SIMULTANEOUS DETERMINATION OF ORGANIC ANALYTES BY REDUCED GRAPHENE OXIDE-AZO DYES/GOLD NANOPARTICLES MODIFIED GLASSY CARBON ELECTRODE

NUSIBA MOHAMMED MODAWE ALSHIK EDRIS

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By

NUSIBA MOHAMMED MODAWE ALSHIK EDRIS

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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September 2019

Chairman : Associate Professor Yusran Sulaiman, PhD
Faculty : Science

The detection of biological molecules and monitoring of environmental contaminants is a demanding aspect in the field of analysis. Electrochemical sensors are one of the tools that attracted great attention in this field due to their applicability. In the present research work, glassy carbon electrode (GCE) was modified by electrochemically reduced graphene oxide (ERGO) with two different azo dyes i.e. poly(eriochrome black T) (pEBT) and poly(Procion Red 5-MX) poly (PR) and gold nanoparticles (AuNPs) to obtain AuNPs/ERGO-pEBT/GCE and AuNPs/ERGO-poly(PR)/GCE electrochemical sensors. The modified sensors were characterised by field scanning electron microscopy (FESEM), Fourier transform infrared spectroscopy (FTIR), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). The resultant modified sensors revealed elevated electrocatalytic properties in comparison to other modified GCEs. The prepared nanocomposites were used to simultaneously determine ascorbic acid (AA), dopamine (DA), and uric acid (UA) as well as hydroquinone (HQ), catechol (CC), and resorcinol (RC) in phosphate buffer solution (PBS). Due to the synergistic effect, the modified electrodes had excellent electrochemical catalytic activity towards the oxidation of two sets of analytes which allowed their simultaneous determination.

The AuNPs/ERGO-pEBT/GCE showed good potential peak separations of 166 and 126 mV for DA-AA and UA-DA respectively when it was immersed in a mixture solution of AA, DA, and UA. Simultaneous detection of HQ, CC, and RC was performed with potentials separation of 111 and 383 mV for HQ-CC and CC-RC, respectively, thus acknowledged the simultaneous detection of these isomers.

In the same manner, AuNPs/ERGO-poly(PR)/GCE was used to simultaneously determine AA, DA, and UA where, the potential separations between DA-AA and UA-DA were found to be 210 and 140 mV, respectively. The modified electrode (AuNPs/ERGO-poly(PR)/GCE) has also been utilised in the simultaneous detection of HQ, CC, and RC. DPV was carried out and the peak potential separation between HQ-CC was 130 mV and CC-RC was 400 mV.

AuNPs/ERGO-pEBT/GCE revealed limit of detections (LODs) of 530, 9, and 46 nM, with sensitivities of 0.003, 0.164, and 0.034 μA/μM, when applied in simultaneous detection of AA, DA, and UA. Whereas, the determination of HQ, CC and RC acquired sensitivity of 0.04, 0.78, and 0.15 μA/μM and LODs of 15, 8, and 39 nM, respectively. The obtained LODs for AA, DA, and UA at AuNPs/ERGO-poly(PR)/GCE were 54, 5.6, and 5.8 nM, respectively. Sensitivity of this electrode in AA, DA, and UA mixture solution was 0.63 μA/μM for UA, 0.40 μA/μM for DA, and 0.48 μA/μM for AA. The three isomers; HQ, CC, and RC showed sensitivies of 4.61, 4.38, and 0.56 μA/μM supplemented by LODs of 53, 53, and 79 nM at the same electrode in their ternary.

Both AuNPs/ERGO-pEBT/GCE and AuNPs/ERGO-poly(PR)/GCE showed prominent selectivity toward the analytes (AA, DA, UA and HQ, CC, RC). In addition, no interference effect with possible co-existence ions and compounds that could hinder the analyte detection as well as presenting good repeatability, reproducibility and favourable stability. The as-proposed electrochemical sensors were applied successfully with outstanding recoveries in urine samples, vitamin C tablets and synthetic wastewaters analysis.

