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ของไทโรซีน



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ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

PREPARATION AND CHARACTERIZATION OF BLENDS OF
CHITOSAN AND TYROSINE-DERIVED POLYCARBONATE



Miss Tidarat Vijithuttagune

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

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ไคโตซานเป็นพอลิเมอร์ชีวภาพประเภทแซคคาไรด์ ซึ่งมีคุณสมบัติที่ดีหลายประการสำหรับนำมาใช้เป็นวัสดุทางชีวภาพ ในงานวิจัยนี้ได้นำไคโตซานมาเบลนด์กับพอลิคาร์บอเนตที่มีไทโรซีนเป็นองค์ประกอบ (ทีพีซี) ซึ่งเป็นพอลิเมอร์กลุ่มใหม่ที่ถูกออกแบบให้มีกรดคาร์บอกซิลิกเป็นหมู่ห้อย ซึ่งเป็นตำแหน่งที่สามารถเกิดอันตรกิริยากับหมู่อะมิโนของไคโตซานได้ การศึกษาอันตรกิริยาที่เกิดขึ้นระหว่างหมู่คาร์บอกซิลิกของทีพีซี กับหมู่อะมิโนของไคโตซาน กระทำได้โดยใช้เทคนิคเอฟที-ไออาร์ เมื่อวิเคราะห์พอลิเมอร์เบลนด์ที่ได้จากการผสมแบบกายภาพไม่พบแรงกระทำแบบอิเล็กทรอนิกส์ จึงได้พยายามทำให้พอลิเมอร์ทั้งสองเชื่อมกันด้วยพันธะเอไมด์โดยใช้ความร้อน แต่พบว่าไม่สามารถทำได้และยังเป็นสาเหตุทำให้เกิดการเปลี่ยนแปลงของสีในเบลนด์ จึงได้นำรีเอเจนต์คู่ควบ 1-เอทิล-3-(3-ไดเมทิลอะมิโนโพรพิล) คาร์โบไดอิมิด (อีดีซีไอ) มาช่วยในการทำปฏิกิริยาเพื่อให้เกิดพันธะเอไมด์เชื่อมระหว่างไคโตซานและทีพีซี ซึ่งพบว่าสามารถทำให้เกิดพันธะเอไมด์ดังกล่าวได้ที่ 1555 ซม.⁻¹ นอกจากนี้ยังได้นำไคโตซานมาเคลือบลงบนฟิล์มทีพีซี แต่การเคลือบแบบธรรมดาไคโตซานสามารถหลุดลอกออกได้ จึงได้นำอีดีซีไอมาช่วยยึดพอลิเมอร์ทั้งสองชั้นให้เชื่อมกันด้วยพันธะเอไมด์ สุดท้ายได้วิเคราะห์การต้านทานแรงดึงขาด, ลักษณะพื้นผิวของฟิล์มโดยใช้เครื่องเอสอีเอ็ม และศึกษาการดูดซับไอออนแคลเซียม ของฟิล์มเบลนด์แบบกายภาพ, เบลนด์แบบใช้อีดีซีไอ และฟิล์มทีพีซีที่เคลือบผิวด้วยไคโตซานโดยใช้อีดีซีไอ

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

ภาควิชา - ลายมือชื่อนิสิต.....

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 TIDARAT VIJITHUTTAGUNE : PREPARATION AND
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 DERIVED POLYCARBONATE. THESIS ADVISOR: VARAWUT
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Chitosan, a saccharide-based biopolymer, exhibits a number of good properties to use as bioactive materials. It was therefore selected to blend with a new class of synthetic polymers, tyrosine-derived polycarbonate (TPC). These polycarbonates contain free carboxyl groups, which can interact with the amino groups on chitosan. Investigation on the interaction between -COOH on TPC and -NH_2 on chitosan was carried out by FT-IR analysis. Nevertheless, such electrostatic interaction in the physical blending between the two polymers could not be identified. Amide linkages were therefore introduced in the blends in order to join the two polymers together. The amide formation was not successfully induced by heat treatment, without causing color change in the blends. A coupling agent, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) was therefore used to form amide bonds between the two polymers and the results show 1555 cm^{-1} . In addition, chitosan was coated on the TPC film. The physical coating of chitosan on TPC did not yield tightly-bound chitosan layer. A coupling agent, EDCI was again used to join the two polymer layers by amide linkages. Analyses of polymer blends with and without EDCI, and chitosan coated TPC with EDCI, were carried out by tensile testing, scanning electron microscopy (SEM) and Ca^{2+} adsorption study.

Department - Student's signature.....

Field of study Petrochemistry and polymer science Advisor's signature.....

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LIST OF ABBREVIATIONS

TPC	Tyrosine-derived polycarbonates
DTE-20% DT	Poly(desaminotyrosyl-tyrosine ethyl esters -co-20% desaminotyrosyl-tyrosine carbonate)
DTE-50% DT	Poly(desaminotyrosyl-tyrosine ethyl esters -co-50% desaminotyrosyl-tyrosine carbonate)
100% DT	Poly(100% desaminotyrosyl-tyrosine carbonate)
DTR	Desaminotyrosyl-tyrosine alkyl esters
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
PAA	Poly(acrylic acid)
FT-IR	Fourier transform-infrared spectrophotometer
ATR-IR	Attenuated total reflectance infrared spectroscopy
AAS	Atomic absorption spectroscopy
SEM	Scanning electron microscopy
DMF	Dimethylformamide
DMSO	Dimethylsulfoxid
THF	Tetrahydrofulan
%DD	Percent degree of deacetylation
h	Hour
min	Minute
\bar{M}_v	Viscosity-average molecular weight
\bar{M}_w	Weight-average molecular weight
ppm	Part per million
RT	Room temperature

CHAPTER I

INTRODUCTION

1.1 Rationale

One of the applications of degradable polymers involves their uses as scaffolds for regeneration of specific tissues such as liver, bone, cartilage, or vascular wall. The use of artificial materials in these advanced applications require a new generation and physicochemical properties that can be adjusted to meet the complex requirements of each specific application.¹

When degradable polymers are used as implant materials in human patients, the potential toxicity of the polymer degradation products and their subsequent metabolites become a major concern. An ideal degradable polymer for a specific medical application must fulfill a stringent set of requirements: The polymer itself must be non-toxic and tissue compatible, the polymer must degrade to fully biocompatible degradation products, and the polymer must possess physicochemical and engineering properties suitable for the intended application. Finally, the polymer must degrade within a clinically useful range of several weeks to several months. Only very few polymers will possess the “correct mix” of all of these properties.²

Blending is one method for developing a new polymeric material which has specific properties different from those of the pure polymers at a lower cost compared to synthesizing a new polymer.

Chitosan, partially deacetylated form of chitin, is a natural polymer. It has been shown a number of good properties,³ *e.g.* biocompatible, antimicrobial, antithrombogenic. It is also found to accelerate wound healing and be capable of chelating metal ions. Structure of chitosan contains a large number of hydroxy and amino groups, which can interact with carboxylic groups of various chemical

entities.⁴ It is therefore, of interest to study polymer blend between chitosan and poly (DTE-co-20% DT carbonate) having carboxylic as a pendant groups.

Poly (DTE-co-20% DT carbonate) was a new polymer containing tyrosine. It was synthesized by a research group of professor Kohn.¹ This polymer family was aimed to use as bone fixation devices, substrates for the growth of mammalian cells, and porous degradable scaffolds for tissue engineering.

1.2 Statement of purpose

The purpose of this research is to prepare polymer blends between poly (DTE-co-20% DT carbonate) and chitosan. The interaction between the amino groups on chitosan and the carboxylic groups on poly (DTE-co-20% DT carbonate) was investigated. Mechanical and physical characterization of the obtained blends was also done.

1.3 Scope of this research

This research was divided into four parts. The first part was the preparation of polymer blends between poly (DTE-co-20% DT carbonate) and chitosan. The second part involved the study of ionic interaction between the amino and carboxylic groups in the blend. In this step poly (acrylic acid) (PAA) was chosen as a model polymer for poly(DTE-co-20% DT carbonate) in this step. The third part was to induce amide bond formation by heat treatment and by the use of a coupling agent (EDCI). In this step, glucosamine and PAA were used as model compounds. In the last part the possibility of preparing chitosan-coated poly (DTE-co-20% DT carbonate) films was investigated.

1.4 Theory

1.4.1 Polymer blends

The term, polymer blend, is used to call any physical mixture of two or more different polymers or copolymers that are not linked by covalent bonds.⁵ The main purpose of blending is to develop a new polymeric material which has specific properties different from those of the pure polymers at a lower cost instead of synthesizing a new polymer. Many preparation methods have been used to form polymer blends.

1.4.2 Preparation of the blends

The preparation of polymer blends can be accomplished by several methods.

Melt mixing

Melt mixing is performed by mixing the polymers in the molten state under shear in the mixing equipment. It is the method of choice for the preparation of polymer blends in a commercial scale because of its simplicity, speed of mixing and the advantage of being free from foreign components (*e.g.* solvents) in the blends. A number of devices are available for laboratory-scale mixing such as brabender mixer, electrically heated two-roller mill, extruder, rotational rheometer.

The primary disadvantage of melt mixing is that both components must be in molten state, which means that the temperature may be high enough to cause degradation. Besides melt mixing is difficult to perform in some pairs of polymers due to a large difference in the melt viscosity of the components. The cost of the equipment is another disadvantage of melt mixing. Melt mixing only works well with large amounts of material, *e.g.* at least 50 grams is required for even laboratory-size mixing equipment. If mixing of quantities of less than 1 g is required, melt mixing is usually not feasible.

Solvent casting

This method is done by dissolving polymers in a suitable solvent. Solution is then cast on a plate into thin films and removal of solvent from the films is performed by evaporating at ambient or elevated temperature. Solvent casting is the simplest mixing method available. Very small quantities of experimental polymers can be handled easily.

The most severe problem with solvent casting is the influence of the solvent and the casting history on resulting product. In spite of the fact that the most of the solvent can be removed from a cast film, the nature of the film depends strongly on the solvent and the conditions used during casting.

Mixing via reaction

Co-crosslinking and interpenetrating polymer networks (IPN) formation are special methods for forming blends. The idea of these methods is to increase a degree of miscibility by reactions between the polymers. Other methods involve the polymerization of a monomer in the presence of a polymer and the introduction of groups onto the polymer chain.

1.4.3 Tyrosine-derived polycarbonate : poly(DTE-co-20% DT carbonate)

Poly (DTE-co-20% DT carbonate) is a polycarbonate, belonging to a new family of pseudo-poly (amino acid) reported by Kohn. The structure of monomer desaminotyrosyl-tyrosine alkyl esters (DTR) is shown in Figure 1.1. These materials possess numerous properties suitable for use as polymeric biomaterials, ranging from degradable bone fixation devices, substrates for the growth of mammalian cells to the fabrication of porous degradable scaffolds for tissue engineering. A main structural feature of tyrosine-derived polycarbonate is the presence of ester pendent chain in every repeating unit. This pendent chain can be varied to achieve a stepwise change in the polymer properties. This polymer system was particularly useful when conducting studies of structure-property correlations.

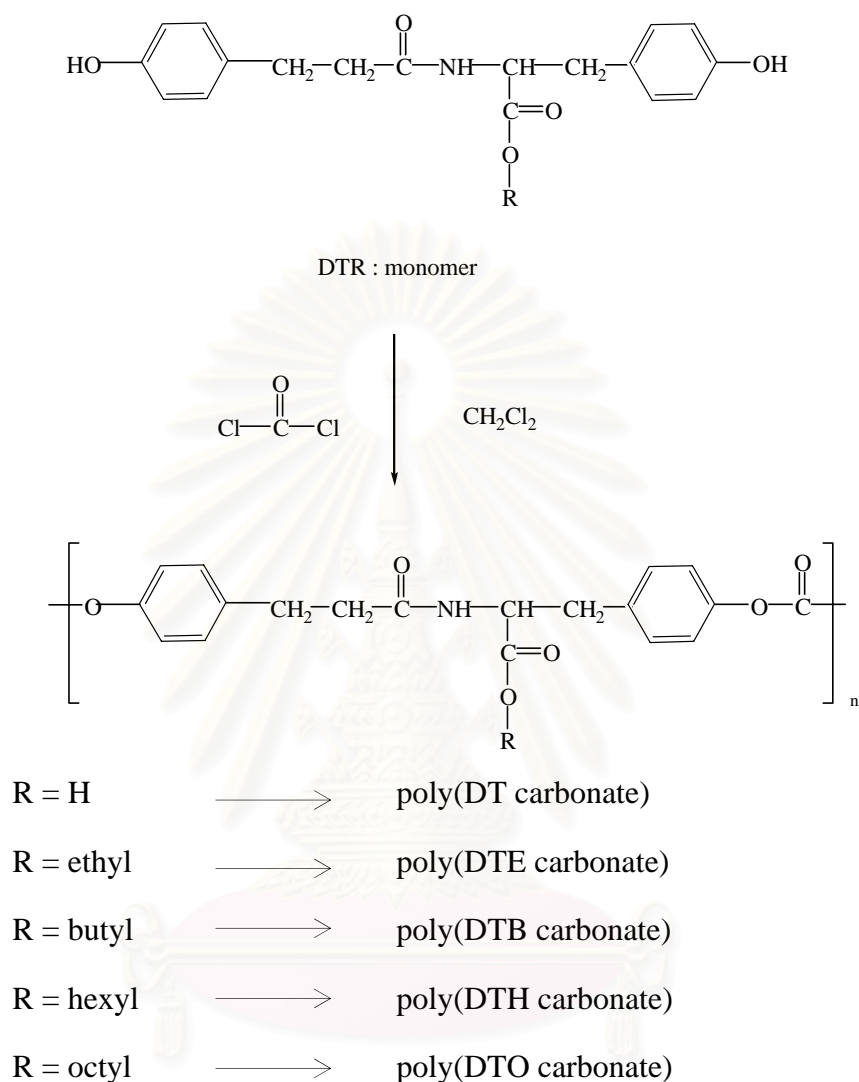


Figure 1.1 Structure of desaminotyrosyl-tyrosine alkyl esters (DTR) and the polycarbonates derived from them.

The degradation of the polycarbonates during *in vitro* incubation can possibly proceed through a sequence of random scissions of the three hydrolyzable bonds, ester, carbonate, and amide, present in the polymer. Carbonate bond cleavage leads to a reduction of the polymer molecular weight, while pendent chain ester bond hydrolysis results in the formation of polymer-bound-free carboxylic acid groups.¹ The backbone carbonate bond cleavage was four times faster than the pendent chain

ester bond.¹ Thus, the postulated degradation mechanism *in vitro* involves the concomitant cleavage of ester bonds and carbonate bonds, leading to the major degradation product. *In vivo*, however, the possible enzymatic cleavage of the amide bonds may accelerate the backbone degradation process and lead to the future cleavage.¹

Varying the pendent chain length affected on polymer properties such as thermal behavior, hydrophilicity, degradation and cell-polymer interaction. The increase in pendent chain length was expected to increase free volume and polymer chain mobility by lowering the packing efficiency. This phenomenon produced systematic variations in macroscopic properties, such as increase solubility, resistance to hydrolysis but decrease in glass transition temperature and its ability to support cell growth. The least hydrophobic polycarbonate (having a short ethyl ester pendent chain) was a more stimulating substrate for cell growth than more hydrophobic polymer (carrying longer alkyl ester pendent chain). Poly(DTE carbonate), has a high degree of osteoconductivity and exhibit's bone apposition when in contact with hard tissue.⁶⁻⁸

Tyrosine-derived polycarbonates rank among the strongest degradable implant materials available today (especially after orientation). This general profile indicates that tyrosine-derived polymers may be potential candidates for orthopedic application such as small bone fixation devices. Because the mechanical properties and rate of degradation can be influenced to some extent by the selection of an appropriate pendent chain, it may be possible in the future to identify materials that offer a maximum of synchronization between degradation and the healing rate of specific tissues. Another advantage presented by the tyrosine-derived polycarbonate over the currently used poly(lactic acid)/poly(glycolic acid) is the absence of acidic degradation products that have been linked in some instances to inflammatory responses at the implant site.

1.4.4 Chitosan

Chitosan, an *N*-deacetylated⁹ derivative of chitin, has a structure similar to cellulose. Chitosan mainly consists of 2-amino-2-deoxy-D-glucose (GlcN) repeating unit with a small amount of 2-acetyl-2-deoxy-D-glucose residues. The amount of GlcN unit in chitosan is generally referred to by percent degree of deacetylation (%DD). Various techniques were used for determination of %DD such as IR,¹⁰ NMR,¹¹ and metachromatic titration.¹² The structures of chitosan and chitin are shown in Figure 1.2.

