UNIVERSITI PUTRA MALAYSIA

STRUCTURAL AND OPTICAL PROPERTIES OF ZINC OXIDE NANOWIRES FOR ITS POTENTIAL APPLICATION IN LACTATE OXIDASE DETERMINATION USING SURFACE PLASMON RESONANCE.

NIK NUR SHAFIKAH BINTI NIK MAT

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By

NIK NUR SHAFIKAH BINTI NIK MAT

Thesis Submitted to the Department of Physics, Universiti Putra Malaysia, in partial Fulfilment of the Requirements for the Degree of Science Physics with Honours

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DEDICATION

Special dedication to:

To my beloved parents Nik Mat Bin Nik Mahmood and Rahimah Binti Che Tom;

For their unconditional love and support from born until now.

To my siblings and family;

For making my life complete and meaningful.

To all my wonderful friend;

For making my life full of joy, laugh and happiness.

ABSTRACT

STRUCTURAL AND OPTICAL PROPERTIES OF ZINC OXIDE NANOWIRES FOR ITS POTENTIAL APPLICATION IN LACTATE OXIDASE DETERMINATION USING SURFACE PLASMON RESONANCE

By

NIK NUR SHAFIKAH BINTI NIK MAT

197678

February 2022

Supervisor: Assoc. Prof. Ts. Dr. Suriati Paiman

Faculty: Faculty of Science, Universiti Putra Malaysia

The purpose of the research is to focus on the characterization of ZnO nanowires growth and its potential application in the Surface Plasmon Resonance (SPR) technique. The HMTA concentration was chosen to study the effect of morphology, structural optical, and SPR properties on the growth of ZnO nanowires for improved better optical sensing. The ZnO seed layer was deposited on the Au coat microscope glass using the spin coating process. ZnO nanowires were grown using the Chemical Bath Deposition (CBD) method and Chromium has been introduced during this method. The morphological and structural of the ZnO nanowires doped with Chromium were characterized by using Field Emission Scanning Electron Microscopy (FESEM). Meanwhile for optical properties, SPR technique has been used. DI water and lactic acid been tested on the active layer to obtain the SPR curve.

ABSTRAK

SIFAT-SIFAT STRUKTUR DAN OPTIK DAWAI NANO ZINC OXIDE UNTUK POTENSI APLIKASINYA DALAM PENENTUAN LAKTAT OKSIDASE MENGGUNAKAN RESONAN PLASMON PERMUKAAN

Oleh

NIK NUR SHAFIKAH BINTI NIK MAT

197678

Februari 2022

Penyelia: Assoc. Prof Ts. Dr Suriati Paiman

Fakulti: Fakulti Sains, Universiti Putra Malaysia

Tujuan penyelidikan adalah untuk memberi tumpuan kepada pencirian pertumbuhan wayar nano ZnO dan potensi aplikasinya dalam teknik Surface Plasmon Resonance (SPR). Kepekatan HMTA telah dipilih untuk mengkaji kesan morfologi, optik struktur, dan sifat SPR pada pertumbuhan wayar nano ZnO untuk penderiaan optik yang lebih baik. Lapisan biji ZnO diendapkan pada kaca mikroskop Au coat menggunakan proses salutan putaran. Wayar nano ZnO telah ditanam menggunakan kaedah Chemical Bath Deposition (CBD) dan kromium telah diperkenalkan semasa kaedah ini. Morfologi dan struktur wayar nano ZnO yang didopkan dengan kromium dicirikan dengan menggunakan mikroskop elektron pengimbasan pelepasan medan (FESEM). Manakala bagi sifat optik, teknik SPR telah digunakan. Air DI dan asid laktik telah diuji pada lapisan aktif untuk mendapatkan keluk SPR.

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In the name of Allah, the Most Gracious, the Most Merciful.

Praise be to Allah for His blessing and giving me the chance and endurance to complete my final year project. Next, I would like to express my gratitude to my project supervisor, Assoc. Prof. Ts. Dr Suriati Paiman for her guidance and support. Her dedication and compassion has empowered me to finish this work. Having her as my supervisor is a true blessing as she helped grasp the technicalities of this research.

Aside from that, I would like to take the opportunity to convey my upmost appreciation to the postgraduate student, Mr Shamsu Abu Bakar, who has helped me comprehend and provide continuous assistance throughout this project.

Note to forget, my beloved parents, Nik Mat and Rahimah, whom without their love and prayer this would be a tougher journey. They offered unwavering support and encouragement during the four years of my degree journey. They have cheered me when I was discouraged. Shout out to my sister Humairah, for her help in putting my ideas into word.

I am extremely grateful to my friend especially Shakirah, Iffah, Adlin, Munawwarah and Hanis for their love, caring, prayers for me. Thanks for the constant support and encouragements.

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LIST OF ABBREVIATIONS

ZnO	Zinc Oxide
NWs	Nanowires
Cr	Chromium
CBD	Chemical Bath Deposition
SPR	Surface Plasmon Resonance
FESEM	Field Emission Scanning Electron Microscope
UV-Vis	Ultraviolet-visible spectrophotometry
DI	Deionized
LOD	Lactate Oxidase
DEA	Diethanolamine
HTMA	Hexamethylenetetramine
ZAD	Zinc Acetate Dehydrate
$ZnO(NO_3)_2$	Zinc Nitrate
$(CrH_{18}N_3O_{18})$	Chromium (III) nitrate nonahydrate

CHAPTER 1

INTRODUCTION

1.1 Background study

One-dimensional semiconductor nanostructures such as nanowires, nanofibers and nanotubes have been of considerable interest in both academic and industrial applications because of their capability to serve as building blocks for other structures (Ghazali et al., 2021). Among the one-dimensional nanostructures, zinc oxide nanowires (ZnO NWs) is one of the most significant nanomaterials for nanotechnology in today's research (Ghazali et al., 2021; Wang et al., 2009). ZnO is a direct band gap n-type semiconductor with a band gap energy (*Eg*) of 3.35 eV because of its unique and great optical and physical qualities. At room temperature, ZnO has a huge band gap and a high exciton binding energy of 60 meV, allowing it to be used in high-efficiency photoelectric devices and lasers in the short wave-length range (F. Lu et al., 2019). This material is predicted to be used in electronic or photoelectric devices (S.K. Kim et al., 2014; H. Wang et al., 2015; S. Xu, Z.L et al., 2011; P. Gutruf et al., 2015). ZnO NWs are well-known for their remarkable optical and electrical capabilities.

Doping with appropriate elements is an efficient method for changing the surface state, energy levels, and transport performance of carriers in semiconductors, hence increasing their optoelectronic capabilities (M. Iqbal et al., 2020). Chromium is a typical transition metal element with a special abundant electron shell structure and a close ionic radius (0.063 nm) to Zn^{2} (0.074 nm), implying that Cr^{3} can easily replace into the ZnO lattice (A. Iqbal et al., 2013). Despite being suitable for doping, chemical stability against etching, and the presence of ferromagnetism at ambient temperature, Cr doped ZnO thin films have received little experimental attention (A. Iqbal et al., 2013). Because Chromium possesses multiple electron shells and an ionic radius near to Zn^{2+}, it is an important dopant in the world of transition metal atoms. As a result, Cr^{3+} ions can easily infiltrate the lattice of ZnO crystals or replace Zn^{2+} in ZnO crystals thus, it is reasonable to expect that Cr doped ZnO materials will improve their visible light absorption properties (M. Iqbal et al., 2020).

