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PREPARATION OF COPOLYMER OF LOW MOLECULAR WEIGHT POLYLACTIC ACID AND CHITOSAN USING CROSSLINKING AGENTS

Miss Supaporn Iabhuay

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Petrochemistry and Polymer Science

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Thesis Title	PREPARATION OF COPOLYMER OF LOW MOLECULAR WEIGHT POLYLACTIC ACID AND CHITOSAN USING CROSSLINKING AGENTS
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สุภาภรณ์ เอี๊ยบฮวย : การเตรียมโคพอลิเมอร์ของพอลิแลกทิกแอซิดน้ำหนักโมเลกุลต่ำและไคโตซานโดยใช้สารเชื่อมขวาง (PREPARATION OF COPOLYMER OF LOW MOLECULAR WEIGHT POLYLACTIC ACID AND CHITOSAN USING CROSSLINKING AGENTS) อ.ที่ปรึกษา: รศ.ดร.พลกฤษณ์ แสงวณิช, อ.ที่ปรึกษาร่วม: ดร.ณัฏฐา ทองจุล, 107 หน้า.

พอลิเมอร์ที่สามารถย่อยสลายได้ทางชีวภาพ เป็นพอลิเมอร์จากธรรมชาติที่ผลิตได้จากวัตถุดิบที่สามารถสร้างขึ้นใหม่ได้ ปัจจุบันได้รับความสนใจมากขึ้นทั้งในด้านวิทยาศาสตร์และการค้า พอลิแลกทิกแอซิดเป็นหนึ่งในพอลิเมอร์ที่สามารถย่อยสลายได้ทางชีวภาพที่มีสมบัติต่างๆ เทียบได้กับพอลิเมอร์ที่ได้จากปิโตรเคมี เหมาะที่จะนำมาประยุกต์ใช้ในด้านต่างๆ แต่อย่างไรก็ตาม การสังเคราะห์พอลิแลกทิกแอซิดให้ได้น้ำหนักโมเลกุลสูงนั้นมีขั้นตอนที่ยุ่งยาก และเนื่องจากไคโตซานเป็นพอลิเมอร์ที่ได้จากธรรมชาติมีสมบัติในการเกิดฟิล์มได้ดี ซึ่งได้มาจากเปลือกกุ้งและเปลือกปู จึงถูกนำมาใช้รวมกับพอลิแลกทิกแอซิดน้ำหนักโมเลกุลต่ำ ดังนั้นในงานวิจัยนี้จึงเป็นการศึกษาสมบัติของฟิล์มโคพอลิเมอร์ระหว่างพอลิแลกทิกแอซิดน้ำหนักโมเลกุลต่ำและไคโตซานโดยใช้สารเชื่อมขวางเพื่อช่วยลดการบวมตัวในน้ำของฟิล์มโดยฟิล์มสามารถเตรียมได้ด้วยวิธีหล่อแบบจากสารละลาย พบว่ากลูทาราลดีไฮด์สามารถลดการบวมตัวของฟิล์มได้และเมื่อใช้ในปริมาณที่มากขึ้นจะให้ค่าการบวมตัวที่ลดลงแต่ให้ฟิล์มที่มีความเปราะมากขึ้น ฟิล์ม CLG3 ซึ่งเตรียมได้จากการกราฟต์ด้วยกรดแลกทิกไปบนไคโตซานโดยตรง จะมีค่าการบวมตัวต่ำสุด คือ 47.02 ± 0.46 % รวมถึงมีค่าความต้านทานแรงดึง ค่ามอดุลัสของยัง และค่าเปอร์เซ็นต์การยืดตัวก่อนขาด คือ 82.83 ± 0.87 MPa 3177.57 ± 18.11 MPa และ 4.88 ± 1.12 % ตามลำดับ ขณะที่ฟิล์ม CLG5-1.0%LPLA ซึ่งเตรียมได้จากการทำโคพอลิเมอไรเซชันของไคโตซานและพอลิแลกทิกแอซิดน้ำหนักโมเลกุลต่ำ จะมีค่าการบวมตัวต่ำสุด คือ 103.64 ± 1.36% รวมถึงมีค่าความต้านทานแรงดึง ค่ามอดุลัสของยัง และค่าเปอร์เซ็นต์การยืดตัวก่อนขาด คือ 99.46 ± 8.59 MPa 4146.31 ± 431.97 MPa และ 5.61 ± 1.04 % ตามลำดับ นอกจากนี้ยังพบว่ากลีเซอรอลมอนอสเตียเรตมีผลทำให้ค่าความต้านทานแรงดึง และค่ามอดุลัสของยังลดลง

สาขาวิชา.....ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์....ลายมือชื่อนิสิต .....สุภาภรณ์ เอี๊ยบฮวย.....

ปีการศึกษา .....2550.....ลายมือชื่ออาจารย์ที่ปรึกษา .....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

487 25236 23: MAJOR PETROCHEMISTRY AND POLYMER SCIENCE

KEYWORD: LOW MOLECULAR WEIGHT POLYLACTIC ACID / CHITOSAN / LACTIC ACID / FILM FORMATION / CROSSLINKING AGENT

SUPAPORN IABHUAY: PREPARATION OF COPOLYMER OF LOW MOLECULAR WEIGHT POLYLACTIC ACID AND CHITOSAN USING CROSSLINKING AGENTS. THESIS ADVISOR: ASSOC.PROF. POLKIT SANGVANICH, Ph.D., THESIS COADVISOR: NUTTHA THONGCHUL, Ph.D., 107 pp.

Biodegradable polymers derived from renewable resources considered as natural polymeric materials have now become increasingly scientific and commercial interest. Polylactic acid is one of such biobased polymer that exhibits many properties equivalent to or better than many petroleum-based plastics; therefore, it is suitable for a variety of applications. However, the synthesis of PLA is somewhat cumbersome. Due to its high film forming property, chitosan, a natural polymer derived from crab and shrimp shell, was used to combine with low molecular weight polylactic acid (LPLA). In this research, we studied the properties of copolymer films between LPLA and chitosan with the help of crosslinking agent and the copolymer film was prepared by solution casting technique. Glutaraldehyde (GA) could effectively reduce the swelling capacity of the copolymer films, the increased GA concentration reduced the swelling capacity but increased the brittleness of copolymer films. The film prepared by grafting lactic acid on chitosan , so called CLG3 film exhibited the lowest swelling capacity of 47.02 ± 0.46 % as well as tensile strength (MPa), Young's modulus (MPa), and % elongation at break value at 82.83 ± 0.87 MPa, 3177.57 ± 18.11 MPa, and 4.88 ± 1.12 %, respectively. While CLG5-1.0%LPLA film prepared by copolymerization of chitosan and LPLA showed the lowest swelling capacity of 103.64 ± 1.36% as well as tensile strength (MPa), Young's modulus (MPa), and % elongation at break value at 99.46 ± 8.59 MPa, 4146.31 ± 431.97 MPa, and 5.61 ± 1.04 %, respectively. Moreover, it was found that glycerol monostearate (GMS) affected the mechanical properties of the copolymer films, resulted in a decreased trend in the tensile strength, Young's modulus, and elongation at break.

Field of Study:..Petrochemistry and Polymer Science..Student's Signature:.....Supaporn Ia

Academic Year :.....2007.....

Advisor's Signature.....

Co-advisor's Signature:.....

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LIST OF ABBREVIATIONS AND SYMBOLS

PLA	Polylactic acid/polylatide
PHAs	Polyhydroxyalkanoates
PCL	Polycarpolactone
PGA	Polyglycolic acid
PET	Polyethylene terephthalate
CL	Chitosan-L-lactic acid
CA	Chitosan-acetic acid
UTM	Universal Testing Machine
DSC	Differential Scanning Calorimetry
XRD	X-ray diffraction
PHB	Polyhydroxybutyrate
T_g	Glass transition temperature
T_m	Melting temperature
^oC	Degree Celsius
GPC	Gel Permeation Chromatograph
NMR	Nuclear Magnetic Resonance Spectrometer
THF	Tetrahydrofuran
LA	L-lactic acid
CS	Chitosan
GA	Glutaraldehyde
SA	Succinic anhydride
MA	Maleic anhydride
Gly	Glycerol
PEG	Polyethylene glycol
GMS	Glycerol monostearate
ATR-IR	Attenuated Total Reflection Infrared Spectroscopy
[η]	Intrinsic viscosity
w/v	weight/volume

LPLA	Low molecular weight polylactic acid
PTSA	Toluene-4-sulfonic acid monohydrate
cm^2	square meter
rpm	round per minute
W	Swelling percentage
W_s	The weight of the saturated swollen
W_o	The weight of the dry
δ	Chemical shifts
$CDCl_3$	Chloroform-d
ppm	parts per million
TS	Tensile strength
Y	Young's modulus
%E	Percent elongation at break
%DD	% degree of deacetylation
M	Molar
aw	water activity
MW	Molecular weight
$\overline{M}_w$	Weight average molar mass
$\overline{M}_n$	Number average molar mass
PDI	Polydispersity
S.D.	Standard deviation
T_d	Thermal degradation
Tr	room temperature

CHAPTER I

INTRODUCTION

Nowadays, global environmental pollution or disruption is becoming a serious problem in the world. In the mean time the polymer industry has numerous problems regarding recycling or disposal of polymeric wastes. Therefore, material developments without an accompanying disruption of the global environment are getting much more important even in the polymer industry. Among them, the natural polymers have undergone a reevaluation regarding their ability to biodegrade.

Biodegradable polymers derived from renewable resources that are natural polymeric materials have become increasingly scientific and commercial interest due to their natural abundance and low costs. Nowadays, biodegradable polymers are used to replace petroleum-based raw materials. There are many biodegradable polymers such as polylactic acid (PLA), polyhydroxyalkanoates (PHAs), polycarpolactone (PCL) and polyglycolic acid (PGA).

Polylactic acid (PLA) is an aliphatic polyester derived from lactic acid, which can be produced from renewable resource such as starch via fermentation process (Garlotta, 2001). The chemical structure of PLA is shown in Figure 1.1. It is a thermoplastic, high strength, high modulus polymer and is considered biodegradable and compostable (Sebastien *et al*., 2006). Polylactic acid exhibits many properties that are equivalent to or better than many petroleum-based plastics, which make it suitable for various applications. PLA is clear and naturally glossy like the polystyrene. It is water and lipid resistant. It has flavor and odor barrier characteristics similar to polyethylene terephthalate (PET) used for soft drinks and many other food products. Tensile strength and modulus of elasticity of PLA are similar to than of PS (Dana, 2005). Not only used in packaging, PLA is also widely used in biomedical applications, such as absorbable sutures, sustained drug delivery systems, implants for orthopedic devices and absorbable fibers (Liu *et al*., 2004). The major route to convert lactic acid to high molecular weight polymers is ring-opening polymerization of lactide. Synthesis of high molecular weight PLA is time consuming and cumbersome.

It consists of 3 steps, i.e., polycondensation of lactic acid, ring formation and ring opening polymerization. To reduce the synthesis time, low molecular weight PLA (LPLA) is blended with other polymers to obtain the similar or better properties.

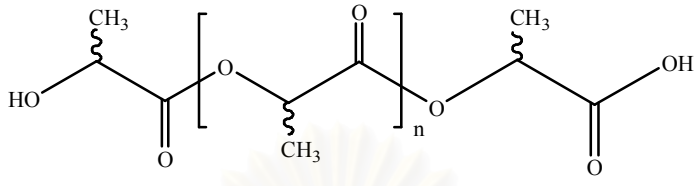


Figure 1.1 The chemical structure of PLA.

Chitosan a natural, nontoxic, edible and biodegradable polymer, derived by deacetylation of chitin (poly-*N*-acetyl-glucosamine), is the second most abundant biopolymer in nature after cellulose (Suyatma *et al*., 2004). The chemical structure of chitosan is shown Figture 1.2. In general, chitin presents in the exoskeleton of arthropods such as insects, crabs, shrimps and certain fungal cell walls (Suyatma *et al*., 2004). At present, several interesting biological properties have been reported for chitosan, such as wound healing, immunological activity and antibacterial effects. Moreover, it has a potential application as a packaging polymer and more particularly, as an edible packaging or coating because of its high film forming capacity. Chitosan film has good oxygen and carbon dioxide permeability, which is lower than that of polyethylene film and good mechanical properties. These properties are comparable with those of many medium-strength commercial polymers (Suyatma *et al*., 2004). However, chitosan films have a poor tensile strength and elasticity due to their brittleness (Wittaya-areekul *et al*., 2006). So, modification of chitosan film with other polymer is necessary to achieve films with better strength and elasticity.

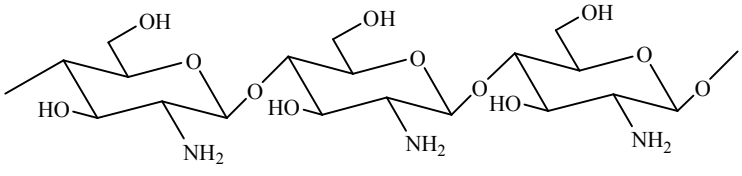


Figure 1.2 The chemical structure of chitosan.

In previous research, graft copolymer of L-lactic acid on chitosan was synthesized without using a catalyst (Yao *et al.*, 2003). In aqueous solution, the CL graft copolymer films could form a pH-sensitive hydrogel due to the aggregation of the hydrophobic side chains, containing swelling and mechanical properties for the packaging application such as packaging. Biodegradable film blends of chitosan and PLA prepared by the solution mixing and film casting (Suyatma *et al.*, 2004), indicated their incompatility to each other. It was found that the miscibility between their molecules is a very significant factor especially for mechanical properties.

Crosslinking is the process of chemically joining two or more molecules by a covalent bond, and a device for modification of polymer properties, such as swelling, strength and solubility. It can help reduce swelling and solubility, and increase mechanical properties of copolymer films. So in this research, use of crosslinking agent to improve copolymer films properties was studied. Crosslinking agents used included glutaraldehyde, succinic anhydride and maleic anhydride.

1.1. Objectives

1. Preparation of film of low molecular weight PLA-chitosan copolymer using crosslinking agent
2. Investigation on physical and mechanical properties of film

1.2. Scope of research

1. Synthesis of low molecular weight PLA-chitosan copolymer using crosslinking agent
2. Preparation of copolymer film by solution casting technique
3. Determination of film properties by attenuated total reflection infrared (ATR-IR) spectroscopy, universal testing machine (UTM), differential scanning calorimetry (DSC) and X-ray diffraction (XRD)
4. Investigation of swelling and solubility properties of copolymer film

CHAPTER II

THEORY AND LITERATURE REVIEWS

2.1 Biodegradation

Nowadays, environmental harm from the production and increased disposal of non-degradable plastic is becoming a serious worldwide problem. So the polymer industry has numerous problems regarding for recycling or disposal of polymeric wastes at present. Environmental concerns have a trigger to develop a polymer without an accompanying disruption of environment. These polymers are called "biodegradable polymer", which derived from a natural resource.

Biodegradation is the process by which organic substances are broken down by other living organisms. Organic material can be degraded into carbon dioxide (CO_2), water and inorganic compounds under aerobically, with oxygen, or into methane, water and inorganic compounds under anaerobically, without oxygen in which the predominant mechanism is the enzymatic action of microorganisms (Krupp *et al*., 1992). Biodegradability can be measured by standardized tests, in a specific period of time, reflecting available disposal conditions. However, many polymers that are claimed to be biodegradable are in fact bioerodable, hydro-biodegradable, or photo-biodegradable. Biodegradable polymers are generally obtained via polymerization of bio-based raw materials, which these raw materials are either isolated from plants and animals or synthesized through modern industrial processes. Biodegradable polymers can be either natural or synthetic. In general, synthetic polymers offer greater advantages than natural materials in that they can be tailored to give a wider range of properties than materials from natural sources. Not all of the biodegradable materials are suitable for production of biodegradable films. Even though there are rapid developments in the manufacturing and marketing areas of biodegradable materials, at least three major commercially available biobased degradable polymers groups from which biodegradable films may be produced can be identified within the main categories (apart from cellulose-based films): starch-based polymers, polyhydroxybutyrate (PHB) polymers, and polylactides (PLA). In the

category of nonbiobased biodegradable polyesters from petrochemical feedstock, commercial films have been developed from biocopolyesters (e.g., Eastar) or from starch-PCL blends. Of course, other biodegradable materials have also been used for film production (e.g., blends of soy protein and biodegradable polyesters, etc.) (Briassoulis *et al*., 2004). Examples of biodegradable polymers are provided in Figure 2.1.

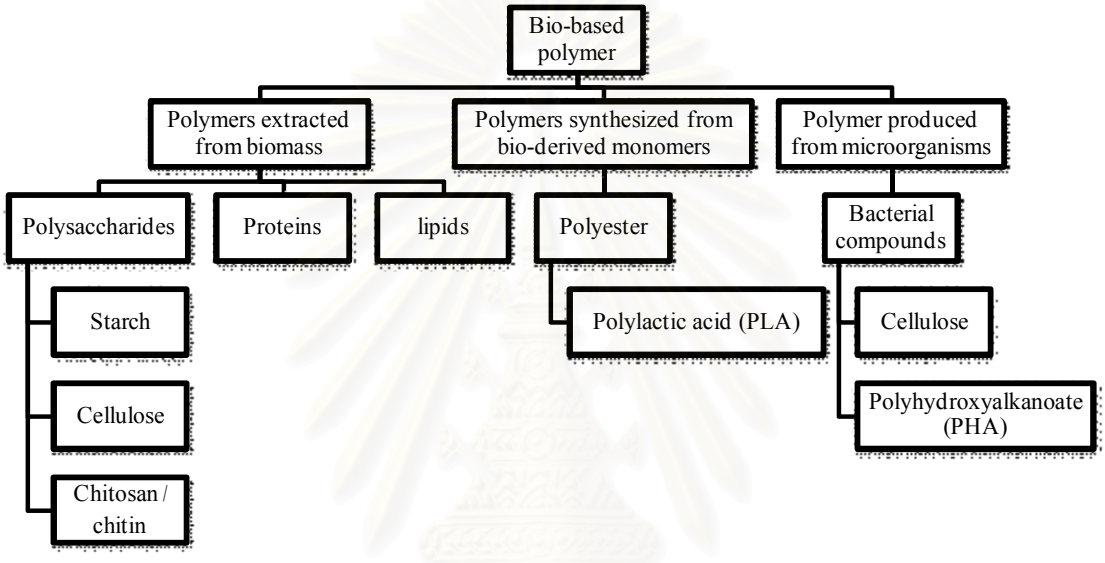


Figure 2.1 Examples of biodegradable polymers (Cutter, 2006).

2.2 Poly(L-lactic acid)

Polylactic acid (PLA) is considered biodegradable and compostable (Sebastien *et al*., 2006). It is an aliphatic polyester derived from lactic acid, which can be produced from either by carbohydrate fermentation or chemical synthesis, although fermentation predominates (Garlotta, 2001). Renewable resource such as corn, sugar beets, wheat and other starch-rich products is the starting material for lactic acid production (Dana, 2005). Polylactic acid consists of mainly lactyl units, of only one stereoisoform or combinations of D and L lactyl units in various ratios.

Lactic acid (2-hydroxypropanoic acid) is a carboxylic acid with a chemical formula of $C_3H_6O_3$: one terminal carbon atom is part of an acid or carboxyl group; the other terminal carbon atom is part of a methyl or hydrocarbon group; and a central

carbon atom having an alcohol carbon group. Lactic acid exists in two optically active isomeric forms. The L(+)-isomer is produced in humans and other mammals, whereas both the D(-)- and L(+)-enantiomers are produced in bacterial systems (Mirdamadi *et al*., 2002), shown in the Figure 2.2.

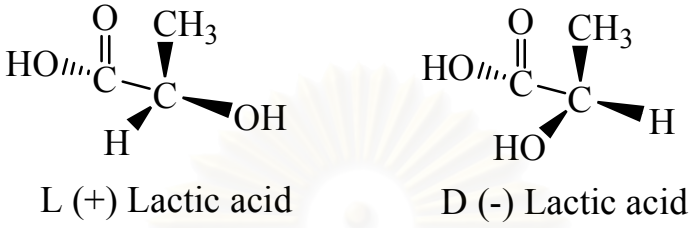


Figure 2.2 Formation of lactic acid isomer (Dorgan *et al*., 2000).

Lactic acid is soluble in water and water miscible organic solvents but insoluble in other organic solvents. It is non-volatile odorless and is classified as GRAS (generally regarded as safe) by FDA in the US. It is a very good preservative and pickling agent. Addition of lactic acid aqueous solution to the packaging of poultry and fish increases their shelf life (Narayanan *et al*., 2004).

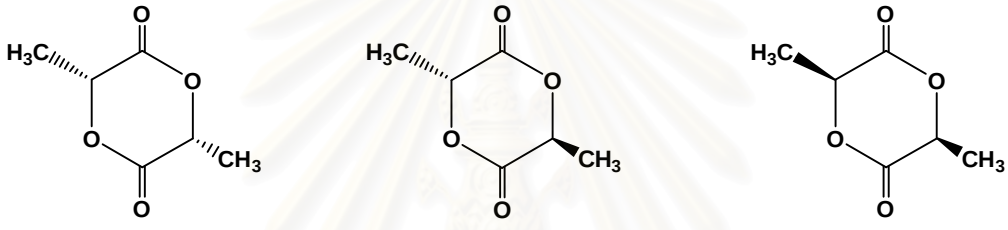
2.2.1 Synthesis of polylactic acid

There are many routes to synthesis of lactic acid into high-molecular-weight PLA: condensation/coupling, or ring-opening polymerization of lactide, which was developed by Cargill Inc. in 1992 and azeotropic dehydrative condensation (Ajioka *et al*., 1995; Garlotta, 2001), depicted in Scheme 2.1. The major route to convert lactic acid to high molecular weight polymers is ring-opening polymerization of lactide. Synthesis of high molecular weight polylactic acid composes of three processes.

The process starts with a polycondensation reaction of lactic acid to produce low molecular weight PLA prepolymer. The condensation polymerization is the least-expensive route, but it is difficult in a solvent-free system to obtain high molecular weight. A disadvantage of polycondensation is that a low-molecular-weight PLA obtained is a brittle, glassy polymer. The molecular weight of condensation polymerization is low due to the viscous polymer melt, the presence of water, impurities, the statistical absence (low concentration) of reactive end-groups

(Garlotta, 2001). Because the direct polycondensation route is an equilibrium reaction, difficulties removing trace amounts of water in the late stages of polymerization generally limit the ultimate molecular weight achievable by this approach (Drumright *et al*., 2000).

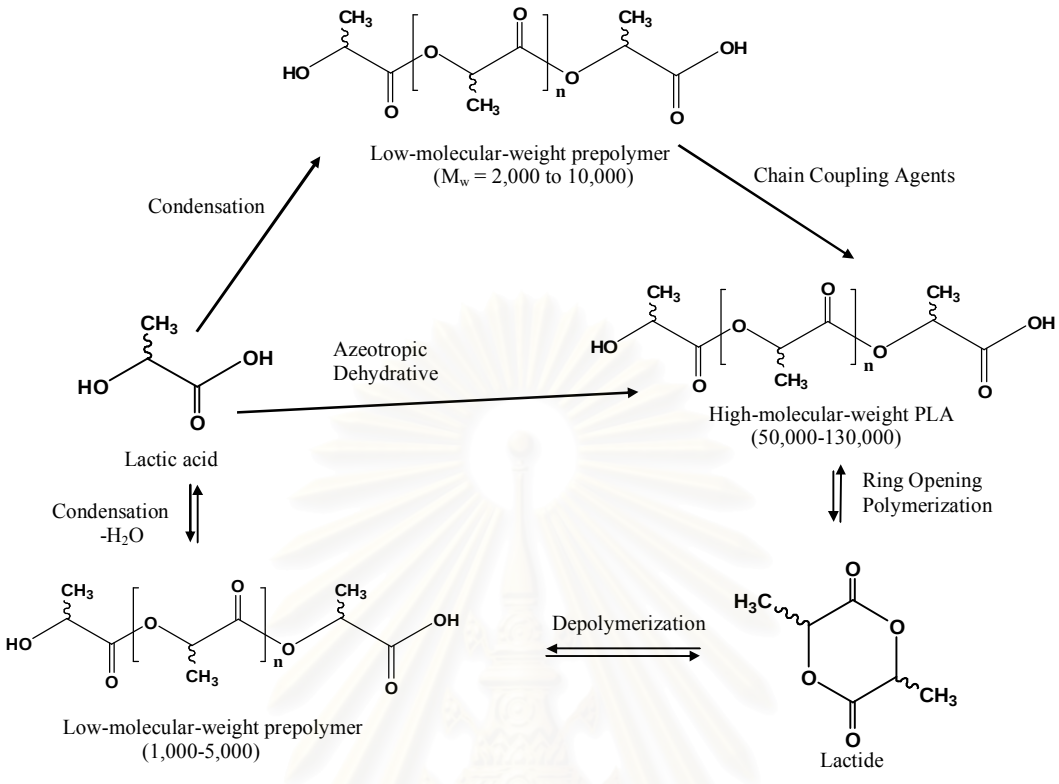
Then, LPLA is converted to lactide, a cyclic diester of lactic acid, by depolymerization under reduced pressure. A mixture of lactide stereoisomers including L-lactide, D-lactide, or *meso*-lactide, is shown in Figure 2.3. The different percentages of the lactide isomers formed depends on the lactic isomer feedstock, temperature and catalyst.



L-lactide *meso*-lactide D-lactide

Figure 2.3 Formation of lactide ring (Garlotta, 2001).

Finally, high molecular weight PLA is produced using a tin-catalyzed, ring-opening polymerization of lactide.



Scheme 2.1 Synthesis of polylactic acid (PLA) (Lunt, 1998; Garlotta, 2001).

2.2.2 Properties of polylactic acid

Properties of PLA, such as glass transition temperature, melting point, mechanical strength, and crystallinity, are determined by different proportions of L, D, or meso-lactide and the molecular mass determined by the proper addition of hydroxylic compounds. In addition, properties of PLA will also depend on compounding and processing conditions. In 1997, Hiltunen *et al.*, studied the effect of catalyst and polymerization conditions on the properties of the resulting low molecular weight lactic acid polymer, which was derived from condensation polymerization and the products were characterized by ^{13}C-NMR, DSC, GPC and titrimetric methods. The properties of low molecular weight lactic acid polyesters can be widely controlled by the use of different catalysts and polymerization temperatures. Resulting polyester can have 49.1% crystallinity. By choosing the catalyst and polymerization conditions, the crystalline behavior of the resulting polyesters can be varied from totally amorphous to highly crystalline. The weight-

average molecular weight can be controlled from 3600 g/mol to 32000 g/mol, and the glass transition temperatures from 34 to over 50 °C.

2.2.2.1 Thermal properties

PLA polymers range from amorphous glassy polymers with a glass transition temperature (T_g) typically between 55–65°C to semicrystalline/highly crystalline polymers with crystalline melting temperature (T_m) of PLA containing either the L- or D-isomeric form alone, between 130 to 180°C depending on its molecular weight (Lunt, 1998; Proikakis *et al*., 2006). It is a thermoplastic, and a stiff polymer at room temperature. The natural and biodegradable thermoplastics are, such as starch, polylactic acid (PLA), polyglycolic acid (PGA), polycarprolactone (PCL), polyhydroxyalkanoate (PHA) and polyhydroxybutyrate (PHB) (Dana, 2005). The glass transition temperature and the melting thermal transition is a change that takes place in a material when it is heated or cooled. Examples of thermal transitions include melting, crystallization, or the glass transition. T_g is a second-order transition, there is no transfer of heat, but the heat capacity does change. T_m is a first-order transition that is a transfer of heat between system and surroundings and the system undergoes an abrupt volume change.

- The glass transition temperature (T_g) : the glass transition temperature (T_g) is the temperature at which the amorphous phase of the polymer is converted between rubbery and glassy states, which the material changes its behavior from being 'glassy' to being 'rubbery'. If a material is at a temperature below its T_g, it behaves like a glass, generally hard, rigid, and brittle. Whereas, at a temperature above its T_g, it behaves like a rubber.

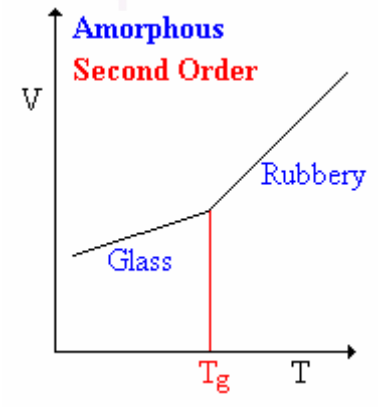


Figure 2.4 Illustrative plot of specific volume vs. temperature of T_g for amorphous polymer.

- **The melting temperature (T_m) :** the temperature at which a substance changes from solid to liquid state. It is a crystalline region property of polymer. If a material is at a temperature below T_m : ordered crystalline solid is observed and above T_m : disordered melt is obtained.

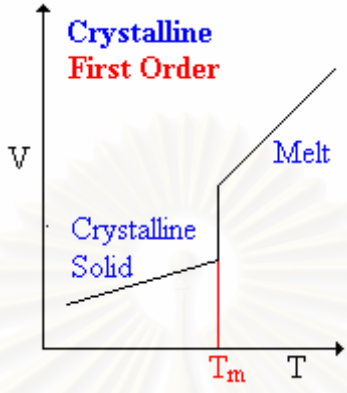


Figure 2.5 Illustrative plot of specific volume vs. temperature of T_m for crystalline polymer.

