acidic aqueous solution. Due to the limit of chitosan solubility, it is difficult to produce oligochitosan in industrial scale. A new method for producing oligochitosan by gamma irradiation in the presence of suitable additives has been developed. Low molecular weight chitosan with narrow molecular weight distribution can be obtained by irradiation at a very low dose, i.e. 2 kGy. Figure 7.1 and Table 7.1 show the relationship between Mw and absorbed dose and the Mw distribution, respectively [7.3]. The Mw distribution is compared with a commercial oligochitosan, which is produced by enzyme degradation in China with 10 times as much as the price of normal chitosan.

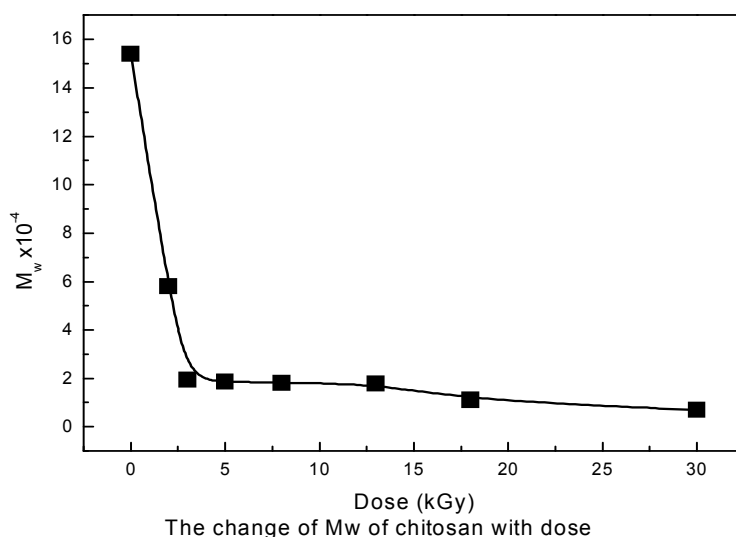


FIG. 7.1. Correlation between the molecular weight and the absorbed dose.

TABLE 7.1. THE MOLECULAR WEIGHT DISTRIBUTION OF RADIATION DEGRADED CHITOSAN AND ENZYME RADIOLYSIS CHITOSAN.

$M_w \times 10^3$		>30	10~30	5~10 (%)	3~5 (%)	1.6~3 (%)	<1.6 (%)
		(%)	(%)				
Radiation chitosan	degraded	0	0.02	0.40	3.9	13.3	82.3
Enzyme radiolysis chitosan		3.5	15.3	24.9	24.2	26.2	5.9

### 7.2.2. Mechanism of radiation degradation of chitosan

The mechanism of radiation degradation of chitosan has been investigated and well elucidated as shown in Figs. 7.2 and 7.3. The main mechanisms are H-abstraction reaction,

fragmentation and/or rearrangement of radicals at C(1), C(2), C(4) and C(5). From t Fig. 7.2, it can be shown that the degradation of CM-chitosan induces the formation of carbonyl groups at the terminal chains of CM-chitosan. The formation of hydroxyl groups at C(2) and partial amino groups is eliminated as shown in Fig. 7.3. In the presence of  $H_2O_2$ , fragmentation occurs more frequently [7.4–7.5].

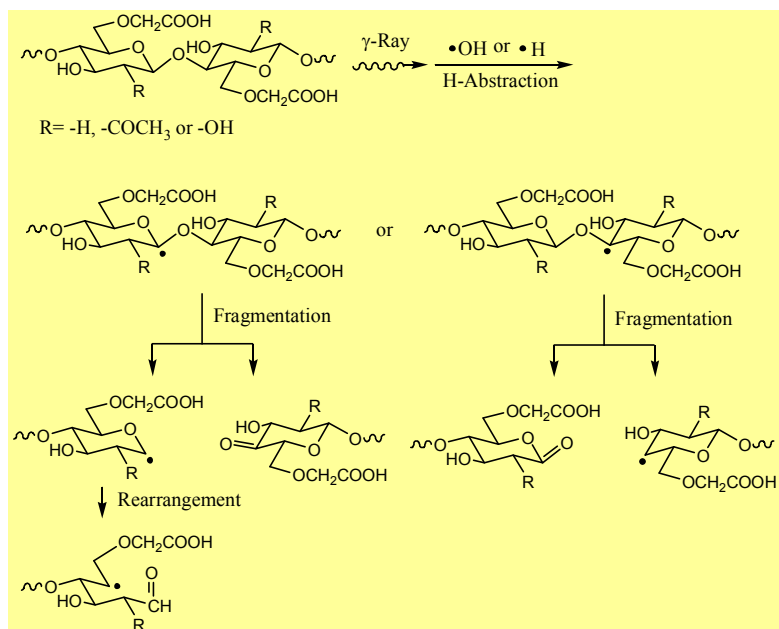


FIG. 7.2. Scheme of the possible mechanism of main chain scission.

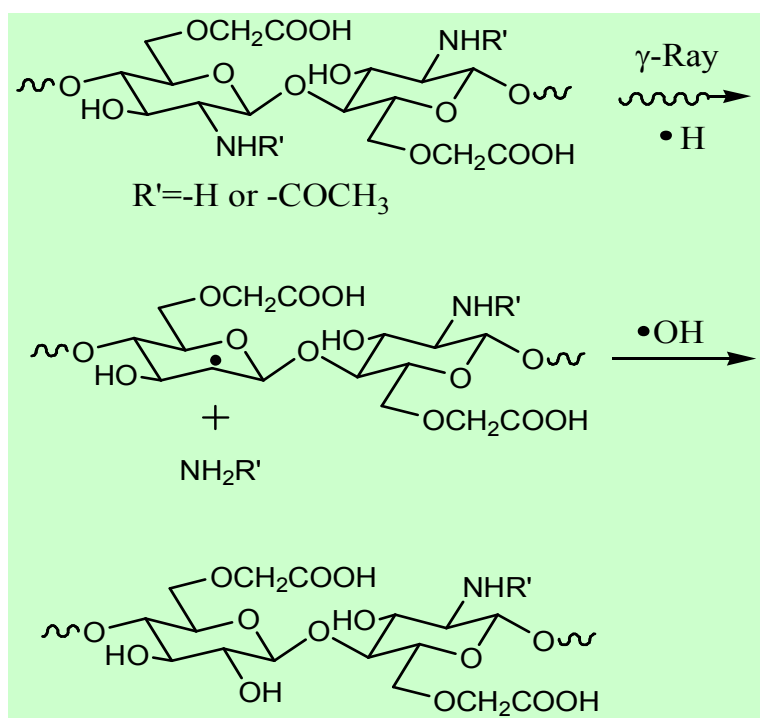


FIG. 7.3. Scheme of the possible mechanism of amino groups eliminated from CM-chitosan during radiation.

### 7.3. COMMERCIAL PRODUCTS OF CHITOSAN IN CHINA

The technology of radiation degradation of chitosan was transferred to Xiamen Blue Bay Co. Ltd and Jiaying Kerui Biology Technology Co. Ltd by Peking University and Shanghai Applied Physics Institute, China Academic respectively. The products of Xiamen Blue Bay Co. Ltd include glucosamine sulphate, chondroitin sulphate, collagen, chitosan and oligochitosan [7.6]. The products of Jiaying Kerui Biology Technology Co. Ltd include Ruikang No.1 (Oligochitosan premix feed for pig), Ruikang No.2 (Oligochitosan premix feed for bird species) and Ruikang No.3 (Oligochitosan premix feed for aquaculture) and Ruishengsu [7.7].

### 7.4. APPLICATIONS OF RADIATION-DEGRADED CHITOSAN

Partial results on the applications of Ruikang series and Ruishengsu on the culture of shrimp (Fig. 7.4), broiler chicken (Figure 7.3, Table 7.2) and piglets (Table 7.3) are illustrated [7.7].

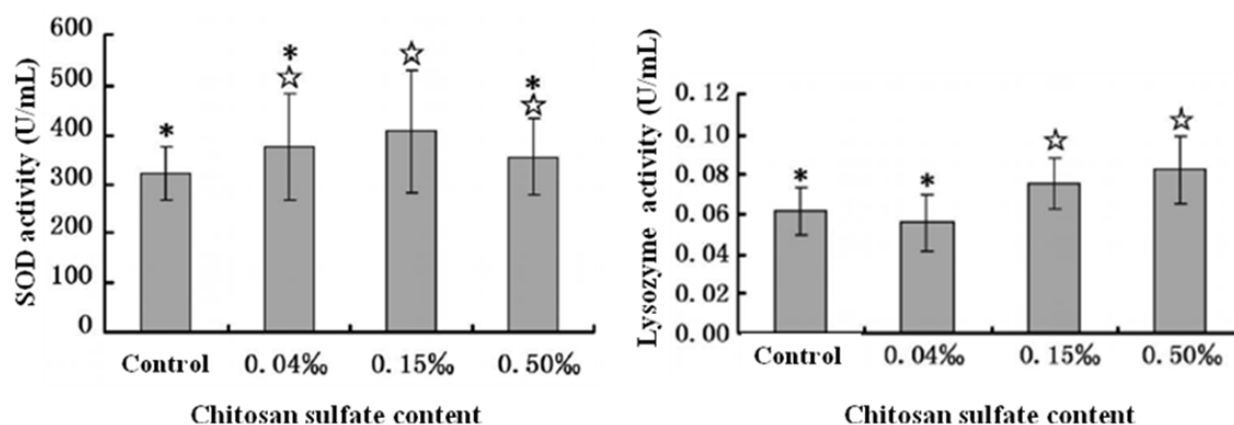


FIG. 7.4. Effect of chitosan sulfate in the serum activity of shrimp.

TABLE 7.2. EFFECT OF RADIATION-DEGRADED CHITOSAN ON BROILER CHICKEN GROWTH PERFORMANCE

	1 d body weight (g)	56 d body weight gains (g)	feed / gain (g/g)
control	49.0±0.21	2936.0	2.07
radiation-degraded chitosan	48.6±0.22	3436.8	2.00



FIG. 7.5. The average weight of chicken after feeding with oligochitosan products for 4-6 weeks.

TABLE 7.3. EFFECT OF RUISHENGUSU ON THE GROWTH OF WEANED PIGLETS IN TERMS OF ANTIOXIDANT CAPACITY

mg/kg	CAT(U/ml)	T-AOC(U/ml)	MDA (nmol/ml)	GSH-PX
Control	4.81±0.32	6.79±0.10	3.02±0.08	808.57±81.50
25	5.30±1.25	6.38±0.34	3.15±0.13	914.29±232.12

50	9.95±1.48	7.09±0.13	4.44±0.65	741.43±52.91
100	8.86±1.65	6.23±0.15	4.24±0.59	915.71±146.93
Synbiotics	9.78±1.76	7.47±0.17	3.75±0.46	775.71±78.95

According to the above mentioned results, radiation-degraded chitosan in animal feed can improve production performance, promote growth, enhance immunity and reduce cholesterol. Being a fine *Bifidobacterium* factor that promotes the incubation of microbial colonies, the feed can make the animals physically stronger and less prone to sickness. The radiation-degraded chitosan can be a new type of feed additive under the classification of “antibiotics-free-feed”. There is a great potential and broad prospect for its commercial application.

## 7.5. CHALLENGES OF COMMERCIALIZATION

Although oligochitosan has been successfully commercialized with competitive cost and quality, the oligochitosan produced by enzyme degradation still dominate and is widely used in China. Thus, more publicity or advertisements will be required for its commercialization. Meanwhile, the cooperation among radiation chemist, company manager and end users will be very important for its commercialization.

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## Chapter 8

### EVALUATION OF RADIATION PROCESSED POLYMERS AS MATERIALS FOR AGRICULTURAL PURPOSES

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#### Abstract

The use of ionizing radiation in the development of natural and synthetic polymers for agricultural purposes has proven to be successful. Polysaccharides such as chitosan and sodium alginate are easily degraded through ionizing radiation which is considered to be a safe, clean, and an environment-friendly method. As a result, different kinds of biologically active fragments possessing growth promotion properties for plant performance and antimicrobial activities against pathogenic microorganisms for food preservation were obtained. Several factors such as preparation conditions, quality of the new materials, safety of the materials especially where the environment is concerned, and economic feasibility and production cost have been studied in its aim to commercialize the products. The required dose for degradation of soluble polysaccharide is relatively low when compared with that of solid polysaccharides. From the economic point of view, the choice to degrade polysaccharide with the use of viscous polysaccharide solutions containing 0.5–1 wt% oxidizing agent such as  $\text{H}_2\text{O}_2$  is necessary to decrease the dose required for degradation in the range of 20–30 kGy. Average molecular weights (MW) as well as molecular weights distributions were accurately determined by using different techniques like gel permeation chromatography (GPC) and viscometer. Large scale field studies of degraded polysaccharide materials as a growth promoter or an elicitor for different crops like wheat, faba bean, zea maze, and other vegetables and fruits (e.g. tomato, hot pepper, and banana crops) have been carried out. It was found that the treatment with low MW alginate of plants such as wheat increased its productivity by 25–30%, improved the quality of the crop, and enhanced the capability of wheat seeds to be used for long-term cultivation. The use of irradiated polysaccharides in stimulating defense reactions against some diseases in some plants was studied. Sodium alginates had a high inhibitory activity against Tobacco Mosaic Virus (TMV). The highest inhibition degree was noticed when the Mw of irradiated alginate was around  $5 \times 10^4$ . The degree of inhibition increased with alginate concentration. On the other hand, it was noticed that degraded chitosan could control the growth of gray mold fungus that infected tomato fruits. High concentrations of degraded chitosan with an Mw range of  $2\text{--}6 \times 10^4$  controlled the increase of gray mold infections and enhanced fungal decay in tomato fruit. Cross-linked fast swelling super porous hydrogels were prepared by irradiating acrylamide-based copolymers in solid and solution states using electron beam irradiation. As the Polyacrylamide/potassium polyacrylate (PAAm/PAAcK) water swellability increased, the PAAm in the copolymer and/or irradiation dose required for the cross-linking process decreased. A field evaluation of PAAm/PAAcK as super water absorbent materials for Zea maze plants revealed that PAAm/PAAcK does not cause salt buildup in the soil, possesses a high ability to absorb water, improves germination of seeds planted in sandy soil, prevents plant losses due to drought and water stress in non-irrigated areas, and increases the Zea maze plant growth and performance. Thus, such a hydrogel could be used for soil amendment to improve the growth of plants and increase their production yield. Field evaluation using polyacrylamide/Na-alginate, copolymer was performed in a range of garden plants using Faba bean and Zea maize. The growth of bean plant cultivated in a polyacrylamide/Na-alginate treated soil was higher than that of polyacrylamide treated soil or in a gel-free soil (control).

#### 8.1. INTRODUCTION

There are numerous potentials for using polymers in agriculture. In the last decades, functionalized polymers revolutionized the agricultural, horticultural, and food industry with new tools for the molecular treatment of diseases, rapid disease detection, the increase in the ability of plants to absorb nutrients, etc. Smart polymeric materials and efficient delivery systems helped the agricultural industry to combat viruses and other crop pathogens. Functionalized polymers were used to increase the efficiency of hormones, pesticides and herbicides while allowing lower doses to be used, protect the environment, and clean-up existing pollutants [8.1–8.5]. The need to improve the physical properties of soils in order to increase productivity in agriculture led to the development of water soluble polymeric soil conditioners. The use of synthetic or natural materials with good water absorption and retention capacities even under high pressure or temperature solves the problem of the water lack and the desertification that compromise agriculture development. Their use in agricultural applications has shown encouraging results. They have been observed to help in the reduction of water consumption in irrigation, minimize the death rate of plants, improve



fertilizer retention in the soil, and increase plant growth rate [8.6-8.9]. During the last decade, there was a great demand for agrochemical, residue-free, and fresh agricultural products. There was a worldwide trend to explore new natural products that act as growth promoters for plants and control of post-harvest pathogenic diseases, giving priority to those that enhance plant productivity, reduce disease incidence and prevent negative side effects on human health as a result of an excessive application of synthetic agrochemicals. Agriculturists and pathologists have shown interest in degradable natural compounds derived from animals and plants. Among these are chitosan, Na-alginate or carrageenan. These are high molecular weight polymers which are nontoxic bioactive agents. They have become useful compounds due to their bio-fertilizer and fungicidal effects or elicitation of defense mechanisms in plant tissues [8.10-8.13].

Degradation is a very important reaction in the chemistry of high-molecular-weight compounds. It is used to determine the structure of polymeric compounds, and to obtain valuable low molecular weight substances from natural polymers [8.14]. Radiation-induced degradation technology is a new and promising application of ionizing radiation used to develop materials for pulp paper, food preservation, and pharmaceutical and natural bioactive agent industries. Polysaccharides and their derivatives exposed to ionizing radiation had long been recognized as degradable type of polymers [8.15-8.17].

The benefits of using radiation technology over conventional chemical methods include its capacity to control the degree of degradation and molecular weight distribution, and its being an environment-friendly process due to the absence of chemicals. However, such technology is not cost-effective because it needs high irradiation doses (hundreds kGy) to degrade natural polymers in solid form. Much has been done to reduce the cost by using low dose irradiation. The process for production of oligosaccharide is carried out by irradiation or by chemical methods (using oxidizing agents). The challenges in the application of irradiated natural polymers as a plant growth promoter include the production of oligosaccharide through a combination of irradiation and oxidizing agents to lower the dose required for degradation. Depending on the kind of polysaccharide, the concentration of oxidizing agents can be selected to enhance the effect of degradation, thus making it more economical to produce oligosaccharides.

The present report deals with the study of the effect of ionizing radiation on the degradation of some natural polymers such as sodium-alginate and chitosan. Field tests on plants like wheat, Zea maize, Faba bean plants, and the possible use of these degraded natural polymers as growth promoter or elicitor were evaluated. The use of synthetic or natural polymers prepared by ionizing radiation as superwater absorbents is also discussed in this report.

## 8.2. EXPERIMENTAL

### 8.2.1. Determination the molecular weight of degraded chitosan

The weight-average molecular weights of the degradable polymers were determined by two methods: viscometry and gel permeation chromatography (GPC).

#### 8.2.1.1. The Viscometric Method

The weight-average molecular weights ( $M_v$ ) of chitosan can be determined by measuring the intrinsic viscosity of polymer solution  $[\eta]$  of chitosan samples using an ubbelohde viscometer [8.18–8.19]. The solvent used was 0.3 M acetic acid with 0.2 M sodium acetate solution.  $M_v$  was computed from Mark-Houwink equation  $[\eta] = kM^a$  where  $[\eta]$  is the intrinsic viscosity of

the polymers with values of  $K = 0.076$  ml/g and  $\alpha = 0.76$ ,  $k$  and  $\alpha$  are the polymer/solvent interaction constants and  $M$  is the average molecular weight.

#### 8.2.1.2. Gel Permeation Chromatography (GPC) Method:

The number average molecular weight ( $M_n$ ) and weight average molecular weights ( $M_w$ ) [8.20] of irradiated samples were obtained through gel permeation chromatography using a 1100 Agilent instrument equipped with organic and aqueous GPC-SEC start up kits with a flow rate of 2 ml/min, maximum pressure 150 bar, minimum pressure 5 bar, injection volume 50  $\mu$ L, and column temperature thermostat 25°C. The eluent was monitored by a refractive index detector with an optical unit temperature of 25°C and peak width of 0.1 min. Polymer concentration was 0.1 (w/%). The molecular weights were determined from a calibration curve using polyethylene oxide standards for aqueous systems.

### 8.3. RESULTS AND DISCUSSION

#### 8.3.1. Usage of degraded alginates and chitosan as growth promoter of plants

Plant growth promoters aid in the better management of nutrients and plant growth. They play a major role in seed germination, fruit ripening, and enhancement of uptake of nutrients. Moreover, they augment immunity, help to withstand stress conditions, reduce flower and fruit drop, and help in better plant growth. Chitosan and alginate can be used as growth promoters for different plants. In general, the proper molecular weights for enhancing the plant growth range from  $1 \times 10^3 - 7 \times 10^4$ . Ionizing radiation may be used to degrade and decrease the  $M_w$  of polysaccharides.

Gamma irradiation as a source of ionizing radiation has been used for degrading alginate and chitosan in solid or in solution. The dose of more than 100 kGy required for solid polysaccharide degradation is relatively high. For polysaccharide in solution form, the dose required for degradation is relatively low (20–50 kGy) and depends on the type and concentration of polysaccharides. To reduce the dose required for polysaccharide degradation, polysaccharides are exposed to ionizing radiation in the presence of oxidizing agents like  $H_2O_2$ . The dose required for polysaccharides degradation is relatively low when compared to those without oxidizing agents. It becomes lower than 100 kGy. For polysaccharide in solution, the degradation dose ranges from 10–30 kGy and depends on the type and concentration of the oxidizing agent. The best method to degrade alginate is to mix 10% by wt alginate with 89% water and 1%  $H_2O_2$ . This highly viscous mixture is irradiated at a dose of 25 kGy to obtain a clear liquid solution.

The effect of oxidizing agents such as hydrogen peroxide, potassium per-sulfate (KPS), ammonium per-sulfate (APS), and/or gamma irradiation on the degradation process of sodium-alginate or chitosan was investigated (Fig. 8.1). It was found that the molecular weight of the sodium-alginate decreased by using gamma radiation or oxidizing agents alone. Combining gamma irradiation and oxidizing agents accelerated the degradation rate of sodium-alginate and reduced the dose required for its degradation. The higher the concentration of the oxidizing agent, the lower the polysaccharide  $M_w$  obtained.

Field studies of alginates and chitosans of different  $M_w$  were carried out as growth regulator for various plants like wheat, zea maize, faba bean, tomato, hot pepper, and banana seedlings. Treatment of wheat plant with degraded alginates at different growth stages improved wheat plant growth and increased its productivity yield. The wheat seeds treated with low  $M_w$  alginate were stored under normal conditions for 2 years. These stored seeds were then

cultivated and most of the seeds were found to still germinate. This implies that the treatment of the plants with low Mw alginate can preserve the embryo of the wheat seed which now has the capability to survive for a longer period of time. Also, the growth rate and productivity of the wheat plant obtained from those treated with low Mw alginate was higher than those obtained from seeds of untreated wheat. The low Mw alginate increased the wheat plant productivity, improved the quality of the crop, and enhanced the wheat seeds' capability to survive longer before cultivation.

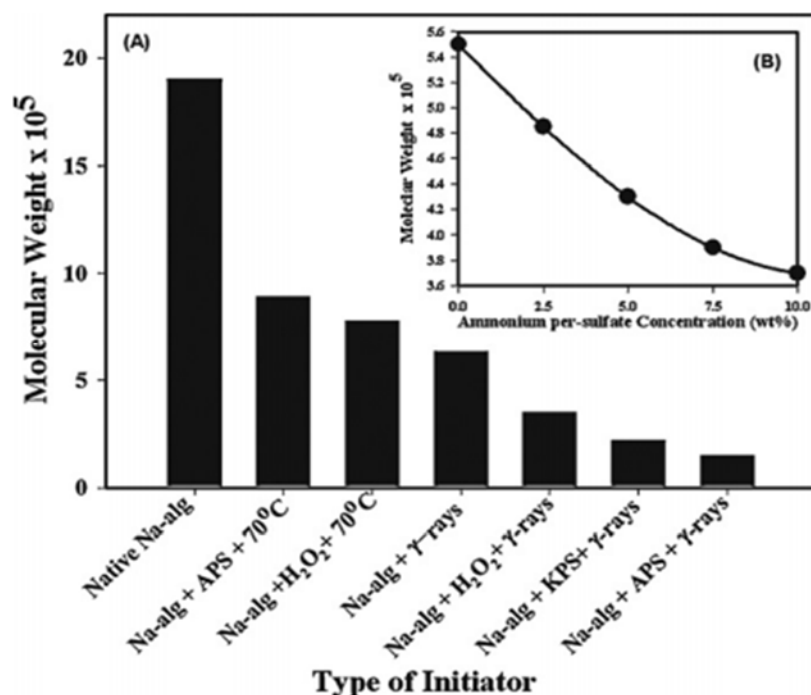


FIG. 8.1. (A) Effect of some oxidizing agents and ionizing radiation on degradation process of sodium-alginate. (B) Effect of ammonium per-sulfate concentration on degradation of molecular weight of sodium-alginate; irradiated dose 80 kGy.

The effects of different molecular weights of chitosan on the growth and productivity of the faba bean plant were investigated. The results showed that the treatment of the faba bean plant with irradiated chitosan not only enhanced the plant growth but also increased its productivity. The treatment of the faba bean plant by degraded chitosan had a positive effect on seed yields (ardab/fad), where ardab = 150 kg and fadan = 4200 square meters, giving a faba bean seed yield of 10.72 (ardab/Acre) as compared to the control with only 8.7 (ardab/Acre). Table 8.1 summarizes the effects of degraded chitosan and alginate on the growth and productivity yields of different plants. The increase in plant performance and production yield when degraded chitosan and alginate are used as growth promoters suggested their possible use for agriculture purposes.

The effects of sodium-alginate and chitosan binary mixtures with different molecular weights and ratios on the growth of the ze m maize plant were investigated (Fig. 8.2). Field test results showed that the spraying of plants with binary mixture of degraded sodium-alginate/chitosan with an average molecular weight of  $1.5 \times 10^4$  and  $9.4 \times 10^3$  Da, respectively, has a promising effect on the plant growth especially at a ratio of 1sodium-alginate / 2chitosan. This binary mixture ratio gave plant length (cm), paper width (cm), ear length (cm), 100 grain wt (g), ear

wt (g) and grain yield/acre (ardab) of 313, 12.2, 27, 40.54, 393 and 36.1, respectively, compared with the control with 266, 9.8, 25, 22.35, 25 and 23.45, respectively.

On a large-scale level, trials were made to incorporate degraded alginate and chitosan in tissue culture media of banana seedlings to enhance their growth and improve plant resistance against diseases like tobacco mosaic virus (TMV). In all media containing degraded alginate and/or chitosan, plant growth was better than those of the control ones. Banana plant growth on the media containing both degraded alginate and chitosan was better than that of media containing alginate or chitosan alone. After the cultivation of such seedling, it was found that the resistivity of cultivated banana plant treated with polysaccharides against TMV increased. This indicates that the incorporation of degraded alginate and chitosan in tissue culture media of banana plants aided in increasing the plant resistivity against TMV and controlling the plant infection by TMV.

TABLE 8.1. EFFECT OF DEGRADED NATURAL POLYMERS (100 PPM) ON CROPS YIELD OF SOME PLANTS

Plant type	Type of polysaccharide	Mwt of polysaccharide	Plant height (cm)	100 seed wt (g)	Biological yield Acre (ardab)	Straw yield Acre (ardab)	Seed yield/ Acre (ardab)
Faba		Control	100	57.25	23.9	15.2	8.7
Faba	Alginate	$7 \times 10^3$	129.85	78.56	33.04	22.11	11.32
Faba		Control	99.51	52.47	22.98	14.87	7.71
Faba	Chitosan	$1.44 \times 10^4$	125.18	72.35	32.34	21.62	10.72
Zea		Control	116.5	27.38	46.24	24.47	21.77
Zea	Alginate	$\times 1037$	229.6	34.85	63.52	33.11	30.41
Zea		Control	113.9	27.33	41.97	23.39	18.58
Zea	Chitosan	$1.44 \times 10^4$	210.1	34.01	54.34	28.55	25.79
Wheat		Control	90.06	4.52	18.52		10.5
Wheat	Chitosan	$\times 1042.1$	108.33	4.50	27.02		15.6

*Ardab~150 kg , fadan = 4200 square meters*

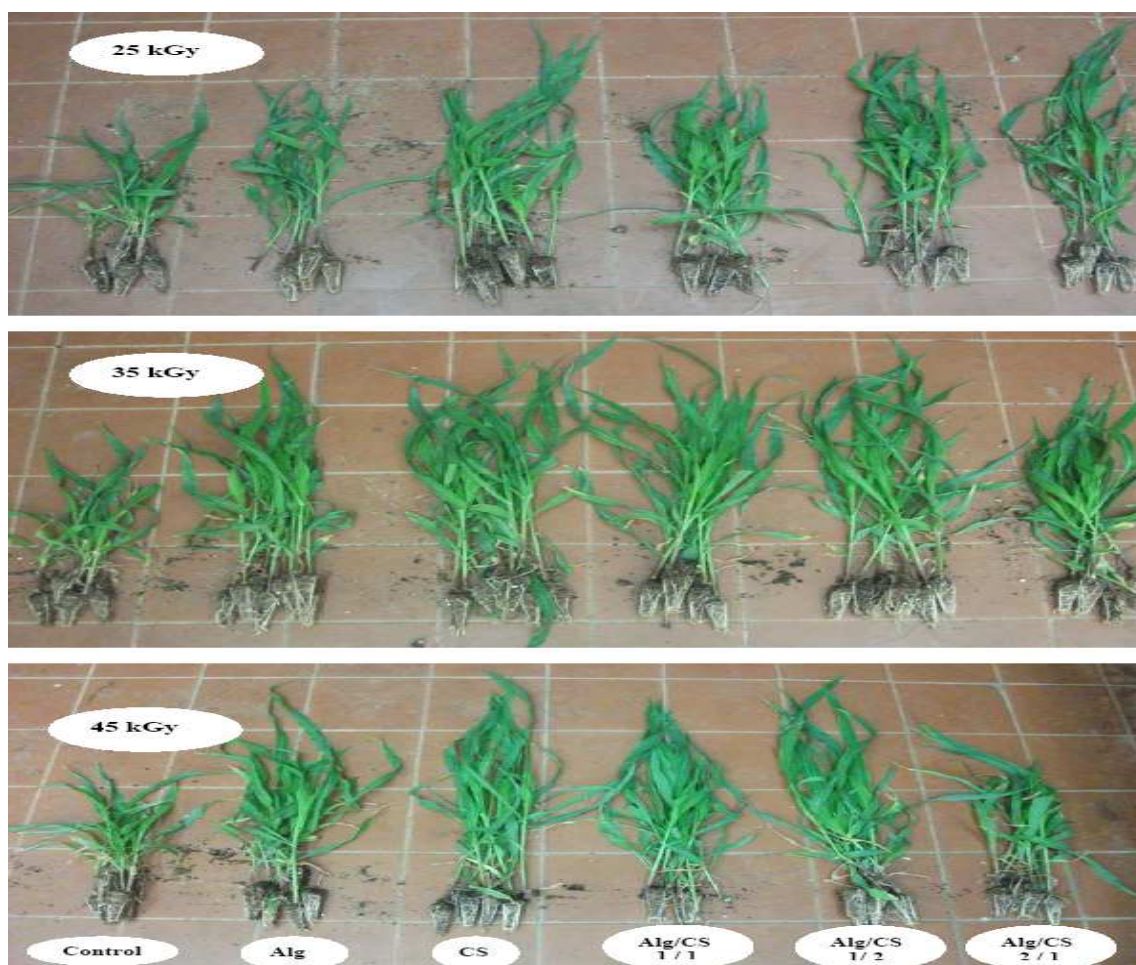


FIG. 8.2. Effect of degraded alginate and chitosan binary mixtures on *Zea mays* plant growth.

### 8.3.2. Usage of irradiated polysaccharides for stimulating defense reactions in some plants against some diseases

Gamma irradiated alginate has been investigated as an antiviral agent. The inhibitory effect of sodium- alginate against Tobacco Mosaic Virus (TMV) was assessed by inoculating leaves with TMV alone and with a TMV and sodium-alginate mixture. The inhibitory activity of sodium-alginate was calculated as the reduction percentage of the number of local regions produced on leaves after 3 days as compared with their controls. It was found that alginates with a relatively high molecular weight had great inhibitory effects against TMV. The highest inhibition degree was noticed when alginate of  $M_w 5 \times 10^4$  was used (chitosan irradiated at dose of 40 kGy). The degree of inhibition increased with alginate concentration. It was also noticed that the addition of alginate caused TMV to form large aggregates. The antiviral activity of alginate against TMV may have been caused by the blocking of the de-capsulation process of TMV protein on the cell membrane surface.

The old third generation banana trees are easily infected by TMV. As a result, many ranchers and farmers prefer to remove and totally wipe out these banana trees from the field and replace them with new banana seedlings. Third generation banana plants were sprayed with alginate solution in the months of March, April and May. It is during these months that there is a high probability of the banana plants being infected by TMV. It was found that the infection of the plant treated with alginate was significantly reduced to 8–10% against the

untreated plants which was in the range of 40–55%. These results indicated that alginates can greatly increase resistance against TMV infection. Treatment of tomato plants with irradiated alginate also enhanced the plant's resistance against infection with TMV. Moreover, treatment of tomato plants with alginate containing nano silver particles prepared by radiation was studied. It was found that the alginate-nano silver composite enhanced the plant's resistance against infection.

### **8.3.3. Effect of degraded chitosan on the controlling of gray mold fungus**

Gray mold disease caused by the *Botrytis* fungus is considered one of the more serious pathogens in the world. It induces decay in a large number of economically important fruits and vegetables during both the growing season and the post-harvest storage. It is also a major problem for long distance transport and storage. This fungal pathogen infects leaves, stems, flowers, and fruit either by direct penetration or through wounds caused by cultivation practices. The use of degraded chitosan to control tomato post-harvest gray mold was investigated. Degraded chitosan of different Mw enhanced and increased the productivity yield of tomato plant. Highest yield was obtained when chitosan with an Mw  $6 \times 10^4$  was used.

Degraded chitosan could also control the gray mold fungus that infected tomato fruits. The control of this disease is especially important during storage because the fungus develops at low temperatures (e.g.  $-0.5^\circ\text{C}$ ) and spreads quickly among fruits and vegetables. From the obtained results, it can be observed that the degraded chitosan enhanced fungal decay when used at high concentration doses. Chitosan with an Mw range of  $2-6 \times 10^4$  provided a good control of gray mold in tomato fruit among all different molecular weight chitosans.

*Fusarium* is a large genus of filamentous fungi widely distributed in soil and affecting many plants. Irradiated chitosan at different doses was tested against the *Fusarium fungus* which causes plant diseases. The chitosan of different Mw:  $7.3 \times 10^6$ ,  $1.4 \times 10^4$ ,  $5.9 \times 10^3$  and  $3.5 \times 10^3$  showed inhibitory effects on the *Fusarium* growth. It was also found that the most effective chitosan had an Mw of  $1.4 \times 10^4$  (Fig. 8.3).



FIG. 8.3. Effect of different MWts of chitosan on *Fusarium* growth (1)  $7.3 \times 10^6$ , (2)  $1.4 \times 10^4$  (3)  $5.9 \times 10^3$  and (4)  $3.5 \times 10^3$ .

#### 8.3.4. Preparation of super-absorbed hydrogel polymers

The preparation and characterization of superabsorbent hydrogels obtained through radiation and which induced the cross-linking of polyacrylamide (PAAm), poly(acrylic acid) (PAAc), poly(vinyl alcohol) (PVA), and potassium polyacrylate (PAAcK) were investigated individually and in (PAAm)– binary systems for possible uses in agricultural fields. The swelling of the investigated super-absorbed hydrogels was mainly related to the type of their hydrophilic functional groups and/or the presence of polarized charges.

The effect of preparation conditions such as irradiation dose and hydrogel blend compositions on water absorbency, gel content, and crosslinking density of PAAm/PAAcK copolymers were investigated. It was found that the higher the irradiation dose, the higher the gel content, and the lower the water absorbency. PAAm/PAAcK copolymer, possessing pore structure and fast swelling, was prepared by mixing the hydrogel components with a gas-forming agent, namely, ammonium carbonate.

The minimum time needed for super porous PAAm /PAAcK hydrogels to achieve the maximum swelling was 5–10 min, depending on the copolymer crosslinking density (Fig. 8.4a). This is due to its highly porous structure (Fig. 8.4b). PAAm/PAAcK irradiated in solid reached its equilibrium swelling within a few minutes, resulting in a drastic increase of its volume as compared to the unirradiated PAAm which dissolved in water (Fig. 8.5a). SEM analyses of irradiated PAAm, unirradiated and irradiated freeze dried PAAm hydrogels are shown in Fig. 8.5b. The irradiated powders show distinct aggregated particles while the unirradiated PAAm lost its particle shape to form a highly porous film. It is clear that the morphology of PAAm was affected by radiation. The network structure prevented the PAAm particles from dissolving in water, and kept their shape, although in distorted form.



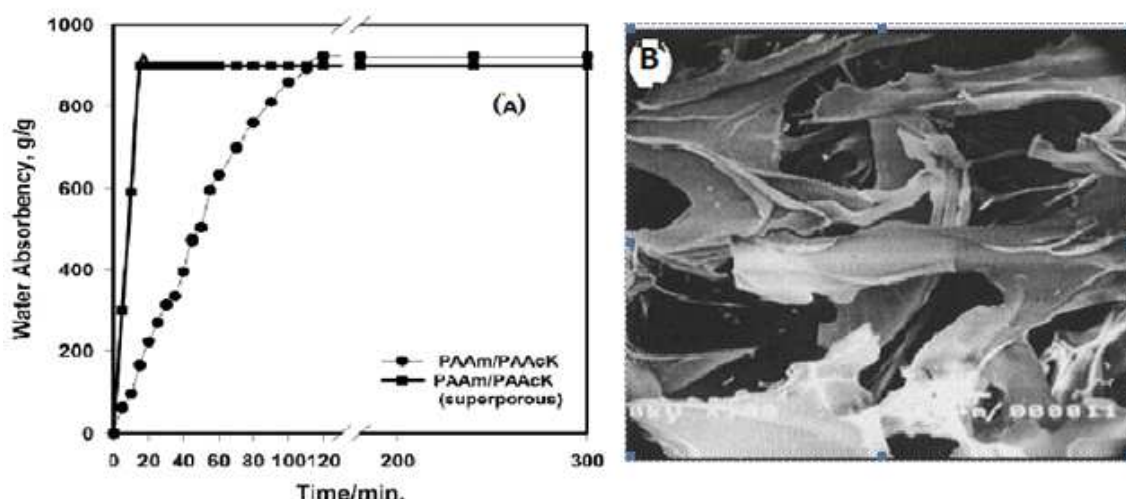


FIG. 8.4. Swelling rate of conventional and superporous PAAm/PAAcK hydrogels in distilled water (A) and morphology of PAAm/PAAcK hydrogel prepared at 30 kGy (B), (copolymer concentration: 4 wt %, copolymer composition: 25/75 wt/wt (PAAm/ PAAcK), dose: 30 kGy).

### 8.3.5. Salt effect on the hydrogel swelling

Fertilizers are often applied with hydrogels for use in landscaping. Mineral nutrients, including nitrogen in ammonium ( $\text{NH}_4^+$ ) or nitrate ( $\text{NO}_3^-$ ) form, potassium ( $\text{K}^+$ ), magnesium ( $\text{Mg}^{2+}$ ), and calcium ( $\text{Ca}^{2+}$ ) etc. exist in the growing medium solution as electrically charged ions.

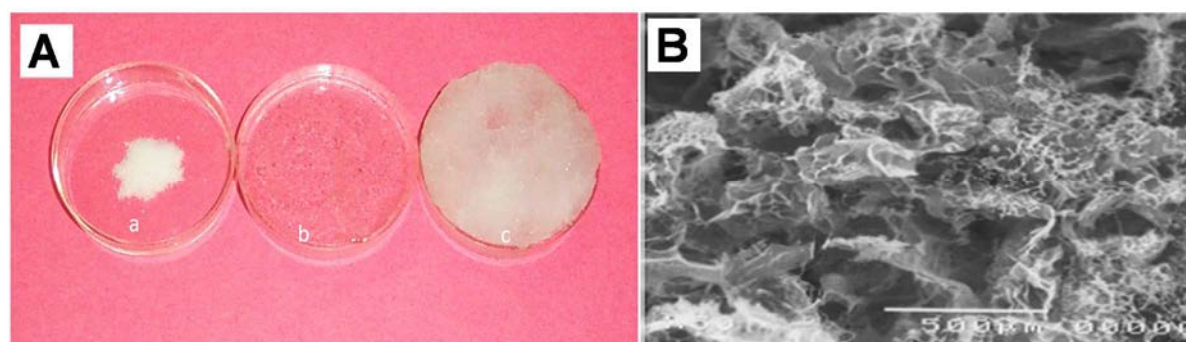


FIG. 8.5. (A) Effect of EB Irradiation on solid PAAm/PAAcK (a- powder , b- un irradiated , c- irradiated ), (B) SEM of PAAm/PAAcNa particles irradiated in a dry form at 30 kGy.

The water-holding capacity of hydrogels drops significantly at sites where the soil or source of irrigation water contains high levels of dissolved salts. PAAm/PAAcK is ionic in nature and undergoes a volume phase transition in response to a change in environmental conditions such as ionic strength and soil pH. The presence of the electrolyte in aqueous medium significantly affected the hydrogel absorption capacity. Type and valence of fertilizer ions had a great influence on PAAm/PAAcK swelling.



PAAm/PAAcK could be further cross-linked with multivalent ions such as  $\text{Fe}^{3+}$ . PAAm/PAAcK water absorbency decreased with an increase in the ionic valence of salt. The lower the atomic radius, the higher the hydrogel water absorbency. It may be recommended that the suitable forms for NPK fertilizers, in the presence of PAAm/PAAcK hydrogels, are urea,  $\text{H}_3\text{PO}_4$ ,  $\text{K}_2\text{SO}_4$  and  $\text{KNO}_3$  at which the PAAm/PAAcK absorption capacity is higher when compared with other inorganic fertilizers (Table 8.2).

TABLE 8.2. EFFECT OF DIFFERENT SALTS AND FERTILIZERS ON THE WATER-HOLDING CAPACITY OF PAAM/PAAcK HYDROGELS

Conc. (M)	Water absorbency (g/g)											
	Urea	NaCl	NH <sub>4</sub>	H <sub>3</sub> PO <sub>4</sub>	KCl	(NH <sub>4</sub> ) <sub>2</sub>	K <sub>2</sub> SO <sub>4</sub>	KH <sub>2</sub>	K <sub>2</sub>	NaH <sub>2</sub>	CaCl <sub>2</sub>	FeSO <sub>4</sub>
			NO <sub>3</sub>			SO <sub>4</sub>		PO <sub>4</sub>	HPO <sub>4</sub>	PO <sub>4</sub>		
10 <sup>-6</sup>	1043.0	615.0	520.0	470.0	480.0	460.0	455.0	440.0	415.0	395.5	368.4	332.5
10 <sup>-5</sup>	613.0	583.0	485.0	410.0	461.0	448.6	446.0	395.0	386.0	372.8	351.7	325.0
10 <sup>-4</sup>	601.0	523.0	376.0	370.0	420.0	420.2	418.0	352.0	345.0	342.9	316.5	318.0
10 <sup>-3</sup>	570.0	475.0	345.0	355.0	332.0	270.0	265.0	322.0	310.0	329.5	37.7	52.0
10 <sup>-2</sup>	490.0	330.0	286.0	265.0	240.0	145.2	143.0	230.0	205.0	150.5	4.5	6.9
10 <sup>-1</sup>	458.0	135.0	179.0	187.0	110.0	94.7	94.0	144.0	122.0	67.8	3.3	5.9
10 <sup>0</sup>	430.0	43.0	96.0	75.0	35.0	50.3	8.5	46.5	42.0	21.2	2.2	5.6

#### 8.4. APPLICATIONS OF COPOLYMER HYDROGELS IN AGRICULTURAL PURPOSES

##### 8.4.1. Effect of paam/paack hydrogels on growth and production of zea maze plant

The growth of a plant can be determined through the changes in its height, leaf width, and total dry weight. The presence of hydrogels in the soil increases the height, leaf width, total dry weight, and corncob production of Zea maze plants as compared with the control. The height average of a Zea maze plant cultivated in soil treated with different types of hydrogels decreases in the order: PAAm/PAAcK < PAAcK < PAAm. Twelve weeks after cultivation, the average plant height and leaf width (cm/week) of Zea maze plants treated with PAAm/PAAcK were almost 1.5 times higher than the control. Total dry weight of Zea maze plants treated with PAAm/PAAcK was also almost twice more than the control. The average double corncob production relative to single corncob production was higher for plants treated with different types of hydrogels as compared with that of control (Table 8.3).

