THIN FILMS HYDROXYETHYL CELLULOSE/ SILVER NANOPARTICLES (HEC/ AGNP) FOR ANTIBACTERIAL STUDIES

NURUL ATIKAH BINTI ABDUL HALIM

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Faculty of Industrial Sciences and Technology
UNIVERSITI MALAYSIA PAHANG

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ABSTRACT

Thin films of hydroxyethyl cellulose (HEC) containing silver nanoparticles were prepared and characterized. Silver nitrate ($AgNO_3$) aqueous solution was reduced by HEC by heating and this solution was used to form the thin film. HEC/AgNP solution and the thin films were characterized using SEM and UV-Vis spectroscopy. The silver nanoparticles showed absorbance peak at 439 nm. Different solutions of HEC/AgNP were prepared with different concentration of silver nitrate. The thin films were cross-linked by heating at different temperatures and the effect of heating the thin films on the antibacterial property was studied. The thin films were used to study the antimicrobial effect on E. coli and S. aureus. The thin films of HEC/AgNP showed strong antimicrobial activity. HEC being a biocompatible and water soluble polymer is a very good candidate for wound dressing material.

ABSTRAK

Filem nipis hydroxylethyl selulosa (HEC) yang mengandungi argentum nanopartikel telah disediakan dan dikaji ciri-cirinya. Larutan akueus argentum nitrat ($AgNO_3$) telah dikurangkan oleh HEC dengan kaedah pemanasan dan larutan ini digunakan untuk membentuk filem nipis. Larutan HEC/AgNP dan filem nipis ini dikaji ciri-cirinya dengan menggunakan SEM dan UV-vis spektroskopi. Argentum nanopartikel telah menunjukkan graf puncak pada 439 nm. Larutan yang berbeza HEC/AgNP telah disediakan dengan kepekatan argentum nitrat yang berbeza. Filem nipis dapat dihubung kaitkan dengan pemanasan pada suhu yang berbeza dan kesan pemanasan terhadap filem nipis ini ke atas antibakteria telah di kaji. Filem nipis ini digunakan untuk mengkaji kesan antibakteria terhadap E. coli dan S.aureus. Filem nipis HEC/AgNP menunjukkan aktiviti antibakteria yang kukuh. HEC digunakan sebagai polimer yang tidak toksik dan air adalah sebagai bahan larut yang sangat sesuai sebagai bahan pembalut luka.

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LIST OF ABBREVIATIONS

Ag^+	Silver ion
$AgNO_3$	Silver nitrate
AgNPs	Silver nanoparticle
E. coli	Escherichia coli
HEC	Hydroxylethyl cellulose
S. aureus	Staphylococcus aureus
SEM	Scanning Electron Microscope
TEM	Transmission Electron Microscope
UV-vis	Ultraviolet Visible Spectroscopy

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Infections by pathogenic microorganisms are the important issues in medical devices, drugs, hospital surfaces/furniture, dental restoration and health care products and hygienic applications, surgery equipment, textiles, water purification systems and others fields involve our lives. More people were killed by infectious diseases more than other cause. For example, Maya was reported in Grage News, she mentioned that "the death toll from the outbreak of E. Coli bacteria in Germany and several other European countries has increased to at least 35 people killed by the bacteria, as disclosed on Sunday, June 12, 2011 local time". These infections are due to bacteria, viruses, fungi and protozoa, which are found everywhere whether in soil, water and air. Other than that, these infections will come from eating, touching, drinking or breathing something those contain that bacteria, viruses, fungi and protozoa. Therefore, these infections would defeated by antimicrobial agents when there are susceptible to their action.

