CHAPTER II

THEORY

2.1 Flow Analysis

Flow analysis is one of automated tools for the analysis of liquid samples, which were injected, processed and then delivered in flow media to detect the considered analytes. The flow analysis concept has generated the large number of publications in many languages since it was introduced by Ruzicka and Hansen in 1974 [23]. Due to the development of this concept, the flow analysis was classified into three generations [24, 25]: flow injection analysis (FIA), sequential injection analysis (SIA) and bead injection–lab-on-valve (BI-LOV).

2.1.1 Flow Injection Analysis

A typical manifold of flow injection analysis (FIA), the first generation, is shown in Figure 2.1 that consisted of a pump, an injection valve, a reaction coil, a flow-through detector and connecting tubing. The sample solution is injected through the injection valve into a continuously flowing stream. In fact, if reagent(s) are added via side-streams, the chemical reaction can occur at the interfaces between the sample and the reagent by the diffusion process along the distance of the reaction coil that increases the generation of the product. The segment of the product will result in a concentration gradient that can be obtained as transient signal by a suitable detector.

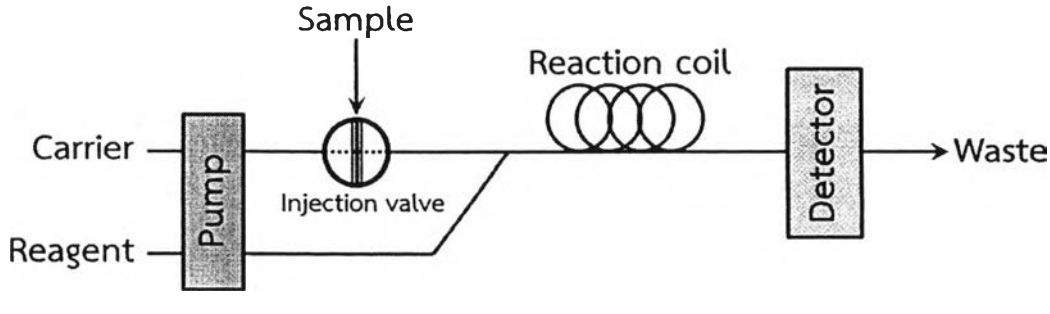

Figure 2.1 Typical manifold of flow injection analysis.

FIA presents many advantages. For example, any number of additional reagents can be added via side-stream, any type of detector can be used, and the FIA can work continuously. However, it might consume the carrier and reagents due to the continuous flow.

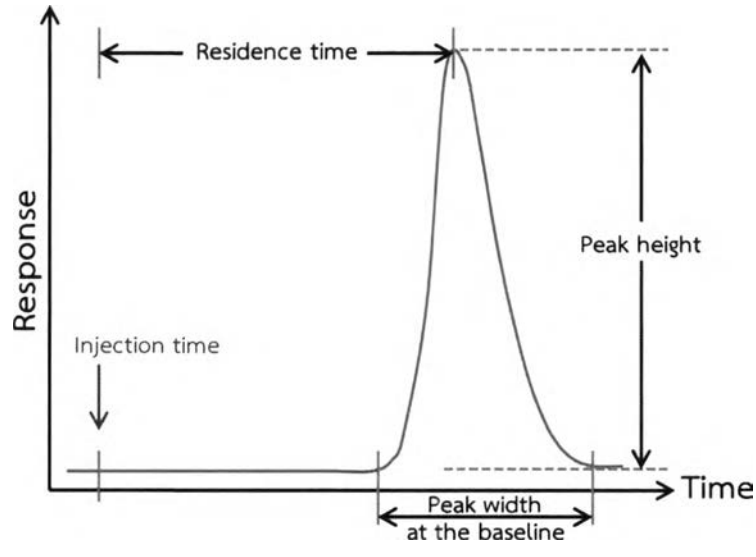

Figure 2.2 Transient signal of FIA output.

