Enantioanalysis of pharmaceutical compounds

by

Tumelo Rameleko Mashile

Submitted in partial fulfillment of the requirements for the degree

MAGISTER SCIENTIAE

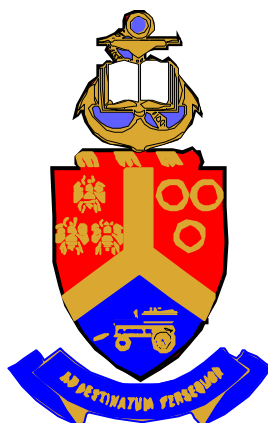In the Faculty of Natural and Agricultural Sciences

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SYNOPSIS

Due to the differences in pharmacokinetics and pharmacodynamics of the enantiomers of the same chiral pharmaceutical substance, there is a high need of reliable analytical methods for enantiopurity tests of them in the raw material as well as in its pharmaceutical formulations. If some of the chiral pharmaceutical compounds can be delivered as racemates, there are many others for which the enantiopurity is essential. Enantioanalysis using enantioselective, potentiometric membrane electrodes became a good alternative of the chromatographic methods due to its high reliability.

To have reliable analytical information it is necessary to use reliable analytical methods and electrodes. The most reliable design for the enantioselective, potentiometric membrane electrodes proved to be the one based on carbon paste. This electrodes are made by mixing graphite powder with paraffin oil to give carbon paste, which is modified by the addition of a chiral selector (e.g., cylodextrins, maltodextrins, macrocyclic antibiotics and fullerenes).

The high sensitivity, selectivity, enantioselectivity, accuracy and precision made the enantioselective, potentiometric membrane electrodes suitable to be used for the enantioanalysis of different pharmaceutical compounds such as S- and R-deprenyl and S-ibuprofen as raw materials and in their pharmaceutical formulations.

Acknowledgements

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Introduction

Chirality is a very important issue for different compounds of pharmaceutical and biological importance. In the modern pharmaceutical industry chirality has become a major concern. This is attributed largely to a heightened awarness that enantiomers of a racemic drug may have different pharmacological activities, as well as different pharmacokinetics and pharmacodynamic effects. The importance of chiral drugs, specifically single isomer drugs in the pharmaceutical market has grown at an exponential rate each year, due to recent developments in the area of chiral drug technologies. The gains in potency, efficacy and selectivity obtained by treatment with single isomer drugs are undeniable. Therefore, enantiopurity is now imperative in the production of most pharmaceutical products with a chiral moiety.

The challenges imposed on the pharmaceutical companies by the problems emanating from the side effects that could be caused by the presence of an undesirable component in racemic drugs, or problems arising from the different pharmacokinetics and pharmacodynamic effects that may be triggered by each of the enantiomer, has further stimulated the imperativeness to find an analytical method that can discriminate between the two enantiomers. These methods will be very helpful and should exhibit reliable analytical information, fast analysis, and could be employed for the enantiopurity tests of pharmaceutical compounds.

In molecular recognition of enantiomers, electrochemical sensors are a very good alternative for chromatographic and structural analysis techniques. Electrochemical sensors have superceded and out classed the other techniques with respect to many analytical aspects, including the high reliability that is given by high precision, high reproducibility, rapidity, and due to the fact that electrochemical sensors can be used directly for measurement of compounds in solution, without any prior separation of the substances that has to be analysed.

The primary aim of this dissertation is to construct reliable enantioselective, potentiometric membrane electrodes to be applied in enantiomeric analysis of pharmaceutical compounds: deprenyl and ibuprofen. Carbon paste are proposed as matrix for the sensors ' design, since it has proved to be the most reliable design, and due to the economic convenience of using carbon which can be easily obtained from the abundantly available and affordable graphite powder. For the selection of the best chiral selector, chiral recognition that is based on selective binding is considered.

Direct potentiometry was employed for the assay of enantiomers in pharmaceutical tablets and raw materials. The selection of the type of electrode and matrix of its membrane was done in accordance with the complexity of the structure of the enantiomer to be determined.

DECLARATIONS

I declare that the dissertation, which I hereby submit for the degree MSc at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Tumelo R. Mashile

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    R.I. Stefan-van Staden, T.R. Mashile, B. Lal, K.C. Mathabate, J.F. van Staden

    J.Pharm.Biomed.Anal., Submitted.

2. Enantioselective assay of S-(+)-ibuprofen using enantioselective, potentiometric membrane electrodes based on maltodextrins

    R.I. Stefan-van Staden, T.R. Mashile

    Sens.Actuators B, In Press (SNB 9191).

3. Determination of R-deprenyl using a maltodextrin based enantioselective, potentiometric membrane electrode

    R.I. Stefan-van Staden, T.R. Mashile

    Instrum.Sci.Technol., Submitted

4. Enantioselective, potentiometric membrane electrodes based on α-, β- and γ-cyclodextrins as chiral selectors for the assay of S-deprenyl.

RI Stefan-van Staden, T.R. Mashile, J.F. van Staden, H.Y. Aboul-Enein

Anal.Chim.Acta, Submitted.

5. Enantioselective, potentiometric membrane electrodes based on C_{60} fullerene derivatives for the assay of deprenyl

R.I. Stefan-van Staden, T.R. Mashile, B. Lal

Anal.Chem., Submitted.

Chapter 1

Chirality in pharmaceutical industry

1.1 Introduction

Nowadays, chirality is extremely important in many fields, such as clinical, pharmaceutical, environmental fields, to mention a few [1]. Enantiomers of the same substance proved to behave different in the body or environment, or to be the product of a totally different metabolic system in the body being markers of different diseases.

Due to these different behaviours of the enantiomers of the same chiral molecule, studies were conducted in order to identify the role of each enantiomers and its pathway in the body. Pharmaceutical industry had to consider the re-evaluation of each of the medicines sailed as racemates or with no control of enantopurity. Accordinly, new patents had to be issued for single enantiomers based pharmaceutical formulations.

1.2 Chirality. Terms and definitions

A molecule is chiral if it is not superimposable on its mirror image regardless of how it is contorted. A chiral molecule exist in two forms, called the R and S isomers which are mirror images of each other. The two non-superimposable, mirror images of chiral molecules are referred to as enantiomers. Enantiomers are therefore related to each other through the reflection by the mirror plane, and are not superimposable. Not all mirror-image pairs constitute enantiomers, but only those which are not superimposable after any rotation of the whole molecule or its mirror image. The

existence of enantiomers is usually (but not always) associated with at least one chiral centre. Chiral compounds exhibit optical activity, so enantiomers are also sometimes called optical isomers. Enantiomers have exactly the same energies, and therefore are not differentiated by physical measurements other than optical rotation (rotation of the plane of polarized light). The two enantiomers of a compound may also be classified as levorotary (L) or dextrorotary (D) depending on whether they rotate plane-polarised light in a left- or right-handed manner, respectively. A 50:50 mixture of the two enantiomers of a chiral compound is called a racemic mixture and does not exhibit optical activity [1].

Symmetry refers to a regular occurrence of certain patterns within a molecule or compound. These patterns are generated by the presence of symmetry elements such as centre of symmetry; symmetry axes and symmetry planes. The symmetry of a molecule determines whether it is chiral or not. A molecule is achiral (i.e not chiral) if and only if it has an axis of improper rotation, that is, an n-fold rotation (rotation by $360^{0}/n$) followed by a reflection in the plane perpendicular to this axis which maps the molecule on to itself. Thus a molecule is chiral if and only if it lacks an improper rotation axis. They are not necessarily asymmetric (i.e. without symmetry), because they can have other types of symmetry, like rotational symmetry. However, all naturally occurring amino acids (except glycine) and many sugars are asymmetric as well as chiral. Enzymes, which themselves are always chiral, often distinguish between two enantiomers of a chiral substrate. Most common chiral molecules have point chirality which centers around a single asymmetric atom (usually a carbon atom). This is the case for chiral amino acids where the α-carbon atom is the stereogenic center, having point chirality. A molecule can have multiple chiral centers

without being chiral overall if there is a symmetry element (mirror plane or inversion center) which relates those chiral centers. Such compounds are referred to as meso compounds. It is also possible for a molecule to be chiral without any specific chiral centers in the molecule. Conformations are temporary positions atoms in a molecule can assume as a result of bond rotation, bending, or stretching as long as no bonds are broken. Configurations are structures of a molecule which are assumed not to be interconvertible under ambient conditions. Enantiomers, and other optically active isomers such as diastereomers, are examples of configurational isomers [2].

1.3 Chiral pharmaceutical compounds

Chirality is a major concern in the modern pharmaceutical industry. A large percentage of commercial and investigational pharmaceutical compounds are chiral and their enantiomers show significant differences in their pharmacokinetics and pharmacodynamics. The importance of chirality of drugs has been increasingly recognized, and the consequences of using them as racemates or as enantiomers has been frequently discussed in the pharmaceutical literature during recent years [3-4]. The biological activity of chiral substances often depends upon their stereochemistry, since the living body is highly chiral environment. The body being amazingly chiral selective, will interact with each racemic drug differently and metabolize each enantiomer by a separate pathway to produce different pharmacological activity. Thus, one isomer may produce the desired therapeutic activities, while the other may be inactive or, in worst cases, produce unwanted effects. For example, the anesthetic ketamine is administered as racemate, and the S(+)-ketamine form is more potent than the R(-) form, the R(-) form being responsible for post-operative effects. A very tragic example is the case of the racemic drug of n-phthalyl-glutamic acid imide that was

marketed in the 1960's as the sedative Thalidomide. Its therapeutic activity resided exclusively in the R(+)-enantiomer. Only after several hundred births of malformed infants was discovered that the S(-)-enantiomer was teratogenic [5]. Lipitor – one of the most sold drug in the world is delivered as single enantiomers; lipitor is well known for its effect on reducing cholesterol. Zocor – another drug used to reduce the cholesterol in the body is also sold as single enantiomers. While these medicines as well as plavix (used as antithrombotic) and nexium (used as antiulcerant) are sold as single enantiomers, there are medicines that are still sold as racemates: norvasec (used as antihypertensive), seretide (used as bronchodilatator), ogastro (used as antiulcerant) and effexor (used as antidepressant) to mention a few.

The current tendency in the pharmaceutical industry is to prepare drugs based on a single enantiomer, because of the side effects that could be caused by the presence of an undesirable component in a racemic drug. Indeed, to avoid the possible undesirable effects of a chiral drug, it is imperative that only the pure, therapeutically active form be prepared and marketed. However, the production of such drugs through stereoselective reaction or preparative enantiomeric separation can provide impure materials. Hence there is a great need to develop the technology for analysis and separation of racemic drugs.

