Chapter 3
Electrochemical Detection of Label-free Immunosensors

Electrochemical detection is the measurements of electrical properties, such as current, potential, or charge, and their relationship to chemical or biological compounds. The technique of electrochemical detection provides a sensitive method for trace analysis of chemical and biological materials. Electrochemical detection for label-free immunosensors employs several transduction principles, \textit{i.e.}, potentiometric (Bush and Rechnitz, 1987; Fu \textit{et al.}, 2004; Keating and Rechnitz, 1984; Tang \textit{et al.}, 2004b; 2004c; 2005; Yao and Rechnitz, 1987; Yuan \textit{et al.}, 2004), amperometric (Ramanaviciene and Ramanavicius, 2004; Sargent and Sadil, 1998; Tang \textit{et al.}, 2005; Zhang \textit{et al.}, 2005), conductimetric (Kanungo \textit{et al.}, 2002; Yagiuda \textit{et al.}, 1996), impedimetric (Bart \textit{et al.}, 2005; Bordi \textit{et al.}, 2002; Fu \textit{et al.}, 2004; Grant \textit{et al.}, 2005; Guiducci \textit{et al.}, 2004; Tang \textit{et al.}, 2004a; Yang \textit{et al.}, 2003; Yang and Li, 2006) and capacitive (Bataillard \textit{et al.}, 1988; Berggren \textit{et al.}, 1998; Bontidean \textit{et al.}, 1998; 2000; Jiang \textit{et al.}, 2003; Hedström \textit{et al.}, 2005; Hu \textit{et al.}, 2002; 2005; Mirsky \textit{et al.}, 1998; Wu \textit{et al.}, 2005(a); 2005(b); Yin \textit{et al.}, 2005).

3.1 Potentiometric immunosensor

Potentiometric transducer electrodes measure surface potential alterations at near-zero current flow. According to Nernst equation, potential changes are logarithmically proportional to the specific ion activity (Luppa \textit{et al.}, 2001; Wang, 2000). Potentiometric immunosensors depend on the immobilization of one component of the immunological pair (\textit{i.e.}, antibody or antigen) onto the electrode (transducer) and monitoring the electrical potential change resulting from the antibody-antigen complex on the electrode surface. The potentiometric response of the immunosensors towards analyte is

\[ \Delta E = E_2 - E_1 \]  \hspace{1cm} (1)
where $E_1$ is the value of the steady-state potentiometric response vs. standard reference electrode (i.e., Ag/AgCl reference electrode, or saturated calomel electrode) in a buffer solution before the antibody–antigen reaction, $E_2$ represents the value of the steady-state potentiometric response vs. standard reference electrode after the antibody–antigen reaction under the same conditions (Fu et al., 2004; Tang et al., 2004b; 2004c; 2005).

Potentiometric response can be detected with ion-selective membrane electrode such as, potassium ion-selective membrane electrode (Keating and Rechnitz, 1984), an indicator electrode capable of selectively measuring the activity of potassium ions. The response of a potassium ion-selective membrane electrode is based on the membrane potential reflects the gradient of potassium ion in the inner and outer solution that can be described as

$$E = \frac{RT}{nF} \ln \left( \frac{a K^+ \text{, sample soln}}{a K^+ \text{, inner soln}} \right)$$  \hspace{1cm} (2)

Where $E$ is the potential produced across the membrane of the analyte ion in the outer and inner solution, $R$ is the universal gas constant (8.134 J k$^{-1}$ mol$^{-1}$), $T$ is the absolute temperature, $n$ is the number of electron, $F$ is the Faraday constant (96,487 coulombs), $a$ is the activity of potassium ion (Bard and Faulkner, 2001; Wang, 2000).

