SYNTHESIS AND CHARACTERIZATION OF ZINC OXIDE NANOPARTICLES USING ASCORBIC ACID FOR EVALUATION OF ANTIOXIDANT ACTIVITY

SYED MUHAMMAD AL HAFIZ BIN SYED ZAKREE

UNIVERSITI SAINS MALAYSIA

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by

SYED MUHAMMAD AL HAFIZ BIN SYED ZAKREE

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LIST OF ABBREVIATION

AA	Ascorbic Acid
ABTS	2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDX	Energy Dispersed X-ray Spectroscopy
FTIR	Fourier-Transform InfraRed Spectroscopy
IC_{50}	Inhibition Concentration
OH	Hydroxyl Ion
SEM	Scanning Electron Microscope Spectroscopy
UV-Vis	Ultra-Violet Visible Spectroscopy
Zn^{2+}	Zinc Ion
Zn	Zinc
ZnO NPs	Zinc Oxide Nanoparticles

SINTESIS DAN KARAKTERISASI NANOPARTIKEL ZINK OKSIDA MENGGUNAKAN ASID ASKORBIK UNTUK PENILAIAN AKTIVITI ANTIOKSIDA

ABSTRAK

Sintesis nanopartikel logam oksida menggunakan asid askorbik telah menjadi salah satu tumpuan penyelidik sejak beberapa dekad yang lalu kerana penggunaan tenaga yang lebih rendah dan alternatif yang lebih murah. Dalam penyelidikan ini, nanopartikel zink oksida (ZnO NPs) disintesis dengan kaedah pemendakan kimia. Prekursor zink asetat dihidrat dan asid askorbik bertindak sebagai agen pengurangan digunakan. Kesan pH larutan dan nisbah kepekatan asid askorbik terhadap zink asetat dihidrat (AA:Zn) adalah parameter yang dipilih untuk dikaji. Lebih dari julat pH yang dikaji pada AA: Zn tetap 1.0, pH 11 didapati yang terbaik berdasarkan puncak penyerapan tertinggi pada 355 nm dan serapan tertinggi 0.92 yang diukur menggunakan spektrometer Ultraviolet (UV-Vis). Didapati bahawa kehadiran ZnO ditunjukkan dari spektrum spektroskopi inframerah transformasi fourier (FTIR) pada julat wavenumber 470 - 520 cm - 1 untuk semua sampel. Analisis SEM menunjukkan bahawa inti dan ukuran zarah menurun ketika pH larutan meningkat. Nisbah kepekatan asid askorbik kepada zink asetat dihidrat (AA:Zn) diubah dari 1.0 hingga 4.0 sambil mengekalkan pH optimum 11. Ukuran zarah, kemurnian dan morfologi dikaji menggunakan spektrometer ultralembayung/nampak (UV-Vis), spektroskopi sinar-x penyebaran tenaga (EDX), dan Mikroskop Elektron Pengimbasan (SEM). Analisis SEM menunjukkan bahawa ukuran dan agregat zarah menurun ketika kepekatan meningkat. Analisis EDX mengesahkan bahawa ZnO NP yang disintesis mempunyai ketulenan yang lebih tinggi kerana komposisi hasil spektrum Zn dan O. UV-Vis spektra yang tinggi menunjukkan jalur

penyerapan pada 355 nm, 365 nm, 359 nm, 355 nm dan 350 nm untuk nisbah kepekatan masing-masing AA: Zn 1.0, 1.5, 3.0, 3.5 dan 4.0. Aktiviti antioksidan ZnO NP yang disintesis ditentukan dengan menggunakan 2,2-diphenyl-1-picrylhydrazyl (DPPH) dan 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). Nilai IC_{50} ZnO NP yang disintesis masing-masing adalah 29.38 dan 27.65 µg / ml untuk DPPH dan ABTS, yang lebih rendah daripada asid askorbik standard. Penyelidikan semasa menunjukkan bahawa ZnO NP yang disintesis boleh menjadi pengganti ubat kimia daripada patogen bawaan makanan tahan antibiotik.

SYNTHESIS AND CHARACTERIZATION OF ZINC OXIDE NANOPARTICLES USING ASCORBIC ACID FOR EVALUATION OF ANTIOXIDANT ACTIVITY

ABSTRACT

Synthesis of metal oxide nanoparticles using ascorbic acid has become one of the focus of researchers over the past decades due to its lower energy consumption and cheaper alternative. In this research, zinc oxide nanoparticles (ZnO NPs) were synthesized by a chemical precipitation method. A precursor zinc acetate dihydrate and ascorbic acid act as reducing agent were used. The effects of solution pH and the concentration ratios of ascorbic acid to zinc acetate dihydrate (AA:Zn) were the chosen parameters to be studies. Over the range of pH studied at a constant AA:Zn of 1.0, pH 11 was found the best based on the highest absorption peak at 355 nm and the highest absorbance of 0.92 that was measured using the Ultraviolet Visible spectrometer (UV-Vis). It was found that the presence of ZnO was displayed from the Fourier-Transform Infrared Spectroscopy (FTIR) spectra at the wavenumber range of 470-520 cm^{-1} for all samples. SEM analysis showed that the nucleation and size of particles were decreased as the solution pH increased. The concentration ratios of ascorbic acid to zinc acetate dihydrate (AA:Zn) were changed from 1.0 to 4.0 while keeping the optimum pH of 11. Particle sizes, purity and morphology were study using Ultraviolet Visible spectrometer (UV-Vis), Energy Dispersed X-ray (EDX), and Scanning Electron Microscope (SEM). SEM analysis showed that the size and aggregation of particles decreased as the concentration increased. EDX analysis verified that the synthesized ZnO NPs has a higher purity because of the high composition of Zn and O. UV-vis spectra results showed the absorption band at 355 nm, 365 nm, 359 nm, 355 nm and 350 nm for

concentration ratio of AA:Zn 1.0, 1.5, 3.0, 3.5 and 4.0, respectively. Antioxidant activity of the synthesized ZnO NPs was determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay. The IC_{50} values of the synthesized ZnO NPs were 29.38 and 27.65 µg/ml for both DPPH and ABTS, respectively, which were lower than the standard ascorbic acid. The current research suggests that the synthesized ZnO NPs can be a substitute to the chemical drugs against antibiotic resistant foodborne pathogens.

