เอนแคปซูเลชันของเพนดิเมทาลินด้วยบีดแอลจิเนตคอมโพสิต

นางสาวเกตุแก้ว กามูณี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ENCAPSULATION OF PENDIMETHALIN BY ALGINATE COMPOSITE BEADS

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
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เกตุแก้ว กามูณี : เอนแคปซูเลชันของเพนดิเมทาลินด้วยบีดแอลจิเนตคอมโพสิต (ENCAPSULATION OF PENDIMETHALIN BY ALGINATE COMPOSITE BEADS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร.วรินทร ชวศิริ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ.ดร.นงนุช เหมืองสิน, 96 หน้า.

งานวิจัยนี้ได้พัฒนาสูตรสำหรับเม็ดบีดไคโทซานและไคโทซาน-แอลจิเนตที่มีเพนดิเมทาลินและเพื่อศึกษาการปลดปล่อยเพนดิเมทาลิน ในเบื้องต้นได้ศึกษาการเตรียมเม็ดบีดไฮโดรเจลของไคโทซาน-เพนดิเมทาลินและพบว่าการปลดปล่อยเพนดิเมทาลินที่เก็บในเม็ดบีดไคโทซานมีปริมาณน้อยไม่เพียงพอเพื่อที่จะเพิ่มอัตราการปลดปล่อยของเพนดิเมทาลิน จึงใช้แอลจิเนตซึ่งเป็นพอลิเมอร์ที่ละลายน้ำได้ดี และสามารถเป็นไฮโดรเจลได้กับไคโทซาน การศึกษาแสดงให้เห็นว่าเม็ดบีดไคโทซาน และไคโทซาน-แอลจิเนตมีอัตราการปลดปล่อยที่ต่ำ ซึ่งทำให้จำเป็นต้องใช้เม็ดบีดมากขึ้นในแปลงเกษตร เพื่อที่จะแก้ปัญหานี้จึงเตรียมเม็ดบีดแอลจิเนตเพื่อใช้ในการควบคุมการปลดปล่อย เมื่อเม็ดบีดแห้งพบว่าเม็ดบีดสูญเสียรูปทรงกลมและหดตัวลง จึงเตรียมเม็ดบีดโดยใช้แอลจิเนตกับแป้ง จาก SEM ยืนยันว่าเม็ดบีดมีรูปทรงกลม และ FTIR ยืนยันความคงตัวทางเคมีของเพนดิเมทาลินในแอลจิเนต-แป้ง ได้เตรียมเม็ดบีดที่ต่างกันโดยการแปรชนิดของแป้ง อัตราส่วนของแอลจิเนตต่อแป้ง ความเข้มข้นของแคลเซียมคลอไรด์และเวลาในการแช่ในสารละลายแคลเซียมคลอไรด์ พบว่าหากความเข้มข้นของแคลเซียมคลอไรด์ และเวลาที่ใช้แช่ในแคลเซียมคลอไรด์มากขึ้น การบวมตัวและการปลดปล่อยลดลง กลไกการปลดปล่อยสารออกฤทธิ์ของเม็ดบีดแอลจิเนต-แป้งสามารถปลดปล่อยเพนดิเมทาลินโดยเริ่มจากเม็ดบีดบวมตัว และสลายตัวพร้อมปลดปล่อยเพนดิเมทาลิน จากการศึกษาแสดงให้เห็นว่าเม็ดบีดแอลจิเนต-แป้ง มีการปลดปล่อยของเพนดิเมทาลินเพียงพอที่สามารถกำจัดวัชพืชได้ จากการศึกษาประสิทธิภาพพบว่าเม็ดบีดแอลจิเนต-แป้งสามารถควบคุมการงอกของวัชพืชได้

สาขาวิชา..ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์...ลายมือชื่อนิสิต.....

ปีการศึกษา..2554..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

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KETKAEW KAMUNEE: ENCAPSULATION OF PENDIMETHALIN BY ALGINATE COMPOSITE BEADS
ADVISOR: ASST.PROF. WARINTHORN CHAVASIRI, Ph.D.,
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In this research, the formulations for chitosan and chitosan-alginate beads containing pendimethalin were developed and explored for their control release ability. The entrapment of pendimethalin in chitosan beads was investigated. The release rate of pendimethalin loaded chitosan was not satisfactory. To increase the release rate of pendimethalin, alginate as a good water soluble polymer and can act as hydrogel was used in combination with chitosan. The study showed that chitosan and chitosan-alginate beads had very low release rates. To solve this problem, alginate beads were prepared to control the release of pendimethalin. During drying process of making beads, the initial spherical shape was lost and shrinkage occurred. The beads were eventually prepared using alginate and starch. SEM images of beads suggested the formation of spherical beads. FTIR confirmed chemical stability of pendimethalin in alginate-starch beads. A series of beads with different compositions were prepared by varying type of starch, ratio of alginate, starch, concentration of $CaCl_2$ and immersion time. When the concentration of $CaCl_2$ and immersion time were increased, the swelling and release rate decreased. The mechanism of alginate-starch beads was believed to release pendimethalin at the beginning once the beads were swelled and then eroded together with pendimethalin release. The study showed that alginate-starch beads had sufficient release of pendimethalin. The efficacy study confirmed that alginate-starch beads could be used to control the germination of weeds.

Field of Study: Petrochemistry and Polymer Science Student's Signature .....
Academic Year: 2011 ..... Advisor's Signature .....
Co-advisor's Signature .....

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CHAPTER I

INTRODUCTION

1.1 Overview

Thailand is an agricultural country. In 2002, more than half of the Thai population (about 34 million people or 55.7%) were agriculturists. (Department of agriculture, ministry of agriculture and cooperatives, agricultural country's data year 2003). Therefore, it is necessary to use pesticides to protect agricultural crops. There are more than 1,000 species of pesticides sold in the agricultural market. The types of pesticides used for controlling and eliminating are insecticides, herbicides, fungicides, mollussicides, acaricides, rodenticides, *etc*. Pendimethalin is a pre-emergence herbicide activated by adsorption inhibiting the cell division and cell elongation of weeds during the first period. Then the treated weeds would die a short time after germination. Pendimethalin, typically is sold in the form of pendimethalin 33%w/v under the brand name "Stomp" or others. Commercially, "Stomp" is imported in a form of emulsifiable concentration (EC) using xylenes as solvent which is highly toxic, expensive and dangerous. In 2010, Thailand imported more than 452 tons of "Stomp" (valued at around 43.15 million Baht), whereas those numbers increased in 2011 to 569 tons (82.40 million Baht). When the residue of solvent is exposed to water and air, it is harmful to living organisms and environment. Moreover, in each subsequent application, the additional amount of active ingredients and solvents cause a lot of waste. It is necessary to maximize the performance and efficiency of pendimethalin in consideration of appropriate climate and environmental factors to control weeds by using a minimal amount of pendimethalin within the required time. This research was intended to reduce the use of pendimethalin and minimize the number of applications by controlling the release of pendimethalin containing in biodegradable polymer.

1.2 Objective of this research

This research focused on the physical characteristics and retained efficiency of pendimethalin in prepared beads. The release rate of pendimethalin, swelling of the beads and the stability of pendimethalin in the beads were explored. The objectives of this research are to search for a suitable formula for the beads using chitosan and alginate for controlling the release of pendimethalin.

CHAPTER II

THEORY AND LITERATURE REVIEWS

2.1 Herbicides [1]

2.1.1 Weeds and weed-related damage

Weeds are unwanted plants or plants that must be eradicated. Weeds can cause both direct and indirect damage as follows:

- Weeds compete for food, water, sunlight and space, *etc.* in the growth of crops.
- Weeds create a habitat for disease and insect pests, thereby increasing costs for prevention of the aforementioned disease and insect pests.
- The quality of crop yields decline due to contamination by weed seeds.
- Weeds can cause damage to irrigation systems due to blocked drainage.
- Weed eradication leads to higher costs for agricultural production.
- Weeds adversely affect health and the environment. Some weeds may even affect the health of people or animals. Moreover, weed eradication, *e.g.* the use of fire or highly toxic herbicides, has a potentially negative effect on the environment.

Weed-related crop damage, particularly in the area of crop yield, caused destruction and loss to plants, such as rice (25-75%), corn (80%), soybean (40-80%), green beans (30-80%), peanuts (30-70%), cassava (20-90%), sugar cane (60%), *etc*.

2.1.2 Critical threshold level of competition between weeds and crops

Weeds which grow in planting fields cause problems for wanted crops by competing for food, nutrients, sunlight and water. Because weeds are able to adapt to the environment more easily than desired crops, weed populations grow higher than crop populations, so competition also increases.

Weeds affect crops by damaging crops or reducing crop yields. This competition has maximum effects on crop during certain growth periods. Thus, weeds should be controlled so crops can have healthy growth and maximum yield. This control can ensure that weeds are unable to compete with crops over the critical threshold level and should be eradicated at the critical period of competition.

Therefore, the competition points and periods are important and necessary for selecting the proper control method in terms of suitability, reasonable cost and safety. The control principle is that competition with weeds should be minimal at the critical period of competition so growth and yield can be maximal.

The critical period of competition for crops, especially annual crops, occurs at the vegetative growth period, or with initial planting up to 25-30% of the harvest age. Soybeans, for example, take 100 days to harvest, so the proper control period (no competition with weeds) should begin on the first day up until 25-30 days after planting. If the control is not carried out at the right period, the weeds will not be controlled and farmers incur higher expenses at the addition coast of time and impact on the environment.

2.1.3 Weed control and prevention

Weed control and prevention methods are numerous. Each method performs differently, depending upon the area conditions and operation methods selected. Weed control and prevention methods can be categorized into the following two groups:

1. Non-chemical weed control comprising the following:

1.1 The soil is plowed 1-2 times in order to prepare the soil before planting in rainy season when crops rely on rainfall. Properly tilled soil will reduce weed growth, so fields should be plowed at least twice, *i.e.* the first round involves rough plowing in order to eradicate the weeds. The objective of plowing is to allow the soil to dry so weeds die and the weed seeds in the soil are brought up to the surface to regrow. These weeds are left to grow in the fields until crops are planted. The ground can then be plowed for the second time in regular furrows or paddies so the newly sprouted seedlings of the weeds following the first plowing can also be eradicated before they germinate. Then crops should be planted immediately to help reduce the amount of weeds competing with growing plants, which may be sufficient for crop growth and maturity.

1.2 Manual or machine labor by using human or mechanical force, such as shovels for mowing, harrows for making grooves and mud piles *etc*. Sometimes, however, the abovementioned cannot be carried out due to various problems, *e.g.*, soil with high humidity, high wages, labor shortages, *etc*. Regardless, the suitable period

for eliminating weeds should be within approximately 15-20 days after weed germination, or after planting. If necessary, the aforementioned can be performed twice, depending upon the amount of weeds.

1.3 The use of fire to burn waste straw before planting can eliminate some weeds or weed seeds. However, the environmental impact, *e.g.* toxic fumes or destruction of animals and beneficial pests, traffic accidents caused by thick smoke and the spreading of uncontrolled fires, of frequent burning consider must be considered.

1.4 The use of mulch to create conditions unsuitable for germination and the growth of weeds can also reduce the problems. Furthermore, mulching also has other advantages, such as saving water, maintaining soil fertility, reducing chemical use and minimizing production costs.

1.5 The use of water level to flood on the soil surface and allow the flooding for a period of time to prevent weed germination or growth.

1.6 Selecting crop types, planting rates and proper planting methods to ensure as much quantity and competitive advantage with weeds as possible.

1.7 Crop rotation or switching crop planting can prevent and control weeds.

1.8 Weed control by biological weeds, such as diseases, insects and some plants, may be studied and extracted for use as organic herbicides in weed control.

2. Chemical weed control comprising the following:

The use of herbicides is a good method for controlling the weeds in a method that can save labor, reduce production costs and control weed during competition period. Although the right herbicides can be selected and used properly with no effect on crops, chemical herbicides can be harmful to the user. Hence, users of chemical herbicides require knowledge and understanding of the beneficial, safe and effective use of herbicides in weed control.

2.1.4 Application methods

There are numerous types of herbicides, each with the ability to destroy weeds in varying degrees according to the manufacturer's instructions, period of use and how to manage the herbicide for effective weed control. Manufacturers, therefore, need to test different types of herbicides in order to know the usage methods and application rates before selling. In addition, academic research needs to be conducted on the use of

herbicides in order to guide farmers in proper application per manufacturer's instructions to achieve optimum weed control. Moreover, herbicide applications must not be harmful to fish or other plants and crops planted nearby. Herbicides can achieve effective weed control if they meet the following requirements:

- Adequately contact plants.
- Sufficiently absorbed by plants.
- Move within the plants to the site of action without deactivating.
- Reach toxic levels at the site of plant. In consideration of the aforementioned limitations, the effective herbicide application requires knowledge of herbicide properties, *e.g.* how to use the substance, how to spray and how long the substance can/should be used.

2.1.5 Effective herbicide use

Herbicides sold on the market have the licensing requirements set forth by the Department of Agriculture (DOA). The DOA tests the performance of herbicides able to eridicate weeds in crop fields at the recommend ratio and instructions. Herbicides must be registered with sales and labels on the containers. The use of herbicide must follow the instruction guide contained on the label in order to meet requirements and be effective. Effective herbicide can be described by the following key practices.

1. Using the correct type of substance according to the instructions. The following considerations are essential to proper herbicide selection:

- Ability to use together with the crops in order to eradicate weeds without damaging crops.
- Ability to control weeds as desired.
- Reasonble prices.

2. Correct Spray Use

Farmers need to know how to use herbicides, *e.g.* knowledge of the right nozzle and spraying machine for spray applications. Sprayers must also exercise caution in preventing the droplets from contact with the crops. Hence, the spray may have a droplet cover or screen to cover the crops during application. All herbicides have different instructions regarding the features and performance of the herbicide.

Spraying is required to comply with instructions or restrictions in order to spray accurately and carefully.

3. Correct Usage Rates

If herbicides are used incorrectly according to the recommendations, the incorrect use will affect the efficiency of weed control and preventive measures to avoid affecting crops. The application rate recommended for eradicating weeds and protecting other plants from harm, therefore, is essential. Rates lower than the spray rate will not harm the plants, but neither will they be fully effective at weed control. Similarly, higher spray rater can eradicate weeds, but may have toxic effects on crops and increase the cost of planting, which may result in residues of substances that are harmful to the environment.

4. Specific Usage Times

Herbicides have the ability to destroy one another in different plants. To be effective, they must be used correctly according to the duration of such substance which may require spraying before weed germination. In addition, the spray delay over a certain period may able to eradicate weeds, but sometimes may not be effective in reducing weed competition and yields of crops or plants.

5. Personal and Environmental Safety

Herbicides are toxic substances for plants, people and animals. The spraying or use of an herbicide demands awareness of safety and security in handling, mixing, spraying, storage, disposal whereby containers must be correctly and carefully closed.

2.1.6 **Pendimethalin** [2]

Pendimethalin (chemical name : *N*-(1-ethylpropyl)-2,6-dinitro-3,4-xylidene (Figure 2.1) is a herbicide used in pre-mergence and post-emergence applications to control annual grasses and certain broadleaf weeds. It inhibits cell division and cell elongation. It is used to control annual grasses and certain broadleaf weeds which interfere with growth, development, yield and quality of agricultural and horticultural crops by competing for nutrients, water and light. Pendimethalin acts during pre-emergence, *i.e.* before weed seedlings have sprouted, and early post-emergence, *i.e.* shortly after weed seedlings have sprouted, pendimethalin inhibits root and shoot growth. It controls the weed population and prevents weeds from emerging,

particularly during the crucial development phase of the crop. Its primary mode of action is to prevent plant cell division and elongation in susceptible species. Physical and chemical properties of pendimethalin are shown in Table 2.1.

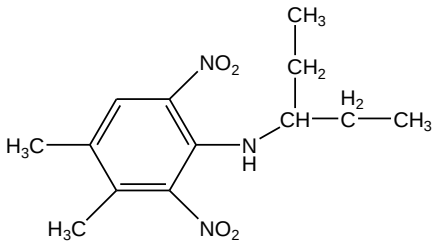


Figure 2.1 Chemical structure of pendimethalin.

Table 2.1 Physical and chemical properties of pendimethalin. [3]

Parameter	**Value**
Physical state	Crystals
Color	Orange-brown solid
Odor	Faint nutty or fruitlike
Melting point	57.7-58 °C (99.6 %)
Boiling point	Estimated 330 °C
Specific gravity	1.19 (25 °C)
Relative density	0.85 g / ml at 25 °C
Vapor pressure	1.94 x 10^{-3} Pa at at 25 °C
Solubility in water	pH 4: 0.54 mg / l at 20 °C pH 7: 0.33 mg / l at 20 °C pH 10: 0.44 mg / l at 20 °C
Solubility in organic solvents	n-Hexane = 48.98 g / l n-Octanol = 66.08 g / l Dichloromethane > 800 g /l 20 °C Xylene > 800 g /l Ethylacetate > 800 g /l Acetone > 800 g /l
Explosive properties	Non-explosive
Flammability	Non-flammable
Chemical name: N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine	

2.2 Encapsulation

2.2.1 The encapsulation [4]

Encapsulation is a process that active ingredient (solid, liquid or gaseous content) are surrounded by coating to form small capsules. The material or active ingredients inside microcapsule is referred to as a core whereas the surrounding material forms a shell. Encapsulation is a technology for controlled release. The advantage of the controlled release pesticides is that they can release active ingredients in the demanded quantity compared with non-controlled release pesticides during the same period of use. Typically, the half-life of these pesticides is short, easily adsorbed and decayed by light, moisture and microbes. If the amount of pesticides used and released can be controlled, the amount of pesticides used can be reduced. Encapsulation technologies with biodegradable polymers are used widely in food, medicine, cosmetic and other industries. Polymers are selected depending upon the properties and objectives. In general, polymers used are those that are easy to find, biodegradable and leave no residues harmful to living creatures or the environment. Frequently selected pesticides also have controlled release properties. Some examples are the polymers used in encapsulations, *e.g.* chitosan, alginate, ethyl cellulose, starch, *etc*. The polymers should be selected as appropriate for the needs of released substance. Other parameters requiring consideration are effects on the release patterns, *e.g.* the amounts and methods of preparation, *etc.* Coacervation is an encapsulation by chemical technique consisted of three phrases of colloids, covering continuous phase, core material, and coating material phase. In this case, the coating is based on reconditioning of two kinds of hydrophillic colloids with different ions to be neutral and coated along the surface of core material.

2.2.2 The reasons for encapsulation [5]

- Protection or isolation of the core materials from the surrounding attributes, such as, light, oxygen, water, chemicals, *etc*.
- Improvement the handling properties, such as the phase transformation inside the shell.
- Control release of the core materials at the targeted application.
- Masking of the odor, taste, and activity of encapsulated materials.

- Separation of incompatible components.
- Controlled time release of active ingredient in the core.

2.2.3 **Biodegradable polymer** [6]

Biodegradable polymers are polymers that break down and lose their initial integrity. Biodegradable polymers are used in medical devices to avoid a second operation to remove them, or to gradually release a drug. Applications of biodegradable polymers include sutures, controlled drug release and tissue engineering. Biodegradable polymers could also be implemented in drug delivery. The polymer slowly degrades into smaller fragments, releasing a natural product, and there is controlled ability to release a drug. The drug slowly releases as polymer degrades. Biodegradable polymers have been used to coat a stent and release drugs in a controlled way. Biodegradable polymers should be non-toxic, capable of maintaining good mechanical integrity until degraded, and capable of controlled rates of degradation.

A goal is not to illicit the immune response, and the products of degradation also need to be non-toxic. In addition, it should control the rate at which water can get into polymers.

Factors controlling the rate of degradation include percent crystallinity, molecular weight and hydrophobicity. The degradation rate depends on the location in the body. The environment surrounding the polymer is different depending on the location in the body.

2.2.3.1 **Chitosan** [7]

Commercial chitosan is derived from the shells of shrimp and other sea crustaceans. Chitosan is manufactured by deacetylation of *N*-acyl group by heating chitin in a highly concentrated (40%) alkali solution or heating powdered chitin in fused $Ca(OH)_2$ at 180◦C for 30 min. Chitosan is positively charged and soluble in acidic to neutral solution, with a charge density that depends on pH and the percentage degree of deacetylation (DA value). It binds to negatively charged surfaces. Chitosan is biocompatible and biodegradable. Purified chitosans are available for biomedical applications. Chitosan and its derivatives have been used in non-viral gene delivery for

the transfection of breast cancer cells; with approximately 50% degree of trimethylation, the derivative is most efficient at gene delivery. Chitosan is a polycation that can be crosslinked with multivalent anions. This option can be used to prepare beads.

A chitosan–glycerol–water gel or gel-like membrane, useful as a carrier for medications to be applied to wounds, was prepared by dissolving chitosan in an acid–water–glycerol solution which when neutralized forms a gel upon standing. A typical simple example of gel formation was provided with chitosan tripolyphosphate and chitosan polyphosphate gel beads. Chitosan gel beads could also be prepared in an amino acid solution at about pH 9, despite the requirement for a pH above 12 for gelation in water. pH-sensitive hydrogels were also synthesized by grafting *D,L*-lactic acid onto the amino groups in chitosan without catalyst. Glutaraldehyde was used as a crosslinking agent for chitosan. A semi-interpenetrating network was synthesized with poly(ethylene oxide) and chitosan and crosslinked with glyoxal. Advances in the field of chitosan gelation promote biomedical applications that use microgel or nanogel particles for drug delivery. The chemical structure of chitosan is shown in Figure 2.2.

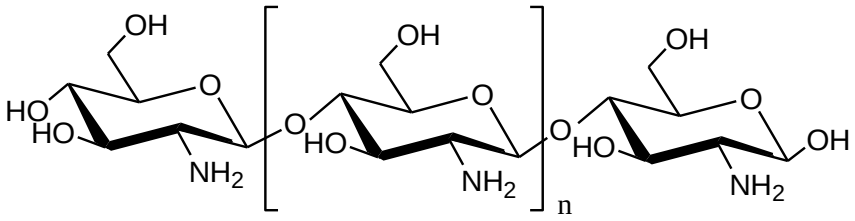


Figure 2.2 Chemical structure of chitosan.

2.2.3.2 Alginate [8]

Alginate is a substance extracted from brown seaweed (Phaeophyceae). For example, *Macrocystis pyrifera* contains alginate at approximately 14-19%, while *Laminaria cloustoni* and *L. digitata* contain alginate at approximately 15-40%. This percentage of alginate contained depends upon types of seaweed, seasonal changes and place. Algae are found worldwide and the countries producing high volumes of alginate are the United States, the United Kingdom, France, Spain, Norway, Canada and Japan.

Alginate is made of unbranched binary copolymers of 1, 4-β-*D*-manuronic acid (M) and *L*-guluronic acid (G) in a molecule including homopolymeric regions of G and M called G-and M-blocks, respectively. A part of the molecular structure of MG-blocks is shown in Figure 2.3. The proportion of copolymers and these structures determine the properties of alginate. If the polymer line has a high amount of G, it will have hard gel properties with high concentration of cationic metals only (polyvalent metal cation). However, if the polymer has a high amount of M, it will have soft gel properties and the conditions of the gel will be wider. Commercial alginate has many derivatives, so the solubility properties in water are different, *e.g.* the derivatives of salt (Ca^{2+}, K^+, Na^+, NH_4^+) are also produced in the form of propylene glycol alginate obtained from the reaction of alginic acid with propylene oxide under pressure. These derivatives are soluble in both hot and cold water. The viscosity of alginate solution depends upon temperature, concentration, molecular weight and positive charged of metal. Not all alginates have gel properties which appear when reacting with Ca^{2+}. The gel structure is similar to an egg box with Ca^{2+} on the line as shown in Figure 2.4. The good properties of alginate is its irreversible gel in cold water, including Ca^{2+}. This property of the gel at low temperatures makes alginate unlike hydrocolloid derived from red seaweed.

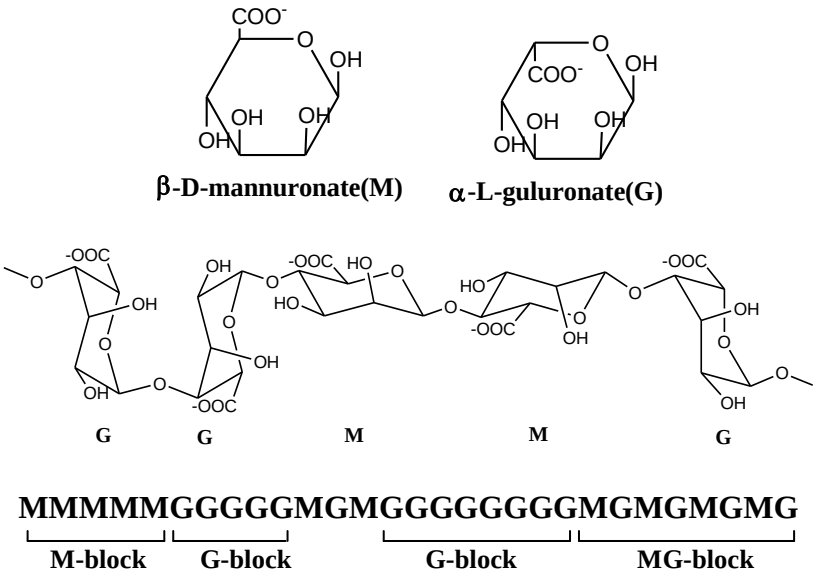


Figure 2.3 Structure of alginate.

