SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING
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DEVELOPMENT OF NEEM MODIFIED ZnO PHOTOCATALYST INCORPORATED LDPE FOR ANTIMICROBIAL APPLICATION

By

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(Materials Engineering)

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DECLARATION

I hereby declare that I have completed my research work and written the dissertation entitled "**Development of Neem Modified ZnO Photocatalyst Incorporated LDPE for Antimicrobial Application**". I also declare that it has not been previously submitted for the award of any degree or diploma or other similar title of this for any other examining body or university.

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LIST OF ABBREVIATIONS

CB	Conduction band
D_c	Crystalline size
e^-	Electron
EDX	Energy dispersive X-ray Spectroscopy
FTIR	Fourier Transmission Electron Microscope
h^+	Hole
HDPE	High Density Polyethylene
OH•	Hydroxyl radical
LDPE	Low density Polyethylene
LLDPE	Linear Low Density Polyethylene
MB	Methylene blue
PE	Polyethylene
PP	Polypropylene
SEM	Scanning electron microscope
UV	Ultraviolet
UV-vis	UV-Visible spectroscopy
VB	Valence band
XRD	X-ray diffraction
FESEM	Field Emission Scanning Electron Microscopy
nm	Nanometer

LIST OF SYMBOLS

^{o}C	Degree Celsius
θ	Angle
%	Percentage
g	Gram
h	Hour
min	Minutes
ml	Milliliter
nm	Nanometer
ppm	Parts per million
rpm	Revolution per minute
eV	Electron Voltage

PERKEMBANGAN UBAH-SUAIAN FOTOKATALIS ZNO DIGABUNGKAN DENGAN LDPE UNTUK APLIKASI ANTIMICROB

ABSTRAK

Kebelakangan tahun ini, isu jangkitan dalam hospital semakin mendapat perhatian orang ramai kerana ini merupakan salah satu sebab yang membawa maut kepada pesakit. Fotopemangkin ZnO telah dikenali dengan fungsi antimikrobnya. Tetapi, penyelidikan tentang aktiviti antimikrob bergabung dengan polimer belum diselidik secara mendalam. Oleh itu, projek ini bertujuan untuk menghasilkan komposit polimer dengan gabungan fotopemangkin ZnO dalam LDPE untuk aplikasi antimikrob. Sintesis ZnO yang stabil adalah melalui cara sonikasi dan ekstrak neem didapati melalui cara reflux. Carian projek ini mendapati bahawa PVA dapat menstabilkan ZnO dan minyak neem boleh mempertingkatkan pengagihan ZnO. Selepas itu, ZnO yang distabilkan digabungkan dengan LDPE melalui acuan suntikan dan LDPE/ZnO/PVA/10% neem mencapai kristalliniti yang paling rendah iaitu 42.61%. Ujian fotopenyahwarnaan dan ujian pembangkai telah mengenalpastikan pengeluarkan ROS dan ion Zn^{2+} dikenalpasti oleh ujian ICP. Pengeluaran partikle ini membantu dalam proses antimikrob. Akhirnya, mengikuti protokol ASTM E2149, komposit LDPE/ZnO/PVA/10% neem menunjukkan aktiviti antimikrob dengan pengurangan E.coli sebanyak 98.56% dalam 72 jam.

DEVELOPMENT OF NEEM MODIFIED ZNO PHOTOCATALYST INCORPORATED LDPE FOR ANTIMICROBIAL APPLICATION

ABSTRACT

In recent years, healthcare-associated infections are rising concerns as the susceptible attack of harmful microbes may cause death to patients. ZnO was known with its antimicrobial properties and LDPE is commonly used in biomedical field but the study on antimicrobial properties of ZnO incorporated LDPE was not comprehensively investigated. Thus, this research project aims to develop stabilized ZnO particles incorporated into LDPE for antimicrobial application. The ZnO is stabilized using PVA by sonication method and neem extraction is added in using methanol reflux method. It is found out that PVA can stabilized ZnO and neem extraction further enhance the distribution of the ZnO. The stabilized particles were incorporated into LDPE matric by injection moulding, and LDPE/ZnO/PVA/10% neem portraits the lowest crystallinity (42.61%). Photocatalytic and scavenger test confirmed release of ROS and Zn^{2+} ions released is confirmed by ICP test, which contributes to the antimicrobial properties. Lastly, under the protocol of ASTM E2149, the LDPE/ZnO/PVA/10% neem portraits antibacterial properties with 98.56% reduction of gram negative *E.coli* bacteria in 72 hours.

CHAPTER 1
INTRODUCTION

1.1 BACKGROUND

Hospitals are the places which having potential risk to acquire an infection during the healthcare delivery. Healthcare-associated infections (HCAI), as known as Nosocomial infections, happens due to the direct medical treatment in hospitals or result of contacting with a healthcare setting. (Suleman et al., 2014) All patients that admitted to the hospital wards are susceptible to such infections. However, due to the complicated environments of hospitals, the factors that caused this risk are different between specific site infections. (Yallew et al., 2017)

According to some conducted researches, there are different risk factors, including longer hospital stays (Al-Rawajfah et al., 2013), age of patients, gender of patients (Deptuła et al., 2015), intubation (Phu et al., 2016), and catheter including intravascular catheter (Deptuła et al., 2015) and urinary catheter (Phu et al., 2016). In this study, the focus will be on the polymeric facilities of the hospitals: the catheter and intubation. According to Gupta et al., the tubings, shunts and catheters that made of low-density polyethylene (LDPE) composites is biocompatible to be used as medical applications. (2018)

