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# OPTIMIZATION SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING FACTORIAL DESIGN AND ITS ANTIBACTERIAL ACTIVITY

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**ABSTRACT**
Due to their chemical stability, potent antibacterial activity, and comparatively low hazardous profile, inorganic metal oxides may be used as efficient antibacterial. Zinc oxide nanoparticles (ZnO NPs) significantly slow down the growth of various bacteria. This study aimed to determine the effect and interaction of pH, calcination temperature, and pectin (capping agent) on the synthesis and antibacterial activity of ZnO NPs. The synthesis was carried out by the precipitation method. Synthesis optimization using Design Expert software (factorial design) with parameters including yield, particle size, polydispersity index, zeta potential, particle shape, and inhibition zone of bacteria (*P.acnes*, *S.aureus*, and *S.epidermidis*). This study proves that the synthesis of ZnO NPs is influenced by pH, calcination temperature, and pectin. Various characteristics of the synthesized ZnO NPs have different antibacterial activity. The results of *Design Expert* analysis with 3 g of precursor obtained the optimum combination at pH 8.8, calcination temperature of 600°C, and pectin 0.76%.
**Keywords:** Synthesis, ZnO Nanoparticles, Antibacterial, pH, Calcination Temperature, Pectin.
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## INTRODUCTION

Nanotechnology has been widely developed and applied in the pharmaceutical, one of which is zinc oxide nanoparticles (ZnO NPs) which have many therapeutic benefits due to their antibacterial activity.[1] The most popular method is the chemical method.[2] Control of synthetic products is very important. The production process of ZnO NPs will have considerable challenges, due to the synthesis-related parameters that can affect the size and shape of nanoparticles.[3] Several parameters in the research technique affect the synthesis. The particle size, shape, and geometry of ZnO NPs will be influenced by pH, capping agent, and calcination temperature.[4,5] pH in the synthesis of ZnO nanoparticles (precipitation technique) is a factor that controls the size and shape of the particles. The synthesis of ZnO NPs with alkaline pH produces pure nanoparticles, but high-purity ZnO nanoparticles tend to bind to each other and agglomerate.[6,7] A capping agent, a stabilizer that limits nanoparticle overgrowth and prevents aggregation in colloid synthesis, is a crucial component in the synthesis of nanomaterials.[8,9] Capping agents are PVA, PVP, gelatin, pectin, and others.[10] Pectin has strong biocompatibility and biodegradability. The temperature of the calcination affects the synthesis. Calcination temperature has an important role in the shrinkage of particle pores. The shrinkage will cause the particles to have a small and uniform size. Calcination removes residual impurities from the synthesis process so that the overall particles become more compact and uniform.[11-13] Controlling ZnO NPs size during synthesis is crucial for achieving the greatest antibacterial effect. Smaller ZnO NPs exhibit the strongest antibacterial activity. Since aggregation might influence the effect and stability, the colloidal stability of nanoparticles is crucial in this context. *P. acnes*, *S. aureus*, and *S. epidermidis*, the bacteria that cause acne vulgaris, are susceptible to the antibacterial effects of ZnO NPs.[4,14,15] The research gap is about pH, calcination temperature, and capping agent interacting and combining effectively in the characterization and application of the synthesized ZnO NPs. This study optimized the synthesis of ZnO


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NPs to ascertain the impact and interaction between factors on particle size, polydispersity index, zeta potential, surface particle shape, and antibacterial activity.

## EXPERIMENTAL

### Materials

Zinc nitrate hexahydrate (Sigma Aldrich), pectin (Focus Herb), NaOH (Merck), *P. acnes* (Nanobio Laboratory), *S. epidermidis* (Nanobio Laboratory), *S. aureus* (UMS).

### Synthesis of ZnO Nanoparticles

The synthesis was carried out by the precipitation method. The synthesis factors are pH (8 and 13), pectin (12 mg (0.4%) and 23 mg (0.76%)), and calcination temperature (250˚C and 600˚C). Synthesis based on suggestions from Design Experts based on Table-1. For each run, 3 g of zinc nitrate hexahydrate was dissolved in 100 mL of demineralized water. The solution was agitated on a hot plate stirrer at 80°C for two hours. 16 g of NaOH dissolved in 100 mL of demineralized water. The mixture was added a drop of NaOH solution gradually until the pH shown in Table-1 was reached. Then the mixture was added pectin. Centrifugation was used to precipitate the solution for 15 minutes at 3000 rpm after it has reached room temperature. The precipitate was dried for an hour in an oven at 120°C. After two hours of calcination at the temperatures shown in Table-1 with a furnace, the nanoparticle powder was cooled to room temperature. ZnO NPs were characterized with FTIR (PerkinElmer, USA), Particle Size Analyzer (Horiba, Japan), and SEM (JSM-6510LA, Japan). Characterization of ZnO NPs using PSA (3 times auto scanning). Particle shape is classified in the range 1-4 (1= spherical, 2=slightly spherical, 3=irregular, and 4=very irregular). Particle shape was categorized based on the subjectivity of the researcher. ZnO NPs were also tested for antibacterial activity. In general, the antibacterial activity test is divided into several stages. It includes sample preparation, bacterial suspension preparation, and antibacterial testing by measuring the inhibition zone.[16,17] The concentration of ZnO NPs for the antibacterial test is 50mg/well. Antibacterial test results include wells with a diameter of 6 mm.

