Sudan University of Science and Technology
College of Graduate Studies

Biosynthesis and Antibacterial Activity of Silver Nanoparticles

التخليق الحيوي لجسيمات الفضة النانوية وتأثيرها المضاد للبكتيريا

A dissertation Submitted in Partial Fulfillment for the Requirement of Mater Degree (M.sc) in General Physics

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Dedication

This study is wholeheartedly dedicated to our beloved parents, who have been our source of inspiration and gave us strength when we thought of giving up, who continually provide their moral, spiritual, emotional, and financial support. To my brothers, sisters, relatives, mentor, friends, and classmates who shared their words of advice and encouragement to finish this study. And lastly, thank you my God for the guidance, strength, power of mind, protection and skills and for giving us a healthy life.

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Furthermore, many thanks are due to staff members of department of Physics at Sudan University of Science and Technology for being as a family.

My heart felt gratitude to my parents, teachers, friends and classmates upon the long journey of education. During this journey, I was extraordinarily fortunate in having many nice teachers who gave interesting feedback and valuable lessons in different knowledge, as well as overcame many hard times. Without them I would never be able to love and carry out my studies. I am sorry that no names written, but indeed no one forgotten.

Last but not least, my greatest debt is to my family for their support, endless patience and encouragement in all forms.

Abstract

Recently, noble metals nanoparticles are gained the interest of the researchers due their unique physical characteristics. Among noble metals nanoparticles, silver nanoparticles exhibit sharp and distinct absorption band in the visible region of electromagnetic spectrum associated with localized surface Plasmon resonance. Therefore, the appearance of absorption band in visible region is considered as confident conformation of the formation of silver nanoparticles.

In this work, silver nanoparticles were synthesized based on Turkevich method using acacia nilotica extract as the reducing agent and silver nitrate as the source of silver. During the preparation process, the color of the solution was changed after adding the acacia nilotica extract, indicating the formation of silver nanoparticles. The UV-Vis spectrometer was further confirming the formation of silver nanoparticles. The effect of the concentration and heating time on the optical properties of silver nanoparticles was investigated. The obtained results showed that both concentration and heating time had no effect on the formation of silver nanoparticles but instead enhanced the reduction rate. The particle size of silver nanoparticles was calculated using Mie theory and found to be about 5nm.

The antibacterial activity of the obtained Ag NPs with optimum parameters was investigated against Escheriachia coli using well diffusion method. The obtained results showed considerable effect against Escheriachia coli.

المستخلص

في الآونة الأخيرة ، اكتسبت الجسيمات النانوية للمعادن النبيلة اهتمام الباحثين بسبب خصائصها الفيزيائية الفريدة. من بين الجسيمات النانوية للمعادن النبيلة ، تُظهر جسيمات الفضة النانوية نطاق امتصاص حاد ومتميز في المنطقة المرئية من الطيف الكهرومغناطيسي المرتبط برنين Plasmon السطحي. لذلك ، يعتبر ظهور ذروة الامتصاص في المنطقة المرئية بمثابة تاكيد قوي لتكوين جسيمات الفضة النانوية .

في هذا العمل ، تم تصنيع جسيمات الفضة النانوية على أساس طريقة Turkevich باستخدام مستخلص الأكاسيا النيلية (القرض) كعامل اختزال ونترات الفضة كمصدر للفضة. أثناء عملية التحضير ، تغير لون المحلول بعد إضافة مستخلص الأكاسيا النيلية (القرض) ، مما يشير إلى تكوين جزيئات الفضة النانوية. تم تاكيد تكوين جسيمات الفضة النانوية باستخدام مطياف الأشعة المرئية وفوق البنفسجية. تم دراسة تأثير التركيز ووقت التسخين على الخواص البصرية لجسيمات الفضة النانوية. أظهرت النتائج المتحصل عليها أن كلا من التركيز ووقت التسخين لم يكن لهما أي تأثير على تكوين جسيمات الفضة النانوية ولكن بدلاً من ذلك عززت معدل الاختزال. تم حساب حجم الجسيمات النانوية الفضية باستخدام نظرية مي ووجد أنها حوالي 7 نانومتر .

تم فحص النشاط المضاد للميكروبات لجسيمات الفضة النانوية التي تم الحصول ضد Escheriachia coli باستخدام طريقة الانتشار. أظهرت النتائج المتحصل عليها تأثيرا معتبرا ضد Escheriachia coli.

Keywords and Acronyms

Keywords

Acacia Nilotica - Turkevich method - Localized surface Plasmon resonance (LSPR) - Mie theory-Silver nanoparticles.

Acronyms

SPP: Surface Plasmon Polariton.

LSPs: Localized Surface Plasmon.

LSPR: Localized Surface Plasmon Resonance.

Ag-NPs: Silver Nanoparticles.

UV-VIS: Ultraviolet-Visible Spectroscopy.

MNPs: Metal Nanoparticles.

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CHAPTER ONE

INTRODUCTION

1.1 Overview

The prefix nano comes from the Greek word "nanos" meaning "dwarf". A nanometer is actually a unit of measure just like inches, feets and miles. By definition, a nanometer is one-billionth of a meter (1 x10^{-9}). Indeed, there are two fields of research associated with the prefix nano called nano science and nanotechnology (Jeremy, J. R., 2011). Nanoscience is the study of the behavior of objects at very small scale, roughly 1 to 100 nm ranges in at least one dimension while nanotechnology deals with using objects in the same size range to develop products with possible practical applications and it is usually based on nanoscience insights (Jeremy, j. R., 2011). Therefore, any material that has dimension in nanoscale particularly between 1 to 100 nm is called nanomaterial. Moreover, nanomaterials can be in zero dimension (0D) (e.g. spheres and clusters), one dimension (1D) (e.g. nanofibres, nanowires and nanorods), two dimensions (2D) (e.g. nanofilms and nanoplates), or three dimensions (3D) (eg. nanomaterils) (Kuno, M., 2005). They can exist in single, fused, aggregated or agglomerated forms with spherical, tubular or any other irregular shapes (Cristina, B., et al., 2007). Common types of nanomaterials include nanotubes, dendrimers, quantum dots and fullerenes and…etc. (Kakani, S. L. and Kakani, A. 2006, Chris, B., 2010).

Actually, the researches in nanoscale was started intensively after the lecture of Richard Feynman on December 1959 at the California Institute of Technology titled, “There’s Plenty of Room at the Bottom.” which he proposed the “possibility of maneuvering things atom by atom” (Jhon, G. and Mary, G., 1997). Although, there was experimental activity in the 1950s and 1960s on small metal particles, but it was not called nanotechnology at that time. The term nanotechnology has been used for the first time in 1974 by Japanese scientist called Taniguchi (Taniguchi, N., 1974). Later on, the field nanotechnology becomes more and more popular and is growing rapidly due to the emergence of nanomaterials, particularly inorganic nanoparticles (NPs) and nanorods with unique size and shape (Hari, S. N., 2001). These nanomaterials have found potential applications in almost all fields of research such as physics (Kakani, S. L. and Kakani, A., 2006), chemistry (Karim, A., et al., 2015), biology (Palaniselvam, K., et al., 2014, Peter, L., 2015), medicine (Singh, M., 2008), engineering (Chris, B., 2010) and ….etc.

Recently, the inorganic NPs have attracted much attention due their wide range of applications in various fields particularly in biological application (Ghodsieh, B., et al., 2017). This is due to their physicochemical properties. Among inorganic NPs, noble metal NPs have attracted the attention of researchers due to their novel physical, chemical and biological properties that are different from their bulk form (Kakani, S. L. and Kakani, A. 2006, Sudipta, P., et al., 2016). Furthermore, noble metal NPs have distinction optical properties owing to localized surface Plasmon resonance (LSPR). LSPR is defined as collective oscillation of conductions electrons in small metal NPs (Sudipta, P., et al., 2016). More recently, silver NPs have drawn more attention due to their fascinating properties such as high thermal and electrical conductivity, surface enhanced Raman scattering, chemical stability, biological and catalytic activities, and nonlinear optical behavior (Sudipta, P., et al., 2016). These properties placed Ag-NPs at the top list to be used in industrial fields, engineering, electronics, medical applications and environmental studies (Santhia, T., et al., 2014). Furthermore, it is well known that Ag-NPs have effective antimicrobial activity both in solution and in components against microbes such as bacteria (Joerger, R., et al., 2000), fungi (Monali, G., et al., 2009), virus (Ghodsieh, B., et al., 2017) and other pathogenic organisms (Peter, L., et al., 2015). In addition, Ag-NPs are widely used in some commercial products, such as plastics, food packaging, soaps, pastes, food, and textiles (Julia, F., et al., 2011).

Actually there are two approaches that can be employed to synthesis metals nanoparticles namely top-down and bottom-up. In top-down, larger molecules are decomposes into small units, which are then converted into suitable nanoparticles (Tapasztó, L., et al., 2008). In bottom-up approach (it is also called building up approach), material build up from atom or molecular species (Murphy, C. J., 2002).

There are two methods that have been used to synthesis metal nanoparticles namely physical and chemical methods (Sharma, V. K., et al., 2009, Khan, I., et al., 2019). Examples of physical methods are discharge, physical vapor condensation, energy ball milling methods,… etc. (Sharma, V. K., et al., 2009). The requirement of high temperatures, space, time, and high energy are the disadvantages of this method while the absence of solvent contamination and uniformity of the distribution of nanoparticles are the advantages of the physical methods in comparison with chemical processes. Example of chemical method is chemical reduction, electrochemical, and pyrolysis methods. Chemical methods have the advantages of higher yield, easy, low temperature and fast compared to physical methods (SSN Fernando, et al., 2018, Colless, J. I., et al., 2018).

