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ROLE OF pH ON ZINC OXIDE NANOPARTICLES SYNTHESIS USING GUM ARABIC FOR SUSTAINABLE AND SAFE ANTIBACTERIAL AGENT

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Abstract: In this study, ZnO nanoparticles were produced via the precipitating method supplemented by the microwave heating method. Zinc nitrate, sodium hydroxide and gum arabic were used as zinc salt, reducing agent and stabilizing agent respectively. The objective of this work is to investigate the pH effect, ranging from 5 to 12 on the ZnO nanoparticle size. By using Dynamic Light Scattering (DLS) method, the average nanoparticle size ranged from 200 nm to 350 nm was obtained. UV-vis absorption spectrum was found in the range of 340 nm – 350 nm indicating the absorption peak of ZnO nanoparticles. FTIR spectra showed peaks range from 412 to 466 cm^{-1} which indicating standard of Zn–O stretching. The presence of (100), (002) and (101) planes were apparent in the XRD result, indicating the crystalline phase of ZnO nanoparticles. At the pH < 7, zinc nitrate hydroxide hydrate was produced, which was similar to ZnO nanoparticle that is in white solution. pH 10 was required to attain the pure phase of ZnO nanoparticles. Smaller nanoparticles synthesized at pH 10 show larger antibacterial effects at both Gram-positive bacterium, *Staphylococcus aureus* and Gram-negative bacterium, *Escherichia coli*.

KEYWORDS: ZnO nanoparticle, pH effect, antibacterial agent, gum arabic

Introduction

In the last few decades, the misuse of antibiotics has resulted in the emergence of antibacterial resistance bacterial (Qiao *et al.,* 2018). Therefore, the new alternative of the antibacterial agent is required to solve this problem. Through chemistry, ZnO nanoparticles are being regarded as one of the possible antibacterial agents. ZnO nanoparticles have shown promising biocidal and biostatic actions against Gram-positive and Gram-negative bacteria (Pauzi *et al.,* 2018). In order to synthesis safe and sustainable ZnO nanoparticles for the biomedical purpose, green synthesis should become the priority.

The current work emphasized the green synthesis of ZnO nanoparticles using gum arabic as a stabilizing agent. Gum arabic is a hydrophilic, non-toxic, biocompatible and totally biodegradable polymer. When the gum arabic is adsorbed on the particle surface, steric hindrance, bridging or charge-patch is formed depending on the solution pH (Barik *et al.,* 2015). In this paper, the effect of pH on the properties of ZnO nanoparticles stabilized with gum arabic was investigated.

The most synthesis method of metal oxide nanoparticle required sophisticated equipment, tedious procedures, a toxic solvent, as well as high temperature (Seabra & Durán, 2015). Conventional synthetic methods often depend on the energy inputs from external heat sources such as water bath, furnace and heating mantel (Kooti & Sedeh, 2013). These heating methods caused intensive energy consumption and low reaction efficiencies (Gray *et al.,* 2018). In order to achieve a green synthesis of nanoparticles, it is not only depending on the selection of environmentally benign chemical but also on the methodological used. Microwave heating method provided thermal uniformity, homogeneous volumetric heating, shorter reaction time and high reaction rate (Mohammadi *et al.,* 2018). Besides that, microwave heating has excellent control of the reaction condition for easy optimization of experimental parameters (Brollo *et al.,* 2017). Therefore, in this study the microwave heating method becomes an environmentally benign technology to tackle the problems in nanoparticles synthesis such as hazardous substances and to reduce intensive energy consumption.

Several researchers had investigated the effect of pH on the morphology and size of nanoparticles (Rayerfrancis *et al.,* 2015; Kim *et al.,* 2016). However, to the author's best knowledge, the number of studies addressing the pH effect on the properties of ZnO nanoparticle stabilized with gum arabic is rather limited. pH is a crucial parameter during the synthesis of metal nanoparticles. It could be used to control the nanoparticle sizes of colloids because the stability of surface charge and particle interactions are dependent on the pH value (Tso *et al.,* 2010).

