การเตรียมบีดไคโทซานที่มีฮอร์โมนออกซิน

นางสาวสมหญิง บุญวรรณ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์
หลักสูตรปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2544

ISBN 974-17-0146-2

PREPARATION OF CHITOSAN BEADS

CONTAINING AUXIN HORMONES

Miss Somying Boonwan

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Petrochemistry and Polymer Science

Program of Petrochemistry and Polymer Science

Faculty of Science

Chulalongkorn University

Academic Year 2001

ISBN 974-17-0146-2

Thesis Title : Preparation of Chitosan Beads Containing Auxin Hormones

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Field of Study : Petrochemistry and Polymer Science

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(PREPARATION OF CHITOSAN BEADS CONTAINING AUXIN HORMONES)
อ.ที่ปรึกษา : ผศ.ดร. มงคล สุขวัฒนาสินิทธิ์, 113 หน้า. ISBN 974-17-0146-2

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หลักสูตร ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต……..
สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่ออาจารย์ที่ปรึกษา……..……...
ปีการศึกษา 2544 ลายมืออาจารย์ที่ปรึกษาร่วม……-……

4272417223 : MAJOR PETROCHEMISTRY AND POLYMER SCIENCE

KEY WORDS : CHITOSAN BEADS / AUXIN HORMONES / 2,4-D / IAA / CONTROLLED RELEASE

SOMYING BOONWAN : PREPARATION OF CHITOSAN BEADS CONTAINING AUXIN HORMONES. THESIS ADVISOR : ASST. PROF. MONGKOL SUKWATTANASINITT, Ph.D., 113 pp. ISBN 974-17-0146-2

Chitosan, a biopolymer, was used for controlled release of auxin hormones, 2,4-dichlorophenoxyacetic acid (2,4-D) and 3-indoleacetic acid (IAA). Polyphosphoric acid and glutaraldehyde were utilized as crosslinking agents in the preparation of beads from chitosan and 2,4-D (or IAA) mixtures. Our results indicated that the significant amount of 2,4-D was lost (only 20% recovery) during the preparation of beads containing chitosan:2,4-D ratio of 1:1 (w/w). Increase of chitosan contents and the use of polyphosphoric acid as crosslinking agent increased percent recovery of 2,4-D to about 69%, while the use of glutaraldehyde as crosslinking agent increased percent recovery of IAA to about 33%. The soil column leaching test indicated that polyphosphoric acid-crosslinked beads sustained the irrigation numbers of over 250% for 2,4-D and over 117% for IAA of the pure active compounds.

Program Petrochemistry and Polymer Science Student's signature……..…...

Field of study Petrochemistry and Polymer Science Advisor's signature……..

Academic year 2001 Co-advisor's signature.…-…….

ACKNOWLEDGMENT

I wish to express gratitude and appreciation to my advisor, Assistant Professor Dr. Mongkol Sukwattanasinitt, for his helpful suggestions, generous guidance and encouragement throughout this research. Gratefully thanks to Associate Professor Dr. Supawan Tantayanon, Assistant Professor Dr. Warinthorn Chavasiri, Assistant Professor Dr. Suwabun Chirachanchai and Dr. Varawut Tangpasuthadol, for their valuable suggestions and advice as thesis committee.

I also would like to gratefully thank to Miss Kitsana Siralerdmukun from Metallurgy and Materials Science Research for making available the facilities of using optical microscope and chitosan supports. Many thanks to the Mongkol' group members for their supports and encouragement. I am also obliged to the Graduate School of Chulalongkorn University and the Department of Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University for their financial supports throughout this research.

Finally, I also thank to my family for their continued support, love and encouragement. Without them this thesis could not have been success.

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FIGURE

LIST OF SYMBOLS AND ABBREVIATIONS

°C	=	degree celcius
g/mL	=	gram per milliliter
mg	=	milligram (s)
mL	=	milliliter (s)
M	=	molarity
mM	=	millimolarity
v/v	=	volume per volume
w/v	=	weight per volume
w/w	=	weight per weight
ppm	=	parts per million
CA	=	crosslinking agent
GA	=	glutaraldehyde
PPA	=	polyphosphoric acid
nm	=	nanometer
rev./min	=	revolve per minute
IR	=	infrared
cm^{-1}	=	unit of wave number

CHAPTER I

INTRODUCTION

1.1 Statement of Problems

In each year, great quantities and varieties of agrochemicals such as herbicides, insecticides, fungicides, plant hormones and fertilizers have been used for increasing crop productions.[1] The efficiencies of these agrochemicals usually decrease when they are swept out of the target by rain and wind. This results in escalation of use of agrochemicals leading to many problems associated to their toxicity. Controlled release technology has emerged as an alternative approach that promise to solve these problems.[2] In a popular controlled release system, polymeric materials are combined with active substances to allow automatic delivery of the agents to the target at controlled rates to maintain its concentration within an optimum concentration range over a long period of time. The degradation rates of the polymers and the residual products from the degraded polymers after the release of the active agents must be considered to avoid possible environmental adverse effects. In this work, chitosan is chosen for controlled release of auxin hormones such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 3-indoleacetic acid (IAA). Chitosan is a natural polymer, biodegradable and nontoxic that its residual products from the degraded polymer backbone are not harmful to human and environment. Futhermore, chitosan is a regeneratable natural resources (e.g., crab and shrimp shells), which are abandoned by sea food processing companies.

1.2 Objectives

The objectives of this thesis are to develop efficient methods for preparation of chitosan beads containing 2,4-dichlorophenoxyacetic acid (2,4-D) or 3-indoleacetic acid (IAA), and to study their releasing behaviors.

1.3 Methods of Study

1. Survey literatures.
2. Prepare or purchase necessary chemicals and equipments.
3. Determine molecular weight of chitosan by intrinsic viscosity.
4. Determine the degree of deacetylation by FT-IR spectroscopy and colloid titration.
5. Prepare chitosan beads containing 2,4-dichlorophenoxyacetic acid (2,4-D) or 3-indoleacetic acid (IAA). The following variable will be studied.
   - Sources of chitosan being Sea Fresh, Mahachai and Japan
   - The ratios of chitosan to auxin hormones in the solution being 1:1 and 5:1
   - Crosslinking agents being glutaraldehyde and polyphosphoric acid.
   - Using techniques of crosslinking agents: none, surface, bulk
   - The concentration of crosslinking agents
   - Crosslinking time
   - Other conditions e.g. temperature and coagulant solutions

6. Characterization of prepared beads.
   - Assay of 2,4-D or IAA contents in prepared beads by using UV spectrophotometer.
   - Study the releasing profile of 2,4-D from the prepared beads by using UV spectrophotometer.
   - Study the swelling behavior of uncrosslinked-beads, glutaraldehyde-crosslinked beads and polyphosphoric-crosslinked beads.
   - Examine the size and surface morphology of the prepared beads by optical microscope.
   - Examine the crosslinked chitosan beads by using FT-IR spectroscopy.
7. Interpret, summarize the data, and write up a thesis.

1.4 Theory

1.4.1 Controlled Release Formulations[2]

Controlled release formulation is designed to protect the supply of the active reagent and to allow its automatic delivery to the target at controlled rates to maintain its concentration within an optimum concentration range over a long period of time.

The controlled release formulations are normally done in two ways; (a) physical mixing and (b) chemical attachments

(a) physical mixing: Two different approaches have been reported in the case of the physical combination of biologically active agents with polymeric materials. First, the biologically active agent can be encapsulated in a polymeric material in which the release of the active agent is controlled by Fick's Law of diffusion through the micropores in the capsule walls.

$$R_d = \mathrm{d}M_t / \mathrm{d}t = (A / h D)(C_s - KC_e)$$

where M_t is the mass of the agent released; $\mathrm{d}M_t/\mathrm{d}t$ is the steady state release rate (R_d) at time t; A is the surface area through which the diffusion takes place; h is the thickness through which the diffusion occurs; D is the diffusion coefficient of the active agent in the polymer; C_s is the saturation solubility of the active agent in the polymeric membrane; K is the partition coefficient of the active agent between the polymer and the medium which surrounds the device; C_e is the concentration of the released active agent in the environment.

In the second approach, biologically active agent is heterogeneously dissolved in a solid polymeric matrix, which can be either biodegradable or non-biodegradable. The release of the active agent is generally controlled by diffusion phenomena through the matrix, by chemical or biological erosion, or by a

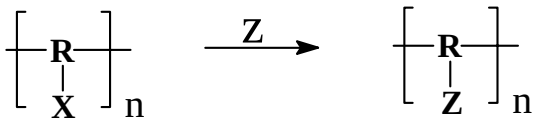
combination of both diffusion and erosion. Release by erosion is a surface area dependent phenomena, and the general expression which describes the rate of release (R_r) by an erosion mechanism.

$$R_r = \mathrm{d}M / \mathrm{d}t = K_E C_o A$$

where K_E is the erosion rate constant; A is the surface area exposed to the environment; C_o is the loading of the active agent in the erodible matrix.

The design of such physical combination is generally not influenced by the structure of the active agent molecule. It is also not strongly influenced by the structure of the polymer matrix, and a broad range of polymer matrices can be used. However, in most cases there are material requirements to the polymer such as: (i) compatibility with the active agent so that it can be readily dissolved or dispersed in the polymer, and so that there is no undesirable reactions or physical interactions; (ii) a low softening point is desirable in order to prevent thermal degradation of the active agent during mixing of an active agent with the molten polymer; (iii) high crystallinity in the polymer should be avoided, as in a highly ordered matrix the release rate of dissolved material would be altered and might be very low. Additionally, polymers must be mechanically stable, easy to fabricate and of low cost.

(b) Chemical attachment: In this type, the active agent is chemically attached to a natural or synthetic polymers by a specific chemical bond, either via an ionic or a covalent linkage. The active agent must contain a structural moiety with at least one reactive functional group suitable for use as a link to the functionalized polymer. Polymer containing chemically bonded active agents can be prepared by two synthetic approaches. The first approach involves chemical modification of a preformed polymer with the desired active agent *via* a chemical bond, leading to a polymer having the active species linked to the main chain as pendant groups.



Z = active agent

The second method requires synthesis and polymerization of a monomer containing an active agent moiety which leads to polymers having the active groups as a repeating unit in the main backbone.

The controlled release formulation can have the following advantages: (i) give satisfactory effect for a long period of time (residue effect); (ii) reduce total dosages (resource-saving, safety) by reducing the number of frequent applications used to achieve longer activity; (iii) reduce toxicity, since toxic material become chemically non-toxic when combined with polymers; (iv) decrease undesirable environmental effects; (v) change liquid into solid materials to facilitate handling and transportation.

1.4.2 Chitosan[3]

Chitosan is a polysaccharide obtained by deacetylating chitin, which is a major constituent of the exoskeleton of crustacean. It is a natural polymer with a structure similar to cellulose (Figure 1.1). It is not only naturally abundant, but it is also nontoxic and biodegradable.

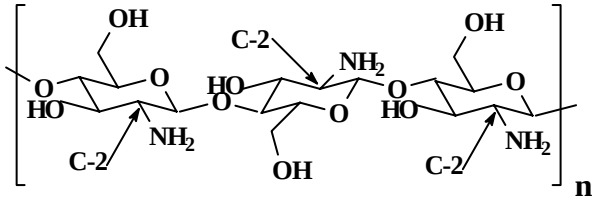


Figure 1.1 Chemical structure of chitosan.

Chitosan was reportedly discovered by Rouget in 1859 when he boiled chitin in a concentrated potassium hydroxide solution, resulting in the deacetylation of chitin (Figure 1.2).

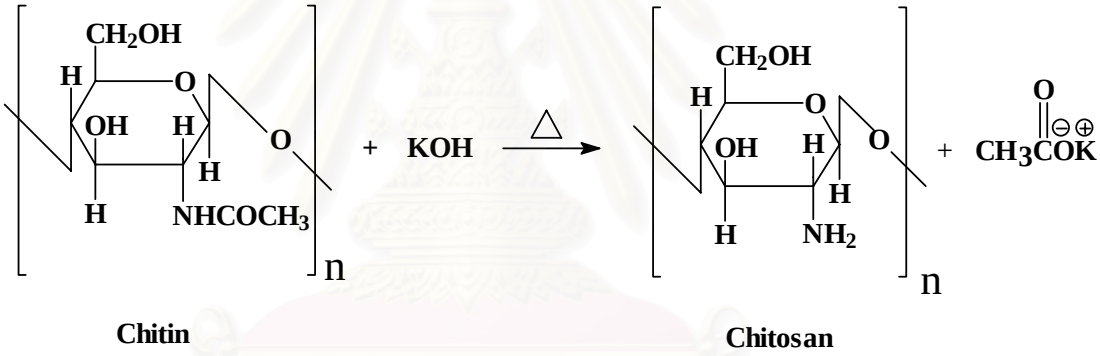


Figure 1.2 Deacetylation of chitin.

The difference between chitin and chitosan lies in the degree of deacetylation. Generally, the deacetylation of chitin in an alkali solution cannot reach completion even under harsh treatment. The degree of deacetylation usually ranges from 70% to 95% depending on the method used. The physicochemical properties of chitosan to depend on factors of structure are the degree of deacetylation and molecular weight.

The degree of deacetylation is one of the more important chemical characteristics of chitosan. It specifies the content of free amino groups in the

polysaccharide. Methods for determining the removal of acetyl groups in chitosan include infrared spectroscopy, titration and chromatography.

The molecular weight of chitosan can be determined by method such as chromatography, light scattering and viscometry. Among these, viscometry is the most convenient and economical method.

Normally, chitosan is insoluble in water, alkali and organic solvents but is soluble in most solutions of organic acids when the pH of the solution is lower than 6. Acetic and formic acids are two of the most widely used acids for dissolving chitosan. The applications of chitosan are shown in Table 1.1.

Table 1.1 Applications of chitosan

Application	Example
Water Treatment	Removal of Matal Ions
	Flocculant/Coagulant: Proteins, Dyes, Amino Acids
	Filtration
Pulp and Paper	Surface Treatment
	Photographic Paper
Medical	Blood Cholesterol Control
	Dental/Plaque Inhibition
	Skin Burns/Artificial Skin
	Contact Lens
	Controlled Release of Drugs
Cosmetics	Make-up Powder
	Moisturizers
	Bath Lotion
	Face, Hand and Body Creams
Biotechnology	Enzyme Immobilization
	Protien Separation
	Cell Immobilization
	Glucose Electrode
Agriculture	Seed Coating
	Leaf Coating
	Hydroponic/Fertilizer
	Controlled Agrochemical Release
Food	Removal of Dyes, Solids, Acids
	Preservatives
	Animal Feed Additive
Membranes	Reverse Osmosis
	Permeability Control
	Solvent Separation

1.4.3 Auxin Hormones[4]

Auxin is a group of plant growth regulating substances responsible for such processes as the promotion of growth by cell enlargement, the maintenance of apical dominance, and the initiation of root formation in cuttings. Auxins are also involved in the abscission of leaves, fruits, or other plant organs and in the development of flowers and fruits. Naturally occurring auxins, principally indoleacetic acid (IAA, Figure 1.2), are synthesized in actively growing regions of the plant, from where they are transported to other parts of the plant. Synthetic auxins, such as 2,4-dichlorophenoxyacetic acid (2,4-D) is used as weedkiller for broad-leaved weeds. The concentration of these plant hormones are normally low because they are constantly being destroyed by IAA acid oxidase, an enzyme system found in intact plants.

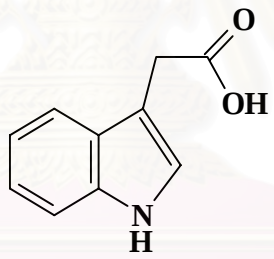


Figure 1.3 Chemical structure of IAA.

Although an auxin stimulates growth, some auxins can nevertheless be used as herbicide. This is because stems, buds, and roots show a two-phase type of response to auxins, with promotion at lower concentrations and inhibition at higher ones. Stems have the highest optimum auxin concentration for growth and so auxin levels which promote stem growth may inhibit bud or root growth. High dosages of auxins disrupt all growth, thus killing or permanently damaging the plants. Many synthetic auxins have been found. Some of these have more biological activity than IAA in the acidic plant cell walls, such as 2,4-D.

The herbicide 2,4-D (2,4-Dichlorophenoxyacetic acid; Figure 1.4) is a widely used member of the phenoxy family. It is a selective herbicide, with highest toxicity to broad leaf plants.[5]

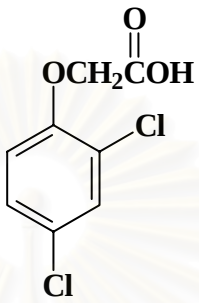


Figure 1.4 Chemical structure of 2,4-D.

Concentrations of auxins normally fluctuate in oder to properly direct growth. In cell exposed to 2,4-D, however, levels of this auxin mimic remain high because 2,4-D is more stable and presistent than IAA. As a result, 2,4-D stimulates the synthesis of nucleic acids and proteins and causes abnormal growth. Death occurs when the plant' s transport system (xylem and phloem) is crushed and plugged by this growth.

The use of 2,4-D as a herbicide has two approaches, preemergence and postemergence herbicide. It is used to control broad leaf weeds in wheat, sod, corn, rangeland/pastureland and sorghum. The toxicity of 2,4-D is LD_{50} (rat oral acute) 375 mg/kg. Trade names of 2,4-D such as Hedonol, Ester-79, Bara-ester, Ratanal or Veedex.

1.4.4 Crosslinking[6, 7]

When polymers are produced in which the polymer molecules are linked to each other at points other than their ends, the polymers are said to be crosslinked. Crosslinking can be made to occur during the polymerization process by the use of

appropiate monomers. It can also be brought about after the polymerization by various chemical reactions. The crosslinks between polymer chains can be of different lengths depending on the crosslinking method and the specific conditions employed. One can also vary the number of crosslinks so as to obtain lightly or highly crosslinked polymers. When the number of crosslinks is sufficiently high, a three-dimensional or space network polymer is produced in which all the polymer chains in a sample have been linked together to form one giant molecule. Low degree of crosslinking is used to impart good recovery (elastic) properties to polymers to be used as rubbers. High degrees of crosslinking are used to impart high rigidity and dimensional stability (under conditions of heat and stress) to polymers.

Crosslinking agents are the reagent that are used to link between two polymeric chains. In 1990, Cardinal proposed that crosslinking agents for chitosan may be devided into two main types as below.

a) Low molecular weight compounds such as glutaraldehyde, glyoxal, succinaldehyde, benzoquinone and epihalohydrin. This type of crosslinking agents usually contains two or more functional groups which can react with amino groups of chitosan.

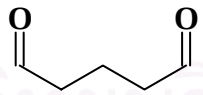


Figure 1.5 Chemical structure of glutaraldehyde.

Chitosan can be crosslinked with glutaradehyde by attributing the formation of imine (C=N) due to a condensation reaction between amino and aldehyde groups (Figure 1.6).

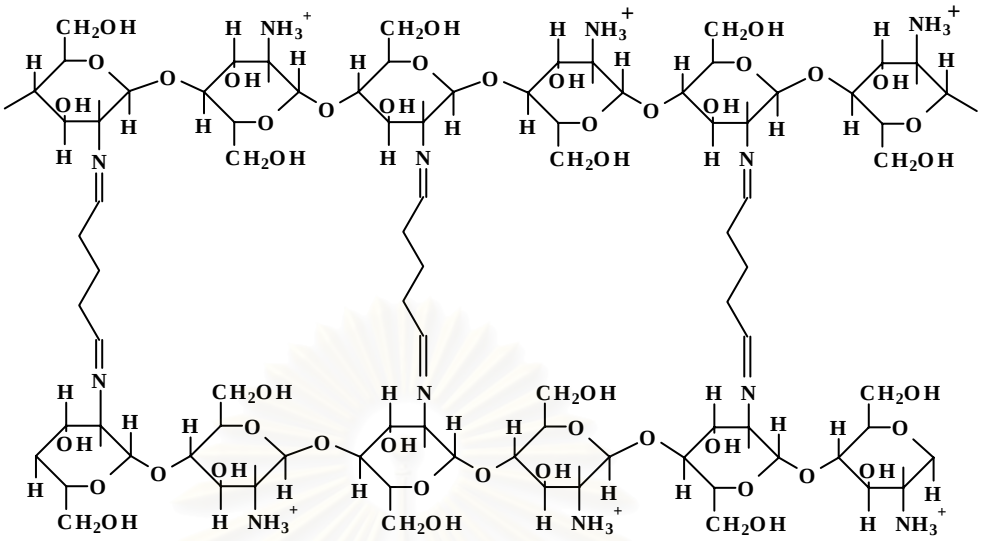


Figure 1.6 Chitosan crosslinked with glutaraldehyde.

b) High molecular weight compounds such as polyphosphoric acid (Figure 1.7). These crosslinking agents contain many active functional groups. Due to their long molecular structure, this type of crosslinking agents cannot diffuse very well. The crosslinking reaction with solid chitosan can thus only occur at the surface.

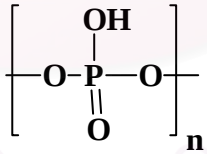


Figure 1.7 Chemical structure of polyphosphoric acid.

Chitosan can be crosslinked with polyphosphoric acid by the ionic interaction between positively charged ammonium groups and negatively charged counterion, phosphate groups. The macromolecular polyelectrolyte, polyphosphoric acid, reacted with chitosan through an interpolymer complex between $–[P_2O_5^{4-}]–$ and $–NH_3^+$ groups to form three-dimensional interpenetrating interpolymer complex networks (Figure 1.8).

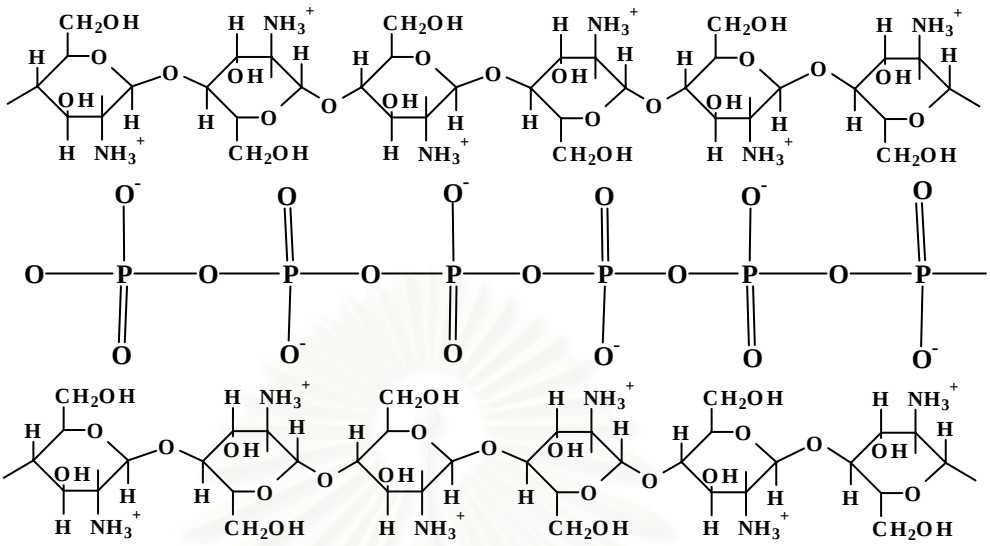


Figure 1.8 Ionic interaction of chitosan in polyphosphoric acid aqueous interpolymer complex.

