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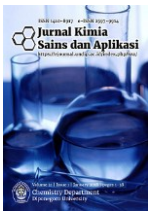

Effect of Calcination Temperature and Heating Rate on Zinc Oxide (ZnO) Synthesis Toward Antibacterial Properties

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Abstract

The increasing resistance of pathogenic bacteria to conventional antibacterial agents poses a significant challenge to global health, driving the need for alternative antibacterial materials with minimal resistance potential. Zinc oxide (ZnO) has gained attention for its antibacterial properties, attributed to its ability to generate reactive oxygen species (ROS), release Zn^{2+} ions, and disrupt bacterial cell membranes. In this study, ZnO was synthesized via thermal decomposition of zinc acetate dihydrate, with different calcination temperatures and heating rates. The structural and morphological characteristics of ZnO were analyzed using X- ray diffraction (XRD) and scanning electron microscopy (SEM), revealing that higher calcination temperatures increased crystallinity while heating rates influenced particle morphology. Antibacterial tests against *Staphylococcus aureus* and *Escherichia coli* showed that ZnO exhibited stronger antibacterial activity against *S. aureus*. The results also indicated that higher calcination temperatures reduced antibacterial efficacy, whereas higher heating rates enhanced bacterial inhibition. Notably, ZnO synthesized at 500°C with a heating rate of 5°C/min demonstrated the highest antibacterial performance, which correlated with its lower crystallinity and rod-like morphology. These findings emphasize the importance of controlling synthesis parameters to optimize ZnO properties for biomedical applications.

1. Introduction

The resistance of pathogenic bacteria to antibacterials has led to an increase in dangerous infection outbreaks. The emergence of multidrug-resistant (MDR) strains of Gram-positive and Gram-negative bacteria complicates the control and spread of infectious diseases. As a result, researchers have conducted extensive studies on antibacterial agents derived from organic and inorganic materials. Inorganic materials, such as semiconductor metal oxides, are often chosen for their stability under challenging processing conditions and their broad antibacterial activity. The development of semiconductor materials to combat pathogenic bacteria is becoming increasingly important, and the photocatalytic process offers an environmentally friendly alternative for pathogen elimination compared to other methods [1, 2].

Zinc oxide (ZnO) has garnered significant attention in various fields due to its unique physicochemical properties, including a wide bandgap (3.37 eV), high exciton binding energy (60 meV), and remarkable chemical stability. These characteristics make ZnO a promising material for applications in optoelectronics, photocatalysis, and biomedical science [2, 3, 4, 5]. In particular, its antibacterial activity has been widely studied, attributed to its ability to generate reactive oxygen species (ROS), release Zn^{2+} ions, and disrupt bacterial membranes, leading to cell damage and death [1, 3, 6, 7].

Numerous synthesis methods have been developed to produce ZnO, including sol-gel, hydrothermal, co-precipitation, and thermal decomposition techniques. Among these, thermal decomposition stands out as a simple, cost-effective, and scalable method that utilizes metal precursors such as zinc acetate dihydrate to

produce ZnO through thermal degradation. This approach offers excellent control over the purity and morphology of ZnO. However, the structural, morphological, and antibacterial properties of ZnO are heavily influenced by synthesis parameters, particularly calcination temperature and heating rate, which govern crystallinity, particle size, and surface area [4, 8, 9].

Previous studies have shown that calcination temperature plays a critical role in determining the phase purity and particle size of ZnO, while the heating rate affects the rate of precursor decomposition and the microstructure of the final product [8]. These factors, in turn, influence ZnO's ability to interact with bacterial cells, as properties like surface area and crystallite size are directly related to its antibacterial activity [1, 3]. Despite the development of ZnO synthesis, a comprehensive understanding of how calcination temperature and heating rate interact to affect its antibacterial performance remains underexplored.

In this study, this study utilized zinc acetate dihydrate as the single precursor to synthesize ZnO via the thermal decomposition method. The effects of varying calcination temperatures and heating rates on the structural, morphological, and antibacterial properties of ZnO were systematically investigated. X-ray diffraction (XRD) was employed to analyze the crystallinity and phase composition, while scanning electron microscopy (SEM) was used to study the particle morphology. The antibacterial activity of the synthesized ZnO was evaluated using disk diffusion against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*).

This work aims to provide valuable insights into optimizing calcination conditions for tailoring ZnO properties for enhanced antibacterial efficacy. By bridging the gap between synthesis parameters and functional performance, this study contributes to advancing the design of ZnO-based antibacterial agents for biomedical and environmental applications.