2.2.2.2 Mechanical and barrier properties of PLA films

The mechanical properties of PLA are dependent on the molecular weight and stereochemical makeup of the backbone. Low-molecular-weight PLA is a brittle, glassy polymer, which is the most unusable for any applications unless external coupling agents used to increase the molecular weight of the polymer (Chen *et al.*, 2006).

Polylactic acid is a thermoplastic, high strength, and high modulus polymer (Dana, 2005). It exhibits many properties that are equivalent to or better than many petroleum-based plastics, which makes it suitable for a variety of applications. Table 2.1, shows a comparison of the mechanical properties of PLA to those of other common commodity plastics. It is shown that PLA most closely resembles polystyrene (clear and naturally glossy) (Dorgan, 2000; Dana, 2005). PLA is water and lipid resistant. It has flavor and odor barrier characteristics similar to polyethylene terephthalate (PET) used for soft drinks and many other food products. Moreover, from the Table 2.1, PLA is characterized by a high modulus and low elongation at break. That is to say, PLA is a relatively brittle plastic but possesses good strength, which tensile strength and modulus of elasticity of PLA is also comparable to PET.

PLA can be formulated to be either rigid and flexible and can be copolymerized with other materials.

Table2.1 Comparision of PLA properties with other important thermoplastics (Dana, 2005)

Property	**PLA**	**PS**	**PVC**	**PP**
Yield strength (MPa)	49	49	35	35
Elongation (%)	2.5	2.5	3.0	10.0
Tensile modulus (GPa)	3.2	3.4	2.6	1.4

PVC = poly (vinyl chloride), PP = polypropylene, PS = polystyrene

2.2.2.3 Solubility

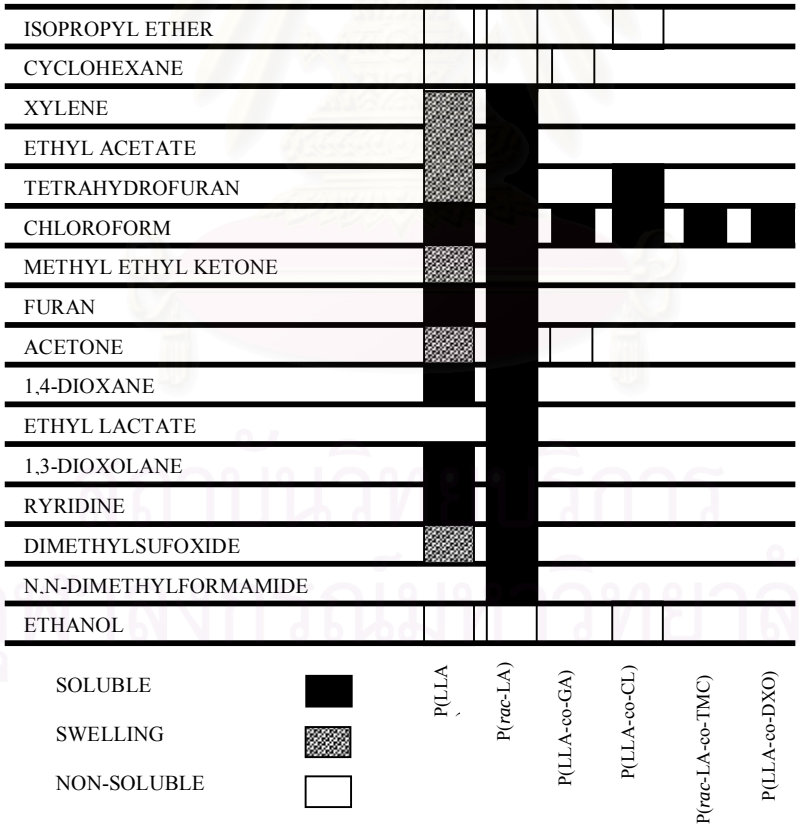


Figure 2.6 The solubility of lactic acid based polymer (Sodergard *et al*., 2002).

The solubility of lactic acid based polymers is highly dependent on the molar mass and degree of crystallinity present in the polymer. From solubility studies of polylactic acid, it is soluble in organic solvents, such as chloroform, 1,4-dioxane, benzene and tetrahydrofuran (THF), reported by Cargill. The organic solvents are used to dissolve amorphous and crystalline PLA such as chlorinated solvents, benzene at elevated temperature. PLA is insoluble in water, alcohols (e.g., methanol, ethanol, propylene glycol and hexane), are shown in Figure 2.6.

2.2.3 Degradation mechanism of polylactic acid

PLA is a potential material for degradable packaging applications. It is non-volatile and odorless and was classified as Generally Recognized As Safe (GRAS) by Food and Drug Administration (FDA). The degradation mechanism includes both chemical hydrolysis and biodegradation (Karjomaa *et al.*, 1998). The hydrolytic degradation of polylactic acid proceeds through random cleavage of the ester bond, which involved to racemization, are shown in Figure 2.7.

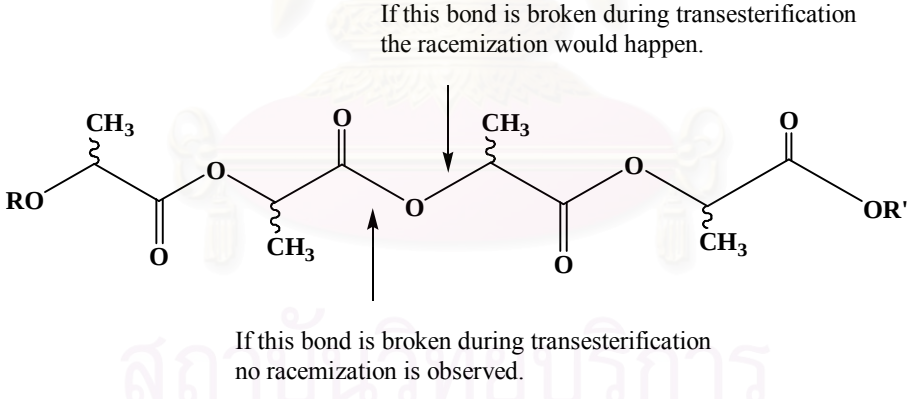
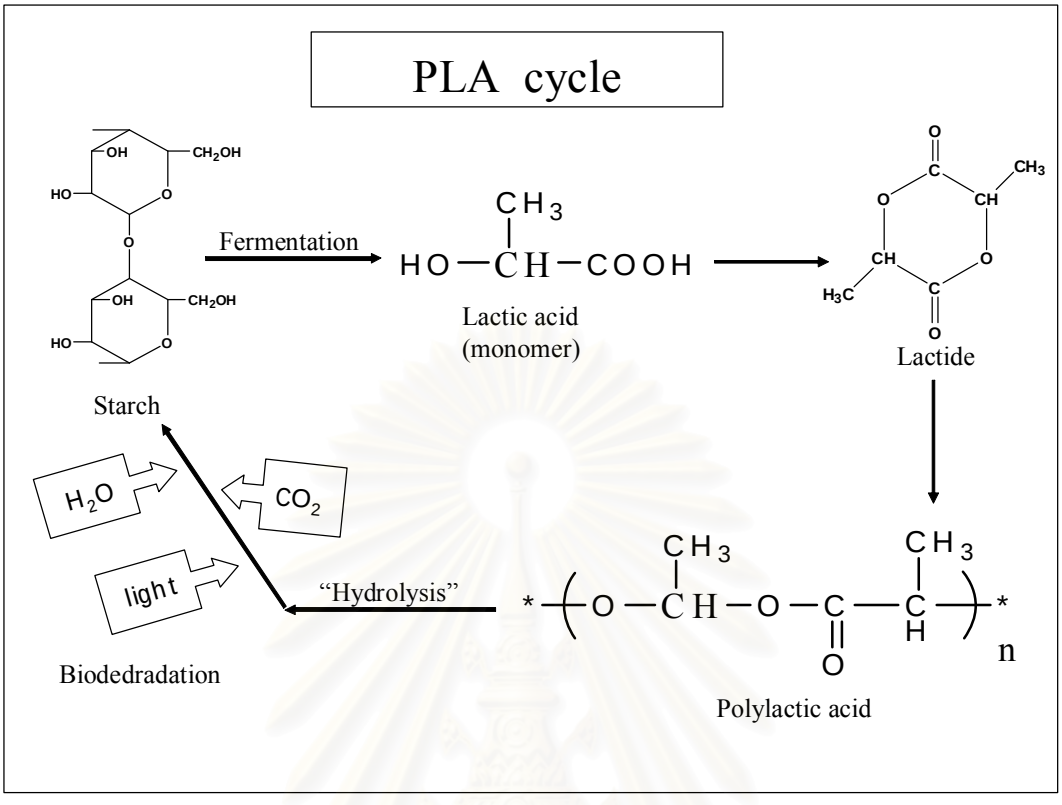


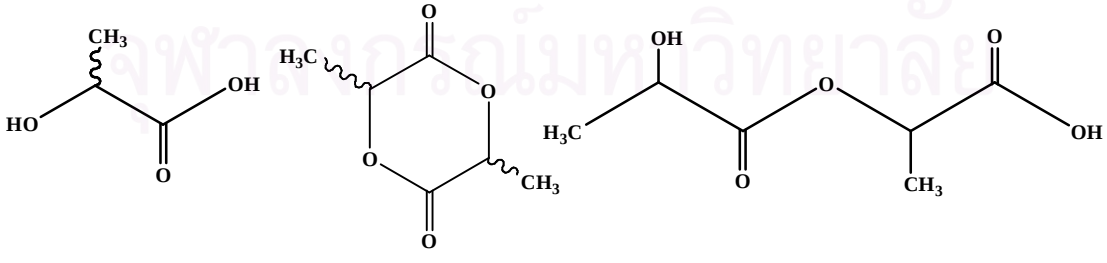
Figure 2.7 The hydrolytic degradation of polylactic acid proceeds through random cleavage of the ester bond (Hiltunen *et al.*, 1997).

PLA which is degraded by hydrolysis is controlled by many parameters such as the rate constant, the amount of absorbed water, the diffusion coefficient of chain fragments within the polymer and solubility of degradation products (Proikakis *et al.*, 2006). PLA can be broken down by other living organisms. The degradation products are carbon dioxide (CO_2), water and inorganic compounds under aerobic condition.



Scheme 2.2 The cycle of PLA in environment.

High molecular weight PLA is a hydrophobic polymer, its hydrophilicity increases with decreasing molecular weight due to chemical hydrolysis. Products from degradation of PLA including lactic acid, lactoyllactic acid (linear dimer of lactic acid), other small oligomers of PLA (trimer etc.), and lactide (cyclic dimmer of lactic acid and the monomer used to build the polymer) are shown in Figure 2.8 (Conn *et al.*, 1995).



lactic acid lactide lactoyllactic acid

Figure 2.8 Chemical structures of lactic acid, lactide, and lactoyllactic acid.

2.2.4 Application of polylactic acid

The unique physical characteristics that PLA possesses make it suitable for many different applications. PLA is resistant to moisture and grease that makes it suitable for food packaging. It is clear and glossy, used in “blister packs” for batteries and toys. As well as using for soft drinks because of it has flavor and odor barrier characteristics. Furthermore, the rheological characteristics of PLA make it suitable for specific manufacturing processes, such as sheet extrusion, film blowing, injection molding, thermoforming, film forming and fiber spinning using most conventional techniques and equipment (Drumright *et al*., 2000).

Application of PLA depending on molecular weight polylactic acid, poly L-lactic acid with low degree of polymerization can help in controlled release. Because of poly(lactic acid) is biocompatible and undergoes scission in the body, to monomeric units of lactic acid, which is a natural intermediate in carbohydrate metabolism. These characteristics make this polymer suitable for use in resorbable sutures, carriers for the controlled release of drugs, implants for orthopaedic surgery or blood vessels and tissue-engineering-scaffolds, which finally can be replaced by the body’s tissues. Morover, PLA is used as a degradable mulch films for large-scale agricultural applications (Datta *et al*., 1995). Mechanical properties that are directly affected by molecular weight of the polymer are more or less critical depending on the application. Such as using in controlled release does not require high strength and, therefore, low molecular weight material can be used. On the other hand, the construction of screws and plates for use as orthopaedic implants needs tough, high molecular weight material. As well as using packaging industry such as plastic cups, cling-film, food containers cutlery, plastic bottles and textile such as carpets, clothing, bedding.

2.3 Chitosan

Chitosan is a linear polysaccharide mainly composed of the β(1-4)-2-amino-2-deoxy-D-glucopyranose (D-glucosamine) repeating unit and includes a small amount (<20%) of *N*-acetyl-D-glucosamine (GlcNAc) residues (Hsu *et al*., 2004). It is the

second abundant biopolymer in nature after cellulose. Chitosan is a natural, nontoxic, edible and biodegradable polymer, derived by the deacetylation of chitin (poly-*N*-acetyl-glucosamine) (Suyatma *et al*., 2004). Chitin is found in the skeleton of arthropods such as insects, crabs, shrimps, lobsters, and certain fungal cell walls. In economically, chitosan is a byproduct of the crab and shrimp processing industries. The structure of chitin is so similar to cellulose because both are β-(1-4) linked. The difference between them is that chitin has an amide group instead of a hydroxyl group, which cellulose. Moreover, the structure of chitosan is also very similar to chitin. The difference is that chitosan has an amine group instead of an amide group. Figure 2.9, shows the comparative structures of cellulose, chitin, and chitosan.

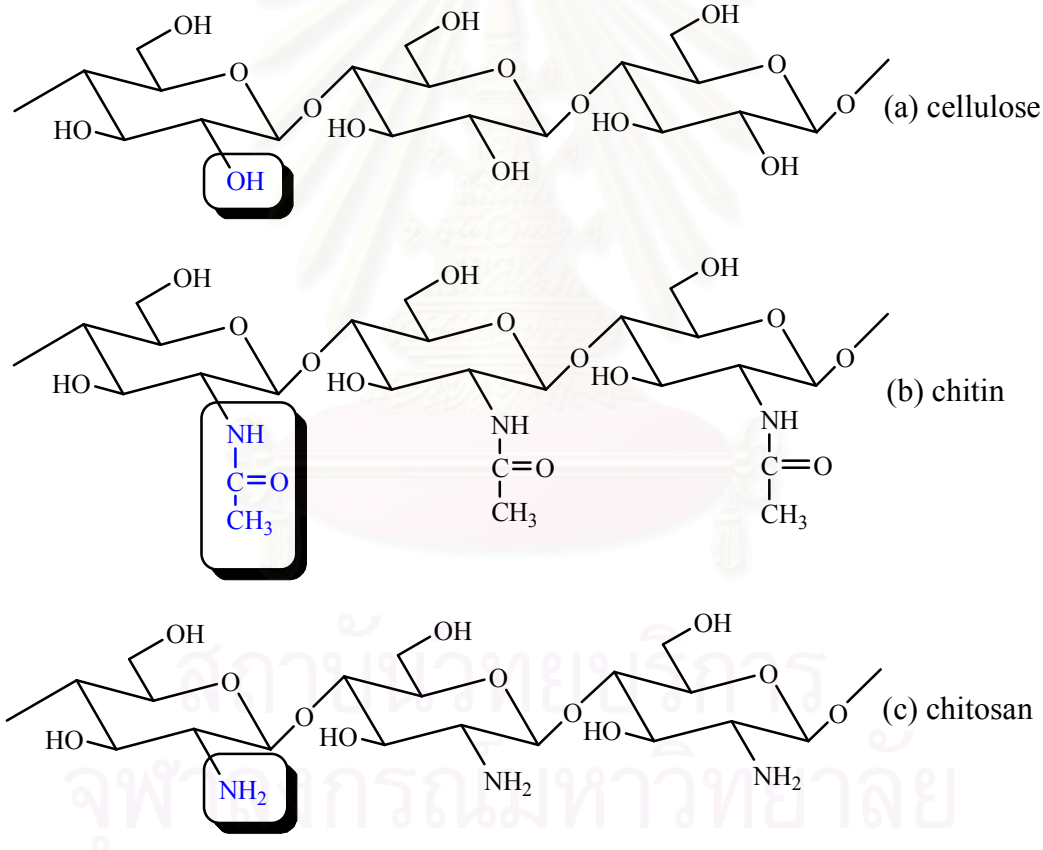


Figure 2.9 The chemical structures of: (a) cellulose, (b) chitin, and (c) chitosan**.**

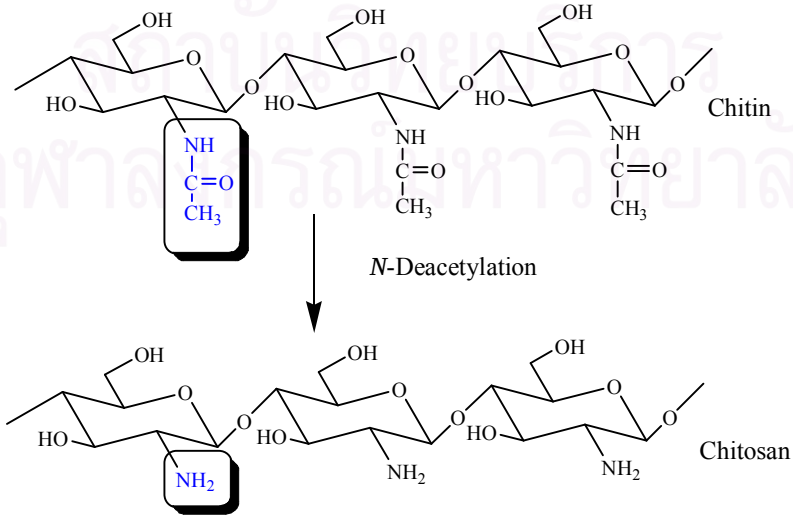
2.3.1 Physical and chemical characterization

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

2.3.1.1 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. 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 < 4-4.5%. Usually, chitosan has degree of deacetylation between 70% and 90%. Properties of chitosan are greatly affected by the degree of deacetylation. As a consequence of the influence of this parameter on the properties of chitosan, various techniques were used to determine the degree of deacetylation of chitosan such as IR, UV and NMR spectroscopy (Hirai *et al.*, 1991).

Since degree of deacetylation depends mainly on the method of purification and reaction conditions, therefore, many routes have been developed to increase the degree of deacetylation values. For example, increase either in temperature or strength of sodium hydroxide solution could enhance the removal of acetyl groups from chitin, resulting in a range of chitosan molecules with different properties and hence its application. Scheme 2.3, the process of deacetylation of chitin, which acetyl groups from the molecular chain of chitin are removed to form amino groups.



Scheme 2.3 The process of deacetylation of chitin.

2.3.1.2 Molecular weight

The random distribution of glucosamine to N-acetyl-glucosamine is often expressed as the degree of deacetylation. Chitosan molecular weight distributions can be determined by HPLC. The weight-average molecular weight (M_w) of chitin and chitosan can also be determined by light scattering.

Viscometry is a simple and rapid method for the determination of molecular weight (Gupta *et al.*, 2006); the constants α and K in the Mark-Houwink equation have been determined in 0.1M acetic acid and 0.2M sodium chloride solution. The intrinsic viscosity is expressed as:

$$[\eta] = KM^{\alpha} \qquad ; [\eta] = \text{intrinsic viscosity}$$

$$\alpha = 1.81 \times 10^{-3}$$

The charged nature of chitosan in acid solvents and chitosan's propensity to form aggregation complexes requires care when applying these constants. Furthermore, converting chitin into chitosan lower molecular weight, changes the degree of deacetylation, and thereby alters the charge contribution, which in turn influences the agglomeration. The weight-average molecular weight (M_w) of chitin is 1.03×10^6 to 2.5×10^6, but the deacetylation reaction reduces this to 1×10^5 to 5×10^5. The increase in the number of deacetylation step promotes an increase in the degree of deacetylation followed by a decrease in the average of molecular weight and polydispersion.

2.3.1.3 Solubility

Chitosan is a cationic polymer having a pKa value of ~6.5 and soluble in organic acid solution with pH less than 6. Its solution's stability is poor above pH 7 due to precipitation or gelation that takes place in alkali pH range (Qin *et al.*, 2006). It is insoluble in water, alcohol, base and organic solvents. Organic acids such as acetic, formic and lactic acids are used for dissolving chitosan, and the most commonly used solvent is 1% acetic acid solution. Its solubility depends on the presence of the free

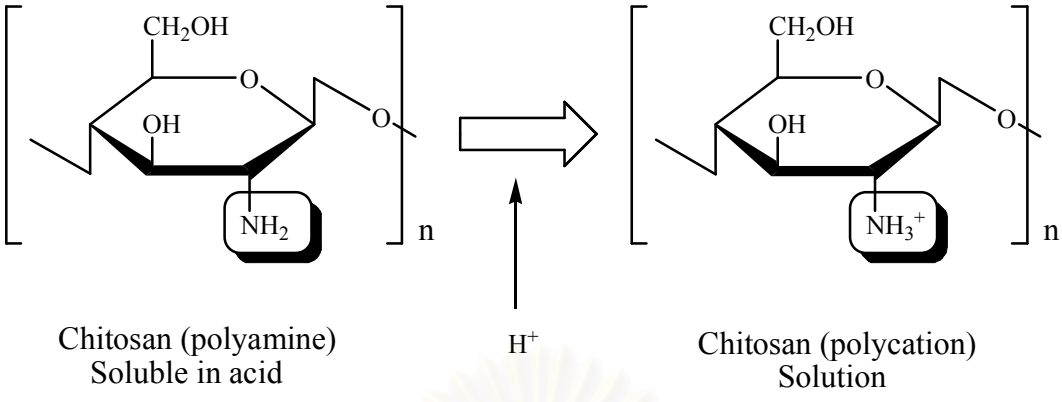
amino groups capable of being protonated by the acid medium. The exact degree of deacetylation required to render the solubility of polymer is not readily determined and undoubtedly varied to such factors as chitosan molecular weight, concentration, impurity and nature of the acid employed. Solubility of chitosan in inorganic acid solvent is quite limited. Chitosan is soluble in 1 % hydrochloric acid but insoluble in sulfuric and phosphoric acids. Most properties of chitosan are related to its polyelectrolyte and polymeric carbohydrate character. In acid solutions, amino groups of chitosan are protonated to $-NH_3^+$ leading to the cationic property as shown in Scheme 2.4. Since most other soluble biopolymers become anionic in water, chitosan cation exhibits good affinity for other biopolymers. Solubility of chitosan in different types of acid solutions is shown in Table 2.2.

Table 2.2 Solubility of chitosan in different concentration of various types of acid solutions

Acid types	**Concentration of acid solution (v/v)**				
	1%	**5%**	**10%**	**50%**	**>50%**
Acetic	+	+	+	+	
Adipic	+				
Citric	-				
Formic	+	+	+	+	+
Lactic	+	+	+		
Malic	+	+	+		
Tartaric	-		+		
Hydrochloric	+	-	-		
Nitric	+	-	-		
Phosphoric	-	-	-		
Sulfuric	-	-	-		

Note: + Soluble

- Insoluble
- Insoluble in sulfuric acid solution and phosphoric acid solution at concentration higher than 0.5%



Scheme 2.4 Cationic property of chitosan (Jun, 2000).

The physicochemical properties of solutions of chitosan are expected to be governed by many factors, such as temperature, degrees of deacetylation, pH, ionic strength, and surfactant concentration. 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.

Chitosan has been considered as a potential polysaccharide resource. Many efforts have been reported to prepare functional derivatives of chitosan by chemical modifications, which lead to improve its solubility in general organic solvents. Because the nitrogen content in chitin varies from 5-8% depending on the extent of deacetylation, whereas, the nitrogen in chitosan is mostly in the form of primary aliphatic amino groups. Chitosan, therefore, undergoes the reactions typical to amines, of which N-acylation and Schiff reaction are the most important. Chitosan derivatives are easily obtain under mild conditions and can be considered as substituted glucans N-acylation with acid anhydrides or acyl halides introduces amino groups at the chitosan nitrogen.

2.3.2 Properties of chitosan films

Preparation of chitosan films by using the different organic acid solution, films have different properties too. It is shown in Table 2.3.

Table 2.3 Film-forming ability of unplasticized crawfish chitosan with different organic acids[a] (Nadarajah, 2005)

Film casting solvent[b]	Film properties
Acetic acid	A yellow tinted, flexible, transparent, non-sticky film with smooth shiny surface and slight acidic odor
Ascorbic acid	A brown colored, highly brittle film
Citric acid	A yellowish, flexible, transparent, non-sticky film with slight brittle and grainier surface without any acidic odor
Formic acid	A yellow tinted, flexible, transparent, non-sticky film with smooth shiny surface without any acidic odor
Lactic acid	Highly sticky films which shrink upon peeling, becoming a sticky mass/clump
Malic acid	Highly sticky films which shrink upon peeling, becoming a sticky mass/clump

[a] 1% chitosan in film casting solvent by w/v, [b] 1% acid by w/v

Chitosan film has good oxygen and carbon dioxide permeability, which is lower than that of polyethylene film and has a poor water vapor resistance. The oxygen permeability of the film was affected by type of acid and molecular weight of chitosan used. Generally, oxygen permeability value of low molecular weight chitosan film was lower than that of chitosan films with high molecular weight chitosan. The oxygen permeability values of chitosan film are comparable with commercial polyvinilidene chloride or ethylene vinyl alcohol copolymer films. Low oxygen permeability of chitosan films can be exploited for food, medical and packaging applications. It has good mechanical properties, which are comparable with those of many medium-strength commercial polymers (Suyatma *et al*., 2004).

Using for unmodified chitosan films are limited because of their high moisture permeability and brittleness. However, the properties of chitosan films can be modified to improve barrier and mechanical properties. Modification of chitosan can be made by synthesis of graft-copolymer of chitosan with other polymers (Liu *et al*.,

2004; Wu *et al*., 2005) or blending of chitosan and other polymers (Sakurai *et al*., 2000; Peeson *et al*., 2005; Park *et al*., 2001), which the miscibility between their molecules is a very significant factor especially for a mechanical property of the blend. Example of previous research of a chitosan blending was reported. Suyatma *et al*., (2004) prepared biodegradable film blends of chitosan with poly(lactic acid) (PLA) by solution mixing and film casting to improve the water vapor barrier of chitosan. Mechanical properties, thermal properties, barrier properties (water vapor permeability) and hydrophobicity were evaluated. The miscibility of a chitosan/PLA blend was discussed by analyzing the change of the thermal properties and mechanical properties of blends as a function of the blend ratio and was confirmed by FTIR analysis. The thermal and mechanical properties of chitosan/PLA blends reveal that a phase separation in the blend has occurred, indicating their incompatibility. The tensile strength and elastic modulus of chitosan decreased with the addition of PLA. Moreover, the FTIR analysis shows there is no specific interaction between chitosan and PLA. In spite of their incompatibility, the incorporation of PLA could improve the water vapor barrier and hydrophobicity of chitosan.

Begin *et al*., (1999) prepared the antimicrobial films by dissolving chitosan into hydrochloric, formic, acetic, lactic and citric acid solutions. Compatibility of the organic acids with chitosan solutions were estimated by viscosimetry at different temperatures and mechanical properties of the resulting films from their resistance to rupture during their elongation. The results indicate that film properties and the influence of the counter ion could not be predicted from solution viscosity measurements. The mechanical resistance of films to elongation was quite different depending on the counter ion. Films made from hydrochloric, formic and acetic acid were hard and brittle, whereas those from lactic and citric acids were soft and could be stretched.

2.3.3 Chitosan applications

At present, several interesting biological properties have been reported for chitosan alone, such as wound healing, immunological activity, and antibacterial effects. Chitosan has received great attention from those developing medical and pharmaceutical applications because of its beneficial intrinsic properties.

Khan *et al*., (2000) investigated the mechanical, bioadhesive strength, and water vapor permeability of chitosan films, prepared using two different solvents, acetic acid (Chitosan-AA) and lactic acid (Chitosan-LA). In addition, biological studies were conducted to investigate the skin irritation and systemic toxicity of the films for wound dressing. The results, Chitosan-LA exhibited a lower tensile strength, but more flexible and bioahesive than Chitosan-AA. Furthermore, Chitosan-LA did not cause erythema, edema and systhemic toxicity. Hence, Chitosan-LA film is more suitable than Chitosan-AA film to be used in the management of wound healing and skin burn.

Wittaya-areekul *et al*., (2006) developed the composite wound dressings based on 2 main requirements: that they help with wound healing and that they simple to produce. Chitosan-based composite films were prepared by adding Eudragit RS 30D as a composite polymer and glutaraldehyde as a crosslinker. Water vapor penetration, water uptake, oxygen penetration, bioahesive properties, and film elasticity were required for wound dressing applications. The results showed that pure chitosan films exhibited relatively high liquid uptake and the adsorption tended to decrease with the addition of Eudragit RS 30D. Moisture vapor and oxygen were found to be able to penetrate through all film formulations in comparable amounts. The film elasticity increased, but the bioadhesiveness test tended to show lower bioadhesive properties with the addition of Eudragit RS 30D. So, the addition of Eudragit RS 30D could improve a film's mechanical properties but lower its bioadhesiveness.

2.4 Crosslinking

Crosslinking is the process of chemically joining two or more molecules by a covalent bond. Crosslinking reagents contain reactive ends to specific functional groups (primary amines, sulfhydryls, etc.) on proteins or other molecules. Crosslinking reagents have been used to assist in determination of near-neighbor relationships, three-dimensional structures of proteins, and molecular associations in cell membranes. They also are useful for solid-phase immobilization, hapten-carrier protein conjugation, preparing antibody-enzyme conjugates, immunotoxins and other labeled protein reagents. Other uses include modification of nucleic acids, drugs and solid surfaces.