Polymeric nanocomposite materials with inorganic nanoparticles gained significant attention from researchers due to their antibacterial properties, allowing them to find applications in biomedical engineering field (Kumar et al., 2018). Polymer matrices embedded with metal particles is a simple way to use the advantages of nanoparticles. However, problem of aggregation of metal particles need to be taken care

in this case (Zare and Shabani, 2016). Instead of dispersing homogeneously, metal oxides tend to form small aggregates of more than four particles (Gonzalez-Benito and Olmos, 2010)

Zinc oxide is a metal oxide that portraits high chemical stability, high electrochemical coupling coefficient, broad range of radiation absorption and high photostability, therefore considered as a multifunction material. Zinc oxide is a semiconductor in group II-VI, whose covalence is on the boundary between ionic and covalent semiconductors. In the wise of medical field, low cytotoxicity with biocompatibility are the two most important properties that make it a material of interest (Zhao et al., 2011). Good antimicrobial activity of ZnO nanoparticles against Gram negative (*E. coli* and *P. aeruginosa*) and Gram-positive (*S. aureus* and *Bacillus subtilis* (*B. subtilis*)) bacteria was discovered. (Azam et al., 2012)

In this research, commercial ZnO powders is stabilized and modified with neem prior to incorporated into LDPE as polymer matirc. The dispersion of ZnO powders in LDPE is controlled due to the modification of ZnO particles using PVA as steric stabilizer. In a recent research, neem leaves (*Azadirachta Indica*) extraction also was reported to show good antimicrobial effect towards gram positive bacterias of *Listeria monocytogenes* and *Staphylococcus aureus*. (Yehia, 2016). Thus, different percentages of neem extract is added into the modified ZnO/PVA particles to observe the antimicrobial activities of LDPE composites. Injection moulding method is used to prepare the antimicrobial LDPE polymer composites and antimicrobial activity against gram-positive bacteria, *S. aureus* and gram-negative bacteria, *E. coli* was evaluated.

1.2 PROBLEM STATEMENT

1.2.1 Agglomeration of metal oxide particles

Intermolecular interactions among the ZnO particles in combination with their high surface area tend to make particles to agglomerate especially during synthesizing process. The powders can either weakly or strongly agglomerated due to the van der Waals forces of interactions between the particles (Mathioudaki et al., 2018). If high temperature is used for synthesizing process, partial sintering may reinforce the agglomerates by forming inter-particle necks (Roth, 2007). Agglomeration is also driven by the capillary forces generated by drying a dispersion; this effect is more pronounced for aqueous dispersions of oxides (Teleki et al., 2008). Furthermore, the contact points between the grains may act as nucleation sites for the condensation of dissolved material, which leads ultimately to the formation of necks between the grains resulting in so-called 'hard' agglomerates (Faure et al., 2013). Therefore stabilization of nanoparticles is essential for avoid of agglomeration issue. In this work, commercial ZnO is stabilized sterically with PVA and modified with neem extraction to see the potential of reducing the particles agglomeration problem.

1.2.2 Non-homogeneous dispersion of ZnO particles in polymer matric

Agglomeration of metal oxide particles will affect their distribution in LDPE as the bonds between the particles are strong and can hardly been broken down therefore cannot distribute homogeneously in LDPE matric. On the other hand, there is difference in surface polarity between LDPE and ZnO which affect the dispersion of ZnO in LDPE as ZnO particles are polar and LDPE matric is non-polar (Hang et al., 2018). Besides that,

the dispersion in the polymer matrix which will cause inconsistent release of antimicrobial particles. This will lead to inefficient antimicrobial activity of the polymer composites produced. Dispersion of hydrophilic particles in non-polar media is conventionally achieved by adding surfactants in the suspension as dispersants or by coating particle surface with organic polymer. Generally, it requires the supplemented surfactant to present a critical concentration allowing sufficient particle dispersion (Lee et al., 2015).

Besides than applying stabilizer to the ZnO powder, blending method can be applied to the mixture of LDPE and ZnO before injection moulding. Blending method can enhance the dispersion of ZnO in LDPE matric before poured into injection moulding machine and formed LDPE composites. During blending process, particles colliding with each other which ultimately decreases agglomerations and increases distribution of particles in polymer (Buruga and Kalathi, 2018). Processing method of incorporating photocatalyst ZnO particles into the LDPE matrix by injection moulding is suggested in this research and blending process is applied before injection moulding.

1.2.3 Release of Zn^{2+} ions and ROS from stabilized ZnO incorporated LDPE.

Antimicrobial properties of ZnO and ZnO nanoparticles are well reported in the researches made. It is believed that Zn^{2+} ions and Reactive Oxide Species (ROS) are the main mechanism contributed to antimicrobial properties of ZnO. However, the release of Zn^{2+} ions and ROS of stabilized ZnO upon incorporating into LDPE are not well addressed (Espitia et al., 2012). In recent years, metal oxide incorporated into polypropylene (PP) is widely explored as PP is commonly used for food packaging in which antibacterial properties enhancement can help in extending the shelflife of food but

LDPE that widely used in biomedical field are not well addressed yet. Therefore the Zn^{2+} ions and ROS release are investigated on LDPE/ZnO/PVA/neem composites. Neem leaves (*Azadirachta Indica*) extraction is added as it shows good antimicrobial effect towards gram positive bacterias of *Listeria monocytogenes* and *Staphylococcus aureus* (Yehia, 2016).

