PRODUCTION OF BIOCOMPOSITE FILM FOR PACKAGING APPPLICATION

(PEMBINAAN FILEM BIOKOMPOSIT UNTUK APPLIKASI PEMBUNGKUSAN)

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Specially dedicated to
our beloved family and friends who have encouraged, guided and inspired
throughout our journey of education

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ABSTRACT

PRODUCTION OF BIOCOMPOSITE FILM FOR PACKAGING APPLICATION

(Keywords: Biodegradable, film, composite, packaging)

This study will be focusing on fabrication of composite biodegradable film from chitosan blends with addition of antimicrobial agent. The film was characterized in term of morphology, physical, chemical and also the antimicrobial analysis. The chitosan solution was prepared by preparing chitosan solution and antimicrobial agent was added to the mixture. The solution was cast onto flat glass plate. The thickness of the film was adjusted using a casting knife. The film was left to dry at room temperature before peeled off. The fabricated film was analyzed and characterized using Scanning electron microscopes (SEM), Fourier transform infrared Spectroscopy (FTIR), Thermogravimetric analyzer (TGA) and Differential scanning calorimeter (DSC). The antimicrobial analyses were performed using Zone inhibition assay and Liquid culture test. As a conclusion, chitosan blend biodegradable films shows the highest thermal resistant compared to control film. Other than that, the film also shows the greatest antimicrobial activity towards *E.coli* and *B.subtilis.*

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ABSTRAK

PEMBINAAN FILEM BIOURAI UNTUK APPLIKASI PEMBUNGKUSAN MAKANAN

(Kata kunci: biourai, filem, komposit,pembungkusan)

Kajian ini memfokuskan fabrikasi filem boleh biodegradasi daripada campuran antara chitosan dengan bahan anti mikrob. Karakter filem ini dikenal pasti daripada segi morfologi, fizikal, kimia dan juga daripada segi analisis anti-mikrob. Dalam menyediakan campuran untuk membentuk filem tersebut, chitosan dilarutkan dan anti mikrob ditambah ke dalam larutan. Larutan dituangkan ke atas plat gelas yang rata. Ketebalan lapisan larutan dikawal menggunakan pisau penipis. Filem tersebut dibiarkan kering sebelum dicabut. Filem tersebut dikarakter menggunakan *Scanning electron microspoce* (SEM), *Fourier transform infrared spectroscopy* (FTIR), *Thermogravimetric analyzer* (TGA) dan juga *Differential scanning calorimeter* (DSC). Analisis anti-mikrob filem tersebut dijalankan dengan mengunakan teknik *Zone inhibition assay* dan juga *Liquid culture test.* Sebagai kesimpulan, filem boleh biodegradasi daripada campuran chitosan menunjukkan ketahanan haba yang paling tinggi berbanding filem kontrol. Selain itu, filem tersebut menunjukkan aktiviti anti-mikrob paling tinggi terhadap *E.coli* dan *B. subtilis.*

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LIST OF ABBREVIATIONS

RH	-	Relative humidity
AFM	-	Atomic Force Microscopy
FTIR	-	Fourier Transform Infrared
DSC	-	Differential Scanning Calorimeter
TGA	-	Thermo Gravimetric Analysis

LIST OF SYMBOLS

^{o}C	-	celcius
g	-	gram
mg	-	miligram
%	-	percent

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Nowadays there are many research related on biodegradable film had been performed in order to help the environment. The majority of engineered plastic materials used today are made from synthetic polymers. Usage of conventional petroleum-based polymer products creates many potential problems due to their non-renewable nature and ultimate disposal (Mali and Grossmann, 2003). Cellulose and its derivatives, for example from the rice starch when used in such applications, offer advantages with respect to sustainability, limited environmental impact and simplified end-of-life disposal issues and this clearly helps to preserve the environment (Mali and Grossmann, 2003). Biodegradable polymer films are not meant to totally replace synthetic packaging films, but to limit moisture, aroma, and lipid migration between food components where traditional packaging cannot function (Xu *et al.*, 2005).

This study is to see how rice starch can work out with chitosan to improve the quality of biodegradable film produced since both of the sources were proven worthy in producing biodegradable plastics which will helps in preserving the quality of living for mankind in nowadays environment. Furthermore this study will improve the quality of the biodegradable film by which antimicrobial agent will also added in order to magnificent and increase the variability of its usage. As we all know nowadays plastic not only used in packaging material and stuff but also used in many other industries such as food industries, plantation and even in clinical and pharmaceutical side. Only few of them in the market contain antimicrobial or antiseptic agent (Xu *et al.*, 2005).

In certain industries such as food packaging and pharmaceutical, hygiene become priority since some of the bacteria and microorganisms such as *E.coli sp.* and *S. typhi sp.* bring harm and unwanted reaction which will cause problems. Thus, if only we can replace the non-biodegradable plastic which will bring pollution to our world with biodegradable plastic which also providing antimicrobial properties, this will bring huge advantages to our environment and many sectors (Lee *et al.*, 2003). For the antimicrobial agent, usage from natural sources seems to be more practical. Since the antimicrobial agent from natural sources bring less harm and side effect to human compared to chemical based antimicrobial agent for example ethanol, it become major choice by consumers.

1.2 Problem statement

The majority of engineered plastic materials used today are made from synthetic polymers. The use of conventional petroleum-based polymer products creates many potential problems due to their non-renewable nature and ultimate disposal. In Malaysia, plastic became the third largest waste volume in Malaysian municipal solid waste (MSW). For the record plastic manufacturing became an important role in many sectors such as food industries, pharmaceutical, clinical and last but not least municipal daily usage (Lee *et al.*, 2005).

Average person use about 350 plastic bags per year. The conventional petroleum-based polymer took around 400 to 500 years to biodegrade by itself. When incinerated, plastic produce dangerous gases that pollute the environment. Even though we recycle plastic to minimize its quantity in order to avoid pollution, the process used during the recycling process still releases heavy, toxic metals into the air and environment. Thus the best solution is to use biodegradable plastic (Bert *et al.*, 2002).

Much research had been done to produce biodegradable polymer. Biopolymer-based materials originated from naturally renewable resources such as polysaccharides, proteins, and lipids have become the best candidates to form this biodegradable film in recent years since such biopolymers have their environmental

advantages of recyclability and reutilization compared to other conventional petroleum-based synthetic polymer (Lee *et al.*, 2007). Biopolymer films can also become barriers of gas and solute barriers and complement other types of packaging by reducing food quality deterioration and extending the life of foods (Debeaufort *et al.*, 1998).

As we speak, chitosan nowadays has high potential in making biodegradable film. Chitosan principally derived from chitin by deacetylation with an alkali which is the natural and low-cost biopolymer. Chitosan possess many excellent properties such as biocompatibility, biodegradability and non-toxicity (Wan *et al.*, 2006).The only flaw of this material is that its hygroscopic properties of the bio-packaging containing polysaccharides are responsible for their weak moisture barrier and thus have little or no influence on the dehydration or rehydration phenomena of the foodstuffs (Sebastien *et al.*, 2006).

Furthermore nowadays biodegradable film mainly does not possess antimicrobial properties which important in certain uses such as food packaging, clinical usage and pharmaceutical usage. The physicochemical properties, composition and antimicrobial activity of cinnamon essential oil (*Cinnamomum zeylanicum*) were studied thoroughly as we speak. Cinnamon essential oil is highly anti-microbial and anti-bacterial for a great diversity of infectious bacteria which lead to many interest of using it as antimicrobial agent in many research. Studies have shown the strength of cinnamon bark oil to eliminate many forms of pathogenic organisms (Fuselli *et al.*, 2005).

1.3 Objective

a. To fabricate composite film from rice starch-chitosan blends with combination of cinnamon oil.
b. To characterize composite biodegradable film in term of morphology, physical and chemical.
c. To analyze the antimicrobial activity of the fabricated biodegradable film.

1.4 Scope of the study

a. Fabrication of composite biodegradable film from rice starch-chitosan with combination of cinnamon essential oil.
b. The characterization of the composite biodegradable film using various analysis method:
    i. Scanning electron microscope (SEM)
    ii. Fourier transform infrared (FTIR) spectroscopy
    iii. Differential scanning calorimetry (DSC)
    iv. Thermogravimetric analysis (TGA)
c. Antimicrobial analysis of biodegradable film:
    i. Agar diffusion method (zone inhibition assay)
    ii. Liquid culture test (optical density measurement)

CHAPTER 2

LITERATURE REVIEW

2.1 Biodegradable film

The majority of engineered plastic materials used today are made from synthetic polymers. The use of conventional petroleum-based polymer products creates many potential problems due to their non-renewable nature and ultimate disposal. Cellulose and its derivatives, when used in such applications, offers advantages with respect to sustainability, limited environmental impact and simplified end-of-life disposal issues (Petersson and Oksman, 2006)

Early studies examined the application of chitosan, starch and cellulose derivatives which were shown have film forming properties (Krochta *et al.*, 1994). There is a considerable interest in biodegradable films made from renewable and natural polymers, such as starch (Lawton, 1996). Biodegradable polymer films are not meant to totally replace synthetic packaging films, but to limit moisture, aroma, and lipid migration between food components where traditional packaging cannot function.

For instance, biodegradable and edible films can be used for versatile food products to reduce loss of moisture, to restrict absorption of oxygen, to lessen migration of lipids, to improve mechanical handling properties, to provide physical protection, or to offer an alternative to the commercial packaging materials (Nelson and Fennema, 1998).

2.2 Chitosan

2.2.1 Overview

A linear β-1, 4-$_D$-glucosamine, it is a biocompatible, nontoxic compound mainly obtained by deacetylation of chitin, a natural structural component present for instance in crustaceans such as crabs shells (Moller *et al.*, 2004). In other word, Chitosan is the N-deacetylated product of chitin, a linear polymer composing primarily of glucosamine and a natural polymer that can be extracted from outer shells of crusastaceans (Feng and Huang, 1996).

This biopolymer presents interesting properties such as excellent film-forming capacity and gas and aroma barrier properties at dry conditions, which makes it a suitable material for designing food coatings and packaging structures (Park *et al.*, 2002).

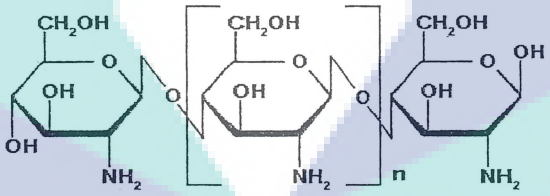


Figure 2.1: Chemical Formula of Chitosan in Haworth's Projection.

2.2.2 History

The origin of chitosan was first discovered by Braconnot in 1811. Braconnot was conducting research on mushrooms, he isolated what was later to be called chitin. The name chitin is derived from Greek, meaning tunic or envelope. Twenty years later, there was a man who wrote an article on insects in which he noted that

similar substance was present in the structure of insects as well as the structure of plants. He then called this astounding substance as chitin (Coma *et al.*, 2003). The name chitin is derived from Greek, meaning tunic or envelope.

The discovery of chitin leads to emerging of chitosan. It was first discovered by Rouget while experimenting with chitin. Rouget observed that the compound of chitin could be manipulated through chemical and temperature treatments for it to become soluble. In 1878, Ledderhose identified chitin to be made of glucosamine and acetic acid. It was not actually until 1894 that Hoppe-Seyler named the tailored chitin, chitosan.

