GROWTH OF ZnO NANORODS ON WIRE VIA SONOCHEMISTRY METHOD FOR GLUCOSE SENSING

by

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LIST OF ABBREVIATIONS

Ag/AgCl	silver/silver chloride
BSA	Bovine Serum Albumin
$(CH_2)_6N_4$	Hexamethylenetetramine
CV	Cyclic voltammetry
CVD	chemical vapor deposition
dH20	double distilled water
DI	Deionized water
DMF	dimethylformamide
DNA	deoxy ribonucleic acid
EDS	Electronic Deposition System
FAD	flavine adenine dinucleotide
$FADH_2$	flavine adenine dinucleotide hydrogen
FESEM	Field Emission Scanning Electron Microscope
GOD	Glucose oxidase
HRTEM	High Resolution Transmission Electron Microscopy
IEP	isoelectric point
KCl	Potassium chloride
MOVPE	metal organic vapor-phase epitaxy

NaOH	Sodium Hydroxide
PBS	Phosphate Buffer Solution
pH	potential of Hydrogen
PLD	pulsed vapor deposition
VLS	vapour-liquid-solid
XRD	X-Ray Diffraction
ZnO	Zinc oxide
$Zn(NO_3)_2.6H_2O$	Zinc nitrate hexahydrate

PERTUMBUHAN ZnO NANORODS ATAS WAYAR MELALUI KAEDAH KIMIASONO UNTUK PENGESANAN GLUKOSA

ABSTRAK

Banyak penyelidikan telah dilakukan keatas pertumbuhan ZnO nanorod atas pelbagai substrat mengunakan pelbagai jenis kaedah. Sintesis ultrasonik merupakan kaedah yang sememangnya diketahui berkesan untuk pertumbuhan nanorod. Wayar logam adalah sejenis substrat yang sesuai untuk digunakan bagi pertumbuhan ZnO dimana ia seterusnya dapat digunakan sebagai elektrod bagi aplikasi pengesanan. Namun, belum terdapat laporan tentang pertumbuhan ZnO nanorod secara terus atas wayar logam menggunakan kaedah sintesis secara ultrasonik. Pertumbuhan ZnO nanorod telah dilakukan ke atas empat jenis wayar logam yang berbeza (argentum (Ag), nikel (Ni), kuprum (Cu) dan tungsten (W)) dan sampel yang terbaik dipilih sebagai elektrod untuk ujian pengesanan glukosa. Zink Nitrat Hexahydrat dan Hexametelenetetramine (HMT) digunakan sebagai pelopor untuk pertumbuhan ZnO nanorod. Keadaan yang optimum untuk pertumbuhan ZnO nanorod ditentukan dengan mempelbagaikan kepekatan larutan pelopor, masa sonikasi dan nisbah larutan pelopor. Pencirian ZnO nanorod telah diperhatikan menggunakan FESEM yang dilengkapi dengan EDS dan kualiti rod dikenalpasti menggunakan XRD. Keputusan yang diperoleh menunjukkan bahawa kepekatan larutan pelopor mempengaruhi struktur pertumbuhan dan ketumpatan nanorod. Morfologi, pengedaran dan orientasi nanorod berubah mengikut perubahan masa sonikasi. Saiz dan ketumpatan nanorod berubah secara ketara dengan perubahan nisbah larutan pelopor. Morfologi, saiz, pengedaran dan orientasi yang terbaik pada wayar didapati pada wayar Ag. Struktur rod atas wayar Ag didapati paling seragam dengan saiz yang hampir serupa keseluruhannya dan mempunyai bentuk heksagon yang jelas. Nanorod tunggal berbentuk heksagon telah diperolehi. Bentuk ini mengesahkan bahawasanya ZnO selari dengan struktur wurtzite heksagon. ZnO nanorod atas wayar Ag menunjukkan pengesanan positif terhadap glukosa di mana tindak balas elektrik meningkat dengan peningkatan kepekatan glukosa dari 0.001 M kepada 0.015 M. Keputusan ini serupa dengan kajian yang dilakukan untuk ZnO nanorods yang tumbuh atas substrat kaca bersalut Au yang diuji sebagai sensor glukosa dan menunjukkan tindak balas dengan peningkatan kepekatan larutan glukosa. Perubahan pada kepekatan gukosa mempengaruhi pengesanan sensor. Kehadiran nanorod ZnO meningkatkan keberkesanan pengesanan glukosa yang dilihat melalui puncak katod yang meningkat daripada 70.19 pA kepada 126.6 pA daripada kepekatan glukosa 0.001 M kepada 0.015 M.

GROWTH OF ZnO NANORODS ON WIRE VIA SONOCHEMISTRY METHOD FOR GLUCOSE SENSING

ABSTRACT

There has been much research on growth of ZnO nanorods on various substrates, and various growth methods. Sonochemical method is a well-known method for growing nanorods. Metal wire is a suitable substrate for the growth of ZnO nanorods as it can then be used as sensor electrode for sensing application. However there has not been any report on growth of ZnO nanorods on metal wire directly using sonochemical method. ZnO nanorods were directly grown on four different wires (silver (Ag), nickel (Ni), copper (Cu), and tungsten (W)) through sonochemical method and the best sample was chosen as electrode and tested for glucose sensing. Zinc nitrate hexahydrate and hexamethylenetetramine (HMT) were used as precursors for the growth of ZnO nanorods. The optimum condition for the growth of ZnO nanorods were determined by varying precursor molarity, sonication time and molar ratio of precursor solution. Characterization of ZnO nanorods grown was observed using FESEM equipped with EDS and the crystal quality was checked using XRD. The results demonstrated that the precursor concentration affected the growth structure and density of the nanorods. The morphology, distribution, and orientation of nanorods changed as the sonication time changed. The size and density of the nanorods changes significantly with the change of molar ratio of precursor. The best morphology, size, distribution, and orientation of the nanorods were observed on the Ag wire. The structure of rods on Ag wire appears to be to most uniformly distributed with stable size and clear hexagonal shaped. The presence of single crystal nanorod with hexagonal shaped was obtained. This shape indicates that the ZnO nanorods corresponded to the hexagonal wurtzite structure. The ZnO nanorods on Ag electrode showed positive detection of glucose whereby the current response with increase of glucose concentration from 0.001 M to 0.015 M. This result is similar to research done for ZnO nanorods grown on Au coated glass substrate tested as glucose sensor which showed that increased in current response with increased of glucose concentration. Changes in glucose concentration do affect the sensor detection. Presence of ZnO nanorods enhances the sensor efficiency for the detection of glucose seen through the cathodic peak current which increased from 70.19 pA to 126.6 pA from 0.001 M to 0.015 M glucose concentration.

