# Enantioselective, potentiometric membrane electrodes based on cyclodextrins for the determination of L-histidine

**Raluca-Ioana Stefan-van Staden[a], and Luxolo Holo[a]**

[a]Department of Chemistry, **University of Pretoria, Pretoria** 0002, South Africa

## Abstract

Four enantioselective, potentiometric membrane electrodes based on carbon paste impregnated with α-, β-, 2-hydroxyl-3-trimethylammoniopropyl-β-(as chloride salt) and γ-cyclodextrins (γ-CDs) are proposed for the assay of L-histidine (L-his). The proposed electrodes showed near-Nernstian response over L-his but not over D-histidine (D-his). The recovery of L-his in the presence of D-his was higher than 99.10% with R.S.D. lower than 0.1%. The surfaces of the electrodes are easily renewable by simply polishing on an alumina paper.

## Article Outline

## 1. Introduction

L-Histidine (L-his) (Fig. 1) is an essential component of many proteins. Due to the high reactivity of its imidazole group, histidine residue is often found at the active site of enzymes and involved directly in catalysis [1]. It controls the transmission of metal elements in biological bases [2], and has been reported to act as a neurotransmitter, or neuromodulator in mammalian central-nervous system, including the retina. Thus, the determination of L-his in biological fluids is of great importance.

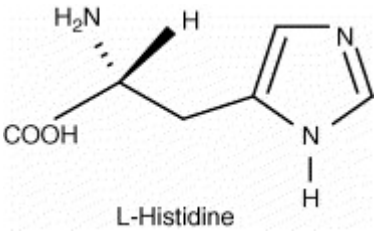


Fig. 1. L-Histidine.

Chiral recognition became an area of considerable research interests because of its importance in almost all fields of biological, chemical, and pharmaceutical sciences [3]. The techniques used until now for enantioanalysis are based on chromatography, capillary zone electrophoresis, mass spectrometry, and electroanalysis. Advantageously, electroanalysis feature relatively high efficiency and low cost [4], [5], [6], [7], [8] and [9].

Cyclodextrins (CDs) are cyclic, non-reducing oligosaccharides of six, seven and eight α-D-glucose units (commonly referred to as the α-, β- and γ-CDs, respectively), obtained from starch by enzymatic degradation using Bacillus amylobacter [10], [11], [12], [13], [14] and [15]. The CDs have the ability to form inclusion complexes. This property made possible the recognition of a single enantiomer through internal and external selectivities assured by the cavity size and the groups bound on it.

HPLC [16] and [17], capillary electrophoresis [18], [19] and [20] and fluorescence [21] were proposed for the assay of histidine. A silver oxide/silver phosphate electrode was proposed for the assay of histidine, but it cannot discriminate between L- and D-enantiomers of histidine [22].

This paper reports four enantioselective, potentiometric membrane electrodes (EPMEs) based on α-, β-, 2-hydroxyl-3-trimethylammoniopropyl-β-(as chloride salt) and γ-CDs impregnated on carbon paste for the assay of L-his.

## 2. Experimental

### 2.1. Reagents and materials

L- and D-his were supplied by Sigma–Aldrich (St. Louis, MO, USA). α-, β-, 2-Hydroxyl-3-trimethylammoniopropyl-β-(as chloride salt) and γ-CDs were supplied by Walker–Chemie GmbH (München, Germany). Graphite powder (1–2 μm, synthetic) was supplied by Aldrich. Paraffin oil was supplied by Fluka (Buchs, Switzerland). Food supplement, energy booster, immunity modulator, capsules (containing 0.72 g L-his) were supplied by Hypo-Plus Naturals, South Africa.

Deionised water from a Modulab system (Continental Water Systems, San Antonio, TX, USA) was used for the preparation of all solutions. The L- and D-his solutions used in the characterization of the enantioselective, potentiometric membrane electrodes were prepared from standard L- and D-his solutions ($10^{-2}$ mol/L), respectively, by serial dilutions. All standard and diluted solutions were prepared using phosphate buffer (pH 5.40, 0.1 mol/L) from Merck (Darmstadt, Germany) (1:1 (v/v), buffer:deionised water).

### 2.2. Apparatus

A 663 VA stand (Metrohm, Herisau, Switzerland) connected to a PGSTAT 20 (Eco Chemie, Utrech, The Netherlands) and a software Version 4.8 was used for all potentiometric measurements. Ag/AgCl (0.1 mol/L KCl) served as a reference electrode in the cell.