2.2.3. Advantages of Chitosan

Chitosan is relatively low cost. They were widespread availability from a stable renewable source, that is, shellfish waste of the sea food industry, along with chitosan's ability to form a good film, are primary reasons to seek new applications of this polymer (Bangyekan *et al.*, 2005).

Chitosan provides unique functional, nutritional, and biomedical properties, and its present and potential uses range from dietary fiber to a functional ingredient and processing aid. Some of the well known applications of chitosan include its use for prevention of water pollution, medicine against hypertension, antimicrobial and hypocholesterolemic activity, flavor encapsulation, seed coating, film-forming, and controlled release of food ingredients and drugs (Muzzarelli and Vincenzi, 1997).

Chitosan had several of special function including enhancing immune, persisting moist, broad spectrum antimicrobial, promoting regeneration of the epithelium and rehabilitation of the tissue and had abroad applicable value in increasing repair of oral tissue, treatments of periodontal or periapical disease and oral ulcer (Hong *et al.*, 2007).

Chitosan prepared by deacetylation of chitin which mainly obtained from crab and shrimp shell and is an aminopolysaccharide that is useful in chemical modification as a result of its reactive amino and hydroxyl groups. Chitin and chitosan can be considered to be an extremely low cost, nonhazardous, and environmentally biopolymer (Hong *et al.*, 2007).

2.3 Characterization

2.3.1 Scanning Electron Microscope

The scanning electron microscope (SEM) is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern (Suzuki, 2002).The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity.

The types of signals produced by an SEM include secondary electrons, back-scattered electrons (BSE), characteristic X-rays, light (cathodoluminescence), specimen current and transmitted electrons (Kiernan, 2000). Secondary electron detectors are common in all SEMs, but it is rare that a single machine would have detectors for all possible signals. The signals result from interactions of the electron beam with atoms at or near the surface of the sample.

In the most common or standard detection mode, secondary electron imaging or SEI, the SEM can produce very high-resolution images of a sample surface, revealing details about less than 1 to 5 nm in size (Danilatos, 1990). Due to the very narrow electron beam, SEM micrographs have a large depth of field yielding a characteristic three-dimensional appearance useful for understanding the surface

structure of a sample (Hindmarsh *et al.*, 2007). This is exemplified by the micrograph of pollen shown to the right.

A wide range of magnifications is possible, from about 10 times (about equivalent to that of a powerful hand-lens) to more than 500,000 times, about 250 times the magnification limit of the best light microscopes. Back-scattered electrons (BSE) are beam electrons that are reflected from the sample by elastic scattering. BSE are often used in analytical SEM along with the spectra made from the characteristic X-rays (Danilatos, 1990).

Because the intensity of the BSE signal is strongly related to the atomic number (Z) of the specimen, BSE images can provide information about the distribution of different elements in the sample. For the same reason, BSE imaging can image colloidal gold immuno-labels of 5 or 10 nm diameter which would otherwise be difficult or impossible to detect in secondary electron images in biological specimens (Faulkner *et al.*, 2008). Characteristic X-rays are emitted when the electron beam removes an inner shell electron from the sample, causing a higher energy electron to fill the shell and release energy. These characteristic X-rays are used to identify the composition and measure the abundance of elements in the sample. (Hindmarsh *et al.*, 2007).

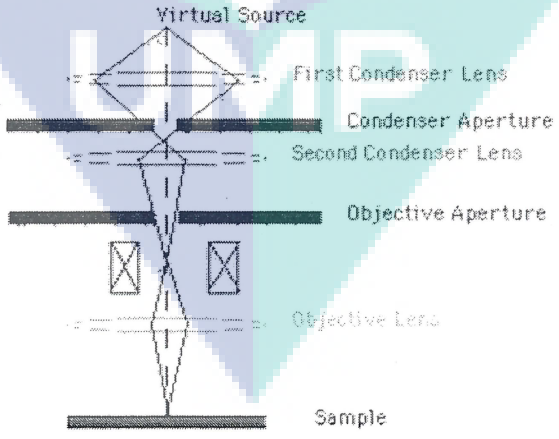


Figure 2.2: The plan of a SEM structure

2.3.2 Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectroscopy is a measurement technique for collecting infrared spectra. Instead of recording the amount of energy absorbed when the frequency of the infra-red light is varied (monochromator), the IR light is guided through an interferometer. After passing through the sample, the measured signal is the interferogram. Performing a mathematical Fourier transform on this signal results in a spectrum identical to that from conventional (dispersive) infrared spectroscopy (Luypaert, 2003).

FTIR spectrometers are cheaper than conventional spectrometers because building of interferometers is easier than the fabrication of a monochromator. In addition, measurement of a single spectrum is faster for the FTIR technique because the information at all frequencies is collected simultaneously. This allows multiple samples to be collected and averaged together resulting in an improvement in sensitivity. Because of its various advantages, virtually all modern infrared spectrometers are FTIR instruments (Tokmakoff *et al.*, 2004).

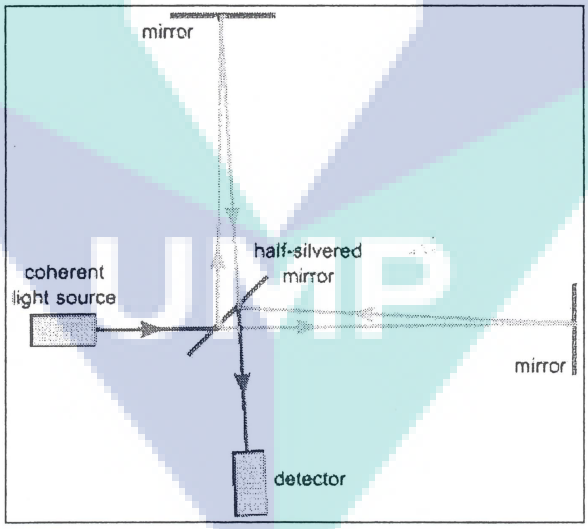


Figure 2.3: The outline of the FTIR process

2.3.3 Thermogravimetric Analysis

Thermogravimetric Analysis or TGA is a type of testing that is performed on samples to determine changes in weight in relation to change in temperature (Liu *et al.*, 2009). Such analysis relies on a high degree of precision in three measurements: weight, temperature, and temperature change. As many weight loss curves look similar, the weight loss curve may require transformation before results may be interpreted. A derivative weight loss curve can be used to tell the point at which weight loss is most apparent. Again, interpretation is limited without further modifications and deconvolution of the overlapping peaks may be required.

TGA is commonly employed in research and testing to determine characteristics of materials such as polymers, to determine degradation temperatures, absorbed moisture content of materials, the level of inorganic and organic components in materials, decomposition points of explosives, and solvent residues (Guan *et al.*,1998). It is also often used to estimate the corrosion kinetics in high temperature oxidation.

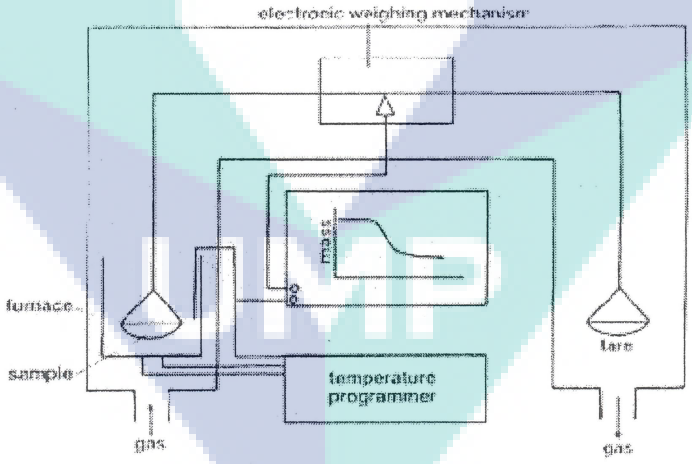


Figure 2.4: Plan structure of TGA

2.3.4 Differential Scanning Calorimeter

Differential scanning calorimeter is a device that using technique developed by E.S. Watson and M.J. O'Neill in 1960, and introduced commercially at the 1963 Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. The term DSC was coined to describe this instrument which measures energy directly and allows precise measurements of heat capacity (Wunderlich, 1990).

DSC is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference are measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment (Pungor and Erno, 1995). Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned (Dean and John, 1995).

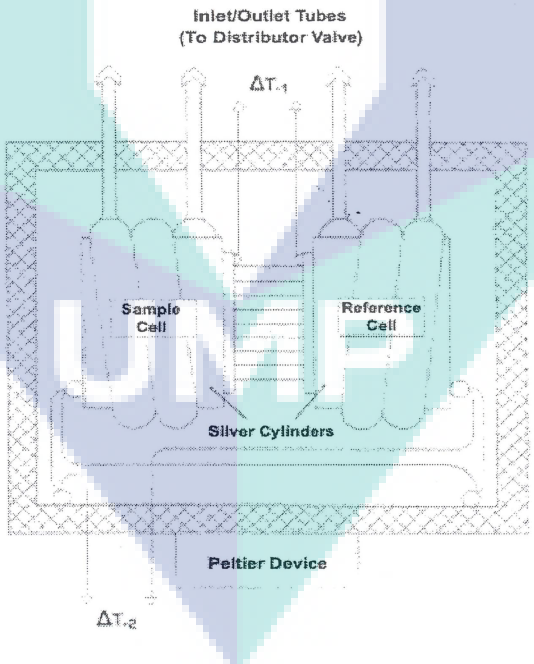


Figure 2.5: Plan structure of DSC

The main application of DSC is in studying phase transitions, such as melting, glass transitions, or exothermic decompositions. These transitions involve energy changes or heat capacity changes that can be detected by DSC with great sensitivity (James and Timothy, 1998).

DSC is used widely for examining polymers to check their composition. Melting points and glass transition temperatures for most polymers are available from standard compilations, and the method can show up possible polymer degradation by the lowering of the expected melting point, Tm, for example. Tm depends on the molecular weight of the polymer, so lower grades will have lower melting points than expected (Skoog and Douglas, 1998). It may also be used to evaluate drug and polymer purities.

CHAPTER 3

METHODOLOGY

3.1 Material and Equipment

The chitosan used was chitosan from crab shell, practical grade from Aldrich. The starch was obtained from the local grocery shop The chitosan and starch were used as the polymer with addition of antimicrobial agent. For the bacteria culture preparation, *Bacillus subtilis* and *Escherichia coli* was used. Bacteria are inoculated into its culture media in order to keep them alive and to study their growth.

3.2 Bacteria Culture Preparation

3.2.1 Transfer of Microbial Culture from Nutrient Broth to Agar Plate Via Streaking Method

Bunsen burner turned on for the flame. Flame the inoculation loop flamed to redness and allowed to cool. A loopful of one broth culture been obtained. The streaking procedure may be done with the petri plate on the table or held by hand. To streak the plate, edge of petri plate been covered, and first sector been streaked by making as many streaks as possible without overlapping previous streaks. It supposedly not to gouge the agar while streaking the plate.

