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BIMETALLIC SILVER-GOLD NANOPARTICLES DECORATED REDUCED GRAPHENE OXIDE FOR NON-ENZYMATIC GLUCOSE DETECTION

NUR ATIKAH BINTI ALI

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By

NUR ATIKAH BINTI ALI

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

July 2020

DEDICATION

In appreciation of their patience, love, sacrifices, and encouragement, I want to dedicate my thesis to my beloved family, husband Mohd Taufik and my son Tariq Luqman for being understanding and support my Master journey.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chair : Faizah MD Yasin, PhD
Institute : Nanoscience and Nanotechnology

This research has employed a chemical reduction method in one pot reaction to obtained a bimetallic silver-gold nanoparticles with varying volume ratio (4:0 to 0:4) decorated reduced graphene oxide for non-enzymatic glucose detection. The morphological observation and other physicochemical properties were characterized using Raman Spectra, Ultra Violet Visible Spectra, X-Ray Powder Diffraction, and High Resolution Transmission Electron Microscopy, respectively.

Raman spectra analysis confirms the formation of GO and rGO. 3 reported band (D, G and 2D band) was observed in the region of 1350- 2713 cm^{-1} in all spectra, in which these band was used to interpreted disorder degree on intensity ratio of GO and rGO. UV-Visible spectra authenticated that, after GO was reduced to rGO, the absorption peak indicated there is an increase in electronic conjugation in the rGO hence suggesting the sp^2 hybridization in carbon network are restored. The colour changes from brown to black dispersion of GO to rGO was another proof of successfully reduction of GO to rGO. UV-Visible spectra of bimetallic silver-gold nanoparticles with varying volume ratio (4:0 to 0:4) decorated reduced graphene oxide, exhibited only one absorption peak and the maximum absorption peak red-shifted from 420 nm to 535 nm in wavelength, with increasing of gold ratio in Ag:Au (1:3), hence revealing the formation of an alloy structure, respectively. X-ray diffraction analysis suggested the formation of crystalline silver and gold nanoparticles at reduced graphene oxide surface, due to the characteristic peaks obtained for both metal have a same standard bragg reflection values. The surface morphology revealed that, GO and rGO both have a wrinkle morphology; meanwhile bimetallic silver-gold nanoparticles with varying volume ratio (4:0 to 0:4) were successfully decorated on the surface of reduced graphene oxide in the particle size ranges of 20 nm to 55 nm. These variations of the size

obtained for the nanoparticles are strongly dependent on the concentration used of two metal between silver and gold.

Finally, a non-enzymatic glucose sensor based on indium tin oxide glass electrode was successfully modified with bimetallic silver-gold nanoparticles decorated reduced graphene oxide with varying the volume ratio in the range 4:0 to 0:4. Cylic voltammetry studies on the modified electrode showed that combination of bimetallic silver-gold nanoparticles exhibited a great electrochemical activities toward glucose oxidation compared to monometallic silver and gold nanoparticles. However, among of the bimetallic silver-gold nanoparticles at 3:1,1:1 and 1:3, it was found that, when the Ag:Au at 1:3 ratio, the modified electrode give a remarkable increase in peak current density, followed by Ag:Au at 1:1 and 3:1 ratio. A chronoamperometry was conducted to study the peak current dependent of the modified electrodes on the glucose concentration, and the current response was found to increase linearly along with the glucose concentration increment from 0-18mM. The results obtained also revealed that, the amperometry response of the modified electrodes with combination of bimetallic silver-gold nanoparticles give a higher value of current response compared to monometallic silver and gold nanoparticles. In addition, these finding strongly indicated that the composition and the average particles size between silver and gold nanoparticles in bimetallic system plays a significant role in determining the electrochemical oxidation of glucose. Among of all modified electrodes, S4 electrode with Ag:Au at 1:3 ratio, the current response were magnify to 765.57 μA mM cm^{-2} and low detection limit of 1.3 μM at signal to noise ratio (*S/N = 3*). Hence, we may concluded this S4 electrode may serve as an ideal candidate to develop an enzymeless, fast and responsive for future glucose biosensors application.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

