การใช้มอนต์มอริลโลไนต์ดัดแปรและผงถ่านไม้ไผ่ดัดแปรเป็นสารตัวเติมในฟิล์มไคโตซาน

นางสาววลัยกร นิตยพัฒน์

UTILIZATION OF MODIFIED MONTMORILLONITE AND MODIFIED
BAMBOO CHARCOAL POWDER AS FILLERS IN CHITOSAN FILMS

Miss Walaikorn Nitayaphat

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วลัยกร นิตยพัฒน์ : การใช้มอนต์มอริลโลไนต์ดัดแปรและผงถ่านไม้ไผ่ดัดแปรเป็นสารตัว
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ฟิล์มคอมพอสิตไคโตซาน/มอนต์มอริลโลไนต์ดัดแปรและฟิล์มคอมพอสิตไคโตซาน/ผง
ถ่านไม้ไผ่ดัดแปรเตรียมโดยการผสมไคโตซานกับมอนต์มอริลโลไนต์ดัดแปรหรือผงถ่านไม้ไผ่ดัด
แปรและขึ้นรูปฟิล์มด้วยเครื่องเคลือบแบบหมุน โดยปริมาณความเข้มข้นของมอนต์มอริลโลไนต์
ดัดแปรหรือผงถ่านไม้ไผ่ดัดแปรในฟิล์มไคโตซานเป็น 0-2 เปอร์เซ็นต์ (โดยน้ำหนัก) ฟิล์มที่เตรียม
ได้จะถูกตรวจสอบลักษณะทางสัญฐานวิทยา โครงสร้าง ตลอดจนศึกษาสมบัติการใช้งานอื่นๆ
จากการวิเคราะห์ด้วยเทคนิคเอ็กซเรย์ดิฟแฟรกชันพบว่าการดัดแปรมอนต์มอริลโลไนต์ด้วยไคโต
ซานที่มีน้ำหนักโมเลกุล 15 กิโลดาลตัน ทำให้เกิดการแทรกสอดขึ้นในมอนต์มอริลโลไนต์ดัดแปร
การวิเคราะห์โครงสร้างทางเคมียืนยันว่าเกิดหมู่คาร์บอกซิลิกขึ้นที่ผิวของอนุภาคของถ่านไม้ไผ่
ขณะทำการรีฟลักซ์ด้วยกรดไนตริก การศึกษาสมบัติเชิงกลของฟิล์มคอมพอสิตพบว่าค่าความทน
แรงดึงและความแข็งเกร็งของทั้งฟิล์มคอมพอสิตไคโตซานมีค่าเพิ่มขึ้น ในขณะที่เปอร์เซ็นต์การยืด
ตัว ณ จุดขาดมีค่าลดลงเมื่อเพิ่มปริมาณของมอนต์มอริลโลไนต์และผงถ่านไม้ไผ่ทั้งที่ดัดแปรและ
ไม่ได้ดัดแปรในฟิล์มคอมพอสิตไคโตซาน การเติมมอนต์มอริลโลไนต์หรือมอนต์มอริลโลไนต์ดัด
แปรในฟิล์มคอมพอสิตไคโตซานลดค่าการดูดซับน้ำและน้ำมันในฟิล์มคอมพอสิตไคโตซาน ค่า
การดูดซับน้ำมันและโดยเฉพาะอย่างยิ่งการดูดซับน้ำของฟิล์มคอมพอสิตไคโตซาน/ผงถ่านไม้ไผ่
ดัดแปรมีค่าเพิ่มขึ้นเมื่อเติมผงถ่านไม้ไผ่ไม่ได้ดัดแปรหรือผงถ่านไม้ไผ่ดัดแปร การเติมมอนต์มอริล
โลไนต์ดัดแปรหรือผงถ่านไม้ไผ่ดัดแปรในฟิล์มไคโตซานมีผลต่อเสถียรภาพทางความร้อนของฟิล์ม
คอมพอสิตไคโตซาน และภาพจากกล้องจุลทรรศน์แบบส่องกราดแสดงถึงการกระจายตัวที่ดีของ
อนุภาคมอนต์มอริลโลไนต์ดัดแปรหรือผงถ่านไม้ไผ่ดัดแปรในเนื้อไคโตซานเมื่อเติมสารตัวเติมทั้ง
สองชนิดไม่เกิน 0.5 เปอร์เซ็นต์โดยน้ำหนัก และเมื่อปริมาณของสารตัวเติมทั้งสองเริ่มตั้งแต่ 1
เปอร์เซ็นต์โดยน้ำหนัก จะสังเกตได้ว่าอนุภาคของสารตัวเติมทั้งสองชนิดเริ่มเกาะตัวเป็นอนุภาค
ใหญ่ขึ้น นอกจากนี้การเกิดและเจริญเติบโตของเชื้อราถูกยับยั้งอย่างสมบูรณ์โดยฟิล์มไคโตซานและ
ฟิล์มคอมพอสิตไคโตซาน

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WALAIKORN NITAYAPHAT : UTILIZATION OF MODIFIED MONTMORILLONITE AND MODIFIED BAMBOO CHARCOAL AS FILLERS IN CHITOSAN FILMS. THESIS ADVISOR : ASST. PROF. SIRIWAN KITTINAOVARAT, THESIS CO-ADVISOR : ASST. PROF. NANTANA JIRATUMNUKUL, ASST. PROF. SIREERAT CHARUCHINDA, 131 pp.

Chitosan/modified montmorillonite composite films and chitosan/modified bamboo charcoal composite films were prepared by blending chitosan with either modified montmorillonite or modified bamboo charcoal and forming the composite film by using a spin coater machine. The chitosan composite films contained contents of modified montmorillonite or modified bamboo charcoal varying from 0 to 2% (w/w). The morphological, structural and functional properties of these composite films were evaluated. The X-ray analysis showed that the modification of montmorillonite with depolymerized chitosan at molecular weight of 15 kDa appeared to induce the intercalation occurred in the modified montmorillonite. FTIR analysis confirmed that nitric acid oxidized some areas of bamboo charcoal particles during refluxing, leading to formation of carboxylic acid groups on the surface of the bamboo charcoal particles. Investigation of the mechanical properties of these composite films revealed that the tensile strength and rigidity were both found to increase, whilst the elongation at break decreased, with increasing either virgin or modified montmorillonite and bamboo charcoal in chitosan composite films. Inclusion of either unmodified montmorillonite or modified montmorillonite in chitosan films decreased the water and oil absorption of the composite films. Oil, and especially water, absorption of the chitosan/modified bamboo charcoal composite films increased with inclusion of either unmodified bamboo charcoal or modified bamboo charcoal in chitosan films. Inclusion of either modified montmorillonite or modified bamboo charcoal into chitosan films enhanced the thermal stability of chitosan composite films. SEM micrographs showed a good dispersion of modified montmorillonite and bamboo charcoal particles in chitosan matrix up to 0.5% (w/w), while some aggregation of montmorillonite or bamboo charcoal were observed at the content starting of 1% (w/w) of the chitosan composite films. The growth of *Colletotrichum gloeosporiodes* was completely inhibited by chitosan and chitosan composite films.

Department : Materials Science   Student's Signature _Walaikorn Nitayaphat_

Field of Study : Materials Science   Advisor's Signature _____

Academic Year : 2009   Co-Advisor's Signature _____

    Co-Advisor's Signature _____

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LIST OF ABBREVIATIONS

CTS	:	Chitosan
MMT	:	Montmorillonite
mMMT	:	Modified montmorillonite
BC	:	Bamboo charcoal
mBC	:	Modified bamboo charcoal
XRD	:	X-ray diffraction
SEM	:	Scanning electron microscope
kDa	:	Kilo Dalton
MPa	:	Mega pascal
rpm	:	Revolution per minute
et al.	:	et alibi
NMR	:	Nuclear magnetic resonance
HPLC	:	High performance liquid chromatography
UV	:	Ultra violet
FTIR	:	Fourier transform infrared spectroscopy
etc.	:	et cetera

CHAPTER I

INTRODUCTION

In recent years, increasing interest in degradable plastics has developed mainly due to awareness of the waste of petroleum-based plastics. Plastic wastes require a long time of degradation and most of them end up over burdening on landfill. Today, incineration is a common method to get rid of synthetic plastic materials, but this unfortunately leads to high emission of CO_2 to the atmosphere. One approach for solving the problem is to use biodegradable materials instead of non-renewable polymers. These materials have the potential to reduce environmental pollution by lowering solid disposal waste and reducing the need for incineration [1].

Among various natural polymers, chitosan is considered as one of the most promising candidates for the future biodegradable material because of its attractive combination of availability, price and performance.

Chitin and chitosan, which are extracted from various animals and plants, are the second most abundant natural biopolymers found on earth next to cellulose. Chitin exists widely in cell walls of some microorganisms such as fungi, molds, and yeasts [2] and in the cuticular and the exoskeletons of invertebrates such as crustaceans, mollusks, crabs, shrimps, lobster, squid and insects (for example beetles). Chitosan exists naturally only in a few species of fungi [3]. Chitin and chitosan consist of 2-acetamido-2-deoxy-**β**-D-glucose and 2-amino-2-deoxy-**β**-D-glucose as repeating units, respectively. Chitin and chitosan are chemically identical to cellulose except that hydroxyl groups in cellulose molecule are substituted with acetamide groups in chitin and amino groups in chitosan. Normally, chitosan dissolves readily in dilute

solutions of most organic acids such as acetic acid, formic acid, etc. In acid solutions, amine groups of chitosan are protonated to NH_3^+ leading to the cationic property. Since most other soluble biopolymers become anionic in water, chitosan cations exhibit good affinity for other biopolymers. Because of its biocompatibility, biodegradability, antimicrobial activity and non-toxicity, chitosan has been extensively investigated for several decades for uses in biomaterials, wastewater treatment, cosmetics, food packaging, and textiles [4]. However, its properties, such as thermal stability, mechanical properties and gas barrier properties are frequently not sufficient to meet the requirements of these wide ranges of applications [5-10].

Several studies have been carried out on chitosan-based films obtained by casting from a solution or gel with addition of plasticizers [11-12]. Even so, chitosan films have poor mechanical properties when compared to those of synthetic polymers. This is due to their hydrophilic nature and their sensitivity to moisture content which are difficult to control. In order to improve mechanical properties and thermal stability, chitosan can be modified by several methods such as blending with synthetic [13-15] or natural polymers [16-17], preparing in the composite form [18-24], and cross-linking [25-26].

The preparation of chitosan composites is aimed to improve the mechanical properties of materials. Commonly, addition of inorganic fillers to a polymer matrix has been investigated.

Montmorillonite, the main component of bentonite, is a layered aluminosilicate mineral that belongs to the montmorillonite/smectite group of clay minerals. The crystals of montmorillonite consist of three layers: a silicon tetrahedron, an aluminum octahedron, and another silicon tetrahedron. The unit structure is a very

thin platelet (about 10 Å [1 nm] thick, but over 200 times that in width). The stacks of clay platelets are held tightly together by electrostatic forces. The structure of montmorillonite contains counterions, which are attracted to the net negative charge within the clay platelets. The cations are not strongly bound to the clay surface. Therefore, the other cations can replace the cations presented in the clay. Under the proper conditions, the gallery spaces can be filled with monomer, oligomer, or polymer. Its advantages of high surface area, large aspect ratio (50-1000), and platelet thickness of 10 Å make it suitable for reinforcement purposes [27].

Bamboo (*Dendrocalamus asper* Backer) charcoal powder contains many pores and gaps in its structure, making it excellent for absorption, electromagnetic shielding and infrared emission [28]. Bamboo charcoal powder also contains ions, such as the anions calcium, sodium and iron, and is used in water and air purification, improving the acidity condition [28-29], and is widely used in the fields of food cooking, baking, stocking, and keeping food produce fresh.

Chitosan, montmorillonite, and bamboo are plentiful, relatively cheap, renewable, biodegradable, and relatively non-toxic and sustainable products. The aim of this research is to evaluate the performance of chitosan composite films which were incorporated with montmorillonite relative to those with bamboo charcoal. Chitosan/montmorillonite composite films and chitosan/bamboo charcoal composite films were prepared by using a spin coater machine. The morphological, structural and functional properties of these hybrid films were investigated.

CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Chitosan

Chitin and chitosan, which are extracted from various animals and plants, are the second most abundant natural biopolymers found on earth next to cellulose. Chitin exists widely in cell walls of some microorganisms such as fungi, molds, and yeasts [2] and in the cuticular and the exoskeletons of invertebrates such as crustaceans, mollusks, crabs, shrimps, lobster, squid and insects (for example beetles), while chitosan exists naturally only in a few species of fungi [3].

Table 2.1 Sources of chitin and chitosan [30].

Arthropods	Insect	Microorganisms
Annelida	Scorpion	Green algae
Molluska	Spider	Yeast (β-type)[b]
Coelentera	Brachiopods	Fungi (cell wall)[b]
Crustaceans		
Lobster[a]	Ant	Penicillium
Shrimp[a]	Cockroach	Brown-green algae
Prawn[a]	Bug	Spore
Krill		Chytridiacee
Crab[a]		Blastocladiaceae
		Ascomydes

[a]sources in the present, [b]sources in the future when the demand increases

Chitin and chitosan consist of 2-acetamido-2-deoxy-**β**-D-glucose and 2-amino-2-deoxy-**β**-D-glucose as repeating units, respectively. Chitin and chitosan are chemically identical to cellulose except that hydroxyl groups at position C-2 in cellulose molecule are substituted with acetamide groups in chitin and amino groups in chitosan.
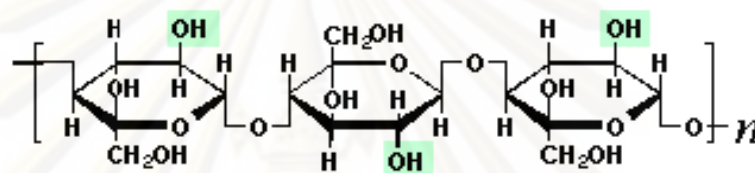
(a) cellulose
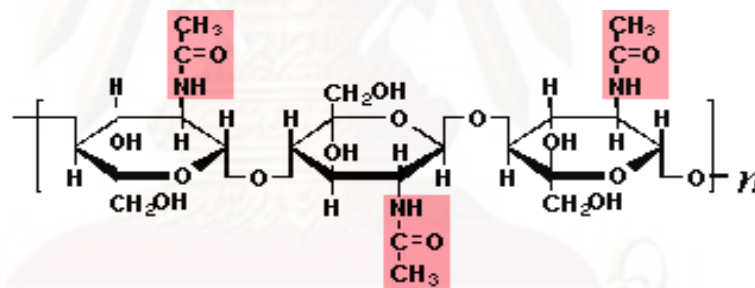
(b) chitin
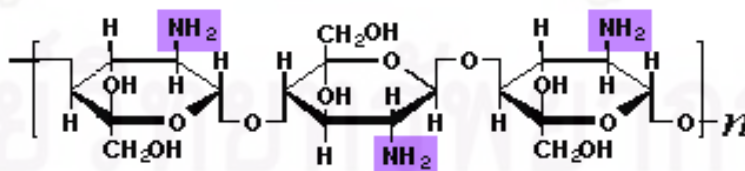
(c) chitosan

Figure 2.1 Similarities of chemical structures of cellulose, chitin, and chitosan [3].

Being a biopolymer, chitosan is recommended as suitable resource material for various applications. It has uniquely excellent properties such as biogradability,

biocompatibility, non-toxicity, absorption property and so on. However, the naturally abundant material also exhibits a limitation in reactivity and processability [31].

Chitosan has been extensively used over a wide range of applications. For example, a biomaterial in medicine either its own or as a blend component, a water membrane filter for treatment, a biodegradable and edible coating or film in food packaging [32].

2.1.1 Procedures of Chitin Extraction [33]

❖ Raw materials preparation

The different local resources used in the extraction of chitin are described in Table 2.1. The shells of these species were scraped free of loose tissue, washed, dried, grounded to pass through a 250 μm sieve, then subjected to demineralization and deproteinization.

❖ Demineralization

Demineralization was carried out in dilute HCl solution. The mineral content in the exoskeleton of crustacean is not the same in each specie. All species except for cuttlefish were treated with 0.25 M HCl solution at ambient temperature for 2 hours with a solution-to-solid ratio of 40 mL/g, whereas 1.0 M HCl was used to demineralize the cuttlefish pens.

❖ Deproteinization

Deproteinization of chitin was carried out using 1.0 M NaOH (20 mL/g) at 70 ºC. The treatment was repeated several times until the medium is colorless.

❖ Decoloration

Decoloration of chitin may be achieved by use of organic solvents such as acetone for 10 min. The use of cold formic acid or mixtures of NH_2SO_4 and H_2SO_4 are alternative was to remove the pigments of chitin.

2.1.2 Procedure of Making Chitosan

There are two methods used to convert chitin to chitosan. First, chitin extracted from different species was treated with 45% NaOH (15 mL/g) at 110 ºC for 96 hours. Microwave radiation is an alternative method in making chitosan to decrease the long processing time required to complete N-acetylation.

A flowchart of chitin extraction and chitosan preparation is shown in Fingure 2.2.
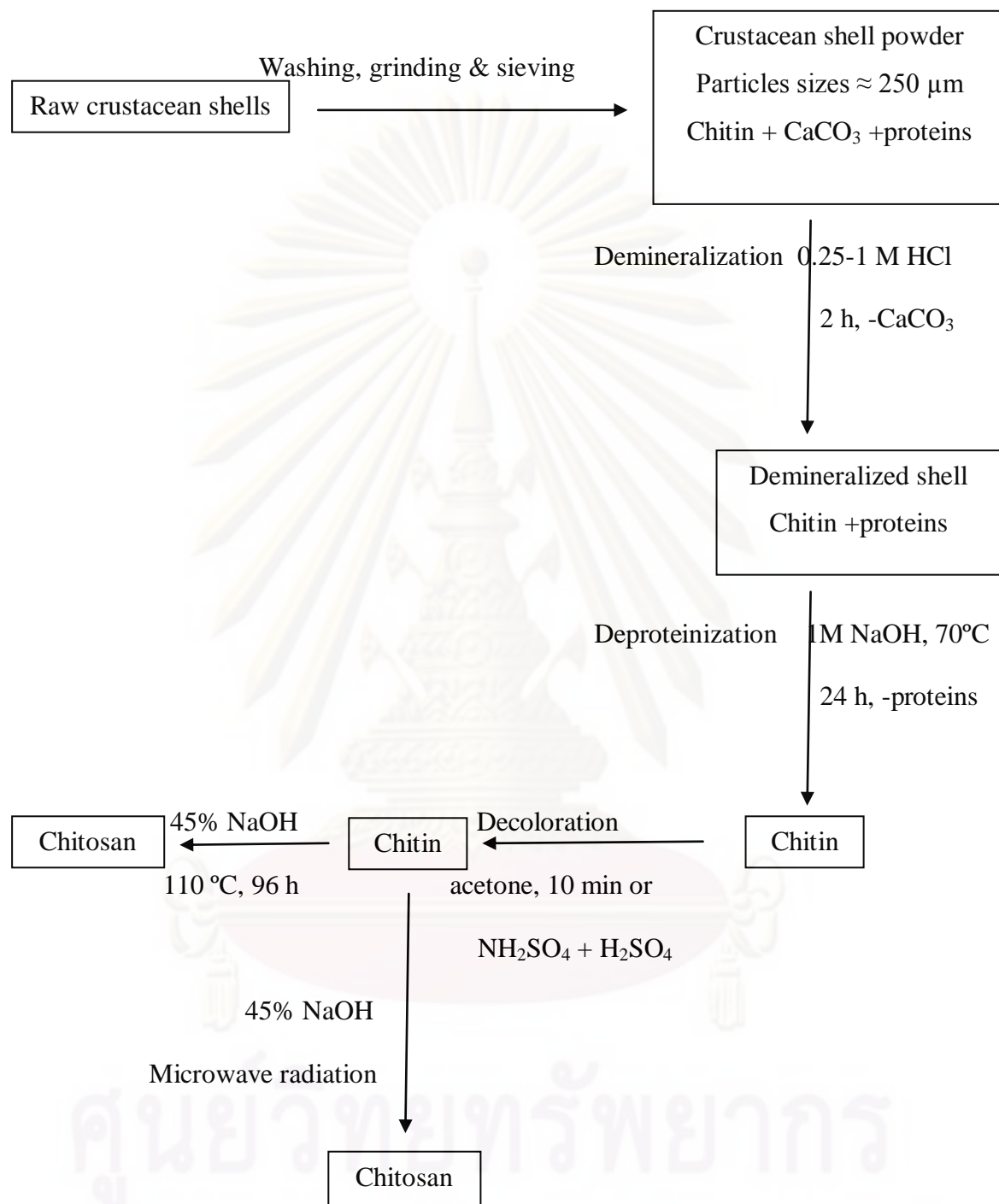
Figure 2.2 Chitin extraction and chitosan preparation [33].

The property of each chitosan product may be varied widely from one another due to many factors used in the manufacturing process of chitosan. Various commercial grades of chitosan contain different molecular weights, viscosities, degrees of deacetylation, and purities.

2.1.3 Physical and Chemical Characterization of Chitosan

Chitosan can be characterized in terms of its quality, intrinsic properties (purity, molecular weight, viscosity, and degree of deacetylation), and physical forms.

Most of the naturally occurring polysaccharides, e.g. cellulose, dextran, pectin, aliginic acid, agar, agarose and carragenans, are neutral or acidic in nature, whereas chitin and chitosan are examples of highly basic polysaccharides. Like cellulose, chitosan functions naturally as a structural polysaccharide, but differs form cellulose in its properties. It especially has amino groups, which posses many properties different from cellulose such as solubility in dilute organic acid, effective capability of binding with metal ions and its biological effect including the unique properties such as polyoxysalt formation, ability to form film, chelate metal ions and optical structural characteristics [34]. The properties of chitosan such as the degree of deacetylation, molecular weight, and solution properties are discussed in brief.

2.1.3.1 Degree of Deacetylation

Degree of deacetylation is one of the important chemical characteristics, which could influence the performance of chitosan in many of its applications [35-36]. The degree of deacetylation determines the content of free

amino groups in chitinous material that can be employed to differentiate between chitin and chitosan. Chitosan could be defined as chitin sufficiently deacetylated to form soluble amine salts. The degree of deacetylation necessary to obtain a soluble product must be 80-85% or higher because the acetyl content of the chitosan product must be less than 4-4.5%. Chitosan products are highly viscous, resembling natural gums.

To define the degree of deacetylation, attempts have been made with many analytical tools, which include IR spectroscopy, pyrolysis gas chromatography, gel permeation chromatography and UV spectrophotometry, first derivative of UV spectrophotometry, ^{1}H NMR spectroscopy, ^{13}C solid state NMR, thermal analysis, various titration schemes, acid hydrolysis and HPLC, separation spectrometry methods and, more recently, near-infrared spectroscopy.

2.1.3.2 Molecular Weight

Chitosan is polydipersed with different chains lengths. The random distribution of glucosamine to N-acetyl-glucosamine is often expressed as the degree of deacetylation. Chitosan molecular weight distributions have been obtained by HPLC. The weight average molecular weight ($\overline{M}_w$) of chitin and chitosan has been determined by light scattering.

Viscometry is a simple and rapid method for the determination of molecular weight; the constants α and K in the Mark-Houwink equation have been determined in 0.1 M acetic acid and 0.2 M sodium chloride solution. The intrinsic viscosity is expressed as shown below

$$[\eta] = KM^{\alpha} = 1.81 \times 10^{-3} M^{0.93}$$

Converting chitin into chitosan lowers the molecular weight, changes the degree of deacetylation, and thereby alters the charge distribution, which in turn influences the agglomeration. The weight-average molecular weight of chitin is 1.03×10^6 to 2.5×10^6, but the N-deacetylation reaction reduces this to 1×10^5 to 5×10^5. Increasing the number of deacetylation step promotes an increase in the degree of deacetylation and a decrease in the average of molecular weight and polydispersion of chitosan.

2.1.3.3 Solution Properties

Chitosan is insoluble in water or in acid solutions at pH about 6.5, or in organic solvents. Normally, chitosan dissolves readily in dilute solutions of most organic acids, including formic, acetic, tartaric, and citric acids. Chitosan is soluble to a limited extent in dilute inorganic acids except phosphoric and sulfuric acids. Most properties of chitosan relate to its polyelectrolyte and polymeric carbohydrate character. In acid solutions, amine groups of chitosan are protonated to NH_3^+ leading to the cationic property as shown in Figure 2.3. Chitosan cations exhibit good affinity for other biopolymers because of most other soluble biopolymers become anionic in water.

Figure 2.3 Cationic property of chitosan [37].

The cationic properties offer good opportunities to take advantage of electron interactions with numerous compounds during processing and incorporating specific properties into the materials. The cationic property may further be used for incorporation and/or slow release of active components, adding to possibilities for the manufacturer to tailor the properties [37].

Chitosan is not one chemical entity, but varies in composition depending on manufacturing. The physicochemical properties of solutions of chiotsan are expected to be governed by many factors, such as temperature, pH, ionic strength, surfactant concentration, and degree of deacetylation. In addition, it is known that the charge density along the chain increases with an increase in degree of deacetylation, and the chain flexibility of chitosan molecules can be manipulated by changing degree of deacetylation.

2.1.4 Chitosan Applications

A wide range of chitosan properties, such as antimicrobial activity, non-toxicity, and biodegradability, attract scientific and industrial interest. Many potential products using chitosan have been developed, including flocculating agents for waste treatment, chelating agents for removal of traces of heavy metals from aqueous solutions, coatings to improve dyeing characteristics of glass fibers, wet strength additives for paper, adhesives photographic and printing applications, thickeners, fibers and films [38]. Especially, in films and coatings applications, it has been used as fruit coatings to prolong conserving period, as preservative, and as wound coating film to accelerate the wound healing process. Some novel applications involving chitosan include biodegradable fish hooks and surgical sutures, coated

paper and transparencies for inkjet ink, biodegradable implants and vascular prostheses, and low-fat whipping cream and ice cream [39-42].

2.1.5 Chitosan Films and Properties

Chitosan has been extensively investigated for several decades for uses in a variety of applications. Chitosan can form transparent films, which may find applications in a variety of packaging needs. Chitosan films can be easily obtained by casting its solution on a desired surface and allowing spontaneous solvent evaporation. The examples of films or membranes produced from chitosan are as follows.

Chitosan membranes can also be formed by making rigid films with crosslinking agent, such as glutaraldehyde and diepoxy compounds [39]. Chemically modified chitosan membranes can be used for separating ethanol from water pervaporation [40], and chitosan membranes are applied to water purification as well [41].

Chitosan films and coatings applied to fruits and vegetables can reduced water loss and extend shelf-life [42]. Chitosan films have been currently used in pharmaceutical industry [41], wastewater separation membranes, and edible protective coatings on minimally processed food.

Most mechanical properties of chitosan films are comparable to those of many medium-strength commercial polymers. The important factors are molecular weight and degree of deacetylation of chitosan. It has been suggested by Muzzarelli and co-worker [43] that tensile strength of chitosan film increased with increasing molecular weight of chitosan. Chitosan forms hydrogen bonds between hydroxyl

groups and amino groups in chitosan film [39]. During the film formation, hydrogen bonding in the chitosan film increased with the increasing amount of amino and hydroxyl groups, due to the increase in concentration of chitosan. In addition, film-making conditions, including solvent pH, ionic strength, type of acid solutions used and annealing treatment are parameters often manipulated to alter the mechanical properties and membrane porosity of chitosan films. Ionic strength or pH can be manipulated in order to reduce inter- and intramolecular electrostatic repulsion between chitosan chains, thus allowing the chains to approach each other and enhance the inter- and intramolecular hydrogen bonding during film formation [44]. Park and co-workers [45] explained the effects of molecular weight and organic acid solution properties on chitosan film properties. For example, molecular weight and molecular dimension of chitosan were measured by light scattering method. They showed that molecular weight of chitosan dissolved in acetic acid was larger than that dissolved in the other three acid solutions. In the acetic solution, chitosan forms dimmers indicating that the intermolecular interaction is relatively strong, which suggests that the chitosan films prepared with acetic acid had tighter structure than those prepared with other acid solutions.

Chitosan film has excellent O_2 and CO_2 barrier properties, but poor water vapor resistance. The O_2 permeability of the film was affected by type of acid and molecular weight of chitosan used. Generally, O_2 permeability value of low molecular weight chitosan film was lower than that of chitosan films with high molecular weight chitosan. As indicated by Butler and co-workers [46], O_2 permeability values of chitosan film are comparable with commercial polyvinilidene

chloride or ethylene vinyl alcohol copolymer films. Low oxygen permeability of chitosan films can exploited for food and medical packaging applications.

