Chapter 3

Amperometric electrodes for enantioanalysis

3.1 Introduction

Amperometric detection has found wide application to measurements in biological media [1-4], environment analysis [5-6] and pharmaceutical industry [7-8]. Amperometric electrodes have higher sensitivity for enantiomer recognition than the best chromatographic technique (e.g., capillary zone electrophoresis in enantiomer recognition (CZE) [9]). The separation step in CZE is not always accurate, therefore the reliability of the analytical information is decreasing. The utilization of amperometric electrodes in molecular recognition of the enantiomers is not laborious if one compares it with structural analysis (IR, NIR, Raman, MS, X-ray Diffraction, and Neutron Diffraction) and with chromatographic techniques [10]. The advantage of using these sensors over the other techniques is the high reliability that is given by high precision, high reproducibility and rapidity [11-12]. High precision can be explained by to the fact that amperometric sensors can be used directly for measurement of the compounds in solution without any prior separation of the enantiomer that has to be determined.

Sensor validation is dependant on the reliable response characteristics and reliable analytical information [13]. The high reliability of analytical information obtained using these electrodes made automation of amperometric techniques possible, by integration of enantioselective sensors as detectors in flow and sequential injection analysis systems [14-15].

Unfortunately, no electrochemical sensor can be reliably used for all types of enantiomers. The type of amperometric electrodes must be selected in concordance with the complexity of the enantiomer structure. The principle of enantiomer recognition using amperometric electrode is catalyst selectivity.

3.2 Design of amperometric electrodes

The design of the amperometric electrode is very important for enantioselective high throughput screening of enantiomers because the reliability of the sensor design determines the quality of the analytical information. Solid membrane electrodes are preferred over liquid membrane electrodes because they can easily be used for environmental, food, and clinical analysis as well as for *in vivo* analysis. Amperometric electrodes design is based on different types of matrices such as molecular imprinting polymers [16], composite polymers [17], sol gel [18-19] and in particular for enantioanalysis, carbon paste [8, 20] and diamond paste [21-22]. Amperometric electrodes based on carbon or diamond paste are the most reliable for enantioanalysis [15].

3.2.1 Design of carbon paste based amperometric electrodes

This design is based on physical mixture of graphite powder with oil (paraffin or nujol oil). These electrodes can be used as they are, or modified with enzyme (amperometric biosensor), or antibody (amperometric immunosensor). The most reproducible design for graphite paste electrode was recommended by Stefan et al. [23]. The carbon paste electrode was prepared by mixing 0.1 g of graphite powder with 20 μL paraffin oil. The

paste was filled then into a plastic pipette tip. The diameter of the sensing part was 3.0 mm. Electrical contact was made by inserting a silver wire into the carbon paste. Before each use, the electrode surface was smoothed by polishing with alumina paper (polishing strips 30144-001, Orion). When not in use the paste was stored at room temperature.

3.2.2 Design of diamond paste based amperometric electrodes

Diamond has some inherent electrochemical properties such as hardness, high thermal conductivity, high electrical resistance, high electron and hole mobilities, variable conductivity via doping and electrode geometry pattern using selective growth methods. Most of electrochemical studies are performed with diamond film based electrodes prepared by chemical vapor deposition (CVD). Synthetic films often possess a polycrystalline and textured microstructure with a small volume fraction of non-diamond impurity [24].

The application of synthetic diamonds, primarily in the area of active electronic components, did not realize to a great extent because of the poor structural quality of most CVD grown films. The resistively of polycrystalline diamond thin-films made by CVD can be decreased by doping diamond with boron [25]. Resistivity as low as 0.01 Ω-cm has been reported for boron doped films, rendering them conductive enough for electrochemical studies.

Boron doped diamond (BDD) exhibits several superior electrochemical properties that are significantly different from those of other carbon allotropes, which have been widely

used as electrode materials. Its attractive features include a wide electrochemical potential window in aqueous media, very low capacitance and extreme electrochemical stability.

In the crystal structure of diamond, each carbon atom is tetrahedrally (Figure 3.1) surrounded by four equidistant neighbors at 154.45 pm, and the tetrahedral are arranged to give a cubic unit cell with a_o = 356.68 pm. Diamond has the following physical properties: a low density (d = 3.51 g/cm^3); it is the hardest material and the best conductor of heat ever known; transparent to visible light and IR and UV radiation.