2. Experimental

2.1. Materials

Zinc acetate dihydrate, $Zn(OAc)\cdot 2H_2O$ (Merck, 99%), was used as the raw material for ZnO synthesis. To test the antibacterial properties, paper disks were employed in the disk diffusion method, with amoxicillin and sterile distilled water used as positive and negative controls, respectively. Bacterial media were prepared using Mueller-Hinton agar, nutrient agar, and bacteriological agar. Sterile distilled water, 70% alcohol, cotton swabs, and parafilm were used to maintain sterility during the tests.

2.2. Instruments

The ZnO samples were characterized using XRD (PANalytical X'Pert PRO series (PW3040/40)) to identify the crystallinity and phase formation of the ZnO, while the morphology of each sample was observed using SEM (JEOL JSM-6360LA). The antibacterial test was prepared in a laminar flow hood that create a sterile work environment.

2.3. Synthesis of Zinc Oxide (ZnO)

ZnO synthesis was synthesized using a simple thermal decomposition method by varying the calcination temperature and heating rate [8] of zinc acetate dihydrate. This study employs a modified version of temperature range and calcination time. The calcination temperatures used were 500, 600, 700, and 800°C, with heating rates of 1, 3, and 5°C/min at each variation of calcination temperature. ZnO synthesis from 8 g of zinc acetate dihydrate was calcinated in a furnace for 3 hours.

2.4. Materials Characterization

Powder X-ray diffraction (XRD) with Cu Kα radiation in the range of 20-80 was performed to identify crystallinity and phase formation of the ZnO using PANalytical X'Pert PRO series (PW3040/40). XRD characterization of ZnO was conducted for samples calcined at 500°C with different heating rates, as well as for samples heated at 5°C/min with varying calcination temperatures, to determine the optimum conditions for antibacterial properties.

The morphology of each sample was observed using SEM. For SEM analysis, the ZnO samples were first coated with a thin layer of gold to improve conductivity and then placed in the SEM chamber for imaging under a voltage of 20 kV and observed at 15,000× magnifications.

For the antibacterial test, all ZnO samples were tested in the form of suspensions prepared by mixing 0.5 g of ZnO into 2 mL of sterile distilled water, which was then homogenized using an ultrasonic bath for 30 minutes. The test suspension was further homogenized using a vortex mixer just before being used for antibacterial testing. Antibacterial activity was evaluated aseptically using the paper disc method against *S. aureus* ATCC 6538 and *E. coli* ATCC 11229. To confirm the antibacterial effect, amoxicillin and sterile distilled water were used as positive and negative controls, respectively.

3. Results and Discussion

3.1. Synthesis of Zinc Oxide (ZnO)

The product of calcined ZnO is a solid powder with a gray-white color gradation variation. The high calcination temperature produces a whiter ZnO powder color, while the high heating rate produces ZnO powder with a gray color. The formation of ZnO occurs with a reduction in weight as the temperature increases. In this study, the yield of ZnO is approximately 2.5 g compared to its initial weight of 8 g. These results are consistent with those obtained by Lin and Li [10], who found a reduction in sample weight occurred in the dehydration process by 16.5% and 64% in the formation of ZnO.

High calcination temperatures and low heating rates result in a longer calcination process or sample burning times. Prolonged calcination increases the number of oxygen atoms on the ZnO surface due to interactions with the atmosphere. The ZnO sample formed a white powder at a calcination temperature of 800°C and a heating rate of 1°C/min. While at a temperature of 500°C and a heating rate of 5°C/min, the ZnO appeared grayish. Previous

studies also observed a color difference in this ZnO product, which Farbod and Jafarpoor [11] attributed to the absence of oxygen and the strong asymmetric stretching of the wurtzite ZnO structure.

3.2. Crystallinity and Phase Formation

The analysis results validate the diffraction peaks aligning with the typical ZnO crystal hexagonal wurtzite phase in the P63mc space group. All of the diffraction peaks were matched to the standard data of ZnO, which has a hexagonal wurtzite structure (JCPDS 36-1451). Diffraction peaks were observed at 2θ values around 31.7070°, 34.0890°, 36.1190°, 47.2410°, 56.4790°, 62.3460°, 66.2350°, 67.6410°, and 68.8980°, respectively, corresponding to (100), (002), (101), (102), (110), (103), (200), (112), and (201) hexagonal crystal planes of ZnO. The XRD analysis revealed no additional peaks, indicating that pure-phase ZnO was successfully synthesized through the thermal decomposition process.