Films made from chitosan have two characteristics highly desirable to the food industry: they are biodegradable and they have low permeability to O_2. At present, those beneficial characteristics of chitosan films come at the expense of other desirable properties such as tensile strength. However, mechanical and barrier properties of chitosan films can be controlled trough choosing appropriate molecular weight of chitosan and solvent system. The chitosan films with tailored properties can be useful in the food and medical packaging applications. It has been recently reported that advanced genetic engineering techniques can potentially change the distribution of molecular weights in chitosan, particularly which derived from fungi. That would permit scientists to change such characteristic of films as tensile strength, flexibility, gas permeability, and rate of degradation in the environment.

2.2 Clay Minerals

Clay is a cheap natural raw material that has been widely used for many years as filler for rubber and plastic to reduce polymer consumption and cost. Geologists have used the word "clay" in two senses: as a size term, to refer to material of any composition whose average grain size is less than approximately 0.004 mm, and as a mineralogical term, to refer to a group of minerals with a specific range of composition and a particular kind of crystallographic structure. The two meanings often overlap or coincide because the fine grained part of a soil or sediment commonly consists largely of clay minerals.

In general, the term clay implies a natural, earthy, fine grained material which develops plasticity when mixed with a limited amount of water. By plasticity, it means that the property of the moistened material is deformed under the application of pressure, with the deformed shape being retained when the deforming pressure is removed. According to chemical analysis of clays, it composes of silica, alumina, and water, frequently with appreciable quantities of iron, alkali, and alkaline earth metals. The two major types of clay minerals are 1:1 and 1:2 type minerals [47].

<u>1:1 type minerals</u>

The 1:1 clay-mineral type consists of one tetrahedral sheet and one octahedral sheet. These two sheets are approximately 7 A° thick as shown in Figure 2.4.

| Tetrahedral |
| Octahedral |

7 Å
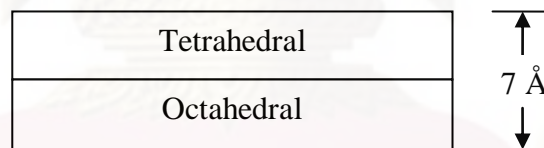
Figure 2.4 1:1 type minerals [46].

A kaolinite mineral is one of 1:1 type minerals. The structure is composed of a single silica tetrahedral sheet and a single alumina octahedral sheet combined in a unit so that the tips of the silica tetrahedrons and one of the layers of the octahedral sheet form a common layer.

2:1 type minerals

The three sheets or 2:1 layer lattice silicates consist of two silica tetrahedral sheets between which is an octahedral sheet. These three sheets form a layer approximately 10 A° thick as shown in Figure 2.5.
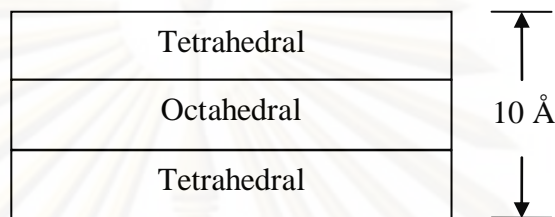
Figure 2.5 2:1 type minerals [47]**.**

Clays are extremely fine grained (<2 µm), creating a large surface area (per unit mass) on which reactions can occur. Most clay minerals have a negative charge within the tetrahedral-octahedral layers owing to isomorphic substitutions. The charge is balanced by cation from the surrounding soil solution that attaches to the surface of the crystallites. These cations exchange easily and are a major source of plant nutrients. Soil scientists define cation exchange capacity (CEC) as the amount of negative charge in the tetrahedral-octahedral layers per 100 g soil. Soil minerals with a higher CEC can hold on the more soil ions and are more reactive, benefiting plants. Examples of surface area and cation exchange capacity of some clays are shown in Table 2.2.

Table 2.2 Surface area and cation exchange capacity of some clay minerals and humus [48].

Mineral	Surface area (10^3 m^2/kg)	Cation exchange capacity (cmol charge/kg)
Kaolinite	10-20	1-10
Chlorite	70-150	20-40
Mica	70-120	20-40
Montmorillonite	600-800	80-120
Vermiculite	600-800	120-150
Humus	900	150-300

The 2:1 layer typed aluminosilicate can be classified into the following subgroups: pyrophylite, smectite, vermiculite, illite, mica, and chlorite. The layer charge density of these minerals are shown in Table 2.3.

Table 2.3 Classification of clay in phyllosilicates types [49].

Layer type	Group	Subgroup	Species
1:1	Kaolinite-serpentine (x~0)	Serpentine (Tr) Kaolinite (Di)	Chrysotile, Amesite, Lizardite, Kaolinite, Dickite, Halloysite
	Pyrophyllite-talc (x~0)	Talc (Tr) Pyrophyllite (Di)	Talc Pyrophyllite
	Smectite (x~0.2-0.6)	Tr smectite Di smectite	Saponite, Hectorite, Sauconite Montmorillonite, Bentonite, Beidellite
	Vermiculite (x~0.6-0.9)	Tr vermiculite Di vermiculite	Trioctahedral vermiculite Dioctahedral vermiculite
2.1	Illite (x<0.9-0.6)	Tr illite Di illite	
	Mica (x~1.0)	Tr mica Di mica	Biotite, Phlogopite, Lepidolite Muscovite, Paragonite
	Brittle mica (x~2.0)	Tr brittle mica Di brittle mica	Clintonite, Anandite Margarite
	Chlorite (x variable)	Tr, Tr chlorites Di, Di chlorites Di, Tr chlorites Tr, Di chlorites	Common name based on Fe3+, Mg2+ Mn2+, Ni2+, Ponbassite Sudoite, Codecite (Li)

Note: x = charge per formula unit, Tr = Trioctahedral, Di = Dioctahedral

Typical property values of some of these clays are shown in Table 2.4 and the chemical formula of commonly used 2:1 type clays are shown in Table 2.5.

Table 2.4 Summary of properties of different clays [47]**.**

Type	Size (mm)	Surface area (m^2/g)		Interlayer spacing (nm)	Cation sorption (meq/100g)
		External	Internal		
Kaolinite	0.1-5.0	10-50	-	0.7	10-50
Smectite	< 0.1	70-150	500-700	1.0-2.0	85-110
Vermiculite	0.1-5.0	50-100	450-600	1.0-1.4	100-120
Illite	0.1-2.0	50-100	5-100	~1.0	15-40

Table 2.5 Chemical formula of commonly used 2:1 layer type aluminosilicate [49].

Clay	General formula
Montmorillonite	$M_x(Al_{4-x}Mg_x)Si_8O_{20}(OH)_4$
Hectorite	$M_x(Mg_{6-x}Li_x)Si_8O_{20}(OH)_4$
Saponite	$M_xMg_6(Si_{8-x}Al_x)O_{20}(OH)_4$

Note: M = Mono valent cation, x = degree of isomorphous substitution

2.2.1 Montmorillonite

Montmorillonite clays are relatively common throughout the world. Deposits of commercial clay are referred to as bentonite, which generally contains in excess of 50% montmorillonite. Conventional purification methods are adequate for the clays used in most common applications, such as binders for metal casting, well-drilling legs, and cosmetics.

Montmorillonite is one in smectite group which has a low thermal expansion coefficient and a high gas barrier property. The crystals of montmorillonite consist of three layers: a silicon tetrahedron, an aluminum octahedron, and another silicon tetrahedron. The unit structure is a very thin platelet (about 10ºA [1 nm] thick and 0.1 to 1 μm wide).
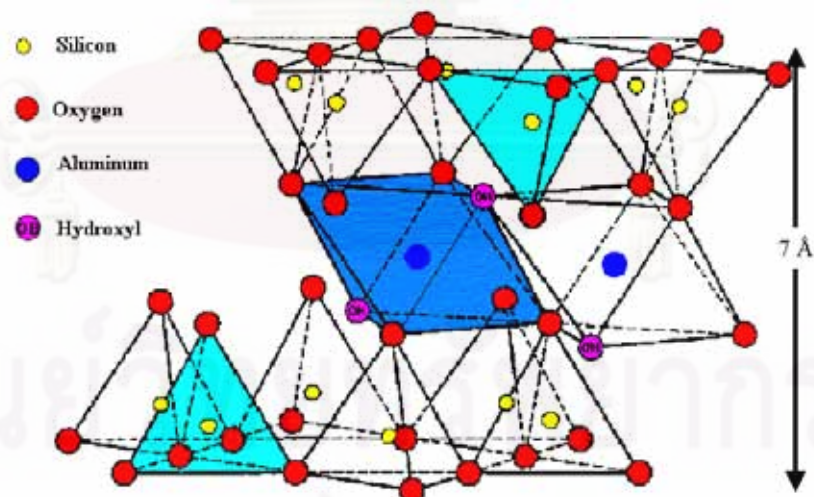
Figure 2.6 The structure of montmorillonite [50].

In montmorillonite, some of the Al ions are substituted by magnesium ions, resulting in a negative charge on the two large surfaces of montmorillonite

platelets. This negative charge is neutralized by an interlayer of cations (e.g., Na^+, K^+, Ca^+, and Mg^{2+}) that separates adjacent layers of platelets. This structure makes montmorillonite chemically stable. The specific characteristics of montmorillonite are due to various actions of its surface negative charge and interlayer cations.

2.2.1.1 Modification of Montmorillonite

Montmorillonite and other layered silicate clay are naturally hydrophilic. This makes them poorly suited to mixing and interacting with most polymer matrices. Moreover, the stacks of clay platelets are held tightly together by electrostatic forces. Figure 2.7 shows the structure of montmorillonite containing counterions, which are attracted to the net negative charge within the clay platelets. The couterions can be shared by two neighboring platelets, resulting in stacks of platelets that are tightly held together.

Figure 2.7 The stacks of montmorillonite platelets [51].

A popular and relatively easy method of modifying the clay surface, making it more compatible with an organic matrix, is ion exchange. The cations are not strongly bound to the clay surface. Therefore, the other cations can

replace the cations presented in the clay, as shown in Figure 2.8. Under the proper conditions, the gallery spaces can be filled with an organic cation molecule, such as cationic surfactant, monomer, oligomer, or polymer.
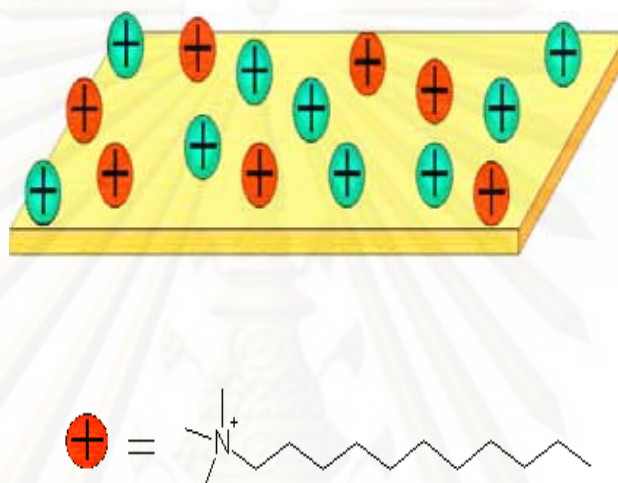
Figure 2.8 Ion-exchange of montmorillonite [51].

The functions of organic cation molecule in organophilic-clay are to lower the surface free energy of the silicate layers and to improve the wettability behavior of hydrophobic polymer matrix. In addition, the organic cation may contain various functional groups that can react with the polymer molecule to improve the adhesion strength between the inorganic phase and the matrix as reported by Giannelis [52].

2.3 Bamboo Charcoal

Charcoal is the blackish residue consisting of impure carbon obtained by removing water and other volatile constituents from animal and vegetation substances. It is usually produced by heating wood in the absence of oxygen, but sugar bone charcoal (which contains a great amount of calcium phosphate), and others can be produced as well. The soft, brittle, light, black, and porous material consists of around 85- 98% of carbon, and a few percentage of volatile chemicals ash, and resembles coal.

The term of "coal" in English was refered to charcoal. This compound term, the prefix "chare-" meant "turn", with the literal meaning being "to turn to coal". The independent use of "char", meaning to scorch, to reduce to carbon, is comparatively recent and must be a back-formation from the earlier charcoal. It may be a use of the word *charren*, meaning to turn, i.e., wood changed or turned to coal; or it may be from the French *charbon*. A person who manufactured charcoal was formerly known as a collier, though the term was used later for those who dealt in coal, and the ships transporting it [53].

2.3.1 Bamboo Charcoal Production

Bamboo charcoal is made of bamboo by pyrolysis process. According to the types of raw material, bamboo charcoal can be classified as raw bamboo charcoal and bamboo briquette charcoal. Raw bamboo charcoal is made of bamboo body such as culms, branch, and root. Bamboo briquette charcoal is made of bamboo residue such as bamboo dust and saw powder by compressing the residue into certain

shape sticks and carbonizing the sticks. Bamboo production process is divided into three stages: raw material preparation, carbonization, finishing process as shown in Figure 2.9.
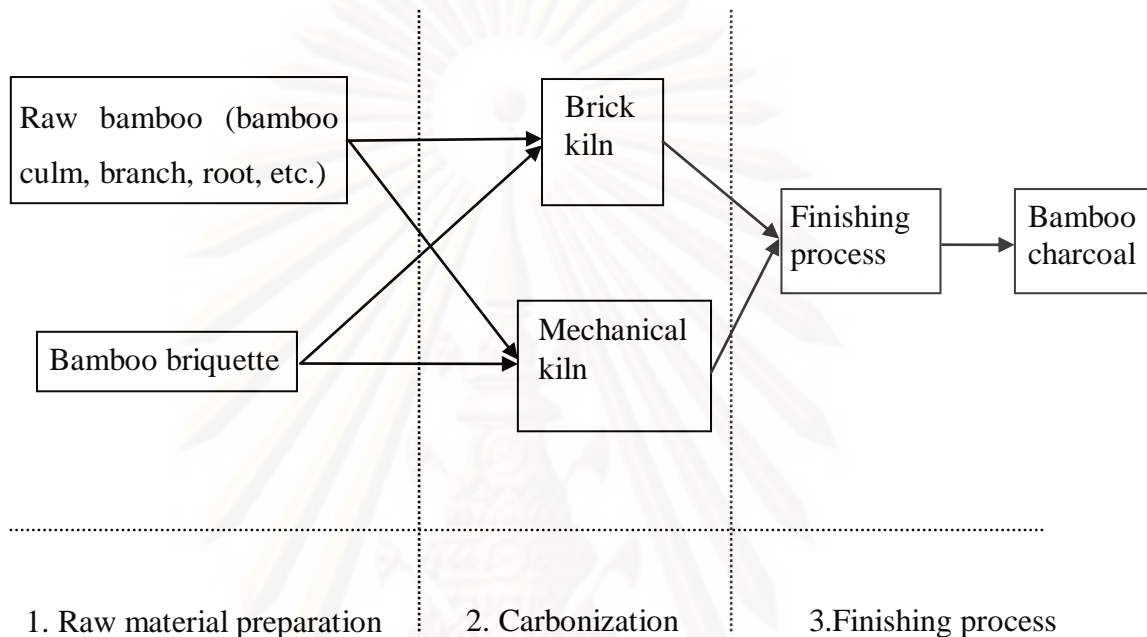
Figure 2.9 Three steps of making bamboo charcoal [54].

2.3.1.1 Raw Bamboo Charcoal Products

Raw material for making bamboo charcoal made of bamboo culms can be divided into bamboo tube, bamboo slice. For making raw charcoal, the bamboo at the age of four years or more should be selected. The culms should be chopped into segments according to the size of kiln before drying. The moisture content of raw bamboo should be 15%-20% by air drying before loading into kiln.

Bamboo clums

Bamboo tube

Bamboo slice

Bamboo charcoal tube

Bamboo charcoal slide

Bamboo charcoal particle
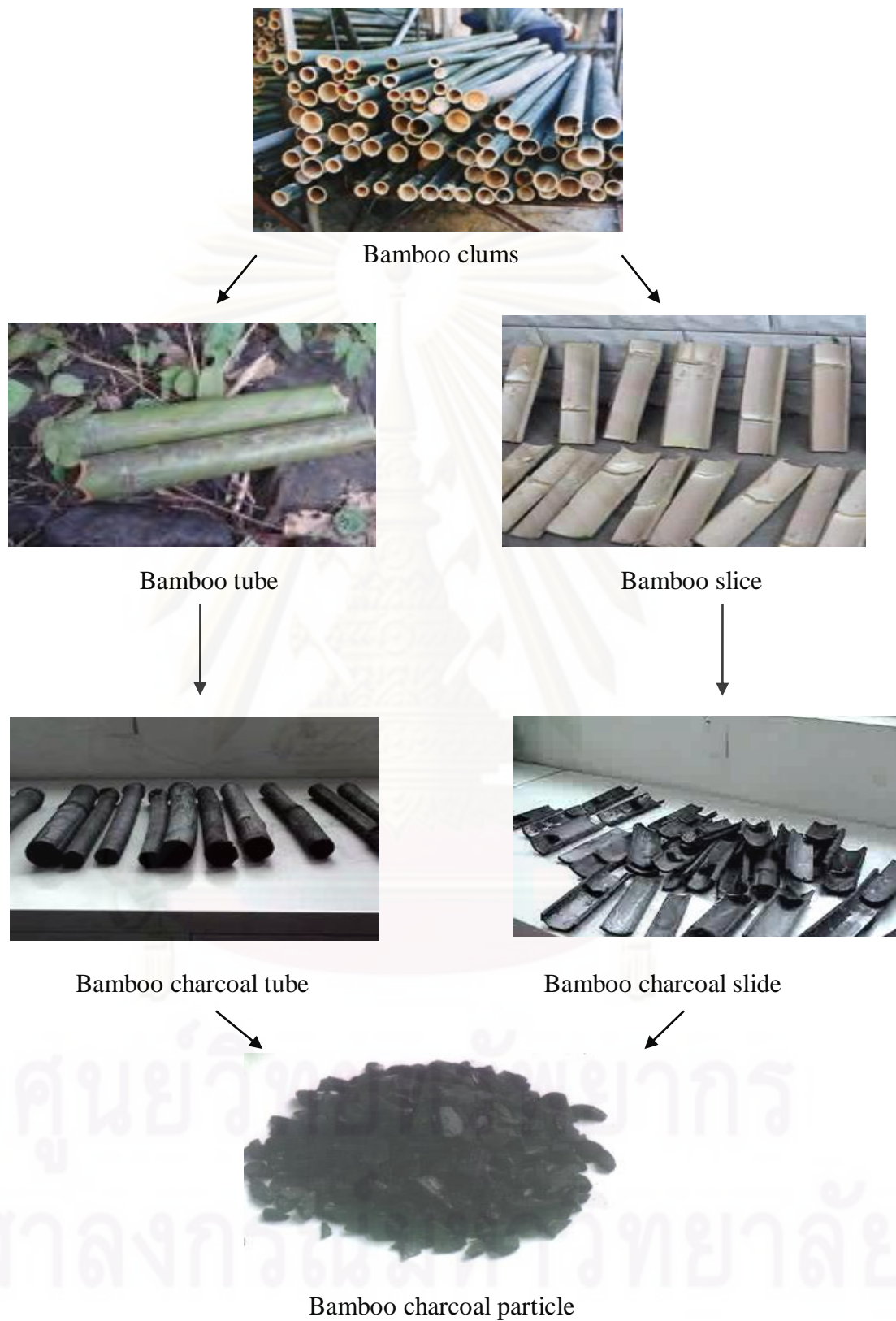
Figure 2.10 Bamboo charcoal particle preparation [54].

2.3.1.2 Carbonization of Bamboo Charcoal

Bamboo carbonization can be divided into four stages according to temperature and product situation in a kiln [55].

First stage of drying: the temperature is below 120ºC and the speed of carbonization is slow. Heat is used to evaporate the water in bamboo, and the chemical composition of the bamboo is still intact.

Second stage of pre-carbonization: the temperature is in the range of 120ºC to 260ºC and there is a distinct chemical reaction in bamboo. The unstable chemical compounds begin to decompose and carbon dioxide and carbon monoxide are released.

Third stage of carbonization: the temperature is in the range of 260ºC to 450ºC, and the bamboo is decomposed into liquid and gas products. Liquid products contain much acetic acid, methanol and bamboo tar. The most flammable methane and ethylene in gas products are increased while carbon dioxide production is reduced.

Fourth stage of calcination (refining stage): the temperature is over 450ºC. The bamboo becomes charcoal by means of providing a mass of heat, emitting the volatile substances and enhancing nonvolatile carbon. Based on the temperature in this stage, the bamboo charcoal can be divided into three groups (low-temperature, middle-temperature and high-temperature charcoal). The quality and properties of bamboo charcoal differs with different temperatures during the refining stage.

Lastly the bamboo charcoal is left to cool down under ambient condition. Cool down process for the big volume of bamboo charcoal needs five to eight days.

During the carbonization process, it can be extracted alcohol, tar, vinegar, medicinal liquid (water form), and many other products. Products from bamboo charcoal have countless uses for skin diseases, allergies, influenza, heart diseases, stomachaches, insecticides, pesticides, germicides, bactericides, deodorants, disinfectants, gardening, cosmetics, cooking, washing, and hundreds of others.

```
┌──────────────┐   ┌──────────────┐   ┌──────────────┐   ┌──────────────┐
│ Feeding raw  │──▶│   Drying     │──▶│Pre-carboniza-│──▶│ Carbonization│
│material into │   │at below 120°C│   │tion at120-   │   │  at120-240°C │
│    kiln      │   │              │   │   240°C      │   │              │
└──────────────┘   └──────────────┘   └──────────────┘   └──────────────┘
                                                                 │
                                                                 ▼
┌──────────────┐   ┌──────────────┐   ┌──────────────┐
│    Bamboo    │◀──│ Cooling down │◀──│Refine at high│
│  briquette   │   │   to 60°C    │   │ temperature  │
└──────────────┘   └──────────────┘   └──────────────┘
```
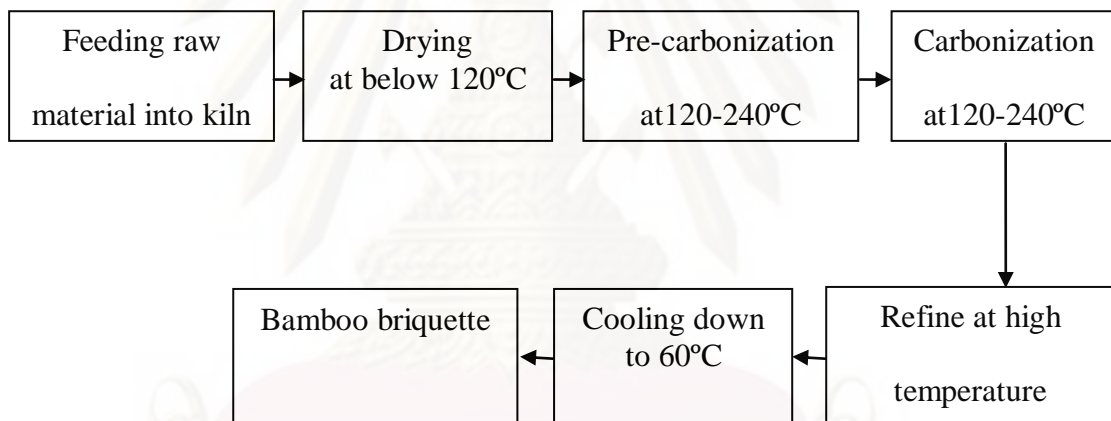
Figure 2.11 Process flowing chart of bamboo carbonization [54].

2.3.1.3 Finishing Process of Bamboo Charcoal

Finishing process of bamboo charcoal starts with sorting, finishing processing including checking and packing.

2.3.2 Basic Properties of Bamboo Charcoal

The effects of carbonization temperature on bamboo charcoal properties are shown in Table 2.6.

Table 2.6 Properties of bamboo charcoal obtained from different temperatures [54].

Item of Properties	Temperature of Carbonization （°C）							
	300	400	500	600	700	800	900	1000
Yield rate (%)	40.70	35.6	29.68	28.96	27.52	27.43	26.39	26.69
Ash (%)	2.93	3.48	3.54	3.92	4.07	4.58	4.69	4.57
Volatile content (%)	30.98	25.24	14.41	7.20	3.24	2.71	2.7	2.11
Fixed carbon (%)	66.69	71.28	82.05	88.88	92.69	92.71	92.61	93.32
Density (g/cm^3)	0.564	0.593	0.599	0.638	0.690	0.687	0.712	0.723
Specific resistance (10^{-2}Ω•m)	18.6	9.5	7.4	3	5.4	2.15	1.45	8.29
Specific area (m^2/g)	23	133	316	360	385	259	133	35

Note: Yield rate means the percentage of final bamboo charcoal made from raw bamboo.

Information provided in Table 2.4 could be concluded as follows:

(1) The higher carbonization temperature, the lower the yield rate.

(2) Ash, fixed carbon and density of bamboo charcoal are positively correlated to the temperature of carbonization.

(3) Increasing carbonization temperature from 300ºC to 900ºC decreased the value of specific resistance from high to low.

(4) Increasing carbonization temperature going up from 300ºC to 700 ºC. The value of specific area increased from 23 to 385, and then gradually decreased down to 35 at 1000ºC of carbonization temperature.

2.3.3 The Major Characteristics of Bamboo Charcoal Powder

The bamboo charcoal power has the ability to absorb natural minerals, filth, and pollutant. The bamboo charcoal powder also contains calcium, sodium, and irons etc., therefore, it can produce anions to purify the air and improve the acidity condition. The major characteristics of the bamboo charcoal powder are as follows [56].

a) Anions

Some anions are not harmful to human body. Sometimes it is found that the more anions in the air, the healthier we will get. In that, anions can not only activate metabolism of the human body but also can stimulate the blood circulation.

As a result, this can prevent aging, eliminate shoulder and lower back pain, stabilize the nerve system, and restore vigor.

b) Far-infrared rays

Far-infrared energy penetrates beyond the skin level and is absorbed efficiently by cells below. Near-infrared is mostly absorbed at the skin level and raises the skin temperature. Far-infrared rays can penetrate up to 4 centimeter (about 1-1/2 inches), excite the vibration of molecules and resonate with cellular frequencies iV as a result, Far-infrared rays promote bioprocesses such as increased metabolism and blood circulation.

Bamboo charcoal powder emits some radiation in the region of far-infrared rays. This radiation renders human body warm and revitalizes human cells.

c) Antiseptic and deodorant

Bamboo charcoal powder contains a lot of natural minerals. It possesses excellent antiseptic, deodorant, insect repellent, mold prevention properties. In addition, it can improve the hygienic environment.

Compared to other wood charcoal powder, bamboo charcoal powder contains more micro-pores, hence absorption property is better due to its larger specific surface area. Absorption of bamboo charcoal powder is classified into physical absorption and chemical absorption [55]. Physical absorption is caused by molecule acting force (Van der Waals force) between adsorbent and adsorbate that doesn't change the surface composition of adsorbent and the situation of the molecule of adsorbate. Chemical absorption is chemical bond between adsorbent and adsorbate in which the exchange and transference of electrons happen to result in rearrangement of atoms and chemical bond formation or destroying.

Physical absorption is faster and reversible. It occurs in lower temperature without selection and acts in monolayer or multilayer because there is Van der Waals force on one layer of molecule of adsorbate. Similar to chemical reactions, chemical absorption needs activation energy. It is not reversible and occurs in higher temperature with chemical output. As opposed to physical absorption, it is monolayer absorption with distinct selection. "In nature, ions are formed in a variety of ways. About half are created by radioactive gases. Radioactive substances in the soil, cosmic rays, ultraviolet rays, air flow friction, falling water and plants all produce the other half". Bamboo charcoal powders also generate negative ions. More accurately, the emission of infrared rays constantly from bamboo charcoal powder reduce moisture in the air into fine particles that naturally change into negative ions.

d) Humidity adjustment

Bamboo charcoal powder has long been well-known in humidity adjustment by absorbing moisture and sweat. By contrast, bamboo charcoal powder releases moisture in arid environment.

e) Limit the electromagnetic wave

Bamboo charcoal powder has a very strong hardness and it will limit the harmful aspects of electromagnetic wave and static electricity in its direct vicinity.

