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Research Article

Antibacterial Effect of Ag Nps/Agar Composite Films Synthesized by Hydrothermal Method

Zainab Sabeeh Sadik†*, Dunya Fareed Salloom‡, Sarmed Salih Mahdi† and Enas Diaa Salah Alden†

†Physics Department, Baghdad University, Al-jadiria – Baghdad, Iraq
‡Biology Department, Baghdad University, Al-jadiria – Baghdad, Iraq

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Abstract

This study developed a rapid, eco-friendly and convenient green method for the biosynthesize of stable silver nanoparticles have been prepared by hot chemical reduction method with using agar as a biopolymer matrix and a capping (reduction) agent to get a Ag - agar hybrid NP (hybrid core / shall NP). We prepared the stable Ag NPs by using high concentration agar gel which prepare from simple hydrothermal method to provide conditions for the preparation of gels of greater homogeneity than conventional methods. The resulting Ag NPs/agar composite films had homogeneous distribution of Ag NPs in the polymer matrix, and their properties were affected by the content of silver nanoparticles added. Characterization of silver nanoparticles was carried out based on UV–Vis, SEM, EDX and FTIR analysis. The SEM suggests that the silver nanoparticles synthesized are spherical in shape, having average mean size of 14.06-15.95 nm depending on the concentration of Ag NPs .The antibacterial activity of biologically synthesized silver NPs was evaluated against E coil showing increasing the effective bactericidal activity with increase the Ag NP concentration.

Keywords: *Silver nanoparticles, Agar, Antimicrobial activity, Hydrothermal process, biopolymer matix.*

1. Introduction

In recent years, noble metal nanoparticles have been the subject of focused researches due to their unique electronic, optical, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials. These special and unique properties could be attributed to their small sizes and large specific surface area. For these reasons, metallic nanoparticles have found uses in many applications in different fields as catalysis, electronics, and photonics (Guzmn, *et al*, 2009). Nanoparticles fall into two categories: mainly organic and inorganic nanoparticles. Organic nanoparticles may include carbon nanoparticles (fullerenes). On the other hand, inorganic nanoparticles may include magnetic nanoparticles, noble metal nanoparticles (like gold and silver) and semiconductor nanoparticles (like titanium dioxide and zinc oxide). There is a growing interest in inorganic nanoparticles as they provide superior material properties with functional versatility and have been examined as potential tools for medical imaging as well as for treating diseases due to their size features and advantages available in chemical imaging drugs agents and drugs. When mesoporous silica combined with molecular machines prove to be excellent imaging and drug releasing systems. Gold nanoparticles have been used extensively in imaging, as drug carriers and in thermo – therapy of biological targets (Cheon, *et al*, 2009). Inorganic nanoparticles (metallic and semiconductor nanoparticles) exhibit intrinsic optical properties which may enhance the transparency of polymer – particle composites. For these reasons, inorganic nanoparticles have found special interest in studies devoted to optical properties in composites. The size dependent color of gold nanoparticles has been used to color glass for centuries (Caseri, 2009). The exact mechanism, which silver nanoparticles employ to cause antimicrobial effect, is not clearly known and is a debated topic. There are however, various theories on the action of silver nanoparticles on microbes to cause the microbicidal effect (Prabhu, 2009). Silver nanoparticles have the ability to anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell. There is formation of 'pits' on the cell surface, and there is accumulation of the nanoparticles on the cell surface (Sondi, *et al*, 2004). The formation of free radicals by the silver nanoparticles may be considered to be another mechanism by which the cells die. There have

*Corresponding author: **Zainab Sabeeh Sadik**

been electron spin resonance spectroscopy studies that suggested that there is a formation of free radicals by the silver nanoparticles when these are in contact with the bacteria, and these free radicals have the ability to damage the cell membrane and make it porous, which can ultimately lead to cell death (Kim, *et al*, 2007). It has also been proposed that there can be a release of silver ions by the nanoparticles, and these ions can interact with the thiol groups of many vital enzymes and inactivate them (Feng, *et al*, 2008), (Matsumura, *et al*, 2003). The bacterial cells in contact with silver take in silver ions, which inhibit several functions in the cell and damage the cells. Then, there is the generation of reactive oxygen species, which are produced possibly through the inhibition of a respiratory enzyme by silver ions and attack the cell itself. Silver is a soft acid, and there is a natural tendency of an acid to react with a base; in this case, a soft acid to react with a soft base.

