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Investigation of the Antibacterial Activity of Silver and Zinc-Containing Solutions and Ag:ZnO Films Against some Pathogenic Bacteria

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Abstract

Multidrug resistant bacteria are well-recognized as one of the greatest threats to human health worldwide. Antibacterial activity of nanoparticles has received significant interest worldwide particularly by the implementation of nanotechnology to synthesize particles in the nanometer region. The antimicrobial effects of Ag, ZnO mixed solutions were examined in this work. Silver nitrate and zinc acetate were precursors for preparing two solutions (referred to as Sol_1 and Sol_2). These solutions contained well-known active antibacterial ions: Zn^{+2} and Ag^{+}. The mixed solutions were made using Sol_1 and Sol_2 at three volume ratios (0.2, 0.4 and 0.8 %). The antibacterial activities of the mixed solutions were studied against *Klebsiella pneumoniae; Acinetobacter baumannii; Escherichia coli; Pseudomonas aeruginosa* and *Staphylococcus aureus* using the agar well diffusion method. All mixtures had an inhibitory effect against all pathogenic bacteria but with different inhibition zones. These zones were compared with those formed by Sol_2 only to investigate the effect of Ag particles. The coexistence of nano and micro Ag particles was one of the important factors used to explain the results of inhibition zones. Utilizing the spin coating method, mixed solutions were deposited on glass substrates to produce three silver doped zinc oxide (Ag: ZnO) films. An experimental study of antiadhesive effects of Ag:ZnO films against pathogenic bacteria *Pseudomonas aeruginosa* has been performed. These effects increased by increasing the silver amount as a dopant material inside ZnO matrix.

Keywords: Antibacterial activities, ZnO films, Pathogenic bacteria, *Acinetobacter baumannii , Klebsiella pneumoniae.*

1. Introduction

Multidrug resistant (MDR) bacteria are one of the most important current threats to public health. Typically, MDR bacteria are associated with nosocomial infections. However, some MDR bacteria have become quite prevalent causes of community-acquired infections (van Duin and Paterson, 2016). The necessity to create active antibacterial materials inspires the researchers in the field of biomaterials to find new materials with efficient antimicrobial properties. The classic antibacterial materials become inoperative and new ones must originate. A logic substitute is the syntheses of new antibacterial materials including inorganic compounds that have unique properties such as a physical and chemical stability, durability, being easy to formulate and extract from available and cheap raw materials (Akindoyo *et al*., 2016).

Zinc oxide (ZnO) and silver compounds have been known as antimicrobial materials for years, and they have applications in different biological fields including antibacterial creams and wound healing creams (Emamifar *et al*., 2011). ZnO matrix has been demonstrated to have inhibition against micro organisms' growth (Salman *et al*., 2018). Also, this material is a well-Known antibacterial agent (Jones *et al*., 2008). Some reports attribute the antibacterial activity of this oxide to the generation of reactive oxygen species on its surface (Sawai and Oshikawa, 2004). The probable mechanism for zinc ion antibacterial action is the binding with the outer shell (membrane) of microorganisms (Jiang *et al*., 2018). Also, zinc may lengthen the period of growth cycle and then increase organisms generation time (cell division will need more time) (Atmaca *et al*.,1998).

As an antibacterial agent, there is yet another element; it is silver which is non-toxic with low concentration in human cells (Pal *et al*., 2007). Ag^{+} ions are released by silver species and kill the bacteria by interacting with its proteins affecting the replication of DNA (Marini *et al*., 2007). These ions may kill bacteria by different ways; they attack the cell wall which has a negative charge and change the permeability of it and then deactivate the cellular enzymes (Feng *et al*., 2000). In this contribution there is an attempt to test the antibacterial activity of a solution (used in sol gel technique for the preparation of

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ZnO thin films) and its mixture with silver colloid, and the anti-adhesive effect of Ag doped ZnO films.

2. Materials and Methods

2.1. Preparation of Silver Nanoparticles

The details of silver nanoparticles preparation was described elsewhere by Ratyakshi and Chauhan (2009). 50 mL of 0.001 M $AgNO_3$ was heated to the point of boiling, and then 5 mL of 1 % trisodium citrate was added drop by drop to this solution. The solution was heated until a change of color was evident (pale yellow); this solution will still be referred to as (*Solution no.1*). To characterize the produced silver, its film was deposited by a spin coating method on glass substrates using solution no.1. The following paragraph shows the details of the spin coating method.