The advances in single-crystal diamond have enabled the development of a wide range of monocrystalline diamond products to meet the requirements of different applications [26]. Room temperature drift mobilities of 4500 cm^2/Vs for electrons and 3800 cm^2/Vs for holes have been measured in high purity single-crystal diamond [19]. These values were determined by using the time-of-flight technique on thick, intrinsic freestanding diamond plates and were verified by current-voltage measurements on p-i junction diodes [27].

Monocrystalline diamond paste amperometric electrodes exhibit several superior electrochemical properties if one compares with carbon paste and glassy carbon electrodes [22]. Wide working concentration range and relatively low limits of detection (10^{-8} to 10^{-11} mol/L) obtained by using amperometric electrodes based on diamond paste proved their high sensitivity [22, 28].

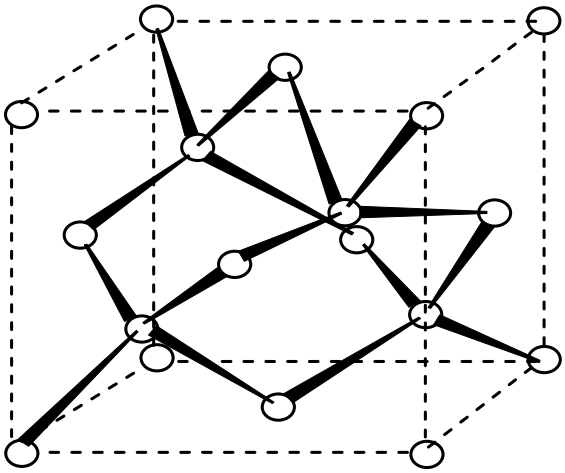

Figure 3.1 Structure of diamond showing the tetrahedral coordination of C.

Three types of monocrystalline diamond powder were used for the design of the diamond paste electrodes used for amperometric sensors, named: natural diamond (1 μm), synthetic diamond (50 μm), and synthetic diamond (1 μm). Amperometric electrodes were prepared by mixing 100 mg of diamond powder with 20 μL paraffin oil. Some of the diamond paste was then filled into a plastic pipette peak. The diameter of the sensing part was approximately 2.3 mm. Electric contact was achieved by inserting a silver wire (0.5 mm in diameter) into the diamond paste. Before each use, the electrode surface was smoothed by polishing with alumina foils (polishing strips 30144-001, Orion). All sensors were stored at room temperature, when not in use.

3.3 Response characteristics of amperometric electrodes

An amperometric electrode is based on the measurement of a developed current in concordance to the controlled applied potential between the working electrode and reference electrode. There are two ways to develop a current in electrochemical cells:

(i) applying a step potential and recording the developed current as a function of time (chronoamperometry);

(ii) applying variable forms of potentials (e.g., triangle potential wave form (cyclic voltammetry), square voltage pulses of constant amplitude with linear ramp (differential pulse voltammetry).

The functional relation between the intensity of the current (I), measured at the certain potential (E) and concentration of the analyte (C) is given by the linear correlation;

$$I = f(c) \tag{3.1}$$

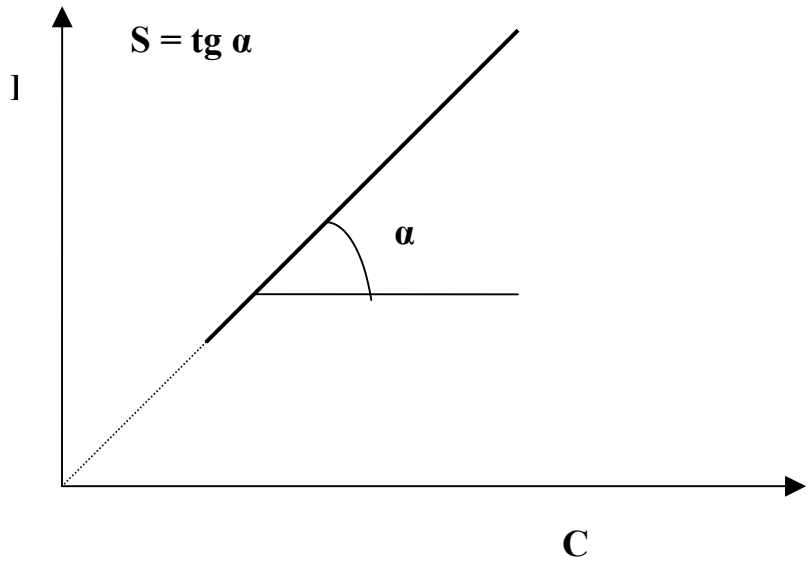

Figure 3.2 Electrode function for amperometric electrodes

The response output of the intensity of current and concentration of the analyte is shown in Figure 3.2. The current of an amperometric electrode is related to the mass transfer of a substrate to the electrode as described by the following equation [29]:

$$i_t = nFAD_E\left(\frac{\partial C_E}{\partial x}\right)_{x=0,t} \tag{3.2}$$

where i_t = current at time t, A; n = number of electrons transferred per molecule; F = Faraday's constant, 96500 C/equiv; A = electrode area, cm^2; C_E = concentration of E, mol/cm^3; D_E = diffusion coefficient of E cm^2/s; t = time, s; X = distance, cm

Thus the current is directly proportional to the slope of the concentration-distance profile at the electrode surface, i.e., to $(\frac{\partial C_E}{\partial x})_{x=0,t}$. This equation can be expressed in terms of the Nernst diffusion layer concept by simply approximating ∂x by δ and ∂C by $C_E - C_E^s$.

$$i_t = nFAD_E \frac{C_E - C_E^s}{\delta} \tag{3.3}$$

The current is determined by the slope of the profile for species E. As the slope increases due to decreasing C_E^s, the current increases. A limiting cathodic current, i_{lc}, is reached when the surface concentration C_E^s becomes effectively zero. Substitution of $C_E^s = 0$ into the previous equation gives the equation of the limiting current [29]:

$$i_{lc} = \frac{nFAD_E C_E}{\delta} \tag{3.4}$$

3.3.1 Response (slope) of the electrode

One of the most important response characteristics for amperometric electrodes is the slope considered for the linear concentration range. The slope is a measure of the electrode sensitivity, where a minimum value of 100 nA/decade of concentration is requested for amperometric electrodes to be considered for bioanalysis. There are two ways to determine the slope of the amperometric electrodes:

(i) from the graphical method, the slope of the amperometric electrodes is equal to the tangent of the angle formed between the calibration line and the concentration or activity axis. Slope can be represented by the following equation:

$$S = tg\alpha \tag{3.5}$$

where S is the slope of the amperometric electrode; tg is the tangent function; α is the angle between the calibration line and concentration axis (Figure 3.2);

(ii) the slope can be determined by linear regression method (which is more recommended).

3.3.2 Limit of detection

The limit of detection is defined by IUPAC as the concentration at which, under specified conditions, the intensity of the current I, deviates from the average value by a multiple of the standard error of a single measurement of the intensity of the current in this region [30]. An experimental determination of the limit of detection is recommended for amperometric electrodes rather than a statistical treatment of the experimental data obtained for the calibration of the electrodes [15]. The amperometric electrode limit of detection can be considered as the concentration:

(i) where the limiting current intensity value is equal with the one obtained for the buffer solution;

(ii) where the limiting current intensity value is double the one obtained for the buffer solution;

(iii) the concentration below the one where the intensity of the limiting current remains constant [31].

Due to the high sensitivity of the amperometric electrodes, their limit of detection can be of pmol/L magnitude order, or even less.

3.3.3 Linear concentration range

The linear concentration range can be determined according to electrode linearity response in the plot of current versus the concentration of the enantiomer. The linear concentration range can be defined by the range of concentration of the substrate over which the sensitivity of the electrode is constant with a specific variation (usually ± 5%). This response characteristic is very important because the activity of concentration of all the solutions to be measured must lie within the linear concentration range. The reproducibility of the linear range is influenced by the working conditions of the amperometric electrode, like stirring rate, composition of the solution, pH of the solution, composition of the solution where the electrode was exposed before the measurement, the temperature, and the preconditioning of the amperometric electrode [31].

3.3.4 pH range

The pH is an important characteristic of the response of amperometric electrodes. The pH can influence the formation of protonated and unprotonated species of the same enantiomer. It can favor certain redox processes at the electrode. The relation between the intensity of the limited current and the pH variation must be determined for amperometric electrodes. It is very important to work at a certain pH value for all the solution to be

determined and to use a certain composition for the buffer. For some electrode a range where the intensity of the limited current does not depend on the pH value was found. For these electrodes, it is necessary to control the pH and buffer all the standard and sample solutions in the suitable pH range. In the case of the electrodes that did not have any range where the intensity is independent of the pH, special care must be taken to the buffering of each solution.

