CHAPTER 5 CONCLUSIONS

The overall goal of this dissertation was to study enzyme amplified labeling using carbon nanomaterials and apply the labels to an electrochemical immunoassay. The construction of enzyme layers on carbon nanomaterials was performed by using layer-by-layer deposition based on electrostatic adsorption. The electrochemical study of enzyme-substrate recyling of both monoenzyme layers of tyrosinase, and multienzyme layers of alkaline phosphatase and diaphorase, have been examined. All of the various experimental conditions were examined for the optimum response. Insights into the kinetics of monoenzyme tyrosinase layers were also achieved. The labels were applied to immunoassay for *Salmonella* Typhimurium by in polystyrene microplates.

In the first section, an amplified labeling system, for an electrochemical immunosensor, consisting of carbon nanotubes and layers of tyrosinase was developed. The label was constructed by a layer-by-layer deposition method, based on the formation of negatively charged carboxylic groups on the MWNTs surface, followed by positively charged PAH, and then negatively charged tyrosinase enzyme. Optical measurement indicated that each charge excess coating on the nanotubes corresponded to approx. 3 monolayers of enzyme. Scanning Electron Microscopy (SEM) images of unmodified and modified MWNTs indicated the successful immoblisation of enzyme on the MWNTs surface. The electrochemical study of the catechol/*o*-quinone redox couple indicated that the system could be recycled by tyrosinase (Tyr). Tyr catalyses the oxidation of catechol to *o*-quinone, which is then reduced back to catechol at the electrode surface. Various factors, including pH, applied potential, number of layers, incubation time, and scan rates were investigated to maximize the efficiency of the detection. The kinetics of T Tyr on MWNTs was also studied and it was found that k_{cat} / K_M was only one order of magnitude less than the value for free tyrosinase, suggesting the catalytic efficiency of the enzyme was not largely altered by this method of immobilisation. The modified labels were coated with streptavidin to enable tagging to anti-salmonella antibodies. Hence, the label was applied to an electrochemical immunoassay. The assay was performed in a polystyrene microplate to eliminate the problems of background current due to the could detect *Salmonella* Typhimurium., down to approximately 340 CFU/ml in pure culture and 10^3 CFU/ml in milk sample. The immunoassay detection system also allows detection of the enzyme product, PQI, at 410 nm. This optical detection method exhibited a limit detection for *Salmonella* Typhimurium of approximately 10^3 CFU/ml.

The second section, alkaline phophatase and diaphorase layers on graphene nanoparticles for enzyme amplified labeling were developed. Both enzymes were immobilized on GO by layer-by-layer deposition. Optical measurements suggested that GO allowed more enzyme adsorption relative to MWNTs. Elctrochemical study of the system proved that alkaline phosphatase could convert *p*-aminophenylphosphate (PAPP) substrate into the p-aminophenol (PAP) product. The electrochemical response to this product could be further enhanced by DI enzyme which reduced PQI back to PAP in the presence of its natural substrate, NADH, forming an amplification cycle. The kinetics of diaphorase immobilized on GO were also examined. However, k_{cat}/K_M of the system could not be obtained due to the fact that the reciprocal plot of the catalytic plateau current versus PAP concentrations exhibited a negative intercept and the current were also not proportional to PAP concentration. Additionally, the labels

could not provide a signal when used in a microplate. Therefore, the label were therefore not applicable for *Salmonella* Typhimurium immunoassay.

Suggestion for Further Work

This thesis has developed enzyme amplified labels by using carbon nanotubes and graphene nanoparticles coupled with enzyme recycling system based on electrochemical techniques. Toward future development, however, future investigations for the monoenzyme-MWNTs labels required include:

1. The stability of the enzyme immobilized on carbon nanomaterials, such as MWNTs and GO, should be futher studied since it is known that enzyme can lose activty over the course of time, depending on temperature and modifications.
2. Since the result indicated that MWNT-(PAH/Tyr) conjugates exhibited excellent response toward catechol in terms of high sensitivity of 320.8 $mA.M^{-1}$ with a short response time (2 seconds), therefore, the system can be examined for use as phenol biosensor.
3. The sensitivity of the electrochemical immunoassay could be improved by reducing background signals by increasing the incubation time between antigen and labels, increasing the blocking time, and increasing the washing steps in order to minimize non-specific binding.
4. The specificity of the immunoassay detection could be further evaluated by comparison of the response to various bacterial species e.g. *Listeria*, *E.coli*, *Campylobacter* and *Clostridium perfringens*.

Future possible development and investigations for multienzyme-GO labels include:

1. The major limitation of this bienzyme system is the activity of diaphorase. From the experiments, a loss of diaphorase activity was found. From this, it could be understood that possibly temperature, light and immobilization method affected the activity of diaphorase. During the drop-drying procedure, diaphorase was exposed to light and higher temperatures, which can hardly be avoided, resulting in reducing enzyme acitivity. The l-b-l method may also cause enzyme deactivity by changing the active site configuration.
2. Layers of enzyme could be immobilized on alternative materials e.g. latex particles may be designed and the current response could be studied. The shape and size of the carrier might affect the response of the system.
3. Since the result indicated the bienzyme system (ALP and DI) could not provide a large enough amplification response in term of PAP oxidation, compared to the mono enzyme system (ALP), due to the reasons described above, selecting a different enzyme combination may provide better results.