Chapter 2

Enantioselective,potentiometric membrane electrodes

2.1 Introduction

Enantioselective, potentiometric membrane electrodes (EPMEs) were particularly developed for enantioanalysis of pharmaceutical compounds. Development of new electrodes materials and more sensitive and stable electronic components has gained momentum in the last two decades and has resulted in an increase in the range of analytical applications utilizing potentiometric electrodes. The use of EPMEs for analysis of chiral compounds is well documented and, considering the enormous increase in publications, it can be expected that in the near future chiral analysis by EPMEs will be widely applied to pharmaceutical and clinical samples [1].

The accuracy obtained when EPMEs were used in clinical analysis made their utilization a valuable alternative for chromatographic techniques [1, 2]. The method is rapid, precise, and not expensive. The high reliability of the analytical information obtained using these electrodes made automation of potentiometric techniques possible, by the integration of enantioselective electrodes as detectors in flow injection analysis (FIA) [3, 4] and sequential injection analysis (SIA) [5, 6] systems. The type of electrode and chiral selector must be selected in concordance with the complexity of the structure of the enantiomer to be determined. The principle of molecular recognition for EPMEs is the selective binding between a molecule with a special chemical architecture (chiral selector) and the enantiomer. The thermodynamics of the reaction between enantiomers and a chiral selector plays the main role in molecular interaction of enantiomers using this type of electrodes.

If L is the chiral selector and S and R the enantiomers to be determined, the following reactions take place:

$$L + S \leftrightarrow LS \qquad K_S$$

$$L + R \leftrightarrow LR \qquad K_R$$

where S and R are enantiomers to be determined, L is the chiral selector, LS and LR are the complexes formed between R(S)-enantiomer and L, respectively, and K_S and K_R are the stability constants of the complexes formed between chiral selector and enantiomers.

The stability constants (K_S and K_R) of the complexes formed between chiral selectors and R- and S-enantiomers are given by the following equations:

$$K_S = e^{-\frac{\Delta G_S}{RT}} \tag{2.1}$$

$$K_R = e^{-\frac{\Delta G_R}{RT}} \tag{2.2}$$

where ΔG_S and ΔG_R are the free energies recorded for the S- and R-enantiomer reactions with the chiral selector, L; R = 8.31 J/mol K is the gas constant and T is the temperature in Kelvin.

The efficiency of the chiral selector is given by the difference between the free energies of reactions (1) and (2):

$$\Delta(\Delta G) = \Delta G_S - \Delta G_R \tag{2.3}$$

The log K_S is directly propotional to ΔG_S and log K_R is directly propotional to ΔG_R, respectively. This means that a difference in the free energies of the reactions will result in a difference of the stability of the complexes formed between the chiral selector and the S- and R-enantiomers. Therefore, the stability of the complexes is directly correlated with the response (slope) of the EPMEs [7]. Accordingly, a large difference between the free energies of the reactions of chiral selector with S- and R-enantiomer will give a large difference between the slopes when S- and R-enantiomers will be determined. The enantioselectivity of the measurement is given by the difference between the two free energies. The slope is a measure of enantiorecognition. The minimum value tolerable for a 1:n stoichiometry between the enantiomer and chiral selector is 50/n mV/decade of concentration [8].

2.2 Selection of chiral selectors for the construction of enantioselective, potentiometric membrane electrodes

The selection of the chiral selector must take into account the structure and the size of the enantiomers that has to be determined. The most utilized chiral selectors for the construction of enantioselective, potentiometric membrane electrodes are from the classes of crown ethers [9-12], cyclodextrins [13-19], and maltodextrins [20]. The enantioselectivity of these chiral selectors is given by an internal selectivity (the size of the cavity of the chiral selectors), and by an external selectivity (due to the arrangement, size and type of radicals, atoms or ions bound on the external chain of the chiral selector). While in the case of crown ethers and cyclodextrins the enantioselectivity is mainly due to the external selectivity of the selectors, when maltodextrins are utilized as chiral selectors, the enantioselectivity is due to the internal selectivity of maltodextrin used, because the size of the cavity is dependent

on the dextrose equivalent of maltodextrin. It was found that the lower dextrose equivalent will give the higher enantioselectivity. In order to select the best chiral selector, the roles of chiral selectors and enantiomers are changed. Therefore, the enantioselective, potentiometric membrane electrode is constructed using the enantiomer in the membrane design, and the chiral selectors are the analytes to be measured. The chiral selectors that give the best slopes when they are analyzed are then considered for the molecular recognition of the enantiomer used in the membrane design.

