

Antibacterial activity of zinc oxide with silver nanoparticles

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Suggested Citation:

Bourfaa, F., Boutelala, A., Aida, M. S., Attaf, N. & Merouane, F. (2015). Antibacterial activity of zinc oxide with silver nanoparticles. *World Journal of Environmental Research*. 7(2), 98-107.

Received June 21, 2017; revised October 21, 2017; accepted December 5, 2017

Selection and peer review under responsibility of Prof. Dr. Haluk Soran, Near East University.

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Abstract

Nanoscale materials and their unique properties make them attractive for research and health-related applications. Silver nanoparticles were synthesised and incorporated in zinc oxide (ZnO) thin films on glass and tissue, in order to study their effect on antibacterial activity. ZnO films with various contents of Ag nanoparticles were prepared by the sol-gel method. X-ray diffraction revealed the polycrystalline structure of the films, scanning electron microscopy exhibited their dense and continuous structure and UV-visible spectroscopy for measurement and transmittance was at more than 87%. The absorbance peak of Ag NPS was centred at 351 nm. The diameter of Ag NPS was analysed by Zeta Sizer and the colloids ranged from 2 to 110 nm. The results indicate that ZnO with Ag NPs on tissue can resist the growth of this kind of bacteria, with the zone of inhibition of the bacteria ATTC 700603 at between 8 and 18 mm.

Keywords : Ag nanoparticles, zinc oxide, tissue, antibacterial activity.

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1. Introduction

Metal thin films used in various technological applications are predominantly governed by their properties. Among these, zinc oxide (ZnO) thin films have been intensely studied as a promoting material for many industrial applications.

ZnO is a unique semiconducting material with a wide direct band gap of 3.37 eV and a large exciton-binding energy of 60 meV at room temperature. It has attracted increasing interest due to its potential applications for optoelectronic devices operating in the visible and near ultraviolet regions (Yu, Li & Hu, 2008). The structural, physical, optical and electrical properties of ZnO films are closely related with the deposition method and its parameters (Ndong, Deannoy, Boyer, Giani & Foucaran, 2003), post-treatment (Alasmar, Ferblantier, Mailly, Gallborrut & Foucaran, 2005) and dopants (Buchholz, Chang, Song & Ketterson, 2005; Gomez, Maldonado, Dela, Olvera & Acosta, 2005; Kang et al., 2006; Lin & Huang, 2006; Ueda, Tabata & Kawai, 2001) such as Al, Ga, Y, Mn, Cu and Ag. Ag is a good electric conductor with a relatively low optical absorption coefficient in the visible region. As a group IB element, if incorporated on substitutional Zn sites, Ag can also act as an acceptor in ZnO (Fan & Freer, 1995).

Nanomaterials often show unique and considerably changed physical, chemical and biological properties compared to their macro-scaled counterparts (Li, Hu, Yang & Alivistos, 2001).

Synthesis of noble metal nanoparticles for applications such as catalysis, electronics, optics, environmental, and biotechnology is an area of constant interest (Albrecht, Evans & Raston, 2006; Burlison, Driessen & Penn, 2005; Cheng, 2005; Hussain, Brust, Papworth & Cooper, 2003; Masciangioli & Zhang, 2003; Obare & Meyer, 2005; Yuan, 2005). Gold, silver and copper have been used mostly for the synthesis of stable dispersions of nanoparticles, which are useful in areas such as photography, catalysis, biological labelling, photonics, optoelectronics and surface-enhanced Raman scattering detection (Kearns, Foster & Hutchison, 2006; Smith, Duan, Rhyner, Ruan & Nie, 2006).