BI-LOGAM PERAK-EMAS DALAM ZARAH NANOPARTIKEL YANG DIHIASI DENGAN GRAPHENE OXIDA UNTUK PENGESANAN GLUKOSA BUKAN ENZIM

Oleh

NUR ATIKAH BINTI ALI

Julai 2020

Pengerusi : Faizah MD Yasin, PhD
Institut : Nanosains dan Nanoteknologi

Penyelidikan ini menggunakan kaedah penurunan kimia dalam reaksi satu periuk untuk mendapatkan bimetallik nanopartikel perak-emas dengan nisbah isipadu yang berbeza-beza (4:0 hingga 0:4) yang dihiasi oksida grafena yang diturunkan untuk pengesanan glukosa bukan enzim. Pemerhatian morfologi dan sifat fizikokimia yang lain dicirikan dengan menggunakan analisis Spektrum Raman, Spektrum Terlihat Ultra Violet, Pembelauan X-Ray, dan Mikroskopi Elektron Transmisi Resolusi Tinggi.

Analisis spektrum Raman mengesahkan pembentukan GO dan rGO. 3 jalur yang dilaporkan (jalur D, G dan 2D) diperhatikan di kawasan 1350-2713 cm^{-1} di semua spektrum, di mana jalur ini digunakan untuk menafsirkan tahap gangguan pada nisbah intensiti GO dan rGO. Spektrum terlihat Ultra Violet mengesahkan bahawa, setelah GO diturunkan menjadi rGO, puncak penyerapan menunjukkan terdapat peningkatan konjugasi elektronik dalam rGO sehingga menunjukkan hibridisasi sp^2 dalam rangkaian karbon telah dipulihkan. Perubahan warna larutan dari coklat ke hitam adalah antara bukti lain yang berjaya menurunkan GO kepada rGO. UV-spektrum bimetalik nanopartikel perak-emas dengan nisbah isipadu yang berbeza-beza (4:0 hingga 0:4) dihiasi oksida grafena yang diturunkan, hanya menunjukkan satu puncak penyerapan dimana puncak penyerapan maksimum berubah dari 420 nm hingga 535 nm di dalam panjang gelombang, dengan peningkatan nisbah emas dalam Ag:Au (1:3), sehingga menunjukkan pembentukan struktur aloi, masing-masing. Analisis pembelauan sinar-X mencadangkan pembentukan Kristal nanopartikel perak dan emas pada permukaan grafena oksida yang diturunkan kerana ciri puncak yang diperolehi untuk kedua-dua logam ini mempunyai nilai pantulan Bragg Standard yang sama. Permukaan morfologi menunjukkan bahawa, GO dan rGO kedua-duanya mempunyai morfologi kedutan; sementara itu bimetalik nanopartikel perak-emas dengan nisbah isipadu yang berbeza-beza (4:0 hingga 0:4) berjaya dihiasi pada permukaan grafena oksida yang diturunkan dalam ukuran zarah antara 20 nm hingga 55 nm. Variasi ukuran

yang diperolehi untuk nanopartikel sangat bergantung pada kepekatan larutan yang digunakan diantara dua logam perak dan emas.