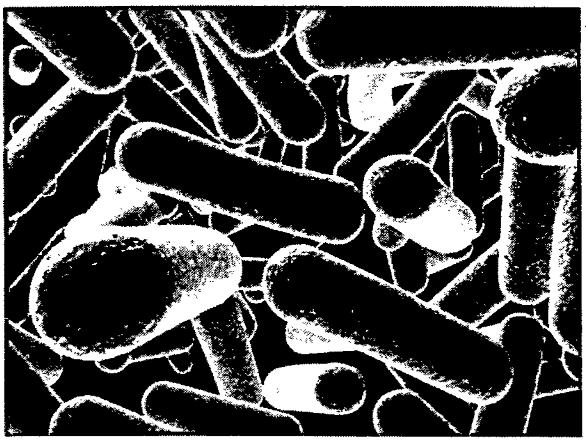


Figure 1.1: Bacteria

Source: ttp://blogs.discovermagazine.com/discoblog/files/2008/04/bacteria.jpg

Bacterial resistance to conventional antibiotics is threatening human health the world over. Medicinal chemists are trying to develop new compounds that can kill strains such as MRSA (methicillin, or multiple-resistant *Staphylococcus aureus*) and E. coli O157. Frontline defenses, such as environmentally benign and cost-effective antibacterial compounds could prevent such infective agents spreading through contact with phones, cars and other devices. However, the microorganism was difficult to eliminate due to their easily to mutate the genes. Staphylococcus aureus for example, is a bacterium that commonly colonizes human skin and mucosa without causing severe problems. However, serious illnesses that range from mild to life-threatening can be developed if the bacteria enter the body. These include skin and wound infections, infected eczema, abscesses infections, heart valves infections or endocarditis, pneumonia and blood stream infection or bacteraemia.

1.2 BACKGROUND RESEARCH

1.2.1 Nanoparticle

In nanotechnology, a small object that behaves as a whole unit in terms of its transport and properties is defined as particle. Particles are classified according their size which is in term of diameter, fine particles cover a range between 100 and

2500 nanometers. Nanoparticle has similar sized as ultrafine which is range between 1 and 100 nanometers. Nanoparticle is widely studied due to a variety of potential applications in biomedical, optical, and electronic fields. Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. A bulk material should have constant physical properties regardless of its size, but at the nano-scale this is often not the case. Size-dependent properties are observed such as quantum confinement in semiconductor particles, surface plasmon resonance in some metal particles and superparamagnetism in magnetic materials. The properties of materials change as their size approaches the nanoscale and as the percentage of atoms at the surface of a material becomes significant. Nanoparticles exhibit a number of special properties relative to bulk material.

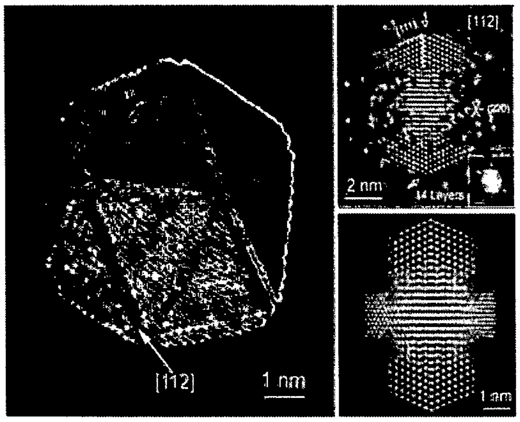


Figure 1.2: Nanostructure at 1 nanometer

Source: http://ncem.lbl.gov/images/OAM/nanoparticle.jpg

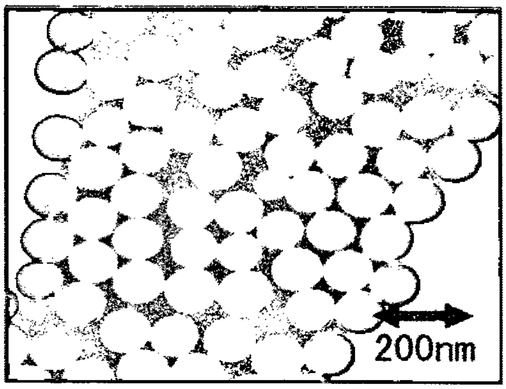


Figure 1.3: Nanostructure at 200 nanometer

Source: http://www.furukawa.co.jp/english/what/2007/images/nanosilica.jpg

1.2.2 Silver nanoparticle

Silver is already been recognized in ancient Greece and Rome for its infection-fighting properties and it has a long and intriguing history as an antibiotic in human health care. Then, silver is use in medicine. The antimicrobial effects of minute silver particles, which were then known as "colloidal silver," were known from the earliest days of its use. The nanoparticles were known as "colloidal silver" is meant as “nano” which is extremely small particles of silver. Silver particles with diameters of seven to nine nm were mentioned as early as 1889. They were used in medications or as biocides to prevent the growth of bacteria on surfaces, for example in antibacterial water filters or in algaecides for swimming pools.