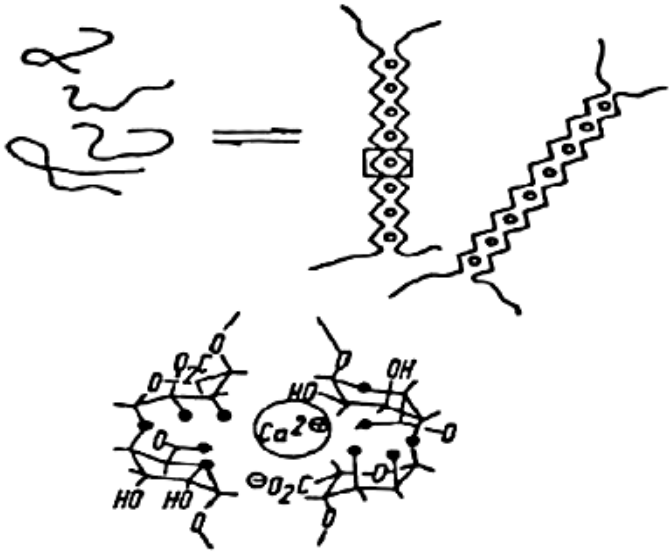


Figure 2.4 Gel structure of calcium alginate (egg-box model).

Alginate has been used in many food products since 1920s as additive to some canned foods to increase the viscosity and stabilizers resulting in emulsion stability. It is also used to make gel and inhibit the syneresis, *e.g.* propylene glycol alginate used in salad dressing and beer, because it is highly soluble at low pH. Sodium alginate is used as an ingredient in lemon pie filling, which is chilled to ensure stability during the freeze-thaw. Other uses include coating on fish surfaces before freezing to prevent freezer burn on the fish meat, use as stabilizers for ice cream, frozen desserts, sherbets and processed cheese in addition to use as alginate gel restructured products such as onion rings and shrimp-like fish products.

2.2.3.3 Starch [9]

Starch is a polymer of glucose with a larger molecule size, the general formula is $(C_6H_{10}O_5)_n$. Starch is the basic unit of anhydroglucose units connected by bonding α-glycosidic linkage at carbon position 1 of the glucose unit and at carbon position 4 of the glucose unit of the next. The ends of starch molecules are anomeric carbon (C1), which is unbound and free. Thus, each molecule of starch has a reducing end. One starch molecule will have one position reducing end. The molecular structure of starch is divided into two kinds of principles according to molecular size and type of arrangement, *i.e*. amylose, which has small sides and small branches in addition to

amylopectin, which is larger and has more branches. Another type of molecular starch, which is larger than amylose, but smaller than amylopectin, has been found in small volumes and called an "intermediate material". Amylose and amylopectin have different properties as shown in Table 2.2.

Table 2.2 - Different properties of amylose and amylopectin. [10]

Amylose	Amylopectin
1. Glucose molecules are connected together by bonding a-1, 4.	1.Glucose molecules are connected together by bonding α-1,4- and the branching insoluble, α-1,6-.
2. Consists of 200 – 6000 units of glucose.	2. Each branch has 20-25 glucose units.
3. Less water solubility.	3. More water solubility.
4. When boiled in water is low viscosity.	4. Viscous and clear when boiled in water.
5. Blue when reacting with iodine solution.	5. Purple, red or reddish brown when reacting with iodine solution.
6. Becomes a solid plate when boiled and setaside.	6. Not binding as gel and strength.

Amylose is a linear polymer containing approximately 1,000-6,000 units of glucose connected by bonding α-1,4-glycosidic linkage as shown in Figure 2.5 where branches in the molecules of amylose may be found in some small amount

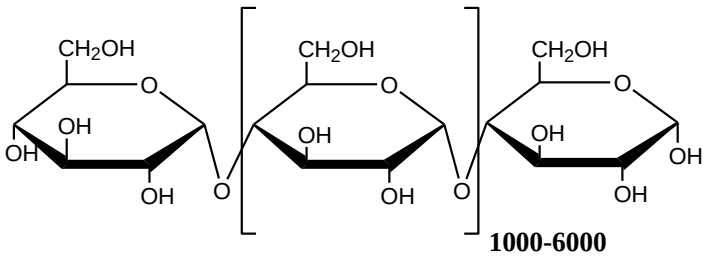


Figure 2.5 Structure of amylase.

In general, starch from cereals such as wheat starch and sorghum starch, has high amylose content of approximately 22-30%. The starch from roots and bulbs, such as cassava and potato starch, has lower amylose within a range of 18-24%. Amylose molecular weight ranges from 105 to 106 daltons. Amylose in starchs is different in molecular weight because each type of starch has different degrees of amylose polymerization (DP). Potato starch with DP of amylose in the range of 1,000 to 6,000 is higher than corn and wheat, which have DP of 200 to 1,200 lines of amylose. Longer lines will decrease the retrogradation. The amount and properties of amylose in each starch are shown in Table 2.1.

Amylopectin polymers are branches of glucose. The linearity of glucose is connected by bonding α-1,4-glycosidic linkage and a sub branch that is a polymer of short chain glucose with DP ranging from 10 to 60 units connected together by bonding α-1,6-glycosidic linkage as shown as Figure 2.6.

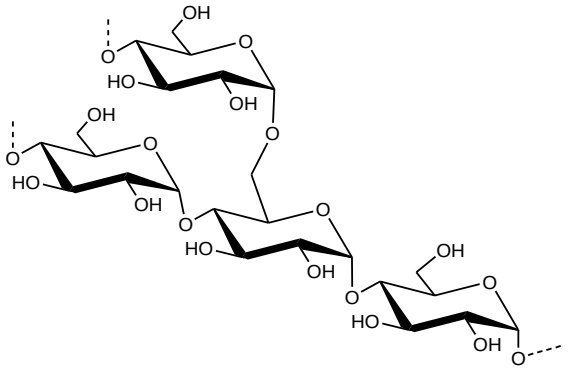


Figure 2.6 Structure of amylopectin.

Glucose units are bound by α-1,6-glycosidic linkage at approximately 5% of the glucose units in amylopectin. Amylopectin has approximately 1,000 times the molecular weight of amylose. Amylopectin has a molecular weight of approximately 107 to 109 kDa and a low recovery because amylopectin has a branch structure. Amylopectin acts as the main structure of starch granules, so only amylopectin molecules can be formed into granular starch.

2.3 Literature Reviews

In 1990, Shukla *et al.* [11] studied the swelling and release of carbofuran by using starch-urea formaldehyde for the encapsulation. The starch-urea formaldehyde matrix shows an inverse relationship between rate of release and extent of crosslink. The release rate was decreased when the cross-linking was increased. If the amount of carbofuran was increased, the release rate was higher compared with the formula using a lower amount of carbofuran. If the amount of carbofuran was increased, the porosity and solubility in the beads were increased. This released study was investigated using different ratios of solvent (0, 50, 70 and 100% MeOH) according to differing degrees of carbofuran solubility. The burst effect will be effective for initial knockdown of pest, and the further slower release for the sustained protection of plant from later attacks for a longer period.

In 1991, Chong *et al*. [12] reported the release of blue dextran from alginate beads which were used as the model of macromolecular drugs. Blue dextran was added to sodium alginate and dissolved completely; this solution was then transferred dropwise to a $CaCl_2$ solution and then alginate beads were separated from the solution and dried. The Na^+ in the droplets was substituted by Ca^{2+}, thus yielding alginate gel. At this point, on inspection of an alginate bead against light, a circular boundary was observed within the bead, constituting the border of ion exchange between Na^+ and Ca^{2+}. The alginate beads were very spherical (Figure 2.7A) and, subsequent to drying, their sizes decreased to approximately l/3 of the radius (Figure 2.7B). The release of blue dextran from alginate beads was considerably affected by drying time and the blue dextran/sodium alginate ratio. Moreover, under preparative conditions that were maintained constant, the release of blue dextran displayed no significant dependence on the other experimental factors, such as sodium alginate concentration, $CaCl_2$ concentration, curing time and drop size. Therefore, alginate beads can be prepared and show reproducible release behavior which indicate that the development of alginate beads hold promise for manufacturing purpose in the future.

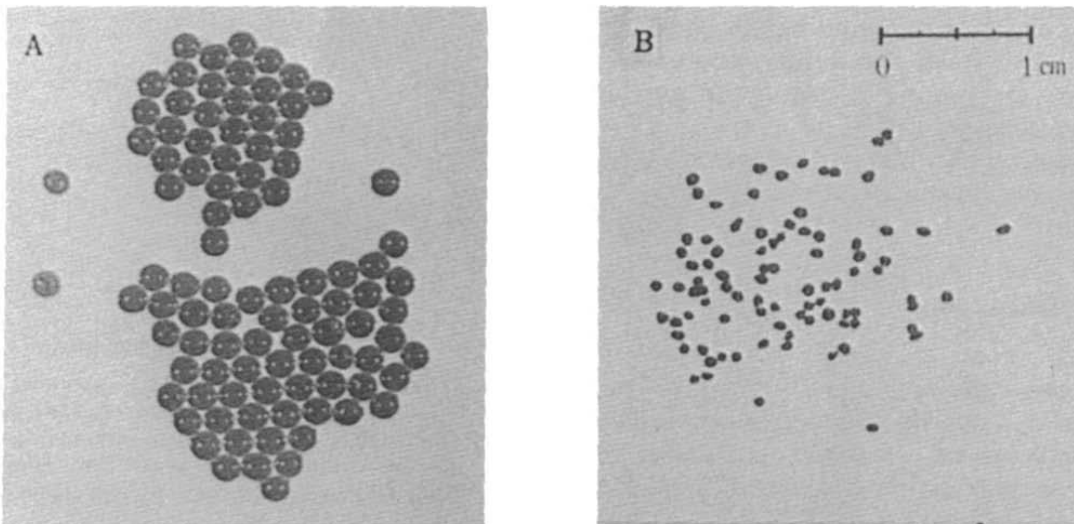


Figure 2.7 Photographs of alginate beads before drying (A), after drying (B).

In 1995, Bergman [13] examined the encapsulation of pendimethalin in the form of microcapsule using poly(vinyl alcohol) (PVA), a film polymer membrane with surfactants. By heating pendimethalin at 60^0C for melting and maintaining a liquid state while preparing, the primary particle size was found to be approximately 1-12 microns. It was then dried using a spray dry at temperatures of 50 and 220^0C. The resulting microcapsule is shown in Figure 2.8.

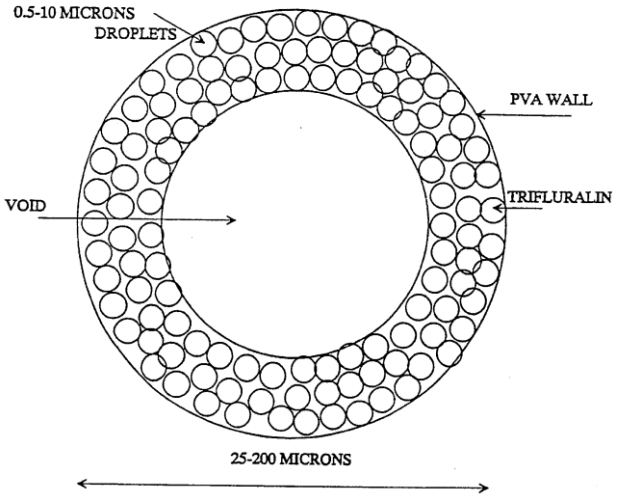


Figure 2.8 Pendimethalin microcapsule.

In 1997, Fernandez-Hervas *et al.* [14] studied *in vitro* evaluation of alginate beads of diclofenac salt. Alginate beads containing diclofenac hydroxyethypyrrolidine were formulated with either Eudragit or chitosan in order to achieve an enteric formulation. In all cases, high entrapment efficiencies were obtained. The examination of fractured beads revealed internal void in the Eudragit-alginate beads. In contrast, a dense homogeneous internal structure was observed in chitosan-alginate beads due to interpolymeric complex. An interaction between chitosan and drug was also observed. Under condition mimicking those in the stomach, a small amount of drug was released. The alginate-chitosan beads showed a release behavior dependent on pH value and alginate-chitosan ratio.

In 2001, Mi *et al.* [15] studied the release of active ingredients in chitosan and alginate beads by using genipin for cross-linking, thereby suggesting that the beads consist of an obvious inner core and outer skin layers. The preparation was done by dropping chitosan into a genipin-alginate solution with various concentrations of alginate. This study disclosed that the reaction between chitosan and alginate happened at the outer surface of the beads, while chitosan and genipin cross-link happened at the inner layer of the beads as shown in Figure 2.9. The swelling ratio of the cross-linking reinforced chitosan-alginate complex beads decreased as pH or concentration of alginate in the gelling solution was decreased. The protonation of amine group of chitosan by hydrogen ions from acid or the shield of charge of ammonium group of chitosan by Cl^- ions resulted in the decrease of cross-linking density due to the inhibition of nucleophilic attack on the dihydropyran ring of genipin. Contrary to the swelling properties, the rate of indomethacin releasing out of chitosan-alginate complex beads increased with the decrease in pH or concentration of alginate in the gelling solution due to the decreased cross-linking density of the beads.

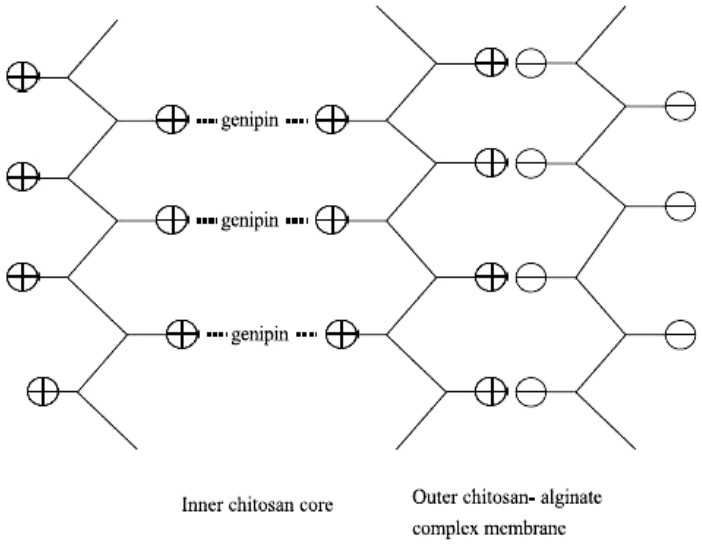


Figure 2.9 Chitosan-alginate beads: outer and inner chitosan-alginate complex.

In 2004, Almeida *et al*. [16] developed a new particulate drug delivery system using sodium alginate matrix containing pindolol as the model drug molecule for prolonged release of intestinal drugs. Calcium alginate beads are known to be unable to control the release of insoluble drugs. Thus, calcium alginate beads were prepared by dropping an aqueous suspension of sodium alginate into a $CaCl_2$ solution. As particles were drying, the initial spherical shape was lost (Figure 2.10).

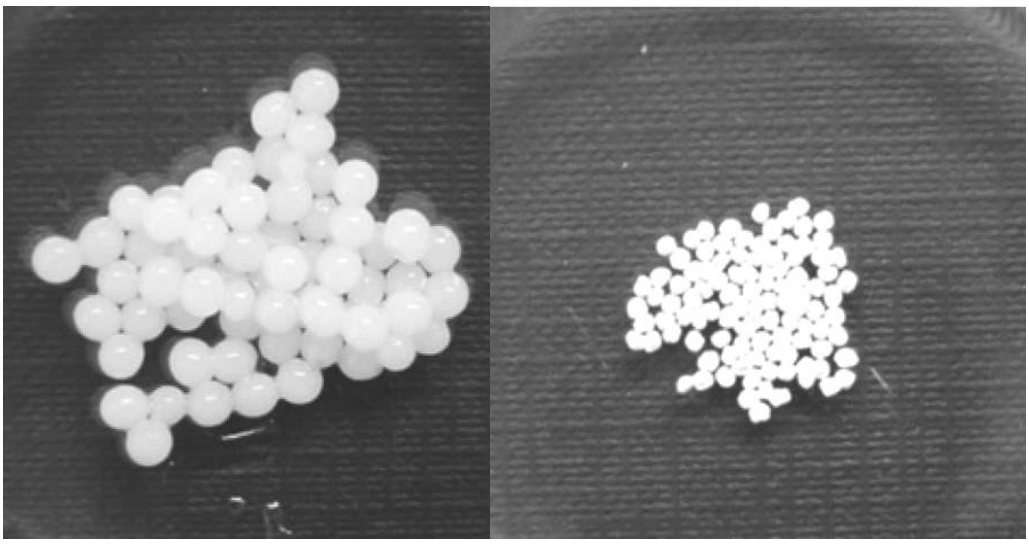


Figure 2.10 Alginate bead (a) before drying; (b) after drying.

Microscopic analysis shows that beads prepared with calcium alginate or calcium alginate-gelatin present an irregular shape. The inclusion of gelatin in the matrix creates beads with a rough surface, which becomes smoother when sodium alginate and gelatin are cross-linked with formaldehyde. High encapsulation efficiency was achieved for all formulations (≥80%, w/w). The drug release from alginate matrices is usually modulated by a swelling-disolution-erosion process. The use of cross-linking agent successfully delays pindolol release, which can be tailored to produce a suitable single dose administration per day by adjusting the quantity of cross-linking agent and obtain the desired time of complete drug release. Therefore, matrix modification by an appropriate method, such as a suitable cross-linking reaction, is crucial in the controlled release of pindolol from alginate beads. Further work is currently being carried out in order to both render the beads gastro resistant and to reduce particle size while maintaining the vitro prolonged release profile.

In 2006, Pasparakis *et al.* [17] studied the swelling behavior and release of the antihypertensive drug, verapamil hydrochloride, from calcium alginate and chitosan-treated calcium alginate beads. The swelling ability of the dry calcium–alginate beads in water was lower when coated with chitosan (Figure 2.11(a-b)). As chitosan membrane reduced the permeability of the beads, a fraction of the hydrophilic groups at the surface of dry calcium alginate beads formed a polyelectrolyte complex with the amino groups of chitosan. Hence, it did not contribute to the entrapment of water molecules within the beads. The phenomenon was more obvious in the case of dry alginate–chitosan mixed beads exhibiting even lower degrees of swelling (Figure 2.11c). Alginate–chitosan mixed beads possess greater swelling resistance when exposed to aqueous media due to the greater physical entanglement of polymer chains.

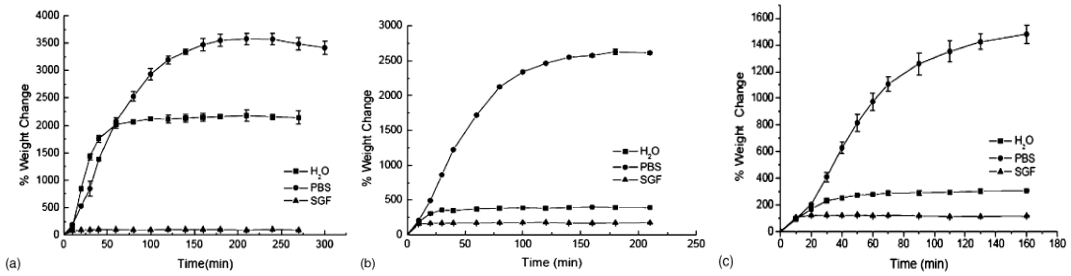


Figure 2.11 Swelling profiles of dry beads in H_2O, PBS and SGF: (a) calcium–alginate beads, (b) chitosan-coated alginate beads and (c) alginate–chitosan mixed beads. Values are the mean ± standard deviation (S.D.) of the three experiments.

The results revealed that the encapsulation of verapamil in both calcium–alginate and calcium alginate–chitosan mixed beads exceeded 80%. Considering the stability of verapamil encapsulating beads, 70% of drug was released from wet and dry plain calcium alginate beads within 1 and 3 h, respectively. The presence of chitosan was found to significantly retard the release from the wet beads. However, in the case of dry beads, the presence of chitosan had no significant effect on the initial release stage and significantly increased the release at the later stage (Figure 2.12).

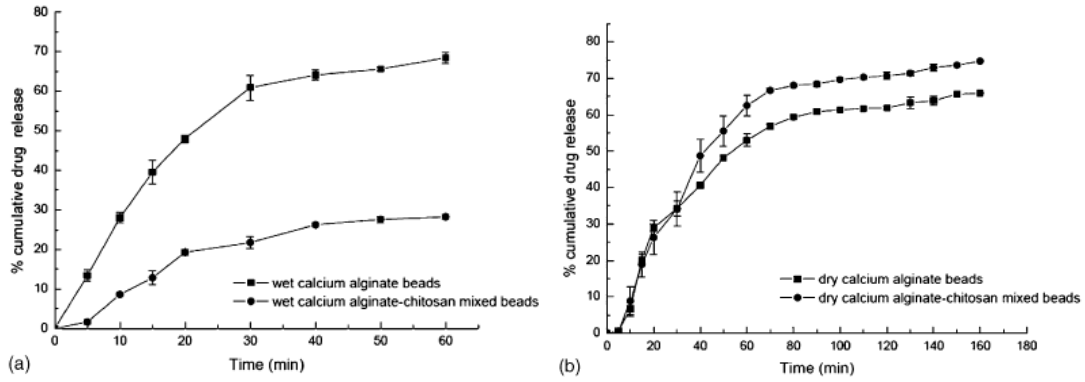


Figure 2.12 Cumulative release of VRP from (a) wet calcium–alginate and alginate–chitosan mixed beads and (b) dry calcium–alginate and alginate–chitosan mixed beads. Values are the mean ± standard deviation (S.D.) of the three experiments.

In 2006, Babu *et al.* [18] prepared a microsphere of nifedipine with sodium alginate, methyl cellulose and glutaraldehyde. These microspheres were cross-linking with glutaraldehyde and loaded with nifedipine, an anti-inflammatory drug. The blend

matrix is cross-linking with glutaraldehyde as shown in Figure 2.13. SEM picture of microspheres suggested the formation of spherical particles. Swelling experiments on the microspheres provided important information on drug diffusion properties. The swelling studies of microspheres have shown that with an increasing amount of methyl cellulose in the microspheres, water uptake has decreased. This effect is correlated with the release rates of the drug though the microspheres containing different amount of methyl cellulose.

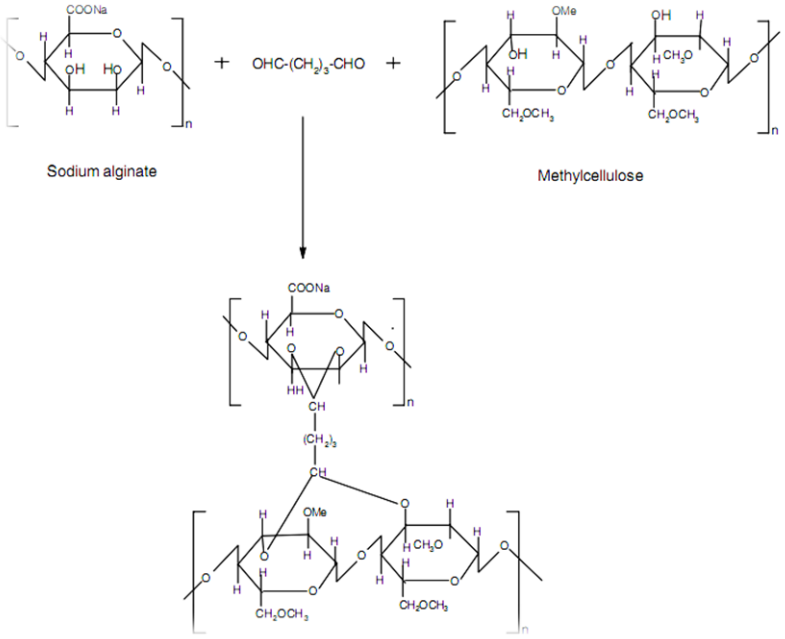


Figure 2.13 Formation of sodium algenate-methyl cellulose blend microsphere crosslinked with glutaraldehyde.

In 2008, Singh *et al.* [19] developed starch- and alginate-based controlled and sustained agrochemical delivery systems in the form of beads using $CaCl_2$ as cross-linker. To study the effects of the composition of the beads on the release dynamics of fungicide (thiram), beads were prepared by varying the amount of starch, alginate and cross-linker. The percentage yield of starch-alginate-Ca^{2+} beads was not affected by the increase in starch content, but it did increase with increases in the alginate content and cross-linker concentrations in the composition of the beads, *i.e.* more and more alginate and Ca^{2+} were available for cross-linking of alginate and calcium, which increased the percentage yield of the beads. These interactions starch-alginate beads

are shown in Figure 2.14. The cumulative release and percentage release of the total entrapped thiram decreased with the increase in cross-linker concentration in the formulation.