Generally, metal nanoparticles can be prepared and stabilised by physical and chemical methods; the chemical approach, such as chemical reduction, electrochemical techniques and photochemical reduction is most widely used (Chen, Cai, Zhang, Wang & Zhang, 2001; Frattini, Pellegrini, Nicastro & de Sanctis, 2005). Studies have shown that the size, morphology, stability and properties (chemical and physical) of the metal nanoparticles are strongly influenced by the experimental conditions, the kinetics of interaction of metal ions with reducing agents and adsorption processes of stabilising agent with metal nanoparticles (Knoll & Keilmann, 1999; Sengupta et al., 2005). Hence, the design of a synthesis method in which the size, morphology, stability and properties are controlled has become a major field of interest (Wiley, Sun & Xia, 2007).

All research works use ZnO in various fields and applications of different size, shape and powder or thin films, but in this study, we are interested in the deposition of ZnO by sol-gel on tissue and the inclusion of Ag nanoparticles at different concentrations with the aim to improve the antibacterial activity.

2. Material and Method

2.1. Synthesis of Ag nanoparticles

The silver colloid was prepared using a simple chemical method. All the solutions of reacting materials were prepared in distilled water. In typical experiments, silver nitrate AgNO_3 was dissolved in 200 ml distilled water and the solution was heated to boiling.

Then trisodium citrate was dissolved in 50 ml distilled water and 2 ml of trisodium citrate was added to the solution of AgNO_3 after boiling (drop by drop). During the process, the solution was mixed vigorously.

The solution was left on a hot plate for 2 hours at 90°C for heating only. Then it was cooled to room temperature, the colour was yellow.

The mechanism of reaction could be expressed as follows:



2.1. Preparation of ZnO thin films by sol-gel

The sol-gel solution was made at room temperature in the following way: Zinc acetate was dissolved in methanol and the solution was left after stirring at 60°C. Then monoethanolamine (C₂H₇NO) was added drop by drop. The solution was left on the hot plate for 2 hours with continuous stirring.

Dip-coating was used as a method for deposition of ZnO thin films onto glass substrates and tissue with two and five dipping. The amount of Ag nanoparticles used for each sample was varied between 5% and 10%. The samples were immersed in the sol-gel solution at a speed of 6 mm/s and dried at 100°C for 15 min, while samples on tissue were dried at 80°C for 10 min. Then ZnO thin films were deposited on glass annealed in furnace at 500°C for 2 hours.

2.2. Preparation of the bacteria

The antibacterial activity of the extract on tissue impregnated is investigated against a Gram-negative test bacterium *Klebsiella pneumoniae* American Type Culture Collection (ATCC) 700603, obtained from the ATCC.

From an 18-hour culture on the trypticase soy agar medium, a suspension of each bacteria-test in physiological water (0.9% NaCl) is prepared.

The cell density of each suspension was adjusted by dilution in sterile physiological water and in comparison with the 0.5 McFarland solutions (an optical density of 0.2–650 nm) so as to obtain a final concentration of 10⁶ UFC/ml after incorporation in Mueller Hinton medium (Cavalla & Eberlin, 1994).

2.3. Antibacterial activity

The pieces of tissues with diameters of 5 and 10 mm are sterilised in Petri dishes made of glass in the Pasteur oven (180°C for 30 min). The tissues are then covered with a thin layer of the Mueller-Hinton medium previously inoculated with the test bacterium.

Before incubation at 37°C, the optimum growth temperature of the test strain, the dishes were left for 2 hours at + 4°C to allow pre-diffusion of the bioactive substances (Tortorano et al., 1979). The results were read after 24 hours of incubation. Any zone of inhibition of bacterial growth around and on pieces of tissue, even of small diameter, is considered as a positive result.

Films structural properties were determined by XRD using Philips X'Pert system with CuK α radiation ($\lambda_{\text{Cu}} = 0.154056$ nm). The diffract meter reflections were taken at room temperature and the 2θ value were varied in the range of 20–70°. Film morphology was analysed using scanning electron microscope (SEM). Optical transmission and absorption in the UV-visible range (200–800nm) measurements were performed using the Shimadzu UV-3101PC spectrophotometer. The film thicknesses were estimated from fitting optical transmission data. The diameter of Ag NPs was measured by Zeta Sizer. The application interesting for study is the antibacterial activity. The *Klebsiella pneumoniae* ATCC 700603 was tested and the results indicated that zinc oxide with Ag NPs on tissue can resist the growth of bacteria.

