Preparation and Characterization of Al-doped ZnO Particle for Antibiotic Materials Application

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Abstract. ZnO and metal (Al)-doped-ZnO were successfully prepared by wet chemical synthetic route. The understanding of substituted Al ions into ZnS leads to significant enhancement on antibacterial properties. The 10-50 wt% was partially substituted into ZnO crystal structure. The structural properties and lattice parameter were investigated by X-ray diffraction. Brunauer-Emmett-Teller and Scanning Electron Microscope were employed to investigate specific surface area and morphological properties. It exhibits the irregular shape with variation in size, while pore volume and specific surface area can be observed. After that preliminary test on antibiotid properties was therefore conducted based on *E. Coli* and *S. Aureus* bacteria species. The result suggested that it can be further developed as antimicrobial agent material.

1. Introduction

In recent year, the developments of metal oxide semiconductors with a variation of size and shape have received considerable interest because of their good chemical resistance and thermal stability. Among these metal oxide semiconductors, ZnO semiconductors have been extensively employed in many industrial sectors [1-4]. The role of ZnO particle can be utilized as photocatalyst for environmental remediation due to excellent chemical stability, large surface area, low cost and high electron mobility. Another application was due to antimicrobial agent in food and textile clothing [5], ZnO particle can be separated into free ions by light and it can be reduced on the growth rate of bacteria [6,7]. Due to the achievement of ZnO utilization, numerous efforts have been synthesized ZnO with the variation in size and shape. It can be prepared as a plate, sheet, rod, tube as well as particle. The size can be lower from bulk particle into nano-scale level. It was therefore gained many interests due to versatility of morphology. From the fundamental point of view, our research group was successfully co-doped Mn and Cu into ZnS particle by conventional synthetic technique [8,9]. The objective of co-doped process was partially substituted Mn and Cu atom into Zn position. It can be altered on energy gap level in ZnS particle for luminescent device. Furthermore, codoped ZnS particle was successfully integrated into polymer matrix in organic light emitting diode [10,11]. The efficiency of as-synthesized ZnS particle can be employed in flexible display device. With the achievement of this project, small amount of Al particle was partially therefore integrated into ZnO particle by conventional technique. Significant alteration of small amount of Al insertion was due to antimicrobial properties enhancement of ZnO. It was further employed as antimicrobial agent in food packaging industry [12].

In this research work, we wish to present on the synthesis of ZnO and Al-doped ZnO powder by conventional and facile synthetic route using zinc chloride and aluminium hydroxide as chemical reagents. Al ions induce impurity level by substituting the position of Zn^{2+} in the lattice of ZnO. The performance of antimicrobial properties of Al-doped ZnO will be reported.

2. Materials and Method

2.1 Chemical reagents

$ZnNO_3$, $6H_2O$ and triethanolamine were used as chemical reagents and the organic ligand. They were purchased from Ajax Finechem, Pyd LTD and Carlo Erba Reagents, respectively. Aluminium hydroxide was purchased from Sigma Aldrich, Co. LTD. Analytical grade of ethylene glycol and methanol were employed as organic solvents which purchased from QREC chemical, Co, LTD. All chemical reagents were used further purification.

2.2 Instruments

The crystal structure of the powders was investigated by X-ray diffraction (XRD, Phillips P.W. 1830), which was employed using nickel-filtered CuKa radiation. The diffraction patterns were recorded over a range from 25 to 80°C. The powders were investigated with an SEM (a JOEL JSM-6301F). The machine was operated with an acceleration voltage of 20 kV at a working distance of 15 mm to identify the morphological properties of the powders. Before the investigation, the samples were sputter-coated with Au to enhance their electrical conductivity. A magnification of 50,000 was used. The specific surface area of the powder was determined by Surface Area and Porosimetry Analyzer (Micromeritics ASAP 2020). The BET surface areas were calculated from the nitrogen adsorption isotherms at -200 °C and a value of 0.164 nm^2 for the cross-section of the nitrogen molecule. Prior to analysis, samples were degassed at 370 °C under high vacuum for 24 h.

