CHAPTER II

THEORY AND LITERATURE SURVEY

2.1 Boron doped diamond thin film electrode

Diamond exhibits several important properties such as high thermal conductivity, extreme hardness, chemical inertness and corrosion resistance. Each carbon atom of diamond is bound into tetrahedral, using sp3-hybrid orbitals. Its microstructure atoms arrange themselves in stacked six-member rings. This structure is different form that of other carbon-based materials (e.g. carbon fibers, glassy carbon and graphite) which have structures consisting of layers of condensed, six member rings with sp^2-hybridized carbon atoms trigonally bound to one another [3].

Traditional carbon electrodes, such as glassy carbon, carbon fiber, carbon cloth, carbon nanotubes, various forms of disordered carbon and graphite are important in electrochemistry because of its low cost, simple preparation methods, possibility of achieving large surface area, and a relatively wide potential window of water stability. These electrodes have been used in various applications, ranging from Li-ion batteries and double layer capacitors to electrochemical sensors. Carbon also plays an important role in fuel cells as a substrate for dispersal of a small amount of precious metal catalyst over a large area. Despite their advantages, traditional carbon electrodes still suffer a number of drawbacks. For example, electrode fouling limits their long term stability and leads to frequent polishing or disposal of the electrode after a few uses. The limit potential window for water electrolysis prevents the detection of compounds that oxidize at relative high anodic potentials. Electrode exhibiting better stability and wider potential window are desired for such applications.

Recently, chemical vapor deposition (CVD) techniques (Figure 2.1) have afforded the possibility to produce synthetic diamond thin films. Generally, CVD techniques can be classified into three groups: plasma-assisted CVD, hot filament-assisted CVD, and combustion flames, as well as combination of these. The plasma-

plasmas used in this technique are (a) the DC plasma, (b) the RF plasma, (c) the microwave plasma, (d) the electron cyclotron resonance microwave plasma, and (e) the high-pressure plasma. The role of the plasma is to generate atomic hydrogen and to produce the appropriate carbon precursors for the growth of diamond. Hydrogen incorporation occurs during the film preparation since the presence of atomic hydrogen in the plasma is necessary to promote diamond formation. It was used to grow on a variety of metal and nonmetal substrates e.g. c-BN, Ni, Cu, Si, Ta, Mo, W and glassy carbon. Diamond is one of the nature's best insulating materials. In order to obtain conductive diamond films for electrochemical studies, the material must be doped. Boron is most commonly used as the dopant, the resulting films are either p-type semiconductors or have semimetal electronic properties, depending on the doping level.

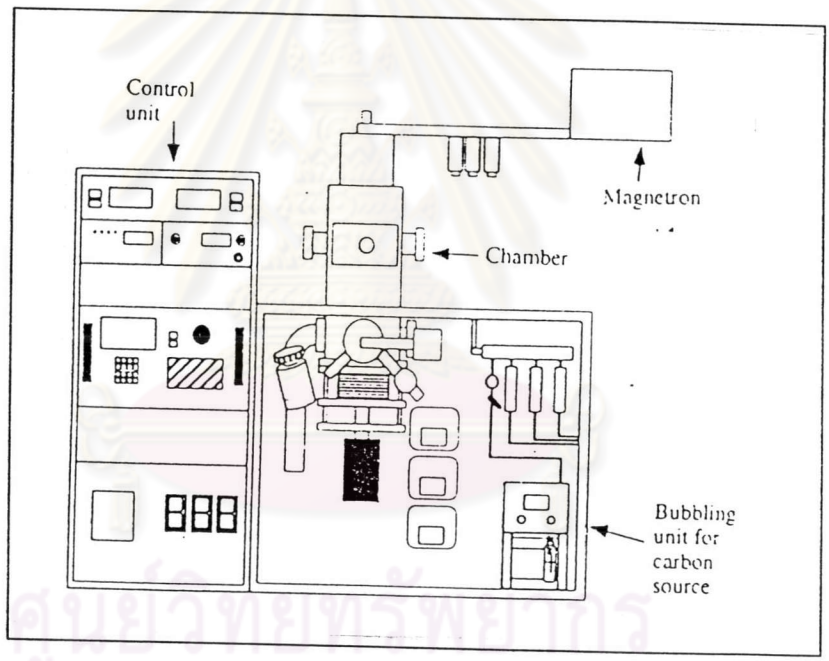


Figure 2.1 Diagram of the microwave plasma-assisted CVD diamond thin film reactor [24]

It has been observed that boron doped diamond thin film posses several unique electrochemical properties. First, diamond electrodes exhibit a lower and more stable background current in both voltammetric and amperometric detection compared to glassy carbon electrodes (Figure2.2). Enhancement of S/B ratios [40] and long term

response stability of several aqueous-based redox analytes were also obtained. Second, diamond provides a wide working potential window in aqueous media (2.5-3 V) due to its large overpotentials for oxygen and particularly, hydrogen evolutions. This property may allow the detection of redox analytes with more positive and negative standard reduction potentials. Third, the diamond surface is resistant to severe morphological damage and corrosion during anodic fluoride, acidic chloride and alkaline media [2, 41]. Fourth, diamond exhibits a quasi-reversible transfer kinetic for redox analytes such as $Fe(CN)_6^{3-}$/ $Fe(CN)_6^{4-}$, $Ru(NH_3)_6^{2+}$/ $Ru(NH_3)_6^{3+}$ and $IrCl_6^{2-}$/ $IrCl_6^{3-}$. Moreover, it was found that slightly polar molecules can be adsorbed on the diamond surface. Swain and co-workers confirmed this property [4, 42].

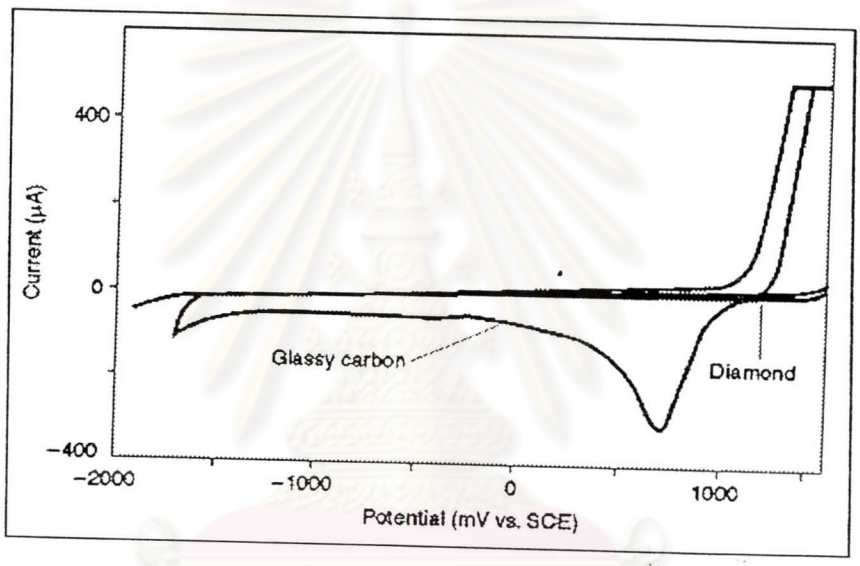


Figure 2.2 Cyclic voltammetric i-E curve for glassy carbon and boron-doped diamond thin film electrodes in 0.1 M KCl. [40]

2.1.1 Metal and metal oxide on diamond surfaces

A variety of metals and metal oxides were reported to have electrocatalytic properties [13, 36, 43] , and upon deposition onto a given electrode, its electrochemical capability can be greatly increased. Platinum has been electrolytically deposited on diamond surfaces and then examined with various techniques [43-45]. The Pt deposit has been found to be quite stable on the polycrystalline diamond surface during potential cycling, more so than on

conventional carbon or graphite substrates itself, particularly at highly positive potentials, at which graphitic carbon undergoes irreversible oxidation.

Other metals that have been studied include copper [46] and nickel [43]. These metals are of interest due to their electrocatalytic activity for glucose oxidation. The copper was deposited electrochemically, whereas the nickel was deposited via ion implantation. The behavior of these metals in the form of nanoparticles on diamond is highly attractive in terms of glucose determination, because the current for glucose oxidation is increased dramatically without increasing the background current substantially.

There has been interest in the possibility that diamond electrodes could be intercalated or inserted with lithium, because there are still problems associated with graphitic materials in terms of stability. Li et al. showed that there is essentially no "underpotential deposition" of lithium on homoepitaxial diamond [43]surfaces under highly controlled conditions, particularly the absence of sp^2-carbon-containing grain boundaries [47]. Pleskov et.al. also concluded that there is negligible Li intercalation into diamond nanoparticles [48]. However, a recent work does suggests that significant insertion of Li into a diamond film drown on carbon is possible [49].

One of the first metal oxides examined electrochemically on a diamond substrate was ruthenium dioxide [50, 51]. This material is important both for electrochemical capacitor and electrocatalytic applications (chlorine evolution). Another example is cobalt hydrous oxide, which has catalytic activity for oxygen evolution [52]. A vary recent example is lead dioxide [53]. Vanadium oxide (V_2O_3) has also been supported on particulate diamond as a catalyst for an organic gas-phase reaction [54].

2.2 High Performance Liquid Chromatography (HPLC) [55-58]

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.

Figure2.3 shows the five most widely used types of high performance liquid chromatography. These include: (1) partition or liquid-liquid chromatography; (2) adsorption or liquid solid chromatography; (3) ion-exchange chromatography, and two types of size-exclusion chromatography which are (4) gel-permeation chromatography; and (5) gel-filtration chromatography.

