CHAPTER III
EXPERIMENTAL

This chapter has provided the information of instruments and equipments, apparatus, chemicals and reagents, and sample preparation employed in this work.

3.1 Instruments and Equipments

The following were the list of instruments utilized in this work.

3.1.1 A Metler AT 200 or a Precisa of 40SM-200A (Switzerland) analytical balance was used for weighing chemicals in the preparation of standard and reagent solutions.

3.1.2 pH meter 744 (Metrohm) was used to measure the pH of prepared buffer solutions.

3.1.3 Milli-Q water system, model Millipore ZMQS 5 VOOY, Millipore, USA. was used to produce deionized-distilled water for preparing chemical and reagent solutions.

3.1.4Autolab Potentiostat (PG-30, Methrom) was used to record the electrochemical response of analytes.

3.1.5 CHI Version 3.27 software (CH Instruments) was used to record the amperometric response of analytes in microchip CE system

3.1.6 Diamond electrodes (compliment of Prof. Akira Fujisjima, Japan), the films were prepared by deposition of the BDD thin films on highly conductive n-Si (1 1 1) substrates by microwave plasma-assisted chemical vapour deposition. Deposition was usually carried out for 10 h to achieve a film thickness of approximately 30 μm. The nominal B/C atomic ratio in the gas phase was 1:100, and the typical boron-doping level in the film was approximately 10^{21} cm^{-3}.

3.1.7 Glassy carbon electrode (0.07 cm^2, Bioanalytical System Inc) was pretreated by polishing with alumina powder (1 and 0.05 micron, respectively) slurries in ultrapure water on felt pads and rinsed thoroughly with an ultrapure water prior to use.

3.1.8 Ag/AgCl electrode (TCI) with a salt bridge was used as a reference electrode in batch analysis.

3.1.9Ag/AgCl electrode (RE-3V aqueous reference electrode, Screw type) was was used as a reference electrode in the flowing system.

3.1.10 Home-made platinum wire was used as a counter electrode.

3.1.11 Home made glass cell was used in batch analysis

3.1.12 Home made brass holder was used to hold diamond electrode and glass cell and for making an ohmic contact

3.1.13 O-ring viton (0.07 cm^2) was placed at the bottom home made glass cell to isolate and control the electrode surface.

3.1.14 Polishing set of 0.05 and 1 micron alumina powder slurry (Bioanalytical System Inc.) was used to polish the glassy carbon electrode.

3.1.15 Thin layer flow cell (Bioanalytical System Inc.) was used as flow through cell for flow injection system.

3.1.16 Teflon cell gasket (Bioanalytical System Inc.) was used to place against diamond electrode and flow cell.

3.1.17 Rheodyne injection valve, Model 7725 (Altech), with a 20 µl stainless steel injection loop (0.5 mm. i.d.) was used in the flow injection to deliver the sample solution into the system.

3.1.18 Peristaltic pump (Ismatic) was used to continuously deliver a carrier solution into the flow system.

3.1.19 A home made pulse dampener was used in series to reduce the pulsation introduced by the alternation of the roller of the peristaltic pump.

3.1.20 Automated LC system consisted of a binary pump (GL Sciences, Inc., PU611), an autosampler (Spark-Holland, Triathlon) for constant 20 mL injections, a thinlayer flow-cell (GL Sciences, Inc.), an amperometric detector (Bioanalytical Systems, LC-4C), and a data acquisition system (EZChrom Elite, Scientific Software, Inc.) was used to in the separation and detection homocyteine and related compounds.

3.1.21 The chromatographic column (Inertsil ODS-3) was obtained from GL Sciences, Inc. and used as analytical comlumn

3.1.22 Solid phase extraction (Phenomenex) was used for trapping organic substance and unwanted particle from sample solution before injection into the system.