1.4 Current trends in enantioanalysis of pharmaceutical compounds

Current methods of enantiomeric analysis include polarimetry, nuclear magnetic resonance, isotopic dilution, calorimetry, enzyme techniques as well as chromatographic techniques such as high performance liquid chromatography (HPLC) [6-8], gas chromatography (GC) [9-10], thin layer chromatography (TLC)

[11] and capillary electrophoresis (CE) [12-20]. Capillary electrophoresis is a fact in enantioanalysis. This technique is the most accurate chromatographic techniques used in enantioanalysis. The main disadvantage of it is the low sensitivity.

The utilization of these techniques involves a lot of steps before the actual analysis of the chiral compounds, that includes, the pre-treatement of the analyte sample with different necessary chemicals, separation steps using the chromatographic column. Therefore, the accuracy of the analytical information is not as good as expected.

The utilization of electrochemical sensors in molecular recognition of the enantiomers is not laborious if one compares it with structural and with chromatographic techniques [21]. The reliability of the response characteristics as well as the analytical information obtained by using electrochemical sensors is strictly correlated with the design of sensors [22]. The most reliable design is based on carbon paste. The method is rapid, precise and not expensive. Accordingly, the enantioanalysis using electrochemical techniques became a good alternative for the enantioseparation techniques.

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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:

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

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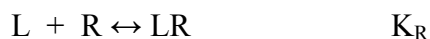
where S and R are enantiomers to be determined, L is the chiral selector, LS and LR are the complexes formed between R(S)-enantiomer and L, respectively, and K_S and K_R are the stability constants of the complexes formed between chiral selector and enantiomers.

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:

$$E = f(-\log a_i) \tag{2.4}$$

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:

$$E = f(pM). \qquad\qquad (2.5)$$
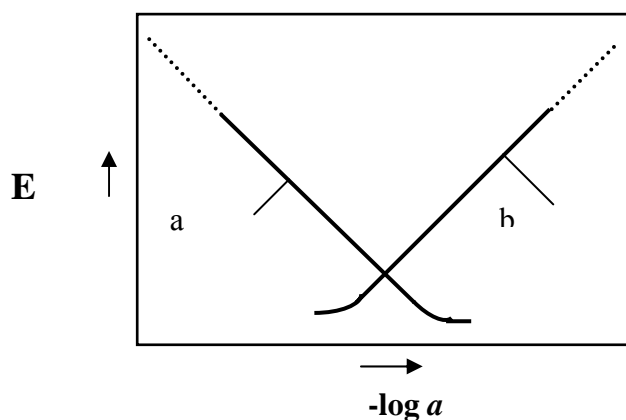
Figure 2.1 EPME function. (a) cation-selective electrode; (b) anion-selective

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:

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

where E is the potential of the electrode, E^0 is the standard electrode potential, S is the slope, and $pM = -\log C_M$.
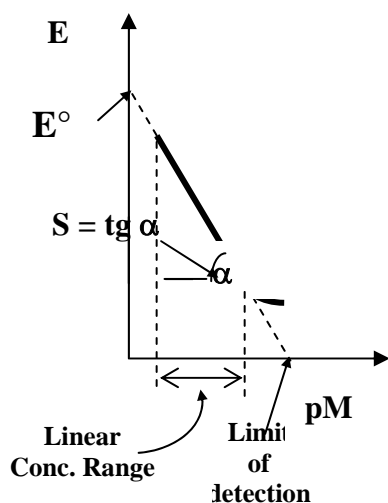
Figure 2.2 Response characteristics of EPME

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:

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

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:

$$S = \frac{RT}{zF} \tag{2.8}$$

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:

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

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:

$$E_{cell} = E_{ind} - E_{ref} + E_{ij} \tag{2.15}$$

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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Chapter 3

The role of the enantiomers of ibuprofen and deprenyl in pharmaceutical industry

3.1 Ibuprofen enantiomers

The IUPAC name for ibuprofen is 2-(4-isobutyl-phenyl)-propionic acid. Figure 3.1 depicts a two dimensional structure of ibuprofen. Ibuprofen is an aromatic compound with a *para*-sustituted benzene ring. In the upper right of the structure is a carboxylic acid functional group, specifically a propionic acid. Attached to the second carbon is a phenyl group. Attached to the phenyl group, *para* to the propionic acid group, is an isobutyl group which leads to the beginning of its common name. Ibuprofen is a small organic compound composed of 13 carbon atoms, 18 hydrogen atoms and 2 oxygen atoms.
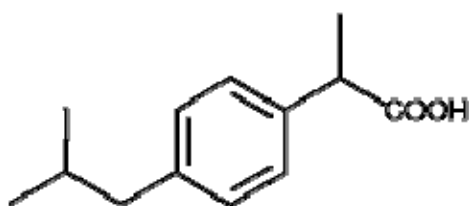
Figure 3.1 Ibuprofen

Ibuprofen, like other 2-arylpropionate derivatives (including ketoprofen, flurbiprofen, naproxen, etc) contains a chiral carbon in the α-position of the propionate moiety. As such there are two possible enantiomers of ibuprofen with the potential for different biological effects and metabolism for each enantiomer. It was found that the S-

enantiomer of ibuprofen was the active form both *in vitro* and *in vivo* [1-3]. However, most of the ibuprofen formulations that are currently marketed are a racemic mixture of both enantiomers (including ibuprofen drug). Figure 3.2 (a) and (b) shows a three-dimensional (3D) representations of S-ibuprofen and R-ibuprofen, respectively.

(a)   (b)
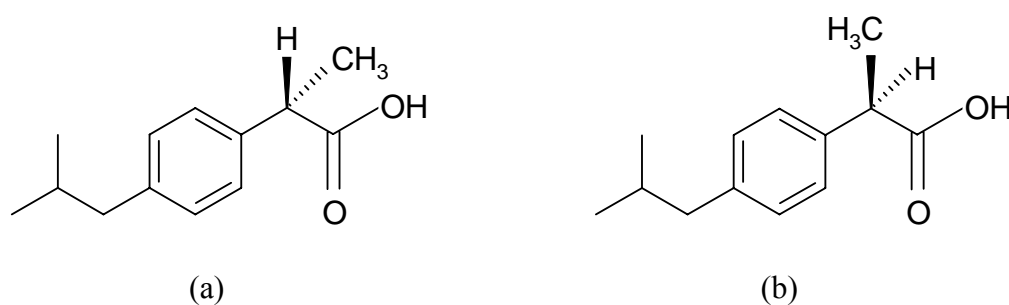
Figure 3.2 Ibuprofen enantiomers (a) S-ibuprofen; (b) R-ibuprofen

Ibuprofen is a nonsteroidal anti-inflamatory drug (NSAID) that is widely marked under various trademarks including Act-3, Advil, Brufen, Mortin, Nuprin, Nurofen and Myprodol. Ibuprofen is used to relieve the pain, tenderness, inflammation (swelling), and stiffness caused by arthritis and gout. It is also used to reduce fever and to relieve headaches, muscle aches, menstrual pain, aches and pains from the common cold, backache, and pain after surgery or dental work. It was shown that only the S-enantiomer is responsible for the desired therapeutic effects [4], while the R-enantiomer displays toxicity due to its storage in fatty tissue as a glycerol ester, whose long-term effects are not known [5]. Despite this fact, the ibuprofen is currently administered as racemate.

3.1.1 Current analytical methods used for the determination of ibuprofen

Proton-NMR [6] HPLC and GC-MS [7] techniques were used for the identification of ibuprofen and its degradation products of ibuprofen. Novel ibuprofen potentiometric membrane sensors based on tetraphenylporphyrinato indium (III) were developed for use in the quantification and quality control assessment of ibuprofen in pharmaceutical preparations, but not for enantioanalysis of ibuprofen [8].

3.2 Deprenyl enantiomers

Deprenyl, an irreversible inhibitor of monoamine oxidase enzyme (MAO), was first described by Knoll *et al* [9]. R-deprenyl selectively inhibits MAO type B [10,11]. Figure 3.3 (a) and (b) shows 3D structures of R-deprenyl and S-deprenyl respectively.

(a)   (b)
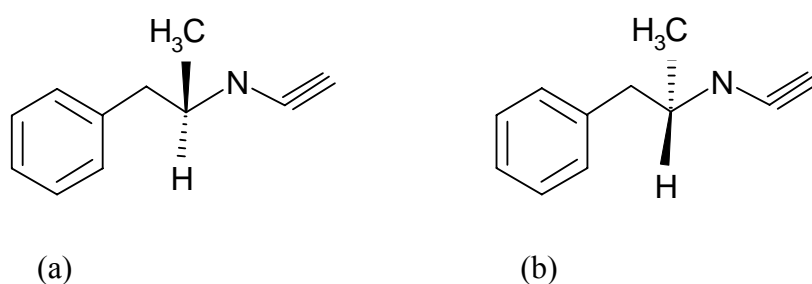
Figure 3.3 Enantiomers of deprenyl. (a) R-deprenyl; (b) S-deprenyl

The main metabolites of deprenyl are desmethyldeprenyl, methamphetamine, and amphetamine formed by N-dealkylation [12-15]. R-deprenyl and its metabolites also inhibit the monoamine transpoters in the brain [16-18]. Based on its dopamine sparing effect, S-deprenyl is used worldwide in the treatment of Parkinson `s disease [19], as well as other neurodegenerative disorders. Neuroprotective and neuronal rescue effects of R-deprenyl, unrelated to MAO-B inhibition but also highly stereoselective,

have also been reported [20-25]. Furthermore, R-deprenyl has been found to exert neuroprotection against toxic insult [26-28], as well as have anti-apoptotic [29,30] and anti-tumor effects [31] and, in low concentrations, it has been shown to increase the lifespan of laboratory animals [32,33]. The S-enantiomer has strong psychostimulant effects than the R-enantiomer [34].

3.2.1 Current analytical methods used for the determination of deprenyl

The methods available for determination of deprenyl are based on the enantioseparation of its enantiomers as well as of the metabolits of S- and R-deprenyl. The chromatographic methods proposed for the enantioanalysis of deprenyl are: thin layer chromatography (TLC) [35,36], High performance liquid chromatography (HPLC) [37,38] and capillary electrophoresis (CE) [39,40].

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Chapter 4

Enantioselective, potentiometric membrane electrodes based on fullerenes

4.1 Fullerenes as chiral selector in the EPMEs design

A long search for molecular allotropic forms of carbon other than graphite and diamond culminated in 1985 in the discovery of a C_{60} molecule. Fullerenes (Figure 4.1) are "cage" molecules that are named after eccentric architect R. Buckminster Fuller, the inventor of the truncated icosahedron-shaped geodesic dome. The soccer-ball shaped C_{60} molecule and the rugby-ball shaped C_{70} were soon followed by higher-order fullerenes of 76, 84, 90, and 94 carbon "cages" as large as C_{240} and C_{540}.