1.4.5 Colloid Titration[8]

The colloid titration method is one way to estimate the net charge density of surfaces, polyelectrolytes, and colloidal materials in an aqueous mixture. What is actually measured is the capacity of the mixture to adsorp a polyelectrolyte of opposite net charge. In a titration of amino groups in chitosan, the chitosan was treated with excess acetic acid to produce polycationic polymer. A small amount of indicator (usually toluidine blue-O) is added to the solution of this polycationic polymer. The blue solution is titrated with an anionic polyelectrolyte such as potassium polyvinylsulfate (PVSK) to a purple-pink endpoint (Figure 1.9).

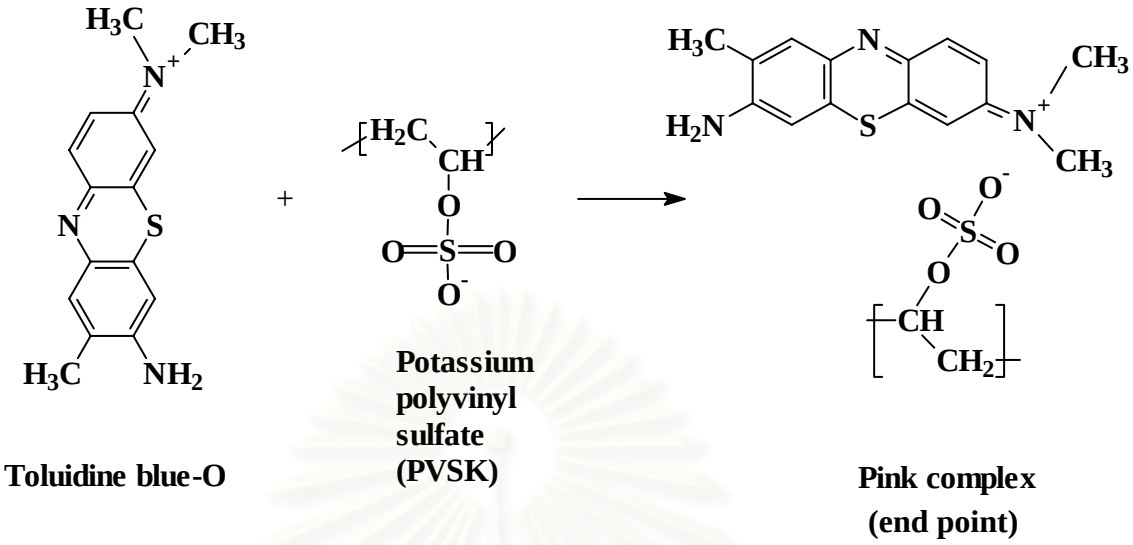
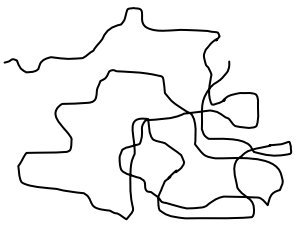


Figure 1.9 Colloid titration to color end point.

1.4.6 Intrinsic Viscosity[9]

The molecular weight is another important structural characteristic of polymer. The molecular weight of a polymer can be determinded by various methods such as light scattering, viscometry, chromatography and mass spectrometry. Among these, viscometry is the most simple and economical method for determination of molecular weight.

A polymer contains many single bonds, around which rotation is possible. If the configurations around successive carbon atoms are independent and unrelated, it will be seen that two parts of the polymer chain more than a few carbon atoms apart are essentially uncorrelated in regard to direction in space. The molecule is then “statistically coiled” and resembles a loose tangle of yarn:



When the polymers behave as statical coils, molecular weights of polymers are related with the intrinsic viscosity [η] according to Mark-Houwink equation,

$$[\eta] = K M_v^a \qquad (1.1)$$

where K and a are empirical parameters characteristic both of the polymer itself and of the solvent. For chitosan in 0.1 M acetic acid containing 0.2 M NaCl at 25 °C, K = 1.8×10^{-3} g/mL and a = 0.93.[10]

The intrinsic viscosity, denoted by [η], is defined as the ratio of the specific viscosity η_{sp} to the weight concentration of solute(C), in the limit of zero concentration, obtained from a Y-intercept in the plot of η_{sp}/C against C.

The defining equation of specific viscosity η_{sp} can be shown that

$$\eta_{sp} = \eta_r - 1$$

where η_r is the relative viscosity, which defined as the ratio of the viscosity of the solution to the viscosity of the solvent (η/η_0) or the ratio of the falling time of the solution to the falling time of solvent (t/t_0).

The molecular weight determination of a polymer by viscometry will give an accurate result when a polymer whose molecules are all of the same molecular weight is said to be monodisperse. Furthermore, the analysis should be done in low concentration solutions in order to decrease interaction between particles of polymer.

Viscosity of dilute polymer solutions are determined by using an Ubbelohde viscometer (Figure1.10) tube.

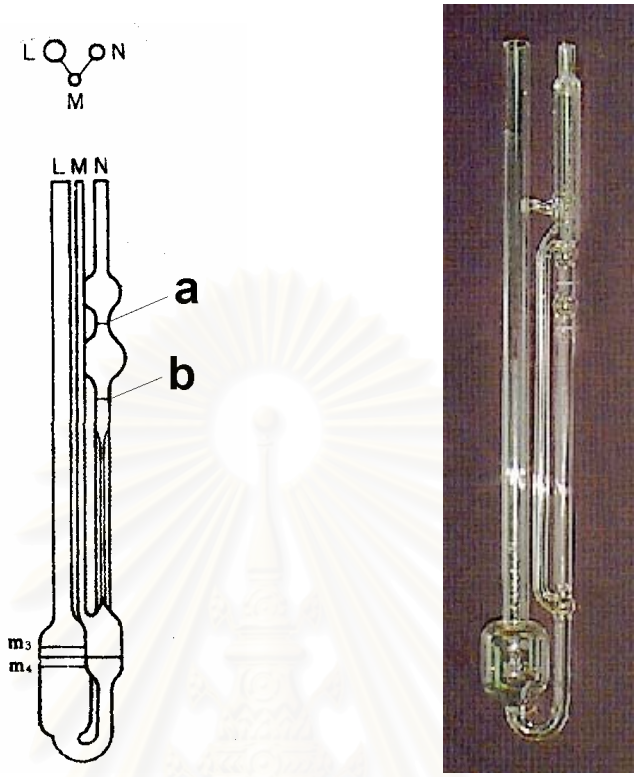


Figure 1.10 Ubbelohde viscometer.

1.5 Literature Review

a) Chemical modification of synthetic polymers with herbicides or herbicidal derivatives.

In 1997, S. W. Kim and co-workers[11] reported studies on the synthesis of 2,4-dichlorophenoxyacetyloxymethylstyrene (DOMS) by a reaction of the sodium salt of 2,4-D with *p*-chloromethylstyrene. DOMS was radically copolymerized with acrylamide (AM) to prepare a hydrophilic poly(DOMS-*co*-AM). From the hydrolysis of the copolymer, it was shown that the release of 2,4-D increased with increasing pH and temperature of the medium. In 1998, the same group of resarchers[12] reported the synthesis of herbicidal polymers by using 2,4-D, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), methyl chlorophenoxy acetic acid (MCPA) and 2-(2,4-dichlorophenoxy)

propionic acid (DCPP) herbicides. The herbicidal monomers were synthesized from the sodium salts of the herbicides and chloromethylstyrene. The herbicidal polymers were prepared by radically copolymerization of acrylamide with the herbicidal monomers. The release rates of the herbicides from the copolymers with about 10 mol% of the herbicidal monomers were controlled more effectively than those of the herbicides from the copolymers with about 5 mol% of the herbicidal monomers.

b) Chemical modification of naturally occurring polymers with herbicides or herbicidal derivatives.

In 1970, S. A. N. Neogi[13] studied system using a number of natural polymers. Kraft lignin and Douglas fir bark were reacted with MCPA to yield formulations with 38 and 51wt% of the herbicide. The reaction of 2,4-D with α-cellulose gave only 7% incorporation of the herbicide. All three systems were reported to have biological activity in lettuce seed germination studies.

In 1981, C. L. McCormick and co-workers[14] reported studies on the synthesis of polymeric herbicides from naturally occurring polysaccharides. Three different herbicides were combined with five polymeric systems, viz. amylose, amylopectin, cellulose, dextran and chitin. The isocyanate derivative of metribuzin and the acid chloride derivatives of 2,4-D were employed. Hydrolysis studies conducted by immersing the samples in an aqueous media and monitoring the aqueous phase by liquid chromatography. The results indicated that the hydrolysis rate greatly depended on the polymer types.

In 1993, J. Tefft and D. R. Friend[15] prepared polymeric microspheres (MSs) and tested for their ability to control the release of an herbicide, Dicamba (DA; 3,6-dichloro-2-methoxybenzoic acid). Microsphere were produced from ethyl cellulose (EtCell), polyarylsulfone (PS), or a combination of the two, in the desired size range of 20-40 μm by solvent evaporation. It was found that EtCell MSs released DA at a greater rate than did comparable PS MSs under sonication and soil column leaching tests. The release rate of DA from the EtCell MSs could be controlled by using

different viscosity grades (i.e., molecular weights) of EtCell. Higher viscosity EtCell led to a lower DA release rate. Microspheres containing EtCell/PS ratio of 1:9 release DA lower rate than the ratio of 1:4 and 2:3 respectively. Futhermore, PS MSs loaded at 18 wt% DA, release DA lower rate than PS MSs loaded at 30 wt% DA and EtCell MSs loaded at 16 wt% DA.

c) Examples of chitosan for controlled release of biological active compounds.

In 1981, P. Daver and J. P. Wightman[16] reported that among organochlorinated pesticides, only acidic pesticides, such as 2,4-D, 2,4,5-T, Dicamba, and MCPA, showed significant uptake on the chitosan.

In 1994, K. D. Yao and co-workers[17] studied the aqueous swelling kinetics of crosslinked chitosan with glutaraldehyde interpenetrating polyether hydrogels and the releasing of drug, chlorhexidini acetas from the semi-IPN discs. They reported that the fastest swelling rate was at pH = 1, then pH = 2-4.84; there was very limited swelling at pH ≥ 6. The release of drug, chlorhexidini acetas, from the semi-IPN discs increased with decreasing pH of the medium.

In 1997, S. S. Shyu and co-workers[18] prepared CM-chitin microsphere for sustained release of anticancer agent, 6-Mercaptopurine (6-MP). The anticancer agent was incorporated into the gel by the ionotropic crosslinking with ferric chloride. The release studies showed that drug release rate of the CM-chitin microsphere decreased with increasing concentration of iron(III)-chloride solution and the curing time. In addition, the release of 6-MP increased with decreasing pH of the medium. In 1999, the same group of resarchers[19] prepared chitosan-tripolyphosphate and chitosan-polyphosphoric acid gel beads using a polyelectrolyte complexation method for the sustained release of 6-MP. The release studies showed that the rate of releasing of 6-MP from the chitosan-tripolyphosphate and chitosan-polyphosphoric acid gel beads decreased when the pH of the medium was increased. Moreover, the rate of 6-MP

from gel beads matrix was significantly increased with the decreased molecular weight of chitosan.

In 1998, H. Yoshimichi and co-workers[20] prepared crop-selective herbicides by mixing a non-selective herbicidal component selected from among glyphosate, glyphosine, bialaphos and glufosinate with a second component selected from among phosphorous acid derivatives, chitosans, metal salts of isopropyl phosphate, metal salts of organic acids and so on, and, if necessary, a third component such as fungicide. By virtue of the second component, the herbicides do not completely kill weeds in spite of the action of the non-selective herbicidal component but can retard the growth of weeds, so that the herbicides are applicable to hillsides and useable as crop-selective herbicides reduced in the chemical damage to useful crops.

In 1999, A. Klaikherd and K. Sakunnee[21] prepared mixtures of chitosan and 2,4-D by mixing different ratios of chitosan to 2,4-D in solutions and cast into films. The release study showed that the films that had high ratio of the chitosan to 2,4-D controlled the releasing of 2,4-D for longer periods.

In 2000, A. Jaiyu and I. Limruangroj[22] used chitosan for controlled release of a herbicide, 2,4-D. Polyphosphoric acid and glutaraldehyde were utilized as crosslinking agents in the preparation of beads, films and fibers from chitosan and 2,4-D mixtures. The controlled release study indicated that films containing glutaraldehyde at 20% equivalent of chitosan can controlled release 2,4-D for about 8-10 days and lower loss of 2,4-D was also observed in the film and fiber preparation compared to the bead preparation.

In 2000, X. Shu and K. J. Zhu[23] prepared tripolyphosphate (TPP)/chitosan beads. Cross-section; analysis indicated that the beads had homogenous crosslinked structure, as a result the beads were strengthened greatly. Furthermore sodium alginate (a polyanion) can interact with cationic chitosan on the surface of these Tpp/chitosan beads to form polyelectrolyte complex film for the improvement of the

drug sustained release performances. The loading efficiency of model drugs (brilliant blue and FICT-dextran) in the beads was very high (more than 90%). Crosslinking time, TPP solution pH and other preparation factors had an effect on the drug release performance of beads.

In 2000, K. C. Gupta and M. N. V. Ravi Kumar[24] studied the *in vitro* release kinetics of diclofenac sodium (DFS) from chitosan beads and microgranules. The *in vitro* release profiles of DFS from chitosan beads and microgranules were monitored using UV-Vis spectrophotometer. The release rate of DFS from the beads had been found to be slower in comparison to the microgranules and amount of the drug release were much higher in acidic solution than in basic solution. In 2000, they[25] prepared semi-interpenetrating polymer network beads of chitosan and glycine, crosslinked with different concentrations of glutaraldehyde for controlled release of drugs, chlorphenramine maleate (CPM). The swelling studies, they reported that the degree of swelling was very high in solution of pH 2.0 compared to that of pH 7.4 and swelling rates of the crosslinked beads decreased when the concentration of glutaraldehyde solution was increased. The release studies, showed that the amount of and percentage of CPM release were much higher in acidic solution (pH 2.0) than in basic solution (pH 7.4), because the release rate depended on swelling of the beads. Furthermore, the release rate of CPM from chitosan beads decreased with increasing concentration of glutaraldehyde solution. In 2001, they[26] reported a preparation of semi-interpenetrating polymer network beads of chitosan and poly(ethylene glycol), crosslinked with different concentrations of glutaraldehyde, for studies on the controlled release of drugs and swelling behavior. It was found that the rate of swelling of the matrix and release of drugs was dependent on the degree of crosslinking and solution pH. The degree of swelling and release rate of drugs were very high in solution of pH 2.0 and they decreased with increasing concentration of glutaraldehyde solution. Moreover, the prepared beads showed 82% drug-loading capacity.

CHAPTER II

EXPERIMENTAL

2.1 Instruments and Apparatus

1. Fourier Transform Infrared Spectrophotometer (Impact 410, Nicolet, USA)
2. UV-Vis Spectrophotometer (Diode Array 8452A, Hewlett Packard)
3. Viscometer
4. Shaker (Metrohm 744)
5. Digital camera (Kodak DC 3400)
6. Optical microscope (Olympus B071, Japan)
7. Syringe filters (0.45 μm PTFE, Alltech)
8. Magnetic stirrers/ Magnetic bars
9. Beakers
10. Cylinders
11. Volumetric flasks
12. Pipets
13. Burettes
14. Stopwatch
15. Spatulas
16. Droppers
17. Heating bath
18. Round bottom flasks
19. Petri dishes
20. Columns
21. Syringes
22. Dessicator

2.2 Chemicals

1. Chitosan, Seafresh (Thailand), Koyo Chemical Co. Ltd. (Japan), Mahachai (Thailand), Ta Ming Enterprises (Thailand)
2. Glacial acetic acid, Analar grade, Merck, Germany
3. Sodium chloride, Analar grade, Merck, Germany
4. Sodium hydroxide, Analar grade, Merck, Germany
5. Methanol, Analar grade, Merck, Germany
6. Sodium carbonate, Analar grade, Carlo Erba, Itali
7. Potassium carbonate, Analar grade, AJAX Chemical, Australia
8. 2,4-Dichlorophenoxyacetic acid, Analar grade, Fluka, Switzerland
9. 3-Indoleacetic acid, Analar grade, Fluka, Switzerland
10. Glutaraldehyde solution, Analar grade, Fluka, Switzerland
11. Polyphosphoric acid, Analar grade, Fluka, Switzerland
12. Potassium polyvinyl sulfate solution, Analar, Wako, Japan
13. Cetyl pyridinium chloride, Analar grade, Kanto Chemical, Japan
14. 0.1% Toluidine blue, Analar grade, Chameleon Reagent, Japan

2.3 Procedures

2.3.1 Determination of Molecular Weight of Chitosan by Intrinsic Viscosity

Solvent: 0.1 M acetic acid and 0.2 M NaCl in water

Dried chitosan sample (ca, 25 mg or depending on molecular weight of chitosan) was weighed accurately in a 50 mL volumetric flask, dissolved in 1 M aqueous acetic acid solution (5 mL) and water (30 mL), and stirred with a magnetic stirrer overnight. To the solution 1 M aq. NaCl solution (10 mL) was added and stirred overnight, then made up the volume to the mark with distilled water (C_1). A portion of solution (10 mL) was taken and diluted with the above solvent (0.2 M

NaCl/0.1 M AcOH, 10 mL) (C_4). The temperature of a thermostatic water bath was maintained at 25 °C.

Chitosan	Weight (mg)	C (mg/mL)					
		C_1	C_2	C_3	C_4	C_5	C_6
Ta Ming	151.40	3.028	2.523	2.163	1.514	1.262	1.081
Mahachai	77.70	1.554	1.295	1.110	0.971	0.809	0.694
Seafresh	51.70	1.034	0.862	0.739	0.646	0.538	0.461
Koyo	38.00	0.760	0.633	0.543	0.217	0.181	0.155

Measurement of solvent viscosity: The solvent (0.2 M NaCl/0.1 M AcOH, 10 mL) was put into a Ubbelohde viscometer (Figure 2.1). The liquid was pushed up above the upper level (a) by a balloon pump. The time of falling between the upper (a) and lower mark (b) was measured by a stopwatch in triplicate.

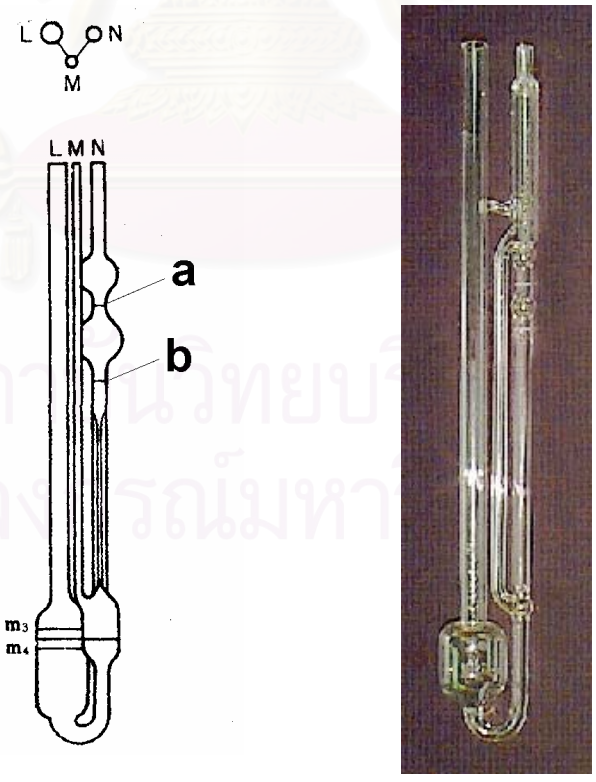


Figure 2.1 Ubbelohde viscometer.

Measurement of chitosan solution viscosity: The solution (C_1, 10 mL) was put into a viscometer. The falling time was measured by a stopwatch in triplicate. The solvent (2 mL) was added through tube L and mixed well to give C_2. The falling time was measured again. The solvent (0.2 M NaCl/0.1 M AcOH, 2 mL) was added again to give C_3 and the falling time was measured. The viscometer was rinsed with the solvent (15 mL), water (15 mL) and solution C_4 (15 mL). The solution C_4 (10 mL) was put into the viscometer. The falling time was measured as described above. The solution was diluted (C_5 and C_6) and the falling time was measured according to the procedure described for C_2 and C_3. Plot a graph of η_{sp}/C and against C, and draw a straight line using the linear least square. The intrinsic viscosity [η] was obtained from the Y-intercept. Viscosity-average molecular weight can be calculated as follows:

$$[\eta] = kM_v^a \quad (k = 1.8x10^{-3}, a = 0.93) \qquad (2.1)$$

2.3.2 Determination of the Degree of Deacetylation

By Using IR Method

Chitosan (20 mg) was dissolved in 0.1 M aqueous acetic acid (3 mL). The solution was cast on petri dish (D, 60 mm) and dried under atmosphere for several days. After drying, 0.1 M aqueous NaOH/methanol (1/1 v/v, 1-2 mL) was poured onto the dried films. The films were peeled off and washed with water/methanol (1/1 v/v) to the neutral. (The films are very thin, so take care on handling.) The wet films were dried in a desicator. The IR spectrum was recorded and the ratio of the peak area of the absorption bands at 1655 and 2867 cm^{-1} (A_{1655}/A_{2867}) were compared against the calibration curve (Figure 2.2).[27]

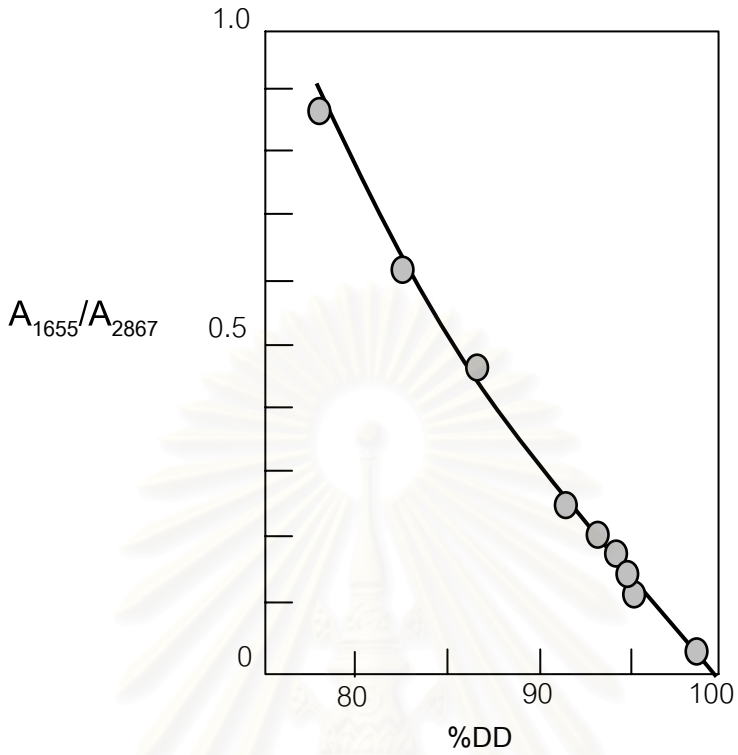


Figure 2.2 Calibration curve for degree of deacetylation using the (A_{1655}/A_{2867}).