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Nanomaterials, especially the 1-dimensional (1D) nanostructures are suitable to be used for nanoelectrics and nanodevices. 1D nanostructure has an anisotropic morphology whereby it grows along a preferred direction. The properties of an anisotropic material are directionally dependent. Their fine-tune in both physical and chemical properties makes them perfect candidate for designing new devices. 1D nanomaterial has been considered the standard example of material with unique physiochemical properties. Their unique properties lie from the large surface to volume ratio and restricted motion and confinement of electrons around the particles. Nanostructure compared to a macrostructure has higher amount of molecules or atoms at its surface leading to larger surface to volume ratio which has effect on its reactivity, magnetic, hardness and opto-electronic properties. Anisotropy in nanoparticle increases its biocompatibility and the coercive field of ferromagnetic particle. Having optical absorption in near-infrared region, enhance photothermal heating capacity which making it a suitable candidate to be used in diagnosis and therapeutic field of research. Anisotropic nanomaterials are suitable for the detection of biomolecules as it has better sensitivity (Sajanlal et al., 2011). 1D nanostructures normally have nanoscale sizes ranging from 1 and 100nm with exception of the length that can be up to a few millimeters long or more. The elongated length allows the nanostructures to have contact with the macroscopic world for the purpose of physical measurements. Therefore this morphology makes the 1D electrical measurements of two terminals easier. Different types of 1D nanostructure that have been synthesized and characterized are such as rods, wires, tubes, belts, needles, tips etc.. These different 1D forms makes it suitable to be applied in various nanodevices as they can enhance the unique properties of the material. Some applications of nanostructures are in biosensors, photodetectors, gas sensors, transistors etc. Metal oxide nanostructures particularly have been widely researched. Metal oxides are ionic compounds that have positive metallic and negative oxygen ions. The electronic interaction between these opposite charges of positive metallic and negative oxygen ions produces a firm and solid ionic bond. Some unique characteristics of metal oxides are having wide bandgaps, good superconductivity, electrical, optical and electrochromic property. These 1D metal oxide nanostructures has elicited interest and produce breakthrough in all areas of electronics. Examples of metal oxide nanostructures are nickel oxide, zinc oxides, tungsten oxides, vanadium oxides, tin oxides etc. (Devan et al., 2012). These metal oxides have become an alternative to the normally used silicon and metal nitride for electronic devices. The huge application potential of metal oxide has opened a bridge up between physicist and biotechnologist to make full use of the material (Singh et al., 2010).

ZnO is a key material with novel applications in the field of nanoscience and nanotechnology. Zinc oxide (ZnO) is a semiconductor material which is extensively researched due to its potential to be used in variety of applications such as photo-detectors, light emitting diodes, piezoelectric transducers, chemical or biosensors, solar cells, actuators etc. ZnO has excellent material properties such as large exciting binding energy of 60 meV, direct band gap of 3.37 eV and high optical gain at room temperature and large saturation velocity of 3.2x 10^7 cm/s (Solis-Pomar et al., 2011). Through years, variations of ZnO nanostructures have been produced. As nanostructure has large potential in many application including devices and sensors, it is important to have a thorough understanding on the growth mechanism of ZnO. This is because the morphology of ZnO influences the properties of ZnO nanostructure in such a way that the properties changes with the change of nanostructure dimension. Therefore this affects their application in the practical fields (Amin et al., 2011). The most sought after form of ZnO is the wurtzite hexagonal as it has diverse morphologies, properties and applications other than its excellent physicochemical properties (Devan et al., 2012 & Wang, 2004). Many parameters are to influence the morphology and size of ZnO nanoparticle formed. Among them are the temperature, time of synthesis, concentration of precursors and pH of solution. ZnO nanoparticle changes in dimension with the changes in the parameter (Wang et al, 2013). Previous researches has shown the use of different reagents for the formation of ZnO nanoparticle such as the use of zinc (II) acetate and N,N-dimethylformamide (DMF) (Bhattacharyya and Gedanken, 2008), zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) and sodium hydroxide (NaOH) (Chu et al, 2012) and zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) and hexamethylenetetramine (HMT) (Malek et al., 2013 & Melendrez & Vargas-Hernandez, 2013). For this research the reagents ($Zn(NO_3)_2.6H_2O$) and HMT has been chosen as the fabrication of ZnO nanorods. There have been a few methods documented on the fabrication of ZnO nanorods such as hydrothermal (Solis-Pomar et al., 2011 & Nozaki et al., 2013), chemical vapor deposition (CVD) (Abd Aziz et al., 2014), pulsed laser deposition (PLD) (Tien et al., 2008) and metal organic vapor-phase epitaxy (MOVPE) (Robin et al., 2007) method. This work focused on the fabrication of ZnO nanorods on wire using sonochemical method for biosensing purpose.

Sonochemical method or ultrasonic irradiation differs from traditional energy sources such as light and heat in terms of duration, pressure and energy per molecule.

