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SCIENCE DIRECT
Talanta 69 (2006) 1049–1053
Talanta
www.elsevier.com/locate/talanta

Short communication

Enantioselective, potentiometric membrane electrodes based on cyclodextrins: Application for the determination of *R*-baclofen in its pharmaceutical formulation

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Received 8 June 2005; received in revised form 3 November 2005; accepted 6 December 2005
Available online 19 January 2006

Abstract

Two enantioselective, potentiometric membrane electrodes based on α- and γ-cyclodextrins were proposed for the assay of *R*-baclofen. The slopes of the electrodes were 59.50 and 51.00 mV/p*R*-baclofen for α- and γ-cyclodextrin-based electrodes, respectively. The detection limits of the proposed electrodes were 7×10^{-9} mol l^{-1} for α-cyclodextrin-based electrode and 1.44×10^{-10} mol l^{-1} for γ-cyclodextrin-based electrode. The enantioselectivity was determined over *S*-baclofen. The proposed electrodes can be employed for the assay of *R*-baclofen raw materials and its pharmaceutical formulation, Norton-Baclofen® tablets. The surfaces of the electrodes are stable and easily renewable by polishing on alumina paper.

Keywords: Enantioselective, potentiometric membrane electrode; Cyclodextrins; *R*-baclofen

1. Introduction

Chiral discrimination is very important for drug development in the pharmaceutical industry. The interaction between the drug and the receptor is highly stereoselective, because in most cases only one of the enantiomers of the certain drug provides desired pharmacological activity, while the other enantiomer may exhibit negative side effects [1]. Therefore, the search for reliable and efficient methods for chiral recognition is of outmost importance for scientists working in pharmaceutical and clinical fields. Popular techniques for the determination of enantiomers are based on chromatographic, capillary electrophoretic, spectrophotometric methods and more recently electrochemistry. Electrochemical techniques offer some advantages over traditional ones such as low cost, short time of analysis, high enantioselectivity and simplicity [2].

Baclofen [4-amino-3-(*p*-chlorophenyl)butyric acid] (pK_a = 3.9), a chemical analogue of inhibitory neurotransmitter γ-amino-butyric acid (GABA), has been widely used for the symptomatic relief of muscular spasm and multiple sclerosis caused by spinal or cerebral injury since its introduction in 1967 [3]. Baclofen exerts its antispastic effects by depressing monosynaptic and polysynaptic transmission in the spinal cord. It reduces excitatory postsynaptic potentials in motoneurons in the ventral horn without affecting their membrane potential or input resistance. Unlike natural aminoacids baclofen is capable of passing a blood–brain barrier. It is rapidly adsorbed in the human body after oral administration and almost completely recovered unchanged in urine [4]. It has been established, that *R*-enantiomer of baclofen (Fig. 1) is 100 more active, than the *S*-enantiomer [5,6]. *R*-enantiomer of baclofen was also found to be stereospecifically active at so-called $GAGA_B$-receptors [7,8]. Consequently, the enantioanalysis of baclofen using reliable analytical methods is necessary.

Several analytical techniques have been introduced for the determination of baclofen in biological fluids: high performance liquid chromatography with electrochemical [9,10], UV [11–14] and fluorescence detection [15–17], gas chromatography with electron capture detection [18], gas chromatography–mass spectrometry (GC–MS) [19], liquid chromatography–mass spectrometry [20,21] and capillary zone electrophoresis [22–25].

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doi:10.1016/j.talanta.2005.12.022

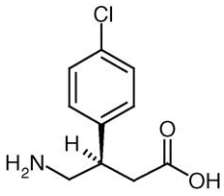

Fig. 1. *R*-baclofen.

Cyclodextrins (CDs) are oligosaccharides obtained as products of enzymatic degradation of starch and glycosyltransferases or cyclodextrinases. They have the shape of a hollow truncated cone with a hydrophobic inner cavity and hydrophobic outer surface. The larger opening of the cavity is lined with secondary hydroxyl groups and the smaller opening with primary hydroxyl groups [26,27]. Cyclodextrins are available at native forms (namely α- and γ-CDs consisting of six, seven and eight glucopyranose units, respectively) as well as charged and uncharged derivatized forms (CDs can be derivatized in 2, 3 or 6 position to receive compounds with new features). Chiral selectivity results from inclusion of a hydrophobic portion of the analyte in the cavity and also from the hydrogen bonding to the hydroxyl moieties.

Cyclodextrins and their derivatives have been widely used as chiral selectors for capillary electrophoresis [26–37], gas chromatography [38], gas chromatography–mass spectrometry [39], capillary electrochromatography [40,41] and liquid chromatography–mass spectrometry [42].