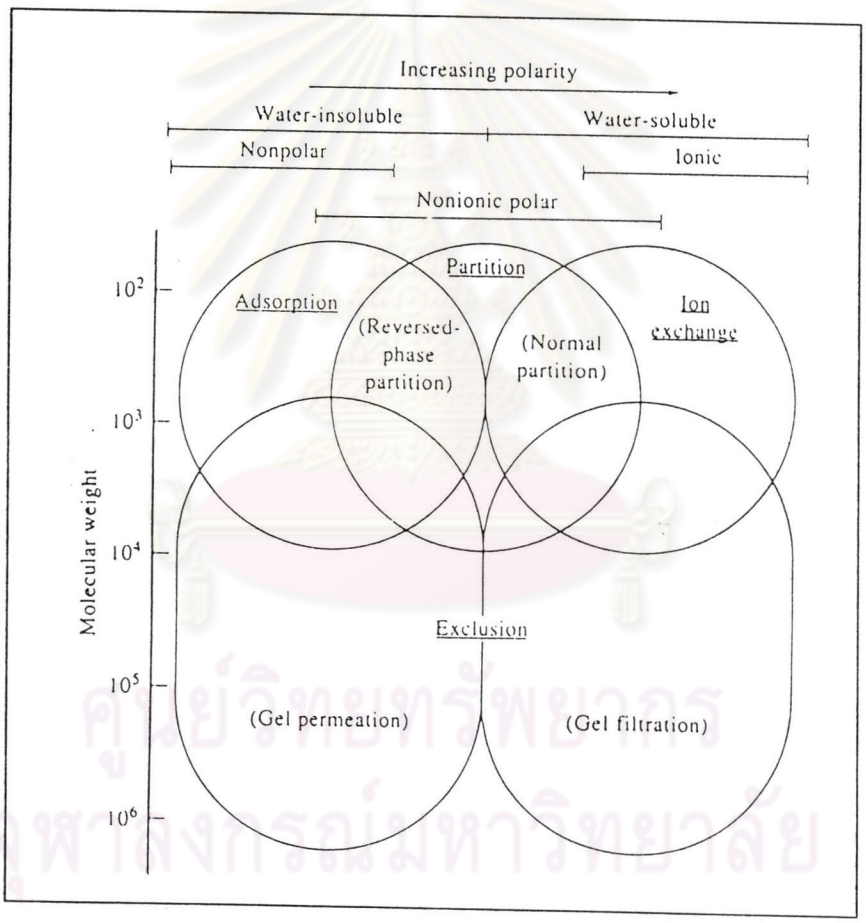


Figure2.3 Application of liquid chromatography [55]

Partition chromatography can be subdivided into liquid-liquid and liquid bonded-phase chromatography. The difference between the two lies in the method by

which the stationary phase is held on the support particles of the packing with liquid-liquid, retention is by physical adsorption, while with bonded-phase, covalent bonds are involved. Early partition chromatography was exclusively liquid-liquid; now, however, bonded-phase packing predominate because of their greater stability, with liquid-liguid packing being relegated to certain special applications.

Adsorption chromatography is based on adsorption of analyte species on a surface of solid stationary phase. The analyte competes with the mobile phase for sites on the solid surface. The analytes interact with the stationary phase according to premise "like likes like": polar solute will be retained longest by polar stationary phase and nonpolar solutes will be retained by nonpolar stationary phases. In adsorption chromatography the solute are in contact with both the stationary and the mobile phase, simultaneously.

Ion-exchange chromatography (IEC) is based on the principle that ions of opposite change opposites attract. IEC is used to separate charged analytes and therefore occurs as a result of interaction between a charge solute and oppositely charged, solid stationary phase. IEC can be applied to any solute that can acquire a charge in solution.

Size-exclusion chromatography (SEC) is based on the sieving principle. In SEC, the stationary phase particles are manufactured with a wide range of pore sizes. Then, the stationary phase behaves like a molecular sieve. From sieving action, the solute are separated on the basis of size, with the larger one elute first.

HPLC instrument

Figure 2.4 is a diagram showing the important components of a typical HPLC instrument.

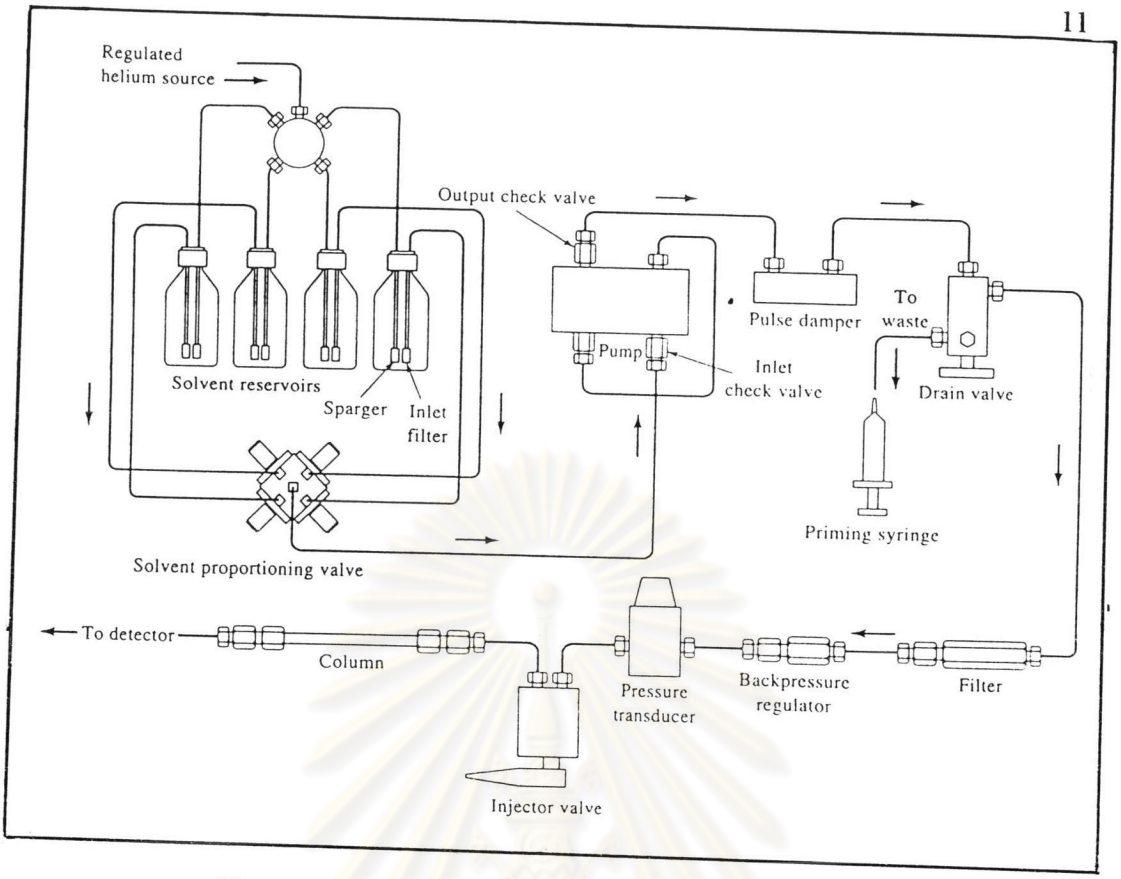


Figure 2.4 Schematic of HPLC instrument. [55]

Generally, a typical HPLC instrument consists of:

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

1) Mobile phase reservoirs and solvent treating systems

A modern HPLC apparatus is equipped with one or more glass or stainless steel reservoirs, each of which contains 500 ml or more of a solvent. The mobile phase preparations often include steps to remove dissolved gases and dust from the liquids. Degassers may consist of a vacuum pumping system, a distillation system, a device for heating and stirring or a system for sparging, in which the dissolved gases, are swept out of solution by fine bubbles of an inert gas that is not soluble in the mobile phase.

2) Pumping system

The requirements for liquid chromatography pumps include (1) the generation of pressures up to 6000 psi, (2) pulse-free output, (3) flow rate ranging from 0.1 to 10 ml/min, (4) flow reproducibilities of 0.5% or better, and (5) resistance to corrosion

There are 2 types of HPLC pumps:

1. Mechanical pumps

There are 2 types of mechanical pumps: a screw-driven syringe type and a reciprocating pump. These pumps produce a pulse delivery and flow rate is readily controlled. Reciprocating pumps are more widely used, usually consisted of small cylindrical chamber that is filled and then emptied by the back and forth motion. The pumping motion produces a pulsed flow that must be subsequently damped. They are adaptable to gradient elution.

2. Pneumatic pumps

Some instruments use a pneumatic pump, which its simplest form consists of a solvent container housed in vessel that can be pressurized by a compressed gas. This pump is simple, inexpensive and pulse-free. The limit of solvent capacity and pressure output are major disadvantages and the pumping rates depend on solvent viscosity. In addition, they are not adaptable to gradient elution.

3) Sample injection system

The most widely used method of sample introduction in liquid chromatography is based on sampling loops. The devices are often an integral part of modern liquid chromatography equipment and have interchangeable loop. The loops provide a choice of sample sizes ranging from 5 to 500 μl.

4) Column

Liquid chromatography columns are usually constructed from stainless steel tubing. Most columns range in length from 10 to 30 cm and have inside diameter of 4 to 10 mm. The column packing typically have particle size of 5 or 10 μm and often contain 40,000 to 60,000 plates/m. The most common packing for liquid chromatography is silica with highly uniform diameters. The particles are coated with thin organic films that chemically or physically bonded to the surface. Other packing materials include alumina particles, porous polymer particles and ion-exchange resins.