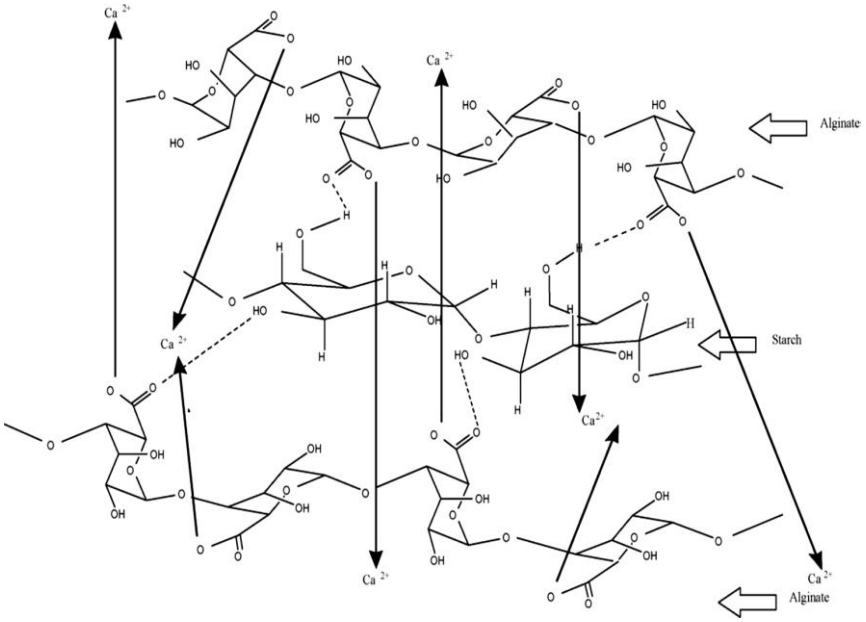


Figure 2.14 Structure of starch–alginate–Ca^{2+} beads.

In 2008, Roy *et al.* [20] studied the biopolymer microsphere of sodium alginate and starch prepared by employing $CaCl_2$ as a cross-linker. A series of starch microspheres of different composition were prepared by varying the amount of sodium alginate, starch, $CaCl_2$ in the feed mixture. The prepared microspheres were loaded with an insecticide, chlorpyrifos. The swelling and erosion controlled release of insecticide was investigated for 8 days taking by distilled water as a release medium. The prepared microspheres showed greater swelling with increasing wt% of alginate and decreasing wt% of starch exhibiting an optimum water uptake at a definite bead composition. The biopolymeric beads showed a significant fall in their swelling ratio with increasing cross-linking. The biopolymeric beads of calcium alginate and starch not only swelled in aqueous environment but also showed erosion of their surface layers. The extent of the erosion expressed as %erosion showed a decrease with increasing and decreasing wt% of alginate and starch, respectively, while a constant fall in %erosion occurred with increasing degrees of cross-linking of beads. The polymer beads show a great potential for release of chlorpyrifos as a model

agrochemical, while fractional release increases with increasing wt% of alginate and decreases with increasing content of starch. However, an optimum fractional release was obtained for a bead composition with more alginate and less starch. The studied release system offers fairly good possibilities for application to agricultural fields as evident from the soil-pot experiments. Although a 50% release was found to occur in one day only when pesticide was applied directly to the soil, it took 5 days for the same extant of release when the release occurred through swelling and eroding of loaded biopolymeric beads.

In 2011, Yao *et al.* [21] studied the preparation and evaluation of a novel gastric floating alginate/poloxamer inner-porous beads using foam solution. The beads were prepared by dropping the foam solution into $CaCl_2$ solution using disposable syring needle, where the foam solution consisting numerous of microbubbles with poloxamer 188 as foaming agent, alginate as foaming stabilizer. Foamability and foam stability of different polymer ratios were evaluated. The visual observation result and the resultant-weight method confirmed that the floating beads showed good buoyancy, most beads could float in the stomach for more than 6 h. The beads release behavior *in vitro* showed that drug release from the beads in a sustained-release fashion for 10 h.

In 2011, Korkiattithaweechai *et al*. [22] studied controlled release of diclofenac from matrix polymer of chitosan and oxidized konjac glucomannan. Konjac glucomannan (KGM) was initially oxidized by sodium periodate and then cross-linked to CTS *via* imine bonds (-C=N-) to form the new CTS-OKG copolymer. The DFNa loaded CTS-OKG polymers were characterized by FT-IR and XRD. Finally, the release profiles of DFNa from the CTS-OKG polymer matrices were evaluated in a simulated gastrointestinal fluid system comprised of two hours in simulated gastric fluid (SGF; pH 1.2) followed by 24 h in simulated intestinal fluid (SIF; pH 7.4). A 1:2:1 (w/w/w) ratio of CTS:OKG:DFNa prepared at room temperature for 3 h gave the highest %encapsulation efficiency (EE) of 95.6±0.6 and resulted in a minimalrelease of DFNa (<1% over 2 h) in SGF (pH 1.2) and a significantly improved sustained release in SIF (pH 7.4) with ~6 and 19% release over 8 and 24 h, respectively, some 15- and five-fold lower than that of the two commercial DFNa preparation, diclosian and voltaren. This formulation may be used for further study as a long term intestine controlled release drug model (at least 3 days).

CHAPTER III

EXPERIMENTAL

3.1 Chemicals and materials

Pendimethalin (technical grade, 99% pure) was provided by Meghmani organics limited. Chitosan (MW 500,000-1,000,000 daltons, 95% min deacetylated) from shrimp shell was purchased from Bonafiles marketing Co., Ltd. (Bangkok, Thailand). Sodium alginate, wheat flour (without pretreatment) and rice flour (without pretreatment) were purchased from Qingdao Xuan group Co., Ltd., China; Thai flour mill industry Co., Ltd., Thailand; and Thai Wah Food Products Public Co., Ltd., Thailand, respectively. Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) (Solvay chemicals international, Belgium) was used as a cross-linker. All other chemicals used in this study were of analytical quality.

3.2 Instruments and apparatus

- Balance (Satorius, ED224S, German).
- Fourier transform infrared spectrometer (FTIR, model 470 FT-IR, Thermo Scientific, USA).
- Scanning electron microscope (SEM, JSM-5410, JEOL, Japan).
- Gas chromatograph (GC, model 6890N GC System, Agilent Technology, USA).
- Oven (Binder, FD53, German).

3.3 Bead preparation:

3.3.1 Chitosan beads loaded with pendimethalin

The chitosan solution was prepared by dissolving chitosan (6 g) in 2.5% acetic acid. Pendimethalin (4 g) was completely dissolved in acetone and then added into the chitosan solution and stirred until homogeneous. The syringe was clamped at a height of 60 cm above glutaraldehyde solution 0.5% v/v. The chitosan-pendimethalin solution

was added dropwise, extruding through a hole (*dia.* 1 mm), then the beads were collected at 1, 2, 3, 6, 24 and 48 h, respectively, washed with water and dried.

3.3.2 Chitosan-alginate bead loaded with pendimethalin

The general protocol was conducted as that in 3.3.1 except for the solution of 0.5%v/v glutaraldehyde was changed to a mixed solution of 0.5%v/v glutaraldehyde and 2%w/v sodium alginate.

3.3.3 Na-alginate beads loaded with pendimethalin

The Na-alginate beads were loaded by dissolving 12 g of sodium alginate with deionized water into a 600 mL beaker. Pendimethalin (8 g) was added and the mixture was heated by a hotplate until pendimethalin melted and stirred until the solution became homogeneous. The solution was added dropwise, extruding through the hole of a syringe (*dia*. 1 mm) into 5.0 %w/v $CaCl_2$ solution, respectively. The syringe was clamped at a height of 60 cm above $CaCl_2$ solution. The beads were collected from $CaCl_2$ solution after 1 min; the beads were then washed with water and placed in open air at ambient temperature until dry.

3.3.4 Na-alginate and wheat flour beads loaded with pendimethalin

Sodium alginate and wheat flour were weighed at the ratio of 5:1 (I), 3:3 (II) and 2:4 (III) into a 600 mL beaker, respectively. Sodium alginate and wheat flour were then completely dissolved in hot deionized water. Pendimethalin (4 g) was added in each ratio and heated by hotplate until pendimethalin melted and the solution was homogeneous. Solutions (I), (II) and (III) were then added dropwise, extruding through the hole of a syringe (dia. 1 mm) into $CaCl_2$ 5.0% w/v solution and solution (I) was added dropwise, extruding through the hole of a syringe (dia. 1 mm) into 10.0% w/v solution. (The syringe was clamped at a height of 60 cm above $CaCl_2$ solution). The beads were collected as follows:

No.	Solution	$CaCl_2$(%w/v)	Time (min)
1	I	5.0	1
2	I	10.0	1
3	I	5.0	30
4	I	5.0	overnight
5	II	5.0	30
6	III	5.0	30

The beads were then washed with deionized water and dried by placing in open air at ambient temperature.

3.3.5 Na-alginate and rice flour beads loaded with pendimethalin

The general procedure was carried out as that in 3.3.4 except for wheat flour was changed to rice flour. The solution was then added dropwise, extruding through the hole of a syringe (*dia*.1 mm) into $CaCl_2$ 5.0% w/v solution. The beads were collected at 1 min and washed with water, then dried by placing in open air at ambient temperature.

3.4 Bead characterization

3.4.1 Surface morphology by SEM

The morphology of the beads was investigated by scanning electron microscopy (SEM). The bead was dropped on a clean glass slide and dried. The specimens were fractured under cryogenic conditions using liquid N_2. Then the specimens were mounted on an SEM stub using double-sided tape and the fracture of the surface of the specimens was sputtered with a thin gold layer. The surface morphologies of the beads were then investigated using SEM operated with 35 and 150 times magnification. The cross-section surface morphologies of the beads were operated with 350 times magnification.

3.4.2 Bead composition by FTIR

Sodium alginate, pendimethalin and prepared beads were analyzed by FTIR scanning from 4000 to 400 cm^{-1}. The sample amounts of 2-3 mg were grinded and mixed with KBr powder. Then pressed into discs using compression using a hydraulic press so that they could be analyzed.

3.5 % Loading of pendimethalin

3.5.1 Preparation of internal standard solution (dibutyl phthalate, DBP)

DBP (2.500 g) was weighed and transferred into a 250 mL volumetric flask and made for the volume with acetone.

3.5.2 Analytical standard solution

Pendimethalin (0.005 and 0.01 g) was weighed and transferred to a 25 mL volumetric flask, respectively. 2 mL of an internal standard solution was pipetted into each volumetric flask and made for the volume with acetone.

3.5.3 Preparation of sample solutions

The beads were prepared as described in topics 3.3.1-3.3.5. The dried beads were weighed then milled in order to achieve a fine particle size. The milled beads (0.0050 g) were weighed into a volumetric flask 25 mL and pipetted 2 mL of internal standard solution into the volumetric flask and then made volume with acetone. The amount of pendimethalin was quantified by gas chromatography.

3.5.4 Setting gas chromatography conditions

- Columns: HP-101 methyl siloxane capillary 5.0 m × 320 µm× 0.32 µm
- Carrier: Nitrogen at 42 cm^3/sec, measured at 200°C
- Oven: 200°C-260°C at 20°C/min
  260°C for 22 min
- Injector: Split (5.0:1), 270°C
- Detector: FID, 280°C

Nitrogen make-up gas at 45.0 mL/min,

3.5.5 Quantification of pendimethalin

The calibration curve was constructed by injecting standard solutions (topic 3.5.2). The samples were injected and calculated the %pendimethalin as follows:

$$\textit{\% Loading} = \frac{\text{Wt of pendimethalin x 100}}{\text{Wt of beads (W)}}$$

$$\textit{\% Encapsulation efficiency} = \frac{\text{actual drug loading}}{\text{Theoretical drug loading}} \times 100$$

3.6 Release studies

The beads (0.15 g) were weighed and transferred into 250 mL screw cap glass bottle and deionized water 50 mL was added. The 5 mL of solution was pipetted at 1, 2, 5, 10, 20, 30, 45, 60, 75, 90, 120, 150 and 180 days (t_x), respectively into 25 mL volumetric flask. Deionized water (5 mL) was then replaced. Next, 2 mL of internal standard solution was pipetted into each volumetric flask and made volume with acetone. Pendimethalin was quantified by gas chromatography.

$$\textit{\% Cumulative release} = \frac{\text{Cumulative release per gram of beads}(t_x)}{\text{Pendimethalin content per gram of beads(W)}} \times 100$$

3.7 Swelling studies

Six dry beads of each formula were weighed (W_d) into 250 mL screw cap glass bottle and added deionized water 20 mL into the glass bottle. The swollen beads were weighed at time intervals (1, 2, 5, and 8 h, 1, 2, 5, 10, 20, and 30 days) (W_s). The solution was filtered to collect the beads. During this process, handling was smooth to avoid mass loss by breaking or eroding the beads. The swelling ratio was calculated by the following equation.

$$\textit{Swelling ratio} = \frac{\text{Wt of swollen beads } (W_s)}{\text{Wt of dry beads } (W_d)}$$

3.8 Accelerated storage testing [23]

Duplicate beads (2.0 g) were weighed into a screw cap glass bottle. The first grass bottle was placed in an incubator at 54°C for 14 days. The second bottle was placed in an incubator at 25°C for 14 days. After 14 days, %loading of pendimethalin in the beads of two samples was assayed (25 and 54°C) by gas chromatograph. %Loss of pendimethalin was determined by the following formula:

$$\%loss\ pendimethalin = \frac{(\%\ loading\ at\ 25°C - \%\ loading\ at\ 54°C)}{\%\ loading\ at\ 25°C} \times 100$$

3.9 Efficacy studies

Tissue paper (1.5 g) was weighed in a transparent plastic box (width 10.0 cm x length 16.0 cm x height 6.0 cm). Water (8.5 g) was weighed and added into the plastic box. The beads (0.06 g) were weighed in transparent plastic box and birdsrice seeds (weed seeds) 10 grains into plastic boxes, closed the plastic boxes and placed them in the shade (indirect sunlight). The controlled plots had been prepared by the same way, but did not have the beads. The germination of birdsrice seeds was examined every month and new 10 birdsrice seeds were added every month until a period of 6 months.

CHAPTER IV

RESULTS AND DISCUSSION

This research was conducted for the purpose of developing formulations of pendimethalin. The new formulation was intended to reduce the use of pendimethalin and minimized the number of application times by controlling the release of pendimethalin. The developed formulation involves pendimethalin encapsulated by biodegradable polymer. The biodegradable polymer studied is chitosan, alginate and starch. The release of pendimethalin was studied in water for maintaining a longer release time of 6 months. The nature and size of particles were determined by SEM. The chemical stability of pendimethalin was confirmed by FT-IR. Accelerated storage test of the beads formulation was intended to produce a shelf life of the product of two years after production and storage in the market. The efficacy study was performed to confirm the efficacy of the loaded beads with pendimethalin that could inhibit cell division at the root and cell elongation at the root of the grass-seed. The results of this study are as follows.

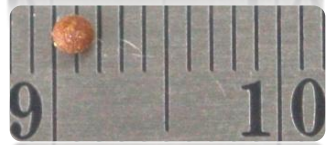
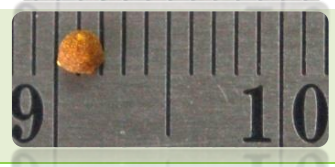
4.1 Pendimethalin containing chitosan beads

The first part of this thesis is the preliminary studies for the hydrogel preparation and the entrapment of pendimethalin in chitosan beads. Chitosan solution containing the desired amount of pendimethalin can be formed into gel upon contact with glutaraldehyde. The aldehyde groups form covalent imine bonds with amino groups of chitosan. Glutaraldehyde can form cross-linking by direct reaction in aqueous media under mild conditions, and does not require the addition of auxiliary molecules such as reducers. It also adds to retain biocompatibility of polymer. Chitosan beads were successfully prepared by using the coacervation technique. For preliminary study, the effect of immersion time of chitosan beads in glutaraldehyde solution was investigated in order to obtain the longer release time of pendimethalin from the beads when the mass ratios of chitosan:pendimethalin and percentage of glutaraldehyde were fixed at 6:4 (wt/wt) and 0.5% (v/v), respectively.

4.1.1 Characterization of beads

Chitosan beads were prepared by the coacervation method between a positively charged amino group of chitosan and glutaraldehyde as crosslinking agent. The average beads size, %encapsulation efficiency of the loaded pendimethalin at different immersion times are shown in Table 4.1. The chitosan beads prepared by this method are spherical in shape and orange-yellow in color. The size of chitosan dried beads was smaller but still spherical in shape than that of chitosan wet beads in glutaraldehyde solution. This is probably due to the loss of water and the strong networks between the chitosan and glutaraldehyde crosslinking agent. The morphology of beads was taken under camera. The size of dried beads was about 2 mm for all formulations indicating that the immersion time was no effect on the bead size. The chitosan beads formed are almost spherical and stiffened. The crosslinking bifunctional agents bind to different reaction sites in chitosan. The aldehyde groups of glutaraldehyde form covalent imine bonds with amino groups of chitosan, due to the resonance established with adjacent double ethylenic bonds *via* Schiff base reaction (Figure 4.1). Table 4.1, the %encapsulation efficiency of pendimethalin loaded chitosan beads, generally, was more than 80%. Since pendimethalin is non-soluble in water, it was not dissolved in the solution during the cross-linking, hardening and washing processes. The percentage of encapsulation efficiency slightly decreased with an increased immersion time. This is probably because pendimethalin is immiscible in water, leading to difficulty penetrating into or being encapsulated with the dense framework of chitosan and glutaraldehyde.

Table 4.1 Compositions of Chitosan beads as a function of immersion time in glutaraldehyde solution.

Formula	Mass ratio (g/g)		Glutaraldehyde (%v/v)	Time (hour)	%Encapsulation efficiency	Photographs
	Chitosan	Pendimethalin				
CTS-1	6	4	0.5	1	86.5±1.4	
CTS-2	6	4	0.5	2	87.4±4.2	
CTS-3	6	4	0.5	3	85.8±2.1	
CTS-4	6	4	0.5	6	82.3±1.1	
CTS-5	6	4	0.5	24	81.4±1.1	
CTS-6	6	4	0.5	48	81.0±0.4	

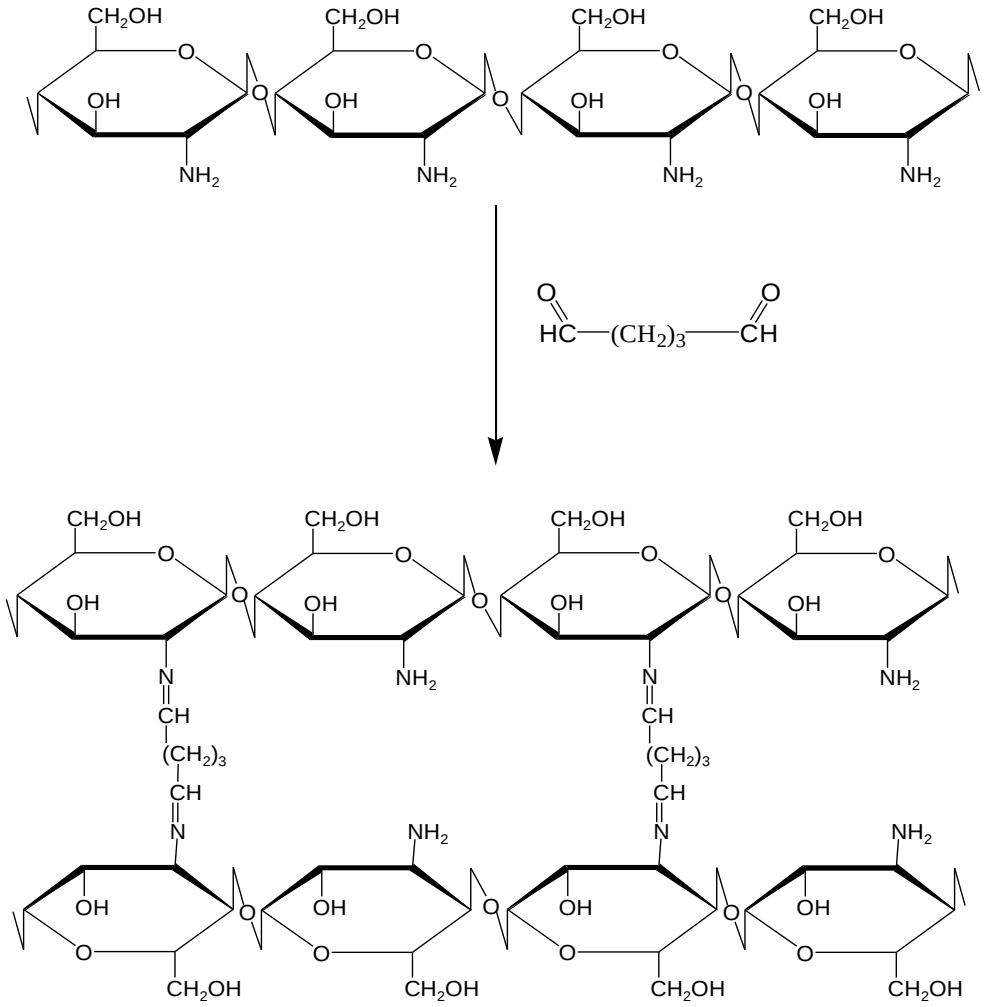


Figure 4.1 Crosslinking scheme of chitosan and glutaraldehyde.

4.1.2 Swelling and release profile studies

The swelling behavior of chitosan beads was studied by immersing them in DI water (pH 7.0) for 30 days. The swelling curves of the beads are shown in Figure 4.2. The water uptake of the beads was relatively fast for the first 3 h and reached the maximal value within approximately 1 day. The swelling ratio of the CTS-6 showed the minimum value, which was due to the fact that the dense networks of chitosan beads increased with an increase of immersion time which was due to increasing in cross-linking density. There are two steps of swelling and erosion. After chitosan beads were fully swollen, the weight of the beads is the highest and then the beads started to erode, resulting in the weight is decreased. Therefore, there were two stages for releasing of pendimethalin from beads. In the initial stage, water was adsorbed (swelling), the network was loosen, hence partial pendimethalin was released from the beads and the second stage or the erosion stage, the weight of beads were rapidly decreased, resulting in rapidly released of pendimethalin.

The drug release studies were performed with pendimethalin loaded chitosan beads in water for at least 90 days (the requirement time for applying the pendimethalin in the field). The amounts of pendimethalin were determined by gas chromatography. The release profiles of pendimethalin from chitosan beads with 0.5% of glutaraldehyde crosslinking as a function immersion time is shown in Figure 4.3. During the first 10 days, CTS-1 (immersion time of 1 h) exhibited a sustained release of pendimethalin up to 0.7% which was 2-fold higher than that of CTS-6 (0.3%) with the immersion time of 2 days. It revealed that the release profiles of pendimethalin from the beads decreased when the time of immersion increased. CTS-1 showed the highest swelling ratio of 1.7 and CTS-6 with the immersion time of 2 days showed the lowest swelling ratio of 1.2. The results suggested that the swelling ratio decreased when the immersion time was increased. With an increment of the degree of crosslinking, polymer becomes much denser with a decrease in the rate of release of active ingredients through the erosion beads. The behavior of the chitosan beads in water could clearly be classified as a two-stage mechanism; swelling and then eroding. At the first stage of the mechanism, chitosan beads were rapidly swollen. For the second stage, after 1 day of swelling, the beads started to erode, the weight decreased rapidly within 10 days and then reached the steady state because the

dense networks that would block the beads from adsorbing water and beads had low erosion.

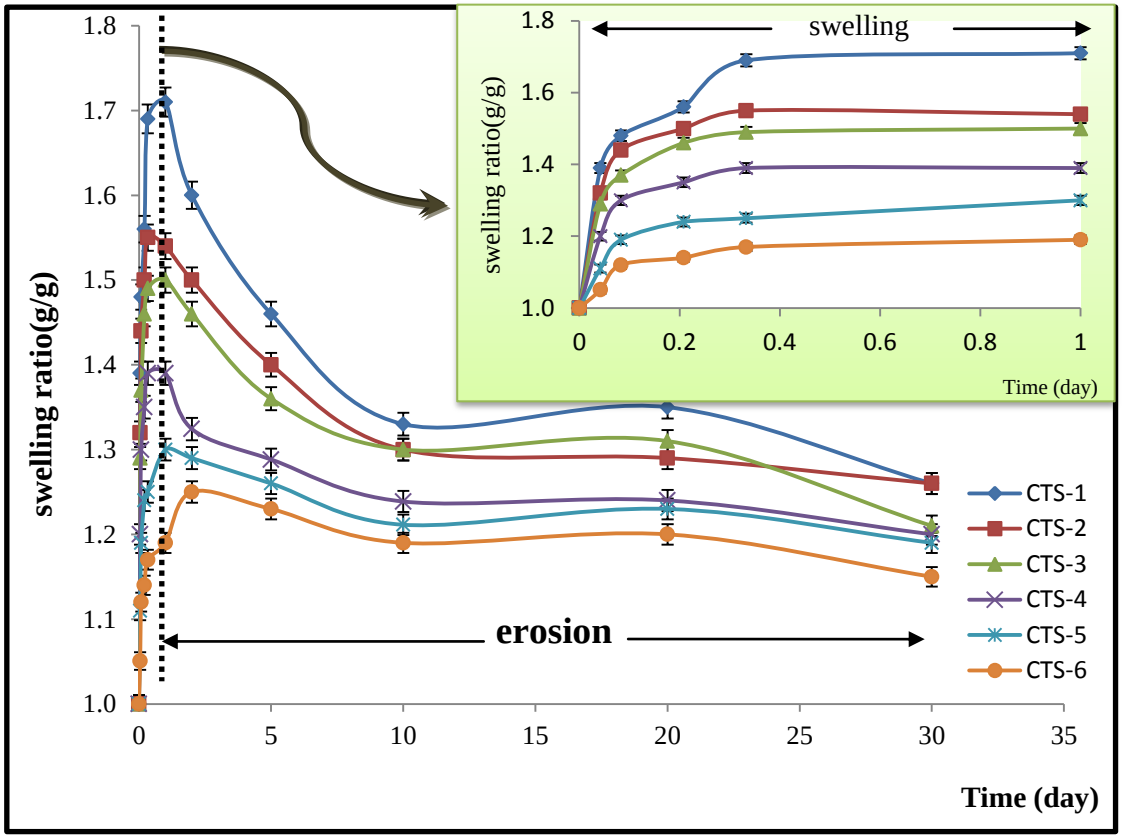


Figure 4.2 Swelling ratio of chitosan beads as a function of immersion time. (CTS-1: immersion time of 1 h, CTS-2: immersion time of 2 h, CTS-3: immersion time of 3 h, CTS-4: immersion time of 6 h, CTS-5: immersion time of 24 h, CTS-6: immersion time of 48 h).