2.3 Design of enantioselective, potentiometric membrane electrodes

The design of enantioselective, potentiometric membrane electrode (EPMEs) plays a very important role in the reliability of analytical information. The evolution concerning the design of EPMEs made their utilization a very accurate and precise alternative for structural analysis techniques [21]. The reliability of the response characteristics as well as the analytical information obtained using EPMEs is strictly correlated to the reliability of the electrodes design [7]. Only a reliable design of EPME will give reliable response characteristics and reliable analytical information.

One of the designs proposed for sensors is based on the impregnation of a chiral selector on a conducting layer such as PVC; imprinting polymers, and a carbon paste matrices. The repartition of chiral selector in the plastic membrane is not homogeneous and not reproducible. The liquid membrane needs a support characterized by certain porosity that assures reliability in construction. Accordingly, the most reliable design is that of EPME based on carbon paste that is preferred due to the simplicity and reliability of the construction of electrode.

2.3.1 Modified paste electrode design

One of the most reproducible designs for EPME based on carbon paste has been proposed by Stefan *et al* [22-24]. Graphite powder proved to be a very good material for electrode design. Mixing paraffin oil with the graphite forms the carbon paste. The paraffin oil and graphite powder were mixed in a ratio of 1:4 (w/w) followed by the addition of a solution of chiral selector (10^{-3} mol/L), 100 µL of chiral selector solution is added to 100 mg of carbon paste. The plain carbon paste was filled into a plastic pipette peak leaving 3 to 4 mm empty space in the top to be filled with the modified carbon paste. The optimum diameter of the EPME is 3 mm. Electrical contact is made by inserting a Ag/AgCl wire in the plain carbon paste. The surface of the electrode can be renewed by simply polishing it with alumina paper. Because the electrode response is directly propotional to the complex formed at the membrane-solution interface, different types of chiral selectors were proposed for the design of EPMEs such as crown ether, cyclodextrins and its derivative, maltodextrins, macrocyclic antibiotics and fullerenes.

2.4 Response characteristics of EPME

The functional relation between the potential, E measured at I = 0, and the activity, a, of the enantiomer gives the electrode function (Figure 2.1). The potential is not dependent on the activity, a of the ion, but on –log a. The same type of function can be deducted from the Nernst equation:

$$E = f(-\log a_i) \qquad (2.4)$$

Usually, the ionic strength is kept constant by the addition of a strong electrolyte to each solution (e.g., NaCl, KCl), or by buffering the solution with a buffer that can also maintain the ionic strength at a constant value. Accordingly, the activity can be

substituted with the concentration, and further more for an ion M^{z+}, pM = -log $C_M{}^{z+}$ is used, and the electrode function is given by:

$$E = f(pM). \quad (2.5)$$

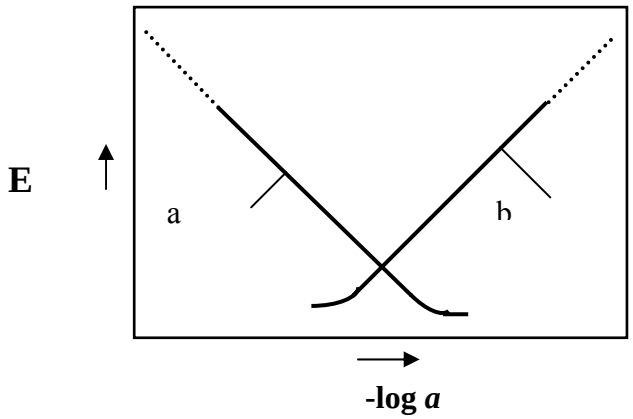

Figure 2.1 EPME function. (a) cation-selective electrode; (b) anion-selective electrode.

2.4.1 Standard electrode potential, E^0

IUPAC defined standard electrode potential as the value of the standard emf of a cell in which molecular hydrogen is oxidized to solvated protons at the left-hand electrode [25]. E^0 does not depend on the concentration of the ions in solution and can be determined graphically from the calibration graph of the potentiometric electrode (Figure 2.2).

