CHAPTER II
THEORY AND LITERATURE SURVEY

2.1 Fundamental of Electrochemistry [6, 7]

Electrochemistry was concerned with charges and their movement and transfer from one medium to another. The ultimate unit of charge was that carried out by the electron; electrons are important in electrochemistry. Electrochemical detection is based on the monitoring of changes in an electrical signal, due to an electrochemical reaction at an electrode surface, usually as a result of an imposed potential or current. Figure 2.1 implies that the interplay of electrochemical process takes place at the electrode-solution interface.

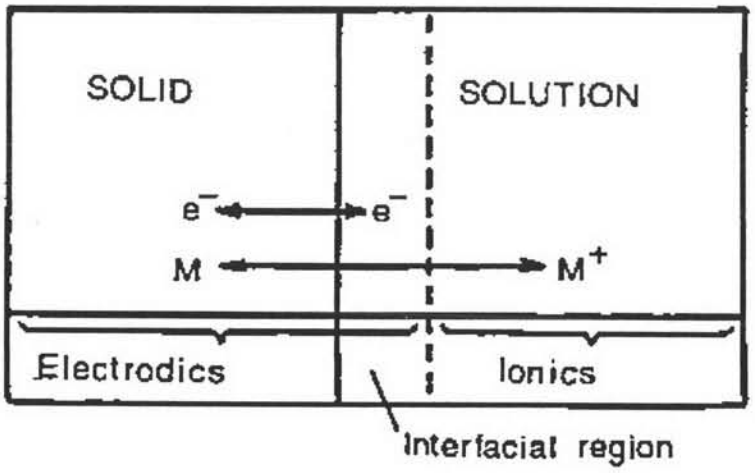


Figure 2.1 Electrodics is concerned with the exchange of electrons between electrodes and species in solution (these are frequently ions). Ionic is the discipline concerned with the behavior of ions in solution [7]

In a solution, the equilibrium concentrations of the reduced and oxidised forms of a redox couple are linked to the potential (E) via the Nernst equation (1),

$$E = E^{o} + \frac{RT}{nF} \ln \frac{c_{ox}}{c_{red}} \qquad (1)$$

where E^0 denotes the standard potential, c_{ox} and c_{red} the concentrations of the oxidised and reduced forms, respectively, while the other symbols have their usual meanings. For each redox couple, a potential exists, known as the standard potential E^0 at which the reduced and oxidised forms are present at equal concentrations. If a potential E, with respect to the reference electrode, is applied to the working electrode, e.g. by the use of a potentiostat, the redox couples present at the electrode respond to this change and adjust their concentration ratios according to Equation (1). In this process, electrons have to be transferred. If the potential of the electrode is made more negative, the energy of the electrons in the electrode has been increased, and eventually an electron can be transferred from the electrode to the lowest unoccupied energy level of a species in the nearby solution. This would be the oxidised form of the redox couple, which is thus reduced. Vice versa, by applying a sufficiently high positive potential, the reduced form can be oxidised. The movement of electrons in or out of the electrode can be measured as an oxidation or reduction current.

The distinction between various electroanalytical techniques reflects the type of electronic signal used for the quantitation. The two types of electroanalytical measurements are potentiometric and potentiostatic. This chapter attempts to give an overview of these methods.

2.1.1 Potentiometry [8, 9]

Potentiometry is the field of electroanalytical chemistry in which potential is measured under the conditions of no current flow. The measured potential may then be used to determine the analytical quantity of interest, generally the concentration of some component of the analyte solution. This concept is typically introduced in quantitative analysis courses in relation to electrochemical cells that contain an anode and a cathode. For these electrochemical cells, the difference in potential between the cathode and the anode electrode is the potential of these electrochemical cells. Since the beginning of the twentieth century, potentiometric

techniques have been used for the endpoint location of titrimetric methods of analysis. In more recent original methods, ion concentrations are obtained directly from the potential of an ion-selective membrane electrode. Such electrodes are relatively free from interference and provide a rapid and convenient means for quantitative estimations of numerous important anions and cations. The equipment required for direct potentiometric measurements includes an ion-selective electrode (ISE), a reference electrode, and a potential-measuring device as shown in Figure 2.2.

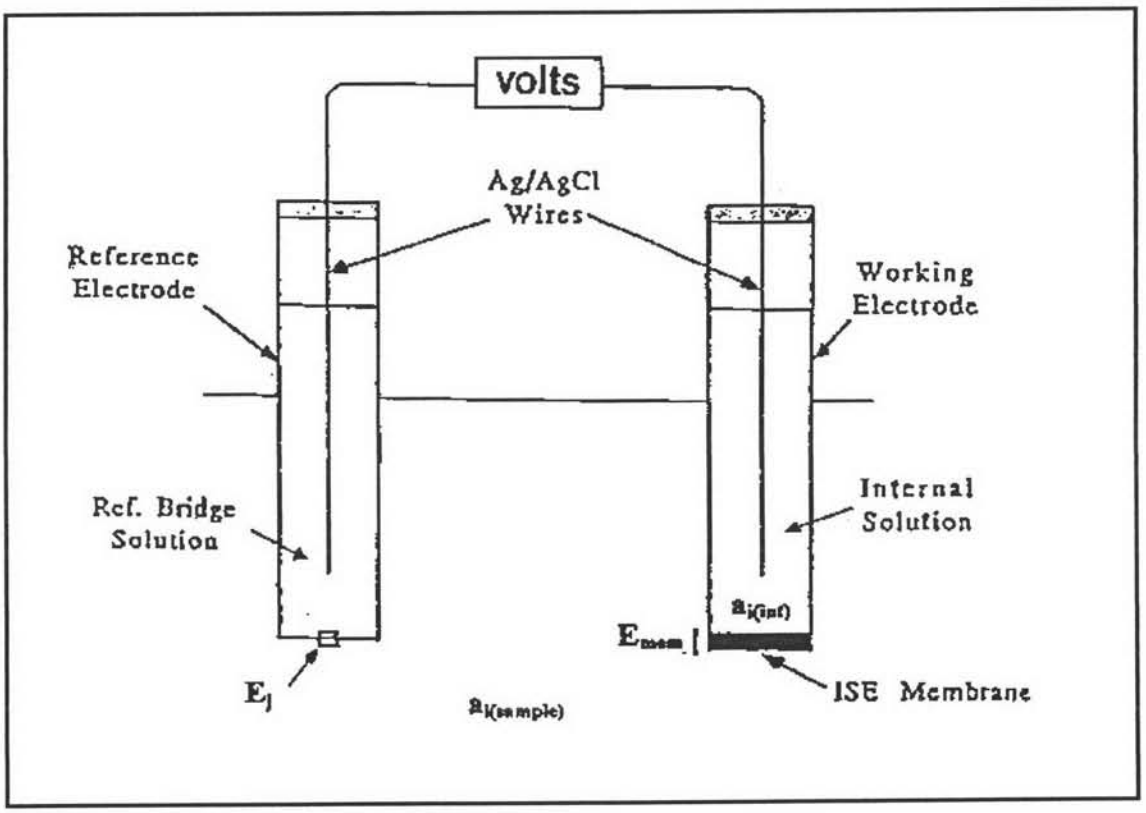


Figure 2.2 Schematic diagram of an electrochemical cell for potentiometric measurement [6].

2.1.2 Voltammetry [6, 8, 9,10]

Voltammetry comprises a group of electroanalytical methods, in which information about the analyte is derived from the measurement of current, as a function of applied potential, obtained under conditions that encourage polarization of an indicator, or working electrode. Voltammetry can be used for both qualitative and

quantitative analysis of a wide variety of molecular and ionic materials. In this method, a set of two or three electrodes are dipped into the analyte solution, and a regularly varying potential is applied to the indicator electrode relative to the reference electrode. The analyte electrochemically reacts at the indicator electrode. The reference electrode is constructed so that its potential is constant regardless of the solution into which it is dipped. Usually, a third electrode (an auxiliary or counter electrode) is placed in the solution for the purpose of carrying most of the current. The potential is controlled between the indicator electrode and the reference electrode, but the current flows between the auxiliary electrode and the indicator electrode. The signal, in the form of current as a function of potential obtained, is called voltammogram. The most common waveforms used in voltammetry are shown in Figure 2.3.

