Preparation of Silver Nanoparticles by Chemical Reaction Method at Different Reaction Temperatures and the Study of their Antibacterial Activity

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Received on: 20/10/2014
Accepted on: 22/06/2016

Abstract- *It is found silver nanoparticles have strong antibacterial activity due to use in medical applications. It can be tuned antibacterial activity through control the shape and size of the synthesized silver nanoparticles. This article, silver nanoparticles were produced as different sizes at different reaction temperatures by chemical reaction method. Morphology and structure have been characterized by Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), X-ray diffraction (XRD) and UV-Visible Spectroscopy. Results indicate that silver nanoparticles have different sizes in range of (30- 97.26 nm). Additionally, antibacterial activity test against the pathogens, namely Escherichia colis hows effective inhibitory activity.*

Keywords- *silver nanoparticles, antibacterial activity and chemical method.*

How to cite this article: K.S. Shaker, M.A. Muhi, M.Sh. Khalaf and H.L. Mansour, "Preparation of Silver Nanoparticles by Chemical Reaction Method at Different Reaction Temperatures and the Study of their Antibacterial Activity" *Engineering and Technology Journal*, Vol. 35, Part B, No. 2, pp. 189-194, 2017.

1. Introduction

In recent years, nanotechnology is considered important and frutable technology to contribute in many fields like physics, chemistry industry, engineering, medicine and biology due to its characteristic, like as; high resistance of oxidation, high thermal conductivity, and antibacterial activity. It shows great promise for providing us in the near future with many breakthroughs that will change the direction of technological advances [1, 2]. Moreover, silver has superior performance in different applications so that it is considered very important [3]. Silver nanoparticles can be synthesized in different shapes, such as, spheres, discs, rods, wires, stars, prisms, right bipyramids, and cubes, moreover it has many applications in catalysis, electronics, surface-enhanced Raman scattering (SERS) and as antimicrobial sterilization to decrease toxicity toward mammalian cells [4,5]. Different methods of Silver nanoparticles preparation have been developed, including physical, chemical, and biological methods, among of prepared methods, There are some advantages that was chose and studied the chemical reduction method due to; yielding nanoparticles without aggregation, high yield, low preparation cost and easy and gentle condition [6,7].

Silver nanoparticles products are found bactericidal effects and strong inhibitory, furthermore a broad spectrum of antimicrobial activities have attracted much attention, so that, for centuries, it has been employed to prevent and treat various diseases, especially infections. Many researchers report the silver nanoparticles to possess anti-fungal, anti-viral, anti-inflammatory and antiangiogenics [8, 9]. One major mechanism of antibacterial properties of silver nanoparticles is represented by adhesion and penetration of AgNPs to cell membrane of bacterial [10]. The silver nanoparticles have antimicrobial capability, which is employed suitably in applications and fields like water filtration materials [11], textile [12] and disinfection or for medicine purposes [13].

https://doi.org/10.30684/etj.2017.138668
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The goal of present study is preparation and characterization Ag-nanoparticles by chemical method and evaluating its bactericidal activities against E.coli pathogen.

2. Experimental Procedure

I. Materials

Silver nitrate ($AgNO_3$) and Sodium citrate ($Na_3C_6H_5O_7$)were supplied by Fluke Company (Germany) E. coli bacteria was supplied from biotechnology Lab., Department of Applied Science, Technology University, Iraq, by using Muller–Hinton agar plates which were seeded with10 ml of suspensions of activated bacterial isolated separately.

II. Preparation of silver nanostructures

Silver colloid has been prepared by using the chemical reduction method, which depends on the reduction of silver nitrate ($AgNO_3$), by sodium citrate ($Na_3C_6H_5O_7$). After preparing 100 ml of stock solution of silver nitrate, 25 ml of stock solution (0.005 M) silver nitrate has taken and added to a 100 ml of distilled water. The resulting 125ml solution has heated temperature of 100℃, covered with a watch glass on a hot plate stir solution with a magnetic stir bar once boiling, add 5mL of a 1% solution of trisodium citrate drop wise, about 1 drop per second. Wait for solution to change to a light golden color or pale yellow. Carefully remove beaker from hot plate and let solution cool at room temperature. The process was repeated until the reaction reached 150 and 200℃.

