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Enantioselective potentiometric membrane electrodes based on α-, β- and γ-cyclodextrins as chiral selectors for the assay of L-proline

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Abstract

Novel enantioselective, potentiometric membrane electrodes based on carbon paste impregnated with α-, β- and γ-cyclodextrins are reported. Response characteristics showed that the proposed electrodes could be reliably used in the assay of L-proline (L-pro), with the best enantioselectivity and time-stability exhibited by α-cyclodextrin. The enantioselective, potentiometric membrane electrodes based on the proposed unsubstituted cyclodextrins (CDs) showed lower detection limits (in the region of 10^{-10} to 10^{-9} mol L^{-1}) than the one previously studied, based on β-cyclodextrin derivative [R.I. Stefan, J.F. van Staden, H.Y. Aboul-Enein, Anal. Lett. 31 (1998) 1787–1794]. The surfaces of the electrodes are easily renewable by simply polishing on an alumina paper.

Keywords: Enantioselective, potentiometric membrane electrode; Enantioanalysis; α-, β- and γ-Cyclodextrins; L-Proline

1. Introduction

Chiral recognition became an area of considerable research interests because of its importance in almost all fields of biological, chemical and pharmaceutical sciences [1]. Popular techniques for chiral discrimination are based on chromatography, capillary zone electrophoresis, mass spectrometry and more recently electrochemistry. Advantageously, electrochemical techniques feature relatively high efficiency and low cost [2]. The use of carbon paste based potentiometric and amperomeric electrodes in the discrimination of chiral molecules of clinical and pharmaceutical importance has been well documented [2]. The design and construction of such electrodes require the impregnation of chiral selector in the carbon-paste matrix. Different types of chiral selectors such as cyclodextrin's (CDs) derivatives [2,3–6], crown ethers [7,8] and maltodextrins [9–12] were used for enantioanalysis; however, cyclodextrins were by far the most commonly used.

Cyclodextrins (Fig. 1a) are cyclic, non-reducing oligosaccharides of six, seven and eight α-D-glucose units (commonly referred to as the α-, β- and γ-cyclodextrins, respectively), obtained from starch by enzymatic degradation using *Bacillus amylobacter* [13–18]. The cyclic linkage of their glucose units, through the C–O–C α-1,4 bonds, gives them the so-called toroidal or truncated cylindrical molecular shape of relatively hydrophobic cavity [13–18]. The most important property of CDs 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 formulation of certain drugs, increasing their bioavailability and solubility in water and reducing side effects [13–18]. The cavities of CDs have been found very suitable for enantioanalysis of chiral compounds, with the possibility of achieving double selectivity: an internal selectivity (i.e., inclusion-type, dependent on size of cavity and guest molecule) and external selectivity (dependent on functional

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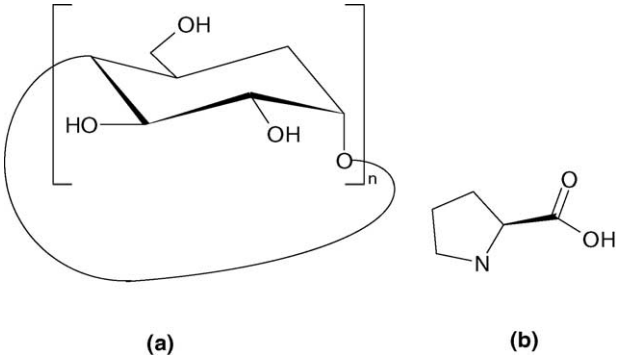

Fig. 1. (a) Cyclodextrins, where $n = 6$, 7 and 8 for α-, β- and γ-cyclodextrins and (b) L-proline.

groups) [13–18]. Many cyclodextrin derivatives have been developed to modify their properties such as hydrophilicity and cavity shape.

Proline is used as a model analyte. Proline has been a subject of recent theoretical and experimental studies [19,20] because of its health implications in humans (e.g., the autosomal recessive disorder of amino acid metabolism known as the hyperprolinemia whose sufferers have been known to have neurological manifestations, seizures and mental retardation [21]) as well as its importance in food and beverage industry [22]. L-Proline (L-pro) (Fig. 1b) functions as an inhibitory neurotransmitter in the mammalian central nervous system [23,24] by inducing oxidative stress in cerebral cortex of mammals [21]; acts as an anti-convulsant [25]; protects plant tissues against stress due to salt or water [26].

