CHAPTER II

THEORY AND LITERATURE SURVEY

2.1 Theory of electroanalytical Chemistry

2.1.1 Voltammetry [8], [9], [10]

Voltammetry comprised a group of the electroanalytical methods. The information about the analyte is derived from the measurement of current under conditions of complete concentration of polarization of working electrode. The potential of the working electrode is varied and the resulting current is recorded as a function of applied potential. 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 impressed on an electrochemical cell containing an electrode. Three waveforms of most common excitation signals used in voltammetry are shown in Figure 2.1. The classical voltammetric excitation signal is a linear scan shown in Figure 2.1a. 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 2.1b and 2.1c. 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.

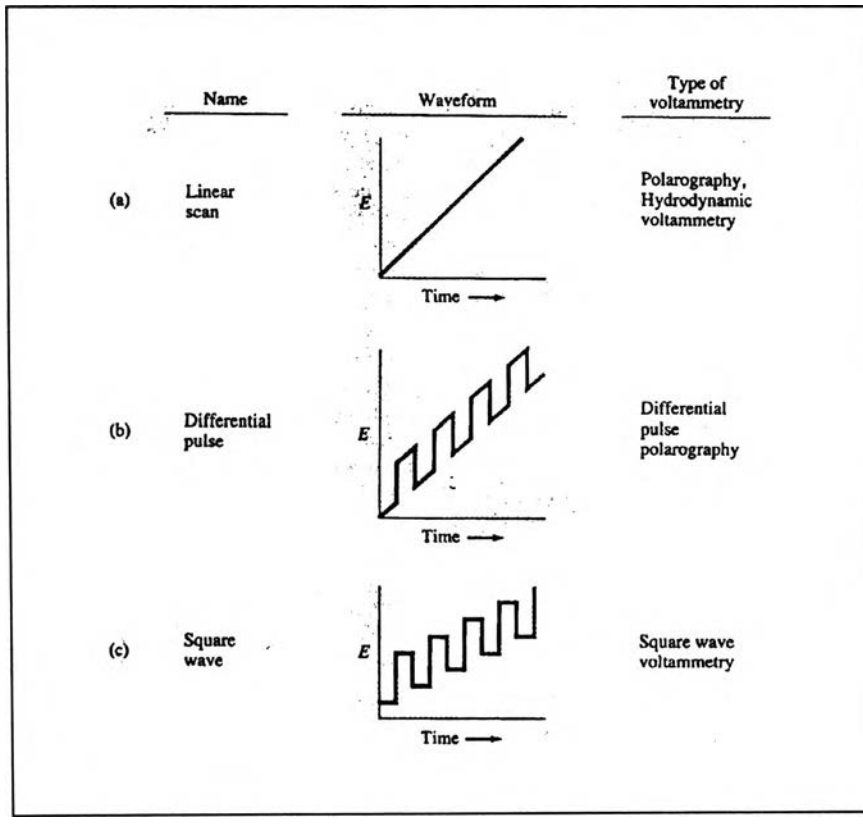

Figure 2.1 Typical excitation signals for voltammetry

2.1.2 Rotating disk voltammetry [11], [12], [13]

Similar to cyclic voltammetry, the working electrode potential in rotating disk voltammetry is swept back and forth across the formal potential of the analyte. The difference between these two techniques is the rotation of working electrode at a high speed for rotating disk voltammetry. The working electrode can be made from metal, graphite, or other conductors. Figure 2.2 shows the diagram of the typical rotating disk electrode.

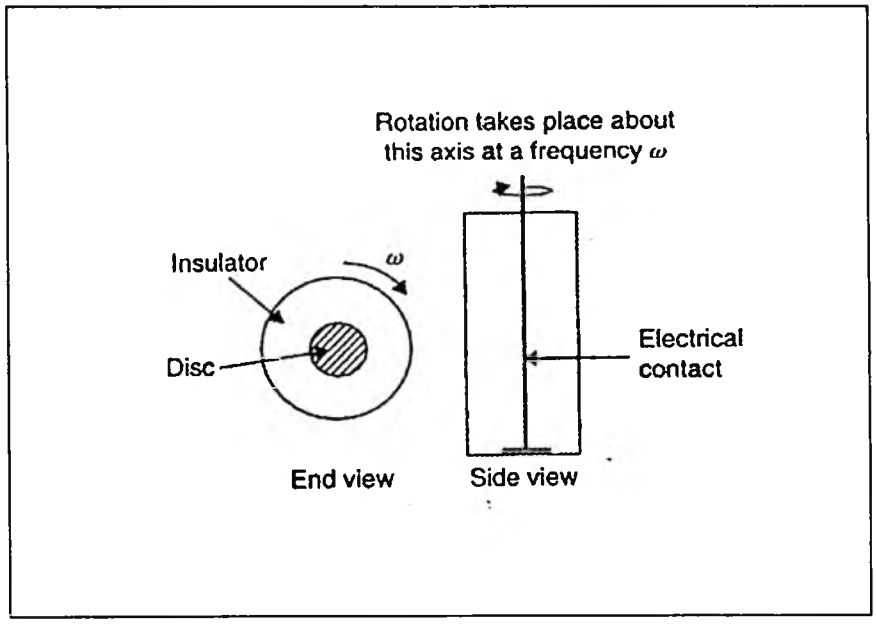

Figure 2.2 The schematic diagram of rotating disk electrode

This electrode is a flat circular disk electrode. The conducting disk is embedded centrally in a rod of insulating materials such as Teflon, epoxy resin, or other plastics. At one flat end of an insulating material, an electrical contact is connected behind the face of the electrode. The potential of the electrode is controlled by the connection between the potentiostat and the electrical contact. The rotating disk electrode (RDE) is rotated at a fixed and known frequency about its central axis. Both the auxiliary and reference electrodes are kept stationary. The RDE is immersed

in the solution of the analyte during the measurement. In order to minimize the eddy currents, the face of the RDE should immerse below the surface of the solution at least 10 mm. The RDE should be 30 or 40 mm above the floor of the electrochemical cell in order to maintain a good and reproducible flow of the solution over the surface of the RDE. The rotational motion set up the well-defined flow pattern of solution toward the surface of the rotating disk electrode. Figure 2.3 shows the schematic diagram of the liquid flow from the bulk solution and toward the surface of the RDE.