Crosslinkers can be either *homobifunctional* or *heterobifunctional*. Homobifunctional crosslinkers have two identical reactive groups and often are used in one-step reaction procedures to crosslink proteins to each other or to stabilize quaternary structure. Even when conjugation of two different proteins is the goal, one-step crosslinking with homobifunctional reagents often results in self-conjugation, intramolecular crosslinking and/or polymerization. Heterobifunctional crosslinkers possess two different reactive groups that allow for sequential (two-stage) conjugations, helping to minimize undesirable polymerization or self-conjugation. Heterobifunctional reagents can be used when modification of amines is problematic.

Crosslinkers are selected on the basis of their chemical reactivities (i.e., specificity for particular functional groups) and compatibility of the reaction with the application. The best crosslinker to use for a specific application must be determined empirically. Crosslinkers are chosen based on the following characteristics.

- Chemical specificity
- Spacer arm length
- Reagent water-solubility and cell membrane permeability
- Same (homobifunctional) or different (heterobifunctional) reactive groups
- Thermoreactive or photoreactive groups
- Reagent crosslinks cleavable or not

- Ø Reagent contains moieties that can be radiolabeled or tagged with another label

Crosslinkers contain at least two reactive groups. Functional groups that can be targeted for crosslinking include primary amines, sulfhydryls, carbohydrates and carboxylic acids.

Types of the crosslinking agents were used in this work such as glutaraldehyde (GA), maleic anhydride (MA), and succinic anhydride (SA). Because of these crosslinking agents contains aldehyde groups (-CHO) which can be reacted with –OH and –COOH of LPLA and $–NH_2$ of chitosan.

2.4.1 Glutaraldehyde

Glutaraldehyde (GA) is a very common crosslinking agent. It is mostly used because it is inexpensive and water-soluble. It has fairly small molecules, each with two aldehyde groups, separated by a flexible chain of 3 methylene bridges (HCO-$(CH_2)_3$-CHO). The potential for crosslinking is obviously much greater than with formaldehyde because it can occur through both the –CHO groups and over variable distances because of the high activity of –CHO groups, which readily form Schiff's base with amino groups make it is used in polypeptide and protein cross-linking (Wang *et al.*, 2004).

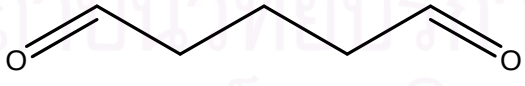


Figure 2.10 The structure of glutaraldehyde.

2.4.2 Succinic anhydride

Succinic anhydride or dihydro-2,5-furandione is an organic compound, a white crystalline solid with the formula $C_4H_4O_3$. The structure of succinic anhydride was shown in Figure 2.11. Succinic Anhydride is mainly used in the manufacture of polymeric materials (alkyd and other special resins). It is used in the manufacture of

pharmaceuticals, agrochemicals, dyes, photographic chemicals, surface active agents, esters, flavors and fragrances. Its application includes a cross-linking agent in ion-exchange membranes, curing agent for epoxy resins and starch modifier in foods and feeds.

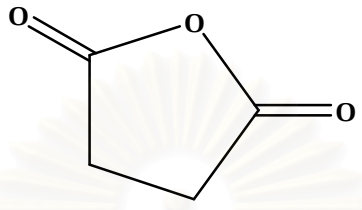


Figure 2.11 The structure of succinic anhydride.

2.4.3 Maleic anhydride

Maleic anhydride (dihydro-2,5-dioxofuran, cis-butenedioic anhydride) is an organic compound with the formula $C_4H_2O_3$. The structure of maleic anhydride was shown in Figure 2.12. It is a colorless or white solid with an acid odour. It is produced commercially by the oxidation of benzene or butane and used in the manufacture of resins such as unsaturated polyester resins, to which it imparts fast-curing and high-strength characteristics.

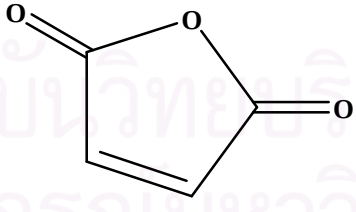


Figure 2.12 The structure of maleic anhydride.

2.4.4 Crosslinking of chitosan

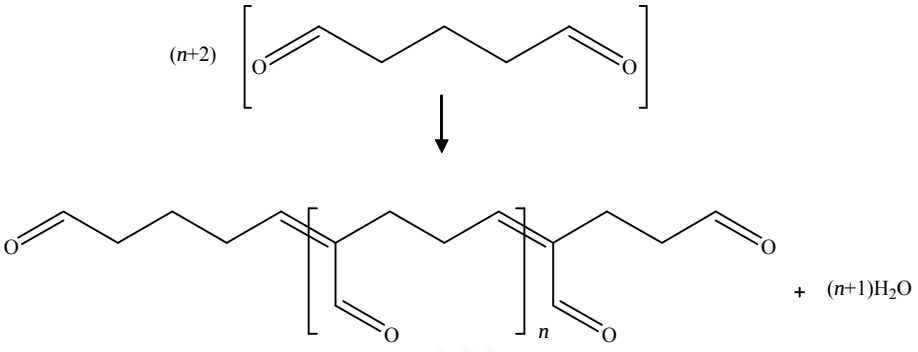
Crosslinking is one of the most popular methods being used to modify chitosan in order to achieve desired properties. Some properties could be altered by

crosslinking such as swelling, permeability, drug releasing, transport properties, water uptake, mechanical properties, chemical stability, sponge structure, and crystallinity.

The swellability of the chitosan salts can be improved further by after-crosslinking. Suitable crosslinking agents include all the polyfunctional substances which are capable of reacting with amino or OH groups, and the number of functional groups should preferably be two. Examples of crosslinking agents in the context of the invention include dicarboxylic acids, dianhydrides, dicarboxylic acid chlorides, diepoxides and dialdehydes. The crosslinking agents are preferably selected so that the crosslinking sites formed can easily be reopened biologically or hydrolytically in order to ensure the desired biodegradation. Since the chitosan salts are very long polymer chains, only a very small amount of crosslinker component is necessary to achieve optimum crosslinking. If the degree of crosslinking is too high, the swellability is adversely influenced and decreases significantly. The amount of crosslinking agent added depends on its nature. In the case of dicarboxylic acids, dianhydrides and diacid chlorides, a comparatively large amount of crosslinking agent of 0.01 to 10 mmol/g of chitosan must be employed, since these crosslinking agents can react partly with the water, which is preferably present in small amounts, or with the alcohols, if these have been used as solvents. On the other hand, diepoxides and dialdehydes react preferentially with the amino groups of the chitosan salt, so that these are used, for example, in amounts of only 10^{-4} to 10^{-2} mmol/g of chitosan

It should be noted that the properties achieved are strongly depending on crosslinking procedure (i.e., crosslinking agent, crosslinking condition, crosslinking technique). Several crosslinking agents for chitosan are employed such as glutaraldehyde, sodiumtripolyphosphate, genepin, copper sulfate, calcium chloride, ethylene glycol diglycidyl ether, and sulfuric acid. However, the most popular crosslinker is glutaraldehyde.

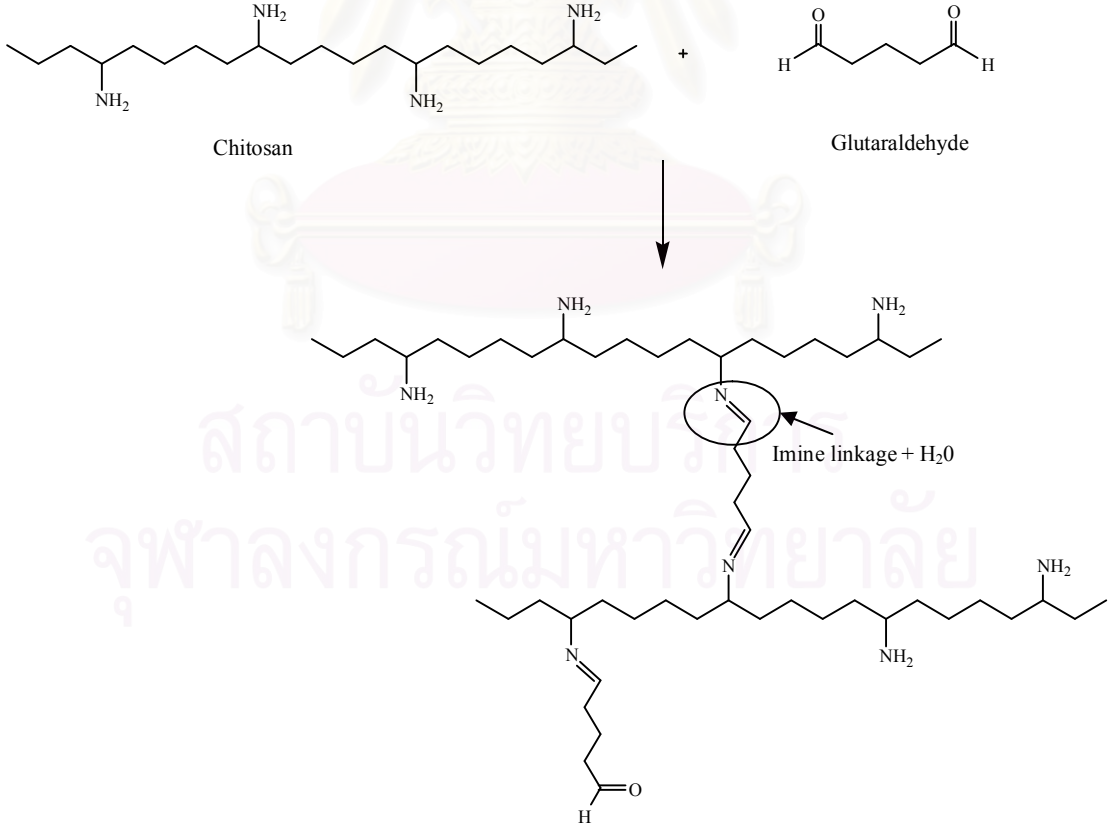
In aqueous solution, glutaraldehyde is present largely as polymer of variable size. There is a free aldehyde group sticking out of the side of each unit of the polymer molecule (Scheme 2.4), as well as one at each end.



Scheme 2.5 Polymerization reaction of glutaraldehyde, showing an aldehyde side-chain on each unit of the polymer.

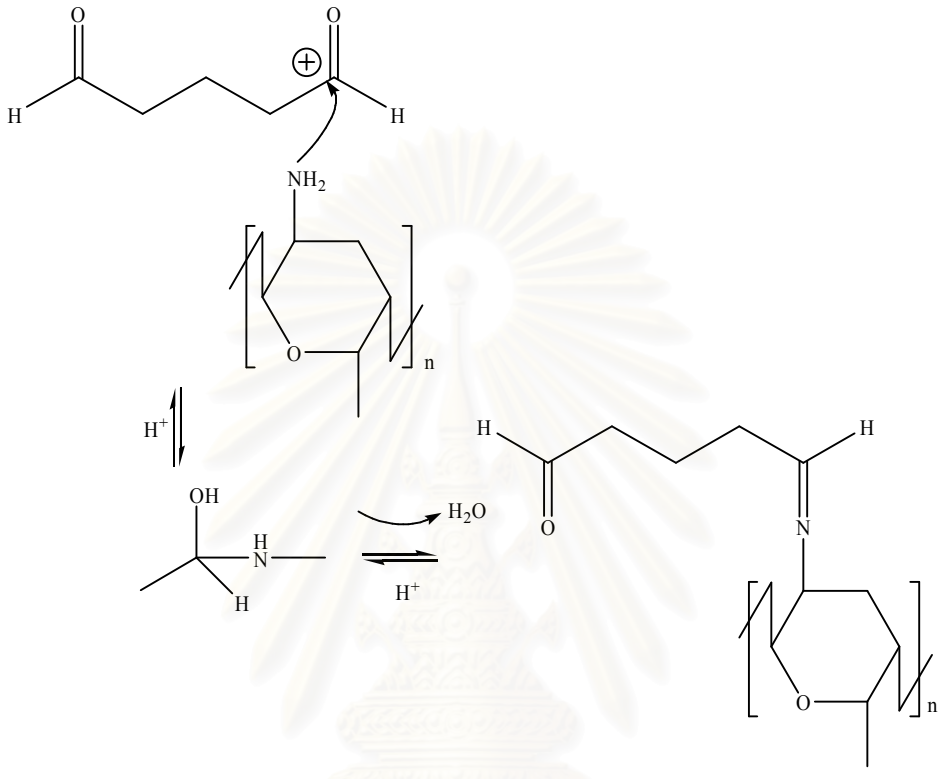
2.4.4.1 Crosslinking reaction of chitosan with glutaraldehyde

Crosslinking reaction between chitosan and glutaraldehyde occurs from amine group of chitosan and aldehyde group of glutaraldehyde (as shown in Scheme 2.6).



Scheme 2.6 Crosslinking reaction between chitosan and glutaraldehyde (Oztop *et al.*, 2002).

From the Scheme 2.6, crosslinking reaction between chitosan and glutaraldehyde occur forming the C=N bond, called “Schiff’s base formation”. The mechanism of this crosslinking reaction is schematically illustrated in Scheme 2.7.



Scheme 2.7 Formation of the schiff’s base (C=N) between amino groups of chitosan and aldehyde groups of glutaraldehyde (Wang *et al*., 2004).

Although imine linkage is mainly formed in the crosslinking reaction, different mechanisms have been proposed in order to explain the crosslinking reaction between chitosan and glutaraldehyde.

1. Schiff’s base formation has been proposed and leads to the formation of imine-type crosslinked according to Figure 2.13(A).
2. Michael-type adducts with amine groups leading to the type of crosslinked proposed by Muzzarelli have also been considered. They lead to the presence of carbonyl groups on the polymer structure (Figure 2.13 (B)).
3. In addition to these crosslinking phenomena, the formation of oligomers presented in Figure 2.13(C) is also proposed.

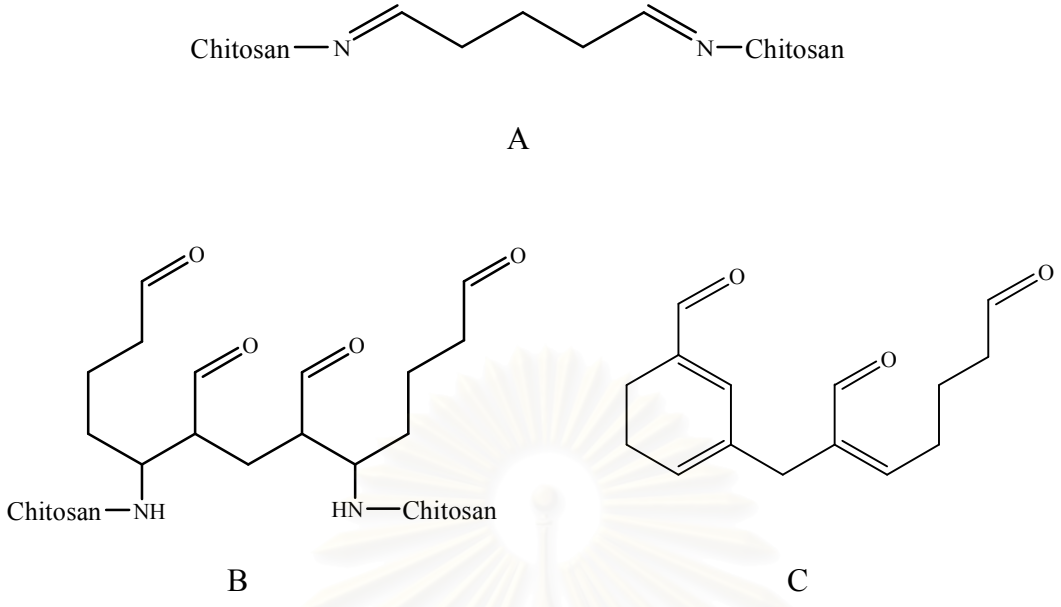


Figure 2.13 Proposed structures for chitosan crosslinked by glutaraldehyde.

2.5 Plasticizing agent

Biodegradable polymers derived from renewable resources considered as natural polymeric materials have now become increasingly scientific and commercial interest due to their natural abundancy and low cost. Biodegradable polymers are used to replace petroleum-based raw materials. However, the most natural polymer have a low mechanical properties, are brittle. The brittleness of natural polymers led to numerous investigations concerning the addition of plasticizers for applications both in industry and medicine.

Plasticizers are additives which, added to a polymeric materials to overcome the brittleness, to increase the flexibility or plasticity of polymers. Brittleness is an inherent quality attributed to the complex/branched primary structure and weak intermolecular forces of natural polymers. Plasticizers act by reducing the intermolecular forces soften the rigidity of the film structure and increase the mobility of the biopolymeric chains.

Hydrophilic plasticizers, such as glycerol (Gly), polyethylene glycol (PEG) and sorbitol (Sor) are generally used for protein-based films to improve mechanical

properties. However, the differences in composition, size, structure and shape of plasticizers directly influence their ability to function in the film network.

2.5.1 Glycerol (Gly)

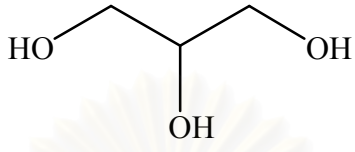


Figure 2.14 The structure of glycerol (Gly).

Glycerol is a chemical compound with the formula $HOCH_2CH(OH)CH_2OH$, which was presented in Figure 2.14. It is a colorless, odorless, and viscous liquid, widely used in pharmaceutical formulations. Also commonly called *glycerin* or *glycerine*, it is a sugar alcohol, and a low toxicity. Glycerol has three hydrophilic alcoholic hydroxyl groups that are responsible for its solubility in water and its hygroscopic nature. Its surface tension is 64.00 mN/m at 20^{o}C, and it has a temperature coefficient of -0.0598 mN/(mK). It is a central component of lipids.

2.5.2 Polyethylene glycol (PEG)

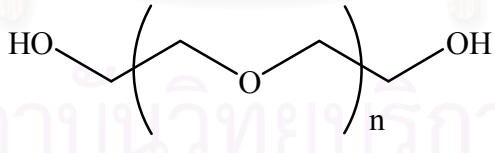


Figure 2.15 The structure of polyethylene glycol (PEG).

Polyethylene glycol (PEG), also known as polyethylene oxide (PEO), is the most commercially important polyethers. PEG or PEO refers to an oligomer or polymer of ethylene oxide, the structure was shown in Figure 2.15. Their melting points vary depending on the formula weight of the polymer. PEG or PEO are liquids or low-melting solids, depending on their molecular weights. While PEG or PEO with different molecular weights find use in different applications and have different

physical properties (e.g., viscosity) due to chain length effects. PEG has a low toxicity and is used in a variety of products.

PEG is soluble in water, methanol, benzene, dichloromethane and is insoluble in diethyl ether and hexane. It is coupled to hydrophobic molecules to produce non-ionic surfactants. It is used in food and food packaging, used as plasticizers, solvents, water-soluble lubricants for rubber molds; wetting or softening agents, antistatics in the production of urethane rubber, components of detergents, etc. In medicine, PEGS are used in cosmetics, ointments, suppositories, in ophthalmic solutions and sustained-released oral pharmaceutical applications.

2.5.3 Glycerol monostearate (GMS)

Figure 2.16 The structure of glycerol monostearate (GMS).

Glycerol monostearate (GMS) is a white flake or powder, and chemical formula is $CH_3(CH_2)_{16}COOCH_2CHOHCH_2OH$, (Figure 2.16). Melting point of GMS is 54º-60ºC. Glycerol monostearate is used as antistatic in polyolefins, softer in textiles and external lubricant for polymer and PVC plactics industry.

2.6 Literature reviews

Novel cytocompatible graft copolymer of chitosan and L-lactic acid (CL) by grafting L-lactic acid onto the amino groups in chitosan without a catalyst was prepared (Yao *et al.*, 2003). The structure of the CL graft copolymer was characterized by FTIR, ^{13}C-NMR and X-ray measurements. The pH-sensitive

swelling properties and mechanistic strength of CL graft copolymers were investigated. The proliferation of fibroblasts on the CL copolymer films was discussed in comparison with chitosan cross-linked by glutaraldehyde. The result showed that crystallinity of chitosan gradually decreased after grafting. The tensile strength of the CL copolymers increased along with the enhancement of feed ratio when LA/CS $\leq$ 2, after that, the raising of LA/CS resulted in a decrease of tensile strength. In aqueous solutions, the CL graft copolymer could form a pH-sensitive hydrogel and the in vitro fibroblast static cultivation on the films showed that the cell growth rate on the copolymers films was faster than chitosan obviously.

Liu *et al*. (2004) synthesized a kind of novel graft copolymer with the natural polysaccharide chitosan as the main chain and the artificial biopolymer poly(DL)-lactide as the side chains using Et_3Al as the catalyst in toluene at 70^{o}C. It was found that a greater lactide content in the feeding ratio results in a higher grafting percentage. FTIR spectrometry, ^{1}H-NMR, DSC scanning and wide-angle X-ray scattering are used to characterize this branch copolymer. A copolymer has a definite melting point when the molar feeding ratio of lactide to chitosan is more than 10:1 and the *DH* of the copolymers increases with the feed ratio of lactide to chitosan in feeding.

Peeson *et al*. (2005) prepared blend films of hexanoyl chitosan (H-chitosan) and polylactide (PLA) by the solution-casting technique from the corresponding blend solution in chloroform. The effect of blend composition on miscibility, morphology, thermal properties and mechanical properties was investigated. The main objective of this work was to find an economical way for improving the applicability of H-chitosan through the blending with PLA. Fourier-transformed infrared spectroscopy results indicated no significant interaction between H-chitosan and PLA molecules. Only the blend film having the H-chitosan content of 20 wt% exhibited the degradation temperature greater than those of the pure components. All of the blend films exhibited one composition-dependent glass transition temperature, indicating partial miscibility between H-chitosan and PLA molecules in the bulk amorphous phase. Both the tensile strength at break and the Young's modulus of the blend films were found to decrease from that of the pure PLA to that of the pure H-chitosan with increasing H-chitosan content.

Sebastien *et al*. (2006) prepared composite films from chitosan and poly(lactic acid) (PLA) by solution mixing and a film casting procedure to study the elaboration and the characterization of chitosan/PLA based bio-packaging for potential food application and the study of antifungal activity of coatings and films on three mycotoxinogen fungal strains, *fusarium proliferatum*, *fusarium moniliforme* and *Aspergillus ochraceus*. Difficulties were however encountered in producing miscible PLA and chitosan film forming solution, leading to heterogeneous films with high water sensitivity. Due to their antifungal activity, the composite films offer a great advantage in preventing the growth of mycotoxinogen strains. However, the physico-chemical properties of such heterogeneous films dramatically limit their development as packaging materials.

CHAPTER III

EXPRIMENTAL

3.1 Materials

Chemical	Company	Grade
1. L-lactic acid (LA) (88% aqueous solution)	Archer Daniels Midland	Commercial
2. Chitosan (CS) (DD = 94.6%, MW 600,000-1,000,000)	Taming Enterprises Co., Ltd.,	Commercial
3. Glutaraldehyde 50% in water	Fluka	AR grade
4. Toluene-4-sulfonic acid monohydrate	Fluka	AR grade
5. Nitrogen gas	Thai Industrial Gas	High purity 99.99%
6. Methanol (MeOH)	Fluka	Commercial
7. Succinic anhydride (SA)	Fluka	AR grade
8. Maleic anhydride (MA)	Fluka	AR grade
9. Sodium hydroxide (NaOH)	Carlo ERBA	AR grade
10. Acetic acid (CH_3COOH)	Merck	AR grade
11. Chloroform-d	Aldrich	AR grade

Chemical	Company	Grade
12. Glycerol (Gly)	BDH Laboratory Supplier	AR grade
13. Polyethylene glycol (PEG)	Aldrich	AR grade
14. Glycerol monostearate (GMS)	Namsiang International Co., Ltd.,	Commercial

3.2 Equipments and instrument

Instrument	Model	Company/Country
Nuclear Magnetic Resonance Spectrometer (NMR)	: Varian mercury-400 spcetrometer	Varian, USA
Gel Permeation Chromatograph (GPC)	: Waters 150-CV Degas: ERC-3415α Column: Waters Styragel HR columns (HR 1, 3, and 4), PL-gel 10 μm Pump: Waters 600 Controller Refractive Index Detector: Waters 2414	Waters, USA
Differential Scanning Calorimeter (DSC)	: NETZSCH DSC204F1 Phoenix	Phoenix, USA
Attenuated Total Reflection Infrared (ATR-IR) spectroscopy	: NICOLET 6700 FT-IR spectrometer with CONTINUμM IR Microscope	USA
X-ray diffraction (XRD)	: Bruker AXS Model D8 Discover	Germany

Instrument	Model	Company/Country
Universal Testing Machine (UTM)	: 5583 Serial#11202H	USA
Vacuum Drying Oven	: DP 41	Yamato Scientific, Japan
Centrifuge	: KR-20000T	Shimadzu, Japan
Hot Air Oven	: FG 32R	Japan
Shaker	: INFORS AG CH-4103 Bottmingen	Switzerland
Homogenizer	: NISSEI AM-11	Japan

3.3 Experimental procedure

3.3.1 Preparation of chitosan film

Chitosan 1% (w/v) was dissolved in 1% aqueous acetic acid solution (w/v). After stirring for 24 hours at room temperature, the solution was filtered through a nylon cloth to remove the insoluble substances. Then, the solution was poured into the glass plate (5 × 6 inch in size) and maintained at 65^{o}C for 8 hours in the hot-air oven to allow water evaporation. Film was peeled off and immersed in 0.1M NaOH:MeOH (1:1) and MeOH:H_2O (1:1) to neutralize the acid. Finally, film was dried under vacuum at 30^{o}C and maintained in the desiccator to characterize film properties.

3.3.2 Study on the effect of crosslinking agents on preparation of L-lactic acid-chitosan copolymer films.

Preparation of L-lactic acid-chitosan copolymer films, crosslinking agents such as glutaraldehyde (GA), succinic anhydride (SA) and maleic anhydride (MA) were used. The kind and ratio of crosslinking agents were studied.

Chitosan was dissolved in the 1% aqueous L-lactic acid solution. A mixture of chitosan and L-lactic acid (the weight ratio of chitosan (CS) to L-lactic acid (LA) is

1.75:1, 1.50:1, 1.25:1, 1:1, 0.75:1, 0.50:1) was stirred overnight at room temperature. After that, the solution was filtered through a nylon cloth to remove the insoluble substances. A crosslinking agent was slowly added into the mixture solution and stirred at room temperature for 1 hour. Then solution was poured into the plate (5 × 6 inch in size) and maintained at 65°C for 8-12 hours in the hot-air oven or at room temperature for 3-5 days, to allow water evaporation. To remove the unreacted L-lactic acid, oligo(L-lactic acid) and crosslinking agent, film was soaked with methanol (MeOH). Then film was dried under vacuum at 30°C and maintained in the desiccator prior to film property characterization. All of the crosslinking agent (GA, SA and MA) were compared, which are shown in Table 3.1 as well as the effect of ratio of CS-LA copolymer films which are shown in Table 3.2.

Table 3.1 The composition of 1% (w/v) chitosan in 1% (w/v) L-lactic acid solution with a different crosslinking agents

Film	**Vol. of CL solution (ml)**	**Mole of crosslinking agents**	**Molar ratio of chitosan : crosslinking agent**
CL	65	-	-
CLG	65	2.0×10^{-4}	1 : 0.05
CLG1	65	6.5×10^{-4}	1 : 0.16
CLS	65	2.0×10^{-4}	1 : 0.05
CLS1	65	6.5×10^{-4}	1 : 0.16
CLM	65	2.0×10^{-4}	1 : 0.05
CLM1	65	6.5×10^{-4}	1 : 0.16

*CLG, CLS and CLM = CL solution with glutaraldehyde, succinic anhydride, and maleic anhydride.

Table 3.2 The weight ratio of chitosan (CS) to L-lactic acid (LA) at 2.0×10^{-4} mole of glutaraldehyde (GA) in 65 ml of CL solution

Film	Vol. of CL solution (ml)	Ratio of CS : LA (w/w)
1.75CLG	65	1.75:1
1.5CLG	65	1.50:1
1.25CLG	65	1.25:1
CLG	65	1.00:1
0.75CLG	65	0.75:1
0.5CLG	65	0.50:1

CLG sample with GA as crosslinking agent was used for varying of concentration of GA, all of sample are shown in Table 3.3.

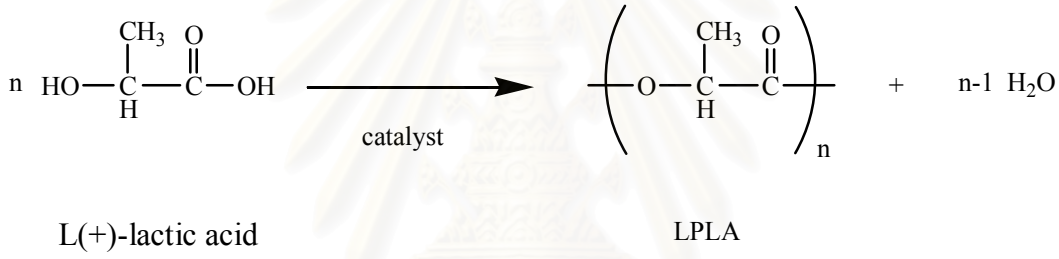
Table 3.3 The composition of 1% (w/v) chitosan in 1% (w/v) L-lactic acid with molar ratio of CS : GA from 1 : 0.16 to 1 : 0.80

Film	Vol. of CL solution (ml)	Amount of glutaraldehyde (mole)	Molar ratio of CS : GA
CLG1	65	6.5×10^{-4}	1 : 0.16
CLG2	65	1.3×10^{-3}	1 : 0.32
CLG3	65	1.95×10^{-3}	1 : 0.48
CLG4	65	2.6×10^{-3}	1 : 0.64
CLG5	65	3.25×10^{-3}	1 : 0.80

3.3.3 Preparation of low molecular weight polylactic acid-chitosan copolymer films using crosslinking agent (CLG-LPLA)

Low molecular weight polylactic acid-chitosan (CS-LPLA) copolymer using crosslinking agent preparation was divided into two steps. The first step was synthesis of low molecular weight polylactic acid (LPLA) using toluene-4-sulfonic acid monohydrate (PTSA) as a catalyst by linear polycondensation of L(+)-lactic acid to LPLA. The second step was the preparation of chitosan (CS) and LPLA copolymer film was prepared by casting technique.