In this present study, Dynamic Light Scattering (DLS) was used to estimate the average size and size distribution of nanoparticle. DLS was preferable in the current study as a larger number of particles could be measured via DLS (in millions) compared to TEM (few hundreds) (Bhattacharjee, 2016). The error in the size determination by the dynamic light scattering method for spherical particles is rather low, not greater than 5–10% (Chirikov, 2016). Therefore, the more reliable size distribution and polydispersity index (PDI) can be obtained by using DLS. DLS techniques are non-invasive, require minimal sample preparation and no pre-experimental calibration (Bhattacharjee, 2016). To date, DLS has not been employed to characterize ZnO nanoparticle stabilized with gum arabic.

For the antibacterial application, Gram-positive bacterium, *Staphylococcus aureus* and Gram-negative bacterium, *Escherichia coli* were used to investigate the antibacterial potential of synthesized ZnO nanoparticles. Hopefully, a green synthesis method using microwave heating and gum arabic as a natural stabilizing agent will produce sustainable and safe antibacterial agent.

Materials and Methods

Materials

Zinc nitrate hexahydrate $Zn(NO_3)_{2.6}H_2O$, sodium hydroxide and gum arabic of analytical grade were used without further chemical treatment and purification. The zinc salt used in this study was zinc nitrate as suggested by Barreto *et al.,* 2013, so that the very pure phase of ZnO nanoparticles of high density could be obtained using microwave heating method.

Method of ZnO Nanoparticles Synthesis

Firstly, 1.0 % (w/v) of gum arabic was dissolved in 100 ml of distilled water and heated for 2 minutes (in a 450W microwave) to fully dissolve the gum arabic. 2.974 g of solid $Zn(NO_3)_{2.6}H_2O$ zinc nitrate was added into 1% of gum arabic solution subjected to continuous stirring. Then the obtained solution was heated for 2 minutes in the microwave running at 450W. In order to adjust the pH, 1 M NaOH solution was dripped into the zinc nitrate and gum arabic solution undergoing vigorous stirring until the desired pH was achieved (pH5-12). Again, the mixture solution was exposed to microwave heating (450 W) for 4 minutes. The white precipitate was cleaned using distilled water before the precipitate was dried in an oven at 80 °C. The formed ZnO nanoparticles were used for the characterization process.

Dynamic Light Scattering (DLS) and Zeta Potential Measurement

Thirty µL of ZnO solution was diluted in 2 mL of de-ionized water. The nanoparticle size and the zeta potential in liquid suspension at 25°C were measured using Zetasizer Nano ZS (Malvern Instruments). Both hydrodynamic diameter and polydispersity index were measured.

Characterization

The band gap of ZnO nanoparticles was analysed using the UV-vis spectrophotometer in the range of 250 nm to 800 nm. The chemical composition of the synthesized nanoparticles was studied using the FTIR spectrometer in the range of 400 cm^{-1}- 4000 cm^{-1}. The crystal structures of the prepared ZnO nanoparticles were studied using the X-ray diffractometer (XRD.) The X-ray powder diffraction patterns were recorded at every 0.01° in the angular range of 20°–80° using monochromatic X-rays. The size and

the morphology of the ZnO nanoparticle were examined using the field emission scanning electron microscope (FESEM).

Antibacterial Assays

The colonies of *E. coli* and *S. aureus* were grown overnight in the Tryptic Soy Broth (TSB) solution at 37 ℃ until the late mid log phase. A total of 50 µl bacterial sample were spread onto the solid Tryptic soy agar medium in petri dishes. Sterilized filter paper disks (1 cm) was placed on the inoculated plates. Meanwhile, the ZnO nanoparticles were prepared at 1 mg/mL in distilled water. Subsequently, a total of 15 µL ZnO nanoparticles mixture solution were pipetted on the equal size autoclave-sterilized filter paper disk and incubated for 1 day (at 37°C). After 1 day, the diameter of the inhibition zone was measured. The size of the inhibition zone indicated the extent of bactericidal activity in the ZnO sample.