The modified electrode AuNPs/ERGO-pEBT/GCE offered enhanced efficiency in the simultaneous determination of dihydroybenzene isomers, while the electrode AuNPs/ERGO-poly(PR)/GCE is demonstrated superior performance towards determination of UA,DA, and AA.

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

PENENTUAN SERENTAK ANALIT ORGANIK OLEH ELEKTROD KARBON KACA TERUBAHSUAI GRAFIN OKSIDA TERTURUN-PEWARNA AZO/ NANOPARTIKEL EMAS

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Pengesanan molekul biologi dan pemantauan pencemaran alam sekitar adalah aspek yang sangat diperlukan dalam bidang analisis. Sensor elektrokimia adalah salah satu alat yang menarik perhatian dalam bidang ini kerana kebolehgunaannya. Dalam penyelidikan ini, elektrod karbon kaca (GCE) diubahsuai dengan mengelektropolimerkan grafen oksida (ERGO) dengan dua pewarna azo yang berbeza iaitu poli(eriochrome black T) (pEBT) dan poli(Procion Red 5-MX) poli(PR) dan nanopartikel emas (AuNPs) untuk mendapatkan sensor elektrokimia AuNPs/ERGO-pEBT/GCE dan AuNPs/ERGO-poli (PR)/GCE. Sensor yang telah diubah suai dicirikan dengan menggunakan pengimbasan medan mikroskop elektron (FESEM), spektroskopi Fourier inframerah (FTIR), spektroskopi impedans elektrokimia (EIS) dan voltammetri kitaran (CV). Sensor yang telah diubahsuai tersebut mendedahkan sifat elektrokatalik yang tinggi berbanding GCE yang diubahsuai lain. Nanokomposit yang dihasilkan telah digunakan untuk menentukan kehadiran asid askorbik (AA), dopamina (DA), dan asid urik (UA) serta hidrokuinon (HQ), katekol (CC) dan resorsinol (RC) dalam larutan fosfat penimbal (PBS) secara serentak. Oleh kerana kesan sinergistik, elektrod yang telah diubahsuai mempunyai aktiviti pemangkin elektrokimia yang sangat baik ke arah pengoksidaan dua set analit yang membolehkan penentuan serentak.

AuNPs/ERGO-pEBT/GCE menunjukkan keupayaan pemisahan puncak 166 dan 126 mV untuk DA-AA dan UA-DA apabila direndam dalam campuran AA, DA, dan UA. Pengesanan serentak HQ, CC, dan RC dilakukan dengan pemisahan keupayaan 111 dan 383 mV untuk HQ-CC dan CC-RC, dengan itu mengakui pengesanan serentak isomer ini.

Menerusi kaedah yang sama, AuNPs/ERGO-poly (PR)/GCE digunakan secara serentak untuk menentukan AA, DA, dan UA di mana, keupayaan pemisahan antara DA-AA dan UA-DA adalah 210 dan 140 mV. Elektrod yang telah diubahsuai (AuNPs/ERGO-poly(PR)/GCE) juga telah digunakan untuk megesan s HQ, CC, dan RC secara serentak. DPV telah dijalankan dan diadapati keupayaan pemisahan puncak di antara HQ-CC adalah 130 mV dan CC-RC adalah 400 mV.

AuNPs/ ERGO-pEBT/GCE menunjukkan had pengesanan (LOD) 530, 9, dan 46 nM, dengan sensitiviti 0.003, 0.164, dan 0.034 μA/μM, apabila digunakan dalam mengesan AA, DA, dan UA secara serentak. Manakala penentuan HQ, CC dan RC memperoleh kepekaan 0.04, 0.78, dan 0.15 μA/μM dan LOD sebanyak 15, 8, dan 39 nM. LOD yang diperolehi untuk AA, DA, dan UA menggunakan elektrod AuNPs/ERGO-poly (PR)/GCE adalah 54, 5.6, dan 5.8 nM. Sensitiviti elektrod ini di dalam campuran AA, DA, dan UA adalah 0.63 μA/μM untuk UA, 0.40 μA/μM untuk DA, dan 0.48 μA/μM untuk AA. Tiga isomer; HQ, CC, dan RC menunjukkan sensitiviti 4.61, 4.38, dan 0.56 μA/μM ditambah oleh LOD 53, 53, dan 79 nM pada elektrod yang sama dalam ternari mereka.