III. Characterization techniques

Structure and lattice parameters of AgNPs have been analyzed by a LabX XRD 6000 SHIMADZU XR–Diffractometer with Cu Kα radiation (wavelength 1.54059 Å, voltage 30 kV, current 15 mA, scanning speed = 4 °/min).Bioreduction of Ag+ ions was tested by UV-Visible spectrophotometer (metertech, UV/VIS SP8001). Silver nanostructures morphology investigated by both scanning electron microscope (SEM, the VEGA EasyProbe) and Transmission Electron Microscopy (TEM) analysis (EM208, Philips, Day Petronic Co., Tehran, Iran). Finally, X-ray diffraction (Philips PW 1050 X-ray diffract meter of 1.5oA from Cu-Kα. Additionally) has been used. To analyses, the samples were prepared as thin films in carbon copper grid and allowed to be dried by mercury lamp for 5 minutes.

IV. Antimicrobial testing of Ag NPs

Antimicrobial property of AgNPs is performed by using disc diffusion test, which is different from viable count method. In this test, 100µL of bacterial suspension is prepared as control equal to 107 CFU/ml. Then, bacterial suspension is spread on to a Nutrient agar (N.agra) solidified plate. Sterile paper discs (like Whatman filter paper, 6 mm diameter) were prepared and dipped in different concentrations sample of silver nanoparticles depending on size and shape along with four standard antibiotic containing discs were placed in each plate. After that, the test plates were put in 37 °C incubation, after 24 h, the zones of inhibition could become clear to calculate.

3. Results and Discussion

I. X-ray diffraction

The X-ray diffraction (XRD) was used to characterize crystallinity of the produced material. This technique could be employed to give information of the grain size and the type of material prepared (i.e. silver nanoparticles). It can be seen only one peak in XRD spectrum shown in Figure 1 at $2\theta = 38$ which indicates to polycrystalline of silver nanoparticles according to the ASTM standards.

II .UV–Vis spectrum analysis

One of important properties to silver particles is optical properties investigated by extinction measurement (UV/VIS) due to the potential of photonic applications. UV-visible spectroscopy (metertech, UV/VIS SP8001) technique is used widely for structure of AgNPs. Absorption spectrum of paled AgNPs colloids (yellow-brown) synthesized at different conditions by using 1% trisodium citrate showed two absorption cut of wave length around (293) & (430) nm. The surface plasmon resonance depends upon the size and shape of nanoparticles. The greatest frequency range is imputed to spherical shape nanoparticles that alloy the metals of silver at different relative concentrations. Various curves of surface plasmon absorption the indicates the presence of different shapes and radii (spherical or approximately spherical Ag nanoparticles) in the range of250 to 500 nm as shown in Figure 2. The SPR bands of silver nanoparticles changes as a function of concentration. The two SPR bands were observed at three curves (1.23, 1.06, and 0.62) for temperatures (200, 150 and 100°C) respectively. That means the two size of nanoparticles in most concentration as spherical shaped. It indicate that the concentration of silver particles had increase with a high temperature of preparation they shift from that of the bulk Ag (421.41 nm) in values of temperature.

