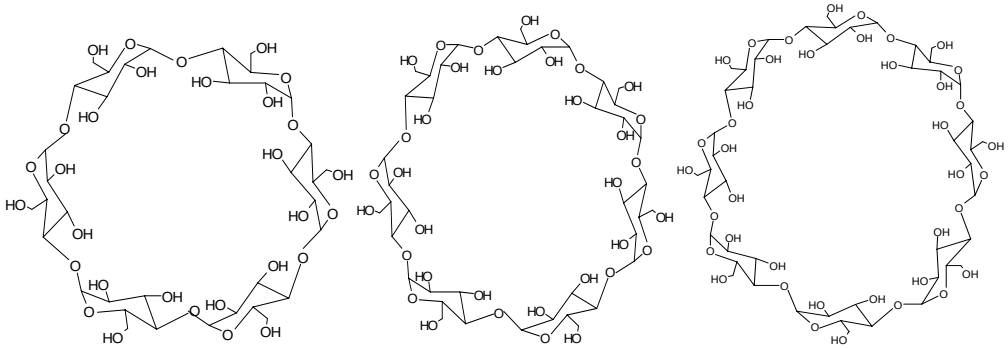
Chapter 6

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 x 10^{-11}
β-CD	57.7	514.4	10^{-8}-10^{-3}	1.2 x 10^{-9}
γ-CD	56.2	581.0	10^{-10}-10^{-3}	4.5 x 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

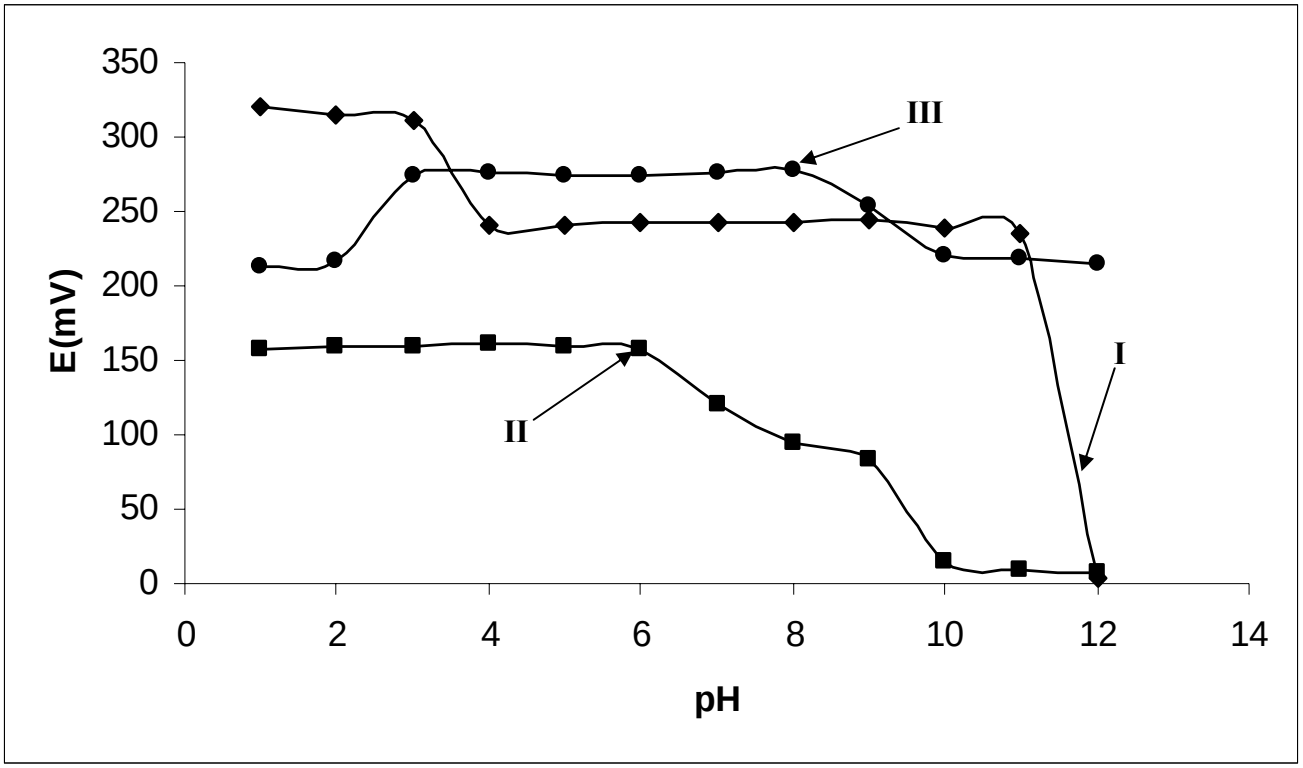


Figure 6.2 Effect of pH on the response of the enantioselective, potentiometric membrane electrodes based on α-cyclodextrin (I), β-cyclodextrin (II) and γ-cyclodextrin (III), respectively, for the assay of S-deprenyl (10^{-5} mol l^{-1} S-deprenyl solution).

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	4.4×10^{-4}	3.2×10^{-3}	$<< 10^{-4}$
PVP	1.9×10^{-3}	$<< 10^{-4}$	$<< 10^{-4}$
Creatine	8.9×10^{-4}	4.1×10^{-4}	$<< 10^{-4}$
Creatinine	2.0×10^{-3}	$<< 10^{-4}$	4.2×10^{-4}
Paracetamol	9.0×10^{-4}	1.7×10^{-3}	1.3×10^{-3}
L-glutamine	$<< 10^{-4}$	2.3×10^{-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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