This method has been explored to obtain novel materials. The chemical reaction in an ultrasonic irradiation involves an acoustic cavitation which is the formation, growth and implosive collapse of bubbles in a liquid medium. This creates an environment with extremely high temperature (5000K-25000K) and pressure (Yadav, Mishra and Pandey, 2008). The phase leading to the growth of the bubble involves diffusion of solute vapour into the volume of the bubble. Subsequently the collapse of the bubble takes place when the bubble size reaches its' maximum. A research by Hu et al. (2004) has demonstrated the applicability of sonochemical method in producing free standing ZnO rods (Hu, Zhu & Wang, 2004). Sonochemical method is a practical and green method which is known to produce novel materials with interesting properties. The advantage of using sonochemical method is the possibility of mass production, large deposition area and short reaction time. This method has so far been used to grow variety different kinds of nanomaterials (Shahidi, Zarei and Elahi, 2014).

Glucose biosensor is considerably the most widely sought after type of biosensor and till now many different design has been offered. This is because monitoring blood glucose level is crucial as it is an important indicator of various diseases and health complications. This brings to an increase necessity to obtain a fast and accurate method for the determination of glucose (Ali et al., 2010). Therefore, many researches have been done to produce a simple, robust and non-invasive glucose sensor with good selectivity, high sensitivity, stable and fast response (Alvi et al., 2012). Glucose oxidase (GOD) base glucose detection has been commonly used in glucose detection. GOD has been used in commercial applications such as for the improvement of colour, flavour and shelf life of food materials; as glucose assay kit and in biosensors for the detection of glucose in body fluids such as blood and urine as well as in industrial solutions (Banker et al., 2009). This is because it has high specificity towards glucose and high activity over a wide range of ionic strength, temperature and pH. Nevertheless, the first generation GOD based biosensor has its downside as it is unstable and has low activity due to the absence of functional materials. Therefore, one promising means is to employ nanomaterials as an effective sensing material. Nanomaterials have novel optical, magnetic and electrical properties, good biocompatibility and large surface to volume ratio (Wang et al., 2013).

The currently frequently used method of ZnO nanorods fabrication are time consuming and requires certain growth conditions such as elevated temperature, and specific pressure for the growth process. Different sensor electrode based on ZnO is usually fabricated on substrates such as silicon or glass substrate. This substrate requires vigorous surface preparation such as cleaning and coating of the substrate before growing ZnO nanorods. The use of wire as electrode is simpler as it does not require such preparation and the wire could readily be use for the growth of ZnO nanorods and then as sensor electrode.

As such, the aim of this work is to produce glucose biosensor based on ZnO nanorods grown in a one-pot sonochemical synthesis method under ambient condition using various thin wires (Ag, Ni, Cu and W) as template for the growth of the nanorods by optimizing the growth parameters. Cross-linking method was used for the immobilization of GOD. The best wire for the growth of ZnO nanorods was chosen as the electrode and the applicability of the sensor electrode was evaluated using different glucose concentrations.

1.2 Research objectives:

1. To explore the feasibility of ZnO nanorods growth on Ag, Ni, Cu & W wire via sonochemistry method.
2. To determine the optimum concentration, ultrasonic power and molar ratios of precursor required for the growth of ZnO nanorods.
3. To determine the efficacy of ZnO nanorods based electrode for glucose sensing.

1.3 Originality of Research

Currently there is no report about using this direct method of growing ZnO nanorods on wire. Previously reported studies are on growth of ZnO nanomaterials on arbitrary substrate and glass substrate. They would normally require two steps in the preparation of substrate surface and growth of nanomaterials. Therefore the direct use of wire as substrate for growth reduces the research time and sonochemical method is a proven method used to produce ZnO nanorods.

As for the application for glucose sensor, there is very few reported research on the use of wire as sensor electrode. The test on glucose sensing efficacy using cyclic voltammetry with ZnO nanorods grown wire has not been reported before.

1.4 Organization of research as follow:

The content of this thesis is divided into 5 chapters. Chapter 1 gives a brief introduction of the topic of study and the research objectives.

Chapter 2 gives the overview of nanostructures, properties of ZnO nanostructures, some background search related to the growth, fabrication and application of ZnO nanostructures. Furthermore, this chapter describes the use of sonochemical method, the theory and mechanism of sonication reaction in the synthesis of nanoparticles and gives a background study on the development of nanostructure based enzymatic biosensor.

Chapter 3 provides the materials used, sample preparation, growth technique, characterization and experimental setup used to grow and evaluate properties and applicability of ZnO nanorods as biosensor.

Chapter 4 presents the data and analysis on ZnO nanorods grown on wire via sonochemical method and the glucose sensing ability of ZnO nanorod based electrode. The growth of ZnO nanorods on wire (Ag, Ni, Cu & W) are optimized by varying the precursor concentration and sonication time and molar ratio of precursor. The properties of ZnO grown are discussed by means of its morphological and crystallographic aspects. The morphology of rods post immobilization is also discussed. The best sample is chosen as electrode for glucose sensing. The effect of absence and presence of rods and glucose and varying glucose concentration and is tested.

Chapter 5 concludes the findings of this work and highlights the potential of ZnO nanorods on wire to be used as biosensor. Other than that, some suggestion is provided for the improvement of the device to be used for future research project.

CHAPTER TWO

LIERATURE REVIEW AND THEORETICAL BACKGROUND

2.0 Introduction

This chapter gives understanding on theoretical and background knowledge on ZnO nanostructures, sonochemical method for synthesis of nanomaterials and biosensing mechanism reaction. Previously done research involving different synthesis method involving different ZnO nanomaterials are also included.

