ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2013, *58*(7-8), 667-671

VANCOMYCIN AND TEICOPLANIN BASED ENANTIOSELECTIVE, POTENTIOMETRIC MEMBRANE ELECTRODES FOR THE ASSAY OF L-CYSTEINE

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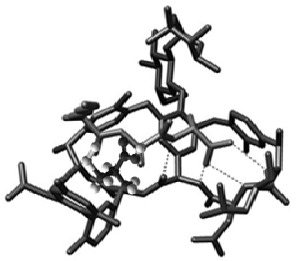
Received December 20, 2012

Two enantioselective, potentiometric membrane electrodes based on the macrocyclic antiobiotic vancomycin and teicoplanin were proposed for the enantioanalysis of L-cysteine. The linear concentration ranges for the proposed electrodes were 10^{-10}-10^{-3} and 10^{-8}-10^{-5} mol/L for vancomycin and teicoplanin based enantioselective, potentiometric membrane electrodes with the slopes of 58.0 and 59.0 mV/decade of concentration, respectively. These electrodes were used reliably for the enantioanalysis of L-cysteine in urine samples. The surfaces of the electrodes could be easily renewable by simply polishing on alumina paper.



INTRODUCTION

Chiral recognition has become an area of considerable research interest because of its importance in the 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 utilization of carbon paste based potentiometric and amperometric electrodes in the discrimination of the enantiomers of chiral molecules improved the sensitivity, accuracy and time of analysis obtained using different chromatographic methods.[2]

Cysteine (CySH) is one of the most important amino acids, being also widely used in the pharmaceutical and food industries. However the couple L-cystine/L-cysteine is generally used as a model for the role of disulfide bond and thiol group in proteins in a variety of biological media. To date, the following analytical methods were reported for the analysis of cysteine: capillary electrophoresis,[3] liquid chromatography,[4-6] reversed-phase chromatography,[7] chemiluminescence,[8] as well as an electrode based on oxovanadium(IV) complex of Salen modified carbon paste electrode.[9]

Vancomycin and teicoplanin are very powerful and efficient chiral selectors in chromatography.[10,11] Macrocyclic antibiotics, vancimycin and teicoplanin are produced as fermentation products of Streptomyces orientalis and Actinoplanes teichomyceticus, respectively. These antibiotics are primary active against aerobic and anaerobic Gram-positive microorganisms both in vitro and in vivo. However, they are known to inhibit cell wall synthesis.

In this paper, vancomycin and teicoplanine are proposed as chiral selectors for the design of enantioselective, potentiometric membrane electrodes (EPMEs) for the enantioanalysis of L-cysteine in urine samples.

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EXPERIMENTAL

Reagents and materials

L- and D-Cysteine, vancomycin and teicoplanin were supplied by Sigma-Aldrich (St. Louis, MO, USA). Graphite powder (1-2 μm, synthetic) was supplied by Aldrich.

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-cysteine solutions necessarily in the characterization of the enantioselective, potentiometric membrane electrodes were prepared from standard L- and D-cysteine solutions (10^{-2} mol/L), respectively, by serial dilutions. All standard and diluted solutions were buffered with phosphate buffer (pH 2.40, 0.1mol/L) from Merck (Darmstadt, Germany) (1:1, v/v, buffer:deionised water).

Apparatus

A 663 VA stand (Metrohm, Herisau, Switzerland) connected to a PGSTAT 20 (Eco Chemie, Utretch, Netherlands) and a software version 4.9 was used for all the potentiometric measurements. Ag/AgCl (0.1 mol/L KCl) served as reference electrode in the cell.

Electrodes 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 vancomycin or teicoplanin (10^{-3} mol/L) (100 μL chiral selector solution to 100 mg carbon paste). A certain quantity of carbon paste free from chiral selector 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 3mm. Electric contact was obtained by inserting 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 electrode was wetted with deionised water and polished with alumina paper (polishing strips 30144-001, Orion) before using them for each experiment.