Results and Discussion

ZnO Nanoparticles Synthesis

Results showed that precipitation occurred when the zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) solid was added into the gum arabic solution. However, upon adding the NaOH solution (drop-wise), a white precipitate (ZnO nanoparticles) formed after a few seconds. It was anticipated that the precipitation of ZnO nuclei started when the concentrations of Zn^{2+} and OH- ions exceeded the critical value (Al-Harbi *et al.,* 2011). This precipitation process is known as the initial nucleation process for the formation of ZnO nanoparticles. Precipitates (in the form of nuclei) would grow to form particles. The pH value of the milk-like colloidal suspensions of ZnO nanoparticles was then adjusted accordingly. The solution was further treated hydrothermally using microwave heating.

Dynamic Light Scattering (DLS)

DLS is used for calculating the hydrodynamic diameters of suspended nanoparticles based on their Brownian movements (Brar & Verma, 2011). As shown in Figure 1, the calculated average hydrodynamic diameter ranged from 200 nm to 300 nm for pH ranging from 5 to 12. The polydisperse nature, the indicator of aggregation, of nanoparticles was characterized by using the polydispersity index value (Masarudin *et al.,* 2015). This index ranged from 0.1 to 0.2 for the pH range of 5 to 12. Higher polydispersity index denotes polydisperse system.

In acidic condition (< pH 7), the OH concentration was low, thus inhibiting the growth of ZnO particle due to limited $Zn(OH)^2$ formation in the solution. An acidic solution contained less OH; therefore the hydrodynamic and crystallite sizes were smaller. As pH was increased to 7, the hydrodynamic and crystallite size increased as well. The initial phase of ZnO growth is slow as the nucleation sites are limited and these smaller ZnO nanoparticles would merge during growth to form bigger ZnO nanoparticles structure (Baruah & Dutta, 2009). However, the hydrodynamic sizes decreased when the pH was further increased to alkaline. Figure 1 shows the nanoparticle size of ZnO synthesized at different pH values. Apparently, the particle size increased when the pH was increased from 5 to 7. The particles sizes were more than 300 nm at pH 7 and pH 8. However, the particles size decreased slightly when the pH was higher than 8.

The addition of more NaOH to the aqueous solution to adjust the pH to 8 and above, will reduce the growth rate of ZnO nanostructures by the large amount of $Zn(OH)_4^{2-}$ because the solubility of OH in this solution was lower. This result was supported by Rayerfrancis *et al.,* 2015 who reported the decreasing size of ZnO nanostructure when the pH changes from neutral to alkaline. The influences of pH on the Zn ion and OH ion from Zinc hydroxide complex which affects the zinc solubility that influence the size of ZnO nanoparticles in alkaline condition (Heidari & Younesi, 2009).

