การเตรียมไมโครแคปซูลไคทินโดยใช้แม่แบบพิกเคอริงอิมัลชัน

นายนิโรจน์ รัตน์เถลิงศักดิ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาวิทยาศาสตร์พอลิเมอร์ประยุกต์และเทคโนโลยีสิ่งทอ ภาควิชาวัสดุศาสตร์
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2555

PREPARATION OF CHITIN MICROCAPSULE BY PICKERING EMULSION TEMPLATE

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Applied Polymer Science and Textile Technology
Department of Materials Science
Faculty of Science
Chulalongkorn University
Academic Year 2012

Thesis Title	PREPARATION OF CHITIN MICROCAPSULE BY PICKERING EMULSION TEMPLATE
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Field of Study	Applied Polymer Science and Textile Technology
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อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ. ดร. วันเพ็ญ เตชะบุญเกียรติ, 86 หน้า.

ไมโครแคปซูลเป็นที่รู้จักและถูกใช้งานมาเป็นเวลานานเนื่องจากประโยชน์นานับประการ โดยส่วนมากไมโครแคปซูลถูกใช้เพื่อกักเก็บสารตั้งต้นหรือวัตถุดิบเพื่อป้องกันไม่ให้เสื่อมสภาพ จุดประสงค์ของงานวิจัยนี้เพื่อศึกษาความเป็นไปได้ในการขึ้นรูปไมโครแคปซูลโดยใช้แม่แบบพิกเคอริงอิมัลชัน อนุภาคไคทินถูกใช้เพื่อทำให้อิมัลชันเสถียรโดยทำให้กระจายตัวในเฟสน้ำ คุณสมบัติการเปียกผิวของอนุภาคไคทินได้ถูกทดสอบด้วยเครื่องทดสอบมุมสัมผัส ผลที่ได้คือ θ = 52.05±3.19 แสดงถึงไคทินมีส่วนของโครงสร้างที่ชอบน้ำมากกว่าส่วนของโครงสร้างที่ชอบน้ำมัน เฟสน้ำถูกนำไปกระทำอิมัลชันกับนอร์มัลเฮกเซนโดยเครื่องอัลตราโซนิคชนิดโพรบ ไมโครแคปซูลไคทินถูกเตรียมขึ้นอย่างสำเร็จ เมื่อความเข้มข้นของอนุภาคไคทินเพิ่มสูงขึ้น (ที่อัตราส่วนของเฟสน้ำต่อเฟสน้ำมันคงที่ที่ 8:2) พบว่าขนาดของหยดอิมัลชันเพิ่มขึ้นแต่ค่าดัชนีอิมัลชันใกล้เคียงกันในทุก ๆ ความเข้มข้น เพราะการเพิ่มขึ้นของอนุภาคไคทินทำให้มีอนุภาคมากพอในการหุ้มหยดอิมัลชันอย่างสมบูรณ์และมีการห่อหุ้มทับกันหลายชั้นที่ผิวหน้าของหยดอิมัลชัน หลังจากนั้นโครงสร้างของไมโครแคปซูลถูกศึกษาโดยกล้องจุลทรรศน์แบบส่องกราด กล้องจุลทรรศน์แรงอะตอม และกล้องจุลทรรศน์แบบส่องผ่าน ภาพระดับจุลภาคพบว่าอนุภาคไคทินมีลักษณะกลมขนาดเล็ก ๆ และมีการรวมตัวกันอย่างหนาแน่นของอนุภาคที่บริเวณผิวหน้า ซึ่งคาดว่าเป็นการรวมตัวกันของอนุภาคไคทิน ผลการทดลองแสดงให้เห็นว่าไมโครแคปซูลถูกสร้างขึ้นมาจากการรวมตัวของอนุภาคไคทิน

ภาควิชา วัสดุศาสตร์ ลายมือชื่อนิสิต.....................................

สาขาวิชา วิทยาศาสตร์พอลิเมอร์ประยุกต์และเทคโนโลยีสิ่งทอ ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.........

ปีการศึกษา 2555

#5272381523 : MAJOR APPLIED POLYMER SCIENCE AND TEXTILE TECHNOLOGY
KEYWORDS : MICROCAPSULE / CHITIN / PICKERING EMULSION / EMULSION TEMPLATED / OIL-IN-WATER EMULSION

NIROJE RATHALOENGSAK : PREPARATION OF CHITIN MICROCAPSULE BY PICKERING EMULSION TEMPLATE
ADVISOR : ASST. PROF. WANPEN TACHABOONYAKIAT, Ph.D., 86 pp.

Microcapsule has been known and used for many years due to its own several advantages. Microcapsule has been mostly used to encapsulate substrate or ingredient to protect from degradation. The aim of this research was to evaluate the possibility of the fabrication of microcapsule using Pickering emulsion template. Chitin particles had been used to stabilize the emulsion by dispersing in water phase. The wettability of chitin particles was investigated using contact angle meter. The contact angle was θ = 52.05±3.19. It showed that chitin particle contains with the hydrophilic part more than the hydrophobic part. The aqueous phase was emulsified with n-hexane using probe type ultrasonic. The stable chitin microcapsule using pickering emulsion templated fabrication was successfully prepared. When the chitin concentration was increased (at the constant water-to-oil ratio 8:2), the emulsion droplets size increased but the emulsion index was almost the same in every concentration because the increasing of the chitin particles gave enough particles for complete coverage and formed the multilayer at the interface of the emulsion droplets. After that, The microcapsule structure was observed using scanning electron microscopy, atomic force microscopy and transmission electron microscopy. The micrographs showed that chitin particles have the round shape like granule. There was the densely packed of the particles at the interface that supposed to be the packing of chitin particles. The results showed that the microcapsule was formed by the assembly of chitin particles.

Department : Materials Science
Field of Study : Applied Polymer Science and Textile Technology
Academic Year : 2012

Student's Signature………………...
Advisor's Signature………………...

ACKNOWLEDGEMENTS

I feel much delighted to being offered a completed thesis and sincerely appreciate the contributions of every people who have helped shaping it.

Firstly, I would like to thank my advisor, Assistant Professor Dr. Wanpen Tachaboonyakiat who has tirelessly guided and supported me every steps of the way in my research, and who was never short on valuable advice and direction when it was needed most. I gratefully thank to my advisor for her accompanying with me in every single testing, the supporting for any data and every discussion and suggestion.

On particular aspects of the thesis committee, I have had the advantage of insight from experts whose specialist advices were much appreciated: Assistant Professor Dr. Usa Sangwatanaroj, Associate Professor Onusa Saravari, Assistant Professor Dr. Rangrong Yoksan.

Also, I would like to express my sincere thanks to the Department of Materials Science, Faculty of Science, Chulalongkorn University for providing me with a good environment and facilities to complete my research.

I gratefully expressed my sincere thank to Prof. Dr. Suwabun Chirachanchai, the Petroleum and Petrochemical College (PPC),Chulalongkorn University for facilitating on places and valuable instruments such as dynamic light scattering and transmission electron microscope testing.

I would also like to thank the other biochemical polymer materials group members for their constant encouragement and valuable suggestions.

Without helps of these particular persons that mentioned above, this thesis would not been success.

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CHAPTER I

Introduction

1.1 Problem and introduction

Microcapsule has the great potential interesting due to many advantages. In this pass few years, microcapsule has been used in many fields such as agriculture, foods and beverages, pharmaceutical, medical [1]. Microcapsule is very useful to protect the substrate from degradation. In the agriculture field, microcapsule has been used to encapsulate the fertilizer or nutrients for plants [2]. For foods and beverages industry, microcapsule has been used to capture the ingredients, tastes or smells [3]. Microcapsule has been used in pharmaceutical and medical as a drug carrier to encapsulate and carry protein, vitamin or the therapeutic and then release them in the body to cure the infection [4]. There are many ways to fabricate microcapsule such as emulsion polymerization [5], controlled gelation at the surface of droplets [6], electrostatic deposition [7] and polymer precipitation [8]. The emulsion templated is one of the most interesting. The interface between oil phase and water phase will be formed as a hard solid shell. Unfortunately, emulsion is thermodynamically unstable system [9] because emulsion consists of two liquid phases that immiscible. Each liquid phase always moves and trends to separate from each other due to the different of the density. The instability is the main problem that should be concerned. The simple way to stabilize the emulsion system is to use surfactant, also referred as emulsifier during emulsification.

Emulsifier is divided into two types, small molecule and macro molecule. Each type has its own advantages. The macromolecule provides the long-term stability emulsion very well but poorly to generates the small emulsion droplets. On the other hand, the small molecule emulsifier has the high efficiency to generate the small emulsion droplets but not so good to give the long-time stability emulsions and they were reported about the toxic and danger for the human cells [10]. To be used in pharmaceutical and medical industry, the products must be safe for everybody. In 1907, the new kind of surfactant was found by S.U. Pickering [11]. The emulsion that stabilized by solid particles was so called Pickering emulsion. It was referred as surfactant free emulsion. Pickering emulsion had the outstanding properties. It can provide the wide range of emulsion droplets size, from nanometer to micrometer depending on the size of solid particles used to emulsify the emulsion [12]. The obtained emulsion has high stability and long-term stability [13], causing by the strong absorption of the solid particles at the interface between liquid phases. Therefore, Pickering emulsion needs high energy to remove the particles from the interfaces between two liquid phases.

In this research, chitin particles were used to stabilize oil-in-water emulsion. Chitin is the second most numerous natural polysaccharides after cellulose. Chitin was chosen according to the unique characteristics. Chitin is biocompatible, biodegradable and non-toxic for living things. So chitin is not irritated to human cells. However, chitin is insoluble in water and usual solvents. This is the problem for using chitin. To be used in this research, chitin was prepared as small particles dispersed in water and used as the water phase to emulsify with n-hexane as an oil phase. The effect of the varying of chitin particles concentration on the emulsification was investigated. The obtained emulsion was characterized to ensure the emulsion formation and to observe the microscopic structure in details. The emulsion droplets size was measured by dynamic light scattering technique. After the emulsion droplets size measurement, the emulsions were dried in the air to evaporate n-hexane before the microscopic structural observation. Scanning electron microscopy, atomic force microscopy and transmission electron microscopy were used to visualize the emulsion droplets structure. The obtain emulsions were expected to keep their structure as microcapsule and retain their properties after drying in the air.

1.2 Objectives

1. To evaluate the possibility for the synthesis of hollow chitin microcapsule using the Pickering emulsion templated fabrication.

2. To determine the structure of the obtained microcapsule

1.3 Expected results

1. The stable hollow chitin microcapsule using pickering emulsion templated fabrication would be successfully prepared.

2. The obtained hollow chitin microcapsule would retain its own integrities that can be used to encapsulate the payloads for the future works.

1.4 Scope of work

The aim of this research is to fabricate the hollow chitin microcapsule via pickering emulsion templated by using chitin as the solid particle emulsifier. The microscopic observation of the obtained hollow chitin microcapsule was investigated. Since chitin is insoluble in water, chitin particles were prepared and dispersed in water. The chitin particle suspension was used as emulsifier in water phase and emulsify with n-hexane as an oil phase. The mixtures were homogenized to form emulsion using the ultrasonic technique. After the emulsification, the particle size of the as-prepared emulsion was determined. Then, the obtained emulsions were dried in the air to evaporate n-hexane inside the core of the emulsions according to the fact that emulsions were used as the template to fabricate the microcapsule. The microscopic structures of the dried emulsions were characterized by scanning electron microscopy (SEM), atomic force microscopy (AFM) and transmission electron microscopy (TEM). The dried emulsions were expected to keep their structures as microcapsule and retain their properties after drying in the air. The properties and stability of the microcapsule will be studied in the future. The microcapsule will also be used as the drug carrier for future work.

CHAPTER II

Literature review

2.1 Emulsion

Several attentions had been paid to emulsion for long time. There are many researches about emulsion in different objectives. Recently, emulsion is widely used in many fields including agriculture [2], foods and beverages [3], cosmetics and pharmaceuticals [1] due to their unique and highly potential advantages. An emulsion is a kind of colloid mixture that contains two or more immiscible liquid phases. One liquid phase dispersed (also referred as disperse phase) in second liquid phase (also known as continuous phase). Since those two liquid phases are immiscible, so it is very simply to separate into two phases mixture. The higher density liquid phase will going downwards and the lower density liquid phase will going upwards depend on their nature. It should be noted that emulsion is thermodynamically unstable system. Preparing emulsion is required emulsifying agents to reduce the surface energy between phases and increase the stability of emulsion. However, emulsion cannot be formed without energy inputs such as shaking, acoustic emulsifying or homogenizing [14].

2.1.1 Emulsion compositions [15]

Generally, emulsion is consists with three components, oil phase, water phase and surface-active agents (often referred as emulsifier).

2.1.1.1 Water phase

Water phase of emulsion is the phase of the polar molecules such as water, solid- or liquid- substance that can be dissolved in water and other materials that can be dissolved in water such as hardeners, antioxidants and surfactants. These kinds of material can be added to the water and used as a water phase.

2.1.1.2 Oil phase

The non-polar molecules are always used as an oil phase in emulsion. Some samples of non-polar molecules such as oil, fat, wax and oil painting.

2.1.1.3 Emulsifying agents

Emulsifying agent is the important part in the emulsifying process. It will absorb at droplet surfaces to increase the surface area and reduced the surface tension energy. Type of emulsifying agent can be effect to the characteristics of emulsion including emulsion type, size of emulsion droplets and emulsion stability.

2.1.2 Emulsion type

Emulsion can be categorized by size or type.

2.1.2.1 Size classification [16]

The emulsion can be divided into 3 types depending on their size. :

1. Microemulsion

Microemulsion is an emulsion which the radius of the droplet is less than 100 nm . It is a small-scale emulsion with a single optically isotropic property.

2. Mini-emulsion

Mini-emulsion is an emulsion which the radius of the droplet is within 100 nm up to 1 μm. In the past decades, the interesting in mini-emulsion has been grown up. Mini-emulsions have the unique application in the market of latex particles.

3. Macro-emulsion

The biggest scale of emulsion is macro-emulsion. Macro-emulsion is an emulsion that have greater radius of droplet than micro- and mini-emulsion (more than 1 μm).

The size classification of emulsion is a guide only. It is not precise. The classification is normally described to be thermodynamically stable system by using Gibb’s free energy.

2.1.2.2 Type classification

Types of emulsion can be classified basically by liquid phases which are dispersed phase or continuous phase.

1. Oil-in-water emulsions (O/W emulsion): Emulsions that contain oil droplets dispersed in water continuous phase. Properties of emulsions are depended on two factors, components in the continuous phase and properties of surfactants.

2. Water-in-oil emulsions (W/O emulsion): Droplets of water are dispersed in an oil continuous phase. The stability of W/O emulsion is based on both the properties of oil or fat and the surfactants. Properties of oil or fat and surfactants are more effect on the emulsion stability than those of aqueous phase.

3. Multiple emulsions: Multiple emulsions represent the emulsion of emulsion droplets. These can be divided into 2 categories, oil-water-oil (O/W/O) emulsion and water-oil-water (W/O/W) emulsion as shown in Figure 2.1. Therefore, O/W/O emulsion consisted of O/W emulsion as dispersed phase with the outer oil as continuous phase. On the other hand, W/O/W emulsion consisted of W/O emulsion as dispersed phase with the outer water as continuous phase. These kinds of emulsion are more complicated, hard to prepare and to control their properties. For example, in the case of O/W/O emulsion, the inside O/W emulsion should be stable as much as the water droplets dispersed in the outer oil continuous phase.

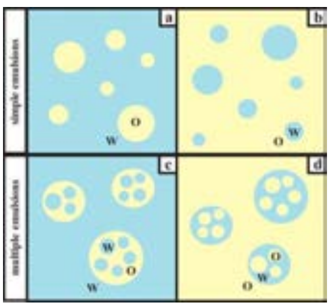

Figure 2.1 Multiple emulsions (a) oil-in-water (O/W) emulsion, (b) water-in-oil (W/O)emulsion,(c) water-in-oil-in-water (W/O/W) emulsion and (d) oil-in-water-in-oil (O/w/O) emulsion [17]

2.1.3 Emulsions stability [18]

The power or force is needed to apply to the mixture to homogenize the water phase and oil phase. Emulsion is the system that composed from two immiscible liquid phases. The liquid phases are always trend to separate from each other. The mechanisms of the breaking of emulsions are shown in Figure 2.2.