Akhirnya, sensor glukosa bukan enzimatik berdasarkan elektrod kaca timah oksida indium berjaya diubah suai dengan bimetalik nanopartikel perak-emas dihiasi grafena oksida yang diturunkan dengan nisbah isipadu yang berbeza-beza dalam lingkungan 4:0 hingga 0:4. Kajian kitaran voltammetri pada elektrod yang diubah menunjukkan bahawa gabungan bimetalik nanopartikel perak-emas menunjukkan aktiviti elektrokimia yang hebat terhadap pengoksidaan glukosa berbanding dengan mono metalik nanopartikel perak dan emas. Walau bagaimanapun, di antara bimetalik nanopartikel perak-emas pada 3:1,1:1 dan 1:3, didapati bahawa, apabila nisbah Ag:Au pada 1:3, elektrod yang diubah memberikan peningkatan yang luar biasa dalam kepadatan arus puncak , diikuti oleh Ag:Au pada nisbah 1:1 dan 3:1. Kronoamperometri dilakukan untuk mengkaji arus puncak yang bergantung pada elektrod yang diubah pada kepekatan glukosa, dan tindak balas semasa didapati meningkat secara linear seiring dengan kenaikan kepekatan glukosa dari 0-18 mM. Hasil yang diperoleh juga menunjukkan bahawa, tindak balas amperometrik elektrod yang diubah dengan kombinasi bimetalik nanopartikel perak-emas memberikan nilai tindak balas arus yang lebih tinggi berbanding mono metalik nanopartikel perak dan emas. Di samping itu, penemuan ini menunjukkan bahawa komposisi dan ukuran zarah antara nanopartikel perak dan emas dalam sistem bimetalik memainkan peranan penting dalam menentukan pengoksidaan elektrokimia glukosa. Di antara semua elektrod yang diubah, elektrod S4 dengan nisbah Ag:Au pada 1:3, mempunyai tindak balas semasa yang paling tinggi, iatu pada 765.57 μA mM cm^{-2} dan had pengesanan rendah 1.3 μM (S/N = 3). Oleh itu, kita dapat menyimpulkan bahawa elektrod S4 ini dapat diaplikasikan sebagai calon ideal dalam penghasilan sensor glukosa tanpa enzim, cepat dan responsif untuk aplikasi biosensor glukosa masa depan.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the Master of Science. The members of the Supervisory Committee were as follows:

Faizah binti Md Yasin, PhD
Senior Lecturer
Faculty of Chemical & Environmental Engineering
Universiti Putra Malaysia
(Chairman)

Shafreeza binti Sobri, PhD
Senior Lecturer
Faculty of Engineering
Universiti Putra Malaysia
(Member)

____

ZALILAH MOHD SHARIFF,PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 14 October 2021

Declaration by graduate student

I hereby confirm that:

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Name and Matric No: Nur Atikah Binti Ali,

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This is to confirm that:

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Signature: ______________________________
Name of Chairman of Supervisory Committee: Dr. Faizah Binti Md Yasin

Signature: ______________________________
Name of Member of Supervisory Committee: Dr. Shafreeza binti Sobri

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LIST OF ABBREVIATIONS AND SYMBOLS

GO	Graphene Oxide
rGO	reduced Graphene Oxide
NPs	Nanoparticles
DM	Diabetes Mellitus
RS	Raman Spectroscopy
UV-VIS	Ultra Violet-Visible Spectroscopy
XRD	X-Ray powder Diffraction
FESEM	Field Emission Scanning Electron
HRTEM	High Resolution Transmission Electron Microscope
ITO	Indium Tin oxide
EDX	Energy Dispersive X-Ray
MWCNTs	Multi-Walled Carbon Nanotubes
SWCNTs	Single-Walled Carbon Nanotubes
CNTs	Carbon Nanotubes
fcc	Face centre cubic
Mm	Micrometer
^{o}C	Degree Celcius
T	Temperature
V	volt
π	Pi
E_p	Peak potential
0D	Zero Dimensional
1D	One Dimensional

2D	Two Dimensional
3D	Three Dimensional
CVD	Chemical Vapor Deposition
cm	centimetre
mg	milligram
mM	milimolar
μM	micromolar
I_D	Intensity of D band
I_G	Intensity of G band
SERS	Surface Enhance Raman Spectroscopy
SPR	Surface plasmon Resonance
LOD	Limit of detection
mΩ cm	milliOhm centimetre
mL	millilitre
nm	nanometer
Ag	Silver
Au	Gold
PBS	Phosphate Buffer Saline
CA	Chronoamperometry
CV	Cyclic Voltammetry
GO_x	Glucose oxidase
ARM	Ames Reflectance Meter
CGM	Continuous glucose monitoring
JCPDS	Joint Committee Powder Diffraction Sheet
Ag/AgCl	Silver/Silver Chloride
CL	Chemiluminescence

