SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING

UNIVERSITI SAINS MALAYSIA

DEVELOPMENT OF TiO_2/ZnO PHOTOCATALYST INCORPORATED LLDPE FOR ANTIMICROBIAL APPLICATION

By

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DECLARATION

I hereby declare that I have completed my research work and written the dissertation entitled "**Development of TiO_2/ZnO photocatalyst incorporated LLDPE 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
PS	Polystyrene
R	Polymer molecule
SEM	Scanning electron microscope
TTIP	Titanium (IV) isopropoxide
UV	Ultraviolet
UV-vis	UV-Visible spectroscopy
VB	Valence band
XRD	X-ray diffraction
ZAD	Zinc acetate dihydrate
FESEM	Field Emission Scanning Electron Microscopy

IR	Infrared
nm	Nanometer
TiO_2	Titanium Oxide
ZnO	Zinc Oxide
μm	Micron meter

LIST OF SYMBOLS

^oC	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

PENGHASILAN KOMPOSIT NANO TiO_2/ZnO TERGABUNG LLDPE UNTUK KEGUNAAN ANTIMIKROB

ABSTRAK

Kebelakangan ini, pertumbuhan dan evolusi pathogen telah mendapat perhatian pengguna tentang serangan mikroorganisma terutamanya dalam bidang yang berkaitan dengan kesihatan termasuk industri perubatan dan makanan. Fotopemangkin TiO_2/ZnO telah dikenali dengan fungsi antimikrobnya. Tetapi, penyelidikan tentang aktiviti antimikrobnya bergabung dengan polimer belum diselidik secara mendalam. Oleh itu, projek ini bertujuan untuk menghasilkan komposit nano gabungan fotopemangkin TiO_2/ZnO dan polimer LLDPE untuk aplikasi antimikrob. Sintesis TiO_2/ZnO melalui cara sol-gel dengan TTIP dan ZAD dalam larutan etanol diikuti dengan pemanasan dalam suhu 500 ^oC selama 2 jam. 25TiO_2/75ZnO didapati mempunyai daya fotopenyahwarnaan sebanyak 95.59% terhadap pewarna metil biru selepas 3 jam sinaran matahari. Melalui spektrum PL, 25TiO_2/75ZnO mempunyai intensiti pancaran yang tertinggi dalam lingkungan pancaran biru (465-485 nm) dan pancaran kuning-oren (570-650 nm) menandakan kehadiran kecacatan struktur elektronik yang tertinggi untuk peningkatan aktiviti fotopemangkinannya. Komposit TiO_2/ZnO tergabung polimer LLDPE melalui cara pengacuan larutan dengan 1,2-Diklorobenzena pada 75 ^oC dan pengeringan dalam oven pada 80 ^oC mempunyai tahap kristalliniti (< 30%) yang sesuai untuk pelepasan ajen antimikrob dan kekuatan mekanikal yang lebih tinggi daripada filem tulen LLDPE. Akhirnya, mengikuti protokol ASTM E2149, pertambahan kuantiti 25TiO_2/75ZnO dalam komposit nano LLDPE menambah peratusan pengurangan *S. aureus*. Aktiviti antimikrob yang tertinggi telah ditunjukkan oleh sampel 10wt% 25TiO_2/75ZnO tergabung LLDPE dengan 100% pengurangan *S. aureus* dalam 24 jam.

DEVELOPMENT OF TiO_2/ZnO INCORPORATED LLDPE FOR ANTIMICROBIAL APPLICATION

ABSTRACT

In recent decades, the rapid growth of pathogens are rising human concern about the susceptible attack of harmful microorganisms in especially human health related sectors including hospitals and dental equipment, food packaging and storage. Coupled TiO_2/ZnO photocatalysts was well known with its antimicrobial properties. However, the study on the antimicrobial activity of TiO_2/ZnO incorporated polymers was not investigated comprehensively. Hence, this work aims to develop TiO_2/ZnO photocatalyst incorporated LLDPE for antimicrobial application. The TiO_2/ZnO photocatalyst was prepared through sol-gel with TTIP and ZAD as precursors in ethanol solution followed by calcination for 2 hours at 500 ^{o}C. It was found that 25TiO_2/75ZnO has the highest photodegradation efficiency of 95.59 % in degrading of 3ppm of methylene blue under 3 hours of sunlight irradiation. Based on PL results, 25TiO_2/75ZnO has the highest emission intensity in blue range (465-485 nm) and yellow-orange range (570-650 nm) indicating the most structural defects present which enhanced the photocatalytic activity. The TiO_2/ZnO photocatalyst incorporated LLDPE films fabricated by solution casting using 1,2-Dichlorobenzene at 75 ^{o}C and oven dried at 80^{o}C have degree of crystallinity (<30%) adequate for antimicrobial agent release and higher tensile strength and young modulus compared to the pure LLDPE film. Lastly, under the protocol of ASTM E2149, the higher the wt% loading of 25TiO_2/75ZnO photocatalyst in the LLDPE composite film, the more percentage of reduction in *S. aureus*. The highest antimicrobial activity was observed with the 10 wt% 25TiO_2/75ZnO loaded LLDPE with 100% reduction of *S. aureus* within 24 hours.