When the antibody (or antigen) immobilized on the membrane electrode binds the antigen (or antibody) from the solution. This antibody-antigen complex on the membrane inhibited the movement of potassium ion into electrode. Therefore, the potential across the membrane due to the potassium ion in the inner and outer solution decreases after the antibody-antigen reaction (Figure 3.1). Potentiometric immunosensors with potassium ion-selective membrane electrode were developed for the measurement digoxin antibody (Bush and Rechnitz, 1987; Keating and Rechnitz, 1984). Flavin adenine dinucleotide-bound membrane electrode has also been developed for the measurement of riboflavin (vitamin B$_2$) (Yao and Rechnitz, 1987).
Potentiometric immunosensors have also been developed using polymer modified electrodes. When antibody is immobilized on the electrode, the surface charge of the immunosensor will depend on the net charge of the immobilized antibody. When antigen is present in the solution, the immunochemical reaction will take place at the interface resulting in a change of the surface charge that provides the potential response (Fu et al., 2004; Tang et al., 2004b; 2004c; Yuan et al., 2004). The increase or decrease of this potential response depends on the isoelectric points of the Ag and Ab. For example, if an Ab with an isoelectric point 8.00 is used in a system with pH 7.00 buffer the charge of the immobilized Ab on the electrode surface is positive. When the antigen that has a negative charge at pH 7.00 was added it binds to the Ab causing the density of the positive electrical charges on the immunosensor surface to decrease (Figure 3.2). Therefore, $\Delta E$ is negative. On the other hand if the
charge on the antigen is positive the density of positive electrical charges on the immunosensor surface increase, giving positive $\Delta E$.

This method has been developed for many different analytes, such as diphtheria antigen (Tang et al., 2004b; 2004c), hepatitis B (Tang et al., 2005; Yuan et al., 2004), and IgG antigen (Fu et al., 2004).

![Potentiometric units diagram](Image)

**Figure 3.2** Schematic diagram of polymer modified electrode potentiometric immunosensor; reference electrode (RE), working electrode (WE) (Modified from Fu et al., 2004; Tang et al., 2004b; 2004c).

The advantage of potentiometric immunosensor system is the simplicity of operation and the small size. However, it is still suffering from major problems, *i.e.*, the change in potential due to antibody-antigen reaction is very small, so a poor sensitivity and low precision is obtained. These systems also suffer from non-specific binding reactions (Luppa et al., 2001; Marco and Barceló, 1996; Marty et al., 1998).
3.2 Amperometric immunosensor

Amperometric immunosensors are designed to measure a current flow generated by an electrochemical reaction at an applied potential (Luppa et al., 2001; Marty et al., 1998). Amperometric immunosensor can be performed by using pulsed amperometric (Ramanaviciene and Ramanavicius, 2004; Sargent and Sadil, 1998) and chronoamperometric technique (Tang et al., 2005; Zhang et al., 2005). These techniques are performed by using a three-electrode system consists of a working electrode (Ab immobilized on electrode surface), a standard reference electrode (i.e., Ag/AgCl reference electrode, or saturated calomel electrode) and an auxiliary electrode (i.e., a platinum wire). All potentials are referenced to standard reference electrode.

Pulsed amperometric detection is based on the change of current in the amperometric response before and after antibody-antigen reaction (Ramanaviciene and Ramanavicius, 2004; Sargent and Sadil, 1998). After the potential is pulsed on the working electrode (with immobilized antibodies), the amperometric response is obtained ($\Delta i_{AB}$) (Figure 3.3). When antigen is added, it binds to antibodies immobilized on the electrode. This antibody-antigen complex on the surface of the electrode inhibits the electron transfer. Therefore, the current response decreases after the antibody–antigen reaction ($\Delta i_{AB-Ag}$). This type of amperometric immunosensors has been developed for human serum albumin (HSA) (Sargent and Sadil, 1998), and bovine leukemia virus (BLV) glycoprotein gp51 (gp51) (Ramanaviciene and Ramanavicius, 2004).
Figure 3.3 Schematic diagram of pulsed amperometric technique for amperometric immunosensor. (a) amperometric response of antibody ($i_{Ab}$), (b) amperometric response of antibody-antigen reaction ($i_{Ab-Ag}$) (Modified from Ramanaviciene and Ramanavicius, 2004; Sargent and Sadil, 1998).
Chronoamperometric detection is also based on the peak current before and after antigen–antibody reaction. When antibodies, immobilized on the electrode, bind with antigens, the peak current decreases (Figure 3.4) (Tang et al., 2005; Zhang et al., 2005). This technique has been applied for the detection of Hepatitis B (Tang et al., 2005), and rubella vaccine (Zhang et al., 2005).

