UNIVERSITI PUTRA MALAYSIA

PREPARATION AND CHARACTERIZATION OF SILVER/CHITOSAN/GELATIN BIONANOCOMPOSITES BY CHEMICAL REDUCING METHOD AND THEIR ANTIBACTERIAL ACTIVITIES

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By

LIM JENN JYE

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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The aim of this study is to investigate the functions of polymers and size of nanoparticles on the antibacterial activity of silver bionanocomposites (Ag BNCs). In this research, silver nanoparticles (Ag NPs) were incorporated into biodegradable polymers, which are chitosan (Cts), gelatin and both polymers via chemical reduction method in solvent in order to produce Ag BNCs. Silver nitrate and sodium borohydride were employed as a metal precursor and reducing agent respectively. In addition, chitosan and gelatin were added as both polymeric matrix and stabilizer. The properties of Ag BNCs were studied based on function of the polymer weight ratio in relation to the use of chitosan and gelatin. These prepared Ag NPs were very stable over a long period (i.e. 4 months) in an aqueous solution without any sign of precipitants. The UV-vis spectra shown maximum absorbance bands for Ag BNCs were detected at 408, 416, 414, 413, and 414 nm, respectively. From the FTIR spectra, the vibrational band of Ag BNCs detected at ~1395 cm^{-1} could indicate the interaction between

Ag NPs with chitosan and gelatin. The morphology of the Ag BNCs films and the distribution of the Ag NPs were characterized using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). From the SEM image, Ag/Cts BNCs shown layered surfaces with small flakes, Ag/Cts/gelatin BNCs showed smooth layered surfaces and Ag/gelatin BNCs showed porous layered surfaces. In addition, the EDX spectra for Ag BNCs had confirmed the presence of elemental compounds in the Cts, gelatin and Ag NPs without any impurity peaks. From the TEM images, the results of average diameters of the Ag NPs in different weight composition of polymers were in the range of 2.90-11.25 nm. However, the TEM results also shown that aggregation of Ag NPs where there is an overlap of particles and larger particle size in chitosan only which contributed to the size of 11.25 nm compared with others samples which are 2.90 nm-4.38 nm. With the aid of XRD pattern, Ag BNCs films confirmed the face-centered cubic (FCC) type crystallographic planes of the Ag crystal. The XRD peaks at 2θ with value of around 38°, 44°, 64°, and 77° are well recognized to the 111, 200, 220, and 311 crystallographic planes of the FCC Ag crystals, respectively. The antibacterial activities of the Ag BNC films were examined against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and Gram-positive (*S. aureus* and *M. luteus*). The silver ions released from the Ag BNCs and their antibacterial activities were scrutinized. From TEM analysis and the result of antibacterial activity, Ag/Cts/gelatin BNCs exhibited better particle distribution as well as having high antibacterial activity. A simple way to prepare Ag/Cts/gelatin bionnanocomposites had been employed as previously there is no research on investigating the antibacterial activities of silver into both chitosan and gelatin.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENYEDIAAN DAN PENCIRIAN BIONANOKOMPOSIT PERAK / KITOSAN / GELATIN DARI KAEDAH PENURUNAN KIMIA DAN AKTIVITI ANTIBAKTERIA

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Tujuan kajian ini adalah untuk menyelidik fungsi polimer dan saiz nanopartikel dalam aktiviti antibakteria perak bionanokomposit (Ag BNCs). Dalam penyelidikan ini, nanopartikel perak (Ag NPs) telah dimasukkan ke dalam polimer yang biodigradasi iaitu kitosan, gelatin dan kedua-dua polimer melalui kaedah penurunan kimia dalam pelarut untuk menghasilkan Ag BNCs. Argentum nitrat dan natrium borohydride telah digunakan sebagai prekursor logam dan ejen penurunan masing-masing. Sebaliknya, kitosan (Cts) dan gelatin telah ditambah sebagai matriks polimer dan penstabil. Ciri-ciri Ag BNCs telah dikaji berdasarkan nisbah berat kitosan dan gelatin. Ag NPs yang disediakan adalah sangat stabil dalam tempoh yang panjang (iaitu 4 bulan) dalam larutan tanpa sebarang tanda mendakan. Spektrum UV-nampak menunjukkan jalur penyerapan yang maximum untuk Ag BNCs telah dikesan pada 408,416,414,413, dan 414nm masing-masing. Dari specktrum jelmaan

