Preparation and Characterization of Antimicrobial PVA/ZnO Nanocomposite for Biomaterial Applications

Ali Kareem Mohammed Al Maamori
University of Babylon, College of material engineering, Department Polymer and Petrochemicals
Sabah N. K. AL-Thomir
University of Babylon, College of Pharmacy
Aliabdahgo@gmail.com

Abstract

Control of microbial infections is a highly important issue in hospitals. Antimicrobial polymers are new kinds of antiseptics, which can be used as alternatives to disinfectants in sometimes. Zinc oxide nanoparticles (90 nm) with polyvinyl-alcohol (PVA) generated by melting route. ZnO NPs was modified by dimethyl sulfoxide (DMSO) to get on uniform distribution within the matrix and then reduce agglomeration. The purpose of this study is to determine the antimicrobial activity of PVA ZnO nanoparticles against *Gram-negative bacteria (Pseudomonas aeruginosa; Escherichia coli)* and *Gram-positive bacteria (Staphylococcus aureus; Staphylococcus epidermidis)* and *fungi (Candida albicans,)*. The effects of concentration and particle size on the antibacterial activity of ZnO nanoparticles was studied using minimum inhibitory concentration (MIC) test and the antimicrobial activity of PVA/ZnO nanocomposite was studied using agar well diffusion method. Results showed that the antimicrobial activity of ZnO nanoparticles with PVA increased with decreasing particle size and increasing concentration of ZnO NPs. Results showed that all microbes were fully inhibited at 3.7 µg / ml concentration for ZnO NPs, but activity against bacteria was not observed at 1.8 µm / ml.
Key words: PVA/ZnO nanocomposite, Antibacterial activity, agar well diffusion and MIC.

الخلاصة

السيطرة على العدوى الميكروبية هي قضية بالغة الأهمية في المستشفيات. البوليمرات المضادة للميكروبات هي أنواع جديدة من المعقمات، والتي يمكن استخدامها كبدائل للمطهرات في بعض الأحيان. تم في هذا البحث تكوين متراكب نانوي من بوليمر بولي فنيل الكحول مع الجسيمات النانوية لأوكسيد الزنك بحجم (90 نانو متر) بطريقة ذوبان المنصهر. حبيبيات أوكسيد الزنك النانوية تم تعديل سطحها بواسطة مادة الداي مثيل سلفوكساید (DMSO) وذلك للحصول تشتيت جيد ضمن المادة الأساس ومن ثم تقليل التكتل. والغرض من هذه الدراسة هو تحديد النشاط المضاد للميكروبات للمادة المركبة النانوية (بولي فنيل الكحول\اوكسيد الزنك) ضد بكتيريا غرام- موجب (Pseudomonas aeruginosa;Escherichia coli) وغرام-سالبة (Staphylococcus aureus;Staphylococcus epidermidis) والفطريات .Candida تم دراسة تأثير تركيز وحجم الجسيمات على النشاط المضاد للبكتيريا لجسيمات أوكسيد الزنك النانوية باستخدام اختبار اقل تركيز يثبط البكتيريا (MIC) وتم دراسة النشاط المضاد للميكروبات للمادة المركبة بواسطة اختبار Agar well diffusion. وأظهرت النتائج أن النشاط المضاد للميكروبات من الجسيمات ZnO مع PVA يزداد مع انخفاض حجم الجسيمات وزيادة تركيز .ZnO.

الكلمات المفتاحية :- مادة مركبة نانوية (PVA/Zuo) ، الفعالية ضد البكتريا ، طريقة اقل تركيز يثبط البكتريا (MIC).

1. Introduction

Biofilm is defined as group of microorganisms that are growing on material surfaces. Preventing of microbial biofilm forming over the surface of materials is a technological major in health care. Many types of bacteria have capacity of formation biofilm on abiotic surfaces are problems menacing in medical and industrial systems (S. M. & Natarajan, 2015). Therefore, microbial cells related to any synthetic surface in humidity environment can grow and continue to live. While the cell number increases on the surface, the microbial cells start to build up a biofilm.

Antimicrobial polymers considered as a kind of biocides that has turned out to be progressively essential as a contrasting option to existing biocides and at times even to disinfectants. The working system for many of different polymers is not understand (Siedenbiedel and Tiller, 2012). Three general kinds of antimicrobial polymers: polymeric biocides, biocidal polymers, and biocide-releasing polymers. Dispersion of useful inorganic Nano-fillers like ZnO inside polymer substrate give good photo bactericidal activity to the composite.