2.3.4 Usage of Bamboo Charcoal

As a kind of carbon material, bamboo charcoal has the following functions shown in the Figure 2.12.
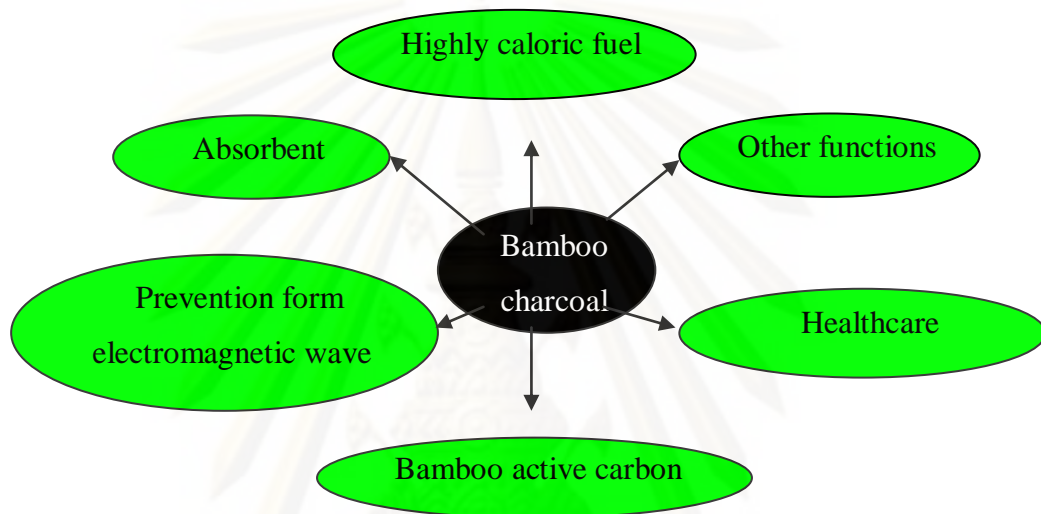
Figure 2.12 Bamboo charcoal functions [54].

The tissue of bamboo charcoal contains much mineral matter with great porosity. The area of pores exceeds the surface area charcoal, reaching 300 m^2/g. Consequently, bamboo charcoal has high absorption ability. The microbes epibiotic on the surface of charcoal molecules decompose the adsorbed matter thoroughly. The charcoal processed at high temperature (1000°C) releases far-infrared, produces anions and absorbs magnetic waves. Due to its special properties and exceptional functions, bamboo charcoal is used widely in fuel, absorption product purifying agent for water and air in dwell environment, prevention from electromagnetic wave, and healthcare [54].

2.4 Literature Reviews

Because of its biocompatibility, biodegradability, antimicrobial activity and non-toxicity, chitosan has been extensively investigated for several decades for uses in biomaterials, wastewater treatment, cosmetics, food packaging, and textiles amongst others [57-61]. The uses of unmodified chitosan films are limited because their properties, such as thermal stability, mechanical properties and gas barrier properties, are frequently not sufficient to meet the requirements of these wide ranges of applications [5-10]. Property improvement of chitosan film has been studied by many researchers as shown below.

Wong et al. [5] prepared chitosan-lipids films and studied their microstructure and surface energy . They reported that the use of chitosan-lipid composite films improves the moisture barrier properties of chitosan film.

Pinotti et al. [6] studied properties of composite films based on chitosan and methylcellulose. They found that water vapor permeabilities of composite films did not differ significantly from chitosan and methylcellulose films. Chitosan films showed rigid characteristics. Flexibility of composite films increased with increasing methylcellulose content.

Hoagland and Parris [7] investigated chitosan/pectin laminated films by casting chitosan/lactic acid films upon pectin films with either glycerol or lactic acid as plasticizers. The storage modulus and loss modulus of chitosan/pectin laminated films were significantly greater than respective moduli of chitosan films alone.

King-Fu et al. [8] examined a novel method to prepare chitosan/montmorillonite nanocomposites. The montmorillonite clay was incorporated with potassium persulfate (KPS) through the intercalation process. The KPS-incorporated MMT was dispersed in the acidified aqueous solution of chitosan. Solutions were casted to form a film of chitosan/MMT nanocomposite. The exfoliated montmorillonite layers were found to flatten out in parallel with the surface, which not only increased the tensile strength of the chitosan film but also hindered degradation in the *in vitro* test.

Yixiang and Milford [9] prepared nanocomposites of chitosan and nanoclays by solvent casting. They reported that tensile strength of chitosan film enhanced but elongation-at-break decreased with addition of clays into the chitosan matrix. Melt behavior and thermal stability did not change significantly with addition of clays.

Dilip et al. [10] prepared nanocomposites by dissolving chitosan and dispersing sodium montmorillonite in aqueous solution of L-lactic acid with subsequent heating and film casting. They found that the film showed enhanced hydrophilicity when compared with polylactic acid (PLA). The nanocomposites exhibited better thermal and physical properties than those of neat chitosan-g-LA and PLA.

Srinivasa et al. [12] studied effect of plasticizers and fatty acids on mechanical and permeability characteristics of chitosan films. They prepared chitosan films by blending with polyols and fatty acid. The result showed that the tensile strength of the blended films decreased with addition of polyols and fatty acids, whereas the percent elongation was increased in polyol blend films. Glycerol blend

films showed decrease, whereas sorbitol and polyethylene glycol blend films showed increase in the water vapor permeability values.

Xu et al. [20] prepared chitosan/starch composite film by combining chitosan solution and thermally gelatinized cornstarch. They found that both tensile strength and elongation at break of composite film increased with starch addition. The addition of starch decreased water vapor transmission rate of composite film.

Hosokawa et al. [21] studied biodegradable film derived from chitosan and homogenized cellulose. They reported that the composite film was hydrophilic but insoluble in water. It had a high oxygen-gas barrier capacity. The strength of the composite film changed with chitosan content. The flexibility of the composite film could be improved by the addition of glycerol as a plasdticizer.

Tao et al. [25] prepared the chitosan/TiO_2 hybrid film by sol-gel method. The result showed that the chitosan/TiO_2 hybrid film fabricated embodied certain tensile strength. TGA demonstrates that the chitosan/TiO_2 hybrid film exhibited better thermal stability.

CHAPTER III

EXPERIMENTAL

3.1 Materials and Chemicals

3.1.1 Chitosan Powder

Chitosan powder, with a deacetylation degree of 95 % and a molecular weight = 100 kDa, used in this investigation was purchased from Seafresh Chitosan (Lab) Co., Ltd. (Thailand). The properties of the bulk chitosan used in this study are summarized in Table 3.1.

Table 3.1 Characteristics of the chitosan.

Item	Specification of standard chitosan	Characteristics of tested chitosan
Appearance	Yellowish	Yellowish
Particle size	Mesh No. 18	Mesh No. 18
Ash content	Less than 1.0%	0.41%
Moisture content	Less than 10.0%	9.0%
Deacetylation degree (%DAC)	At least 95%	95%
Solution (1% in 1% acetic acid)		
Insoluble (%)	Less than 1.0%	0.30%
Viscosity	Less than 200 cps	19 cps
Molecular Weight	100,000-200,000 Da	110,000 Da
Heavy metal	Less than 20 ppm	0 ppm
Microbial content		
Total Plate Count	Less than 1,000 cfu/g	10
Yeast & Mold	Less than 100 cfu/g	10
E. coil	Nil	Nil
Salmonella	Nil	Nil

Depolymerized chitosan having molecular weight at 15 kDa was provided by dissolving 90 g of chitosan powder in 1,800 ml of 20% (v/v) hydrochloric acid solution and stirring at 60°C for 24 hours.

3.1.2 Montmorillonite

Premium grade montmorillonite clay under trade name of SAC-1 was supplied from Thai Nippon Chemical Industry Ltd. (Thailand). The properties of the montmorillonite used in this study are summarized in Table 3.2.

Table 3.2 Characteristics of the montmorillonite.

Item	Montmorillonite
Moisture content	<12%
pH at 5% suspension	9.5-11.0
Swelling	12 min ml/2g
Cation exchange capacity (CEC)	55 meq/100g

3.1.3 Bamboo Charcoal Powder

Bamboo charcoal powder used in this study was supplied by Charcoal Home Co., Ltd. Bamboo charcoal powders have the particle sizes in the range of 7-13 µm.

3.1.4 Acetic Acid

Glacial acetic acid used as a solvent for chitosan was purchased from J. T. Baker (Thailand).

3.1.5 Nitric Acid

Nitric acid (70% (v/v)) used for surface modification of bamboo charcoal was purchased from J. T. Baker (Thailand).

3.2 Instruments

The instruments listed consecutively based on the experimental procedure are shown in Table 3.3.

Table 3.3 Experimental instruments.

Instruments	Model	Manufacturer
Spin coater machine	P6700	SPINCOATER®
Magnetic stirrer	D-38678	IKA®
Hi-speed mixer	75C1S-7A	MAGNA
Centrifuge	P1115B	MULTEX
Fourier transform infrared spectroscopy	Spectrum One	Perkin-Elmer
Laser particle size analyzer	Materziser S	MALVERN
X-ray diffractometer	PW3710	Philips
Gel permeation chromatography	Water 600E	Waters
Scanning electron microscope	JSM-5410 LV	JEOL
Universal testing machine	LR 100K	LLOYD
Thermogravimetric analysis (TGA)	TGA/SDTA851	METTLER TOLEDO
Micrometer	Model G	Peacock
Contact angle meter	CAM-PLUS MICRO	Tactec Inc.

3.3 Methods

3.3.1 Preparation of Chitosan Solution

Pure chitosan aqueous solution was prepared by dissolving 0.5 g of chitosan powder in 100 ml of 1% (v/v) acetic acid solution and stirring for 24 hours. The chitosan solution was filtered to remove foam and undissolved impurities.

3.3.2 Modification of Montmorillonite

An aqueous solution of chitosan having molecular weight 15 kDa was prepared by dissolving 0.5 g of chitosan in 100 ml of distilled water containing 20 ml of conc. HCl and stirred with magnetic stirrer for 24 hours until the chitosan was completely dissolved. One gram of montmorillonite was dispersed in 10 ml of distilled water and stirred for 30 min. The chitosan solution was then added to the montmorillonite suspension, and the mixture was stirred in a hi-speed mixer at room temperature for 1 hour to separate the montmorillonite-chitosan complex from the solution, and the precipitate was washed with distilled water and then dried at 50°C for 48 hours, prior to being ground and sieved through a 200 mesh sieve.

Montmorillonite modified with bulk chitosan was prepared by using the same procedure as mentioned above except for changing depolymerized chitosan to bulk chitosan having molecular weight 100 kDa.

3.3.3 Modification of Bamboo Charcoal Powder

Eight grams of bamboo charcoal powder was suspended in 600 ml of 70% (v/v) nitric acid in a 1000 ml flask and refluxed at 130 °C for 2 hours. The charcoal was recovered from the cooled mixture by centrifugation at 4000 rpm for 15

min with decanting of the supernatant. The charcoal pellet was washed by thorough resuspension in distilled water and pelleted by centrifugation as before. The pelleted washed charcoal powders were then harvested, dried in an oven at 80 °C overnight and further heated at 130 °C for 2 hours to remove any residual nitrogen oxides.

3.3.4 Preparation of Chitosan Films

Pure chitosan films were prepared by dissolving 5 g of chitosan powder in 100 ml of 30% (v/v) acetic acid solution and stirring in a hi-speed mixer for 1 hour. Chitosan films were then derived from this solution using a spin coater machine and dried at room temperature for 72 hours.

3.3.5 Preparation of Chitosan/Montmorillonite Composite Films

Chitosan/montmorillonite composite films were prepared by mixing 5% (w/v) chitosan solution with 30% (v/v) acetic acid with various amounts (0.1%, 0.25%, 0.5%, 1% and 2% (w/w)) of montmorillonite and then stirred at 2500 rpm for 1 hour. The chitosan/montmorillonite composite films were likewise derived from the solution using the spin coater machine and dried at room temperature for 72 hours.

Chitosan/modified montmorillonite composite films were prepared as above, except using modified montmorillonite in place of virgin montmorillonite.

3.3.6 Preparation of Chitosan/Bamboo Charcoal Composite Films

Chitosan/bamboo charcoal composite films were prepared by mixing 5% (w/v) chitosan solution with 30% (v/v) acetic acid with various amounts (0.1%, 0.25%, 0.5%, 1% and 2% (w/w)) of bamboo charcoal powder and then stirred at 2500 rpm for 1 hour. The chitosan/bamboo charcoal composite films were likewise derived

from the solution using the spin coater machine and dried at room temperature for 72 hours.

Chitosan/modified bamboo charcoal composite films were prepared as above, except using modified bamboo charcoal in place of virgin bamboo charcoal.

3.3.7 Characterization

3.3.7.1 Characterization of Bamboo Charcoal Powder by Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectra (transmission) were recorded on a Perkin-Elmer Model Spectrum One in the range of 4000-450 cm^{-1} at a resolution of 1 cm^{-1}. Bamboo charcoal powders were thoroughly mixed with KBr and pressed into a pellet form.

3.3.7.2 Particle Size of Bamboo Charcoal Powder and Montmorillonite

Particle sizes of both virgin and modified bamboo charcoal powder and montmorillonite were measured by the laser particle size analyzer (Water 600E, Waters). Water was used as the dispersing medium.

3.3.7.3 X-ray Diffraction (XRD)

X-ray diffraction measurements were made directly from montmorillonite, modified montmorillonite, and chitosan powder. The test was performed using a PW3710 Philips diffractrometer with CuK$_\alpha$ radiation (λ = 0.1542 nm) in sealed tube operated at 40 kV and 30 mA. The diffraction curves were obtained from 0 to 40° at a scanning rate of 2°min^{-1}.

3.3.7.4 Thickness

Film thickness was measured by a micrometer. Five thickness values were taken along the length of the filmstrip and mean value was used for tensile strength, water absorption, and oil absorption calculation.

3.3.7.5 Mechanical Properties

The tensile strength at maximum load, tensile modulus, and percent elongation at break of the film samples were measured using a Universal Testing Machine (Model LLOYD LR 100K). Film specimens of 150 mm × 15 mm were measured for thickness, and then conditioned for 24 hours at 25 °C and 60% RH before testing. The test was carried out according to the ASTM D 882 standard method, with an initial grip separation of 100 mm, a crosshead speed of 10 mm min^{-1}, and load cell of 100 N.

At least five specimens of each sample were tested and the results were averaged to obtain a mean value.

3.3.7.6 Water Absorption

Water absorption of films was determined by following the standard method of ASTM D 570-95. For water absorption, specimens of 76.2 mm × 25.4 mm were placed in a desiccator for 24 hours, weighed, and immersed in distilled water at an ambient temperature for 24 hours. The test specimens were removed, patted dry with a dry lint cloth and weighed immediately. Water absorption is expressed as the percentage increase in weight. The percentage of increase in sample weight was calculated by using the below formula.

$$\text{Water absorption (\%)} = \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100$$

3.3.7.6 Oil Absorption

Oil absorption was determined as the amount of sunflower oil absorbed by film samples under the same testing procedures as mention earlier for water absorption, except for changing distilled water to sunflower oil.

3.3.7.8 Water Contact Angle

The water contact angles of film samples were measured by using a CAM-PLUS MICRO (Tantec, Inc.) according to Tantec's Half-Angle method. To calibrate the device, the height of the syringe was adjusted so that the needle tip image was at the lower border of the grid on the screen. The needle's image was then focused on by moving the lens plate back and forth. The given specimen was placed onto the specimen holder under the syringe needle, and the knob was released clockwise to release the droplet of water. One droplet of water was obtained and then focused the droplet by moving specimen holder up and down slowly. The line was then adjusted so that it was lined up with the left edge of the droplet. The line was then moved till it crossing the apex of the droplet, and the angle was measured on the protractor. A zero contact angle means the surface wetting, while an angle between 0º and 90º means the droplet spreading (due to molecular attraction). Contact angles greater than 90º indicates the liquid tendinf to bead or shrink away from the solid surface.

3.3.7.9 Oil Contact Angle

Oil contact angle of film samples was determined under the same testing procedures as mention earlier for water contact angle, except for changing distilled water to sunflower oil.

3.3.7.10 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) of films was performed with TGA/SDTA851, METTLER TELDDO. Approximately, 8 mg of each sample was heated from 25 ℃ to 800 ℃ under nitrogen atmosphere at a heating rate of 20 ℃/min.

Prior to the experiment, the samples were dried in a vacuum oven at 50 ℃ for 24 hours. The decomposition temperature (Td) for each sample was taken as the onset of significant weight loss from the heated sample, after the moisture loss.

3.3.7.11 Scanning Electron Microscopy (SEM)

Scanning electron microscope (SEM) was used to study the morphology of film samples. The SEM samples were cut from composite films. The film samples were mounted on stub with double-sided adhesive tape and coated with a thin layer of gold to enhance their conductivity. Images were taken using JEOL scanning electron microscope, JSM-5410LV and scanned at an accelerating voltage of 15 kV.

3.3.7.12 Antifungal of *Colletotrichum gloeosporioides*

Colletotrichum gloeosporioides was directly isolated from infected chili pepper (*Capsicum annuum* Linn.) and maintained on potato-dextrose-agar (PDA). The culture was derived from single spore and also available at the plant pathology laboratory. The isolates were identified using morphology and colony growth characteristics. After growth, the fungus was maintained on PDA dishes at room temperature for 7 days. The isolates were corked and transfer to the slide then place the film specimen and cover slide, respectively. After growth, the dish was maintained on PDA dishes at room temperature for 7 days. Afterward, the fungus germination through film was investigated under microscope by covering film specimen with slide.

Table 3.4 The composition of each formula.

Sample code	CTS weight percent (based on solution)	MMT weight percent (based on solution)	mMMT weight percent (based on solution)	BC weight percent (based on solution)	mBC weight percent (based on solution)
CTS	5.0	-	-	-	-
CTS/MMT 0.1	5.0	0.1	-	-	-
CTS/MMT 0.25	5.0	0.25	-	-	-
CTS/MMT 0.5	5.0	0.5	-	-	-
CTS/MMT 1	5.0	1.0	-	-	-
CTS/MMT 2	5.0	2.0	-	-	-
CTS/mMMT 0.1	5.0	-	0.1	-	-
CTS/mMMT 0.25	5.0	-	0.25	-	-
CTS/mMMT 0.5	5.0	-	0.5	-	-
CTS/mMMT 1	5.0	-	1.0	-	-
CTS/mMMT 2	5.0	-	2.0	-	-
CTS/BC 0.1	5.0	-	-	0.1	-
CTS/BC 0.25	5.0	-	-	0.25	-
CTS/BC 0.5	5.0	-	-	0.5	-
CTS/BC 1	5.0	-	-	1.0	-
CTS/BC 2	5.0	-	-	2.0	-
CTS/mBC 0.1	5.0	-	-	-	0.1
CTS/mBC 0.25	5.0	-	-	-	0.25
CTS/mBC 0.5	5.0	-	-	-	0.5
CTS/mBC 1	5.0	-	-	-	1.0
CTS/mBC 2	5.0	-	-	-	2.0

3.4 Experimental Procedure

The flow chart of the experimental process is shown in Figure 3.1.
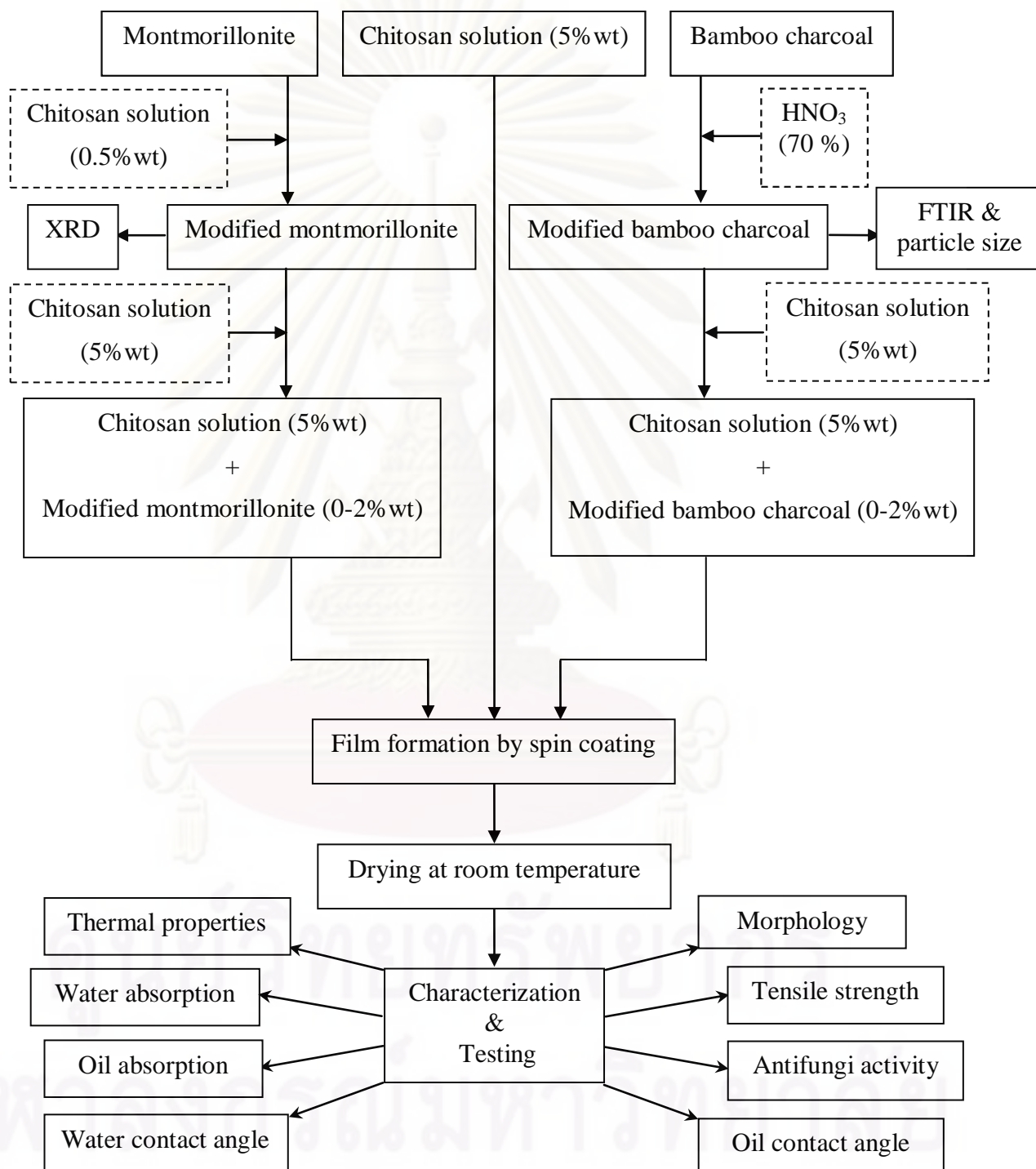
Figure 3.1 The flow chart of experimental procedure.

CHAPTER IV

RESULTS AND DISCUSSION

There were two kinds of chitosan composite films were investigated in their mechanical properties. One was chitosan/montmorillonite composite films, and the other was chitosan/bamboo charcoal composite films. The results and discussion of each property of these two different chitosan composite films were reported side by side.

4.1 X-ray Diffraction (XRD) of Chitosan and Montmorillonite

The basal peak position, d-spacing, and X-ray diffraction patterns of bulk chiotsan, virgin montmorillonite, bulk chitosan modified montmorillonite, and depolymerized chitosan modified montmorillonite are summarized in Figure 4.1.
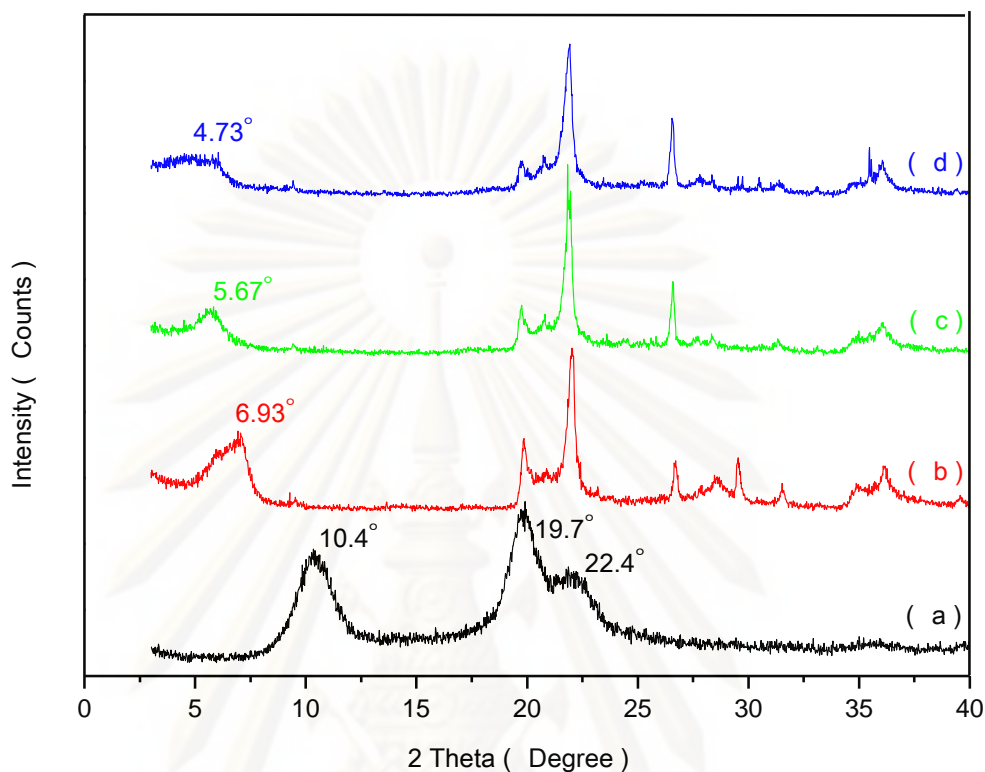
Figure 4.1 X-ray diffraction patterns of: (a) Bulk chitosan, (b) Virgin montmorillonite, (c) Bulk chitosan modified montmorillonite, and (d) Depolymerized chitosan modified montmorillonite.

The semicrystalline character of chitosan was revealed from the diffractogram illustrated in Figure 4.1 (a). The diffractogram of chiotsan shows three major characteristic peaks at $2\theta = 10.4°$, $19.7°$ and $22.4°$, which are fingerprint for chitosan and corresponding with Samuels' report [62]. It was noted that a peak at approximately $2\theta = 10.4°$ was assigned to crystal forms I, and the reflection appeared at around $2\theta = 19.7°$ corresponding to crystal forms II. The unit cell of crystal forms I is characterized by $a = 7.76$, $b = 10.91$, $c = 10.30$ A°, and $\beta = 90°$, and it is larger than that of crystal forms II, whose unit cell is characterized by $a = 4.4$, $b = 10.0$, $c = 10.30$

Å, and β = 90º. In this study, the intensity of the crystal peak at about 2θ = 19.7º is very strong. It implied that chitosan is in crystalline state. It was noted that the degree of deacetylation had obviously affected crystallinity of chitosan, according to the previous research [63]. It was observed that the crystallinity of the chitosan increased gradually with increasing degree of deacetylation ranging from 70 to 92%. This may be attributed to the fact that chains of chitosan with higher degree of deacetylation are more compact leading to facilitate the hydrogen-bonding formation and in turn crystallinity formation in the chitosan. Furthermore, higher degree of deacetylation chitosan contains more glucosamine groups that also facilitate hydrogen-bonding formation. On the other hand, lower degree of deacetylation chitosan has more acetyl groups that hinder the hydrogen-bonding formation due to their rigidity and steric effect. Therefore, the high deacetylation chitosan contains a larger crystalline region.

The X-ray diffraction patterns of virgin montmorillonite, bulk chitosan modified montmorillonite, and depolymerized chitosan modified montmorillonite are shown in Figure 4.1 (b), (c), and (d), respectively. Virgin montmorillonite shows a strong diffraction peak at 2θ = 6.93º, corresponding to 001 lattice spacing of silicate layer of 12.75 Å in montmorillonite as suggested by Changyu et al. [64]. Since the polycationic characteristic of chitosan in acidic environment, this polymer could easily be adsorbed onto the negative charge surface of montmorillonite when cationic-exchange between cationic chitosan and Na^+ and Ca^+ ions residing in interspacing layer can take place. The X-ray diffraction pattern of bulk chitosan modified montmorillonite shows the diffraction peak of 001 lattice spacing of silicate layer slightly shifting to 2θ = 5.67º, corresponding to a basal spacing layer of 15.57 Å. The interlayer distance of depolymerized chitosan modified montmorillonite is larger than

that of virgin montmorillonite. The depolymerized chitosan modified montmorillonite displays the diffraction peak of 001 lattice spacing of silicate layer at $2\theta = 4.73°$, corresponding to a basal spacing layer of 18.67 Å. The larger interlayer values for these two different modified montmorillonite, compared to virgin montmorillonite, indicated the likely formation of an intercalated nanocomposite. In general, the higher degree of basal spacing expansion usually results in the higher chance of polymer intercalation which leads to the more possibility of layered-silicate delamination in the polymer matrix. These results showed that the modified montmorillonite derived from depolymerized chitosan molecule had a lower diffraction peak indicating that the depolymerized chitosan chains were intercalated into the silicate layers and the coherent order of virgin montmorillonite was destroyed more.

The peak at $2\theta = 10.4°$ was only found in bulk chitosan XRD profile. It illustrated that the depolymerization of chitosan and addition depolymerized chitosan in montmorillonite decreased the crystalline of chitosan.