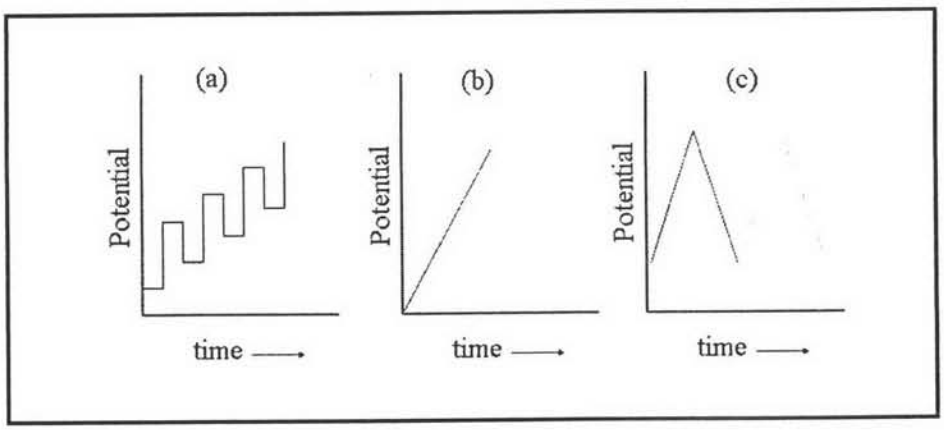


Figure 2.3 Potential-time waveforms are used in various electroanalytical techniques. Waveforms based on (a) square, (b) linear, and (c) triangular potential-time patterns are used in square wave, linear sweep, and cyclic voltammetry, respectively. [10]

2.1.2.1 Cyclic Voltammetry

Cyclic voltammetry is the most widely used technique for acquiring quantitative information about electrochemical reactions. The power of cyclic voltammetry results from its ability to rapidly provide considerable information on the thermodynamics of redox processes, the kinetics of heterogeneous electron-transfer reactions, and coupled chemical reactions or adsorption processes. Cyclic

voltammetry is often the first experiment performed in an electroanalytical study. In addition, it offers a rapid of redox potential location of the electroactive species, and convenient evaluation of the effect of media upon the redox process.

Cyclic voltammetry consists of scanning the potential of a stationary working electrode linearly using a triangular potential wavefrom (Figure 2.3c). Depending on the information sought, single or multiple cycles can be used. During the potential sweep, the potentiostat measures the current resulting from the applied potential. The resulting plot of current versus potential is termed a cyclic voltammogram. When the potential is applied, electroactive species approach the electrode surface. This movement, known as mass transport, occurs via three separate mechanisms, namely migration, convection and diffusion. Basically, if a solution is quiescent and contains supporting electrolyte, then electroactive species approach the electrode surface by diffusion mode.

Figure 2.4 illustrates the expected response of a reversible redox couple during a single potential cycle.

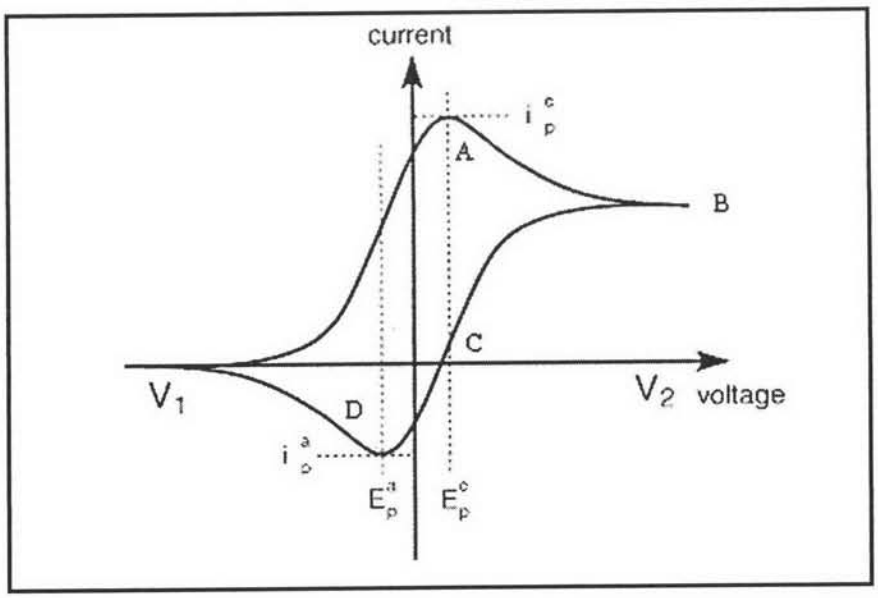


Figure 2.4 The current-potential (i-E) plot is called a cyclic voltammogram.

2.1.3 Amperometry [8, 11-12]

Amperometry is an electrochemical technique, in which a constant potential is applied at the working electrode. A simple potential-time waveform is shown in Figure 2.5. This technique is normally carried out in stirred or flowing solutions or at the working electrode. The potential of a chosen working electrode, with respect to a reference electrode is set at a fixed potential to detect the change in current response. At this potential, the electroactive species undergo an oxidation or reduction at the electrode. The amperometric current is a function of the number of molecules or ions that have been removed by the reaction at the electrode. Hence, the resultant amperometeric signal is proportional to the concentration of the analyte. In addition, the current response is directly proportional to the number of moles of analyte oxidized or reduced at the working electrode surface, as described by Faraday's law:

$$i_t = \frac{dQ}{dt} = nF\frac{dN}{dt} \qquad (2)$$

where i_t is the current generated at the electrode surface at time t, Q is the charge at the electrode surface, t is time, n is the number of electron moles transferred per mole of analyte, N is the number of moles of analyte oxidized or reduced, and F is the Faraday constant (96 485 C/mol).

Amperometry is a popular electrochemical detection mode, due to its ease of operation and minimal background current contributions. Tunable selectivity is achieved through judicious choice of detection potential, with the optimum potential being selected by constructing a hydrodynamic voltammogram.

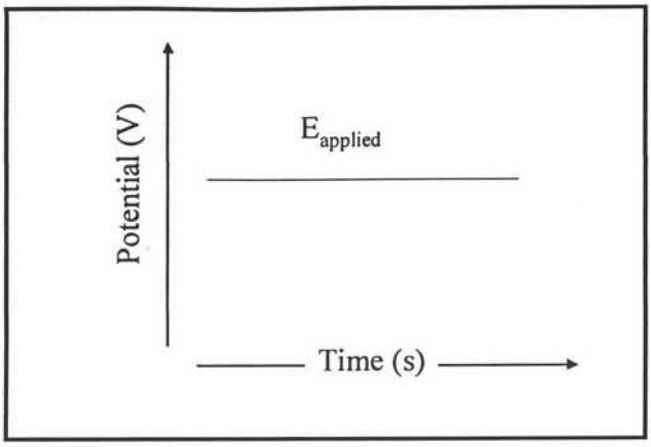


Figure 2.5 Amperometry (E-t) waveform.

2.1.4 Hydrodynamic Voltammetry [8, 13]

Hydrodynamic voltammetry is performed in several ways. Hydrodynamic voltammetry differs from normal voltammetry in terms of mass transport mode. A principal mode of mass transport in a typical voltammetry is the diffusion. Mass transport by migration is minimized by adding an inert ionic salt to the sample solution and convection is totally eliminated by keeping the solution still (quiescent). In the hydrodynamic system, the principal mode of mass transport is convection. Mass transport by migration can be eliminated in the same way as that in typical voltammetry. Mass transport by diffusion can never be totally eliminated if there are differences in concentration throughout the solution (e.g. as caused by current flow), but convection is a very effective form of mass transport when compared with either migration or diffusion. In the hydrodynamic system, convection can arise from the movement of the electrode, or stirring or flowing the solution past the electrode surface. Convection is only effective for bringing electroactive species more rapidly to the electrode surface, where the concentration gradient has already occurred. In electrochemistry, the concentration gradients always occur in the boundary layer close to the electrode surface. When convection is present in the system, current densities are 3-100 times greater than the steady state diffusion limit.

2.1.5 Working Electrode: Boron Doped Diamond Thin Film Electrode [14]

The choice of the working electrode material is an important factor in amperometric detection. This stems from the required electron transfer between the electrode and the analyte.

The conductive boron-doped diamond thin film electrode is an alternative to traditional carbon electrodes by providing superior electrochemical properties. The boron-doped diamond (BDD) thin film electrode has been realized as the outstanding electrode material for several electrochemical applications, including electrochemical waste treatment [15] and electroanalysis [16-18].

Diamond possesses several technologically important properties including extreme hardness, high electrical resistance, chemical inertness, high thermal conductivity, high electron and hole mobilities, and optical transparency [19-20]. The material offers several advantages for electronic applications under extreme environmental conditions. Diamond is one of nature's best insulators; but when doped with boron, the material possesses semimetal electronic properties, making it useful for electrochemical measurements. For example, synthetic diamond thin films, grown using hot-filament or microwave-assisted chemical vapor deposition (CVD), can be doped resulting in films with resistivities $<0.1\ \Omega$-cm, and the electrical conductivity of the film surface and bulk is influenced by the boron-doping level, grain boundaries, and impurities.