Zinc oxide nanoparticles have attraction interesting because they have different physical and chemical properties that are differ from bulk. ZnO is generally utilized for cosmetic application since it is a chemically stable and friendly material that has good transparency and UV-blocking properties (Jong-hun *et.al.,* 2011).

Zinc oxide is described as an inorganic substance with a wide range of uses. It was known as the II-VI semiconductors, to Zn and O arranged in the second and sixth groups in the periodic table, respectively. The zinc oxide carries an interesting optical, chemical sensor, semiconductor, electrical conductivity, and piezoelectric properties (Fan Z1, 2005). It is characterized by a large direct (3.3 eV) gap in near-term ultraviolet radiation, high exciton binding energy (60 m eV) at room temperature and natural-type electric transporters(W. J. S. M., B., & I., 2008) These properties make zinc oxide have large array applications in different fields.The aim of this study is to determine the antimicrobial activity of PVA/ZnO nanoparticles against group of different microorganisms.

2. Mechanism of Antimicrobial Activity of ZnO-NPs

The killing mechanism of nano zinc oxide indicates to different issues, where the correct toxicity system is not very clear and still controversial. There are also a few inquiries into the activity against bacteria that require deep explanations. The specific and recorded killing mechanisms are as follows: direct contact of ZnO-NPs with cell walls, or destruction of bacterial cells (Brayner *et.al.,* 2006), or the release of Zn ions as antibodies (Kasemets *et.al.,* 2009), or the formation of effective oxygen ions (Jalal et al., 2010).

It demonstrates generation of high activity groups such as OH^-, H_2O_2, and O_2^{-2} as follows. If zinc oxide has an electron-gap pair (e- h +). The energy gap in zinc oxide divides the H_2O molecules into OH^- and H^+. Thus, oxygen molecules are split into free radicals ($\cdot O^{-2}$), which in turn interact with H^+ to create free radicals ($\cdot HO_2$), which collide with the electrons that produce hydrogen peroxide (HO_2). They then interact with hydrogen ions to produce H_2O_2 molecules. H_2O_2 can generate cell membrane penetration and kill bacteria (Fang *et.al.,* 2006).

$$ZnO + hV \longrightarrow e^- + h^+,\ h^+ + H_2O \longrightarrow \cdot OH + H^+$$
$$e^- + O_2 \rightarrow \bullet O^{-2};\ \cdot O_2 + H^+ \rightarrow HO_2^{\bullet}$$
$$HO_2^{\bullet} + H^+ + e^- \rightarrow H_2O_2$$

3. Experimental Procedure

3.1 Preparation of ZnO for Minimum Inhibitory Concentration (MIC)

Depending on study (Hadi and Dawood, 2016), 10 mg of ZnO-NPs were dissolved in 10 ml dimethyl sulfoxide (DMSO) (Note: this solvent does not affect bacteria) yielding stock solutions of (1 mg/ml) concentration, after that (1 ml) of this solution was diluted to (10 ml) with DMSO again giving a solution of 100 mg/ml concentration, then from this solution, the dilutions: 30-15-7.5-3.7-1.8 were obtained.

3.2 Preparation of PVA / ZnO nanoparteciales solutions for well diffusion Method

ZnO / PVA nanocomposite have been prepared via melting route of PVA with ZnO-NPs solution in dimethyl sulfoxide (DMSO). ZnO nanoparticles was added with concentration: (0.07, 0.035, 0.017 and 0.008 wt % mg/ml) to DMSO in magnetic stirring and dispersed in ultrasonic for a period of 2h to all solutions. Then, PVA was add to all solutions in percentages of (1, 0.993, 0.995 and 0.998 g) respectively for a period of 1h in magnetic starring again in temperature of 90 ^{o}C. Thereafter, the solution was poured in culture plate of get films.