4.2 FTIR Spectroscopy

Bamboo charcoal particles are hydrophobic and stay in aggregated form in aqueous environments, which makes it difficult to be adsorbed onto the surface and then diffuse into the chitosan film, particularly the more hydrophilic ones. Oxidative treatment of bamboo charcoal particles is one effective way to increase the hydrophilicity due to the generation of carboxylic acid groups (as well as other minor changes such as hydroxyl groups) on the particles' surface. Nitric acid, a strong oxidizing agent, can attack some imperfect areas of the bamboo charcoal particles

during refluxing, which leads to the formation of carboxylic acid groups (-COOH) at these surfaces [65].
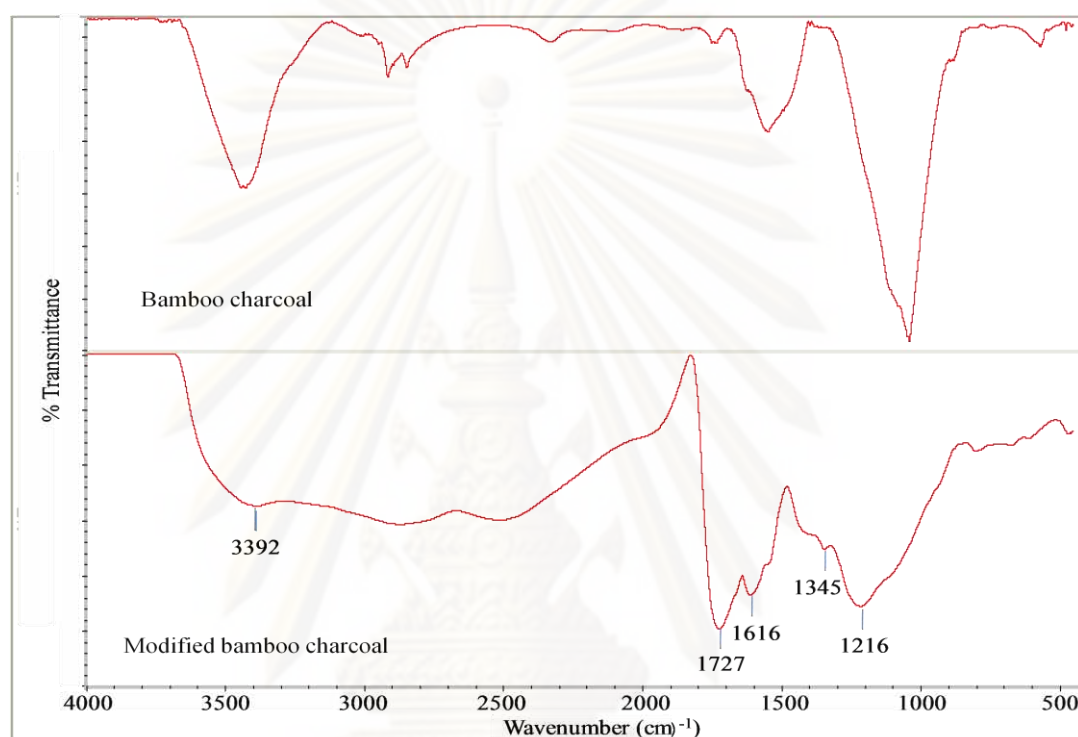
Figure 4.2 FTIR spectra of unmodified and modified bamboo charcoal particles**.**

The surface functional groups of unmodified and modified bamboo charcoal particles were characterized with infrared transmission spectroscopy, as shown in Figure 4.2. The signature of surface hydroxylic (-OH) groups is evident at 3445 cm^{-1}, and lactone groups appear at 1041 cm^{-1}. FTIR spectra of modified (70% (v/v) nitric acid treated) bamboo charcoal particles revealed the likely presence of COOH (~1727 cm^{-1}), as well as, other typical features of oxidation including -OH at ~3392 cm^{-1}, C=C at 1616 cm^{-1}, N=O at 1345 cm^{-1} and C-O at ~1216 cm^{-1}, in the modified bamboo charcoal particles (Figure 4.2). These results supports that nitric acid, used as an

oxidizing agent, had oxidized some areas of bamboo charcoal particles during refluxing leading to formation of carboxylic acid groups (and other moieties) on the surface of the bamboo charcoal particles.

4.3 Mechanical Properties of Chitosan Composite Films

The mechanical properties of two different chitosan composite films were measured in terms of tensile strength, Young's modulus, and % elongation at break.

The first part of this section reported the mechanical properties of chitosan/montmorillonite composite films. Mechanical properties of chitosan, chitosan/virgin montmorillonite and chitosan/modified montmorillonite of composite films are given in Table 4.1.

Table 4.1 Mechanical properties of chitosan and chitosan/montmorillonite composite films.

Composition of films	Thickness (mm)	Tensile strength (MPa)	Young's modulus (MPa)	Elongation at break (%)
CTS	0.015±0.00	73.23±2.94	5330.74±416.91	2.63±0.26
CTS/MMT 0.1	0.015±0.00	75.08±2.32	5308.88±38.02	2.45±0.21
CTS/MMT 0.25	0.016±0.00	77.03±3.41	5683.29±60.24	1.90±0.05
CTS/MMT 0.5	0.017±0.00	82.96±4.26	5936.60±302.33	1.54±0.09
CTS/MMT 1	0.019±0.00	92.86±1.24	6079.89±443.76	1.25±0.10
CTS/MMT 2	0.020±0.00	91.29±0.70	6018.01±34.90	0.86±0.06
CTS/mMMT 0.1	0.015±0.00	83.31±4.42	6766.90±112.34	2.54±0.08
CTS/mMMT 0.25	0.016±0.00	94.01±3.04	7636.10±85.72	2.39±0.16
CTS/mMMT 0.5	0.017±0.00	104.87±.06	8038.34±25.62	1.98±0.18
CTS/mMMT 1	0.019±0.00	123.26±5.19	10438.04±688.77	1.91±0.07
CTS/mMMT 2	0.020±0.00	108.09±2.85	9581.74±493.91	1.61±0.09

The effect of the inclusion of different montmorillonite contents on the tensile strength, Young's modulus, and elongation at break of the composite films are summarized in Figures 4.3, 4.4 and 4.5, respectively.

Figure 4.3 The effect of unmodified and modified montmorillonite on the tensile strength of chitosan films.

The tensile strength of the chitosan/unmodified montmorillonite composite films slightly increased with increasing unmodified montmorillonite contents in chitosan composite films up to 1% (w/w), at which level the increase in tensile strength attained over pure chitosan films was around 27%, and then slightly decreased at 2% (w/w) montmorillonite levels. This result may conclude that the unmodified montmorillonite presented interfacial adhesion with chitosan film and could insert into the chitosan matrix and reinforce the tensile strength of the

chitosan/unmodified montmorillonite composite films. However, that 2% (w/w) unmodified montmorillonite in the composite film slightly reduced the tensile strength, compared to 1% (w/w), may be due to the effect of heterogeneous dispersion of unmodified montmorillonite in chitosan matrix when higher montmorillonite loading in the matrix, with a resulting reduced stress distribution from chitosan to the montmorillonite.

Similarly to chitosan/unmodified montmorillonite composite films, the tensile strength of the chitosan/modified montmorillonite composite films increased with increasing levels of modified montmorillonite up to a maximum at 1% (w/w), at which level the increase in tensile strength attained over pure chitosan films was ~ 68%, and then slightly decreased at 2% (w/w) modified montmorillonite. In addition, the tensile strengths of the chitosan/modified montmorillonite composite films were higher than those of chitosan/unmodified montmorillonite composite films at the same content of montmorillonite. It is noteworthy that modified montmorillonite has a more homogeneous dispersion in chitosan matrix than unmodified montmorillonite.

Figure 4.4 The effect of unmodified and modified montmorillonite on the Young's modulus of chitosan films.
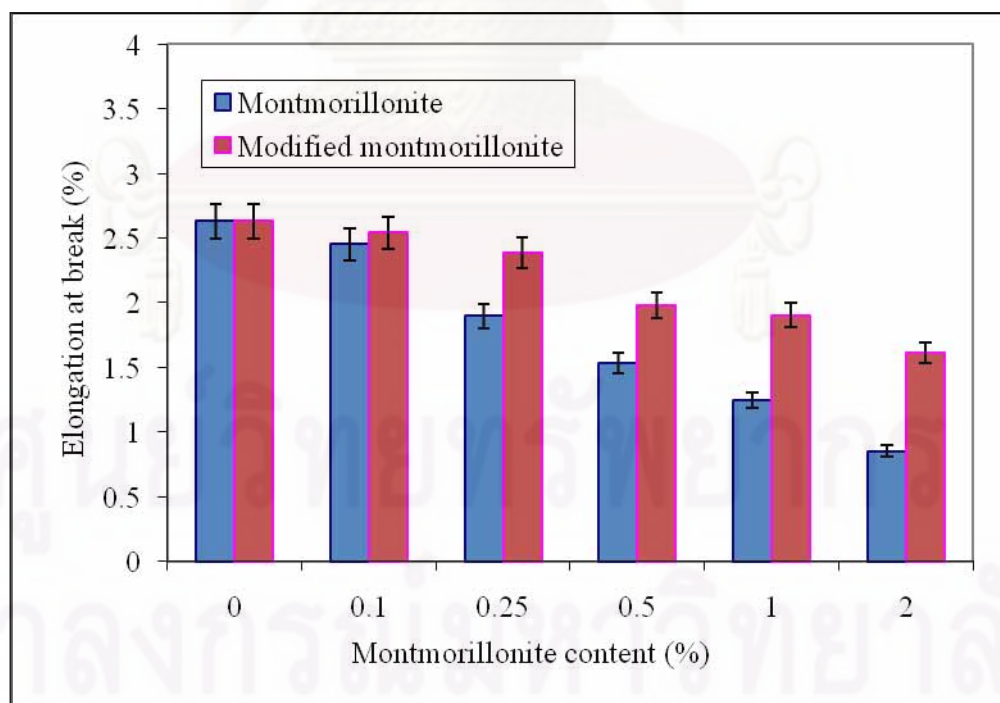
Figure 4.5 The effect of unmodified and modified montmorillonite on the elongation at break value of chitosan films.

Using Young's modulus as a marker of the rigidity of these films, the rigidity of the chitosan/montmorillonite composite films, somewhat akin to the tensile strength, was slightly increased with increasing unmodified and modified montmorillonite content up to 1% (w/w), at which level an ~ 14% increase in rigidity was observed compared to pure chitosan films. Thereafter, a slight reduction, compared to 1% (w/w) modified montmorillonite, in rigidity was observed with 2% (w/w) modified montmorillonite. This result is similar to chitosan/modified montmorillonite, the rigidity of the chitosan films was increased with increasing modified montmorillonite content up to a maximum at 1% (w/w), at which level the increase in rigidity attained over pure chitosan films was ~ 98%, and then decreased at 2% (w/w) montmorillonite contents. Furthermore, the rigidity of the chitosan/modified montmorillonite composite films is higher than those of chitosan/unmodified montmorillonite composite films at the same content of montmorillonite. This may be because the montmorillonite modified with depolymerized chitosan provide the better compatibility with the chitosan and also increase the interfacial adhesion between modified montmorillonite and chitosan, and thus leading to a higher rigidity of their composite film compared to either pure chitosan or chitosan/unmodified montmorillonite composite films.

The elongation at break of chitosan/unmodified montmorillonite composite and chitosan/modified montmorillonite composite films showed a slightly different trend to those seen for composite rigidity and tensile strength. Thus, the elongation at break of both unmodified and modified montmorillonite decreased with increasing level of montmorillonite content in composites. This may be because the montmorillonite either unmodified or modified, once incorporated with chitosan

reduces the extensibility of the matrix in the film composites. The resistance created by montmorillonite would reduce the deformability of chitosan molecules.

The second part of this section reported the mechanical properties of chitosan/bamboo charcoal composite films and the results of mechanical properties of these composite films are summarized in Table 4.2

Table 4.2 Mechanical properties of chitosan and chitosan/bamboo charcoal composite films.

Composition of films	Thickness (mm)	Tensile strength (MPa)	Young's modulus (MPa)	Elongation at break (%)
CTS	0.015±0.00	73.23±2.94	5330.74±416.91	2.63±0.26
CTS/BC 0.1	0.015±0.00	67.03±3.01	5190.11±130.80	2.59±0.23
CTS/BC 0.25	0.016±0.00	66.50±6.65	5057.68±211.36	2.39±0.56
CTS/BC 0.5	0.017±0.00	63.42±8.00	5092.64±432.51	1.33±0.13
CTS/BC 1	0.019±0.00	25.08±8.96	5048.65±1097.68	1.35±0.35
CTS/BC 2	0.020±0.00	25.32±6.69	4625.96±786.69	1.31±0.27
CTS/mBC 0.1	0.015±0.00	81.24±1.49	5622.44±252.89	3.74±1.01
CTS/mBC 0.25	0.016±0.00	85.87±1.47	5700.62±112.36	3.70±1.16
CTS/mBC 0.5	0.017±0.00	86.42±3.26	5771.15±606.00	3.66±0.50
CTS/mBC 1	0.019±0.00	106.38±2.98	7036.74±426.70	2.81±0.23
CTS/mBC 2	0.020±0.00	87.89±3.06	6763.33±207.15	1.99±0.11

The effect of the inclusion of different bamboo charcoal contents on the tensile strength, Young's modulus, and elongation at break of the composite films are summarized in Figures 4.6, 4.7 and 4.8, respectively.
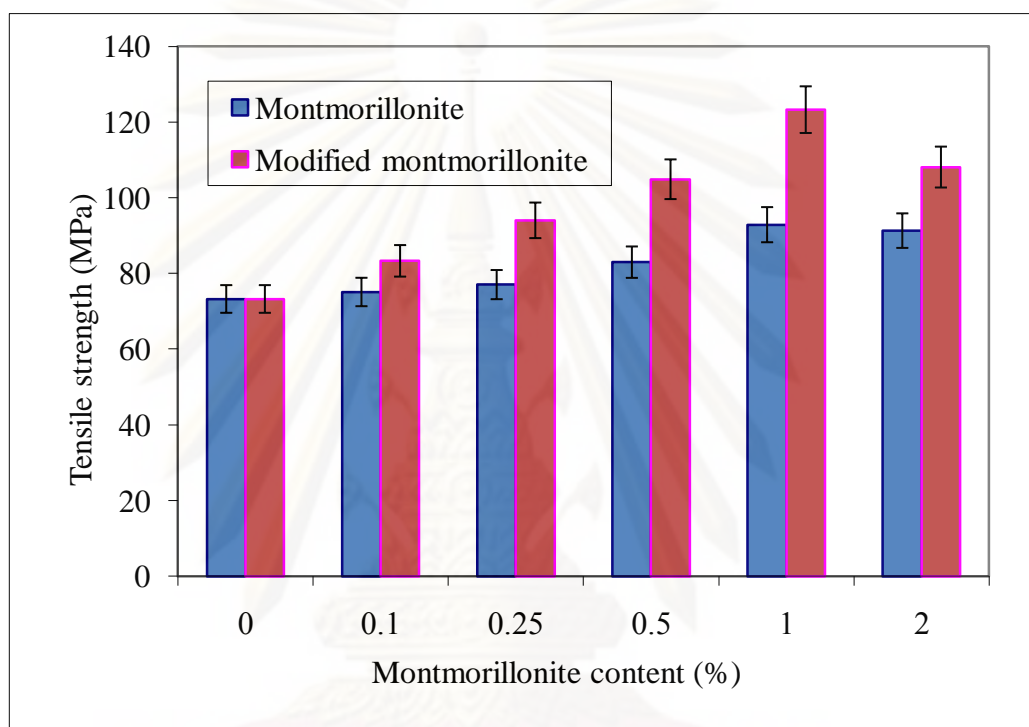
The tensile strength of the chitosan/unmodified bamboo charcoal composite films slightly decreased but insignificantly with increasing unmodified bamboo charcoal contents in composite films up to 0.5% (w/w), and then decreased dramatically (~ 66%) at 1 and 2% (w/w) unmodified bamboo charcoal contents. This result may suggest that no reinforcement was obtained by the addition of unmodified bamboo charcoal due to the poor interfacial adhesion between unmodified bamboo charcoal and the composite matrix. It may be because of more agglomeration at 1 and 2% (w/w) of unmodified bamboo charcoal, which weakens the stress distribution in the chitosan/unmodified bamboo charcoal composite film.
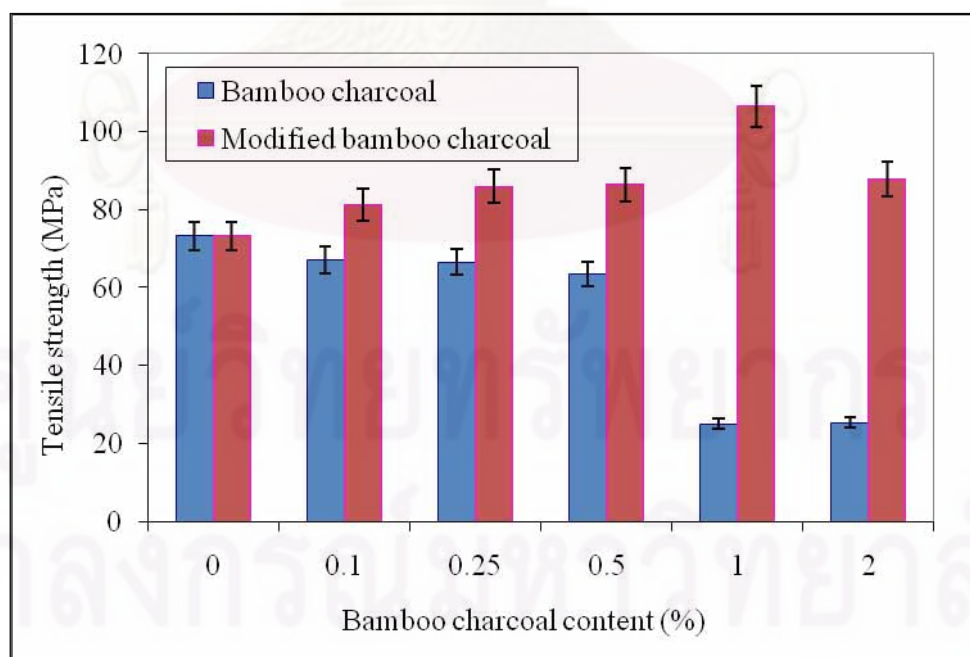
Figure 4.6 The effect of unmodified and modified bamboo charcoal on the tensile strength of chitosan films.
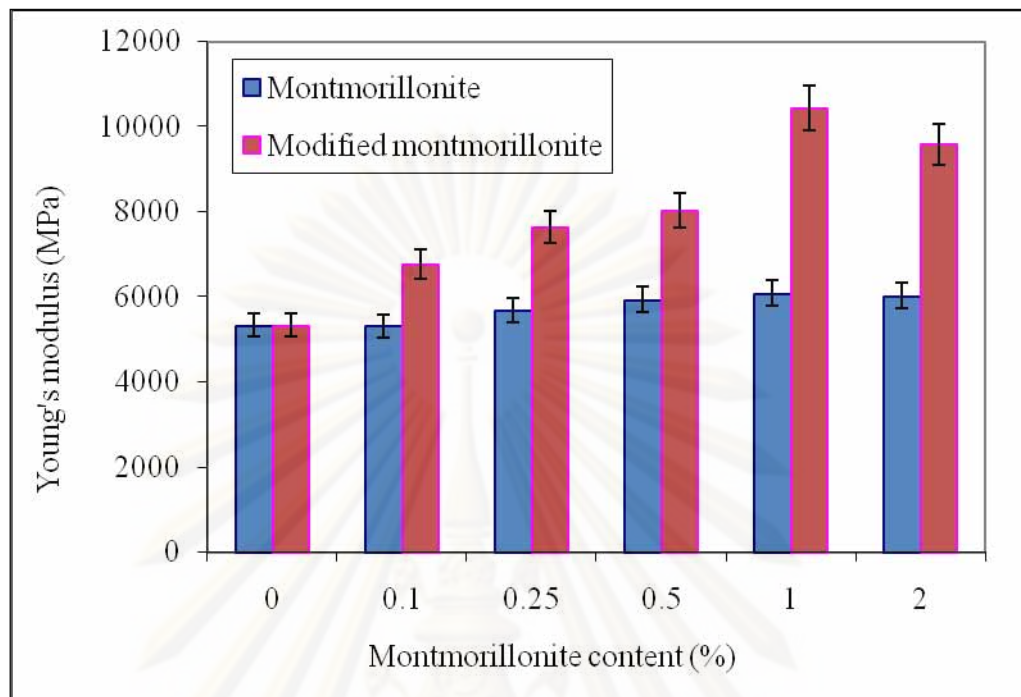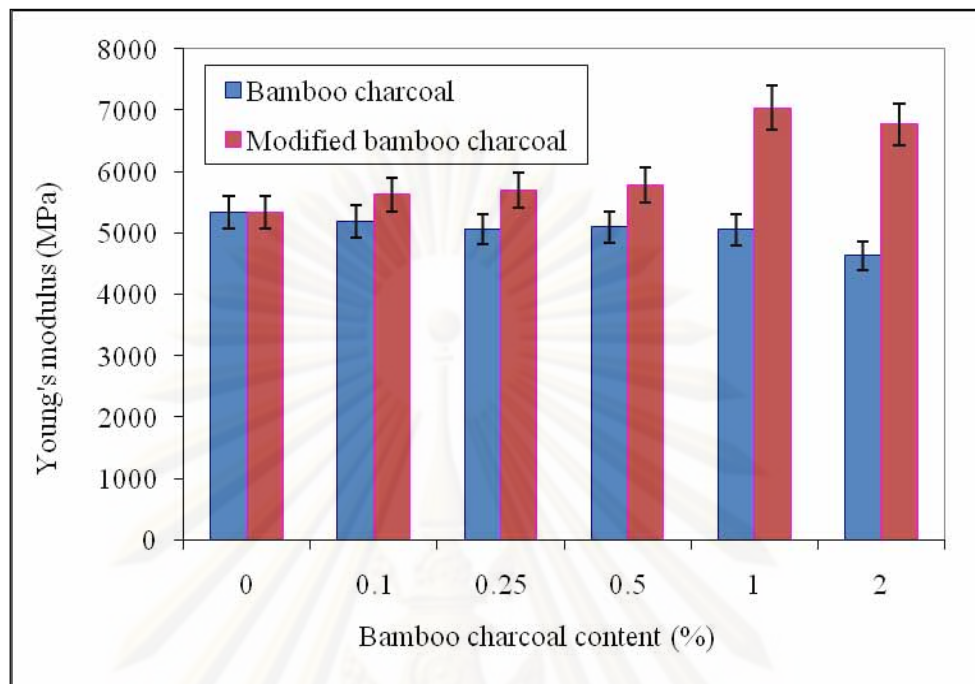
Figure 4.7 The effect of unmodified and modified bamboo charcoal on the Young's modulus of chitosan films.
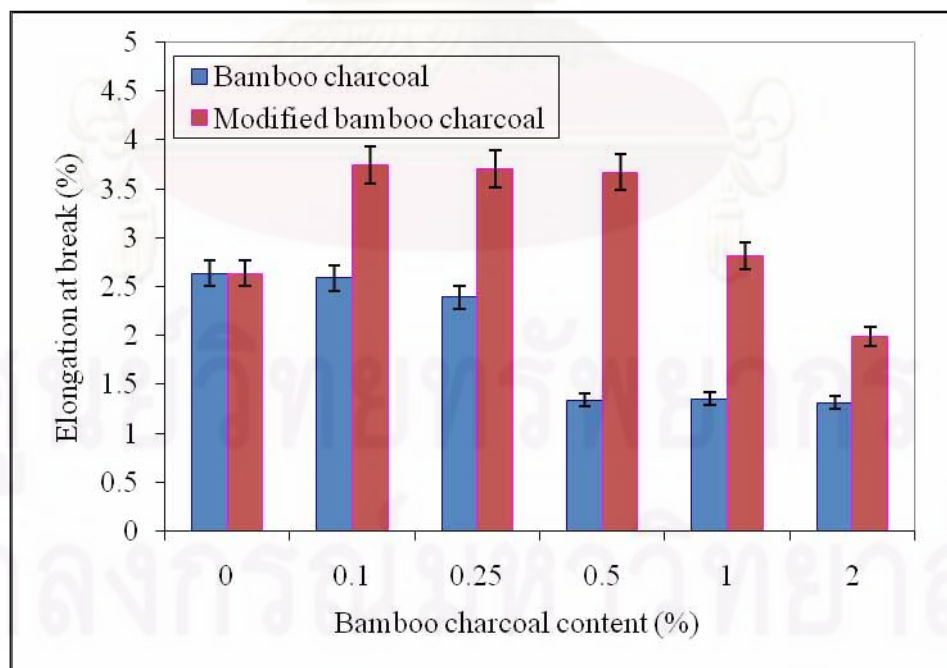
Figure 4.8 The effect of unmodified and modified bamboo charcoal on the elongation at break value of chitosan films.

In contrast, the tensile strength of the chitosan/modified bamboo charcoal composite films increased with increasing levels of modified bamboo charcoal up to a maximum at 1% (w/w), at which level the increase in tensile strength attained over pure chitosan films was ~ 46%, and then fell slightly at 2% (w/w) modified bamboo charcoal. This result could be attributed to the presence of carboxylic acid groups (and perhaps to a lesser extent the other polar modifications such as hydroxyl groups) formed on the surface of the bamboo charcoal by nitric acid oxidation which provide a better interfacial adhesion with the chitosan through hydrogen and electrostatic bonding. However, that 2% (w/w) modified bamboo charcoal in the composite film slightly reduced the tensile strength, compared to 1% (w/w), may be due to agglomeration of bamboo charcoal in the chitosan composite film.

Using Young's modulus as a marker of the rigidity of these films, the rigidity of the chitosan/modified bamboo charcoal composite films, somewhat akin to the tensile strength, was increased with increasing modified bamboo charcoal content up to 1% (w/w), at which level an ~ 30% increase in rigidity was observed compared to pure chitosan films. Thereafter, a slight reduction, compared to 1% (w/w) modified bamboo charcoal, in rigidity was observed with 2% (w/w) modified bamboo charcoal. In contrast, the rigidity of the chitosan films was slightly decreased with increasing unmodified bamboo charcoal content up to 1% (w/w), and more so at 2% (w/w). The changes of the composite film rigidity, as determined by Young's modulus, could suggest that the oxidative treatment of bamboo charcoal particles was effective in increasing the interfacial adhesion between bamboo charcoal and chitosan (such as electrostatic forces between amine and carboxylic groups), and also in providing more H-bonding between them. As a result, this leads to a higher rigidity of the composite

film compared to either pure chitosan or chitosan/unmodified bamboo charcoal composite films.

The elongation at break of chitosan/modified bamboo charcoal films showed a slightly different trend to those seen in composite rigidity and tensile strength. Thus, the elongation at break increased essentially the same amount (~ 38%) with the addition of 0.1 to 0.5% (w/w) modified bamboo charcoal content, and then decreased drastically with higher contents (1 and 2% (w/w)) of modified bamboo charcoal. At 2% (w/w) modified bamboo charcoal content, elongation at break value was lower than that of pure chitosan film. This may be due to the reduction of extensible matrix when the content of modified bamboo charcoal is increased to 1% (w/w) and above. The elongation at break of chitosan/unmodified bamboo charcoal showed no increase relative to pure chitosan films but slightly decreased with increasing unmodified bamboo charcoal content up to 0.25% (w/w), and then markedly at 0.5% (w/w) where a reduction of ~ 46% compared to pure chitosan films was observed, and then remained at this level with higher unmodified bamboo charcoal contents (at least up to 2% (w/w)). Presumably, the unmodified bamboo charcoal, without any enhanced compatibility (electrostatic and hydrogen bonds) with chitosan, will be less able to resist deformation of these composite films.

According to above results, those could be concluded that montmorillonite either virgin or modified used as filler in chitosan composite films provided better results of tensile strength and Young's modulus than those of bamboo charcoal used as a filler in chitosan film. However, the chitosan/bamboo charcoal composite films could have better results of tensile strength and Young's modulus by modification the bamboo charcoal with nitric acid. This was because the oxidation treatment of

bamboo charcoal particles increased the interfacial adhesion between chitosan and bamboo charcoal. While the elongation at break of these two different chitosan composite films was not much different form one another.

4.4 Water Absorption

The effect of the different types and levels of montmorillonite content on water absorption of chitosan composite films is summarized in Table 4.3 and Figure 4.9.