The cells are majorly made up of sulfur and phosphorus which are soft bases (Morones, *et al*, 2005). The action of these nanoparticles on the cell can cause the reaction to take place and subsequently lead to cell death. Another fact is that the DNA has sulfur and phosphorus as its major components; the nanoparticles can act on these soft bases and destroy the DNA which would definitely lead to cell death. The interaction of the silver nanoparticles with the sulfur and phosphorus of the DNA can lead to problems in the DNA replication of the bacteria and thus terminate the microbes (Prabhu, *et al,* 2009). It has also been found that the nanoparticles can modulate the signal transduction in bacteria. It is a well established fact that phosphorylation of protein substrates in bacteria influences bacterial signal transduction.

Dephosphorylation is noted only in the tyrosine residues of gram-negative bacteria. The phosphotyrosine profile of bacterial peptides is altered by the nanoparticles. It was found that the nanoparticles dephosphorylate the peptide substrates on tyrosine residues, which leads to signal transduction inhibition and thus the stoppage of growth (Shrivastava, *et al*, 2007).

2. Experimental

2.1 Synthesis of Ag NPs by Hot Chemical Reduction Method

A 50 ml as a starting solution of ~ 5.0 x 10^{-3} M $AgNO_3$ in water (0.0425 g in 50 mL deionized H_2O) was prepared, in order to reach 10^{-3} M concentration, 25 mL of $AgNO_3$ solution was added to 100 mL of H_2O (now ~1.0 x 10^{-3} M). On the other hand, a solution of 1% sodium citrate (0.5 g in 50 mL of H_2O) has been made, then heated 125 mL solution of $AgNO_3$ until it begins to boil at 97 ˚C. At a certain moment of boiling, 5 mL of 1% sodium citrate solution has been added drop-by-drop, as soon as boiling commences. Heating was continued for 5 minutes until color change became evident (pale yellow). As shown in figure 1. This process of the Ag NPs production is eco-friendly as it is free from any solvent or toxic chemicals.

Fig.1 Ag NPs which is prepared by hot chemical reduction method

2.2 Preparation of Ag NPs / hydrothermal Agar composite films by hydrothermal method

At first hydrothermal agar solution has been prepared by dissolving agar powder in deionized water via hydrothermal method. The hydrothermal synthesis is performed by placing the Ager solution (1 g agar powder dissolved in 60 ml deionized water) in the autoclave, then applying the voltage of about 43V (current = 2 A) and set the temperature to be 60 ^{o}C by temperature controller. After 30 min to put the solution in autoclave under high pressure and temperature higher than room temperature, Turn off the system and leave the prepared gel for 10 sec). After that, cast the gel in the Petri dish and leave it dry at room temperature, Figure 2 illustrates the hydrothermal setup.

Fig. 2 Hydrothermal setup

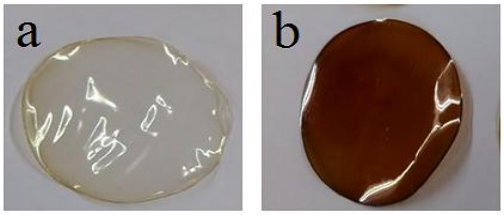


Fig. 3 Preparation of Ag NPs / hydrothermal agar composite films by hydrothermal method with two concentrations of Ag NPs: hydrothermal agar, (a) 1.5:3.5 and (b) 2.5:2.5

2.3 Antibacterial Assay Procedure

Agar diffusion technique was applied to study the antimicrobial effect of the Ag NPs. At first, a total of 0.1 ml of bacterial suspension that were cultured over night at 37 °C in the nutrient broth and used as inoculums then the turbidimetry of the suspension was adjusted to the McFarland 0.5 turbidity standard (10^8cfu/ml) was poured on each plate containing nutrient broth (Owen, *et al*, 2007). The lawn culture were prepared by sterile cotton swab and allowed to remain in contact for 1 min. Ag NPs were prepared by hot chemical reduction according to the description given in section 2.1 then volumetric ratio of Ag/Agar were prepared by hydrothermal method according to the description given in section 2.2. A well method was employed by making holes using cork borer in the nutrient broth (5 mm in diameter and 4 mm in depth). Then we placed in incubation at 37°C for 24 hrs. Inhibition zones were across the diameter of each well. Complete resistance of bacterial isolates to the tested agent was indicated when there were no zones of inhibition The Petri dishes were incubated at 37 °C for 24 h and the inhibition zone around each well was measured in mm. Percentage of bacterial growth inhibition was calculated as per the equation of Shahi *et al* (Shahi, *et al*, 2003).

BGI % = (BC –BT) x 100 / BC
Where, BGI = Bacterial Growth Inhibition.
BC = Number of Bacterial Colonies in the Control.
BT = Number of Bacterial Colonies in the Treatment.