The loop been hold as holding a pencil or paintbrush, and the surface of the agar touched gently. The loop been flamed and let cooled. The plate been turned so the next sector is on top. One area of the first sector streaked out and then streak a few times away from the first sector. Loop been flamed and the plate turned again, and streaked through one area of the second sector. The third sector then streaked out. Loop been flamed again and streaked through one area of the third sector, and then streaked the remaining area of the agar surface, being careful not to make additional contact with any streaks in the previous sections. Loop been flamed before setting it down. Each of petri plate labeled with marker pen and incubated at 30°C for 24 to 48 hours. Plates incubated in an inverted position (Chen *et al.* 2002).

3.2.2 Transfer of Microbial Culture from Agar Plate to Broth Bottle

Loop flamed until it is red and let cooled. Inoculating loop holds in dominant hand. Aseptically a loopful of one broth culture obtained from the plate of broth culture of bacteria. Using the other hand and the dominant hand's small finger, the broth bottle cap opened and the mouth flamed of the bottle to avoid contamination and been recap. Again, using the other hand and the dominant hand's small finger, the broth bottle cap opened and the mouth of the bottle flamed to avoid contamination. Using dominant hand with the inoculating loop, the slant inoculated by moving the loop gently across the agar surface from the bottom of the slant to the top, being careful not to gouge the agar. The mouth of the tube flamed and the cap replaced. Loop flamed and let cooled. Broth bottle marked with marker pen and incubated at 30°C for 24 to 48 hours (Chen *et al.*, 2002).

3.3 Solution Preparation

Starch was dissolved in distilled water heating the mixtures on hot plates and stirring until it gelatinized The mixtures were then cooled to 27°C. The chitosan solution was prepared by dispersing chitosan solution. After the chitosan dissolved completely, the solutions were filtered through a polyester screen (mesh no. 140 with

mesh opening of 106 mm) by vacuum aspiration to remove any small lumps in the solution.

While pulling a vacuum with an aspirator, the vacuum flask containing the chitosan solutions was sealed with a rubber stopper and held for a few hours to de-gas the solution, which prevented the formation of air bubbles in the films when the casting solvent evaporated. Lastly antimicrobial agent was added to the solution. The solution was degasses to remove bubbles for about 24 hours.

3.4 Film Casting

The mixtures were cast onto flat, leveled, non-stick glass plate. The layer thickness of the solution during casting process adjusted using the casting knife. The casted film was held overnight at room temperature until completely dried.

3.5 Film Peeling

After held overnight at room temperature and completely dried, the fabricated film was ready to be peel off from the tray. Peeling off the fabricated film can be done by hand or other necessary equipment. Fabricated film then been cut to several pieces for further test.

3.6 Film characterization

3.6.1 Scanning Electron Microscopy (SEM)

The dried film samples are mounted on a metal stub with double-sided adhesive tape. 2 cm in diameter of the fabricated film's morphological structures are studied under the JSM-5600 LV scanning electron microscope of JEOL, Tokyo, Japan and the images are taken at accelerating voltage 5 kV and a magnification 500

and 1000 times of origin specimen size for both cross section and surface morphology.

3.6.2 Fourier Transform Infrared (FTIR)

The chemical bonds in a molecule can be identified using FTIR by the infrared absorption spectrum produced by the bonds. The FTIR generates an infrared spectral scan of samples that absorb infrared light. For this test, the wave numbers were recorded in the range of 500 to 4000 cm^{-1} with a piece of film 2 cm in diameter.

3.6.3 Thermogravimetric Analyzer (TGA)

Thermogravimetric Analysis was performed on samples to determine changes in weight in relation to change in temperature Thermal stability of each sample was determined using TGA Q500 series Thermogravimetric analyzer (TA Instruments) with a heating rate of 10°C/min in a nitrogen environment with a maximum temperature of 600°C.

3.6.4 Differential Scanning Calorimeter (DSC)

DSC is mainly used to identify the melting temperature of the composite biodegradable film. With a sample weight from 10 mg to 20 mg, the test was done by a DSC Q1000 series (TA Instrument) under nitrogen atmosphere, with a flow capacity of 25 ml/min from 20 to 300°C at a heating rate of 10°C/min. The temperature range is from 20°C up to 300°C was selected for two reasons because to avoid endothermic signals related to the melting of frozen water around 0°C and to limit possible sample degradation.

3.6.5 Agar Diffusion Test (Zone Inhibition Assay)

This method is to test the antimicrobial properties of the fabricated film. The *Bacillus subtilis* representing Gram-positive bacteria and *Escherichia coli*

representing Gram-negative bacteria. Each film is cut into squares (1cm x 1cm) and is placed on the bacterial lawns in the agar plate containing bacteria. Duplicate agar plates are prepared for each type of film. The plates are incubated for 48 h at 37°C in the appropriate incubation chamber (aerobic chamber for *E. coli*). The plates are then examined visually to see the zones of inhibition around the film disc, and the size of the zone diameter is measured at two cross sectional points and the average is taken as the inhibition zone.

3.6.6 Liquid Culture Test (Optical Density Measurements)

The second method to test the antimicrobial properties of the fabricated film is by using liquid culture test. This method was using optical density measurement to detect the effect of antimicrobial properties of the fabricated film. The lower the optical density shows the greater effect of antimicrobial activity of the fabricated film. To perform the test, each film is cut into squares (1cm x 1cm). Three sample squares are immersed in 20 ml nutrient broth in a 25 ml universal bottle. The medium is inoculated with 200µl of *Escherichia coli* or *Bacillus subtilis* in its late exponential phase, and then transferred to an orbital shaker and rotated at 37°C at 200 rpm. The culture is sampled periodically (0, 2, 4, 8, 12, 24 hours) during the incubation to obtain microbial growth profiles. The same procedure is repeated for the control starch-based film. The optical density (OD) 600 is measured at λ= 600nm using a spectrophotometer (Model UV-160, Shimadzu, Japan.

CHAPTER 4

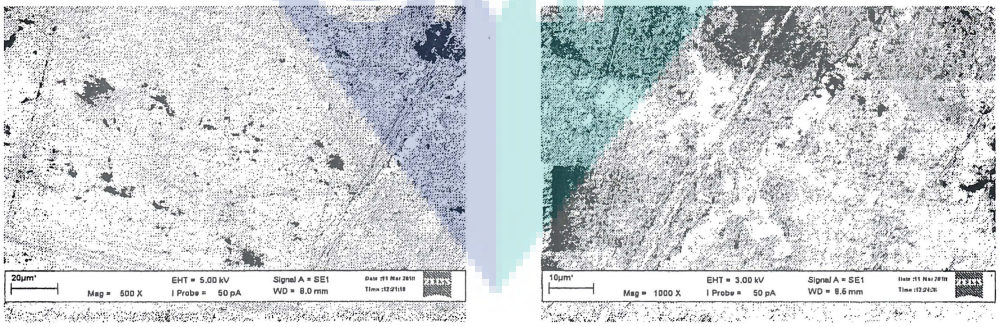
RESULTS AND DISCUSSION

In the chapter, different films were analyzed and discussed namely sample A, B and C. Sample A was chitosan based film, sample B was starch chitosan blend film and sample C was rice chitosan blend film with addition of antimicrobial agent.

4.1 Scanning Electron Microscope (SEM)

In this study the SEM was used to see the surface and cross section morphology of each sample. The morphology will show the structure across the film. A compact structure shows better strength and heat resistance. Comparison between each sample will determine the better surface and cross section morphology.

4.1.1 Surface Morphology of Sample A

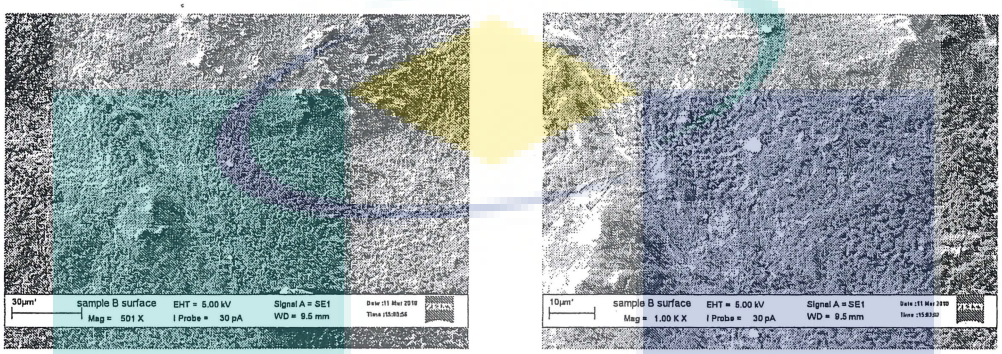


(a) (b)

Figure 4.1: Surface morphology of sample A at magnificent (a) 500X, (b) 1000X

From the Figure 4.1, we can see a smooth surface where no observation of large granular structure. Since sample A was fabricated from chitosan powder only, the mixture between chitosan molecules shows good homogeneity.

4.1.2 Surface Morphology of Sample B

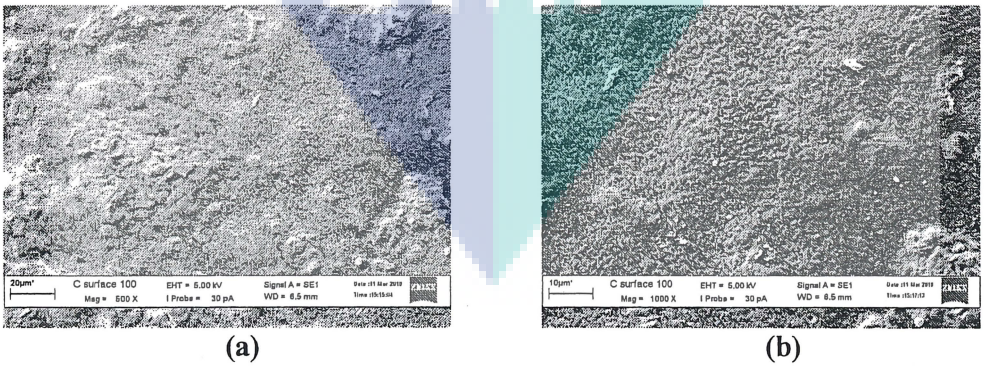


(a) (b)

Figure 4.2: Surface morphology of sample B at magnificent (a) 500X, (b) 1000X

From the Figure 4.2, observation shows the surface morphology of sample B was rougher compared to sample A. Large granular structure of starch was observed. This was due to composite factor since sample B was fabricated from chitosan blend. Larger granular structure of starch cause less homogeneity towards chitosan thus resulting rougher surface

4.1.3 Surface Morphology of Sample C

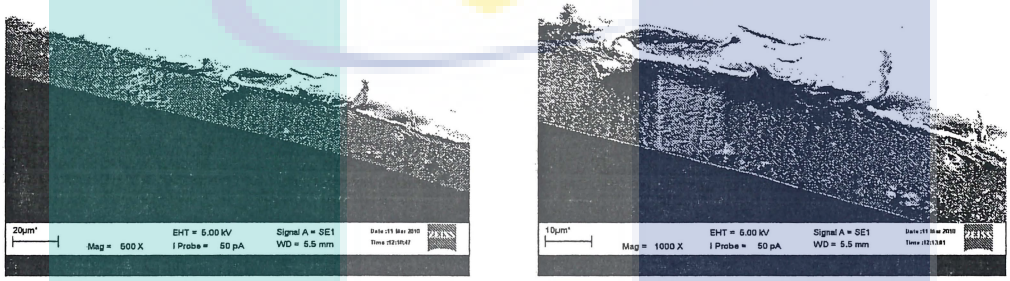


(a) (b)

Figure 4.3: Surface morphology of sample C at magnificent (a) 500X, (b) 1000X

From the Figure 4.3, observation shows the surface morphology of sample C was rougher than sample A. Due to composite factor, sample C shows large granular structure of starch. Larger granular structure of starch cause less homogeneity toward chitosan thus resulting rougher surface. Sample C shows smoother surface compared to sample B.