2.3 Preparation

The Zn-O precursor was prepared. A mixture of 10 mmol of $ZnNO_3$ $6H_2O$ and 10 mmol of triethanolamine was added to 50 ml of ethylene glycol. The chemical reaction was held at 120°C for 6 h. After the reaction was complete, the Zn-O precursor was calcined at 700°C for 3 h. The ZnO powder was examined by X-ray diffraction, scanning electron microscopy with energy dispersive analysis and Brunauer-Emmett-Teller. The Zn-Al-O precursor was prepared. A mixture of 10 mmol of $ZnNO_3$ $6H_2O$ and 10 mmol of triethanolamine was added to 50 ml of ethylene glycol. The 10, 20, 30, 40 and 50 wt% of $Al(OH)_3$ was added into the mixture. The reaction was held at 120°C for 6 h. After the reaction was complete, the Zn-Al-O precursor was calcined at 700°C for 3 h. The ZnO powder was examined by X-ray diffraction and scanning electron microscopy with energy dispersive analysis and Brunauer-Emmett-Teller. The antimicrobial activity of ZnO and Al-doped ZnO was evaluated by agar diffusion method. Test method was controlled by AATCC 147 (Modified): Antimicrobial activity assessment. Gram negative bacteria E. coli (ATCC 8739) and Gram positive bacteria S. aureus (ATCC 6538) were used as test organisms. Sterile plates were prepared and 0.1 mL of the inoculums of test organism was spread uniformly over. Samples were prepared by using a sterile borer of diameter 6 mm and Al-doped ZnO particle was separately added. The zone of inhibition of microbial growth around was observed. The antimicrobial efficacy of samples against clinical bacteria (*E. Coli* and *S. Aureus*) was evaluated by disc diffusion method. To evaluate the antimicrobial performance, a modification of the ISO/IEC 17025 was used. The antimicrobial test was reported as a clear zone. Three replicate experiments were performed for each of the samples in order to ensure on the reproductivity.

3. Results and discussion

ZnO powder and Al-doped ZnO were successfully synthesized from wet chemical synthetic route. It presented as a fine powder with white-color. There is no change on physical characteristic after Al doping process. Small amount of Al was successfully inserted into Zn position. XRD pattern of ZnO and Al-doped ZnO powders were exhibited eight well-defined diffraction peaks corresponding to the lattice planes of (100), (002), (101), (102), (110), (103), (200) and (112) can be observed for ZnO sample. The peaks matched very well with the cubic zinc blended structure, confirming the purity of the as-synthesized ZnO. As ZnO was doped with Al, the (101) peak became broadened in all cases, along with smaller relative intensity of peak (101). It can be noted that the crystallinity of ZnO was degraded as a result of doping. It was due to the size effect, XRD peaks broaden as the particle become smaller. However, only 30 wt% of Al was successfully inserted into ZnO particle. The incomplete substitution occurred during experimental reaction step. The excess Al can be occurred if 30 wt% was prepared. The reason of incomplete substitution was due to non-sufficient energy. Excess Al can be led to alter the structure of ZnO from cubic to hexagonal phase.

Fig. 1 exhibits the typical microstructure FESEM image of as-synthesized ZnO and Al-doped ZnO. The powders exhibited blocky particles with irregular shapes possibly due to the agglomeration among particle. The particle size was estimated to be 20-50 nm with a connected spherical shape. There is no alteration on morphological properties due to Al insertion. However, with spherical shape of particle, slightly less on size of Al-ZnO can be observed. Al was partially substituted into Zn lattice. It may involve on surface energy during crystal formation step. On the other hand, the occurrence of porosity can be observed due to the pathway of water and solvent evaporation. With the elevation of calcination temperature, water and solvent was therefore evaporated, subsequently the decomposition of TEA complex. This decomposition step can be led to volatile gas evaporation. The existence of porosity can be led to be high specific surface area. Due to the significant discovery on high specific surface, the application of Al-doped ZnO can be employed in many areas of industrial research such as catalyst support and antimicrobial agent.