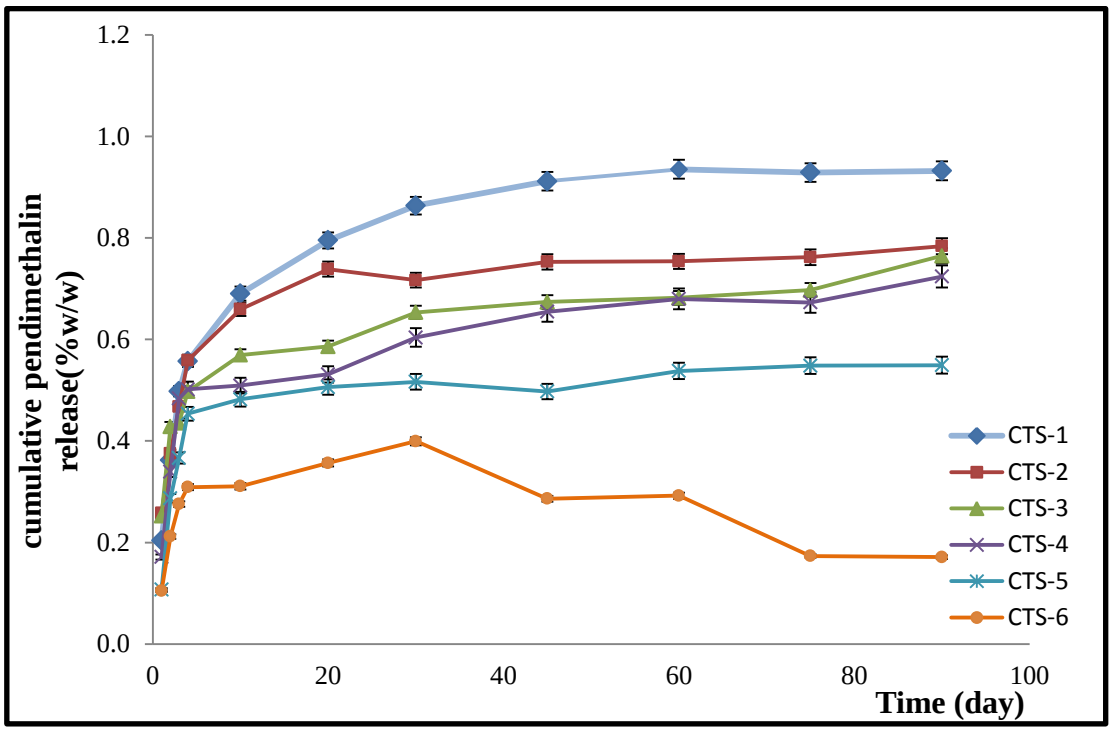


Figure 4.3 Release profiles of cumulative pendimethalin released from the various chitosan bead formulations as a function of time. (CTS-1: immersion time of 1 h, CTS-2: immersion time of 2 h, CTS-3: immersion time of 3 h, CTS-4: immersion time of 6 h, CTS-5: immersion time of 24 h, CTS-6: immersion time of 48 h).

However, the release rate of pendimethalin was not satisfied, only 0.8% of pendimethalin was released from chitosan beads for 90 days. This might be resulted from the fact that chitosan was not a suitable polymer for carrying water insoluble pendimethalin. In order to improve the release rate of pendimethalin, the hydrogel composites between chitosan-alginate were formulated. Because alginate could be more swollen in water than chitosan and could act as hydrogel.

4.2. Pendimethalin containing chitosan-alginate beads.

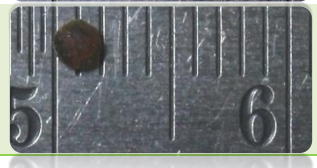
To increase the release rate of pendimethalin, alginate, which is a good water soluble polymer and can act as hydrogel, was used to composite with chitosan. Polyelectrolyte complexes (PECs) were obtained by mixing aqueous solutions of two polymers carrying opposite charges. The preparation of an alternative alginate-

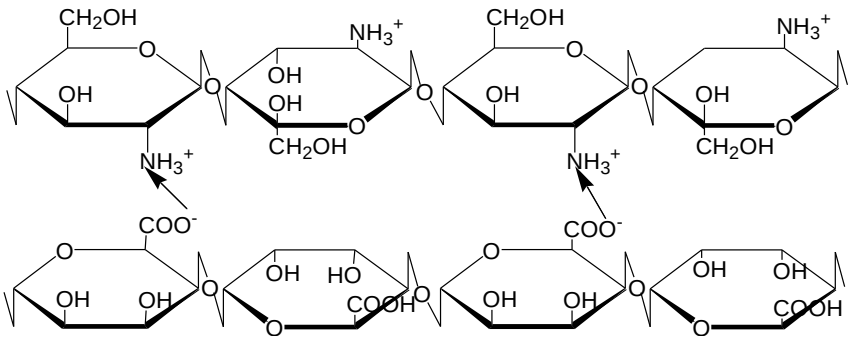
chitosan matrix beads was investigated using a crosslinking reaction with glutaraldehyde. The beads were prepared by dropping chitosan containing pendimethalin into a mixture of glutaraldehyde and sodium alginate solution. The electrostatic attraction between the cationic amino groups of chitosan and the anionic carboxyl groups of alginate was the main interaction leading to the formation of polyelectrolyte complex. The beads were prepared using the constant mass feeding ratios of chitosan to alginate, but vary the immersion times from 1 to 48 h to investigate the effect of release profiles. The composites of chitosan, alginate, pendimethalin and glutaraldehyde used are given in Table 4.2.

4.2.1 Characterization of the beads

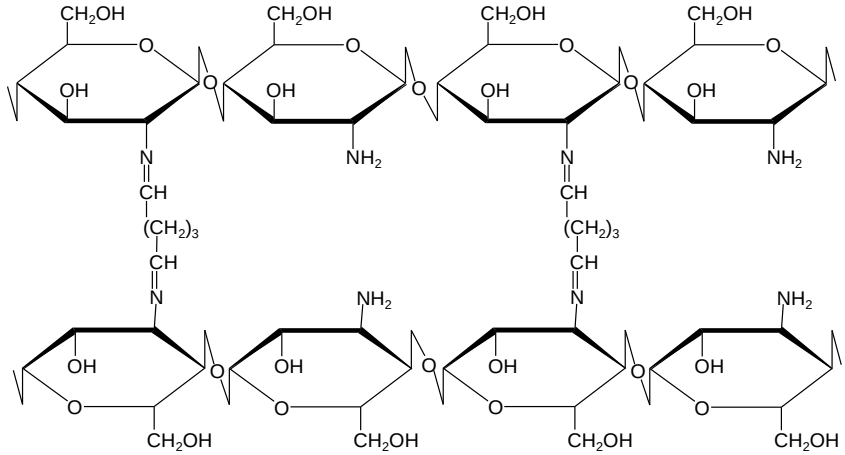
Table 4.2 shows the representative photograph of crosslinking reinforced chitosan-alginate beads. The crosslinking reinforced chitosan-alginate beads formed instantaneously after dropping chitosan droplets into alginate-glutaraldehyde mixed solution and the beads produced in this work had diameters about 2 mm with a narrow size distribution. The color of chitosan-alginate beads was dark brown in color. The chitosan-alginate beads were spherical in shape and hard, but brittle. The formation of interphasic membrane could be attributed to the interaction of anionic alginate with cationic chitosan. Contrary to the skin layer, the inner core was formed due to the crosslinking of chitosan by glutaraldehyde (Figure 4.4). Both the formations of outer chitosan-alginate complex membrane and cured inner chitosan core led to the fixing of sphericity of beads. The percentage of encapsulation efficiency slightly decreased with an increase of immersion time. The beads immersed for 1 h in glutaraldehyde showed the highest encapsulation efficiency of 77.8% and the lowest encapsulation efficiency (73.4%) was observed in the formulation that glutaraldehyde was immersed for 48 h.

Table 4.2 Chitosan-alginate beads as function of immersion time in glutaraldehyde solution.

Formula	Mass ratio (g/g)		%Glutaraldehyde (%v/v)	Alginate (%w/v)	Time (hour)	%Encapsulation efficiency	Photographs
	Chitosan	Pendimethalin					
CTS-Alg-1	6	4	0.5	2	1	77.8±3.2	
CTS-Alg-2	6	4	0.5	2	2	76.4±1.1	
CTS-Alg-3	6	4	0.5	2	3	76.5±2.1	
CTS-Alg-4	6	4	0.5	2	6	75.9±0.7	
CTS-Alg-5	6	4	0.5	2	24	74.6±0.0	
CTS-Alg-6	6	4	0.5	2	48	73.4±0.7	



Chitosan-alginate polyelectrolyte complex



Chitosan crosslinked by glutaraldehyde

Figure 4.4 A proposed mechanism for chitosan gelled in alginate-glutaraldehyde mixed solution.

4.2.3 Swelling and release profile studies

The swelling ability of the crosslinked reinforced chitosan-alginate beads depended on the process for preparation of beads, such as immersion times in glutaraldehyde solution. The swelling ratio of prepared beads decreased with the increase in immersion time in glutaraldehyde solution (Figure 4.6). The crosslinked chitosan-alginate beads showed the obviously highest swelling ratio at the time immersion of 1 h and lowest swelling ratio at the time immersion of 48 h. This results may be ascribed to the fact that the deprotonatation of inner chitosan core increased the nucleophilicity of amino group, which in turn led to the increase in the crosslinking degree due to nucleophilic attack of chitosan on the aldehyde group of glutaraldehyde.

In general, the release profile of pendimethalin from chitosan-alginate beads decreased with the increased immersion time. As crosslinking time increased from 1 to 48 h, the release of drug also decreased. As shown in Figure 4.7, the beads prepared in glutaraldehyde solution for 1 h showed faster release behavior than those of beads prepared by longer immersing time (48 h). The release of pendimethalin was extended over a longer period of time especially for beads with post-coagulation treatment by chitosan and alginate. The lower rate of release coincided with the delay in the erosion of the multilayer beads. The delayed erosion and concomitantly the more sustained release of pendimethalin from chitosan-reinforced alginate beads probably reflected the strengthening of the beads by ionic interaction of chitosan (NH_3^+) with alginate (COO^-) ions. The structure of pendimethalin loaded chitosan-alginate beads is shown in Figure 4.5. The release profile from alginate matrix was usually modulated by a swelling-dissolution-erosion process. The release of pendimethalin seemed to occur by erosion of the beads. The swelling behavior of beads indicated the speed and easiness of a liquid to penetrate the chitosan-alginate matrix, as a necessary step for release. Chitosan-alginate beads of different formulations displayed that 1.8% pendimethalin released from the beads after 1 month from an immersion time of 1 h (CTS-Alg-1) and 0.9% pendimethalin released from the beads after 1 month from an immersion time of 48 h (CTS-Alg-6). The observed small differences in the amount of released pendimethalin at different immersion times (1, 2, 3, 6, 24 and 48 h) could be attributed to a tight alginate network that formed. Approximately 3.5% of total

pendimethalin was released after 3 months from an immersion time of 1 hour (CTS-Alg-1).

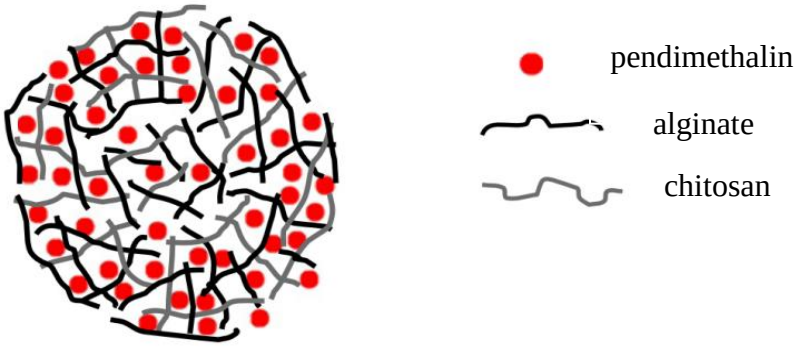


Figure 4.5 Schematic structure of pendimethalin-loaded chitosan-alginate beads.

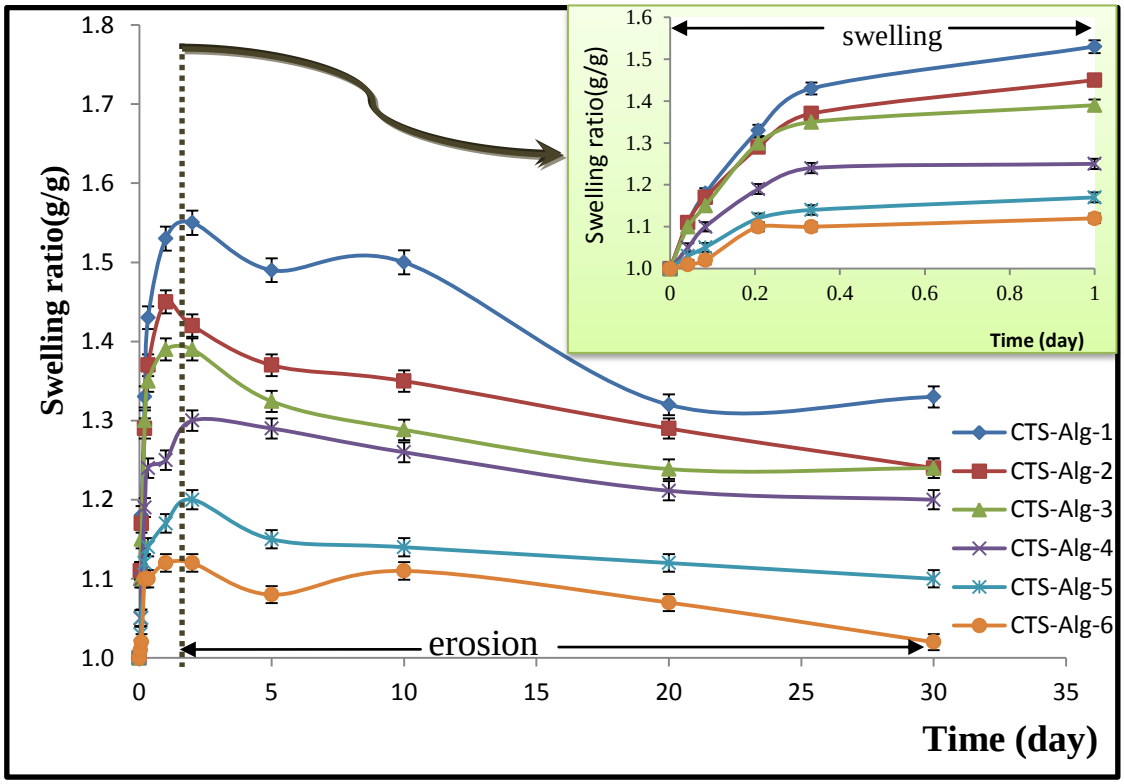


Figure 4.6 Swelling ratio of chitosan-alginate beads as a function of immersion time. (CTS-Alg-1: immersion time of 1 h, CTS-Alg-2: immersion time of 2 h, CTS-Alg-3: immersion time of 3 h, CTS-Alg-4: immersion time of 6 h, CTS-Alg-5: immersion time of 24 h, CTS-Alg-6: immersion time of 48 h).

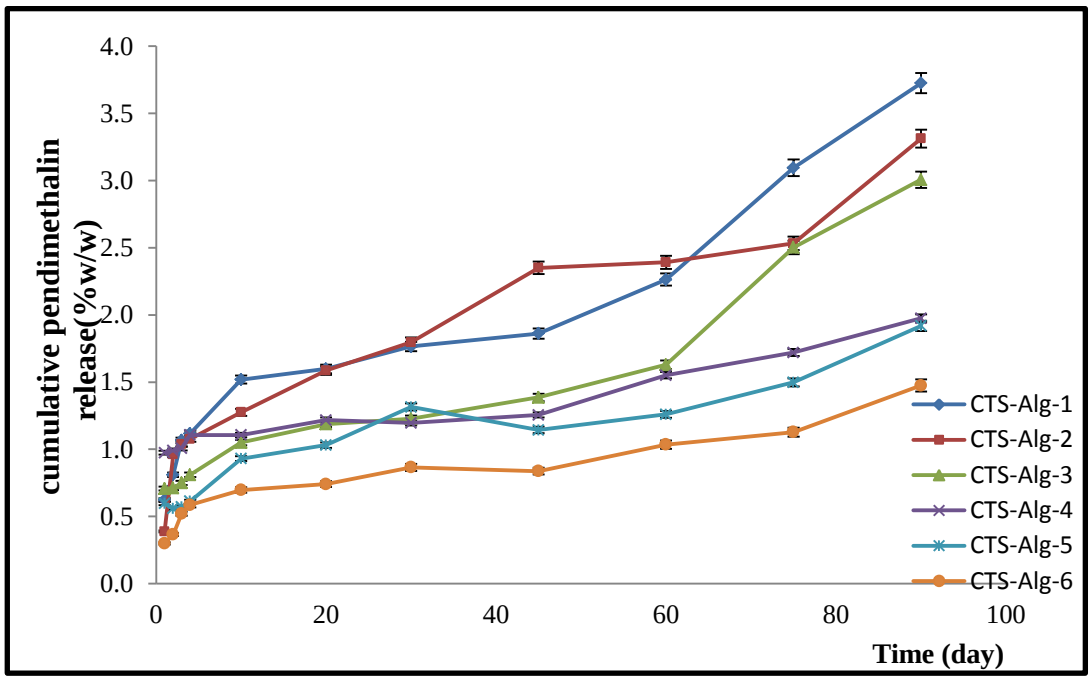


Figure 4.7 Release profiles of cumulative pendimethalin from chitosan-alginate beads as a function of time. (CTS-Alg-1: immersion time of 1 h, CTS-Alg-2: immersion time of 2 h, CTS-Alg-3: immersion time of 3 h, CTS-Alg-4: immersion time of 6 h, CTS-Alg-5: immersion time of 24 h, CTS-Alg-6: immersion time of 48 h).

This study showed that chitosan and chitosan-alginate beads had very slow release rates. Chitosan beads immersed 1 h (CTS-1) had released less than 1% pendimethalin (the active ingredient contented in the beads at more than 99%) and the chitosan-alginate beads (CTS-Alg-1) had the cumulative of released for 3.5% pendimethalin (the active ingredient contented in the beads at more than 96.5%) after 3 months. The swelling ratio of chitosan beads (1.7% in 1 day for CTS-1) was higher than that of chitosan-alginate (1.5% in 1 day for CTS-Alg-1), but showed slower release rate because chitosan-alginate beads were brittle which allowed more pendimethalin to be released. However, chitosan and chitosan-alginate beads showed unsatisfied release profiled of pendimethalin from the beads, only 3.5% was released within 3 months. That caused to use more beads applied in the field. To solve this problem, chitosan which is non-water soluble polymer was omitted. The next experiments were prepared by using alginate for an increased percentage of

pendimethalin release and still maintained a longer release time of 6 months. The results of this study are as follows.

4.3 Pendimethalin containing alginate and alginate-starch beads

In the previous work, chitosan and chitosan-alginate beads showed 3.5% pendimethalin released because of non-water soluble of pendimethalin and high degree of crosslink and density of chitosan and chitosan-alginate. Therefore, in the present work, alginate beads were prepared by dropping an aqueous suspension of pendimethalin-alginate into a $CaCl_2$ solution. During the step of drying beads, the initial spherical shape is lost and shrinkage. The beads were thus further prepared using alginate composite with another polymer that can swell very well in water. So, starches, an alternative polymer that can easily biodegrade in water were selected to composite with alginate. In order to reinforce the beads in a spherical shape, starch was used as filler. Two types of starches: wheat flour and rice flour were selected because starch is cheap, widely available, non-toxic and biodegradable. For preliminary studies, the mass ratios (wt/wt/wt) of alginate: wheat flour (W): pendimethalin and %$CaCl_2$ were fixed at 5:1:4 and 5% $CaCl_2$, respectively, and vary the immersion time from 1, 30 min and overnight.

4.3.1 Characterization of beads

Conventional calcium alginate beads were prepared by dropping an aqueous suspension of sodium alginate into a $CaCl_2$ solution, which produced particles with a mean diameter of approximately 3 mm (Alg-1). When sodium alginate reacted with Ca^{2+}, cross-links were formed giving large three dimensional networks that become viscous/gel-like and holds water (Figure 4.8). During particle drying, the initial spherical shape was lost (Table 4.2) and particle shape did not improve significantly with pendimethalin encapsulation, which was confirmed by photographs. The characteristics of the beads prepared by this method had an orange-yellow color. The alginate bead loaded with pendimethalin found the spherical shape in $CaCl_2$ solution. But when the beads dried, they became flat beads because water evaporated from the beads leading to corrugations and the loss of the spherical shape. The alginate loaded with pendimethalin and added starch was spherical shaped beads in $CaCl_2$ solution

and were still spherical shaped beads when dried. The particles of alginate-starch were generally spherical in shape with sizes approximately 3 mm and are presented in Table 4.3 except for the beads immersed for a long time (Alg-W-3) and concentration of $CaCl_2$ 10% (Alg-W-6) approximately 2 mm. A series of such beads of different compositions were prepared by varying types of starch (wheat flour and rice flour), ratio of alginate:wheat flour, concentration of $CaCl_2$ and immersion time.

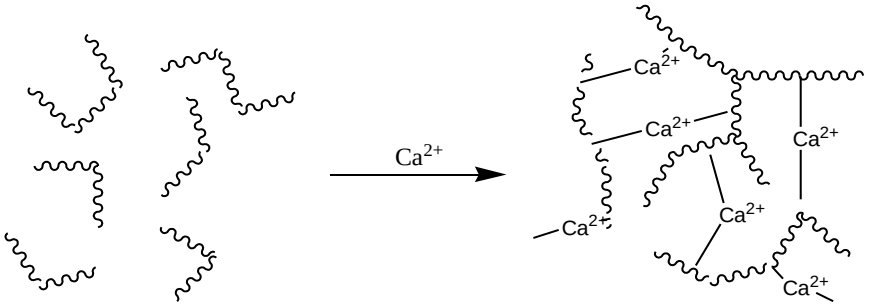
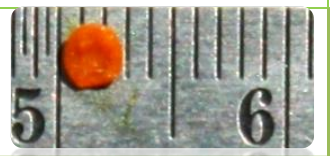


Figure 4.8 Crosslinking process of alginate treated with $CaCl_2$.

This preparation technique could highly entrap pendimethalin in beads at more than 70-90%. The results of %encapsulation efficiency are given in Table 4.3. The formula Alg-W-6 prepared from a high concentration of 10% of $CaCl_2$, resulted in high crosslinking degree that made the wheat flour could not be entrapped inside the beads. This can be seen from the solution being turbid and the wheat flour dispersed in 10% $CaCl_2$ solution. Therefore, the percentage of encapsulation efficiency of formulation Alg-W-6 was the lowest (70.8%). For alginate beads (Alg-1), the percentage of encapsulation efficiency was 85.1% and when wheat flour was added, the encapsulation efficiency was decreased to 83.3% at the same preparation condition. The formulas of Alg-W-2 and Alg-W-3 had higher percentage of encapsulation efficiency of 88.8 and 90.4%, respectively, due to Ca^{2+} ($CaCl_2$) could react completely when the time immersed increases. Therefore, the optimum immersion time was selected for 30 min (Alg-W-4) when the mass ratio of alginate was increased: wheat flour from 5:1 to 3:3 and 2:4, the %encapsulation efficiency was not significantly different. The ratio of alginate: wheat flour 3:3 (Alg-W-4) provided the percentage of encapsulation efficiency as 91.4%. It is possible that a reasonable amount of wheat flour remained in alginate beads.

Table 4.3 Compositions of alginate and alginate-starch beads.

Formula	Mass ratio(g/g)				$\%CaCl_2$ (%w/v)	Time (minutes)	%Encapsulation efficiency	Photographs
	Alginate	Wheat flour	Rice flour	Pendimethalin				
Alg-1	6	-	-	4	5.0	1	85.1±2.1	
Alg-W-1	5	1	-	4	5.0	1	83.3±5.0	
Alg-W-2	5	1	-	4	5.0	30	88.8±1.8	
Alg-W-3	5	1	-	4	5.0	Overnight	90.4±1.4	
Alg-W-4	3	3	-	4	5.0	30	91.4±1.8	

Formula	Mass ratio(g/g)				%$CaCl_2$ (%w/v)	Time (minutes)	%Encapsulation efficiency	Photographs
	Alginate	Wheat flour	Rice flour	Pendimethalin				
Alg-W-5	2	4	-	4	5.0	30	78.8±1.1	
Alg-W-6	5	1	-	4	10.0	1	70.8±2.5	
Alg-R-1	5	-	1	4	5.0	1	88.4±0.4	

4.3.2 Swelling and release profile studies

This section focused on the investigation of appropriate formula containing pendimethalin with different variables.

