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# Potentiometric enantioselective membrane electrode for s-enalapril assay

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**Abstract.** A novel potentiometric, enantioselective membrane electrode based on graphite paste (graphite powder and paraffin oil), impregnated with $10^{-3}$ mol/L 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin (as chloride salt) solution, has been constructed. The potentiometric, enantioselective membrane electrode can be used for the reliable assay of S-enalapril as raw material and from its pharmaceutical formulations in the concentration range of $3.6 \times 10^{-5}$ – $6.4 \times 10^{-2}$ mol/L, with a detection limit of $1.0 \times 10^{-5}$ mol/L and with an average recovery of 99.96% (RSD = 0.098%), using a chronopotentiometric (zero current) technique. The enantioselectivity was determined *versus* D-proline and it was shown that only L-proline is the main interfering compound. The surface of the electrode can be regenerated by simply polishing to obtain a fresh surface which is ready to be used in a new assay.

**Key words.** Potentiometry – enantioselective membrane electrode – enantioselective analysis – 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin – S-enalapril.

## Introduction

The discrimination between enantiomers has become one of the most important fields of modern analytical chemistry, especially for pharmaceutical products and biomedical research, since the stereochemistry has a significant influence on the biological activity [1].

Several chromatographic techniques for enantioanalysis, involving liquid chromatography [2], thin-layer chromatography [3], gas chromatography [4], and capillary zone electrophoresis [5], using β-cyclodextrin derivatives as chiral selectors have been reported. The reliability of these separation techniques is sometimes low, especially due to the low affinity of the enantiomers for a chiral selector.

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To improve the quality of enantioselective analysis, enantioselective amperometric biosensors [6,7], and potentiometric, enantioselective membrane electrodes [8,9] were proposed. The best reliability for enantioselective sensors was obtained by using carbon paste as support for the electroactive material [10].

This paper describes a new type of potentiometric enantioselective membrane electrode based on 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin. The β-cyclodextrin derivative is impregnated in a carbon paste. The potentiometric enantioselective membrane electrode is used for the enantioselective assay of S-enalapril.

S-enalapril, (S)-1-[N-[1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline is an angiotensin-converting enzyme (ACE) inhibitor which is extensively used for the treatment of hypertention and congestive heart failure. Due to the fact that only the S enantiomer possesses the ACE inhibitory activity, it is therefore of interest to develop reliable methods for the S-enalapril enantiopurity assay.

## Experimental section

### Electrode design

The paraffin oil and graphite powder were mixed in a ratio of 1:4 (w/w) followed by the addition of the solution containing $10^{-3}$ mol/L of 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin (100 μL chiral selector solution to 100 mg carbon paste). The graphite - paraffin oil paste was filled into a plastic pipette peak leaving 3 to 4 mm empty in the top to be filled with the carbon paste that contains the chiral selector. The diameter of the potentiometric enantioselective membrane sensor was 3 mm. Electric contact was made by inserting a silver wire into the carbon paste.

The surface of the electrode was wetted with deionised water and then polished with an alumina paper (polishing strips 30144-001, Orion) before starting a new batch of analysis. When is not in use, the electrode was immersed in a $10^{-3}$ mol/L S-enalapril solution.

### Apparatus

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

### Reagents and materials

S-enalapril (Elp) was supplied by Merck Sharp & Dohme (West Point, PA, USA). Renitec tablets (10 mg S-enalapril/tablet) were supplied by Merck Sharp & Dome, Haarlem, Netherlands.

2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin was supplied by Wacker-Chemie GmbH (Germany).

Deionised water from a Modulab system (Continental Water Systems, San Antonio, TX, USA) was used for all solutions. The S-enalapril solutions were prepared from a standard S-enalapril solution ($10^{-2}$ mol/L) by serial dilutions.

### Procedures

#### Direct potentiometry

Chronopotentiometry (zero current) is a well known electronic technique used for testing potentiometric sensors as well as direct and indirect (titration) determination of ions in solutions. The time had the meaning of response time, and is the interval of time necessary to reach the steady-state value of potential.