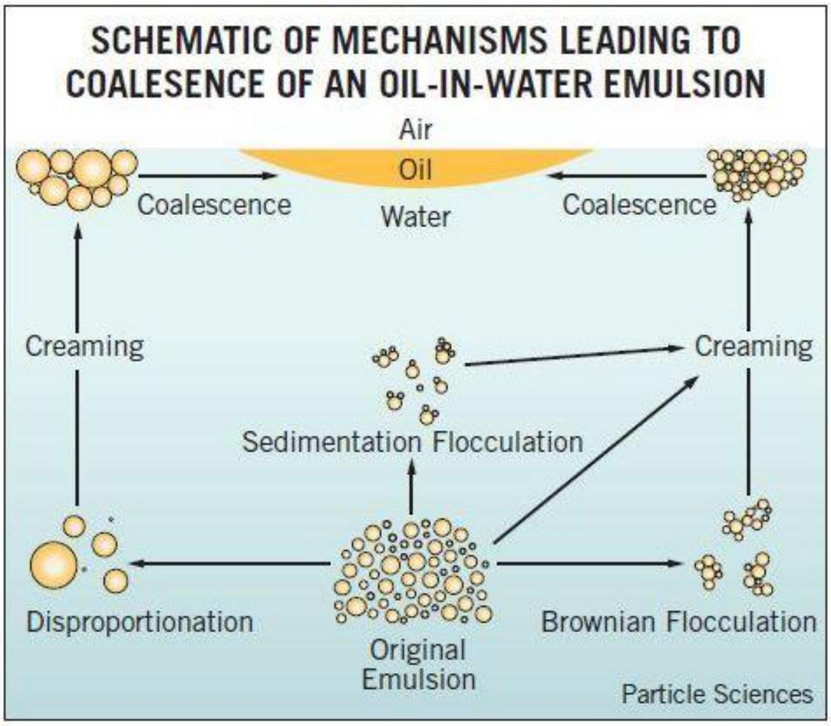

Figure 2.2 Schematic of mechanisms leading to coalescence of and oil-in-water emulsion

1. Creaming

Creaming is the de-homogenizing process. It is the initiating mechanism of coalescence. The liquid phase with the low density separated from another liquid phase and go upwards to the surface. Creaming is not the completely separated. So it is reversible mechanism. Creaming could be reversed to the emulsion by apply forces like stirring, agitating, shaking or homogenizing. The extension of the homogenizing time can prevent creaming.

2. Sedimentation

The mechanism of sedimentation is as similar as creaming process, but the liquid phase with higher density separated and aggregated. Then the aggregated particles move downwards to the bottom of the emulsion. Forces can be used to prevent sedimentation like the prevention of creaming as well.

3. Flocculation

Flocculation is the aggregation of the droplets without coalescence. All droplets still maintain their own boundary and remain separate from each other. Flocculation happened because the attraction between droplets and they moves upwards or downwards together. Flocculation is divided into two categories, Brownian flocculation and sedimentation flocculation. For Brownian flocculation, the flocculated droplets move randomly according to random Brownian movement. Sedimentation flocculation is the result of the vertical movement. It is assumed that the flocculated droplets move vertically all the path.

4. Disproportionation

Disproportionation is often referred to as Ostward ripening. The dispersed droplets diffused from the small molecules to the larger molecules through the continuous phase.

5. Coalescence

Coalescence is the result of phase separation of the dispersed phase from the continuous phase. The dispersed droplets diffused and merged together. Coalescence is the completely separated process and irreversible. The emulsion unstability will be observed.

2.1.4 Emulsifying agents

Emulsifying agents are necessary in emulsifying procedure to prevent phase separation in emulsion. A classification was made into three classes.

1. Surface-active materials (Surfactants)

The surface-active materials (always referred as surfactant) are the molecule that contains hydrophilic part and hydrophobic part as shown in Figure 2.3. Surface-active materials consist of hydrophilic heads which can be soluble in water or polar solvents and hydrophobic tails (long chain hydrocarbon) which can be soluble in non-polar solvents or organic solvents. Surface-active materials are used to prevent the phase separation or droplets coalescence.

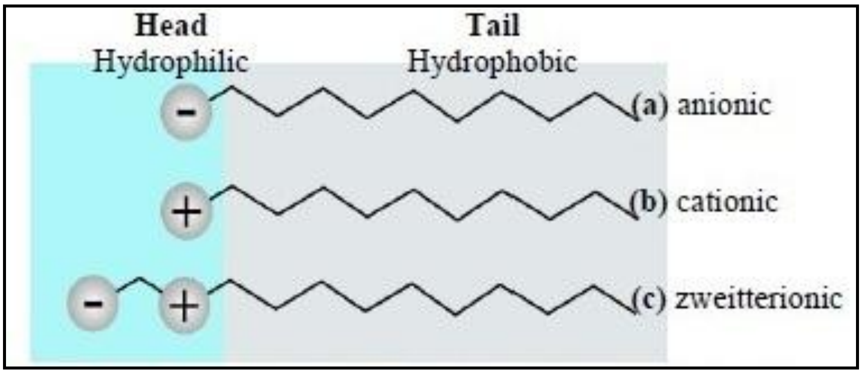

Figure 2.3 Surfactant structures (a) anionic surfactant (containing negatively charged head), (b) cationic (containing positively charged head) and (c) zweitterionic (containing two oppositely charged head). [19]

2. Naturally occurring emulsifiers

The naturally occurring emulsifers were divided into 2 categories, protein-based emulsifiers (sodium caseinate, lysozome, apomyoglobin, lactoglobulin, whey protein) and polysaccharide based emulsifiers (dextran, β-cyclodextran, carboxylmethyl cellulose, chitosan)

3. Fine solid particles

The fine solid particles stabilized emulsion is a new category of emulsion that referred as pickering emulsion. The emulsion was stabilized by the absorption of the solid particles at the interface between the liquid phases. Then the densely pack of solid particles layer is formed. The layer of particles may prevent the emulsion droplets from the phase separation such as flocculation or coalescence by steric mechanism [20, 21]. The strong adsorption at the oil-water interface result as the high stability of the emulsion. [13, 17-18]

2.1.5 Emulsion stabilized by solid particles (Pickering emulsion)

Numerous studies had been investigated about the fine solid stabilized emulsion. Small solid particles can effectively emulsify the emulsion similar to emulsifiers. Solid particles can attach to the interface between oil-water (usually attach at the oil phase first) to form the closely packed layer. In these recent years, a variety of solid particles were used to stabilize emulsion such as clays [22], hydrophobic silica particles [23-25], latexes [24, 26] and carbon nanotubes [27].

2.1.5.1. Mechanism of stabilization [28]

The usual used solid particles must be amphipathic particles. The amphipathic particle is the particle that wetted by oil phase and aqueous phase or using Janus particles (Janus particle is the objects which consist of two parts with different properties or compositions) [29]. The previous works showed that type of the obtained emulsion is depended on the wetting of the solid particles. The type could be determined using Young's equation.

$$\gamma_{wo}\,\mathrm{Cos}\theta = (\gamma_{so} - \gamma_{sw})$$

γ_{wo} : The contact angle between water phase and oil phase

γ_{so} : The contact angle between solid phase and oil phase

γ_{Sw} : The contact angle between solid phase and water phase

According to Young's equation, there are three situations due to the contact angle of the droplets.

1. $\gamma_{so} > \gamma_{wo} + \gamma_{sw}$, The solid particles remaining suspended in the aqueous phase.

2. $\gamma_{sw} > \gamma_{wo} + \gamma_{so}$, The solid particles remaining suspended in the oil phase.

3. $\gamma_{wo} > \gamma_{sw} + \gamma_{so}$, The solid particles will densely at the interface.

The contact angle of the solid particles effectively to the type of emulsion formed. If $\gamma_{so} < \gamma_{sw}$, Cos$\theta$ is negative and $\theta > 90°$, solid particles will put their own larger part in oil phase. That means solid particle is hydrophobic particles. So this category of solid particle is suit to stabilize water-in-oil emulsion (W/O emulsion). On the other hands, If $\gamma_{sw} < \gamma_{so}$, Cos$\theta$ is positive and $\theta < 90°$, this showed that solid particles is hydrophilic particles. The solid particles will lay their own larger part in aqueous phase. The hydrophilic particle is suit to stabilize the oil-in-water emulsion (O/W emulsion). In the case of $\theta = 90°$, solid particles are wetted by oil phase as good as wetted by aqueous phase. Importantly, this category of emulsion has highly stability caused by solid particle is wetted equally by both phases. The distributions of solid particle at the interface between oil and water are shown in Figure 2.4.

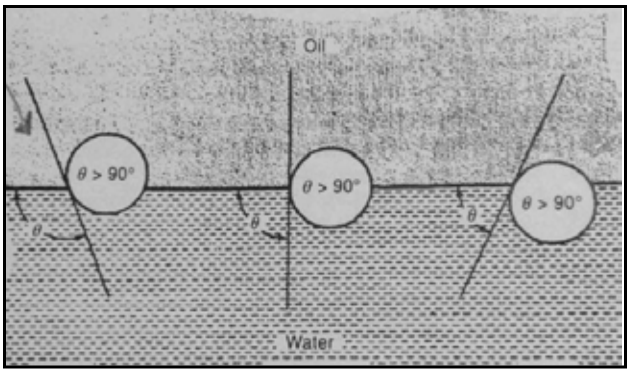

Figure 2.4 Distributions of solid particle at the interface between oil and water, (left) the solid particle is wetted by oil better than aqueous, (right) the solid particle is wetted by water better than oil, (middle) solid particle is wetted equally both in oil and water.

2.1.5.2 Basic factors affecting the stability of emulsion [30]

There are many factors that made pickering emulsion outstanding from the conventional emulsion. The following conditions are the guide to obtain the stable emulsions.

1. Since type of the obtain emulsion can be controlled by solid particles used. So the particles should be selectively wetted by one of the phases.

2. The particle concentration have affected to the packing at the interfaces between liquid phases. The higher concentration should be sufficiently assembled densier.

3. Pickering emulsion can be the nanometer to several micrometer depending on the size of the solid particles used. The size of the solid particles supposed to be smaller than the emulsion droplets size.

4. The solid particles should be the high monodisperse. It should be coagulated to some extent.

2.2 Chitin [31]

One of the most influence natural polysaccharide recently is chitin due to the many of the advantages. Chitin is the second most abundant naturally occurring polysaccharide after cellulose. Chitin could be found in nature in the fungi or yeast as the cell walls or the other living organisms. It also could be found as the structural components in arthropods. Figure 2.5 shows the chemical structure of chitin comparing to cellulose.

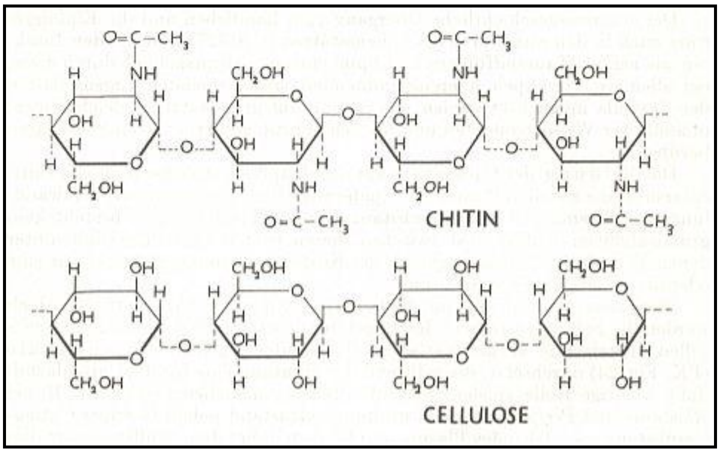

Figure 2.5 Chemical structure of chitin and cellulose [32]

Chitin or poly[ß-(1-4)-N-acetyl-D-glucosamine] is growing interests and has been using in many fields. The main sources of chitin in the industrial are shrimp or crab shells. Chitin is extracted using acid treatment and alkaline extraction respectively. From Figure 2.5, the structure of chitin show that chitin is non-polar. So chitin is insoluble in water and all the usual solvents. The insoluble of chitin is the major drawback for using chitin. In the past few years, the scientists have studied and modified chitin to many derivatives. The well known chitin derivative is chitosan which can be dissolved in diluted acids.

2.2.1 Type of chitin

Chitin occurs as three allomorphs, α-, β- and γ- form. The γ-chitin seems to be just a variant of α-chitin. The α-chitin is the most abundant chitin so far. It could be found in fungi, yeast, shrimp or crab shells, tendons and the insect cuticle. The α-chitin could be synthesized in many ways such as using the *in vitro* biosynthesis or recrystallization.

The chitin β-form was found in squid pens, seaweeds or worms. The β-chitin is more reactive than the α-chitin. But the β-chitin could not be synthesized like the α-chitin. So the α-chitin is the most abundant nowadays.

2.2.2. Chitin preparation

According to the main sources of chitin in the industrial is the waste from sea food industry. Both type of chitin (α-chitin and β-chitin) occur in nature with inorganic salts such as calcium carbonate and phosphate, protein and lipids. Chitin occurs as ordered crystalline as the microfibrils in the matrix of protein. Before using chitin, we need to extract from those sea food waste. There are 3 steps to extract chitin as shown in Figure 2.6. The main process is acid treatment and alkaline extraction. The addition process is decolorization. This process was usually added to remove the residue pigments and to obtain the colorless chitin.

1. Demineralization

Calcium carbonate and other inorganic salts were eliminated by acid treatment. Diluted hydrochloric acid (HCl) was used to dissolve the inorganic salts from the sea food wastes. For the industrial process, diluted sulfurous acid (H_2SO_3) was used due to H_2SO_3 is a weak acid that wouldn't damage the structure of chitin. So chitin would not degrade in H_2SO_3.

2. Deproteinization

To eliminate protein from the raw materials, diluted alkaline solution was used. The higher surface area makes the rate of reaction increase. So chitin is usually grinded to reduce the particles size. On the other hands, enzymatic extraction can be used to eliminate protein as well. The enzymatic extraction will also reduce the viscosity of chitin.

3. Decolorization

The decolorization is the optional process to get the colorless chitin and remove the remaining pigments or lipids from the raw materials. The decolorization can be done during demineralization or deproteinization steps.

2.2.3 Applications of chitin

Chitin is inert, biodegradable, biocompatible and non-toxic but it is insoluble in water and common solvents. However, chitin is widely used in many ways such as;

1. In chromatography, chitin is used as the stationary phase for isolation of lectins testing [33]

2. Chitin is used to immobilize the whole cells or enzyme that very useful in foods industry. [34]

3. Chitin-based materials can be used for industrial pollutants treatment.

4. Chitin can be processed in many ways such as films and fibers [35]. Chitin fibers can be blend with other fibers such as silk [36] and cellulose [37]

5. For the pharmaceutical and medical, chitin can be generated in the form of wound-dressing [38] or used as controlled drug release carriers [39, 40].

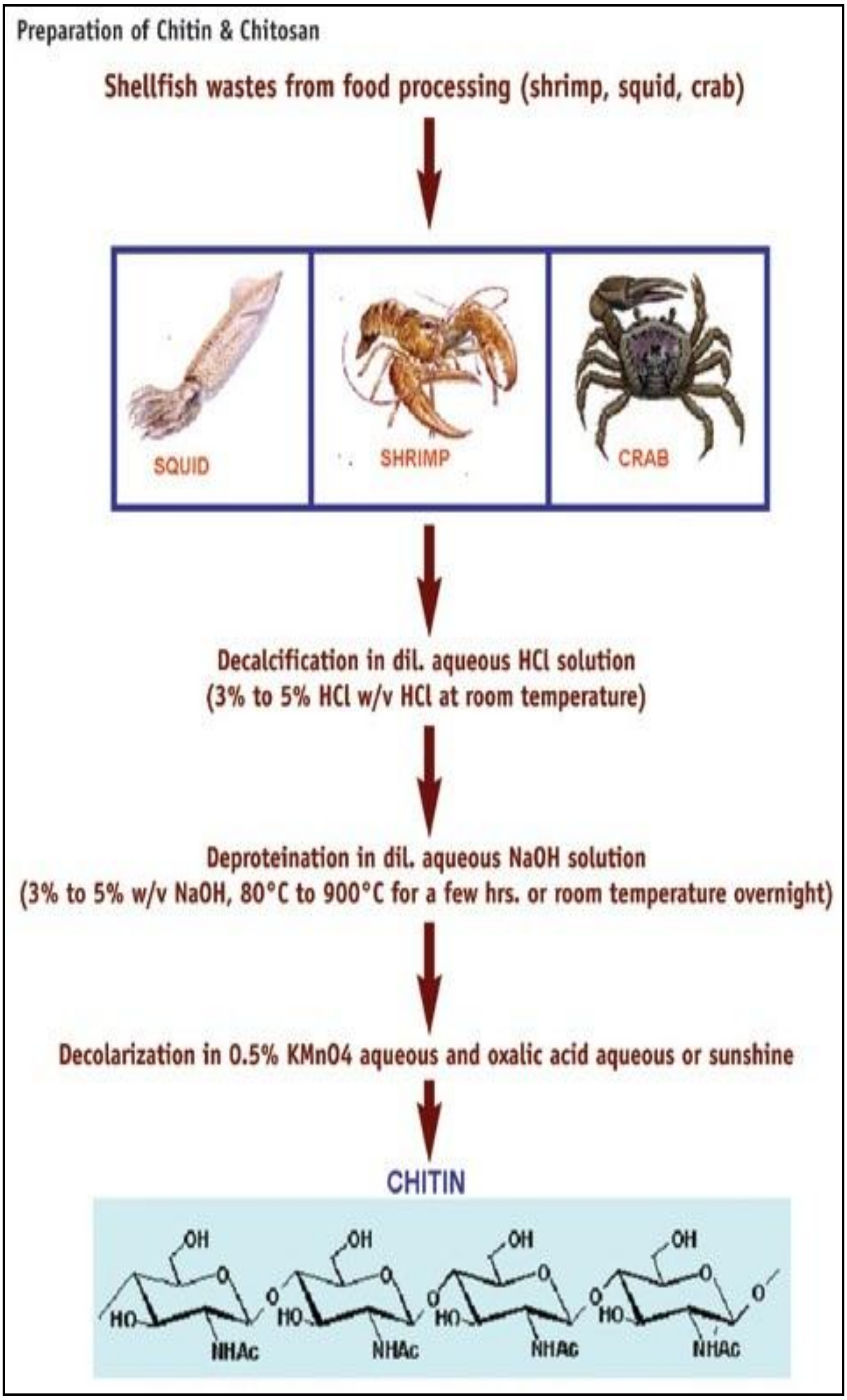

Figure 2.6 Schematic of chitin extraction [41]

2.3 Related works

Pichot [42] and his co-workers studied about the stability of O/W emulsions using surfactants and colloidal particles in 2010. Two categories of surfactants were chosen, the naturally stabilize oil-in-water emulsion (Tween 60 and Sodium Caseinate) and the surfactants that have the ability to stabilize water-to-oil emulsion (lecithin). Also, they studied the mix emulsifiers by added silica particles. The results showed that the oil-in-water emulsions were stabilized by any of three emulsifiers but depend on the type of emulsifier and emulsifier concentration. The increasing in emulsifier concentration caused the smaller emulsion droplets and the longer stability. For the mix emulsifiers, the emulsion droplets were smaller than using only emulsifier or particle. The increasing of O/W emulsifier made the emulsion droplets bigger because the higher fraction of O/W emulsifier caused particles desorption from the interfacial. In contrast, when W/O emulsifier increased, there was no particles desorption observed.

