CHAPTER 1

Introduction

1.1 Overview

Determination of hydroquinone is very important because this chemical is harmful to human, animal and environment. Several methods have been employed for hydroquinone determination including spectrophotometry, chemiluminescence, and high performance liquid chromatography. Although these methods provide high sensitivity and accuracy, they have many disadvantages such as higher cost, more complicated and much time consuming compared with electrochemical methods. However, electrochemical method in batch manner still use a lot of chemical reagents and sample, so in this work, we are interested in applying flow injection system (FI-system) to solve this problem.

In order to improve selectivity for determination of hydroquinone and avoid complicated sample preparation, biosensor has been used with FI-system. Biosensor combines biological component with a physicochemical detector. Biological component exhibits high selectivity which is controlled by specificity of the biocomponent to react with the analyte. Amperometric detection provides additional selectivity because the electrochemical reaction of interest will be taken place at a constant voltage applied to the working electrode, producing electrical current related to concentration of the

analyte.

In this work, a simple screen-printed carbon electrode (SPCE) modified with various nanomaterials, i.e., carbon nanotubes (CNTs), CNTs with gold and platinum nanoparticles have been investigated. The CNTs modified SPCE and laccase column were incorporated to FI-system to develop flow-based amperometric sensor for the determination of hydroquinone. Laccase was immobilized on silica gel using a cross-linking method by glutaraldehyde modification. The parameters such as applied potential, flow rate, pH, buffer concentration and temperature were optimized. The proposed system offered advantages such as rapid, simple and high sensitivity.

1.2 Hydroquinone

Hydroquinone (HQ, 1, 4-dihydroxybenzene) is an aromatic organic compound that is a type of phenol and widely applied as a developer in black and white photography, a polymerization inhibitor, varnishes, motor fuels, stabilizer in paints, depigmenters, rubber and food antioxidant. Hence, it exists widely in industrial effluents such as the waste from plastic, leather, rubber, cosmetic and pharmaceutical industry [Zou, 2014]. Moreover, this chemical is harmful to humans and the environment. It can incur cough, spew, pigmentation of eye, headache, fatigue and the damage to kidney [Zhou, 2014]. The maximum amount of hydroquinone in surface water allowed by the European Community is lower than 1.8 µM. The analytical methods for determination of hydroquinone are summarized in Table 1.1.

Table 1.1 Some analytical methods for determination of hydroquinone

Title	Method	Linear range	LOD (μM)	Sample	Ref.
A hydroquinone sensor based on a new nanocrystals modified electrode	electrochemistry	0.005–4.5 mM	1.5	tap water	[Zou, 2014]
Fast and sensitive high performance liquid chromatography analysis of cosmetic creams for hydroquinone, phenol and six preservatives	HPLC	0.004–1.8 mM	1.82	cosmetic creams	[Gao, 2011]
Simultaneous determination of hydroquinone and catechol at an activated glassy carbon electrode	electrochemistry	0.5–200 μM	0.16	tap water	[Ahammad, 2010]
Determination of optimum wavelength and derivative order in spectrophotometry for quantitation of hydroquinone in creams	spectrophotometry	0.09–0.24 mM	-	cream	[García, 2007]
Ultra–trace level determination of hydroquinone in waste photographic solutions by UV–vis spectrophotometry	spectrophotometry	0.60–18.2 μM	0.19	waste photographic solutions	[Sirajuddin, 2007]
A sensitive chemiluminescence method for determination of hydroquinone and catechol	chemiluminescence	9.08–90 nM	0.91 nM	river water	[Zhao, 2007]

1.3 Flow injection analysis

Flow injection analysis (FIA) was firstly introduced by Ruzicka and Hansen in 1975. Then this technique was quickly gained worldwide acceptance in many laboratories. The principle of FIA can be concluded that the sample is injected into a flowing carrier stream and mixed together. Then the mixed zone is flowed to the detector. This zone is measured by the detector and recorded a physical parameter such as absorbance, pH, current, potential and so on. The signal was obtained in term of a peak that changes continuously as a function of time as the sample passes through the flow cell. The peak height and peak area are proportional to concentration of the analyte and the peak height are usually used to evaluate the concentration of the sample being determined by calculating from the calibration graph of standard solution [Parikh, 2010]. The peak shape is dependent on the physical process of dispersion of sample zone.