3.1.23 Auto pipette and tips were obtained from Eppendrof, Germany

3.1.24 Cutting set (Altech)

3.1.25 0.2 μm Nylon membrane filter (Altech)

3.1.26 0.45 μm Nylon membrane syringe filter with polypropylene (PP) housing (Orange Scientific filter)

3.2 Apparatus for Microchip Capillary Electrophoresis System

Details of the integrated CE-EC glass chip microsystem were described previously [176]. Briefly, the homemade high-voltage power supply had an adjustable voltage range between 0 and +4000 V. The simple-cross single-separation channel glass microchip was obtained from Micralyne (model MC-BF4-001, Edmonton, Canada). The detection reservoir was cut off to facilitate the end-column electrochemical detection. The chip had a 75-mm-long separation channel (from injection cross to the channel outlet) and a 5-mm-long injection channel (between the sample reservoir and injection cross). The channels had a maximum depth of 20 μm and a width of 50 μm at the top. Short pipette tips were inserted into the holes of the various reservoirs. A Plexiglass holder was fabricated for housing the separation chip and the detector and allowing their convenient replacement and reproducible positioning. Platinum wires, inserted into the individual reservoirs on the holder, served as contacts to the high-voltage power supply.

3.3 Chemicals and Reagents

All chemicals were of analytical grade or better and were used without further purification. Deionized-distilled water obtained from a Milli-Q-system was used for the preparation of chemical and reagent solutions. List of chemicals and their suppliers was summarized in Table 3.1

Table 3.1 List of Chemicals and Reagents and Their Suppliers

Chemicals/ Reagents	Formula	Suppliers
Tiopronin	$C_5H_9NO_3S$	Sigma
Captopril	$C_9H_{15}NO_3S$	Sigma
Homocysteine	$C_4H_9NO_2S$	Sigma
Cystine	$C_6H_{12}N_2O_4S_2$	Wako
Cysteine	$C_3H_7NO_2S$	Wako
Reduced glutathione	$C_{10}H_{17}N_3O_6S$	Sigma
Methionine	$C_4H_6N_2S$	Wako
Homocystine	$C_8H_{16}N_2O_4S_2$	Wako
Oxidized glutathione	$C_{20}H_{32}N_6O_{12}S_2$	Wako
Hydrazine sulfate	$H_6N_2O_4S$	Aldrich
1,1 dimethylhydrazine	$C_2H_8N_2$	Aldrich
Phenylhydrazine hydrochloride	$C_6H_8N_2$	Sigma
Phenol	C_6H_6O	Aldrich
2-chlorophenol	C_6H_5ClO	Aldrich
2,3-dichlorophenol	$C_6H_4Cl_2O$	Aldrich
4-nitrophenol	$C_6H_5NO_3$	Aldrich
Sudan I	$C_{16}H_{12}N_2O$	Aldrich
Sudan II	$C_{18}H_{16}N_2O$	Aldrich
Sudan III	$C_{22}H_{16}N_4O$	Flika
Sudan IV	$C_{24}H_{20}N_4O$	Aldrich
Cyclotrimetylene trinitramine (RDX)	$C_3H_6N_6O_6$	Cerilliant Inc.
pentaerythol tetranitrate (PETN)	$C_5H_8N_4O_{12}$	Cerilliant Inc.
2-(*N*-Morpholino)-ethanesulfonic acid (MES)	$C_6H_{13}NO_4S$	Sigma
Sodium dodecyl sulfate (SDS)	$C_{12}H_{25}NaO_4S$	Sigma

Table 3.1 Cont.

Chemicals/ Reagents	Formula	Suppliers
Sodium phosphate monobasic monohydrate	H_4NaO_5P	Sigma
Sodium phosphate dibasic	HNa_2O_4P	Sigma
Sodium hydroxide	NaOH	Merck
Hydrochloric acid	HCl	Merck
Phosphoric acid 85%	H_3O_4P	Merck
Graphite powder	-	Johnson Mallthey
Gold atomic absorption standard solution	-	Aldrich
Borax (sodium tetraborate)	$B_4H_{20}Na_2O_{17}$	Sigma
Acetonitrile	C_2H_3N	Merck
1-octanesulfonic acid sodium salt	$C_8H_{19}NaO_4S$	Wako
Tetrabutylammonium perchlorate	$C_{16}H_{36}ClNO_4$	Sigma

3.4 Preparation of Solutions

These successive parts included the preparation procedures of supporting electrolyte and standard solutions employed in this work.

