UNIVERSITI PUTRA MALAYSIA

SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES MEDIATED IN GLUTATHIONE AND SODIUM ALGINATE

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By

SEPIDEH KESHAN BALAVANDY

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

June 2014

DEDICATION

To my beloved family

Thank for their support, understanding, love and encouragement.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES MEDIATED IN GLUTATHIONE AND SODIUM ALGINATE

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June 2014

Chairman: Associated Professor Zurina Zainal Abidin - PhD
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Synthesis of silver nanoparticles (Ag NPs) has attracted huge interest from scientists due to its wide applications. Different pathways, such as physical and chemical reduction has been employed regarding the synthesis of Ag NPs. Many of them contain highly reactive chemicals and can cause potential environmental and biological risks. In comparison to those methods, green synthesis of nanoparticles using biopolymer provides a safe way of nanoparticle production with the size and shape of our interest. Nanoparticles formation depends on various factors such as metal ion concentration, pH, time and temperature as well as nature of reducing and stabilizing agent. There is a lack of a comprehensive study in optimizing important parameters for the green synthesis of Ag NPs in the biopolymer substrates. In this study, silver nanoparticles with the small size of 1 to 30 nm were successfully synthesized by green method in the substrate of sodium alginate (Na-Alg) and glutathione (GSH). Specific percentage of $AgNO_3$ and biopolymeric substance were mixed together and stirred for different time at various temperature. Moreover, rapid synthesis of Ag NPs was achieved using an environmental friendly and biodegradable solvent that act as, stabilizing and reducing agent without use of high pressure or temperature, with the help of the accelerator. The silver nitrate, sodium alginate/glutathione, and sodium hydroxide were used as the silver precursor, stabilizer/ reducing agent and accelerator respectively.

The crystalline structure of Ag NPs for all of the samples, the average size and size distributions, surface Plasmon resonance (SPR), surface morphology, and functional groups were studied using X-ray diffraction (XRD), transmission electron microscopy (TEM), UV-visible spectroscopy (UV-vis), scanning electron microscopy (SEM) and Fourier transform infrared (FT-IR) respectively. The XRD analysis confirmed that the crystallographic planes of the silver crystals were the face-centered cubic (fcc) types. The UV-visible absorption spectra showed the peaks characteristic of the surface plasmon resonance (SPR) bands of Ag NPs. The antibacterial activities of Ag NPs were investigated against Gram-negative and Gram-positive bacteria by the disk diffusion method using Mueller-Hinton Agar (MHA) that show highly antibacterial activity of Ag NPs.

The properties of Ag/Na-Alg and Ag/GSH were studied as the function of time, temperature, concentration and pH until a relatively stable size and size distribution were achieved. The results from the UV-visible spectroscopy and TEM demonstrated that the initial concentration of 0.1 M for $AgNO_3$,1.5 wt% for Na-Alg and GSH, in 90°C after 12h stirring time of reaction are the optimum for the synthesis of Ag Nps incorporate in Na-Alg and GSH. In addition, 5 ml is the optimum amount of sodium hydroxide (NaOH) for synthesis of Ag Nps in short time (30 minutes) of the reaction. From the results, all objective was achieved.

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

SINTESIS DAN PENCIRIAN NANOPARTICLE PERAK MENGGUNAKAN GLUTATHIONE DAN SODIUM ALGINATE

Oleh

SEPIDEH KESHAN BALAVANDY

Jun 2014

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Sintesis nanopartikel perak (Ag NPs) telah menarik minat sebahagian besar saintis disebabkan kegunaannya yang meluas. Pelbagai kaedah berbesa seperti secara pengurangan fizikal dan kimia telah digunakan dalam sintesis Ag NPs. Kebanyakan kaedah ini mengunakan bahan kimia yang sangat reaktif dan boleh menyebabkan risiko pencemaran alam sekitar dan biologikal. Berbanding dengan kaedah-kaedah ini, sintesis "hijau" atau mesra alam menyediakan cara yang selamat untuk menghasilkan nanopartikel dengan menggunakan biopolymer mengikut saiz dan bentuk yang dikehendaki. Pembentukan nanopartikel bergantung kepada pelbagai faktor seperti kepekatan ion logam, pH, masa, suhu dan juga sifat ejen pengurangan dan penstabilan. Daripada kajian, terdapat kekurangan kajian yang komprehensif mengenai keadaan optima bagi penghasilan Ag Nps secara "hijau" dengan menggunakan biopolimer.