4.1.4 Cross Section Morphology of Sample A

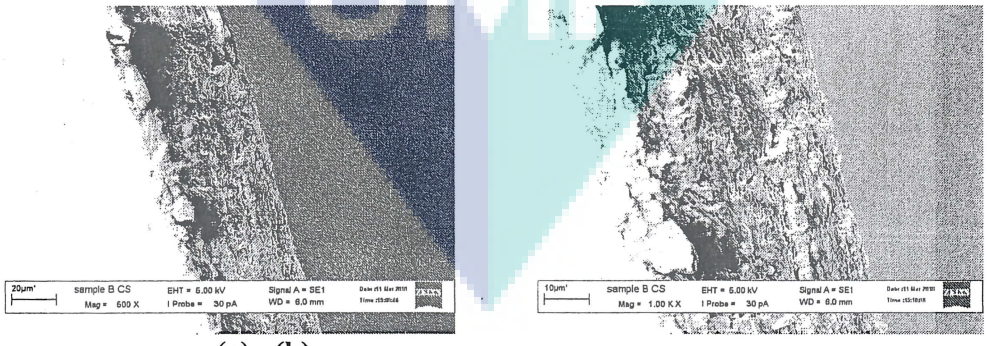


(a) (b)

Figure 4.4: Cross section morphology of sample A at magnificent (a) 500X, (b) 1000X

Figure 4.4 shows cross section morphology of sample A. Compact structure of cross section was observed. Sample A was fabricated from chitosan only thus resulting good homogeneity between molecules.

4.1.5 Cross Section Morphology of Sample B

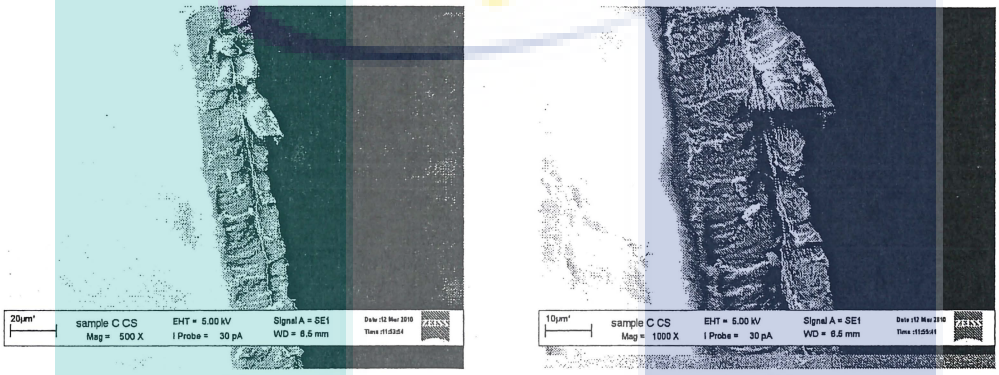


(a) (b)

Figure 4.5: Cross section morphology of sample B at magnificent (a) 500X, (b) 1000X

From the Figure 4.5, observation shows cross section of sample B was less compact compared to sample A. Due to composite factor, larger granular structure of starch cause less homogeneity towards chitosan thus resulting less compact structure of cross section.

4.1.6 Cross Section Morphology of Sample C



(a) (b)

Figure 4.6: Cross section morphology of sample C at magnificent (a) 500X, (b) 1000X

From the Figure 4.6, sample C has compact structure compared to sample B although they were formed by the same mixture of composite. Due to the present of additive, the homogeneity between larger granular structures of starch toward chitosan was increased.