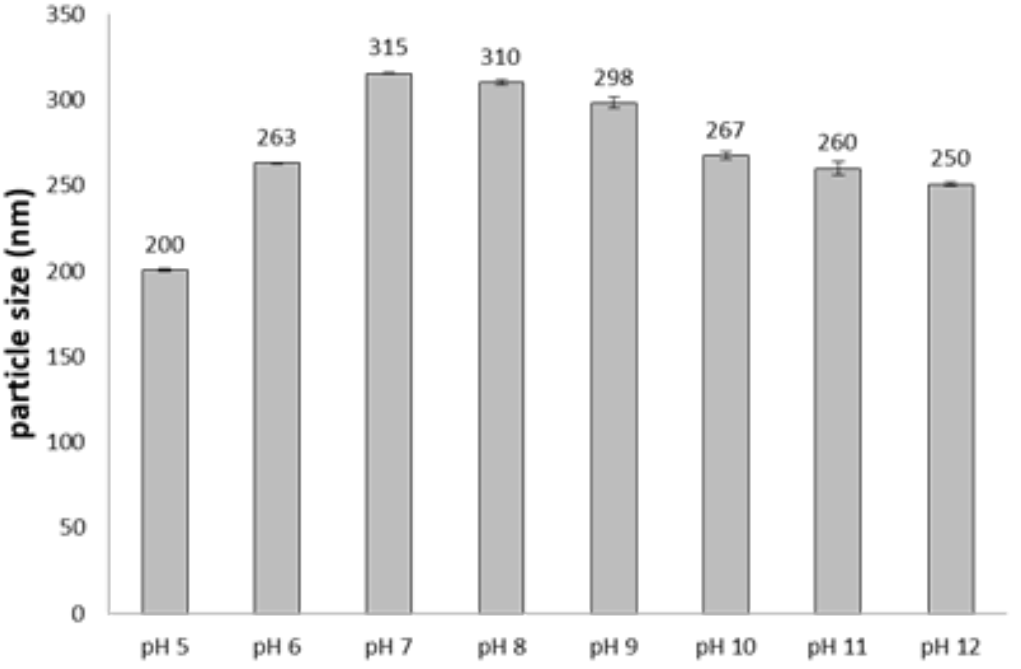

Figure 1: ZnO nanoparticles size at different pH.

Table 1 demonstrated the stability of ZnO nanoparticles at different pH value. In this experiment, the surface charge of ZnO was affected by the chemical reaction between the surface of the zinc oxide and the stabilizer solution. The molecules of gum arabic as a stabilizer were highly negatively charged (Klein *et al.,* 2010). The zeta potential charges were negative in all solution. It might be caused by gum arabic as a negatively chargeg polymer. Absolute zeta potential value of more than 60 mV indicates excellent stability, 30 mV shows physically stable, below 20 mV denotes limited stability and below 5 mV indicates agglomeration (Zain *et al.,* 2014). Although smaller nanoparticles size can be produced at lower pH, the stability of the small-sized nanoparticle is low as well (zeta potential value < 30 mV). At higher pH, the zeta potential was more than 30 mV, indicating the stability of the ZnO solution. At higher pH, the ZnO nanoparticle is more stable due to the complex formation between the oxygen of the hydroxyl group of gum arabic and the ZnO nanoparticle. At high pH, it is possible that the complexation of Zn^{2+} ions by NaOH decreases its reactivity and decelerates the rate of nucleation and growth.

Table 1: DLS measurement on ZnO nanoparticles synthesis at pH of 5 to 12

pH	Hydrodynamic size (nm)	PDI index	Zetapotential (mV)
5	200	0.245	-13.5
6	263	021	-16.8
7	315	0.149	-24.9
8	310	0.189	-27.3
9	298	0.226	-33.8
10	267	0.111	-34.1
11	260	0.109	-36.4
12	250	0.107	-36.8

UV-Vis Analysis

The optical properties of ZnO nanoparticles synthesized at different pH conditions were studied using by UV-vis spectroscopy at room temperature. Figure 2 shows that the absorbance increased with the increased in pH. This is because of a slight decrease in surface roughness. The formation of larger particles on the surface of ZnO nanoparticles with the increase of pH values which causes scattering of light (Fatehah *et al.,* 2014). For pH values ranging from 7 to 12, a sharp peak was formed in the range of 340 nm – 350 nm, representing the hexagonal phase ZnO.

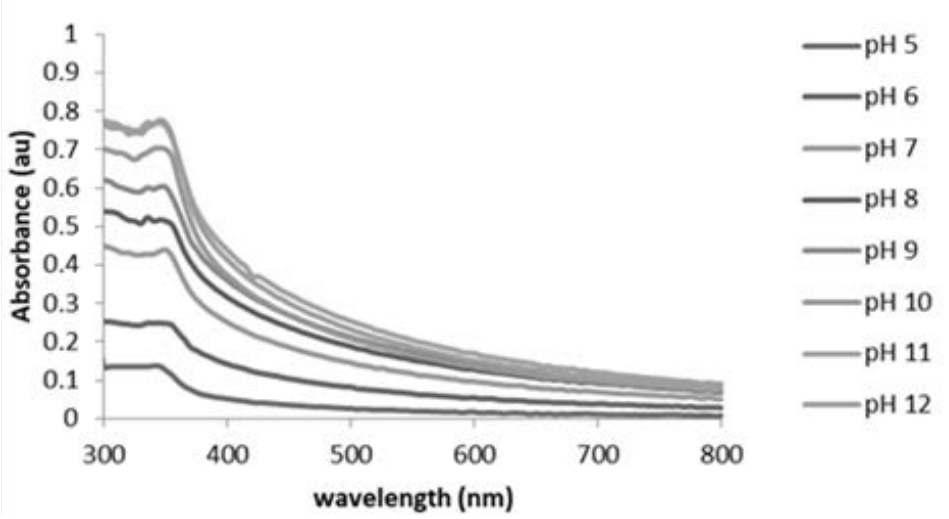

Figure 2: UV-vis spectra of ZnO nanoparticles synthesis at different pH.

