



UNIVERSITI PUTRA MALAYSIA

***OPTICAL PROPERTIES OF ZNO NANOWIRES AND ITS SENSING
POTENTIAL FOR LACTATE OXIDASE DETERMINATION USING
SURFACE PLASMON RESONANCE SPECTROSCOPY***

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RESONANCE SPECTROSCOPY**

By

IFFAH NABILAH BT ISMAYUDIN

**Thesis Submitted to the Department of Physics, Faculty of Science, Universiti Putra
Malaysia, in partial Fulfillment of the Requirements for the Bachelor of Science in
Physics with Honours**

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DEDICATION

Special dedication to:

To my beloved parents Ismayudin bin Salimin and Siti Zaleha binti Bakri

For their unconditional love and support.

To my siblings and family:

For their existence that make my whole life contented.

To all my amazing friends:

Thank you for being such a wonderful friend.

ABSTRACT

OPTICAL PROPERTIES OF ZnO NANOWIRES AND ITS SENSING POTENTIAL FOR LACTATE OXIDASE DETERMINATION USING SURFACE PLASMON RESONANCE SPECTROSCOPY

By

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FEBRUARY 2022

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Faculty: Faculty of Science, Universiti Putra Malaysia

Zinc oxide nanowires (ZnO NWs) has become one of the important metal oxide nanostructures that is widely use nowadays due to their high sensitivity characteristic and it is a low-cost material. Therefore, the purpose of this project is to focus on the characterization of ZnO nanowires growth and its sensing properties in the surface plasmon resonance (SPR) technique. The condition of seed layer of the ZnO nanowires is chosen to study the effect on the structural optical and sensing properties for better optical sensing. The optical and sensing properties of these ZnO thin films have been investigated by UV-Visible spectroscopy (UV-Vis) and surface plasmon resonance (SPR) technique. The UV-Vis study indicate that both annealed and unannealed ZnO nanowires have absorption peak at the range of 278 nm to 280 nm and thus, proved the absorption of the ZnO bandgap. From the Tauc plot, the band gap energy of both annealed and unannealed ZnO nanowires were determined to be 4.03 eV. The SPR results showed that the right shift of incident angle from 53.56° to 54.39° for the samples that are tested with the deionized water when the sample is annealed. However, a left shift of SPR

incident angle from 57.47° to 56.35° is observed for the lactic acid detection when the sample is annealed. This project proved that the ZnO nanowires can act as an active layer for the SPR biosensor.



ABSTRAK

SIFAT OPTIKAL ZnO NANOWAYAR DAN POTENSI PENDERIANNYA DALAM PENENTUAN LAKTAT OKSIDASE DENGAN MENGGUNAKAN SPEKTROSKOPI PERMUKAAN PLASMON RESONANS

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Zink oksida nanowayar (ZnO Nws) telah menjadi salah satu nanostruktur oksida logam terpenting yang digunakan secara meluas pada masa kini kerana ciri kepekaannya yang tinggi dan kos bahannya yang rendah. Oleh itu, tujuan projek ini adalah untuk memberi tumpuan terhadap ciri pertumbuhan ZnO nanowayar dan sifat penderiaannya dalam teknik permukaan plasmon resonans (SPR). Keadaan lapisan benih ZnO nanowayar dipilih untuk mengkaji kesannya terhadap struktur optikal dan sifat penderiaannya untuk menghasilkan deria optikal yang lebih baik. Sifat optikal dan penderiaan bagi lapisan filem nipis ZnO ini telah disiasat dengan menggunakan spektroskopi ultralembayung (UV-Vis) dan teknik permukaan plasmon resonans (SPR). Kajian UV-Vis menunjukkan bahawa kedua-dua ZnO nanowayar yang telah disepuhlandap dan tidak disepuhlandap mempunyai puncak penyerapan pada julat 278 nm hingga 280 nm dan dengan itu membuktikan bahawa terdapat penyerapan pada celah jalur ZnO. Daripada plot Tauc, tenaga jurang jalur bagi kedua-dua ZnO nanowayar yang telah disepuhlandap dan tidak disepuhlandap ditentukan pada 4.03 eV. Keputusan SPR menunjukkan

terdapat anjakan sudut tuju ke kanan daripada 53.56° kepada 54.39° bagi sampel yang diuji dengan air ternyahion apabila sampel disepuhlindapkan. Namun, anjakan ke kiri sudut tuju SPR daripada 57.47° kepada 56.35° dapat diperhatikan untuk pengesanan asid laktik apabila sampel disepuhlindapkan. Projek ini membuktikan bahawa ZnO nanowayar boleh bertindak sebagai lapisan aktif untuk SPR biosensor.



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ZnO nanowires tested with both deionized water and pure lactic acid



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LIST OF ABBREVIATIONS

ZnO	Zinc Oxide
NWs	Nanowires
IEP	Isoelectric Point
CBD	Chemical Bath Deposition
SPR	Surface Plasmon Resonance
FESEM	Field Emission Scanning Electron Microscope
UV-Vis	Ultraviolet-Visible spectrophotometry
LOD	Lactate Oxidase
DI	Deionized Water
DEA	Diethanolamine
HTMA	Hexamethylenetetramine
ZAD	Zinc Acetate Dehydrate
ZnO(NO ₃) ₂	Zinc Nitrate
PBS	Phosphate Buffer Solution
NaCl	Sodium Chloride
LDH	Lactate Dehydrogenase
NAD ⁺	Nicotinamide Dinucleotide
IGZO	Indium Gallium Zinc Oxide

CHAPTER 1

INTRODUCTION

1.1. Background

Lactic acid detection is very importance as it is widely used in the cosmetic, food, chemical and many other industries (Borshchevskaya et al., 2016). Therefore, it is essentials to develop an easy and inexpensive method to detect the lactic acid. Commonly, enzyme lactate oxidase (LOD) is used due to its simple catalytic process and low isoelectric point (IEP) which is 4.6. Studies have shown that enzyme lactate oxidase (LOD) helps to show an excellent sensitivity in the solution which is in the range of 150 nM to 1.1 mM and can be further use to improve the detection or management of disease (Shimomura et al., 2012). There are many biosensing instrument available nowadays, however, the surface plasmon resonance (SPR) makes observing binding behavior on a molecular level a lot easier and more accurate.

SPR is an optical technique for detecting and monitor the interaction of two different molecules in which one is mobile analyte solution and one is immobile biospecific partner on a thin gold film (Drescher et al., 2009; Homola et al., 2008). A thin gold film is used to reduce the absorption of photons and gives more accurate result. In order to get a strong signal from SPR, gold is commonly used. SPR is widely used due to its distinct advantages such as label free detection, small size samples, real time monitoring, reusable sensor chips, and the ability to handle complex samples (Erickson, 2008). Sensing layer of SPR also plays an important part in order to achieve an excellent result from the SPR and to know the sensing potential of the nanostructures. The sensitivity of the nanostructures can be defined as the ratio between the shift of resonance angle and measured target concentration (Hashim et al.,

2020a). The commonly used sensing layer of SPR are the metal oxide nanostructures that are known for its distinct characteristics.

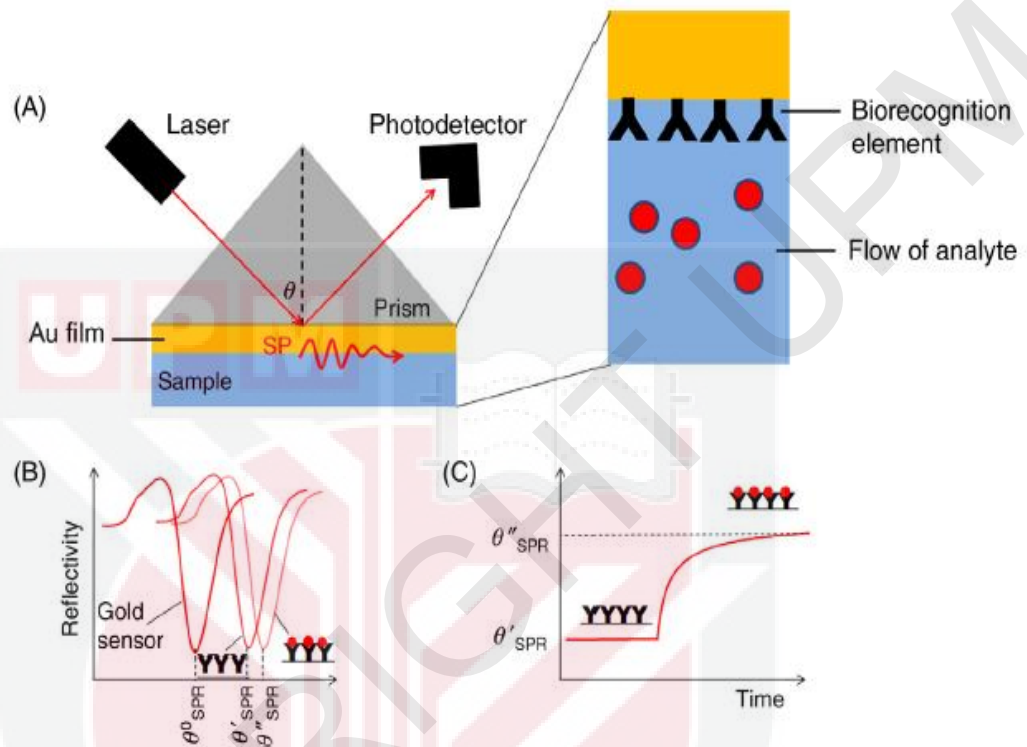


Figure 1.1 (A) The schematic diagram of the SPR setup with the graph of reflectivity that show the before and after the antibody immobilization of the sensor surface as shown in (B). As in (C), the sensorgram show the real time interaction between antibody and antigen on the sensor surface (Miyazaki et al., 2017).