Kedua- dua AuNPs/ERGO-pEBT/GCE dan AuNPs/ERGO-poli(PR)/GCE menunjukkan selektiviti yang bagus terhadap semua analit yang digunakan (AA, DA, UA dan HQ, CC, RC). Sebagai tambahan, tiada kesan gangguan dengan kemungkinan kehadiran ion-ion dan sebatian yang boleh menganggu pengesanan analit serta kedua – dua elektrot tersebut menujukkan kebolehan ulangan, kebolehan penghasilan semula dan kestabilan yang bagus. Sensor elektrokimia yang dicadangkan telah berjaya digunakan dengan jayanya dalam sampel air kencing, tablet vitamin C dan analisis air buangan sintetik.

Elektrod AuNPs/ERGO-pEBT/GCE menawarkan kecekapan yang lebih baik dalam penentuan serentak isomer dihidrobenzena, manakala elektrod AuNPs/ERGO-poli(PR)/GCE menunjukkan prestasi unggul terhadap penentuan UA, DA, dan AA.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment 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

0 D	zero dimensional
1 D	one dimensional
2 D	two dimensional
3 D	three dimensional
3-TPA	3-thiophenemalonic acid
3-DGH	three dimensional graphene hydrogel
[C12mim]Br	1-dodecyl-3-methylimidazolium bromide
AGR	activated graphene
AA	ascorbic acid
AAS	atomic absorption spectroscopy
Ar	Argon gas
Aza	azure
ß-CD	ß-cyclodextrin
BPB	bromophenol blue
CC	catechol
CDDA acid	3-(5-chloro-2-hydroxyphenylazo)-4,5-dihydroxynaphthalene-2,7-disulfonic
CDs	carbon dots
CDs	carbon dots
CFP	carbon fiber paper
CILE	carbon ionic liquid electrode
CTAB	hexadecyl trimethyl ammonium bromide
CV	cyclic voltammetry
DA	dopamine
DHA	dehydroascorbic acid
DPV	differential pulse voltammetry
EBT	eriochrome black T
EIS	electrochemical impedance spectroscopy
Fc	ferrocene
FESEM	field scanning electron microscopy
ERGO	electrochemically reduced graphene oxide
FTIR	Fourier transform infrared
GCE	glassy carbon electrode
GF	graphene fibers
Au-NPs	gold nanoparticles
AuNCs	gold nanocluster
Gr	graphene
r-GO	reduced graphene oxide
RGO	reduced graphene oxide
GO	graphene oxide
GNR	Graphene nanoribon
GPE	Graphene paste electrode
GNS	graphene nanosheets
GS	graphene sheet
HQ	hydroquinone
HPLC	high performance spectroscopy
MFGSs	modified functionalised graphene sheets

MWCNTs	multiwalled carbon nanotubes
Nano-Au	N anogold
NRCu	nanospberry copper
NSs	nanosheets
OPFP	N-octyl-pyridinium hexafluorophosphate
P-4-ABA	poly 4-aminobutyric acid
p-AMT	poly 5-amino-2-mercapto-1,3,4-thiadiazole
PAR	4-(2-Pyridylazo)-Resorcinol
PB	Prussian blue
PBS	phosphate buffer solution
PDDA	poly(diallyldimethylammonium chloride)
Poly(EBT)	poly eriochrome black T
PSA III	poly(sulfonazo III)
PR	Procion Red 5-MX
PVA	poly vinyl alcohol
POCT	point-of-care testing
PPy	polypyrrole
PANI	polyaniline
PG	porous graphene
PEDOT	poly(3,4-ethylenedioxythiophene)
P-rGO	pristine reduced graphene oxide
PGA	polyglutamic acid
Poly ACBK	acid chrome blue K
PSS	poly-(styrenesulfonate)
PSFM	sulfonazo III,2,7-bis(2-sulfophenylazo) chromotropic acid tetra sodium salt
RC	resorcinol
RF	ratio frequency
SPE	screen-printed electrode
SWV	square-wave voltammetry
SWCNT	single walled carbon nanotubes
TH	thionine
TMDC	transition metal dichalcogenides
UA	uric acid