2.2. Preparation of ZnO Films

To deposit the ZnO film, a microscope glass slide was used as substrate with the dimensions (75 × 25 × 1 mm). The cleaning of the substrates was done by washing them with ethanol and then distilled water. Sol gel solution was prepared by adding 3.1 g of Zinc acetate dihydrate Zn $(CH_3COO)_2$. $2H_2O$ to isopropanol alcohol. After that, 0.86 g of monoethanolamine (MEA) was added to yield a homogeneous and clear solution. The solution was, then, aged at room temperature for one day (Habibi and Sardashti, 2008). This solution will be referred to as (*Solution no.2*). A spin coating machine was utilized with the speed of 2000 rpm. Using a micropipette, 100 µL of the prepared Solution no.2 was injected on the substrate surface when the speed reaches the 2000 rpm. Then under ambient conditions, the substrate was heated to 400 ^{0}C for two hours.

2.3. Characterization Techniques

To determine the orientations of the deposited films, Shimadzu X-ray diffractometer was used. Optical properties of the samples were characterized using UV-Visible 1800 spectrophotometer (Kumar and Rani, 2013).

2.4. Preparation of the Samples with Antibacterial Activities

Antibacterial tests were done on two sample groups; group one was prepared as follows: Three beakers were filled with 5mL of solution no.2 and then solution no.1 was added to them with different volumes as tabulated in Table 1.The mixture was stirred for fifteen minute to obtain homogeneous solutions. The tests on these samples were compared with the test on the control sample containing solution no.2 only.

Table 1. The samples' codes of group one and their components

Sample code	Solution no.1	Solution no.2
1	100µL	50mL
2	200µL	50mL
3	400µL	50mL

Group two was prepared as follows: Three silver doped ZnO (Ag:ZO) films were deposited on glass substrates by spin coating (the same procedure of ZnO thin film preparation). The solutions of samples 1, 2, and 3 were injected respectively (each had the volume of 150 µL) on rotating glass substrates at 2000 rpm. The deposited films were annealed at 400 0C for two hours. These films will be referred to as SZO1, SZO2, and SZO3 (Singh *et al*., 2017).

2.5. Evaluation of Samples' Antibacterial Activities

Samples 1, 2 and 3 were screened for their antibacterial effects against pathogenic bacteria (*Klebsiella pneumoniae; Acinetobacter baumannii; Escherichia coli; Pseudomonas aeruginosa and Staphylococcus aureus*). These isolates were obtained from the Department of Biology, College of Science, Mustansiriyah University. The plates were prepared by spreading approximately 10^5 CFU/mL of the culture broth of each indicator bacterial isolates on the nutrient agar surface. The agar plates were left for about fifteen minute before aseptically dispensing the 50µL of the tested samples into the agar wells already bored in the agar plates. The plates were then incubated at 37 °C for eighteen – twenty-four hours. Zones of inhibition were measured and recorded in a millimeter diameter (Salman, 2013).

2.6. Anti-adhesive Effects

The anti-adhesive effects of the SZO1, SZO2, and SZO3 samples against *Pseudomonas aeruginosa* were determined. The bacterial suspensions were poured onto the samples and were allowed to settle on the top of the film; the control was the ZnO film without silver. All the coated samples and control were incubated for twenty-four hours at 37^0 C. The unattached bacterial cells were removed by being washed with water three times. They were then dried for fifteen minutes at room temperature. After drying, 1 % of the crystal violet stain was added to the plates for twenty minutes. The stained attached bacterial cells were rinsed with distilled water three times, and were allowed to dry for fifteen minutes at room temperature. They were extracted twice with ethanol (95 %) (Ali, 2012; Salman *et al*., 2014), and the absorbance was measured by a spectrophotometer at 590 nm using the equation bellow:

$$\text{Adhesion formation \%} = [1- (A/A_o)] \times 100$$

Where A is the absorbance of the coated slide and A_o is the absorbance of the control slide

3. Results

3.1. Structural and Optical Properties

Figure 1 shows the XRD pattern of the deposited Ag films by spin coating using solution no.1 with three different degrees of thickness 1µm, 2µm, and 3µm. The indexing of the data was carried out using a standard PDF file number (411402). Peaks of figure 1 confirm the formation of the Ag film. The intensity of the dominant peak (004) was increased with increasing the film thickness.

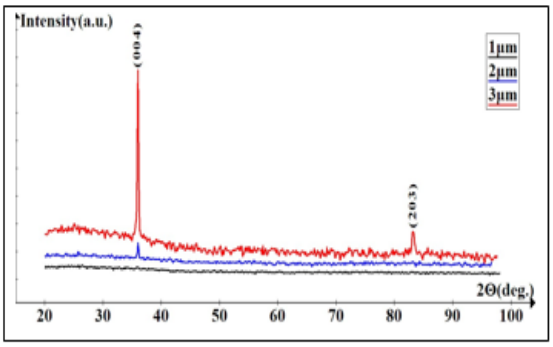

Figure 1. XRD of Ag films with different thicknesses.