The term nanoparticle is referring to particles that have dimensions are less than 100 nm. That is because of their minute size nanoparticles have different properties than those of larger particles of the same material. The bulk material is less reactive than volume of nanoparticle because this nanoparticle has much greater surface area, so that, makes it more reactive than bulk material. In addition, small quantities nanosilver produces more silver ions than solid silver. Therefore, it will give these silver ions are toxicity to bacteria. Then, it will suitable to apply as antibacterial activity.

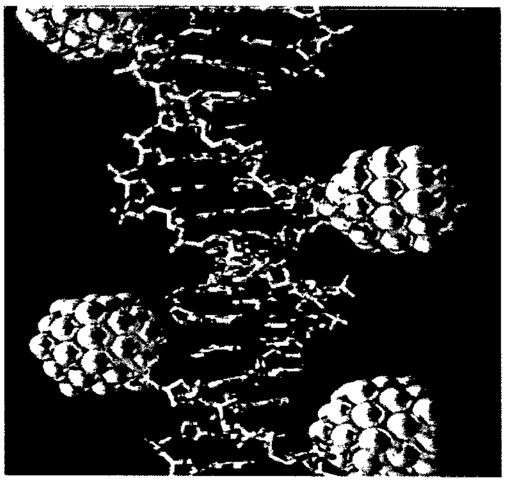


Figure 1.4: Colloidal silver nanoparticles

Source: http://altered-states.net/barry/graphics/cs7.jpg

Silver metal is very ductile and malleable. It has the highest electrical and heat conductivity of all metals and also have the lowest contact resistance. It has been widely used in medicine for curing disease and helps to wound healing since a long time ago. Therefore, the common silver used to treat infections is silver nitrate. Silver nanoparticle has been introduces into medical field due to new advancement in technology. Silver nanoparticles improvement in medical applications has been developed to help prevent the onset of infection and encourage the better wound healing. The size of nanoparticles gives the different properties when compared with the bulk material. The small size of nanoparticles generates the particles having a large surface area relative to their volume. The silver ions are bioactive and have broad spectrum antimicrobial properties against a wide range of bacteria. This silver nanoparticle will allow to easily interacting with other particles and increases their antibacterial efficiency. By controlling the size, shape, surface and agglomeration state of the nanoparticles, specific silver ion release profiles can be developed for its application.

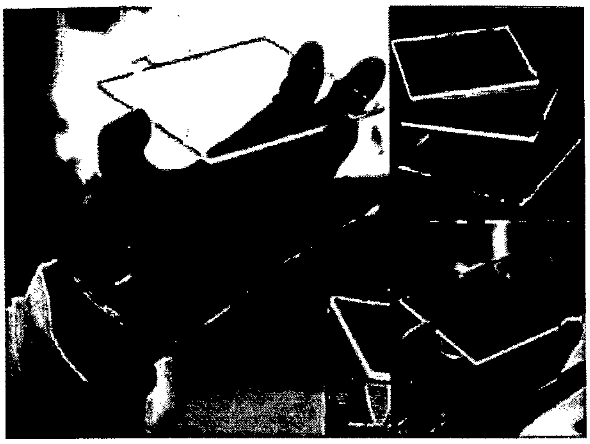


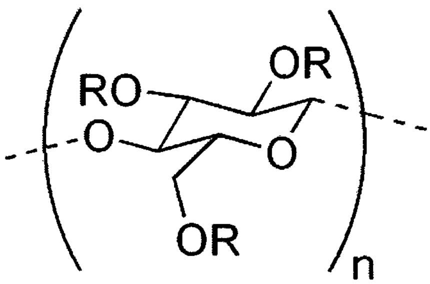
Figure 1.5: Thin film

Source: http://metallurgyfordummies.com/wp-content/uploads/2011/11/thin-film6.jpg