Recently green synthesis (or Biosynthesis) becomes more popular due to their environment friendly, cost effective, easily scaled up for large scale synthesis. Basically green synthesis is associated with the used of herbal extracts in synthesis of nanoparticles. Actually there are different sources of plants part that has been used to synthesis metal nanoparticles (Sharma, V. K., et al., 2009, Peter, L., et al., 2015, Ghodsieh, B., et al., 2017).

Biosynthesis of nanoparticles is a kind of a bottom up approach where the main reaction occurring is reduction/oxidation. The main reason that made the researchers prefer the green synthesis is that the physical methods are very expensive while chemical methods leads to presence of some of the toxic chemical absorbed on the surface that may have adverse effect in the medical applications (Parashar, V., et al., 2009). This is not an issue when it comes to biosynthesized nanoparticles via green synthesis route (Begum, N. A., et al., 2009). So, in the search of cheaper pathways for nanoparticles synthesis, scientist used microbial enzymes and plant extracts (phytochemicals). With their antioxidant or reducing properties they are usually responsible for the reduction of metal compounds into their respective nanoparticles.

Biologically synthesized nanoparticles with antimicrobial, antioxidant, and anticancer properties are possible through the collaboration of different natural science sectors. These nanotechnologies may provide novel resources for the evaluation and development of newer, safer, and effective drug formulations (Palaniselvam, K., et al., 2014, Peter, L., et al., 2015, Ghodsieh, B., et al., 2017).

In this work, silver nanoparticles were synthesized using green method. Silver nitrate was used as source for silver while acacia nilotica fruits extract was used as reducing agent for the first time. The obtained Ag NPs was used to study their antibacterial activity.

1.2 Literature Review

The green synthesis, a bottom up approach, is similar to chemical reduction where an expensive chemical reducing agent is replaced by extract of a natural product such as leaves of trees\crops or fruits for the synthesis of metals or metals oxide nanoparticles (Hussain, I., et al., 2016).

It is an emerging area in the field of bio-nanotechnology and provides economic and environmental benefits as an alternative to chemical and physical methods. In this method, nontoxic safe reagents which are eco-friendly and bio-safe are used.

Biological methods of synthesis have thus paved way for the “greener synthesis” of nanoparticles and these have proven to be better methods due to slower kinetics (Elumalai, E. K., et al., 2010). They offer better manipulation, control over crystal growth and their stabilization. This has motivated an upsurge in research on the synthetic routes that allows better control of shape and size for various nano-technological applications. The use of environmentally beningn materials like plant extracts (Jain, D., et al., 2009), bacteria (Sharma, V. K., et al., 2009), fungi (Bhainsa, K. C., 2006) and enzymes (Willner, I., et al., 2007) for the synthesis of silver nanoparticles offer numerous benefits of eco-friendly and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol.

1.3 Problem Statement

Actually there are various chemical methods that have successfully been used to synthesis Ag NPs with different sizes and shapes, but the most chemicals material used in these methods were toxic and expensive. Due to increases of applications of Ag NPs in medical, biological and food additives fields, the researchers replace these toxic chemicals by natural plants. Therefore, this work conducted to use an acacia nilotica fruits extract to be used for the first time instead of harm chemicals to produce cheap and safe nanoparticles.

1.4 Research Objectives

1.4.1 General Objective

To synthesis Ag NPs by biosynthesis method(green method) and investigate its biological activity.

1.4.2 Specific Objectives

- To synthesis silver nanoparticles using acacia nilotica extract as reducing agent and Ag nitrate as the source of silver.
- To optimize the synthesis parameters (concentrations and heating time).

- To determine the absorption spectra of Ag-NPs by using UV-Vis spectrometer in order to confirm the formation of Ag-NPs.
- To investigate the biological activity of the obtained Ag NPs.

1.5 Thesis Layout

This thesis contains five chapters. In chapter one, brief introduction, the aim of this study and the problem statement are expressed. Chapter two focuses on the theoretical background and physical concept of Plasmon types, effects of nanoparticles shape, size, and environmental, applications of Ag-NPs. Chapter three focuses on the material, method and characterization techniques. The results and discussion were presented in chapter four. Chapter five focuses on the conclusion, Recommendation, and references.

CHAPTER TWO

THEORETICAL BACKGROUND

2.1 Overview

This chapter focus on the definition and synthesis of nanoparticles. In addition, the theoretical background was also presented.

2.2 Nanoparticles

Nanoparticles (NPs) are defined as an objects having size between 1 to 100 nm (Jeremy, J. R., 2011). Nanoparticles are divided into two groups namely organic and inorganic NPs. Example of organic NPs is carbon NPs (fullerness) while an examples of some inorganic NPs are magnetic NPs, noble metal NPs (I.e., gold and silver) and semi-conductor NPs (I.e., titanium oxide and zinc oxide). Recently, there is a growing interest in inorganic nanoparticles particularly noble metal NPs (Gold and silver) as they provide superior material properties with functional versatility. As this work is focus on the noble metal NPs particularly silver NPs, more details about their synthesis, properties and applications will be given in the following sections.

2.3 Synthesis of Metals Nanoparticles

Synthesis of metal nanoparticle is categorized into two approaches namely, top-down approach and bottom-top approach (see Fig.2.1 right hand side) (Murphy, C. J., 2002). The top-down approach includes a process of breaking down of large structure to create small structure. Physical techniques such as lithography, laser ablation, sputtering deposition, chemical etching, mechanical milling, and electro-explosion are most commonly used in this approach (Tapasztó, L., et al., 2008). Bottom-up approach is refer to the build-up of material from the bottom atom by atom, or molecule by molecule to create big structure (see Fig.2.1 lift hand side). The most common methods used in the bottom-up approach are sol-gel processing, chemical vapor deposition, laser pyrolysis, spray pyrolysis, atomic/molecular condensation, and aerosol processing (Pokropivny, V., et al., 2007).

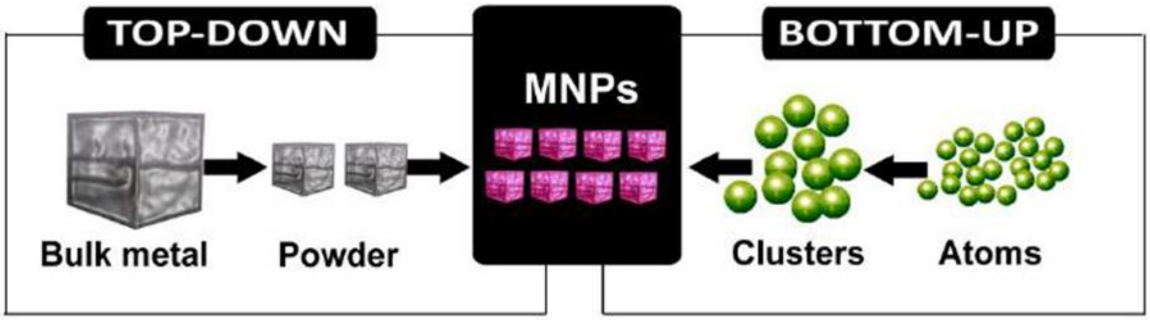


Fig.2.1 Schematic diagram explaining approaches that used to synthesis nanomaterials.

Actually, the synthesis methods mentioned above are categorized into two group namely chemical and physical methods. All these methods produce pure and well-defined nanoparticles, but the chemicals used during synthesis processes are toxic, energy consuming, expensive, and not suitable for biological applications. Since silver NPs proved its activity in biological applications such as antimicrobial agents in wound dressings, as topical creams to prevent wound infections, and as anticancer agents, the researchers replaced these toxic chemical with natural plants. The method that used these natural plants is called green method or biosynthesis method.

2.4 Silver Nanoparticles

Silver is a soft, white and lustrous belonging to transition metals. It is possessing high electrical and thermal conductivity. It has been known since ancient time due to its medical and therapeutic application even before the realization that microbes are agents for infections. Moreover, it was used in many forms as coins, vessels, solutions, foils, sutures, and colloids as lotions, ointments, and so forth (Firdhouse, M. J., and Lalitha, P., 2015). It is the foremost therapeutic agent in medicine for infectious diseases and surgical infections. After discovery of the promising properties of nanomaterial, silver NPs have found new applications in almost all fields of research (Khan, I., Saeed, K. and Idrees, K., 2019). Metal nanoparticles particularly silver and gold shows special optical properties owing to phenomenon called surface plasmon resonance (SPR). This phenomenon is occur due to a collective oscillation of free electrons in metal nanoparticles when interact with electromagnetic radiation. As the result, strong absorption of electromagnetic radiation occurs at visible and infrared regions.

2.5 Plasmon

Plasmon is defined as the collective oscillations of the free electrons in the conduction band of metals due to existence of an internal electric field. According to the dimensions of metal there are two main types of Plasmon namely bulk Plasmon (three dimensions (3D) and surface Plasmon (two or one dimension (2D or 1D)). More details about these types of plasmon will be given in the following sections.