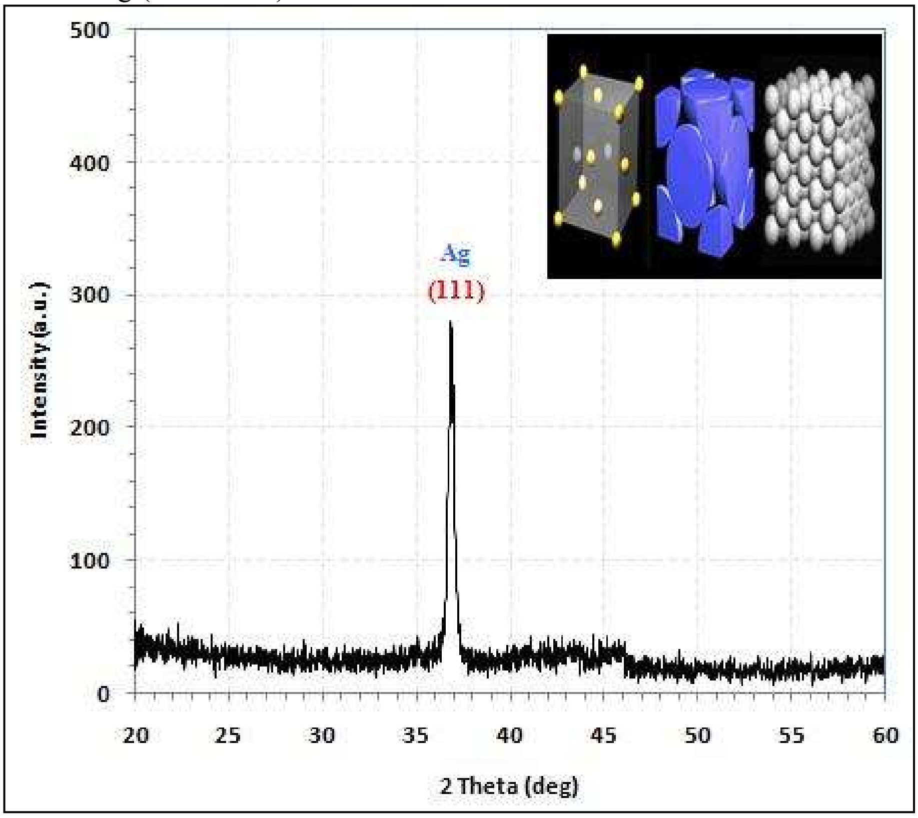

Figure 1: of silver nanoparticles synthesized by chemical method

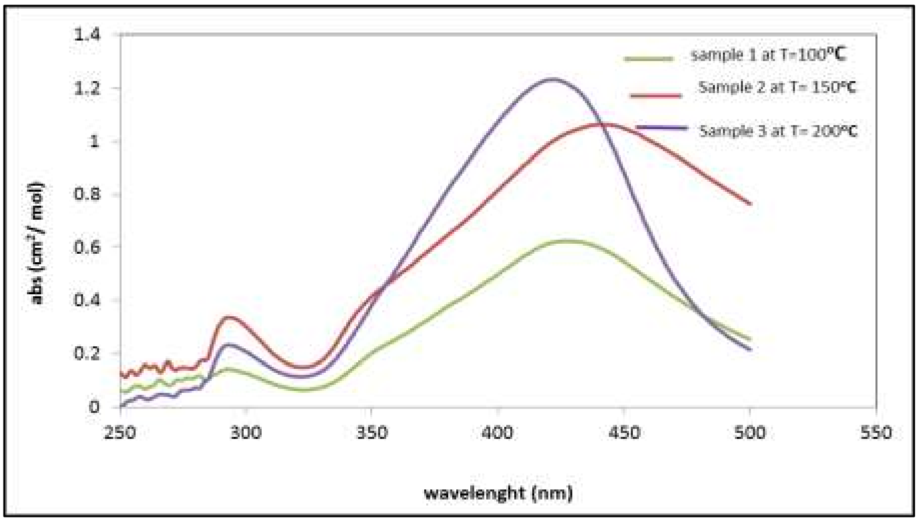

Figure 2: UV-Vis absorption spectrum of silver nanoparticles synthesized by chemical method 0.005 M of AgNO3 at different Temperatures (100, 150 and 200) °C.

