UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF NANOCRYSTALLINE CELLULOSE/GRAPHENE OXIDE BASED COMPOSITE THIN FILM FOR METAL IONS DETECTION USING SURFACE PLASMON RESONANCE SPECTROSCOPY

WAN MOHD EBTISYAM MUSTAQIM BIN MOHD DANIYAL

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June 2022

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Increasing metal ion contamination in the environment can cause severe effects towards human health. Hence, it is important to detect metal ion at low concentration for continuous monitoring of the surrounding environment. In this study, nanocrystalline cellulose/graphene oxide (NCC/GO) based thin film has been developed for metal ion sensing using surface plasmon resonance (SPR) technique. The properties of the NCC/GO thin film was confirmed by Fourier transform infrared (FTIR) and X-ray photoelectron spectroscopy (XPS) by showing all the functional groups of the composite. Moreover, the atomic force microscopy (AFM) result shows that the thin film roughness increased after the combination of NCC and GO. Conversely, the optical properties of the NCC/GO thin film were characterized by Ultraviolet-Visible spectroscopy (UV-Vis) where the absorbance peak can be observed in the range of 280 to 300 nm. Based on the band gap energy analysis, the NCC/GO thin film has optical band gap of 4.00 eV. Meanwhile, the sensing properties of the NCC/GO thin film shows a good result where metal ions such as copper, zinc, and nickel ion can be detected as low as 0.01 ppm using SPR. Fitting of the SPR curves also reveals the real part refractive index, n and the imaginary part refractive index, k values found for the NCC/GO layer were 1.4240 and 0.2520, respectively with thickness of 9.5 nm. Interestingly when the NCC/GO thin film was exposed to metal ion, a clear change of refractive index and the thickness was observed. The sensitivity of the NCC/GO thin film for copper, zinc, and nickel ion detection was 3.271° ppm^{-1}, 2.579° ppm^{-1}, and 1.509° ppm^{-1} respectively. In addition, the NCC/GO thin film also has high affinity towards copper ion with binding affinity constant of 4.075×10^3 M^{-1} compared to zinc and nickel ion at 2.579° ppm^{-1} and 1.509° ppm^{-1} respectively. Furthermore, the NCC/GO thin film incorporated with copper ionophore has successfully increased the sensitivity and selectivity for sensing copper ion. When compared with NCC/GO thin film, the NCC/GO-ionophore thin film has much higher sensitivity value of 59.9150° ppm^{-1} from 0.001 to 0.01 ppm and 2.2261° ppm^{-1} from 0.01 to 0.1 ppm. Besides that, the NCC/GO-ionophore thin film was able to detect copper ion in spiked water sample as low as 0.5 ppm with sensitivity of 0.01418°

ppm^{-1} from 0.5 to 20 ppm and $9.815 \times 10^{-3\circ}$ ppm^{-1} from 20 to 100 ppm. The above results conclude that the developed optical sensor using NCC/GO-ionophore thin film has a good sensitivity and selectivity for copper ion sensing.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENGHASILAN FILEM NIPIS BERASASKAN SELULOSA NANOKRISTAL/GRAFIN OKSIDA KOMPOSIT UNTUK PENGESANAN ION LOGAM MENGGUNAKAN SPEKTROSKOPI RESONANS PLASMON PERMUKAAN

