BIODEGRADABLE FILMS FROM POLY (LACTIC ACID) (PLA)-CHITOSAN-SILVER NANOPARTICLES: PREPARATION AND CHARACTERIZATION

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SUPERVISOR'S DECLARATION

I hereby declare that I have read this project and in my opinion, this project is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering.

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STUDENT'S DECLARATION

I hereby declare that the work in this project is my own except for quotations and summaries which have been duly acknowledged. The project has not been accepted for any degree and is not concurrently submitted for award of other degree.

Signature

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Special dedication to my beloved family especially my father (Azli Abdullah),

my mother (Safna Asaruddin) and my grandmother (Jamiah) for their love and encouragement.

And,

Thanks to my friends, my fellow course mates and all faculty members.

For all your care, support and best wishes

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BIODEGRADABLE FILMS FROM POLY (LACTIC ACID) (PLA)-CHITOSAN-SILVER NANOPARTICLES: PREPARATION AND CHARACTERIZATION

ABSTRACT

The biodegradable food packaging films were produced from biopolymers poly (lactic acid) (PLA), chitosan, and silver nanoparticles. The objectives of this research are to produce the biodegradable food packaging films from biopolymers poly (lactic acid) (PLA), chitosan, and silver nanoparticles and also to study the effects silver nanoparticles on the antimicrobial qualities of silver nanoparticles loaded with chitosan-PLA based films. Other objective in this research is to study the characterization in chemical, mechanical and biodegradability of biodegradable food packaging films. The characteristics of biodegradable food packaging films were evaluated by using equipments like Fourier transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM). The film also was characterized in term of moisture content and water absorption test and also biodegradability test using soil burial degradation method. The biodegradable food packaging films based of PLA-chitosan loaded with silver nanoparticles also have antimicrobial properties towards any bacteria due to the present of silver nanoparticles that have characteristics of antifungal and antibacterial. In this study, the antimicrobial properties of the films were investigated through the antimicrobial activities by using agar disc diffusion methods and test the films with the microbial *E.coli* bacteria. The performance of films were optimized by manipulating the concentration of silver nanoparticles in order to obtain the biodegradable food packaging films that have high biodegradability and high qualities for food packaging application. The results shows that, the films were show positive response towards degradation process due to the weight loss within 12 days and the poly(lactic acid) (PLA) film show high resistivity towards water compared to the chitosan (CS) film. The concentration of silver nanoparticles at (23.1 % w/w SNP) shows the 100% of inhibition against Gram-negative bacterium *E.coli* and Gram-positive bacterium *Micrococcus.* Thus, it show that silver nanoparticles were successfully inhibit the bacteria grow and improve the antimicrobial properties of poly(lactic acid) (PLA)-loaded chitosan-silver nanoparticles blend films.

BIODEGRADASI FILEM DARIPADA POLI (ASID LAKTIK) (PLA)-CHITOSAN-NANOPARTIKEL PERAK: PENGHASILAN DAN PENCIRIAN

ABSTRAK

Pembungkusan makanan biodegradasi filem dihasilkan dari biopolimer poli (asid laktik) (PLA), chitosan, dan nanopartikel perak. Objektif kajian ini adalah untuk menghasilkan filem pembungkusan makanan terbiodegradasi daripada biopolimer poli (laktik asid) (PLA), chitosan, dan nanopartikel perak dan juga untuk mengkaji kesan nanopartikel perak pada sifat daya tahan terhadap bakteria melalui campuran nanopartikel perak dengan chitosan dan PLA filem. Objektif lain dalam kajian ini adalah untuk mengkaji sifat kimia, mekanikal dan biodegredasi filem pembungkusan makanan terbiodegradasi. Filem pembungkusan makanan terbiodegradasi telah dinilai dan dicirikan dengan menggunakan peralatan seperti spektroskopi jelmaan Fourier inframerah (FTIR) dan mikroskop imbasan elektron (SEM). Makanan terbiodegradasi pembungkusan filem juga dicirikan dalam sifat penyerapan terhadap air melalui kaedah serapan dan biodegredasi menggunakan kaedah kambusan tanah. Terbiodegradasikan filem pembungkusan makanan berasaskan PLA-chitosan sarat dengan nanopartikel perak juga mempunyai ciri-ciri daya tahan terhadap bacteria kerana kehadiran nanopartikel perak yang mempunyai ciri-ciri antikulat dan antibakteria. Sifat antimikrob pada pembungkusan biodegradasi filem makanan berasaskan PLA-chitosan-silver nanopartikel disiasat melalui aktiviti antimikrobial dengan menguji filem dengan bakteria E.coli mikrob. Prestasi filem pembungkusan biodegradasi makanan dioptimumkan dengan memanipulasi kepekatan nanopartikel perak untuk mendapatkan filem pembungkusan makanan terbiodegradasi yang mempunyai biodegredasi yang tinggi. Keputusan menunjukkan filem yang dihasilkan daripada chitosan, PLA dan nanopartikel perak terbiodegradasi dalam masa yang singkat iaitu 12 hari sahaja dan PLA filem menunjukkan daya tahan keserapan air yang tinggi berbanding chitosan filem. Kepekatan nanopartikel perak pada peratus 23.1 menunjukkan seratus peratus perencatan terhadap bakteria positif *E.coli* dan bakteria negatif *Micrococcus*. Ini menunjukkan nanopartikel perak berkesan dalam menjadikan filem mempunyai daya tahan yang tinggi terhadap bakteria.

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LIST OF SYMBOLS

°C	Degree celcius
g	grams
H	hour
L	litre
ml	millilitre
mm	millimetre
min	minutes
w/w	Weight per weight
v/v	Volume per volume
%	Percentage
$\bar{\upsilon}$	wavenumbers
λ	wavelengths

LIST OF ABBREVIATIONS

Ag-nanoparticles	Silver nanoparticles
CH_3COOH	Acetic Acid
CS	Chitosan
DD	Degree Deacetylation
DLS	Dynamic Light Scattering
E.Coli	Escherichia coli
FTIR	Fourier transform infrared spectroscopy
IR	Infrared
PCL	Poly (e-caprolactone)
PEK	Polyethylketone
PGA	Poly (glycolic acid)
PLA	Poly (lactic acid)
PHB	Poly (3-hydroxybutyrate)
PHBV	Polyhydroxyvalerate
PVC	Polyvinyl chloride
PVOH	Polyvinyl alcohol
PVAC	Polyvinyl acetate
SEM	Scanning Electron Microscopy
SNP	Silver nanoparticles
SPR	Surface Plasmon Resonance
UV	Ultra-violet

CHAPTER 1

INTRODUCTION

1.1 Background of Proposed Study

The development of plastics or packaging films based on biopolymers has wide applications due to their environmentally friendly nature and their potential use in the food packaging industry (Marcos *et al.*, 2010). The biodegradable and biocompatible polymers have caused significant attention from both ecological and biological perspectives in the past decade. In general, synthetic polymers produced from petrochemical are not easily degraded in the environment and also contains substances of carcinogen that is an agent directly involved in causing cancer (Momani, 2009).

Packaging films based on biopolymers driving efforts towards biodegradable and biocomposite polymers that can be used as renewable and non-toxic resources. The most popular and important biodegradable polymers are aliphatic polyesters such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA),

poly(e-caprolactone) (PCL) and poly(3-hydroxybutyrate) (PHB), and among of them, PLA has received the most attention due to its renewable resources and biocompatibility (Tokiwa *et al.,* 2009).

In addition, due to environmental considerations, the elaboration of new edible or biodegradable bioactive packaging constitutes a very interesting option to recycle. Biodegradation poly(lactic acid) generates acidic degradation products so chitosan may be combined with acid producing biodegradable polymers so that local toxicity due to the acid by products can be reduced (Yao *et al.,* 2003). Chitosan was thus used to produce films from renewable resources and have multipurpose material such as food packaging, drug release component and for environmental pollutants.

Chitin that derived from polysaccharide is a chitosan that is one of the natural polymers and largely widespread in living organisms such as shellfish, shellcrab, insects and mushrooms (Sebastien *et al.,* 2006). Chitosan is monocomponent antimicrobial agent is not already full filling the requirements of some conditions. For instance, the combination of chitosan with other inorganic agents such as Ag, Zn, SiO_2, and TiO_2 and among them chitosan-Ag nanoparticles composite had significantly have high antibacterial activity with only small presence of Ag-nanoparticles which exhibit potential antifungal properties (Li *et. al.,* 2010).

1.2 Problem Statement

Recently, the materials of food packaging from the synthetic polymers have become increasingly important due to its high demands and low cost of manufacturing. The increasing of the production of synthetic polymers can cause many environmental problems including waste accumulation and pollutions. Synthetic polymers are not biodegradable and its end up in landfill sites and produce very harmful gases that can cause environmental pollutions and soil contaminations. A synthetic polymer takes a long time to degrade due to the molecular bonds of the polymer makes the polymer so durable and resistant to biodegradation. The burning of synthetic polymer also can release dioxin which highly toxic and will cause chronic disease like cancer through inhalation. Hence, it is very crucial to find other biodegradable polymers that are environmental friendly to nature and will degrade without ends up in landfill by using renewable sources of biodegradable polymer such as poly(lactic acid) (PLA) and chitosan to substitute the using of synthetic polymer.

1.3 Research Objectives

1. To produce the biodegradable of food packaging films from biopolymers poly (lactic acid) (PLA), chitosan, and silver nanoparticles.

2. To investigate the effects of silver nanoparticles on antimicrobial properties of poly(lactic acid) (PLA)-loaded chitosan-silver nanoparticles blend films
3. To study the characterization of poly(lactic) acid (PLA)-loaded chitosan-silver nanoparticles blend films in chemical properties and biodegradability of the films.

1.4 Scope of Proposed Study

To achieve the objective of this research, scopes have been identified

i. Preparations of poly(lactic acid) (PLA)-loaded chitosan-silver nanoparticles blend films
ii. Characterization of poly(lactic acid) (PLA)-loaded chitosan-silver nanoparticles blend films by using Fourier Transforms Infrared Spectroscopy (FTIR), UV-Vis absorption spectrophotometer and Scanning Electron Microscope (SEM).
iii. Characterization of poly(lactic acid) (PLA)-loaded chitosan-silver nanoparticles blend films on antimicrobial properties against Gram-negative bacterium *E.coli* and Gram-positive bacterium *Micrococcus* by using the colony forming count method.
iv. Characterization of poly(lactic acid) (PLA)-loaded chitosan-silver nanoparticles blend films on biodegradability by using soil burial degradation

v. Analyzing the moisture and water absorption of poly(lactic acid) (PLA)-loaded chitosan-silver nanoparticles blend films by using swelling method.

1.5 Significance of Proposed Study

The significant of this study is to produce biodegradable food packaging films from PLA and also modified those biopolymers that made up from PLA with chitosan and silver nanoparticles that have better antimicrobial and chemical properties and biodegradability.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

2.1.1 Plastics and environment

Plastics are synthetic substances consisting very large of molecules that produced by chemical reaction. Plastics are one of the organic polymers that have high molecular weight. They are usually synthetics and almost all of them are made from petroleum. Plastics can be remoulded, extruded or processed into others forms including solid object, film and filaments. The structural molecules of the polymers that are plastics are linked together by chemical bonds. Plastics can be divided into two which are thermosetting or thermoplastics materials. The difference between the thermoplastic and thermosetting is their characteristics. The thermoplastic is flexible and becomes soft when heated and hard when cooled while thermosetting is hard and brittle and cannot be heated and remoulded like thermoplastic when heated (Van *et al,* 2000).

Recently, plastics are widely used because of its properties that easy and low cost of manufacturing and processing. Approximately 140 million tons of synthetic polymers are produced worldwide each year to replace more traditional materials and particularly packaging (Swift & Baciu, 2006). Plastics are manufactured to resist the environmental degradation. Over 60% of plastics wastes are produced by households and most of it as single use packaging. Plastics packaging has a cycle less than year and continuously giving the bad effect to the environment. The growing rate of plastics in industries has lead to the increasing of plastic wastes in landfill (Zhen & Yanful, 2005).The solid waste disposal causes serious problem with billions of tons of waste disposed every year. Landfill used to be one of the main routes of disposal in everywhere but landfill capacity is now dimishing (Moore & Sauders, 1997).