There are three important concepts of FIA consisting of reproducible sample injection volume, controlled dispersion of the injected sample zone, which is promoted the reproducible mixing between sample and carrier and reproducible timing of the movement of the injected zone from the injection valve to the detector, so it can be precisely detected at non equilibrium reaction.

The simplest flow injection system is illustrated in Figure 1.1. It consists of a pump, which is used for propelling the carrier stream and the most common is a peristaltic pump, an injection valve, typically 5–200 μL loop sample, for introduction of a well-defined volume of standard or sample into the system, a reaction coil or

mixing coil which is employed for mixing of the sample and the carrier stream. After that this zone is automatically flowed to detect at a detector and producing a signal such as absorbance, current, potential and so on, to be recorded at a recorder.

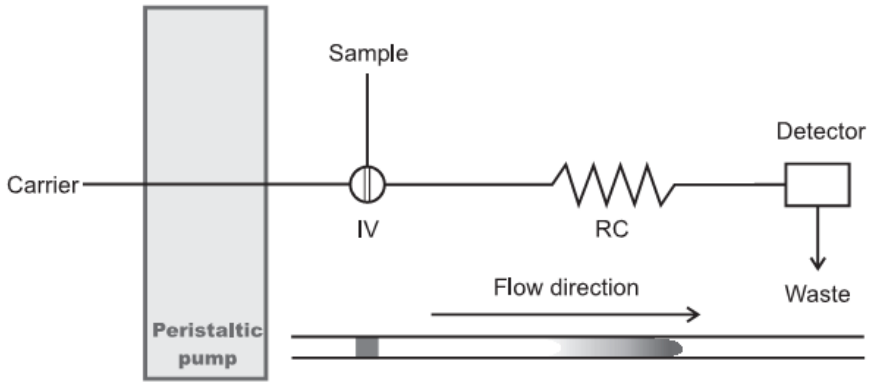

Figure 1.1 The simplest flow injection system [Cerda, 2014]

FIA technique have been applied in many applications because it provides many advantages including of low amount of chemical reagent and sample consumption compared to conventional batch methods, short analysis time, simple, low cost, the reproducible detection of the product and high sample throughput. Furthermore, the contamination during the measurement from the environment can be avoided because of being the closed-system. FIA method is more flexible equipment as it can be incorporated with a variety of detectors such as spectrophotometer, fluorometer, chemiluminescence, atomic absorption, electrochemical including amperometer, conductometer and potentiometer.

1.4 Amperometric method

Electrochemical methods are the techniques in analytical chemistry which is powerful and versatile analytical techniques that offer high sensitivity, robust, good precision and low-cost instrumentation [Farghaly, 2014]. Moreover, there is advantage over spectrophotometric method due to the ability to measure the analyte in colored and turbid samples. Amperometry is a kind of electrochemical technique and it is very popular techniques to couple with biosensor because the product from the reaction between biological component (enzyme) and some substrate give electrochemical active species which can be detected by amperometric method.

The principle of amperometry is based on the measurement of the electrical current which is generated by a redox reaction at the working electrode. A constant voltage is applied to the working electrode and the current is proportional to the concentration of electroactive species in the solution. This technique consists of electrolyte which is needed in order to provide electrical conductivity such as buffer solution and three electrodes including of working electrode (WE), auxiliary electrode (AE) and reference electrode (RE). The system using three electrodes offers advantage over the system using two electrodes which are working electrode and reference electrode. The advantage of auxiliary electrode is that it provides circuit over which current is measured. Furthermore, auxiliary electrode is adjusted to balance the reaction occurring at working electrode that leads to allow the potential of working electrode to be measured against a known reference electrode without compromising the stability of reference electrode due to passing current over it [Auxiliary electrode, 2015].