3.4.1 0.1 M Phosphate Buffer

The buffer solutions were prepared with boiled distilled, CO_2-free water. The weight of sodium di-hyrogen phosphate and di-sodium hydrogen phosphate of various pH values were given in Table 3.1. Phosphate buffer (pH 2.5) was prepared from 0.1 M potassium dihydrogen phosphate and the pH was adjusted with orthophosphoric acid (85%, Carlo Erba). Phosphate buffer (pH 9 and pH 10), 0.1 M, was prepared from 0.1 M potassium dihydrogen phosphate and the pH was adjusted with 0.1 M sodium hydroxide.

Table 3.2 Compositions of 0.1 M Phosphate Buffer Solutions

pH Values	$NaH_2PO_4.H_2O$(g)	$Na_2HPO_4.2H_2O$(g)	Volume of Stock Solution (mL)
2.5	6.8995	0	500
5.0	6.8443	0.0712	500
6.0	6.1350	0.9878	500
7.0	2.8495	5.2064	500
8.0	0.2553	8.5702	500
9.0*	0	8.8995	500
10.0*	0	8.8995	500

* The solutions were prepared from 0.1 M di-sodium hydrogen phosphate and pH was adjusted with 0.1 M sodium hydroxide.

3.4.2 10 mM Phosphate Buffer

The buffer solutions were prepared in the same fashion as shown in 3.4.1. Table 3.3 shows the list for various pH solutions used in microchip CE system.

Table 3.3 The Composition of 10 mM Phosphate Buffer Solutions

pH Values	$NaH_2PO_4.H_2O$(g)	$Na_2HPO_4.2H_2O$(g)	Volume of Stock Solution (mL)
5	0.1379	0.0014	100
6	0.1227	0.0518	100
6.5	0.0901	0.0493	100
6.8	0.0737	0.0662	100
7.0	0.0570	0.0833	100
7.3	0.0544	0.2280	100
7.6	0.0353	0.2476	100
8.0	0.0051	0.1714	100

3.4.3 100 mM MES Buffer pH 4

3.904 g of MES was dissolved in 200 mL of deionized water and then adjusted with 0.1 M sodium hydroxide or 85% phosphoric acid to the required pH.

3.4.4 100 mM Borate Buffer pH 9

Borate 9.5343 g was dissolved and diluted with deionized water into 250 mL volumetric flask to give a stock solution of 100 mM.

3.4.5 30 mM SDS

0.865 g of SDS powder was dissolved in 100 mL of 20 mM MES buffer (pH 4). Working solutions were obtained by appropriate dilution with 20 mM MES buffer (pH 4).

3.4.6 Stock Standard Solution of Thiol-Containing Drugs

The 10 mM tiopronin solution was prepared by weighing 0.4080 g tiopronin powder and transferring into 250 mL volumetric flask. The 0.1 M phosphate buffer (pH 8) was used for diluting this aliquot to the mark. This solution was used for studying pH dependence and investigating the oxidation of tetracycline by cyclic voltammetry. The 1 mM tiopronin solution for FIA study was prepared by an appropriate amount and dilution. The captopril stock standard solution was prepared in the same manner as described for preparation of tiopronin.

3.4.7 Thiol-Containing Drugs for Calibration

The standard solution of tiopronin solution at various concentrations, for calibration experiment, were prepared by appropriately diluting of 1000 μM stock solution with 0.1 M phosphate buffer (pH 8) into 10 mL volumetric flask. The concentration and volumes required for these preparations are shown in Table 3.4

Table 3.4 Compositions of Tiopronin Standard Solutions

Final Concentration of Tiopronin Solution (μM)	Concentration of Tiopronin Solution (μM)	Volume of Stock Solution (mL)	Volume of 0.1 M Phosphate Buffer (mL)
0.01	0.025	10	6
0.025	0.05	10	5
0.05	0.1	10	5
0.1	0.5	10	8
0.5	1	10	5
1	5	10	8
5	10	10	5
10	25	10	6
25	50	10	5
50	100	10	5

Table 3.4 Cont.

Final Concentration of Tiopronin Solution (μM)	Concentration of Tiopronin Solution (μM)	Volume of Stock Solution (mL)	Volume of 0.1 M Phosphate Buffer (mL)
100	250	10	6
250	500	10	5
500	1000	10	5
1000	-	10	-

The captopril stock standard solutions, they were also prepared in the same way as described for the preparation of tiopronin.