The SPR technique has become prominent in biosensors for sensing (J. Homola, 2003). Biosensors based on the SPR approach have gotten a lot of attention from researchers in recent years because of their wide variety of applications, such as biomolecule detection, environmental monitoring, biosensing, food safety, and medical diagnostics (A. S. Kushwaha et al., 2018). SPR sensing is based on label-free detection (J. Homola & M. Piliarik, 2006). These sensors are used to change the value of the SPR curve's resonance angle when the refractive index (RI) of the sensing medium changes (J. Homola & M. Piliarik, 2006; J. Homola et al., 1999). In response to molecular interactions, SPR measures mainly changes in the refractive index (RI) close to a thin metal surface. The RI is directly linked to substance concentration near the surface in the medium. Typically, the detection system consists of a single, linearly polarised light source, a metallic film which is in contact with a prism base and a photodetector (J. Homola, 2003). Various studies have indicated that nanocomposite gold and zinc oxides (Au/ZnO) were effective in enhancing the performance of SPR for the detection of molecules (C.C. Chang et al., 2010).

Lactic acid biosensors are in high demand for rapid lactic acid measurement in clinical analysis, biotechnology, food sectors, and sports. Amperometric biosensors commonly use enzymes for lactate determination such as hydrogen peroxide, lactate oxidase (LOx) and flavocytochrome b2 (Leonida M.D et al., 2003; Spohn U., 1996; Staskeviciene S.L et al., 1991). The isoelectric point (IEP) of lactate Oxidase is 4.6 and ZnO is (9.5). For ZnO nanomaterials to be more compatible with the immobilisation of low IEP proteins through strong electrostatic bonds, this significant IEP difference between ZnO and lactate oxidase is obvious (Ali, S.M.U.; Nur, O.; Willander, M.; Danielsson, B., 2009).

1.2 Problem statement

Over recent years, ZnO nanowires have been receiving continuous attention from the scientific community. Significant amount of research and methods had been done for the development of this material matching with the intended application. Thus, the deposition of Au layer is significant as it is the material required for SPR excitation, because there is no effect in the ZnO layer toward the SPR signal. Many research have also reported that the seed layer will improve nucleation for growth due to lowering of the thermodynamic barrier. Then, because it is a low-cost and low-temperature approach, chemical bath deposition will be used for the growth of ZnO nanowires. In order to enhance the surface area for sensing performance especially in the biosensor, ZnO must be immobilize with the enzymes.

In this project, the sputter coating technique is being used due of its simplicity and ease of deposition when compared to other procedures. To grow the ZnO nanowires with the desired properties, the seed layer was deposited using spin coating, followed by the chemical bath deposition (CBD) method. The CBD technique was used to grow ZnO nanowires throughout this experiment. Then the sample will be characterized to examine structural and optical properties for optical sensing parameters. So, to investigate the potential application of sensors, lactic acid and deionized (DI) water have been used toward the sensor using the SPR technique.

1.3 Research Objectives

i. To deposit the ZnO seed layer on gold coated microscope coverslip by sol-gel.

ii. To grow ZnO nanowires doped with chromium on ZnO seed layer using chemical bath deposition method (CBD).

iii. To study the structural and optical properties of the ZnO NWs active layer.

iv. To investigate the ZnO NWs potential application in lactate oxidase determination using Surface Plasmon Resonance (SPR).

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Several studies on the synthesis, characterization, and growth of various ZnO-based nanostructures have recently been published. The preparation of ZnO nanostructured thin films have been utilise by several physical and chemical deposition techniques. Since chemical bath deposition (CBD) is one of the solution phase methods useful for the preparation of compound semiconductors from aqueous solutions, so this chapter will discuss the growth of ZnO nanowires using CBD and its potential application on SPR. The measured parameter under consideration is the ZnO nanowire's for different conditions which are unnealing and un-annealing. Then, the effect of the structural and morphology properties of the ZnO nanowires was explored for the optical parameter and its possible uses in the detection of lactic acid using the surface plasmon resonance technique.

2.2 Zinc Oxide (ZnO)

Zinc oxide (ZnO) stands out among the candidate scintillator materials as an inorganic II-VI semiconductor compound with a large and direct band gap (3.3 eV) and a high exciton binding energy (60 meV). ZnO as an n-type semiconducting metal oxide is well known for its wide and direct band gap (~3.4 eV) and high exciton energy (60 meV) at room temperature (Liu BC et al., 2003). Because of the increased surface area to volume ratio, ZnO nanocrystals can achieve higher sensitivity and efficiency, which is beneficial to light absorption/emission or electron transfer (T. ora et al., 2014). ZnO nanostructures with various morphologies are easily synthesised, making them ideal for sensing application (C. Wang et al., 2010). Hojiri et al. used the Sol-Gel process to generate ZnO nanostructures for gas sensing in 2013. Hydrothermal development of ZnO nanostructure is another method to grow ZnO nanostructured. Because of its simplicity, low cost, and great efficiency, this method is one of the most promising and adaptable techniques for nanostructure creation (Shi Weidong et al., 2013).

2.3 Zinc Oxide Nanowires (ZnO NWs)

For high-quality ZnO NWs, seed layer properties such as nucleation site density, surface roughness, and thickness should be optimised by seed layer annealing with higher density, orientation, and aspect ratio. The seed layer properties required for high-quality growth of ZnO NWs can be highly dependent on the surface condition of a substrate for active nucleation site adsorption (Ana M. Peir´o et al., 2005). 1-D ZnO (nanowires) offers specific sensing advantages over 0-D ZnO as it has a high surface-to-volume ratio and provides a clear and stable path for easy transportation of electrons (Wei et al., 2017).

ZnO nanowires can be used for a wide range of applications in a variety of fields due to their excellent electrical, optical, and mechanical properties (Zhang et al., 2012). Vertically aligned ZnO NWs are grown using a low-temperature hydrothermal method. The quality of the produced nanowires is investigated using SEM (morphology), XRD, and TEM (crystallinity). The presence of a thin-film of (0001)-oriented ZnO compels the ZnO NWs to bend (A.S. Dahiya et al., 2017).

2.4 Chromium (Cr)

Using an uncomplicated and friendly solvothermal approach, Cr doped ZnO nanospheres with good optical properties were obtained. SEM, XRD, and UV–Vis were used to evaluate the structural and optical properties of Cr doped ZnO NSs (M. Iqbal et al., 2020). The band gaps modulation was detected from 3.35 eV to 3.26 eV and indicates the sp–d exchange in ZnO with Cr. Spectrophotometry and ellipsometry were used to compare band gaps. The energy of band gaps derived from transmission data and ellipsometry is very similar. With increasing Cr content, the refractive index and extinction coefficient increase. AFM analysis demonstrates the production of thin films and confirms the rise in surface roughness with increasing Cr content (A. Iqbal et al., 2013).

2.5 Synthesis of Zinc Oxide Nanowires

There are many methods used for synthesis of ZnO nanostructures. To synthesize the different morphologies and sizes of ZnO nanostructures, methods such as hydrothermal, Radio frequency magnetron (RF) sputtering, electrospinning, Chemical vapor deposition, sol-gel, molecular beam epitaxy and metal-organic chemical vapor deposition were used (Bhati et al., 2020). Carbothermal reduction method (Wang et al., 2017), hydrothermal (Zhou et al., 2017), chemical bath deposition (Chen & Ting, 2016) were the methods used for synthesis of ZnO NWs.

Lee, B et al (2021) reported the purity and crystallinity of the synthesized ZnO nanowires were confirmed using X-ray diffraction (XRD) while Ahmad M. et al (2010) reported Pb-doped ZnO nanowires (NWs) have been synthesized by modified thermal evaporation method.