CHAPTER 1

INTRODUCTION

1.1 Background

As technology advances, antimicrobial plastics has become a vital raw materials used in various applications including mainly the food packaging and the medical industries. This is due to the fact that plastics are cheap, lightweight, chemical inert when in contact with food and easy in manufacturing (Arora and Padua, 2010). However, plain plastics no longer sustain the current needs. The rapid growth of harmful pathogenic microorganisms has posed challenges in maintaining the freshness of packaged food and preventing the bacterial infection (Jain *et al.*, 2014). Microbial infection in polymer based products are intense due to insufficient antimicrobial power for individual nanoparticles in the polymer matrix.

Ravishankar Rai and Jamuna Bai (2011) proved antibacterial effect of TiO_2 nanoparticles against four foodborne pathogens namely *Listeria monocytogenes*, *Escherichia coli (E. coli)*, and *S. aureus*. On the other hand, Azam *et al.* (2012) reported 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. Different metal or metal oxides nanoparticles have different degree of antimicrobial effects on different microbes. Therefore, it is important to investigate the ability to kill broad spectrum of microbes by developing coupled catalyst such as TiO_2/ZnO. Several studies have been directed to the use of TiO_2/ZnO but most of them are focused on the use of loose metal oxide particles instead of polymer embedded coupled oxides.

Furthermore, most of the studies are focused on the use of coupled oxides to treat pollution in water and air and its generation via water electrolysis. Only a few investigation can be found for antimicrobial application in coupled catalyst polymer composites.

Also, the high surface-to-volume ratio of nanoparticles with large interfacial areas, provide the nanocomposites different matrix-filler interactions on nano-scale compared to the micro-scale particles of the same type. However, it is challenging to control the nanoparticle dispersion within the polymer matrices. Instead of homogeneous dispersion, they usually form small aggregates of more than four particles (Gonzalez-Benito and Olmos, 2010). Therefore, an appropriate nanoparticle incorporation method could lead to homogeneous dispersion of antimicrobial agent nanoparticles to ensure well control of antimicrobial release need to be investigated. In this work, the use of coupled TiO_2/ZnO nanoparticles and the use of solution casting method to prepare antimicrobial LLDPE films to overcome the aforementioned drawbacks are proposed. The ratio of coupled oxides, concentration processing parameter and antimicrobial activity against the common gram-positive bacteria, *S. aureus* was investigated as a pre-screening test before further studies on gram-negative bacteria such as *E. coli* due to time constraint. The optimal ratio of coupled TiO_2/ZnO polymer nanocomposites for best antimicrobial activity will be recommended.

1.2 Problem Statement

1.2.1 Agglomeration of nanoparticles and its dispersion in polymer matrix

Agglomeration of nanoparticles during synthesis affect their distribution and dispersion in the polymer matrix which produce inconsistent release of antimicrobial particles. This causes the inefficient antimicrobial activity of the photocatalyst nanoparticles, TiO_2/ZnO. Thus, the growth of bacteria in the packaged food cannot be controlled persistently. Suitable processing method of incorporating TiO_2/ZnO nanoparticles into the LLDPE matrix by solution casting is suggested in this project.

1.2.2 Effect of crystallinity of TiO_2/ZnO nanoparticles on the antimicrobial activity

The release of antimicrobial agents depends on the degree of crystallinity of the nanoparticles embedded in the polymer matrix. Calcination temperature of the nanoparticles affects its degree of crystallinity that consequently affects its photocatalytic ability. At higher calcination temperature, higher crystallinity of nanoparticles give more defined ZnO and TiO_2 phases resulting in more efficient electron transfer between the particles, thus hindering the electron-hole pair recombination which improves the photocatalytic activity (Ullah *et al.*, 2014). On top of that, based on Gupta (2015), ZnO nanowires (ZnO NW) show better crystallinity, high specific area, increased area of contact, and lower transmittance than ZnO nanoparticles (NP). Thus, suitable size of nanoparticles at the adequate calcination temperature and profile should be practised to obtain nano-photocatalyst with optimum photocatalytic activity.