4.2 Fourier Transform Infrared (FTIR)

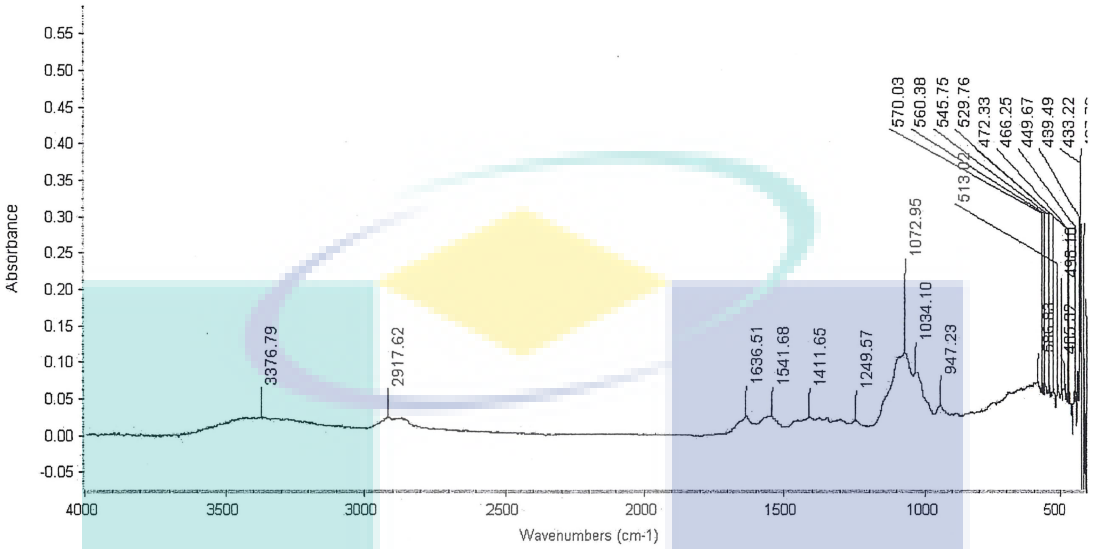


Figure 4.7: FTIR result of sample A

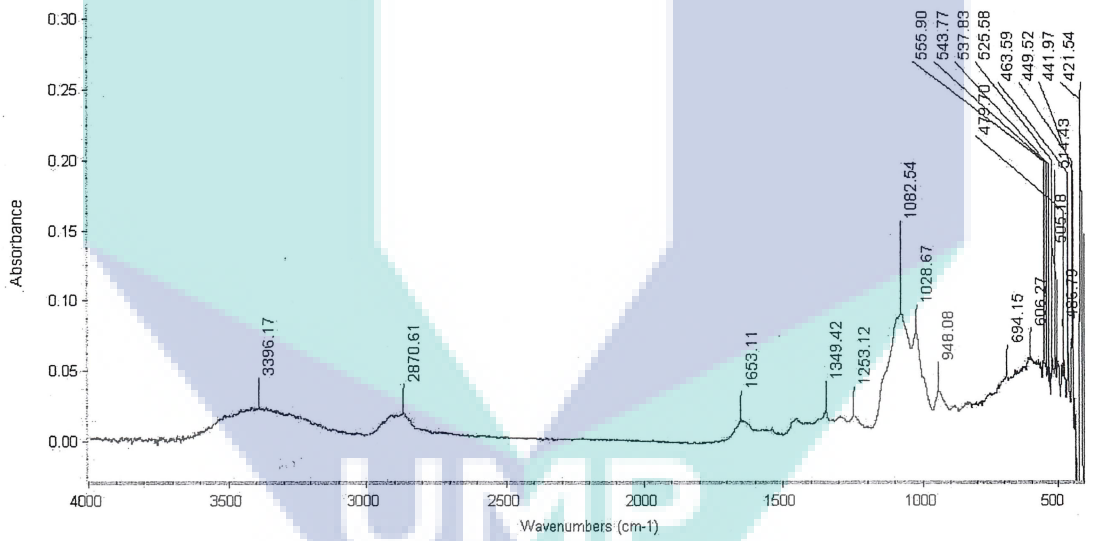


Figure 4.8: FTIR result of sample B

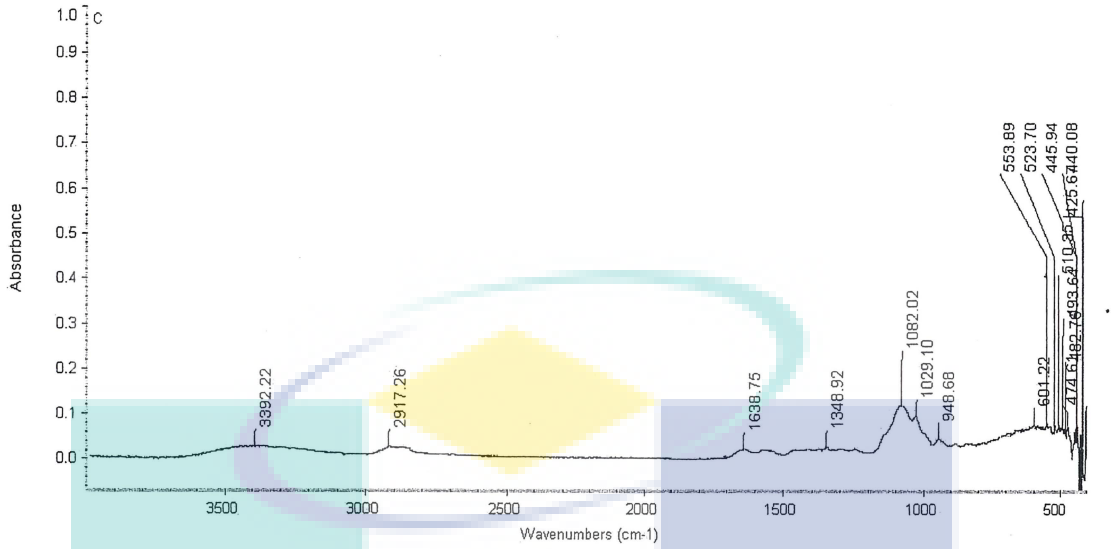


Figure 4.9: FTIR result of Sample C

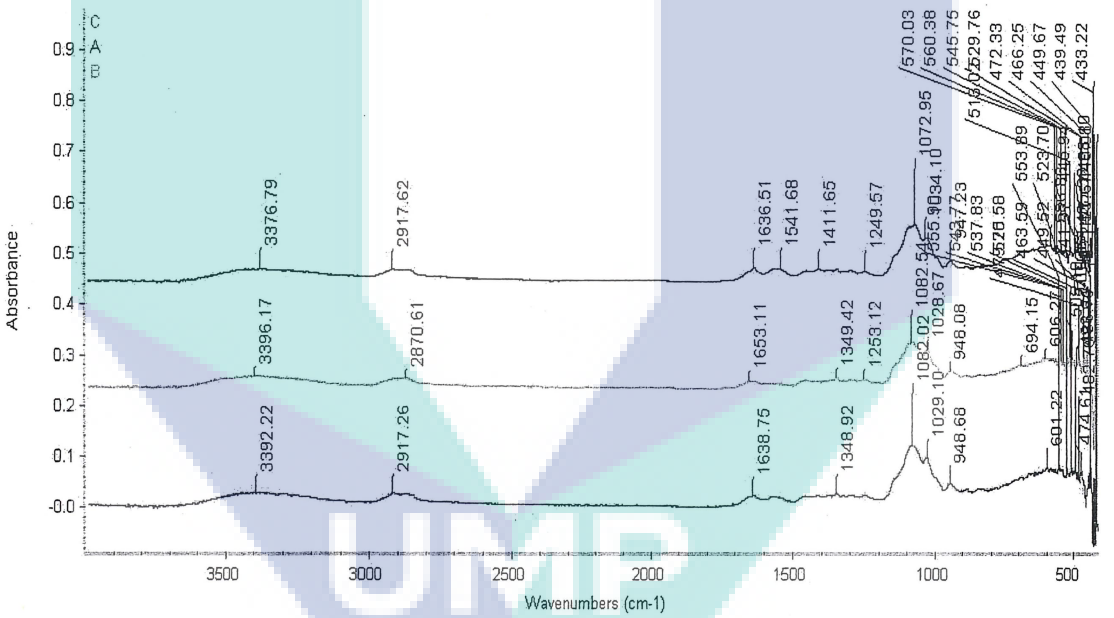


Figure 4.10: FTIR result of Sample A, B and C

Figure 4.10 shows the result for the FTIR for the sample A, B and C respectively. As we can see, the wave numbers value for sample A, B and C are almost similar. This situation can be explained due to the presence of similar interactions between the three samples. The value of the wavenumber in range of 500 to 600 cm^{-1} explained that there are aromatic rings in the samples. The characteristic absorption band around 1030 to 1155 cm^{-1} which confirmed the presence of C-O in the sample. In the figure, there is NH2 interaction at 1550 to 1650 cm^{-1} which shows presence of amine in the chitosan.

One of the peaks in the graph shows read at 1660-1850 cm^{-1} which shows that the carbonyl group which is in antimicrobial agent. Other than that, there is O-H absorption occurs at 2500- 3500 cm^{-1}. The bands due to C-H stretching vibrations overlapped in the absorption peaks at 2280 cm^{-1}.

The broad band area of 3000-3500 cm^{-1} show that chitosan and starch shared a high composition in this film as the absorbance in that range is quite high. This O-H and N-H stretching band shows up secondary amine and hydroxyl group in chitosan and starch. In other words, there are only some slight differences between these three samples.

