DEVELOPMENT OF SURFACE PLASMON RESONANCE SENSOR BASED ON GRAPHENE QUANTUM DOTS AND CELLULOSE-BASED BIOPOLYMERS FOR PHENOL DETECTION

By

HAZWANI SUHAILA BINTI HASHIM

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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The extensive discharge of phenol into aquatic environments from industrial sectors has led to adverse effects on living organisms due to its high toxicity. Hence, various detection methods have been developed, with surface plasmon resonance (SPR) standing out for its label-free analysis and real-time detection. However, this sensor struggles with poor sensitivity to low concentrations of target analytes. In this regard, SPR sensor has been integrated with graphene quantum dots (GQDs), sodium carboxymethyl cellulose (NaCMC), nanocrystalline cellulose (NCC), NaCMC-GQDs, and NCC-GQDs thin films for phenol detection, for the first time. The structural properties of the NaCMC-GQDs and NCC-GQDs thin films, determined using Fourier transform infrared spectroscopy and atomic force microscopy, confirmed the presence of O–H, C–H, C=O, and C=C stretching, as well as C–H bending, with an additional C–O stretching peak for NaCMC-GQDs, and showed that the surfaces of the thin films were covered with NaCMC and NCC, respectively. Moreover, the optical properties, examined using ultraviolet-visible spectroscopy, revealed energy band gap values of

4.088 eV and 4.094 eV for the NaCMC-GQDs and NCC-GQDs thin films, respectively. The sensing performance of the thin films coated with sensing materials (GQDs, NaCMC, NCC, NaCMC-GQDs, and NCC-GQDs) for phenol detection was analyzed using the SPR sensor. Limits of detection (LOD) of 0.1 µM for GQDs, 0.01 fM for NaCMC and NCC, and 0.001 fM for NaCMC-GQDs and NCC-GQDs were achieved, outperforming the gold thin film with a LOD of 1 µM. This can be attributed to phenol binding to the sensing materials through hydrogen bonds, π-π stacking interactions, and carbohydrate-aromatic interactions. Among all the thin films, the NCC-GQDs thin film showed the best sensing performance with the lowest LOD of 0.001 fM and a sensitivity of 0.02038° M^{-1}, surpassing the NaCMC-GQDs thin film's sensitivity of 0.01353° M^{-1} with the same LOD value. Integration with tyrosinase (Tyr) enzyme further enhanced its sensitivity to 0.04657° M^{-1}. The NCC-GQDs-Tyr thin film showed outstanding selectivity for phenol, evidenced by significant resonance angle shifts for the mixtures of interferents containing phenol compared to those without. The sensor also demonstrated good stability over a 14-day storage period and excellent recovery rates for detecting phenol in spiked water samples. The NCC-GQDs-Tyr thin film incorporated SPR sensor has shown great potential as a reliable tool for sensitive and selective phenol detection.

Keywords: Graphene quantum dots, nanocrystalline cellulose, phenol, sodium carboxymethyl cellulose, surface plasmon resonance

SDG: GOAL 6: Clean Water and Sanitation

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

PENGHASILAN SENSOR RESONANS PLASMON PERMUKAAN BERDASARKAN TITIK KUANTUM GRAFIN DAN BIOPOLIMER BERASASKAN SELULOSA UNTUK PENGESANAN FENOL

Oleh

HAZWANI SUHAILA BINTI HASHIM

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Pelepasan fenol yang meluas ke dalam persekitaran akuatik daripada sektor perindustrian telah membawa kepada kesan buruk terhadap organisma hidup kerana ketoksikannya yang tinggi. Oleh itu, pelbagai kaedah pengesanan telah dibangunkan, dengan resonans plasmon permukaan (SPR) menonjol untuk analisis tanpa label dan pengesanan masa nyatanya. Walau bagaimanapun, sensor ini bergelut dengan sensitiviti yang lemah terhadap kepekatan rendah bahan analisis sasaran. Dalam hal ini, sensor SPR telah diintegrasikan dengan filem-filem nipis titik kuantum grafin (GQDs), natrium karboksimetil selulosa (NaCMC), selulosa nanohabluran (NCC), NaCMC-GQDs, dan NCC-GQDs untuk pengesanan fenol, buat kali pertama. Sifat-sifat struktural filem-filem nipis NaCMC-GQDs dan NCC-GQDs, ditentukan menggunakan spektroskopi inframerah transformasi Fourier dan mikroskopi daya atom, mengesahkan kehadiran regangan O–H, C–H, C=O, dan C=C, serta lenturan C–H, dengan puncak regangan C–O tambahan untuk NaCMC-GQDs, dan menunjukkan bahawa permukaan filem-filem nipis telah ditutup dengan NaCMC dan NCC, masing-