2.1.2 Sequential Injection Analysis

SIA it the 2nd generation of flow analysis which was introduced in the early 1990s by J. Ruzicka and G.D. Marshall from the University of Washington [26]. While FIA is based on using continuous flow by a uni-directional pump, SIA employs discontinuous flow using a bi-directional pump and a multi-position selection valve. Therefore, this variant of FIA drastically reduces the consumption of carrier and reagent solutions.

A diagram of a typical SIA manifold is presents in Figure 2.3. The heart of SIA system is a multi-port selection valve (shown as a 8-port valve), which there is a central communication channel inside that can be rotate to select each of the ports (number 1-8). A holding coil is a central communication line of the selection valve that is connected to a syringe pump. The sample and reagent solutions at the individual ports of selection valve are sequentially aspirated into the holding coil

where they are stacked the adjacent segment. After the central communication channel is addressed to the outlet port (here port number 2) and then the segments are propelled in the reverse-direction towards the detector, the sample and reagent solutions are thereby mixed to promote the chemical reaction. The detectable product is generated and propelled through the flow cell in which a suitable detector is integrated. In addition, a reaction coil might be added in the SIA system to give rise of diffusion process or to extend the reaction time.

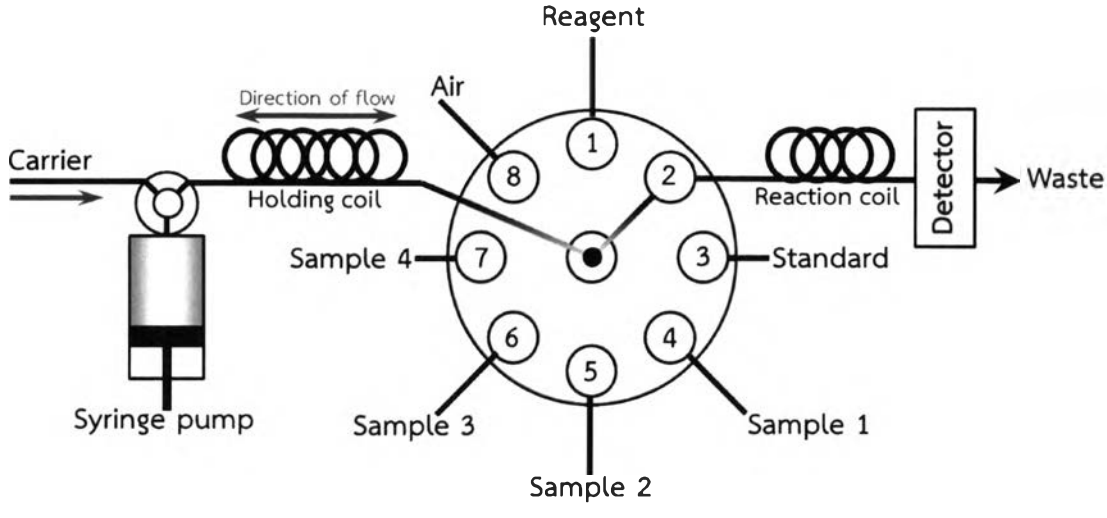

Figure 2.3 Typical manifold of sequential injection analysis.

The advantages of SIA are that it is an extremely economical system as a low-consumption of sample and reagents and reducing of waste generation, and that it can precisely and reproducibly handle the liquid solutions in a micro-scale level via the computer-controlled syringe pump. Furthermore, there are many published methods having proven that the SIA can be incorporated with sample treatment methods such as solid-phase extraction [27-30], separation on mini-columns [31-35], pH adjustment and even immunoassays [36-40].

2.1.3 Lab-on-Valve

A basic lab-on-valve (LOV), the 3rd generation of flow analysis, is illustrated in Figure 2.4. Although the LOV is consisted of many features like SIA system, the variant of LOV is that the selection valve of the LOV was designed by

placement of an integrated micro-channel inside it. Any required operation units for a considered assay are also incorporated with the selection valve; hence the developed device will act as a laboratory on a valve.