5) Detector

The final component of the HPLC is the detector. There are wide ranges of detector available. In this part, we will describe only UV-visible detector, and electrochemical detector

1. UV-visible detector

The basic UV-visible detector works by measuring the difference in intensity between an incoming beam of light and the same signal attenuated according to the concentration and absorbing power of the analyte. The two basic configurations of UV-visible detector are the fixed-wavelength and the photodiode array (PDA). The diagram of both types of UV-visible detector is shown in Figure 2.5

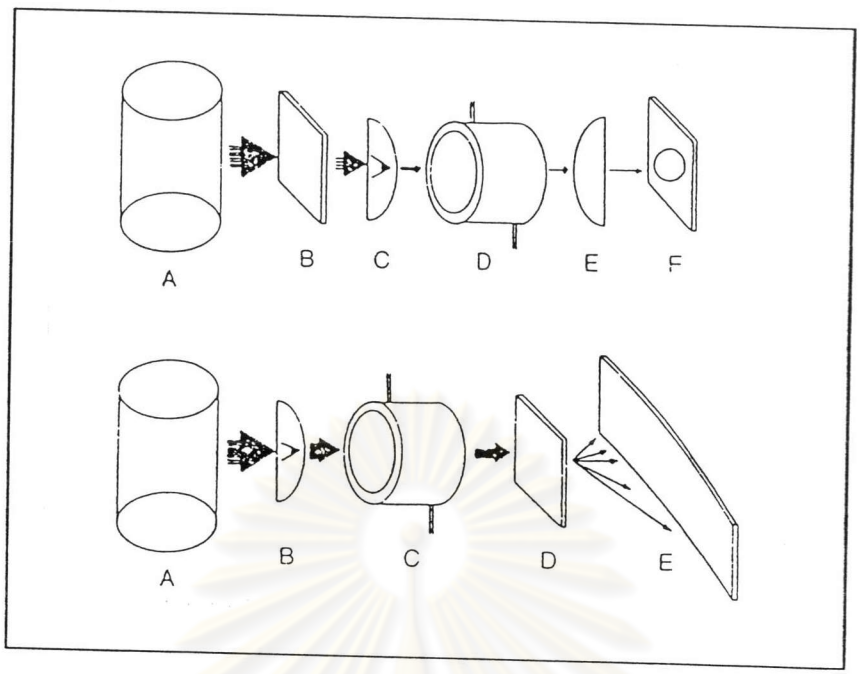


Figure 2.5 Top: Schematic for single-wavelength UV-visible detector: (A) source; (B) monochromator; (C) focusing lens; (D) flow cell; (E) focusing lens; (F) photodiode. Bottom: Schematic photodiode-array detector: (A) source; (B) focusing element; (C) flow cell; (D) dispersing element (typically grating); (E) photodiode array. [55]

The fixed-wavelength detector has a series of lenses and slits to focus the source beam on the flow cell and then focus the transmitted beam onto the diode. The basis resolution is achieved through the properties of monochromators and filters. A source monochromator is used to select the wavelength of the source output. A monochromator placed after the sample help exclude scattered light source.

The same principles apply for the PDA detector except there is no monochromator. The entire source light is focused on the detector cell. The transmitted light passes through a dispersive object (e.g. prism) that spreads the light into different wavelength regions. Photodiodes of specific width are placed along the spreading light curve. Each diode corresponds to specific band of radiation determination by its width. PDA detectors are powerful tool for the analyst. They not only are excellent in quantitation of the analyte but also can generate an absorbance-

vs.-wavelength curve for each analyte, and thus can be used to confirm an analyte's identity.

2. Electrochemical detector

Electrochemical detectors of several types are currently available from the instrument manufacturers. These devices are based upon amperometry, polarography, coulometry, and conductometry.

Although electroanalytical procedures have as yet not been exploited to the extent of optical detectors, they appear to offer advantages, in many instances, of high sensitivity, simplicity, convenience, and wide-spread applicability. A variety of HPLC/electrochemical detector cells have been described in the literature and several are available from commercial sources. Figure 2.6 is an example of a simple thin-layer type of flow-through cell for amperometric detection. Here, the electrode surface is part of a channel wall formed by sandwiching a 50 μM Teflon gasket between two machined blocks of Kel-F plastic. The indicator electrode is platinum, gold, glassy carbon, or carbon paste. A reference electrode, and often a counter electrode, is located downstream from the indicator electrode block. The cell volume is 1 to 5 μL. A useful modification of this cell, which is available commercially, includes two working electrodes, which can be operated in series or in parallel. The former configuration, in which the fluent flows first over one electrode and then over the second, requires that the analyte undergo a reversible oxidation (or reduction) at the upstream electrode. The second electrode then operates as a cathode (or an anode) to determine the oxidation (or reduction) product. The two can then be operated at different potentials (relative to a downstream reference electrode), which often gives an indication of peak purity. Alternatively, one electrode can be operated as a cathode and the other as an anode, thus making possible for a simultaneous detection of both oxidants and reductants.

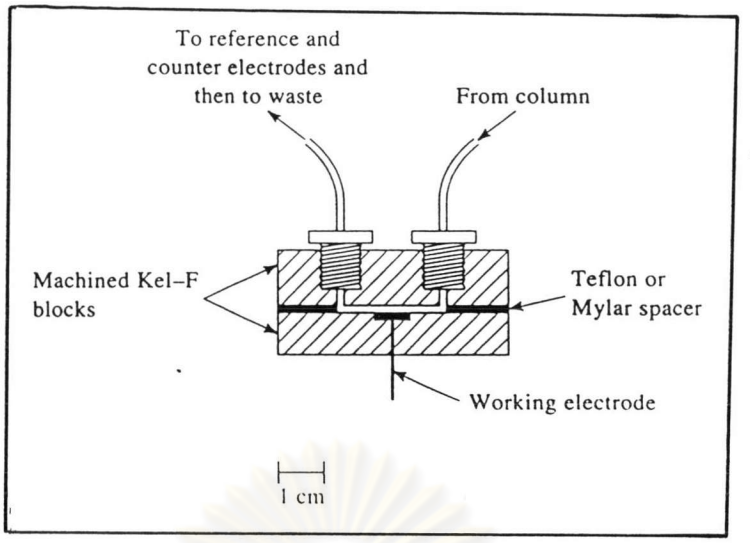


Figure 2.6 Amperometric thin-layer detector cell for HPLC [55]

2.3 Electroanalytical Chemistry

2.3.1 Voltammetry [55, 58, 59]

Voltammetry comprises a group of the electroanalytical methods in which information about the analyte is derived from the measurement of current as a function of applied potential. It is besed on the measurment of a current that develops in an electrochemical cell under conditions of complete concentration of polarization of working electrode. In the presence of the electroactive (reducible or oxidizable) species, a current will be recorded when the applied potential becomes sufficiently negative or positive for it to electrolyze. The recording result is called a voltammogram. The potential excitation signal is imposed on an electrochemical cell containing an electrode. Three waveforms of most common excitation signals used in voltammetry are shown in Figure 2.7. The classical voltammetric excitation signal is a linear scan shown in Figure 2.7a. The potential applied to the cell of this excitation increases linearly as a function of time. The two pulse excitation signals are shown in Figure 2.7b and 2.7c. The current responses of the pulse type are measured at various times during the lifetime of these pulses.

Voltammetry is widely used for the fundamental studies of oxidation and reduction processes in various media, adsorption process on electrode surfaces, and electron transfer mechanisms at electrode surfaces. In the mid-1960s, several major

modifications of classical voltammetric techniques were developed that enhanced the sensitivity and selectivity of the method.

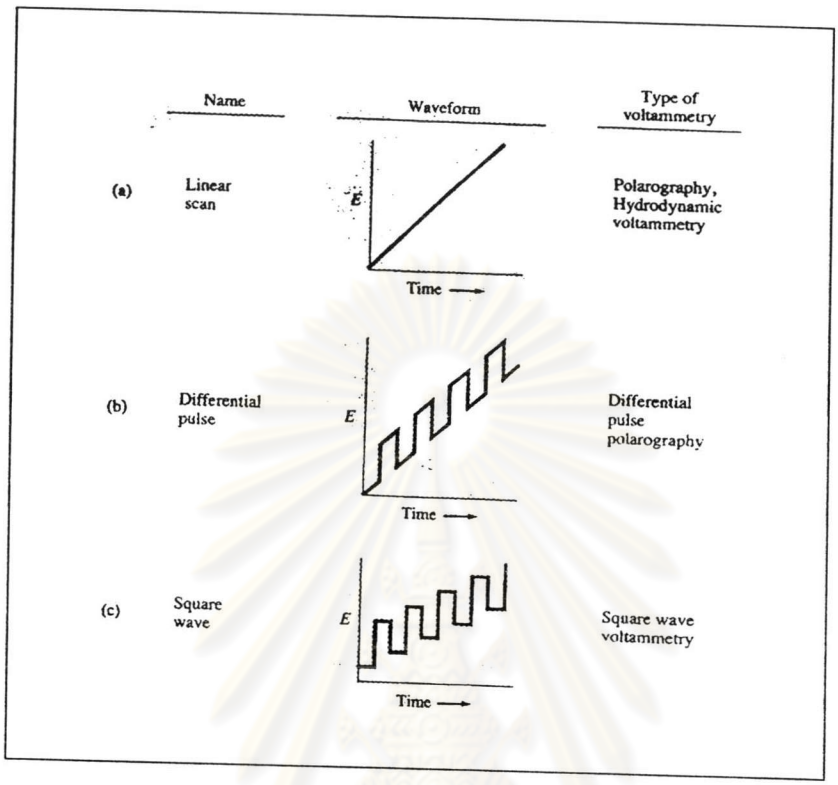


Figure 2.7 Typical excitation signals for voltammetry [55]

2.3.2 Cyclic voltammetry [55, 60, 61]

Cyclic voltammetry is the most widely used technique for acquiring qualitative information about electrochemical reactions. The power of cyclic voltammetry result from its ability to rapidly provide considerable information on the thermodynamics of redox processes, on the kinetics of heterogeneous electron-transfer reactions, and on coupled chemical reaction or adsorption processes. Cyclic voltammetry is often the first experiment performed in an electroanalytical study. In particular, it offers a rapid location of redox potentials of the electroactive species, and convenient evaluation of the effect of media upon the redox process.