Metal oxide nanostructures are widely known as the material for sensor ranging from gas to humidity sensors, including ultraviolet sensors and biosensors as these materials have a low produce cost and simple preparation methods. The common example of metal oxide nanostructures that is used in sensing application are zinc oxide, tin oxide, titanium dioxide, copper oxide, tungsten trioxide, and vanadium oxide

due to their high sensitivity, high selectivity, adjustable reliability and low cost. (Nunes et al., 2019; Viter et al., 2019; Wang et al., 2019). However, these materials do not gain much attention due to their characteristic such as limited binding sites and low utilization of visible light (Korotcenkov & Cho, 2012; Sun et al., 2018). Compared to other metal oxide nanostructures, zinc oxide (ZnO) has many distinct characteristics that make it famous among the researchers and scientists.

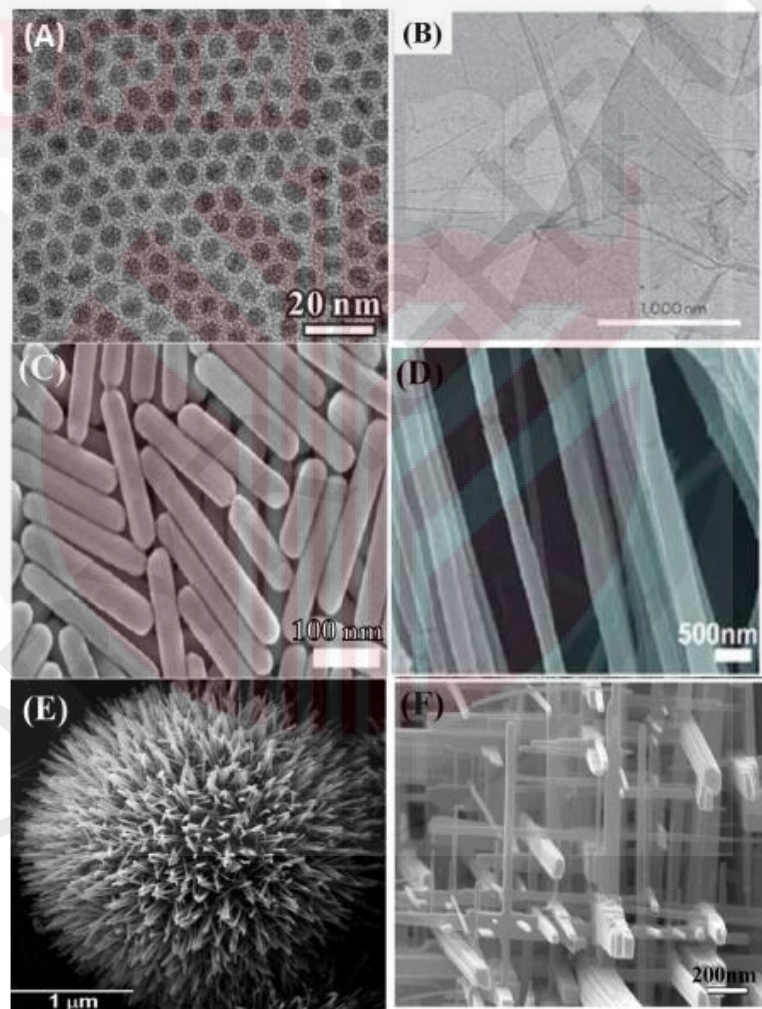


Figure 1.2 The nanomaterials that exist with different morphologies which are nonporous Pd nanoparticles (A), Graphene nanosheets (B), Ag nanorods (C), polyethylene oxide nanofibers (D), ZnO nanowires (E), and WO₃ nanowire network (F) (Jeevanandam et al., 2018).

Nowadays, the nanoscale materials such as zinc oxide is the most significant semiconductor due to its unique chemical properties and structure (Kołodziejczak-Radzimska & Jesionowski, 2014). Due to wide band gap which is 3.37 eV, high excitation energy at 60 meV, high electron mobility and high thermal conductivity make ZnO suitable to be used in sensing application (Janotti & van de Walle, 2009; Kołodziejczak-Radzimska & Jesionowski, 2014; Yang et al., 2002). In biosensor application, zinc oxide nanowires have attracted a lot of interest due to its distinct advantages such as large specific surface area, nonlethal, biosafety, good biocompatibility, high combination with immobilized enzymes, high isoelectric point which is 9.5 and high electron-transfer rates. All of these advantages are very useful in providing efficient electron transfer channels, absorbing more enzyme, and a good medium for electrostatic interactions with high binding stability to immobilize low isoelectric point acidic proteins or DNA (Yang et al., 2002; Y. Zhang et al., 2012). Therefore, there are various of research about ZnO nanowires as sensor to detect glucose (Ahmad et al., 2017), nitrogen dioxide (NO₂) gas (Ahn et al., 2008) and lactic acid (Borshchevskaya et al., 2016; Shimomura et al., 2012; Y. Zhao et al., 2014) and the annealing of seed layer of the ZnO nanowires play a very important role for the well-aligned nanowires growth.

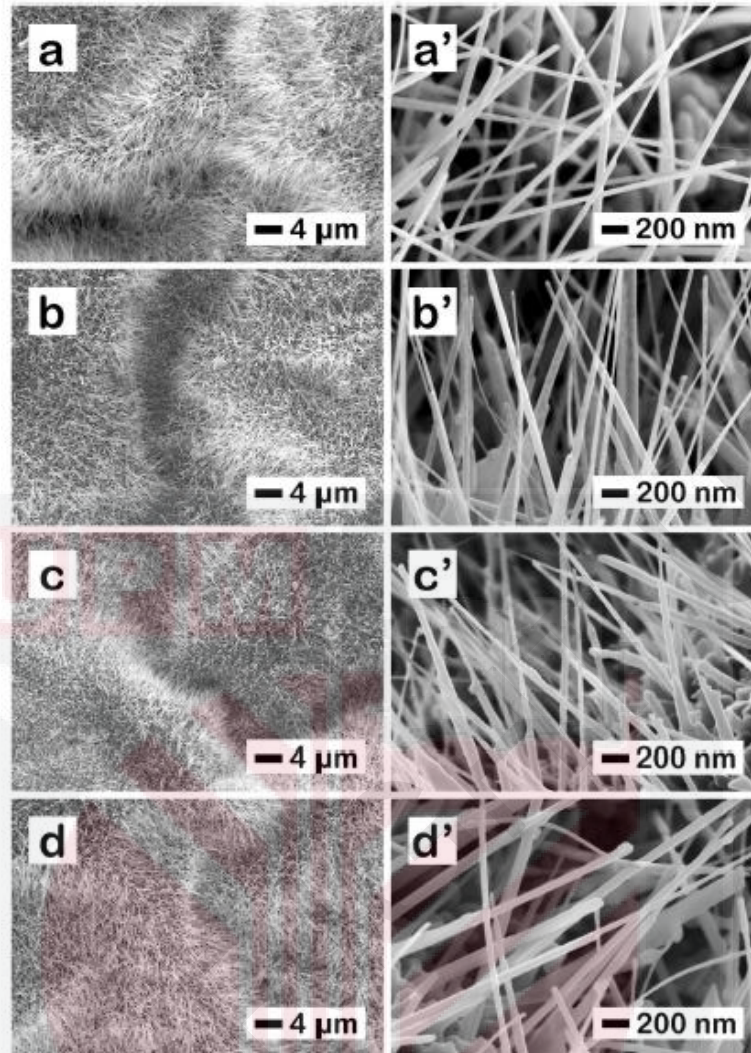


Figure 1.3 The morphologies of ZnO nanowires at different magnification and deposition time of $\text{Cu}_x\text{O} : \text{ZnO}$ nanowires (a, a'), ZnO- Cu_xO at 6 min (b, b'), ZnO- Cu_xO at 18 min (c, c') and ZnO- Cu_xO at 30 min (d, d') (Florica et al., 2019).

In order to obtain a preferable optical and structural properties of the ZnO nanowires, annealing process is very important as it can improved the adhesion on the gold thin film and also play a part in the grown of the nanowires (Kim et al., 2013). The annealing of the seed layer has the potential to significantly affect its morphology and thus improve the quality of the grown ZnO nanowires. The benefit of the seed layer annealing process toward the ZnO nanowires properties including its

morphology, size, shape and growth orientation that will affect the structural, optical and sensing properties of the ZnO nanowires (Gu et al., 2015; Paul et al., 2021). Park et al. (2016) reported that the parameters such as thickness, density of the nucleation sites and surface roughness are highly affected by the temperature of seed layer annealing (Park et al., 2016). Therefore, the annealing process is a very important step to improve the growth of ZnO nanowires that will be used for the lactic acid detection.