2.5.1Bulk Plasmon

Bulk Plasmons can be described in the classical picture as an oscillation of electron density with respect to the fixed positive ions in a metal. To visualize a plasma oscillation, imagine a cube of metal placed in an external electric field pointing to the right (see Fig.2.2). Electrons will move to the left side (uncovering positive ions on the right side) until they cancel the field inside the metal. If the electric field is removed, the electrons move to the right, repelled by each other and attracted to the positive ions left on the right side. They oscillate back and forth at the plasma frequency until the energy is lost in some kind of resistance or damping. The quantization of this kind of oscillations is called bulk Plasmon. Bulk Plasmon can be well defined using Durde model through the following equation (Ashcroft, N.W. and Mermin, N. D. 1976).

$$\omega_{\mathrm{p}} = \sqrt{\mathrm{N}\,\mathrm{e}^2/\varepsilon_0 \mathrm{m_e}} \qquad (2.1)$$

where N is the conduction electron density, e is the electron charge, m_e is the electron effective mass and ε_0 is the vacuum dielectric permittivity. Actually, most of bulk metals have plasmon frequency with corresponding energy above 5 eV. Moreover, bulk Plasmon cannot be excited by photons, because the electrons in the bulk metals have longitudinal oscillations while photons have transfers oscillations which are not coupled together. Due to these mentioned properties, bulk Plasmon has no real practical in photonic applications.

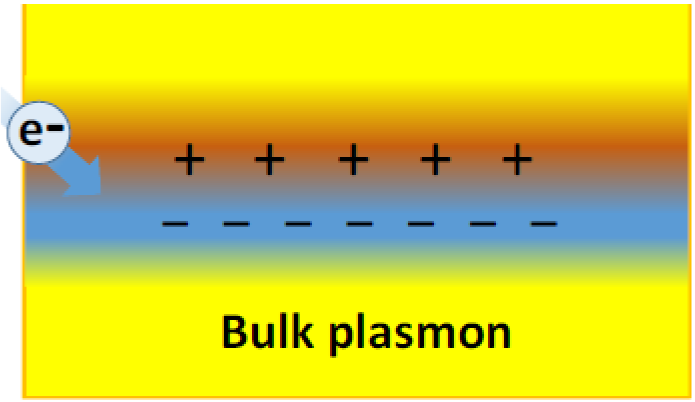


Fig.2.2 Schematic diagram illustrating Bulk Plasmon (Amendola, V., et al., 2010).

2.5.2 Surface Plasmon Resonance

Surface Plasmon Resonance (SPR) is referring to the resonant oscillation of conduction electrons in metals when their size reduced to nano scale. SPR is the basis of many standard tools for measuring adsorption of material onto planar metal (typically gold or silver) surfaces or onto the surface of metal nanoparticles. It is the fundamentals principle behind many color-based biosensor applications, different lab on- a-chip sensors and diatom photosynthesis. Actually, there are two types of SPR namely Surface Plasmon Plariton (SPP) and Localized Surface Plasmon (LSP). Details about these types will be discussed below.

2.5.2.1 Surface Plasmon Polaritons (SPPs)

Surface Plasmon Plariton (SPP) occurs when electromagnetic waves practically in the infrared or visible region travel along a metal–dielectric or metal–air interface (see Fig.2.3) (Amendola, V., et al., 2010). The term "surface plasmon polariton" explains that the wave involves both charge motion in the metal "surface plasmon" and electromagnetic waves in the air or dielectric "polariton".

They are a type of surface wave, guided along the interface in the same way that light guided by an optical fiber. SPPs are shorter in wavelength than the incident light (photons). Hence, SPPs can have tighter spatial confinement and higher local field intensity. Perpendicular to the interface, they have sub wavelength-scale confinement. The SPP propagates along the interface until its energy is lost either by absorption in the metal or scattering into other directions (I, e., into free space). Common Applications of this type of Plasmon are photonic data storage, light generation, and bio-photonics (William, L. B., et al., 2003).

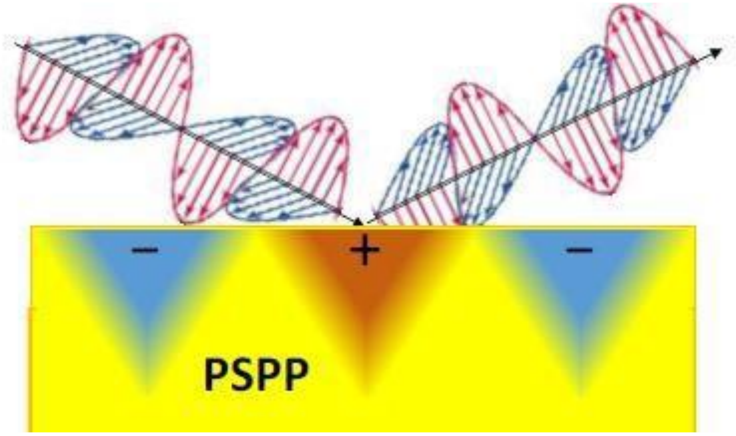


Fig.2.3 Schematic diagrams illustrating SPP (Amendola, V., et al., 2010).

2.5.2.2 Localized Surface Plasmon (LSP)

Localized Surface Plasmon Resonances (LSPRs) is defined as collective electron charge oscillations in metallic nanoparticles that are excited by light (see Fig.2.4). In case of small NPs compare to the wavelength of electromagnetic radiation (EMR), the electrons in these NPs oscillate transforming the energy from EMR into thermal energy in an absorption process.

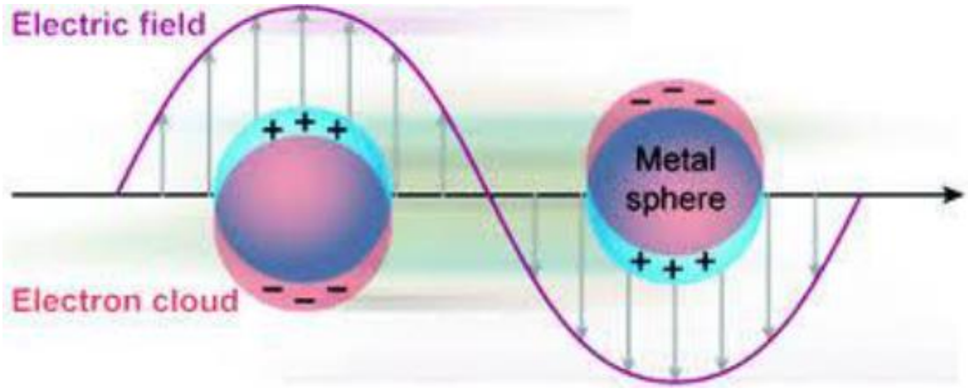


Fig.2.4 Schematic diagram illustrating localized surface Plasmon (Borys, 2011, Sarah, U., et al., 2015).

The enhancement of this process is called near-field enhancement. Of interest is that this field is highly localized at the nanoparticles surfaces and decays rapidly away from the nanoparticles. For big NPs, these electrons can be accelerated, and they radiate energy in scattering process. The enhancement of this process is called far-field enhancement. Actually, the theory of the interaction between metal NPs and electromagnetic radiation (EMR) was developed by scientist Mie 1908 and Later on called by Mie theory (Hergert, W., and Wriedt, T., 2012). In general, this theory expressed the electromagnetic extinction C_{ext} as the sum of absorption C_{abs} and scattering C_{sca} (Kakani, S. L. and Kakani, A. 2006).

$$C_{ext} = C_{abs} + C_{sca} \tag{2.2}$$

Light intensity enhancement (in both cases of absorption or scattering) is a very important aspect of LSPRs. The localization means that the LSPR has very high spatial resolution (sub wavelength), limited only by the size of nanoparticles. Because of the enhanced field amplitude, effects that depend on the amplitude such as magneto-optical effect are also enhanced by LSPRs.

2.6 Tuning of the Localized Surface Plasmon Resonance

When metal nanoparticles are excited by electromagnetic radiation, they exhibit collective oscillations of their conduction electrons known as localized surface plasmons. The wavelength corresponding to the extinction maximum (λmax), of the LSPR is highly dependent on the sizes and shapes of metal NPs and dielectric function of the environment. Details about these parameters will be discussed below.

2.6.1 Effect of Shape

It is well known that the shape of metal nanoparticles (MNPs) strongly affects their SPR. For many metal NPs, there are indications of the presence of polyhedral shapes with well-defined facets and vertices, including icosahedral (Ih), decahedral (dh), face-centered cubic (fcc), and truncated cubes (tc). The SPRs for polyhedral nanoparticles have been recently studied and a general relationship between the SPR and the morphology or the shape of each nanoparticle is shown in figure 2.5. For instance, the optical properties have been investigated computationally for cubes and decahedra as well as for their different truncations. As can be seen from figure 2.5 that the number peaks in spectra are increases as the symmetry of a structure decreases. For example, the spectrum of the cube shows a couple of peaks or shoulders beyond the strong dipole peak near 450 nm, whereas the spectrum of the sphere shows only one resonance peak. The appearance of these additional peaks is due to lower symmetry of a cube relative to a sphere which in turn leads to possible polarization of the electrons in more than one way. This trend is also seen for others shapes. It can also be seen that the size play an important role in the magnitude of the scattering and absorption cross sections of a nanoparticles (for more details see section 2.6.2).