1.3 RESEARCH OBJECTIVES

The research objectives of this project are:

(i) To convert the commercial ZnO into stabilized ZnO via steric stabilization using PVA;

(ii) To investigate the release of Zn^{2+} ions and ROS from LDPE composites containing stabilized ZnO with neem extraction;

(iii) To determine the antimicrobial activity of LDPE composites towards *S. aureus* and *E.Coli*.

1.4 RESEARCH SCOPE

The research scopes firstly involved the preparation of ZnO/PVA/neem particles. This is done by mixing commercial ZnO powders with solution of PVA using sonication method and then subsequently reflux the ZnO/PVA with different percentage (2%-10%) of neem extraction. The particles synthesized is evaluated using X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), High Resolution Transmission Electron Microscopy (HRTEM), Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric Analysis (TGA), Dynamic Light Scattering and Zeta Potential to

determine the phase presence, surface morphology, internal structures, functional groups, thermal stability, particle size distribution and surface charge respectively. The ZnO/PVA/neem powders are incorporated into LDPE matric by blending method and then injection molding. The structural characteristic of LDPE composites were determined via Scanning Electron Microscopy (SEM), Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectroscopy (FTIR). The photocatalytic and scavenger test are used to understand the ROS release whereas Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is used to evaluate the ion release and finally the antimicrobial properties was determined via Colony count method (CCM).

1.5 THESIS OUTLINE

This thesis consists of five chapters. Chapter 1 highlights the introduction, problem statement, research objectives and research scope of this project. Chapter 2 describes the infections caused to human by medical devices in hospitals, the antimicrobial activity mechanisms of nanoparticles as antimicrobial agents through photocatalysis, stabilization mechanisms and selection of optimized stabilizer for effective dispersion of ZnO in LDPE. Chapter 3 explains the detailed raw materials used, the experimental procedures of fabrication of stabilized ZnO and LDPE/ZnO composite films and their respective characterization methods. Chapter 4 discuss about the analysis of results obtained for stabilized ZnO in terms of the phase presence, surface morphology, internal structures, functional groups, thermal stability, particle size distribution and surface charge. As for LDPE/ZnO, the discussion of analysis is about the surface morphology, thermal stability,

degree of crystallinity of LDPE, the interaction of stabilized ZnO with the LDPE, ROS group that released during antimicrobial process, concentration of metal ions released during process, and the antimicrobial activity of stabilized ZnO incorporated LDPE. Lastly, Chapter 5 concludes this research works and recommends suggestions for future improvements.

CHAPTER 2
LITERATURE REVIEW

2.1 HEALTHCARE-ASSOCIATED INFECTIONS (HCAI)

In hospitals, medical devices including ventilators, catheters, shunts and endoscopes can cause patients to have infections due to the contaminated surfaces of medical equipments with air-borne fungal spores. Among the infections, the most common HCAI included Ventilator-associated pneumonia (VAP), Central-line-associated septicaemia, Catheter-associated urinary tract infection, Clostridium difficile infection, and surgical site infection. Catheter-associated urinary tract infection is our main focus as catheter is a medical device that made from polymeric materials including LDPE.

HCAI happens when a number of micro-organisms infected human. Besides than common being bacteria, HCAI can also arise from infection by viruses, fungi, parasites or prions. Infection is caused when microorganisms reach the new host. This can be done by either directly contact with the infected person, or indirectly contact with contaminated surfaces. The microorganisms will transmission to a new host too via airborne contamination or consumption of contaminated food. The new host will now carry the microorganisms, which become a potential infecting new host towards other people. Thus, the cycle continues.

The most typical HCAI have been reported to be associated with Gram-negative bacteria. A report on the most commonly isolated bacteria from a range of HCAI

showed that Gram-negative bacteria (e.g., *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*) are among the more prevalent.

Table 2.1: Common Microbes found in HCAI.

Medical Device	Microbes	References
Urinary catheters	*Escherichia coli* *Klebsiella pneumonia* *Pseudomonas aeruginosa* *Staphylococcus aureus* *Staphylococcus epidermidis*	(Wang et al., 2010)
Intravascular catheter	*Candida albicans* *Klebsiella pneumonia* *Pseudomonas aeruginosa* *Staphylococcus aureus* *Staphylococcus epidermidis*	(Larsen et al., 2008)
Endotracheal tubes	*Klebsiella pneumonia, Pseudomonas aeruginosa* *Staphylococcus aureus* *Staphylococcus epidermidis*	(Singhai et al., 2012)

Table 2.1 shows the related microbes for the catheters and tubes used in hospital. In a clinical setting, *P. aeruginosa* is an opportunistic pathogen that affects mainly immuno-compromised patients and it is potential to cause mild illness towards healthy individuals. This microbe can be found in hospital water systems, and patient exposure can be due to contaminated tap water through bathing, showering, drinking or tap water-contaminated medical equipment.

Besides that, as part of the *Enterobacteriaceae* family, *Klebsiella pneumoniae* is a Gram-negative microbe. *K. pneumoniae* has primarily been implicated in urinary and respiratory tract infections. *K. pneumoniae* can form biofilms through usage of fimbriae which act as appendages. This microbe can be found on urinary catheter tubing too. Another member of the *Enterobacteriaceae* family is *E. coli,* a Gram-negative pathogen that is commonly associated with urinary tract infections and septicaemia. Another

common bacteria is *S. aureus*, a Gram-positive bacterium that can reside asymptomatically on the skin and in the nostrils of approximately one-third of human beings. This infection will indwelling medical devices such as catheters which cause poor vascularisation at implantation sites which impedes host defences against colonisation. In addition, *S. aureus* has strong ability to fight against multiple antibiotics, including penicillin and methicillin.

