DEVELOPMENT OF ELECTROCHEMICAL METHOD FOR THE DETERMINATION OF AZO DYES BASED COLORANTS IN FOOD AND BEVERAGE PRODUCTS

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I hereby declare that the material in this thesis is of my own effort, except for quotations, excerpts, equations, references and summaries, which have been duly acknowledged and cited clearly its sources.

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ABSTRACT

Development of a rapid and sensitive electrochemical method is important for monitoring synthetic colorants in food and beverage products which caused toxicity and pathogenicity to human health when excessively consumed. Herein, an electrochemical sensor was developed based on the modifications of glassy carbon electrode (GCE) with chitosan (CHIT), graphene oxide (GO), multi-walled carbon nanotubes (MWCNTs) and gold nanoparticles (AuNPs) for the determination of azo colorants (Amaranth (AM), Allura Red (AR), Sunset Yellow (SY) and Tartrazine (TZ)) in food and beverage products. The hybrid nanomaterials of CHIT/MWCNTs/GO/AuNPs were effectively enhanced electron-transfer and promoted the current response of azo colorants. Cyclic voltammetry and differential pulse voltammetry were used to investigate the electrochemical behavior with the modified GCE in the present of methylene blue as a redox indicator. The morphological characteristics of nanomaterials were observed high porosity, under scanning electron microscope (SEM), energy-dispersive X-ray spectroscopy (EDX) and transmission electron microscope (TEM). Potential peak currents were found in the order of bare GCE>CHIT/GO>CHIT/GO/MWCNTs> CHIT/GO/MWCNTs/AuNPs. The modified-GCE showed optimum response when operated at 25 ±1 °C at pH 7 of PBS as working solution (0.1 M) for all tested colorants. Meanwhile, the scan rate was found of 0.35 V s^{-1} for AM, 0.2 V s^{-1} for AR, 0.25 V s^{-1} for SY and 0.3 V s^{-1} for TZ, within 5, 10, 30 and 50 sec, respectively. Under optimal conditions, the developed sensor was tested with different concentrations of standard AM, AR, SY and TZ in the ranged of 10 to 90 mg mL^{-1}. The limits of detection and quantification ranges were found to be 0.032 to 0.5 mg mL^{-1} and 0.096 to 0.92 mg mL^{-1}, respectively. Sensitivity value of AM, AR, SY and TZ were calculated to be 0.01, 0.02, 0.06 and 0.05 µA/mg mL^{-1} mm^3, respectively. The developed sensor was successfully applied to determine AM, AR, SY and TZ in candy, jelly and soft drinks samples, and good recovery values range from 93.19 to 104.03 %. This simple and sensitive sensor offers low cost and rapid detection of specific colorants without skilled operators and sophisticated instruments.

ABSTRAK

PEMBANGUNAN KAEDAH ELEKTROKIMIA UNTUK MENGESAN PEWARNA AZO DALAM PRODUK MAKANAN DAN MINUMAN

Pembangunan kaedah elektrokimia yang pantas dan sensitif adalah penting untuk memantau pewarna sintetik dalam produk makanan yang menyebabkan ketoksikan dan patogenik kepada kesihatan manusia apabila digunakan secara berlebihan. Di sini, sensor elektrokimia dibangunkan berdasarkan pengubahsuaian elektrod karbon berkaca keras (GCE) dengan campuran chitosan (CHIT), graphene oxide (GO), nanotube karbon multi-walled (MWCNTs) dan nanopartikel emas (AuNPs) untuk mengesan pewarna azo (Amaranth (AM), Allura Red (AR), Sunset Yellow (SY) dan Tartrazine (TZ)) dalam produk makanan dan minuman. Penggabungan bahan nano CHIT/MWCNTs/GO/AuNPs berkesan untuk meningkatkan pemindahan elektron dan mencetus tindak balas terhadap pewarna sintetik yang disasarkan. Voltammetry kitaran dan voltammetri pulse-berbeza digunakan untuk mengkaji tingkah laku elektrokimia dengan GCE yang telah diubah suai dengan kehadiran metilena biru sebagai petunjuk redoks. Ciri-ciri morfologi nanomaterial diperhatikan menggunakan mikroskop elektron (SEM), spektroskopi sinar-X pembiasan tenaga (EDX) dan mikroskop elektron penghantaran (TEM). Arus elektrik meningkat naik daripada GCE>CHIT/GO>CHIT/GO/MWCNTs>CHIT/GO/MWCNTs/AuNPs. GCE yang diubahsuai menunjukkan tindak balas optimum apabila dikendalikan pada 25 ± 1 ^{o}C pada pH 7 PBS (0.1 M) untuk semua sintetik warna yang diuji. Sementara itu, kadar imbasan masing-masing mendapati untuk warna AM ialah 0.35 V s^{-1}, AR ialah 0.2 V s^{-1}, SY ialah 0.25 V s^{-1} dan TZ 0.3 V s^{-1} untuk TZ, dalam 5, 10, 30 dan 50 saat. Dalam keadaan yang optimum, teknik mengesan yang dibangunkan diuji dengan kepekatan AM, AR, SY dan TZ yang berbeza di antara 10 hingga 90 mg mL^{-1}. Had pengesanan dan limit kuantifikasi didapati sebanyak 0.032 hingga 0.5 mg mL^{-1} dan 0.096 hingga 0.92 mg mL^{-1}. Nilai sensitif terhadap kepekaan AM, AR, SY dan TZ telah dikira, masing-masing 0.01, 0.02, 0.06 dan 0.05 µA/mg mL^{-1} mm^3. Teknik mengesan yang dibangunkan berjaya digunakan untuk menentukan AM, AR, SY dan TZ dalam sampel gula-gula, jeli dan minuman ringan, dan menunjukkan tahap pemulihan yang sangat baik bernilai dari 93.19 hingga 104.03 %. Teknik pengesanan yang sensitif dan seletiviti ini menawarkan kos yang rendah, prosedur yang mudah, pantas, mengesan dalam kepekaan tinggi sehingga rendah, dan tertentu tanpamenggunakan pengendali yang mahir dan instrumen yang canggih.

