Chapter 2

Enantioselective, potentiometric membrane electrodes

2.1. Introduction

Enantioselective, potentiometric membrane electrodes (EPMEs) were particularly developed for enantioanalysis. EPMEs based on 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin were proposed for the assay of angiotension-converting enzyme inhibitors [1-5] as well as for L-proline [6].

Rapid development of new electrodes materials and more sensitive and stable electronic components in the last two decades has increased the range of analytical applications utilizing potentiometric electrodes. The fast development of this field is a scale of the degree to which potentiometric measurements satisfy the need of the clinical chemist for rapid, low cost and accurate analysis.

The accuracy obtained when EPMEs were used in clinical analysis made their utilization a valuable alternative for chromatographic techniques [7, 8]. The method is rapid, precise, and not expensive. The high reliability of the information obtained using these electrodes made automation of potentiometric technique possible, by the integration of enantioselective electrodes as detectors in FIA [9, 10] and SIA [11, 12] 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. Therefore, the chiral selector plays the main role in molecular interaction.

$$L + CS \rightleftharpoons L\text{-}CS \qquad K_L$$

$$D + CS \rightleftharpoons D\text{-}CS \qquad K_D$$

where L and D are enantiomers to be determined, CS is chiral selector, L-CS and D-CS are the complexes formed between L(D)-enantiomer and CS, respectively, and K_L and K_D are the stability constants of the complexes formed between chiral selector and enantiomers.

The stability constants (K_L and K_D) of the complexes formed between chiral selectors and L- and D-enantiomers are given by the following equations:

$$K_L = e^{-\frac{\Delta G_L}{RT}} \tag{2.1}$$

$$K_D = e^{-\frac{\Delta G_D}{RT}} \tag{2.2}$$

where ΔGL and ΔG_D are the free energies recorded for the L- and D-enantiomer reactions with the chiral selector, CS. R is the gas constant = 8.31 J/mol K and T is the temperature in Kelvin.

The log K_L is directly proportional to ΔG_L and log K_D is directly proportional to ΔG_D, 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 L and D enantiomers. Therefore, the stability of the complexes is directly correlated with the response (slope) of the EPMEs [13]. Accordingly, a large difference between the

free energies of the reactions of chiral selector with L- and D-enantiomers will give a large difference between the slopes when L and D enantiomers will be determined. The enantioselectivity of the measurements is given by the difference between the two free energies. Also, 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 [14].

2.2. Design of enantioselective, potentiometric membrane electrodes

The design of enantioselective, potentiometric membrane electrodes (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 [15]. 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 [13]. 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 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.

2.2.1. Modified paste electrode design

Graphite powder proved to be a very good material for electrode design. Mixing oil (paraffin or nujol oil) with the graphite powder is forming carbon paste. One of the most reproducible designs for EPME based on carbon paste has been proposed by Stefan et al [16-18]. 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 (ligand) (10^{-3} mol/L) (100 μL of chiral selector solution is added to 100 mg carbon paste). The plain carbon paste was filled into a plastic pipette peak leaving 3 to 4 mm empty in the top to be filled with the modified carbon paste. The optimum diameter of the designed EPME is 3 mm. Electrical contact is made by inserting a silver 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 proportional 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 derivatives, maltodextrins and macrocyclic antibiotics.

2.2.1.1 Cyclodextrins as chiral selector in the EPMEs design

Cyclodextrins (CDs) are oligosaccharides prepared by enzyme degradation of starch and glycosyltransferases of cyclodextrinases producing a mixture of different CDs [19]. The most frequent used CDs as chiral selectors are those consisting of six (α-CD), seven (β-CD) and eight (γ-CD) glucopyranose units with a truncated cone shape providing a hydrophobic cavity (Figure 2.1). Due to the presence of hydroxyl groups (position 2, 3 and 6 of glucopyranose), the outside ring of CD is hydrophilic [20]. The inner diameter

of α-, β- and γ-CDs is increasing as a number of glucose units increases from 0.57 to 0.78 and 0.95 mm, respectively. CDs have a suitable solubility in aqueous medium. β-CD has the lowest solubility (1.85 g/100ml water), caused by the existence of intermolecular hydrogen bonding [21].

