UNIVERSITI PUTRA MALAYSIA

PREPARATION, CHARACTERIZATION AND ANTIMICROBIAL ANALYSES OF CHITOSAN AND STARCH MEDIATED COPPER NANOPARTICLES

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PREPARATION, CHARACTERIZATION AND ANTIMICROBIAL ANALYSES OF CHITOSAN AND STARCH MEDIATED COPPER NANOPARTICLES

By

MUHAMMAD SANI USMAN

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

November 2013

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DEDICATION

To my parents, Dr. S.D Usman and Dr. (Mrs) Yalwa Usman.

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

PREPARATION, CHARACTERIZATION AND ANTIMICROBIAL ANALYSIS OF CHITOSAN AND STARCH MEDIATED COPPER NANOPARTICLES

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MUHAMMAD SANI USMAN

November 2013

Chairperson: Nor Azowa Ibrahim, PhD

Faculty: Science

In this study, chemical synthesis and characterization of Cu-NPs was carried out in the presence of two different stabilizers i.e. chitosan and starch polymer. The aim of this research is to individually synthesize, characterize, and test the antimicrobial activity of starch stabilized and chitosan stabilized Cu-NPs.

The synthesis was carried out using aqueous medium in the presence of hydrazine as reducing agent, ascorbic acid as anti-oxidant, and sodium hydroxide as pH moderator. In determining the optimum conditions for the nanomaterials synthesis, temperature, stabilizer concentration, and reductant concentration were varied accordingly, i.e. 0.05, 0.1, 0.2 and 0.5 weight (*wt*)% by volume for stabilizers, 0.5, 1.0, 1.5 and 2.0 mL for hydrazine, range of 40, 80, 100 and 120°C for temperature. All the Cts/Cu-NPs and Stc/Cu-NPs synthesized were characterized using TEM, FESEM, FT-IR, UV-Vis, XRD and EDX. Following synthesis of the nanoparticles, antimicrobial analysis of the synthesized particles was investigated using different stabilizer concentrations.

The UV−Vis spectroscopy results obtained for all samples showed significant surface plasmon resonance (SPR) peaks in the range of 500−600 nm, which indicated Cu-NPs formation. The molecular surface bonding of the nanoparticles and stabilizing media showed good interaction, which showed new bands at lower wave numbers (410, 600, 610 and 425), as revealed by the FT−IR spectra of all the samples. This indicates the capping of the nanoparticles by the polymers. The XRD diffractograms of samples obtained indicated high purity of the nanoparticles synthesized. No obvious peaks of CuO or Cu_2O were observed. TEM analysis indicated size and size distribution of the nanoparticles differed based on the various parameters studied. FESEM micrographs of the synthesized Cu-NPs showed morphology of particles embedded within the polymer matrix. Spherical shaped nanoparticles were dominant while few hexagonal and rod-shaped were observed;

depending on the conditions. Agglomeration was also noticed in samples with low stabilizer concentration. EDX spectra showed pure copper signal and carbon signal. Antimicrobial analysis conducted on the synthesized nanoparticles particles showed activity against the entire tested microorganism. Comparison between Cts/Cu-NPs and Stc/Cu-NPs showed starch as favourite among the two.

With the optimization of the reaction parameters, the optimum concentrations of the reductant (0.5 mL), stabilizers (0.2 *wt*% for starch and 0.5 *wt*% for chitosan) and temperatures of 100°C for starch and 120°C for chitosan. Based on the results obtained from this experiment, the nanoparticles synthesized showed small sized nanomaterials with superb antimicrobial activity. The nanoparticles concept can serve as positive step in the fight against pathogenic microorganisms.

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

PENYEDIAAN, PENCIRIAN DAN ANALISIS ANTIMIKROB DISTAKILIKAN KITOSAN DAN KANJI NANOPARTIKEL KUPRUM YANG

Oleh

MUHAMMAD SANI USMAN

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Dalam kajian ini, sintesis kimia and pencirian ke atas Cu-NPs telah dijalankan dengan kehadiran dua jenis penstabil berbeza iaitu kitosan dan polimer kanji. Matlamat kajian ini adalah untuk sintesis secara individu, melakukan pencirian, dan menguji aktiviti antimikrob ke atas kanji dan chitosan Cu-NPs yang telah distabilkan. Sintesis telah dijalankan dengan menggunakan akueus media dalam kehadiran hidrazin sebagai ejen pengurang, asid askorbik sebagai anti-oksida, dan natrium hidroksida sebagai moderator pH. Dalam penentuan kondisi optimum untuk sintesis nanomaterial, suhu, kepekatan penstabil, dan kepekatan pengurang telah dimanipulasikan sewajarnya, contoh 0.05, 0.1, 0.2, dan 0.5 berat (wt)% dengan isipadu untuk penstabil, 0.5, 1.0, 1.5, dan 2.0ml untuk hidrazin, dalam julat 40, 80, 100 dan 120°C untuk suhu. Semua Cts/Cu-NPs dan Stc/Cu-NPs yang telah disintesis, dicirikan menggunakan TEM, FESEM, FT-IR, UV-Vis, XRD and EDX. Berikutnya, sintesis nanopartikel, analisis antimikrob telah dijalankan menggunakan kepekatan penstabil yang berbeza.