To overcome this problem, the 3R are alternative ways which are reduced, reuse and recycle. The word reduced means the reduction of the amounts of materials entering the waste streams by redesigning patterns of consumption and production. The word reuse means transformation of the materials that are no longer need and make its reusable again. But reuse also has limitations. This is because most the plastics that are used are not redesigned to reuse again due to its impurities and contamination. The most common plastics products are food packaging, disposable diapers, agricultural mulch bags and medical appliances are not suitable to reuse again. Recycling of plastics after used is possible but the plastics product for example plastic bags are rarely to be recycled. The technology of the recycling like collecting and sorting of the plastics is expensive and time-consuming process (Tollinski, 2009)

2.1.2 Polymers used in Biodegradable Packaging

With the growing of the environmental pollution that caused by plastics wastes need immediate resolution. Biodegradable plastics have been intensively studied in recent years. (Khabbaz *et al*, 1998) have been commercialized into several of product such as garbage bags, grocery bags, and waste bags that will decompose in proper ways. Plastic packaging demand will increase more rapidly based on the good opportunity of both flexible and rigid packaging. In rigid plastic packaging, the best opportunities are anticipated for trays, cups and tubes.

2.1.3 Development in Biodegradable Packaging Materials

Over the last three decades, there has been a growing interest in biodegradable polymers. Initial interests were in the fields of medical such as producing degradable fibers for sutures and agriculture for mulch films and controlled pesticide release. In more recent years attention has been focused on the rising concern for the environment. Biodegradable plastics are plastics that can undergo a degradation process known as biodegradation (Scott *et al.,* 2007). Biodegradation of plastics materials are leading to change in its chemical structure caused by biological activity leading to naturally occurring metabolic end products. Rate of biodegradation is determined by standardized test systems. Biodegradable plastics have similar properties of conventional plastics but it can be decomposed by the activity of the microorganisms after disposal to the environment (Tharanathan, 2003).

Biodegradable plastics also can be used in hygiene products, households, agriculture and horticultural products and also in medicine. The production of biodegradable polymers will decreases the solid waste problems and environmental pollutions (Bovea *et al.,* 2003)Biodegradable polymers can be divided into two main categories which are naturally occurring biodegradable polymers and synthetic biodegradable polymers (Bovea *et al.,* 2003). Naturally occurs of biodegradable polymers including polysaccharides such as starch, cellulose and chitin/chitosan. Some polyester such as PLA is also naturally biodegradable polymers (Yu *et al.,* 2006). The most attractive feature of the biopolymer based materials is their total biodegradability. As the result they fit perfectly well in the ecosystems and save the world from the growing ecological pollution caused by non-biodegradable plastics (Garlotta, 2002).

Synthetic biodegradable polymers are usually polymers with hydrolysable backbone and polymers that are sensitive to photodegradation or UV-degradation. Examples of polymers that in the group of polyesters are poly(glycolic) acid, poly(glycolic acid-co-lactic acid), polycaprolactone, polyether-polyurethane and poly(amide-enamide). Some common synthetic biodegradable and its description are listed in Table 2.1 (Garthe & Kowal, 1994).

Table 2.1 Common Synthetic Biodegradable Plastics

Plastic Type	Name	Description
Polyester	Polyglycolic acid (PGA)	Hydrolyzable polyhydroxy acid.
	Polylactic acid (PLA)	Hydrolyzable polyhydroxy acid; polymers derived from fermenting crops and dairy products; compostable.
	Polycaprolactone (PCL)	Hydrolyzable; low softening and melting points; compostable; long time to grade.
	Polyhydroxybutyrate (PHB)	Hydrolyzable; produced as storage material by microorganisms; possibly degrades in aerobic conditions; stiff; brittle; poor solvent resistance.
	Polyhydroxyvalerate (PHBV)	Hydrolyzable copolymer; processes similar to PHB; contains a substances ton increase degradability; melting point; toughness; compostable; low volume and costly production.
Vinyl	Polyvinyl alcohol (PVOH)	Water soluble, dissolve during compositing.
	Polyvinyl acetate (PVAC)	Water soluble, predecessor to PVOH; has shown no significant property loss during compositing tests.
	Polyethylketone (PEK)	Water soluble; derived from PVOH; possibly degrades in aerobic and anaerobic conditions.

(Source: Garthe & Kowal, 1994)

2.2 FILM FORMING MATERIAL

2.2.1 Poly(lactic) acid (PLA)

Poly(lactic acid) belongs to the family of the aliphatic polyesters is most important of the biodegradable polymers such as poly(lactic acid) (PLA), poly(3-hydroxybutylene) (PHB), poly(glycolic acid) (PGA) and poly(e-caprolactone) (PCL) but among of them PLA has received most attention due to its biocompatibility,

biodegradation, excellent thermal/mechanical properties and superior transparency of the materials (Urayama *et al.,* 2002). PLA is biodegradable through hydrolytic and enzymatic reaction. PLA is produced from the linear aliphatic thermoplastic polyester and also can be synthesized by condensation polymerization of the lactic acid monomers or by ring-opening polymerizations of monomers that those monomers can be obtained from fermentation of corn, potato, sugar beat and sugar cane (Sawai *et al.,* 2007)

The copolymers of PLA are poly ($_{L}$-lactide) and poly ($_{DL}$-lactide). The amounts of monomers affect the physical properties of PLA such as melting point, degree of crystallinity and the mechanical properties of the PLA (Wu, 2006). High molecular weight of PLA will appear colorless and has glossy looks with properties similar to polystyrene. The amorphous PLA is soluble in most organic solvents such as tetrahydrofuran (THF), chlorinated solvents, benzene, chloroform, and 1, 4-dioxane PLA behaved like thermoplastics and will dispose harmless. PLA is degrade by using simple hydrolysis of the ester bond and does not require the presence of enzymes to catalyze this hydrolysis.

The rate of degradation of PLA will depend on the size and shape of PLA and also the isomer ratio and temperature of hydrolysis. PLA degradation is dependent on the time, temperature and molecular weight (Garlotta, 2002).PLA present one of the renewable biodegradable thermoplastics due to its applications in packaging, textile industry, biomedical field and fibre reinforced composite manufacturing (Gregorova *et al.,* 2011). To enhance the impact resistance of PLA and compete with low cost commodity of polymers, considerable progress has been made by blending the PLA

with other biodegradable polymers (Martin & Averous, 2002), For biomedical applications, PLA has weak in high loading bearing application, so it is necessary to incorporate the PLA with reinforced fillers (Bleach *et al.*, 2002). PLA or known as poly(**α**-hydroxy acids) generates acidic degradation products at the implanted site which cause the undesirable tissue reaction (Campus, 2002). The acid by product will lead to the local disturbance due to poor vascularisation in the surrounding tissue but the incorporating the PLA with other biodegradable polymers will reduce the toxicity (Yao *et al.*, 2005).

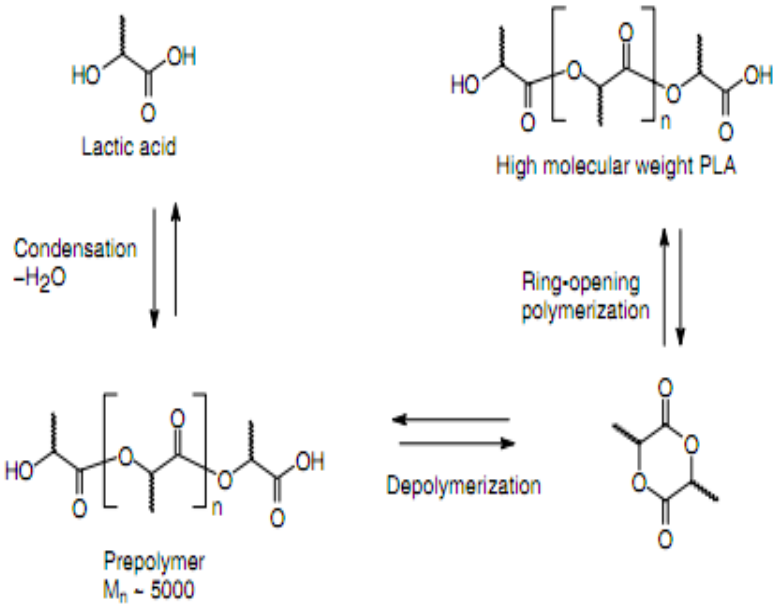


Figure 2.1 Schematic of PLA production via prepolymer and lactide (Gruber *et al.*, 2006)

The synthesis of lactic acid into PLA consists of two different ways as illustrated in Figure 2.1 which are by direct condensation of lactic acid, or ring-opening polymerization of cyclic lactide dimer. The condensation polymerization is low cost but it's difficult in solvent free system in order to obtain high molecular weight. Coupling agents that have been used to increase the molecular weight and the chain-coupling agent will reacts with either hydroxyl or carboxyl groups that leads to different kinetic reaction rates of coupling. Hydroxyl terminated PLA synthesize by condensation of the lactic acid in the presence of the hydroxyl compounds such as glycerol or 1, 4-butanediol which leads hydroxyl ends group by condensation reactions of epoxide to convert to hydroxyl group. And on the others ways is esterification process which has been used to increase the molecular weight of the PLA (Gruber *et al.*, 2006).

Among the various aliphatic degradable polyesters, polylactide (PLA) has been considered as one of the most interesting and promising biodegradable materials and has been used in medical applications, such as surgical implants, culturing of tissue and also closuring wounds (Khang *et al.*, 2003). Polymers of lactic acid are biodegradable thermoplastics poly(lactic acid) (PLA) with low degree of polymerization can help in controlled release or degradable films for large-scale agricultural applications. PLA is commercially and largely scale production due to the remarkable properties that make it suitable for different applications (Averous, 2008).

2.2.2 Chitosan

Chitosan is a linear polysaccharide and biodegradable of copolymer that composed of N-acetyl glucosamine and D-glucosamine. Chitosan is produced by deacetylation of chitin which have the exoskeleton of crustacean's structural element such as crabs and shrimps, (Peesan *et al.,* 2005). Chitosan is a deacetylated product of chitin ß-(1-4)-2-acetamido-2-deoxy-D-glucan. Chitosan has gained attention by researchers due to its functional properties such as film-forming capabilities, mineral binding properties, hypolipidemic activity, biodegradability, antimicrobial activity and acceleration of wound healing (Dutta *et al.,* 2012).

Chitosan is soluble in acidic condition and the free amino group on its polymeric chains contributes to its positive charge (Phaechamud, 2008) and also known to be non-toxic, odourless, biocompatible to animal tissue and biodegradability (Zong *et al.,* 2007). Chitosan is the second most plentiful natural biopolymer and relatively cheap (Dutta *et al.,* 2012). Chitosan has very unique biological properties such as antimicrobial activity and antitumor activity. The antimicrobial activity of chitosan is influenced by species of the bacteria, concentration, pH, solvent and molecular weight of chitosan (Lauzardo *et al.*, 2008). In the antimicrobial action, the binding to the negatively charged wall will destabilize the cell envelope and altered permeability, followed by attachment to DNA with inhibition (Halender *et al.,* 2001). Due to the excellent antimicrobial properties of chitosan, chitosan film may used in food packaging (Triphati *et al.,* 2009).

Methods that are used to synthesis the chitosan from the deacetylation of chitin are by using sodium hydroxide in excess as a reagent and water as a solvent. The amino groups in chitosan can lead the protonation of acidic conditions to neutral solution. This is because its pKa value of ~6.5 and its charge density dependent on pH. Chitosan is water soluble and biodhesive which can binds to negative charge. Chitosan also is biocompatible and biodegradable. Amino group in the structure of the chitosan have been used in transfect breast cancer cells by trymetylation process. The increasing of cytotoxicity can leads the increasing of the degree of trimethylation. At 50% trimethylation, it is the best and efficient condition to deliver gene. Chitosan is now widely available, is low cost and toxicity. Chitosan also shows great potential for varied chemical derivatizations and multiple physical forms. Thus, it is an interesting compound to study in different applications (Pillai *et al.*, 2009).