Table 4.3 Water absorption of chitosan/montmorillonite composite films**.**

Composition of films	Water absorption
CTS	112.39±5.02
CTS/MMT 0.1	111.26±5.48
CTS/MMT 0.25	109.96±3.18
CTS/MMT 0.5	97.26±0.63
CTS/MMT 1	96.27±2.32
CTS/MMT 2	93.49±1.80
CTS/mMMT 0.1	101.01±3.35
CTS/mMMT 0.25	97.34±1.97
CTS/mMMT 0.5	89.75±3.23
CTS/mMMT 1	85.31±0.53
CTS/mMMT 2	77.25±2.89
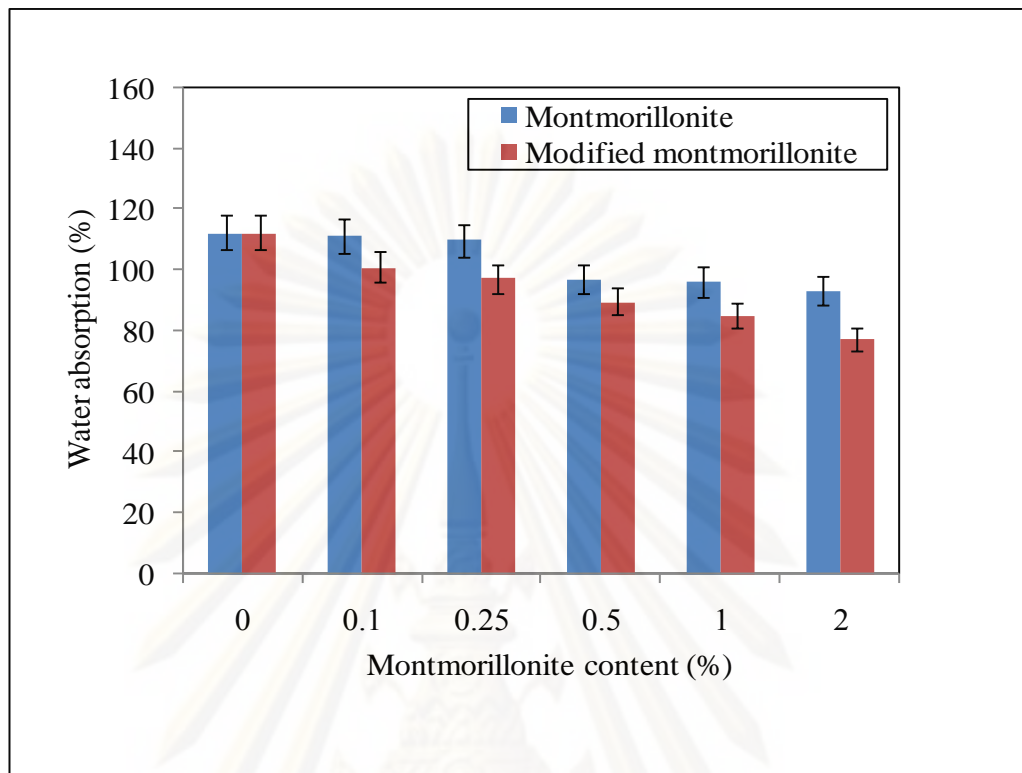
Figure 4.9 The effect of unmodified and modified montmorillonite contents on water absorption of chitosan films.

According to the results in both Table 4.3 and Figure 4.9, those could be concluded that water absorption of the pure chitosan film was about 112% by weight. Chitosan film had be able to hold the water molecules due to hydrophilic nature of chitosan. The water absorptions of composite films were slightly decreased by inclusion of either unmodified or modified montmorillonite. These results indicated that the addition of montmorillonite improved the water resistance of chitosan film. In addition, the decrease in water absorption was more marked in modified than that of unmodified montmorillonite composite films. It could be concluded that the larger interlayer distance between the individual silicate layers of modified montmorillonite could not be able to hold the water. Therefore, addition of modified montmorillonite

having this characteristic in chitosan film could not enhance the water absorption of chitosan composite films. In addition, inclusion of unmodified and modified montmorillonite in chitosan film reduced the water absorption of chitosan. This may be because both unmodified and modified montmorillonite blocked the area on the chitosan film to hold and interact with water molecule.

Water absorption of chitosan/bamboo charcoal composite films are summarized in Table 4.4 and Figure 4.10.

Table 4.4 Water absorption of chitosan/bamboo charcoal composite films**.**

Composition of films	Water absorption
CTS	112.39±5.02
CTS/BC 0.1	113.02±6.76
CTS/BC 0.25	129.40±7.12
CTS/BC 0.5	163.91±16.64
CTS/BC 1	269.45±14.70
CTS/BC 2	157.44±41.19
CTS/mBC 0.1	119.05±4.94
CTS/mBC 0.25	150.62±2.95
CTS/mBC 0.5	175.76±4.80
CTS/mBC 1	343.83±31.74
CTS/mBC 2	331.04±37.12

Figure 4.10 The effect of unmodified and modified bamboo charcoal contents on water absorption of chitosan films.

Water absorption was increased by inclusion of either unmodified or modified bamboo charcoal up to 1% (w/w), although the increase in water absorption was more marked in modified than unmodified bamboo charcoal composite films. This may be attributed to the formation of hydrophilic carboxylic groups on the surface of the modified bamboo charcoal by nitric acid. As a result, this leads to stronger hydrogen bonding to water and thus more hydrophilic surface nature than those of chitosan/unmodified bamboo charcoal film. When the content of unmodified or modified bamboo charcoal was increased to 2% (w/w), however, a marked and slight decrease in water absorption levels, with respect to that observed at 1% (w/w) was seen, respectively. The amount of bamboo charcoal contents of 2% (w/w) in composite films may cause significant agglomeration of the bamboo charcoal

reducing the pores and gaps in the bamboo charcoal structure. These may reduce the ability of water absorption of both types of composite films, compared to that seen at 1% (w/w). Bamboo charcoal was not considered as a hydrophilic material, but the significantly increased water absorption in both types of chitosan/bamboo charcoal composite films was likely to be attributed to many pores and gaps in the bamboo charcoal structure.

4.5 Oil Absorption

The effect of the different types and levels of montmorillonite content on oil absorption of chitosan composite films is summarized in Table 4.5 and Figure 4.11.

Table 4.5 Oil absorption of chitosan/montmorillonite composite films**.**

Composition of films	Oil absorption
CTS	6.06±0.80
CTS/MMT 0.1	6.00±0.22
CTS/MMT 0.25	5.68±0.17
CTS/MMT 0.5	5.62±0.16
CTS/MMT 1	5.10±0.30
CTS/MMT 2	5.15±0.38
CTS/mMMT 0.1	5.89±0.03
CTS/mMMT 0.25	5.66±0.23
CTS/mMMT 0.5	5.14±0.66
CTS/mMMT 1	5.17±0.42
CTS/mMMT 2	4.45±0.27
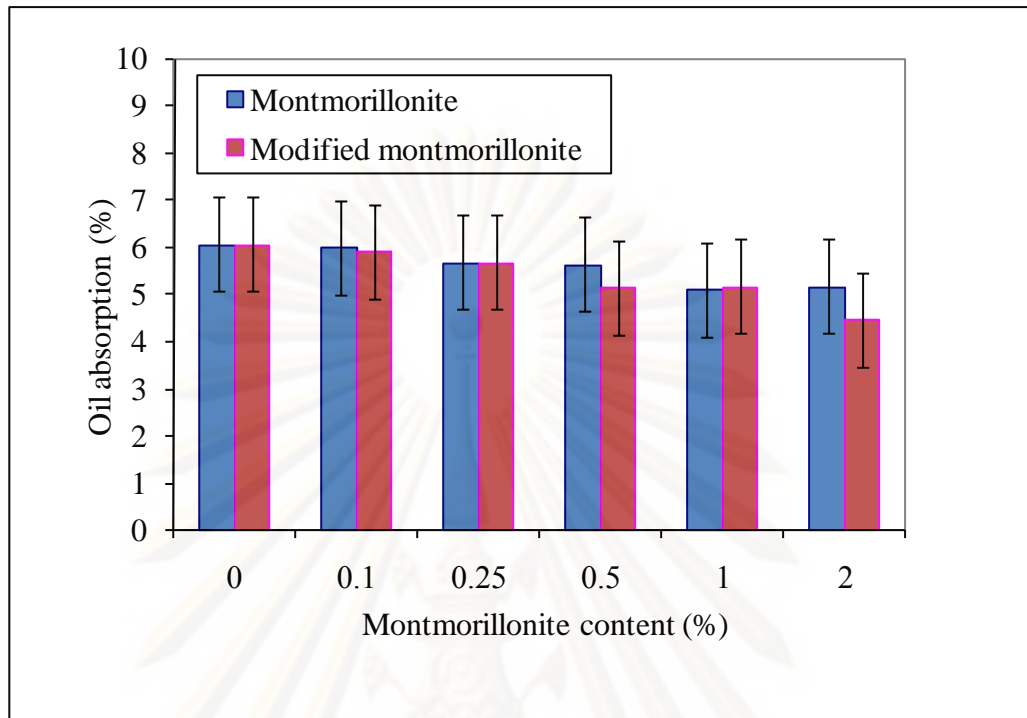
Figure 4.11 The effect of unmodified and modified montmorillonite contents on oil absorption of chitosan films.

Although, chitosan itself has a good affinity with oil, when inclusion of either unmodified or modified montmorillonite in chitosan film reduced the oil absorption of chitosan composite film. The oil absorption of chitosan/montmorillonite composite films decreased slightly but significantly with increasing montmorillonite contents in composite films at 1% (w/w) unmodified montmorillonite and at 0.5% (w/w) modified montmorillonite. These results could be attributed to the particles of unmodified or modified montmorillonite inserted into chitosan film blocking the ability of chitosan to adhere with oil.

Oil absorption of chitosan/bamboo charcoal composite films is summarized in Table 4.6 and Figure 4.12.

Table 4.6 Oil absorption of chitosan/bamboo charcoal composite films.

Composition of films	Oil absorption
CTS	6.06±0.80
CTS/BC 0.1	6.84±0.80
CTS/BC 0.25	7.43±0.26
CTS/BC 0.5	7.83±1.14
CTS/BC 1	9.30±0.52
CTS/BC 2	8.97±0.30
CTS/mBC 0.1	7.65±0.22
CTS/mBC 0.25	8.07±0.54
CTS/mBC 0.5	8.20±0.70
CTS/mBC 1	9.71±0.41
CTS/mBC 2	9.45±0.10

Inclusion of bamboo charcoal in chitosan composite films could enhance better oil absorption than that of pure chitosan film. This was because of good affinity with the oil of chitosan and the hydrophobic materials of bamboo charcoal. The results shown in Figure 4.12 could be concluded that oil absorption in the pure chitosan films was about 6% by weight, and both modified and unmodified bamboo charcoal composite films revealed increase in oil absorption up to 1% (w/w) charcoal

composition. The results of oil absorption of both types of bamboo charcoal composite films were in broad accordance with the results obtained for water absorption, except the magnitude was much lower. This again may reflect the presence of pores and gaps that bamboo charcoal introduces into the composite structure and also due to hydrophobic character of bamboo charcoal. Chitosan/modified bamboo charcoal composite film providing more oil absorption than that of chitosan/unmodified bamboo charcoal composite film would presumably be due to the better interfacial adhesion of modified bamboo charcoal with the chitosan film and also providing more modified bamboo charcoal particles throughout the composite matrix.
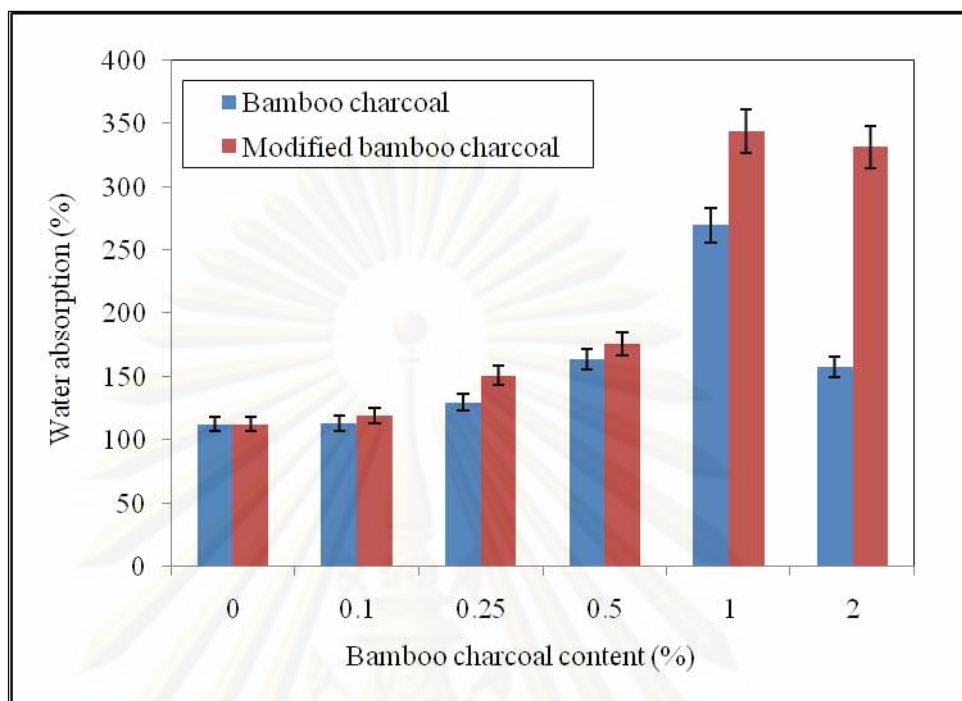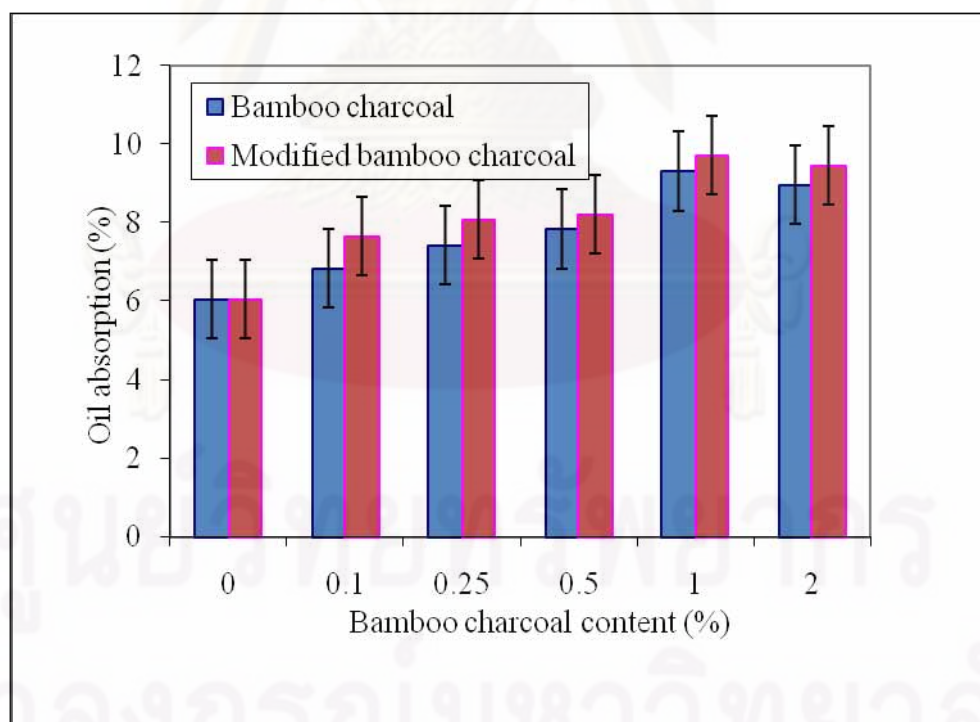

Figure 4.12 The effect of unmodified and modified bamboo charcoal contents on oil absorption of chitosan films.

Bamboo charcoal increased water and oil absorption in both types of chitosan/bamboo charcoal composite film. This may be due to the pores and gaps in the bamboo charcoal structure. On the other hand, montmorillonite decreased water and oil absorption in both types of chitosan/monmorillonite composite films. This may be due to the particle of montmorillonite blocking the chitosan to adhere water and oil. Moreover, the larger interlayer distance between the individual silicate layers of modified montmorillonite could not be able to hold or capture water or oil.

4.6 Water Contact Angle

The surface hydrophilicity of film was evaluated by means of water contact angle determination. The effect of the different types and levels of montmorillonite content on water contact angle of chitosan composite films is summarized in Table 4.7 and Figure 4.13.

Table 4.7 Water contact angle of chitosan/montmorillonite composite films**.**

Composition of films	Water contact angle (º)
CTS	110.00±0.00
CTS/MMT 0.1	120.33±0.58
CTS/MMT 0.25	129.33±1.15
CTS/MMT 0.5	133.33±1.15
CTS/MMT 1	134.00±0.00
CTS/MMT 2	136.00±0.00
CTS/mMMT 0.1	120.00±0.00
CTS/mMMT 0.25	130.00±0.00
CTS/mMMT 0.5	134.00±0.00
CTS/mMMT 1	136.00±0.00
CTS/mMMT 2	138.00±0.00
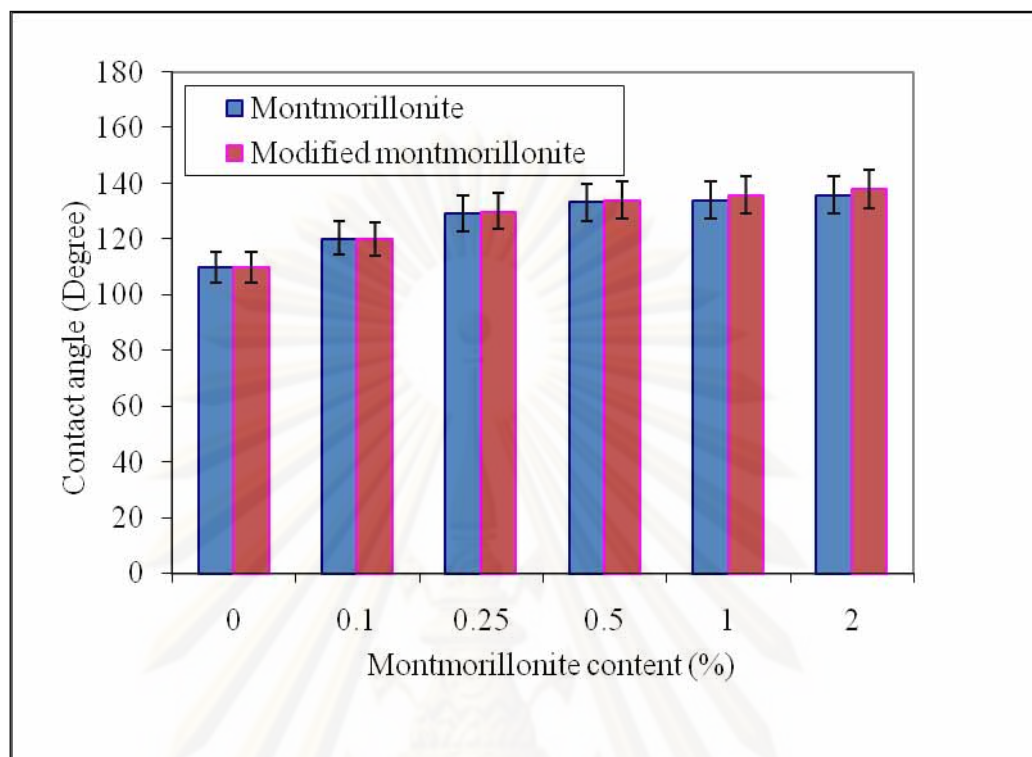
Figure 4.13 The effect of unmodified and modified montmorillonite contents on water contact angle of chitosan films.

Water contact angle was used as a marker of the surface wettability of these films. According to the results in both Table 4.7 and Figure 4.13, those could be concluded that water contact angle of the pure chitosan film was about 110º. Chitosan film had good water surface wettability due to hydrophilic nature of chitosan. The water contact angles of composite films were slightly increased by inclusion of either unmodified or modified montmorillonite. These results indicated that the addition of montmorillonite led to a decreasing of hydrophilicity of chitosan film. In addition, the increase in water contact angle was more marked in modified than that of unmodified montmorillonite composite films. It could be concluded that the larger interlayer distance between the individual silicate layers of modified montmorillonite could not

be able to adsorb the water. Therefore, addition of modified montmorillonite having this characteristic in chitosan film could not enhance the surface hydrophilicity of chitosan composite films. In addition, inclusion of unmodified and modified montmorillonite in chitosan film increased the water contact angle of chitosan. This may be because both unmodified and modified montmorillonite blocked the area on the chitosan film to hold and interact with water molecule.

Water contact angle of chitosan/bamboo charcoal composite films are summarized in Table 4.8 and Figure 4.14.

Table 4.8 Water contact angle of chitosan/bamboo charcoal composite films**.**

Composition of films	Water contact angle (º)
CTS	110.00±0.00
CTS/BC 0.1	99.33±1.15
CTS/BC 0.25	91.33±1.15
CTS/BC 0.5	88.00±0.00
CTS/BC 1	84.00±0.00
CTS/BC 2	86.00±0.00
CTS/mBC 0.1	98.00±0.00
CTS/mBC 0.25	88.67±1.15
CTS/mBC 0.5	86.67±1.15
CTS/mBC 1	82.67±1.15
CTS/mBC 2	88.00±0.00
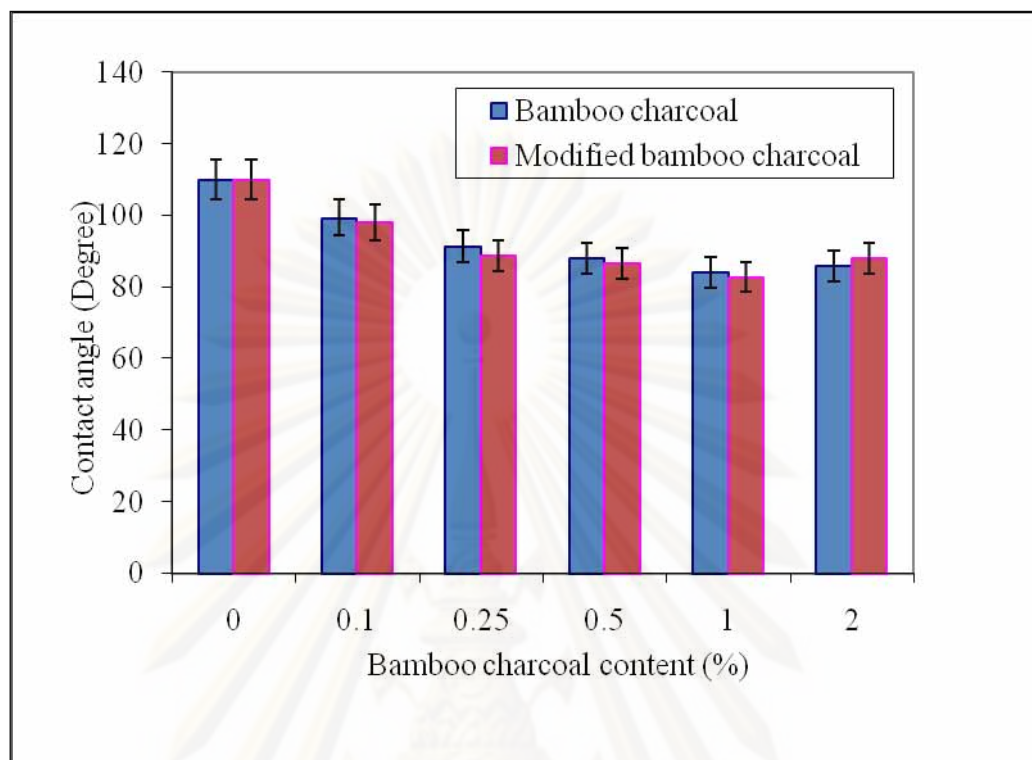
Figure 4.14 The effect of unmodified and modified bamboo charcoal contents on water contact angle of chitosan films.

Water contact angle was decreased by inclusion of either unmodified or modified bamboo charcoal up to 1% (w/w), although the decrease in water absorption was more marked in modified than unmodified bamboo charcoal composite films. This may be attributed to the formation of hydrophilic carboxylic groups on the surface of the modified bamboo charcoal by nitric acid. As a result, this leads to stronger hydrogen bonding to water and thus more hydrophilic surface nature than those of chitosan/unmodified bamboo charcoal film. When the content of unmodified or modified bamboo charcoal was decreased to 2% (w/w), however, a marked and slight increase in water contact angle levels, with respect to that observed at 1% (w/w) was seen, respectively. The amount of bamboo charcoal contents of 2% (w/w) in

composite films may cause significant agglomeration of the bamboo charcoal reducing the pores and gaps in the bamboo charcoal structure.

4.7 Oil Contact Angle

The effect of the different types and levels of montmorillonite content on oil contact angle of chitosan composite films is summarized in Table 4.9 and Figure 4.15.

Table 4.9 Oil contact angle of chitosan/montmorillonite composite films**.**

Composition of films	Oil contact angle (º)
CTS	36.00±0.00
CTS/MMT 0.1	46.00±0.00
CTS/MMT 0.25	46.67±1.15
CTS/MMT 0.5	52.00±0.00
CTS/MMT 1	56.00±0.00
CTS/MMT 2	57.33±1.15
CTS/mMMT 0.1	47.33±1.15
CTS/mMMT 0.25	50.00±0.00
CTS/mMMT 0.5	54.00±0.00
CTS/mMMT 1	58.00±0.00
CTS/mMMT 2	70.00±0.00
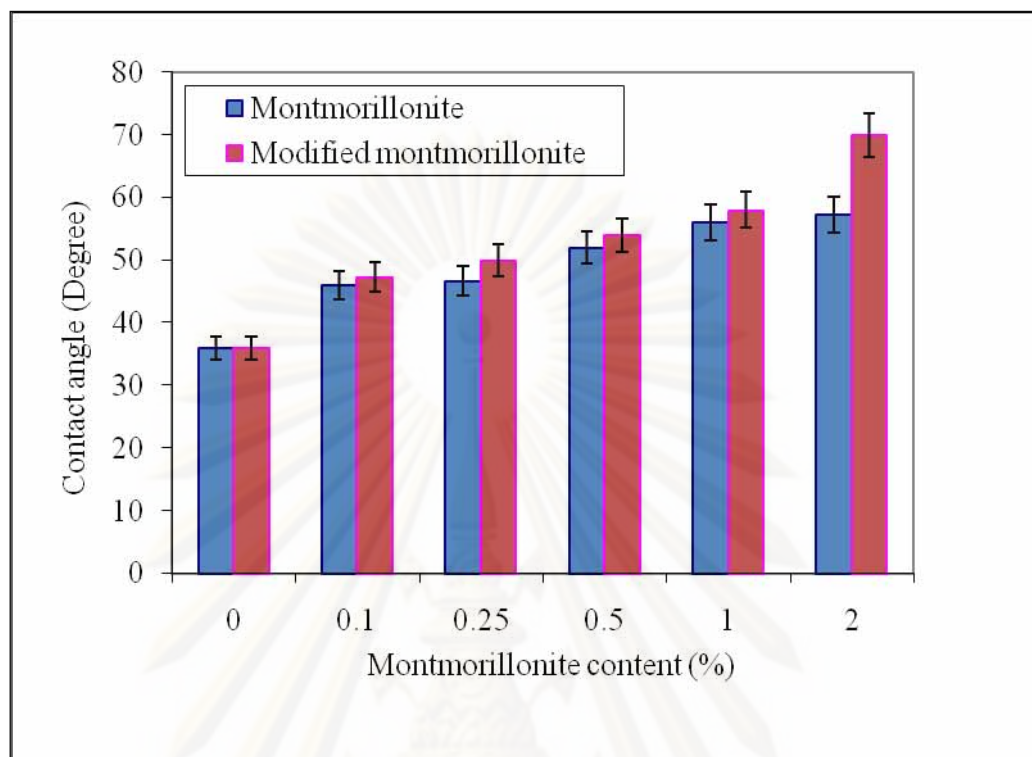
Figure 4.15 The effect of unmodified and modified montmorillonite contents on oil contact angle of chitosan films.

The oil contact angle of chitosan/montmorillonite composite films increased with increasing montmorillonite contents in composite. These results could be attributed to the particles of unmodified or modified montmorillonite inserted into chitosan film blocking the ability of chitosan to adhere with oil.

Oil contact angle of chitosan/bamboo charcoal composite films is summarized in Table 4.10 and Figure 4.16.

Table 4.10 Oil contact angle of chitosan/bamboo charcoal composite films.