The chronopotentiometric (zero current) technique was used for potential determination of each standard solution ($10^{-8}$ – $10^{-2}$ mol/L). Figure 1 shows a graph of *E* (mV) *vs*. time. The electrodes were placed in the stirred standard solutions and a graph of *E* (mV) *vs*. pElp was plotted. The unknown concentrations were determined from the calibration graphs.

#### Content uniformity assay of Renitec tablets

Ten tablets were placed in ten separate calibrated flasks (25 mL), shaking with 10 mL deionised water and 12.5 mL citrate buffer (pH = 4.0); after tablet dissolution the solution was diluted up to the mark with deionised water.

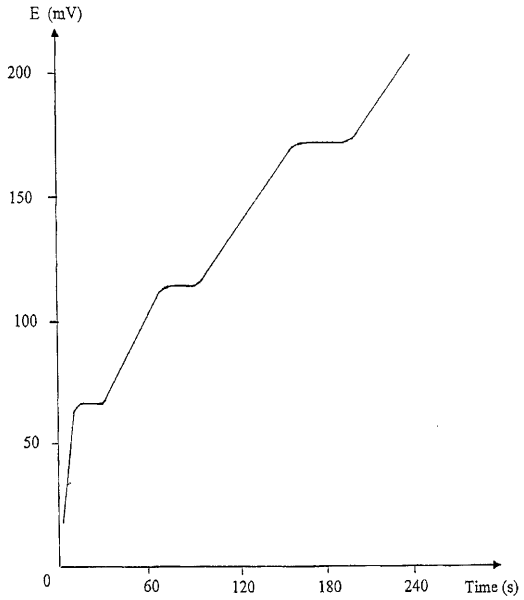


**Fig. 1.** The chronopotentiogram obtained for S-enalapril solutions of: $3.6 \times 10^{-3}$, $3.6 \times 10^{-4}$, and $3.6 \times 10^{-5}$ mol/L concentrations.

The *E* (mV) was determined for each solution through the chronopotentiometric (zero current) technique. The unknown concentration was determined from the calibration graph, as described above.

## Results and discussion

### *Electrode response*

The calibration equation obtained for S-enalapril is:

$$E = -65.40 + 55.00\ p_{Elp}$$

where *E* (mV) is the cell potential, $p_{Elp} = -\log[Elp]$. The correlation coefficient is 0.9998.

The response characteristics of the electrode are shown in table I.

The limits of detection is low: $1.0 \times 10^{-5}$ mol/L. As shown from the table I and seen from the calibration equation, the membrane electrode has a linear and a near-Nernstian response. It displayed a good stability and reproducibility over the tests performed, as shown by the relative standard deviation values.

The response time is <1 min. for concentration range $10^{-4} - 10^{-2}$ mol/L, and >1 min. for the $10^{-6} - 10^{-5}$ mol/L concentration range.

**Table I.** Response characteristics of potentiometric enantioselective membrane electrode for S-enalapril.

| *Slope (mV/pElp)* | *Intercept, E° (mV)* | *Linear range (mol/L)* | *Detection limit (mol/L)* |
|---|---|---|---|
| 55.00 ± 0.30 | –65.40 ± 3.00 | $3.6 \times 10^{-5} - 6.4 \times 10^{-2}$ | $1.0 \times 10^{-5}$ |

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

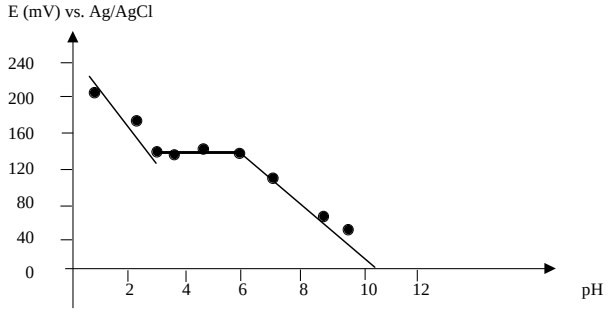


**Fig. 2.** Effect of pH on the response of the potentiometric enantioselective membrane electrode for *S*-enalapril ($3.6 \times 10^{-4}$ mol/L *S*-enalapril solution).