Dalam kajian ini, nanopartikel perak bersaiz kecil (1 to 30 nm) telah berjaya dihasilkan melalui kaedah "hijau"dalam subtract natrium alginate (Na-Alg) dan glutasion (GSH). $AgNO_3$ dan bahan biopolymer dicampur bersama pada peratusan tertentu dan dikacau pada masa dan suhu yang tertentu. Tambahan pula, sintesis Ag NPS telah dicapai melalui pengunaan pelarut yang mesra alam yang bertindak sebagai ejen penstabil dan pengurang tanpa mengunakan suhu atau tekanan yang tinggi dengan bantuan ejen peluncur. Argentum Nitrat, natrium alginat / glutasion dan natrium hidroksida adalah merupakan ejen-ejen "precursor" perak, penstabil/pengurang dan peluncur mengikut urutan yang telah digunakan.

Struktur kristal Ag NPS untuk semua sampel, pengagihan saiz dan saiz purata, permukaan "Plasmon" resonan (SPR), morfologi permukaan dan kumpulan berfungsi telah dikaji dengan teknik pembelauan sinar-X (XRD), mikroskopi electron penghantaran (TEM), UV – spektroskopi (UV -vis), mikroskopi pengimbasan elektron (SEM) dan Fourier inframerah (FT- IR) masing-masing . Analisis XRD mengesahkan bahawa permukaan kristalografi Kristal perak itu adalah jenis berpusat-muka padu (fcc) . UV-spektrum penyerapan menunjukkan persamaan dengan cirri puncak resonan plasma permukaan untuk Ag NPs. Aktiviti antibakteria Ag NPS menunjukkan kadar aktivit yang tinggi setelah dikaji terhadap bakteria Gram –negatif dan Gram- positif melalui kaedah penyebaran "disk" dan Mueller-

Hinton Agar (MHA). Sifat-sifat Ag/ Na- Alg dan Ag/GSH telah dikaji untuk fungsi masa, suhu, kepekatan dan pH sehingga saiz dan taburan saiz yang agak stabil dicapai. Keputusan daripada spektroskopi UV- vis dan TEM menunjukkanbahawa kepekatan awal 0.1 M untuk $AgNO3$, 1.5 % berat untuk Na- ALG dan GSH, dalam 90°C selepas dikacau 12 jam adalah keadaan yang optima untuk sintesis Ag NPs dalam menggabungkan Na- Alg dan GSH. Di samping itu, 5 ml adalah jumlah natrium hidroksida(NaOH) yang optima untuk sintesis Ag Nps dalam masa tindakbalas yang singkat (30 minit).

ACKNOWLEDGEMENTS

At first I want to thank Allah for all of the things that he has given in my life and then I offer my sincerest gratitude to my chairman, Associate Professor Dr. Zurina Zainal Abidin, who has supported me throughout my thesis from the initial to the final level with her patience and knowledge. I also would like to acknowledge Dr. Dayang Radiah binti Awang Biak and Dr.Mohd Nizar b Hamidon my co-supervisors for their invaluable support and advice. Their encouraging, detailed and constructive comments have enabled me to develop an understanding of the subject. My appreciation goes especially to Dr. Kamyar Shameli, for his guidance and advice throughout the project.

Sincere thanks to Esra Ahmadi for her endless support; we have been studying and working together since September 1998 and she is my best And also Dr. Shima Shayesteh for being my friend, colleagues, and advisor.