2.1 Nanostructures (ZnO)

Nanostructures are divided into four morphologies that are the 0D, 1D, 2D and 3D nanostructures. Nanostructures with different dimensions have different physical and chemical properties. Nanomaterials of 1D, with width and thickness between 1 and 100nm and length up to a few millimeters are most suitable to be applied in nanodevices and nanoelectronics. This characteristic of the 1D nanostructure allows contact in macroscopic level in terms of physical measurement making electrical measurement with two terminals easier. Extensive research has been done to fabricate and characterize metal oxide 1D nanostructure such as zinc oxides, tungsten oxides, nickel oxides, tin oxides, vanadium oxides etc.. Examples of different forms of 1D nanostructures are needles, rods, ribbons, tubes, belts etc.. The applications of this material are in the area of biosensor, gas sensors, solar cells, photodetectors, transistors and capacitors. For 0D structure, they include nanocluster materials and nanodispersions in which nanoparticles are isolated from each other. One of the examples is quantum dots. As for 2D structure the examples are nanoplates while for 3D is nanotetrapods. There are various known techniques for the synthesis of these nanostructures through direct physical deposition techniques and direct chemical deposition technique (Devan et al., 2012; Gomez and Tigli, 2013). Some of the methods in direct physical deposition techniques are physical vapour deposition (PVD), confinement growth and lithographic techniques which involve no chemical reactions during the synthesis process (Abd Aziz et al., 2014). Several methods of fabricating ZnO nanorods such as hydrothermal (Amin et al., 2011), vapour-liquid-solid (VLS) (Yang et al., 2008), pulsed laser deposition (PLD) (Tien et al., 2008), metal organic vapour-phase epitaxy (MOVPE) (Robin et al., 2007) and sonochemical method (Hu et a., 2004) have been reported. Oxygen plays an important role in the synthesis method.

ZnO has been the material of interest in semiconductor research since 1945 for its growth, band structure, luminescence and electronic transport. Moving on to year 2000s the attention moved to the potential of ZnO as nanostructures, quantum wells and for epitaxial layer growth. Recent years has shown the grown attention in ZnO in area of biosensing and optoelectronics applying the ZnO nanostructures. ZnO has been the material of interest in the area of nanotechnology due to its unique properties and the ability to be synthesized with great control and precision. ZnO has a stable polar surface making it suitable to induce the formation of nanostructures such as nanorods, nanowires, nanobelts, nanotubes etc. (Gomez and Tigli, 2014).

ZnO nanostructure has two crystalline forms which is the hexagonal wurtzite and zinc blend. The three crystal structures of ZnO are wurtzite, zinc-blend and rock-salt. In general condition ZnO follows the hexagonal wurtzite structure (Figure 1) which is the thermodynamically stable phase. The hexagonal structure of ZnO can be defined as "a number of alternating planes composed of tetrahedrally coordinated O^{2-} and Zn^{2+} stacked alternately along the c-axis". The entire structure is said to lack symmetry (Zhang et al., 2012). This lack of symmetry results in the piezoelectric effect, whereby the mechanical stress will be converted to electrical voltage causing the cations and anions to be displaced. Existence of polar charges at the surface which are the Zn^{2+} (0001) and O^{2-} $(000\bar{1})$ results in the growth of various unique nanostructures such as nanorings, nanobelts, nanosprings and nanohelices. Even though the ZnO unit cell is neutral, the cations and anions might be distributed to a specific configuration based on crystallography thus some surfaces can be filled entirely by cations or anions causing the existence of polar charges of either positively or negatively charged. In order to maintain a stable structure, the facets undergo surface reconstruction except for the ZnO ±(0001) as they are stable, atomically flat and do not need reconstruction. They have equal number of zinc and oxygen atoms present at the non-polar facets. Other than the common basal plane (0001), there are also other secondary planes exist in the crystal structure (Fulati, 2010). The zinc blend ZnO structure is metastable and can only be stabilized by growing on cubic substrates such as ZnS in order to overcome the tendency of forming wurtzite phase. The wurtzite structure of ZnO can be converted to the rock-salt structure under slight external hydrostatic pressures (Morkoc and Uzgur, 2008).

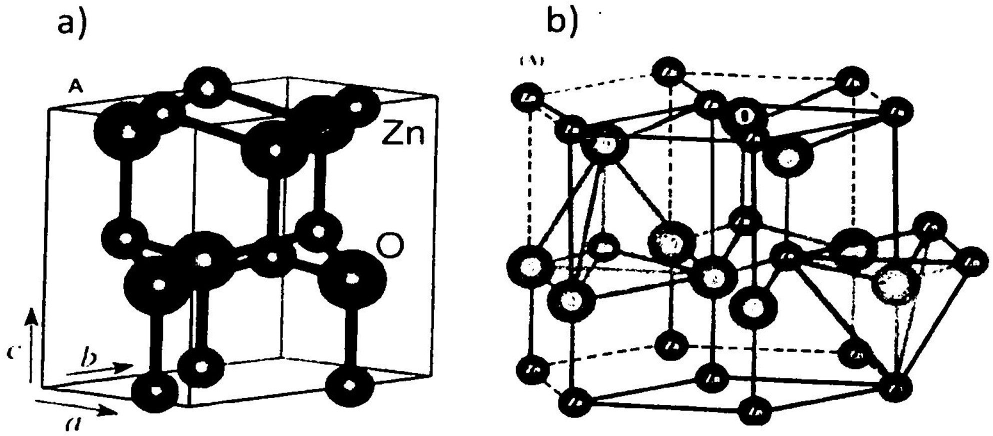

Figure 1: ZnO wurtzite unit cell on the left and ZnO wurtzite structure on the right (Sahoo et al., 2015; Kamble et al, 2014).

The wurtzite structure is calculated using its two lattice parameters a and c in the ratio of *c*/*a* whereby the ideal value to be *c*/*a*=1.633. There is also parameter *u*, known as the "length of bond parallel to the c-axis" having value *u*=0.375 for ideal structure. It is calculated from the bond length or nearest neighbour distance, parameter *b* divided by parameter *c*. In reality the wurtzite structure of a ZnO crystal would deviate slightly from the ideal arrangement as the ratio appears to be smaller than the ideal value. This is cause by the lattice stability and iconicity. There is a strong correlation between the c/a ratio and u parameter in such a way that when the ratio decreases, u parameter increases in order to maintain the distances between the four tetrahedral angles to be nearly constant even with distortions. The lattice parameters can be affected by variables such as stress, free charge, impurities and temperature (Morkoc and Uzgur, 2008).