fourier inframerah, gelombang getaran Ag BNCs dikesan pada ~1395 cm^{-1} menunjukkan interaksi antara Ag NPs dengan kitosan dan gelatin. Morfologi filem Ag BNCs dan pengedaran Ag NPS turut dipercirikan menggunakan Mikroskop Pengimbasan Elektron (SEM) dan Mikroskop transmisi elektron (TEM). Dari gambar SEM, Ag/kitosan BNCs menunjukkan permukaan berlapis dengan serpihan kecil, Ag/kitosan/gelatin BNCs menunjukkan permukaan licin berlapis dan Ag/gelatin BNCs menunjukkan permukaan lapisan berliang. Sebagai tambahan, spektrum EDX bagi Ag BNCs telah mengesahkan kehadiran sebatian unsur dalam kitosan, gelatin dan Ag NPs tanpa sebarang puncak bendasing. Dari imej TEM, hasil diameter purata Ag NPs dalam komposisi berat polimer yang berbeza adalah dalam lingkungan 2.90-11.25 nm. Walau bagaimanapun, hasil TEM juga menunjukkan agregasi Ag NPs di mana terdapat pertindihan partikel dan saiz partikel yang lebih besar dalam kitosan sahaja yang menymbang kepada saiz 11.25 nm berbanding dengan sampel lain yang bersaiz 2.90-4.38 nm. Dengan bantuan corak XRD, Ag BNCs mengesahkan Ag NPs memiliki jenis satah kristalografi yang muka berpusat padu (FCC). Puncak-puncak XRD di paksi 2θ dengan nilai kira kira 38 °, 44 °, 64 °, dan 77 ° telah diiktiraf kepada 111, 200, 220, dan 311 satah kristalografi FCC masing-masing daripada Ag kristal. Aktiviti antibakteria yang Ag BNC filem dikaji terhadap bakteria Gram-negatif (*E. coli* dan *P. aeruginosa*) dan Gram-positif (*S. aureus* dan *M. luteus*). Ion perak dikeluarkan dari BNCs Ag dan aktiviti antibakteria mereka telah diteliti. Daripada analisis TEM dan hasil aktiviti antibakteria, Ag/kitosan/gelatin BNCs mempamerkan pengagihan zarah yang lebih baik serta mempunyai aktiviti antibakteria yang tinggi. Satu cara mudah untuk menyediakan Ag / Cts / gelatin bionnanocomposites telah digunakan sebab sebelum ini tidak ada penyelidikan yang mengkaji aktiviti antibakteria perak ke dalam kedua-dua kitosan dan gelatin.

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I certify that a Thesis Examination Committee has met on 3 January 2013 to conduct the final examination of Lim Jenn Jye on his thesis entitled "Preparation And Characterization Of Silver/Chitosan/ Gelatin Bionanocomposites By Chemical Reducing Method And Their Antibacterial Activities" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

LIM JENN JYE

Date: 3 January 2013

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LIST OF ABBREVIATIONS

Ag/Cts	silver/chitosan
Ag/Cts/gelatin	silver /chitosan/gelatin
Ag BNCs	silver bionanocomposites
$AgNO_3$	silver nitrate
Ag NPs	silver nanoparticles
BNCs	bionanocomposites
Cts	chitosan
Cts/gelatin	chitosan/gelatin
E. coli	*Escherichia coli*
EDX	energy dispersive X-ray
FTIR	Fourier transform infrared
HAC	acetic acid
MHA	Mueller-Hinton Agar
M. luteus	*Micrococcus luteus*
MMT	montmorillonite
$NaBH_4$	Sodium borohydride
NCs	nanocomposites

NPs	nanoparticles
SPR	Surface Plasmon Resonance
S. aureus	*Staphylococcus aureus*
SEM	scanning electron microscopy
P.aeruginosa	*Pseudomonas aeruginosa*
PVP	Poly(vinylpyrrolidone)
PVA	Poly(vinyl alcohol)
TEM	transmission electron microscopy
UV-vis	ultraviolent visible
XRD	X-ray diffraction

CHAPTER 1

INTRODUCTION

1.1 Background of study

Nanotechnology is becoming a significantly important field, offering massive improvements to standards of living by developing the new nano-scaled materials. The term nanotechnology was first introduced by Professor Norio Taniguchi in the year 1974 to describe the accurate processing of a material to nano scale (Taniguchi, 1983). The term nanotechnology comes from the combination of two words: the Greek numerical prefix nano referring to a billionth and the word technology. As an outcome, nanotechnology is generally considered to be at a size below 100 nm. Nanoscience, sometimes defined as 'the science underlying nanotechnology' is related to the study of nanostructured materials which included the process of synthesis, characterization, exploration, interrogation, exploitation, and utilization. Nanomaterials are materials that have structural components with at least one dimension smaller than 100 nm.

