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ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

ENCAPSULATION OF GIBBERELLIC ACID BY HYDROPHOBIC MODIFIED STARCH FOR ACCELERATING GROWTH OF PLANTS

Miss Chatdaw Datchanchaiwong

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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งานวิจัยนี้มีวัตถุประสงค์ที่จะกักเก็บกรดจิบเบอเรลลิก (GA_3) ด้วยแป้งที่ดัดแปร (HB-Starch) ให้มีส่วนไม่ชอบน้ำ โดยการคอนจูเกตกับออกตะเดกซิลโบรไมด์ ส่วนของแป้งที่ดัดแปรสามารถจัดวางโมเลกุลได้ด้วยตัวเอง (self-assembled) ในขนาดนาโนซึ่งจะนำไปประยุกต์ใช้ทางการเกษตรเพื่อควบคุมการปลดปล่อย โครงสร้างโมเลกุล, ลักษณะทางสัณฐานวิทยาและขนาดอนุภาคทำการพิสูจน์เอกลักษณ์ด้วยเทคนิค ^{1}H-NMR, FTIR, SEM และการวิเคราะห์ด้วย zetasizer ที่เห็นได้ชัดดีกรีการแทนที่ของสายโซ่ออกตะเดกซิลคือ 8.5 เปอร์เซ็นต์ คำนวณโดยเทคนิค ^{1}H-NMR ซึ่งแสดงว่าการคอนจูเกตด้วยสายโซ่ออกตะเดกซิลบนสายโซ่หลักของแป้งและให้สมบัติไม่ชอบน้ำทำได้สำเร็จ นอกจากนี้ค่าความเข้มข้นวิกฤติที่ทำให้เกิดไมเซลล์เท่ากับ 0.25 ไมโครกรัมต่อมิลลิลิตรซึ่งหาได้จากเทคนิค fluorescence โดยเฉพาะอย่างยิ่งพบว่า 1 เปอร์เซ็นต์ตามน้ำหนักของกรดจิบเบอเรลลิกต่อแป้งที่ดัดแปรก่อให้เกิดการกักเก็บที่มีประสิทธิภาพสูงสุดเช่นเดียวกับสามารถขยายเวลาการปลดปล่อยเมื่อเทียบกับกรดจิบเบอเรลลิกบริสุทธิ์ซึ่งทั้งนี้นำไปสู่การเร่งการเจริญเติบโตของพืชที่ความเข้มข้นที่เหมาะสม (0.5 ppm)

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สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต ....

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CHATDAW DATCHANCHAIWONG: ENCAPSULATION OF GIBBERELLIC ACID BY HYDROPHOBIC MODIFIED STARCH FOR ACCELERATING GROWTH OF PLANTS. ADVISOR: ASSOC. PROF. NATTAYA NGAMROJANAVANICH, Ph.D., 78 pp.

The objective of this research is to encapsulate of gibberellic acid (GA_3) by hydrophobic modified starch (HB-Starch). It was prepared by octadecyl bromide conjugation. The HB part was able to perform self-assembled nano micelles which used in agricultural applications for the controlled release. The molecular structure, surface morphology and particle size were characterized by ^{1}H-NMR, FTIR, SEM and zetasizer analysis techniques. Evidently, the degree of octadecyl chain substitution was 8.5 % calculated by ^{1}H-NMR. The evidences illustrated that the octadecyl chain was successfully conjugated on the starch backbone and provided the hydrophobic properties. Additionally, the CMC value of micelle was 0.25 μg/mL determined by fluorescence technique. Furthermore, 1% (w/w) of GA_3-HB-Starch produced high entrapment efficiency as well as prolonged the release than the pure GA_3, which this led to an increase of plant growth acceleration at the optimum concentration (0.5 ppm).

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Field of Study:	Petrochemistry and Polymer Science	Student's Signature ....
		Advisor's Signature ....
Academic Year:	2015	

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LIST OF ABBREVIATIONS

cm	Centimeter
°C	Degree Celsius (centigrade)
%DS	Degree substitution
EE	Entrapment efficiency
FTIR	Fourier Transform Infrared Spectrophotometer
GA_3	Gibberellic acid
g	Gram
^{1}H-NMR	^{1}H Nuclear Magnetic Resonance spectroscopy
h	Hour
HB-starch	Hydrophobic modified starch
kDa	Kilo Dalton
kV	Kilovolt
L	Lite
µm	Micrometer
mg	Milligram
mL	Milliliter
MW	Molecular weight
nm	Nanometer
ppm	Part Per Million
%	Percentage
PDI	Polydispersity Index
rpm	Round Per Minute
SEM	Scanning Electron Microscope
S.D.	Standard Deviation
UV-Vis	Ultraviolet-visible spectroscopy
cm^{-1}	Wavenumber (unit)
v/v	Volume/Volume
w/w	Weight/Weight

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CHAPTER I
INTRODUCTION

1.1 Introduction

Plant growth regulators are controlled or modified plant growth processes in modern agriculture which also called plant hormones. They are used to increase the benefits of products in agriculture filed, such as elongation of stems, development and ripening of fruit, formation of leaves, and flowers. There are currently seven recognized groups of plant hormones: auxins, gibberellins, cytokinins, ethylene, miscellaneous, plant growth inhibitors, and plant growth retardants [1, 2].

Gibberellic acid (GA_3) (Figure 1.1) is one of plant growth regulators which was used to control or improve plant growth and increase the benefits of products in the modern agriculture, such as gene expression, seed germination, epidermal cell elongation, left expansion, flower development, and senescence [3, 4]. It is able to stimulate elongation of plant shoots and induce growth of plants, such as barley, orchids and garlics [5-7]. Moreover, GA_3 was reported to reduce the salinity (decreasing the plant growth, accumulation of toxic ion) in nutrient solution of the hydroponic system [8-10]. Jennifer (2010) has reported that the GA_3 using in hydroponic system to increase *Artemisia annua* growth [11]. GA_3 can dissolve in alcohol exhibiting limited solubility in water. It is also sensitive to heat, light and can degrade under neutral or alkaline condition [12]. Thus, some studies attempted to

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improve its stability property for achieving the high maximum utilization efficiency. Recently, Liu (2013) had developed the high utilization efficiency of GA_3 by gibberellin-chitosan conjugate for controlled-release application that could protect the GA_3 [13]. However, the controlled release of GA_3 may be achieved by using the cheaper different carrier materials. The suitable carrier materials should be biodegradable, compatible and non-toxicity to the environment [14]. These properties indicated that the natural polymer is a good carrier.

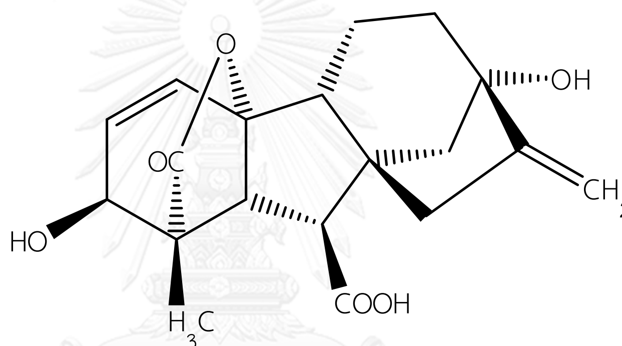

Figure 1.1 Chemical structure of GA_3

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Natural polymer is a naturally occurring polymer from renewable resource, such as starch, silk, wool, and cellulose [15]. The starch is a well-known inexpensive natural polymer and biodegradable carbohydrate. It is widely used in food and industrial fields [16]. The low-priced of starch make it very suitable as a using in agriculture field and compatible with plant because it has in many different plant organs, such as roots, leaves, seeds, and stems. Starch has two polymers consisting of glucose units, amylose and amylopectin. Amylose is a linear polymer, while amylopectin is a branch polymer [17] as seen in Figure 1.2.

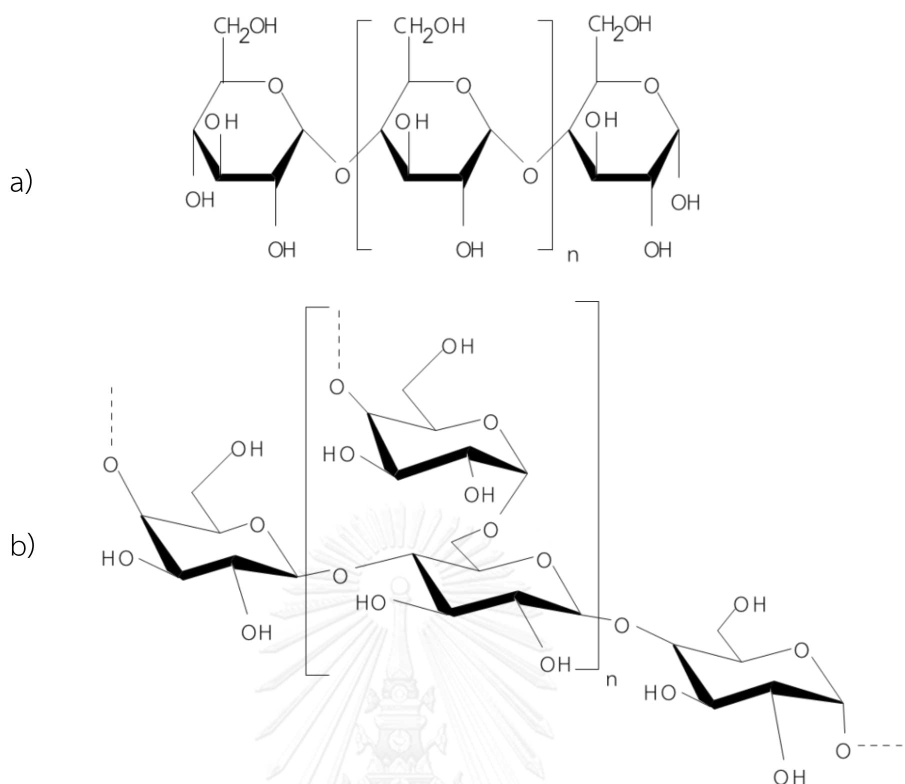

Figure 1.2 Chemical structures of starch a) amylose and b) amylopectin

In the recent years, the researchers have attended to the controlled-release system for agricultural applications by starch, encapsulation of plant oils in porous starch microspheres [18], encapsulation of diazinon in starch xanthate (SX) cross-linking [19] and urea encapsulation with starch-g-poly (L-lactide) [20]. Generally, the modified starch presents some hydroxyl groups which derived from substitution of native starch with alternative functionalities for low hydrophilicity and improvable properties [21].

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Self-assembly hydrophobic modified starch is one of methods to synthesize the nanostructure materials for encapsulation and controlled-release drug delivery system. For examples, the hydroxyethyl starch was modified with lauric, palmitic,

and stearic acids by esterification by using DCC and DMAP [22], the 2-hydroxy-3-butoxypropyl starch micelles were prepared by using butyl glycidyl ether [23] and the starch-*g*-PEG copolymers were synthesized by grafting starch with carboxyl group terminated PEG, and then conjugated with lipoic acid for disulfide crosslinking [24]. However, the structure of polymer micelles (Figure 1.3) is the appearance of a hydrophobic core and hydrophilic exterior, which also entraps in the hydrophobic drugs [25].

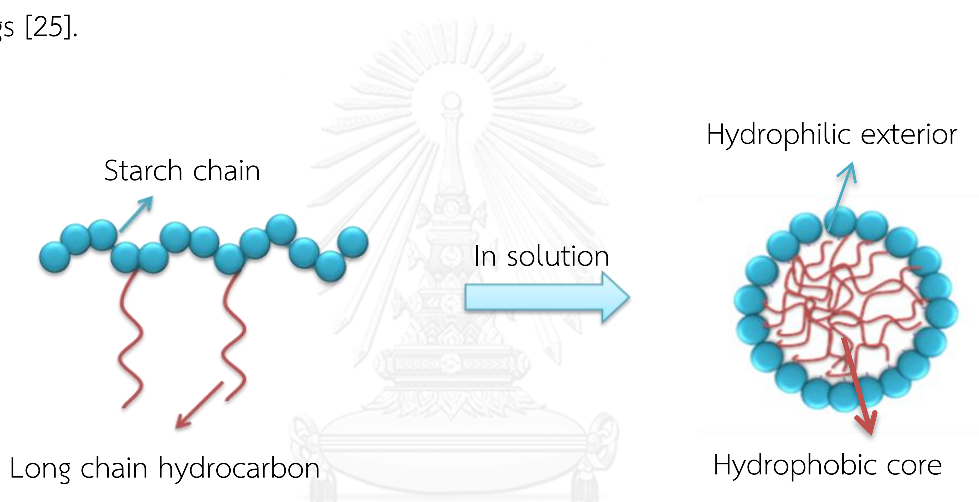

Figure 1.3 The structure of polymer micelles

Nowadays, the hydroponic system is a method for growth plants in nutrient solutions (water containing fertilizers) without soil [26]. In 1950, the hydroponic system was widely used for the growth of high-value plant products in the greenhouses. The advantages of hydroponic systems are easy to harvest, non-toxic, able to generate higher yield, and moreover it can control the nutrient content, pH and growing environment. Hydroponics have been known as a viable method of various producing vegetables, such as tomato, green-oak, lettuce and celery [27]. The

vegetable consumption has grown over the last few years because the consumers have been growing more health conscious. A strong demand for organic vegetables caused production to be insufficient.

Therefore, the aim of this work was to prepare the optimal HB-starch to encapsulate GA_3 which is activated by sodium hydroxide and subsequently synthesized with octadecyl chain (Figure 1.4). The encapsulation of GA_3 derived by hydrophobic modified starch (GA_3-HB-Starch) was characterized by ^{1}H-NMR, FTIR, scanning electron microscopy (SEM) and zetasizer analysis techniques, and also was evaluated the in vitro controlled-release behavior of GA_3 in nutrient solution in hydroponic system. Moreover, the utilization of the GA_3 encapsulation was investigated and then compared with the pure GA_3 for acceleration of plant growth (bunching onion, ***Allium fistulosum***).

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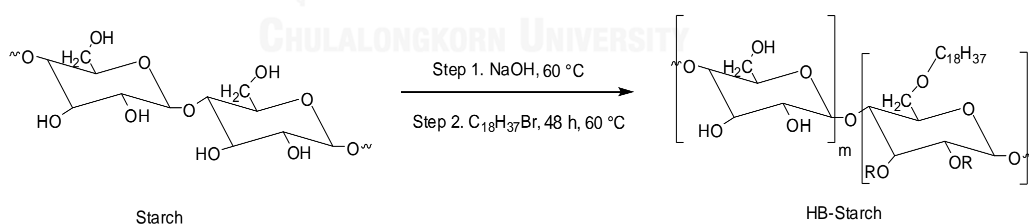

Figure 1.4 Synthesis scheme of HB-Starch

1.2 The objectives of this research

1) To synthesize and prepare the optimal HB-starch for encapsulated GA_3

2) To study the *in vitro* controlled-release behavior of GA_3-HB-Starch in nutrient solution on hydroponic system

3) To investigate the optimal GA_3-HB-Starch for accelerating growth of plants

1.3 The scope of research

The scope of this research was carried out by stepwise methodology as follows:

1) Review literature for related researches

2) Part I: Preparation of the optimal HB-Starch for encapsulated GA_3

a. Synthesis of hydrophobic modified starch

b. Characterization of the physical and chemical properties of starch and hydrophobic modified starch by using ^{1}H-NMR and FTIR

c. Determination of degree of hydrophobic chain substitution by ^{1}H-NMR

d. Determination of critical micelle concentration (CMC) by fluorescence technique

e. Characterization of the obtained HB-Starch micelles in terms of morphology, size, size distribution and zeta potential by SEM and particle size analyzer

3.) Part II: Fabrication and evaluation of the GA_3-HB-Starch

a. Preparation of the GA_3-HB-Starch by dialysis method

b. Characterization of the obtained GA_3-HB-Starch in terms of morphology, size, size distribution and zeta potential by SEM and particle size analyzer

c. Determination of the GA_3 encapsulation efficiency (%EE)

d. Study the *In vitro* release behavior of the GA_3-HB-Starch in nutrient solution on hydroponic system for 14 days by using UV-Vis method

e. Investigation of the GA_3-HB-Starch for accelerating growth of plants (bunching onion, ***Allium fistulosum***) in nutrient solution on hydroponic system for 14 days

