**UNIVERSITI PUTRA MALAYSIA**

# *SYNTHESIS AND CHARACTERIZATION OF SILVER /KAPPA-CARRAGEENAN NANOPARTICLES USING GREEN METHODS AND EVALUATION OF THEIR ANTIBACTERIAL ACTIVITIES*

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By

**RANDA FAWZI ELSUPIKHE**

**Thesis Submitted to the School of Graduates Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy**

**March 2017**

# DEDICATION

I dedicate this work to Allah for his tender unlimited and to my precious parents, my dear mum **Fatma Elgandoz** and my darling dad **Fawzi Elsupikhe**, which without their giving and their education, I could not make any success. I dedicate it with special thanks to my dear's Husband **Taha Husin** for his encouragements, understandings and helping during my study, which without him I would never able to finish my PhD and to my sweetheart daughters **Touka and Jana** for giving me the hope. I owe my loving thanks to my dear sister **Hanin**.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in Fulfillment of the requirement for the Degree of Doctor of Philosophy

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Nanoscale materials have received extensive attention because their unusual properties that differ significantly from bulk sample of the same material. Nanoparticles are particles with size less than 100 nm which small in diameter, but larger in surface area. Silver nanoparticles (Ag-NPs) are being increasingly used in consumer products such as water purification, household cleaning agents and huge in current many exclusive medical applications such as biological engineering. Synthesis of Ag-NPs has attracted the scientists' attention in recent years due to the huge advantages and applications of Ag-NPs especially as antimicrobial agent. Chemical methods have been used for the synthesis of Ag-NPs, but these methods have a lot of disadvantages because most of the chemical that have been used for synthesis the nanoparticles are too expensive and toxic, which are responsible for various biological risks. Also, most of the chemical methods for synthesis Ag-NPs are not able to control the size of the NPs. Furthermore, the agglomeration between the nanoparticles lead to bad results in antibacterial application. In this work, the green methods for synthesis Ag-NPs have been used for solving these problems and κ-carrageenan polymer has been used as a stabilizer to prevent this agglomeration. Ag-NPs in κ-carrageenan synthesized by different green methods (stirring method, UV- irradiation ultrasonic-irradiation) at room temperature were developed to prepare and control the size of Ag-NPs. Parameters such as the time of stirring, time of irradiation, ultrasonic amplitude, concentration of $AgNO_3$ and concentration of κ-carrageenan have been optimized. κ-carrageenan was used as an eco-friendly stabilizer and $AgNO_3$ as producer. Formation of Ag/κ-carrageenan was determined by the UV–visible spectra, which improved the formation of Ag-NPs by surface plasmon resonance in range 300-450 nm. The FT-IR spectra indicated the presence of κ-carrageenan in capping with Ag-NPs. The XRD analysis showed that the Ag-NPs were of face-centred cubic structure. TEM images illustrated the well dispersed of Ag-NPs with similar particle size. SEM images displayed the change on the surface morphology of the κ-carrageenan and illustrated the shape of the Ag-NPs.

EDXRF spectra of Ag-NPs in κ-carrageenan confirmed the presence of elemental compounds without any impurity peak. The antibacterial properties of the synthesized nanoparticles were evaluated using agar diffusion methods. Four species of bacteria were used in this study, including two Gram-positive and two Gram-negative bacteria: Methicillin Resistant *Staphylococcus aureus* (MRSA), *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* (*E-coli*). Optimized parameters in the stirring method for synthesis Ag-NPs were: 48 h of stirring times, 0.2 M of $AgNO_3$ and 0.3% κ-carrageenan, which produced, the size and the concentration of Ag-NPs of 32 nm and 0.065 M, respectively. The good condition of UV-irradiation method for synthesis Ag-NPs were 60 min irradiation time, 0.2 M $AgNO_3$ and 0.3% κ-carrageenan, which produced, the size of and the concentration Ag-NPs of 14 nm and 0.12 M, respectively. The conditions of the ultrasonic-irradiation method for synthesis Ag-NPs that give the best results were 90 min irradiation time, 0.15 M $AgNO_3$, 0.3 % κ-carrageenan and 60 amplitude, which produced the size and the concentration of Ag-NPs of 1.21 nm and 0.22 M, respectively. All Ag-NPs from the above methods were in spherical shape. The different methods demonstrated different results on anti-bacterial activity, which depended on the size and concentration of Ag-NPs. The stability test by using zeta potential analysis proved the Ag-NPs that synthesized by stirring method, UV-irradiation and ultrasonic irradiation were stable. The comparison between the three methods according to the size and concentration of Ag-NPs and the effect on the bacterial activity showed that, the ultrasonic irradiation synthesis was the best method for synthesis of Ag-NPs because the high yield and with a small size of Ag-NPs which lead to a high effect on the bacterial activity.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**SINTESIS DAN PENCIRIAN NANOPARTIKEL PERAK/KAPPA-KARAGEENAN MENGGUNAKAN KAEDAH HIJAU DAN PENILAIAN AKTIVITI ANTIBAKTERIA**