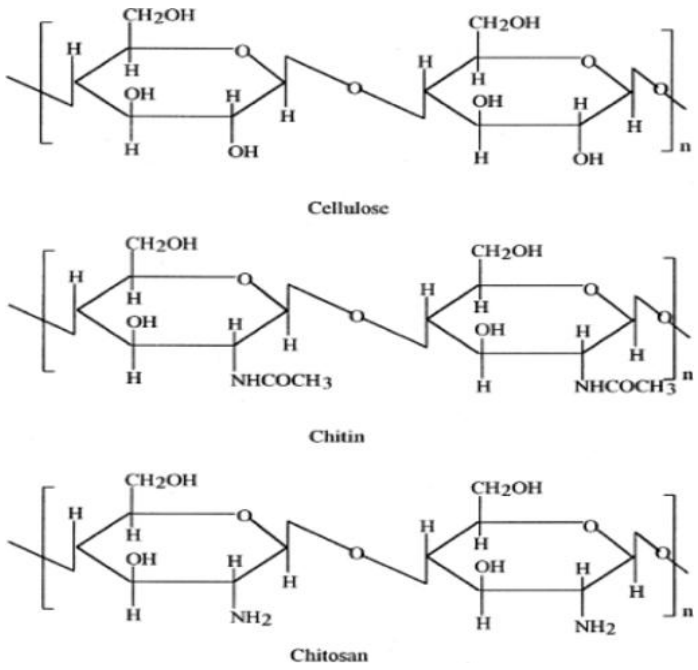


Figure 2.2 Structures of Chitin, Chitosan, and Cellulose, (Zhang *et al.*, 2011).

The film-forming capacity of chitosan has been the object of many studies that have led to its various industrial uses for example, in photographic films, cosmetics, and reverse osmosis membranes. The structural and stereochemical similarity between cellulose and chitosan suggests that chitosan-cellulose combinations form a chemical continuum as shown in Figure 2.2. Like cellulose, chitosan contains no active chromophoric groups. The presence of the amino groups makes chitosan soluble in dilute aqueous solutions of monobasic acids. Chitosan has been shown to be even more compatible with cellulose because the differences in chemical structure between cellulose and chitosan are minimal. Cellulose usually has a negative charge due to the presence of some carboxylate groups and it's strongly interacts with the positive charges in chitosan (Zhang *et al.*, 2011).

Chitosan has a shorter chain than a chitin length because of its extreme alkaline deacetylation conditions. It is insoluble in water, soluble in some aqueous monobasic acids, and insoluble in dilute or concentrated alkali solutions. Its solubility in aqueous mineral and organic acids is due to the formation of positively charged ammonium ions. Chitosan is insoluble in pure organic solvents, is insensitive to humidity, and contains 10–14% water. The solubility of chitosan in aqueous acid solutions and the viscosity of these solutions depend on the degree of acetylation and polymerization (Lu *et al.*, 2009).

2.2.3 Silver nanoparticles.

Silver nanoparticles (Ag-nanoparticles) are small objects that have particle size of 1-100 nanometers that behave like a whole unit. Silver nanoparticles contain about 10 000-15 000 silver atoms (Warheit *et al.,* 2007). For nanoparticles, size is often directly proportional to the number of atoms. For example, the addition of nanoparticles to the materials of photovoltaic cells and solar radiation, the materials that present of nanoparticles in that materials has high efficiency absorb more solar energy compared to others conventional materials. Silver nanoparticles are prepared by using several physical methods which are spark discharging, electrochemical reduction, solution irradiation and cycrochemical synthesis (Chen & Schluesner, 2008). The silver nanoparticles exhibit important physical and chemical properties such as pH-dependent partitioning to solid and dissolved particulate matter (Pal *et al*., 2007).

Silver nanoparticles also have important biological properties which are effectively bactericidal agents against bacteria, including antibiotic resistant strains (Percival *et al.,* 2007). Silver nanoparticles have several characteristics and one of the useful characteristics is its antimicrobial property. When the silver is transformed into nanoparticles, it's have strong inhibitory and bactericidal effects and this antimicrobial property is becomes effective in eliminating fungus, bacteria and viruses. Silver nanoparticles release low level of silver ions to provide protection against bacteria as the use of silver nanoparticles of many applications of antimicrobial coatings, wound dressings and biomedical devices (Cho *et al.,* 2005).

Silver ions will bind to the bacterial cell wall and alternating the function of the bacterial cell membrane (Percival *et al.,* 2005). Silver nanoparticles are also termed as new-generation of antimicrobials (Rai *et al.,* 2009) and was proved to have bactericidal potential (Feng *et al.*, 2000) as potential of silver ions that showing strong biocidal effects on as many as 16 species include *E.coli* (Spadaro *et al.*, 1974). Thus the possible use of silver nanoparticles as antibacterial agent has been investigated as a means of arresting increasing bacterial resistance to conventional bactericides and antibiotics (Gogoi *et al.,* 2006). Different types of metal like copper, zinc and titanium (Schabes *et al.,* 2006), magnesium and gold (Gu *et al.,* 2003), alginate (Ahmad *et al.,* 2006) but silver have proved to be the most effective in antimicrobial efficiency against bacteria, viruses and other microorganisms (Gong *et al.,* 2007). Silver nanoparticles used as drug disinfectant have some risk assessment of health which exposure to the silver can cause agyrosis and also toxic to mammalian cells (Gong *et al.,* 2007).

The biodegradable polymer that contains metal nanoparticles has great attention due to its antimicrobial properties. The biodegradable polymer nanocomposites can be produced by blending the biodegradable polymers with metal nanoparticles through several of methods which are mechanical mixing of polymer with silver nanoparticles, in situ polymerization of a monomer in the presence silver nanoparticles or in situ reduction of silver salts or complexes in a polymer. The presence of silver nanoparticles in the biodegradable polymers will improve the antimicrobial properties of the polymers (Shanmugam *et al.,* 2006).

2.3 ANALYSIS EQUIPMENT

2.3.1 Fourier Transform Infrared (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy is equipment of vibrational spectroscopic that use infrared radiation to vibrate molecular bonds within the sample absorb it. Different samples contain different molecular bonds and FTIR will be used to obtain chemical information of the molecules within the sample (Baker *et al.*, 2008). Infrared (IR) spectroscopy is common spectroscopy techniques that use absorption measurement at different IR frequencies by sample positioned in the path of an IR beam. IR spectroscopy analysis is to determine the chemical functional groups in the sample because different functional groups will absorb different characteristic frequencies of IR radiation as shown in Table 2.2 (Spectroscopy, 2009)

Table 2.2 The IR-Spectra of the functional group (Spectroscopy, 2009)

IR spectra	Functional Group
3200-3600	O-H, N-H stretching
2930-2939	C-H stretching
1630-1660	C=O stretching of amide
1440-1460	O-H bending of water
1050-1150	C-O stretching

IR absorption positions are usually presented as either wavenumbers ($\bar{\upsilon}$) or wavelengths (λ). Wavenumbers is representing the number of waves per unit length. The wavenumbers are directly proportional to frequency as the energy of the IR absorption in unit (cm^{-1}) scale (Spectroscopy, 2002)

2.3.2 Scanning Electron Microscope

The scanning electron microscope (SEM) use focused beam of high energy electrons to produce variety of signals at the surface of solid samples. The signals that derive from the interaction of the electrons will reveal the information about the sample including the texture of the sample through the external morphology, chemical composition and crystalline structure. Kinetic energy that derived from the accelerated electrons dissipated a variety signals that produced from electron sample interactions. These signals include the secondary electrons that produce image of scanning electron microscope (SEM) (Goldstein, 2003).

2.3.3 UV-Vis absorption Spectrophotometer

A spectrophotometer is an instrument which measures the reflection or absorbance characteristics of the sample. If there absorption of the radiation, a particular and discrete wavelength is used to illuminate the sample. Absorption of radiation can be illustrated in a graph of absorption versus wavelength that is called spectrum (Schoonheydt, 2010).

Table 2.3 The predetermined electromagnetic radiation wavelengths of ultra-violet (uv), visible (vis) and near infra-red (nir) radiation (Schoonheydt, 2010).

Radiation	Wavelengths (nm)
Uv radiation	300-400
Vis radiation	400-765
Nir radiation	765-320

The absorption spectroscopy uses the technique of the interaction of electromagnetic radiation with matter. Electrons in atoms or molecules can be raised or “excited” from one energy state to another by the absorption of electromagnetic radiation. The range of electromagnetic radiation is use to analyze the sample depends on the energy required to cause transitions within the absorbing species of the samples (Förster, 2004).

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

This chapter presents the detail procedures for producing the biodegradable poly(lactic acid) (PLA)-loaded chitosan-silver nanoparticles blend films including the material required. The step involve in this research include the preparation of biodegradable film from poly(lactic acid) (PLA) and loaded with chitosan and silver nanoparticles and characterization of biodegradable film by using the equipment such as FTIR spectrophotometer, SEM, and UV-Vis spectrum. This step also involve analysis of biodegradable film on antimicrobial properties by using the colony forming count method, characterization of biodegradable film for biodegradability by using soil burial degradation method and analysis of biodegradable film on the moisture and water absorption by using swelling method.

3.2 MATERIALS

Poly(lactic acid) (PLA) were supplied from the Sigma Aldrich. Chitosan flakes from crab shells were purchased from Fisher Scientific. Polyethylene glycol (PEG) 400 was supplied by Chemical Laboratory, Universiti Malaysia Pahang. Acetic acid (CH_3COOH) and Chloroform ($CHCl_3$) were obtained from the Sigma Aldrich. Silver nitrate ($AgNO_3$) was purchased from System. Tea powder was purchased from nearby market. Mueller-Hinton broth and Nutrient Broth were product of Oxoid and supplied by Thermo Scientific. *Escherichia coli (E.coli)* and *Micrococcus* bacteria were prepared by Microorganisms Laboratory of Universiti Malaysia Pahang. Soils for the biodegradable test were taken from the field near university campus.

3.3 METHODS

3.3.1 Preparation of silver nanoparticles.

The silver nanoparticles (Ag-nanoparticles) were prepared from the tea extract and silver nitrate ($AgNO_3$). The extraction from the tea powder was prepared by weighted 5 grams of tea grounds then dissolved with 500 ml of distilled water. The solution of tea was stirred for 1 hour until all the tea grounds completely dissolve in the water then leave to settle. After that, the solution of the tea was filtered by using filter paper before filtered by using filter membrane with 0.2µm (Cronus membrane nylon). 10 ml of the solution of the tea extract was added with 2

ml of 0.1 M silver nitrate and incubated at room temperature. The solution was stirred with 50 rpm to ensure the solution through mixing for about 24 hours. The present of synthesized silver nanoparticles in the solution was detected by the observation of the colour change from light brown to the dark brown. The solution of silver nanoparticles was separated and concentrated by repeated the centrifuge of mixture at 10000 rpm for 10 minutes. The supernatant was altered by distilled water each time.

3.3.2 Preparation of biodegradable poly(lactic) acid (PLA)-loaded chitosan-silver nanoparticles blend films.

The biodegradable poly(lactic acid) (PLA)-loaded chitosan-silver nanoparticles blend films was prepared by using three different solutions which are Poly (lactic acid) (PLA) solution, chitosan solution and silver nanoparticles solution. The (PLA) solution was prepared by dissolved 1 g of the (PLA) with 100 ml chloroform to obtain 1% w/w concentration. The preparation of (PLA) solution was handled in fume chamber to avoid direct inhalation with chloroform. The chitosan solution was prepared by dissolving 1 g of chitosan powder with 100 ml acetic acid (1% v/v) to obtain 1% w/w concentration. Both solution were stirred and heated on the hot plate magnetic stirrer for 2 hours until the (PLA) pellet and chitosan powder were completely dissolved. After 2 hours, 18% w/w concentration of poly(ethylene glycol) (PEG) was added into both (PLA) and chitosan solution and stirred for 1 hour. After 1 hour, 9 ml of (PLA) solution was blend with 21 ml of chitosan solution and silver nanoparticles solution at different concentration of silver nanoparticles (

4.7%, 9.1%, 16.7% and 23.1% w/w SNP) .The solution was stirred and heated on the hot plate magnetic stirrer about 30 min before put into glass Petri dish. The stirring was used to homogenize the mixture prior to pouring onto the dish. The blend films of different composition prepared by casting a mixture in glass Petri dish in 1 mm thick uniform layers. The casting film was left dried in the oven at temperature of 35 ^{o}C for 1 day. Then, the dried film was exposed in room temperature for 1-2 min and after that the film was peeled off from the glass Petri dish.