In the chemical procedure, the 'sol' (or solution) gradually evolves towards the formation of a gel-like diphasic system containing both a liquid phase and solid phase whose morphologies range from discrete particles to continuous polymer networks. Sol-gel derived materials have diverse applications in optics, electronics, energy, space, biosensors, medicine, reactive material and also separation technology. The precursor sol can be either deposited on a substrate to form a film cast into a suitable container with the desired shape or used to synthesize powders. The sol-gel approach is a cheap and low temperature technique that allows for the fine control of the product's chemical composition. Thin film has thickness of material ranging from fractions of a nanometer (monolayer) to several micrometers. Thin-film is used in pharmaceuticals industry which is thin film drug delivery. This is due to its relatively high hardness and inertness that making it for protection of substrate material against corrosion and oxidation.

1.2.3 Hydroxyethyl Cellulose

Hydroxylethyl cellulose is a gelling and thickening agent derived from cellulose. Hydroxyethyl cellulose (HEC) is a nonionic, water-soluble polymer that can thicken, suspend, bind, emulsify, form films, stabilize, disperse, retain water, and provide protective colloid action. It is readily soluble in hot or cold water and can be used to prepare solutions with a wide range of viscosities. It has outstanding tolerance for dissolved electrolytes. Hydroxyethyl cellulose and methyl cellulose are frequently used with hydrophobic drugs in capsule formulations, to improve the drugs' dissolution in the gastrointestinal fluids. This process is known as "Hydrophilization".



$R = H$ or CH_2CH_2OH

Figure 1.6: Hydroxyethyl Cellulose Formula

Source:
http://upload.wikimedia.org/wikipedia/commons/1/13/Hydroxyethyl_cellulose.png

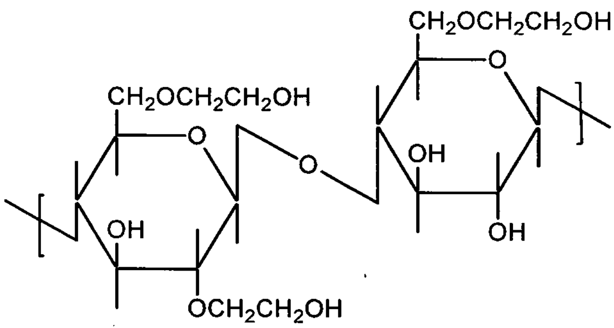


Figure 1.7: Structure of Hydroxyethyl cellulose (HEC)

HEC is a non-ionic polymer with β (1→4) glycosidic linkage held together with H-bonds. It is a biocompatible water soluble polysaccharide material with protective colloidal action. It is non-expensive and widely used in various pharmaceutical compositions, wound dressing and wound healing applications

1.2.4 Ultraviolet Visible Spectroscopy

Ultraviolet-visible spectroscopy (UV- Vis or UV/Vis) is on instruments that monitor the absorption or reflectance in the ultraviolet-visible spectral region. This absorption or reflectance in the visible range directly affects by perceived color of the chemicals involved. Solutions of transition metal ions can be colored due to *d* electrons within the metal atoms can be excited from one electronic state to another. In this study, UV-vis is use for the determination the absorption of silver. Otherwise, silver has a wavelength around 390 nm to 430 nm. The peak wavelength is a unique spectral fingerprint for a plasmonic nanoparticle with a specific size and shape. Other than that, UV-vis spectroscopy provides a mechanism to monitor how the nanoparticles change over time. When silver nanoparticles aggregate, the metal particles become electronically coupled and this coupled system has a different surface plasmon resonance than the individual particles. For the case of a multi-nanoparticle aggregate, the plasmon resonance will be a longer wavelength than the resonance of an individual nanoparticle.