3) Report, Discussion and Writing up thesis

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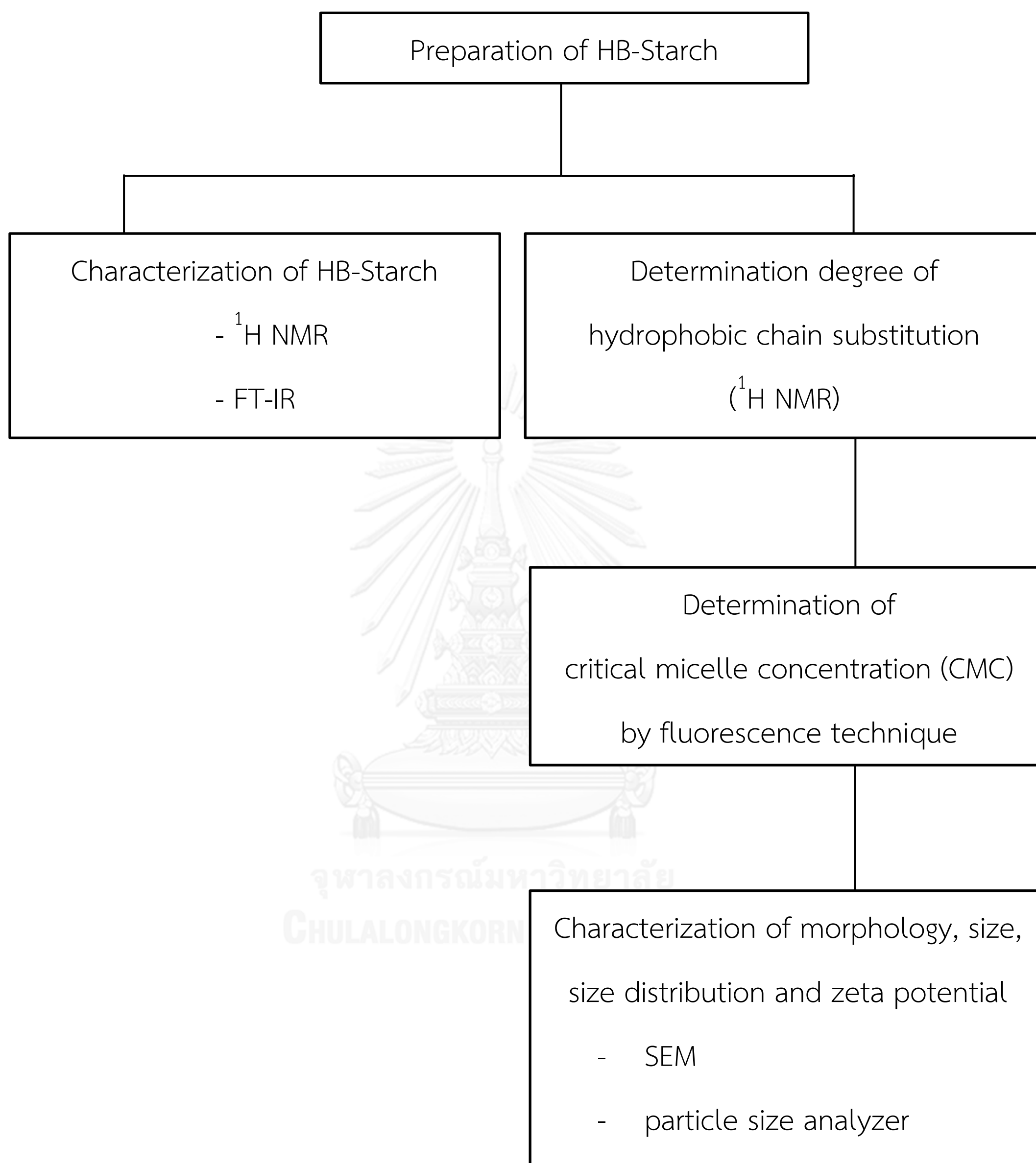
Part I: Flow chart for synthesis and characteristic



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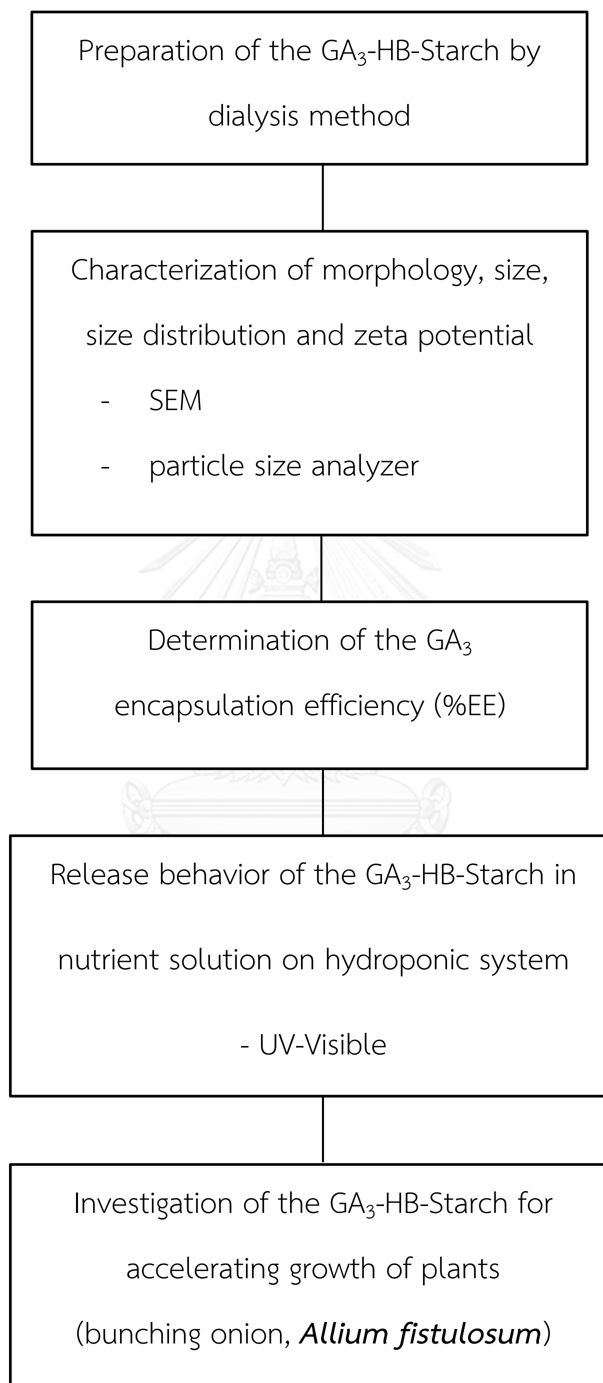
Part II: Flow chart for fabrication and evaluation of the GA_3-HB-Starch



CHAPTER II
THEORY AND LITERATURE REVIEWS

2.1 Plant growth regulators

Plant growth regulators (also known as plant hormones) are chemical signals produced within the plant that regulate the plant growth. It occurs in highly low concentrations at targeted cells locally and moved to other locations in other functional part of the plant [28, 29].

In modern agriculture, they are used to increasing the benefits of products in such as stimulates cell elongation, induce cell division, increase in size of leaves and fruits, development and ripening of fruit, formation of leaves and flowers [30, 31]. There are currently seven types of plant hormones: auxins, gibberellins, cytokinins, ethylene, miscellaneous, plant growth inhibitors and plant growth retardants as seen in Figure 2.1.

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2.2 Gibberellic acid (GA_3)

In 1926, Kurosawa discovered that filtrates from the pathogenic fungus *Gibberella fujikuroi* were able to induce increased growth similar to that occuring when the stems of rice plants are infected with this fungus in the field. In 1935, Yabuta and Sumuki were found the first isolate as named gibberellin. As of 2003, the gibberellins had occurred 126 GA_s identified from plants, fungi and bacteria [32]. In

general, the structural GA_s has the gibbane ring system (Figure 2.2) that it changes to be some of the early gibberellin compounds such as GA_1, GA_2, GA_3 and GA_4 [33].

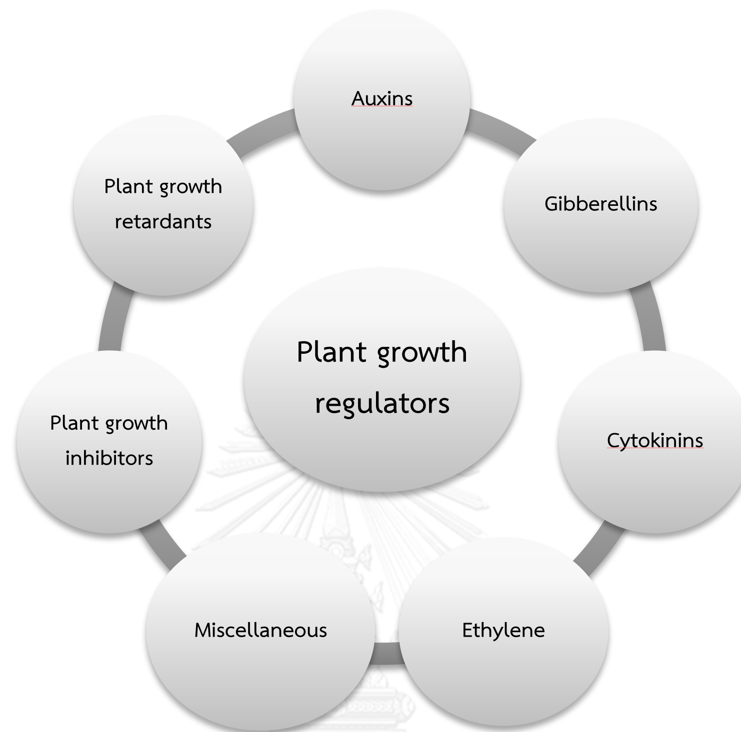

Figure 2.1 Schematic representation of plant growth regulators

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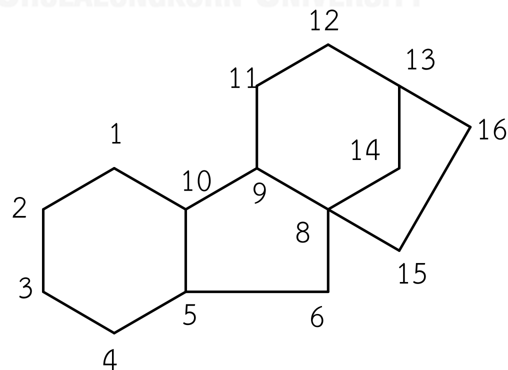

Figure 2.2 The gibbane ring system of gibberellins

Normally, the most active of the naturally occurring gibberellins is gibberellic acid (GA_3) which can be used to control or improve plant growth and increase the benefits of products in the modern agriculture such as gene expression, seed germination, epidermal cell elongation, cell division, left expansion, flower development and senescence [3, 4]. It is ability to stimulate elongation of plant shoots and induce growth of plants. For examples;

Cardoso et al. presented that the application of GA_3 at 125 mg L^{-1} showed the best results for the promotion of flowering and flower quality of this orchid hybrid [6].

Rahman et al. showed that application of GA_3 has the potentiality to break dormancy and accelerates the sprouting in the local cultivar of garlic [7].

Mahmoud et al. was reported that GA_3 could reduce the salinity (decreasing the plant growth, accumulation of toxic ion) in nutrient solution of the hydroponic system [10].

Jehnsen has reported that the GA_3 using in hydroponic system for increased *Artemisia annua* growth compared to the control [11].

Burge et al. reported that the GA_3 treatment had no effect on fruit length but reduced fruit diameters and weight, and increased pedicel length [34].

Núñez-Elisea et al. found that the GA_3 prevent initiation of reproductive shoots of mango rather than inhibiting floral induction [35].

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Casanova et al. reported that GA_3 increases berry size of 'Emperatriz' seedless grape, the response depending on the phenological stage of vine at treatment date and on the concentration applied [36].

Awad et al. found that GA_3 have promotive effects on bunch and fruit weight and improve quality of 'Barhee' dates under hot arid conditions [37].

For physical and chemical properties, GA_3 is soluble in alcohol and has a limited solubility in water (5 g/L). It is sensitive to heat, light and can degrade under neutral or alkaline condition [12]. Moreover, It is stable when dry but unstable in aqueous-alcoholic solutions.

The stages in decomposition of gibberellic acid in water have been reported to follow the pathway illustrated in Figure 2.3.

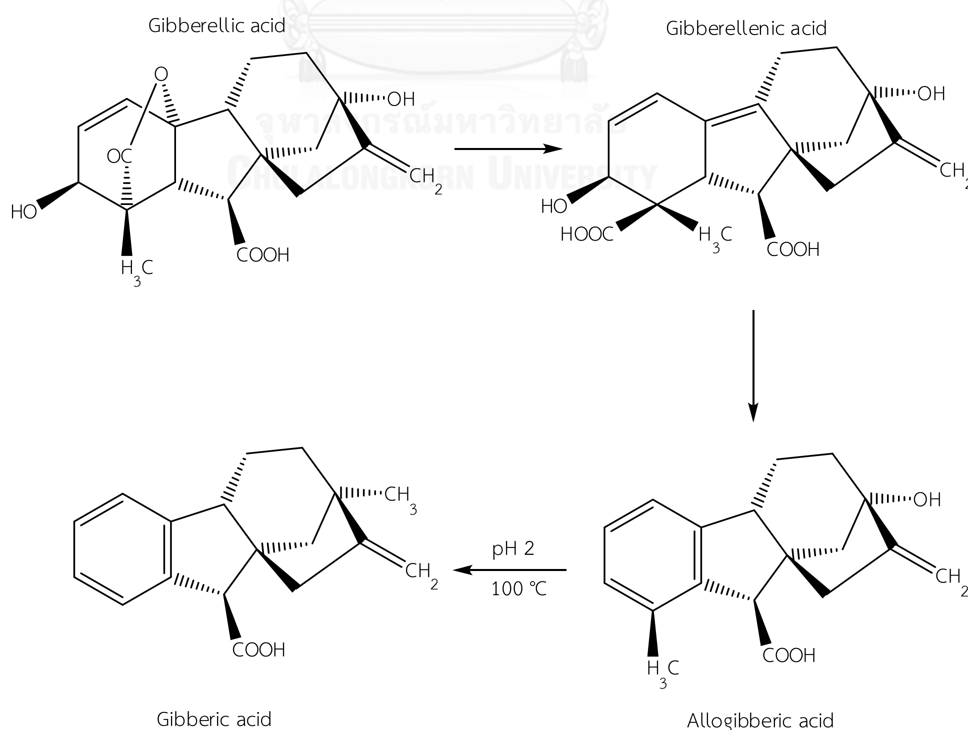

Figure 2.3 Degradation pathway of gibberellic acid in water

2.3 Controlled release system

Controlled release system is based upon macromolecules (usually polymers) and they can entrap small and large molecules such as fertilizer and pesticides. The formulation with polymers provides the construct needed to entrap the fertilizer and to build into the resulting depot device the mechanism for reliable release rates. It aims to make available fertilizer at rates suitable for efficient control of pests under properly conditions. The releasing systems are usually solid and can vary in size from micro particles to large devices several centimeters across. Controlled release system can be used with a wide range of fertilizer, including conventional low molecular weight organic substances, high molecular weight substances and inorganic substances. In agricultural applications are used for controlling a variety of pest organisms such as insects, mites, rodents, nematodes, weeds, microorganisms and improving crop production with plant growth regulators. [38]. As for all fertilizer formulations, controlled release system need to be applied, or placed, in the field appropriately for targeting the pests. In crop protection, this usually means application to the crop, or crop area, by means that achieve good distribution. Such distribution depends on how the pesticide moves to the target organism following application and often needs small particle size to provide this. Thus, the standard application methods of spraying and granules are important in agricultural fertilizer delivery, which in turn limits the device size of the controlled release systems deployed [39].

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2.3.1 Types of controlled release formulation

For environmental application of fertilizer delivery, chemical and physical types are traditionally divided. In recently, the biological approach has appeared. The types controlled release formulation was described as Table 2.1 [40].

Table 2.1 Types of controlled release formulation

Chemical	Physical	Biological
Backbone linking Side-chain bonding Matrix degradation Carrier molecules	Reservoir with membrane Reservoir without membrane Monolith or matrix	Living, or dead, cells (microorganisms) as delivery mechanisms

Many researchers are improvability and developing controlled release system. The basic configurations of controlled release system were shown in Figure 2.4.