TABLE 8.3. THE AVERAGE PLANT HEIGHT, LEAF WIDTH, TOTAL DRY WEIGHT, AND COB PRODUCTION OF CORN (ZEA MAYS) PLANT PLANTED IN SOIL CONTAINING DIFFERENT TYPES AND AMOUNTS OF HYDROGELS

Hydrogel Types	Hydrogel rate (g/m <sup>3</sup> )	Average total dry weight (g)	Average plant height (cm) at 12 weeks	Average leaf width (cm) at 12 weeks	Double corn cob (%)
Control	0	18.6	95	5.3	8.3
PAAm	6	24.0	115	7.0	41.6
PAAcK	6	26.6	120	7.7	41.6
PAAm/PAAcK	6	34.0	143	8.7	50.0
PAAm/PAAcK	9	41.0	170	10.6	58.0
PAAm/PAAcK	15	47.0	176	11.4	58.0

#### 8.4.2. Effect of hydrogels on the time to wilting for plant

The shelf-life (time to wilting) of Zea maize plants grown in sandy soil treated with different types of hydrogels such as PAAm, PAAcK, and PAAm/PAAcK was investigated and results are shown in Table (8.4). The time to wilting of Zea maize plants cultivated in the presence of hydrogels was longer than that of the control and increased in the order of PAAm > PAAcK > PAAcK/PAAm. Increasing the amount of gel in the soil led to a significant increase in the time to wilting of the Zea maize plants. The use of PAAm/PAAcK hydrogel at 6 g/m<sup>3</sup> increased the time to wilting of Zea maize by 50% against the wilting time of the control. The time to wilting for Zea maize plants planted in sandy soil treated with PAAm/PAAcK hydrogel at 15 g/m<sup>3</sup> increased by almost 65% as compared to the control. The magnitude of the plant wilting response may be due to the fact that, as the amount of gel increases, soil water retention also increases, which results in an increase of the available water uptake for plants, thereby delaying the wilting of the Zea maize plant.

TABLE 8.4. TIME TO WILTING OF CORN (ZEA MAYS) PLANT PLANTED IN SANDY SOIL TREATED WITH DIFFERENT TYPES AND RATES OF HYDROGELS

Hydrogel Types	Hydrogel rate (g/m <sup>3</sup> )	Time to wilting (h)
Control	0	288
PAAm	6	360
PAAcK	6	384
PAAm/PAAcK	6	432
PAAm/PAAcK	9	456
PAAm/PAAcK	15	474

The effect of pure sandy soil (control) and those treated with P(AAm–Na–AAc) microgels on the vegetative growth and time to wilting of radish plants was also investigated. It is clear that the presence of hydrogels in the soil enhances radish plant growth and performance (Fig. 8.6). Also, radish plants planted in sandy soil treated with P(AAm–Na–AAc) microgel took a long time to wilt (14 days) as compared with the control (9 days). This means that the soil treated with poly(acrylamide-sodium-acrylic) P(AAm–Na–AAc) microgel possesses a high ability to absorb and retain water, and significantly delays plant losses due to drought and water stress conditions. Thus, the survival rate of the plants is much improved.



FIG. 8.6. Radish plants planted in soil: (A) untreated (control), (B) treated with  $P(AAm-Na-AAc)$ .

Radiation induced cross-linking of PAAm and PAAm-alginate copolymers was investigated for their use as a possible soil conditioner. The effect of preparation conditions on the properties of the obtained hydrogel was studied. The addition of cross-linked PAAm and PAAm/sodium-alginate copolymer in small amounts to sandy soils could increase the retention of water and minimize evaporation losses. The water retention of different amounts of PAAm/sodium-alginate mixed with sand was investigated at various times (Fig. 8.7). Water retention of sandy soil was much lower than those added with hydrogel. As the amount of gel in soil increased, water retention increased.

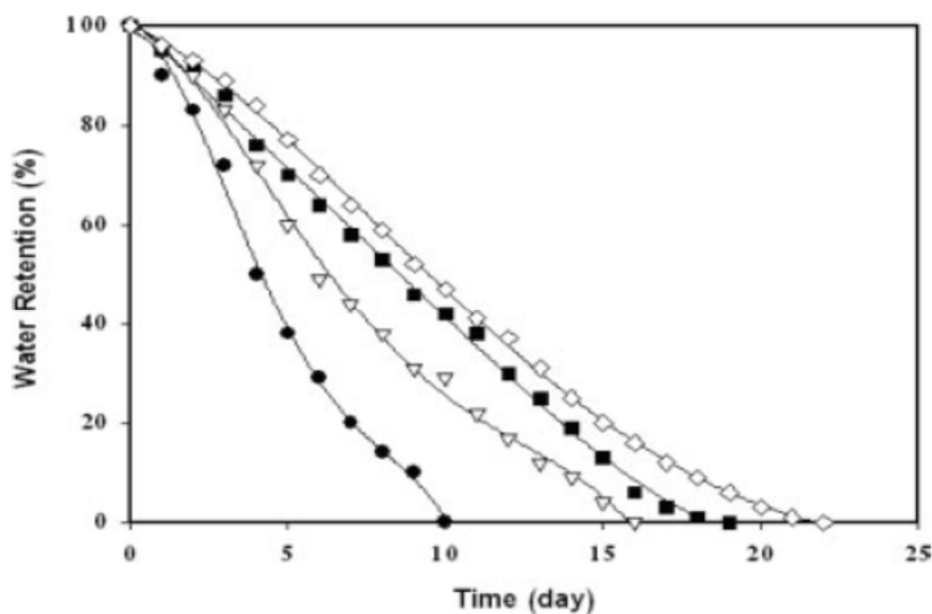


FIG. 8.7. Water retention of sandy soil containing different amounts of PAAm/Na-alginate hydrogel: (●) sandy soil without hydrogel (control); (Δ) containing 0.5%, (■) containing 1%, and (◊) containing 2% PAAm/Na-alginate composition (80/20 wt/wt).

Field evaluation of the PAAm/sodium-alginate copolymer for its possible uses in agriculture as a soil conditioner was performed in garden plants using bean as a model plant. As shown

in Table 8.5 and Fig. 8.8, it is clear that the growth and total dry weight of the bean plant cultivated in the soil treated with PAAm and PAAm sodium-alginate hydrogels were higher than those of the bean plant cultivated in gel-free soil (control). Also, the growth of the plant treated with PAAm sodium-alginate was greater than that of the plant treated only with PAAm. The most significant difference between the PAAm and PAAm-alginate copolymer is that the copolymer is partially undergoing degradation to produce oligo-alginate, which acts as a plant growth promoter and greatly improves plant performance.

TABLE 8.5. TOTAL DRY WEIGHT OF FABA BEAN PLANT PLANTED IN SOIL CONTAINING DIFFERENT TYPES OF HYDROGELS, 12 WEEKS AFTER PLANTING

Hydrogels	Total dry weight/g
Control	21
PAAm	30
PAAm/Na-alginate	39



FIG. 8.8. Faba bean plant planted in soil: (a) untreated (control); (b) treated with PAAm/sodium-alginate gel. PAAm/sodium-alginate of composition (80/20, wt/wt) was prepared at 10 kGy irradiation dose.

Field test evaluation of copolymers based PAAm containing alginate (Alg) and/or chitosan (Cs) was performed using Zea maize as a model plant for their possible uses as soil conditioner. The plant response can be detected through their change in height, leaf width, and total plant dry weight. As shown in Fig. 8.9 and Table 8.6, the presence of hydrogels mixed with the soil improved the plant length and leaf width. The growth of the Zea maize plant cultivated in soil containing PAAm/Alg was higher than the plant cultivated in gel-free soil (control). Response of plants cultivated in soil mixed with PAAm/Cs was lowest as compared to that of plants planted in soil containing PAAm/Alg/Cs and PAAm/Alg. The response of plants towards the different hydrogels used followed the order of PAAm/Alg >

PAAm/Alg/CS > PAAm/CS. The Grain yield/acre for the plant cultivated in soil mixed with PAAm/Alg increased by 50% as compared with the control. The use of PAAm as soil conditioner can be increased and its efficiency on plants can greatly be improved when mixed with natural polymers like Na-alginate or chitosan. The copolymers supply the plant with water and growth regulator. The cost of PAAm/Na-alginate hydrogel is considered to be low as compared to other commercial superabsorbent considering its effect on the increase in the productivity in plants.



FIG. 8.9. (a) control, (b) AAm-ALg, (c) AAm-Cs, (d) AAm-ALg/CS.

TABLE 8.6. EFFECT OF DIFFERENT TYPES OF HYDROGELS ON GROWTH AND PRODUCTIVITY OF ZEA MAIZE PLANTS PLANTED IN SOIL

	Plant length (cm)	Leaf width (cm)	Ear length (cm)	Ear radius (cm)	No. of rows/ear	100 grain wt (g)	Ear wt (g)	Grain yield/acre (ardab)
Control	240	9.0	24.5	7.0	12	28.05	225	24.05
PAAm/Alg	302	13.0	28.2	9.2	14	39.39	345	36.88
PAAm/CS	282	10.5	24.2	7.5	12	34.31	255	27.26
PAAm/Alg/CS	297	11.4	27.0	8.7	14	37.65	295	31.50

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## Chapter 9

### PRODUCTS BASED ON BIO-RESOURCED MATERIALS FOR AGRICULTURE. RADIATION PROCESSED BIODEGRADABLE POLYMERS, PLANT GROWTH PROMOTERS AND SUPERABSORBENT POLYMERS

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#### Abstract

Radiation-processed natural polymers and their derivatives, namely starch, alginate, chitosan and carboxymethyl cellulose (CMC) were explored for different agricultural applications such as biodegradable mulch films, super adsorbent polymers (SAPs), and plant growth promoters (PGPs). It was observed that gamma radiation-processed starch can lead to a better processability of starch/synthetic polymer alloys, and can offer tuneable biodegradability (as low as one month) with acceptable physico-mechanical properties. Acrylic acid/CMC-based SAP was prepared using  $^{60}\text{Co}$  gamma radiation, for soil conditioning. The equilibrium degree of swelling (EDS) of the acrylic acid/CMC SAP was found to be 460 g/g. The field trial of the SAP was conducted on sorghum. It was found that, with the use of 20 kg/ha of SAP, the crop yield can be increased by almost 18.5% whereas the increase in plant height was 8.5%. A new super adsorbent polymer with a much higher water uptake capacity was also developed by adding a small fraction of carrageenan to neutralized acrylic acid (AA). This SAP had EDS of 800 g/g, with the addition of only 1% carrageenan. Experiments to check the soil conditioning efficacy of AA/carrageenan SAP are in progress. Oligomers of chitosan and alginates were prepared by gamma irradiation and were tried as plant growth promoters in wheat (*Triticum aestivum*), mung bean (*Vigna radiata*), linseed (*Linum usitatissimum*), mentha (*Mentha arvensis*), and lemon grass. The results suggest that these oligomers have a significant impact on the grain and oil yield. Large scale field trials on *Mentha arvensis* in collaboration with an industry are in progress, and efforts are going on to formulate a policy framework for the use of oligosaccharides as plant growth promoters.

#### 9.1. INTRODUCTION

Chitosan, starch, and sodium alginate are natural polysaccharides derived from plants, crustacean shells, and algae, respectively. These polymers are available in large quantities and can be used in different applications after gamma or electron beam irradiation. Sodium alginate and chitosan in degraded form have been reported to aid in plant growth promotion; on the other hand, it is expected that radiation degradation of starch might affect its compatibility with various synthetic polymers, leading to reduction in the cost and improvement in the process parameters [9.1–9.8].

High energy radiation is an effective technology used to develop radiation degraded polysaccharides. Compared to the conventional techniques such as acid, base or enzymatic hydrolysis, radiation processing has several process advantages. It is an additive-free single-step process used for the degradation of these polymers and the production of low molecular weight oligomers. The low operation cost, additive-free technique, and room temperature operations are among the added advantages of radiation technology over other techniques [9.9–9.10].

Mulch films are widely used in agriculture [9.11]. The demand to develop biodegradable mulch films is increasing, and these can have tuneable biodegradability, good mechanical properties, and cost effectiveness [9.12–9.14]. The development of a partially or fully biodegradable polymer material with good physico-mechanical properties, desired biodegradation behaviour, and a low cost has been a challenge until now [9.8, 9.15–9.19]. This is mainly due to the unavailability of a natural metabolism route for synthetic polymers and the inherent incompatibility of natural and synthetic polymers.



Polyolefin/natural polymer blends have gained considerable interest in this regard, as these blends are expected to be partially biodegradable and cost effective. Moreover, microbial consumption of the natural polymer component in polyolefin/natural polymer blends is expected to show increased porosity, void formation, and the loss of integrity in the polyolefin phase, eventually increasing the rate of oxidative degradation of polyolefin. Starch is easily metabolized by a wide range of microorganisms and, unlike other natural polymers, can be processed as a thermoplastic material after suitable plasticization. Further studies are needed to explore the applications of high-energy radiation in this important area.

Radiation-degraded starch is used in developing biodegradable blends, alloys, and composites. In recent years, the blending of thermoplastic starch (TPS) with synthetic polymers has gained considerable importance in enhancing the biodegradation of LDPE at an acceptable cost without many inputs in synthetic polymers processing technology. However, on blending TPS with synthetic polymers, processability and mechanical properties of synthetic polymers deteriorate significantly due to poor interfacial compatibility. To some extent, such drawbacks are expected to be overcome using high energy radiation for blend modification, since high energy radiation can be effectively used to modify the interface and bulk properties of polymers. Solvation and other chemical reactions of cellulosic polymers have been reported to be less energy- and chemically intensive with the use of ionizing radiations. As stated above, high energy radiation can also be used to reduce or increase the molecular weight of polymeric material.

In this report, the use of high-energy radiation to modify the chain length and microstructure of starch, sodium alginate, and chitosan has been discussed. Efforts have also been made to establish the morphology–property correlation using differential scanning calorimetry (DSC), melt flow, scanning electron microscope (SEM), and soil burial studies. Special emphasis has been given to investigate whether the application of irradiated sodium alginate and chitosan could be used to enhance the growth, physiological activities, yield attributes, and the production of essential oils and other active constituents in wheat (*Triticum aestivum*), mung bean (*Vigna radiata*), linseed (*Linum usitatissimum*), mentha (*Mentha arvensis*), and lemon grass.

## 9.2. MATERIAL AND METHODS

### 9.2.1. Plant materials and growth conditions

For activities concerning plant growth promoters (PGP), the experiment was conducted in earthen pots (25 cm diameter × 25 cm height) in the natural conditions of the net house. Healthy rhizomes of plants were procured from a local supplier. They were surface-sterilized with HgCl<sub>2</sub> and then thoroughly washed. The soil samples were initially collected at random from different pots, and soil characteristics were analysed. A uniform recommended basal dose of N, P and K (25.0, 11.0 and 21.0 mg per kg soil, respectively) was applied in the form of urea, single superphosphate, and muriate of potash, respectively. Similarly, the experiments were conducted for wheat (*Triticum aestivum*), mung bean (*Vigna radiata*), linseed (*Linum usitatissimum*), and lemon grass. Field trials of SAPs were conducted on Sorghum (JOWAR) in Rajsathan, India.

### 9.2.2. Sample preparation of biodegradable blends

Starch samples were irradiated with different radiation doses in the dose range 5–50 kGy, and then plasticized with glycerol and water at 100°C to prepare thermoplastic starch (TPS).

Blends of TPS (made from irradiated and unirradiated starch) and synthetic polymers were prepared by mixing components in a Brabender plasticorder. The homogeneously mixed samples consequently formed were cut into small pieces, and compressed into sheets of size 12x12 cm<sup>2</sup> with different thicknesses in ranges 0.2–1 mm using a compression-molding machine at 150 kg/cm<sup>2</sup> pressure for 2 min at 130°C.

### **9.2.3. Irradiation**

Irradiation was performed under aerated conditions in a gamma chamber 5000 (GC-5000) with a <sup>60</sup>Co gamma source supplied by M/s BRIT, India. The dose rate of the gamma chamber was ascertained to be 2.5 kGy h<sup>-1</sup> by a Fricke dosimetry before the irradiation of samples. The starch samples in powder form were initially dried at 60°C for 24 h and later irradiated under sealed conditions.

### **9.2.4. Pot culture**

The pot experiment was conducted according to a simple randomized block design. Different concentrations of ISA were applied, using distilled water and unirradiated chitosan or alginate as absolute-control and sodium alginate-control, respectively. The ISA treatments were applied as foliar sprays to the crop using a hand sprayer. Plants were grown under naturally illuminated environmental conditions. Each treatment was replicated five times. Each pot contained a single healthy plant. The pots were watered as required. The crop performance was assessed in terms of growth attributes, physiological activities, herbage yield, and content as well as yield of the active constituents of the plant.

### **9.2.5. Determination of growth attributes**

The growth attributes such as plant height, leaf-area, leaf-yield per plant, and fresh and dry weights of the plant were determined. All leaves of the plant were weighed to determine leaf-yield. At 100 and 120 DAP, five plants of each treatment pot were uprooted and their roots were washed carefully with tap water to remove all adhering foreign particles. The height and fresh weight of the plants were measured. After taking each of the plant's fresh weight, the plants were dried at 80°C for 24 h and dry weight was recorded thereafter. Only 10% of the total leaves of each plant sample (consisting of five plants) were used to determine the leaf area using a graph paper. The mean area per leaf was then multiplied with the total number of leaves to obtain the leaf-area per plant.

### **9.2.6. X ray diffraction studies**

XRD patterns were recorded with a Philips X ray diffractometer PW 1710 (Almelo, Netherlands) using monochromatized CuK $\alpha$  radiation from an X ray generator operated at 30 kV and 20 mA.

### **9.2.7. Morphological studies**

Cryogenically fractured surfaces were examined by SEM. Acceleration voltages of 30 kV with a magnification range of from 200–5000 were used. The fractured surfaces were coated with a thin layer of gold before SEM examination.

### 9.2.8. Differential scanning calorimetry

Differential scanning calorimetry (DSC-822 from M/sMettler) of blends was performed to estimate changes in crystallinity of the blends. All specimens were initially scanned from 0–150 °C. Heat capacity thermograms for the second run have been reported. All thermograms were recorded under inert argon atmosphere. Before DSC run, the instrument was calibrated for temperature and heat flow using high purity indium standard. The crystallinity of the blends was estimated using the equation below:

$$X_c(\%) = \frac{\Delta H}{\Delta H^o} \times 100 \quad (1)$$

where  $\Delta H$  and  $\Delta H^o$  are the melting enthalpies of the sample and 100% crystallized sample, respectively.  $\Delta H$  was acquired from the integral area of a DSC heating curve associated with melting event and  $\Delta H^o$  for LDPE (100% crystalline) was assumed at 279 J/g.

### 9.2.9. Soil burial studies

The biodegradation behaviour of the blends was investigated through soil burial testing under natural flora and fauna conditions. Samples with a size of  $5 \times 3\text{ cm}^2$  and 0.2 mm thick were buried in the soil at a depth of 20 cm in properly marked plastic mesh trays, to allow the access of microorganisms and moisture. The plastic mesh allowed for the safe retrieval of samples after 3 months. After removal, samples were thoroughly washed first with tap water, then with distilled water, and dried at 60°C in a vacuum oven for 24 h. Weight difference (before and after burial) was determined gravimetrically.

## 9.3. RESULTS AND DISCUSSION

### 9.3.1. Growth, yield and quality attributes

The application of different concentrations of ISA significantly ( $P \leq 0.05$ ) improved the growth attributes of *Mentha arvensis* L. both at 100 and 120 DAP. There was a progressive increase in values with the increase in ISA concentration up to 100 mgL<sup>-1</sup>. Thereafter, the values declined significantly. The maximum values of growth characteristics were attained at 120 DAP with 100 mgL<sup>-1</sup> ISA. As compared to the control, application of 100 mg L<sup>-1</sup> ISA significantly increased the shoot length, leaf area, leaf yield, and fresh and dry weights of the plant.

Several exogenous and endogenous factors regulate the growth, development, and yield of a plant. Among the exogenous factors, various plant growth promoters are known to have direct or indirect influence on growth and development of the plant. The ISA treatment enhanced the leaf-area, which could provide an increased opportunity for light harvesting. This could eventually manifest itself in a significantly higher dry matter in the present investigation as compared to the control.

The irradiation of SA by gamma rays affects the overall polymer degradation process. Consequently, its application influences the biological properties of the plant cells. Previous studies have claimed that a range of concentrations of radiation-degraded sodium alginate depends on the source and dose of irradiation for a particular plant. However, the phenomenon that stimulates the processes related to the promotion of plant growth still needs further investigation. The application of ISA significantly increased its essential oil content and yield as when compared to the un-treated plants (control). Compared to the control, the

application of ISA at 100 mgL<sup>-1</sup> concentration significantly enhanced the herbage yield as well as content and yield of essential oil at 100 and 120 DAP, respectively (Table 9.1).

TABLE 9.1. EFFECT OF DIFFERENT CONCENTRATIONS OF IRRADIATED SODIUM ALGinate ON OIL CONTENT, OIL YIELD, MENTHOL CONTENT AND MENTHOL YIELD PER PLANT

Treatments (ppm)	Oil content (%)	Shoot fresh weight (g)	Oil yield (mL)	Menthol content (%)	Menthol yield (mL)
Control	0.65	38.6	0.25	75.98	0.18
UN – 20	0.64	38.0	0.24	71.16	0.17
IR – 20	0.69	42.0	0.28	71.68	0.20
IR – 40	0.74	49.2	0.36	69.51	0.25
IR – 60	0.81	55.4	0.44	69.89	0.30
IR – 80	0.94	58.4	0.54	67.30	0.36
IR – 100	0.95	62.2	0.59	68.59	0.40
IR – 120	0.87	37.0	0.32	68.38	0.21

Specific gravity and refractive index of the essential oil were not improved by ISA treatments at any sampling stage. As compared to the control, there was no progressive increase in the active components of the essential oils (particularly menthol content), when various ISA concentrations were applied to the foliage. However, application of ISA significantly enhanced other active constituents of the essential oil. The spray of ISA at 100 mg L<sup>-1</sup> increased the contents of L-menthone, isomenthone, and methyl acetate at 100 and 120 DAP.

Furthermore, 100 mg L<sup>-1</sup> ISA considerably increased the yields of menthol and the content of L-menthone, isomenthone, and menthyl acetate at both the stages. For the lemon grass, the effect of different concentrations of irradiated sodium alginate on oil content, oil yield, citral content, and citral yield per plant has been shown in Table 9.2. It is evident that its effect on plant growth is very high in lemon grass.

TABLE 9.2. EFFECT OF DIFFERENT CONCENTRATIONS OF IRRADIATED SODIUM ALGinate ON OIL CONTENT, OIL YIELD, CITRAL CONTENT AND CITRAL YIELD PER PLANT

Treatments (ppm)	Oil content (%)	Shoot fresh Weight (g)	Oil yield (mL)	Citral content (%)	Citral yield (mL)
Control	0.61	28.0	0.17	40.39	0.07
UN – 20	0.60	30.2	0.18	47.10	0.08
IR – 20	0.64	37.5	0.24	44.70	0.10
IR – 30	0.64	49.2	0.31	37.07	0.11
IR – 40	0.66	53.1	0.35	40.90	0.14
IR – 50	0.68	56.3	0.38	38.25	0.15

IR – 60	0.71	67.3	0.47	36.80	0.17
IR – 70	0.70	46.6	0.33	40.24	0.13
IR – 80	0.65	38.3	0.25	43.05	0.11

### 9.3.2. Poly (acrylic acid)-carrageenan superabsorbent hydrogels

A highly efficient super absorbent was developed by 15% partially neutralized acrylic acid and 1–5% carrageenan. Even 1% carrageenan (sodium salt) increases the equilibrium degree of swelling (EDS) of the hydrogels from 320–800 g/g. The response has been presented in the Fig. 9.1.

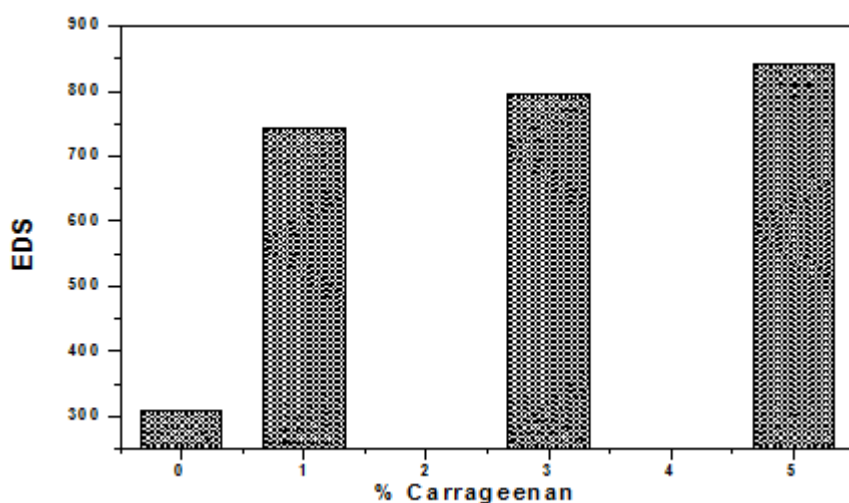


FIG. 9.1. Variation of equilibrium degree of swelling of poly (acrylic acid)-carrageenan superabsorbent hydrogels with carrageenan concentration.

### 9.3.3. Effect of irradiation on molecular weight of starch and mfi

The variation in molecular weight of starch with absorbed radiation doses is presented in Figure 9.2. The molecular weight of starch decreased sharply to up to 25 kGy followed by slower reduction at higher doses, indicating a predominantly radiation-induced degradation of starch on irradiation. Similar molecular weight reduction on irradiation to different extents of starch with different origins has been reported earlier. Figure 9.2 also shows the effect of addition of RTPS on MFI of LDPE matrix. MFI of LDPE/RTPS blend was higher than that of LDPE/TPS blend, and it showed a sustained increase together with an increase in the radiation dose. This suggests a higher mobility of polymer chains of blends in the presence of RTPS. This again can be explained on the basis of the discussion provided in earlier sections, that is: with an increase in the radiation dose, molecular weight of starch decreases, resulting in less hindrance from starch in the diffusion of LDPE polymer chains. As MFI can be correlated to material viscosity, these results also indicated that LDPE/RTPS had a lower viscosity than LDPE/TPS.

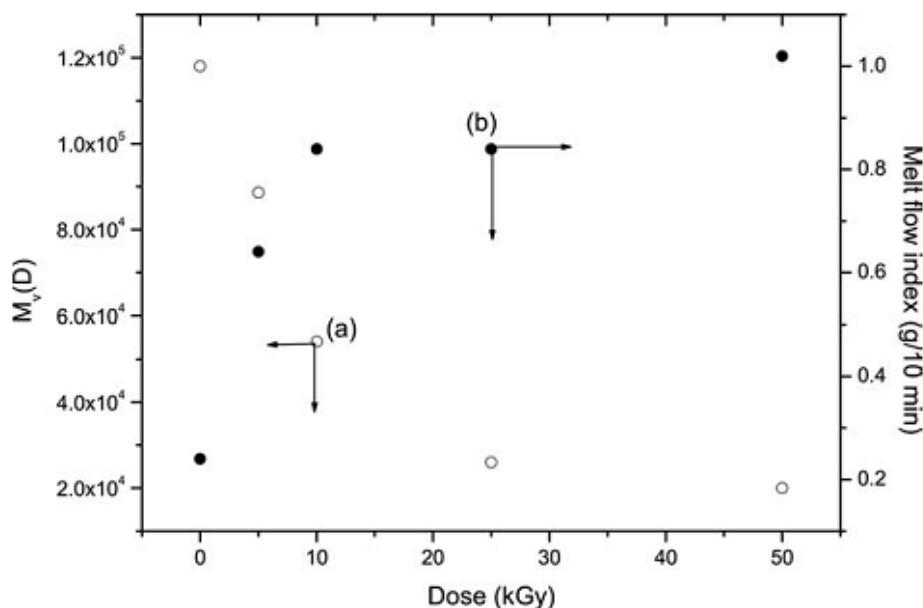


FIG. 9.2. Effect of irradiation on (a) change in the molecular weight and (b) MFI of LDPE/TPS.

#### 9.3.4. Thermal behaviour of ldpe/tps blends

Figure 3 depicts the crystallization and melting thermo-grams of LDPE/TPS blends. The crystallization temperature of LDPE shifts from 92.3°C in the neat state to 93.9°C in the blends. The shift of  $T_c$  to a higher temperature indicates an increase of the LDPE's crystallization rate on the incorporation of irradiated TPS, as the blend system is non-interactive. Crystallinity of LDPE also substantially increased when using irradiated starch. The result suggests a disruption in the crystallization at the LDPE phase due to the intrusion and intermingling of starch chains that had been reduced when starch that was irradiated to a higher dose was used. A similar increase in the crystallinity of the parent matrix with fillers has been reported earlier for other systems. This observation may be understood when taken with the fact that starch, being predominantly a degrading type of polymer, will undergo substantial chain scission after irradiation. Consequently, the low molecular weight starch produced on radiation degradation may not show any crystallization inhibitory effect as compared to the unirradiated starch of comparatively high molecular weight. On the other hand, the decrease in the melting temperature when irradiated starch was used suggested the low thermodynamic stability of LDPE crystallites. The low stability of LDPE crystallites in RTPS blends may be attributed to the wider molecular weight distribution of irradiated starch which results in imperfect crystallization, although it increases the crystalline content of LDPE. It may also be noted that a maximum change in the crystallinity was observed at high doses (50 kGy), suggesting that a critical radiation dose might be necessary to observe such a phenomenon.

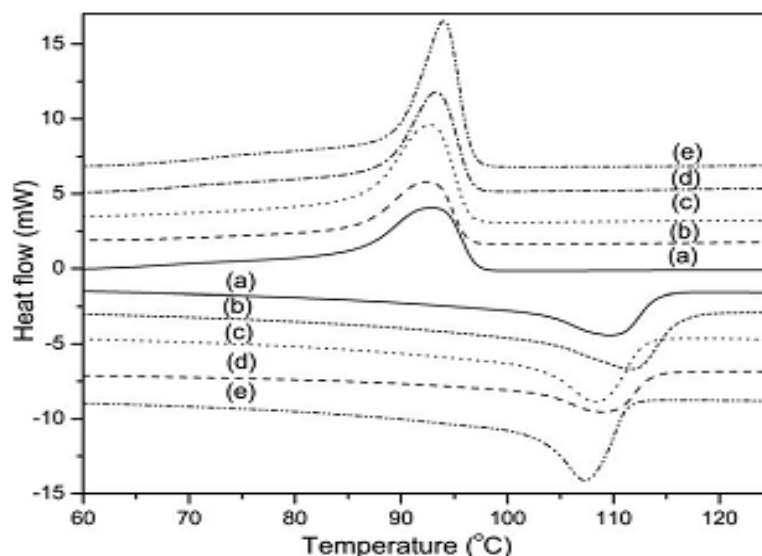


FIG. 9.3. Crystallization and melting thermograms of (a) LT0, (b) LT5, (c) LT10, (d) LT25, and (e) LT50.

### 9.3.5. Morphology of ldpe/tps blend

Scanning electron micrographs of fractured blend surfaces is shown in Figure 9.4. SEM of LDPE/TPS blend (0 kGy) showed substantial phase separation, whereas starch irradiated to 50 kGy in a matrix was uniform. The figures show a gradual improvement in the blend morphology. For samples in which starch is irradiated from 0–25 kGy, LDPE clearly exists as a continuous phase with TPS as dispersed phase, whereas at 50 kGy, it was relatively homogeneous. From SEM studies, it is difficult to draw a quantitative estimate due to asymmetric shapes and distribution of starch granules. The variation in the dispersion pattern of TPS in LDPE with the use of RTPS can be attributed to lower molecular weight starch chains formed upon radiation-induced degradation, which are easier to disperse. As discussed in the next section, the degradation pattern of 50 kGy samples buried in soil may indicate a fine dispersal of starch phase in LDPE phase, but the bond morphology is still not continuous.

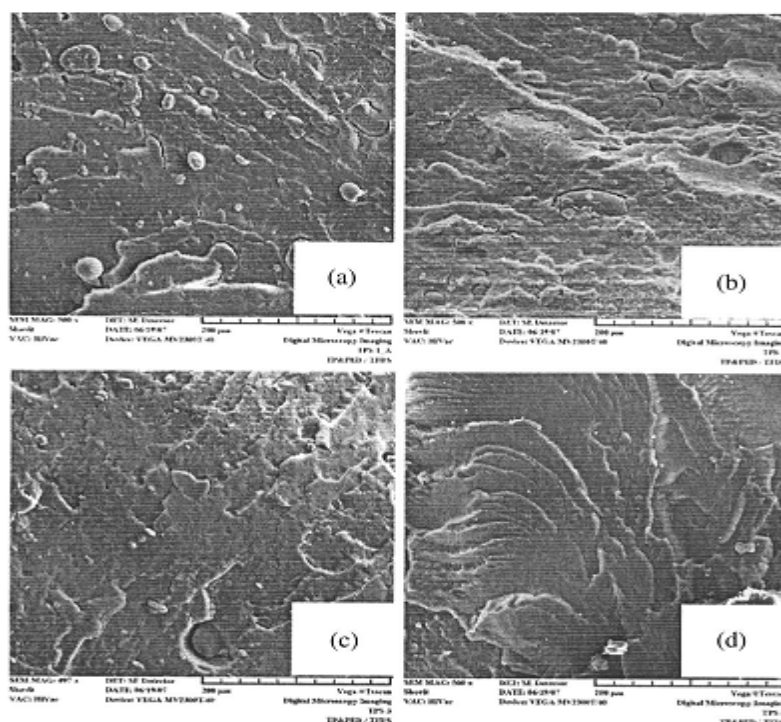


FIG. 9.4. Scanning electron micrographs of LDPE/RTPS blends (a) 0 kGy, (b) 10 kGy (c) 25 kGy and (d) 50 kGy.

### 9.3.6. Field trial of cmc based super absorbent

A super absorbent was developed using carboxymethyl cellulose (CMC) and acrylic acid. The equilibrium degree of swelling was 460 g/g. The effect of different amounts of this hydrogel on the crop yield of sorghum was investigated. The crop yield significantly increased when hydrogel was used. As can be seen in Table 9.3, both plant height and crop yield were dependent on the amount of hydrogel used.

TABLE 9.3. EFFECT OF CMC BASED SUPER ABSORBENT ON THE CROP YIELD OF SORGHUM

Treatments	Plant height	Crop yield
	(cm)	(q/ha)
Control	174.2	24.08
Hydrogel – 5 kg/ha	176.4	25.20
Hydrogel – 10 kg/ha	183.5	27.36
Hydrogel – 20 kg/ha	189.1	28.53

## 9.4. CONCLUSION

Recent work carried out on radiation-processed natural polymers and their derivatives suggests that oligomers produced after radiolytic degradation may be utilized in different agricultural applications. They can be used in biodegradable mulch films, super adsorbent polymers (SAPs) and plant growth promoters (PGPs). It was observed that gamma radiation-



processed starch can lead to better processability and improve the mechanical properties of starch/synthetic polymer alloys. It can also offer tuneable biodegradability with acceptable physicochemical properties. The field trial of the acrylic acid/ CMC SAP on sorghum suggested that, with the use of 20 kg/ha of SAP, the crop yield can be increased by almost 18.5%. A new superabsorbent polymer with a much higher water uptake capacity was also developed when a small fraction of carrageenan was added to a neutralized acrylic acid (AA). This SAP had an EDS of 800 g/g. Experiments to check the soil conditioning efficacy of AA/carrageenan SAP are in progress. Oligomers of chitosan and alginates were prepared by gamma irradiation. These radiation-processed oligomers demonstrated a plant growth promoter effect in wheat (*Triticum aestivum*), mung bean (*Vigna radiata*), linseed (*Linum usitatissimum*), mentha (*Mentha arvensis*), and lemon grass. The results also suggested that these oligomers have a significant impact on the grain and oil yield. Large-scale field trials in collaboration with an industry on *Mentha arvensis* are in progress. Considerable work in the conduct of additional large-scale trials on different crops, and in the formulation of a policy framework in the use of oligosaccharides as plant growth promoters is currently being carried out.

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## Chapter 10

### PREPARATION OF LOW MOLECULAR WEIGHT CHITOSAN BY RADIATION AND ITS APPLICATION FOR PLANT GROWTH PROMOTER

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#### Abstract

Chitosan was prepared through the alkaline deacetylation of chitin from shrimp shell waste. Chitosan with a degree of deacetylation of about 70% was produced by hot alkaline deacetylation (DDA) at 90°C for 8 hours or at room temperature deacetylation for 7 days. Through these processes, chitosan with an average molecular weight (Mw) of 141 k Dalton was obtained. Low molecular weight chitosan, Mw 14 k Dalton called “Fitosan” was prepared by irradiating chitosan using gamma rays at a dose of 75 kGy. The results showed that gamma irradiation is an effective method of degrading chitosan by cleavages of glycosidic bond. To improve crop yields and suppress diseases due to virus, bacteria, and fungi, Fitosan was successfully applied to chili, potato, and soybean. Socio-economic impacts of the use of Fitosan on the plants include increased income and improvement of the welfare of farmers.

#### 10.1. INTRODUCTION

Among the polysaccharides, cellulose and chitin are the two most abundant biopolymers in the biosphere [10.1–10.2]. Chitin is a copolymer of N-acetyl-D-glucosamine and D-glucosamine units links (1,4- $\beta$ ) glycosidic bond where N-acetyl-D-glucosamine is predominant in the polymeric chain [10.3]. Chitin commonly exists in crab and shrimp shells. It is also a major component of the cuticles of insects, fungal cell walls, yeast, and green algae [10.4–10.5]. The deacetylated form of chitin is known as chitosan.

In the past few decades, chitin and chitosan have attracted significant interest because of their wide range of applications. Chitosan has been largely employed in many areas, such as photography, biotechnology, cosmetics, food processing, biomedical products (artificial skin, wound dressing, contact lens, etc.), a system of controlled liberation of medicines (capsules and microcapsules), and the treatment of industrial effluents to remove metallic and coloring ions [10.6]. Due to the antifungal, antibacterial, and antiviral properties of chitosan, it is used as a plant protection, plant growth promoter, in soil correction, as an enhancer of secondary metabolites production, and activator of defense mechanisms in agriculture.

Indonesia is an archipelago that comprises approximately 17 508 islands [10.7]. Shrimp is considered as the “prima donna” commodity in the fisheries subsector, and it is hoped that its role as a major source of income in the country will be increased. This is because shrimp, which comprises a greater part of the fisheries product export, has a large potential both as an export product where the trend continues to increase, and in the local Indonesian market where it is commonly available [10.8]. Production of shrimp has increased significantly in the last 3 years. In 2012, it increased by about 32.87%, from 400 385 tons in 2011 to 457 600 tons in 2012 [10.9–10.10].

During the industrial processing of shrimps for human food, about 50% of the total mass is waste, of which half is chitin, a clear indication that Indonesia has so much source of chitin, especially from shrimp shell. It is believed that chitosan plays an important role in plant protection against diseases. Unfortunately, this waste product is not fully utilized.

Chitosan induces the phytoalexins' secondary metabolites accumulation by antifungal responses and increases their protection against further infections. Chitosan also has great potential to become an effective green biopesticide. As a biopesticide, chitin and chitosan can function as a seed-soaking agent, a root-applying agent, and a spray agent; all of these play an important role in plant disease control and stress resistance [10.11]. However, chitin is insoluble in common solvents and chitosan is likewise insoluble in either organic solvents or aqueous media at neutral and basic conditions, but is soluble in aqueous diluted acids [10.11–10.12].

The biological activity of chitosan depends significantly on its physico-chemical properties such as molecular weight and the degree of deacetylation. These factors can influence chitosan solubility and its interaction with the cell walls of target microorganisms [10.11]. It was reported that low molecular weight chitosan showed high activity against bacteria, yeast, and fungus and has a stronger growth stimulation effect in plants as compared to high molecular weight chitosan [10.13]. Low molecular weight chitosan can be produced through chemical or enzymatic depolymerization of chitosan. The chemical method can be performed by acidic or oxidative depolymerization. The acidic depolymerization is carried out through the use of a high concentration of acids such as hydrochloric, phosphoric, or sulfuric acid [10.14]. This method has several disadvantages such as harsh conditions for hydrolysis, low yields of low molecular weight chitosan, formation of by-products, and the need to remove used strong acids after purification [10.14].

Another method to depolymerize chitosan is high-energy radiation. Irradiation of chitosan using gamma ray or electron beam accelerator led to the reduction of molecular weight by scission of glycosidic linkages. As compared to the conventional techniques such as acid or base hydrolysis or enzymatic methods, radiation processing offers a clean one-step method for the formation of low molecular weight polysaccharides.

## 10.2. PREPARATION OF LOW MOLECULAR WEIGHT CHITOSAN

Low molecular weight chitosan was prepared by irradiating chitosan using gamma rays from Cobalt-60 source. Chitosan was produced by deacetylation of chitin using sodium hydroxide (NaOH) solution. All of the chemicals used were of technical grade.

### 10.2.1. Extraction of chitin from waste of shrimp shell

Shrimp shell waste materials were collected from Cirebon, West Java, Indonesia. The extraction of chitin from shrimp shell was done using 2 steps, namely: deproteination and demineralization. Chitin was extracted from shrimp shell by removing protein with 1 N NaOH aqueous solution at room temperature for 24 hours. The shells were then rinsed with water up to neutral pH. The deproteinated shells were then suspended in 1 N of hydrochloric acid (HCl) aqueous solution at room temperature for 24 hours to remove minerals, especially calcium carbonate. The residue was then collected and washed to neutrality in running tap water. It was then dried under the sun and the product obtained was chitin.

### 10.2.2. Deacetylation of chitin

Before the deacetylation process, chitin was cut using a mixer to produce chitin flakes with size of 1 mm. Chitosan was produced by the deacetylation of chitin under base condition using 50% of NaOH solution at room temperature or at 90°C. The chitosan produced was then collected and washed with hot water up to neutral pH followed by immersion in distilled water for 1 hour.

### 10.2.3. Irradiation of chitosan

Low molecular weight chitosan was prepared by irradiating chitosan flakes using gamma rays at various doses with a dose rate of 10 kGy/h.

### 10.2.4. Characterization of chitosan

#### 10.2.4.1. Determination of degree of deacetylation (DDA)

The deacetylation degree of chitosan was determined using an FTIR spectrometer (Shimadzu, Prestige 21). Infrared spectra were recorded using the FTIR spectrometer at a frequency range of 4000–400 cm<sup>-1</sup>. DDA-values were calculated using equation 1 [10.15],

$$DD = 100 - \frac{\left( A_{1665 \text{ cm}^{-1}} / A_{3450 \text{ cm}^{-1}} \right)}{1.33} \times 100 \quad (10.1)$$

where A<sub>1655</sub> and A<sub>3450</sub> are the absorbencies at 1655 cm<sup>-1</sup> (N-acetyl content) and 3450 cm<sup>-1</sup> (assigned to the hydroxyl group), respectively. A<sub>3450</sub> was used as an internal standard to correct any differences in the chitosan concentration in the KBr disk. Duplicate FTIR measurements were made for each sample.

#### 10.2.4.2. Determination of molecular weight and viscosity

The molecular weight of chitosan was determined by gel permeation chromatography. The column used was PL Aquagel-OH 30 with a flow rate of 0.5 ml/min and the solvent was acetic acid/Na acetate buffer. Poly(ethylene oxide) (PEO) was used as a standard. Viscosity of chitosan was measured using an Oswald capillary viscometer (Cannon 150/P863) at 25°C having flow time for pure solvent (distilled water),  $T_0 = 31$  seconds. Chitosan solutions of different concentrations in 0.2 M acetic acid and 0.1 M sodium acetate were prepared. During the preparation, all the solutions were magnetically stirred for 1 hour to ensure the proper dissolution of chitosan and were filtered using stainless steel 200 mesh screen. The flow times of chitosan solutions and solvent were recorded in triplicate and the average value was calculated. The relative viscosity  $\eta_r$  for a diluted polymer solution can be calculated from equation 2 [10.16],

$$\eta_r = \frac{\eta}{\eta_0} = \frac{t}{t_0} \quad (10.2)$$

where  $\eta$  and  $\eta_0$  are viscosities of the polymer solution and the pure solvent, respectively,  $t$  is the outflow time of the polymer solution, and  $t_0$  is the outflow time for the pure solvent. The specific viscosity,  $\eta_{sp}$ , was obtained from the relative increment in the viscosity of the polymer solution to the viscosity of the solvent as can be calculated from equation 3.

$$\eta_{sp} = \frac{\eta - \eta_0}{\eta_0} = \eta_r - 1 \quad (3)$$

The reduced viscosity (viscosity number),  $\eta_{red}$  is the specific viscosity per unit concentration  $C$  and can be calculated from equation 4.

$$\eta_{red} = \frac{\eta_{sp}}{c} = \frac{(\eta_r - 1)}{c} \quad (4)$$

The intrinsic viscosity,  $[\eta]$  can be determined from the reduced viscosity, equal to  $\frac{\eta_{sp}}{c}$  by extrapolation to zero concentration. To obtain an accurate value of the intrinsic viscosity of

the chitosan solution, therefore, its average extrapolated value to  $C = 0$  from the linear graphs of  $\ln nr/C$  and  $\frac{\eta_{sp}}{c}$  versus concentration was taken. [10.15–10.16].