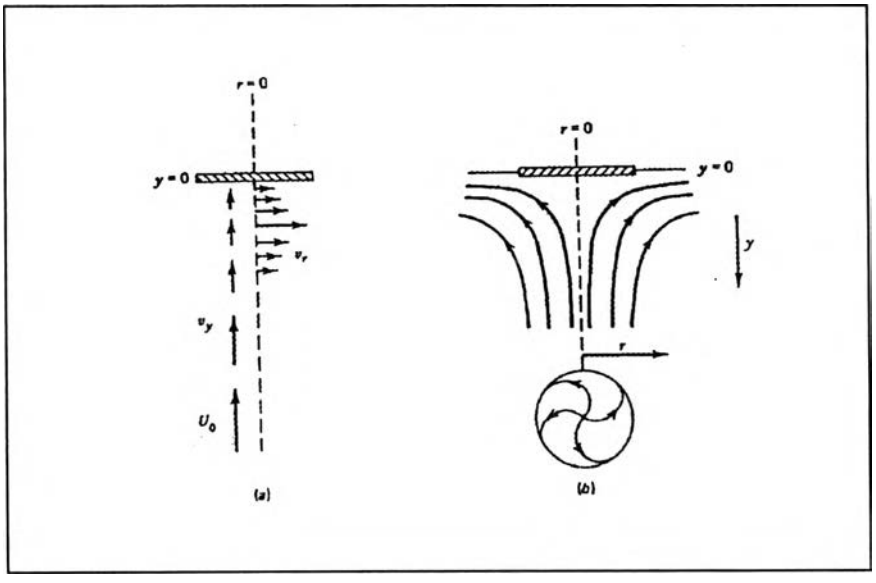

Figure 2.3 Variation of i with $\omega^{1/2}$ at RDE for electrode reaction

During rotation, the solution is radially moved away the face of the RDE. This movement caused the drawing of the bulk solution toward the electrode surface and then backward into the bulk solution. The current is measured as a function of the applied potential and the rotation speed. The relationship between the current at the RDE and the concentration of analyte is expressed by the Levich equation, as follows:

$$i = 0.602nFA\,D^{2/3}\,\nu^{-1/2}\,\omega^{1/2}\,C$$

where i : current response (ampere)

F: Faraday constant (96,484.6 C equiv^{-1}.)

A: area of electrode (cm^2)

D: diffusion coefficient (cm^2 s^{-1})

ν: kinematic viscosity of the liquid (cm^2 s^{-1})

ω: angular velocity of the disk ($\omega = 2\pi N$, N = revolution per second)

C: solution concentration (mol dm$^{3-1}$)

The plot of the current (as 'y') against the square root of the rotation speed (as 'x') is the Levich plot. This plot is used to indicate the relationship between the type of solution flow and the rotation speed. From the Levich plot displayed in Figure 2.4, the laminar flow is indicated by the linear part of the plot at the lower rotation speed, whereas the turbulent flow is indicated by the non-linear portion at the higher rotation speed values. The deviations from the Levich line are caused by the insufficient analyte brought to the electrode surface per unit time. In order to obtain the good quality analytical results, the rotation speeds that produced the turbulent flow must be avoided. The Levich equation is applied to the totally mass transfer controlled condition at the RDE. It is also used to predict that the current is proportional to the analyte concentration and rotation speed. The linearly plot of the current with the square root of the rotation speed is the characteristic of a fast heterogeneous kinetics. Thus, these responses are under the control of the convective-diffusion mass transport.

The mass transportation of reactant to the electrode surface consists of three sources; migration, convection, and diffusion mass transport. The overall extent of mass transport is defined as the flux of the electroactive species to an electrode.

Migration mass transport is the movement of the charged species (cations or anions) to the negatively charged (cathode) or positively charged (anode) electrode. The effects of this mass transport may be neglected by using the supporting electrolyte which contains an excess of unreactive ionic salt. It usually means that the concentration of an electrolyte is about 100 times greater than the concentration of the analyte.

Convective mass transport is the movement of the solution relative to the face of an electrode. There are two extremes of the convection mass transport as follow:

(i) movement of an electrode through the quiescent solution;

(ii) movement of solution passed a stationary electrode

The convection mass transport is the principal mode of mass transport for rotating disk voltammetry. The most effective form of mass transport is convection mass transport because of the very thin of the diffusion layer near the electrode surface. This mass transport can be eliminated by keeping the solution quiet.

Diffusion mass transport is the most important of mass transport process. This mass transport is contributed by the concentration gradient of the electroanalyte around the electrode. The diffusion will be present even in the absence of migration and convection mass transport. In rotating disk voltammetry, the diffusion also occurs at the vicinity of the electrode surface.

A deviation of this plot from a straight line that intersects the origin suggests some kinetic step involved in the electron transfer reaction. The equations derived for the RDE will not apply at very low or very high values of rotation speed. The upper limit of rotation speed is controlled by the onset of turbulent flow. The turbulent flow can occur at lower values of rotation speed when the electrode surface is not perfectly polished, when there are small bends or eccentricities in the RDE shafts, or when the

walls of the electrochemical cell are too close to the electrode surface. In practice, the maximum rotation rates are frequently set at 10,000 r.p.m. or at $\omega \approx 1{,}000\ sec^{-1}$. In most RDE studies, the ranges of ω and frequencies are given by: $10\ sec^{-1} < \omega < 1{,}000\ sec^{-1}$ or 100 r.p.m.< frequency <10,000 r.p.m.

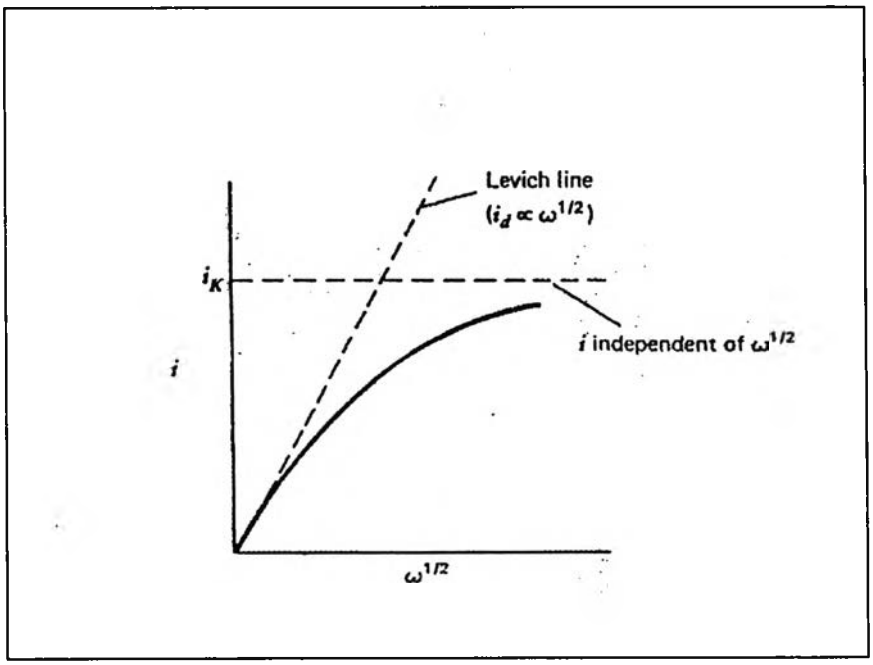

Figure 2.4 Variation of i with $\omega^{1/2}$ at RDE for electrode reaction

2.1.3 Amperometry [14, 15, 16]

Amperometry is a one of the controlled-potential technique. It is normally carried out in stirred or flowing solutions or at a rotating disk 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 are underwent an oxidation or reduction at the electrode. The resultant amperometeric signal is directly proportion to the concentration of the analyte. The amperometric current is a function of the number of the molecules or ions that have been removed by the reaction at the electrode.