Cyclic voltammetry consists of scanning linearly the potential of a stationary working electrode (in an unstirred solution) using a triangular potential waveform

(Figure 2.8a). The triangular waveform produces the forward and then the reverse scan. Depending on the information sought, single or multiple cycles can be used. During the potential sweep, the potentiostat measures the current resulting from applied potential. The resulting plot of current versus potential (i-E plot) is termed a cyclic voltammogram (Figure 2.8b). The significant parameters in cyclic voltammogram are the cathodic peak potential (E_{pc}), the anodic peak potential (E_{pa}), the cathodic peak current (i_{pc}), and the anodic peak current (i_{pa}). The cyclic voltammogram is a complicated, time-dependent function of a large number of physical and chemical parameters.

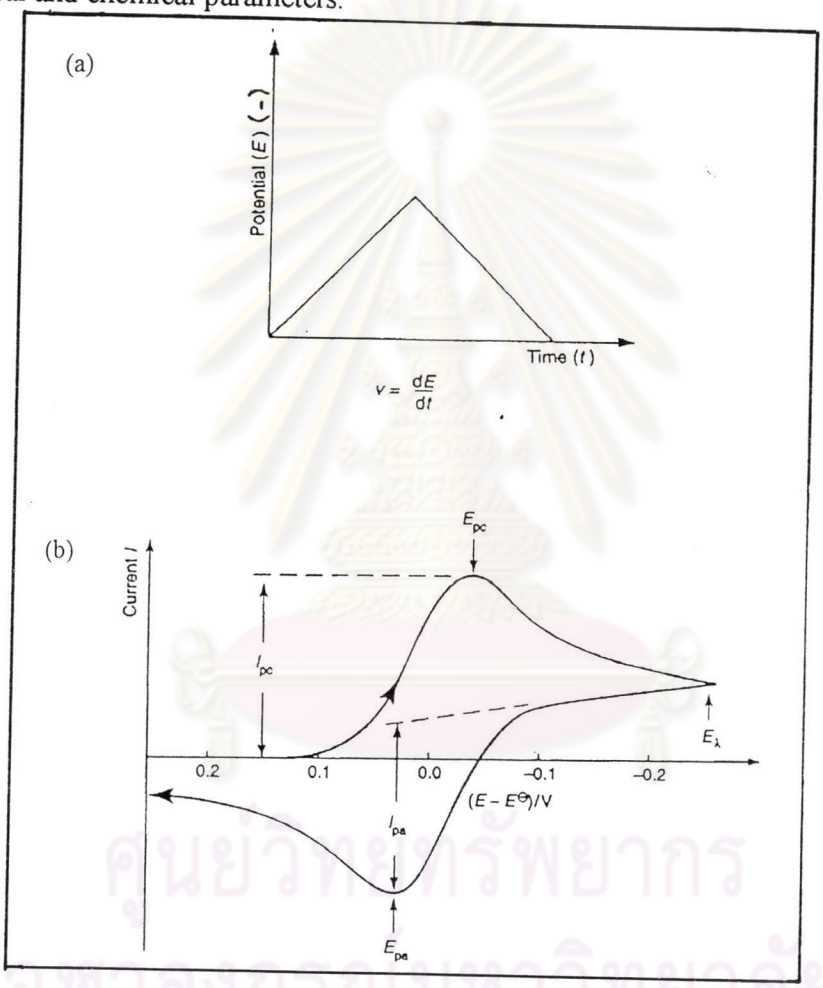


Figure 2.8 Schematic of (a) a potential wave form used in cyclic voltammetry, and (b) a cyclic voltammogram [56]

2.3.3 Amperometry [55, 60-63]

Amperometry is used in this technique one of the controlled-potential electrochemical techniques. A simple potential-time waveform is shown in Figure 2.9. It is normally carried out in stirred or flowing solutions or at 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 the molecules or ions that have been removed by the reaction at the electrode. Hence, the resultant amperometeric signal is directly proportion to the concentration of the analyte.

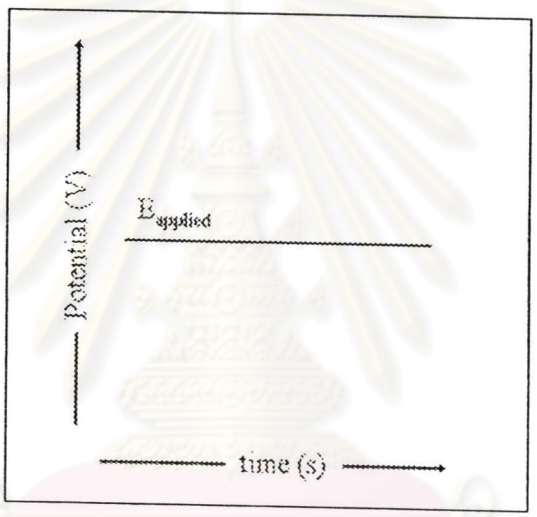


Figure 2.9 A typical waveform employed in amperometry [55]

2.4 Flow Injection Analysis (FIA) [64]

In 1975, the concept of flow injection analysis (FIA) was introduced by Ruzicka and Hansen. This technique is based on the injection of a liquid sample into a moving stream of a suitable carrier solution. The zone of injected sample is formed in a narrow tube and then transported toward a detector. In order to propel the carrier solution through a narrow tube, a pump is used as a propelling device. The analyte of interest is then detected and the detector response such as absorbance, electrode potential or current is recorded as it continuously changes due to the movement of the analyte sample through the flow cell.

The schematic diagram below (Figure 2.10) groups the FIA process into three stages to help visualize how the FIA performs a method or analysis.

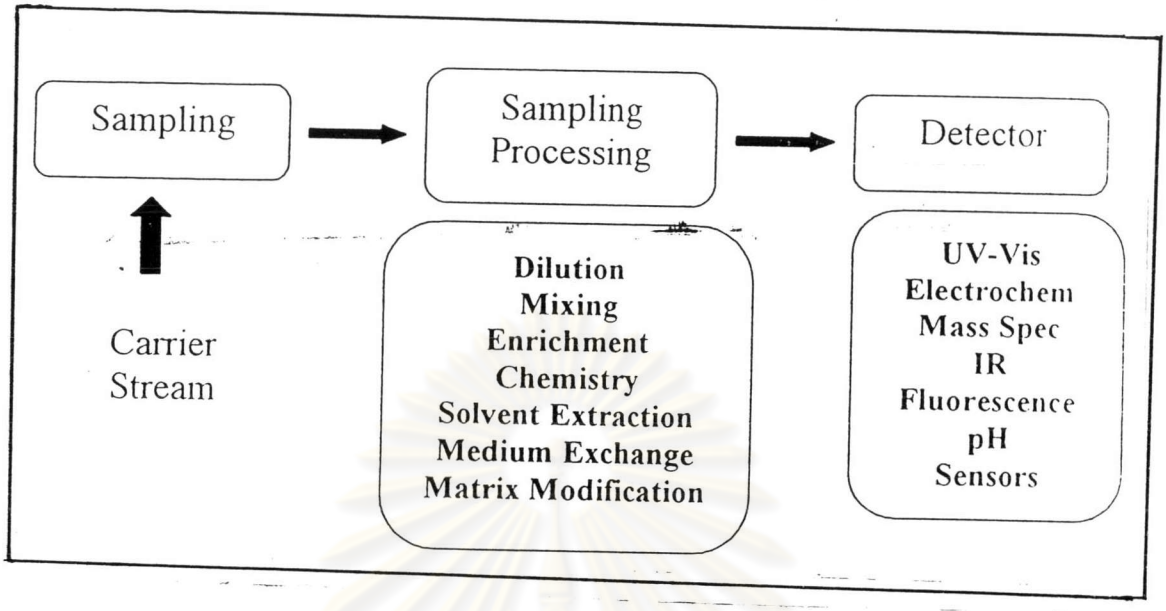


Figure 2.10 Schematic diagram of a generic description of FIA [64]

The first step is sampling where the sample is injected into the flowing carrier steam. This step is generally performed with a sample injection value. The second stage is called sample processing. The purpose of this step is to transform the analyte into a species that can be measured by the detector and manipulate its concentration into a range that is compatible with the detector, using one or more of the indicated processes. The third stage is detection where the analyte, or its derivative, generates a signal which is used for quantitation. As indicated, a large variety of detectors can be used in FIA.

The FIA manifold can be designed in order to obtain the best analytical results. The simplest FIA manifold is the single-line FIA manifold. In this case, the sample solution is rigorously and precisely transported to the flow cell in undiluted form. The example of the single-line FIA manifold is shown in Figure 2.11

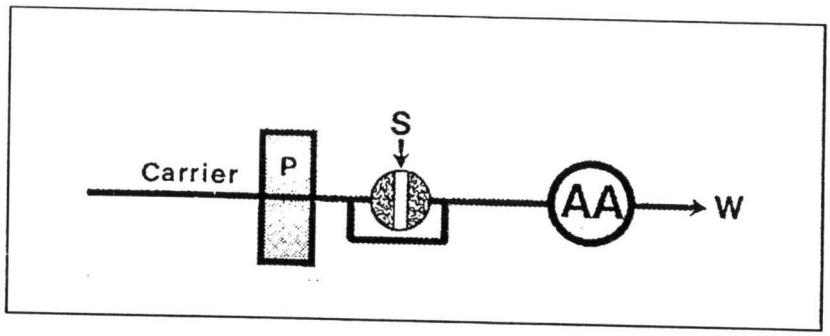


Figure 2.11 Single-line FIA manifold. P: pump, S: sample, AA: atomic absorption spectrometry (detector), W: waste [64]

The 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 methologies rapidly and automatically within a few minutes and with low amount of sample.