Oleh

WAN MOHD EBTISYAM MUSTAQIM BIN MOHD DANIYAL

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Peningkatan pencemaran ion logam dalam alam sekitar boleh menyebabkan kesan yang teruk terhadap kesihatan manusia. Oleh itu, adalah penting untuk mengesan ion logam pada kepekatan rendah untuk memantau alam sekitar secara berterusan. Dalam kajian ini, filem nipis berasaskan selulosa/grafin oksida (NCC/GO) telah dibangunkan untuk pengesanan ion logam menggunakan teknik resonans plasmon permukaan (SPR). Ciri-ciri filem nipis NCC/GO telah disahkan oleh Fourier Transform Infrared spectroscopy (FTIR) dan X-ray Photoelectron spectroscopy (XPS) dengan menunjukkan semua kumpulan berfungsi dalam komposit tersebut. Selain itu, Atomic Force Microscope (AFM) menunjukkan bahawa kekasaran filem nipis meningkat selepas gabungan NCC dan GO. Seterusnya, sifat optik filem nipis NCC/GO dicirikan oleh Ultraviolet-Visible spectroscopy (UV-Vis) di mana puncak penyerapan boleh diperhatikan dalam julat 280 hingga 300 nm. Berdasarkan analisis jurang tenaga jalur, filem nipis NCC/GO mempunyai jurang jalur optik sebanyak 4.00 eV. Sementara itu, sifat penderiaan filem nipis NCC/GO menunjukkan hasil yang baik di mana ion logam seperti ion kuprum, zink, dan nikel boleh dikesan serendah 0.01 ppm menggunakan SPR. Pemadanan lengkung SPR juga mendedahkan nilai indeks biasan bahagian sebenar, *n* dan nilai indeks biasan bahagian khayalan, *k* yang ditemui untuk filem nipis ini ialah masing-masing 1.4240 dan 0.2520 dengan ketebalan 9.5 nm. Menariknya apabila filem nipis NCC/GO terdedah kepada ion logam, perubahan yang jelas pada nilai indeks biasan nilai ketebalan telah diperhatikan. Kepekaan filem nipis NCC/GO untuk pengesanan ion kuprum, zink dan nikel ialah masing-masing 3.271° ppm^{-1}, 2.579° ppm^{-1}, dan 1.509° ppm^{-1}. Selain itu, filem nipis NCC/GO juga mempunyai pertalian tinggi terhadap ion kuprum dengan pemalar pertalian pengikatan 4.075×10^3 M^{-1} berbanding ion zink dan nikel masing-masing pada 2.579° ppm^{-1} and 1.509° ppm^{-1}. Tambahan pula, filem nipis NCC/GO yang digabungkan dengan ionofor kuprum telah berjaya meningkatkan sensitiviti dan selektiviti untuk mengesan ion kuprum. Jika dibandingkan dengan filem nipis NCC/GO, filem nipis NCC/GO-ionofor mempunyai nilai sensitiviti yang lebih

tinggi iaitu 59.9150° ppm^{-1} daripada 0.001 hingga 0.01 ppm dan 2.2261° ppm^{-1} daripada 0.01 hingga 0.1 ppm. Selain itu, filem nipis NCC/GO-ionophore dapat mengesan ion kuprum dalam sampel air berpancang serendah 0.5 ppm dengan kepekaan 0.01418° ppm^{-1} daripada 0.5 hingga 20 ppm dan 9.815×10^{-3}° ppm^{-1} daripada 20 hingga 100 ppm. Keputusan di atas menyimpulkan bahawa sensor optik yang dibangunkan menggunakan filem nipis NCC/GO-ionofor mempunyai kepekaan dan selektiviti yang baik untuk penderiaan ion kuprum.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

NCC	Nanocrystalline cellulose
GO	Graphene oxide
SPR	Surface plasmon resonance
CTAB	Hexadecyltrimethylammonium bromide
FTIR	Fourier-transform infrared spectroscopy
XPS	X-Ray photoelectron spectroscopy
AFM	Atomic force microscopy
UV-Vis	Ultraviolet-visible spectroscopy
A	Absorbance
α	Absorbance c0efficient
n	Real part refractive index
k	Imaginary part refractive index
K	Binding affinity constant
θspr	Resonance angle
FWHM	Full width half maximum
DA	Detection accuracy
SNR	Signal-to-noise ratio
ppm	Parts per million

CHAPTER 1

INTRODUCTION

1.1 Metal Ion

Metal ions are important element in biological systems to maintain the lifespan of all organisms. It is increasingly recognized that essential metal ions are involved in many structural and functional roles, participating in biochemical reactions, and also can arise in several forms. For instance, copper is the third most abundant transition metal ion and very crucial to several critical biological functions in humans. Although copper is limited to 23.6 μM in human body, it contributes to the human bone production, cellular respiration, connective tissue formation, and brain function (Chang and Yang 2013; Korin et al., 2011; Lin et al., 2013; Zhang et al., 2014). Another crucial metal ion is zinc and it is the second most abundant metal ion in human body about 2-3 g in total (Jiang & Guo, 2004). Zinc is important for human immune systems, development during pregnancy, and support normal growth (Jung et al., 2017; Treska et al., 2015) while Ni^{2+} is essential in adrenaline and glucose metabolism, iron absorption, and aids in red blood cell production (Kumar and Trivedi, 2016). The deficiency of these essential metal ions in human body may cause several diseases such as anemia, brain and heart disease, growth retardation and skin changes (Gupta, 2018).