2.1.4 Pulsed amperometric detection (PAD) [17, 18, 19, 20]

Pulsed amperometric detection (PAD) is the amperometric detection under the control of a multistep potential-time waveform. The typical waveform for PAD is the triple step waveform. This waveform consists of three potential steps, which are detection, cleaning, and reactivation steps. In detection step, the oxidation reaction of the electroactive species of analyte interest is occurred. The potential applied in this step is called a detection potential (E_{det}). The time duration for the application of this potential is called a detection time (t_{det}). This time duration consists of two timing parameters, namely, delay time (t_{del}) and integration time (t_{int}). The delay time is necessary to overcome the double-layer charging currents. The current response is sampled during a short integration time period after a delay of delay time. The adsorption of the adsorbed detection products and/or solution impurities is occurred in the step of detection. These adsorbed species are necessary desorbed from the electrode surface before the next detection process. The desorption process is performed at the oxidation step (cleaning step). In cleaning step, the electrode

potential is set at the potential value more positive than the detection potential. The timing parameter for the application of this potential is oxidation time, t_{oxd}. Besides the removal of the adsorbed molecule from the electrode surface, the oxide layer is also developed in this cleaning step. This oxide layer covering the electrode surface can be deactivated the electrode activity. Thus, the electrode activity must be regenerated by a subsequent negative potential to reduction potential, E_{red} for a duration of reduction time, t_{red}. This last step is called the reactivation step. In the last step, the oxide film is dissolved from the electrode surface and the active surface is ready for the next cycle of PAD waveform. The typical PAD waveform is shown in Figure 2.5. From the schematic diagram of PAD waveform displayed in Figure 2.5, the PAD waveform parameters can be divided into two categories; potential and timing parameters. The overall PAD waveform parameters are E_{det}, t_{det} (t_{del} and t_{int}), E_{oxd}, t_{oxd}, E_{red}, and t_{red}. Therefore, there are seven waveform parameters which the optimization must be performed.

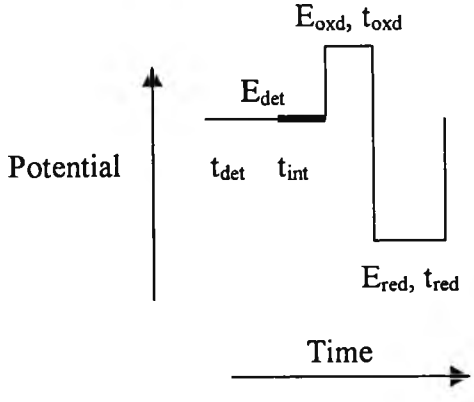

Figure 2.5 Typical PAD waveform

The components of a typical FIA apparatus consist of solution reservoir, pump, injector, detector, and recorder. The most commonly pump used in FIA is peristaltic pump. The speed of this pump can be varied to obtain the optimal flow rate. Thus, the solution flow rate is controlled by the pump speed and the internal diameter of the used tube. There are several injection techniques including volume-based injection, split and nested sample loops, hydrodynamic injection, and time-based injection. The volumetric-based FIA injection is the oldest injection technique. In this technique, the sample solution is introduced into the carrier stream by the insertion of the syringe through the injector. The injection volume is controlled by the sample loop. The injection valve for the volumetric injection is shown in Figure 2.7.

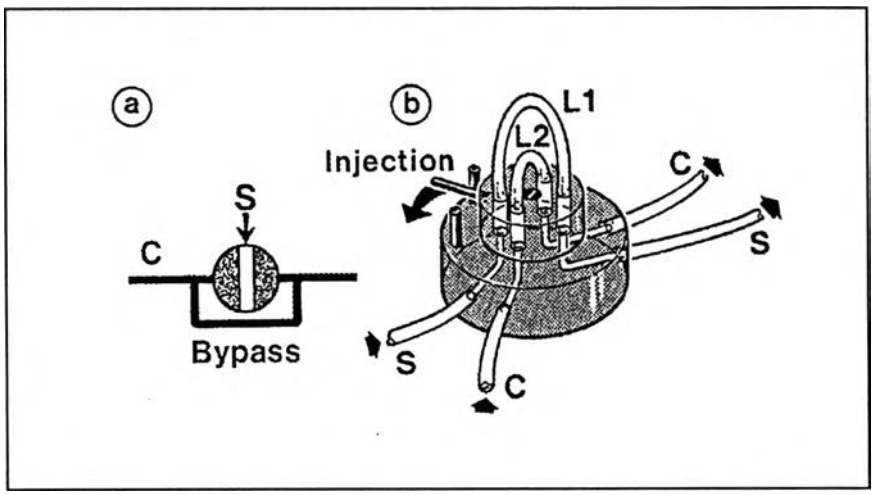

Figure 2.7 (a) schematic drawing of FIA rotary injection valve. (b) four-port rotary injection valve. S: sample, C: carrier stream, L1: sample loop, L2: by-pass loop

The valve has two positions, the sampling and injection positions. The sample is introduced into the sample loop (L1) while the carrier stream (C) is shunted via a by-pass loop (L2) in order to prevent the set up of back pressure. When the sample loop is filled with sample, the valve is rapidly turned to the injection position and left

2.2 Flow Injection Analysis [21]

In 1975, the concept of flow injection analysis (FIA) is 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. At the detection part, the analyte of interest is detected and then 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. A typical recorder output has the form of a peak as shown in Figure 2.6. The peak height, peak area, or peak width is related to the concentration of the analyte.