3.3 Determination of antimicrobial activity of ZnO by MIC method

Antimicrobial activities of ZnO-NPs were calculated against bacteria of: E.coli, S.aureus and Pseudomonas and Candida fungus by serial dilution during minimum inhibitory concentration (MIC) in culture broth. In this method, they used a series of diluents called twofold where the concentration was diluted in half. 1 mL of media was taken into the test tube, add 1 ml of ZnO solution (100 mg / ml), and then, 0.1 mL of all bacterial strains used were prepared in 0.9% NaCl was added to the test tube containing media and solution of composite material. It was diluted to five times and gave concentrations of 30-15-7.5-3.7-1.8 mg / mL. Nutrient broth, where samples test and control for 24-hour, it was incubated at 37 ° C. Note that control samples contain only the media and bacteria. In addition, DMSO solvent test was also performed to insure that the solvent does not affect the growth of bacteria

To calculate the bacterial effectiveness ratio for ZnO by MIC, microplate was placed inside the Eliza device (BioTek, El×800) and exposed to light at 600 nm. Then antibacterial activity values are determined by the following equation:

$$Percentage\ antibacterial\ activity\ = \frac{OD\ of\ control}{OD\ of\ control - OD\ of\ test} \times 100 \quad ...(1)$$

(Banjara *et.al.*, 2012)

3.4 Determination of antimicrobial activity of PVA / ZnO by well diffusion method

The antibacterial activity of the sample solutions was determined in accordance with the agar-well diffusion method described by (Irobi *et.al.*, 1994 ; Irobi, Moo-Young, Anderson, & Daramola, 1994).

The studied bacterial isolates included Gram-negative bacteria (Pseudomonas aeruginosa; Escherichia coli) and Gram-positive bacteria (Staphylococcus aureus; Staphylococcus epidermidis). 0.1 mL of bacteria suspensions 0.5 McFarland tube (1.5 X 108 CFU/ml) standards of gram-negative and positive were distributed into the nutrient agar (NA) medium.

A sterile swab was used to obtain an inoculum from the bacterial suspension and spreader on a Muller-Hinton agar plate. Using a sterile plastic pipette, five holes were punched in each of the culture plates with a diameter of (6) mm. One of the holes was punched in the center of the plate where 50 µl of PVA was added as positive control; 50 µl of composite solution was added as a negative control in the other hole at the Muller-Hinton agar plate. One-hour pre - diffusion time was allowed, after which the plates were incubated at 37°C for 18 hrs. The zones of inhibition were then measured in millimeter. The above method was carried out in duplicates and the mean of the duplicate results were taken.

4. Results and Discussions

4.1 Minimum Inhibitory Concentration (MIC) of ZnO

In this study, anti-bacterial activity of ZnO against E. coli, S.aureus, Pseudomonas and Candida albicans was studied in Nutrient broth by calculating MIC values. These values were taken at the least concentration necessary for the growth of bacteria in the test tube after incubation. This method depends on the change in the turbidity of the solution as shown in Figure 1, where No. 1: E.coli No. 2: Pseudomonas No. 3: S.aureus No. 4: Candida.

Five concentrations of ZnO were taken in different concentrations in twofold as follows (30-15-7.5-3.7-1.8 mg / ml) and the results were given in table (1) where the MIC value shows the least concentration that kills the bacteria. Results showed that all microbes were fully inhibited at 3.7 mg / ml concentration for ZnO NPs, but activity against bacteria was not observed at 1.8 mg / ml. These results were consistent with the results of Noor Hadi Issa and Hala Dawood (Hadi & Dawood, 2016). The values of MIC for ZnO was also compared to E. coli, Pseudomonas, S.aureus, and Candida at 3.7 mg / ml concentration with Jehad Youssef and Enas Daniel (Yousef & Danial, 2012). MIC values found of ZnO against Pseudomonas and E.coli were 0.5 mg / ml and S.aureus were 1μm / ml and Candida were 10 mg / ml.

These differences from current result may be due to use of preserved bacterial strain and fungi that not isolated from burn or isolation of clinical isolates that were able to form an effective biofilm. Other reasons that may lead to different results may be the differences in the preparation methods of nano ZnO and may be due to the size of ZnO nanoparticles such as (30-90) nm in (Saadat *et.al.*,) study (Mina Saadat1, 2013). While we used ZnO nanoparticles of 90 nm.

The growth rate of bacteria and fungus was calculated by calculating the optical density (OD) by the Eliza device at 600 nm and the rate of inhibition of growth of bacteria is determined by equation (1).