Composition of films	Oil contact angle (°)
CTS	36.00±0.00
CTS/BC 0.1	32.00±0.00
CTS/BC 0.25	29.33±1.15
CTS/BC 0.5	28.00±0.00
CTS/BC 1	26.67±1.15
CTS/BC 2	27.33±1.15
CTS/mBC 0.1	30.00±0.00
CTS/mBC 0.25	29.33±1.15
CTS/mBC 0.5	26.00±0.00
CTS/mBC 1	22.67±1.15
CTS/mBC 2	24.00±0.00

Inclusion of bamboo charcoal in chitosan composite films could enhance better surface wettability with oil than that of pure chitosan film. This was because of good affinity with the oil of chitosan and the hydrophobic materials of bamboo charcoal. The results shown in Figure 4.16 could be concluded that oil contact angle in the pure chitosan films was about 36°, and both modified and unmodified bamboo charcoal composite films revealed decrease in oil contact angle up to 1% (w/w) charcoal composition. The results of oil contact angle of both types of bamboo charcoal composite films were in broad accordance with the results obtained for water contact angle. This again may reflect the presence of pores and gaps that bamboo

charcoal introduces into the composite structure and also due to hydrophobic character of bamboo charcoal.
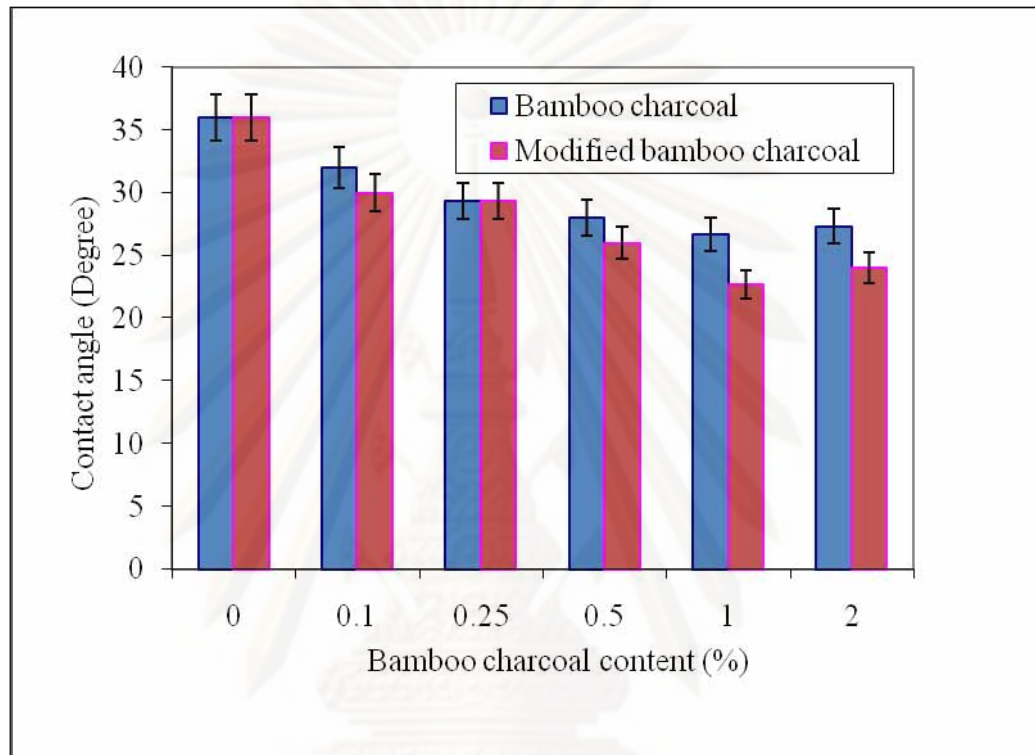
Figure 4.16 The effect of unmodified and modified bamboo charcoal contents on oil contact angle of chitosan films.

Bamboo charcoal decreased water and oil contact angle in both types of chitosan/bamboo charcoal composite film. This may be due to the pores and gaps in the bamboo charcoal structure. On the other hand, montmorillonite increased water and oil contact angle in both types of chitosan/monmorillonite composite films. This may be due to the particle of montmorillonite blocking the chitosan to adhere water and oil. Moreover, the larger interlayer distance between the individual silicate layers of modified montmorillonite could not be able to hold or capture water or oil.

4.8 Thermogravimetric Analysis (TGA)

The thermal stability of the chitosan/montmorillonite, chitosan/modified monmorillonite, chitosan/bamboo charcoal, and chitosan/modified bamboo charcoal composite films was investigated by TGA under nitrogen atmosphere, as shown in Figures 4.17-4.20, respectively. The degradation of chitosan and chitosan composite films in the nitrogen atmosphere is different as seen in the Figures 4.17-4.18. The use of nitrogen flow allows non-oxidative degradation occurring in the samples. From the Figure 4.17, there are two steps of degradation of chitosan. The first range (50-150 ℃) is associated with the loss of water about 12 wt%, whereas the second range (150-350 ℃) corresponds to the degradation and deacetylation of chitosan and left about 31wt% solid residue. This is similar to the results reported by other researchers [69].

The effect of the montmorillonite content on thermal stability of chitosan/montmorillonite composite films is summarized in Table 4.11 and Figure 4.17.

Table 4.11 Temperature and weight in TG during the thermal degradation of chitosan/montmorillonite composite films.

Composition of films	Temperature at 60% weight remaining (ºC)	Weight remaining (%) at 750 ºC
CTS	307	31
CTS/MMT 0.1	311	32
CTS/MMT 0.25	305	30
CTS/MMT 0.5	309	34
CTS/MMT 1	336	33
CTS/MMT 2	356	34
CTS/mMMT 0.1	298	35
CTS/mMMT 0.25	312	35
CTS/mMMT 0.5	333	36
CTS/mMMT 1	352	36
CTS/mMMT 2	378	37
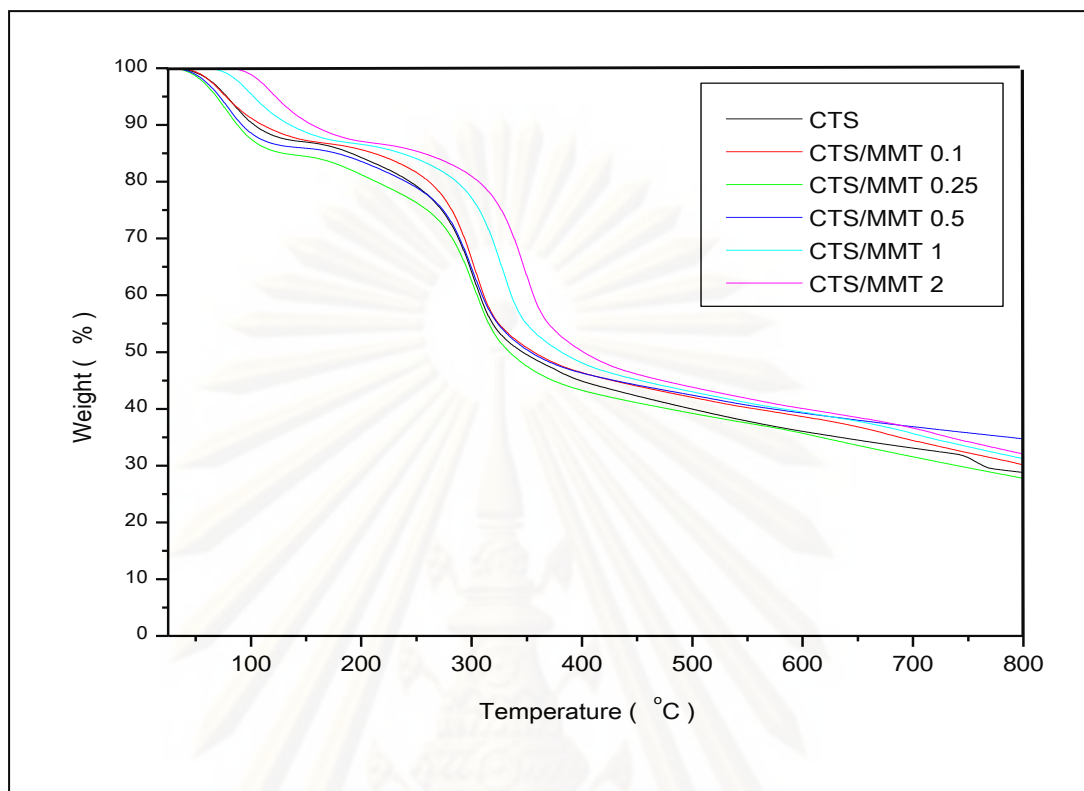
Figure 4.17 Thermogravimetric curves of chitosan and chitosan/montmorillonite composite films.

The dispersed montmorillonite in chitosan matrix exhibits a delay in weight loss, especially at 1 and 2% (w/w) of montmorillonite content. Chitosan composite films containing montmorillonite at 1 and 2% (w/w) had the percent weight remaining at 750 ºC around 33 and 34% (w/w), respectively. The content of weight remaining at water decomposition stage increased with increasing montmorillonite content of the film samples. This implied that the formation of chitosan-silicate complex would decrease the water absorbability of the films, which is decreasing the hydrophilicity of the films. After pyrolysis, the composite films form char with a multilayered carbonaceous-silicate structure, which may keep its multilayered structure in the polymer matrix even at 600ºC [67]. This high-performance carbonaceous-silicate char

builds up on the surface during burning, thus insulating the underlying material and slowing the escape of the volatile products generated during decomposition. The clay acts as a heat barrier as well as assists in the formation of char after thermal decomposition. The dispersed lamellae of clay in chitosan matrix will result qualitatively in a spatially more uniform and thicker char during decomposition. The formation of char on the surface of polymer matrix, as a consequence, reduces the rate of decomposition.

The effect of the modified montmorillonite content on thermal stability of chitosan/modified montmorillonite composite films is summarized in Figure 4.18.
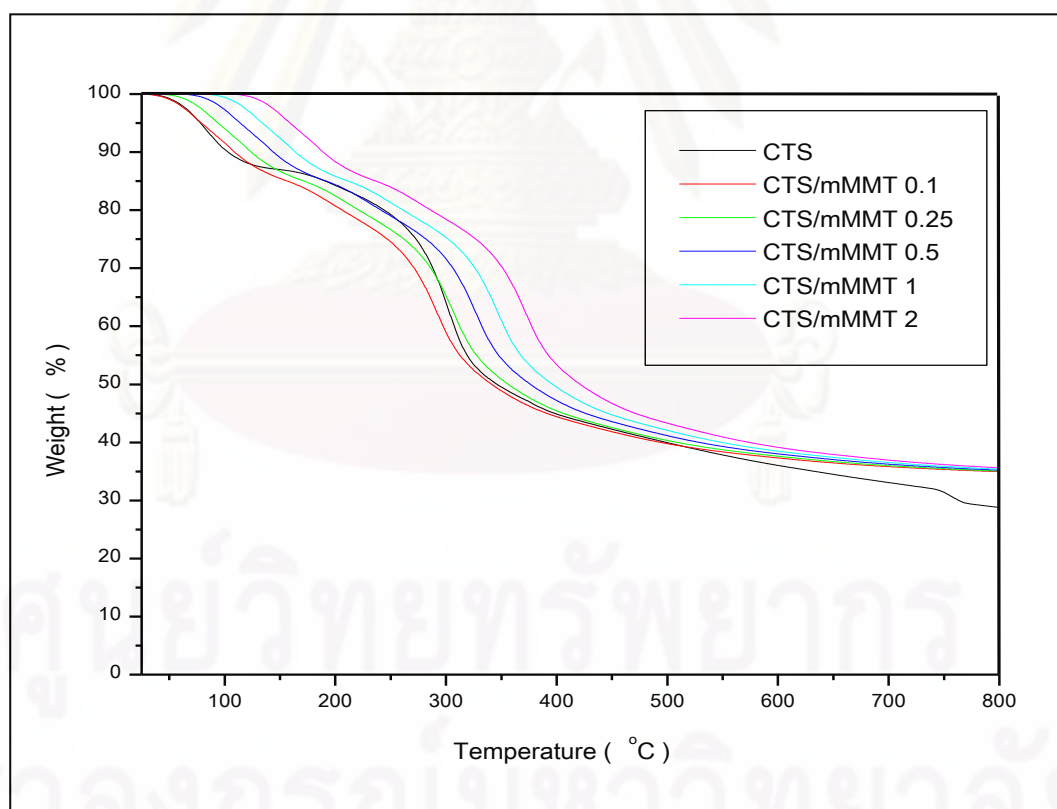
Figure 4.18 Thermogravimetric curves of chitosan and chitosan/modified montmorillonite composite films.

The thermal stability of chitosan/modified montmorillonite composite film showed a slightly different trend to those of chitosan/unmodified montmorillonite composite films. The thermal stability of chitosan/modified montmorillonite composite films at 60% weight remaining slightly decreased with the addition of 0.1 to 0.5% (w/w) modified montmorillonite content, and then increased with higher levels (1 and 2% (w/w)) of modified montmorillonite content. At lower modified montmorillonite content, the thermal stability of chitosan/modified montmorillonite composite films were reduced. This may be because the content of modified montmorillonite is not enough to form uniform and thick carbonaceous-silicate char. When the content of modified montmorillonite increased with higher levels (1 and 2% (w/w)), thermal stability was increased. The dispersed lamellae of montmorillonite in chitosan matrix will result qualitatively in a spatially more uniform and thicker carbonaceous-silicate char during decomposition.

The effect of the bamboo charcoal content on thermal stability of chitosan/bamboo charcoal composite films is summarized in Table 4.12 and Figure 4.19.

Table 4.12 Temperature and weight in TG during the thermal degradation of chitosan/bamboo charcoal composite films.

Composition of films	Temperature at 60% weight remaining (ºC)	Weight remaining (%) at 750 ºC
CTS	307	31
CTS/BC 0.1	317	41
CTS/BC 0.25	328	41
CTS/BC 0.5	348	42
CTS/BC 1	339	42
CTS/BC 2	379	43
CTS/mBC 0.1	302	27
CTS/mBC 0.25	302	34
CTS/mBC 0.5	312	34
CTS/mBC 1	292	33
CTS/mBC 2	282	32
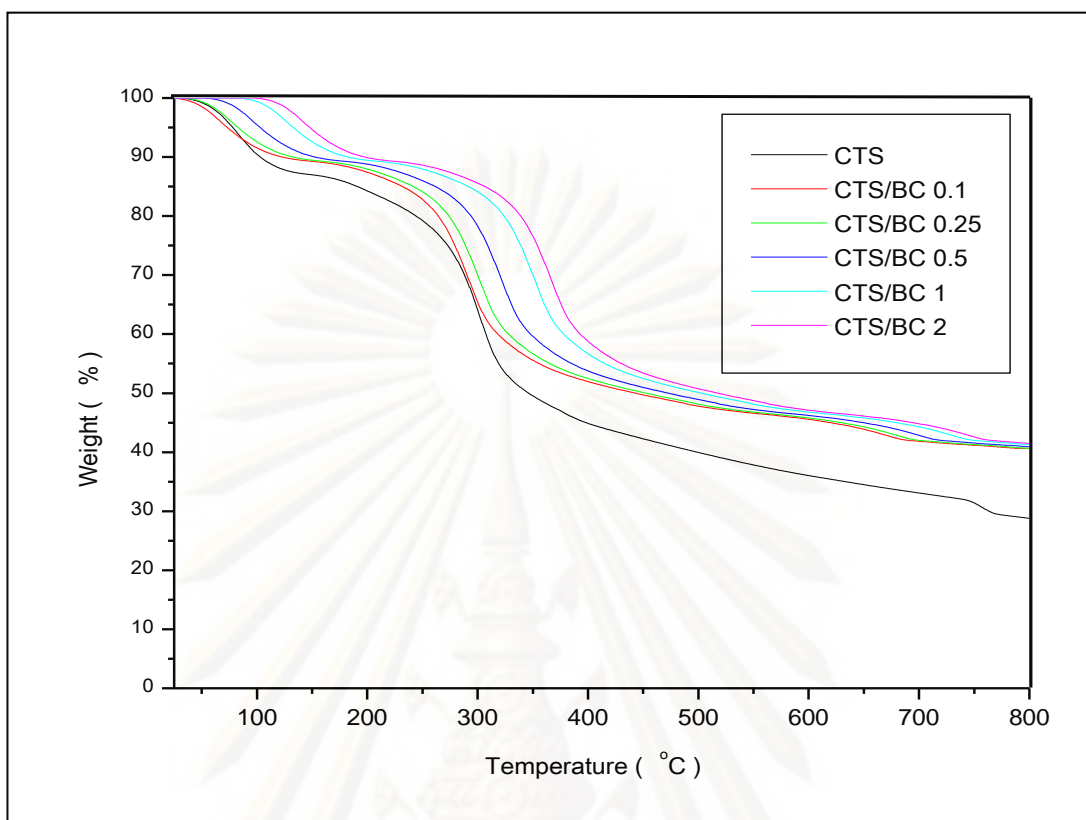
Figure 4.19 Thermogravimetric curves of chitosan and chitosan/bamboo charcoal composite films.

The results show that introducing the bamboo charcoal into chitosan matrix can enhance the thermal stability of chitosan/bamboo charcoal composite films. The chitosan/bamboo charcoal composite films decompose slower and thoroughly and thus are more stable than chitosan film. The chitosan/bamboo charcoal composite films show increased thermal stability with increasing the bamboo charcoal content. This is probably due to the bamboo charcoal acts as a heat barrier, which could enhance the overall thermal stability of the film during thermal decomposition.

The effect of the modified bamboo charcoal content on thermal stability of chitosan/modified bamboo charcoal composite films is summarized in Figure 4.20.
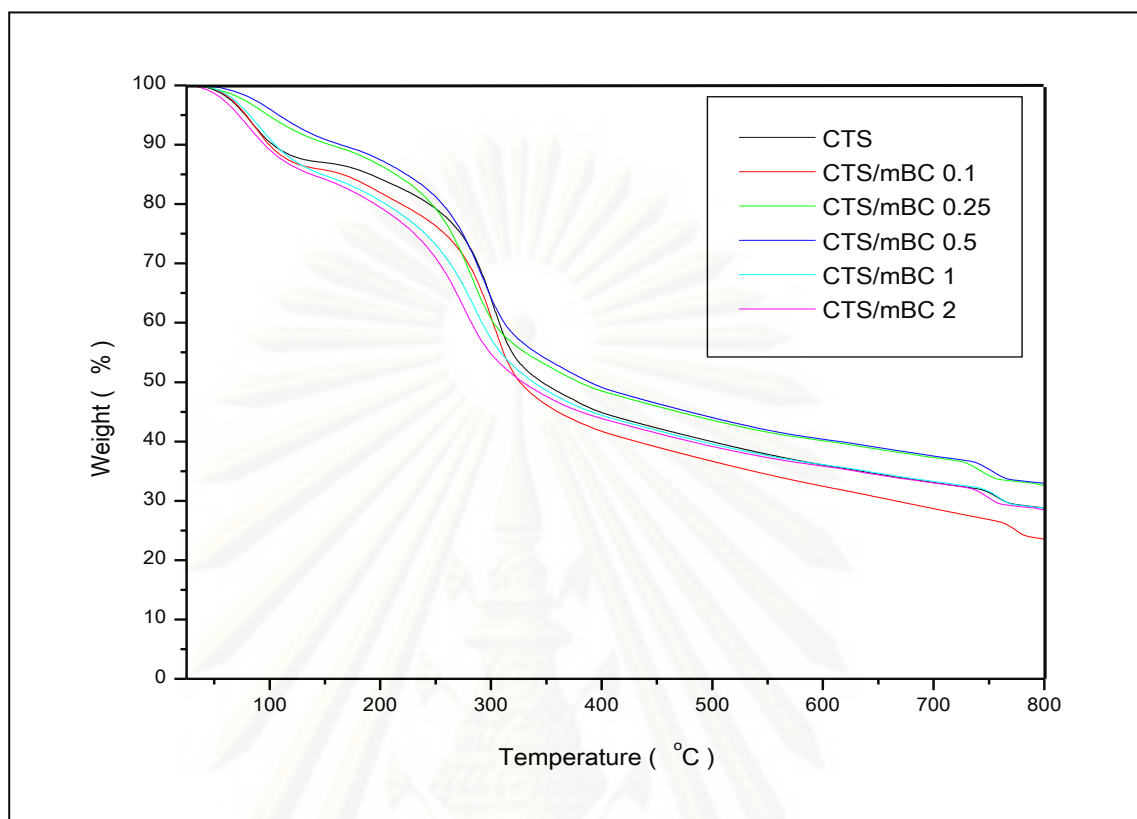
Figure 4.20 Thermogravimetric curves of chitosan and chitosan/modified bamboo charcoal composite films.

The chitosan/modified bamboo charcoal composite films show lower thermal stability compare to chitosan and chitosan/bamboo charcoal composite films. The thermal stability of chitosan/modified bamboo charcoal composite films decreased with increasing bamboo charcoal content of the composite films. The residue of acid, which is by product of oxidation of bamboo charcoal, in chitosan strongly induces the thermal degradation of the matrix. A similar phenomenon was also observed by Wang et al. [66]. They reported the existence of acetic acid residue in chitosan matrix would induce the thermal decomposition of the chitosan matrix.

4.9 Scanning Electron Microscopy (SEM)

Surface morphology of composite films under scanning electron microscopy was undertaken in order to investigate filler aggregation in the chitosan matrix. Scanning electron micrographs (SEMs) at 100x magnification of the outer surface of the chitosan film sample is shown in Figure 4.21 and those of chitosan/montmorillonite composite, chitosan/modified montmorillonite composite, chitosan/bamboo charcoal composite, and chitosan/modified bamboo charcoal composite film samples are shown in Figure 4.22, 4.23, 4.24, and 4.25, respectively.
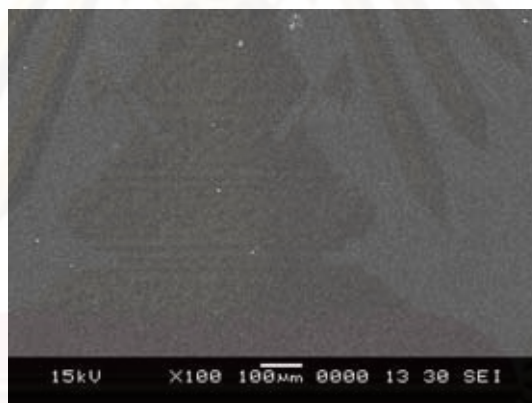
Figure 4.21 SEM micrographs (x100) of the pure chitosan film**.**

Figure 4.21 displays the surface of chitosan film to be relatively smooth, to be homogeneous and to be a continuous matrix without any pores or cracks with good structural integrity. It is flat and compact with vary sparsely distributed small particles without any phase separation.

(a) CTS/MMT 0.1

(b) CTS/MMT 0.25

(c) CTS/MMT 0.5
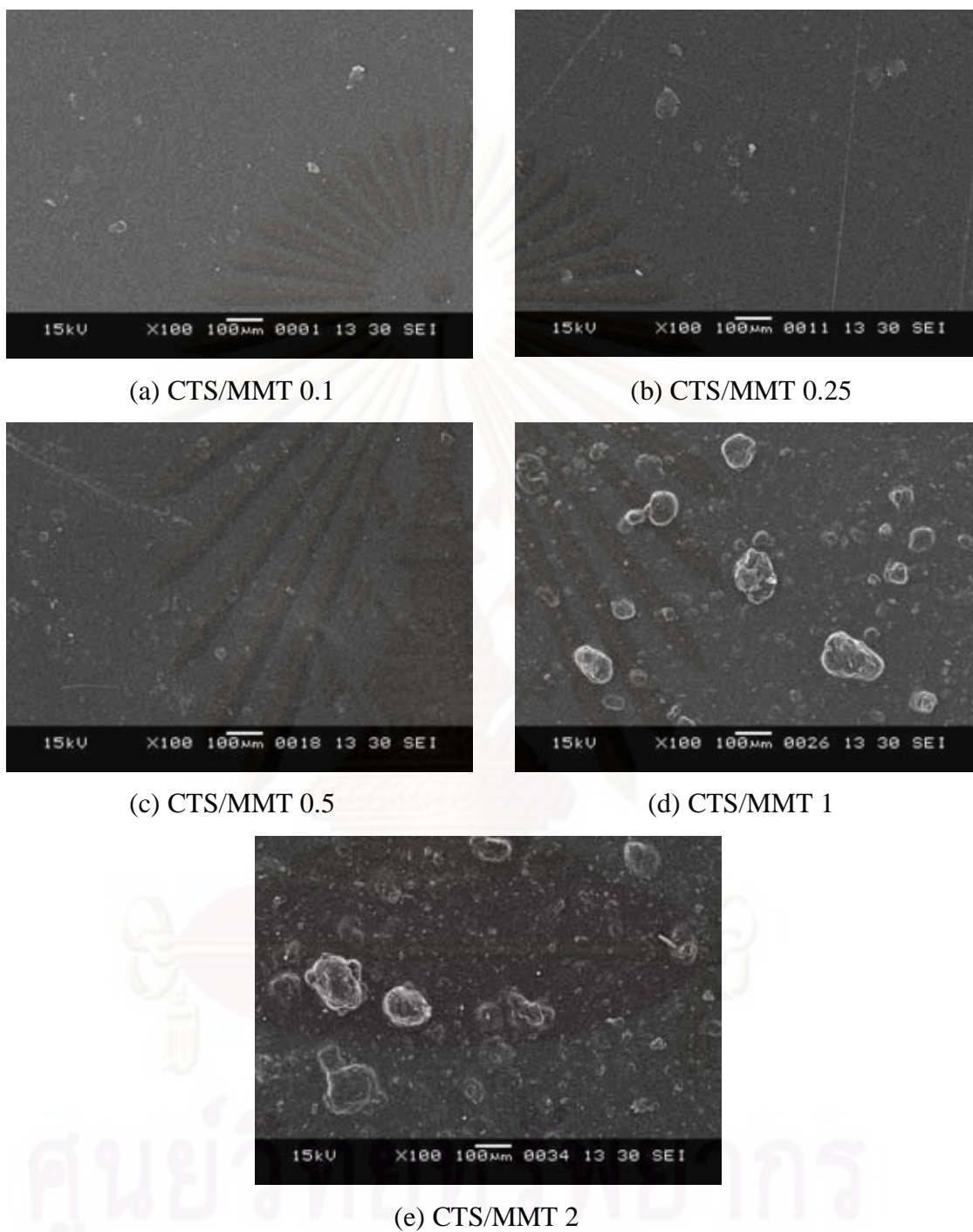
(d) CTS/MMT 1

(e) CTS/MMT 2

Figure 4.22 SEM micrographs (x100) of the chitosan/montmorillonite composite films.
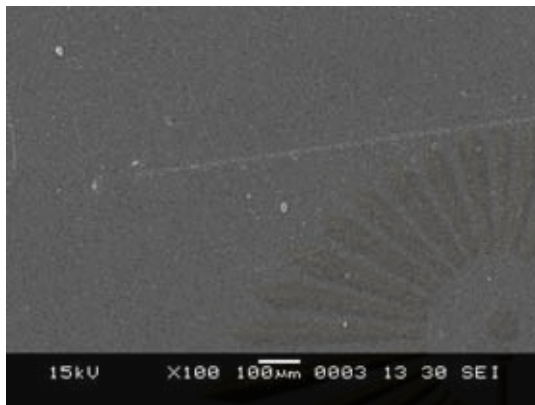
(a) CTS/mMMT 0.1
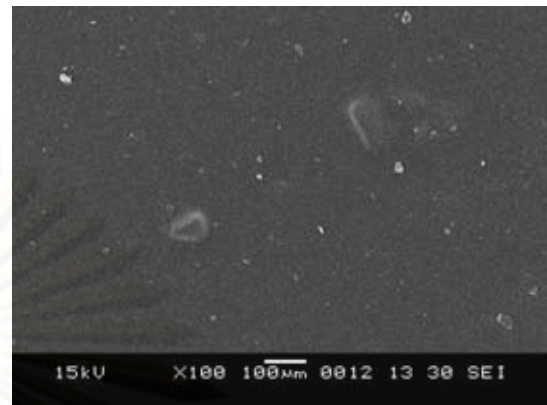
(b) CTS/mMMT 0.25
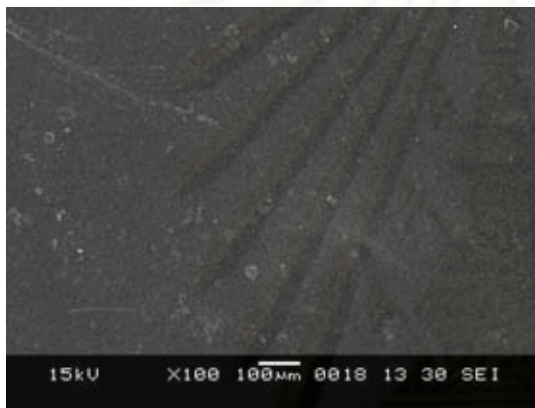
(c) CTS/mMMT 0.5
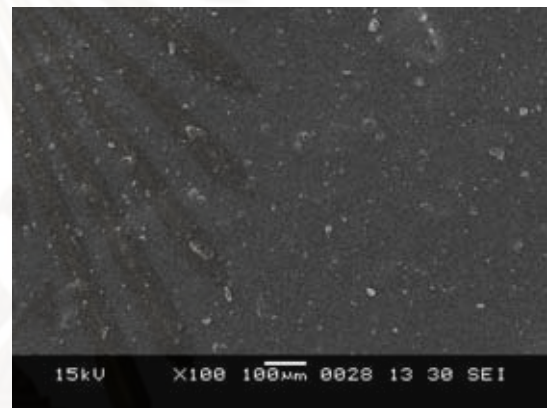
(d) CTS/mMMT 1
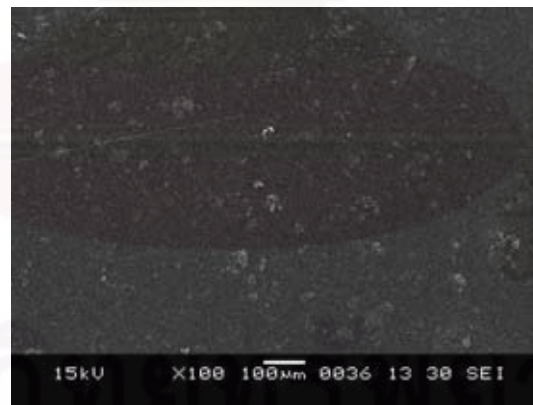
(e) CTS/mMMT 2

Figure 4.23 SEM micrographs (x100) of the chitosan/modified montmorillonite composite films.