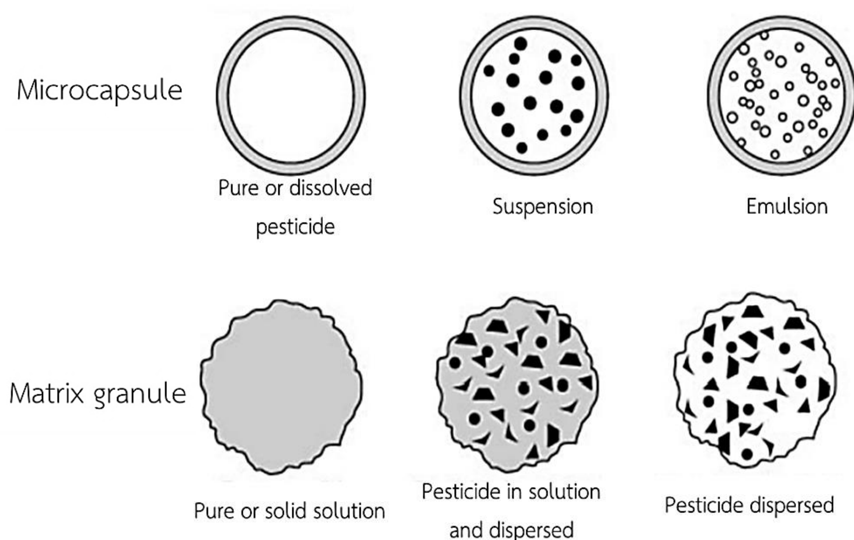

Figure 2.4 Representation of controlled release system [38]

2.3.2 Mechanisms of release from matrix

1. Diffusion controlled delivery

Diffusion of the fertilizer occurs from the interior of the particle to the external environment. This system must be a longer diffusion time to progressively release fertilizer through a polymeric matrix [41].

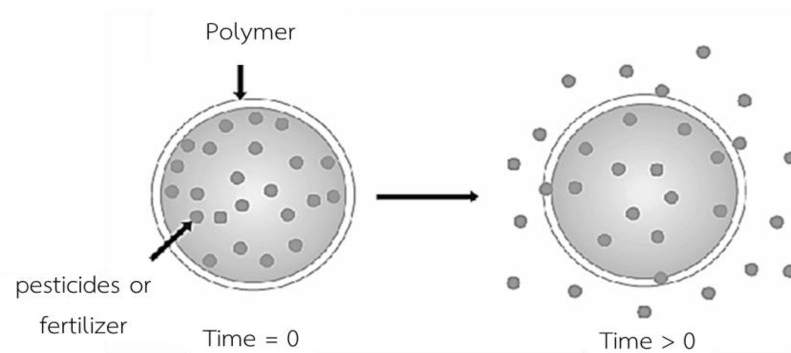

Figure 2.5 Representation of diffusion controlled release [42]

2. Swelling controlled delivery

Swelling controlled release occurs in matrix systems where water uptake and then, fertilizer diffuse through the swollen carrier into the external environment. The factor of swelling of the fertilizer carrier is a change of the environment surrounding such as pH, temperature, ionic strength, etc.

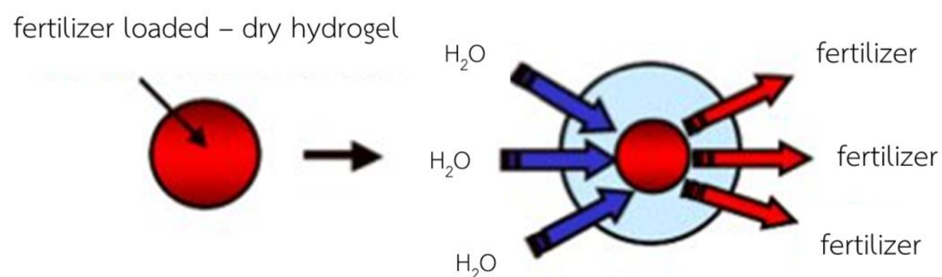

Figure 2.6 Representation of swelling controlled release [43]

3. Erosion controlled delivery

Erosion controlled release has 2 types including bulk erosion and surface erosion. The Bulk erosion is the polymer degradation in a fairly uniform manner throughout the polymer matrix. Surface erosion is the occurrence of degradation at the surface of the polymer device.

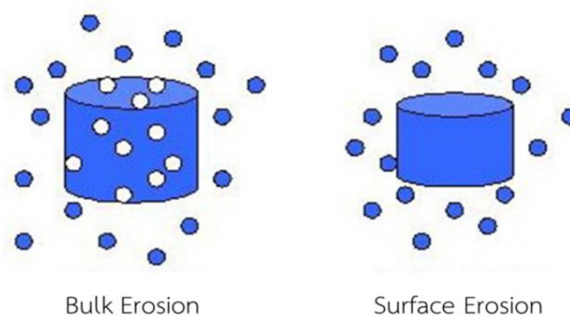

Figure 2.7 Representation of erosion controlled release [44]

2.4 Polymer in Agriculture field

For polymers in agriculture field, it should be the best degradable as remove them from the environment following their use. In selecting polymers for formulation cost is of great importance in agriculture, when compared to medical drug delivery systems where benefits are considered more commercially valuable. Thus, natural polymer is one alternative for using in agriculture field such as starch, chitosan and cellulose [15]. They were degraded by the enzymatic action of microorganisms such as bacteria, fungi, and algae. In addition, their polymer chains can be broken down by non-enzymatic processes such as chemical hydrolysis. However, the natural polymer must be blending with synthetic polymers for desired properties because it

does not fit the needs of specific applications [45]. Many researchers are interested in controlled-released system for agricultural applications by starch. For example;

In 1984, White et al. was prepared the starch encapsulated trifluralin. They are reported that it provided good control and reduced the loss of trifluralin [46].

In 2001, Zhu et al, was prepared the starch-g-poly(vinyl alcohol) for slow release of 2,4,5-trichlorophenoxyacetic acid which the rate of the herbicide released from the matrix in wet environment can be reduced [47].

In 2016, Wilpiszewska et al. was performed the novel high substituted carboxymethyl starch-based microparticles containing sodium montmorillonite for encapsulated isoproturon herbicide. It could reduce the potential leaching of herbicide [48].

2.5 Principles of molecular self-assembly

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Self-assembly is a process which molecules arrange by themselves without guidance or management from an outside source. The success of self-assembly in a molecular system was investigated by five characteristics of the system.

- Components
- Interactions
- Reversibility (or adjustability)
- Environment
- Mass Transport and Agitation

Molecular aggregation occurs when they had a net attraction and an equilibrium separation between the components. Generally, the equilibrium separation represents a balance between attraction and repulsion which two interactions are fixed in molecular self-assembly but can be engineered independently in macroscopic self-assembly (Figure 2.8).

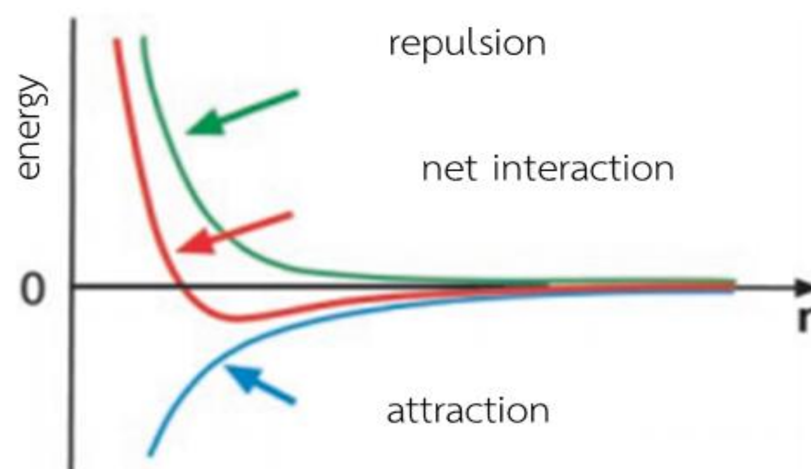

Figure 2.8 Representation of principles of molecular aggregation [49]

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2.6 Hydroponic system

Hydroponic is a technology for growing plants in water and fertilizers without soil include sand, gravel, vermiculite, rockwool, peat, coir and sawdust. All plants must have the same basic needs of light, carbon dioxide (CO_2), oxygen, water, heat and food in the form of nutrients [50]. The advantages and disadvantages of hydroponic system are presented as follows.

Table 2.2 The advantages and disadvantages of hydroponic system

Advantages	Disadvantages
- High-density maximum crop yield - More efficient use of water and fertilizers minimal use of land area - Suitability for mechanization, disease and pest control - Reduce the problems of diseases, pests, salinity	- High costs of capital and energy inputs - High degree of management skills required for successful production

There are 6 basic types of hydroponic system; Basic Wick, Water Culture, Ebb and Flow (Flood & Drain), Drip (recovery or non-recovery), N.F.T. (Nutrient Film Technique) and Aeroponic (Figure 2.9) [51]. In this study, Basic Wick was selected because it is very basic and easily for preparation.

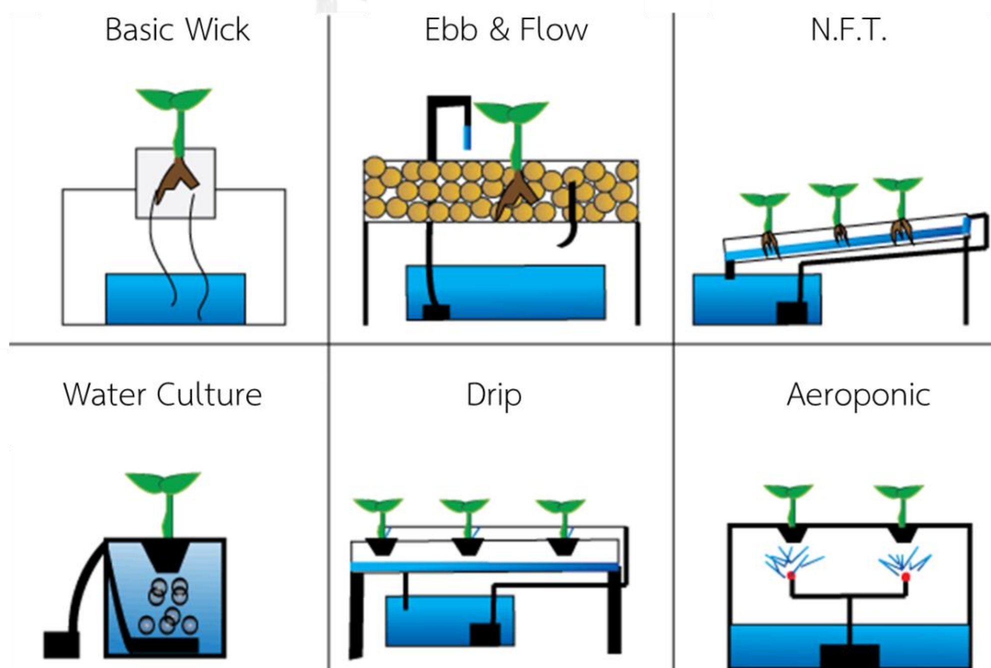

Figure 2.9 Types of hydroponic system [52]

CHAPTER III

EXPERIMENTAL

3.1 Materials

3.1.1 Model plant hormones

- Gibberellic acid (GA_3, CAS number: 77-06-5, Lot No.62HO383, powder obtained by Sigma Aldrich, USA)

3.1.2 Polymers

- Cornstarch (food grade, powder purchased from Unilever Thai Holding Limited.)

3.1.3 Plant materials

- Bunching onion seeds (FARGRANT 961, seeds purchased from Known-You Seed (THAILAND) CO., LTD.)

- Hydroponic nutrient solution purchased from Hydrowork, Thailand

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3.1.4 Solvent and chemicals

- Acetone, commercial grade (Merck, Germany)

- Dichloromethane, commercial grade (Merck, Germany)

- Dialysis membrane, Mw cut-off 12-14 kDa (Membrane Filtration Products,Inc.)

- Ethanol 95%, commercial grade (Merck, Germany)

- Octadecyl bromide (1-bromooctadecane), AR grade (Sigma-Aldrich, USA)

- Pyrene for fluorescence, ≥99.0% (Sigma Aldrich, USA)

- Potassium bromide, AR grade (Merck, Germany)

- Sodium hydroxide, pellets for analysis grade (Merck, Germany)

3.2 Instruments

Table 3.1 The instruments used in this study

Instrument	Manufacture	Model
FTIR spectrometer	Nicolet	6700
Horizontal shanking water-bath	Lab-line instrument	3575-1
Micropipette	Mettler Toledo	Volumate
NMR spectrometer	Varian	400 MHz
Scanning Electron Microscope	Philips	XL30CP
Small bench centrifuge	Hettich	EBA20
UV-VIS spectrometer	PerkinElmer	Lambda 80
Fluorescence spectrometer	PerkinElmer	LS 55
Vortex	Vortex-genie	V2
Zetasizer nano series	Malvern Instruments	S4700
Freeze dryer	Labconco	Freeze 6

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3.3 Methods

3.3.1 Synthesis of HB-Starch

Cornstarch (5 g) was suspended in distilled water (75 mL) at 60 °C under stirring within 1 hour and cooled down to a room temperature. Then, 0.40 M of a sodium hydroxide (30 mL) and octadecyl bromide (2.5 mL) were added to the gelation and stirred for 48 hours at 60 °C, respectively. The solution was extracted

with dichloromethane for three times (30 mL × 3), dialyzed in dichloromethane (1 L × 2) for 48 hours. The distilled water (1 L) was washed the solution for 24 h again to remove hydrobromic acid, unreacted octadecyl bromide and sodium hydroxide. After dialysis, the HB-Starch was precipitated in acetone (300 mL) and dried in oven at 60 °C.

3.3.2 Preparation of GA_3-HB-starch micelles

The HB-Starch (100 mg) was prepared in distilled water (0.4 mg/mL) to micelle formation. Next, GA_3 (0.8, 1, 1.5, 5 and 10 mg) were dissolved in small quantities of ethanol for loading in each of sample under stirring and the mixtures were dialyzed in distilled water at room temperature for 24 h using a dialysis membrane (molecular weight cut-off of 3500 g mol^{-1}). Subsequently, the GA_3-HB-Starch micelles were collected by centrifugation at 5000 rpm for 20 min and the final products were washed for several times with ethanol to remove unloaded GA_3 on the surface of micelles. At last, the product was dried by freeze dry.

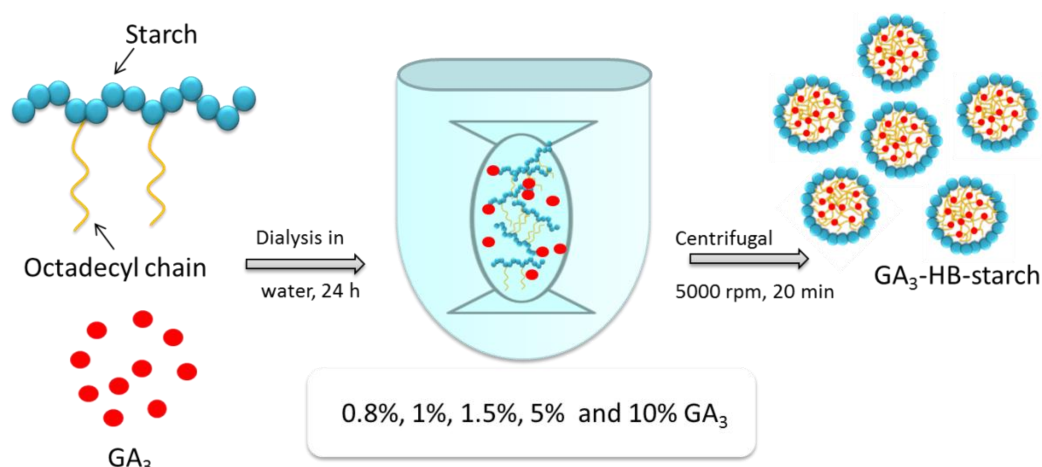

Figure 3.1 Preparation of GA_3-HB-starch micelles

3.4 Characterization

3.4.1 ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy

The chemical structures of starch, octadecyl bromide and HB-Starch were recorded on Mercury Varian NMR spectrometer operated at 400 MHz (Agilent Technologies, CA, USA), using pulse accumulating of 64 scans. Ten milligram of each sample was dissolved in DMSO. The degree of hydrophobic chain substitution (DS) can be calculated by using the equation:

$$\mathrm{DS}\ (\%) = \left[\frac{I_{Hc}}{I_{H1}} \times \frac{1}{2}\right] \times 100 \qquad (1)$$

Where, I_{Hc} is the integral area of protons at the c-position and I_{H1} is the integral area of proton at 1-position of HB-Starch, respectively.