masing. Tambahan pula, sifat-sifat optikal, diperiksa menggunakan spektroskopi ultraungu-tampak, mendedahkan nilai jurang jalur tenaga 4.088 eV dan 4.094 eV untuk filem-filem nipis NaCMC-GQDs dan NCC-GQDs, masing-masing. Prestasi penderiaan filem-filem nipis yang disalut dengan bahan-bahan penderiaan (GQDs, NaCMC, NCC, NaCMC-GQDs, dan NCC-GQDs) untuk pengesanan fenol telah dianalisis menggunakan sensor SPR. Had pengesanan (LOD) sebanyak 0.1 µM untuk GQDs, 0.01 fM untuk NaCMC dan NCC, dan 0.001 fM untuk NaCMC-GQDs dan NCC-GQDs telah dicapai, mengatasi prestasi filem nipis emas dengan LOD 1 µM. Ini boleh dikaitkan dengan pengikatan fenol kepada bahan-bahan penderiaan melalui ikatan hidrogen, interaksi penyusunan π-π, dan interaksi karbohidrat-aromatik. Di antara semua filem-filem nipis, filem nipis NCC-GQDs menunjukkan prestasi penderiaan terbaik dengan LOD terendah 0.001 fM dan sensitiviti 0.02038° M^{-1}, mengatasi sensitiviti filem nipis NaCMC-GQDs 0.01353° M^{-1} dengan nilai LOD yang sama. Perintegrasian dengan enzim tirosinase (Tyr) meningkatkan lagi sensitivitinya kepada 0.04657° M^{-1}. Filem nipis NCC-GQDs-Tyr menunjukkan kepilihan yang luar biasa untuk fenol, dibuktikan oleh anjakan sudut resonans yang ketara untuk campuran gangguan yang mengandungi fenol berbanding dengan yang tidak. Sensor itu juga menunjukkan kestabilan yang baik selama tempoh penyimpanan 14 hari dan kadar pemulihan yang sangat baik untuk mengesan fenol dalam sampel air berpancang. Filem nipis NCC-GQDs-Tyr yang menggabungkan sensor SPR telah menunjukkan potensi besar sebagai alat yang boleh dipercayai untuk pengesanan fenol yang sensitif dan terpilih.

Kata Kunci: Fenol, natrium karboksimetil selulosa, resonans plasmon permukaan, selulosa nanohabluran, titik kuantum grafin

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LIST OF ABBREVIATIONS

2D	Two-dimensional
3D	Three-dimensional
α	Absorbance coefficient
θ_{SPR}	Resonance angle
A	Absorbance
AA	Ascorbic acid
AFM	Atomic force microscopy
Cu^{2+}	Copper ion
DA	Detection accuracy
DW	Deionized water
E_g	Energy band gap
Fe^{2+}	Iron ion
FTIR	Fourier-transform infrared
FWHM	Full width at half maximum
GQDs	Graphene quantum dots
Hg^{2+}	Mercury ion
hv	Photon energy
k	Imaginary part of refractive index
K_{sp}	Wave vector of surface plasmon
K_x	Wave vector of incident light
LOD	Limit of detection
n	Real part of refractive index
NaCMC	Sodium carboxymethyl cellulose
NCC	Nanocrystalline cellulose

PBS	Phosphate buffer solution
R^2	Correlation coefficient
RMS	Root mean square
SNR	Signal-to-noise ratio
SPR	Surface plasmon resonance
Tyr	Tyrosinase
UA	Uric acid
UV-Vis	Ultraviolet-visible