The fullerenes appear as a whole system able to form a completely new type of chemistry with many surprising arrangements and applications, including analytical ones and it is also said that, when a new atom is put in a fullerene cage, or when an atom (or group) is attached to the outside, a new molecule is formed [1,2].

       (a)   (b)
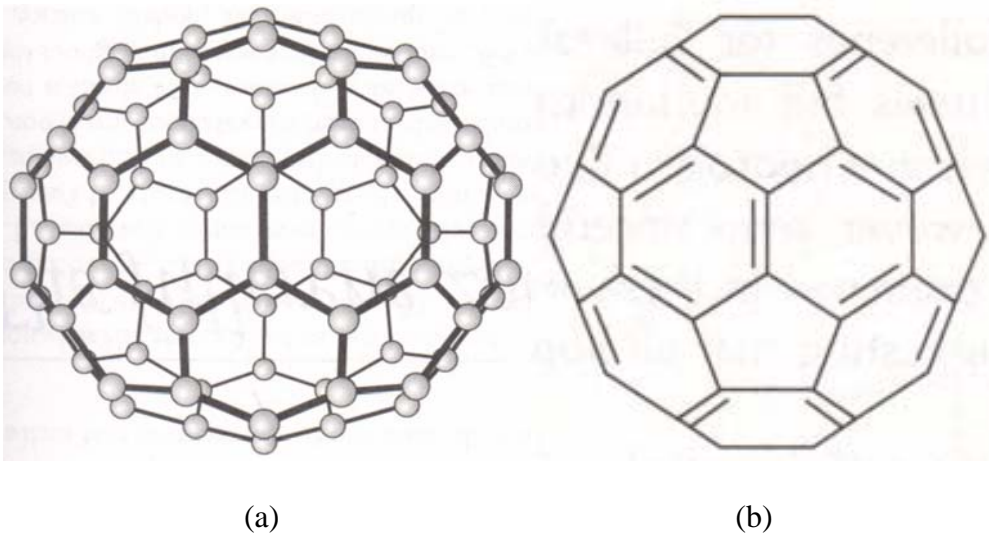
Figure 4.1 Fullerenes (a) C_{70} and (b) C_{60}

Since the discovery of the fullerenes (C_{60}, C_{70} and higher fullerenes), electrochemistry has played a significant part in investigations of the properties of these fascinating new types of carbon. Initially, some important physiochemical properties, including the standard redox potentials, and confirming theoretical predictions of these molecules [3,4] were considered. Fullerenes (C_{60} and C_{70}) display anomalous behaviour in solution due to the formation of aggregates [5-13]. Since the specific surface energies of interactions of fullerene molecules are very close in magnitude [5], fullerenes in solution often tend to form aggregates.

4.2 Enantioanalysis of S-deprenyl using enantioselective, potentiometric membrane electrodes based on C_{60} fullerenes

Three enantioselective, potentiometric membrane electrodes based on C_{60} fullerene derivatives impregnated on carbon-paste matrices as chiral selector are proposed for the enantioanalysis of S-deprenyl (dep) [14].

4.2.1 Reagents and materials

(1,2-methanofullerene C_{60})- 61- carboxylic acid (I), diethyl (1,2-methanofullerene C_{60})-61-61-dicarboxylate (II) and tert-butyl (1,2-methanofullerene C_{60})-61-carboxylic acid (III) were supplied by Fluka (Buchs, Switzerland). Phosphate buffer of pH 5.82 was supplied by Merck (Darmstadt). Deionized water from a Modulab system (Continental Water Systems, San Antonio, TX, USA) was used for all solutions preparations. The S- and R-dep solutions necessarily in the characterization of the enantioselective potentiometric membrane electrodes were prepared from standard S- and R-dep solutions (10^{-2} mol/L), respectively, by serial dilutions. All standard and diluted solutions were buffered with phosphate buffer (pH 5.82 mol/L) from Merck (Darmstadt, Germany) (1:1,v/v, buffer: deionised water). Lentogesic tablets (65 mg deprenyl per tablet) were obtained from Adcoc Ingram Limited (Johannesburg, South Africa).

4.2.2 Apparatus

A 663 VA Stand (Metrohm, Herisau, Switzerland) combined with a PGSTAT 20 and a software (Eco Chemie version 4.4) were used for all potentiometric measurements. A glassy carbon electrode and a Ag/AgCl (0.1 mol/L KCl) served as the counter and reference electrodes in the cell.

4.2.3 Electrodes design

Paraffin oil and graphite powder were mixed in a ratio of 1:4 (w/w) followed by the addition of chiral selector (fullerene (I), (II) or (III)) to carbon paste. A certain quantity of carbon paste free of chiral selector was prepared and it was placed into a plastic pipette peak leaving 3–4mm empty in the top to be filled with the carbon paste that contains the chiral selector. The diameter of the potentiometric, enantioselective membrane electrode was 3 mm. Electric contact was obtained by inserting a Ag/AgCl wire in the carbon paste. 0.1mol/L of KCl was used as internal solution. The surface of the electrodes was wetted with deionised water and polished with alumina paper (polishing strips 30144-001, Orion) before using them for each experiment. When it was not in use, the electrode was immersed in a 10^{-3} mol/L S-dep solution.

4.2.4 Recommended procedure: Direct potentiometry

The potentiometric method was used for the potential determination of each standard solution (10^{-10}- 10^{-3} mol/L, pH 5.82). The electrodes were placed in the stirred standard

solutions and graphs of E (mV) versus pS-dep were plotted. The unknown concentrations of S-dep were determined from the calibration plots.

4.2.4.1 Content uniform assay of Lentogesic tablets

Each of the ten tablets were placed into 100 ml calibrated flask, dissolved and diluted to the mark using a phosphate buffer (pH 5.8) : deionized water 1:1. The unknown concentration of S-deprenyl was determined using the direct potentiometric method.

4.2.5 Results and discussion

4.2.5.1 Response characteristics of the electrodes

The response characteristics exhibited by the proposed electrodes towards S-dep are summarized in Table 4.1. For all the calibration plots, the potentiometric membrane electrodes showed linear near Nernstian responses for S-dep, with correlation coefficients for the equations of calibration of 0.9998 (I), 0.9996 (II) and 0.9999 (III), respectively. R-dep on the other hand, showed non-Nernstian response.

Table 4.1. Response characteristics of the potentiometric, enantioselective membrane electrode based on C_{60} and its derivative.

Chiral Selectors	Slope [mV/S-Dep]	Intercept, E° [mV]	Linear range [mol/L]	Detection limit [mol/L]
(I)	55.30	559.00	10^{-10}-10^{-4}	7.6×10^{-11}
(II)	56.30	560.40	10^{-9}-10^{-3}	1.12×10^{-10}
(III)	58.30	595.20	10^{-10}-10^{-3}	6.1×10^{-11}

All measurements were made at room temperature; all values are the average of ten determinations.

The response time was 1min for the fullerene (I) based electrode in concentration range 10^{-10}-10^{-9} mol/L and less than 30s between 10^{-8} and 10^{-5} mol/L. The response time for fullerene (II) based electrode was 2 min between 10^{-9} and 10^{-5} mol/L and 30s between 10^{-4} and 10^{-3} mol/L. The response times for fullerene (III) based electrode in the concentration range 10^{-9}-10^{-8} mol/L was higher 1.5 min and 20s in the concentration range 10^{-7}-10^{-5} mol/L. All electrodes displayed good stability and reproducibility over the test period (6 months) (RSD<0.1%) when used every day for measurements.

4.2.5.2 Effect of pH on the response of the electrodes

The influence of pH on the response of the EPME was checked by recording the emf of the cell for solutions containing 10^{-5} mol/L S-dep at different pH values (pH, 1-12).
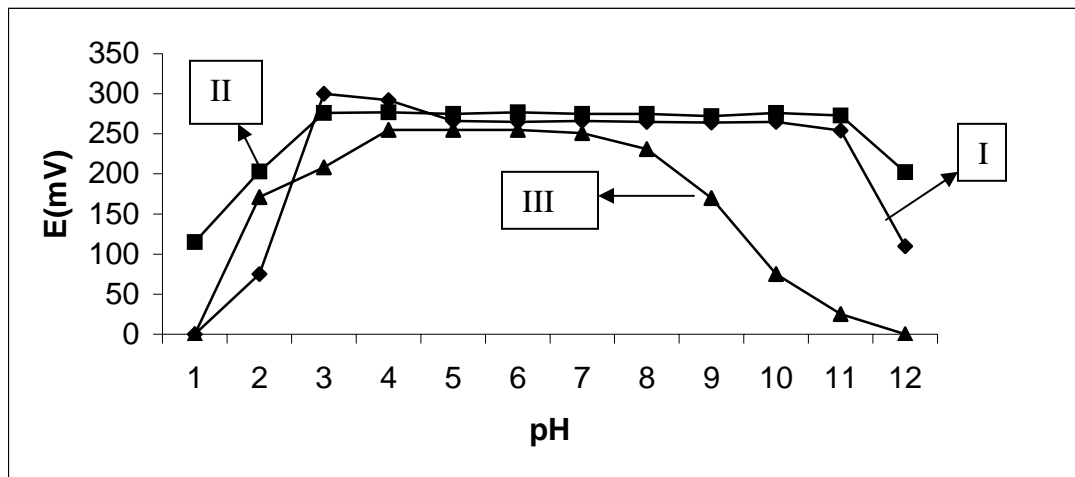
Figure. 4.2. The influence of pH on the response of the enantioselective potentiometric membrane electrodes (S-dep $=10^{-6}$ mol/L); for fullerenes I, II, and III based enantioselective, potentiometric membrane electrodes.

These solutions were prepared by adding very small volume of HCl/ NaOH solution (0.1 mol/L or 1 mol/L of each) to S-dep solution. The plots of E (mV) versus pH (Figure 4.2) indicate that the response of the electrodes does not depend upon the pH in the following range: 5.0-10.0 (I) 3.0-11.0 (II) and 4.0-7.0 (III).

4.2.5.3 Selectivity of the electrodes

The selectivity of the potentiometric membrane electrode was checked using the mixed solutions method. The concentrations of interfering ions and S-dep were 10^{-4} and 10^{-5} mol/L, respectively. The values obtained for the potentiometric selectivity coefficients for R-dep, PVP, creatine and creatinine demonstrated the enantioselectivity and selectivity properties of the proposed EPMEs for the assay of S-dep (Table 4.2).