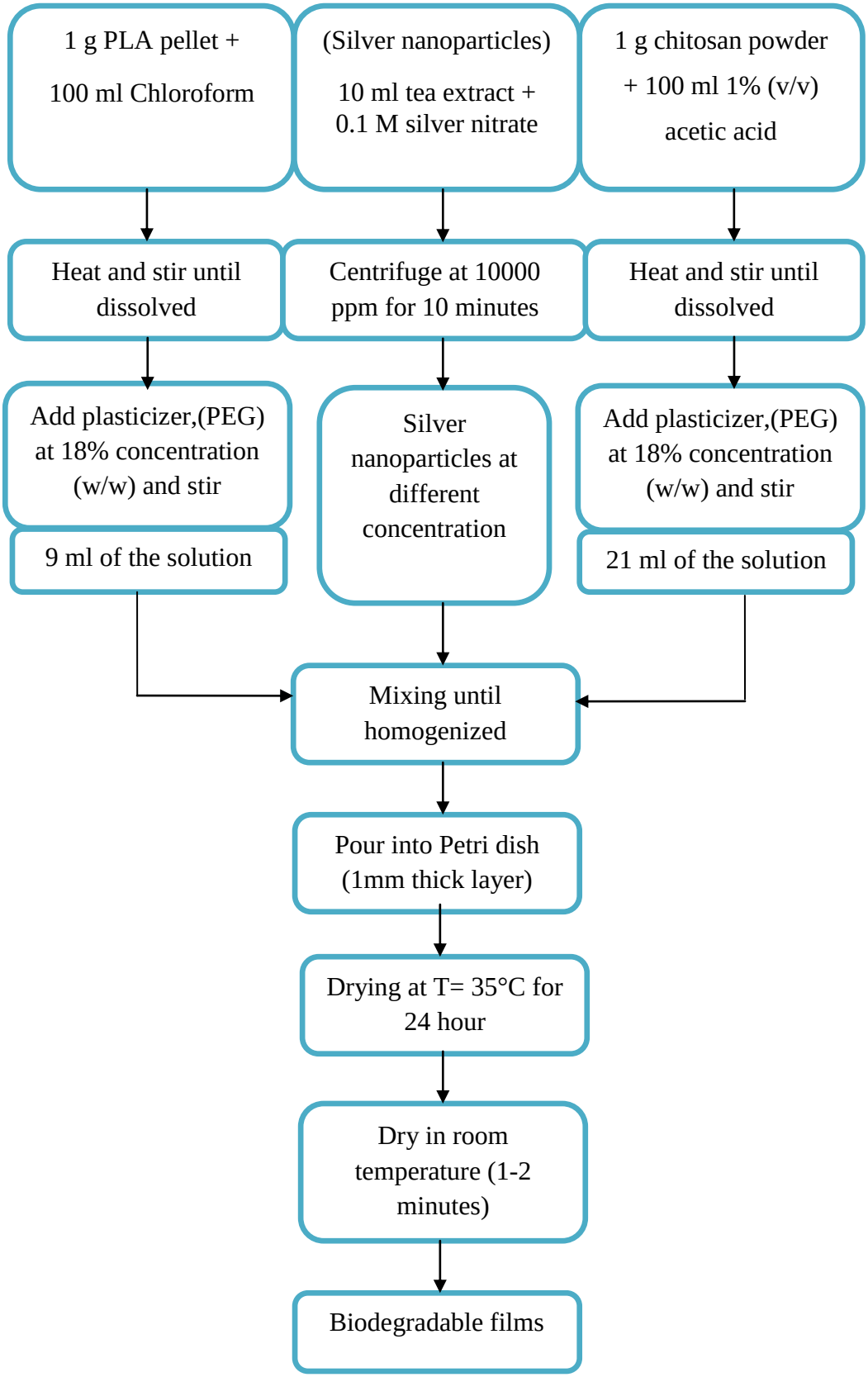


Figure 3.1 Film preparation process

3.3.3 Characterization

3.3.3.1 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was used to characterize the chemical functional groups of as-prepared biodegradable poly(lactic acid) (PLA)-loaded chitosan-silver nanoparticles blend films by producing an infrared absorption spectrum. The FTIR spectroscopy was performed by using FTIR Nicolet Avatar 370 DTGS in the range between 500 and 4000 cm^{-1}. The spectra obtained were used to determine functional groups of pure chitosan (CS) film, blends of chitosan and poly(lactic acid) (PLA) (CS/PLA) film and blends of chitosan, poly(lactic) acid (PLA) and silver nanoparticles (CS/PLA/SNP) film.

Figure 3.2 FTIR Nicolet Avatar 370 DTGS

3.3.3.2 Scanning Electron Microscopy (SEM)

The morphological investigation on the surface and cross section of the films were investigated using SEM (EDX Spectrometer EVO 50). This equipment was operated at an acceleration voltage of 15KV. Prior testing, the films were cut into 3mm wide and immersed into liquid nitrogen before coated with white gold under vacuum condition. The films were place at 90° position on the stage in order to get cross section view.

3.3.3.3 UV-Vis Absorption Spectrophotometer

The UV-Vis spectrum was used to investigate the spectrum of composites by investigate the single peak of surface plasmon vibrations of Ag atoms in the blends of chitosan, poly(lactic acid) (PLA) and silver nanoparticles (CS/PLA/SNP) film. The UV-Vis spectrum of the (CS/PLA/SNP) film was recorded by insert the liquid blended film in the UV-Vis spectroscopy cell.

Figure 3.3 UV-Vis Absorption Spectrophotometer

3.3.4 Antimicrobial activity

3.3.4.1 Preparation of Culture Medium.

The nutrient broth was prepared by weigh out 13g of nutrient broth powder and dissolved with 1L of distilled water in a 1L Schott bottle. The nutrient agar was prepared by weigh out 23g of nutrient agar powder and dissolved with 1L of distilled water in a 1L Schott bottle. Both solutions were completely stirred and heated on the hot plate magnetic stirrer until both nutrient broth and nutrient agar completely dissolves and sterile at 121°C for 20 min. lastly 15-20mL of a warm sterile nutrient agar was poured into glass petri dish. The nutrient agar was then allowed to harden.

3.3.4.2 Preparation of Microbial Culture

The flame of Bunsen burner turned on. The nutrient broth bottle cap was opened and the mouth of the bottle flamed to avoid contamination and recap it. The inoculating loop was hold and the inoculation loop was flamed to redness and allowed to cool. A loop of *E.coli* and *Micrococcus* bacteria was obtained from the plate and was inoculated by immersing the loop of *E.coli* and *Micrococcus* bacteria into different nutrient broth bottle. Lastly the *E.coli* and *Micrococcus* bacteria culture was let in the nutrient broth for 2 days.

3.3.4.3 Antimicrobial Test

The agar plate method was taken to evaluate the antimicrobial activities of chitosan (CS) film, poly(lactic acid) (PLA) film, blends of chitosan and poly(lactic acid) (PLA) (CS/PLA) film and blends of chitosan, poly(lactic acid) (PLA) and silver nanoparticles (CS/PLA/SNP) film at different concentration of silver nanoparticles (4.7%, 9.1%, 16.7% and 23.1% w/w SNP). The antimicrobial activity was investigated against negative gram of bacteria *(E.coli)* and positive gram of bacteria (*Micrococcus).* The sample films were cut into square (2x2 cm). Then 1µl of negative gram (*E.coli)* bacteria was pipette carefully on the hardened nutrient agar then the sample films were placed on it. The sample film was sealed with parafilm to avoid any contamination of other bacteria. Then, the sample film was incubated at 30°C for 2 days. The antimicrobial properties were investigated by counted the number of colonies of bacteria that survived against the antimicrobial agent of silver nanoparticles. The antimicrobial activities of the sample films were then investigated against positive gram of bacteria (*Micrococcus)* by repeating the same procedure (Li *et al.,* 2010)

3.3.5 Soil Burial Degradation

Soil burial degradation was preformed according to Yun et al., (2008) with minor adjustment. The soil burial degradation test was carried to evaluate the biodegradation rate of pure chitosan (CS) film, poly(lactic) acid (PLA) film, blends of chitosan and poly(lactic acid) (PLA) (CS/PLA) film and blends of chitosan,

poly(lactic acid) (PLA) and silver nanoparticles (CS/PLA/SNP) film at different concentration of silver nanoparticles (4.7%, 9.1%, 16.7% and 23.1% w/w SNP). The garden pots were filled with soil taken from field around the Universiti Malaysia Pahang. The films were cut into 2x2 cm pieces and weight to obtain the initial weight (W1). The films were buried in the soil at depth of 5 cm. The pots were placed in an uncovered place near the laboratory. The soil was kept moist by sprinkling water at regular time interval to maintain humidity. The excess water was drained through the hole of the bottom of the pot. The degradation of the films was determined at a regular time interval (12 days) by taking the films carefully from the soil and washing it gently with distilled water to remove the soil. The films were dried in the oven at temperature 60°C for 20 minutes until the constant weight was obtained (W2). Weight loss of the sample with time was used to indicate the degradation rate in the soil burial test. The degradation rate was calculated as Eq. (3.1)

$$\text{Degradation rate} = (W2-W1/W1) \times 100 \quad (3.1)$$

3.3.6 Water Absorption

Water absorption was performed as described by Cao et al., (2007) with slight modification. The films of pure chitosan (CS) film, poly(lactic acid) (PLA) film, blends of chitosan and poly(lactic acid) (PLA) (CS/PLA) film and blends of chitosan, poly(lactic acid) (PLA) and silver nanoparticles (CS/PLA/SNP) film at different concentration of silver nanoparticles (4.7%, 9.1%, 16.7% and 23.1% w/w SNP) were cut into rectangular size (2x2 cm) and placed in the oven at temperature 60°C for 30 minutes until the weight was constant (W1). Then the films were immersed in

30 ml of distilled water in different 50 ml of beaker for 10 minutes time interval. Wet samples were wiped with soft tissue to remove excess liquid. The films were weighted and the step above was repeated until the weight of the films become constant (W2). The amount of absorbed water was determined by using Eq. (3.2)

Maximum water absorption = (W2-W1/W1) x 100 (3.2)

3.3.7 Moisture Absorption

Moisture absorption was performed as described by S. Karnnet et al., (2005) with slight modification. The films of pure chitosan (CS) film, poly(lactic acid) (PLA) film, blends of chitosan and poly(lactic acid) (PLA) (CS/PLA) film and blends of chitosan, poly(lactic acid) (PLA) and silver nanoparticles (CS/PLA/SNP) film at different concentration of silver nanoparticles (4.7%, 9.1%, 16.7% and 23.1% w/w SNP) were cut into rectangular size (2x2 cm) and placed in the oven at temperature 35°C for 30 minutes until the weight was constant (W1). These samples were then placed in moisture saturated atmosphere for 7 days. After that, the samples were weighed (W2). Moisture absorption was then calculated by following the Eq. (3.3)

Moisture Absorption = (W2-W1/W1) x 100 (3.3)

CHAPTER 4

RESULT AND DISCUSSION

4.1 INTRODUCTION

This chapter provides a detail discussion on the analysis result of biodegradable of poly(lactic) acid (PLA) -loaded chitosan -silver nanoparticles blend films.

