

SYNTHESIS AND CHARACTERIZATION OF BIONANOCOMPOSITE SILVER
NANOPARTICLES (AgNPs)-CHITOSAN/POLYLACTIC ACID (PLA) BIOFILM

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ABSTRACT

Biobased polymers derived from renewable resources are increasingly important due to intense concerns about the environmental issues and limited petroleum resources. Poly(lactic acid) (PLA) is such polymer that has shown good potential to produce biodegradable plastics. However, poor antimicrobial and mechanical properties restrict its broad applications. The thesis pursues to overcome these limitations by blending PLA with chitosan and reinforcing them with inorganic nanoparticles. The performances of antimicrobial biodegradable films with enhanced properties have potential good for many applications. Firstly, the silver nanoparticles have been synthesized with tea extract leaves. Characterization of silver nanoparticles by UV-vis, TEM, XRD and FTIR indicate that silver nanoparticles were present in the tea extract solution with the range of size 10-150 nm. Antimicrobial analysis of silver nanoparticles against gram negative (*Escherichia coli*) and gram positive (*Micrococcus luteus*) microorganisms had proved that silver nanoparticles is a good antimicrobial agent although incorporated with blending of PLA/chitosan films. The best concentration of silver nanoparticles which incorporated with PLA/chitosan films was 23.08% w/w. It means that films have showed slight growth of microorganism at 23.08% w/w of silver nanoparticles. The films were characterized by FESEM and EDX. The morphological images presented the shape of silver nanoparticles incorporated into the films was almost spherical. Tensile measurement was represented by tensile strength and elongations at break which were used as a response for choosing of the best conditions bionanocomposite films fabrication (BFF) in the mechanical properties films. The interaction and main effect of two factors with the range (0-30%) concentration of plasticizer PEG and (0-100%) percentage volume PLA/chitosan were studied using 2-level full factorial design. P-value for factor A = 0.0269, B = <0.0001 significant influence to this response. While P-value only for factor A = 0.0034 and B = <0.000. Both factors were given the effect to the response based on higher percentage contribution (>5%) and lower p-values (<0.05). The best conditions BFF had been chose using rotatable central composite design under Response Surface Methodology (RSM) with with the range (5-25%) concentration of plasticizer PEG and (20-80%) percentage volume PLA/chitosan. The mechanical properties have improved at this the best condition (7.93% w/w concentration of PEG and 28.79% / 71.21% percent volume of PLA/chitosan). While the best responses (8.32 MPa tensile strength and 32.15% elongation at break) were obtained, also using the best condition. The validation experiment proved the empirical model developed were considerably accurate (error between the actual and predicted values = between 5%) with the percentage errors are ranging from 2.08% for tensile strength and 3.89% elongation at break. These results showed that there were in agreement with the other previous studies findings.

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LIST OF SYMBOLS

Ag^0	Argentum colloid (silver colloid)
Ag^+	Argentum ion (silver ion)
Å	Armstrong
cm	Centimeter
°C	Degree Celsius
e^-	Electron
g	Gram
H^+	Hydrogen ion
kPa	Kilo pascal
kV	Kilovolt
L	Liter
MPa	Mega pascal
M^0	Metal colloid
M^+	Metal ion
m	Meter
μg	Micro gram
μl	Micro Liter
μm	Micro meter
ml	Milliliter
mm	Millimeter
min	Minute
M	Mole

nm	Nanometer
cm ⁻¹	One per centimeter
%	Percent
rpm	Revolution per minute
sec	Second
v/v	Volume per volume
w/v	Weight per volume
w/w	Weight per weight

LIST OF ABBREVIATIONS

ATP	Adenosine-5'-triphosphate
ANOVA	Analysis of variance
B-B	Box-Behnken
BFF	Bionanocomposite films fabrication
s-CNC	Cellulose nanocrystals
CCD	Central Composite Design
CFU	Colony Forming Unit
Conc	Concentration
DNA	Deoxyribonucleic acid
EDX	Energy dispersive X-ray spectrometer
EPA	Environment Protection Authority
EC	Epicatechin
ECG	Epicatechin-3-gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin-3-gallate
E. coli	Escherichia coli
FESEM	Field emission scanning microscope
FDA	Food and Drug Administration
FTIR	Fourier transform infrared spectroscopy
FFD	Full Factorial Design
GC	Gallocatechin
HPMC	Hydroxypropyl methylcellulose
ICDD	International Centre for Diffraction Data