In 2000, Binks and Lumsdon [43] investigated about the influence of the wettability of silica particles on the stability and type of resulting emulsion. The particles were studied at rang from hydrophobic to hydrophilic by varying silane groups on their surfaces. They found that the emulsion stabilized by the very high hydrophilic or highly hydrophobic particles were not stable and not resist to coalescence. But using of the partial hydrophilic and hydrophobic particles gave the emulsions that stable to coalescence. They also reported that the phase inversion was occurred by increasing aqueous phase. The higher amount of water inverted the emulsion from water-in-oil emulsion to oil-in-water emulsion.

In 2009, Zhu Ao [9] and his co-workers studied about the formation of microcapsule using emulsion-templated. The emulsion was stabilized using polystyrene latex particles (the particle surfaces were treated and coated with carboxylic group (-COO^--)). Polystyrene latex particles were put in the aqueous which is ethyl acetate saturated. The oil phase contained n-octanol oil, ethyl acetate (EA) and EA-soluble or water-insoluble polymer (poly (lactic-co-glycolic acid) (PLGA)). After emulsifying, oil-in-water emulsion formed which the oil phase consisted of PLGA. The aqueous continuous phase which saturated with EA and polystyrene latex formed the monolayer at the interfacial between both phases. After that, the large amount of pure water was poured to the water phase. This lead the saturated water phase turned into the unsaturated water phase. Since EA is immiscible with n-octanol but more miscible with water, EA would diffuse from the oil phase at the core of the droplets into the water continuous phase. Also, PLGA would undergo outward from the core of the emulsion droplets and assembled with poly styrene latex at the interfacial. The solid polymer shell formed that caused by the assembled of the PLGA and poly styrene. The microcapsule formation has succeeded. The resulting microcapsule retained the integrity even drying in the air. The important mechanism is the locking of polymer at the interfacial. The effective locking gave the robust microcapsule structure.

In 2011, Tzoumaki and her co-workers [44] investigated the chitin nanocrystals (colloidal rod-like particles) stabilized oil-in-water emulsion and the factors that may affected to the properties. They also studied about the other factors that affected to the stability of the emulsion such as temperature, ionic strength and pH. The corn oil and water was homogenized using ultra-sonic homogenizer to generate oil-in-water emulsion. They reported that chitin nanocrystals had highly effect to stabilize oil-in-water emulsion against coalescence. The stability of the emulsion increased when the concentration of chitin nanocrystal was increased. The rheological measurement showed the formation of network at the interfacial by increasing chitin nanocrystal concentration.

CHAPTER III

Experimental

3.1 Materials and chemical

3.1.1 Chitin particles

Chitin flake (DD~29 determined by Fourier Transform Infrared Spectroscopy, FT-IR) was obtained from A.N. Lab, Thailand. Chitin was ground into powder by ball-mill. In this experiment, chitin particles were prepared as solid particles and utilized to stabilize emulsion. The chemical structure of chitin was shown in Figure 3.1.

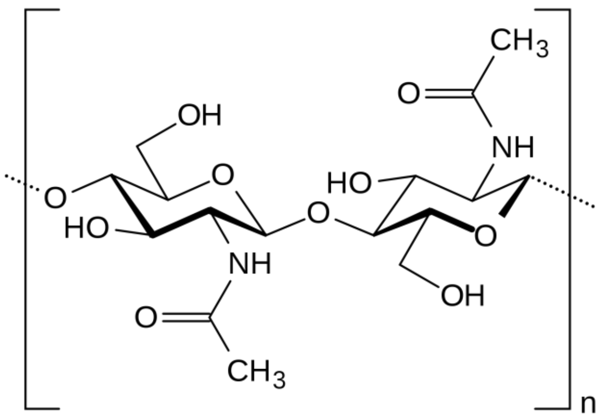

Figure 3.1 Chemical structure of chitin

3.1.2 Methanesulfonic acid, anhydrous

Methanesulfonic acid, anhydrous, R&D grade was purchased from SIGMA-ALDRICH® INC., St. Louis, USA. Due to the fact that chitin cannot be dissolved in water and oil but it can be dissolved in strong acid. So, chitin was dissolved by methanesulfonic acid, anhydrous.

3.1.3 n-Hexane

To prepare oil-in-water emulsion, n-hexane 99% AR grade was used as an oil phase. It was purchased from QREC Co,Ltd., Malaysia.

3.1.4 Distilled water

Distilled water was used in the whole experiment

3.2 Instruments

3.2.1 Emulsion preparation instruments

The instruments that used for preparing emulsion are shown in Table 3.1

Table 3.1 Emulsion preparation instruments

Instruments	Manufacturer	Model
Dialysis tube (Regenerated cellulose tubular membrane)	Membrane Filtration Products, Inc., Texas, USA	Cellu•Sep® T2 (MWCO 6000-8000)
Vortex machine (Figure 3.2)	Scientific Industries, New York, USA.	Vortex-Genie®2
Ultrasonic : bath type (Figure 3.3)	Crest ultrasonics, Malaysia	TRU-SWEEP™ (575HTAE)
Ultrasonic probe or High intensity ultrasonic processor (Figure 3.4)	Betatek INC., Toronto, USA.	VCX 130

Figure 3.2 Vortex machine

Figure 3.3 Ultrasonic : bath type

Figure 3.4 High intensity ultrasonic processor

3.2.2 Characterization instruments

The characterization instruments are shown in Table 3.2

Table 3.2 Characterization instruments

Instruments	Manufacturer	Model
Contact angle meter (Figure 3.5)	Tantec, USA	CAM-PLUS Tantec
Zetasizer (Figure 3.6)	Malvern Instrument Ltd., Worcestershire,UK	ZS nano series
Scanning Electron Microscopy (SEM) (Figure 3.7)	JEOL Ltd., Japan	JSM-6400
Atomic Force Microscope (AFM) (Figure 3.8)	Veeco Instruments Inc., New York, USA.	Nanoscope® IV
Transmission Electron Microscope (TEM) (Figure 3.9)	Hitachi, Japan	S-4800

Figure 3.5 Tantec contact angle meter

Figure 3.6 Malvern Zetasizer ZS nano series

Figure 3.7 Scanning electron microscope (JSM-6400)

Figure 3.8 Atomic force microscope

Figure 3.9 Transmission electron microscope (Hitachi H-9500)

3.3 Emulsion preparation procedure

3.3.1 Experimental flow chart

The process of this research was shown as a flow chart in Figure 3.10

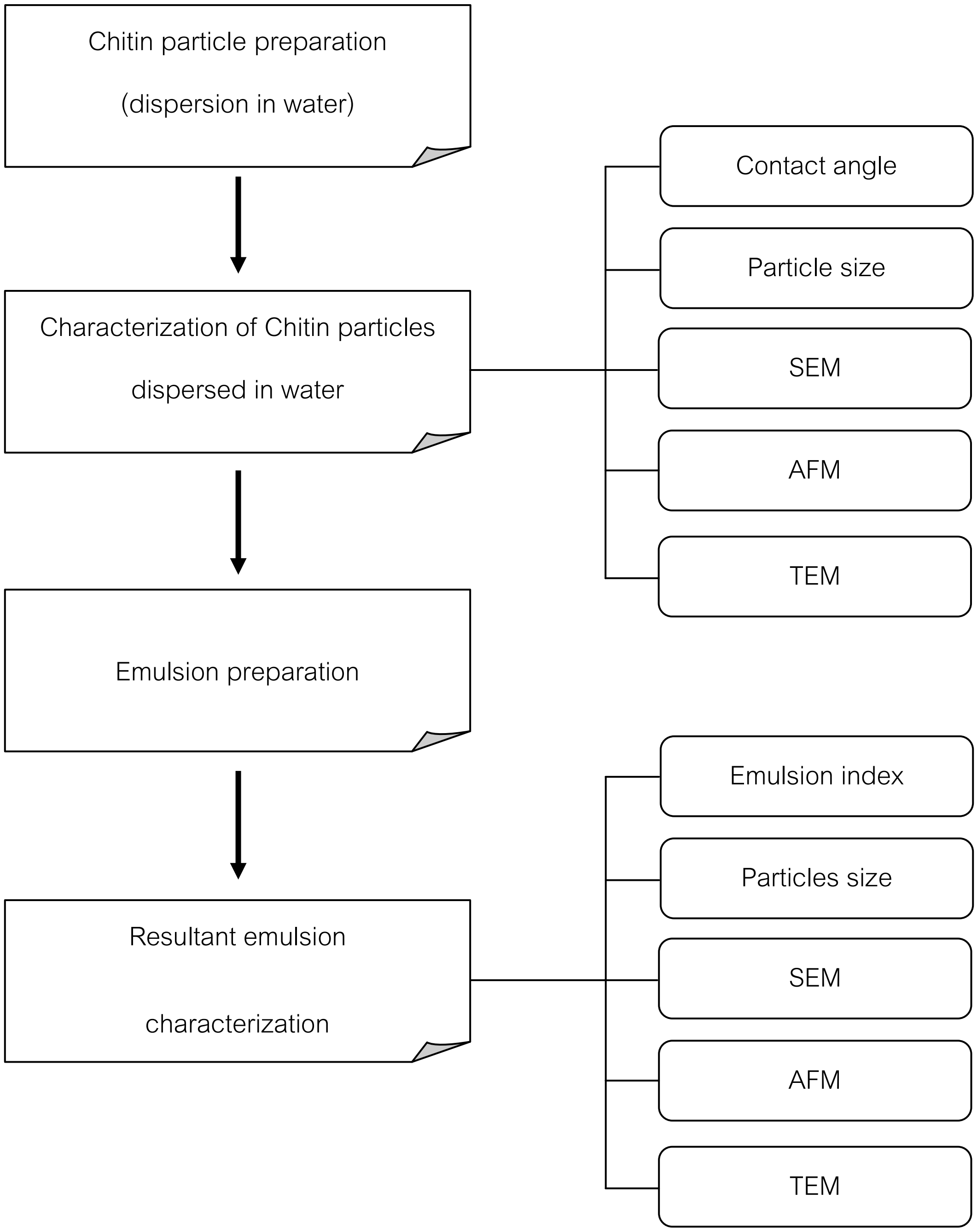

Figure 3.10 Experimental procedure

3.3.2 Chitin particles preparation

Since chitin is not able to be dissolved in either water or oil. In this research, chitin particles were prepared and utilized to stabilize the emulsion. Chitin particles dispersed in distilled water were prepared in several concentrations of 0.1% w/v, 0.5% w/v, 1% w/v, 2% w/v, 3% w/v and 4% w/v in order to study the effect of concentration of chitin particles on the stabilization of emulsion. The formulas were shown in Table 3.3. At the beginning, Chitin was dissolved in small aliquot of methanesulfonic acid, anhydrous (as shown in Table 3.3), and shaken by vortex machine until completely dissolved. Then, distilled water was gradually dropped into the mixture under sonication for 15 minutes. Then, the as-prepared chitin particles were poured into dialysis tube (nominal molecular weight cut off (MWCO) : 6,000-8,000, wall thickness : 28μm, dry cylinder diameter 15.9mm, flat width : 25mm, vol/cm 1.98ml) and dialyzed against distilled water to eliminate acid for 2 days.

Table 3.3 Chitin particles preparation formulas at various concentrations

Sample	Chitin particle Concentration (% w/v)	Chitin powder (mg)	Methanesulfonic acid (μl)	Distilled water (μl)
1	0.1	8	32	7968
2	0.5	40	160	7840
3	1	80	320	7680
4	2	160	640	7360
5	3	240	960	7040
6	4	320	1280	6720

3.3.3 Emulsion preparation

Chitin particles were used as emulsifier dispersed in water phase to emulsify with n-hexane, an oil phase. The effect of concentration of chitin particles dispersed in water (emulsifier concentrations) and the effect of oil-to-water ratios on the emulsification of the emulsion were evaluated. In order to evaluate the effect of chitin particles concentrations on the emulsification of the emulsion, oil-to-water ratio was fixed at 2:8, whilst chitin particles concentrations were varied at 0.1% w/v, 0.5% w/v, 1% w/v, 2% w/v, 3% w/v and 4% w/v On the other hand, in order to evaluate the effect of oil-to-water ratios on the emulsification of the emulsion, chitin particles dispersed in water was fixed at concentration of 3%w/v and used as emulsifier dispersed in water phase, whilst n-hexane was used as oil phase. The oil-to-water ratios were shown in Table 3.4.

Table 3.4 Chitin particles preparation formulas at various oil-to-water ratio

Oil-to-water ratio	Distilled water dispersed with chitin particles (μL)	Distilled water (μL)	n-Hexane (μL)	Total volume (μL)
8:2	2	-	8	10
6:4	2	2	6	10
4:6	2	4	4	10
2:8	2	6	2	10

The amount of distilled water dispersed with chitin particle was fixed to control the amount of chitin particles. Emulsification had been done by using ultrasonic probe for 30 minutes with pulse on 30 second and pulse off 60 second in order to avoid heat. Probe tip was set at the interface between water phase and oil phase.

3.4 Characterization

3.4.1 Contact angle

The hydrophilicity or hydrophobicity of chitin particles dispersed in water was measured by using contact angle meter. The hydrophilicity or hydrophobicity of chitin particles can be used to predict a type of emulsion that will be obtained after emulsifying by chitin particles. Chitin particles dispersed in water was dropped multilayer on a glass slide. Chitin particles dispersed in water were covered the surface of glass slide and formed chitin layer after dried in the air. Glass slide with chitin surface was placed on the sample holder. Then, small aliquot of distilled water (5 μL) was dropped on the surface of chitin layer by using needle. The contact angle was measured after dropping distilled water for 20 seconds. Contact angle was measured between the chitin layer surface and tangent to the water drop surface and measured for 20 times to calculate the average value and standard.

3.4.2 Emulsion Index

After emulsifying, the as prepared emulsion was kept in a standard glass tube (diameter 13 mm with 120 mm height) for 1 day. The height of emulsion phase and residue oil phase were measured to calculate the emulsion index from equation (1).

$$\text{Emulsion index} = \frac{\text{Emulsion Volume}}{\text{Total Volume}} \quad (1)$$

3.4.3 Particles size measurement

$$d_{32} = \frac{\sum_i n_i d_i^3}{\sum_i n_i d_i^2} \quad (2)$$

where d_{32} = mean particle size

n_i = intensity

d_i = individual particle size

The size of chitin particles dispersed in water and that of emulsion droplet was measured by using Zetasizer®. All samples were prepared by diluting with distilled water. The ratio of sample to distilled water was 1:10000. Each formula was prepared into 3 samples and measured for 3 times in each sample. The mean diameter of emulsion droplet was calculated from equation (2) and the size distribution graph was plotted.

3.4.4 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was used to observe the surfaces of chitin particles and emulsion droplets. All samples were diluted by using distilled water at the ratio of 1:10000. Then 2 µl of samples were dropped on a glass cover slip. Samples were dried in the air. Samples were fixed on a specimen stub using carbon tape and were coated with gold before the observation. The SEM micrographs were observed under vacuum at magnification of 35,000 and 15kV voltage.

3.4.5 Atomic force microscope (AFM)

Atomic force microscope (AFM) was used to observe how chitin particles rearrange themselves for forming the emulsion. The as-prepared emulsions were diluted in distilled water at the sample to distilled water ratios of 1:10000. Then, 2 µl of samples were dropped on a glass cover slip and dried in the air for 1 day. Finally, glass cover slip was broken into a small piece for the observation. AFM micrographs were observed using a tapping mode with silicon probe Al-coating at 204-497µHz (resonance high frequency).