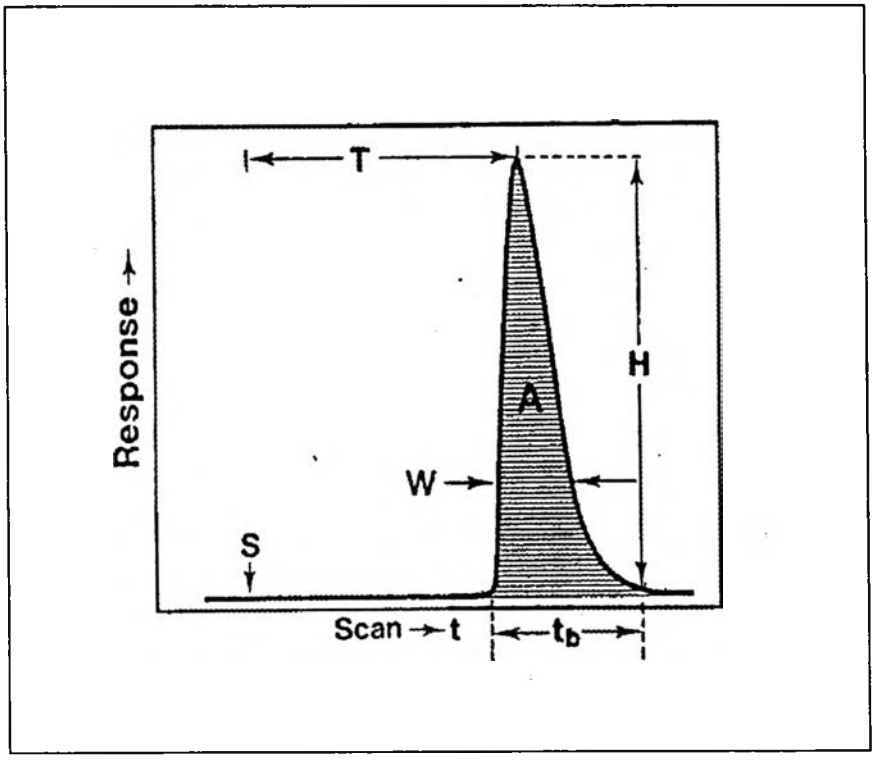

Figure 2.6 The recorder output in the form of peak. S: time of injection, W: peak width, A: peak area, H: peak height, T: residence time

there until the sample is completely swept by the carrier stream out of the sample loop.

In the split and nested sample loops injection, the sample loop is split or nested in order to perform more sophisticated functions. The nested loop and split loop injections are shown in Figure 2.8.

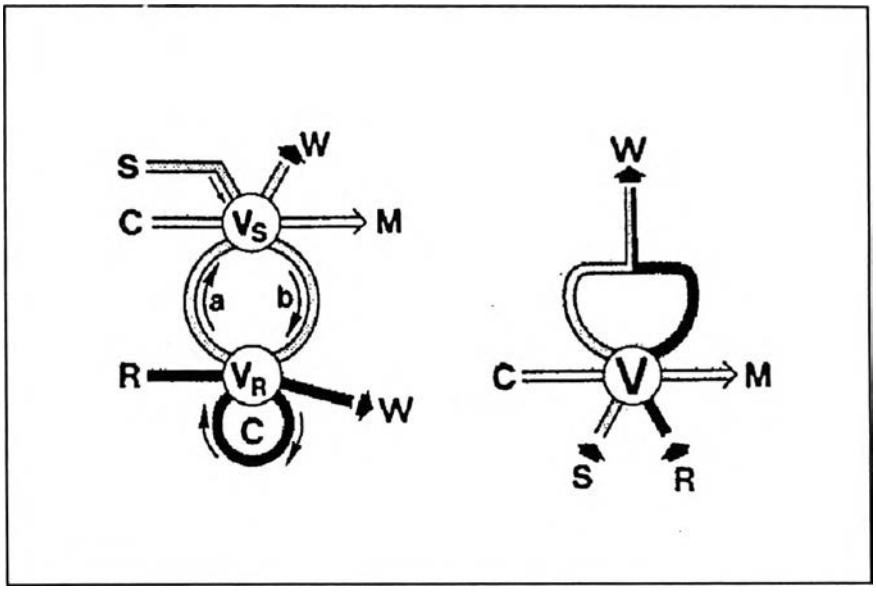

Figure 2.8 Nested loop injection (left) and split loop injection (right)

C: carrier stream, V: rotary valve (V_S: sample valve and V_R: reagent valve), S: sample loop, R: reagent loop, W: waste, M: manifold

In order to obtain simultaneous injection of the analyte and reagent, the nesting loop and the splitting loop are suggested. The another loop is nested by mean of a second valve in the way as shown in Figure 2.8 (left). The two-valve system is used to entrap a zone of reagent between the two zones of sample (a and b). The merging zones are propelled into the manifold by the same pump tube. This technique yields a sample-reagent-sample or a reagent-sample-reagent sequence. The advantage of this injection technique is a very high volumetric and merging precision.

In splitting loop, the external loop is divided into two sections accommodating sample and reagent solutions, which upon injection propel and merge into each other. The resulting sequence is sample- (sample + reagent) or sample-reagent. As shown below, in the load position the two sections of the split loop are filled with sample and reagent via a common outlet. When the valve is turned, the two zones are swept down the manifold, propelling and gradually penetrating into each other.

The hydrodynamic injection employs the hydrodynamic force to insert the well-defined sample zone into the carrier stream. The movement of sample and carrier solutions is controlled by a peristaltic pump. The manifold for hydrodynamic injection is shown in Figure 2.9.

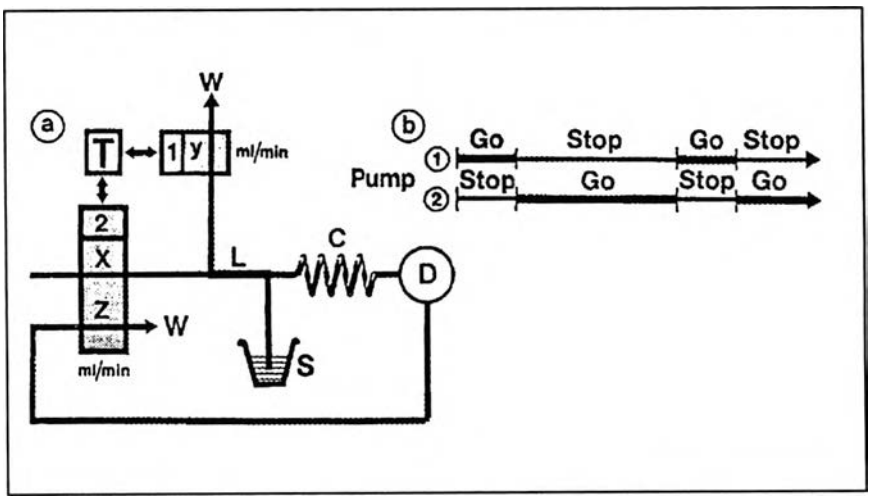

Figure 2.9 (a) FIA manifold for hydrodynamic injection (b) time sequence of the pump events. T: timer, L: sample conduit, C: reactor, D: detector, S: sample reservoir, W: waste, x, y, z: pumping rate (ml min^{-1})

In order to control the movement of sample and carrier solutions, the two peristaltic pumps are used. When both peristaltic pumps operated, if pumping rates x and y is equal and if the pump follow the sequence indicated in Figure 2.9b, the sample conduit (L) will be filled by the solution from the sample reservoir (S). When pump 1 is stopped and pump 2 continuously operates, the carrier stream will carry the exactly metered volume of sample from conduit toward the detector (T).