Table 4.2 Potentiometric selectivity coefficients for the enantioselective, potentiometric membrane electrode for S-dep.

EPMEs	Interferering species					
	R-dep	PVP	Creatine	Creatinine	Paracetamol	L-glutamine
(1)	5.1×10^{-3}	4.0×10^{-3}	$<< \times 10^{-4}$	1.8×10^{-3}	4.3×10^{-4}	6.5×10^{-3}
(II)	$<< \times 10^{-4}$	2.3×10^{-3}	3.3×10^{-3}	6.3×10^{-3}	3.9×10^{-3}	1.4×10^{-3}
(III)	8.2×10^{-4}	1.3×10^{-3}	2.0×10^{-3}	1.2×10^{-3}	2.5×10^{-3}	7.9×10^{-3}

All measurements were made at room temperature; all values are the average of ten determinations.

4.2.5.4 Analytical applications

To assess the feasibility of the proposed direct potentiometry method for enantioanalysis of S-deprenyl, recovery tests were performed for S-deprenyl in the presence of its

antipode. The assay of S-dep in the presence of R-dep was conducted by using different ratios between S-dep and R-dep. The results obtained (Table 4.3) demonstrated the suitability for the proposed enantioselective, potentiometric membrane electrodes for testing the enantiopurity of deprenyl tablets due to the good recovery values obtained for the assay of one of the enantiomers in the presence of its antipode. No significant differences in the recovery values were recorded for the ratios between S:R enantiomers varying from 1:9 to 1:99.9.

Table 4.3 Determination of S-deprenyl in the presence of R-deprenyl.

Sample	S-Dep, Recovery (% of nominal value)		
S:R	[I]	[II]	[III]
2:1	99.60 ± 0.02	100.00 ± 0.01	99.99 ± 0.02
1:1	99.59 ± 0.02	99.05 ± 0.02	99.98 ± 0.02
1:2	99.62 ± 0.01	99.32 ± 0.02	99.98 ± 0.01
1:4	99.98 ± 0.01	100.00 ± 0.02	100.00 ± 0.01
1:9	99.45 ± 0.02	99.99 ± 0.01	99.99 ± 0.02

All measurements were made at room temperature; all values are average of ten measurements.

The proposed enantioselective, potentiometric membrane electrodes proved to be useful for the uniformity content test of Lentogesic tablets. The results obtained for content uniformity test of Lentogesic tablets using the proposed electrode showed, that the tested pharmaceutical formulations contain 98.12±0.14, 98.13±0.15, and 98.20±0.17% S-deprenyl, when electrodes based on fullerenes I, II, and III, respectively were used.

4.3 Conclusions

This chapter describes new enantioselective, potentiometric membrane electrodes designed using (I), (II) and (III) as chiral selectors for the enantioanalysis of deprenyl. The electrodes can be successfully used for the assay of S-deprenyl in the presence of R-deprenyl. The enantioselectivity is good for all the three proposed electrodes; the best being recorded when tert-butyl (1,2-methanofullerene C_{60})-61-carboxylic acid is used as chiral selector. The proposed EPME based on fullerene derivatives have good feature in enantioselective analysis. The simple, fast and reproducible construction of the electrode assures the reliable response characteristics. The good enantioselectivity of designed electrodes is allowed to perform the enantiopurity assay of S-deprenyl raw material and in its pharmaceutical formulation Lentogesic.

4.4 References

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Chapter 5

Enantioselective, potentiometric membrane electrodes based on antibiotics

5.1 Macrocyclic antibiotics as chiral selectors for EPMEs design

Enantiorecognition of several classes of pharmaceutical drugs and molecules of biological importance has been successfully achieved by using macrocyclic antibiotics as chiral selectors. Macrocyclic antibiotics have several functional groups that are responsible for multiple stereoselective interaction.

Many macrocyclic antibiotics exhibit similar physico-chemical properties, on the hand showing different stereoselective power [1]. The most commonly used macrocyclic antibiotics are vancomycin and teicoplanin [2-4].

(a)   (b)
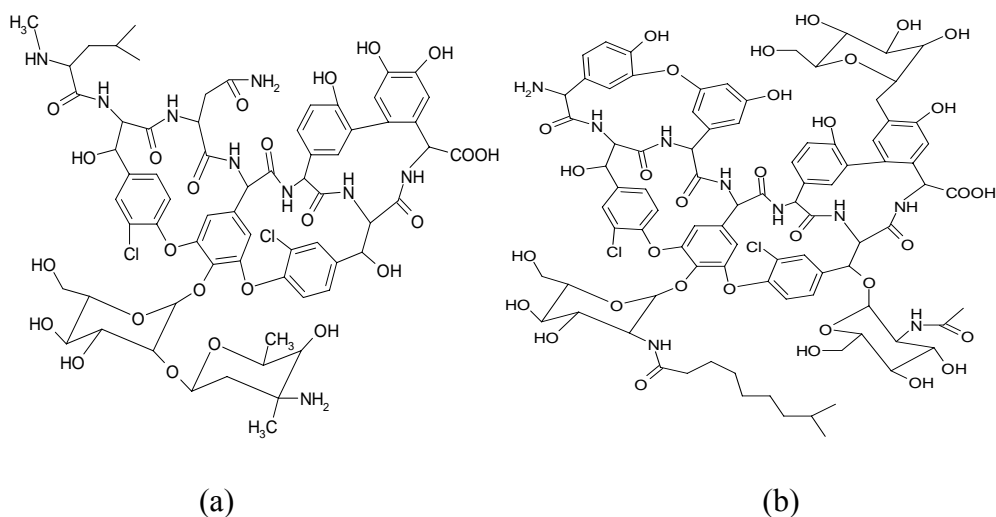
Figure 5.1 Chemical structures of (a) Vancomycin and (b) Teicoplanin

Vancomycin is "basket" shaped (Figure 5.1a) having three fused macrocyclic rings and two side chains, a carbohydrate dimmer and a N-methyl leucine moiety [4]. There are 18 asymmetric centers and several and several functional groups such as carboxylic, hydroxyl, amino, amido, and aromatic rings [1].

It is very soluble in water and can dimerize in aqueous solutions depending on vancomycin concentration [5]. Vancomycin solutions are very stable at low temperatures and in buffered solutions (pH 3.0-6.0) [5,6].

Teicoplanin (Figure 5.1b) is obtained from fermentation by Actinoplanes teichomyceticus. It is structurally related to the antibiotics vancomycin, and ristocetin A, but differs from these antibiotics in several ways [7], it contains the carbohydrates D-glucoseamine and D-mannose, and the amino group of the glucoseamine is substituted with a long fatty acid chain that contains 10 or 11 carbons, and is more hydrophobic than vancomycin and it aggregates in aqueous solution [8].

Teicoplanin contains one free amine and one free carboxylic acid group. However it contains 23 stereogenic centers, four phenolic groups and seven aromatic groups and is obtained as a mixture of five analogous compounds containing different fatty acid chains (C_{10}-C_{11}) attached to the amine of 2-amino-2-deoxy-β-D-glucopyranozyl groups. Self-association of teicoplanin with micellization favoured by lower pH [8] is caused by this hydrophobic tail.

The most important functional groups for amino acid recognition are the $-NH_2$ and the $-COOH$ groups ionised over pH 3.5-8.0 range [9]. Teicoplanin is soluble in water, slightly soluble in methanol and ethanol and insoluble in non-polar organic solvents.

5.2 Enantioanalysis of S-ibuprofen using enantioselective, potentiometric membrane electrodes based on antibiotics

Two enantioselective, potentiometric membrane electrodes based on vancomycin and teicoplanine modified with acetonitrile are proposed for the enantioanalysis of S-ibuprofen [10].

5.2.1 Reagents and materials

Graphite powder (1-2μm, synthetic) was purchased from Aldrich. Paraffin oil was purchased from Fluka (Buchs, Switzerland). Vancomycin and teicoplanin were purchased from Sigma-Aldrich. (S)-(+)-Ibuprofen was purchased from Sigma-Aldrich. Phosphate buffer (pH 4.00) was obtained from Merck (Darmstadt, Germany). Deionized water from a Modulab system (Continental Water Systems, San Antonio, TX, USA) was used for all solutions preparations.

The solution of vancomycin (2×10^{-3} mol/L) was prepared in phosphate buffer (pH 4.00). The solution of teicoplanin (2×10^{-3} mol/L) containing acetonitrile was prepared using pH 6.00 phosphate buffer containing 40% (v/v) of acetonitrile. All standard and diluted

solutions were buffered with phosphate buffer pH 4.00 using the ratio buffer:distilled water 1:1 (v/v).

Myprodol capsules (200mg (S)-(+)-ibuprofen per capsule) and Nurofen tablets (200mg (S)-(+)-ibuprofen per tablet) were obtained from Nutrent (Sandton, South Africa). The Myprodol capsules and Nurofen tablets contain (S)-(+)-ibuprofen as an active compound, paracetamol and usual additives such as starch.

5.2.2 Apparatus

A 663 VA Stand (Metrohm, Herisau, Switzerland) in combination with a μAutolab and Ecochemie (Utrech, The Nertherlands) Software version 4.9 were used for all potentiometric measurements. An Ag/AgCl (0.1 mol l^{-1} KCl) electrode served as reference electrode in the cell.

5.2.3 Electrode design

The paraffin oil and graphite powder were mixed in a ratio of 1:4 (w/w) followed by the addition of solution of vancomycin or teicoplanin modified with acetonitrile (100μl of chiral selector solution to 100mg of carbon paste). A certain quantity of carbon paste free from chiral selector was prepared and placed in a plastic pipette peak, leaving 3-4mm empty in the top to be filled with the modified carbon paste. The diameter of the electrode was 3 mm. Electric contact was made by inserting an Ag/AgCl wire into the carbon paste. The internal solution was 0.1mol l^{-1} KCl. Before each set of measurement the surface of the electrode was "refreshed" with a new portion of carbon paste,

containing the chiral selector and then polished with alumina paper (polishing strips 30144-001 Orion). The carbon paste prevents the leaching of the antibiotic from the membrane into the solution.

5.2.4 Recommended procedures

5.2.4.1 Direct potentiometry

The potentiometric method was used for potential determination of each standard solution (10^{-10}-10^{-4}mol/L). The electrodes were placed into stirred standard solutions and graphs of E (mV) versus pS-Ibuprofin were plotted. The unknown concentrations were determined from the calibration graphs.