By using Colloid Titration Method

At first the solution of potassium polyvinyl sulfate solution (PVSK) must be titrated to determine the concentration. Cetyl pyridinium chloride (CPC, 12.5 mg) was weighed precisely into a 25 mL volumetric flask and the volume was made up to the mark with 0.1 M aqueous acetic acid solution. The solution (precise 5 mL) was placed in a 25 mL beaker, three drops of 0.1% toluidine blue were added as an indicator, and the mixture was continuously stirred with a magnetic bar. The PVSK solution was added titrimetrically from a burette until the blue color of the indicator changed into reddish purple. The titration was repeated three times. In a blank titration, 0.1 M aqueous acetic acid solution was used in place of the CPC solution. The other procedures are the same as the above.

For the determination of the concentration of PVSK (N, equiv./L):

$$N = 50\,C' / 358\,D \quad (2.2)$$

C′ : concentration of CPC (%)

D : difference between CPC and blank titration volumes (mL)

Dried chitosan (8.33 mg) was weighed precisely in a 25 mL volumetric flask and the volume was made up to the mark with 0.1 M aqueous acetic acid solution. The solution (precise 5 mL) was pipetted and placed in a 25 mL beaker and titrated as described above.

For the determination of the degree of N-acetylation (DA):

$$DA\ (\%) = 100x(50C-161ND)/(42ND+50C) \quad (2.3)$$

N : concentration of PVSK (equiv./L)

D : difference between chitosan and blank titration volumes (mL)

C : concentration of chitosan (%)

For the determination of the degree of deacetylation (DD):

$$DD\ (\%) = 100-DA\ (\%) \quad (2.4)$$

2.3.3 Preparation of Calibration Curve of 2,4-D

Calibration Curve for 2,4-D Assaying

2,4-D (6 mg and 10 mg) were weighed into a 50 mL volumetric flask and a 100 mL volumetric flask. The volume was made up to the mark with aqueous acetic acid solution (5% v/v) to obtain 120 ppm and 100 ppm stock solutions.

Various volumes of 100 ppm stock solution were pipetted into a 25 mL volumetric flask. Aqueous acetic acid solution (5% v/v) was added to the volume mark (Table 2.1). The absorbance of each solution was measured by using UV

spectrophotometer at 284 nm. A calibration line was obtained by plotting the absorbance (A) against the concentration of 2,4-D.

Table 2.1 Preparation of standard solution of 2,4-D in aqueous acetic acid (5% v/v).

Concentration of 2,4-D (ppm)	**Volumes of 100 ppm Stock Solution (mL)**	**Total Volume (mL)**
80	20	25
60	15	25
40	10	25
20	5	25
10	2.5	25

Calibration Curve of 2,4-D for Releasing Studies

2,4-D (6 mg and 10 mg) were weighed into a 50 mL volumetric flask and a 100 mL volumetric flask. The volume was made up to the mark with distilled water to obtain 120 ppm and 100 ppm stock solutions.

Various volumes of 100 ppm stock solution were pipetted into a 25 mL volumetric flask. Distilled water was added to the volume mark (Table 2.2). The absorbance of each solution was measured by using UV spectrophotometer at 284 nm. A calibration line was obtained by plotting the absorbance (A) against the concentration of 2,4-D.

Table 2.2 Preparation of standard solution of 2,4-D in distilled water.

Concentration of 2,4-D (ppm)	Volumes of 100 ppm Stock Solution (mL)	Total Volume (mL)
80	20	25
60	15	25
40	10	25
20	5	25
10	2.5	25

2.3.4 Preparation of Calibration Curve of IAA

Calibration curve for Assaying

IAA (25 mg) was weighed into a 500 mL volumetric flask. The volume was made up to the mark with aqueous acetic acid (5% v/v) to obtain a 50 ppm stock solution.

Various volumes of 50 ppm stock solution were pipetted into a volumetric flask. Aqueous acetic acid (5% v/v) was added to the volume mark (Table 2.3). The absorbance of each solution was measured by using UV spectrophotometer at 280 nm. A calibration line was obtained by plotting the absorbance (A) against the concentration of IAA.

Table 2.3 Preparation of standard solution of IAA in aqueous acetic acid (5% v/v).

Concentration of IAA (ppm)	Volumes of 50 ppm Stock Solution (mL)	Total Volume (mL)
30	15	25
25	20	50
20	10	25
15	15	50
10	5	25
5	5	50
2.5	5	100

Calibration curve for Releasing Studies

IAA (25 mg) was weighed into a 500 mL volumetric flask. The volume was made up to the mark with distilled water to obtain a 50 ppm stock solution.

Various volumes of 50 ppm stock solution were pipetted into a volumetric flask. Distilled water was added to the volume mark (Table 2.4). The absorbance of each solution was measured by using UV spectrophotometer at 280 nm. A calibration line was obtained by plotting the absorbance (A) against the concentration of IAA.

Table 2.4 Preparation of standard solution of IAA in distilled water.

Concentration of IAA (ppm)	Volumes of 50 ppm Stock Solution (mL)	Total Volume (mL)
30	15	25
25	20	50
20	10	25
15	15	50
10	5	25
5	5	50
2.5	5	100

2.3.5 Preparation of Chitosan Beads Containing 2,4-D

Chitosan was dissolved in aqueous acetic acid solution (1% v/v) under stirring with a magnetic bar for 24 h at room temperature to prepare a stock solution of the chitosan with possible maximum concentration. Types of chitosan used for preparation of beads were obtained from Seafresh (Thailand), Mahachai (Thailand), and Koyo Chemical Co. Ltd. (Japan). The concentrations of solution are shown in Table 2.5.

Table 2.5 The concentration of stock solution of various chitosan types.

Chitosan Source	Concentration (% w/v)
Seafresh	2
Mahachai	3
Koyo Chemical	1

The solution of chitosan was mixed with solution of 2,4-D. The solution of 2,4-D was prepared by dissolving 2,4-D in sodium carbonate solution (1.5 % w/v). A mixture of chitosan and 2,4-D was formed for the beads by non-crosslinking, surface crosslinking and bulk crosslinking methods. The appropriate condition was investigated by varying the parameters.

Non-Crosslinked Beads

A mixture of chitosan and 2,4-D was dropped into a potassium carbonate solution (1.5 M, 200 mL) to form uncrosslinked beads. The uncrosslinked beads were filtered and washed by stirring beads in distilled water for 30 seconds (100 x 2 mL), then dried under vacuum. The dried beads were analyzed for the 2,4-D content and studied for releasing of 2,4-D. Chitosan from different sources were used in the bead preparation under varied conditions (Table 2.6).

Table 2.6 Conditions used in the preparation of non-crosslinked beads.

No.	Chitosan Source	Ratio of Chitosan:2,4-D (w/w)	Temp of Coagulant
1	Seafresh	5:1	room temp
2	Seafresh	1:1	room temp
3	Seafresh	1:1	started at 8 ^{o}C
4	Mahachai	5:1	room temp
5	Mahachai	1:1	room temp
6	Mahachai	1:1	started at 8 ^{o}C
7	Koyo	5:1	room temp

Surface-Crosslinked Beads

Glutaraldehyde and polyphosphoric acid were utilized as crosslinking agents in the preparation of surface-crosslinked beads from the solution of chitosan and 2,4-D mixtures.

The glutaraldehyde-crosslinked beads were prepared by putting uncrosslinked beads, prepared as previously described, in a glutaraldehyde solution (5% w/v, 100 mL) for 5 minutes. The polyphosphoric acid-crosslinked beads were prepared by direct dropping of the chitosan-2,4-D mixture into the various concentrations of polyphosphoric acid solution (Table 2.5). The beads were filtered and washed by stirring beads in distilled water for 30 seconds (100 x 2 mL), then dried under vacuum. The 2,4-D content and the releasing profile of 2,4-D were determined. Chitosan from different sources were used in the bead preparation under varied conditions (Table 2.7).

Table 2.7 Conditions used in the preparation of surface-crosslinked beads.

No.	Chitosan Source	CA	Ratio of the Chitosan:2,4-D (w/w)	Temp of Coagulant	Coagulant
1	Seafresh	GA	5:1	room temp	1.5 M K_2CO_3
2	Seafresh	GA	1:1	room temp	1.5 M K_2CO_3
3	Seafresh	GA	1:1	started at 8 ^oC	1.5 M K_2CO_3
4	Seafresh	PPA	1:1	room temp	5% (w/v) PPA
5	Seafresh	PPA	1:1	started at 8 ^oC	5% (w/v) PPA
7	Seafresh	PPA	5:1	room temp	5% (w/v) PPA
8	Seafresh	PPA	5:1	room temp	5% (w/v) PPA
9	Seafresh	PPA	5:1	room temp	5% (w/v) PPA
10	Mahachai	GA	5:1	room temp	1.5 M K_2CO_3
11	Mahachai	GA	1:1	room temp	1.5 M K_2CO_3
12	Mahachai	GA	1:1	started at 8 ^oC	1.5 M K_2CO_3
13	Mahachai	PPA	5:1	room temp	5% (w/v) PPA
14	Mahachai	PPA	1:1	room temp	5% (w/v) PPA
15	Mahachai	PPA	1:1	started at 8 ^oC	5% (w/v) PPA

Bulk-Crosslinked Beads

Glutaraldehyde and polyphosphoric acid were utilized as crosslinking agents in the preparation of bulk-crosslinked beads from solution of chitosan and 2,4-D mixtures (5:1 w/w).

The glutaraldehyde-crosslinked beads were prepared by mixing the glutaraldehyde solution (0.56 M) at 2, 4 and 6% equivalent of chitosan (Table 2.7). The mixtures were stirred for the various time (Table 2.8) and dropped into potassium carbonate solution (1.5 M, 100 mL) at room temperature. The polyphosphoric acid-crosslinked beads were prepared by addition of polyphosphoric acid solution (0.53 M,

0.35 mL) into a mixture of chitosan and 2,4-D. This mixture was stirred and dropped into polyphosphoric acid solution (5% w/v, 100 mL). The beads were filtered and washed by stirring beads in distilled water for 30 seconds (100 x 2 mL), then dried under vacuum. The 2,4-D content and the releasing profile of 2,4-D were determined. Chitosan from different sources were used in the bead preparation under varied conditions (Table 2.8).

Table 2.8 Conditions used in the preparation of bulk-crosslinked beads.

No.	Chitosan Source	CA	Concentration of CA	Crosslinking Time (min)
1	Seafresh	GA	2% eq	5, 15, 30, 60
2	Seafresh	GA	4% eq	5
3	Seafresh	GA	6% eq	5
4	Seafresh	PPA	5% (w/v)	5
5	Mahachai	GA	2% eq	5
6	Mahachai	GA	4% eq	5
7	Mahachai	GA	6% eq	5
8	Ta Ming	GA	2% eq	5
9	Ta Ming	GA	4% eq	5
10	Ta Ming	GA	6% eq	5

2.3.6 Preparation of Chitosan Beads Containing IAA

IAA (95 mg) was weighed and dissolved in water (4 mL) and mixed with the solution of chitosan in acetic acid (1% v/v, 30 mL). The mixture was dropped into a potassium carbonate solution (1.5 M, 100 mL) to form the uncrosslinked beads. The glutaraldehyde-crosslinked beads were prepared by mixing the glutaraldehyde solution (0.56 M, 0.06 mL) with the chitosan-IAA mixture. This mixture was stirred for 5 minutes and dropped into a potassium carbonate solution (1.5 M, 100 mL). The

polyphosphoric acid-crosslinked beads were prepared by direct dropping of the chitosan-IAA mixture into polyphosphoric acid solution (5% w/v, 100 mL). The beads were filtered and washed by stirring beads in distilled water for 30 seconds (100 x 2 mL), then dried under vacuum. The IAA content and the releasing profile of IAA were determined.

2.4 Characterization of Chitosan Beads

2.4.1 Swelling Studies

The water sorption capacity of uncrosslinked beads, glutaraldehyde-crosslinked beads and polyphosphoric acid-crosslinked beads were determined by swelling the beads in distilled water at room temperature. A known weight (0.1 g) of various chitosan beads without 2,4-D (or IAA) were placed into vial containing distilled water (10 mL) for the required period of time. The wet weight of the swollen beads were determined by first blotting the beads with filter paper to remove excess water on the surface and then weighed immediately on an electronic balance. The degree of swelling for each sample at time *t* was calculated by using the following expression:

$$(W_t - W_0) / W_0 \qquad (2.7)$$

where W_t and W_0 are the weights of the beads at time *t* and in the dry state, respectively. Each swelling experiment was repeated two times, and the average value was taken as the degree of swelling value.

2.4.2 Determination of 2,4-D (or IAA) Content

Chitosan beads were weighed and placed in a mortar and triturated thoroughly, then dissolved in 100 mL of aqueous acetic acid solution (5% v/v). After

stirred the mixture at room temperature for 24 h, the mixture was filtered through a syringe filter (0.45 μm, PTFE). The 2,4-D (or IAA) was assayed by using UV spectrophotometer detected at 284 nm (or 280 nm for IAA) and calculated for concentration of 2,4-D (or IAA) by using a calibration curve. The percentage of 2,4-D (or IAA) content and the percent recovery of 2,4-D (or IAA) were calculated from the following expression (2.5) and (2.6), respectively:

$$\text{2,4-D (or IAA) content} = \frac{\text{weight of 2,4-D (or IAA) (g)}}{\text{weight of beads (g)}} \times 100 \qquad (2.5)$$

$$\text{Recovery of 2,4-D (or IAA)} = \frac{\text{weight of 2,4-D (or IAA) in the beads}}{\text{starting weight of 2,4-D (or IAA) in the solution}} \times 100 \qquad (2.6)$$

2.4.3 The 2,4-D (or IAA) Release Studies

Dissolution Test

The dissolution medium was distilled water (500 mL). The medium was placed in a 1 L flask and shaked with a shaker at a rate of 60 rev./min. The dissolution medium temperature was maintained at room temperature. Chitosan beads were dispersed in the disssolution medium, after a predetermined period, 5 mL of the medium was removed, filtered and the amount of 2,4-D (or IAA) was analyzed spectrophotometrically at 284 nm (or 280 nm for IAA). The released amount of 2,4-D (or IAA) was calculated by using calibration curves. The percentage of the releasing of 2,4-D (or IAA) was plotted against time (day).

Soil Column Leaching Test

The soil leaching studies were performed in column (15 cm deep and 4 cm in diameter). The soil used was a mixture of course sand and fine sand. The sand

was placed in the column (3 cm deep). Chitosan beads were spread evenly on the top of the column. The amount of irrigation of water was placed on the column: 30 mL. The water passing through the column was collected and analysis for the released 2,4-D (or IAA) by UV spectrophotometer. All the water passing through the column was collected before the next irrigation was applied.

2.4.4 Determination of Crosslinked of Beads by IR Spectra Analysis

IR spectra of the glutaraldehyde-crosslinked beads and polyphosphoric acid-crosslinked beads were recorded on KBr pellets by using a Impact 410 Fourier Transform Infrared Spectrophotometer at 200-350 cm^{-1} scanning ranges.

2.4.5 Determination of Size and Surface Morphology of Beads by Optical Microscope

Size and surface morphology of beads were examined by using optical microscope (Olympus B071, Japan).

CHAPTER III

RESULTS AND DISCUSSION

3.1 Determination of Molecular Weight of Chitosan by Viscometry

The molecular weights of the chitosan from four differenct sources, Seafresh Company (Thailand), Koyo Chemical Co. Ltd. (Japan), Mahachai District (Thailand) and Ta Ming Enterprise (Thailand) were determined by viscometry in solutions of 0.2 M NaCl/ 0.1 M AcOH at 25 °C (Table 3.1). The falling times in a Ubbelohde tube were recorded (Tables A1-A4). The intrinsic viscosities [η] (Table 3.1) were obtained from the Y-intercept of the plots of η_{sp} /C against C using linear least square (Figure 3.1, see also Appendix C).

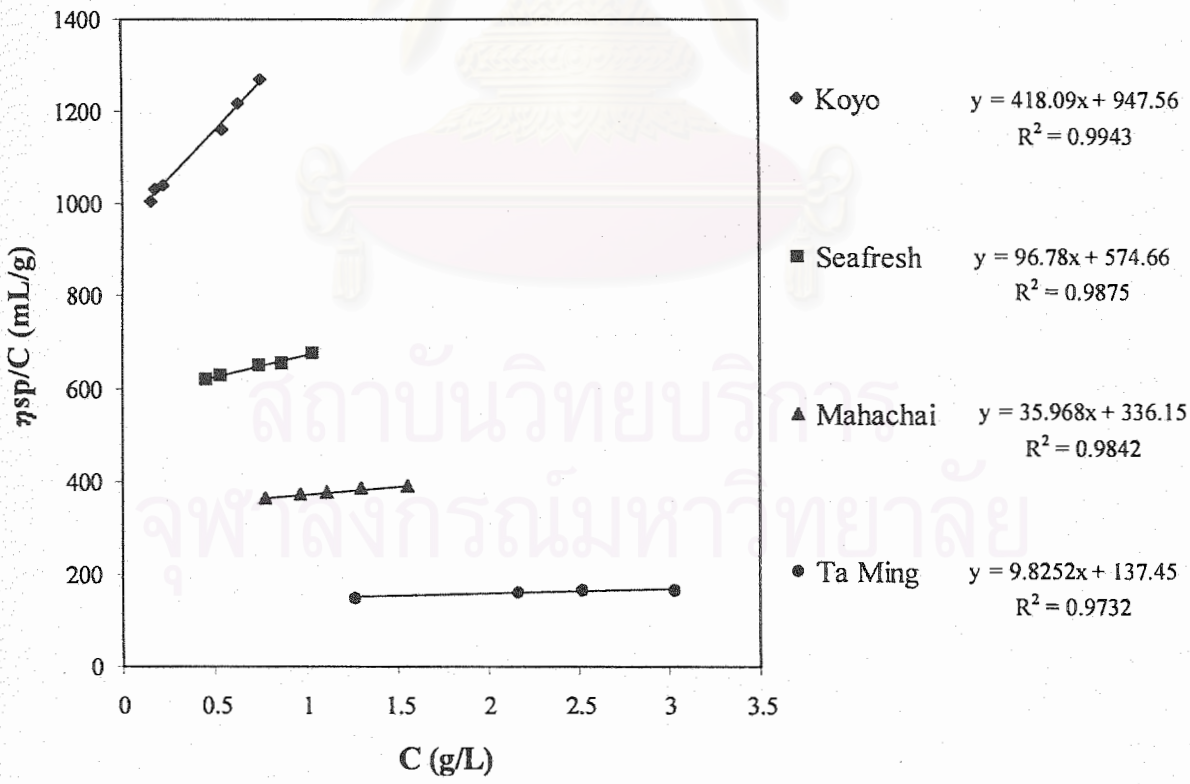


Figure 3.1 Plots of η_{sp}/C against C and molecular weight of the various types of chitosan.

Table 3.1 Molecular weights of chitosan calculated from $[\eta] = KM_v^a$ ($K = 1.8x10^{-3}$, $a = 0.93$).

Chitosan	$[\eta]$	log M_v	M_v
Ta Ming	137.5	5.251	178,062
Mahachai	336.2	5.668	465,684
Seafresh	574.7	5.918	828,821
Koyo	947.6	6.152	1,419,031

Chitosan from Ta Ming Enterprise had the lowest molecular weight, while chitosan from Koyo Chemical Co. Ltd. had the highest molecular weight. It is also worth noting here that the plots of η_{sp}/C against C of the chitosan with higher molecular weights gave better correlation factors (R^2) than those of the chitosan with lower molecular weights.

3.2 Determination of the Degree of Deacetylation

The degrees of deacetylation were determined by using infrared spectroscopy and colloid titration methods. The IR spectra were recorded (Figures B1-B3) and the degrees of deacetylation were determined from the ratio of the area (A_{1655}/A_{2867}) of the absorption bands at 1655 and 2867 cm^{-1} (Table A5) using the literature calibration curve (Figure 2.2).[27] In the colloid titration method, the PVSK solution was used to titrate the chitosan solution (Table A7) and the degree of deacetylation was calculated from the equation (2.3) and (2.4).

Table 3.2 Degree of deacetylation (%DD) of chitosan determined from IR method and colloid titration.

Chitosan Sources	%DD	
	(IR)	(Colliod Titration)
Ta Ming	-	87
Mahachai	84	66
Seafresh	89	84
Koyo	85	79

Chitosan from Ta Ming Enterprise and Seafresh Company had comparable degrees of deacetylation, while chitosan from Mahachai had relatively low degree of deacetylation. However, there was significant difference in the degree of deacetylation of Mahachai chitosan obtained from IR method and colloid titration. The lower molecular weight of Mahachai chitosan caused the prepared film to be too thick to get a good IR spectrum. The IR signals appeared rather broad resulting in inaccurate integration. The lowest molecular weight of Ta Ming chitosan prevented any film preparation suitable for the IR method.

3.3 Preparation of Calibration Curve

Two calibration lines for 2,4-D, one in water and another in acetic acid solutions, were required for assaying and releasing studies. The UV-Vis absorption of standard 2,4-D solutions was measured at 284 nm (Tables A8-A9) to give linear calibration lines (Figure 3.2 and Figure 3.3). For IAA, the calibration lines were obtained from acetic acid and aqueous solutions (Figure 3.4 and Figure 3.5). The UV-Vis absorption of standard IAA solutions was measured at 280 nm (Tables A10-A11).