3. Results and Discussion

3.1. Film structure

Figure 1 shows the XRD spectra of ZnO thin films deposited with different inclusions of Ag nanoparticles. As can be seen, the films deposited without Ag NPs are polycrystalline in nature with the crystal growth according to the plane (002) having a hexagonal wurtzite structure. Among the thin films with 5% Ag NPs, diffracted three peaks are recorded at 2θ angles of 31.65° ; 34.5° and 36.32° corresponding to (100), (002) and (101), respectively, of the hexagonal wurtzite structure. Moreover, the ZnO film with 10% Ag and 5 dipping exhibited a strong preference to (100) plane and a small peak at $2\theta = 66.27^\circ$, corresponding to the plane (200). This indicated that the obtained films have a hexagonal wurtzite structure. We observed that the inclusion of Ag nanoparticles with different concentrations improved the crystallisation of ZnO thin films prepared by sol-gel, especially the films elaborated with 10% Ag and five dipping.

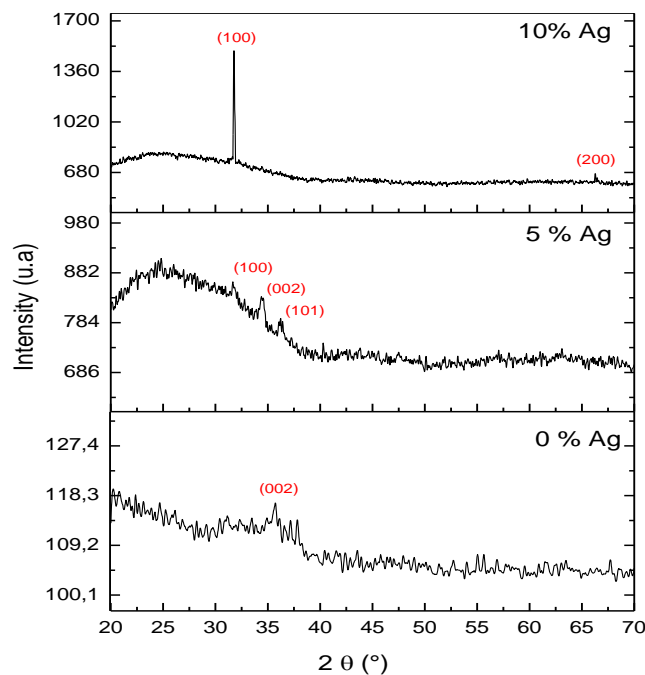


Figure 1. XRD spectrum of ZnO thin films with and without inclusion of Ag NPs

Figure 2 represents typical SEM images of ZnO thin films after incorporation of 10% Ag NPS and five dipping with two magnifications. The surface morphology of films obtained was dense and compact with the appearance of a small white spot, which is possibly related to silver nanoparticles. As a result, these characteristic of surface make the films more suitable against the growth of bacteria. So the antibacterial activity is more important when the films have a large specific surface, which is dense and compact.