3.4.5 Transmission electron microscopy (TEM)

Structure of chitin particles and emulsion were observed under transmission electron microscopy (TEM). Chitin particles dispersed in water and emulsion were diluted by distilled water at the ratio of 1:100. Samples were dropped on a sample grid that was covered by polymer (Formvar™ (Polyvinylformal, PVF)). Then, samples were dried under vacuum for 1 day before the observation. TEM micrographs were observed at the voltage of 100kV and various magnifications to find the most clearly images.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Chitin particle preparation

Chitin particles dispersed in distilled water were prepared in several concentrations of 1% w/v, 2% w/v, 3% w/v and 4% w/v. Chitin was dissolved in small aliquot of methanesulfonic acid, anhydrous and shaken by vortex machine until completely dissolved. Then, distilled water was gradually dropped into the mixture under sonication for 15 minutes. Then, the as-prepared chitin particles were dialyzed against distilled water to eliminate acid for 2 days. Each sample was diluted in distilled water for 10,000 times before particles size determination. Each sample was measured for 3 times. Chitin particle size was measured by zetasizer and calculated the average surface mean diameter (d_{32}). The d32 was shown in Figure 4.1.

Figure 4.1 showed that chitin particles have been found around 350 – 450 nm in diameter. The coalescence might be happened with some particles. However, the diameters were not significantly different in any concentration of chitin particles. So, the size of chitin particles was not depended on the concentration.

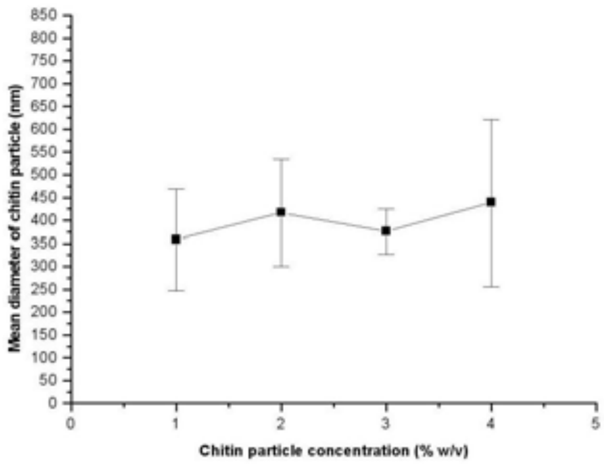

Figure 4.1 Particle size (mean±SD) of chitin with various chitin particle concentrations

4.2 Hydrophilic-hydrophobic property of chitin particles

The hydrophilic-hydrophobic property of solid chitin particles should predict the emulsion type. The hydrophilic-hydrophobic property of solid chitin particles was investigated using contact angle meter. Contact angle was measured for 20 times to find the average value. The contact angle was 52.05±3.19. Since the contact angle was less than 90 degree, indicating that chitin particles were hydrophilic. Chitin particles would lay their large part in the water phase and the small part in oil phase. Hydrophilic particles were suit to prepare oil-in-water emulsion. Therefore, chitin particles can be used to stabilize oil-in-water emulsion.

4.3 Emulsifying Efficiency of chitin particles

Emulsion was prepared by using chitin particles as emulsifier, Chitin particles were dispersed in water and used as water phase, whilst n-hexane was used as oil phase. Emulsification had been done by using ultrasonic probe for 30 minutes with pulse on 30 second and pulse off 60 second in order to avoid heat. Probe tip was set at the interface between water phase and oil phase. Emulsifying efficiency and emulsion stability were determined by measuring emulsion index and emulsion droplet size and size distribution.

4.3.1 Effect of chitin particles concentration to emulsion index

In order to evaluate the effect of chitin particles concentrations on the emulsification and stabilization of the emulsion, oil-to-water ratio was fixed at 2:8 because there was the previous work reported that the oil-to-water ratio at 2:8 was sufficiency for the preparing of stable emulsion, whilst chitin particles concentrations were varied at 0.1%w/v, 0.5%w/v, 1% w/v, 2% w/v, 3% w/v and 4% w/v. The as-prepared emulsions were kept in the standard glass tube for one day before measuring the emulsion index. Emulsion index was an indicator to evaluate the emulsifying efficiency of chitin particles. The higher the emulsion index, the higher the efficiency was.

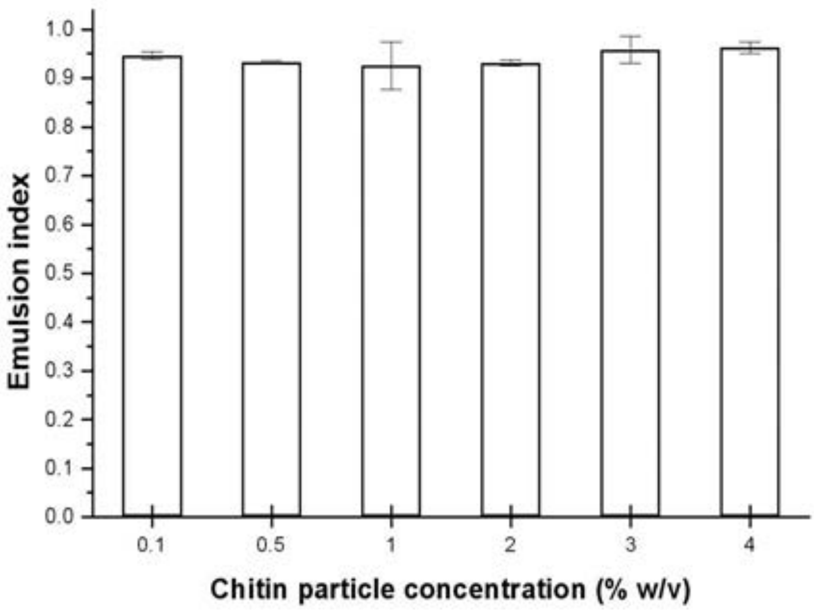

Figure 4.2 Emulsion index of the as-prepared emulsions from various chitin particle concentrations.

Figure 4.2 showed the emulsion indexes of the as-prepared emulsions from various chitin particle concentrations. It was found that all as-prepared emulsion showed almost the same emulsion indexes, indicating that the concentrations of chitin particles was not influenced to the emulsifying efficiency when fixed oil-to-water ratio at 2:8.

Figure 4.3 showed the volume fractions of the as-prepared emulsions preparing from various chitin particle concentration. This figure showed that chitin particles concentration did not significantly influence to the volume fraction of the emulsion as same as emulsion index. This indicated that chitin particle concentration from 0.1%w/v was high enough over the critical aggregation concentration (CAC) for chitin particles forming micelles. However, using chitin particle concentration of 3 %(w/v) gave the less oil residue than the emulsion that were prepared using the chitin particle concentration at 0.1 %w/v, 0.5 %w/v, 1 %w/v and 2 %w/v. This might be due to the arrangement of chitin particles in thicker layer. The results of chitin particles concentration at 3 %w/v and 4 %w/v were similar but the as-prepared emulsion at 3 %w/v exhibited the higher emulsion phase volume than using chitin particle concentration of 4 %w/v. However, the as-prepared emulsion using 0.1 %w/v and 0.5 %w/v of chitin particle concentration exhibited too small of emulsion phase volume, indicating that the chitin particle concentration at 0.1 %w/v and 0.5 %w/v were not good enough to prepare the stable emulsion because there were not enough of the chitin particle to stabilize the emulsion droplets.

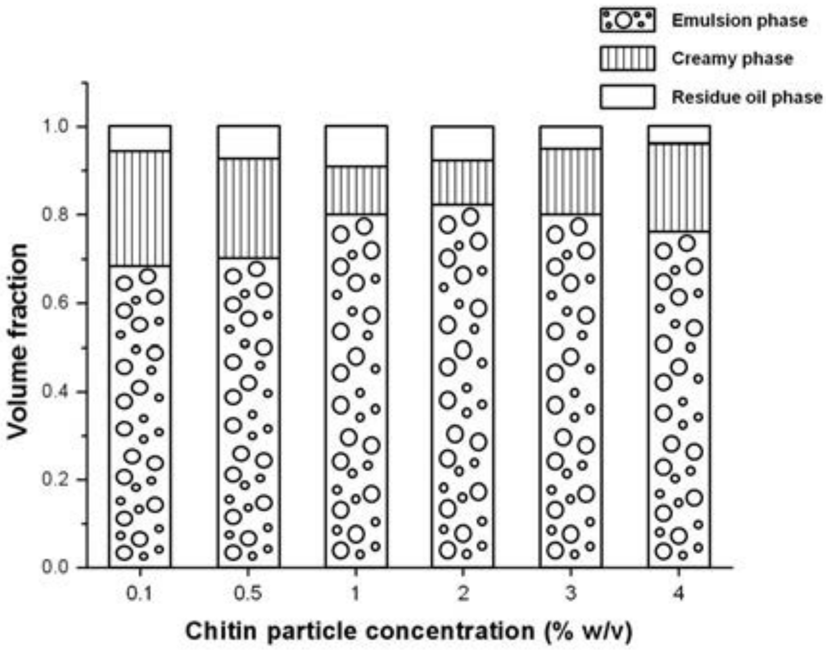

Figure 4.3 Volume fractions of the as-prepared emulsions from various chitin particle concentrations

4.3.2 Effect of oil-to-water ratio to the emulsion index

Emulsions were prepared at the oil-to-water ratio of 8:2, 6:4, 4:6 and 2:8 at the fixed chitin particle concentration of 3 %w/v to study the effect of oil-to-water ratio to the emulsion index. The results were showed in Figure 4.4.

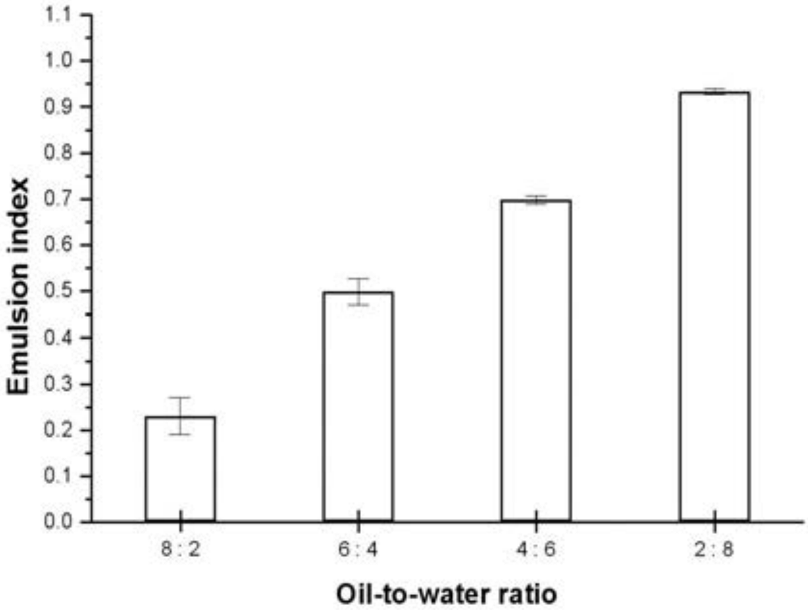

Figure 4.4 Emulsion index of the as-prepared emulsions from various oil-to-water ratio

Figure 4.4 showed that the oil-to-water ratio has the highly effect to the emulsion index. When the amount of water was increased, the emulsion phase and emulsion index increased. The as-prepared emulsions were oil in water emulsion since chitin particle got more hydrophilic than hydrophobic in their own particle. The increasing in the amount of water made the emulsion more stable that showed in the increasing of the emulsion index. The oil-to-water of 2:8 exhibited the highest emulsion index (about 0.93), indicating the most stable as-prepared emulsion. Due to the results above, the as-prepared emulsion using 3 %w/v chitin concentration and the oil-to-water ratio of 2:8 was selected for the macroscopic observation.

4.3.3 Effect of chitin particles concentration to emulsion droplet size and size distribution

The emulsion droplet size was determined by dynamic light scattering (DLS) technique, using Zetasizer© Each sample was diluted in distilled water for 10,000 times before particles size determination. Each sample was measured for 3 times.

Figure 4.5 showed that when chitin particles concentration increased, the mean diameter of droplet was increased from 420 nm to 570 nm. The emulsion droplets were formed by the assembly of chitin particles at the surface between oil phase and water phase. Chitin particles assembled by aggregation densely at the interface. The multilayer of chitin particles were formed when the amount of chitin particles increased by increasing chitin particle concentration. The aggregation of particles is the reason for the steric barrier against the droplets coalescence. Chitin particles formed a network at the interfacial between dispersed phase and continuous phase by inter-particle attraction. The excess chitin particles for the droplet covered meant the more particles to adsorb. The addition layers were formed at the interface. This may be the reason why the emulsion droplets get bigger with higher chitin particle concentrations. Unlike the classical emulsion (the emulsions that was formed and stabilized by using surfactant), the smaller emulsion droplets showed the more stability of emulsions.

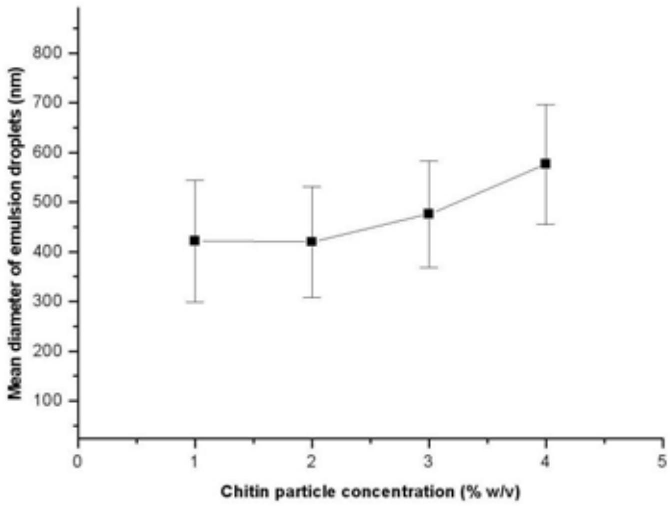

Figure 4.5 Emulsion droplet size by vary chitin particle concentrations

Figure 4.6 showed the size distribution of emulsion droplets at the chitin concentration of 3 (% w/v). The emulsion droplets size was around 396.1-531.2 nm. The peak of the size distribution graph was 458.7 nm (43.9%). The result showed that the as-prepared emulsions had the narrow size distribution and exhibited the unimodal distribution.

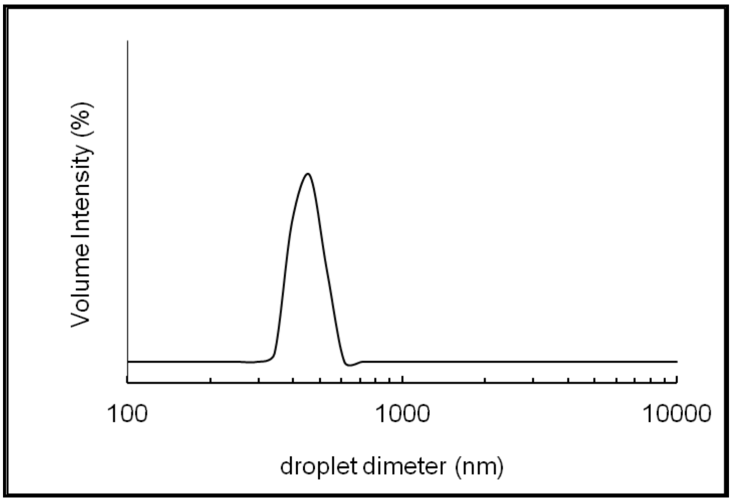

Figure 4.6 Size distributions of emulsion droplets at chitin concentration of 3 %w/v

4.4 Microstructure of chitin particles and the emulsions

4.4.1 Scanning Electron Microscopic (SEM) observation

Chitin particles and the emulsions microstructure were observed by using scanning electron microscope (SEM). Each sample was diluted by distilled water (1:10,000) before the observation. The individual particle was expected to be observed at high dilution condition. Chitin particles were observed at the magnification of 30000 X. The SEM micrograph showed that chitin particles exhibited round shape like granule (Figure 4.7).