Figure 2 illustrates UV-VIS absorption spectra for solution no.1. There are three intense peaks observed in the ultraviolet region (200, 247 and 275 nm). The cut off wavelength of the absorption of silver particles is 320 nm. A peak around 420 nm is a characteristic of the spherical silver nanoparticles.

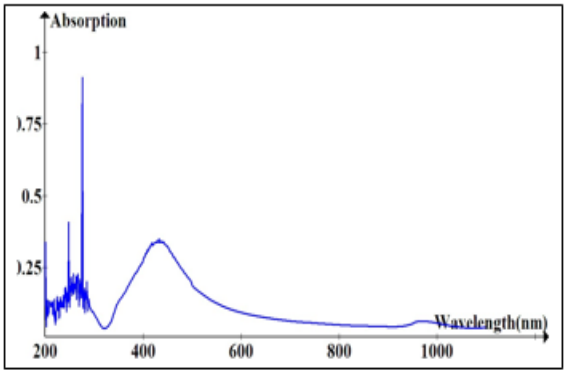

Figure 2. UV-VIS absorption spectra of solution number**.**

Figure 3 shows the XRD patterns of the pure ZnO and SZO films. The SZO films had an amorphous structure with formation beginning of the ZnO peaks (100), (002), (101), and (102). These peaks are relatively strong for SZO_1 (where there was a little silver amount), and then their intensities decreased for the SZO_2 and SZO_3 samples (where there was much silver amount).

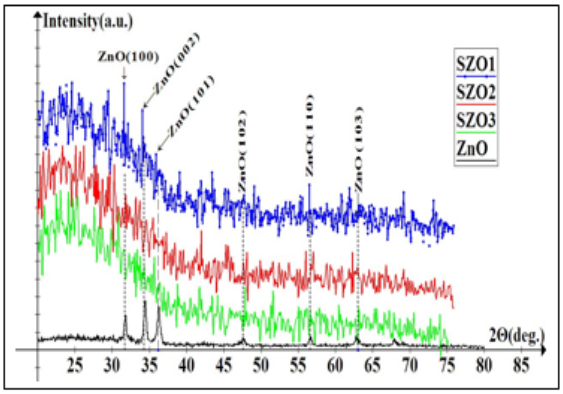

Figure 3. XRD patterns for pure ZnO and Ag doped ZnO films.

The antibacterial activities of synthesized samples, inhibition zones created by sample 1, 2, and 3, and the control samples are shown in table 2 and Figure 4.

Table 2. Diameters of inhibition zones for 1, 2, 3 and the control samples.

Bacterial Isolates	Inhibition zone (mm)			
	1	2	3	Control sample
K. pneumonia (Gram –ve)	29	35	29	42
E.coli (Gram –ve)	26	27	32	36
A. baumannii (Gram –ve)	26	27	32	40
S. aureus (Gram +ve)	38	30	37	33
P.aeruginosa (Gram –ve)	30	41	28	36

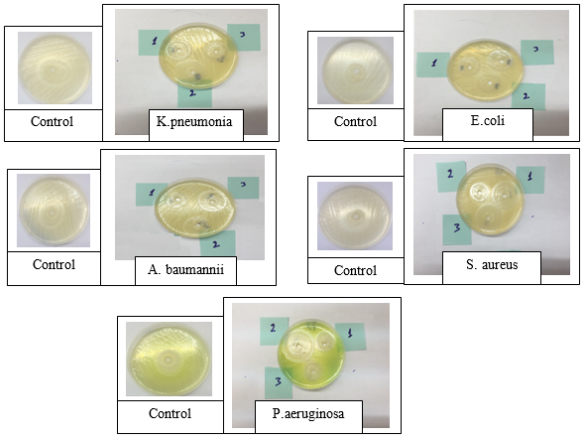

Figure 4. Inhibition zones of 1, 2, 3, and the control samples.

1= (sample 1) , 2= (sample 2) ,3= (sample 3)

The addition of Sol_1 to Sol_2 increases the inhibition against *S. aureus* bacteria. To understand the effects of this addition on the values of inhibition zones in table 2, The SEM image in Figure 5 may give more explanation.

The anti-adhesive effects (%) of the SZO_1, SZO2, and SZO_3 samples against pathogenic bacteria *Pseudomonas aeruginosa* were 45 % , 57.7 %, and 59.29 % respectively .