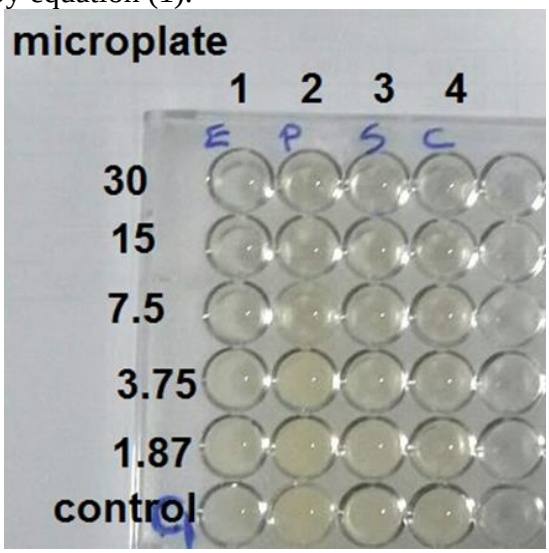

Figure (1) Turbidity as a function of concentration of ZnO-NPs.

Table (1) Antimicrobial activity (MIC values in mg / mL) for ZnO-NPs against certtain microorganisms.

Type of pathogenic	microorganisms	Minimum inhibitory concentration (mg/mL)
Gram^{+} positive bacteria	*S. aureus*	≥ 3.7
Gram^{-} negative bacteria	*E. coli Pseudomonas*	
Fungi	*Candida albicans*	

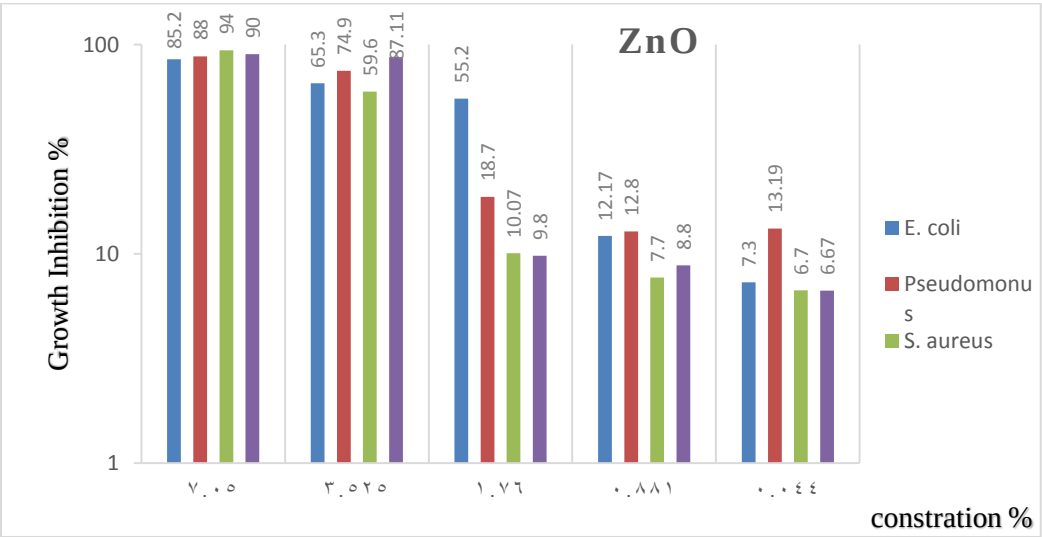

Figure (2) Percent growth inhibition of ZnO-NPs against microorganisms

The growth of these bacteria was measured at 600 nm after 24 hours of injection by optical density measurement. The measurement of control was conducted without the addition of zinc oxide (only media and bacteria). Test results for all gram-negative and gram-positive bacteria were observed, at optical density as 7.05%, 3.525%, 1.76% wt and 0.044 % wt mg/ml respectively for bacteria E.coli, S. aureus, Pseudomonas and candida.

The growth inhibition ratio was indicative of the optical density values that were reduced by decrease turbidity to resistance bacteria and increased inhibition with concentration. Figure (2) explain growth inhibition ratio. The results showed that of all Gram positive and negative bacteria and fungi had highest percentage growth inhibition for ZnO (94%) was obtained for bacteria S.aureus, 90% for candida, 88% for pseudomonas and 85.2% for E.coli at maximum concentration 7.05 wt% mg/ml. Some optical densities were found to be similar and stable at different concentrations of all bacteria.