MNP	Metal nanoparticles
OFAT	One factor -at-a-time
PHB	Poly(3hydroxybutyrate)
PCL	Poly(ϵ -caprolactone)
PGA	Poly(glycolic acid)
PEG	Polyethylene glycol
PLA	Polylactic acid
ROS	Reactive oxygen species
RSM	Response surface methodology
AgNP	Silver nanoparticles
SNP	Silver nanoparticles
AgNO ₃	Silver nitrate
SS	Sum of squares
SPR	Surface plasmon resonance
SERS	Surface-enhanced Raman scattering
TEM	Transmission electron microscopy
XRD	X-ray diffraction

CHAPTER 1

INTRODUCTION

1.1 Background of study

The field of nanotechnology is expected to be one of the most active areas of research and development in modern material science. It has long been known that silver nanoparticles have come up as one of the most effective antimicrobial agents in a number of fields, including medical and pharmaceutical (Tozuka et al., 2011), dental (Chan et al., 1999) coating and paint (Aldraihem et al., 2009), membrane (Xue et al., 2006) and food packaging purposes (Yoksan and Chirachanchai, 2010).

Due to application of silver nanoparticles which act as antimicrobial agent, many efforts have been made to incorporate silver nanoparticles into acceptable material. Numerous biodegradable materials, for example polysaccharides such as chitosan (Yoksan and Chirachanchai, 2010), polylactic acid (Sebastien et al., 2006) and polyethylene (Xing et al., 2012) have been used to fabricate silver nanoparticle-based composite films.

Yoksan and Chirachanchai (2010) fabricated silver nanoparticles loaded chitosan and starch based film which aims to determine the minimum concentration of silver nanoparticles dispersed gas barrier properties, the antimicrobial qualities of silver nanoparticle-loaded chitosan–starch based films in chitosan solution, and to investigate the effects of silver nanoparticle content on the tensile properties, the water vapor and oxygen. Then, Wei et al. (2009) synthesized chitosan- silver nanoparticles based films and characterized their antibacterial activity. The chitosan-based silver nanoparticles in

this research thus obtained demonstrate high antibacterial activity against Gram-positive and Gram-negative bacteria.

Subsequently, the multifunctional bionanocomposite films of combination poly(lactic acid), cellulose nanocrystals and silver nanoparticles have been fabricated and reported by Fortunati et al. (2012). The researchers' success in present the influence combination of cellulose structures with silver nanoparticles in mechanical, thermal and antimicrobial properties.

However, many researchers using silver nanoparticles which synthesizing by physical and chemical method then contributing in accumulating an enormous quantity of toxic. There are several number of methods are available for synthesis of silver nanoparticle such as chemical reduction (Mukherjee and Mahapatra, 2009), photochemical reduction (Tapley et al., 2011), microwave (Jiang et al., 2011) and recently via green chemistry method (Dubey et al., 2010). In continuation of our efforts for search of synthesizing silver nanoparticles by green chemistry approaches, we have utilized the tea leaves extraction as biomimetic material.

There are many important factors which influencing the quality of bionanocomposite such as antimicrobial, mechanical, thermal and water vapor properties, the focus of this study is to investigate antimicrobial and mechanical properties. Realizing that much blending composites with silver nanoparticles which synthesis from chemical method show good results in antimicrobial and mechanical properties, this study is needed to investigate the same responses when composites incorporate with silver nanoparticles which is synthesis using biomimetic material.

Polymer blend is an interesting alternative to produce new polymeric materials with customizable features. Other advantages for polymer blends are versatile, simple, and inexpensiveness. Nowadays, natural polymers have become increasingly important due to lots of their natural abundances and with lower cost. Biofilms products based on one types of biofilms, possess many disadvantages, mainly attributed to brittle nature of films and poor mechanical properties. To overcome these problems, the blending of the

biodegradable chitosan and polylactic acid (PLA) with antimicrobial agent, silver nanoparticles have been produced through this research.

This then is the intention of this study; to synthesis silver nanoparticles in greener chemical method and incorporate it in blending polylactic acid (PLA)/chitosan then to test its antimicrobial and mechanical properties of bionanocomposite.

1.2 PROBLEM STATEMENTS

Recently, high demands and low cost of production are the reasons of synthetic polymer has become increasingly valuable (Wu, T.M. and Wu, C.Y., 2006) especially in food packaging and medical field. These synthetic polymers are not biodegradable; it is finally end up in landfill sites and produce very harmful gases that can cause environmental pollutions and soil contaminations. The burning of synthetic polymers can cause the release of dioxin which is highly toxic that will cause chronic disease (i.e: cancer) through inhalation.

Comprehensive waste composition in Malaysia is influenced by municipal solid waste (64%), followed by industrial waste (25%), commercial waste (8%) and 3% consists of construction waste. About 9.7% of municipal waste are plastic based which are nondegradable (Yiing and Latifah, 2014).