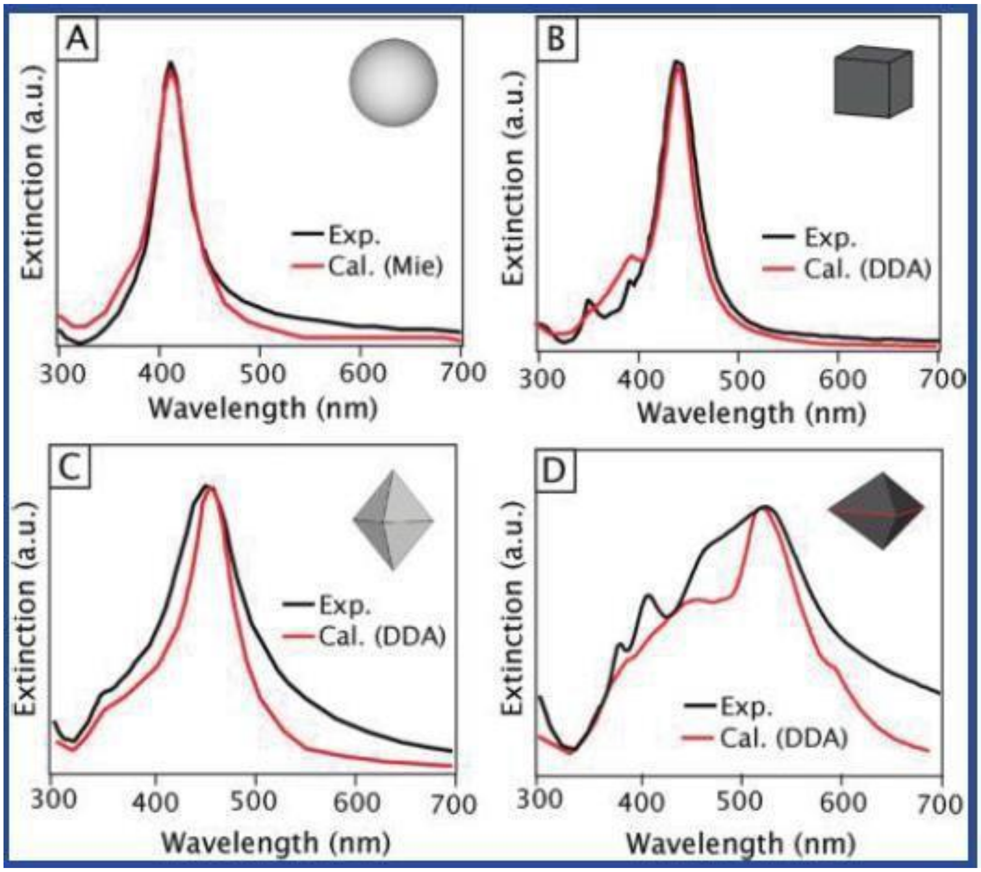


Fig.2.5 Extinction spectra of Ag nanoparticles with (A) Ag spheres 40 nm in diameter, (B) cubes 40 nm in edge length, (C) octahedrons 40 nm in edge length, (D) and right bipyramids 75 nm in edge length in ethylene glycol and different shapes (Rycenga, M., et al., 2011).

2.6.2 Effect of Size

As mentioned before, the extinction coefficient responsible for the measured UV-vis spectrum of metal nanoparticles has contributions from electronic absorption and scattering. The scattering effect becomes increasingly more important for larger particles. For metallic nanoparticles of less than about 30 nm of diameter, the scattering processes are usually negligible, and the particle mainly absorbs light energy through either SPR or inters band electronic transitions. SPR is dependent on the particle shape, size, embedding environment and chemical nature of the material. Due to the small size and thereby large surface-to-volume (S/V) ratio, surface dispersion or scattering of the “free” electrons, especially when their mean free path is comparable to the dimension of the nanoparticles, is an important process to consider, e.g. in electronic energy relaxation or dissipation. For metal NPs smaller than ~10 nm, collision of electrons with the particle surface becomes important, which usually results in broadening and blue-shift of the SPR absorption band (Hodak, J. H., et al., 2000, Hergert, W. and Wriedt, T., 2012). From a quantum viewpoint, for particles containing about 100 atoms the electronic energy bands are quantized and energy level spacing may become comparable to thermal energy $k\ T$. This affects intra band transitions of the conduction electrons and leads to a damping of electron motion, which, in turn, influences the dielectric constant (Taleb, A., et al., 1998).

One way to account for the size or surface effect is to divide the contribution to the dielectric constant into two parts: one from interband transitions and the second from intraband transitions including the surface effect (Johnson, P. B., et al., 1972, Ashcroft, N.W. and Mermin, N. D. 1976).

$$\mathcal{E}_1(\omega) = \mathcal{E}_1^{intra}(\omega) + \mathcal{E}_1^{inter}(\omega) \qquad (2.1)$$

$$\mathcal{E}_2(\omega) = \mathcal{E}_2^{intra}(\omega) + \mathcal{E}_2^{inter}(\omega) \qquad (2.2)$$

where ε_1 and ε_2 are the real and imaginary parts of the complex dielectric constant of the metal particle with a radius R.

The intraband contribution can be calculated using the Drude model for the nearly free electrons (Ashcroft, N.W. and Mermin, N. D. 1976).

$$\mathcal{E}_1^{intra}(\omega) = 1 - \frac{\omega_p^2}{\omega^2 + \Gamma^2} \qquad (2.3)$$

$$\mathcal{E}_2^{intra}(\omega) = 1 - \frac{\omega_p^2 \Gamma}{\omega(\omega^2 + \Gamma^2)} \qquad (2.4)$$

where $\boldsymbol{\omega}_p$ is the plasmon frequency, which is related to the free electron density N, and $\boldsymbol{\Gamma}$ is a damping constant. For metals such as Al and Ag, where the onset of interband transitions is well separated from the plasmon band, the plasmon band width is controlled by $\boldsymbol{\Gamma}$. For metals such as Au and Cu, where the interband transitions occur in the same spectral region as the plasmon band, the bandwidth is determined by both $\boldsymbol{\Gamma}$ and $\mathcal{E}^{\text{intra}}(\omega)$, which makes the analysis more difficult. In either case, however, the dependence of the bandwidth on particle size can be accounted using the following expressing (Vollmer, M. and Kreibig, U., 1995, Hodak, J. H., et al., 2000, Johnson, P. B., et al., 1972).

$$\Gamma = \Gamma_o + A\frac{V_f}{R} \qquad (2.5)$$

where R is the particle radius, V_f is the Fermi velocity of the electrons and is 1.4× 10^8 cm/s for Ag (Kakani, S. L. and Kakani, A. 2006) , A is a constant dependent on the electron- surface interaction and is usually on the order of unity (Kakani, S. L. and Kakani, A. 2006) and Γ_0 stands for the frequency of inelastic collisions of free electrons within the bulk metal, e.g. electron–phonon coupling or defects, and is on the order of hundredths of an electron

Violate (Kakani, S. L. and Kakani, A. 2006). For small-size particles, the second term in equation 2.5 which accounts for dephasing due to electron-surface scattering, can greatly exceed the bulk scattering frequency Γ_0, which accounts for bulk contribution to the electronic dephasing. For example, it has been determined experimentally for Ag nanoparticles that $A = 0.43$ and $\Gamma_0 = 4.4 \times 10^{14}$ Hz (Hodak, J. H., et al., 2000).

In general, the SPR depends weakly on the particle radius R. As R increases, one generally expects a shift and broadening of the SPR and the absorption cross section per particle will increase mainly due to an increase in particle volume.

2.6.3 Effect of Dielectric of the Environment

Besides the influence of nanoparticle morphology such as size and shape, the physical environment also modifies SPRs. It has been shown that SPRs are shifted if the dielectric properties of the surrounding media are changed (Noguez, C., 2007). In particular, the SPRs in a medium with density more than one are red-shifted with respect to those in vacuum. The SPR is also dependent on the geometry of the system including distance between the metal NP and substrate. The effects of both the dielectric properties and the physical environment of the nanoparticles are often of interest, since they have implications in processes such as SPR detection and surface enhanced raman scattering (SERS), catalytic processes, and plasmonic devices.

2.7 Applications of Silver Nanoparticles

Recently, silver nanoparticles have found wide range of applications in all most all fields of research. For examples, their applications in medicine, industrial, and scientific will be discussed in details below:

2.7.1 Applications in Medicine

For long time ago, silver was used for a variety of clinical conditions including epilepsy, venereal infections, acnes and leg ulcers. Moreover, silver foil was applied to surgical wounds for improved healing and reduced post-operative infections, while silver and ‘lunar caustic’ (pencil containing silver nitrate mitigated with potassium nitrate) was used for wart removal and ulcer debridement. Although some centers still use these solutions, they have been shown to be very impractical to use on large wounds or for extended time periods due to instability. With nanotechnology, the availability of silver nanoparticles has enabled the use of pure silver to achieve a rapid growth in medical practice (Singh, M., 2008). Since the size, shape and composition of silver nanoparticles can have a significant effect on their efficacy; extensive research has gone into synthesizing and characterizing silver nanoparticles. The application of nanosilver can be broadly divided into diagnostic and therapeutic uses.

2.7.2 Applications in Industrial

Ag-NPs have been in use for more than 150 years and are recognized as antimicrobial agent in United States (USA) since 1954.There are many assumptions about the use of silver by the ancient Egyptian and Romans. The most stable oxidation state of Ag is 0 and +1 although it can exist in other oxidation states as well and can form various complexes. The size and geometry of Ag-NPs are dependent on the synthetic route adopted for its synthesis; however it can be found in spherical, rod, and triangular shape, or coated with polymer, biomolecules, and sugars. Ag-NPs has numerous chemical, physical and biological functions and therefore are used as antimicrobial agents in a diverse range of applications, including air sanitizer sprays, socks, pillows, slippers, face masks, wet wipes, detergent, soap, shampoo, toothpaste, air filters, coatings of refrigerators, vacuum cleaners, washing machines, food storage containers, cellular phones.