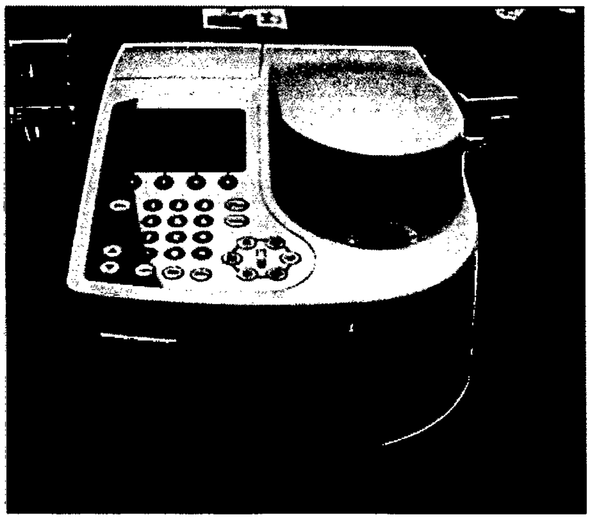


Figure 1.8: UV-vis Instrument

Source: http://parikh.lawr.ucdavis.edu/images/UvVis.jpg

1.2.5 Transmission Electron Microscope (TEM)

Transmission electron microscopy (TEM) is a microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen. It is interact with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen. Then, the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a CCD camera. TEMs are capable of imaging at a significantly higher resolution than light microscopes, owing to the small de Broglie wavelength of electrons. This enables the instrument's user to examine fine detail, even though as small as a single column of atoms, which is tens of thousands times smaller than the smallest resolvable object in a light microscope.

TEM forms a major analysis method in a range of scientific fields, in both physical and biological sciences. At smaller magnifications TEM image contrast is due to absorption of electrons in the material, due to the thickness and composition of the material. At higher magnifications complex wave interactions modulate the intensity of the image, requiring expert analysis of observed images. Alternate modes of use allow for the TEM to observe modulations in chemical identity, crystal orientation, electronic structure and sample induced electron phase shift as well as the regular absorption based imaging.

1.3 PROBLEM STATEMENT

Most of the nanoparticle work is done using solvents which are harmful to the environment. In this study, the main approach is in a green chemistry and environmental friendly way. For this research, the only solvent is water. The polymer used is biocompatible. The nanoparticles showed excellent stability for many months without aggregation.

1.4 RESEARCH OBJECTIVES

This experiment had carried out some objectives:

a) To study the efficiency of thin film of HEC/Silver nanoparticles in antibacterial studies.
b) To study the effect of heating the thin film on the antibacterial property.

1.5 SCOPE OF STUDY

In this research, the effectiveness of silver nanoparticles (Ag NP) using HEC solution in antibacterial studies focused. The optimum concentrations of silver nanoparticles in curing it also have to be focused. These two things are very important because during the experimental work, the exact amount of concentration used have to be recorded to show the effectiveness.

1.6 SIGNIFICANCE OF STUDY

Thin films of hydroxyethyl cellulose embedded with silver nanoparticles for antibacterial studies will have many uses and advantages in many fields especially in health care applications. It will be environmentally benign. It gives a cost effective way of producing the antibacterial thin film.

CHAPTER 2

LITERATURE REVIEW

Bacterial infection is to prevent healing of wounds and burns. Therefore, it's resulting in delayed healing and potential of the wound to become a chronic state (Schultz et al., 2004). Antibacterial coating is important not just on the environment pollution prevention but also to human health. In fact, antibacterial effects of silver salts have been known since ancient times (Dibrov et al., 2002). Silver is important used in the treatment of wounds and also in the treatment of major burn injuries due to its antimicrobial properties. Other than that, silver also was used to control bacterial growth in various applications such as dental work (Yoshida et al., 2002) and catheters (Zan and Su, 2010). The antimicrobial efficiency of topical silver has encouraged the development of several silver-functionalized 'active' wound care dressings (Atiyeh et al., 2007; Mooney et al., 2006; Ip et al., 2006). This silver is eluted from the dressing and transported by diffusion to the surface of the wound bed to establish antimicrobial activity (Mooney et al., 2006).