3. Results and Discussion

3.1 UV–Vis spectroscopy

A simple hydrothermal method has been used to prepare agar gel which has led us to prepare high concentration, micro structurally agar hydrogels. The resulting UV/vis absorption spectra of the Ag NPs / hydrothermal agar films at two different concentrations of Ag NP: hydrothermal agar is shown in Figure 4The silver colloidal was prepared by using hot chemical reduction method according to the description given in section 1. All solutions of reacting materials were prepared in distilled water. Then Ag colloidal solution has been mixed with agar solution at two different concentration of Ag NPs : Agar (1.5:3.5, 2.5:2.5), in order to study the nature and structure of metallic Ag nucleated and grown within hydrothermal agar gels and then study its antibacterial effect. Figure 3 shows two Samples of prepared Ag NPs with agar solution by hydrothermal method.

The characteristic properties of the composite films were also greatly influenced by changing the concentration of Ag NPs added. It can be concluded that optical plasmon resonance absorption peak of Ag NPs / hydrothermal agar films fixed with a maximum of 431.6 nm and increasing the SPR peak intensity with increase in the concentration of Ag NPs, i.e., a smaller particle size obtained when we use the hydrothermal agar, avoid the continuing growth of Ag NPs. It is worth mentioning the reduction of silver ions by hydrothermal agar was taking place from the beginning, before gel formation i.e., that the hydrothermal agar works here as a perfect capping agent, and the results showed that the smallest particle sizes of Ag NPs were obtained within the high concentrated agar obtained hydrothermally, which means that the denser hydrogel networks with high homogeneity and low defect lead to the formation of the lower sized of Ag NPs networks with high homogeneity and low defect lead to the formation of the lower sized of Ag NPs.

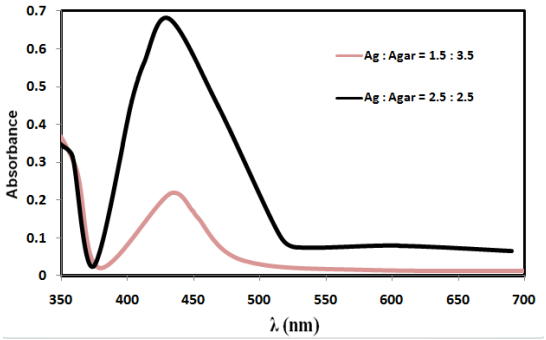


Fig.4 The absorption spectrum of Ag NPs / hydrothermal agar at two different concentration

3.2 SEM Analysis of Ag NPs / Hydrothermal Agar

Ag NPs /hydrothermal agar films was observed and analyzed by the FE-SEM and these SEM images showed relatively spherical shape nanoparticles as shown in Figure 5. Thus we can observed that the hydrothermal agar as a dense polymer matrix, produce smaller Ag NPs since it could be avoid the formation of larger AgNPs. The size of the Ag NPs in the organic polymer matrix (hydrothermal) increased as the content of Ag NPs increased. These results are consistent with the results of optical property test.

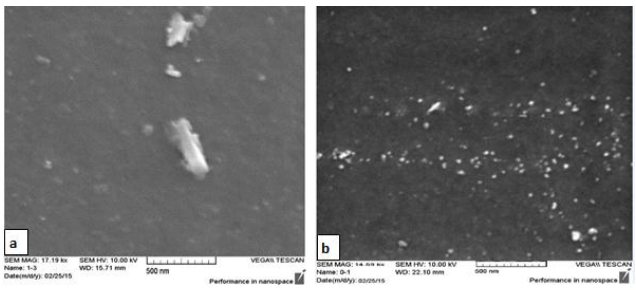


Fig.5 Shows the SEM image with magnification force = 14.59 kx of Ag NPs / hydrothermal agar film with concentration of Ag NP / agar : (a) 1.5 : 3.5 (b) 2.5:2.5

3.3 AFM Analysis of Hydrothermal Agar

A simple hydrothermal method has been used to prepare for high concentration, microstructurally agar

hydrogels. The hydrothermal treatment produces a sol dispersion ideally suited for preparation of dense agar gels at temperatures little higher than room temperature. The microstructure of these gels was studied by AFM analysis. Figures 6 show the AFM images corresponding to hydrothermal agar gel. It can be seen how the number and size of pores formed by the hydrothermal agar polymer change substantially getting homogenous. From the figure 6, we clearly observed that the hydrothermal method presents certain unique characteristics thus, it allows to easily obtain well mixed, very homogeneous, bubble free hydrogels.

