

## **CHAPTER VI**

### **CONCLUSIONS AND FUTURE PERSPECTIVES**

The major work in this dissertation was focused on the use of electrochemical detectors for flowing liquid system consisting of flow injection analysis, high performance liquid chromatography, and microchip capillary electrophoresis. While understanding the behavior of electrochemical detection process, boron-doped diamond thin film electrode was tested for importance thiol-containing drugs and homocysteine. In the next section, several electrode materials employed as a detector for microchip capillary electrophoresis for detection of hazardous contaminants in environmental assays were evaluated.

#### **6.1 Boron-Doped Diamond Thin Film Electrode for Thiol-Containing Drugs Analysis Coupled with Flow Injection Analysis System**

The use of BDD electrode for the detection of organic species made particular use of the extremely wide potential range. Such electrodes have been used as working electrodes to detect various kinds of organic compounds. Thiol-containing drugs represent an important class of thio-compounds due to their clinical significance. Cyclic voltammetry was first used to examine the electron transfer reactions of thiol-containing drug analytes. In an early section, the effect of pH buffers were studied at BDD electrode and the experiments were compared with those of glassy carbon electrode. The measurement of the background current response of interfaces formed at BDD electrodes in contact with aqueous electrolytes was also performed. It has been suggested that the oxidation of cysteine taken place on the diamond surface has been used as a model for other thiol-containing compounds [162]. We believed that all thiol-containing drugs behave on the BDD electrode in the same way as cysteine. The electrochemical oxidation of thiol-containing drugs occurred by the dissociation of proton from the thiol group, followed by the electrochemical oxidation of thiol anion ( $RS^-$  ion). It was found that the electrochemical oxidation reaction at BDD electrode was the rate determination step, whereas at GC electrode the overall oxidation reaction was controlled by the desorption of reaction products. These studies

explained why BDD electrode was better than GC electrode for the electroanalysis of thiol-containing drugs. In order to study the adsorption on the electrode surface, the cyclic voltammograms were recorded during variation of the scan rate for the BDD electrode. It can be seen the peak currents were linearly proportional to the square root of the scan rate within the examined ranges. The linearity suggested that the current was limited by semi-infinite linear diffusion of thiol-containing compounds in the interfacial reaction zone and that rate limiting adsorption step and specific surface interactions can be neglected. Moreover, the concentration dependences were studied at BDD electrode. The response of the BDD electrode was appropriate quantitative determination of thiol-containing drugs even in the millimolar concentration ranges.

Next, FIA with diamond thin-film detectors was employed to determine thiol-containing drugs in both standard chemical form and commercially available formula. These redox reactions were studied by hydrodynamic voltammetry, and FIA with electrochemical detection (FIA- EC) at a BDD electrode. The aim of the use of FIA- EC was to improve the speed, the sensitivity, the reproducibility, and the detection limits. The FIA amperometric response obtained for a BDD electrode provided the reproducible peak currents, with a peak variability of less than 5 % (n=10), which confirms a lack of adsorption of oxidation products on the electrode surface. Furthermore, this approach has been applied for determinations thiol-containing drugs in pharmaceutical formula. The results demonstrated the promise of the use of BDD as an amperometric detector in FIA for the analysis of these types of compounds.

## **6.2 The Electrochemical Oxidation of Homocysteine at Anodized Boron-Doped Diamond Thin Film Electrode with Amperometric HPLC**

An increasing number of publications have described the usefulness of boron-doped diamond thin film electrode for electroanalysis, which results from their unique electrochemical properties. The modification of diamond surfaces were becoming more and more extensively used recently because it provided enhanced sensitivity and selectivity for the detection of variety compounds. The anodic polarization in alkaline solution was one simple method for chemically modifying BDD electrode. These treatments resulted in the incorporation of substantial amounts of oxygen on the electrode surface. In order to enhance the sensitivity for homocysteine detection,

cyclic voltammetric and amperometric measurements were carried out at anodized BDD electrode in acidic solution. The concentration and scan rate dependence on the voltammetric responses were thoroughly examined including the hydrodynamic voltammetry also was used to study the detection potentials. In addition, to increase the selectivity and efficiently eliminate interference anodized BDD electrode was applied as a detector in HPLC system. The results discussed demonstrated that the use of anodized BDD electrode clearly provided an effective methodology for the determination of homocysteine with high sensitivity, reproducibility and low detection limit. In addition, these behaviors have been demonstrated to be generic in nature with cystine, cysteine, reduced glutathione, methionine, homocystine, and oxidized glutathione providing the similar electrochemical responses. Thus, the developed electrochemical method would be competent for the reliable determination of homocysteine in biological system and for studying homocysteine-associated biological events.