Stefan et al. reported previously the use of a potentiometric sensor based on 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin (as chloride salt) [43] for the analysis of *S*-perindopril while unsubstituted α-, β- and γ-cyclodextrins were used by Ozoemena et al. for the determination of *S*-perindopril in pharmaceutical formulations [44]. The slopes of the proposed electrodes were near-Nernstian and good results were recorded for the enantioselectivity and recovery tests. Fortunately, these substances of pharmaceutical importance are not formulated in the same tablet or injection. Therefore, the enantioselectivity tests coupled with the uniformity content test will be always reliable. One is only interested to check for the pure chiral substance if it also has the enantiopurity quality.

This paper reports the applications of two enantioselective, potentiometric membrane electrodes (EPMEs) based on α- and γ-cyclodextrins for the determination of *R*-baclofen in raw materials and from its pharmaceutical formulation, Norton-Baclofen® tablets.

2. Experimental

2.1. Reagents and solutions

Graphite powder (1–2 μm, synthetic) was purchased from Aldrich. Paraffin oil was purchased from Fluka (Buchs, Switzerland). α- and γ-cyclodextrins were supplied by Wacker Chemie GMBH (München, Germany). *R*-(+)-baclofen hydrochloride and *S*-(−)-baclofen hydrochloride (99% enantiopurity) were purchased from Sigma–Aldrich. Phosphate buffer (pH 2.00, 0.1 mol l^{-1}) was obtained from Merck (Darmstadt, Germany). Deionized water from a Modulab system (Continental Water Systems, San Antonio, TX, USA) was used for all solutions preparations.

All standard and diluted solutions of baclofen were buffered with phosphate buffer pH 2.00 (containing also NaCl 0.1 mol l^{-1}) using the ratio buffer:distilled water 1:1 (v/v) in order to keep the pH and ionic strength constant. The pH was determined for each standard and sample solution, and it was always found to be equal with 2.00.

Norton-Baclofen® tablets (10 mg baclofen/tablet) were obtained from Norton Healthcare (Pty) Limited. Norton-Baclofen® tablets contain *S*-baclofen as a main component and only usual additives such as starch.

2.2. Apparatus

A 663 VA Stand (Metrohm, Herisau, Switzerland) in connection with PGSTAT 20 and Eco Chemie (Utrecht, The Netherlands) Software version 4.9 were used for all potentiometric (zero current) measurements. An Ag/AgCl (0.1 mol l^{-1} KCl) electrode served as reference electrode in the cell.

All pH measurements were performed using an Orion pH-meter, model 420A.

2.3. Enantioselective, potentiometric membrane electrodes design

The paraffin oil and graphite powder were mixed in a ratio of 1:4 (w/w) followed by the addition of aqueous solution of cyclodextrin (α- or γ-cyclodextrins, 10^{-3} mol l^{-1}) (100 μl of chiral selector solution to 150 mg of carbon paste) to form the modified carbon paste. A certain quantity of carbon paste obtained by mixing paraffin oil and graphite powder, was prepared and placed in a plastic pipette peak, leaving 3–4 mm empty in the top to be filled with the modified carbon paste (Fig. 2). The diameter of the enantioselective, potentiometric membrane electrode was 3 mm. Electric contact was obtained by inserting Ag/AgCl wire into the carbon paste. The internal solution was 0.1 mol l^{-1} KCl.

Before each series of measurements, the surface of the electrode was "refreshed" with a new portion of carbon paste, containing the chiral selector and then polished with alumina paper (polishing strips 30144-001 Orion). The oil from the carbon

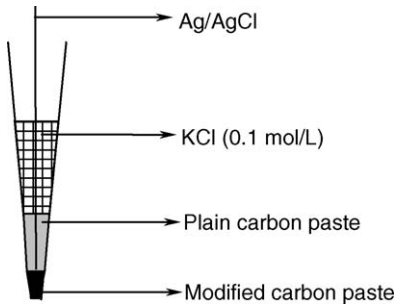

Fig. 2. The design of the enantioselective, potentiometric membrane electrodes.

paste prevents the leaching of the cyclodextrin from the membrane into the solution. No decrease on the sensitivity of the electrode was recorded during working period (while used for measurements daily) which was 6 months.

2.4. Recommended procedures

2.4.1. Direct potentiometry

The potentiometric (zero current) technique was used for potential (E, mV) determination of each standard solution of baclofen (10^{-10} to 10^{-3} mol l^{-1}). The electrodes were placed into stirred standard solutions and graphs of E (mV) versus pR-baclofen (pR-baclofen = −log[R-baclofen]) were plotted. The unknown concentrations were determined from the calibration graphs.