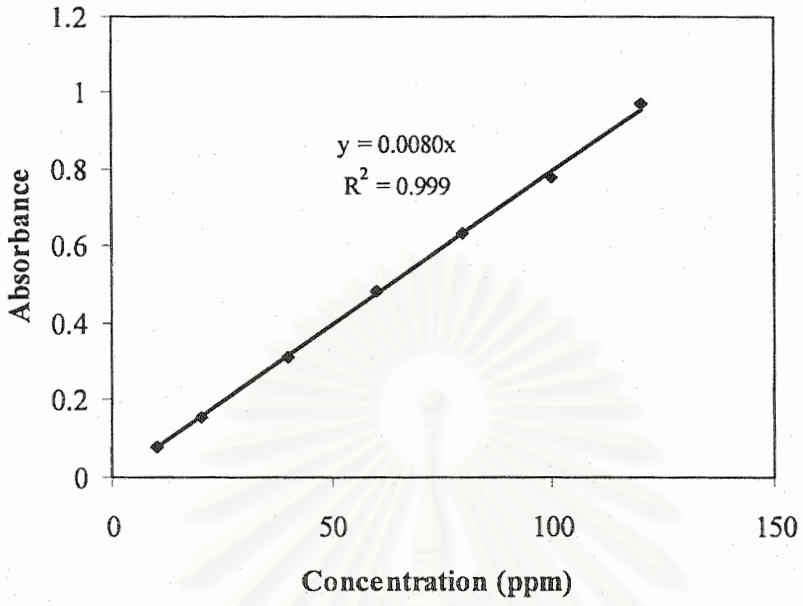


Figure 3.2 Calibration curve of 2,4-D in 5% (v/v) acetic acid for assaying.

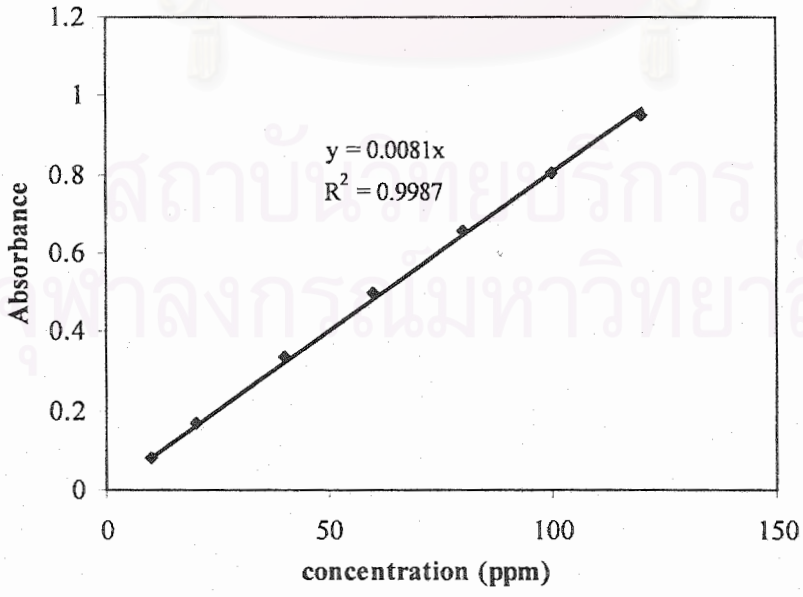


Figure 3.3 Calibration curve of 2,4-D in water for releasing studies.

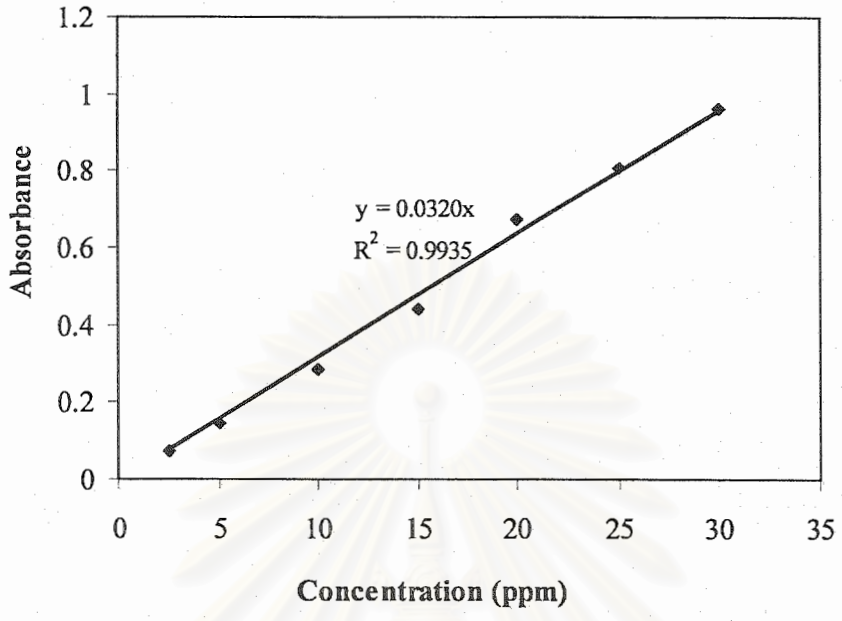


Figure 3.4 Calibration curve of IAA in 5% (v/v) acetic acid for assaying.

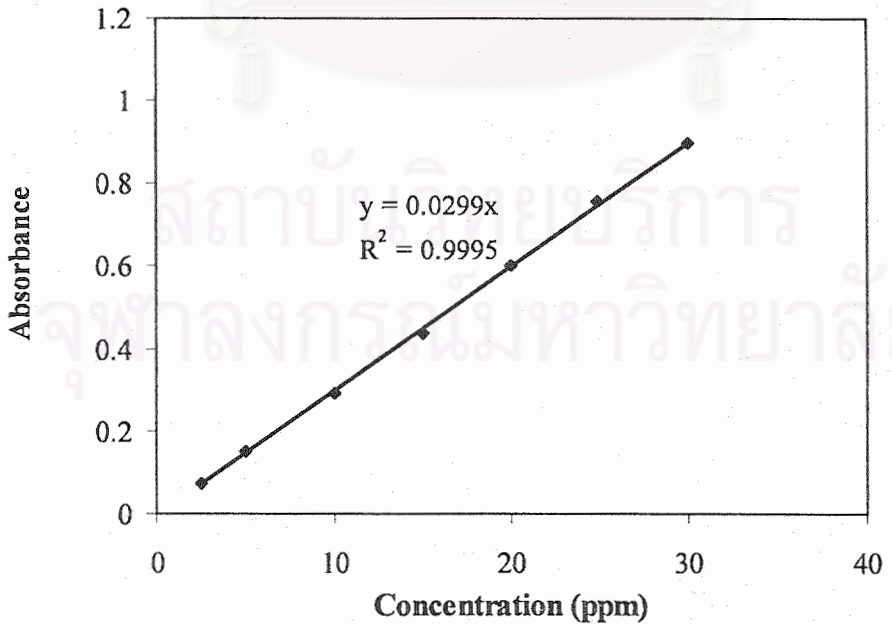


Figure 3.5 Calibration curve of IAA in water for releasing studies.

3.4 Preparation of Chitosan Beads Containing 2,4-D

In this study, the chitosan beads containing 2,4-D were prepared by using glutaraldehyde and polyphosphoric acid as crosslinking agents. The parameter variation including types of coagulants, chitosan sources, types of crosslinking agents and techniques of crosslinking, temperature of coagulants, ratio of chitosan to 2,4-D, molecular weight of chitosan, crosslinking time and concentration of the crosslinking agents, were studied. The prepared beads were assayed for the 2,4-D content and studied for the releasing profile of 2,4-D by using UV spectrophotometer. The swelling behavior of these beads were also investigated. FT-IR spectroscopy was used to obtain the nature of crosslinking and interaction between 2,4-D and chitosan. The sizes and surface morphology of beads were characterized by optical microscope.

3.4.1 Effect of Types of Coagulants

The experiment was started out by searching for suitable conditions for bead formation. A mixture of chitosan and 2,4-D in the ratio of 5:1 was dropped into various coagulants. The appearance of beads prepared from different coagulants was recorded (Table 3.3).

Table 3.3 Appearance of beads containing chitosan:2,4-D (5:1 w/w) prepared from different coagulants.

Coagulants	Bead Appearance*
10% NaOH	broken
5% NaOH	broken
5% NaOH + Methanol	broken
Buffer KH_2PO_4 + NaOH (pH = 8)	not coagulated
Buffer Na_2HPO_4 + NaOH (pH = 11)	not coagulated
1 M K_2CO_3	broken
1.5 M K_2CO_3	spherical

* see samples of bead picture in appendix B4-B5

The preparation of chitosan:2,4-D mixture beads was not as simple as it was originally thought out. The use of well known coagulant such as NaOH (aq) and NaOH (aq)/ CH_3OH gave only beads with broken shapes. These results may be attributed to the strong basicity of sodium hydroxide that promoted the diffusion of 2,4-D from the beads into the coagulants. However, when the buffer pH = 8 and pH = 11 were used, the beads could not be formed at all. Fortunately, beads with minimal defects could be formed by using potassium carbonate solution (1.5 M, pH = 13) as a coagulant.

3.4.2 Effect of Chitosan Sources on Bead Preparation

Chitosan used for preparation of beads were obtained from different sources, Seafresh Company (Thailand), Mahachai District (Thailand), and Koyo Chemical Co. Ltd. (Japan). The beads were prepared by dropping a solution of chitosan and 2,4-D (5:1 w/w) into potassium carbonate solution (1.5 M) at room temperature to form beads. The bead appearance was recorded and the percent recovery of 2,4-D was determined (Table 3.4).

Table 3.4 Effect of chitosan sources on bead preparation[a]

Chitosan Source	Bead Appearance*	% Recovery of 2,4-D
Seafresh	spherical	31
Mahachai	spherical	29
Koyo	broken	-

[a] Beads were prepared from solution with [chitosan] = 108.9, 163.4 and 54.5 mM for Seafresh, Mahachai and Koyo, respectively; [AcOH] = 153.5 mM; [2,4-D] = 16.0, 24.0 and 8.0 mM for Seafresh, Mahachai and Koyo, respectively.

* see samples of bead picture in appendix B6-B7

In this experiment, the saturated solution of each chitosan in 1% acetic acid was used. These saturated solutions had different concentrations in terms of molarity. Beads prepared from Koyo chitosan were not stable and broken during

washing. The poor bead formation might be attributed to the low concentration of this saturated chitosan solution due to its high molecular weight. While, beads prepared from Seafresh and Mahachai chitosan had spherical shape. The amount of 2,4-D left in the beads were however only around 30% of the amount of 2,4-D originally presented in the solution indicating significant loss of 2,4-D during the bead preparation.

3.4.3 Effect of Crosslinking Agent and Techniques of Crosslinking on Bead Preparation

In order to minimize the loss during the preparation of 2,4-D, glutaraldehyde and polyphosphoric acid were utilized as crosslinking agents in the preparation of crosslinked beads. First, the glutaraldehyde-crosslinked beads were prepared by dropping the chitosan-2,4-D mixtures (5:1 w/w) into various concentration of glutaraldehyde solutions (10-40% v/v) but the beads could not be formed at all. Therefore, they were prepared by immersing the uncrosslinked beads (prepared as described in section 3.4.2) in a glutaraldehyde solution (5% v/v for 5 minutes) for surface crosslinking and by addition of glutaraldehyde solution directly into the mixture of chitosan and 2,4-D (5:1 w/w) before being coagulated in potassium carbonate solution (1.5 M) for bulk crosslinking. The polyphosphoric acid-crosslinked beads were prepared by direct dropping of the solution of the chitosan-2,4-D mixture (5:1 w/w) into polyphosphoric acid solution (5% w/v) for surface crosslinking and by addition of polyphosphoric acid solution directly into the mixture of chitosan and 2,4-D before being coagulated in a more concentrated polyphosphoric acid solution (5% w/v) for bulk crosslinking. All the beads were prepared at room temperature.

Table 3.5 Effect of crosslinking agents and techniques of crosslinking on bead preparation[a]

Chitosan Source	CA	Techniques of Crosslinking	Bead Appearance*	% Recovery of 2,4-D
Seafresh	-	none	spherical	31
	GA	surface	spherical	21
		bulk	spherical	46
	PPA	surface	flatten	48
		bulk	flatten	35
Mahachai	-	none	spherical	29
	GA	surface	spherical	24
		bulk	spherical	39
	PPA	surface	flatten	69

[a] Beads were prepared from solution with [chitosan] = 108.9 and 163.4 mM for Seafresh and Mahachai, respectively; [AcOH] = 153.5 mM; [2,4-D] = 16.0 and 24.0 mM for Seafresh and Mahachai, respectively.

* see samples of bead picture in appendix B6-B14

The use of polyphosphoric acid as a coagulant and crosslinking agent increased percent recovery of 2,4-D (Table 3.5). The polyphosphoric acid-crosslinked beads prepared by the surface crosslinking loss 2,4-D during the bead preparation lower than the beads prepared by the bulk crosslinking. On the other hand, in case of glutaraldehyde-crosslinked beads, the bulk crosslinking was more efficient than the surface crosslinking in maintaining 2,4-D inside the beads. The glutaraldehyde-crosslinked beads prepared by the surface crosslinking gave lower percent recovery of 2,4-D comparing to the non-crosslinked beads. The lower percent recovery of 2,4-D in the beads crosslinked with glutaraldehyde by surface crosslinking can be attributed to the loss of 2,4-D during the immersion in the glutaraldehyde solution.

Another interesting observation in this experiment was the shape of the prepared beads. Polyphosphoric acid caused some deformation of the spherical beads

into flatten round beads. This deformation might occur in oder to maximize the ionic interaction between the polymeric chains of chitosan and polyphosphoric acid.

3.4.4 Effect of Temperature of Coagulants on Bead Preparation

The effect of the temperature of the coagulants was carried out by preparing the beads in cold coagulants (8 °C) from the solution of chitosan and 2,4-D (1:1 w/w) in aqueous acetic acid (1% v/v). The uncrosslinked beads were prepared by dropping the solution of chitosan and 2,4-D into a potassium carbonate solution (1.5 M). The glutaraldehyde and polyphosphoric acid crosslinked beads were prepared by the surface crosslinking technique.

Table 3.6 Effect of temperature of coagulants on bead preparation[a]

Chitosan Source	CA	Temp of Coagulant Solutions	Bead Appearance	% Recovery of 2,4-D
Seafresh	-	room temp	flatten	15
		started at 8 °C	flatten	11
	GA	room temp	spherical	8
		started at 8 °C	spherical	3
	PPA	room temp	flatten	42
		started at 8 °C	flatten	36
Mahachai	-	room temp	spherical	21
		started at 8 °C	spherical	18
	GA	room temp	spherical	9
		started at 8 °C	spherical	5
	PPA	room temp	flatten	51
		started at 8 °C	flatten	50

[a] Beads were prepared from solution with [chitosan] = 74.1 and 111.1 mM for Seafresh and Mahachai, respectively; [AcOH] = 104.4 mM; [2,4-D] = 54.3 and 81.4 mM for Seafresh and Mahachai, respectively.

The percent recoveries of 2,4-D in the beads prepared by using cold coagulants slightly lower than those in the beads prepared by using room temperature coagulants in all cases (Table 3.6). Therefore the room temperature was the preferred condition for bead preparation. The clear explanation for higher lost of 2,4-D in the cold coagulants remains elusive to us. The slower rate of coagulation of chitosan could be one possible reason.

3.4.5 Effect of Ratio of Chitosan:2,4-D on Bead Preparation

Two chitosan:2,4-D ratios, 1:1 (w/w) and 5:1 (w/w), were used in the preparation of the uncrosslinked beads, glutaraldehyde-crosslinked beads and polyphosphoric acid-crosslinked beads. The uncrosslinked beads were prepared by dropping solution of chitosan and 2,4-D into potassium carbonate solution (1.5 M). The glutaraldehyde and polyphosphoric acid crosslinked beads were prepared by the surface crosslinking technique. All the beads were prepared at room temperature.

Table 3.7 Effect of ratio of chitosan:2,4-D on bead preparation[a]

Chitosan Source	CA	Ratio of Chitosan:2,4-D (w/w)	Bead Appearance*	% Weight Content of 2,4-D	% Recovery of 2,4-D
Seafresh	-	1:1	flatten	6.0	15
		5:1	spherical	2.3	31
	GA	1:1	spherical	5.0	8
		5:1	spherical	2.3	21
	PPA	1:1	flatten	25.1	42
		5:1	flatten	5.3	48
Mahachai	-	1:1	spherical	7.0	21
		5:1	spherical	2.8	29
	GA	1:1	spherical	4.1	9
		5:1	spherical	2.8	24
	PPA	1:1	flatten	25.6	51
		5:1	flatten	8.2	69

[a] For a 1:1 ratio the beads were prepared from solution of [chitosan] = 74.1 and 111.1 mM for Seafresh and Mahachai, respectively; [AcOH] = 104.4 mM; [2,4-D] = 54.3 and 81.4 mM for Seafresh and Mahachai, respectively.

For a 5:1 ratio the beads were prepared from solution of [chitosan] = 108.9 and 163.4 mM for Seafresh and Mahachai, respectively; [AcOH] = 153.5 mM; [2,4-D] = 16.0 and 24.0 mM for Seafresh and Mahachai, respectively.

* see samples of bead picture in appendix B6-B9, B12-B13 and B15-B20

The significant amount of 2,4-D was lost during the preparation of beads from the solution containing chitosan:2,4-D ratio of 1:1 (w/w). The percent recovery of 2,4-D in chitosan beads increased with the increasing ratio of chitosan:2,4-D in all cases. However the percent content of 2,4-D decreased with the increasing ratio of chitosan:2,4-D.

3.4.6 Effect of Molecular Weight of Chitosan on Bead Preparation

Chitosan had different molecular weight from Seafresh (MW. = 828,821), Mahachai (MW. = 465,684) and Ta Ming (MW. = 178,062) which were used to prepare glutaraldehyde-crosslinked beads. The glutaraldehyde concentration at 4% equivalent to chitosan:2,4-D mixture (5:1 w/w) for preparation of glutaraldehyde-crosslinked beads by using bulk crosslinking method at room temperature.

Table 3.8 Effect of molecular weight of chitosan on bead preparation[a]

Chitosan Source	Molecular Weight (M_v)	Bead Appearance*	% Recovery of 2,4-D
Seafresh	828,821	spherical	52
Mahachai	465,684	spherical	41
Ta Ming	178,062	flatten	32

[a] Beads were prepared from solution with [chitosan] = 108.9 mM; [AcOH] = 153.5 mM; [2,4-D] = 16.0 mM.

* see samples of bead picture in appendix B10-B11 and B21

In this experiment, the solution of each chitosan in 1% acetic acid was used. These solutions had same concentrations in terms of molarity. Beads prepared from Seafresh chitosan had spherical shape and had the highest percent recovery of 2,4-D. While, beads prepared from Ta Ming chitosan had flatten shape and had the lowest percent recovery of 2,4-D due to its low molecular weight. The percent recovery of 2,4-D in chitosan beads increased with the increasing molecular weight of chitosan.

3.4.7 Effect of Crosslinking Time in Bulk Glutaraldehyde-Crosslinked Beads

The effect of crosslinking time was investigated by varying the crosslinking time: 5, 15, 30 and 60 minutes, in the preparation of the bulk glutaraldehyde-crosslinked beads. The beads were prepared by mixing the glutaraldehyde solution (0.56 M) at 2% equivalent of chitosan-2,4-D mixtures (5:1 w/w), allowed to stand for the periods specified above, and dropping the mixture into a potassium carbonate solution (1.5 M) at room temperature. Seafresh chitosan was used.

Table 3.9 Effect of crosslinking time on bead preparation[a]

Crosslinking Time (minutes)	Bead Appearance	% Recovery of 2,4-D
5	spherical	46
15	spherical	46
30	spherical	52
60	spherical	44

[a] Beads were prepared from solution with [chitosan] = 108.9 mM; [AcOH] = 153.5 mM; [2,4-D] = 16.0 mM.

From this experiment, there was no significant difference in the percent recovery of 2,4-D when the crosslinking time was varied. Therefore, the crosslinking time at 5 minute was used in the subsequent experiments.

3.4.8 Effect of Concentration of Crosslinking Agent

By Using Glutaraldehyde as a Crosslinking Agent in Bulk-Crosslinked Beads

The glutaraldehyde concentration was varied at 2, 4 and 6% equivalent to chitosan:2,4-D mixture (5:1 w/w) for preparation of glutaraldehyde-crosslinked beads by using bulk crosslinking method at room temperature.

Table 3.10 Effect of glutaraldehyde concentration on bead preparation[a]

Chitosan Source	Concentration of GA	Bead Appearance	% Recovery of 2,4-D
Seafresh	2% eq	spherical	46
	4% eq	spherical	52
	6% eq	spherical	53
Mahachai	2% eq	spherical	39
	4% eq	spherical	41
	6% eq	spherical	39

[a] Beads were prepared from solution with [chitosan] = 108.9 mM; [AcOH] = 153.5 mM; [2,4-D] = 16.0 mM.

The increase of the amount of glutaraldehyde in the preparation of bulk crosslinking beads seems to increase the percent recovery of 2,4-D. When using the glutaraldehyde greater than 6% equivalent, the mixture of chitosan:2,4-D turned into gel before the beads could be prepared. Even when using glutaraldehyde at 6% equivalent, a great deal of difficulty was encountered during the bead preparation, the solution became very viscous. The use of glutaraldehyde at 4% equivalent was thus preferable.

By Using Polyphosphoric acid as a Crosslinking Agent in Surface-Crosslinked Beads

The polyphosphoric acid was varied at 5, 10 and 20% w/v for preparing polyphosphoric acid-crosslinked beads by using the surface crosslinking method at room temperature. Seafresh chitosan was used.

Table 3.11 Effect of polyphosphoric acid concentration on bead preparation[a]

Concentration of PPA	Bead Appearance	% Recovery of 2,4-D
5% w/v	flatten	48
10% w/v	flatten	56
20% w/v	broken	-

[a] Beads were prepared from solution with [chitosan] = 108.9 mM; [AcOH] = 153.5 mM; [2,4-D] = 16.0 mM.

The increase of polyphosphoric acid concentration from 5% to 10% (w/v) resulted in higher percent recovery of 2,4-D. When the concentration of polyphosphoric acid solution increased to 20% w/v, the beads formed were rather weak and broken up during washing . The poor bead formation might be attributed to some dissolution of chitosan at this highly acidic condition. Furthermore, at high concentration of polyphosphoric acid, the complexation ratio between the polyphosphoric acid and chitosan chain was not suitable for effective crosslinking.

3.5 Preparation of Chitosan Beads Containing IAA

A mixture of chitosan and IAA (5:1 w/w) was dropped into coagulants at room temperature formed uncrosslinked beads, glutaraldehyde-crosslinked beads and polyphosphoric acid-crosslinked beads. The uncrosslinked beads were prepared by dropping solution of chitosan and IAA into a potassium carbonate solution (1.5 M). The glutaraldehyde-crosslinked beads beads were prepared by bulk crosslinking technique, mixing the glutaraldehyde solution (0.56 M) at 4% equivalent of chitosan and dropping the mixture into a potassium carbonate solution (1.5 M). The polyphosphoric acid-crosslinked beads were prepared by direct dropping a solution of the chitosan-IAA mixture into polyphosphoric acid solution (5% w/v). Seafresh chitosan was used in the experiment.