CHAPTER 1

INTRODUCTION

1.1Nanoparticle

Nanotechnology is a collection of disciplines, techniques, and devices that are used to manipulate, restructure, and design matter at the nanoscale level (one billionth of a meter). Nanomaterial refers to a material that contains particles, aggregates, or filaments with dimensions smaller than 100 nm. Nanomaterials can occur naturally, be produced purposefully through engineering, or be created as the by-products of combustion reactions to perform a specific function. As a result, nanotechnology produces a wide range of new structures and systems known as nanoparticles, nano dispersions, nanolaminates, nanotubes, nanowires, buckyballs, quantum dots, and other terms (Scrinis & Lyons, 2007). The nanoscale modification and fabrication of materials results in small-sized particles with a very large surface area to volume ratio. This has resulted in enhanced optical, electrical, mechanical, and functional properties of matter, which is responsible for the current and future success of this relatively new technology (Neethirajan & Jayas, 2011). The basic factors which influence the unique properties of nanoparticles are the size of nanoparticles, their distribution, the number of interfaces or grain boundaries, the chemical composition of the constituent phases, and their interactions. These naturally occurring or engineered particles of the twenty-first century have been labelled "magic bullets" because they can be targeted to deliver in a specific manner and thus have a high potential in a variety of applications, including drug, textile, and food manufacturing (Naseer et al., 2018). Nanomaterial is used in a wide range of industries, from healthcare and cosmetics to environmental preservation and air purification due to its ability to generate materials in a specific method to play

a specific role. For an example in healthcare field, nanomaterials are used as a drug delivery, where the nanoparticles are being developed in order to assist the transportation of chemotherapy drug directly to cancerous cell growths and to deliver the drugs to the damaged arteries to fight cardiovascular disease. Other than that, carbon nanotubes are used in aerospace industry, to morphing of aircraft wings. The nanotubes are used in a composite to bend in response to the application of an electric voltage. Other processes, such as environmental preservation, used zinc oxide nanowires which are application of nanomaterials to be used in flexible solar cells and role in the treatment of polluted water.

Nanoparticle is a small particle that have ranges in size from 1 to 100 nanometres. Nanoparticles cannot be seen by human naked eyes, have a significant different physical and chemical properties than their larger material. According to the definition by European Commission, it states that the particle size of at least half of the particles in the number size distribution must have a particle size of 100 nm or less. The nanoparticles are composed of a few hundred atoms. When the size of particles approaches the atomic scale, the material properties also changed. The reason behind this is that the surface area to volume ratio increasing, causing the material's surface atoms dominating the material performance. Due to its small size, nanoparticles have a larger surface area to volume ratio when compared to bulk material such as plate, sheet, and powders. Also, because they are small enough to confine their electrons and produce quantum effects, this allows nanoparticles to possess astonishing physical, chemical and optical properties. For example, copper with bulk copper bending at the 50 nm scale is considered as a soft material. Whereas copper nanoparticles that is smaller than 50 nm are considered as a very hard material and has a drastically different in ductility and malleability performance. The change in size of a particle can affect the

melting characteristics. In example, bulk gold melt at very high temperature which is 1064 ^{o}C than gold nanoparticles which is at 300 ^{o}C. Furthermore, nanoparticle material has a higher absorption of solar radiation compared in thin films of continuous sheets of material.

There are different types of metal oxide such as indium (III) oxide (In_2O_3), tin (IV) oxide (SnO_2), silicon dioxide (SiO_2), zinc oxide (ZnO) and titanium dioxide (TiO_2). Zinc oxide is one of the most produced metal oxides after silicon dioxide and titanium oxide. Zinc oxide is an organic substance with unique features such as semiconductor, broad radiation absorption, piezoelectricity, pyroelectricity, and high catalytic activity. Because of zinc oxide is known as its non-toxic properties, it has been considered to be one of the safest metal oxides for use in food processing by The Food and Drug Administration (FDA) (Bettini et al., 2016). Zinc oxide nanoparticles (ZnO NPs) have gained popularity in recent years. This is mostly owing to their microscopic size which increases the chemical reactivity. As a result, zinc oxide nanoparticles are now widely used in electronics, optics, biomedicine, and agriculture. ZnO NPS are one of the most important metal oxides materials in materials science because of its unique physical, chemical, and biological qualities such as biocompatibility, environmental friendliness, inexpensive, and non-toxic nature (Alwan et al., 2015) . ZnO NPs have been used as a functional advanced material to solve a variety of social challenges, including wastewater treatment catalysis, cosmetics, and antibacterial compounds (Ruszkiewicz et al., 2017). ZnO NPs have various advantages, including chemical and thermal stability, toughness, and long shelf life.

In general, many physical and chemical methods can be used to synthesize zinc oxide nanoparticles. Chemical synthesis method performance in controlling the properties of nanoparticles such as structural and morphological properties is more

convenient and low expressive cost than physical process. Anisotropic, face-specific growth kinetics are commonly used to describe the modification of ZnO NPs growth (Garcia and Semancik, 2007). Ascorbic acid (AA) or vitamin C is an organic material derived from the decomposition of organic matter that can be extracted from plant roots, aquatic macrophytes, and phytoplankton. Ascorbic acid is widely used as a reducing agent in the synthesis of nanomaterials such as metal oxide and metal nanoparticles (Cho et al., 2010; Tan et al., 2014). Ascorbic acid can form complexes in solution with various metal ions such as $Fe^{3+,}$ Cu^{2+}, Co^{3+}, and Zn^{2+}.