4.2 Agar Well Diffusion Method PVA / ZnO Nanocomposite

Antimicrobial activity of PVA/ZnO solution determined by inhibition zone measurements. In comparison with control PAV, It is noted that inhibition zone for PVA/ZnO increase with increasing ZnO concertation as shown in figure (3), which no. 1,2,3 and 4 represent concentrations. In addition, figure (4) shows the minimum concentration to kill bacteria *S. aureus and S. epidermidis*, it was at (70.5 % mg/ml) and little concentration at (8.81 mg/ml), this results identical with result of MIC of ZnO test.

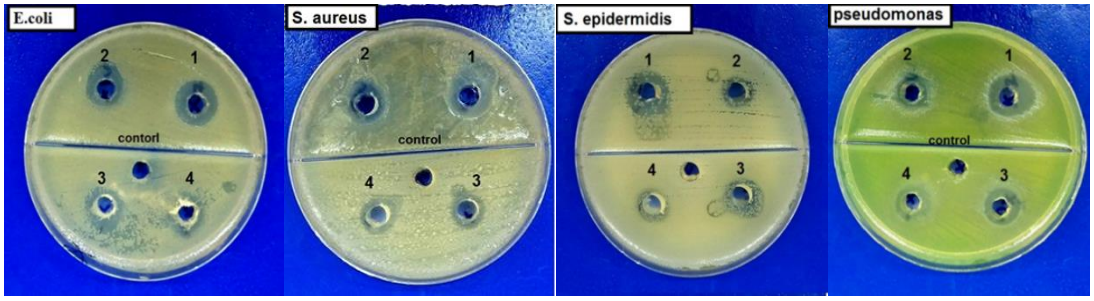

Figure (3) culture plate for PVA/ZnO NPs by agar well diffusion.

This conclusion is evaluated in terms of the diameter of the inhibition zone presented in table (2) which shows the diameter of the inhibition zone (mm) of PVA/ZnO at four concentrations, and PVA used as a control sample against E. coli, S. aureus, S.epidermidis and Pseudomonas. The results show that, the antimicrobial activity of the tested samples is dependent on concertation ZnO-NPs. Thus, the *S. aureus and S. epidermidis* is found be much more sensitive to the PVA/ZnO at percentage 7.05 % wt mg/ml than other types as show in figure (4).

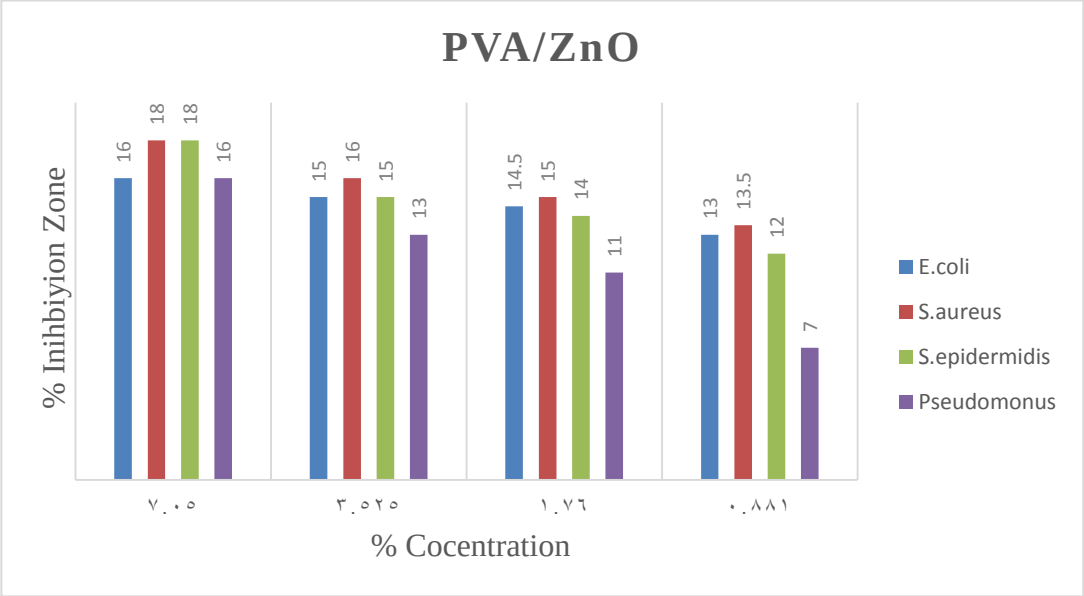

Figure (4): inhibition zone with different concentrations of PVA/ZnO nonocomposite.