The characteristic of zinc oxide (ZnO) is that it is a type of semiconductor having wide band gap energy of around 3.37eV (Chu et al., 2012; Abd Aziz et al., 2014). This is much higher than other known semiconductors such as silicon (1.12 eV), gallium phosphide (2.25 eV, indium phosphide (1.27 eV) and germanium (0.66 eV). This characteristic makes ZnO suitable to be used in photonic application (Nayak et al, 2012). At room temperature, ZnO also have large excitation binding energy of 60 meV. With these unique characteristics, ZnO nanostructures such as nanobelts, nanofibres and nanorods have become the focus for research (Abd Aziz et al, 2014). As ZnO is tetragonally coordinated, the positive charges overlap with the negative charges at the center. Therefore whenever an external force is applied, the tetrahedron is distorted creating a dipole moment which generates the piezoelectric properties. The growth of hexagonal tubular nanostructures would start off as ZnO nanorods at the beginning before transforming further to their respective shapes. This is because the surface of the ZnO shell would undergo oxidation followed by sublimation, making the core hollow. Other method to produce more unique structures of ZnO is by modifying the composition of as synthesized ZnO, diffusion rate and temperature (Gomez and Tigli, 2013). One of the uses of ZnO nanostructures is in the application of biosensor as they are nontoxic, biologically compatible, easy preparation and fast electron transfer rate. Other than that they also have high surface to volume ratio, good chemical stability and are electrochemically active. ZnO have an isoelectric point (IEP) around 9.5 which allows it to immobilize molecules with low isoelectric point such as DNA, proteins by electrostatic adsorption in proper buffer solutions (Ali et al, 2010; Kong et al, 2009). The high IEP also allows sustainability of higher electric fields and a higher breakdown voltage. In addition adsorption of proteins is much easier with high IEP due to stronger electrostatic interactions. Biocompatibility is another important characteristic of ZnO for biosensing application (Gomez and Tigli, 2013). This is because ZnO nanoparticles are able to supply suitable environment for the immobilization of glucose oxidase and aid the electron transfer between glucose oxidase and electrode (Salimi et al., 2007). There are many applications which have used ZnO as part of its components such as in electronics, acoustics, sensing and photonics. ZnO is known to have high optimal transmissivity and high conductivity which is useful in electronic side. Due to large electromechanical coupling, this inorganic oxide is highly promising in area of acoustic wave devices. It is relatively easy to produce ZnO nanostructures which have good charge carrier transport and high crystalline quality hence many devices are incorporating the use of nanowires/nonorods in biosensor and gas sensors. ZnO also is used in device applications due to its low production cost and superior optical properties (Umit et al., 2010).

2.1.1 Synthesis of ZnO

ZnO nanostructures can be grown either independently or on a substrate. Different substrates that have been used for the growth of ZnO nanostructures include Si substrate (Nayak et al, 2010), gold, indium tin oxide, glass (Breedon et al., 2008) and wire (Ali et al., 2010). When grown on a substrate, the crystallinity of the substrate is said not an important factor for the assembly of the nanoparticles. The surface chemistry indicated to play major role on the alignment but the exact reaction is unclear. The nanorods grown could be positively attracted and not repelled by the hydrophobic substrate surface. The nanorods prefer to maintain in the solvent when the substrate surface is hydrophilic (Sajanlal et al., 2011). A research by Breedon (2008) showed the growth of ZnO nanostructures on variety of substrates such as glass, quartz, gold etc. The substrates were coated with ZnO seed layer to improve the orientation and uniformity of ZnO grown. The different substrate exhibit different growth orientation of ZnO especially the glass substrates which showed no preferential growth angle. The theory was that the surface roughness of the glass might have affected the orientation causing the nanorods to grow disorderedly. However, the use of seed layer might cause etching during the reaction process increasing the concentration of free Zn^{2+} ions (Breedon et al, 2008). The presence of seed layer acts as a homoepitaxial nucleation site and is usually generated through sputtering or sol-gel method. In order to ensure a good adhesion of seed particles to the substrate, high temperature annealing is required. So far a seedless approach for the fabrication of well-aligned ZnO nanorods on different substrate in still looked-for (Zheng et al., 2013).

Different solution based routes for the growth of ZnO nanostructures has been reported over the years. They are hydrothermal (Amin et al, 2009), sol-gel (Askarinejad et al., 2011) and sonochemical (Hu et al., 2004; Nayak et al., 2012; Wahab et al., 2013). Hydrothermal is the commonly used method for the synthesis of ZnO nanostructures. It is a reaction whereby the aqueous precursor solution is placed in a thermal reactor (a stainless steel chamber). The most important factor in this reaction process is to consider the temperature of reaction solution and the time of reaction to determine the different types of ZnO nanostructure produced. Other factors which influence the morphology and crystallinity of the nanoparticles formed are the type and concentration of precursors and the pH of reaction solution. The sol-gel method involves a precursor solution which forms a gel of discrete particles. The precursor solution such as zinc chloride undergoes poly-condensation and hydrolysis reactions. ZnO forms in such a way that the zinc centers are connected with hydroxo bridges. Thus the gel like system with both of liquid and solid phase exists in which the morphologies varies from discrete particles to continuous polymer of network. As for sonochemical method, the synthesis method involves precursor solution as the starting material. The solution will be ultrasonically synthesized to produce variety types of ZnO nanostructures depending on the growth parameters employed (Singh et al., 2010).