Recommended procedures

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

Determination of L-cysteine in urine samples

Urine samples were collected from different patients and buffered with phosphate buffer (pH 2.40, 0.1mol/L) (1:1, v/v, buffer:urine sample). Direct potentiometry was applied to determine L-cysteine in urine samples.

RESULTS AND DISCUSSION

Electrodes response

The response characteristics exhibited by the two carbon paste modified with vancomycin and teicoplanin electrodes towards the detection of L-cysteine are summarized in Table 1. For all the calibration plots, the membrane electrodes showed linear and near-Nernstian responses for L-cysteine, with correlation coefficients of 0.9999. The proposed electrodes showed non-Nernstian responses over D-cysteine. Amongst the two electrodes, teicoplanin showed to be the best electrode with a slope of 59.0 mV/decade of concentration.

Table 1

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

EPME based on	Slope (mV/pL-cys)	Intercept E^0 (mV)	Linear concentration range (mol/L)	Detection limit (mol/L)
Vancomycin	58.0	601.5	10^{-3}–10^{-10}	4.3×10^{-11}
Teicoplanin	59.0	480.0	10^{-5}–10^{-8}	7.3×10^{-9}

All measurements were made at 25^0C. All values are average of 10 determinations.

Table 2

Potentiometric selectivity coefficients of the proposed enantioselective, potentiometric membrane electrodes for the assay of L-cysteine

Interference species (J)	K_{sel}^{pot}	
	Vancomycin	Teicoplanin
D-Cys	2.2×10^{-3}	1.2×10^{-3}
PVP	1.7×10^{-3}	3.8×10^{-4}
Creatine	4.0×10^{-4}	2.2×10^{-3}
Creatinine	$<< 1.0\times10^{-4}$	4.0×10^{-4}

All measurements were made at 25^0C. All values are average of 10 determinations.

The proposed electrodes were highly stable and reproducible over a month test period. The response time was 30s for concentration range 10^{-5} – 10^{-7} mol/L and 1min for concentrations lower than 10^{-7} mol/L.

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-cysteine at different pH values (pH 1-12). These solutions were prepared by adding small volumes of HCl and/or NaOH solutions (0.1-1 mol/L of each) to the L-cysteine solution. The plots of E (mV) versus pH (Fig. 1) showed that the response of the electrode is not depending on the pH, in the ranges 2.0-4.0 (I) and 2.0-6.0 (II) for the EPMEs based on vancomycin and teicoplanin, respectively.

The selectivity of the electrodes

The selectivity of the proposed electrodes was investigated over D-Cys, PVP, creatine and creatinine, using mixed solution method. The concentration of the interfering ions and L-Cys were 10^{-4} and 10^{-5} mol/L, respectively. The potentiometric, selectivity coefficients shown that the electrodes are selective and enantioselective (Table 2). Inorganic ions such as Na^+, K^+, Ca^{2+} did not interfere with the analysis of L-cysteine.

Analytical applications

Determination of L-cysteine in the presence of D-cysteine was performed in order to prove if the electrodes can be used for the enantioanalysis of L-cysteine. The assay of L-cysteine in the presence of D-cysteine was conducted by use of different ratios between L- and D-cysteine. The results obtained (Table 3) demonstrated the suitability for the proposed enantioselective, potentiometric membrane electrodes for testing the enantiopurity of cysteine in urine samples (Table 4) 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.