Table 3.12 The percentage recovery and weight content of IAA on bead preparation[a]

CA	Bead Appearance*	% Recovery of IAA
-	spherical	20
GA	spherical	33
PPA	flatten	13

[a] Beads were prepared from solution contained [chitosan] = 108.9 mM; [AcOH] = 153.5 mM; [IAA] = 16.0 mM.

* see samples of bead picture in appendix B22-B24

The significant amount of IAA was lost during the preparation of beads when no crosslinking agent was used (Table 3.11). When using the glutaraldehyde as crosslinking agent, the percent recoveries of IAA in the beads increased. However, beads prepared by using polyphosphoic acid as crosslinking agent had percent recovery of IAA lower than uncrosslinked beads.

3.6 Characterization of Chitosan Beads

3.6.1 Swelling Studies

The swelling of beads without 2,4-D (or IAA) prepared by using non-crosslinking, surface crosslinking and bulk crosslinking methods were studied (Tables A12-A16).

The beads prepared by non-crosslinking method were swollen more than beads prepared by using crosslinking agents. It is a well-known fact that the swelling degree of the polymer depends on the degree of crosslinking. Surprisingly, the glutaraldehyde-crosslinked beads prepared by bulk crosslinking had higher degree of swelling than the non-crosslinked beads. This results suggested that the bulk crosslinking might reduce the tight packing between the chitosan chain during the coagulating process. It is noteworthy to point out here that the swelling of the bulk glutaraldehyde-crosslinked beads contained two steps. First, the swelling of beads took place rapidly up to one point, then the swelling began to decline after the highest swelling. The swelling of the uncrosslinked beads was more gradual and the polyphosphoric acid-crosslinked beads and the surface glutaraldehyde-crosslinked beads barely swelled.

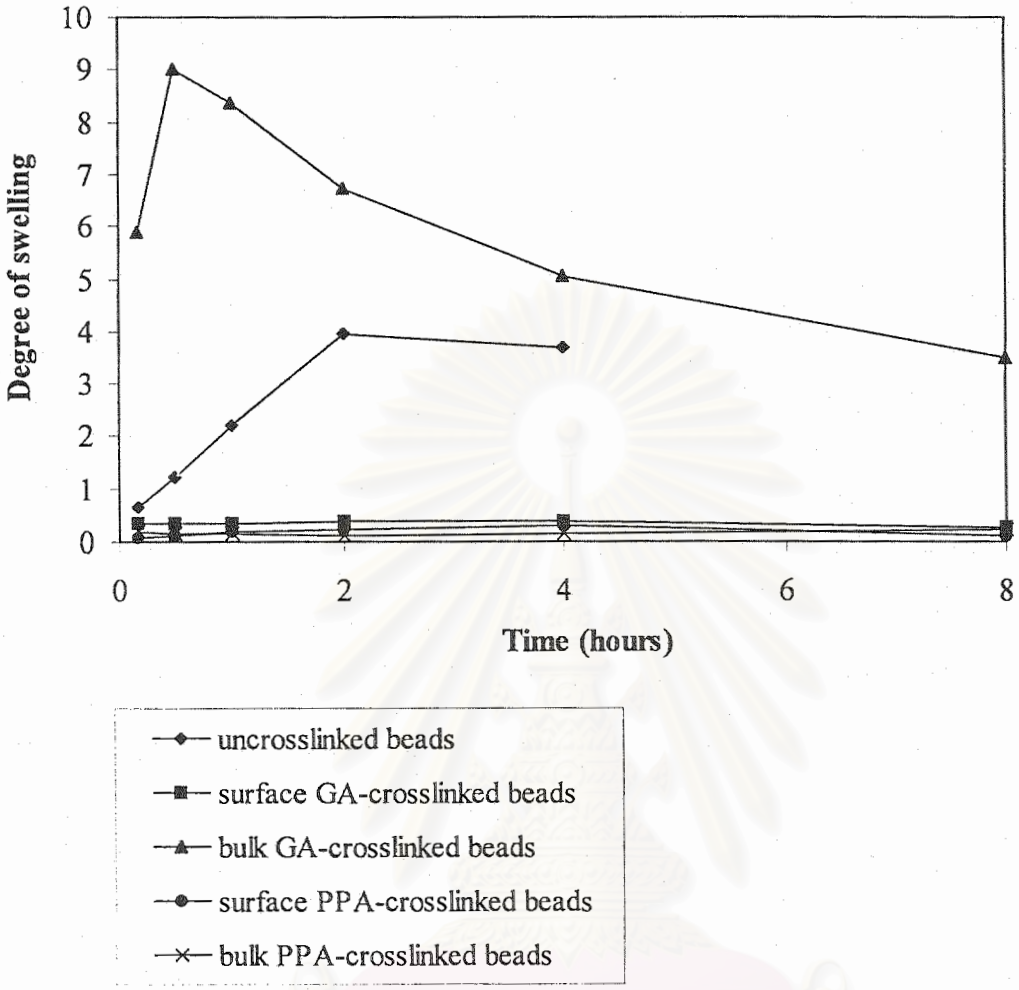


Figure 3.6 Swelling behavior of prepared beads measured as a function of time in distilled water at room temperature.

3.6.2 The 2,4-D Release Studies

Dissolution Test

The release of 2,4-D from the beads was performed in distilled water at room temperature. The amount of 2,4-D was analyzed spectrophotometrically at 284 nm. The released amount of 2,4-D was calculated by using a calibration curve. The effects of various parameters including, molecular weight, the ratio of chitosan:2,4-D, crosslinking agents and the method for crosslinking on the releasing profiles were investigated.

The uncrosslinked beads prepared from chitosan with two different molecular weights, Seafresh (MW. = 828,821) and Mahachai (MW. = 465,684) containing chitosan:2,4-D (5:1 w/w), which were measured for the release of 2,4-D. The amounts of 2,4-D released as a function of time were recorded (Tables A17-A18). The percentage release of 2,4-D was plotted against time (Figure 3.7).

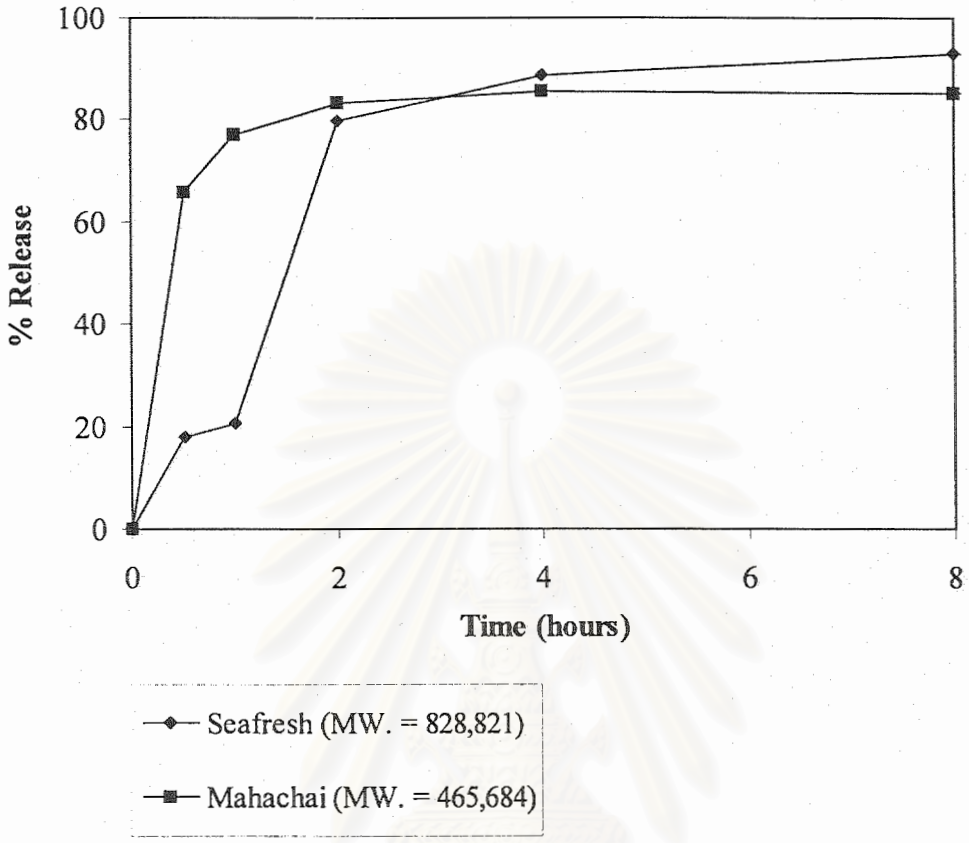


Figure 3.7 Effect of molecular weight on the releasing profile of 2,4-D from the uncrosslinked beads.

The initial release of 2,4-D from the beads prepared from the lower molecular weight chitosan, Mahachai chitosan, was faster than the release from the beads prepared from the higher molecular weight chitosan, Seafresh chitosan. The results implied that higher molecular weight chitosan controlled the release of 2,4-D for longer period.

The effect of chitosan:2,4-D ratio (1:1 and 5:1 w/w) on the releasing behaviors of the uncrosslinked beads were studied. The amounts of 2,4-D released as a function of time were recorded (Tables A18-A19) and the percent release of 2,4-D was plotted against time (Figure 3.8).

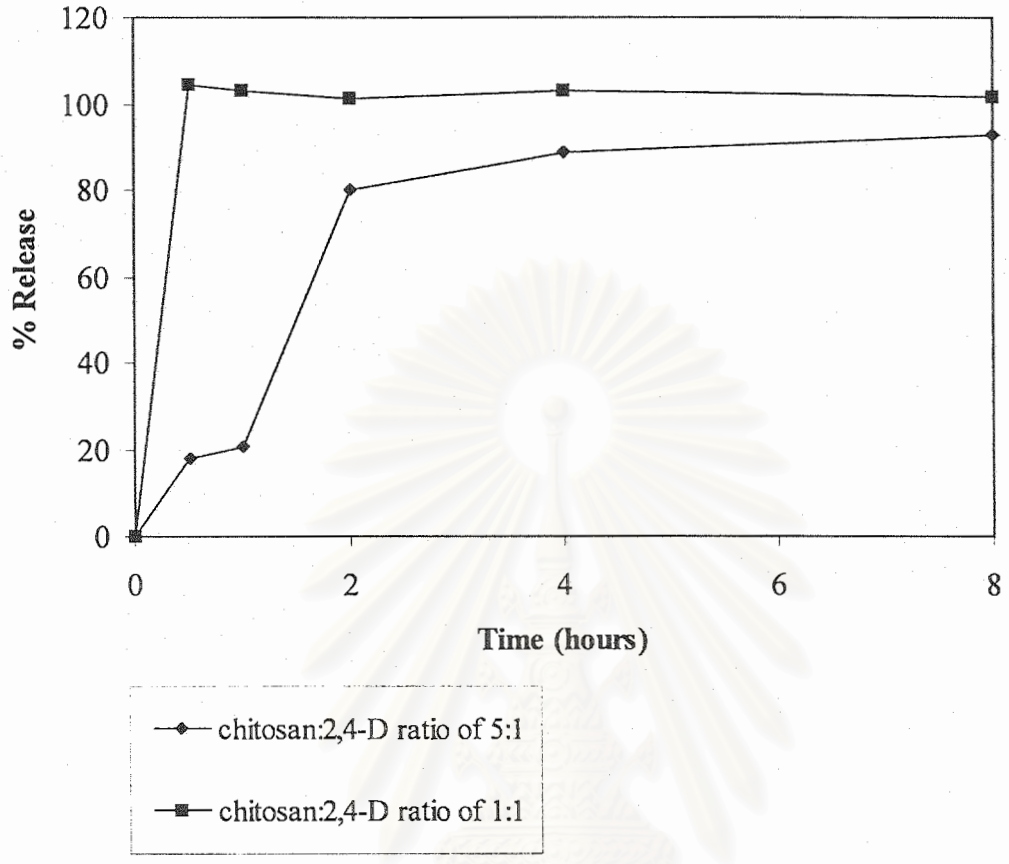


Figure 3.8 Effect of the ratio of chitosan to 2,4-D on the releasing profile of 2,4-D from uncrosslinked beads.

The initial release of 2,4-D from the uncrosslinked beads containing chitosan:2,4-D of ratio 1:1 was faster than the release from the beads containing chitosan:2,4-D ratio of 5:1. The results suggested that chitosan retarded the release of 2,4-D at the lower loading better than that at the higher loading.

The releases of 2,4-D from uncrosslinked beads, glutaraldehyde-crosslinked beads and polyphosphoric acid-crosslinked beads prepared by the surface crosslinking containing chitosan:2,4-D (5:1 w/w) were studied. The amounts of 2,4-D released as a function of time were recorded (Tables A18, A20 and A22). The percent release of 2,4-D was plotted against time (Figure 3.9).

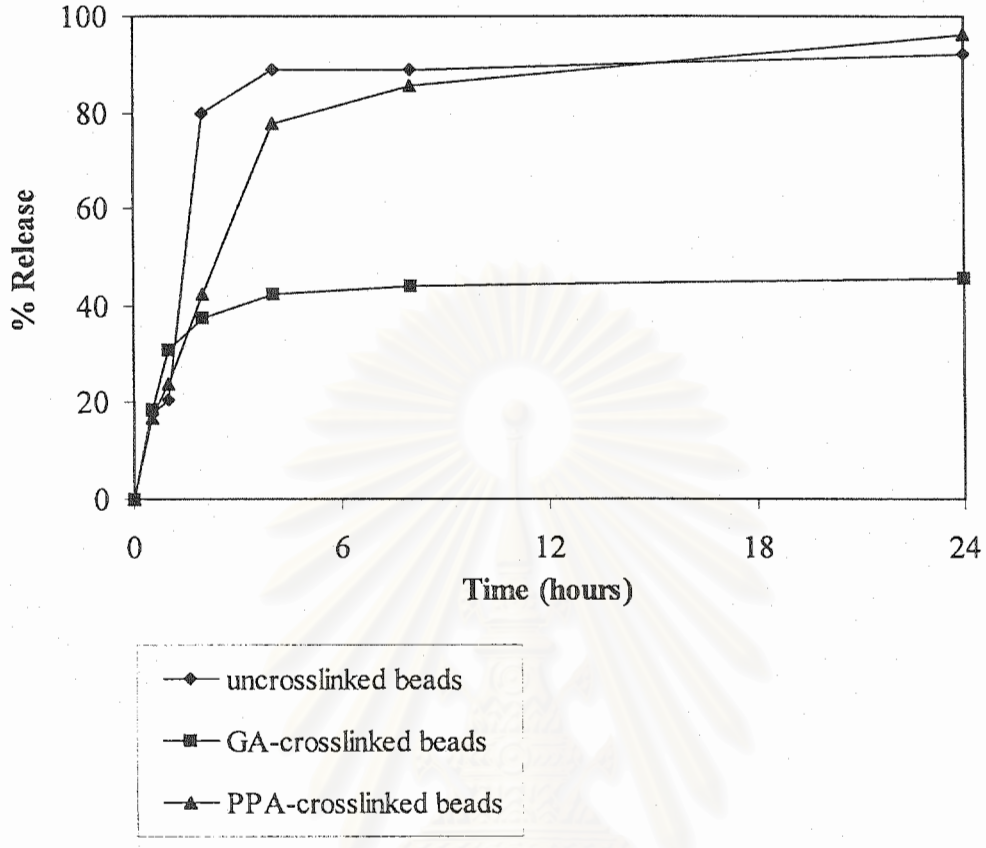


Figure 3.9 Effect of crosslinking agents on the releasing profile of 2,4-D from Seafresh chitosan beads containing chitosan:2,4-D ratio of 5:1 (w/w).

The release of 2,4-D from crosslinked beads was slower in comparison to uncrosslinked beads corresponding to the swelling behaviors of the beads. The glutaraldehyde-crosslinked beads released 2,4-D in the first 4 hours before they stopped releasing at about 40%. In the case of polyphosphoric acid-crosslinked beads, there were almost two distinct steps of releasing. The first fast step accounted for 80% of releasing finished within 4 hours. The second slow step prolonged the release to over 24 hours for the remaining 20% of release.

The glutaraldehyde-crosslinked beads and polyphosphoric acid-crosslinked beads prepared by the surface and bulk crosslinking containing chitosan:2,4-D (5:1 w/w) were measured for the release of 2,4-D. The amounts of

2,4-D release as a function of time were recorded (Tables A20-A23). The percent release of 2,4-D was plotted against time (Figure 3.10).

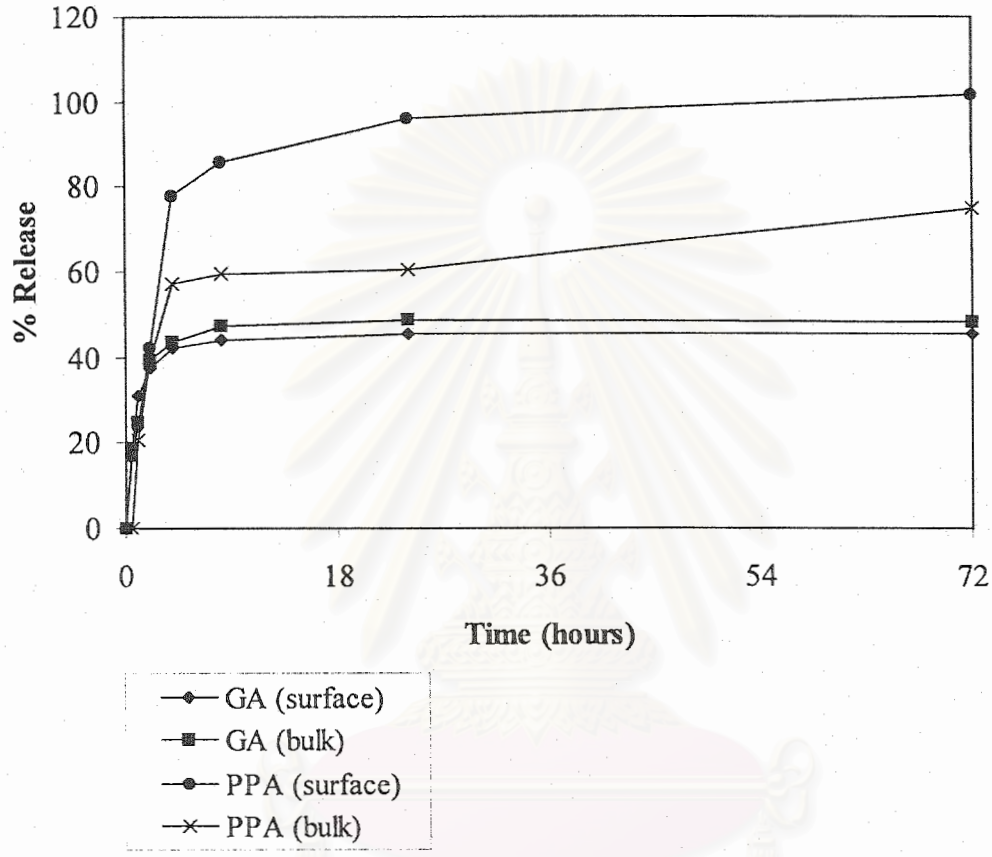


Figure 3.10 Effect of the method for crosslinking on the releasing profile of 2,4-D from GA-crosslinked beads and PPA-crosslinked beads prepared from Seafresh chitosan.

Both surface and bulk glutaraldehyde-crosslinked beads behaved very similarly. They released 2,4-D up to 40% within 4 hours and then the releasing rate became very slow to nearly stopped. For polyphosphoric acid-crosslinked beads, the surface-crosslinked beads released 2,4-D considerably faster than the bulk-crosslinked beads for both fast and slow steps.

Soil Column Leaching Test

The results from dissolution test did not provide appropriate data for interpretation of controlled release effect on agrochemicals which are normally used in a dry condition or, at most, in a damp condition. A more practical test for studying the controlled release of agrochemicals is the soil column leaching test. In the leaching test, the bead formulations were spread evenly on the top of wet sand filled in a column. A measured amount of water was then added to the column to simulate irrigation. The water (30 mL) passing through the column was collected and analysis for the released 2,4-D by UV spectrophotometer at 284 nm. The resulted are discussed as follows.

Pure 2,4-D, non-crosslinked beads, glutaraldehyde-crosslinked beads and polyphosphoric acid-crosslinked beads were compared for the release of 2,4-D in the column leaching test. The amounts of 2,4-D released as a function of irrigation numbers were recorded (Tables A24-A27). The percentage of the releasing of 2,4-D was plotted against the irrigation numbers (Figure 3.11).

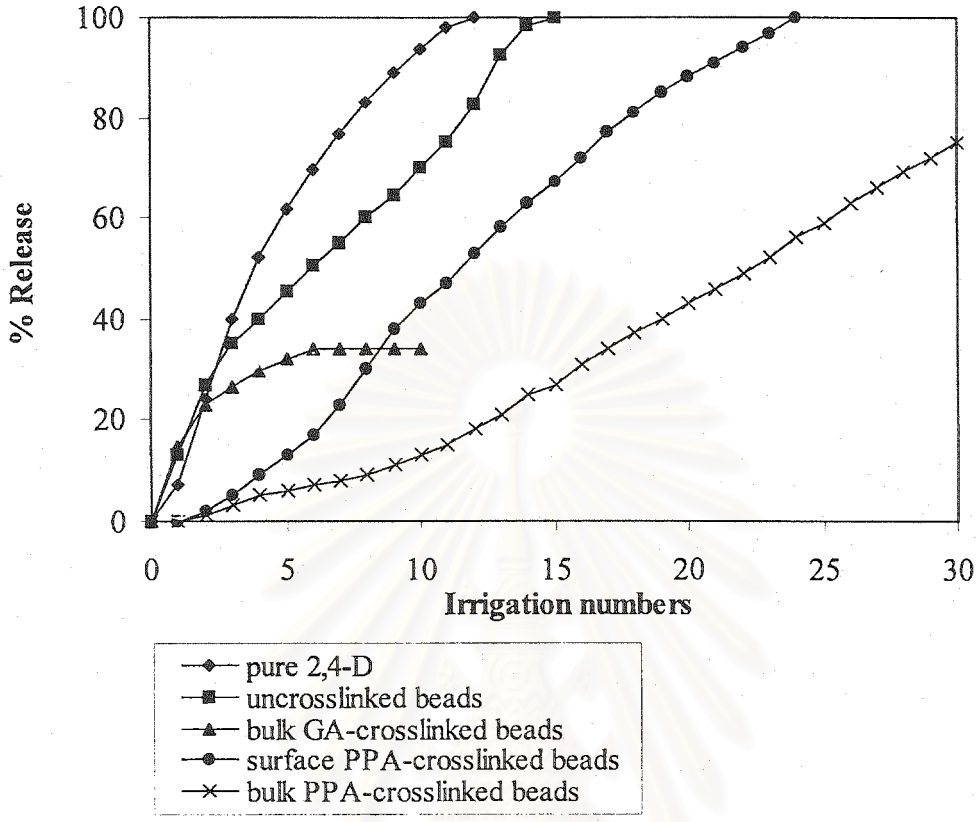


Figure 3.11 Percent release of 2,4-D from pure 2,4-D and the uncrosslinked beads, glutaraldehyde-crosslinked beads and polyphosphoric acid-crosslinked beads in the leaching test (30 mL irrigation).