Keputusan UV-Vis yang diperolehi untuk semua sampel menunjukkan puncak plasmon permukaan resonans (SPR) ketara dalam julat 500-600nm, iaitu menunjukkan penghasilan Cu-NPs. Ikatan permukaan molekul nanoprtikel dan penstabilan media menunjukkan interaksi yang baik, di mana mempamerkan pita baru pada nombor gelombang yang rendah (410, 600, 610 dan 425), bagaimana didedahkan oleh spektra FT-IR dalam semua sampel. Ini menunjukkan nanopartikel diselaputi oleh polimer. Belauan XRD bagi sampel nanopartikel yang disintesis didapati menunjukkan ketulenan yang tinggi. Tiada puncak yang ketara bagi CuO atau Cu_2O. Analisis TEM menunjukkan saiz and taburan saiz nanopartikel berbeza berdasarkan pelbagai parameter yang dikaji. Mikrograf FESEM bagi Cu-NPs yang disintesis menunjukkan morfologi partikel tertanam di antara matrik polimer. Nanopartikel yang berbentuk sfera adalah dominen, ada segelintir yang berbentuk heksagon dan rod, bergantung kepada keadaan. Penumpuan juga didapati di dalam sampel dengan kepekatan penstabil yang rendah. Spektra EDX menunjukkan isyarat bagi tembaga asli dan karbon. Analisis antimikrob dijalankan ke atas nanopartikel

yang telah disintesis mempamerkan aktiviti menentang segala mikroorganisma yang diuji. Perbandingan di antara Cts/Cu-NPs dan Stc/Cu-NPs menunjukkan kanji sebagai kegemaran antara dua tersebut.

Dengan pengoptimuman parameter tindak balas, kepekatan optimum bagi pengurang (0.5mL), penstabil (0.2 *wt*% untuk kanji dan 0.5 *wt* % untuk kitosan) dan suhu 100°C untuk kanji dan 120°C untuk kitosan. Berdasarkan keputusan yang didapati di dalam ujikaji ini, nanopartikel yang disintesis menunjukkan saiz nanomaterial yang kecil dengan aktiviti antimikrob yang hebat. Konsep nanopartikel boleh berkhidmat sebagai langkap positif dalam memerangi mikroorganisma patogenik.

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I certify that a Thesis Examination Committee has met on 29 November 2013 to conduct the final examination of Muhammad Sani Usman on his thesis entitled "Preparation, Characterization and Antimicrobial Analyses of Chitosan and Starch Mediated Copper Nanoparticles" 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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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Cts	Chitosan
Stc	Starch
NPs	Nanoparticles
Cu-NPs	Copper nanoparticles
PEG	Poly Ethylene Glycol
CTAB	Cetyltrimethyl ammonium bromide
di-HCF_4 Bis	(2,2,3,3,4,4,5,5-octafluoro-1-pentyl)-2-sulfosuccinate
AOT	Sodium bis(2-ethylhexyl) sulfosuccinate
DIZ	Diameter of inhibition zone
SPR	Surface Plasmon Resonance
S.d	Standard deviation

CHAPTER 1

INTRODUCTION

1.1 Background of Nanotechnology

Metal nanoparticles have become attractive throughout recent decades due to their size and shape-dependent physical and chemical properties, which are influenced by their large surface areas. Even though there are different definitions of nanoparticles, they are commonly defined as materials with at least one of their dimensions in the range of 1-100 nm. Nanomaterials vary from their bulk materials due to their unique atomic properties and quantum effect that change with sizes and morphologies. Despite the fact that the concept was introduced as far back as 1959 by an American physicist known as Richard Feynman; the idea remains an open research area (Edwards, 2006).