SEM micrographs as shown in Figure 4.22 show the good dispersion of montmorillonite in the chitosan matrix at lower montmorillonite content (0.1-0.5% (w/w)). Typically, there is an attractive force (hydrogen bonding attraction) between chitosan and montmorillonite, resulting in good distribution of montmorillonite in the chitosan matrix. Chitosan composite films containing 1 and 2% (w/w) montmorillonite had aggregation of montmorillonite more than that in chitosan films containing lower montmorillonite at 0.1-0.5% (w/w). The poor distribution of montmorillonite particles at high content in chitosan matrix could be attributed to the formation of a flocculated structure in the composite which is due to the hydroxylated edge-edge interaction of the silicate layers because of the hydrogen bonding interaction between the silicate hydroxylated edge groups and the amino or hydroxyl functional groups in long chains of chitosan [68].

In contrast, inclusion of montmorillonite modified with depolymerized chitosan in chitosan film showed better homogenous dispersion of modified montmorillonite in chitosan matrix. This may result in the better compatibility of modified montmorillonite in chitosan matrix than that of unmodified montmorillonite in chitosan matrix.

(a) CTS/BC 0.1

(b) CTS/BC 0.25

(c) CTS/BC 0.5

(d) CTS/BC 1

(e) CTS/BC 2
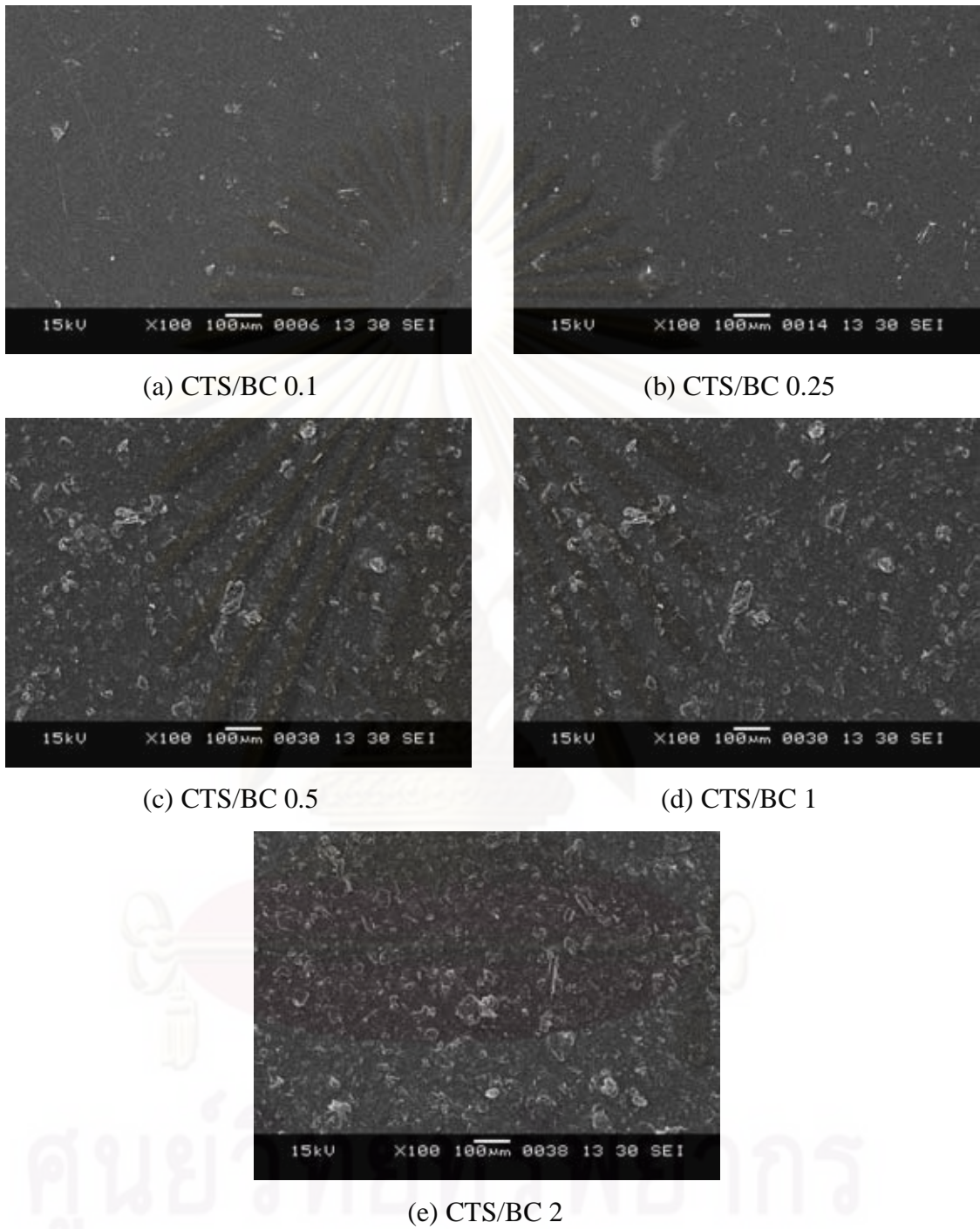
Figure 4.24 SEM micrographs (x100) of the chitosan/bamboo charcoal composite films.
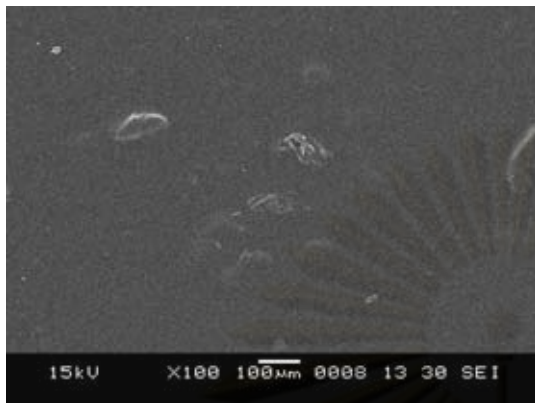
(a) CTS/mBC0.1
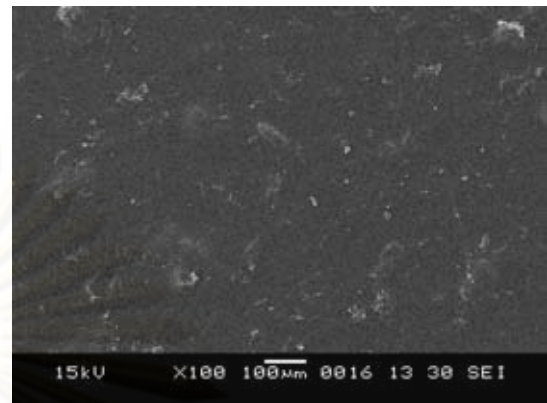
(b) CTS/mBC 0.25
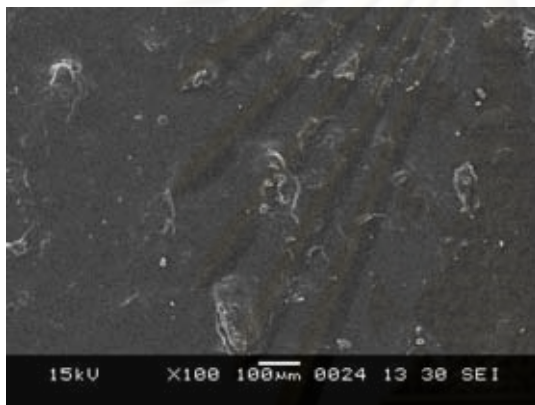
(c) CTS/mBC 0.5
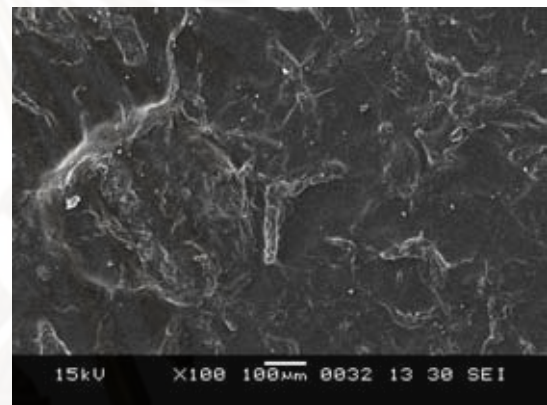
(d) CTS/mBC 1
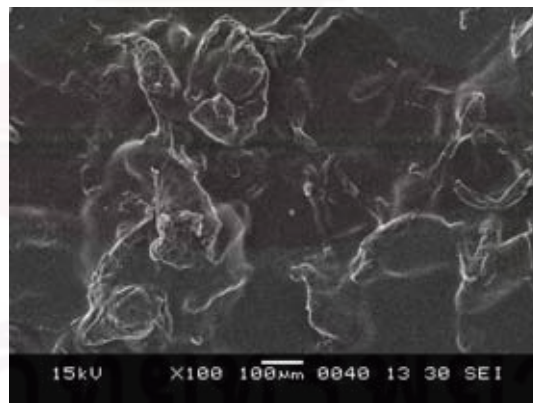
(e) CTS/mBC 2

Figure 4.25 SEM micrographs (x100) of the chitosan/modified bamboo charcoal composite films.

Microphase separation and roughness appearances were observed in chitosan/bamboo charcoal composite films (Figure 4.24) and chitosan/modified bamboo charcoal composite films (Figure 4.25). With an increase in the amount of bamboo charcoal or modified bamboo charcoal in the composite films, the smooth surface gradually disappeared. The flat dense surface of the chitosan became progressively undulating. For chitosan/bamboo charcoal composite films, bamboo charcoal particles were not diffused into the chitosan film. This result could be attributed to the bamboo charcoal particles were large and stayed in aggregated form in chitosan matrix, which made it difficult to be embedded into the surface of and then diffused into the chitosan film.

Oxidative treatment of bamboo charcoal particles was effective in increasing the interfacial adhesion between bamboo charcoal and chitosan, and also in providing more H-bonding between them, and thus bamboo charcoal particles were dispersed within chitosan matrix in the composite film with relatively good interfacial adhesion between the two components. A good dispersion of modified bamboo charcoal in chitosan matrix was observed at 0.25% and 0.5% (w/w) modified bamboo charcoal, whilst 1% and 2% (w/w) modified bamboo charcoal contents displayed more aggregation of modified bamboo charcoal particles on chitosan composite films.

4.10 Antifungal of *Colletotrichum gloeosporioides*

The inhibitory effect of chitosan and chitosan composite films on spore germination was studied. The results of fungus germination on film specimens of chitosan, chitosan/montmorillonite composite, and chitosan/modified montmorillonite composite films are shown in Figures 4.26 and 4.27.

Chitosan films inhibited *Colletotrichum gloeosporiodes* growth. Chitosan possesses several biological properties in addition to its ability to form a semi-permeable film due to its polymeric nature. Agullo et al. [67] observed that the use of a chitosan-based coating delayed *Alternaria sp*, *Penicillium sp* and *Cladosporium sp* growth. Preliminary tests were carried out to verify the antifungal activity of the chitosan film on *Colletotrichum gloeosporiodes*. Inhibitory effect of a chitosan film forming solution was observed by using a coating process of an inoculated agar medium. The chitosan coating of inoculated agar medium inhibited the growth of fungi. This result showed that chitosan film could be used to control the development of mycotoxinogen fungi on plants. The polycationic property allows chitosan to interact with negatively charged substances, thereby exhibiting antifungal on molds. As specified by Agullo et al. [69], three mechanisms have been proposed for chitosan's antifungal property. The biopolymer appears to work both by interfering directly with fungal growth on plants, and also by activating many defense responses. The chelating properties of chitosan make it an antifungal agent [68]. Moreover, Sebti et al. [70] mentioned that a strong inhibition of *Aspergillus niger* spore germination on a solid medium supplemented with chitosan. They also observed that chitosan produced spore aggregation and morphological anomalies.
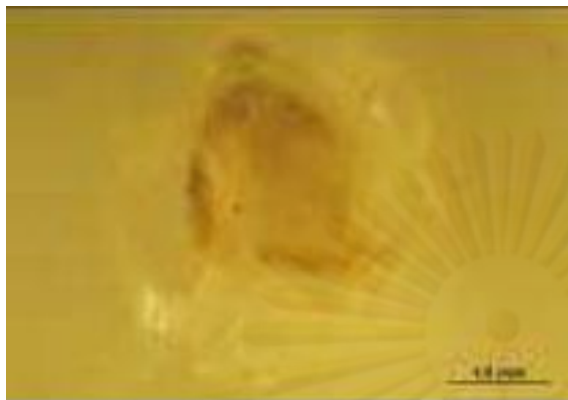
(a) CTS

(b) CTS/MMT 0.1

(c) CTS/MMT 0.25

(d) CTS/MMT 0.5

(e) CTS/MMT 1

(f) CTS/MMT 2

Figure 4.26 The fungus germination of the chitosan/montmorillonite composite films.

(a) CTS

(b) CTS/mMMT 0.1

(c) CTS/mMMT 0.25

(d) CTS/mMMT 0.5

(e) CTS/mMMT 1

(f) CTS/mMMT 2
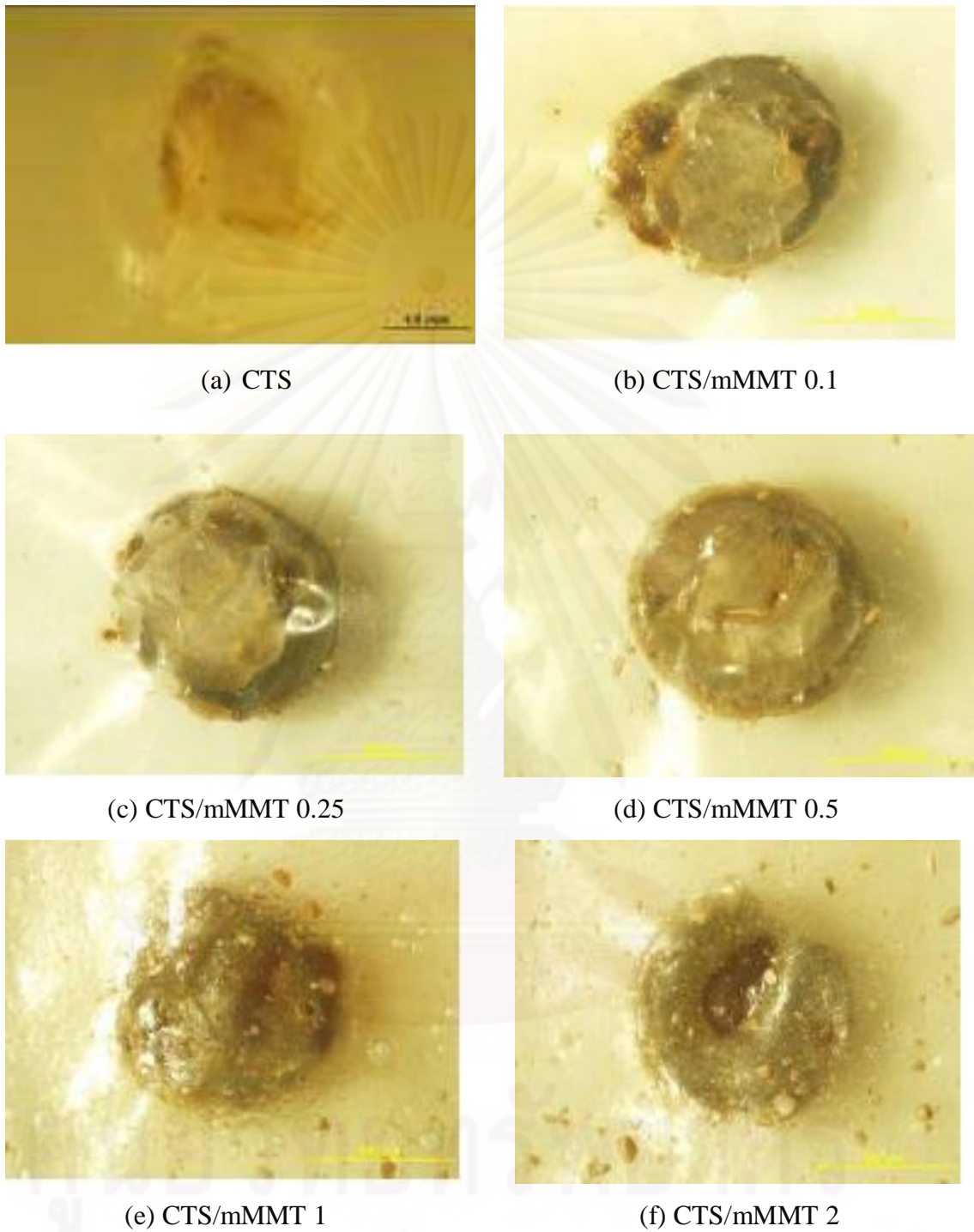
Figure 4.27 The fungus germination of the chitosan/modified montmorillonite composite films.

Furthermore, the germination and growth of *Colletotrichum gloeosporiodes* was completely inhibited by chitosan composite films. This was because the films underneath chitosan composite film were clear from the spores of *Colletotrichum gloeosporiodes*. This implied that chitosan composite films inhibited the growth of *Colletotrichum gloeosporiodes* pass through them, therefore, the film underneath of those chitosan composite films could not be investigated any spores of *Colletotrichum gloeosporiodes*. Thus, it can be concluded that the incorporation of bamboo charcoal and montmorillonite in chitosan films had an outstanding in antifungal activity. In addition, the strong acetic acid used to dissolve chitosan may also have ability of antifungal and needed more investigation in futher research.

The inhibitory effect of fungus germination on film specimens of chitosan, chitosan/bamboo charcoal, and chitosan/modified bamboo charcoal composite films are shown in Figures 4.28 and 4.29.

(a) CTS

(b) CTS/BC 0.1

(c) CTS/BC 0.25

(d) CTS/BC 0.5

(e) CTS/BC 1

(f) CTS/BC 2
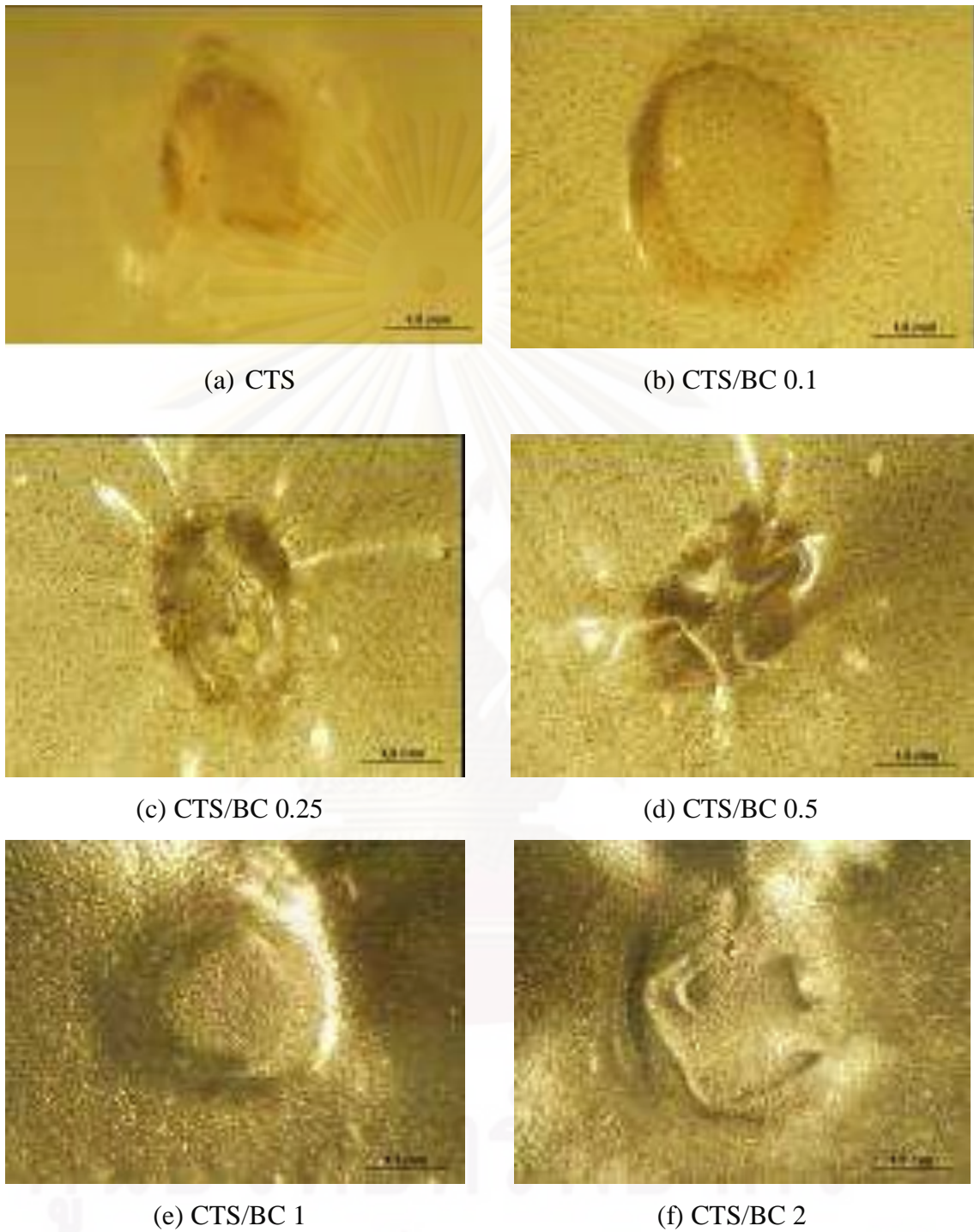
Figure 4.28 The fungus germination of the chitosan/bamboo charcoal composite films.

(a) CTS

(b) CTS/mBC 0.1

(c) CTS/mBC 0.25

(d) CTS/mBC 0.5

(e) CTS/mBC 1

(f) CTS/mBC 2
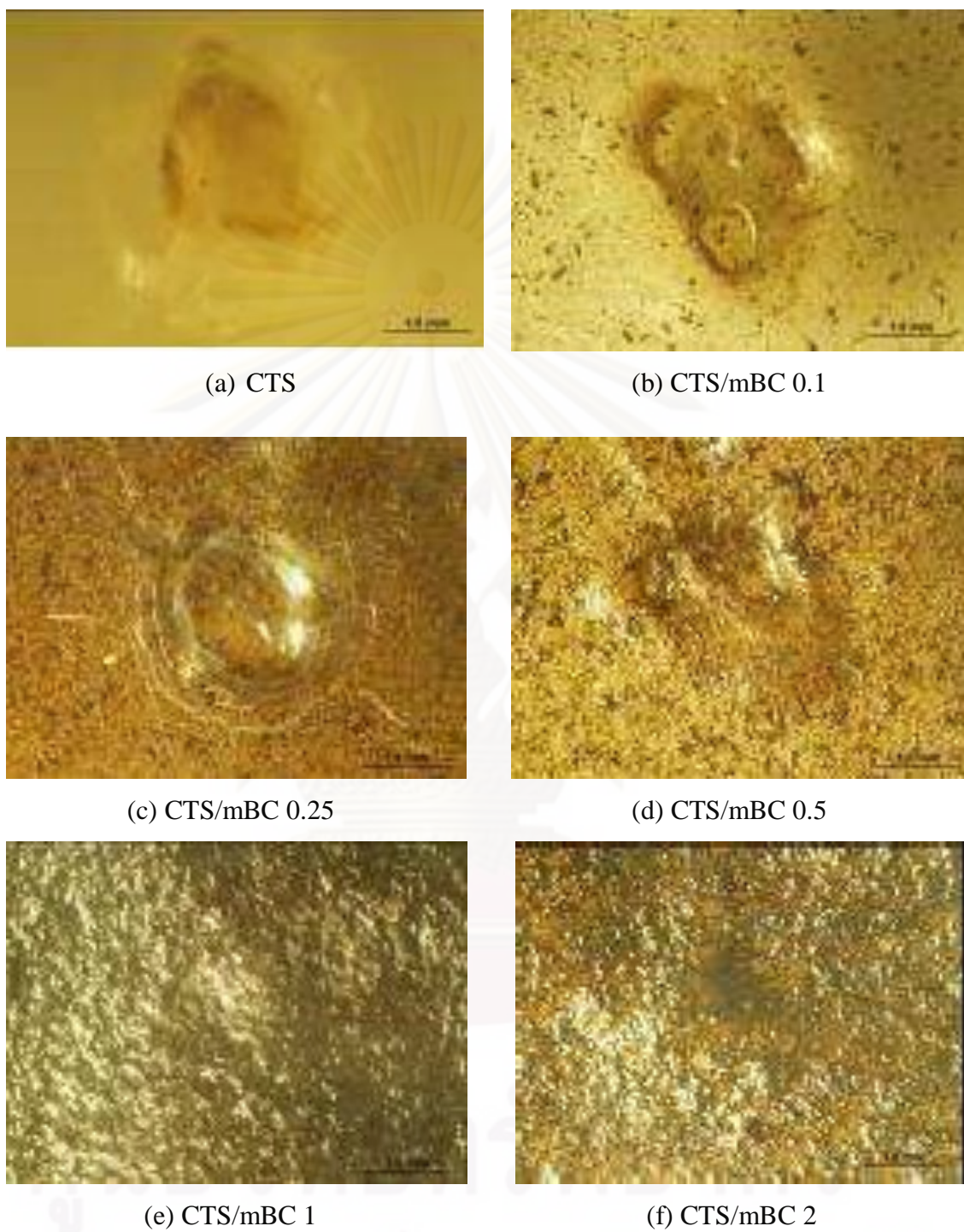
Figure 4.29 The fungus germination of the chitosan/modified bamboo charcoal composite films.

CHAPTER V

CONCLUSIONS

5.1 Conclusions

Chitosan/montmorillonite composite films and chitosan/bamboo charcoal composite films were made by adding either virgin or modification of montmorillonite and bamboo charcoal into chitosan solution and forming the composite film by spin coating. The chitosan composite films contained contents of montmorillonite or bamboo charcoal varying from 0 to 2% (w/w). The morphological, structural and functional properties of these composite films were evaluated. The results were summarized as follows.

1. The X-ray analysis showed that the modification of montmorillonite with depolymerized chitosan at molecular weight of 15 kDa appeared to induce the intercalation occurred in the modified montmorillonite.

2. FTIR analysis confirmed that nitric acid, used as an oxidizing agent, oxidized some areas of bamboo charcoal particles during refluxing leading to formation of carboxylic acid groups on the surface of the bamboo charcoal particles.

3. Mechanical properties analysis showed that the tensile strength and rigidity were both found to increase, whilst the elongation at break decreased, with increasing either virgin or modified montmorillonite and bamboo charcoal in chitosan composite films.

4. Inclusion of either unmodified montmorillonite or modified montmorillonite in chitosan films decreased the water absorption of the composite

films. These effects would be attributed to montmorillonite particles embedded in the chitosan film obstructing ability of chitosan to adherencing the water.

Inclusion of either unmodified or modified bamboo charcoal in chitosan films enhanced the water absorption of the composite films. These effects would be attributed to structure and hydrophilic character of chitosan itself and many pores and gaps in the bamboo charcoal. The enhanced water absorption in modified bamboo charcoal composite films relative to the same (w/w) unmodified bamboo charcoal composite films would be attributed to the formation of hydrophilic carboxylic groups on the surface of modified bamboo charcoal by oxidative treatment with nitric acid.

5.   Inclusion of either unmodified montmorillonite or modified montmorillonite in chitosan films decreased the oil absorption of the composite films as well. This may also because montmorillonite particles embedded in the chitosan film obstructing ability of chitosan to adherencing the oil.