The pure 2,4-D completed the release of 2,4-D within 12 irrigation. The uncrosslinked beads sustained 3 (125%) more irrigations than the pure of 2,4-D. The surface polyphosphoric acid-crosslinked beads sustained 12 (200%) more irrigations than the pure 2,4-D, while the bulk polyphosphoric acid-crosslinked beads released 2,4-D well over 30 irrigations (>250% increase). The glutaraldehyde-crosslinked beads released 2,4-D up to about 34% of their 2,4-D content within 6 irrigations before they stopped releasing.

3.6.3 The IAA Release Studies

The dissolution studies of the beads containing IAA showed no meaning full data as mentioned previously that the dissolution process was too fast and inappropriate for studying the controlled release of agrochemicals. Therefore, the releasing of IAA from the beads was studied by using soil column leaching test.

The leaching test was studied on the pure IAA, uncrosslinked beads, bulk glutaraldehyde-crosslinked beads and surface polyphosphoric acid-crosslinked beads. The water (30 mL) passing through the column was collected and analysis for the released IAA by UV spectrophotometer at 280 nm. The amounts of IAA released as a function of irrigation numbers were recorded (Tables A28-A31). The percentage of the releasing of IAA was plotted against the irrigation numbers (Figure 3.12).

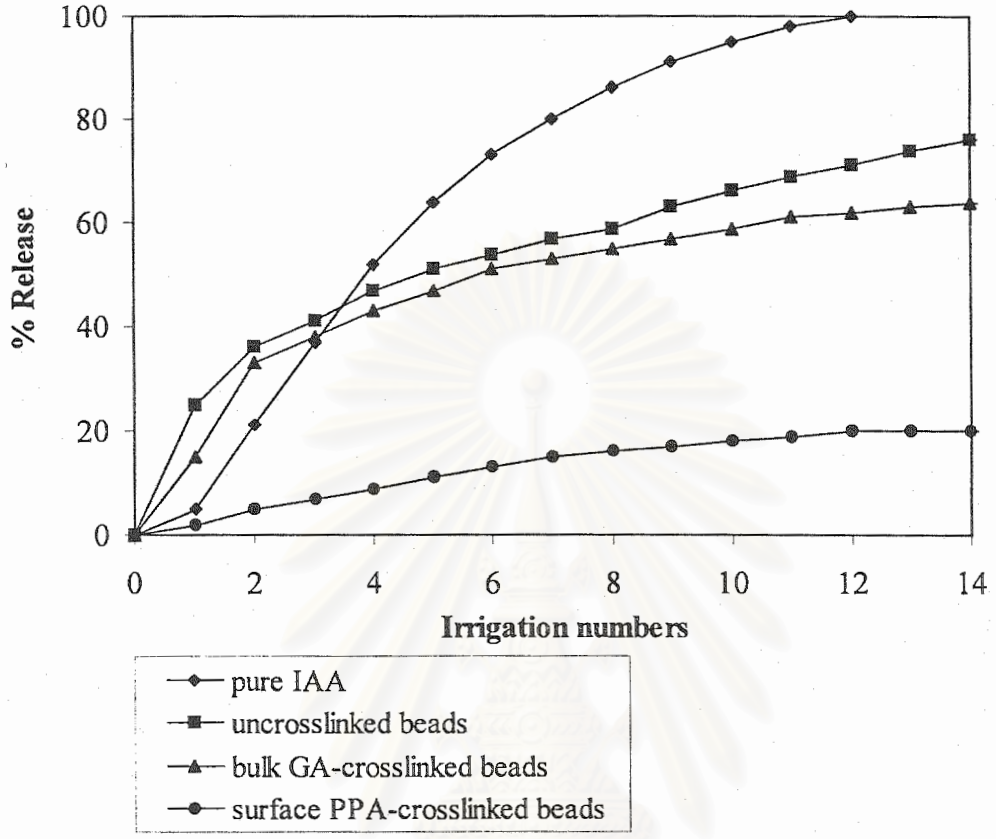


Figure 3.12 Percent release of IAA from pure IAA and the uncrosslinked beads, glutaraldehyde-crosslinked beads and polyphosphoric acid-crosslinked beads in the leaching test (30 mL irrigation).

The release of IAA from uncrosslinked beads, bulk glutaraldehyde-crosslinked beads and surface polyphosphoric acid-crosslinked beads could be studied up to 14 irrigations where the percent release were 76%, 64% and 20% respectively. After 14 irrigations, the concentration of IAA released became very low and undetectable by UV-Vis spectrometer. However, these results suggested variable degree of controlled release of IAA by the chitosan beads and the crosslinked beads. Unfortunately, the beads had run out before the more reliable results could be confirmed by using the larger of beads.

3.6.4 Determination of Crosslinked at Surface of Beads by IR Spectra Analysis

IR spectra of the 2,4-D and beads prepared from Seafresh chitosan were recorded on KBr pellets by using a Fourier Transform Infrared Spectrophotometer (Figure 3.13).

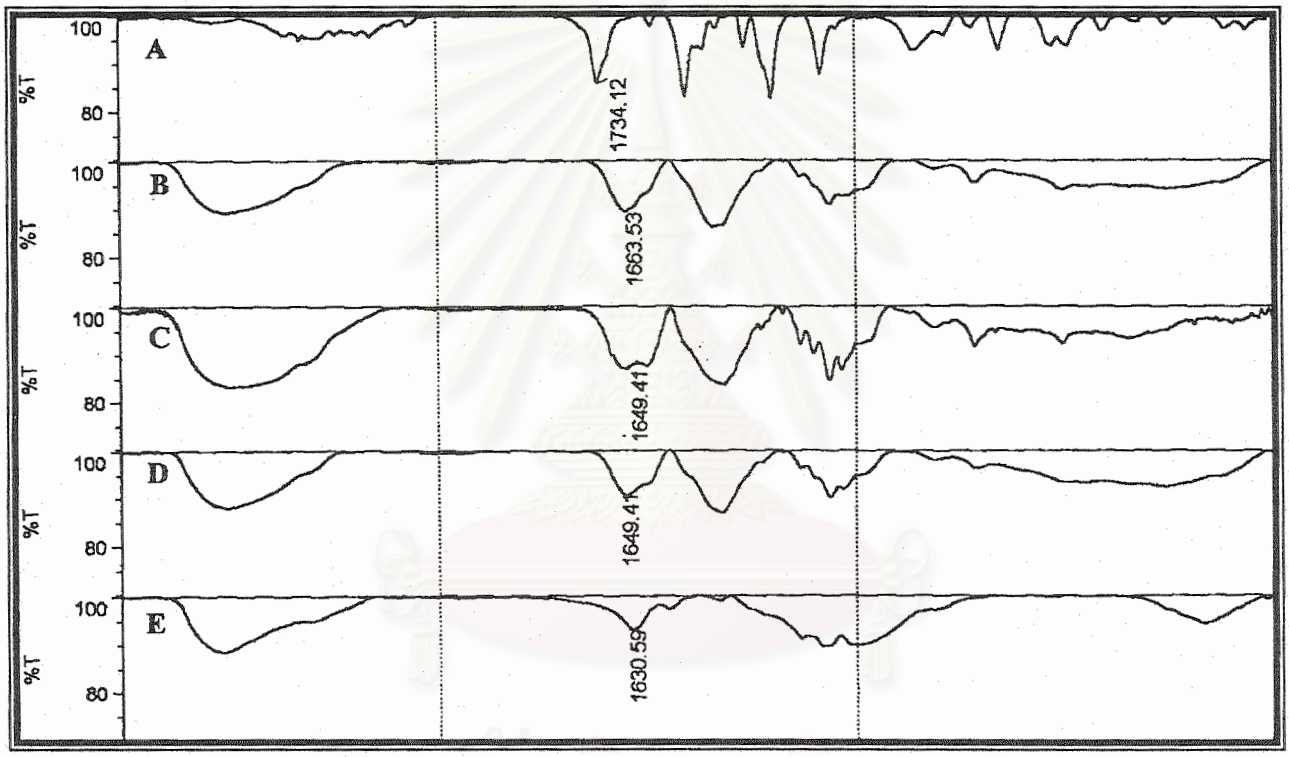


Figure 3.13 FTIR spectra of 2,4-D (A), beads without 2,4-D (B), uncrosslinked chitosan-2,4-D beads (C), bulk GA-crosslinked chitosan-2,4-D beads (D) and bulk PPA-crosslinked chitosan-2,4-D beads (E).

The IR spectra for both sets of beads containing 2,4-D and IAA were very similar. This is reasonable since 2,4-D and IAA contained the same IR active functional groups, carboxylic groups and aromatic rings. In comparison between each spectrum within each set (2,4-D or IAA), the following information could be drawn. The carboxylic group of auxin hormones, 2,4-D and IAA, probably reacted with the amino group to form carboxylate and ammonium salt. This is evidenced by the shift of C=O stretching from ~ 1700 cm^{-1} (A) into the same range of N-H bending signal around 1650 cm^{-1} (C, D and E). The N-H bending signal around 1650 cm^{-1} (B) also obscured the C=N stretching in the spectra of glutaraldehyde-crosslinked beads (D). The polyphosphoric acid-crosslinked beads showed characteristic band at 1100-1200 cm^{-1} of P-O adsorption.

3.6.5 Determination of Size and Surface Morphology of Beads by Optical Microscope

Size and surface appearance of beads were examined by using an optical microscope (Figure 3.14).

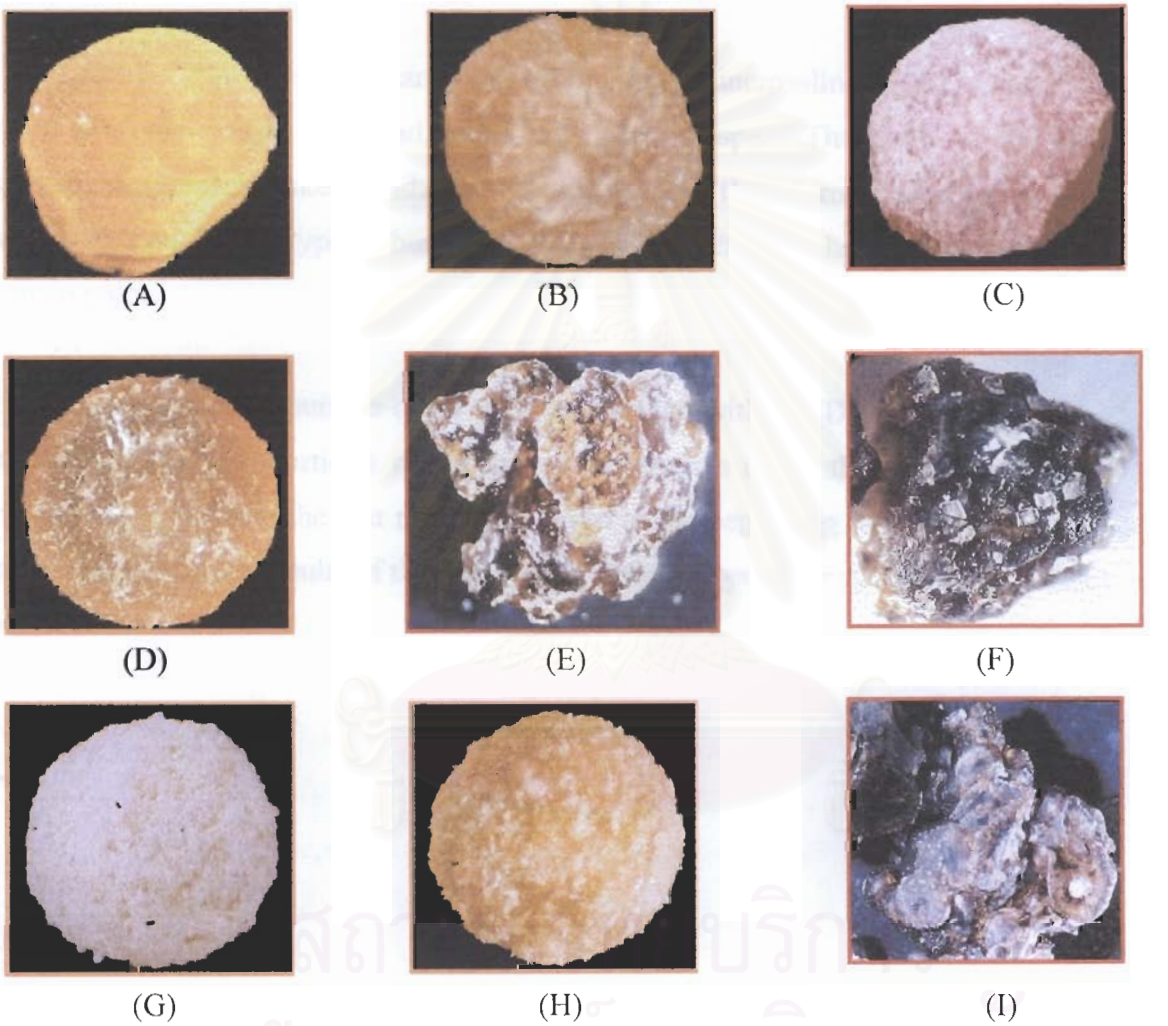


Figure 3.14 Optical microscope photographs of the beads without auxin hormones (A), uncrosslinked chitosan-2,4-D beads (B), surface GA-crosslinked chitosan-2,4-D beads (C), bulk GA-crosslinked chitosan-2,4-D beads (D), surface PPA-crosslinked chitosan-2,4-D beads (E), bulk PPA-crosslinked chitosan-2,4-D beads (F), uncrosslinked chitosan-IAA beads (G), bulk GA-crosslinked chitosan-IAA beads (H) and surface PPA-crosslinked chitosan-IAA beads (I). Chitosan:2,4-D (or IAA) ratio = 5:1 (w/w).

The chitosan-2,4-D beads, uncrosslinked beads (B) and bulk glutaraldehyde-crosslinked beads (D), had sphere shape and yellow color. The surface glutaraldehyde-crosslinked beads (C) were also spherical but the color of the beads were red. Both surface (E) and bulk polyphosphoric acid-crosslinked beads (F) had flatten shape and brown color.

For the chitosan-IAA beads, both uncrosslinked (G) and bulk glutaraldehyde-crosslinked beads (H), had sphere shape. The color of the bulk glutaraldehyde-crosslinked beads were yellow, while the uncrosslinked beads were white. The surface polyphosphoric acid-crosslinked beads (I) had flatten shape and brown color.

On the surface of all the beads loaded with 2,4-D or IAA, there were apparent crystals or particles of the loaded compounds under the microscope. This results suggested that the fast release observed at the beginning of dissolution study may partially be the results of the dissolution of these crystals or particles on the bead surface.

CHAPTER IV

CONCLUSION

4.1 Conclusion

This thesis has described the study of parameters affecting bead preparation and controlled release behaviors of the prepared beads. Chitosan was used as a bead matrix while 2,4-dichlorophenoxyacetic acid (2,4-D) and 3-indoleacetic acid (IAA) were selected as auxin hormone representatives. The parameters being varied were molecular weight of chitosan, chitosan concentration, chitosan:2,4-D ratio, types of crosslinking agents, concentration of crosslinking agents and method of crosslinking. From the results of this study, it is impossible to define the effect for each parameter since these parameters are highly interrelated. However, it is logical to conclude from this study that the prefered conditions for successful bead preparation and longer period of controlled release are listed below.

Condition for higher 2,4-D recovery.

- Higher molecular weight of chitosan (at the same chitosan concentration): Seafresh better than Mahachai and Ta Ming
- Higher chitosan concentration: 3% (w/v) better than 2% (w/v) better than 1% (w/v)
- Higher chitosan:2,4-D ratio: 5:1 (w/w) better than 1:1 (w/w)
- Type of crosslinking agent: polyphosphoric acid better than glutaraldehyde better than none
- Concentration of polyphosphoric acid: 10% (w/v) better than 5%(w/v)
- Method of crosslinking of polyphosphoric acid: suface better than bulk

Condition for longer period of controlled release of 2,4-D.

- Higher molecular weight of chitosan: Seafresh better than Mahachai
- Higher chitosan:2,4-D ratio: 5:1 (w/w) better than 1:1 (w/w)

- Type of crosslinking agent: polyphosphoric acid better than glutaraldehyde better than none
- Method of crosslinking of polyphosphoric acid: bulk better than suface

It is important to emphasize here that the set of conditions presented above is quite empirical and may not be applicable to every active compounds. Significant discrepancy in the preferred conditions between the beads containing 2,4-D and the beads containing IAA were also observed especially for the last three parameters, (types of crosslinking agents, concentration of crosslinking agent and method of croslinking).

4.2 Suggestion for Further Work

Improvement in bead preparation for higher efficiency of 2,4-D incorporation must be developed. In this study, the time consuming in the bead preparation was the major obstacle. It is also important to find a faster way to prepare beads. The pot and field biological test of the prepared beads should also be carried out.

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APPENDIX A

Table A1 Time of Ta Ming chitosan solution travelling through the Ubbelohde Viscometer.

C	time (sec)				η_r	η_{sp}	η_{sp}/C	$(\ln \eta_r)/C$
(g/L)	t_1	t_2	t_3	t			(mL/g)	(mL/g)
0.000	88.67	88.86	88.92	88.82	1.000	0.000	-	-
3.028	133.02	133.63	133.61	133.42	1.502	0.502	166	134
2.523	125.44	125.17	125.19	125.27	1.410	0.410	163	136
2.163	119.10	119.72	119.80	119.54	1.346	0.346	160	137
1.262	105.93	105.38	105.11	105.47	1.188	0.188	149	137

Table A2 Time of Mahachai chitosan solution travelling through the Ubbelohde Viscometer.

C	time (sec)				η_r	η_{sp}	η_{sp}/C	$(\ln \eta_r)/C$
(g/L)	t_1	t_2	t_3	t			(mL/g)	(mL/g)
0.000	93.22	93.00	93.11	93.11	1.000	0.000	-	-
1.554	149.58	149.80	149.77	149.72	1.608	0.608	391	306
1.295	139.79	139.21	139.52	139.51	1.498	0.498	385	312
1.110	131.73	131.95	132.03	131.90	1.417	0.417	375	314
0.971	126.99	126.59	126.41	126.66	1.360	0.360	371	317
0.777	119.27	119.51	119.54	119.44	1.283	0.283	364	321

Table A3 Time of Seafresh chitosan solution travelling through the Ubbelohde Viscometer.

C	time (sec)				η_r	η_{sp}	η_{sp}/C	$(\ln \eta_r)/C$
(g/L)	t_1	t_2	t_3	t			(mL/g)	(mL/g)
0.000	91.59	91.65	91.64	91.63	1.000	0.000	-	-
1.034	155.46	155.52	155.63	155.54	1.698	0.698	675	512
0.862	143.48	143.34	143.17	143.33	1.564	0.564	655	519
0.739	135.67	135.78	135.53	135.66	1.481	0.481	650	531
0.538	122.64	122.74	122.20	122.53	1.337	0.337	627	540
0.461	117.88	117.84	117.43	117.72	1.285	0.285	618	544

Table A4 Time of Koyo chitosan solution travelling through the Ubbelohde Viscometer.

C	time (sec)				η_r	η_{sp}	η_{sp}/C	$(\ln \eta_r)/C$
(g/L)	t_1	t_2	t_3	t			(mL/g)	(mL/g)
0.000	91.60	91.68	91.97	91.75	1.000	0.000	-	-
0.760	180.24	180.44	180.37	180.35	1.966	0.966	1271	889
0.633	162.60	162.50	162.06	162.39	1.770	0.770	1216	902
0.543	149.52	149.50	149.56	149.53	1.630	0.630	1160	899
0.217	112.77	112.47	112.27	112.50	1.226	0.226	1042	940
0.181	108.90	108.82	108.82	108.85	1.186	0.186	1030	944
0.155	106.04	106.16	106.00	106.07	1.156	0.156	1007	935

Table A5 Degree of deacetylation (%DD) of the various types of chitosan (IR Method).

Chitosan Source	Peak Area of Absorbtion Band at 1655 cm^{-1}	Peak Area of Absorbtion Band at 2867 cm^{-1}	Absorbtion Band Ratio A_{1655}/A_{2867}	%DD
Ta Ming	-	-	-	-
Mahachai	1128	2138	0.53	84
Seafresh	176	484	0.36	89
Koyo	481	938	0.51	85

Table A6 Volumes of the PVSK solution for blank and CPC titration.

Time	PVSK (mL)	
	CPC Titration	Blank Titration
1	3.5	0.1
2	3.5	0.1
3	3.45	0.1
AVG.	3.48	0.1

Table A7 Volumes of the PVSK solution for titration of chitosan samples.

Chitosan Source	Weight (mg)	PVSK (mL)			
		1	2	3	AVG.
Ta Ming	9.5	3.90	3.80	3.90	3.87
Mahachai	8.5	2.50	2.50	2.40	2.47
Seafresh	8.7	3.50	3.40	3.40	3.43
Koyo	8.4	3.00	3.10	3.10	3.07

Table A8 Absorbance of the standard 2,4-D solution in acetic acid solution (5% v/v) at 284 nm.

Concentration (ppm)	Absorbance
10	0.0786
20	0.1584
40	0.3133
60	0.4825
80	0.6320
100	0.7814
120	0.9738

Table A9 Absorbance of the standard 2,4-D solution in distilled water at 284 nm.

Concentration (ppm)	Absorbance
10	0.0821
20	0.1675
40	0.3343
60	0.4960
80	0.6569
100	0.8058
120	0.9486

Table A10 Absorbance of the standard IAA solution in acetic acid solution (5% v/v) at 280 nm.

Concentration (ppm)	Absorbance
2.5	0.0733
5	0.1456
10	0.2849
15	0.4402
20	0.6743
25	0.8054
30	0.9609

Table A11 Absorbance of the standard IAA solution in distilled water at 280 nm.

Concentration (ppm)	Absorbance
2.5	0.0752
5	0.1488
10	0.2918
15	0.4363
20	0.602
25	0.7569
30	0.8975

Table A12 The degree of swelling for uncrosslinked beads as a function of time.

Time (hours)	Degree of swelling		
	1	2	AVG.
0.17	0.55	0.75	0.65
0.50	1.21	1.19	1.20
1	2.05	2.38	2.22
2	3.68	4.22	3.95
4	3.49	3.86	3.68

Table A13 The degree of swelling for surface glutaraldehyde-crosslinked beads as a function of time.