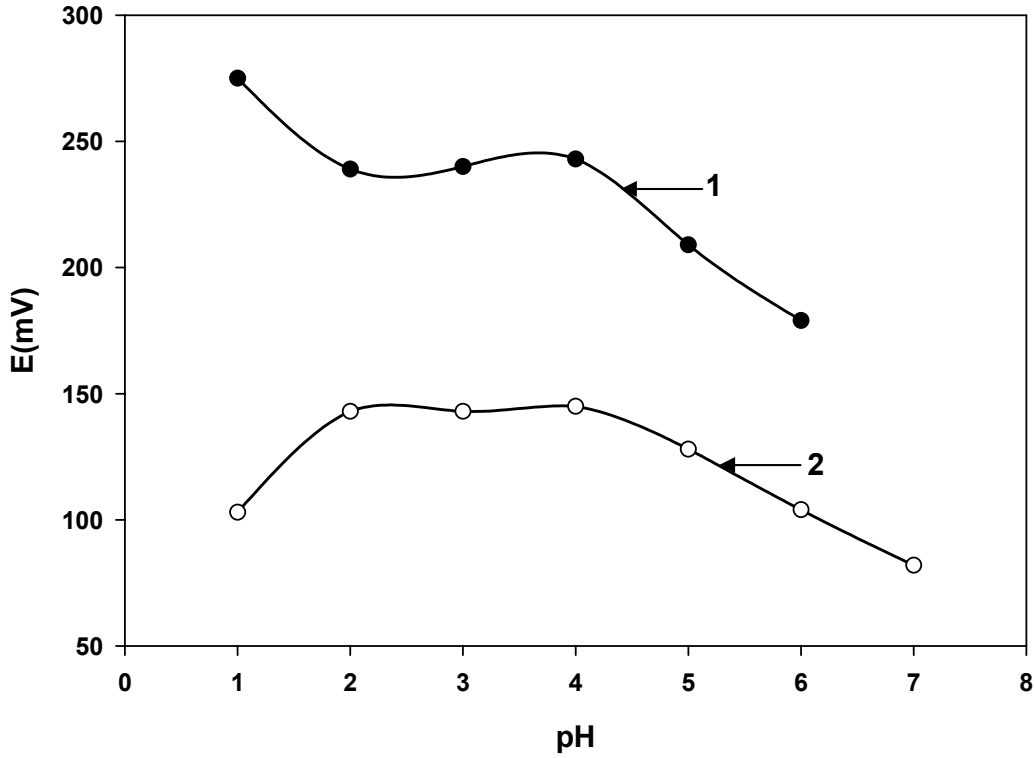

Fig. 1 – The influence of pH on the response of the enantioselective potentiometric membrane electrodes ($C_{L\text{-}cys}=10^{-5}$) based on vancomycin (1) and teicoplanin (2).

Table 3

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

L: D (mol/mol)	L-Cysteine, Recovery (%)	
	EPME based on	
	Vancomycin	Teicoplanin
2:1	100.00±0.01	99.27±0.01
1:1	99.65±0.02	99.09±0.02
1:2	99.65±0.01	99.55±0.02
1:4	99.82±0.02	99.53±0.02
1:9	99.87±0.02	99.55±0.02

All measurements were made at 25^0C. All values are average of 10 determinations.

Table 4

Determination of L-cysteine in urine samples

Sample No.	L-Cysteine, Recovery (%)	
	EPME based on	
	Vancomycin	Teicoplanin
1	99.82±0.15	99.98±0.12
2	100.00±0.12	101.02±0.25
3	99.67±0.15	99.87±0.13
4	99.58±0.20	99.74±0.15

All measurements were made at 25^0C. All values are average of 10 determinations.

L-cysteine was reliable determined in urine samples using the proposed enantioselective, potentiometric membrane electrodes (Table 4). The average recovery of L-cysteine in urine samples was higher that 99.00% from the amount of L-cysteine determined using a standard method.[12]

CONCLUSIONS

This paper describes two enantioselective, potentiometric membrane electrodes designed using vancomycin and teicoplanin as chiral selectors used in the enantioanalysis of L-cysteine. The electrodes exhibited near-Nernstian slopes, good enantioselectivity and very low limits of detection. While the results obtained for the enantioselectivity and recovery tests are comparable, parameters that make the difference are the slopes and the linear concentration ranges of the electrodes. Although, the slope of the electrode based on teicoplanine is 1 mV/decade of concentration higher than the one obtained for the vancomycin based electrode, the vancomycin based electrode has a wider linear concentration range, that made him applicable for the enantioanalysis of L-cysteine in raw material, pharmaceutical formulations, food supplements as well as biological fluids without being necessary any dilution of preconcentration. Accordingly, vancomycin based enantioselective, potentiometric membrane electrode will be the electrode of choice for the enantioanalysis of L-cysteine.

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