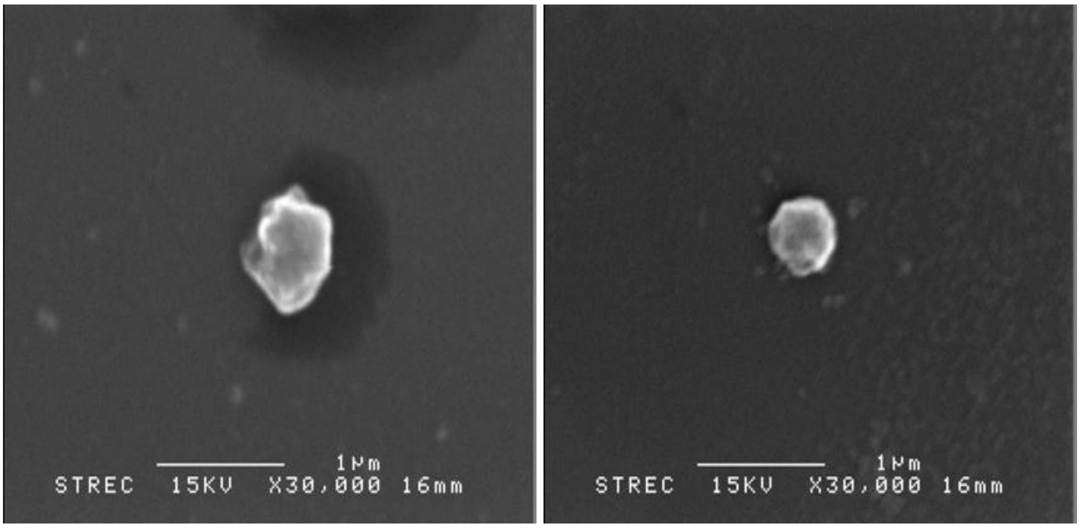

Figure 4.7 SEM micrographs of chitin particles

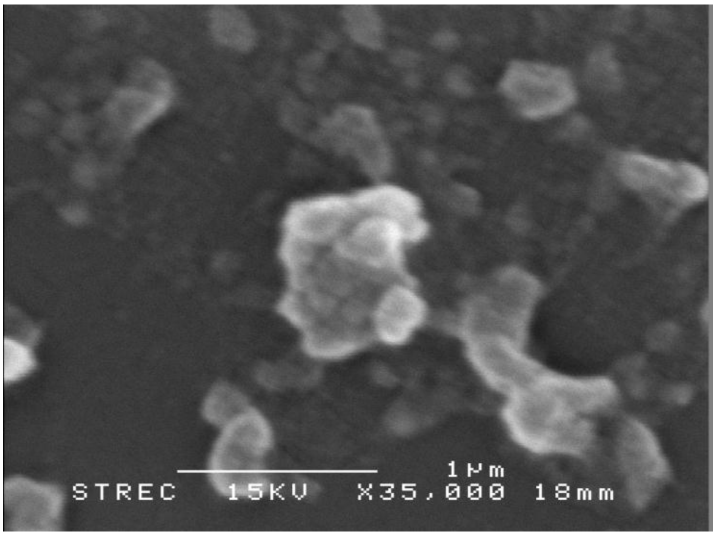

Figure 4.8 SEM micrographs of the emulsion droplets

The emulsions at constants concentration of chitin particles of 3 %(w/v) with oil-to-water ratio of 2:8 was observed under SEM at the magnification of 35000 X. The emulsion was dropped on the glass cover slip and dried in the air for 1 day before the observation to evaporate water and n-hexane. Figure 4.8 showed SEM micrograph of the as-prepared emulsion. The SEM micrograph showed that chitin particle densely pack to form emulsion that can be seen in the round shape of small particles agglomeration. Due to the very small size of as-prepared emulsion droplets, SEM may not well show the structure details of the droplets. At least the assembly of chitin particles was observed from SEM micrograph. To ensure about the result, other techniques have been used to observe the emulsion.

4.4.2 Atomic Force Microscope (AFM) observation

AFM was used to improve the results from SEM. The micrograph of chitin particles and emulsion prepared from using chitin particle concentration of 3%(w/v) at oil-to-water ratio of 2:8 was observed under AFM. The chitin particles and emulsion were diluted in distilled water at the ratio 1:100 before observation. Then, 2 μl of samples were dropped on a glass cover slip and dried in the air for 1 day. AFM micrographs were observed using a tapping mode with silicon probe Al-coating at 204-497μHz (resonance high frequency). A tip was set at 20 nm far from sample.

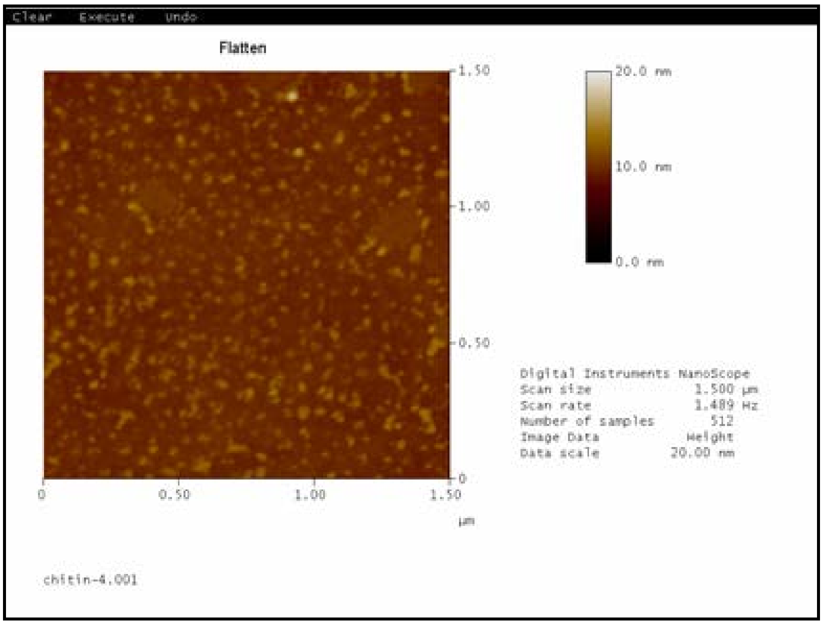

Figure 4.9 AFM micrographs of chitin particles

Figure 4.9 showed that chitin particles were not regular in shape with a diameter around 100 nm and well dispersed in water. The AFM result does not correlate with the result from particles size measurement using dynamic light scattering (DLS) technique. This may because the sample for DLS was in the solution, chitin particles were hydrophilic that might be swollen in water, whilst the sample for AFM was dried before observed, chitin particle was dried and collapse to be smaller in size.

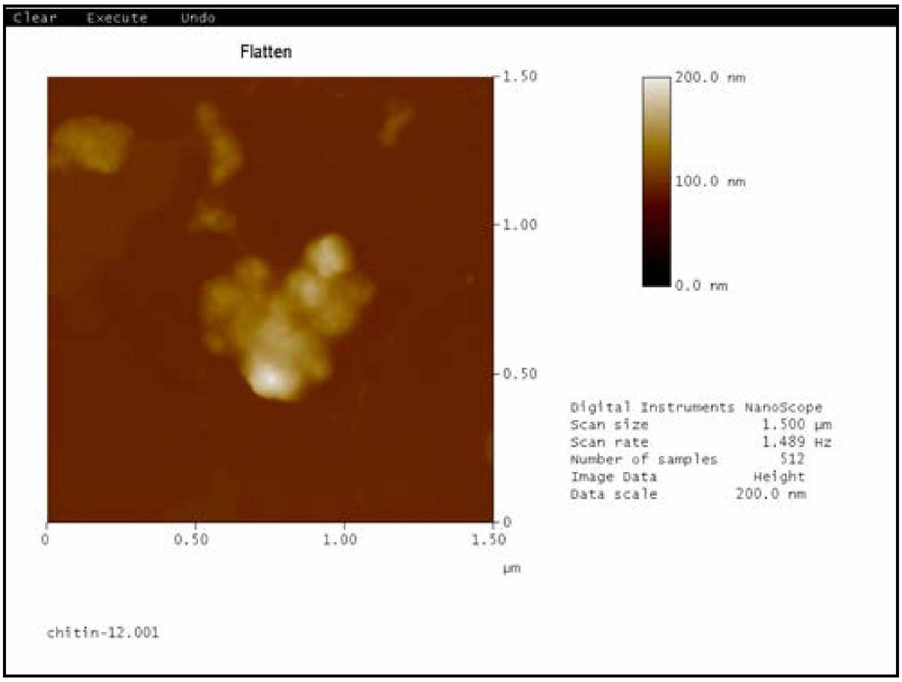

Figure 4.10 AFM micrograph of the emulsion

Figure 4.10 showed the assembly of small particles that supposed to be chitin particles. It was found that the emulsion droplet was formed by the assembly of chitin particles. The small particles of chitin assembled to form the emulsion droplet with dense packing. However, the AFM micrograph showed that the emulsion droplet might be collapsed that caused by drying samples in the air to evaporate water and n-hexane before AFM observation. This might cause the collapse in emulsion droplets and size changing to small size than the size measuring from DLS.

4.4.3 Transmission Electron Microscopy (TEM) observation

TEM was used to improve the results due to the high efficiency of this technique. It can be used to visualize a sample in a very small scale and determined the internal structure.

Chitin particles dispersed in water and the obtained emulsions were first diluted by 10000 times using distilled water. Then samples were fixed on sample grid and dried in vacuum for one day before TEM visualization.

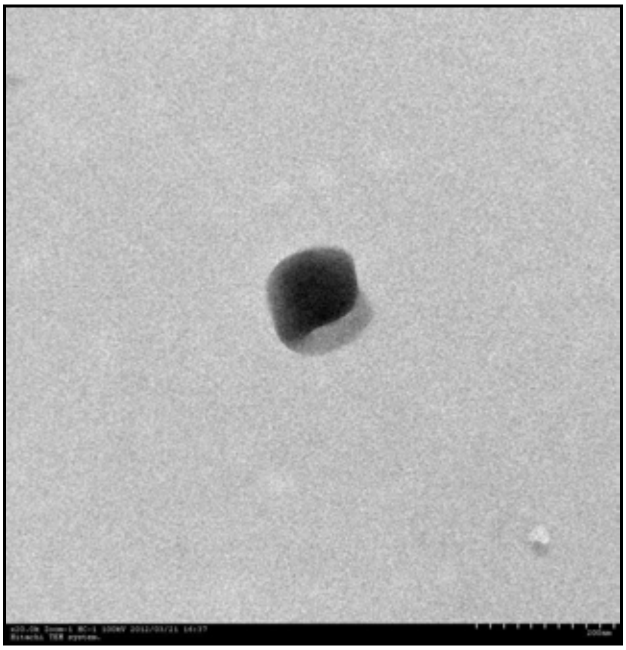

Figure 4.11 TEM micrograph of chitin particle

Figure 4.11 showed the chitin particle structure observed under transmission electron microscope. The micrograph showed that chitin has the round shape.

From figure 4.12, the resulting emulsions were observed under TEM. The micrograph showed that there were the small particles assembled closely. The emulsions were generated by the assembled of chitin particles at the interfacial.

The microscopic observation by SEM, AFM exhibited the surface details that chitin particles assembled closed pack to form small round shape emulsion droplets. Whilst, TEM gave the information throughout the emulsion droplet since the electron beam passed throughout the emulsion droplet. TEM gave clearer information that chitin particles assembled together to form a round shape emulsion droplet. However, the hollow structure detail cannot be observed among these methods. It should be noted that the sample pre-cooled in liquid nitrogen and freeze-dried could maintain the microcapsule structure and cross-sections could be made to determine the internal structure. These techniques would be recommended to determine the hollow structure detail.

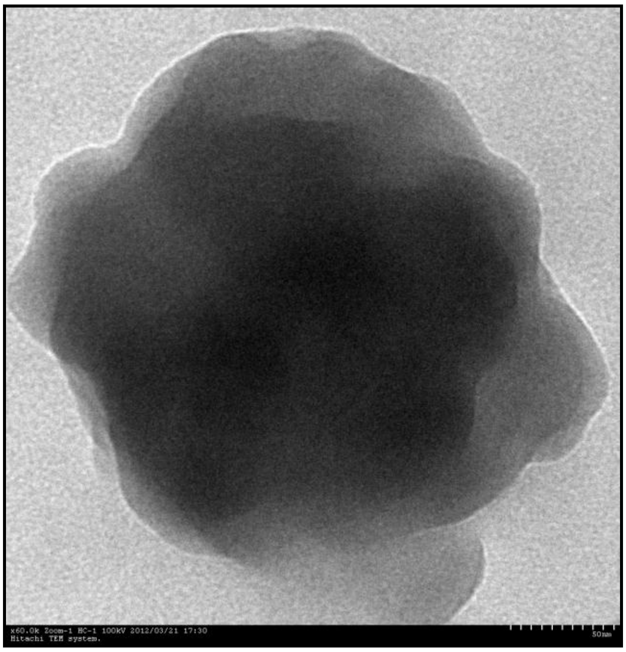

Figure 4.12 TEM micrograph of emulsion droplet at the magnification of 60 X

CHAPTER V

Conclusions

5.1 Conclusions

5.1.1 Preparation of chitin particles dispersed in water

Chitin particles dispersed in water were successful prepared by dissolving chitin particles in methanesulfonic acid then dialysis in distilled water until pH turned to neutral.

Chitin particle dispersed in distilled water exhibited the particle size (d_{32}) around 350-450 nm.

5.1.2 Chitin particles hydrophilic-hydrophobic property

The contact angle of chitin particles was 52.05 ± 3.19, indicating that chitin particles were hydrophilic which was suitable to stabilize oil-in-water emulsion.

5.1.3 Emulsifying Efficiency of chitin particles

5.1.3.1 Effect of chitin particles concentration to emulsion index

With varying concentrations of chitin particles (emulsifier concentrations), all as-prepared emulsion showed almost the same emulsion indexes, indicating that the concentrations of chitin particles was not influenced to the emulsifying efficiency when fixed oil-to-water ratio at 2:8.

The concentration of chitin particles dispersed in water at 3%(w/v) was the optimal concentration in this research due to the least residue oil phase.

5.1.3.2 Effect of oil-to-water ratio to the emulsion index

At the fixed chitin concentration of 3% w/v (2mL) , water and oil ratios were varied. The emulsion index was increased when the amount of water was increased. The oil-to-water ratio of 2:8 exhibited the highest emulsion index (about 0.93) indicating highly stable emulsion.

5.1.3.3 Effect of chitin particles concentration to emulsion droplet size and size distribution

The mean diameter of droplet was increased from 420 nm to 570 nm when chitin particles concentration increased. Unlike the classical emulsion (the emulsions that was formed and stabilized by using surfactant), the smaller emulsion droplets showed the more stability of emulsions. This may be due to chitin particles assembled densely at the interface as many layer as increasing chitin particle concentration.

The emulsion droplets size was around 396.1-531.2 nm. The highest intense was 458.7 nm (43.9%). The result showed that the as-prepared emulsions had the narrow size distribution.

5.1.4 Microstructure of chitin particles and the emulsions

The microscopic observation by SEM, AFM exhibited the surface details that chitin particles assembled closed pack to form small round shape emulsion droplets. Whilst, TEM gave the information throughout the emulsion droplet since the electron beam passed throughout the emulsion droplet. TEM gave clearer information that chitin particles assembled together to form a round shape emulsion droplet. However, the hollow structure detail cannot be observed among these methods.

5.2 Suggestions

Owing to increasing in emulsion droplet size as increasing chitin particle concentration. Therefore, chitin particles may absorb multilayer at the interface between oil and water phases. In order to investigate the absorption of chitin particle, low-temperature field emission scanning electron microscopy (LTFESEM) could be used to observe the interfacial structure of emulsion droplet.

In order to investigate the hollow structure of the emulsion, the sample should be pre-cooled in liquid nitrogen and freeze-dried because the techniques could maintain the microcapsule structure and cross-sections could be made to determine the internal structure.

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Appendices

Appendix A

Contact Angle

x_i	contact angle	x_i	contact angle
1	56	11	47
2	56	12	50
3	57	13	51
4	54	14	50
5	55	15	52
6	57	16	48
7	50	17	50
8	52	18	56
9	48	19	52
10	50	20	50

The mean average was calculated using the equation below

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

$\bar{X}$: The mean average

x_i : data

n : number of data

Emulsion index

The emulsion index by varying chitin particle concentration (constant oil-to-water ratio at 2:8)

Chitin concentration (%w/v)	1	2	3	Average	STDEV
1	0.92405	0.97468	0.87805	0.92559	0.04834
2	0.93421	0.925	0.9359	0.9317	0.00587
3	0.97333	0.92683	0.97531	0.95849	0.02744
4	0.97531	0.96154	0.95181	0.96288	0.01181

Particles size calculation

All of the results for particles size were calculated by the equation below.

$$d_{32} = \frac{\sum_i n_i d_i^3}{\sum_i n_i d_i^2}$$

d_{32} = mean particle size

n_i = intensity

d_i = individual particle size

Size of chitin particles by vary chitin concentration

Conc. (%w/v)	Sample						Average	STDEV
	1	2	3	4	5	6		
1	327.991	301.592	424.307	541.947	224.863	327.8	358.0833	110.411
2	492.289	323.503	582.922	303.498	318.613	481.593	417.0697	117.1966
3	436.809	417.037	337.108	405.766	317.61	341.982	376.052	49.693
4	591.148	723.824	288.128	282.916	432.1	316.216	439.0553	182.4948

Chitin size results at chitin concentration of 1 %w/v - Sample 1

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	42.2	141.8	20107.24	2851206.632	848525.528	120320919.9
2	57.8	164.2	26961.64	4427101.288	1558382.792	255886454.4
	100				2406908.32	376207374.3
						156.3031592

EX 2	ni	di	di^2	di^3	nidi2	$nidi^3$
1	36.5	295.3	87202.09	25750777.18	3182876.285	939903367
2	63.5	342	116964	40001688	7427214	2540107188
	100				10610090.29	3480010555
						327.9906637

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	5.7	32.67	1067.3289	34869.63516	6083.77473	198756.9204
2	1.6	37.84	1431.8656	54181.7943	2290.98496	86690.87089
3	8.3	531.2	282173.44	149890531.3	2342039.552	1244091410
4	41.2	615.1	378348.01	232721861	15587938.01	9588140671
5	38.8	712.4	507513.76	361552802.6	19691533.89	14028248742
6	4.6	825	680625	561515625	3130875	2582971875
	100.2				40760761.21	27443738146
						673.2881656

Chitin size results at chitin concentration of 1 %w/v - Sample 2

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	3.4	255	65025	16581375	221085	56376675
2	84.6	295.3	87202.09	25750777.18	7377296.814	2178515749
3	12	342	116964	40001688	1403568	480020256
	100				9001949.814	2714912680
						301.5916258

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	4.6	342	116964	40001688	538034.4	184007764.8
2	55.4	396.1	156895.21	62146192.68	8691994.634	3442899075
3	40	458.7	210405.69	96513090	8416227.6	3860523600
	100				17646256.63	7487430439
						424.3070128