The main advantages of amperometric immunosensor are the low cost, ease of operation and disposable sensor. However it has some disadvantages such as, low linear range, very noisy response and non-specific binding of other components in the sample (Byfield and Abuknesha, 1994; Gooding et al, 2001).

![Diagram](image)

Figure 3.4 Schematic diagram of chronoamperometric technique for amperometric immunosensor. (a) amperometric response of antibody ($i_{Ab}$), (b) amperometric response of antibody-antigen reaction ($i_{Ab-Ag}$).
3.3 Conductimetric immunosensor

A conductimetric immunosensor device measures the change of conductivity of the layer between the electrodes. Antibodies are immobilized onto the surface of one of the two electrodes. After antigen reacts to antibody immobilized surface, the antibody–antigen complex causes a decrease in the conductivity between the electrodes (Figure 3.5) (Yagiuda et al., 1996). This device has been developed for methamphetamine (MA) (Yagiuda et al., 1996).

![Diagram of conductimetric immunosensor](image)

Figure 3.5 Schematic diagram of a conductimetric immunosensor measures the change of the conductivity of the layer between the electrodes due to antibody-antigen reaction (Modified from Yagiuda et al., 1996).

Another approach has been developed for rabbit IgG (Kanungo et al., 2002) by measuring the change in the conformation of the polymer due to the formation of antibody–antigen (Figure 3.6). This immunosensor device is fabricated by immobilizing antibody on the polymer matrix during polymerization. The device is then exposed to solution containing the antigen. The conductimetric response of the immunosensor is represented by $\Delta g/g$ where $\Delta g = g - g_0$; $g_0$ is the conductance of the sensor without any antigen and $g$ is the conductance of the sensor in presence of the antigen (Kanungo et al., 2002).

The advantages of conductimetric immunosensor are inexpensive, reproducible and disposable sensors. The main disadvantages are the interferences of
ionic strength of the sample and non-specific binding of other components in the sample (Byfield and Abuknesha, 1994).

Figure 3.6 Schematic diagram of conductimetric immunosensor measures the change of the polymer modified electrode due to antibody-antigen reaction; reference electrode (RE), working electrode (WE).
3.4 Impedimetric immunosensor

For label-free electrochemical immunosensor that relies on the detection of impedance. Electrochemical impedance spectroscopy (EIS) is most often applied. In this technique the total impedance was determined by several parameters which include (1) the electrolyte resistance, $R_s$; (2) the double-layer capacitance, $C_{dl}$; (3) the electron-transfer resistance, $R_{et}$ and (4) the Warburg impedance, $Z_w$. The complex impedance can be presented as the sum of the real, $Z_{re}$, and imaginary, $Z_{im}$ components that originate mainly from the resistance and capacitance of the cell (Grant et al., 2005; Katz and Willner, 2003). To give more detailed information about the impedance of the modified electrode, a modified Randles' equivalent circuit (Figure 3.7) was chosen to fit the measured results. The two components of the scheme, $R_s$ and $Z_w$, represent bulk properties of the electrolyte solution and diffusion of the applied redox probe, respectively. Thus, they are not affected by chemical transformations occurring at the electrode interface. The other two components of the circuit, $C_{dl}$ and $R_{et}$, depend on the dielectric and insulating features at the electrode/electrolyte interface (Grant et al., 2005; Katz and Willner, 2003; Kharitonov et al., 2000).