In this project, the ZnO nanowires are grown on the gold thin film in order to act as a sensing medium for the SPR. The UV spectra have been obtained to study the optical properties of the ZnO thin films. Next, the focus is on the application of ZnO nanowires as a sensor to detect lactic acid through the SPR.

1.2. Problem statement

Nowadays, surface plasmon resonance (SPR) has been widely known as one of the versatile optical sensors for lactic acid detection. One of the essential parts in optical sensor is the active layer thin film and each thin film was fabricated to have its own sensitivity and selectivity towards specific metal ion. The annealing of the seed layer is the important parameter that allow ZnO nanowires to be a very good active layer thin film for the optical sensor. To further improve the reliability and versatility of this sensor, some novel material is needed to be incorporated in the development of the SPR sensor (Shamim Anower et al., 2021). The development of SPR as an optical sensor for lactic acid sensing have been receiving growing attention from the scientific community including the detection of phenol and glucose (Hashim et al., 2020b; Rosddi et al., 2020). However, to the best of our knowledge, there is only a few reports on the detection of lactic acid via SPR technique. Lactate oxidase enzyme (LOD) is a trace of

element that is widely found in the lactic acid. Although it has a biological need as a substrate to be oxidized to carbon dioxide (CO₂) and water and involve in the glucogenesis process, the high level of it causes bad effects such as lactic acidosis disease and muscle cramp (Lacramioara Pavel et al., 2017; Stades et al., 2004). Different concentration of LOD allows the optical sensor to determine the limit of concentration of lactic acid that it can detect. Therefore, it is of interest to develop different condition of seed layer for the active layer with different concentration of LOD that will be used for the detection of lactic acid via SPR technique.

1.3. Hypothesis

Several hypotheses were made for this research. ZnO is immobilize with the enzyme lactase oxidase (LOD) because LOD has low isoelectric point (4.6) while ZnO has high isoelectric point (9.5) which is a good combination to achieve a high sensitivity to detect the lactic acid. To obtain the SPR signal, the gold layer deposition is very important and the sputter coater technologies is used. Thus, an excellent sample of ZnO nanowires with gold thin film can be produce in order to use it as a sensor in the lactic acid detection. The optical and structural properties of ZnO nanowires can be achieve by properly controlling the deposition time and the deposition technique.

1.4. Research question

- i. What is the influence of active layer thickness on the SPR sensitivity?

1.5. Objectives

- i. To deposit the seed layer of ZnO nanowires on gold-coated microscope coverslip by sol-gel technique.
- ii. To grow ZnO nanowires on gold-deposited glass cover slip using chemical bath deposition method (CBD).
- iii. To characterize the sensing properties of the ZnO nanowires active layer with lactase oxidase using surface plasmon resonance (SPR).



CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

In the recent years, ZnO nanostructures have gained many attentions from the researchers due to its distinct characteristics such as wide band gap and high excitation energy. Various studies have reported its growth, characterization, optical properties and the potential as a sensor. This chapter discusses the effect of active layer thickness toward the sensitivity of the sensor and the potential applications of ZnO in the lactic acid detection using SPR.

2.2. Zinc oxide nanowires

Nanostructures are the material or structures that have one dimension and the size are smaller than 100 nm (Minagar et al., 2017). One-dimensional structures have a wide variety of nanostructures, such as nanorods, nanoparticles, nanowires, nanotubes, nanoribbons, nanopores, and nanobelts. (Nasrollahzadeh et al., 2019). Over the past few years, nanostructures have a very important role in the advancement of nanoscale technologies due to their unique physical, electrical, and chemical properties. Metal oxide is one of the examples of composition in the nanostructures that have frequently used and gained attention from the researchers nowadays (Nasrollahzadeh et al., 2019).

The commonly used metal oxide nanostructures among the researchers nowadays are the zinc oxide nanowires (ZnO NWs). One-dimensional structure of zinc oxide nanowires makes it a useful material in the application of piezoelectric devices, optical sensor, gas detector and solar cells (Ghazali et al., 2021; Shahi, 2017). Owing to its unique properties such as wide band gap and high excitation energy, zinc oxide

nanowires are the most important nanostructures for nanotechnology in research nowadays.

2.3. Effect of annealing process on ZnO seed layer.

At present, various studies show that the quality of the ZnO nanowires' growth is very dependent to the condition of the seed layer. One of the important parameters that affect the seed layer properties is the annealing of the seed layer. The significant of seed layer annealing include improve the crystallinity and enhance the adhesion of thin film on the substrate that will affect the growth of the nanostructures (Shewale et al., 2019). Muchuweni et al. (2018) reported that the ZnO nanowires that have annealed seed layer have better electrical properties than the unannealed ZnO nanowires' seed layer. In the previous studies, the annealing of seed layer affect the growth and surface roughness of the of the seed layer. The grain size of the ZnO nanorods increase significantly with the increase of the annealing temperature and thus, the influence the growth of ZnO nanorods (Kamruzzaman & Zapien, 2018). Lastly, the band gap energy of the materials also influenced by the annealing of the seed layer where the optical band gap decreases with the increasing of the annealing temperature given to the seed layer (Nandani et al., 2018).

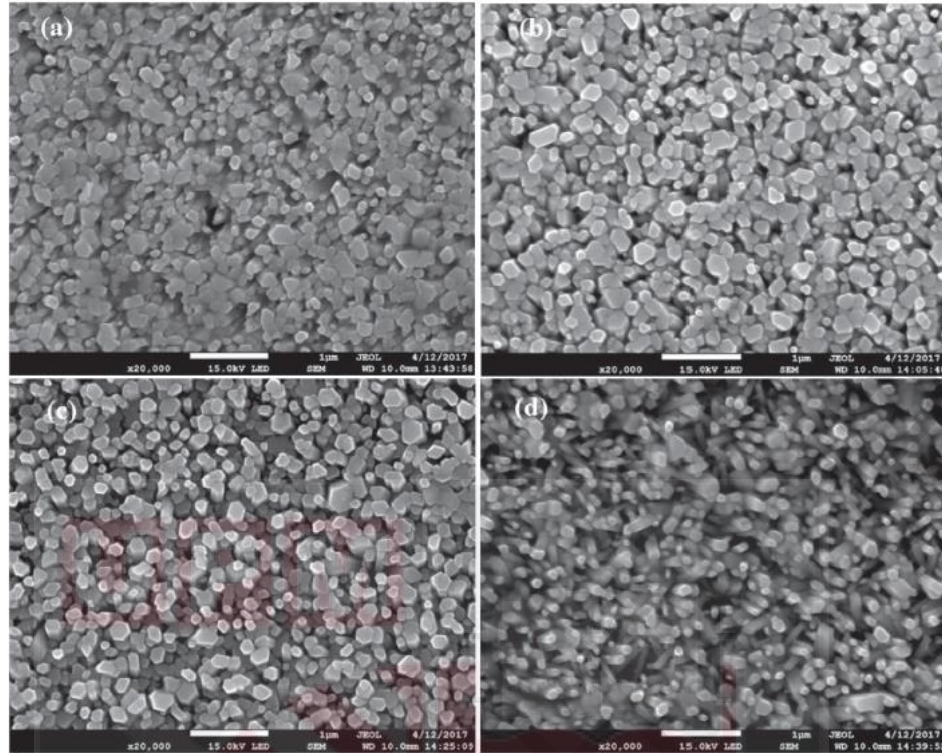


Figure 2.1 FESEM images of ZnO nanowires (a) unannealed seed layer (b) annealed seed layer at 150°C (c) annealed seed layer at 250°C and (d) annealed seed layer at 350°C.

2.4. Detection method using ZnO nanostructures

ZnO have been widely used in the application of gas sensing (S. Zhao et al., 2020), piezoelectric devices (Ghazali et al., 2021) electrochemical and electromechanical nano-devices (Barzinjy et al., 2020). ZnO nanostructures play an important role in the sensing application due to their good physical and chemical properties. Bhati et al. (2018) successfully doped nickel (Ni) precursor into ZnO nanostructures by radio frequency (RF) sputtering technique to enhance the hydrogen sensor for lower concentration hydrogen detection at the lower temperature range. Previous studies also mention that the surface area and structural defects of the metal oxide semiconductors

also influenced the gas sensing properties. Habib et al. (2019) also managed to dope chromium (Cr) precursor into ZnO films through the sol-gel method and successfully proved that the sensitivity of ZnO films increases with the presence of Cr doping. Therefore, this research proved that ZnO films can be used as sensor to detect carbon monoxide even at low temperature. Then, other research development that enhance the NO₂ sensing also using ZnO nanowires that operated with the ultra-fine In₂O₃ nanoparticles (S. Zhao et al., 2020). From the previous studies that been studied, it proved that ZnO nanostructures really help in enhancing the gas sensing response.