FET	Field Effect Transistors
FRET	Fluoroscence Resonance Energy Transfer
CCG	Chemically converted graphene
CMG	Chemically modified graphene

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Carbon-based materials become a vital role in nanoscience and nanotechnology in which they primarily engaged with the synthesis, characterization, exploration and exploitation of nanomaterial's (Arthi G & BD, 2015). Carbon not only has a generous non-metal elements quantity available in the earth crust, but its extraordinary properties make it become sufficiently notable. There are many forms of carbon allotropes such as amorphous carbon, graphite, diamond, and continue with fullerenes, carbon nanotubes and graphene. They have a different physical and chemical properties depending on the hybridization of their carbon atoms. For example, diamond has sp^3 and graphite has sp^2 hybridized carbon atoms in which diamond may form 3D network meanwhile graphite form layer of 2D planar sheets (Ravi & Vadukumpully, 2015).

An interesting carbon allotrope, graphene has made significant progress among researchers following the discovery of graphene by Geim and Novoselov in 2004. Graphene is a flat monolayer of sp^2 bonded carbon atom that tightly integrated into the two-dimensional (2D) honeycomb lattice structure and considered to be thin material in the universe (Novoselov et al., 2004; Rather, Pilehvar, & De Wael, 2014). Due to outstanding properties such as large surface area ($2630 m^2/g$), high electrical conductivity, chemical stability, and low cost (W. Zhou et al., 2015), it has made graphene an ideal substrate for stabilizing, anchoring metal nanoparticles (J. Luo, Zhang, Lai, Liu, & Liu, 2015) and a rising element for electrochemical sensors. In sensing application, graphene is not only considered an ideal material, its high surface area and superior electrical conductivity plays a vital role to improve the sensing performances (Qiao & Zheng, 2012). However, graphene sheets have a tendency to form the interaction between sheets, which may affect the application in electrochemistry, and electro analysis. Therefore, a reduced graphene oxide (rGO) has been introduced to become a suitable support for metal nanoparticles as they offering high surface area to distributed more number of nanoparticles to immobilized the biomolecules and prevent them from agglomerates, hence improving the catalytic, optical, electronic, and magnetic properties of metallic nanoparticles (Boomi, Prabu, & Mathiyarasu, 2013; L. Li et al., 2014).

In recent years, various articles related to electrochemical sensors especially glucose biosensors have been published abruptly in research community due to their unique features such as high sensitivity, low cost of fabrication, ease operation, fast response time and excellent potential for miniaturization and construction for portable glucose sensing in point-of-care application (Dutta, Sahoo, Chattopadhyay, & Ghosh, 2016). In 1962, Clark and Lyons become the first researchers developed the enzymes electrode. They modified the electrode surface with a glucose sensitive enzyme that reduces oxygen to hydrogen peroxide which is observed amperometrically (L.C. Clark &

Lyons, 1962). Due to it succeeds in the study, in-depth research on the glucose biosensors have got a very deep concern among researchers and have been used widely in glucose biosensors fabrication.

Even though enzymatic glucose biosensors known to have a good selectivity and high sensitivity, it can be affected easily against environmental factor such as temperature, pH, humidity, toxic chemical and have a short life span (Thanh, Balamurugan, Hwang, Kim, & Lee, 2016). In order to overcome these issues, much attention has been focused on the development of non-enzymatic glucose biosensors, which directly oxidized glucose at various metal electrodes. It has many benefits that will prevent the drawbacks of enzyme glucose sensors.