Figure 1(a) shows the XRD pattern of ZnO samples with calcination temperature variations of 500, 600, 700, and 800°C at a heating rate of 5°C/min. The high calcination temperature increases the intensity of the diffraction peaks. The increased intensity of the diffraction peaks suggests a strengthened ZnO phase [11]. These findings indicate that as the calcination temperature rises, the crystals become more structured and well-ordered, leading to improved crystallinity. In contrast, at lower calcination temperatures, ZnO crystal formation is incomplete, leaving some material in an amorphous state.

Variations in the heating rate during synthesis have an opposite effect to variations in the calcination temperature. Figure 1(b) shows that higher heating rates result in lower diffraction peak intensities. This effect occurs because a high heating rate influences the movement of constituent atoms in the crystal, causing stronger thermal vibrations. In contrast, a low heating rate provides a more stable environment for crystal formation, promoting a well-ordered structure. When the heating rate is high, the increased thermal energy induces significant atomic movement, leading to structural stretching within the crystal.

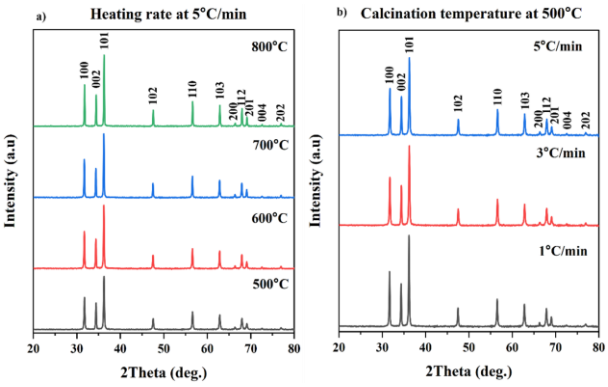

Figure 1. Diffractogram of ZnO samples: (a) at a heating rate of 5°C/min with varying calcination temperatures and (b) at a calcination temperature of 500°C with varying heating rates

According to the data, low calcination temperature and high heating rate have a low crystallinity effect on ZnO structure. This low crystallinity indicates that crystal formation is still not optimal, or some crystals remain in an amorphous state. The presence of an amorphous phase allows oxygen vacancies in the ZnO crystal structure. In this study, synthesis control through calcination temperature and heating rate plays a crucial role in determining the peak intensity in the diffractogram. Previous research also highlights that thermal treatment parameters affect ZnO's crystallinity and defect structure, impacting its functional properties [8, 12].

3.2.1. Morphology

SEM analysis was conducted to examine the morphology of ZnO crystals. In this study, the synthesized ZnO was observed using SEM at a magnification of 15,000×. The analysis revealed that the ZnO crystals exhibited wire- and rod-like structures. The morphological characteristics of the ZnO were influenced by synthesis parameters, particularly calcination temperature and heating rate, which played a crucial role in determining the final crystal structure.

The transformation of the ZnO structure from wire to rod occurs as the calcination temperature increases. The high calcination temperature causes the compression of the wire structure into the rod, which can be seen in Figure 2(a). When the calcination temperature increases, the wire becomes shorter and thicker. Furthermore, the effect of heating rate variation on the morphology at a calcination temperature of 500°C is given in Figure 2(b). The results indicate that the ZnO wire structure becomes shorter as the heating rate increases. The reason for collecting data on calcination temperature at 500°C and heating rate at 5°C/min is the optimum condition for antibacterial properties, which will be discussed later.

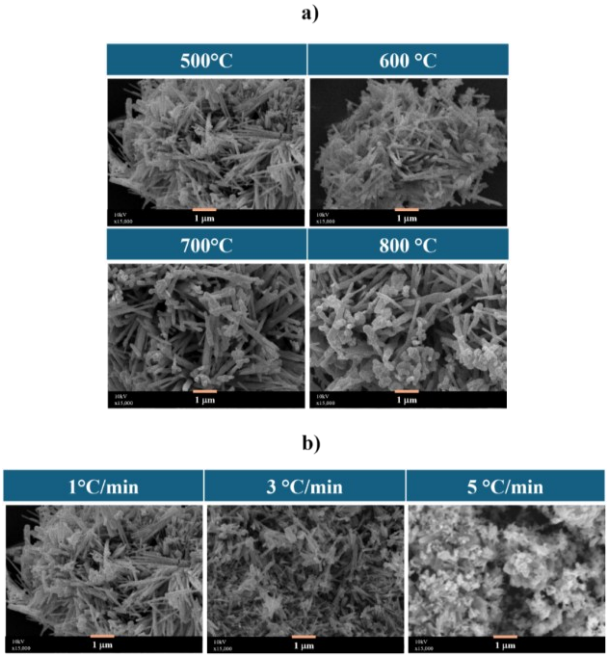

Figure 2. SEM images of ZnO samples: (a) at a heating rate of 5°C/min with varying calcination temperatures and (b) at a calcination temperature of 500°C with varying heating rates