TYPE OF SENSOR	SENSING MATERIAL	REFERENCES
Hydrogen sensor	Ni/ZnO nanostructures	(Bhati et al., 2018)
Carbon monoxide sensor	Cr/Zno films	(Habib et al., 2019)
NO₂ sensor	In ₂ O ₃ /ZnO nanowires.	(S. Zhao et al., 2020)

Table 2.1 The comparison between different type of sensors that use ZnO nanostructures.

2.5. Detection of lactic acid

In the last decade, lactate detection plays an important role in the healthcare, food industries, cosmetic, chemical and many other industries (Borshchevskaya et al., 2016). The lactate biosensor was developed in the combination of lactate dehydrogenase (LDH) and nicotinamide adenine dinucleotide (NAD^+) that act as a linker on the indium gallium zinc oxide (IGZO) to measure the lactic acid (Chou et al., 2017) They also claimed that the lactate biosensor has simple fabrication process, high stability and low cost in production. A rapid fluorometric method is defined using ZnO nanowires (ZnO NWs) for the determination of lactate and cholesterol and the identification of the hydrogen peroxide produced by lactate during the enzymatic reactions by using the electrostatic interactions between the enzymes and the ZnO NWs, two bioconjugates were prepared by combining the nanomaterial and the lactate oxidase enzymes (LOD) (Briones et al., 2020) Alam et al. (2019) reported that the combination of ZnO nanoflakes (ZnO NFs) and lactate oxidase as the enzyme for lactic acid detection present a highly sensitive electrochemical sensors to be used in the detection of lactic acid in different sample of human sweat.

TECHNIQUE	SENSING LAYER	IMMOBILIZED MATERIAL	FINDING	REFERENCES
Arrayed flexible lactate biosensor	Indium gallium zinc oxide (IGZO)	Nicotinamide Adenine Dinucleotide (NAD ⁺)	Concentration limit of detection: 3 mM	(Chou et al., 2017)
	Zinc oxide nanowires (ZnO NWs)	Lactate oxidase (LOD)	Limit of detection: 0.54 μM	
	Zinc oxide nanoflakes (ZnO NFs)	Lactate oxidase (LOD)	Concentration limit of detection: 10 pM	

Table 2.2 Comparison between the technique used to detect the lactic acid using ZnO nanostructures as sensing layer.

CHAPTER 3

METHODOLOGY

3.1. Introduction

In this chapter, the methods and process involved in the preparation of ZnO nanowires growth are explained. Firstly, the method used to deposit the ZnO seed layer is sol-gel technique and the ZnO nanowires are growth through the chemical bath deposition method. In order to ZnO nanowires able to detect the lactic acid, the lactate oxidase (LOD) enzyme is prepared. Next, the ZnO nanowires will be tested through the SPR method to study the sensing properties.

3.2. Preparation of gold thin layer

Firstly, the glass slips need to be clean using the acetone to remove any finger mark and dirt on the surface of the glass slips. Next, after the glass slips are cleaned, the glass slips are coated with gold using the Sputter Coater for 67 seconds to produce a gold thin film with thickness of 50 nm in order to obtain an optimum dip in SPR curve and using the current of 20mA.



Figure 3.1 Sputter coater machine at Institute of Nanoscience and Nanotechnology (ION2), UPM.



Figure 3.2 Thin Au layer coated coverslip.

3.3. Deposition of ZnO seed layer

3.3.1. Preparation of sol-gel solution.

The sol-gel method is used to prepare the ZnO seed layer. The sol-gel solution is prepared by zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$, ZAD) as a precursor, ethanol as solvent, and Diethanolamine ($\text{HN}(\text{CH}_2\text{CH}_2\text{OH})_2$, DEA) as a stabilizer. 175.6 mg and 0.1 M of ZAD is needed to dissolve in 8 mL of ethanol. After the ZAD and the ethanol are mixed, the magnetic stirrer is inserted into the mixing and the magnetic stirrer stirred the solution on the hot plate. The rotation takes place at about 300 rpm. Once the rotation started, drops of diethanolamine (DEA) approximately 154 μL are added drop by drop into the solution until the solution turns into a clear and colorless solution. The solution is rotating until the ZAD is dissolved completely while increasing the temperature until 56°C to 58°C. Once it reaches the optimum temperature, the solution is left at 45 minutes at the same temperature and rotation. Next, the vial needed to be wrapped in aluminum foil and kept in a dry place to avoid any reaction takes place. The solutions were kept within 18 to 24 hours in a dry place.



Figure 3.3 Sol-gel technique

3.3.2. Spin-coating technique.

Next, the sol-gel solution with 0.1 M of ZAD is dropped onto the Au SPR substrate using a pipette until it covered all surfaces of the substrate with an approximation of 30 μ L. The substrate is rotated using the spin coater machine at 3000 rpm in 30 seconds. Once the rotating finished, the sample is left to dry on the hot plate at 100°C in 10 minutes. This step was repeated three-time, to get three layers of the ZnO seed layer.



Figure 3.4 Spin coater machine.

3.3.3. Annealing of ZnO seed layer

After the deposition of the seed layer, the seed layer of the ZnO are annealed at 150°C for 60 minutes. The annealing process took place inside the furnace that located at the nano SEM Lab, Department of Physics, Universiti Putra Malaysia.

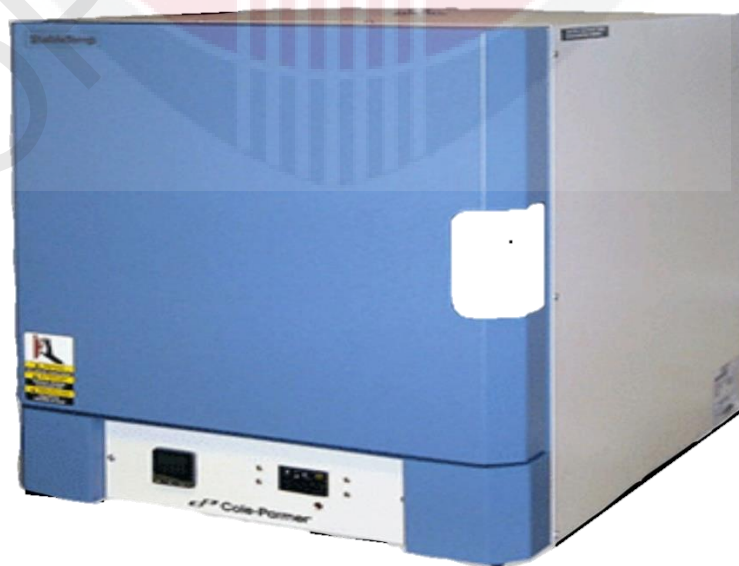


Figure 3.5 Furnace for annealing of the Zno seed layer

3.4. Chemical bath deposition technique

3.4.1. Preparation of chemical bath deposition solution

In order to synthesize the ZnO nanowires, the chemical bath deposition (CBD) is used. The chemical bath solution prepares with a ratio of 1:1 of zinc nitrate (ZnNO_3) with hexamethylenetetramine (HMTA)($\text{C}_6\text{H}_{12}\text{N}_4$). Mix the 0.03 M zinc nitrate (ZnNO_3) solution with a few drops of deionized water and stirred the solution until zinc nitrate dissolved. Then deionized water was added again until 50 mL and continue to dilute 0.015 M HMTA solution with few drops of DI water also and stirred until the solution and the HMTA dissolved. The 100 mL of mixed solution is being transferred into the beaker.



Figure 3.6 The chemical bath deposition (CBD) solution.

3.4.2. Growth of ZnO nanowires

When the chemical bath solution is ready, the substrate is immersed vertically into the beaker that contains the chemical bath solution using the sample holder. The substrate deposited in two deposition period which are one and two hours to get two different thickness of thin film. The beaker containing the chemical bath solution and substrate needs to be cover with aluminum foil to avoid contamination and evaporation of the

solution. The samples then place in the oven for 1h 30 min at 90 °C. After the growth, the samples must be rinse using deionized water to remove any excess salt on the surface and dried in the air. Then the samples were kept in an open atmosphere for one day for the complete drying process. The substrates were kept in a desiccator to absorb the moisture before immobilizing with the enzyme.



Figure 3.7 Oven for the growth of ZnO nanowires.