Among all metal, silver is more interesting due to its application and properties. Silver acts as the stabilizer and reducing agent. Silver nanoparticles are one of the most commonly utilized nanomaterials due to their anti-microbial properties, high electrical conductivity, and unique optical properties. Silver has such advantages as broad spectrum antibacterial activity, non-toxicity to human cells and long-lasting effect (Yuranova et al., 2003). Thus, in recent years it has been widely used in medical devices ranging from wound dressings to urinary catheters. The deposition time is affected the antibacterial properties and also its grain size. The increased deposition time led to the increased coverage of silver film and the increased silver weight percentage per unit surface, leading to an increase in release rate of silver ions from the coating, which resulted in the improved antibacterial properties. This statement is proved by research of the correlation between the sputtering parameters and antibacterial properties (Bo et al., 2007). Their result show that deposition time affected the antibacterial properties and grain size significantly, while the sputtering power and argon pressure did not show an obvious effect on the antibacterial properties.

Various antimicrobial agents have been developed for curing and preventing diseases in public health hygiene and antifouling in biomedical industry (Vigo et al., 2001). The possible mechanism of killing microorganisms by silver ions may be explained as follows: (1) silver ion inhibits ATP synthesis via binding to the ATP synthesis enzyme molecules in the cell wall, (2) silver ion enters the cell and binds with DNA, leading to the DNA denaturation, (3) silver ion blocks the respiratory chain of microorganisms in the cytochrome oxidase and NADH-succinate-dehydrogenase region (Klueh et al., 2000; Kumar et al., 2005). It has been reported that the mode of antibacterial action of silver nanoparticles is similar to that of silver ion (Dibrov et al., 2002). However, the effective biocidal concentration of silver nanoparticles is at a nanomolar level in contrast to a micromolar level of silver ions. Silver-impregnated polymer matrix provides antimicrobial efficacy with a sustained release of silver. The silver/polymer fabric minimizes the transmission of infective agents and enhances patient comfort as well as facile application for health care. They reported that the synthesized fiber encapsulating silver (I) N-heterocyclic carbene complexes facilitated the release of silver ions with maximum bactericidal activity over a longer period of time than that of aqueous silver. (Kluech et al., 2000) demonstrated that silver-coated poly (ethylene terephthalate) fabric was fabricated by the deposition of silver onto the polymeric substrate, and the resultant fabric. Other than that, the development a simple and reliable approach to synthesize polymer nanofibers with enhanced antimicrobial activities. The silver nanoparticle-embedded polymer nanofiber was fabricated by radical-mediated dispersion polymerization, and this novel polymerization technique was found to be a facile one step method for polymer nanofiber synthesis. They concluded that the silver nanoparticles were released from the polymer nanofiber as a function of immersion time. Other than that, the silver/polymer nanofiber had an enhanced killing rate and effective antimicrobial activity than that of AgNO3 by three times and that of silver sulfadiazine by nine times.

Moreover, its multilevel antimicrobial mode has been well known (Ip et al., 2006; Sanchez-Valdes et al., 2009), which ensures that silver has a very broad antibacterial spectrum even at low concentrations (Dibrov et al., 2002). A broad range of silver treatments is available, from silver salts to silver dressings containing various forms of silver often used as antimicrobial agents in medicine and biocides in hospital and other human settings. Compared to other metal ions, silver has the highest toxicity to microoganisms and is least toxic to animal cells (Dibrov et al., 2002; Alt et al., 2004; Shi et al., 2006; Lee et al., 2005; Podsiadlo et al., 2005). Silver ions have been reported to accumulate in epithelial cells, macrophages, fibroblasts and connective tissue (Klasen, 2000; Kristiansen et al., 2008) and it have been shown to cause tissue toxicity and impaired wound healing (Atiyeh et al., 2007; Cho Lee, 2002; Trop et al., 2006). This have been demonstrated in vitro studies, where the concentrations of silver ions incorporated into wound healing products can be cytotoxic to mammalian cells involved in wound healing, including fibroblasts (Poon and Burd, 2004; Hidalgo et al., 1998; Lee and Moon, 2003), keratinocytes (Poon and Burd, 2004) and lymphocytes (Hussain et al., 1992). One of the recent studies has reported that rapid induction of differential cell death by all the leading silver dressings that were tested in vitro (Van Den Plas et al., 2008), while another study reported elevation of hepatic enzymes and argyria-like symptoms in burn patients treated with a leading silver dressing (Trop et al., 2006). Eventhough the silver is proven the effectiveness as antimicrobial agent, it also leads to dose-related toxicity in tissue. Typically, when diffused to the surface of a wound bed from a macroscopic silver reservoir such as a dressing or ointment, the high loadings and concentrations of silver necessary to establish antimicrobial activity in the wound bed are not well-controlled and can become cytotoxic and impair wound healing (Atiyeh et al., 2007; Cho Lee et al., 2005; Mintz et al., 2008). In the other way, the dressings serve as macroscopic reservoirs of high concentrations of silver.