On the other hand, an X-ray energy dispersive spectroscopy (EDX) spectrum acquired from the metal-doped ZnO confirm that the particles consist of Zn, O and related Al-dope with mainly a stoichiometric ZnO composition. The investigation was conducted based on qualitative and quantitative analysis. The technical data is exhibited in Table 1. The successive substitution of Al-doped into ZnO cubic structure was successfully prepared. The amount of Al atom was indicated on EDX spectrum which is consistent with added-amount in preparation step. In addition, in Table 2, it presents the element content of ZnO and Al-doped ZnO powder. The major elements of all samples are Zn and O, while the minor elements are Al. This result can confirm the presence of Al-doped ZnO. This analysis method is accurate with an experimental error of 10%. This is due to the counting statistic during measurement.

Table 2 exhibits BET analysis of ZnO and Al-doped ZnO particle. The technical data was reported based on specific surface area, pore volume and pore diameter. It was remarkable to note that significant enhancement on specific surface, pore size and pore diameter has been observed. With the integration of Al into ZnO particle, the alteration of specific surface tension and surface energy was changed. It was subsequently provided for the longer nucleation time of Al-doped ZnO crystal. The size of particle was therefore enlarged. However, formation of Al-doped ZnO crystal was presented as porosity. It was related to increment on pore volume and pore diameter. This discussion on crystal size and pore volume was strongly associated with SEM analysis.

Table 1 Quantitative analysis by energy dispersive analysis of ZnO and Al-doped ZnO particle

Sample	Zn	Al	O
ZnO	64.46	-	35.54
10wt% ZnO/Al	30.21	7.78	30.21
20wt% ZnO/Al	53.23	13.85	32.92
30wt% ZnO/Al	30.02	27.50	42.48
40wt% ZnO/Al	25.12	38.86	36.02
50wt% ZnO/Al	8.51	43.05	48.43

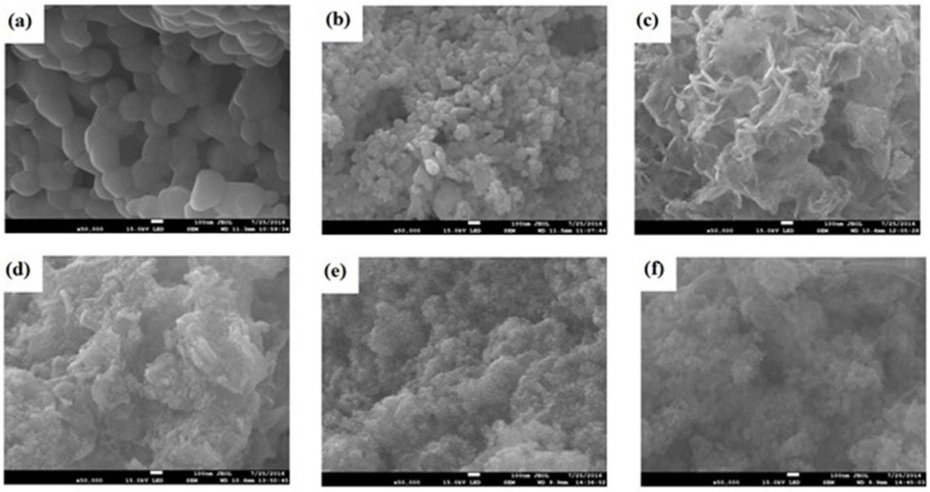

Fig. 1. Field emission scanning electron microscope (a) ZnO (b) 10 wt% Al-doped ZnO (c) 20 wt% Al-doped ZnO (d) 30 wt% Al-doped ZnO (e) 40 wt% Al-doped ZnO (f) 50 wt% Al-doped ZnO