Method of synthesizing ZnO nanostructures using different reagents has been reported. It is usually the use of one solution containing zinc source and the other solution the alkaline reagent (Singh et al., 2010). Among them are the use of zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) and hexamethylenetetramine (HMT) (Ali et al., 2013; Nayak et al., 2010) and zinc acetate dehydrate and sodium hydroxide (Wahab et al., 2013). Reagents $Zn(NO_3)_2.6H_2O$ and HMT are the most commonly used starting precursor for the formation of ZnO due to its advantage in producing high quality ZnO. For the fabrication of ZnO, $Zn(NO_3)_2.6H_2O$ supplies the main material, Zn^{2+} ions while HMT provides O^{2-} ions. The zinc nitrate is the precursor while HMT is the stabilizer in the reaction process. HMT acts as a pH buffer and as an OH^- supplier. The product of HMT, NH_3 provides basic environment for the formation of Zn(OH), a ZnO intermediate. This is important as divalent metal ions such as ZnO does not hydrolyse in an acidic environment. Under alkali environment, the reaction can take place at room temperature to give rise to ZnO nanoparticle. In the reaction HMT will decompose forming hydroxyl ions for building of ZnO. HMT also ensures the growth of ZnO nanorods are controlled to a specific structure and rate. HMT is said to act as a bridge between two Zn^{2+} ions. This is done as HMT attaches to the Zn^{2+} ions. In turn the growth of ZnO is structured along the polar c-axis. As HMT attaches to the nonpolar facets of ZnO, it facilitates growth along the polar surface of ZnO, which is referred to as anisotropic growth. Once supersaturation is achieved, the Zn^{2+} reacts with the OH^- ions to form ZnO nuclei on the surface of the wire. Then the Zn^{2+} and OH^- ions will continuously attached one after another to form ZnO nanorods (Devan et al, 2012; Wang, 2004). As the O^{2-} comes from the base solution, the solvent does not necessarily have to be H_2O. Several organic solvents that have been used for the synthesis of ZnO are such as ethanol and acetone (Khoza et al., 2012). Dissolved oxygen in the growth solution also plays an important role in determining the quality of ZnO nanostructures produced. Extra H_2O_2 produces higher quality of ZnO nanoparticle while insufficient oxygen such as the use of boiled DI would lead to the formation of ragged ZnO surface (Xu and Wang, 2011).

The formation of ZnO in solution starts off with the nucleation stage. Nucleation is the stage whereby the nuclei act as a template which aids in crystal growth. Primary nucleation is the starting phase of nucleation in absence of any other crystalline matter. There are two types of nucleation which is the homogenous and heterogeneous nucleation. In homogenous nucleation, the nuclei forms first directly over the parent body while in heterogeneous nucleation there are structural irregularity such as impurities and dislocations in which the nucleation forms on. Heterogeneous nucleation is said to be easier to occur in liquid as there are already a stable nucleating surface present. This causes a decrease in the thermodynamic barrier that has to be overcome for the nucleation to occur (Thanh et al., 2014). The nucleation normally takes place rapidly and succeeding growth occurs by two primary mechanisms which is Ostwald ripening and oriented attachment. In Ostwald ripening, the growth of larger nanoparticle occurs at expense of the smaller crystals. This is also called coarsening. Due to higher solubility and surface energy near a smaller crystal, it increases the chemical potential causing it to dissolve allowing the larger crystals to grow more. Oriented attachment is the growth of crystals by aggregation. In this mechanism, particles are assembled from randomly oriented to highly oriented nanoparticles. The primary nanoparticles may aggregate in an oriented manner to form larger crystals or aggregate randomly causing the particles to recrystallize, reorient or change phase to form larger crystals. However, aggregation initiated growth may cause defects such as edge and screw dislocations (Genget al., 2012). The effect from the Ostwald ripening is said as "the last stage of a condensation transition from liquid to solid" whereby the smaller particles can vanish due to the thermodynamic instability. The smaller ZnO crystals are dissolved to form larger and more thermodynamically stable nanorods (Breedon et al., 2008).

2.2 Sonochemistry

Sonochemistry is defined as " the chemistry associated with ultrasound which is governed by parameters such as amplitude and frequency of an applied sound field, temperature, surface tension, gas content, vapour pressure and nuclei density of the solution as well as vessel and probe geometry"(Gong and Hart, 1998). Ultrasonic wave is a branch of sound waves consist of cyclic sound pressure (compression and expansion) at a certain frequency. Depending on frequency, it is divided into three categories as shown in Figure 2; power ultrasound (20-100 kHz), high frequency ultrasound (100 kHz-1 MHz) and diagnostic ultrasound (1-500 MHz). The sound range of 20 to 100 kHz is normally in systems which require chemical and physical changes as it has the ability to induce cavitation bubbles. Ultrasonic range of 1 to 10MHZ is for the purpose of animal navigation and communication and also to detect underwater echo plus in diagnostic areas (Pilli et al., 2011).

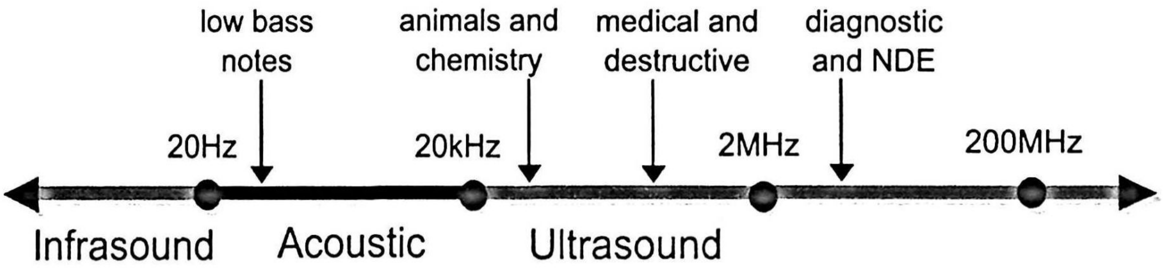

Figure 2: Diagram of ultrasound range (https://en.wikipedia.org/wiki/Ultrasound)