Recently, published papers on glucose sensors introduced various nanostructures such as nanoparticles, nanowires, nanorods, nanotubes and films. However, noble metal nanoparticles such as gold, silver, platinum, copper, etc have been extensively used for electrode surface modification as they serve as an active site to enhance the electro catalysis of the sensors (Hwang, Lee, Seo, & Chung, 2018a). Although noble metal nanoparticles offer good features, however, bimetallic nanoparticles also capable of promising multi-functional features, as they comprise a combination of two different metals. Therefore, it has great potential from both technological points and scientific advances (Mandal, Baranwal, Srivastava, & Chandra, 2018; Zaleska-Medynska, Marchelek, Diak, & Grabowska, 2016). The synergistic effect that exists between the two metals in bimetallic nanoparticles not only enhance existing properties, but also creates new features (Gu et al., 2011; Wang et al., 2008). For example, An investigation of bimetallic nanoparticles such as (PtAu, PtPb, PtIr) have been applied to adjust the electrodes as they are not only owing a higher selectivity and catalytic properties (Y. Li, Niu, Tang, Lan, & Zhao, 2014), but also stimulated electron transfer, and magnified the conductivity (Long, Zhang, Wang, Yan, & Zhou, 2015). Hence, these advantages could greatly improve the sensitivity of the fabricated non-enzymatic glucose biosensors.

It is known that gold (Au) and silver (Ag) nanoparticles have received a remarkable evolution in the area of biosensors, optics, drug delivery, catalysis and electrochemistry (Meena Kumari, Jacob, & Philip, 2015). Silver nanoparticles not only own a local surface plasmon resonance but they also have distinctive application prospect in surface enhanced Raman spectroscopy (SERS) and nonlinear optics (L. Zhou et al., 2013). Furthermore, gold and silver have the same lattice parameter and the face-centered crystal structure (fcc) of both nanostructures promote the easy synthesis of Au-Ag alloy/core-shell structures. Therefore, investigations on the use of Ag and Au bimetallic nanoparticles (NPs) for decoration on reduced graphene oxide are needed to determine the sensitivity of glucose detection as Ag and Au shown a remarkable catalytic performance due to their synergistic effects and electronic effects (P. C. Pandey, Singh, & Pandey, 2015). Hence, this study aims to investigate the synthesis, characterization, and optimization of the ratio between silver-gold nanoparticles decorated reduced graphene oxide towards various glucose concentrations for sensor detection.

1.2 Problem Statement

Nowadays, bimetallic nanoparticles decorated rGO has been the main focus of studies relating to sensing applications especially in glucose biosensors due to the superior synergistic effects occurred between two metals compared to the monometallic nanoparticles (Y. Liu et al., 2015). However, in depth study on the different composition ratio between bimetallic silver and gold nanoparticles decorated rGO in sensing applications has not yet received a comprehensive attention in literature. Research has shown that this parameter very important to study the growth of bimetallic silver and gold nanoparticles such as morphology and particle sizes at the surface of reduced graphene oxide, in which this parameter later on contributes to the glucose sensors performance liability and selectivity of the modified electrode. Thus, the investigation of the composition ratio between two metal nanoparticles (silver and gold) have been a subject of considerable interest to make a comparison between monometallic silver, monometallic gold, bimetallic silver-gold with more silver content, bimetallic silver-gold with equal amount and bimetallic silver-gold with more gold content for the control of nanoparticles growth and yield at rGO surface.

Recently, increasing health-related issues especially Diabetes Mellitus (DM) has threatens the lives of peoples around the world. In the biosensor market, this problem become a major milestone for the development of fast, responsive and accurate monitoring glucose biosensors and become importance in diabetic management. Therefore, this study proposed the used of the synthesized mono and bimetallic silver gold nanoparticles decorated rGO at different composition ratio, modified indium tin oxide glass electrode as a sensor materials for non-enzymatic glucose detection. As we acknowledge, to date there is only a few study on this parameter towards non-enzymatic glucose detection. It was found, the combination of bimetallic nanoparticles actually possessing an excellent electrochemical properties due to their synergy effect compared to the monometallic nanoparticles and widely studied and applied in the fabrication of electrochemical sensors (Arvinte, Crudu, Doroftei, Timpu, & Pinteala, 2018). Therefore, with this focus of the study, we will be able to determine the selectivity and sensitivity of each modified electrodes toward various glucose concentration, hence suggesting which bimetallic silver-gold nanoparticles composition ratio modified indium tin oxide electrode are most sensitive toward glucose detection and to be serve as a platform for future glucose biosensors development and fabrication.