#### 10.2.4.3.Evaluation of in vivo Toxicity of chitosan

Toxicity evaluation of low molecular weight of chitosan was done using the Sprague Dawley rat. The chitosan solutions were prepared by irradiating chitosan with gamma ray at a dose of 75 kGy. Two types of toxicity were evaluated, namely, acute oral toxicity and acute dermal toxicity. In the oral acute toxicity test, about 200 gram weights of the male Sprague Dawley rat were used. A maximum single dose of chitosan of 5000 mg/kg body weight and control were administered orally to each of 5 male Sprague Dawley rats. Examinations regarding clinical signs, body weights, and number of dead animals were conducted daily up to 14 days. After 14 days, pathological examinations of the visceral organ were carried out [10.17]. An acute dermal toxicity test was done using 200 gram weights of the male Sprague Dawley rat. A maximum single dose of chitosan of 2000 mg/kg body weight and control was administered dermally to each of the 5 male Sprague Dawley rats. Approximately 24 hours before the test, the fur of the test animals were shaved off from the dorsal area of the trunk and the animals were left for 24 hours [10.18]. A certain weight of chitosan solution in acetic acid was applied to the skin of the animal and covered with polyethylene (PE) plastic and gauze and left for 24 hours. After 24 hours, the cover was removed, and the skin was rinsed with water and evaluated for any changes. Examinations regarding clinical signs, body weights, and number of dead animals were conducted daily up to 14 days. After 14 days, pathological examinations of the visceral organ were carried out.

#### 10.2.4.4.Measurement of elemental content of chitosan

The elemental composition in chitosan was measured using appropriate methods. Nitrogen was analyzed using a Kjaldhal apparatus and phosphorus using a spectrophotometry. Other elements such as Fe, Mn, Cu, Na, K, Ca, Mg and Zn were analyzed using an atomic absorption spectroscopy (AAS). [10.19-10.20].

#### 10.2.4.5.Application of chitosan to plants

Irradiated chitosan with 75 kGy dose and concentration of 5% (w/v) was dissolved in dilute acetic acid. It was then packed in bottles with volumes of 250 mL, 500 mL and 1 L and named “FITOSAN” as can be seen in Figure 1. Fitosan was applied to several plants such as chili, potato, soybean, and rice as growth promoter, and also to reduce disease.



FIG. 10.1. Irradiated chitosan (“Fitosan”) in acetic acid solution for plant growth promoter.

### 10.3. RESULTS AND DISCUSSION

#### 10.3.1. Degree of deacetylation (DDA)

The DDA values of chitosan are summarized in Table 10.1. Chitosan was prepared by deacetylation of chitin using 50% of NaOH solution at room temperature. It took 7 days to get a DDA value of 69% while it took only 8 hours when the reaction temperature was raised to 90°C. The DDA value was also affected by the reaction time. Increasing the reaction time will increase the DDA value of chitosan. Form these results, it can be said that a relatively high temperature (90°C) of deacetylation is more effective than la ower temperature with regards to the time factor. Consequently, for the analysis of other characteristics of chitosan, deacetylation was done in alkaline media at 90°C for 8 hours.

TABLE 10.1. DDA VALUE OF CHITOSAN PREPARED AT DIFFERENT DEACETYLATION CONDITIONS

Deacetylation condition	Reaction time	DDA value (%)
At room temperature	4 days	64
	5 days	65
	7 days	69
At 90°C	5 hours	50
	8 hours	69
	15 hors	69
	20 hours	68

#### 10.3.2. Molecular weight and viscosity of chitosan

The weight average molecular weight and number average molecular weight of non-irradiated and irradiated chitosan by gamma ray at a dose of 75 kGy are shown in Table 10.2. The initial weight average molecular weight and number average molecular weight of chitosan are 141 kD and 41 kD, respectively. Irradiation of the chitosan flake with a dose of 75 kGy resulted in a reduction of weight average molecular weight and number average molecular weight to 16 kD and 7 kD, respectively. The decrease in molecular weight of chitosan is due to scission of 1-4 glycosidic bond of chitosan chain [10.13].

To evaluate the role of radiation on the degradation of chitosan, the viscosity of irradiated chitosan was measured. Figure 2 illustrates the relationship between the viscosity of the chitosan solution and the irradiation dose. The viscosity of chitosan decreased sharply up until 75 kGy and then gradually decreased with increasing doses. From these results, it can be said that irradiation is an effective method for chitosan degradation. The low molecular weight of chitosan may have several advantages for application in agriculture, one of which is to accelerate the growth of plants. Nagasawa et al. and Hien et al. reported that alginate degraded by radiation could accelerate the growth of plants [10.21–10.22].

TABLE 10.2. WEIGHT AVERAGE MOLECULAR WEIGHT AND NUMBER AVERAGE MOLECULAR WEIGHT OF CHITOSAN FOR DIFFERENT IRRADIATION DOSES

Irradiation dose (kGy)	Weight average molecular weight (kD)	Number average molecular weight (kD)
0	141	41
75	16	7

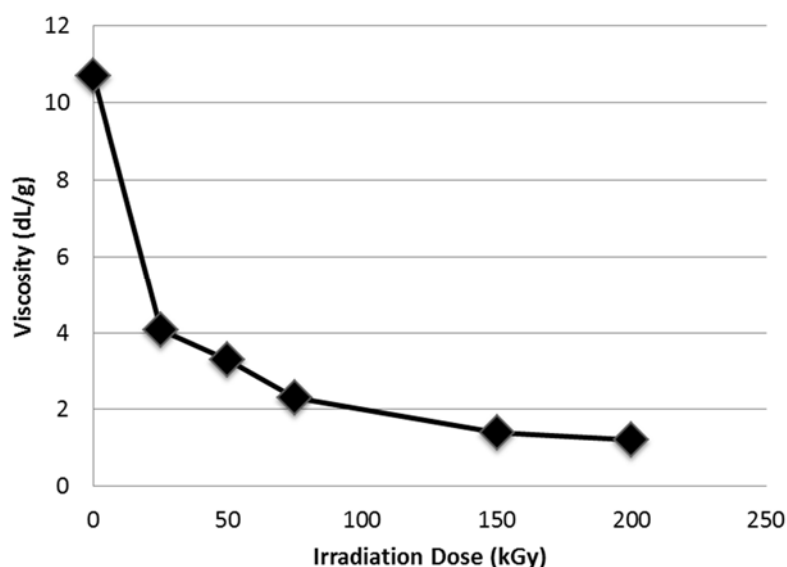


FIG. 10.2. Effect of irradiation dose on viscosity of chitosan.

### 10.3.3. Toxicity of chitosan

Toxicity is an important parameter when chitosan is used in agriculture and medical fields. According to the Indonesian Regulation on Pesticide, a plant growth regulator/promoter is categorized as a pesticide [10.23]. Acute oral toxicity and acute dermal toxicity are two types of toxicity which should be evaluated when chitosan will eventually be used as a pesticide (plant growth regulator or antibacterial) in agriculture. The in vivo acute oral toxicity and acute dermal toxicity of irradiated chitosan with 75 kGy was evaluated based on the OECD Series on testing and assessment number 24 and 402 [10.19–10.20]. The results showed that none of the animals died from acute oral toxicity and acute dermal toxicity. This is an indication that irradiated chitosan is categorized safe (nontoxic) for use as a pesticide (PGP) in agriculture.



#### 10.3.4. Elemental content of chitosan

The elemental content of chitosan was determined using the AAS as outlined in Table 3. It can be seen that chitosan contains all macronutrients (nitrogen, phosphorus, potassium) in relatively high amounts, secondary nutrients (sulfur, calcium, and magnesium), and micronutrients (cobalt, copper, iron, manganese, molybdenum and zinc) that are needed by plants to grow, and these nutrients are normally available in fertilizers. The availability of nutrients in chitosan probably increases plant growth. From the toxic metal analysis, it was confirmed that there is no toxic metal such as Pb and Cd in chitosan.

TABLE 10.3.ESSENTIAL OF MINERAL ELEMENTS CONTAINED IN CHITOSAN

Element	Quantity (ppm)
N	3356
P	0.5
K,	31
Ca	18.2
Mg	6.1
Fe	55.7
Mn	0
Cu	2.56
Zn	3.6
Na	153
Co	2
Cd	Not detected
Pb	Not detected

#### 10.3.5. Field test of chitosan

Fitosan is a chitosan irradiated at 75 kGy and prepared from the deacetylation of chitin using an NaOH solution at 90°C for 8 hours. Fitosan was tested in the field for several plants such as chili, potato, soybean, and rice.

##### 10.3.5.1.Chili plant

A field test was done at the Kerinci District, Jambi Province, Sumatra. About 4000 red chili plants were cultivated in 3200 square meters (m<sup>2</sup>). Chili seeds were coated by immersion into 50–100 ppm of Fitosan for 24 hours. After about 20 days seeding, the chilies were transplanted to the soil hole that had been previously prepared. During planting, Fitosan with a concentration of 50 ppm was foliar sprayed every 1 to 2 weeks. The Fitosan concentration was increased to up to 100 ppm when diseases started to attack the plants.

The results showed that the germination rates increased significantly and seedlings germinate quicker, better, and vigorously as can be seen in Fig. 10.3. It was also reported that the use of chitosan as seed coating for maize resulted in an enhanced germination index, reduced the mean germination time (MGT), and increased shoot height, root length, and shoot and root dry weights. The 0.50% chitosan seems to be a suitable concentration for the seed priming of

maize [10.24]. The results showed that applying 50 ppm Fitosan by foliar spray once a week could repress the incident of disease up to 85%. On the other hand, almost 100% of the chili without the treatment with Fitosan suffered from diseases resulting in zero crop yields.

Figure 4 shows chili treated with Fitosan and control (without Fitosan). Fitosan was also used as an anti-virus to the chlilli plants which were attacked by the leaf curl virus at the age of 25 days after planting. By spraying 100 ppm Fitosan 8 times, the diseases were reduced to 80% as can be seen in Fig. 10.5. Several advantages of using Fitosan in chili were observed. Among these were the significant increase in crop yields, improved crop quality, increase in harvesting times by as much as 23–25 times (without Fitosan, normal harvest of chili is 10–12 times), suppressed diseases, and improved appearance of chili fruits.



*FIG. 10.3. Seedling of chili after 2 weeks germination.*



FIG. 10.4. Chili treated with Fitosan (left) and without treatment (right).

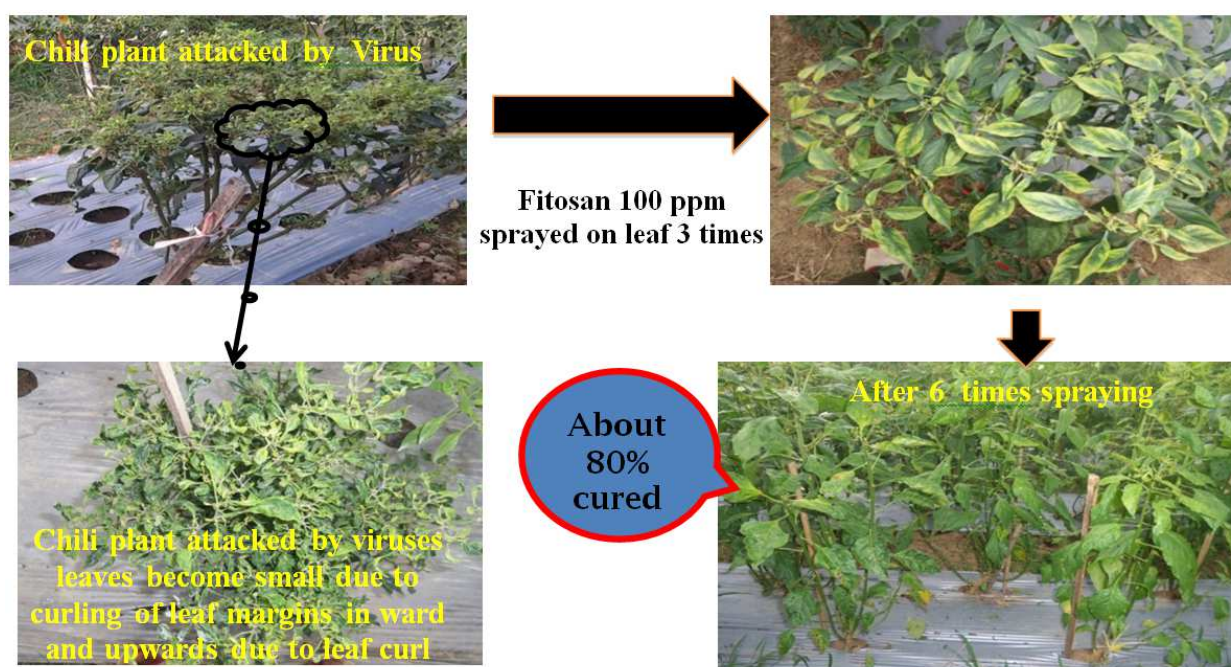


FIG. 10.5. Fitosan used as antiviral to chili plants. At 25 days, chili was attacked by leaves curl virus (left) and Fitosan administered 6 times (right).



#### 10.3.5.1.1. Socio-economy impact

The application of Fitosan to the chili plant has created a significant impact on the socio-economy of the country, and can be translated to an increase in the farmers' income. From the planting area of about 3200 m<sup>2</sup> with 4000 chili plants, the total production cost was US\$ 1200, including the US\$ 20 cost for Fitosan. The total crop yields from overall harvesting (24 harvesting times) was 1680 kg. If 1 kg of red chili is priced at US\$ 2, the farmers can get revenues of US\$ 3360 and the profit of farmers is US\$ 2160. On the contrary, there is almost no crop yield when Fitosan is not used. It can be estimated that, for a 1 hectare area, a farmer can already earn about US\$ 3360 × 3 = US\$ 10080. From this simple calculation, it can be said that Fitosan can give a significant impact on improving the welfare of farmers.

#### 10.3.5.2. Potato plant

Fitosan has been tested for the potato plant at Ciwide, West Java to improve production yields. Total plantation area is 0.6 Ha. Fitosan with a concentration of 100 ppm was foliar sprayed every 2 weeks. The production yield of potato treated with Fitosan and control is shown in Table 10.4 and Figure 10.6. The results showed that the yield increased by 33% and the tuber size was bigger than the control. In addition, the harvesting time was one month shorter for potato treated with Fitosan. Normally, potato can be harvested after 4 months of planting while potato treated by Fitosan can be harvested within only 3 months after planting.

TABLE 10.4. YIELD OF CROPS AND HARVESTING AGE OF POTATO TREATED BY FITOSAN AND CONTROL

Treated with Fitosan	Control	% Increase in Yield	Treated with Fitosan	Control
2.1	1.58	33	3	4



FIG. 10.6. Appearance potato treated by Fitosan and control.

#### 10.3.5.3. Soybean plant

A field test of Fitosan on soybean has been done in the Indralaya District, South Sumatra Province. In this experiment, the soybean Mitani variety was used and planting area was 1

hectare (ha). Fitosan with a concentration of 100–150 ppm was foliar sprayed. Fitosan was administered 4 times during one season planting. The production of soybean treated by Fitosan against control is shown in Table 10.5 and the demonstration plot of soybean is illustrated in Fig. 10.7. It can be shown that the soybean plant treated with Fitosan increased the yield to up to 38%, increased the number of pod/plant, and improved the quality of the pod.

TABLE 10.5. TOTAL PRODUCTION OF SOYBEAN MITANI VARIETY

Crop yields of soybean		
Treated with Fitosan (ton/ha)	Control (ton/ha)	% Increase in Yield
2.42	1.75	38



FIG. 10.7. Demonstration plots of soybean at Indralaya District, South Sumatra Province.

#### 10.4. CONCLUSION

Chitosan was prepared through the alkaline deacetylation of chitin obtained from shrimp shell waste. Chitosan with a degree of deacetylation of about 70% was produced through the hot alkaline deacetylation (DDA) at 90°C for 8 hours or at room temperature deacetylation for 7 days. Through these processes, chitosan with an average molecular weight (Mw) of 141 k Dalton was obtained. A low molecular weight chitosan, Mw 14 k Dalton called “Fitosan” was prepared by irradiating chitosan using gamma rays at a dose of 75 kGy. Gamma irradiation is an effective method used to degrade chitosan through the cleavage of glycosidic bonds. Fitosan was successfully applied to chili, potato, and soybean to improve crop yields

and suppress diseases due to virus, bacteria and fungi. The socio-economic impact of the use of Fitosan on the country shows an increase in income and an improvement in the welfare of farmers.

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## CHAPTER 11

### EXPLOITING NANOTECHNOLOGY AND RADIATION TECHNOLOGIES TO DEVELOP NEW ECO-SUSTAINABLE NANOMATERIALS FOR FOOD PACKAGING SUITABLE FOR STERILIZATION BY IRRADIATION

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#### Abstract

The objective of the study is to develop new food packaging nanomaterials based on biopolymers (PLA) to be used in pre-packaged foods intended for radiation. The processing conditions were optimized, and the properties of the systems before irradiation were studied. From these studies, the following conclusions were drawn: 1) The final properties of polymer nanocomposites depend on their structure and morphology, and 2) Improved properties can be achieved only if a good dispersion and distribution are realized in the polymer nanomaterials. The team at Istituto di Chimica e Tecnologia dei Polimeri, Consiglio Nazionale delle Ricerche (ICTP/CNR) participated in the IAEA CRP on “*Application of Radiation Processing Technology in the Development of Advanced Packaging Materials for Food Products*”.

#### 11.1. INTRODUCTION

Recent reports by the Food and Agriculture Organization of the United Nations (FAO) [11.1, 11.2] state that a huge amount of food (30–40% of the total) is lost between the production and consumption stages mainly because food is not handled and packed with care and can be easily contaminated with pathogenic microorganisms. The challenge now is to synergistically exploit pre-packaged food using two emerging technologies: irradiation procedures and packaging nanotechnology. Radiation technology, which has been endorsed as safe by the World Health Organization (WHO) and the European Food Safety Authority (EFSA) [11.3, 11.4], provides a drastic reduction of food microbial contamination. Packaging nanotechnology, on the other hand, can increase package performance further adding safety, economical and environmental advantages.

Recently, biodegradable polymers from renewable sources, along with polymers derived from oil, have been introduced in the market as food packaging materials. This has also partially solved the ecological problems caused by plastic waste accumulation. These polymers can be degraded after their disposal through the enzymatic action of microorganisms such as bacteria, fungi, and algae. One of the most common biodegradable polymers used in the packaging industry is polylactic acid (PLA), a thermoplastic polymer that belongs to the family of linear aliphatic polyesters and derived from renewable resources such as corn starch, tapioca roots, sugarcane, etc. Although these biodegradable polymers have excellent commercial potential as bio-plastics, some of their properties such as brittleness, low heat distortion temperature, high gas permeability, and low melt viscosity for further processing, etc. restrict their use for packaging. The improvement of the properties of PLA using nano reinforcement of pristine polymer in order to obtain nanocomposite has proven to be an effective way of improving its mechanical and barrier properties [11.5–11.7].

The ICTP/CNR is developing new nanomaterials based on PLA and titanium dioxide (TiO<sub>2</sub>) nanoparticles for food packaging that is suitable for irradiation to improve food safety, enhance shelf life, reduce post-harvest losses, and promote international trade. This paper reports the initial step of this work. It will describe in detail the optimization of the



processing conditions, the modification procedure for the TiO<sub>2</sub> nanoparticles, and the influence of the kind (unmodified, modified) and concentration of nanoparticles on the PLA properties. The properties of these samples after irradiation are also reported.

## 11.2. EXPERIMENTAL

### 11.2.1. Materials

The following materials were used for the PLA-based nanocomposites:

- Polylactic Acid (PLA 4032 Resinex , Mn  $1.3 \times 10^5$  and Mw  $2.1 \times 10^5$ )
- Hydrophilic titanium dioxide nanoparticles (TiO<sub>2</sub>) (Degussa P-25 standard, 99.5% purity, with average particle size 25 nm, 80% anatase and 20% rutile). TiO<sub>2</sub> was dried in the oven at 120°C under vacuum prior to use.

The modification of the nanoparticles was performed using a perfluorohexane monomer in the plasma depositions. For the synthesis of perfluoro functionalized TiO<sub>2</sub> nanoparticles, a custom-built 3600 rotating plasma reactor, operable under vacuum conditions, was used to functionalize the surface of pure TiO<sub>2</sub> nanoparticles. A schematic diagram of the apparatus is shown in Figure 1. The reactor details are similar to that previously described in reference 1. The plasma was generated using a radio frequency (RF, 13.56 MHz frequency) power input. One of the important steps in the successful surface functionalization of the TiO<sub>2</sub> involved an initial treatment of the particles with a short, high power O<sub>2</sub> plasma discharge to remove any adsorbed carbaceous materials and simultaneously activate the surfaces of these crystals.

The perfluorocarbon monomer was immediately introduced after the oxygen treatment. A high power continuous wave plasma discharge was employed for 1 min to graft a thin, strongly adherent, amorphous carbon-like layer to the TiO<sub>2</sub> particles. The function of this layer was to overcome the inherent chemical incompatibility between the inorganic particles and the plasma-deposited organic films. Subsequently, the RF power input was converted to a pulse operational mode, and the plasma duty cycle, i.e. ratio of plasma on to plasma off, was slowly decreased. This created a gradient layered structure in which the fluorine content of the plasma polymer film was slowly increased as the pulsed plasma duty cycle was decreased. The process was terminated at a plasma duty of 10 ms on and 100 ms off and peak power input of 200 W [11.8].

## 360° Rotating Pulsed RF Plasma Reactor

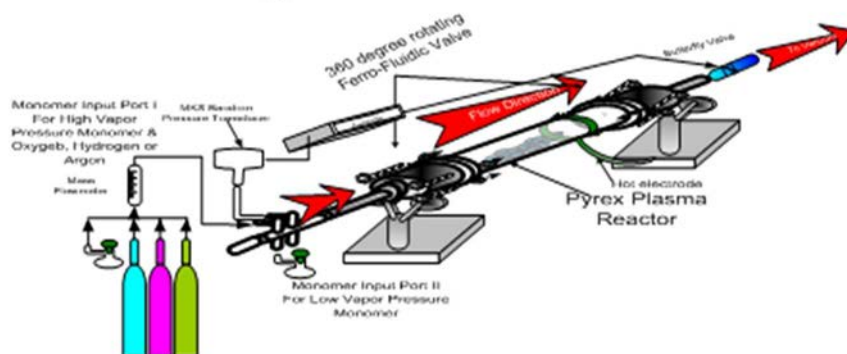


FIG. 11.1. Plasma Reactor.

### 11.2.2. Processing conditions

Polylactic acid and nanoparticles were compounded in a batch mixer at 180°C, 50 rpm for 15 minutes. The composition used is reported in Table 11.1.

TABLE 11.1. COMPOSITIONS OF THE PLA BASED SYSTEM

Sample	PLA (g)	TiO <sub>2</sub> (g)	TiO <sub>2</sub> Modified (g)
PLA	100	-	-
PLA/2%TiO <sub>2</sub>	100	2	-
PLA/2%TiO <sub>2</sub> Modified	100	2	-
PLA/5%TiO <sub>2</sub>	100	-	5
PLA/5%TiO <sub>2</sub> Modified	100	-	5

## 11.3. RESULTS AND DISCUSSION

### 11.3.1. Modification of tio<sub>2</sub> nanoparticles

The surface modification of the TiO<sub>2</sub> nanoparticles was successfully done as shown in Fig. 11.2. The spontaneous dispersion of the nanoparticles, before and after plasma treatment, with respect to a two layer liquid mixture consisting of water (bottom layer) and hexane (top layer) is shown here. The initial addition of untreated TiO<sub>2</sub> particles which are hydrophilic in nature clearly shows that they are dispersed selectively in the water layer (vial A). This conforms to expectations based on surface energy considerations. In sharp contrast, the particles exhibited absolute preference for the nonpolar hexane layer (vial C) following the plasma treatment that involved the deposition of the perfluorocarbon films on the TiO<sub>2</sub> substrates. A further illustration of the excellent controllability of surface energy modifications provided by this plasma approach is shown in vial B. TiO<sub>2</sub> samples which were treated with short perfluorocarbon plasma exhibited an intermediate surface energy. The TiO<sub>2</sub> modified particles indicated a virtual equal preference for the water and hexane layers.

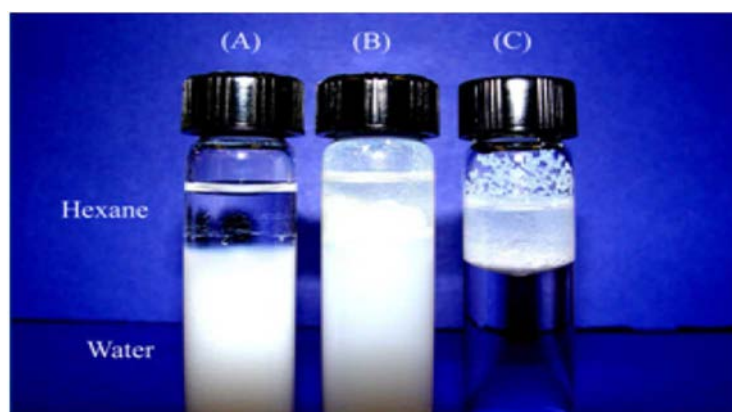


FIG. 11.2. Demonstration of the change in the surface energy of the  $\text{TiO}_2$  with plasma treatment.

### 11.3.2. Morphology

The SEM micrographs (Fig. 11.3) show a good dispersion and distribution of nanoparticles in the polymer matrix.

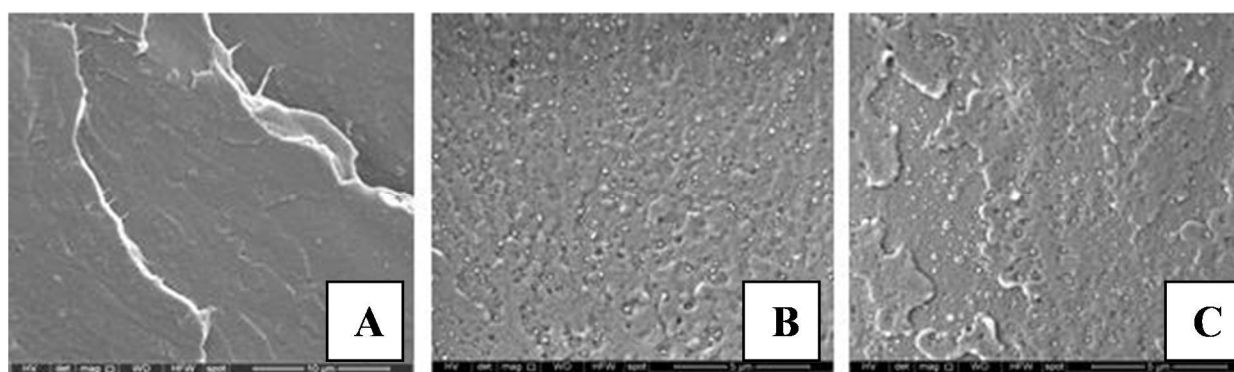


FIG. 11.3. SEM micrographs of PLA (a), (b) PLA/ $\text{TiO}_2$  5% (unmodified), (c) PLA/ $\text{TiO}_2$  5% (unmodified).

### 11.3.3. Thermal stability

Thermal Gravimetric Analysis (TGA) results are reported below. It is clear from the curves that the thermal stability of nanocomposites improved as compared to a neat PLA, and increased with increasing  $\text{TiO}_2$  and modified  $\text{TiO}_2$  concentrations.

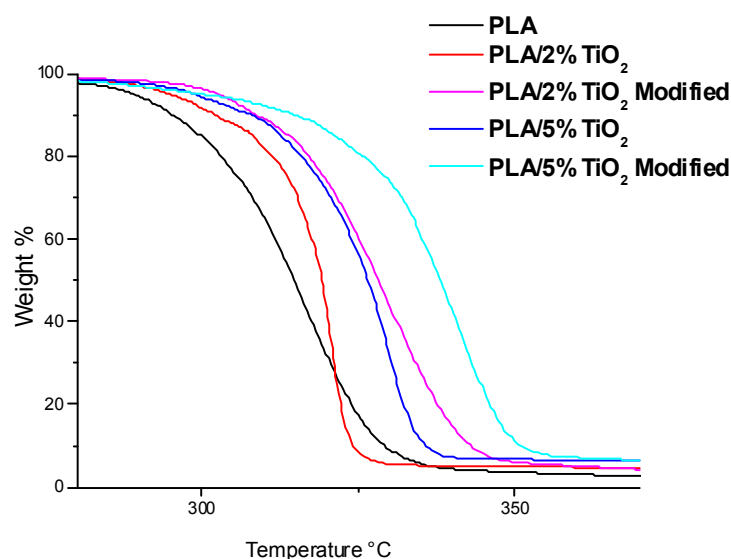


FIG. 11.4. Thermogravimetric curve of PLA and PLA nanocomposites.

#### 11.3.4. MECHANICAL AND BARRIER PROPERTIES

The impact test was determined by the Charpy impact test and the results are shown in Table 11.2. The samples with TiO<sub>2</sub> increased in toughness compared to neat PLA, and maximum increase was observed in 2% PLA/TiO<sub>2</sub> (unmodified and modified).

TABLE 11.2. IMPACT TEST

Sample	MW	Impact strength (KJ/m <sup>2</sup> )
PLA	12.3	1.0±0.1
PLA/ 2%TiO <sub>2</sub>	13.2	2.5±0.6
PLA/ 2%TiO <sub>2</sub> modified	14.3	2.7±0.3
PLA/ 5%TiO <sub>2</sub>	6.9	1.5±0.4
PLA/ 5%TiO <sub>2</sub> modified	8.4	2.0±0.2

The O<sub>2</sub> permeability measured by multiperm on nanocomposites films did not present any variation compared to neat PLA.

#### 11.4. CONCLUSIONS

From the results, the following conclusions can be made: a) despite the polarity mismatching between PLA and TiO<sub>2</sub>, a good distribution of particles in all nanocomposites has been obtained; b) the thermal stability of PLA/TiO<sub>2</sub> nanocomposites has been improved compared

to neat PLA as it increases with TiO<sub>2</sub> content and is better with modified TiO<sub>2</sub>; c) the nanocomposites increased in toughness compared to neat PLA; d) the best values were found for 2% PLA/TiO<sub>2</sub> (unmodified and modified); and e) TiO<sub>2</sub> nanoparticles do not modify the PLA O<sub>2</sub> permeability.

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## Chapter 12

### APPLICATIONS OF BIOPOLYMERS MODIFIED BY RADIATION PROCESSING

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#### Abstract

Radiation processing using quantum beam such as electron beam and gamma rays is a clean process. Using this process, biopolymers with low environmental burden were modified for agricultural and environmental applications. High performance materials such as soil conditioner for arid area, spray coating Washi (Japanese paper), biodegradable dummy lens, chemically-induced biodegradable plastic, biodiesel catalyst, and plant growth promoter were developed by radiation-induced crosslinking, graft polymerization, and degradation.

#### 12.1. INTRODUCTION

Products for agricultural and environmental applications in Japan are described with the end view of producing environmental-friendly processing and raw materials. Radiation processing of polymers is initiated by high-energy irradiation using quantum beam. Quantum beams are highly controlled artificial radiation such as ion/electron accelerators, artificial radioisotopes, ultra-high intensity laser systems, nuclear reactors etc. Takasaki Advanced Radiation Research Institute, Japan Atomic Energy Agency, Takasaki, Japan (TARRI, JAEA) has three kinds of the following quantum beam facilities for R&D of material science and biotechnology.

Gamma ray irradiation facility was installed in 1964 and the total activity of  $^{60}\text{Co}$  of  $3.2 \times 10^{16}$  Bq was stored in water pools in eight irradiation rooms. Dose rate is in the range of 0.04–20 kGy/h. The electron beam accelerator was installed in 1964 and renewed in 1981. This 2 MeV electron accelerator, Cockcroft-Walton circuit type, has horizontal and vertical acceleration tubes. The horizontal electron beam, 60 cm in scanning width, is used for irradiation of large volume gas. The vertical electron beam, 120 cm wide, irradiates sample on conveyors. Ion beam facility called TIARA (Takasaki Ion Accelerators for Advanced Radiation Application) consists of four accelerators, K110 AVF (Azimuthally Varying Field) cyclotron, 3MV tandem accelerator, 3 MV single-ended accelerator and 400 kV ion implanter. TIARA complex provides a great variety of ion species from proton to gold ion with a wide range of ion energy from keV to MeV. Quantum beams have various functions, such as "observe", "create (modify)" and "cure". Typical applications according to functions are shown in Fig.12.1.

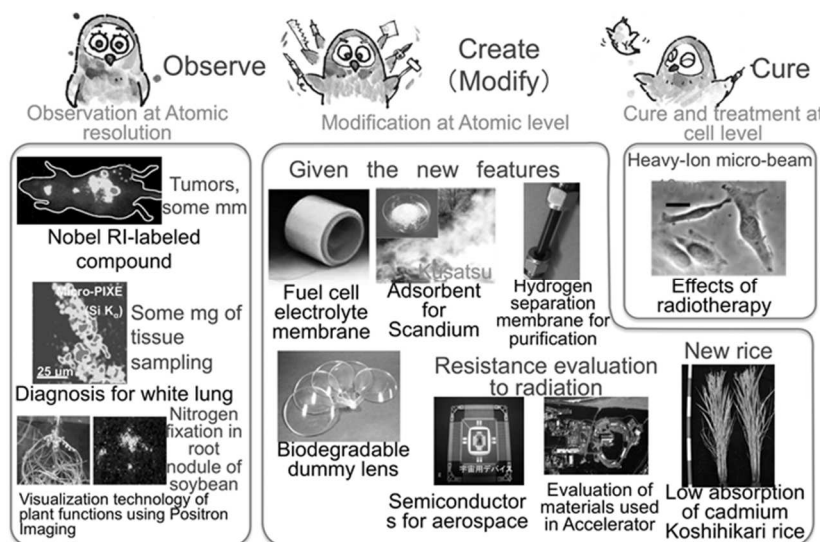


FIG. 12.1. Functions of quantum beam and corresponding applications in TARRI, JAEA.

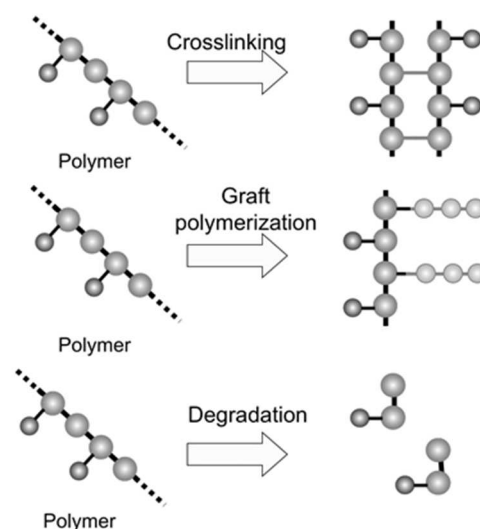
Radiation processing can modify the function of the commercially available polymers in an environmental-friendly way. In the initial stage, reactive radicals are created in the radiation processing of polymers. The radicals in the irradiated polymers can lead to chemical reactions within  $10^{-6}$  s after high-energy irradiation. This process proceeds simultaneously without any catalyst. Hence, the radiation processing of polymers is environment-friendly since it does not need any chemicals to initiate the reaction. Additionally, modified materials have little thermal damages due to radical creation in the polymers either at room or at lower temperatures. In radiation processing, even distribution of radicals creates homogeneous reaction in all parts of the polymer. If the radicals are created by thermal reaction, the temperature difference from the surface of the polymers causes heterogeneous reaction. It is very difficult for thermal reaction to achieve even reaction in the whole polymer. For these reasons, commercially available polymers can be modified using radiation processing and versatile applications are expected. This advantage makes possible the technology transfer of radiation-processed materials to private companies. Environment-friendly radiation processing of polymers is quite a promising R & D for the development of advanced materials [12.1].

For the agricultural and environmental applications, biopolymers have been selected as target materials for radiation processing using quantum beam. Biopolymer is produced from naturally occurring biomass and  $\text{CO}_2$  released is reabsorbed by biomass growth. Biopolymer is renewable, biodegradable and carbon neutral. These properties fit the concept of a zero carbon footprint.

## 12.2. RADIATION PROCESSING OF BIOPOLYMER

Typical technologies of biopolymer modifications with radiation processing using quantum beams are crosslinking, graft polymerization, and degradation as shown in Fig. 12.2. Crosslinking takes place in the radicals of the neighboring molecules. The degree of crosslinking is affected by molecular weight, chemical structure, and irradiation conditions such as temperature and atmosphere.  $\Gamma$  Radiation-induced graft polymerization is a convenient method to impart a desired function into the polymers. The important factor for graft polymerization is to select a functional monomer and trunk polymer and to optimize the conditions such as dose, solvent, reaction temperature, reaction time etc. The trunk polymer is

pre-irradiated and then immersed into monomer solution. Degradation is caused simultaneously with crosslinking. The general classification of crosslinkable and degradable polymers depends on the chemical structure of biopolymers.



*FIG. 12.2. Major modification of polymers using radiation processing.*

### 12.2.1. Crosslinking

Crosslinking is the most well-known radiation effects on polymers in the industrial applications e.g. crosslinking of rubber tire and cable wire. Crosslinking improves the mechanical strength of the rubber and reduces the tack strength. The thermal resistance of polyethylene and polyvinylchloride covering electric wire can also be improved. Strong interaction with polymer chains can induce increase in viscosity, molecular weight, and branching depending on the degree of crosslinking.

In the case of biopolymers, cellulose derivative in the form of carboxymethyl cellulose (CMC) can be crosslinked when irradiated in paste-like condition without any additives. CMC is a water-soluble polymer and is used for food additives such as viscosity modifier, and thickener. When CMC in paste-like state is irradiated by electron beam or gamma rays, crosslinking takes place and biodegradable hydrogels are formed [12.2]. If the CMC concentration is less than 10% or higher than 70%, degradation is expected and no gel fraction is obtained as shown in Fig. 12.3.



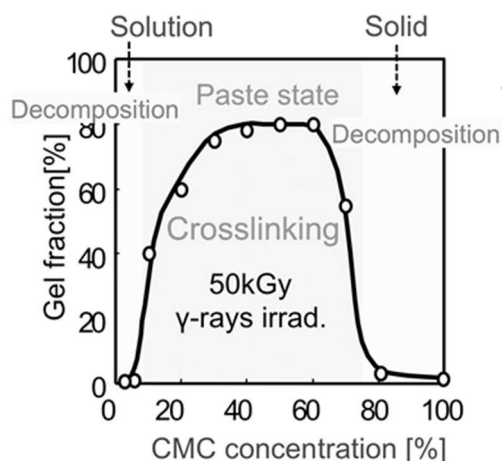


FIG. 12.3. Effects of irradiation on carboxy-methyl cellulose (CMC) in various states.

This result indicates that the distances among CMC molecules are not enough for crosslinking reaction for concentrations less than 10%. At concentration higher than 70%, CMC is not homogenously distributed in water. Some insoluble portions of CMC behave like being in a solid state. When the obtained hydrogel was soaked in water, swelling reached equilibrium after soaking in 4 h with a degree of swelling of 400.

CMC gel can be applied as super water absorbent (SWA) for soil conditioner in arid area. Sandy soil in arid area has too low capacity to keep water in the soil. Available water content increased 2 times in an irrigated system of tomato plants when 3% dry CMC gel was incorporated in sandy soil. As a result, tomato fresh yield increased from 19 to 31 Mg/ha. This application revealed that the combination of drip irrigation system and SWA can significantly enhance efficiency of water utilization.

Washi is traditional paper in Japan and is prepared by spreading Washi fibril from its aqueous suspension onto screen, as shown in Fig. 12.4. Addition of CMC gel into the aqueous suspension of Washi fibril enables spray coating of Washi. This new spray coating technique for Washi can also coat interior walls and 3D structural surfaces. For example, the walls of the meeting room in TARRI were coated by this technique. Other applications of novel coatings of CMC-containing Washi suspensions are lampshades and washable dishes. If CMC gel was not added to Washi suspension, sprayed coated lampshade cannot maintain the original shape due to insufficient mechanical strength.

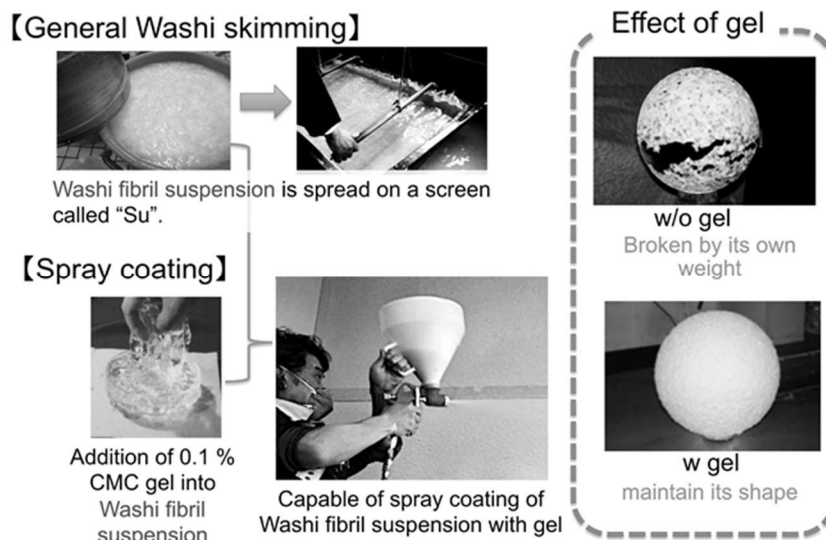


FIG. 12.4. Coating of Washi incorporated with carboxy-methyl cellulose (CMC).

Similar cellulose derivatives, hydroxypropylcellulose (HPC) in paste state can be crosslinked by irradiation. HPC gives highly transparent gel [12.3]. When dried HPC gel was soaked in solution of irradiation-sensitive monomer, gel dosimeter was obtained. This transparent gel sheet became clouded at around 2 Gy. Degree of white turbidity was in the range of 1–15 Gy which can be controlled by monomer compositions. This range is suitable for quantitative determination of dose for cancer therapy. When HPC gel sheets including monomer (some mm thick) were stacked up to a certain thickness, 3D dosimeter was formed. Figure 12.5 shows performance of 3D dosimeter which confirmed the measurement of 3D dose as applied in dummy sample of human body in cancer therapy.

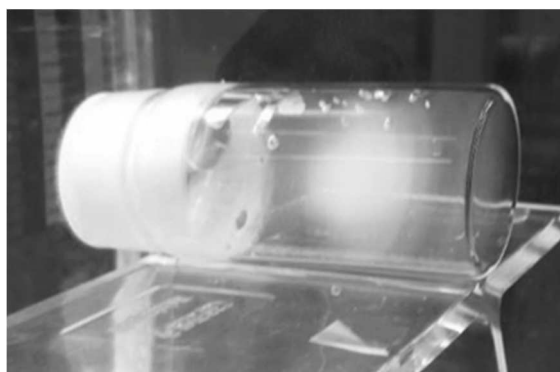
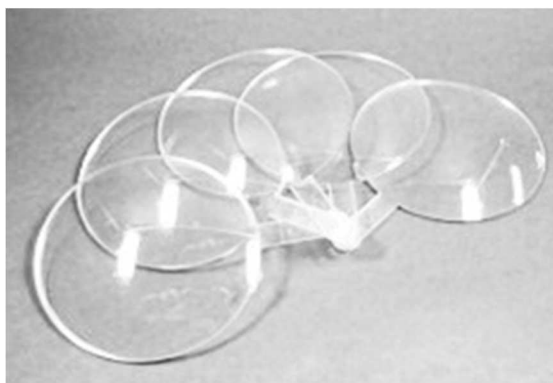


FIG. 12.5. 3D dosimeter assembled by gel sheets of hydroxypropyl-cellulose (HPC) after dipping into monomers.

Poly(L-lactic acid), PLA, which is a transparent and hard plastic is produced by condensation polymerization of lactic acid obtained by fermentation of starch. PLA is a typical renewable plastic. One of the promising applications of PLA is thermally molded products such as optical lens, cabinets for electrical applications, and regeneration medicine which require the property of high thermal stability. However, PLA thermally deforms at temperatures higher than its glass transition temperature of 60°C though it has high melting point of 175°C. PLA cannot be crosslinked by irradiation without a crosslinker since PLA is degraded by high energy radiation. It was found that addition of polyfunctional monomers (PFM) as

crosslinker, triallyl isocyanurate (TAIC) to PLA could induce its crosslinking [12.4]. The gel fraction reached 83.3% with the addition of 3% of TAIC after irradiation at 50 kGy. Without crosslinking, the PLA became clouded due to crystallization after heating at 100°C for 1 h. To avoid this turbidity, 3 - 5% TAIC is added at doses of 50 - 100 kGy. Crosslinking of PLA by radiation processing maintains high transparency which can be used as dummy lens of display frame as shown in Fig. 12.6. Dummy lens are discarded after corrective lens is set in the frame. They should be biodegradable to reduce the environmental burdens. Dummy lens in the frames are exported by surface. The temperature in hatch cargo reaches 70°C. Hence, the thermal property must be improved by crosslinking to avoid distortion. Degradation studies by enzyme degradability indicated that it still maintained half of its intrinsic PLA after crosslinking. This result implies that PLA is an environmentally-acceptable lens material even after crosslinking.