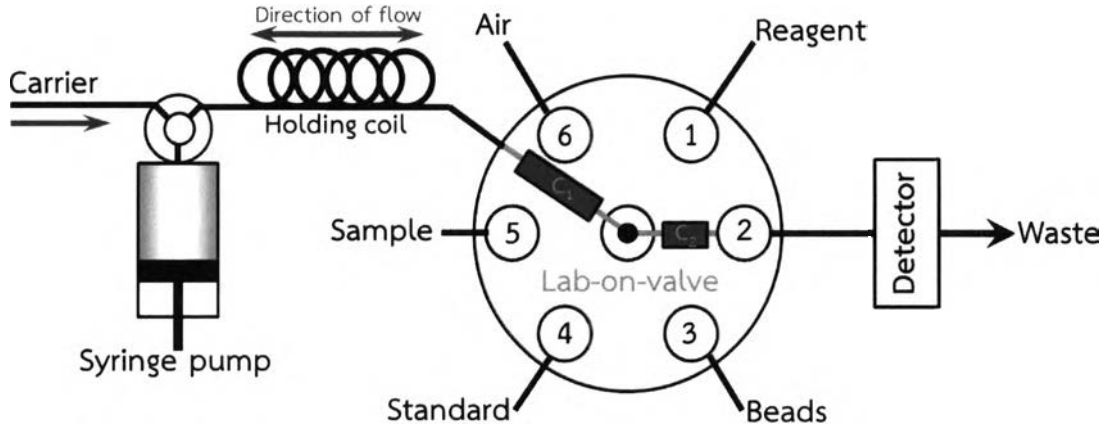

Figure 2.4 Typical manifold of Lab-on-valve.

The LOV is an example of the novel flow analysis, moreover, recently there are many publications reporting the hybrid flow analyses for the individual approach. These emphasize that the flow analysis is an important tool that can be continuously moved forward for the numerous approaches for liquid sample analysis due to its versatility.

2.2 Electrochemical Technique

Basically, electrochemistry can be classified as two techniques of potentiometric and potentiostatic techniques [41]. The minimum features required for the both techniques are a working electrode, a reference electrode and a sample solution containing the supporting electrolyte which encompass in the electrochemical cell. The working electrode responds to the target electrochemically active analytes, whilst the reference electrode is of constant potential. Two electrochemical cells are well-known as galvanic cell in which electrical energy is produced, and electrolytic cell in which the chemical reaction occurs when external electrical energy was applied.

Potentiometric technique relies on the use of a static electric circuit, in which the current is zero, to obtain the information of the sample composition by measuring the potential established across membrane. Potentiometric techniques have been widely used for direct detection of ionic species such as calcium ion, fluoride ion, potassium ion and even hydronium ion in the sample solutions.

In this technique, the potential is applied from an external source to drive the transfer of electrons in the electric circuit. The change of current in this dynamic circuit can be measured. The resulting current is related to the transfer of electrons during the redox reaction of the target analyte, hence, the concentration of the analyte can be calculated from the current response.

$$O + ne^- \rightleftharpoons R \qquad \text{(Equation 2.1)}$$

O and R are the oxidized and reduced forms of the redox couple, respectively. The redox reaction occurs in a potential range that makes the transfer of electrons thermodynamically kinetically favorable. In the case of thermodynamically controlled system, the observed potential depends linearly on the logarithm of the concentration of the electroactive specie at the electrode surface that represented by $C_O(0,t)$ and $C_R(0,t)$ according to the equation introduced by Walther Nernst:

$$E = E^0 + \frac{2.3RT}{nF} \log \frac{C_O(0,t)}{C_R(0,t)} \qquad \text{(Equation 2.2)}$$

where E^0 is the standard-state potential for the redox reaction. R is the universal gas constant (8.314 J K^{-1} mol^{-1}), T is the temperature in Kelvin, n is the number of electrons in the redox reaction, F is Faraday's contant (96,487 coulombs), and $C_O(0,t)/C_R(0,t)$ is the concentration quotient of the oxidized form (subscript O) and reduced form (subscript R) at the electrode surface at the individual time (t).