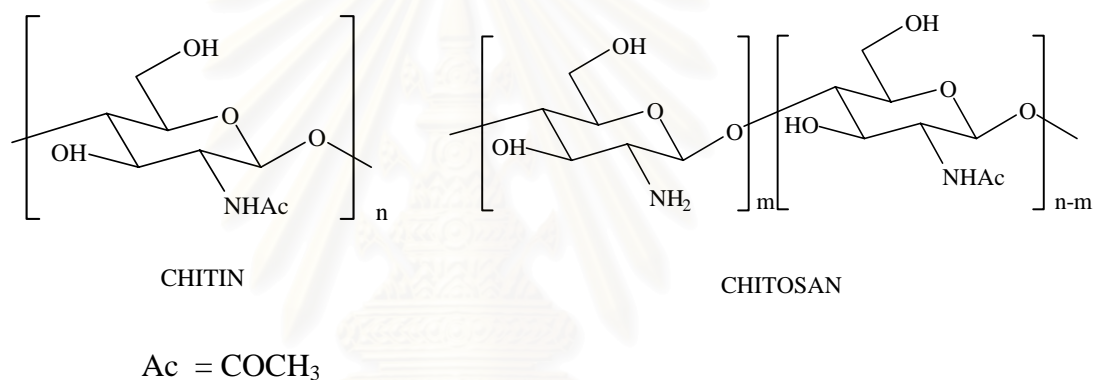


Figure 1.2 Structures of chitin and chitosan

Chitosan is dissolved in inorganic acids such as HCl, HNO₃, and organic acids, preferably acetic acid and formic acid. Pure chitosan precipitates from solutions if the pH rises above 6.

Since chitin is the main component of the exoskeleton of crustaceans, such as crabs, lobsters, shrimps, and cray fish, chitin and chitosan have recently been interesting choices of materials for various types of health related applications. Both chitin and chitosan are proved to be biologically safe for use in human. Chitosan was also found to be an antimicrobial and antifungal agent. It shows good properties that have biocompatibility for most tissues including skin and bone. It accelerates wound healing and exhibits good blood compatibility and hemostatic activity.¹³ It has been expected to be used in such industrial and biomedical applications¹⁴ as an

antibacterial fiber, drug carrier, immobilizer of biological molecules, membrane, wound-healing agent, artificial skin. It is also used in food industries.¹⁵ Besides chitosan can chelate metal ions so it is used as a metal capture substance¹⁶ in waste water treatment.

However, applications of chitosan fibers and films are limited mainly due to its poor mechanical properties such as brittleness and rigidity. Therefore, it is interesting to prepare a biomaterial having both good biocompatibility and mechanical properties.

1.4.5 Utilization of chitosan

The amino groups in chitosan could facilitate the formation of hydrogen bonding, ionic bonding, and possibly the covalent bonding with other functional groups.¹⁷ Many researchers have been interested to study the interaction between the $-NH_2$ groups and the $-COOH$ groups in other polymers as a way to modify polymer properties either by physical blending or chemical reaction.

Electrostatic Interaction between amino and carboxylic group in blend

- Mixtures of chitosan and carboxyl-containing polymers, e.g. xanthan¹⁸ (Figure 1.3) and PAA¹⁹ (Figure 1.4), were reported as new blends for enzyme immobilization and separation of water-ethanol mixtures respectively. Spectroscopic analysis of the blends lead to evidences confirming the electrostatic attraction between the ammonium groups of chitosan and the carboxylate group.

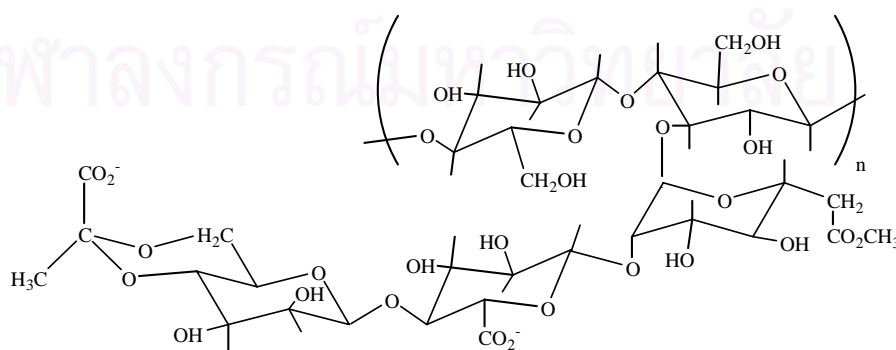


Figure 1.3 Structure of xanthan

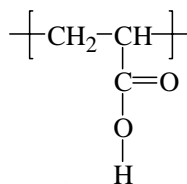


Figure 1.4 Structure of PAA

Amide bond formation

- The reaction between the amino and the carboxylic groups could be induced by either thermal or chemical methods. By heating the blends of chitosan and PAA in 180-260°C, the formation of amide linkage was identified.^{19, 20} While using thermal method, the blend might risk thermal degradation, an alternative method to create an amide linkage at a lower temperature is the use of coupling agent. A water soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide or EDCI, was introduced to link deoxycholic acid to chitosan^{21,22} (Figure 1.5). The hydrophobically modified chitosan was able to self-aggregate in aqueous media and form charge complexes when mixed with plasmid DNA. This modified chitosan can be used as a vehicle to transfer genes into cells.

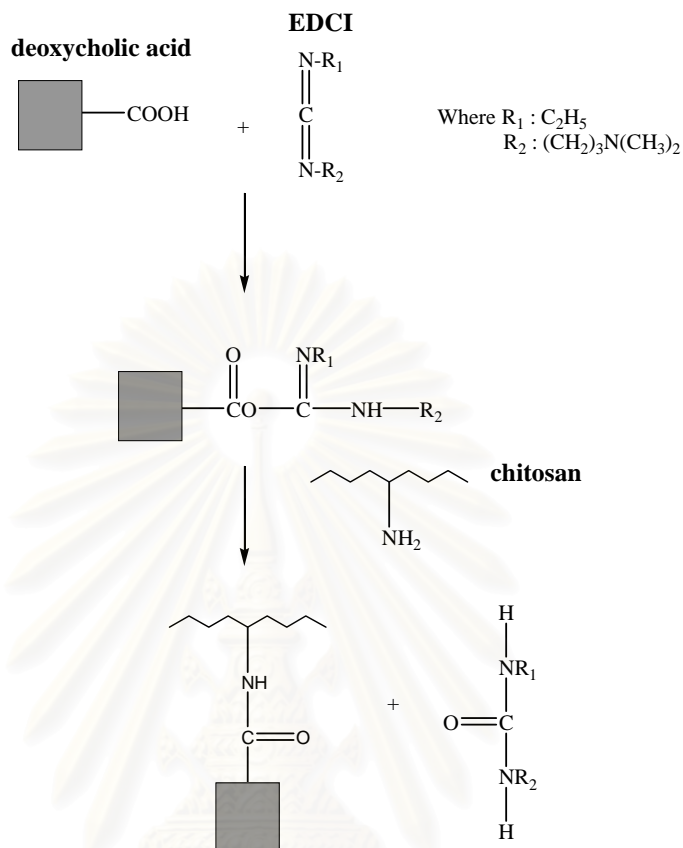


Figure 1.5 A scheme of coupling mechanism between chitosan and deoxycholic acid using EDCI through amide linkage formation

The use of chitosan to modify surface properties of other polymers

- Since chitosan is biocompatible, it was used to immobilize on the surface of other polymers in order to enhance their biological activity. In 2001, Xin and coworkers coated chitosan on the modified-HDPE tubes.²³ This tube was later coated with heparin that interpenetrated into the pre-coated chitosan layer. They found that the blood compatibility of this coated tubes improved significantly. Another work by Cai involved the chemical grafting of chitosan onto polylactide films in the presence of EDCI.²⁴ The amide linkage formed between the two polymers. This modified polylactide was

found to enhance cell biocompatibility. From these examples, it is clear that chitosan is a useful macromolecule for enhancing biological activity of other polymeric devices, that is less bioactive or even bio-inert.

1.4.6 Methods for characterizing the interaction between amino and carboxylic groups

Fourier transform-infrared spectroscopy (FT-IR)

Molecules are able to absorb electromagnetic energy in the infrared (IR) region of the electromagnetic spectrum. The IR radiation has the appropriate level of energy to excite vibrational movement of molecular bonds. Each type of bond will absorb IR radiation at slightly different frequency from one to another. The structure of a molecule can be determined by observing the different vibrational signals. An IR spectra is obtained by irradiating a sample with an IR source of light. The light passes through the sample, which can be in solution or contains within a salt plate, and then onto an IR detector. The spectrum is analyzed by examining at which frequency the peaks occur. Different peak frequencies indicate different type of vibrational motion and hence, different types of chemical bonds. The peak intensities are usually denoted as percent transmittance (%T), which compares the amount of light absorbed compared to the amount of IR light that was not absorbed. The frequencies are normally listed in wavenumbers (in units of reciprocal centimeters, cm^{-1}).

Attenuated total reflectance infrared spectroscopy (ATR-IR)

This is a method to analyze functional group on the surface of a substrate, which is usually in the form of the films or flat solids. The infrared beam from the spectrometer is focused onto the beveled edge of an internal reflection element (IRE). The beam is then reflected, generally numerous times, through the IRE crystal, and directed to a detector (Figure 1.6).

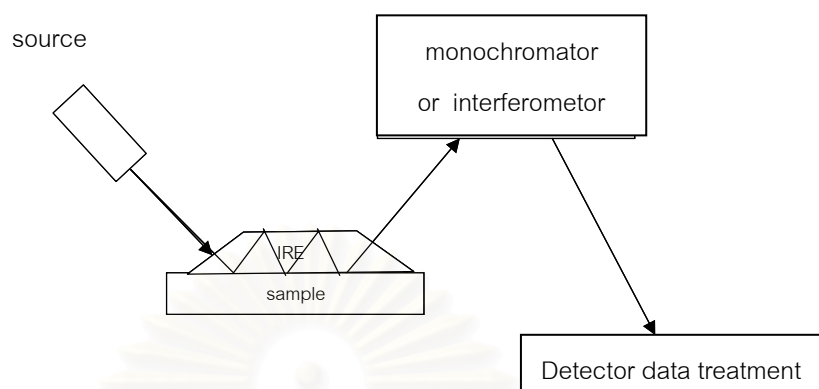


Figure 1.6 Diagram of ATR-IR

The radiation can penetrate a short distance into the sample, thus interact with any functionalities existed within that depth. The depth of penetration (d_p , defined as the distance from the IRE-sample interface where the intensity of the evanescent wave decays to $1/e$ of its original value) can be calculated using the formula in Equation:

$$d_p = \frac{\lambda}{2\pi n_p (\sin^2 \theta - n_{sp}^2)^{1/2}}$$

where λ = wavelength of the radiation in the IRE, θ = angle of incidence, n_{sp} = ratio of the refractive indices of the sample vs. IRE, and n_p = refractive index of the IRE. Practically, the sample is placed in close optical contact with one of the crystal. In this study, ATR-IR was used for identifying functional groups on the surface of poly(DTE-co-20% DT carbonate) film before and after coated with chitosan. Sampling depth of characterization is 1-1.5 μm .

1.4.7 Mechanical properties

Mechanical properties of polymer blends were characterized by tensile testing

Tensile testing

Polymers are divided into five categories according to a qualitative description of their mechanical behavior and corresponding stress-strain characteristics as shown in the Table 1.1 and Figure 1.7.

A typical stress-strain data curve is shown in Figure 1.8. The elongation is directly proportional to the applied stress. Along the linear portion has relation between stress and strain. following Hooke's law given in equation 1.

$$\delta = E \varepsilon \quad \text{Eq..... (1)}$$

where δ , ε and E represent tensile stress, strain and Young's modulus, respectively.

Table 1.1 Qualitative description of polymer and it's stress-strain characteristics

Polymer Description	Characteristics of stress-strain curve			
	Young's modulus	Yield Stress	Tensile strength	Elongation At break
Soft, weak	Low	Low	Low	Low to moderate
Soft, tough	Low	Low	Moderate	Very high (20-100%)
Hard, brittle	High	None (break around yield point)	Moderate to high	Very low (<2%)
Hard, strong	High	High	High	Moderate (\approx 5%)
Hard, tough	High	High	High	High

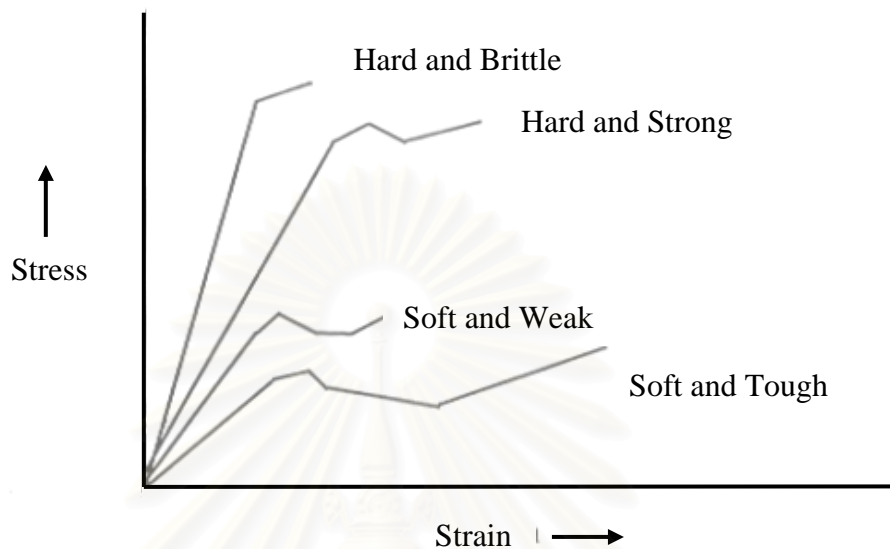


Figure 1.7 Influence of polymer properties on the stress-strain curves of several polymeric films

Tensile strength (δ), ultimate strength or breaking stress is the maximum stress applied to a point at which the film specimen breaks. Tensile strength can be computed from the applied load at rupture advised by the cross sectional area of fractured film. The determination of tensile strength alone is not very useful in predicting mechanical performance of the films, however higher values of tensile strength of the films are desirable for abrasion resistance.

Strain or elongation (ϵ) is a measure of ductility of the film. Strain is the deformation of the materials after applied stress. It is the increase in length relative to the original length.

Young's modulus or modulus of elasticity (E) is a measure of stiffness and rigidity of the film. It is calculated as applied stress divided by the corresponding strain in the region of linear elastic deformation (slope). The greater slope of the curve the higher the elastic modulus. The high value of the elastic modulus indicates the stiffness and strength of film and more stress will be required to produce a given amount of deformation.²⁵

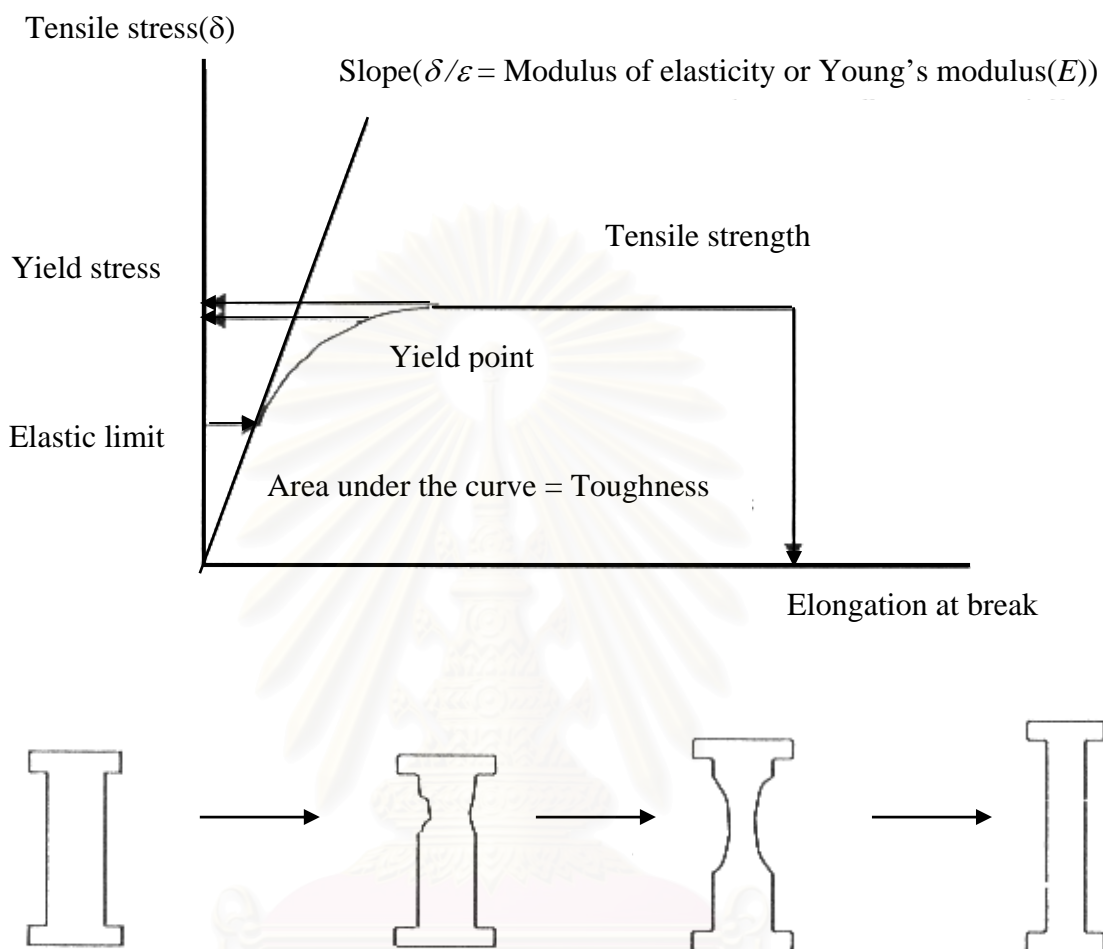


Figure 1.8 Stress-strain curve obtained from a free film of a plasticized polymer

Yield point is the point that there is an increase in strain with no increase in stress at this point called yield stress. When the applied stresses exceed yield stress the specimen exhibits plasticity, becoming ductile and flowing or creeping under nearly constant stress, resembling a highly viscous liquid and the sample undergoes permanent elongation.