Several interesting and important electrochemical properties distinguish boron-doped diamond (BDD) thin films from conventional carbon electrodes. The films exhibit voltammetric background currents and double-layer capacitances up to an order of magnitude lower than that for glassy carbon [21]. While there is an increase in the double-layer capacitance towards more positive potentials, the capacitance versus potential profile shape and magnitude for diamond is largely independent of the electrolyte composition (0.1 M NaF, NaCl, NaBr, and NaI) and solution pH [21-22]. These two observations suggest that the specific adsorption of these electrolyte ions does not occur to any appreciable extent and that the surface is relatively void of ionizable surface carbon-oxygen functionalities. The residual background current is about an order of magnitude lower than that for freshly

polished glassy carbon of similar geometric area, leading to a low and stable background in voltammetric measurements, which result in improved signal-to-noise characteristics in electrochemical assays.

2.1.5.1 Preparation and Availability of Conductive Diamond Films by CVD Processes

There are several possible methods that have been reviewed for preparing CVD diamond films [23]. The principal methods are hot-filament CVD [24], microwave plasma CVD [25], radio-frequency plasma CVD [26] and arc-jet [27]. The hot filament and microwave methods have been used extensively to prepare boron-doped films. Boron can be introduced in any of several forms, e.g., solids that are placed in the CVD chamber, including boron metal [28], boron nitride and boron-containing ceramics [29]; gases introduced into the chamber, including diborane [30] or trimethylborane [31]; or as a compound dissolved in a spray of liquid droplets, e.g., boron oxide dissolved in a mixture of methanol and acetone entrained in the hydrogen feed gas [32]. Films can be prepared in one of several possible particle sizes, depending upon growth conditions that range in typical crystallites from microcrystalline (MCD), in the 1-10 μm range [33] to nanocrystalline (NCD), with particles in the range below 1 μm [30,34] and ultrananocrystalline (UNDC), with particles in the range down to 1 nm [35-36], respectively.

2.1.5.2 Characterization of Conductive Diamond Films

2.1.5.2.1 Microscopy

The quality of microcrystalline CVD films is conveniently assessed with SEM, to ensure that the crystallinity is good. Typically, the (111)-type grains are darker, which correlates with the previously mentioned tendency of this grain type to incorporate higher levels of boron; the (100)-type grains are lighter. This produces a characteristic pattern of light and dark areas. SEM is also a good technique with which to examine the surface after the deposition of metal particles [37-40].

2.1.5.2.2 Raman Spectroscopy

Raman is an essential characterization technique. One of the routine way of using it is used is to gauge the presence of sp^2 carbon, which shows up typically as a very broad peak centered at ca. 1600 cm^{-1}, while diamond itself has a highly characteristic peak, due to the principal phonon mode that is very close to 1332 cm^{-1} [41-43]. This peak is extremely intense for highly crystalline diamond. The presence of a high concentration of boron as a dopant leads to an increase in a broad peak at ca. 1200 cm^{-1}. This peak is thought to be due to the presence of either very small crystalline domains of diamond or disordered diamond [41], either of which could result from the perturbation of the structure by the boron dopant atoms.

2.1.5.2.3 X-ray Photoelectron Spectroscopy

The surface termination of the diamond film, whether hydrogen or oxygen, can greatly affect the electrochemical properties [44-50]. This can be established through the use of x-ray photoelectron spectroscopy (XPS). The O 1s/C 1s peak area ratio typically reaches a maximum of 0.10 to 0.15, with increased time of oxidizing treatment, for example, electrochemical oxidation [46]. This may correspond approximately to a monolayer coverage with oxygen-containing functional groups. It is possible to attain higher O/C ratios with oxygen plasma treatment, but this type of aggressive treatment may lead to surface damage, e.g. graphitization [51], which is similar to the type of damage observed even of bombardment with light ions such as D^+ and He^+ [51-52]. XPS is a useful technique for the examination of chemically modified diamond surfaces, particularly if the modifier contains characteristic elements, e.g., N, Si [51] or F [53].

2.2 Flow Injection Analysis [54]

Flow analysis is recommended as the generic name for all analytical techniques that are based on the introduction, processing and detection of liquid samples in flowing medium. The sample processing may involve sample transport,

in-flow separation, chemical reaction, heat treatment, etc., under diffusion and/or convection dispersion conditions. Therefore, the term flow analysis may be considered as an equivalent to controlled dispersion analysis. Flow analysis modes are classified according to (i) the basic character of the flow, which is either continuous or segmented, and (ii) the method of sample introduction, which can be continuous or intermittent.

The concept of flow injection analysis (FIA) is introduced by Ruzicka and Hansen in 1975. Flow-injection analysis (FIA) implies a nonsegmented FA in which the liquid analyte and/or reagent is injected into a nonsegmented flowing stream of inert or reacting carrier solution (often called carrier solution) and the analyte, reagent, or a chemical reaction product is detected downstream. Injection means forming a well-defined zone of the analyte or reagent sample within the analyzer channel, where the zone disperses in a controlled manner on its way toward and through the detection cell. For injecting a small volume of the sample as a narrow plug, mechanical injection valves (rotary valves and, in the early history of FIA, syringes) or hydrodynamic injection techniques are utilized. Both can be made as volume- or time-based injections or a combination of the two. The concentration profile of the analytes entering the detection cell (i.e. the dispersion of the detected species) depends upon the mode of the sample introduction, flow parameters, and geometry of the FIA channel situated between the sampling point and the detection site (often called the reaction or dilution section). Therefore, a symmetric or asymmetric peak-shaped transient signal (rather than a steady-state plateau) is obtained as the detection signal. The extent of sample dispersion determines the analysis frequency (or throughput), i.e. the number of analyses per time unit. The schematic diagram in Figure 2.6 groups the FIA process into three stages to help visualize how the FIA performs a method or analysis.

Such power of FIA as an analytical tool lies in its ability to combine these analytical functions in a wide variety of different ways to create a broad range of different methodologies, and perform these methodologies rapidly and automatically within a few minutes and with a low amount of sample.

The thin-layer cell design is commonly used as an amperometric detector for liquid chromatography. An electrochemically active substance passes over an electrode held at a potential sufficiently great (positive or negative) for an electron

transfer (either oxidation or reduction) to occur. An amperometric current is produced that is proportional to a concentration of analyte entering the thin-layer cell.

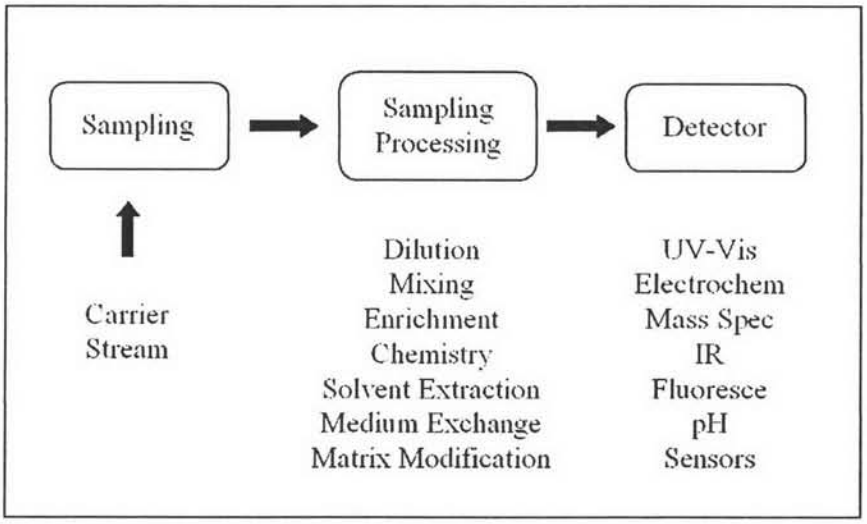


Figure 2.6 Schematic diagram of a generic description of FIA.

2.3 High Performance Liquid Chromatography [55-57]

Liquid Chromatography (LC) is an analytical technique that is used to separate a mixture in solution into individual components. The separation relies on the use of two different "phases" or "immiscible layers", one of which is held stationary while the other moves over it. Liquid chromatography is the generic name used to describe any chromatographic procedure in which the mobile phase is liquid. High performance liquid chromatography (HPLC) is the term used to describe liquid chromatography in which the liquid mobile phase is mechanically pumped through a column that contains the stationary phase. Figure 2.7 is a diagram showing the important components of a typical HPLC instrument.