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LIST OF SYMBOLS

%	Percentage
±	Plus minus
°C	Degree Celsius
µg	Microgram
µg/mL	Microgram per milliliter
µL	Microliter
µm	Micrometer
cm	Centimeter
g	Gram
h	Hour
M	Molar
mg	Milligram
mg/mL	Milligram per milliliter
min	Minute
mL	Milliliter
mM	Milimolar
mm	Millimeter
nm	Nanometer

LIST OF ABBREVATIONS

AM	Amaranth
AR	Allura red
AuNPs	Gold nanoparticles
CHIT	Chitosan
CV	Cyclic voltammetry
dH_20	Distilled water
DPV	Differential pulse voltammetry
EDX	Energy dispersive x-ray
GCE	Glassy carbon electrode
GO	Graphene oxide
LOD	Limit of detection
LOQ	Limit of quantification
MB	Methylene blue
MWCNTs	Multi-walled carbon nanotubes
NPs	Nanoparticles
PBS	Phosphate buffer saline
RP-HPLC	Reverse phase-high performance liquid chromatography
SEM	Scanning electron microscope
SY	Sunset yellow
SPE	Solid phase extraction catridge
TZ	Tartrazine
TEM	Transmission electron microscope

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CHAPTER 1

INTRODUCTION

1.1 Research background

Color provides the first impression of a food taste, texture and freshness to consumers that will influence their choices on food. Generally, food colorants are categorized into two types which are natural and synthetic colors. Natural colors are known as natural-identical that extracted from plant, fungi and insect. However, natural colors have some drawback such as easy to degrade, more sensitive to light, temperature, pH and costly. Prior to that, synthetic dyes have been discovered as the most reliable and economical compound because of their several advantages such as high stability to light, oxygen, pH, color uniformity, less microbiological contamination and low production cost (Wu *et al.*, 2013a; Llamas *et al.*, 2009). Synthetic colors are mainly involved as azo colors, triphenylmethane colors, xanthene colors, indigotine colors, and quinolone colors.

Azo colors with the azo group (-N=N-) as the chromophore in the molecular structure is the largest group of colors accounting more than half of global dyes production while approximately 65 % of colors used as food additives came from azo dyes (Yamjala *et al.*, 2016; Li *et al.*, 2015; Rebane *et al.*, 2010). Synthetic colorants have been used to substitute natural food color due to more stable during food processing. Regrettably, it is more concern synthetic food colorants due to pathogenicity, particularly when they are excessively consumed (Wang *et al.*, 2010b; Yadav *et al.*, 2013). In this research project focused azo colorants which are Amaranth (AM), Allura Red (AR), Sunset Yellow (SY) and Tartrazine (TZ). Those synthetic colorants are belonging to azo dyes and widely used in food products such as carbonated beverage, candies, prawn slice, cakes and many more (Gómez *et al.*, 2012). Unfortunately, high concentration of these colorants in food products can lead to severe problems to human health due to the presence of azo group (-N=N-) (de

Campos Ventura-Camargo, and Marin-Morales, 2013; Muhd Julkapli *et al.*, 2014). The usages of the azo colorants in food products are strictly controlled by World Health Organization (WHO) laws and regulations. The EU and Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1982 and EU Scientific Committee for Food (SCF) in 1984 have standardized an acceptable daily intake (ADI) for each azo colorant in food and beverage products (Aguilar *et al.* 2014). Previously, several analytical methods have been applied for the determination of azo colorants, including spectrophotometry (Oakley *et al.*, 2012), high performance liquid chromatography (HPLC) (Ma *et al.*, 2006), column chromatography (Alves *et al.*, 2008), and capillary electrophoresis (Jager *et al.*, 2005).