2.6 Seed Layer Deposition

The seed layer is extremely important and plays a significant role in the growth of high quality ZnO NWs in their properties (Boubenia et al., 2017). ZnO seed layer must be used in order to synthesize continuous and well aligned ZnO NWs (Tlemcani et al., 2019). However, there are different effects to the ZnO NWs for the condition of the seed layer for annealing and un-annealing process. To get a larger aspect ratio and higher density of well-oriented ZnO NWs using the hydrothermal growth process, a ZnO seed layer with higher covering density of the active nucleation sites, optimum thickness, and decreased surface roughness is required (K.A. Wahid et al., 2013; T.A.N. Peiris et al., 2013). Furthermore, films may be formed at low temperatures on a variety of substrates, and the thickness of deposited layers can be easily adjusted by varying the chemical composition of the bath and the deposition period (A. Bayer, at al., 2000).

Seeds are used to lower the surface energy at the substrate-ZnO contact, allowing for heterogeneous nucleation on ZnO crystals on the substrate surface and vertical ZnO growth that would otherwise be impossible. To deposit sol-gel seeds on the substrate surface, spin coating was used.

2.7 Chemical Bath Deposition (CBD)

The CBD method has numerous advantages for synthesising ZnO nanowires because it is a simple process that uses low temperatures and can produce large areas (S. Yue et al., 2013). In CBD, Zinc nitrate and hexamethylenetetramine (HMTA) have been used to provide Zn2+ ions and 0 -2 ions to facilitate ZnO formation (Yang et al., 2009). The function of HMTA is to release the OHions gradually by thermal decomposition of formaldehyde and ammonia (Govender et al., 2004). The pH of a solution was found to be critical in determining the morphology of ZnO nanostructures (Govender et al., 2004). The concentration of the precursors affected the growth rate of the nanowires (Sugunan et al., 2006). H. Xu et al (2009) and J.A. Gerbec et al (2005) reported that the CBD process with microwave irradiation is a quick and effective method. There are no thermal gradients and it delivers quick volumetric heating. Microwaves ensure homogeneous heat distribution within the synthesis vessel, which is critical for increasing the synthesised material's quality. Microwave irradiation causes the dipole moment of polar molecules to interact with alternating electronic and magnetic fields, resulting in molecular-level heating and rapid thermal reactions. The hexagonal wurtzite structure dominates the formation of ZnO nanowires grown by chemical bath deposition, with a preferential (002) orientation with a vertical direction to the substrates (Ghazali, M. N. I et al., 2021).

2.8 Structural properties of ZnO Nanowires

2.8.1 Field Emission Scanning Electron Microscope (FESEM)

Field emission scanning electron microscope (FE-SEM) has been used to characterize the morphological properties of ZnO Nws by observing the diameter and length of the sample. The obtained ZnO nanowires can be seen to be vertically oriented for the substrates. S.Y. Liu et al 2009 reported the average diameter and length of the ZnO nanowires increased with increasing deposition time of the seed layer, where the diameter increased from 81 ± 4.08 nm to 205 ± 10.26 nm and the length of the nanowires decreased from 1084 ±56.04 nm to 875 ± 25.64 nm.

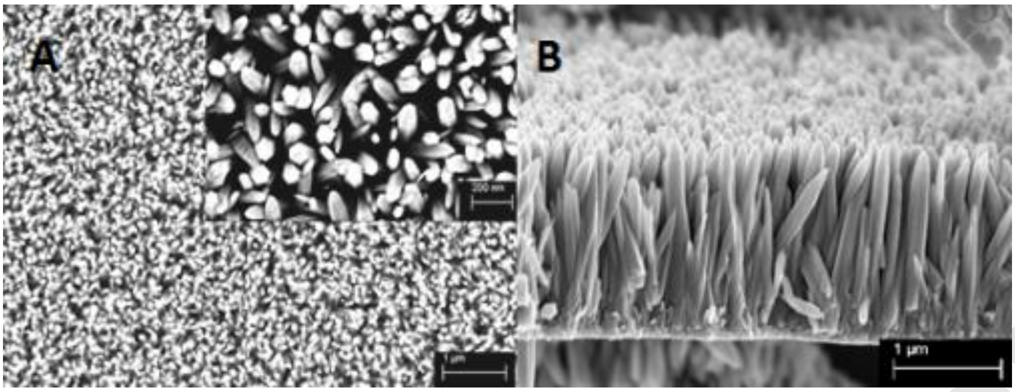

Figure 2.1 FESEM image of ZnO nanowires arrays (Zhou et al., 2017)

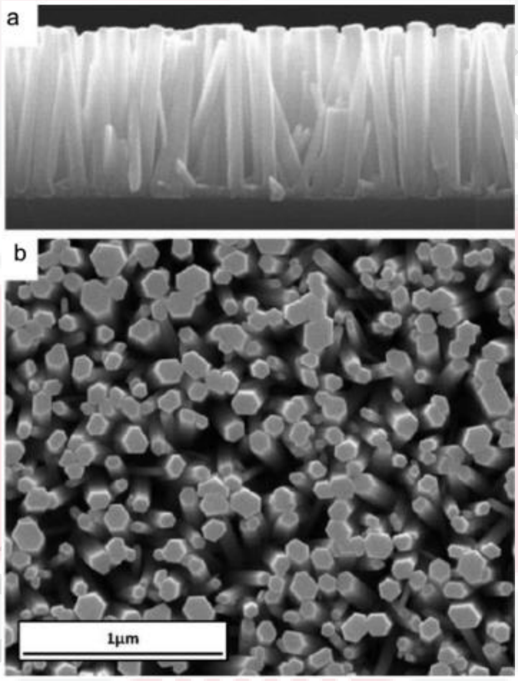

Figure 2.2 The FESEM image of the side view and top view of ZnO nanowires (Apostoluk et al., 2016)

2.9 Optical properties of ZnO Nanowires

2.9.1 UV visible spectra photometer (UV-vis)

Transmission, absorbance, reflection, and bandgap are commonly affected by crystallinity, surface morphology, seed layer thickness, and roughness across the sample region (Farhad et al., 2018). The optical absorption of the sample was determined using UV-vis analysis. According to Singh et al., 2020 analysis as a synthesis of ZnO found out the ZnO absorption peak is positioned at 359 nm and is attributable to excitons recombination at room temperature, as shown in Figure 2.3.

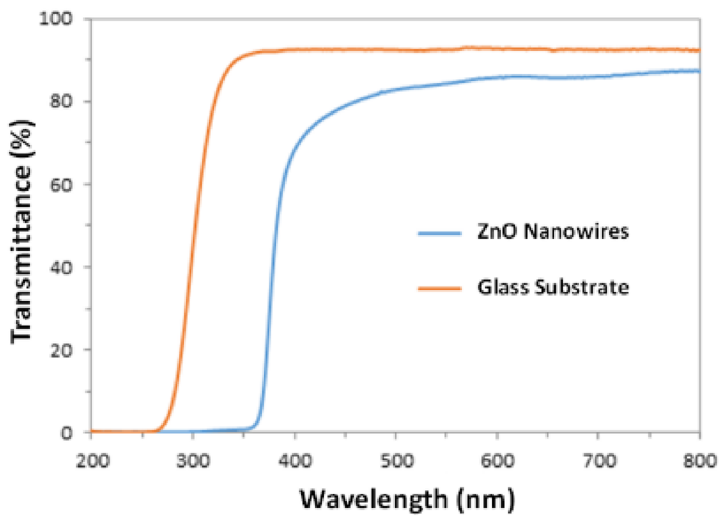

Figure 2.3 The UV-vis spectra of transmittance of ZnO nanowires (Zhou et al., 2017)

2.9.2 Incorporation of ZnO nanowires with SPR sensor

Surface plasmon resonance is a charge-density oscillation that can occur at the interface of two media with opposite-sign dielectric constants, such as metal and dielectric (Khansili et al., 2018). In SPR biosensor, It has proven to be one of the most promising methods for incorporating affinity, specificity, and kinetic factors into macromolecular binding (protein-

protein, enzyme-substrate or inhibitor, protein-DNA, receptor-drug, lipid membrane-protein, and so on (Khansili et al., 2018). Kim et al. 2019 create three-dimensional (3D) nanostructured fibre optic localised surface plasmon resonance (FO-LSPR) sensors using zinc oxide nanowires and gold nanoparticles for highly sensitive plasmonic biosensing.