3.3.5 Ionic strength and activity coefficients

The accuracy of amperometric electrode measurements is influenced by the ionic strength and activity coefficients. It is necessary to work at the same ionic strength otherwise another error may occur due to the variations of the activity coefficients of the ions in the solution [32]. From physiological point of view, the activity is the relevant quantity because equilibrium between different phases depends on activity and not on concentration alone.

3.3.6 Response time

IUPAC defines the response time as the time which elapses between the instant when the electrodes of the amperometric cells are brought into contact with a sample solution, or at which the activity of the ion of interest in solution is changed, and the first instant at which the slope of the working electrode becomes equal to a limiting value selected on the basis of the experimental conditions and/or requirements concerning the accuracy [30]. The response time of amperometric electrodes is a function of the kinetics of the processes that takes place at the surface of the electrode. It increases with decrease in

concentration of the substrate that has to be determined. Amperometric electrodes with short response times are preferred for the use in bioanalysis. The response time is influenced by the presence of other interferences due to the competitive equilibrium that takes place at the electrode interface.

3.3.7 The influence of temperature on the response of the electrode

The response of the electrode is highly influenced by the temperature. The increase of the temperature will favor the kinetics and the thermodynamics of the processes that take place at the electrode surface and as a result the slope will increase. During the measurements of standard and sample solution using amperometric electrodes, the temperature must be maintained constant. The recommended temperature for electrodes characterization is 25^{o}C.

3.4 Selectivity of the amperometric electrodes

Amperometric electrodes are affected by two classes of interfering substances: electrode/electrochemical interferences include substances whose response is similar to the molecule being determined or electrolyte present at high concentration level; chemical interferences interacts with the ion/molecule being determined, so as to decrease its activity or apparent concentration (e.g., H^+, HO^-), or substances that interact with the membrane surface. The selectivity degree of amperometric electrodes is given by the values of the amperometric selectivity coefficients, $K_{i,j}^{amp}$, respectively as follows

(i) if a magnitude order is higher than 10^{-3}, the ion tested for interference does interfere;

(ii) if a magnitude order is 10^{-3}, the ion tested for interference is not a strong interfering species;

(iii) if a magnitude order is less than 10^{-3}, the ion tested for inferences does not interfere.

The following equation was proposed for the correlation of the total current response and amperometric selectivity coefficients by Wang [33].

$$I_t = b + \left(c_i + \sum_{j=1}^{N} K_{i,j}^{amp} c_j \right) \tag{3.6}$$

where I_t is the total current response, c_i and c_j are the concentration of the main and interfering species, respectively, N is the number of interfering species and b is given by the following equation:

$$b = \frac{n_i AFD_i}{\delta_i} \tag{3.7}$$

where n_i is the number of electrons transferred per mole of analyte, A is the surface of the electrode (cm^2), F is Faraday number (96500 C), D_i is the diffusion coefficient of the analyte (cm^2/s), and δ_i is the thickness of diffusion layer (cm). There are two methods for the determination of amperometric selectivity coefficients: mixed and separate solutions methods.

3.4.1. Mixed solution method

The mixed solution method is recommended for the determination of amperometric selectivity coefficients because it shows the actual conditions under which the electrode is used. In this method the current recorded for a solution that contains only the main enantiomer is compared with the total current given by the equation proposed by Wang

for a solution containing both the enantiomer and the interfering species [33]. The selectivity coefficient, $K_{i,j}^{amp}$, can be calculated using the following equation:

$$K_{i,j}^{amp} = \left[\frac{\Delta I_t}{\Delta I_i} - 1\right] x \frac{c_i}{c_j} \qquad (3.8)$$

where $\Delta I_t = I_t - I_b$ and $\Delta_i = I_i - I_b$, I_t = the current recorded for the mixed solution; I_b = the current recorded for the blank solution; I_i = the current recorded for the solution containing only the enantiomer for which the electrode was designed; c_i = the concentration of the main species; c_j= the concentration of the interfering species.