2.1.1 Gram Positive and Gram Negative Bacterias

Table 2.2: Comparison of Gram Positive and Gram Negative Bacteria.

	Gram Negative Bacteria	Gram Positive Bacteria
Gram Reaction	Can be decolourized to accept counter stain (Safranin or Fuchsine); stain red or pink, they don't retain the Gram stain when washed with absolute alcohol and acetone.	Retain crystal violet dye and stain dark violet or purple, they remain coloured blue or purple with gram stain when washed with absolute alcohol and water.
Peptidoglycan	This (single-layered)	Thick
Teichoic acids	Absent	Present in many
Periplasmic space	present	Absent
Outer membrane	Present	Absent
Lipopolysaccharide (LPS) content	High	Virtually none
Lipid and lipoprotein content	High (due to presence of outer membrane)	Low (acid-fast bacteria have lipids linked to peptidoglycan)

Flagellar structure	4 rings in basal body	2 rings in basal body
Toxins produced	Primarily Endotoxins	Primarily Exotoxins
Cell wall composition	The cell wall is 70-120 Å (ångström) thick; two layered. Lipid content is 20-30% (high), Murein content is 10-20% (low).	The cell wall is 100-120 Å thick; single layered. Lipid content of the cell wall is low , whereas Murein content is 70-80% (higher).
Antibiotic Resistance	More resistant to antibiotics.	More susceptible to antibiotics

Table 2.2 gives the summary of the difference between Gram Negative and Gram Positive bacteria. Danish scientist Hans Christian Gram devised a method to differentiate two types of bacteria based on the structural differences in their cell walls. In his test, bacteria that retain the crystal violet dye do so because of a thick layer of peptidoglycan and are called gram positive bacteria. In contrast, gram negative bacteria do not retain the violet dye and are colored red or pink. Compared with gram positive bacteria, gram negative bacteria are more resistant against antibodies because of their impenetrable cell wall. (Pommerville, 2004)

As shown in Figure 2.1, in the gram positive bacteria, the cell wall consist of many layers of peptidoglycan which forms a thick and rigid structure. The cell wall of the gram-positive also contains teichhoic acids which is made up of alcohol (glycerol or ribitol) and phosphate. Two types of teichoic acids are found in the gram positive bacteria: lipoteichoic acid which spans the peptidoglycan layer and is linked to the plasma membrane, and the other is teichoic wall acid, which is connected to the peptidoglycan layer. They have negative charge on them, and thus they can bind and regulate the movement of cations across the cell membrane. The cell wall is called murein. The peptidoglycan is made from repeating units of NAG (N-acetylglucosamine) and

NAM (N-acetylmuramic acid). The repeating units of NAM and NAG make thick interlinked parallel layers to form a cell wall. (Tortora et al., 2004). The common gram positive bacteria including *Staphylococcus aureus* and *Bacillus subtilis*.

As for gram negative bacteria, as shown in Figure 2.1, the cell wall is made up of few layers of peptidoglycan and an outer membrane. The outer membrane is made up of LPS (Lipopolysaccharides), lipoproteins, and phospholipids. The peptidoglycan remains bound to lipoproteins of the outer membrane. It is present in the periplasm which is a gel-like fluid between the outer membrane and the plasma membrane. The periplasm is filled with degrading enzymes and proteins aiding in the transportation of the molecules. The cell wall of gram-negative bacteria lacks the teichoic acid. The cell is more susceptible to mechanical breakage as compared to the gram-positive bacteria as the cell wall has thin layer of peptidoglycan. However, due to the presence of the outer membrane made up of lipoproteins and other components, the cell is not easily affected by antibodies, enzymes, metals, detergent, salts (bile salts), and dyes. The outer membrane is permeable due to the presence of porins. The membrane is permeable to food, nutrition, H_2O, Vitamin B12, and Iron. The LPS of the outer membrane is made up of a large complex molecules. They consist of lipids and carbohydrates. It acts as an antigen and is useful for the identification of the species (Pommerville, 2004). The common gram negative bacteria including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Campylobacter jejuni*.