An enantioselective potentiometric membrane electrode (EPME) based on a β-cyclodextrin derivative had previously been used by Stefan et al. for the assay of L-proline [3]. Other methods that have been proposed for L-proline assay are based on fluorescence spectrometry [27], thin layer chromatography [28], high-pressure liquid chromatography [29,30] and electrochemiluminescence [31,32]. The use of chromatographic and spectrophotometric techniques is laborious, time-consuming, requiring several chemicals and chemical pre-treatments of samples, yet with certain problems inherent with selective retention of one of the enantiomers in the column. The major disadvantages easily observable with electrochemiluminescence, include high relative errors, use of expensive and toxic reagents, high cost of instrumentation and possible high limit of detection and interferences from impurities in real sample analysis. Thus, the use of a low-cost electrochemical technique that is simple, highly sensitive, precise and reliable is needed for the enantioanalysis of chiral molecules.

This paper reports three new enantioselective potentiometric membrane electrodes based on α-, β- and γ-cyclodextrins impregnated on carbon paste for the assay of L-proline.

2. Experimental

2.1. Reagents and materials

L-Proline and D-proline were supplied by Sigma–Aldrich (St. Louis, MO, USA). α-, β- and γ-Cyclodextrins were supplied by Wacker–Chemie GmbH (München, Germany). Graphite powder (1–2 μm, synthetic) was supplied by Aldrich. Paraffin oil was supplied by Fluka (Buchs, Switzerland). Deionised water from a Modulab system (Continental Water Systems, San Antonio, TX, USA) was used for the preparation of all solutions. The L-proline and D-proline solutions necessarily in the characterization of the enantioselective potentiometric membrane electrodes were prepared from standard L-proline and D-proline solutions (10^{-2} mol/L), respectively, by serial dilutions. All standard and diluted solutions were buffered with phosphate buffer (pH 4.00, 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 100 (Eco Chemie, Utretch, The Netherlands) and a software version 4.8 were used for all potentiometric measurements. Ag/AgCl (0.1 mol/L KCl) served as reference electrodes 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 cyclodextrin (α-(I), β-(II) or γ-(III)cyclodextrins) (10^{-3} mol/L) (100 μL chiral selector solution to 100 mg carbon paste) as described before [3]. A certain quantity of carbon paste free of cyclodextrin 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 potentiometric, enantioselective membrane electrode was 3 mm. Electric contact was obtained by inserting a Ag/AgCl wire in the carbon paste. As internal solution it was utilized a solution of 0.1 mol/L KCl.

The surface of the electrodes was wetted with deionised water and polished with alumina paper (polishing strips 30144-001, Orion) before using them for each experiment. The carbon paste prevents the leach of the cyclodextrin from the membrane into solution. When it was not in use, the electrode was immersed in a 10^{-3} mol/L L-proline solution.

2.4. Recommended procedure: direct potentiometry

The direct potentiometry was used for measurement of the potential of each standard solution (10^{-8}–10^{-2} mol/L). The electrodes were placed in stirred standard solutions and graphs of E (mV) versus pL-pro were plotted. The unknown concentrations were determined from the calibration graphs.

Table 1
Response characteristics of enantioselective, potentiometric membrane electrodes

Cyclo-dextrin	Slope (mV/pL-pro)	Intercept, E° (mV)	Linear concentration range (mol/L)	Detection limit (mol/L)
α-CD	−53.66	566.88	10^{-8}–10^{-5}	9×10^{-10}
β-CD	−54.00	544.83	10^{-8}–6.3×10^{-5}	5.5×10^{-10}
γ-CD	−59.50	586.39	10^{-8}–10^{-3}	10^{-10}

All measurements were made at 25 °C. All values are averages of ten measurements performed during one month (R.S.D < 0.1%).

3. Results and discussion

3.1. Electrode response

The response characteristics exhibited by the three carbon pastes modified with cyclodextrin (α-(I), β-(II) or γ-(III)cyclodextrins) electrodes towards the detection of L-proline are summarized in Table 1. For all the calibration plots, the membrane electrodes showed linear and near-Nernstian responses for L-proline, with correlation coefficients for the equations of calibration of 0.9999, 0.9994 and 0.9982 for (I), (II) and (III), respectively. D-Proline, on the other hand, showed non-Nernstian responses (slopes found in the 25–30 mV/pD-pro range). The linear ranges and the limits of detection recorded for L-proline are in the lower concentration ranges than those obtained for L-proline using the electrode impregnated with 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin (as chloride salt) (5.0×10^{-5} to 1.5×10^{-1} mol/L concentration range (detection limit 1.0×10^{-5} mol/L) with a slope of 52 mV/decade of concentration and an average recovery of 99.90% (R.S.D. = 0.12%)) [3]. Although enantioselectivity of substituted β-cyclodextrin derivative is always difficult to interpret [19], the results found may be connected with the combined effect of enhanced hydrophilicity, cavity enlargement and increased steric restrictions usually introduced by bulky substituents. All these factors affect the approach of an interacting molecule.