1.2 Problem Statement

From literature, we know that the major challenges faced by zinc oxide nanoparticles are the shape of nanoparticle that has a different functionality and the method used to synthesise the nanoparticles can affect the size of the nanoparticles. These factors make zinc oxide nanoparticle cannot be fully utilized on general purposes. Thus, the key for having the high-quality zinc oxide nanoparticle is by finding the optimum conditions such as pH, method used for synthesis of zinc oxide nanoparticle, reaction time, and etc. In this paper, for synthesis of zinc oxide nanoparticles, chemical precipitation method has been proposed. Chemical precipitation method synthesis can take place over a wide range of temperatures, from ambient temperature to extremely high temperatures. The parameters such as pH value and concentration of ascorbic acid are to be manipulated to find the optimum condition for synthesized zinc oxide nanoparticle. Whereas the characterization of synthesized zinc oxide nanoparticles will be examined by using several equipment which are Ultraviolet-Visible (UV-Vis) Absorption Spectrum, Fourier Transform Infrared Spectroscopy (FT-IR), Energy Dispersion X-ray (EDX) and Scanning Electron Microscope (SEM). Besides, this paper also studies about the antioxidant activity by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS).

1.3 Research Objective

i. To investigate the most influential parameters to control the size and shape of the synthesized zinc oxide nanoparticles.

ii. To determine characterization method to evaluate the synthesized zinc oxide nanoparticles.

iii. To find the methods to be used to evaluate antioxidant activity of synthesized zinc oxide nanoparticles.

CHAPTER 2

LITERATURE REVIEW

2.1 Synthesize Method of Zinc Oxide Nanoparticles

Zinc oxide nanoparticles have been synthesised using a variety of chemical and physical methods, such as sol–gel, hydrothermal, precipitation and co-precipitation, chemical vapour deposition, spray pyrolysis, magnetic sputtering, microwave-assisted technique, solvothermal, and biological routes (Naveed Ul Haq et al., 2017). Each of the methods listed has advantages and disadvantages, and as a result, different morphologies of zinc oxide nanoparticles have been reported based on characterization tools ranging from nanoplates, nano stars, nanobelts, and nanotubes (Ambika & Sundrarajan, 2015).

2.1.1 Sol-Gel

Sol-gel is a very advantageous method for synthesizing materials due to its reproducibility, effortlessness, consistency, and economical nature. The sol-gel method in synthesizing nanoparticles offer good optical properties. In this method, an inorganic compound is synthesized through a chemical reaction that is being produced in the solution at low temperature. This method offers a number of advantages such as has an excellent homogeneity and purity of synthesized product and found to be economical and convenient. Sol-gel method has a higher popularity and industrial application than other existing methods and due to its special properties and characteristics, this method is capable to produce high quality nanoparticle of the same size. Sol-gel is a simple and convenient method for producing zinc oxide nanoparticles with controllable shape and

size. The sol-gel method is a common approach for producing metal oxides in a variety of forms such as xerogels, aerogels, fibres, thin films, nanoparticles, microparticles, and so on. At low processing temperatures, this technique of synthesis yields homogeneous and porous oxides. The materials that are synthesised take several shapes. The resultant materials from the sol-gel technique of synthesis are used in a variety of applications, including protective coatings, heterogeneous catalysts, optoelectronic materials, and many more (Arya et al., 2021).

This approach allows for precise control over the texture and surface qualities of the materials. In general, the sol–gel process may be broken down into five distinct steps: hydrolysis, polycondensation, ageing, drying, and heat breakdown. Hydrolysis and condensation processes in an organometallic precursor such as alkoxides, chloride, beta-diketonate, or nitrate under aqueous circumstances generate a solid substance. In the beginning, the precursor solution is hydrolysed and condensation reactions occur, resulting in the production of gel; this is followed by ageing, solvent extraction, and drying treatment of the synthesised product to provide the needed material. The sol–gel process is a well-established synthetic strategy for producing high-quality metal oxide nanoparticles and mixed oxide composites (Parashar et al., 2020).

Hydrolysis of the precursors such as metal alkoxides takes place in water or alcohols. The production of metal oxide requires oxygen, which is provided by water or organic solvents (e.g., alcohols) during the synthesis of metal oxide nanoparticles. The aqueous sol–gel technique uses water as the reaction medium, whereas the nonaqueous sol–gel method uses an organic solvent as the reaction media for the sol–gel process. An acid or a base, in addition to water and alcohol, assists in the hydrolysis of the precursors. Next, Condensation of neighbouring molecules occurs in this stage, when water/alcohol are removed and metal oxide bonds are produced, and polymeric

networks develop to colloidal dimensions in the liquid state. Condensation takes place through two processes: olation and oxolation. Olation is the formation of a hydroxyl (–OH–) bridge between two metal centres (metal–hydroxy-metal bonds), whereas oxolation is the formation of an oxo (–O–) bridge between two metal centres (metal–oxometal links). Condensation or polycondensation eventually results in an increase in the viscosity of the solvent, resulting in the formation of a porous structure with a liquid phase known as gel. The ageing process causes changes in the structure and characteristics of the gel. Polycondensation continues inside the concentrated solution during the ageing process, along with reprecipitation of the gel network, resulting in decreased porosity and increased thickness between colloidal particles. The drying process is difficult because water and organic components separate to produce gel, causing its structure to be disrupted. There are three types of drying processes: atmospheric/thermal drying, supercritical drying, and freeze-drying, each of which has a different effect on the structure of the gel network. Finally, heat treatment/calcination is used to remove residues and water molecules from the desired sample; the calcination temperature is a critical parameter in determining pore size and density (Parashar et al., 2020).