SPR also can be use as a gas detector. Next, ZnO thin film for the SPR sensor was the first used as a sensing layer for gas detection (Mei & Menon, 2020). Another study used a ZnO sensing film placed on Au coated prisms to measure CO gas using an indigenously built surface plasmon resonance (SPR) sensor at ambient temperature (Paliwal et al., 2017). Fallah et al. 2020 demonstrated a simple method for detecting carbon monoxide gas using Plasmon resonance peak shift in gold-coated ZnO nanorods produced on the SiO2 surface.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter will explain briefly the methods used and the process involved to prepare ZnO nanowires growth on microscope coverslip. Firstly, the seed layer of ZnO is deposited using sol-gel methods. The ZnO nanowires were then grown using chemical bath deposition (CBD). The active layer of the ZnO nanowire was then enhanced with lactate oxidase (LOx) for lactic acid detection. The morphologies of the ZnO NWs active layer were studied using a field emission scanning electron microscope (FESEM). Next, the sample will be evaluated using the SPR technique for potential in the sensing mechanism. This project also investigates the optical properties using a UV visible spectrophotometer (UV-Vis).

3.2 Thin gold layer

3.2.1 Preparation for sample of microscope coverslip

The coverslip 22 x 22 mm from brand Menzel Glaser has been used. Firstly, the finger mark and the dirt on the surface of the glass slips need to be removed using acetone in order to avoid impurities that can affect the whole process.

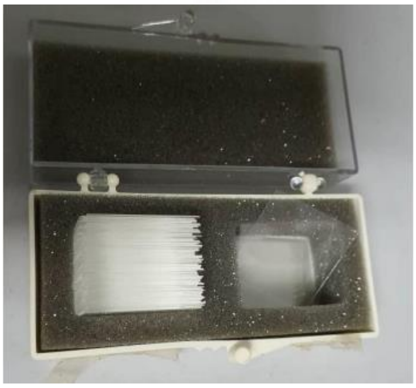

Figure 3.1 Microscope coverslip sample (Noorul, 2021)

3.2.2 Coating Technique

This technique is used once the coverslip has been cleaned. Using the Sputter Coater, which is located at the Institute of Nanoscience and Nanotechnology (ION2), Universiti Putra Malaysia. With a deposition time of 67 seconds, the thin gold layer coated the coverslip. This approach uses a current of 20 mA and a vacuum of $\mathbf{7 \times 10^{-3}}$ mbar.

Figure 3.2 Sputter Coater machine at ION2, UPM

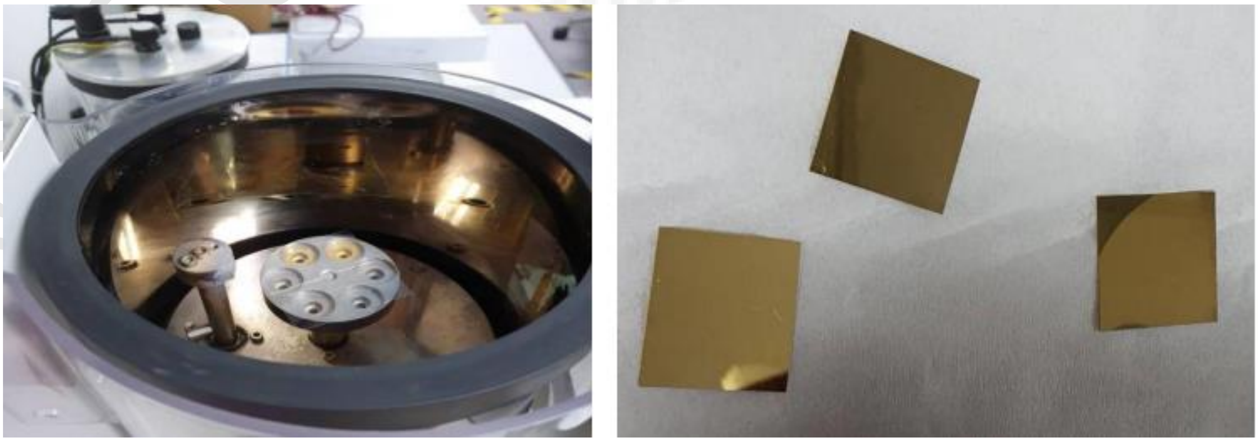

Figure 3.3 Thin Au layer coated coverslip

3.3 Deposition of ZnO seed layer

3.3.1 Preparation of sol-gel solution

The sol-gel procedure was used to make a ZnO seed layer by combining a precursor, a stabilising agent, and a solvent. ZnO seed layers are prepared on the Au SPR substrate using the sol-gel spin coating process in this experiment. Zinc acetate dihydrate ($Zn(CH_3COO)_2.2H_2O, ZAD$), ethanol as the solvent, and diethanolamine ($HN(CH_2CH_2OH), DEA$) as the stabiliser are used to make the sol-gel solution.

The concentration of ZAD used is 0.1 M. According to the formula, for 0.1 M of ZAD where 175.6 mg g of ZAD is needed and then dissolved in 8 mL of ethanol. The magnetic stirrer with a diameter of 1 cm is inserted into the mixing after the ZAD and ethanol have been thoroughly mixed. On the hot plate, the solution is then stirred with a magnetic stirrer. The rotation occurs at a speed of about 300 rpm. Once the rotation began and the solutions began to mix, approximately 154 L of diethanolamine (DEA) was added drop by drop into the solution until the solution became clear and colourless. While increasing the temperature to 56 °C-58 °C, the solution is rotated until the ZAD is completely dissolved.

When the solution reaches the optimal temperature, it is left for 45 minutes at the same temperature and rotation. The vial was then wrapped in aluminium foil and stored in a dry place to prevent any reaction from occurring. The solutions were kept in a dry place for 18-24 hours.