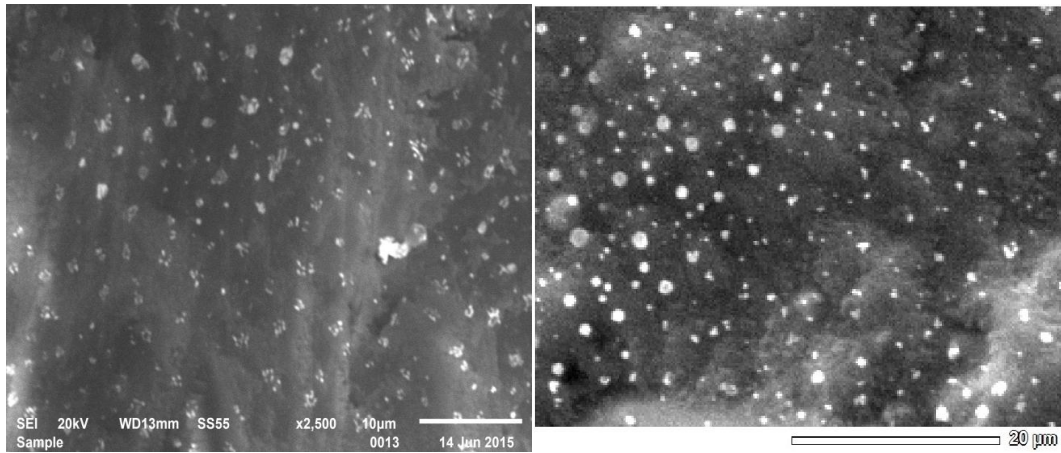


Figure 2. SEM images of ZnO films with 5% Au and five dipping

3.2. Optical properties

The optical properties of thin films of ZnO: Ag were determined from the transmission measurement in the range of 200–800 nm. Figure 3 shows the transmittance spectra of zinc acetate deposited by dip-coating onto the glass substrates with 0–5 and 10% Ag NPs for five dipping. As can be seen, the average transmittance values lies between 81% and 88%. The pure ZnO thin films obtained are highly transparent with a transmittance reaching 88% in the visible wavelength range. While the transmittance of thin films decrease with the rise in the number of dipping and with the variation of concentration of Ag nanoparticles. All the films have a steeper absorption around 300 nm.

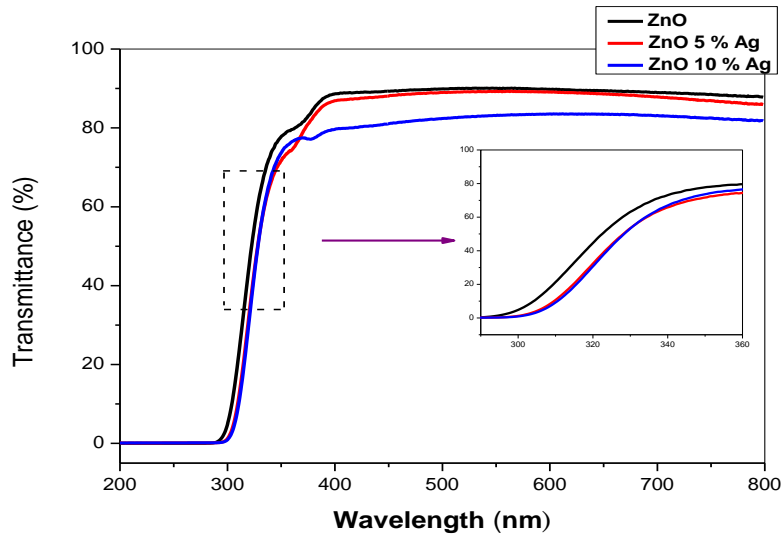


Figure 3. Transmittance spectra of ZnO thin films annealing at 500 C with five dipping and 5% and 10% Ag NPs

Optical band gaps of the films were obtained using the Tauc (1974) formula:

$$(\alpha h\nu)^n = B(h\nu - E_g) \quad (1)$$

where B is a constant, E_g is the band gap energy and n is equal to 2 for direct transition and 1/2 for indirect transition.

Figure 4 exhibits the plot of $(\alpha h\nu)^2$ versus photon energy $h\nu$ for the obtained ZnO films. The plot linearity indicates the materials of direct band gap nature. The extrapolation of the straight line to $(\alpha h\nu)^2 = 0$ axis gives the energy band gap of the film material. The obtained optical band gaps (E_g) are shown in Figure 5.

The band gap energy is ranged from 3.06 eV to 3.20 eV for the deposited ZnO thin films with the variation of Ag NPs content for five dipping. The decrease in the band gap energy of thin films with increase of the Ag nanoparticles content is mainly due to the inclusion of silver nanoparticles. From the enlarged area of the absorption band edges in the inset, it can be seen that when Ag doping concentration increases, the absorption band edge is shifted to a long wavelength. The red shift demonstrates that the band gap of ZnO decreases when Ag doping density increases (Xian et al., 2013).