In liquid, mechanical vibration causes ultrasonic waves to undergo series of compression and expansion phases creating pressure in the liquid. During compression, a positive pressure pushes liquid molecules together while in expansion liquid molecules are pulled part. When the pressure amplitude exceeds the tensile strength of liquid during the expansion cycle, it is then that cavitation bubbles (vapour-filled bubbles) are formed. This cavitation phenomenon as shown in Figure 3 consists of creation, growth and collapse of the cavitation bubbles in the liquid (Wu et al., 2012). Suslick (1990) stated that as the bubble collapses, each bubble acts as a hotspot creating extreme environment in the core of the bubbles with temperature of 5000 K and pressure up to 500 atm and cooling rate as fast as 10^9 K/s. According to hot spot theory, "each microbubble acts as a small microreactor which produces different reactive species and heat during its collapse". This extreme condition from the collapsing bubble causes mechanical and chemical effects of ultrasonic energy. The sound wave in the reaction also provides intensive mixing in media (Suslick, 1990). As ultrasonic irradiation occurs in liquid medium, example when in water, it induces sonolysis of water molecules and thermal dissociation of molecules of oxygen. This forms different reactive species such as H•, O•, OH• and radicals OOH•. Through sonolysis of water, there are also production of H_2O_2 and H_2 gas from OH• and H•. Other than chemical effects, there are also physical effects in which when the liquid medium is irradiated, energy from the sound waves will be absorbed along the propagation direction of the wave. Physical effects that are produced by the cavitation bubbles include microstreaming, microstreamers, microjets and shock waves. This causes turbulent movement of fluid and velocity gradient at microscale level within the area of the cavitation bubbles (Wu et al., 2012).

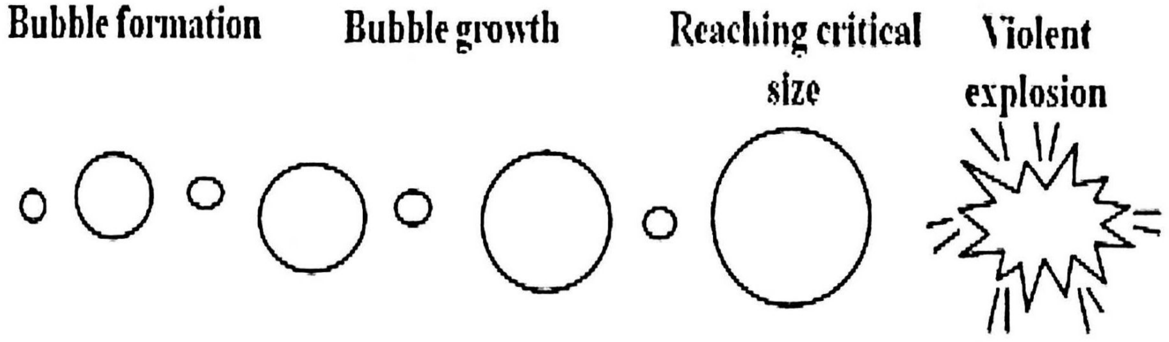

Figure 3: Cavitation bubble formation, growth and collapse (Badday et al, 2012)

There are a few parameters which affect the physical performance of ultrasonic cavitation producing different properties of the final product. They are frequency, intensity, solvent, temperature, external pressure and the application of ultrasonic being direct or indirect. For effect of frequency, the production of cavitation bubbles is more difficult at high sonic frequencies (MHz) compared to low frequencies (kHz). This is because at high frequency compression and expansion becomes too short that cavitation could not be obtained. Intensity is proportional to the amplitude vibration of the ultrasound. Amplitude that is very high is not necessarily good as it might lead to deterioration of the ultrasonic transducer, inducing agitation of the liquid medium instead of cavitation. Solvent used is important in terms of type and viscosity as if it is high, it could inhibit cavitation. Temperature has two roles in sonication. High temperature is required to break the bonds of the solute molecules which involve hydrogen bonding, Van Der Waals forces and dipole attractions. However, low temperature is more desirable to produce cavitation. There should be a balance between temperature and cavitation. Generally research using ultrasonic applications in analytical chemistry is carried out under atmospheric pressure. If external pressure were to be increased, greater ultrasonic energy is required to induce cavitation. Direct sonication is by using ultrasonic probes whereby the probe is immersed into the sample solution without any barrier for the sonication wave to cross and sonication is performed directly. Indirect application is the use of ultrasonic bath whereby the sonication waves have to cross the liquid in the ultrasonic device and the wall of the sample container before reaching the sample solution (Santoset al., 2009).

According to Suslick et al, (1999), sonochemistry has been applied for the synthesis and modification of inorganic materials. As the acoustic cavitation causes bubble collapse when the liquid is irradiated, it concentrates the diffuse energy of sound into several unique situations to produce different materials from the dissolved solution precursors. The cavitation and shockwaves created from ultrasonic irradiation of liquid-powder slurries could hasten solid particles to high velocities. The interparticle collision causes changes in the surface morphology, reactivity and composition (Suslick, 1999).

Over the years, the application of ultrasonic energy has been widely applied in the field of science. This ultrasonic method has been employed in various organic and inorganic reactions and in the fabrication of nanostructured materials. Researches have been using this method to synthesize different shapes of ZnO nanostructures. Melendrez (2013) has used ultrasound to synthesize ZnO nanorods on flexible substrate (Melendrez and Vargas-Hernandez, 2013), Nayak et al., (2010) has grown ZnO nanowire on arbitrary substrate (Nayak et al., 2010), Kandjani et al., (2008) synthesized ZnO nanoparticles (Kandjaniet al., 2008), Bhattacharyya & Gedaken, (2008) used sonochemical method to grow ZnO nano-disk (Bhattacharyya and Gedanken, 2008). By using sonochemical method, the conversion rate of precursor to nanometer sized ZnO is greatly enhanced even without heating at high temperature and surfactants (Wahab et al., 2013). Ultrasound method is said to be able to enhance crystal nucleation and reduce the nucleation time to a much faster rate (Genget al., 2012). Other than differences in shape, nanomaterials obtain in sonochemical method also vary in their solid phase being whether in crystalline or amorphous form. The advantage of using sonication is that it provides good dispersion of precursor molecules in the liquid medium, preventing agglomeration thus ensuring a homogenous and uniform reaction process for the formation of nanomaterials. This prevents the nanomaterials from growing too fast only in a certain direction (Novik, 2010; Mamat et al., 2012). For growth of ZnO using sonochemical method, several zinc precursors that are used are zinc nitrate hexahydrate, zinc chloride and zinc acetate. For hydroxide anion precursor hexamethylenetetramine (HMT) is commonly used as the starting material. The physical parameters that affect the ultrasonic irradiation controls the morphology, size and shape of zinc oxide nanostructures (Singh et al, 2010).