Oleh

**RANDA FAWZI ELSUPIKHE**

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Bahan nano telah mendapat perhatian meluas kerana sifat yang luar biasa mereka yang berbeza dengan ketara daripada sampel pukal daripada bahan yang sama. Nanopartikel adalah zarah dengan saiz kurang daripada 100 nm, iaitu diameter yang kecil tetapi kawasan permukaan yang lebih besar. Nanopartikel perak (Ag-NPs) semakin banyak digunakan dalam produk pengguna seperti ejen pembersihan air, ejen pembersihan rumah, dan besar dalam aplikasi perubatan eksklusif seperti kejuruteraan biologi. Sintesis $Ag\text{-}NP_S$ telah menarik perhatian para saintis dalam tahun-tahun kebelakangan ini disebabkan oleh kelebihan yang besar dan aplikasi Ag-NPs terutamanya sebagai ejen anti-mikrob. Kaedah kimia telah digunakan untuk sintesis $Ag\text{-}NP_S$, tetapi kaedah ini mempunyai banyak kelemahan kerana kebanyakan bahan kimia yang digunakan untuk sintesis nanopartikel terlalu mahal dan toksik, yang bertanggungjawab untuk pelbagai risiko biologi. Juga sebahagian besar daripada kaedah kimia untuk sintesis $Ag\text{-}NP_S$ tidak dapat mengawal saiz NPs. Tambahan pula, pengumpalan zarah nano membawa kepada keputusan yang buruk dalam kegunaan sebagai anti-bakteria. Dalam kajian ini, kaedah hijau untuk sintesis Ag-NPs telah digunakan bagi menyelesaikan masalah ini dan polimer κ-karageenan telah digunakan sebagai penstabil untuk mengelakkan pengumpalan. Ag-NPs dalam κ-karageenan disintesis oleh kaedah hijau yang berbeza (kaedah kacau, penyinaran-UV dan penyinaran-ultrasonik) pada suhu bilik dibangunkan untuk menyedia dan mengawal saiz nanopartikel Ag-NPs. Parameter seperti waktu kacau, masa penyinaran, amplitud ultrasonik, kepekatan $AgNO_3$ dan kepekatan κ-karageenan telah dioptimumkan. κ-karageenan digunakan sebagai penstabil mesra alam dan $AgNO_3$ sebagai pengeluar. Bentukan Ag/κ-karrageenan ditentukan oleh spektra UV-boleh dilihat yang dipertingkatkan pembentukan Ag-NPs oleh resonans plasmon permukaan dalam julat 300-450 nm. Spektra FT-IR menunjukkan kehadiran κ-karageenan dalam liputan dengan $Ag\text{-}NP_S$. Analisis XRD menunjukkan Ag-NPs adalah berstruktur kiub berpusatkan muka. Imej TEM menggambarkan juga Ag-NPs tersebar dengan saiz zarah yang sekata. Imej SEM memaparkan perubahan pada morfologi permukaan κ-karageenan dan menggambarkan bentuk Ag-NPs. Spektra EDXRF Ag-NPs dalam κ-karageenan mengesahkan kehadiran sebatian unsur tanpa puncak junub. Sifat antibakteria nanopartikel yang disintesis dinilai menggunakan kaedah penyebaran agar. Empat spesies bakteria telah digunakan dalam kajian ini, termasuk dua bakteria Gram-positif dan dua bakteria Gram-negatif: *Staphylococcus aureus tahan-Methicillin* (MRSA), *subtilis Bacillus*, *Pseudomonas aeruginosa* dan *Escherichia coli* (*E-coli*). Parameter optimum bagi kaedah kacau untuk sintesis Ag-NPs adalah: 48 j masa kacau, 0.2 M $AgNO_3$ dan 0.3% κ-carrageenan, yang menghasilkan saiz dan kepekatan Ag-NP$_S$ masing-masing 32 nm dan 0.065 M. Keadaan terbaik bagi kaedah penyinaran-UV untuk sintesis Ag-NP$_S$ adalah 60 min masa penyinaran, 0.2 M $AgNO_3$ dan 0.3% κ-carrageenan, yang menghasilkan saiz dan kepekatan Ag-NPs masing-masing 14 nm dan 0.12 M. Keadaan bagi kaedah penyinaran-ultrasonik untuk sintesis Ag-NP$_S$ yang memberikan hasil yang terbaik adalah 90 min masa penyinaran, 0.15 M $AgNO_3$, 0.3% κ-carrageenan dan 60 amplitud yang menhasilkan saiz dan kepekatan Ag-NPs masing-masing 1.21 nm dan 0.22 M. Semua Ag-NPs dari kaedah di atas adalah dalam bentuk sfera. Kaedah yang berbeza menunjukkan keputusan yang berbeza pada aktiviti anti-bakteria bergantung kepada saiz dan kepekatan Ag-NPs. Ujian kestabilan dengan menggunakan analisis potensi zeta membuktikan Ag-NPs yang disintesis oleh kaedah kacau, UV-sinaran dan penyinaran-ultrasonik adalah stabil. Perbandingan antara ketiga-tiga kaedah mengikut saiz dan kepekatan Ag-NPs dan kesan ke atas aktiviti bakteria menunjukkan, sintesis penyinaran-ultrasonik adalah kaedah terbaik untuk sintesis Ag-NPS kerana hasil yang tinggi dengan saiz Ag-NPs kecil yang membawa kepada kesan yang tinggi kepada aktiviti bakteria