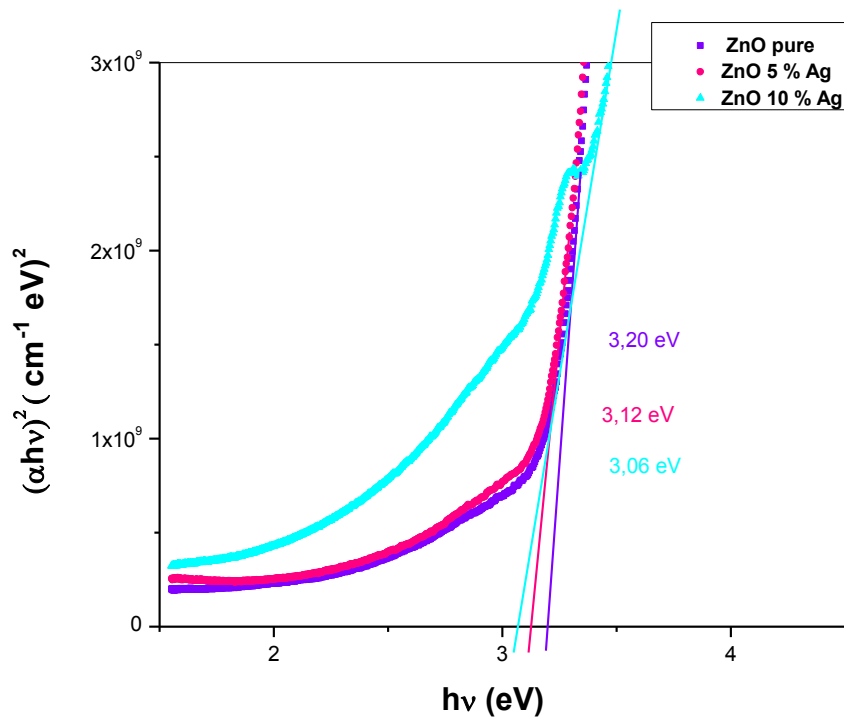


Figure 4. The typical variation of $(\alpha h\nu)^2$ versus photon energy of ZnO thin films annealed at 500 C

Figure 5 shows the absorbance spectra of silver nanoparticles. As observed, the spectra reveal a characteristic absorption peak of Ag nanocomposite material at 351 nm. The presence of this peak was a result of the surface plasmon resonance of silver nanoparticles (Jianshen, Zhang, Wang & Tang, 2008). Many studies have found the peak of absorbance to be centred at 360 nm (Karami & Fakoori, 2011; Ni et al., 2007). So Ag nanoparticles strongly depend on the experimental conditions: the quantity of Ag in the solution, the temperature, the reducing agent and the stabiliser or protecting agent according to Amany, El-Kheshen and Gad El-Rab (2012).

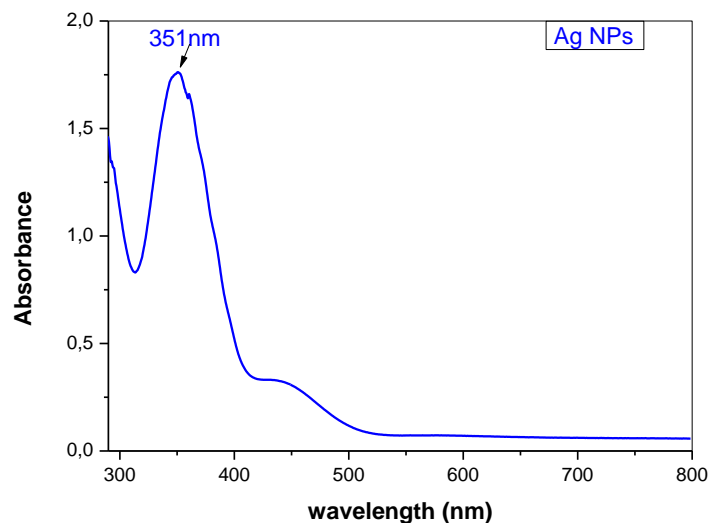


Figure 5. Absorbance spectra of Ag nanoparticles

Figure 6 presents the size distribution by intensity of Ag nanoparticles measured by Zeta Sizer. As shown, the colloids obtained have a small size of particles ranging from 2 to 110 nm.

