UNIVERSITI PUTRA MALAYSIA

STRUCTURAL, OPTICAL AND ANTIBACTERIAL PROPERTIES OF ZINC OXIDE NANOPARTICLES SYNTHESIZED VIA CHEMICAL AND GREEN SYNTHESIS METHODS

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By

SITI HUZAIMAH BINTI RIBUT

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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May 2019

Chair: Che Azurahanim Che Abdullah, PhD
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Zinc oxide nanoparticles (ZnONPs) are subjected to intense research due to their wide application in various fields such as electronics, solar cells and optics, and another impressive property. In terms of biological application, in wastewater treatment, chemical disinfection like chlorination can respond with various components in natural water yielding to the by-product formation that possibly hazardous to the environment which could harmfully affect human health. Metal oxide nanoparticles can be one of the options to enhance the wastewater treatment since it is partly neutral to water and not a strong oxidant. To reduce the use of harmful chemicals and by-product formation in wastewater treatment, the use of plant extract in the synthesis of nanoparticles are cost-effective and environmentally friendly. Therefore, the ZnONPs and biogenic ZnONPs was successfully synthesized using chemical and green synthesis method. The structural, optical and the antibacterial properties of both ZnONPs and biogenic ZnONPs was revealed by characterization and antibacterial activity against *Escherichia coli* bacteria. For structural properties, XRD test showed that both ZnONPs and biogenic ZnONPs have a wurtzite structure as the peaks of zinc oxide (ZnO) dominated in (002) plane. Then, the FESEM result revealed that the ZnO formed in a spherical shape and the nanoparticles sizes measured is a range around 60 ± 12 nm - 24 ± 4 nm for both ZnONPs and biogenic ZnONPs. For optical properties, the energy band gap obtained is between 3.33 – 3.37 eV. For ZnONPs synthesis by chemical method, the antibacterial test discovered that ZnONPs prepared at pH 10 & 11 shows the significant inhibition compared to other pH values. Meanwhile, the biogenic ZnONPs at 0.1 M concentration exhibit the highest inhibition of *E. coli* bacteria due to their smallest size of nanoparticles. The results obtained indicated that both ZnONPs and biogenic ZnONPs exhibit the significant inhibition towards bacteria growth due to their great properties in structural and optical.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

CIRI-CIRI STRUKTURAL, OPTIKAL DAN ANTIBAKTERIAL NANOPARTIKEL ZINK OKSIDA SINTESIS MELALUI KAEDAH KIMIA DAN SINTESIS HIJAU