Area under the stress-strain curve is a function of work done in breaking the film specimen and is representative of the film toughness. The sample that has more area under the stress-strain curve also has more hardness and toughness.

1.4.8 Physical appearance

Usually physical appearance of polymer blends can be characterized by scanning electron microscopy.

Scanning electron microscopy (SEM)

Scanning electron microscope (SEM) uses the technique of a focus electron beam to scan the sample surface. The electron beam is controlled by lenses consisting of magnetic fields. The polymer specimen to be examined by SEM was first coated with a thin layer of gold to provide a conductive layer.

SEM provides more detailed information on the morphology; domains down to a size of 10 nm can be resolved. SEM is becoming the most popular method of observation of polymer blends. Sometimes, different domains of blends can be observed.

1.4.9 Calcium adsorption on the surface of polymer films

Chitosan has already been described as a suitable natural polymer for the collection of metal ions, since the amine group on the chitosan chain can act as chelation sites for metal ions. Many researchers study chelation of metal ion by chitosan such as adsorption of gold(III) ions,²⁶ removal of chromium(III) from aqueous solutions by chitosan.²⁷

Calcium ion was chosen for studying metal adsorption by polymer films. Calcium is the most abundant mineral in the human body. Of the body's total calcium, about 99% is in the bones and teeth where it plays a structural role. The remaining 1% is present in body tissues and fluids where it is essential for cell metabolism, muscle contraction and nerve impulse transmission.²⁸

Determination of Ca^{2+} by atomic absorption spectroscopy

Atomic absorption spectrophotometry (AAS) (Figure 1.9) is an analytical technique used to measure a wide range of elements in materials such as metals, pottery and glass. Although it is a destructive technique, the sample size needed is very small (typically about 10 milligrams) and its removal causes little damage. The

sample is accurately weighed and then dissolved, often using strong acids. The resulting solution is sprayed into the flame of the instrument and atomised (see schematic diagram). Light of a suitable wavelength for a particular element is shone through the flame, and some of this light is absorbed by the atoms of the sample. The amount of light absorbed is proportional to the concentration of the element in the solution.

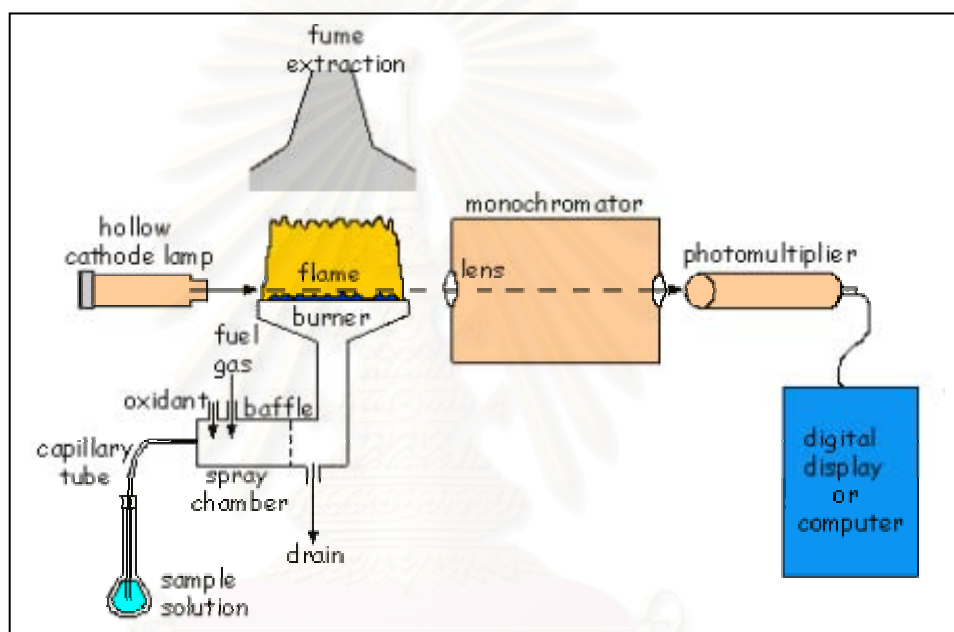


Figure 1.9 Diagram of AAS

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CHAPTER II

EXPERIMENTAL

2.1 Materials

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and glucosamine hydrochloride were purchased from Fluka Chemika. PAA $\bar{M}_v = 450,000$ g/mol were purchased from Aldrich Chemical Co. Poly(DTE-co-20% DT carbonate) $\bar{M}_w = 158,000$ g/mol, poly(DTE-co-50% DT carbonate) $\bar{M}_w = 39,900$ g/mol and poly(100% DT carbonate) $\bar{M}_w = 34,500$ g/mol were supplied by Professor Joachim Kohn of Rutgers University, USA. Chitosan $\bar{M}_v = 100,000$ g/mol (degree of deacetylation = 95 %DD) was purchased from Seafresh Chitosan (Lab) Co., Ltd., Thailand.

2.2 Equipments

2.2.1 Fourier transform-infrared spectroscopy (FT-IR)

The FT-IR spectra were recorded on a Nicolet Impact 410 Spectrophotometer. The sample was prepared as a KBr pellet by enishing the polymer with KBr and pressed into a pellet.

2.2.2 Attenuated total reflectance infrared spectroscopy (ATR- IR)

All spectra were collected at a resolution of 4 cm^{-1} and 16 scan co-addition using Bruker vector 33 FT-IR spectrometer equipped with a DTGS detector. A multiple attenuated total reflection (MATR) accessory with 45° zinc selenide (ZnSe) IRE (Spectra Tech, USA) and a variable angle reflection accessory (SeagullTM, Harrick Scientific, USA) with a hemispherical ZnSe IRE were employed for all ATR spectral acquisitions.

2.2.3 Atomic Absorption Spectroscopy (AAS)

Flame atomization technique was performed by using air/acetylene gas the flame, Shimadzu Atomic Absorption / Flem Emission Spectrophotometer AA-670.

2.2.4 Mechanical stirrer

A mechanical stirrer, BEC THAI Rw 20 DZM.n IKA LABORTECHNIX, was used to mix two polymers suspended in the solution at the speed of 1,000 rpm.

2.3 Blend preparation

Table 2.1 Blend preparation

<i>Blend preparation between</i>	<i>Ratio (by mole)</i>
<i>PAA and chitosan</i>	<i>1:1</i>
<i>DTE-20% DT and chitosan</i>	<i>1:1</i>
<i>100% DT and chitosan</i>	<i>1:1</i>

2.3.1 Preparation of PAA and chitosan blend

PAA 5 wt% and chitosan 0.5 wt% solution were prepared separately by dissolving the polymers into 30 wt% acetic acid aqueous solution at room temperature. The solutions of PAA and chitosan were mixed together at a molar ratio 1:1 with stirring by a mechanical stirrer for 1 h to be cloudy solution. The resulting solution was cast onto a Teflon coated mold (8x8 inch in size). After 5 days, the film was peeled off and immersed in distilled water for 24 h. The film was dried under vacuum before characterization.

2.3.2 Preparation of DTE-20% DT and chitosan blend

DTE-20% DT (0.2 mmol) was dissolved in THF (4 mL). Chitosan (0.2 mmol) was dissolved in 1% acetic acid (4 mL). This was followed by the addition of 1 mL THF. DTE-20% DT and chitosan solutions were mixed together by a mechanical stirrer for 1 h to form a casting solution. The solution was cast onto a Teflon-coated petridish. After 1 day, the film was peeled off and washed with distilled water. The film was dried under vacuum before characterization.

2.3.3 Degradation study of DTE-20% DT in THF-acetic acid mixed solvent

The stability towards hydrolysis of DTE-20% DT was studied. DTE-20% DT 0.5 g was dissolved in THF (25 mL). To the solution, 25 mL 1% acetic acid was added. After 1 h, the solution mixture was poured into ethanol to precipitate DTE-20% DT. The precipitates were filtered off, washed thoroughly with distilled water and vacuum-dried at room temperature. Samples of different incubation periods (4, 8, 16 and 24 h) were analyzed by GPC. The \bar{M}_w of DTE-20% DT was determined in THF, using polystyrene as standards.

2.3.4 Preparation of poly(100% DT carbonate) (100% DT) and chitosan blend

100% DT (2 mmol) was dissolved in THF (15 mL). Chitosan (2 mmol) was dissolved in 1% acetic acid (15 mL). This was followed by the addition of 1 mL THF. 100% DT and chitosan solutions were mixed together by a mechanical stirrer for 1 h to form a casting solution. The solution was cast onto a Teflon-coated petridish. After 1 day, the film was peeled off and washed with distilled water. The film was dried under vacuum before characterization.

2.4 Amide bond formation

2.4.1 By heat treatment

Table 2.2 Amide bond formation by heat treatment

<i>Heat treatment between</i>	<i>ratio(by mole)</i>
<i>PAA and chitosan</i>	<i>1:1</i>
<i>DTE-20% DT and chitosan</i>	<i>1:1</i>

Solvent cast films of PAA and chitosan or DTE-20% DT and chitosan blends were heated in oven, present at 180 °C, for 1, 4, 8 h. The treated films were subjected to FT-IR analysis in order to determine the amide bond formation.

2.4.2 By EDCI (a coupling agent)

Table 2.3 Amide bond formation by EDCI

<i>Reaction between</i>	<i>ratio(by mole)</i>
<i>PAA and glucosamine</i>	<i>1:1</i>
<i>DTE-20% DT and chitosan</i>	<i>1:1</i>

PAA and glucosamine

PAA (0.5 mmol) and glucosamine (0.5 mmol) were dissolved in distilled water followed by dropwise addition of EDCI (0.5 mmol) solution in distilled water. After 24 h stirring at room temperature, the reaction mixture was precipitated in ethanol. The precipitates were filtered off, washed thoroughly with distilled water and vacuum dried at room temperature before characterization.

DTE-20% DT and chitosan

DTE-20% DT (0.2 mmol) was dissolved in THF (4 mL) and chitosan (0.2 mmol) was dissolved in 1% acetic acid (4 mL). This was followed by the addition of 1 mL THF. DTE-20% DT and chitosan solutions were mixed together by a mechanical stirrer and followed by the dropwise addition of EDCI (0.2 mmol) aqueous solution. After 1 h, stirring at room temperature, the reaction mixture was cast onto a Teflon coated petridish. After 1 day, the film was peeled off and washed with distilled water. The film was dried under vacuum before characterization.

2.5 Preparation of chitosan coated on DTE-20% DT film

2.5.1 Without EDCI-coupling agent

DTE-20% DT (92 mg) was dissolved in THF (2mL). After stirring for 10 min, the solution was then cast into film on a Teflon-coated petridish. The film was peeled off. The thickness was between 40 to 100 μm .

Chitosan (1.7 mmol) was dissolved in 1% acetic acid (10 mL). The solution was transferred into a flask containing DTE-20% DT film. The film was suspended in the chitosan solution at room temperature for 24 h. The film was rinsed with distilled water and were dried under vacuum before characterization.

2.5.2 With EDCI-coupling agent

Method I

The DTE-20% DT or DTE-50% DT film ($1 \times 1 \text{ cm}^2$) was immersed in a 0.1% (w/v) solution of EDCI in distilled water at room temperature for 24 h. After that, chitosan solution (2% w/v, 10 mL) was added to the reaction mixture and let stand for another 24 h. The films were dried in air. After that, the film was dipped in distilled water and dried in vacuum before characterization.

Method II

The DTE-20% DT or DTE-50% DT film was treated with a 0.1% (w/v) solution of EDCI in distilled water. Each film was immersed at 4°C for 24 h. The film was rinsed with distilled water. The activated DTE-20% DT film was immersed into 2 % chitosan solution for 48 h at 4°C. After the immobilization reaction, the polymer film was washed with distilled water and then dried and stored.

2.6 Tensile testing

The ultimate tensile strength and percentage of elongation were examined by using an universal testing machine (Tensometer, Instron (model 5565, serial No. H333, Instron Corp., Canton, MA, USA.) equipped with 10 Newton tension load cell. The relative humidity of operating room was about 55 % and temperature was 25 ± 1 °C. The tensile properties were determined by the following procedure. Polymer films were cut into small strips by using a standard knife. The thickness of each strip was the mean value of five separate measurements taken along the length of the films using a micrometer to ensure the thickness range 50-100 μm . The accurate 2 cm. in length was marked in the middle section of each strips. Then the strip was carefully clamped by an upper and lower pneumatic flat-faced grip and extended by the test machine at a speed of 1.5 mm/min until the film was ruptured. The breaking force and the change in the length at moment of rupture were record. The film strips of polymer films such as DTE-20% DT, blend with EDCI, blend without EDCI, chitosan coated on DTE-20% DT film with EDCI and chitosan coated on DTE-20% DT film without EDCI were cut 2 mm x 2 cm (width x lenght). The ultimate tensile strength and percentage of elongation were calculated using the following equations.

The ultimate tensile strength = breaking force/cross section area

The percentage of elongation = $\Delta L / L_0$

ΔL = the difference of length

L_0 = the initial length

The mean value and standard deviation of %elongation and tensile strength were obtained from 5 specimens of each polymer films. All test parameters such as %strain at break, stress at break and Young's modulus were automatically computed by the Merlin Series IX material testing software.

2.7 Physical appearance analysis by scanning electron microscopy (SEM)

Polymer films, adhesive layer and backing layer, were posted on SEM stub and coated with gold. The sample was examined for surface appearance using a SEM model : Jeol, JSM-5410 L, Japan.

2.8 Calcium adsorption on the surface of polymer films

The standard of calcium solution

Stock solution (10,00 ppm) of calcium was prepared by dissolving CaCl_2 41.621 mg into 15 mL ultrapure distilled water was provided by the MilliQ Labo system. This stock solution was then diluted to give a standard solution of 10 ppm. A series of calcium solution at the concentration of 0.2, 0.4, 0.6, 0.8 and 1 ppm was prepared by further dilution of the 10.0 ppm solution.

Calcium absorption on the surface of chitosan films

The absorption experiments were performed in a close polyethylene bottle, by immersing the chitosan film ($1 \times 1 \text{ cm}^2$) in 10 ppm 10 mL of calcium solution. After 20 min the film was removed. The concentration of calcium in supernatant was analyzed using atomic absorption

spectrophotometer. The procedure was repeated for aqueous samples after 40, 60, 80 and 1380 min of chitosan film immersion time.

Calcium adsorption on the surface of polymer films

The absorption experiments were performed in a close polyethylene bottle, by immersing the polymer film (1x1 cm²) in 10 mL of 10 ppm calcium solution. After 80 min the film was removed. The concentration of calcium in supernatant was analyzed using atomic absorption spectrophotometer.



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CHAPTER III

RESULTS AND DISCUSSIONS

3.1 Electrostatic interaction between amino and carboxylic groups in blends

3.1.1 Blend of chitosan and PAA

Since PAA contains carboxylic acid group, as in DTE-20% DT, it was used as a model polymer for studying the interaction between the amino groups on chitosan and the carboxylic acid groups. Moreover, the structure of PAA is simpler to be characterized than that of DTE-20% DT.

After mixing the solution of PAA and chitosan, a white gel-like precipitate was obtained. It is well known that mixing of oppositely charged polyelectrolytes leads to the formation of a complex by electrostatic interaction, and usually causes a precipitate in an aqueous medium. More dispersed mixtures were obtained when using a mechanical stirrer, at a speed of 1,000 rpm. Adding acetic acid helped dissolving all precipitate. It was necessary to use a high concentration of acetic acid in order to dissociate the precipitate.

During the film-forming step of the chitosan and PAA blend, an interesting phenomenon was observed. When water/acetic acid solvents were allowed to evaporate in air, some areas of the film turned from transparent to opaque. With further devolatilization the entire film appeared milky white. Finally, the milky white gradually disappeared and became transparent again with golden-yellow color. This phenomenon did not occur during the film-forming process of pure chitosan. The observed opacity might be related to aggregative state of two polymers in solution. The concentration of the casting solution increased with the volatilization of the solvents. When the concentration reached a critical value, the chitosan chains were close to the PAA chains. The aggregated chains probably caused the refraction

change of the light, so casting solution turned milky-white. Meanwhile, it should be noted that chitosan is a polymer with rigid chains which make it possible to form liquid crystal-like structure.¹

The IR spectrum of chitosan (Figure 3.1) shows the characteristic peak at 1589 and 1650 cm^{-1} , assigned to N-H bending vibrations of 2-aminoglucosamine primary amines and C=O stretching of secondary amides, respectively.¹³ The strongest peak, at 1719 cm^{-1} in the IR spectrum of PAA (Figure 3.2), has been assigned to C=O stretching of carboxylic acid groups. In the spectrum of chitosan and PAA blend (Figure 3.3) the strongest peak at 1404 cm^{-1} was observed. The appearance of this peak could be due to symmetrical stretching of COO^- groups, while the peak at 1630 was attributed to the formation of NH_3^+ groups.¹³ These facts confirmed the presence of carboxylate groups of PAA and ammonium groups of chitosan in the blends. This therefore leads to an electrostatic interaction between the two polymers (Scheme 3.1). Furthermore, the carboxylic group of PAA cannot electrostatic interaction was remained when using PAA more than chitosan blend (10:1). The results showed peaks at 1714 and 1404 cm^{-1} (Figure 3.4) assigned to C=O stretching of carboxylic acid and COO^- groups of PAA, respectively.