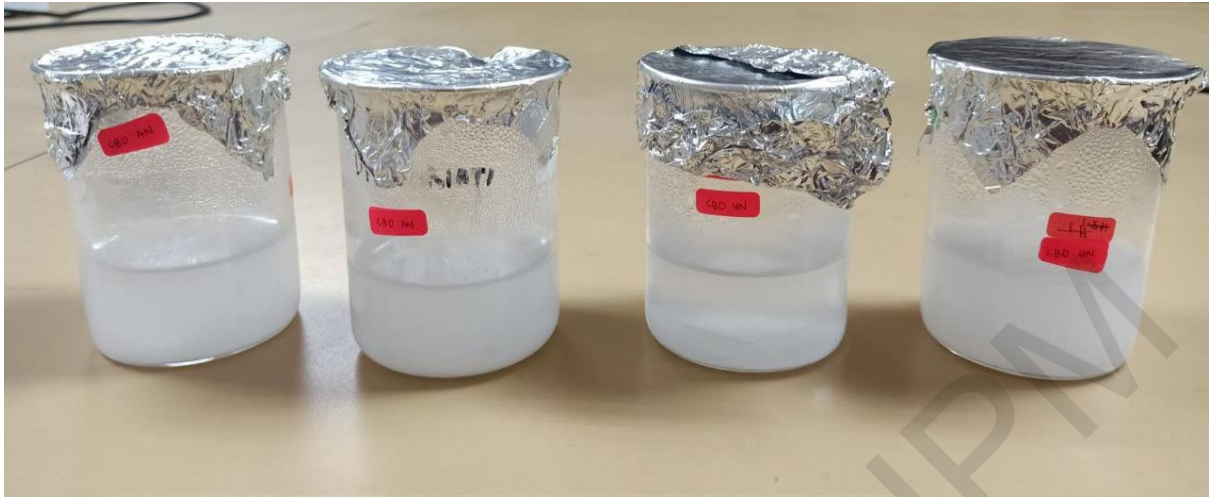


Figure 3.8 The chemical bath solution after the growth of ZnO nanowires.

3.5. Preparation of lactate oxidase (LOD) solution

For the lactate oxidase solution, 1.3 mg of LOD dissolved in 250 μL of a 0.1 M phosphate buffer solution (PBS) with a pH value of 7.4 containing 0.9 % of NaCl. Then the solution must be stored at 4°C. To introduce the enzyme on the ZnO sensing layer, take 3 μL of LOD solution. Then drop it on ZnO nanowires layer growth over the gold SPR substrate. Next, the sample will store at room temperature for 3 hours to attach LOD /ZnO nanowires.

3.6. Determination of optical properties of ZnO nanowires using Uv-Vis.

An atom inside a material can react to the photon or incoming ray from a light source. The atom can either absorb or scatter the photon. The UV-Visible spectrophotometry is a technique based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra. The matter will undergo excitation and de-excitation when it absorbs the light. This action would be resulting in the production of a spectrum. The crucial point is that the difference in the energies

of the ground state and the excited state of the electron is always equal to the amount of ultraviolet radiation or visible radiation absorbed by it. Ultraviolet and visible absorption spectroscopy is a type of spectroscopy that involved the calculation of a light beam's attenuation after it passes through a sample or reflects from a sample surface. The range of wavelength that UV-Visible can measured is from 190 nm to 1100 nm. However, in this project, the range of wavelength that will be used is from 220 to 800 nm. The UV-vis machine of model Lambda 35 is being used where it is located at the Institute of Advanced Technology, Universiti Putra Malaysia.

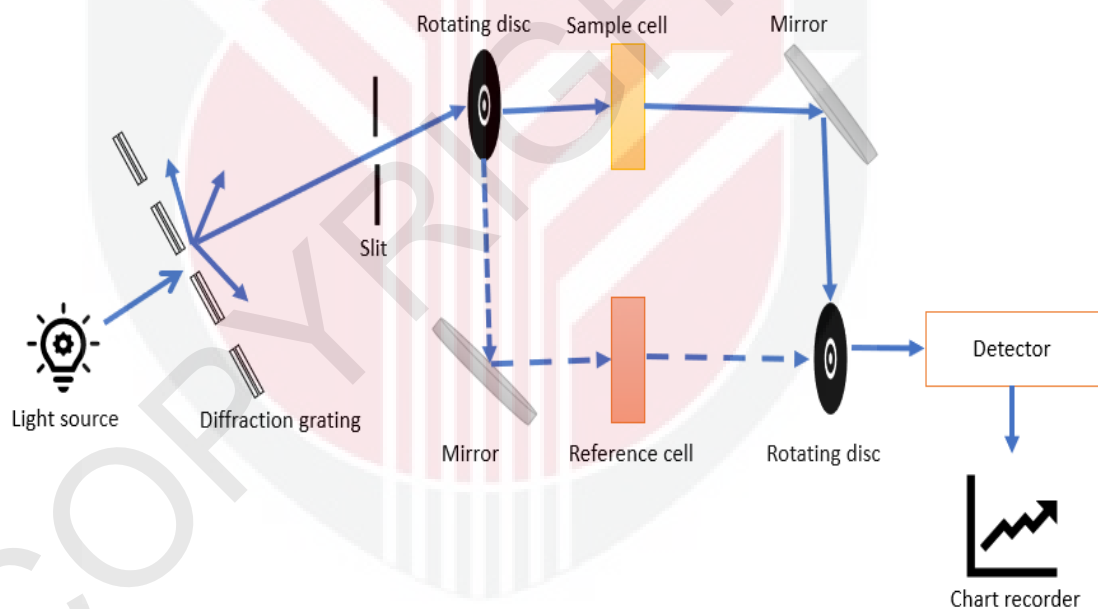


Figure 3.9 Schematic diagram of UV-Visible Spectrophotometer (Bordoloi, 2018).



Figure 3.10 UV-Visible spectrophotometer at the Institute of Advanced Technology, University Putra Malaysia.

3.7. Detection of lactic acid using SPR method

Surface plasmon resonance is a phenomenon that occurs when a polarized light strikes the surface of metal at a particular angle. This technique excites the electron and it detects the oscillations of free electrons that are known as surface plasmons. The schematic diagram of the setup of the SPR technique that is used in this project is shown in Figure 3.11 and 3.12 respectively.

Firstly, the sample being tested with deionized water (DI) water to determine the SPR curve for that active layer, 2 ml of DI water volume was injected into the hollow, which was attached to the prism. The sample was temporarily glued to the prism. To avoid contamination, the prism is cleaned with acetone before using it immediately after each step. Next, for the lactic acid detection, the LOD /ZnO nanowires active layer will be tested with different concentrations of lactic acid in the range of 0 mM to 10 mM to find the sensitivity of the sensing medium toward lactic acid concentration.

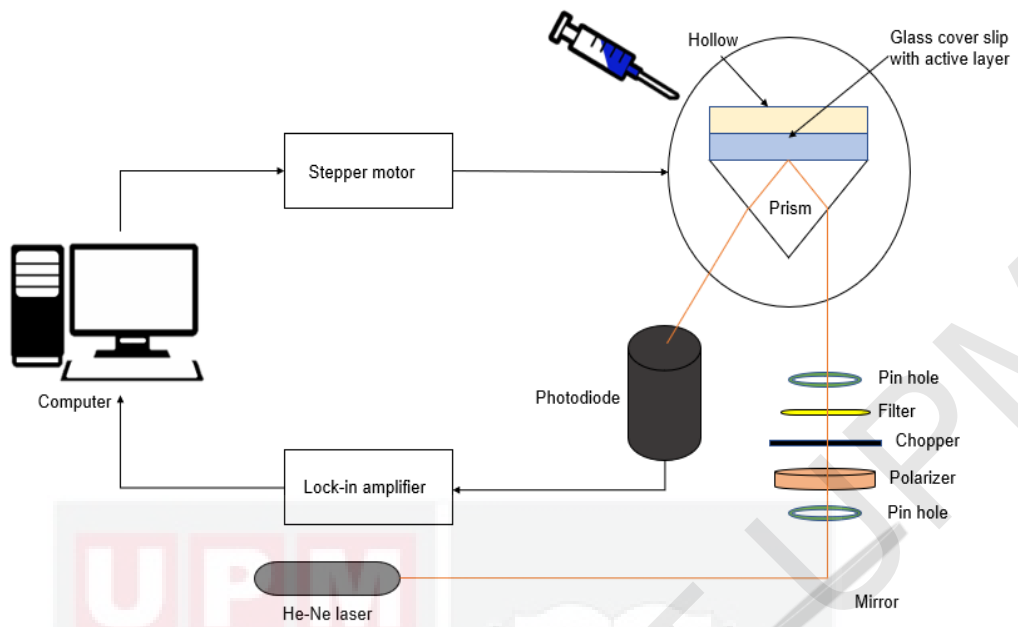


Figure 3.11 Schematic diagram of the experimental setup of SPR (Saleviter et al., 2020).



Figure 3.12 The SPR setup at Applied Optics Laboratory, Physics Department, Faculty of Science.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Introduction

In this chapter, the obtained characterization results of the ZnO nanowires were analyzed and discussed. The optical and sensing properties of ZnO nanowires are studied from the results of UV-Visible spectrophotometry and surface plasmon resonance (SPR) respectively.

4.2. Optical studies of ZnO nanowires.

ZnO has four oxygen atoms which are coordinated to the tetrahedral Zn atoms. The absorption occurs when the Zn d electrons hybridize with the oxygen p electrons to form an energy band gap (Samavati et al., 2016). The optical properties of the annealed and unannealed seed layer of the ZnO nanowires are studied by UV-visible spectroscopy. The optical absorption spectra of the annealed and unannealed seed layer can be observed for the wavelength range of 220 nm to 800 nm. However, the absorption spectrum of annealed ZnO nanowires is found to be the same with the unannealed ZnO sample as observe in Figure 4.1. Hence, from the figure, the absorption peak of ZnO was located at the range of 278 to 280 nm at the room temperature due to its exciton recombination (Rathee et al., 2016).