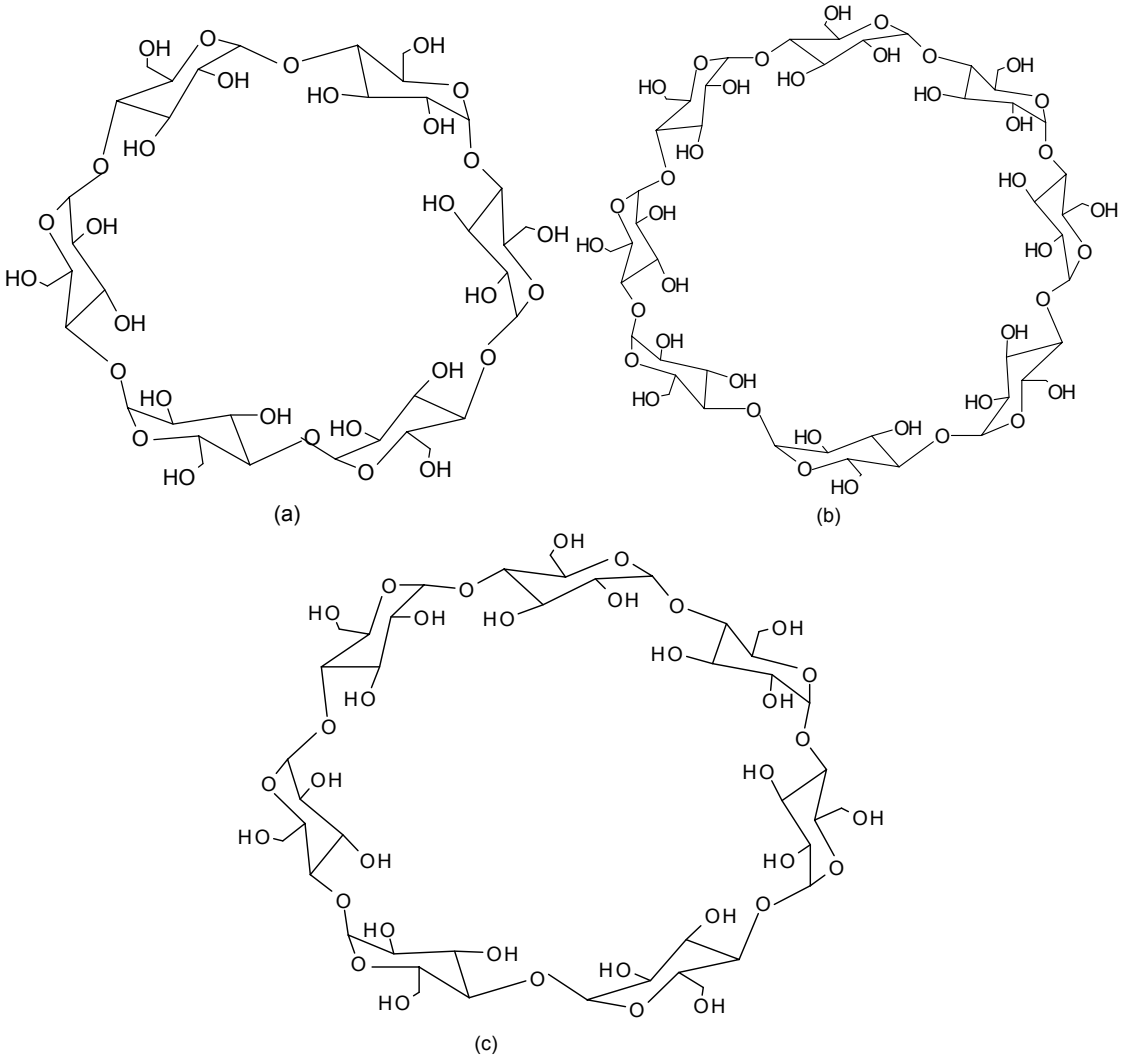


Figure 2.1 (a) α-cyclodextrin, (b) β-cyclodextrin and (c) γ-cyclodextrin

CDs have the enantioselectivity property because of its cavity dimension, providing sufficient interactions with analytes to form the host-guest complex inclusion [22].