### 2.3. Electrode design

Paraffin oil and graphite powder were mixed in a ratio of 1:4 (w/w) followed by the addition of the aqueous solution of CD (α-, β-, 2-hydroxyl-3-trimethylammoniopropyl-β-(as chloride salt) (β-CD-derivative) or γ) ($10^{-3}$ mol/L) (100 μL chiral selector solution to 100 mg carbon paste). A certain quantity of carbon paste free of CD was prepared and it was placed into a plastic pipette peak leaving 3–4 mm empty in the top to be filled with the carbon paste that contains the chiral selector. The diameter of the EPMEs was 3 mm. Electric contact was obtained by inserting Ag/AgCl wire in the carbon paste. A 0.1 mol/L KCl solution was used as internal solution.

The surface of the electrode was wetted with deionised water and polished with alumina paper (polishing strips 30144-001, Orion) before using them for each experiment. The oil from the carbon paste prevents the leach of the CD from the membrane into solution.

### 2.4. Recommended procedure: direct potentiometry

The direct potentiometry was used for measurements of the potentials of each standard solution ($10^{-10}$ to $10^{-2}$ mol/L). The electrodes were placed in stirred standard solutions and graphs of $E$ (mV) versus $p$L-his were plotted. The unknown concentrations were determined from the calibration graphs.

### 2.5. Uniformity content test

Each of 10 capsules (0.72 g histidine/tablet) was placed in a 250 mL volumetric flask and dissolve in a 1:1 distilled water:buffer (pH 5.4) solution. The unknown concentration was determined using the direct potentiometric method.

## 3. Results and discussion

### 3.1. Electrodes response

The response characteristics exhibited by the proposed EPMEs towards the detection of L-his are shown in Table 1. The responses of all the electrodes was near-Nernstian for L-his and non-Nernstian for D-his. The working concentration ranges are large, and the correlation coefficients for the calibration plots are 0.9999. The response time (the time which elapses between the instant when the electrodes of the potentiometric cells are brought into contact with a 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) of the proposed electrodes was 30 s for concentrations between $10^{-5}$ and $10^{-3}$ mol/L and 1 min for concentrations lower than $10^{-5}$ mol/L.

Table 1.

Response characteristics of enantioselective, potentiometric membrane electrodes designed for the assay of L-histidine

| EPME based on | Slope (mV/decade of concentration) | Intercept $E^0$ (mV) | Linear concentration range (mol/L) | Detection limit (mol/L) |
|---|---|---|---|---|
| α-CD | 57.30 | 413.10 | $10^{-3}$ to $10^{-7}$ | $6.17 \times 10^{-8}$ |
| β-CD | 57.76 | 587.95 | $10^{-3}$ to $10^{-10}$ | $6.62 \times 10^{-11}$ |
| β-CD-derivative | 53.89 | 556.25 | $10^{-4}$ to $10^{-10}$ | $4.77 \times 10^{-11}$ |
| γ-CD | 56.36 | 576.21 | $10^{-3}$ to $10^{-10}$ | $5.97 \times 10^{-11}$ |

All measurements were made at 25 °C. All values are average of measurements performed during 1 month.

The proposed electrodes were highly stable and reproducible over a month test period. α- and β-cyclodextrin based enantioselective, potentiometric membrane electrodes showed better time stability, their standard potentials varying by ±0.10 mV, compared to that of γ-CD based enantioselective, potentiometric membrane electrode, varying by ±4.0 mV during the 1 month test period.

### 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 the solutions containing $10^{-5}$ mol/L L-his at different pH values (pH 1–12). These solutions were prepared by adding small volumes of HCl and/or NaOH solutions (0.1 or 1 mol/L of each) to an L-his solution. The plots of *E* (mV) versus pH (Fig. 2) showed that the response of the electrodes is not depending on pH, in the ranges 4.0–9.0 (β-CD and β-CD-derivative based EPMEs), 4.0–7.0 (γ-CD based EPME), and 3.0–8.0 (α-CD based EPME). This proves the basic behaviour of L-his at pH <4 and its acidic behaviour at pH >8.

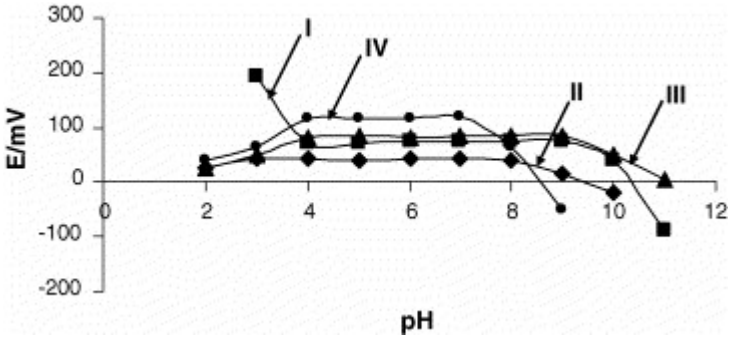


Fig. 2. The influence of pH on the response of the enantioselective potentiometric membrane electrodes ($C_{\text{L-his}} = 10^{-5}$); I—β-CD based EPME, II—γ-CD based EPME, III—β-CD-derivative based EPME, and IV—α-CD based EPME.