In recent years there has been growing interest in the preparation and study of silver nanoparticles (AgNPs), because those nanoparticles have been found to exhibit interesting antibacterial activities (Shahverdi et al., 2007; Pal et al., 2007). The use of AgNPs as an antibacterial agent is relatively new. In contrast, the antimicrobial activity of colloidal silver particles is influenced by the dimensions of the particles which is the smaller size of the particles will give the greater of antimicrobial effect (Zhang et al., 2003). Silver (Ag) particles of size less than 10 nm are more toxic to bacteria such as Escherichia coli (Xu et al., 2004; Gogoi et al., 2006). Nanocrystalline silver has a unique structure that was specifically developed for use in equilibrating wound dressings that could be left on a wound for multiple days (Burrell and Morris, 1998). The distinct properties of nanocrystalline silver include a combination of antimicrobial and anti-inflammatory (Nadworny et al., 2008) properties that improve the wound healing process. Production of nanosized metallic silver particles with different morphologies and sizes using different routes has been reported (Bae et al., 2002; Patel et al., 2006; Zhang et al., 2006; Xie et al., 2006; Patel et al., 2003; Salkar et al., 1999; Rosemary and Pradeep, 2003; Starowicz et al., 2006; Liu et al., 2001). Along with those methods, the simple process involving a reduction of silver salts has already been well developed (Chen and Gao, 2007; Šileikaitė et al., 2006). It is generally believed Ag^+ can bind to bacterial cell wall membrane (slightly negative), damage it and so alter its functionality (Yuranova et al., 2003; Feng et al., 2000; Sondi et al., 2004). The reduction of silver ions (Ag^+) in aqueous solution generally yields colloidal silver with particle diameters of several nanometers (Wiley et al., 2005). This chemical reduction is the most frequently applied method for the preparation of silver nanoparticles (Ag NPs) as stable, colloidal dispersions in water or organic solvents (Wiley et al., 2005). Commonly used reductants are borohydride, citrate, ascorbate, and elemental hydrogen (Chou and Ren, 2000; Nickel et al., 2000; Shirtcliffe et al., 1999; Frattini et al., 2005.

The properties of silver nanoparticles have been intensively studied for the past few decades due to their exceptional optical as well as catalytic properties (Suber et al., 2005; Ullah et al., 2006; Mallick et al., 2006). Initially, the reduction of various complexes with Ag^+ ions leads to the formation of silver atoms (Ag), which is followed by agglomeration into oligomeric clusters (Kapoor et al., 1994). These clusters eventually lead to the formation of colloidal Ag particles. When the colloidal particles are much smaller than the wavelength of visible light, the solutions have a yellow color with an intense band in the 380–400nm range and other less intense or smaller bands at longer wavelength in the absorption spectrum. The strong absorbance of silver nanoparticles is due to the coupling of the nanoparticles conduction electrons with the incident electromagnetic waves leading to the appearance of a strong plasmon absorbance band (Widoniak et al., 2005). This band is attributed to collective excitation of the electron gas in the particles, with a periodic change in electron density at the surface (surface plasmon absorption) (Henglein, 1989; Tao et al., 2006). The position of the plasmon absorbance band is the function of a few parameters such as the dielectric constant of the surrounding medium as well as the size and shape of the nanoparticles (Sun and Luo, 2005; Steiner et al., 1998; Patakfalvi et al., 2004).

Figure 2.1: Spherical silver nanoparticle

Source: Zhu et. al. (2000)