Fourier Transforms Infrared Spectroscopy (FTIR) Analysis

The FTIR measurement was conducted at room temperature for wavenumber ranging from 4000 cm^{-1} to 400 cm^{-1}. Figure 3 shows the compositional analysis of ZnO nanoparticles synthesized at different pH values. At pH 5, the band at 3447 cm^{-1} corresponded to the O-H mode of vibration within the hydroxyl groups. As seen, the O-H peaks were no longer visible at both pH 7 and 10, showing that the synthesized ZnO nanoparticles were not fully developed yet at pH 5. As pH increased, the peak of C=O due to carboxyl group oscillated due to the structural variation in the morphology. This symmetric stretching occurred between 1363 cm-1 and 632 cm^{-1}. Also, when pH increased, the vibration band of ZnO decreased from 466 cm^{-1} to 419 cm^{-1} due to the morphological changes in ZnO.

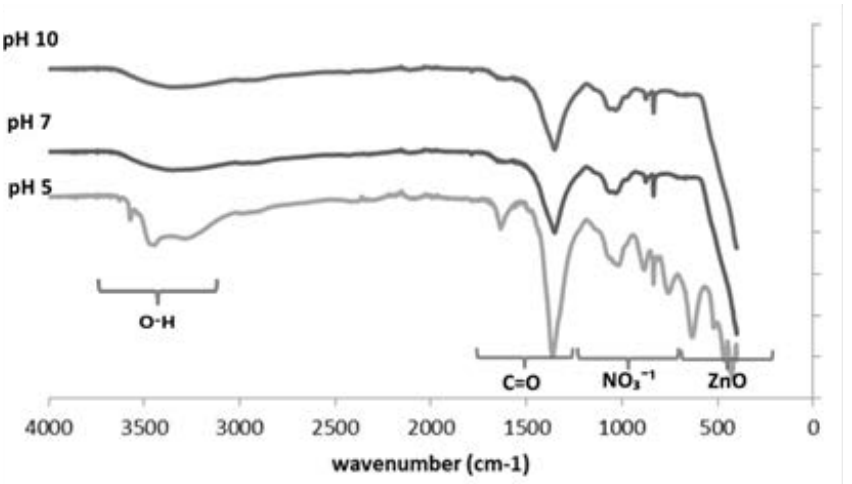

Figure 3: FTIR spectra of ZnO nanoparticles synthesis at pH 5, 7 and 10.

X-ray Diffraction (XRD) Analysis

Figure 4 shows the XRD patterns of ZnO powder synthesized at pH 5, 7 and 10. All reflection peaks can be indexed as pure hexagonal ZnO. The changes in pattern during the transformation from the Zinc nitrate hydroxide hydrate to the hexagonal structure of ZnO were observed. At pH 5, several impurities existed within the ZnO nanoparticles such as Zinc nitrate hydroxide hydrate with PDF no 00-024-1460 (dominant peak), $NaNO_3$ (Nitratine) with PDF no 00-036-1474 and amino isobutyric acid with PDF no 00-030-1511. The intensity of the diffraction peak of ZnO nanoparticles increased and became narrower as pH increased due to the grain growth of the zinc oxide (Ikono *et al.,* 2012). The intensities of XRD peak of (101) plane at pH 7 and 10 were more apparent than that at pH 5. The diffraction peak intensity was the highest at pH 10 due to the adequate amount of OH- were released into the synthesis solution, which would promote hydrolysis and nucleation.