CHAPTER 1

INTRODUCTION

1.1 Phenol

Phenol is one of the phenolic compounds that plays an important role in the rapid development of the industrial sector. Phenol consists of a phenyl group bonded to a hydroxyl group as depicted in Figure 1.1. It has been used as the raw material in the production and manufacturing of resins, plastics, germicides, pharmaceutical, textiles, dyes, and petrochemical products (Ahmad et al., 2016; Belekbir et al., 2020; Nezhad et al., 2008). Hence, it is widely distributed throughout the environment as the by-products contaminant from the industrial wastewater (Pino et al., 2016). However, phenol has been listed as dangerous substance or priority pollutant by United States Environmental Protection Agency (US-EPA) and the European Commission (Oriero et al., 2015). Phenol is able to exert harmful effects to living things including human due to their inherent toxicity and persistence in the environment (Adamski et al., 2010; Guan et al., 2013). In particular, acute exposure of phenol towards human usually results in dryness in throat and mouth as well as excretion of urine with dark colour, meanwhile chronic exposure can lead to fatigue, lung problems and even cancer (Olujimi et al., 2010; Villegas et al., 2016).

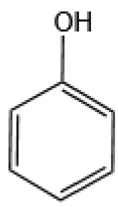

Figure 1.1: Chemical structure of phenol, consisting of a phenyl group bonded to a hydroxyl group.

1.2 Graphene Quantum Dots

Graphene is a two-dimensional nanostructure that consists of single layer of graphite or in other words, a single layer of sp^2 hybridized carbon atoms arranged in a honeycomb-like lattice (Nurrohman & Chiu, 2021; Popov et al., 2021). Graphene has been extensively exploited in a lot of applications due to its unique properties. However, graphene encountered several limitations such as zero bandgap and low absorptivity (Duan et al., 2015). Thus, in order to overcome these limitations, several modifications of graphene have been done. Graphene quantum dots (GQDs) then was successfully introduced in 2008 by Ponomarenko and Geim (Ponomarenko et al., 2008).

GQDs is the zero-dimensional monolayer graphene sheet with a size of nanometer (Abbas et al., 2020). GQDs is unique since it carries both the properties of graphene and carbon dots (Sun et al., 2013). GQDs possesses excellent optical and electrical properties as a result of its prominent quantum confinement and edge effects (Mansuriya & Altintas, 2020). Furthermore, GQDs also displays some exceptional characteristics such as large surface area, high stability, low toxicity, good biocompatibility, and good solubility (Kumar et al., 2020; Meng et al., 2019). Hence, GQDs has appeared as an interesting class of nanomaterial in various disciplines including optoelectronics, bioimaging, drug delivery, sensors, and photocatalysis applications (Kadian & Manik, 2020). Figure 1.2 displays the chemical structure of GQDs that contains numerous oxygen-containing functional groups such as hydroxyl, carbonyl, and carboxyl groups. These functional groups enhance the solubility of GQDs in water and other solvents, making them versatile for various applications.

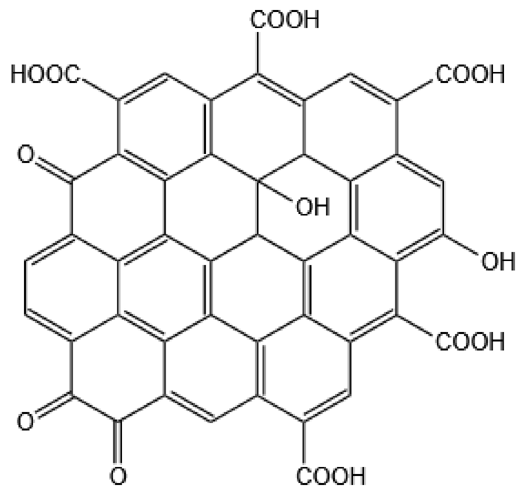

Figure 1.2: Chemical structure of GQDs, highlighting the presence of various oxygen-containing functional groups such as hydroxyl, carbonyl, and carboxyl groups.