The nanomaterials enclose different group of nanostructured materials including quantum dots, clusters, nanocrystals, nanowires, and nanotubes (Logothetidis, 2012). Nanoparticles (NPs) may exhibit size-related properties that significantly diverge from those found in those molecular or bulk materials with the same chemical composition. As particles become increase smaller and enter the nanosize range, the numbers of atoms at the surface of the particle are far greater and more significant, thus altering the physical and chemical properties of the nanoparticles. Also, their physical-chemical properties can be significantly different mainly due to the nanometer size of the materials causing them to have high surface energy, spatial confinement, and reduced imperfections which these properties do not exist in the corresponding bulk materials (Cao, 2004). The fundamental of nanoscience and nanotechnology has two approaches. First, the bottom-up approach where materials are built from molecular components which assemble themselves chemically by principles of molecular recognition. Second, the top-down approach of the self assembly of molecular components, where each nanostructured component plugs itself into a suprastructure (Jortner *et al.*, 2002).

Metal nanoparticles play important part in different research fields due to its unique chemical, optical, electrical, and thermal properties when their size is confined to the nanometer length range. They represent in the development of novel materials that can be used in various physical, chemical, biological (Zhang *et al.*, 2001), biomedical and pharmaceutical applications. The intrinsic properties of metal nanoparticles are mainly determined by its size, composition, shape, structure, and crystallinity. Despite this, the challenge of synthetically controlling the parameter to tailor the antibacterial, electrical, and optical behaviours of metal nanoparticles has been met with limited success. Perfectly monodispersed metal NPs are ideal, but their unique properties are to be expected even if the ideality is not perfectly realized.

Composites can be defined consist of at least two materials with rather different properties, revealing heterogeneous microstructure and have improved properties with respect to their components. Nanocomposites (NCs) are multiphase solid material or structures where one of the phases has dimensions within nanometer range. Nanocomposites can be considered as solid structures with nanometer- scale dimensional repeat distance between the different phases. Typically, nanocomposites are classified as inorganic matrix (inorganic-inorganic), organic filler in organic (organic-organic), and hybrid materials, i.e., organic in inorganic or inorganic in organic matrix (Ajayan *et al.*, 2003). The mechanical, optical, thermal, catalytic and others properties of the NCs will differ noticeably from that of the component materials. Bionanocomposites (BNCs) has become a common term to designate those NCs involving a naturally occurring biopolymer with organic/inorganic nanomaterials. BNCs, an original invention of NCs materials, indicate a promising field in nanotechnology, materials and life sciences due to their extraordinary advantages of biocompatibility and biodegradability (Darder *et al.*, 2007).

1.2 Problem statement

In previous research done by other researchers, there were reports on preparation of silver/chitosan bionanocomposites and silver/gelatin bionanocomposites. However, reports on the effect of antibacterial activity of Ag NPs in chitosan/gelatin which has potential application in biomedical field had yet to be investigated. Moreover, there has been no attempt to compare the properties of Ag NPs in Cts, gelatin and Cts/gelatin. In this study, Ag NPs were effectively prepared in the chitosan, gelatin and Cts/gelatin composite by using chemical reducing method. The Ag ions were successfully reduced by sodium borohydride ($NaBH_4$) into the polymers matrix forming Ag NPs. The characterization will be based on the different ratios of the polymer weights and the antibacterial activities will be carried out using MHA diffusion method.

1.3 Scope

The present research is an attempt to study the size, surface morphology, and antibacterial effect of Ag NPs in different weight ratio of polymer matrix. The crystalline structure, average size, particles distributions, surface morphology, surface Plasmon resonance and functional groups of all the samples were characterized using X-ray diffraction (XRD), UV-visible spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), Energy-dispersive X-ray spectroscopy (EDX) and Fourier transform infrared (FTIR). The antibacterial activities for Ag NPs nanocomposites were investigated against Gram positive and Gram negative bacteria using Muller-Hinton Agar method.

1.4 Objectives

The main objectives of this research are to:

1. prepare Ag NPs by using chemical reducing agent in different weight ratios of Cts and gelatin.
2. characterize the crystalline structure, particle size and distribution, surface morphology, functional groups and the surface plasmon resonance of Ag BNCs obtained
3. investigate the antibacterial behaviour for different Ag NPs size against Gram positive and Gram negative bacteria by Mueller-Hinton Agar (MHA) test.

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