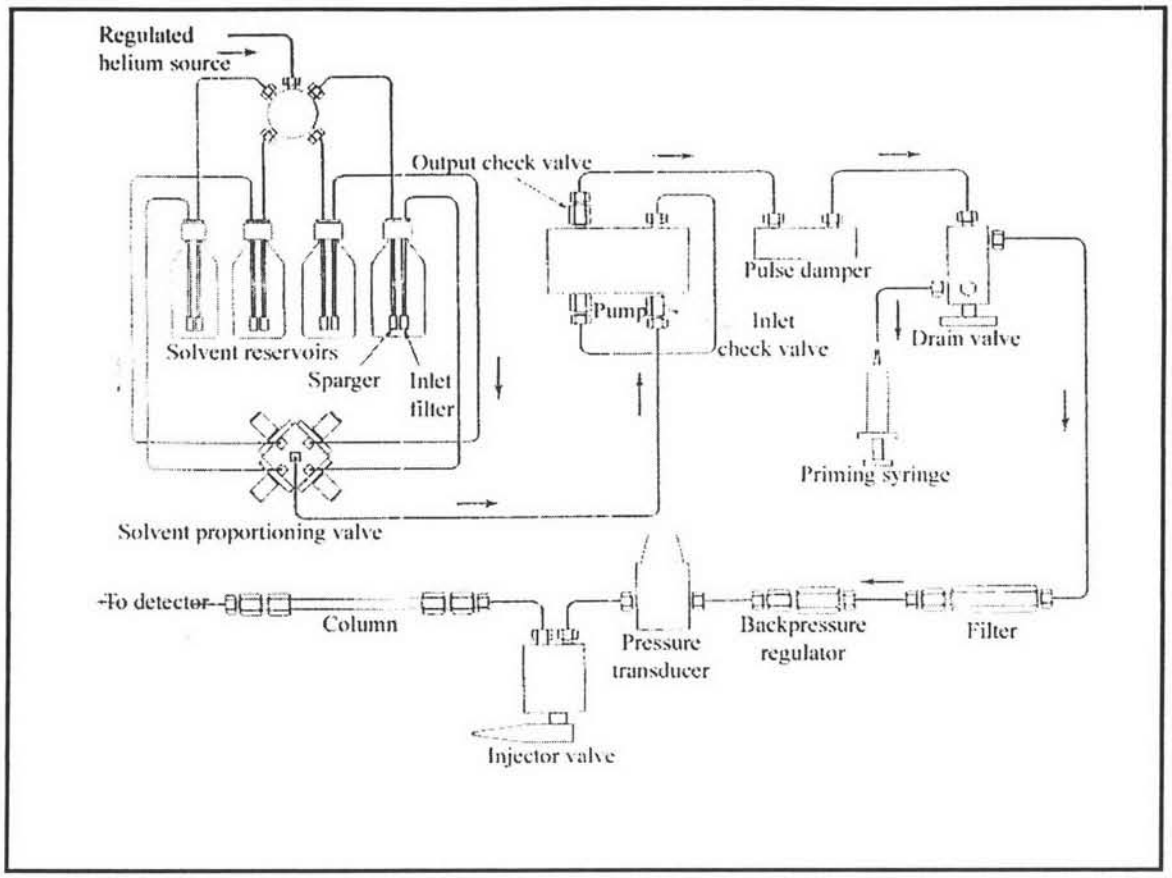


Figure 2.7 Schematic diagram of the HPLC instrument. Typically, the HPLC instrument consists of:

1) Mobile phase reservoirs and solvent treating systems
2) Pumping system
3) Sample injection system
4) Column
5) Detector.

2.3.1 Ion-Pair Chromatography

Ion-pair chromatography represents an alternative to ion-exchange chromatography. Ionic samples may be separated by reverse-phase chromatography, provided that they contain only weak acid or only weak basic compounds (in additional to neutral compounds) present in undissociated form, as determined by the chosen pH; that is known as "ion suppression". Ion-pair chromatography is an extension of this principle. An organic ionic substance is added to the mobile phase

and forms an ion pair with a sample component of opposite charge. In fact this is a salt, but its chromatographic behavior is that of a non-ionic organic substance:

$$\text{sample}^{+} + \text{counter ion}^{-} \longrightarrow [\text{sample}^{+} + \text{counter ion}^{-}]\ \text{pair}$$

$$\text{sample}^{--} + \text{counter ion}^{+} \longrightarrow [\text{sample}^{--} + \text{counter ion}^{+}]\ \text{pair}$$

Reverse-phase chromatography can be used in this instance for separation.

2.4 Microchip Capillary Electrophoresis

2.4.1 Capillary Eectrophoresis

2.4.1.1 Background Theory [58-61]

Electrophoresis is the movement of electrically charged particles or molecules in a conductive liquid medium, which is usually aqueous under the influence of an electric field. Electrophoresis can be performed using an apparatus as shown in Figure 2.8. The ends of the glass tube that is filled with an aqueous buffer, called the electrolyte or run buffer, are connected to containers filled with the same buffer. Also, in these containers contain electrodes that are connected to a power supply.

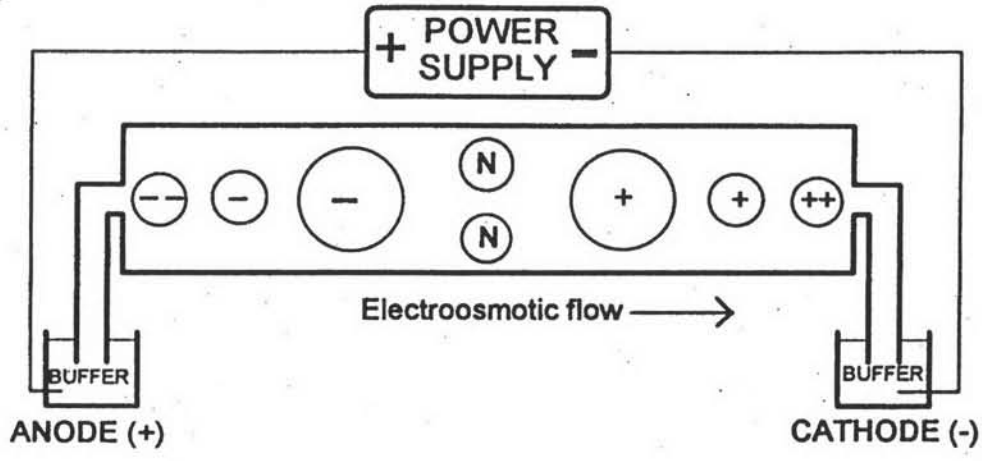


Figure 2.8 Schematic representation of electrophoresis [61].

Imagine a sample containing a mixture of neutral and electrically charged molecules, and that the electrically charged molecules, the ions, have different charges and sizes.

If the sample is placed in the anodic end of the tube, which is that with a positive charge, and an electric field is then applied across the liquid; the ions in the sample will tend to migrate through the tube at different rates and in different directions. The rates and directions of migrations depend on the sizes of the ions and the magnitudes and signs of their charges [61].

Positively charged cations will migrate towards the negatively charged electrode, the cathode, and negatively charged anions will migrate towards the positively charged electrode, the anode. The rates at which they migrate depend on their charge-to-size ratios. A smaller ion will migrate faster than a larger one of the same charge. An ion with a higher charge will migrate faster than one with a lower charge, if they are of the same size. Thus, a divalent ion migrates faster than a monovalent ion of the same size. Neutral molecules are not influenced by the electric field.

Under the influence of an electric field, the buffer and neutral molecules also move through the tube, due to electroosmosis. This electroosmotic flow of the buffer moves towards the negative electrode and carries the solutes with it. In the electroosmotic flow the anions try to migrate towards the positive electrode, usually they are "swim upstream" against a force that is too strong and most of them are carried to the negative electrode. Cations move faster than the electroosmotic flow, neutrals at the same rate, and the anions moves slower, so the order at which the molecules reach the negative electrode is cations first, neutral second, and finally, anions. The divalent cations move faster than monovalent cations of the same size, and both move faster than larger monovalent cations. The rate of anions movement towards the negative electrode is just the opposite to that of cations, with small divalent anions moving the slowest.