![Figure 3.7 Randles' equivalent circuit; (Rs) the ohmic resistance of the electrolyte resistance resulting from the diffusion of redox-probe, (Cdl) the double-layer capacitance, (Ret) the electron-transfer resistance, and (Zw) the Warburg impedance (Grant et al., 2005; Katz and Willner, 2003; Kharitonov et al., 2000; Tang et al., 2004a; Yang et al., 2003).](image-url)
In electrochemical impedance spectroscopy (EIS), the semicircle diameter of EIS equals the electron-transfer resistance, $R_{et}$, as shown in a Nyquist plot arising from Randles' circuit (Figure 3.8). From Figure 3.8(a), $R_s$ and $R_{et}$ can be calculated from the intercept with the $Z_{re}$-axis. The resistance of $R_{et}$ controls the electron transfer kinetics of the redox-probe at electrode interface, which is relative to the concentration of antigen. When antigen bind to the surface-immobilized antibodies the electron transfer resistance will increase (Figure 3.8(b)) (Katz and Willner, 2003; Kharitonov et al., 2000; Tang et al., 2004a). The change of electron-transfer resistance ($\Delta R_{et}$) is calculated by

$$\Delta R_{et} = \Delta R_{Ag-Ab} - \Delta R_{Ab} \quad (3)$$

Where $R_{Ag-Ab}$ is the value of electron-transfer resistance after Ag binding to Ab. $R_{Ab}$ is the value of electron-transfer resistance of the immobilized Ab.

The impedimetric immunosensors have been developed for many different analytes, such as, Bovine serum albumin (BSA) (Grant et al., 2005), Hepatitis B (Tang et al., 2004a), IgG antigen (Fu et al., 2004), and Interferon-γ (IFN-γ) (Bart et al., 2005). However the data of this immunosensor is difficult to interpret and it is not suitable for real-time measurements. Furthermore, the instrumentation is expensive (Berggren et al., 1997; 1999; Bindi et al., 2002; Guiducci et al., 2004).
Figure 3.8 Typical Nyquist plot of immobilized Ab on the surface of the electrode (a) and Ab-Ag complex on the surface of the electrode (b), \( R_s \) the electrolyte resistance, \( R_{et(\text{Ab})} \) the electron-transfer resistance of immobilized Ab, and \( R_{et(\text{Ab-Ag})} \) the electron-transfer resistance of Ab-Ag complex (Modified from Katz and Willner, 2003; Kharitonov et al., 2000; Tang et al., 2004a).
3.5 Capacitive immunosensor

The emergence of novel transducers opens new scopes for the development of biosensors. Recently, a capacitive transducer, based on the theory of the electrical double-layer, has been developed to detect compound at low concentration (Bataillard et al., 1988; Berggren and Johansson, 1997; Berggren et al., 1998; 2001; Billard et al., 1991; Bontidean et al 1998; Gebbert et al., 1992; Hedström et al., 2005; Hu et al., 2002; 2005; Jiang et al., 2003; Steltze and Sackmann, 1989; Wu et al., 2005(a); 2005(b); Yang et al., 2005).

The theory of the electrical double layer is an array of charged particles and/or oriented dipoles that exists at every material interface (Mark, 1991; Wang, 2000). A metal electrode immersed in an electrolyte solution can generally be described as resembling a capacitor in its ability to store charge. For a given potential the metal electrode will possess a charge $q_m$ and the solution another charge $q_s$, where $q_m$ will be equal to $-q_s$. Charged species and dipoles will be oriented at the metal/solution interface, hence making up the electrical double-layer (Figure 3.9(a)) (Bard and Faulkner, 2001).
Figure 3.9 Schematic representation of (a) model of the double-layer region (IHP is inner Helmholtz plane; OHP is outer Helmholtz plane) and (b) potential profile across the double layer (Redrawn from Bard and Faulkner, 2001; Mark, 1991; Wang, 2000).
This double-layer consists of several regions (Figure 3.9(b)). The inner layer (closest to the electrode, at distance $X_1$), known as the inner Helmholtz plane (IHP), contains solvent molecules and specifically adsorbed ions (which are not fully solvated). It is defined by the locus of points for the specifically adsorbed ions. The next layer, the outer Helmholtz plane (OHP) (at distance $X_2$), reflects the imaginary plane passing through the center of solvated ions at their closest approach to the surface. The solvated ions are nonspecifically adsorbed and are attracted to the surface by long-range electrostatic forces; in fact they are said to be non-specifically adsorbed ions since their interaction is independent of the chemical properties. Both Helmholtz layers represent the compact layer (at distance $X_H$). Such a compact layer of charges is strongly held by the electrode and can survive even when the electrode is pulled out of the solution. The Helmholtz model does not take into account the thermal motion of ions, which loosens them from the compact layer. The outer layer (beyond the compact layer) is the diffuse layer (or Gouy layer), which extends from the OHP to the bulk solution. The thickness of this layer depends on the total ionic concentration of the solution. The total charge of the compact and diffuse layers equals (and is opposite in sign to) the net charge on the electrode side. The potential-distance profile across the double-layer region involves two segments, with a linear increase up to the OHP and an exponential increase within the diffuse layer. These potential drops are displayed in Figure 3.9(b) (Bard and Faulkner, 2001; Mark, 1991; Wang, 2000).