The SEM analysis revealed that ZnO crystals exhibited non-uniform shapes and sizes due to agglomeration during crystallization. The crystal formation process involves nucleation followed by crystal growth, where crystal-forming molecules systematically attach to the crystal core, increasing crystal size. A longer formation time generally results in larger particle sizes. Consequently, a high calcination temperature promotes the formation of shorter and thicker wire-like structures, eventually developing into rod-shaped crystals. This finding aligns with previous studies that reported ZnO morphology characterized by elongated structures with reduced diameters, resembling wires [8].

The ImageJ application processed SEM image data to determine the crystal particle size. The analysis showed that the particle size increased with increasing calcination temperature and decreased with increasing heating rate. The particle sizes of each sample calcined at temperatures of 500, 600, 700, and 800°C with a heating rate of 5°C/min are 0.1121 µm, 0.1930 µm, 0.2285 µm, and 0.3500 µm, respectively. At the same time, the particle sizes synthesized at heating rates of 1, 3, and 5°C/min at a calcination temperature of 500°C are 0.1235 µm, 0.1110 µm, and 0.1101 µm, respectively. These results showed that high calcination temperatures led to an increase in particle size, whereas high heating rates decreased particle size.

3.2.2. Antibacterial Activity Test

The antibacterial activity test was analyzed qualitatively and quantitatively. Qualitative analysis was carried out by observing the clear zone formed around the disc paper in both *S. aureus* and *E. coli* bacteria, while quantitative analysis was carried out by measuring using a caliper and obtaining data on the diameter of the inhibition power expressed in millimeters (mm). Figure 3 presents the results of the antibacterial activity test on ZnO.

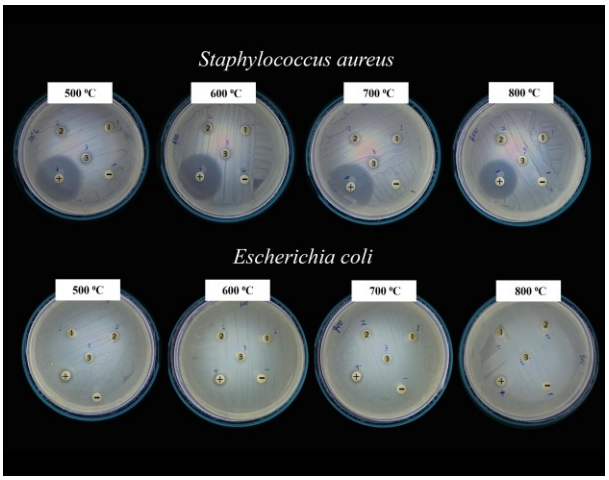

Figure 3. Antibacterial activity test results of ZnO against a) *S. aureus* and b) *E. coli*

The test results revealed that ZnO was more sensitive to *S. aureus* than *E. coli*, as evidenced by the clarity of the clear zone and the diameter of the formed inhibition force. ZnO's antibacterial activity is more sensitive to *S. aureus*, a Gram-positive bacteria than to *E. coli*, a Gram-negative bacteria. Several studies also show that the sensitivity of ZnO against gram-positive is higher than gram-negative, showing that *S. aureus* bacteria are more susceptible than *E. coli*. This is associated with differences in cell wall structure, cell physiology, and metabolism [3, 6, 9].

Gram-positive bacteria, such as *S. aureus*, possess a single plasma membrane encircled by a peptidoglycan-based cell wall, which constitutes about 90% of the wall's composition, with the remainder consisting of molecules like teichoic acid. In contrast, Gram-negative bacteria possess a double membrane system, where a permeable outer membrane encloses the plasma membrane, and a thinner peptidoglycan layer lies in between. This structural complexity in Gram-negative bacteria helps prevent lipid peroxidation caused by ROS generated by ZnO.