3.4.2 Fourier transformed infrared (FTIR) spectroscopy

The HB-Starch and GA_3-HB-Starch were dried, mixed with potassium bromide pellets at 1:100 (w/w) and pressed into a disc. The sample was measured by a Perken Elmer Fourier transformed infrared (FTIR) spectrometer system in the region from 4000-400 cm^{-1}.

3.4.3 Scanning Electron Microscope (SEM)

The morphology of samples were mounted on an aluminum stub using double-sided carbon adhesive tape and coated with gold-palladium. The HB-Starch and GA_3-HB-Starch were investigated by SEM using Philips XL30CP and performed under high vacuum and ambient temperature with beam voltage of 15 kV. The

samples were dispersed in 1 mL distilled water at room temperature and vortexed for 2 h. The solutions were dropped on a clean surface of glass slide to make self-assembly process for 2 days.

3.4.4 Particle size measurement

The particle size and size distribution of micelles were evaluated with a particle size analyzer (Zetasizer nano series, Malvern instuments). The HB-Starch and GA_3-HB-Starch micelles were prepared in distilled water with the concentration of 0.4 mg/mL. The sample size calculation based on dynamic light scattering (DLS) method as a software protocol. The scattered light was collected at an angle of 90° through fiber optics and converted to an electrical signal by an avalanche photodiode (APDS). All samples were vortexed and run in triplicate with the number of runs set to 5 seconds and run duration set to 10 seconds.

3.4.5 Zeta potential

The zeta potential of the micelle was determined using particle sizer. The analysis was performed at a scattering angle of 90°. All samples were vortexed and run in triplicate with the number of runs set to 5 and run duration set to 10 seconds.

3.5 Determination of critical micelle concentration (CMC)

The (CMC) value was examined by fluorescence technique with an excitation wavelength of 340 nm. The acetone (25 mL) was added to 0.5 mg of pyrene(9.9×10^{-5} M). Then, 0.5 g of HB-Starch was dissolved in 50 mL water and diluted until it has the different concentrations from 1.0×10^{-5} to 5.0 mg/mL. The pyrene solution (20 µL)

was added in 2.0 mL of each HB-Starch concentration and vortexed for 5 min. The CMC of HB-Starch was determined from the cross-over value between the intensity (peak height) ratios of I_{374}/I_{384} and HB-Starch concentrations [53].

3.6 Determination of GA_3 encapsulation efficiency (EE)

Twenty milligrams of GA_3-HB-Starch *(*0.8*,* 1*,* 1.5, 5 and 10 *%*wt*)* was immersed into 1 ml of 30 %v/v EtOH. The mixture was stirred at room temperature for 24 hours and centrifuged at 5000 rpm for 20 min to precipitate HB-starch. Finally, the supernatant of solution was obtained and determined by UV-Visible spectroscopy at 254 nm.

The GA_3 encapsulation efficiency (EE) of the micelles was calculated as the equation:

$$\text{EE (\%)} = \frac{\text{Actual } GA_3 \text{content}}{\text{Theoritcal } GA_3 \text{content}} \times 100 \qquad (2)$$

3.7 *In vitro* release studies of GA_3 and GA_3 from GA_3-HB-Starch micelles

Thirty milligrams of pure GA_3, 1.2 g and 1.5 g of GA_3-HB-Starch (0.8 and 1%wt) were placed into 10 ml of nutrient solution at room temperature. The mixture was centrifuged at specific time after that 2 ml of the mixture was withdrawn and replaced with 2 ml of nutrient solution. The experiment was performed for all samples before starting the analysis. The each sample was analyzed by UV-Vis spectrophotometer at 254 nm in triplicate. The amount of GA_3 released in solution

was calculated by interpolation from a calibration curves. Therefore, the actual amount of GA_3 released without degradation at time t (A_t) can calculate by the equation [54]:

$$A_t = D_t + C_0(1 - e^{-k_2 t}) \qquad (3)$$

Where, D_t is measured amount of GA_3 released at time t, C_0 is the initial amount of GA_3 prior to degradation and k_2 is the degradation rate constant.

The percentage of cumulative GA_3 release was calculated from this equation:

$$\text{Cumulative release (\%)} = \frac{\text{Actual amount } GA_3 \text{release}}{\text{Theoritcal amount } GA_3 \text{release}} \times 100 \qquad (4)$$

3.8 Agricultural applications

In order to study an agricultural application potentiels of the GA_3 encapsulation as a drug delivery system, the GA_3 encapsulation were prepared by dialysis method and the properties of the the GA_3 encapsulation, e.g. morphology, particle size, zeta potential, encapsulation efficiency and GA_3 release profiles were investigated.

The bunching onions were dipped in water for 20-24 h before the water drained and keeping the seeds in wet cloth. Then, the pre-geminated seeds (4-5 seeds) were grew in the sponge off-cuts (1×1 inch). Three days later, the seedlings having 0.3-1.0 cm height were transplanted to a bigger seedling container (10 L) in a hydroponic system.

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The nutrient solution was mixed in each container and the seedlings in a sponge were set up. The various concentrations of pure GA_3 (0, 0.1, 0.5, 1 and 5 ppm) was added into the nutrient solution in each container to examine for the optimum of stimulation of elongation and induce in the growth of bunching onions within 14 days.

Ultimately, the GA_3-HB-Starch micelles (500 mg/ 10 L) were compared the efficiency with the optimal pure GA_3 in hydroponic system and transplanted the bunching onions on ground. The growth rates of plants were measured with the length of plants in triplicates.

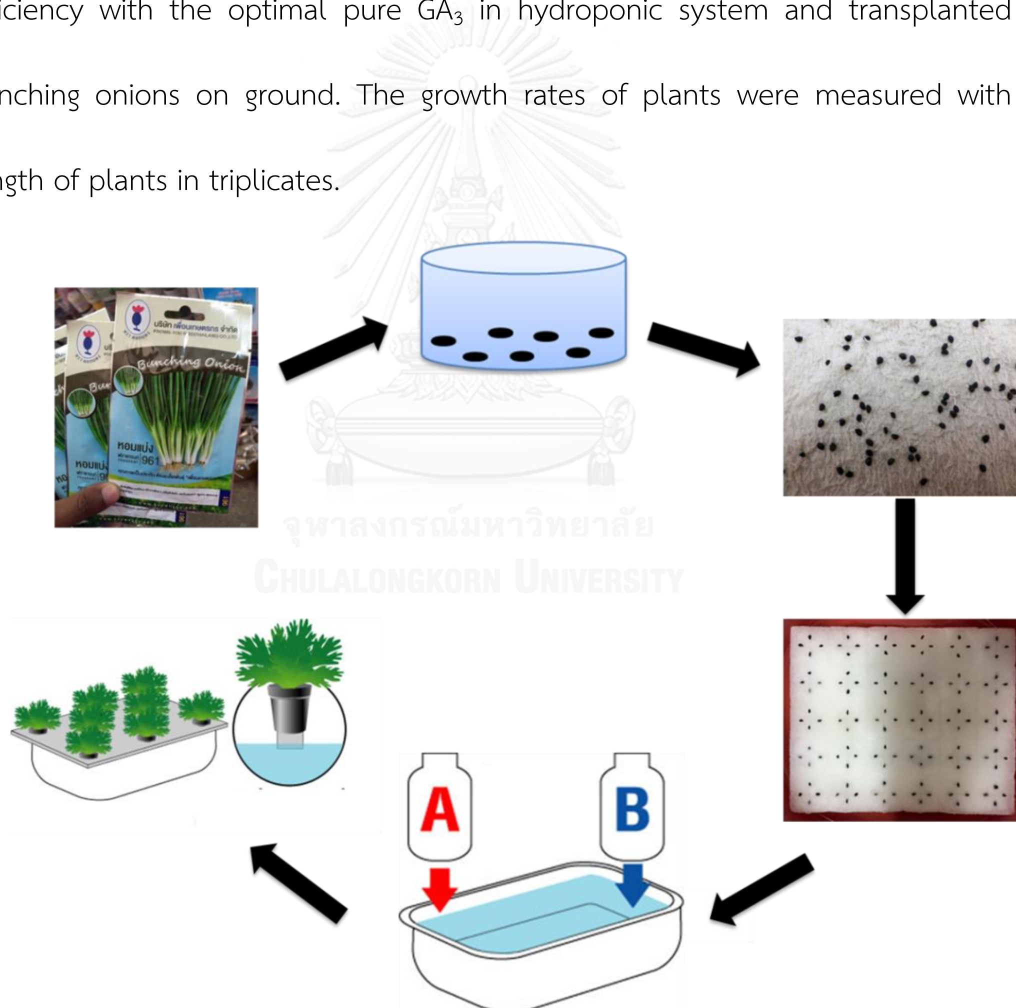

Figure 3.2 Preparation of agricultural application

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CHAPTER IV
RESULTS AND DISCUSSION

4.1 Synthesis and formation of HB-Starch

In general, the reaction of native starch granule cannot process to be a starch polymer directly, it must be modified. This is because starch has the multilevel structures including alternating amorphous and semicrystalline shells, crystalline and amorphous lamellae, and macromolecular chains [55]. Thus, it is the reason that we have to break down the intermolecular bonds of starch molecules through starch gelatinization. The starch gelatinization is a physical modification of the swollen starch granules in the presence of water and heat. As the result, the chemical modification can be easily prepared by a reaction of starch (St-OH) and octadecyl bromide in sodium hydroxide (NaOH) solution.

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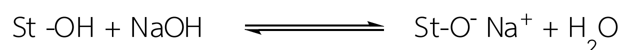
The first step is an alkalization, which the native starch was activated and transformed into an alkoxide form (St-O-) using sodium hydroxide [56].



$$St\text{-}OH + NaOH \rightleftharpoons St\text{-}O^- Na^+ + H_2O$$

The second step, the alkoxide form was reacted with octadecyl bromide. The nucleophilic substitution of alkyl halides can attack the starch and the halogen atom can leave as a halide ion. The result of HB-Starch form ($St\text{-}O\text{-}(CH_2)_{17}CH_3$) is shown below (Figure 4.1).

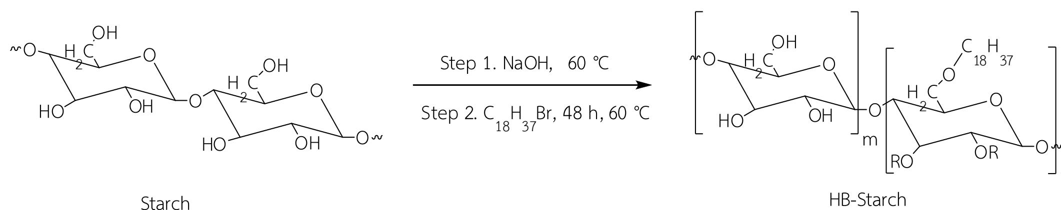


Figure 4.1 Synthesis of HB-Starch

When considering the formation of HB-Starch, it suggested that the starch and octadecyl chain consists of the hydrophilic backbone and hydrophobic side chains, respectively. Thus, it can entrap the GA_3 which has a low solubility in water by its hydrophobic bond. While the hydrophobic bond is present between GA_3 and starch side chain, the hydrophilic part, which is a backbone of the starch, formed hydrogen bonds with water molecules.

For this reason, the GA_3 encapsulation is generated and be able to use for controlled-release system. The GA_3 could be released from a HB-Starch by swelling-controlled delivery when immersed in aqueous solution. For the swelling of the carrier, it can increase the aqueous solvent content within polymer matrix and enables the GA_3 to diffuse through the swollen matrix into the external environment. The swell can change by the condition of the environmental surrounding such as pH, temperature and ionic strength. For the release, the rate will commonly decrease with the variety of system.

4.2 ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy

The result obtained from these reactions appeared as pale light brown powder. The products were charaterized by ^{1}H NMR and FTIR.

The ^{1}H NMR spectra in DMSO of a cornstarch and HB-Starch are shown in Figure 4.2. The peaks at 5.07 and 3.19-3.85 ppm chemical shifts were attributed to the H_1 and H_{2-6} in the starch (Figure 4.2a), respectively [57].

For the ^{1}H NMR spectra of octadecyl bromide, it was showed the proton at chemical shift 3.48-3.51, 1.72-1.77, 1.32, 1.16-1.21 and 0.77-0.83 ppm. They were ascribed to the H_a, H_b, H_c, H_d and H_e as seen in Figure 4.2b.

After conjugated, all peaks were shifted and the appearances of new proton positions of HB-Starch were observed at 1.90-1.99, 1.37-1.46, 1.21 and 0.80-0.85 ppm. They were assigned to the H_b, H_c, H_d and H_e, respectively (Figure 4.2c). However, the H_a peak of *O*-alkyl group of hydrophobic chain ($-O-CH_2-CH_2-CH_2-(CH_2)_{14}-CH_3$,) overlapped with the chemical shift of starch signal between 3.45 and 3.68 ppm.

The substitution of octadecyl chain on the starch in form of %DS calculated by using Eq. (1) was 8.5%. These results indicated that the octadecyl chain was successfully introduced in the starch backbone to give the HB-Starch.

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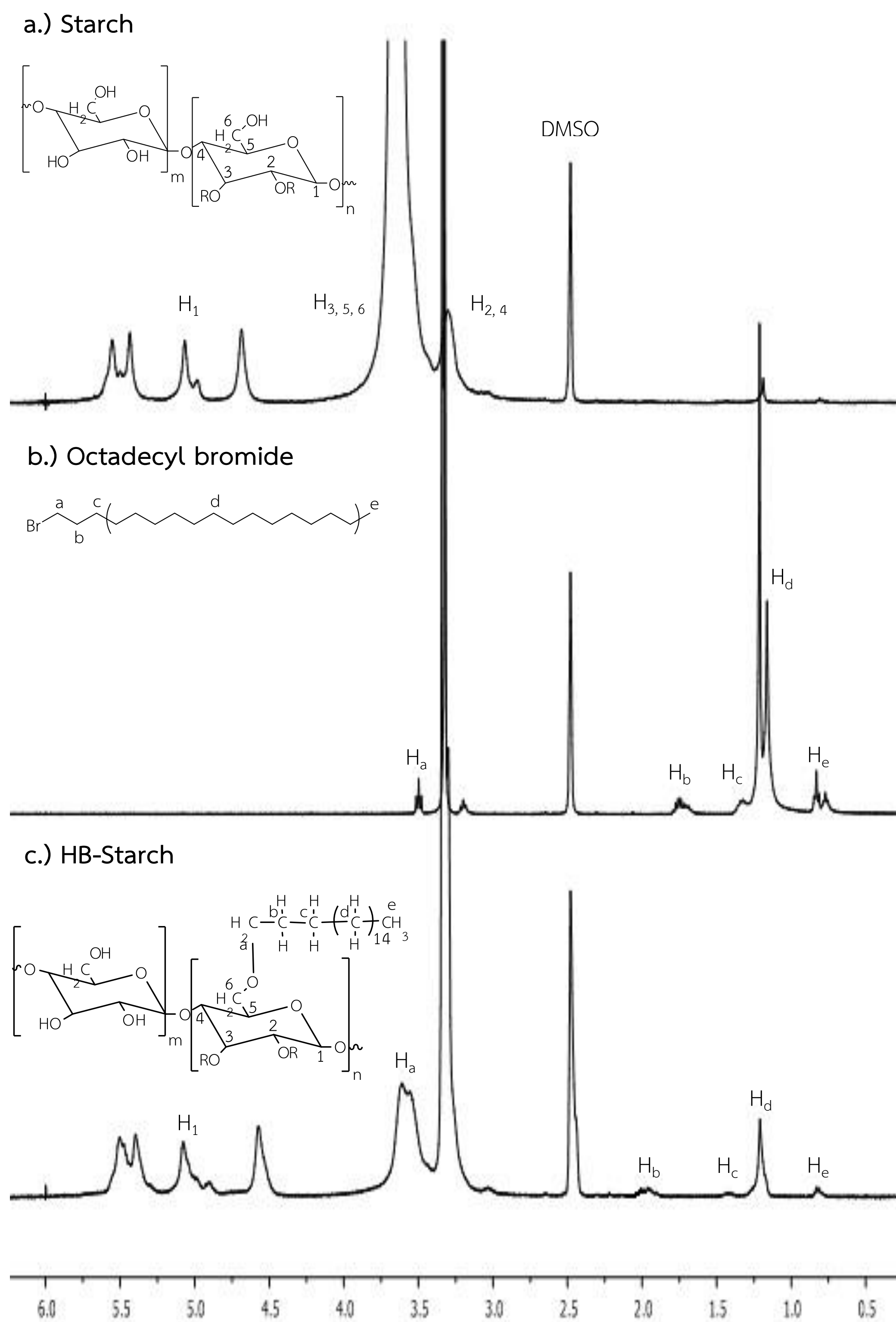

Figure 4.2 Representative ^{1}H NMR spectra of (a) starch, (b) octadecyl bromide and (c) HB-Starch

The degree substitution of octadecyl chain on the starch was calculated by using Eq. (1). The results in Table 4.1, it showed that the %DS values were not statistically significant when the amount of octadecyl bromide increased with reaction time during a 48 hours period. The results suggest that the hydroxyl groups of starch have limited ability of transformation into an alkoxide form (St-O-) due to NaOH content. Moreover, the structure of the long-chain hydrocarbon leads to the effect of steric factor. Therefore, HB-Starch was synthesized at the 1 : 0.5 (weight ratio) of Starch : Octadecyl bromide was selected for this study.