Table-1: Run Analysis on Factorial Design with Two Times Replication

| Run | pH | Calcination temperature (ºC) | Pectin (mg) |
|---|---|---|---|
| 1 | 8 | 250 | 23 |
| 2 | 8 | 250 | 12 |
| 3 | 13 | 250 | 23 |
| 4 | 8 | 600 | 12 |
| 5 | 8 | 250 | 12 |
| 6 | 8 | 600 | 23 |
| 7 | 8 | 250 | 23 |
| 8 | 13 | 250 | 23 |
| 9 | 13 | 250 | 12 |
| 10 | 8 | 600 | 12 |
| 11 | 13 | 600 | 12 |
| 12 | 13 | 250 | 12 |
| 13 | 8 | 600 | 23 |
| 14 | 13 | 600 | 23 |
| 15 | 13 | 600 | 23 |
| 16 | 13 | 600 | 12 |

### Design Expert Analysis

The data (yield, particle size, polydispersity index, zeta potential, particle shape, inhibition zone of *P.acnes*, *S. aureus*, and *S. epidermidis*) were processed using *Design Expert* version 13 with the factorial design method. Because three factors were analyzed, cube graphs are used to determine the effects and interactions of all these factors. Prediction and verification data were analyzed using a one-sample t-test with a confidence level of 95%.

## RESULTS AND DISCUSSION

The synthesis product of ZnO nanoparticles is affected by factors such as pH, calcination temperature, and pectin (capping agent). These factors play a role in the characteristics of the nanoparticles and their

antibacterial activity. Therefore, controlling the desired synthesis product is an important thing that must be considered. Characterization and antibacterial activity of ZnO NPs are listed in Table-2. The appearance of ZnO peaks in the region of 358.7-383.47 $cm^{-1}$ in the study demonstrated the success of the synthesis.

Table-2: Results of Analysis Response and Antibacterial Test of ZnO Nanoparticles (run 1-16)

| Run | Yield (%) | Particle size (nm) | Polydispersity index | Zeta Potential (mV) | Particle shape | Inhibition Zone | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | *P.acnes* (mm) | *S.aureus* (mm) | *S.epidermidis* (mm) |
| 1 | 25.92 | 182.07±0.17 | 0.23±0.02 | -56.00±1.34 | 3 | 13.45±0.75 | 18.60±0.40 | 11.80±0.30 |
| 2 | 26.13 | 161.77±15.23 | 0.25±0.01 | -55.77±0.61 | 2 | 16.45±1.05 | 20.55±0.55 | 12.20±0.80 |
| 3 | 26.76 | 175.50±9.55 | 0.34±0.03 | -49.47±0.42 | 3 | 17.25±0.75 | 17.70±0.90 | 11.25±0.25 |
| 4 | 26.36 | 168.60±18.46 | 0.42±0.15 | -57.33±0.26 | 3 | 15.90±0.30 | 16.35±0.45 | 12.55±0.35 |
| 5 | 27.00 | 161.90±15.43 | 0.28±0.06 | -48.27±6.04 | 2 | 15.60±0.40 | 19.15±0.85 | 14.45±0.25 |
| 6 | 27.39 | 181.07±0.31 | 0.18±0.04 | -52.00±0.16 | 1 | 13.80±0.60 | 19.00±1.00 | 14.20±0.10 |
| 7 | 26.98 | 174.87±9.45 | 0.32±0.09 | -50.13±0.31 | 3 | 12.75±0.25 | 17.55±0.25 | 11.75±0.25 |
| 8 | 28.23 | 380.17±38.66 | 0.69±0.23 | -56.10±0.67 | 4 | 18.10±0.90 | 20.15±0.55 | 11.90±0.10 |
| 9 | 26.77 | 182.97±0.40 | 0.18±0.03 | -56.40±0.57 | 3 | 13.95±0.15 | 20.50±0.50 | 12.55±0.15 |
| 10 | 26.37 | 168.07±9.60 | 0.29±0.04 | -61.63±0.56 | 3 | 15.65±0.35 | 18.10±1.10 | 10.10±0.10 |
| 11 | 27.20 | 214.73±11.46 | 0.26±0.01 | -51.10±1.34 | 1 | 13.55±0.45 | 14.80±1.80 | 9.75±0.55 |
| 12 | 26.45 | 168.23±8.96 | 0.18±0.03 | -57.80±1.28 | 3 | 11.75±0.25 | 17.55±1.45 | 13.00±0.10 |
| 13 | 27.34 | 177.07±25.03 | 0.33±0.03 | -49.10±0.51 | 2 | 15.90±0.40 | 22.55±1.85 | 11.70±0.10 |
| 14 | 25.99 | 175.13±9.43 | 0.24±0.01 | -57.33±0.17 | 3 | 10.80±0.40 | 19.25±1.25 | 16.85±0.55 |
| 15 | 26.11 | 196.67±11.15 | 0.26±0.07 | -64.40±0.14 | 3 | 14.30±0.20 | 15.55±0.55 | 16.70±1.00 |
| 16 | 26.34 | 205.73±0.74 | 0.24±0.08 | -54.43±1.05 | 3 | 15.95±0.35 | 13.75±0.65 | 9.45±0.05 |