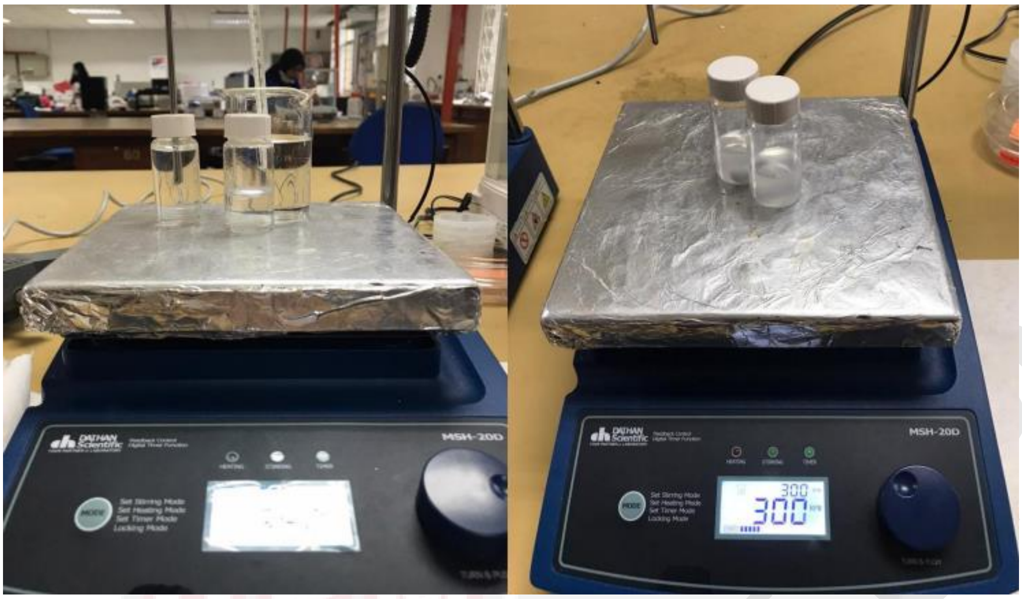

Figure 3.4 Sol-gel technique

3.3.2 Spin coating technique

After ageing the solution for 18-24 hours, this method takes place. The Au SPR substrate is attached to the glass slide so that it remains in place while rotating. The sol-gel solution containing 0.1 M of ZAD is then pipetted onto the Au SPR substrate until it covers all surfaces of the substrate with an approximate volume of 30 L. The substrate is rotated at 3000 rpm for 30 seconds by the spin coater machine (SPIN-3000 Series) at applied optic laboratory in Faculty of Science UPM. After the rotating process is completed, the sample is left to dry on a hot plate at 100 °C for 10 minutes. This procedure was repeated three times to obtain three layers of the ZnO seed layer.

While carrying the process, the vacuum must be clean and the spin coater is in good condition. If the vacuum is clogged with dirt or liquid impurities, the spin coater may not function properly.

Figure 3.5 Spin coating machine

Figure 3.6 Hot plate

3.3.3 Annealing

Right after the three layers of zinc oxide seed layers obtained, two samples were annealed at 150ºC using furnace for one hour.

Figure 3.7 Furnace

3.4 Chemical Bath Deposition

3.4.1 Preparation of Chemical Bath Solution

Chemical bath deposition (CBD) was commonly used to synthesize nanostructures such as nanorods due to controllability and low costs. The chemical bath solution is made by measuring the zinc nitrate ($ZnNO_3$) and hexamethylenetetramine (HMTA)($C_6H_{12}N_4$) in a 1:1 ratio and put into the separate beakers. To begin, combine the 0.03 M zinc nitrate ($ZnNO_3$) solution and HMTA with 50 mL of DI water in each beaker and stir until it is dissolved. After that, the magnetic stirrer with 1 cm is inserted into each of the beakers. The solution is then stirred together with a magnetic stirrer on the hot plate for 30 minutes.

Figure 3.8 Preparation of the chemical bath solution

3.4.2 Preparation of ZnO Doping with Cr

When the solutions from the chemical bath deposition is being stirred, the 0.02 M Chromium (III) nitrate nonahydrate ($CrH_{18}N_3O_{18}$) has been introduced into the beaker. Then, it is left for 30 minutes for it to dissolved.

Figure 3.9 Solutions of ZnO doped with Cr

3.4.3 Growth of ZnO nanowires

The substrate is then vertically plunged into the beaker with the chemical bath solution using the sample holder following the preparation of the chemical bath solution. In order to avoid contamination and evaporation of the solution the beaker containing the chemical bath solution and the substratum needs to be covered with aluminium foil. Put the samples in the oven at 90°C for 1 h 30 min. After the growth, the sample for a whole drying process was kept in an open atmosphere for one day. It is stored in a desiccator to absorb humidity before the enzyme is immobilised. Samples should be rinsed on the surface and dried into the air using DI water.

Figure 3.10 Oven for growth

3.6 Characterization

3.6.1 Determination of structural properties using FESEM

The image is created by scanning the sample surface with a high-energy electron beam in a FESEM electron microscope. The electrons accelerated in the gradient of the high electric field are emitted by the field emission source. The electron beam is subsequently focused on the samples after passing via the electromagnetic lens. Based on the image obtained, the diameter and length of the ZnO nanowires may be measured using ImageJ software. The Nova Nanosem 230 type FESEM machine is in use at the Universiti of Putra Malaysia's Institute of Nanoscience and Nanotechnology.

Figure 3.11 FESEM machine

3.6.2 Determination of phase properties using XRD

The orientation and crystalline structure of the ZnO nanowires was determined by X-ray diffraction (XRD) at the UPM Faculty of Science. The brand used is Philips brand (PW 3040/60 MPD X'Pert High 25 Pro PAN-Analytical) located at the Physics Department. The type of X-ray radiation used was wavelength Cu Kα (λ = 1,54056 A°) working at 100 mA and 40 keV. XRD can be used to determine crystalline materials' orientation, phase identification, crystallographic structure, and grain size. For detecting crystalline phase development on a sample, the XRD spectrum test might be equal to the International 30 Center for Diffraction Data (ICDD) standard.

3.6.3 Determination of optical properties using UV-Vis

UV-Visible Spectroscopy is based on the absorption of ultraviolet or visible light by chemical molecules, then resulting in the production of unique spectra. When matter absorbs light, it experiences excitation and de-excitation, which results in the formation of a spectrum. It is important to remember that the difference in energy between the electron's ground and excited states is always equal to the quantity of ultraviolet or visible radiation received by it. Ultraviolet and visible absorption spectroscopy is a type of spectroscopy that involves calculating the attenuation of a light beam after it passes through or reflects from a sample surface. The UV-Vis spectrometer measured wavelengths ranging from 190 nm to 1100 nm. However, in this project, the range of wavelength that will be use is 220 nm to 800 nm for UV-Vis machine, type Lambda 35, which located at the Institute of Nanoscience and Nanotechnology (ION2), Universiti Putra Malaysia.

Figure 3.12 UV-visible spectrometer

3.6.4 Detection of lactic acid using SPR method

The deionized water on the ZnO nanowires active layer is tested using a surface plasmon resonance optical sensor. The SPR is calculated by measuring the reflected laser beam He-Ne as a function of the incident angle. The light sources in the device include a p-polarized He-Ne laser with a wavelength of 632.8 nm, a prism table, and a photodiode. When a monochromatic, p-polarized light beam strikes a metal surface, usually gold, this phase occurs. The incidence angle shapes and, eventually, the frequency of the incident light matches the frequency of the plasmon surface wave as light matches the resonance conditions. The incidence angle shapes and, eventually, the frequency of the incident light matches the frequency of the plasmon surface wave as light matches the resonance conditions. Light energy would be partially transferred from the metallic surface to the electron packets as a result. As a result, when reflected light is detected, it will show a drop in intensity. The optical configuration shown in Figure 3.13.

To begin testing the sample with DI water to determine the SPR curve for that active layer, 2 ml of DI water volume was injected into the hollow that was attached to the prism. The prism was briefly bonded to the sample. To prevent contamination, the prism is washed with acetone immediately after each operation. The LOD/ZnO nanowires active layer will next be tested with different concentrations of lactic acid ranging from 0 mM to 10 mM to determine the sensitivity of the sensing medium toward lactic acid concentration.