4.3.2.1 Types of starch

Alginate beads were spherical when starch was added. It could be clearly seen in Table 4.3 that starch had significant influence on bead shape and %encapsulation efficiency. The additional starch in the beads was not significantly affected the swelling of the beads compared to those without starch as shown in Figure 4.8. The different types of starch such as wheat flour and rice flour did not significantly affect the swelling of beads as shown in Figure 4.9. The swelling ratio, at an immersion time of 1 min, Alg-W-1 with wheat flour was higher than that of Alg-R-1 with rice flour. The release of pendimethalin, entrapped in an alginate and alginate/starch beads, occurred after water was penetrated the network to swell polymer and erode polymer. The release profiles of pendimethalin were closely related to swelling and erode characteristics of polymers, which in turn, is a key function of chemical architecture of polymers. In the present study, the release of pendimethalin from the beads has been studied at the interval time and the result is presented in Figure 4.10 for different types of starch in beads. The cumulative release of pendimethalin from the beads occurred in very controlled and sustained manner, which was the primary requisite for the use of agrochemicals to control the environment and health hazard. The wheat flour had percentage release of pendimethalin more than rice flour as the results from Figure 4.10 showed the percentage release at an immersion period of 180 days; wheat flour (Alg-W-1) was higher than rice flour (Alg-R-1). The perentage of pendimethalin cumulative released decreased slightly when starch was added because the anionic nature of the alginate was shielded by nonionic starch and caused an enhanced repulsion among the molecular chains. Therefore, if the amount of feeding starch was increased, the swelling and release would also be lightly affected, but that assumed it would not impact the efficiency of the beads because the beads had a spherical shape and still controlled the release properties.

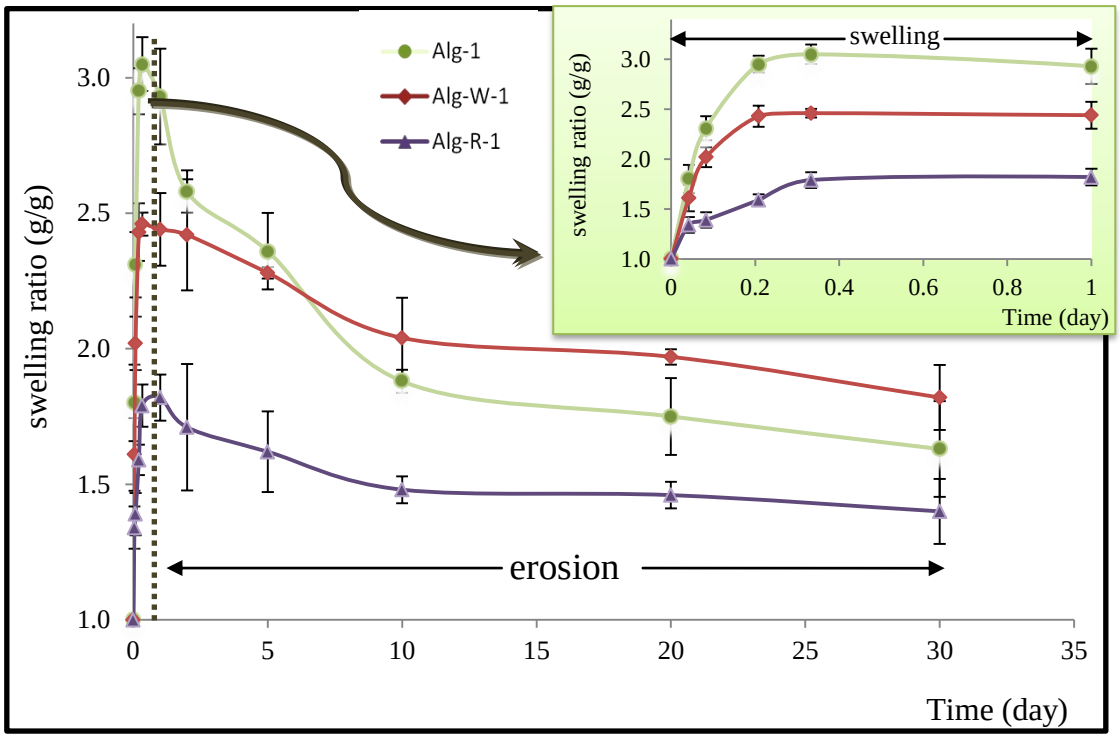


Figure 4.9 Swelling ratio of alginate-starch beads with as a function of types of starch. (Alg-1: no starch, Alg-W-1: wheat flour, Alg-R-1: rice flour.).

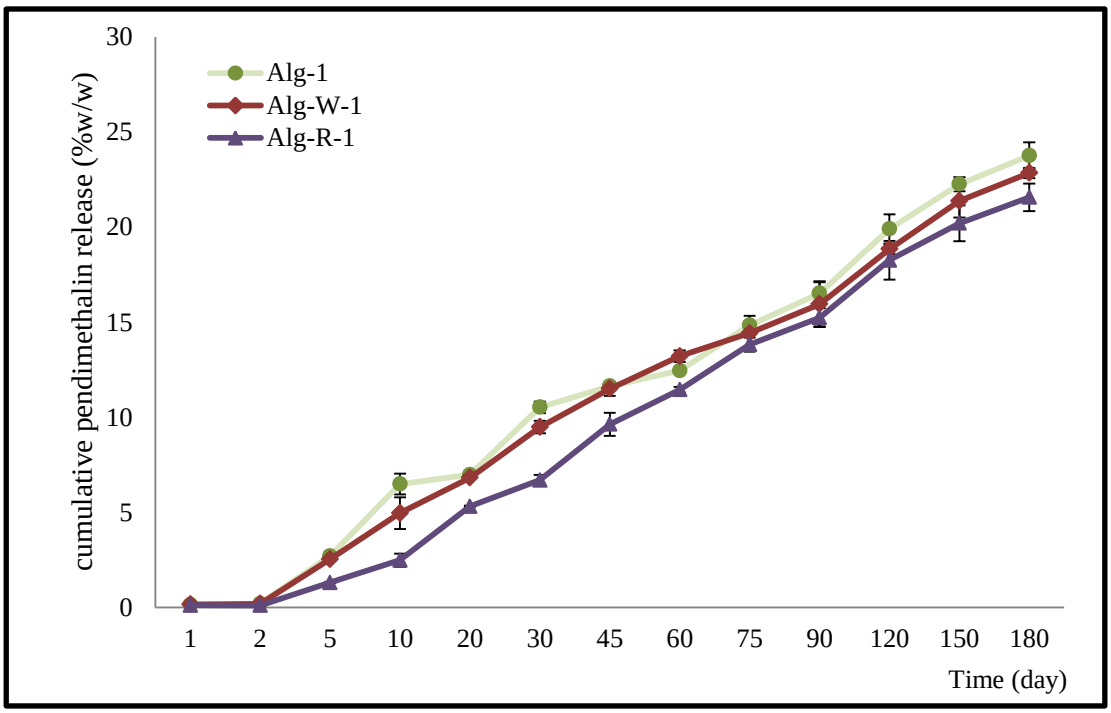


Figure 4.10 Cumulative pendimethalin released profiles from alginate-starch beads as a function of types of starch. (Alg-1: no starch, Alg-W-1: wheat flour, Alg-R-1: rice flour.).

4.3.2.2 Ratio of alginate:wheat flour

The effect of sodium alginate on the swelling ratio of beads has been studied by varying its ratio of alginate: wheat flour. When the ratio of alginate: wheat flour decreased, there was not a significant effect to the swelling of beads and percentage release of pendimethalin. It was shown that the ratio of alginate:wheat flour in formulas Alg-W-2 (5:1), Alg-W-4 (1:1) and Alg-W-5 (2:4). The maximum swelling of formula Alg-W-2 (5:1), Alg-W-4 (1:1) and Alg-W-5 (2:4) were respectively (Figure 4.11) and the percentage release of pendimethalin of Alg-W-2 (5:1), Alg-W-4 (1:1) and Alg-W-5 (2:4), respectively (Figure 4.12), which clearly showed that the swelling ratio constantly decreased when the weight of starch increased. The observed decrease in swelling ratio could be attributed to the fact that due to the anionic nature of alginate, its decreasing amount in the beads would cause enhanced repulsion among the molecular chains and this result decreased in the swelling ratio. Therefore, the ratio of starch was increased, the swelling and release would be lightly affected, but assumed it would not have impacted to the efficiency of the beads. Nonetheless, if the amount of starch was used in an excess, the amount of starch was found to suspend on the crosslinking solution.

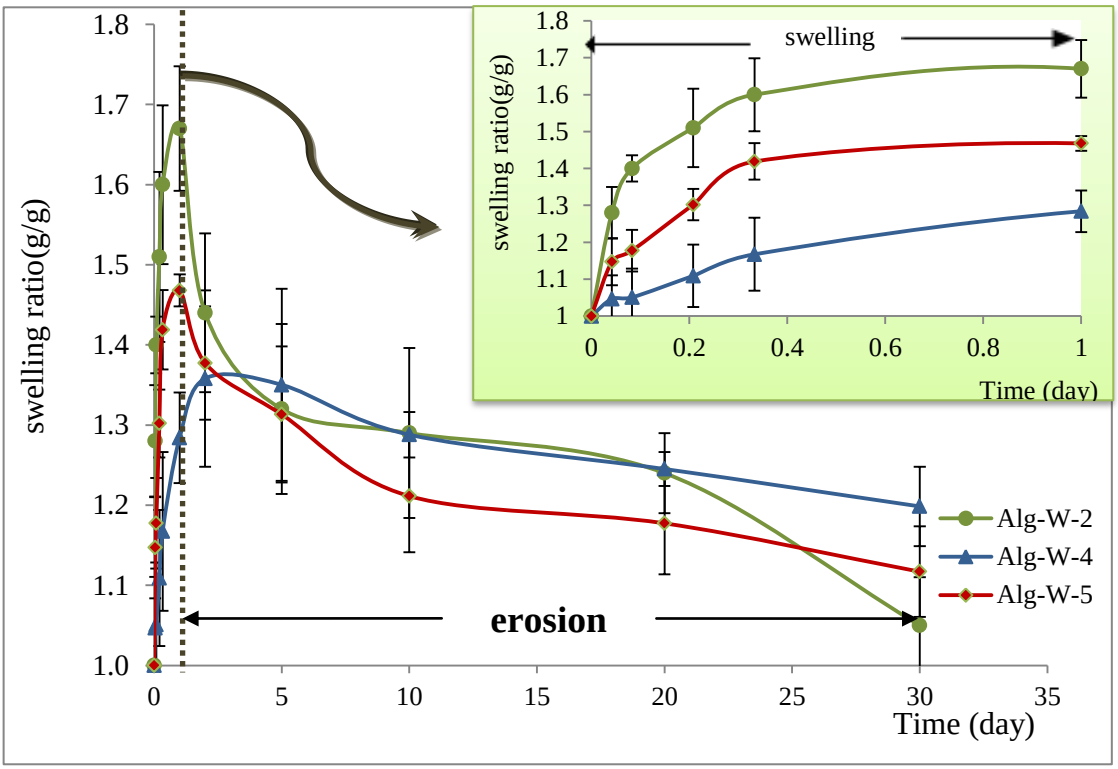


Figure 4.11 Swelling ratio of alginate-starch beads as a function of ratios of alginate: wheat flour. (Alg-W-2: 5:1, Alg-W-4: 3:3, Alg-W-5: 2:4.).

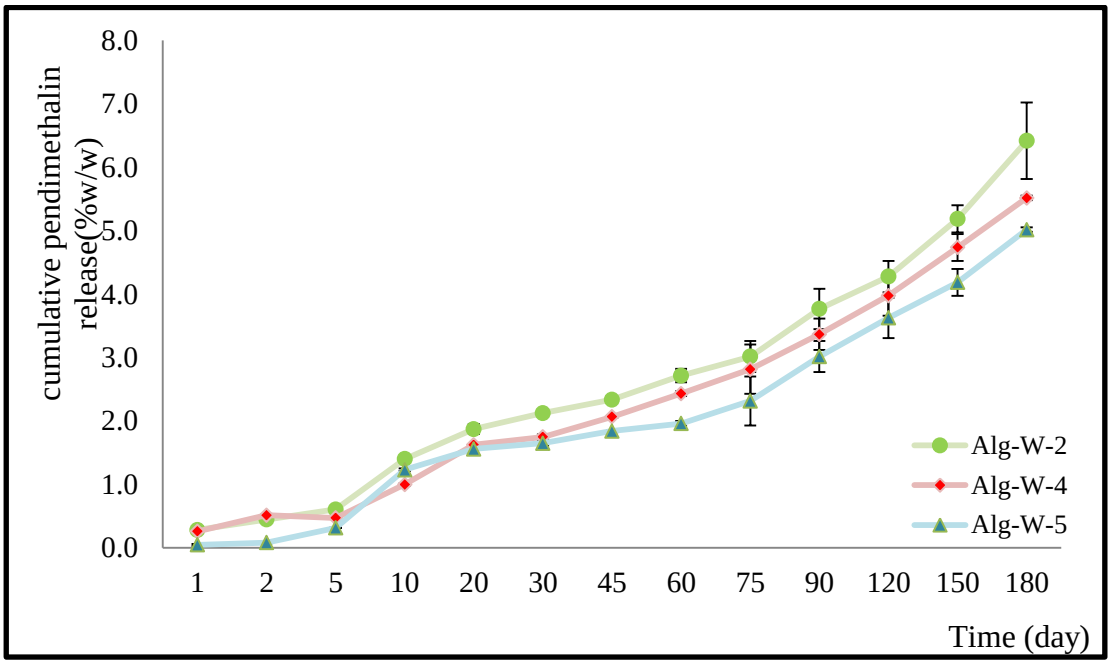


Figure 4.12 Percentage of cumulative pendimethalin released from alginate-starch beads as a function of ratios of alginate:wheat flour. (Alg-W-2: 5:1, Alg-W-4: 3:3, Alg-W-5: 2:4.)

4.3.2.3 Concentration of the crosslinking agent

$CaCl_2$, a very well known crosslinking agent for alginate, was assumed to act by its bivalent Ca^{2+}, forming a cavity type of network. In this study, the effect of $CaCl_2$ was investigated by varying its concentration between 5.0 and 10.0% w/w. The results given in Figures. 4.13 and 4.14 showed that the degree of water adsorption constantly decreased with increasing the concentration of crosslinker. The observation of the decrease in the swelling ratio may be because at high concentration, the crosslink would be stronger or had a higher intensity of crosslink than at lower concentration. When the swelling property decreased, the percentage of release of pendimethalin would decrease. The concentration of crosslinking agent also affected the swelling property. If the concentration of the crosslinking agent was increased, the swelling would decrease. Figure 4.13 shows the swelling at the immersion period of 1 minute of Alg-W-1 was higher than that of Alg-W-6.

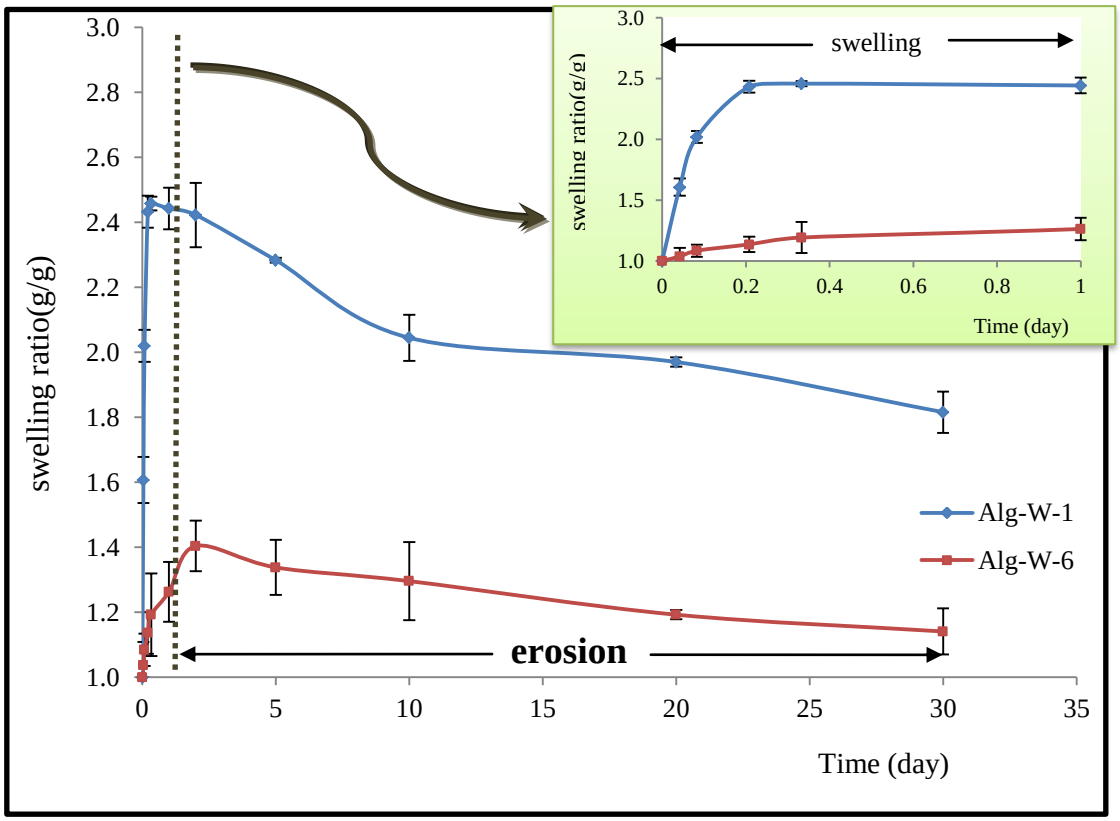


Figure 4.13 Swelling ratio of alginate-starch beads as a function of concentrations of $CaCl_2$. (Alg-W-1: 5%w/w, Alg-W-6: 10%w/w.)

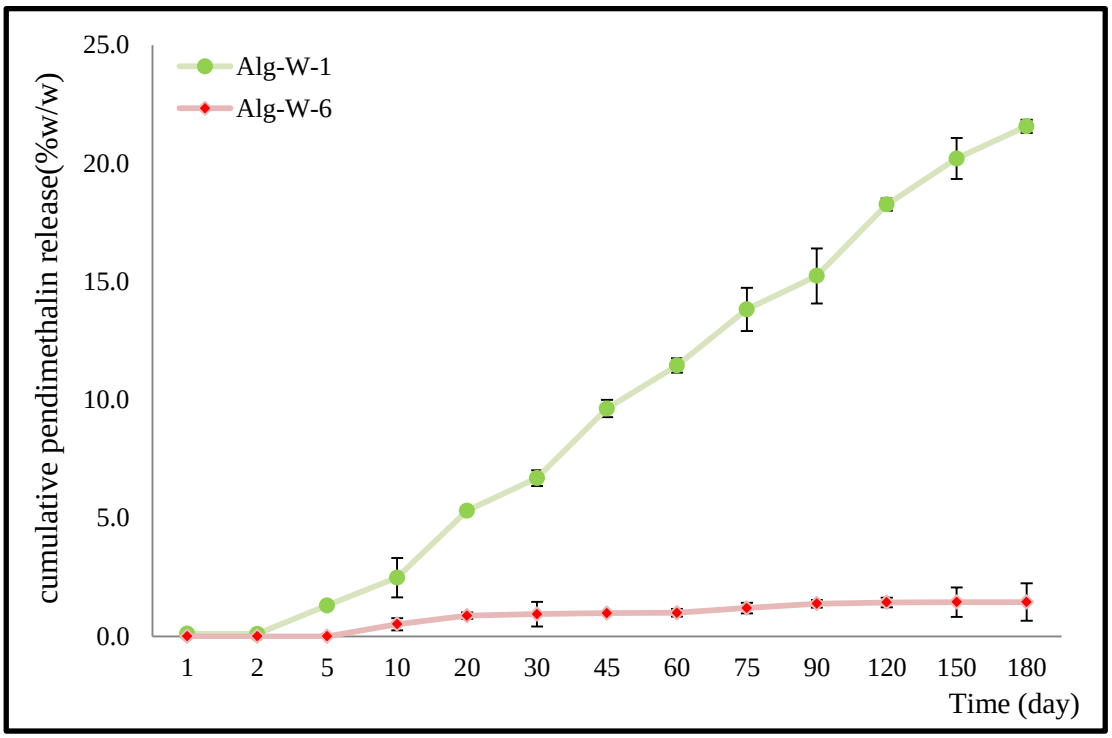


Figure 4.14 Percentage of cumulative pendimethalin released from alginate-starch beads as a function of concentrations of $CaCl_2$. (Alg-W-1: 5%w/w, Alg-W-6: 10%w/w)

4.3.2.4 Immersion time

The length of the immersion time affected the swelling of beads and percentage of pendimethalin released from the beads. The time of immersion is important for Ca^{2+} to act with alginate. The solution would contain Ca^{2+} from $CaCl_2$ which dissolved in solution. Two negatively-charged $-COO^-$ groups, one on one alginate chain and one on the other could associate with each other because both may be attracted to a positive ion. Because of their double charges, Ca^{2+} was better than singly charged ions such as Na^+ from sodium alginate. This had the effect of cross-linking alginate molecules together with a three dimensional network as shown in Figure 4.15. This was called the alginate 'raft'. In fact the cross-linking took place between regions of alginate polymer chains consisting of G monomers. This was because of the zig-zag shape of these regions of the chain which allowed Ca^{2+} to sit neatly between polymer chains. When the immersion period was longer, the crosslink had a higher intensity because Ca^{2+} could react to a greater degree with alginate, then there was a stronger crosslink (Figure 4.15). The stronger crosslink would then block

the beads from adsorbing water and the beads had low rate of erosion. Thus, the immersion period affected the swelling property. If the immersion time of the beads increased from 1 min (Alg-W-1) to 30 min (Alg-W-2) and 24 h (Alg-W-3), the swelling of the beads decreased (Figure 4.16). If the immersion time of the beads increased from 1 min (Alg-W-1) to 30 min (Alg-W-2) and 24 h (Alg-W-3), the percentage of pendimethalin released from the beads would decrease from 22.85 to 6.42 and 0.63%, respectively (Figure 4.17).

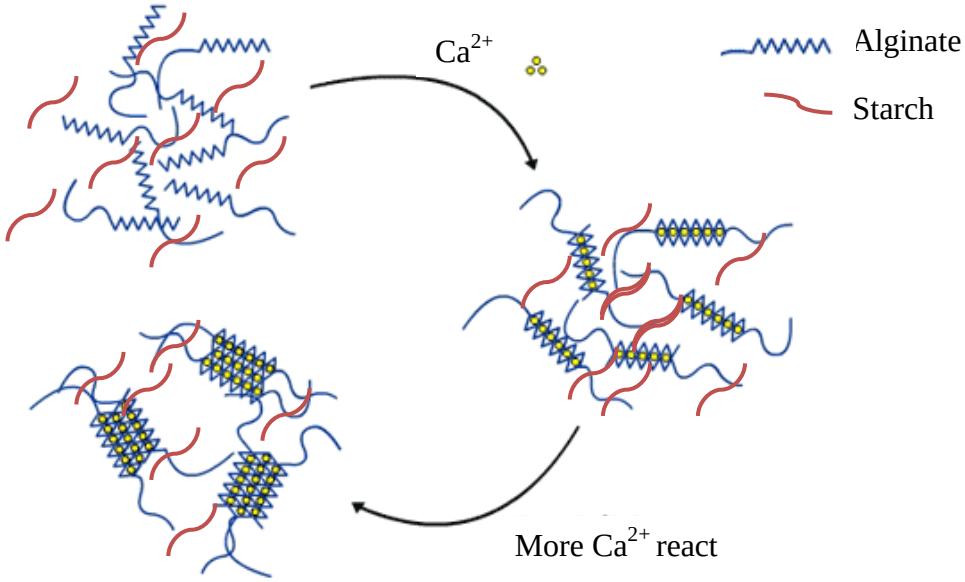


Figure 4.15 Crosslinking of alginate molecules by Ca^{2+} forms an 'alginate raft' when time of immersion was increased in $CaCl_2$ solution.

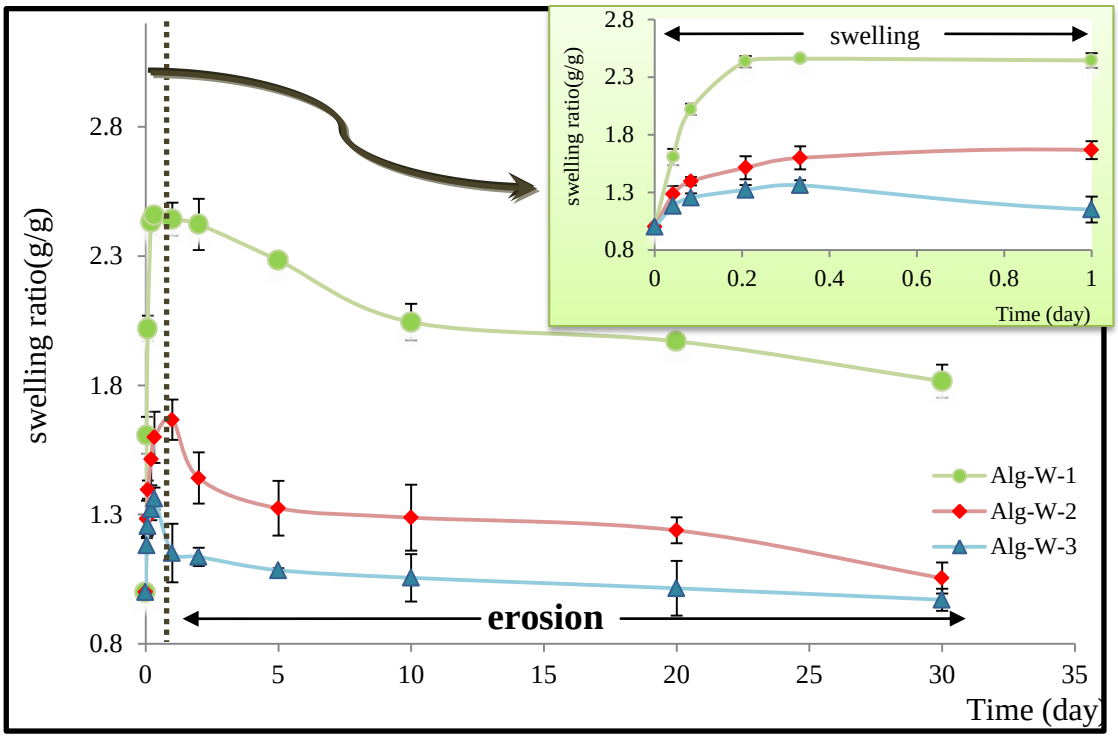


Figure 4.16 Swelling ratio of alginate-starch beads as a function of times of immersion. (Alg-W-1: 1 min, Alg-W-2: 30 min, Alg-W-3: overnight).