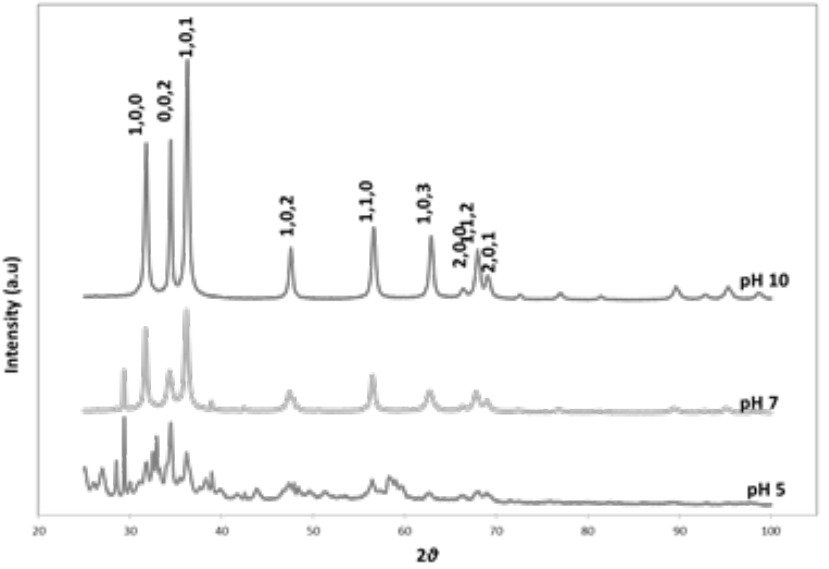

Figure 4: XRD patterns of ZnO nanoparticle synthesis at pH 5, 7 and 10.

Field Emission Scanning Electron Microscopy (FESEM)

Figure 5 showed that the spherical nano-plates structure consisted of many smaller nanoparticles that were spherical in shapes were observed at pH 5, pH 7 and pH 10. However many researchers reported the changes in morphology and the characteristics of the ZnO nanostructures were investigated at pH ranging from 2 to 12 (Rayerfrancis *et al.,* 2015 and Hosseinian *et al.,* 2017). The reason the impact of pH on the morphology of ZnO nanoparticles was not noticeably detected in this current study is may be due to the gum arabic as a stabilizing agent that controls the spherical shape morphology throughout the pH range.