2.3 Electroanalytical Chemistry

Electroanalytical chemistry [42] is a branch of analytical chemistry that studies the interesting specie in the solution by using established electrochemical methods of potentiometry, voltammetry and coulometry. Additionally, electroanalytical chemistry also includes the studies of electrochemical reduction/oxidation processes

at the atomic or molecular level. An example device that has been successfully established by using an electrochemical method is the glucose biosensor [43-45] which uses an electrode immobilized with glucose oxidase to facilitate the glucose detection. Electrochemical technique is widely applied in the heavy metal detection, neurotransmitters detection and gas sensing using the unmodified or modified electrode. Furthermore, electrochemical methods can be integrated with luminescence spectroscopy as known in a field of electrochemiluminescence (luminescence emitted by stimulation from the electrochemical technique) that providing good selectivity and an extremely low detection limit [46].

In this Chapter, some potentiostatic techniques in terms of electroanalytical method used in this dissertation including cyclic voltammetry and anodic stripping voltammetry are described.

2.3.1 Voltammetry

Voltammetry, one of electroanalytical measurements, has been introduced since 1992 by Jaroslav Heyrovsky, who was awarded the Nobel Prize in Chemistry in 1959 [47]. The electrochemical cell is comprised of a three-electrode system (a working electrode, a reference electrode and a counter electrode) immersed in a sample solution containing an excess of a supporting electrolyte, which is a nonreactive electrolyte. A time-dependent potential (shown in Figure 2.5) is applied to a working electrode that changes its potential related to a constant potential of a reference electrode [48-50]. The resulting current that flows between the working electrode and a counter electrode is measured as a function of the applied potential. The observed signal is called voltammogram.