4.3 Thermo Gravimetric Analysis (TGA)

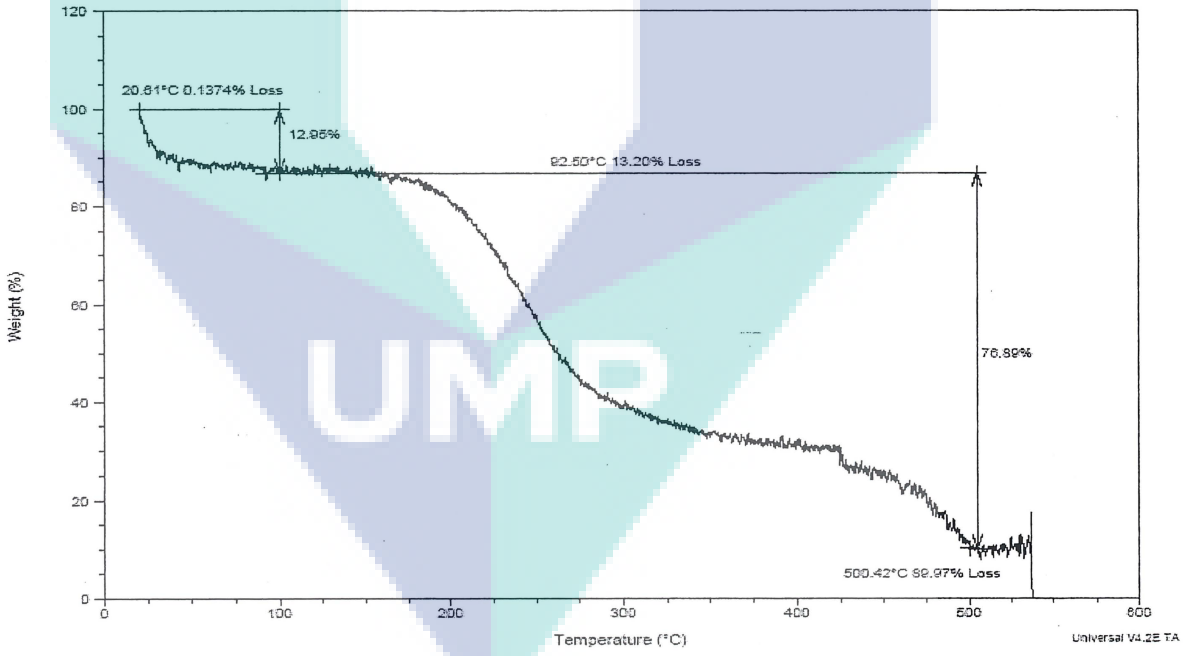


Figure 4.11: TGA result of sample A

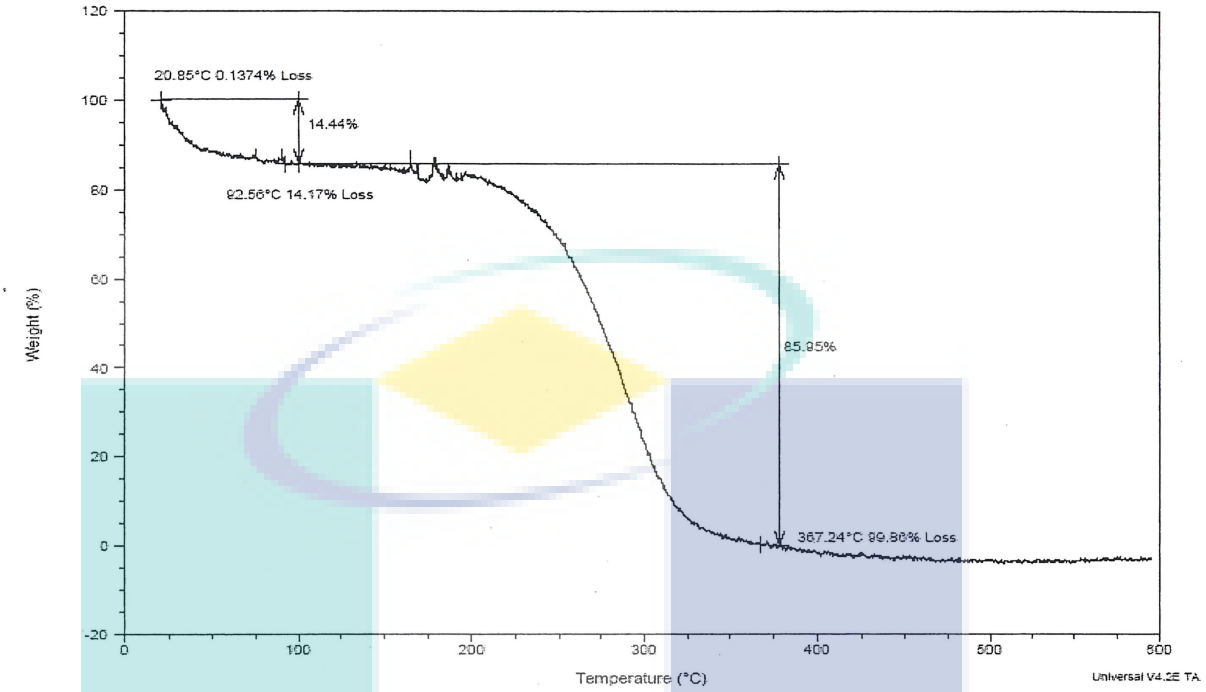


Figure 4.12: TGA result of sample B

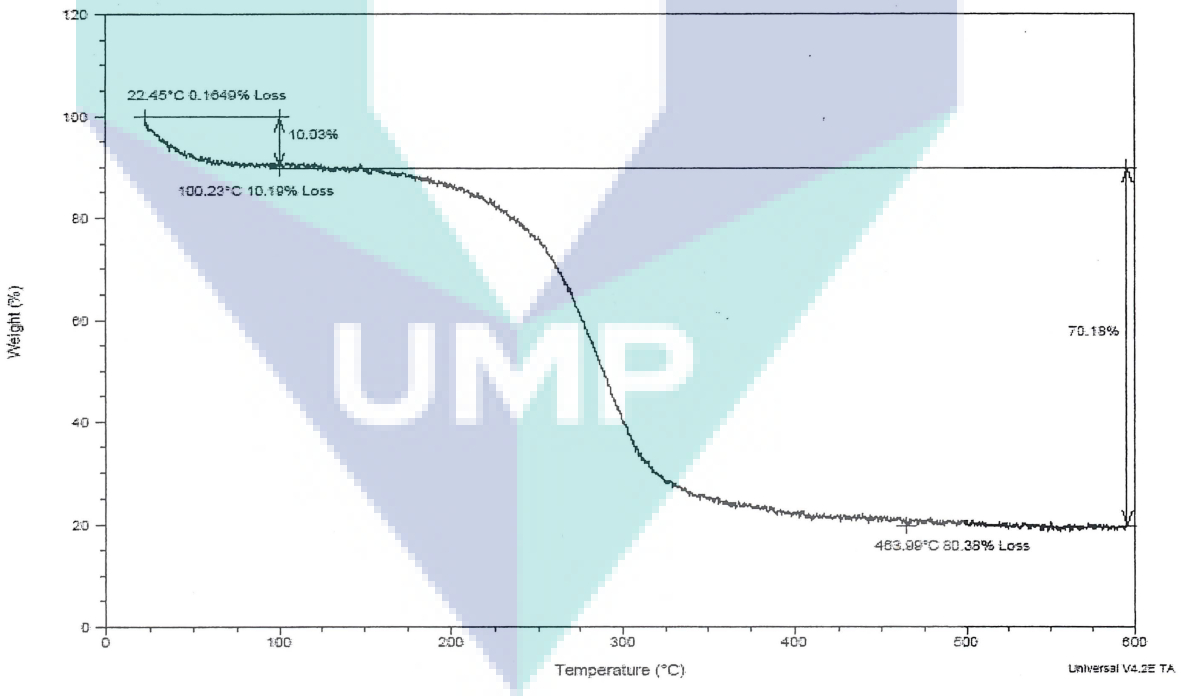


Figure 4.13: TGA result of sample C

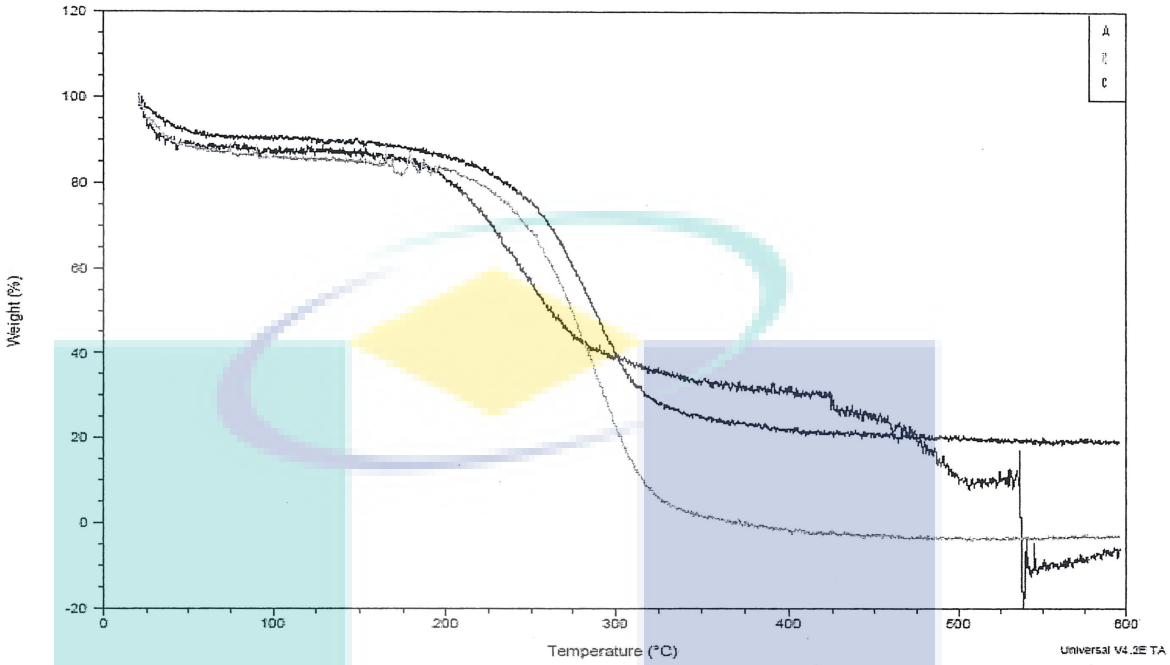


Figure 4.14: TGA result of sample A, B and C

Thermogravimetric analysis was performed to the three samples in order to determine their thermal decomposition behavior. Figure 4.11 shows thermal decomposition behavior of sample A. From the figure, at 100°C there was 12.95% mass loss at the temperature. Approximately at 530°C, film A was totally degrades and thus shows us the sample degradation temperature. The heat resistance of sample A was considered high. From previous analysis using SEM, a compact structure of sample A was observed which shows strong molecular bond between the molecule thus resulting high thermal resistances.

Figure 4.12 shows thermal decomposition behavior of sample B. From the figure, observation shows at 100°C sample B loss 14.14% of its total mass. At temperature 370°C sample B was totally degrade and thus show its degradation temperature. Compared to sample A, sample B shows relatively low heat resistance. Due to the previous analysis by SEM, the cross section of sample B was less compact compared sample A. thus resulting lower heat resistance

The thermal decomposition behavior of sample C was observed from the Figure 4.13. From the figure, at 100°C there was 10.03% mass lost. This was the least mass lost among the 3 samples analyzed. From the figure, at 600°C there was 70.18% mass lost and degradation temperature was not reached. This shows that sample C has a very good heat resistance compared to the sample A and B. This was shown by the cross section morphology of sample C which shows compact structure thus resulting high thermal resistance.

Figure 4.14 shows the combination graph for the thermal decomposition behavior of sample A, B and C for clear observation and comparison of the three samples. From the figure we can clearly see that sample C has the highest thermal resistance compared to sample A and B since it has less mass lost at temperature. This shows the present of cinnamon essential oil help to improve the bond formed between the composite thus increase its heat resistance.