2.1.2 Hydrothermal

Hydrothermal synthesis method is one of many techniques to crystallize substances. Nanoparticles is one of the most critical factors in it. In the future, technology progress brings us closer to becoming more innovative. Nanotechnology is one of the driving forces with the potential to revolutionize material in this current era. In terms of synthesis of nanoparticles, the hydrothermal method is being increasingly used globally, by industry and research and development labs alike. Hydrothermal synthesis method usually does at a using a high-temperature aqueous solution and high

vapor pressure level. Hence, it is named as Hydro + Thermal = Hydrothermal method. Hydrothermal synthesis method in an easily described are as an artificial way to synthesize nanoparticles(single-crystal). It also depends on the solubility of the solution under higher temperature levels and hot water. A strong vessel within the hydrothermal reactor 'Autoclave' and fill with a solution. The process wants a constant maintenance of temperature difference between the opposing ends of the crystallizing compartment. The end with higher temperature is wherever the solvent was dissolved. At the same time in the other side, which is comparatively cooler, where the nanoparticle growth takes place (Gan et al., 2020).

In order to synthesise nanoparticle in hydrothermal method, it needs a special instrumentation which called as hydrothermal autoclave reactor. The hydrothermal autoclave is a specialised strong vessel that intended to withstand high temperatures and better pressure levels from inside. In hydrothermal synthesis, the formation of nanomaterial can happen in a wide temperature range from room temperature to very high temperatures. Depending on the vapour pressure of the primary component in the reaction, either low-pressure or high-pressure conditions can be applied to regulate the morphology of the materials to be synthesised. Using this method, several different types of nanomaterials have been successfully produced. The hydrothermal synthesis process has major benefits over other methods. Hydrothermal synthesis can produce nanomaterials that are unstable at high temperatures. The hydrothermal process can generate nanomaterials with high vapour pressures with minimal material loss. Whether using a liquid phase or multiphase chemical reactions, the compositions of nanomaterials to be synthesised may be carefully controlled in hydrothermal synthesis (Gan et al., 2020).

2.1.3 Green Synthesis

An alternative method to chemical and physical methods, which is biological method that provide an environmentally friendly way of synthesizing nanoparticles. Furthermore, biological does not require any harmful, expensive, and toxic chemicals. Metallic nanoparticle with different shape, sizes, compositions, and physiochemical qualities can be manufactured using biological technique that has been popular in recent years. Synthesis of nanoparticles can be completed in one step by using biological organisms such as actinobacteria, yeast, moulds, algae, bacteria and plants, or their products. Molecules in microorganisms and plants, such as enzymes, protein, phenolic compounds, amines, alkaloids, and pigments perform nanoparticle synthesis by reduction (Shah et al., 2015).

In conventional chemical and physical methods, reducing agent involved in the reduction of metal ions, and stabilizing agents are required to prevent undesired agglomeration of the produced nanoparticles carry a risk of toxicity to the cell and environment. Also, the contents of the synthesize nanoparticles by conventional chemical and physical method are thought to be toxic in terms of shape, size, and surface chemistry. In green synthesis method in which nanoparticle with biocompatibility are produced, these agents are naturally present in the employed biological organisms (Hussain et al., 2016).

Bacteria are obvious candidates in the synthesis of nanoparticles because to their fast development, low culture costs, and ease of control and manipulation of the growing environment. Simultaneously, it is known that some bacteria species have particular mechanisms for suppressing the toxicity of metals or heavy metals. Bacteria are favoured for these features because they can synthesise nanoparticles both in-situ and ex-situ. Metal ions can be reduced and precipitated for nanoparticle production by

using metabolic pathways and reducing agents found in bacteria such as proteins, enzymes, and so on (Gao et al., 2014).

Actinobacteria are aerobic, stationary, and primarily filamentous gram-positive bacteria that produce secondary metabolites such as antibiotics. Because of their detoxification ability, they are resistant to the most poisonous heavy metals. Toxic metal ions that are soluble are detoxified by either intracellular or extracellular reduction or precipitation. As a result, nanoparticles with antibacterial, antifungal, anticancer, antioxidant, anti-bio contamination, and catalytic activities can be synthesized. Nanoparticles can be synthesised extracellularly or intracellularly with enzymes using easily cultured and fast-breeding eukaryotic yeasts and moulds with simple biomass design. The size of the nanoparticles produced is influenced by the incubation conditions and the metallic ion solutions employed. Some moulds are harmful to humans, which restricts their usage in nanoparticle synthesis (Manivasagan et al., 2016).

Algae are eukaryotic aquatic photo sites that use pigments, proteins, carbohydrates, lipids, nucleic acid, and secondary metabolites to break down metallic ions into nanoparticles. The algae extract, which exists in an aqueous medium at a certain temperature, is supplemented with metal solutions of the appropriate pH and concentration, and thus the synthesis of nanoparticles with antimicrobial properties is achieved without the production of any toxic by-products during the synthesis. Algae are additionally advantageous to this synthesis technique due to their ease of availability and utility. Furthermore, effective biomolecules in the reaction media are less extinguished by the nanoparticles generated when bacteria and plant extracts are used (Karaduman et al., 2017).

Plants, which have a high capacity for metal detoxification, reduction, and accumulation, are promising, rapid, and environmentally friendly in the removal of trace metals. Metallic nanoparticles with a variety of morphological properties can be created both intracellularly and extracellularly. The synthesis process begins with the addition of extracts from plant components such as leaves, roots, and fruits to an aqueous solution containing metal ions. The plant extract's components, such as sugar, flavonoid, protein, enzyme, polymer, and organic acid, operate as a reducing agent in the bio induction of metal ions into nanoparticles (Nadaroglu et al., 2017).