In the time-based injection procedure, the sample volume is measured as a function of time. Thus, the injected sample volume is controlled by the selected time interval. The sample solution is aspirated into the carrier stream by the action of the pump which installed after the detection part. The manifold for time-based injection is shown in Figure 2.10.

From this Figure, a system comprises two pumps (1 and 2) pumping at volumetric rates x and y, where $x > y$. In the standby position (a), both pumps are operated and the carrier solution is propelled toward the manifold. The excess solution is expelled via channel (L). For the sampling step (b), the sample reservoir is contacted to the inlet channel and then pump 1 is stopped while the operation of pump 2 is maintained. In this step, a defined zone of sample solution is aspirated into the manifold. In the injection step, the sample reservoir is removed and pump 1 is restarted. Thereby the carrier stream forces the sample zone through the FIA manifold and into the detector.

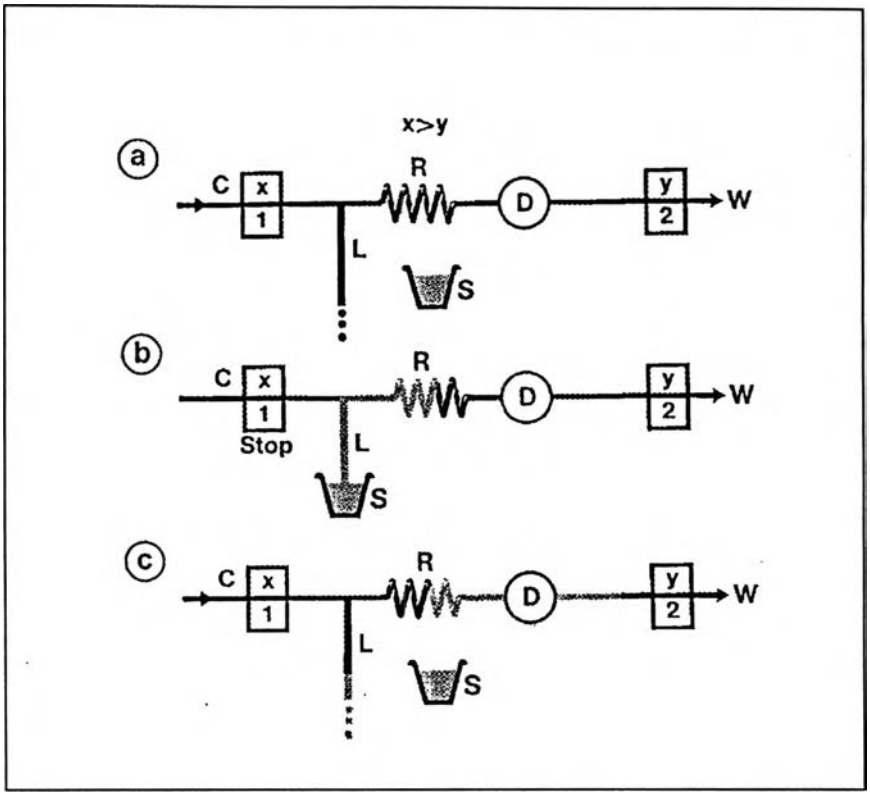

Figure 2.10 Time-base injection FIA system. (a) standby position, (b) sampling step, and (c) injection step. C: carrier stream, L: inlet channel, R: reactor, S: sample reservoir, D: detector, W: waste, x and y: pumping rates (ml min^{-1})

The conventional detection techniques in HPLC such as spectrophotometric, fluorimetric, chemiluminometric, atomic absorption, flame photometric, inductively coupled plasma spectroscopic (ICP) and electrochemical detection can be used for the detection part of 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.

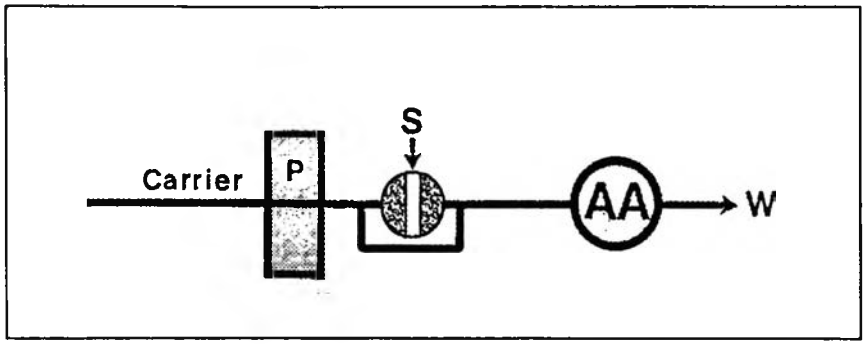

Figure 2.11 Single-line FIA manifold. P: pump, S: sample, AA: atomic absorption spectrometry (detector), W: waste

If there are two or several reagent solutions, the two-line or multiline FIA manifolds are employed. The homogeneous mixing of sample and reagents is performed at the reaction coil. Now, the two-line and multiline manifolds are commonly placed for FIA methods. The example of the multiline FIA manifold is shown in Figure 2.12.