Oleh

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Nanopartikel zink oksida (ZnONPs) menjadi penyelidikan yang sengit kerana aplikasinya yang luas dalam pelbagai bidang seperti elektronik, sel suria dan optik, dan sifat lainnya yang menonjol. Dari segi aplikasi biologi, dalam merawat air kumbahan, pembasmian kuman kimia seperti pengklorinan boleh bertindak balas dengan pelbagai komponen di dalam air semulajadi yang menyebabkan terhasil kesan sampingan yang berbahaya kepada alam sekitar yang boleh menjejaskan kesihatan manusia. Nanopartikel oksida logam adalah salah satu alternatif untuk memperbaiki sistem rawatan air kumbahan, memandangkan ia bukan oksidan yang kuat dan cirinya yang neutral seperti air. Untuk mengurangkan penggunaan bahan kimia berbahaya dan kesan sampingan dalam merawat air kumbahan, penggunaan ekstrak tumbuhan dalam sintesis nanopartikel boleh menjadi kos efektif dan mesra alam. Oleh itu, ZnONPs dan ZnONPs biogenik telah berjaya disintesis menggunakan kaedah sintesis kimia dan hijau. Sifat-sifat struktural, optik dan antibakteria ZnONPs dan biogenik ZnONPs dilakukan dengan pencirian dan aktiviti antibakteria terhadap bakteria *Escherichia coli*. Untuk sifat-sifat struktur, ujian XRD menunjukkan bahawa kedua-dua ZnONPs dan biogenic ZnONPs mempunyai struktur wurtzite kerana puncak zink oksida (ZnO) muncul di satah (002). Kemudian, keputusan FESEM mendedahkan bahawa ZnO terbentuk dalam bentuk sfera dan saiz nanopartikel diukur adalah sekitar 60 ± 12 nm - 24 ± 4 nm untuk kedua-dua ZnONPs dan biogenik ZnONPs. Jurang band tenaga yang diperoleh adalah antara 3.33-3.37 eV. Untuk ZnONPs sintesis dengan kaedah kimia, ujian bakteria mendapati bahawa ZnONPs pada pH 11 menunjukkan perencatan yang signifikan berbanding dengan nilai pH yang lain. Sementara itu, biogenik ZnONPs pada kepekatan 0.1 M menunjukkan perencatan tertinggi terhadap *E. coli* bakteria disebabkan ia mempunyai saiz nanopartikel yang paling kecil. Keputusan yang diperoleh menunjukkan bahawa kedua-dua ZnONPs dan ZnONP biogenic menunjukkan perencatan yang signifikan terhadap pertumbuhan bakteria kerana sifatnya yang unggul dalam struktur dan optik.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ZnONPs	Zinc Oxide Nanoparticles
CB	Conduction Band
VB	Valence band
WHO	World Health Organization
DBPs	Halogenated Disinfection by Products
NaOH	Sodium Hydroxide
NA	Nutrient Agar
NB	Nutrient Broth
XRD	X-Ray Diffraction
FTIR	Fourier Transform Infrared
FESEM	Field Emission Scanning Electron Microscopy
EDX	Energy Dispersive X-Ray
HRTEM	High-Resolution Transmission Electron Microscopy
PL	Photoluminescence
UV	Ultraviolet
UV-Vis	Ultraviolet-Visible
CuK_{α}	Copper K-Alpha
ROS	Reactive Oxygen Species
FWHM	Full Width at Half Maximum
MO	Methylene Orange
MB	Methylene Blue
eV	Electron Volt
E. coli	*Escherichia coli*
C. hystrix	*Citrus hystrix*

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CHAPTER 1

INTRODUCTION

1.1 Structure of the Thesis

The first chapter, **Chapter 1**, gives an overview of this thesis. It includes a brief introduction, the scope of research, thesis structure and the novelty of the study undertaken is also briefly explained. **Chapter 2** of this thesis presents a review of the structure and properties of zinc oxide nanoparticles (ZnONPs). This chapter also details the biological applications of ZnONPs, particularly in structural, optical and antibacterial properties of ZnONPs. The facile method used to synthesis the ZnONPs also has been stated in this chapter. The main experimental procedures used during the research and the instrumentation used to characterize the ZnONPs are explained in **Chapter 3**.

In **Chapter 4**, the prepared ZnONPs were characterized by XRD, FTIR, EDX, FESEM, PSA and HRTEM to investigate the crystalline quality, the functional group, the surface morphology, the particle size and the internal nanostructure formation. Photoluminescence and UV-Vis spectroscopies techniques will be used to analyse their optical properties. The photocatalytic and antibacterial activity also conducted to exhibit the properties of ZnONPs as an antibacterial material. **Chapter 5** summarizes the results of the entire thesis research. At the end of this chapter, an outcome, challenges and future work inspired by this research are presented.

1.2 Background

In the last decades, development and advanced discovery related to nanomaterials have been marked. Among the generation of novel semiconductor compounds, metal oxide nanomaterials have emerged and attracted an increasing number of attention worldwide researchers for several reasons. Metal oxide nanomaterial semiconductor like zinc oxide (ZnO), titanium dioxide (TiO_2) and cadmium oxide (CdO) have been taken into consideration as scientific finding for numerous applications. These nanomaterials grew much interest because of their well-known performance in electronics, optics, quantum applications as well as their ability in bioactivity. Nanomaterials offer large specific surface areas to volume ratio which improved both chemical and physical properties to be applied in various applications including biological application.