III. Scanning electron microscopy and Transmission electron microscopy

According on reducing agent type, the reduction of silver nitrate by using 1% trisodium citrate in distal water and at various conditions resulted dispersions of silver particles with diverse shapes and sizes. The elemental analysis of the silver nanoparticles (AgNPs) had been performed by scanning electron micrographs (SEM) (the VEGA Easy Probe) images. As shown in figs.3, 4, and 5 respectively.

Figure 3 shows that the AgNPs were predominantly dendritic provides a clear shape in SEM microscope with varies magnifying of first method using citrate-AgNPs, that were synthesized by 0.005 M of $AgNO_3$ reduction at T=1000C for 2h and the particles with a size ranging from 34.5 to 160 nm (mean 97.25 nm). Figure 4 shows the biggest agglomerations of particles while, in Figure 5, the particles are dispersed individually. The size of silver particles are about 20–80 nm with spherical shape which were produced by reducing silver nitrate with second method at T=150^{0}C.

Figure 5 shows SEM micrograph of sphericaland irregular Ag nanoparticles using the third method with 5ml of citrate for 2h at T=200 ^{0}C, from which it can be observed the aggregation state of nanoparticles by increasing the temperature above 200 ^{o}C and the particle size ranges from 30 to 40 nm**.** It can be noticed that when the reaction temperature increases, the ($AgNO_3$) molecules might be broken and in this case the silver nanoparticles become smaller and whereby the nanoparticles become more efficient, as it is a wall known phenol, when the temperature increased, the particle size and the narrower size distribution decreased [14]. Figure 6. shows the size and size distribution of Ag nanoparticles produced at T= 200^{o}C which shows sphere shape with average particles size of 80 nm is in fact appears large due to effects of nanoparticles agglomeration.

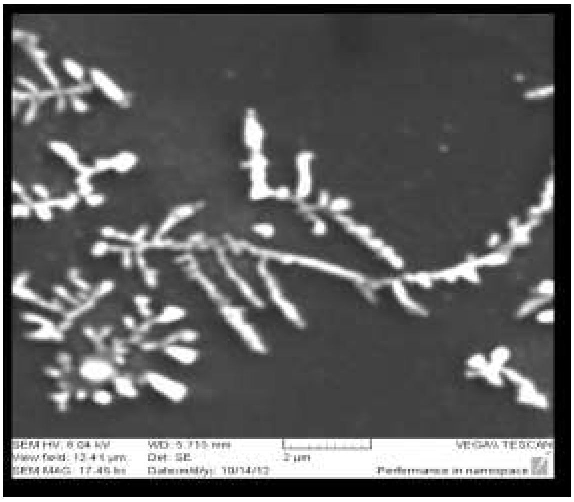

Figure 3: SEM micrographs of the prepared AgNPs using first method (AgNPs) at T= 100℃

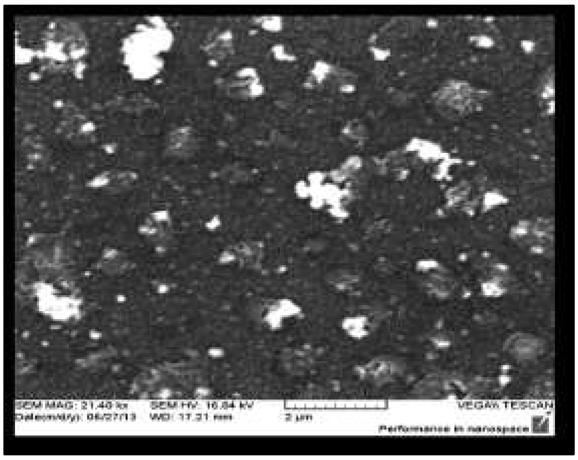

Figure 4: SEM micrographs of the prepared AgNPs using second method (AgNPs) at T= 150℃

