CHAPTER 1 INTRODUCTION

1.1 Background

In the field of biotechnology and medical biodiagnostics, biosensor devices that provide fast, cheap, accurate, are highly sensitive and highly selective are usually required [1]. This is especially true for, recent trends in immunosensors which have been focused on ultrasensitive measurements in order to monitor a very small amount of target molecules in the sample [2, 3] which the classical methods such as Enzyme Linked Immunosorbant Assay (ELISA) cannot provide. Therefore, an electrochemical method based immunosensors has been developed to meet this standard.

Electrochemical transducers are a very attractive option for an alternative biosensor method, because they provide high sensitivity, are simple, can be miniaturized, and are inexpensive and portable [4]. In the past many years, label-based electrochemical biosensors have been developed by using various promising nanoparticles [5-7] coupled with various methods of deposition/immobilization of biomolecules such as enzyme, proteins, DNA. The use of enzyme labels to generate electrical signals has been useful for ultrasensitive electrochemical bioassay of target biomolecules [8]. However, signal amplification from such labeling still remains a major challenge in this field.

Therefore, a new platform based on coupling the biocatalytic amplification of enzyme tags with nanoparticle carriers is of interest to achieve high sensitivity demands of electrochemical detection of biomolecules. Among various deposition techniques to construct a nano-label, layer-by-layer (LBL) deposition is considered to be simple and effective method to immobilise biomolecules such as enzyme, antibodies, polymers, and DNA on the surface and/or inside nanoparticles [9, 10]. In the past decade, the LBL method for ultrathin film assembly on nanoparticles by using alternate adsorption of oppositely charged polyions and proteins on a variety of charged substrate materials, for instance, carbon nanotubes [11], graphene nanoparticles [12], has been developed.

Carbon nanotubes (CNTs) are one of the most promising materials for electrochemical biosensors due to their special physical and chemical properties [13, 14]. There have been a number of studies reporting that CNTs help promote electron transfer and are capable of being carriers for bio-molecular label according to their huge surface-to-volume ratio [11, 15]. Therefore, CNTs have become an attractive nanomaterial in ultrasensitive labeling platform design for electrochemical biosensors. Combining CNTs with enzymes has also become one of the most promising strategies for the design of labels. The challenge in this design is to achieve of a high loading of enzyme on the CNT surface and a high rate of electron-transfer between the enzyme active site and the electrode.

Graphene oxide (GO) is a novel class of 2D carbon-based nanomaterials and currently considered to be one of the most promising materials for biosensors due to its special physical and chemical properties. In particular, GO nanoparticles are soluble in water and many other common polar solvents which allows to the formation of large-scale uniform films on various substrates [16-18]. Additionally, the presence of oxygen-containing functional groups provide potential advantages for GO for numerous applications because they can be used to introduce various types of biomolecules on its surface, especially for enzyme immobilization [19].

As mentioned before, in order to archive high sensitivity in electrochemical biosensor, signal amplification by enzyme-substrate recycling is one key factor to be considered. This is due to the recycling property, which enhances the electrical signal. For example, the catechol/o-quinone redox couple has been involved in many signal amplification systems on account of its reversibility and its ability to be recycled by many enzymes, such as tyrosinase (Tyr), which catalyses the oxidation of catechol to o-quinone, followed by reduction back to catechol at the electrode surface. Another example is the system that provides an amplification cycle due to the activity of ALP (alkaline phosphatase) by converting p-aminophenylphosphate (PAPP) substrate into the p-aminophenol (PAP) product. An amperometric current response resulted from the oxidation of PAP into p-quinoneimine (PQI). The electrochemical response is then further enhanced by DI (Diaphorase) enzyme which reduces PQI back to PAP. The resulting oxidized form of DI is regenerated in its reduced native state by its natural substrate, NADH, present in the solution.

Interestingly, there is no report of using these enzymes for amplified labeling before. Hence, our interest in signal amplified labeling systems based on multi-enzyme layers on both CNTs and graphene nanoparticles has arisen due to its perceived suitability as a means of constructing an ultrasensitive immunosensor.

1.2 Objective

The objective of this research is to develop a highly sensitive platform for immunoassay by using enzyme amplified labels via the carbon-based nanomaterials, CNTs and GO. The work aims to provide information regarding the construction of enzyme on the surface of CNTs and GO by using layer-by-layer deposition to enhance the detection ability of the target molecule, *Salmonella* Typhimurium, by electrochemical immunoassay.

1.3 Outline of the dissertation

In this dissertation, the electrochemical immunoassay based on enzyme amplified labeling is described. The first component consists of the construction of multiple enzyme layers on carbon nanomaterials, CNTs and graphene oxide nanoparticles by using layer-by-layer deposition technique. This is followed by the electrochemistry of enzyme recycling system towards their substrates. The analytical method focuses on the measurement of the current response from the enzyme using cyclic voltammetry, amperometry and differential pulse voltammetry are also explained. The last part focused on the application of enzyme amplified labeling in immunoassay method for detection of *Salmonella* Typhimurium detection.

In chapter 3, an amplified labeling platform for an *electrochemical immunoassay* was described. The platform consists of multi-wall carbon nanotubes (MWNTs) and layers of the enzyme tyrosinase (Tyr), which provides an amplification cycle due to the substrate recycling property. The immobilization of multi-layered Tyr on the surface of MWNTs by using a stepwise layer-by-layer deposition technique was described. Various conditions affecting the current response including pH, and applied potential

and numbers of T Tyr layers on the MWNTs were optimized. Kinetic studies of the Tyr on MWNTs for catechol substrate were also undertaken. Finally, MWNTs- Tyr conjugation was applied for amplified labels for electrochemical immunoassay for *Salmonella* Typhimurium. Simple electrochemical method in micro-well plates using disposable screen-printed electrodes (SPE) was also introduced.

In chapter 4, an amplified labeling immunoassay platform consisting of graphene nanoparticles (GO) and layers of bi-enzyme, alkaline phosphatase (ALP) and diaphorase (DI) was presented. The immobilization of multi-layered enzymes on the surface of GO by using a stepwise layer-by-layer deposition technique was demonstrated. The GO and enzyme conjugation was then applied as a label for immunoassay detection of *Salmonella* Typhimurium.

In the last chapter, the conclusions for enzyme amplified labeling on CNTs and GO for immunoassay detection of *Salmonella* Typhimurium and suggestion for further work are described.