5.2.4.2 Content uniform assay of Ibuprofen capsules and tablets

Each of the five capsules and five tablets (200mg (S)-(+)-ibuprofen per capsule and per tablet) were placed into 100ml calibrated flask, dissolved and diluted to the mark using a phosphate buffer (pH 5.34):deionized water = 1:1 (v/v). The unknown concentration of (S)-(+)-ibuprofen was determined using the direct potentiometric method.

5.2.5 Results and discussion

5.2.5.1 Electrodes response

The responses of the electrodes were determined for both enantiomers S- and R-ibuprofen, at pH = 4.0 (phosphate buffer) using potentiomatric method. The responses obtained for R-ibuprofen were not linear and non-Nernstian. That proved that the

electrodes cannot be used for the assay of R-ibuprofen. The equations of calibration obtained for S-ibuprofen are as follows:

(I) E =235.0 - 58.5 pS-ibuprofen $\qquad$ r = 0.9997

(II) E = 370.0 - 57.0 pS-ibuprofen $\qquad$ r = 0.9999

where E(mV) is the cell potential, pS-ibuprofen = -log[S-ibuprofen], and (I), (II) correspond to the EPMEs based on vancomycin and teicoplanine modified with acetonitrile. The linear concentration ranges were 10^{-4} to 10^{-10} and 10^{-8} to 10^{-10} mol l^{-1} for electrodes based on vancomycin and acetonitrile modified teicoplanin, respectively, with detection limits of 9.6×10^{-5} and 3.2×10^{-7} mol/L, respectively. The responses of the electrodes showed good stability and reproducibility for all the performed tests for 6 months, when they are used daily for measurements (RSD<1.0%).

The response time was 30s for the concentration range between 10^{-7} and 10^{-4} mol/L, and 1min for the concentration range between 10^{-8} and 10^{-10} mol/L for both electrodes.

5.2.5.2 Effect of pH on the response of the electrodes

Potentiometry was used to determine the effect of pH on the response of the proposed electrodes. The solution used for measurements were containing (S)-(+)-ibuprofen (C = 10^{-7} mol l^{-1}) at different pH values. The plots of E (mV) versus pH (fig. 5.2) indicate, that the response of the electrodes does not depend on the pH changes in the following pH ranges: 3.0-8.0 for vancomycin based electrode and 4.0-10.0 for teicoplanin, modified by acetonitrile based electrode.
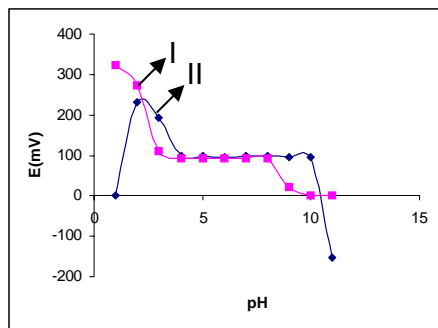
Figure 5.2 Effect of pH on the response of the potentiometric enantioselective membrane electrodes for (S)-(+)-ibuprofen (10^{-7} mol l^{-1}) (I) vancomycin based electrode; (II) teicoplanin, modified with acetonitrile based electrode.

5.2.5.3 Selectivity of the electrodes

The selectivity of the electrodes was investigated using the mixed solution method. The ratios between the concentrations of (S)-(+)-ibuprofen and the concentration of interferent were 1:10. As it follows from the values of the potentiometric selectivity coefficients that are shown in table 5.1, the electrodes are selective over all possible interfering compounds.

Also they are enantioselective because the values of the potentiometric selectivity coefficients over R-ibuprofen were lower than 10^{-4}. These results proves that the two electrodes can be used for (S)-(+)-ibuprofen enantioanalysis.

Table 5.1. Selectivity coefficients $\left(K_{sel}^{pot}\right)$ for the enatioselective, potentiometric membrane electrodes.

Interfering species	K_{sel}^{pot}	
	EPME based on	
	Vancomycin	Teicoplanin
PVP	1.7×10^{-3}	6.2×10^{-3}
Creatine	$\ll 10^{-4}$	5.0×10^{-4}
Creatinine	2.0×10^{-3}	4.0×10^{-4}
Paracetamol	$\ll 10^{-4}$	3.9×10^{-4}

All measurements were made at room temperature; all values are average of 10 determinations.

5.2.5.4 Analytical applications

The response characteristics and the selectivity and enantioselectivity of the proposed electrodes indicate that the electrodes are suitable for the enantiopurity tests of (S)-(+)-ibuprofen raw materials and for uniformity content of Myprodol capsules and Nurofen tablets. The uniformity content test for Myprodol capsules and Nurofen tablets shows that (S)-(+)-ibuprofen can be reliably assayed in the tablets with average recoveries of 98.79 ± 0.10 and 98.96 ± 0.12% for Myprodol capsules and Nurofen tablets respectively when vancomycin based electrode was used, and 98.77 ± 0.12 and 98.45 ± 0.13% for Myprodol capsules and Nurofen tablets when teicoplanin, modified with acetonitrile based electrode was used. These results show that the tested pharmaceutical compounds contain (S)-(+)-ibuprofen as main component. They are also in good agreement with the results obtained using a HPLC method: 98.80% and 98.50% (S)-(+)-ibuprofen in Myprodol capsules and Nurofen tablets, respectively.

5.3 Conclusions

The proposed enantioselective, potentiometric membrane electrodes based on macrocyclic antibiotics vancomycin and teicoplanin have attractive features in enantioselective analysis. The construction of the electrodes is simple, fast and reproducible with reliable response characteristics for the proposed enatioselective membrane electrodes. The electrodes can be successfully used for enantiopurity tests of pharmaceutical formulations of (S)-(+)-ibuprofen.

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Chapter 6

Enantioselective, potentiometric membrane electrodes based on cyclodextrins

6.1 Cyclodextrins as chiral selectors in the EPMEs design

Cyclodextrins (Fig. 6.1) are cyclic, non-reducing oligosaccharides of six, seven and eight α-D-glucose units, which are commonly referred to as α-, β-, and γ-cyclodextrins respectively and obtained from starch by enzymatic degradation by Bacillus Amylobacter [1-6].

(a)   (b)   (c)

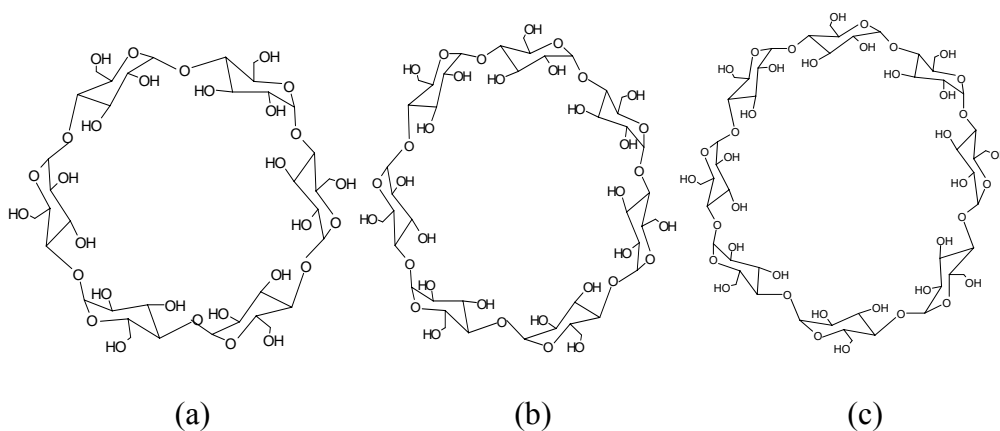**Figure 6.1** Cyclodextrins: (a) α-, (b) β-, (c) γ-cyclodextrin.

Their cyclic linkage of their glucose units is through C-O-C α-1,4 bonds that gives them a toroidal or truncated molecular shape of relative hydrophobic cavity [1-6]. An important property of cyclodextrins is their ability to form inclusion complexes with a large number of organic and inorganic compounds, an important property that has been

extensively exploited in pharmaceutical formulations of certain drugs thereby reducing their side effects and increasing the bioavailability and solubility in water [1-6]. Their cavities are suitable for enantioanalysis of chiral compounds, with the possibility of achieving double selectivity: an internal selectivity (i.e., inclusion type, dependent on the cavity size and guest molecule) and external selectivity (dependent on functional groups) [1-6]. Cyclodextrin derivative are developed to modify their properties such as cavity shape and hydrophilicity.

6.2 Enantioanalysis of S-deprenyl using enantioselective, potentiometric membrane electrodes based on cyclodextrins

Three enantioselective, potentiometric membrane electrodes based on α-, β- and γ-cyclodextrins are proposed for the enantioselective assay of S-deprenyl.

6.2.1 Reagents and materials

Graphite powder (1-2 μm) was purchased from Aldrich (Milwaukee, WI, USA). Paraffin oil was purchased from Fluka (Buchs, Switzerland). S- and R-deprenyl were purchased from Sigma-Aldrich. α-, β- and γ-cyclodextrins were supplied by Wacker-Chemie GmbH (Munchen, Germany). Phosphate buffer (pH 5.8) was obtained from Merck (Darmstadt, Germany). Lentogesic tablets (65 mg deprenyl per tablet) were obtained from Adcoc Ingram Limited (Johannesburg, South Africa). Deionized water from a Modulab system (Continental Water System, Sand Antonio, TX, USA) was used for all solutions preparations. The solution of cyclodextrin (10^{-3} mol l^{-1}) was prepared using deionized

water. All standard and diluted solutions were buffered with phosphate buffer pH 5.8 using the ratio buffer:distilled water 1:1 (v/v).

6.2.2 Apparatus

A 663 VA Stand (Metrohm, Herisau, Switzerland) in combination with a μAutolab and Ecochemie (Utrech, The Nertherlands) Software version 4.9 were used for all potentiometric measurements. An Ag/AgCl (0.1 mol l^{-1} KCl) electrode served as reference electrode in the cell.

6.2.3 Electrode design

Paraffin oil and graphite powder in a ratio of 1:4 (w/w), were first thoroughly mixed, followed by the addition of an aqueous solution of cyclodextrin (α-(I), β-(II) or γ-(III) cyclodextrins) from a 10^{-3} mol.l^{-1} cyclodextrin solutions. A quantity of carbon paste, without cyclodextrin, was also prepared and placed in a plastic pipette peak, leaving 3-4mm empty in the top to be filled with carbon paste containing the chiral selector. The diameter of the EPMEs was 3mm. Electric contact was obtained by inserting a Ag/AgCl wire into the carbon paste. The internal solution was 0.1 mol.l^{-1} KCl. Prior to use, the surface of the electrode was wetted with deionised water and polished with alumina paper (polishing strips 30144-001, Orion).

