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Fabrication of Cu_2O-ZnO Nanocomposite by the Sol-gel Technique and its Antibacterial Activity

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Abstract

In this work, the Cu_2O-ZnO nanocomposite having the high antibacterial activity was synthesized through the sol-gel method. The effects of calcination temperature and duration on the formation of Cu_2O-ZnO composite were emphasized. The most suitable condition of calcination was found to be 500 ºC and 2 hours. Synthesized Cu_2O-ZnO nanocomposite was were characterized by powder X-Ray Diffraction, BET nitrogen adsorption isotherms, field emission scanning electron microscopy, and transmission electron microscopy, and evaluated its antibacterial against human pathogenic bacteria such as *Staphylococcus aureus* (gram-positive bacteria) and *Escherichia coli* (gram-negative bacteria). The results showed that the particles of Cu_2O-ZnO composite existed spherical shapes with a wide variation in the size of 10 – 60 nm. Cu_2O-ZnO nanocomposite had the high antibacterial activity against both the gram-positive bacteria (*S. aureus*) and the gram-negative bacteria *(E. coli*) with the values of minimum inhibitory concentration (MIC) of 0.16 $mg.mL^{-1}$ and 1.25 $mg.mL^{-1}$ correspond to *S. aureus* and *E. coli*. The stability of the antibacterial activity of the sample was also surveyed. The antibacterial activity of Cu_2O-ZnO nanocomposite was reduced after 45 days when stored at room temperature in a becher without cover.

Key words: Cu_2O-ZnO; nanocomposite; sol-gel; antibacterial activity

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Introduction

Recently, the rapid development of nanotechnology with controlled particle size and shape led to many results of new antibacterial materials were published. There are some studies proved that nanomaterials have high antibacterial potential with low concentration (Balta et al., 2012; Chen et al., 2012; Xu et al., 2016; Almutairi, 2019). Among nanoparticles, nano metal oxides having antibacterial property are promising materials used in medicine, textile, or anti UV light. The antibacterial effect of nanomaterials is depended on their shape, size, chemical composition, or concentration (Negi et al., 2012). There are many antibacterial materials were studied such as transition metals Ag (Chen et al., 2017; Chatterjee et al., 2014), Cu (Chatterjee et al., 2014; Pham and Lee, 2014), Au (Sharma et al., 2009) and their oxides ZnO (Li et al., 2017; Haghighi et al., 2011; Chauhan et al., 2015), TiO_2 (Li et al., 2016), and CeO_2 (Lu et al., 2014). Some metal oxide nanoparticles such as CuO, ZnO, TiO_2 are well recognized because of their good bacterial and fungal inhibition (Jin et al., 2017; Hamza and AlSolami, 2018). While many studies have been focused on antimicrobial properties of the composite of nano metals Ag, Zn and Cu to enhance the antibacterial property of each metal separately (Chen et al., 2017; Pham and Lee, 2014; Ren et al., 2018). ZnO is a kind of cheap semiconductor, non-toxic and has a high antibacterial property with low concentration, even without light (Jin et al., 2017; Ma et al., 2014). Several previous studies proved that ZnO can be killed both negative and positive gram of bacteria such as *Escherichia coli*, *Klebsiella pneumoniae, Staphylococcus aureus,* and *Enterococcus faecalis* (Jiang et al., 2009). Besides, Cu_2O particle which has highly efficient, broadspectrum and economical (Wu et al., 2019) is a promising material for solar energy conversion, catalysis, and antibacterial (Hassan et al., 2004; Ruparelia et al., 2008; Santo et al., 2010; Ferhat et al., 2009).

However, some kinds of bacteria cannot be inhibited by Cu_2O or ZnO separately. Therefore, a composite of Cu_2O and ZnO is not only expected to enhance its antibacterial property but also can be improved both Cu_2O and ZnO functionality. ZnO has been known with strong chemical-thermal stability and its ability for resisting microbial activity, even without sunlight (Zhang et al., 2008). Also, the advantage of Cu_2O combined with ZnO is the easy incorporation and improvement of antibacterial properties comparing to other metal oxide materials (Hong et al., 2017). Besides, both materials are rather cheap and environmentally friendly. Also, there are few kinds of literature focusing on the

study of synthesis as well as the antibacterial properties of Cu_2O/ZnO nanoparticles. Composites of two metal oxides can be synthesized with various methods such as electrodeposition (Sharma et al., 2009), hydrothermal process (Li et al., 2017), electrospinning (Haghighi et al., 2011) and co-precipitation (Chauhan et al., 2015), and many previous types of research have synthesized nanoparticles material of metal oxide using the sol-gel method (Stoyanova et al., 2011; Shalaby et al., 2015; Lee et al., 2005; Amin et al., 2009; Zhang and Chen, 2009) because of uniform nanosize and low energy consumption. In this study, Cu_2O-/ZnO nanocomposite was synthesized by the sol-gel method with various calcination condition and its physicochemical properties and antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* (abbreviated as *E. coli* and *S. aureus*) were tested. The results have high potential in applications for many fields such as medical or construction materials.