3.4.2. Separate solution method

The separate solution method is based on recording the intensity of current separately, for the solution containing the enantiomer and the interfering species solution. Both recorded currents and concentrations of main and interfering species are compared with each other to calculate the amperometric selectivity coefficient, $K_{i,j}^{amp}$ [34]:

$$K_{i,j}^{amp} = \frac{\frac{\Delta I_j}{C_j}}{\frac{\Delta I_i}{C_i}} \qquad (3.9)$$

where $\Delta I_j = I_j - I_b$ and $\Delta_i = I_i - I_b$; I_j = the current recorded for the main species; I_i = the current recorded for the interfering species; I_b = the current recorded for the blank solution; c_i = the concentration of the main species; c_j= the concentration of the interfering species.

3.5 Direct amperometric method

Direct amperometry is a very simple method to be applied for the direct analysis of enantiomers in their sample solutions without any or minmum prior preparations. To obtain the best precision of measurement it is necessary to calibrate the working electrode just before the assay of sample. This can be performed by standard addition method. Standard aliquots of the analyte are added to the solution and plotting steady-state response possibly corrected for the blank signal versus the enantiomer concentration [35]. To avoid any error in measurements, pH and the ionic strength of the samples must be adjusted to the same values as the solutions used for calibration. The values of current intensity obtained for the samples are interpolated on the calibration plot and the unknown concentrations are determined.

3.6. Differential pulse voltammetry

Voltammetry comprises a group of electroanalytical methods in which information about the analyte is derived from the measurement of current as a function of applied potential under conditions that encourage polarization of an indicator electrode. These methods are classified according to the type of potential (AC, DC) and form of applied potential with time (linear scan, differential pulse, square wave, triangle …etc.). Polarography is a particular case of voltammetry that differs in the respect that the working electrode takes the form of a dropping mercury electrode. A plot of current flowing in the cell as a function of the applied potential is called a voltammogram. A residual current flows in the cell is developed at small applied potentials which is caused by the reduction of trace impurities in the sample solution. An electroactive species are initiated by reduction

when a decomposition potential is applied, a developed current (limiting current) increases with applied potential until it levels off a limiting value. The difference between the limiting current and the residual current is known as the diffusion current, i_d. Voltammetry is useful for quantitative analysis (diffusion current is proportional to analyte concentration and the height of a voltammetric wave tells how much analyte is present) and qualitative analysis of an unknown substance (enantiomer) using the half-wave potential $E_{½}$ of the particular electroactive species to compare with known values of $E_{½}$.

Diffusion current is directly proportional to the concentration of electroactive species. If the applied potential exceeds the decomposition potential of the electroactive species, its concentration at the surface of the electrode is immediately dimensioned and a concentration gradient is established and more of that species diffuses from the bulk solution to the electrode surface (Fick's law of diffusion). The resulting current flow is proportional to the rate of diffusion, which in turns is determined by the concentration gradient,

$$i = k\,(C-C_o) \qquad (3.10)$$

where C and C_o are the concentrations of the electroactive species in the bulk solution and at the surface of the working electrode, respectively. The current flowing in the cell reaches the limiting value by progressively increasing the applied potential; reduction occurs more rapidly and C_o eventually becomes virtually zero:

$$I_d = kC \qquad (3.11)$$

Half-wave potential of the electroactive species are given by the following equation:

$$E_{1/2} = E^{o} - \frac{0.059V}{n}\log\frac{k}{k_r} - E_{reference} \qquad (3.12)$$

where E^{o} is the standard electrode potential; $E_{1/2}$ is the half wave-potential, $E_{reference}$ is the potential of the reference electrode and n is the number of electrons changed per mole of analyte; k and k_r are proportionality constants relating cell current to the rates of diffusion of oxidized and reduced forms of the electroactive species.

Differential pulse polarography is the most used technique in analysis that differs from normal pulse in that after the potential pulse, the potential does not return to a constant base value, Figure 3.4. The potential pulse is superimposed on a conventional rising linear dc voltage ramp. The pulse is also imposed for about 60 ms near the end of the drop lifetime when the growth of the drop almost ceased.