4.2 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was used to identify chemical bonds in a molecule by producing an infrared absorption spectrum. The infrared spectra of pure chitosan (CS) film, blends of chitosan and poly(lactic acid) (PLA) (CS/PLA) film and blends of chitosan, poly(lactic acid) (PLA) and silver nanoparticles (CS/PLA/SNP) film were shown in Figure 4.1. For chitosan (CS) film and (CS/PLA) blend film spectrum (Figure 4.1a and 41b), the characteristic peaks assignment of chitosan are 3284 cm^{-1} and 3364 cm^{-1} (O-H stretch overlapped with N-H stretch), 2877 cm^{-1} and 2881 cm^{-1} (C-H stretch), 1567 cm^{-1} (amide II band, N-H

stretch), 1407-1380 cm^{-1} (asymmetric C-H bending of CH_2 group), and 1073 cm^{-1} and 1075 cm^{-1} (skeletal vibration involving the bridge C-O stretch). While for the blends of chitosan, poly(lactic) acid (PLA) and silver nanoparticles (CS/PLA/SNP) film at different concentration of silver nanoparticles (4.7% and 9.1%, w/w SNP) were shown in (Figure 4.1c and 4.1d) respectively. The spectral band appears at 3364 cm^{-1} corresponding to OH/NH_2 groups has shifted to 3442 cm^{-1} indicating that the silver nanoparticles are bounded to functional groups chitosan and PLA.

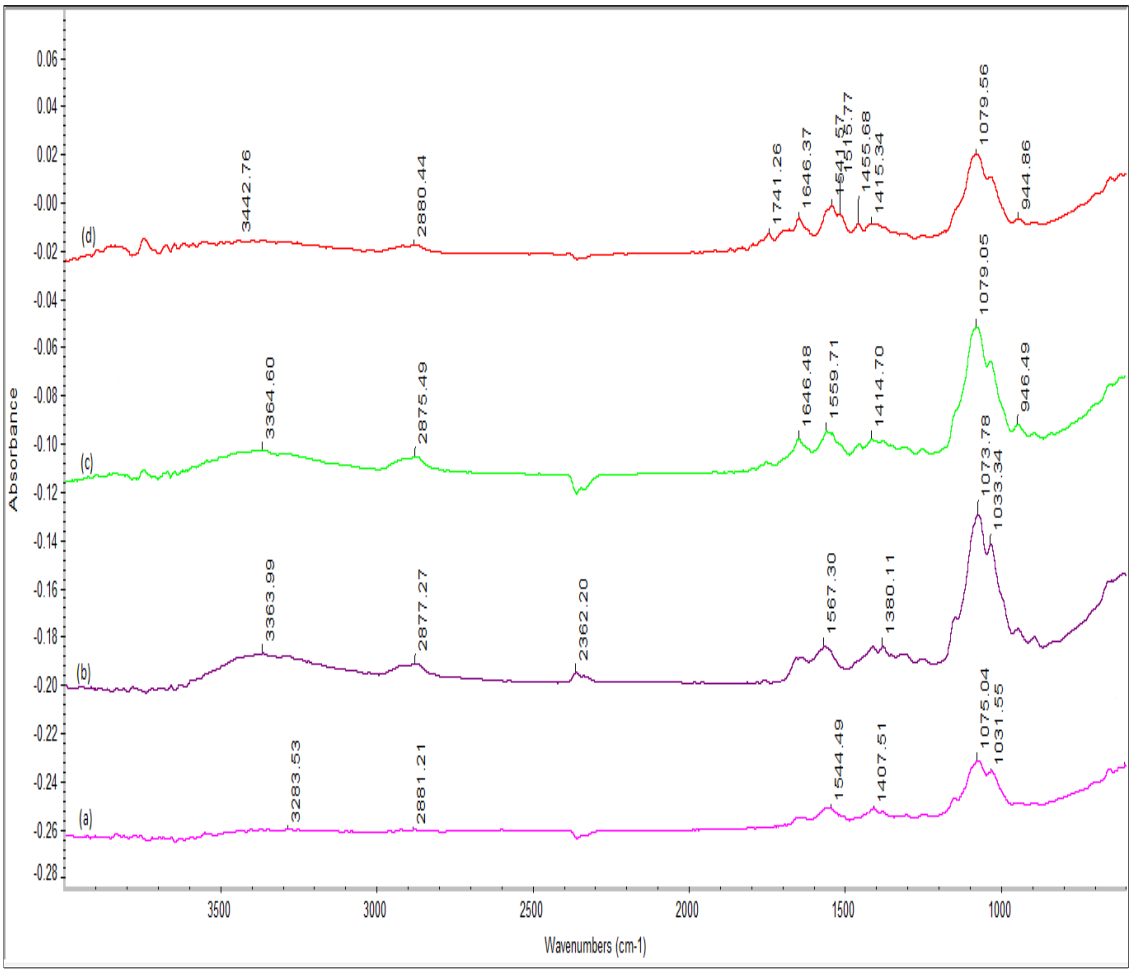


Figure 4.1 FTIR spectra of the samples: (a) pure CS film, (b) CS/PLA film, (c) blends film of (CS/PLA/ 4.7 % w/w SNP), (d) blends film of (CS/PLA/ 9.1% w/w SNP).

The shifting of the peak is due to the formation of co-ordination bond between the silver atom and electron rich groups (oxygen/nitrogen) present in chitosan. This causes the bond length and frequency. The spectral bands at 1741 cm^{-1} corresponding to the (C=O carbonyl group vibration), 1646 cm^{-1} (amide I of C=O stretching, N-H,C-N stretching and CH_2 wagging coupled with OH groups of chitosan respectively,1415 cm^{-1} (C-C stretching vibration and asymmetric C-H bending of CH_2 group) and 1079 (skeletal vibration involving the bridge C-O stretch). FTIR study indicates (-C=O), hydroxyl (-OH) and amine (N-H) groups shows the present of silver nanoparticles bounded to the chitosan and PLA.

4.3 Scanning Electron Microscopy (SEM)

Surface morphological investigation of the surface and cross section of the sample films were investigated by using SEM as shown in Figure 4.2. The image of SEM show the addition of silver nanoparticles into the solution in film making, gave the different surface of films morphologies between incorporated with silver nanoparticles (a, c ,d) and without silver nanoparticles films (b, d, e) at different magnification. The presence of silver nanoparticles with uniform distribution on film surface can be obviously detected on films (a, c, d). The films load with silver nanoparticles show more rough surface compared to films without silver nanoparticles. The films loaded with silver nanoparticles show the strong aggregation of particles in granulate with an average diameter of 20-100 *nm* compared to the film without silver nanoparticles, which is relatively smooth view with no sign of silver nanoparticles.

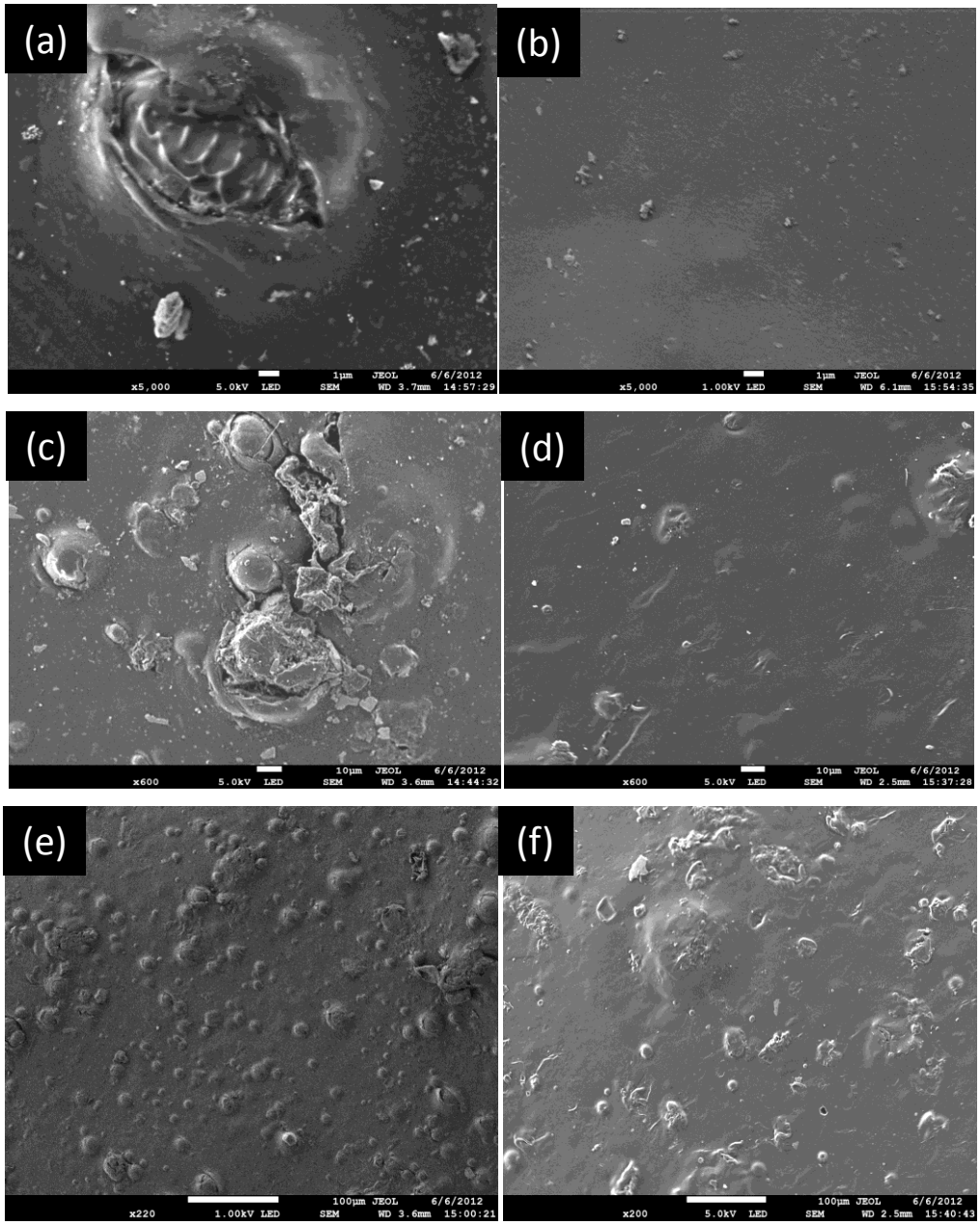


Figure 4.2 SEM images of comparison between incorporated with and without silver nanoparticles film.(a) Film with silver nanoparticles at magnification 5000x (b) Film without silver nanoparticles at magnification 5000x (c) Film with silver nanoparticles at magnification 600x (d) Film without silver nanoparticles at magnification 600x (e) Film with silver nanoparticles at magnification 200x (f) Film without silver nanoparticles at magnification 200x.

4.4 UV-Vis Absorption Spectrophotometer

The UV-Vis absorption spectrophotometer was used to investigate the surface plasmon resonance (SPR) of silver nanoparticles (SNP) in the blends of chitosan, poly(lactic acid) (PLA) and silver nanoparticles (CS/PLA/SNP) film. The UV-Vis absorption spectra are quite sensitive to the formation of silver colloids because silver nanoparticles exhibit an intense peak due to the surface plasmon of the collective excitation of conduction electrons in a metal itself (Šileikaitė, *et al.*, 2006). The UV-Vis spectrum of the silver colloids (Ag colloids) in the blends of chitosan, poly(lactic acid) (PLA) and silver nanoparticles (CS/PLA/SNP) film in the range 300 to 800 nm was shown in Figure 4.3.