2.1.4 Co-precipitation

Metal nanoparticles could be synthesised through co-precipitation, in which a metal nanoparticle solution is combined with a base in an inert environment at normal temperature or increased temperature. The type of bases utilised has a considerable impact on the metal nanoparticles formed. Co-precipitation is a simpler and more convenient method of synthesis than other methods. Following the production of a metal nanoparticle, a surfactant is applied dropwise to shield the newly formed metal nanoparticle from oxidation. They act as a stabilising agent, preventing agglomeration (Adam et al., 2018).

The coprecipitation reaction involve the simultaneous occurrence of growth, nucleation, coarsening, and/or agglomeration process. Coprecipitation reactions exhibit various characteristic which are the product produced are generally insoluble species formed under high supersaturation conditions, nucleation is a key step where a large number of small particles will be formed, secondary processes such as Ostwald aggregation and ripening can dramatically affect the size, morphology, and properties of the products, and the supersaturation conditions necessary to induce precipitation are usually result of a chemical reaction (Rane et al., 2018).

Typical coprecipitation synthetic method are metal can be formed from aqueous solution, by reduction from nonaqueous solutions, electrochemical reduction, and decomposition of metalorganic precursors. Oxides are formed from aqueous and nonaqueous solutions. Metal chelonids formed by reaction of molecular precursors. Microwave/sonication-assisted coprecipitation. Some of advantages of co-precipitation method are simple and rapid preparation, easy control of the particle size and composition, low temperature used compared to other method, energy efficient, does not involve used of organic solvent (Adam et al., 2018).

2.1.5 Acid Reduction

Chemical reduction is one of the most commonly used in research lab for synthesis of nanoparticles. This method has been used to synthesis many types of nanoparticles with different complexities and different surface changes. The chemical reduction usually consists of three essential stages which are reduction of metallic salts by reducing agents, stabilization of the ionic complexes, and controlling of the size by the capping agent. There is various type of metallic nanoparticles have been synthesized using this method. The reaction involves heating the metallic salt solution in the presence of a reducing agent. The stabilizing agent or known as capping agent is used to control the size of the nanoparticles. Both the reducing agent and capping agent are added to the reaction at different time. In some circumstances, a single chemical compound can act as a reducing agent as well as a capping agent (Yousefi & Cheraghizade, 2018).

The examples of reducing agent are sodium borate, ascorbic acid, sodium triethyl borohydride, or some organic compounds such as toluene or hydrazine. Whereas, capping agents' example are sodium citrate, surfactants, etc. For instance, citrate ions bind on the surface of the organic nanoparticles, leading to prevention of

further aggregating as well as further nucleation. The rule of thumb is that when citrate ions are added earlier in the reduction reaction, the smaller nanoparticles can be synthesized. The most common metals prepared by using this chemical reduction are cooper, silver, aluminium, zinc, iron, gold, and platinum nanoparticles. As there are no more citrate ions on the surface to prevent aggregation, the washing of the nanoparticle prepared by using citrate ions tends to cause them to aggregate (Krishna et al., 2021).

2.1.6 Precipitation

The control of physical and chemical features such size, size dispersity, shape, surface state, crystal structure, organisation onto a support, and dispensability is proven to be essential for the many uses of ZnO nanoparticles (Wahab et al., 2007). As a result, several different methods for synthesising the chemical have been developed. A controlled precipitation approach was employed by (Hong et al., 2006) Utilizing zinc acetate ($Zn(CH_3COO)2.H_2O$) and ammonium carbonate $(NH_4)_2CO_3$, zinc oxide was precipitated. (Lanje et al., 2013) carried conducted an easy precipitation technique for the production of zinc oxide. The cost-effective preparation of ZnO nanoparticles requires a one-step procedure with large-scale manufacturing without undesirable impurities. Another method of regulated zinc oxide precipitation was described by (Y. Wang et al., 2010). By precipitating NH_4HCO_3 and $ZnSO_4.7H_2O$ from aqueous solutions, nanometric zinc oxide was produced. ZnO powder was created by (Benhebal et al., 2013) using the sol-gel process using zinc acetate dihydrate, oxalic acid, and ethanol as the solvent.

2.2 Selection of Synthesis Method

The most suitable synthesis method to produce zinc oxide nanoparticles is chemical precipitation method. This is because chemical precipitation method is the simplest and can be accessed in our school of chemical engineering lab compared to

other method. Other than that, the preparation for synthesizing zinc oxide nanoparticles is easy. Also, the particle size and composition of zinc oxide nanoparticle is easy to control by adjusting or optimize the important parameter in the process. Moreover, chemical precipitation method using low temperature compared to other method.

2.3 Precursor

As a precursor, zinc salts such as zinc acetate dihydrate ($ZN(C_2H_3O_2)22H_2O$), zinc nitrate hexahydrate ($Zn(NO_3)26H_2O$), zinc sulphate ($Zn(SO_4)27H_2O$), and zinc chloride ($ZnCl_2$) have been used to prepare zinc oxide nanoparticles based on (Ambika & Sundrarajan, 2015). The use of different zinc salt precursors influenced the textural, morphological, and optical properties of zinc oxide nanoparticles. In this paper, zinc acetate dihydrate was used.