Experimental

To synthesize of Cu_2O-ZnO composite, 23.76 grams of $Zn(NO_3).6H_2O$ (Xilong, >99%) was dissolved into distilled water, then 37.80 grams of oxalic acid (Merck, >99%) was added and the mixture was heated about 80 ^oC with stirring until a change to a transparent solution. Besides, 4.84 grams of $Cu(NO_3).3H_3O$ (Xilong, >99%) was also dissolved in 11 mL of ethylene glycol (Xilong, >99.8%) and dropped into the previous solution, then distilled water was added in this mixture to reach 100 mL totally with stirring continuously and the solution has light blue color. The solution was kept at 80 ^oC in 2 hrs, then the solution was changed to gel state and temperature was increased to reach paste state. The mixture was dried at 200^oC within 2 hrs and changed to ash mixture. This mixture then was calcined in pure N_2 flow (3 $L.h^{-1}$) at different temperatures (450 ^oC, 500 ^oC, and 550 ^oC) at a heating rate of 10 $°C.min^{-1}$ for various durations (1, 2 and 3 hrs) to reach Cu_2O-ZnO composites. The last sample was ball grinded in 12 hrs and very fine powder of product was obtained for other analyses. The obtained samples were denoted as Cu-Zn-T-t, where T and t represent calcination temperature (^oC) and duration (hr), respectively.

The characteristics of obtained Cu_2O-ZnO composites were investigated by thermogravimetric analysis (TGA, Setaram LABSYS Evo TG-DSC 1600C), powder X-Ray Diffraction (XRD, Bruker D2 Phaser), BET nitrogen adsorption isotherms (Nova 2200e instrument), field emission scanning electron microscopy (SEM, Hitachi S4800), and transmission electron microscopy (TEM, Jeol Jem 1400).

The Cu_2O-ZnO nanocomposite synthesized by the most suitable process has been tested for antibacterial activity against *E.coli* ATCC 25922 and *S. aureus* ATCC 43300 (MRSA). To examine the minimum inhibitory concentration of Cu_2O-ZnO against two bacteria, different concentrations of Cu_2O-ZnO (N, N/2, N/4, N/8, N/16, N/32, N/64, N/128, N/256, and N/512 with N was the initial concentration of solution, N = 20 $mg.mL^{-1}$) were prepared by diluting Cu_2O/ZnO solution with deionized water. Subsequently, the diluted samples were mixed with the sterile nutrient agar. By using sterile sticks, the standardized inoculum of each selected bacteria with 1.5×10^7 $CFU.ml^{-1}$ was inoculated on agar plates mixed with Cu_2O-ZnO samples from low to high concentrations. A plate of the sterile nutrient agar was not mixed with Cu_2O-ZnO for control (Wayne, 2013). Each strain of bacteria was inoculated at three points on a plate with the same location on the plates. Finally, the plates were incubated at 37 °C for 24 hours. The lowest concentration of Cu_2O-ZnO that inhibits the growth of tested bacteria was considered as the minimum inhibitory concentration (MIC) (Washington and Wood, 1995).

Results and Discussion

To determine the range of calcination temperature, the ash sample which was dried at 170 – 200 °C within 2 hrs after sol-gel synthesis was tested by TGA. The result in Figure 1 shows that from 50 °C to 400 °C the total mass was lost about 30% which was mainly due to the evaporation of water and ethylene glycol remaining in ash sample. But in the second stage at around 407 °C to 450 °C, the significant mass reduction of about 35% was observed due to the decomposition of NO_3^- in ash sample to produce metal oxides to form the Cu_2O-ZnO composite. Then the mass of the sample nearly not change when the temperature was increased to higher than 450 °C proved that the product's structure was stable. So, the calcination temperature will be served from 450 °C in the process.