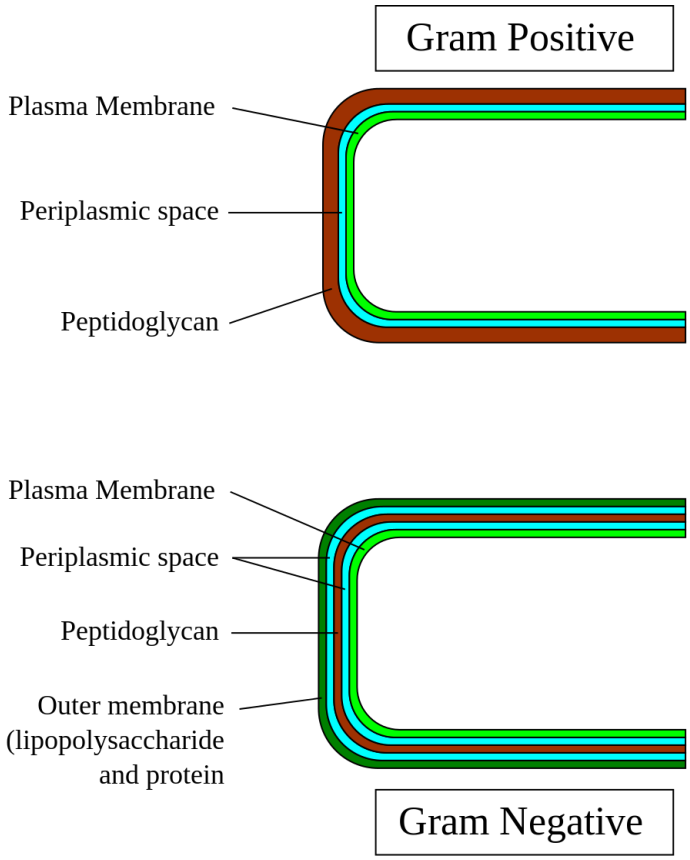

Figure 2.1: Difference of cell wall between Gram Positive and Gram Negative Bacteria. (Tortora et al., 2004)

2.2 STABILIZATION OF METAL OXIDE

Various studies has reported nano-sized or micron-sized ZnO will agglomerate and formed large aggregates. Agglomeration of the metal oxide will affect the performance of the polymer as the dispersion inside polymer is directly affecting the antimicrobial particles (Yung et al., 2017). Thus, the first problem to overcome in the preparation of antimicrobial polymer composite is the agglomeration of the incorporated particles used. Since the metal oxide particles are having strong and long range of attractive forces between each other, stability can only gained by giving long range repulsion to the particles (Shi, 2002). There are 3 methods of stabilization: electrostatic stabilization, electrosteric stabilization, and steric stabilization, as shown in Figure 2.1.

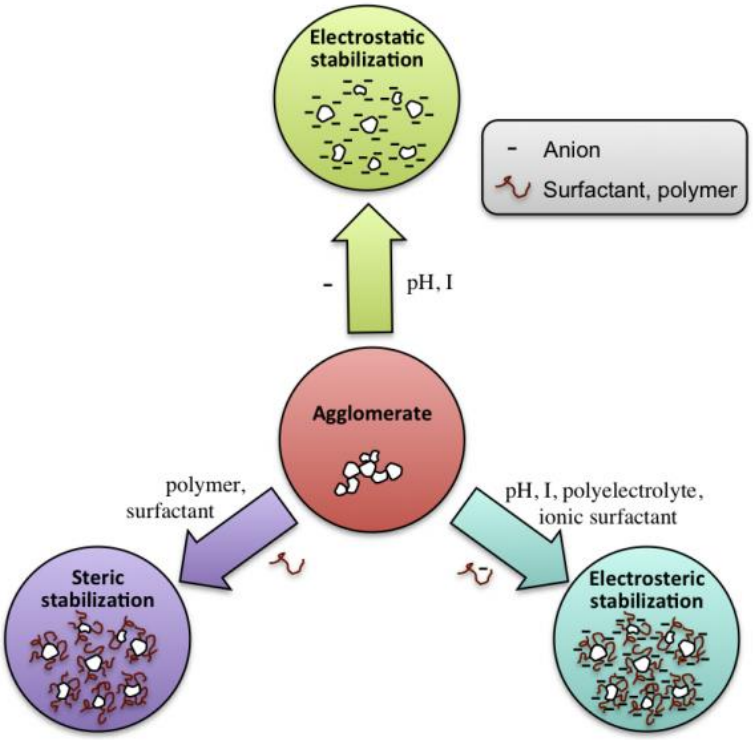

Figure 2.2: Schematic diagram of stabilization methods. (Faure et al., 2013)

2.2.1 Electrostatic stabilization

When the metal oxide particles interact with each other, there will be Van der Waals forces arise which contributes to the agglomeration. In order to counterbalance the forces of attraction in polar liquids, Coulombic repulsion is introduced. Charged layer is formed via adsorbtion of ionic groups to the surface of the particles. Thus, equal number of counterions with opposite charges is needed to neutral the charges. (Israelachvili, 2011) In electrostatic stabilization, this mutual repulsion that embedding the particles will provides the stability. According to research of Peng et al. (2015), the aggregation of ZnO nanoparticles was due to weak electrostatic repulsion between particles. The disadvantage of this electrostatic stabilization is the great sensitivity of particles towards the ionic strength of dispersion medium. Besides that, this stabilization can only works in polar liquid that can dissolve electrolytes which gives limitation to this stabilization. (Shi,

2002). Furthermore, pH is needed to be fixed for each metal oxide used in order to achieve this stabilization.

2.2.2 Electrosteric Stabilization

Electrosteric stabilization is the combination of electrostatic stabilization and steric stabilization. This combination is used when there is high concentrations of free polymer in the dispersion medium. If the polyelectrolyte adsorbs in a flat conformation, the polymeric repulsion is short range, and the stabilization mechanism is mainly electrostatic. With thicker adsorbed layers, having chains protruding into the solution, the polymeric contribution will become more important. In addition to the steric contribution, there is always an electrostatic contribution since the adsorption of a highly charged polyelectrolyte on a weakly charged, amphoteric oxide surface usually results in an increase of the net surface charge density (Liufu et al., 2005). Dispersants containing carboxylic groups (e.g., polyacrylic acid (PAA) and polyacrylamide (PAM)) interact with the surface through electrostatic interactions and H-bonding. Such dispersants have been used successfully to stabilize TiO_2 and ZnO below their Isoelectric Point (IEP), where the net surface charge is neutral (Dange et al., 2007). This stabilization is not commonly chosen as the procedure is complicated and a fixed pH too is needed for this stabilization to achieved. In many nanoparticle systems, it is not possible to create a stable dispersion simply by controlling the pH.

