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S-Perindopril Assay Using a Potentiometric, Enantioselective Membrane Electrode

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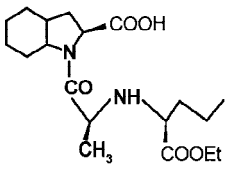
ABSTRACT A potentiometric, enantioselective membrane electrode based on graphite paste (graphite powder and paraffin oil) has been constructed. The graphite paste is impregnated with a 10^{-3} mol/L 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin (as chloride salt) solution. The potentiometric, enantioselective membrane electrode can be used reliably for enantiopurity tests of *S*-perindopril using a chronopotentiometric (zero current) technique, in the 10^{-5}–10^{-2} mol/L concentration range (detection limit 5×10^{-6} mol/L), with an average recovery of 99.58% (RSD = 0.33%). The enantioselectivity was determined over *R*-perindopril and D-proline. The response characteristics of the enantioselective, potentiometric membrane electrode were also determined for *R*-perindopril. It was shown that L-proline is the main interfering compound. The surface of the electrode can be regenerated simply by polishing, obtaining a fresh surface ready to be used in a new assay. *Chirality 11:631–634, 1999.*

KEY WORDS: *S*-perindopril; enantioselective analysis; potentiometric, enantioselective membrane electrode

S-Perindopril (1-[2-[(1-carboxybutyl)amino]-1-oxopropyl]octahydro-1*H*-indole-2-carboxylic acid) is a long-acting angiotensin-converting enzyme inhibitor with a perhydroindole group and no sulfydryl radical. It is a pro-drug that is hydrolized to the active metabolite perindoprilat. Clinical trials have indicated that *S*-perindopril is at least as effective as usual therapeutic doses of *S*-captopril, atenolol, or a combination of hydrochlorothiazide plus amiloride in mild to moderate essential hypertention.[1]



Despite the significant differences in pharmacological, pharmacodynamics, and pharmacokinetics of the individual enantiomers, the methods reported for perindopril assay did not describe the chiral discrimination of its enantiomers. These methods are based particularly on gas chromatography (GC) as a separation technique for perindopril and its metabolites coupled with different detection systems such as MS,[2] electron-capture,[3] and FID.[4] A radioimmunoassay technique is also proposed for perindopril assay, but it cannot discriminate between its enantiomers.[5]

Taking into account the requirements necessary for high-precision methods, reproducibility, reliability, and rapidity, it is easy to see that chromatographic methods cannot always assure the best precision in quantitative enantiopurity tests for chiral drugs. The main reasons for this are necessity of derivatization processes (especially in HPLC and GC); low differences of stability of complexes obtained between enantiomers and chiral selector; and lower sensitivity of detection systems (e.g., CZE and MECK need amplification systems for the diode array detector). The best accuracy in quantitative enantioselective analysis is obtained by using direct methods of analysis, characterized through high sensitivity and enantioselectivity. Due to their long life times, potentiometric, enantioselective membrane electrodes are preferred in chiral discrimination of chiral drugs. Cyclodextrin derivatives[6] and crown-ethers,[7] are used as chiral selectors in plastic (PVC matrix) membranes. The main disadvantage of these types of electrodes is the non-reproducibility of their construction.[8]

To obtain reliable analytical information, the reliability of electrodes construction is essential. Carbon paste electrodes are well known for their reliable construction.[9–11] 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 into a carbon paste. The po-

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TABLE 1. Response characteristics of potentiometric, enantioselective membrane electrode for *S*-perindopril and *R*-perindopril (all measurements were made at room temperature; all values are the average of ten determinations)

Enantiomer	Slope (mV/*p*S(R)Pdp)	Intercept, E° (mV)	Linear range (mol/L)	Detection limit (mol/L)
S-Perindopril	54.23 ± 0.20	−117.78 ± 3.00	10^{-5}–10^{-2}	5.0×10^{-6}
R-Perindopril	38.00 ± 0.50	256.00 ± 4.00	10^{-4}–10^{-2}	1.8×10^{-5}

tentiometric, enantioselective membrane electrode is used for the enantioselective assay of *S*-perindopril.