2.7.3 Scientific Applications

Silver nanoparticles have a wide range of applications in automotive catalyst, membranes, fuel cells, photocatalysts, propellants, scratch-resistant coatings, structural ceramics and solar cells (Sharma, A. and Bhargava, M. 2013). High surface area and high surface energy predetermine metal nanoparticles for being effective catalytic medium. The growing small silver particles have been proved to be more effective catalysts than stable colloidal particles. These growing particles catalysed the borohydride reduction of several organic dyes. As compared with the stable and larger silver particles, the rate of reduction catalysed by growing particles was found to be faster.

Catalysis is due to efficient particle-mediated electron transfer from the BH4- ion to the dye. The catalytic activity of the particles depends on their size, *E*1/2 of the dye, and the dye-particle interaction. By controlling the size of nanoparticles, its catalytic activity can be controlled, as the redox potential depends on the nanoparticle size (Jana, N. R., *et al*., 2004). Using Kashayam and Guggulutiktham an ayurvedic medicine, the size-dependent catalytic activity of the synthesized Ag NPs was observed in the reduction of methylene blue dye by using NaBH4 (Suvith, V. S., and Philip, D., 2014). The synthesized Ag NPs from the plant extract of *Gloriosa superb* has the electron relay effect which influences the degradation of methylene blue at the end of the 30 min (Ashokkumar, S., *et al*., 2013). The catalytic activities of Ag-NPs synthesized by *Dimocarpus longan* seed extract were assessed against the photocatalytic degradation of methylene blue and chemo-catalytic reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP). The results suggest that the prepared Ag-NPs have strong chemo-catalytic activity with a complete reduction of 4-NP to 4-AP within 10min (Khan, I., et al., 2019). Hence, it can be said that silver nanoparticles find good applications in catalysis.

2.7.4 Biological Applications

Silver is being used from centuries as a non-toxic, safe inorganic antibacterial agent capable of killing about 650 microorganisms that cause various diseases (Annamalai, J. and Nallamuthu, T. 2016). It has a significant potential for a wide range of biological application such as antibacterial agents for antibiotic resistant bacteria, preventing infections, healing wounds and anti-inflammatory (Atiyeh, B.S., et al., 2007). Silver ions and the compounds made from it have a very strong destructive effect on many bacterial species and also have low toxicity towards animal cells. Therefore, silver ions, being an antibacterial component, are employed in the formulation of dental resin composites, bone cement, ion exchange fibres and coatings for medical devices (Panáček, A., et al., 2006, Alta, V., et al., 2004).

Silver nanoparticles also have antimicrobial properties. It was reported that Ag in nanoforms is considered less toxic than Ag^+ ions (Pattabia, R. M. and Pattabi, M., 2013). The antimicrobial properties of silver nanoparticles caused the employment of those nano-metals in numerous fields of medication, numerous industries, agriculture, packaging, accessories, cosmetics, health and military (Roh, H. Y., and Cho, M. 2005).

Silver nanoparticles with their large surface to volume ratio have been widely studied as a valuable material for their strong antimicrobial effect (Song, B. J., et al., 2011). The toxicity of silver nanoparticles has been well-known to a wide range of microorganisms. The antibacterial property of silver nanoparticles against *Staphyloccocus aureus, Pseudomonas aeruginosa* and *Escherichia coli* has been investigated (Birla, S. S., et al., 2009). It was considered that silver nanoparticles of 1 -10 nm range attach to the surface of the cell membrane and disturb its proper function like permeability and respiration (Morones, J. R., et al., 2005).

The biosynthesized silver nanoparticles displayed antimicrobial activity against a range of pathogenic microorganisms, such as *C. albicans*, *V. parahaemolyticus*, *S. enterica*, *B. anthracis*, *B. cereus*, and *E. coli* (Singh, P., *et al*., 2015). Biogenic synthesis of silver nanoparticles(Antibacterial and cytotoxic potential) (Algebaly, A. S., et al., 2020). Antimicrobial activity of the synthesized Ag NP's from the leaf extracts of *C. torulosa D*.Don and fungal endophytes *Pestalotiopsis versicolo* was done against three pathogenic bacteria *Bacillus subtilis, Salmonella entrica* and *Pseudomonas aeruginosa* showed promising medicinal drug activity against all the pathogens (Rajput, V. D., *et al*., 2018, Tao, A., et al., 2006, Baletto, F. and Ferrando, R., 2005).

The exact mechanism of antimicrobial action is not clearly known, but studies suggest that silver nanoparticles have the ability to damage the cell membrane permeability, damage the respiration functions of the cell, and encourage the formation of free radicals. The antimicrobial activity of silver nanoparticles was investigated by using fluorescent bacteria (Gogoi, S. K., et al., 2006, Nikoobakht, B. and El-Sayed, M. A. 2003). A different conclusion was drawn from the study that silver nanoparticles get attached to the sulfur containing bacterial cell which causes the death of the bacteria. The fluorescent measurements of the cell-free supernatant reflected the effect of silver on recombination of bacteria.

Several studies have shown that the combination of silver nanoparticles with antibiotics leads to an enhanced effect of the antibiotics against microorganisms (Fayaz, A. M., et al., 2010, Vazquez-Muñoz, R., et al., 2019). This is probably due to the increase in cell wall penetration by antibiotics with the nanoparticles. The synergistic effect of the antibiotics (vancomycin, novobiocin lincomycin, oleandomycin, penicillin G, and rifampicin) in association with biosynthesized silver nanoparticles increased the sensitivity of the tested microorganisms (S. enterica, E. coli, V. parahaemolyticus, B. anthracis, and B. Cereus) (Singh, P., et al., 2015, Ghodsieh, B., et al., 2017).

Many researchers have demonstrated the synergistic effect of biosynthesized silver nanoparticles with commercially available antibiotics (Dar, M. A., et al., 2013, Fayaz, A. M., et al., 2010). However, the exact mechanism behind this activity is still not well understood and must be explored.

CHAPTER THREE

METHODS AND TECHNIQUES

3.1 Introduction

In this chapter, the experimental method and materials used were described in details. At the end of the chapter, a brief description of the UV-Vis spectrometer used in the characterization of Ag-NPs is presented.

3.2 Materials and Tools

Materials and tools used to synthesis Ag-NPs were:

- Silver nitrate ($AgNO_3$) with purity 98%.
- Distilled water and ethanol.
- Beakers and hot plate with magnetic stirrer (see Fig.3.1)
- Acacia Nilotica 10g.
- Sensitive balance (accuracy=0.0001g) (see Fig.3.2a).
- B E L engineering SRL via carlo carra, 5monza 20900 Italia (see Fig.3.2b).

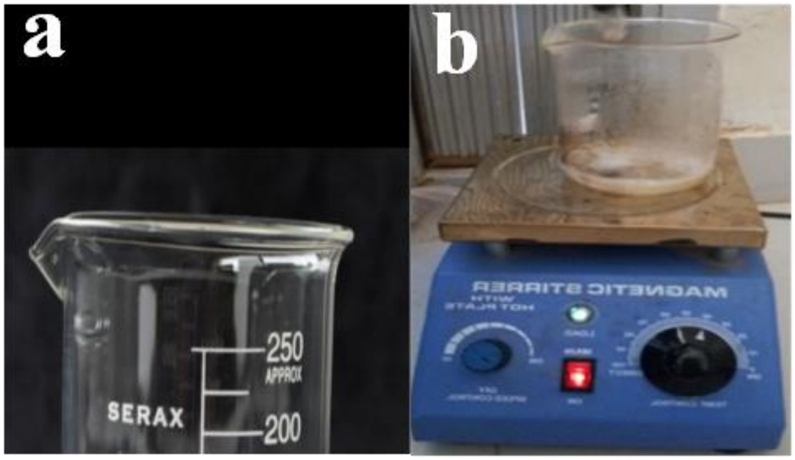


Fig.3.1 (a) beaker (b) Magnetic stirrer with hot plat.

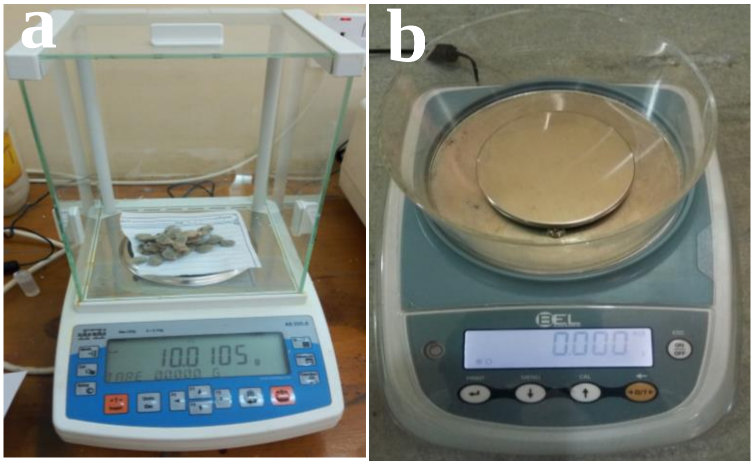


Fig.3.2 (a) Sensitive balance (b) BEL engineering SRL.