2.2.1 Sonication reaction

The shapes of the ZnO nanorods are dependent on the concentration of zinc nitrate hexahydrate and HMT. Alkaline reagent of hexamethylenetetramine (HMT) and zinc salt from zinc nitrate hexahydrate Zn(NO3)2.6H2O were used as the precursor solution in the sonochemical synthesis. These two reagents play an important role in the formation of ZnO nanorods with the help of sonication which disperse and mixes the solution initiating the reaction activity in the solution. The chemical reaction which takes place during the sonication process is as following (Malek et al., 2013; Melendrez and Vargas-Hernandez, 2013):

1. $ZnO(NO_3)_2 \rightarrow Zn^{2+} + 2NO_3^-$
2. $(C_6H_{12}N_4) + 6H_2O \rightarrow HCHO + 4NH_3$
3. $NH_4OH \leftrightarrow NH_3 + H2O$
4. $Zn^{2+} + 4NH_3 \rightarrow Zn[(NH_3)_4]^{2+}$
5. $2H_2O \leftrightarrow H_3O^+ + OH^-$
6. $Zn^{2+} + 2OH^- \leftrightarrow Zn(OH)_2$
7. $Zn(OH)_2 \rightarrow ZnO + H_2O$

Implosive collapse of bubble from acoustic cavitation during sonication breaks the chemical bonds of precursor molecules Zn(NO3)2.6H2O (1) and HMT (2). Decomposition of HMT (2) produces amine which controls the release of Zn in the growth solution which affects the growth rate of ZnO nanorods. With the help of of NH_3 (3), a product from decomposition of HMT, OH^- (5) forms complex with Zn^{2+} (6) forming intermediate $Zn(OH)_2$ (6) which then dehydrates into ZnO (7). The reaction are in equilibrium and can be controlled by regulating the parameters such as precursor concentration, growth time and growth temperature. The above reaction takes place very slowly at room temperature and would remain unchanged for months. Sonication speeds up the reaction process (Xu and Wang, 2011).

2.3 Biosensor

Biosensor is an analytical device which is used to detect and quantify a certain biological molecules using a recognition element linked to a transducer. Biosensors normally consist of two part which is the biological (enzyme based) and physical part (physicochemical transducer). A good biosensor has to have fast detection capacity, low cost and stable to be able to selectively detect target molecule. Different methods have been adopted in order to develop a new biosensor through the use of novel materials and immobilization strategies. Biosensor is used to monitor in the change of activity of bounded enzyme due to the presence of an analyte. Biosensor so far has been used in areas such as environmental and bioprocess monitoring, clinical diagnosis and food analysis. Glucose sensors are mostly favoured due to its use in clinical and experimental application. As for the detection of glucose, enzymatic and non-enzymatic method has been employed (Saei et al, 2013; Wong and Jameson, 2011). Enzymatic electrochemical sensor compared to a non-enzymatic technique requires short execution time for the experiment, simple experimental setup and cheap chemicals (Alvi et al., 2012).

2.3.1 Enzymatic sensor

Enzymes are referred to as proteins compounds with high selectivity and catalytic activity towards substrates (reactant molecules). The availability of enzymes at high purity makes it desirable to be used for enzymatic sensors. They function to assay concentration of analytes. However there are some limitations when using enzymes as it might lose activity at temperature higher than 60 ^{0}C. Several other factors that might affect the enzyme activity other than temperature are humidity, pH and toxic chemicals. Commonly used enzymes are oxidases that consume dissolved oxygen. Usually enzyme will be immobilized on the transducer surface (insoluble material) (De Corcuera and Cavalieri 2003). Full potential of an enzyme can be harness through immobilization. Immobilization could either be through physical or chemical interaction. Different methods have been adopted for this immobilization to take place such as cross linking (Ali et al., 2010), physical adsorption (Ahmad et al., 2012) and sol-gel method (Malek et al., 2015). Immobilization creates a barrier between the enzyme catalyst and reaction mixture which increases enzyme turnover. This gives higher efficiencies and lower operational cost. Other than that, immobilized enzyme seems to have better operational and thermal stability compared to soluble enzymes. Studies showed that enzyme immobilization also offers a well-balanced performance, a low mass transfer limitation and have a reasonable yield (Chu et al., 2012; Aini et al., 2015). The enzymes will be coupled to an electrochemical transducer. Enzymes are normally covalently linked to a support with the help of functional groups (Bakar et al., 2009). Cross linking is among the approach which is used to effectively improve the activity and stability of an enzyme.

2.3.2 Type of sensor

It is crucial to determine the concentration of glucose in areas such as biology, chemistry, food processing, as well as clinical area for diagnosing diabetics. Research has been carried out to develop the most reliable glucose biosensors. Out of all, the enzyme involved electrochemical glucose biosensor has been studied intensively due to its simplicity, high selectivity and low cost. Glucose sensing also can be applied in experimental and clinical area. It is important to have suitable matrix for the immobilization of enzyme to improve the electron transfer in developing a biosensor (Chu et al., 2012). By far, there have been three generations of glucose biosensor based on GOD. They can be identified using (i) natural oxygen as co-substrate and generation and detection of hydrogen peroxide, (ii) synthetic electron mediator and (iii) direct electron transfer between glucose oxidase and the electrode. However it is not an easy task to convert biological signal to electronic signal. There are advantages of electrochemical biosensors such as their robustness, outstanding detection limits, easy miniaturization, also with small sample volumes and ability to be used in turbid bio-fluids (Wang, 2008).