1.2.3 Effect of crystallinity of LLDPE matrix on the antimicrobial activity

For antimicrobial plastics to work, the moisture has to enter the LLDPE matrix before reaching the photocatalyst nanoparticles to initiate the photocatalysis of TiO_2/ZnO and effectively generate key species namely the trapped electrons, superoxide radical (O^{2-}), hydroxyl radical (OH•), hydrogen peroxide (H_2O_2), and singlet oxygen ($1O_2$) for antimicrobial reactions (Nosaka and Nosaka, 2013). Good water absorption capability is essential for effective antimicrobial activity of photocatalysts. Based on the investigation by Hodge *et al.* (1996) on the semi crystalline poly (vinyl alcohol) samples, water encountered the amorphous region of the material first. If only excess water was available after the penetration into amorphous region, the moisture would enter the crystalline region by the amorphous/crystalline interface. Therefore, the fabrication of LLDPE should instil certain amorphous structure in it for good water absorption without compensating the mechanical properties of the crystalline structure.

1.2.4 Inadequacy of single photocatalyst for growth inhibition of broad spectra of microbes

According to Addis and Sisay (2015), vulnerable bacteria that causes food borne illnesses are *E. coli*, *Samonella*, *Listeria monocytogenes*, *Clostridium perfringens*, *Campylobacter spp.* and *Norovirus*. TiO_2 is effective in killing *E. coli*, *S. aureus*, *Listeria monocytogenes* while ZnO can inhibit food-borne bacteria *E.coli 0157:H7*, *B. Subtilis*, *Pseudomonas fluorescens*, *L. monocytogenes*, *Samonella enteritidis*, *S. aureus* and *S. typhimurium* (Ravishankar Rai and Jamuna Bai, 2011). However, ZnO and TiO_2 alone is not capable of inhibiting the growth of a broad spectra of microbes. This is because single photocatalyst has rapid electron-hole pair recombination rate and TiO_2 has restricted photocatalytic activity under UV irradiation only due to its low visible light response (Hussein *et al.*, 2013). Coupling of TiO_2 and ZnO is expected to improve the photocatalytic activity of the resulting coupled metal oxides nanoparticles. Studies on the antimicrobial activity of optimum ratio and concentration of TiO_2/ZnO is essential to address broad spectrum of bacteria which were not investigated comprehensively.

1.3 Research Objectives

The research objectives of this project are:

(i) To synthesise and determine the appropriate ratio of coupled TiO_2/ZnO for excellent photocatalytic activity;

(ii) To investigate the processing parameter of solution casting to ensure homogeneous distribution of particle in the LLDPE matrix;

(iii) To determine the antimicrobial activity of the optimized TiO_2/ZnO for *S. aureus*.

1.4 Research Scope

The research scopes involved are:

(i) To synthesise the TiO_2/ZnO antimicrobial agent particles with sol-gel method.

The TiO_2/ZnO sol of composition 100TiO_2/0ZnO, 75TiO_2/25ZnO, 50TiO_2/50ZnO, 25TiO_2/75ZnO and 0TiO_2/100ZnO were produced by mixing the TiO_2 sol produced with the precursor Titanium Isopropoxide (TTIP), ethanol (95%) and deionized water and the ZnO sol produced with the precursor Zinc acetate dehydrate (ZAD), ethanol (95%) and deionized water. Then, the TiO_2/ZnO sol was centrifuged to obtain the precipitate of TiO_2/ZnO particles which was dried overnight at 80 ^oC, grinded with agate mortar and calcined at 500 ^oC for 2 hours.

To select the ratio of TiO_2/ZnO with the best photocatalytic activity, necessary characterizations including X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS), Fourier Transform Infrared Spectroscopy (FTIR), UV-Visible Spectroscopy (Diffusive Reflectance Spectroscopy and photocatalytic activity tests with sunlight radiation) and photoluminescence study were carried out to determine the surface morphology, stoichiometry, crystalline phases present, the chemical bonding, optical band gap and photocatalytic activity and also the structural defects present in each samples of TiO_2/ZnO.

(ii) To incorporate the coupled TiO_2/ZnO in LLDPE matrix by solution casting

After obtaining TiO_2/ZnO powder calcined with the composition of 100TiO_2/0ZnO, 75TiO_2/25ZnO, 50TiO_2/50ZnO, 25TiO_2/75ZnO and 0TiO_2/100ZnO, 5 wt% of each were sonicated in 1,2-dichlorobenzene solvent and added dropwise into the LLDPE melt, stirred until homogeneous and then poured onto a petri dish to be left dried at 80^oC. Next, the same procedures were repeated with the optimized ratio of TiO_2/ZnO with different weight percentages (1wt%, 3wt%, 7wt% and 10wt%).

To determine the LLDPE composite film with the best antimicrobial activity, Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectroscopy (FTIR), tensile testing and Colony count method (CCM) were carried out to determine the surface morphology, the availability of TiO_2 and ZnO crystalline phases after incorporation into LLDPE matrix, degree of crystallinity of LLDPE, the interaction of TiO_2/ZnO with the LLDPE polymeric chains, the mechanical tensile strength and the antimicrobial activity of LLDPE/TiO_2/ZnO.