Both unmodified and modified bamboo charcoal in chitosan films enhanced the oil absorption of the composite films. These results could be concluded that the presence of pores and gaps in bamboo charcoal providing more oil absorption than that of pure chitosan film.

6.   Inclusion of either unmodified montmorillonite or modified montmorillonite in chitosan films increased the water contact angle of the composite films.

On the other hand, inclusion of either unmodified or modified bamboo charcoal in chitosan films decreased the water contact angle of the composite films.

7.   Inclusion of either unmodified montmorillonite or modified montmorillonite in chitosan films increased the oil contact angle of the composite films as well.

Both unmodified and modified bamboo charcoal in chitosan films decresed the oil contact angle of the composite films. Inclusion of either modified montmorillonite or modified bamboo charcoal into chitosan films enhanced the thermal stability of chitosan composite films.

8.   SEM micrographs demonstrated a good dispersion of modified montmorillonite or bamboo charcoal particles in chitosan matrix up to 0.5% (w/w), while some aggregation of montmorillonite or bamboo charcoal were observed at the content starting of 1% (w/w) of the chitosan composite films.

9.   *Colletotrichum gloeosporiodes* was completely inhibited by chitosan and chitosan composite films.

5.2 Recommendations

1.   The shrinkage of chitosan films and chitosan composite films after drying needs to be solved by using crosslinking agent.

2.   Comparing the quaternary ammonium modified montmorillonite with the depolymerized chitosan modified montmorillonite in chitosan composite films in terms of physical properties and water and oil absorption. This is because quaternary ammonium normally has been used to separate the layer of montmorillonite.

3.   Chitosan and chitosan composite films formed by spin coater in this study were too thin. Their thickness of these films were not be able to use as a film packaging.

4.   Air permeability and water vapor permeation are important properties for the film. The films formed by the spin coating in this study were not appropriate to use for testing air permeability and water vapor permeation. For further research, the suitable size and thickness of the film need to be form for testing these two properties. The other methods that can be used to form a stronger and thicker film need to be studied further.
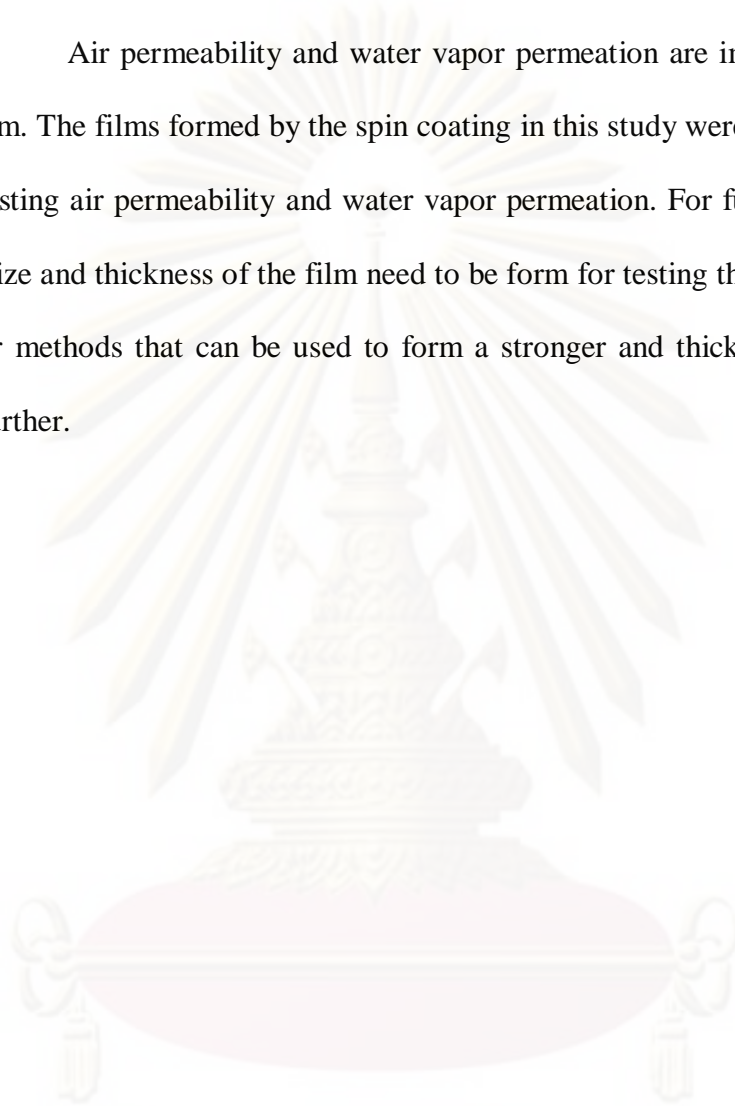
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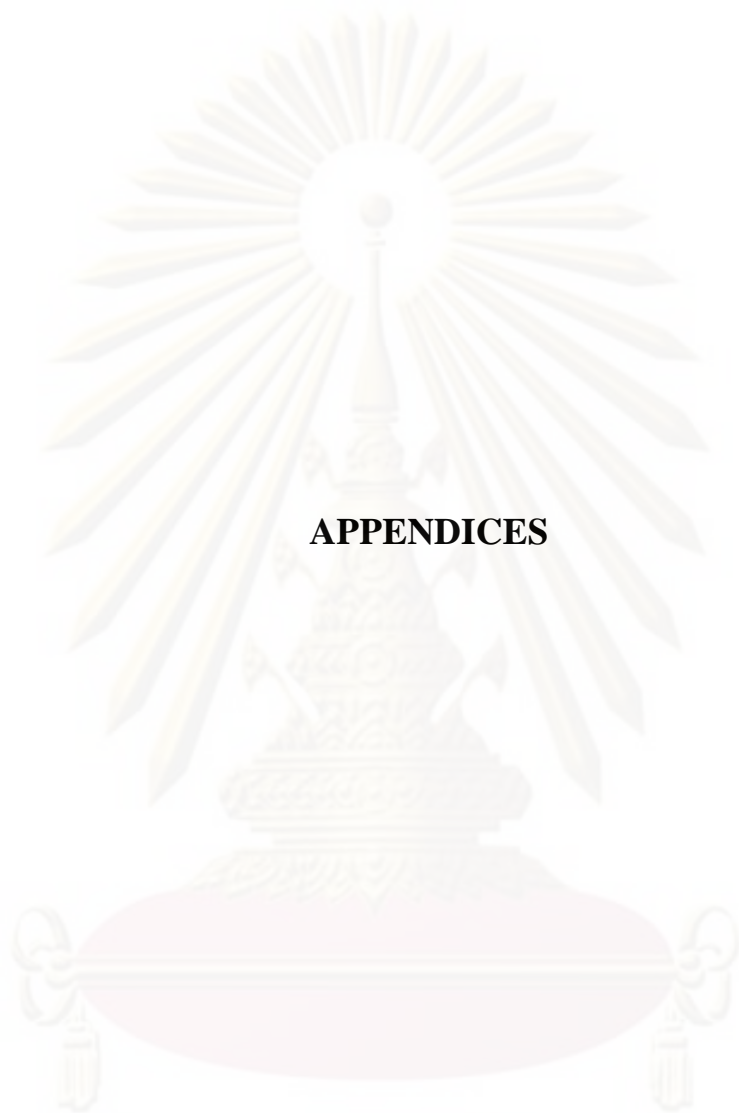
APPENDICES

APPENDIX A

PARTICLE SIZE CHARACTERIZATION

A1 Particle size of bamboo charcoal powder

MALVERN INSTRUMENTS

MASTERSIZER 2000

Result Analysis Report

Sample Name: bc - Average	**SOP Name:**	**Measured:** 03 October 2008 14:25:16
Sample Source & type:	**Measured by:** Toommee	**Analysed:** 03 October 2008 14:25:17
Sample bulk lot ref:	**Result Source:** Averaged	

Particle Name: Carbon	**Accessory Name:** Hydro 2000SM (A)	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 2.420	**Absorption:** 1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 13.68 %
Dispersant Name: Water	**Dispersant RI:** 1.330	**Weighted Residual:** 0.378 %	**Result Emulation:** Off

Concentration: 0.0252 %Vol	**Span :** 2.843	**Uniformity:** 1.18	**Result units:** Volume
Specific Surface Area: 0.461 m²/g	**Surface Weighted Mean D[3,2]:** 13.029 um	**Vol. Weighted Mean D[4,3]:** 35.231 um	

d(0.1): 6.392 um   **d(0.5):** 20.374 um   **d(0.9):** 64.314 um
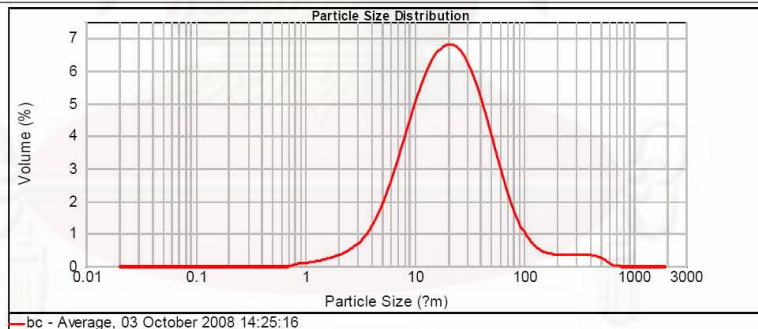
Particle Size Distribution — bc - Average, 03 October 2008 14:25:16

Size (?m)	Volume In %	Size (?m)	Volume In %	Size (?m)	Volume In %	Size (?m)	Volume In %	Size (?m)	Volume In %	Size (?m)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.12	11.482	5.24	120.226	0.52	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.16	13.183	5.64	138.038	0.40	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.20	15.136	5.94	158.489	0.35	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.25	17.378	6.11	181.970	0.33	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.31	19.953	6.14	208.930	0.32	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.39	22.909	6.05	239.883	0.32	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.50	26.303	5.81	275.423	0.32	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.65	30.200	5.45	316.228	0.32	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.86	34.674	4.98	363.078	0.32	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	1.14	39.811	4.40	416.869	0.31	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	1.49	45.709	3.76	478.630	0.28	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	1.93	52.481	3.10	549.541	0.24	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	2.44	60.256	2.45	630.957	0.09	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	3.00	69.183	1.87	724.436	0.01	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	3.59	79.433	1.38	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.03	8.710	4.18	91.201	0.99	954.993	0.00	10000.000	0.00
0.091	0.00	0.955	0.07	10.000	4.75	104.713	0.71	1096.478	0.00		
0.105	0.00	1.096	0.09	11.482		120.226		1258.925	0.00		

Operator notes:

A2 Particle size of modified bamboo charcoal powder

MALVERN INSTRUMENTS

MASTERSIZER 2000

Result Analysis Report

Sample Name: mbc - Average	**SOP Name:**	**Measured:** 08 July 2008 16:58:19
Sample Source & type:	**Measured by:** Unknown	**Analysed:** 08 July 2008 16:58:20
Sample bulk lot ref:	**Result Source:** Averaged	

Particle Name: Carbon	**Accessory Name:** Hydro 2000SM (A)	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 2.420	**Absorption:** 1	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 17.31 %
Dispersant Name: Water	**Dispersant RI:** 1.330	**Weighted Residual:** 0.314 %	**Result Emulation:** Off

Concentration: 0.0284 %Vol	**Span :** 2.406	**Uniformity:** 0.745	**Result units:** Volume
Specific Surface Area: 0.523 m²/g	**Surface Weighted Mean D[3,2]:** 11.479 um	**Vol. Weighted Mean D[4,3]:** 23.819 um	

d(0.1): 5.510 um   d(0.5): 18.519 um   d(0.9): 50.064 um

Particle Size Distribution

—mbc - Average, 08 July 2008 16:58:19

Size (?m)	Volume In %	Size (?m)	Volume In %	Size (?m)	Volume In %	Size (?m)	Volume In %	Size (?m)	Volume In %	Size (?m)	Volume In %
0.010		0.105		1.096		11.482		120.226		1258.925	
0.011	0.00	0.120	0.00	1.259	0.15	13.183	5.33	138.038	0.00	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.20	15.136	5.71	158.489	0.00	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.26	17.378	6.00	181.970	0.00	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.34	19.953	6.20	208.930	0.00	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	0.43	22.909	6.28	239.883	0.00	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	0.56	26.303	6.23	275.423	0.00	2884.032	0.00
0.026	0.00	0.275	0.00	2.884	0.73	30.200	6.04	316.228	0.00	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.94	34.674	5.69	363.078	0.00	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	1.20	39.811	5.20	416.869	0.00	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	1.52	45.709	4.58	478.630	0.00	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	1.91	52.481	3.86	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	2.34	60.256	3.11	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	2.83	69.183	2.36	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.03	7.586	3.35	79.433	1.68	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.08	8.710	3.88	91.201	1.07	954.993	0.00	10000.000	0.00
0.091	0.00	0.955	0.10	10.000	4.39	104.713	0.49	1096.478	0.00		
0.105		1.096		11.482	4.89	120.226	0.06	1258.925	0.00		

Operator notes:

APPENDIX B

MECHANICAL PROPERTIES CHARACTERIZATION

B1. Tensile strength

Table B1.1 Tensile strength of chitosan, chitosan/unmodified montmorillonite composite, and chitosan/modified montmorillonite composite films.

Sample	Trial No.					Mean	SD
	1	2	3	4	5		
CTS	77.55	72.82	73.07	69.28	73.45	73.23	2.94
CTS/MMT 0.1	68.70	63.06	70.72	67.57	67.11	67.03	3.01
CTS/MMT 0.25	71.65	69.43	70.71	55.41	65.32	66.50	6.65
CTS/MMT 0.5	54.87	68.17	70.93	54.59	68.52	63.42	8.00
CTS/MMT 1	35.53	22.88	32.99	14.30	19.71	25.08	8.96
CTS/MMT 2	35.27	24.31	27.86	21.38	17.77	25.32	6.69
CTS/mMMT 0.1	81.45	83.69	79.90	80.92	80.27	81.24	1.49
CTS/mMMT 0.25	85.13	86.12	84.85	88.29	84.94	85.87	1.45
CTS/mMMT 0.5	85.95	89.35	83.07	83.53	90.19	86.42	3.26
CTS/mMMT 1	107.76	102.57	105.06	110.52	105.97	106.38	2.98
CTS/mMMT 2	87.59	88.65	83.45	87.77	92.00	87.89	3.06

Table B**1.2** Tensile strength of chitosan, chitosan/unmodified bamboo charcoal composite, and chitosan/modified bamboo charcoal composite films.

Sample	Trial No.					Mean	SD
	1	2	3	4	5		
CTS	77.55	72.82	73.07	69.28	73.45	73.23	2.94
CTS/BC 0.1	68.70	63.06	70.72	67.57	65.11	67.03	3.01
CTS/BC 0.25	71.65	69.43	70.71	55.41	65.32	66.50	6.65
CTS/BC 0.5	54.87	68.17	70.93	54.59	68.52	63.42	8.00
CTS/BC 1	35.53	24.31	27.86	21.38	17.77	25.32	6.69
CTS/BC 2	35.27	24.31	27.86	21.38	17.77	25.32	6.69
CTS/mBC 0.1	81.45	83.69	79.90	80.92	80.27	81.24	1.49
CTS/mBC 0.25	85.13	86.12	84.85	88.29	84.94	85.87	1.45
CTS/mBC 0.5	85.95	89.35	83.08	83.53	90.194	86.42	3.26
CTS/mBC 1	107.76	102.57	105.06	110.52	105.97	106.38	2.98
CTS/mBC 2	87.59	88.65	83.45	87.77	92.00	87.89	3.06

Table B1.3 Young's modulus of chitosan, chitosan/unmodified montmorillonite composite, and chitosan/modified montmorillonite composite films.

Sample	Trial No.					Mean	SD
	1	2	3	4	5		
CTS	5543.13	5509.73	4966.98	4824.28	5809.57	5330.74	416.91
CTS/MMT 0.1	5335.03	5243.33	5308.68	5325.89	5331.48	5308.88	38.02
CTS/MMT 0.25	5672.57	5653.72	5675.47	5628.89	5785.79	5683.29	60.24
CTS/MMT 0.5	6135.92	6071.41	6181.15	5847.87	5446.66	5936.60	302.33
CTS/MMT 1	6529.65	6596.99	5697.49	5765.50	5809.84	6079.89	443.76
CTS/MMT 2	6045.00	5967.44	6053.81	6022.62	6001.18	6018.01	34.90
CTS/mMMT 0.1	6719.95	6885.99	6886.79	6645.56	6696.20	6766.90	112.34
CTS/mMMT 0.25	7666.20	7760.33	7642.98	7553.61	7557.36	7636.10	85.72
CTS/mMMT 0.5	8044.24	8067.44	8011.88	8011.62	8056.51	8038.34	25.62
CTS/mMMT 1	11646.4	10359.3	10027.2	10125.0	10032.3	10438.0	668.77
CTS/mMMT 2	9218.83	9473.14	9166.38	10390.2	9660.18	9581.74	493.91

Table B1.4 Young's modulus of chitosan, chitosan/unmodified bamboo charcoal composite, and chitosan/modified bamboo charcoal composite films.

Sample	Trial No.					Mean	SD
	1	2	3	4	5		
CTS	5543.13	5509.73	4966.98	4824.28	5809.57	5330.74	416.91
CTS/BC 0.1	5392.18	5118.89	5171.90	5044.76	5222.84	5190.11	130.80
CTS/BC 0.25	4965.29	5245.97	5310.11	4956.90	4810.14	5057.68	211.63
CTS/BC 0.5	5570.23	5063.54	5488.27	4613.43	4727.75	5092.64	432.51
CTS/BC 1	5135.82	5584.07	6313.88	4860.59	3348.90	5048.65	1097.68
CTS/BC 2	4310.82	5467.40	5463.52	4079.44	3808.63	4625.96	786.69
CTS/mBC 0.1	5257.24	5730.16	5635.67	5945.61	5543.54	5622.44	252.89
CTS/mBC 0.25	5547.59	5738.94	5657.84	5703.95	5854.78	5700.62	112.36
CTS/mBC 0.5	5530.09	5478.79	5758.03	5277.66	6811.18	5771.15	606.00
CTS/mBC 1	7556.21	6589.83	6800.08	7426.39	6811.18	7036.74	426.70
CTS/mBC 2	6594.17	6626.12	6939.38	6622.69	7034.31	6763.33	207.15

Table B1.5 Elongation at break of chitosan, chitosan/unmodified montmorillonite composite, and chitosan/modified montmorillonite composite films.

Sample	Trial No.					Mean	SD
	1	2	3	4	5		
CTS	2.45	2.44	3.07	2.56	2.63	2.63	0.26
CTS/MMT 0.1	2.36	2.44	2.44	2.79	2.21	2.45	0.21
CTS/MMT 0.25	1.90	1.84	1.91	1.98	1.88	1.90	0.05
CTS/MMT 0.5	1.42	1.52	1.52	1.60	1.66	1.54	0.09
CTS/MMT 1	1.26	1.21	1.37	1.29	1.11	1.25	0.10
CTS/MMT 2	0.94	0.88	0.84	0.79	0.84	0.86	0.06
CTS/mMMT 0.1	2.44	2.60	2.48	2.59	2.60	2.54	0.08
CTS/mMMT 0.25	2.43	2.49	2.48	2.44	2.10	2.39	0.16
CTS/mMMT 0.5	2.00	1.96	1.84	2.08	2.02	1.98	0.90
CTS/mMMT 1	1.84	1.98	1.92	1.96	1.84	1.91	0.07
CTS/mMMT 2	1.59	1.58	1.63	1.52	1.75	1.61	0.17

Table B1.6 Elongation at break of chitosan, chitosan/unmodified bamboo charcoal composite, and chitosan/modified bamboo charcoal composite films.

Sample	Trial No.					Mean	SD
	1	2	3	4	5		
CTS	2.45	2.44	3.07	2.56	2.63	2.63	0.26
CTS/BC 0.1	2.27	2.61	2.89	2.66	2.50	2.59	0.23
CTS/BC 0.25	2.43	2.24	2.49	1.61	3.17	2.39	0.56
CTS/BC 0.5	1.18	1.51	1.35	1.23	1.40	1.33	0.13
CTS/BC 1	1.43	1.52	0.80	1.28	1.73	1.35	0.35
CTS/BC 2	1.16	0.99	1.45	1.70	1.28	1.31	0.27
CTS/mBC 0.1	5.35	3.83	2.84	2.92	3.75	3.74	1.01
CTS/mBC 0.25	2.51	4.24	4.61	4.76	2.39	3.70	1.16
CTS/mBC 0.5	3.65	3.31	3.23	3.63	4.50	3.66	0.50
CTS/mBC 1	2.82	2.81	3.01	2.43	3.00	2.81	0.23
CTS/mBC 2	2.00	2.08	1.80	2.05	2.03	1.99	0.11

APPENDIX C

WATER ABSORPTION CHARACTERIZATION

Table C1.1 Water absorption of chitosan, chitosan/unmodified montmorillonite composite, and chitosan/modified montmorillonite composite films.

Sample	Trial			Mean	SD
	1	2	3		
CTS	116.22	114.24	106.71	112.39	5.02
CTS/MMT 0.1	102.05	103.71	97.27	101.01	3.35
CTS/MMT 0.25	95.07	98.67	98.26	97.34	1.97
CTS/MMT 0.5	93.11	89.47	86.66	89.75	3.23
CTS/MMT 1	84.75	85.40	85.79	85.31	0.53
CTS/MMT 2	73.48	78.28	78.68	77.25	2.89
CTS/mMMT 0.1	114.52	104.94	114.33	111.26	5.48
CTS/mMMT 0.25	106.34	112.32	111.22	109.96	3.18
CTS/mMMT 0.5	97.71	96.54	97.52	97.25	0.63
CTS/mMMT 1	95.92	94.20	98.78	96.27	2.32
CTS/mMMT 2	92.21	92.71	95.55	93.49	1.80

Table C1.2 Water absorption of chitosan, chitosan/unmodified bamboo charcoal composite, and chitosan/modified bamboo charcoal composite films.

Sample	Trial			Mean	SD
	1	2	3		
CTS	116.22	114.24	106.71	112.39	5.02
CTS/BC 0.1	120.85	108.14	110.51	113.02	6.76
CTS/BC 0.25	125.55	111.31	118.09	129.40	7.12
CTS/BC 0.5	179.36	146.30	166.09	163.91	16.64
CTS/BC 1	277.19	252.50	278.65	269.45	14.70
CTS/BC 2	124.13	203.37	144.81	157.44	41.10
CTS/mBC 0.1	124.51	114.89	117.75	119.05	4.94
CTS/mBC 0.25	154.03	148.92	148.91	150.62	2.95
CTS/mBC 0.5	176.27	170.72	180.29	175.76	4.80
CTS/mBC 1	359.22	307.33	364.95	343.83	31.74
CTS/mBC 2	370.47	296.76	325.90	331.04	37.12

APPENDIX D

OIL ABSORPTION CHARACTERIZATION

Table D1.1 Oil absorption of chitosan, chitosan/unmodified montmorillonite composite, and chitosan/modified montmorillonite composite films.

Sample	Trial			Mean	SD
	1	2	3		
CTS	6.98	5.52	5.68	6.06	0.80
CTS/MMT 0.1	5.93	5.82	6.25	6.00	0.22
CTS/MMT 0.25	5.64	5.53	5.86	5.68	0.17
CTS/MMT 0.5	5.76	5.45	5.66	5.62	0.16
CTS/MMT 1	5.45	4.95	4.90	5.10	0.30
CTS/MMT 2	5.07	5.57	4.82	5.15	0.38
CTS/mMMT 0.1	5.60	5.92	5.87	5.89	0.03
CTS/mMMT 0.25	5.42	5.88	5.69	5.66	0.23
CTS/mMMT 0.5	5.78	5.17	4.46	5.14	0.66
CTS/mMMT 1	5.44	4.69	5.38	5.17	0.42
CTS/mMMT 2	4.18	4.73	4.45	4.45	0.27

Table D1.2 Oil absorption of chitosan, chitosan/unmodified bamboo charcoal composite, and chitosan/modified bamboo charcoal composite films.

Sample	Trial			Mean	SD
	1	2	3		
CTS	6.98	5.51	5.68	6.06	0.80
CTS/BC 0.1	6.59	7.04	6.88	6.84	0.23
CTS/BC 0.25	7.73	7.27	7.28	7.43	0.26
CTS/BC 0.5	7.78	8.95	6.68	7.83	1.14
CTS/BC 1	9.91	8.99	9.01	9.30	0.52
CTS/BC 2	8.95	8.68	9.28	8.97	0.30
CTS/mBC 0.1	7.82	7.79	7.34	7.65	0.27
CTS/mBC 0.25	7.53	8.09	8.60	8.07	0.54
CTS/mBC 0.5	7.71	9.00	7.89	8.20	0.70
CTS/mBC 1	9.50	10.18	9.45	9.71	0.41
CTS/mBC 2	9.25	9.46	9.37	9.45	0.10

APPENDIX E

WATER CONTACT ANGLE CHARACTERIZATION

Table E1.1 Water contact angle of chitosan, chitosan/unmodified montmorillonite composite, and chitosan/modified montmorillonite composite films.

Sample	Trial			Mean	SD
	1	2	3		
CTS	110	110	110	110	0
CTS/MMT 0.1	120	120	121	120.33	0.58
CTS/MMT 0.25	130	130	128	129.33	1.15
CTS/MMT 0.5	132	134	134	133.33	1.15
CTS/MMT 1	134	134	134	134	0
CTS/MMT 2	136	136	136	136	0
CTS/mMMT 0.1	120	120	120	120	0
CTS/mMMT 0.25	130	130	130	130	0
CTS/mMMT 0.5	134	134	134	134	0
CTS/mMMT 1	136	136	136	136	0
CTS/mMMT 2	138	138	138	138	0

Table E1.2 Water contact angle of chitosan, chitosan/unmodified bamboo charcoal composite, and chitosan/modified bamboo charcoal composite films.

Sample	Trial			Mean	SD
	1	2	3		
CTS	110	110	110	110	0
CTS/BC 0.1	100	98	100	99.33	1.15
CTS/BC 0.25	90	92	92	91.33	1.15
CTS/BC 0.5	88	88	88	88	0
CTS/BC 1	84	84	84	84	0
CTS/BC 2	86	86	86	86	0
CTS/mBC 0.1	98	98	98	98	0
CTS/mBC 0.25	88	88	90	88.67	1.15
CTS/mBC 0.5	88	86	86	86.67	1.15
CTS/mBC 1	84	82	82	82.67	1.15
CTS/mBC 2	88	88	88	88	0

APPENDIX F

OIL CONTACT ANGLE CHARACTERIZATION

Table F1.1 Oil contact angle of chitosan, chitosan/unmodified montmorillonite composite, and chitosan/modified montmorillonite composite films.

Sample	Trial			Mean	SD
	1	2	3		
CTS	36	36	36	36	0
CTS/MMT 0.1	46	46	46	46	0
CTS/MMT 0.25	46	46	48	46.67	1.15
CTS/MMT 0.5	52	52	52	52	0
CTS/MMT 1	56	56	56	56	0
CTS/MMT 2	56	58	58	57.33	1.15
CTS/mMMT 0.1	46	48	48	47.33	1.15
CTS/mMMT 0.25	50	50	50	50	0
CTS/mMMT 0.5	54	54	54	54	0
CTS/mMMT 1	58	58	58	58	0
CTS/mMMT 2	70	70	70	70	0

Table F1.2 Oil contact angle of chitosan, chitosan/unmodified bamboo charcoal composite, and chitosan/modified bamboo charcoal composite films.

Sample	Trial			Mean	SD
	1	2	3		
CTS	36	36	36	36	0
CTS/BC 0.1	32	32	32	32	0
CTS/BC 0.25	30	30	28	29.33	1.15
CTS/BC 0.5	28	28	28	28	0
CTS/BC 1	26	28	26	26.67	1.15
CTS/BC 2	26	28	28	27.33	1.15
CTS/mBC 0.1	30	30	30	30	0
CTS/mBC 0.25	30	28	30	29.33	1.15
CTS/mBC 0.5	26	26	26	26	0
CTS/mBC 1	24	22	22	22.67	1.15
CTS/mBC 2	24	24	24	24	0

BIOGRAPHY

Miss Walaikorn Nitayaphat was born in Saraburi, Thailand, on July 4th, 1979. She received a Bachelor of Science with a major in Materials Science from the Department of Materials Science, Faculty of Science, Chulalongkorn University in March 2001. And she graduated a Master of Science with a major in Applied Polymer Science and Textile Technology from the Department of Materials Science, Faculty of Science, Chulalongkorn University in October 2004. She started as a graduate student in the field of Materials Science, Department of Materials Science, Chulalongkorn University in 2004 and completed the program in 2009.