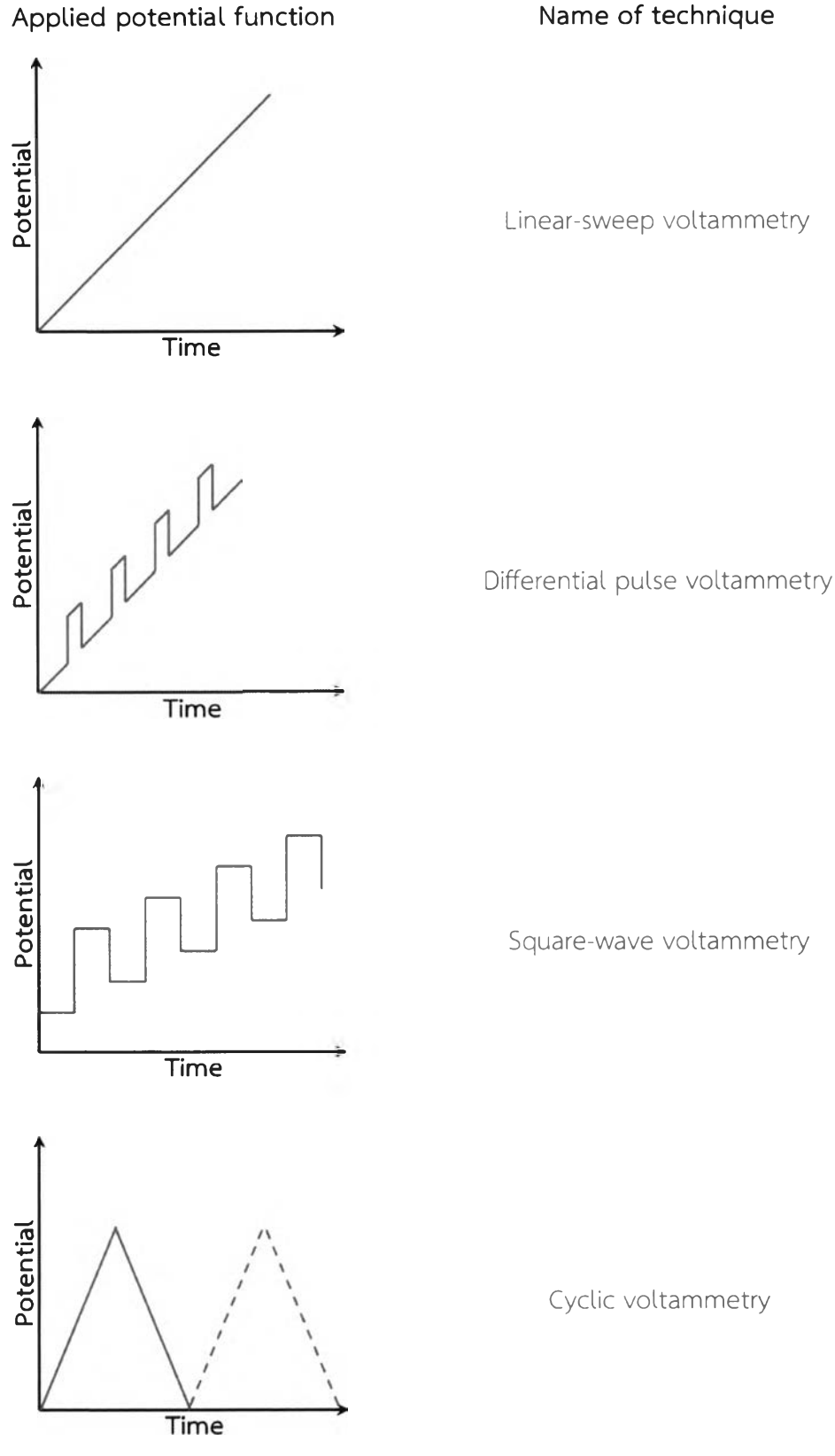

Figure 2.5 Time-dependent potential waveforms used in various voltammetry.

2.3.2 Cyclic Voltammetry

Cyclic voltammetry (CV) is the most widely used electrochemical technique for acquiring qualitative data of the redox reactions such as a number of intermediates, a number of reaction steps, and stability of the product from the electrochemical reaction, although CV is rarely used in quantitative method [49, 51]. Due to its ability to rapidly provide the significant qualitative information, CV is commonly used at the preliminary for the characterization of the developed electrochemical detection and for the study the redox reaction of the target analyte.

CV is based on the potential, which is in a linear function of time, applied through a stationary working electrode (immersed in an unstirred solution) in both forward direction and reverse direction as a triangular potential wave form (see Figure 2.5). In fact, the potential wave form can be applied as single cycle or multiple cycles, depending on of the interesting information. While the potential is being scanned, the resulting current is measured by a potentiostat. The plot of the measured current versus the applied potential is called cyclic voltammogram.

The expected voltamogram of a reversible redox couple during a single cycle of scanned potential is illustrated in Figure 2.6. This considered condition is assumed that there is only the oxidized form (O) contained in the initial solution. The potential is initially scanned in a negative-direction for the first half cycle, starting from a potential that any reduction does not occur. When the scanned potential approaches to the standard-state potential (E^0) for the redox process, the measured current increases in the cathodic region and then it is reached as a peak. During this step, the reduced form (R) is generated and accumulated near the electrode surface. After the scanned potential traverses commonly at least $90/n$ mV beyond the observed cathodic peak, the potential for another half cycle is swept in the reverse-direction. The R molecules from the first half cycle are oxidized back to O that it results as the anodic peak shown in the voltammogram.