2.2.3 Steric Stabilization

The pH value portraited at net neutral surface charge of ZnO particles is pH9. It is difficult to create a stable dispersion simply by control the pH value, therefore

surfactants addition or polymeric dispersants is used to stabilized the particles as this method is easier. The addition of polymeric dispersant as stabilizer will embedded the particles by adsorbtion. As two particles getting closer to each other, it will not agglomerate as the adsorbed polymer act as a barrier. (Alexander, 1977)

Several conditions should be fulfilled for efficient steric stabilization: the adsorbed layer should be thick enough to screen the Van der Waals interaction of the particles, the adsorbed molecules should be strongly adsorbed and cover the entire nanoparticle surface, the segments protruding into the solvent should be in so-called good solvent conditions. The polymer layers induce an increase in the pair-interaction energy when the adsorbed segments overlap at short separation distances (Napper, 1977). For metal oxide nanoparticles, the high surface density of hydroxyl groups is commonly used as the specific surface groups that are targeted for the adsorption groups of the surfactants or the polymer dispersants.

PVA can act as a steric stabilizer for ZnO particles by adsorbtion. ZnO with OH functional group on its surface can react with OH functional group of PVA, so hydrogen bonding can be formed between them and reduce accumulation of the ZnO particles. Furthermore, the NPs surface was changed from hydrophilicity to hydrophobicity and the dispersion of the modified NPs was improved in the organic matrix. Organic PVA molecules created steric hindrance between inorganic ZnO particles to reduce their agglomeration. The stabilized ZnO powder is predicted to be as Figure 2.3.

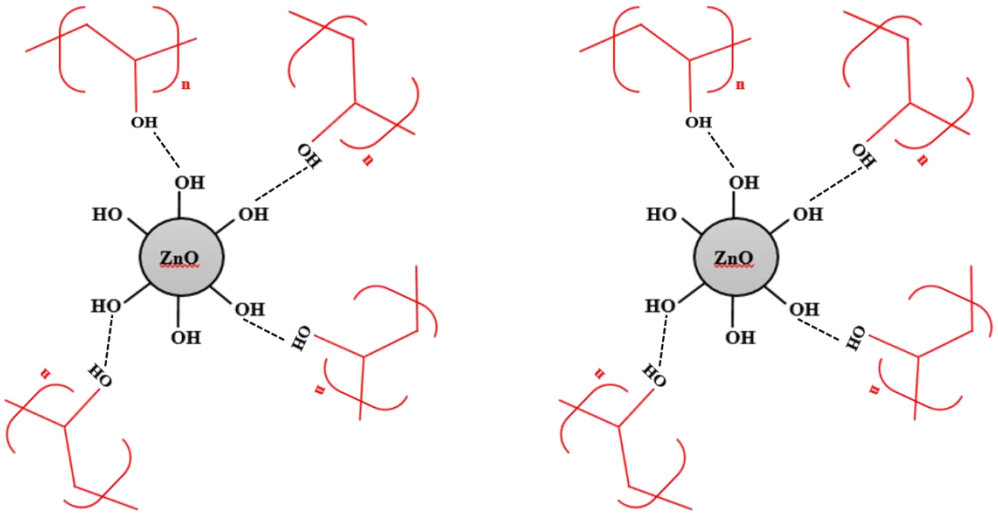

Figure 2.3: Schematic diagram of ZnO/PVA whereby PVA act as barrier to prevent ZnO particles to agglomerate.

2.3 ANTIMICROBIAL POLYMERS

To solve this problem, many antimicrobial polymers have been reported in recent years. The new developments for antimicrobial polymers in the past few years can basically categorized into 3 types: polymeric biocides, biocidal polymers, and biocide-releasing polymers.

2.3.1 Polymeric biocides

Polymeric biocides are polymers that covalently link bioactive repeating units with antimicrobial activity such as amino, carboxyl, or hydroxyl groups. However, the drawback polymerization of biocidal monomers does not lead to active antimicrobial polymers because the polymers are water-insoluble or the biocidal functions do not reach their target.

For instance, Dizman et al. (2005) reported the possibility of polymerizing antibiotics that retaining their activity at the polymer backbone by copolymerization of methacrylate modified Norfloxacin and PEG-methacrylates. Furthermore, according to the findings of Lawson et al. (2009), direct modification of Vancomycin with PEG-methacrylate and subsequent polymerization resulted in active polymers that are less active when comparing them to the unmodified antibiotic.

Despite of the negative effects reported, Turos et al. (2007) reported that penicillin attached to polyacrylate nanoparticles showed higher activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) than the free antibiotic in solution. Polymers with antimicrobial side groups, based on hydrophobic quaternary ammonium functions can be considered as polymeric biocides as well (Tatsuo, 2001). However, some of those polymers are more active than the respective monomers, indicating that the mode of action is not that of the biocidal group alone.