Time (hours)	Degree of swelling		
	1	2	AVG.
0.17	0.35	0.36	0.355
0.50	0.34	0.34	0.340
1	0.35	0.33	0.340
2	0.35	0.38	0.365
4	0.37	0.40	0.385
8	0.28	0.23	0.255

Table A14 The degree of swelling for bulk glutaraldehyde-crosslinked beads as a function of time.

Time (hours)	Degree of swelling		
	1	2	AVG.
0.17	6.09	5.69	5.890
0.50	9.01	9.02	9.015
1	8.32	8.40	8.360
2	6.74	6.69	6.720
4	5.03	5.06	5.045
8	3.49	3.53	3.510

Table A15 The degree of swelling for surface polyphosphoric acid-crosslinked beads as a function of time.

Time (hours)	Degree of swelling		
	1	2	AVG.
0.17	0.08	0.08	0.080
0.50	0.10	0.10	0.100
1	0.21	0.17	0.190
2	0.22	0.23	0.225
4	0.27	0.33	0.300
8	0.11	0.12	0.115

Table A16 The degree of swelling for bulk polyphosphoric acid-crosslinked beads as a function of time.

Time (hours)	Degree of swelling		
	1	2	AVG.
0.17	0.19	0.16	0.175
0.50	0.13	0.17	0.150
1	0.11	0.17	0.140
2	0.08	0.12	0.100
4	0.11	0.17	0.140
8	0.21	0.21	0.210

Table A17 The percentage release of 2,4-D from uncrosslinked beads prepared from Mahachai chitosan containing chitosan:2,4-D (5:1 w/w) as a function of time by dissolution test.

Time (hours)	% Release		
	1	2	AVG.
0.5	65.95	65.71	65.83
1	76.02	77.70	76.86
2	82.97	83.21	83.09
4	86.09	84.89	85.49
8	86.54	83.92	85.23
24	87.04	86.04	86.54

Table A18 The percentage release of 2,4-D from uncrosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (5:1 w/w) as a function of time by dissolution test.

Time (hours)	% Release		
	1	2	AVG.
0.5	18.45	17.02	17.74
1	21.80	19.57	20.69
2	81.34	78.51	79.93
4	89.10	88.72	88.91
8	94.97	90.85	92.91
24	90.78	93.83	92.31

Table A19 The percentage release of 2,4-D from uncrosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (1:1 w/w) as a function of time by dissolution test.

Time (hours)	% Release		
	1	2	AVG.
0.5	102.41	106.02	104.22
1	101.20	104.82	103.01
2	98.80	103.61	101.21
4	104.82	101.20	103.01
8	103.61	100.00	101.81
24	104.82	101.20	103.01

Table A20 The percentage release of 2,4-D from surface GA-crosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (5:1 w/w) as a function of time by dissolution test.

Time (hours)	% Release		
	1	2	AVG.
0.5	17.06	20.05	18.56
1	29.16	32.88	31.02
2	37.87	36.71	37.29
4	42.78	41.64	42.21
8	42.51	45.75	44.13
24	44.96	46.03	45.50
72	44.65	47.72	45.38

Table A21 The percentage release of 2,4-D from bulk GA-crosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (5:1 w/w) as a function of time by dissolution test.

Time (hours)	% Release		
	1	2	AVG.
0.5	18.01	19.72	18.87
1	23.80	25.96	24.88
2	39.04	40.87	39.40
4	45.84	41.39	43.62
8	51.64	42.67	47.16
24	47.10	50.64	48.87
72	46.85	49.87	48.36

Table A22 The percentage release of 2,4-D from surface PPA-crosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (5:1 w/w) as a function of time by dissolution test.

Time (hours)	% Release		
	1	2	AVG.
0.5	15.18	18.44	16.81
1	23.71	24.06	23.89
2	41.63	42.80	42.22
4	77.09	78.50	77.80
8	87.56	83.83	85.74
24	95.42	96.84	96.13
72	101.99	101.38	101.69

Table A23 The percentage release of 2,4-D from bulk PPA-crosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (5:1 w/w) as a function of time by dissolution test.

Time (hours)	% Release		
	1	2	AVG.
0.5	0.00	0.00	0.00
1	19.33	21.54	20.44
2	39.31	39.69	39.50
4	59.45	55.35	57.40
8	57.28	64.83	59.56
24	60.19	60.59	60.39
72	74.93	74.60	74.77

Table A24 The percentage release of 2,4-D from pure 2,4-D as a function of time by soil column leaching test.

Irrigation Numbers	% Release		
	1	**2**	**AVG.**
1	7	7	7.0
2	24	24	24.0
3	40	40	40.0
4	51	53	52.0
5	61	62	61.5
6	69	70	69.5
7	76	77	76.5
8	83	83	83.0
9	89	89	89.0
10	93	94	93.5
11	97	99	98.0
12	100	102	101.0

Table A25 The percentage release of 2,4-D from uncrosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (5:1 w/w) as a function of

time by soil column leaching test.

Irrigation Numbers	% Release		
	1	2	AVG.
1	12	14	13.0
2	25	29	27.0
3	33	37	35.0
4	38	42	40.0
5	43	48	45.5
6	48	53	50.5
7	53	57	55.0
8	58	62	60.0
9	63	66	64.5
10	70	70	70.0
11	76	74	75.0
12	85	80	82.5
13	95	90	92.5
14	99	98	98.5
15	101	100	100.5

Table A26 The percentage release of 2,4-D from bulk GA-crosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (5:1 w/w) as a function of time by soil column leaching test.

Irrigation Numbers	% Release		
	1	2	AVG.
1	15	14	14.5
2	22	24	23.0
3	25	28	26.5
4	28	31	29.5
5	30	34	32.0
6	32	36	34.0
7	33	35	34.0

Table A27 The percentage release of 2,4-D from surface PPA-crosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (5:1 w/w) as a function of time by soil column leaching test.

Irrigation Numbers	% Release		
	1	2	AVG.
1	0	0	0.0
2	2	3	2.5
3	5	5	5.0
4	9	9	9.0
5	13	15	14.0
6	17	17	17.0
7	23	22	22.5
8	30	29	29.5
9	38	36	37.0
10	43	42	42.5
11	47	47	47.0
12	53	54	53.5
13	58	58	58.0
14	63	65	64
15	67	69	68.0
16	72	74	73.0
17	77	77	77.0
18	81	81	81.0
19	85	84	84.5
20	88	88	88.0
21	91	92	91.5
22	94	94	94.0
23	97	97	97.0
24	100	100	100.0

Table A28 The percentage release of 2,4-D from bulk PPA-crosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (5:1 w/w) as a function of time by soil column leaching test.

Irrigation Numbers	% Release		
	1	2	AVG.
1	0	0	0.0
2	1	1	1.0
3	3	2	2.5
4	5	5	5.0
5	6	6	6.0
6	7	7	7.0
7	8	8	8.0
8	9	9	9.0
9	11	10	10.5
10	13	12	12.5
11	15	14	14.5
12	18	17	17.5
13	21	21	21.0
14	25	25	25.0
15	27	27	27.0
16	31	31	31.0
17	34	34	34.0
18	37	37	37.0
19	40	39	39.5
20	43	42	42.5
21	46	45	45.5
22	49	49	49.0
23	52	52	52.0
24	56	56	56.0
25	59	59	59.0

Table A28 (Continuted)

Irrigation Numbers	% Release		
	1	2	AVG.
26	63	62	62.5
27	66	65	65.5
28	69	69	69.0
29	72	72	72.0
30	75	75	75.0

Table A29 The percentage release of IAA from pure IAA as a function of time by soil column leaching test.

Irrigation Numbers	% Release		
	1	2	AVG.
1	6	5	5.5
2	23	21	22.0
3	36	37	36.5
4	52	52	52.0
5	64	64	64.0
6	72	73	72.5
7	79	80	79.5
8	85	86	85.5
9	91	91	91.0
10	95	95	95.0
11	98	98	98.0
12	100	101	100.5

Table A30 The percentage release of IAA from uncrosslinked beads prepared from Seafresh chitosan containing chitosan:IAA (5:1 w/w) as a function of time by soil column leaching test.

Irrigation Numbers	% Release		
	1	2	AVG.
1	24	25	24.5
2	35	36	35.5
3	41	41	41.0
4	46	46	46.0
5	49	50	49.5
6	52	53	52.5
7	56	56	56.0
8	60	59	59.5
9	64	63	63.0
10	66	66	66.0
11	68	68	68.0
12	71	71	71.0
13	74	73	73.5
14	76	76	76.0
15	76	76	76.0
16	76	76	76.0
17	76	76	76.0
18	76	76	76.0

Table A31 The percentage release of IAA from bulk GA-crosslinked beads prepared from Seafresh chitosan containing chitosan:IAA (5:1 w/w) as a function of time by soil column leaching test.

Irrigation Numbers	% Release		
	1	2	AVG.
1	13	15	14.0
2	32	33	32.5
3	37	38	37.5
4	43	43	43.0
5	46	47	46.5
6	51	51	51.0
7	53	53	53.0
8	55	55	55.0
9	57	57	57.0
10	59	59	59.0
11	61	61	61.0
12	62	62	62.0
13	63	63	63.0
14	64	64	64.0
15	64	64	64.0
16	64	64	64.0
17	64	64	64.0
18	64	64	64.0

Table A32 The percentage release of IAA from surface PPA-crosslinked beads prepared from Seafresh chitosan containing chitosan:IAA (5:1 w/w) as a function of time by soil column leaching test.

Irrigation Numbers	% Release		
	1	**2**	**AVG.**
1	1	2	1.5
2	4	5	3.0
3	6	7	6.5
4	9	9	9.0
5	11	11	11.0
6	13	13	13.0
7	15	15	15.0
8	16	16	16.0
9	17	17	17.0
10	18	18	18.0
11	19	19	19.0
12	20	20	20.0
13	20	20	20.0
14	20	20	20.0
15	20	20	20.0
16	20	20	20.0
17	20	20	20.0
18	20	20	20.0

APPENDIX B

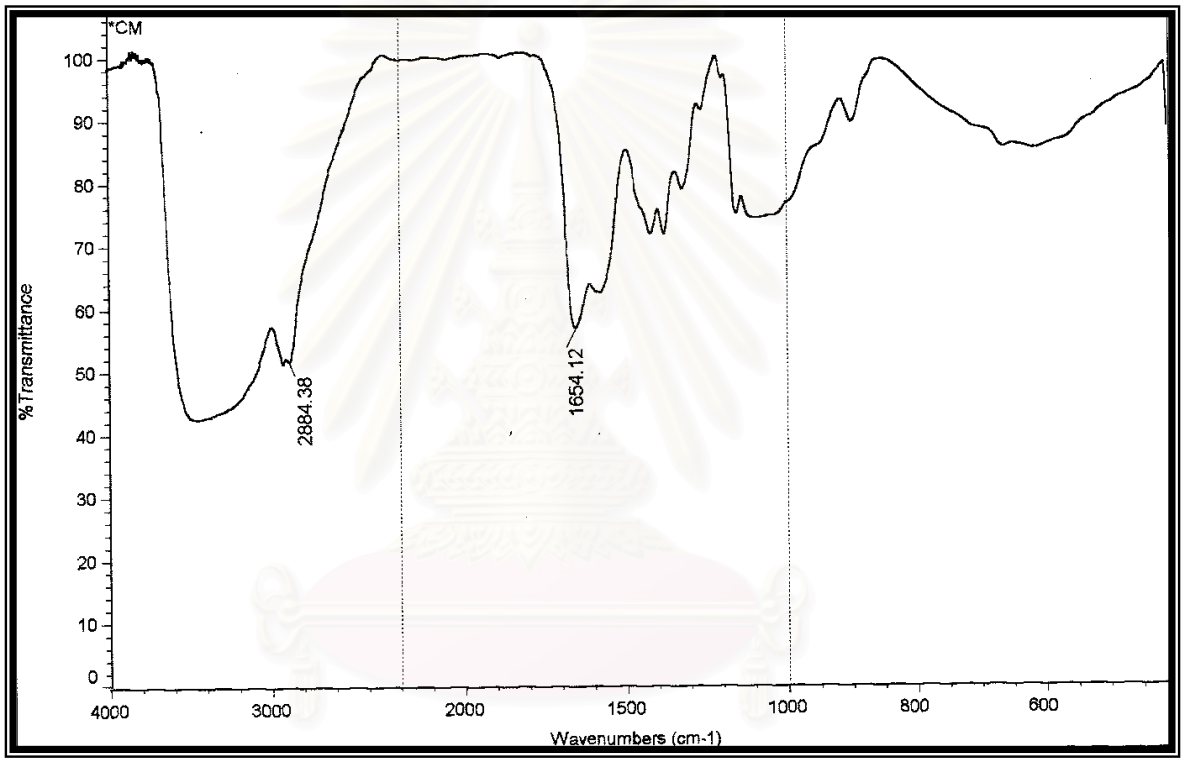

Figure B1 IR spectra of Mahachai chitosan.

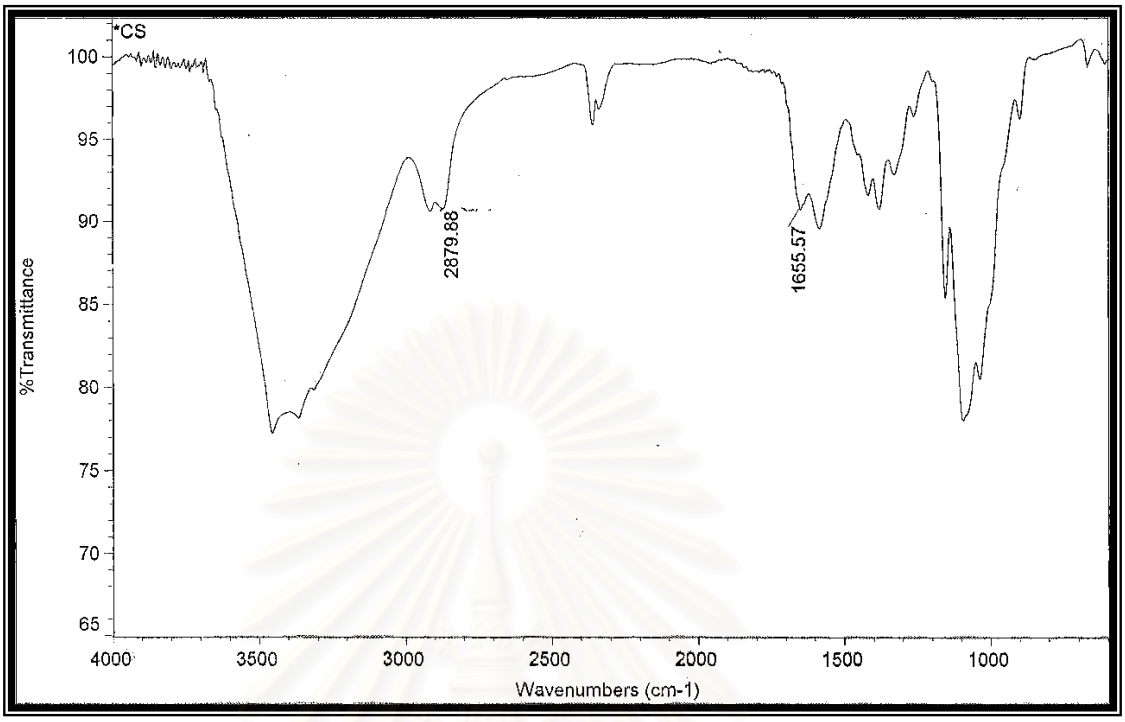

Figure B2 IR spectra of Seafresh chitosan.

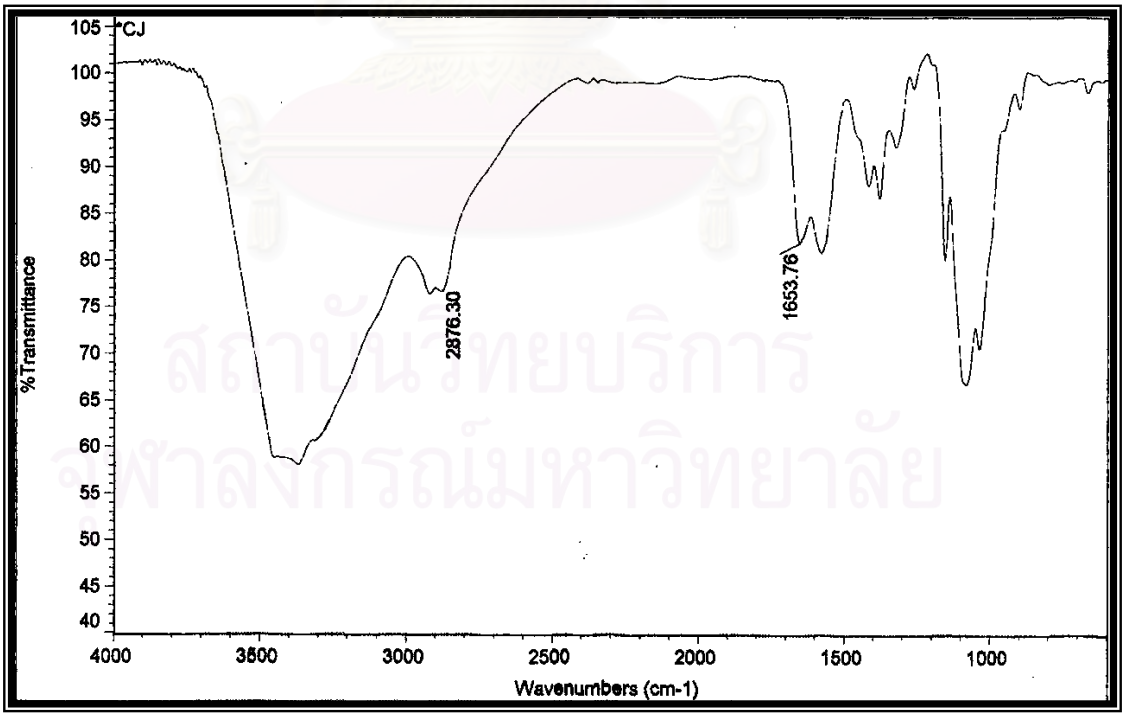

Figure B3 IR spectra of Koyo chitosan.

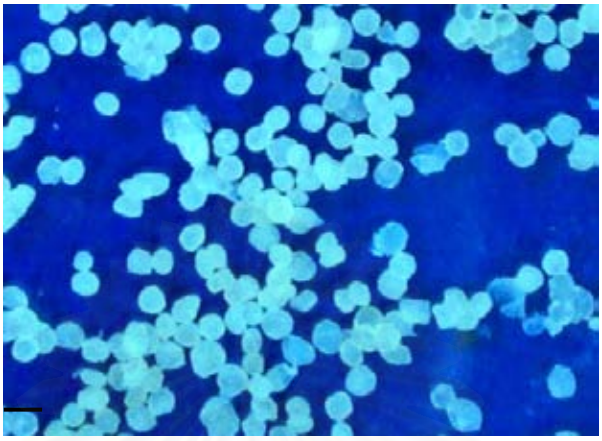

Figure B4 Wet beads prepared from NaOH solution.

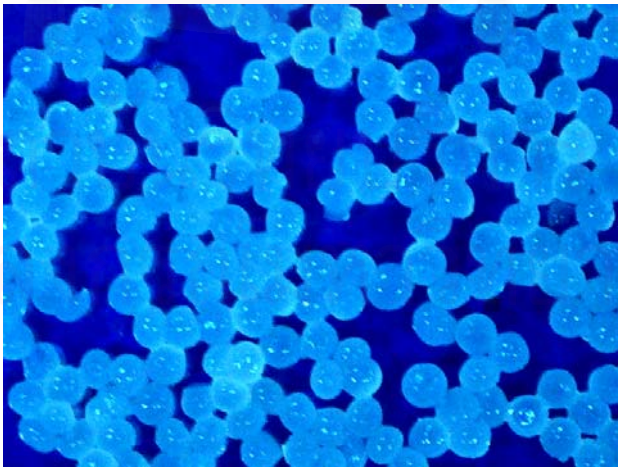

Figure B5 Wet beads prepared from K_2CO_3 solution (1.5 M).

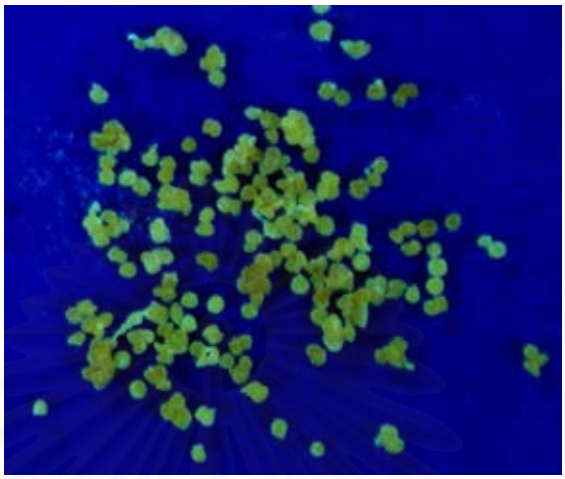

Figure B6 Dry uncrosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (5:1 w/w).

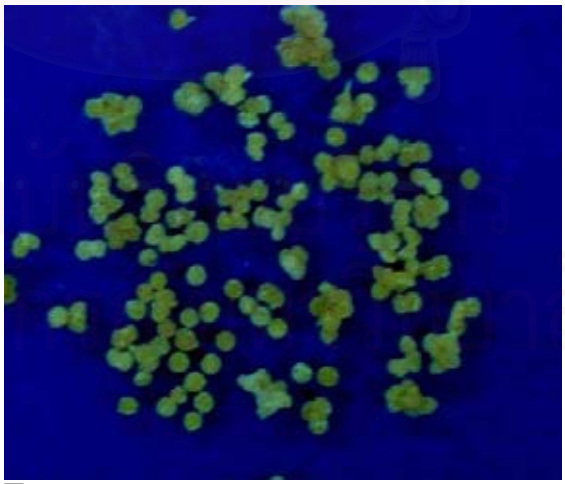

Figure B7 Dry uncrosslinked beads prepared from Mahachai chitosan containing chitosan:2,4-D (5:1 w/w).

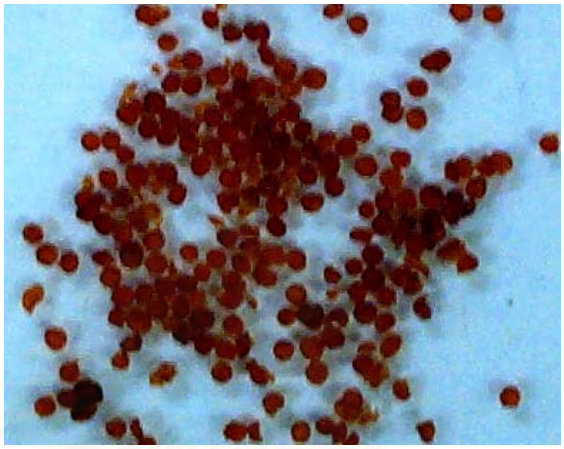

Figure B8 Dry surface GA-crosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (5:1 w/w).