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 of 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin (10^{-3} mol/L) (100 µL chiral selector solution to 100 mg carbon paste). The graphite–paraffin oil paste was filled into a plastic pipette peak leaving empty a space of 3–4 mm into the top to be filled with the carbon paste that contains the chiral selector. The diameter of the potentiometric, enantioselective membrane sensor used was 3 mm. Electric contact was obtained by inserting a silver wire into the carbon paste. The surface of the electrode was wetted with deionized water and then polished with alumina paper (polishing strips 30144-001, Orion) before use. When not in use, the electrode was immersed in a 10^{-3} mol/L *S*-perindopril solution.

Apparatus

A 663 VA Stand (Metrohm, Herisau, Switzerland) in combination with a PGSTAT 20 and software (Eco Chemie 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-Perindopril (S Pdp) and *R*-perindopril (RPdp) were supplied by the Institute de Researches Servier, France. 2-Hydroxy-3-trimethylammoniopropyl-β-cyclodextrin was supplied by Wacker-Chemie GmbH (Germany), graphite powder, 1–2 µm (synthetic) was from Aldrich, and paraffin oil was from Fluka.

Deionized water from a Modulab system (Continental Water Systems, San Antonio, TX) was used for the preparation of all solutions. Buffer Titrisol (citrate), pH = 4.00, was supplied by Merck (Darmstadt). The *S*- and *R*-perindopril solutions (pH = 4.00 (citrate)) were prepared by serial dilutions from standard *S*- and *R*-perindopril solutions (10^{-2} mol/L, pH = 4.00 (citrate)).

Recommended Procedures

Direct potentiometry. The chronopotentiometric (zero current) technique was used for potential determination of each standard solution (10^{-8}–10^{-2} mol/L, pH = 4.00 (citrate)). The electrodes were placed in stirred standard solutions, and graphs of E (mV) vs pSPdp and pRPdp were plotted, respectively. The unknown concentrations were determined from the calibration graphs.

RESULTS AND DISCUSSION

Electrode Response

The electrode response was determined for both enantiomers: *S*-perindopril and *R*-perindopril at pH = 4.00 (citrate). The equations of calibration obtained are as follows:

S-Perindopril: $E = -117.78 + 54.23\ p\text{SPdp}$; $r = 0.9999$
R-Perindopril: $E = 256.00 - 38.00\ p\text{RPdp}$; $r = 0.9980$

where E (mV) is the cell potential, pSPdp = −log[SPdp], pRPdp = −log[RPdp].

The response characteristics of the electrode for both enantiomers are shown in the Table 1.

The direct dependence between the slope of the electrodes and the stability of complexes formed at the membrane interface[12,13] was also proved for this type of electrodes because it is well known that, for the chiral selector utilized, the best stability of the complex is obtained for the *S* enantiomers.

The limits of detection are low: 5.0×10^{-6} and 1.8×10^{-5} mol/L for *S*-perindopril and *R*-perindopril, respectively. As can be seen from Table 1 and from the equations of calibration, the membrane electrode has a linear response for both enantiomers, but the response is near-Nernstian only for the *S* enantiomer.

The electrode response displayed a good stability and reproducibility for the tests performed as shown by the relative standard deviation values.

The response time is lower for the *S*-enantiomer than for the *R*-enantiomer. *S*-Perindopril: <1 min for the 10^{-3}–10^{-2} mol/L concentration range, and >1 min between 10^{-7} and 10^{-4} mol/L. *R*-Perindopril: 3 min for 10^{-3}–10^{-2} mol/L concentration range and >3 min between 10^{-6} and 10^{-4} mol/L.

Effect of pH on the Response of the Electrode

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

The E (mV) vs pH graph presented in Fig. 1 shows the pH independence in the range 2.35–6.00. It also proved the basic behavior of *S*-perindopril at the pH < 2.30, and its acidic behavior at pH > 6.0.

Selectivity of the Electrode

The selectivity of the potentiometric membrane electrode was checked by the mixed solutions method. The