Table 4.1 Comparison of %DS in each sample

Starch : Octadecyl bromide (weight ratio)	Degree substitution (%DS)
1 : 0.5	8.5
1 : 1	8.5
1 : 2	9
1 : 4	11.5

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4.3 Fourier transformed infrared (FTIR) spectroscopy

The FTIR spectra of (a) starch, (b) HB-Starch, (c) GA_3 and (d) GA_3-HB-Starch are shown in Figure 4.3. The FTIR spectrums of the starch (Figure 4.3a) show a broad peak at 3400 cm^{-1}, the peak at 2932 cm^{-1}, 1644 cm^{-1}, and 926 – 1163 cm^{-1} which attribute to the O-H stretching, C-H stretching, hydroxyl groups of surface and C-O-C of starch, respectively. After the preparation of HB-Starch (Figure 4.3b), the FTIR

spectrum shows a new peak at 2851 cm^{-1} which attributes to long chain hydrocarbon so it can indicate the attachment of long chain onto the hydroxyl groups of starch. In order to define the chemical interaction between GA_3 and HB-Starch in the GA_3 encapsulations, the samples of GA_3 and GA_3-HB-Starch were investigated. The FTIR spectrum of GA_3 (Figure 4.3c) appeared the strong peak at 1732 cm^{-1} that attributed to the stretching of carbonyl group. Moreover, the FTIR spectrum of GA_3-HB-Starch (Figure 4.3d) showed the peak at 1729 cm^{-1} as C=O ester stretching to confirm the successful loading of GA_3 into HB-Starch.

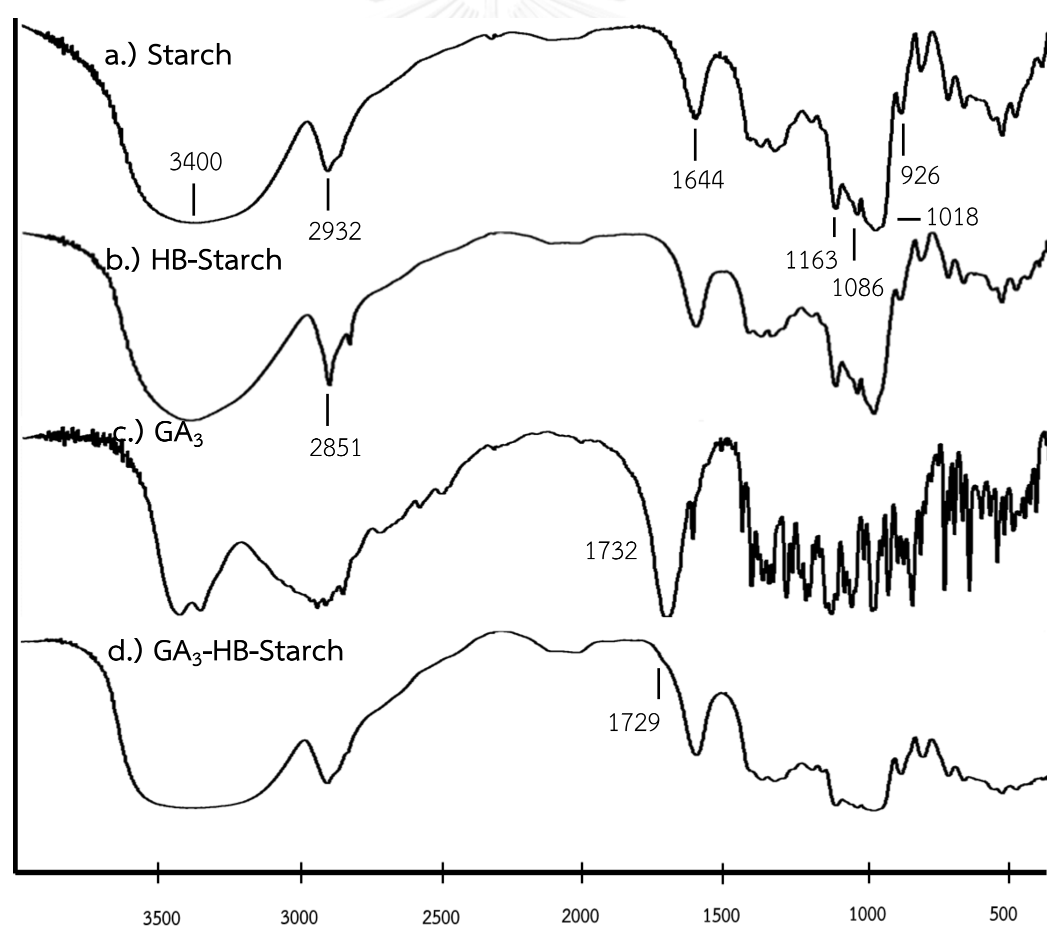

Figure 4.3 Representative FTIR spectra of (a) starch, (b) HB-Starch, (c) GA_3 and (d) GA_3-HB-Strach

4.4 Morphology

The morphology of samples were investigated by SEM. The samples (0.4 mg) were dispersed in 1 mL distilled water at room temperature and vortexed for 2 h. The solutions were dropped on a clean glass slide to self-assembly process in desiccators for 2 days.

Self-assembly is a process which molecules were arranged by themselves without guidance or management from an outside source. However, concentration factor of self-assembly process is importantly to the formation and morphological appearance of HB-Starch nanoparticles. Thus, the concentration of samples for SEM analysis was prepared above the CMC value of HB-Starch because the HB-Starch micelles have started forming since the CMC point.

The results from the studies of self-assembly process showed that the HB-Starch and GA_3-HB-Starch micelles were successfully prepared. The representative scanning electron microscope (SEM) images of HB-Starch and GA_3-HB-Starch are shown in Figure 4.4.

As can be seen, the morphology of HB-Starch micelles without GA_3 was compared with HB-Starch micelles loaded GA_3, both SEM images showed the nanoparticles in the spherical shapes without the visible pores. This may be attributed to the fact that the modified starch successfully formed by self-assembly process.

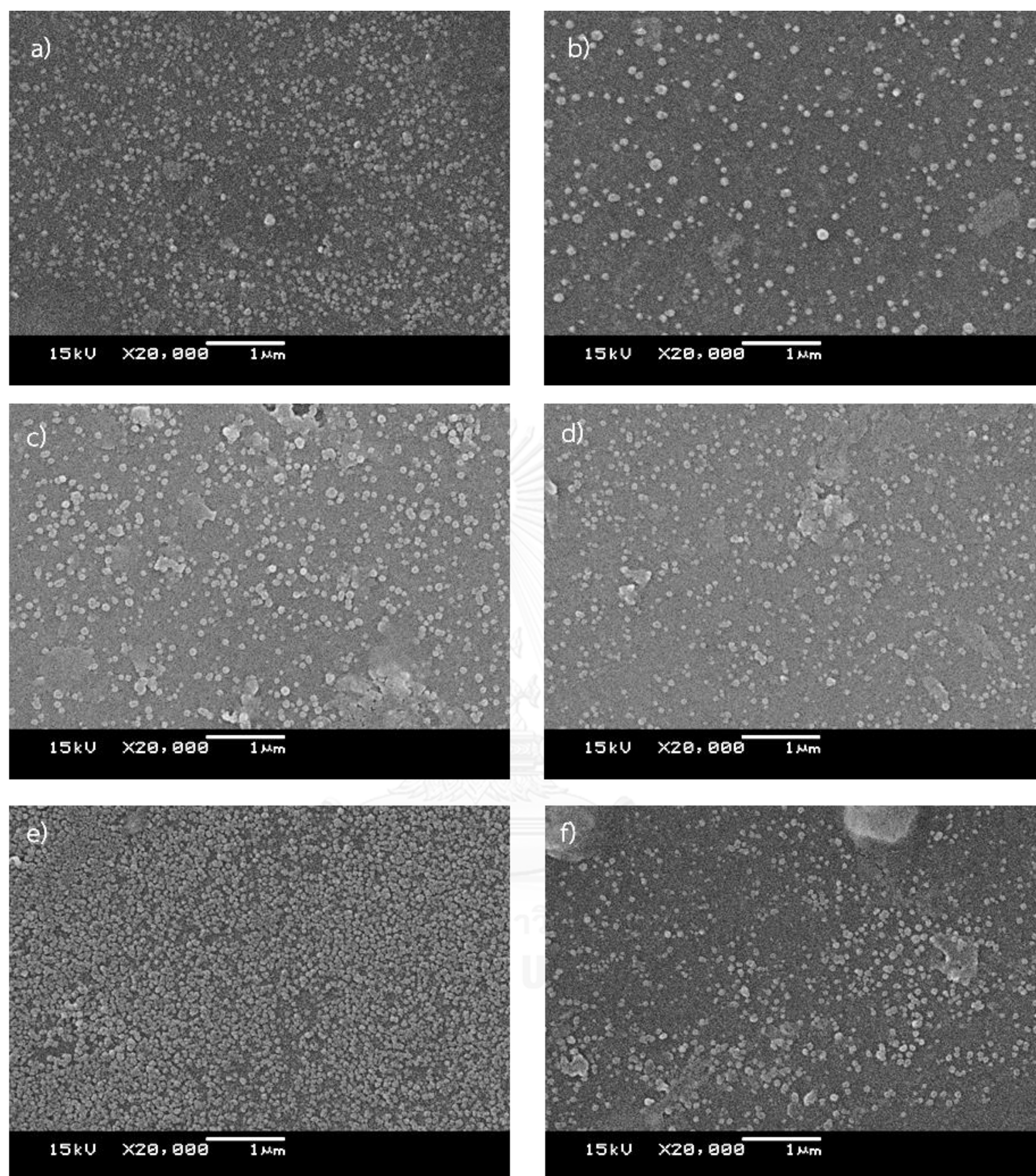

Figure 4.4 Representative SEM images of (a) HB-Starch, (b) 0.8 % GA_3-HB-Strach, (c) 1 % GA_3-HB-Strach, (d) 1.5 % GA_3-HB-Strach, (e) 5 % GA_3-HB-Strach and (f) 10 % GA_3-HB-Strach

4.5 Particle size, size distribution

The particle size and size distribution of HB-Starch and GA_3-HB-Starch nanoparticles are presented in Table 4.2.

When considering the particle size and average size of nanoparticles by SEM, it showed that the HB-Starch without GA_3 has 91 nm of size (Figure 4.4a). The HB-Starch loaded 0.8, 1, 1.5, 5 and 10% of dried GA_3 was in the range of 113 nm (Figure 4.4b), 114 nm (Figure 4.4c), 108 nm (Figure 4.4d), 98 nm (Figure 4.4e) and 97 nm (Figure 4.4f), respectively. The diameter values after distribution of the swollen nanoparticles was measured by DLS, it was found that the size of HB-Starch before loading the GA_3 was about 1101 nm. After loading of GA_3, the size of each sample was 1160, 1174, 1157, 1135 and 1120 nm, respectively.

The polydispersity index (PDI) was used to measure for the width of the particle size distribution (from 0 = monodisperse to 1 = polydisperse). All samples showed that they have PDI in narrow range of 0.390 to 0.520.

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From the results, it suggestes that the mean of particle size and size distribution of nanoparticles increase when the GA_3 is loaded. One percentage of GA_3 loaded into HB-Starch micelles has the large particle size and wide size distribution. It assumes that the large size gives a wide size distribution and effects to the high value of polydispersity index.

Furthermore, the sizes measured by DLS in aqueous solution are in the swollen state leading to the swelling of polymer, whereas the sizes observed by SEM

are in the dried state. This is a reason that the sizes of swollen nanoparticles are larger than the dried nanoparticles.

Table 4.2 The particle size and size distribution of nanoparticles

Abbreviations	Particle size ± SD (nm) by SEM	Particle size ± SD (nm) by zetasizer	Polydispersity Index (PDI)
HB-Starch	91 ± 10.83	1101 ± 30.53	0.39 ± 0.10
0.8% GA_3-HB-Starch	113 ± 15.58	1160 ± 41.14	0.51 ± 0.12
1% GA_3-HB-Starch	114 ± 13.02	1174 ± 42.67	0.52 ± 0.17
1.5% GA_3-HB-Starch	108 ± 11.14	1157 ± 21.52	0.49 ± 0.10
5% GA_3-HB-Starch	98 ± 10.71	1135 ± 92.93	0.46 ± 0.07
10% GA_3-HB-Starch	97 ± 10.04	1120 ± 43.71	0.42 ± 0.29

*Data are shown as the mean ± SD and derived from three independent repeats

4.6 Zeta potential

Zeta potential analysis is a technique for measuring the overall surface charge of the particles in solution. The magnitude of the zeta potential is predictive of the colloidal stability. The obtained results of surface of HB-Starch micelles measured about -6.74 mV by the negative charges. After the GA_3 loading, the surface charges of GA_3-HB-Starch nanoparticles were found that -5.75, -5.89, -5.65, -5.22 and -5.15 mV (0.8%, 1%, 1.5%, 5%, 10% GA_3-HB-Starch, respectively) have decreased as seen in

Table 4.3. The decrease of zeta potential of micelles after GA_3 loading in all formulations shows that the micelles have poor stability.

In the general case, the nanoparticles with Zeta Potential values are higher than +25 mV or lower than -25 mV typically having the high degrees of stability [58]. As the results suggest that the dispersion of nanoparticle with the low zeta potential values (typically range from +25 to -25 mV) could presumably aggregate due to the interparticle attractions or Van Der Waal forces. Therefore, the maximum of zeta potential is 1 % of GA_3 loading so it was the most stable formulation.

Table 4.3 The zeta potentials of nanoparticles

Abbreviations	Zeta potential (mV)
HB-Starch	-6.74 ± 0.22
0.8% GA3-HB-Starch	-5.75 ± 0.02
1% GA3-HB-Starch	-5.89 ± 0.08
1.5% GA3-HB-Starch	-5.65 ± 0.04
5% GA3-HB-Starch	-5.22 ± 0.50
10% GA3-HB-Starch	-5.15 ± 0.09

*Data are shown as the mean ± SD and derived from three independent repeats

4.6 Determination of critical micelle concentration (CMC)

The critical micelle concentration (CMC) is an important characteristic for nanoparticles and indicating the stability property. Generally, the CMC was used for selection of micelles in specific applications or properties [59]. In the study, the CMC value of HB-Starch in distilled water was examined by the fluorescence probe technique using pyrene. At the fixed excitation wavelength of 337 nm, the emission spectras were scanned from 350 to 600 nm. As exhibited in Figure 4.5, the emission fluorescent intensity of pyrene is decreased when the polymer concentration increases from 1.0×10^{-5} to 5.0 mg/mL. The result suggested that the pyrene molecules transfer into the hydrophobic cores of the micelles in order to micelle formation.

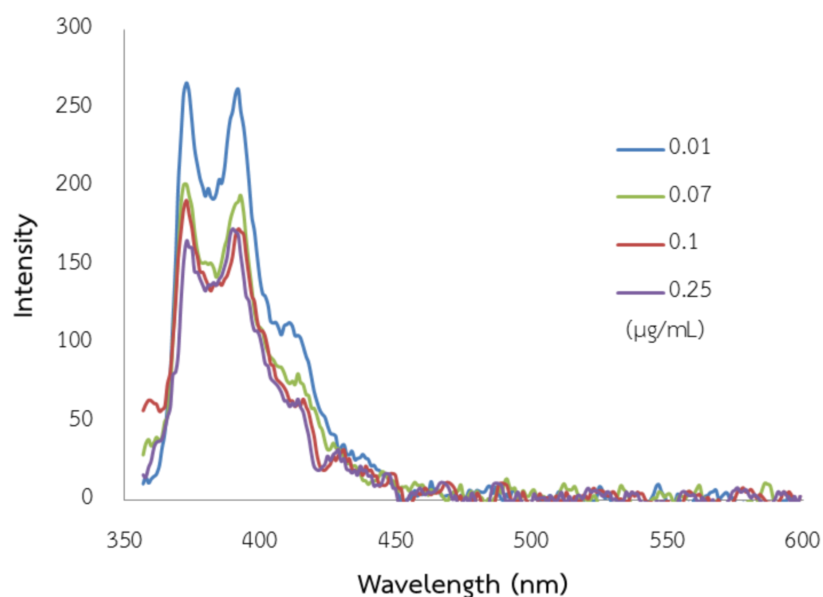

Figure 4.5 Fluorescence spectra of pyrene in water at different concentrations of HB-Starch

The fluorescence intensity ratios of pyrene at 374 and 384 nm (I_{374}/I_{384}) were calculated and plotted with the polymer concentration. The CMC value of HB-Starch is 0.25 µg/mL as seen in Figure 4.6. At the CMC point, the monomer molecules of HB-Starch in the bulk started to form to the spherical shapes.