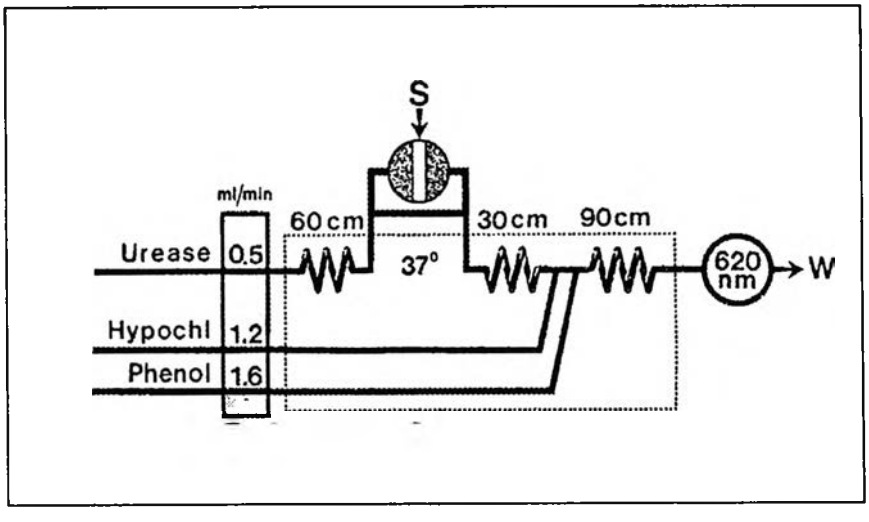

Figure 2.12 The multiline FIA manifold for determination of urea in serum

2.3 Gold electrode [22]

Glod atom has an d^{10}-electronic configuration. Gold electrode is a noble-metal electrode, which is inert under the typical amphoteric conditions. The electrochemical profiles of gold electrode is quite active. Figure 2.13 shows the cyclic voltammetric response (i-E) for an Au RDE in 0.1 M NaOH with (....) and without (__) dissolved oxygen. The formation of surface oxide occurs in the positive scan providing the anodic signal (wave a, *ca.* + 150 to +700 mV vs. Ag/AgCl). The evolution of oxygen is observed at *ca.* above +700 mV vs. Ag/AgCl corresponding to the discharge of water to produce oxygen. The cathodic peak signal for the negative scan corresponds to the surface oxide reduction (wave c, *ca.* +300 to –100 mV vs. Ag/AgCl). The reduction of dissolved oxygen during positive and negative scans is observed at *ca.* less than –50 mV vs. Ag/AgCl (wave d). The electrochemical equilibria of gold in aqueous media is proposed by Pourbaix as follows:

$$2Au + 3H_2O \rightleftharpoons Au_2O_3 + 6H^+ + 6e^- \quad \text{--------- (1)}$$

$$Au_2O_3 + H_2O \rightleftharpoons 2AuO_2 + 2H^+ + 2e^- \quad \text{--------- (2)}$$

The hydrate oxide, Au_2O_3, is considered as hydrate gold oxide or hydroxide, $Au(OH)_3$. The gold (I) oxide, Au_2O, is considered as gold (I) hydroxide, AuOH. The gold peroxide, AuO_2, is unstable and decomposed to form Au_2O_3 and oxygen. In acid solution, the formation of oxide monolayer is occurred at potential more positive than the one in basic solution. In addition, the evolution of oxygen gas in acid case is also occurred at more positive potential than in the case of basic solution. During negative potential scan, the sharp cathodic peak of oxide reduction is obtained.

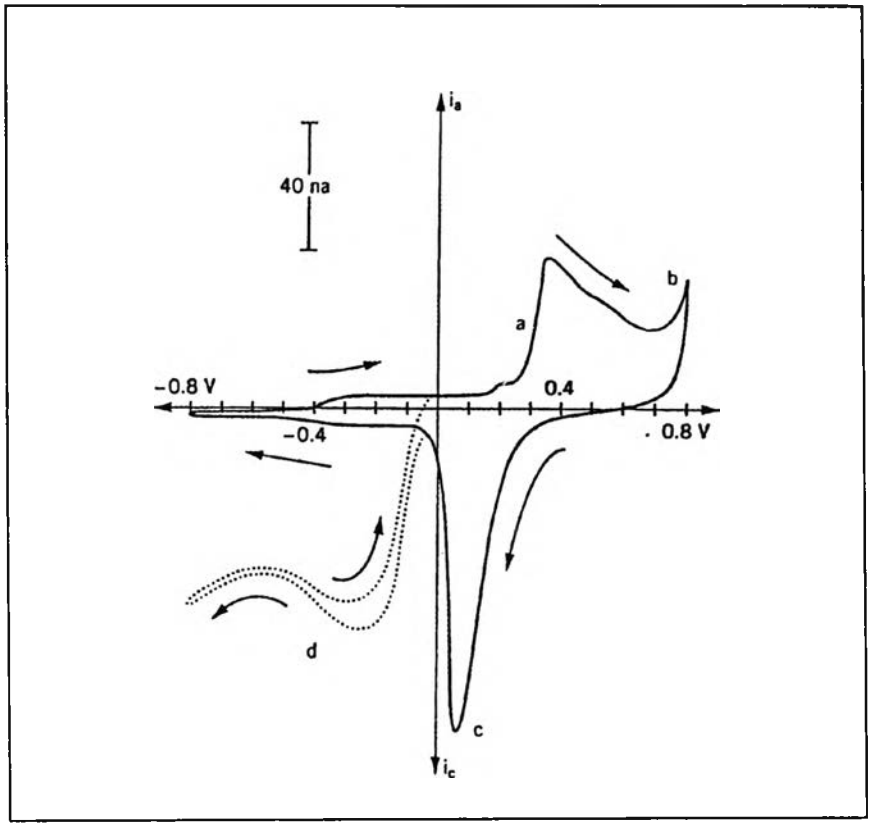

Figure 2.13 Rotating disk cyclic voltammetric response for a Au RDE in 0.1 M NaOH solution. Condition: electrode rotation speed 900 r. p. m.; scan rate 200 mV s^{-1}; Ag/AgCl reference electrode. Solution: (.....) 0.1 M NaOH; (___) deaerated 0.1 M NaOH.

2.4 Literature surveys

Tetracycline antibiotics are the group of the widely used drugs for the treatment of various infectious diseases. A wide range of applications in therapeutics may be due to the highly effective against a number of gram-positive and gram-negative bacteria. From these applications, they are considered as the broad-spectrum antibiotic drugs.

2.4.1 Chlortetracycline

In 1948, the first member of the tetracycline antibiotics, chlortetracycline or 7-chloro-4-(dimethylamino)-1, 4, 4a, 5, 5a, 6, 11, 12a-octahydro-3, 6, 10, 12, 12a-pentahydroxy-6-methyl-1, 11-dioxo-2-naphthacene-carboxamide, was discovered by Benjamin M. Dugger. This antibiotic drug was used to treat the typical symptoms such as ear, nose, and throat infections. Moreover, this drug also treats various infections including acute and chronic bronchitis, pneumonia, gastro-intestinal infections, amoebic hepatitis, urinary tract infections, soft tissue infections, venereal diseases, typhus fever, and acne. However, the adverse reaction can be occurred during long term use of the drug. The typical occurrence is the permanent discoloration of the teeth (yellow-gray-brown). This symptom occurred due to the used of this drug during tooth development (last half of the pregnancy, infancy, and children to the age of 8 years). The side effects of the usage of this drug are gastro-intestinal disturbances, stomatitis, vaginitis, glossitis, staphylococcal enterocolitis, headache, blurred vision, and enamel hypoplasia. The structure of chlortetracycline hydrochloride is shown in Figure 2.14.