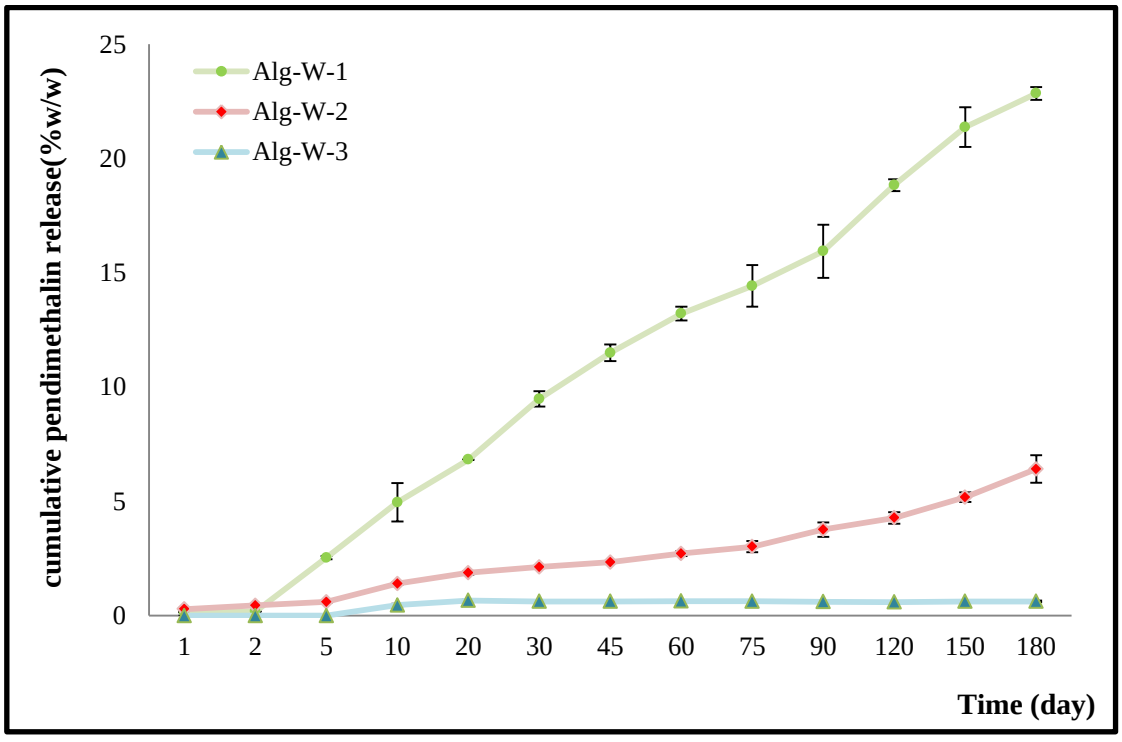


Figure 4.17 Percentage of cumulative pendimethalin released from alginate-starch beads as a function of immersion time. (Alg-W-1: 1 min, Alg-W-2: 30 min, Alg-W-3: overnight).

The results herein indicated that a new pendimethalin-loaded cross-linked alginate–starch formulation was obtained, with high encapsulation efficiency, presenting promising characteristics as compared to conventional calcium alginate beads. The use of a cross-linking agent successfully delayed pendimethalin release, which could be tailored to produce a suitable long time release by adjusting the quantity of cross-linking agent and to obtain the desired time of complete pendimethalin release. Therefore, matrix modification by an appropriate method, such as a suitable type of starch, ratio of alginate:starch, immersion time, concentration of cross-linking reaction, are crucial in the controlled release of pendimethalin from alginate beads. The study showed that chitosan and chitosan-alginate beads had scanty release pendimethalin while alginate and alginate-starch beads had sufficient release pendimethalin. Alginate and alginate-starch beads released pendimethalin by beads swelling and then eroding together with pendimethalin release (Figure 4.18).

(a) swelling beads (1 day).

(b) partially eroding. (10 days)

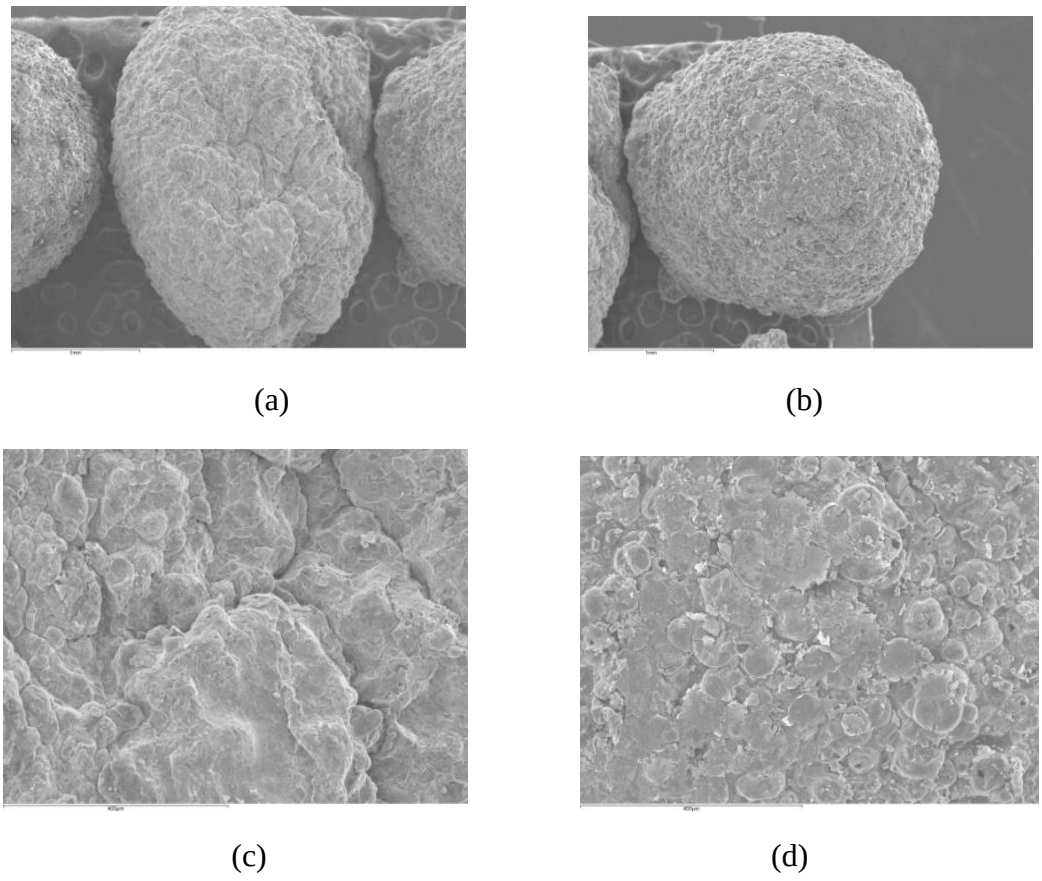
(c) eroding (180 days).

Figure 4.18 Alginate-wheat flour beads were immersed in water for (a) alginate-wheat flour beads were swelling after 1 day, (b) alginate-wheat flour beads were partially eroding after 10 days and (c) alginate-wheat flour beads were eroding after 180 days.

4.3.3 Morphology of beads

4.3.3.1 The surface morphology by SEM

The morphological features of beads have been studied by recording their SEM images as shown in Figure 4.19. In order to gain a clear surface of biopolymer beads, the images were recorded at two different magnifications as shown in the images. The alginate, starch and pendimethalin were dissolved in homogenous solutions. The images showed homogenous bead surface. The images at the lower magnification (x35) of the beads appeared spherical shape for alginate-starch beads, while alginate beads appeared rather flat shape which indicated that the beads prepared with alginate-starch were more spherical than pure alginate. Microscopy analysis showed that beads prepared with calcium alginate or calcium alginate–starch presented an irregular shape irrespective of the composition and preparation method (Figures 19a and b). The surface showed minor cracks and small clusters of alginate-pendimethalin blends and alginate-starch-pendimethalin blends. The images at the larger magnification (x150) of the beads showed that the surfaces of native beads contained irregular shaped bundles of alginate, starch and pendimethalin blends. On the other hand, when starch was added in the beads to reduce the roughness of the alginate beads and eliminated the cracks, the adhesion of the alginate resulted in a smooth outer surface. The beads prepared with chitosan-alginate were smooth in morphology (Figure 19d) compared with alginate beads (Figure 19c). In addition, no particle aggregation was found in the cross-linked formulations, owing to the cross-linking reaction that was carried out before the dropping step into $CaCl_2$ solution. Instead, in the methods previously described, the reaction with the crosslinking agent was usually carried out as a last step for particle formation or hardening, which may lead to particle aggregation.



(a) (b)

(c) (d)

Figure 4.19 Scanning electron micrographs of (a) alginate beads (Alg-1)(x35), (b) alginate-starch beads (Alg-W-1)(x35), (c) alginate beads (Alg-1)(x150) and (d) alginate-starch beads (Alg-W-1)(x150)

4.3.3.2 The composition of beads by FTIR

FTIR was used to confirm the stability of pendimethalin in alginate-starch beads. FTIR spectra of pure alginate (a), and pendimethalin (b) are compared in Figure 4.20. In case of pure pendimethalin, the peaks at 3316 cm^{-1} due to N-H stretching and at 2969, 2932 and 2873 cm^{-1} due to C-H stretching, at 1618 cm^{-1} for N-H bending, and at 1486 cm^{-1} due to N=O stretching (Fig 4.20a) were observed. The spectra clearly marked the presence of alginate as confirmed by O-H stretching of hydroxyl at 3415 cm^{-1} and C=O stretching of carboxylate ion (Figure 4.20b).

The spectra of alginate-starch beads from different types of starch (Figure 4.21) showed that there were the spectra corresponding to N-H stretching at 3322 cm^{-1}, C-H stretching at 2959, 2928, 2873 cm^{-1}, N-H bending at 1623 cm^{-1} and N=O stretching at 1476 cm^{-1} for the formula using wheat flour (Alg-W-1) and for that using rice flour, the peaks were observed at 3322 cm^{-1} due to N-H stretching and 2965, 2931 and 2876 cm^{-1} due to C-H stretching, 1620 cm^{-1} N-H bending, and 1482 cm^{-1} due to N=O stretching. FTIR clearly verified that pendimethalin was encapsulated in beads.

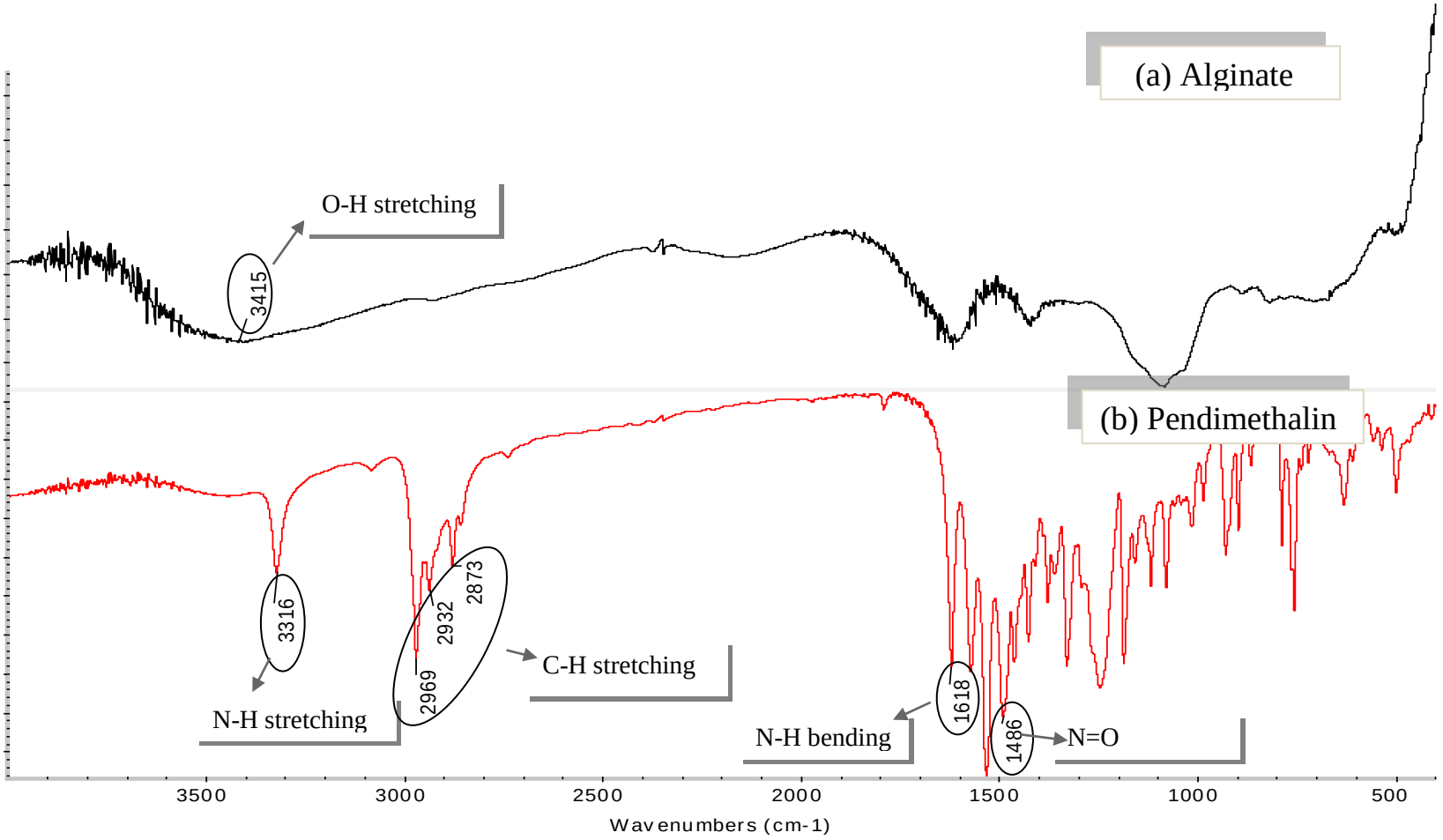


Figure 4.20 FTIR spectra of (a): alginate and (b):pendimethalin**.**

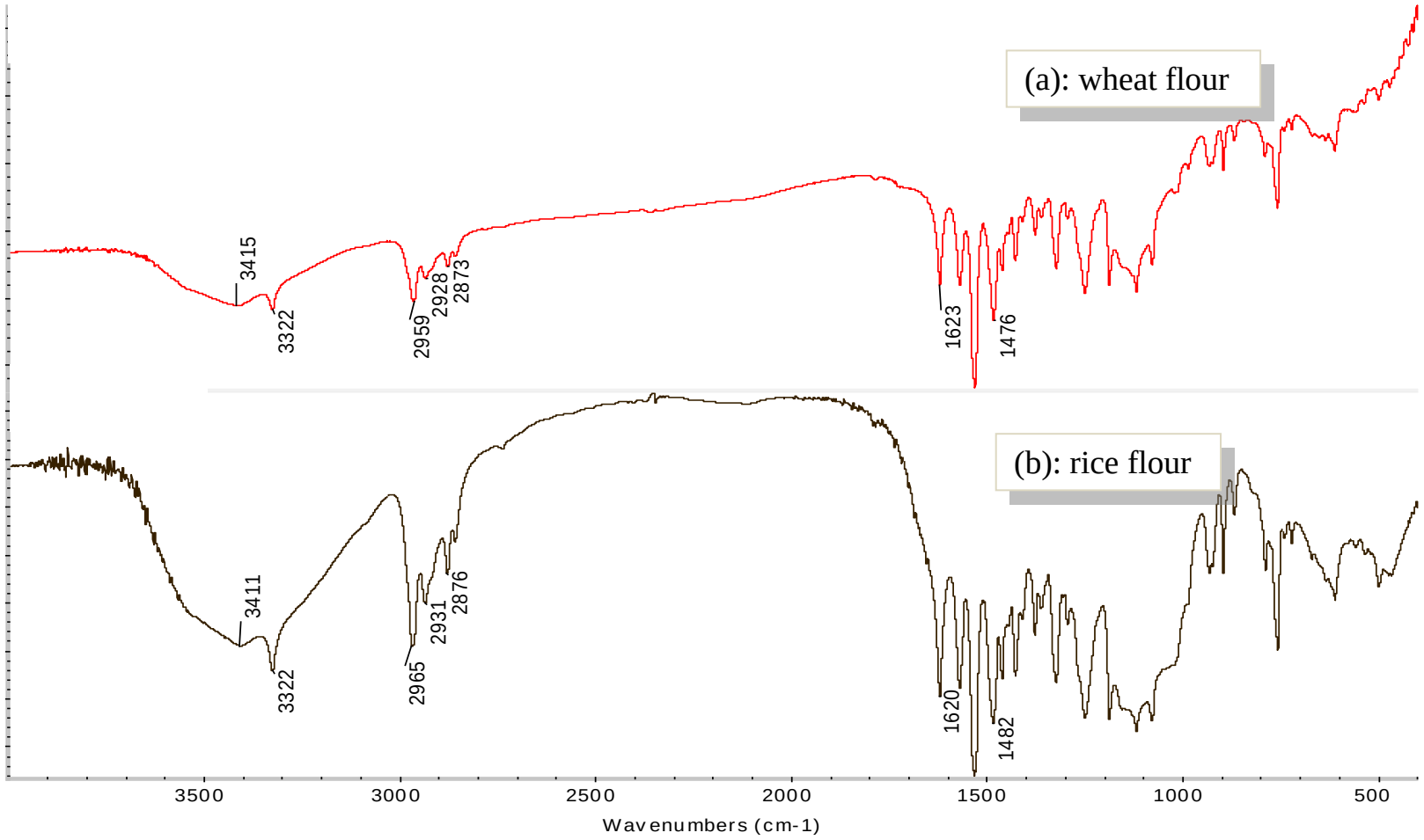


Figure 4.21 FTIR spectra of alginate-starch beads with the different types of starch (a): wheat flour, (b): rice flour.

The spectra of alginate-starch beads with different ratios of starch and alginate (Figure 4.22) showed that there were the peaks corresponding to the hydroxyl group (3430, 3415 and 3405 cm^{-1} for beads with formulated with different ratios of alginate and starch 5:1 (Alg-W-2), 1:1 (Alg-W-4) and 1:2 (Alg-W-5), respectively. For the spectrum of pendimethalin: the peaks belong to N-H stretching (3319, 3319 and 3322 cm^{-1}), C-H stretching ((2971, 2928, 2873 cm^{-1}), (2968, 2928, 2870 cm^{-1}) and (2971, 2934, 2873cm^{-1})), N-H bending (1617, 1623 and 1623cm^{-1}) and N=O stretching (1485, 1482 and 1485 cm^{-1}) for Alg-W-2, Alg-W-4 and Al-W-5, respectively were observed.

The peaks of a hydroxyl group of alginate-starch beads with different immersion times is shown in Figure 4.23. FTIR confirmed that pendimethalin contained in beads. The peaks appeared at 3322 cm^{-1} due to N-H stretching and 2962, 2922, 2873 cm^{-1} due to C-H stretching, 1617 cm^{-1} N-H bending, and 1479 cm^{-1} due to N=O stretching for the formula with an immersion time of 1 min. For alginate-starch beads immersed for 30 min, the peaks appeared at 3319 cm^{-1} due to N-H stretching and 2971, 2931, 2873 cm^{-1} due to C-H stretching, 1623 cm^{-1} N-H bending, and 1485 cm^{-1} due to N=O stretching and showed the peaks corresponding to N-H stretching at 3322 cm^{-1}, C-H stretching at 2968, 2931, 2873 cm^{-1}, N-H bending at 1620 cm^{-1} and N=O stretching at 1485 cm^{-1} for the formula immersed overnight.

The FTIR spectra of alginate-starch beads with different concentrations of $CaCl_2$ (Figure 4.24) showed that there were the peaks corresponding to the hydroxyl group (3408 and 3411 cm^{-1}), 5%w/v (Alg-W-2) and 10%w/v (Alg-W-6), respectively and those of pendimethalin: N-H stretching (3325 and 3325 cm^{-1}), C-H stretching (2962, 2925, 2870 cm^{-1}) and (2965, 2928, 2873 cm^{-1})), N-H bending (1620 and 1617cm^{-1}) and N=O stretching (1479 and 1476 cm^{-1}) for Alg-W-2 and Al-W-6, respectively. The FTIR spectra confirmed that pendimethalin contained in the beads.

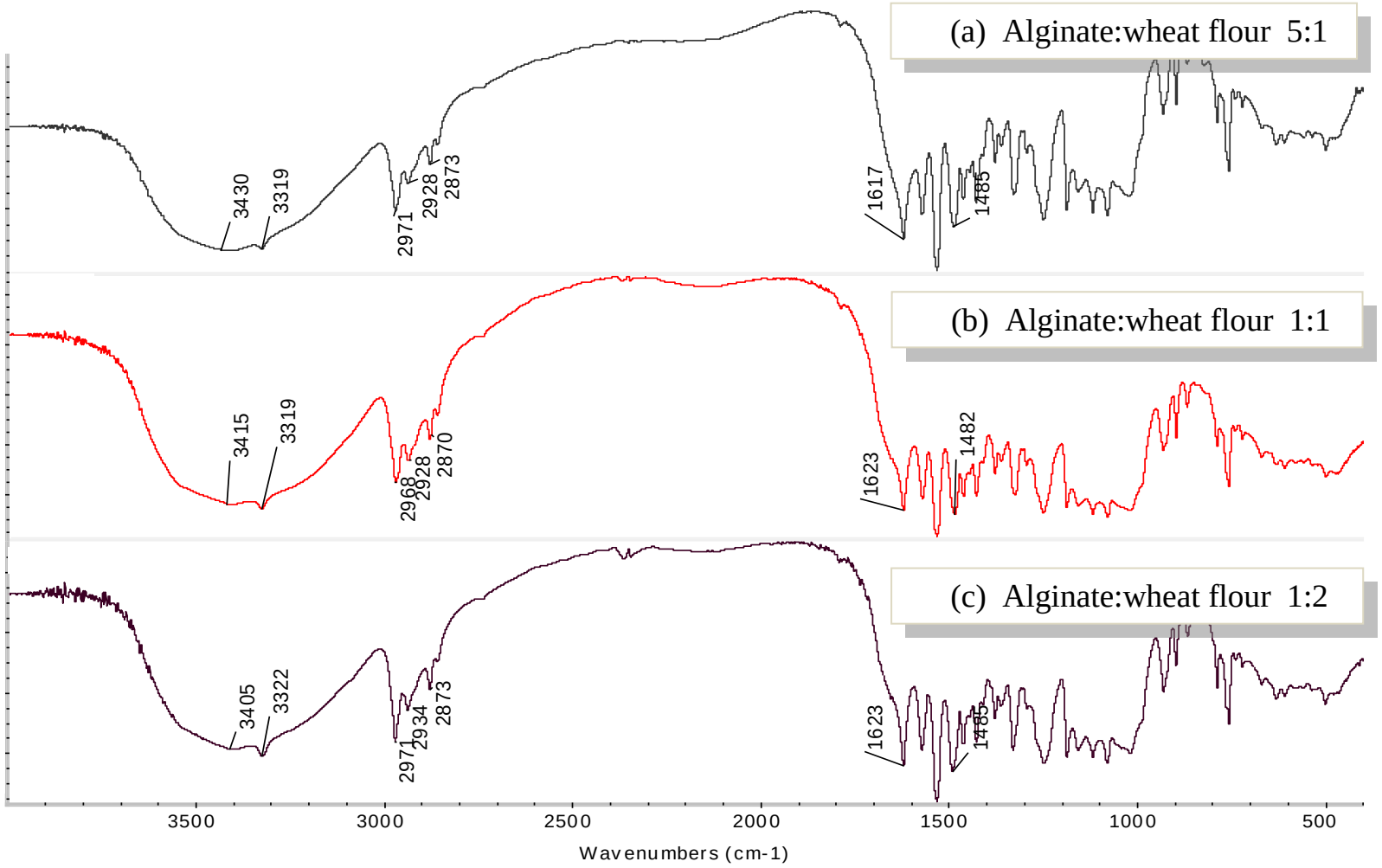


Figure 4.22 FTIR spectra of alginate-starch beads with different ratios of alginate and wheat flour (a) 5:1, (b) 1:1 and (c) 1:2.