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	30.8	458.7	210405.69	96513090	6480495.252	2972603172
2	44.8	531.2	282173.44	149890531.3	12641370.11	6715095803
3	24.4	615.1	378348.01	232721861	9231691.444	5678413407
	100				28353556.81	15366112383
						541.9465532

Chitin size results at chitin concentration of 1 %w/v - Sample 3

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	12	190.1	36138.01	6869835.701	433656.12	82438028.41
2	72	220.2	48488.04	10677066.41	3491138.88	768748781.4
3	16	255	65025	16581375	1040400	265302000
	100				4965195	1116488810
						224.8630335

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	11.6	164.2	26961.64	4427101.288	312755.024	51354374.94
2	38	190.1	36138.01	6869835.701	1373244.38	261053756.6
3	37.5	220.2	48488.04	10677066.41	1818301.5	400389990.3
4	12.9	255	65025	16581375	838822.5	213899737.5
	100				4343123.404	926697859.4
						213.3712937

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	14.7	255	65025	16581375	955867.5	243746212.5
2	38.6	295.3	87202.09	25750777.18	3366000.674	993979999
3	35.5	342	116964	40001688	4152222	1420059924
4	11.2	396.1	156895.21	62146192.68	1757226.352	696037358
	100				10231316.53	3353823494
						327.7997983

Chitin size results at chitin concentration of 2 %w/v - Sample 1

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	76	190.1	36138.01	6869835.701	2746488.76	522107513.3
2	24	220.2	48488.04	10677066.41	1163712.96	256249593.8
	100				3910201.72	778357107.1
						199.0580443

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	21.9	396.1	156895.21	62146192.68	3436005.099	1361001620
2	41	458.7	210405.69	96513090	8626633.29	3957036690
3	31.1	531.2	282173.44	149890531.3	8775593.984	4661595524
4	6	615.1	378348.01	232721861	2270088.06	1396331166
	100				23108320.43	11375965000
						492.2886989

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	15.6	458.7	210405.69	96513090	3282328.764	1505604204
2	38.5	531.2	282173.44	149890531.3	10863677.44	5770785456
3	35.3	615.1	378348.01	232721861	13355684.75	8215081692
4	10.6	712.4	507513.76	361552802.6	5379645.856	3832459708
	100				32881336.81	19323931060
						587.6869049

Chitin size results at chitin concentration of 2 %w/v - Sample 2

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	46.8	295.3	87202.09	25750777.18	4081057.812	1205136372
2	53.2	342	116964	40001688	6222484.8	2128089802
	100				10303542.61	3333226173
						323.5029251

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	67.9	220.2	48488.04	10677066.41	3292337.916	724972809.1
2	32.1	295.3	87202.09	25750777.18	2799187.089	826599947.4
	100				6091525.005	1551572756
						254.7100694

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	14.1	458.7	210405.69	96513090	2966720.229	1360834569
2	41.1	531.2	282173.44	149890531.3	11597328.38	6160500838
3	36.9	615.1	378348.01	232721861	13961041.57	8587436669
4	7.9	712.4	507513.76	361552802.6	4009358.704	2856267141
	100				32534448.89	18965039216
						582.9217911

Chitin size results at chitin concentration of 2 %w/v - Sample 3

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	86.3	295.3	87202.09	25750777.18	7525540.367	2222292070
2	13.7	342	116964	40001688	1602406.8	548023125.6
	100				9127947.167	2770315196
						303.4981629

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	4.1	255	65025	16581375	266602.5	67983637.5
2	51.4	295.3	87202.09	25750777.18	4482187.426	1323589947
3	44.4	342	116964	40001688	5193201.6	1776074947
	99.9				9941991.526	3167648532
						318.613079

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	7.9	190.1	36138.01	6869835.701	285490.279	54271702.04
2	36.8	220.2	48488.04	10677066.41	1784359.872	392916043.8
3	37.7	255	65025	16581375	2451442.5	625117837.5
4	13.2	295.3	87202.09	25750777.18	1151067.588	339910258.7
5	2.5	825	680625	561515625	1701562.5	1403789063
6	1.7	955.4	912789.16	872078763.5	1551741.572	1482533898
	99.8				8925664.311	4298538802
						481.5931512

Chitin size results at chitin concentration of 3 %w/v - Sample 1

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	41.9	396.1	156895.21	62146192.68	6573909.299	2603925473
2	58.1	458.7	210405.69	96513090	12224570.59	5607410529
	100				18798479.89	8211336003
						436.8085107

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	62.7	615.1	378348.01	232721861	23722420.23	14591660682
2	37.3	712.4	507513.76	361552802.6	18930263.25	13485919538
	100				42652683.48	28077580220
						658.2840265

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	38.7	615.1	378348.01	232721861	14642067.99	9006336019
2	58.5	712.4	507513.76	361552802.6	29689554.96	21150838954
3	2.8	825	680625	561515625	1905750	1572243750
	100				46237372.95	31729418722
						686.2288383

Chitin size results at chitin concentration of 3 %w/v - Sample 2

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	21.2	342	116964	40001688	2479636.8	848035785.6
2	44.8	396.1	156895.21	62146192.68	7028905.408	2784149432
3	32.5	458.7	210405.69	96513090	6838184.925	3136675425
4	1.5	531.2	282173.44	149890531.3	423260.16	224835797
	100				16769987.29	6993696440
						417.0364782

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	39.3	295.3	87202.09	25750777.18	3427042.137	1012005543
2	48.2	342	116964	40001688	5637664.8	1928081362
3	12.5	396.1	156895.21	62146192.68	1961190.125	776827408.5
	100				11025897.06	3716914313
						337.1076559

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	1.4	295.3	87202.09	25750777.18	122082.926	36051088.05
2	28.6	342	116964	40001688	3345170.4	1144048277
3	43.6	396.1	156895.21	62146192.68	6840631.156	2709574001
4	26.3	458.7	210405.69	96513090	5533669.647	2538294267
	99.9				15841554.13	6427967633
						405.766226

Chitin size results at chitin concentration of 3 %w/v - Sample 3

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	67.2	255	65025	16581375	4369680	1114268400
2	32.8	295.3	87202.09	25750777.18	2860228.552	844625491.4
	100				7229908.552	1958893891
						270.9431077

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	11.5	255	65025	16581375	747787.5	190685812.5
2	47	295.3	87202.09	25750777.18	4098498.23	1210286527
3	39.4	342	116964	40001688	4608381.6	1576066507
4	2.1	396.1	156895.21	62146192.68	329479.941	130507004.6
	100				9784147.271	3107545852
						317.6102899

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	35.9	295.3	87202.09	25750777.18	3130555.031	924452900.7
2	46.9	342	116964	40001688	5485611.6	1876079167
3	17.2	396.1	156895.21	62146192.68	2698597.612	1068914514
	100				11314764.24	3869446582
						341.9820775

Chitin size results at chitin concentration of 4 %w/v - Sample 1

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	36.5	531.2	282173.44	149890531.3	10299330.56	5471004393
2	62.7	615.1	378348.01	232721861	23722420.23	14591660682
3	0.8	712.4	507513.76	361552802.6	406011.008	289242242.1
	100				34427761.8	20351907317
						591.1481391

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	13	615.1	378348.01	232721861	4918524.13	3025384192
2	61.3	712.4	507513.76	361552802.6	31110593.49	22163186801
3	25.7	825	680625	561515625	17492062.5	14430951563
	100				53521180.12	39619522556
						740.2587624

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	21.2	615.1	378348.01	232721861	8020977.812	4933703452
2	61	712.4	507513.76	361552802.6	30958339.36	22054720960
3	17.8	825	680625	561515625	12115125	9994978125
	100				51094442.17	36983402537
						723.8243724

Chitin size results at chitin concentration of 4 %w/v - Sample 2

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	22.5	255	65025	16581375	1463062.5	373080937.5
2	77.5	295.3	87202.09	25750777.18	6758161.975	1995685231
	100				8221224.475	2368766169
						288.1281464

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	37.3	255	65025	16581375	2425432.5	618485287.5
2	62.7	295.3	87202.09	25750777.18	5467571.043	1614573729
	100				7893003.543	2233059016
						282.9162567

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	3	342	116964	40001688	350892	120005064
2	48.6	396.1	156895.21	62146192.68	7625107.206	3020304964
3	46.7	458.7	210405.69	96513090	9825945.723	4507161303
4	1.6	531.2	282173.44	149890531.3	451477.504	239824850.1
	99.9				18253422.43	7887296182
						432.0995808

Chitin size results at chitin concentration of 4 %w/v - Sample 3

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	6.4	255	65025	16581375	416160	106120800
2	53	295.3	87202.09	25750777.18	4621710.77	1364791190
3	40.5	342	116964	40001688	4737042	1620068364
	99.9				9774912.77	3090980354
						316.2156458

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	39.6	141.8	20107.24	2851206.632	796246.704	112907782.6
2	36.8	164.2	26961.64	4427101.288	992188.352	162917327.4
3	0.2	190.1	36138.01	6869835.701	7227.602	1373967.14
4	6.9	712.4	507513.76	361552802.6	3501844.944	2494714338
5	12	825	680625	561515625	8167500	6738187500
6	4.4	955.4	912789.16	872078763.5	4016272.304	3837146559
	99.9				17481279.91	13347247475
						763.5166044

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	6.4	141.8	20107.24	2851206.632	128686.336	18247722.44
2	39.6	164.2	26961.64	4427101.288	1067680.944	175313211
3	41.4	190.1	36138.01	6869835.701	1496113.614	284411198
4	12.6	220.2	48488.04	10677066.41	610949.304	134531036.7
	100				3303430.198	612503168.2
						185.4142911

Size of emulsions by vary chitin concentration

Conc. (%w/v)	Sample						Average	STDEV
	1	2	3	4	5	6		
1	328.851	536.567	491.341	564.084	320.839	285.847	421.2548	122.9359
2	279.067	393.286	507.676	590.128	361.04	386.547	419.624	111.1763
3	463.046	653.595	388.014	480.56	395.12	N/A	476.067	107.2517
4	428.214	429.477	638.7	657.858	590.989	711.983	576.2035	120.5467

Emulsion droplets size results at chitin concentration of 1 %w/v - Sample 1

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	39.1	295.3	87202.09	25750777.18	3409601.719	1006855388
2	58.6	342	116964	40001688	6854090.4	2344098917
3	2.3	396.1	156895.21	62146192.68	360858.983	142936243.2
	100				10624551.1	3493890548
						328.8506511

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	10.5	396.1	156895.21	62146192.68	1647399.705	652535023.2
2	30.6	458.7	210405.69	96513090	6438414.114	2953300554
3	36.2	531.2	282173.44	149890531.3	10214678.53	5426037234
4	20.8	615.1	378348.01	232721861	7869638.608	4840614708
5	1.9	712.4	507513.76	361552802.6	964276.144	686950325
	100				27134407.1	14559437844
						536.5673844

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	1.7	531.2	282173.44	149890531.3	479694.848	254813903.3
2	21.9	615.1	378348.01	232721861	8285821.419	5096608755
3	36.1	712.4	507513.76	361552802.6	18321246.74	13052056175
4	29.5	825	680625	561515625	20078437.5	16564710938
5	10.8	955.4	912789.16	872078763.5	9858122.928	9418450645
	100				57023323.43	44386640416
						778.394484

Emulsion droplets size results at chitin concentration of 1 %w/v - Sample 2

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	2.6	255	65025	16581375	169065	43111575
2	9.2	295.3	87202.09	25750777.18	802259.228	236907150
3	16.7	342	116964	40001688	1953298.8	668028189.6
4	21.5	396.1	156895.21	62146192.68	3373247.015	1336143143
5	21.3	458.7	210405.69	96513090	4481641.197	2055728817
6	16.4	531.2	282173.44	149890531.3	4627644.416	2458204714
7	9.3	615.1	378348.01	232721861	3518636.493	2164313307
8	3	712.4	507513.76	361552802.6	1522541.28	1084658408
	100				20448333.43	10047095303
						491.3405456

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	0.1	220.2	48488.04	10677066.41	4848.804	1067706.641
2	2.4	255	65025	16581375	156060	39795300
3	6.6	295.3	87202.09	25750777.18	575533.794	169955129.4
4	11.3	342	116964	40001688	1321693.2	452019074.4
5	15.1	396.1	156895.21	62146192.68	2369117.671	938407509.5
6	16.8	458.7	210405.69	96513090	3534815.592	1621419912
7	16	531.2	282173.44	149890531.3	4514775.04	2398248501
8	13.2	615.1	378348.01	232721861	4994193.732	3071928565
9	9.2	712.4	507513.76	361552802.6	4669126.592	3326285784
10	5	825	680625	561515625	3403125	2807578125
11	1.9	955.4	912789.16	872078763.5	1734299.404	1656949651
12	0.2	1106	1223236	1352899016	244647.2	270579803.2
13	0.6	4801	23049601	1.10661E+11	13829760.6	66396680641
14	1.6	5560	30913600	1.7188E+11	49461760	2.75007E+11
	100				90813756.63	3.58158E+11
						3943.877168

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	5.9	342	116964	40001688	690087.6	236009959.2
2	16.2	396.1	156895.21	62146192.68	2541702.402	1006768321
3	24.1	458.7	210405.69	96513090	5070777.129	2325965469
4	24.8	531.2	282173.44	149890531.3	6997901.312	3717285177
5	18.4	615.1	378348.01	232721861	6961603.384	4282082241
6	8.9	712.4	507513.76	361552802.6	4516872.464	3217819943
7	1.8	825	680625	561515625	1225125	1010728125
	100.1				28004069.29	15796659236
						564.0844219

Emulsion droplets size results at chitin concentration of 1 %w/v - Sample 3

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	2.6	220.2	48488.04	10677066.41	126068.904	27760372.66
2	22.7	255	65025	16581375	1476067.5	376397212.5
3	36	295.3	87202.09	25750777.18	3139275.24	927027978.4
4	28.6	342	116964	40001688	3345170.4	1144048277
5	10.1	396.1	156895.21	62146192.68	1584641.621	627676546.1
	100				9671223.665	3102910386
						320.8394815

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	18.7	220.2	48488.04	10677066.41	906726.348	199661141.8
2	34.4	255	65025	16581375	2236860	570399300
3	31.7	295.3	87202.09	25750777.18	2764306.253	816299636.5
4	14.8	342	116964	40001688	1731067.2	592024982.4
5	0.3	396.1	156895.21	62146192.68	47068.563	18643857.8
	99.9				7686028.364	2197028919
						285.8471

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	10.8	220.2	48488.04	10677066.41	523670.832	115312317.2
2	23.2	255	65025	16581375	1508580	384687900
3	27.6	295.3	87202.09	25750777.18	2406777.684	710721450.1
4	22	342	116964	40001688	2573208	880037136
5	11.2	396.1	156895.21	62146192.68	1757226.352	696037358
6	2.5	458.7	210405.69	96513090	526014.225	241282725
7	0.3	4801	23049601	1.10661E+11	6914880.3	33198340320
8	2.4	5560	30913600	1.7188E+11	74192640	4.12511E+11
	100				90402997.39	4.48737E+11
						4963.745789

Emulsion droplets size results at chitin concentration of 2 %w/v - Sample 1

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	19.3	164.2	26961.64	4427101.288	520359.652	85443054.86
2	72.5	190.1	36138.01	6869835.701	2620005.725	498063088.3
3	8.2	220.2	48488.04	10677066.41	397601.928	87551944.55
	100				3537967.305	671058087.7
						189.6733434

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	11.7	220.2	48488.04	10677066.41	567310.068	124921677
2	42.7	255	65025	16581375	2776567.5	708024712.5
3	39.6	295.3	87202.09	25750777.18	3453202.764	1019730776
4	6	342	116964	40001688	701784	240010128
	100				7498864.332	2092687294
						279.0672295

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	0.9	255	65025	16581375	58522.5	14923237.5
2	16.8	295.3	87202.09	25750777.18	1464995.112	432613056.6
3	31.6	342	116964	40001688	3696062.4	1264053341
4	31.3	396.1	156895.21	62146192.68	4910820.073	1945175831
5	17	458.7	210405.69	96513090	3576896.73	1640722530
6	2.4	531.2	282173.44	149890531.3	677216.256	359737275.2
	100				14384513.07	5657225271
						393.2858376

Emulsion droplets size results at chitin concentration of 2 %w/v - Sample 2

EX 1	ni	di	di^2	di^3	nidi^2	nidi^3
1	2.8	295.3	87202.09	25750777.18	244165.852	72102176.1
2	12.5	342	116964	40001688	1462050	500021100
3	22.4	396.1	156895.21	62146192.68	3514452.704	1392074716
4	26	458.7	210405.69	96513090	5470547.94	2509340340
5	21.4	531.2	282173.44	149890531.3	6038511.616	3207657370
6	11.8	615.1	378348.01	232721861	4464506.518	2746117959
7	3.2	712.4	507513.76	361552802.6	1624044.032	1156968968
	100.1				22818278.66	11584282630
						507.6755702

EX 2	ni	di	di^2	di^3	nidi^2	nidi^3
1	4.2	342	116964	40001688	491248.8	168007089.6
2	13.3	396.1	156895.21	62146192.68	2086706.293	826544362.7
3	21.8	458.7	210405.69	96513090	4586844.042	2103985362
4	24.6	531.2	282173.44	149890531.3	6941466.624	3687307071
5	20.4	615.1	378348.01	232721861	7718299.404	4747525963
6	11.9	712.4	507513.76	361552802.6	6039413.744	4302478351
7	3.8	825	680625	561515625	2586375	2133759375
	100				30450353.91	17969607575
						590.1280369