3.4.8 Stock Standard Solution of Aminothiol Solutions

Homocysteine 0.16900 g was weighted and dissolved in 0.05 M phosphate solution pH 2.7 or 0.1 M phosphate buffer pH 9 for cyclic voltammetric study. The desired concentrations were obtained from dilution of stock solution with suitable phosphate buffer. The reset of aminothiols (Cystine, Cysteine, Homocystine, Reduced glutathione, Oxidized glutathione, and Methionine) were also prepared in the same direction as mentioned for homocysteine. All solutions were prepared daily.

3.4.9 Stock Standard Solution of Phenolic Compounds

Stock solutions of phenolic compounds (phenol, 2-chlorophenol, 2,3-dichlorophenol and 4-nitrophenol, 100 mM) were prepared daily in deionized water. The accurate amounts of 0.0941 g of phenol, 0.1286 g of 2-chlorophenol, 0.1630 g of 2,3-dichlorophenol, 0.1391 g of 4-nitrophenol were diluted into 10 mL volumetric flask.

3.4.10 Stock Standard Solution of Explosive Compounds

Stock solutions (1000 mg/ L in acetonitrile) of Cyclotrimetylene trinitramine (RDX) and pentaerythol tetranitrate (PETN) were diluted to required concentration by dilution with 20 mM MES buffer (pH 4).

3.4.11 Stock Standard Solution of Hydrazine Compounds

0.0032 g of hydrazine, 0.0601 g of 1,1 dimethylhydrazine, and 0.1081 g of phenylhydrazine were weighted and dissolved in deionized water and then transferred into 10.00 mL volumetric flask. The working standard solutions were diluted with suitable volume phosphate buffer to obtain the desired concentration.

3.4.12 Mobile Phase for HPLC-ECD

0.70 mL of 85% phosphoric acid was pipetted and transferred into 1.0 L volumetric flask. Then, pH was adjusted to 2.7 by adding drop-wise 0.1 M sodium di-hydrogen orthophosphate. 970 mL of this phosphate buffer solution was combined with 30 mL acetonitrile solution in 1.0 L flask. 0.0433 g of 1-octanesulfonic acid sodium salt (OSA) was added and then mixed thoroughly to complete dissolution and degas by ultrasonic bath.

3.4.13 Aminothiol Mixture Standard Solutions for Calibration

The standard solution of 7-standard aminothiol solution mixture at various concentrations, for calibration purpose, were prepared by appropriately diluting 500 μM stock solution with 0.1 M phosphate buffer (pH 2.5) into 10 mL volumetric flask. The concentration and volumes required for these preparations are shown in Table 3.5

Table 3.5 Compositions of Mixture Standard Solutions of Seven Aminothiols

Final Concentration of 7-Standard Aminothiol Solution (μM)	Concentration of 7-Standard Aminothiol Solution (μM)	Volume of Stock Solution (mL)	Volume of 0.1 M Phosphate Buffer (mL)
0.001	0.005	10	8
0.0025	0.005	10	5
0.005	0.01	10	5
0.01	0.05	10	8
0.025	0.05	10	5
0.05	0.1	10	5
0.1	0.5	10	8
0.25	0.5	10	5
0.5	1	10	5
1	5	10	8
2.5	5	10	5
5	10	10	5
10	50	10	8
25	50	10	5
50	100	10	5
100	500	10	8
250	500	10	5

3.5 Real Sample Analysis

Tablets containing tiopronin (Mission pharmacal) were analyzed. The drug tablet was homogenized in an agar mortar. The amount of the powdered mass analyte was dissolved in 100 ml of 0.1 M phosphate buffer (pH 8). This solution was diluted in such a way that the concentration of tiopronin in the final test solution was within the linear dynamic range (0.5–50 μM). Thiol-containing drug tablets used in this study available in the market such as captropril and tiopronin summarized in Table

3.6. The sample preparations were also carried out in the same manner as those of tiopronin, except that the final concentration set within the linear dynamic range.