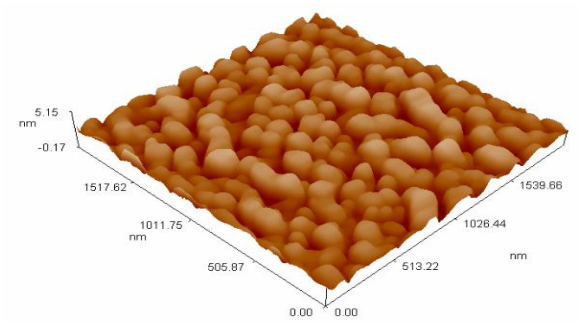


Fig.6 AFM result for Hydrothermal Agar with Average Diameter 89.12 nm

3.4 EDX analysis

Energy dispersive X-ray spectroscopy (EDX) explain the chemical analysis of synthesized silver nanoparticles. Figure 7 show the Ag NPs / hydrothermal agar film, the strong signals was obtained (at the energy of 3 keV) for silver, carbon, Sulfur and sodium and weak signals of Iron, Phosphorus and Chlorine silver. Table shows the Element analysis results Ag NPs / hydrothermal agar film determined by EDX. Similarly according to an earlier report, silver nanoparticles showed an EDX spectrum, with emission energy at 3 keV for silver and weak signals for other elements (M. Vanaja, *et al*, 2013) ,(Gaddam, *et al* ,2014).

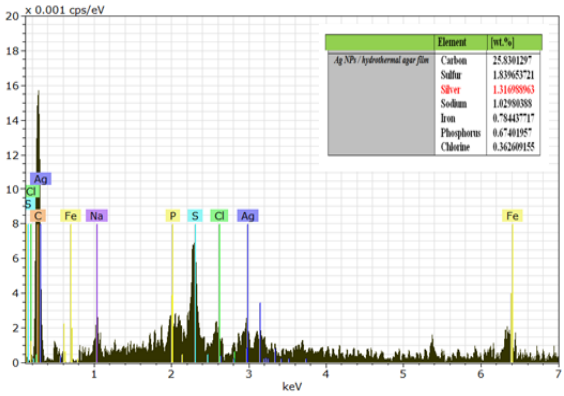
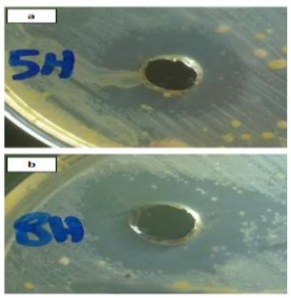


Fig.7 EDX spectrum of synthesized Ag NPs / hydrothermal agar film

3.5 Antibacterial Activity of Ag NPs / hydrothermal Agar

Silver has long been considered as a powerful and natural antibiotic and antibacterial agent. Silver nanoparticles exhibit antimicrobial properties against bacterial pathogens with close attachment of the nanoparticles themselves with the microbial cells. The antimicrobial activity of the hydrothermal agar and Ag NPs / agar films was tested against Gram-negative (E. coli urea tract infection bacteria using well diffusion method, and the results are shown in table and figure (8). As expected, the control agar film did not show any antimicrobial activity against both Gram-positive and Gram-negative bacteria. However, the Ag NPs containing composite films exhibited clear antimicrobial activity and their antimicrobial activity was dependent on the concentration of Ag NPs and the type of microorganisms tested. The studied Ag NPs were completely inactive toward Gram-positive bacteria.

While the studied Ag NPs exhibited excellent antibacterial activity which observed by increased zone of inhibition against Gram-negative bacteria depending on the concentration of Ag NPs.The results of antimicrobial activity of Ag NPs / hydrothermal agar (are increased with increased the Ag NPs concentration. The present result is also consistent with the well known belief that the smallest particle size of Ag NPs has stronger antimicrobial activity. This result agrees well with previously reported result of Rhim *et al*.[Rhim, *et al*, 2014][Rhim, *et al*, 2013].



Concentration of AgNPs :hydrothermal agar	Zone of inhibition (mm) of AgNPs: hydrothermal agar
1.5:3.5	20
2.5:2.5	24

Fig.8 Zone of inhibition for Ag NPs against E. coli At two different concentration of Ag NPs :a)1. 5:3.5, b) 2.5:2.5 .

Conclusions

The stability of Ag NPs which is prepared by fast, convenient and environment friendly method for the synthesis of silver NP by reducing silver nitrate with agar as a biopolymer matrix is stronger when we use the high concentrated agar (which prepare from simple hydrothermal method) The smallest particle size of Ag NPs in hydrothermal agar has stronger antimicrobial activity. Higher concentration of Ag NPs exhibited strong antimicrobial activity against the Gram-negative pathogenic bacteria.

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