The electrical double layer resembles an ordinary (parallel-plate) capacitor. For an ideal capacitor, the change ($q$) is directly proportional to the potential difference ($E$)

$$q = CE$$  \hspace{2cm} (4)

Where, $C$ is the capacitance (in farads, F), the ratio of the charge stored to the applied potential. The potential-charge relationship for the electrical double layer is
\[ q = C_{dl} A (E - E_{pzc}) \]  

(5)

Where, \( C_{dl} \) is the double layer capacitance per unit area and \( E_{pzc} \) is the potential of zero charge (i.e. where the sign of the electrode charge reverses and no net charge exists in the double layer).

The capacitance of the double layer consists of combination of the capacitance of the compact layer in series with that of the diffuse layer. For two capacitors in series, the total capacitance is given by

\[
\frac{1}{C_{dl}} = \frac{1}{C_H} + \frac{1}{C_D}
\]  

(6)

Where, \( C_H \) and \( C_D \) represent that capacitance of the compact and diffuse layers, respectively (Wang, 2000).

The measuring principle of capacitance is based on changes in dielectric properties or charge distribution when an antibody-antigen complex forms on the surface (Berggren, 2001; Gebbert et al., 1992). Capacitance measurements can be made in two approaches, either by measuring the capacitance change between parallel metal plates (Gebbert et al., 1992) or by measuring the capacitance at the electrode/solution interface (Bataillard et al., 1988; 1998; Hedström et al., 2005; Hu et al., 2002; 2005; Jiang et al., 2003; Mirsky et al., 1998; Wu et al., 2005(a); 2005(b); Yin et al., 2005).

In the first approach, the capacitance between parallel metal plates can be measured with a high precision capacitance bridge. The capacitance is described by

\[ C = \frac{\varepsilon \varepsilon_0 A}{d} \]  

(7)
Where, $\varepsilon$ is the dielectric constant of the material between the plates, $\varepsilon_0$ is the dielectric constant for vacuum, $A$ is the area and $d$ the distance between the plates (Gebbert et al., 1992). When the antibody (or antigen) is immobilized on one plate the capacitance change can be detected as a change of the thickness of a layer immobilized on the transducer and/or as a change in the dielectric constant causes by the binding or releasing of an analyte (Figure 3.10a). In the case of interdigitated electrodes, with the antibody (or antigen) immobilized between them, the forming of the antibody-antigen complex will change the dielectric properties between the electrode causing the capacitance to change (Figure 3.10b). The disadvantages associated with this approach are difficulties in producing a short and reproducible distance between the two plates, and its sensitivity to changes in the bulk solution. To overcome the last problem, a reference cell without recognition element can be used (Tayor et al., 1991).
(a) Parallel metal plates

![Diagram of parallel metal plates with electrolyte between them.]

(b) Interdigitated electrode

![Diagram of interdigitated electrode with electrolyte between conductors on the substrate.]