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I certify that a Thesis Examination Committee has met on 21 March 2017 to conduct the final examination of Randa Fawzi El Supikhe on her thesis entitled "Synthesis and Characterization of Silver/Kappa-Carrageenan Nanoparticles using Green Methods and Evalution of 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 Doctor of Philosophy.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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| | |
|---|---|
| Ag -NPs | Silver nanoparticles |
| fcc | face-centred cubic |
| SPR | Surface Plasmon Resonance |
| MHA | Mueller-Hinton Agar |
| MRSA | methicillin-resistant *Staphylococcus aureus* |
| NPs | Nanoparticles |
| *E. coli* | *Escherichia coli* |
| PNP | Polymer nanoparticles |
| $AgNO_3$ | Silver nitrate |
| TEM | Transmission electron microscopy |
| UV-Vis | UV-Visible spectroscopy |
| SEM | Scanning electron microscopy |
| FT-IR | Fourier transform infrared |
| PXRD | Powder X-ray diffraction |
| EDXRF | Energy dispersive X-ray fluorescence spectrometer |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background study

Publications in the field of nanotechnology have grown dramatically in the last two decades. Nanotechnology is the process of engineering of functional systems at the nanoscale level (1-100 nm ) through structural modifications of their shapes and sizes (Silva, *et al.*, 2004). The conceptual underpinnings of nanotechnologies are first laid out in 1959 by a physicist Richard Feynman in his lecture entitled "There's plenty of room at the bottom". Feynman explored the possibility of manipulating material at the scale of individual atoms and molecules (Sahoo, *et al.*, 2007). Nanofabrication methods are divided into two major categories. "Top–down" and "Bottom–up" methods, according to the processes involved in creating the nanoscale structures. A top–down approach corresponds to using nanofabrication tools that are controlled by external experimental parameters to create nanoscaled structures/ functional devices with the desired shape and characteristics starting from larger dimensions and reducing them to the required values. On the other hand, bottom–up approaches seek to have molecular or atomic components built up into more complex nanoscale assemblies or self directed assemblies based on complex mechanisms and technologies (Biswas *et al*., 2012).