2.4.1.2 Electroosmotic Flow

When a buffer is placed inside the capillary, the inner surface of the capillary acquires a charge. This may be due to ionization of the capillary surface or adsorption of ions from the buffer onto the capillary. Even Teflon capillaries exhibit electroosmotic flow, which is probably due to adsorption of the electrically charged ions in the buffer onto the capillary wall. In the case of fused

silica, the surface silinol (Si-OH) groups are ionized to negatively charged silanoate ($Si-O^-$) groups at a pH of above approximately three. This ionization can be enhanced by first passing a basic solution through the capillary followed by the buffer. Often, a new capillary is conditioned by treatment with a KOH or NaOH solution. The negatively charged silanoate groups then attract positively charged cations from the buffer, which form an inner layer of cations at the capillary wall. These cations are not of sufficient density to neutralize all the negative charges, so a second, outer layer of cations forms. The inner layer is tightly held by the $Si-O^-$ groups and is referred to as the fixed layer. The outer layer of cations is not tightly held because it is further away from the silanoate groups, and it is referred to as the mobile layer. These two layers make up the diffuse double layer of cations (figure 2.9). When an electric field is applied, the mobile, outer layer of cations is pulled towards the negatively charged cathode. Since, these cations are solvated, they drag the bulk buffer solution with them, thus causing electroosmotic flow.

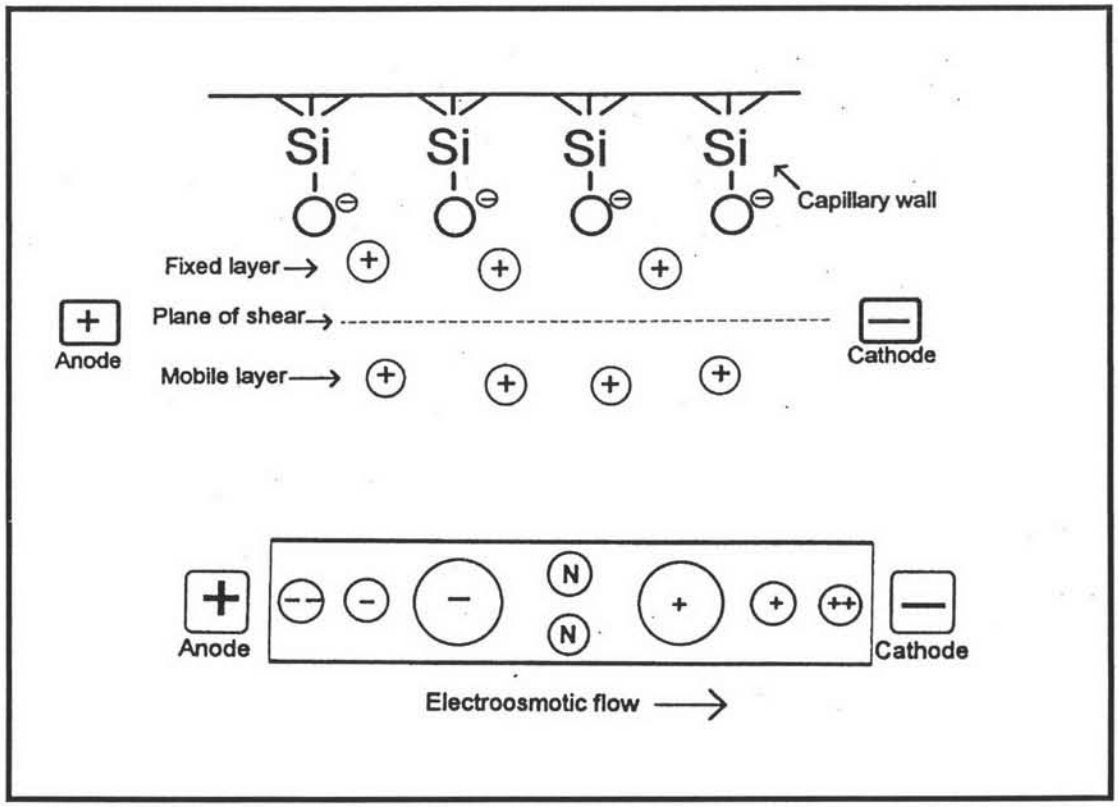


Figure 2.9 Representation of osmotic flow and migration of neutral, cationic, and anionic solutes in a capillary, due to electroosmotic flow and electrophoretic mobility [61].

2.4.1.3 Injection Schemes

In HPLC and GC, the sample is usually injected directly into a flowing liquid or gas stream through the use of loop-valve injectors or syringes. In capillary electrophoresis, the sample is ordinarily introduced into the capillary, while there is no flow of buffer through the capillary. Samples are loaded into the sample vials. The inlet of the capillary is placed into the sample container; the sample is introduced into the capillary; the capillary inlet is placed into the source vial, which contains the buffer; an electric field is applied; and the electrophoretic separation proceeds. In capillary electrophoresis, there are two major injection schemes commonly used, the hydrodynamic (hydrostatic) injection and the electrokinetic (electromigration) injection.

2.4.1.3.1 Hydrodynamic Injection

Hydrodynamic injection can be done either by pressure or gravity. Pressure injections can be made by placing the inlet of the capillary into a sample vial and applying pressure to the vial or vacuum (gravity injection) and the destination vial. To minimize any siphoning of sample or buffer during the injection, the liquid levels in the sample and destination vials should be kept even and the injection time should be as short as possible. Since the injection volume is dependant on the applied pressure, a constant pressure source is required for reproducible results.

Hydrodynamic injections give reproducible injection volumes as long as the variables are kept constant. Since the volumes injected are dependent on the viscosity of the sample solution, any changes in sample temperature will also affect reproducibility.

2.4.1.3.2 Electrokinetic Injection

Electrokinetic injection is made by placing the capillary and the anode into the source vial and applying a voltage for a given period of time. After the sample is introduced, the anode and capillary are placed back into the source

vial, an electric field is applied, and electrophoresis proceeds. Solutes are carried into the capillary by electroosmotic flow and electrophoretic mobility.

Neutral solutes are pulled into the capillary by the electroosmotic flow only. Ionic solutes are injected as a result of electroosmotic flow and their electrophoretic mobilities. If the capillary has been treated to eliminate electroosmotic flow and the voltage is such that the cathode is at the detector end of the capillary, only cations are injected because the anions are reversed, and when osmotic flow is reversed, only anions are injected. In either case, neutral solutes are not injected. The electrophoretic sample loading, in which a treated capillary is used produces no electroosmotic flow, which results in ions being injected due to just their electrophoretic mobilities.

2.4.1.4 Separation Modes

The modes of CE are classified by the different separation mechanisms allowing CE techniques to be used for a wide variety of substances. These modes include;

Capillary zone electrophoresis (CZE)

Micellar electrokinetic chromatography (MEKC)

Capillary electrochromatography (CEC)

Capillary gel electrophoresis (CGE)

Capillary isoelectric focusing (CIEF)

Capillary isotachophoresis (CITP).

The first two techniques, CZE and MEKC, used in this research were briefly discussed. Figure 2.10 shows the separation mechanism in CZE and MEKC.

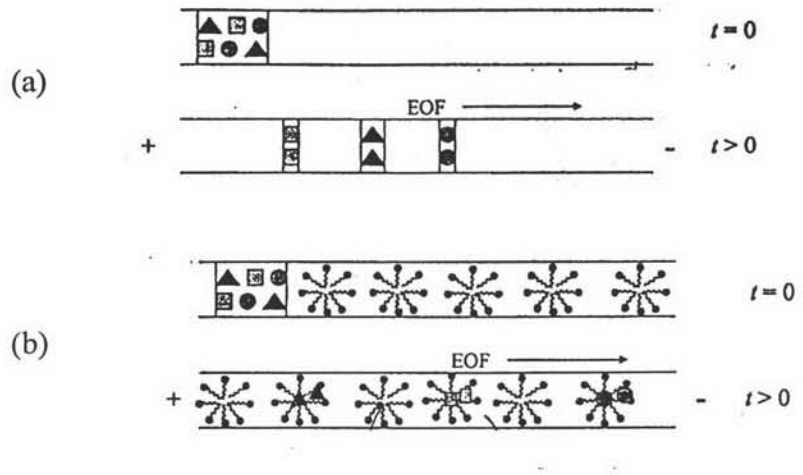


Figure 2.10 Separation mechanisms in (a) CZE and (b) MEKC.

2.4.1.4.1 Capillary Zone Electrophoresis (CZE)

CZE is a widely used mode of electrophoresis because it is applicable to separations of cations and anions, but not neutrals, in the same run, and it is relatively simple. In CZE, the capillary is filled with a buffer of constant composition, and the source and destination vials are filled with the same buffer. The order of elution in this method is cations, neutrals and anions. Neutral compounds are not separated in CZE, whereas ions are separated on the basis of their charge-to-size ratios. Electrophoretic mobility is dependant to same extent on the shape of the ions. The method can be used to separate almost any ionized compounds that are soluble in the buffer.