I owe great thanks to my precious parents that leave me soon before my appreciation for all things that they gave me or taught me. And also thanks to my dearest sister Saiedeh and my darling brother Saman, for love and support they gave me always. I owe great thanks to my family in-law specially my father in-law Valiollah from distances far away; this dissertation would not have been possible without their love and encouragement during this tedious journey.

Finally, my greatest appreciation will always go to my loving family for their sacrifices, love, patience, and supports. My dear’s husband Bahador Dastorian and my sweetheart daughter Diana for unending support and all their helping in during my study without them I would never able to finish my master.

At long last, I would like to dedicate this thesis to my daughter Diana.

Diana, you will always be the source of my inspiration and a part of me.

I certify that a Thesis Examination Committee has met on 26 June 2014 to conduct the final examination of Sepideh Keshan Balavandy on her thesis entitled "Synthesis and Characterization of Silver Nanoparticles Mediated in Glutathione and Sodium Alginate" 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 fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

Declaration by the student

I hereby confirm that:

this thesis is my original work

quotations, illustrations and citations have been duly referenced

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LIST OF ABBREVIATIONS

Ag NPs	Silver nanoparticles
Na-Alg	Sodium alginate
GSH	Glutathione
Ag/Na-Alg	Silver/Sodium alginate
Ag/GSH	Silver/Glutathione
fcc	face-centred cubic
SPR	Surface Plasmon Resonance
MHA	Mueller-Hinton Agar
S. aureus	*Staphylococcus aureus*
MRSA	*methicillin-resistant Staphylococcus aureus*
E. coli	*Escherichia coli*
TEM	Transmission electron microscopy
UV-vis	UV-visible spectroscopy
SEM	Scanning electron microscopy
FT-IR	Fourier transform infrared
PXRD	powder X-ray diffraction
XRD	X-ray diffraction
EDXRF	energy dispersive X-ray fluorescence spectrometer
SPR	Surface pelasmon resonance

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Nanotechnology is a rapidly growing field with its aim in science and technology for expanding new materials at the nano-scale level (Albrecht et al., 2006). The term nanotechnology was created by Professor Norio Taniguchi of Tokyo Science University in the year 1974 to indicate accuracy manufacturing of materials at the nanometer level (Rai et al., 2009) Nanotechnology and Nanoscience relate to the creation, interrogation, characterization, utilization and exploitation of nanostructured materials, which, at least are characterized by one dimension in the nanometer (1 nm = 10^{-9} m) range. Therefore, nanotechnology has been established as a new multidisciplinary science (Shabatina and Sergeev, 2007). The nanomaterials envelop different categories of nanostructured materials, including nanocrystals, nanowires, nanotubes, clusters, and quantum dots, while collections of nanostructures involve arrays, assemblies, and superlatives of the individual nanostructures (Rao and Cheetham, 2001).

The properties of materials with nanometer sizes are meaningfully altered from those of atoms and bulk materials. Due to their small dimensions, nanomaterials have an extremely large surface area to volume ratio, which results in more desirable properties of surface dependent material. Table 1.1 lists some of the typical dimensions of nanostructures (Jortner and Rao, 2002). In nanotechnology, a particle is described as a small object that acts as a full unit in the relations of its properties and transport. It is more classified according to size: in terms of diameter, fine particles cover a range between 100 and 2500 nm, while nanoparticles (NPs) and ultrafine particles, are between 1 and 100 nm. The nanocrystals may exhibit size-related properties that differ significantly from those detected in fine particles or bulk materials (Buzea et al., 2007).

Table1.1:Typical nanostructure categories (Jortner and Rao, 2002).