Finally, LLDPE/TiO_2/ZnO with optimized parameter was selected.

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 emerging of antimicrobial plastics, the mechanism of antimicrobial activity of nanoparticles as antimicrobial agents through photocatalysis, selection of coupled TiO_2/ZnO as antimicrobial agents and LLDPE as polymer matrix of antimicrobial plastics, homogeneous dispersion of optimized ratio and concentration of TiO_2/ZnO in LLDPE for effective antimicrobial agent release. Chapter 3 explains in details the raw materials used, the experimental procedures of fabrication of TiO_2/ZnO and LLDPE/TiO_2/ZnO nanocomposite films and their respective characterization methods. Chapter 4 discuss about the analysis of results obtained for TiO_2/ZnO in terms of the surface morphology, stoichiometry, crystalline phases present, the chemical bonding, optical band gap, structural defects present and the photocatalytic activity; and for LLDPE/TiO_2/ZnO in terms of the surface morphology, the availability of TiO_2 and ZnO crystalline phases after incorporation into LLDPE matrix, degree of crystallinity of LLDPE, the interaction of TiO_2/ZnO with the LLDPE polymeric chains, the mechanical tensile strength and antimicrobial activity of LLDPE/TiO_2/ZnO to select the optimized LLDPE/TiO_2/ZnO samples. Lastly, Chapter 5 concludes the project works and recommends suggestions for future studies.

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CHAPTER 2

LITERATURE REVIEW

2.1 Popularity of plastics in daily life

In recent decades, polymers are widely used to replace conventional packaging materials including metals, glass or paper. This is due to their low price and weight, functionality especially their chemical stability and inertness in contact with food, ease in manufacturing, light sterilisation and aesthetic design that can be achieved easily with polymers (Arora and Padua, 2010).

For over 50 years, the plastics industry has continuously grown globally. According to Plastics Europe (PEMRG) / Consultic / ECEBD, from 2012 to 2017, there is a steady increase in demand for plastic products by about 3.7 percent per year with a total world plastics production of about 300 Mtonne in 2013.

In United States, the plastics industry is the third largest manufacturing industry at 10.9 billion dollars. Among all the industrial plastics, plastics for food packaging comprises almost a fifth of the net revenue of the plastic industry (Chin, 2010).

Based on Malaysia Plastics Manufacturers Association (MPMA), the plastics industry registered a total sales turnover of RM24.77 billion in 2015 from RM19.46 billion in 2014 with the actual growth of 5% to 6%.

2.1.1 Type of plastics

Plastics are used basically in various manufacturing sectors including food, beverages, chemicals, electronic packaging, automotive, household products, medical and health care. Examples of polymers used for food packaging are various grades of polyethylene (PE) such as high density polyethylene (HDPE) and low density polyethylene (LDPE), polypropylene (PP) polyethylene terephthalate (PET), polystyrene (PS) and polyvinyl chloride (PVC). Each of them has their pros and cons in terms of their characteristics. Their characteristics are basically summarised in Table 2.1.

Table 2.1: Characteristics and usage of food packaging plastics

Type of plastic	**Characteristics**	**Application or Usage**	**References**
PET	Clear, strong, good barrier to gases and moisture, resistant to heat, mineral oils, solvents and acids	Plastics bottles for carbonated drinks	Bratovčić *et al.*, 2015
PP	Strong, excellent chemical resistance and low density	Packaging film	Bratovčić *et al.*, 2015
LDPE	Very low cost, inert, large stretch ability, heat sealable, odour free and shrinks when heated, good moisture barrier but relatively permeable to oxygen.	Plastic bags and containers for general purposes, for coating papers or boards and as a component in laminates	Bratovčić *et al.*, 2015; Allahvaisi, 2012; Chin, 2010
HDPE	Cheap, stronger, thicker, less flexible and more brittle than LDPE and has a better barrier to gases and moisture.	Clouded containers or bottles for foods such as milk where strength is required but not clarity.	Allahvaisi, 2012; Chin, 2010
PS	Rigid, heat resistance	Styrofoam food containers and cups as well as meat and egg trays	Chin, 2010
PVC	Cheap and capable of stretching	Packaging films, containers and structural containers	Chin, 2010; Bratovčić *et al.*, 2015

2.1.2 The use of LLDPE in food packaging

About 70% of foil packaging in the European Union are originated from different polyethylenes (Fellows and Axtell, 2003). This indicates the significance of polyethylenes in food packaging sectors. There are different types of polyethylenes (PE), high density polyethylene (HDPE), low density polyethylene (LDPE) and linear low density polyethylene (LLDPE). Table 2.2 shows the usage of different PE in the industries.