2.4.1.4.2 Micellar Electrokinetic Chromatography (MEKC)

MEKC combines the separation mechanism of chromatography with the electrophoretic and electroosmotic movement of solutes and solutions. By using MEKC even extremely hydrophobic or electrically neutral compounds can be separated.

In MEKC, a buffer solution that contains micelles is used as the run buffer. This mode of capillary electrophoresis is based on the partitioning of solutes between micelles and the run buffer. Micelles are generally spherical in shape and form so that hydrophobic groups are on the outside of the micelle, towards the aqueous buffer. The hydrophobic carbon molecules are in the center of the micelle, away from the aqueous buffer. The solutes are separated based on their interaction with the hydrophobic groups on the surface of the micelle.

Electrokinetic chromatography can also be done using substances other than micelles. For example, cyclodextrins (CD's) can be used, solutes distribute themselves, and cyclodextrins the in run buffer and then separated due to differences in their tendency to form inclusion complexes. Solutes that spend a lot of time included in the complex elute later than solutes that spend more time in the buffer. While this approach is similar to MEKC, it is not the same because the CD's do not form micelles.

2.4.2 Introduction to Micro Total Analysis Systems (μTAS) [62-63]

A miniaturised analytical system, which is also called a lab-on-a-chip or micro Total Analysis System (μTAS) includes at least the separation and detection of components present in, typically liquid, samples in a chip-based device. Additional sample pre-treatment, filtering, or derivatisation steps can also be implemented. The typical dimensions of the structures used for the various steps are in the range of a few micrometers to several millimetres in length or width, and between 100 nm and 100 μm in depth or height. It has been considered that the μTAS is becoming one of the most powerful tools in analytical chemistry.

2.4.3 Personal Laboratories for the 21st Century [64]

Micro miniaturization of analytical procedures is having a significant impact on all aspects of diagnostic testing as we move into the 21st century. It enables highly complex clinical testing to be miniaturized, and hence permits future testing to move from the central laboratory into non-laboratory settings. These new personal laboratories will enable relatively unskilled operators to perform highly complex

clinical tests, at one time only available from large specialized central laboratories. Many factors will determine to what extent of this type of the testing is implemented, including prevailing regulations that govern laboratory testing, cost-benefit considerations, and the interest by the members of the general public in performing self-testing.

There is already a diverse range of micro analytical devices, e.g. microchips, gene chips, and bio-electronic chips. These silicon, silicon-glass, quartz, or plastic devices are fabricated using techniques borrowed mainly from the microelectronics industry. The main advantages of the new devices are integration of multiple steps in complex analytical procedures (particularly sample preparation); diversity of application; sub-μL consumption of reagents and sample; and due to their small size and light weight, portability. The latter feature makes possible devices that would serve as personal laboratories. Based on the current state of the art, the user would still have to collect a sample (e.g., blood, urine, saliva), but once introduced into the personal laboratory, all subsequent analytical steps would be performed automatically and a result displayed and stored in memory. A two way wireless communication feature would allow the results to be communicated to a physician for comment or interpretation. A further degree of simplification can be envisaged, with the development and miniaturization of non-invasive testing, but this type of technology is still at a very early stage of development.

2.4.4 Capillary Electrophoresis on Microchip [65]

In recent years, capillary electrophoretic methods have enjoyed gaining popularity primarily due to the observed high separation efficiencies, peak resolution, and wide dynamic ranges of molecular weights that may be analyzed [66-67]. Furthermore, the simple open-tubular capillary design has lent itself to a variety of automation, injection and detection strategies developed previously for more conventional analytical technologies. As a result, interest in capillary electrophoresis (technique and instrumentation) has significantly increased in the field of analytical chemistry during the last two decades [68-70] and several books have been written [61, 71-73].

Capillary electrophoresis on microchip is an emerging new technology that promises to lead the next revolution in chemical analysis. It has the potential to simultaneously analyze hundreds of samples in a matter of minutes or less. The rapid analysis combined with massively parallel arrays should yield high throughput. Microchips typically consume only picoliters of samples. These samples may be prepared potentially on-board for a complete integration of the sample preparation and analysis. These features make microchips an attractive technology for the next generation of capillary electrophoresis instrumentation.

The first instrument on a microchip was an integrated gas chromatograph [74]. While this device never succeeded commercially, it initiated the application of micromachining technology to build chemical analysis devices. By the early 1990's, chemical analysis on microchip has been demonstrated for many capillary electrophoresis applications [75-78]. Miniaturization of liquid handling components such as pumps or valves is technically problematic in those dimensions [79]. However, in microfluidic capillary electrophoresis devices, the generation of EOF on the charged substrate is beneficial and may be used for liquid handling control [80]. This method of flow control provides many advantages for microfabricated chemical analysis platforms, due to the ease of automation, great reproducibility, and short analysis times associated with its use. The characteristics of electrokinetically controlled fluid flow in microchannels have been studied in a systematic way [81-83]. If external potentials are applied to a system of several interconnected channels, the respective field strength in each channel will be determined by Kirchoff's law in an analogy to an electrical network of resisters [84]. Ideally, electrokientically driven mass transport in each of the channels would take place according to the magnitude and direction of these fields. This allows convenient manipulation of complex fluid operations in the nanoliters to picoliter range without the need of any active control elements, such as external pumps or valves. This is of particular relevance to the demanding limitations with respect to void volumes in the system.

Apart from simple electrophoretic separations, an increasing number of μ-TAS applications have been shown in the last few years, especially in the field of bioanalysis. Full DNA analyzers have been implemented in a single device, with a polymerized chain reaction (PCR) chamber, followed by an electrophoretic separation

[85]. Continuous flow PCR has also been shown, where the analyte solution is driven through a capillary with crossing zones of different temperatures. Other genetic analyses have also been demonstrated comprising high-speed DNA sequencing [86], high density parallel separation [87] or single DNA molecule detection [88]. Another application of μTAS has been shown in electrochromatography. Open-channel electrochromatography in combination with solvent programming has been demonstrated using a microchip device [89]. Other researches have successfully used μ-TAS to conduct immunoassays involving competitive markers, noting several advantages over more traditional formats including (a) high efficiency separations between competitive markers and antibody-marker complexes, (b) excellent mass detection limits (0.3-0.4 amol injected) at high speed, and (c) good potential for automation. This has first been demonstrated in a micromachined capillary electrophoresis device by Koutny *et al.* [90]. Cortisol was determined in serum subsequently using a competitive immunoassay. Separation of immunoassay components from antibody-analyte complexes is accomplished in under 30 seconds with a resolution of 400 theoretical plates per second. Harrison and co-workers have also shown a fully automated immunoassay device where electroosmotic pumping was used firstly to control first the mixing of a 50-fold diluted serum sample with labeled theophylline tracer in a 1:1 ratio, followed by 1:1 mixing and reaction with an anti-theophylline antibody [91]. A microfluidic system was fabricated on a glass chip to study mobilization of biological cells on-chip. Electroosmotic and/or electrophoretic pumping were used to drive the cell transport within a network of capillary channels [92]. An automated enzyme assay was performed within a microfabricated channel network. Precise concentrations of substrate, enzyme and inhibitor were mixed in nanoliter volumes using electrokinetic flow [93]. Finally, the new insight into the use of a microfabricated system has been combining the advantage of parallel reactions and liquid handling in extremely small volumes, with an electrospray or nanospray interface for mass spectrometry analysis [94-95]. This last application opens the way to efficient use of the microchip format not only for genetic analysis, where it is already recognized but also in protein sequencing [96-97].

Electrically driven separations such as capillary electrophoresis [80, 83-84], synchronized cyclic electrophoresis [98], free-flow electrophoresis [99], open-channel electrochromatography [100], micellar electrokinetic capillary

chromatography [101], capillary gel electrophoresis [86] and a two-dimensional obstacle course for electrophoretic sizing of DNA fragments have been demonstrated. Structures that perform chemical reactions include arrays for solid-phase chemistry reaction, wells for polymerase chain reactions, channels with immobilized enzymes for flow injection analysis [102], and stacked modules for flow injection analysis [103] The first monolithic devices that integrated reactions with analysis are pre-column [104] and post-column [105] derivatization with capillary electrophoresis and DNA restriction digestions with fragment sizing [106], and a hybridized device for PCR amplification and capillary electrophoresis analysis was reported [107]. Recently, a high speed free flow electrophoresis on microchip platform has been demonstrated [108]. Many of these devices have demonstrated advantages analogous to those of microelectronics including compact geometries and high-speed analysis, while having a simple and reliable operation.