3.3 Silver Nitrate

It is an inorganic compound with chemical formula Ag NO_3 and molar mass: 169.87 g/mol, density: 4.35 g/cm³,melting point: 413.6°F (212°C), boiling point: 824°F (440°C), soluble in: water, glycerol. It is a versatile precursor to many other silver compounds, such as those used in photography. It is far less sensitive to light than the halides. It was once called *lunar caustic* because silver was called *luna* by the ancient alchemists. It can be prepared by reacting silver, such as silver bullion or silver foil, with nitric acid, resulting in silver nitrate, water, and oxides of nitrogen. Reactions byproducts depend upon the concentration of nitric acid used see the following equations.

$$3\,\mathrm{Ag} + 4\,\mathrm{HNO_3}\ \text{(cold and diluted)} \rightarrow 3\,\mathrm{AgNO_3} + 2\,\mathrm{H_2O} + \mathrm{NO} \quad (3.1)$$

$$Ag + 2H\mathrm{NO_3}\ \text{(hot and concentrated)} \rightarrow \mathrm{AgNO_3} + \mathrm{H_2O} + \mathrm{NO_3} \quad (3.2)$$

This is performed under a fume hood because of toxic nitrogen oxides evolved during the reaction. Figure 3.3 shows the chemical formula of Ag NO_3

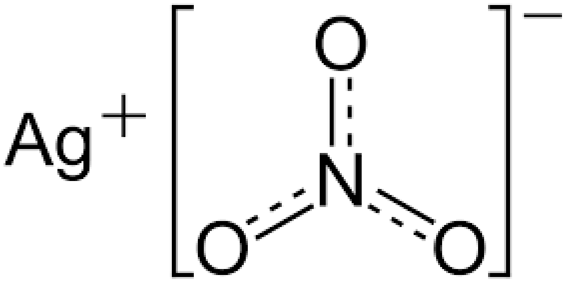


Fig.3.3 chemical formula silver nitrate $AgNO_3$.

3.4 Acacia Nilotica

Acacia is a genus of shrubs and trees belonging to the subfamily mimosoideae of the family fabaceae or leguminosae. It is indigenously known as ‘babul’ or ‘kikar’ is moderate sized with a spreading crown tree and is broadly scattered in tropical and subtropical countries. It is indigenous to the indian sub-continent as also in tropical Africa, Burma, Srilanka, Saudi arabia, Egypt and in west and east of Sudan. In India, natural babul forests are generally found in Maharashtra, Gujarat, Andhra Pradesh, Rajasthan, Haryana and Karnataka. However, scattered trees in groups occur naturally and also widely planted in almost all states and Union territories except north-eastern states, Kashmir and Kerala. A. *nilotica* is truly a multipurpose tree and extensively used in traditional agro-forestry system. In the present scenario of climate change, agro-forestry practices, emerging as a viable option for combating negative impacts of climate change (Kumar, V., et al., 2017). It is reported to be well nodulated with rhizobium species (Dreyfus, B. L. and dommergues, Y. R., 1981). This nodulation behavior helps in biological nitrogen fixation which helps to meet the nitrogen requirement in nutrient-poor soils and application of nitrogen. In addition, this species form symbiotic associations with naturally occurring soil fungi called vesicular arbuscular mycorrhizae (vam) (Abiala, M. A., et al., 2013). This association assists the roots to exploit more soil volume and to gain improved access to available nutrients especially phosphorus under stress and also makes the unavailable forms of nutrients into utilizable forms (Rengel, Z., 2008). Its timber is valued by rural folks, its leaves and pod are used as fodder and gum has a number of uses. acacia nilotica is an imperative multipurpose plant that has been used broadly for the treatment of various diseases (Ali, A., et al., 2012, Asgarpanah, J., et al., 2015). Furthermore, acacia nilotica, butea monosperma, terminalia arjuna, albizia procera and zizyphus mauritiana are an integral part of the rural agroforestry practices of the region and have tremendous importance in poverty alleviation and income generation (Rao, G. R., et al., 2018). The tree has a special significance to resource-poor farmers, who have a long tradition of growing it on their farms along with crops (Viswanath, S., et al., 2000). Moreover gum produced from this resource is very valuable in pharmaceutical purpose, calico-printing, sizing paper, cloth and textiles and encapsulation etc.

3.5 Experimental Procedure to Synthesize Ag Nanoparticles

Silver nitrate ($AgNO_3$, 98%) was obtained from chemical laboratory of Sudan University science and Technology. Acacia (10 g) was purchased from Omdurman market. It was picked in 2017 from Al hawatia western Sudan in al Gatareef state, and kept in local store. All glass ware was cleaned with distilled water and dried before used. To prepare Acacia extract, it was washed for several times with distilled water to remove dust and dried at room temperature. Then 10g of Acacia was added to 100 ml of distil water in beaker and boiled for 25 min. The obtained mixture was filtered and stored at room temperature for further use.

To prepare homogenous solution of $AgNO_3$ with concentration of 1mM, 0.169g of $AgNO_3$ was added to 1 litter of distilled water according to equation (3.1) and stirred for 30 min at room temperature.

$$\boldsymbol{Weight(gram)} = \frac{\boldsymbol{concentration(mM) \times molecular\ weight\ (g/mol) \times volume(ml)}}{\boldsymbol{1000}} \quad \boldsymbol{(3.1)}$$

To optimize the synthesis parameters, different heating time and different ratios between acacia nilotica extract and silver nitrate solution were applied. In the first experiment, different amount of acacia nilotica extract 1 ml, 2 ml, 3 ml, and 4 ml was added separately to 20 ml of silver nitrate solution to get different ratios (1:20, 2:20. 3:20 and 4:20). This mixture was heated at fixed temperature of 70°C for 10 minutes. In the second experiment, the ratio 1:20 for acacia nilotica extract and silver nitrate solution, respectively was used to optimize the heating time. This ratio is used because it showed highest intensity of the absorption band of LSPR compared to other ratios. Similarly, the mixture of nilotica extract and silver nitrate solution was heated at 70°C for different time (10 min, 20 min, 30 min, 50 min and 70 min). The most interesting for both experiments, the color was changed after 5 min, indicating the formation of Ag-NPs. Figure 3.4 showed the preparation flowchart of Ag NPs.

3.6 Examination of Ag NPs Against Escheriachia Coli

A plate of Mueller Hinton agar had inoculated by spreading a volume of the Escheriachia coli, then a hole of diameter 6 mm was punched spherically with sterile cork borer and a volume 100 ml of the obtained Ag NPs solution at desired concentration was introduced into the well. Then agar plates were incubated at 37^0C for 24 hrs.

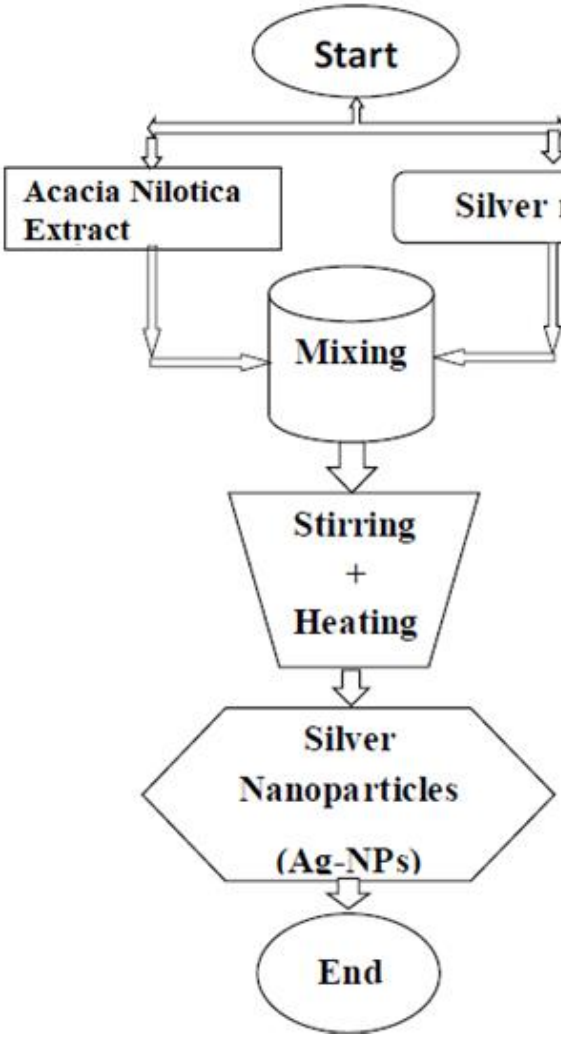


Fig.3.4 Preparation flowchart of silver nanoparticles.

3.7 Characterization Techniques

3.7.1 UV-VIS Spectrophotometer

The instrument used in ultraviolet-visible spectroscopy is called a UV-Vis spectrophotometer. It measures the intensity of light passing through a sample (I), and compares it to the intensity of light before it passes through the sample (Io).The ratio (I/Io) is called the transmittance.

The UV-visible spectrophotometer can also be configured with an integrated sphere to measure reflectance. In this case, the spectrophotometer measures the intensity of light reflected from a sample (I), and compares it to the intensity of light reflected from a reference material (Io). The ratio (I/Io) is called the reflectance. In a UV-Vis (ultraviolet-visible light) spectroscopic measurement, light absorption as a function of wavelength provides information about electronic transitions occurring in the material (Förster H., 2004). There are two types of UV spectrophotometer (single beam and double beam).

In a single beam UV spectrophotometer, all of the light passes through the sample cell (Chen, T. W., et al., 2013). Io must be measured by removing the sample. This was the earliest design, but is still in common use in both teaching and industrial labs. In a double-beam UV spectrophotometer, the light is split into two beams before it reaches the sample (see Figure 3.5). One beam is used as the reference and the other beam passes through the sample. The measured UV-Vis spectrum can be obtained as absorption, transmission and reflection spectrum. Absorption and transmission apply for gas and solution or transparent glass samples, while reflection applies only for solid samples. Samples are typically placed in a transparent cell, known as a cuvette. Cuvettes are typically rectangular in shape and commonly with an internal width of 1 cm. Cuvettes are made of high quality fused silica or quartz glass because these are transparent throughout the UV, visible and near infrared regions.