The XRD patterns of the samples which were calcinated at 450 °C, 500 °C, and 550 °C within 2 hrs are shown in Figure 2. The results show 2 theta values of main peaks of ZnO at 31.88°, 34.55°, 36.37°, 56.63°, and 68.04° (JCPDS PDF #800075), main peaks of Cu_2O at 31.88°, 36.37°, 43.41°, 62.90° (JCPDS # 782076), mail peaks of Cu at 43.41°, 50.51°, 74.13° (JCPDS # 040836), respectively. When the calcination temperature of the samples is increased leads to the intensity of the characteristic diffraction peaks of Cu_2O and ZnO are also gradually enhanced, that is showing the improvement in the crystallinity for various particles. But there is no significant difference from 500 °C and 550 °C results, so the calcination temperature of 500 °C is chosen for energy saving.

Fig. 3 shows the XRD patterns of the samples prepared at the calcination temperature of 500 °C and the calcination times of 1, 2 and 3 hrs, respectively. All the XRD results show the characteristic diffraction peaks of Cu_2O and ZnO. But the main peaks of these compounds are higher and sharper since 2 hrs of calcination time.

BET result of the synthesized sample which was prepared at 500 °C of calcination temperature and 2 hrs of calcination time is 67.7 m^2/g, this result is higher than that of works (Shi et al., 2011; Wang et al., 2007). The sample with the same synthesis conditions was used to receive SEM and TEM results.

The morphology and particle size of Cu_2O-ZnO composite determined by SEM and TEM are shown in Figure 4. From the SEM image (Figure 4a), it can be seen that Cu_2O-ZnO composite existed in nanoparticles with a diameter of 20 – 50 nm were adhesive on the surfaces. The result by TEM (Figure 4b) shows the presence of spherical particles of about 15 – 60 nm.

Figure 5 presented the inhibition zone against *S. aureus* and *E. coli* treated with the solutions of Cu_2O-ZnO nanocomposite under different concentrations. It was observed that the exponential phase of bacteria delayed in the presence of Cu_2O-ZnO nanocomposite and this phenomenon was more obvious with the increase of nanocomposite concentration. The Cu_2O-ZnO nanocomposite could delay the exponential phase of both bacteria *S. aureus* and *E. coli* and could completely inhibit the bacterial growth at the MIC value of 0.16 mg/mL (N/128) and 1.25 mg/mL (N/16) correspond to *S. aureus* and *E. coli*. The antibacterial efficiency of Cu_2O-ZnO nanocomposite is different for gram-positive and gram-negative bacteria. That is, it shows better antibacterial activity for *S. aureus* (gram-positive) than *E. coli* (gram-negative). This phenomenon could be explained that on the surface of ZnO nanoparticles gram-positive bacteria are more susceptible to inhibition compared to gram-negative bacteria. In more detail, the growth inhibition for the gram-negative bacteria occurred at higher ZnO concentrations (Hu et al., 2012). In the component of Cu_2O-ZnO nanocomposite, the ratio Zn/Cu is high. So, the results of this work were consistent with those obtained by authors (Sirelkhatim et al., 2015; Reddy et al., 2007) studying the antibacterial activity against *S. aureus* and *E. coli* on ZnO nanoparticles. But, Cu_2O-ZnO nanocomposite had antibacterial activity against both bacteria much higher than ZnO nanoparticles. Comparing to Ag-ZnO nanocomposite, its antibacterial activity against S. aureus is almost equivalent, meanwhile, that against *E. coli* is lower (seen in Table 1). However, in terms of economics, Cu_2O-ZnO nanocomposite has a much better advantage.

To assess the stability of antibacterial activity, the minimum inhibitory concentration against *S. aureus* on the Cu_2O-ZnO nanocomposite the sample assessed results from after stored at room condition in a becher without cover for 45 days. The obtained result in Figure 6 shows that the MIC of the samples stored after 45 days at room condition is 0.63 $mg.mL^{-1}$ (N/32) with *S. aureus.* This value is lower than that of the initial sample, which was 0.16 $mg.mL^{-1}$. It can be explained because the samples were inactivated by moisture and may be partly oxidized by oxygen in the air (Kurapov et al., 2018; Yu et al., 2011).