Table 2 BET analysis of ZnO and Al-doped ZnO particle

Sample	Specific surface area (m^2/g)	Pore volume (cc/g)	Pore diameter (nm)
ZnO	10.120	0.026	3.413
10wt% ZnO/Al	76.543	0.257	3.819
20wt% ZnO/Al	30.270	0.183	12.293
30wt% ZnO/Al	43.717	0.170	12.247
40wt% ZnO/Al	91.731	0.532	9.550
50wt% ZnO/Al	74.453	0.377	5.604

The ISO/IEC 17025 was employed to investigate the antibacterial properties of ZnO and Al-doped ZnO particle. The technique was involved on the assessment of antimicrobial activity of bacteria on the surface of antimicrobial products. After 24 h of exposure, no viable counts of E. *coli* and S. *aureus* were recorded. These results indicated that ZnO particle and Al-doped ZnO particle provided a biocide effect at high concentrations 10 ± 0.005 wt%. The antimicrobial effect of ZnO and Al-doped ZnO was ascribed to the fact that particles were well-dispersed and stabilized in solution. Fig. 2 exhibits the antibacterial properties of ZnO and Al-doped ZnO. The existence of clear zone was estimated to be 25 mm surrounding both ZnO and Al-doped ZnO particle. No significant alteration can be observed over variation of Al-doped ZnO particle. From the fundamental point of view, it was notable that antimicrobial effects of ZnO and Al-doped ZnO particle can be explained by many mechanisms. One of the most interests was due to release phenomena of Zn and Al ions. These ions can be inserted to membrane of microorganisms. However, the toxicity of ZnO and Al-doped ZnO particle was not directly related to their diffusion to cell membrane. It may contact onto the cell causes changes in the microenvironment in the vicinity of the organism-particle contact area to either increase metal solubility or generate reactive oxygen species. It can ultimately cause the damage cell membrane. Moreover, the toxicity of ZnO and Al-doped ZnO particles was not only affected by the light via reactive oxygen species production. It occurred in the dark due to prevention of light sensitivity.

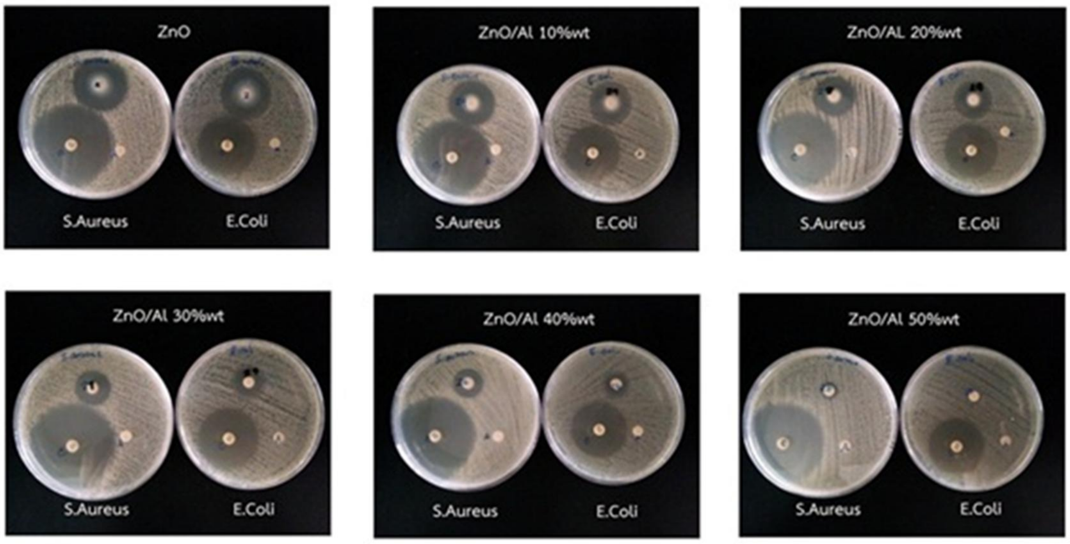

Fig. 2. Antibacterial test ZnO, 10 wt% Al-doped ZnO, 20 wt% Al-doped ZnO, 30 wt% Al-doped ZnO, 40 wt% Al-doped ZnO, 50 wt% Al-doped ZnO