<u>Step 1.</u> Synthesis of low molecular weight polylactic acid using toluene-4-sulfonic acid monohydrate (PTSA) as a catalyst



Scheme 3.1 Synthesis of low molecular weight polylactic acid (LPLA)**.**

First L-lactic acid solution (50 g) was heated with toluene-4-sulfonic acid monohydrate (1% by weight) at 140^{o}C under nitrogen atmosphere for two hours. High vacuum was applied in this step for 30 minutes to remove water which was initially presented in L-lactic acid solution and obtained from polymerization. Later, the reaction mixture was heated to 160^{o}C until no water was condensed. Low molecular weight polylactic acid (Mn<5,000) was obtained.

<u>Step 2</u>. Preparation of chitosan and LPLA copolymer film

LPLA from the first step was heated at 70^{o}C. Glutaraldehyde was slowly added and the reaction mixture stirred until brownish yellow color solution was obtained. After that the obtained brownish yellow color solution was added into the 1% (w/v) chitosan in 1% (w/v) L-lactic acid solution. The mixture was stirred at 70^{o}C for 15 mins and at room temperature for 45 mins. The solution was centrifuged and the

supernatant was then poured into the plate (5 × 6 inch in size) and incubated at 65°C for 8 hours or room temperature for 3-5 days. The free LPLA present on film surface was removed by soaking the film into chloroform or methanol. The film was then dried under vacuum at 30°C and maintained in the desiccator prior to film characterization. Varying of LPLA weight and glutaraldehyde concentration of preparation of chitosan and LPLA copolymer films with GA were presented in Table 3.4 and 3.5, respectively.

Table 3.4 Composition of chitosan and LPLA copolymer films with different weight of LPLA

Film	Vol. of chitosan (ml)	Weight of LPLA (g)	Mole of GA	Molar ratio (chitosan : GA : LPLA)
CLG1-0.5%LPLA	65	0.33	6.5×10^{-4}	1 : 0.16 : 0.9
CLG1-1.0%LPLA	65	0.65	6.5×10^{-4}	1 : 0.16 : 1.8
CLG1-1.5%LPLA	65	0.98	6.5×10^{-4}	1 : 0.16 : 2.7
CLG1-2.0%LPLA	65	1.30	6.5×10^{-4}	1 : 0.16 : 3.6
CLG1-2.5%LPLA	65	1.63	6.5×10^{-4}	1 : 0.16 : 4.5

Table 3.5 Composition of chitosan and LPLA copolymer films with a different concentration of glutaraldehyde

Film	Vol. of chitosan (ml)	Weight of LPLA (g)	Mole of GA	Molar ratio (chitosan : GA : LPLA)
CLG1-1.0%LPLA	65	0.65	6.5×10^{-4}	1 : 0.16 : 1.8
CLG2-1.0%LPLA	65	0.65	1.3×10^{-3}	1 : 0.32 : 1.8
CLG3-1.0%LPLA	65	0.65	1.95×10^{-3}	1 : 0.48 : 1.8
CLG4-1.0%LPLA	65	0.65	2.6×10^{-3}	1 : 0.64 : 1.8
CLG5-1.0%LPLA	65	0.65	3.25×10^{-3}	1 : 0.80 : 1.8

3.3.4 Modification of the copolymer films

The CLG3 and CLG5-1.0%LPLA copolymer films obtained from 3.3.2 and 3.3.3 were modified using plasticizing agent. In case of the copolymer films were prepared from the first method, glycerol (Gly) and polyethylene glycol (PEG) as a plasticizing agents, was added into CL solution with glutaraldehyde (CLG3 solution), after stirring at room temperature for 1 hour. The plasticized CLG3 solution was heated at 70^{o}C for 15 mins. After that the solution was poured into the plate and incubated at 65^{o}C. The film was soaked in the methanol to removed unreacted substance. The film was then dried under vacuum and maintained in the desiccator prior to film characterization.

For the copolymer films prepared from the second method, after the solution was centrifuged, plasticizing agent (Gly and PEG) was added into CLG5-1.0%LPLA solution. The plasticized CLG5-1.0%LPLA solution was heated at 70^{o}C for 15 mins. After that the solution was poured into the plate and incubated at 65^{o}C. The film was soaked in the methanol to remove unreacted substance. The film was then dried under vacuum and maintained in the desiccator prior to film characterization.

Glycerol monostearate (GMS) was added into CLG3 solution and CLG5-1.0%LPLA solution. To avoid phase separation, homogenizer was used. The solutions were heated at 70-90^{o}C for 15 mins and then were homogenized at 12,000 rpm for 5 mins. The solution was heated and homogenized for 3 times. Finally, it was heated for 15 mins and incubated at room temperature, the remaining GMS was precipitated. The plasticized solutions were centrifuged. The supernatant was poured into the plate and incubated at 65^{o}C or room temperature for 3-5 days. The film was soaked in the methanol to remove unreacted substance. The film was then dried under vacuum and maintained in the desiccator prior to film characterization. Quantities of glycerol, polyethylene glycol, and glycerol monostearate used in the preparation of copolymer films are shown in Table 3.6.

Table 3.6 Composition of the CLG3 and CLG5-1.0%LPLA copolymer films

Copolymer film	GA(%)	Plasticizer (%)		
		Glycerol (Gly)	Polyethylene glycol (PEG)	Glycerol monostearate (GMS)
1	3	-	-	-
2	3	0.25	-	-
3	3	0.50	-	-
4	3	-	0.50	-
5	3	-	1.00	-
6	3	-	-	0.50
7	5	-	-	-
8	5	0.50	-	-
9	5	-	0.50	-
10	5	-	-	0.50
11	3[a]	-	-	-
12	3[a]	-	-	0.50
13	5[a]	-	-	-
14	5[a]	-	-	0.50

Copolymer films 1-6, and 11-12 : CLG3

Copolymer films 7-10 and 13-14 : CLG5-1.0%LPLA

[a] Film formation temperature = room temperature

3.4 Copolymer film characterization

3.4.1 Determination of solubility (%)

The solubility of the film was determined. The preweighed films (2×2.5 cm^2) were soaked into deionized water at 200 rpm, 30°C, 1 h. After discarding the deionized water, the films were dried in a hot air oven until constant weight. The percentage of total soluble matter (% solubility) was calculated as follows :

$$\% \ Solubility = \left(\frac{Initial\ dry\ weight - Final\ dry\ weight}{Initial\ dry\ weight} \right) \times 100$$

3.4.2 Swelling capacity (%)

Swelling property was analyzed gravimetrically. Dry chitosan and copolymer films were preweighed and soaked into deionized water at certain time. The resulting swollen films were removed and dry on filter paper to remove free water on the film surface. The films were then weighed. After that the films were resoaked, dried, and reweighed until the constant weight. The swelling percentage (%) was calculated as follows :

$$W = \frac{W_s - W_o}{W_o} \times 100$$

Where W is the swelling percentage of swollen films. W_s is the weight of the saturated swollen film. W_o is the weight of the dry film.

3.4.3 Nuclear magnetic resonance spectrometer (NMR)

Proton (^1H) and carbon (^{13}C) nuclear magnetic resonance analysis were used to characterize chitosan and copolymer films. The sample was dissolved in chloroform-d ($CDCl_3$). Chemical shifts (δ) were reported in parts per million (ppm) relative to the residual protonated solvent signal as a reference.

3.4.4 Gel permeation chromatograph (GPC)

Molecular weight of LPLA was determined by gel permeation chromatography (GPC). The LPLA sample (15 mg) was dissolved in tetrahydrofuran (THF) (5 ml) and filtered by syringe filter (diameter 13 mm, 0.45 μm. nylon). GPC chromatogram of LPLA was obtained from Waters 150-CV chromatography equipped with PL-gel 10 μm mixed B 2 columns (MW resolving range = 500-10,000,000) at 35^{o}C. Tetrahydrofuran was used as an eluent and was delivered at the flow rate of 1.0 mL/min. Degassed THF mobile phase was flowed through the column for 20 minutes before injection. The sample volume of 100 μl was injected and run for 40 minutes. Polystyrenes (MW = 5,460-1,290,000) were used as standards for calibration. The molecular weight was determined by a reflection index detector.

3.4.5 Attenuated total reflection infrared (ATR-IR) spectroscopy

Attenuated total reflection infrared (ATR-IR) spectroscopy technique is used to analyze material surface. It is also suitable for characterization of materials which are either too thick or too strong absorbing to be analyzed by transmission spectroscopy. A single attenuated total reflection accessory with 45^{o}germanium (Ge) IRE (Spectra Tech, U.S.A.) and a variable angle reflection accessory (seagull^{TM}, Harrick Scientific, U.S.A.) with a hemispherical Ge IRE were employed for all ATR spectral acquisitions. All spectra were collected at a resolution of 4 cm^{-1} and 128 scans using a NICOLET 6700 FT-IR spectrometer with CONTINUμM IR Microscope equipped with a liquid-nitrogen-cooled mercury-cadmium-telluride (MCT) detector.

3.4.6 Differential scanning calorimeter (DSC)

Differential scanning calorimeter was determined by NETZSCH DSC204F1 Phoenix. Technique in which the difference in energy input into a substance and a reference material is measured as a function of temperature, while the substance and reference material are subjected to a controlled temperature program.

10-13 mg film was weighed in an aluminum pan. The temperature was increased from 20°C to 180°C (heating rate 20°C/min) and remained at 180°C for 3 minutes. In this step, the film was completely molten. Liquid nitrogen was used to reduce temperature to -50°C (cooling rate 20°C/min) and remained constant for 3 minutes. During the step, semi-crystalline substances were precipitated quickly but the amorphous substances were not precipitated. Then the sample was heated to 200°C (heating rate 20°C/min) until completely molten again. The empty pan was used as standard calibration. The amorphous structure was then changed from glass-like to rubber-like at glass transition temperature.

3.4.7 X-ray diffraction (XRD)

The crystalline structure of film samples was analyzed by an X-ray diffractometer ; Bruker AXS Model D8 Discover. The film sample (1×2 cm in size) was cut and placed on the mirror. X-ray diffraction investigation of the film was carried out with CuKα radiation was generated at 40 kV, 40 mA and the scattering range (2θ) of 5-40°.

3.4.8 Universal testing machine (UTM)

The mechanical properties of films were studied. The tensile strength (TS), Young's modulus (Y) and percent elongation at break (%E) of the film samples were measured by Universal testing machine (UTM). The film samples (0.5×4 inch in size) were maintained in the desiccator prior to the test. The width and the thickness of the film were measured prior to testing. The test was carried out according to ASTM D 882 standard method, at 25°C and 50% humidity, with crosshead speed of 12.5 mm/min, and load cell of 5.0 kN.

CHAPTER IV

RESULTS AND DISCUSSION

Chitosan (CS) was generally prepared via the partial deacetylation of chitin in a hot alkali solution, contains 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose groups (Liu *et al*., 2004). The process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin, leaving behind a complete amino group (-NH_2).

4.1 Determination of % degree of deacetylation (%DD) of chitosan by ^{1}H NMR

Degree of deacetylation (%DD) is determined from the ratio of glucosamine and N-acetyl glucosamine units. ^{1}H NMR was used to determine %DD of chitosan (Figure 4.1). The signal for –CH_3 of N-acetyl glucosamine units at δ = 2.94 ppm and the proton at C-2 of glucosamine units at δ = 1.84 ppm were integrated to determine %DD (Table 4.1).

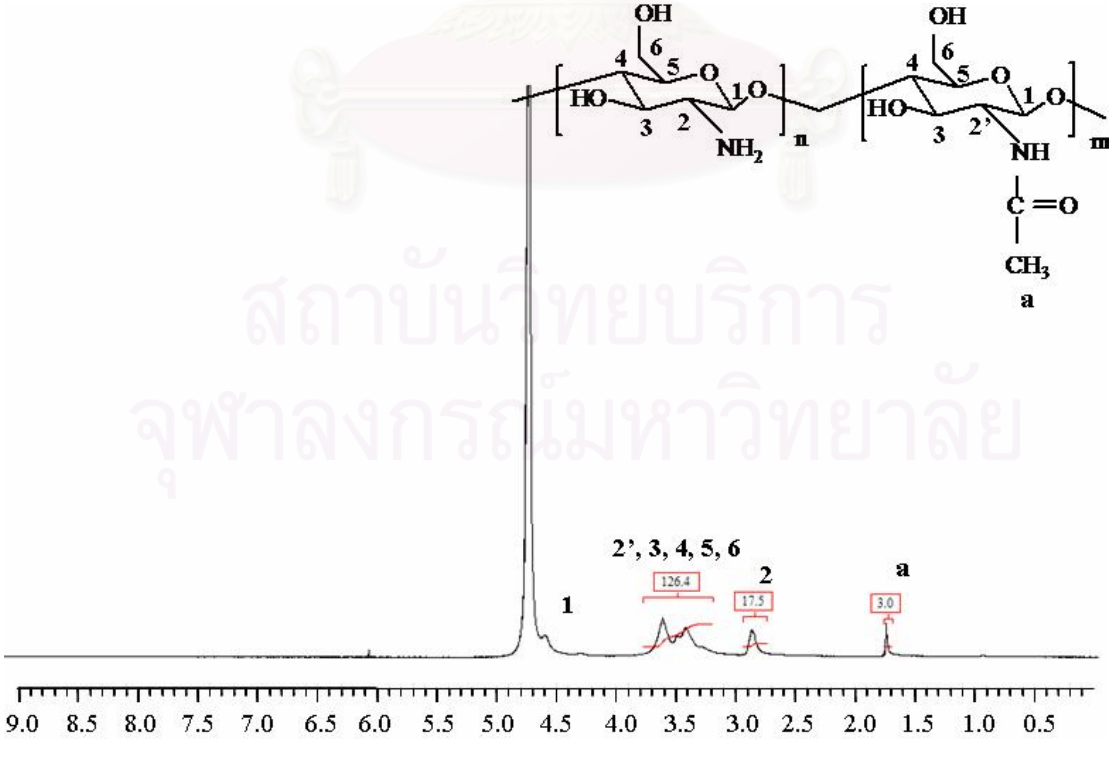


Figure 4.1 ^{1}H NMR spectrum of chitosan obtained from Taming company (solvent : 1%CF_3COOH in D_2O, 25°C).

Table 4.1 Information from ^{1}H NMR spectroscopy of chitosan

	d **ppm**	**Integration**	**Amount of units in chitosan**
-C<u>H</u>NH_2 of GlcN	2.89	17.5	17.5
-C<u>H_3</u> of GlcNAc	1.78	3	3/3

From the data in Table 4.1, %DD could be calculated as follows. The total amount of GlcN and GlcNAc units, in chitosan, are equal to 17.5 + 3/3 = 18.5 units. If the total repeating units in chitosan are 100%, thus %DD is $\frac{(17.5/1) \times 100}{(17.5/1) + (3/3)} = 94.6\%$.

4.2 Preparation of film samples

4.2.1. Preparation of chitosan film

Chitosan film was prepared in acetic acid solution, later so called CA film, by casting technique. It was found that film prepared from 1% (w/v) chitosan dissolved in 1% (w/v) aqueous acetic acid solution was rigid, transparent yellow and could be easily peeled off from the casting surface. After chitosan film was neutralized by 0.1M NaOH:MeOH (1:1) and MeOH:H_2O (1:1), the color of the film was changed from transparent yellow to cloudy white.

4.2.2. Effect of crosslinking agents on preparation of L-lactic acid and chitosan copolymer film

The chitosan film was prepared in L-lactic acid solution, so called CL film, by casting technique. The CL film was characterized and compared with CA film. CL film was prepared from 1% (w/v) chitosan dissolved in 1% (w/v) aqueous L-lactic acid solution. The resulting film was transparent and yellow which was similar to the non neutralized CA film but CL film was more difficult to be peeled off from the casting surface than CA film, since the film was sticky. After soaking in methanol to remove unreacted L-lactic acid and oligo-L-lactic acid, CL film became soft and flexible.

In this research, 3 crosslinking agents (glutaraldehyde (GA), succinic anhydride (SA) and maleic anhydride (MA)) were used. It was found that the presence of glutaraldehyde affected the film color and appearance. The film color was from pale yellow to brown depending on the concentration of glutaraldehyde used. The color of crosslinked films using a different crosslinking agent is shown in Figure 4.2.

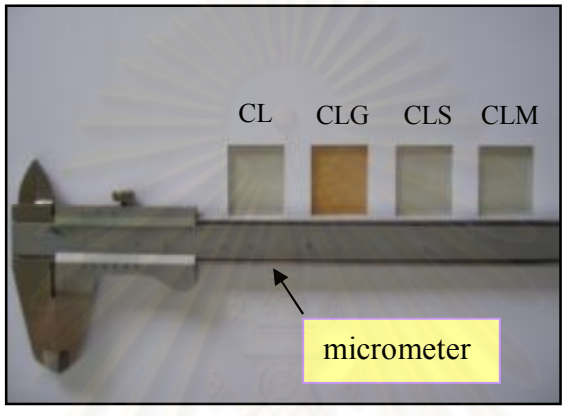
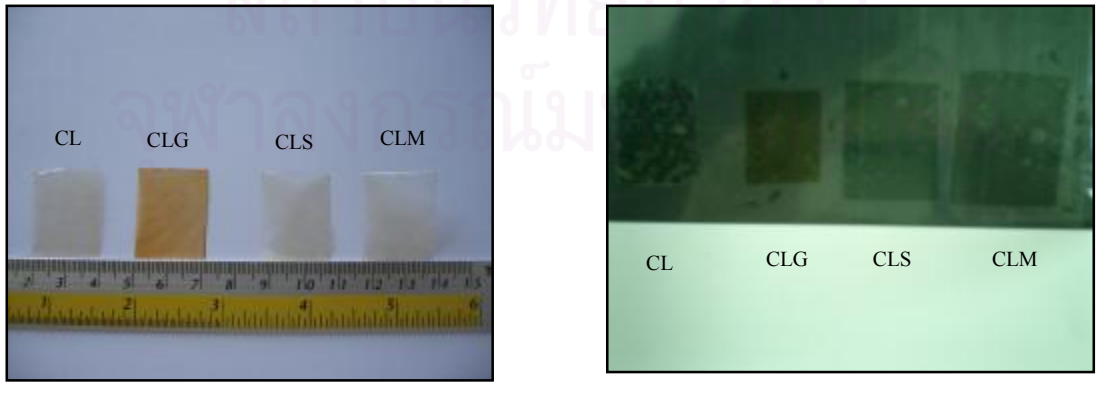


Figure 4.2 The color of chitosan-lactic acid (CL film) and crosslinked films with glutaraldehyde (CLG), succinic anhydride (CLS), and maleic anhydride (CLM) at molar ratio of chitosan to crosslinking agent at 1:0.05.

The swelling capacity of the films was determined. Figure 4.3 shows the physical appearance of the films before and after the swelling test. It was found that the swelling capacity was decreased with the increased glutaraldehyde concentration.



(a) (b)

Figure 4.3 The CL, CLG, CLS, and CLM films physical appearance before the swelling (a) and the swelled films (b).

Table 4.2 The physical appearance, and swelling capacity (%) of films with 0.05-0.10 mm thickness

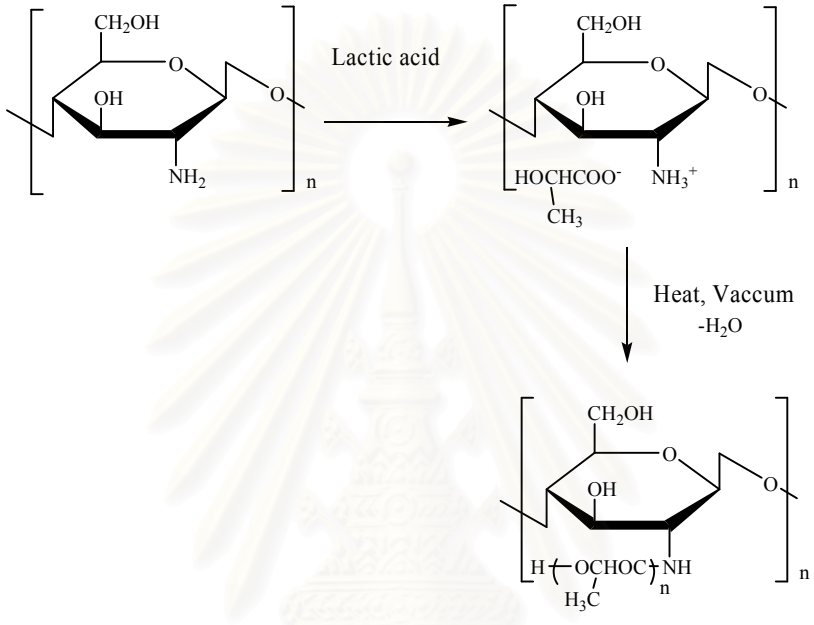
Film	Physical appearance of films		Swelling capacity (%)
	Before swelling	After swelling	
CA	Transparent, rough	Turbid, and rubbery	107.94 ± 1.36
CL	Transparent, smooth	Transparent, flexible, and sticky	204.61 ± 0.73
CLG[a]	Yellow, smooth	Yellow and brittle	172.67 ± 1.76
CLG1[b]	Yellow, smooth	Yellow and brittle	82.32 ± 0.69
CLS[a]	Transparent, smooth	Transparent, flexible, and sticky	279.32 ± 2.85
CLS1[b]	Transparent, smooth	Transparent, flexible, and sticky	Not available
CLM[a]	Transparent, smooth	Transparent, flexible, and sticky	313.55 ± 2.15
CLM1[b]	Transparent, smooth	Transparent, flexible, and sticky	Not available

Remarks :

- [a,b] Molar ratio of chitosan to crosslinking agent (a = 1:0.05, b = 1:0.16)
- Not available due to films were dispersed in water.

Compared with CA film, the swelling capacity of CL film was greater since lactic acid composes of –OH and –COOH groups. When CL film was soaked in aqueous solution, some lactic acid grafted on chitosan chains formed lactate salt resulted in CL film was soluble in aqueous solution (Table 4.2, and Scheme 4.1). When chitosan was dissolved in L-lactic acid solution, graft copolymer was generated by which the carboxylic group of lactic acid reacting with the amine group of chitosan. The mechanism is shown in Scheme 4.1 (Yao *et al*., 2003). Amino groups of chitosan were protonated and chitosan amino was formed once chitosan was dissolved

in L-lactic acid aqueous solution. As salt dehydration occurred by heating, the amide groups between L-lactic acid and chitosan was formed. Graft chitosan also formed hydrogel (3-dimensional structural network) which was swollen in aqueous solution due to the physical crosslinking through hydrogen bonding and dipole-dipole interaction between the neighboring ester groups and chitosan chains, as well as the hydrophobic side chain aggregation (Qu *et al*., 2000).



Scheme 4.1 Graft copolymerization of chitosan and L-lactic acid (Yao *et al*., 2003).

Crosslinking agent was added into CL solution to improve the swelling capacity. It was found that glutaraldehyde (GA) could effectively reduce the swelling capacity of the CL film. Increasing concentration of GA led to the decrease in swelling capacity from 172.67% to 82.32%. However, succinic anhydride (SA), and maleic anhydride (MA) showed no significance in improving swelling capacity. CLS and CLM films were swollen in water. This could be explained by the vinyl carboxylic groups and carboxylic groups generated by MA and SA, respectively were introduced to the film surface resulting in more hydrophilic surface which was in favor of water adsorption (Zhang *et al*., 2007; Don *et al*., 2005). Therefore, glutaraldehyde was used as a crosslinking agent for preparation of L-lactic acid and chitosan copolymer film and low molecular weight polylactic acid-chitosan copolymer.

I. Effect of weight ratio of chitosan (CS) to L-lactic acid

Different weight ratio of chitosan/L-lactic acid copolymer film was prepared using GA as the crosslinking agent (CS : GA = 1 : 0.05). Figure 4.4 indicates the swelling capacity of the copolymer films, where 1.75CLG was prepared from 1.75:1 of chitosan to L-lactic acid weight ratio, 1.5CLG was prepared from 1.50:1 of chitosan to L-lactic acid weight ratio, 1.25CLG was prepared from 1.25:1 of chitosan to L-lactic acid weight ratio, CLG was prepared from 1:1 of chitosan to L-lactic acid weight ratio, 0.75CLG was prepared from 0.75:1 of chitosan to L-lactic acid weight ratio, 0.5CLG was prepared from 0.5:1 of chitosan to L-lactic acid weight ratio. It was found that when the chitosan content decreased, it resulted in the sticky solution and the film made from the solution was difficult to be peeled off the casting surface (from CLG to 0.5CLG film). The high chitosan content films (1.75CLG, 1.5CLG, and 1.25CLG) were easily peeled off and brittle. Considering the film appearance, it was found that when the chitosan content decreased, the film color was changed and the thickness and the swelling capacity were decreased. The film solubility in water is an important property which governs potential applications of these materials to food preservation, the swelling property of CLG film was determined. Films with low water solubility are necessary for the protection of food with high or intermediate water activity (aw). From Figure 4.4, the solubility of the films was approximately 12-13%.

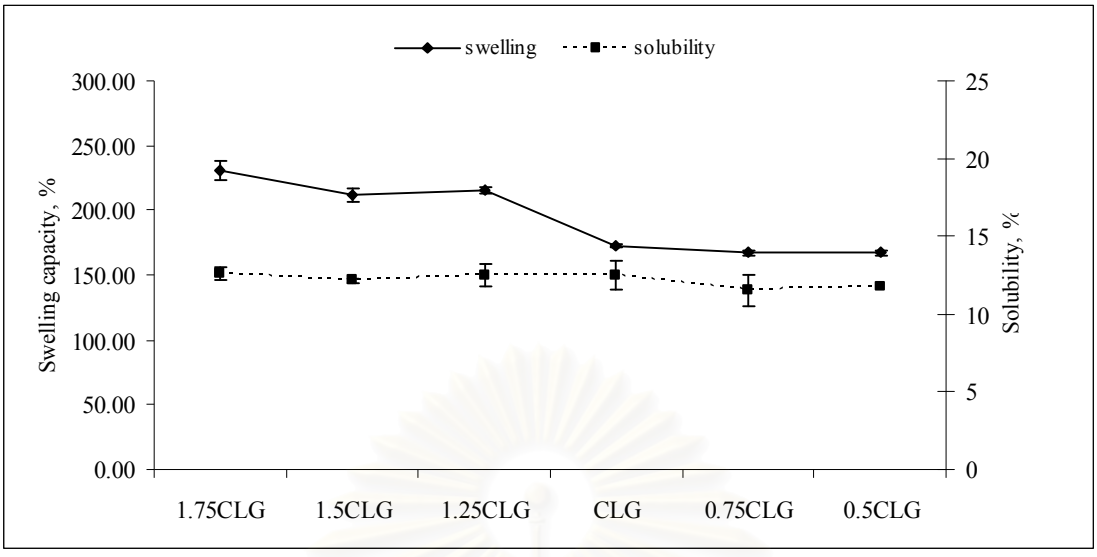


Figure 4.4 Swelling capacity and solubility of variation in weight ratio of the lactic acid-chitosan copolymer films (1.75CLG-0.5CLG films) at molar ratio of chitosan to crosslinking agent at 1:0.05.

II. Effect of GA concentration

Chitosan/L-lactic acid copolymer was prepared from 1% (w/v) chitosan in 1% (w/v) aqueous L-lactic acid solution using different GA concentration. Increasing concentration of GA resulted in the decrease in solubility of chitosan and the formation of hydrogel (Table 4.3).

Table 4.3 Effect of glutaraldehyde concentration on the solubility of 1% (w/v) chitosan in 1% (w/v) aqueous L-lactic acid solution, physical appearance, and swelling capacity of copolymer films

Film	Molar ratio of CS : GA	Solubility	Appearance of film	Swelling capacity, %
CLG1	1 : 0.16	Soluble	Pale orange, transparent, and smooth	81.87 ± 1.38
CLG2	1 : 0.32	Soluble	Orange, transparent, And smooth	56.04 ± 1.55
CLG3	1 : 0.48	Soluble	Brown, transparent, smooth, and shrink	47.02 ± 0.46
CLG4	1 : 0.64	Swell	Brown, transparent, smooth, shrink, and brittle	Not available
CLG5	1 : 0.80	Swell	Brown, transparent, smooth, shrink, and brittle	Not available

Remarks :

- Not available due to films were dispersed in water.

During swelling study, it was found that CLG3, CLG4, and CLG5 films were swelling and became brittle especially when the concentration of GA was higher than 3.25×10^{-3} mol (molar ratio of CS:GA = 1:0.8), the film solution turned into hydrogel.