1.3 Cellulose-Based Biopolymers

Biopolymers also can be known as natural polymers as they are formed under natural conditions during the growth cycles of all organisms. Cellulose is the most abundant, broadly-distributed natural polymer in the world (Heinze, 2015). It is composed of several hundred to ten thousand linear chains of ᴅ-glucose units which are linked by β-1,4-glycosidic bonds with the formula of $(C_6H_{10}O_5)_n$ (Sofla et al., 2016; Tang et al., 2009). Cellulose has been widely utilized in many important applications including sensors, supercapacitors, flexible electronics, and batteries due to its properties of being inexpensive, biocompatible, biodegradable, and renewable material (Alamry et al., 2022; Kawalerczyk et al., 2020; Liu et al., 2021).

One of the cellulose-based biopolymers which is sodium carboxymethyl cellulose (NaCMC) is a water-soluble anionic linear polysaccharide and semi-synthetic derivative of cellulose produced by reacting monochloroacetic acid with cellulose in the presence of sodium hydroxide (Zhang et al., 2014). It is loaded with CH_2COONa

groups that were bounded to some of the hydroxyl groups on the cellulose backbone, as shown in Figure 1.3 (Kumar et al., 2018). Owing to its exclusive characteristics including high water solubility, good film-forming properties, and ease of modification, it has been widely employed in biomedical and industrial applications such as binders, paper, foods, and drug formulations (Ebrahimzadeh et al., 2016; Son & Park, 2018). It also has been identified as an excellent adsorbent in the field of wastewater treatment due to the presence of many active functional groups that can interact with metal ions and organic compounds (Abdulkhani et al., 2016; Eltaweil et al., 2020).

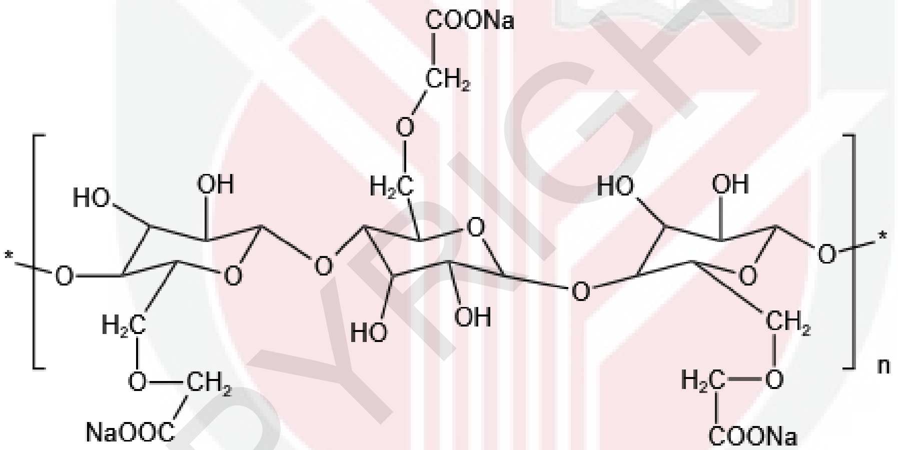

Figure 1.3: Chemical structure of NaCMC, showing CH_2COONa groups bonded to some of the hydroxyl groups on the cellulose backbone.

Besides, nanocrystalline cellulose (NCC) is also a part of the cellulose-based biopolymers that can be derived from native cellulose through acid hydrolysis process (Dorieh et al., 2022). It is a rod-shaped crystalline cellulose with lengths ranging from tens to hundreds of nanometers and diameters typically falling within the range of 1 to 100 nm (Abitbol et al., 2014; Fan et al., 2014). It has abundance of surface hydroxyl groups, as depicted in Figure 1.4, and exhibits unique characteristics such as large surface area and nanoscale dimension (Xu et al., 2015; Zainuddin et al., 2017). Hence, NCC has shown significant potential for applications across various fields such as composite material, enzyme immobilization, drug delivery, and tissue engineering (Jia et al., 2017; Nguyen et al., 2019).

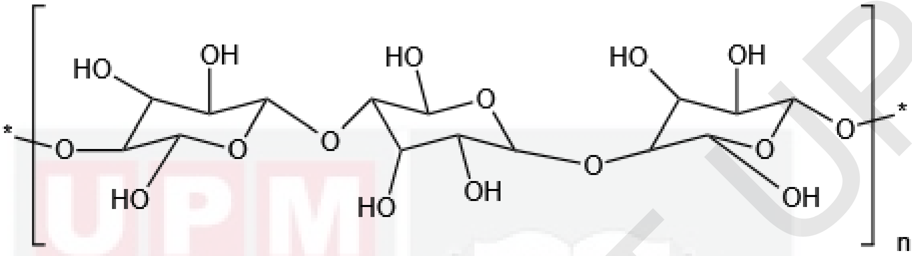

Figure 1.4: Chemical structure of NCC, highlighting the abundance of surface hydroxyl groups.