The proposed electrodes were highly stable and reproducible over a month test period. α- and β-Cyclodextrin showed better time stability, their measured potentials varying by ±0.10 mV, compared to that of γ-cyclodextrin found to be ±4.0 mV during one month test period.

The response times of α- and β-cyclodextrins are approximately similar, being higher than 1 min for concentrations of L-proline between 10^{-8} and 10^{-6} mol/L and lower than 1 min for concentrations between 10^{-5} and 10^{-4} mol/L. For γ-cyclodextrin, the response times higher than 1 min were recorded for concentrations of L-proline between 10^{-8} and 10^{-5} mol/L and lower than 1 min for concentrations between 10^{-4} and 10^{-3} mol/L.

3.2. The 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^{-6} mol/L L-proline at different pH values (pH 1–10). These solutions were prepared by adding very small volumes of HCl and/or NaOH solution (0.1 mol/L of each) to a L-proline solution.

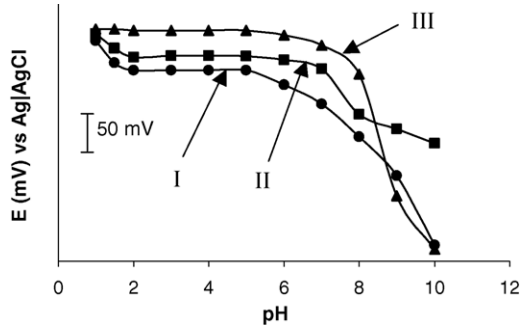

Fig. 2. The influence of pH on the response of the enantioselective potentiometric membrane electrodes ($c_{\text{L-pro}} = 10^{-6}$ mol/L); (I) for α-cyclodextrin based electrode, (II) for β-cyclodextrin based electrode and (III) for γ-cyclodextrin based electrode.

The plots of E (mV) versus pH (Fig. 2) show that the response of the electrodes is not depending on pH, in the following pH ranges 2.0–5.0 (I), 2.0–6.0 (II) and 2.0–6.5 (III). This proves the basic behaviour of L-pro at pH < 2 and its acidic behaviour at pH > 5.0.

3.3. The enantioselectivity of the electrodes

The enantioselectivity of the electrodes was investigated over D-proline, using mixed solutions method. The concentration of the interfering ions and L-proline were 10^{-5} and 10^{-6} mol/L, respectively. The values of pK_{pot} (p$K_{\text{pot}} = -\log K_{\text{pot}}$) (where K_{pot} is the potentiometric selectivity coefficient) were 2.96, 2.52 and 2.05 for (I), (II) and (III), respectively. The enantioselectivity decreases with increasing cavity size, the best enantioselectivity being shown by α-cyclodextrin based electrode. Inorganic ions such as Na^+, K^+ and Ca^+ did not interfere with the analysis of L-proline.

3.4. Analytical applications

To assess the feasibility of the proposed direct potentiometry procedure, recovery tests were performed for proline-raw material. The assay of L-proline in the presence of D-proline was conducted by use of different ratios between L-proline and D-proline. The results obtained (Table 2) demonstrated the suitability for the proposed enantioselective potentiometric membrane electrodes for testing the enantiopurity of proline-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
Results obtained for the assay of L-proline in the presence of D-proline

L-Pro:D-pro (mol/mol)	Recovery (%)		
	α-CD	β-CD	γ-CD
2:1	100.00 ± 0.00	99.87 ± 0.04	99.65 ± 0.22
1:1	100.00 ± 0.00	100.13 ± 0.01	99.88 ± 0.18
1:2	100.00 ± 0.00	101.29 ± 0.02	100.51 ± 0.03
1:4	100.00 ± 0.00	100.00 ± 0.01	99.96 ± 0.10
1:9	100.79 ± 0.01	100.90 ± 0.01	100.78 ± 0.11

All measurements were made at 25 °C. All values are averages of ten measurements.

4. Conclusions

This paper describes new enantioselective, potentiometric membrane electrodes designed using α-, β- and γ-cyclodextrins as chiral selectors used in the enantioanalysis of proline. The electrodes can be successfully used for the assay of L-proline in the presence of D-proline. The enantioselectivity is good for all the three electrodes proposed, the best being recorded when α-cyclodextrin is used as chiral selector.

If one is comparing the results obtained using these chiral selectors and those obtained previously for L-proline using a derivative of β-cyclodextrin [3], one can easily see that the improvements were found for the limits of detection (which are lower when the unsubstituted cyclodextrins are used as chiral selectors) as well as enantioselectivity (higher especially when the α-cyclodextrin is used as chiral selector).

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