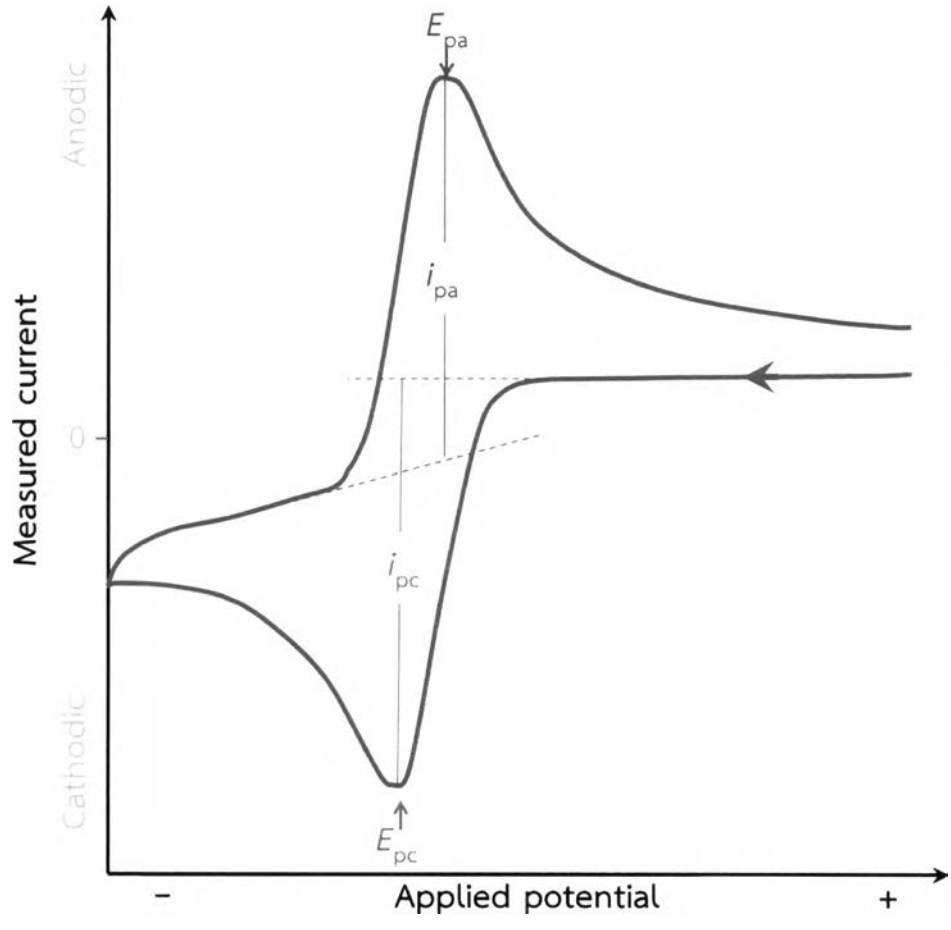

Figure 2.6 Typical cyclic voltammogram of a reversible redox couple.

i_{pa} and i_{pc} are an anodic peak current and a cathodic peak current, respectively, that are the maximum currents commonly measured at by baseline extrapolation as shown in Figure 2.6. The voltammogram can be characterized by an anodic peak potential (E_{pa}) and a cathodic peak potential (E_{pc}), at which the i_{pa} and i_{pc} are acquired, respectively. The peak current for a reversible reaction is described by Randles-Sevick equation [52]:

$$i_p = (2.69\times10^5)n^{3/2}ACD^{1/2}\nu^{1/2} \qquad \text{at 25 °C (Equation 2.3)}$$

where i_p is a peak potential (A), n is a number of transferring electrons in the redox process, A is an electrode area (cm^2), D is diffusion coefficient ($cm^2\ s^{-1}$), C is a concentration of the electroactive species (mol cm^{-3}) and ν is a potential scan rate ($V\ s^{-1}$)

According to Equation 2.3 based on the reversible process in the diffusion control, the i_p is directly proportional to the square root of the potential scan rate. Hence, a linear relation plot between i_p and $\nu^{1/2}$ could be used to prove that the electrochemical reaction on the electrode surface is controlled by the diffusion process [53-55]. In addition, the ratio between i_{pa} and i_{pc} is unity for a simple reversible process ($i_{pa} = i_{pc}$). The peak potential is related to the formal potential of the redox reaction. The formal potential for a reversible process (E^0) is centered between E_{pa} and E_{pc} as shown in following equation:

$$E^0 = \frac{(E_{pa}+E_{pc})}{2} \qquad \text{(Equation 2.4)}$$

Furthermore, the separation between E_{pa} and E_{pc} for a reversible process is given as ΔE_p in V unit by:

$$\Delta E_p = E_{pa} - E_{pc} = \frac{2.303RT}{nF} = \frac{0.059}{n} \qquad \text{(Equation 2.5)}$$

2.3.3 Square Wave Voltammetry

To improve sensitivity and speed of the voltammetry technique, pulse voltammetry techniques have been developed since 1960s such as a normal pulse voltammetry, differential pulse voltammetry and square wave voltammetry [56]. Pulse voltammetry is based on a measurement of sampling current on a potential waveform. Moreover, pulse voltammetry also presented the possibility to be a quantitative method.