The generation of highly reactive species such as OH^{-}, H_2O_2 and $O_2{}^{2-}$ was explained. Since ZnO and Al-doped ZnO can be activated by both UV and visible light, electron-hole pairs ($e^{-}h^{+}$) can be created. The holes split H_2O molecules (from the suspension of ZnO and Al-doped ZnO) into OH^{-} and H^{+}. Dissolved oxygen molecules are transformed to superoxide radical anions ($^{\bullet}O^{-}{}_2$), which in turn react with H+ to generate ($HO_2{}^{\bullet}$) radicals, which upon subsequent collision with electrons produce hydrogen peroxide anions ($HO_2{}^{-}$). They react with hydrogen ions to produce molecules of H_2O_2. The generated H_2O_2 can penetrate the cell membrane and kill the bacteria. Since, the hydroxyl radicals and super oxides are negatively charged particles, they cannot penetrate into the cell membrane and must remain in direct contact with the outer surface of the bacteria; however, H_2O_2 can penetrate into the cell. It can be assumed that the concentration of H_2O_2 generated from the surface increases with decreasing particle size, because the number of ZnO and Al-doped ZnO particle per unit volume of powder slurry increases with decreasing particle size. Based on the above, the increase in antibacterial activity is assumed to be due to the increase in H_2O_2 generated from the surface of ZnO and Al-doped ZnO on reducing the particle size of the sample.

Furthermore, in order to evaluate on the performance of antibacterial properties of ZnO and Al-doped ZnO particle, comparison on antibacterial properties over bacterial types has been observed. The effect on antibacterial properties for *S. aureus* was inferior to *E.coli*. Due to the difference on structure and chemical composition for both bacterial types, thin layers of lipid A, lipopolysaccharide and peptidoglycan are presented on the cell surface of *E. coli*, whereas there is only a peptido-glycan layer for *S. aureus*. However, antibacterial activity towards *S. aureus* and *E. coli* are assumed to be different due to sensitivity towards H_2O_2. On the other hand, it was remarkable to note that ZnO and Al-doped ZnO particle can be externally used to control the spreading of bacterial infections. It would be interesting to determine if any derivatives of ZnO and Al-doped ZnO particle with various chemical groups were more effective at eliminating various microorganisms. In the prevention and control of bacterial spreading and infections, the main target was focused on cell wall structure. It can be enrolled on the charge of macromolecules; therefore, a specific interaction to disrupt main function and location was triggered by introducing specific groups on the surface of the particles. Thus, Gram-positive bacteria may allow less ZnO and Al-doped ZnO particle to reach the cytoplasmic membrane than Gram-negative bacteria and may therefore be less susceptible.

4. Conclusion

ZnO and Al-doped ZnO particle were successfully synthesized via wet chemical synthetic method. X-ray diffraction can be used to identify the chemical bonding and crystal structure. Only 30 wt% of Al was partially substituted into ZnO crystal structure. The Al ion can be completely substituted to ZnO lattice. Scanning electron microscope revealed that ZnO and Al-doped ZnO particle was blocky particle with irregular sharp and variation in size. The specific surface area, pore volume and pore size distribution of Al-doped ZnO was inferior to neat ZnO due to surface tension and surface energy. Antibacterial properties of ZnO and Al-ZnO were conducted based on E. Coli and S. Aureus bacterial specie test. The existence of clear zone was observed as a 25 mm in radius surrounding ZnO and Al-doped ZnO particle. ZnO and Al-doped ZnO particle was promising as a good candidate as the antimicrobial agent material.

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5. References

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