Several cyclodextrin derivatives have been developed in order to improve the external enantioselectivity of CDs [23]. EPMEs based cyclodextrin derivatives proved good enantioselectivity and reliable analytical information. Stefan et al. proved the suitability of 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin based EPME for the enantioanalysis of the S-enantiomer of angiotension-converting enzyme inhibitors [2, 3].

2.2.1.2 Maltodextrins as chiral selectors in the EPMEs design

Maltodextrins proved to be suitable chiral selectors for compounds with acidic moieties [24]. Hydrolysis of starch by means of heat and acid or specific enzymatic treatments or combined acid and enzymatic hydrolysis yields a spectrum of depolimerized oligomers [25]. These hydrolyzates are described in terms of their dextrose equivalent (DE) value, which is a measure of the total reducing power of all sugar present relative on a dry weight basis. Maltodextrins are hydrolyses products of starch with DE lower than 20. They are produced be enzyme-catalyzed conversion using α-amylase (1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1) from *Bacillus subtilis* and pullulanase (pullulan 6-glucanohydrolase, EC 3.2.1.41) [26]. In general, linear maltodextrins consists of D-(+)-glucose units connected through Glu-(1-4)-α-D-Glu linkages (Figure 2.2). Up to now, three types of maltodextrins with different DE values (I (4.0-7.0), II (13.0-17.0) and III (16.5-19.5)) were used as chiral selectors in enantioanalysis. Hygroscopicity, solubility, osmolarity, and their effectiveness to reduce the freezing point increase with increasing DE, while viscosity, cohesiveness and coarse-crystal prevention increase as DE decreases. Enantioselective, potentiometric membrane electrodes based on maltodextrins have been applied for the enantioanalysis of several drugs [27].

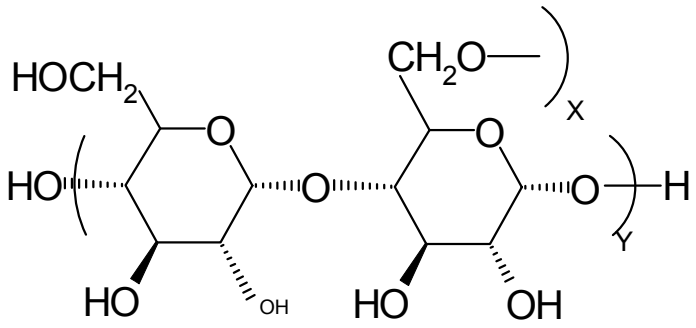
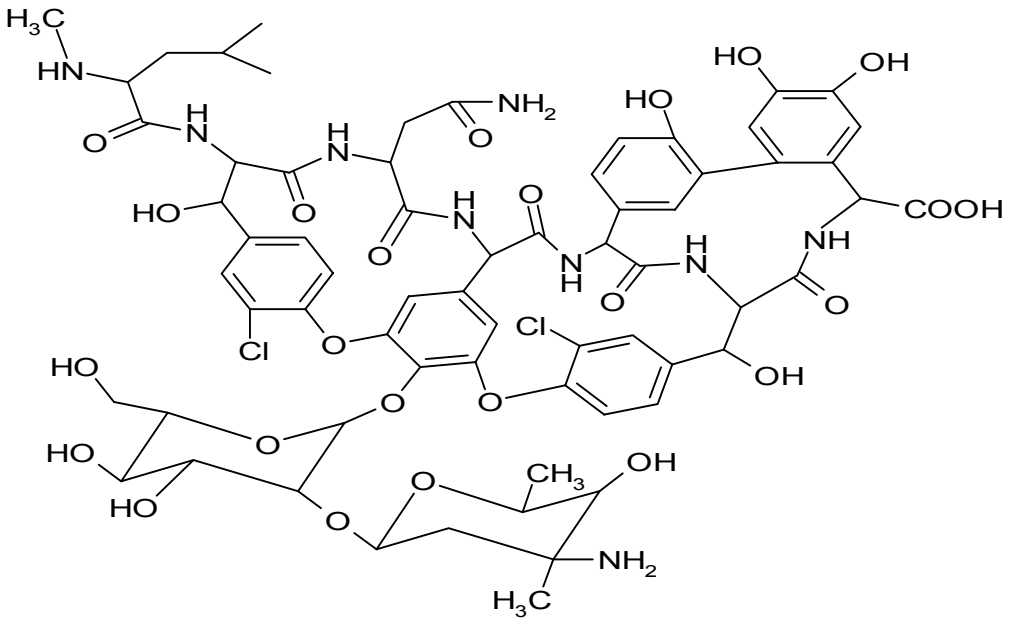