4.4 Differential Scanning Calorimeter (DSC)

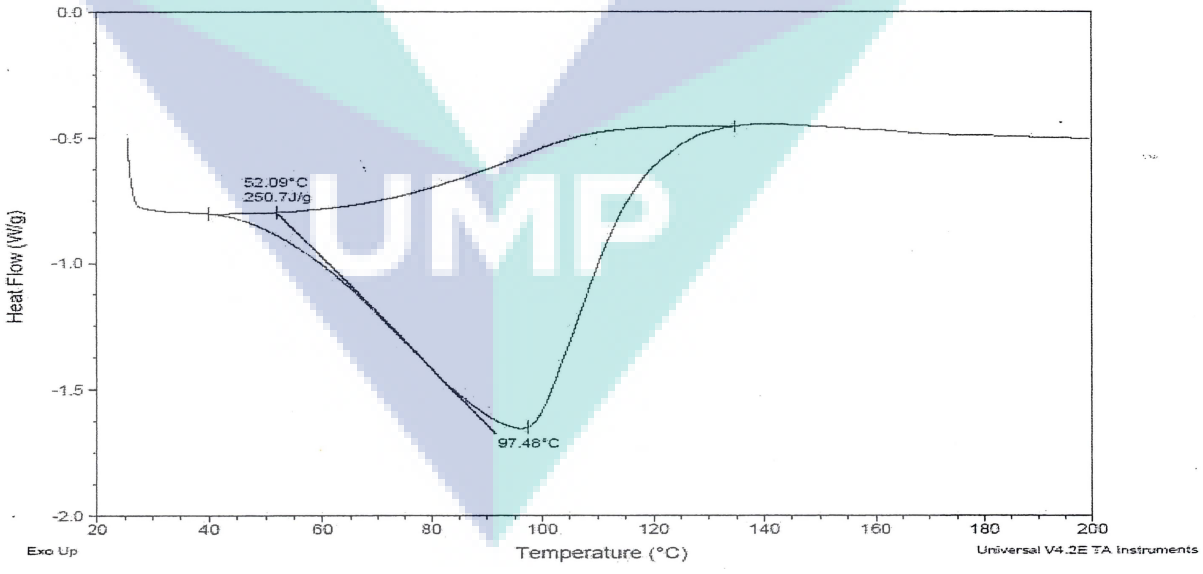


Figure 4.15: DSC result of sample A

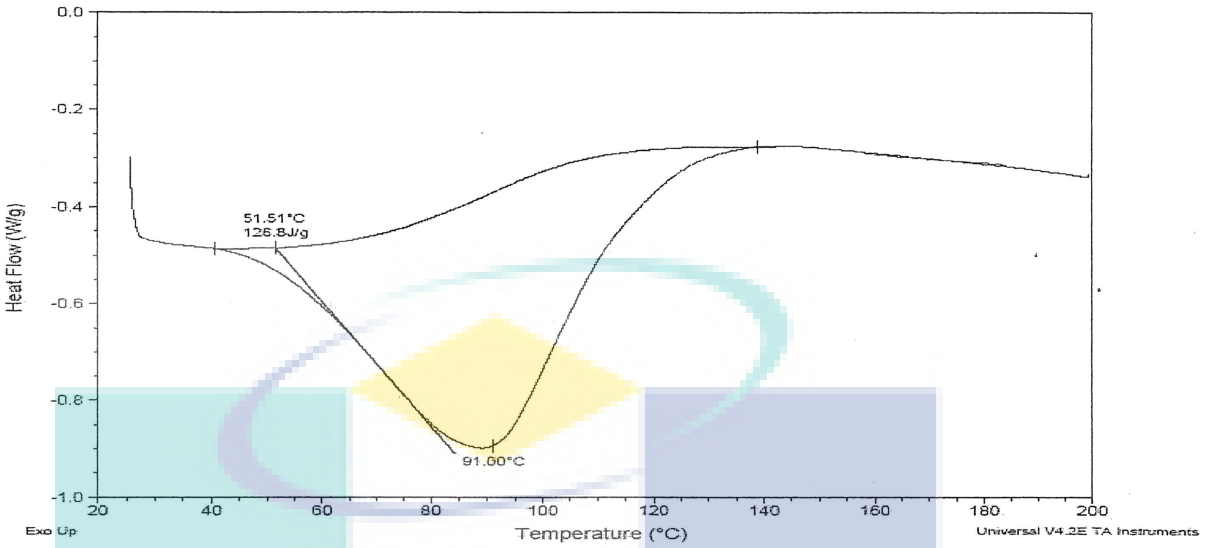


Figure 4.16: DSC result of sample B

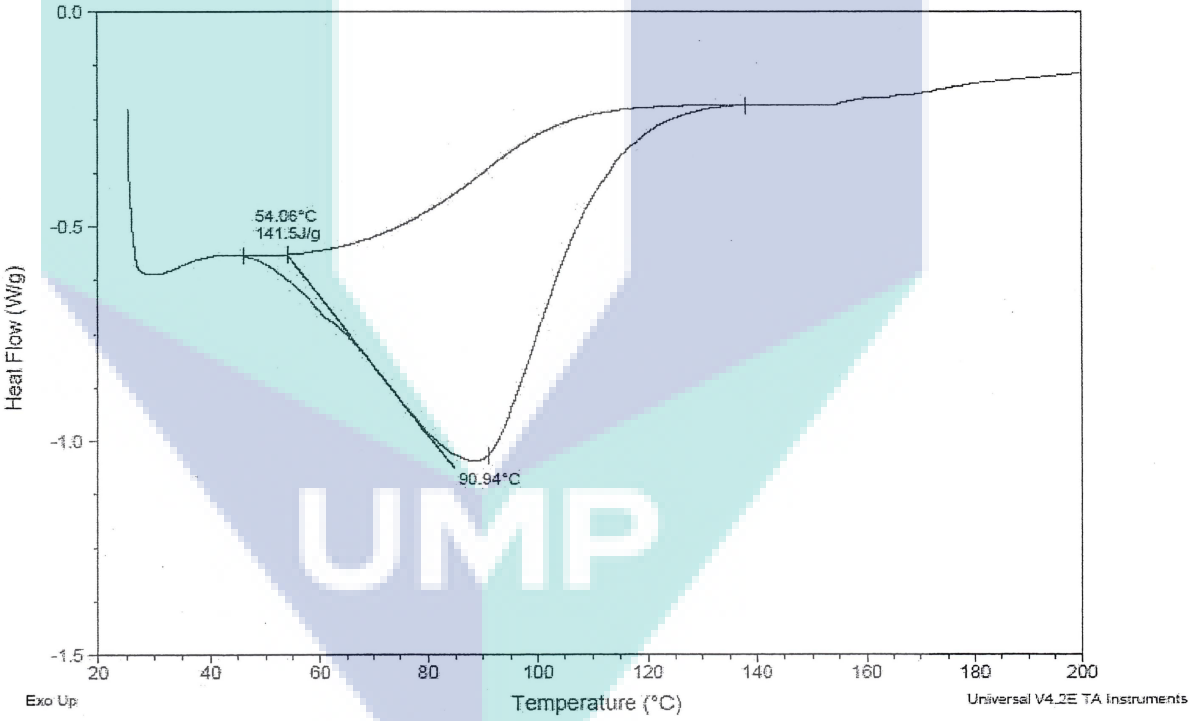


Figure 4.17: DSC result for sample C

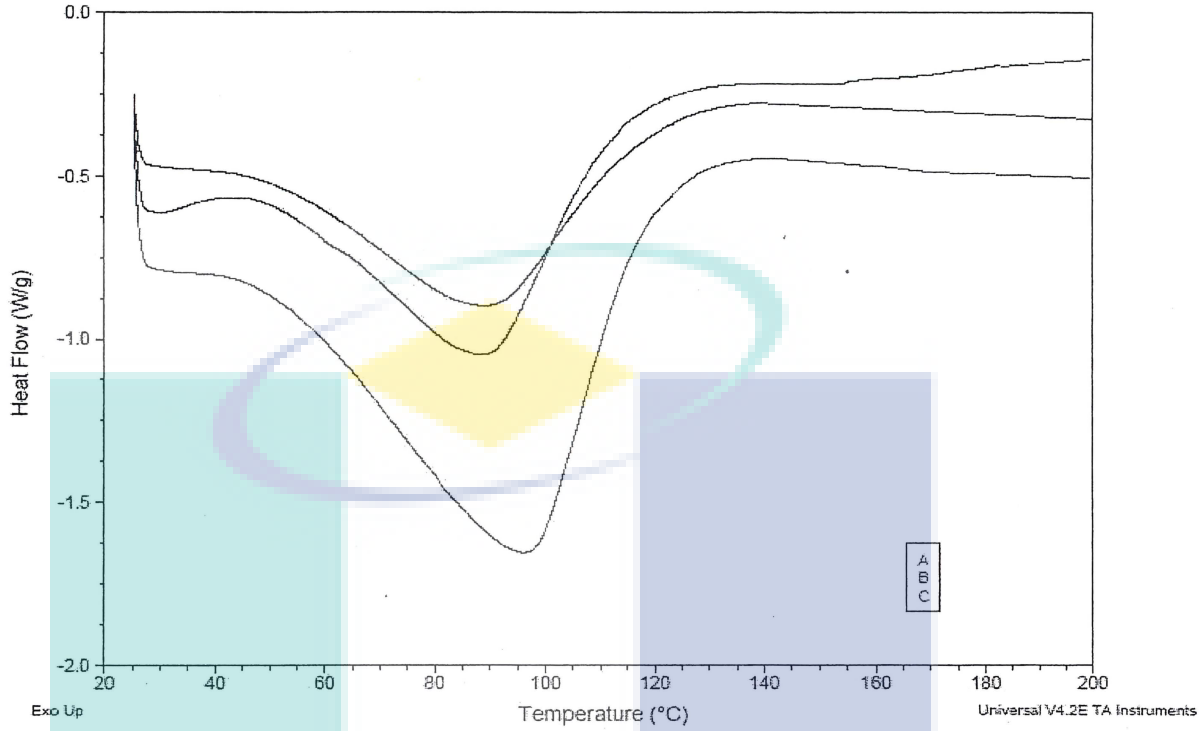


Figure 4.18: DSC result of sample A, B and C

Differential Scanning Calorimeter (DSC) analysis was performed in order to characterize the thermal properties of the three fabricated films. From the result of the DSC analysis, we can determine the melting point of the fabricated films. Two stage heating process was conducted for the DSC analysis. The first stage heating is used to decrease the water content in the blend films and release the stress of blend films. Since chitosan contains NH_2 and OH functional groups, the hydrogen bonding force is strongly formed among molecular (Chen *et al.*, 2008).

Figure 4.15 shows the thermal properties of sample A. The endothermic peak of the figure shows the melting point (T_m) of the sample A. As shown in the figure, the melting points of sample A is at 52.09°C and completely melt at 97.48°C. Meanwhile, Figure 4.16 shows that the melting temperature of sample B was 51.51°C and completely melts at 91°C. For sample C, Figure 4.17 shows that the melting temperature of sample C was at 54.06°C and completely melts at 91°C.

From Figure 4.18, the combination of the three graph shows clearly that sample C has the higher melting point compare to other sample which are sample A and sample B. This may be due to the composite factor and addition of antimicrobial agent that help to strengthen the bond between composite molecules which are the hydrogen bond between the hydroxyl groups. This proven by the SEM analysis before which sample C shows good compact cross section structure compared to the sample B although they were fabricated from the same composite material.

4.5 Antimicrobial Analysis

4.5.1 Zone Inhibition Assay

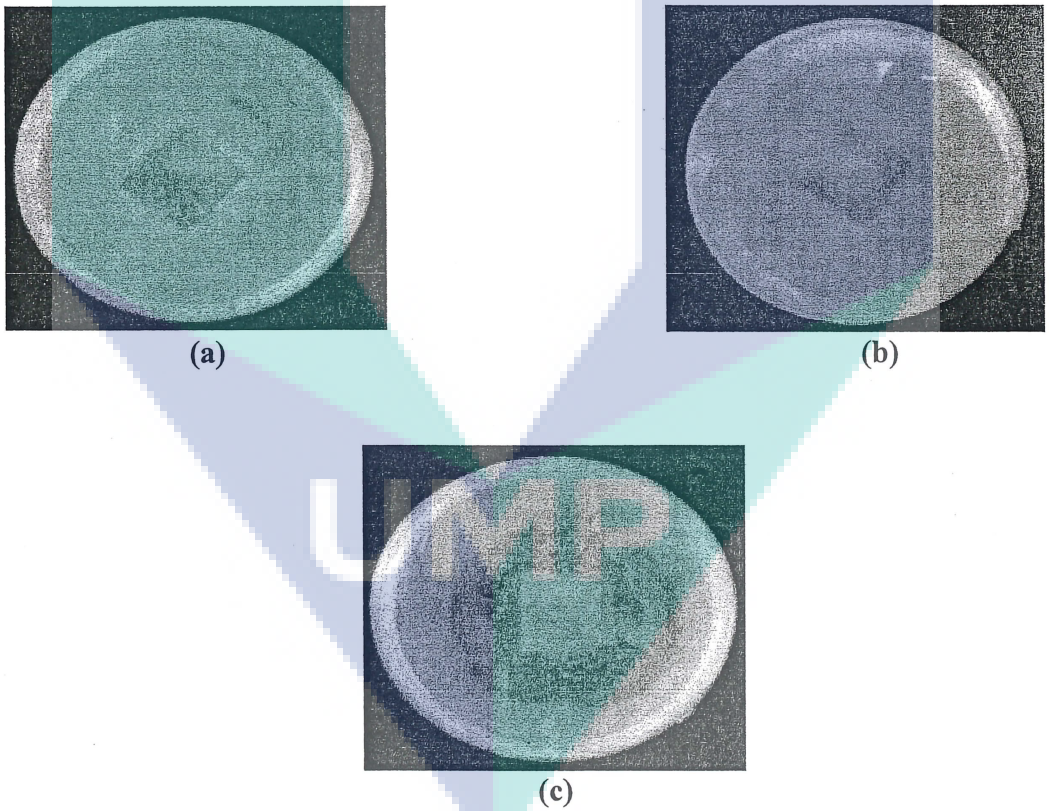


Figure 4.19: Zone inhibitory of sample (a), (b) and (c) toward *E.coli*

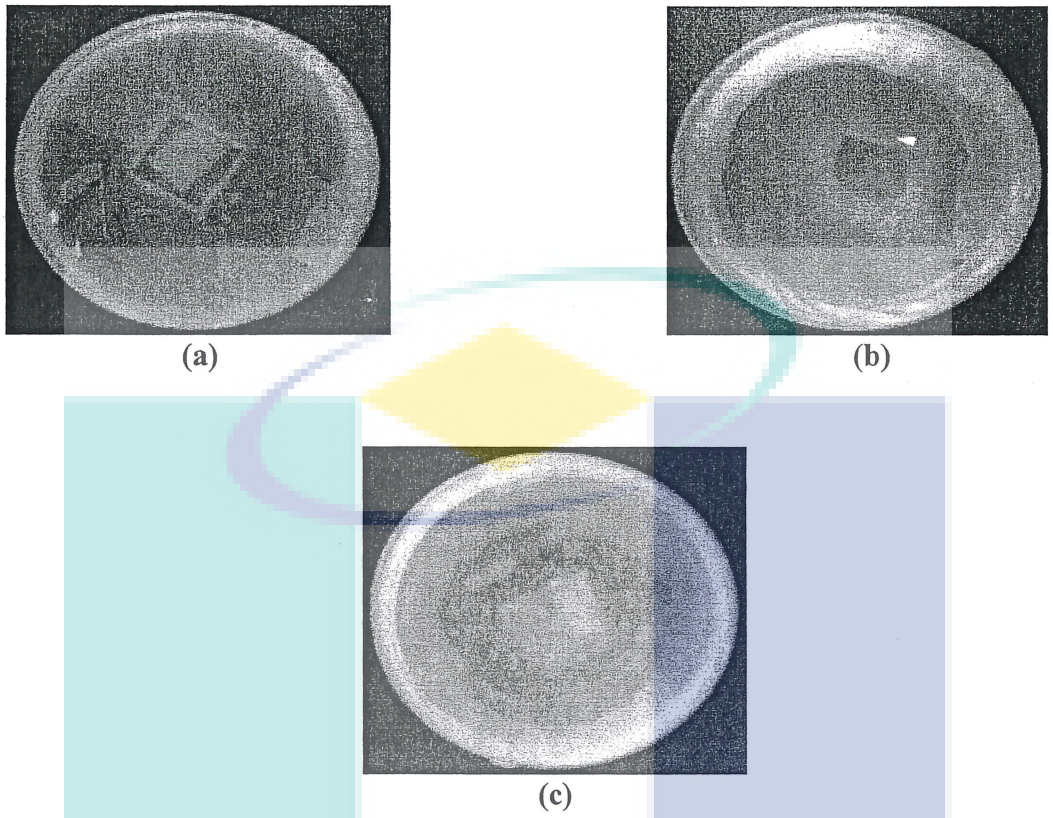


Figure 4.20: Zone inhibitory of sample (a), (b) and (c) toward *B.subtilis*

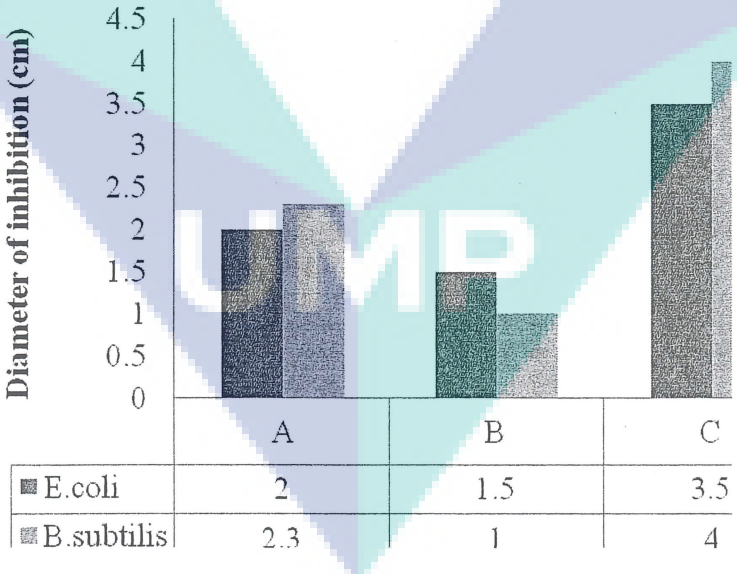


Figure 4.21: Zone inhibition diameter of Sample A, B and C

The greater the size of diameter zone inhibition shows the greater antimicrobial activity of the samples (Pranoto *et al.*, 2005). Figure 4.19 shows the zone of inhibition toward *E.coli* by the sample A, B and C. From the Figure 4.21 the diameter of inhibition by sample A was 2 cm while sample B was 1.5 cm. Sample C has the highest diameter of zone inhibition which was 3.5 cm.