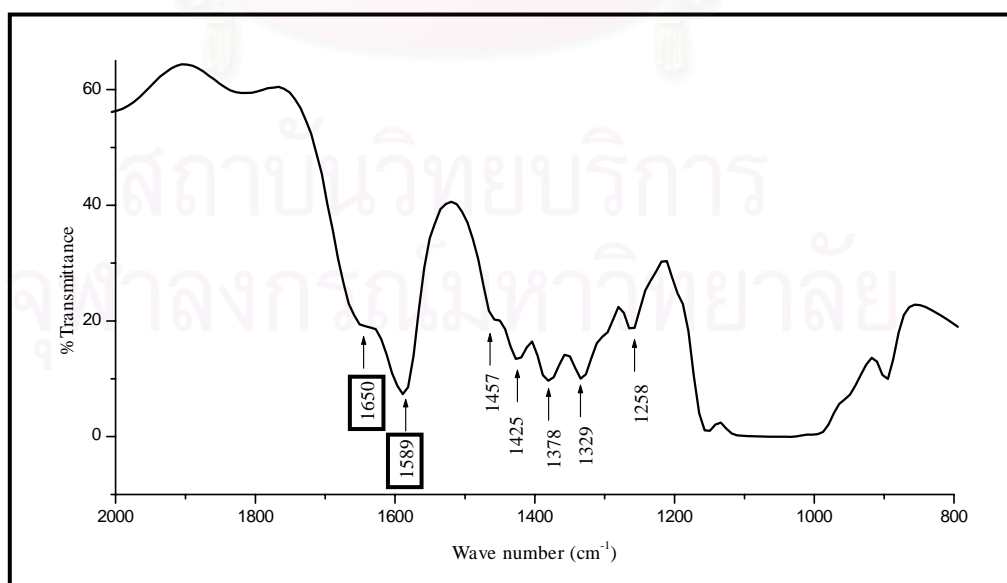


Figure 3.1 FT-IR spectrum of chitosan film

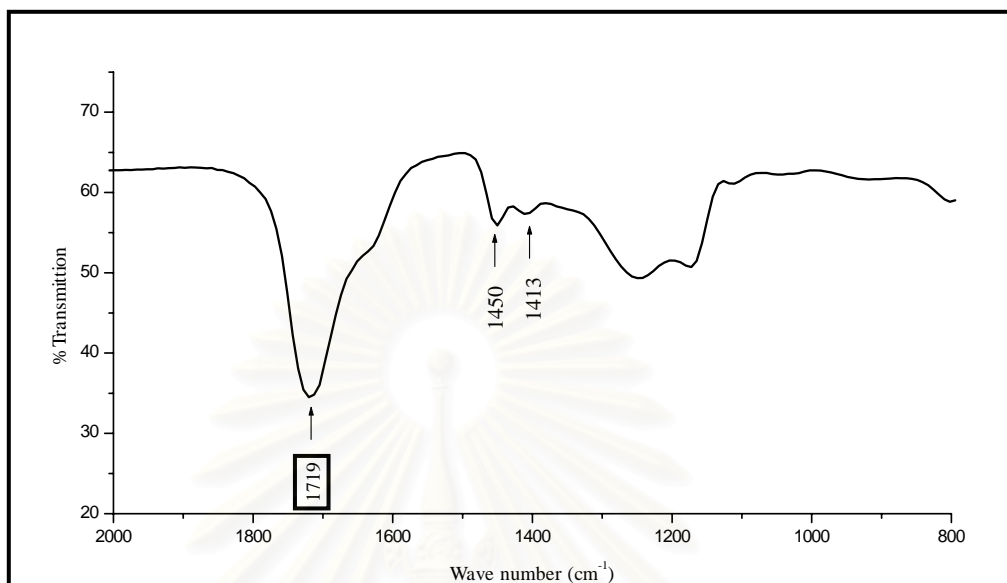


Figure 3.2 FT-IR spectrum of PAA

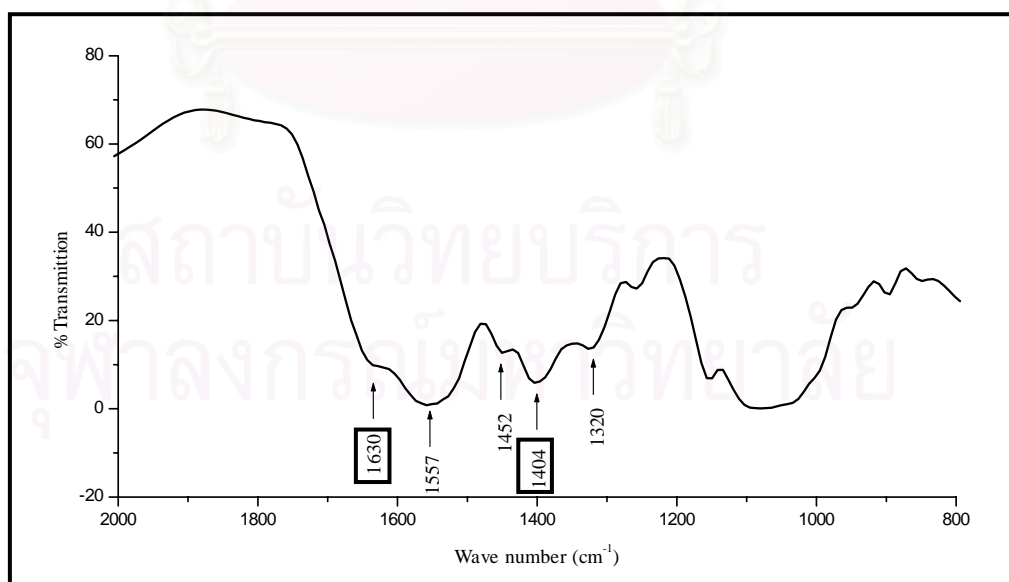


Figure 3.3 FT-IR spectrum of chitosan and PAA blend

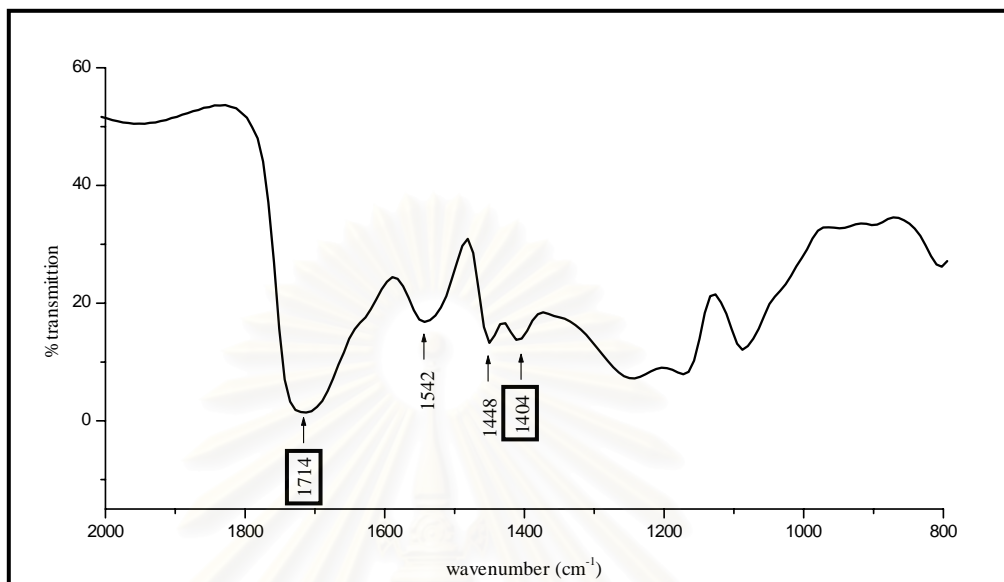
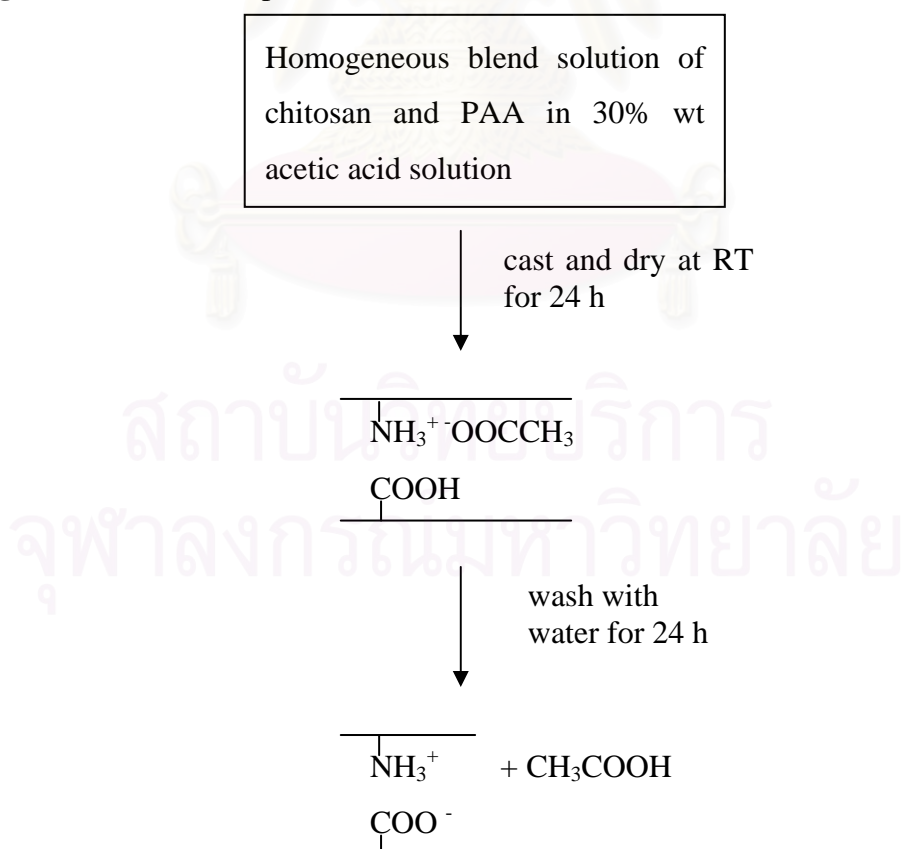


Figure 3.4 FT-IR spectrum of PAA more than chitosan blend (10:1)



Scheme 3.1 Electrostatic interaction in the blend between chitosan and PAA

3.1.2 Blend of chitosan and DTE-20% DT

Several solvents such as CH₂Cl₂, DMF, DMSO, acetone, MeOH, THF, acetic acid 1%(v/v) and NaOH 40% (w/v) were investigated to find a suitable solvent for preparing the blend between chitosan and DTE-20% DT.

Table 3.1 Solubility of chitosan and DTE-20% DT

Solvent	Chitosan	DTE-20% DT
CH ₂ Cl ₂	N	Y
DMF	N	Y
DMSO	N	N
Acetone	N	N
MeOH	N	N
THF	N	Y
1% acetic acid	Y	N
NaOH	N	N

N = not dissolved

Y = dissolved

From Table 3.1, there was not any good solvent that could dissolve both chitosan and DTE-20% DT. The most suitable solvents to prepare the blends are acetic acid (1%) and THF, used to dissolve chitosan and DTE-20% DT respectively. Nevertheless, one disadvantage remained. The polycarbonate slowly degraded in the mixed THF:acetic acid (1:1) solution, as shown in Table 3.2. After 24 h, its molecular weight dropped by 13%. An averaged blend preparation time was 24 h. Film neutralization using basic solution was also included in order to remove all acid to prevent further degradation.

Table 3.2 Changes of molecular weight of DTE-20% DT after various incubation times at RT 30 °C in THF:acetic acid (1:1)

Time(h)	0	1	4	8	16	24
\bar{M}_w	48044	52856	54918	48010	43437	41637

It was found that when the two polymer solutions were mixed, lots of solid was formed. To increase its homogeneity, THF was also added in the chitosan solution before mixing with DTE-20% DT solution. The mechanical stirrer was also used to stir the solution at a speed of 1,000 rpm. The solution obtained was milky white. The appearance of dry film was opaque white.

In the IR spectrum of DTE-20% DT (Figure 3.5), peaks at 1770, 1738 and 1650 cm^{-1} were assigned to C=O stretching of the carbonate, the carboxylic acid groups and the secondary amides, respectively. The blend peaks at 1589 and 1738 cm^{-1} were assigned to the N-H bending vibrations of $-\text{NH}_2$ of chitosan and C=O stretching of carboxylic acid groups of DTE-20% DT (Figure 3.6), respectively. But the peaks at 1630 and 1404 cm^{-1} , evidences for NH_3^+ and COO^- formation, were not found in this blend.

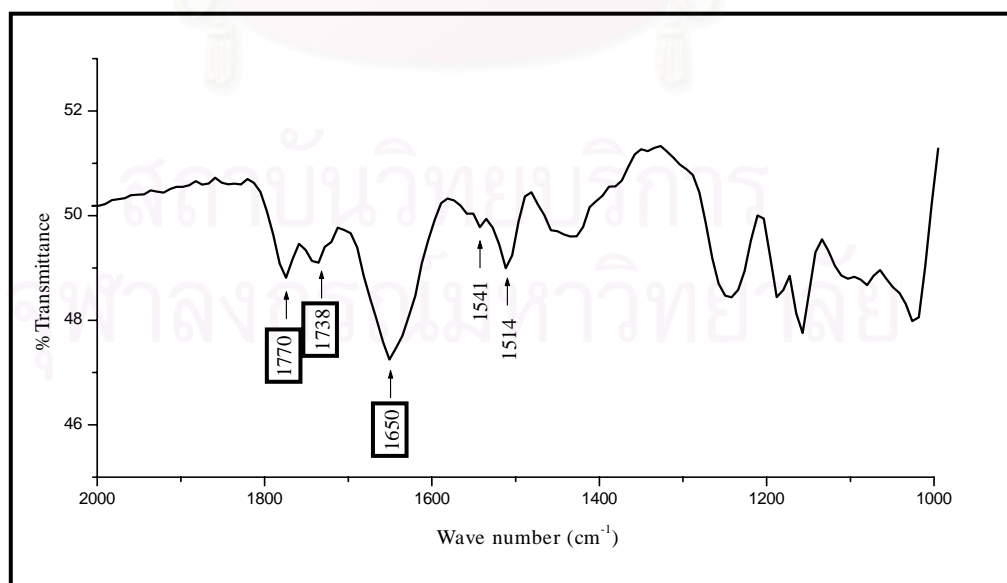


Figure 3.5 FT-IR spectrum of DTE-20% DT

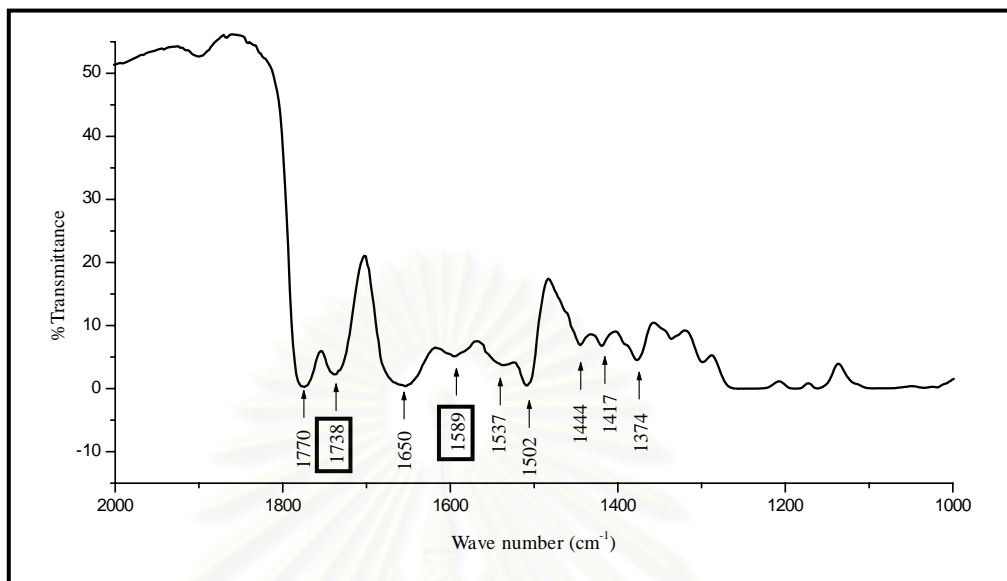


Figure 3.6 FT-IR spectrum of chitosan and DTE-20% DT blend

Using SEM technique, two different features were revealed from the chitosan and DTE-20% DT film. For the homopolymers, the surface of chitosan is rough with distinct flakelike morphology (Figure 3.7). On the other hand, the surface of DTE-20% DT film was rather smooth (Figure 3.8). For the blend (Figure 3.9) the surface appeared to be rough and grainy. This suggests that both polymers cannot mix very well together, possibly due to the difference in functional groups and structures.