1.4 Tyrosinase

Tyrosinase (Tyr), which is also referred to polyphenol oxidase, is a copper containing metalloprotein that is widely distributed in nature (Apetrei et al., 2013; Vedrine et al., 2003). This enzyme possesses the ability to catalyze the hydroxylation of monophenols to *o*-diphenols, and their subsequent oxidation to *o*-quinones (Perez et al., 2006; Wu et al., 2017b). Tyr also can serves as a rate-limiting enzyme in the regulation of melanin production (Mulla et al., 2018). Moreover, Tyr has been extensively utilized in the fabrication of sensors for the detection of various phenolic compounds due to its high sensitivity, effectiveness, simplicity, and broad substrate specificity (Jang et al., 2010; Liu et al., 2015; Zhao et al., 2009).

1.5 Surface Plasmon Resonance

Surface plasmon is a phenomenon where the free electrons that present at a metal-dielectric interface collectively oscillate when they interact with incident electromagnetic wave (Abdulhalim et al., 2008). As the momentum of the surface plasmon and the momentum of the incident electromagnetic wave are matched, resonance will occur, hence the term surface plasmon resonance (SPR) (Singh & Hillier, 2006). There have been a lot of extensive researches done on SPR technique in the last few decades due to their possible application in biotechnology, biomedical sciences, chemical, biochemical sensing and environmental monitoring fields (Chau et al., 2018). This is owing to the beneficial features that it offers including simple sample preparation, high sensitivity, fast detection capability, and real-time detection (Rahman et al., 2018; Xue et al., 2019).

There are several approaches that have been introduced to generate SPR which includes grating coupled system, optical fiber system, and prism coupled system. Due to its high sensitivity and easy to be used, prism-based SPR is the most commonly used approach. This prism-based SPR can be categorized into two configurations which are Otto configuration and Kretschmann configuration. The Kretschmann configuration is normally used in most SPR applications, where a noble metal that carries a large number of free electrons, is placed at the interface of two dielectric media, as shown in Figure 1.5. Copper, gold, aluminum, and silver are some options of the noble metals in the fabrication of SPR sensor (Gupta & Verma, 2009). Among them, gold has been preferred by a lot of researchers as it is chemically stable. When monochromatic and p-polarized light beam hits on the metal thin film under total internal reflection conditions, SPR will eventually occur that can be observed by the reduction of the intensity of the reflected light at a certain incident angle (Pirvu & Manole, 2013).

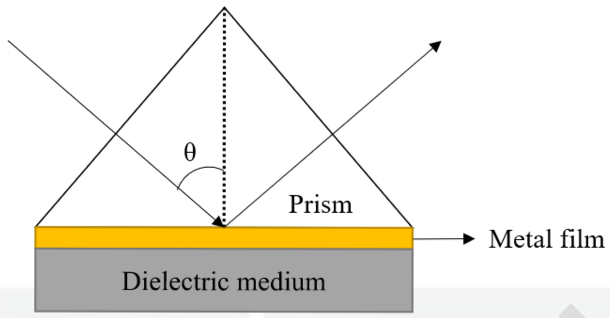

Figure 1.5: Kretschmann configuration of prism-based SPR, where a noble metal film is placed at the interface between prism and dielectric medium.

One of the most crucial things in the development of SPR sensor is the development of the active layer adjacent to the metal layer. The selection of a suitable material to be deposited on the surface of metal film is really important in order to improve the effectiveness and efficiency of the sensor as it will determines the sensitivity, specificity, and several other sensing parameters (Matsui et al., 2005).