Meanwhile, Figure 4.20 shows the zone of inhibition by the sample A, B and C toward *B. subtilis*. Figure 4.21 shows the diameter of inhibition by sample A was 2.3 cm and sample B was 1 cm. Sample C once again has the highest zone of inhibition which is 4 cm.

From the result we can analyze that sample A has antimicrobial properties but poor toward *E.coli* and *B. subtilis*. Chitosan was proven to have antimicrobial properties in the previous research (Pranoto *et al.*, 2005) thus it shows some antimicrobial activity toward both bacteria but poor. For the sample B, the sample shows poorer antimicrobial compared to the sample A. this is because sample B has half of the amount of chitosan in sample A since sample B fabricated from starch combined with chitosan. Sample C has the highest antimicrobial activity towards both bacteria. Its chemical properties damaged the outer bacteria cell wall and prevent them to grow and dividing (Liesel *et al.*, 2008). Sample A has poorer antimicrobial properties since chitosan is in a solid form, therefore, only organisms in direct contact with the active sites of chitosan is inhibited (Pranoto *et al.*, 2005).

4.5.2 Liquid Culture Test (Optical Density Measurement)

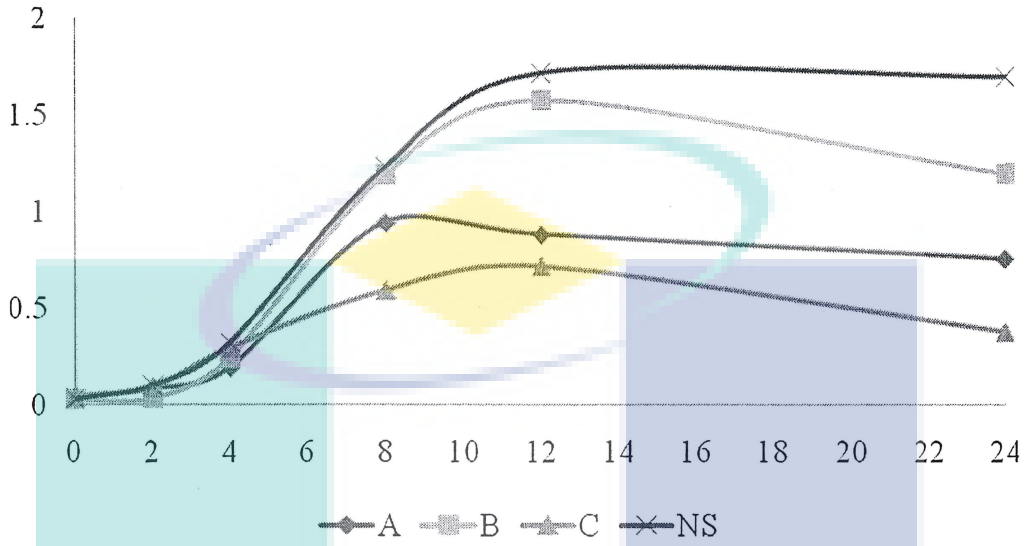


Figure 4.22: OD measurement of Samples toward *E.coli*

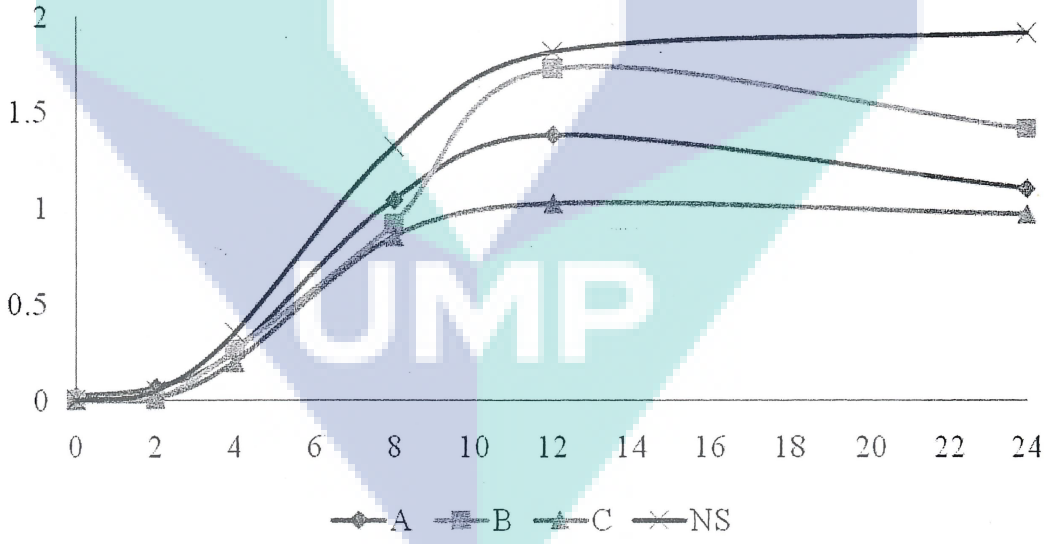


Figure 4.23: OD measurement of Samples toward *B.subtilis*

Table 4.1: OD reads of Sample A, B, and C

Period (hr)	*E.coli*				*B.subtilis*			
	Sample							
	A	B	C	NS	A	B	C	NS
	Optical Density (OD)							
0	0.045	0.028	0.044	0.030	0.023	0.0040	0.003	0.003
2	0.087	0.033	0.092	0.102	0.068	0.009	0.008	0.052
4	0.194	0.244	0.294	0.320	0.256	0.264	0.199	0.352
8	0.942	1.193	0.591	1.230	1.046	0.923	0.856	1.324
12	0.883	1.580	0.717	1.720	1.386	1.731	1.031	1.820
24	0.756	1.198	0.379	1.700	1.107	1.420	0.975	1.920

The result shown by the liquid culture test respectively similar to the zone inhibition assay analysis which sample C has the best antimicrobial activity compared to other sample. In the Table 4.1, the antimicrobial film was more effective against Gram- a negative bacterium which is *E. coli* than the Gram- positive bacteria which is *B. subtilis*. This study indicated that the mechanisms of the antimicrobial activity were different between those different types of bacteria.

For other sample there were also showing antimicrobial properties but poor. One of the reasons for the antimicrobial character is because of chitosan positively charged amino group which interacts with negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms. In the Gram-positive bacteria, the major constituent of its cell wall is peptidoglycan and there is very little protein. The cell wall of Gram-negative bacteria also has an outer membrane, which constitutes the outer surface of the wall. Previous study, observed that from electron micrographs for Gram-positive and Gram-negative bacteria in the presence of chitosan show the cell membrane of Gram-positive bacteria was weakened or even broken, while the cytoplasma of Gram-negative bacteria was concentrated and the interstice of the cell were clearly enlarged (Salleh and Muhamad, 2001).

CHAPTER 5

CONCLUSION

5.1 Conclusion

As a conclusion, all of the objectives of this experiment were successfully achieved. The surface and cross section morphology of the sample was successfully analyzed by SEM. The bond exist in the fabricated film has successfully determine using the FTIR. Furthermore, the thermal biodegradation behavior of the fabricated film was successfully analyzed by TGA and also the melting point by DSC. Other than that, the antimicrobial analysis of liquid culture test and zone inhibition assay was successfully performed in order to analyze the fabricated film antimicrobial properties.

From the result and discussion, we can conclude that the starch-chitosan blend biodegradable film has very good characteristic in term of morphology, physical and chemical. Although the surface morphology did not smooth as chitosan only based film due to large granular structure of starch, the cross section of the film shows good compact structure. Other than that, the thermal resistance of the fabricated composite film was the highest compare to the control film. Most importantly, the fabricated film shows a very good antimicrobial property compared to the control film.

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APPENDIX A

Patent Application

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YOUR REF :
DATE : 3 May 2010

Universiti Malaysia Pahang
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Office of Deputy Vice Chancellor (Research & Innovation)
Lebuhraya Tun Razak, 26300 Gambang,
Kuantan, Pahang.

By Post

Attn : Ms. Nor Ilma

Re : New Patent Application in Malaysia – No. PI 2010001963
Applicant : Universiti Malaysia Pahang
Invention : Biodegradable Composite Material Based on Plant Starch and The Method for Preparation Thereof

We refer to the above matter.

We confirm having filed the above-mentioned patent application in accordance with your instructions in Malaysia on **29 April 2010**. We enclose a copy of the request form as filed for your records.

The Patent Office will shortly issue a Certificate of Filing confirming the date of filing and reflecting the official application number, which we shall forward to you upon receipt thereof.

We also enclose herewith our invoice No. Inv/UMP/2496/10 for the said matter.

Further progress will be reported.

Yours faithfully,

MOHAN K / PC YAN
PATENT AGENT REGN. NO. PA04/0141

ADASTRA

An Intellectual Property Firm

Year in Operation serving Clients Satisfactorily

PATENT
MOHAN K
B.ENG (HONS) U.K.
PC YAN
B.SC (HONS) M.
ELINA MASZURA ISA
B.SC BIOTECHNOLOGY
TAI YOKE YEE
B.SC (HONS) AGRI TECHNOLOGY UM
TRADEMARK/LEGAL
NOOR AZIAH YAHAYA LUDDIN
LL.B (HONS) UITM
EZARINA YAAKUB
DIP. IN FASHION & DESIGN UITM
FRANCHISE/GRANTS
ZAFFAN MD YASSIN
BS (HONS) UITM
HEAMA.S
BRANDING/DESIGN
JULIA KOH
B.BUSINESS ADMINISTRATION

OUR REF : P/UMP/10MY19/Tai
YOUR REF :
DATE : 3 May 2010

Universiti Malaysia Pahang
Intellectual Property Management Unit
Office of Deputy Vice Chancellor (Research & Innovation)
Lebuhraya Tun Razak, 26300 Gambang,
Kuantan, Pahang.

By Post

Attn : Ms. Nor Ilma

Re : New Patent Application in Malaysia – No. PI 2010001964
Applicant : Universiti Malaysia Pahang
Invention : Method for Preparing Biodegradable Composite Polymeric Material

We refer to the above matter.

We confirm having filed the above-mentioned patent application in accordance with your instructions in Malaysia on **29 April 2010**. We enclose a copy of the request form as filed for your records.

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Further progress will be reported.

Yours faithfully,

MOHAN K / PC YAN
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APPENDIX B

Award Certificate

International Invention, Innovation and Technology Exhibition

ITEX 2010

MINISTRY OF SCIENCE, TECHNOLOGY & INNOVATION

ITEX MALAYSIA — INTERNATIONAL INVENTION, INNOVATION AND TECHNOLOGY EXHIBITION

MINDS

Certificate of Award

This is to certify that

NORASHIKIN BINTI MOHD ZAIN,MOHD ZAMRI BIN IBRAHIM,NUR ADILAH BINTI ISMAIL

has been awarded the

ITEX GOLD MEDAL

for the invention

NOVEL GREEN, ECONOMICALAND ENVIRONMENTALLY FRIENDLY BIODEGRADABLE COMPOSITE FILMS FROM CALADIUM

at the

21st International Invention, Innovation & Technology Exhibition
ITEX 2010
Kuala Lumpur, Malaysia

held from

14th – 16th May 2010

Academician Emeritus Professor Tan Sri Datuk
Dr Augustine Ong Soon Hock
President
Malaysian Invention and Design Society