Table (2): Antimicrobial activity expressed by the diameter of the inhibition zone (mm) of PVA/ZnO nanocomposite.

No. inhibition zone on image	concentration % (mg/ml)	Inhibition Zone (mm)				Control (PVA)
		E.coli	S.aureus	S. epid.	P.	
1	7.05 %	16	18	18	16	0
2	3.525 %	15	16	15	13	0
3	1.762 %	14.5	15	14	11	0
4	0.881 %	13	13.5	12	7	0

4.3 Scanning Electron Microcopy

Calculating the dispersion of ZnO NPs is an important factor in improving the properties of PVA. A good dispersion system gives much of the desired properties of composite material. Figure (5) shows a SEM image for a sample of PVA / ZnO at a concentration of 3.5 wt% mg / ml. As observe that the ZnO particles were distributed uniformly in the PVA base material. Good distribution is obtained from ultrasonic waves and good mixing under magnetic stirrer.

Good dispersion of nanoparticles is noticeable at low concentrations, whereas high concentrations occur agglomeration. The uniformity obtained at this level by SEM technique is an indication that the composite material PVA / ZnO nanoparticles possess a homogeneous nanostructure obtained by the mixing process. The other factor of this test is to know the particle size of the nanoparticles if it is found to be within the range (90-110) nm as shown in the picture.

Figure (6) shows a SEM image for a sample of PVA / ZnO at a concentration of 7.05 wt% mg / ml. As observe that the ZnO particles were distributed nonuniformly in the PVA base material because that percent 7.05 was higher than 3.5 with magnitude half.