Square wave voltammetry (SWV) is one of pulse voltammetry techniques, that the potential is applied as a time-function in a symmetrical square wave-wave form which is superimposed on a staircase; the information details are shown in Figure 2.7. The forward current (i_f) and reverse current (i_r) are sampled for each square wave cycle at the end of forward pulse and at the end of reverse pulse, respectively. The forward pulse drives a forward electrochemical reaction to generate the product which then will be back to the former specie that following the reverse reaction by the reverse pulse. The net current (i_{net}), calculated from the

difference between i_f and i_r, is plotted versus the applied potential from the base staircase potential value. Based on the theory, a plot of i_f, i_r and i_{net} for a reversible process is presented in Figure 2.8. The peak height of i_{net} is directly proportional to the concentration of the analyte.

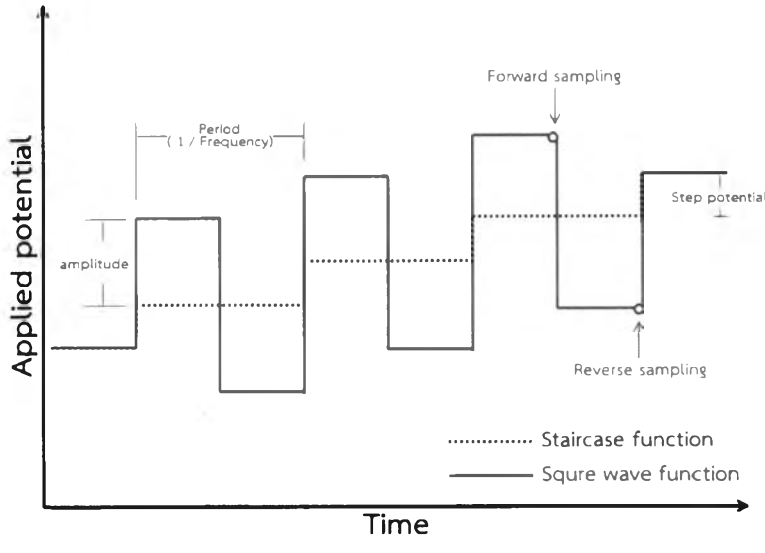

Figure 2.7 Waveform and measurement scheme for a square wave voltammetry.

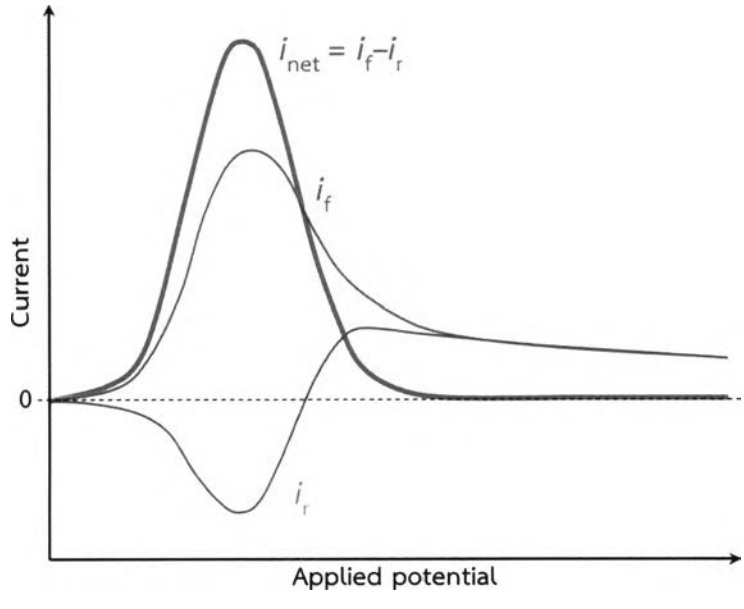

Figure 2.8 Theoretical forward current (i_f), reverse current (i_r) and net current (i_{net}) of a square wave voltammogram for a reversible process.