Table 3.6 List of Thiol-Containing Drugs Employed in This Work

Sample Name	Supplier	Weight of Tablet (mg)	Active Ingredient (mg/tablet)
THIOLA ™	Mission	150	100
Captropril	Bristol-Myers	50	12.5

3.6 Preparation of CoPc-Modified Carbon Paste

Unmodified carbon paste was prepared by hand-mixing 1 g of graphite and 0.6 mL mineral oil. CoPc-modified paste was prepared in a similar fashion except that the graphite powder was mixed with desired weight of CoPc powder prior to the addition of mineral oil. 10 mL of diethyl ether was added to complete mixing of these two components. The compositions of CoPc-modified carbon paste were displayed in Table 3.7.

Table 3.7 Compositions of %CoPc Loading in Carbon Paste

% CoPc	Weight of CoPc (g)	Weight of Graphite Powder (g)	Mineral Oil (mL)	Diethyl Ether (mL)
0	0.0	1.0	0.6	2.0
1	0.01	0.99	0.6	2.0
2	0.02	0.08	0.6	2.0
4	0.04	0.96	0.6	2.0
5	0.05	0.95	0.6	2.0
10	0.1	0.90	0.6	2.0

3.7 Procedures of Electrochemical Detection

3.7.1 Batch Analysis

3.7.1.1 Cyclic Voltammetry

The electrochemical measurements were performed in a single compartment glass cell using a potentiostat. Figure 3.1 showed an electrochemical cell for cyclic voltammetric experiment. An Ag/AgCl (A) was used as the reference electrode and platinum wire (B) was employed as the counter electrode. The boron-doped diamond electrodes (0.07 cm^2) were used as working electrodes (C). The working electrodes were pressed against a smooth ground joint at the bottom of the cell isolated by O-ring (area 0.07 cm^2). The exposed geometric area was 0.07 cm^2 Ohmic contact was made by placing the backside of the Si substrate on a brass plate.

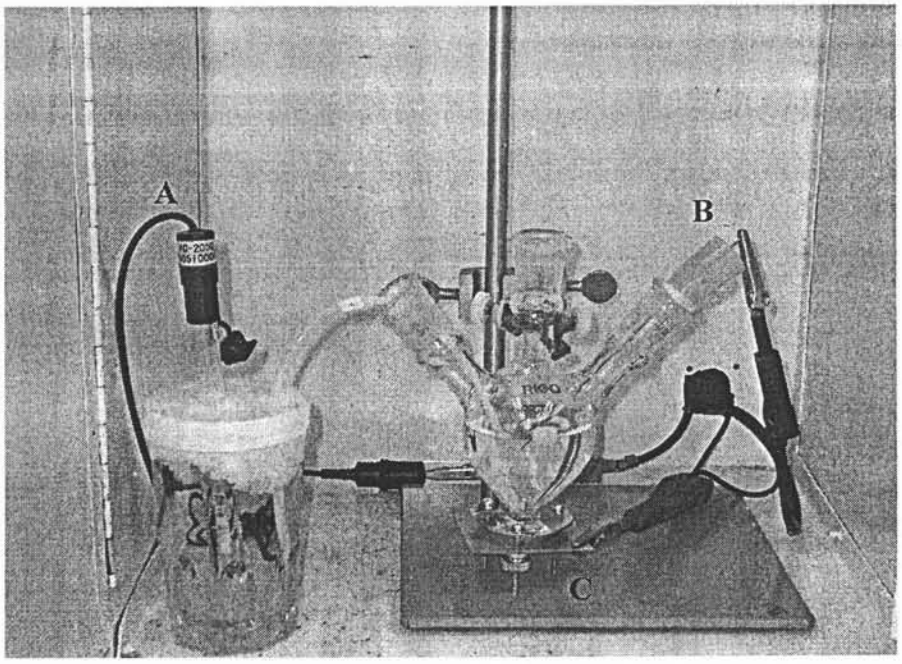

Figure 3.1 The electrochemical cell for cyclic voltammetric study.

3.7.1.2 Background Current

The experiment was carried out in 0.1 M phosphate buffer using boron-doped diamond electrode at the scan rate of 50 mV/ s. The results were compared with thoseglassy carbon electrodes.

3.7.1.3 pH Dependence

These experiments were performed to obtain the optimum pH for captopril and tiopronin at the scan rate of 50 mV/s. 1 mM captopril solutions were prepared in pH 2.5, 5, 6, 7, 8, and 9 phosphate buffer, respectively. These solutions were studied by cyclic voltammetry.