6.2.4 Recommended procedures

6.2.4.1 Direct potentiometry

The potentiometric technique was used for potential determination of each standard solutions 10^{-10}-10^{-4} mol.l^{-1}. The electrodes were placed into stirred standard solutions, and graphs of E(mV) versus pS-deprenyl were plotted. The unknown concentrations were determined from the calibration graphs.

6.2.4.2 Content uniform assay of Lentogesic tablets

Each of the ten tablets were placed into 100 ml calibrated flask, dissolved and diluted to the mark using a phosphate buffer (pH 5.8) : deionized water 1:1. The unknown concentration of deprenyl was determined using the direct potentiometric method.

6.2.5 Results and discussion

6.2.5.1 Electrodes response

The response characteristics exhibited by proposed cyclodextrins based EPMEs for the enantioanalysis of S-deprenyl are summarized in Table 6.1. All the proposed membrane electrodes exhibited linear and near-Nernestian responses (53-58 mV per decade of concentration) for S-deprenyl, with correlation coefficients of 0.9998 for α-CD based EPME and 0.9999 for β- and γ-CD based EPMEs. The best response was recorded for the EPME based on β-cyclodextrin. The electrodes responses were highly stable and reproducible over the tests when used daily for six months (RSD<0.1%). The same electrodes shown non-Nernstian responses when used for R-deprenyl.

Table 6.1 Response characteristics of enantioselective, potentiometric membrane electrodes.

Cyclodextrin	Slope (mV/decade of conc.)	Intercept, E^0 (mV)	Linear conc. range (mol.l^{-1})	Detection limit (mol l^{-1})
α-CD	53.9	568.5	10^{-10}-10^{-4}	2.8×10^{-11}
β-CD	57.7	514.4	10^{-8}-10^{-3}	1.2×10^{-9}
γ-CD	56.2	581.0	10^{-10}-10^{-3}	4.5×10^{-11}

All measurements were made at room temperature; all values are average of 10 determinations.

6.2.5.2 Effect of pH on the response of the electrodes

The influence of pH on the response of the proposed electrodes was investigated by
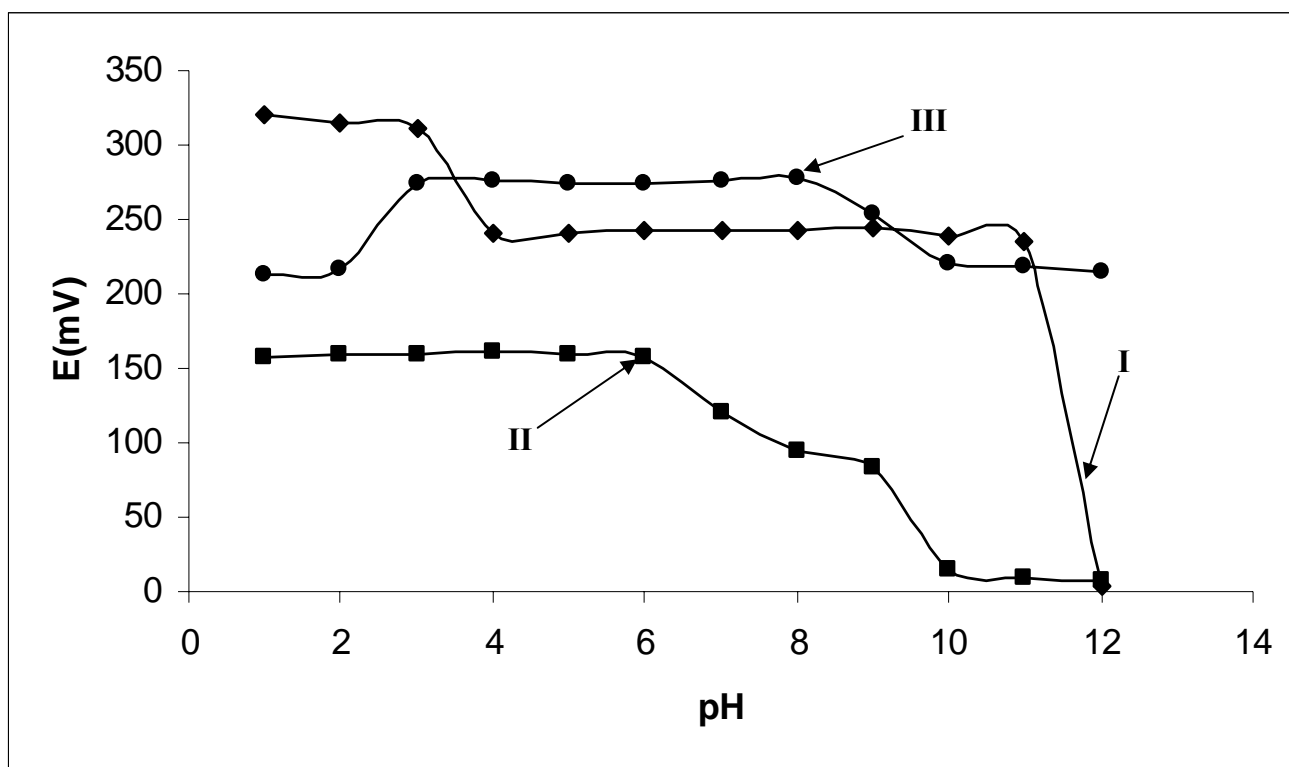

Figure 6.2 Effect of pH on the response of the enantioselective, potentiometric membrane electrodes based on α-cyclodextrin   (I), β-cyclodextrin (II) and γ-cyclodextrin (III), respectively, for the assay of S-deprenyl (10^{-5} mol l^{-1} S-deprenyl solution).

recording the emf of the cell for solutions containing 10^{-5} mol.l^{-1} S-deprenyl at different pH values (pH 1-12). The E (mV) versus pH plots presented in fig. 2 shows that the response of the EPMEs are pH-independent in the following pH ranges: 4.0-9.0, 1.0-6.0 and 3.0-8.0, for EPMEs based on α-, β- and γ-CD, respectively.

6.2.5.3 Selectivity of the electrodes

The selectivity of the potentiometric membrane electrodes was investigated using the mixed solutions method. The concentrations of interfering ions and S-deprenyl were 10^{-4} mol.l^{-1} and 10^{-5} mol.l^{-1}, respectively.

Table 6.2 Potentiometric selectivity coefficients for the enantioselective, potentiometric membrane electrodes.

Interfering species (J)	K_{sel}^{pot}		
	EPME based on		
	α-CD	β-CD	γ-CD
R-deprenyl	4.4 x 10^{-4}	3.2 x 10^{-3}	<< 10^{-4}
PVP	1.9 x 10^{-3}	<< 10^{-4}	<< 10^{-4}
Creatine	8.9 x 10^{-4}	4.1 x 10^{-4}	<< 10^{-4}
Creatinine	2.0 x 10^{-3}	<< 10^{-4}	4.2 x 10^{-4}
Paracetamol	9.0 x 10^{-4}	1.7 x 10^{-3}	1.3 x 10^{-3}
L-glutamine	<< 10^{-4}	2.3 x 10^{-3}	<< 10^{-4}

All measurements were made at room temperature; all values are average of 10 determinations

The values shown in Table 6.2 proved that the proposed electrodes are enantioselective and selective over polyvinylpyrolidone (PVP), creatine, creatinine, paracetamol and L-

glutamine. Therefore it can be used for enantioanalysis of S-deprenyl in Lentogesic tablets as well as in biological fluids.

6.2.5.4 Analytical applications

The assay of S-deprenyl in the presence of R-deprenyl was conducted by useing different ratios between S- and R-enantiomers of deprenyl. The good recovery values obtained (Table 6.3) for the assay of S-deprenyl in the presence of R-deprenyl, demonstrated the suitability for the proposed enantioselective potentiometric membrane electrodes for the enantiopurity tests of deprenyl raw material as well as in its pharmaceutical formulations. No significant difference in the recovery values were recorded for the different ratios between the enantiomers.

Table 6.3 Determination of S-deprenyl in the presence of R-deprenyl.

S : R (mol:mol)	S-Deprenyl, % Recovery		
	EPME based on		
	α-CD	β-CD	γ-CD
2 : 1	99.94 ± 0.02	99.92 ± 0.02	99.92 ± 0.02
1 : 1	99.98 ± 0.01	99.90 ± 0.01	99.90 ± 0.02
1 : 2	99.96 ± 0.02	99.95 ± 0.02	99.96 ± 0.01
1 : 4	99.96 ± 0.02	99.93 ± 0.02	99.98 ± 0.02
1 : 9	99.98 ± 0.01	99.91 ± 0.02	99.97 ± 0.01

All measurements were made at room temperature; all values are average of 10 determinations

The results obtained for the content uniformity test of Lentogesic tablets shown that S-deprenyl can be reliably assayed from its pharmaceutical formulation with average recoveries (n = 10) of 98.47 ± 0.17 %, 98.48 ± 0.24 %, and 98.62 ± 0.29 %, when EPMEs

based on α-, β- and γ-CD, respectively, were used. These results are correlating very good with those obtained when a HPLC method was used (98.50% S-deprenyl).

6.3 Conclusions

The proposed enantioselective, potentiometric membrane electrodes designed using α-, β- and γ-cyclodextrins as chiral selectors can be successfully used in the enantioanalysis of S-deprenyl raw material as well as in its pharmaceutical formulation. The analysis is far more simple, fast, and reliable than the chiral separations using chromatographic techniques. One of the features is the enantioanalysis of S-deprenyl in biological fluids, as creatine and creatinine did not interfere.

6.4 Refereces

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Chapter 7

Enantioselective, potentiometric membrane electrodes based on maltodextrins

7.1 Maltodextrins as chiral selectors in the EPMEs design

Maltodextrins represent a class of very powerful chiral selectors among the chiral selective substances, e.g., cyclodextrins, crown ethers, macrocyclic antibiotics, proteins. Maltodextrins (Fig. 7.1) are complex malto-, oligo-, and polysaccharide mixtures formed by hydrolysis of starch, with DE lower than 20 [1-3].
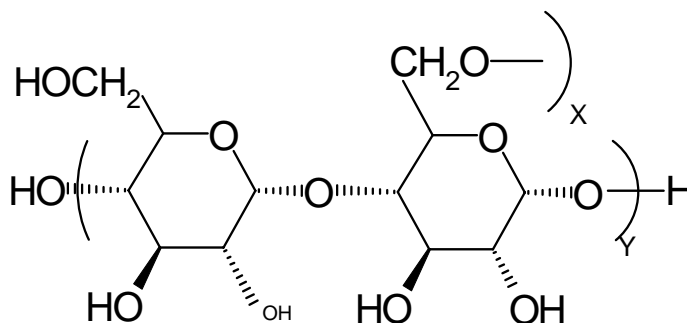

Figure 7.1 Maltodextrins

Possible types of maltodextrins have different DE values [I (4.0-7.0), II (13.0-17.0), and III (16.5-19.5)]. Variations in DE values result in maltodextrins with varying physico-chemical properties: solubility, hydroscopicity, osmolality and their effectiveness to reduce the freezing point increase with increasing DE, while viscosity, cohesiveness and coarse-crystal prevention increase as DE decreases [4,5]. Maltodextrins were intensively investigated as chiral selectors for enantiomeric separations by capillary zone electrophoresis, the maltodextrins with the highest DE values being the best chiral selectors [2,3,6-10], and they were also used in the design of enantioselective, potentiometric membrane electrode for the enantioanalysis of several drugs.