2.4.2. Determination of R-baclofen in Norton-Baclofen® tablets

Each Norton-Baclofen® tablet (10 mg R-baclofen/tablet) was placed into 100 ml calibrated flask, dissolved and diluted to the mark using a phosphate buffer (pH 2.00):deionized water ratio 1:1. The unknown concentration of R-baclofen was determined using the direct potentiometric method.

3. Results and discussion

3.1. Electrode response

The response characteristics exhibited by the two cyclodextrin-based carbon paste electrodes are summarized in Table 1. For all the calibration plots the membrane electrodes showed linear and near-Nernstian responses towards R-baclofen with correlation coefficients of 0.9994 and 0.9999 for α- and γ-cyclodextrin-based electrodes, respectively (Fig. 3). As it follows from data presented in Table 1, α-cyclodextrin-based electrode shows higher value of response of electrode function and wider linear concentration range than γ-cyclodextrin-based electrode, and γ-cyclodextrin-based electrode is characterized by a lower detection limit. The detection limit was calculated from the equation of calibration of the electrode, at $E = 0$ mV.

The mechanism of chiral recognition is based on formation of inclusion (host-guest) complexes between R-baclofen and cyclodextrins. The slope of the electrode is directly proportional with the log K_f, where K_f is the formation constant of the complex between the enantiomer and chiral selector.

The electrodes showed non-Nernstian responses towards S-(−)-baclofen (slopes found in 12–20 mV-decade of concentration) proving that they can only be used for the determination of R-(+)-baclofen.

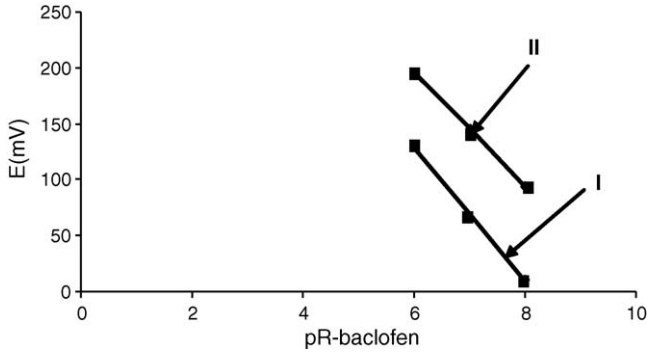

Fig. 3. Calibration graphs for: (I) α-cyclodextrin-based enantioselective, potentiometric membrane electrode; (II) γ-cyclodextrin-based enantioselective, potentiometric membrane electrode.

The electrodes were tested for concentrations between 10^{-10} and 10^{-3} mol l^{-1}. The response times of α- and γ-cyclodextrins-based electrodes are 1 min for R-baclofen concentrations between 10^{-10} and 10^{-6} mol l^{-1} and lower than 30 s for concentrations between 10^{-5} and 10^{-3} mol l^{-1}.

The proposed electrodes are characterized by a smaller value of the detection limit than teicoplanin-based electrodes previously described for the assay of R-baclofen [45].

The response characteristics of the electrodes (when the same modified paste is used) were stable and reproducible over a month test period (R.S.D. < 0.1%).

3.2. Effect of pH on the response of the electrodes

The effect of pH on the response of the proposed electrodes was examined by recording the e.m.f. of the cell, using direct potentiometric technique, which contain 10^{-7} mol l^{-1} R-baclofen at different pH values (pH 1–10). These solutions were prepared by adding very small volumes of HCl and/or NaOH solutions (10 or 1 mol l^{-1} of each) to a R-baclofen solution. Orion Labotec 420A pH-meter with combined electrode was used for the control of pH of the solutions used for measurements.

The plots of E (mV) versus pH (Fig. 4) indicate that the response of both electrodes does not depend on pH in pH interval 2.00–7.20. From pH 1.00 to 2.00, the potential of the electrodes increased proving the acidic behavior of baclofen in this pH range, while after pH 7.20 the potential of both electrodes decreased fast, thus, proving the basic behavior of the electrodes after a pH higher than 7.20.