*FIG. 12.6. Transparent dummy lens produced by crosslinked poly-lactic acid with crosslinking agent.*

#### **12.2.2. Graft polymerization**

Radiation-induced graft polymerization can impart a desired function into the biopolymers. Various shapes of trunk polymers such as fabric, film hollow fiber, particle, and fiber are available. Grafting needs the chemical propagation reaction of monomer on trunk polymer. Preirradiation grafting is a practical technique wherein the trunk polymer is irradiated in advance and then soaked in monomer solution. Less homopolymer is created in the process.

Poly(3-hydroxybutyrate), PHB, is a biodegradable thermoplastic polyester which is synthesized by microorganisms. Control of the enzymatic degradability of PHB is an important requirement to enable practical utilization. If the enzymatic degradability in surface layer of PHB is controlled, many applications of PHB will be expected. To realize this idea, nonbiodegradable vinyl acetate was grafted onto PHB and the resulting layer of poly vinyl acetate was hydrolized to biodegradable poly(vinyl alcohol) [12.5] as shown in Fig. 12.7.

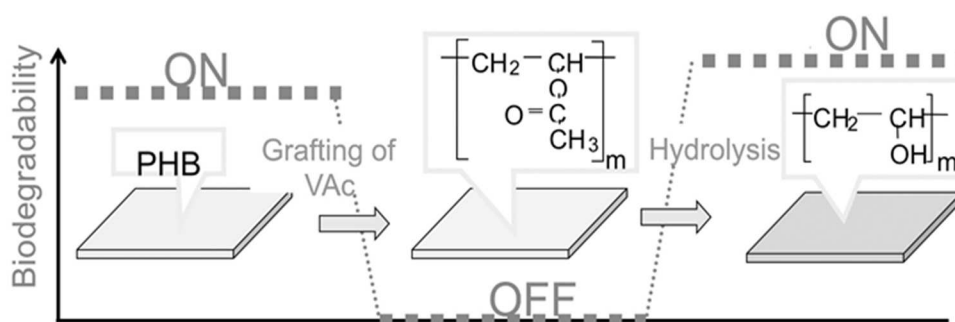


FIG. 12.7. Chemically induced biodegradable polymer to combine of VAc grafting and saponification on Poly(3-hydroxybutyrate), PHB.

When degree of grafting (Dg) is higher than 5%, the grafted films (PHB-g-VAc) completely lost its enzymatic degradability. In this state the surfaces of these PHB-g-VAc films were completely covered with graft chains, and the enzyme, PHB depolymerase, could not attack the PHB substrate. The biodegradability of the PHB-g-VAc films was recovered when the films were saponified in alkali solution under optimum conditions. BOD-biodegradability test showed the degradation of saponified PHB-g-VAc films in the environmental water. The optimum saponification conditions of PHB-g-VAc (Dg 5%) films and PHB-g-VAc (Dg 15%) films were at 0.05 M NaOH methanol solution for 8 h at 25°C. The combination of VAc grafting and saponification can produce chemically induced biodegradable plastics.

One of the well-known natural fibers is kenaf fiber which is used for a variety of applications such as paper products, building materials, and composites. Since it was introduced in Malaysia as alternative crop for tobacco areas, 2005, 42.2 ha of land in the east of Peninsular Malaysia were planted with kenaf for pre-commercialization development. The kenaf fiber was tested as a trunk polymer for graft polymerization instead of the synthetic polyethylene and nylon fibers. Dg does not increase more than 2% when natural kenaf fibers were used as a trunk polymer of grafting. Delignification of kenaf fibers is necessary to impart desired function into kenaf fibers. Lignin content was reduced from 14.2% to 1.1% using the optimum conditions of 0.5% NaClO<sub>2</sub>, a temperature of 80°C, and after 6 h treatment [12.6].

The delignification can substantially improve Dg of the kenaf fiber. Using a preirradiation dose of 150 kGy and 3% chloromethylstyrene aqua-emulsion state, the Dg of grafting reached up to 134% at 24 h. Kenaf-based graft material can be used as catalyst of biodiesel production. Biodiesel is produced from triglycerides such as vegetable oils, waste oils, animal fats and waste greases through catalyzed transesterification with alcohol. Various catalysts, including enzyme catalyst, have been used in the conversion processes of the triglycerides. Base and acid catalysts are essential for biodiesel production. Base catalysts such as KOH or NaOH in alcohol facilitate better the conversion of triglycerides into biodiesel than acid catalysts. Residual base catalyst in biodiesel must be rinsed to enhance the quality of final products. Hence, kenaf fiber is grafted and successively functionalized with a base catalyst. Chloromethylstyrene was grafted on kenaf fiber [12.7] and the obtained graft material was treated with trimethylamine and subsequently with NaOH. Figure 8 shows the performance of biodiesel conversions of triolein using kenaf-based and commercial resin (Diaion PA306) catalysts having the same trimethylamine functions. Kenaf catalyst showed 92% conversion which is two times as that of commercial resin catalyst. Fibrous catalyst prepared from kenaf fiber has higher activity than commercial resin catalyst. Graft polymerization produced an environment-friendly and high performance catalyst for biodiesel production from kenaf fiber.

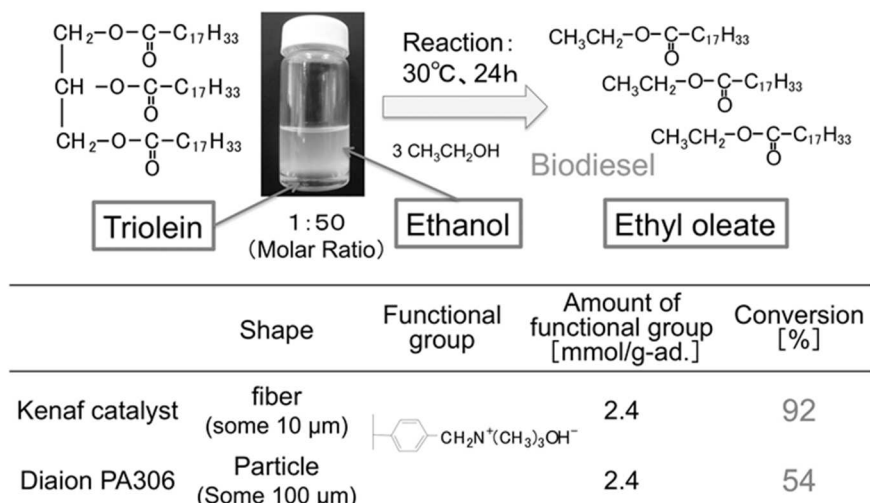


FIG. 12.8. Comparison of biodiesel conversions using kenaf catalyst with commercial resin catalyst (diaion PA306).

### 12.2.3. Degradation

The polysaccharide is an attractive material for agricultural applications of degraded polymers. Decomposed marine polysaccharides such as chitosan from crab shrimp shells play an important role as plant growth promoter (PGP) in shoot elongation and yield. Low molecular weight chitosan called oligochitosan can be degraded by acid hydrolysis and enzymatic reaction. However, radiation degradation of oligochitosan shows higher efficiency than that obtained by acid hydrolysis. Lower molecular weights with higher PGP activity are obtained from oligochitosan degraded by radiation degradation than acid hydrolysis[12.8]. Solution of chitosan is degraded effectively at lower dose than that in solid phase. This enhancement is caused by the formation of hydroxyl radicals from the radiolysis of water. Foliar spray of PGP induced 30–60% increase in yield of rice, chilli, potato, carrot, etc. in field tests carried out in Asian countries. Additionally, oligochitosan initiated the elicitor effect against fungal infection as shown in Fig.12.9. Synergetic effect of PGP and biofertilizer increased nitrogen fixation in soybean as experimented by a collaborative work with FNCA (Forum for Nuclear Cooperation in Asia) biofertilizer project. Electron Accelerator Application Project in FNCA provided the guideline for R & D on PGP/elicitor for the degradation of natural polymers as well as applications of radiation crosslinked hydrogel for super water absorbent as shown in the FNCA home page.

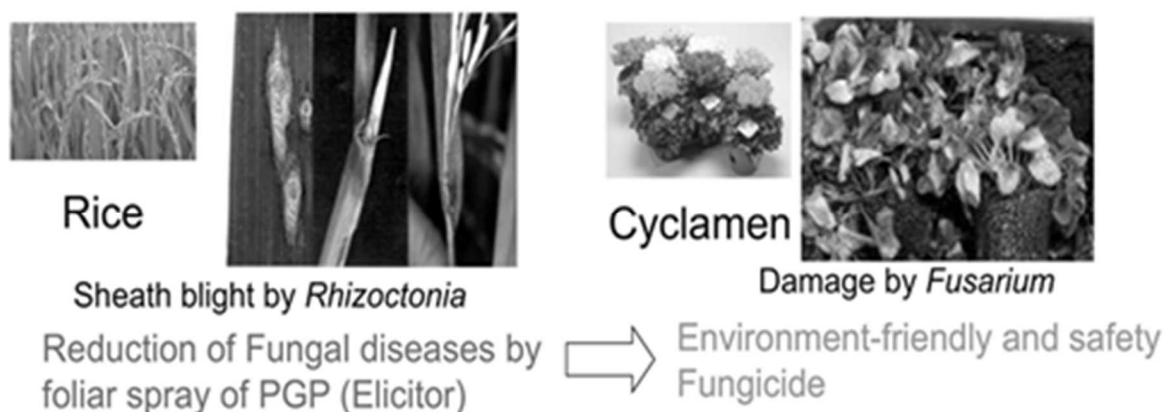


FIG. 12.9. Elicitor functions of oligochitosan prepared by deprecating chitosan with radiation.

### 12.3. CONCLUSIONS

Radiation processing of polymer using quantum beams such as electron beam and gamma rays has produced unique materials which can not be prepared by chemical reactions. Radiation processing is an environment-friendly process due to the absence of chemical initiators. Versatile applications from radiation processed biomaterials have been the target in the agricultural and environmental fields. These developed products can only be useful if it reaches the technology transfer stage to private companies. The commercialization of commodities by radiation processing often faces the barrier of public acceptance. To overcome this problem, education and dissemination of information about radiation technology even at the early research stage is necessary. Fortunately, products from radiation processing of polymers are easily acceptable owing to its high performance. Japan will continue its R & D on radiation processing of polymers as a promising technology for the improvement of welfare of people all over the world.

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## Chapter 13

### UP-SCALING RADIATION-PROCESSED OLIGOCHITOSAN AND ITS APPLICATION IN THE PLANTATION OF RICE

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#### Abstract

The up-scaling production of oligochitosan using a continuous gamma irradiation facility at Nuclear Malaysia has been established. Over 2 000 L of 20 000 ppm of oligochitosan at molecular weight of  $\leq 10\,000$  D can be produced per cycle. Subsequently, the oligochitosan has been used in field trials at two different rice plantations during the wet and dry seasons. Both field trials showed remarkable effects on the growth of rice seedlings as well as rice yields. The use of oligochitosan has proven to shorten the period of the rice seedlings from 15 days to 10–12 days. In addition, the cost of this procedure has been greatly reduced since no additional nutrients were used. The growth of the rice seedlings increased by 22.8–23.3% on burned rice husk substrate and by 13.0% on commercial soil when sprayed with oligochitosan as compared to those sprayed with commercial nutrients. With the introduction of oligochitosan, the yield of rice also increased from 2.0–20.0%, depending on the seasons.

#### 13.1. INTRODUCTION

Because of their many potential applications in different fields, the preparation of low molecular weight chitosan and oligochitosan either through gamma ray or electron beam irradiation has been carried out in many radiation processing research centres. Chitosan, which is prepared from chitin by the deacetylation process, has generally high molecular weight that, in many cases, limits its applications. The low molecular weight chitosan and its oligomer have some special biological properties such as antioxidant property [13.1–13.2], antimicrobial property [13.3–13.6], antitumor activity [13.7], immunity stimulation for animal [13.8–13.9] and for plants [13.10–13.12], and so on. These properties are vastly different from that of the ordinary high molecular weight chitosan.

Articles reviewed by Kim & Rajapakse [13.13] and Xia et al [13.14] contain comprehensive information on the biological activities of chitosan and oligochitosan. A variety of techniques, including chemical and enzymatic hydrolysis and radiation degradation processes, can be used to prepare low molecular weight (MW) chitosan and its oligomer, as described by Makuuchi [13.15]. However, radiation (gamma ray, electron beam) is a useful tool for the degradation of polymer from the viewpoint of being an environment- friendly technology [13.16]). Consequently, in most cases, oligochitosan has been produced by the irradiation of chitosan in solution [13.17–13.18].

In Asia and the Pacific Region, several pilot scale facilities for the gamma irradiation of liquids such as natural rubber latex and aqueous solution of chitosan have been established in countries like Malaysia, Indonesia, India, and Vietnam. All the pilot scale plants are batch-type irradiation plants except for the one in Malaysia. This particular plant has been designed for the continuous irradiation of natural rubber latex and, subsequently, for the production of radiation-processed oligochitosan as a plant growth promoter and plant elicitor. In this paper, the preparation of oligochitosan in a continuous gamma irradiation plant at Nuclear Malaysia is described. The field trial study on the application of oligochitosan as a plant growth promoter of rice is also presented.



## 13.2. EXPERIMENTS

### 13.2.1. Materials

Chitosan powder with the following properties 90.6% of DDA, 10% water content and containing 0.1 ppm As, 0.1 ppm Cd, 0.8 ppm Cu, 3.16 ppm Ni, and 0.1 ppm Pb was purchased from China. Lactic acid (90%), NaOH solution (2M), hydrogen peroxide (30%), and ethanol are all industrial grades and supplied by local companies.

### 13.2.2. Production of oligochitosan

A  $^{60}\text{Co}$  plant of  $\sim 800$  kCi was used to partially degrade chitosan powder at a dose of 50 kGy and dose rate of 2.51 kGy/h. Subsequently, 3% of radiation-degraded chitosan in 2% lactic acid solution was prepared as follows:

Chitosan powder (10% moisture):	45 kg
Lactic acid (90%):	33 L
Water:	1 485 L
Hydrogen Peroxide (30%):	5 L
NaOH solution (2 M):	15 L
Ethanol:	750 L
TOTAL VOLUME = 2288 L (1.97% chitosan $\sim$ 19 700 ppm)	

Hydrogen peroxide 0.1% was added to the chitosan solution to provide for the synergetic effects on the radiation degradation of chitosan. Meanwhile, the sodium hydroxide solution of 2M and ethanol was added after the chitosan solution was irradiated to neutralize the oligochitosan solution for agriculture usage and to preserve the solution upon storage.

The chitosan solution was irradiated by using a liquid (Natural Rubber Latex) gamma irradiator of an activity, 94 818 Ci. The cycle time for 1500 L solution (56 strokes  $\times$  15 min/stroke) is  $\sim$ 14hrs for 12 kGy. Dose rate is 0.86 kGy/h.

### 13.2.3. Characterization of chitosan and oligochitosan

The viscosities of 50 kGy irradiated chitosan powder solution and 12 kGy irradiated chitosan solution were determined at 3% chitosan solution in 2% lactic acid using a spindle type viscometer model Brookfield DV II+.

The irradiated chitosan was purified by adding 2.5% ammonium hydroxides until the pH reached 6.5. The precipitated oligochitosan was washed with ethanol and air-dried. Subsequently, the purified oligochitosan was prepared in acetic acid/ammonium acetate buffer solution at a concentration of 1.0 g/L (0.20M/0.15M at 25°C). The intrinsic viscosity was then measured using an ubbelohde viscometer capillary 531/10/I of Schott instrument, model AVS 440. Data of the viscosity average molecular weight was taken from the average of the three measurements. The pH of the solution was measured using a pH meter Mettler Toledo.

## 13.3. RESULTS AND DISCUSSION

### 13.3.1. Pilot scale production of oligochitosan

In the pilot scale production of oligochitosan in Malaysia, two steps in gamma irradiation were employed. Currently, the liquid gamma irradiator is set to deliver an irradiation dose of

12 kGy. In this condition, the chitosan powder has to be partially degraded at 50 kGy using another gamma irradiation plant. The viscosity of 3% chitosan in 2% lactic acid after irradiation at 50 kGy, which was selected for this study, is shown in Table 13.1. At a dose rate of 0.86 kGy/h, the cycle time for irradiation of 1500 cm<sup>3</sup> of oligochitosan at 12 kGy is 14.0 h. The irradiated chitosan solution in the form of oligochitosan is continuously pumped into the storage tank. Oligochitosan was in an acidic condition but was neutralized with a sodium hydroxide solution before being applied to the field as shown in Table 13.1. For a longer storage period, ethanol was added to the oligochitosan solution. A total volume of 2 300 cm<sup>3</sup> of oligochitosan with the concentration of ~20 000 ppm per cycle was prepared.

TABLE 13.1. VISCOSITY AND pH OF CHITOSAN IN 2% LACTIC ACID BEFORE AND AFTER IRRADIATION AT 50 kGy

Formulation	Dose (kGy)	Viscosity (cPs)	pH at 50 kGy	pH at 12 kGy	Final pH (adjusted with NaOH)
3CL2	0	315.26	3.40	3.58	5.03
	50	35.5			

In Table 13.2 and Fig. 13.1, the viscosity average molecular weight of irradiated chitosan dropped significantly, i.e. 55% from 81 574 – 36 564 when the irradiation dose increased from 25–50 kGy. However, the drop in molecular weight was reduced to only 9% when the irradiation dose was further increased to 75 kGy. Subsequent increases in the irradiation dose did not compensate for the decrease in molecular weight of chitosan. Therefore, an irradiation dose of 50 kGy was chosen to degrade the chitosan for further processing in liquid form using a gamma irradiator.

TABLE 13.2. MOLECULAR WEIGHT OF CHITOSAN AND OLIGOCHITOSAN: PRE-IRRADIATED CHITOSAN POWDER AND OLIGOCHITOSAN AT 12 kGy

Chitosan (kGy)	Powder Preirradiation	Mw	Mw after irradiation at 12 kGy (3CL2 + 0.3% H <sub>2</sub> O <sub>2</sub> )
0		218 269	-
25		81 574	12 684
50		36 564	10 453
75		33 358	10 000
100		32 219	6 657

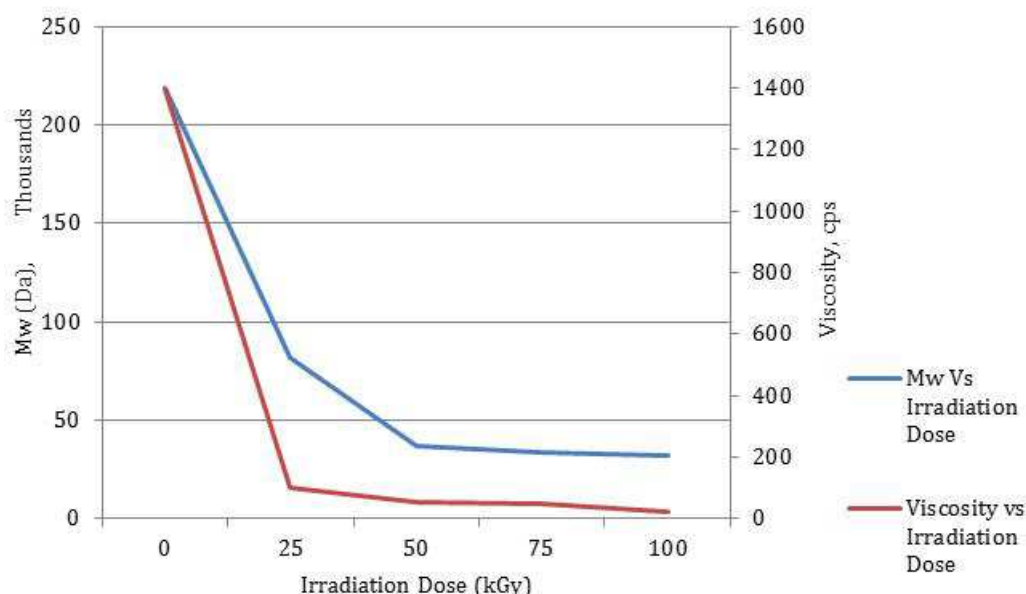


FIG. 13.1. Molecular weight of chitosan at various irradiation doses.

After the degradation of chitosan powder at 50 kGy, the chitosan was further degraded to produce small molecules of oligochitosan with a molecular weight of 10 000 or below. In this process, the irradiation was carried out on the degraded chitosan, 3% in 2% lactic acid (3CL2) in the presence of 0.1–0.3% hydrogen peroxides. Hydrogen peroxides are used to impact a synergetic effect that facilitates the reduction of the molecular weight of degraded chitosan powder in aqueous solution when exposed to 12 kGy gamma radiation. Table 13.2 shows the molecular weights of degraded chitosan and oligochitosan produced after the irradiation at different doses of chitosan powder and 3CL2 aqueous solution made from chitosan powder. There was a slight reduction in the molecular weight of the oligochitosan (3CL2) after 12 kGy. The Mw of oligochitosan from degraded chitosan of 50 kGy and 75 kGy were not much different, which was around 10 000, as shown in Fig. 13.1. In this study, the preirradiation process of chitosan powder was carried out at 50 kGy to produce low molecular weight chitosan, followed by the irradiation of lactic acid solution of low molecular weight chitosan at 12 kGy to produce oligochitosan.

### 13.3.2. Field trial of rice seedling for transplanting

The following chart shows the comparison of growth rates of rice seedlings when oligochitosan and a commercial product were applied to rice seedlings within 15 days prior to being transferred to the field.

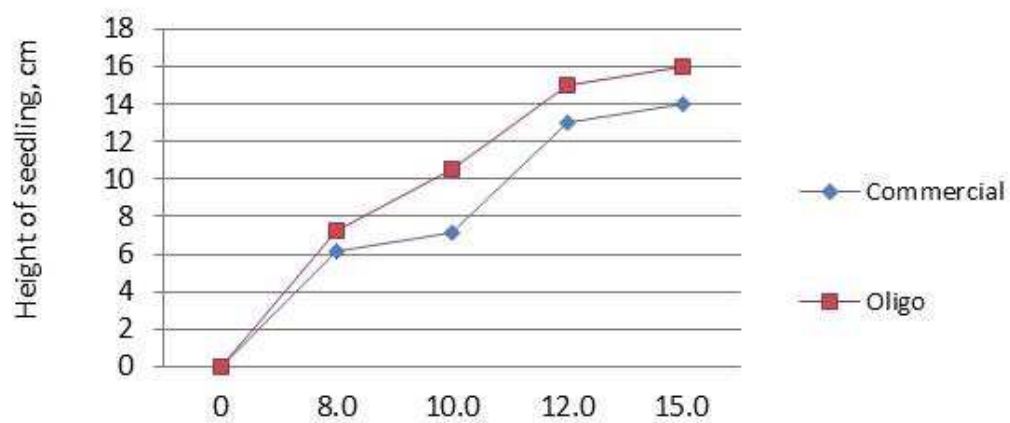


FIG. 13.2. Growth rate of rice seedling in wet season (Oct – Feb).



FIG. 13.3. Height of rice seedling at 8 Days.

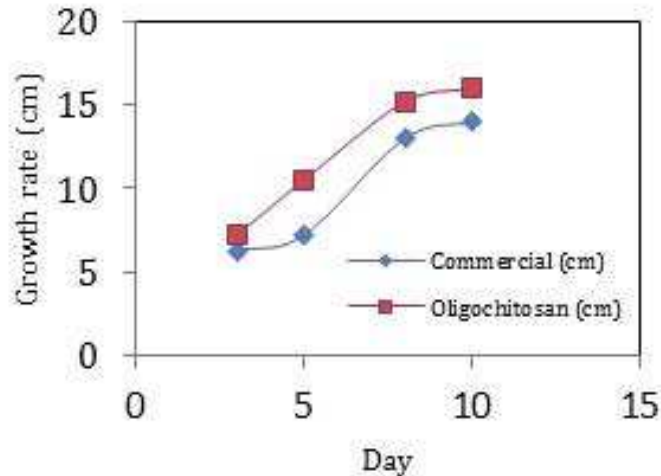


FIG. 13.4. Growth rate of rice seedling during dry season (March – July).

The effect of oligochitosan on the growth of rice seedlings was very significant, as shown in Figs 13.2, 13.3, and 13.4, and can be summarized as follows:

The growth of rice seedlings increased by 22.8–23.3% on burned rice husk substrate and by 13.0% on commercial soil when the rice seedlings were sprayed with oligochitosan as compared to those sprayed with commercial nutrients. This also indicates that the type of soil or substrate significantly affected the growth rate of rice seedlings. The growth of rice seedlings in any soil/substrate and sprayed with oligochitosan is much faster than when it is sprayed with commercial nutrients, although oligochitosan has no nutrients that can provide essential elements to the seedlings. Figure 13.3 clearly shows that, after 8 days, the rice seedlings that were treated with oligochitosan were much taller than those treated with commercial nutrients. Similarly, the root growth of the rice seedlings treated with oligochitosan was significant compared to those treated with commercial nutrients as shown in Fig. 13.5.

The usual 15-day growth duration of rice seedlings can be shortened to 10-12 days. The root growth was faster and well spread on the substrates. This allowed for the rice seedlings to be transferred to the field on the 10-12<sup>th</sup> day instead of on the 15<sup>th</sup>. From these trials, it can be concluded that oligochitosan enhanced the growth of rice seedlings without adding nutrients which reduces the growth duration and save on cost.

Commercial Promoter



Treated with oligochitosan



*FIG. 13.5. Roots growth at 8 Days of seedling.*



*FIG. 13.6. Rice seedling.*



*FIG. 13.7. Transplanting of rice seedling.*

Figure 13.6 shows the rice seedlings at the site ready for transfer to the fields on day 15. Fig. 13.7 shows the method of transplanting the rice seedlings to the fields using a transplanter.

### 13.3.3. Field trials of rice planting

The field trial of rice planting was carried out in two different areas, at the FELCRA Seberang Perak and at the Agriculture Department, Tanjung Karang, and their rice yields are given in Tables 13.3 and 13.4, respectively. Rice seeds were harvested 112 days after planting. The FELCRA Seberang Perak rice plantation area is considered a low-yield rice area as indicated in the yield/hectare of not more than 5.9 metric ton/hectare in the past three years, 2008–2010. Unfortunately, the rice yields kept decreasing from 2008–2010. The average increase of rice yield when sprayed with oligochitosan was about 3.5–7.0% higher than the control's plots of T1 (no oligo and no fungicides) and T2 (no oligo but with fungicides) for a large field trial area of 24 hectares.

However, when the field trial was conducted in a smaller plot area of about 1 hectare, the increase of from 9.0–20.0% in rice yield was quite significant. The vast difference in yield was due to the poor management of the field trial plots against the rice enemies like rats, golden snails, birds, weedy rice, and weather. A 24-hectare trial field would require more workers to ably monitor and manage it as compared to a 1.0-hectare field. The lack of manpower also contributes to the low percentage of increase in yields.

Nevertheless, most of the rice plots sprayed with oligochitosan have higher yields than those sprayed with fungicides (T2). This indicates the effectiveness of oligochitosan as a plant elicitor that induces the rice crops to protect themselves against fungi.

TABLE 13.3. RICE YIELDS IN THE PAST 3 YEARS, 2008–2010 FOR FIELD TRIALS CONDUCTED AT FELCRA (M) BERHAD, SEBERANG PERAK

Rice Yield, spraying with 40 ppm oligochitosan	Field trial at FELCRA Seberang Perak			
	Large Area, 24 hectares	Small area, 1 hectare		
			Season 2/2008	Season 1/2009
			Season 1/2010	Season 2/2010
% increase, over Control T1	7.3	5.5	9.14	20
% increase, over Control T2	3.5	3.6	4.1	13.6

On the other hand, the average rice yield at Tanjung Karang during the dry season was about 6.0 metric tons/hectare and up to 9.0 metric ton/hectare during the wet season. Tanjung Karang is one of the high-yield rice areas in Malaysia. During the low season, the rice plots that were sprayed with oligochitosan had a 15% increase in yield compared to the control. However, during the high season, when the yield of rice can reach an optimum of 9.0 metric tons, the effectiveness of the application of oligochitosan was not significant, but still, 2.4–3.2% higher than control.

From the results of the above field trials, it can be concluded that:

- oligochitosan enhances the yield of rice crops,
- an oligochitosan concentration of 40 ppm is sufficient to enhance yield,
- the effectiveness of oligochitosan as a plant growth promoter can be clearly observed during the wet season (off season), and
- oligochitosan seems capable of acting as a plant elicitor against fungus, hence the use of chemical fungicides may become unnecessary.

TABLE 13.4. RICE YIELDS FOR 2 SEASONS IN 2011 FOR FIELD TRIALS CONDUCTED AT AGRICULTURE DEPARTMENT, TANJUNG KARANG, SELANGOR

Sub Lot	Treatment	Area/ hectare	2 <sup>nd</sup> trial, July – Dec 2011 (wet season)	1 <sup>st</sup> trial, Jan – June 2011 (dry season)					
			Weight (Ton)	Weight/ hectare (Ton)	% increase	Weight (Ton)	Weight/ hectare (Ton)	% increase	
T1	Control, fungicides no oligo	no and 0.0437	0.420	9.6110	-	0.240	5.4920	-	
T2	40 ppm oligo	0.0437	0.433	9.9161	3.17	0.276	6.3310	15.28	
T3	100 ppm oligo	0.0437	0.430	9.8398	2.38	0.276	6.3310	15.28	
	Average hectare	per		9.7890			6.0513		

#### 13.3.4. COST OF PRODUCTION AND USAGE OF OLIGOCHITOSAN

In Table 13.5, the cost of materials and irradiation services were used as the basis for calculating the cost of production of oligochitosan and its use.

The total volume of oligochitosan produced per cycle is 2 288 liters with a concentration of 19 668 ppm. Consequently, at 40 ppm, 1 126 840 liters of oligochitosan can be prepared and can cover 2 504 hectares of rice plantation. The estimated cost of production of oligochitosan is RM 8.50 (US \$2.83) per liter of 19 668ppm.

The usage and cost of oligochitosan for rice plantation is shown as follows:

450 L/hectare oligochitosan

40 ppm × 450 L = 19 668 ppm × 0.915 L (hectare)

4 × spray per season, 3.66 L per season per hectare of 19 668 ppm oligochitosan.

The cost of usage of oligochitosan per hectare per season,

US \$2.83 × 3.66 L, = US \$10.36

Costing during seedling for 15 days:

Commercial plant growth enhancer, US \$4.0/500 ml



Spraying with oligochitosan, 40 ppm for 20 liter, the cost is US \$0.115. The costing during plantation per season and the cost of commercial fungicides as described below are the following as compared to oligochitosan:

Blast = US \$43.00/500 ml/ha  
 Score = US \$26.67/500 ml/ha  
 Benang Perang = US \$40.00/500 ml/ha  
 Krate = US \$20.00/500 ml/ha  
 Oligochitosan = US \$10.36/ha

The above list shows the difference in cost of commercially available chemicals that can either be replaced or reduced by using oligochitosan, which costs lower than the others.

TABLE 13.5. COSTING FOR PRODUCTION OF OLIGOCHITOSAN

No.	Item	Amount use	Cost (*RM)/unit	Total cost (*RM)
1	Chitosan	45 kg	212.00 per kg	9540.00
2	Ethanol industrial grade (95%)	750 liter	11.27 per liter	8 452.50
3.	Lactic Acid	33 liter	13.00 per liter	429.00
4.	Sodium hydroxide	1.2 kg (15.0 L)	119.00 per kg	142.80
5	Hydrogen peroxide (30%)	5.0 liter	60/liter	300.00
6.	Water	1485 liter	1.5/1000liter	2.20
7.	Irradiation services for 12 kGy (Raymintex)	1500 liter	0.36/kg	540.00
8.	Irradiation services for 50 kGy (Sinagama)	50 kg	0.90/kg	45.00
Total				451.50

\*RM = Ringgit Malaysia. The exchange rate used in this article is RM3.00 equivalent to US \$1.00

### 13.4. CONCLUSION

The application of oligochitosan as a plant growth promoter for rice crops has been tested in a large trial field within a complete cycle of dry and wet seasons. Oligochitosan has been shown to enhance the growth of rice seedlings and increase rice yields. The concentration of oligochitosan of 40 ppm is sufficient to produce such effects on rice crops. The application of oligochitosan, a natural and an environment-friendly product, should be further developed and promoted as an alternative to chemical-based products, and as a plant growth promoter and plant elicitor in the agriculture sector.

### Acknowledgement

The authors wish to thank the government of Malaysia for fully supporting the field trials that were conducted at FELCRA Sbg. Perak and Tanjung Karang under the Ninth Malaysian Plan (2005 - 2010) with the support and cooperation of the staff of the Division of Radiation Processing Technology of Nuclear Malaysia. The authors also wish to express their deepest

appreciation to the management of FELCRA (Malaysia) Berhad and the Agriculture Department of Tanjung Karang for their cooperation in conducting the field trials.

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## Chapter 14

### INFLUENCE OF IRRADIATED CHITOSAN ON GROWTH AND FLOWER QUALITY OF GLADIOLUS AT DIFFERENT SOWING DATES AND SYNTHESIS OF RADIATION CROSS-LINKED POLY(ACRYLIC ACID) HYDROGEL FOR AGRICULTURE APPLICATIONS

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#### Abstract

The plant growth promoter activity of irradiated chitosan on *Gladiolus hortulanus* cv. Amsterdam was studied. Chitosan was applied in the form of foliar spray at third leaf stage. Corms were sown at three different dates with 15-day intervals. Data on several parameters such as survival percentage, leaf area, plant height, number of florets per spike, and vase life were collected. Chitosan-treated plants showed superior results as compared to the control samples. Acrylic acid-based superabsorbent hydrogel was prepared using phenyltriethoxysilane (PTES) as cross-linker. Different amounts of PTES were incorporated and irradiated at different doses of up to a maximum of 30 kGy. The cross-linked acrylic acid showed hydrogel properties, and its swelling kinetics, gel fraction, and equilibrium degree of swelling (EDS) were studied. The swelling of hydrogel was also affected by pH, ionic strength, and temperature. These hydrogels can be further explored as a super water absorbent material in semi-arid and drought prone areas.

#### 14.1. INTRODUCTION

*Gladiolus* (*Gladiolus hortulanus*), a member of the Iridaceae family, produces a great diversity of flowers in varied shapes and colors [14.1]. *Gladiolus* is very popular as a cut flower with the consumer and the florist. In Pakistan, it is cultivated in areas with tropical, subtropical and temperate weather. *Gladiolus* grows 2-6 feet high and is topped by a flower spike bearing a double row of trumpet-shaped florets. The flowers measure from 1-8 inches in diameter. Observing the correct planting schedule of the *gladiolus* can greatly improve its vegetative growth and flower quality which, in turn, could mean better and more beautiful blooms to satisfy the consumers. Low molecular weight chitosan showed post-harvest preservative and elicitor properties [14.2-14.4]. The present study was designed to evaluate the influence of irradiated chitosan on the growth and flower quality of *Gladiolus*.

#### 14.2. MATERIALS AND METHODS

Chitosan in solution form was irradiated under gamma rays. Irradiated chitosan was applied in the form of foliar spray at third leaf stage. Corms were sown at three different dates at intervals of 15 days. Data on the number of days to sprouting, sprouting percentage, survival percentage, leaf area, plant height, number of florets per spike, spike length, time of spike initiation, time of first floret opening, crop maturity, fresh weight of spike, and vase life were collected. Table 14.1 shows the time of sowing, code, and concentration of chitosan used in this study.

TABLE 14.1. CODE AND CONCENTRATION OF CHITOSAN USED

Sowing dates	Chitosan (ppm)			
	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
S <sub>1</sub> 15 <sup>th</sup> Oct.	0	200	300	400
S <sub>2</sub> 1 <sup>st</sup> Oct.	0	200	300	400
S <sub>3</sub> 15 <sup>th</sup> Nov.	0	200	300	400

### 14.3. RESULTS

Figure 14.1 shows the effect of irradiated chitosan on the plant height and survival percentage. It can be observed that the plants treated with 200 ppm (T<sub>1</sub>) gave the best results in plant height (82.00 cm) and survival percentage (81.22).

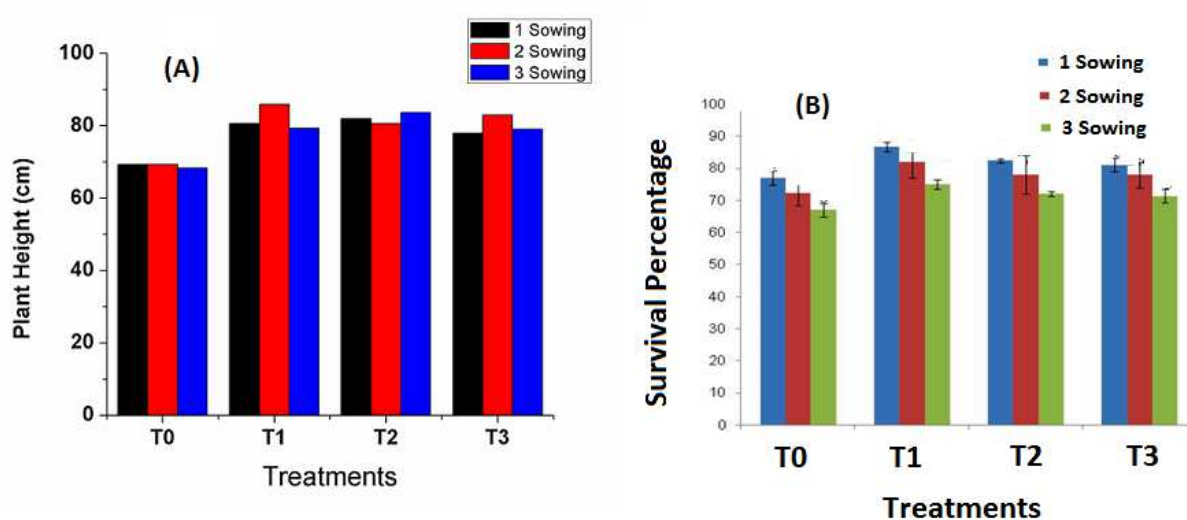


FIG. 14.1. Effect of irradiated chitosan on plant height (A) and Survival percentage (B).

The effect of different concentrations of irradiated chitosan on the number of leaves per plant and florets per spike is shown in Figure 14.2. This figure shows that the best results were obtained in plants sprayed with 300 ppm (T<sub>2</sub>) chitosan.

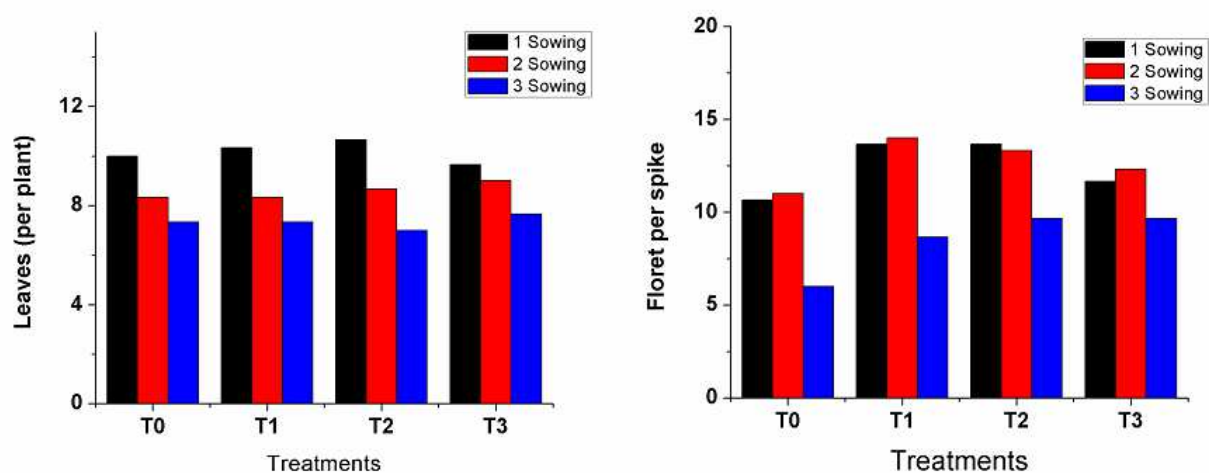


FIG. 14.2. Effect of irradiated chitosan on number of leaves per plant (A) and floret per spike (B).

Best results were obtained from the post-harvest foliar application of chitosan on Gladiolus cut flowers at higher concentrations as shown in Fig. 14.3.

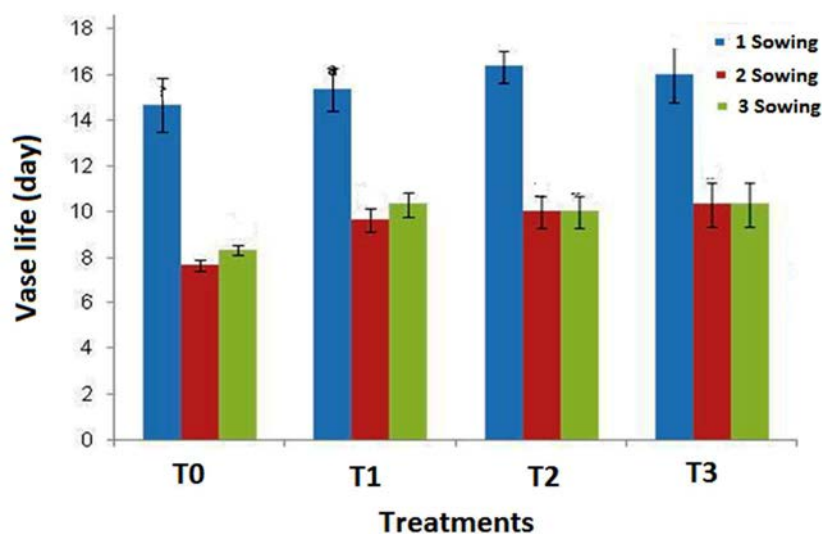


FIG. 14.3. Effect of post harvest foliar application of chitosan Gladiolus cut flower.

In addition, the effect of the use of chitosan as a plant elicitor in other plants such as Zinnia, chrysanthemum, and bell pepper was also studied. The irradiated chitosan showed very good results in these plants. The reproducibility of the obtained result is still being processed before the product is eventually introduced in the local market.

## 14.4. SYNTHESIS OF RADIATION CROSS-LINKED POLY(ACRYLIC ACID) HYDROGEL FOR AGRICULTURE APPLICATIONS

### 14.4.1. Introduction

The increasing population and climatic changes strongly affect the water resources. There is a need to develop a material that can retain water and important nutrients for the growth promotion in plants [14.5]. To minimize crop loss, hydration strategies are essential to reduce the frequency of irrigation during crop production. Poly(acrylic acid) and polyacrylamide are mostly used for polymeric-based hydrogel to reduce the water runoff. This hydrogel plays a very important role in horticulture and agriculture since it increases the water holding capacity of soil [14.6-14.10].

Poly(acrylic acid) (PAA) is a versatile synthetic polymer which is used in many commercial applications [14.11-14.12]. The cross-linked PAA exhibits pH sensitivity and hydrogel properties. Generally, the high water solubility of PAA in an aqueous medium results in the breakdown of the gel structure. Thus, there is a need to find cross-linking agents or methods to obtain a stable poly(acrylic acid) gel.

The objective of the present study is to prepare a radiation cross-linked PAA hydrogel with good stability and pH sensitivity properties. PTES is selected as the polyfunctional monomer (PFM) to cross-link acrylic acid. In acrylic acid, PTES reacts in two steps. In the first step, ionizing radiation generates free radicals that induce polymerization in PTES. In the second step, the silanol group of PTES forms a cross-linked network.

### 14.4.2. Materials and methods

Acrylic acid, phenyltriethoxysilane (PTES), sodium hydroxide, hydrochloric acid, potassium chloride, acetic acid, and sodium acetate were purchased from Sigma-Aldrich. All others chemicals were of analytical grade.

Acrylic acid was first neutralized with NaOH up to 75%. A 100 ml amount of acrylic acid was transferred into the flasks, after which an appropriate amount of PTES was slowly added. The samples were irradiated under  $^{60}\text{Co}$  gamma source at a dose rate of 1.05 kGy/h. The resultant hydrogel was then washed and dried. The compositions and codes of the synthesized hydrogels are shown in Table 14.2.

TABLE 14.2. COMPOSITION AND CODES OF FORMULATIONS

Code	AA40/15	AA60/15	AA80/15	AA40/30	AA60/30	AA80/30
Dose (kGy)	15	15	15	30	30	30
PTES amount <sup>1</sup>	0.83	1.25	1.65	0.83	1.25	1.65

<sup>1</sup>  $\mu\text{mol}/100\text{ ml of acrylic acid}$

The gel content of dried hydrogels was extracted with water for eight hours using the Soxhlet apparatus. The swelling response of cross-linked acrylic acid was studied under different conditions. A sample (50 mg) in uniform size was placed in a beaker filled with solvent (100 mL) at a specific temperature. At fixed time intervals, the weight of the swollen sample was measured after the excess surface solvent was removed. After weighing, the sample was again placed in the same solution and weighed again. The swelling of the sample was determined gravimetrically by using the following equation:

$$\text{Swelling} = (W_s - W_i) / W_i \quad (1)$$

where  $W_i$  is the initial weight of the sample and  $W_s$  is the swollen weight of the sample after time  $t$ .