4.2.3 Preparation of low molecular weight polylactide-co-chitosan copolymer film (CLG-LPLA)

Low molecular weight polylactide was synthesized using toluene-4-sulfonic acid monohydrate (PTSA) as a catalyst. The resulting low molecular weight polylactide (LPLA) was a sticky yellow substance at the room temperature. The synthesis of polylactic acid by polycondensation reaction is shown in Figure 4.5.

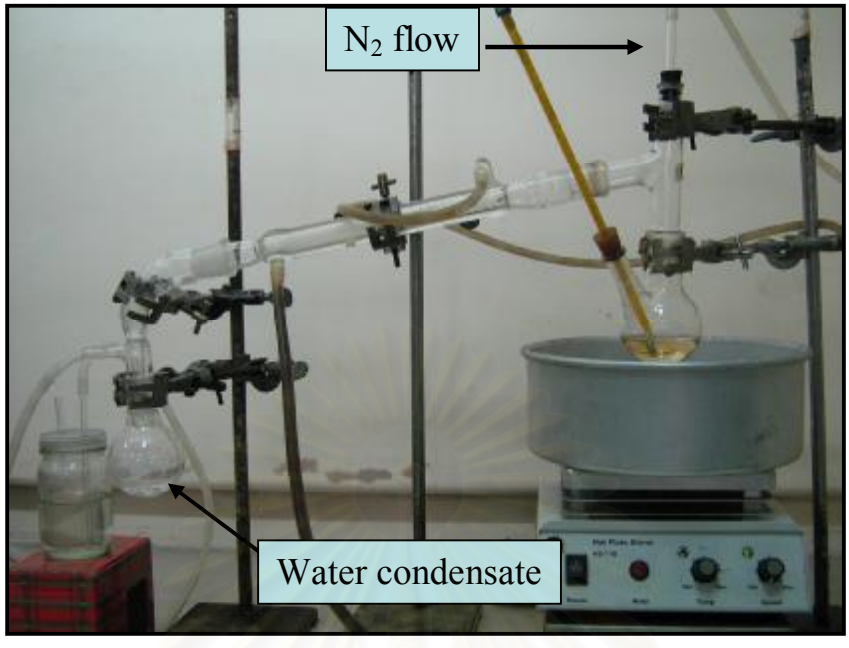


Figure 4.5 Experimental set up for low molecular weight PLA synthesis.

The MW of LPLA was determined by gel permeation chromatography (GPC). Effects of the catalyst and reaction temperature on PLA molecular weight are described in Table 4.4. It was found that the higher MW was obtained when using PTSA as a catalyst. The increased reaction temperature resulted in the increase in MW.

According to GPC chromatogram shown in Figures 4.6 and 4.7, high molecular weight PLA cannot pass through the porous structure of gel in column; therefore, it is eluted from column prior to low molecular weight PLA. For this reason, shorter retention time indicates higher molecular weight of PLA as the highest peak in the chromatogram shifts to the left side of the x-axis. From GPC chromatograms, the synthesized LPLA composed of PLA with different molecular weights.

Table 4.4 Molecular weights and polydispersity index of PLA obtained by polycondensation at 140-160°C without and with a catalyst

Polycondensation at 140-160°C	GPC			Physical Appearance
	$\overline{M}_w$	$\overline{M}_n$	PDI	
Polylactic acid	260	228	1.14	Clear viscous
Polylactic acid+1%PTSA	1379	723	1.90	Yellow viscous
Polylactic acid+1%$Sn(Oct)_2$	668	425	1.57	Yellow viscous
Polylactic acid+1%GA	430	321	1.34	Yellow viscous
Polylactic acid+70°C	715	463	1.54	Clear viscous

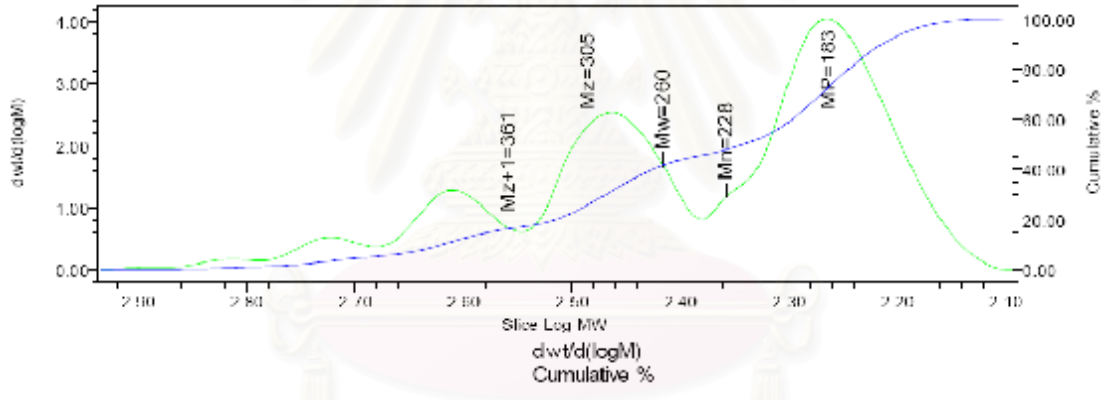


Figure 4.6 The chromatogram of polylactic acid obtained from polycondensation polymerization.

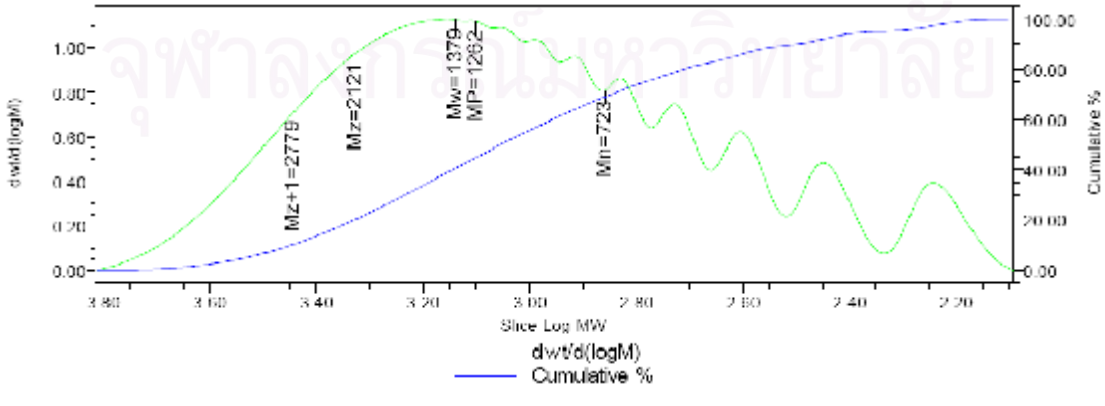


Figure 4.7 The chromatogram of polylactic acid using 1% toluene-4-sulfonic acid monohydrate (PTSA), ($\overline{M}_w$= 1379).

It was observed that increasing the amount of LPLA in copolymer solution (0.5% w/v-2.5% w/v) resulted in the increase in copolymer MW. However, the higher amount of LPLA led to the change in film appearance, resulting in stickier film. The swelling capacity and solubility of copolymer films using different amount of LPLA are shown in Figure 4.8. CLG1-1.0% LPLA film showed the lowest swelling capacity of 177.14%. This was perhaps because of pore size of CLG1-1.0%LPLA film is smaller than other film so that water penetration through the film was more difficult compared to others. The solubility in water was approximately 4-5%.

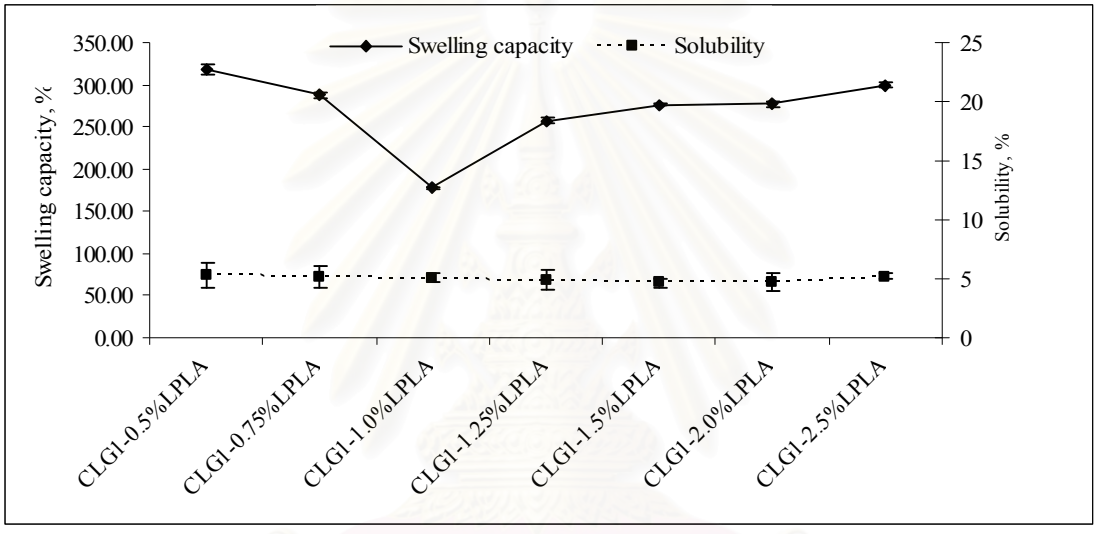


Figure 4.8 Swelling capacity, and solubility of the CLG1-LPLA copolymer films with variation in amount of LPLA from 0.5% (w/v) to 2.5% (w/v) while the GA concentration constant (molar ratio of CS:GA = 1:0.16).

The appearance and properties of CLG-LPLA copolymer films are given in Table 4.5. Compared with the CLG films (Table 4.3), CLG-LPLA films were more flexible when the concentration of GA was increased. The color of the film changed from yellow to brown like that observed in CLG films at high GA concentration.

Table 4.5 The appearance of CLG-LPLA copolymer films with molar ratio of CS:GA from 1:0.16 to 1:0.80 in 1.0% (w/v) LPLA in CL solution

Sample	Molar ratio of CS : GA	Solubility	Appearance of film	Swelling capacity, %
CLG1-1.0%LPLA	1 : 0.16	Soluble	Orange, transparent, and smooth	177.14 ± 0.73
CLG2-1.0%LPLA	1 : 0.32	Soluble	Orange, transparent, and smooth	171.51 ± 1.28
CLG3-1.0%LPLA	1 : 0.48	Soluble	Orange, transparent, smooth ,and brittle	163.65 ± 2.55
CLG4-1.0%LPLA	1 : 0.64	Soluble	Brown, transparent, smooth, and brittle	160.87 ± 1.67
CLG5-1.0%LPLA	1 : 0.80	Soluble	Brown, transparent, smooth, and brittle	103.64 ± 1.36

4.3 Crosslinking reaction of polylactic acid, glutaraldehyde and chitosan

4.3.1 Crosslinking reaction between polylactic acid and glutaraldehyde

A sticky solution of LPLA was heated and stirred at 70°C to reduce viscosity. GA was slowly added into LPLA solution and stirred for 1 hour. The resulting solution was yellow to brown depending on the concentration of GA. Reaction of polylactic acid and glutaraldehyde was characterized by ^{1}H and ^{13}C-NMR spectroscopy, using chloroform-d ($CDCl_3$) as a solvent.

From the molecular structure of L-lactic acid, the highest signal intensity signal appears at δ = 1.23 and 4.03 ppm attributed to the methyl (CH_3) and methine

(C*H*) carbons, respectively. It is well-known that esterification of an alcohol results in deshielding of the H_α resonance and, to a less extent, that of the H_β resonance (Espartero *et al.*, 1996).

In this research, ^1H and ^{13}C-NMR spectra of LPLA are shown in Figure 4.9. ^1H-NMR spectrum (Figure 4.9 (a)) of LPLA is clearly constituted of two chemically different units, a. and b. Therefore, downfield signals (δ = 1.52 and 5.15 ppm) are assigned to C*H* and CH_3 protons in unit b, and upfield resonances (δ = 1.43 and 4.40 ppm) to C*H* and CH_3 protons in unit a. ^{13}C-NMR spectrum of LPLA consists of a different 3 carbon atoms, the signal at 179.2 ppm is assigned to the carbonyl carbon atom of lactic acid units. In addition, signal at δ = 20.1 and 77.5 ppm correspond to the methyl, methine carbon atoms, respectively (Figure 4.9 (b)).

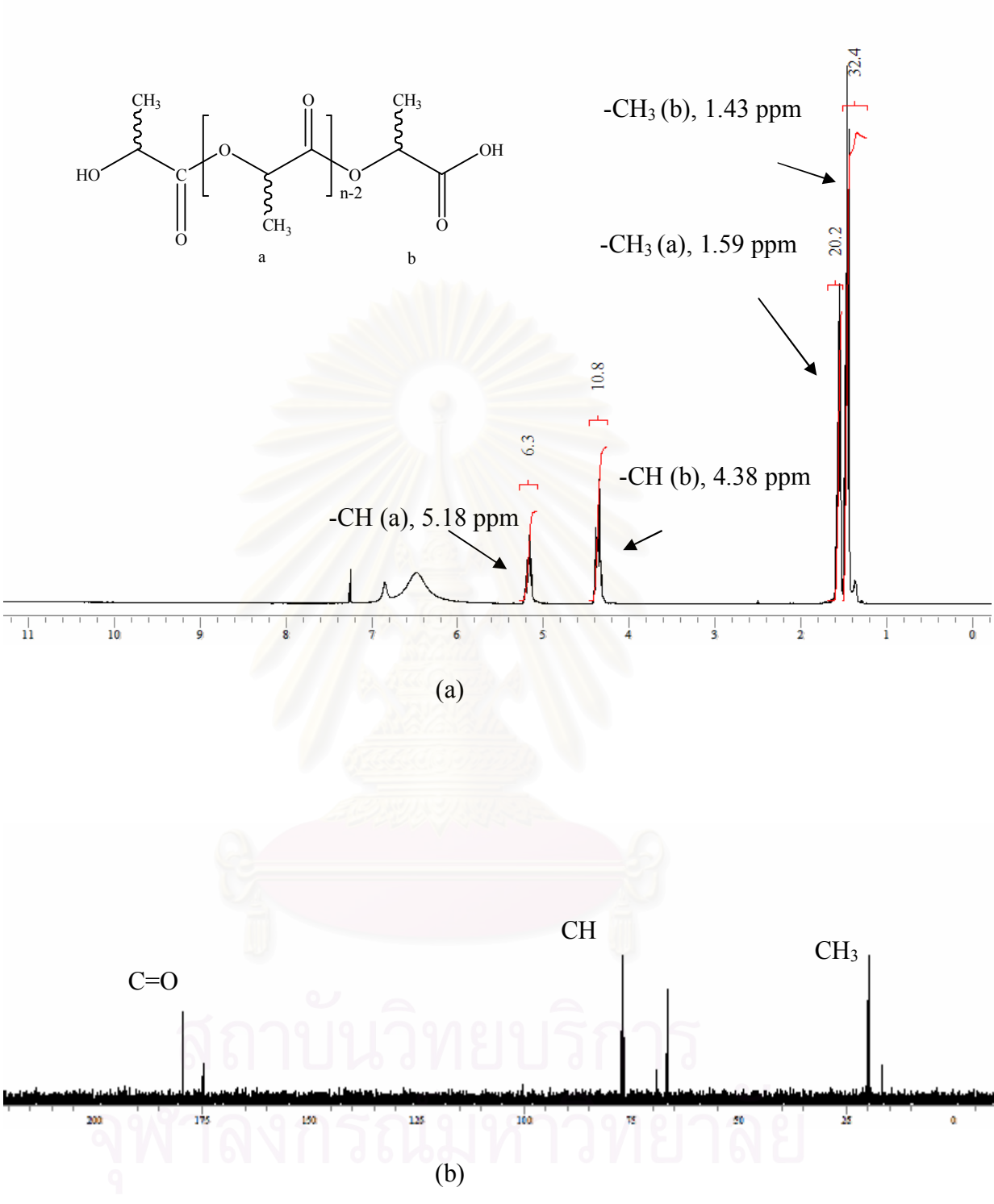


Figure 4.9 (a) ^{1}H-NMR and (b) ^{13}C-NMR spectra of low molecular weight polylactic acid (LPLA).

In the ^{1}H and ^{13}C-NMR spectra of LPLA reacted with GA (GA-LPLA), ^{1}H-NMR spectrum shows three new peaks consisting of signals at δ = 1.83, 5.6 and 9.7 ppm which are assigned to the methylene groups of glutaraldehyde, methine carbon,

directly attaching to two O_2 atoms and proton of aldehyde group, respectively (Figure 4.10). ^{13}C-NMR spectrum found at δ = 34.0 and 103.5 ppm are attributed to the carbon atom of methylene group and carbon atom, directly attaching to two O_2.

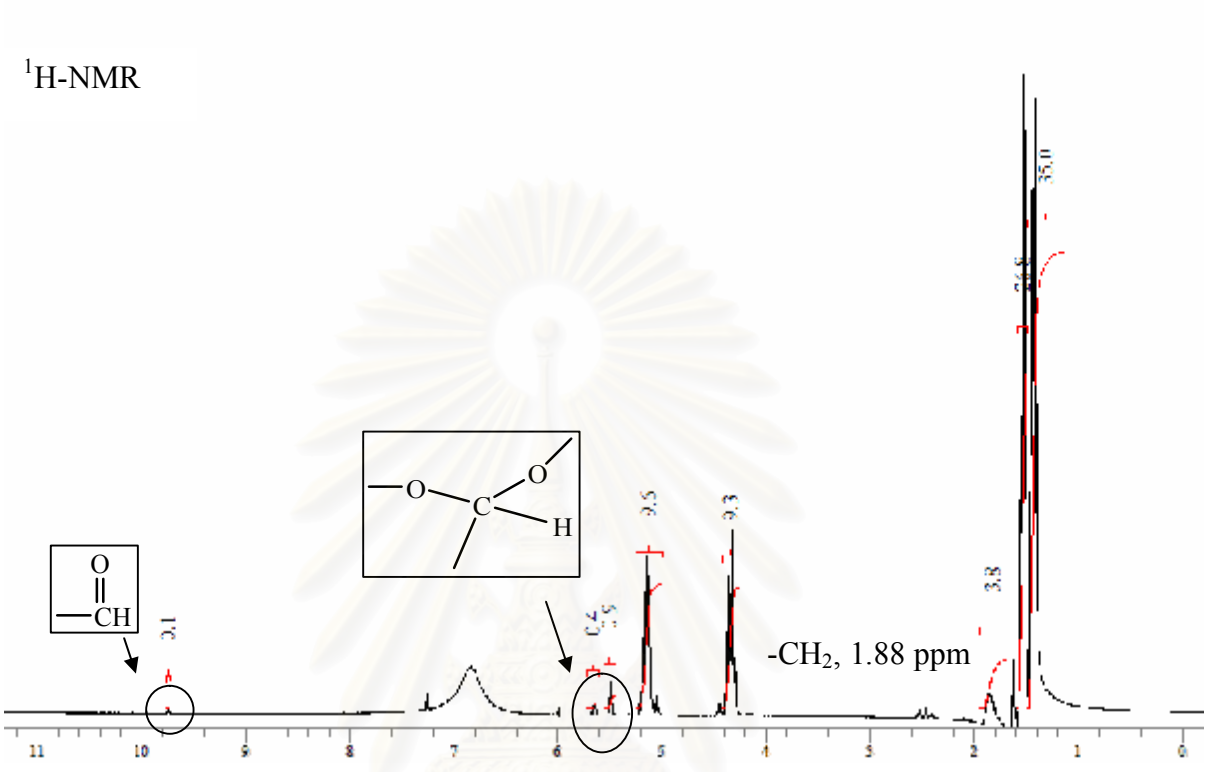
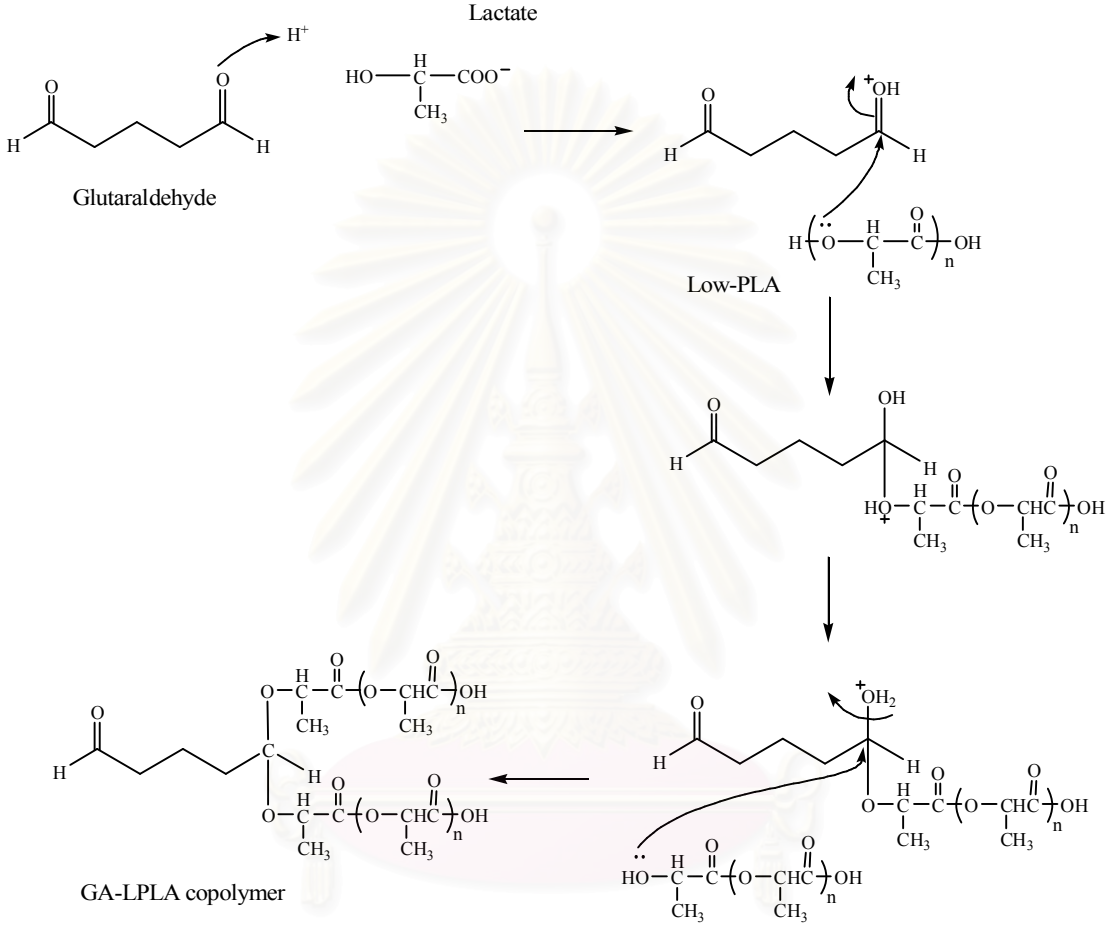


Figure 4.10 ^{1}H and ^{13}C-NMR spectra of polylactic acid reacting with glutaraldehyde.

The molecular structure of PLA composes of hydroxyl (-OH) and carboxylic acid (-COOH) functional groups. Aldehyde groups of glutaraldehyde are protonated and then hydroxyl group of PLA attracts carbon atom. Mechanism of LPLA and GA is shown in Scheme 4.2.



Scheme 4.2 The crosslinking mechanism of polylactic acid and glutaraldehyde (LPLA-GA).

4.3.2 Crosslinking reaction between chitosan and glutaraldehyde

The swelling capacity of chitosan can be improved by crosslinking, one of the most popular methods being used to modify chitosan in order to achieve desired properties. Some properties could be altered by crosslinking such as swelling, permeability, drug releasing, transport properties, water uptake, mechanical properties, chemical stability, sponge structure, and crystallinity.

Crosslinking reaction between chitosan and glutaraldehyde occurs from amino group of chitosan and aldehyde group of glutaraldehyde to form Schiff's base (-C=N- linkage). There are three distinct structures ; including formation of one Schiff's base, with one aldehyde group of the glutaraldehyde, two Schiff's bases involving both aldehyde groups of the glutaraldehyde molecule, and the glutaraldehyde undergoing an aldol condensation to polymerize in an aqueous environment, forming a greater crosslinking chain. After crosslinking, the glutaraldehyde-crosslinked-chitosan film turned yellow. The mechanism of crosslinking of chitosan with glutaraldehyde is presented by Wang ***et al.***, (2004). In this research, the copolymer film was analyzed by ^{13}C-NMR solid state spectroscopy to confirm a crosslinking of between chitosan and glutaraldehyde.

^{13}C-NMR solid state analysis, ^{13}C-NMR spectra of PLA, chitosan and CLG film are shown in Figure 4.11. The peak of chitosan found at 105.1 ppm belongs to C1 carbon, directly attaching to two oxygen atoms, while the peak at 23.7 and 174.0 ppm, are attributed to methyl and carbonyl groups. These peaks remain in the chain of chitosan, as a consequence of the incomplete deacetylation of the chitin. In the copolymer film, the peaks at 19.0 and 21.9 ppm are attributed to different chemical environments of the CH_2 group. The peak at 140.95 ppm is assigned to the double ethylenic bond (C=C) and the peaks at 174.3 and 182.2 ppm are assigned to the double imine bond (N=C) (Oyrton ***et al.***, 1999).

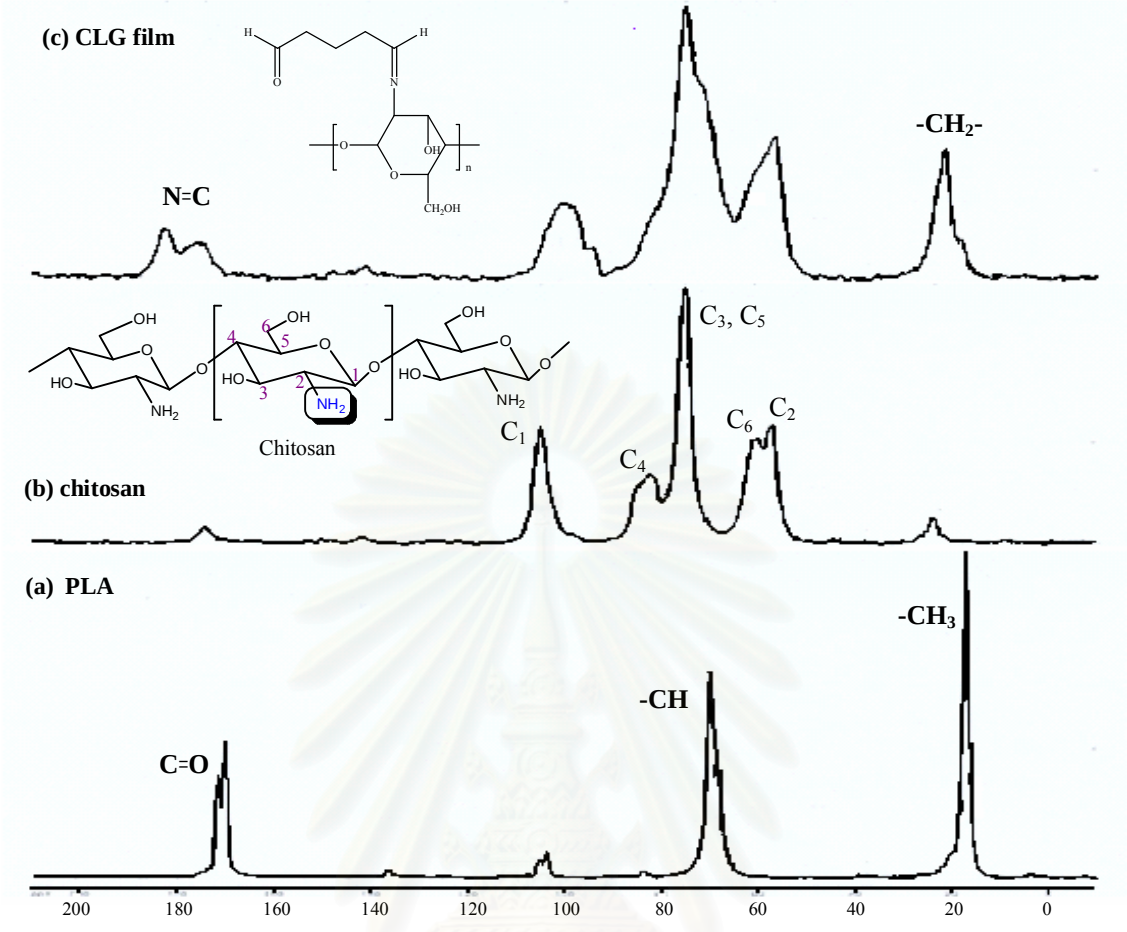


Figure 4.11 ^{13}C-NMR solid state spectra of (a) PLA, (b) chitosan and (c) CLG film.

4.4 Effect of a plasticizing agent on the copolymer films

It was found that the films with low swelling capacity (molar ratio of CS:GA = 1:0.48, called CLG3 copolymer film and molar ratio of CS:GA:LPLA = 1:0.8:1.8, called CLG5-1.0%LPLA copolymer film) were brittle (Table 4.3 and 4.5). To improve the film flexibility, plasticizers have been used so far. Plasticizing agents are essential generally to overcome the brittleness of the copolymer films. Brittleness is an inherent quality attributed to the complex/branched primary structure and weak intermolecular forces of natural polymers. Plasticizers by reducing the intermolecular forces soften the rigidity of the film structure and increase the mobility of the biopolymeric chains, thus in this research, glycerol (Gly), polyethylene glycol (PEG), and glycerol monostearate (GMS) were used as plasticizer to improve copolymer films flexibility. The surface of copolymer films with the addition of Gly and PEG were fragile after soaking in methanol because both Gly and PEG were soluble in

methanol. The effect of plasticizer on physical appearance of copolymer films are shown in Figure 4.12 and Table 4.6.