Conclusion

In summary, Cu_2O-ZnO nanocomposite has been successfully synthesized through the sol-gel method using metal nitrates and ethylene glycol. In which, ethylene glycol played like a solvent as well as a ligand reagent in the synthesis of nanocomposites. The favorable regime of calcination to obtain Cu_2O-ZnO nanocomposite was proposed to be 500 ºC for 2 hours. The best one has a uniform structure with a particle size of lower than 60 nm and the surface area of 67.7 $m^2.g^{-1}$ exhibited high antibacterial activity and stability against human pathogenic bacteria. It could be a cheap and excellent antibacterial material with high usability in practical applications.

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Table 1. Comparison of antibacterial activity between Cu_2O-ZnO nanocomposite and other antibacterial materials.

Samples	Minimum inhibitory concentration (mg/mL)		References
	S. aureus	*E. coli*	
Cu_2O-ZnO	0.16	1.25	This work
ZnO	1.50	3.10	(Shi et al., 2011)
	1.00	3.40	(Wang et al., 2007)
Ag-ZnO	0.06	0.55	(Reddy et al., 2007)
Ag-ZnO	0.40	0.60	(Kurapov et al., 2018)

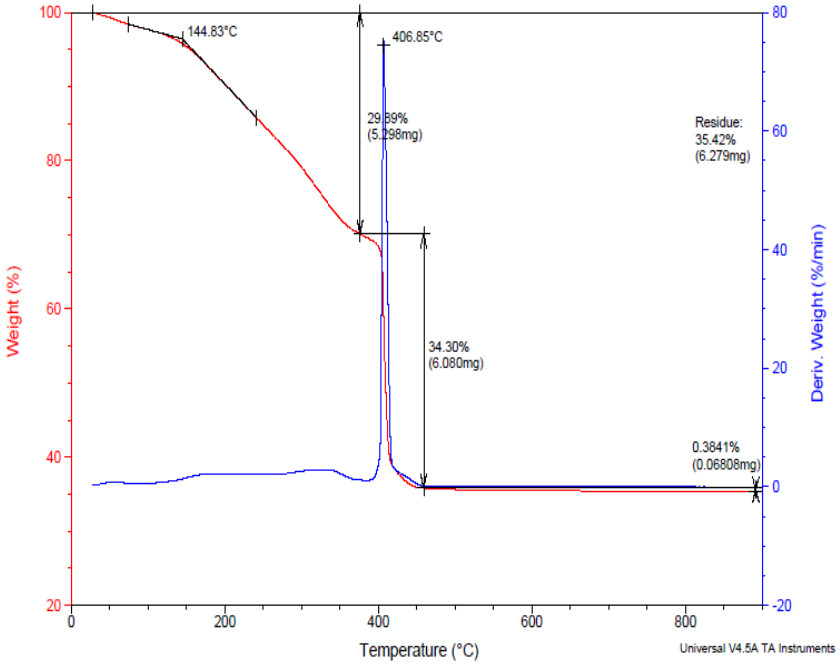


Figure 1. TG-TGA curve of the synthesized gel dried at 200 °C for 2 hrs.

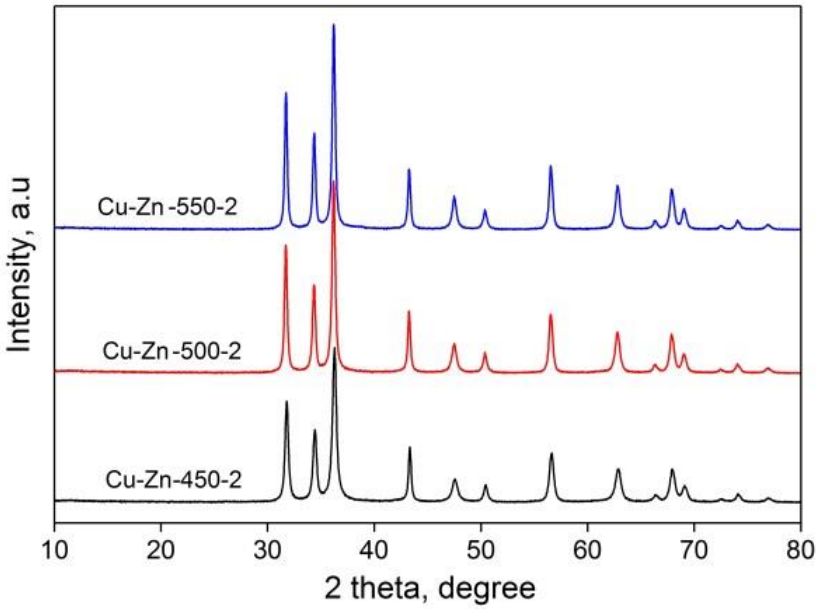


Figure 2. Effect of calcination temperature on synthesized material structure.