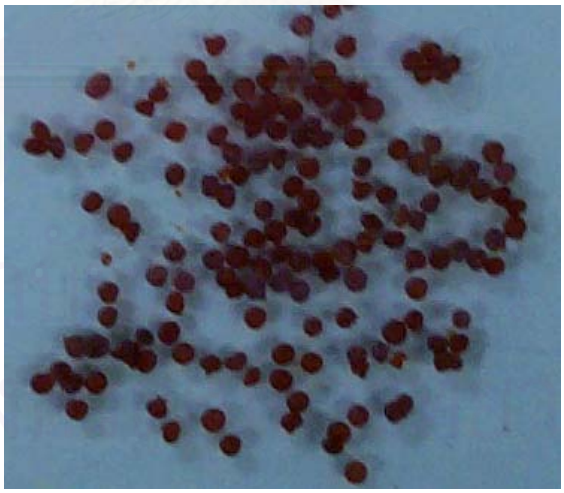

Figure B9 Dry surface GA-crosslinked beads prepared from Mahachai chitosan containing chitosan:2,4-D (5:1 w/w).

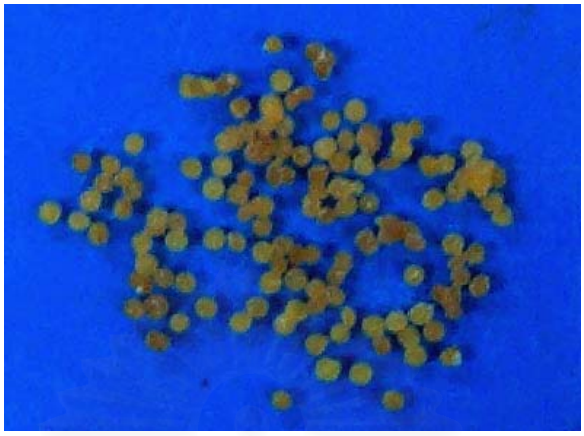

Figure B10 Dry bulk GA-crosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (5:1 w/w).

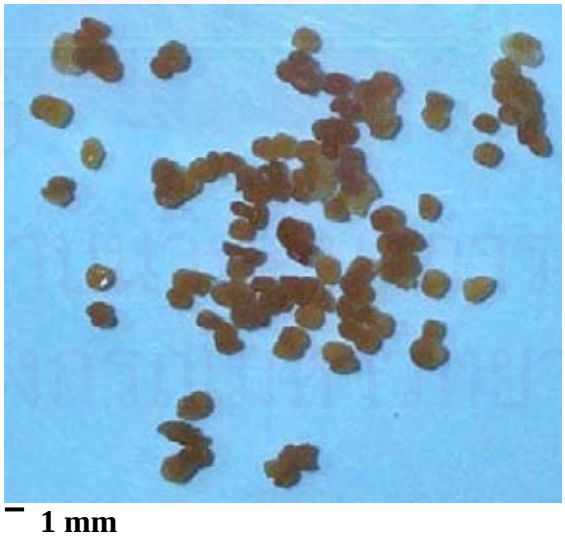

Figure B11 Dry bulk GA-crosslinked beads prepared from Mahachai chitosan containing chitosan:2,4-D (5:1 w/w).

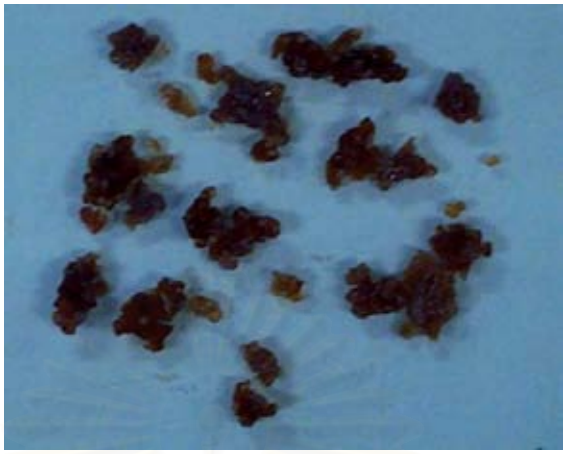

Figure B12 Dry surface PPA-crosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (5:1 w/w).

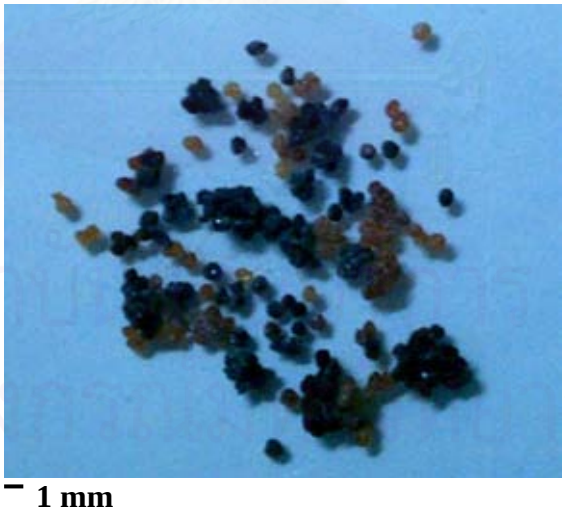

Figure B13 Dry surface PPA-crosslinked beads prepared from Mahachai chitosan containing chitosan:2,4-D (5:1 w/w).

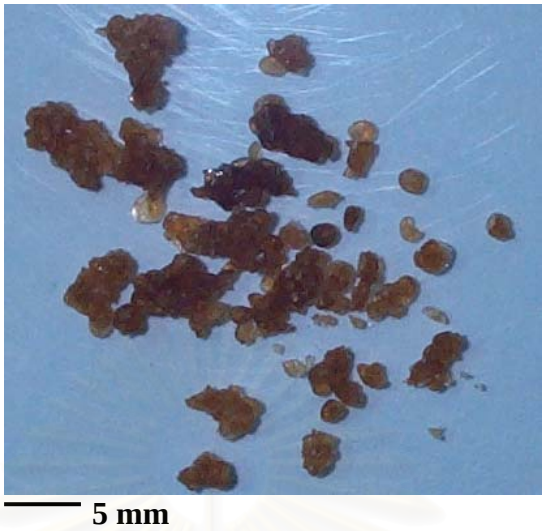

Figure B14 Dry bulk PPA-crosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (5:1 w/w).

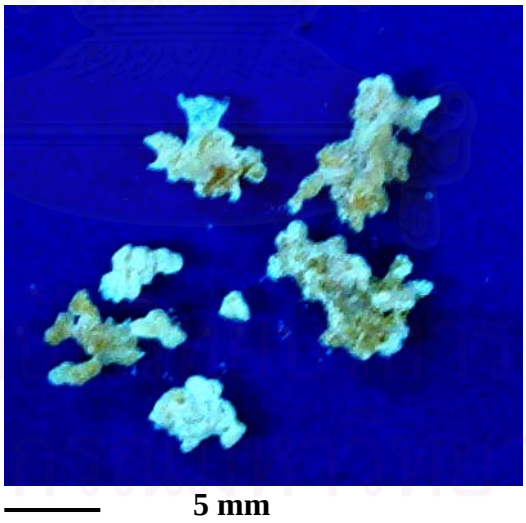

Figure B15 Dry uncrosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (1:1 w/w).

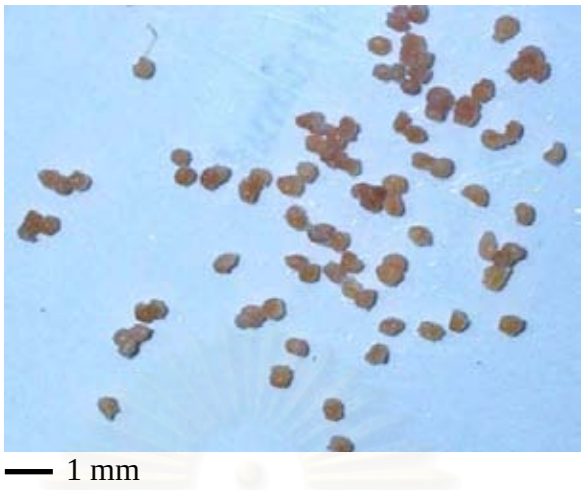

Figure B16 Dry uncrosslinked beads prepared from Mahachai chitosan containing chitosan:2,4-D (1:1 w/w).

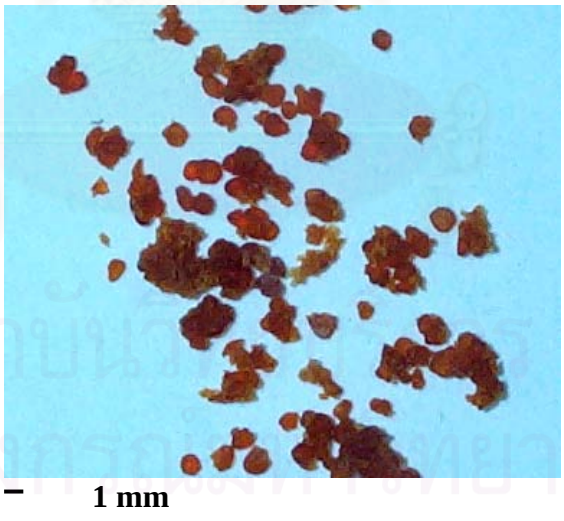

Figure B17 Dry surface GA-crosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (1:1 w/w).

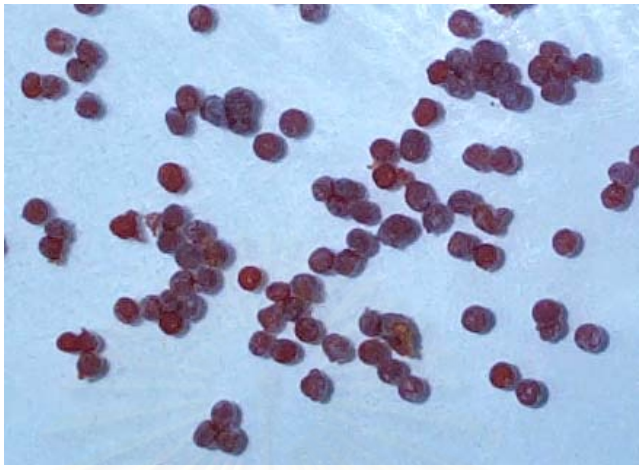

Figure B18 Dry surface GA-crosslinked beads prepared from Mahachai chitosan containing chitosan:2,4-D (1:1 w/w).

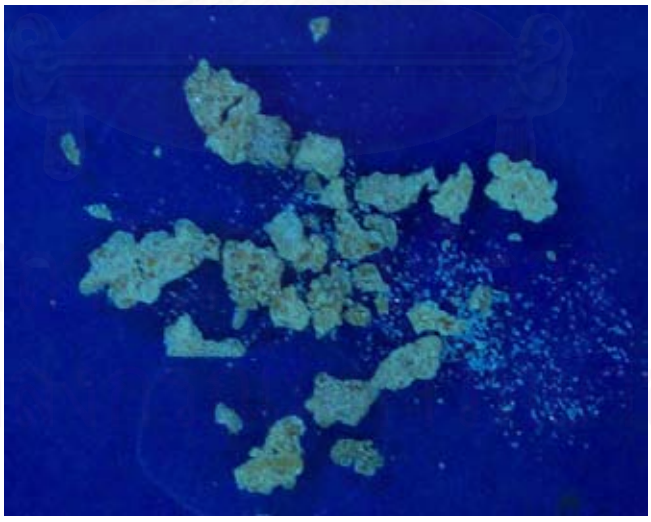

Figure B19 Dry surface PPA-crosslinked beads prepared from Seafresh chitosan containing chitosan:2,4-D (1:1 w/w).

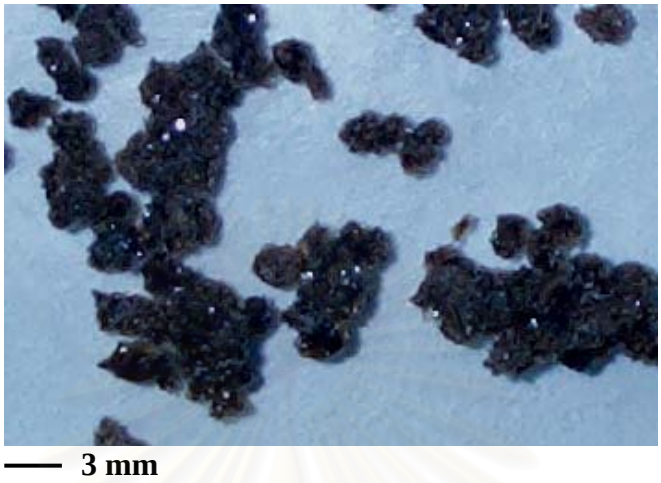

Figure B20 Dry surface PPA-crosslinked beads prepared from Mahachai chitosan containing chitosan:2,4-D (1:1 w/w).

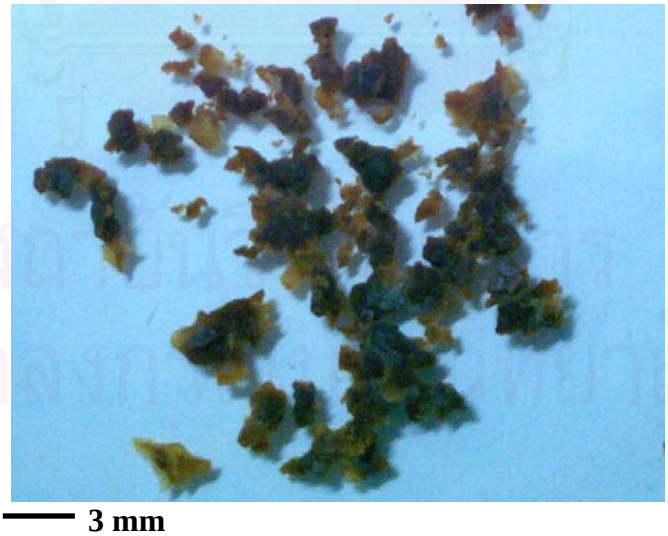

Figure B21 Dry bulk GA-crosslinked beads prepared from Taming chitosan containing chitosan:2,4-D (1:1 w/w).

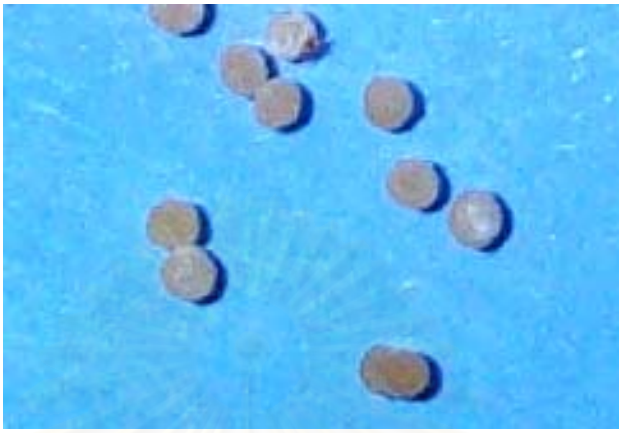

Figure B22 Dry uncrosslinked beads prepared from Seafresh chitosan containing chitosan:IAA (5:1 w/w).

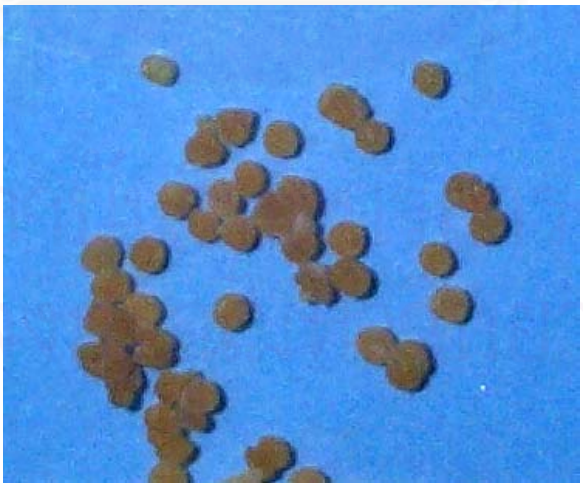

Figure B23 Dry bulk GA-crosslinked beads prepared from Seafresh chitosan containing chitosan:IAA (5:1 w/w).

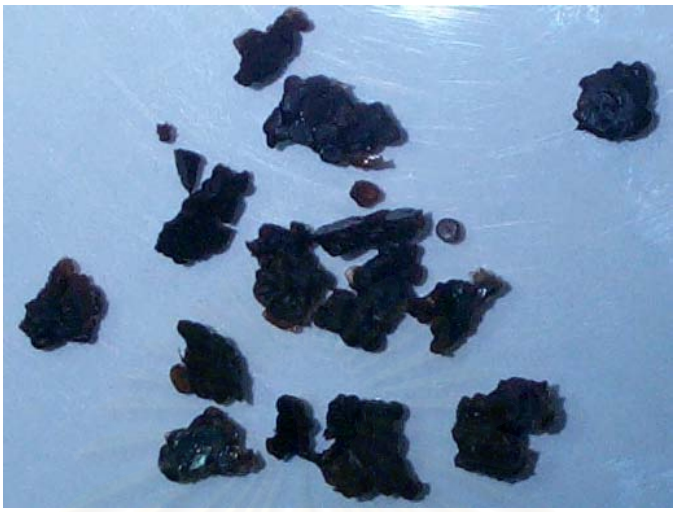

Figure B24 Dry surface PPA-crosslinked beads prepared from Seafresh chitosan containing chitosan:IAA (5:1 w/w).

APPENDIX C

The falling times in a Ubbelohde tube were recorded (Tables A1-A4). The intrinsic viscosities [η] (Table C1) were obtained from the Y-intercept of the plots of (ln η_r) /C against C using linear least square (Figure C1).

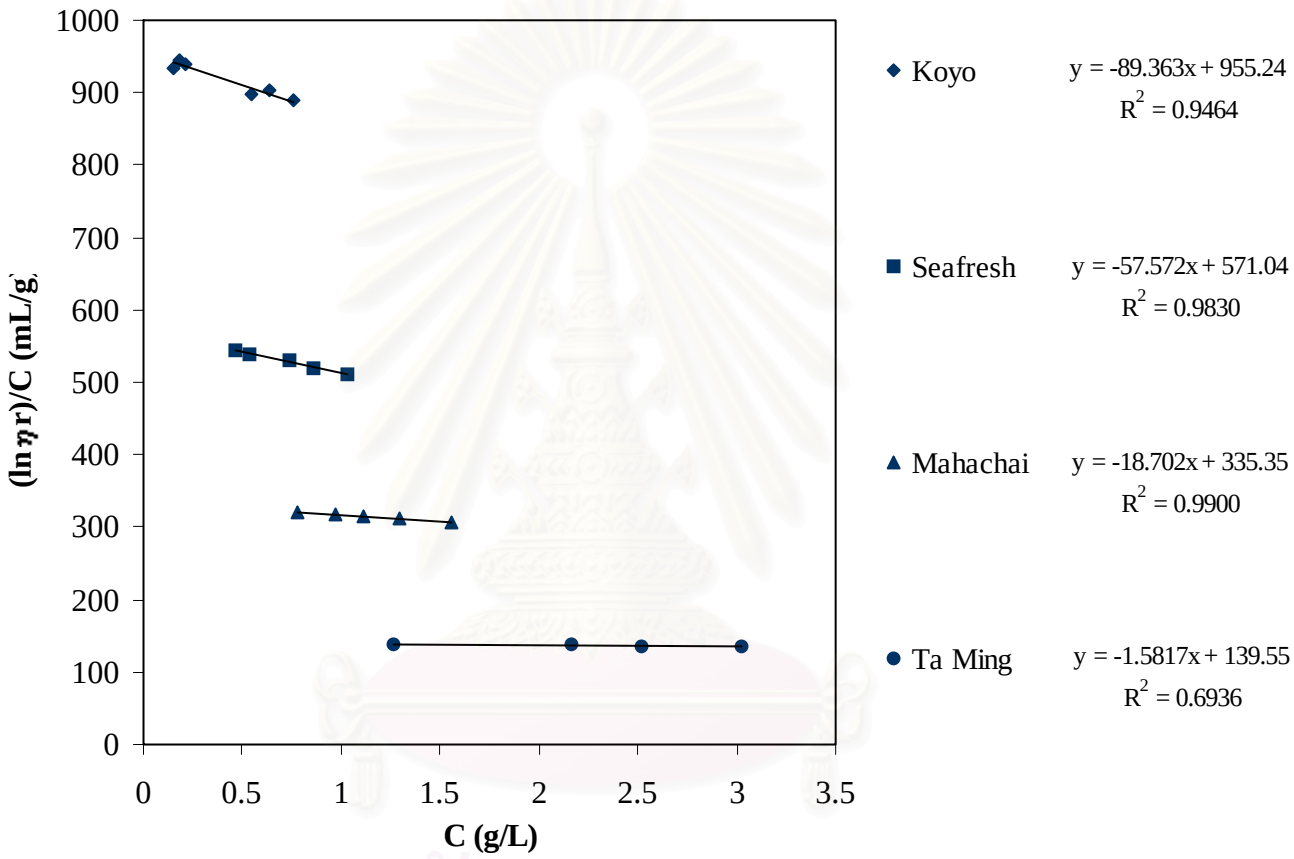

Figure C1 Plots of (ln η_r)/C against C and molecular weight of the various types of chitosan.

Table C1 Molecular weights of chitosan calculated from . = KM_v^a ($K = 1.8x10^{-3}$, $a = 0.93$).

Chitosan		log M_v	M_v (x 10^5)
Ta Ming	139.6	5.258	1.810 (1.781)[a]
Mahachai	335.4	5.667	4.645 (4.657)[a]
Seafresh	571.0	5.915	8.231 (8.288)[a]
Koyo	955.2	6.156	14.31 (14.19)[a]

[a] The M_v values in parentheses were calculated from . obtained from the plot of η_{sp}/C against C.

Molecular weights of chitosan calculated from the intrinsic viscosities [η] derived from the Y-intercept of the plots of η_{sp} /C and (ln η_r) /C against C had vicinal values. However, the plots of (ln η_r) /C against C of the chitosan from Ta Ming gave poor correlation factors (R^2) of about 0.6936, probably due to low molecular weight.

VITAE

Miss Somying Boonwan was born on November 23, 1977, in Nakornpathom, Thailand. She recieved her Bachelor of Science degree in Chemistry, Chulalongkorn University, in 1998. Since 1999, she has been a graduate student under the Program of Petrochemistry and Polymer Science at Chulalongkorn University, and completed her Master of Science degree in 2001.