#### 14.4.3. Results and discussion

Radiation technology has been effectively used in the preparation of hydrogels through the cross-linking of hydrophilic polymers. The properties of these hydrogels can be controlled by changing the dose that influences the structure and cross-linking density of the final product [14.13].

The effect of the absorbed dose on the gel fraction of PAA is shown in Table 14.3. It was observed that the gel fraction is slightly affected by the PTES concentration but greatly affected by the absorbed dose. The increase in the dose from 15–30 kGy almost doubled the gel fraction even with a low PTES concentration. This shows that, at high doses, more PTES chains are incorporated into the growing polymer chain that subsequently gives a network of PAA and PTES [14.14].

The swelling behavior of cross-linked PAA hydrogel in water in relation to time was studied and the results are shown in Fig. 14.4. This figure shows that the swelling of hydrogel increased linearly with an increase in time and reached the equilibrium value between 25–30 hours. It is also observed that, at a given dose, the swelling of hydrogel decreased as the concentration of PTES increased. This indicates that a higher PTES concentration increases the number of links between polymer chains. As a result, the size of pores in the hydrogels decreased, which resulted in a marked decrease in the swelling. For example, the swelling of AA40/15 is 246 g/g and was decreased to 198 g/g in AA80/15. Similarly, the swelling is also affected by the dose. Swelling decreased with an increase in dose from 15–30 kGy in samples having the same amount of cross-linker. At high doses, more radicals are generated in the sample, producing more links between the polymer chains. Thus, a reduction in the mobility of the polymer chain also reduces the EDS value of the hydrogel. A similar decreasing trend in EDS values is observed in poly(acrylic acid) hydrogel prepared by electron beam [14.15].

Swelling is the result of the diffusion of solvent molecules from the outer medium into the hydrogel structure. The diffusion mechanism of the solvent in the hydrogel can be explained by using the following equation:

$$F = k t^n \quad (2)$$

where  $F$  is the swelling at time  $t$  (min),  $k$  is the rate constant for swelling which is characteristic of water and the polymer network, and  $n$  is the diffusion exponent. The mechanisms of diffusion, release, and transport are explained by the value of  $n$  [14.9]. The swelling data from Fig. 14.4a was used to obtain the values of  $n$  and  $k$ . These values of  $n$  and  $k$  are given in Table 14.3 and plot of  $\ln F$  versus  $\ln t$  is shown in Fig. 14.4b. It is observed that the value of  $n$  is decreased with increasing amounts of PTES in the sample. The diffusion of water in all hydrogel follows the non-Fickian mechanism. For non-Fickian diffusion, the value of  $n$  is  $0.5 < n < 1$  [14.16-14.17].



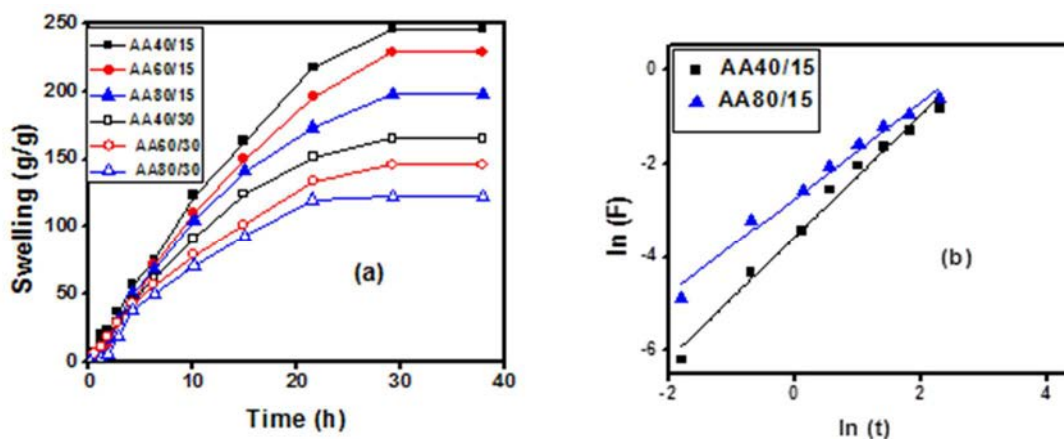


FIG. 14.4. (a) Swelling kinetics of cross-linked hydrogel in water (b)  $\ln(F)$  plotted versus  $\ln(t)$  for hydrogels.

TABLE 14.3. GEL CONTENT AND DIFFUSION PARAMETERS OF CROSS-LINKED HYDROGELS

Samples	Gel content (%)	N	$K \times 10^2$
AA40/15	35	0.88	6.1
AA60/15	36	0.85	6.2
AA80/15	38	0.81	8.1
AA40/30	55	0.78	6.1
AA60/30	65	0.73	6.2
AA80/30	82	0.71	8.1

The swelling response of ionic hydrogel is greatly affected by the pH of the external medium. The changes in the pH of the external medium can disturb the charge of the ionizable groups present in the hydrogel network. Ionization can control the penetration of solvent molecules into the hydrogels structure [14.17]. The swelling response of the hydrogels at different pH plays an important role in industrial and biomedical applications. In this study, the swelling response of the hydrogels against pH in buffer and non-buffer media was investigated.

The effect on swelling of hydrogel in non-buffer media was studied and results are shown in Fig. 14.5. This figure shows a switchable trend in the swelling of hydrogel at different pH. Maximum swelling was obtained around neutral pH while low swelling was observed in basic and acidic pH range. The swelling of AA40/15 hydrogels was negligible at pH 2. It gradually increased and reached the maximum value of 247 g/g at pH 7. Further increase in pH lowered the swelling of the hydrogel and reached 68 g/g at pH 10. These pH dependent swelling and deswelling behaviors are probably due to the ionization of the carboxylic groups of AA. When the pH of the medium is less than the pKa of AA ( $pK_a = 4.7$ ), the ionization of carboxylic acid group is suppressed due to the presence of higher amount of  $H^+$  ions [14.18]. As a result, a compact structure is formed by the intra and inert hydrogen bonds within the hydrogel. However, when the pH of the medium is around the pKa value of the AA, then the carboxylic group dissociates into carboxylate and hydrogen ions. The repulsion between the carboxylate ions increased the free spaces and results in the high swelling of the hydrogel. Whereas in basic pH range, all carboxylic groups are deprotonated and the concentration of negative ions is maximum on the chain. As a result, maximum repulsion between ions is observed and water molecules are not able to stand in the pores that reduce the swelling [14.19].

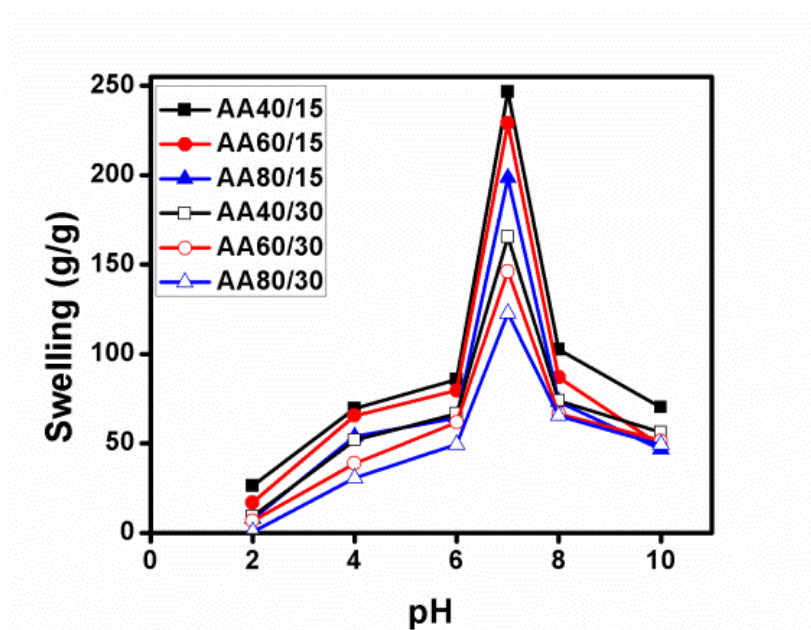


FIG. 14.5. Effect of pH on the swelling behavior of the hydrogel in non-buffer solution.

The swelling behavior of hydrogels as a function of buffer pH is shown in Fig. 14.6. It shows that the swelling behavior is similar as observed in non-buffer media. All the hydrogels showed maximum swelling at neutral pH and low swelling in acidic and basic pH range. However, at same pH, the swelling values for these hydrogels are three times less as compared to the swelling in non-buffer media. The swelling ratio of AA40/15 was 47 g/g at pH 7 in buffer media, whereas, in non-buffer media at same pH, it was 247 g/g. The possible reduction in swelling in buffer media might be due to the high ionic strength of the buffer media as compared to the non-buffer media [14.19].

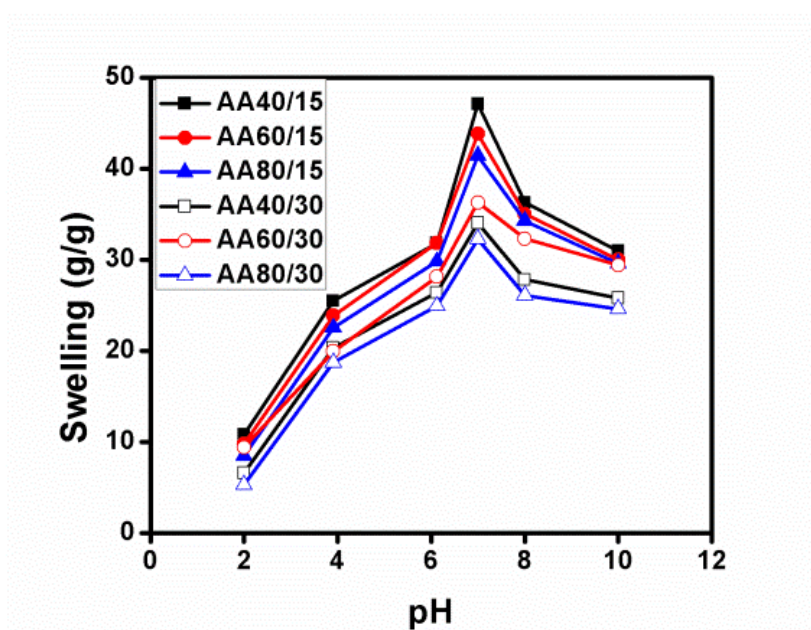


FIG. 14.6. Effect of pH on swelling behavior of the hydrogel in buffer solution.

## 14.5. CONCLUSION

Radiation cross-linked poly(acrylic acid) hydrogels were successfully prepared by incorporating various concentrations of PTES. It was found that the increased PTES concentration decreased the EDS of the hydrogels. The swelling of the hydrogel was affected by the pH, ionic strength, and temperature. These hydrogels showed low swelling in acidic and basic pH range and high swelling in around neutral pH. These hydrogels can be exploited as super water absorbent material in semi- arid and drought prone areas.

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## Chapter 15

### ROADMAP TOWARDS REGISTRATION AND TECHNOLOGY TRANSFER OF RADIATION PROCESSED PLANT GROWTH PROMOTERS/ELICITORS: THE PHILIPPINE EXPERIENCE

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#### Abstract

Pot experiments to test the effectivity of radiation-processed oligocarrageenans as a plant growth promoter/elicitor have been done on rice, soybean, tomatoes, and corn. Though many data from IAEA RCA and CRP participating countries have proven the efficacy of radiation modified oligomers as bio-stimulants and elicitor, certain R & D gaps need to be addressed in order that these products can reach the commercialization stage. A more integrated and systematic study of each crop with the following components need to be conducted: a) timing of application (plant growth stages such as seedling, active, vegetative, and reproductive stages); b) dosage (concentration of oligomers and volume of spray); c) effect in different seasons of the year (dry or wet); d) efficacy in photosynthetic activities (greening of leaves); and e) induction of resistance to pests and diseases (sturdiness, color of leaves, plant height, enzyme systems etc.). Some R & D work on the effect of radiation processed oligocarrageenans as plant growth promoter/elicitor are discussed. It also discusses R & D gaps that need to be addressed to make these oligomers reach the market. The Fertilizer and Pesticide Authority of the Philippines categorizes plant growth promoters and elicitors as fertilizers, requiring very stringent regulations for its registration. The paper proposes a roadmap towards the commercialization of plant growth promoter/elicitors.

#### 15.1. INTRODUCTION

The *Eucheuma* seaweed, which produces carrageenan, is a red algae grown in culture farms located mostly in the Eastern Visayas and Mindanao areas of the Philippines. The Philippines remains the world's top producer of seaweed, according to SIAP (Seaweed Industry Association of the Philippines) President Benson U. Dakay, with an estimated production of 92 700 metric tons per year (MTPY) (46% of the *Eucheuma* seaweed world production) in 2007 [15.1]. A total yield of 34 500 MTPY of processed carrageenan was also produced in the same year. This accounts for 41% of the processed carrageenan world production. These data easily make the *Eucheuma* seaweed and the Philippine processed carrageenan as priority export products of the country. The Philippines should maintain this competitive advantage through R&D that will diversify the applications of carrageenan and open new markets for it.

Carrageenans are hydrophilic polymers that comprise the main structural polysaccharides of numerous species of seaweed e.g., *Eucheuma*, *Chondrus*, *Gigartina*, *Fucellaria*. They are composed of D-galactose units linked alternately with  $\alpha(1,3)$ -D-galactose-4-sulfated and  $\beta(1-4)$ -3,6-anhydro-D-galactose. Due to their half-ester sulfate moieties, they are strongly anionic polymers. These sulfated galactans are classified according to the presence of the 3,6 anhydrogalactose on the 4-linked residue, and in the number and position of the sulfate group. Carrageenan that can create gels have a 3,6-anhydrogalactose unit. The most well-known of

these are kappa ( $\kappa$ )-carrageenans (A-G4S), iota ( $\iota$ )-carrageenans (A2S-G4S), and lambda ( $\lambda$ )-carrageenan.

Chitosan is derived from chitin whose structural element comes from the exoskeleton of crustaceans (such as crabs and shrimp) and cell walls of fungi. Chitosan, a cationic polysaccharide produced by the N-deacetylation of chitin under alkaline conditions, contains a linear sugar backbone of chitosan composed of  $\beta$ -1,4-linked glucosamine units. It exhibits a wide variety of biological activities, including antitumor activities, immunostimulating effects, cholesterol-lowering, antimicrobial effects, wound healing effects, antifungal activities, and free radical scavenging activities [15.2].

The Philippines exports crab and shrimp shells used for the production of chitin and chitosan. However, due to the low demand for these materials in the country, no current commercial facility for the processing of chitin and chitosan exists. The demand for chitosan in the Philippines has so far been limited to its use as food supplements, and health and cosmetic products. Exploiting its utilization in agriculture will certainly increase the market demand and boost the marine shell industry in the country.

While both the polysaccharides carrageenan and chitosan exhibit several biological functions, their utilizations are, in most cases, in the form of their oligomers. Oligochitosan has shown a wide range of biological applications, including health food, plant growth stimulator, feed additive, antimicrobial agent, etc. In addition, oligochitosan is effective at eliciting plant innate immunity against plant diseases in several plants like tobacco, rapeseed, rice, and grapevine. Oligo- $\kappa$ -carrageenan induces the secretion of laminarinase from *Rubus* cells and protoplast [15.3]. Degraded  $\lambda$ -carrageenan is reported to have tumour-inhibiting activities [15.4–5]. Oligomers from carrageenan suggest promising antiherpetic and anti-HIV (human immunodeficiency virus) activities [15.6–9].

## 15.2. RESEARCH AND DEVELOPMENT ON RADIATION PROCESSED OLIGOCHITOSAN AND OLIGOCARRAGEENAN AS PLANT GROWTH PROMOTER/ELICITOR

Low molecular weight naturally occurring polysaccharides, prepared through conventional methods (enzymatic or acid/base hydrolysis), have been reported to possess novel features such as the promotion of germination and shoot elongation and the stimulation of growth of *Bifidiobacteria* [15.10]. It is reported that the oligosaccharides act on plants as phytohormone-like compounds in regulation processes of morphogenesis, growth, and development [15.11–15.14].

Recently, degradation by radiation processing of polysaccharides has gained much attention owing to its technological effectiveness in producing low molecular weight oligomers. Upon irradiation, polysaccharides, e.g. carrageenan and chitosan, can be depolymerized to form shorter fragments. 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.

Under the auspices of the International Atomic Energy Agency (IAEA) and the Forum for Nuclear Cooperation in Asia (FNCA), a regional cooperative project on “Radiation Processing of Natural Polymers” has demonstrated the usefulness of radiation modified polymers (chitosan, alginates and carrageenan) as growth promoters and protectors of crops. A lot of studies have been carried out in Member States, including the Philippines, to

investigate the plant growth promotion and plant protection effect of radiation processed polysaccharides in a variety of crops under different environmental conditions.

When solutions of the irradiated  $\kappa$ -carrageenan and  $\iota$ -carrageenan are mixed with the growth medium for rice seedlings under hydroponics conditions, stimulation of growth is observed [15.15–15.16]. The maximum % weight gain of rice seedlings is obtained with  $\kappa$ -carrageenan irradiated at 100 kGy. Unirradiated  $\kappa$ -carrageenan and  $\iota$ -carrageenan also stimulate the growth of rice to some extent. This suggests that the radiation product should be within a certain range of Mw for it to exhibit the growth promoting effect on plants. Thus, carrageenan exhibits various levels of growth promoting effects. At 100 kGy and with a Mw of 24 000,  $\kappa$ -carrageenan shows the highest effect. On the other hand,  $\iota$ -carrageenan exhibits less growth promoting properties than  $\kappa$ -carrageenan. The growth promoting activity of  $\kappa$ -carrageenan on vegetables like bok-choi and mustard has also been tested, wherein  $\kappa$  carrageenan is applied through hydroponics for the cultivation of bok-choi, while foliar spraying is used for mustard. Growth promoting effects are observed for both cultivation conditions.

The effect of irradiated  $\kappa$ -carrageenan on the growth of chrysanthemum in tissue culture has previously been studied. The optimum growth promoting effect of irradiated  $\kappa$ -carrageenan is observed to be at 200 kGy. Based on the radiation degradation yield (Gd) of  $\kappa$ -carrageenan, the corresponding Mw of  $\kappa$ -carrageenan irradiated at 200 kGy is 2000. The biological activity of oligosaccharides derived from irradiated  $\kappa$ -carrageenan at different doses in potato tissue culture bioassay has also been tested. Unirradiated carrageenan inhibits the growth of potato in tissue culture while carrageenan irradiated at 30 kGy (Mw=10 000) shows the highest growth promoting effect. Compared to the control, the fresh biomass and shoot height increase by 35% and 15%, respectively, when supplemented with oligosaccharide with an Mw of 10 000 [15.17].

Chitosan irradiated at suitable doses (ca. 100 kGy) is effective as plant growth promoters (PGP) and heavy metal eliminators in crop production. Radiation modified chitosan has been demonstrated to increase yield in crops against control (with recommended rate of fertilizer) such as rice (10–23%), mungbean (30%), chili (34–50%), potato (25%), carrot (55%), tomato (25–31%), strawberry (71%), cucumber (23%), and sugar cane (13%) in pot and field experiments [15.18–16.21].

### 15.2.1. Preliminary pot experiment on rice

Preliminary experiments on the effect of oligocarrageenan on rice using pot experiments were conducted by the Philippine Rice Research Institute (PhilRice). Two factorial experiments arranged in randomized complete block design were conducted under greenhouse conditions. Factor A was Fertilizer Level: No fertilizer application as control,  $\frac{1}{2}$  of the recommended rate (RR) of fertilizer (60–20–20 kg NPK/ha) application and RR. Factor B was application of radiation modified carrageenan at 50 ppm: No Carrageenan, refined carrageenan, semi-refined carrageenan, and seaweed. The results are summarized in Table 15.1.

TABLE 15.1. TWO-WAY TABLE ON THE YIELD OF RICE AS INFLUENCED BY APPLICATION VARYING LEVELS OF FERTILIZER AND SOURCES OF CARRAGEENAN UNDER GREENHOUSE CONDITIONS

Fertilizer	No	Refined	Semi-refined	Seaweed	Mean	% Increase over
	Carrageenan	Carrageenan	Carrageenan	Carrageenan		Control
No Fertilizer	17.23	17.03	20.16	19.42	18.46	0

1/2 RR	17.52	24.69	21.26	22.76	21.56	16.78
RR	19.32	26.11	26.25	25.89	24.39	32.14
Mean	18.02	22.61	22.56	22.69		
% Increase over Control	0	25.45	25.15	25.89		

Across carrageenan application, plants in pots applied with RR (120-40-40 kg NPK/ha) had higher yield, produced more tiller, panicle number, and heavier straw weight than those plants applied with 60-20-20 kg NPK/ha and those without fertilizer application, as shown in Figure 15.1.

Figure 2 indicates that, compared with the control, application of refined, semi-refined, and seaweed extract enhanced the growth of rice but did not vary among types of carrageenan.

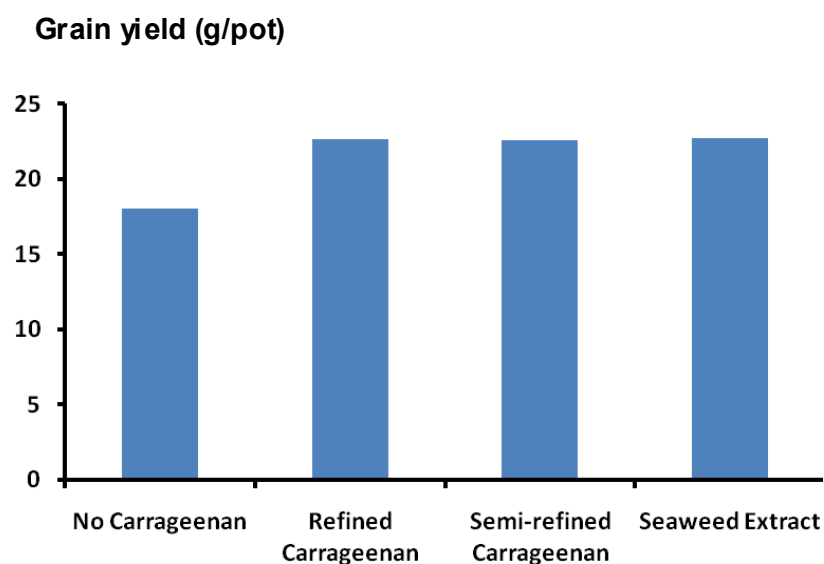


FIG. 15.1. Effect of fertilizer application, across carrageenan application on grain yield of rice under greenhouse conditions.



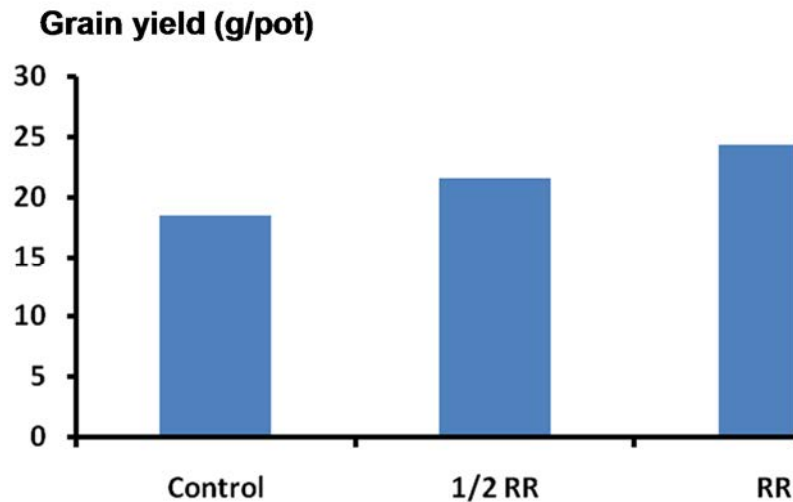


FIG. 15.2. Effect of carrageenan application, across fertilizer levels, on grain yield of rice under greenhouse conditions.

### 15.2.2. Preliminary pot test on mungbean

Preliminary experiments were conducted to assess the effect of oligo-carrageenan concentration on the yield of mungbean using a completely randomized design under greenhouse conditions. Table 15.2 shows a significant difference in yield between those added with oligo-carrageenan and control which was highest at a concentration of 75 ppm. However, no significant difference was observed in plant height among treatments.

TABLE 15.2. EFFECT OF OLIGO-CARRAGEENAN APPLICATION ON YIELD AND PLANT HEIGHT IN MUNGBEAN

Oligo-κ-carrageenan concentration (ppm)	Plant height (cm)	Yield (g)
0	60 <sup>a</sup>	14 <sup>a</sup>
50	69 <sup>a</sup>	23 <sup>bc</sup>
75	74 <sup>a</sup>	27 <sup>c</sup>
100	69 <sup>a</sup>	24 <sup>bc</sup>
150	64 <sup>a</sup>	18 <sup>ab</sup>

Values of different letters (a), (b), and (c) in columns are significantly different at  $P < 0.05$ .

Values of the same letters in columns are not significantly different at  $P < 0.05$ .

### 15.3. RESEARCH AND DEVELOPMENT GAPS ON RADIATION PROCESSED OLIGOMERS AS PLANT GROWTH PROMOTER/ELICITOR

Several IAEA RCA member states have proven the efficacy of radiation modified oligomers as bio-stimulants and elicitor. Some of them have, in fact, commercialized their own products. In Vietnam, three such formulations are already in the market, namely, Olicide and Gold Rice (chitosan-based) and T&D (alginate based). China has also commercialized several radiation modified oligochitosan products. Other countries like Malaysia, Thailand, and Indonesia are now heading in the direction towards the technology transfer of their radiation modified polysaccharide products.

In the Philippines, the commercialization of the plant growth promoters and elicitors do not depend only in their efficacy, e.g. increase in yield and increase in disease resistance. For technology transfer purposes, what is important in companies is the science to support claims of its effectiveness. Thus, it is vital to determine the mechanism and the contributory factors to the plant growth promoter and elicitor effects. Although some researches have been conducted on certain mechanisms such as induction of Phytoalexin Momilactone A in rice leaves and increase P uptake in soybean plant, no systematic experiments have been done so far to make a definite conclusion. It is essential that research on the following areas be clearly established:

- Timing of application (plant growth stages such as seedling, active vegetative and reproductive stages),
- Dosage (concentration of oligomers and volume of spray),
- Effect in different seasons of the year (dry or wet),
- Efficacy in photosynthetic activities (greening of leaves), and
- Induction of resistance to pests and diseases (sturdiness, color of leaves, plant height, enzyme systems etc.).

To address the above gaps in research, the Philippines is currently undertaking a research program entitled “Plant Bio-Stimulants and Elicitor from Radiation Modified Natural Polymers”. This has three (3) component projects implemented by different institutes: a) Evaluation of the effects of Radiation - Modified Carrageenan on the Growth and Yield of Mungbean (*Vigna radiata* [L.] R. Wilczek) and Peanut (*Arachis hypogaea* L.) by the Philippine Nuclear Research Institute; b) Elucidation of Growth Promotion Mechanisms of Radiation-Modified Carrageenan and Chitosan on Rice by the Philippine Rice Research Institute; and c) Biological Efficacy Evaluation of Radiation-Modified Kappa Carrageenan and Chitosan as Inducers of Resistance Against Major Pests and Diseases in Rice by the National Crop Protection Center, College of Agriculture, UP Los Baños. The program is being funded by the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD), Department of Science and Technology (DOST) with a total budget of around US \$400 000 for three years.

### 15.4. PHILIPPINE REGULATION FOR THE REGISTRATION OF PLANT GROWTH PROMOTERS/ELICITORS

The Fertilizer and Pesticide Authority (FPA) is the regulating agency in the Philippines in charge of product registration of fertilizers and pesticides. It has a stringent policy that follows regulation set by the Food and Agriculture Organization. In most Asian countries, registration of PGPs requires only its chemical composition for labelling purposes and some proof for the effectiveness of the product as plant growth promoter/elicitor. However, in the

Philippines where the PGPs are categorized as fertilizers, their registration would require all related information, including product composition, physical and chemical properties, list of material/raw materials used, actual production process, target users/crops (for what specific crop), the handling of the experiment/field test to determine efficacy of the product by an accredited researcher of the FPA, information on the cost of components, among other requirements [15.22]. Considering the financial implication that the process of registration for PGPs would entail, a solid proof of concept for the effectivity of the product has to be done prior to registration. This can only be achieved through a more systematic R & D (both greenhouse and field experiments for different seasons) that would include the mode of action for its plant growth promoter and elicitor effects.

#### 15.5. PHILIPPINE ROADMAP TOWARDS COMMERCIALIZATION OF PGPS

In order for the PGP to reach commercialization, it should have some proven technical benefits such as the following:

- Reduced NPK (e.g. for rice up to 65%, for corn up to 30%),
- Increased resistance to water, temperature and disease stress, and
- Ultra low volume and easy to adopt.

In addition, it has to perform some product capability analysis with the inclusion of the following components:

- Differentiation compared to competitor products,
- Features and expected benefits, and
- Cost-benefit efficiency.

Considering all the requirements for the registration of PGPs and their eventual commercialization, Figure 15.3 shows the Philippine roadmap towards the commercialization of PGPs. It is hoped that, with the support given by the DOST on the R & D work, PGPs that are made up of radiation modified chitosan and carrageenan oligomers will reach successful commercialization.

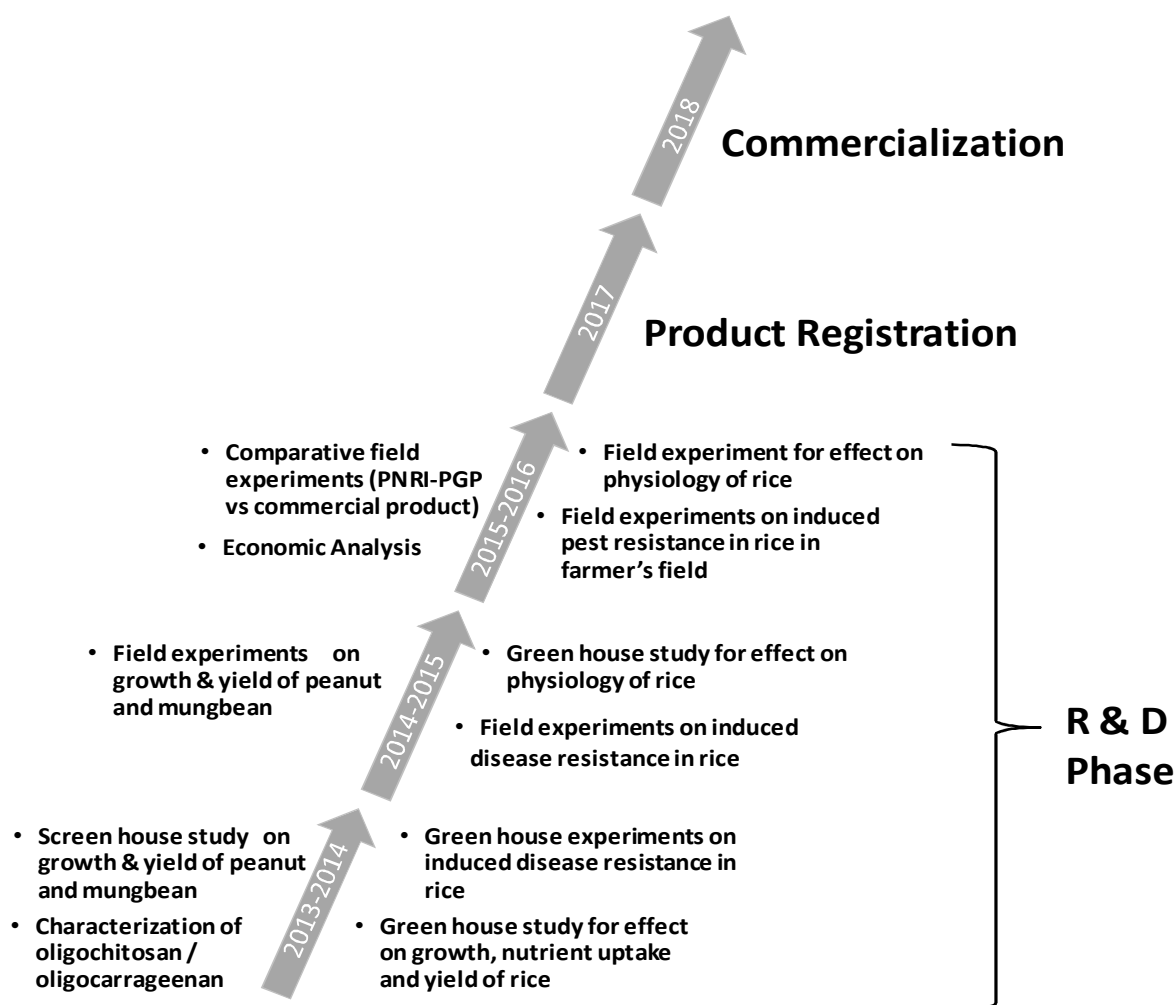


FIG. 15.3. Philippine roadmap towards the commercialization of radiation processed chitosan and carrageenan oligomers as plant growth promoter/elicitor.

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## Chapter 16

### STUDIES ON THE RADIATION-CHEMICAL BASIS OF SYNTHESIZING OLIGOSACCHARIDES AND POLYMER MICROGELS, AND ANALYTICAL METHODS FOR THEIR CHARACTERIZATION

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#### Abstract

This report summarizes recent studies performed at the Institute of Applied Radiation Chemistry (IARC) at the Lodz University of Technology in fields related to radiation synthesis and the modification of polymers for agricultural applications. These projects included both natural and synthetic polymers. Based on the extensive studies conducted on the mechanism and kinetics of radiation-induced reactions in polysaccharides within the CRP's "Development of Radiation-Processed Products of Natural Polymers for Application in Agriculture, Healthcare, Industry, and Environment", interlaboratory activities to assess existing methods in determining the molecular weight and degree of deacetylation of chitosan, and to refine protocols for performing these measurements have been planned, performed and evaluated. Synthetic polymers have been used in the basic and application-oriented research on the radiation synthesis of hydrogels, microgels, and nanogels (> 50 papers). IARC is ready to support research teams from member states that work on the elaboration and implementation of polymers for agricultural applications, using the existing experience in basic studies and in analytical methods to evaluate the physicochemical properties of polysaccharides.

#### 16.1. CONTRIBUTIONS TO THE RADIATION CHEMISTRY OF POLYSACCHARIDES

Members of the Applied Radiation Chemistry Group at the IARC have been involved in studies on the radiation chemistry of polysaccharides since the early 1990s, with 20 published papers in this field [16.1-20]. Focus of these studies has been two-fold, namely: a) the elucidation of the mechanism of radiolysis of polysaccharides using techniques and approach previously developed for water-soluble synthetic polymers, and b) new practical applications of polysaccharides. To some extent, this work has been based on an even earlier experience at the IARC that is related to the radiation modification of cellulose. Within the last few years, it has concentrated on research work on chitosan and on carboxymethylated derivatives of water-soluble polysaccharides.

Early studies on the radiolysis of chitosan in the solid state where focus was on the degradation processes, oxidation, and loss of side groups [16.1-3, 16.8, 16.10] have been performed at the IARC. Recently, however, the focus has shifted to irradiation in aqueous solution [16.4, 16.5, 16.8]. Kinetics and mechanisms of elementary reactions induced by ionizing radiation for selected polysaccharides have been studied, including reactions with OH radicals that led to the formation of carbon-centered radicals at the polysaccharide chain. The subsequent transformations of these radicals, in turn, led to the breakage of the glycosidic bond and resulted in the splitting of the chain into two shorter fragments (degradation, Fig. 16.1). Using pulse radiolysis with conductometric detection made it possible to follow the chain breakage process in chitosan and to determine the rate constants of this reaction (Fig. 16.2). In oxygen-containing solutions, the initially formed carbon-centered radicals reacted rapidly with oxygen. The resulting peroxy radicals can initiate chain scission or rearrange with  $\text{HO}_2^\cdot$  elimination (Fig. 16.3).

As a result of radiation-induced degradation in the solid state and in solution, the average molecular weight of a polysaccharide decreases (Figs. 16.4a, b), leading to significant

changes in properties. Interestingly, in many aspects, the biological activity of polysaccharides significantly becomes more pronounced with decreasing molecular weight. This effect becomes the basis for the application of radiation technology in the modification of polysaccharides to make them suitable as plant growth promoters, elicitors, diet additives [16.8, 16.10], biomaterial components [16.2], etc.

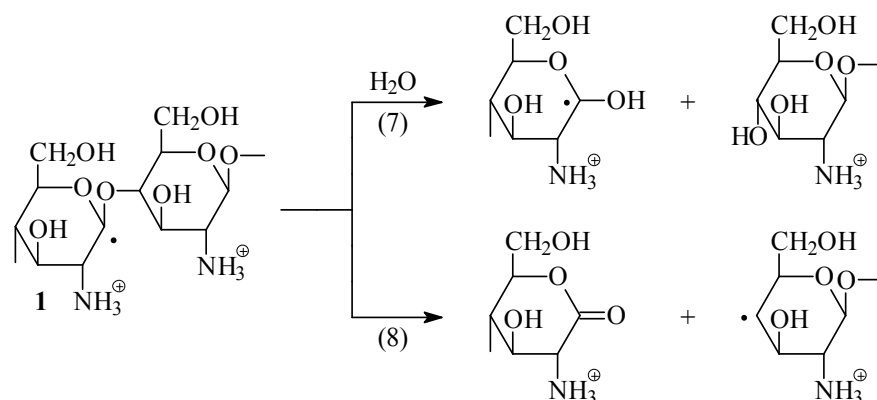


FIG. 16.1. Exemplary hydrolysis, rearrangement, and fragmentation reactions during radiolysis of chitosan in oxygen-free aqueous solution.

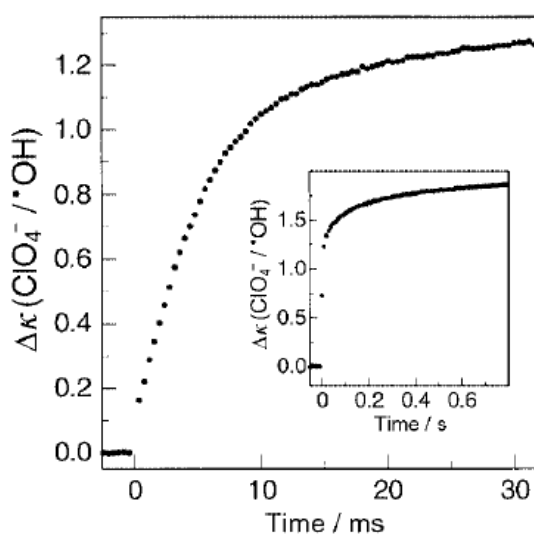


FIG. 16.2. Pulse radiolysis of chitosan ( $4.0 \times 10^5$  Da, DD = 90.5 %,  $0.5 \text{ mmol dm}^{-3}$ ) in  $\text{N}_2\text{O}$ -saturated solution, pH 3.3 ( $\text{HClO}_4$ ), dose 9 Gy. Conductivity increase (in mol  $\text{ClO}_4^-$  released per mol of  $\bullet\text{OH}$  radicals reacted) as a function of time after pulse.

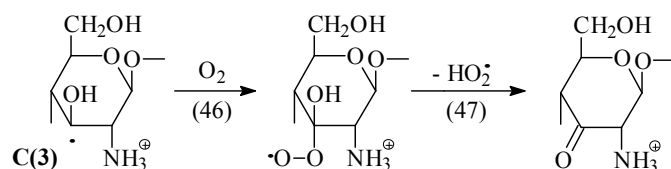


FIG. 16.3. Exemplary reactions in the radiolysis of chitosan in aqueous solution in the presence of oxygen.

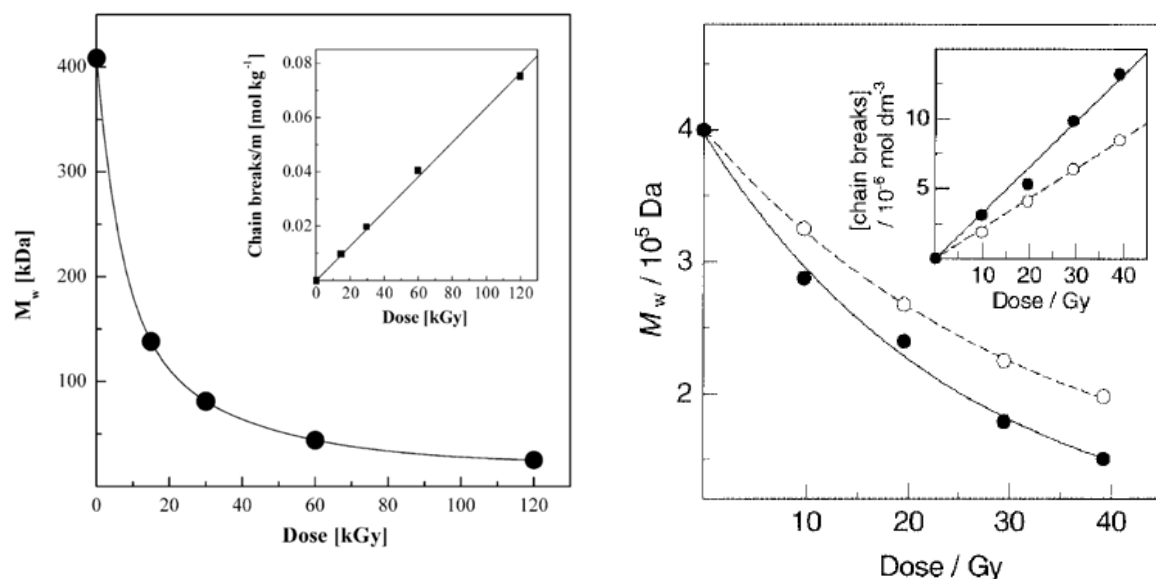


FIG. 16.4. (a)  $\gamma$ -Radiolysis of chitosan in the solid state in open vessels. Weight-average molecular weight as a function of absorbed dose. Inset: number of chain breaks per kg as a function of dose. (b)  $\gamma$ -Radiolysis of chitosan ( $1 \times 10^{-2} \text{ mol dm}^{-3}$ , pH 3.0) in  $\text{N}_2\text{O}$ -saturated ( $\bullet$ ) and  $\text{N}_2\text{O}/\text{O}_2$ -saturated ( $\circ$ ) aqueous solution. Dose rate  $0.082 \text{ Gy s}^{-1}$ . Weight-average molecular weight as a function of dose. Inset: concentration of chain breaks as a function of dose.

Irradiation of polysaccharides in any form is inevitably accompanied by side reactions, one of which is oxidation that manifests itself in the formation of carbonyl groups. There may be other effects that are specific to particular polysaccharides and their chemical structures (*e.g.*, decay of amino groups in chitosan). Formation of carbonyl functions is one of the reasons for the change in color (darkening) of polysaccharides caused by irradiation (see the spectral changes presented in Fig. 16.5 [16.3, 16.8]). While color change is not a problem for preparations intended for agricultural applications, the underlying chemical changes may be crucial, since they may influence chemical properties and biological activity of the polysaccharide.



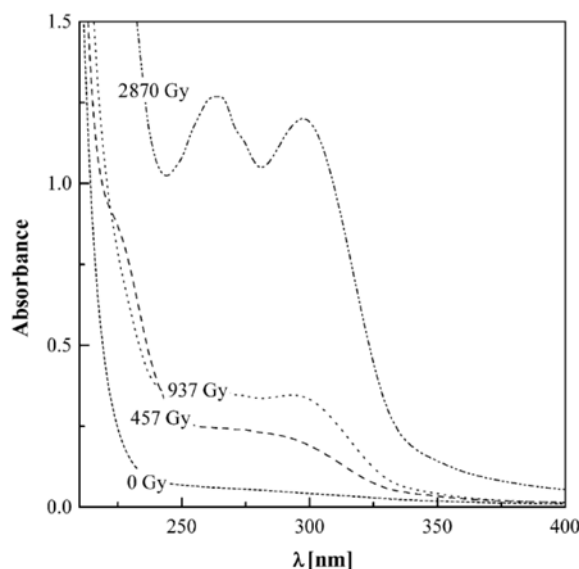


FIG. 16.5.  $\gamma$ -Radiolysis of chitosan ( $M_w = 3.2 \times 10^5$  Da, DD = 88 %,  $2 \times 10^{-2}$  mol dm $^{-3}$ , pH 3.0, HClO $_4$ ) in N $_2$ O-saturated aqueous solution. Dose rate 0.023 Gy s $^{-1}$ . Absorption spectra as a function of dose.