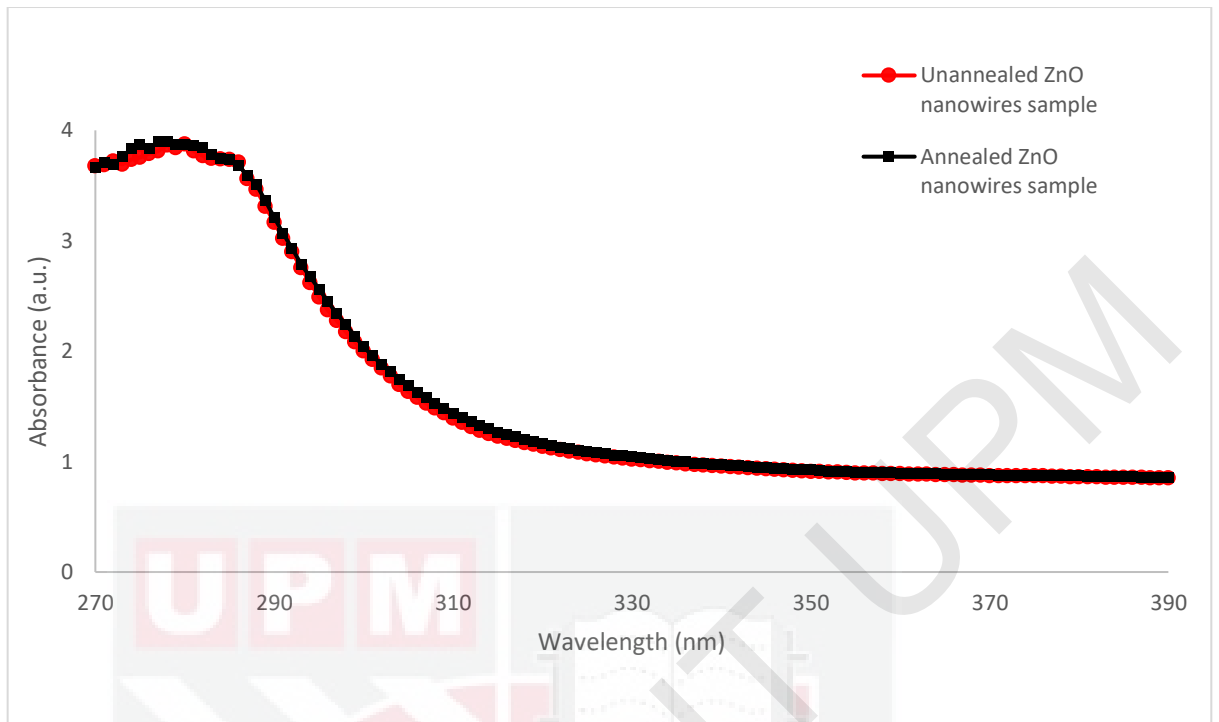


Figure 4.1 UV-Visible absorption spectra of the ZnO nanowires of annealed and unannealed seed layer of the ZnO nanowires.

The relationship of the absorption coefficient and the incident photon energy of ZnO nanowires exist from the UV- visible results in Figure 4.3 (Z. Zhang et al., 2008). The relationship is given by the equation of

$$\alpha(h\nu) \propto (h\nu - E_0)^n$$

where α is the absorption coefficient, $h\nu$ is the energy of the incident photon, n is 0.5 for the semiconductor with direct band gap and 2 for semiconductor with indirect band gap, and lastly, E_0 is the value of energy gap (Z. Zhang et al., 2008). According to the above equation, a Tauc plot is plotted. The graph of $(\alpha h\nu)^2$ versus $h\nu$ for the annealed ZnO sample is shown in Figure 5. From the figure, the energy band gap, E_g of the ZnO nanowires was determined as 4.03 eV for annealed and unannealed seed layer of the

ZnO nanowires. This represents the blue shift in the band gap of ZnO nanowires from its theoretical band gap energy, E_g which is 3.37 eV (Goswami & Sharma, 2010).

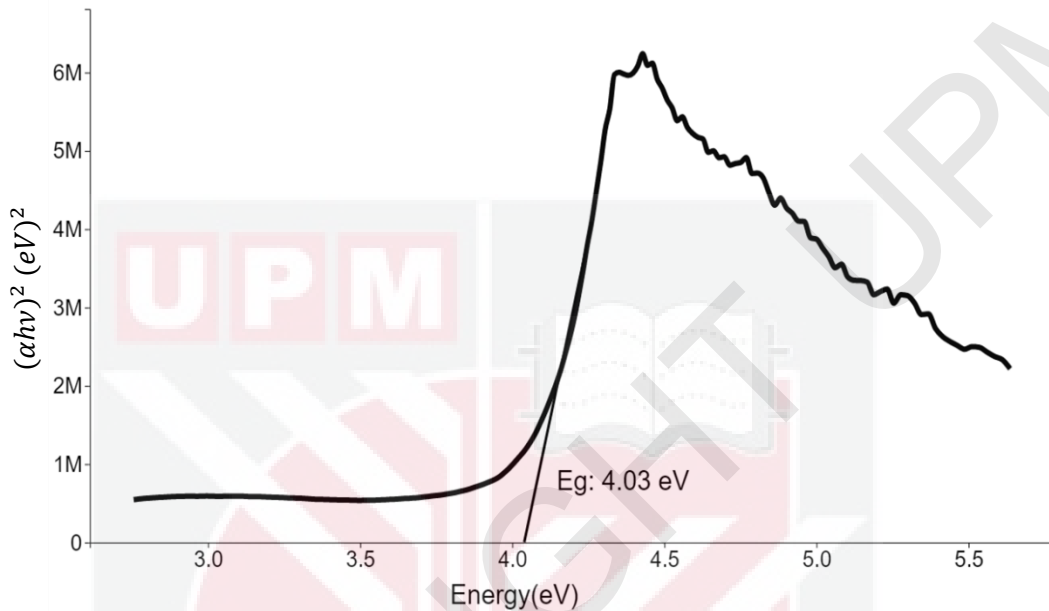


Fig. 4.2 Energy band gap of annealed seed layer of the ZnO nanowires.

4.3. SPR properties

The sensing potential of ZnO nanowires is studied from the surface plasmon resonance technique. The ZnO nanowires were grown on gold coated microscope glass with different condition of the seed layer which are annealed and unannealed 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.

A preliminary 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 ZnO nanowires is shown in Figure 4.3. From the figure, the resonance angle for unannealed seed layer is 53.56° while for annealed seed layer is from 54.12 to 54.39° . The SPR resonance angle (θ_{SPR}) is found to shift from 53.56° to 54.39° with the annealing of the seed layer of ZnO nanowires as shown in Figure 4.3. This is due to the annealing of the ZnO seed layer where it enhances the growth of ZnO nanowires and thus, 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 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.

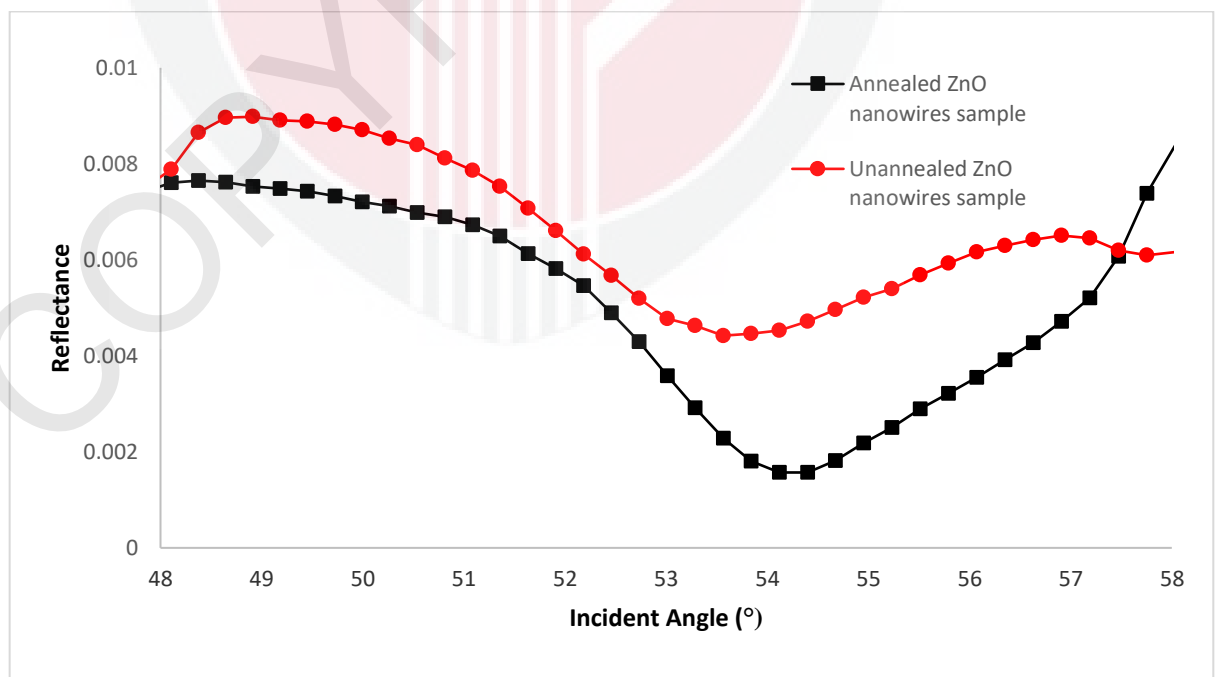


Figure 4.3 The SPR curve comparison of annealed and unannealed seed layer of the ZnO nanowires tested with deionized water.