### **6.3 Microchip Capillary Electrophoresis Coupled with Electrochemical Detection**

Following growing interest in the development of electrochemical detectors, an effort has been made to understand the electrode kinetics in CE-based separations on microchip platforms. Judicious selection of detection parameters was critical in minimizing / eliminating the high voltage effects on the detection process and to obtain high efficiency separations.

Two factors have been affected the reaction rate: detection potential and the feature of the electrode surface. Favorable detection potential resulted in efficient electro-reaction and favorable electrode-surface offered fast reaction. Various materials were explored and evaluated in detail while developing sensitive detection schemes, including screen-printed carbon electrode and its surface modified with gold, glassy carbon electrode, and cobalt phthalocyanine modified carbon paste electrode. Sensitive detection and efficient separations were achieved for variety of group of compounds (including hydrazine compounds, phenolic compounds, and nitroaromatic explosives). An attempt was made in this study to look at the effect of electron transfer rate (either by modulating the detection potential or the electrode material). It was found that the electron transfer rate played an all-important role in improving the

resolution in micromachined capillary electrophoresis microchips. When an analyte passes from the channel outlet and the applied potential was set on the working electrode at a sufficiently positive value for its oxidation (for example), as the analyte band passed over the electrode surface, those molecules immediately adjacent to the electrode surface will be oxidized in a heterogeneous transfer of electrons. The current that results from this exchange of electrons with the surface was measured as a function of time. Since the rate of material conversion by the EC reaction was proportional to the instantaneous concentration, the current will be directly related to the amount of compound eluted as a function of time. The optimization of detection potential was necessary while developing a sensitive detection protocol for microchip applications by constructing hydrodynamic voltammogram (HDV). HDV's represent a rapidly achieved mass transport steady state and the magnitude of current was directly proportional to bulk concentration at every point along the voltammetric curve. Furthermore, it was demonstrated that the separation voltage (at a given detection potential) also must be optimized as it severely limits the potential applied onto the electrode (due to high separation voltages involved).

Electrochemical detection was ideally suited for environmental assays as most of the relevant compounds are electroactive, for example, hydrazines, phenolic compounds, and nitroaromatic compounds. Sensitive detection schemes were developed after electrophoretic separation on microchip platforms. Separation parameters were studied and optimized. Excellent reproducibility of signals was achieved with high sensitivities and fast separations. Due to their importance in security scenario, nitroaromatic explosives determination was carried out by employing MEKC separation. Finally, we reported on the direct coupling of hydrodynamically flowing stream to a microchip capillary electrophoresis (CE) for continuous assays of liquid samples. The new interface relied on mounting the sample tubing onto a sharp inlet tip and allows rapid, convenient and reproducible electrokinetic loading from a continuously flowing stream directly into the narrow separation microchannel. Such simple and effective interface facilitates and the high sensitive electrochemical detection, it could lead to portable fully automated devices for on-site environmental monitoring.

#### 6.4 Suggestion for Future Work

Based on its range of superior characteristics for sensitive electroanalytical measurements, BDD as an electrode material and electrochemical sensor material may constitute one of the more important developments in this area, which further support the development of new applications from flowing liquid system. Miniaturization of analytical procedures will have a significant impact on all aspects of diagnostic testing as we move into the 21<sup>st</sup> century. It will enable highly complex clinical testing to be miniaturized, and hence permit testing to move from the central laboratory into non-laboratory settings. Compared to previous analytical technologies, microchips offer an enlarged and unified menu of tests, and this will modify thinking on deployment of such devices and may have far reaching effects on the future of central laboratories.

Compared to already existing detection schemes, electrochemical detection was shown to be excellent in view of the integrated system requirements (such as low power, low-cost, sensitivity, and selectivity etc). The majority of the research described is still basic work and further studies are needed for application to practical chemical and/or biochemical micro total analysis system.