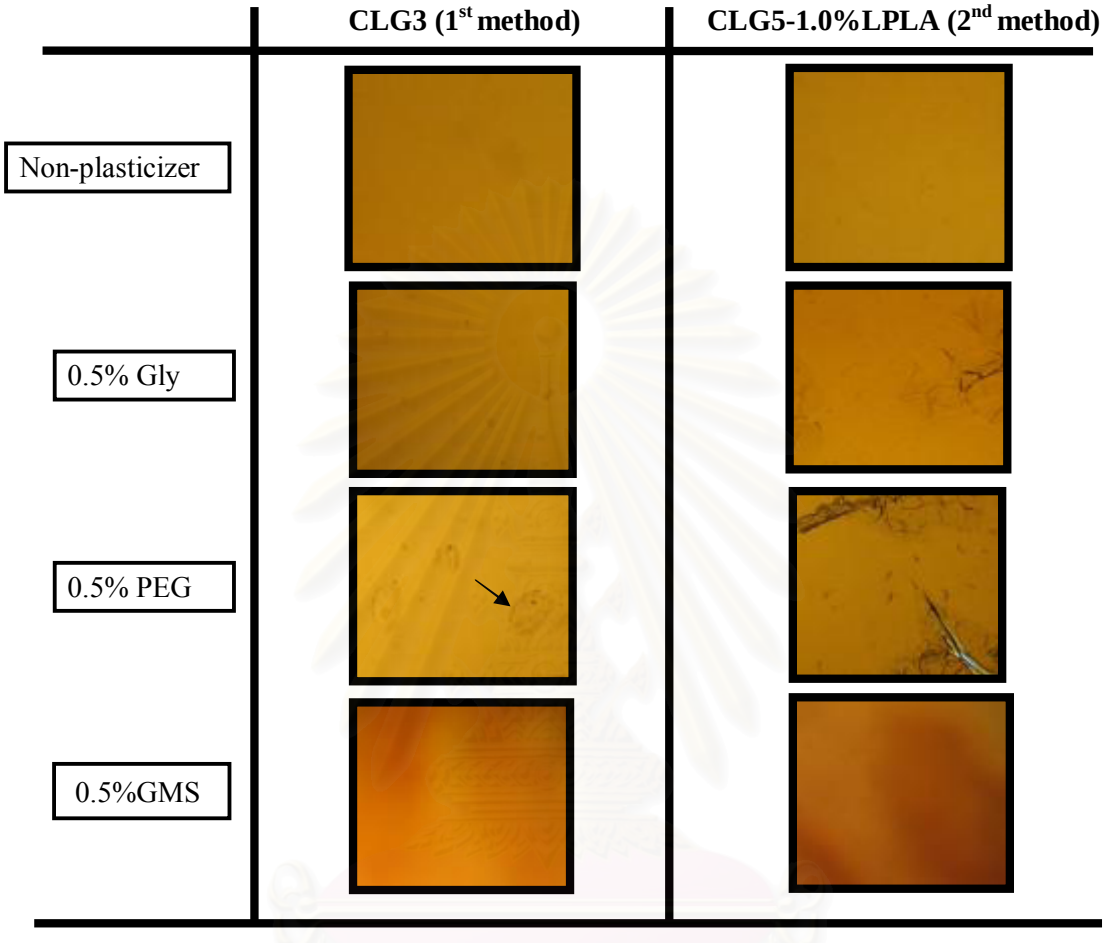


Figure 4.12 Effect of plasticizing agent on the L-lactic acid-chitosan (CLG3) and low molecular weight polylactide-co-chitosan (CLG5-1.0%LPLA) copolymer films surface.

When GMS was used in film preparation, homogeneous film solution was obtained with the help of homogenizer since GMS was insoluble in methanol. After centrifugation, the solution turned from turbid yellow to clear yellow. After casting, the copolymer film was orange-brown with a wavy surface.

Table 4.6 Effect of plasticizers on film appearance of copolymer films (CLG3 and CLG5-1.0%LPLA) at casting temperature of 65°C

Copolymer film	GA (%)	Plasticizer (%)			Film appearance
		Glycerol (Gly)	Polyethylene glycol (PEG)	Glycerol monostearate (GMS)	
CLG3	3	-	-	-	Orange film with smooth surface
CLG3-0.25%Gly	3	0.25	-	-	Orange film with fragile surface, and heterogeneous film
CLG3-0.5%Gly	3	0.50	-	-	Orange film with fragile surface, and heterogeneous film
CLG3-0.5%PEG	3	-	0.50	-	Orange film with very fragile surface, and heterogeneous film
CLG3-1.0%PEG	3	-	1.00	-	Orange film with very fragile surface, and heterogeneous film
CLG3-0.5%GMS	3	-	-	0.50	Orange and heterogeneous film with not smooth thickness
CLG5-1.0%LPLA	5	-	-	-	Orange film with smooth surface
CLG5-1.0%LPLA-0.5%Gly	5	0.50	-	-	Orange film with fragile surface, and heterogeneous film
CLG5-1.0%LPLA-0.5%PEG	5	-	0.50	-	Orange film with very fragile surface, and heterogeneous film
CLG5-1.0%LPLA-0.5%GMS	5	-	-	0.50	Orange and heterogeneous film with not smooth thickness

Swelling property of the CLG3 and CLG5-1.0% (w/v) LPLA copolymer films with and without GMS at casting temperature of 65°C and room temperature, are compared in Figure 4.13. It was observed that GMS and casting temperature had no significant in decreasing film swelling capacity.

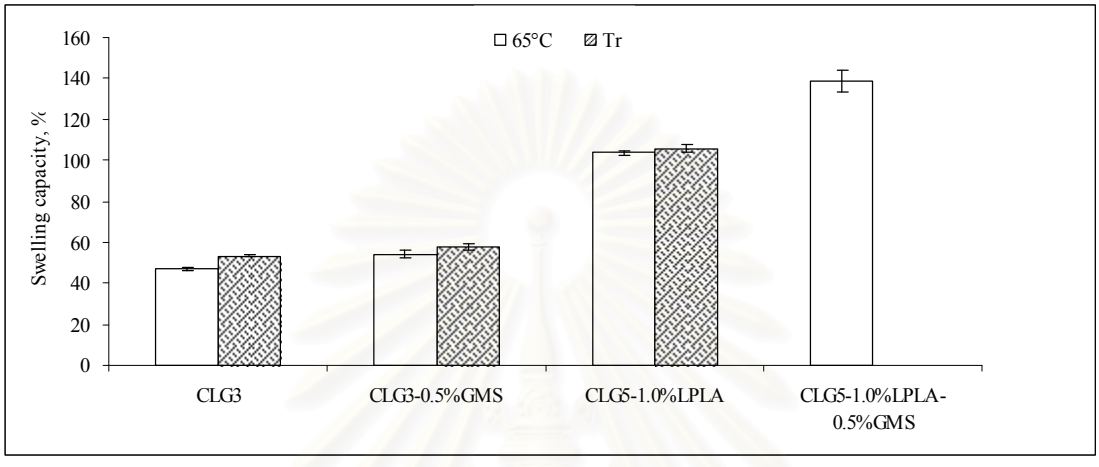


Figure 4.13 Swelling capacity of the CLG3, CLG3-0.5%GMS, CLG5-1.0%LPLA, and CLG5-1.0%LPLA-0.5%GMS copolymer films at casting temperature of 65°C and room temperature.

4.5 Copolymer film characterization

Surface characterization is a method for analyzing chemical and physical properties of material surface. In this research, surface of copolymer films, before and after modification, were analyzed for functional groups using ATR-IR. DSC technique was used to study glass transition temperature (T_g) and melting temperature (T_m) of copolymer films. While the crystalline structure of the film samples were analyzed by an X-ray diffraction (XRD) technique.

4.5.1 IR spectral analysis of film samples

4.5.1.1 L-lactic acid-chitosan copolymer film using glutaraldehyde (CLG film)

IR spectra of L-lactic acid-chitosan (CL) copolymers were compared with IR spectra of chitosan and PLA in Figure 4.14. IR spectrum of chitosan shows peaks assigned to the saccharine structure at 894 and 1150 cm^{-1}. The strong peak at 1577 cm^{-1} is attributed to amino characteristic (NH_2 deformation) and the absorption bands at 1653 and 1318 cm^{-1} are attributed to the amide I and III bands of *N*-acetylated chitosan, respectively. Compared with the IR spectrum of chitosan, IR spectrum of the CL copolymer and crosslinked CL copolymer with glutaraldehyde (CLG) generate a new peak appearing around 1732 cm^{-1}, corresponding to the ester or carboxylic groups of oligomer-L-lactic acid (OLLA) existing as freedom or side chain (Yao *et al.*, 2003). An obvious shift to a lower wave number in comparison with PLA, appearing at 1747 cm^{-1}, and peak of the remaining amino groups that shifted from 1577 to 1580 cm^{-1} can be observed and are attributed to the formation of hydrogen bonds between the ester groups of OLLA and amino or hydroxyl groups of chitosan. Moreover, the methyl asymmetric deformation of polylactic acid side chains appears at ~ 1452 cm^{-1}.

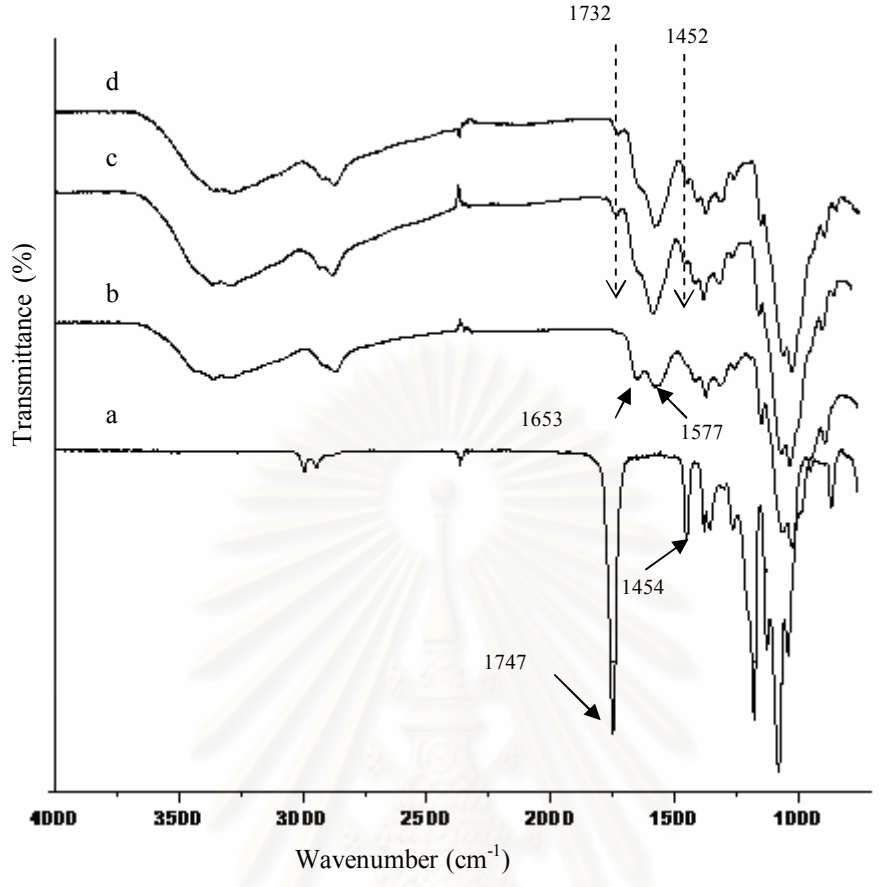


Figure 4.14 The ATR-IR spectra of (a) PLA, (b) chitosan, (c) CL and (d) CLG1 copolymers film.

4.5.1.2 Low molecular weight polylactic acid-chitosan copolymer film using glutaraldehyde (CLG-LPLA film)

The IR spectra of CLG1-0.5%LPLA copolymer film and that of with CLG1 copolymer film, indicate the absorption peak at 1736 and 1732 cm^{-1}, respectively corresponding to the ester or carboxylic groups of OLLA existing as freedom or side chain. The peak at 1454 cm^{-1} is assigned to the methyl asymmetric deformation of polylactic acid side chains. IR spectrum of CLG1-0.5%LPLA film shows a new peak at 1210 cm^{-1} assigned to the symmetric C-O-C stretching of the ester group (Figure 4.15). IR spectrum of PLA shows peaks at 1177 and 1210 cm^{-1}, a doublet peak, corresponding to C-O-C stretching of ester group in side chain. So, both of the peaks at ~1736 and ~ 1210 cm^{-1} of CLG1-0.5%LPLA copolymer film are stronger than CLG1 copolymer because of the increased molecular weight of polylactic acid.

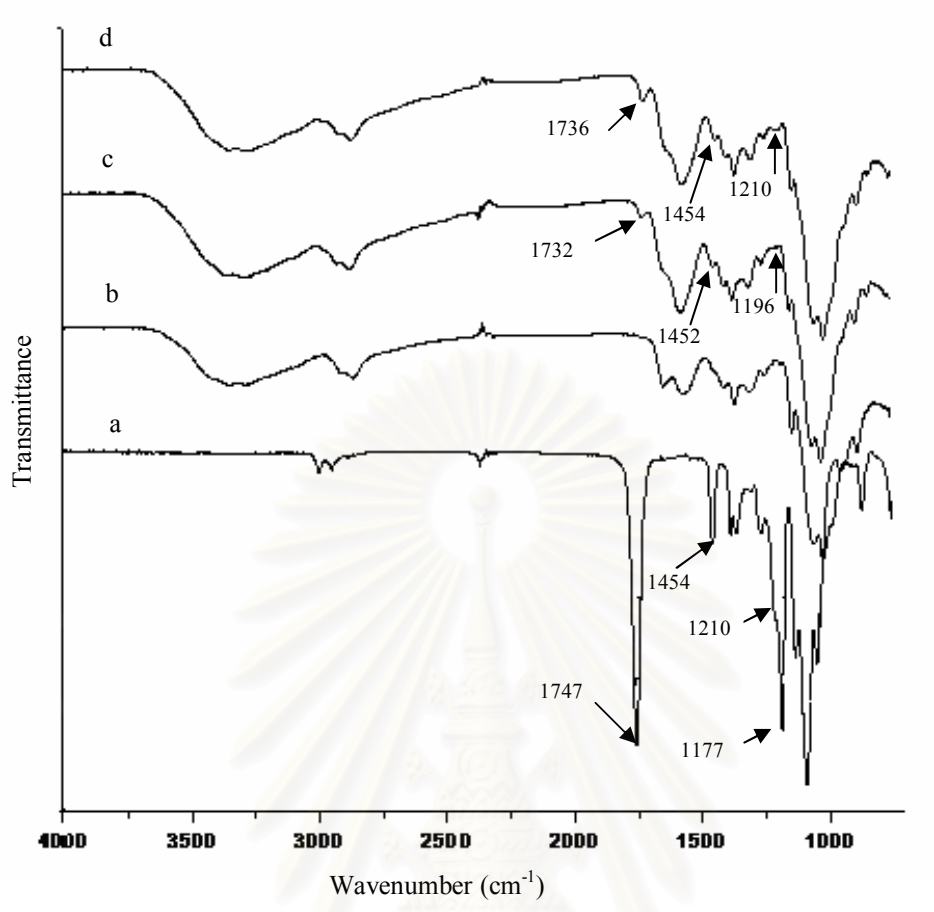


Figure 4.15 The IR spectra for (a) PLA, (b) chitosan, (c) CLG1 (d) and CLG1-0.5%LPLA copolymer films.

4.5.2 Differential scanning calorimeter (DSC)

Glass transition temperature (T_g) is an important parameter used to investigate whether two polymer are miscible in the amorphous phase. It is also well known that, for semicrystalline polymers, T_g is quite difficult to measure.

The miscibility of two polymers in the bulk amorphous phase can be evaluated by the presence of a single, composition-dependent T_g value between those of the constituent polymers.

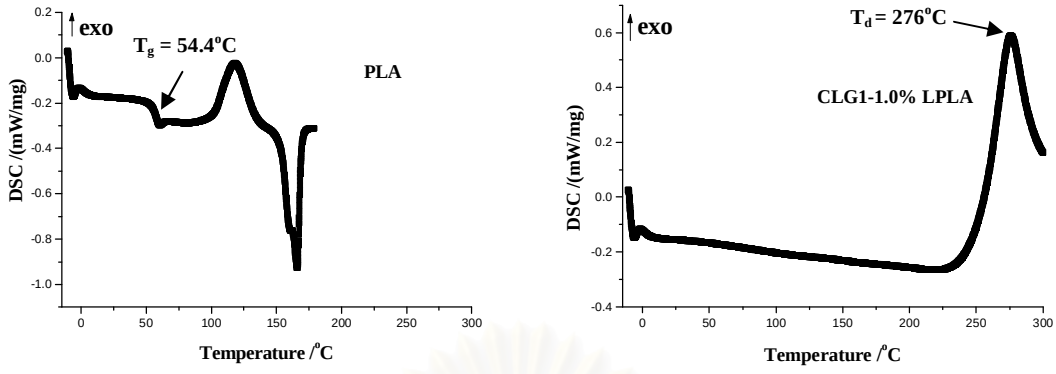


Figure 4.16 DSC thermograms of (a) PLA and (b) CLG1-1.0%LPLA copolymer film in the second heating scan.

T_g is taken at the initial change in the slope of the change in heat capacity of the DSC thermogram. Analysis of T_g of chitosan, determined though the baseline steps, was small for chitosan. This small baseline step is due to the molecular structure of chitosan consisting of rigid β-1,4-linked D-glucosamine units, so the change in heat capacity corresponding to the change in specific volume (or molecular mobility) at T_g is equally small (Suyatma *et al.,* 2004; Sakurai *et al.,* 2000).

The results of the second heating scan are shown in Figure 4.16. PLA is an amorphous polymer with glass transition temperature (T_g) of approximately 54.4°C, while T_g of both of the copolymer films prepared by two different methods could not be determined. Moreover, increasing of the second heating scan temperature indicated the thermal degradation (T_d) of CLG1-1.0%LPLA copolymer film at approximately 276°C.

4.5.3 X-ray diffraction (XRD)

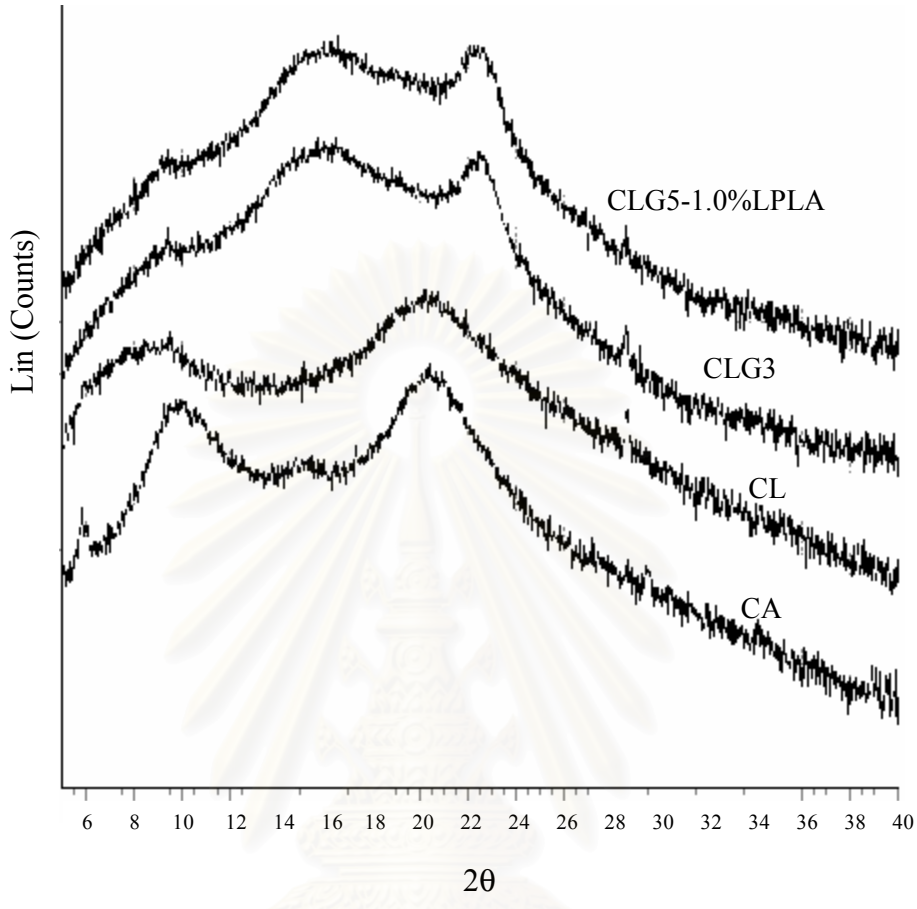


Figure 4.17 X-ray diffraction profiles of the chitosan, CL, CLG3 and CLG5-1.0%LPLA copolymer films.

The crystallinity of chitosan can be seen in the X-ray diffraction (XRD) analysis. X-ray diffraction profiles of chitosan, CL, CLG3 and CLG5-1.0%LPLA copolymer films are shown in Fig. 4.17. The strongest reflection of chitosan appears at $2\theta = 9.95^{o}$ and 20.36^{o} which are assigned to crystal forms I and forms II (Wu *et al., 2005*), respectively. Poly(L-lactic acid) crystallized in a pseudo-orthorhombic unit cell (dimensions a = 1.07 nm, b = 0.595 nm and c = 2.78 nm) contains two of 10^3 helices (α-form), the main peaks in X-ray diffraction profile appeared at 2θ = 15.00, 17.00 and 19.00 (Yao *et al., 2003*). Compared with chitosan (CA), CL film, lactic acid grafted onto chitosan appeared the reflection shift to $2\theta = 9.51^{o}$ and 20.25^{o}. In addition, it was found that the CL intensity at $2\theta = 9.51^{o}$ decreased. Since lactic acid was reacted with chitosan in homogeneous solution, therefore the grafting by lactic

acid took place at random along the chitosan chain, giving rise to a random copolymer.

Moreover, the crystallinity decreases with the addition of glutaraldehyde (GA) into CL solution, the peaks at $2\theta = 9.42^{o}$, 16.01^{o}, 19.12^{o} and 22.48^{o} appeared in CLG3 film, while in CLG5-1.0%LPLA films the peaks at $2\theta = 9.43^{o}$, 15.91^{o}, 18.99^{o} and 22.41^{o} appeared. The peak intensity of CLG5-1.0%LPLA film was higher than that of CLG3 film because of the longer PLA side chains. The peaks at 2θ values of 15^{o}, 17^{o}, 19^{o} and 22.34^{o} are attributed to the crystallization of PLA (Yao *et al.,* 2003). These results indicated that when lactic acid, and LPLA were grafted as well as GA was added onto chitosan, the crystalline patterns were different from chitosan, since the original crystallinity was destroyed.

4.5.4 Mechanical properties

The mechanical properties of the copolymer films were studied. Those properties including tensile strength (TS), Young's modulus (Y) and percent elongation at break (%E), are given in Figure 4.18.

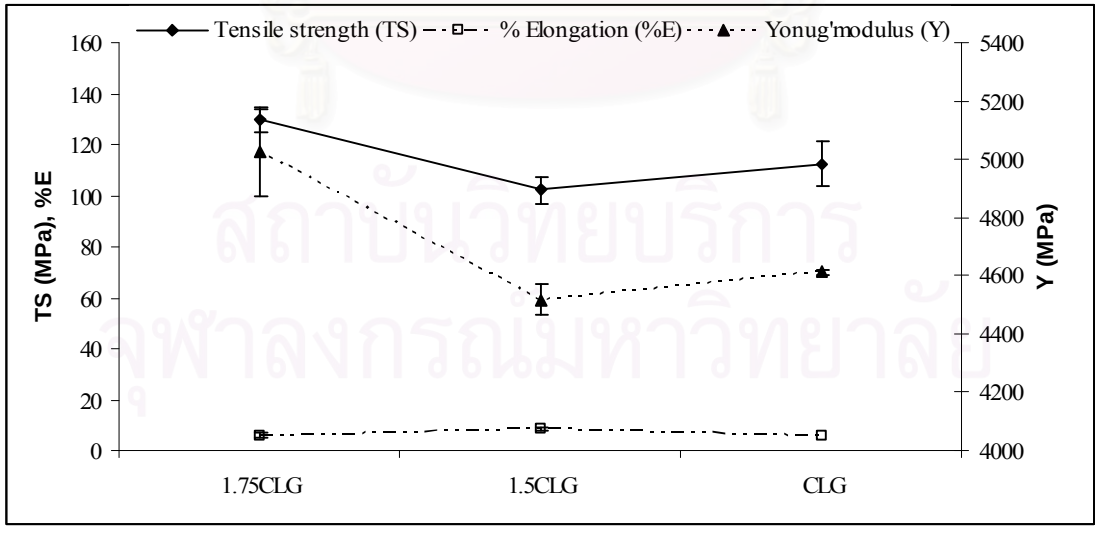
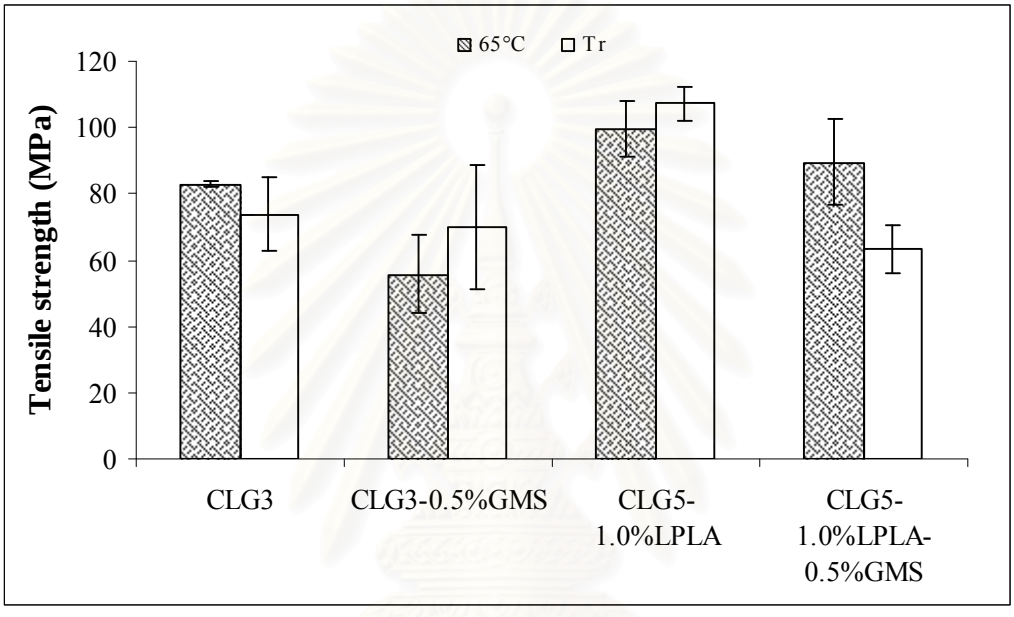
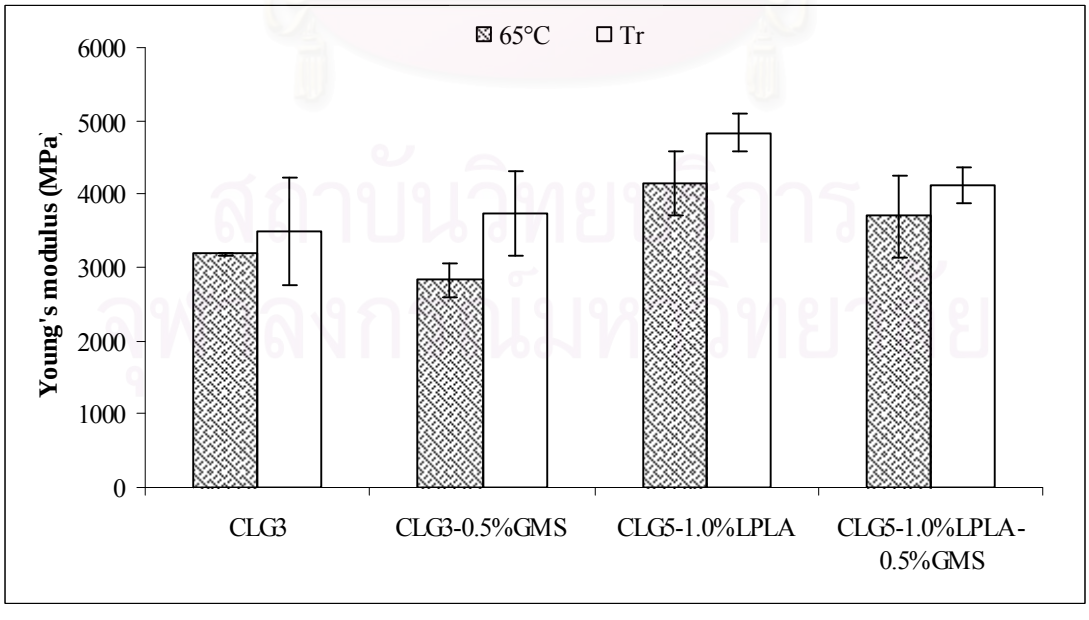


Figure 4.18 Tensile strength (MPa), Young's modulus (MPa), and % elongation at break of CLG copolymer films with a different weight ratio of chitosan : lactic acid.