2.3.2 Biocidal Polymers

Microbial cells generally carry a negative net charge at the surface. Gram-positive bacteria has membrane proteins and teichoic acids which is negatively charged whereas outer membrane of Gram-negative bacteria is negatively charged too due to the phospholipids. This way, polycations are attracted and if they have a proportionate amphiphilic character, they are able to disrupt the outer as well as the cytoplasmic membrane and afford lysis of the cell resulting in cell death. Therefore the antimicrobial activity of the cationic polymers is related to the charge density of cationic groups. However, the drawback of this method is that the polycations cannot sufficiently separate the hydrophobic side (backbone or side groups) from cationic groups, which cause the

low activity of most biocidal polymers. Table 2.3 summarize some examples of the biocidal polymers with the targeted bacterias.

Table 2.3: Examples of biocidal polymer synthesized for antimicrobial activity.

Polymer	Target	Remark	Reference
Quaternary ammonium polyethyleneimine	Gram-positive and Gram-negative bacteria	Shows effective antimicrobial activity, but dependent on the hydrophobic and positively charged immobilized long polymeric chains	(Beyth et al., 2014)
Arginine–tryptophan-rich peptide	Gram-positive and Gram-negative bacteria	Retain antimicrobial functionality for 21 days	(Lim et al., 2015)
Ammonium ethyl methacrylate homopolymers	*S.aureus, E.coli*	Higher inhibitory effects against Gram-positive bacteria than Gram-negative bacteria	(Thoma et al., 2014)
Quaternary phosphonium modified epoxidized natural rubber	*S.aureus, E.coli*	Growth inhibition of microbes is moderate	(Li et al., 2013)

2.3.3 Biocide-releasing Polymers

Biocide-releasing polymers is produced by either bonding the biocide-releasing molecules to the backbone of host polymer or incorporate biocide-releasing molecules into polymer matric to form polymer composites. In this system, the polymer matric act as a carrier for biocides to be released. Thus, polymers of this type display antimicrobial properties by incorporating antibiotic or antiseptic compounds. The biocide-releasing polymers demonstrates great potential for medical applications because it portraits tendency in maintaining great biocides concentration and can facilitates the short in vivo

half-lives biocides to be delivered. In Table 2.4, the biocide-releasing polymer system is shown.

Table 2.4: Incorporation of organic particles into polymer matric.

Material		Surface modification	Incorporation Method	References
Polymer	Particles			
PP	Nano-TiO_2	Vinyltrimeth-oxysilane (VTMS, Aldrich)	Compounding and injection moulding	(Altan and Yildirim, 2014)
	Nano ZnO	-	Melt Mixing	(Bustos-Torres et al., 2017)
	Nano ZnO	NA	Roll Mill	(Omar et al., 2014)
	Nano ZnO	NA	Melt Mixing	(Hadi et al., 2016)
HDPE	Nano-TiO_2	Vinyltrimeth-oxysilane (VTMS, Aldrich)	Compounding and injection moulding	(Altan and Yildirim, 2014)
	Nano ZnO	3-methacyloxyprop yltrimethoxy silane, KH570	Melt mixing	(Mwafy et al., 2015)
LDPE	Nano TiO_2	HDTS	Melt Blending	(Alvarado et al., 2016)
	Nano ZnO	Vermiculite	Melt compounding technique	(Barabaszová et al., 2017)
PVC	Commercial ZnO	PVA	Melt Mixing	(Mallakpour and Javadpour, 2017)
Diglycidyl Ether of Bisphenol-A (Epoxy)	Nano ZnO	PVA	Chemical mixing	(Mohan and Renjanadevi, 2016a)
NA	Self synthesized ZnO, Commercial ZnO	PVP PVA PGA	Hydrothermal	(A.Stanković, 2013)

	Commercial ZnO	PEG and PVA	Mixing	(Nabiyouni et al., 2011)
	ZnO	PVA	Synthesis: sol-gel, then mix with PVA	(Etefagh et al., 2017)
	ZnO	NA	Biosynthesis using Leaf Extract	(Khalil et al., 2017)

According to Table 2.4, the types of polymers that is widely used are PP. This is because PP is commonly used for food packaging. Researches are done to lengthen the shelftime of food. As for biomedical field, LDPE is widely used due to their easy formability, clarity and low production costs. Even though, low-density polyethylene (LDPE) materials are not biodegradable (Protonotariou et al., 2010), in combination with bioactive materials, they create bioactive and biocompatible nanocomposites which are of great importance in wide range of applications in the health care and medical products, especially as disposable materials as syringe, catheters for percutaneous transluminal coronary angioplasty, pharmaceutical bottles, polyethylene bags, health foils or nonwovens (Popelka et al., 2012). Therefore, LDPE is the main focus of this research.

Besides that, Table 2.4 shows that the commonly used nanoparticles in recent years is ZnO nanoparticles. This is because ZnO portraits strong antimicrobial effect against a broad spectrum of microorganisms. The antimicrobial activity of ZnO nanoparticles may be related to several mechanisms including the induction of oxidative stress in microorganisms and release of Zn ions from the surface of nanoparticles that bind to the cell membrane, which may cause its degradation. However, the mechanism of toxicity is still only partially understood. Moreover, ZnO is recognized as safe material by Food and Drug Administration (FDA) and it is thermally stable which indicates that it

is suitable to be used for thermal processing therefore ZnO is used in this research project. (Emamifar and Mohammadizadeh, 2015)

According to the Table 2.4, there are various methods used to prepare the polymer. The most common method is the melt mixing method. This method is commonly applied in polymer industry based on usual compounding devices including extruders or mixers. Direct mixing is simple, economic and environmental friendly as solvents are not required. However, this method cannot produce polymer composites with good dispersion of metal oxide incorporated. Conditions during process need to be tested many times using trial-and-error based to get the optimized conditions for better dispersion (Nabiyouni et al., 2011). Therefore, injection moulding method is chosen and the polymer powders with ZnO is blended using rotary mixer before injection moulding process so that the dispersion of ZnO in LDPE can be enhanced.