1.5 Screen-printed electrode

Carbon electrodes are widely applied as working electrode in electrochemistry because they offer advantages including wide potential window, good electrical conductivity, low and stable background noise and cost effective. Carbon is also a biocompatible and chemically inert material [Gao, 2008]. Moreover, there are various formats of carbon such as glassy carbon electrode (GCE), carbon paste electrode (CPE), graphite electrode (GE) and screen-printed carbon electrode (SPCE). Nowadays, Screen-printed electrodes (SPE) have attracted considerable attention in recent years due to their advantages comparing with the traditional electrodes. For example, they are portable, field-based size, disposable, inexpensive, reliable, simple to perform with high sensitivity, selectivity and highly reproducible [Gupta, 2015].

SPEs are planar electrodes including of plastic substrates coating with layers of conducting materials. In addition, SPEs can be easily modified with different materials to improve the properties such as metals, nanomaterials, enzymes and polymers [Tukur, 2015]. Some researches using carbon electrode as working electrode modified with various materials are summarized in Table 1.2. Metallic nanoparticles have received great attention in electrode modification because of their physical and chemical properties like high surface area, good electrical properties, strong adsorption ability and good catalytic ability [Mistry, 2014]. Moreover, carbon nanotubes (CNTs) have attracted increasing interest in the application of CNTs based sensors in the detection and determination of phenols and phenolic compounds due to their unique morphology, nanosized scale, novel physico-chemical properties [Negash, 2014]. Carbon nanotubes

could be visualized as rolled sheets of graphene which is sp^2 carbon arranged in a honeycomb lattice. There are two types of carbon nanotubes including single-wall carbon nanotubes (SWCNTs) and multi-wall carbon nanotubes (MWCNTs) [Elrouby, 2013]. A SWCNTs and MWCNTs are a single graphene sheet rolled seamlessly and multiple rolled layers of graphene, respectively [Carbon nanotube, 2015]. The procedure of MWCNTs synthesis is easier than SWCNTs synthesis and their cost is also lower. Furthermore, they exhibit good electrical conductivity, excellent electron transfer rate, chemical stability, strong electrocatalytic activity and high surface area [Oliveira, 2013].

In this work, we interested in CNTs and metal nanoparticles decorated CNTs to develop simple screen-printed electrodes for application in electrochemical sensor for determination of hydroquinone.

Table 1.2 Some reports on application of carbon electrode modified with different materials

Title	Working electrode	Analyte	Sample	Ref.
Effective electrochemical sensor based on screen-printed electrodes modified with a carbon black-Au nanoparticles composite	SPE modified with carbon black–gold nanoparticles	Glucose H_2O_2 Hydroquinone Ascorbic acid	-	[Arduini, 2015]
Electrochemical behavior of ascorbate oxidase immobilized on graphite electrode modified with Au-nanoparticles	Graphite electrode modified with gold nanoparticles	Ascorbic acid	-	[Dodevska, 2013]
Laccase biosensor based on screen-printed electrode modified with thionine–carbon black nanocomposite, for Bisphenol A detection	SPE modified with thionine–carbon black nanocomposite	Bisphenol A	Tomato juice	[Portaccio, 2013]
Electrochemical sensor for epinephrine based on a glassy carbon electrode modified with graphene/gold nanocomposites	GCE modified with graphene/ gold nanocomposites	Epinephrine	Drug	[Cui, 2012]
Voltammetric determination of resorcinol on the surface of a glassy carbon electrode modified with multi-walled carbon nanotube	GCE modified with multi-walled carbon nanotube	Resorcinol	Artificial wastewater	[Ghoreishi, 2012]

Table 1.2 (continued)

Title	Working electrode	Analyte	Sample	Ref.
Gold electrodeposition on carbon nanotubes for the enhanced electrochemical detection of homocysteine	CPE modified with gold on carbon nanotube	Homocysteine	-	[Hung, 2011]
Laccase-based biosensor for the determination of polyphenol index in wine	SPE modified with multi–walled carbon nanotubes	Gallic acid	Wine	[Fusco, 2010]
A hydroquinone biosensor using modified core–shell magnetic nanoparticles supported on carbon paste electrode	CPE modified with core–shell magnetic nanoparticles	Hydroquinone	compost extracts	[Zhang, 2007]