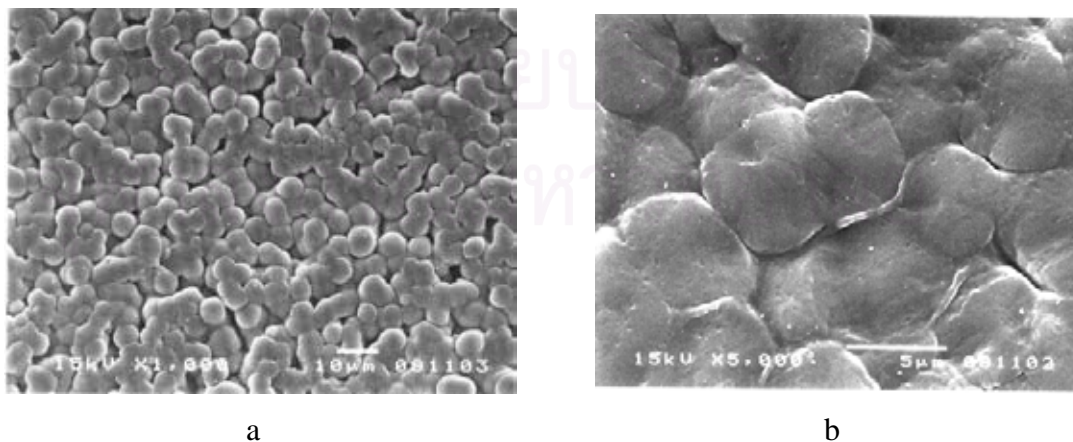


Figure 3.7 SEM micrograph of the chitosan film surface (a x 1,000 and b x 5,000)

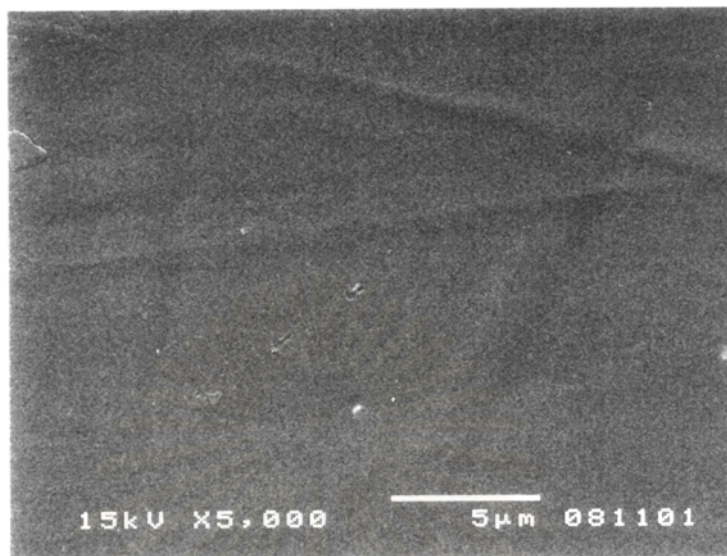


Figure 3.8 SEM micrograph of the DTE-20% DT film surface (x 5,000)

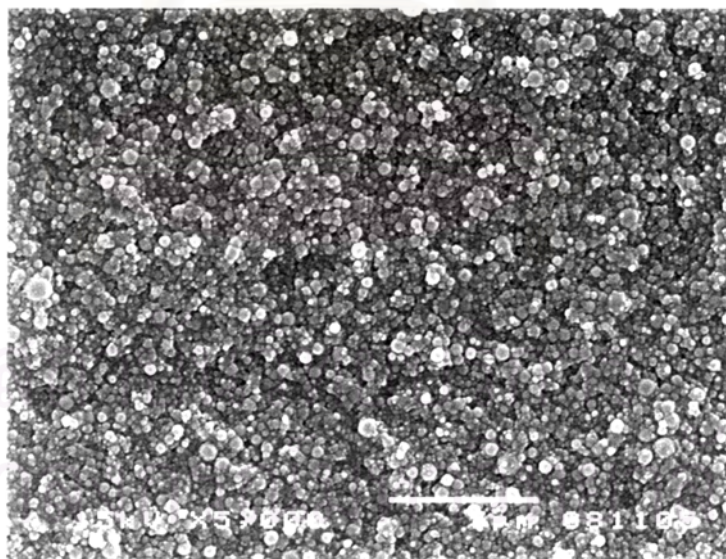


Figure 3.9 SEM micrograph surface of the chitosan blend with DTE-20% DT film
(x 5,000)

3.1.3 Blend of chitosan and 100% DT

The interaction in the blend between chitosan and DTE-20% DT was further analyzed by using poly(100% DT carbonate) (100% DT) (Figure 3.10) this polymer contains only carboxylic acid pendent group, similar to PAA.

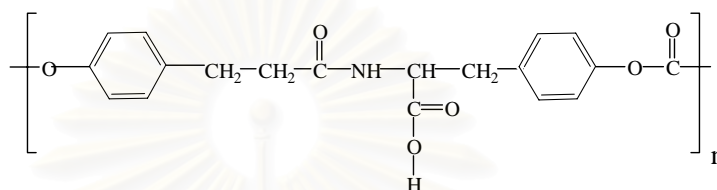


Figure 3.10 Structure of 100% DT

In the IR spectrum of 100% DT (Figure 3.11), peaks at 1770, 1738 and 1650 cm^{-1} were assigned to C=O stretching of the carbonate, the carboxylic acid groups and the secondary amides, respectively. Analysis of the blend revealed that the peak at 1738 cm^{-1} assigned to the C=O stretching of carboxylic acid groups was missing (Figure 3.12). The peaks at 1589 cm^{-1} (N-H bending of $-\text{NH}_2$ of chitosan), and at 1634 and 1398 cm^{-1} evidences for NH_3^+ and COO^- formation, were observed.

These results suggested that electrostatic interaction between chitosan and tyrosine-derived 100% DT was possible, but only when polycarbonate with high $-\text{COOH}$ content was used to prepare the blend.

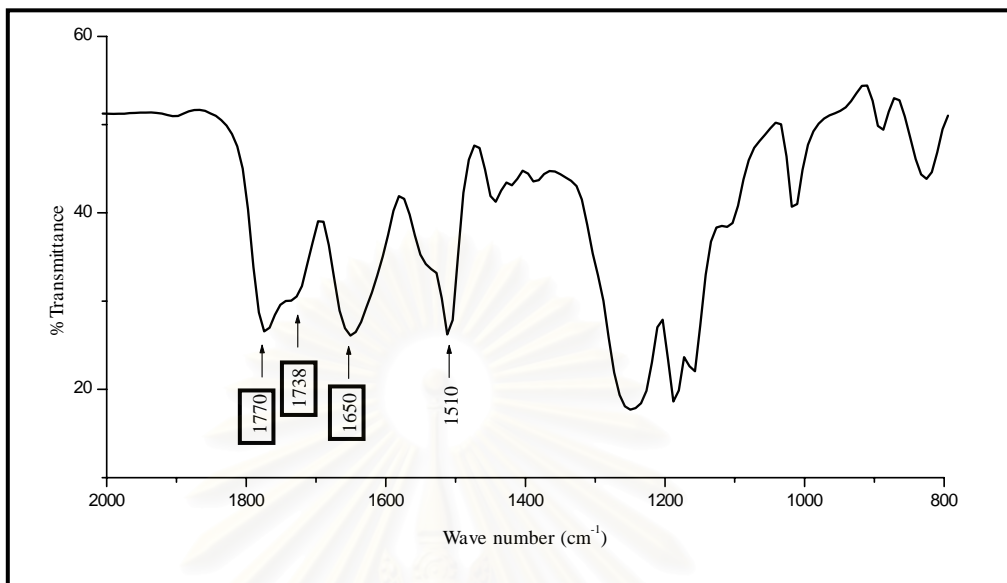


Figure 3.11 FT-IR spectrum of 100% DT

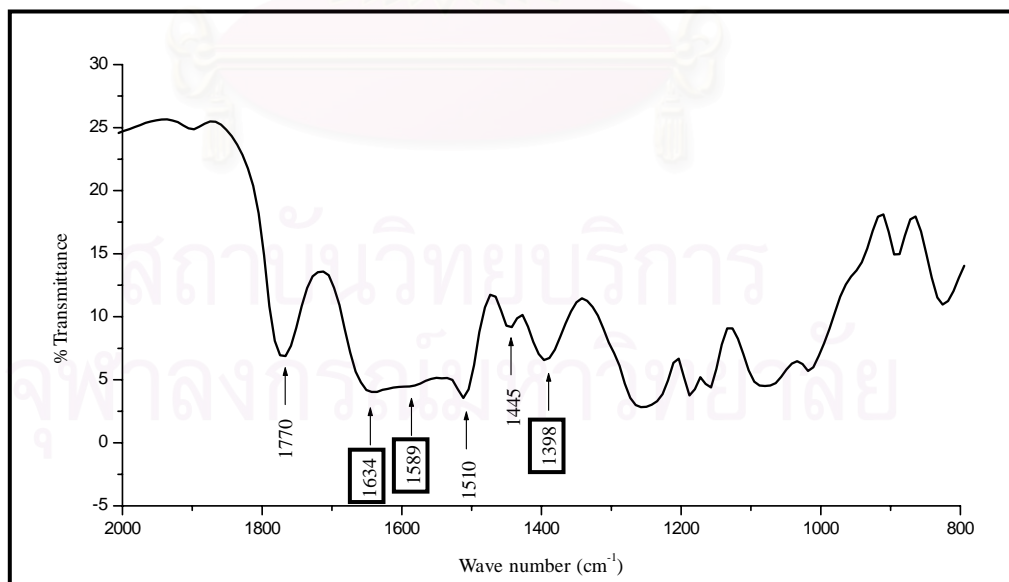


Figure 3.12 FT-IR spectrum of chitosan and 100% DT blend

3.2 Amides bond formation in blends

From the previous section, the electrostatic interaction between chitosan and DTE-20% DT blend could not be detected by IR. It was, therefore, turning the focus of this work to create amide linkage between these two polymers. Two methods were investigated; they were by heat treatment and by using a coupling agent.

3.2.1 Heat induced formation of amide bond

Blends of chitosan and PAA

Films of PAA and chitosan blend were heated in an oven, preset at 180 °C. After heating for 1, 4 and 8 h, all samples were subjected to IR analysis. IR spectrum shows (Figure 3.13) peaks at 1641, 1646 and 1650 cm^{-1} . The intensity at 1650 cm^{-1} increased with heating time, and were assigned to the C=O stretching of secondary amide (amide I band). Moreover, the peak at 1630 and 1404 cm^{-1} for NH_3^+ and COO^- were disappeared. This result suggested that amide bond be formed after heat treatment (Scheme 3.2).

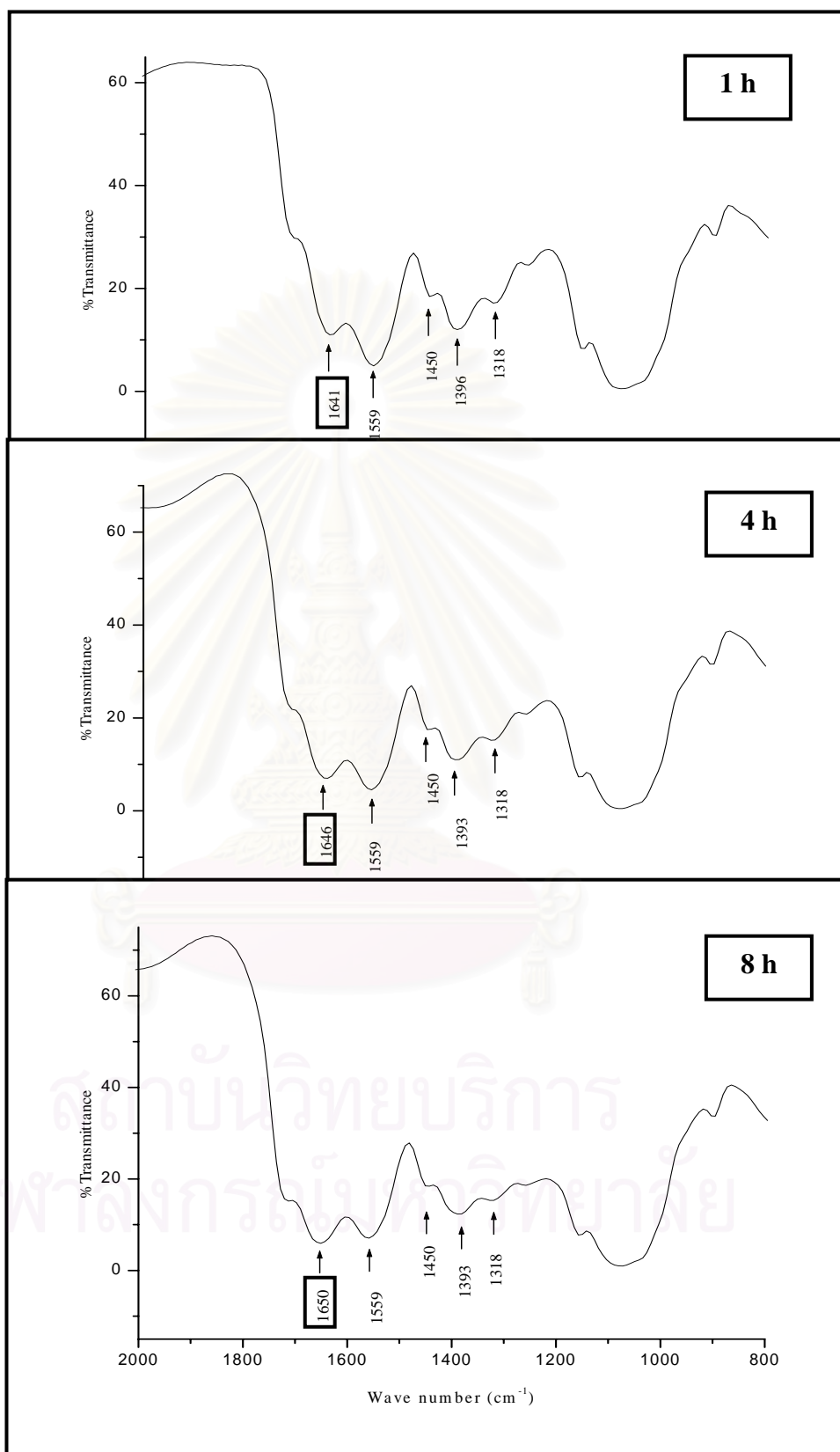
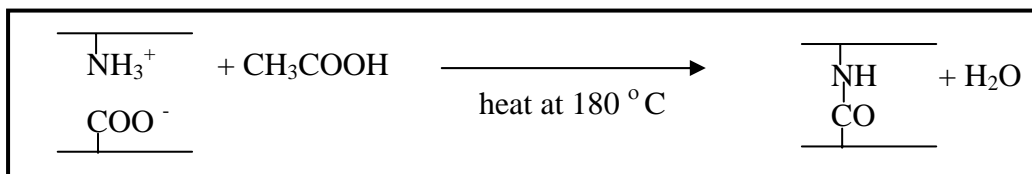


Figure 3.13 FT-IR spectrum of chitosan and PAA blend was heated in oven at 180°C for 1, 4 and 8 h.



Scheme 3.2 Amide bond formation between chitosan and PAA by heat treatment

Blend of chitosan and DTE-20% DT

The blending films of DTE-20% DT and chitosan were heated in an oven, preset at 180°C . The films were kept in the oven for upto 8 h. When compared with the IR spectrum of the blend before heat treatment (Figure 3.6), samples obtained after 1,4 and 8 h (Figure 3.14) of heat produced similar IR spectra. No conclusive evidence about new amide bond formation between chitosan and DTE-20% DT was observed. The film also appeared to be more brittle and darker in color than the original blends. Changes of brittleness and color were caused by chitosan, as was shown by a separate test, performed by heating the chitosan film in the same temperature and condition.

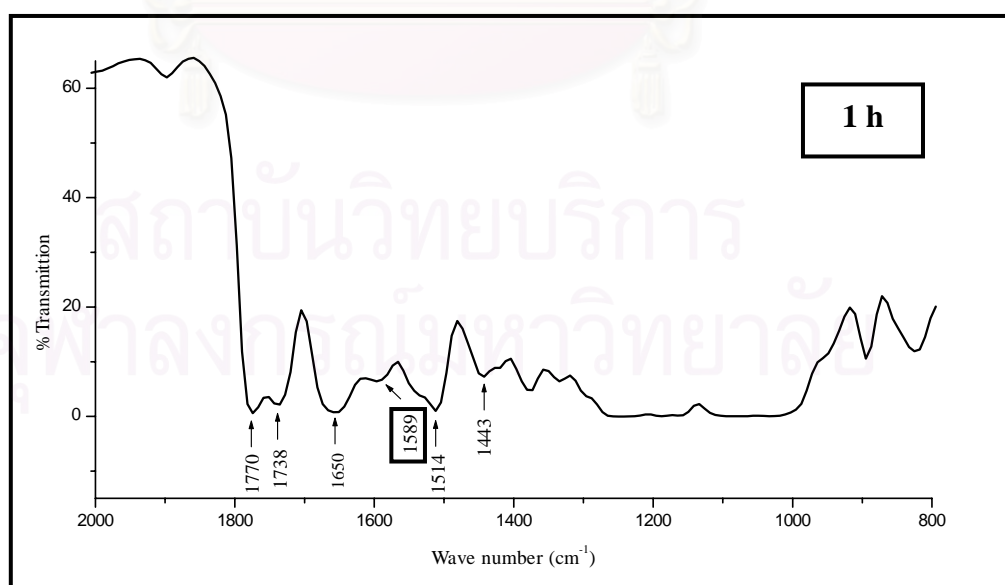


Figure 3.14 FT-IR spectrum of chitosan and DTE-20% DT blend, heated in oven at 180°C for 1, 4 and 8 h.