Nanotechnology is an area that offers insights on materials at nano-scale. It deals with technology at atomic, molecular and macromolecular level that is in nano range. It also provides the fundamental knowledge of nanomaterials, their effect in relation to their sizes and applications. The sizes of nanomaterials are particularly of interest in nanotechnology research, as the sizes influence most of their unique properties.
Properties of metals vary from one metal to another. For instance, copper nanoparticles differ in properties such as catalytic, electrical, and biological, from silver or gold nanoparticles. The difference may be as a result of their different surface area and surface area to volume ratio, which play major vital role in influencing their applications in various fields. Copper nanoparticles specifically are interesting due to their superb properties and availability. Figure 1.1 shows schematic Illustration of the typical copper nanoparticles in the presence of capping agents.

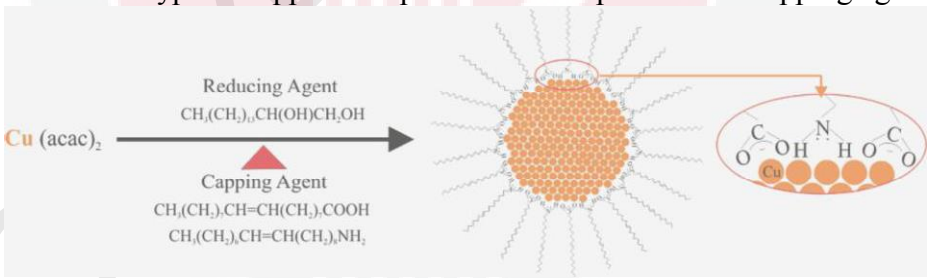

Figure 1.1: Schematic illustration of the synthesis of copper nanoparticles in the presence of capping agents in organic solvent (Mott *et al.* 2007)

Such abilities will also lead to an increased use of copper in many other areas of nanotechnology that are currently dominated by the use of gold, silver, and platinum nanoparticles. Copper, one of the cheapest metals around has attracted a lot of research attention through its nanoparticle synthesis because of similarity in properties with gold and silver, which are much more expensive. Copper is in fact considered as an alternative to silver, gold and platinum in areas like thermal conducting and microelectronic. More so, it has been reported in literature of anti-microbial susceptibility tests, that copper nanoparticles were observed to show higher anti-bacterial properties than its silver counterpart. This makes copper nanoparticle synthesis one of the most researched areas of nanotechnology and nano-science. The synthesis of copper nanoparticles of different compositions with preferred sizes and

shapes is vital in exploring their applications in nanotechnology. Areas like catalysis, sensors, microelectronics, are largely dependent on the nanoparticle sizes and shapes. The synthesis of copper nanoparticles with controllable sizes, shapes, and surface properties is important to exploring copper-based properties such as catalysis (Hoover *et al*. 2006) and biological applications (Cioffi *et al*. 2005, Kumar *et al*. 2000 and Wei *et al*. 2010).

This research focuses on effect of stabilizing medium on the antimicrobial properties of the synthesized copper nanoparticles. In doing so, optimum synthesis conditions were obtained by varying certain parameters such as temperature and reducing agent. A brief comparison between the two different stabilizers used in this effort was made on the antibacterial activity and sizes of the nanoparticles.

1.2 Problem statement/Significance of study

The rate at which microorganisms resist antibiotics is alarming. Pathogenic bacteria are becoming more and more resistant to antibiotics, which are continuously being produced to combat the microorganism. Presently all existing antibiotics have been resisted by different pathogenic organism, which makes the fight a challenging one (Raffi *et al*. 2010).

Nanotechnology is a promising research area with vast potentials in nanomedicine. Nanoparticles properties, including antimicrobial and small size, make them applicable in different areas of biology and medicine. They display an extensive range of properties, including biological properties which differ from their bulk materials. Thus, synthesis of copper nanoparticles especially in bio-based stabilizers can serve as antimicrobial agent, which in turn could fight pathogenic microorganisms.

1.3 Objectives

The synthesis of copper nanoparticles is advantageous due to its potential for use in different industries. Its similarity in properties with noble expensive metals (gold, silver and platinum), makes is more or less an alternative to these metals because it is cheaply and readily available. Though there are various methods of Cu-NPs synthesis. The chemical approach is commonly used by researchers, in view of the fact that it has allowed size and shape control, which can be done through the manipulation of reaction parameters, such as reducing agent, temperature, stabilizers and metal salt concentration. The objectives of this work are as follows:

I. To synthesize chitosan stabilized Cu-NPs and starch stabilized Cu-NPs

II. To characterize the synthesized Cu-NPs using UV-Vis, TEM, XRD, FESEM, EDX and FT–IR

III. To optimise the parameters used in the synthesis

IV. To evaluate the antimicrobial activity of the synthesized nanoparticles

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