Nanocomposite membrane of chitosan (CHIT) is an interesting natural biopolymer that containing of reactive amino and hydroxyl functional groups which commonly used for immobilization process of electrochemical sensor combined with nanomaterial because of the high absorption, excellent film-forming ability, high permeability, high heat-stability, mechanical strength, non-toxicity, biocompatibility, low cost and availability. Moreover, chemically modifications of the amino group of CHIT have provided hydrophilic environment for the biomolecules and soluble in diverse acids that able to interact with polyanions to form complexes and gels (Shukla *et al.*, 2013; Lin *et al.*, 2007). However, hybrid materials based on CHIT has been developed such as conducting polymers, carbon nanotubes, redox mediators, metal nanoparticles and oxide reagents due to excellent properties of individual components and outstanding synergistic effects simultaneously for electrochemical sensing platforms (Li *et al.*, 2010; Lin *et al.*, 2009).

Graphene has been widely used as carbon nanomaterials-based sensor because of the superior mechanical strength, thermal stability, low density and high heat conductance, good electrical conductivity and high aspect ratio properties (Punetha *et al.*, 2017; Novoselov *et al.,* 2012). Graphene is a highly promising material for biosensors due to its excellent physical and chemical properties which facilitate electron transfer. It is a single sheet of carbon atoms arranged in a honey-comb lattice which effectively enhanced their electronic and electrochemical properties (Wisitsoraat *et al.*, 2017; Hla, 2012). Various applications referring to in electrochemical sensing are phytohormone (Gan *et al.*, 2011), biomolecular (Huang *et al.*, 2012), pharmaceuticals (Wei *et al.*, 2012), toxic food additives (Wu *et al.*, 2012), and environment pollutants (Gan *et al.*, 2012a). In chemical and biosensor applications, graphene has shown superior performances compared with other carbon-based materials including its nearest counterpart, carbon nanotubes (CNTs), because of its double surface area compared to CNTs, higher chemical reactivity due to larger number of edge plane per unit mass and higher electron mobility and conductivity (Wisitsoraat *et al.,* 2017; Ratinac *et al.*, 2011; Pumera, 2010).

Furthermore, carbon nanotubes have extraordinary tensile strength, excellent electrical conductivity, and high chemical stability that can create good electron transfer rates (Ajayan, 1999). There are two types of carbon nanotubes available which are multi-walled carbon nanotubes (MWCNTs) and single-walled carbon nanotubes (SWCNTs). MWCNTs and gold nanoparticles (AuNPs) are extremely attractive materials in electrochemical sensor (Messaoud *et al.,* 2017; Saha *et al.,* 2012). Metal nanoparticles having broad range of dimensions are endowed with size-dependent optical, magnetic, electronic and chemical properties appropriate for catalysts, optoelectronic devices, as well as for chemical and biosensor applications (Ioniţă *et al.,* 2017; Stassen *et al.,* 2017). In this study, the candidate was used AuNPs for modification of glassy carbon electrode in electrochemical system. AuNPs are exploited as a potential material for sensing due to high surface area, strong adsorption ability, good biocompatibility, chemical stability and nontoxicity. AuNPs are more suitable for the determination of food coloring *via* the development of electrochemical and colorimetric sensors. One of the considerable advantages of using AuNPs based assay, the molecular recognition event can be translated into visible color changes (Wu *et al.*, 2011; Li *et al.*, 2010).

1.2 Problem statement

Nowadays, various additives including synthetic food colorants are widely applied in food industry to make food attractive to consumers. However, some synthetic food colorants can be toxic to aquatic organisms and carcinogenic effects on humans, particularly when they are extremely consumed (Ceyhan *et al.*, 2013). Food colorant can induce food intolerance (non-immune mediated) and allergic reactions (immune mediated, ranging from urticaria and/or angioedema and asthma to anaphylaxis). Previous studies reported that AM, AR, SY and TZ can cause the appearance of allergies and asthma and childhood hyperactivity (Silva *et al.*, 2007; Nevado *et al.*, 1997).