1.6 Problem Statements

In recent years, interest in GQDs has surged due to their intriguing characteristics (Erkmen et al., 2021; Zhu et al., 2023). Similarly, cellulose-based biopolymers such as NaCMC and NCC have garnered significant attention for their applications in adsorbing toxic effluents and sensor development (Fu et al., 2015a; Kafy et al., 2016; Khalid et al., 2019; Tao et al., 2020). Thus, the synergy between GQDs and NaCMC or NCC has been explored in various studies. For instance, Javanbakht & Namazi (2018a) developed a hydrogel nanocomposite film using GQDs and NaCMC for applications in anticancer films and drug delivery systems. Additionally, a fluorescent

NCC-GQDs hydrogel has been used as an injectable material in 3D printing (Khabibullin et al., 2017). Despite extensive research on GQDs integrated with NaCMC and NCC, there remains a lack of comprehensive studies on the structural and optical properties of these composites. This limitation has inspired the exploration of the structural and optical properties of thin films comprising NaCMC-GQDs and NCC-GQDs.

Due to the widespread distribution of phenol, a toxic pollutant, in water systems, there has been a growing interest in developing various methods for its detection over the past decade. Optical methods have gained significant attention for detecting various analytes owing to their simplicity, speed, cost-effectiveness, and high sensitivity and selectivity compared to electrochemical and other modern techniques (Kumar et al., 2017). Among optical sensors, SPR stands out due to its real-time sensing capabilities, simple sample preparation, and label-free detection (Choi et al., 2014; Yanase et al., 2019). Nevertheless, SPR lacks the sensitivity needed for detecting low concentrations of phenol due to its similarity in refractive index. Hence, modifying the surface of the gold thin film with an active layer can overcome this limitation. GQDs- and NCC-based materials have demonstrated the capability to detect phenol at micromolar levels, while NaCMC-based materials can effectively detect industrial phenolic compounds with similar sensitivity. Thus, incorporating these materials, GQDs, NaCMC, NCC, NaCMC-GQDs, and NCC-GQDs, into SPR sensor could enhance its sensitivity for phenol detection. To date, no studies have reported on phenol detection using SPR sensor incorporated with these materials. Hence, it is of interest to study the potential sensing properties of GQDs, NaCMC, NCC, NaCMC-GQDs, and NCC-GQDs thin films for phenol detection using SPR technique.

One of the most important features to evaluate a sensor is the selectivity, which is the ability of a sensing material to identify a particular target analyte within a sample containing various mixtures and contaminants (Bhalla et al., 2016). However, the studies on the selectivity of SPR-based sensor for phenol detection are greatly limited. Only one study has reported the good selectivity of SPR sensor for phenol detection so far, achieved using a phenol-imprinted polymeric film (Derazshamshir, 2021). Therefore, this has become an initiative to investigate the selectivity of thin film-based SPR sensor towards phenol. The thin film with the best sensing performance for phenol detection among the developed GQDs, NaCMC, NCC, NaCMC-GQDs, and NCC-GQDs thin films, will be firstly verified. Subsequently, the best sensor thin film will be incorporated with Tyr, an enzyme known for its specificity in detecting phenolic compounds, to confirm the selectivity of the thin film for phenol detection using SPR spectroscopy.

1.7 Research Objectives

The main objectives of this study are summarized as follows:

1. To determine the structural and optical properties of the NaCMC-GQDs and NCC-GQDs thin films.
2. To analyze the sensing performance of the GQDs, NaCMC, NCC, NaCMC-GQDs, and NCC-GQDs thin films for phenol detection using SPR technique.
3. To verify the selectivity of the thin film with best sensing performance incorporated Tyr for phenol detection using SPR technique.

1.8 Thesis Outline

This thesis consists of five chapters. Chapter 1 introduces phenol, GQDs, cellulose-based biopolymers (NaCMC and NCC), Tyr, and SPR, along with the problem statements and objectives of this study. Chapter 2 explores the structural and optical properties of NaCMC and NCC modified graphene-based materials. Besides, this chapter also covers the past studies on GQDs-based materials, cellulose-based biopolymers, and SPR incorporated various materials for the detection of industrial phenolic compounds. Moving on, Chapter 3 explains the methodology of this study including the preparation of all materials and thin films, characterization techniques and experimental procedure. Next, Chapter 4 analyzes all of the obtained results from the characterization of the developed thin films for its structural, optical, and sensing properties and also includes its comprehensive explanations. Finally, Chapter 5 presents the conclusion of this study and offers the recommendations for future work.

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