Figure 2.2 Maltodextrin

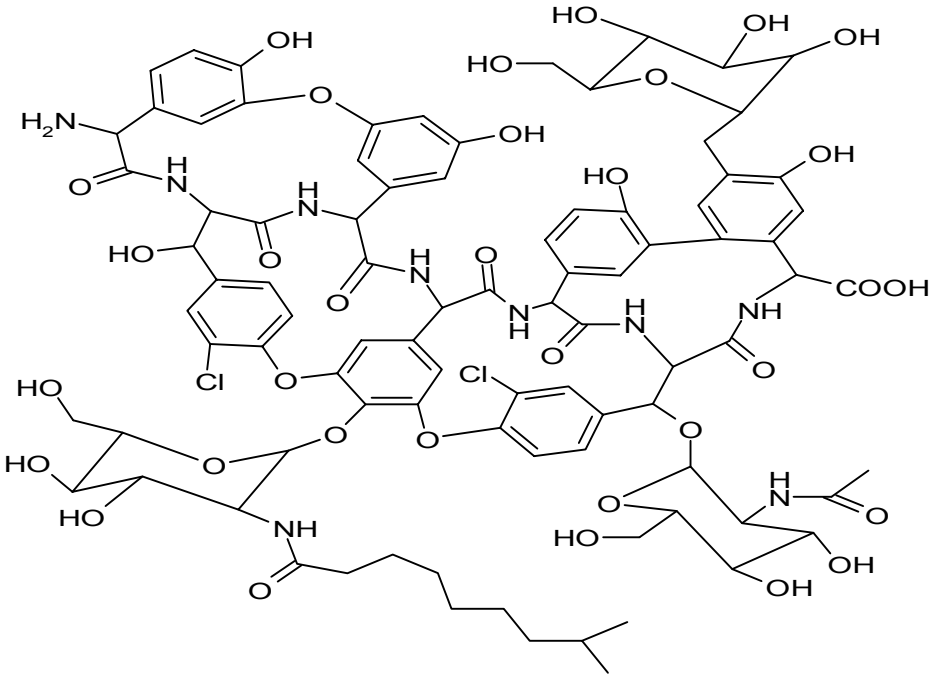
2.2.1.3. Macrocyclic antibiotics as chiral selectors for EPMEs design

Macrocyclic antibiotics have been successfully used as chiral selectors for the enantiorecognition of several classes of pharmaceutical enantiomers of drugs and molecules with biological importance. The high selectivity and efficiency in molecule discrimination make antibiotics a typical chiral selector of biological origin. Macrocyclic antibiotic contains several functional groups responsible for multiple stereoselective interactions. All macrocyclic antibiotics exhibit very similar physico-chemical properties, but they show a different stereoselective power [28]. The most used macrocyclic antibiotics in enantioanalysis are vancomycin and teicoplanin [29, 30].

Vancomycin is "basket" shaped (Figure 2.3a) with three fused macrocyclic rings and two side chains, a carbohydrate dimmer and a N-methyl leucine moiety [31]. It has 18 asymmetric centers and several functional groups such as carboxylic, hydroxyl, amino, amido and aromatic rings [28]. Vancomycin is very soluble in water and can dimerize in aqueous solutions depending on vancomycin concentration [32].