ZnO is the most widely used nanomaterials in nanotechnology due to their outstanding properties. The enormous attention has arisen due to its unique physical properties consists wide energy band gap of 3.37 eV at ambient temperature and large binding energy of 60 meV (Saleem et al., 2012), which

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give development to an extensive range of potential applications in many areas such as electronics, solar cells and biological applications. As biological applications of these novel nanomaterials continue to increase, concerns have been raised about the ability of zinc oxide nanoparticles (ZnONPs) in the biological activity to treat wastewater. The biological activity of ZnONPs was investigated through the antibacterial activity that identified the effect of ZnONPs on the bacteria inhibition. The method used to prepare the ZnONPs also take an important part which is to reduce the by-product formation when applied in wastewater treatment. Thus, it is necessary to undertake careful antibacterial studies and the synthesis method used to prepare the ZnONPs.

A broad procedure of synthesis ZnONPs with toxic chemicals may lead to the probably hazardous the environment which could badly affect human health. Apart from using the chemical method, green synthesis is a new method to synthesis ZnONPs. In order to minimize or eliminate the use of hazardous chemicals in preparing the ZnONPs, the use of plant extract in the synthesis of nanoparticles can be facile, low cost, renewable and environmentally friendly approach.

Currently, ZnONPs have been developed as adsorbent nanomaterials in wastewater treatment due to their brilliance in antimicrobial and photocatalyst properties. ZnONPs verifies itself as a very promising photocatalyst that suitable for water treatment as water pollution is a danger issue that we face today. Several studies have confirmed that ZnONPs exhibits a better efficiency than TiO_2 in photocatalytic degradation of some dyes, even in aqueous solution (Li & Haneda, 2003). In addition, ZnONPs also shows attractive antimicrobial properties that are utilized in several pharmaceutical and cosmetic products to overcome microbial infection problem.

Therefore, the purpose of the current research reported in this thesis is to explore the structural, optical and antibacterial properties of ZnONPs synthesis with both chemical and green synthesis method. The findings from this research also revealed the novel production of ZnONPs based on green synthesis method synthesized using plant extract of interest. The various pH and concentration of ZnONPs were verified in order to optimize the amount of sample to be applied in antibacterial activity. In the current research, the final yield of ZnONPs synthesized with chemical method grows as a white powder while the biogenic ZnONPs synthesized with green synthesis method appears as a pale green powder (Appendix 1). The ZnONPs is almost insoluble in water but soluble in any alcohol solvent like ethanol.

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1.3 Problem Statement

In wastewater treatment, conventional methods including chlorination have their own harmful side effect to the human health as they produced the by-product formation when react with various components in water. It has been discovered that nanotechnology, especially metal oxide nanoparticles used for wastewater treatment is a promising method to overcome the disadvantage of the current treatment (Masoumbaigi et al., 2014). Because of the factors like small sizes and large surface areas, nanoparticles exhibit sturdy reactivity and adsorption capacities. Previously, Lu et al., (2016) stated that TiO_2 nanoparticles is a strong candidate to treat the wastewater due to high photocatalytic activity and large bandgap energy. However, the large bandgap energy of TiO_2 nanoparticles required them to use the UV light instead of visible light. Besides, the antibacterial properties of TiO_2 nanoparticles is also questionable in visible light as they efficient when expose under UV light.

ZnO is a very promising material for many applications in physics, materials science, chemistry, biology and biomedical applications. ZnONPs was not much investigated in research for application in wastewater treatment. The synthesis product of ZnONPs can be contributed as a photocatalyst to overcome the water pollution through the degradation colour. The bandgap energy of the ZnONPs also is possible to control during the synthesis process. Besides, the ZnONPs also possess the antibacterial properties as they can kill bacteria especially in wastewater (Espitia et al., 2012). There are several advantages of biogenic ZnONPs to be applied in wastewater treatment, such as have lower energy and synthesized costs, reduction of heavy metal content, by-product formation, nitrates and sulphates, and the degradation colour of wastewater (Patil, 2015).