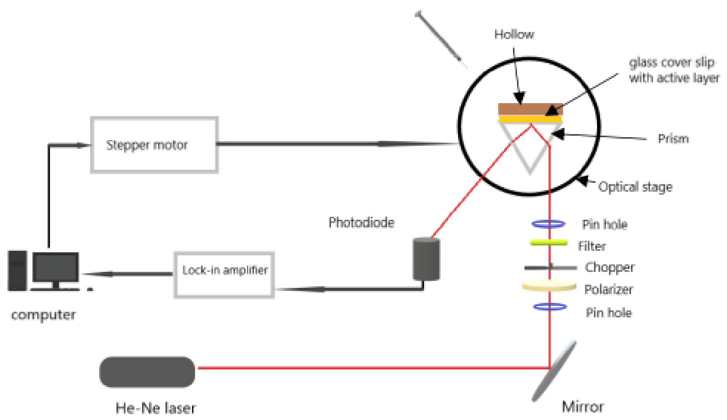

Figure 3.13 Schematic diagram of the experimental setup of SPR

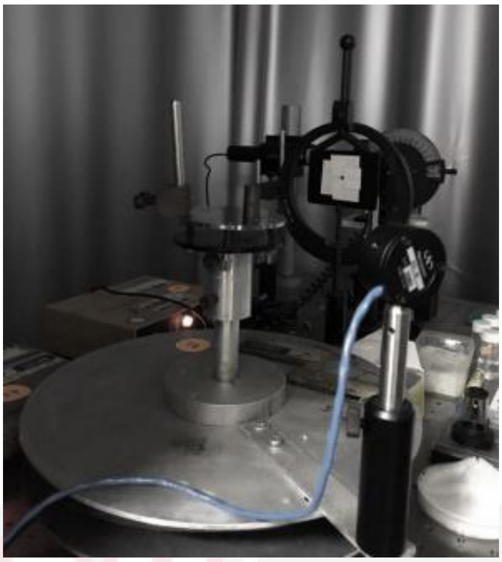

Figure 3.14 Experimental setup of SPR located at Applied Optics Laboratory, Physics Department, Faculty of Science

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

In this chapter, the characterization results of the Cr-doped ZnO NWs were analyzed and discussed. The structural properties of ZnO nanowires are studied from the results of FESEM. The optical and sensing properties of ZnO nanowires are studied from the results of UV-visible spectroscopy and surface plasmon resonance respectively. The SPR properties are studied from the result of detection using deionized water and lactic acid in SPR graph.

4.2 ZnO nanowires morphology

Figure 4.1 and 4.2 shows the FESEM image of Cr-doped ZnO NWs formed on the Au film surface with a different condition which are annealed and un-annealed. As its diameter, length and density could be monitored for future study, the functions of determining the morphology and structural properties of ZnO NWs by FESEM are crucial. From the observation in figures below, the dark region should be attributed to the Au and ZnO thin film surface while the white structure was attributed to the ZnO nanowires. However, the growth of Cr-doped ZnO NWs cannot be seen from the image of FESEM below.

(a) (b)

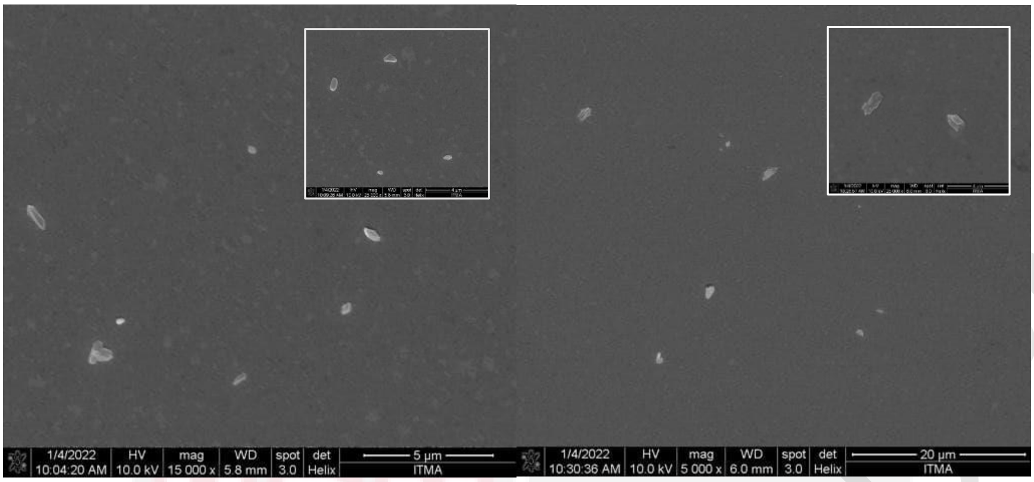

Figure 4.1 FESEM images of the top-viewed of ZnO Nanowires grown for sample

(a) un-annealed and (b) annealed

The FESEM imaging technique is used to visualise the morphology and aggregation of the produced nanoparticles. It can be seen from the photos that the pure Zinc Oxide nanoparticles have spherical morphology. It has also been discovered that as the chromium dopant concentration increases, there is greater agglomeration, resulting in an increase in crystallite size and a change in morphology from spherical to a spindle-like shape (S. Janet Priscilla et al., 2020). FESEM images of Cr-doped ZnO NWs should be like image below.

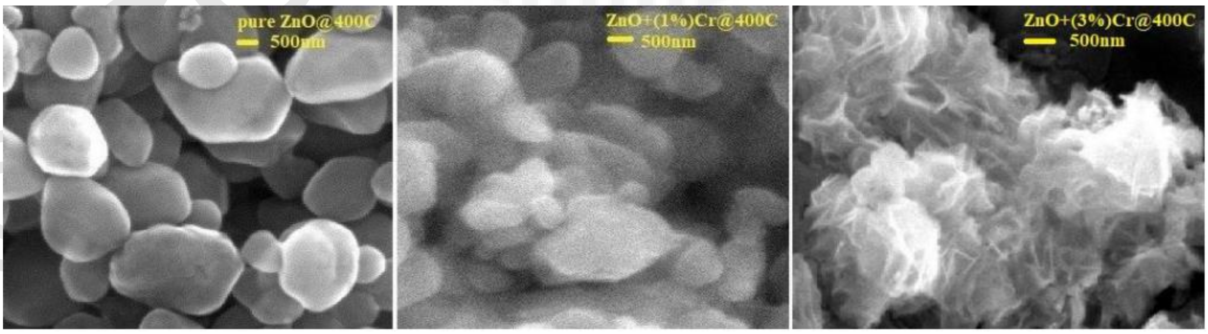

Figure 4.2 FESEM image of Cr-doped ZnO NWs (Janet Priscilla et al., 2020)

4.3 Determination of optical properties from UV-Vis

Figure 4.3 show the absorbance peak of ZnO doped Cr with different conditions which are annealed and un-annealed. The optical properties of the ZnO NWs doped Cr for this work was obtained from UV-vis analysis. To examine the UV-vis spectrum of the synthesized ZnO NWs doped Cr and the wavelength range of 200 - 800 nm using a double-beam UV-VIS NIR spectrophotometer, ethanolic dispersion was prepared and measurements were made. The absorption peak of of both annealed and un-annealed was located at 282 nm and is at room temperature due to exciton recombination.