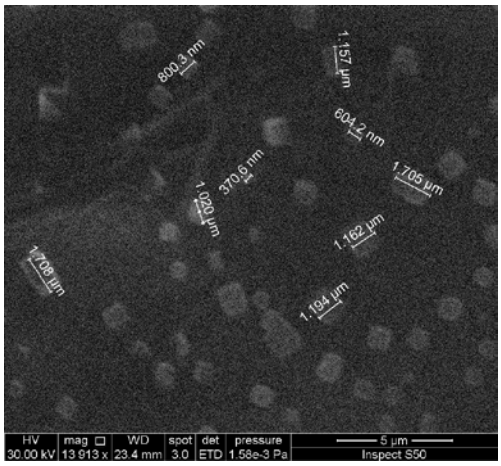

Figure 5. The deposited Ag particles on glass substrate.

4. Discussion

Inorganic antibacterial agents such as metal and metal oxides are advantageous compared to organic compounds due to their stability. Among these metal oxides, ZnO has attracted a special attention as an antibacterial agent (Salem *et al*., 2015). In the current study, silver and zinc-containing solutions and Ag:ZnO film were prepared, and the orientations of deposited films were determined. The

intensity of the dominant peak (004) was increased with increasing the film thickness; this refers to increasing the crystallinity of the deposited Ag films with the increasing of the thickness. The cut off wavelength of the absorption of silver particles was 320 nm. This result is similar to that obtained by Budhiraja *et al*. (2013), who studied optical properties of silver nanoparticles. A peak around 420 nm is characteristic of spherical silver nanoparticles. The decreasing of the XRD pattern intensities of SZO with the increasing of the silver amount was observed by Jeong *et al*. (2005), who attributed this decreasing to the Ag^{+} substitution into the ZnO^{+} site. The following notes can be observed from Table (2): For *K. pneumonia, E.coli and A. baumannii*; control sample has a wider inhibition zone than that of the samples 1, 2, and 3. The control sample had a minimum inhibition zone for *S. aureus.* The increasing of silver widens the inhibition zones against *E.coli* and *A. baumannii* bacteria for sample 1, 2, and 3. All of the samples had different inhibitory effects against the used pathogenic bacteria.

Zinc acetate was the source of zinc ion (Zn^{+2}) due to the easy dissolution inside water (Atmaca *et al*., 1998). This ion has antibacterial properties due to its binding to the cell wall of the bacteria allowing for cytotoxic effects **(**Jeong *et al*., 2005). The comparatively wider inhibition zones for control (the sample without silver ions) refer to the higher activity of zinc ion in killing Gram-negative bacteria (*K. pneumonia, E.coli, A. baumannii and P. aeruginosa*). This result disagrees with that obtained by Södeberg *et al.* (1990), who confirmed the activity of zinc ion against Gram positive bacteria. These researchers found that Gram-positive bacteria were the most susceptible bacterial group to zinc ion. The addition of Sol_1 to Sol_2 increased the inhibition against *S. aureus* bacteria. This result is in agreement with that obtained by Mirzajani *et al* (2011)**.**

The average size of the Ag particles was 1μm; this size is not the perfect size to kill all bacteria species (compared with that of nanoparticles). When Sol_1 was added to Sol_2, the relatively big silver particle sizes may resist the antibacterial activity of Zn ions against Gram-ve bacterial isolates. As a result, the inhibition zones for these bacteria species are minimized. On the other hand, the increasing of inhibition zones against *E.coli* and *A. baumannii* with increasing the amount of Sol_1 (increasing of silver ions) may be attributed to the increasing of Ag nanoparticles that coexist with the Ag microparticles. The existence of Ag nanoparticles is confirmed by UV-VIS absorption spectra in Fig (2). The unstable behavior of inhibition zones for *K. pneumonia* and *P. aeruginosa* may attribute to inhomogeneous distribution of Ag nanoparticles inside the samples. Many reports refer to antibacterial activity of ZnO, oxygen species on the surface of this oxide as the possible reason behind this merit. Stoimenov *et al.* (2002) clarified that the electrostatic forces bind ZnO nanoparticles with the bacteria and then kill them**.** Sawai (2003) revealed that the generation of hydrogen peroxide from the zinc oxide surface is an active mean for the inhibition of the bacterial growth.

5. Conclusions

The antimicrobial effects of Ag, ZnO-mixed solutions were investigated in this study. These solutions were synthesized through wet chemistry and were dispersed in aqueous solution. All mixtures showed different inhibition zones against pathogenic bacteria. These zones were compared with those formed by the ZnO solution only to investigate the effect of Ag particles. The coexistence of nano and micro Ag particles was one of the important factors used to explain the results of inhibition zones. Although the coexistence of Zn and Ag ions is not always an appropriate choice to have effects on current used bacteria, the increase of silver had widened the inhibition zones against *E.coli* and *A. baumannii* bacteria. Also, the experimental results indicated that the anti-adhesive effects of Ag:ZnO- coated films against pathogenic bacteria *Pseudomonas aeruginosa* were increased with the increment of silver as a dopant material inside the ZnO matrix.

Acknowledgments

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