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The 21st century has been accompanied by various challenges. Some of these challenges include; the need to promote the health and well-being of an increasing number of populations, which can be resolved by providing clinical treatments and implementing clean water resources free of contaminants. Environmental conflicts are becoming a key focus for governments and researchers as a result of the growth in the world´s population, the increase in rural and manufacturing movement, air contamination, soil, and marine environment, and worldwide climatic variation. Global potential work has been developed to recognise the stimulation of human's behaviour toward the planet and integrates modern technologies. These efforts tend to minimise food garbage, and industrial by-products, which have influenced in both the environment and human health.

The detection of biomolecules, such as neurotransmitters and vitamin biomarkers provide a basic understanding of various biological and physiological processes that, in turn, help in the diagnosis of the diseases. Ascorbic acid (AA), dopamine (DA), and uric acid (UA) are essential molecules that significantly affect the biological activities of most mammalians. Therefore, the development of a fast and reliable approach to monitor and detect these compounds in biological fluids is a demand (Mazloum-Ardakani *et al.*, 2009, Tığ, 2017).

Environmental pollution is one of the main concerns in modern times, it is essential to find unusual platforms for rapid detection of contamination. Dihydroxybenzene isomers are non-biodegradable phenolic isomers that are very toxic even at very low concentrations. These compounds are among the major environmental pollutants (Li *et al.*, 2014). Hydroquinone (HQ), catechol (CC), and resorcinol (RC) have been found in effluents and could release to water from various industries for example textile, paper, and pulp, steel, petrochemical, petroleum refinery, rubber, dye, plastic, pharmaceutical, cosmetic, etc. and within the wastewater of manufactured coal fuel transformation operations (Kumar *et al.*, 2003). Therefore, the Environmental Protection Agency (EPA) and European Union (EU), are considering these compounds as environmental pollutants and risks to human health due to their toxicity (Huang *et al.*, 2015). Hence, it is essential to explore advanced analytical techniques in order to detect these phenolic compounds.

Simultaneous determination of various analytes is a challenging aspect although it offers many advantages such as minimised application time and economic benefits. In this regard, the simultaneous determination of biomolecules and hazardous organic compounds is becoming an interesting subject.

1.2 Problem Statement

Traditional analytical techniques such as high-performance liquid chromatography (HPLC) and atomic absorption spectroscopy (AAS) provide accurate and sensitive analysis of multi-analytes. However, these techniques are sophisticated; need pre-treatment process and huge amounts of organic solvents. On the other hand, electrochemical methods have gained significant attention of researchers for the determination of multiple analytes of interest, mainly because of their numerous features. These features include simple operation, time-saving, low cost, and most importantly reasonable sensitivity and selectivity. AA, DA, and UA are one of the necessary compounds that take part in the human metabolism function (Damier *et al.*, 1999). These molecules are usually coexisting in biological samples and therefore, observing their concentration in biologic fluids such as blood and urine can anticipate and control numerous diseases. In the biological samples normally AA and UA concentration are considered higher than DA (100–1000 times). Thus, developing of a straightforward and fast approach for the determination of each of these molecules with high selectivity and sensitivity is eligible for diagnostic applications. Since AA, DA and UA are electroactive compounds, electrochemical techniques for their detection have drawn impressive interest (Kim *et al.*, 2010). The primary obstacle for the electrochemical determination of DA is the interference from AA. Overlapping oxidation of DA and AA occurred because the oxidation potential of DA and AA are extremely overlapped at conventional electrodes such as Au, Pt, and glassy carbon electrode (Deng *et al.*, 2009). Additionally, the oxidation product of DA can catalyse the oxidation of AA, which causes electrode fouling with poor selectivity and reproducibility (Liu *et al.*, 2008).

