# Enantioselective Potentiometric Membrane Eectrodes Based on α-, β- and γ-Cyclodextrins as Chiral Selectors for the Assay of *S*-Deprenyl

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**Abstract:** Enantioselective, potentiometric membrane electrodes (EPMEs) based on α-, β- and γ-cyclodextrins (CDs) as chiral selectors were proposed for the assay of *S*-deprenyl. The response characteristics proved that the proposed EPMEs could be reliably used in the assay of *S*-deprenyl. The best slope is exhibited by the EPME based on β-cyclodextrin. The limits of detection are very low: $10^{-9}$ mol $L^{-1}$ magnitude order for β-cyclodextrin based EPME, and $10^{-11}$ mol $L^{-1}$ magnitude order for α-, and γ-cyclodextrins based EPMEs. The surfaces of the electrodes are stable and easily renewable by polishing on alumina paper.

**Keywords:** Enantioselectivity, potentiometric membrane electrode; enantioanalysis, cyclodextrins; S-deprenyl.

## 1. INTRODUCTION

Enantioanalysis of pharmaceutical compounds with a chiral moiety is essential, because usually, only one of the enantiomers may have the correct pathway in the body, the other being non-active, or toxic or having a different pathway in the body. Therefore it is a need of high reliable analytical methods for enantiopurity tests of both raw material and pharmaceutical formulations.

Cyclodextrins (CDs) are cyclic oligosaccharides being formed by six (α-), seven (β-), or eight (γ-) glucose units [1]. In all cases, they display a torus-like or hollow truncated cone shape, with a cavity and two hydrophilic rims in which the primary and secondary hydroxyl groups are inserted. The main feature that makes CDs of interest is their ability to form inclusion compounds with a variety of guest molecules, including enantiomers [2-10].

S-Deprenyl (Fig. **1**) is a drug used worldwide in the treatment of Parkinson`s disease [11]. S-deprenyl selectively inhibits MAO type A and R-deprenyl selectively inhibits MAO type B [12]. The "A" form is responsible for breaking down the neurotransmitters serotonin, adrenalin and noradrenalin, while "B" form is breaking down the dopamine. The main metabolites of deprenyl are desmethyldeprenyl, methamphetamine, and amphetamine formed by N-dealkylation [13-16].

Enantioanalysis of deprenyl and its metabolits was done using only chromatographic methods, such as: TLC [17, 18], HPLC [19, 20] and CE [21, 22]. Electrochemical methods are a very good alternative in terms of accuracy and reproducibility to the chromatographic techniques, for the enantioanalysis of pharmaceutical compounds [23]. They are also very simple, rapid and they are not expensive. Therefore, we proposed three enantioselective, potentiometric membrane electrodes based on α-, β- and γ-cyclodextrins for the enantioselective assay of S-deprenyl.

## 2. EXPERIMENTAL

### 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).

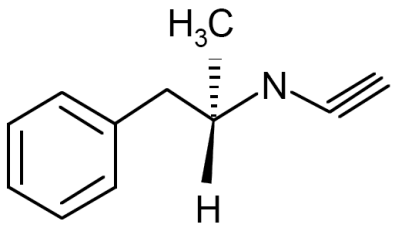


**Fig. (1).** The structure of S-Deprenyl.

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### 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.

### 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).

### 2.4. Recommended Procedures

#### *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.

#### *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.

## 3. RESULTS AND DISCUSSION

### 3.1. Electrodes Response

The response characteristics exhibited by proposed cyclodextrins based EPMEs for the enantioanalysis of S-deprenyl are summarized in Table **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 have shown non-Nernstian responses when used for R-deprenyl.

### 3.2. Effect of pH on the Response of the Electrodes

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

### 3.3. 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. The values shown in Table **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.

### 3.4. 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 **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.

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).

## 4. 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

**Table 1. Response Characteristics of Enantioselective Potentiometric Membrane Electrodes**

| EPME Based on | 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 | $10^{-10}$ - $10^{-4}$ | $2.8 \times 10^{-11}$ |
| β-CD | 57.7 | 514.4 | $10^{-8}$ - $10^{-3}$ | $1.2 \times 10^{-9}$ |
| γ-CD | 56.2 | 581.0 | $10^{-10}$ - $10^{-3}$ | $4.5 \times 10^{-11}$ |

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

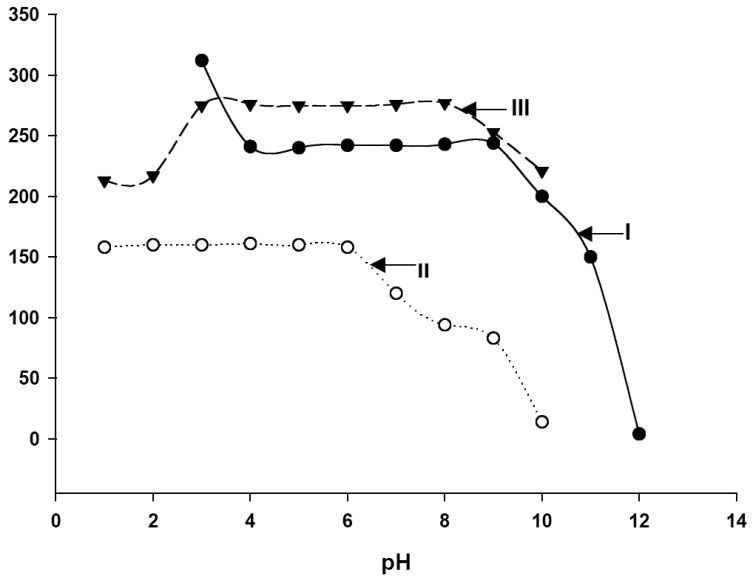


**Fig. (2).** Effect of pH on the response of the enantioselective, potentiometric membrane electrodes based on α-cyclodextrin (I), β-cyclodextrin (II) and γ-cyclodextrin (III), respectively, for the assay of S-deprenyl ($10^{-5}$ mol $L^{-1}$ S-deprenyl solution).

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

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

**Table 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 |

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

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.

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