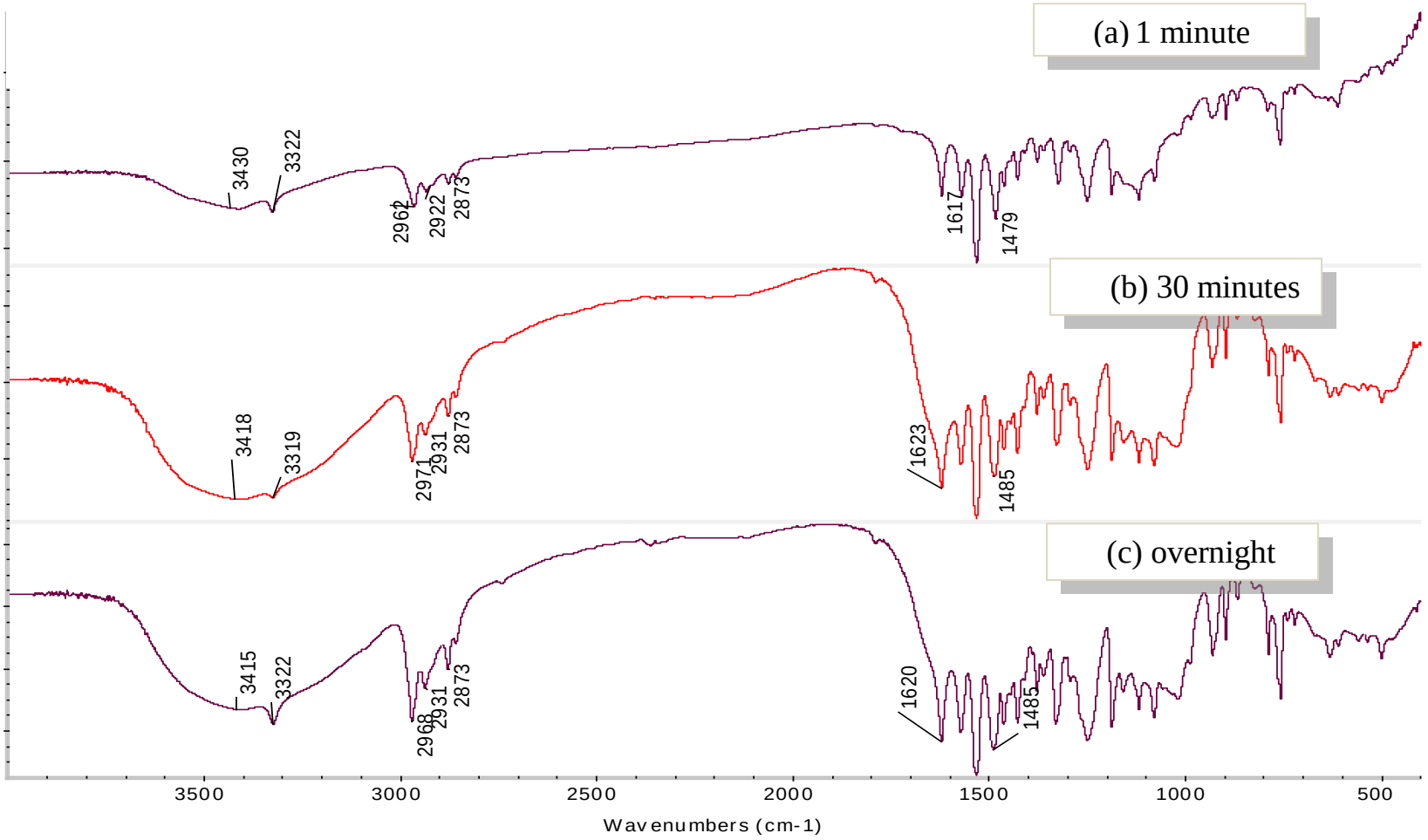


Figure 4.23 FTIR spectra of alginate-starch beads with different immersion times (a): 1 minute, (b): 30 minutes and (c):overnight.

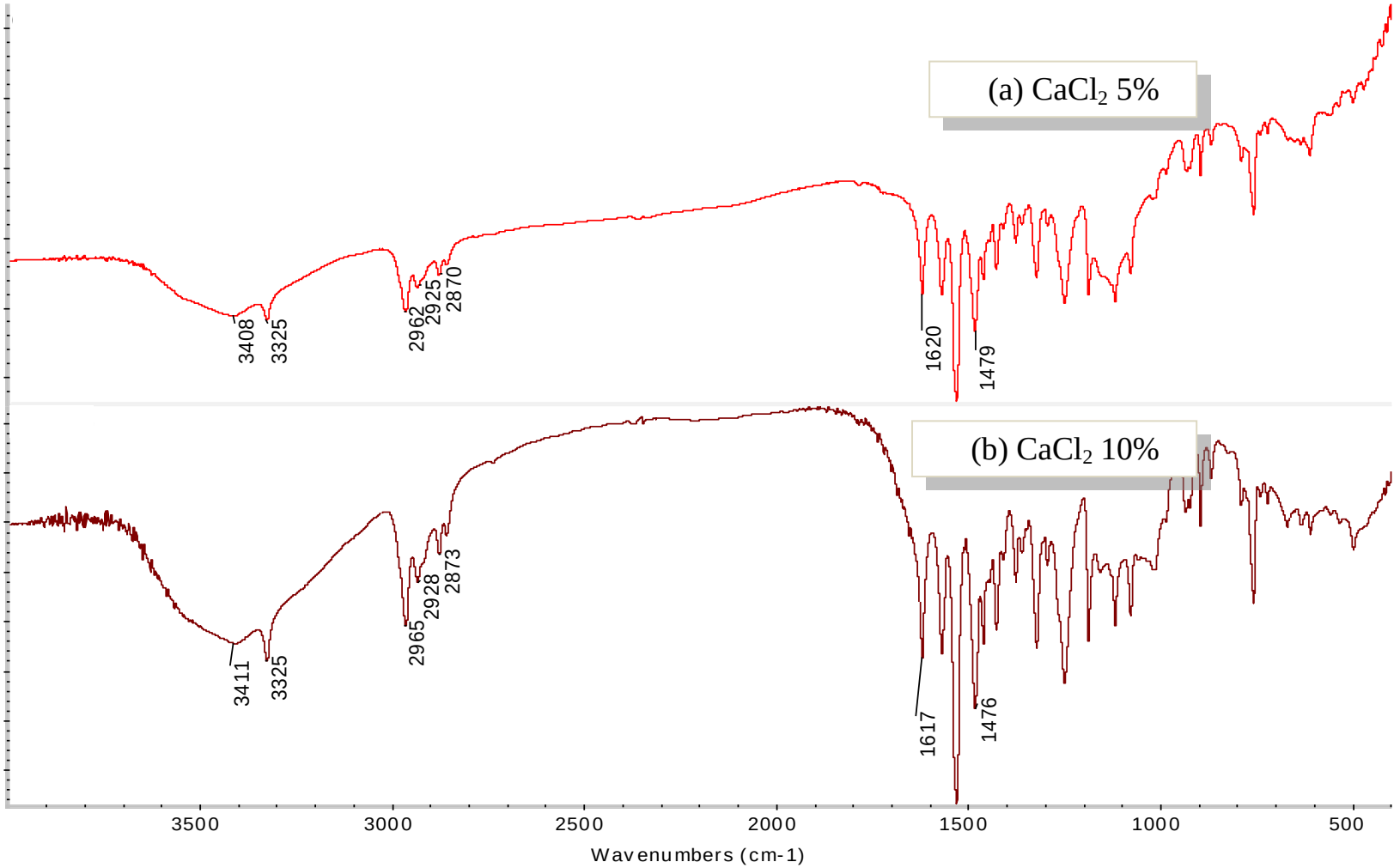


Figure 4.24 FTIR spectra of alginate beads with different concentrations of $CaCl_2$ (a): 5% and (b): 10%.

Table 4.4 % Loading of pendimethalin at 25 and 45°C after 2 weeks and %pendimethalin lost in different formulas.

Formula	% pendimethalin (after 2 weeks) (%w/w)		% loss of pendimethalin (%w/w)
	at 25°C	at 54°C	
Alg-1	34.0±0.8	33.2±0.6	2.3
Alg-W-1	33.3±2.0	32.6±0.8	2.2
Alg-W-2	35.5±0.7	35.0±0.6	1.4
Alg-W-3	36.2±0.6	36.1±1.1	0.2
Alg-W-4	36.6±0.7	35.8±0.3	2.1
Alg-W-5	31.5±0.4	31.2±1.1	0.9
Alg-W-6	28.3±1.0	28.3±0.4	0.1
Alg-R-1	35.4±0.1	34.6±0.6	2.3

4.3.4 Accelerated storage test (Shelf life)

This test method referred to shelf life of the product for two years after production and storage in the market. The physical properties such as color and beads characteristics and %loss of pendimethalin were investigated by comparing the beads stored at 25 and 54^0C for 14 days. The beads that were stored in an oven at 54^0C for 14 days represented that the beads had a shelf-life of two years. The color and characteristics of the beads that were stored at 25 and 54^0C for 14 days were the same. This showed that the beads could be stable and stored in the market for two years. The parameters that impacted the percentage of pendimethalin lost in the beads were the concentration of the crosslinking agent and immersion period. If the concentration of crosslinking agent was higher, the percentage of pendimethalin lost would decrease. Table 4.4 shows that the formula Alg-W-3 (0.2%w/w) had a lower % loss than formula Alg-W-1 (2.2%w/w). If the immersion time of the beads increased from a period of 1 min (Alg-W-1) to 30 min (Alg-W-2) and 24 h (Alg-W-3), the percentage of pendimethalin lost in the beads would decrease from 2.2, 1.4 and 0.2%w/w, respectively. The results of the swelling and release in each formula were in accordance with the percentage lost. Therefore, if the beads had hard beads, the swelling and release decreased and then the percentage loss also decreased. If the swelling and release increased, the percentage loss also increased. The experiment found the formula that had the lowest swelling and release rate used a high concentration of $CaCl_2$, as formula Alg-W-6, had 0.1%w/w loss and formula Alg-W-3 had 0.2%w/w loss. For the opposite result, the formula that had the highest swelling and release rate, Alg-1, had the highest percentage loss (2.2%w/w). The acceptable storage at two years is 5.0 %w/w loss.

4.3.5 Efficacy studies

The objective of this study was to confirm the efficacy of the loaded beads with pendimethalin that could inhibit cell division at root and cell elongation at the root of the grass-seed, control the weed population and prevent weeds from emerging. The target of the weed control should effectively control the weeds for more than 6 months. Thus, the new grass-seed had to be added in the plot every month to observe the efficacy for 6 months. The control plot was used for confirming the germination

rate of the grass-seed. Two formulas of the loaded beads selected were Alg-W-1 and Alg-R-1 because they had the maximum percentage release of pendimethalin and showed high release rate of pendimethalin. The selected grass-seed in this work was birdsrice seed because it could be found throughout Thailand, and normally needed chemicals to control in the field, for example, golf field. The results from Figure 4.25 showed the followings:

- The birdsrice seeds of the control set (without our beads) had germinated at day four, but the seeds in the plot with our beads of Alg-R-1 and Alg-W-1 started to germinate at the fifth day.
- The germination rate of birdsrice seed was 75% in the two control sets.
- In the plot with the Alg-W-1 and Alg-R-1, the germination rate was found to be 15 and 20%, respectively.

The results suggested that the plots with the beads could delay and decreased %germinations of weed seeds. According to Table 4.5, birdrice seeds had continuously grown in the control set, while in the plots with the prepared beads, they stopped growing because pendimethalin could release from the beads, and inhibited cell division at root and cell elongation at the root of the seed. At the 15^{th} day, the birdrice in all plots with the beads died, whereas the birdrice in the control set are grow normally. At the 30^{th}, 60^{th}, 90^{th}, 120^{th}, 150^{th} and 180^{th} days, the birdrice seeds were added into the plots. Five days later, there were only one or two germinations of birdsrice seeds. At the 15^{th} day, all birdsrice seeds died (Figure 4.25).

The loaded beads (alginate or alginate-wheat flour) with pendimethalin (Alg-W-1 or Alg-R-1) could inhibit cell division and cell elongation of the root of the birdsrice seed, and then controlled the weed population and prevented weed from emerging for six months (180 days).

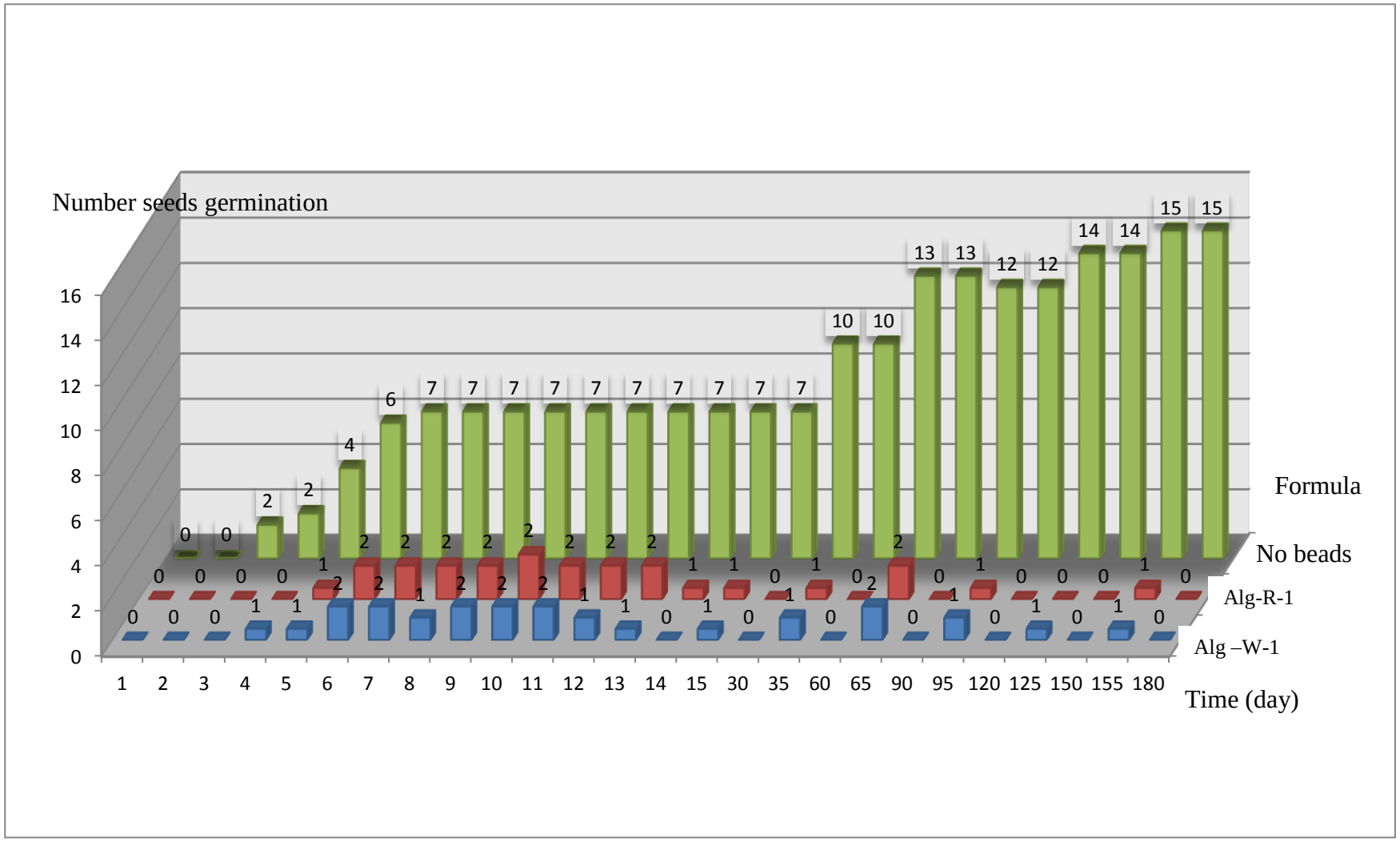


Figure 4.25 Number of birdsrice seed germinations by comparing control set for alginate-wheat flour and alginate-rice flour beads.

Table 4.5 The efficacy study results of loaded beads with pendimethalin for use in controlling the birdsrice seed germination in the plot.

Time / Formula	6 day	8 day	10 day
Control set			
Alg-W-1			
Alg-R-1			

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Time / formula	15 day	30 day	60 day
Control set			
Alg-W-1			
Alg-R-1			

Time / formula	90 day	120 day
Control set		
Alg-W-1		
Alg-R-1		

Time / formula	150 day	180 day
Control set		
Alg-W-1		
Alg-R-1		

4.3.6 Worthiness of using alginate beads

Comparing to the market price of pendimethalin 33%w/v in 2012, it is found that the EC formula is 280 Baht/L, whereas the bead formula produced from alginates and starch is 280 Baht/Kg. The market price will be higher if the products are placed at the market. However, the bead formula is worth using. The normal formula is needed to be sprayed every month to restrict germination of weeds. Moreover, the spraying equipment also requires maintenance expenses every month. In contrast, the bead formula can restrict germination of weeds for six months. So, it can be referred that the bead formula is worthier to use in long term.

CHAPTER V

CONCLUSIONS

5.1 Conclusion

This research is related to encapsulation of pendimethalin by biodegradable polymer. The preliminary studies for the hydrogel preparation and the entrapment of pendimethalin in chitosan beads showed that the release rate of pendimethalin is not satisfied, only 0.8% of pendimethalin was released from the chitosan beads for 90 days. To increase the release rates of pendimethalin, alginate as good water soluble polymer and can act as hydrogel was used to composite with chitosan. The study showed that chitosan and chitosan-alginate beads also showed very slow release rates that will cause to use more beads applied in the field. The results were still not satisfied. To solve this problem, chitosan which is non-water soluble polymer was omitted from the formulation. Alginate was a choice of encapsulated pendimethalin because it could swell very well in water. The next experiments involved the preparation using alginate for increased percentage of pendimethalin release and still maintain a longer release time of 6 months. Therefore, alginate beads were prepared by dropping an aqueous suspension of pendimethalin-alginate into a $CaCl_2$ solution. During bead drying, the initial spherical shape is lost and shrinkage occurs. So, starches are an alternative polymer that can easily biodegrade in water were selected to composite with alginate. Then, the beads were prepared using alginate with two types of starches; wheat and rice. SEM of microspheres suggested the formation of spherical beads. FTIR spectral data were used to confirm chemical stability of pendimethalin in alginate-starch beads. A series of such beads of different compositions were prepared by varying type of starch, ratio of alginate: starch, concentration of $CaCl_2$ and immersion time in $CaCl_2$ solution. The concentration of crosslinking agent and immersion time also affected the swelling property and release profile. If the concentrations of the crosslinking agent and immersion times were increased the swelling and releasing would be decreased. The release mechanism of

alginate-starch beads released pendimethalin at the beginning of the beads swelling and then eroding together with pendimethalin release. Accelerated storage test showed that the alginate-starch beads containing pendimethalin were stable at least for 24 months. Efficacy study in transparent plastic box confirmed that the efficacy of loaded beads with pendimethalin could be use to made weeds die in a short time after germination. It showed that the alginate-starch beads sufficiently released pendimethalin in a sustained-release fashion for 6 months.

5.2 Recommendation for the future work

This formulation may be used for further study as a long term controlled release drug model by decreasing crosslink agents, immersion time and amount of pendimethalin to decrease amount of beads applied in the field.

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APPENDICES

Appendix A

The weight and value of swelling ratio of beads

Table A-1 The weight of chitotan-alginate beads.

Formulation	No.	Weight of beads (g)										
		0 hr	1 hr	2 hrs	5 hrs	8 hrs	1 day	2 days	5 days	10 days	20 days	30 days
CTS-Alg-1	1	0.0324	0.0369	0.0399	0.0454	0.0499	0.0528	0.0518	0.0509	0.0499	0.0470	0.0460
	2	0.0243	0.0262	0.0275	0.0306	0.0321	0.0347	0.0365	0.0343	0.0355	0.0289	0.0301
CTS-Alg-2	1	0.0276	0.0301	0.0309	0.0331	0.0356	0.0381	0.0378	0.0359	0.0356	0.0342	0.0317
	2	0.0254	0.0287	0.0310	0.0351	0.0368	0.0386	0.0373	0.0366	0.0358	0.0340	0.0338
CTS-Alg-3	1	0.0267	0.0286	0.0291	0.0323	0.0331	0.0336	0.0339	0.0326	0.0320	0.0315	0.0312
	2	0.0203	0.0229	0.0246	0.0282	0.0296	0.0309	0.0307	0.0288	0.0280	0.0264	0.0266
CTS-Alg-4	1	0.0265	0.0273	0.0284	0.0294	0.0315	0.0321	0.0329	0.0337	0.0329	0.0310	0.0318
	2	0.0312	0.0334	0.0353	0.0396	0.0402	0.0402	0.0424	0.0409	0.0399	0.0390	0.0393
CTS-Alg-5	1	0.0246	0.0251	0.0263	0.0283	0.0285	0.0293	0.0303	0.0288	0.0303	0.0308	0.0303
	2	0.0276	0.0287	0.0284	0.0301	0.0309	0.0317	0.0323	0.0312	0.0323	0.0334	0.0323
CTS-Alg-6	1	0.0218	0.0218	0.0220	0.0238	0.0235	0.0240	0.0240	0.0235	0.0233	0.0229	0.0229
	2	0.0236	0.0241	0.0243	0.0262	0.0264	0.0269	0.0269	0.0255	0.0271	0.0257	0.0281

Table A-2 The value of swelling ratio of chitotan-alginate beads.

Formulation	No.	Swelling ratio (g/g)										
		0 hr	1 hr	2 hrs	5 hrs	8 hrs	1 day	2 days	5 days	10 days	20 days	30 days
CTS-Alg-1	1	1.00	1.14	1.23	1.4	1.54	1.63	1.6	1.57	1.54	1.45	1.42
	2	1.00	1.08	1.13	1.26	1.32	1.43	1.5	1.41	1.46	1.19	1.24
	Average	1.00	1.11	1.18	1.33	1.43	1.53	1.55	1.49	1.50	1.32	1.33
CTS-Alg-2	1	1.00	1.09	1.12	1.20	1.29	1.38	1.37	1.30	1.29	1.24	1.15
	2	1.00	1.13	1.22	1.38	1.45	1.52	1.47	1.44	1.41	1.34	1.33
	Average	1.00	1.11	1.17	1.29	1.37	1.45	1.42	1.37	1.35	1.29	1.24
CTS-Alg-3	1	1.00	1.07	1.09	1.21	1.24	1.26	1.27	1.22	1.20	1.18	1.17
	2	1.00	1.13	1.21	1.39	1.46	1.52	1.51	1.42	1.38	1.3	1.31
	Average	1.00	1.1	1.15	1.30	1.35	1.39	1.39	1.32	1.29	1.24	1.24
CTS-Alg-4	1	1.00	1.03	1.07	1.11	1.19	1.21	1.24	1.27	1.24	1.17	1.2
	2	1.00	1.07	1.13	1.27	1.29	1.29	1.36	1.31	1.28	1.25	1.26
	Average	1.00	1.05	1.10	1.19	1.24	1.25	1.30	1.29	1.26	1.21	1.23
CTS-Alg-5	1	1.00	1.02	1.07	1.15	1.16	1.19	1.23	1.17	1.23	1.25	1.23
	2	1.00	1.04	1.03	1.09	1.12	1.15	1.17	1.13	1.17	1.21	1.17
	Average	1.00	1.03	1.05	1.12	1.14	1.17	1.20	1.15	1.20	1.23	1.20
CTS-Alg-6	1	1.00	1.00	1.01	1.09	1.08	1.10	1.10	1.08	1.07	1.05	1.05
	2	1.00	1.02	1.03	1.11	1.12	1.14	1.14	1.08	1.15	1.09	1.19
	Average	1.00	1.01	1.02	1.1	1.1	1.12	1.12	1.08	1.11	1.07	1.12

Table A-3 The weight of chitotan beads.

Formulation	No.	Weight of beads (g)										
		0 hr	1 hr	2 hrs	5 hrs	8 hrs	1 day	2 days	5 days	10 days	20 days	30 days
CTS-1	1	0.0297	0.0422	0.0463	0.0484	0.0514	0.0520	0.0490	0.0457	0.0416	0.0422	0.0386
	2	0.0233	0.0317	0.0326	0.0347	0.0384	0.0389	0.0361	0.0322	0.0294	0.0298	0.0284
CTS-2	1	0.0249	0.0334	0.0383	0.0396	0.0398	0.0401	0.0378	0.0364	0.0334	0.0329	0.0324
	2	0.0206	0.0268	0.0276	0.0290	0.0309	0.0303	0.0305	0.0276	0.0260	0.0260	0.0251
CTS-3	1	0.0265	0.0318	0.0350	0.0376	0.0384	0.0390	0.0371	0.0350	0.0334	0.0331	0.0315
	2	0.0237	0.0327	0.0337	0.0356	0.0363	0.0363	0.0360	0.0332	0.0318	0.0325	0.0292
CTS-4	1	0.0203	0.0238	0.0248	0.0262	0.0268	0.0264	0.0262	0.0256	0.0242	0.0244	0.0240
	2	0.0227	0.0279	0.0313	0.0320	0.0331	0.0336	0.0308	0.0299	0.0292	0.0291	0.0277
CTS-5	1	0.0287	0.0330	0.0347	0.0373	0.0362	0.0364	0.0362	0.0350	0.0339	0.0344	0.0330
	2	0.022	0.0235	0.0257	0.0260	0.0273	0.0293	0.0290	0.0286	0.0273	0.0277	0.0271
CTS-6	1	0.0268	0.0292	0.0308	0.0314	0.0054	0.0330	0.0348	0.0322	0.0330	0.0335	0.0316
	2	0.0212	0.0214	0.0231	0.0235	0.0454	0.0244	0.0254	0.0267	0.0244	0.0244	0.0237

Table A-4 The value of swelling ratio of chitotan beads.