Structure	Size Diameter (nm)	Materials
Nanocrystals and clusters (quantum dots)	Radius: 1-10 nm	Insulators, Metals, Semiconductors, Magnetic materials
Other Nanoparticles	Radius: 1-100 nm	Ceramic oxides
Nanowires	Radius: 1-100 nm	Metals, Semiconductors, oxide, sulphides, nitrides
Nanotubes	Radius: 1-100 nm	Carbon, Layered, Chalcogenides
Nanoporous solids (pore)	0.5-30 nm	Zeolite, phosphates (etc.)
2-Dimensional arrays of Nanoparticles	Area: Several nm2- µm2	Metals, semiconductors, magnetic materials
Surfaces and thin films	thickness 1-1000 nm	Insulators, metal, DNA
3-Dimensional structures	several nm	Metals, semiconductors, magnetic materials

In addition, metallic NPs demonstrate unusual properties that are as a result of an arrangement of high energy surface of atoms compared to bulk solid or isolated atoms (Murphy, 2008). A challenge in nanotechnology is to modify the antibacterial, electrical, and optical behaviors of nanoparticles by monitoring their size and shape. Monodisperse metal NPs are of course ideal, but unusual properties are to be expected, even if the ideality is not perfectly realized (Panigrahi et al., 2004).

Benefits of compatibility and eco-friendliness for pharmaceutical and other biomedical applications as they do not usage toxic chemicals for the synthesis procedure, grab significant attention.

Silver has long been recognized as having an inhibitory effect on microbes and bacteria present in industrial and medical process (Rai et al., 2009). The most significant application of silver nanoparticles and silver is in the medical industry such as topical ointments to prevent infection against burn and open wounds (Singh et al., 2010).
Herein, this research reports for the first time synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by sodium alginate and glutathione. Further, these biologically synthesized nanoparticles were found highly toxic against different pathogenic bacteria.

1.2 Research Problems

Silver nanoparticles often synthesized using chemical and physical methods that involves toxic chemical reagents, hazardous procedures or toxic and potentially harmful by-products involved in most of these methods thus; it has been difficult to employ these methods on large scale production (Panigrahi et al., 2004). There is a strong requirement for green, economic, commercially possible as well as environmental friendly procedure for synthesis of Ag NPs. In order to approach green synthetic techniques, several methods have been developed for the synthesis of desired Ag NPs. In spite of uncountable research, comprehensive investigations in green chemistry frame work are much more demanded. So far there has been no thoroughly investigation to optimize the most important factor (time, temperature, concentration and PH) on the green synthesis of Ag NPs by the ecofriendly and organic polymer substrates that exhibit highly antibacterial properties.

1.2 Research Approach

Green synthesis offers improvement over physical and chemical method as it is environment friendly, cost effective, easily scaled up for large scale production and in this method there is no need to use high temperature, high pressure, toxic chemicals and energy (Panigrahi et al., 2004). In these researches, using the green method Ag NPs was effectively prepared in aqueous solution of Glutathione (GSH) and Sodium Alginate (Na-Alg). The possibilities for manipulating the geometry of silver nanoparticles by altering the key growth parameters such as pH, temperature, concentrations and time have been explored. The Ag ions were reduced to the Ag NPs into the space of GSH and Na-Alg by using thermal condition. Furthermore, different time, temperature, concentrations of either silver nitrate or polymers completely investigated as well as pH effect.

Finally, Ag NPs synthesized into the biodegradable and organic mediator via green method in H_2O solvent. The crystalline structure of NPs average size, size distributions, surface morphology, surface Plasmon resonance and functional groups were characterized using powder X-ray diffraction (PXRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), UV-visible spectroscopy and Fourier transform infrared (FT-IR) respectivly.

The antibacterial activities for Ag NPs in organic substrates were investigated against Gram positive and Gram negative bacterium at different size and amount of Ag NPs.

1.3 Objectives

The main objectives of this research are:

1. To optimize effects of time, temperature, concentration and PH on the synthesis of Ag NPs by two different biopolymers, Glutathione and Sodium Alginate via green frame work of chemical reduction method.

2. To characterize the crystalline structure, average size and size distribution, surface morphology, functional groups and the surface plasmon resonance of nano particles in the optimum point

3. To investigate the antibacterial behavior of synthesized Ag NPs against Gram negative and Gram positive bacteria by Mueller-Hinton Agar (MHA) test.

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