As a result, the HB-Starch forms through self-assembly at a low concentration. The HB-Starch with a low CMC value is less trigger than high CMC value. Thus, the HB-Starch generally has high solubility and be stable to the circulation in hydroponic system so it is a suitable substance for the controlled-release.

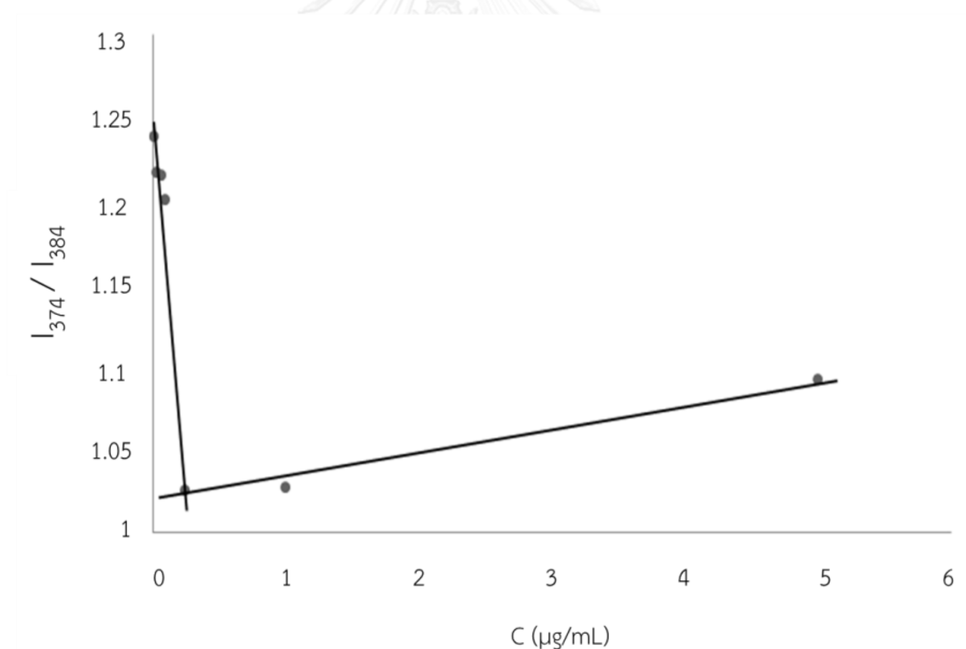

Figure 4.6 Plot of intensity ratio I_{374}/I_{384} as HB-Starch concentrations

4.7 Determination of GA_3 encapsulation efficiency (EE)

Gibberellic acid (GA_3) should be used in a limit because of its poor water solubility and instability. In the study, the GA_3 was loaded into the HB-Starch nanoparticles by dialysis method and the percentages of encapsulation efficiency (EE) of the GA_3 loaded are shown in Table 4.4.

The results were analyzed by an Ultraviolet (UV) spectrophotometer at 254 nm using the linear equation and the standard curve ($y= 0.0008x - 0.0011$, $R^2 = 0.9996$). One percentage of GA_3 loaded into HB-Starch micelles showed the high encapsulation efficiency (over 90%) and the GA_3 loading ratio of 0.8, 1.5, 5 and 10% GA_3 loaded HB-Starch were 81.76%, 73.26%, 14.04% and 11.25%, respectively.

The result suggested that the optimum of GA_3 loading ratio is 1%wt of GA_3 and the entrapped GA_3 is more effectively stabilized in HB-Starch micelles.

Table 4.4 Encapsulation of GA_3 loaded into the HB-Starch

Abbreviations	%Encapsulation
0.8% GA3-HB-Starch	81.76 ± 0.21
1% GA3-HB-Starch	93.19 ± 0.68
1.5% GA3-HB-Starch	73.26 ± 0.20
5% GA3-HB-Starch	14.04 ± 0.03
10% GA3-HB-Starch	11.25 ± 0.01

*Data are shown as the mean ± SD and derived from three independent repeats

4.8 *In vitro* release studies of GA_3 and GA_3 from HB-Starch micelles

The *in vitro* GA_3 release behavior of HB-Starch nanoencapsulation was performed in nutrient solution at room temperature. The linear equation of GA_3 calibration curve is y = 0.0009x – 0.0003 and R^2 = 0.9993.

However, the stability of GA_3 has reported that it could be limited solubility in water and under neutral or alkaline conditions. Moreover, it decomposed in water and aqueous-alcoholic solutions [33]. Thus, the degradation is an important factor affecting the corrected release value of GA_3.

From the experiment, the typical degradation behavior of GA_3 in nutrient solution was shown in Figure 4.7.

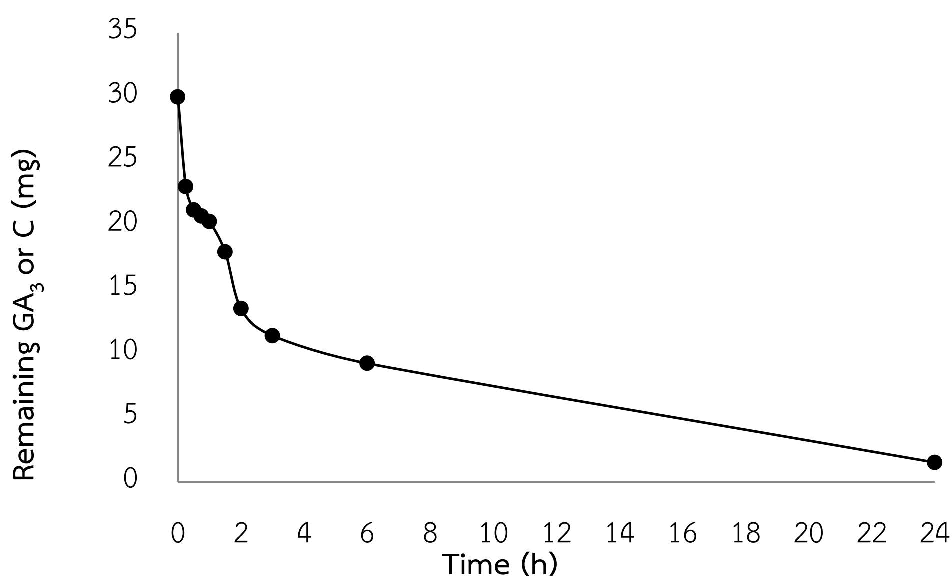

Figure 4.7 Degradation of GA_3 in nutrient solution

The value of the degradation rate constant in days (k_2) can be calculated from the slope of the straight line plot. The plot in Figure 4.8 showed the degradation rate constant in days (k_2) (-0.1139) following the first-order kinetics [60].

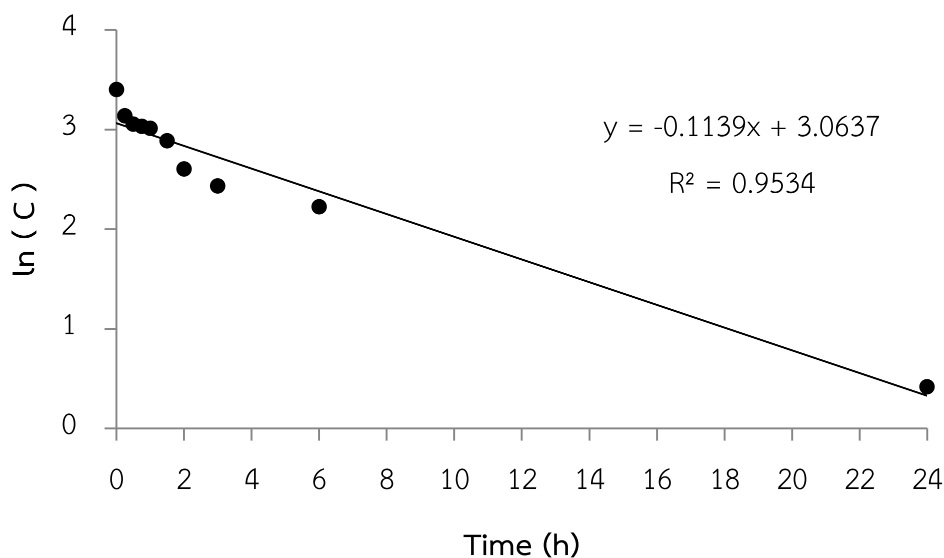

Figure 4.8 The degradation rate constant of GA_3 in days

The plots of the release of GA_3 in nutrient solution before correction by the degradation factor are shown in Figure 4.9 and the release profile of GA_3 in nutrient solution corrected with the degradation factor is shown in Figure 4.10.

The comparison of the release profiles of GA_3 loaded HB-Starch and pure GA_3 as a function of time for 14 days was reported that the pure GA_3 presented a complete release with 100% in 1 day (24 h). Then, the cumulative release was decreased within 14 days due to the decomposition of GA_3 in nutrient solution having water and aqueous-alcoholic solutions. On the other hand, the GA_3-HB-Starch nanoparticles was observed that the release rate of 1% GA_3-HB-Starch and 0.8% GA_3-

HB-Starch showed the initial burst release about 50 and 55 % within the first 6 hours, respectively and then gradually slow down. The GA_3 encapsulation showed better results than the pure GA_3 because it could prolong the release from 1 to 7 days. The burst release might be relative to the presence of more-free GA_3 on the nanoparticles surface.

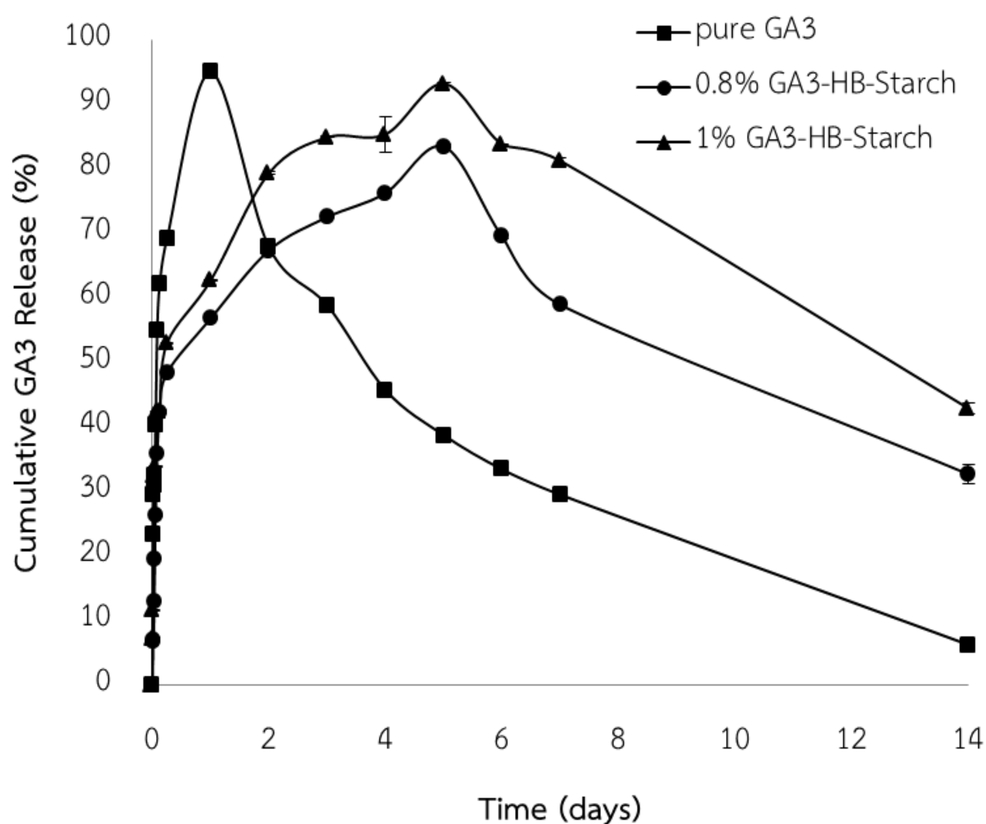

Figure 4.9 Release profiles of 0.8% and 1% GA_3 from HB-Starch micelles in nutrient solution prior to the correction by the degradation factor (average ± SD, n=3).

Moreover, 1% GA_3-HB-Starch obviously showed that the cumulative release was also higher than 0.8% GA_3-HB-Starch because of the amount of GA_3 entrapping into micelle.

All the results indicated that HB-Starch micelles could protect GA_3 and also sustain the release more than pure GA_3. Therefore, the 1% GA_3-HB-Starch was selected for agricultural application to accelerative growth rate.

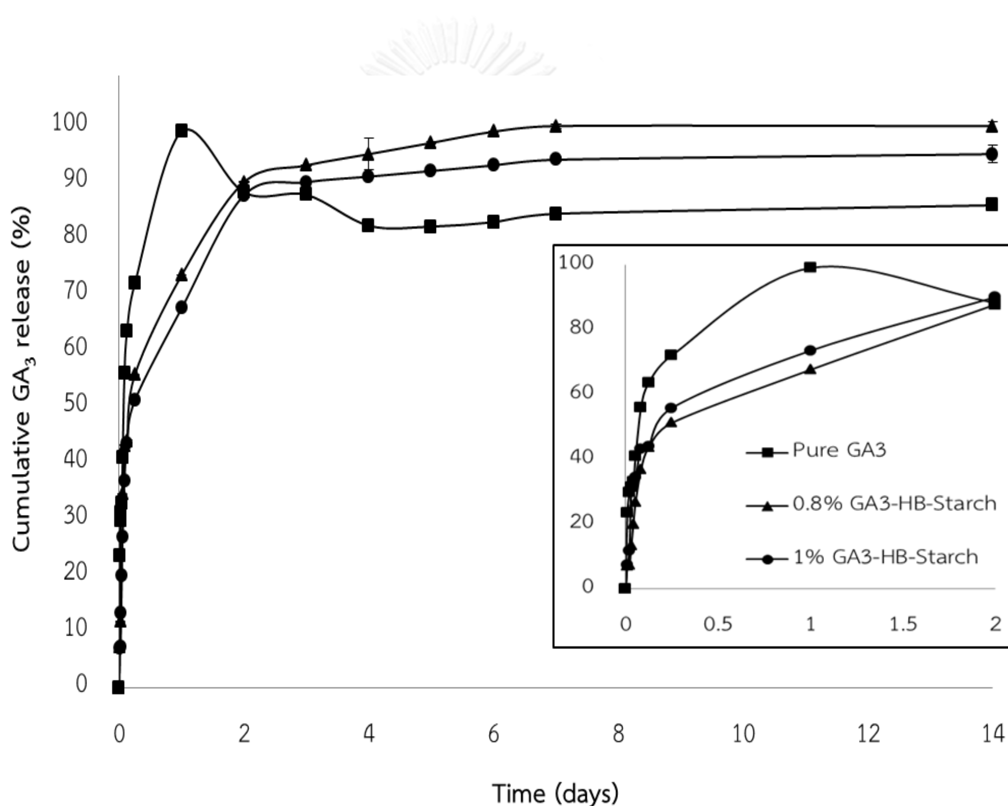

Figure 4.10 Release profiles of 0.8% and 1% GA_3 from HB-Starch micelles In nutrient solution (average ± SD, n=3).

4.9 Agricultural application on growth of bunching onion

For the gibberellic acid treatment, the graph of all concentrations (Figure 4.11) showed the continuing trend of the growth rates higher than the control. The addition of GA_3 on plant can increase the cell enlargement and accelerated the plant growth.

Moreover, Brian described that the low concentrations of GA_3 had a large increase in plant growth rate, while the high concentrations of GA_3 had a lower plant growth rate than the other one may be explained due to the phytotoxicity of GA_3 applied in high concentrations [4]. Phytotoxicity is a toxic effect by a compound on plant growth as a wide variety of compounds such as pesticides, salinity [61].