7.2 Enantioanalysis of R-deprenyl using enantioselective, potentiometric membrane electrodes based on maltodextrins

One enantioselective, potentiometric membrane electrode based on a maltodextrin is proposed for the enantioselective assay of R-deprenyl [11].

7.2.1 Reagents and materials

Graphite powder (1-2 μm), maltodextrin (DE 16.5-19.5) was purchased from Aldrich (Milwaukee, WI, USA). Paraffin oil was purchased from Fluka (Buchs, Switzerland). R-deprenyl was purchased from Sigma-Aldrich. Phosphate buffer (pH 5.8) was obtained from Merck (Darmstadt, Germany). Deionized water from a Modulab system (Continental Water System, Sand Antonio, TX, USA) was used for all solutions preparations. Lentogesic tablets (65 mg deprenyl per tablet) were obtained from Adcoc Ingram Limited (Johannesburg, South Africa).

The solution of maltodextrin (10^{-3} mol.l^{-1}) was prepared using deionized water. All standard and diluted solutions were buffered with phosphate buffer pH 5.8 using the ratio buffer:distilled water 1:1 (v/v).

7.2.2 Apparatus

A 663 VA Stand (Metrohm, Herisau, Switzerland) in combination with a µAutolab and Ecochemie (Utrech, The Nertherlands) Software version 4.9 were used for all potentiometric measurements. An Ag/AgCl (0.1 mol l^{-1} KCl) electrode served as reference electrode in the cell.

7.2.3 Electrode design

Paraffin oil and graphite powder in a ratio of 1:4 (w/w), were first thoroughly mixed, followed by the addition of an aqueous solution of maltodextrin with DE 16.5-19.5 (solution 10^{-3} mol l^{-1}). A quantity of carbon paste, free of maltodextrin, was also prepared and placed in a plastic pipette peak, leaving 3-4mm empty in the top to be filled with carbon paste containing the chiral selector. The diameter of the EPME was 3mm. Electric contact was obtained by inserting a Ag/AgCl wire into the carbon paste. The internal solution was 0.1 mol l^{-1} KCl. Prior to use, the surface of the electrode was wetted with deionised water and polished with alumina paper (polishing strips 30144-001, Orion).

7.2.4 Recommended procedure

7.2.4.1. Direct potentiometry

The potentiometric technique was used for potential determination of each standard solutions 10^{-10}-10^{-3} mol l^{-1}. The electrode was placed into stirred standard solutions and graphs of E(mV) versus pR-deprenyl were plotted. The unknown concentrations were determined from the calibration graphs.

7.2.4.2. Content uniform assay of deprenyl tablets

Each of the ten tablets were placed into 100 ml calibrated flask, dissolved and diluted to the mark using a phosphate buffer (pH 5.85):deionized water 1:1. The unknown concentration of deprenyl was determined using the direct potentiometric method.

7.2.5 Results and discussion

7.2.5.1 Electrode response

The response characteristics of the proposed electrode are as follows: slope 53.1 mV/decade of concentration, intercept, E° 554.3 mV, linear concentration range 10^{-10} – 10^{-3} mol/L, and detection limit 3.6 x 10^{-11} mol/L, when all measurements were performed at room temperature. All values are average of ten determinations. For the calibration equation, the correlation coefficient was 0.9999. The electrode showed a non-Nernstian response for S-deprenyl proving that it can be used only for the assay of R-deprenyl.

7.2.5.2 Effect of pH on the response of the electrode

The influence of the pH values on the response of the proposed electrode was investigated by recording the emf of the cell for solutions containing 10^{-5} mol.l^{-1} R-deprenyl at pH values between 1 and 12. The plot of E (mV) versus pH (Fig. 7.2) indicates, that the response of the electrode does not depend on the pH changes in the pH ranges 2.0-7.0.
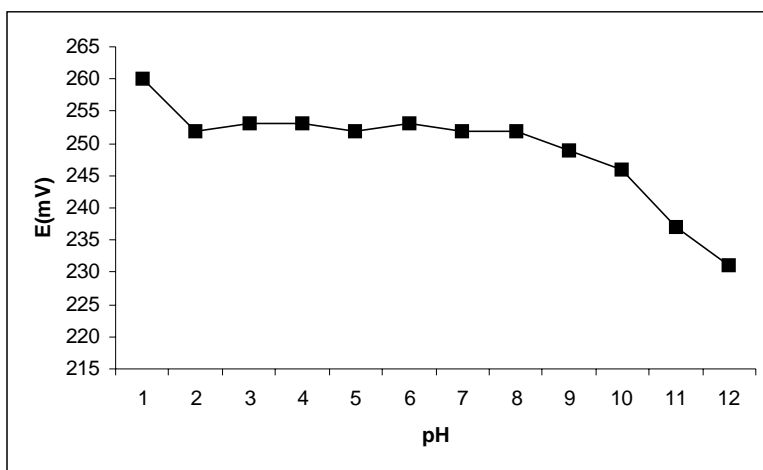
Figure 7.2 Effect of pH on the response of the enantioselective, potentiometric membrane electrode used for the assay of R-deprenyl (10^{-5} mol l^{-1} R-deprenyl solution).

7.2.5.3 Selectivity of the electrode

The selectivity of the potentiometric membrane electrode was investigated using the mixed solution method. The concentrations of interfering ions and R-deprenyl were 10^{-4} mol l^{-1} and 10^{-5} mol l^{-1}, respectively. The values of the potentiometric selectivity coefficients (Table 7.1) indicate that the electrode can be reliably used for enantioanalysis of R-deprenyl due to its good selectivity and enantioselectivity.

Table 7.1 Selectivity coefficient for the EPME based on maltodextrin used for the assay of R-deprenyl.

Interfering species (J)	K_{sel}^{pot}
S-Deprenyl	4.4×10^{-3}
PVP	3.5×10^{-3}
Creatine	4.0×10^{-3}
Creatinine	3.8×10^{-3}
Paracetamol	3.0×10^{-3}
L-glutamine	1.4×10^{-3}

All measurements were made at room temperature; all values are average of 10 determinations

7.2.5.4 Analytical applications

The assay of R-deprenyl in the presence of S-deprenyl was carried out using different ratios between R- and S-deprenyl. The results obtained (Table 7.2) confirmed once more the suitability of the proposed potentiometric membrane electrode for the enantioanalysis of R-deprenyl. No significant difference in the recovery values were recorded for the different ratios between the enantiomers.

Table 7.2 Determination of R-deprenyl in the presence of S-deprenyl.

R : S (mol/mol)	% R-deprenyl, Recovery
2 : 1	99.14 ± 0.02
1 : 1	99.78 ± 0.01
1 : 2	99.25 ± 0.02
1 : 4	99.78 ± 0.02
1 : 9	99.80 ± 0.01

All measurements were made at room temperature; all values are average of 10 determinations

The results obtained for content uniformity test of Lentogesic tablets using the proposed electrode showed, that the tested pharmaceutical formulations contain 1.36 % R-deprenyl (RSD = 0.11%, (n = 10)). The average recovery value determined was in agreement with that obtained using a HPLC method: $1.49 \pm 0.17\%$.

7.3 Enantioanalysis of S-ibuprofen using enantioselective, potentiometric membrane electrodes based on maltodextrins

Three enantioselective, potentiometric membrane electrodes (EPMEs) based on maltodextrin with different values of dextrose equivalence(DE) (maltodextrin I: DE 4.0-7.0; maltodextrin II: DE 13.0-17.0; maltodextrin III: DE 16.5-19.5) were proposed for the assay of S-ibuprofen [12].

7.3.1 Reagents and materials

Graphite powder (1-2 µm), maltodextrins (DE 4.0-7.0 (I), 13.0-17.0 (II) and 16.5-19.5 (III)) were purchased from Aldrich (Milwaukee, WI, USA). Paraffin oil was purchased from Fluka (Buchs, Switzerland). S-ibuprofen was purchased from Sigma-Aldrich. Phosphate buffer (pH 4.00) was obtained from Merck (Darmstadt, Germany). Deionized water from a Modulab system (Continental Water System, Sand Antonio, TX, USA) was used for all solutions preparations. Myprodol capsules (200 mg ibuprofen/capsule) and Nurofen tablets (200 mg ibuprofen/tablet) were obtained from Nutrent (Sandton, South Africa).

The solutions of maltodextrins (10^{-3} mol l^{-1}) were prepared using deionized water. All standard and diluted solutions were buffered with phosphate buffer pH 4.00 using the ratio buffer:distilled water 1:1 (v/v).

7.3.2 Apparatus

A 663 VA Stand (Metrohm, Herisau, Switzerland) in combination with a μAutolab and Ecochemie (Utrech, The Nertherlands) Software version 4.9 were used for all potentiometric measurements. An Ag/AgCl (0.1 mol l^{-1} KCl) electrode served as reference electrode in the cell.

7.3.3 Electrode design

Paraffin oil and graphite powder in a ratio of 1:4 (w/w), were first thoroughly mixed, followed by the addition of an aqueous solution of maltodextrin (DE 4.0-7.0 (I), 13.0-17.0 (II) and 16.5-19.5 (III)) from 10^{-3} mol l^{-1} maltodextrin solution. A quantity of carbon paste, without maltodextrin, was also prepared and placed in a plastic pipette peak, leaving 3-4mm empty in the top to be filled with carbon paste containing the chiral selector. The diameter of the EPMEs was 3mm. Electric contact was obtained by inserting a Ag/AgCl wire into the carbon paste. The internal solution was 0.1 mol l^{-1} KCl. Prior to use, the surface of the electrode was wetted with deionised water and polished with alumina paper (polishing strips 30144-001, Orion).

7.3.4 Recommended procedures

7.3.4.1   Direct potentiometry

The potentiometric method was used for potential determination of each standard solution 10^{-10} - 10^{-3} mol l^{-1}. The electrodes were placed into stirred standard solutions and graphs of E(mV) versus pS-ibuprofen were plotted. The unknown concentrations were determined from the calibration graphs.