Table 2.2: Type of polyethylenes (PE) and its usage

Type of polymer	**Characteristics**	**Packaging usage**	**References**
Linear low-density polyethylene (LLDPE)	Well-mechanical properties; linear polymer with significant numbers of short branches	transparent thin films used in food package and agricultural application	Wang *et al.*, 2005
Low-density polyethylene (LDPE)	Very low cost, excellent processing property, large stretch capacity and excellent barrier properties	food storage bags, plastic bags and containers for general purposes	Alexander, 2010
High density polyethylene (HDPE)	Better barrier against water vapour than polyethylene terephthalate (PET)	packaging milk in bags and bottles	Bratovčić *et al.*, 2015

HDPE has a low degree of branching and thus greater intermolecular forces and tensile strength (Vidya and Eby Thomas, 2012). LDPE has a high degree of short & long chain branching while LLDPE is a linear polymer with significant numbers of short branches. Through the copolymerisation of ethylene with short-chain alpha olefins LLDPE is produced. High-density polyethylene (HDPE) is stronger, thicker, less flexible and more brittle than LDPE and a better barrier to gases and moisture (Allahvaisi, 2012). Although with less barrier to gases and moisture, LLDPE is preferred for producing sheets

& films in packaging, shopping bags & agriculture due to its toughness, flexibility & relative transparency (Vidya and Eby Thomas, 2012). As an intermediate between LDPE and HDPE in terms of mechanical properties namely the strength, toughness and flexibility, LLDPE can be a good candidate for versatile application purpose (Gulmine *et al.*, 2003).

2.2 Emerging of antimicrobial plastics

As the polymer nanocomposites technology advances, the demand of antimicrobial plastics has emerged tremendously. Requirements of just plain plastics can no longer sustain the need of keeping food fresh and hygiene. The necessity of plastics products has evolved from just merely food wrapping package to active packaging that enables the tracking of freshness and quality of food or antimicrobial packaging with extended shelf life. Market demand for active packaging made up of plastics added with additives is getting attention. Therefore, active packaging is now widely introduced to combat food borne illnesses which are most likely to outbreak without proper care or maintenance.

Now, active packaging such as polymer film that incorporates antimicrobial metal or metal oxide nanoparticles is becoming popular among the food manufacturers and packaging industry. Mechanisms involved in the nanoparticles allow the production of secondary products such as reactive oxygen species (ROS) or dissolved heavy metal ions to interrupt transmembrane electron transfer or damage the cell membranes and DNA of microbes/microorganism. This functions to extend the log phase and reduce the growth rate of microorganisms in order to extend shelf life and to maintain product quality and safety (Emamifar, 2011).

2.2.1 Mechanism of antimicrobial activity

Basically, nanoparticles can behave as antimicrobial agent that reach the important target sites in the bacterial metabolism to damage the bacterial cells. This can be done by cell membrane damaging, the release of toxic ions, the interruption of electron transport, protein oxidation and membrane collapse or the generation of Reactive Oxygen Species (ROS) (Santos *et al.*, 2013). Figure 2.1 demonstrates the mechanisms of antimicrobial activity.

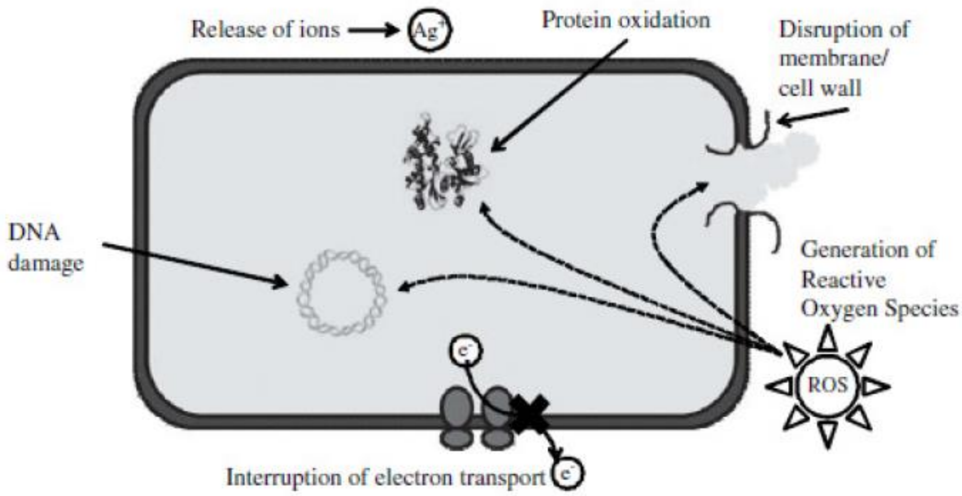

Figure 2.1: Illustration on the mechanism of antimicrobial activity (Emamifar, 2011)

2.2.1.1 Cell membrane damage

The mechanism of cell membrane damage by the action of nanoparticles is non-specific and it is unsure if polymixins has any involvement on this process. However, polymixins antibiotics is capable to break the vital barriers of microorganisms by attacking its cell membrane (Aruguete *et al.*, 2013). When the cell of microorganism is in contact with the nanoparticles, the cell permeability is altered. The possible hypothesis from experiments is the nanoparticles induce the formation of a "hole" or "pore" in the living cell membranes causing cell damage. For more severe cases, a hole will exist in the bilayer membrane and promotes the complete loss of the plasma membrane (Leroueil *et al.*, 2007).