The thin-layer cell design [65] is commonly used as an amperometric detector for liquid chromatography and was also employed in this study. The basic functioning of this mode of detection is depicted schematically in Figure 2.12 As illustrated, 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 concentration of analyte entering the thin-layer cell.

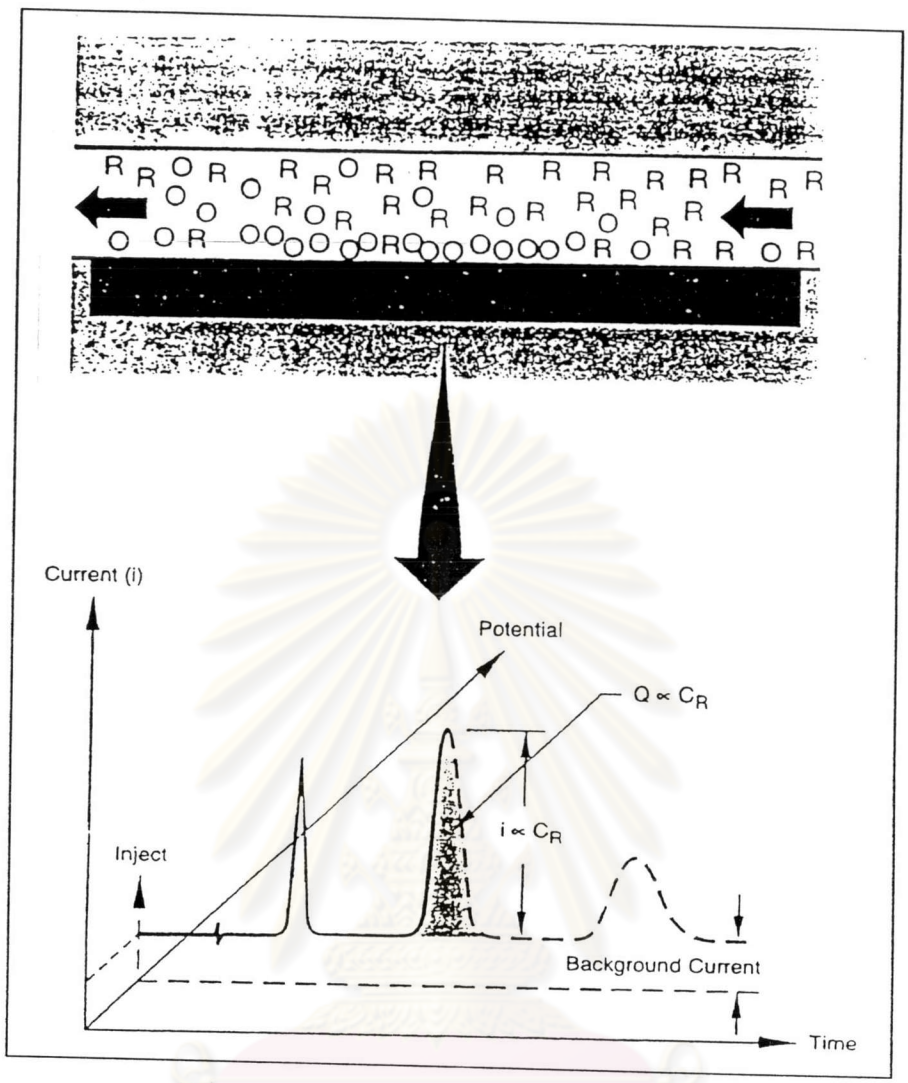


Figure 2.12 At any instant in time, the current in an LCEC experiment reflects the rate of conversion of reactant (R) to product (O) [65]

2.5 Sample Preparation [66]

Sample preparation is a technique used to clean-up a sample before analyzing it and/or to concentrate a sample to improve its detection. In carry out this process properly, three critical criterias must be concerned, namely:

Sample Concentration

Frequently, the component of interest is present in level too low a for detection. Sample preparation can make the component to become concentrated to an adequate level for measurement.

Contaminations

The presence of interfering matrix elements can mask the analysis of the component of interest. Sample preparation can remove excess contaminants to yield clean, informative chromatograms.

In Solution

For most analyses (HPLC, GC, Spectrophotometer, etc.), the sample must be properly prepared in solution for subsequent analysis.

Classification of extraction methods depend on the type of samples (solids, liquids and gases) and their preparations are as follow:

2.5.1 Volatile Samples

There are many sampling techniques for volatile samples. These include gas-solid adsorption, headspace (HS) analysis, purge and trap.

2.5.2 Liquid Samples

Liquid samples are much easier to prepare for analytical measurement relative to volatile compounds or solids, because dissolution or an extraction step may not be involved. Often, dilution in a compatible solvent is all that is required. The major considerations for liquid samples are the matrix interferences, the concentration of analytes, and compatibility with the analytical technique.

2.5.2.1 Liquid-Liquid Extraction (LLE)

Liquid - liquid extraction is a separation process that takes advantage of the relative solubilities of solutes in immiscible solvents. The solute dissolves more readily and becomes more concentrated in the solvent in which it has a higher solubility. A partial separation occurs when a number of solutes have different relative solubilities in the two solvents used.

2.5.2.2 Dilution

Sample is diluted with solvent that is compatible with analytical measurement technique to avoid chromatographic column overload or to be in linear range of detector or spectrophotometer. Solvent should be compatible with analytical measurement technique; solvent should not be too strong for HPLC mobile phase conditions so that injection causes unacceptable band broadening.

2.5.2.3 Evaporation

Liquid is removed by gentle heating at atmospheric pressure with flowing air or inert gas or under vacuum. Cautions should be taken in that the samples must not be overheated or evaporated too quickly and it is favorably performed under inert gas such as nitrogen. The schematic of a typical evaporator is shown in Figure 2.13

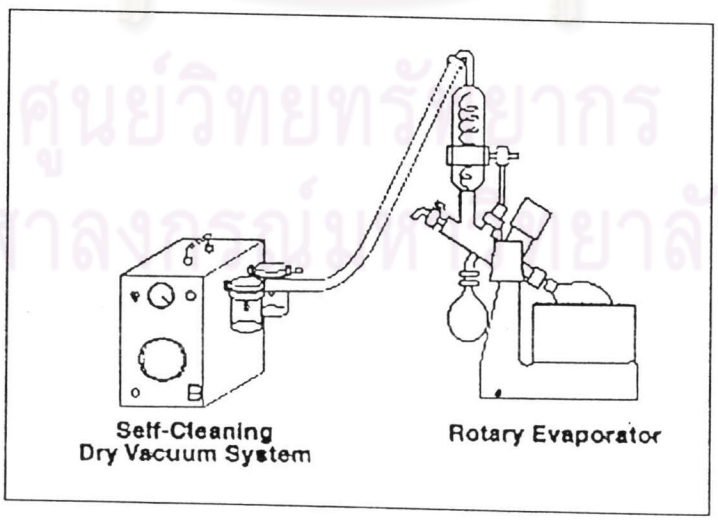


Figure 2.13 Instrument of evaporator [66]

2.5.2.4 Distillation

Sample is heated to boiling point of solvent and volatile analytes are concentrated in vapor phase, condensed, and collected; steam distillation involves boiling with water or purging with steam and collecting distillate. Mainly for samples that can be volatilized; sample can decompose if heated too high; vacuum distillation can be used for nonvolatile compounds. The instrument of distillation as shown in Figure 2.14

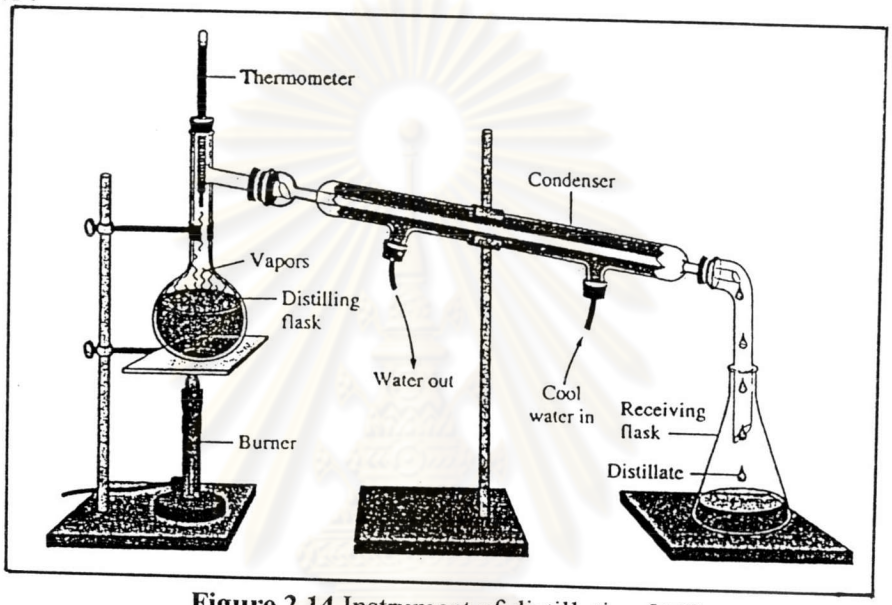


Figure 2.14 Instrument of distillation [66]