The value of standard electrode potential is also recommended to be determined using the linear regression method as one of the parameters of the equation of calibration of EPME:

$$E = E^0 \pm S \times pM \quad (2.6)$$

where E is the potential of the electrode, E^0 is the standard electrode potential, S is the slope, and pM = -log C_M.

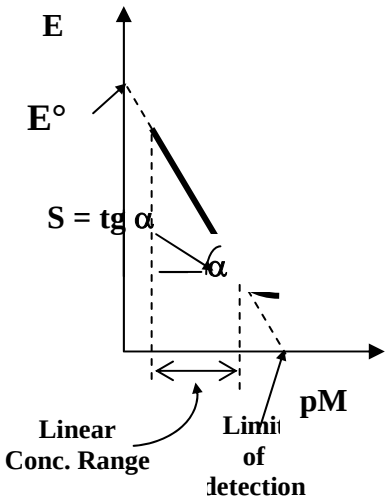

Figure 2.2 Response characteristics of EPME

2.4.2 Response of EPME

The slope, S (also called response of the electrode), is the main characteristic of the potentiometric electrodes. The ideal value of the slope is given by Nernst: 59.16/z mV/decade of concentration, where z is the charge of the ion that has to be determined. This value can be computed from the equation of Nernst:

$$E = E^0 \pm \left(\frac{RT}{zF}\right) \log a \qquad (2.7)$$

where E is the potential of the electrode, E^0 is the standard electrode potential, R = 8.31 J/mol K, F = 96500 C, T = 298 K, and a is the activity of the ion. From this equation, the slope of the potentiometric electrode is given by:

$$S = \frac{RT}{zF} \qquad (2.8)$$

Nernstian response implies ideal sensitivity, but not necessarily ideal selectivity since interfering ions may also give Nernstian response when present as the sole potential determining species. The minimum acceptable value of the slope of potentiometric electrodes for bioanalysis is 50/z mV/ decade of concentration [26]. The slope is dependent on the stability of the compound formed at the membrane-solution interface [27]. The value of the slope can be deducted using the equation of dependence of slope on the stability of the compound formed at the membrane-solution interface [27].

$$S = S^o - a + \left(\frac{b}{S^o}\right)\log \beta_S \qquad (2.9)$$

where S is the slope of the electrodes (mV/decade of concentration), S^0 is the Nernstian slope (59.16 mV/decade of concentration), β_S is the stability constant of ion-pair complex, and a and b are two coefficients depending on the membrane composition [27].

The slope can be determined experimentally as follows:

1. tangent of the angle made by the calibration curve and pM axis (Figure 2.6);
2. as a parameter of the equation of calibration by using the linear regression method.

The slope depends on some parameters which characterize the matrix such as polarity of the plasticizer, oil or solvent. The slope of the potentiometric electrodes could be improved by selecting the suitable chiral selector that forms a compound with higher stability or by changing the composition of the matrix.

2.4.3 Limit of detection

IUPAC defined the limit of detection as the concentration at which, under specified conditions, the cell potential, E, deviates from the average value by a multiple of the standard error of a single measurement of the cell potential in this region [63]. The limit of detection of EPME depends on the values of standard electrode potential, slope and the stability of the compounds formed at membrane-solution interface. The internal solution of EPME influences the value of the limit of detection. By using 0.1 mol/L KCl as internal solution, the detection limits obtained for EPMEs are very low.

The value of the limit of detection can be deducted from the calibration graph of EPME, as the concentration (activity) of the ions at the point of intersection of the extrapolated linear calibration curve and activity (or concentration) axis.

2.4.4 Linear concentration range

The linear concentration range represents the range of concentration of an analyte (or an ion) over which the sensitivity of the electrode is constant within a specific variation, usually ± 5 %. The linear concentration range can be determined from the plot of the cell potential difference versus the logarithm of responsive ionic activity (or concentration) (Figure 2.2). The linear response range is very important for EPME because all the solutions required for measurement must have the activity (concentration) of the substances within the linear range. The reproducibility of the linear range is influenced by stirring rate of the solution, composition of the solution containing the proposed substance for measurement, pH of the solution, the precondition of the electrode, temperature, composition of the solution where the electrode was exposed before the measurement [28].