2.2.1.2 Release of toxic ions

Besides, metal nanoparticles can form toxic ions and react with the different groups of proteins in microorganisms, for instance, Cd^{2+}, Zn^{2+} and Ag^{+} ions. For the case of silver (Ag), it can form sparingly soluble Ag^{+} salts to attack the bacterial cells. When Ag^{+} ions were formed, the precipitation of chloride ions inhibit the cell respiration of cytoplasm of the cells. Besides, Ag nanoparticles, the well-known antimicrobial agent against Gram-negative E. coli, Cd^{2+} and Zn^{2+} ions can also bind to sulphur-containing proteins of the cell membrane and interfere in cell permeability (Niskanen *et al.*, 2010).

2.2.1.3 The interruption of electron transport, protein oxidation and membrane collapse

Basically, the death in targeted cells can be initiated with the introduction of positive charge nanoparticles since the bacterial cell membrane is negatively charged. The Ag^{+} ions can affect the membrane-bound respiratory enzymes and the efflux bombs of ions leading to cell death (Allaker, 2010). For CeO_2 or nC60, when it is in contact with bacteria, oxidation of respiratory enzymes can help to facilitate the production of Reactive

Oxygen Species (ROS) which eventually disturb the cell physiology and promote DNA degradation (Allaker, 2010; Xia *et al.*, 2008).

2.2.1.4 The generation of Reactive Oxygen Species (ROS)

When ROS is generated, it can harm bacterial components such as proteins and nucleic acids (Xia *et al.*, 2008). The generation of Reactive Oxygen Species namely the small molecules like H_2O_2, free radicals like $OH^{\bullet}$, highly reactive triplet oxygen ($3O_2$) or even singlet oxygen ($1O_2$) and superoxide ions such as O^{2-} at the surface of the metal oxide nanoparticles induces the bacterial cell damages or even spontaneous death. The damages or death of bacterial cells is due to oxidative stress, oxidative lesions and membrane lipid peroxidation. The bacterial components such as proteins and nucleic acids are easily harmed by the ROS. For example, the cells' respiratory burst can consume O_2 and form hydrogen peroxide (H_2O_2) and then generate the free hydroxyl radicals leading to oxidation of DNA, proteins and membrane lipids (Santos *et al.*, 2013). Also, the Ag_2O nanoparticles was shown to be able to damage the DNA of *E. coli* by inducing the oxidative stress which then led to the interruption of the bacterial cell cycle and induction of the cell death. The formed ROS induced bactericidal effect in both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria.

2.3 The Use of metal oxides (TiO_2 and ZnO) photocatalyst as antimicrobial agents

Previously, antimicrobial plastics made use of metal nanoparticles, Silver (Ag), Gold (Au), Copper (Cu) and Zinc (Zn) nanoparticles due to their effective inhibition of microbes' growth (Parham *et al.*, 2016). Reported as non-toxic to human cells (Sirelkhatim *et al.*, 2015), the photocatalysts, the inorganic oxides nanoparticles such as

Titania (TiO_2) and Zinc oxide (ZnO) are then discovered to be useful in both antimicrobial and photodegradation application due to its well-known photocatalysis activity. The summary of TiO_2, ZnO and other oxides for antimicrobial application is discussed in detail in Section 2.4.

2.3.1 Mechanism of Photocatalysis

Photocatalysts are the metal oxides that functions as catalyst when radiated or exposed to sunlight or UV-radiation. Depending on the photocatalyst itself, when it is radiated with incident photons of energy greater than its energy band gap, 3.2 eV (TiO_2) and 3.37 eV (ZnO) (Hussein *et al.*, 2013), electrons will be excited from the valence band (VB) to the conduction band (CB) forming an electron-pair. The negative-electron reacts with oxygen molecule to form super oxide anion (1.1) while the positive-hole of titanium dioxide breaks apart the water molecule to form hydrogen gas and hydroxyl radical (1.4) (Nosaka and Nosaka, 2013). Based on Padmavathy and Vijayaraghavan (2008), the photo reaction and a series of antibacterial reactions produce hydrogen peroxide (H_2O_2) molecules which penetrates the membrane, causing fatal damage. On the other hand, according to Sawai (2003) the photocatalytic prompted H_2O_2 attributes the disruption of the cell membrane to peroxidation of the unsaturated phospholipids. The equations below show how the generated electrons and holes help in the generation of ROS, namely the oxygen radicals, $O^{\bullet}{}_2{}^{-}$, the hydrogen peroxides, H_2O_2 and the hydroxide radicals, $OH^{\bullet}$.