(a)



(b)

Figure 2.3 (a) Vancomycin and (b) Teicoplanin

Vancomycin solutions are stable at low temperature and in buffered solutions (pH 3.0-6.0) [32, 33]. This antibiotic is very efficient for the enantiorecognition of anionic compounds containing carboxylic groups in their structure, which could be explained by the presence of amine groups [28].

Teicoplanin (Figure 2.3b) has a long hydrophobic tail, which behaves like surfactant properties. The molecular structure of teicoplanin shows a slightly higher solubility in water than vancomycin [30]. The amide and carboxylic groups are the most important functional groups of teicoplanin used for the enantiorecognition of molecules containing carboxylic groups. These groups are ionized over the 3.5-8.0 pH range [34, 35]. Teicoplanin exhibits a very slight basic behavior even at acidic pH. At low pH, teicoplanin favors aggregation and micelle formation [36].The most common teicoplanin glycopeptide (A_2-2) has a molecular weight of 1877 [34]. Addition of organic modifiers to teicoplanin, such as acetonitrile, improves the resolution ability. These modifiers enhance the enantioselectivity by alerting and/or inhibiting aggregation of teicoplanin monomer producing more teicoplanin molecules available to interact with solutes [28].

2.2.2. Plastic membrane based electrode design

Nowadays, polyvinylchloride (PVC) and its derivatives are the most used in sensor technology. PVC membranes have some disadvantages such as:

1- It is not possible to control the uniformity distribution of the electroactive species in the membrane;
2- PVC membranes have low thermal durability and low mechanical strength.

PVC membranes are prepared by dissolving a polymer (PVC), a large amount of plasticizer, and the sensing compound in an organic solvent. Tetrahydrofuran and cyclohexane can be used as organic solvents. The solvent is allowed to evaporate, leaving a dry membrane attached to the body of the electrode. The ratio between plasticizer and PVC is 70:30 (w/w) [37, 38]. The PVC and plasticizer form the medium of electroactive compound formation. The most used plasticizers are dioctyl phthalate (DOP), dioctyl sebacate (DOS), dinonyl phthalate (DNP) and ortho-nitrophenyl octyl ether (o-NPOE). Crown ethers [39-42] and lipophilic cyclodextrins [43] are used as chiral selectors for the design of PVC membrane based EPMEs

2.3. 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 2.4).

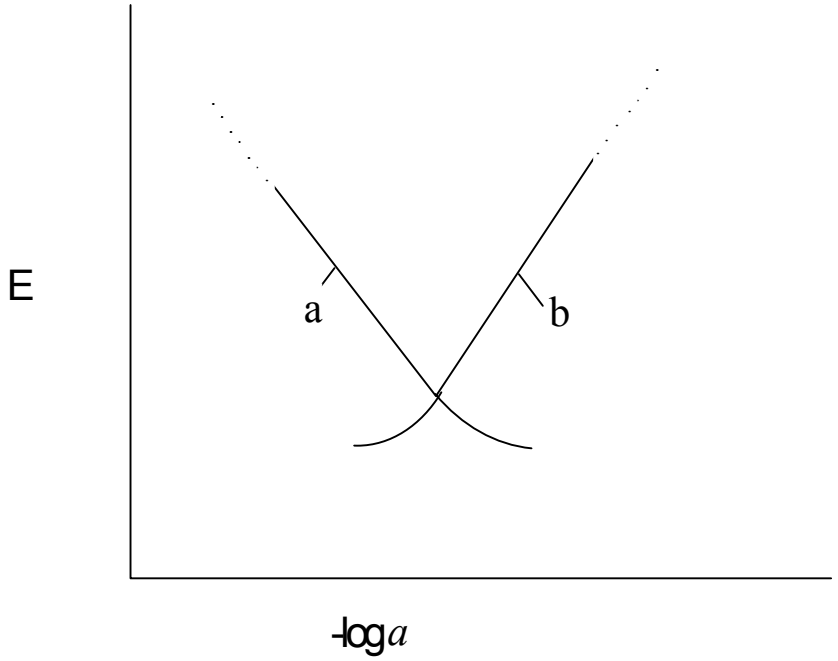


Figure 2.4 EPME function. (a) cation-selective electrode; (b) anion-selective electrode.