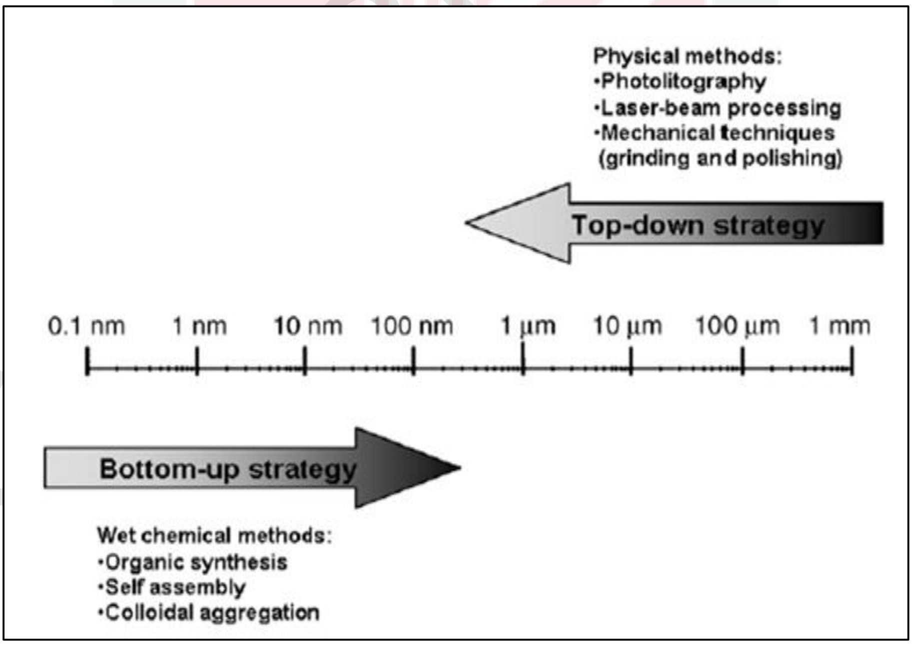


**Figure 1.1 : Top-down and Bottom-up Strategies** (Source: Nam and Lead, 2008)

The properties of materials with nanometer dimensions are completely different from those of atomic and bulky materials and this is mainly due to the nanometer size of the materials which cause to have unique properties, for example, a large fraction of surface atoms, high surface energy and reduced imperfections which do not exist in the corresponding bulk materials. Table 1.1 lists some typical dimensions of nanostructures (Rao and Cheetham, 2010).

A nanoparticle is the most fundamental component in the fabrication of a nanostructure, and is far smaller than the average everyday object, that is described by Newton's laws of motion, but are bigger than an atom or a simple molecule that are governed by quantum mechanics (Horikoshi *et al.*, 2013).

**Table 1.1 : Typical nanostructure categories** (Rao & Cheetham, 2010)**.**

| 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, nitrites |
| Nanotubes | Radius. 1-100 nm | Carbon layered chalcogenides |
| Nanoporous solids (pore) | Radius. 0.5-30 nm | Zeolites, phosphates etc. |
| 2-Dimensional arrays of Nanoparticles | Area. Several $nm^2$-$\mu m^2$ | Metals, semiconductors, magnetic materials |
| Surfaces and thin films | Thickness 1–1000 nm | Insulators, metal, DNA |
| 3-Dimensional structures | Several nm | Semiconductors, magnetic material |

Today, metal nanoparticles are important in a variety of scientific fields. Metal nanoparticles especially those containing gold (Au), silver (Ag), platinum (Pt) etc. have been of particular interest in recent years because of their unique and attractive optical and electronic properties., which are significantly different from those of bulk materials (Mohan *et al.*, 2014).

Metal nanoparticles are important due to their interesting and unusual properties such as. large optical fields, strong and well pronounced Raman scattering and light absorption effects (Jain *et al.,* 2008). Size, shap and surface morphology play vital roles in controlling the physical, chemical, optical, and electronic properties of the metal nanoparticles (Raveendran *et al.,* 2003). Metallic nanoparticles find excellent potential in biomedical sciences and engineering fields because of their huge potential in nanotechnology, hence opening a wide range of potential applications in biotechnology (Mohan *et al.,* 2010).