Reactions of excited electrons (e^-),

$$O_2 + e^- \rightarrow O^{\bullet}{}_2{}^{-} \tag{1.1}$$

$$O^{\bullet}{}_2{}^{-} + H^+ \rightarrow HO^{\bullet}{}_2 \tag{1.2a}$$

$$HO^{\bullet}{}_2 + H^+ + e^- \rightarrow H_2O_2 \tag{1.2b}$$

$$H_2O_2 + H^+ + e^- \rightarrow OH^{\bullet} + H_2O \tag{1.3}$$

Reaction of holes (h^+),

$h^+ + H_2O \rightarrow H^+ + \bullet OH$ (1.4)

$2\ h^+ + 2\ H_2O \rightarrow 2\ H^+ + H_2O_2$ (1.5)

$H_2O_2 \rightarrow 2\ \bullet OH$ (1.6)

2.4 Tackling broad spectra of microbes in food packaging

According to Addis and Sisay (2015), vulnerable bacteria that cause food borne illnesses are *E. coli*, *Samonella*, *Listeria monocytogenes*, *Clostridium perfringens, Campylobacter spp.* and *Norovirus*. Table 2.3 demonstrates that TiO_2 showed antibacterial effect to *E. coli*, *S.aureus, Listeria monocytogenes* while ZnO showed antibacterial effect to *E. coli* 0157:H7, *B.subtilis, Pseudomonas fluorescens, L. monocytogenes, Salmonella enteritidis, S. aureus, S. typhimurium* (Bratovčić *et al.*, 2015).

Table 2.3: Antibacterial activity of Metal and metal oxides nanoparticles and its nanocomposites

Material		Surface modification	Microbes	Killing efficiency	References
Nano-particles	**Polymer**				
TiO_2/ZnO	**-**		*E. coli*	90 TiO_2/10 ZnO bactericidal process complete in less than 20 min while 50 TiO_2/50ZnO after 45 min	Stoyanova *et al.*, 2013
TiO_2	**-**		*E. coli, S.aureus, Listeria monocytogenes*	effective in killing the stated microbes	Ravishankar Rai and Jamuna Bai, 2011
TiO_2	**PP**	Vinyltrimeth-oxysilane (VTMS, Aldrich)	E. coli	no bacterial growth (1% & 3% silane in total composition of dry granules composite)	Altan and Yildirim, 2014

Table 2.3 (con't)**:** Antibacterial activity of Metal and metal oxides nanoparticles and its nanocomposites

Material		**Surface modification**	**Microbes**	**Killing efficiency**	**References**
Nano-particles	**Polymer**				
TiO_2	**HDPE**	Vinyltrimeth-oxysilane (VTMS, Aldrich)	E. coli	no bacterial growth (1% silane in total composition of dry granules composite)	Altan and Yildirim, 2014
ZnO	**-**		Food-borne bacteria *E. coli 0157:H7*, *B.subtilis*, *Pseudomonas fluorescens*, *L. monocytogenes*, *Salmonella enteritidis*, *S. aureus*, *S. typhimurium*	Food borne bacteria inhibition	Ravishankar Rai and Jamuna Bai, 2011
ZnO	**PP**	Vinyltrimeth-oxysilane (VTMS, Aldrich)	*E. coli*	no bacterial growth (3% silane in total composition of dry granules composite)	Altan and Yildirim, 2014
ZnO	**HDPE**	Vinyltrimeth-oxysilane (VTMS, Aldrich)	*E. coli*	log reduction of 2.71, 2.85 and 2.38 cfu/ml for 1,3, and 5% silane in total composition of dry granules composite	Altan and Yildirim, 2014
ZnO	**UHMW PE**	3-aminoproply-triethoxysilane, (3-APTES, Sigma Aldrich (M) Sdn. Bhd.)	*E. coli and S. aureus*	20 wt% filler exhibited better antibacterial activity compared to 5, 10 and 15 wt%	Chang *et al.*, 2014
ZnO	**HDPE**	γ-aminopropyltriethoxysilane, Shuguang chemicals company of Nanjing, China	*E. coli and S. aureus*	2wt% ZnO has 97.7% and 99.9% antibacterial rate for E. coli and S.aureus respectively	Li and Li 2010

Table 2.3 (con't): Antibacterial activity of Metal and metal oxides nanoparticles and its nanocomposites