The potential is not dependant on the activity, a of the ion, but on –log a:

$$E = f(-\log a_i) \tag{2.3}$$

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).

2.3.1 Standard electrode potential, E^o

The standard electrode potential is defined by IUPAC as the value of standard emf of a cell in which molecular hydrogen is oxidized to solvated protons at the left-handed electrode [44].

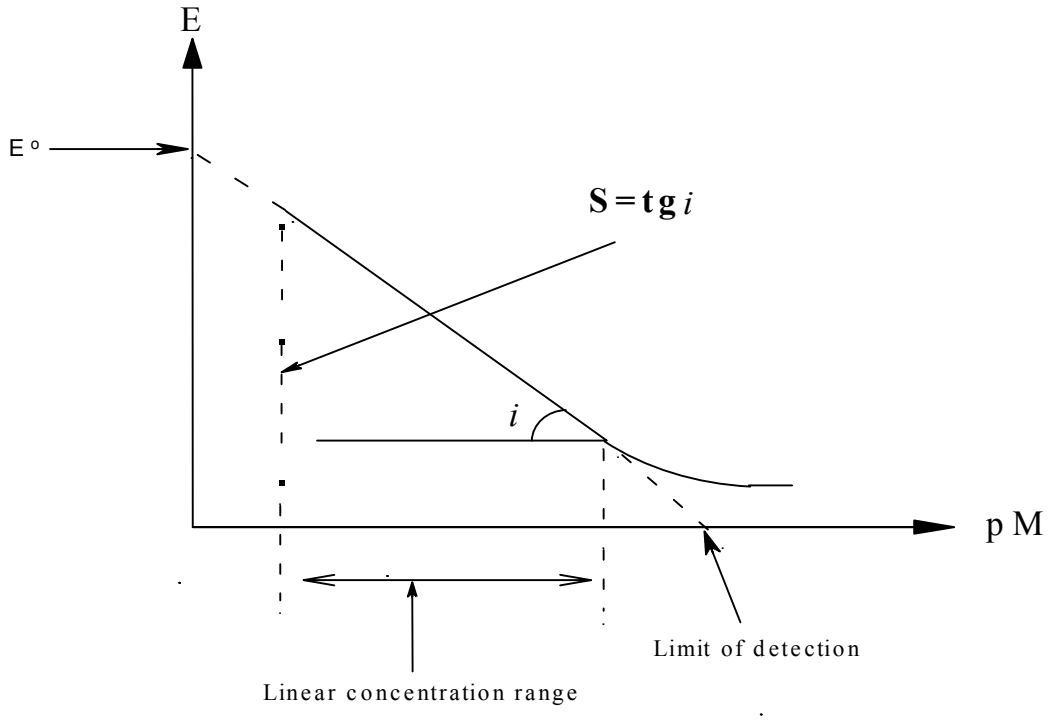


Figure 2.5 Response characteristics of EPME

E^o does not depending on the concentration of the ions in solution and can be determined graphically from the calibration graph of the potentiometric electrode (Figure 2.5).

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:

$$E = E^o \pm SxpM \tag{2.4}$$

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

2.3.2 Response of EPME

The response of EPME is dependant on the slope of the linear part of the calibration graph. It is the main characteristic of the potentiometric electrodes. This value can be computed from the Nernst equation:

$$E = E^o \pm S(\log a) \tag{2.5}$$

where E is the potential of the electrode, E^o is the standard electrode potential, S is the slope, and a is the activity of the ion. The slope of the potentiometric electrode has ideal value given by Nernst (59.16/z mV/decade of concentration) where $S = \frac{RT}{zF}$ (R = 8.31 J/K mol, T = 298 K, z is the charge of the ion that has determined, F = 96500 C). The minimum accepted value of the slope of potentiometric electrodes for bioanalysis is 50/z [45]. 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 response is dependant on the stability of the compound formed at the membrane-solution interface [13]. 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 [13].

$$S = S^o - a + \left(\frac{b}{S^o}\right)\log \beta_S \qquad (2.6)$$

where S is the slope of the electrodes (mV/decade of concentration), S^o 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 [13]. The slope can be determined experimentally as follows:

1- in figure 2.5, tangent of the angle made by the linear part of the calibration curve and pM axis;
2- as a parameter of the equation of calibration by using the linear regression method.