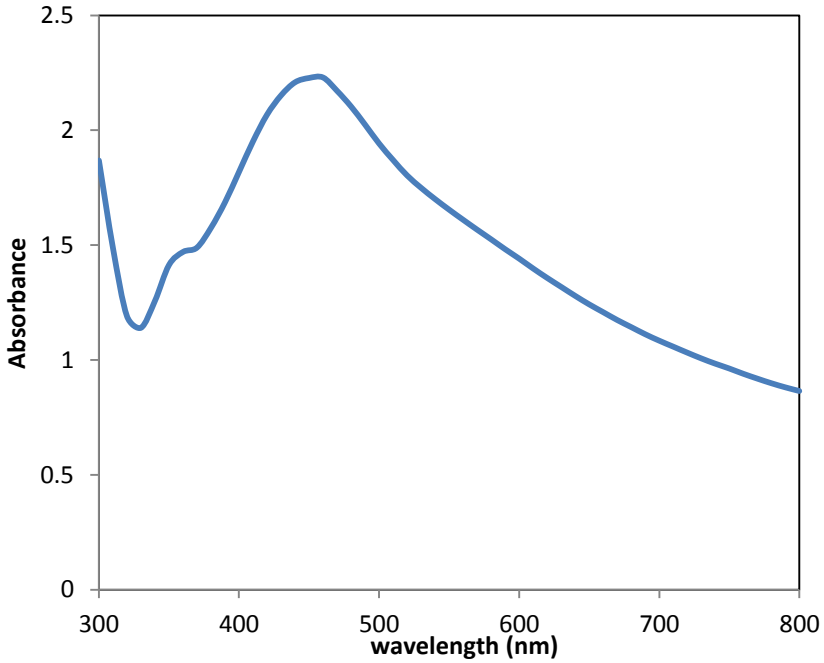


Figure 4.3 UV-Vis spectrum of blends chitosan, poly(lactic) acid (PLA) and silver nanoparticles (CS/PLA/SNP) film.

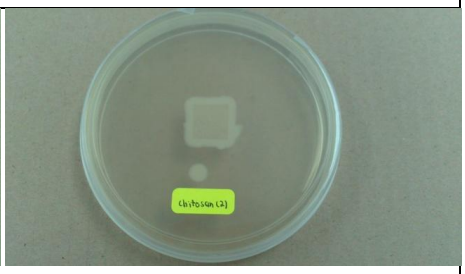
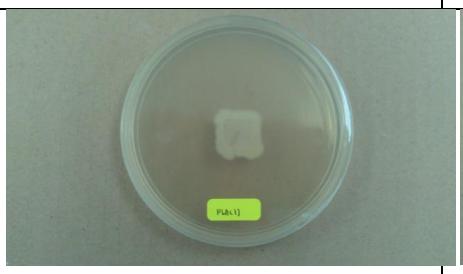
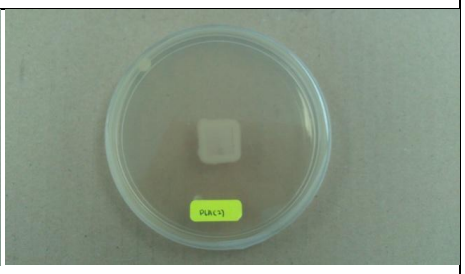
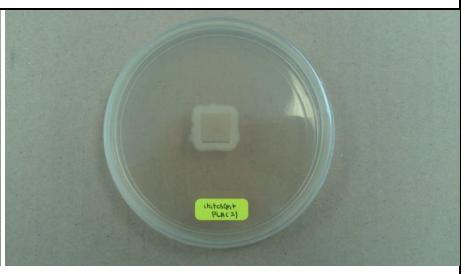
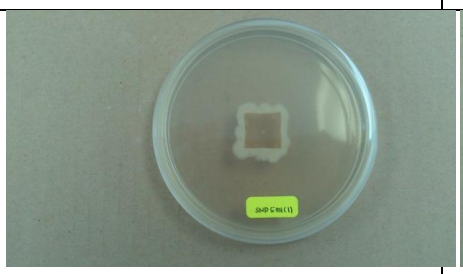
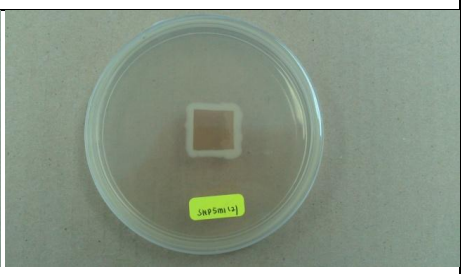
The absorption spectrum is plot of absorbance of electromagnetic radiation through a sample versus wavelength (energy). Colored solutions that contain species absorb in the visible region of the electromagnetic spectrum at 350 to 750 nm. The absorption band in visible light region is seen at wavelength 370-580 nm with plasmon peak at 452 nm that is typical for silver nanoparticles (Šileikaitė, *et al.,* 2006). The solution of blends chitosan, poly(lactic acid) (PLA) and silver nanoparticles (CS/PLA/SNP) film that contains silver nanoparticles (SNP) strongly absorbs light at 452 nm (blue light). A single peak at 452 nm in the spectrum of the composites arises due to the excitation of surface plasmon vibrations of Ag atoms (Tripathi *et al.,* 2009)

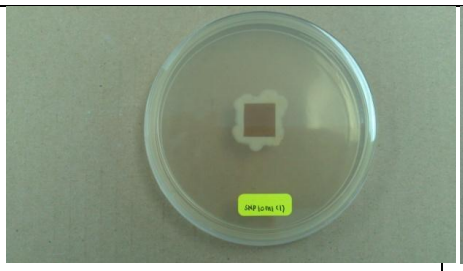
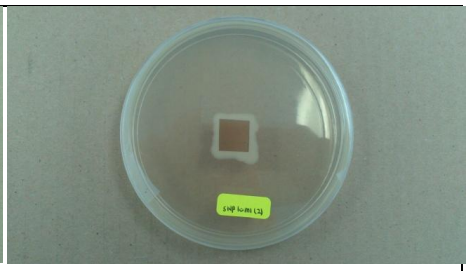
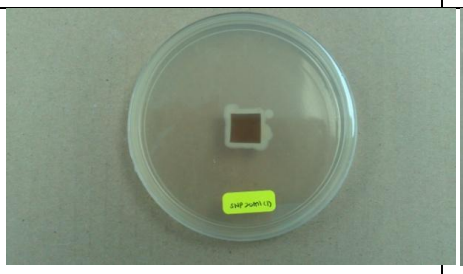
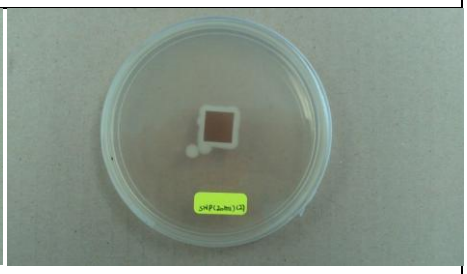
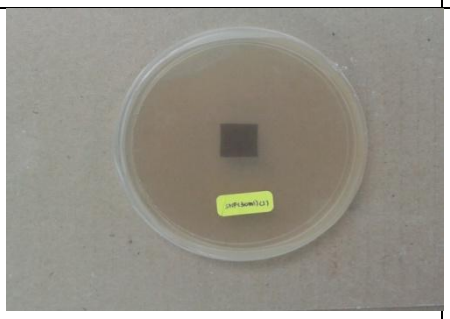
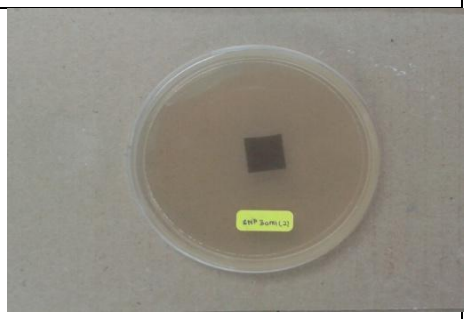
4.5 Antimicrobial test

Antimicrobial test is to investigate the antimicrobial activity of the films by using the colony forming count method (Li *et al.,* 2007). The antimicrobial activity were performed on the sample films of pure chitosan (CS) film, poly(lactic) acid (PLA) film, blends of chitosan and poly(lactic acid) (PLA) (CS/PLA) film and blends of chitosan, poly(lactic acid) (PLA) and silver nanoparticles (CS/PLA/SNP) film at different concentration of silver nanoparticles (4.7%, 9.1%, 16.7% and 23.1% w/w SNP) against the Gram-negative bacterium *E.coli* and Gram-positive bacterium *Micrococcus.*

Table 4.1 shows the number of bacterial colonies grow on the sample films. The number of bacterial colonies grow were counted by measured the diameters of the bacteria colonies that survived against antimicrobial of silver nanoparticles (SNP) as shown in the Table 4.2.

Table 4.1 Antimicrobial activity on the different sample films against the Gram-negative bacterium *E.coli* and Gram-positive bacterium *Micrococcus.*

Sample	E.coli (-ve gram bacteria)	Micrococcus (+ve gram bacteria)
CS		
PLA		
CS/PLA		
CS/PLA/ 4.7% w/w SNP		

CS/PLA/ 9.1% w/w SNP		
CS/PLA/ 16.7% w/w SNP		
CS/PLA/4 .23.1% w/w SNP		

There are several methods to prepare antimicrobial activity of the films. The colony forming count method is one of the methods to investigate the antimicrobial activity of the sample films against two strains of bacteria which are Gram-negative bacterium *E.coli* and Gram-positive bacterium *Micrococcus.* The antimicrobial activity of the films without silver nanoparticles (SNP) and with silver nanoparticles (SNP) is shown in Table 4.1. After incubation at 30°C for 48 h, the growth of bacteria underneath of sample films was observed and the diameter of bacteria colonies that survived was measured as shown in Table 4.2.

Table 4.2 The diameters of the bacteria colonies on the sample films.

Sample film	D (mm)	
	E.coli (-ve gram bacteria)	Micrococcus (+ve gram bacteria)
CS	3.5 ± 0.2	3.0 ± 0.2
PLA	4.0 ± 0.2	3.5 ± 0.2
CS/PLA	4.0 ± 0.2	3.5 ± 0.2
CS/PLA/ 4.7% w/w SNP	3.0 ± 0.1	2.5 ± 0.1
CS/PLA/9.1% w/w SNP	3.0 ± 0.1	2.5 ± 0.1
CS/PLA/16.7% w/w SNP	2.5 ± 0.1	2.0 ± 0.1
CS/PLA/23.1% w/w SNP	-	-

The chitosan (CS) film also show the effectiveness in inhibition of bacteria growth due to the properties of chitosan itself that have antimicrobial properties towards bacteria. Chitosan in its free polymer has been proved to have antimicrobial activity against Gram-negative and Gram-positive bacteria (Chung *et al.,* 2011). The antimicrobial activity of chitosan is due to the interaction between positively charged chitosan molecules and negatively charged microbial cell membranes leads to the leakage of intracellular constituents. Binding of chitosan to DNA triggers inhibition of mRNA synthesis through penetration of the microbial nuclei by chitosan and interfering with the synthesis of mRNA and proteins (Chung *et al.,* 2011). The chitooligomers caused the formation of pore and permeabilization of the cell wall of bacteria, whereas nutrient flow will blocked due to aggregation of chitooligomers thus responsible for the growth inhibition and lysis of *E.coli* and *Micrococcus* (Kumar *et al.*, 2005).

Chitosan acetate acted on the bacterial membrane and the antibacterial activity of chitosan acetate may be due to the disruption or penetration of cell membranes (Li *et al.,* 2007). It is approved that the chitosan itself have antimicrobial activity towards bacteria and it is shown in the Table 4.2, the diameter of bacteria colonies reduced compared to the diameter of bacteria colonies that growth at edges of poly(lactic acid) (PLA) film because poly(lactic acid) (PLA) film not have resistivity towards negative-gram bacteria and positive-gram bacteria. The presence of silver nanoparticles in the films was effective at inhibiting the growth of Gram-negative bacterium *E.coli* and Gram-positive bacterium *Micrococcus.* The size of bacteria colonies grown on the blends of chitosan, poly(lactic acid) (PLA) and silver nanoparticles (CS/PLA/SNP) film at different concentration of silver nanoparticles (4.7%, 9.1%, 16.7% and 23.1% w/w SNP) was significantly reduce by measured the diameter of bacteria colonies at the edges of the sample films. A concentration of silver nanoparticles at 23.1 % w/w was caused 100% inhibition of bacterial growth.

The antimicrobial activity of the films against Gram-positive bacterium Micrococcus was greater than that against the Gram-negative bacterium E.coli. This might be a result of the difference in cell wall structure of those microorganisms, especially a possession of the outer membrane. The cell wall of the Gram-negative consists of lipids, proteins and lipopolysaccharides (LPS) that provide effective protection against biocides whereas that of the Gram-positive does not consists of LPS (Maneerung *et al.,* 2010). The Gram-positive bacteria have a single layer that is (~20-80 nm) thick and does not possess an outer membrane which anchoring and penetrating of the positively charged silver nanoparticles (SNP) through the cell wall which results in weak-end cell walls and cell lysis and death.