Table 1.1 potrays the generation of municipal solid waste by individual states in Malaysia from 1996 to 2009. Johor, Kuala Lumpur and Selangor states are the top three ranked in the municipal solid waste generated. The amount of solid waste plastic based was caused continues to rise over the years because the growth in living standards and most of them are non degradable plastic.

Table 1.1: Generation of municipal solid waste in Malaysia according to states from 1996 to 2009

States	Solid waste generated (tons/day)							
	1996	1998	2000	2002	2004 ^a	2006 ^a	2008 ^a	2009 ^a
Johor	1613	1786	1915	2093	2255	2430	2578	2655
Kedah	1114	1215	1324	1447	1559	1680	1782	1835
Kelantan	871	950	1034	1131	1213	1320	1382	1423
Melaka	433	480	515	563	605	650	690	711
Negeri Sembilan	637	695	757	828	890	957	1015	1046
Pahang	806	879	957	1046	1125	1210	1284	1322
Perak	1286	1402	1527	1669	1795	1930	2048	2109
Perlis	165	180	196	214	230	247	262	270
Pulau Pinang	916	999	1088	1189	1278	1375	1458	1502
Selangor	2380	2595	2827	3090	3322	3573	3790	3904
Terengganu	743	811	883	965	1038	1116	1184	1219
Kuala Lumpur	2105	2305	2520	2755	3025	3323	3525	3631
WP Labuan	NA	NA	46	70	74.3	81.2	86.1	88.7
Sabah	NA	NA	NA	2490	2642	2887	3062	3154
Sarawak	NA	NA	NA	1905	2012	2208	2343	2413
Total	13,069	14,297	15,589	21,455	23,063	24,969	26,489	27,283

NA: not available.

Sources: Agamuthu et al. (2009)

^a Extrapolated figures.

Therefore, it is very crucial to discover other biodegradable films to replace the use of synthetic polymer. The biodegradable films were not meant to totally substitute the synthetic polymers. Nevertheless, the using of biodegradable films as packaging materials is still one of the most hopeful approaches for effective methods of maintaining in food quality and medical application.

By using the biodegradable films for food product packaging and medical field, the industry are facing with the problems of short shelf life period due to low antimicrobial resistance (Aider, 2010). Due to that, inclusion of the active molecules in the packaging films becomes an innovative option to prevent microbial contamination of food product (Guiga et al., 2009). International journal of food microbiology (Sanpui et al., 2008) reported that interactions between silver nanoparticle –chitosan composite material and both prokaryotic and eukaryotic cells are needed.

Silver nanoparticles have been broadly used as antimicrobial agents in food packaging purposes (Yoksan and Chirachanchai, 2010). The synthesis of silver nanoparticle through the chemical methods will accumulate very large quantity of toxic and redundant of chemicals in solid, liquid and gaseous form in the environment (Dubey et al., 2010). Therefore, simple and greener chemical approaches for the synthesis of silver nanoparticle by using biomimetic material such as plant extract will be more encouraging.

However, biodegradable films with incorporation of silver nanoparticle products based on one types of biofilms, possess many disadvantages, mainly attributed to brittle nature of films and poor mechanical properties. In order to improve mechanical properties, these films can be modified by several methods such as blending with other natural polymers such as polylactic acid and chitosan (Salleh et al., 2007).

Thus, the focuses on this research are to synthesis silver nanoparticles in greener chemical approaches and incorporate it in blending nonsynthetic polymer polylactic acid (PLA)/chitosan, then test its antimicrobial and mechanical properties of bionanocomposite.

1.3 OBJECTIVES

The objectives of this research are listed as blow:

1. To synthesize silver nanoparticle colloids from tea leaf extract (*Camellia sinensis*).
2. To synthesize mixed bionanocomposite from chitosan, polylactic acid (PLA) and silver nanoparticles.
3. To characterize bionanocomposite film in terms of antibacterial properties and choosing the best condition of its mechanical properties by using Response Surface Methodology (RSM).

1.4 SCOPES

In order to achieve the stated objectives, the following scopes have been identified:

1. The production of silver nanoparticle acting as antimicrobial agent from biomimetic material tea leaf extract by using greener chemical method.
2. The characterization of silver nanoparticles Transmission electron microscopy (TEM), X-ray diffraction (XRD), UV-Vis Spectrophotometer to prove the present of silver nanoparticles in solution and Fourier transform infrared spectroscopy (FTIR) as to determine the functional groups on the colloid surface and their possible involvement in the synthesis of silver nanoparticles.
3. The effects of silver nanoparticles colloids as antimicrobial agent on growth of *Escherichia coli* (*E. coli*) and *Micrococcus luteus* through agar well diffusion method.
4. The effects of antimicrobial activity of the bionanocomposite were investigated using microbial culture of *Escherichia coli* (*E. coli*) and *Micrococcus luteus* through agar diffusion method (zone inhibition assay).
5. Analysis of the chemical functional groups and examination of the effects of silver nanoparticles loading on synthesized film's morphology as prepared blend bionanocomposite by using Fourier Transform Infrared Spectroscopy (FTIR) and Field Emission Scanning Microscope (FESEM), respectively.
6. The interaction effect of factors in mixed bionanocomposite production from literature studies were screened using 2-level full factorial design (FFD). The factors are including concentration of Polyethylene glycol (PEG) and ratio of percentage volume of polylactic acid (PLA) and chitosan while tensile strength and elongation at break were chosen as the responses and then further the choosing of the best films process was done by Central Composite Design (CCD) approach. Experiments were carried out again to

validate the result of response surface methodology (RSM) from Design Expert software.

1.5 SIGNIFICANT OF THE RESEARCH

The benefit of this research is to identify how the silver nanoparticle colloids can be synthesized from the tea leaves, to find out whether silver nanoparticles can be incorporated in the polylactic acid and chitosan based bionanocomposite in order to prevent the microbial contamination. Besides that, this research is also aim to recognize the suitable concentration of silver nanoparticles to be incorporated into the bionanocomposite and also to recognize the best mechanical strength of bionanocomposite.

In this research, some test were being carried out, such as the characterization of silver nanoparticle test by using Transmission electron microscopy (TEM), X-ray diffraction (XRD), UV-Vis Spectrophotometer and transform infrared spectroscopy (FTIR); antimicrobial test of silver nanoparticle colloid and blended films by using *Escherichia coli* (*E. coli*) and *Micrococcus luteus*; analysis of film's morphology by using Field emission scanning microscope (FESEM) and choosing the best conditions of films in mechanical strength of films by using response surface methodology (RSM) from Design Expert software.

CHAPTER 2

LITERATURE REVIEW

2.1 SILVER NANOPARTICLES

“Nano-” means nanometer (10^{-9} m). The idea of nanotechnology was presented by Richard Feynman in 1959 at a conference of the American Physical Society (Khademhosseini and Langer, 2009). Since then, nanotechnology has spread its usage into multidisciplinary fields. Frequently, nanoparticles which are in the range 1-100 nm show unique properties compared to macro scaled due to their high surface-to-volume ratio, quantum confinement and can dispersed without agglomeration (Azeredo, 2009).

Recently, silver nanoparticles have received extensive use in medical and food industrial process among other noble metals. There is a great interest in using the silver nanoparticles due to several reasons such as an effective antimicrobial agent (Satyavani et al., 2011b) and exhibit low toxicity (Dwivedi and Gopal, 2010). Besides, silver nanoparticles can be as the nanosize filler or nanoreinforcement in the making of nanocomposite to improve the poor mechanical and barrier properties of biofilm (Azeredo, 2009).

2.1.1 Application of silver nanoparticles

The ancient people had used the silver nanoparticles as an antimicrobial agent in food and beverage storage applications and home appliances to water treatment. They stored water and wine in silver vessels, placed silver spoons at the bottom of milk and water bottles to extend shelf life especially during their seafaring moment (Duncan, 2011).

Moreover, silver is effective in penetrating biofilms by killing or damaging many molecular antimicrobials and act as the nanosize filler or nanoreinforcement in the making of nanocomposite to improve the poor mechanical and barrier properties of biofilm (Azeredo, 2009). Furthermore, silver nanoparticles are easily incorporated into many materials, which are the reason for it to be as the largest advantage with broad applications such as textiles and plastics (Tolaymat et al., 2010).

Likewise, the silver nanoparticles not only use in food application but in medical fields too. Silver has long been used as antiseptic. Therefore, silver nanoparticles are used in medical and supplies devices such as wound dressings, scaffold, skin donation, recipient sites, sterilized materials in hospitals, medical catheters, contraceptive devices, surgical instruments, bone prostheses, artificial teeth and bone coating (Tolaymat et al., 2010). Other than that, by combining membrane electrophoresis with silver nanoparticle, it can be used in cancer detection analysis (Lin et al., 2011), accelerate the development of new diagnostics and therapeutics for human pathological conditions (Saini et al., 2006) and as the biosensor in to enhance the electrode conductivity in detecting the allergy disease (Yi-Fen and Liu, 2012).

The unique properties of silver nanoparticles involve their performance in fluorescence and surface plasmon resonance (SPR). Due to that, it has been widely used in photonics, electronics, surface-enhanced Raman scattering (SERS) and biosensors (Bian et al., 2011). Table 2.1 shows summarization of silver nanoparticles's applications.