Even though metal ion is very crucial to the biological systems, excessive intake of the metal ion can cause severe damages and even lead to death. Copper was reported can cause various diseases such as hypoglycemia, Parkinson, Alzheimer, dyslexia, and Wilson (Liu et al., 2013; Yang et al., 2012; Zhang et al., 2014). On the other hand, zinc at higher level, can cause health damages such as vomiting, nausea, stomach ache, anemia, and skin problems (Moyo, 2014) while excess exposure to nickel can cause dermatitis, asthma, acute pneumonitis, disorders of central nervous system, and also can lead to lung cancer (Chervona et al., 2012; Harasim and Filipek, 2015).

1.2 Nanocrystalline Cellulose

Cellulose is the most abundant natural material in the world that was first found in 1838 and has been shown to be a long-chain of polymer with repeating units of D-glucose (Kalia et al., 2011). Cellulose is widely present in various forms of biomasses such as wood, stalks, and some bacteria that can produce cellulose. Cellulose is composed with linear polysaccharides consisted of two anhydroglucose rings linked by repeated β-1,4 glycoside bonds (Eichhorn et al., 2010). Cellulose also is an important raw material for various kind of industries such as for pulp and paper industries. When cellulose is subjected to a pure mechanical shearing and a combination of chemical, mechanical or enzymatic treatment, the amorphous region is selectively hydrolyzed (Beck-Candanedo et al., 2005). Consequently, cellulose break into a shorter crystalline parts, generally referred as nanocrystalline cellulose or NCC (Habibi et al., 2008).

NCC has been realized as a new class of nano-material since the past decades. NCC has many advantages compared to cellulose that includes high surface area, nanoscale dimensions, and high specific strength. NCC has a rigid rod-shaped nanoparticle with diameter in the range of 5-20 nm and length in the range of 10-100 nm (Peng et al., 2011). Besides that, the surface of NCC contained hydroxyl functional group as shown in Figure 1.1. The presence of this functional group enables further modification of NCC to prepare the material for targeted application (Goussé et al., 2004; Grunert and Winter 2002; Junior de Menezes et al., 2009; Salajková et al., 2012; Yuan et al., 2006).

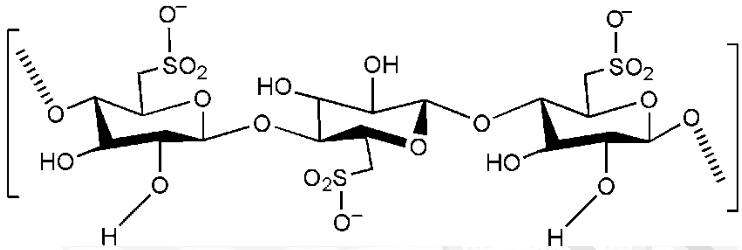

Figure 1.1: Chemical structure for NCC (Zainuddin et al., 2017)**.**

1.3 Graphene Oxide

Carbon is one of the most common atoms on earth that has many forms and also exists as a component in most substances (Paulchamy et al., 2015). A single layer of sp^2-hybridized carbon atom is known as graphene and has been explored theoretically since 1940s (Brownson et al., 2012). Graphene is arranged in a honeycomb pattern or hexagonal structure with a zero band gap (Loryuenyong et al., 2013). These structures provide unique mechanical, thermal, optical, and electrical properties for graphene (Balandin et al., 2008; C. Lee et al., 2008; Orlita et al., 2018). Owing to its unique properties, graphene has been widely explored by researchers for application in electronics, catalysis, sensors, energy conversion, energy storage, optics, optoelectronics, biomedical engineering, tissue engineering, medical implants and medical devices (Chua and Pumera, 2014; Yin et al., 2011).