The Gram-negative bacteria have more complex cell wall compared to cell wall of Gram-positive bacteria. The Gram-negative bacteria posses an outer membrane that lies outside of thin peptidoglycan layer which limited the penetrating the positively charged silver nanoparticles (SNP) (R. Yoksan *et al.,* 2010). The free radicals formed at the surface at the surface of silver nanoparticles induced membrane damage. The nanoparticles may attached to the surface membrane of the cell and penetrate into the bacteria cell, thus disturbing permeability and respiration functions of the cells (Kim *et al.,* 2007). The antimicrobial properties of chitosan (CS) film and poly(lactic acid) (PLA) film was improved by blending with silver nanoparticles (SNP) as shown in Table 4.2, there is no bacteria colonies on the film of (CS/PLA/23.1% w/w SNP) for both Gram-negative bacteria and Gram-positive bacteria which shows that the silver nanoparticles was approved have antimicrobial properties.

4.6 Soil Burial Degradation Test

The soil burial degradation test was using soil that is similar to the environment of disposal area of plastic. Any physical or chemical change in polymer as a result of environmental factors such as light, heat, moisture, chemical conditions or biological activity which lead to induce changes in polymer properties due to chemical, physical or biological reactions resulting in bond scission and subsequent chemical transformations have been categorized as polymer degradation (Shah, 2008).

The soil burial degradation test was using the soils that have acidic condition with pH 4.93. The soil was a medium to degrade the sample films via biological reactions.

Table 4.3 The degradation rates of the sample films

Sample films	Weight before degradation process (g) (W1)	Weight after degradation process within 12 days (g) (W2)	% of degradation rate (w2-w1/w1)x100
CS	0.0065	0.0049	24.62
PLA	0.0162	0.0136	16.05
CS/PLA	0.0205	0.0158	22.93
CS/PLA/ 4.7% w/w SNP	0.0085	0.0061	28.23
CS/PLA/9.1% w/w SNP	0.0145	0.0115	20.69
CS/PLA/16.7% w/w SNP	0.0150	0.0121	19.33
CS/PLA/23.1% w/w SNP	0.0169	0.0141	16.51

Biodegradability of the samples was studied by evaluating the weight loss of the sample films of pure chitosan (CS) film, poly(lactic acid) (PLA) film, blends of chitosan and poly(lactic acid) (PLA) (CS/PLA) film and blends of chitosan, poly(lactic acid) (PLA) and silver nanoparticles (CS/PLA/SNP) film at different concentration of silver nanoparticles (4.7%, 9.1%, 16.7% and 23.1% w/w SNP) within 12 days in a soil environment. From the Table 4.3 all the sample films degrade by biological degradation within a short time compared to plastic bottles that needs 100 years to degrade. PLA is biodegradable and biocompatible thermoplastic which can be produced by renewable resources. PLA-degraders affected to microbial attack compared to other microbial and synthetic aliphatic polymers. The degradation of PLA in soil is slow and takes a long time for degradation to start (Tokiwa *et al.*, 2009).

It is shown in Table 4.3, poly(lactic) acid (PLA) film has slow degradation rate compared to other sample films. The degradation rates of chitosan (CS) film show higher rate compared to degradation rate of poly(lactic acid) (PLA) film. This is due to biological activity of chitosan (CS) film appears faster in acidic medium. Chitosan is known to be non toxic, odourless, biocompatible with living tissue and biodegradable and due to these advantages, chitosan and its derivatives are seen in applications such as biodegradable packaging (Peesan *et al.*, 2006).

Chitosan is a biodegradable, biocompatible, positively charged nontoxic biopolymer. Since chitosan contains primary amino groups in the main backbone that make surfaces positively charge in biological fluids, biodegradable nanoparticles can be readily prepared by treating chitosan with a variety of biocompatible polyanionic complexants in soil (Sonia & Sharma, 2011). Biodegradable polyesters such as poly(lactic) acid (PLA) generate acidic degradation products at the implanted site which cause undesirable tissue reaction. Chitosan may be combined with acid producing biodegradable polymers so that local toxicity due to the acid by-products can be reduced (Yao *et al*., 2005). The biodegradability of poly(lactic acid) (PLA) film will be improved by blending with chitosan producing the blends of chitosan and poly(lactic acid) (PLA) (CS/PLA) film.

The nanocomposite films based on a biodegradable chitosan and poly(lactic) acid (PLA) (CS/PLA) film with different concentration of silver nanoparticles (SNP) also conducted under this degradation studies. From this degradation studies, the blends of chitosan, poly(lactic acid) (PLA) and silver nanoparticles (CS/PLA/SNP) film at different concentration of silver nanoparticles (4.7%, 9.1%, 16.7% and 23.1% w/w SNP) shows the degradation process but at slow rate. At high concentration of silver nanoparticles (SNP), the degradation rate will be slower compare to small concentration of silver nanoparticles (SNP). This is due to releasing of silver ions (Ag+) that effect the polymer degradation processes (Feng *et al.,* 2000). The biodegradation process is chemical dissolution by microorganisms so releasing of silver ions (Ag+) will limits the degradation reactions by microorganisms.

4.7 Moisture and Water Absorption

Table 4.4 The moisture absorption rates of the sample films

	Sample films	Weight before moisture absorption (g) (W1)	Weight after moisture absorption (g) (W2)	% of moisture absorption (w2-w1/w1)x100
(a)	CS	0.0149	0.0158	6.04
(b)	PLA	0.0131	0.0135	3.05
(c)	CS/PLA	0.0172	0.0180	4.65
(d)	CS/PLA/ 4.7% w/w SNP	0.0218	0.0230	5.50
(e)	CS/PLA/9.1% w/w SNP	0.0101	0.0107	5.94
(f)	CS/PLA/16.7% w/w SNP	0.0113	0.0121	7.07
(g)	CS/PLA/23.1% w/w SNP	0.0216	0.0235	8.79

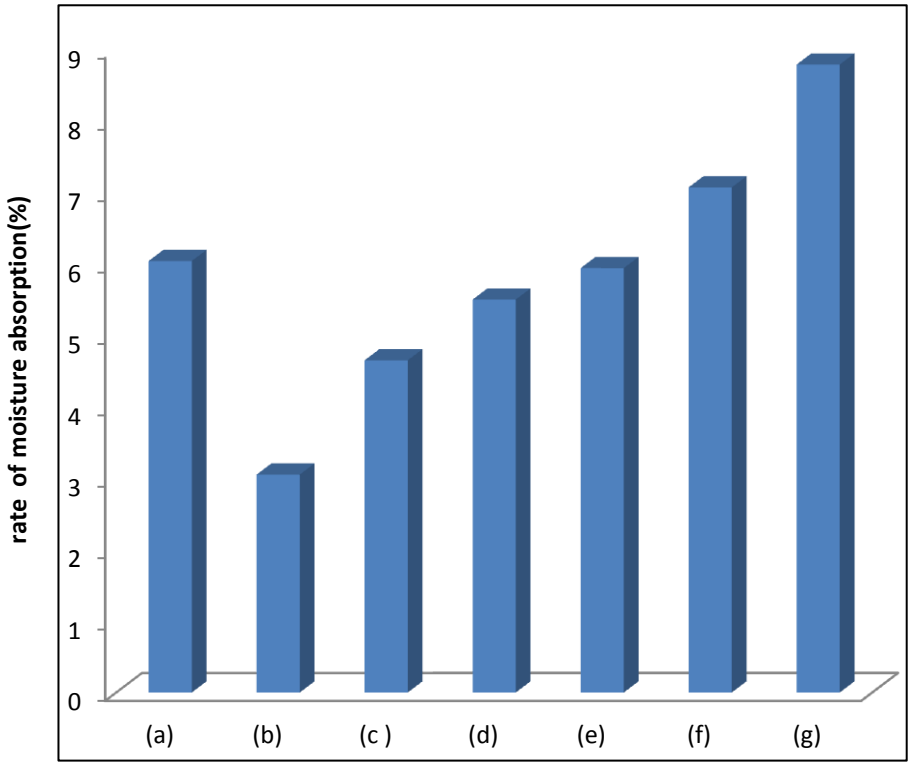


Figure 4.4 The moisture absorption rate of the sample films

Table 4.5 The water absorption rates of the sample films.

	Sample films	Weight before water absorption (g) (W1)	Constant weight after immersed in distilled water (g) (W2)	% of water absorption (w2-w1/w1)x100
(a)	CS	0.0189	0.0247	30.68
(b)	PLA	0.0165	0.0195	18.18
(c)	CS/PLA	0.0261	0.0332	27.20
(d)	CS/PLA/ 4.7% w/w SNP	0.0096	0.0137	42.70
(e)	CS/PLA/9.1% w/w SNP	0.0128	0.0200	56.25
(f)	CS/PLA/16.7% w/w SNP	0.0156	0.0251	60.10
(g)	CS/PLA/23.1% w/w SNP	0.0270	0.0473	75.18

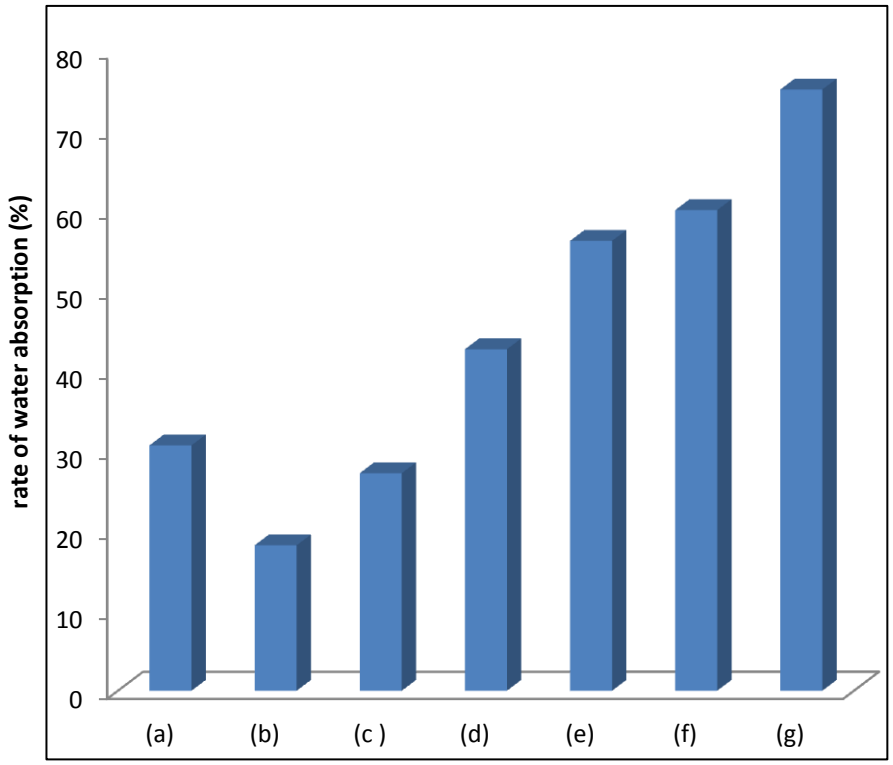


Figure 4.5 The water absorption rate of the sample films

The water absorption and moisture absorption for different type of sample films are shown in Table 4.4 and 4.5. From the results, the poly(lactic acid) (PLA) film show good resistivity towards water and moisture absorption compared to other sample films. Concerning the solubility, the addition of PLA decreased the solubility in water compared to pure chitosan (CS) film. The water solubility of composite films was close to 30-35%, depending on the PLA content in a study related to the properties of PLA, it is expected that the exposed pure chitosan absorb moisture faster than those coated of chitosan with PLA.