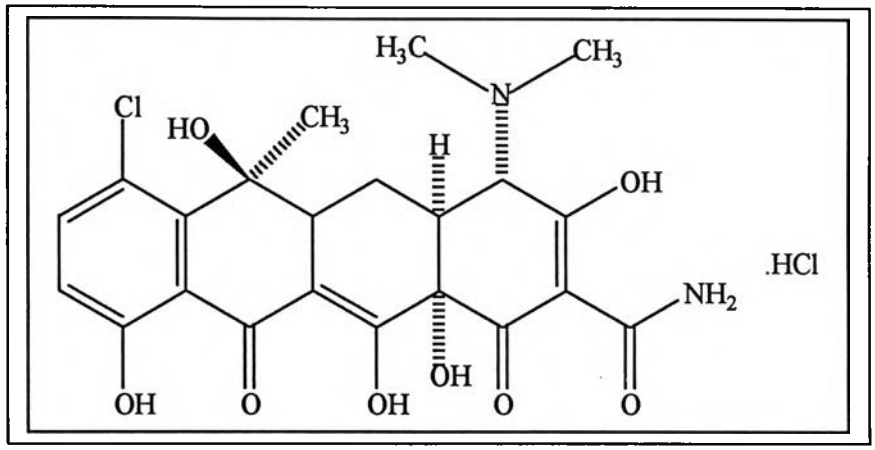

Figure 2.14 Structure of chlortetracycline hydrochloride

Various methods have been proposed for the determination of chlortetracycline. In 1992, Sultan, et al. [23] used flow injection analysis with spectrophotometry for the determination of chlortetracycline in drug formulations. This method is based on the oxidation of chlortetracycline with ammonium iron (III) sulfate. In 1995, McCracken and co-workers [24] determined chlortetracycline in animal tissues using liquid chromatography with fluorescence detection. The post column derivatization process with aluminium chloride is required for this method. Oungpipat, et al. [25] reported that flow injection analysis with amperometry could be used to detect chlortetracycline by electrocatalytic oxidation at a nickel-modified glassy carbon electrode. In 1997, Houglum and Larson [26] analyzed chlortetracycline animal feed by liquid chromatography with fluorescence detection. Webster, et al. [27] investigated the effect of the interference on the microbiological determination of chlortetracycline in feed grade and premixed sample. These investigations confirmed that the result did not affect by the low level of the interference. In 1998, Couto, et al. [28] proposed the flow injection analysis with potentiometry to determine chlortetracycline. This proposed method is based on the

monitoring of the complexation between chlortetracycline and Cu (II). In 2000, Zurhelle and co-workers [29] presented a method for the confirmation of chlortetracycline in egg white, egg yolk, and hen plasma by high performance liquid chromatography (HPLC) with mass spectrometry. In 2001, Zheng, et al. [30] used flow injection analysis with chemiluminescence for the chlortetracycline determination. The chemiluminescent reaction of chlortetracycline with bromine which electrogenerated by hydrogen peroxide is required. Feng, et al. [31] proposed the total internal reflected resonance light scattering for the determination of chlortetracycline in body fluid. The formation of chlortetracycline-europium-trioctyl phosphine oxide at the water/tetrachloromethane interface is required.

Mass spectrometry offers high sensitivity, but its limitations are the requirement of special skills and high instrumental price. The determinations of chlortetracycline by the microbiological methods are commonly used, but they are complicated, time consuming, lack of specific and sensitivity, expensive, and tedious. In order to overcome the drawbacks described above, the determination method providing cheap, fast, simple, and high sensitivity is required. Flow injection analysis with electrochemical method is therefore introduced to maximize the sensitivity and to optimize the sample throughput and economy of reagents.

2.4.2 Tetracycline

Tetracycline or 4-dimethylamino-1, 4, 4a, 5, 5a, 6, 11, 12a-octahydro-3, 6, 10, 12, 12a-pentahydroxy-6-methyl-1, 11-dioxo-2-naphthacenecarboxamide is a bacteriostatic antibiotic with a broad spectrum of activity against bacteria properties. It is used for many different infections such as respiratory tract infection, nongonococcal urethritis, pneumonia, hemophilus influenza, anthrax, syphilis, and

severe acne. It also has a role in the treatment of multidrug resistant malaria. The side effects of the usage of this drug are nausea, vomiting, diarrhea, temporary blurring of vision or stinging following administration, oral candidiasis, glossitis, staining of tongue, throat irritation, gastrointestinal disturbances, renal dysfunction, hepatotoxicity, and abdominal pain. The structure of tetracycline hydrochloride is shown in Figure 2.15.

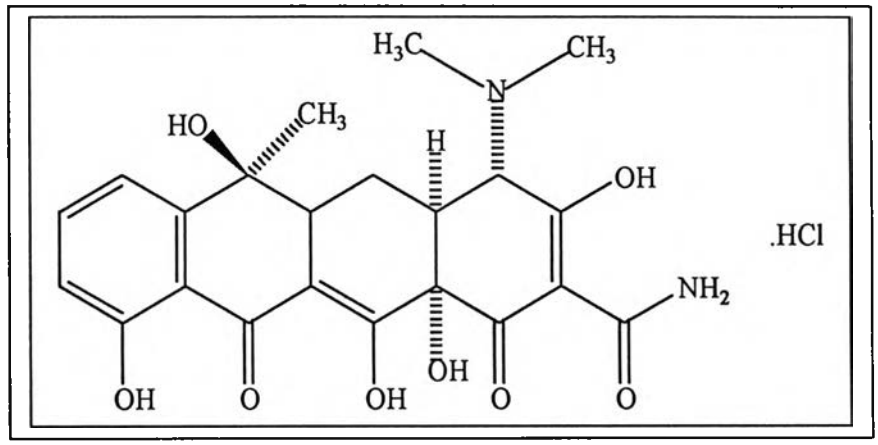

Figure 2.15 Structure of tetracycline hydrochloride

Various methods are employed for identification and determination of tetracycline. In 1979, Jochsberger, et al. [32] used differential pulse poralography for the determination of tetracycline. The complexation of tetracycline with cations is required. In 1986, Sultan [33] determined tetracycline by the spectrophotometric method that based on the derivatization with sodium molybdate. In 1988, Ji and Wang [34] used flow injection analysis with amperometry for the determination of tetracycline that based on ion transfer across a water-solidified nitrobenzene interface. In 1994, Oka and co-workers [35] proposed the method for the identification of tetracycline in honey by frit FAB/LC/MS using a volatile mobile phase. Izquierdo, et al. [36] determined tetracycline in serum by the stopped-flow technique with