The characteristics of the nanoparticles formed are affected by pH, calcination temperature, and pectin content. Nucleation and production of ZnO particles are affected by the release of $OH^-$ ions, so pH has an important role here.[18] The temperature of the calcination affects the synthesis. Calcination can affect particle size, remove contaminants, purify synthetic products, and increase the crystallinity of ZnO nanoparticles.[19,20] Capping agent has a role as a stabilizer or binding molecule that prevents agglomeration and stabilizes nanoparticle interactions.[21] It can also stabilize particles through steric and electrostatic interactions, resulting in nanoparticles with small, uniform sizes and non-aggregated shapes.[22] According to previous studies, ZnO NPs have antibacterial activity.[23] Zinc oxide nanoparticles' antibacterial properties arise from three distinct mechanisms. When zinc oxide nanoparticles come into contact with bacteria, they will enter the cell through protrusions and pores in the cell wall, where they will then cause lysis. ZnO nanoparticles cause the release of antimicrobial ions, particularly $Zn^{2+}$ ions, which significantly impact active transport inhibition, amino acid metabolism, and enzyme system disruption. Reactive oxygen species (ROS) formation is the third pathway. The reactive oxygen species are superoxide anions ($O_2^-$), hydrogen peroxide ($H_2O_2$), and hydroxides ($OH^-$). This reactive oxygen damages cellular components such as fats, DNA, and proteins, resulting in damaged cells.[24] The characteristics of ZnO NPs (particle size, polydispersity index, zeta potential, and particle shape) have different inhibition zones for each bacterium. This indicates that the synthesized characteristics have an effect on the antibacterial power of ZnO NPs.

The cube graph in Fig.-1 demonstrates how the interaction of these three factors (pH, calcination temperature, and pectin) influences the outcomes of the test parameters. The mathematical equations were mentioned in Table-3.

Based on the Design Expert study, the equations of all the responses listed in Table-3 were obtained. Code A is the value of pH, B is the value of the calcination temperature, and C is the value of the pectin content. The equation also shows the interaction between factors. A positive value (+) indicates that the factor has a dominant role in increasing the response value and a negative value (-) indicates that the factor has a dominant role in decreasing the response value. Synthesis optimization resulted in ~~a~~ desirability of 0.607, predicted optimum formula with pH 8.8, calcination temperature 600 °C, and pectin 23 mg (0.76%). The desirability value predicts parameters of yield 27.148%, particle size 180.195 nm, polydispersity index 0.254, zeta potential -52.245 mV, particle shape 1.746, *P.acnes* inhibition zone 14.471 mm, *S.aureus* inhibition zone 20.220 mm, and *S.epidermidis* inhibition zone 13.578 mm. Statistical results between the

predicted and verification showed predictions were reliable with p>0.05 on yield parameters, particle size, particle shape, and inhibition zone of *S.epidermidis* showed no significant results. While the statistical results on the polydispersity index, zeta potential, and inhibition zones of *P. acnes* and *S. aureus* showed predictions could not be trusted with a p-value <0.05. The results of the synthesis product with its characteristics and antibacterial activity can be used as a reference in the desired synthesis of ZnO nanoparticles.