From the results of water and moisture absorption, the blends of chitosan and poly(lactic acid) (PLA) (CS/PLA) film reduced the percent of absorption. It is due to the properties of PLA that is insoluble in water coated at the surface of chitosan film but for short exposure times, water molecules saturated at the surface of the blends films and penetrate into the blends film through void (Shukla *et al.*, 2012). PLA lead to strong reduction of water solubility due to non-soluble properties of PLA that decrease the hydroxyl groups of the chitosan matrix and makes the blends of chitosan and poly(lactic acid) (PLA) (CS/PLA) film weak sensitivity to water compared to pure chitosan (CS) film. The blends of chitosan, poly(lactic) acid (PLA) and silver nanoparticles (CS/PLA/SNP) film at different concentration of silver nanoparticles (4.7%, 9.1%, 16.7% and 23.1% w/w SNP) increase the solubility of water as the concentration of silver nanoparticles (SNP) increase. It's happened due present of molecules silver nanoparticles (SNP) between the porosity of chitosan and PLA matrix makes the blends film strong sensitivity to water.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The poly(lactic acid) (PLA)-loaded chitosan-silver nanoparticles blend films were successfully produced by using mechanical mixing process and casting method. The films were characterized by using Fourier transform infrared spectroscopy (FTIR), Scanning electron microscope (SEM) and UV-Vis absorption spectrophotometer. The FTIR test identified the molecular bonds exist between the material used and the present of the silver nanoparticles was detected by using UV-Vis absorption spectrophotometer due to the excitation of surface plasmon at single peak 452 nm. Through the SEM, it can be seen that, the morphological surface of the films containing silver nanoparticles show rough surface compared to the film that do not contains silver nanoparticles. The films were further analyzed for biodegradability by using soil burial biodegradation. The poly(lactic acid) (PLA)-loaded chitosan-silver nanoparticles blend films were show positive response towards degradation process due to the weight loss within 12 days. The moisture and water absorption of the films

were also analyzed by using swelling method and the poly(lactic acid) (PLA) film show high resistivity towards water compared to the chitosan (CS) film. The blending both of poly(lactic) acid (PLA) film and chitosan (CS) film improved the weakness of chitosan films towards water absorption..

The blends of poly(lactic acid) (PLA) and chitosan (CS) film was further improved by blending with silver nanoparticles. The antimicrobial properties of silver nanoparticles make the film perfect for food packaging film. The concentration of silver nanoparticles at (23.1 % w/w SNP) shows the 100% of inhibition against Gram-negative bacterium *E.coli* and Gram-positive bacterium *Micrococcus.* Thus, it show that silver nanoparticles were successfully inhibit the bacteria grow and improve the antimicrobial properties of poly(lactic acid) (PLA)-loaded chitosan-silver nanoparticles blend films.

5.2 Recommendations

Previously, the limitations of chitosan by high solubility of chitosan can be improved through introduction of specific monosaccharide (especially glucosamine). The water-soluble chitosan derivatives effectively enhanced the solubility of chitosan over the preparations of chitosan derivatives through Maillard reactions between chitosan and reducing sugars (glucose or glucosamine). Glucosamine, like chitosan, contains active amino and hydrophilic a relatively wide pH range (Chung *et al.,* 2011). The chitosan derivatives through Maillard reactions may overcome the solubility limitations of chitosan.

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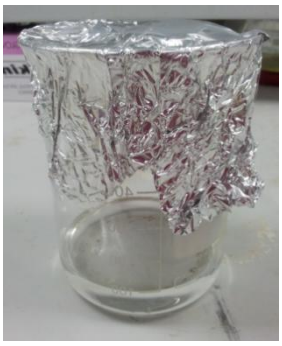
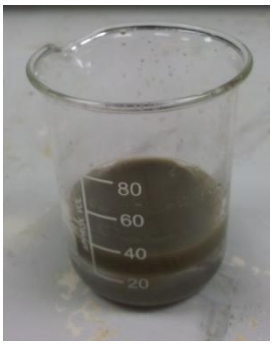
APPENDIX A

FILM PREPARATION

Preparation Chitosan solution

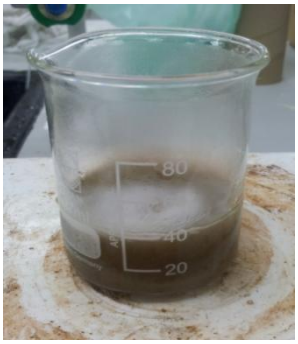


Preparation PLA solution



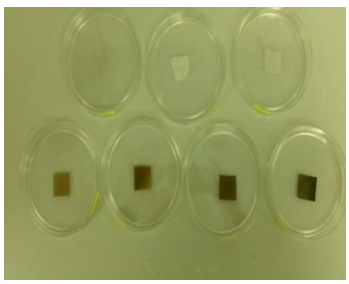
Preparation Silver nanoparticles solution

Mix chitosan & (PLA) solution
+ 18% PEG



Blends solution
+ Silver nanoparticles

Casting of film in glass Petri dish



Biodegradable Films

APPENDIX B

1) Calculation in preparation of biodegradable CS/PLA/SNP film at different concentration of SNP

Chitosan solution = 1g/100 ml x 21 ml = 0.21
Poly(lactic acid) (PLA) solution = 1g/100 ml x 9 ml = 0.09

Concentration of 23.1% w/w SNP

$$\frac{SNP}{CS + PLA + SNP} = 0.231$$

$$\frac{SNP}{0.09 + 0.21 + SNP} = 0.231$$

SNP = 0.231(0.3) + 0.231SNP
0.0769 SNP = 0.0693
SNP = 0.09

1ml of SNP = 3000 µg
SNP = (1 ml /3000x 10^{-6}) x 0.09 = 30 ml

At concentration of 23.1% w/w SNP
Volume of blending solutions required;
Chitosan solution = 21 ml
PLA solution = 9 ml
Silver nanoparticles solution = 30 ml

The volume of the SNP solution required;
23.1% w/w SNP = 30 ml
16.7% w/w SNP = 20 ml
9.1% w/w SNP = 10 ml
4.7% w/w SNP = 5 ml

ANALYSIS RESULT

Fourier Transform Infrared Spectroscopy (FTIR)

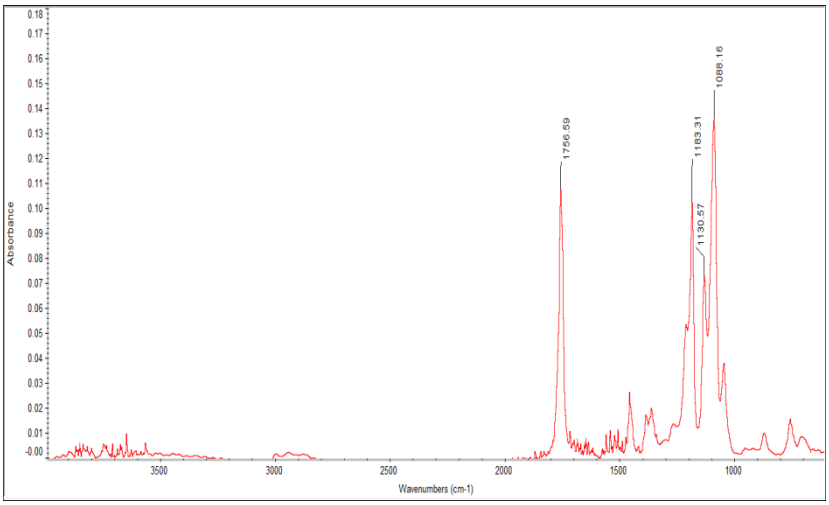


Figure B.1 The analysis of FTIR on PLA film

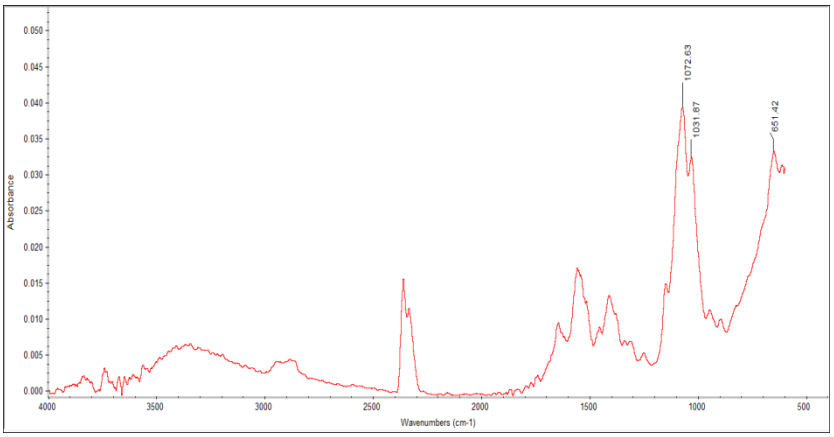


Figure B.2 The analysis of FTIR on chitosan (CS) film

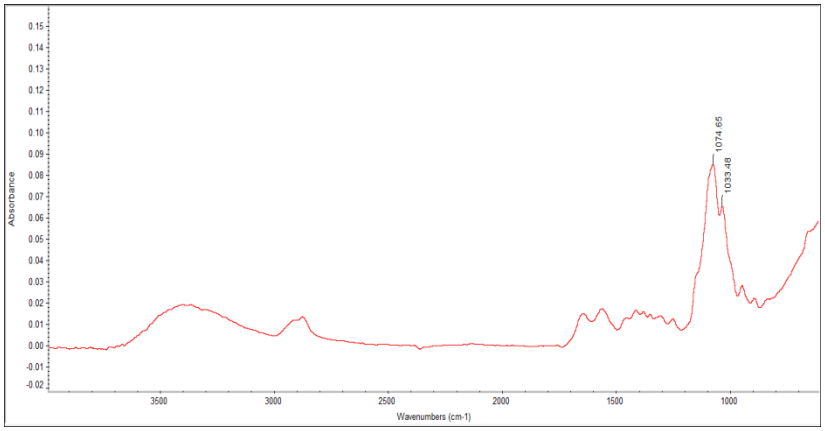


Figure B.3 The analysis of FTIR on CS/PLA film

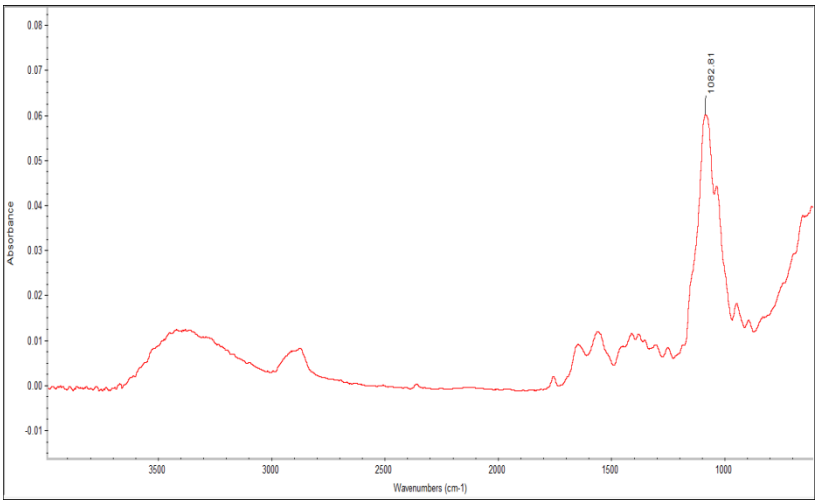


Figure B.5 The analysis of CS/PLA/SNP films

UV-Vis Absorption Spectrophotometer

Wavelength versus absorbance

300	1.868	470	2.171	640	1.279
310	1.488	480	2.103	650	1.242
320	1.191	490	2.024	660	1.208
330	1.14	500	1.942	670	1.174
340	1.257	510	1.87	680	1.143
350	1.413	520	1.803	690	1.112
360	1.47	530	1.749	700	1.084
370	1.489	540	1.7	710	1.058
380	1.575	550	1.654	720	1.032
390	1.686	560	1.61	730	1.007
400	1.818	570	1.567	740	0.984
410	1.951	580	1.525	750	0.963
420	2.067	590	1.482	760	0.94
430	2.151	600	1.441	770	0.919
440	2.208	610	1.398	780	0.899
450	2.227	620	1.357	790	0.881
460	2.229	630	1.318	800	0.865

Table B.1 Data of UV-Vis Absorption spectrophotometer of CS/PLA/SNP film