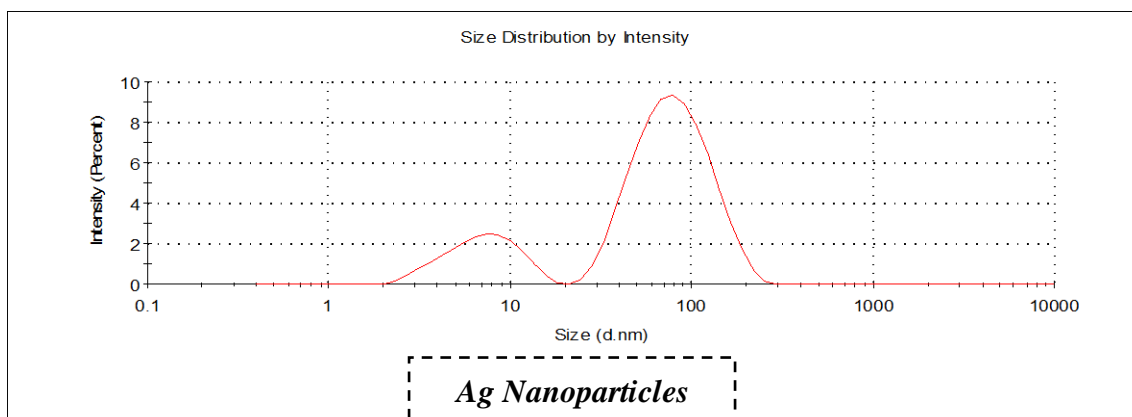


Figure 6. Size distribution by intensity of Ag nanoparticles

4. Antibacterial Activity

In this work, the antibacterial effect of the prepared samples of ZnO with Ag NPs on tissue was studied on Gram-negative bacteria of *Klebsiellapneumoniae* ATCC 700603.

Table 1 and Figure 7 exhibit the inhibition zone of different pieces of tissue with and without ZnO and Ag NPs for five dipping for two diameters, 5 and 10 mm. The results indicate that the zone of inhibition (mm) is about 0, 12, 14 and 18 mm in diameter for reference, ZnO pure with five dipping, ZnO 5% Ag with five dipping and ZnO 10% Ag with five dipping, respectively. So the results are positive. As can be seen, the ZnO samples on tissue with diameter of 10 mm can resist the growth of bacteria, while in the species without ZnO as a reference the bacteria grow on its surface.

However, the samples of ZnO with Ag nanoparticles have a bigger zone of inhibition than ZnO pure. This means that the addition of Ag nanoparticles in the solution of ZnO has an effect on the bacteria, so silver nanoparticles are harmful to bacteria (Chwalibog, Sawosz & Hotowy, 2010). Consequently, zinc oxide and silver nanoparticles could resist the growth of this bacteria ATCC 700603, but together have more efficiency on *Klebsiellapneumoniae*. Finally, smaller particles of Ag NPs having a larger

surface area for interaction and have efficient bactericidal effect. These antibacterial properties can be used to prevent and reduce the bacteria used in this work.