3.7.1.4 The Electrochemical Oxidation of Thiol-Containing Drugs

The 1 mM solutions of target analytes in the chosen buffer solution from the previous experiment (Section 3.7.3) were studied using boron-doped diamond electrode by cyclic voltammetry. The experiments were repeated with the use of glassy carbon electrode. Results obtained from both set of experiments were compared. A scan rate was fixed at 50 mV/s

3.7.1.5 Effect of Scan Rate

Using 1 mM solutions of analytes, experiments were performed to investigate the adsorption of the analytes on the electrode surface at various scan rates. The utilized scan rates in these experiments were 10, 20, 50, 100, 200 and 300 mV/ s.

3.7.1.6 The Analytical Performance

Stock solution of 10 mM of captopril and tiopronin were

freshly prepared and diluted to a concentration range between 0.0025 and 10 mM. A scan rate of 50 mV/ s was used. These studies were carried out to find the linear ranges and detection limits.

3.7.2 Flow Injection with Amperometric Detection

The flow injection analysis system used in this experiment consisted of a thin layer flow cell, an injection port with a 20 μL injection loop, a reagent delivery module or a peristaltic pump and an electrochemical detector. The mobile phase, 0.1 M phosphate buffer, was regulated by a reagent delivery module at a flow rate of 1 mL/min. A pulse dampener was used in series to reduce the pulsation introduced by the alternation of the roller of the peristaltic pump. Figure 3.2 shows a thin layer flow cell for the flow injection system. The thin layer flow cell consisted of a silicon rubber gasket as a spacer, an Ag/AgCl electrode (A) as the reference electrode and a stainless steel tube as an auxiliary electrode (B) and an outlet of the flow cell. The experiments were performed in a copper faradaic cage to reduce electric noise.

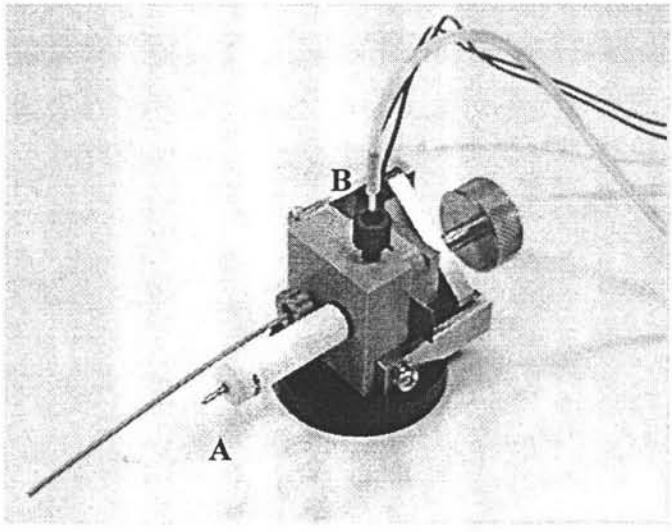

Figure 3.2 The thin layer flow cell.

3.7.2.1 Hydrodynamic Voltammetry

Hydrodynamic voltammetry was performed for each analyte

before the amperometric determination to find the optimum operating potential. The data were obtained by recording the background current at each potential and then injecting a series of three replicate of 20 μL of 100 μM analyte solutions, respectively. The peak current after each injection was recorded, together with the corresponding background current. These data were plotted as a function of applied potential to obtain hydrodynamic voltammograms.

3.7.2.2 Calibration and Linear Range

1 mM stock solutions of each analyte were freshly prepared and then diluted to a concentration range from 10 nM to 1 mM. The experiments were carried out by injection of three replicates of each concentration. The obtained results were used to plot the calibration graph and the linear dynamic range was obtained.

3.7.2.3 Limit of Detection (LOD)

The limit of detection (LOD) was carried out by injection flow concentrations of analyte solutions for three replicates under the optimal potential. The detection limit was defined as the concentration that provided a current response three times higher than the noise (S/N ≥3).