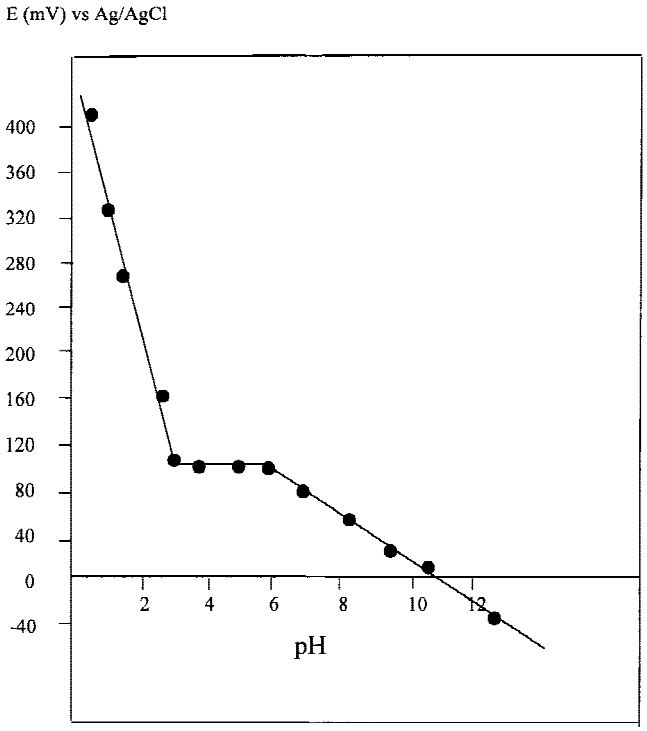

Fig. 1. Effect of pH on the response of the potentiometric, enantioselective membrane electrode for *S*-perindopril (10^{-4} mol/L *S*-perindopril solution).

concentrations of interfering ions and *S*-perindopril were 10^{-3} and 10^{-4} mol/L, respectively. The enantioselectivity was checked against *R*-perindopril and D-proline. D-Proline can be one from the byproducts in *S*-perindopril synthesis. As is shown in Table 2, *R*-perindopril and D-proline do not interfere, thus demonstrating the enantioselectivity property of the constructed potentiometric membrane electrode. Furthermore, the selectivity of the electrode was also tested for polyvinylpyrolidone (PVP) a commonly used compound for tablet compression; the results in Table 2 shows that PVP did not interfere. Also inorganic cations like Na^+, K^+, and Ca^{2+} did not interfere in the analysis of *S*-perindopril.

Analytical Applications

The electrode proved to be useful for the enantiopurity tests of *S*-perindopril by chronopotentiometric (zero current) technique. The recovery tests performed in the absence (Table 3) and in the presence (Table 4) of *R*-perindopril demonstrated the suitability of this potentiometric, enantioselective membrane electrode for enantiopurity tests of *S*-perindopril.

CONCLUSIONS

The construction of electrode is simple, fast, and reproducible. The reliability of the analytical information is assured by the RSD values obtained in the recovery test.

The electrode enantioselectivity as well as its response characteristics made it possible for it to be used successfully for enantiopurity tests of *S*-perindopril.

TABLE 2. Selectivity coefficients for the potentiometric, enantioselective membrane electrode for *S*-perindopril (all measurements were made at room temperature; all values are the average of ten determinations)

Interfering species (J)	K_{sel}
R-Perindopril	4.2×10^{-4}
D-Proline	4.0×10^{-4}
Polyvinylpyrolidone	3.8×10^{-4}
L-Proline	9.8×10^{-2}

TABLE 3. Results obtained for recovery test of *S*-perindopril

Sample	Recovery, % *S*-perindopril[a]
1	99.24 ± 0.02
2	100.00 ± 0.01
3	99.24 ± 0.01
4	100.00 ± 0.02
5	99.12 ± 0.02
6	100.00 ± 0.01
7	99.44 ± 0.05
8	99.65 ± 0.03
9	99.82 ± 0.04
10	99.30 ± 0.03

[a]All values are average of ten determinations.

TABLE 4. Results obtained for recovery test of *S*-perindopril in the presence of *R*-perindopril

S:R (mol/mol)	Recovery, % *S*-perindopril[a]
1:0.50	100.27 ± 0.03
1:0.75	100.01 ± 0.04
1:1	100.00 ± 0.01
1:1.25	99.98 ± 0.01
1:1.50	100.01 ± 0.02
1:1.75	100.00 ± 0.01
1:2	99.99 ± 0.02
1:3	99.97 ± 0.03
1:4	100.02 ± 0.02

[a]All values are average of ten determinations.

Taking into account the advantages of the potentiometric, enantioselective electrode (high precision, rapidity, low cost of analysis) over the chromatographic techniques used for enantiomers separation (time consuming, high cost, high purity of solvents used, loose on precision), the proposed enantioselective sensor opens a new perspective in enantioselective analysis.

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