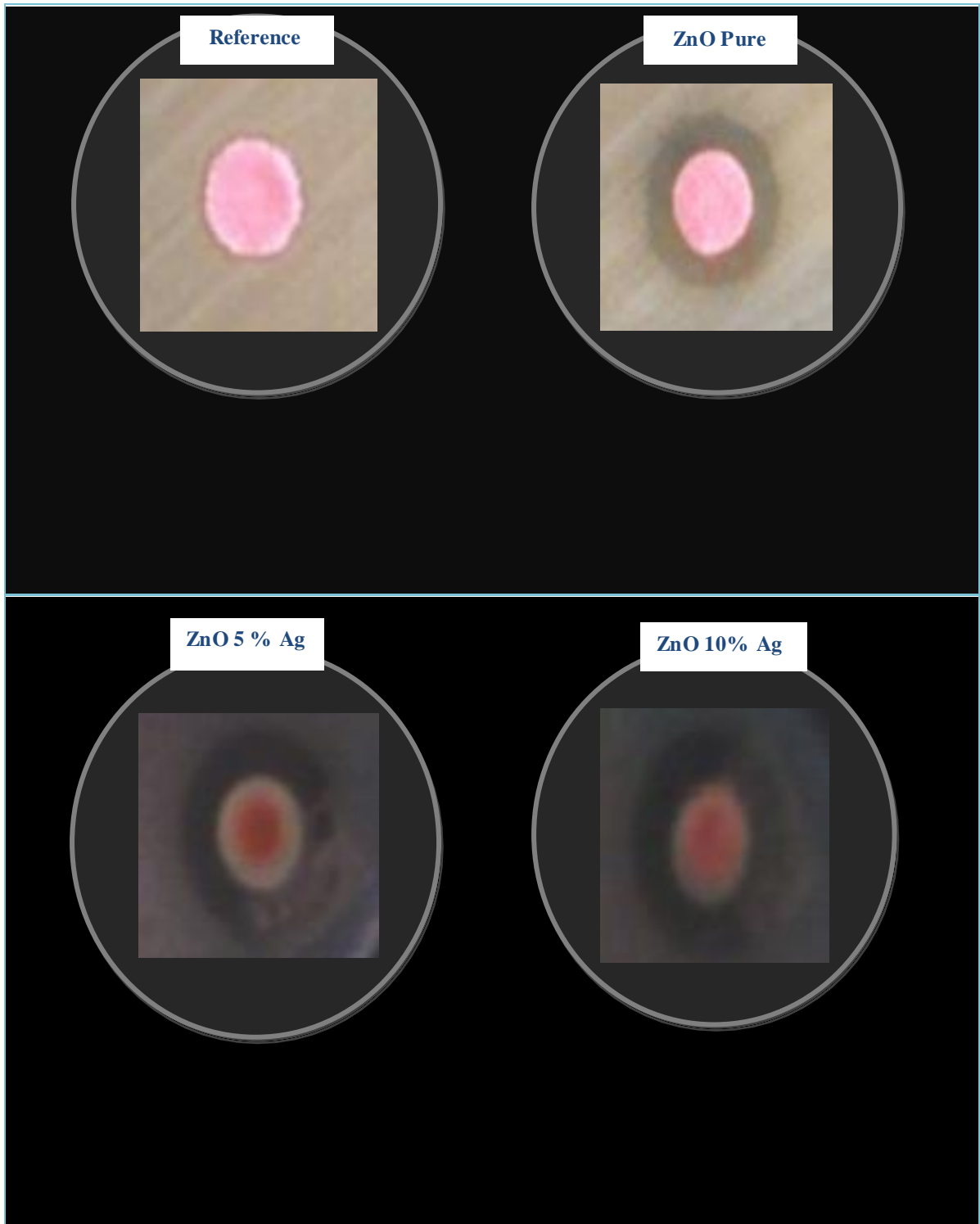


Figure 7. ZnO with inclusion of Ag NPs deposited on tissue for five dipping and tested by *Klebsiellapneumoniae* bacteria

Table 1. Zone of inhibition of ZnO with Ag nanoparticles for five dipping against bacteria tested

Samples	Zone of inhibition
Reference	0
ZnO Pure	12
ZnO 5% Ag NPs	14
ZnO 10% Ag NPs	18

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