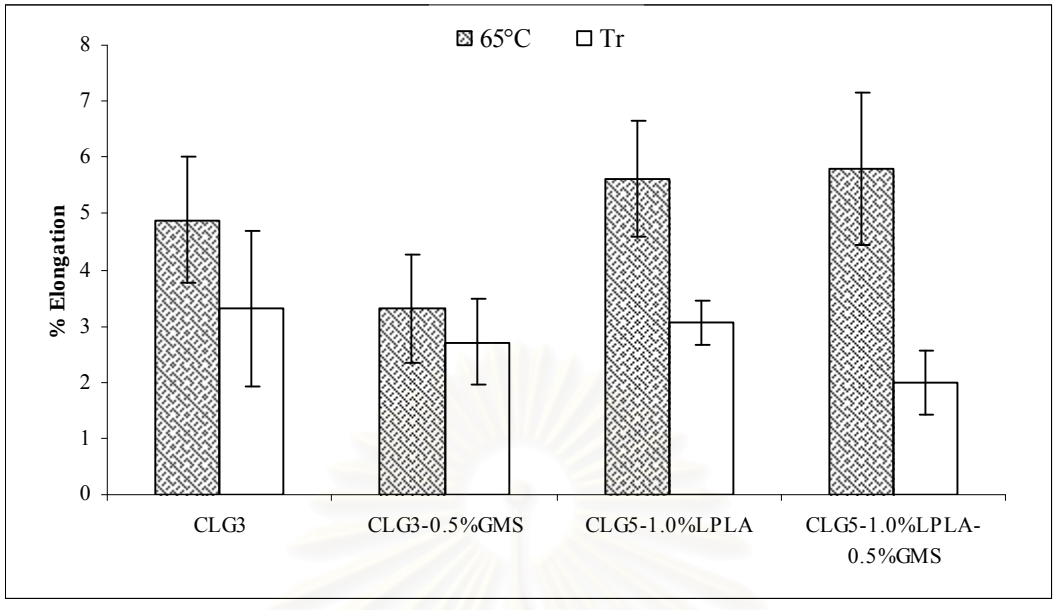
The tensile strength (TS), and Young's modulus (Y) of the CLG film were lower than those of the 1.75CLG film but higher than those of 1.5CLG film while %elongation at break of the 1.5CLG film was the highest. However, mechanical properties of these films were no significant difference in the 1.75CLG, 1.5CLG, and CLG films. Due to breakage at the tensiometer grip, the physical properties of 0.75CLG and 0.5CLG films could not be determined.



(a)



(b)



(c)

Figure 4.19 Mechanical properties of CLG3 and CLG3-1.0%LPLA films with and without GMS; (a) tensile strength (MPa), (b) Young's modulus (MPa), and (c) % elongation at break.

Comparing the CLG5-1.0%LPLA copolymer film with the CLG3 copolymer film without LPLA, the CLG5-1.0%LPLA copolymer film showed an increase in the tensile strength, and Young's modulus but no significant difference was found in the elongation at break between CLG3 and CLG5-1.0%LPLA copolymer films. While, it was observed that GMS affected the mechanical properties of copolymer films as shown in Figure 4.19. That is when GMS was present, the decrease trend in the tensile strength, Young's modulus, and elongation at break because of the difficult miscibility of LPLA and chitosan with GMS film forming solution, leading to heterogeneous film with a rough surface. Although, adding of GMS can not be improve the brittleness of copolymer films but LPLA was added, affected the increase in the tensile strength value from 82.83 ± 0.87 MPa to 99.46 ± 8.59 MPa and in the Young's modulus value from 3177.57 ± 18.11 MPa to 4146.31 ± 431.97 MPa. Comparison of mechanical properties of CLG3 and CLG5-1.0%LPLA copolymer films with other research were shown in Table 4.7.

Table 4.7 Comparison of mechanical properties of CLG3 and CLG5-1.0%LPLA copolymer films with other research

Film	Conditions	TS (MPa)	Y (MPa)	%E	Note
Cassava starch blend	GLY: 1.0 g PEG: 0.3 g	0.473± 0.024	-	27.60 ± 1.33	Parra *et al*., 2004
CS-PLA blends	Chit/PLA: 100/0 (w/w)	82.4 ± 8.5	534 ± 44	5.2 ± 0.9	Suyatma *et al*., 2004
	Chit/PLA: 90/10 (w/w)	72.7 ± 1.8	470 ± 20	4.9 ± 0.5	
	Chit/PLA: 80/20 (w/w)	64.4 ± 5.1	433 ± 35	4.2 ± 0.9	
	Chit/PLA: 70/30 (w/w)	54.5 ± 2.9	406 ± 51	4.1 ± 0.5	
	Chit/PLA: 0/100 (w/w)	52.5 ± 5.9	384 ± 35	3.6 ± 0.5	
H-chitosan/PLA blends	H-chitosan/PLA: 100/0 (w/w)	1.8	240	890	Peesan *et al*., 2005
	H-chitosan/PLA: 80/20 (w/w)	2.5	270	40	
	H-chitosan/PLA: 50/50 (w/w)	2.6	550	6.5	
	H-chitosan/PLA: 20/80 (w/w)	17.5	950	14.5	
	H-chitosan/PLA: 0/100 (w/w)	33.3	1390	10	

Table 4.7 Comparison of mechanical properties of CLG3 and CLG5-1.0%LPLA copolymer films with other research (Continuous)

Film	Conditions	TS (MPa)	Y (MPa)	%E	Note
CL	LA/CS = 0.5 (w/w)	29.0	-	-	Yao *et al*., 2003
	LA/CS = 1.0 (w/w)	77.0	-	-	
	LA/CS = 2.0 (w/w)	92.5	-	-	
	LA/CS = 3.0 (w/w)	55.0	-	-	
	LA/CS = 4.0 (w/w)	46.0	-	-	
CLG3	CS: 1.0 g LA: 1.0 g GA: 3.0%	82.83 ± 0.87	3177.57 ± 18.11	4.88 ± 1.12	
CLG5-1.0%LPLA	CS: 1.0 g LA: 1.0 g GA: 3.0% LPLA: 1.0%	99.46 ± 8.59	4146.31 ± 431.97	5.61 ± 1.04	

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In this work the copolymer films of low molecular weight polylactic acid and chitosan using crosslinking agent were prepared by 2 methods. In the first method, L-lactic acid was directly grafted onto chitosan. Whereas in the second method, low molecular weight polylactic acid obtained by linear polycondensation using toluene-4-sulfonic acid monohydrate (PTSA) as a catalyst and chitosan were copolymerized with the help of crosslinking agent. The copolymer film was prepared by solution casting technique.

In the first method, the effect of the weight ratio of chitosan to lactic acid (CS:LA) from 1.75:1 to 0.50:1 while molar ratio of chitosan to glutaraldehyde (CS:GA) was kept constant at 1:0.05 on swelling capacity were studied. It was found that all films soaking in methanol exhibited flexible and transparent yellow. Decreasing weight ratio of CS:LA resulted in the decrease in swelling capacity (shown in Figure 4.4). So, weight ratio of chitosan to lactic acid (CS:LA) at 1:1 was used in further copolymer films preparation.

It was found that increasing GA concentration helped reduce swelling capacity (%) of films. CLG3 film with molar ratio of CS:GA at 1:0.48 exhibited the lowest swelling capacity (47.02 ± 0.46 % in Table 4.3), as well as tensile strength (MPa), Young's modulus (MPa), and % elongation at break value (82.83 ± 0.87 MPa, 3177.57 ± 18.11 MPa, and 4.88 ± 1.12 %, respectively). However, GA concentration higher than 1.95×10^{-3} mole (molar ratio of CS:GA at 1:0.48) yielded very brittle films.

In the second method, low molecular weight polylactic acid was prepared by linear polycondensation. Toluene-4-sulfonic acid monohydrate (PTSA) was used as a catalyst to increase molecular weight of polylactic acid ($\overline{M}_w$ changed from 260 to

1379 in Table 4.4). From preliminary test of swelling capacity of the film with different amount of LPLA (from 0.5% (w/v) to 2.5% (w/v)), CLG5-1.0%LPLA film showed the lowest swelling capacity (103.64 ± 1.36% in Table 4.5). Tensile strength (MPa), Young's modulus (MPa), and % elongation at break value (99.46 ± 8.59 MPa, 4146.31 ± 431.97 MPa, and 5.61 ± 1.04 %, respectively).

Crosslinking agents including glutaraldehyde (GA), maleic anhydride (MA), and succinic anhydride (SA) were used as the crosslinking agent for copolymer film preparation. It can be seen that glutaraldehyde could effectively reduce the swelling capacity of the CL film; resulting in the crosslinked film that exhibited the lowest swelling capacity. On the other hand, MA and SA as crosslinking agents did not help improve the swelling capacity of the copolymer films.

In conclusion, it was found that CLG5-1.0%LPLA film prepared by the second method showed a higher tensile strength and Young's modulus value than the CLG3 film prepared by the first method but no significant difference was observed in the elongation at break between CLG3 and CLG5-1.0%LPLA copolymer films.

Glycerol (Gly), polyethylene glycol (PEG), and glycerol monostearate (GMS) were used as plasticizing agents to improve the brittleness of the copolymer films. It was found that GMS affected the mechanical properties of the copolymer films, resulted in a decrease in the tensile strength, Young's modulus, and elongation at break.

5.2 Recommendation

In this work, it was found that with the help of glutaraldehyde as the crosslinking agent, copolymer films were prepared at various swelling capacity depending on CS-GA molar ratio. Beside, mechanical properties of the prepared films were dependent on the CS-GA molar ratio as well as the addition of LPLA. Two methods in preparing copolymer films obtained in this study can be used to prepare and manipulate the copolymer film to desired properties (swelling capacity and

mechanical properties) for certain application including drug delivery and controlled release.

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APPENDICES

APPENDIX A

MOLECULAR WEIGHT OF

POLYLACTIC ACID DETERMINATION

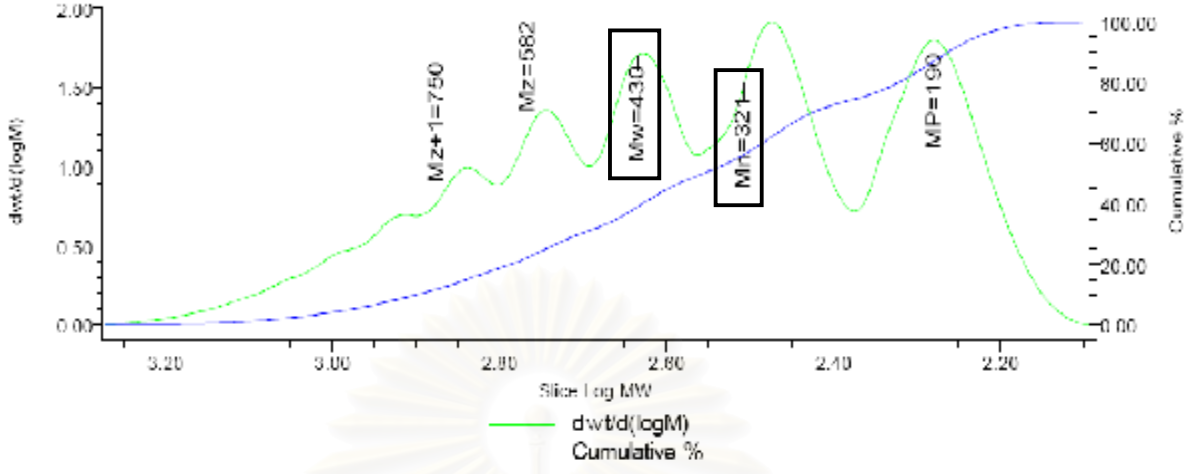


Figure A1 The chromatogram of polylactic acid using 1%(v/v) glutaraldehyde, 70°C, for 15 mins ($\overline{M}_w$ = 430, and $\overline{M}_n$=321).

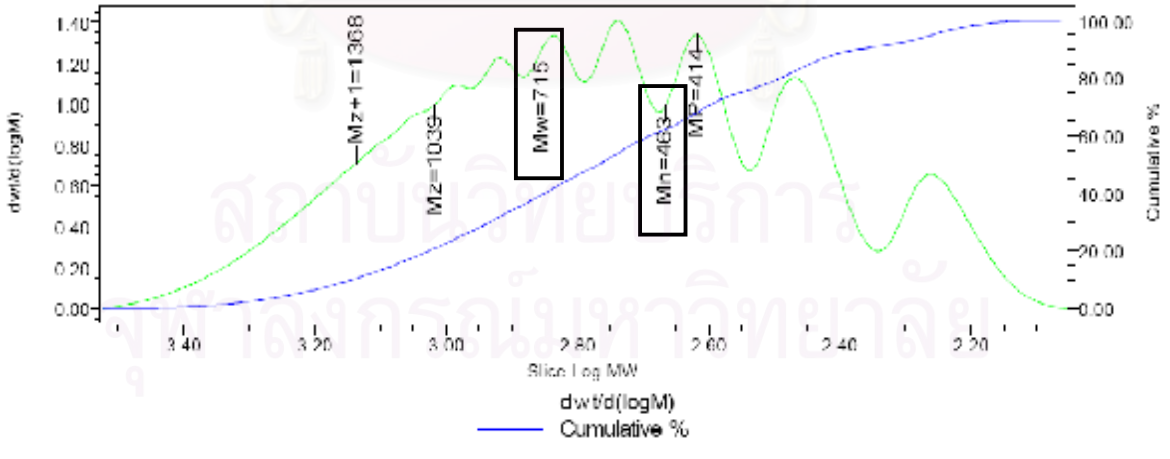


Figure A2 The chromatogram of polylactic acid under stirring at 70°C, for 15 mins ($\overline{M}_w$ = 715, and $\overline{M}_n$=463).

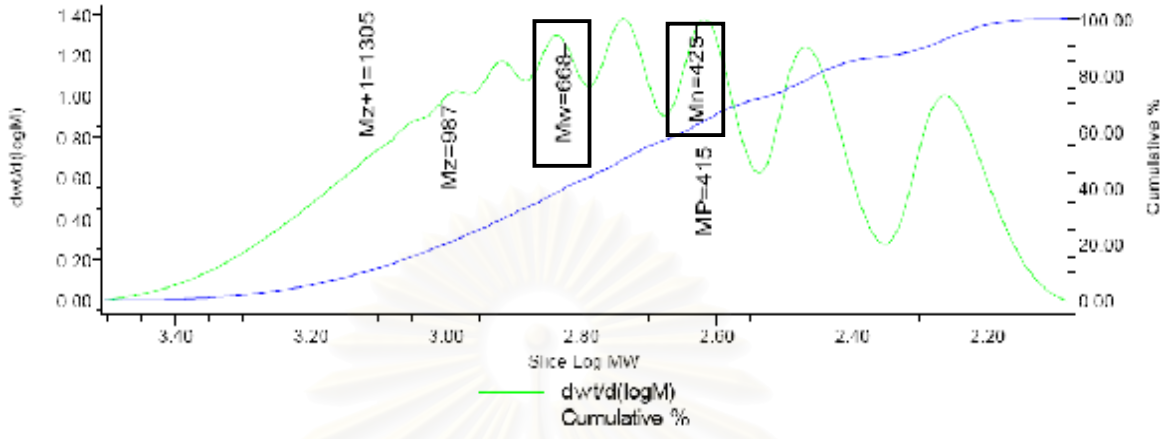


Figure A3 The chromatogram of polylactic acid using 1% stannous(II) 2-ethylhexanoate ($Sn(Oct)_2$) ($\overline{M}_w$ = 715, and $\overline{M}_n$=463).

APPENDIX B

DETERMINATION OF SWELLING CAPACITY OF COPOLYMER FILMS

Table B1 Raw data of swelling capacity of copolymer film with molar ratio of chitosan to glutaraldehyde at 1:0.48

Time	Swelling capacity (%)						Swelling capacity (%) ± S.D.
	1		2		3		
	Weight (g)	Swelling (%)	Weight (g)	Swelling (%)	Weight (g)	Swelling (%)	
0	0.0457	-	0.0416	-	0.0431	-	-
1	0.0574	25.60	0.0525	26.20	0.0579	34.34	28.71 ± 4.88
2	0.0607	32.82	0.0606	45.67	0.0590	36.89	38.46 ± 6.57
3	0.0628	37.42	0.0617	48.32	0.0579	34.34	40.02 ± 7.34
4	0.0630	37.86	0.0617	48.32	0.0585	35.73	40.63 ± 6.74
5	0.0636	39.17	0.0620	49.04	0.0603	39.91	42.70 ± 5.50
7	0.0643	40.70	0.0620	49.04	0.0615	42.69	44.14 ± 4.35
10	0.0656	43.54	0.0628	50.96	0.0617	43.16	45.89 ± 4.40
15	0.0656	43.54	0.0622	49.52	0.0613	42.23	45.10 ± 3.89
20	0.0653	42.89	0.0616	48.08	0.0626	45.24	45.40 ± 2.60
30	0.0654	43.11	0.0618	48.56	0.0630	46.17	45.95 ± 2.73
40	0.0650	42.23	0.0622	49.52	0.0615	42.69	44.81 ± 4.08
60	0.0657	43.76	0.0622	49.52	0.0631	46.40	46.56 ± 2.88
90	0.0667	45.95	0.0623	49.76	0.0625	45.01	46.91 ± 2.51
120	0.0663	45.08	0.0625	50.24	0.0632	46.64	47.32 ± 2.65
150	0.0660	44.42	0.0628	50.96	0.0623	44.55	46.64 ± 3.74
180	0.0662	44.86	0.0634	52.40	0.0628	45.71	47.66 ± 4.13

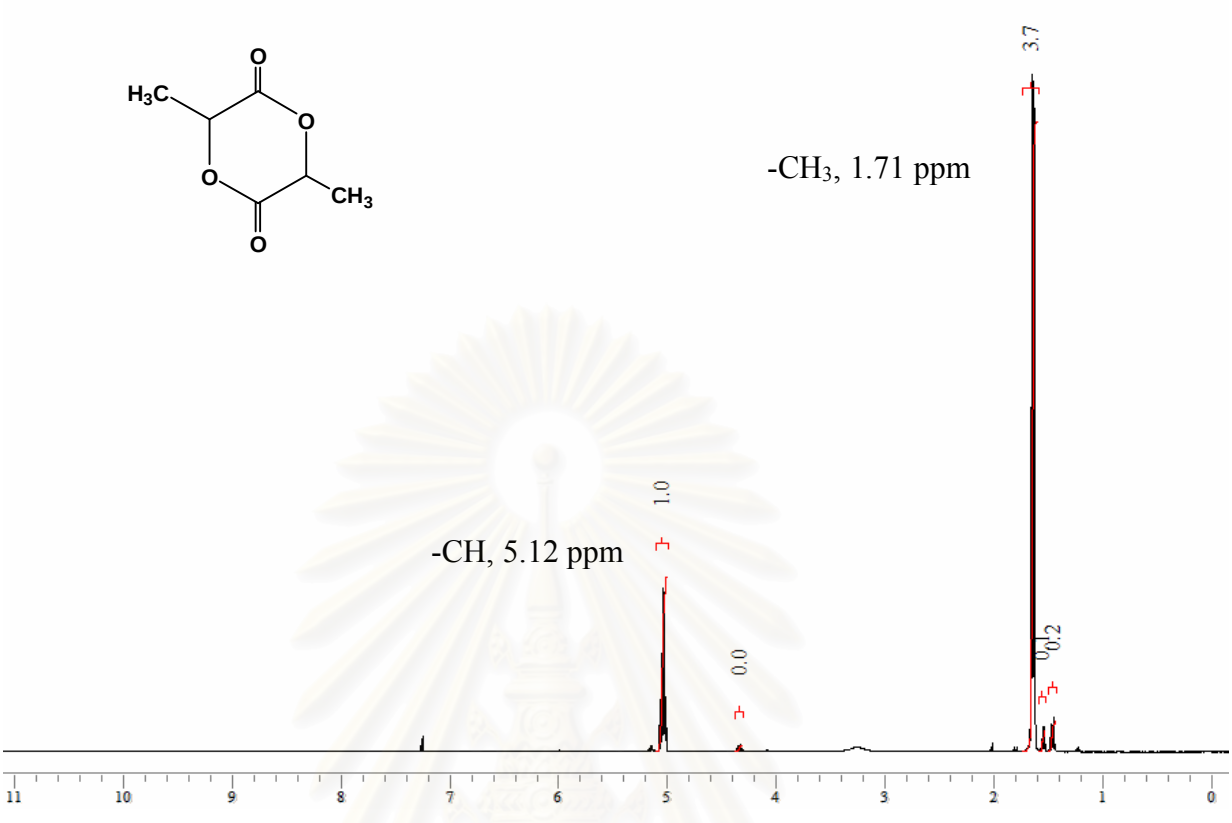
Table B2 Raw data of swelling capacity of copolymer film with molar ratio of chitosan : glutaraldehyde : LPLA at 1:0.80:1.8

Time	Swelling capacity (%)						Swelling capacity (%) ± S.D.
	1		2		3		
	Weight (g)	Swelling (%)	Weight (g)	Swelling (%)	Weight (g)	Swelling (%)	
0	0.0576		0.0486		0.0470		-
1	0.0834	44.79	0.0741	52.47	0.0679	44.47	47.24 ± 4.53
2	0.0971	68.58	0.0850	74.90	0.0810	72.34	71.94 ± 3.18
3	0.1057	83.51	0.0902	85.60	0.0858	82.55	83.89 ± 1.56
4	0.1074	86.46	0.0913	87.86	0.0876	86.38	86.90 ± 0.83
5	0.1108	92.36	0.0935	92.39	0.0891	89.57	91.44 ± 1.62
7	0.1120	94.44	0.0944	94.24	0.0914	94.47	94.38 ± 0.13
10	0.1125	95.31	0.0964	98.35	0.0907	92.98	95.55 ± 2.70
15	0.1145	98.78	0.0981	101.85	0.0907	92.98	97.87 ± 4.51
20	0.1146	98.96	0.0945	94.44	0.0955	103.19	98.86 ± 4.37
30	0.1163	101.91	0.0974	100.41	0.0965	105.32	102.55 ± 2.52
40	0.1173	103.65	0.0990	103.70	0.0926	97.02	101.46 ± 3.84
60	0.1174	103.82	0.0992	104.12	0.0946	101.28	103.07 ± 1.56
90	0.1196	107.64	0.1000	105.76	0.0935	98.94	104.11 ± 4.58
120	0.1170	103.13	0.1007	107.20	0.0951	102.34	104.22 ± 2.61
150	0.1202	108.68	0.0993	104.32	0.0956	103.40	105.47 ± 2.82
180	0.1192	106.94	0.1002	106.17	0.0943	100.64	104.59 ± 3.44

APPENDIX C

^{1}H-NMR, ^{13}C-NMR SPECTROSCOPY

(a)



(b)

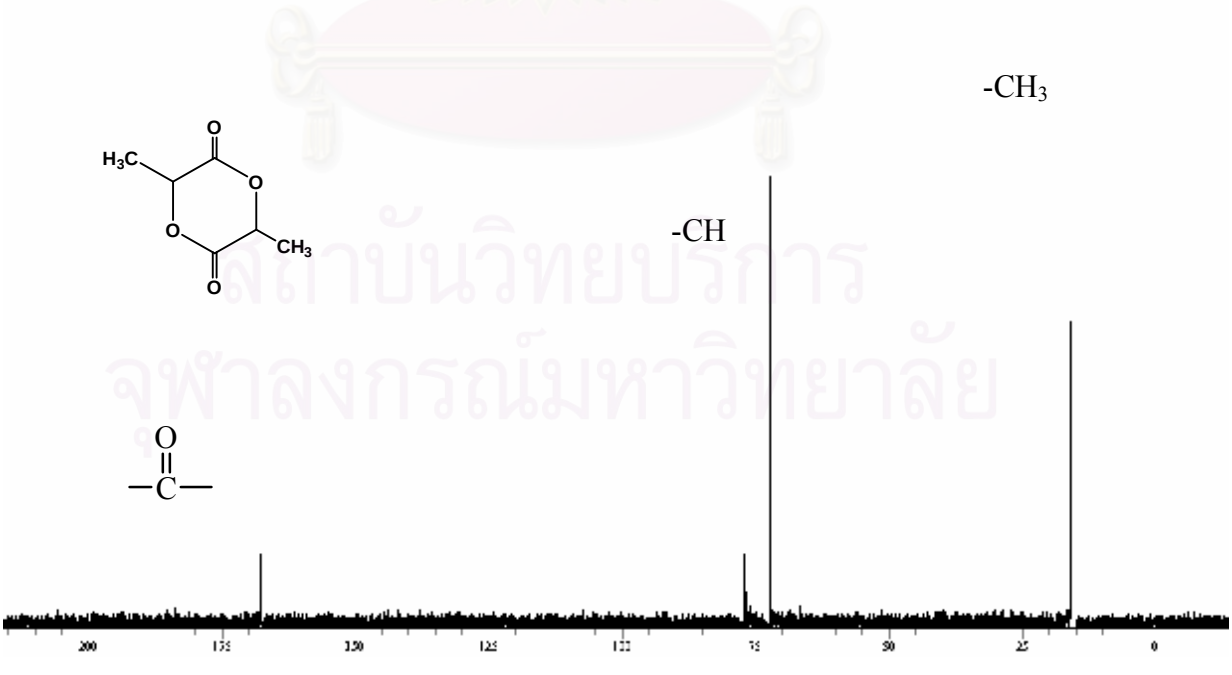
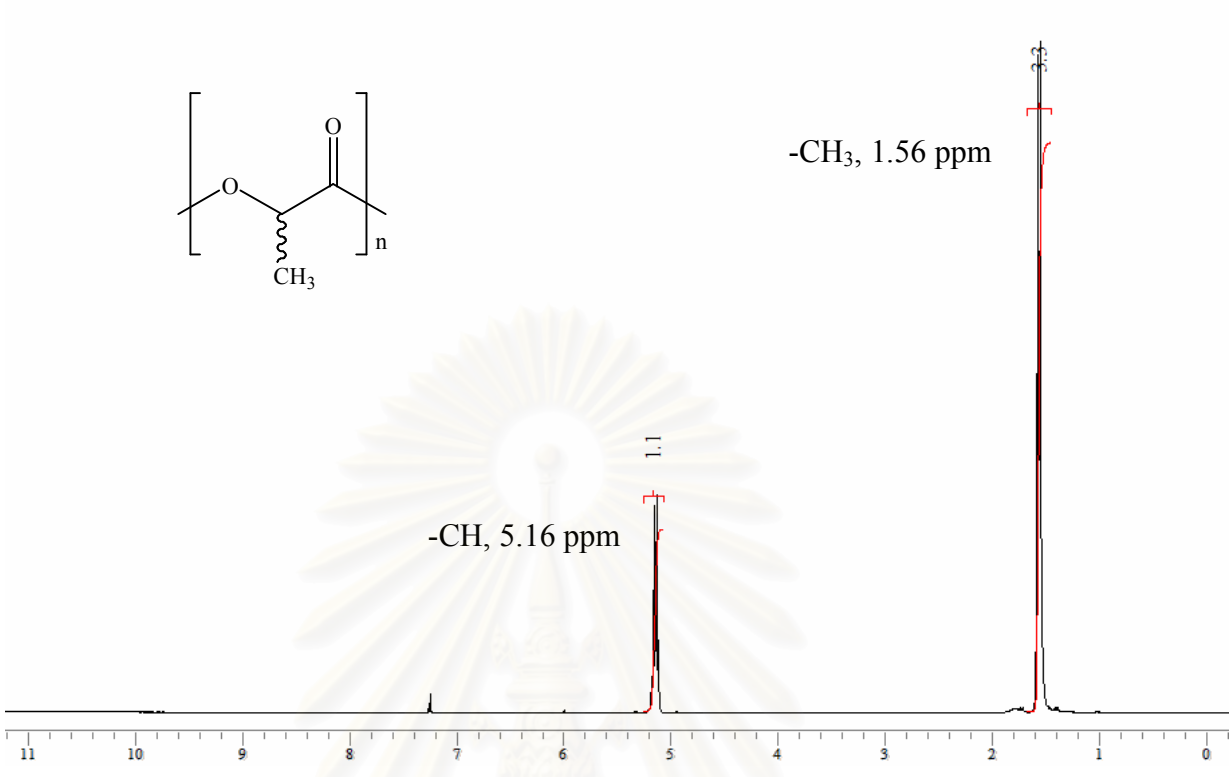


Figure C1 (a) ^{1}H-NMR and (b) ^{13}C-NMR spectra of Lactide.