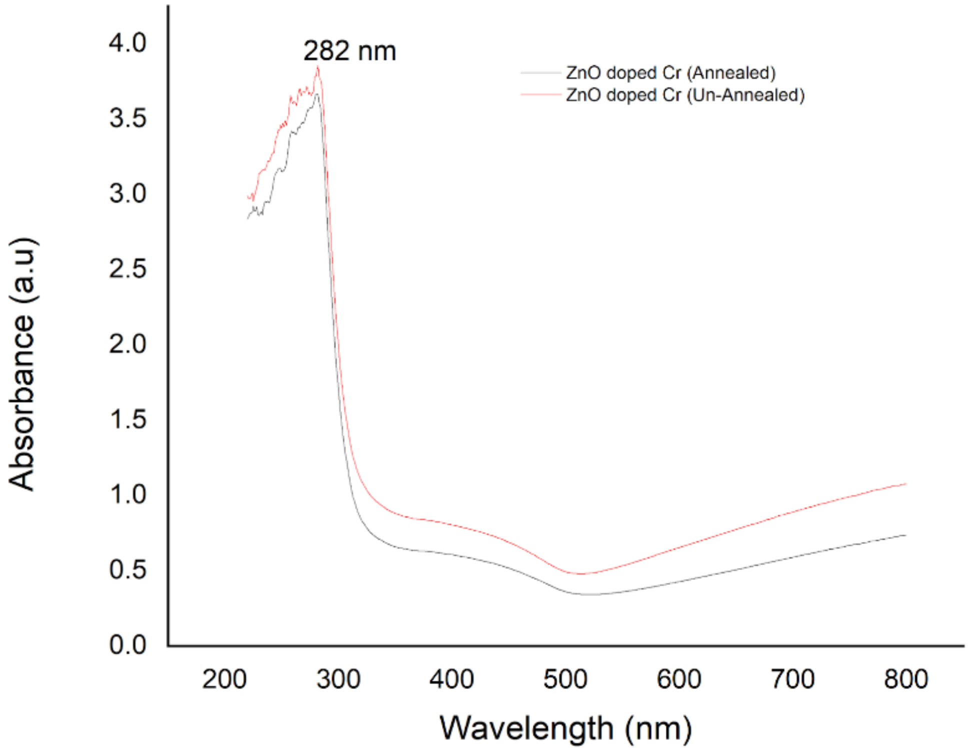

Figure 4.3 UV-Visible absorption spectra of annealed and unannealed seed layer of the Cr-doped ZnO nanowires.

4.4 Determination of optical properties from SPR

For SPR studies, ZnO nanowires were grown on Au coated microscope glass. SPR measurements were carried out at λ = 632.8 nm in angular interrogation mode for prism/Au/ZnO system having ZnO nanowires grown under different conditions of seed layer which are annealed and un-annealed seed layer. The measurement of the SPR were carried out at 60° angle and 1.77 refractive index of the prism. The Kretschmann configuration has been utilized to measure the variation of reflectance with the change in incident angle.

SPR test is carried out with deionized water in contact with a gold/ZnO layer. The deionized water is injected into the cell in contact with the gold/ZnO layer film. The SPR reflectivity curve for the gold/ZnO layer film in contact with deionized water for annealed and unannealed seed layer of the Cr-doped ZnO nanowires is shown in Figure 4.4. From the figure, the resonance angle for unannealed seed layer is 54.95° to 55.23° while for annealed seed layer is from 55.51 to 55.79°. The SPR resonance angle (θ_{SPR}) is found to shift from 55.23° to 55.79° when the seed layer of Cr-doped ZnO nanowires has been annealed as shown in Figure 4.5. This occured due to the annealing of the ZnO seed layer that enhances the growth of ZnO nanowires and also enhance the sensing properties of ZnO nanowires by increasing the grain size and its crystallinity. The changes of resonance angle between the annealed and unannealed Cr-doped ZnO nanowires proved that the ZnO nanowires active layer film has the affinity towards lactic acid molecule and has the potential to be use with the SPR to detect the lactic acid.

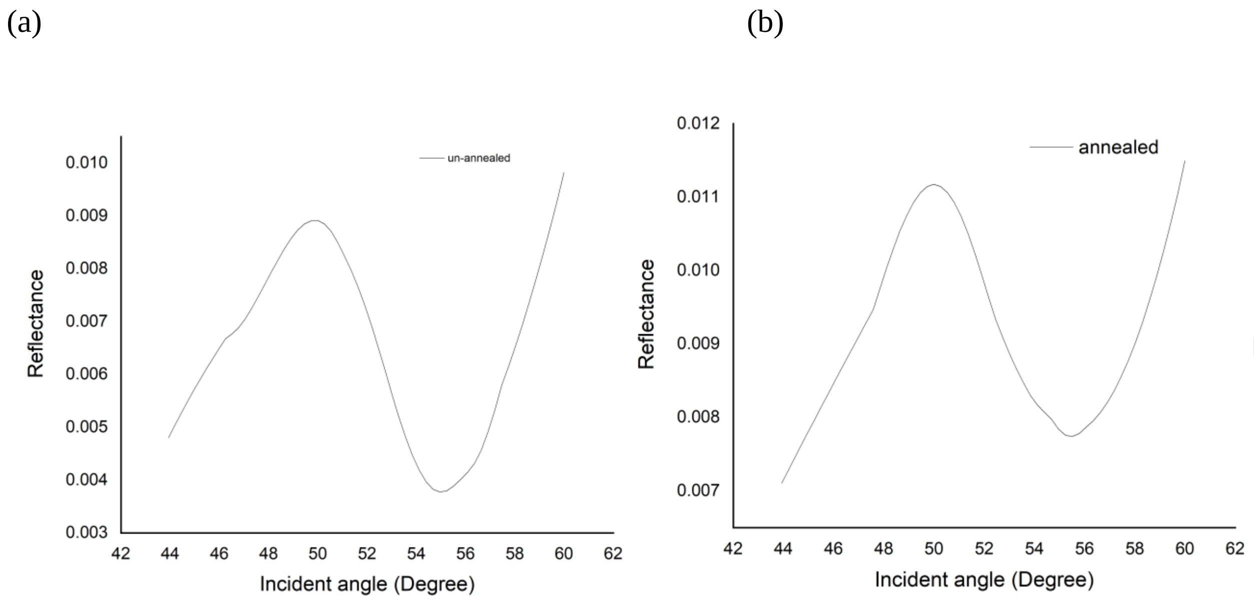

Figure 4.4 SPR curve of Cr-doped ZNO nanowires tested with deionized water for (a) un-annealed and (b) annealed

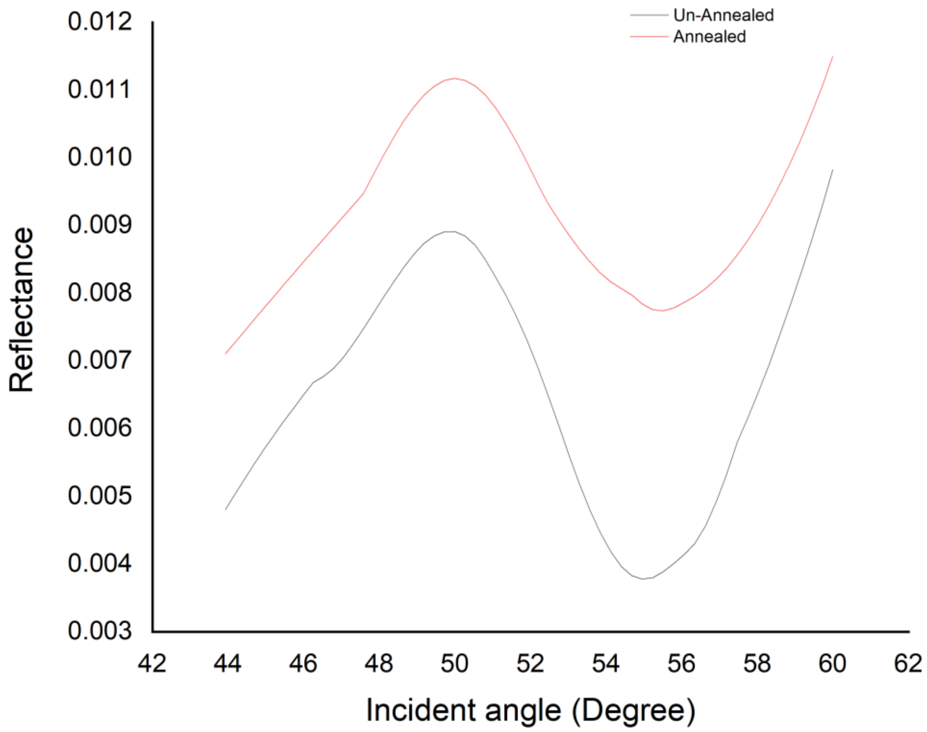

Figure 4.5 The comparison of SPR curve of the unannealed and annealed of Cr-doped ZnO nanowires tested with deionized water.