2.4 Factor influencing the synthesis of Zinc Oxide Nanoparticles

2.4.1 pH of Reaction Mixture

The varieties of ZnO nanoparticles produced are determined by the pH of the reaction mixture (Aziz & Jassim et al). The number of positively and negatively charged ions present in the medium during the preparation greatly influences the crystallite size, shape, phases, and surface areas of ZnO nanoparticles (Chithra et al., 2015). This is due to the fact that solution pH changes the electrical charge of molecules, which affects their reduction (Hasan et al., 2018). The number of hydroxyl ions (OH) in the solution during the synthesis of ZnO in an acidic medium (pH < 7) is generally low, which hampers hydrolysis and condensation processes, resulting in smaller aggregates at the conclusion of the poly-condensation process (Tourné-Péteilh et al., 2019). The faster breakdown of the ZnO crystal structure was due to the decrease in crystallite size of the zinc nanoparticle in an acidic solution (Rafaie et al., 2014a). At a pH of 7 (neutral), the

amounts of hydrogen ion (H+) and hydroxyl ion (OH) are equal, resulting in the solution having little or no impact at the surfaces of zinc oxide crystals (Mohammadi & Ghasemi, 2018). When the pH of the reaction mixture exceeds 7, the quantity of OH ions is generally large, generating strong attraction between the positively charged Zn^{2+} and OH ion; this results in increased crystallisation and the production of a smaller ZnO nanoparticle.

2.4.2 Concentration of Ascorbic Acid

Ascorbic acid (AA) is a naturally occurring compound that is frequently utilised as a reducing agent in the synthesis of nanoparticles such as metal oxide nanoparticles (Yusof et al., 2019). In addition, AA may form in solution with various complex metal ions (Co^{3+}, Fe^{3+}, and Zn^{2+}, etc) and functions as an inhibitor of Zn^{2+} hydrolysis, resulting in a decrease in the concentration of ZnO crystal development units (Khoshhesab et al., 2011). Ascorbic acid is an organic acid that is water soluble (Fukui et al., 2017). Many research on the catalytic oxidation of ascorbic acid have been published (Yusof et al., 2019). In the study by Al-Salem & Seoudi, 2020, AA was investigated as a stabiliser for altering the appropriate characteristics of ZnO NPs. The chemical precipitation approach was used to create ZnO nanoparticles at various concentration of ascorbic acid (AA). The AA complex with Zn^{2+} and cooperative effects are critical. Furthermore, growth kinetics is important in the synthesis of high-quality ZnO NPs. The effect of AA concentration on optical characteristics and crystallisation has been investigated. Because of its sensitivity with Zn^{2+}, ascorbic acid has been utilised as a reducing agent, and it works as chelating ligands, lowering the availability of Zn^{2+} to form a nanoparticle. Furthermore, a low quantity of ascorbic acid promotes the production of ZnO NPs, but a high concentration lowers the crystal size (Al-Salem & Seoudi, 2020).

2.5 Characterization Method for Synthesised Zinc Oxide Nanoparticles

The characterisation of nanoparticles provides the foundation for their further use. The fundamental factor in the engineering of nanoparticles as drug delivery systems is determining their particle size, surface characteristics, and internal structures to meet particular objectives. UV-Vis absorption spectrum is used for verification of metal oxide nanoparticles. Fourier Transform Infrared Spectroscopy is used to study the presence of zinc oxide functional group in the sample. Energy Dispersion X-ray is used to study the purity of the synthesized zinc oxide nanoparticles. Scanning Electron Microscopy is used to better understand the properties of nanoparticles.

2.5.1 UV-Vis Absorption Spectrum

To further verify the production of ZnO NPs, UV-Vis's spectroscopy was used. After dispersing the Zinc Oxide Nanoparticles in ultrapure water, the UV-Vis measurement was carried out. Previous research yielded a similar result for the absorption band that represents synthesized ZnO NPs, with the absorption band ranging from 355 to 380 nm, as summarised in Table 1. Because the absorption bands measured are identical, these supporting data establish the presence of ZnO NPs. Similar results were achieved by (Wang, Sun and Yu, 2011), who concluded that the obtained peak exhibited greater UV absorption for ZnO NPs.

Table 2.1 UV absorption peak from different researchers.

Author	UV absorption peak (nm)
(Talam et al., 2012)	355
(Khorsand Zak et al., 2011)	370
(Bian et al., 2011)	371

(Lavand & Malghe, 2018a)	375
(Akhil & Sudheer Khan, 2017)	370

2.5.2 Fourier Transform Infrared Spectroscopy (FT-IR)

The functional groups of produced ZnO NPs were studied and determined using FT-IR. (Yang & Xing, 2009) reported a wide absorption band at 414 cm^{-1} that was attributed to Zn-O stretching vibration in their FT-IR spectrum analysis. Other researchers were also able to observe an FT-IR spectra with a band around 400 cm^{-1} in prior work on ZnO NPs (Yang and Xing, 2009; Khorsand Zak et al., 2011; Lavand and Malghe, 2018). Also, (Alias et al., 2010) state that the peak of ZnO NPs lies between 464 and 419 cm^{-1}. Previous investigations on the production and characterisation of zinc oxide nanoparticles yielded similar results. The symmetric and asymmetric O-C-O stretching vibrations of adsorbed carbonate anion had peaks at 1339 and 1556 cm^{-1}, respectively. Meanwhile, absorption peaks were created by the peaks at 1047 cm-1, which show the lattice vibration of carbonate. Furthermore, at the absorption peak of 3417 cm^{-1}, hydroxyl group stretching may be noticed. Many various types of synthesis methods have been used to make ZnO NPs, however the resultant FT-IR spectrum for ZnO NPs synthesis has showed similarities (Jayaseelan et al., 2012). As a result, the FT-IR results revealed that the produced ZnO NPs were of high purity. According to (C. M. Wu et al., 2011) this technique gives information on surface functional groups that are present on the surface, which can be used to describe surface speciation.

2.5.3 Energy-Dispersive X-ray spectroscopy

The energy dispersive X-ray analysis is an x-ray technique used to determine the elemental composition of samples. Usually, EDX system are attached to electron microscopy instruments such as scanning electron microscopy and transmission

electron microscopy where the imaging capability of the microscope determines the specimen of interest. The data obtained from the EDX consists of spectra showing peaks corresponding to the elements making up the true composition of the sample being analysed.