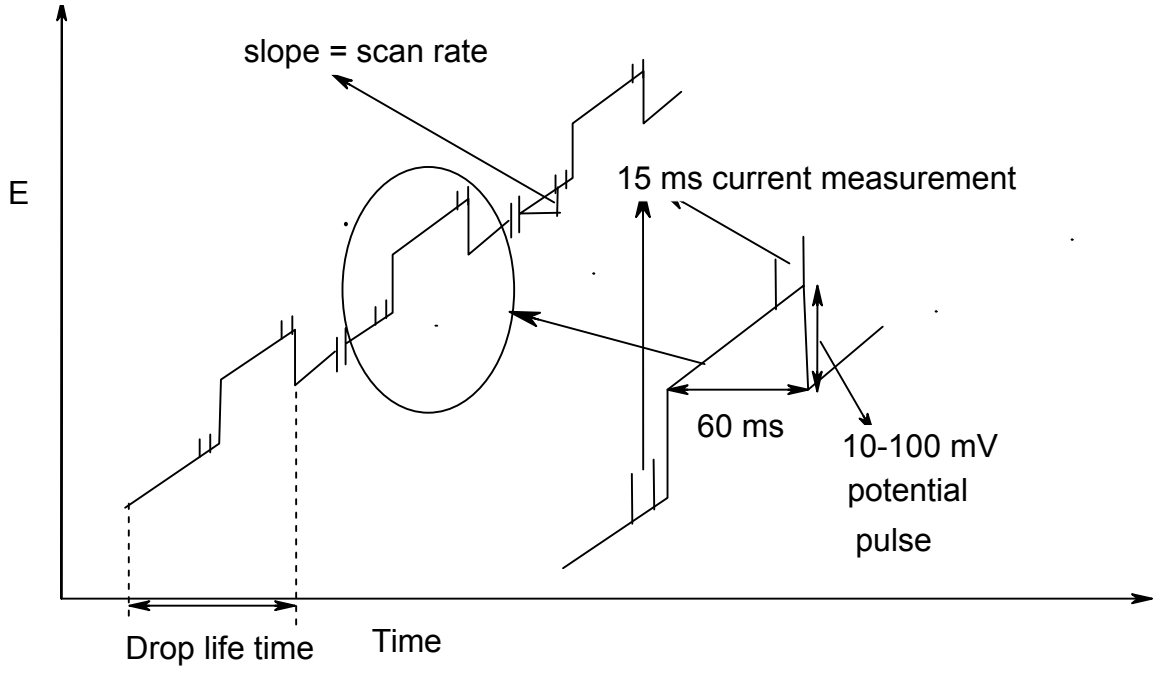

Figure 3.4 The profile of the potential pulse and current measurement in differential pulse polarography [36].

Current is measured once before the pulse and again for the last 15 ms of the pulse. The polarograph subtracts the first current from the second and plots this difference versus the applied potential (measured just before the voltage pulse). The resulting differential pulse polarogram is nearly the derivative for a direct current polarogram as shown in Figure 3.5. The two current represents the current at two potential values separated about 10-100 mV (the pulse amplitude). This technique produces a peak with highest current signal at roughly the half wave potential of the classical dc and normal pulse polarography.