Silver nanoparticles (Ag NPs) have been used as antimicrobial agents, usually in the form of polymer nanocomposites, this bactericidal effect of Ag-NPs has resulted in their global application in various consumer products, e.g., deodorants, toys, humidifiers, filters and also the food and feed industry as instance, packaging materials and nursing bottles (Morones *et al.,* 2005). The broad range of targets within the bacteria makes metal nanoparticles a novel substitute for traditional antibacterial drugs, due to the significantly lower skin absorption and internal organ deposition and their relatively lower toxicity compared to silver sulfadiazine (Galdiero *et al.,* 2011).

The most common chemical approaches, including chemical reduction using a variety of inorganic and organic reducing agents, physicochemical reduction, and radiolysis are broadly used for the synthesis of silver nanoparticles. Recently, nanoparticle synthesis has been among the most interesting scientific areas of inquiry and there is growing attention to produce nanoparticles using environmentally friendly methods (green chemistry). Green synthesis approaches include polysaccharides, biological, and irradiation method which has advantages over conventional methods involving chemical agents related to environmental toxicity (Korbekandi & Iravani, 2012).

The field of polymer nanoparticles (PNP) is quickly expanding and playing a pivotal role in a wide spectrum of areas ranging from electronics to photonics, conducting materials to sensors, medicine to biotechnology, pollution control to environmental technology, and so forth, during the past decade (Rao *et al.*, 2011). Alternative synthetic strategies based on using polymers as both the reducing and stabilizing agents for the generation of stable metal nanoparticles without the use of an additional stabilizing agent have been developed recently (Sardar *et al.*, 2007).

## 1.2 Problem statement

Nanotechnology is able to create new materials and devices with a huge range of applications, such as in electronics, medicine, biomaterials, and energy production. Furthermore, nanotechnology raises many of the same issues as any new technology, including concerns about the toxicity and the environmental impact of nanomaterials, Most of the chemical methods that have been used for the synthesis of nanoparticles are too expensive and involve the uses of toxic and hazardous chemicals which are responsible for various biological risks. Furthermore, the environment is undergoing great damage because a large amount of hazardous and unwanted chemical, gases or substances are released by man-made processes. Furthermore, most of chemical methods for the synthesis of silver nanoparticles cannot control the size and the distribution of nanoparticles. On the other hand, Ag- nanoparticles have regained importance due to increasing bacterial resistance to antibiotics. Pathogenic bacteria are becoming much resistant to antibiotics, which are produced on a continuous basis for combating infections caused by microorganisms. At present, antibiotics that are resisted by every single pathogenic organism, makes the fight much more challenging and is a problem that needs to be addressed (Raffi *et al.*, 2010).

## 1.3 Objectives

The specific objectives of this study are

1- To study the synthesis of Ag-NPs in κ-carrageenan by stirring time and characterize their physical, chemical and morphological properties.
2- To study the synthesis of Ag-NPs in κ-carrageenan by using physical methods as a reducing agent (UV- irradiation and ultrasonic irradiation) and characterize their physical, chemical and morphological properties.
3- To evaluate the antibacterial activity of Ag-NPs by using Mueller-Hinton Agar diffusion (MHA) test.

## 1.4 Research hypothesis

If the preparation of Ag-Nps has done by green methods, it will be safer to the environment. However, when the polymer has used as a stabilizer in the synthesis of Ag-NPs, the Ag-NPs will not agglomerate with each other. Furthe more, if the time of reactions, concentrations of the stabilizer and the concentrations of metal producer have optimized, the size of Ag-NPs will be controlled. Also, when the size of Ag-NPs decreased, it will give good results as an antibiotic.

## 1.5 Research questions

1- Are the green methods can synthesize Ag-NPs?
2- Is the polymer (κ-carrageenan) able to coat the Ag-NPs?
3- Are optimized the experimental parameters (time of reaction, concentrations of the κ-carrageenan and the $AgNO_3$) can control the size of Ag-NPs?
4- Does the size of Ag-NPs influence their antibacterial properties?
5- Are the Ag-NPs that synthesize using green methods stable?

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