EX 3	ni	di	di^2	di^3	nidi^2	nidi^3
1	2	295.3	87202.09	25750777.18	174404.18	51501554.35
2	6.7	342	116964	40001688	783658.8	268011309.6
3	12.4	396.1	156895.21	62146192.68	1945500.604	770612789.2
4	17	458.7	210405.69	96513090	3576896.73	1640722530
5	18.8	531.2	282173.44	149890531.3	5304860.672	2817941989
6	17.3	615.1	378348.01	232721861	6545420.573	4026088194
7	13.2	712.4	507513.76	361552802.6	6699181.632	4772496995
8	7.9	825	680625	561515625	5376937.5	4435973438
9	3.2	955.4	912789.16	872078763.5	2920925.312	2790652043
10	0.5	1106	1223236	1352899016	611618	676449508
11	1	5560	30913600	1.7188E+11	30913600	1.7188E+11
	100				64853004	1.9413E+11
						2993.385878

Emulsion droplets size results at chitin concentration of 2 %w/v - Sample 3

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	0.6	220.2	48488.04	10677066.41	29092.824	6406239.845
2	12.5	255	65025	16581375	812812.5	207267187.5
3	25.8	295.3	87202.09	25750777.18	2249813.922	664370051.2
4	30	342	116964	40001688	3508920	1200050640
5	22.2	396.1	156895.21	62146192.68	3483073.662	1379645478
6	8.9	458.7	210405.69	96513090	1872610.641	858966501
	100				11956323.55	4316706097
						361.0395854

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	4.2	220.2	48488.04	10677066.41	203649.768	44843678.91
2	13.1	255	65025	16581375	851827.5	217216012.5
3	21.1	295.3	87202.09	25750777.18	1839964.099	543341398.4
4	23.8	342	116964	40001688	2783743.2	952040174.4
5	20.2	396.1	156895.21	62146192.68	3169283.242	1255353092
6	12.5	458.7	210405.69	96513090	2630071.125	1206413625
7	4.7	531.2	282173.44	149890531.3	1326215.168	704485497.2
8	0.3	615.1	378348.01	232721861	113504.403	69816558.29
	99.9				12918258.51	4993510037
						386.5466878

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	1	220.2	48488.04	10677066.41	48488.04	10677066.41
2	10.2	255	65025	16581375	663255	169130025
3	20.7	295.3	87202.09	25750777.18	1805083.263	533041087.6
4	25.5	342	116964	40001688	2982582	1020043044
5	22.2	396.1	156895.21	62146192.68	3483073.662	1379645478
6	13.5	458.7	210405.69	96513090	2840476.815	1302926715
7	4.6	531.2	282173.44	149890531.3	1297997.824	689496444.1
8	0.4	4801	23049601	1.10661E+11	9219840.4	44264453760
9	1.9	5560	30913600	1.7188E+11	58735840	3.26571E+11
	100				81076637	3.75941E+11
						4636.855917

Emulsion droplets size results at chitin concentration of 3 %w/v - Sample 1

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	1.9	342	116964	40001688	222231.6	76003207.2
2	32.8	396.1	156895.21	62146192.68	5146162.888	2038395120
3	43.9	458.7	210405.69	96513090	9236809.791	4236924651
4	21.4	531.2	282173.44	149890531.3	6038511.616	3207657370
	100				20643715.9	9558980349
						463.0455291

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	10.4	458.7	210405.69	96513090	2188219.176	1003736136
2	25.5	531.2	282173.44	149890531.3	7195422.72	3822208549
3	31.5	615.1	378348.01	232721861	11917962.32	7330738620
4	23.6	712.4	507513.76	361552802.6	11977324.74	8532646142
5	9.1	825	680625	561515625	6193687.5	5109792188
	100.1				39472616.45	25799121634
						653.5954278

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	0.3	295.3	87202.09	25750777.18	26160.627	7725233.153
2	2.4	342	116964	40001688	280713.6	96004051.2
3	6	396.1	156895.21	62146192.68	941371.26	372877156.1
4	10.2	458.7	210405.69	96513090	2146138.038	984433518
5	13.9	531.2	282173.44	149890531.3	3922210.816	2083478385
6	16.1	615.1	378348.01	232721861	6091402.961	3746821961
7	16.1	712.4	507513.76	361552802.6	8170971.536	5821000122
8	14	825	680625	561515625	9528750	7861218750
9	10.4	955.4	912789.16	872078763.5	9493007.264	9069619140
10	6.1	1106	1223236	1352899016	7461739.6	8252683998
11	2.5	1281	1640961	2102071041	4102402.5	5255177603
12	0.5	1484	2202256	3268147904	1101128	1634073952
13	1.4	5560	30913600	1.7188E+11	43279040	2.40631E+11
	99.9				96545036.2	2.85817E+11
						2960.448175

Emulsion droplets size results at chitin concentration of 3 %w/v - Sample 2

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	3.7	220.2	48488.04	10677066.41	179405.748	39505145.71
2	11.9	255	65025	16581375	773797.5	197318362.5
3	20.3	295.3	87202.09	25750777.18	1770202.427	522740776.7
4	24.2	342	116964	40001688	2830528.8	968040849.6
5	21.4	396.1	156895.21	62146192.68	3357557.494	1329928523
6	13.6	458.7	210405.69	96513090	2861517.384	1312578024
7	4.9	531.2	282173.44	149890531.3	1382649.856	734463603.5
	100				13155659.21	5104575285
						388.0136453

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	1.7	190.1	36138.01	6869835.701	61434.617	11678720.69
2	5.5	220.2	48488.04	10677066.41	266684.22	58723865.24
3	10.3	255	65025	16581375	669757.5	170788162.5
4	14.4	295.3	87202.09	25750777.18	1255710.096	370811191.3
5	16.7	342	116964	40001688	1953298.8	668028189.6
6	16.3	396.1	156895.21	62146192.68	2557391.923	1012982941
7	13.6	458.7	210405.69	96513090	2861517.384	1312578024
8	9.4	531.2	282173.44	149890531.3	2652430.336	1408970994
9	5	615.1	378348.01	232721861	1891740.05	1163609305
10	1.6	712.4	507513.76	361552802.6	812022.016	578484484.2
11	1.4	4801	23049601	1.10661E+11	32269441.4	1.54926E+11
12	4.1	5560	30913600	1.7188E+11	126745760	7.04706E+11
	100				173997188.3	8.66389E+11
						4979.325689

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	0.1	43.82	1920.1924	84142.83097	192.01924	8414.283097
2	0.7	50.75	2575.5625	130709.7969	1802.89375	91496.85781
3	1.4	58.77	3453.9129	202986.4611	4835.47806	284181.0456
4	1.5	68.06	4632.1636	315265.0546	6948.2454	472897.5819
5	1.1	78.82	6212.5924	489676.533	6833.85164	538644.1863
6	0.4	91.28	8332.0384	760548.4652	3332.81536	304219.3861
7	0.3	190.1	36138.01	6869835.701	10841.403	2060950.71
8	2	220.2	48488.04	10677066.41	96976.08	21354132.82
9	5.1	255	65025	16581375	331627.5	84565012.5
10	9.1	295.3	87202.09	25750777.18	793539.019	234332072.3
11	12.7	342	116964	40001688	1485442.8	508021437.6
12	15.1	396.1	156895.21	62146192.68	2369117.671	938407509.5
13	15.4	458.7	210405.69	96513090	3240247.626	1486301586
14	13.8	531.2	282173.44	149890531.3	3893993.472	2068489332
15	10.6	615.1	378348.01	232721861	4010488.906	2466851726
16	6.7	712.4	507513.76	361552802.6	3400342.192	2422403778
17	3.2	825	680625	561515625	2178000	1796850000
18	0.8	955.4	912789.16	872078763.5	730231.328	697663010.8
	100				22564793.3	12729000402
						564.1088856

Emulsion droplets size results at chitin concentration of 3 %w/v - Sample 3

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	4.1	220.2	48488.04	10677066.41	198800.964	43775972.27
2	11.8	255	65025	16581375	767295	195660225
3	19.5	295.3	87202.09	25750777.18	1700440.755	502140155
4	23.2	342	116964	40001688	2713564.8	928039161.6
5	21	396.1	156895.21	62146192.68	3294799.41	1305070046
6	14.1	458.7	210405.69	96513090	2966720.229	1360834569
7	5.8	531.2	282173.44	149890531.3	1636605.952	869365081.7
8	0.5	615.1	378348.01	232721861	189174.005	116360930.5
	100				13467401.12	5321246141
						395.120491

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	10.9	141.8	20107.24	2851206.632	219168.916	31078152.29
2	23.7	164.2	26961.64	4427101.288	638990.868	104922300.5
3	25	190.1	36138.01	6869835.701	903450.25	171745892.5
4	14.6	220.2	48488.04	10677066.41	707925.384	155885169.6
5	3	255	65025	16581375	195075	49744125
6	0.2	2669	7123561	19012784309	1424712.2	3802556862
7	1.5	3091	9554281	29532282571	14331421.5	44298423857
8	3.4	3580	12816400	45882712000	43575760	1.56001E+11
9	5.2	4145	17181025	71215348625	89341330	3.7032E+11
10	6.3	4801	23049601	1.10661E+11	145212486.3	6.97165E+11
11	6.3	5560	30913600	1.7188E+11	194755680	1.08284E+12
	100.1				491306000.4	2.35494E+12
						4793.228895

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	4	122.4	14981.76	1833767.424	59927.04	7335069.696
2	14.2	141.8	20107.24	2851206.632	285522.808	40487134.17
3	17.3	164.2	26961.64	4427101.288	466436.372	76588852.28
4	10.5	190.1	36138.01	6869835.701	379449.105	72133274.86
5	2	220.2	48488.04	10677066.41	96976.08	21354132.82
6	1.9	825	680625	561515625	1293187.5	1066879688
7	6	955.4	912789.16	872078763.5	5476734.96	5232472581
8	10	1106	1223236	1352899016	12232360	13528990160
9	11.8	1281	1640961	2102071041	19363339.8	24804438284
10	10.7	1484	2202256	3268147904	23564139.2	34969182573
11	7.4	1718	2951524	5070718232	21841277.6	37523314917
12	3.4	1990	3960100	7880599000	13464340	26794036600
13	0.7	2305	5313025	12246522625	3719117.5	8572565838
	99.9				102242808	1.5271E+11
						1493.599229

Emulsion droplets size results at chitin concentration of 4 %w/v - Sample 1

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	17.4	255	65025	16581375	1131435	288515925
2	34.6	295.3	87202.09	25750777.18	3017192.314	890976890.3
3	33	342	116964	40001688	3859812	1320055704
4	15	396.1	156895.21	62146192.68	2353428.15	932192890.2
	100				10361867.46	3431741410
						331.1894715

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	0.6	220.2	48488.04	10677066.41	29092.824	6406239.845
2	6.7	255	65025	16581375	435667.5	111095212.5
3	15.6	295.3	87202.09	25750777.18	1360352.604	401712124
4	22.4	342	116964	40001688	2619993.6	896037811.2
5	23.5	396.1	156895.21	62146192.68	3687037.435	1460435528
6	18.4	458.7	210405.69	96513090	3871464.696	1775840856
7	10	531.2	282173.44	149890531.3	2821734.4	1498905313
8	2.8	615.1	378348.01	232721861	1059374.428	651621210.7
	100				15884717.49	6802054296
						428.2137407

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	0.4	220.2	48488.04	10677066.41	19395.216	4270826.563
2	6.4	255	65025	16581375	416160	106120800
3	15.5	295.3	87202.09	25750777.18	1351632.395	399137046.2
4	22.4	342	116964	40001688	2619993.6	896037811.2
5	23.6	396.1	156895.21	62146192.68	3702726.956	1466650147
6	18.6	458.7	210405.69	96513090	3913545.834	1795143474
7	10.2	531.2	282173.44	149890531.3	2878169.088	1528883420
8	2.9	615.1	378348.01	232721861	1097209.229	674893396.8
	100				15998832.32	6871136922
						429.4774009

Emulsion droplets size results at chitin concentration of 4 %w/v - Sample 2

EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	7.3	396.1	156895.21	62146192.68	1145335.033	453667206.6
2	17.8	458.7	210405.69	96513090	3745221.282	1717933002
3	24.8	531.2	282173.44	149890531.3	6997901.312	3717285177
4	24.3	615.1	378348.01	232721861	9193856.643	5655141221
5	17.1	712.4	507513.76	361552802.6	8678485.296	6182552925
6	7.6	825	680625	561515625	5172750	4267518750
7	1.1	955.4	912789.16	872078763.5	1004068.076	959286639.8
	100				35937617.64	22953384921
						638.700794

EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	1.8	342	116964	40001688	210535.2	72003038.4
2	6.5	396.1	156895.21	62146192.68	1019818.865	403950252.4
3	12.6	458.7	210405.69	96513090	2651111.694	1216064934
4	17.5	531.2	282173.44	149890531.3	4938035.2	2623084298
5	19.5	615.1	378348.01	232721861	7377786.195	4538076289
6	17.9	712.4	507513.76	361552802.6	9084496.304	6471795167
7	13.4	825	680625	561515625	9120375	7524309375
8	7.8	955.4	912789.16	872078763.5	7119755.448	6802214355
9	2.8	1106	1223236	1352899016	3425060.8	3788117245
10	0.2	1281	1640961	2102071041	328192.2	420414208.2
	100				45275166.91	33860029162
						747.8719898

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	1.2	342	116964	40001688	140356.8	48002025.6
2	7.8	396.1	156895.21	62146192.68	1223782.638	484740302.9
3	16.6	458.7	210405.69	96513090	3492734.454	1602117294
4	22.5	531.2	282173.44	149890531.3	6348902.4	3372536955
5	22.7	615.1	378348.01	232721861	8588499.827	5282786244
6	17.3	712.4	507513.76	361552802.6	8779988.048	6254863485
7	9.2	825	680625	561515625	6261750	5165943750
8	2.6	955.4	912789.16	872078763.5	2373251.816	2267404785
	99.9				37209265.98	24478394841
						657.8575039

Emulsion droplets size results at chitin concentration of 4 %w/v - Sample 3

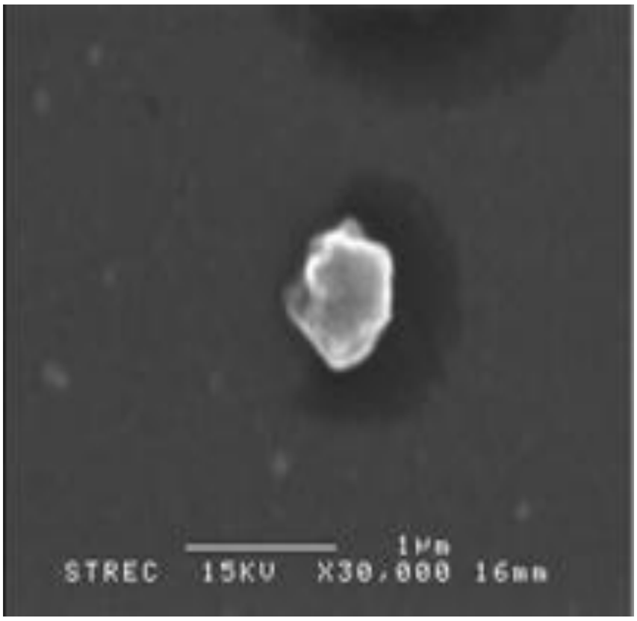
EX 1	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	1.4	396.1	156895.21	62146192.68	219653.294	87004669.75
2	19.2	458.7	210405.69	96513090	4039789.248	1853051328
3	34.1	531.2	282173.44	149890531.3	9622114.304	5111267118
4	31.1	615.1	378348.01	232721861	11766623.11	7237649876
5	14.1	712.4	507513.76	361552802.6	7155944.016	5097894517
	99.9				32804123.97	19386867509
						590.9887283

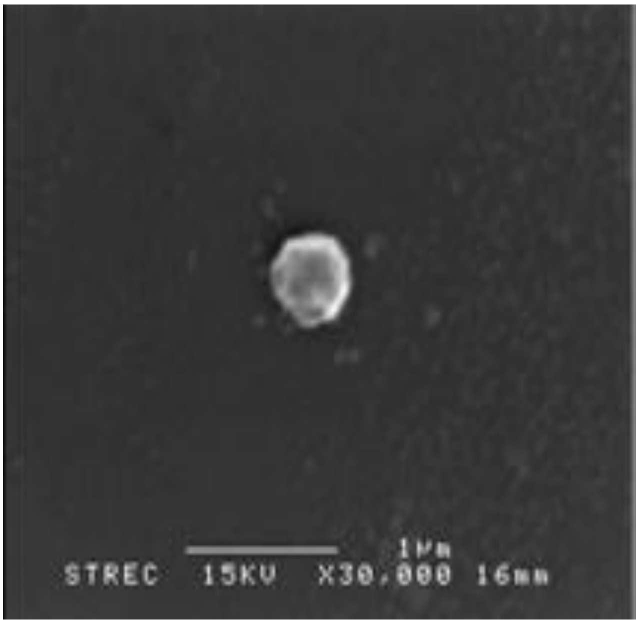
EX 2	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	5.1	458.7	210405.69	96513090	1073069.019	492216759
2	14.7	531.2	282173.44	149890531.3	4147949.568	2203390811
3	22.9	615.1	378348.01	232721861	8664169.429	5329330616
4	24.9	712.4	507513.76	361552802.6	12637092.62	9002664785
5	19.5	825	680625	561515625	13272187.5	10949554688
6	10.3	955.4	912789.16	872078763.5	9401728.348	8982411264
7	2.5	1106	1223236	1352899016	3058090	3382247540
	99.9				52254286.49	40341816462
						772.0288453