Dihydroxybenzene isomers; HQ, CC, and RC are found together in most samples. The absorption of CC or HQ from the gastrointestinal tract can initiate certain infection like renal tube degeneration and liver disfunction. Moreover, inhalation of high concentration of RC can instantly cause death of human beings. Therefore, a highly sensitive and selective analytical method is needed for the simultaneous determination of the three dihydroxybenzene isomers. For most electrochemical methods, more consideration is paid to the simultaneous determination of CC and HQ because their redox peaks overlapping on an ordinary electrode. Despite, the simultaneous determination of HQ, CC, and RC by electrochemical methods is less reported (Ma and Zhao, 2012). Therefore, it is essential to provide electrode modifiers in order to separate the oxidation potentials of these analytes and to improve their voltammetric response as well and then applied for simultaneous determination in routine analyses.

Au nanoparticles (AuNPs) dragged considerable interests because of their advantages of catalysis, large effective surface area and control over surrounding environment. It has shown that the catalytic performance of AuNPs is usually depends on the structure of their surrounding microenvironment, which is greatly influenced by the supporting material on which they are deposited (Welch and Compton, 2006). Graphene as a promising two dimensional material of carbon family opens a new approach to create highly acceptable nanocomposite sensing materials owing to its unique properties(Harraz *et al.*, 2019). The flexible surface properties by chemical modification would increase its demand as a compatible material for nanocomposite formation .(Ismail *et al.*, 2013). However, the individual graphene oxide (GO) sheets would easily get agglomerated or restacked during the preparing process because of the π–π stacking, which would lead to a dramatic decrease in the accessible surface area of graphene sheets, thus hinder electrolyte ions transport and affect the electrochemical performance (Lu *et al.*, 2015). An electrochemical reduction of GO, which is a simple, low-cost, rapid, efficient and green technique is used to produce electrochemically reduced graphene oxide (ERGO) which explored for its potential utility in electrochemical biosensing applications (Haque *et al.*, 2012, Toh *et al.*, 2012). Electrodes modified with electropolymerised polymers have received extensive interest in the detection of analytes due to its high selectivity, sensitivity and homogeneity in electrochemical deposition (Ohnuki *et al.*, 1983). Azo dyes with high concentration of negatively charges functional groups and electron rich oxygen atom provide suitable fabrication of electrode surface (Yao *et al.*, 2007a). Fabrication of AuNPs on diazo molecules to produce stable nanoparticles due to formation of of metal-carbon bond (Mirkhalaf *et al.*, 2006).Benefiting from the synergistic effects, AuNPs, ERGO and azo-dye have been utilized as biosensors due to the excellent catalytic performance in many applications.

1.3 Objectives of the Study

This research is aimed to develop simple and sensitive electrochemical sensors with an excellent low detection limit for simultaneous determination of two sets of analytes; AA, DA, and UA; and HQ, CC, and RC. The proposed electrochemical sensors are based on an electrochemically reduced graphene oxide-azo dye incorporated with gold nanoparticles (AuNPs). Two azo dyes i.e. eriochrome black T (EBT) and Procion Red 5MX (PR) were electropolymerised with electrochemically reduced graphene oxide (ERGO). The electrocatalytic properties of the synthesised sensors are achieved through the synergistic effect of the nanocomposite components. In order to accomplish the aim of the research, the following specific objectives are addressed:

i. To modify glassy carbon electrode (GCE) using electrochemical reduced graphene oxide-azo dye decorated by AuNPs (AuNPs/ERGO-pEBT/GCE and AuNPs/ERGO-poly(PR)/GCE) via electropolymerisation characterise by Fourier-transform infrared spectroscopy (FTIR), Field Emission Scanning Electron Microscope (FESEM), cyclic voltammetry (CV), and electron

impedance spectroscopy (EIS) and to investigate their electrocatalytic performance.

ii. To optimise the variable experimental parameters to promote the sensing ability of the modified electrodes towards oxidation of the mentioned analytes.

iii. To simultaneously detecting AA, DA, UA, and HQ, CC, RC using both sensors AuNPs/ERGO-pEBT/GCE and AuNPs/ERGO-poly(PR)/GCE.

iv. To evaluate the sensing ability of the developed sensors in real samples.

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