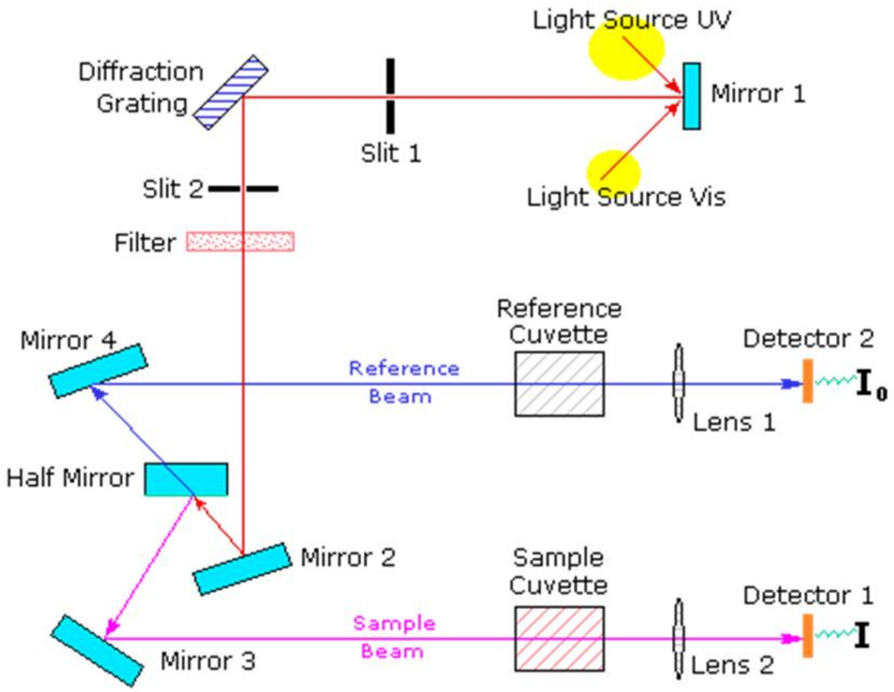


Fig.3.5 UV-Vis spectroscopy diagram.

The main objective of this instrument is to determine the absorption band of LSPR which confirm the formation of Ag NPs.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Synthesis of Silver Nanoparticles

Recently, green synthesis becomes more popular due to their low cost, easy and friendly. The green synthesis or biosynthesis is refers to use the extract of some plants instead of toxic chemicals to reduce silver ions into Ag NPs. It is important to note that the UV-Vis spectrometer is commonly used to confirm the formation of Ag NPs due to appearance of an absorption band in the visible region owing to the localized surface Plasmon resonance.

In this work an aqueous extract of Sudanese acacia nilotica was used as reduction agent and silver nitrate as the source of silver. To optimize the concentration, different ratio between acacia nilotica extract and silver nitrate solution was used while heating temperature and heating time was kept constant. During synthesis, the colour was changed to yellowish after 5 minutes and then to brown. This is the first indication that the silver ions convert to silver nanoparticles (Peter, L., et al., 2015). To confirm the formation of silver nanoparticles, the UV-vis spectrometer was applied (see fig. 4.1).

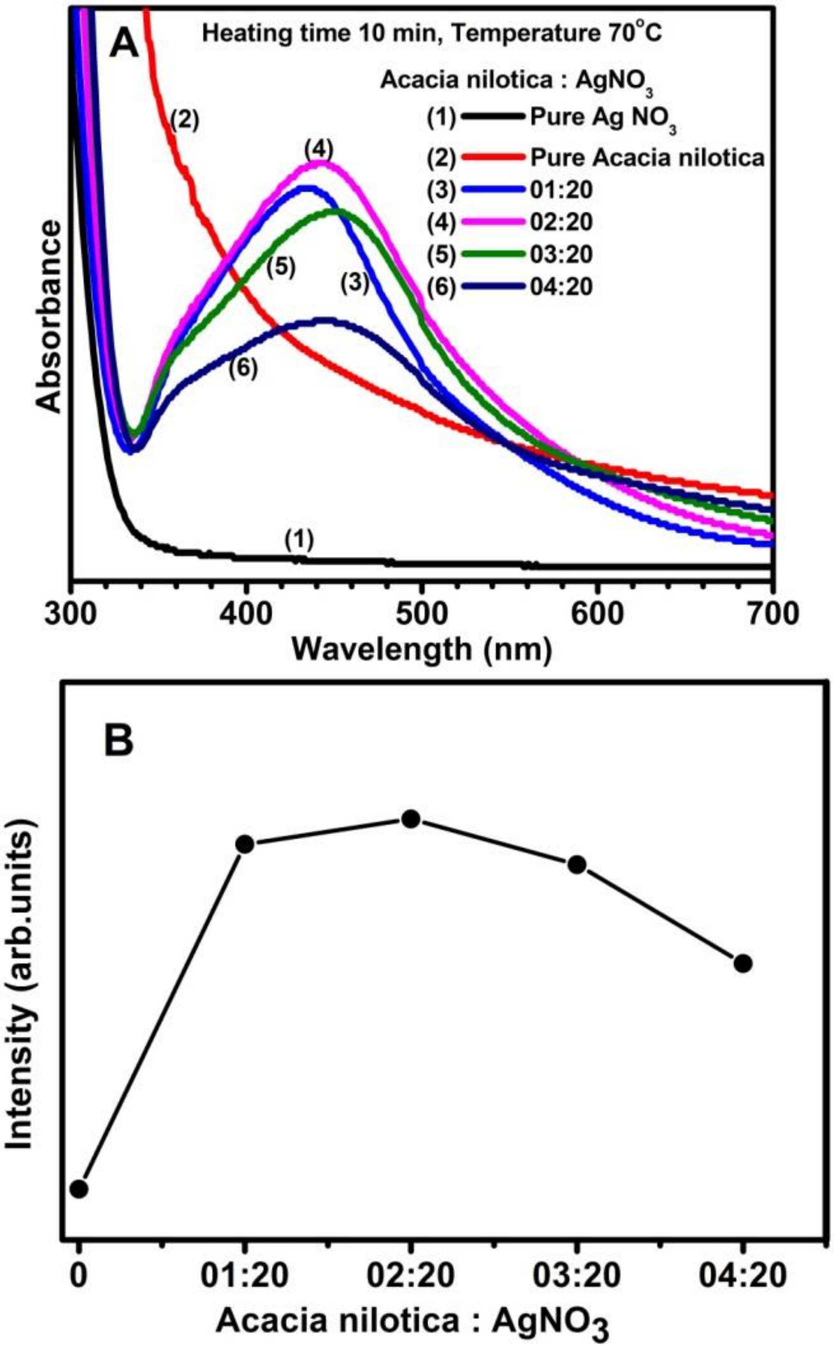
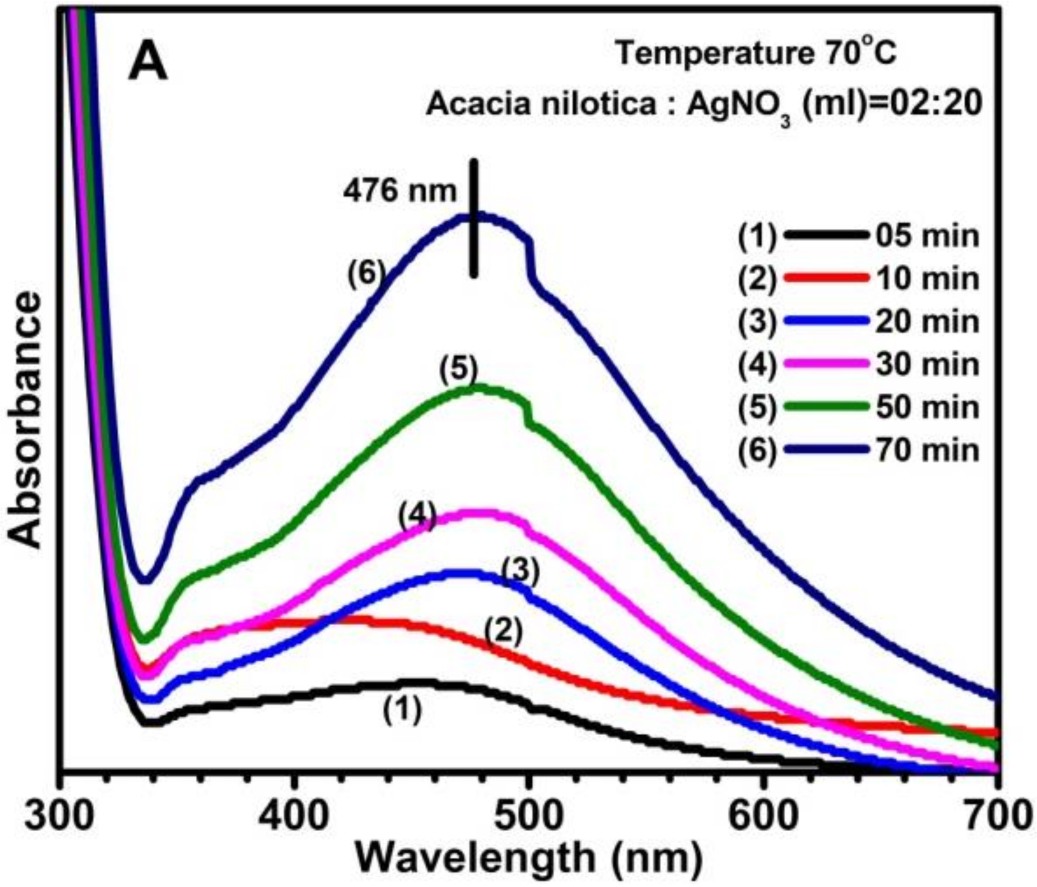


Fig.4.1 (A) diffuse absorption for the pure silver nitrate and Acacia fruits extract and their mixture with different ratio (B) Effect of the amount of Acacia fruits extract on the intensity of the absorption band.