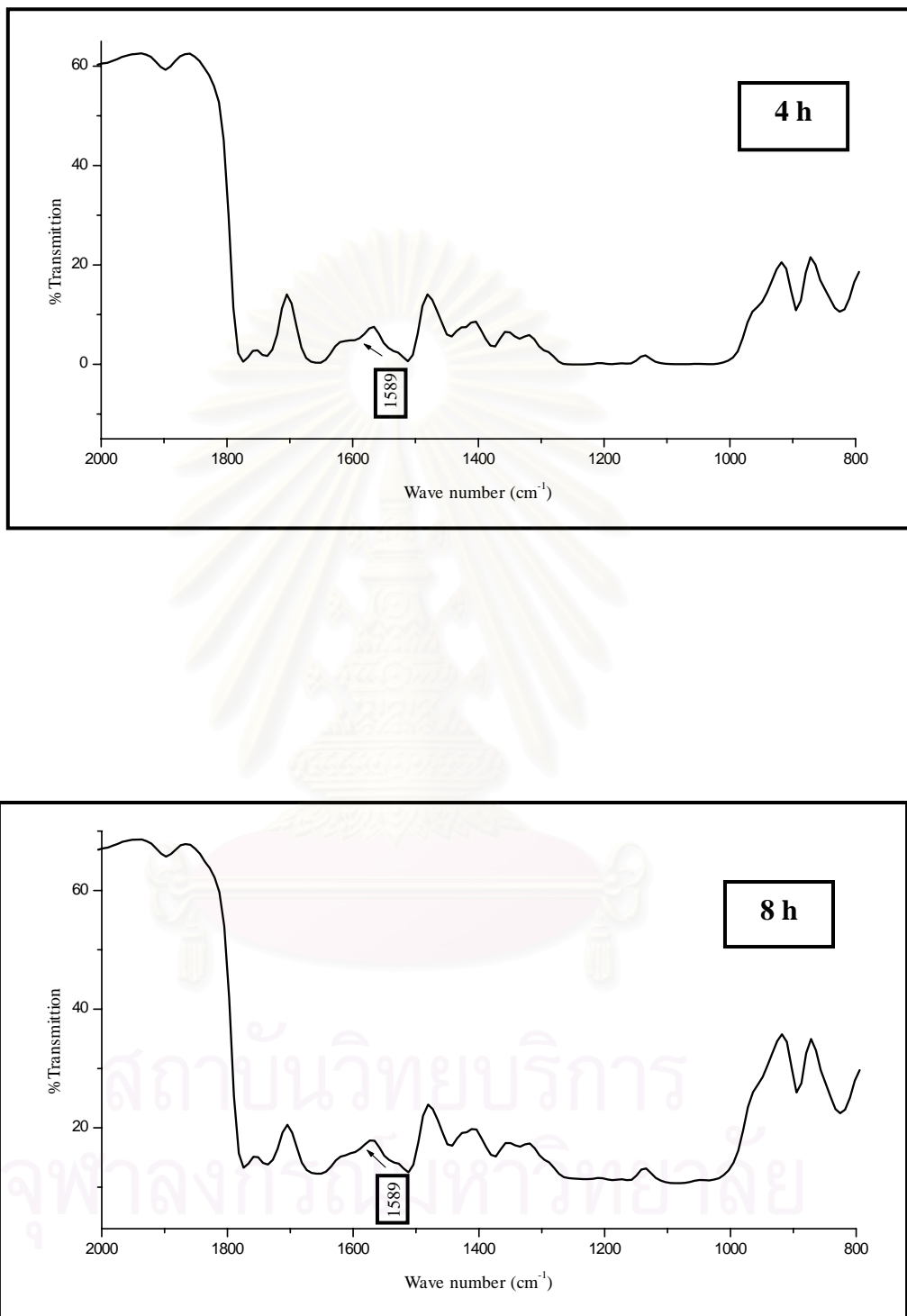


Figure 3.14 FT-IR spectrum of chitosan and DTE-20% DT blend, heated in oven at 180 °C for 1, 4 and 8 h. (continued)

3.2.2 Amide bond formation assisted by a coupling agent-EDCI

Reaction between PAA and glucosamine

This part of the research utilized glucosamine as a model compound for chitosan, because glucosamine has only an amine group that could simplify the characterization of amide bond formation. The IR spectrum of glucosamine shows absorption peak of amine at 1589 cm^{-1} (Figure 3.15). After PAA reacted with glucosamine by using EDCI as a coupling agent (Scheme 3.3), the resulting polymers produced IR absorption peak at 1650 and 1555 cm^{-1} (Figure 3.16). These two signals were assigned to the carbonyl stretching of secondary amides (amide I band) and NH bending vibration of secondary amides (amide II band), indicating the formation of amide bond. Moreover, absorption peak of amine at 1589 was disappeared.

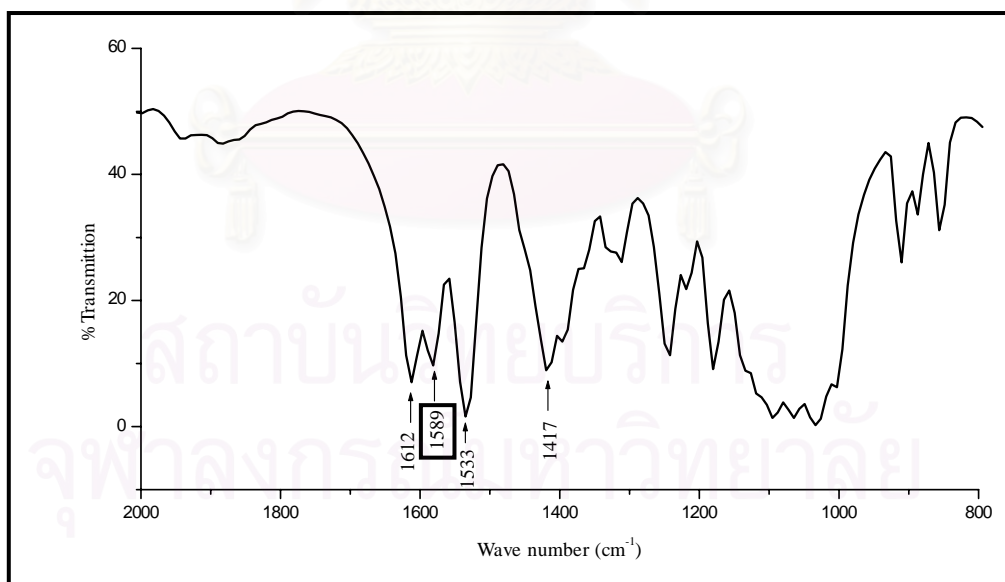


Figure 3.15 FT-IR spectrum of glucosamine

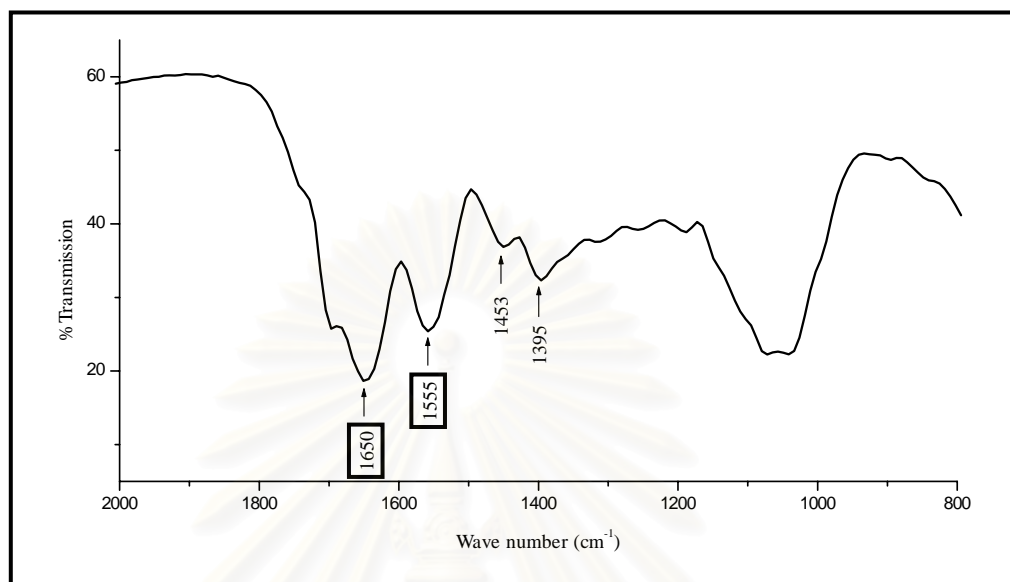
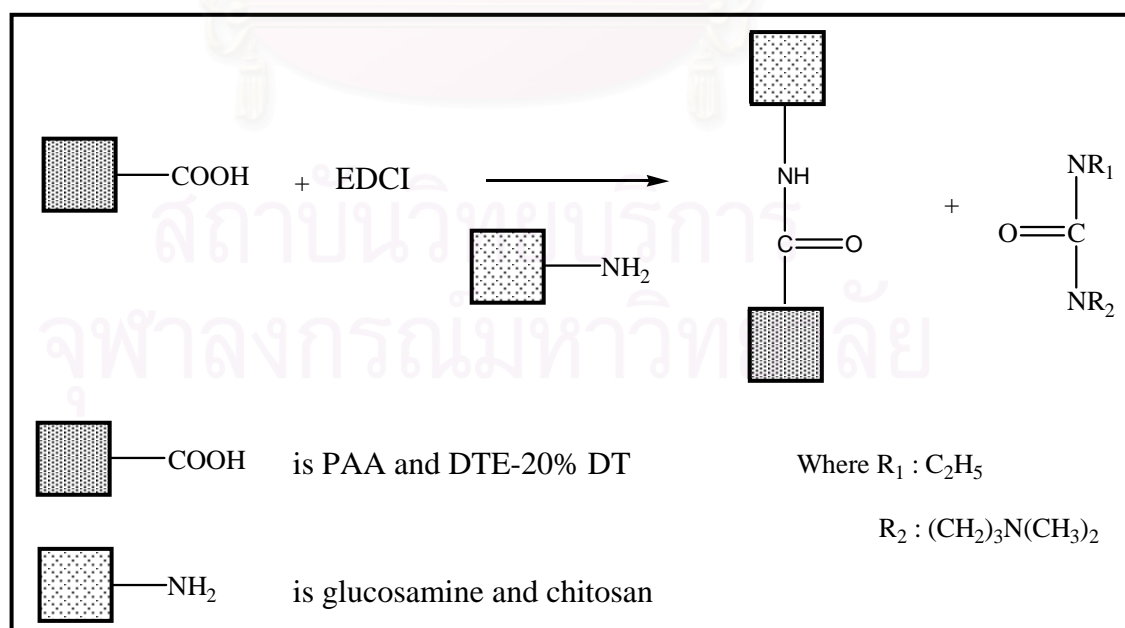


Figure 3.16 FT-IR spectrum of reaction between PAA and glucosamine by using EDCI as a coupling agent



Scheme 3.3 The coupling mechanism using EDCI through amide linkage formation.

Reaction between DTE-20% DT and chitosan

It was shown in the previous section that the amine group of glucosamine could react with the carboxylic groups of PAA in the presence of EDCI to form amide bond. One question remained : could the amine of chitosan react with the carboxylic group of DTE-20% DT? The reaction between chitosan and DTE-20% DT was carried out using EDCI as a coupling agent. IR analysis revealed that the EDCI-coupled polymer blend produced some what different IR spectrum in the range of 1650-1500 cm^{-1} (Figure 3.17) from the blend without EDCI (Figure 3.6). There is an appearance of a peak at 1555 cm^{-1} . Furthermore, absorption peak of the amine of chitosan at 1589 cm^{-1} disappeared. This little change in the IR spectrum could possibly be explained by the reaction between $-\text{NH}_2$ of chitosan and $-\text{COOH}$ of DTE-20% DT to form amide bonds.

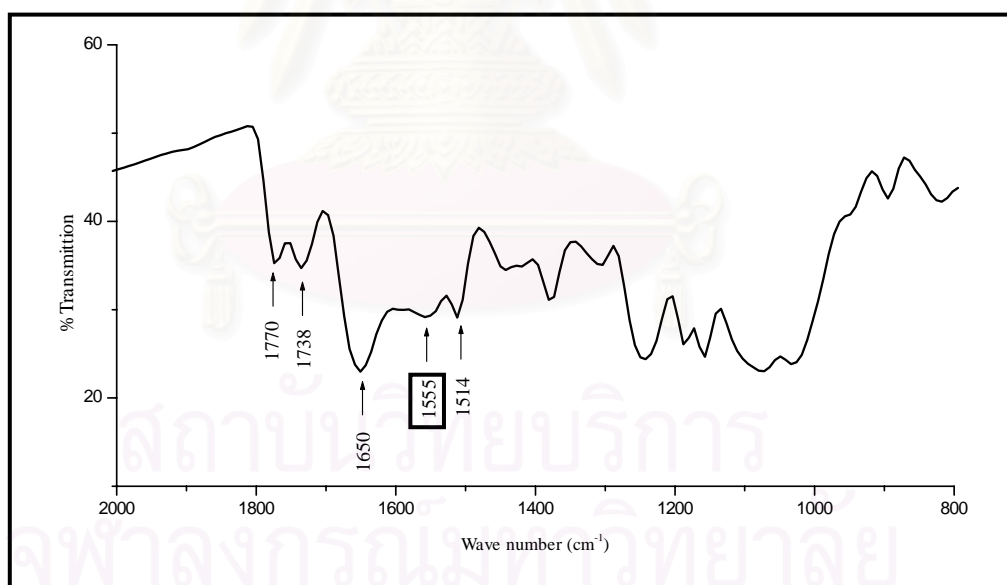


Figure 3.17 FT-IR spectrum of reaction between DTE-20% DT and chitosan by using EDCI

The physical appearance of blend film was characterized by SEM technique. After EDCI was used to link the two polymers, the grain like feature was joined

together by connective matrix (Figure 3.18), different from the blend without EDCI (Figure 3.9).

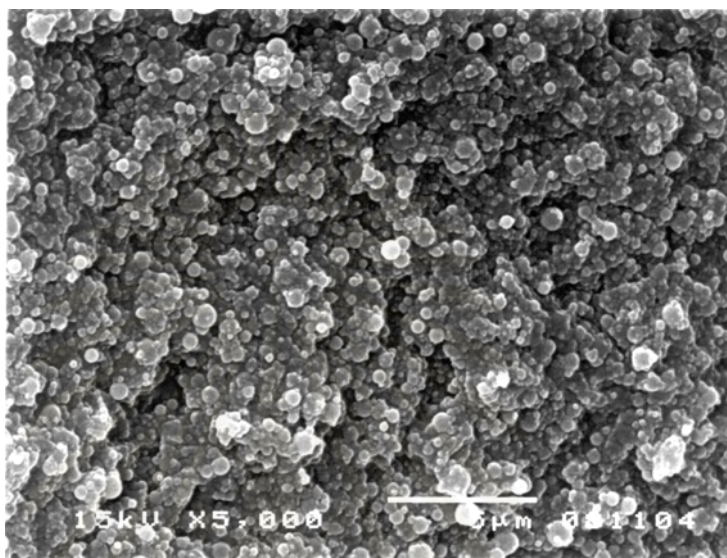


Figure 3.18 SEM micrograph surface of the chitosan blend with DTE-20% DT film by using EDCI as a coupling agent (x 5,000)

Characterization of thermal properties

Thermal properties of reaction between DTE-20% DT and chitosan was characterized by differential scanning calorimeter (DSC 200 NETZSCH thermische analyse). It was found that chitosan did not have glass transition temperature (T_g). Because it have inter and intramolecular hydrogen bonding by $-NH_2$ and $-OH$. If it obtains heat at 320 °C. It will degrade. On the other hand, DTE-20% DT had a T_g at 103.5 °C. The resulting polymer from the reaction between DTE-20% DT and chitosan (Figure 3.17) had a T_g at 65.6 °C, was much lower than the polycarbonate homopolymer. The decrease of T_g was caused by the presence of chitosan mixed with DTE-20% DT. It could also imply that there was only a few linkages between both polymers, otherwise the T_g should increased higher if they were highly crosslinked.

3.3 Chitosan coated on DTE-20% DT

After DTE-20% DT film was suspended in the chitosan solution at room temperature (RT) for 24 h and rinsed with distilled water. The coated chitosan layer expanded and detached from DTE-20% DT film. ATR spectrum showed only signals that belonged to DTE-20% DT (Figure 3.19) at 1770, 1738 and 1650 cm^{-1} , assigned to C=O stretching of the carbonate, the carboxylic acid groups and the secondary amides, respectively. No characteristic peak of chitosan (1589 and 1650 cm^{-1}) was observed. A coupling agent, EDCI, was then used to link the two polymer layers together, as discussed in the next section.