Another important issue, although often overlooked, is post-irradiation effects. This is critical in the practical applications of ionizing radiation for modifying polysaccharides of semi-crystalline structure, since it may affect the shelf life of some products. During irradiation in the solid state or any other state where the partially crystalline structure is retained, some of the initially formed radicals may become trapped in crystalline regions and remain there for long periods of time (hours to months or even longer) after irradiation. These “frozen” radicals may slowly migrate to the boundaries of crystalline regions, where they can undergo reactions of similar mechanisms as those occurring directly under irradiation. Aside from the very slow migration, other processes such as changes in crystalline structure due to external conditions and the migration of traces of water may make these dormant radicals available for reaction. Post-irradiation effects for samples irradiated and stored both in the presence and absence of oxygen may occur. This may result in a slow but distinct decrease of molecular weight occurring for at least a few weeks after irradiation [16.2, 16.3].

Another broad topic in our studies is the additive-free cross-linking of polysaccharides to form hydrogels. For additional information, the reader is referred to other relevant studies [16.11–16.16]. It is possible to use such hydrogels as an interesting alternative to microgels made of synthetic polymers for soil moisture retention (see below).

## 16.2. DETERMINATION OF BASIC PHYSICOCHEMICAL PROPERTIES OF POLYSACCHARIDES

Polysaccharides are polymers of natural origin, most of which may be obtained from various sources (e.g., different species of plants or animals). Therefore, they may differ in their chemical, molecular, structural, and application-oriented properties [16.21–23]. Even if the starting material is obtained from the same source, there may be natural variations in parameters from batch to batch. On the other hand, a final product intended to be marketed should be uniform, reproducible from batch to batch, and of well-defined properties. This means that a careful quantitative analysis of substrates and products should be performed, and the processing protocols need to be adjusted to accommodate potential batch-to-batch variations of the substrates.

While there are many sophisticated techniques in existence that can be used to analyze polymers, they may not be available in a quality control laboratory of a processing site. Therefore, there is a need to develop methods and procedures which would allow researchers to determine the most important parameters of substrates and products using low-to-medium cost methods, and yet yield consistent and reliable results.

To solve this problem, 10 participating laboratories from various member states have been organized within the CRP “Development of Radiation-Processed Products of Natural Polymers for Application in Agriculture, Healthcare, Industry and Environment” to determine the average molecular weight and degree of deacetylation (DD) of chitosan, with the IARC as the coordinating center. A comprehensive manual for viscometric determination of intrinsic viscosity and average molecular weight of chitosan has also been prepared and distributed among the participating laboratories. Detailed descriptions of these studies and their results can be found in the relevant CRP reports.

The interlaboratory study has been considered a success; the standard deviation of intrinsic viscosity results among all the participating laboratories was 7.5% (Fig. 16.6). Through the cooperation of the Hacettepe University (Ankara, Turkey), Glyndwr University (Wrexham, U.K.) and IARC, many methods for DD determination described in literature have been screened and laboratory tested. The best simple methods have been selected, and a detailed manual for one of them has been elaborated to serve as a guide for laboratories in the member states.

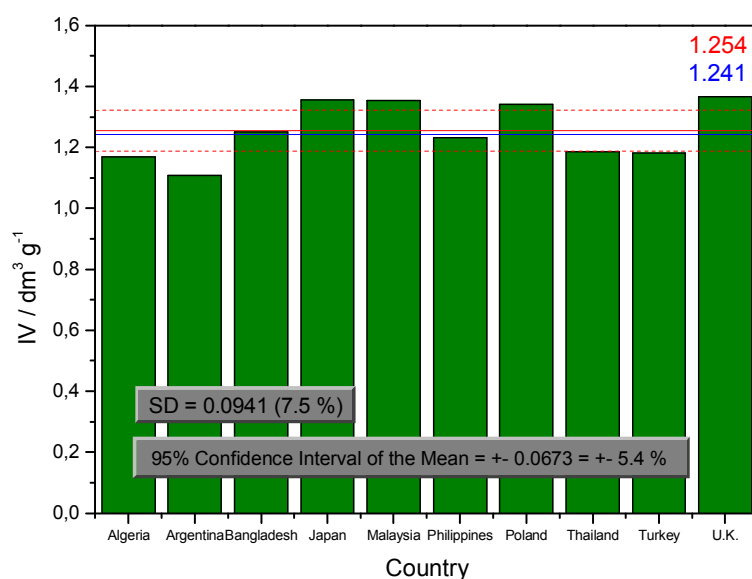


FIG. 16.6. Intrinsic viscosity data for non-irradiated chitosan, obtained in the participating laboratories. Solid red line and number – mean. Broken red lines – 95% confidence interval of the mean. Solid blue line and number – median.

Quantitative interpretation of analytical results may require not just the availability of raw data, but also proper calculation methods. In the case of radiation-induced degradation of polymers, including polysaccharides, one of the basic parameters allowing quantifying degradation is the radiation-chemical yield of degradation, Gs.

The radiation-chemical yield of degradation, G(s) [mol J<sup>-1</sup>] is defined as:

$$G(s) = \frac{n_{cb}}{E} \quad (1)$$

where  $n_{cb}$  [mol] is the number of chain break events which took place in the polymer sample (whether it is dry polymer, solution, etc.) upon absorption by the sample of energy  $E$  [J] of ionizing radiation.

While the relevant equations relating  $G_s$  to the changes in molecular weight have been developed by Charlesby in the 1950s and 1960s [16.24, 16.25] and cited later in some review works [16.26], there seems to be some confusion in many papers reporting original data on the proper form and usage of these equations, especially in multicomponent systems (e.g. polymer solutions). A discussion and derivation of basic equations will soon be published in an IAEA book on radiation chemistry of polysaccharides. Here, the equations are cited for the case where no cross-linking takes place, i.e., when the only radiation-induced process influencing the molecular weight of the polymer is degradation.

For solid state irradiation:

$$G(s) = \frac{1}{D} \left( \frac{1}{M_n} - \frac{1}{M_{n0}} \right) \quad (2)$$

$$G(s) = \frac{2}{D} \left( \frac{1}{M_w} - \frac{1}{M_{w0}} \right) \quad (3)$$

where  $D$  [ $\text{J kg}^{-1}$ ] denotes the dose of ionizing radiation absorbed by the sample,  $M_{n0}$  and  $M_n$  [ $\text{kg mol}^{-1}$ ] are number-average molecular weights before and after absorbing the dose  $D$ , respectively,  $M_{w0}$  and  $M_w$  [ $\text{kg mol}^{-1}$ ] are the weight-average molecular weights before and after absorbing the dose  $D$ , respectively. Equation (2) is valid for any initial molecular weight distribution of the polymer; while equation (3) is valid for the most probable distribution where  $M_{w0} / M_{n0} = 2$ .

For irradiation in a multicomponent system (e.g., in solution), the polymer concentration is denoted as  $c$  [ $\text{kg dm}^{-3}$ ],  $c = m / V$ , then:

$$G(s) = \frac{c}{D \times \rho} \left( \frac{1}{M_n} - \frac{1}{M_{n0}} \right) \quad (4)$$

$$G(s) = \frac{2c}{D \times \rho} \left( \frac{1}{M_w} - \frac{1}{M_{w0}} \right) \quad (5)$$

### 16.3. RADIATION-SYNTHESIZED MICROGELS FOR SOIL MOISTURE RETENTION

Since the 1980s, the IARC has been involved in the application of radiation technique for synthesizing hydrogels, mainly for medical purposes, with over 50 published papers. Besides a broad scope of basic research on the radiation chemistry of water-soluble polymers considered the building blocks for hydrogels, a complete radiation technology for synthesis and simultaneous sterilization of hydrogel wound dressings [16.27] has been developed. This technology has been implemented in various industries in many countries [16.28, 16.29].

Besides research on macroscopic hydrogels, new methods aimed at synthesizing nanogels (internally cross-linked macromolecules) and microgels have been developed [16.30–34]. A standard version of this method is based on intramolecular cross-linking of polymer chains in dilute aqueous solution, typically initiated by one or many intense pulses of fast electrons. The method is versatile; it has been tested on a number of water-soluble polymers, including pH- and temperature-sensitive polymers and interpolymer complexes [16.32, 16.35].

Alternative procedures consisting of two-step irradiation leads to nano or microgels of independently chosen molecular weight and dimensions [16.36]. This allows for the control of the internal density of the product.

While the above-described techniques are being further developed and applied in specialty products, mainly in medicine [16.37–16.42], it may be too complex and expensive to synthesize microgels for agriculture. For this particular purpose, it may be reasonable to use a simple and potentially high-throughput method of irradiating the monomer or a monomer mixture in the bulk where addition of a suitable cross-linking agent may reduce the dose necessary to obtain products of desired cross-link density. This can be followed by grinding and fractionation on sieves to obtain a relatively uniform material [16.10]. An alternative may be to perform cross-linking in monomer- or polymer solution followed by drying, grinding, and fractionation or irradiation of monomer with or without cross-linking agent in reverse emulsion (i.e., water-in-oil emulsion). When performing cross-linking polymerization in aqueous media, one may consider selecting a cross-linking agent. Poly(ethylene glycol) diacrylate was found to be a very efficient water-soluble cross-linking agent [16.43–16.45].

Choice of substrates for large-scale synthesis of microgels for agricultural applications is a matter of compromise among many factors. When it comes to price, acrylamide is generally considered to be the cheapest choice. However, if a very high water-binding capacity is required, ionic functions are desirable (e.g., acrylic acid). On the other hand, gels based to a large extent on polyelectrolytes are typically very sensitive to ionic strength and pH. Ionic strength effects should be taken into consideration since microgels are designed to perform well in the soil in the presence of various minerals, i.e., at a certain level of ion concentration. Taking all these factors into consideration, there is no universal “ideal” recipe for water-retention microgels; the factors should be balanced to best fit the local environmental and economic conditions.

Microgels can be characterized by a variety of methods. Simple tests, such as equilibrium degree of swelling, equilibrium and kinetics of uptake, and release of simple model substances as dyes, etc., can be determined using basic laboratory equipment. Kinetic studies on the dynamics of swelling of single microgel grains may require a good optical microscope with a camera. Uptake and release of particular nutrient ions can be studied using a potentiometry with ion-selective electrodes or by ion chromatography. Microgel grain size in dry state can be determined by sieve analysis (only for relatively large grains, typically > 50  $\mu\text{m}$ ) or by using a laser diffraction particle size analyzer with the grains suspended in a non-solvent. The latter can also be used to measure the size distribution in the swollen state in aqueous systems. Small cross-linked particles - nanogels - can be conveniently analyzed by dynamic and static light scattering, while their surface charge can be assessed by determining the Zeta potential. Visualization of individual microgel or nanogel particles by Scanning Electron microscopy or Atomic Force Microscopy is also possible, although care should be taken to avoid artifacts which may be caused by drying or lyophilization procedure.

Useful general information on microgels, including synthesis methods, analysis, and applications, can be found in recent review papers and book chapters [16.46–16.51].

#### 16.4. CONCLUDING REMARKS

This report summarizes the activities of the IARC in research areas relevant to the topic of the TM. Some contributions have been made to the current state of knowledge on the mechanism and kinetics of radiation-induced reactions in polysaccharides. Activities were conducted by

the IARC within the CRP "Development of Radiation-Processed Products of Natural Polymers for Application in Agriculture, Healthcare, Industry and Environment". These activities included the planning, coordination and evaluation of interlaboratory activities aimed at assessing existing methods to determine the molecular weight and degree of deacetylation of chitosan, and the elaboration of precise protocols for performing these measurements. Active basic and application-oriented studies have been performed in the field of radiation synthesis of hydrogels, microgels and nanogels. The IARC is ready to support research teams from member states working on the elaboration and implementation of polymers for agricultural applications using their experience in basic studies and analytical methods for evaluation of physicochemical properties of polysaccharides.

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## Chapter 17

### SEALED, COMPACT, ECONOMICAL, LOW-ENERGY EBEAM ENGINES —WELCOME TO THE EBEAM RENAISSANCE

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#### Abstract

The advent of commercially viable, sealed, ebeam sources promises game-changing technological, ecological, and economic gains in a wide variety of applications.

#### 17.1. INTRODUCTION

Imagine a world where there exists a way to bring opposites together: oil and water, polar and nonpolar molecules, polymers and enzymes. Some high-tech magic that could get two substances to bond that would not do so otherwise, and then disappear without a trace would give mankind a real tool to solve some real world problems. There would be a technology that drives UPcycling instead of DOWNcycling (which is what 95% of recycling really is).

Countless crops are grown where 95% of their mass have zero or negative value. It is grown for the corn and the rest of it are either discarded as waste or burned. What if mankind had an industrial process that would flip that value ratio on its head? What if the rest of the plant had more value than the seeds?

As William Hague, Foreign Secretary of the United Kingdom, said, “You cannot have food, water, or energy security without climate security. They are interconnected and inseparable.” [17.1]. We have about five or ten years to find some real solutions to *the* dilemma of our time—climate security.

By the year 2050, the planet will be home to some nine billion human beings. How will we feed them? With the demand for energy increasing exponentially, not linearly, how do we provide that energy? We will have 29% more people demanding >50% more energy in that time frame.

Low-energy ebeam, now enabled by the availability of industrialized, low-cost, compact, and sealed emitters, will be an important tool to solve this dilemma –the ultimate challenge of our generation.

##### 17.1.1. Ebeam background

Electrons have since been the first few hundred seconds of the universe. That was around 13.7 billion years ago. Fast-forward 10 billion years to the time when planets were formed. With planets came atmospheres and the first electron beams... lightning. Fast forward another 4.5 billion years to the year 1895 when the work of Dr. Wilhelm Roentgen and his contemporaries resulted in a focused ebeam, first within a vacuum tube, later through air.



*FIG. 17.1. Dynamitron DC electron accelerator [17.2].*

By the 1950s, there were various electron accelerator concepts: Van de Graaf, RF, DC. The accelerator shown in *Figure 1* is a Dynamitron developed by the company RDI, which was later acquired by IBA.

The earliest known industrial use of ebeam was by the Boise-Cascade Company in the mid-1960s, which it employed to cure coatings and inks on wood tiles. By 1970, Ford used ebeam to cure colored lacquer on the dashboard plastics of the Cougar, Mercury, and Grenada. Systems like Ford's were, and still are, bulky and expensive. Implementing ebeam in a manufacturing process is not simply a matter of buying the machine, wheeling it on to the shop floor, and installing it in the production line. It requires major investments in infrastructure. These systems are constantly pumped and require regular maintenance. Prices range from \$0.5 – 5 Million, and this does not even include changes to the infrastructure.

#### **17.1.2. Ebeam now: the ebeam renaissance**

Fifty years later, Comet developed the ebeam lamp (Fig. 17.2). The joint development project of COMET AG and TETRA PAK was started at Comet in 2004, with the goal of producing a sealed, compact, ebeam source that can be easily integrated into inline processes at a price of less than € 50 000. Comet will reach this price target within 3 years.



*FIG. 17.2. Comet ebeam lamp EBA-200/400/EG.*

By 2008, Comet was already producing a second-generation emitter. In 2009, Comet won the Swiss Innovation Prize for this technology and began selling ebeam sources—some call them engines—to clients. Finally, after >13.7 billion years of electrons and 50+ years of electron

accelerators, electron beams can now be produced by the equivalent of a high-tech light bulb for an investment of under \$100 000.

The concept of a sealed electron emitter was not created by Comet. The company AEB already had a product in the market in 2004. So what is different now? Why is this a “renaissance”? The difference is that the Comet ebeam lamps are reliable. AEB went out of business because the lifetime of the emitters was completely unpredictable, sometimes failing after just a few hundred hours. To date, the average lifetime of Comet ebeam lamps is over 8 000 hours and rising (Fig. 17.3). They can survive harsh conditions such as chambers full of ozone, hot H<sub>2</sub>O<sub>2</sub>, and abusive cycling. In the cycle testing of the newest 300 keV lamp, the emitter survived more than 100,000 on/off cycles.

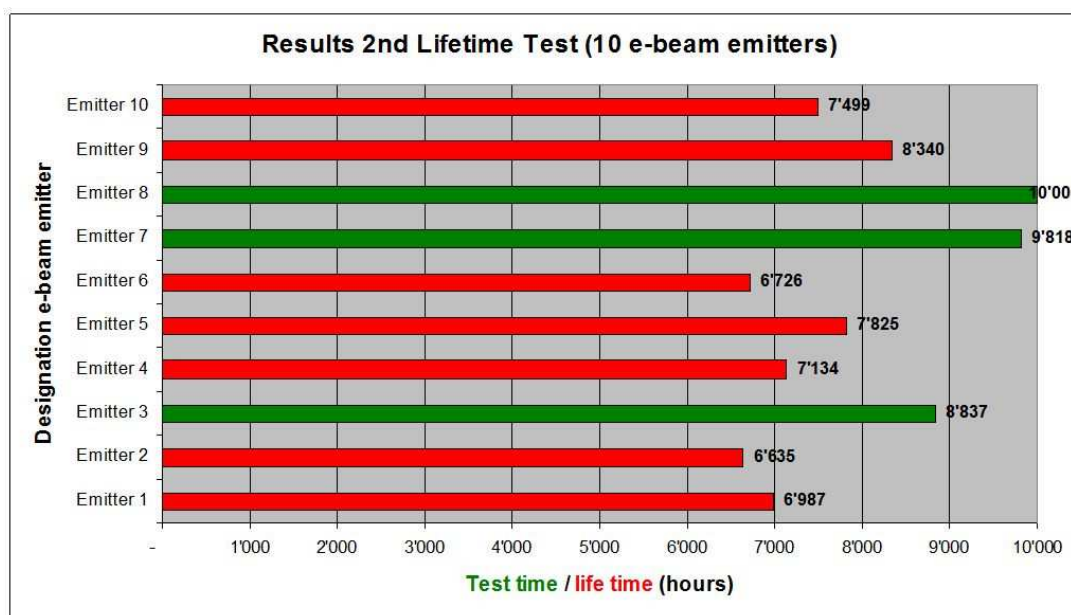


FIG. 17.3. Average lifetime of Comet ebeam lamps.

Consider that, over the past thirty years, hundreds, if not thousands, of applications in which ebeam was investigated and proved to be ideal for the process, had to be abandoned because economic and/or infrastructure issues made introducing ebeam technology impractical. Over the next decade, many of these projects will be revisited and thousands more will begin, now that researchers have access to a compact, low-cost, and industrialized ebeam lamp.

### 17.1.3. Ebeam now: available products

Table 17.1 shows the portfolio of available ebeam lamps.

TABLE 17.1. EBEAM LAMP PRODUCT PORTFOLIO

	LENGTH (mm)	ENERGY (keV)			
		80	120	200	300
270	x	x	x	x	Q4 2014
400	x	x	x	x	Q4 2014
460				upon request	
600				upon request	

About 300 kV engines will be available in Q4 of 2014. Comet ebeam has the infrastructure to produce lamps with a beam length of up to 600 mm. Longer lamps will be developed if there is a demand for it.

Application development with ebeam is very difficult without the proper tools. Bringing samples to a service center equipped with a big accelerator is time-consuming and impractical. The machine shown in Figure 4 solves this problem.



FIG. 17.4. EBLab 200.

The EBLab is a fully programmable, fully shielded lab instrument for beaming samples with 200 keV electrons. The sample tray is the size of an A4 sheet.

If the inerting requirement is moderate (100 – 250 ppm of Oxygen), it is possible to run several samples per minute even with completely different kV and dose parameters.

This unit can either be purchased or rented. It is also possible to have samples run in any of the applications laboratories. By March 2014, there will be labs in Flamatt, Switzerland; Stamford, CT, USA; Davenport, IA USA; and Shanghai, China.

**Cylindrical lamps** (Figure 17.5) emit a cylindrical electron beam and can be used to beam the insides of bottles or pipes.

The diameter of the emitter is 65mm, as shown in Figure 17.5. A design exists for a 35mm version and will be produced if the market demands it.

The same concept is viable down to a diameter of about 18mm.



*FIG. 17.5. Miniature, cylindrical ebeam lamp.*

## 17.2. RESULTS AND DISCUSSION

### 17.2.1. Aseptic packaging

Comet has been working on aseptic packaging for nine years, but worldwide implementation of ebeam technology for this purpose is still very new. Still, it has produced substantial benefits. Consider Tetra Pak's experience.

Tetra Pak announced the launch of ebeam sterilization in April 2012 at the Anuga trade fair. Tetra Pak's mission is to make food safe and available everywhere. One of their corporate targets is to improve their operations over their entire value chain (to include their suppliers) such that their carbon footprint in 2020 is not greater than it was in 2010. When taking into account their expected growth, this amounts to a 40% reduction in CO<sub>2</sub> output [17.4].

The results of using ebeam are as follows, (1) the sterilization process using ebeam requires 80% less energy than the current method, hydrogen peroxide; (2) the carbon footprint of ebeam-equipped filling machines is 40% less than that of the existing machines, and (3) the ebeam-equipped machines draw 33% less power from the mains than the H<sub>2</sub>O<sub>2</sub> machines. Given that Tetra Pak now produces over 170 billion packages per year with the number rising, the conversion to ebeam is a significant step toward solving the population-climate dilemma [17.5].

### 17.2.2. FUNCTIONALIZATION OF MEMBRANE SURFACES

Ebeam makes it possible to bond materials that normally would never bond, and thus makes it possible to immobilize molecules to the surfaces of membranes and do it without the use of toxic chemicals [17.6–17.14]. The benefits include: a) membranes that do not foul; b) membranes that require far less energy for fluid transport; and c) biocompatibility.

Humans in the advanced stage of renal failure must undergo regular dialysis. When blood cells pass through the dialyzing membrane, they get in contact with the membrane surfaces. The cells recognize that the membrane material is not part of the body. They react to it as they would to a foreign body within the bloodstream. The surfaces of the blood cells change, return to the body in immune-defense mode, and proceed to cause a whole list of autoimmune disorders. These immunological reactions are the main reason for the high mortality rate among patients with advanced stage kidney failure, and also for the increase in medications prescribed to the patient.

With ebeam, it is possible to bond biocompatible molecules to the surface of dialysis membranes. When blood cells rub against these surfaces, they do not even realize they are outside the body. As a result, the dialysis can be done without triggering the immune response, and thus increasing the quality of life and life expectancy of the patient. Of course, it also eliminates the need for the use of additional immunosuppressive chemicals. This is not science fiction. Researchers at Leibniz IoM in Leipzig are doing it. They can also immobilize enzymes on membranes without the use of additional chemistry.

### 17.2.3. EBEAM REACTIVE COMPOUNDING

The experts in this field are Dr. Uwe Gohs and his team at the Leibniz IPF in Dresden. Their paper, “Reactive EB Processing of Polymer Compounds” [17.15] discusses the use of ebeam to create a generic, halogen-free, flame retardant PE. The scanning electron microscope (SEM) images in the paper show that the PE and the flame retardant (magnesium hydroxide) actually bond. The data on the mechanical properties also support this interpretation of the result. There was a 3-fold increase in notch impact strength. For those experiments, Dr. Gohs and his colleagues used a batch process Brabender compounder. While the results are impressive, the process itself will remain an esoteric piece of trivia until it can be performed inline. If a production rate of  $\geq 500$  kg per hour per machine was possible, however, the process would undoubtedly interest specialty compounders. Such a machine is in the works.

## 17.3. CONCLUSION

The current position of ebeam as a technology can be compared to that of the laser and the computer 50 years ago. Lasers then existed only as large, constantly pumped tubes. But they both evolved. Pumped lasers became sealed lasers, then solid-state lasers, and then diodes. Mainframe computers became minicomputers, then personal computers, and now smartphones. ebeam has just made the first jump along the price/performance curve, and it will continue to do so. Now that ebeam is in a format and cost range that makes it accessible, there has been an increase in the number of proposals submitted for research and development funding from public sources. There has also been a jump in the number of inquiries at Comet for trials, access to equipment, as well as development partnerships.

“The ebeam Renaissance” will enable acceleration in innovation in many scientific and industrial fields. Those who have the ability to innovate now have the duty to do so. Comet is happy to do its part.

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## Chapter 18

### **SUPERABSORBENT PREPARED BY RADIATION INDUCED GRAFT COPOLYMERIZATION OF ACRYLIC ACID ONTO CASSAVA STARCH**

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#### **Abstract**

Superabsorbent was synthesized by radiation induced graft polymerization of acrylic acid onto cassava starch. Parameters such as the absorbed dose and the amount of monomer were investigated in order to determine the optimum conditions for the grafting polymerization. Water retention, germination percentage, and germination energy were also determined in order to evaluate the possibility of superabsorbent in agricultural applications, especially in arid regions. The graft copolymer was characterized by the Fourier transform infrared spectroscopy (FTIR). Results indicated that the sand mixed with 0.1% wt superabsorbent could absorb more water than the sand without superabsorbent. The germination energy of corn seeds mixed with 0.5% superabsorbent was obviously higher than those without superabsorbent. These experimental results showed that the superabsorbent has considerable effects on seed germination and the growth of young plants.

#### **18.1. INTRODUCTION**

The northeastern part of Thailand is the largest region of the country covering about 170 000 square kilometers, which comprises one-third of the country. The rainfall characteristics in this area are dominated by the southwest monsoon from the Indian Ocean and tropical cyclones from the South China Sea. This zone also suffers from a large number of drought days and, more importantly, the rainfall is highly irregular. The water holding capacity of soils in the Northeast is generally low because of poor level of organic matter and clay contents. Erratic rainfall and the low water-holding capacity of soils often create water stress, affecting the stability of rice production as well as that of other crops. The low fertility of the soils generally results in low crop productivity while the erratic rainfall leads to the instability of rice fields.

The application of superabsorbent polymers to agricultural development especially in the arid rural areas where water is scarce has greatly affected the socioeconomic community. In fact, the physical properties of such superabsorbents are indeed very attractive to farmers and reforesters. When sufficient water is in contact with the superabsorbent granules, they transform themselves into water-laden gel chunks. These gels then act as a local reservoir, releasing water vapor into the soil and plants as needed and also maintaining moisture balance. In addition, these superabsorbents also prevent the leaching of nutrients while generating more nutrients within the soil to seeds, which allows faster germination, promotes earlier emergence, improves stand, and gives a greater crop yield.

Natural polymers such as starches, cellulose, chitin, and chitosan have high potential for various applications in the medical, environmental and agricultural areas due to their unique properties such as biodegradability and biocompatibility. Thailand ranks ninth in the world's producer of cassava starch roots. It is also the world's largest exporter of cassava products. The production of cassava starch often exceeds the export and consumption requirements, which results in Thailand's surplus of unused cassava starch. The development of superabsorbent by radiation processing is, therefore, a promising method to increase the values of cassava starch that is abundant and inexpensive. The use of this natural polymer as a superabsorbent can definitely alleviate the lack of water in arid rural areas of Thailand and also decrease the importation of expensive synthetic superabsorbent from foreign countries.



Radiation processing has been widely utilized to synthesize a large number of new materials. This is simply due to the fact that radiation processing is convenient, environment-friendly, effective, able to initiate reactions at ambient temperature, easier to control compared with chemical processes, and requires no initiators. It has been proven that radiation processing can also be utilized for the synthesis of superabsorbents [18.1–18.4].

This research project aims to use radiation processing to synthesize superabsorbent. The superabsorbent will be synthesized from radiation-induced graft polymerization of acrylic acid onto cassava starch. Various factors to determine the optimum conditions for the grafting polymerization will be investigated. The criteria are emphasized by the optimum conditions of important parameters to give a maximum amount of water absorption.

## 18.2. EXPERIMENTAL

Cassava starch containing 12.8% moisture was supplied by Siam Quality Starch Co. Ltd., Thailand. Acrylic acid (AA) of 99% purity from Aldrich was used without further purification. Methanol, a commercial grade from BDH, was fractionally distilled. In a gelatinization reactor, 10 g of cassava starch was mixed with 190 ml of distilled water. The mixture was continuously stirred at 450 rpm using a mechanical stirrer under nitrogen atmosphere. The mixture was then gradually heated from ambient temperature to 80°C and held at this temperature for one hour. It was left to cool down to room temperature to yield the gelatinized starch. The obtained gelatinized cassava starch was mixed with acrylic acid monomer. The mixture was stirred under nitrogen atmosphere at room temperature for 30 min to form a homogeneous mixture. The gelatinized starch-acrylic acid mixture was transferred into a glass bottle and purged with nitrogen gas for 5 min. Then, it was irradiated under gamma rays from  $^{60}\text{Co}$ , Gamma Chamber 5000 of 14 000 curies from the Department of Atomic Energy, Government of India, Mumbai. After irradiation, the mixture turned into a gel. Finally, the gel was dried in a vacuum oven at 50°C for 24 h. The crude product was weighed and extracted with methanol in a Soxhlet extractor for 24 h to remove poly(acrylic acid) (PAA) homopolymer. Then, pure copolymer was dried in a vacuum oven at 50°C for 24 h.

Homopolymer percentage was determined. The gel samples were immersed directly in distilled water for 72 hours at ambient temperature to reach the equilibrium state of swelling. The weight of the swollen gel ( $W_s$ ) was then measured. The swollen gels were later dried at 50°C until the weight of the dried gels ( $W_d$ ) remained constant. Equilibrium Degree of Swelling (EDS) was identified. The degree of grafting was determined by the percentage increase in weight. The dried superabsorbent with particle diameters between 0.7–0.3 mm was left to swell in distilled water for some time and then, the swollen superabsorbent was weighed after removal of excess water. The percentage of water absorption at the certain time was calculated. Water retention was calculated from the following method. A 500 g of dry sand mixed with 0.5 g superabsorbent was placed in a container, and the other 500 g sand without superabsorbent was placed in an identical container. One liter of water was added into both containers, and then the containers were kept under identical conditions at room temperature (about 30±3°C) for 15 days. The initial mass of the mixture in the two containers were measured after removal of excess water and their masses were recorded daily to compare the water retention of the superabsorbent. Water retention was then evaluated. Germination percentage of the seeds was also calculated. The same amount of soil with 15% moisture was placed into two identical baskets (30 × 25 × 12 cm). The depths of the soil were 10 cm. One basket was irrigated with 1500 ml of water, while the other was irrigated with 1500 ml water mixed with 10 g superabsorbent. The same amount of healthy corn seeds was

placed in each basket. Germination percentages were calculated to testify that the seed qualities of the two samples were identical and germination energies were determined to compare the effect of the superabsorbent on seed germination. Germination percentage of the seeds was then analyzed. A Bruker Tensor 27 FTIR spectrophotometer equipped with an attenuated total reflection (ATR) accessory was used to record the infrared spectra. The scanning used was 16 times at a resolution of  $4\text{ cm}^{-1}$ .

### 18.3. RESULTS AND DISCUSSION

The Fourier transform infrared (FTIR) spectra of cassava starch grafted PAA and cassava starch are shown in Figure 1. The FTIR spectrum of cassava starch shows the characteristic absorption bands of starch at  $3300$  and  $1650\text{ cm}^{-1}$  due to O-H stretching and bending modes, respectively. Additional characteristic absorption bands of cassava starch appear at  $2916$ ,  $1150$  and  $1016\text{ cm}^{-1}$  due to C-H stretching, tertiary C-OH, and C-H bending, respectively, while that of cassava starch grafted PAA shows a similar pattern with an extra band at  $1730\text{ cm}^{-1}$  attributed to  $-\text{COOH}$  group in the acrylic acid.

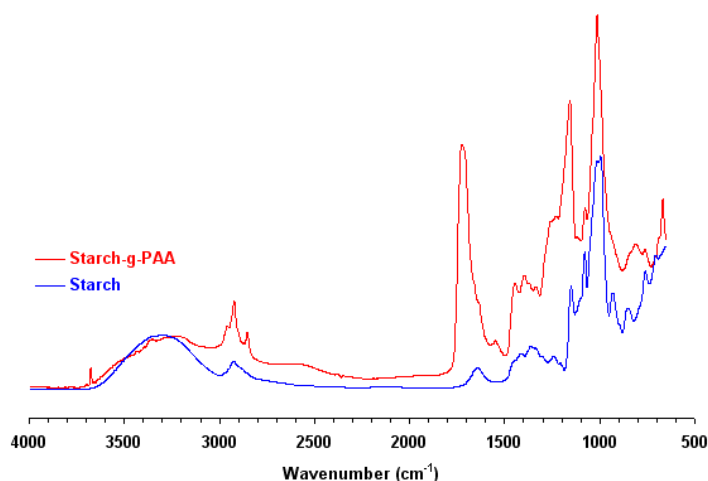


FIG. 18.1. FTIR spectra of starch grafted PAA and cassava starch.

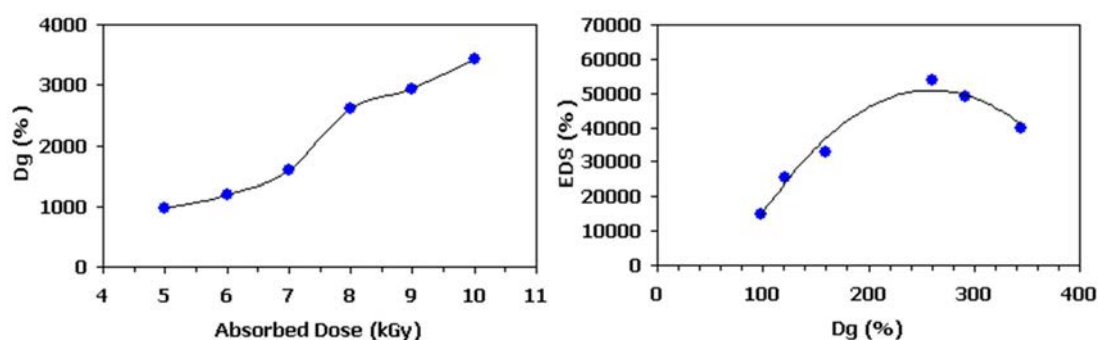


FIG. 18.2. Effect of total dose on degree of grafting and effect of degree of grafting on EDS.

The effect of the absorbed dose on the degree of grafting is shown in Figure 2. The degree of grafting increased with the increasing absorbed dose. An increase in dose enhances the formation of radicals, resulting in higher degree of grafting. There is a limited cross-linking

density for the maximal EDS according to Flory's theory [18.5]. When the cross-linking density is less than the appropriate density, the EDS of superabsorbent increases as the degree of cross-linking increases. However, when the cross-linking is higher than the appropriate one, the EDS of superabsorbent decreases as the cross-linking density increases [18.6]. It can also be observed from Figure 18.2 that the effect of % Dg on % ESD of superabsorbent has the same trend as that of absorbed dose on % EDS of the superabsorbent, which is shown in Figure 18.3.

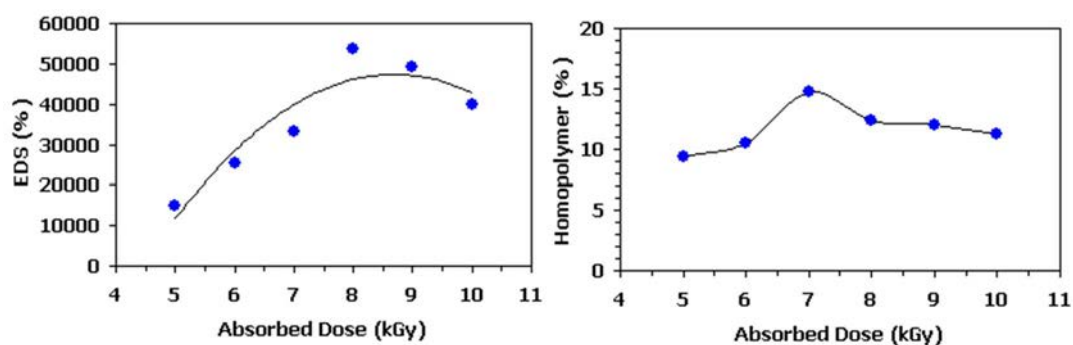


FIG. 18.3. Effect of absorbed dose on equilibrium degree of swelling and effect of absorbed dose on % homopolymer.

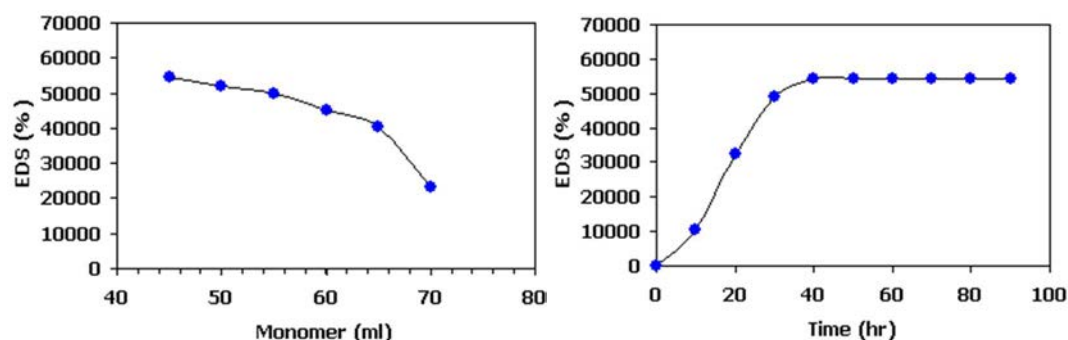


FIG. 18.4. Effect of the amount of monomer on equilibrium degree of swelling and the swelling kinetics of superabsorbent.

From Figure 18.3, it can be seen that % homopolymer increased with increasing absorbed dose up to 7 kGy, then decreased with increasing dose. At the absorbed higher than 7 kGy, % homopolymer decreased. This is due to the fact that high dose can induce ample active grafting sites on the cassava starch backbone for the grafting monomer. The effect of the amount of monomer on EDS is presented in Figure 18.4. EDS decreased with the increasing amount of monomer. This is due to the fact that the networks of the superabsorbent become denser with high monomer concentration, and therefore, would retain a lot of heat produced by polymerization. Hence, the temperature of the reaction system would increase and in turn accelerate the chain termination and transfer speeds. The high reaction temperature decreases the water absorbency of the superabsorbent [18.7]. Figure 4 also shows the swelling kinetics of superabsorbent. The equilibrium degree of swelling initially increased with time, but started to level off at 40 h, indicating that the superabsorbent attained the equilibrium in 40 h.

The water retention of the sand mixed with superabsorbent and the sand without superabsorbent were studied. The masses of two samples were compared within 15 days. The results were presented in Figure 18.5. Initially, the sand mixed with 0.1% wt superabsorbent can absorb more water than the sand without superabsorbent. After seven days, the sand without superabsorbent had nearly given off all water, while the sand with 0.1% wt superabsorbent still retained 50% water.

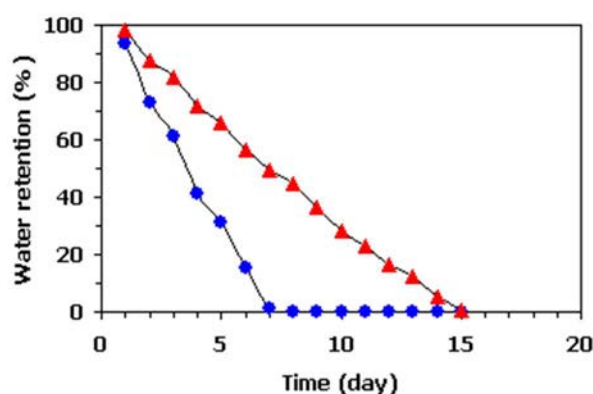


FIG. 18.5. Water retention of sand with (red triangle) and without (blue circle) 0.1% wt superabsorbent.

The germination energy of the corn seeds with 0.5% wt superabsorbent (Table 18.1) was obviously higher than that of the seeds without superabsorbent. This is due to the fact that the superabsorbent not only absorbs large amounts of water but also has good water retention capability, which supplies plentiful water to promote the seed growth. The germination percentage of seeds with 0.5% wt superabsorbent was also higher than that of the seeds without superabsorbent. After 15 days, soil with 0.5% wt superabsorbent showed a favorable effect on weights of leafages and roots of plants (Table 18.2).

TABLE 18.1. EFFECT OF SUPERABSORBENT ON GERMINATION OF CORN SEEDS

Superabsorbent content (%)	Germination percentage (%)	Germination energy (%)
0	63.0 ± 0.2	49.0 ± 0.1
0.5	98.0 ± 0.2	89.5 ± 0.1

TABLE 18.2. EFFECT OF SUPERABSORBENT ON GROWTH OF YOUNG CORN PLANTS

Superabsorbent Content (%)	Plant height (cm)	Leafage weight (g)	Root weight (g)
0	18.3 ± 0.1	5.5 ± 0.1	1.2 ± 0.1
0.5	27.5 ± 0.1	10.6 ± 0.1	2.7 ± 0.1

#### 18.4. CONCLUSION

The experimental results indicated that the sand mixed with 0.1% wt superabsorbent can absorb water longer than the sand without superabsorbent. The germination energy of corn

seeds mixed with 0.5% superabsorbent was clearly higher than those without superabsorbent. The plant height, leafage weight, and root weight of the corn plants with 0.5% superabsorbent were also obviously higher than those without superabsorbent. These experimental results showed that the superabsorbent has considerable effects on seed germination and the growth of young plants. From this preliminary study, it can be concluded that the superabsorbent has potential for applications in agriculture, especially in arid regions.

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## Chapter 19

### **RADIATION SYNTHESIS AND CHARACTERIZATION OF NATURAL AND NATURAL-SYNTHETIC HYBRID SUPER ABSORBENT POLYMERS FOR AGRICULTURAL APPLICATIONS**

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#### **Abstract**

The experimental studies carried out in Hacettepe University, Laboratories of Radiation and Polymers Science (LRPS) in the past ten years, which focused mainly on the synthesis of synthetic and natural-synthetic super absorbent polymers in various irradiation conditions, are summarized in the first part of the presentation. Studies conducted on the following areas: (1) the controlled release of fertilizers and herbicides and the effect of the natural polymer type, (2) the neutralization degree of poly(acrylic acid), (3) the temperature and pressure on the swelling kinetics, and (4) the maximum water absorption capacity of the potential soil conditional hydrogels, were explained. The results were then compared with those obtained from commercial super absorbent polymers prepared through conventional techniques. In the third part of the presentation, basic and advanced techniques in the characterization of the network structure of super water absorbents were presented.

#### **19.1. INTRODUCTION**

Superabsorbent polymers (SAPs) are moderately cross-linked 3-D hydrophilic network polymers that can absorb and conserve considerable amounts of aqueous fluids even under certain heat or pressure. Because their unique properties are superior to conventional absorbents, SAPs have found potential applications in many fields such as hygienic products, disposable diapers, in agriculture and horticulture, gel actuators, drug-delivery systems, water-blocking tapes, coal dewatering, water managing materials for the renewal of arid and desert environment, and many others [19.1]. The worldwide demand for single-use, disposable, and non-biodegradable synthetic superabsorbent polymers has increased dramatically since 1986 and currently amounts to about 280 000 metric tons/ year. The highest demand comes from the United States with 130 000 metric tons/year, followed by Europe at 100 000 metric tons, and Asia and Oceania at 20 000 metric tons. Based on this trend, the demand for superabsorbent polymers is expected to reach 350–400 000 metric tons/year within a few years. The use of biodegradable hydrogels based on natural superabsorbent polymers, especially as diapers and as a soil conditioning material, will be a new approach in the solution of environment problems in the world.

It is common knowledge that polysaccharides, in dry form or in solution, degrade when exposed to ionizing radiation [19.2, 19.3]. So far, the results of the radiation-induced degradation of polysaccharides indicated that the chain scission yield strongly depends on the relative concentration of polymer. It has also been recently found that, when the concentration of polysaccharide is increased beyond viscous solution to paste-like condition, or when some additives like carbon tetrachloride are present, the cross-linking effect starts to dominate, and chain scissioning polymers typically turn into the cross-linking type [19.4, 19.5]. After this development, available natural resources have, in recent years, drawn considerable attention in the preparation of SAPs. Since the mechanical properties of polysaccharide based-natural polymers are low, researchers have mainly focused on natural/synthetic polymer/ monomer mixtures to obtain novel SAPs [19.6] for different applications such as plant growth promotion.

## 19.2. SYNTHESIS AND CHARACTERIZATION OF THE SWELLING PROPERTIES OF ACRYLIC ACID SODIUM SALT/LOCUST BEAN GUM HYDROGELS

Recently, Şen and Hayrabolu [19.7] synthesized a novel superabsorbent polymer with high mechanical strength through radiation-induced polymerization and the cross-linking of AAcNa in the presence of natural polymer locust bean gum. They also characterized the network structures. Liquid retention capacities and absorbency under load (AUL) analysis of the synthesized SAPs were performed in water in order to determine their SAP character. In the characterization of the network structure of the semi-IPN hydrogels, the average molecular weight between cross-links ( $\overline{M}_c$ ) was evaluated through uniaxial compression and oscillatory rheological measurements, and the advantages and disadvantages of these two techniques were compared.

In the characterization of the network structure and the determination of the effective cross-link density of the hydrogels prepared, their swelling properties in water were first investigated. In the identification of the effect of DN and pressure on the swelling behaviour of the hydrogels, the swelling experiments were carried out under the pressures of 0.0 and 2.1 kPa.