Figure 3.10 Schematic diagram of capacitive immunosensor measures the capacitance change due to the change in distance between two plates and/or the change in dielectric constant (a) due to change in the dielectric constant using interdigitated electrode (b) (Modified from Gebbert et al., 1992; Berggren et al., 2001).
In another approach the capacitance is obtained from the current response measured at the electrode/solution interface in a potentiostatically controlled three electrode system by applying an electrical perturbation signal to the electrode (Figure 3.11). A potentiostat is used to control and keep a certain value of the potential at the working electrode (WE) relative to the reference electrode (RE). This is achieved by comparing the working and reference potentials and changing the potential of the auxiliary electrode (Aux) in order to keep the WE at the desired potential, a task that is performed by a control amplifier (A). A rough description of a common adder potentiostat is shown in Figure 3.12. This approach can be constructed by immobilizing biorecognition elements (i.e., antibody) on an electrode surface and measuring changes in the dielectric properties when an analyte (antigen) binds to the antibody on the electrode, causing capacitance to decrease (Figure 3.11).

![Figure 3.11 Schematic diagram of capacitive immunosensor measures the change in the capacitance at the electrode/solution interface; reference electrode (RE), working electrode (WE), auxiliary electrode (AE) (Modified from Hu et al., 2002; Jiang et al., 2003; Wu et al., 2005; Yin et al., 2005(a)).]
There are several ways to determine capacitances at the solution-electrode interface such as, current step (Bard and Faulkner, 2001), potential sweep (Akram, et al., 2004; Azumi and Seo, 2001; Sung et al., 2004; Yang et al., 2003) and potential step (Berggren et al., 1998; Hedström et al., 2005; Hu et al., 2002; 2005; Jiang et al., 2003; Mirsky et al., 1998; Wu et al., 2005(a); 2005(b); Yin et al., 2005).

Two equivalent circuits are commonly used to evaluate the capacitance from the electrode/solution interface, i.e., the Randles's circuit (Figure 3.13(a)) and $R_C$ circuit (Figure 3.13(b)). However, the simple model of a resistor and a capacitor in series has been used successfully for evaluation of the capacitance for the electrode/solution interface system (Berggren et al., 1998; Hedström et al., 2005; Hu et al., 2002; 2005; Jiang et al., 2003).
Figure 3.13 Models of an electrochemical cell for capacitance detection. (a) Randle’s circuit; \((R_s)\) the resistance of the solution, \((C_{dl})\) the double layer capacitance, \((C_p)\) a pseudo capacitance, due to movement of ions at the interface, \((R_{ct})\) the charge transfer resistance at the interface. (b) a series \(R_sC_{dl}\) equivalent circuit (Berggren and Johansson, 1997; Jiang et al., 2003; Yang et al., 2003).
3.5.1 Current step

In the current step method, a pulse of constant current $i$ is applied to the series $R_s C_{dl}$ circuit (Figure 3.13(b)) and the resulting potential $E$ is linear with time $t$ (equation 8) (Figure 3.14).

$$ E = i \left( R_s + \frac{t}{C_{dl}} \right) \quad (8) $$

Where $R_s$ is the resistance of the solution and $C_{dl}$ is the double layer capacitance (Bard and Faulkner, 2001).

The capacitance can easily be obtained from the slope of the resulting potential (Figure 3.14). Also the resistance of the solution can be extrapolated by linear regression. The determination of capacitance via current step has been used to study the formation of silver nanoparticles on thin insulator layer (thin SiO$_2$ layer) (Tsuji et al., 2004).
Figure 3.14 $E-t$ behavior resulting from a current step applied to an $R_sC_{dl}$ circuit (Redrawn from Bard and Faulkner, 2001).
3.5.2 Potential sweep (Voltage ramp)

Linear potential sweep or voltage ramp was also used for capacitance measurement (Akram et al., 2004). In this method a potential is increased linearly with time starting at some initial value at a sweep rate $v$ (V s$^{-1}$). If such a ramp is applied to the series $R_sC_{dl}$ circuit (Figure 3.13(b)), the resulting current $i$ contains a transient part, which responds to a steady-state (Figure 3.15) and it is expressed by equation 9:

$$i = vC_{dl} \left[ 1 - \exp \left( \frac{-t}{R_sC_{dl}} \right) \right]$$  \hspace{1cm} (9)

Where $v$ is the potential scan rate, $R_s$ is the resistance of the solution and $C_{dl}$ is the double layer capacitance. The value of $C_{dl}$ is easily obtained from the steady-state part (Figure 3.15) (Bard and Faulkner, 2001).