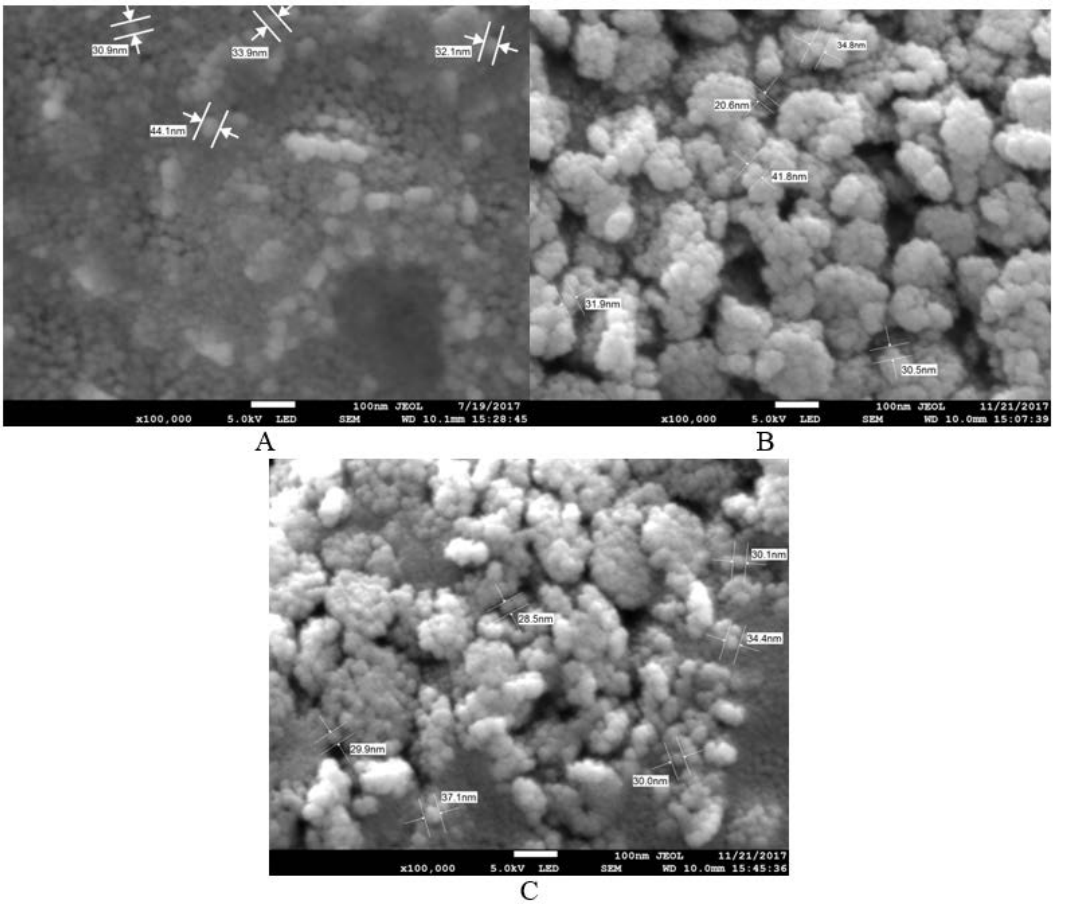

Figure 5: FESEM image of the ZnO nanoparticles synthesized at pH 5 (A), pH 7 (B) and pH 10 (C) at 100k magnification.

Antibacterial studies

The antibacterial activities of ZnO nanoparticles were evaluated by measuring the zones of inhibition of the Gram-positive bacterium *S. aureus* and Gram-negative bacterium *E. coli*. The sizes of the inhibition zones were reported in Table 4. As seen, the antibacterial activities of ZnO nanoparticles against both bacteria were apparent. As pH increased, the inhibitory effect of ZnO increased as well. In this study, smaller nanoparticles were synthesized at pH 10. Both *S. aureus* and *E. coli* showed larger inhibition zones when the ZnO nanoparticles synthesized

at pH 10 were employed. The *S. aureus* was more sensitive against ZnO nanoparticles compared to Gram-negative bacterium *E. coli*. The structures of Gram-negative bacteria are more complex, their outer membranes consist of a high concentration of lipids, polysaccharides and protein. The exterior part of the central membrane contains the periplasmic region, and the peptidoglycan region surrounded by an additional membrane (Sirelkhatim *et al.,* 2015). The lipopolysaccharide layer (LPS) found in Gram-negative bacterial cells could protect the cell membrane from toxic molecules. As compared to Gram-positive bacteria, Gram-negative bacteria contain higher amounts of lipids and lipoproteins. Furthermore, the outer membrane exists in Gram-negative bacteria only (Grumezescu & Andrew, 2017), which make them more resistant to ZnO nanoparticles. Thus, Gram-positive bacteria are more sensitive to ZnO nanoparticles.

Table 2: Inhibition zones against *S. aureus* and *E. coli* at different pH

pH	*S. aureus*	*E. coli*
5	1.1 (± 0.12) cm	1.2 (± 0.4) cm
7	1.6 (± 0.09) cm	1.4 (± 0.16)cm
10	1.7 (± 0.04) cm	1.4(± 0.09)cm

Note: Experiments are in triplicates and the results are presented as a mean ± standard deviation

Conclusion

In this study, the green synthesis of ZnO nanoparticle using gum arabic as a stabilizing agent has been demonstrated. The zeta potential, the size and the evolution phase of ZnO nanoparticles have been found to be quiet sensitive to the pH value. However, the morphology of ZnO nanoparticles was not sensitive to the change of pH. The particles were smaller and more stable in alkaline synthesis condition. Seemingly, in order to get the pure phase of ZnO nanoparticles, alkaline synthesis condition was preferable. In acidic synthesis condition, zinc nitrate hydroxide hydrate was detected by XRD analysis. The synthesized of safe and sustainable ZnO nanoparticles are spherical in shape have an antibacterial effect in both *S. aureus* and *E. coli.*

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