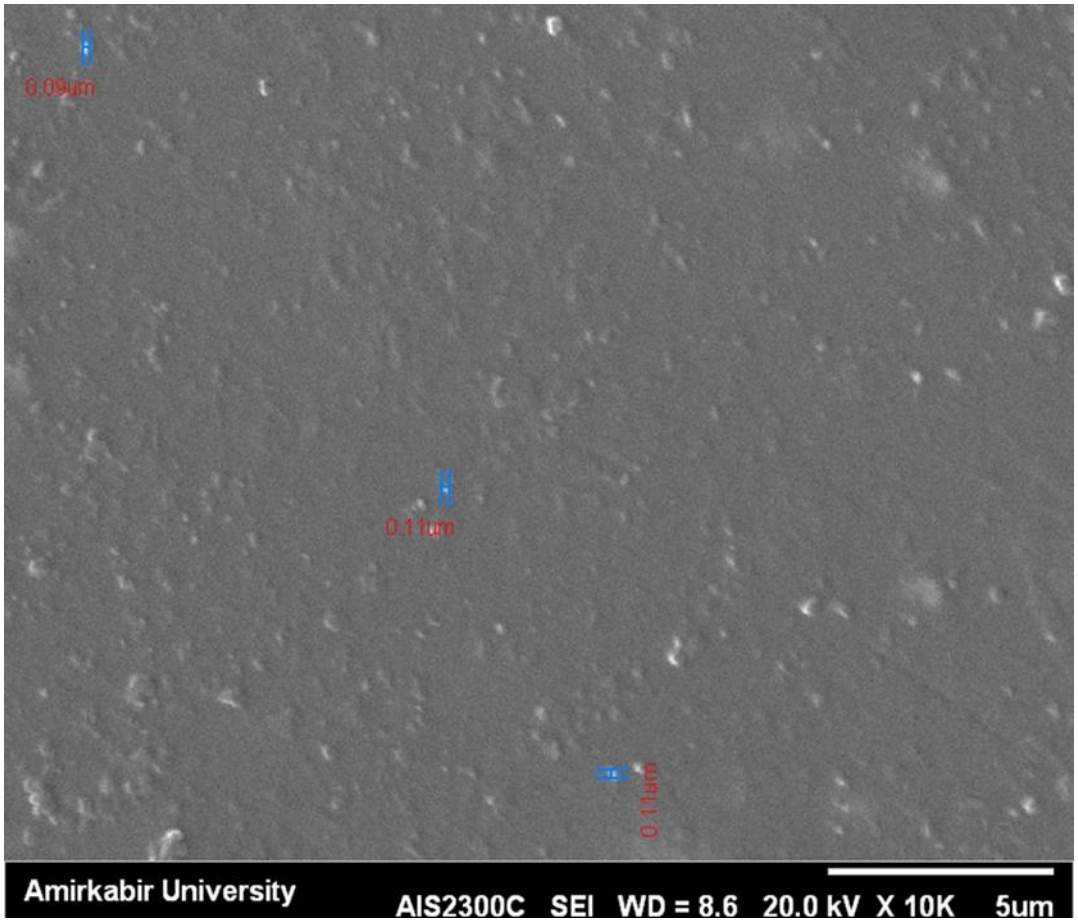

Figure (5) SEM image of composite material PVA / ZnO at concentration of 3.52 wt% mg/ml

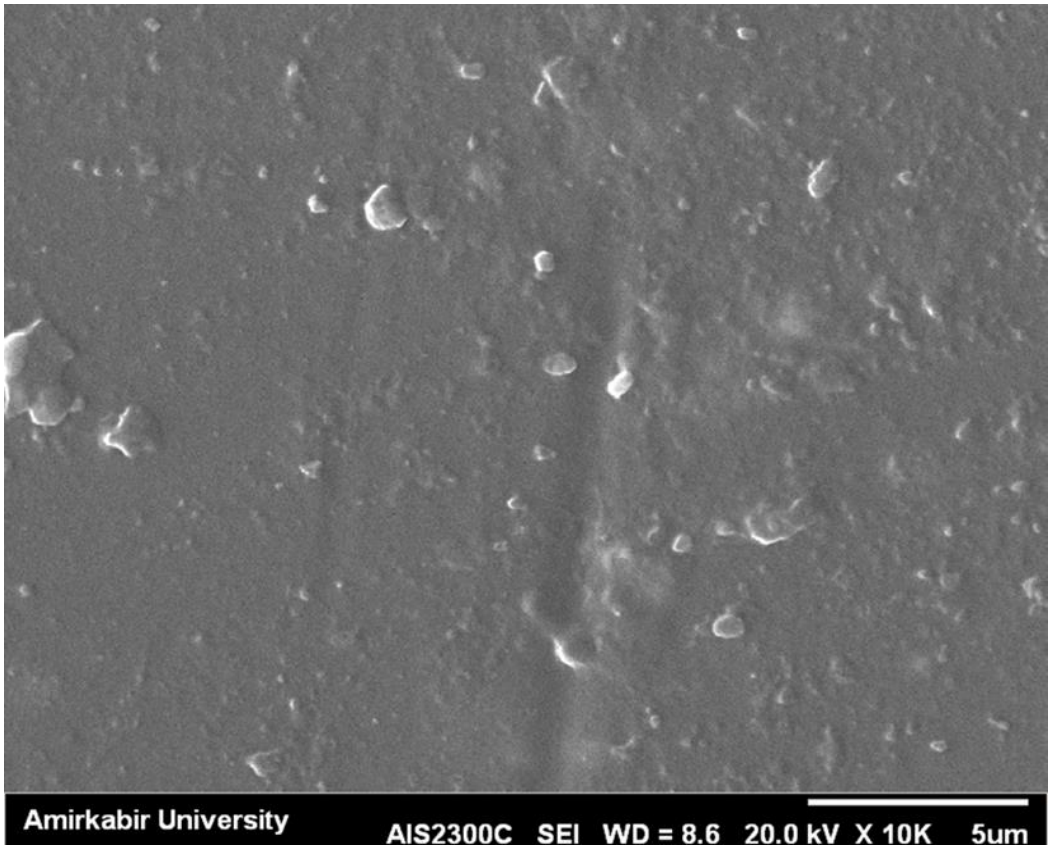

Figure (6) SEM image of composite material PVA / ZnO at concentration of 7.05 wt% mg/ml

5. Conclusions

In conclusion, show that ZnO nanoparticles have highly antimicrobial properties with little percentages 0.07 %, 0.035%, 0.017% and 0.0088% alone and with polymer PVA. This result are clear by Minimum Inhibitory concertation (MIC) test for ZnO and agar well diffusion test for composite PVA/ZnO. This composite material can be used in many applications medical, pharmacy and food, with less cost and ease of manufacturing.

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