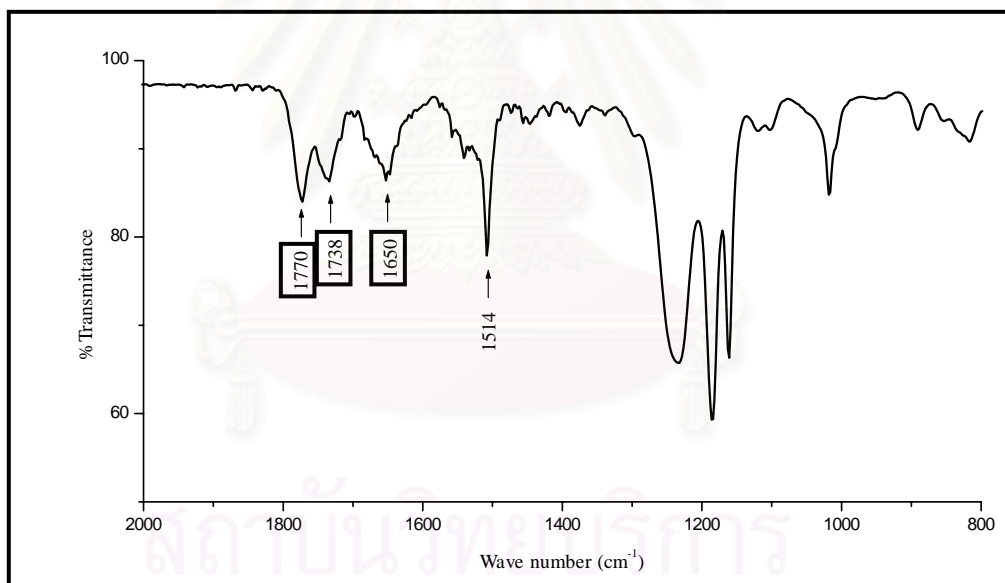


Figure 3.19 ATR-IR spectrum of chitosan coated on DTE-20% DT

Chitosan coated on DTE-20% DT film with EDCI-coupling agent using method I

ATR spectrum showed peaks of chitosan (Figure 3.20) at 1589 and 1650 cm^{-1} , assigned to N-H bending vibrations of $-\text{NH}_2$ and C=O stretching of secondary amides, respectively. The signals at 1770, 1730 and 1650 cm^{-1} which belong to DTE-20% DT were not present in the spectrum.

Using SEM technique, visual observation of the coated film could be made. Most area of the coated film was smooth (Figure 3.21a). But in some area, rippled marks and loose pieces of polymer were present (Fig 3.21b). The rippled mark was also observed by naked eyes, and was caused by layer of chitosan on the DTE-20% DT films. The evidence from ATR-IR and SEM thus lead us to confirm the presence of chitosan on the DTE-20% DT films.

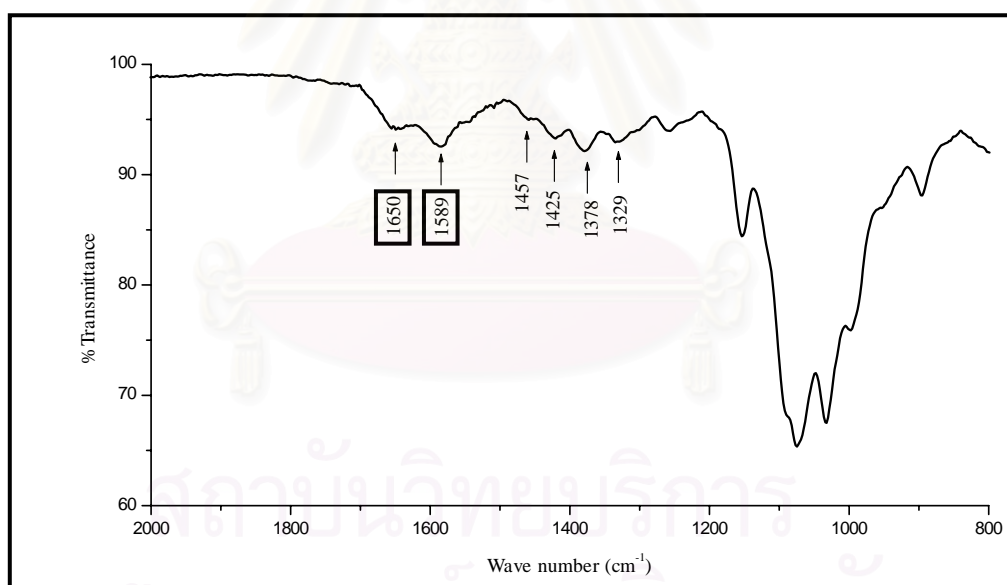


Figure 3.20 ATR-IR spectrum of chitosan coated on DTE-20% DT films with EDCI-coupling agent using method I

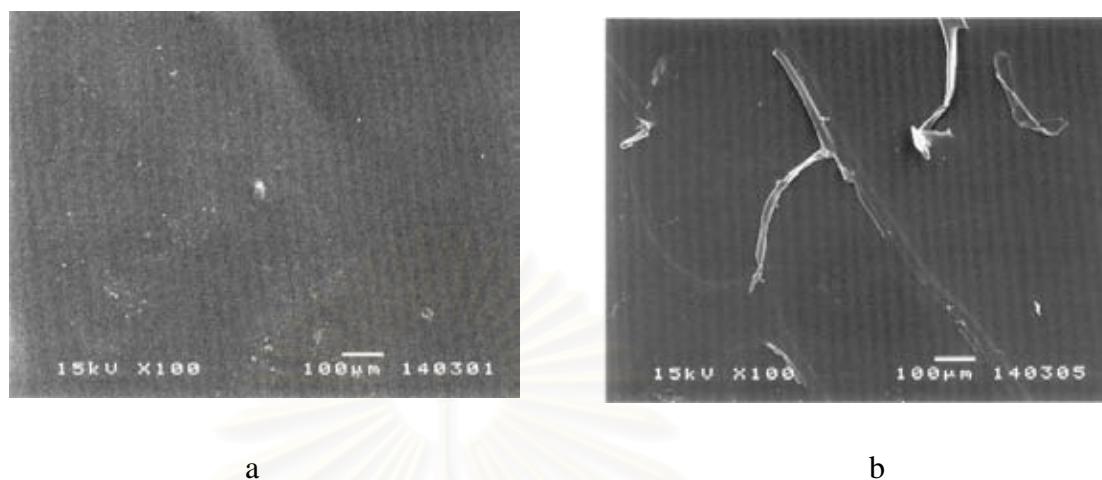


Figure 3.21 SEM micrograph surface of the chitosan coated on DTE-20% DT film by method I (x100)

Chitosan coated on DTE-20% DT film with EDCI-coupling agent using method II

The IR spectrum of coated films prepared by method II was shown in Figure 3.22. Signals from both DTE-20% DT and chitosan were present. Using SEM technique, It was found that pieces of chitosan spread around the film surface (Figure 3.23) These results suggested that the content of chitosan coated on the DTE-20% DT film was less than the sample prepared by method I.

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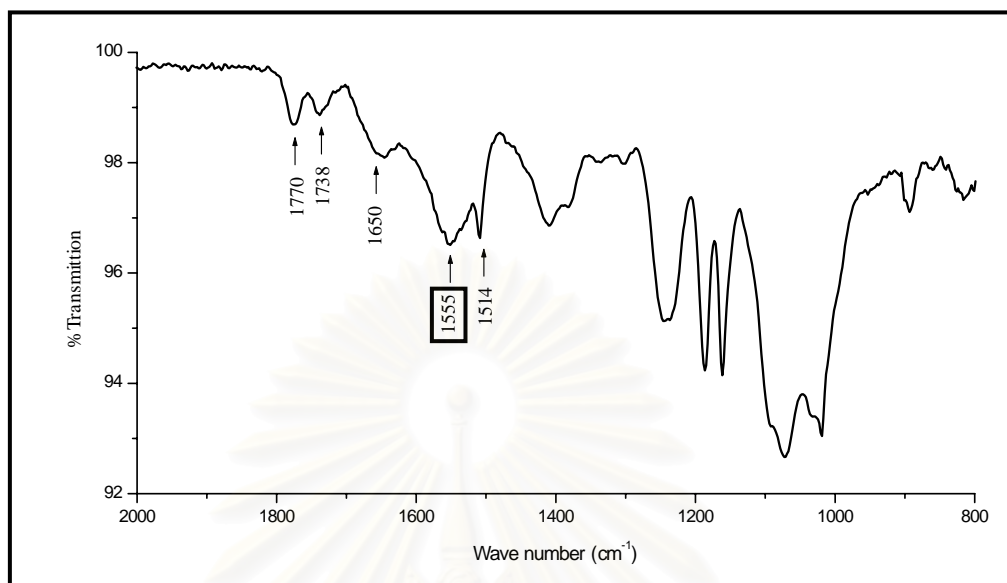


Figure 3.22 ATR-IR spectrum of chitosan coated on DTE-20% DT films by using EDCI as a coupling agent (method II)

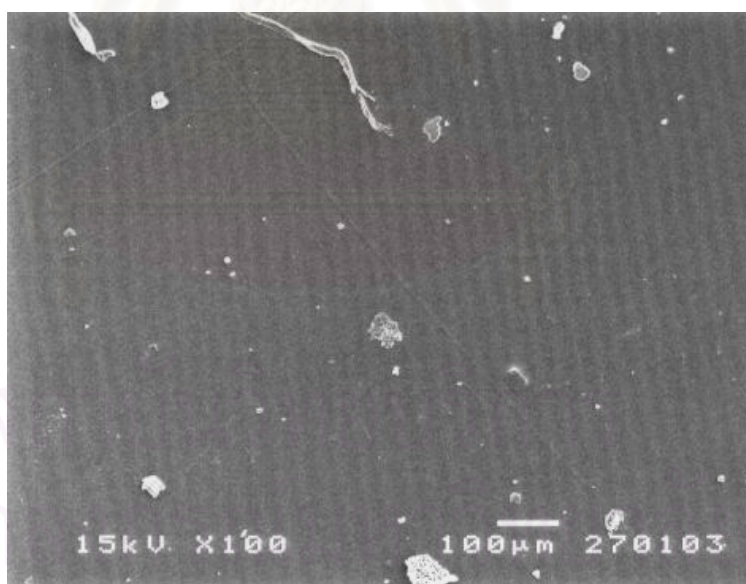


Figure 3.23 SEM micrograph surface of the chitosan coated on DTE-20% DT film by using EDCI as a coupling agent (method II)

Coating chitosan on polycarbonate film was also performed on DTE-50% DT films. Figure 3.24 reveals structure of DTE-50% DT. The IR spectra obtained (Figure 3.25 and 3.26) were similar to the DTE-20% DT films.

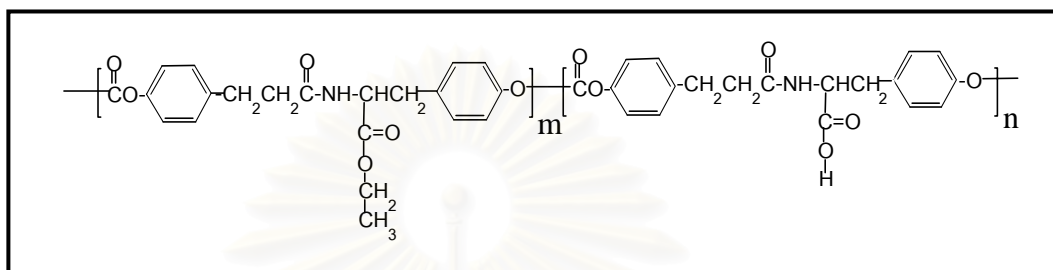


Figure 3.24 Structure of DTE-co-50% DT ($m = 50$, $n = 50$)

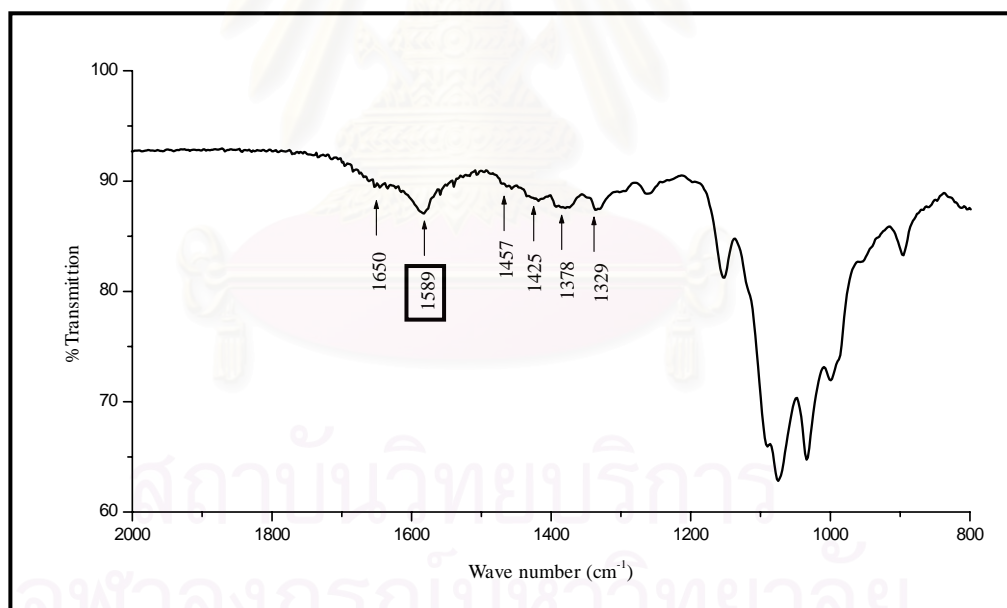


Figure 3.25 ATR-IR spectrum of chitosan coated on DTE-50% DT films by using EDCI as coupling agent by method I

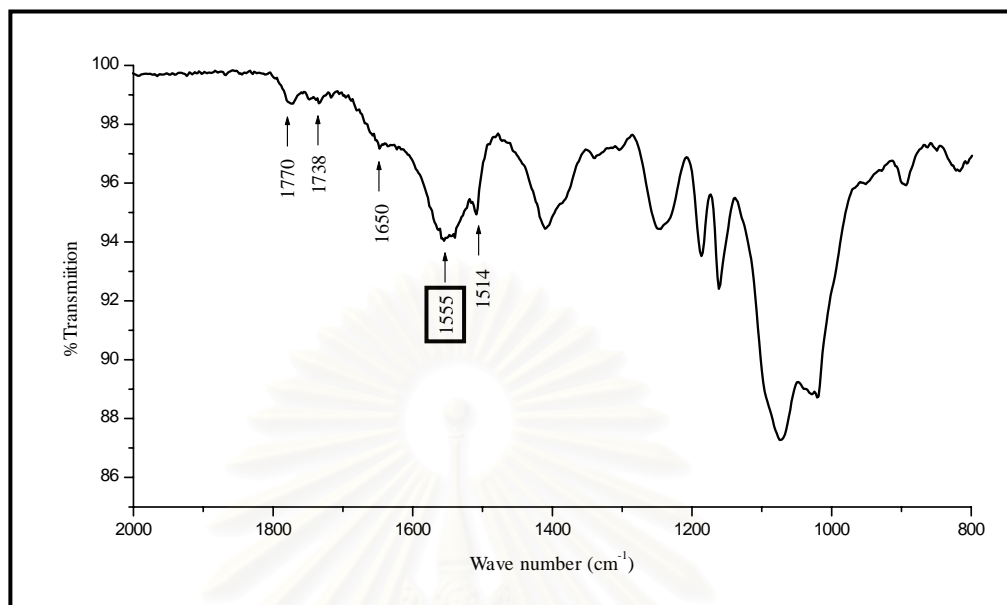


Figure 3.26 ATR-IR spectrum of chitosan coated on DTE-50% DT films by using EDCI as coupling agent by method II

3.4 Mechanical properties of polymer films by tensile strength

The mechanical properties of polymer films, were demonstrated in Table 3.3 and Figures 3.27 to 3.30. The profiles of % strain at break, stress at break (MPa), Young's modulus of elasticity (MPa) and toughness were evaluated. The results from DTE-20%DT, chitosan, polymer blends with EDCI and without EDCI, and then chitosan coated DTE-20%DT film using EDCI as a coupling agent by method I and II were compared.

Percent strain at break or elongation which is defined as distance at break related to the original distance of free film, represents the ductility of the polymer films. The % strain at break, stress at break, Young's modulus and toughness of DTE-20%DT film are higher than those of chitosan film. The blend with EDCI showed stronger film characteristics than blend without EDCI, except its toughness. However, the homopolymer films (DTE-20%DT or chitosan) possessed higher tensile strength than blend films. The DTE-20% DT film coated with chitosan by method I retained its tensile strength closely to the one prepared by method II, except

its Young's modulus. Moreover, the coated films (method I and II) possessed higher tensile strength than blend films.