To produce efficient ZnONPs, there are significant challenges that have to be overcome like to control the nanoparticles size, the purity of the sample, the ability of the sample to emit and absorb the light and their antibacterial properties when modified with various synthesis method. Recently, previous studies have explored the effects of pH value in synthesis, characterization of structural and optical properties of ZnONPs using various methods (Alias et al., 2010; Singh and Haque, 2016). However, the potential of ZnONPs to be used in the antibacterial activity using the different pH value still has not much investigated. Based on our knowledge and literature search, so far there is no further research reported the effect of pH values from natural alkaline of ZnONPs on antibacterial activity. To evaluate that the ZnONPs is one of the nanoparticles that have the antibacterial properties for wastewater treatment, the study on antibacterial activity was conducted to observe the inhibition growth of the bacteria.

In current research, besides using the chemical method to synthesis the ZnONPs, the green synthesis technique was chosen to prepare the ZnONPs or known as biogenic ZnONPs to reduce the chemical usage as chemical are

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possibly hazardous and toxic to the environment which could harmfully disturb the human healthiness. The use of plant extract in the synthesis nanoparticles also are abundant in nature, low cost and environmental friendly approach. As far as the author concern, to date, there is no work reported the synthesis of biogenic ZnONPs using *Citrus hystrix (C. hystrix)* extracts based on the green synthesis technique. To control the growth condition of the synthesis ZnONPs, *C. hystrix* plant can act as bio-capping and stabilizing agent at room temperature.

The expected outcome from this research is to provide a new production of ZnONPs and biogenic ZnONPs based on green synthesis technique as the plant can act as the bio-capping agent to enhance their structural, optical and antibacterial properties.

1.4 Thesis Objectives

The main objectives of current research are to produce both ZnONPs and biogenic ZnONPs. Optimization of the best condition was determined by means of structural, optical and antibacterial properties of both ZnONPs and biogenic ZnONPs. The main objectives of this research are:

1. To synthesis and characterize ZnONPs by using both chemical and green synthesis methods.
2. To study the structural and optical properties of both ZnONPs and biogenic ZnONPs under different pH values and concentrations of precursor.
3. To measure the inhibition zone of the antibacterial activity of both ZnONPs and biogenic ZnONPs against *Escherichia coli* bacteria.

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Siti Huzaimah Binti Ribut was born in Batu Pahat, Johor on October 26, 1993. She completed her primary education at Sekolah Kebangsaan Seri Sejati and attended Sekolah Menengah Kebangsaan (Perempuan) Temenggong Ibrahim for her secondary education and graduate in 2010. After that, she pursues her matriculation study for one year at Johor Matriculation College.

In 2012, she was offered to further her study at Universiti Putra Malaysia for 4 years in Bachelors of Science (Honours) in Physics. During her Bachelor, she accomplished her industrial training at Malaysian Sheet Glass (MSG), Pasir Gudang, Johor for 3 months and gather valuable knowledge about the technology used to produce glass used by people. Her final year project (FYP) was entitle “Structural and Optical Properties of Zinc Oxide Thin Films Growth on Various Substrates” and supervise by Dr. Che Azurahanim Che Abdullah. She discovered her passion during her FYP and decided to pursue Master of Science.

In her Master research, she had successfully synthesized and investigate the structural, optical and antibacterial properties of zinc oxide nanoparticles using chemical and green method. In the future, she plans to continue her research as a way for her to contribute to the community.

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LIST OF PUBLICATIONS

Published Paper

Ribut, S. H., Abdullah, C. A. C., Mustafa, M., Yusoff, M. Z. M., & Azman, S. N. A. (2018). Influence of pH variations on zinc oxide nanoparticles and their antibacterial activity. *Materials Research Express*, *6*(2), 025016.

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Ribut, S. H., Abdullah, C. A. C., & Yusoff, M. Z. M. Review on Green and Chemical Fabrications of Zinc Oxide Nanoparticles: Synthesis and Its Biological Activity. *International Journal of Technical Research and Applications*

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S. H. Ribut, C. A. C. Abdullah, Muskhazli Mustafa, M. Z. M. Yusoff (2018) *Structural, Optical and Antibacterial Activity of Biogenic Zinc Oxide Nanoparticles (ZnONPs)*. Paper presented at Asian Federation of Biotechnology Malaysia Chapter International Symposiums 2018 (AFOBMCIS 2018), Pullman Hotel, Kuching, Sarawak, Malaysia.

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