Then, the SPR experiment was carried out using the pure 88% concentration of lactic acid in an aqueous solution that was injected into the cell attached to the composite layer. The SPR reflectivity curves for the pure concentration of lactic acid in contact with the gold/ZnO layer are shown in Figure 4.6 and Figure 4.7 for the annealed seed layer and unannealed seed layer, respectively. The resonance angle determined from the SPR curve for annealed seed layer of the ZnO nanowires is 56.35° while the unannealed seed layer of the ZnO nanowires is 57.75° . The shift of resonance angle is from 57.75° to 56.35° with the annealing of the seed layer of ZnO nanowires as shown in Figure 4.8. From the results, the resonance angle shifted to the left owing to the surface roughness of the ZnO nanowires where the annealed ZnO nanowires have a smooth surface compared to unannealed ZnO nanowires. This condition allows the annealed ZnO nanowires to improve its sensing properties to detect lactic acid (Shewale et al., 2019).

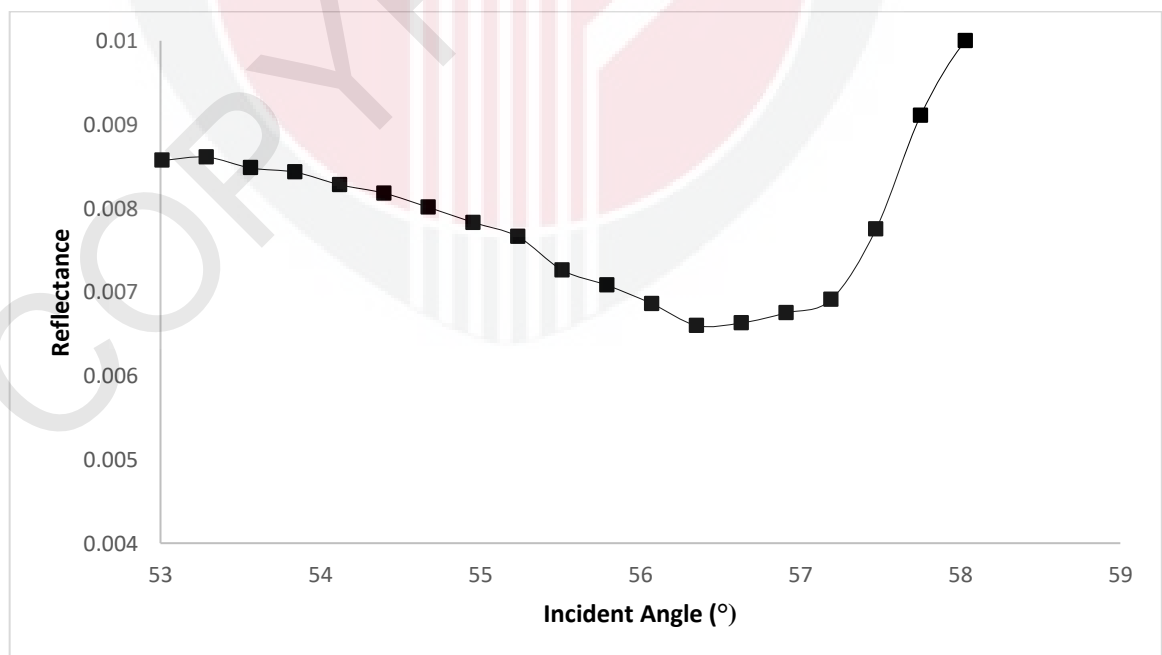


Figure 4.4 SPR curve for annealed seed layer of the ZnO nanowires tested with pure lactic acid.

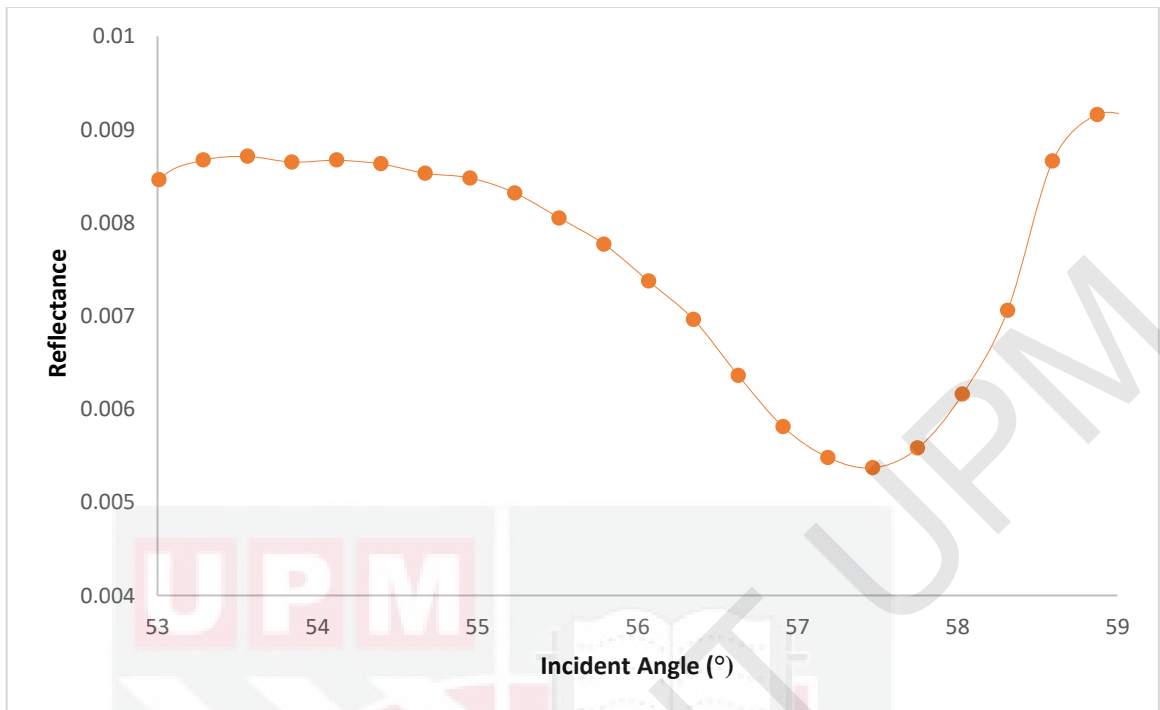


Figure 4.5 SPR curve for unannealed seed layer of the ZnO nanowires tested with pure lactic acid.

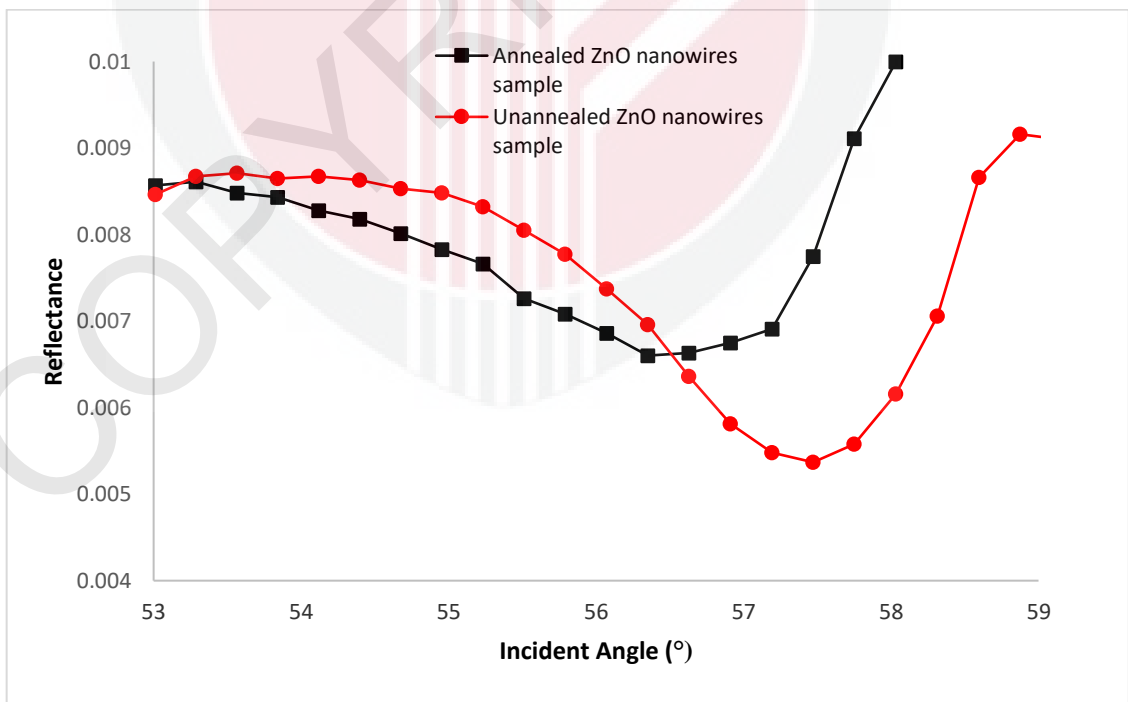


Figure 4.6 SPR curves comparison of annealed and unannealed seed layer tested with pure concentration of lactic acid.