Table 3.3. Mechanical properties of polymer films; data are expressed as means and SD in parentheses; all data were averaged from 5 repetitions (n = 5)

Sample film	Mechanical properties of polymer film			
	% Strain at break	Stress at break (MPa)	Young's Modulus (MPa)	Toughness
DTE-20%DT	14.27±5.98	31.35±10.12	1477±144.80	4.520±1.10
Chitosan	4.66±1.15	25.93±8.59	943±103.16	0.658±0.29
Blend with EDCI	1.761±0.83	0.7183±0.21	172.1±49.80	0.0090±0.00
Blend without EDCI	0.707±0.23	0.114±0.10	106.5±35.69	0.0253±0.01
Coated by method I	8.3114±2.08	10.922±6.44	740.4±97.68	1.09282±0.30
Coated by method II	8.09±2.46	14.45±3.77	1184.76±236.68	1.9858±1.21

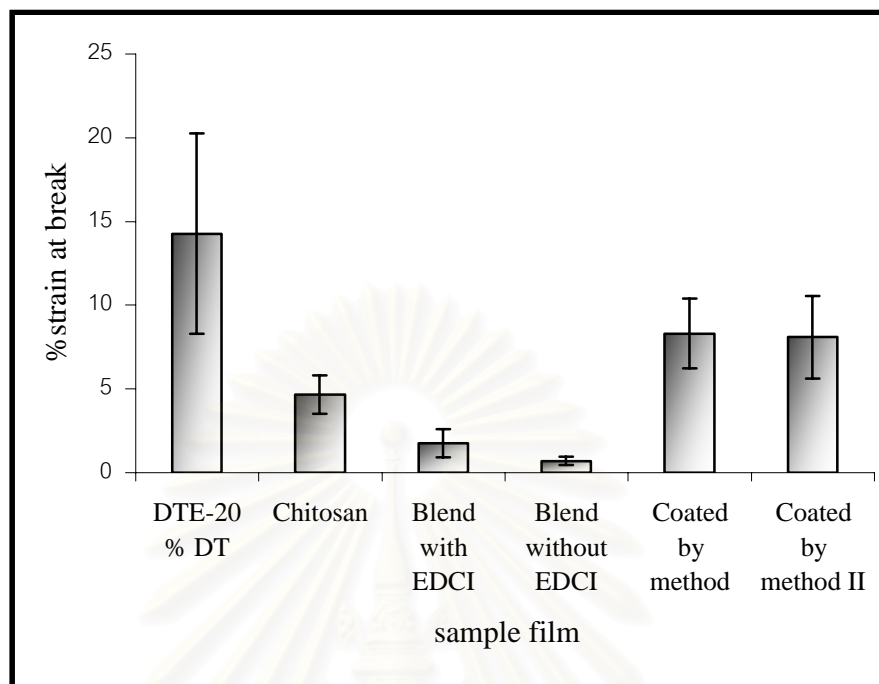


Figure 3.27 Show % strain at break of polymer films; data are expressed as means and SD in parentheses; n = 5

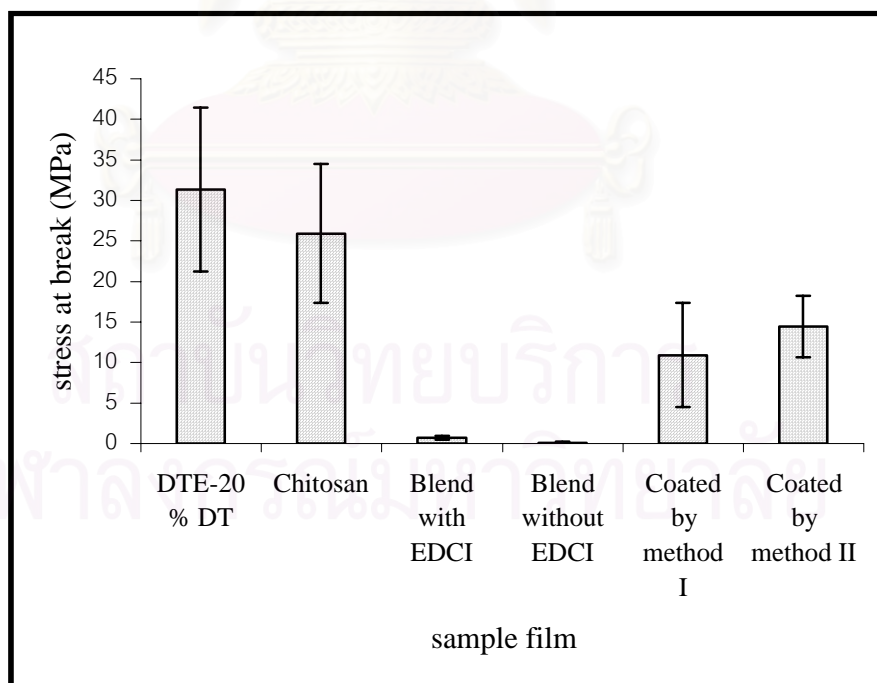


Figure 3.28 Show stress at break (MPa) of polymer films; data are expressed as means and SD in parentheses; n = 5

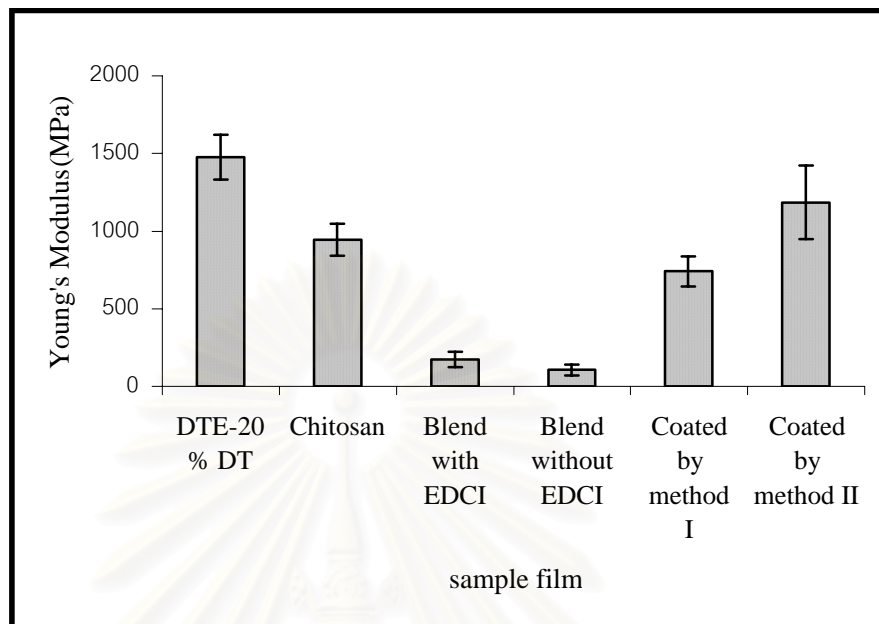


Figure 3.29 Show young's modulus (MPa) of polymer films; data are expressed as means and SD in parentheses; $n = 5$

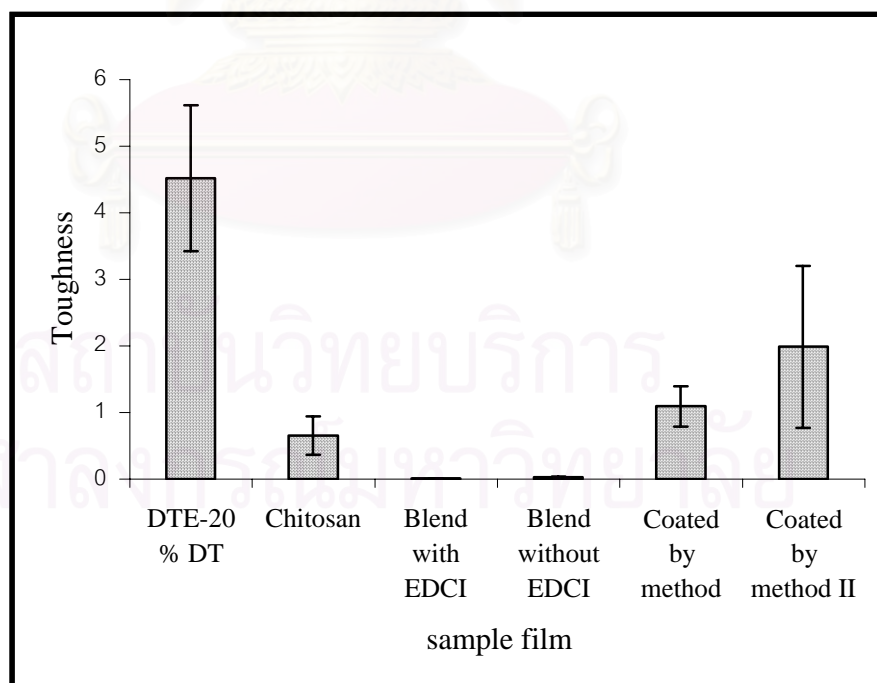


Figure 3.30 Show toughness (MPa) of polymer films; data are expressed as means and SD in parentheses; $n = 5$

3.5 Calcium adsorption study

Calcium ion was chosen for the metal adsorption study on the surface of polymer film. Table 3.4 and Figure 3.31 show the effect of incubation period on the adsorption of Ca^{2+} , by the chitosan films ($1 \times 1 \text{ cm}^2$). Initial pH of Ca^{2+} solution was 5.49. The amount of Ca^{2+} adsorbed increase with time and attained equilibrium after about 80 min. Therefore, the optimum incubation periods for adsorption of Ca^{2+} were about 80 min for chitosan films. Table 3.4 and Figure 3.32 reveal percentage weight increase of chitosan film ($1 \times 1 \text{ cm}^2$) after immerse in calcium solution 10 ppm.

Table 3.4 Calcium adsorption amount by the chitosan films ($1 \times 1 \text{ cm}^2$) (All data were averaged from 3 experiment sets)

<i>Time (min)</i>	<i>Amount of Ca^{2+} adsorbed^a (mg / $1 \times 1 \text{ cm}^2$ of chitosan film)</i>	<i>% adsorption^b</i>	<i>% weight increase of chitosan^c film</i>
20	0.0439±0.0049	43.9±0.49	5.85±0.16
40	0.0481±0.0012	48.1±0.12	9.27±0.12
60	0.0490±0.0049	49.0±0.49	15.20±0.28
80	0.0505±0.0072	50.5±0.72	15.37±0.25
1380	0.0506±0.0012	50.6±0.12	15.39±0.17

^aAmount of Ca^{2+} adsorbed on chitosan film ($1 \times 1 \text{ cm}^2$) was calculated according to

$$\text{Amount of } \text{Ca}^{2+} \text{ adsorbed} = (C - C_0)$$

^bThe percent adsorption of Ca^{2+} was calculated according to

$$\text{Percent of } \text{Ca}^{2+} \text{ adsorped} = \frac{(C_0 - C) \times 100}{C_0}$$

C_0 = the initial Ca^{2+} concentration (ppm).

C = the final Ca^{2+} concentration (ppm).

^cThe Percent weight increase of chitosan film was calculated according to

$$\text{Percent weight increase} = \frac{(W - W_0) \times 100}{W_0}$$

W_0 = the initial weight of chitosan film ($1 \times 1 \text{ cm}^2$)

W = the final weight of chitosan film ($1 \times 1 \text{ cm}^2$)

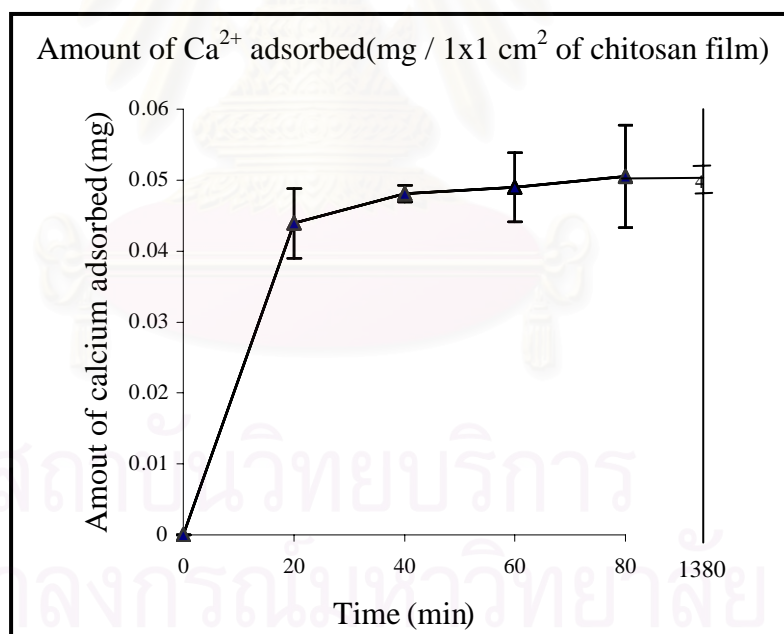


Figure 3.31 Effect of the agitation period for the amount of Ca^{2+} adsorbed by the chitosan films ($1 \times 1 \text{ cm}^2$) (All data were averaged from 3 experiment sets)

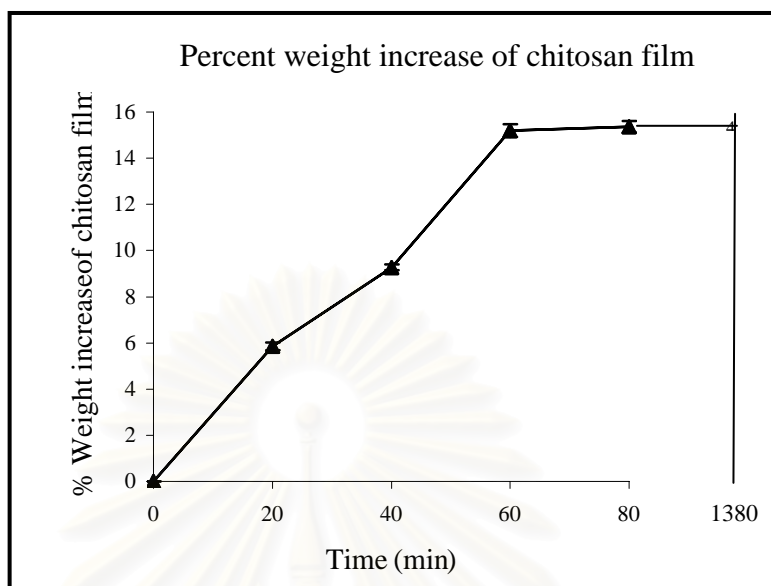


Figure 3.32 Percent weight increase of chitosan film (1x1 cm²) after immersion in calcium solution 10 ppm. (All data were averaged from 3 experiment sets)

The film (1x1 cm²) used for Ca²⁺ adsorption study was DTE-20% DT, blend of chitosan and DTE-20% DT, blend of chitosan and DTE-20% DT by using EDCI as a coupling agent, and chitosan coated on DTE-20% DT film using EDCI by method I and II.

From Table 3.5, it was found that DTE-20% DT film and the chitosan coated DTE-20% DT film with EDCI by method II did not adsorb any Ca²⁺. But the other samples adsorbed some Ca²⁺ to a detectable level. From these data, it seems that the presence of chitosan in the blend or as a coated layer could enhance the adsorption of Ca²⁺ from the solution.

Table 3.5 Amount of calcium adsorption by the polymer film (1x1 cm²) at 80 min

(All data were averaged from 3 experiment sets)

<i>Samples</i>	<i>Amount of Ca²⁺ adsorbed (mg / (1x1 cm²) polymer film)</i>	<i>% adsorption</i>
DTE-20% DT	0	0
chitosan	0.0505±0.0072	50.5±0.72
Blend without EDCI	0.0332±0.00263	33.2±2.63
Blend with EDCI	0.0235±0.00237	23.5±2.37
Coated by method I	0.0239±0.00204	23.9±2.04
Coated by method II	0	0

CHAPTER IV

CONCLUSIONS

The work has focused on the use of chitosan in combination with tyrosine-derived polycarbonate, a new class of biodegradable polymers. Since it was the first time, chitosan was introduced into this polymer class, various preparation methods of these polymer mixtures were evaluated. Initially, PAA was used as a model polymer for DTE-20% DT to study the interaction between –COOH group of PAA and –NH₂ group of chitosan. By using FT-IR, signals for carboxylate and ammonium ion were found at 1404 and 1630 cm⁻¹ indicating an electrostatic interaction between the two polymers. Nevertheless, the electrostatic interaction in the physical blend between chitosan and DTE-20% DT could not be identified. A coupling agent, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) was used to create amide linkages between the two polymers.

Coating of chitosan on DTE-20% DT did not yield tightly-bound chitosan layer. This problem was solved by using EDCI to create amide linkages. Analyses of the physical blend, blend with EDCI and chitosan coated DTE-20% DT with EDCI, were carried out by tensile testing, SEM and Ca²⁺ adsorption study. The blend films had a lower tensile characteristic than the original DTE-20% DT films did. It was also found that EDCI helped increasing the tensile strength of the blend possibly due to the formation of amide linkages between the two polymers. Furthermore, the DTE-20% DT film coated with chitosan using EDCI possessed higher tensile strength than blend films, but lower than that of the non-coated polycarbonate. In the Ca²⁺ adsorption study, it seems that the presence of chitosan in the blend or as a coated layer could enhance the adsorption of Ca²⁺ from aqueous solution.

Future work

In this work, the preparation of blends and coated polymers were introduced with the initial study on the Ca²⁺ adsorption by the polymers. The

Ca^{2+} adsorption could be the first step towards bone formation mechanisms in animals and humans. The next step should be a study on the activity of chitosan-polycarbonate blends and coated films on osteoblasts, a type of cells involving bone formation. This cell study can be performed *in vitro* in order to assess the possibility of using this polymer system for bone regeneration.



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