Each swelling experiment was performed until a constant value of swelling for the sample in question was reached. Based on the results, the swelling kinetics of the AAcNa/LBG hydrogels in water under 0.0 and 2.1 kPa at 25°C are presented in Fig. 19.1 and Fig. 19.2. The data show that the AAcNa/LBG hydrogels reached the equilibrium swelling in 10–20 minutes. On the other hand, the equilibrium swelling degree was lower under pressure than in pure water in each DN value (Fig. 19.3). This decrease was attributed to the increase in the osmotic pressure on the gel system.

The experiment results indicated that one of the most important parameters that affected the swelling behaviour of the AAcNa/LBG hydrogels was DN. The swelling degree of the gels was increased from 245 to 587, together with an increase of from 50 to 90 in the degree of neutralization (DN) in the absence of force. This was attributed to the increase in the number of the carboxylate groups in the gel with DN and the changing of the Donnan equilibrium. As the DN value of the gel increases, the carboxylate groups in the gel also increase. This means that, as the concentration of the fixed charge on the gel network increased, the negative charge repulsion inside the polymeric network became stronger. The water absorbency of the completely neutralized gel (AAcNa/LBG-DN100) was lower than that of the AAcNa/LBG-DN90 gel. This decrease was probably due to the buffer action and the screen effect caused by the counter ions inside the gel [19.8].

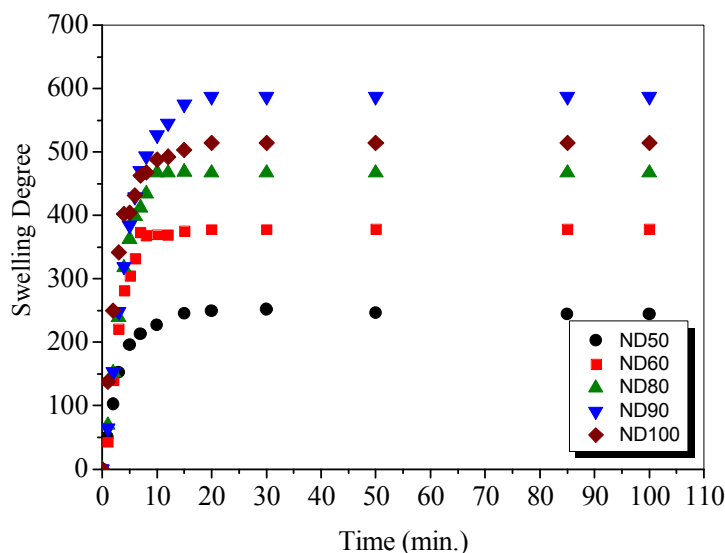


FIG. 19.1. Swelling kinetics of the AAcNa/LBG double network hydrogels under no pressure. The DN of AAc is indicated in legends.

### 19.2.1. Characterization of the network structure of acrylic acid sodium salt/locust bean gum hydrogels

In order to determine the molecular weight between cross-links of the hydrogels prepared through the shear modulus value, two different evaluation techniques were used in this study. The first technique was the uniaxial compression analysis. After the swelling experiments, the uniaxial compression was applied using the Universal Testing Instrument on the swollen gels typical stress–strain curves of the AAcNa/LBG hydrogels. The results are presented in Fig. 19.4. Shear modulus values of the hydrogels ( $G_m$ ) were calculated using the elastic deformation theory and Eq. (1) [19.9–19.11].

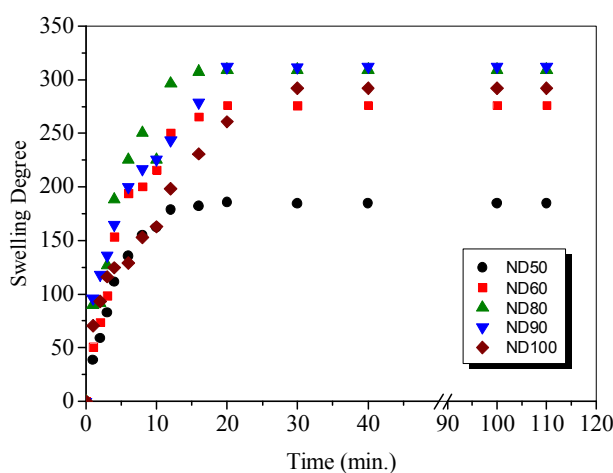


FIG. 19.2. Swelling kinetics of the AAcNa/LBG double network hydrogels under a pressure of 2.1 kPa. The DN of AAc is indicated in legends.



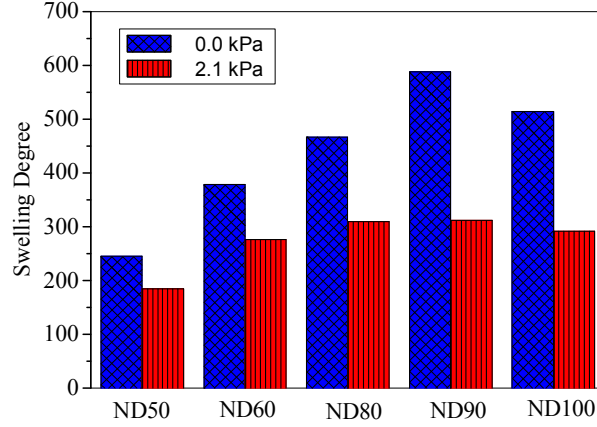


FIG. 19.3. Effects of pressure and DN on the equilibrium swelling of the AAcNa/LBG double network hydrogels.

$$f = G_m(\lambda - \lambda^{-2}) \quad (1)$$

where  $\lambda$  is deformation ratio and equal to  $L/L_0$ .  $L_0$  and  $L$  are the initial length and the length of the deformed hydrogels, respectively, during compression. When the equation is applied to the initial stages of deformation, plots of  $f$  vs.  $-(\lambda - \lambda^{-2})$  yield straight lines (small figure in Fig. 19.4). The  $G_m$  value was calculated from the slope of the lines and is listed in Table 19.1. Using the  $G_m$  values and other relevant experimental parameters, molecular weight between cross-links  $(\overline{M}_c)_m$  values were calculated using Eq. (2) and collected in Table 19.1:

$$G_m = G'_R = A \frac{\rho}{\overline{M}_c} RT (v_{2r})^{2/3} (v_{2m})^{1/3} \quad (2)$$

where  $\rho$  was the polymer density,  $\overline{M}_c$  was the average molecular weight of the network chains,  $v_{2m}$  was the polymer volume fraction of the cross-linked polymer in equilibrium with the swollen gel, and  $v_{2r}$  was the polymer volume fraction in the relaxed state, i.e. after cross-linking but before swelling. The prefactor  $A$  equals 1 for an affine network and  $1-2/\Phi$  for a phantom network [19.9].

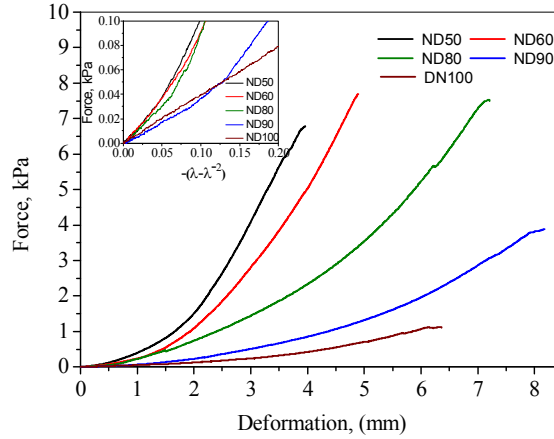


FIG. 19.4. Curves of strain vs. stress and  $-(\lambda - \lambda^{-2})$  vs. stress for the AAcNa/LBG hydrogels.

The polymer volume fractions  $v_{2x}$  ( $v_{2r}$  and  $v_{2m}$ ) could be determined by Eq. 3,

$$Q = 1/v_{2x} = (1 + \rho/\rho_w(w^{-1} - 1)) \quad (3)$$

where  $\rho$  and  $\rho_w$  were the densities of the swollen gel and water, and  $w$  was the weight fraction of the polymer in the relaxed and swollen gel for the determination of  $v_{2r}$  and  $v_{2m}$ , respectively.

As can be seen in Table 19.1, the molecular weights between cross-links and the distance between the two entanglement points of the AAcNa/LBG hydrogels changed with the amount of DN of AAc. The results clearly indicated that DN was one of the important parameters affecting the radiation-induced polymerization and the cross-linking of AAcNa/LBG mixtures. As explained previously, the decrease in the cross-link reactions was probably due to the increase in the macroradical stability of the ionization degree of AAc.

The second technique for the determination of the shear modules of the hydrogels used in this study was rheometry [19.12]. The oscillatory frequency sweep test was performed in the linear viscoelastic region. Stress sweep was performed to determine the linear viscoelastic region (LVR) at a frequency of 1 Hz. The influence of the shear rate  $\gamma$  on the elastic modules of the hydrogels (Fig. 19.5) revealed that the elastic deformation at low shear rates followed by viscose deformation behaviour above a critical shear rate depended on the network structure. The shear stress was constant between 0.01–0.5% to ensure the linearity, and all frequency sweep tests were carried out at  $\gamma = 0.01\%$ .

The storage or elastic moduli, ( $G'_R$ ), of the double networks, exhibited a very low frequency dependence, and the slopes of  $G'_R$  vs. frequency curves were almost zero in the frequency range of 0.01–1 Hz (Fig. 19.6). This figure shows that, for the cross-linked hydrogels, the magnitude of  $G'_R$  decreased with increasing DN up to 90%, and then to 100% similar to the case of the equilibrium swelling.

It is a fact that an ideal oscillatory curve shows a rubber-elastic plateau in the intermediate frequency region. Here, the larger molecular chains cannot slide by one another since the relaxation ability of the segments is smaller than the stress velocity [19.13]. In the linear

viscoelastic range, the network parameters, i.e. the molar mass between the two entanglement points  $(\overline{M}_c)_R$ , the cross-link density  $(\nu_e)$ , and the mesh size  $(\xi)$  were calculated using Eqs. 1 and 4–5. For a homogenous network of Gaussian chains, the elastic modules of the gels swollen to equilibrium  $G'_R$  were related to the network cross-link density through Eq.1. The mesh size broadly defines the space between the macromolecular chains in a cross-linked network, and is usually characterized by the correlation length, or the distance between two adjacent cross-links [19.12, 19.14–19.15].

$$\nu_e = \frac{\rho}{\overline{M}_c} \quad (4)$$

$$\xi = \nu_{2m}^{-1/3} \left[ C_n \left( \frac{2\overline{M}_c}{M_r} \right) \right]^{1/2} l \quad (5)$$

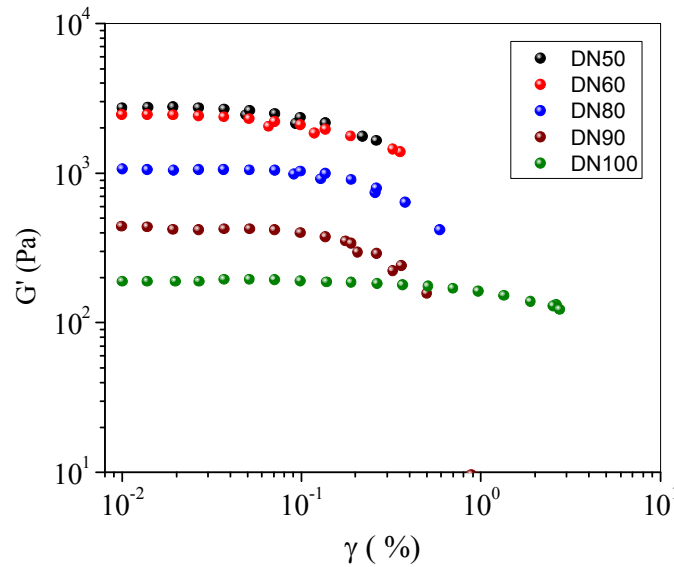


FIG. 19.5. Variation shear moduli of the AAcNa/LBG hydrogels as a function of the strain at  $\omega = 1$  Hz for various DNs.

The symbol  $\xi$  in eq. 5 represents the Flory characteristic ratio or rigidity factor of the hydrogel (6.7) [19.16],  $l$  is the carbon-carbon length (154 pm), and  $M_r$  is the molecular weight of the monomeric unit. The elastic modules  $G'_R$  values determined are listed in Table 19.1. Using the  $G'_R$  values and other relevant experimental parameters,  $(\overline{M}_c)_R$  and  $\nu_e$  were calculated and collected in Table 19.1. Mesh size results clearly indicated that changing the DN of AAc (Table 19.1) could easily control the distance between two adjacent cross-links in the AAc/LBG networks. The magnitudes of  $\overline{M}_c$  calculated from the mechanical properties were very close to those obtained from the rheological properties, but excluding the completely neutralized gel system. A large difference in the  $\overline{M}_c$  values in this system was attributed to their very low toughness and the difficulties in the preparation of perfectly smooth cylindrical gel pieces for mechanical analyses due to their soft structures. Because of the detection difficulty of very small force values in mechanical analyses for the relatively rough surfaces, it was not easy to match the actual onset point of the compression and viscoelastic

deformation range of the gel system. On the other hand, the rheological analyses were independent of the size and shape of the gel. It was observed that the swollen and disintegrated gels provided more reproducible results in the rheological analyses than the non-disintegrated block gels. Owing to the ease in adjustments of the gap distance between the plates, the user could control the initial preload value on the gels prior to the experiment. The viscoelastic deformation range could also be easily measured by simply subjecting the gel system to a strain sweep test.

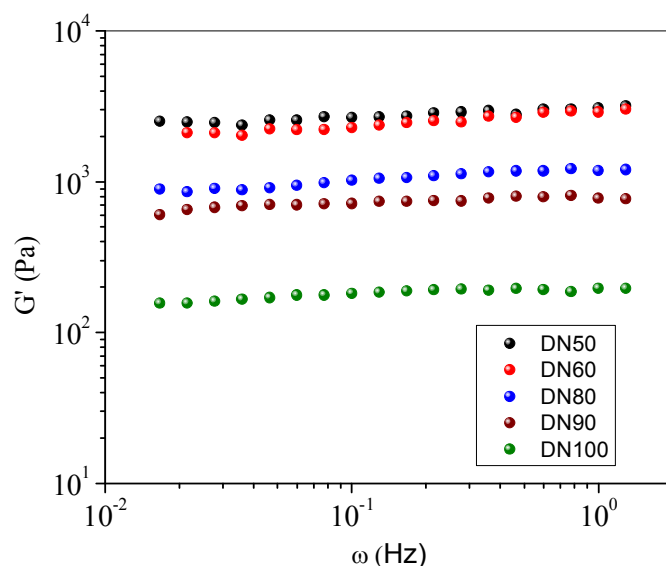


FIG. 19.6. Variation shear moduli of the AAcNa/LBG hydrogels as a function of the frequency at  $\gamma = 0.01\%$  for various DNs.

As a result of all these analyses, it is possible to conclude that the uniaxial compression technique could be used for the characterization of the network structure of a hydrogel along with the rheological analyses. However, a very precise control on the gel size was also needed. Since a very small amount of deformation force could be applied in the linear viscoelastic deformation range on the gel system, regardless of their shape, the rheology was an easier and more reliable technique for the characterization of the network structural parameters of the gel systems.

### 19.2.2. Swelling properties of acrylic acid sodium salt/locust bean gum hydrogels in urine solutions

Swelling behaviour of SAP in salt, urea, or urine solutions is very important in the identification of their use in hygiene products and soil conditional applications. The swelling kinetics of the AAcNa/LBG hydrogels in synthetic urine solutions (67% is urea) at different temperatures and under different loads is given in Fig 19.7. As can be seen from the figures, AAcNa/LBG hydrogels reach the equilibrium swelling in ten minutes at all pressures. On the other hand, the equilibrium swelling degree is lower than that in pure water. This decrease was attributed to the Donnan effect. For the comparison of the swelling capacity of SAP (see Fig. 19.8) prepared in this study with that of commercial products already in use in hygiene products such as napkins or diapers, swelling analyses were performed, and the maximum swelling capacity of AAcNa-based SAP from these products was determined [19.17].

The maximum swelling or water retention capacities of the commercial SAPs in water and pseudo urine solution are given in Fig. 19.9. As in these SAP systems, the swelling degree of the commercial gels also decreased abruptly with the ionic strength of the swelling medium. This decrease was attributed to the high ionic nature of the SAP system. Fig. 19.10 shows the swelling degree in urine solution of AAc/Na/LBG SAP as compared to the commercial SAP. After Şen et al. compared the equilibrium swelling capacities of the figure in several experiments, the results clearly indicate that the hydrogels prepared in this study (the LBG natural polymer interpenetrated with a synthetic monomer) can absorb the aqueous solution as much as the commercial SAP does, especially at high pressures.

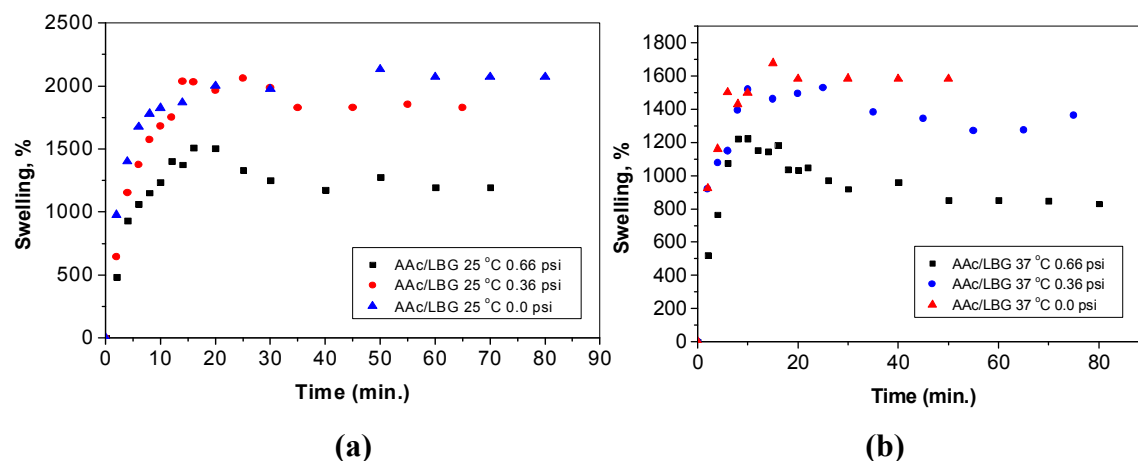


FIG. 19.7. Variation of the swelling kinetics of the AAcNa/LBG hydrogels with load in pseudo urine solutions a) 25°C b) 37°C.

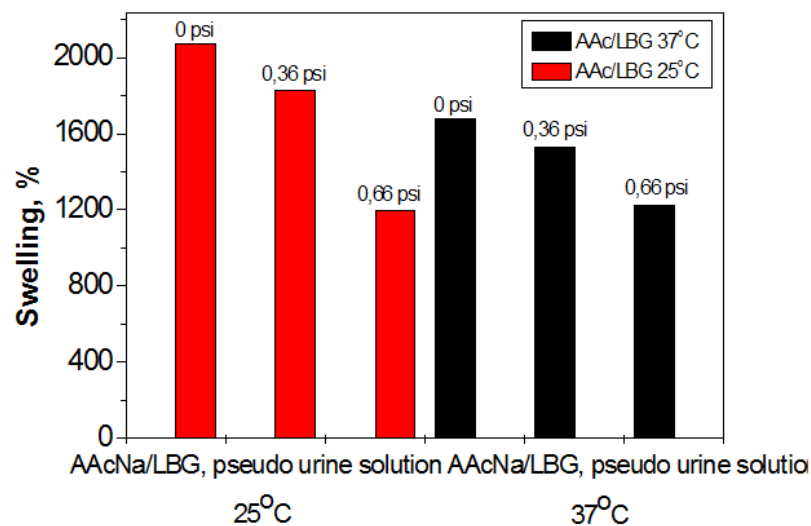


FIG. 19.8. AUL values of AAcNa/LBG SAPs in a pseudo urine solution. The loads applied on the gels and temperatures are given in the figure.

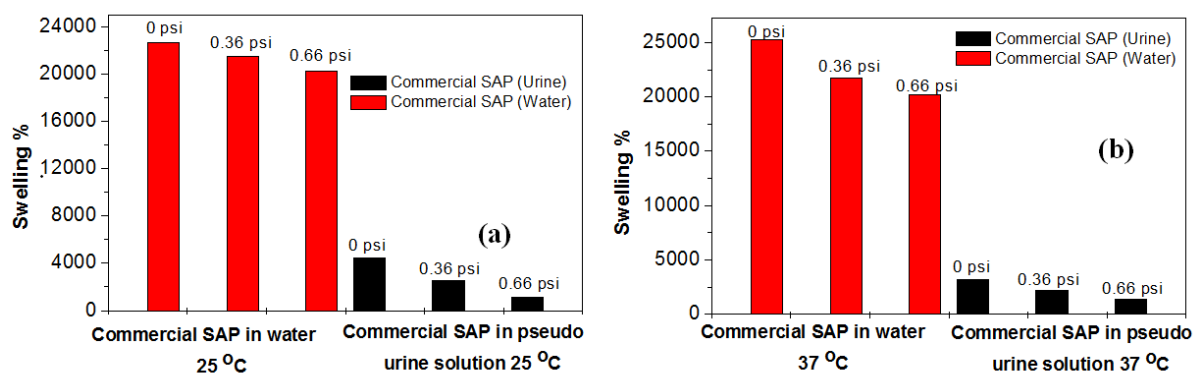


FIG. 19.9. AUL values of the commercial SAPs in water and pseudo urine solution. The loads applied on the gels are given in the figures, a) 25 °C and b) 37 °C.

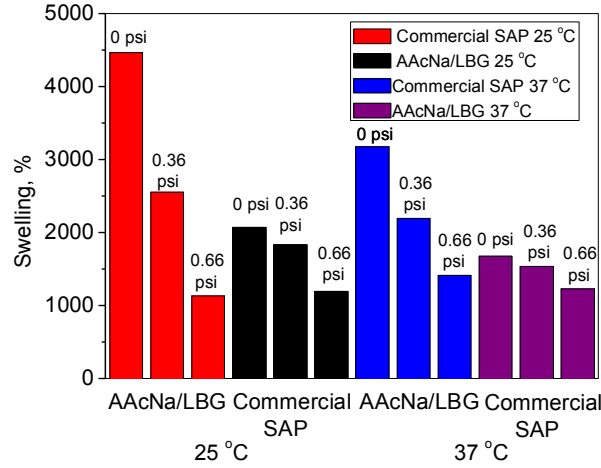


FIG. 19.10. AUL values of AAcNa/LBG and a commercial SAP in pseudo urine solution at 25°C and 37°C.

TABLE 19.1. STRUCTURAL PROPERTIES OF THE AAcNa/LBG HYDROGELS

Gel Name	$(v_{2m})^{1/3}$	$(v_{2r})^{2/3}$	$\rho$ , (kg/m <sup>3</sup> )	$G_m$ (Pa)	$G_R$ (Pa)	$(\overline{M}_c)_m$ (g/mol)	$(\overline{M}_c)_R$ g/mol	$(v_e)_R$ (mol/cm <sup>3</sup> ) $\times 10^{-5}$	$(\square\square)_R$ (nm)
AAcNa/LBG-DN50	0.1479	0.2482	1072	2305	2665	42 320	36 570	2533	92
AAcNa/LBG-DN60	0.1440	0.2349	1100	2120	2290	43 570	40 280	2525	96
AAcNa/LBG-DN80	0.1241	0.2334	1118	990	1020	81 050	78 820	1379	152
AAcNa/LBG-DN90	0.1104	0.2010	1263	850	715	81 500	97 120	1550	172
AAcNa/LBG-DN100	0.0932	0.1401	1494	390	180	124 580	265 580	1199	252

### 19.3. SYNTHESIS AND CHARACTERIZATION OF CARBOXYLATED LOCUST BEAN GUM HYDROGELS

In one of the recent studies of Hayrabolulu, Şen et al. [19.18], carboxylated locust bean gum (CLBG) hydrogels were prepared in paste-like condition in the presence of acetylene through ionizing radiation. In that study, LBG was first carboxylated through a technique known as TEMPO. The CLBG was then irradiated by gamma rays utilizing <sup>60</sup>Co gamma ray sources with dose rates of 30 Gy/h and 300 Gy/h up to 10 kGy in paste-like condition and in the presence of acetylene. A sol-gel analysis was performed in order to determine the polymer to gel conversion ratios. It was found that the swelling capacity of the hydrogels prepared in the presence of acetylene increased with a dose of from 19 000 – 34 000%, and the gelation percentage increased rapidly to up to 5.0 kGy, which then slowed down at higher doses. While a lower gelation was observed, the cross-link density of the CLBG hydrogels prepared in paste-like conditions was high. For the characterization of the network structure of the hydrogels, the average molecular weight between cross links ( $M_c$ ) was evaluated by means of rheological analyses.

### 19.3.1. Preparation and characterization of CLBG

LBG was first carboxylated through a technique known as TEMPO by Şen et al. A 0.5 g of LBG was suspended in a 250 ml of deionized water overnight and then stirred for two hours at 60°C. The solution was then cooled to room temperature, and catalytic amounts of (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) and NaBr were added. The oxidation reaction was initiated by the drop wise addition of NaOCl solution (the active Cl value was 11%) at 4°C. In order to obtain various oxidation ratios, 4.5, 7.0, 13.5, 18.0 or 35 ml of the NaOCl solution were added into the mixture. NaOCl/primary alcohol at molar ratios of 1.2, 1.9, 3.6, 4.8 or 9.3, respectively. For the selective oxidation of polysaccharides, pH should be around 10.2 [19.19]. The pH in this study was kept from 9–10 during the reaction by adding a 0.5 M of aqueous NaOH solution. When the pH decrease was very slow, the reaction was quenched by adding MeOH. Finally, the CLBG was precipitated through the use of acetone. The chemical structures of LBG and CLBG are given in Fig. 19.11.

In order to change the carboxylation degree, the NaOCl/primary alcohol mol ratio was controlled during the oxidation reaction. The effect of NaOCl/primary alcohol mol ratio on the structural changes was first monitored by FTIR. In Fig. 12, a is the spectrum of the virgin LBG, and the others are the spectra of the samples synthesized using a 4.5, 7.0, 13.5, 18.0, or 28 ml of the NaOCl solution in the carboxylation reaction. For the pure LBG (Fig. 19.12, a), the band at  $3430\text{ cm}^{-1}$  represents the O–H stretching vibration. The band at  $2924\text{ cm}^{-1}$  is due to the C–H stretching of the  $-\text{CH}_2$  groups. The bands at  $1641$  and  $1657\text{ cm}^{-1}$  are due to the ring stretching of galactose and mannose. In addition, the bands in the region of  $1350\text{--}1450\text{ cm}^{-1}$  are due to the symmetrical deformations of the  $\text{CH}_2$  and COH groups. The bands at  $1078$  and  $1021\text{ cm}^{-1}$  are due to the primary alcoholic  $-\text{CH}_2\text{OH}$  stretching mode and  $\text{CH}_2$  twisting vibrations, respectively. The weaker bands around  $770\text{ cm}^{-1}$  are due to the ring stretching and ring deformation of the  $\alpha\text{-D-(1-4)}$  and  $\alpha\text{-D-(1-6)}$  linkages. The peak at  $1729\text{ cm}^{-1}$  in the b-f spectra is the stretching bond of the C=O groups in the carboxylic acids. As shown in Fig. 19.11, the peak intensity of the C=O groups continuously increased with the amount of NaOCl. The carboxylation of the LBG was also evidenced by  $^{13}\text{C}$  NMR analyses [19.20].

The effect of the carboxylation or the amount of NaOCl added in the reaction mixture on the molecular weight of the LBG was investigated through the Size Exclusion Chromatography (SEC). The chromatograph was combined with the refractive index (RI) and the Multi-Angle Light Scattering (MALS) detectors. The variations of the weight and number average molecular weights of the CLBG as a function of the NaOCl/primary alcohol mol ratios are given in Table 19.2. As shown in Table 19.2, the molecular weight of the CLBG increased with the NaOCl amount. This increase was attributed to the side esterification reactions and partial cross-linking of the LBG structure. Gel formation in the reaction mixture was observed when the amount of NaOCl was increased to 35 ml. Due to this gelation and the filtration of only the low molecular weight fraction of CLBG, the molecular weight of CLBG-35 is lower than that of CLBG-18. Due to the solubility problem in water of the CLBG-18 and CLBG-35, the CLBG-4, CLBG-7, and CLBG-13 systems were used in the gelation experiments.



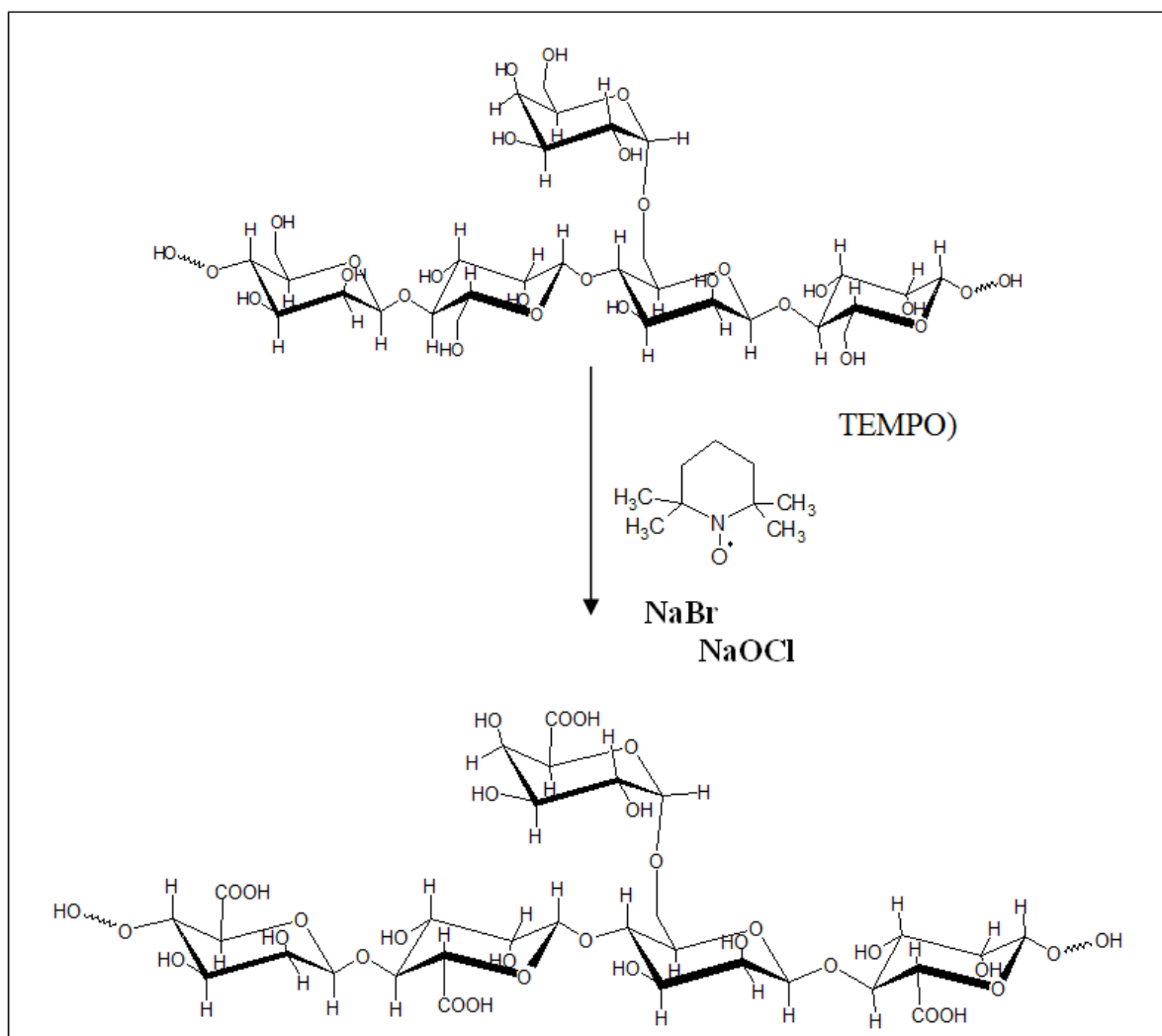


FIG. 19.11. Chemical structures of LBG and CLBG.

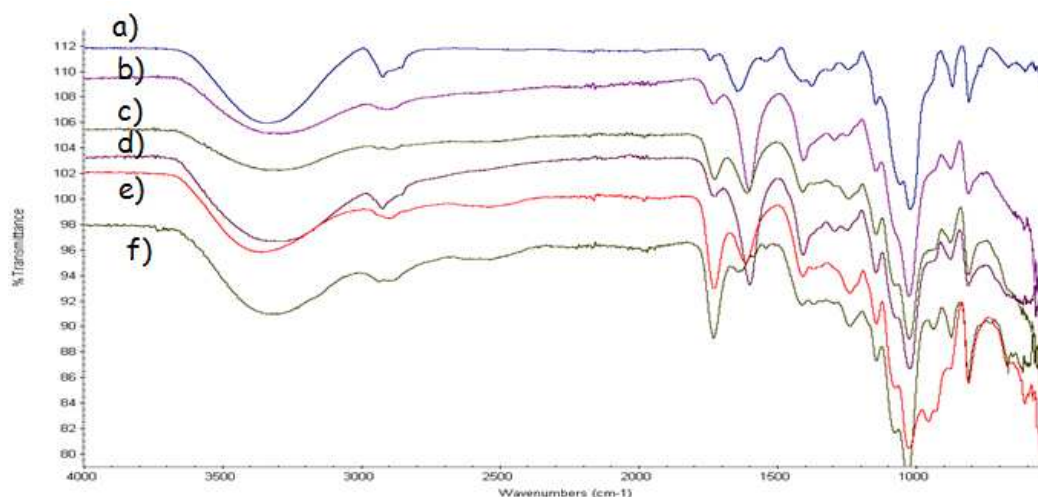


FIG. 19.12. FTIR spectrum of virgin LBG (a) and CLBGs prepared by using b) 4.5 ml, c) 7 ml, d) 13.5 ml, e) 18 ml, and f) 28 ml NaOCl in the carboxylation experiments.

TABLE 19.2. NUMBER AND WEIGHT AVERAGE MOLECULAR WEIGHTS OF CLBG POLYMERS

Sample name	The amount of NaOCl (ml)	$M_w$	$M_n$	PDI
LBG	0.0	510 430	390 200	1.31
LBG-4	4.5	613 000	291 000	2.10
LBG-7	7.0	780 000	456 000	1.71
LBG-13	13.5	1.200 000	630 000	1.90
LBG-18	18.0	1.570 000	870 000	1.80
LBG-28	28.0	385 000	228 000	1.69

### 19.3.2. Preparation of clbg gels in paste-like conditions

For the preparation of CLBG hydrogels in the paste-like state, the solutions of 20% (w/v) LBG and CLBG were placed in tightly closed tubes and irradiated at a dose of 5.0 kGy in a Gammacell 220 type  $^{60}\text{Co}$ -gamma irradiator at room temperature, in air. A sol-gel analysis and swelling experiments were performed using the sintered gooch crucible apparatus, as shown in Fig. 19.13. [19.21].

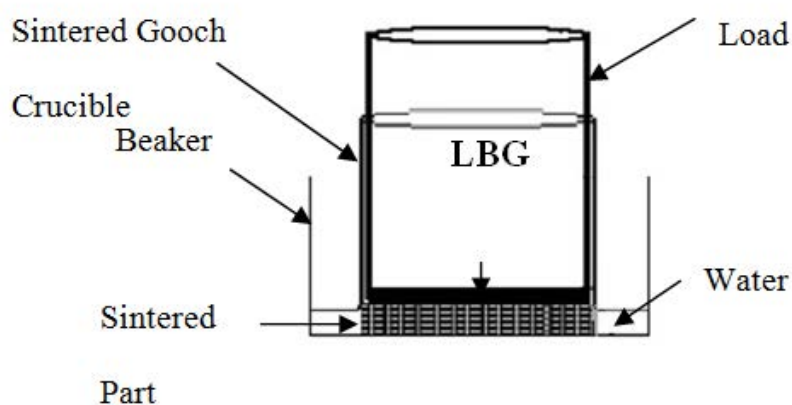


FIG. 19.13. Apparatus used for the determination of the gelation and swelling ratios of the gels.

The gelation and swelling percentages of the prepared gels were calculated, and the results were collected and shown in Table 19.3. A gelation of 71.6% was observed for the non-carboxylated LBG and the swelling degree of this gel was only 1225%. As shown in Table 19.3, the swelling degree (4700%) of the CLBG of 26% was approximately four times higher than that of the LBG. On the other hand, a reduction in the gelation ratio of the system was observed with the increase of carboxylation degree.

When the carboxylation degree of the LBG was increased from 26–40 and 70%, neither a regular increase nor a decrease was observed in the gelation and swelling ratios of the CLBG samples. This behavior was attributed to the partial and local cross-links formed during the carboxylation reactions that led to the inhomogeneous diffusion of water inside the gels.

TABLE 19.3. EFFECT OF NaClO/PRIMER ALCOHOL MOL RATIO ON THE CARBOXYLATION DEGREE AND GELATION AND SWELLING DEGREE OF CLBG GELS PREPARED AT PASTE-LIKE CONDITIONS

NaClO/primer mol ratio	DC	Dose 5 kGy	
		Gelation	Swelling
		%	%
0.0	0.0	71.6	1225
1.2	26	25.5	4700
1.9	40	45.1	1090
3.6	70	35.8	2020

### 19.3.3. Preparation of clbg gels in the presence of acetylene

In order to prepare the CLBG gels in the presence of acetylene gas, a 0.5 g of the ground dry carboxylated polymer samples was placed in glass tubes with rubber lids. The glass tubes were first filled with nitrogen gas and then with acetylene gas for 5 minutes. The samples were irradiated in the gamma source of up to 10 kGy. The sol-gel and swelling analyses of the prepared gels were performed as described above.

Due to the inhomogeneous cross-linking at paste-like conditions, the experiments were focused on the gelation of the CLBG in the presence of acetylene gas. The powdered CLBG was irradiated in the presence of acetylene to up to 10 kGy at a dose rate of 30 Gy/h. The effects of the dose and degree of carboxylation on the gelation and percentage swelling of the gels are presented in Table 19.4.

TABLE 19.4. GELATION AND SWELLING % OF CLBG GELS PREPARED IN THE PRESENCE OF ACETYLENE GAS IN RELATION TO NaOCl/PRIMARY ALCOHOL MOL RATIO AND IRRADIATION DOSE

NaClO/primary alcohol mol ratio	2 kG		5 kGy		10 kGy	
	Gelation	Swelling %	Gelation	Swelling %	Gelation	Swelling %
	%		%		%	
0.0	85.0	490	88.3	443	70	700
1.2	61.3	19 020	99.5	17 730	46	33 945
1.9	58.0	2600	64.5	2415	35	1244
3.6	50.0	975	48.6	935	45	4270

As shown in Table 19.4, higher gelation ratios for the gels prepared in the presence of acetylene gas were observed as compared to the ones prepared in paste-like conditions. The results showed that the increase in the carboxylation degree had a reverse effect on the gelation, as the gelation and swelling percentages decreased with the carboxylation ratio. The results also demonstrated that the cross-linking reactions occurred via different mechanisms,

depending on the extent of the carboxylation of the LBG when irradiations in the presence of acetylene gas were performed [19.22–19.23]. When the irradiation dose was increased from 5 to 10 kGy for all carboxylation degrees, the degrees of gelation were observed to decrease. As is commonly known, polysaccharides in dry and solution forms degrade when exposed to ionizing radiation [19.24–19.29]. The decrease in gelation and increase in swelling at relatively high irradiation doses (at 10 kGy, for instance) showed that the chain scission yield was more dominant than the cross-linking reactions.

Dose rate is one of the most important parameters that control the degradation and cross-linking of natural polymers. In the investigation of the effect of the dose rate on the gelation and swelling of the CLBG gels in the presence of acetylene gas, a CLBG sample that had a carboxylation degree of 26% was also irradiated with gamma rays at a dose rate of 300 Gy/h at 2 kGy or 5 kGy. The gelation and swelling values were calculated and shown in Table 19.5.

TABLE 19.5. GELATION AND SWELLING % OF CLBG GELS PREPARED IN THE PRESENCE OF ACETYLENE GAS AS A FUNCTION OF IRRADIATION DOSE

NaClO/primary alcohol mol ratio/Carboxylation degree	2 kGy		5 kGy	
	Gelation	Swelling	Gelation	Swelling
	%	%	%	%
1.2/26	81	2250	89.5	2115

When the gelation and swelling ratios of the gels prepared at different dose rates were compared, it was found that the dose rate was also an important parameter for the CLBG gels prepared in the presence of acetylene gas. For both dose rates, the gelation values increased as the irradiation dose increased. When the dose rate increased from 30 Gy/h to 300, the swelling percentages of the gels decreased from 17 000 – 19 000 to 2000. These results indicated that the dose rate had a positive effect on the gelation and a significant detractive effect on the swelling values.

As a result of the irradiation studies performed in the presence of acetylene, it was concluded that an irradiation dose of 5 kGy and a carboxylation degree of 26% were the optimal conditions in the preparation of the super absorbent polymer of a CLBG.

#### 19.4. CONCLUSIONS

In this report, the radiation synthesis, characterization of swelling behavior, and network structure of synthetic-natural hybrid (acrylic acid sodium salt/galactomannan) and natural (carboxylated locust bean gum or CLBG) super water absorbents were given in detail. Synthetic-natural hybrid AAcNa/LBG hydrogels were prepared by irradiating the ternary mixtures of natural polymer locust bean gum and synthetic monomer acrylic acid sodium salt in the presence of the cross-linking agent, N,N'-methylene bisacrylamide (MBAAm) and natural CLBG hydrogels in paste-like conditions. This was done through gamma rays at ambient temperature at different dose rates in order to develop a new super absorbent system especially for use in soil conditional applications.

The swelling behaviors of the prepared hydrogels and their sensitivity to the solvents were studied. So far, in most of the related literature, the swelling capacity values of super absorbent polymers are reported as free-swelling data, i.e. load free swelling. It is obvious

that the swelling conditions, and hence, the data are not real, because, in all of the super absorbent polymer applications (agricultural, hygienic, etc.), the swelling particles must absorb aqueous solutions while they are under pressure.

The swelling capacity of prepared hydrogel in water and pseudo (synthetic) urine solution (67% is urea) under different pressures and temperatures were measured. The results show that the temperature, pressure and ionic strength of the swelling solution are the main parameters affecting the swelling capacity of natural/synthetic super absorbent polymers as commercial SAPs. After the experiments were done, it was observed that the degree of neutralization (DN) of acrylic acid is another important factor controlling the equilibrium swelling values of AAcNa/LBG super absorbents. The optimum DN was determined as 80 mol % for the acrylic acid. The UAL values and swelling factor of AAc/LBG hydrogels prepared by using this DN are very close to commercial SAPs values.

It was demonstrated that the LBG polymer could be carboxylated by using the TEMPO-mediated oxidation method. It was also determined that, by controlling the NaOCl amount, CLBG polymers could be prepared with various carboxylation degrees. In the preparation of the hydrogel systems using the so-synthesized CLBG polymers, two main approaches were employed. The first approach was the radiation in paste-like state wherein the cross-linking of hydrocolloids is performed in highly concentrated solutions, and the second was the irradiation of the dry polysaccharide in the presence of acetylene gas. It is assumed that the cross-linked gel systems could be prepared by irradiating the so-synthesized CLBGs at 5 kGy in the paste-like state, and at 2 or 5kGy, in the presence of acetylene gas. The highest swelling could be obtained using the second approach, i.e. in the presence of acetylene gas, (highest swelling was ~34 000%) while a swelling percentage of 4700% could be obtained in the paste-like state.

All these results show that the natural and natural-synthetic hybrid hydrogels prepared through radiation technology can become potential super absorbents. Because of their ease of production and relatively low cost, they can be used as soil conditioning materials instead of using those synthetic SAPs that are synthesized by conventional polymerization techniques.

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## Chapter 20

### NEW OPPORTUNITIES FOR EBEAM TECHNOLOGIES IN ONE HEALTH

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#### Abstract

Globally, there is a growing recognition that, in order to address current and emerging risks and provide system-level solutions, one has to look at public health, animal health, and environmental health at a holistic level. Several international and federal agencies such as the United Nations, World Health Organization, the Centers for Disease Control and Prevention (CDC), and the United States Department of Agriculture (USDA) have come to embrace the One Health concept. The One Health concept is based on the premise that the health of humans, animals, and the environment are interconnected. Ionizing radiation technology, especially eBeam (electron beam) technology, can play a major role in providing solutions pertinent to the One Health concept. There can be no discussion of public health without confronting the issue of food safety and quality. There can be no discussion of animal health without tackling the pre-harvest pathogen protection strategies involving vaccination. And there can be no discussion of environmental health without discussing the proper management of the burgeoning levels of animal and human wastes. The salient features of eBeam technology such as high dose rate, economic feasibility, and that it is the ultimate “green technology”, can be exploited commercially to develop materials from natural and man-made sources that can be used for high value agricultural, industrial and therapeutic applications.