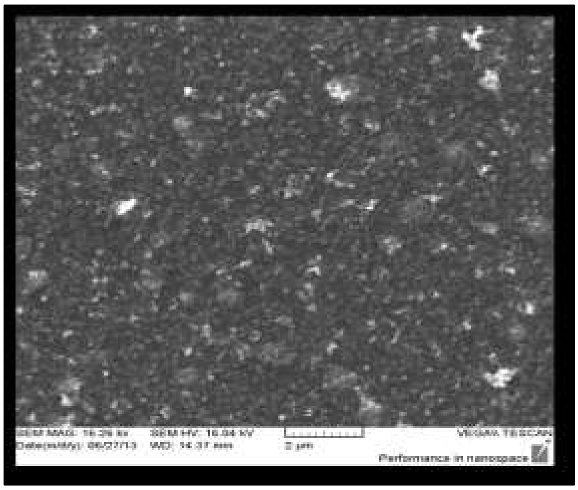

Figure 5: SEM micrographs of prepared AgNPs using third method (citrate-AgNPs) at T= 200℃

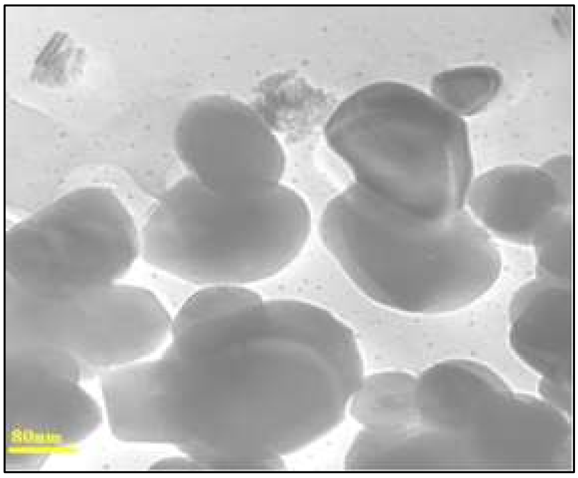

Figure 6: TEM nanographs of prepared AgNPs using third method (citrate-AgNPs) at T= 200℃ .

IV. Antimicrobial activity study

Prepared colloidal silver nanoparticles with different temperatures are tested antibacterial activity via inhibiting Zone using well diffusion assay and determining the diameter of inhibition

Zone. It can be observed from Figure 7, a clear inhibitory zone appeared around samples (3) and (4) (i.e. both samples prepared at1500C and 200 0C continuously) with diameters of about 2mm and 3mm, respectively, which may indicates that synthesized nanoparticles have phenomenal bactericidal effect, furthermore there was no inhibitory zone appeared around samples (1) and (2) this may be interpreted to the temperatures of reaction at which silver nanoparticles prepared since the reaction occurred at temperature lower than 150℃ as compared with two other samples (i.e. sample 3 and 4).

Figure 7: Show Effected AgNps prepared by chemical method for different temperatures on bacteria *E.coil*

4. Conclusion

It has been clearly demonstrated that clear inhibition zone treated with silver nanoparticles highly depends on temperature of reaction, sizing and shaping of AgNPs in all microbes. The AgNPs that produced by using different chemical reduction methods result in increased the inhibition zone treated with AgNPs nanoparticles by penetrating with cell membrane and interact with amino acids, proteins, and nucleic acids. Besides, the results promote the establishment of oxygen species interactive and take the increased oxidative stress and stress. Oxidative stress is one of the indicators that allow the monitoring of the toxic effects of heavy metals on microorganisms. This is based on the toxic effect on the binding of silver ions in the bacterial cell wall and plasma membrane, leading to inhibition of respiration by bacteria and this will disturb the function of strength such as permeability and breathing. It might be reasonable to state that the relationship between the particles and the bacteria depends on the surface area the particle available for interaction. In addition, it was found that smaller particle have larger surface area than larger one; so it will give more bactericidal effect for interaction than the larger particles.

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