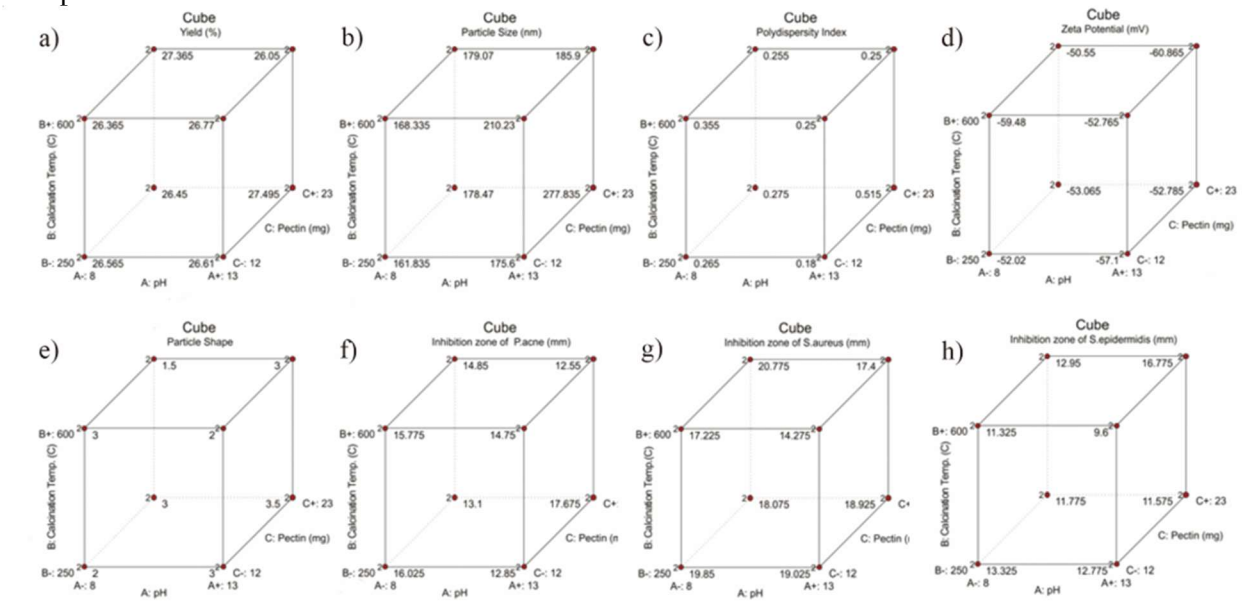


Fig.-1: Results of Design Expert Analysis (a) yield, (b) Particle Size, (c) Polydispersity Index, (d) Zeta Potential, (e) Particle Shape, Inhibition Zone of (f) *P. acnes*, (g) *S. aureus*, and (h) *S. epidermidis*

Table-3: The Results of the Analysis of the Parameter Equation Design Expert

| Parameter | Equation |
|---|---|
| Yield | +26.71+0.023A-0.071B+0.13C-0.25AB-0.090AC-0.061BC-0.34ABC |
| Particle size | +192.16+20.23A-6.28B+13.16C-8.05AB+6.32AC-16.56BC-15.08ABC |
| Polydispersity index | +0.29+0,005625A-0.016B+0.031C -0.033AB +0.053AC-0.056BC-0.028ABC |
| Zeta potential | -54.83-1.05A-1.09B+0.51C+0.15AB-1.46AC-0.31BC-2.80ABC |
| Particle shape | +2.63+0.25A-0.25B+0.12C-0.13AB+0.25AC-0.25BC+0.38ABC |
| Inhibition zone of *P. acnes* | +14.70-0.24A-0.22B-0.15C-0.59AB+0.81AC-0.63BC-1.13ABC |
| Inhibition zone of *S. aureus* | +18.19-0.79A-0.78B+0.60C-0.79AB+0.16AC+1.07BC-0.26ABC |
| Inhibition zone of *S. epidermidis* | +12.51+0.17A+0.15B+0.76C+0.36AB+0.74AC+1.44BC+0.65ABC |

## CONCLUSION

This study proves that the synthesis of ZnO NPs is influenced by pH, calcination temperature, and pectin. Various characteristics of the synthesized ZnO NPs have different antibacterial activity. The optimized synthesis condition of ZnO nanoparticles (precipitation method) with *Design Expert* software yielded pH of 8.8, calcination temperature of 600°C, and pectin concentration of 0.76% for 3 g precursor.

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## CONFLICT OF INTERESTS

All authors confirm that they have no conflict of interest.

## AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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