In order to understand these SPR curve better, the data in Figure 4.9 and Figure 4.10 show the comparison between the curve of annealed and unannealed ZnO nanowires, respectively when being tested with deionized water and pure concentration of lactic acid. In Figure 4.9, the SPR resonance angle for annealed ZnO nanowires is found to shift from 54.11° to 56.35° with the increasing of concentration of the lactic acid while in Figure 4.10, the unannealed ZnO nanowires has a shift of resonance angle from 53.56° to 57.47° . The shift in resonance angle has been introduced as a parameter to measure the sensitivity of a sensor by taking the difference between resonance angle of the sample and deionized water as reference.

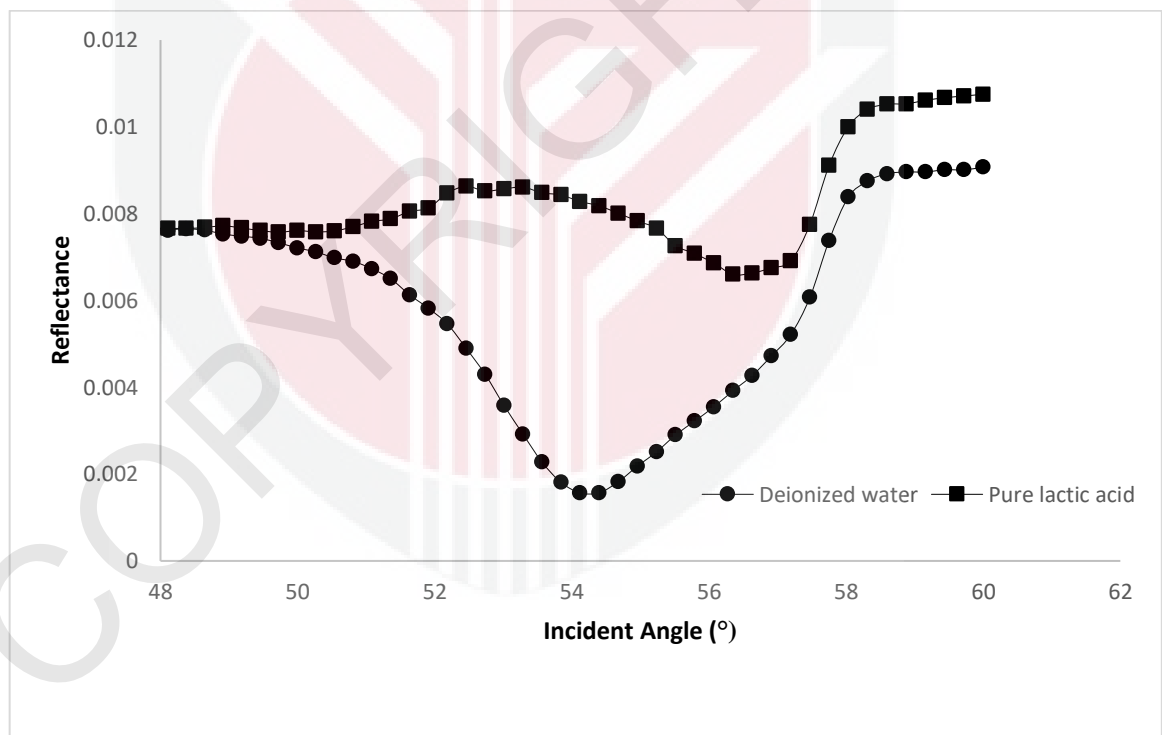


Figure 4.7 The comparison of SPR curves of the annealed seed layer of the ZnO nanowires tested with both deionized water and pure lactic acid.

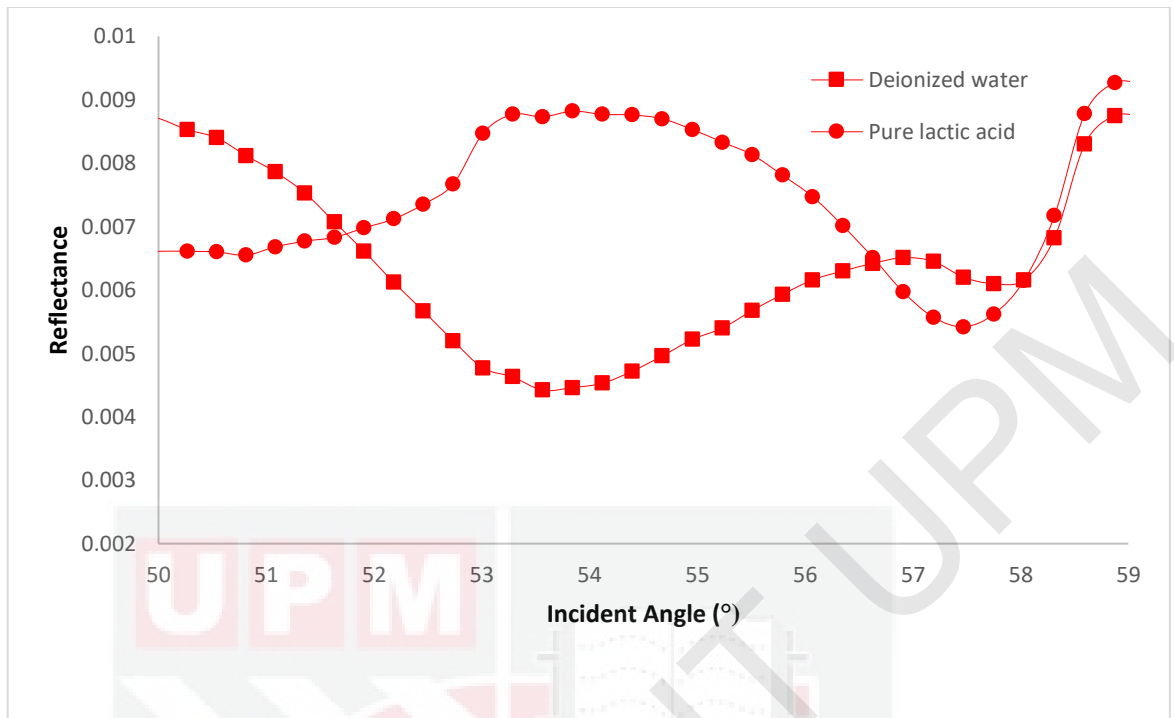


Figure 4.8 The comparison of SPR curves of the unannealed seed layer of the ZnO nanowires tested with both deionized water and pure lactic acid.

Lastly, due to the limitation of time and material, we cannot immobilize the active layer of ZnO nanowires with LOD and also tested the SPR properties with different concentration of lactic acid. However, we used 88% pure concentration of lactic acid. When the active layer of ZnO nanowires consists of LOD, 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 so that the immobilized biomolecules are stable and maintain their biological functionality is the key consideration for improving the sensitivity and efficiency of the SPR biosensor (Mei et al., 2020).

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. Future study recommendations in a similar area are also presented.

5.2. Conclusion

In this project, the seed layer of ZnO nanowires is deposited on the gold-coated microscope coverslip by the sol-gel technique. After the sol-gel technique, the condition of the seed layer is varied into two conditions which are annealed seed layer and unannealed seed layer of ZnO nanowires. The seed layer is annealed at 150°C for 60 minutes. Next, the ZnO nanowires are synthesized on the gold-deposited microscope coverslip by using chemical bath deposition method or also known as CBD technique.

Next, the UV-Visible spectrophotometer data showed that the absorption peak of the annealed and unannealed seed layer of the ZnO nanowires is range from 278 nm to 280 nm due to the electron excitation from valence band to the conduction band. From the Tauc plot, the band gap energy of the ZnO nanowires is determined to be 4.03 eV compared to the reported band gap which is 3.3 eV. This is due to the less compactness of the ZnO nanowires that grown on the thin film.

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 seed layer of the ZnO nanowires. The shift of the SPR curve showed that the deionized water and pure lactic acid have different refractive index which are 1.3242 for deionized water and 1.425 for pure lactic acid. Therefore, in this project, the annealed seed layer of the ZnO nanowires showed the best optimized of SPR curve compared to the unannealed seed layer. It also showed the difference of reflectance angle from 54.12° to 56.35° .

In conclusion, this project showed that the variation of the the condition of 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.

5.3. Future recommendation

There are some recommendations for future projects and proposed as follow:

- I. Immobilize the LOD on the ZnO nanowires' active layer in order to activate the layer of enzyme that will be useful for detection of lactic acid using SPR technique.
- II. Make sure the angle of the sample is 45° in the CBD solution before the sample is put in the oven for the growth of ZnO nanowires.

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