fluorescence detection. The requirement of the complexation of tetracycline with europium (III) is presented. In 1996, Tanase, et al. [37] used alternating current poralography for the analysis of tetracycline. In 1998, Savage and co-workers [38] combined fluorescence and immunoaffinity for the detection of tetracyclinein milk. Wang et al. [39] used fluorescence optical fiber sensor for tetracycline determination. In 1999, Han et al. [40] determined tetracycline in pharmaceutical formulation by chemiluminescence method. Potassium permanganate and tris (2,2′-bipyridine) ruthenium (II) are used for the derivatization of this compound before chemiluminescence detection. In 2000, Kurittu, et al. [41] used microbiological method for the determination of tetracycline in raw milk. In 2001, Lindsey, et al. [42] determined tetracycline in ground water and surface water by liquid chromatography with mass spectrometry.

The microbiological assays are commonly used as the official method, but they are complicate and time consuming. The electrochemical methods are more interest than the other due to the high sensitivity. In order to obtain the specific determination of tetracycline in the contaminants, the optical fiber sensor is proposed. Polarographic methods offer high sensitivity but their drawback is the use of mercury, which is a toxic substance and may be undesirable for many practical applications, including flow injection. The same as chlortetracycline, potentiometry is used for detection of this compound by using the modified electrode. Although voltammetry and amperometry also offer high sensitivity, their major disadvantage is deposition of detection products and/or solution impurities on electrode surface. Thus mechanical polishing which is known to temporarily alter the response of a working electrode, often requiring 60-90 min before a stable baseline, is used to reactivate electrode surface [43].

2.4.3 Doxycycline

Doxycycline or α-6-Doexy-5-oxytetracycline is one of the newer tetracyclines. Doxycycline is a drug of choices in the treatment of lyme disease, brucellosis and several Rickettsial infections. It is also used to treat sexually transmitted diseases and a variety of bacterial infections of the respiratory and genitourinary tracts and soft tissue infections.

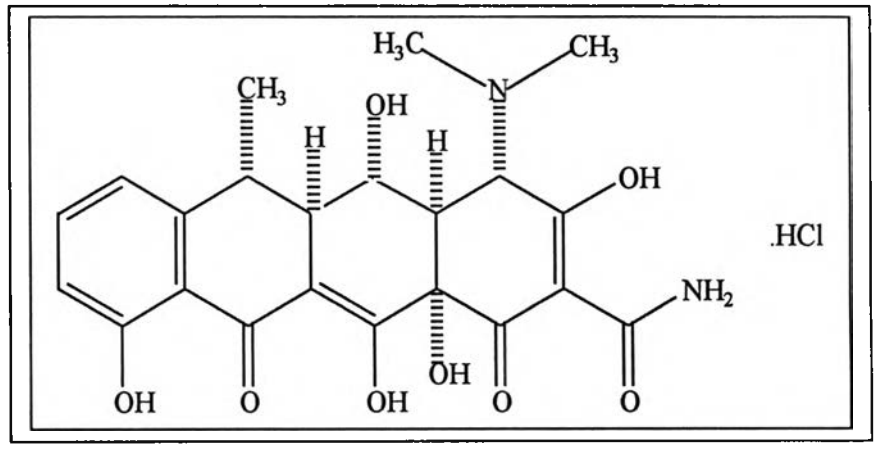

Figure 2.16 Structure of doxycycline hydrochloride

Many methods have been suggested for the determination of doxycycline. In 1977, Olliff and Chatten [44] used alternating current poralography for the determination of doxycycline. In 1980, Booker [45] determined doxycycline in biological samples by HPLC based on the derivatization process before spectrophotometric detection. In 1983, Bocker [46] identified doxycycline in mice, rats, and humans by HPLC with spectrophotometric detection. In 1989, Salinas, et al. [47] used derivative spectrophotometric detection for the determination of doxycycline in pharmaceutical formulations. In 1991, Naidong, et al. [48] isolated doxycycline from methacycline by preparative column liquid chromatography and confirmed the structure by nuclear magnetic resonance spectroscopy (NMR). In 1994,

Croubels and peteghem [49] used spectrophotometry for the determination of doxycycline residues in bovine milk. The method required a post column derivatization process. Karlicek and Solich [50] used flow injection analysis with spectrophotometry for the determination of doxycycline in pharmaceutical preparations. Its reaction with 4-aminophenazone and hexacyanoferrate (III) is required. In 1996, Santos, et al. validated the HPLC method for the determination of doxycycline in turkey plasma. In 1997, Oka, et al. [51] identified the residual doxycycline in bovine tissues by HPLC-tandem mass spectrometry. Kazemifard and Moore [52] used liquid chromatography with amperometry for the determination of doxycycline in pharmaceutical formulations. In 1998, Korpela, et al. [53] detected doxycycline *by Escherichia coli* sensor strain. Liu, et al. [54] used the optical fiber sensor for doxycycline determination. In 1999, Choma, et al. [55] used the simple thin layer chromatographic method (TLC) for the determination of doxycycline in milk. In 2000, Gil and co-workers developed the capillary electrophoresis for the analysis of doxycycline.

The drawbacks of the detection methods above based on high price instrumentation, complicate, and requirement of trained personnel. TLC is simple and cheap method, but its major disadvantage is the lack of sensitivity. The requirement of special skills and quite expensive are the limitations of mass spectrometry. The difficulties of microbiological assays are relatively slow and nonspecific. In the view of these disadvantages, the simple, fast, inexpensive, and sensitive determination methods are required for the doxycycline determination. Flow injection analysis has been proposed to achieve high sample throughput and shorter times. The electrochemical methods are the determination choices for doxycycline due to they are cheap, simple, and sensitive. Ampeormetric detection at glassy carbon electrode is

the alternative method for sensitive detection, but the adsorption of detection products or other species at the electrode surface are the major disadvantages. The mercury electrode used limits the polarographic method. The optical fiber sensor provides high sensitivity and good selectivity. However, the sensor technique requires long time for one measuring cycle and the complicate sensor preparation. In addition, the reproducibility of sensor preparation is difficult to obtain.