The results suggest that the optimum for the stimulation of elongation and the induced growth of bunching onion was 0.5 ppm in 14 days. The application of this plant growth regulator in this concentration allowed that the length of plant obtained about 7 cm higher than control (1 cm).

Thus, the addition of 0.5 ppm GA_3 could increase the growth rate of bunching onion 7 times compared with control at the same time.

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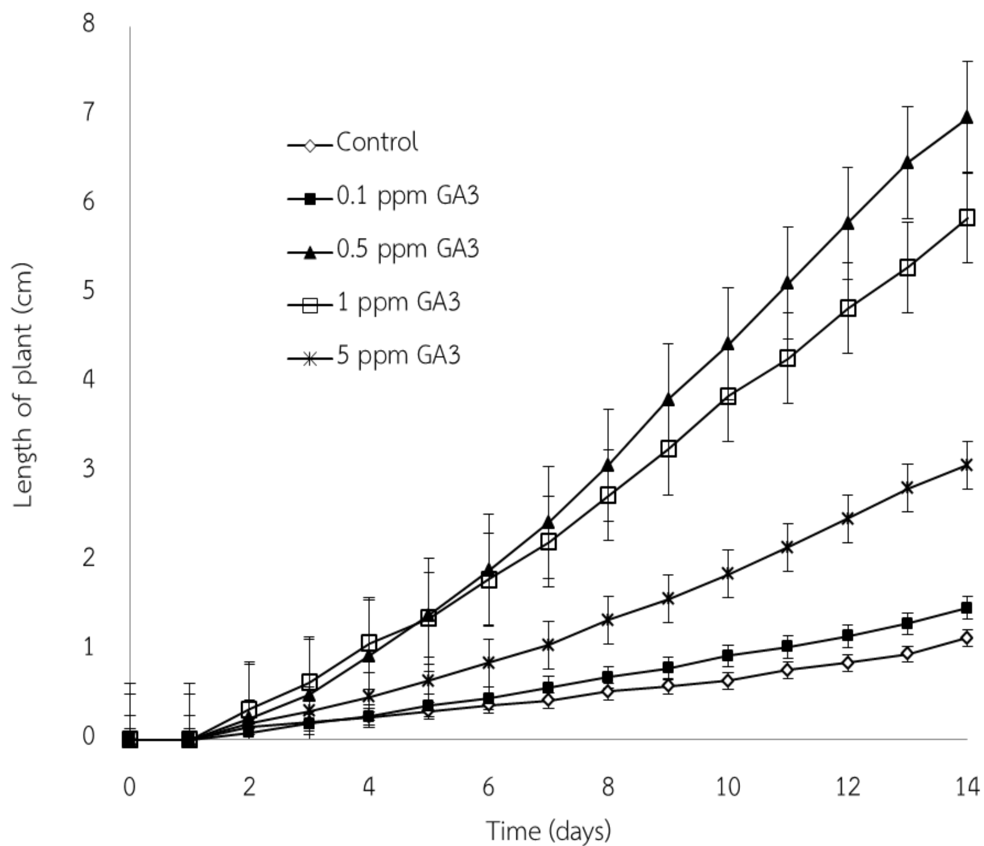

Figure 4.11 Effect of GA_3 treatment at all concentrations

When considering the effect of GA_3-HB-Starch at 0.5 ppm, the results suggested that 1% of GA_3-HB-Starch nanoparticles had the greatest growth rate in 14 days. Moreover, the nanoparticles gave the high growth rate approximately 1.5 cm at the same time compare with pure GA_3 because of the steady release of GA_3 from HB-Starch (Figure 4.13).

According to the results, we found that the nanoparticles could decrease the waste of growing time around 3 days from 14 to 11 days at the same length of

bunching onion. The GA_3 can help to promote the cell elongation and stimulate cell division which leads to the growth rate increase.

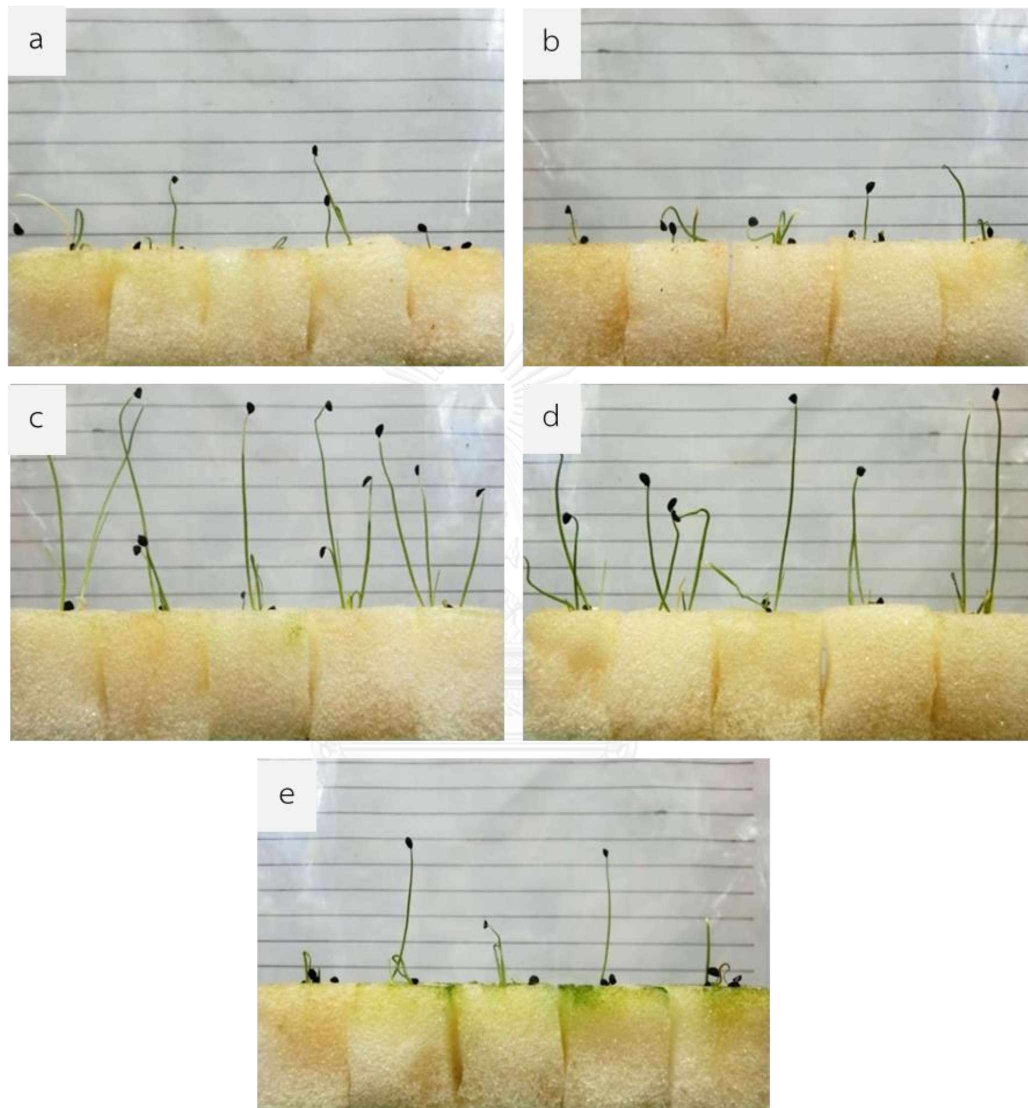

Figure 4.12 Effect of GA_3 concentration on growth of bunching onion

a) control, b) 0.1 ppm GA_3, c) 0.5 ppm GA_3, d) 1 ppm GA_3 and e) 5 ppm GA_3

However, the bunching onions on ground have the higher growth rate than the control in hydroponics system because the soil has the high essential mineral nutrients at the beginning.

The result from the investigation exhibited the influence of GA_3 on the growth rate of bunching onion was implied that HB-Starch micelles can be protect and prolong release the GA_3 in nutrient solution for the utilization in agricultural application of nanoencapsulation.

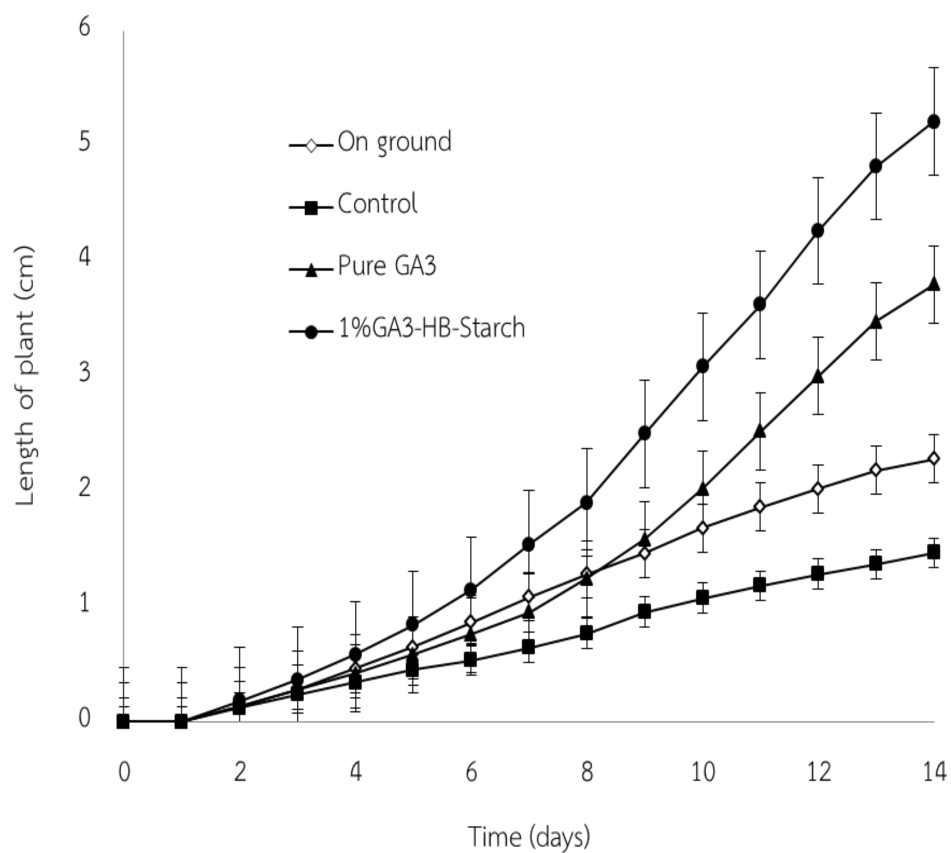

Figure 4.13 The effect of GA_3-HB-Starch at 0.5 ppm

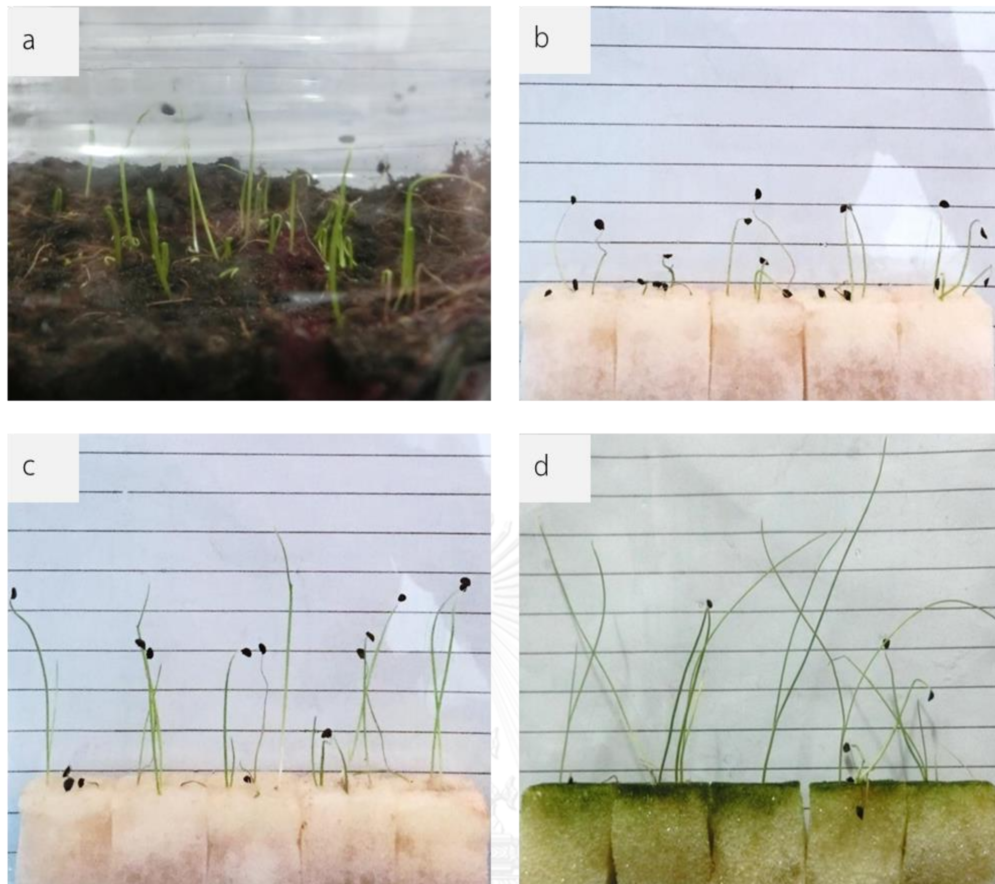

Figure 4.14 Effect of GA_3-HB-Starch on growth of bunching onion at 0.5 ppm

a) On ground, b) Control, c) pure GA_3 and d) 1 %GA_3-HB-Starch

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CHAPTER V
CONCLUSION AND SUGGESTION

5.1 Conclusion

In this work, the HB-Starch was successfully synthesized. First, the native starch was activated and changed to an alkoxide form (St-O-) by sodium hydroxide. Thereafter, the alkoxide form was reacted with octadecyl bromide. The degree substitution of octadecyl chain on the starch was calculated by ^{1}H NMR about 8.5%. The CMC value of HB-Starch was around 0.25 µg/mL examined by the fluorescence probe technique using pyrene.

Gibberellic acid (GA_3) was successfully loaded into HB-Starch micelles by dialysis method. The encapsulation of 1% GA_3 loaded HB-Starch nanoparticles exhibited the optimum efficiency around 93.19 ± 0.68 and the GA_3 loading ratio of 0.8, 1.5, 5 and 10% GA_3 loaded HB-Starch were 81.76 ± 0.21, 73.26 ± 0.20, 14.04 ± 0.03 and 11.25 ± 0.01%, respectively. The morphology of HB-Starch with and without GA_3 was obtained by SEM, presented a spherical shape without the visible pores.

Moreover, The GA_3 loaded HB-Starch nanoparticles could prolong and sustain the release profiles of GA_3 which was longer when compared to the pure GA_3. Hence, it can be properly used in nutrient solution on the hydroponic system in term of nanoparticles structure and acceleration of plant growth in bunching onion.

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5.2 Suggestions for the future work

In this study, we are only used the GA_3-HB-Starch in nutrient solution on the hydroponic system for acceleration of plant growth in bunching onion. In other case, it should be investigated for accelerating growth of another plant and other conditions.

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APPENDIX

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APPENDIX A

The degree substitution of octadecyl chain

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Determination of the degree of Octadecyl chain substitution (%DS)

$$\mathrm{DS}\ (\%) = \left[\frac{\mathrm{I_{Hc}}}{\mathrm{I_{H1}}} \times \frac{1}{2}\right] \times 100 \qquad (1)$$

Where, I_{Hc} is the integral area of protons at the c-position and I_{H1} is the integral area of proton at 1-position of HB-Starch, respectively.