The determination of capacitance via potential sweep has been used to study the behavior of the modified electrode surface, such as aerogel on carbon (Yang et al., 2003), film formed on titanium (Azumi and Seo, 2001), polyaniline films on gold (Sung et al., 2004), and protein A immobilized on gold (Akram et al., 2004).
Figure 3.15  $i$-$t$ behavior resulting from a linear potential sweep applied to an $R_sC_{dl}$ circuit (Redrawn from Bard and Faulkner, 2001).
3.5.3 Potential step

Another way to evaluate the capacitance is to perturb the system with a potential step and this has been used in several work (Berggren and Johansson, 1997; Berggren et al., 1998; 2001; Bontidean et al., 1998; 2000; Jiang et al., 2003). A potentiostatic step with an amplitude of 50 mV was applied (Figure 3.16(a)) to obtain the current response (Fig. 3.16(b)).

![Graph](image)

**Figure 3.16** Potentiostatic step method to evaluate capacitance (a) shows a potential step and (b) the corresponding current response (Redrawn from Bard and Faulkner, 2001; Wang, 2000).
The most simple model to describe the interface is a resistor and a capacitor in series (see $R_sC_{dl}$ circuit in Figure 3.13(b)). This model was a good fit in the beginning of the current transient where signal-to-noise ratios were most favorable (Berggren et al., 1999). The current decay, evoked by a potential step will in this case follow

$$i(t) = \frac{u}{R_s} \exp \left( -\frac{t}{R_s C_{Total}} \right) \quad (10)$$

Where, $i(t)$ is the current in the circuit as a function of time, $u$ is the pulse potential applied, $R_s$ is the dynamic resistance of the recognition layer, $t$ is the time elapsed after the potential step was applied, and $C_{total}$ is the total capacitance measured at the working electrode/solution interface ($C_{total} = C_{dl}$ in equation 6). Taking the logarithm of equation 10 the relationship becomes

$$\ln i(t) = \ln \frac{u}{R_s} - \frac{t}{R_s C_{Total}} \quad (11)$$

Then, $C_{total}$ and $R_s$ can be obtained from the slope and intercept of the linear least-square fitting of $\ln i(t)$ versus $t$ (Berggren and Johansson, 1997; Berggren et al., 1998; 2001; Bontidean et al., 1998; 2000; Jiang et al., 2003).

Several research works have used this potential step technique to develop capacitive affinity biosensors such as, capacitive immunosensors for human serum albumin (HSA) (Hedström et al., 2005; Wu et al., 2005(a); 2005(b)), hyaluronan (Jiang et al., 2003), interleukin-6 (Berggren et al., 1998), phospholipase (Mirskey et al., 1998), and transferrin (Hu et al., 2002; 2005; Yin et al., 2005).

Compared with other electrochemical methods, capacitive detection has several advantages. It can be used for some special purposes i.e., thickness measurements, investigation of formation processes. It is also suitable for studying
insulating material, such as the interaction between antibody-antigen, biotin-avidin, enzyme-substrate interaction, and for detection of small molecules at very low detection limits (Bontidean et al., 1998; Dong and Chen, 2002). In addition the method is quick, can be used to follow bio-recognition events at the electrode surfaces or directly detect the interaction between an analyte and a biorecognition element on the surface of a biosensor system (Berggren et al., 1998; Jiang et al., 2003).

Using capacitance detection, a special immobilization technique is required, since the electrode surface has to be electrically insulated. Self assembled monolayer (SAM) is a particularly suitable immobilization technique for capacitive biosensor (Riepl et al., 1999). SAM can be formed by spontaneous adsorption of alkanethiol on gold surface that takes place at room temperature (Nuzzo and Allara, 1983; Porter et al., 1987). The affinity between sulfur atom of alkanethiol and gold atom is allows electrochemical insulation of the gold surface (to avoid faradic processes). It is also an excellent immobilization technique for protein, it shields proteins from direct contact with solid surface, thus, reduces the risk of the sensing element denaturation (Wadu-Mesthrige et al., 2000). Therefore, SAM is preferred for immobilization technique in this work.