Graphene can also be oxidized to synthesis a much more hydrophilic material i.e., graphene oxides (GO) (Eda and Chhowalla, 2010). The oxidation process introduces a large oxygen atom on the surface of GO. Hence, GO has several oxygen-containing functional groups that includes hydroxyl, carboxyl, and epoxy groups as shown in Figure 1.2. These functional groups provide GO with functionalities such as hydrophilicity, high surface activity, and antifouling properties. Besides that, the oxygen-containing functional groups on the surface of GO have a lone electron pair that can efficiently bind to a metal ion through sharing of electron pair (Sitko et al., 2013).

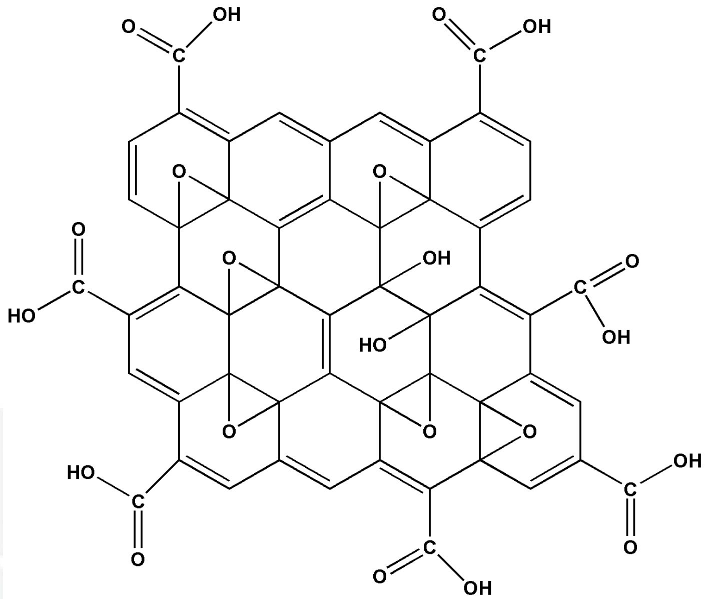

Figure 1.2: Chemical structure of GO (He et al., 1998)**.**

1.4 Surface Plasmon Resonance

Biosensor or sensor is an analytical tool that was used for determination of an analytes based on biocatalyst type of sensors (Khansili et al., 2018). Sensor can be dependent on magnetic, mechanical, electrical, and optical principles. Among all, optical sensor is the simpler in their setup for data acquisition and working activity. Optical sensor has many advantages over electrical and mechanical sensor such as the optical sensor does not modify nor destruct the measurand or the surrounding environment (Ciminelli et al., 2013). Basically, optical sensors required recognition element that can interact specifically with the desired target analytes and the sensor will detect the signal of the binding event (Yan et al., 2018). For instance, reflection interference spectroscopy (RIFS) (Schwarz et al., 2010), surface-enhanced raman scattering (SERS) spectroscopy (Santinom et al., 2018), and surface plasmon resonance (SPR) are optical sensors that required recognition element. SPR is one of the favourable optical sensors that is widely used in sensing biochemical reaction. The very first SPR phenomenon was observed by Wood in 1902. Since then, SPR has been diligently studied and made vast advances in the development of technology and its application for the learning of processes at the surfaces of metals and sensing of gases (Homola, 2008).

There are few approaches to excites surface plasmon grating coupled systems, optical waveguide systems, optical fibres, and prism coupled system. Prism-based SPR is the most widely used approach in current SPR systems can be divided into two arrangements which are Otto configuration and Kretschmann configuration. Among these two configurations, Kretschmann configuration is normally used in most SPR applications where a metal usually gold, is placed at the interface of two dielectric media as shown in Figure 1.3. When plane-polarized light hits the gold-coated film prism under total internal reflection conditions, SPR will occur resulting in reflected beam that will be detected for processing. One of the most findings in SPR sensor is the development of active layers or recognition element on the surface of the metal layer. The development of an improved active layer is crucial as it determines the optical sensor sensitivity, selectivity, and other parameters of sensors. SPR sensors with different active layers have has been studied for various sensing applications including environment monitoring and clinical diagnosis (Firdous et al., 2018; Omar et al., 2019; R. Verma and Gupta, 2015).