Moreover, most of the glucose sensors in the market employed glucose oxidase enzymes due to their high selectivity and stability toward glucose molecules (Hwang, Lee, Seo, & Chung, 2018b). However, below pH 2 or above pH 8, it quickly loses its activity and permanently damaged at temperature over 40^0C. Furthermore, exposure of GO_x enzymes to unstable humidity due to low activity causes it to depend on the concentration of oxygen around it and will seriously influence the sensor performance (Wilson & Turner, 1992). Meanwhile most of the non-enzymatic glucose sensors are free of oxygen limitations and beneficial from structural simplicity and quality control for mass production. To overcome these shortcomings, a continuous research effort on non-enzymatic glucose sensors have been employ and use in this study in order to improve the weakness mentioned in enzymatic glucose sensors.

1.3 Objectives

The objectives of this study are as follows:

i. To synthesis and characterized silver-gold nanoparticles decorated reduced graphene oxide at different volume ratio in the range of 4:0 until 0:4 on the shapes, size and morphologies.
ii. To investigate the effect of silver-gold nanoparticles decorated reduced graphene oxide at different volume ratio in the range of 4:0 until 0:4 on electrochemical properties toward glucose detection.

1.4 Scope of the Study

i. To synthesis graphene oxide (GO) using Improved Hummers method.
ii. To synthesis reduced graphene oxide (rGO) and silver-gold nanoparticles decorated reduced graphene oxide with various of Ag:Au volume ratio using chemical reduction method; in which sodium citrate was used as a reducing agent.
iii. To characterized the physicochemical properties of graphene oxide, reduced graphene oxide, silver-gold nanoparticles with various of Ag:Au volume ratio decorated reduced graphene oxide by Raman spectroscopy (RS), UV-Visible spectra (UV-Vis), X-ray powder diffraction (XRD), and High resolution transmission electron microscopy (HRTEM).
iv. To develop silver-gold nanoparticles decorated reduced graphene oxide on indium tin oxide (ITO) glass electrode surface by drop casting method.
v. To apply the fabricated working electrode in the detection of glucose by varying its concentration.

1.5 Outline of the thesis

This thesis is divided into six chapters. Chapter 1 is a brief introduction of the related research field and gives background on the study of graphene, graphene oxide (GO), reduced graphene oxide (rGO), bimetallic nanoparticles (NPs), decoration of bimetallic nanoparticles on reduced graphene oxide, synthesis and their applications in glucose biosensors. The problem statement, objectives and scope of the study are also stated in this chapter. Chapter 2 will be presented a literature review relating to the definition, formation, and properties of graphene, structure and method to synthesis graphene oxide and reduced graphene oxide and their applications. Apart from that, an introduction on bimetallic nanoparticles, silver and gold nanoparticles, fact about diabetes mellitus, the development of the electrochemical sensors, indium tin oxide working electrode, and application of the bimetallic nanoparticles based reduced graphene oxide in glucose biosensor will be discussed.

In chapter 3, the detailed on the material and chemical used, instrumentation, experimental methodology to synthesis graphene oxide from graphite powder, synthesis of reduced graphene oxide, synthesis of silver-gold nanoparticles at various volume ratio (0:4 to 4:0) decorated reduced graphene oxide, fabrication of indium tin oxide glass as working electrode with nanohybrids, how to prepare phosphate buffer saline solution, and electrochemical studies by using cyclic voltammetry and amperometry for the working electrode will be presented. Chapter 4 will discuss the characterization of the synthesized graphene oxide, reduced graphene oxide; and silver-gold (0:4 to 4:0) nanoparticles decorated reduced graphene oxide hybrids by using UV-Visible spectroscopy, Raman spectra, XRD analysis and HRTEM image with EDX analysis.

Chapter 5 will study and discuss the electrochemical activity of the nanohybrids decorated reduced graphene oxide with and without glucose by using cyclic voltammetry technique. Meanwhile, a various concentration range between 0 mM to 18 mM will be used to study the amperometry detection toward glucose concentration. In this part, a linear range, limit of detection and sensitivity of the glucose sensors will be determined. Finally, chapter 6 will includes a general conclusions and recommendations for future research.

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