### 3.3. The selectivity of the electrodes

The enantioselectivity of the electrodes was investigated over D-his, using mixed solution method. The concentration of the interfering ion and L-his were $10^{-4}$ and $10^{-5}$ mol/L, respectively. The values of $pK^{pot}$ ($pK^{pot} = -\log K^{pot}$, where $K^{pot}$ is the potentiometric selectivity coefficient) were 3.0, 2.2, 2.4, and 2.7 for EPMEs based on β-, γ-, β-CD-derivative and α-CD. Inorganic ions such as $Na^+$, $K^+$, $Ca^{2+}$ as well as polyvinylpyrolidone did not interfere with the analysis of L-his ($pK^{pot} \gg 4$).

### 3.4. Analytical application

To assess the feasibility of the proposed direct potentiometric procedure, recovery tests were performed for histidine-raw material. The assay of L-his in the presence of D-his was conducted using different ratios between L- and D-his. The results obtained (Table 2) demonstrated the suitability for the proposed enantioselective, potentiometric membrane electrodes for testing the enantiopurity of histidine-raw material 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 L:D enantiomers varying from 1:9 to 1:99.9.

Table 2.

Determination of L-histidine in the presence of D-histidine

| **L:D (mol/mol)** | **L-histidine, recovery (%)** | | | |
|---|---|---|---|---|
| | **EPME based on** | | | |
| | **α-CD** | **β-CD** | **γ-CD** | **β-CD-derivative** |
| 2:1 | 99.14 ± 0.03 | 99.98 ± 0.02 | 99.95 ± 0.03 | 99.59 ± 0.02 |
| 1:1 | 99.12 ± 0.02 | 99.94 ± 0.02 | 99.97 ± 0.03 | 99.47 ± 0.02 |
| 1:2 | 99.12 ± 0.02 | 99.96 ± 0.02 | 99.98 ± 0.02 | 99.52 ± 0.03 |
| 1:4 | 99.16 ± 0.03 | 99.95 ± 0.03 | 99.96 ± 0.02 | 99.47 ± 0.03 |
| 1:9 | 99.14 ± 0.02 | 99.96 ± 0.03 | 99.97 ± 0.02 | 99.57 ± 0.02 |

All measurements were made at 25 °C. All values are average of 10 measurements.

Uniformity content tests were performed for the capsules containing histidine. The results shown that the capsules contain: 96.78 ± 0.21, 96.07 ± 0.14, 96.95 ± 0.19, and 96.93 ± 0.20% L-his when. α-, β-, 2-Hydroxyl-3-trimethylammoniopropyl-β-(as chloride salt) and γ-CDs based electrodes were used for the recovery tests.

### 4. Conclusions

This paper describes new enantioselective, potentiometric membrane electrodes designed using α-, β-, 2-hydroxyl-3-trimethylammoniopropyl-β-(as chloride salt) and γ-CDs as chiral selectors used in the enantioselective analysis of L-his. The electrodes can be

successfully used in the assay of L-his in the presence of D-his. The electrodes exhibited near-Nernstian slope, good enantioselectivity and very low limits of detection.

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Corresponding author. Tel.: +27 12 420 2510; fax: +27 12 362 5297.

**Vitae**

**Raluca-Ioana Stefan-van Staden** is Associate Professor of Analytical and Bioanalytical Chemistry at the University of Pretoria, South Africa. The author or coauthor of over 150 journal articles book chapters, and three books, she serves on the Editorial Board of *Preparative Biochemistry & Biotechnology, Sensor Letters and Enyclopedia of Sensors* and on numerous Scientific Committees and has given more than 200 presentations at conferences and symposiums in Europe, North America, Australia, Asia and Africa. Her main research fields are development of new electrochemical sensors for diagnosis and pharmaceutical analysis, quality and reliability in chemical analysis and flow systems (FIA and SIA). She received in 2002 the *President Award from National Research Foundation* (South Africa) and the *Raikes Medal from South African Chemical Institute*.

**Luxolo Holo** is a PhD student at the University of Pretoria. He obtained the MSc degree from the University of Pretoria in 2006. During the MSc studies he developed and characterized new enantioselective sensors for enantioanalysis of amino acids of clinical and pharmaceutical importance.