3.7.2.4 Repeatability

The repeatability was studied by injecting ten replicates of analyte solutions. The repeatability was assessed in terms of the relative standard deviation (%RSD), using the following formula:

$$\% \text{ RSD} = \frac{\text{standard deviation}}{\text{Mean}} \times 100$$

3.7.3 High Performance Liquid Chromatography (HPLC)

A HPLC equipped with a C18 column and phosphate buffer and acetonitrile as mobile phase were used to develop optimal separation. The HPLC system consisted of a thin layer flow cell, an injection port with a 20 μL injection loop, a reagent delivery module or a peristaltic pump and an electrochemical detector. The mobile phase was regulated by peristaltic pump at a flow rate of 1 mL/min Figure 3.3 shows a thin layer flow cell for the HPLC system. The flow cell was assembled with a silicon rubber gasket as a spacer, a Ag/AgCl electrode (A) as the reference electrode and a stainless steel tube as an auxiliary electrode (B) and outlet of the flow cell. The experiments were performed in a copper faradaic cage to minimize electric noise. The separation of all compounds was first tested with standard mixture and the condition was later tested with shrimp spiked matrix standard. Table 3.8 summarizes this optimal HPLC condition.

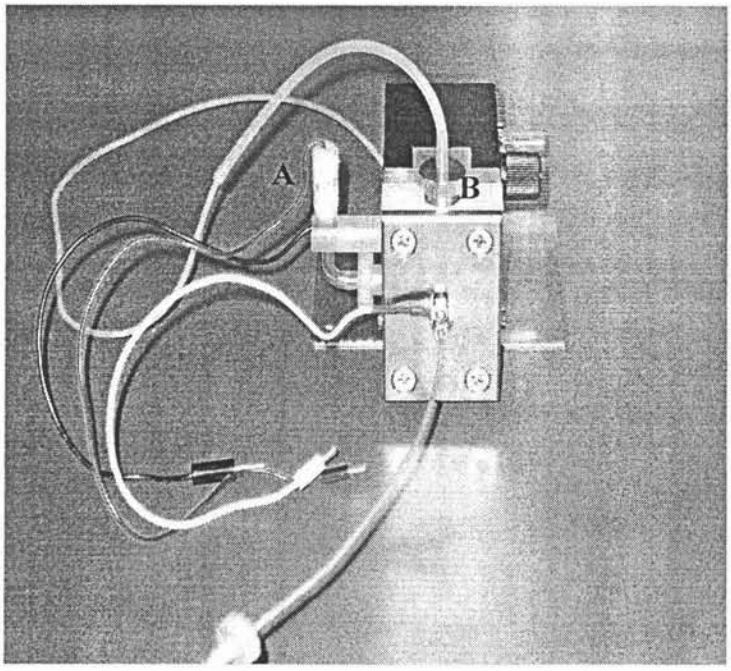

Figure 3.3 The thin layer flow cell for HPLC system.

Table 3.8 Parameters of the HPLC Condition

Parameter	Condition
Column	ODS-3 Inertsil C18, 5 μM 4.6 x 250 mm i.d.
Mobile phase	Isocratic elution at 3% acetonitril-0.05 M phosphate buffer(pH 2.7) and 0.2 mM 1-octane-sulfonic acid
Flow rate	1 min/mL
Injection volumn	20 μL
Detector	Electrochemical detector

3.7.3.1 Optimum Potential for HPLC/ ECD

To obtain an optimal potential for the HPLC- amperometric detection, mixture standard solution of seven aminothiol was injected into the system to find the optimum operating potential to be set the instrument for HPLC system. The data were obtained by recording the background current at each potential and then injecting a series of two replicate of 20 μL of 20 μM aminothiol mixture standard solution using the condition in Table 3.8. The peak current after each injection was recorded, together with the corresponding background current. These data were plotted as a function of applied potential and oxidation currents to obtain hydrodynamic voltammograms.

3.7.3.2 Calibration Curve and Linear Range of HPLC

Each concentration of mixed 7 standard aminothiol solutions in Table 3.5 were measured. The peak areas were plotted as a function of concentration. Each point was the average of two replicates. The value of slope, retention time, intercept and correlation coefficient (R^2) of each tetracycline in the mixed standard were obtained using the conditions in Table 3.8.

3.7.3.3 Limit of Detection (LOD)

The limit of detection (LOD) was determined by injection of various analyte solutions to get the lowest concentration providing the signal higher than the buffer about three times. The analytes were injected into HPLC system under optimum condition listed in Table 3.8. The detection limit was defined as the concentration that provided a current response three times higher than the noise (S/N $\geq$3).