Surfactants are used to improve the dispersion of metal oxides in polymer matric. The commonly used surfactants are VTMS, PVP, PVA, PGA and PEG. Addition of the surfactants will embedded the particles by adsorbtion hence act as a barrier between the metal oxides. By using PVA as example as shown in Figure 2.3, hydrogen bonding is formed between OH groups of ZnO and PVA which act as a barrier between the ZnO particles. According to the SEM and TEM imaging results from the findings of Mohan and Renjanadevi (2016b), ZnO nanoparticles can be deagglomerate after synthesizing with PVA as stabilizer. According to the characterization results, it is clear that the ZnO nanoparticles without any surfactant is highly affected by particle agglomeration and the particle separation is not good. Vice versa, when prepared the ZnO using PVA as surfactant, the particle agglomeration is very less and the particle separation is good. Furthermore, from the research of Stanković et al. (2013), the ZnO nanoparticles that

hydrothermally synthesized with PVA as steric stabilizer shows the highest microbial cell reduction of *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)* as compared to ZnO nanoparticles that synthesized using PVP and PGA as stabilizers. The analysis of the results has also shown that the ZnO powder synthesized using PVA portraits largest specific surface area and the smallest particle size, has the highest antibacterial activity. Hence, PVA is chosen as stabilizer to stabilize ZnO powders in this research project.

2.4 ANTIMICROBIAL ACTIVITY

Previous reports had published regarding the antimicrobial activity of the metal oxide and the mechanism proposed to be due to the release of antimicrobial Zn^{2+} ions, electrostatic reaction between particles and microbes, and release of Reactive Oxygen Species (ROS). Figure 2.4 illustrates the schematic diagram of different antimicrobial mechanism of ZnO nanoparticles.

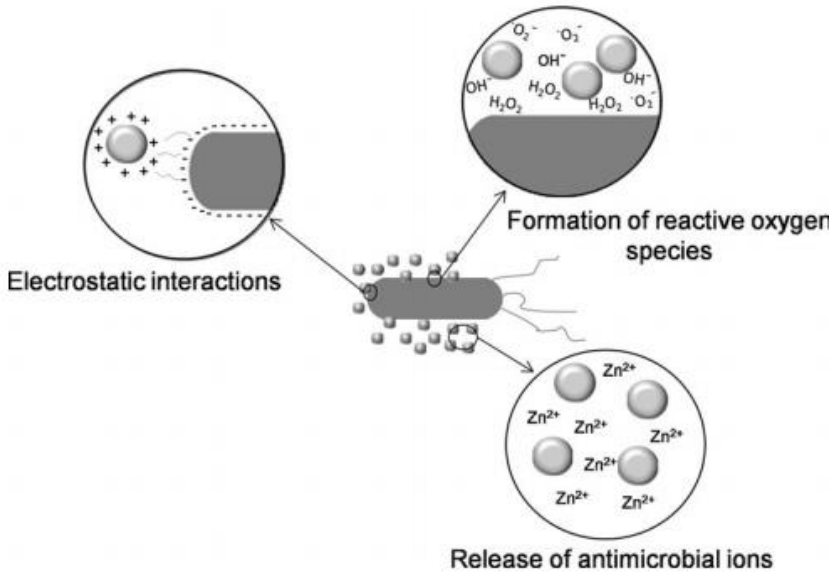

Figure 2.4: Different antimicrobial mechanisms of ZnO nanoparticles. (Espitia et al., 2012)

2.4.1 Electrostatic Interactions

The interaction between ZnO and *E.coli* cell is studied by (Zhang et al., 2008) and it is found out that the interaction between them is electrostatic forces. According to Stoimenov et al. (2002), the global charge of bacterial cells at biological pH values is negative, due to the excess of carboxylic groups, which are dissociated and provide a negative charge to the cell surface. The opposite charges between the microbes cell and Zn^{2+} ions that released from ZnO will generate electrostatic forces that strongly bind them together then produce cell damage to the microbes. There will be disruption of the cell wall towards the microbes, causing the internalization of nanoparticles in bacterial cells. The microbes cell presented considerable damage, with disorganized cell walls, altered morphology and intracellular content leakage.

2.4.2 Formation of Reactive Oxygen Species (ROS)

According to the researches of Gordon et al. (2011), Jalal et al. (2010), and Zhang et al. (2008), they proposed that ROS is exist and responsible for the antimicrobial mechanism of ZnO nanoparticles. After gaining energy from visible light and UV, ROS of hydroxyl radical (•OH), hydrogen peroxide (H_2O_2) and superoxide ($O_2•^-$) will be produced due to activation.

As mentioned, ZnO is a semiconductor material, therefore when the incident radiation with photon energy higher than the value of its band gap (~3.3 eV) is applied, it will cause the movement of electrons from the valence band (V_b) to the conduction band (C_b). The result of this process is the formation of a positive area, known as hole (h^+) in the valence band and a free electron (e^-) in the conduction band. Illustration of the movement is shown in Figure 2.5.