Generally, when it comes to analysing synthesized zinc oxide nanoparticle, the purity of zinc oxide nanoparticles will have a very little impurities seen in the sample. Based on experiment conducted by (Hasnidawani et al., 2016), the theoretical mass percent of Zn and O was 80.3% and 19.7% respectively. While studies conducted by (Brintha & Ajitha, 2015) had obtained the mass percentage of Zn and O are 73.9% and 26.1%.

2.5.4 Scanning Electron Microscopy (SEM)

The scanning electron microscope (SEM) is one of the most often used tools for characterising nanomaterials and nanostructures. The signals produced by electron-sample interactions provide information about the sample such as surface shape and chemical composition. The size of nano objects that can be assessed accurately depends on the sample, the desired uncertainty, and the SEM's capability. Some SEMs' resolving power may not be sufficient to distinguish size and shape variations among tiny nanoparticles, such as sub-10 nm nanoparticles. For example, if the size of the place where the electron-optical column concentrates the main electron beam is 5 nm, the difference between pictures of 3- and 4-nm size particles might be merely in signal intensity at the particle sites, increasing the size measurement error of these particles. Small particles cannot be detected in SEM pictures in some circumstances due to noise, making size and shape measurements impossible. The number of nanoobjects that must be assessed for good-quality findings is determined by the sample, the desired uncertainty, and the SEM's capabilities. For statistically valid size and shape

characterisation, several hundreds or thousands of particles must typically be measured. Image capture and data processing may be optimised and automated to decrease costs while improving results quality (Swapp et. al. 2007).

2.6 Antioxidant Activity

Antioxidants are substances that opposed oxidants. Antioxidants (ROS, RNS, free radicals, and other unstable molecules) may prevent or delay cell damage caused by oxidants (ROS, RNS, free radicals, and other unstable molecules) (Azeez et al., 2017). Any agent that delays, stops, or removes oxidative damage to a target molecule is defined as an antioxidant (Piao et al., 2011). To be considered an antioxidant, a substance must be active at low concentrations (phenolic antioxidants often lose activity at high concentrations and act as prooxidants), have a sufficient amount of activity to deactivate the target molecule, react with oxygen or nitrogen free radicals, and have a less toxic final product than the removed radical. There is no universal antioxidant, as different antioxidants react with different reactive species by various mechanisms, at various locations and protect specific molecular targets (Babior, 2000; Piao et al., 2011). Generally, the antioxidant defence can become active either by in vivo processes or by supplying missing substances in the form of a diet (Cillard et al., 1969; Piao et al., 2011).

2.6.1 Spectrometric Method Used To Evaluate Antioxidant Activity Of The Synthesized Zinc Oxide Nanoparticles.

2.6.1.1 2,2-diphenyl-1-picrylhydrazyl (DPPH)

DPPH is classified as a stable free radical due to the delocalisation of the spare electron over the molecule as a whole, preventing dimerization, like most of other free radical. The delocalization produces the deep violet colour, with a 520 nm absorption in ethanol solution. When DPPH solution is mixed with a substance that may donate a

hydrogen atom, the reduced form loses its violet colour. DPPH can absorb an electron or a hydrogen radical to form a stable, diamagnetic molecule, but it can only be oxidised slowly and permanently. Due to its odd electron, DPPH exhibits a significant absorption band at 517 nm, and the solution appears a deep violet colour; however, the absorption fades when the electron pairs off. The decolorization that results are stoichiometric in terms of the quantity of electrons taken up. The alcoholic solutions of 0.5 mM are highly coloured, and the Lambert-Beer rule is obeyed across the relevant range of absorption at this concentration (Liu et al., 2011).

It is a quick, simple, low-cost, and commonly used technique for determining the potential of substances to serve as free radical scavengers or hydrogen donors, as well as assessing the antioxidant activity of meals. It may also be used to measure antioxidants in complicated biological systems, whether the materials are solid or liquid. The technique is distinct when the sample is reacted with DPPH in methanol/water, facilitating the extraction of antioxidant chemicals from the sample. The use of DPPH to determine the antioxidant activity of various types of food is comparable to other approaches. Other techniques of antioxidant analysis may be limited to chemicals that are soluble in the chosen solvents. The benefit of this approach is that DPPH is allowed to react with the entire sample, and the method allows DPPH to respond slowly even with weak antioxidants (Prakash & Baskaran, 2018).

2.6.1.2 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)

The ABTS/Potassium Persulfate (PP) analysis is based on the interaction of an antioxidant with the pre-generated ABTS•+ radical cation. Due to the bleaching of absorption spectra characteristic peaks at 414, 417, 645, 734, and 815 nm, ABTS•+ scavenging may be easily quantified. The most commonly used characteristic peaks for

monitoring are 414-417 nm and 730-734 nm. However, the latter is the suggested range due to the possibility of sample interference at lower wavelengths, which might result in an underestimated antioxidant capacity (Arnao et al.) . Because of the solvatochromic effect, the maximum bands of ABTS•+ absorbance shift a little different in various solvents: methanol (744-745 nm), ethanol (753 nm), and propanol-1 (757 nm) (Dong et al.. Endpoints of 4 or 6 minutes are usually employed for ABTS•+ loss their detection ability. ABTS•+ is often made a day ahead of time by allowing PP and ABTS to stand overnight (for 12-16 h). PP stoichiometrically oxidises ABTS to yield ABTS•+, which may be clearly identified by a colour shift from almost colourless to deep bluish-green. Under these parameters, the ABTS to ABTS•+ conversion degree is roughly 60%. Although ABTS is predicted to be colourless, the compound is frequently pale greenish. This appears to be related to traces of ABTS contaminants. This is demonstrated indirectly by the fact that after dissolving native ABTS in water, the resulting light greenish solution turns colourless with addition of ascorbate or any other antioxidant agent.