7.3.4.2 Content uniform assay of ibuprofen capsules and tablets

Each of the five capsules and five tablets were placed into 100 ml calibrated flask, dissolved and diluted to the mark using a phosphate buffer (pH 5.34):deionized water 1:1 (v/v) solution. The unknown concentration of ibuprofen was determined using the direct potentiometric method.

7.3.5 Results and Discussion

7.3.5.1 Electrodes response

The response characteristis exhibited by the proposed EPMEs for the assay of S-ibuprofen are summarized in Table 7.3.

Table 7.3 Response characteristics of enantioselective, potentiometric membrane electrodes based on maltodextrins I, II and III for the assay of S-ibuprofen.

Maltodextrin	Slope (mV/decade of conc.)	Intercept, E^0 (mV)	Linear concentration range (mol l^{-1})	Detection limit (mol l^{-1})
I	59.0	593.4	10^{-10} - 10^{-3}	5.5×10^{-11}
II	58.4	473.2	10^{-8} - 10^{-3}	8.0×10^{-9}
III	55.6	632.5	10^{-10} - 10^{-3}	4.1×10^{-12}

All measurements were made at room temperature; all values are average of ten determinations.

All the proposed membrane electrodes exhibited linear and near Nernestian responses (55-59 mV per decade of concentration) for S-ibuprofen while a non-Nernstian response was recorded for R-ibuprofen. The correlation coefficients for all the calibration graphs was 0.9999. The limits of detection were very low. The low working concentration

ranges and limits of detection may be explained by the structural conformation of the maltodextrin that can change from flexible coil at higher DE values to helix at lower DE values. The stability of the complexes formed between the chiral selector and analytes is increasing with the value of DE, because increasing the DE value will result in an increase in the diameter of the helix leading to less steric hindrance of the approach of an interacting molecule, hence more inclusion.

7.3.5.2 Effect of pH on the response of the electrodes

The effect of the pH variation on the response of the EPMEs based on maltodextrin I, II and III has been tested by recording the emf of the cell.

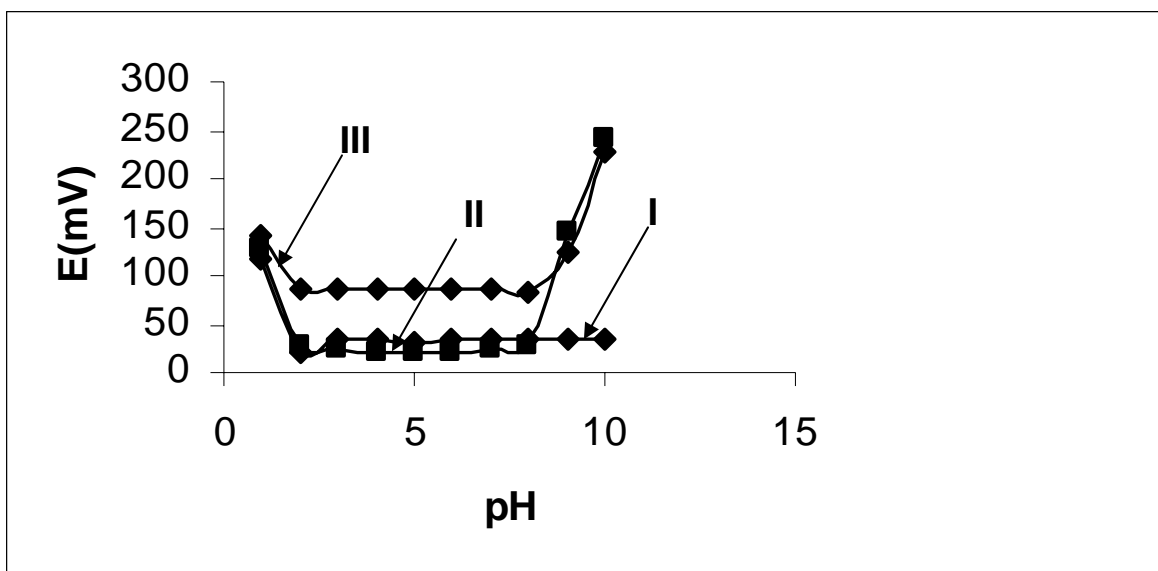

Figure 7.3 Effect of pH on the response of the enantioselective, potentiometric membrane electrodes based on maltodextrin I (I), II (II) and III (III), respectively, for the assay of S-ibuprofen (10^{-7} mol.l^{-1} S-ibuprofen solution).

All measurements were performed for a concentration of 10^{-7} mol l^{-1} of S-ibuprofen, at different pH values selected between 1 and 10. The E (mV) versus pH plots presented in fig. 7.3 shows that the response of the EPMEs are pH-independent in the pH ranges of 3.0-9.0 (maltodextrin I based EPME) and 2.0-8.0 (maltodextrin II and III based EPMEs).

7.3.5.3 Selectivity of the electrodes

The selectivity of the potentiometric membrane electrode was checked using the mixed solutions method. The concentrations of interfering ions and S-ibuprofen were 10^{-5} mol/L and 10^{-6} mol/L, respectively. The values obtained for the potentiometric selectivity coefficients show that the EPMEs are selective over paracetamol, PVP, creatine and creatinine (Table 7.4). They are also enantioselective, since the values recorded for potentiometric selectivity coefficients over R-ibuprofen are lower than 10^{-4}.

Table 7.4 Potentiometric selectivity coefficients for the enantioselective, potentiometric membrane electrodes used for the assay of S-ibuprofen.

Iterference species (J)	K^{pot}		
	EPME based on maltodextrin		
	I	II	III
Paracetamol	4.2×10^{-3}	1.5×10^{-3}	8.6×10^{-3}
PVP	1.2×10^{-3}	4.0×10^{-4}	$<<10^{-4}$
Creatine	1.1×10^{-3}	$<<10^{-4}$	$<<10^{-4}$
Creatinine	1.1×10^{-3}	$<<10^{-4}$	8.6×10^{-4}

All measurements were made at room temperature; all values are average of ten determinations.

7.3.5.4 Analytical applications

The proposed enantioselective, potentiometric membrane electrodes based on maltodextrin are useful for the determination of the enantiopurity of S-ibuprofen raw

material and for testing the content uniformity of Myprodol capsules and Nurofen tablets, by direct potentiometry. The results obtained for the uniformity content test shown that S-ibuprofen can be reliably assayed in pharmaceutical formulations, with average recoveries of $99.35 \pm 0.23\%$, $99.32 \pm 0.17\%$ and $99.34 \pm 0.19\%$ from Myprodol capsules and average recoveries of $98.55 \pm 0.31\%$, $98.43 \pm 0.17\%$ and $98.53 \pm 0.09\%$ from Nurofen tablets, when EPMEs based on maltodextrins I, II and III, respectively. These results are in good agreement with those obtained using a chromatographic method: 99.30% and 98.40% S-ibuprofen in Myprodol capsules and Nurofen tablets, respectively. The results showed that tested pharmaceutical formulations contain S-ibuprofen as a main component.

7.4 Conclusion

The potentiometric, enantioselective membrane electrodes proved to be good for enantioselective analyses of R-deprenyl and S-ibuprofen. The reliability of the analytical information is evident from the RSD values obtained from recovery and content uniformity tests. The electrodes enantioselectivity made it suitable for enantiopurity assay of R-deprenyl and S-ibuprofen as both raw material and pharmaceutical tablet formulations. The construction of the electrodes is simple, fast and reproducible. Due to their good selectivity over creatine and creatinine, the proposed electrodes can also be used for the determination of R-deprenyl and S-ibuprofen in biological fluids.

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Chapter 8

Conclusions

Enantioanalysis of pharmaceutical compounds is necessary for chiral compounds that must be formulated as single enantiomers. For this purpose, highly reliable analytical techniques are required. If chromatographic techniques may be used for qualitative purposes, enantioselective, potentiometric membrane electrodes proved to provide high accuracy, precision, reliability of the analytical information, simplicity, and low cost for the quantitative enantioanalysis of pharmaceutical compounds.

The key of a high reliable enantioanalysis using enantioselective, potentiometric membrane electrodes is to find the best chiral selector for the enantiomers to be determined. The most used chiral selectors for the design of these electrodes are cyclodextrins and maltodextrins. C_{60} fullerenes proved to be good chiral selectors for the enantioanalysis of certain enantiomers.

Accordingly, for the enantioanalysis of S-ibuprofen the chiral selectors of choice were maltodextrins and antibiotics (vancomycin and teicolplanin), for S-deprenyl, fullerenes and cyclodextrins based electrodes were chosen while for the enantioanalysis of R-deprenyl, one maltodextrin proved to be the best for the enantioselective electrode design.

Five electrodes based on maltodextrins (DE 4.0-7.0, 13.0-17.0 and 16.5-19.5) and antibiotics (vancomycin and teicoplanin) were design for the enantioanalysis of S-ibuprofen. These electrodes did not show a Nernstian or near-Nernstian response for R-ibuprofen. The electrodes had good response characteristics, and they could have been reliable used for the enantioanalysis of S-ibuprofen in its pharmaceutical formulations, Myprodol and Nurofen.

Six enantioselective, potentiometric membrane electrodes based on fullerenes ((1,2-methanofullerene C_{60})- 61- carboxylic acid, diethyl (1,2-methanofullerene C_{60})-61-61-dicarboxylate and tert-butyl (1,2-methanofullerene C_{60})-61-carboxylic acid) and cyclodextrins (α-, β-, and γ-cyclodextrin) have been designed for the enantioanalysis of S-deprenyl and one enantioselective, potentiometric membrane electrode have been designed for the enantioanalysis of R-deprenyl. The utilization of fullerenes in the electrodes design improved the response characteristics, reliability and accuracy of the analytical information obtained using the proposed electrodes.

For the enantioanalysis of S-deprenyl the best slopes were obtained when the fullerenes based enantioselective, potentiometric membrane electrodes were used. The lowest limits of detections and the best slopes were obtained when maltodextrins were used for the enantioanalysis of S-ibuprofen.

The design of the electrodes is simple, fast and reproducible. One of the main advantages of the proposed method is that the sample did only need to be dissolved in distilled water

and buffered before the assay of any of the enantiomers and that makes the method simple, fast and highly reliable.

The proposed enantioselective, potentiometric membrane electrodes can be reliable used for the enantioanalysis of the proposed enantiomers as raw materials and in their pharmaceutical formulations. Their good selectivity over compounds such as creatine and creatinine proved that they can also be used for the enantioanalysis of the enantiomers in biological samples such as urine.