2.5.2.5 Centrifugation

Sample is placed in tapered centrifuge tube and spun at high force, liquid is decanted. Quantitatively removing solid sample from tube sometimes presents practical problem: ultracentrifuge normally not used for simple particulate removal. The instrument of distillation is shown in Figure 2.15

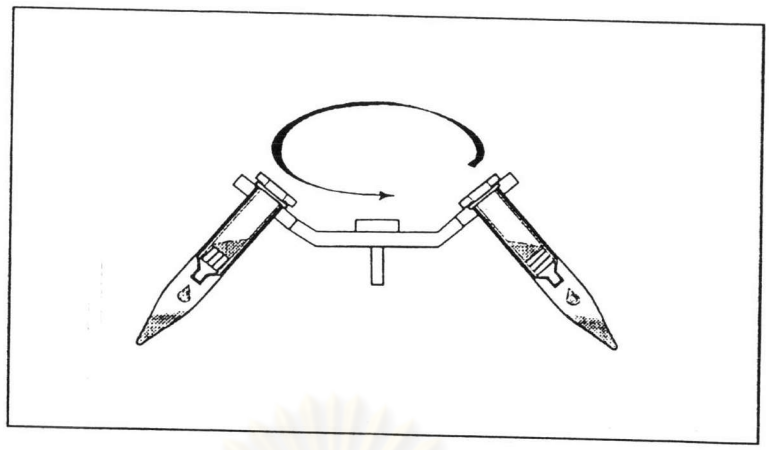


Figure 2.15 Instrument of centrifugation [66]

2.5.2.6 Solid Phase Extraction (SPE)

Solid Phase Extraction is an extraction technique based on the selective partitioning of one or more components between two phases, one of which is a solid sorbent. The second phase typically is a liquid, but it may also be an emulsion, a gas or a supercritical fluid. The components of interest may either preferentially adsorb to the solid, or they may remain in the second, non-solid phase. Once equilibrium has been reached, the two phases are physically separated by decanting, filtration, centrifugation or a similar process. If the desired analytes are adsorbed on the solid phase, they can then be selectively desorbed by washing with an appropriate solvent. If the components of interest remain in a liquid phase, they can by recovered via concentration, evaporation, chromatographic separation, and/or recrystallization.

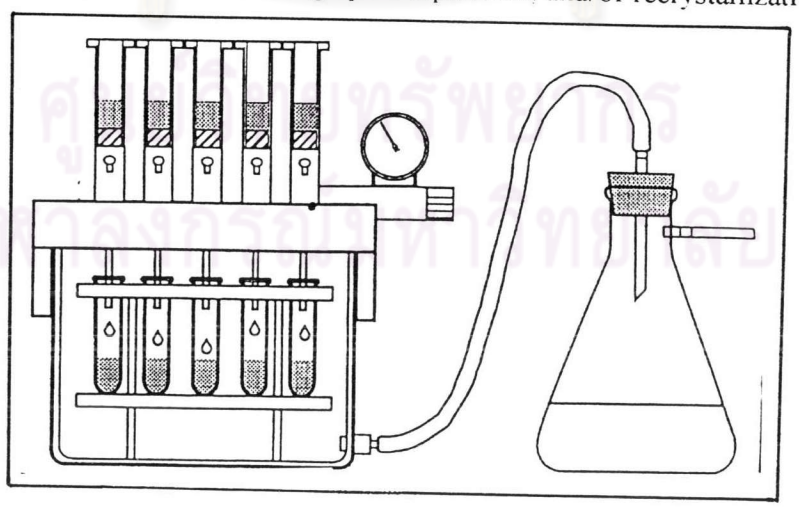


Figure 2.16 Instrument of Solid Phase Extraction [66]

2.5.2.6.1 Mechanisms of SPE

There are three mechanisms of separation and isolation in SPE.

(1) Reversed Phase

This mechanism involves the partitioning of organic solutes from a polar mobile phase, such as water, into a nonploar stationary phase, such as the C-18 sorbent (Figure 2.17). The mechanism of isolation is a nonpolar interaction, called Vander Waals, dispersion forces, or partitioning. The partitioning mechanism is a low-energy process (5 vs. 80 kcal/mol for ion exchange) and is analogous to a molecule being removed from water in a liquid-liquid extraction. The difference being that the organic phase is chemically bonded to the silica. The mechanism is called reversed phase because it was the opposite of early work where the stationary phase was polar and the mobile phase was non-polar. The analyte that is adsorbed on the stationary phase is due to the attractive forces between the carbon-hydrogen bonds in the analyte and the functional group of the stationary phase. To elute the analytes from a reversed phase SPE, a nonpolar solvent is used. Many types of functional group of stationary phase in this mode are C_2, C_4, C_8, C_{18}, cyclohexyl and phenyl groups.

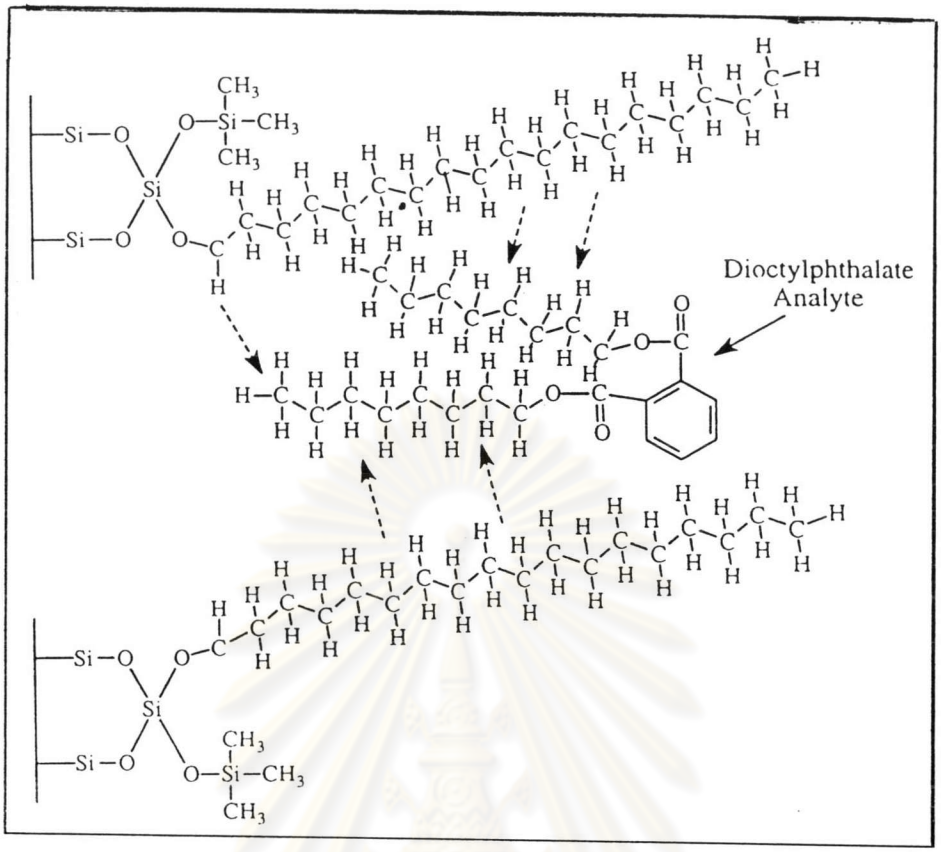


Figure 2.17 Reversed-phase mechanism of sorption of dioctylphthalate in SPE [66]

(2) Normal Phase

Normal-phase SPE refers to the sorption of an analyte by a polar surface. It is called "normal" because this was the standard type of separation (prior to 1960) that was done by classical liquid chromatography. The mechanism of isolation is a polar interaction, such as hydrogen bonding, dipole-dipole interactions, π-π interactions, and induced dipole-dipole interactions. The mechanism involves the sorption of the functional groups of the solute to the polar sites of the packing material versus the solubility of the solute in the mobile phase of the column. The sorption by normal phase is a low to moderately strong interaction (Figure2.18). Figure2.18 shows the sorption of a nitrobenzene molecule to the surface of a silica-bonded cyanopropyl sorbent. The mechanism of sorption is through hydrogen bonding of the cyano group through the amino groups of the nitroaminobenzene analyte.

The types of nonbonded phases used for normal-phase SPE are silica, alumina, and magnesium silicate (Florisil). The most popular phase is silica. Several bonded phases may also be used for normal-phase SPE, including aminopropyl, cyanopropyl, and propyldiol. Water is not used in the mobile phase in normal-phase SPE because it will sorb to the active sites of the sorbent and reduce the interaction between analyte and sorbent.Typically, normal-phase SPE is used as a clean-up procedure for organic extracts of water, soil, food, or other materials. Normal-phase SPE is also used for the isolation of analytes from organic liquids, such as oils.