The antibacterial properties of ZnO are primarily attributed to its ability to generate ROS, including superoxide anions, hydroxyl radicals, and peroxides. These ROS contribute to bacterial cell damage through multiple mechanisms. ZnO nanoparticles accumulate on the bacterial outer membrane or within the cytoplasm, leading to the release of Zn^{2+} ions. This process disrupts the cell membrane, damages proteins, and induces genomic instability, ultimately resulting in bacterial cell death [1, 2, 3, 6]. However, further research is still needed to fully elucidate the antibacterial mechanism of ZnO.

In this study, synthesis control through calcination temperature and heating rate played a crucial role in determining the antibacterial performance of ZnO. The synthesis parameters were adjusted to enhance ZnO's antibacterial activity. The results of the antibacterial activity test indicated that higher calcination temperatures led to weaker bacterial growth inhibition. As shown in Figure 4(a), the inhibition effect decreased with increasing calcination temperature, as reflected by the sequential reduction in inhibition zone diameter: 800<700<600<500°C.

Synthesis control enhances ZnO's antibacterial performance by regulating the heating rate. The ZnO antibacterial activity test results revealed that regulating the heating rate during the thermal decomposition process influenced the inhibition of bacterial growth. A higher heating rate led to stronger antibacterial activity, as evidenced by the increasing diameter of the inhibition zone in Figure 4(b). Among the tested samples, ZnO synthesized at a heating rate of 5°C/min exhibited the highest antibacterial effectiveness compared to those synthesized at 1 and 3°C/min.

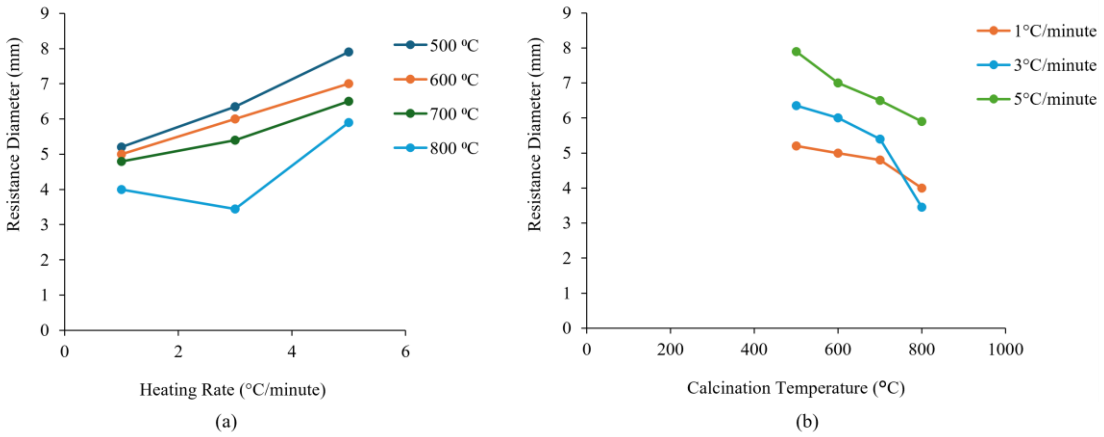

Figure 4. *S. aureus* growth inhibition as a function of the inhibition zone diameter under varying (a) calcination temperatures and (b) heating rates

In this study, the low crystallinity of ZnO suggests the presence of an amorphous phase, which plays a crucial role in enhancing its antibacterial properties. Amorphous ZnO exhibits superior antibacterial activity compared to its crystalline counterpart due to a higher concentration of surface defects and oxygen vacancies [9]. These structural defects promote the generation of ROS, such as hydroxyl radicals (·OH) and superoxide anions (O_2^-), which contribute to bacterial cell damage. Additionally, the increased surface area of amorphous ZnO enhances its interaction with bacterial cells, leading to more effective membrane disruption. Therefore, optimizing synthesis parameters to maintain a partially amorphous structure could be a promising strategy for improving ZnO-based antibacterial materials.

4. Conclusion

Synthesis control, in the form of calcination temperature and heating rate, plays a role in the performance of ZnO as an antibacterial. The calcination temperature and heating rate affect crystallinity, morphology, and size. Calcination temperature is inversely proportional to the inhibition of bacterial growth, while the heating rate is directly proportional to the inhibition of bacterial growth. ZnO samples synthesized at a heating rate of 5°C/min and a calcination temperature of 500°C produce maximum antibacterial activity. This optimal performance is characterized by low crystallinity, a short wire-like ZnO structure, and the smallest particle size among the tested samples.

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