As can be seen from figure 4.1 that the pure silver nitrate and acacia nilotica extract have showed no peaks at the visible range. But when pure acacia nilotica extract solution mixed with silver nitrate with ratio 1:20, respectively under heating of 70℃ for 10 min, a pronounce peak appeared in the visible region. This peak is assigned to localized surface Plasmon resonance (LSPR) (Sherry, L. J., et al., 2006), which confirm the formation of Ag NPs. It is important to note that the intensity of this peak is increases with increasing the amount of acacia extract up to ratio of 2:20 and then decreased when the amount of acacia extract was further increased (see Fig.4.1B). This indicates that the optimum ratio between Acacia fruits extract and silver salt solution is 02:20. It is also seen from Fig.4.1A that there is small random shift in the peak position of the LSPR. It is well known that the shift in the peak position is due to change in the size or shape of NPs or the refractive index of the environment (Klaus B. M. and Kneipp, K., 2014). The change in the particle shape produces more than one peak in the absorption spectrum due to high order mode which is not seen while the change in the particle size produces systematic shift to blue side if the size of NPs is decreased and to red side if the size increased. Therefore, this small random shift may be due change of the refractive index during the preparation of the samples for measurements with the UV-Vis technique, since the samples were diluted with different amount of distil water.

It is reported in literature that the plants metabolites such as sugars, terpenoids, polyphenols, alkaloids, phenolic acids, and proteins play an important role in the reduction of metal ions into nanoparticles (Makarov, V. V., et al., 2014). The acacia nilotica contains crude protein, crude fibers, fats, starch, ashes and moisture content in addition to some minerals namely; P, Ca, Mg, Na, K, Cu and Fe (Mohammed, S. A., et al., 2013). Therefore, crude protein may be responsible for reducing Ag ions into NPs.

To optimize the heating time, different heating times were applied while the ration between Acacia fruits extract and silver nitrate was kept as 02:20 (the optimum ratio obtained from the results above (see Fig 4.1)).



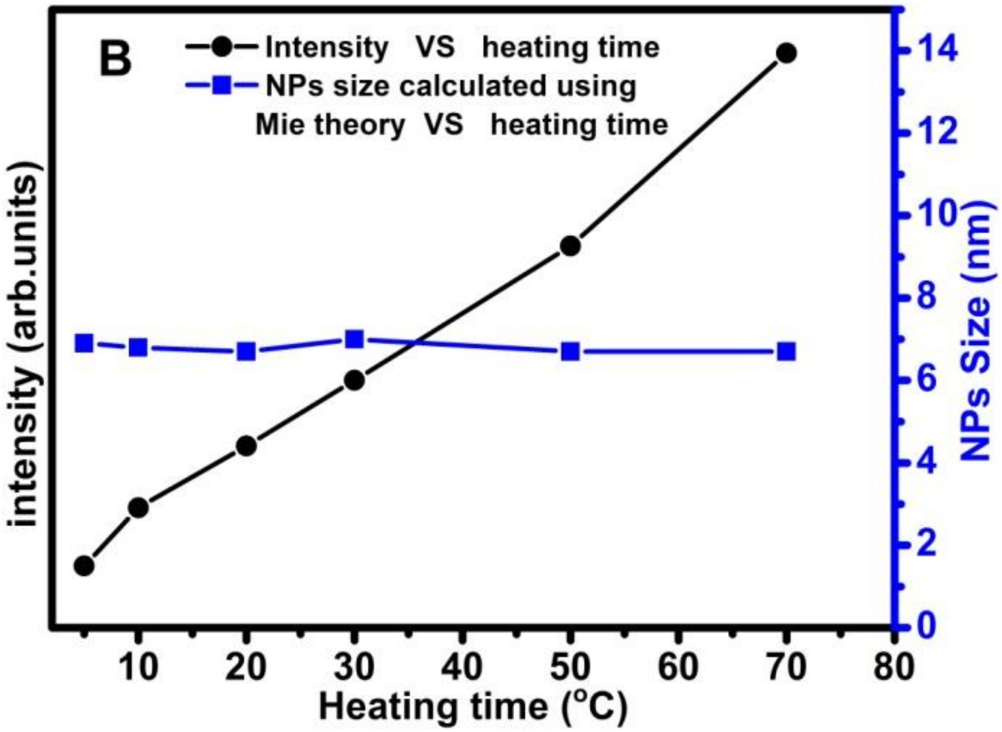


Fig.4.2 (A) Diffuse absorption of the mixture of silver salt and acacia fruits extract with ratio of 02:20 heated at different time (B) Effect of heating time on the intensity of LSPR band and the NPs size.

Figure 4.2 A shows the absorption spectra of the samples heated at 70°C for different time while figure 4.2B shows the effect of heating time on the absorption intensity and the NPs size. As can be seen from figure 4.2 (A&B) that the intensity of LSPR band increases with increasing heating time. This indicates that the heating time enhanced the reaction rate and therefore more silver ions were reduced to Ag NPs. It worth noting that there is no significant shift in the peak position, indicating that the heating time has no effect on the particle size. To confirm that, the Mie theory was used to calculate the particle size (see chapter 2 section 2.6.2, equation 2.5). The obtained result was plotted against the heating time as shown in figure 4.2B.

As can be seen there is no significant change in the particle size. The average particle size was found to be 7 nm.

4.2 Anti-bacterial Activity by Agar Well Diffusion Method

The antibacterial activity of the obtained Ag NPs with optimum parameters (concentration and heating time) as discussed in section 4.2 was investigated against Escheriachia coli using well diffusion method. Different concentrations were applied as shown in figure 4.3.

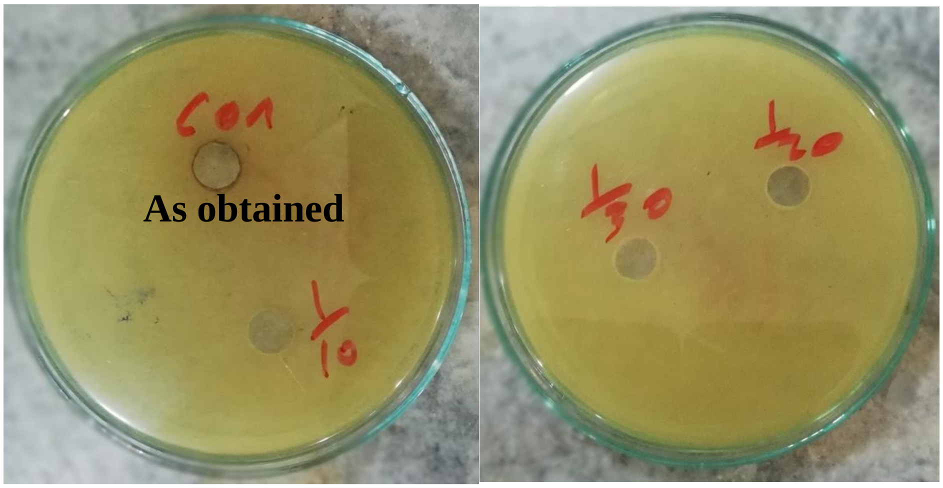


Fig.4.3 effect of Ag NPs with different concentrations on Escheriachia coli.

The Ag NPs diffuses in the agar medium (Mueller Hinton agar) and the growth of Escheriachia coli tested the result inhibition growth zone of E. coli observed a round Ag NPs. As can be seen from figure 4.3 that the as obtained sample show significant effect compare to other diluted samples (1/10, 1/20 and 1/30). Although the effect is weak but is considerable as started test.

CHAPTER FIVE

CONCLUSION AND A FUTURE WORK

5.1 Conclusion

Silver nanoparticles were synthesized based on Turkevich chemical method using acacia nilotica extract as reducing agent. The obtained samples were characterized using UV-visible spectrometer. The change of color during experimental process and the appearance of absorption band in the visible region in the diffuse absorption spectra confirm the formation of silver nanoparticles. Increasing the amount of acacia nilotica extract and heating time showed no effect on the particle size of silver nanoparticles but instead enhanced the reduction rate. The obtained silver nanoparticles with the size about 5 nm showed considerable effect against Escheriachia coli.

5.2 AFuture Work and Recommendation

- In this work, Acacia nilotica is used as reducing agent. Many plants extract can be used to reduce silver ion into silver nanoparticles.
- Acacia extract is used to reduce silver ions only. In future work other metals such as gold, copper…etc. should be used.
- Only UV-Vis spectrometer is used to confirm the formation of Ag-NPs. Appling other characterization techniques such transmission electron microscope (TEM) and x-ray diffractometer (XRD) for further information (I.e., morphology, distribution, size and shape) should be done.
- Ag-NPs were investigated against Escheriachia coli. In future work Ag-NPs will be tested against other microbial.

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