(a)



(b)

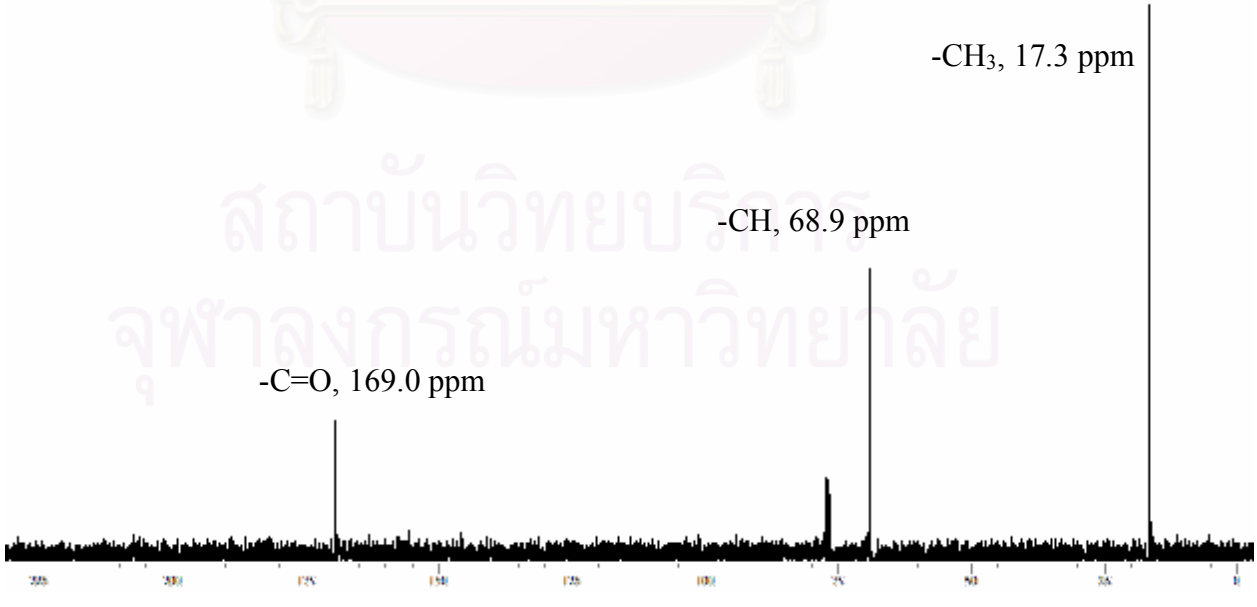
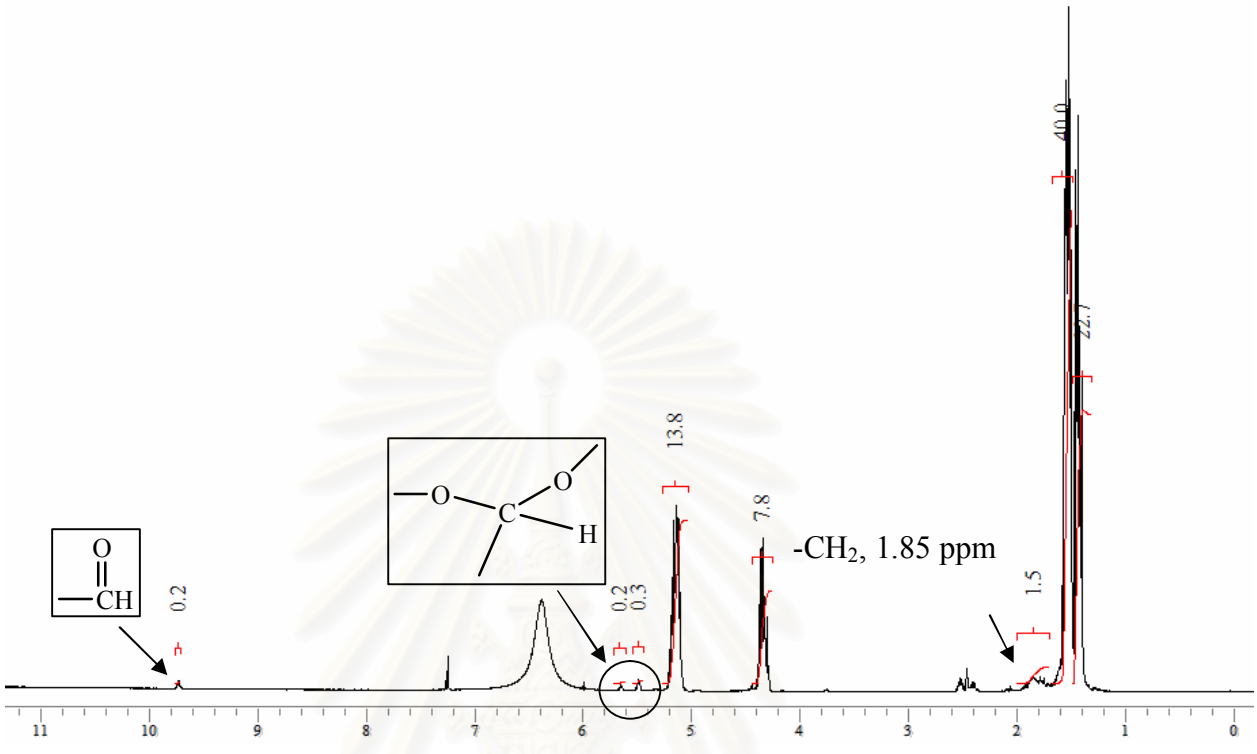


Figure C2 (a) ^{1}H-NMR and (b) ^{13}C-NMR spectra of polylactide (PLA).

(a)



(b)

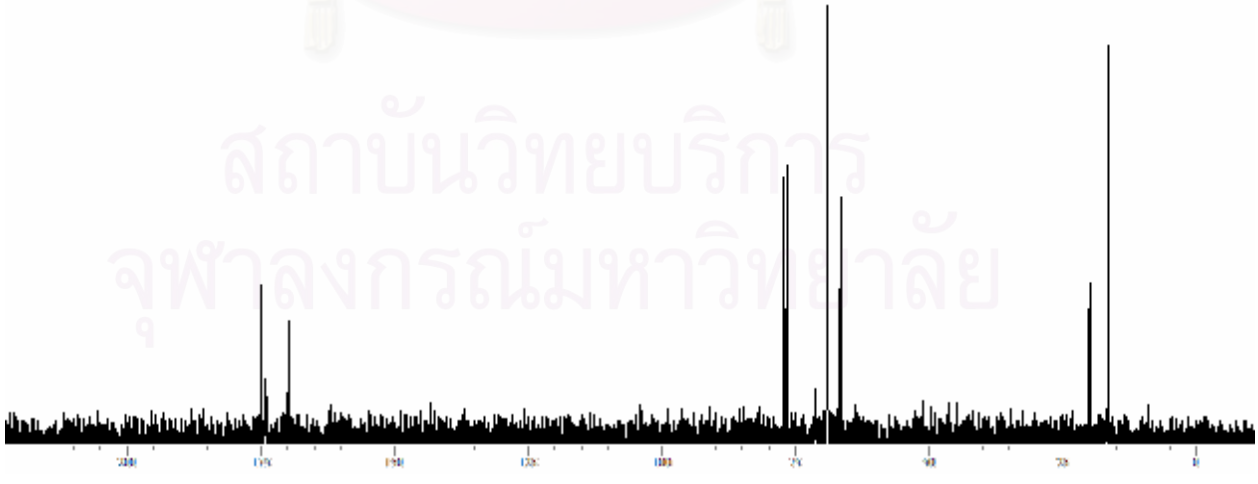
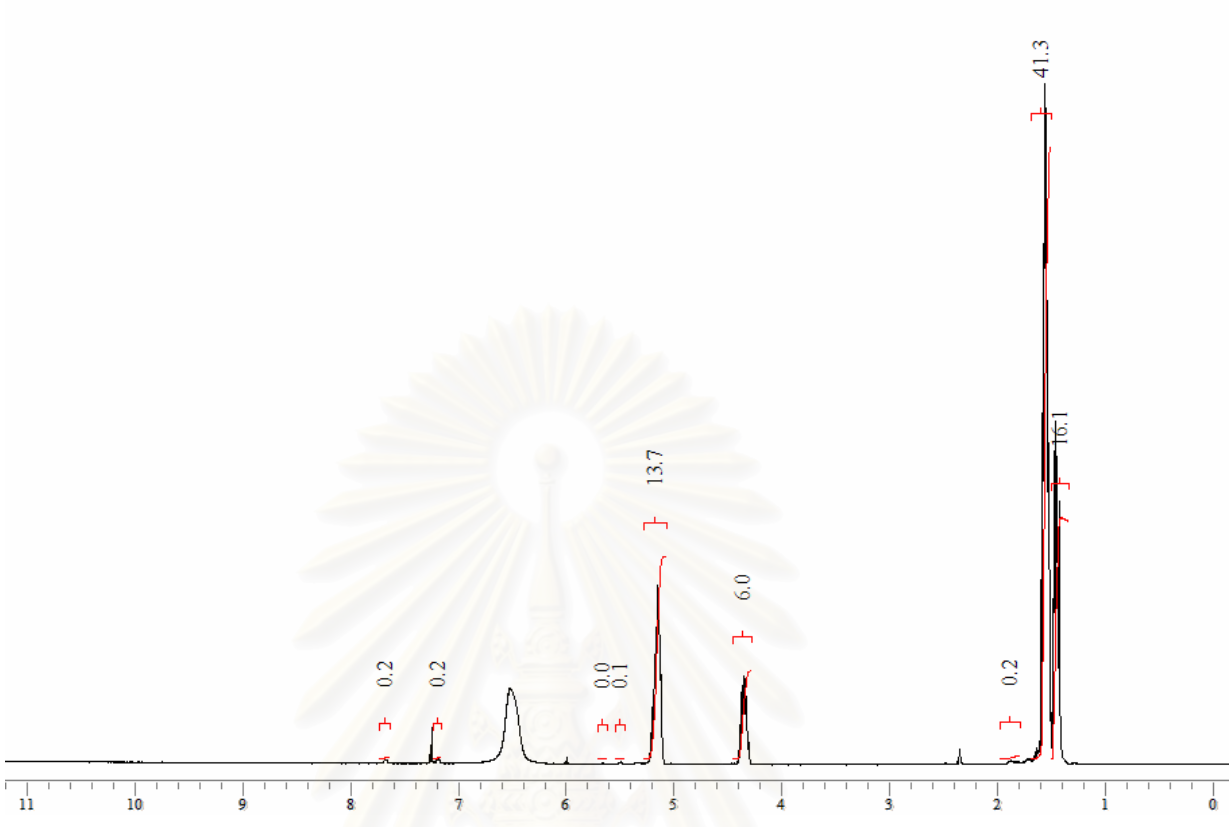


Figure C3 (a) ^{1}H-NMR and (b) ^{13}C-NMR spectra of low molecular weight polylactic acid (LPLA) using 1% glutaraldehyde (GA) in water.

(a)



(b)

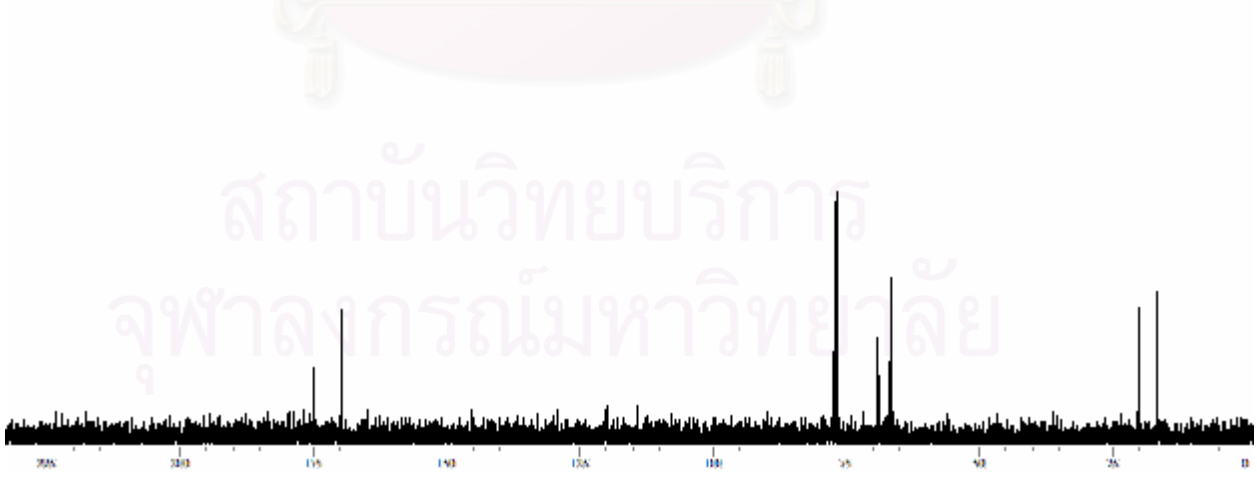
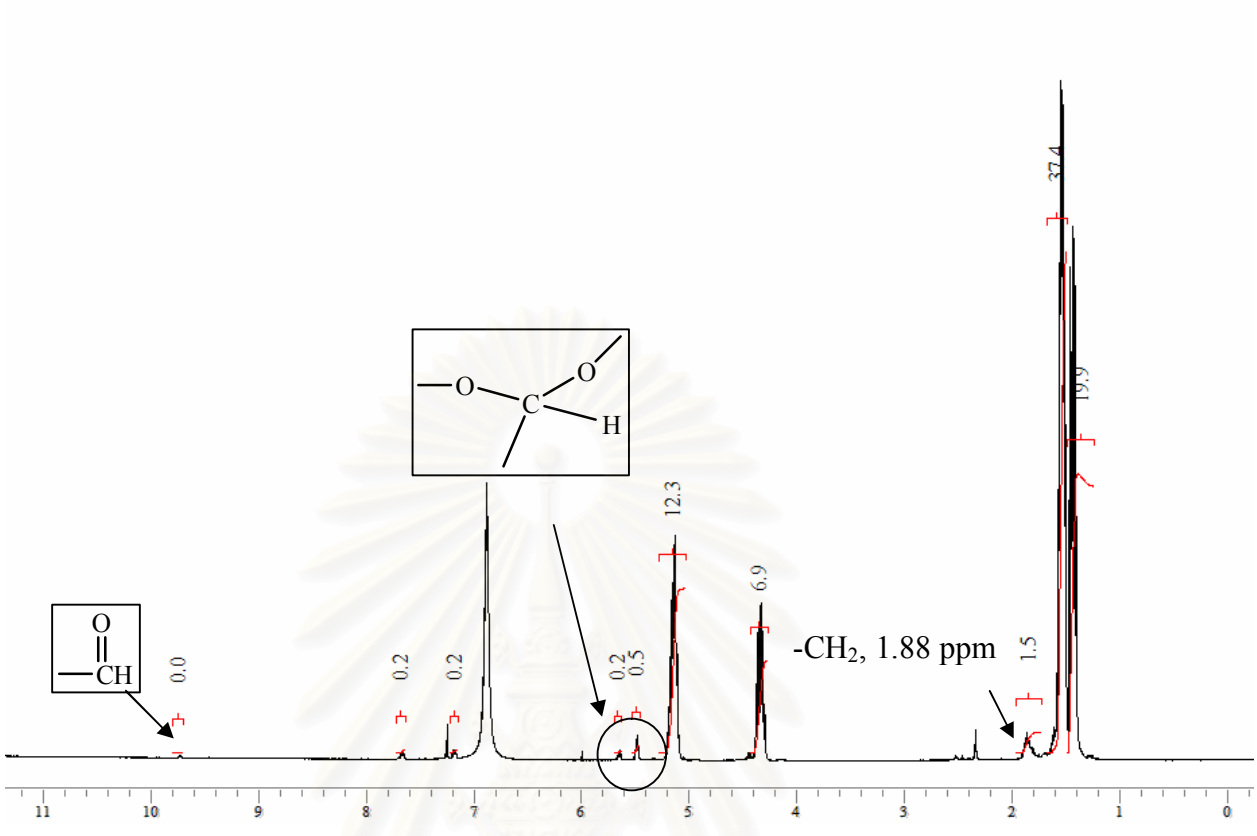


Figure C4 (a) ^{1}H-NMR and (b) ^{13}C-NMR spectra of low molecular weight polylactic acid (LPLA) using toluene-4-sulfonic acid monohydrate (PTSA) and 1% glutaraldehyde (GA) in water.

(a)



(b)

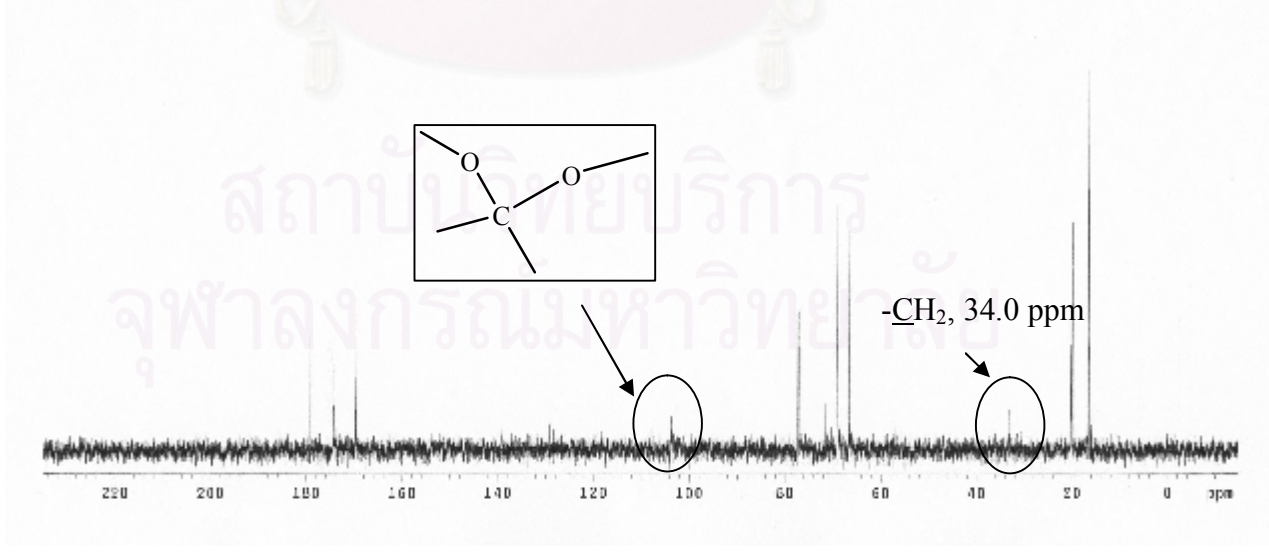


Figure C5 (a) ^{1}H-NMR and (b) ^{13}C-NMR spectra of low molecular weight polylactic acid (LPLA) using toluene-4-sulfonic acid monohydrate (PTSA) and 5% glutaraldehyde (GA) in water.

APPENDIX D

ATR-IR SPECTROSCOPY

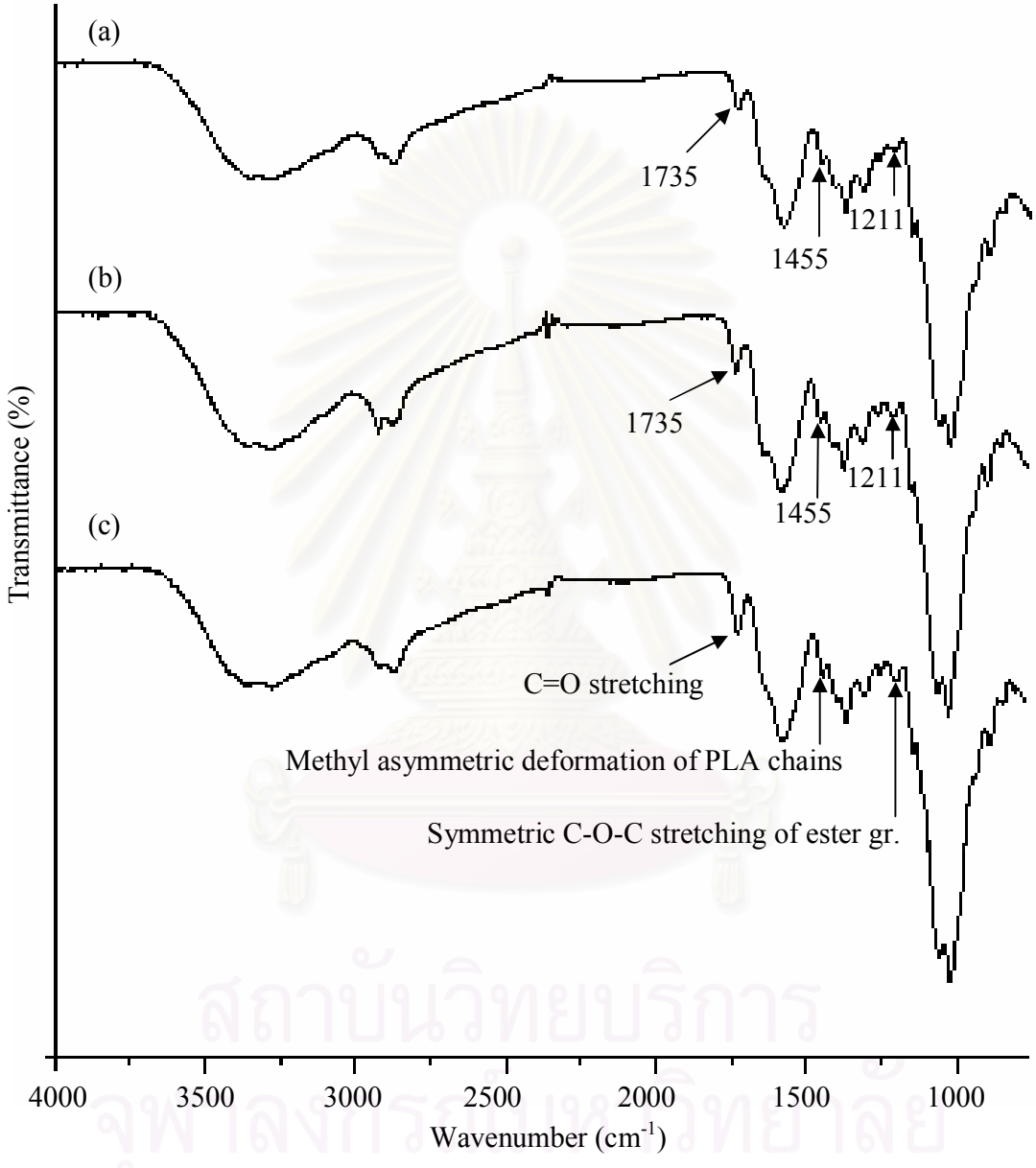


Figure D1 The IR spectra of (a) CLG1-1.0%LPLA, (b) CLG-1.5%LPLA, and (c) CLG1-2.0%LPLA copolymer films.

APPENDIX E

DETERMINATION OF COPOLYMER FILM MECHANICAL PROPERTY RESULTS

Example :

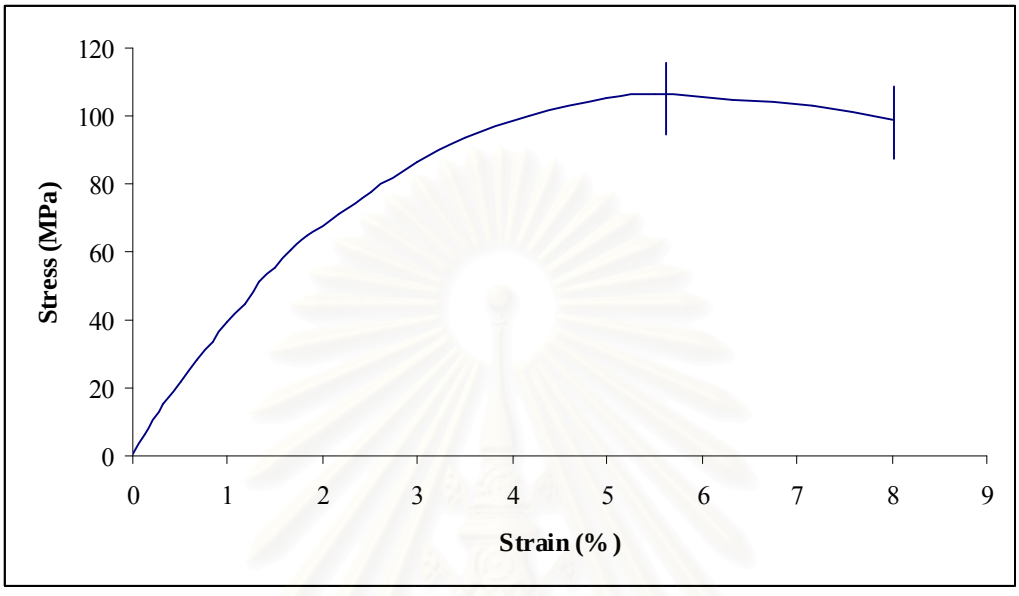


Figure E1 Mechanical properties of CLG film.

Sample : CLG film
Width : 5.0 mm
Thickness : 0.03 mm
Test type : Tensile
Sample rate : 5.0 pts/secs
Crosshead Speed : 12.5 mm/min
Specimen gauge length : 25 mm
Grip distance : 25 mm
Full Scale Load Range : 5.0 kN
Number of data point : 23
Maximum load : 0.0159722 kN (point 19)
Maximum extension : 2.208194 mm (point 23)
Humidity : 50 %
Temperature : 25^{o}C

Table E1 Raw data of mechanical properties of CLG film

point	Load (N)	Strain (%)	Stress (MPa)
1	-	0.00	0.33
2	0.9458	0.14	6.31
3	2.284	0.32	15.23
4	3.232	0.50	21.55
5	4.244	0.67	28.29
6	5.048	0.83	33.65
7	5.925	1.00	39.50
8	6.721	1.17	44.81
9	7.676	1.33	51.17
10	8.326	1.50	55.51
11	9.066	1.67	60.44
12	9.678	1.83	64.52
13	10.66	2.17	71.06
14	11.67	2.50	77.81
15	12.97	3.00	86.46
16	14.07	3.50	93.77
17	14.99	4.17	99.97
18	15.77	5.00	105.20
19	15.97	5.50	106.50
20	15.73	6.33	104.90
21	15.45	7.17	103.00
22	14.84	8.00	98.93
23	-2.372	8.83	-15.81

Calculation of mechanical properties of copolymer films

The mechanical properties of films were studied according to three parameters : tensile strength (TS), Young's modulus (Y) and percent elongation at break (%E) of

the film samples were measured by Universal Testing Machine (UTM) Model 5583 Serial#11202H.

Calculations :

The strength of material can be determined from tensile strength which is calculated by dividing maximum force by film cross-section (thickness x width), given by the following equation :

$$\text{Tensile strength } (N/mm^2) = \frac{\text{Breaking force (N)}}{\text{Cross-sectional area of sample } (mm^2)}$$

Elongation at break is the strain on a sample when it breaks. This usually is expressed as a percent elongation, is defined as the maximum elongation of the gage length divided by the original gage length, given by the following equation :

$$\text{Elongation at break (\%)} = \frac{\text{Increase in length at breaking point (mm)} \times 100\%}{\text{Original length (mm)}}$$

Young's modulus is a measurement of the stiffness of a given material. It is defined as the slope of the tangent to the stress-strain curve, which can be calculated by dividing the tensile stress by the tensile strain. Tensile stress at maximum load is the value of the stress on the stress-strain curve where the curve occurred at maximum load, given by the following equation :

$$E = \frac{\text{Tensile stress}}{\text{Tensile strain}} = \frac{F/A_o}{\Delta L_o/L_o}$$

Table E2 Mechanical properties, and the thickness of CLG copolymer films with a different weight ratio of chitosan:lactic acid

Film[a]	TS (MPa)	Y (MPa)	%E	Thickness (mm)
1.75CLG	129.61 ± 4.48	5026.51 ± 153.74	5.89 ± 1.26	0.05
1.5CLG	102.11 ± 4.96	4518.78 ± 53.38	8.50 ± 0.71	0.05
1.25CLG	Not available	Not available	Not available	Not available
CLG	112.63 ± 8.70	4611.20 ± 9.61	5.50 ± 0	0.03
0.75CLG	Not available	Not available	Not available	0.02
0.5CLG	Not available	Not available	Not available	0.02

Remarks:

- [a]1.75CLG, 1.5CLG, 1.25CLG, CLG, 0.75 CLG, and 0.5CLG films (weight ratio of chitosan (CH) to L-lactic acid (LA) was 1.75:1, 1.50:1, 1.25:1, 1:1, 0.75:1, and 0.5:1, respectively)
- Not available due to breakage at the tensiometer grip

Table E3 Mechanical properties of the CLG3 and CLG5-1.0%LPLA copolymer films with and without glycerol monostearate (GMS) using a plasticizing agent.

Copolymer film	TS (MPa)		Young's modulus (MPa)		%Elongation at break	
	65°C	Tr.	65°C	Tr.	65°C	Tr.
CLG3	82.83 ± 0.87	73.82 ± 11.15	3177.57 ± 18.11	3486.90 ± 744.92	4.88 ± 1.12	3.31 ± 1.38
CLG3-0.5%GMS	55.67 ± 11.62	70.00 ± 18.80	2830.82 ± 233.11	3733.79 ± 526.91	3.31 ± 0.96	2.72 ± 0.77
CLG5-1.0%LPLA	99.46 ± 8.59	107.26 ± 5.07	4146.31 ± 431.97	4835.49 ± 260.20	5.61 ± 1.04	3.06 ± 0.39
CLG5-1.0%LPLA-0.5%GMS	89.52 ± 12.96	63.29 ± 7.36	3695.87 ± 549.31	4116.03 ± 248.05	5.79 ± 1.36	1.98 ± 0.57

VITA

Ms. Supaporn Iabhuay was born on Thursday 24th March, 1983, in Angthong, Thailand. In 2005, she graduated with a Bachelor's degree of Science in Chemistry, from Chulalongkorn University. After that, she has studied for a Master's degree of Science in Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University, and completed the program in 2007. Address : 9/26 Seri Thai Rd., Khannayao, Khannayao, Bangkok Thailand 10230.