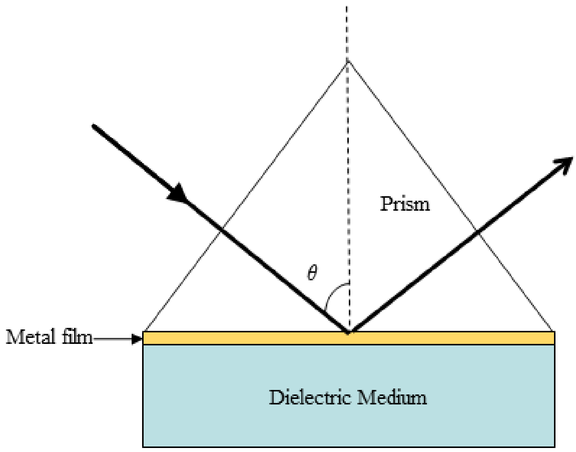

Figure 1.3: Kretschmann configuration.

1.5 Problem Statement

Over the past few years, many researches have been conducted related to the preparation of nanocrystalline cellulose (NCC) for metal ion adsorption owing to its attractive characteristics (Tao et al., 2020). Besides that, modification of NCC with various material has also been carried out to improve the material mechanical and chemical properties (Abitbol et al., 2014). On the other hand, graphene oxide (GO) has also drawn attention owing to its advantages and have been used in chemosensor, optoelectronics, and drug delivery (Li et al., 2015). However, even though there are a lot of works on NCC and GO, to the best of our knowledge, there was still lack of studies on the NCC/GO composite thin film properties thus, this limitation gives the inspiration to explore its structural and optical properties.

It is very challenging to determine metal ion optically because all metal ions are transparent and have the same refractive index at low concentration. Metal ion in environmental has also been reported to increase rapidly due to the industrial sources (Verma and Kaur, 2016). To avoid the main health problem caused by metal ions, the concentration of metal ions in the environment has been limited by the World Health Organization (WHO). For example, the concentration limit of Cu^{2+}, Zn^{2+}, and Ni^{2+} was 2 ppm, 3 ppm, and 0.07 ppm respectively (Braga et al., 2017). Therefore, it is crucial to determine metal ion at trace level owing to its harmful effect to human and its toxicity that can lead to various short-term and long-term diseases. SPR is a well-known optical sensor that has gained attention from the scientific community for various applications since the past two decades owing to its advantages such as label-free, cost-effective, low mass, high sensitivity, rapid detection, and fast measurement with no reference necessity (Zhao et al., 2020). However, SPR has limitation as it cannot distinguish solutions with the same refractive index. To overcome this limit, the surface of gold thin film can be modified with active layer or sensing element. To the best of knowledge, there is still no report on metal ion sensing using SPR optical sensor incorporated with NCC/GO thin film. It is from this matter which initiates the idea to study the ability of NCC/GO thin film for metal ion sensing using surface plasmon resonance technique.

1.6 Objectives

The main objectives of this study are summarized as follows:

1. To analyze the structural and optical properties of the nanocrystalline cellulose/graphene oxide thin film.

2. To calculate the sensitivity and binding affinity of nanocrystalline cellulose/graphene oxide thin film towards various metal ions based on SPR reflectivity curves.

3. To verify the selectivity of the nanocrystalline cellulose/graphene oxide based thin film for sensing a specific metal ion using SPR technique.

1.7 Thesis Outline

This thesis starts with the introduction of the nanocrystalline cellulose, graphene oxide, metal ions, and surface plasmon resonance in Chapter 1. In Chapter 2, previous and current researches by other researchers will be reviewed to give more information related to this research. Next in Chapter 3, the methodology of this research including all materials preparation, characterization, and experiment procedure. Then in chapter 4, each and every result obtained from the characterization will be analysed and elaborated comprehensively in this chapter. Finally, the last chapter will conclude this study and suggestions for any future works will be given in Chapter 5.

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