2.7 Gaps in Knowledge

Zinc oxide nanoparticles have chemical and thermal stability, unsaturated surfaces, and excellent adsorption behaviour against organic and inorganic contaminants in an aqueous medium. In comparison to other semiconductors, ZnO NPs have a higher photon absorption efficiency, surface area, and oxidising power, and are readily available, non-toxic, and cost-effective for wastewater treatment (Ray & Shipley, 2015). Many scientists have been studying the adsorptive potentials of ZnO NPs for the removal of heavy metals from industrial wastewater due to these features in recent years (Yuvaraja et al., 2018). Heavy metal removal effectiveness has been reported for different forms of ZnO NPs. Different process parameters such as pH, stirring speed,

reaction time, reaction temperature, mixing ratio, calcination temperature, and precursor concentration have been used by several studies to synthesis ZnO NPs. However, there is a limitation of information on the impact of these synthesis factors on the shape, size, and phase of zinc oxides in a comprehensive review.

However, problems with the stability, dispersion, and crystalline structure control of ZnO NPs in aqueous medium represent a significant barrier to the material's industrial application. To overcome the difficulties, many researchers have concentrated on the study of factors influencing the properties of stable ZnO NPs (Yu & Dong, 2016). Among these factors are the pH of the reaction mixture, synthesis and calcination temperatures, reaction time, precursor concentrations, solvent medium, and surfactant concentration (Hajiashrafi & Motakef-Kazemi, 2018). The aforementioned variables have a direct impact on the particle size, morphology, phase, and surface area of ZnO NPs (Hajiashrafi & Motakef-Kazemi, 2018; Jamal et al., 2019; Perillo et al., 2018).

CHAPTER 3

METHODOLOGY

3.1 Research Flow

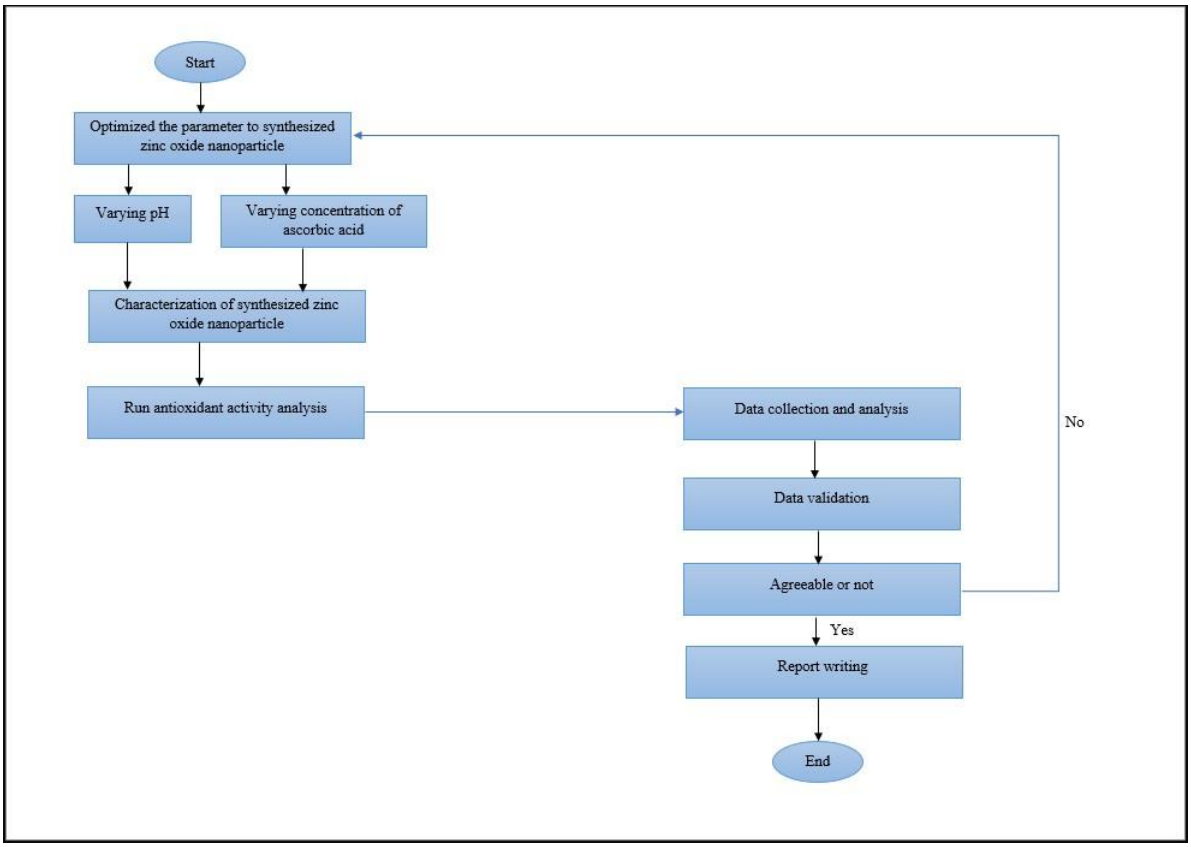

Figure 3.1 Flow diagram on research project for synthesis and characterization of zinc oxide nanoparticles

Figure 3.1 indicates the flow diagram of the research project for synthesis and characterization of zinc oxide nanoparticles. The synthesis of ZnO NPs will be using chemical precipitation method which will used ascorbic acid as reduction agent. The synthesis of ZnO NPs will be carried out in the chemical laboratory in Universiti Sains Malaysia (USM). After the synthesis process is done, the synthesized ZnO NPs will be characterized by using a few equipment which are Fourier transform infrared spectroscopy (FT-IR), Ultraviolet visible spectroscopy (UV-vis), Energy Dispersive X-ray (EDX) and Scanning electron microscopy (SEM). When the characterisation is