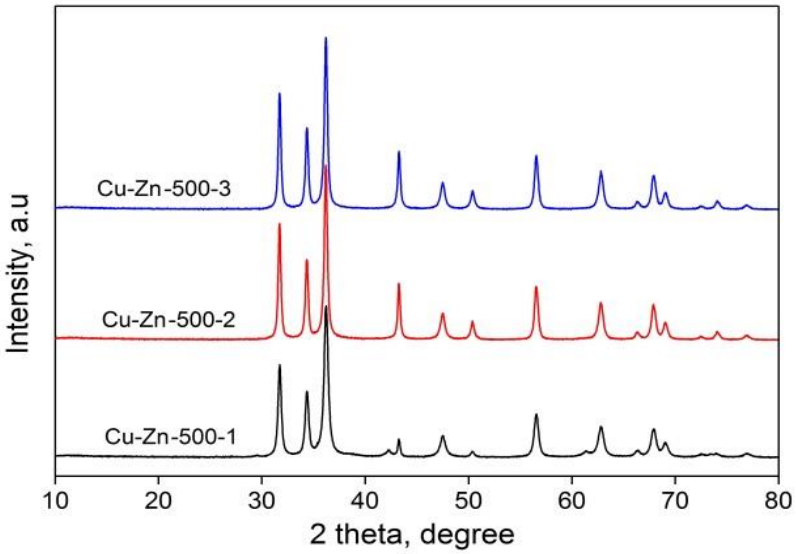
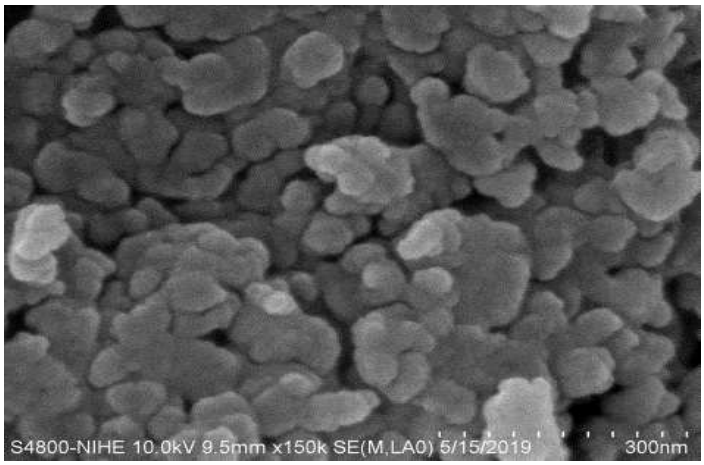
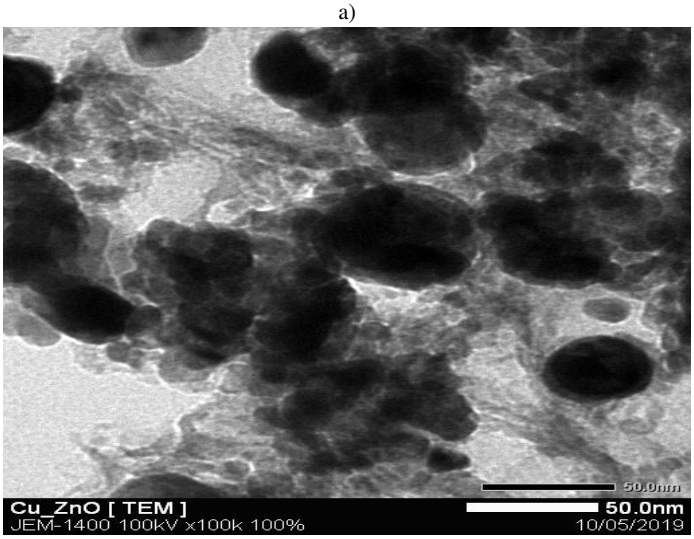


Figure 3. Effect of calcination duration on synthesized material structure.



a)



b)

Figure 4. SEM (a) and TEM (b) images of Cu_2O-ZnO nanocomposite synthesized at the most suitable condition.