2.3.4 Stripping Voltammetry

Stripping voltammetry is the most popular electrochemical technique for determination of trace metals due to its extremely high sensitivity. Stripping voltammetry consists of two steps of a preconcentration step and a measurement step. First, preconcentration step or deposition step, the analyte in a sample solution is electrodeposited onto the electrode surface with a usually preconcentration factor of up to 1000. And then in measurement step, the result is carried out by voltammetry from the accumulated analytes. The limit of detection can be lowered by 2-3 orders of magnitude compared to using a direct measurement from the solution phase [57]. In fact, the different type of voltammetry can be employed, depending upon the nature of the preconcentration step and measurement step.

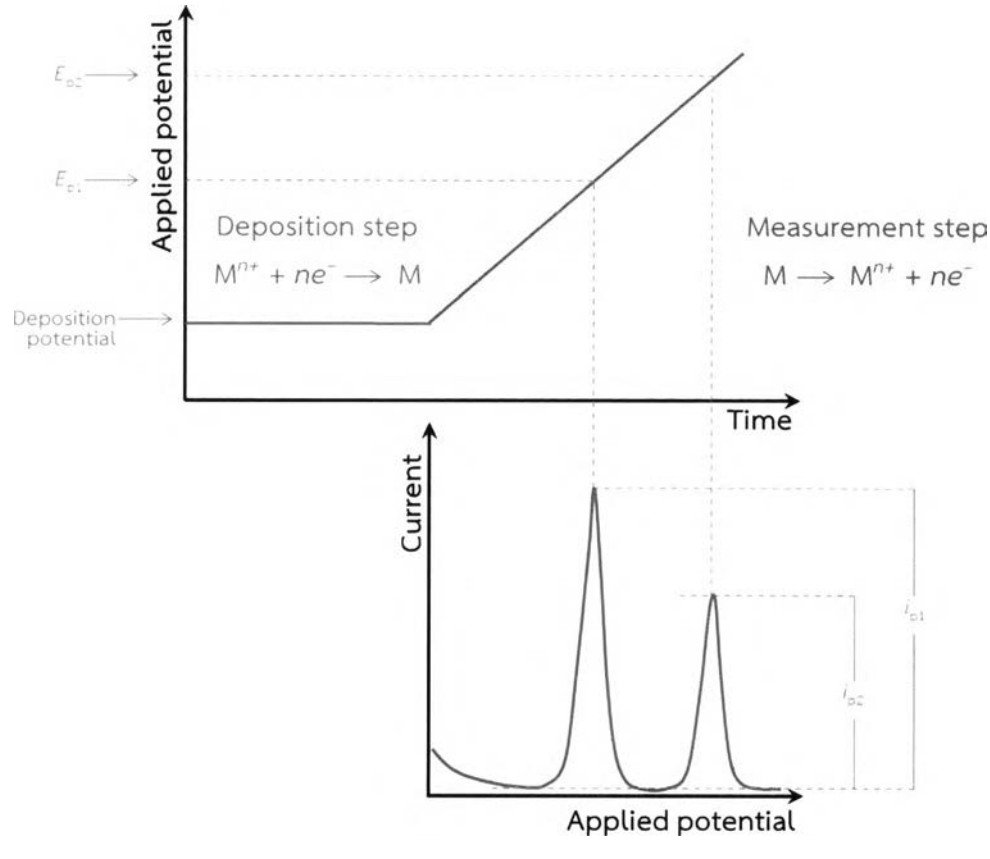

Figure 2.9 Summary diagram of anodic stripping voltammetry for determination of metals: the potential-time waveform (top), along with the typical square wave voltammogram (bottom)

To determination of trace metals, metal ions can be reduced to metallic or solid form and deposited onto the electrode surface. The deposition potential of 0.3-0.5 V more negative than E^0 of the interesting metals ion is usually applied to reduce the metal ions. After that the deposited metals are oxidized and dissolved into the solution when the anodic potential is applied in the measurement step. This technique is called anodic stripping voltammetry. The summary of the anodic stripping voltammetry including a deposition step and a measurement step for determination of metals is displayed in Figure 2.9.