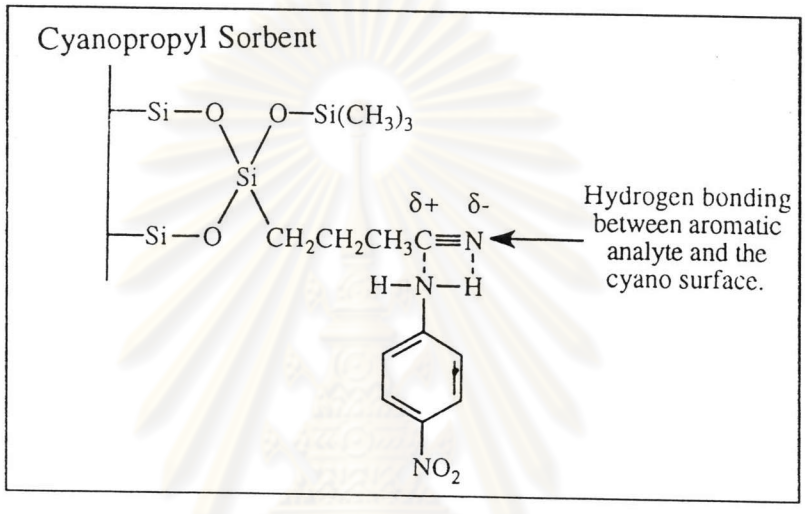


Figure 2.18 Normal-phase mechanism in SPE for the sorption of nitrobenzene. [66]

(3) Ion Exchange

This mechanism involves the ion exchange of a charged organic solute from either a polar or nonpolar solvent onto the oppositely charged ion-exchange sorbent. This mechanism of isolation is a high-energy, ionic interaction; thus, polar solutes may be effectively removed from polar solvents, including water as well as less polar organic solvents.

Sorbets are termed strong cation or anion exchangers if they have a permanent fixed charge, either positive or negative, respectively. In the case of the strong cation-exchange site, a sulfonic-acid functional group is present with a proton as its counter ion. When another cation enters the vicinity of the cation-exchange site, there is a competition or exchanging of ions that depends on the selectivity of the

cation for the site and the number or mass of cations that are competing for the site. Thus, the analyte to be concentrated must compete for sorption sites with other cations in the sample. The mechanism is similar for anions, except that the strong anion site is a quaternary nitrogen atom with chloride as the most common counter ion (Figure 2.19). The example in Figure 2.18 shows the anion exchange of 2, 4-D, an anionic herbicide, onto a strong anion-exchange resin. The resin is in its chloride form and exchanges one chloride ion for the single negative charge on a 2, 4-D molecule.

In order to retain the analytes by the ion exchange, the pH of the sample matrix must be one at which both analytes and the functional groups on the bonded phases are charged. Other species that have the same charge as the analyte may interfere with the adsorption of analyte.

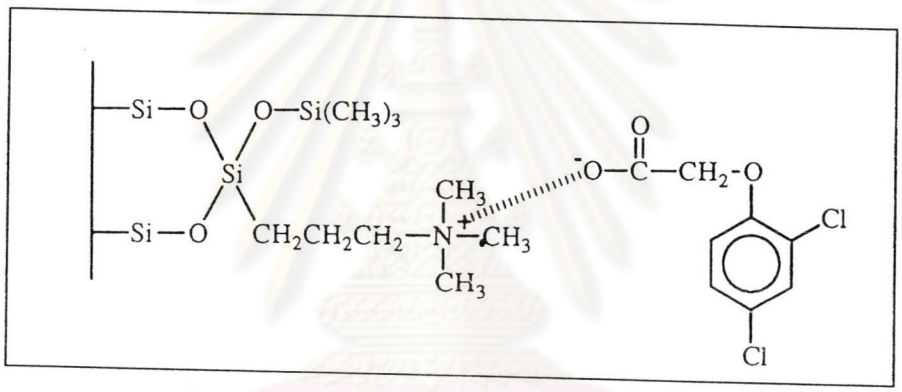


Figure 2.19 Mechanism of ion-exchange SPE for 2, 4-D. [66]

2.5.2.6.2 Steps of SPE

There are four steps contributing to the process of SPE as showed in Figure 2.20

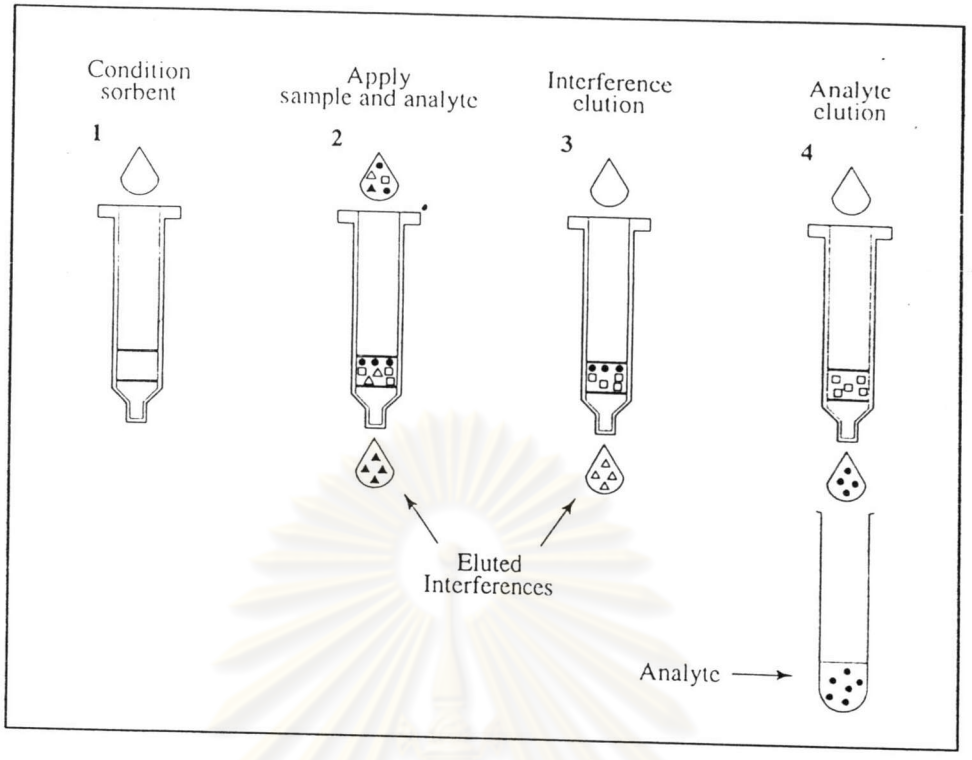


Figure.2.20 Steps of solid-phase extraction [66]

Step I (SPE Tube or Disk Conditioning)

The type silica and nonpolar adsorption media usually are conditioned with a water-miscible organic solvent such as methanol, followed by water or an aqueous buffer. Methanol wets the surface of the sorbent and penetrates bonded alkyl phases, allowing water to wet the silica surface efficiently. Sometimes a pre-conditioning solvent is used before the methanol step. This solvent is usually the same as the elution solvent, and is used to remove any impurities on the SPE tube that could interfere with the analysis, and may be solute only in a strong elution solvent.

Step II (Addition of Sample)

The sample is tranfered to the tube or reservoir, using a volumetric pipette or micropipette. The sample must be in a form that is compatible with SPE. The sample volume can range from microliters to liters. When excessive volumes of aqueous solutions are extracted, reversed phase silica packings gradually lose the solvent layer acquired through the conditioning process. This reduces extraction efficiency and

sample recovery. For samples >250 mL, small amounts of water-miscible solvents (up to 10%) are added to maintain proper wetting of reversed phase packings. Maximum sample capacity is specific to each application and the conditions used. An analyte breakthrough is tested using the following technique in case recoveries are low or irreproducible. Two conditioned SPE tubes of the same packing are attached together using an adapter. The sample is passed through both tubes. Each tube is detached and eluted separately. If the analyte is found in the extract of the bottom tube, the sample volume is too great or the weight is too small, resulting in analyte breakthrough. The pH, salt concentration, and/or organic solvent content of the solution are adjusted to enhance retention of appropriate compounds on the packing, and elution or recipitation of unwanted compounds. Samples are filtered or centrifuged prior to extraction to avoid clogging SPE tube frits or the SPE disk. The sample solution is slowly passed through the extraction device, using either vacuum or positive pressure. The flow rate can affect the retention of certain compounds. Generally, the flow rate should not exceed 2 mL/min for ion exchange SPE tubes, 5 mL/min for other SPE tubes, and may be up to 50 mL/min for disks. Dropwise flow is best, when time is not a factor.

Step III (Washing)

If compounds of interest are retained on the packing, unwanted and unretained materials can be washed off using the same solution in which the sample was dissolved, or another solution that will not remove the desired compounds. Usually no more than a tube volume of wash solution is needed, or 5-10 mL for SPE disks. The packing is washed to remove unwanted, weakly retained materials with solutions that are stronger than the sample matrix, but weaker than needed to remove compounds of interest. A typical solution may contain less organic or inorganic salt than the final eluent. It also may be adjusted to a different pH. Pure solvents or mixtures of solvents differing sufficiently in polarity from the final eluent may be useful wash solutions. About one tube volume of the sample solvent is used to remove any residual, desired components from the tube, or 5-10 mL to remove the material from a disk in case compounds of interest are not retained on the packing. This rinse serves as the elution step to complete the extraction process in this case.

Step IV (Eluting the Compounds of Interest)

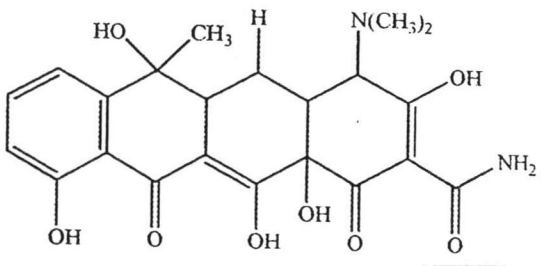
The packing is rinsed with a small volume (typically 200 µL to 2 mL depending on the tube size, or 5-10 mL depending on the disk size) of a solution that removes compounds of interest, but leaves behind any impurities not removed in the wash step. The eluate is collected and further prepared. Two small aliquots generally elute compounds of interest more efficiently than one larger aliquot. Recovery of analytes is best when each aliquot remains in contact with the tube packing or disk for 20 seconds to 1 minute. Slow or drop-wise flow rates in this step are beneficial.