From ^{1}H-NMR spectrum of HB-Starch

1 : 0.5 $\mathrm{DS}\ (\%) = \left[\frac{0.17}{1.00} \times \frac{1}{2}\right] \times 100$ = 8.5

1 : 1 $\mathrm{DS}\ (\%) = \left[\frac{0.17}{1.00} \times \frac{1}{2}\right] \times 100$ = 8.5

1 : 2 $\mathrm{DS}\ (\%) = \left[\frac{0.18}{1.00} \times \frac{1}{2}\right] \times 100$ = 9

1 : 4 $\mathrm{DS}\ (\%) = \left[\frac{0.23}{1.00} \times \frac{1}{2}\right] \times 100$ = 11.5

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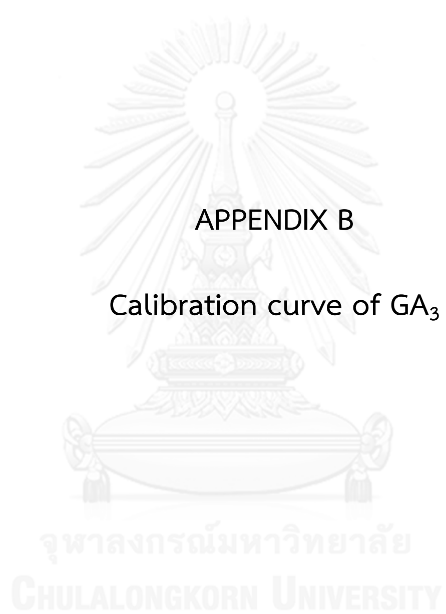

APPENDIX B

Calibration curve of GA_3

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Table 1B Absorbance of gibberellic acid in 30% (v/v) EtOH determined in 254 nm

Concentration (ppm)	Abs	Abs	Abs	AVG	SD
50	0.041	0.040	0.040	0.040	0.000
100	0.075	0.075	0.075	0.075	0.000
200	0.162	0.163	0.163	0.163	0.000
400	0.328	0.329	0.328	0.328	0.000
600	0.495	0.494	0.494	0.494	0.000
800	0.643	0.644	0.645	0.644	0.001

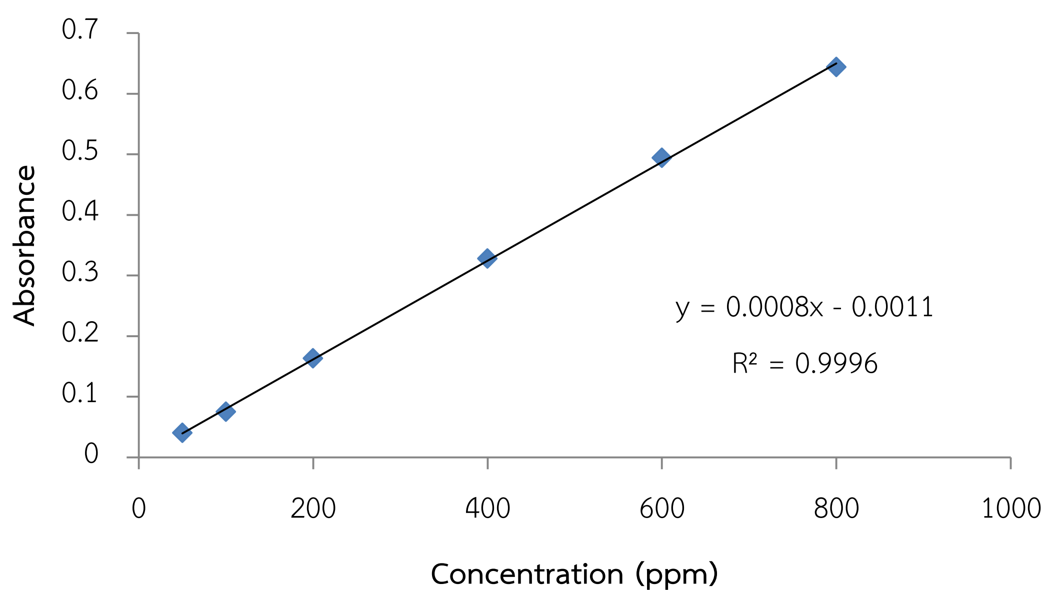

Figure 1B Calibration curve of gibberellic acid in 30% (v/v) EtOH determined in 254 nm

Table 2B Absorbance of gibberellic acid in nutrient solution determined in 254 nm

Concentration (ppm)	Abs	Abs	Abs	AVG	SD
50	0.042	0.042	0.042	0.042	0.000
100	0.081	0.081	0.081	0.081	0.000
200	0.178	0.179	0.179	0.179	0.001
300	0.276	0.276	0.277	0.276	0.001
400	0.347	0.347	0.347	0.347	0.000
500	0.438	0.438	0.438	0.438	0.000
600	0.533	0.534	0.534	0.534	0.001
700	0.617	0.617	0.617	0.617	0.000
800	0.700	0.701	0.700	0.700	0.000

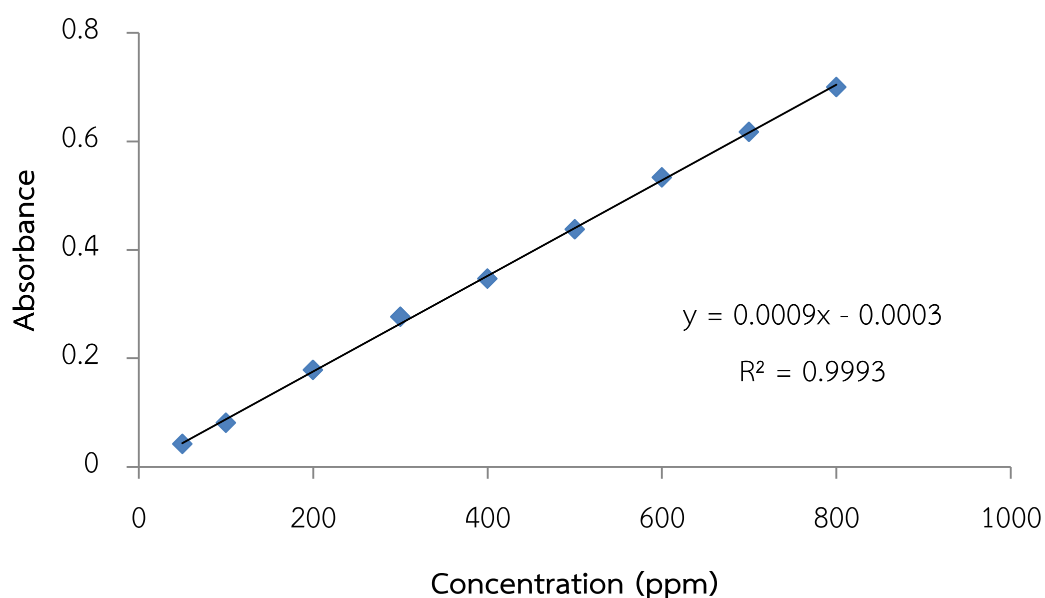

Figure 2B Calibration curve of gibberellic acid in nutrient solution determined in 254 nm

APPENDIX C

Encapsulation and Cumulative Drug Release

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Solution of encapsulation efficiency

20 mg of GA_3-HB-Starch (0.8, 1, 5 and 10 %wt) in 1 mL of 30% (v/v) EtOH were determined the encapsulation of gibberellic acid loaded into HB-Starch micelles.

Table 1C Absorbance of gibberellic acid for determined the encapsulation

Abbreviations	Abs	Abs	Abs	AVG	SD
0.8% GA_3-HB-Starch	0.129	0.128	0.128	0.129	0.001
1% GA_3-HB-Starch	0.146	0.146	0.148	0.148	0.001
1.5% GA_3-HB-Starch	0.174	0.175	0.174	0.175	0.001
5% GA_3-HB-Starch	0.133	0.132	0.133	0.133	0.001
10% GA_3-HB-Starch	0.204	0.203	0.203	0.204	0.000

For example…(1% GA_3-HB-Starch)

From $y = 0.0008x - 0.0011$

$0.148 = 0.0008x - 0.0011$

$x = 184.54$ ppm

Changes a unit from ppm to mg

1000 mL with 184.54 mg

If 1 mL with $(184.54\times1)/1000 = 0.185$ mg

From mass 202 mg of 1% GA_3-HB-Starch has the drug 2 mg

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If 20 mg equal to (2×202)/20 = 0.198 mg

From

$$EE\ (\%) = \frac{\text{Actual } GA_3 \text{content}}{\text{Theoritcal } GA_3 \text{content}} \times 100$$

So the encapsulation of 1% GA_3-HB-Starch

EE% = (0.185/0.198) × 100 = 93 %

Table 2C Cumulative of pure gibberellic acid in nutrient solution prior to the correction by the degradation factor

Time (days)	Amount	Amount	Amount	AVG	SD
0	0.000	0.000	0.000	0.000	0.000
0.00972	23.278	23.352	23.370	23.333	0.049
0.02083	29.370	29.389	29.426	29.395	0.028
0.03125	30.870	30.796	31.093	30.920	0.154
0.0417	32.352	32.519	32.370	32.414	0.091
0.0542	40.352	40.296	40.278	40.309	0.039
0.0833	54.907	54.667	55.278	54.951	0.308
0.125	62.037	61.981	62.093	62.037	0.056
0.25	69.074	69.259	69.259	69.198	0.107
1	95.077	94.690	95.034	94.934	0.212
2	67.778	67.556	68.093	67.809	0.270
3	58.870	58.815	58.722	58.802	0.075
4	45.541	45.674	45.778	45.664	0.119
5	38.756	38.741	38.504	38.667	0.141
6	33.511	33.289	33.333	33.378	0.118
7	29.481	29.378	29.452	29.437	0.053
14	6.196	6.192	6.189	6.193	0.004

Table 3C Cumulative of 0.8 % GA_3-HB-Starch in nutrient solution prior to the correction by the degradation factor

Time (days)	Amount	Amount	Amount	AVG	SD
0	0.000	0.000	0.000	0.000	0.000
0.00972	6.909	6.760	6.788	6.819	0.079
0.02083	7.068	7.049	6.993	7.037	0.039
0.03125	12.979	13.035	12.894	12.969	0.070
0.0417	19.505	19.533	19.533	19.524	0.016
0.0542	26.200	26.134	26.246	26.194	0.056
0.0833	35.789	35.836	35.752	35.792	0.042
0.125	42.232	41.961	42.120	42.104	0.136
0.25	48.282	48.413	48.357	48.350	0.066
1	56.807	56.984	56.601	56.797	0.192
2	67.367	67.255	67.339	67.320	0.058
3	72.549	72.344	72.558	72.484	0.121
4	76.359	76.237	76.069	76.222	0.145
5	83.557	83.576	83.081	83.405	0.280
6	69.636	69.514	69.216	69.455	0.216
7	58.711	58.599	59.384	58.898	0.424
14	33.389	30.906	33.585	32.627	1.494

Table 4C Cumulative of 1 % GA_3-HB-Starch in nutrient solution prior to the correction by the degradation factor

Time (days)	Amount	Amount	Amount	AVG	SD
0	0.000	0.000	0.000	0.000	0.000
0.00972	7.314	7.258	6.996	7.189	0.170
0.02083	11.523	11.597	11.448	11.523	0.075
0.03125	31.528	31.472	31.351	31.450	0.091
0.0417	32.370	32.379	32.323	32.358	0.030
0.0542	33.969	33.773	33.773	33.838	0.113
0.0833	42.284	42.256	42.116	42.218	0.090
0.125	42.443	42.593	42.359	42.465	0.118
0.25	53.030	52.984	52.815	52.943	0.113
1	62.738	62.654	62.673	62.689	0.044
2	79.424	79.106	79.396	79.309	0.176
3	84.933	84.727	84.877	84.845	0.106
4	88.496	83.866	83.642	85.335	2.740
5	93.369	93.201	92.976	93.182	0.197
6	83.707	83.866	83.642	83.739	0.115
7	81.659	80.836	81.098	81.198	0.420
14	42.508	42.181	43.771	42.820	0.840

Table 5C Cumulative of pure gibberellic acid in nutrient solution corrected with the degradation factor

Time (days)	Amount	Amount	Amount	AVG	SD
0	0.000	0.000	0.000	0.000	0.000
0.00972	23.388	23.463	23.481	23.444	0.049
0.02083	29.607	29.626	29.663	29.632	0.028
0.03125	31.226	31.152	31.448	31.275	0.154
0.0417	32.826	32.992	32.844	32.887	0.091
0.0542	40.967	40.912	40.893	40.924	0.039
0.0833	55.852	55.611	56.222	55.895	0.308
0.125	63.451	63.395	63.506	63.451	0.056
0.25	71.881	72.067	72.067	72.005	0.107
1	105.842	105.456	105.799	105.699	0.212
2	88.149	87.927	88.464	88.180	0.270
3	87.814	87.759	87.666	87.746	0.075
4	82.134	82.267	82.371	82.257	0.119
5	82.175	82.160	81.923	82.086	0.141
6	83.021	82.799	82.844	82.888	0.118
7	84.427	84.323	84.397	84.383	0.053
14	85.897	85.894	85.890	85.894	0.004

Table 6C Cumulative of 0.8 % GA_3-HB-Starch in nutrient solution corrected with the degradation factor

Time (days)	Amount	Amount	Amount	AVG	SD
0	0.000	0.000	0.000	0.000	0.000
0.00972	7.020	6.871	6.899	6.930	0.079
0.02083	7.305	7.286	7.230	7.274	0.039
0.03125	13.334	13.390	13.250	13.324	0.070
0.0417	19.979	20.007	20.007	19.998	0.016
0.0542	26.815	26.750	26.862	26.809	0.056
0.0833	36.733	36.780	36.696	36.736	0.042
0.125	43.645	43.374	43.533	43.518	0.136
0.25	51.089	51.220	51.164	51.158	0.066
1	67.572	67.749	67.367	67.563	0.192
2	87.739	87.627	87.711	87.692	0.058
3	90.493	90.287	90.502	90.428	0.121
4	91.952	91.830	91.662	91.815	0.145
5	92.977	92.995	92.500	92.824	0.280
6	93.146	93.025	93.726	93.966	0.216
7	94.657	94.545	94.329	94.844	0.424
14	95.090	95.607	95.286	95.328	1.494

Table 7C Cumulative of 1 % GA_3-HB-Starch in nutrient solution corrected with the degradation factor

Time (days)	Amount	Amount	Amount	AVG	SD
0	0.000	0.000	0.000	0.000	0.000
0.00972	7.425	7.368	7.107	7.300	0.170
0.02083	11.760	11.834	11.685	11.760	0.075
0.03125	31.884	31.827	31.706	31.806	0.091
0.0417	32.844	32.853	32.797	32.831	0.030
0.0542	34.585	34.388	34.388	34.454	0.113
0.0833	43.228	43.200	43.060	43.163	0.090
0.125	43.857	44.006	43.772	43.878	0.118
0.25	55.838	55.791	55.623	55.750	0.113
1	73.504	73.420	73.438	73.454	0.044
2	90.796	90.478	90.767	90.680	0.176
3	93.877	93.671	93.820	93.789	0.106
4	95.089	95.460	95.235	95.928	2.740
5	97.788	97.620	97.395	97.601	0.197
6	99.218	99.377	99.152	99.249	0.115
7	100.000	100.000	100.000	100.000	0.000
14	100.000	100.000	100.000	100.000	0.000

APPENDIX D

Growth of bunching onion

Table 1D Length of plant with the GA_3 treatment of all concentrations

Time (days)	Control (0 ppm)	0.1 (ppm)	0.5 (ppm)	1 (ppm)	5 (ppm)
0	0.00	0.00	0.00	0.00	0.00
1	0.00	0.00	0.00	0.00	0.00
2	0.14	0.08	0.24	0.34	0.18
3	0.19	0.18	0.50	0.64	0.32
4	0.25	0.26	0.94	1.08	0.48
5	0.32	0.38	1.40	1.36	0.66
6	0.39	0.46	1.90	1.80	0.86
7	0.44	0.58	2.44	2.22	1.06
8	0.54	0.70	3.08	2.74	1.34
9	0.60	0.80	3.82	3.26	1.58
10	0.66	0.94	4.44	3.86	1.86
11	0.78	1.04	5.12	4.28	2.16
12	0.86	1.16	5.80	4.84	2.48
13	0.96	1.30	6.48	5.30	2.82
14	1.14	1.48	6.98	5.86	3.08

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Table 2D Length of plant with effect of GA_3-HB-Starch at 0.5 ppm

Time (days)	Ground	Control (0 ppm)	Pure GA_3 (0.5 ppm)	1%GA_3-HB-Starch (0.5 ppm)
0	0.00	0.00	0.00	0.00
1	0.00	0.00	0.00	0.00
2	0.14	0.12	0.14	0.18
3	0.28	0.24	0.28	0.36
4	0.46	0.34	0.42	0.58
5	0.65	0.45	0.58	0.84
6	0.86	0.53	0.76	1.14
7	1.08	0.64	0.95	1.54
8	1.28	0.77	1.24	1.90
9	1.46	0.95	1.58	2.50
10	1.68	1.07	2.02	3.08
11	1.86	1.18	2.52	3.62
12	2.02	1.28	3.00	4.26
13	2.18	1.37	3.47	4.82
14	2.28	1.47	3.80	5.21

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Conference attendance

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