2.4.5 Influence of pH

The pH can influence the formation of protonated and unprotonated species of the same substance. It is very important to determine for EPME the dependence of their potential on the pH variation. Special care must be accorded to the buffering of solutions, because a small difference on pH may cause a significant change in the potential, and that will result in an error in the measurement.

2.4.6 Influence of the temperature on the response of the electrode

The slope of the electrode is highly affected by the temperature. The kinetics and thermodynamics of the processes that take place at the electrode surface are favoured by the increase of temperature, and accordingly the slope will increase. The temperature must be maintained at a constant value during the measurements of standard sample solutions. A temperature of 298 K is recommended for electrode characterization.

2.4.7 Response time

IUPAC defined the response time as the time which elapses between the instant when the electrodes of the potentiometric cells are brought into contact with the 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 [25]. EPME response time is influenced by the membrane-solution interface processes. This response time equals the sum between the time required for the ion or molecule to be extracted in the membrane-solution interface and the required time for ions/molecules to reach equilibrium stage of complexation

or precipitation or redox. For EPME, the response time depends on the concentration and the stability of the complex formed between the analyte molecules and the chiral selector at the EPME surface-solution interface. The response time increases with decreasing the concentration of the molecule that has to be assayed. EPME of short response times are preferred to be used in bioanalysis.

2.4.8 Ionic strength and activity coefficients

The ionic strength and the activity coefficients are also playing a very important role in the accuracy of the measurements. To avoid the differences in the potential readings, which can cause another source of error due to the variations of the activity coefficients of the ions in solution, it is necessary to work at the same ionic strength [28]. The utilization of strong electrolytes (NaCl, KCl) and some of the buffers in the standard and sample solutions preparation can ensure a constant ionic strength.

2.5 Selectivity of enantioselective, potentiometric membrane electrodes

Selectivity is one of the basic characteristics of the electrochemical sensors. It depends on the composition of the membrane (active sites as well as matrix), ratio between the activities of the main ion and interfering ion in the solution, complexity of the matrix of the sample that is analysed, current applied, and the pH of the solution. This property of electrochemical sensors restricts their utilization for the assay of an ion from a complex matrix (e.g., environment). Usually, these electrodes have group selectivity. EPME selectivity is high when utilized for clinical analysis including pharmaceutical analysis.

IUPAC defined the interfering substance as any substance, other than the ion being determined, whose presence in the sample solution affects the measured emf of a cell. There are two classes of interfering substances that affect the EPME potential signal:

(i) "electrode/electrochemical" (substances whose response is similar to that ion being determined, or electrolytes present at high concentration) interferences;

(ii) "chemical" interferences (substances that interact with the ion being determined, so as to decrease its activity or apparent concentration, e.g., H^+, OH^-, or substances that interact with the membrane surface).

The selectivity degree of EPME is given by the values of the potentiometric ($K_{i,j}^{pot}$) selectivity coefficients respectively, as follows:

(i) For magnitude order higher than 10^{-3}, the ion tested for interference interfere strongly;

(ii) For a magnitude order of 10^{-3}, the ion tested for interference is not a strong interferent;

(iii) For a magnitude order less than 10^{-3}, the ion does not interfere.

The Nicolsky-Eisenman equation is the main equation that gives the relation between the potentials of the electrode measured in the presence of the interfering ions and the potentiometric selectivity coefficients:

$$E = \text{constant} + \left(\frac{2.303RT}{z_j F}\right) \log\left(a_i + \sum_{j=1}^{N} K_{i,j}^{pot}\, a_j^{\frac{z_i}{z_j}}\right) \qquad (2.10)$$

where E is the experimentally recorded emf of the cell when the only variables are the activities in the test solution; R is the gas constant that equals to 8.314 J/Kmol; T is the temperature (in degrees Kelvin), F is the Faraday constant which equals to 96500 C/mol; a_i is the activity of the main ion and a_j is the activity of the interfering ion; N is the number of the interfering species in the solution, $K_{i,j}^{pot}$ is the potentiometric selectivity coefficient.