2.5.3 Soild Samples

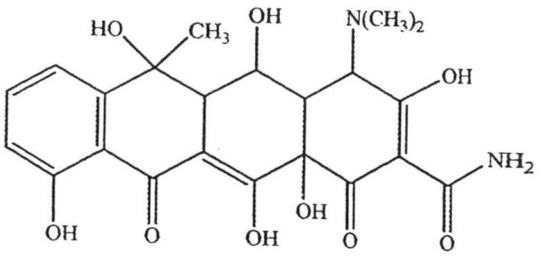
When a sample is a soild, the sample pretreatment process is more complicated. There are two specific cases: the entire sample is of interest and must be solubilized, or only a part of the soild is of interest and analyte must be selectively removed. If the soild sample is a soluble salt or drug tablet formulation, the only sample preparation that may be required is finding a suitable solvent that will totally dissolve the sample and the components of interest. If the sample matrix is insoluble in common solvent but the analytes of interest can be removed or leached out, then sample preparation can also be rather straightforward. In these cases, techniques such as filtration, Soxhlet extraction, supercritical fluid extraction (SFE), ultrasonication, or solid-liquid extraction may be useful.

2.6 Literature surveys

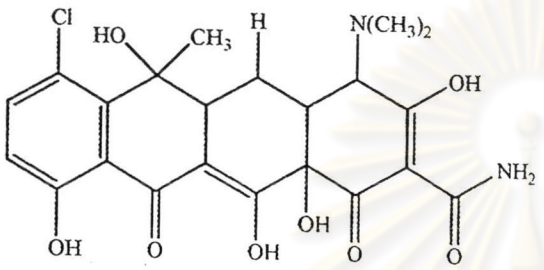
Tetracyclines produced by *Streptomyces* are broad spectrum antibiotics that are active against both gram-positive and gram-negative bacteria, as well as are especially effective against *spirochete, Actinomyces, Ricketsia and Mycoplasma.* Because of a broad spectrum antibiotic, commercial availability and low price, the use of tetracyclines is rising in veterinary and aquaculture. From these applications, they are considered as the broad-spectrum antibiotic drugs. There are four compounds (Tetracycline, Oxyteracycline, Chlortetracycline and Doxycycline) as shown in Figure 2.21



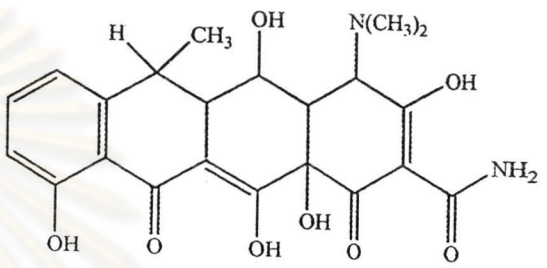
Tetracycline



Oxytetracycline



Chlortetracycline



Doxycycline

Figure 2.21 Structures of 4 tetracyclines

Tetracyclines are the most important ones of antibiotics generally used in the shrimp farming. For detecting the tetracycline residues, the microbiological assays, the official methods, are most commonly used, but they are complicated, time-consuming and non-specific. Therefore, sensitive and specific analytical methods for identification and quantitation of tetracyclines are required. High performance liquid chromatography (HPLC) is normally used for this purpose.

HPLC with different detection methods such as spectrophotometry, fluorometry, chemiluminometry, and mass spectrometry has been described to determine tetracyclines. Some drawbacks of these detection methods are complication of derivatization procedure, long time analysis, and experience. Electrochemical methods are very attractive alternatives because they are simple, fast and low cost. One of the electrochemical methods generally used as detector in HPLC system is amperometric detection.

Various methods have been proposed for the determination of tetracyclines.

In 1988, Sabharwal, et al. [39] determined tetracycline hydrochloride in the presence of anhydrotetracycline by differential pulse polarography.The results obtained with this method are in close agreement with those from the spectrophotometric absorbance ratio method.

In 1995, Oungpipat et al. [36] reported that the flow injection analysis with amperometry could be used to detect tetracyclines by electrocatalytic oxidation at a nickel-modified glassy carbon electrode. The preparation of the modified electrode is carried out by simple deposition of Ni^{2+} solution onto the glassy carbon-based electrode. The detection is based on the measurement of anodic current generated by the catalytic oxidation of the antibiotics at the surface of the electrode through the formation of a high-valent, oxyhydroxide species (NiOOH).

In 1996, Tanase, et al. [67] studied the electrochemical reduction on dropping mercury electrode by alternating current polarography (ACP) of tetracycline.

In 1997, Gong and Zhang [68] reported the determination of tetracyclines with a modified β–cyclodextrin based fluorosensor. An ethyl substituted β–cyclodextrin based fluorosensor was prepared for the determination of tetracyclines in clinical samples. The proposed optosensor has a higher selectivity. Species existing in biological samples hardly interfered, and the main interferences were caused by some metal ions which can complex with Tetracycline and enhance its fluorescence. They could be masked by adding excess of EDTA. The recommended method was successfully tested for the determination of tetracycline in clinical samples (urine and pharmaceutical preparations).

In 2002, Ohnishi et al. [43] reported the determination of glucose by using Nickel-implanted boron-doped diamond electrodes. This electrode exhibited excellent electrochemical stability with low background current even after ultrasonic treatment, indicating the strong bonding of nickel with carbon.

It can clearly be seen from the above that a great deal of methods have been developed for the detection of tetracyclines. However, these techniques alone are not applicable to matrix-prone samples such as food, animal tissues as well as environmental samples due to its lack of selectivity. To overcome this problem, the chromatographic methods are commonly used to remove any interfering species prior to the detection as illustrated by the following.

In 1985, Oka et al. used current standard method for the extraction of tetracyclines from animal tissues. Isolation of tetracyclines from various tissues was followed by homogenization of sample in the presence of extracting solvent. Then the supernatant is put through a series of manipulations to remove interferences while keeping the target tetracyclines. There is an enormous variety of extraction methods for tetracycline analysis. The most common one of these methods is the use of aqueous solutions containing chelating agents to reduce the binding of tetracyclines with cations in the matrix. EDTA, oxalic acid and citric acid are commonly used as masking agents.

In 1997, Kazemifard and Moore [37] reported the use of amperometric detection at the glassy carbon electrode for the detection of tetracyclines, following liquid chromatography. Although amperometric detection can provide a high sensitivity, its major drawback is the deposition of detection products or impurities on the electrode surface.

In 2000, Oka et al. [69] reported the chromatographic analysis of tetracycline antibiotics in foods. Many analytical techniques like thin layer chromatography, capillary electrophoresis, high-performance liquid chromatography, and sample preparation including extraction and clean up procedures of tetracyclines, therefore, have been reported to monitor their residues in foods such as fish, honey, milk, egg, shrimp, etc.

In 2003, Naidong et al. [70] used TLC densitometry with fluorescence detection for the assay and purity control of tetracycline, chlortetracycline and oxytetracycline in animal feed and premixes. Several methods for the detection of

tetracyclines on a TLC plate, fluorescence detection, UV detection and fast atom bombardment mass spectrometry (FAB-MS) [71, 72] have been reported.

The detection of tetracyclines using capillary (CE) have been described.[73, 74] Compared with HPLC, the CE offers many advantages such as the use of much less organic solvent, short run time for separation, high efficiency, etc. In 1996, J.J Pesek et al. [73] described the separations of various tetracycline mixtures by high-performance capillary electrophoresis (HPCE) and a new form of electrochromatography (CEC) was compared. And in 1997, Tjornelund et al. [74] reported the determination of tetracycline by using laser-induced fluorescence detection in non-aqueous capillary electrophoresis systems.

HPLC is commonly used for the detection of tetracyclines in various matrices. The tetracyclines have been analyzed via separation on reversed-phase (C_8- C_{18}) modified silica solid supports, polymer- or resin-based noninorganic solid supports and ion-exchange solid support as the stationary phase. The reversed-phase systems, especially C_8, and C_{18}, have many applications for the detection of tetracyclines in some pharmaceuticals, animal tissues, milk, and environment. A variety of buffers in mobile phase systems have been described [75, 76] for the separation of tetracyclines, such as EDTA, phosphate, citric acid, oxalic acid, imidazole buffer and glycine buffer.

In 2003, Cinquina et al. [76] employed HPLC with diode-array detection for the determination of tetracyclines in bovine milk and tissues by using C_8 column with 60:25:15 of 0.01 M oxalic acid: acetonitrile: methanol and ultraviolet detection at 365 nm. The method was successfully validated for bovine milk and muscle in compliance with requirements set by draft SANCO/1805/2000 European Decision.

In 2003, Palaharn et al. [38] reported the use of pulsed amperometric detection (PAD) at a gold electrode for the detection of tetracycline and applied to a flow injection system. In 2004, Loetanantawong et al. [77] reported the use amperometric detection at ruthenium cyanide- modified glassy carbon electrode for the detection of tetracyclines in shrimp sample.

In 2005, Anderson et al. [75] reported the determination of tetracycline residues in shrimp and whole milk by using C_8 column with 75:15:10 of 0.1% formic acid: acetonitrile: methanol and ultraviolet detection at 370 nm. Several detection methods for HPLC have been described for the detection of tetracyclines, for example: ultraviolet (UV), fluorescence, electrochemistry, mass spectrometry.

In this study, Ni-DIA electrode was used in a FIA with amperometric detection for the determination of tetracycline in commercially available capsules. In addition, the Ni-DIA was coupled to a HPLC for the determination of tetracyclines in real samples.