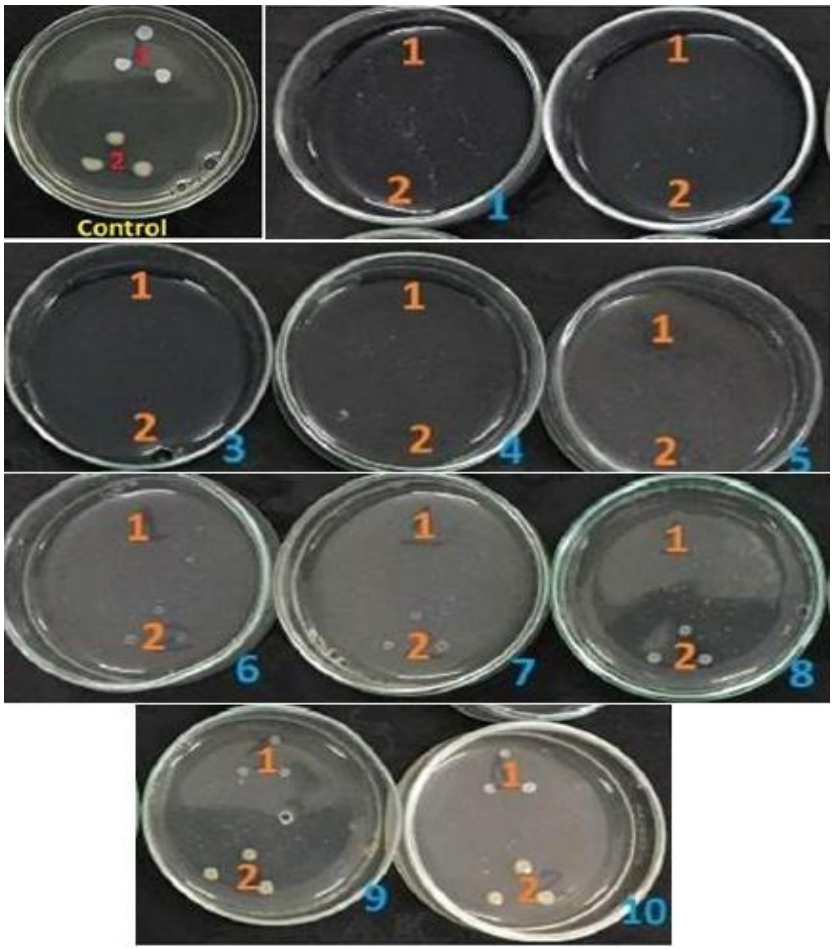


Figure 5. Image of MIC result of Cu_2O-ZnO nanocomposite against *S. aureus* (C) and *E. coli* (2) with different concentrations of N, N/2, N/4, N/8, N/16, N/32, N/64, N/128, N/256, and N/512 corresponding to 1^{st} – 10^{th} plates with N = 20 mg/mL.

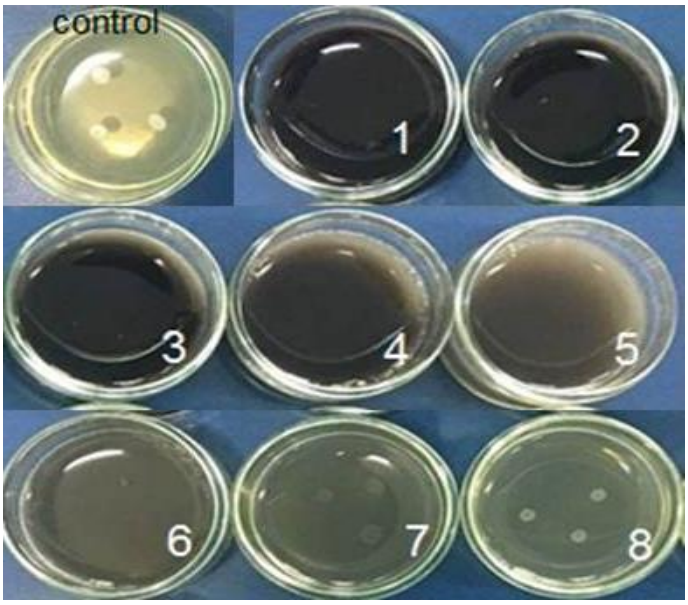


Figure 6. Image of MIC result of Cu_2O-ZnO nanocomposite preserved for 45 days at room condition against *S. auereus* with different concentrations of N, N/2, N/4, N/8, N/16, N/32, N/64, and N/128 corresponding to 1^{st} – 8^{th} plates with N = 20 $mg.mL^{-1}$.