1.6 Hydroquinone biosensor

The first biosensor was described in 1962 by Clark and Lyons who immobilized glucose oxidase (GOD) on an amperometric oxygen electrode surface semipermeable dialysis membrane in order to quantify glucose concentration in a sample [Sassolas, 2012]. Afterward, biosensors have been developed for determination of various substances such as glucose [Samphao, 2015], uric acid [Azmi, 2015], glutamate in food and clinical applications [Hughes, 2015], urea [Ramesh, 2015] and dopamine [Canbay, 2014]. According to IUPAC recommendations 1999, a biosensor is an independently integrated receptor transducer device, which is capable of providing selective quantitative or semi-quantitative analytical information using a biological recognition element [Perumal, 2014]. A biosensor consists of two main elements. First, a bioreceptor that is an immobilized sensitive biological element (e.g. enzyme, DNA, antibody, nucleic acids and cell) recognizing the specific target analyte. Second, a transducer is used to convert biochemical signal resulting from the interaction between the analyte and bioreceptor into an electrical signal. Electrochemical transducers are often used to develop biosensors. This system offers some advantages such as low cost, simple design and small dimensions.

Laccase enzyme was employed as bioreceptor in hydroquinone biosensor. Laccase (EC 1.10.3.2, oxygen oxidoreductase) belongs to a family of multicopper oxidases. This enzyme is able to catalyze the oxidation of various phenolic compounds with the concomitant reduction of oxygen. It includes one type-1 (T1) copper ion and three additional copper atoms including one type-2 (T2) and two type-3 (T3) copper

ions which form a trinuclear copper cluster. The T1 site of the enzyme is involved in substrate binding and oxidation as well as the transfer of electrons to the T2/T3 cluster, where oxygen is reduced to water [Brondani, 2013]. The principle of hydroquinone biosensor is shown in Figure 1.2. Laccase directly catalyze oxidation of hydroquinone to quinone which is electroactive species. When the optimum electrical potential was applied to working electrode, quinone was reduced to hydroquinone again and the current can be measured which is proportion with concentration of hydroquinone. Therefore, quinone could be used in the experiment for optimization of parameters in the system without laccase enzyme. Moreover, the performances of biosensor depend on physical parameters such as pH, buffer concentration and temperature, so these parameters should be optimized.