Next, The SPR experiment was conducted with a pure 88 % concentration of lactic acid in an aqueous solution that was introduced into the cell attached to the composite layer. Figures 4.6 and 4.7 show the SPR reflectivity curves for the pure concentration of lactic acid in contact with the gold/ZnO layer for the annealed seed layer and unannealed seed layer, respectively. The resonance angle determined from the SPR curve for annealed seed layer of the Cr-doped ZnO nanowires is 57.19° while the unannealed seed layer of the ZnO nanowires is 57.75°. The shift of resonance angle is from 57.75° to 56.35° when the seed layer of Cr-doped ZnO nanowires has been annealed as shown in Figure 4.8. The resonance angle shifted to the left as a result of the surface roughness of the Cr-doped ZnO nanowires, with annealed Cr-doped ZnO nanowires having a smoother surface than unannealed Cr-doped ZnO nanowires. This condition allows the annealed Cr-doped ZnO nanowires to improve its sensing properties to detect lactic acid (Shewale et al., 2019).

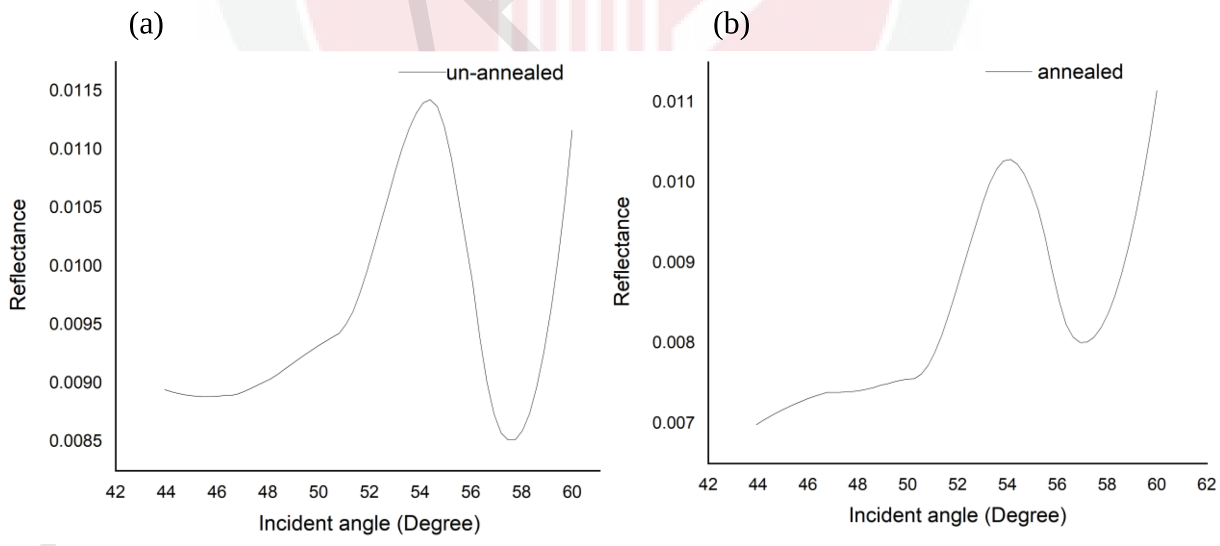

Figure 4.6 SPR curve of Cr-doped ZnO nanowires tested with pure lactic acid for (a) un-annealed and (b) annealed

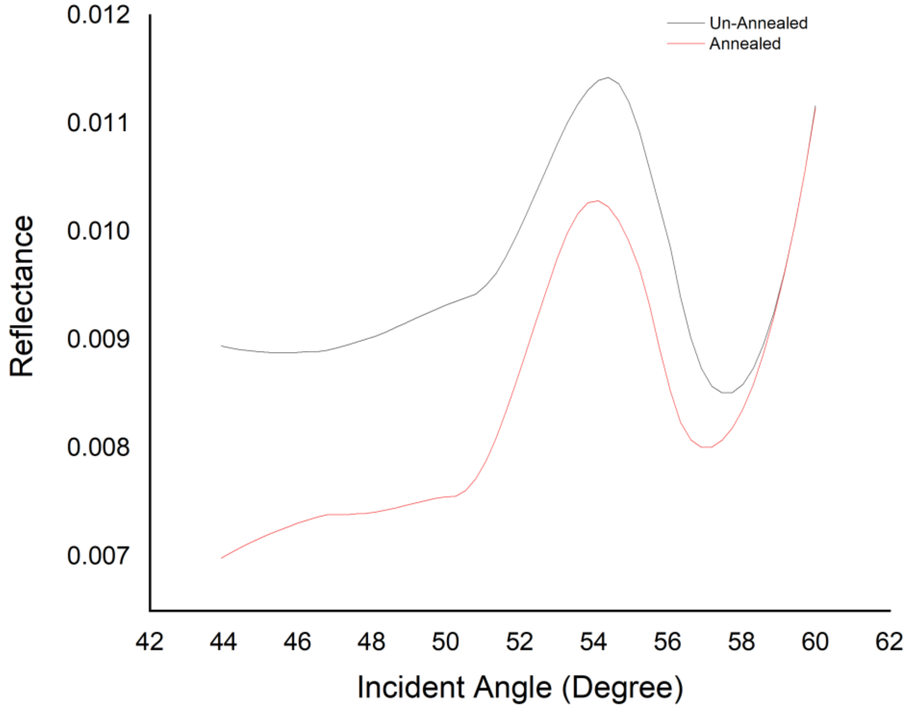

Figure 4.7 The comparison of SPR curve of the unannealed and annealed of Cr-doped ZnO nanowires tested with pure lactic acid.

Since we have limitation of time and material, we cannot proceed to the last step which is immobilize the active layer of ZnO nanowires with Lox and also tested the SPR properties with different concentration of lactic acid. However, Mei et al., (2020) stated that when the active layer of ZnO nanowires consists of LOx, which is the enzyme for the lactate determination, the SPR curve will show a better shift of resonance angle as the enzyme will provide a clear route to pyruvic acid for the oxidation of L-lactic acid and also due to the selection of an effective matrix for the immobilization. Thus, the immobilized biomolecules are stable and maintain their biological functionality is the key consideration for improving the sensitivity and efficiency.

CHAPTER 5

CONCLUSION

5.1 Introduction

This chapter concludes the research discovery and contribution in the field of ZnO nanowires in the SPR technique for detection.

5.2 Conclusion

In this project, Au is coated by using sputter coater and ZnO seed layer is deposited by using sol gel spin coating method by 3 layers before synthesis process. ZnO nanowires are synthesized on the Au microscope glass which is very thin by using the chemical bath deposition with a different conditions which are annealed seed layer and unannealed seed layer of ZnO nanowires. The chromium has been introduced during chemical bath deposition method. The seed layer is annealed at temperature of 150°C for 60 minutes. Next, the ZnO nanowires are synthesized on the gold-deposited microscope coverslip by using CBD technique.

For FESEM, the growth of ZnO NWs cannot be seen from the FESEM image. Next for the UV-Visible spectrophotometer data showed that the absorption peak of the annealed and unannealed ZnO NWs is range from 280 nm to 282 nm.

Lastly, the sensing properties of ZnO nanowires active layer toward deionized water and lactic acid are tested using SPR technique. A sharp curve of SPR reflectance is detected when the ZnO nanowires has been deposited on the gold/prism system and a shift to the right of the incident angle is observe for both annealed and unannealed ZnO nanowires.

In conclusion, this project showed that the variation of the the condition of Cr-doped ZnO seed layer would improve the optical and sensing properties of ZnO NWs and proved that ZnO nanowires showed a significant SPR curve when it acts as an active layer for the lactic acid biosensor.

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