The Nicolsky-Eisenman equation was modified by Buck, by substituting the charge numbers through their absolute values [66]:

$$E = \text{constant} + \left(\frac{2.303RT}{z_j F}\right) \log\left(a_i^{\frac{1}{|z_i|}} + \sum_{j=1}^{N} K_{i,j}^{pot}\, a_j^{\frac{1}{|z_j|}}\right) \qquad (2.11)$$

For cation sensors the sign of the log term is positive and for negative sensors the sign is negative and the standard electrode potential is represented by the constant term.

The potentiometric, selectivity coefficient, $K_{i,j}^{pot}$ can be determined experimentally using two methods, mixed solution method and separate solution method. The potentiometric selectivity coefficients is recommended to be determined at a ratio between main and interfering species of 1 : 10.

2.5.1 Mixed solution method

The potential of the solution that contains both the main and interfering ion, is compared with the one recorded for the solution that contains only the main ion provided that the main ion has the same activity in both solutions.

The equation used for the calculation of the potentiometric selectivity coefficient is:

$$K_{i,j}^{pot} = (10^{\frac{\Delta S}{E}} - 1) \times \frac{a_i}{a_j^{\frac{z_i}{z_j}}} \qquad (2.12)$$

where ΔE is the difference between the potentials recorded for mixed solution ($E_{i,j}$) and for the solution that contains only the main ion (E_i), $\Delta E = E_{i,j} - E_i$ (all in mV); S is the slope of the electrode from the calibration graph or from the linear regression equation (in mV/decade of concentration); a_i and a_j are the activities of both the main ion and the interfering ion, i, and j; z_i and z_j are the charges of both the main and interfering species, i, and j.

2.5.2 Separate solution method

There are two ways to determine the potentiometric selectivity coefficient using the separate solution method:

(i) The emf of a cell comprising an ion-selective electrode and a reference electrode is measured for each of two separate solutions, one containing the main ion of the activity, a_i, and the other one containing interfering ion at the same activity, a_j as the main ion from the first solution ($a_i=a_j$).

The potentiometric selectivity coefficient is given by the equation:

$$\log K_{i,j}^{pot} = \frac{\Delta E}{S} + (1 - \frac{z_i}{z_j}) \log a_i \qquad (2.13)$$

where ΔE is the difference between the potentials recorded for the soluti on of only the interferent, E_j, and for the solution that contains the main ion only, E_i and $\Delta E = E_j - E_i$ (all in mV) and all other terms have the same significance definition as in equation (2.12).

(ii) The activities of two different solutions that are introduced separately into the cell comprised of the enantioselective, potentiometric membrane electrode and a reference electrode are adjusted with each of two different solutions, one containing only the main ion of the activity a_i, and the other containing only the interfering ion, of the activity a_j, with the aim of measuring the same potential. The following equation can be used to calculate the potentiometric selectivity coefficient:

$$K_{i,j}^{pot} = \frac{a_i}{a_j^{\frac{z_i}{z_j}}} \qquad (2.14)$$

where all the terms have the same significance as in equation (2.12).

2.6 Direct potentiometric method

Direct potentiometry is a very simple method to be applied. Potentiometric methods are based on the measurement of a potential difference beween two electrodes (indicator and reference electrode) immersed in a solution containing the analyte. The indicator electrode is chosen to respond to a a particular enantiomer in solution. The

reference electrode is the one for which the half-cell potential is constant. The potential of an electrochemical cell is given by the following equation:

$$E_{cell} = E_{ind} - E_{ref} + E_{ij} \quad (2.15)$$

where E_{cell} is potential of the electrochemical cell; E_{ind} is half-cell potential of the indicator electrode (cathode); E_{ref} is half-cell of potential of the reference electrode and E_{ij} is the liquid-junction potential.

Calibration procedure of EPME assumes that during the measurements the slope of the electrode is constant and the concentration of the determined enantiomer is propotional to the developed potential. To obtain the best precision of measurements it is necessary to calibrate the working electrode just before the assay of the samples. The pH and ionic strength for the samples must be adjusted to the same values of the solutions used for calibration of the electrodes. Direct potentiometry is applied for the analysis of substance with chiral centers. The solutions used for calibration are obtained from standard solutions, by serial dilution. All solution must be buffered. A calibration curve is obtained by plotting the emf of the cell against the negative logarithm of the main species concentration. The values of emf obtained for the samples are interpolated on the calibration plot from where the unknown concentration of the enantiomer can be determined

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