Table 1
Response characteristics of cyclodextrin-based enantioselective, potentiometric membrane electrodes designed for the assay of R-baclofen

EPME based on	Parameter			
	Slope (mV/pR-baclofen)	Intercept, E^0 (mV)	Linear concentration range (mol l^{-1})	Detection limit (mol l^{-1})
α-cyclodextrin	59.50	484.83	10^{-8} to 10^{-6}	7.00×10^{-9}
γ-cyclodextrin	51.00	502.00	10^{-8} to 10^{-7}	1.44×10^{-10}

Table 2
Determination of *R*-baclofen in the presence of *S*-baclofen (*R*:*S*)

Chiral selector	Recovery *R*-baclofen (%)				
	2:1	1:1	1:2	1:4	1:9
α-cyclodextrin	98.92 ± 0.03	98.99 ± 0.02	98.99 ± 0.03	100.00 ± 0.00	100.00 ± 0.00
γ-cyclodextrin	100.00 ± 0.00	100.00 ± 0.00	99.96 ± 0.02	99.98 ± 0.03	99.99 ± 0.02

All values are the average of 10 determinations.

3.3. Selectivity of the electrodes

The selectivity of the electrodes was investigated using the mixed solution method. The concentrations of *R*-baclofen and the interferent, *S*-baclofen, were 10^{-7} and 10^{-8} mol l^{-1}, respectively. The values of the potentiometric selectivity coefficients (K_{sel}^{pot}) obtained are 5.23×10^{-3} and 4.50×10^{-5} for α- and γ-cyclodextrin-based electrodes, respectively (each value is average of 10 determinations). These results proved that enantioselectivity towards *R*-baclofen increases by more than two orders of magnitude with increasing of cyclodextrins cavity size and that *S*-baclofen does not interfere with the analysis of *R*-baclofen.

3.4. Analytical applications

The response characteristics, selectivity and working pH ranges of the proposed electrodes proved their suitability for the determination of enantiopurity of *R*-baclofen raw materials and for the determination of *R*-baclofen in pharmaceutical formulation. The assay of *R*-baclofen was conducted in presence of its antipode (*S*-baclofen) by using different ratios between *R*- and *S*-baclofen. The good recovery values obtained for the assay of *R*-baclofen in the presence of *S*-baclofen (Table 2) clearly demonstrate the suitability of the proposed enantioselective, potentiometric membrane electrodes for the enantioanalysis of *R*-baclofen raw material.

The results obtained for the uniformity content test of Norton-Baclofen® tablets showed that tested pharmaceutical formulations contain *S*-baclofen as a main component, and only small amounts of *R*-baclofen: the average recovery value for *R*-baclofen determined using α-cyclodextrin-based electrode was 2.65 ± 0.09% and average recovery value determined using γ-cyclodextrin-based electrode was 2.89 ± 0.06% (both values are average of 10 determinations). These results are in good agreement with those obtained using a LC method [13]: 2.70%.

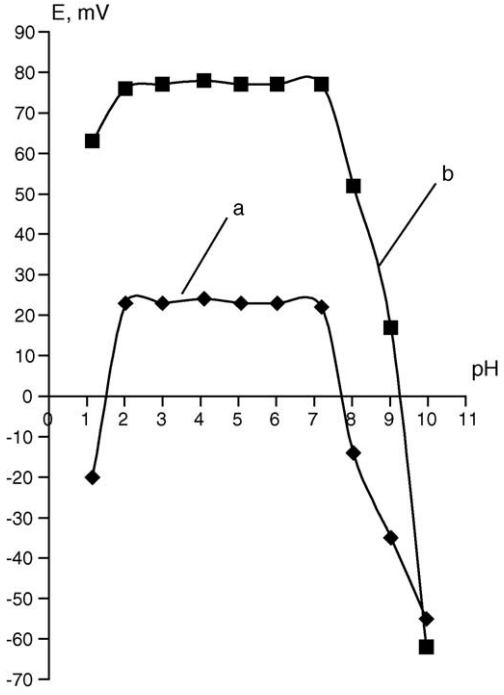

Fig. 4. Effect of pH on the response of the cyclodextrin-based enantioselective, potentiometric membrane electrodes designed for the assay of *R*-baclofen (10^{-7} mol l^{-1} *R*-baclofen solution): (a) α-cyclodextrin-based enantioselective, potentiometric membrane electrode; (b) γ-cyclodextrin-based enantioselective, potentiometric membrane electrode.

4. Conclusions

The proposed enantioselective, potentiometric membrane electrodes based on α- and γ-cyclodextrins showed good features for enantioselective analysis of *R*-baclofen. The construction of the electrodes is simple, fast and reproducible. The proposed electrodes exhibited stable and reliable response characteristics. Good enantioselective properties of the proposed electrodes allowed to conduct enantioanalysis of *R*-baclofen in its raw materials and pharmaceutical formulation, Norton-Baclofen® tablets.

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