Formulation	No.	Swelling ratio(g/g)										
		0 hr	1 hr	2 hrs	5 hrs	8 hrs	1 day	2 days	5 days	10 days	20 days	30 days
CTS-1	1	1.00	1.42	1.56	1.63	1.73	1.75	1.65	1.54	1.40	1.42	1.30
	2	1.00	1.36	1.40	1.49	1.65	1.67	1.55	1.38	1.26	1.28	1.22
	Average	1.00	1.39	1.48	1.56	1.69	1.71	1.60	1.46	1.33	1.35	1.26
CTS -2	1	1.00	1.34	1.54	1.59	1.60	1.61	1.52	1.46	1.34	1.32	1.30
	2	1.00	1.30	1.34	1.41	1.50	1.47	1.48	1.34	1.26	1.26	1.22
	Average	1.00	1.32	1.44	1.50	1.55	1.54	1.50	1.40	1.30	1.29	1.26
CTS-3	1	1.00	1.20	1.32	1.42	1.45	1.47	1.40	1.32	1.26	1.25	1.19
	2	1.00	1.38	1.42	1.50	1.53	1.53	1.52	1.40	1.34	1.37	1.23
	Average	1.00	1.29	1.37	1.46	1.49	1.50	1.46	1.36	1.30	1.31	1.21
CTS-4	1	1.00	1.17	1.22	1.29	1.32	1.30	1.29	1.26	1.19	1.20	1.18
	2	1.00	1.23	1.38	1.41	1.46	1.48	1.36	1.32	1.29	1.28	1.22
	Average	1.00	1.20	1.30	1.35	1.39	1.39	1.32	1.29	1.24	1.24	1.20
CTS-5	1	1.00	1.15	1.21	1.30	1.26	1.27	1.26	1.22	1.18	1.20	1.15
	2	1.00	1.07	1.17	1.18	1.24	1.33	1.32	1.30	1.24	1.26	1.23
	Average	1.00	1.11	1.19	1.24	1.25	1.30	1.29	1.26	1.21	1.23	1.19
CTS-6	1	1.00	1.09	1.15	1.17	0.20	1.23	1.30	1.20	1.23	1.25	1.18
	2	1.00	1.01	1.09	1.11	2.14	1.15	1.20	1.26	1.15	1.15	1.12
	Average	1.00	1.05	1.12	1.14	1.17	1.19	1.25	1.23	1.19	1.20	1.15

Table A-5 The weight of alginate and alginate-starch beads.

Formulation	No.	Weight of beads (g)										
		0 hr	1 hr	2 hrs	5 hrs	8 hrs	1 day	2 days	5 days	10 days	20 days	30 days
Alg-1	1	0.0256	0.0486	0.0614	0.0771	0.0799	0.0783	0.0676	0.0630	0.0489	0.0474	0.0451
	2	0.0204	0.0347	0.0454	0.0589	0.0607	0.0573	0.0515	0.0461	0.0377	0.0336	0.0308
Alg-W-1	1	0.0225	0.0383	0.0470	0.0565	0.0560	0.0572	0.0578	0.0518	0.0484	0.0448	0.0428
	2	0.0177	0.0268	0.0345	0.0417	0.0429	0.0415	0.0403	0.0401	0.0343	0.0345	0.0307
Alg-W-2	1	0.0217	0.0263	0.0297	0.0302	0.0332	0.0349	0.0297	0.0271	0.0260	0.0252	0.0215
	2	0.0227	0.0308	0.0323	0.0372	0.0379	0.0391	0.0343	0.0317	0.0312	0.0299	0.0254
Alg-W-3	1	0.0264	0.0317	0.0338	0.0356	0.0367	0.0325	0.0306	0.0285	0.0296	0.0288	0.0264
	2	0.0278	0.0323	0.0342	0.0359	0.0371	0.0298	0.0309	0.0303	0.0275	0.0261	0.0262
Alg-W-4	1	0.0276	0.0276	0.0279	0.0298	0.0312	0.0331	0.0362	0.0356	0.0326	0.0331	0.0320
	2	0.0238	0.0260	0.0260	0.0271	0.0287	0.0326	0.0335	0.0336	0.0332	0.0307	0.0294
Alg-W-5	1	0.0245	0.0292	0.0301	0.0333	0.0365	0.0370	0.0365	0.0350	0.0301	0.0292	0.0282
	2	0.0285	0.0315	0.0321	0.0354	0.0384	0.0406	0.0360	0.0341	0.0340	0.0332	0.0309
Alg-W-6	1	0.0243	0.0241	0.0255	0.0265	0.0267	0.0292	0.0328	0.0311	0.0294	0.0287	0.0265
	2	0.0183	0.0199	0.0205	0.0216	0.0235	0.0243	0.0267	0.0255	0.0253	0.0221	0.0218
Alg-R-1	1	0.0266	0.0372	0.0386	0.0434	0.0489	0.0500	0.0497	0.0460	0.0404	0.0396	0.0394
	2	0.0304	0.0391	0.0406	0.0471	0.0527	0.0534	0.0468	0.0462	0.0440	0.0432	0.0399

Table A-6 The value of swelling ratio of alginate and alginate-starch beads.

Formulation	No.	Swelling ratio (g/g)										
		0 hr	1 hr	2 hrs	5 hrs	8 hrs	1 day	2 days	5 days	10 days	20 days	30 days
Alg-1	1	1.00	1.90	2.40	3.01	3.12	3.06	2.64	2.46	1.91	1.85	1.76
	2	1.00	1.70	2.23	2.89	2.98	2.81	2.53	2.26	1.85	1.65	1.51
	Average	1.00	1.80	2.31	2.95	3.05	2.93	2.58	2.36	1.88	1.75	1.63
Alg-W -1	1	1.00	1.70	2.09	2.51	2.49	2.54	2.57	2.30	2.15	1.99	1.90
	2	1.00	1.51	1.95	2.36	2.43	2.35	2.28	2.27	1.94	1.95	1.73
	Average	1.00	1.61	2.02	2.43	2.46	2.44	2.42	2.28	2.04	1.97	1.82
Alg-W-2	1	1.00	1.21	1.37	1.39	1.53	1.61	1.37	1.25	1.20	1.16	0.99
	2	1.00	1.36	1.42	1.64	1.67	1.72	1.51	1.40	1.38	1.32	1.12
	Average	1.00	1.28	1.40	1.51	1.60	1.67	1.44	1.32	1.29	1.24	1.05
Alg-W-3	1	1.00	1.20	1.28	1.35	1.39	1.23	1.16	1.08	1.12	1.09	1.00
	2	1.00	1.16	1.23	1.29	1.33	1.07	1.11	1.09	0.99	0.94	0.94
	Average	1.00	1.18	1.25	1.32	1.36	1.15	1.14	1.08	1.06	1.01	0.97
Alg-W-4	1	1.00	1.00	1.01	1.08	1.13	1.20	1.31	1.29	1.18	1.20	1.16
	2	1.00	1.09	1.09	1.14	1.20	1.37	1.41	1.41	1.40	1.29	1.24
	Average	1.00	1.05	1.05	1.11	1.17	1.28	1.36	1.35	1.29	1.25	1.20
Alg-W-5	1	1.00	1.19	1.23	1.36	1.49	1.51	1.49	1.43	1.23	1.19	1.15
	2	1.00	1.10	1.12	1.24	1.35	1.43	1.26	1.20	1.19	1.16	1.08
	Average	1.00	1.15	1.18	1.30	1.42	1.47	1.38	1.31	1.21	1.18	1.12
Alg-W-6	1	1.00	0.99	1.05	1.09	1.10	1.20	1.35	1.28	1.21	1.18	1.09
	2	1.00	1.09	1.12	1.18	1.28	1.33	1.46	1.40	1.38	1.20	1.19
	Average	1.00	1.04	1.08	1.14	1.19	1.26	1.40	1.34	1.30	1.19	1.14
Alg-R-1	1	1.00	1.40	1.45	1.63	1.84	1.88	1.87	1.73	1.52	1.49	1.48
	2	1.00	1.29	1.34	1.55	1.73	1.76	1.54	1.52	1.45	1.42	1.31
	Average	1.00	1.34	1.39	1.59	1.79	1.82	1.71	1.62	1.48	1.46	1.40

Appendix B

Pendimethalin release from GC DATA

Table B-1 The data analysis of percentage of pendimethalin from GC technique(chitosan beads).

Formulation	No.	%Pendimethalin from GC (g/g)									
		1 Day	2 Days	5 Days	10 Days	20 Days	30 Days	45 Days	60 Days	75 Days	90 Days
CTS-1	1	0.06	0.11	0.17	0.18	0.23	0.26	0.29	0.30	0.31	0.31
	2	0.08	0.14	0.18	0.20	0.25	0.29	0.30	0.33	0.34	0.33
CTS -2	1	0.07	0.12	0.17	0.19	0.24	0.28	0.30	0.31	0.33	0.32
	2	0.08	0.11	0.15	0.19	0.22	0.24	0.23	0.25	0.25	0.25
CTS-3	1	0.10	0.16	0.18	0.21	0.24	0.28	0.27	0.28	0.28	0.28
	2	0.09	0.13	0.16	0.20	0.23	0.26	0.25	0.26	0.26	0.27
CTS-4	1	0.08	0.14	0.14	0.17	0.19	0.20	0.22	0.22	0.22	0.23
	2	0.09	0.15	0.16	0.17	0.21	0.21	0.23	0.24	0.25	0.25
CTS-5	1	0.09	0.15	0.15	0.17	0.20	0.20	0.22	0.23	0.23	0.24
	2	0.05	0.10	0.15	0.17	0.16	0.17	0.19	0.20	0.21	0.22
CTS-6	1	0.06	0.12	0.16	0.16	0.17	0.18	0.21	0.23	0.24	0.23
	2	0.06	0.11	0.16	0.16	0.17	0.17	0.20	0.21	0.22	0.22

Table B-2 The data of cumulative pendimethalin release from chitosan beads analysis by GC technique.

Formulation	No.	%Pendimethalin release (g/g)									
		1 Day	2 Days	5 Days	10 Days	20 Days	30 Days	45 Days	60 Days	75 Days	90 Days
CTS-1	1	0.18	0.32	0.49	0.54	0.68	0.77	0.86	0.89	0.92	0.91
	2	0.22	0.40	0.51	0.58	0.70	0.83	0.86	0.93	0.96	0.95
	Average	0.20	0.36	0.50	0.56	0.69	0.80	0.86	0.91	0.94	0.93
CTS -2	1	0.24	0.32	0.45	0.55	0.65	0.71	0.69	0.73	0.74	0.74
	2	0.28	0.44	0.49	0.57	0.67	0.77	0.75	0.77	0.76	0.78
	Average	0.26	0.38	0.47	0.56	0.66	0.74	0.72	0.75	0.75	0.76
CTS-3	1	0.23	0.42	0.43	0.51	0.55	0.58	0.64	0.65	0.65	0.69
	2	0.27	0.44	0.45	0.49	0.59	0.60	0.66	0.69	0.71	0.71
	Average	0.25	0.43	0.44	0.50	0.57	0.59	0.65	0.67	0.68	0.70
CTS-4	1	0.15	0.32	0.47	0.51	0.50	0.52	0.58	0.60	0.65	0.66
	2	0.19	0.36	0.49	0.49	0.52	0.54	0.62	0.70	0.71	0.68
	Average	0.17	0.34	0.48	0.50	0.51	0.53	0.60	0.65	0.68	0.67
CTS-5	1	0.12	0.30	0.40	0.46	0.50	0.53	0.53	0.51	0.57	0.59
	2	0.10	0.28	0.34	0.44	0.46	0.49	0.51	0.49	0.51	0.51
	Average	0.11	0.29	0.37	0.45	0.48	0.51	0.52	0.50	0.54	0.55
CTS-6	1	0.09	0.20	0.25	0.30	0.31	0.35	0.39	0.27	0.28	0.18
	2	0.11	0.22	0.31	0.32	0.31	0.37	0.41	0.31	0.30	0.16
	Average	0.10	0.21	0.28	0.31	0.31	0.36	0.40	0.29	0.29	0.17

Table B-3 The data analysis of percentage of pendimethalin from GC technique(chitosan-alginate beads).

Formulation	No.	%Pendimethalin from GC (g/g)									
		1 Day	2 Days	5 Days	10 Days	20 Days	30 Days	45 Days	60 Days	75 Days	90 Days
CTS-Alg-1	1	0.21	0.27	0.35	0.36	0.50	0.51	0.54	0.61	0.74	1.06
	2	0.18	0.24	0.31	0.33	0.45	0.48	0.55	0.55	0.66	0.87
CTS-Alg-2	1	0.19	0.25	0.33	0.35	0.47	0.50	0.55	0.58	0.70	0.96
	2	0.12	0.29	0.31	0.33	0.37	0.48	0.53	0.71	0.72	0.75
CTS-Alg-3	1	0.12	0.30	0.33	0.33	0.41	0.49	0.57	0.73	0.75	0.79
	2	0.12	0.29	0.32	0.33	0.39	0.49	0.55	0.72	0.73	0.77
CTS-Alg-4	1	0.21	0.21	0.22	0.24	0.31	0.35	0.36	0.40	0.49	0.75
	2	0.23	0.23	0.24	0.26	0.34	0.38	0.40	0.45	0.51	0.78
CTS-Alg-5	1	0.22	0.22	0.23	0.25	0.32	0.36	0.38	0.43	0.50	0.77
	2	0.28	0.30	0.31	0.33	0.33	0.37	0.36	0.38	0.47	0.50
CTS-Alg-6	1	0.31	0.30	0.31	0.35	0.35	0.38	0.37	0.39	0.48	0.54
	2	0.29	0.30	0.31	0.34	0.34	0.37	0.36	0.38	0.47	0.52

Table B-4 The data of cumulative pendimethalin release from chitosan-alginate beads analysis by GC technique.

Formulation	No.	%Pendimethalin from GC (g/g)									
		1 Day	2 Days	5 Days	10 Days	20 Days	30 Days	45 Days	60 Days	75 Days	90 Days
CTS-Alg-1	1	0.65	0.83	1.10	1.14	1.56	1.60	1.70	1.91	2.32	3.31
	2	0.59	0.79	1.04	1.10	1.48	1.60	1.82	1.81	2.20	2.89
	Average	0.62	0.81	1.07	1.12	1.52	1.60	1.76	1.86	2.26	3.10
CTS-Alg-2	1	0.38	0.95	1.01	1.10	1.23	1.58	1.76	2.34	2.36	2.49
	2	0.40	0.97	1.07	1.06	1.33	1.60	1.84	2.36	2.42	2.57
	Average	0.39	0.96	1.04	1.08	1.28	1.59	1.80	2.35	2.39	2.53
CTS-Alg-3	1	0.69	0.69	0.72	0.80	1.02	1.17	1.19	1.34	1.62	2.49
	2	0.73	0.73	0.78	0.82	1.08	1.21	1.27	1.44	1.64	2.51
	Average	0.71	0.71	0.75	0.81	1.05	1.19	1.23	1.39	1.63	2.50
CTS-Alg-4	1	0.94	1.00	1.01	1.08	1.08	1.21	1.19	1.25	1.54	1.67
	2	1.00	0.98	1.01	1.14	1.14	1.23	1.21	1.27	1.56	1.77
	Average	0.97	0.99	1.01	1.11	1.11	1.22	1.20	1.26	1.55	1.72
CTS-Alg-5	1	0.56	0.54	0.58	0.59	0.90	1.02	1.29	1.17	1.20	1.47
	2	0.64	0.58	0.56	0.63	0.96	1.04	1.35	1.11	1.32	1.53
	Average	0.60	0.56	0.57	0.61	0.93	1.03	1.32	1.14	1.26	1.50
CTS-Alg-6	1	0.28	0.34	0.50	0.57	0.68	0.71	0.88	0.83	1.00	1.14
	2	0.32	0.40	0.54	0.61	0.72	0.77	0.86	0.85	1.06	1.12
	Average	0.30	0.37	0.52	0.59	0.70	0.74	0.87	0.84	1.03	1.13

Table B-5 The data analysis of percentage of pendimethalin from GC technique(alginate and alginate-starch beads).

Formulation	No.	%Pendimethalin from GC (g/g)												
		1 Day	2 Days	5 Days	10 Days	20 Days	30 Days	45 Days	60 Days	75 Days	90 Days	120 Days	150 Days	180 Days
Alg-1	1	0.05	0.07	0.88	2.23	2.38	3.46	3.92	4.35	4.97	5.85	6.59	7.45	8.14
	2	0.05	0.07	0.96	2.18	2.37	3.69	3.99	4.12	5.12	5.39	6.94	7.68	8.01
Alg-W -1	1	0.05	0.07	0.86	1.93	2.37	3.21	3.90	4.66	4.78	5.25	6.48	7.21	7.99
	2	0.05	0.06	0.83	1.39	2.18	3.10	3.75	4.15	4.81	5.35	6.07	7.01	7.23
Alg-W-2	1	0.11	0.16	0.22	0.48	0.64	0.74	0.82	0.92	0.99	1.24	1.44	1.87	2.39
	2	0.09	0.15	0.21	0.51	0.69	0.77	0.84	1.00	1.15	1.44	1.60	1.81	2.16
Alg-W-3	1	0.00	0.00	0.00	0.18	0.25	0.22	0.23	0.22	0.23	0.21	0.21	0.22	0.21
	2	0.00	0.00	0.00	0.15	0.23	0.23	0.22	0.24	0.22	0.23	0.22	0.23	0.24
Alg-W-4	1	0.11	0.19	0.10	0.36	0.57	0.64	0.74	0.92	0.95	1.13	1.41	1.78	2.11
	2	0.08	0.18	0.25	0.37	0.62	0.64	0.77	0.86	1.11	1.33	1.50	1.68	1.93
Alg-W-5	1	0.02	0.03	0.10	0.38	0.49	0.51	0.57	0.62	0.64	0.89	1.06	1.26	1.57
	2	0.01	0.03	0.10	0.40	0.50	0.53	0.59	0.61	0.82	1.01	1.22	1.38	1.59
Alg-W-6	1	0.00	0.00	0.00	0.16	0.26	0.28	0.29	0.29	0.38	0.45	0.47	0.47	0.47
	2	0.00	0.00	0.00	0.14	0.23	0.26	0.26	0.27	0.30	0.33	0.35	0.36	0.36
Alg-R-1	1	0.03	0.04	0.47	0.79	1.87	2.29	3.25	4.08	4.79	5.26	6.19	6.90	7.43
	2	0.05	0.04	0.46	0.97	1.90	2.45	3.57	4.03	5.00	5.54	6.74	7.41	7.84

Table B-6 The data analysis of percentage of pendimethalin from GC technique(alginate and alginate-starch beads).

Formulation	No.	%Pendimethalin from GC (g/g)												
		1 Day	2 Days	5 Days	10 Days	20 Days	30 Days	45 Days	60 Days	75 Days	90 Days	120 Days	150 Days	180 Days
Alg-1	1	0.14	0.20	2.54	6.44	6.88	9.99	11.32	12.56	14.36	16.91	19.04	21.54	23.54
	2	0.15	0.20	2.88	6.54	7.10	11.06	11.96	12.35	15.32	16.13	20.77	22.98	23.98
	Average	0.15	0.20	2.71	6.49	6.99	10.53	11.64	12.46	14.84	16.52	19.91	22.26	23.76
Alg-W -1	1	0.14	0.20	2.49	5.55	6.83	9.25	11.24	13.43	13.78	15.12	18.66	20.77	23.04
	2	0.16	0.18	2.59	4.37	6.82	9.72	11.76	13.00	15.07	16.77	19.02	21.99	22.65
	Average	0.15	0.19	2.54	4.96	6.83	9.48	11.50	13.21	14.42	15.95	18.84	21.38	22.85
Alg-W-2	1	0.32	0.46	0.63	1.37	1.82	2.10	2.34	2.64	2.84	3.54	4.10	5.34	6.84
	2	0.25	0.43	0.58	1.43	1.93	2.15	2.34	2.79	3.19	3.99	4.45	5.03	5.99
	Average	0.28	0.45	0.60	1.40	1.87	2.13	2.34	2.72	3.02	3.77	4.28	5.18	6.42
Alg-W-3	1	0.00	0.00	0.00	0.50	0.71	0.62	0.63	0.62	0.65	0.60	0.59	0.62	0.60
	2	0.00	0.00	0.00	0.42	0.62	0.62	0.61	0.65	0.60	0.62	0.60	0.62	0.65
	Average	0.00	0.00	0.00	0.46	0.67	0.62	0.62	0.64	0.63	0.61	0.60	0.62	0.63
Alg-W-4	1	0.30	0.54	0.28	0.99	1.59	1.78	2.05	2.55	2.64	3.14	3.90	4.94	5.84
	2	0.22	0.49	0.67	1.01	1.66	1.72	2.08	2.32	2.99	3.59	4.05	4.53	5.19
	Average	0.26	0.51	0.47	1.00	1.62	1.75	2.07	2.43	2.82	3.37	3.98	4.73	5.52
Alg-W-5	1	0.06	0.09	0.31	1.22	1.56	1.62	1.83	1.99	2.04	2.84	3.40	4.04	5.04
	2	0.04	0.08	0.32	1.25	1.56	1.68	1.85	1.93	2.59	3.19	3.85	4.34	4.99
	Average	0.05	0.08	0.32	1.23	1.56	1.65	1.84	1.96	2.32	3.02	3.63	4.19	5.02
Alg-W-6	1	0.00	0.00	0.00	0.55	0.90	0.96	1.01	1.01	1.31	1.56	1.61	1.61	1.61
	2	0.00	0.00	0.00	0.49	0.85	0.93	0.96	0.99	1.09	1.21	1.26	1.29	1.29
	Average	0.00	0.00	0.00	0.52	0.88	0.94	0.99	1.00	1.20	1.38	1.44	1.45	1.45
Alg-R-1	1	0.08	0.11	1.34	2.24	5.29	6.50	9.21	11.56	13.57	14.89	17.54	19.54	21.06
	2	0.15	0.10	1.29	2.73	5.34	6.89	10.06	11.35	14.09	15.60	18.98	20.87	22.09
	Average	0.11	0.10	1.31	2.48	5.32	6.70	9.64	11.46	13.83	15.25	18.26	20.21	21.57

Appendix C

Percentage pendimethalin from GC DATA

Table C-1 The data of percentage encapsulation pendimethalin in chitosan beads analysis by GC technique.

Formulation	No.	Pendimethalin	
		%Loading	%Encapsulation
CTS-1	1	34.2	85.5
	2	35.0	87.5
	Average	34.6	86.5
CTS -2	1	33.8	84.5
	2	36.2	90.5
	Average	35.0	87.5
CTS-3	1	33.7	84.3
	2	34.9	87.3
	Average	34.3	85.8
CTS-4	1	32.6	81.5
	2	33.2	83.0
	Average	32.9	82.3
CTS-5	1	32.9	82.3
	2	32.3	80.8
	Average	32.6	81.5
CTS-6	1	32.3	80.8
	2	32.5	81.3
	Average	32.4	81.0

Table C-2 The data of percentage encapsulation pendimethalin in chitosan-alginate beads analysis by GC technique.

Formulation	No.	Pendimethalin	
		%Loading	%Encapsulation
CTS-Alg-1	1	32.0	80.0
	2	30.2	75.5
	Average	31.1	77.8
CTS-Alg-2	1	30.3	75.8
	2	30.9	77.3
	Average	30.6	76.5
CTS-Alg-3	1	30.0	75.0
	2	31.2	78.0
	Average	30.6	76.5
CTS-Alg-4	1	30.2	75.5
	2	30.6	76.5
	Average	30.4	76.0
CTS-Alg-5	1	29.8	74.5
	2	29.8	74.5
	Average	29.8	74.5
CTS-Alg-6	1	29.2	73.0
	2	29.6	74.0
	Average	29.4	73.5

Table C-3 The data of percentage encapsulation pendimethalin in alginate and alginate-starch beads analysis by GC technique.

Formulation	No.	Pendimethalin		
		%Loading at 25°C After 2 weeks	%Loading at 54°C After 2 weeks	%Encapsulation
Alg-1	1	34.6	33.6	86.5
	2	33.4	32.8	83.5
	Average	34.0	33.2	85.0
Alg-W-1	1	34.7	32.0	86.8
	2	31.9	33.2	79.8
	Average	33.3	32.6	83.3
Alg-W-2	1	35.0	34.6	87.5
	2	36.0	35.4	90.0
	Average	35.5	35.0	88.8
Alg-W-3	1	35.8	35.3	89.5
	2	36.6	36.9	91.5
	Average	36.2	36.1	90.5
Alg-W-4	1	36.1	36.0	90.3
	2	37.1	35.6	92.8
	Average	36.6	35.8	91.5
Alg-W-5	1	31.2	32.0	78.0
	2	31.8	30.4	79.5
	Average	31.5	31.2	78.8
Alg-W-6	1	29.0	28.0	72.5
	2	27.6	28.6	69.0
	Average	28.3	28.3	70.8
Alg-R-6	1	35.3	34.2	88.3
	2	35.5	35.0	88.8
	Average	35.4	34.6	88.5

VITA

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