McCann *et al.* (2007) have reported that two mixtures of synthetic azo colors had caused hyperactivity to increase in children age 3 to 9 years old. As for previous study conducted by Hashem *et al.* (2010) on the toxicological impact of three types food coloring caused depressing effect on cellular immune response on albino rats. AR has caused DNA damage in pregnant mice's colon after three hours of administration at concentration 0 to 2000 mg kg^{-1} body weight (Sasaki *et al.*, 2002; Tsuda *et al.*, 2001). Besides, SY and AR can cause binding of human serum albumin that lead to complex formation which may interfere their function in human body (Masone and Chanforan, 2015). AM is reduced rapidly by gastrointestinal microflora to naphthionic acid and 1-amino-2-hydroxy-3,6-naphthalene disulphonic acid that may cause caecal enlargement and renal pelvic nephrocalcinosis in rats, because of the effects on mineral absorption and increased faecal water loss (König, 2015: EFSA, 2010).

Facing with increasing legal restrictions, food dye determination became an analytical challenge. Until now, several researches have been conducted for the determination of azo colorants in food and beverage products by using various analytical methods such as high performance liquid chromatography (HPLC) (Li *et al.*, 2015; Shen *et al.*, 2014), thin layer chromatography (TLC) (Andrade *et al.*, 2014), spectrophotometric (Sha and Zhu, 2015; Llamas *et al.*, 2009), capillary electrophoresis (Patsovskii *et al.*, 2004) as well as liquid chromatography/tandem mass spectrometry (LC-MS/MS) (Martin *et al.*, 2016; Tsai *et al.*, 2015). However, these above methods have some drawback such as time consuming, required high skilled manpower and high-tech instruments. Thus, a rapid and reliable method is needed for the determination of synthetic colorants in food and beverage products.

1.3 Research objectives

The aim of this research was to develop a simple and effective way for the determination of azo colors in commercial food and beverage products. Chitosan nanocomposite membrane, graphene oxide, multi-walled carbon nanotubes, and gold nanoparticles were incorporated onto the GCE and enhanced the sensitivity for the determination of azo colorants.

The following specific objectives were formulated to achieve the overall objectives of this study:

a) To modify glassy carbon electrode (GCE) with chitosan nanocomposite membrane, graphene oxide, multi-walled carbon nanotubes, and gold nanoparticles.
b) To characterize the modified GCE by using SEM, EDX, TEM and UV-Vis.
c) To evaluate the analytical performance of the developed sensor.
d) To validate the performance of the developed sensor with RP-HPLC method for the determination of azo dyes.

1.4 Scope of work

Advances in chemistry, physics, biochemistry and molecular biology have led to the developed electrochemical sensor that can able to detect wider range of biochemical elements due to their simplicity, low cost, sensitivity, selectivity, and possibility for miniaturization (Farre *et al.*, 2009). An electrochemical sensor is developed based on nanomaterials and nanocomposite membrane, a small amount of sample requires for rapid detection of synthetic colorants. Electrochemical system found efficient method in the analysis of compound, especially identifying and quantifying the specific food colorant. This technique has been received high attention in the world due to high efficiency and sensitivity, quick response, simple procedure and relatively low costs (Ansari *et al.*, 2017; Zhu *et al.*, 2014). The performance of the electrochemical system is critically dependent on the properties of the presence of nanomaterials and nanocomposite membrane which increased active site and conductivity for the determination of specific compound (Ahmed *et al.*, 2016).

The main aim of this study was to develop an electrochemical sensor based on CHIT nanocomposite membrane, graphene oxide, multi-walled carbon nanotubes and gold nanoparticles modified glassy carbon electrode (CHIT/GO/MWCNTs/AuNPs/GCE) in the presence of methylene blue as a redox indicator for the determination of azo colorants (AM, AR, SY and TZ) (Scheme 1.1). To the best of our knowledge, highly sensitive electrochemical method was firstly developed and applied for the determination of azo colorants in commercial food and beverage products using the developed glassy carbon electrode (CHIT/GO/MWCNTs/AuNPs). The prepared nanomaterials were characterized by SEM, TEM and DPV. The experimental parameters were optimized as pH, interaction time and scan rate, and investigated the interaction mechanism between azo colorants and the modified GCE. The combination of nanomaterials is more precise, accurate, sensitive, rapid, cheap and simple with effective protocol for the determination at the presence level of AM, AR, SY and TZ. The modified electrode has emerged as powerful platforms in electrochemical sensor for identifying and quantifying the presences of azo colorants. Subsequently, the developed method was successfully applied and compared with RP-HPLC technique for the determination of AM, AR, SY and TZ in candy, jelly and soft drinks with satisfactory levels.