#### 20.1. INTRODUCTION

The use of ionizing irradiation technology for food preservation and sanitation is over 115 years old. The technology has come a long way since its early days. Today, this technology is making significant improvements in the sterility of medical devices and products, in the development of new materials, in preventing the spread of agriculturally damaging insects and pathogens, and in enhancing the microbiological safety of foods. Consumers around the world are getting sophisticated in terms of their food needs and expectations. These demands cover the entire gamut from availability of fresh produce year-round, to pesticide-free food and fiber, minimally processed, and organically grown foods.

People all over the world has started to recognize the importance of looking at public health, animal health, and environmental health in a holistic manner when addressing current and emerging risks in order to provide system level solutions. Several international organizations and federal agencies such as the United Nations, World Health Organization, the CDC and the USDA have come to embrace the One Health concept, which is based on the premise that the health of humans, animals, and the environment are interconnected. The One Health concept also provides a framework for addressing risks that entail a multifaceted and coordinated response.

Ionizing radiation, especially eBeam technology, can provide solutions to problems that may plague the One Health concept. Public health will always involve the issue of food safety. Any discussion of animal health will inevitably touch on pre-harvest pathogen protection strategies involving vaccination. Environmental health issues will never be complete unless the proper management of the burgeoning levels of animal and human wastes is given the attention.

Any discussion on food irradiation will always involve the topic of food packaging, since both food packaging and ionizing irradiation technology are interconnected. It is expected that food irradiation will be used as a final step in the comprehensive Hazard Analysis and Critical Control Point (HACCP) program. Thus, any packaging used for food should be



compatible with this technology. In the United States, there are federal regulations governing the type of polymers that are acceptable for use with pre-packaged food intended for irradiation.

This article provides a high level discussion on the emerging opportunities for eBeam technology in One Health. Specific needs of One Health are identified, and the strategies in which the eBeam technology can be deployed are discussed. To help the reader understand the science of this technology and the regulations that govern its use in foods in the United States, relevant background information is provided.

## 20.2. GLOBAL DISTRIBUTION AND CONSUMPTION OF FOODS

The globalization of foods has resulted in fresh produce being grown in one country and consumed in another country. This development can be attributed to rising consumer incomes, international trade agreements, and improved technologies. U.S. fresh fruit and vegetable imports between 1990–92 and 2004–2006 increased from \$ 2.7 billion to \$7.9 billion [20.1]. Tomatoes, peppers, and cucumbers account for 60% of the total volume of fresh vegetables that are imported by the U.S. from developed countries. Today, 13% of vegetables consumed in the United States are grown outside of the United States. According to recent estimates, illnesses caused by food-borne diseases linked to domestic and imported fresh fruits and vegetables cost the US economy over \$ 150 billion per year [20.2].

The International Plant Protection Convention (IPPC) is an international agreement that aims to preserve the standardization of plant health practices around the world. The IPPC provides countries with a framework to assess risks to their national plant resources and to use science-based measures to safeguard their cultivated and wild plant systems. Countries employ a wide variety of technologies to prevent the spread of insects and pests associated with fresh produce and agricultural commodities in international trade. These technologies include chemical treatments with methyl bromide, heat treatments (hot air, hot water dip and steam), cold treatments, fumigation (phosphine gas, methyl bromide) and ionizing radiation. There is a growing trend in employing ionizing radiation as a phytosanitary treatment technology [20.3]. In the United States, the USDA-APHIS has been encouraging the use of methyl-bromide alternatives including ionizing radiation for phytosanitary treatments. In 2006, it established 400 Gy as the generic dose for all insects and 150 Gy for all fruit flies. The USDA-APHIS has approved the use of ionizing radiation as the only viable treatment for new commodities. It has also stipulated that only ionizing radiation should be used in the import of carambola, sweet lime, guava, manzano pepper, sweet orange, and tangelo from Mexico [20.3]. There is a need to expand the technology portfolio that is currently available to improve the microbiological safety and quality of fresh-cut fruits and vegetables. Recent studies have indicated that foodborne illnesses linked to fresh fruits and vegetables cost the US economy over US \$ 150 billion a year. Additionally, there is a need to optimize irradiation technologies to prevent the accidental importation/exportation of insects and pests that can adversely affect the local agriculture.

## 20.3. IONIZING RADIATION TECHNOLOGY

Ionizing radiation can be delivered using electrons, X ray photons, and gamma ray photons. The methods to generate these types of ionizing radiation are very different.

The irradiation sources that have been internationally approved for food processing are gamma rays produced from radioisotopes Cobalt-60 (1.17 MeV and 1.33 MeV) or Cesium-

<sup>137</sup> (0.662 MeV), machine generated electron beams (max. energy 10 MeV), and X ray photons (maximum energy 5 or 7.5 MeV in the U.S. while 5meV for the rest of the world). It is necessary for irradiation technologies that rely on gamma ray photons to involve the radioactive material Cobalt-60 or Cesium-137. The source is radioactive and cannot be switched off. The source's strength also decays with time as part of the natural radioactive decaying process, and the time of exposure needs to be adjusted to take this into account. Thus, facilities using gamma irradiation have inherently different designs to accommodate the normal operation, protection, shielding, and safeguarding of the radioactive materials. In contrast, irradiation facilities that employ eBeam or X ray are machine-based and use high-energy linear accelerators (linacs) to generate the electrons or the X ray. Furthermore, since the accelerators can be turned on and off, no radioactive residue is generated and/or needs to be protected.

### 20.3.1. Electron beam and x ray technology

Electron beam and X ray technologies do not involve radioactive isotope sources. Electron beams and X rays are generated from regular electricity using linear accelerators. The singular most important value of this technology is that it can be switched on and off, unlike gamma irradiators. Because this technology relies on electricity, issues of safety and security in radioactive isotope transportation, and disposal are non-existent. As a result, the number of eBeam irradiation facilities has grown to 1500 worldwide and has outnumbered gamma irradiation facilities by almost 10:1 [20.4]. Most recent estimates suggest that eBeam systems account for approximately US \$ 80 billion of added value to commercial products. A variety of linear accelerator configurations have been developed since the 1950s. Different applications of the electron beam technology require different linear accelerator configurations. The ability to penetrate materials and products is dictated by the energy of the electrons that are generated by the accelerator. The higher the eBeam energy, the greater is its penetration into the material. The penetrating potential depends not only on the thickness of the package product, but also on the bulk density of the material. This makes it possible to calculate whether eBeam can be used for irradiating the product based on packaging-box dimensions and the product density [20.5]. Table 20.1 provides a general description of the applications for accelerators of different energy profiles. It is important to note that eBeam energies that are greater than 10 MeV cannot be used for any food or food-related applications [20.6, 20.7]. The upper threshold is kept at 10 MeV to eliminate any possibility of temporary radioactivity that may be imparted to the products being irradiated with excessive energy. Table 20.2 shows the dose limits established by the FDA for the application of ionizing irradiation technologies.

TABLE 20.1. COMMERCIAL APPLICATIONS OF ACCELERATORS OF VARYING ENERGY RANGE\*

Application	Preferred electron beam energy
Surface curing of materials	80 – 300 (keV)
Shrink film treatment	300 – 800 (keV)
Wire and cable cross linking	0.4 – 3 (MeV)
Food Pasteurization and Medical sterilization	3 – 10 (MeV)

\*adapted from [8]

TABLE 20.2: IONIZING IRRADIATION DOSES APPROVED BY THE FDA FOR FOODS IN THE U.S.

Food	Purpose	Maximum Allowable Dose (kGy)
Fresh, non-heated processed pork	Control of <i>Trichinella spiralis</i>	0.3 kGy min. to 1 kGy max.
Fresh foods	Growth and maturation inhibition	1
Foods	Arthropod disinfection	1
Dry or dehydrated Enzyme preparations	Microbial disinfection	10
Dry or dehydrated spices/seasonings	Microbial disinfection	30
Fresh or frozen, uncooked poultry products	Pathogen control	3
Refrigerated, uncooked meat products	Pathogen control	4.5
Frozen uncooked meat products	Pathogen control	7
Fresh shell eggs	Control of Salmonella	3
Seeds for sprouting	Control of microbial pathogens	8
Fresh or frozen molluscan shellfish	Control of <i>Vibrio</i> species and other foodborne pathogens	5.5
Fresh iceberg lettuce and fresh spinach	Control of food-borne pathogens, and extension of shelf-life	4.0

Table 20.3 shows the FDA-approved dose limits and fresh produce commodities that can be treated with ionizing radiation. It should be emphasized that there was never any intention to use post-harvest technologies such as eBeam irradiation as clean-up technologies [20.9]. These technologies are meant to be used only as the final step in a comprehensive food safety program that starts with Good Agricultural Practices (GAP) in the field and Good Manufacturing Practices (GMP) in packing facilities. Unless the fresh produce commodity has manageable levels of contaminants to begin with, the use of eBeam or other such post-harvest technologies cannot be expected to make significant reductions in the infection rates among the general public.

TABLE 20.3. U.S. FDA-APPROVED IRRADIATION DOSE LIMITS FOR FRESH PRODUCE AND VEGETABLES

Commodity	Intended Application	Maximum Dose limit (kGy)
lettuce	Pathogen control	4.0
spinach	Pathogen control	4.0
Seeds for sprouting	Pathogen control	8.0
Fresh fruits	Growth and maturation inhibition	1.0
Fresh fruits	Arthropod disinfestation	1.0

### 20.3.2. Ionizing radiation, modified atmosphere packaging, and packaging materials

A variety of polymers used for pre-packaged irradiated foods have already been approved by the FDA. Table 20.4 provides a listing of polymers that are approved as packaging material for irradiated foods.

TABLE 20.4. A SELECTED LIST OF PACKAGING MATERIALS APPROVED BY THE FDA FOR USE WITH IRRADIATED FOODS

Packaging Materials	Max Dose (kGy)
Nitrocellulose-coated cellophane	10
Glassine paper	10
Wax-coated paperboard	10
Polyolefin film	10
Kraft paper	0.5
Polyethylene terephthalate film (basic polymer)	10
Polystyrene film	10
Rubber hydrochloride film	10
Vinylidene chloride-vinyl chloride copolymer film	10
Nylon 11 [polyamide-11]	10
Ethylene-vinyl acetate copolymer	30
Vegetable parchment	60
Polyethylene film (basic polymer)	60
Polyethylene terephthalate film	60
Nylon 6 [polyamide-6]	60
Vinyl chloride-vinyl acetate copolymer film	60

### 20.3.3. Modified atmosphere packaging (map) and food irradiation

In addition to ionizing irradiation technology, modified atmosphere packaging (MAP) can be used to improve the retention of quality and to prolong shelf life. There are a number of knowledge gaps related to MAP and ionizing radiation technology. Though there is a lot of published information on the role of MAP to extend the shelf-life of meat and poultry products, there is limited information on the combined use of MAP and ionizing radiation. More specifically, the effects of MAP and e-beam technology on the shelf life of fresh produce are particularly lacking. The MAP technology is used to extend shelf life by reducing water loss, respiration rate, enzymatic browning, microbial growth, and ethylene production. The oxygen content is lowered from the normal 20% found in air to 5% or less. This slows down respiration rate and enzymatic browning as well as ethylene production by limiting the substrate oxygen that is needed for these reactions. The carbon dioxide content is

elevated from <1% in the air to 3% or higher, depending on the fruit. Elevated carbon dioxide atmospheres have an antimicrobial effect on microorganisms as it lowers the pH to a level below that suitable for the growth of many bacteria. Nitrogen is used as a filler gas to complete the gas mixture to 100%. There is a strong rationale for evaluating the value of MAP in combination with e-beam phytosanitary and food safety relevant doses.

#### **20.3.4. Packaging films and map**

Packaging films are a vital parameter in the success of an MAP system. The polymeric films used for these systems all have different permeability and durability properties. Films used alone may not encompass all of the physical properties desired for a product. They are often laminated or co-extruded together to produce films that have a more desirable trait for the food product. Film types include polystyrene, poly amide (nylon), cellulose, polypropylene, and polyethylene. There are several factors that can influence the properties of packaging films and gas mixtures within the package. Increased temperatures cause an increase in respiration rate of the produce and also increase the film permeability. As a result, the produce consumes more O<sub>2</sub> and gives off higher amounts of CO<sub>2</sub>. This leads to a loss of quality of the food product because the film cannot effectively balance the gas mixtures inside the package. Models have been developed to determine the levels of O<sub>2</sub> and CO<sub>2</sub> to be incorporated into a package. The most widely used mathematical model is the Michaelis-Menten respiratory model. This respiratory model is typically used alongside the Arrhenius equation which considers the temperature sensitivity of the film permeability to estimate O<sub>2</sub> partial pressure as a function of other parameters (temperature, product mass, surface area, and film thickness).

It is often easier to customize packaging for lower respiring foods. High respiring commodities such as broccoli need a film with a high gas transmission rate so that gasses enter or leave the package at sufficient rates to support the aerobic respiration needs. This kind of film that improves gas transmission rates can be achieved through 2 or 3 layers of different films. Each layer may provide a specific benefit such as strength, durability, or a better gas transmission ability. These materials can be blended or laminated together to produce the desired materials. Also, airtight packages are not advised because the O<sub>2</sub> levels will be depleted and anaerobic respiration that results in off flavors and smells could result. It is also just as important that the packaging material should not be too porous or the modified atmosphere would escape and not be able to exert its benefits on the product. It is best to keep in mind that the FDA has approved certain packaging materials (Table 20.4) for use in irradiation. This includes polyethylene, nylon, and polyolefin, among others. Thus, it is vital that the materials have been approved before the produce are packaged and irradiated.

Most gas barrier films are composed of multiple layers. Packaging used in this study contained two films: polypropylene on the inside and nylon on the outside. Polypropylene is a vinyl polymer that is very much like polyethylene (the most commonly used plastic). Polypropylene, however, is very useful as it does not melt below 160°C, unlike polyethylene that will anneal at temperatures around 100°C. Polypropylene can be produced from propylene (a monomer) through processes called the Ziegler-Natta polymerization and metallocene catalysis polymerization.

The outside layer of nylon (polyamide) is used to protect the bag from tears, making it more durable and able to hold in the gas mixtures. Nylon can be made from diacid chlorides and diamines. The oxygen transmission rate (OTR) is the rate at which oxygen can permeate through a film and is expressed within a 24-hour time span. The lower the value, the slower

the oxygen is able to migrate into the package and cause undesirable reactions. The poly nylon bags used had an oxygen transmission rate of 63 cc/m<sup>2</sup> (per 24 hours). Polypropylene film alone has an OTR of 3 500 cc/m<sup>2</sup>, thus demonstrating how multiple layers can have an effect on gas transmission properties. The moisture vapor transmission rate (MVTR) was 4.8 g/m<sup>2</sup>/day, meaning that the lower the rate, the longer the package is able to shelter the contents from moisture and maintain the moisture content of the product throughout storage.

#### **20.3.5. Novel distribution channels for ebeam-treated map packed fresh produce**

There is a national urgency to improve the nutritional choices available in convenience foods offered through vending machines and in convenience stores. Convenience foods sold through such venues are particularly high in sugar, sodium, and saturated fat. It is estimated that approximately 23 million children in the U.S. are either obese or overweight. The vending machine market is estimated to be around US \$ 20 billion. There is an untapped business opportunity to positively influence nutrition and health by positioning fruits and vegetable in the vending machine channel, particularly in schools, universities, workplaces, and in public areas. Ionizing irradiation can play a major role in developing such fruit and vegetable products with enhanced quality and guaranteed microbiological safety intended for the vending machine industry. Developing new food packaging materials and evaluating commercially available packaging materials for ionizing radiation compatibility for use in the vending machine distribution channel have significant commercial potential. This potential that can be realized when combining novel packaging material with modified atmosphere packaging and eBeam cannot be over emphasized.

#### **20.3.6. Ebeam based animal vaccines**

*Salmonella enterica* serovars consists of a group of gram-negative facultative intracellular bacteria that causes food poisoning in humans and other associated diseases in livestock. Poultry is not the only possible source of salmonellosis in humans, but it is known to be a major global reservoir of *Salmonella* spp., given that *Salmonella* is a leading cause of human food poisoning even in industrialized countries such as the United States. Controlling *Salmonella* in poultry farmers is a key intervention strategy in protecting public health. Among the serovars of *Salmonella*, *S. Typhimurium* gives rise to systemic infection and enteric fever in different animal models, and, hence, is accepted as an experimental model for more virulent *Salmonella* serovars. Several attenuated strains of *Salmonella* are being considered as live vaccines since they are capable of inducing humoral as well as cellular immune responses. But, safety concerns limit the utilization of live serovars as vaccine. Current inactivation strategies by chemical treatment or heat render safer vaccines, but these approaches suffer from key issues related to reproducibility, immunogenicity, efficiency, and reliability.

The basis of eBeam vaccines is that ionizing radiation will only destroy the target microorganism's nucleic acid without affecting any of the surface macromolecules. Ionizing radiation inactivates microorganisms by directly causing breaks in DNA strands or indirectly by the generation of radiolytic byproducts that interact with DNA, causing DNA breakages. Irradiation has been used for decades for microbial inactivation for sterilization and pasteurization and, at appropriate doses, can be used to inactivate large volumes of microbial cultures or sterilize materials. Though there are reports of attenuated vaccines (attenuated malarial sporozoites) being developed using Cobalt-60, there are no reports of eBeam-based vaccines other than what has been developed at Texas A&M University. There are a number of salient advantages of eBeam-based vaccines, including its suitability for vaccine

formulations that are either in a liquid, lyophilized, or semi-solid state. Also importantly, the technology to accurately deliver and measure varying ebeam energies and doses provides the added possibility of being able to customize this technology for scaling up vaccine production processes.

### **20.3.7. Ebeam-based wastewater treatment options**

Previous studies at the National Center for Electron Beam Research Texas A&M University in collaboration with scientists at Tulane University have shown that high-energy (10 MeV) eBeam is effective as a pathogen disinfectant and for destroying estrogenic activity in wastewater effluent. When eBeam irradiation is combined with chemical oxidants such as ferrate and chlorine dioxide, it is possible to disinfect the sludges and effluent, destroy estrogenic activity as well as achieve the stabilization of sludges. We term this technology the eBeam Chemical Oxidant (EchO) technology. The EChO technology is capable of destroying estrogenic activity in solids and sewage sludges. The EChO technology can also be used in a pre-conditioning treatment prior to anaerobic digesters.

### **20.3.8. Commercialization opportunities**

There are opportunities to take eBeam research products to the marketplace. The ability to target eBeam-based solutions to specific commercial markets under the One Health framework can be valuable to the commercial industry. Using the One Health framework can provide a roadmap for communicating value proposition of specific technologies to decision makers within the industry and governmental agencies.

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## Chapter 21

### RADIATION PROCESSING OF BIO-POLYMERS FOR AGRICULTURE APPLICATIONS

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#### Abstract

Antimicrobial activity of chitosan, a well-known natural polysaccharide has been greatly improved by gamma irradiation. The highest antibacterial activity for *E.coli* and antifungal activity for *Fusarium dimerum* Penzig was obtained with chitosan irradiated at about 50–75 kGy. Other chemical properties of chitosan e.g. viscosity, molecular weight, and degree of deacetylation (DD) were also changed by radiation. The number average molecular weight of chitosan significantly reduced from 300 000–100 000 g.mol<sup>-1</sup>, whereas its DD slightly increased. A study of the chitosan membrane indicated that radiation treatment strongly influenced its mechanical properties. Both tensile and elongation at break of the irradiated chitosan were reduced with radiation dose. Irradiated chitosan can be applied as a preservative solution for the coating of fresh fruits. Also, antimicrobial packaging films from irradiated chitosan can be produced for ready-to-eat foods. Recent studies on radiation cross-linked cassava starch based hydrogels suggest that superabsorbent materials can absorb not only water but also nutrient solutions containing N, P, and K ions. This can be prepared through gamma radiation using a suitable formulation. Superabsorbent materials, swollen in NPK solution, can be used as slow-release fertilizers and considered as one of the technological advances in agricultural production.

#### 21.1. INTRODUCTION

Radiation technology has been utilized to modify the properties of various materials as well as create new materials that can be applied in many fields [21.1]. Radiation treatment is a very useful tool in improving the properties of polymer. Depending on radiation conditions and the chemical structure of treated polymers, either degradation or cross-linking will take place and will determine the final outcome of the radiation process. For the degrading type of polymers, random chains scissions are favored by irradiation, resulting in the reduction of length of polymer chains. On the other hand, the cross-linking type of polymers converts linear polymers into branched polymers and ultimately forms three-dimensional network structures [21.2–21.5]. The radiation grafting of functional monomer on polymer backbone can also be performed either at the surface of a polymer, or both at the surface and in bulk of polymers, depending on the penetration capacity of the radiation and type of monomer [21.6–21.8].

Compared to chemical treatments, radiation processing can reduce energy consumption, be performed at or near room temperature for large volumes of products, improve product quality, eliminate the use of chemicals, eliminate residues of undesired chemicals that can affect the quality of products, and cause the disposal of environmentally harmful substances [21.9]. The applications of radiation processing for polymers have been widely used in industrial, medical, agricultural and environmental areas. To date, there are many gamma irradiator and electron beam facilities being operated all over the world for the radiation processing of polymers.

Chitosan is the deacetylation product of chitin, an abundant polysaccharide found in the exoskeleton of crustaceans and the cell walls of fungi. The main sources are shells of shrimp, crab, and lobster, considered waste materials in the shrimp processing industry. At present, there is an estimated 200 000 metric tons of shrimp waste per year, which can be considered an important source for chitosan production in Vietnam. With the amino and hydroxy groups in its molecule, chitosan can be easily modified through physical and chemical processes.

The high density positive charge in chitosan can interact with negative charged substances such as proteins that exist in the wall of the microbial cell, thereby inhibiting the growth of microorganism [21.10]. It is also known that the antimicrobial activity of chitosan usually depends on their molecular weight and solubility. Radiation treatment can produce radiation-degraded chitosan with lower molecular weight and higher antimicrobial activity [21.11]. These effects of irradiated chitosan can be applied for production of antimicrobial coating layers or packaging films for the preservation of fresh fruits and other foods.

Starch is also considered one of the most promising natural polymers for packaging application because of its availability and low cost, biodegradability, and great potential as edible films for food preservation. Moreover, starch can be used as a main component in various products. Recently, some starch-based products have been studied as superabsorbent materials [21.12]. The gel fraction of the cross-linked hydrogels increased, while their swelling degree reduced with the radiation dose. In nutrient solutions, the degree of swelling of the hydrogels for water is much reduced due to the presence of dissolved ions. The degree of swelling for solutions containing N, P, and K ions was also affected by solution temperature. The nutrient absorbed hydrogels can be used as slow release fertilizers for seedlings.

## 21.2. EXPERIMENTAL

### 21.2.1. Materials and sampling methods

Three kinds of chitosan with number average molecular weights of 350, 180 and 70 kDa of similar DD (80%) were purchased from MTV Chitosan Vietnam Ltd., Co. Cassava starch was bought from Viet Delta Co. (Vietnam). Other chemicals were purchased from Sigma Aldrich. The microbial strains were received from the Institute of Biotechnology, Vietnam Academy of Science and Technology.

Different chitosan dried samples and paste-like solutions from various formulates of cassava starch have been irradiated at varying radiation doses using the gamma source at Hanoi Irradiation Center. The chemical properties of irradiated chitosan were determined with radiation dose, and their antimicrobial activities were investigated. The optimal radiation dose that could improve the antimicrobial activity of chitosan was determined.

### 21.2.2. Measurements

The radiation cross-linked hydrogels obtained were extracted in de-ionized water for 72 h at room temperature, then dried at 60°C overnight under vacuum to constant weight. The gel fraction was calculated as:

$$\text{Gel fraction (\%)} = (G_d / G_i) \times 100 \quad (1)$$

where  $G_i$  and  $G_d$  are the dried weight of the initial sample and dried gel after extraction, respectively.

Weighed hydrogels were immersed in distilled water and in the nutrient (NPK) solution containing 36.4 ppm N, 37.7 ppm P, 88 ppm K, and Murashine and Skoog (MS) medium for 72 h. The swollen hydrogels were taken out and swelling degree was calculated as follows:

$$\text{Swelling degree} = (G_t - G_d) / G_d \quad (2)$$

where  $G_t$  is the weight of the swollen hydrogel after swelling.

The performance and field application of the resulting hydrogels were done as follows: 1g of dried hydrogels was swollen in NPK solution. This was immersed in distilled water and stirred at room temperature for release test. After certain intervals, the water was replaced. The amounts of N, P, K released to water were determined and the remaining nutrients (%) in the hydrogel were plotted against time. Seedlings of black bean and maize were grown in pots contained 200 g cultivation soil and 1 g dried hydrogel swelled with NPK. The cultivation soil was used as a positive control. The root length, plant height, and fresh biomass were determined after two weeks.

## 21.3. IMPROVING ANTIMICROBIAL ACTIVITY OF CHITOSAN BY GAMMA RADIATION FOR FOOD PRESERVATION

### 21.3.1. Viscosity and molecular weight of the irradiated chitosan

Figure 1 shows the changes in intrinsic viscosity ( $[\eta]$ ) and number average molecular weight ( $M_n$ ) of chitosan irradiated by gamma radiation. The viscosity and molecular weight of all chitosan samples sharply decreased with increasing radiation dose of up to 50 kGy and levelled off at dose higher than 200 kGy. Radiation processing can degrade polymer chains by breaking the glycosidic bonds to produce various low molecular weight fragments [21.13]. Hydrogen and other functional groups may be abstracted from the polymer to form labile radicals. When radiation dose increases, the numbers of free radicals and probability for recombination between them also increases. As a result, the molecular weight of irradiated chitosan slowly decreased with a further increase in dose (over 200 kGy). Using this method, chitosan with a predetermined molecular weight can be prepared by radiation treatment.

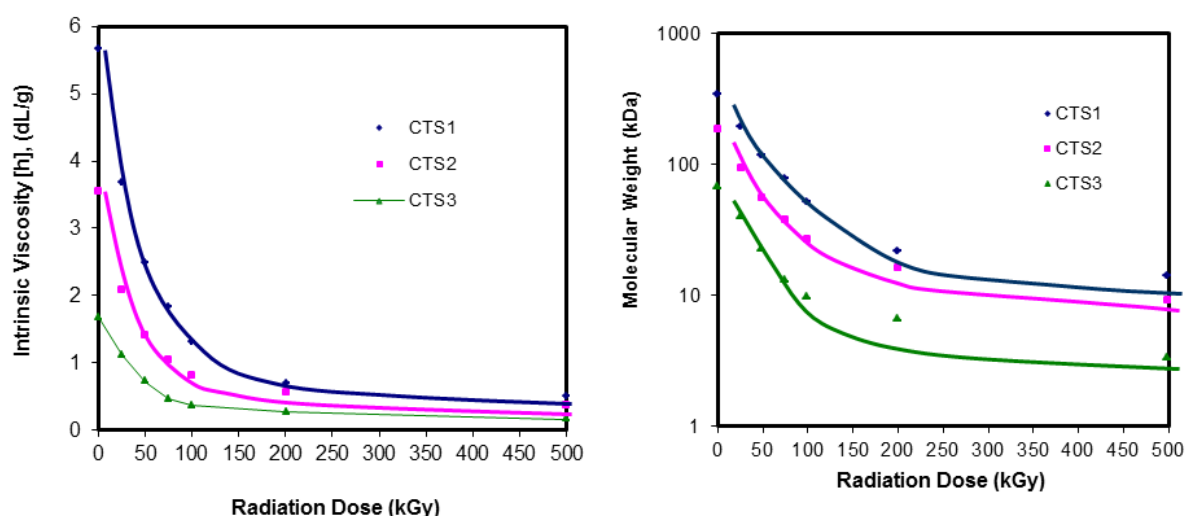
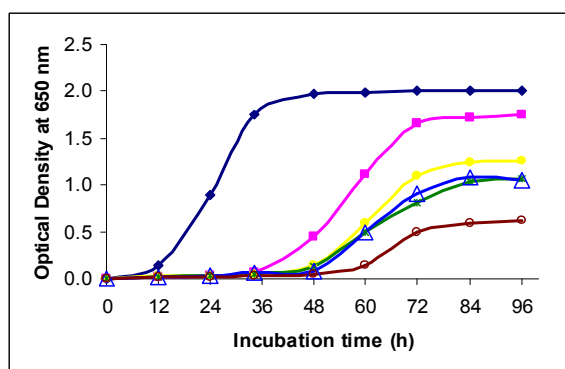


FIG. 21.1. Intrinsic viscosity and number average molecular weight of the irradiated chitosan with radiation dose.

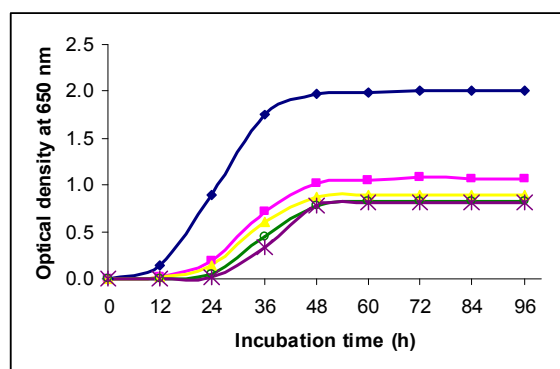
### 21.3.2. Antimicrobial activity

The antimicrobial activity of chitosan was evaluated through minimal inhibition concentration (MIC). Agar-plate method was carried out in petri dishes containing Nutrient Agar. Antibacterial activities of chitosan and the irradiated chitosan were investigated with chitosan having  $M_n$  of 350 kDa [21.14]. The growth of E.coli in the liquid medium containing different chitosan samples are presented in Figure 21.2. Bacterial growth produces turbidity

in the culture medium such that the optical density of agar medium becomes smaller as observed. Figure 2a shows the growth of *E.coli* in the presence of non-irradiated and irradiated chitosan in the initial 24 h. Bacterial growth in Nutrient Broth containing the same amount of chitosan was reduced with an increasing radiation dose. This suggests that the antibacterial activity of chitosan can be improved through radiation. The antibacterial activity of chitosan also depended on the amount of chitosan in the culture medium as shown in Figure 21.2b. The results revealed that 100 ppm is enough to suppress the growth of *E.coli*.



Growth of *E.Coli* B/r in Nutrient Broth at 37°C without chitosan (◆), with 100 ppm chitosan (■) and chitosan irradiated at 25 (●), 50 (△), 75 (\*), and 100 kGy (○)



Growth of *E.Coli* B/r in Nutrient Broth at 37°C without chitosan (◆), with 50 (■), 100 (▲), 150 (●), and 200 ppm (\*) of the chitosan irradiated at 50 kGy

FIG. 21.2. The growth of *E.coli* B/r in nutrient broth supplemented with 100 ppm chitosan irradiated at different dose (a) and with different amount of chitosan irradiated at 50 kGy (b).

The growth of *E.coli* in a solid medium was also determined. Figure 21.3 shows that the growth of *E.coli* was inhibited by the presence of non-irradiated and irradiated chitosan. The bacteria can not grow in the agar medium containing 100 ppm chitosan irradiated at 50 kGy. Growth of colony was still observed in the medium containing non-irradiated chitosan. Thus, antibacterial activity of chitosan was much enhanced by radiation.

The antifungal activity of chitosan for *Fusarium dimerum* Penzig was also investigated. This is a fungus that causes spoilage in many tropical fruits. The growth rate of this fungus was evaluated by the increase in its biomass in liquid medium or increase of mycelia diameter in agar-plate medium. The liquid method is normally used for bacteria. As seen in Figure 4, chitosan has a great effect in reducing the microbial growth in a liquid medium. Using the same incubation time for all samples, fungal mycelia grew in agar, but the size of micelium was reduced by the presence of chitosan. Maximum effect was observed at 150 ppm at a dose of 50 kGy with the absence of the growth of *F. dimerum* Penzig in agar. These results again demonstrate that the antimicrobial activity of chitosan can be improved through the radiation process. Irradiated chitosan can be applied as coating materials for food preservation. Figure 5 shows the differences among fresh fruits coated with non-irradiated and irradiated chitosan at ambient conditions for 5 days.

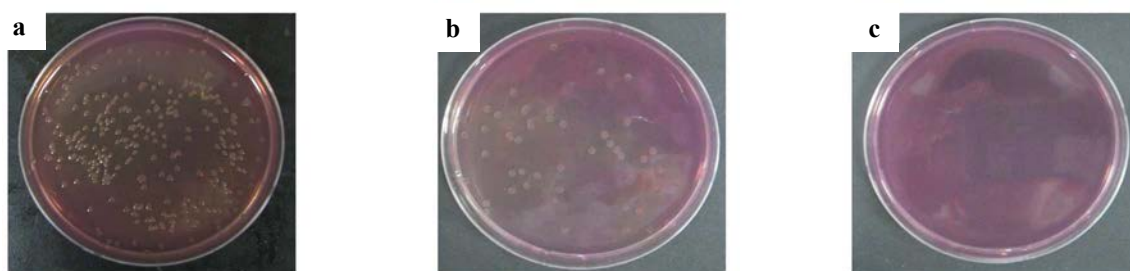


FIG. 21.3. The growth of *E.coli* B/r population ( $5 \times 10^6$  CFU) in nutrient agar without chitosan (a), with 100 ppm chitosan (b) and chitosan irradiated at 50 kGy (c).

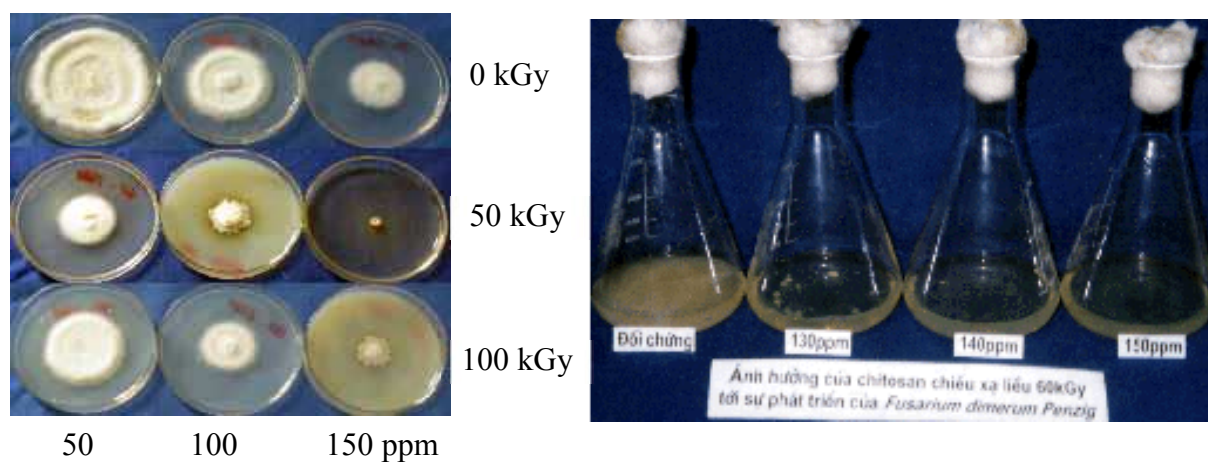


FIG. 21.4. The effect of chitosan and solid radiation treatment on mycelial growth of *Fusarium dimerum* Penzig incubated in agar-plate medium (left) and liquid medium (right).

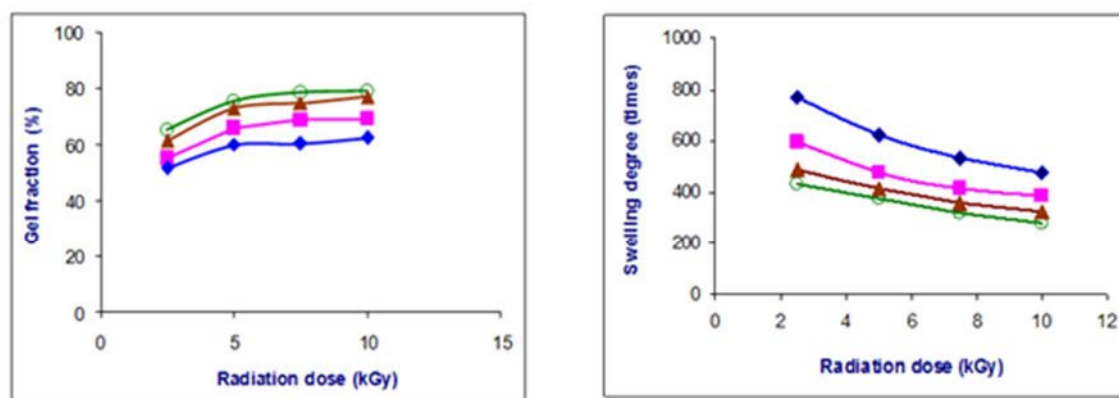


FIG. 21.5. The mangos coated without chitosan (a), with chitosan (b) and irradiated (c) after 5 days preserved at ambient conditions.

## 21.4. PREPARATION OF SUPERABSORBENT HYDROGELS BASED ON STARCH

### 21.4.1. Effects of the amount of starch and radiation doses on the behaviour of hydrogels

As previously reported; cassava starch with other synthetic polymers such as polyvinyl alcohol (PVA), polyacrylamide, and polyethylene glycol; can cross-link in paste-like condition [21.15]. As shown in Figure 6, gel fractions of the cross-linking hydrogels increased with starch concentration and radiation dose which leveled off at 5 kGy, whereas their swelling degrees (DS) decreased.



The crosslinked hydrogels obtained from paste-like solutions of 20 (◆); 30 (■); 35 (▲) and 40 % (○)

FIG. 21.6. Gel fractions and swelling degrees of starch based hydrogels with radiation dose.

### 21.4.2. Effects of the nutrient (n, p, k) medium on the swelling behaviour of superabsorbent hydrogel and in-vitro release of n, p, k from the swollen hydrogels

Figure 21.7a shows the degree of swelling behaviour of the hydrogels in distilled water and nutrient solutions containing N, P, and K ions and Murashige and Skoog (MS) medium. The swelling behaviour of the hydrogels had similar trends with an increasing radiation dose. DS decreased with increasing radiation dose. Expectedly, highest DS was obtained in hydrogels swollen in distilled water and lowest in MS solution. Figure 21.7b also indicates that the release of nutrient ions increased with incubation time. N, P, and K ions were quickly released after the first 5 days, and slowly released thereafter. The release of the N ion was slower than the P and K ions, which may be due to their differences in affinity with the hydrogel.

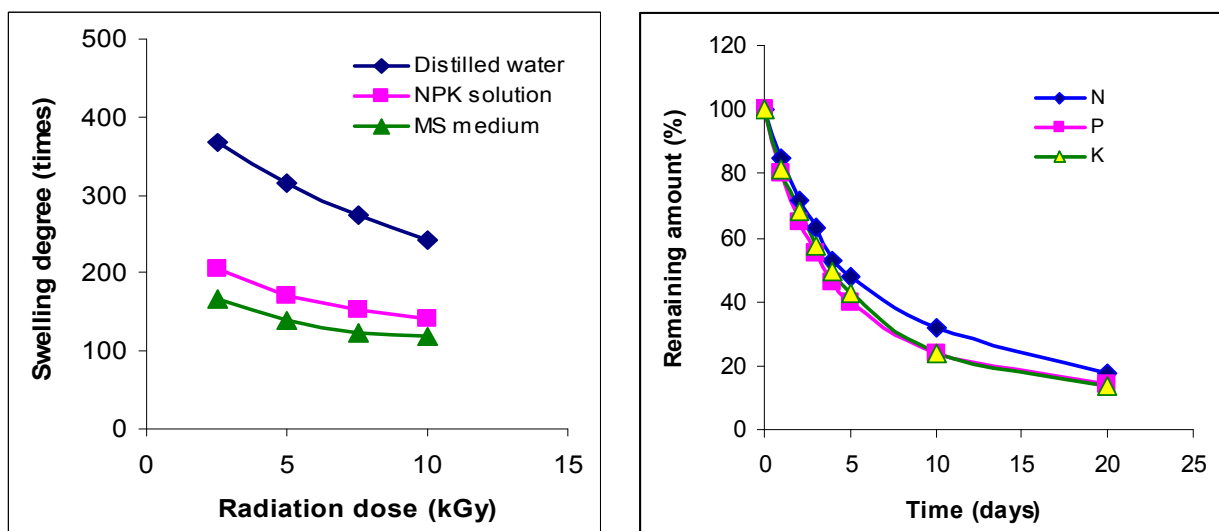


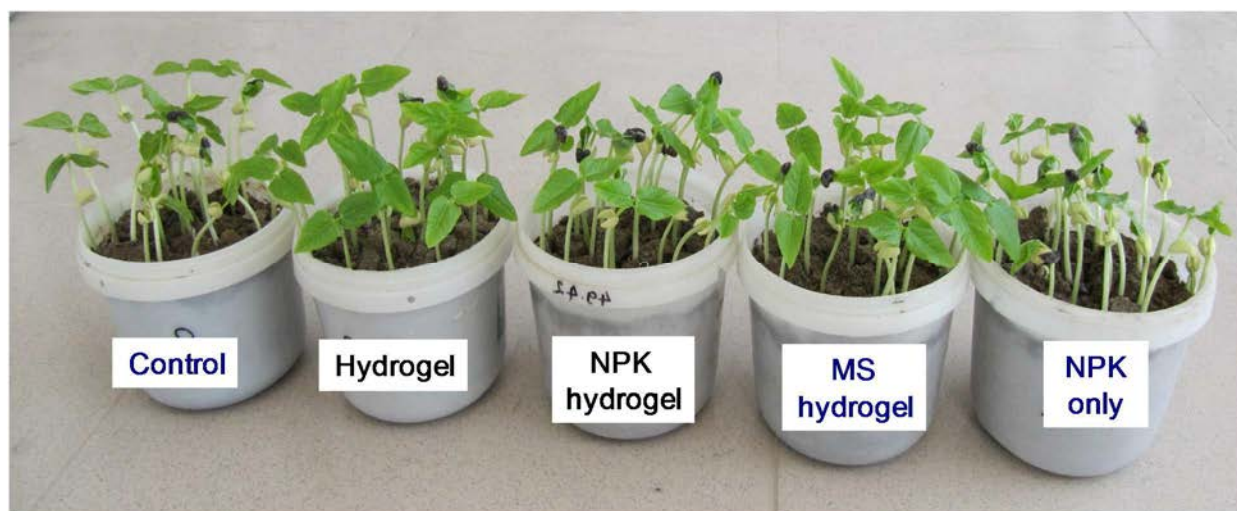
FIG. 21.7. Swelling behaviour of the hydrogels with distilled water and nutrient solution (a), in-vitro release of N, P, K from the hydrogels at room temperature (b).

The effects of the nutrient-containing superabsorbent hydrogel on the growth of black beans seedlings are shown in Table 21.1 and Figure 21.8. Plant height, root length, and fresh biomass of seedlings of plants containing hydrogels in soil were higher compared to those of the control. These parameters were at its maximum in plants containing hydrogels with NPK or MS solution. This suggests that there is an uptake of N, P, and K by the seedlings and plants.

TABLE 21.1. THE EFFECTS OF WATER AND NUTRIENT ABSORBED HYDROGELS ON THE GROWTH OF BLACK BEANS

Treatment	Plant height		Root length		Fresh biomass	
	Average (cm)	Difference with control (%)	Average (cm)	Difference with control (%)	Average (g)	Difference with control (%)
Control	14.6	100.00	8.2	100.00	3.6	100.00
Hydrogel	17.4	119.18	10.8	131.71	4.23	261.11
Hydrogels contain NPK solution	19.8	135.62	12.3	150.00	4.9	136.11
Hydrogel contain MS solution	22.3	152.74	12.9	157.32	5.3	147.22





*FIG. 21.8. The growth of black beans seedlings in cultivation soils containing hydrogel and the nutrient absorbed hydrogels.*

## 21.5. CONCLUSION

Intrinsic viscosity and the number average molecular weight of chitosan can be modified through gamma radiation. The viscosity and molecular weight of the irradiated chitosan were substantially reduced by radiation at a dose of 50 kGy.

Radiation treatment can improve the antibacterial and antifungal activities of chitosan with concrete application as antimicrobial coating films for the preservation of fresh fruits and ready-to-eat foods.

Superabsorbent hydrogels for water and nutrient solutions can be obtained from cassava starch cross-linked with synthetic polymers. The gel fraction of the cross-linked hydrogels increased with dose, whereas their degree of swelling decreased.

The N, P, and K ions can be released from the nutrient absorbed hydrogels for a long time. The superabsorbent hydrogels with NPK solution can be applied as slow-release fertilizers in plant cultivation, especially in drought-hit areas.

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**ISSN 1011-4289**