2.5 Electrochemical Detection under Flow-Through Conditions [6]

Electrochemical detection is usually performed by controlling the potential of the working electrode at a fixed value and monitoring the current as a function of time. The current response thus generated reflects the concentration profiles of these compounds as they pass through the detectors. Detection for liquid chromatography or flow injection analysis or microchip capillary electrophoresis results in sharp current peaks. Accordingly, the magnitude of the peak current serves as a measure of the concentration. A typical response peak recorded during an automated flow injection operation is displayed in Figure 2.11. The current peaks are superimposed on a constant background current (caused by the redox reactions of the mobile phase or carrier solution).

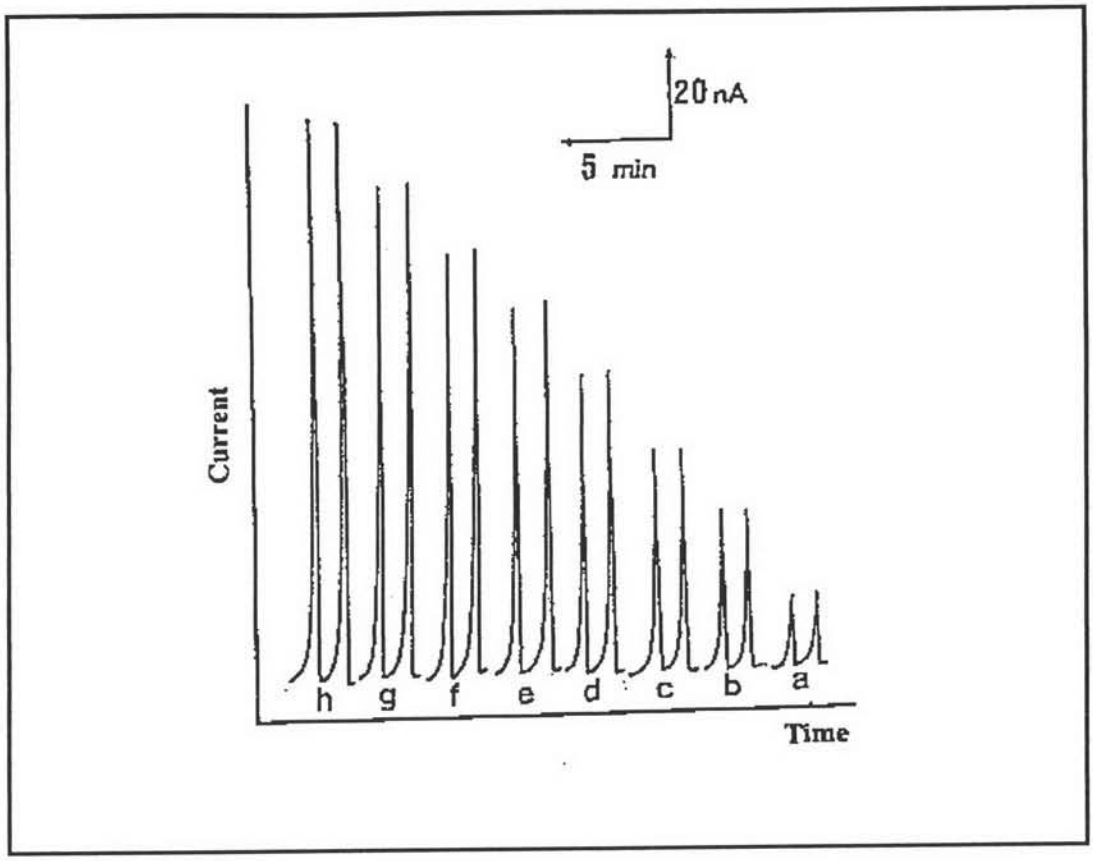


Figure 2.11 Typical amperometric readout during automated flow injection assays of ethanol at an enzyme carbon-paste electrode. Peak a through h: 2×10^{-5} M to 1.6×10^{-4} M ethanol [6].

2.6 Thiol-Containing Drugs

Thiocompounds play an important role in the biological and pharmacological process, and especially thiol-containing drugs hold a particular fascination. They form an important class of compounds with an extensive and interesting chemistry [109-110]. Thiol-containing drugs are well known to possess a multitude of roles within the clinical applications. In addition to the helpful effects of these medicines, however, they may have side effects that can be very serious. If any of the side effects mentioned later occur, the patient should be checked by a doctor immediately. Therefore, these medicines are available only with a doctor's prescription, in the dosage form described later. Because of their side effects, quality control of thiol-

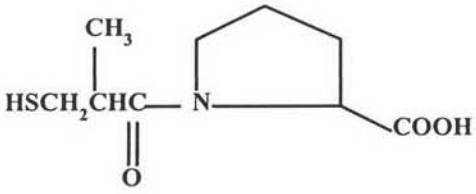
containing drugs is required to prevent unwanted effects from using these medicines. Moreover, in pharmacokinetic study, amount of drugs in biological fluids can provide information to biomedical scientist with a versatile diagnostic handle through which to form treatment. Successful exploitation of such an approach, however, is dependent on the availability of analytical techniques that can provide fast quantitative measurements of the various therapeutic concentrations.

From the literature survey, several methods have been used to determine thiol-containing drugs. Earlier, spectrophotometric methods listed nearly 200 "specific" reagents and reactions for the thiol group. Many assays depend on the ability of the –SH group to form colored complexes with heavy metals. Therefore, many thiols spectrophotometric methods are available, e.g. cysteine gives a specific reaction with ninhydrin in concentrated acid and glutathione reacts with *o*-phthaladehyde giving a fluorescent product [111-112].

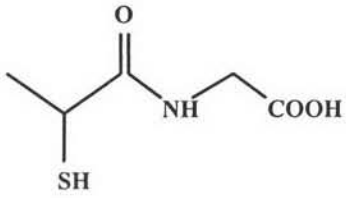
The occurrence of many –SH-containing compounds in physiological fluids prevents many of the simple methods mentioned above from being applied for analysis of individual thiols. This is particularly true in the quantitation of thiol-containing drugs at therapeutic levels, which are substantially lower than endogenous thiol concentrations. Generally, thiocompound analyzers were less successful in the separation of thiols, since the elevated temperatures at which the analytical columns were maintained led to rapid oxidation. There was one early but important attempt to circumvent this problem. In 1960, Stein and Moore, the inventors of the amino acid analyzer, recognized that oxidation could be prevented by prior derivatization of the –SH group. Moreover, the need for high sensitivity analysis led to the development of procedures for the production of volatile derivatives suitable for separation by gas liquid chromatography (GLC). Various GLC derivatives have been employed for determination of the mucolytic agents based on cysteine [113], or after derivatization with fluorophores and chemiluminescence [114]. The flame photometric detector in sulphur mode has been employed [115]. As well as the problems associated with sample collection and stability, the quantitation of free thiols presents two important technical difficulties. Chromatography is poor because of the reactivity of the thiol group, and detection is difficult in the absence of strong chromophores or fluorophore.

For the electrochemical method, in theory, direct oxidation of the thiols at bare electrodes is the simplest approach to electrochemical measurement. However, large

over potential is usually required before sufficient sensitivity can be attained. Moreover, in complex media, selectivity can often suffer as a consequence. Nevertheless, numerous substrates have been investigated for the following electrodes: mercury [116], carbon [117], modified platinum [118], gold [119], and silver [120]. Some examples of certain analytes, captopril and tiopronin, are shown in Figure 2.12



Captopril



Tiopronin

Figure 2.12 Thiol-containing drugs structure.

2.6.1 Tiopronin

Tiopronin [*N*-(2-mercaptopropionyl)-glycine] is a drug with a free thiol group that is used in clinical applications. It is effective in the treatment of cystinuria, rheumatoid arthritis as well as hepatic disorders, and as an antidote to heavy metal poisoning [117, 121-122]. Along with its needed effects, it may cause some unwanted ones such as muscle pain, yellow skin or eyes, sore throat and fever, change in taste or smell, etc. Moreover, this drug produces a dose-related nephritic syndrome. Tiopronin is available only with a doctor's prescription [123-125]. Therefore, various analytical methods were developed for the quantitation of tiopronin. In 1993, K. Matsuura and H. Takashina [126] reported a gas chromatographic- mass spectrometry

method for determination of tiopronin, which had a thiol group, in human blood. To prevent the oxidative degradation of tiopronin in the blood, its thiol group was protected by treatment with isobutyl acrylate. The derivative of tiopronin and an internal standard were analyzed. The method was satisfactorily precise, and the calibration curve was linear in a concentration range of 1-1000 ng/mL. The limit of determination of tiopronin in blood was estimated at 1 ng/mL.