The response depends on some parameters which characterize the matrix such as polarity of the plasticizer, oil or solvent. The response could be improved by selecting the suitable chiral selector (that forms a compound with higher stability) and matrix.

2.3.3 Limit of detection

The limit of detection is defined by IUPAC 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 [44]. The limit of detection of EMPE 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.

2.3.4 Linear concentration range

The definition of linear concentration range is the range of concentration of an analyte over which the sensitivity of the electrode is constant with a specified uncertainty (±5%). It can be determined form the plot of the cell potential difference versus the logarithm of responsive ionic activity (or concentration) (Figure 2.4) 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 solution, composition of the solution containing the proposed substance for measurement, pH of the solution, the preconditioning of the electrode, temperature, composition of the solution where the electrode was exposed before the measurement [46].

2.3.5 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 taken for the buffering of solutions because a small difference on pH may cause a significant error in the potential value.

2.3.6 Influence of the temperature on the response of the electrode

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

2.3.7 Response time

The response time is defined as the elapsed time between the period when the electrodes are immersed in a sample solution and the first time when 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 [44]. 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 EMPE 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.

2.3.8 Ionic strength and activity coefficients

A source of error can be caused due to the variations of the activity coefficients of the ions in the solution containing the molecules required for analysis. For this reason, in EPME optimization it is necessary to run all the measurements at the same ionic strength. The utilization of a strong electrolyte and some of the buffers in the standard and sample solutions preparation can ensure a constant ionic strength.

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2.4 Selectivity of enantioselective potentiometric membrane electrodes

IUPAC defined the interfering species as substance whose presence in the sample solution influence the measured emf of the cell relative to the specie being determined. Unfortunately, no analytical method is totally selective for the analyte species and unaffected by other species in the assay of analyte. Selectivity depends on ratio between the activities of the main molecule/ion and interfering species in the solution, the composition of the membrane, pH of the solution, developed current, and the complexity of the sample matrix to be analyzed. EPME selectivity is high when utilized for clinical analysis including pharmaceutical analysis. Two classes of interfering substances affect the EPME potential signal. First, electrode/electrochemical interferences include substances whose response is similar to the molecule being determined or electrolyte present at high concentration level. Chemical interferences are the second class where it 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 EPME is given by the values of the potentiometric selectivity coefficients 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.

Nicolsky and Eisenman proposed an equation (2.7) that gives the relation between the potential of the electrode recorded in the presence of the interfering ion and potentiometric selectivity coefficients ($K_{i,j}^{pot}$): *constant*

$$E = Q + \frac{2.303RT}{z_i F}\log\left[a_i + \sum_{j=1}^{N} K_{i,j}^{pot} a_j^{z_i/zj}\right] \qquad (2.7)$$

E is the recorded emf of the cell when the only variables are activities in the test solution; Q is constant; R is the gas constant, 8.314 J/K mol; T is the absolute temperature in Kelvin; F is Faraday constant, 96500 C/mol; a_i is the activity of ion/molecule I, a_j is the activity of interfering species J, N is the number of the interfering species in the solution. Nicolsky-Eisenman equation was modified by substituting the charge number through their absolute values [46], as follows:

$$E = Q \pm \frac{2.303RT}{z_i F}\log\left(a_i^{1/|z_i|} + \sum_{j=1}^{N} K_{i,j}^{pot} a_j^{1/|z_j|}\right) \qquad (2.8)$$

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

2.4.1 Mixed solution method

The potentiometric, selectivity coefficient, $K_{i,j}^{pot}$, is given by the difference between the potentials recorded for a mixed solution (containing the main ion and the interfering ions) and the potential recorded for the solution containing only the main specie, I. The main species must have the same activities in both solutions. $K_{i,j}^{pot}$ can be calculated using the following equation:

$$K_{i,j}^{pot} = \left(10^{(\Delta E/S)} - 1\right) x \frac{a_i}{a_j^{(z_i/z_j)}} \qquad (2.9)$$

where $\Delta E = E_{i,j} - E_i$; $E_{i,j}$, is the potential recorded for mixed solution, E_i is the potential recorded for the solution containing only the main species, i (all recorded in mV); S is the slope of the electrode computed from the equation of calibration in mV/decade of concentration; a_i is the activity of the main species, i; a_j is the activity of interfering species, j; z_i and z_j are the charges of the main species (i) and interfering species (j).

