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DEVELOPMENT OF A GRAPHENE BASED IMMUNO-BIOSENSOR FOR HEPATITIS B VIRUS SURFACE ANTIGEN DETECTION

GERALDINE CHAN SUE CHING

Thesis submitted in fulfilment of the requirements for the award of the degree Master of Engineering (Chemical)

Faculty of Chemical and Natural Resources Engineering

UNIVERSITI MALAYSIA PAHANG

APRIL 2016

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LIST OF ABBREVATIONS

Ab	Antibody
ACH	Adrenal cortical hormones
AFP	α-1-fetoprotein
AH	Aluminium hydroxide
APTES	3-Aminopropyl-triethoxysilane
Au@MGN	Multifunctional graphene nanocomposites
AuNP	Gold nanoparticles
BSA	Bovine serum albumin
BoNT/A	Botulinum neurotoxin serotype A
CEA	Carcinoembryonic antigen
CMG	Chemically modified graphene
CNT	Carbon nanotube
CuS	Copper monosulfide
CV	Cyclic voltammetry
CVD	Chemical vapour deposition
DI	Deionized
DNA	Deoxyribonucleic acid
DPV	Differential pulse voltammetry
EBA	Expanded bed adsorption chromatography
EIA	Enzyme immunoassay
EIS	Electrochemical impedance spectroscopy
ELISA	Enzyme-linked immunosorbent assay
FESEM	Field emission scanning electron microscopy
FET	Field effect transistor

FRET	Fluorescence resonance energy transfer
FTIR	Fourier transform infrared spectroscopy
GBP	Gold-binding polypeptide
GCE	Glassy carbon electrode
GO	Graphite Oxide
GOx	Glucose oxidase
GNWs/GO	Gold nanowire functionalized graphene sheets
HBcAg	Hepatitis B core antigen
HBsAb	Hepatitis B surface antigen antibody
HBsAb-HRP	Hepatitis B surface antigen antibody conjugated with HRP
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
hCG	Human chorionic gonadotropin
HRP	Horseradish peroxidase
Ig	Immunoglobulin
IL	Ionic liquid
IR	Infrared
ITO	Indium tin oxide
MGN	Magnetic graphene nanocomposites
MLG	Multilayer graphene
MWCNT	Multiwalled carbon nanotube
NADH	H_2O_2/β-nicotinamide adenine dinucleotide
NaPBS	Sodium phosphate buffered saline
NASBA	Nucleic acid sequence-based amplification
NG	Nitrogen doped graphene

NMR	Nuclear magnetic resonance
OPV	Organic photovoltaic cells
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDDA	Polydiallyldimethylammonium
PDMS	Poly(dimethysiloxane)
PSE	1-pyrenebutyric acid N-hydroxysuccinimide ester
PTFE	Polytetrafluoroethylene
RGO	Reduced graphene oxide
RIA	Radio immunoassay
RNA	Ribonucleic acid
RT	Room temperature
SEM	Scanning electron microscopy
SPR	Surface plasmon resonance
TEM	Transmission electron microscopy
TMB	3,3’,5,5’-Tetramethylbenzidine
TPA	Tissue polypeptide antigen
USA	United States of America
UV	Ultraviolet
UV-Vis	Ultraviolet-visible spectroscopy
WHO	World Health Organisation

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ABSTRACT

Hepatitis B virus (HBV) is a blood-borne and transfusion-transmitted human pathogen that has a large impact on blood safety and public health worldwide whereas reduced graphene oxide (RGO) is a derivatives of graphene which has gain much attention in electrochemical immunosensors. HBV infects the liver and causes chronic and acute Hepatitis. The best treatment for this disease is for the early detection before the occurrence of severe liver damages. The presence of Hepatitis B surface antigen (HBsAg) is evidence of presence of HBV infections. Presently, the conventional methods shows weakness in terms of time and efficiency. In this research, it aims to immobilize the HBsAg antibody (HBsAb) onto functionalized RGO as well as to study the interaction between HBsAb and HBsAg by electrochemical probe of the functionalized RGO. RGO was synthesized via modified Hummer's methods and reduced using hydrazine hydrate. RGO is functionalized with nafion and thionine prior to immobilization of HBsAb. Qualitative analysis of functionalized RGO was conducted via ultraviolet-visible spectrophotometry (UV-Vis), fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and field emission scanning electron microscopy (FESEM). Electrochemical analysis of functionalized RGO probe electrode on the detection of HBsAg was done via cyclic voltammetry (CV). As a conclusion, successful immobilization of HBsAg on functionalized RGO was proven to obey Freundlich adsorption isotherm with maximum adsorption capacity of 31 ng HBsAg/g and further confirmed with the change in surface structure observed as well as the presents of functional groups detected. Interaction between HBsAg and HBsAb causes the colour change when tetramethylbenzidine (TMB) was added and step changes to occur during CV with a limit of detection at 0.5 ng/mL.

ABSTRAK

HBV berjangkit melalui pemindahan dan transfusi darah yang mempunyai kesan yang besar terhadap keselamatan darah dan kesihatan awam di seluruh dunia manakala RGO adalah variasi graphene yang telah menarik perhatian ramai para penyelidik dalam pelbagai bidang. HBV menjangkiti hati dan menyebabkan Hepatitis kronik dan akut. Rawatan yang terbaik untuk penyakit ini adalah pengesanan awal sebelum berlakunya kerosakan hati yang teruk. Jangkitan HBV dikesan melalui pengesanan HBsAg. Pada masa kini, kaedah pengesanan HBV konvensional menunjukkan kelemahan dari segi masa dan kecekapan. Kajian ini bertujuan untuk mempekenalkan HBsAb ke atas RGO yang telah difungsikan serta untuk mengkaji interaksi antara HBsAb dan HBsAg dengan menggunakan kaedah electrokimia. RGO telah dihasilkan melalui kaedah Hummer yang telah diubahsuaikan dan melalui penurunan dengan menggunakan agen penurunan hydrazine hidrat. RGO difungsikan dengan nafion dan thionine sebelum diperkenalkan dengan HBsAb. Analisis kualitatif ke atas RGO yang telah difungsikan adalah melalui ultraviolet-visible spektrofotometri (UV-Vis), spektrokopi Fourier transform infrared (FTIR), mikroskop imbasan elektron (SEM), dan 'field' emisi mikroskop imbasan elektron (FESEM). Analisis elektrokimia dijalankan dengan menggunakan elektrod yang mengandungi RGO yang telah difungsikan untuk mengesan HBsAg melalui voltammetry kitaran (CV). Had pengesanan diperolehi ialah kira-kira 0.5 ng / mL. Tambahan pula, ekuilibrium penjerapan isoterma RGO yang telah difungsikan dan HBsAg didapati mematuhi Freundlich penjerapan isoterma dengan kapasiti maksimum, 31 ng HBsAg / g.