EX 3	ni	di	di^2	di^3	$nidi^2$	$nidi^3$
1	1.6	396.1	156895.21	62146192.68	251032.336	99433908.29
2	9.7	458.7	210405.69	96513090	2040935.193	936176973
3	19.5	531.2	282173.44	149890531.3	5502382.08	2922865361
4	25.1	615.1	378348.01	232721861	9496535.051	5841318710
5	23.2	712.4	507513.76	361552802.6	11774319.23	8388025021
6	15.2	825	680625	561515625	10345500	8535037500
7	5.8	955.4	912789.16	872078763.5	5294177.128	5058056828
8	0.1	1106	1223236	1352899016	122323.6	135289901.6
	100.2				44827204.62	31916204203
						711.9829236

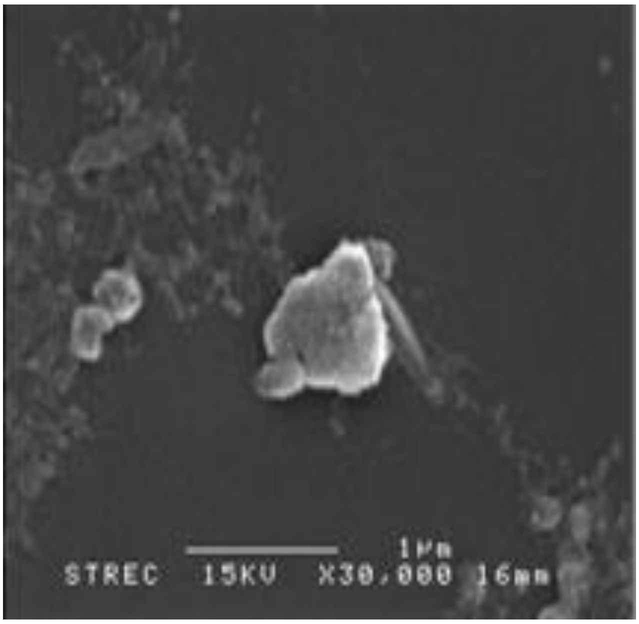
Appendix B

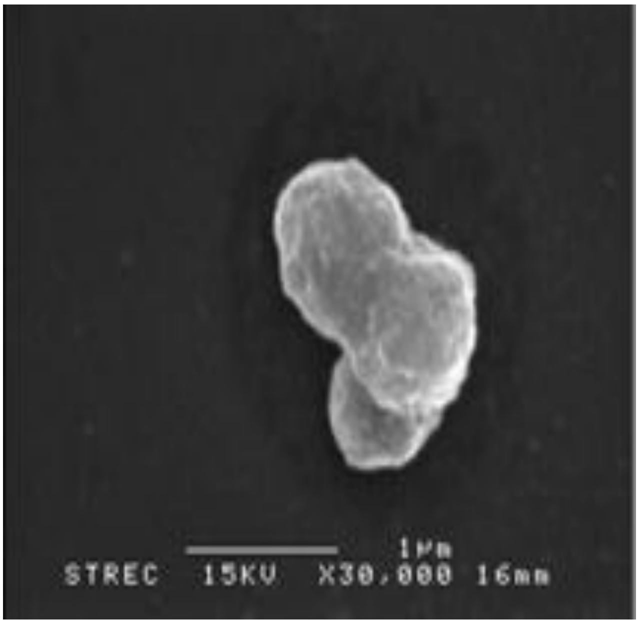
SEM micrograph of chitin particles



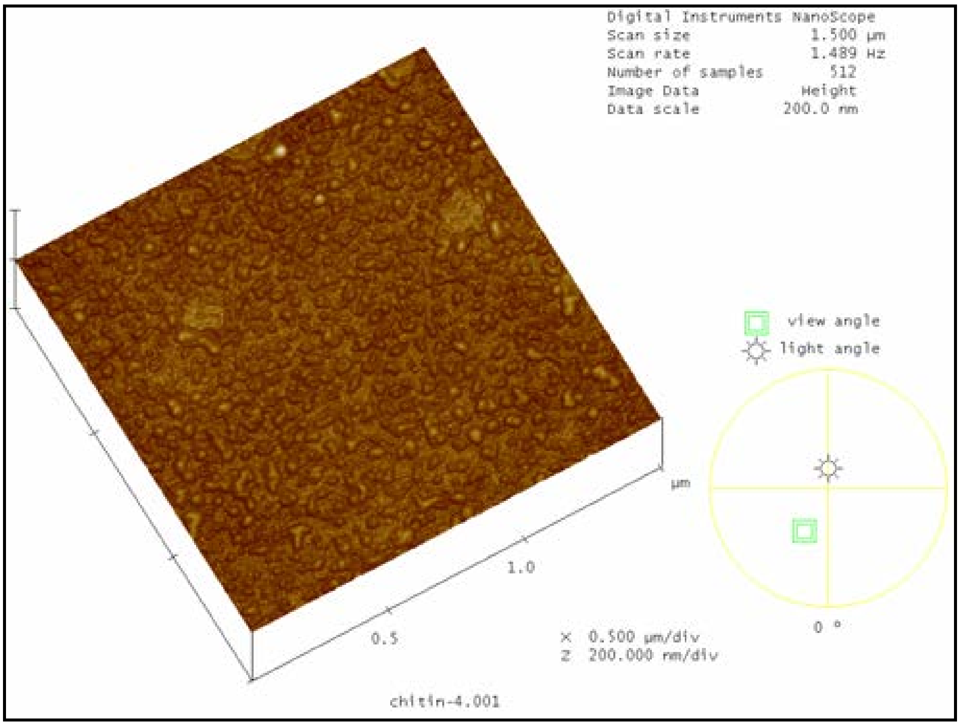


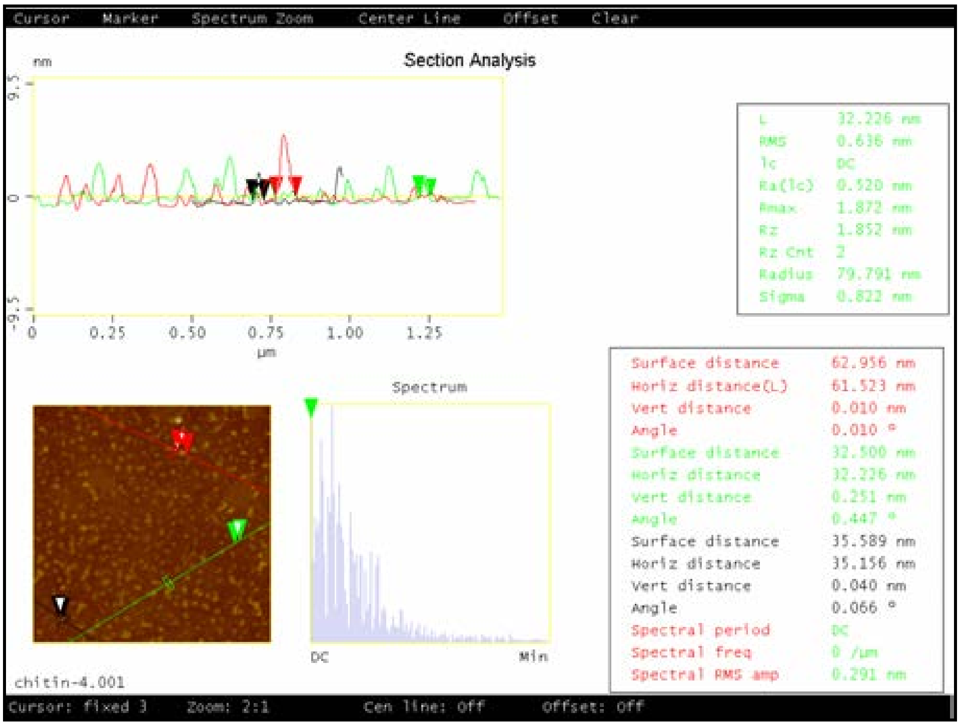
SEM micrograph of emulsion at 3%(w/v) chitin concentration, oil-to-water ratio 2:8



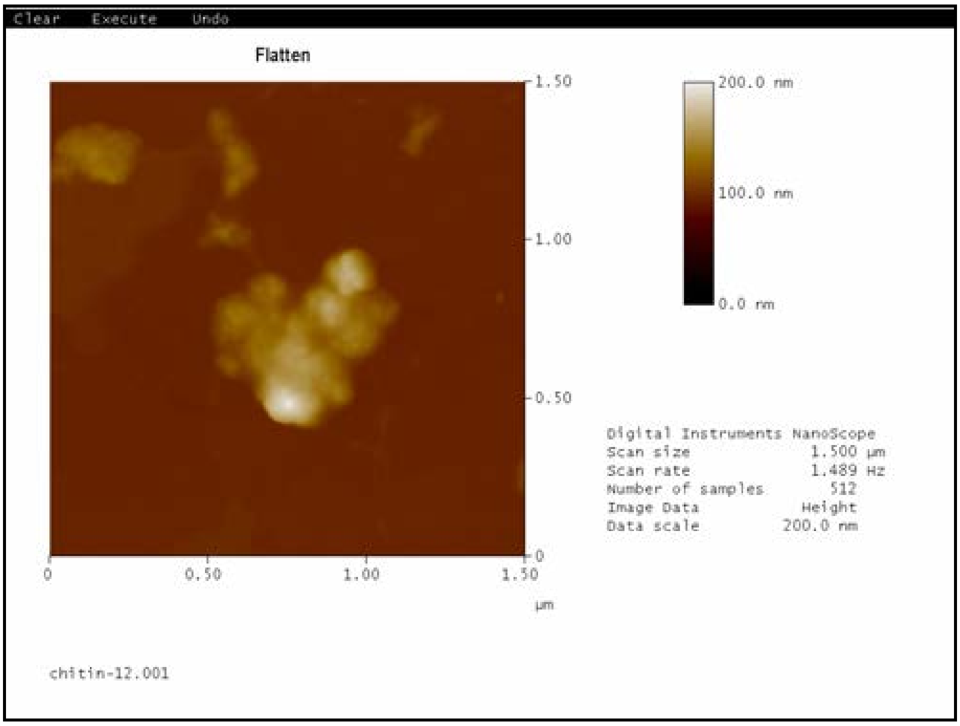


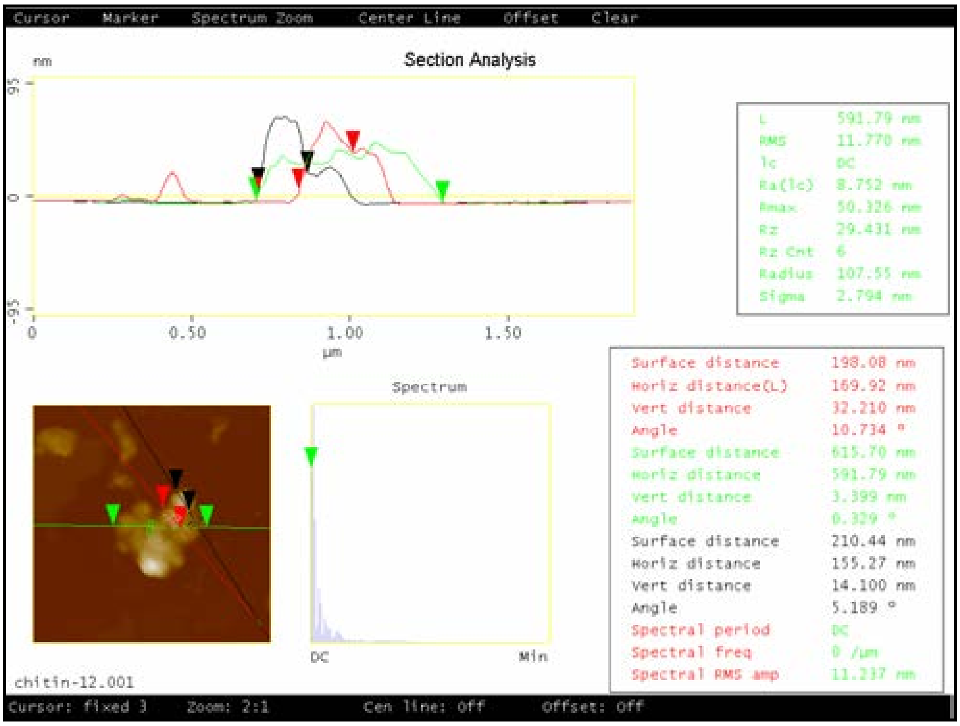
AFM micrograph of chitin particles



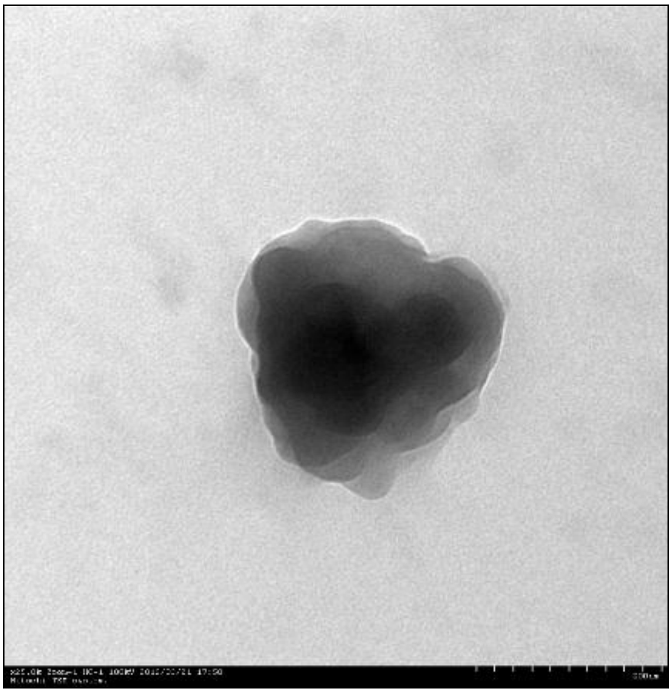


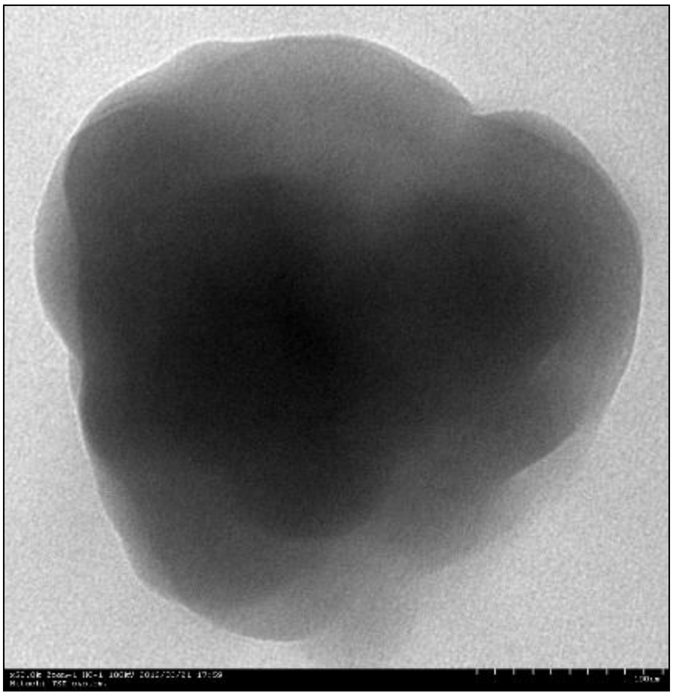
AFM micrograph of emulsion at 3%(w/v) chitin concentration, oil-to-water ratio 2:8





TEM micrograph of emulsion at 3%(w/v) chitin concentration, oil-to-water ratio 2:8





Biography

Mister Niroje Rathaloengsak was born in Bangkok, Thailand on September 25^{th}, 1985. He received his Bachelor's Degree of Engineering in Petrochemical and Polymeric Materials from Faculty of Engineering and Industrial, Silpakorn University in 2008. After that, he continued a further study in a Master Degree in the field of Applied Polymer Science and Textile Technology at the Department of Materials Science, Faculty of science, Chulalongkorn University in 2009, and ultimately completed the Degree of Master of Science in Applied Polymer Science and Textile Technology in May 2013.

Publication:

International Presentation:

- N. Rathaloengsak, and W. Tachaboonyakiat, 2011. "Chitin Microcapsule Fabrication via Pickering Emulsion", The Seventh Mathermatics and Physical Sciences Graduate Congress (MPSGC-7), Singapore, December 12-14, 2011
- N. Rathaloengsak, and W. Tachaboonyakiat, 2012. "Fabrication And Characterization Of Chitin Microparticle via Oil-in-Water Emulsion", The Sixth Pure and Applied Chemistry International Conference 2012 (PACCON 2012), Chiang Mai, Thailand, January 11-13, 2012