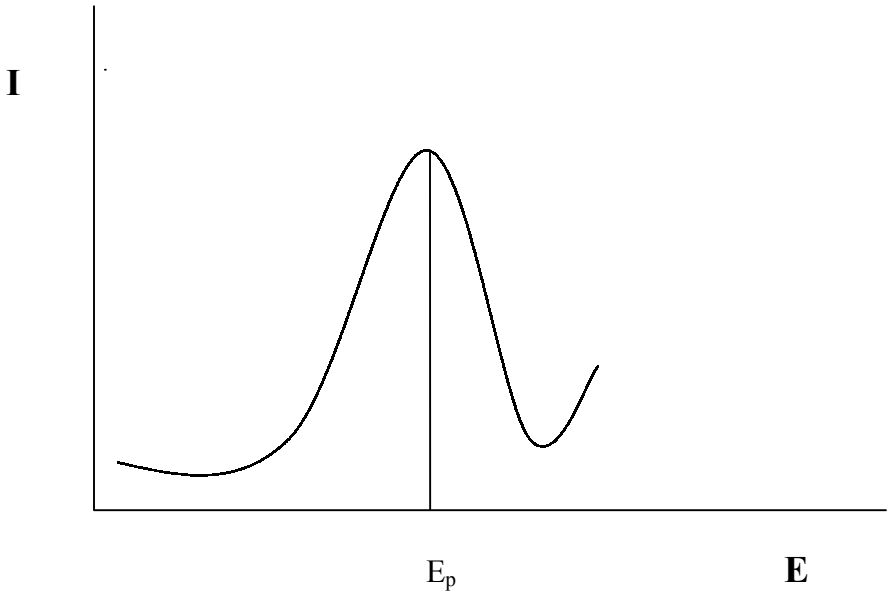

Figure 3.5 Voltamograms for a differential pulse polarography

The potential of the peak E_p is indicative of which species is involved. The concentration of the species controls the current in case the redox mechanism is diffusion controlled. The area under peak and its height are proportional to the concentration of species under reduction (or oxidation) mechanism.

The enhanced sensitivity of differential pulse polarography relative to direct current polarography is due to an increase in faradaic current and a decrease in condenser current. Differential pulse polarography provides better resolution of adjacent waves than does normal pulse polarography, because it is easier to distinguish partially overlapping derivative maxima than partially overlapping polarographic waves. For quantitative analysis, the current at the height of a peak is measured in differential pulse polarogram and subtracted from the residual current at the same potential measured in the absence of analyte. The differential pulse technique has typical limits of detection 10^{-8}-10^{-6} mol/L magnitude order for the normal pulse technique while for the classical dc it would be only about 10^{-4} mol/L magnitude order.

Differential pulse polarography can increase the sensitivity if the signal has been reduced by depletion because it reduce the noise and show better resolution. At low concentration levels the favorable signal to noise ratio of differential pulse polarography gives well-defined peaks where no dc response can be recovered. The current measurement is only taken in very brief pulses at the end of the drop lifetime, although depletion occurs throughout the drop lifetime. A capacitive current will be reduced to its almost constant and lowest level at the end of the drop lifetime. The capacitive and nonfaradaic currents have very little effect due to the potential jump 10-100mV, while a small potential jump will cause a large Faradaic current change specially at the peak potential. Thus the differential pulse mode allows the maximum differentiation of the analytical signals from the background ones.

Differential pulse voltammetry output signal is influenced by many factors like potential pulse amplitude and scan rate. So these variables must be selected carefully to have a good peak resolution that is related directly to the concentration of the enantiomer.

3.6.1. Potential pulse amplitude

Potential pulse amplitude was selected in the range of 10-100mV. The fixed pulse amplitude (usually 50 mV) is the only choice to be made before the analysis. Parry and Osteryoung have derived an equation of the peak current I_p for a totally thermodynamically reversible electrode process controlled by diffusion:

$$I_p = \frac{n^2 F^2 Ac}{4RT}\left(\frac{D}{\pi t}\right)\Delta E \qquad (3.13)$$

where ΔE is the amplitude of the potential pulse, n is the number of electrons changed per mole of analyte, A is the area under the peak, c is the concentration, t is the drop lifetime, R is gas constant, T is the temperature and F is Faraday's number. The equation shows that the peak height is proportional to the concentration and the potential pulse amplitude. The higher potential pulse amplitude produces the greater sensitivity, but increasing the potential pulse amplitude results in peaks broadening and loss of resolution. Two close lying peaks will not be resolved unless the pulse amplitude is significantly smaller than the separation in the two peak potentials. The choice of potential pulse amplitude must be a compromise between a higher value for increased sensitivity and lower amplitude for increased resolution. This is particularly true for thermodynamically irreversible process that produces broader, lower and less well formed peaks than do reversible processes.

3.6.2. The scan rate

The scan rate (mV s^{-1}) plays an important role in the peak resolution. The suitable scan rate must compromise between the adequate resolution and required time for analysis. High scan rate will be too coarse for adequate resolution and the slowest scan rate gives the best resolution results with longer time of analysis.

3.6.3 Peak area and peak height

Peak height and peak area are directly proportional to the current of electrolysis and hence to the concentration of analytical species to be determined. The area under peak will remain constant for the determination of the same concentration of the enantiomer; in most of the cases even though the adsorption of other inactive species can alter the reversibility and electrode kinetics of the process producing sometimes huge change in the shape of the peak. Because DPV is particularly susceptible to surface active phenomena, the utilization of diamond as electroactive material in the design of amperometric electrodes minimizes the contamination of the surface by adsorptive processes favoring a long term of utilization of the electrode and a higher sensitivity of the measurement.

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