3.7.3.4 Precision

Two types of precision were evaluated, namely, the intra-day Precision obtained by repeat analysis of spiked sample in one day and the inter-day precision obtained by analysis on different days. The study was carried out at the spiking level of three different concentration levels. Two (for HPLC) or three injections of each concentration were analysed in the same day to assess the intra-day precison and were then repeated on a different day to obtain the inter-day precision.

3.7.3.5 Accuracy

The accuracy of the developed method was assessed by calculating the average of % recovery, SD and %RSD that were obtained from each spiking level on two different days.

3.7.4 Microchip CE Layout

Figure 3.4 showed the microchip CE system used in this work. The system consisted of glass chip, chip holder, high voltage power supply, and electrochemical detection. The glass chip was placed in a laboratory-bulit Plexiglass holder. The holder contained a sample, a buffer, and a detection reservoir. The platinum wires, inserted into each reservoir, served as contacts for the high voltage power supply to create sample injection and separation. A platinum wire and an Ag/AgCl wire were inserted into the detection reservoir, served as the counter and reference electrodes.

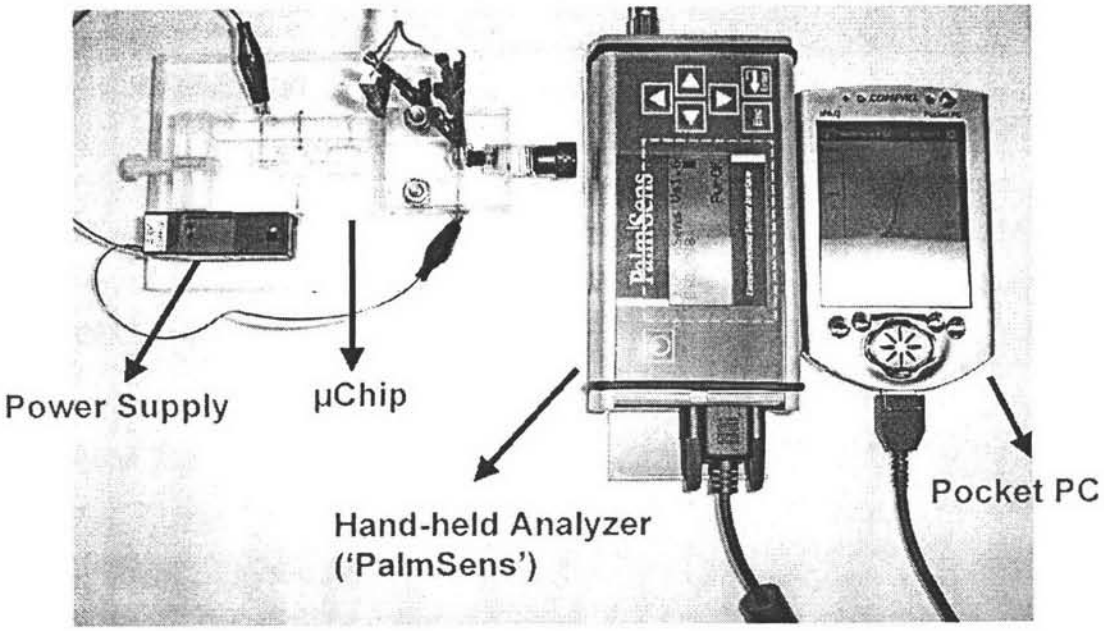

Figure 3.4 Microchip capillary electrophoresis coupled with electrochemical detection layout.

3.7.5 End-Column Amperometric Detection

The detector performance relies on reproducible positioning of working electrode. This was accomplished by inserting the working electrode in a special groove into which the strip fits exactly. The electrode was further held in place by a plastic screw pressing the strip or disc against the channel outlet. Amperometric detection was performed with an Electrochemical Analyzer 621A (CH Instruments, Austin, TX) using the "amperometric *i-t* curve" mode. The electropherograms were recorded with a time resolution of 0.1 s while applying a desired detection potential versus Ag/AgCl wire. Sample injections were performed after stabilization of the baseline. The raw data of electropherograms were digitally filtered using built-in 15-point least-square smoothing by CHI Version 3.27 software (CH Instruments).