2.4.2 Separate solution method

There are two ways to determine $K_{i,j}^{pot}$ 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 only the main ion of the activity, a_i, and the other containing the interfering species at the same activity, a_j, ($a_i = a_j$). The potentiometric selectivity coefficient is given by the equation:

$$\log K_{i.j}^{pot} = \frac{\Delta E}{S} + \left(1 - \frac{Z_i}{Z_j}\right) \log a_i \qquad (2.10)$$

where $\Delta E = E_{i,j} - E_i$; $E_{i,j}$ is the potential recorded for mixed solution, E_i is the potential recorded for the solution containing only the main species, i (all recorded in mV); S is the slope of the electrode computed from the equation of calibration in mV/decade of concentration; a_i is the activity of the main species, i; z_i and z_j are the charges of the main species (i) and interfering species (j).

(ii) The activities of two different solutions introduced into the cell comprised of an EPME and a reference cell, are adjusted with each of two different solutions, one is containing only the main species of the activity a_i, while the other is containing only the interfering ion of the activity a_j, as much as required to achieve the same cell potential measured. From any pairs of activities a_i and a_j for which the cell potential is the same, the $K_{i,j}^{pot}$ may be calculated from the following equation:

$$K_{i.j}^{pot} = \frac{a_i}{a_j^{(z_i / z_j)}} \tag{2.11}$$

where a_i is the activity of the main species, i; a_j is the activity of interfering species, j;z_i and z_j are the charges of the main species (i) and interfering species (j).

2.5 Direct potentiometric method

Potentiometric methods are based on the measurement of a potential difference between two electrodes (indicator and reference electrode) immersed in a solution containing the analyte. The indicator electrode is chosen to respond to a particular enantiomer in

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

$$E_{cell} = E_{ind} - E_{ref} + E_{ij} \qquad (2.12)$$

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 potential of the reference electrode; E_{ij} is liquid-junction potential.

The interface between two solutions containing different electrolytes or different concentrations of the same electrolyte is called liquid junction. A junction potential arises when the two electrolyte solutions of the different composition are brought together into contact with one another and a potential developed at the interface. The potential arises from an unequal distribution of cations and anions across the boundary due to the difference in the rate at which these species migrate. Calibration procedure of EPME assumes that during the measurements the slope of the electrode is constant and the concentration of the determined enantiomer is proportional to the developed potential. However, because the consumption of the electrolyte to be measured can differ from the solutions used in the calibration process, this assumption is fulfilled very unlikely, if ever. The total relative error expected in EPME measurements with respect to the liquid junction uncertainty can be evaluated by differentiating the following equation [47]:

$$\text{Relative error of measured activity [\%]} = 4n\Delta E_i \qquad (2.13)$$

where n is the charge of the determined species and $n\Delta E_i$ is the uncertainty in liquid junction potential (mV). The minimal $n\Delta E_i$ uncertainty is in the ±1 mV magnitude order, which results in a relative error in the measured activity of ± 4% for univalent ions.

Direct potentiometry is applied for the enantioanalysis of substance with chiral centers. The EPME must be calibrated before the analysis of samples. The solutions used for calibration are obtained from standard solutions, by serial dilution. All solutions must be buffered. The pH and ionic strength of the sample solutions must be adjusted to the same values of the solutions used for calibration of the electrodes. A curve of calibration is obtained by plotting the emf of the cell comprising the working electrode and reference electrode versus the negative logarithm of the main species concentration. The values of emf for the samples are interpolated on the calibration graph and the unknown concentration of the enantiomer is determined.

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