### *Effect of pH on the response of the electrode*

The effect of pH on the response of the potential readings of S-enalapril was checked by recording the emf of the cell, through the chronopotentiometric (zero current) technique, which contained $3.6 \times 10^{-4}$ mol/L S-enalapril solution at various pH values, which were obtained by the addition of very small volumes of HCl and/or NaOH solution ($10^{-1}$ mol/L or 1 mol/L of each).

The *E* (mV) *vs.* pH graph presented in figure 2 shows the pH independence in the range 3.0 – 6.0. Taking into account the $pK_a$ values of enalapril, it was shown that the basic behavior of S-enalapril was at a pH < 3, and its acidic behavior was at pH > 6.0.

### *Selectivity of the electrode*

It is well known that cyclodextrins and their derivatives are potential and powerful chiral selectors which are widely used in various modes of chiral chromataography [11–13]. Cyclodextin derivatives are able to interact with chiral compounds stereoselectively *via* (a) intermolecular forces and (b) formation of inclusion complexes. The selectivity of the potentiometric membrane electrode was checked through the mixed solutions method. The concentrations of interfering ions and S-enalapril were $10^{-3}$ mol/L and $10^{-4}$ mol/L, respectively. The enantioselectivity was checked *versus* D-proline. It is of interest to mention that the stereochemical configuration of the proline moiety in S-enalapril is of the L-(S-)-configuration, thus D-(R-) stereochemical configuration at this site of the S-enalapril would represent an impurity in the drug bulk material (raw material). Due to this the enantioselectivity of the constructed potentiometric membrane electrode was evaluated *versus* D-proline to check its enantioselective property. As shown in table II, D-proline does not interfere, and this indicates that the electrode is enantioselective. Furthermore, the electrode showed a low selectivity coefficient value for polyvinylpyrolidone (PVP), a commonly used agent for tablet compression. Inorganic cations e.g. $Na^+$, $K^+$, $Ca^{2+}$ do not interfere.

### *Analytical applications*

The electrode proved to be useful for the determination of the enantiopurity of S-enalapril - raw material as well as for the content uniformity test of Renitec tablets by the chronopotentiometry (zero current) technique. The recovery test demonstrated the suitability of this potentiometric enantioselective membrane electrode for the enantiopurity of S-enalapril assay: 99.96%- average recovery and RSD of 0.098%.

The results obtained for the uniformity content test are presented in table III. S-enalapril can be reliable assayed from the tablets with an average recovery of 99.59%, and a RSD of 0.20%. The results are in good concordance with those requested by The United States Pharmacopoeia XXII [14]: average recovery 85 – 115%, and RSD < 6%.

**Table II.** Selectivity coefficients for the potentiometric enantioselective membrane electrode for S-enalapril.

| *Interfering species (J)* | $K_{sel}$ |
|---|---|
| D-proline | $6.5 \times 10^{-4}$ |
| Polyvinylpyrrolidone | $1.5 \times 10^{-4}$ |
| L - proline | 2.0 |

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

**Table III.** Determination of S-enalapril from Renitec tablets.

| *Sample* | *Recovery (% of nominal value)* | *RSD (%)* |
|---|---|---|
| 1 | 99 | |
| 2 | 98.13 | |
| 3 | 102.3 | |
| 4 | 99.81 | |
| 5 | 99.8 | 0.2 |
| 6 | 99.7 | |
| 7 | 98.12 | |
| 8 | 100.1 | |
| 9 | 99.2 | |
| 10 | 99.7 | |

RSD value refers to all determinations.

## Conclusions

The potentiometric enantioselective membrane electrode presented in this paper has been successfully used for the enantioselective analysis of S-enalapril in raw material and pharmaceutical formulations. Its construction is simple, fast and reproducible. The reliability of the analytical information is assured by the RSD values obtained in the recovery test and in the uniformity content test.

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