In 1996, T. P. Ruiz et al. [121] described the method for determination of tiopronin in pharmaceutical preparations. The procedure was based on the oxidation of this drug by thallium (III). In hydrochloric medium the fluorescence of thallium (I) that forms in the oxidation of tiopronin was monitored. The results showed linearity from 0.8 to 20 μM and the detection limit was 0.098 μM.

Next, in 1998, a research group also developed a sensitive method for determination of tiopronin using FI-chemiluminescent [122]. The method was based on the chemiluminescent reaction of tiopronin with cerium (IV) in sulfuric medium using rhodamine 6G and quinine as fluorophor. The method was applied to the determination of tiopronin in pharmaceutical preparations. The proposed method provided a wider range of linearity (0.1-70 μM) and a better detection limit (0.036μM).

In 1996, G. Favaro et al. [127] reported a method for determination of tiopronin using liquid chromatography with electrochemical detection. This detection was performed with conductive carbon cement matrix, chemically modified with cobalt phthalocyanin. This method provided a wide linear range from 5 to 20 μM and the detection limit was 0.710 μM.

In 1997, Y. Zhao et al. [128] published a work based on chemiluminescent HPLC coupled to flow injection for quantitative analysis of tiopronin. The detection limit was 0.80 μM and linearity was achieved from 1 to 100 μM.

Recently, a sensitive and selective LC-MS or LC-MS-MS method for determination of tiopronin and its metabolites in blood was developed. One of these methods was reported in 2000 by K. Matsuura et al. [129]. A linearity of calibration curve was observed over a range of 0.5-1000 ng/mL and the limit of quantitation estimated for tiopronin was 0.5 ng/mL.

2.6.2 Captopril

Captopril belongs to the group of anti-hypertensive drugs that affect the renin-angiotensin system, commonly referred to as angiotensin converting enzyme (ACE) inhibitors. The chemical name of captopril is (*S*)-1-(3-Mercapto-2-methyl-L-oxo-propyl)-L-proline, and it is widely used for the treatment of arterial hypertension. Recent studies suggest that it may also act as a scavenger of free radicals because of its thiol group [130-132]. Several methods have been proposed for the determination of captopril and in 1999, J. Ouyang et al. [133] developed a narrow-bore liquid chromatography coupled to chemiluminescent for detection of captopril in tablets. Chemiluminescence detection was carried out based on the reaction with Ce (IV) in sulfuric medium, sensitized by rhodamine 6G. The response was linearly related to concentration in the range of 8-300 μmol/l. The detection limit was 67 pmol per injection.

S. Hillaert et al. [134] proposed a capillary electrophoresis for determination of captopril and its degradation products in pharmaceutical formulations. This method was used to evaluate the compound's stability and could also be applied to the control of the raw materials and quantification. The UV absorbance at 214 nm was employed for detection. The response was found to be linear in a concentration range of 80-400 μg/mL.

In 2000, M. A. El Reis et al. [135] developed an indirect method for determination of captopril by AAS. The procedure was based on the complexation of captopril with excess of Pd (II). The unreacted Pd (II) was measured by AAS. The absorbance increased linearity with an increasing concentration from 1-40 μg/mL.

Some research groups developed sequential injection analysis methods that employed electrochemical detection. These methods involved the potentiometric titration using a crystalline membrane electrode [136] or they concerned the amperometric biosensor using a graphite paste electrode [137]. Both methods gave linear calibration: 2.5×10^{-4}-1.0×10^{-3} M (for potentiometric titration) and 1×10^{-6}-1.0×10^{-3} M (for amperometric biosensor).

In 2001, T. Mirza and H. S. I. Tan [132] reported a method for determination of captopril in tablets by anion-exchange HPLC. The photometric detection in indirect mode was employed to detect the mobile phase masker. The

detection limit was a 0.7 ng injected. The results showed that a linear response was obtained over the concentration range of 5-50 μg of injected captopril.

As reported previously, the detection of these thiol-containing drugs by spectrophotometric detection is not effective because this thiol group do not preferentially adsorb light [138] leading to lack of sensitivity. Derivatizations of thiol-containing compounds are often required to overcome this problem. Therefore, these techniques required expensive apparatus and reagents as well as skilled operators and they are also time-consuming. Electrochemical was taken to be a candidate for a novel analysis system for the determination of thiol-containing drugs because they are cheap, simple, fast and sensitive method. As also mentioned, the electrochemical detection of thiol-containing drugs has been reported, using glassy carbon, carbon, graphite, platinum, gold, and silver electrode. Nevertheless, poor voltammetric responses are common among these electrodes and a comprehensive review on the electrochemical response to cysteine and cystine has been previously conducted [139]. Improvements in response have been sought and found with bismuth-doped lead dioxide [140] and conducting salts [141] providing enhanced responses to cysteine and glutathione respectively. Mercury has historically been among the more successful substrates for the study of thiols from both fundamental and analytical perspective [142-143]. However, the method based on mercury has a limitation due to its toxicity and rapid deterioration of the electrode response. In order to improve on these weak points, many researches have tried to develop the new electrode material. Boron-doped diamond (BDD) electrodes have been studied intensely over recent years owing to their attractive properties [21]. These properties include a wide potential window; low and stable background currents, negligible adsorption of organic compounds, and low sensitivity to oxygen. Diamond electrodes have thus proved extremely useful for a wide range of electroanalytical applications, including glucose-based biosensors [144], electrical detection of DNA [145], stripping analysis of heavy metals [146], voltammetric measurements of organic acids [147], NADH [148], or polyamine [149], or amperometric detection of aminonaphthalenes and aminobiphenyls [150], or amperomertic detection of chlorophenol in HPLC system [151].

2.7 Homocysteine

Homocysteine is an important sulfhydryl thiol (RSH) found in the human body. The accumulating data from epidemiological studies have suggested that individuals with elevated blood levels of homocysteine have increases in risks of cardiovascular disease and that even moderately elevated homocysteine levels are associated with heart attacks, strokes, problems in pregnancy, and weakened cognition in middle and old age. In biological environments, homocysteine is formed when methionine is converted to cysteine. Once formed, it may either be irreversibly metabolized by the transsulfuration pathway to cysteine or remethylated to methionine. Deficiencies of the enzymes involved in these pathways and/or cofactors necessary for the metabolism of homocysteine can result in aberrant intracellular processing of homocysteine, leading to hyperhomocysteinemia [152-153]. In humans, the plasma homocysteine level is regulated and the normal basal concentration ranks from 5 to 15 μM. For upper levels, hyperhomocysteinemia is described; an increment in fasting plasma homocysteine of 1 μM has been associated with an increase in risk of coronary heart disease of 10–20% [154]. Therefore, the development of a simple assay for reliable and durable for the determination of homocysteine is of great physiological and pathological importance.

Several comprehensive reviews have been put considerable efforts on the determination of homocysteine. As demonstrated, the detection of homocysteine by spectrophotometric means is not effective, because it lacks a strong chromophore [155]. Derivatization is one option to circumvent this problem, but these results in increased cost and complication [156]. Electrochemical methods are more attractive, because they are inexpensive, highly sensitive and have long-term reliability and reproducibility. White *et al.* have summarized various electrochemical techniques for the detection of thiols and have compared the merits and demerits of different methods [110]. Electrochemical methodologies utilized for the determination of homocysteine are usually divided into two categories: either by a "direct" means or detection after a separating technique has been applied to the sample. The "direct" determination of the species usually requires a mediator or a derivatisation agent to produce a selective voltammetric signal, whereas the post-separation detection processes can be carried out by direct oxidation of the analyte at the electro surface.

The electrochemical detection of homocysteine has been reported, using carbon, platinum, mercury and gold as working electrodes [157-158]. However, the severe detection conditions, *i.e.*, highly positive potential, can damage the electrode and cause fluctuating background currents [155]. BDD electrode can be used to eliminate this problem because of the very high resistance to surface oxidation after it has become oxygen-terminated [159]. Even though the BDD surface is oxygen-terminated, it still exhibits little tendency to adsorb polar molecules, as reported in this paper. Recently, we found that the BDD electrode can provide a well-defined cyclic voltammetric peak for the oxidation of homocysteine and cysteine oxidation in alkaline solution [138,160-162]. These preliminary results have also shown that BDD has better sensitivity than GC. In the present work, we have further investigated the selectivity, sensitivity and detection limits using an HPLC system with an amperometric detector. From the literature, the separation of thiol and disulfide compounds typically utilizes reverse-phase HPLC in acidic media, for example, acidic phosphate solution. Therefore, the electrooxidation of homocysteine in such a solution was investigated. In addition, the HPLC separation of homocysteine from other aminothiols and disulfide amino acids with amperometric detection with BDD electrodes was also examined.