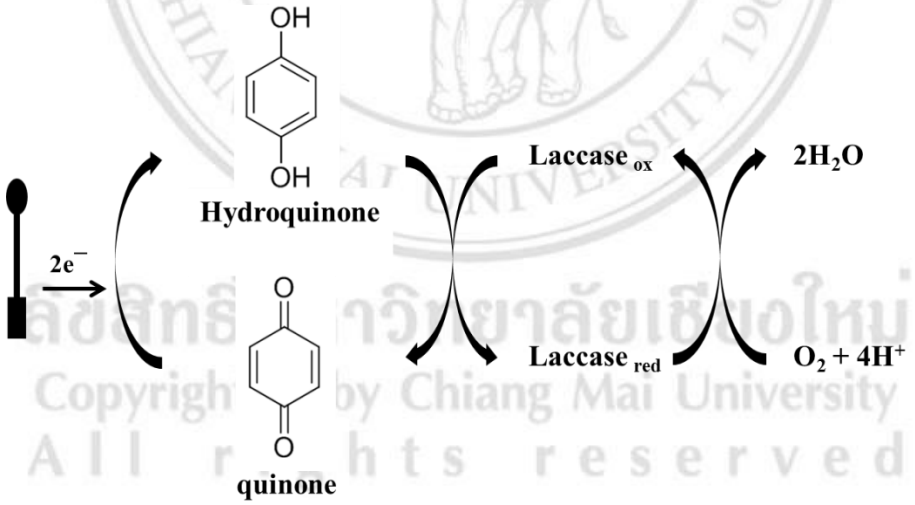

Figure 1.2 The mechanism of hydroquinone biosensor

1.7 Enzyme immobilization method

The enzyme immobilization method is necessary to develop for enhancing almost all enzyme properties i.e., stability, activity and specificity. Several different methods have been employed for enzyme immobilization, for example, adsorption, entrapment, embedment, covalent bonding and linking to an insoluble carrier [Zhang, 2009]. Table 1.3 summaries different method for enzyme immobilization. The enzyme immobilization using covalent bonding has been widely studied. Covalent bonding provides strongly bonding between the enzyme and its carrier. Enzyme immobilized through covalent bonding can be reused more often than other available immobilization methods, such as adsorption and entrapment. Enzyme can be immobilized directly on the surface of electrode of amperometric sensor [Hayat, 2014, Casero, 2013].

However, in flow- based biosensor the enzyme column should provide higher capacity and offer better catalytic efficiency. The column can be repeatedly used for several samples and the reaction product is easily transported by flowing to the transducer. The enzyme column consists of enzyme immobilized on the substrate. There are many kinds of substrate for enzyme immobilization such as porous glass, cellulose, chitosan, silica gels, polystyrene colloidal particles and hydrogel [Wang, 2005]. We interested silica gel because there are high surface area, controllable pore size, high chemical stability, low cost and easy to modify. The large surface areas and greater pore volumes could enhance the loading capacity of enzyme. Silanization to activate substrates and subsequent covalent binding of enzyme to the carrier can be done by using a coupling reagent i.e., glutaraldehyde, carbodiimide and succinimide ester.

Glutaraldehyde coupling is a common method used for immobilization. Silica gel composed of siloxane groups (Si-O-Si) and silanol groups (Si-OH) distributed on its surface. Surface modification can increase the loading amount of enzyme and it is usually achieved with silanization using suitable organosilane agents [Lee, 2006].

Table 1.3 Some methods for enzyme immobilization

Title	Immobilization method	Analyte	Linear range (µM)	Stability (day)	Ref.
Laccase biosensor based on graphene-chitosan composite film for determination of hydroquinone	Entrapment: entrapped laccase into the composite of graphene-chitosan and the mixture was spread on the GCE	Hydroquinone	2–100	10	[Qu, 2014]
Fabrication of a novel laccase biosensor based on silica nanoparticles modified with phytic acid for sensitive detection of dopamine	Adsorption: laccase was coated on the GCE modified with SiO_2-phytic acid	Dopamine	0.99–03	40	[Wang, 2014]
Rapid screening method for assessing total phenolic content using simple flow injection system with laccase based-biosensor	Covalent: laccase-BSA solution was deposited on the AuNPs/ CNTs-NH_2/GCE and coated with glutaraldehyde	Gallic acid	3–60	-	[Amatatongchai, 2013]
Amperometric enzyme carbon paste-based biosensor for quantification of hydroquinone and polyphenolic antioxidant capacity	Entrapment: nafion entrapped tyrosinase and dropped onto the CPE	Hydroquinone	20 – 120	-	[Sýs, 2013]

Table 1.3 (continued)

Title	Immobilization method	Analyte	Linear range (μM)	Stability (day)	Ref.
Electrochemical biosensor for the detection of formaldehyde based on enzyme immobili-zation in mesoporous silica materials	Adsorption: formaldehyde dehydrogenase adsorbed into the pores of silica and confined in nylon mesh and placed on the GCE	Formaldehyde	1.2 – 617	80	[Shimomura, 2008]
Simple laccase-based biosensor for formetanate hydrochloride quantification in fruits	Covalent: glutaraldehyde was used for cross-linking laccase and AuNPs on gold electrode	Formetanate hydrochloride	0.94–11.3	28	[Ribeiro, 2006]

1.8 Research objectives

The objectives of this research are listed as follows:

1. To develop simple screen-printed electrodes modified with nanomaterials for application in electrochemical sensor

2. To apply the flow injection amperometric sensor system for determination of Hydroquinone in water samples.