Material		Surface modification	Microbes	Killing efficiency	References
Nano-particles	**Polymer**				
ZnO	**LLDPE**	γ-aminopropyltriethoxy-silane, Shuguang chemicals company of Nanjing, China	*E. coli and S. aureus*	0.8 wt% ZnO has the antibacterial rate of 91.7% and 95.6% against E. coli and S. aureus respectively	Li *et al.*, 2010
CuO	**-**		*B. subtilis*	Strong inhibition effect	Ravishankar Rai and Jamuna Bai, 2011
MgO	**-**		*E. coli*, *B. subtilis* and *B. megaterium*	Excellent inhibition effect	Ravishankar Rai and Jamuna Bai, 2011
CaO	**-**		*E. coli*, *S. typhimurium*, *S. aureus* and *B. subtilis*	Antibacterial activity	Dizaj *et al.*, 2014
Al_2O_3	**-**		*E. coli*	Growth inhibitory effect	Ravishankar Rai and Jamuna Bai, 2011
Ag	**-**		*E. coli*, *B. subtilis*, *S. aureus*, *methicillin-resistant coagulase-negative staphylococci*, *vancomycin-resistant Enterococcus faecium*, *ESBL-positive K. pneumonia*, *S. typhi*, *Vibri cholera*	Inhibitory activity	Ravishankar Rai and Jamuna Bai, 2011

Table 2.3 (con't): Antibacterial activity of Metal and metal oxides nanoparticles and its nanocomposites

Material		**Surface modification**	**Microbes**	**Killing efficiency**	**References**
Nano-particles	**Polymer**				
Au	-		MRSA, VRE, *E. coli*, *Pseudomonas aeruginosa*	Antibacterial activity	Ravishankar Rai and Jamuna Bai, 2011
Cu	-		*Methicillin resistant S. aureus*, *B. subtilis*, *P. aeruginosa*, *Salmonella choleraesuis*, and *C. albicans*.	Antibacterial and antifungal activities	Ravishankar Rai and Jamuna Bai, 2011

Different photocatalysts were shown to be able to tackle different microbes. As demonstrated by Table 2.3, the antimicrobial activity of single metal or metal oxide nanoparticles is not sufficient for inhibition of a broad range of microbes. Stoyanova *et al.* (2013) have studied on the effect of 2 different ratios 90 TiO_2/10ZnO and 50 TiO_2/50ZnO on the antibacterial activity towards *E. coli* under UV illumination. Similarly other researchers have worked on the effect of different ratio of coupled TiO_2/ZnO on the applications involving photocatalytic activity namely the hydrogen production (Hussein *et al.*, 2013) and photodegradation of methyl orange (Tian *et al.*, 2009). However, studies on the antimicrobial activity of polymer incorporated coupled TiO_2/ZnO was not yet well established. Therefore, the incorporation of hybrid oxide TiO_2/ZnO in LLDPE matrix is investigated in this work to study its antimicrobial activity towards common gram positive bacteria, *S. aureus*. Section 2.5 will elaborate in details the incorporation of TiO_2/ZnO in LLDPE matrix.

2.4.1 Inadequacy of single photocatalyst for growth inhibition of broad spectra of microbes

Although both TiO_2 and ZnO can tackle a few food-borne microbes, TiO_2 and ZnO alone are not capable of inhibiting the growth of a broad spectra of microbes. Often, the inadequacy of antimicrobial activity of single photocatalyst is due to the rapid recombination rate of electron hole pairs that lowers the photocatalytic efficiency. Also, unlike ZnO which has high photocatalytic efficiency, TiO_2 has restricted photocatalytic activity under UV irradiation. TiO_2 has low visible light response due to its indirect band gap which does not allow it to absorb light well (Hussein *et al.*, 2013).

Coupling of TiO_2 and ZnO is expected to improve the photocatalytic activity of the resulting coupled metal oxides nanoparticles.

2.4.2 The Use of Coupled TiO_2/ZnO as antimicrobial agents

The electron/hole separation process at coupled TiO_2/ZnO heterojunction interface proposed by Hussein *et al.* (2013) are illustrated in Figure 2.2. When light incident on the surface of coupled TiO_2/ZnO, a charge separation between excited electron and its valence band hole is induced (Process 1), where electrons transfer from the ZnO (with more negative onset flat band potential) (Garcia-Belmontea and Bisquert, 2010) conduction band to the TiO_2 conduction band (Process 2). Conversely, holes transfer from the TiO_2 valence band to the ZnO valence band (Process 3). With the presence of TiO_2-ZnO heterojunction as the potential barrier, the probability of electron/hole recombination can be limited. Thus, the availability of the electrons or holes to migrate to the TiO_2 or ZnO surface of the TiO_2/ZnO composite photocatalysts is increased (Hussein *et al.*, 2013). Besides, with the presence of ZnO, the visible light