Maltodextrins Based Solid Membranes for the Enantioanalysis of L-Cysteine

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Abstract: Three enantioselective membranes based on maltodextrins with different values of dextrose equivalent (DE) were proposed for the enantioanalysis of L-cysteine. The membranes were used for the design of potentiometric sensors. The slopes of the sensors were near-Nernstian (higher than 58.00mV/decade of concentration) with limits of detection of magnitude order of 10^{-11} and 10^{-12} mol/L. The surfaces of the membranes were stable for more than 6 months of continuous use. They can be renewed by polishing on alumina paper.

Keywords: L-cysteine, solid enantioselective membrane, maltodextrin, enantioselective, potentiometric membrane electrode.

1. INTRODUCTION

L-Cysteine (L-Cys) is a sulphur-containing amino acid R-SH and one of the twenty basic proteins [1]. It can be used as a prospective radiation protector and cancer indicator [2-5]. The electrochemical study of cysteine has been reported [6-9]. The development of chemically modified amperometric electrodes for detection of cysteine is a growing field [10-16]. Fluorescence was also employed for the assay of cysteine [17].

Chiral recognition is an area of considerable research interest due to its importance in biological, chemical and pharmaceutical sciences [18]. Techniques, which are intensively used for chiral recognition are: chromatography, capillary zone electrophoresis, mass spectrometry and electrochemistry. Utilization of electrochemical techniques will make the enantioanalysis highly reliable [19].

Maltodextrins represents a class of very powerful chiral selectors [20-22]. Variations in DE values result in maltodextrins with varying physico-chemical properties: solubility, hydroscopicity, osmolality and their effectiveness to reduce the freezing point increase with increasing DE, while viscosity, cohesiveness and coarse-crystal prevention increase as DE decreases [23, 24]. Maltodextrins were used as chiral selectors for enantiomeric separations by capillary zone electrophoresis [21, 22, 25-29], and they were also used for the design of enantioselective, potentiometric membrane electrodes [19, 30-33]. Although, the HPLC (standard method) method, and fluorescence based method are highly used for biomedical analysis, they cannot always be high reliable especially for urine samples, when the complexity of the sample is very high.

This paper proposed three solid enantioselective membranes used in the design of enantioselective, potentiometric membrane electrodes (EPMEs) for the enantioanalysis of L-cysteine. The membranes were based on maltodextrins with different DE.

2. EXPERIMENTAL

2.1. Reagents and Materials

L- and D-Cysteine were bought from Sigma-Aldrich (St. Louis, MO, USA). Maltodextrins (DE 4.0-7.0, 13.0-17.0, 16.5-19.5) and graphite powder (1-2 μm, synthetic) were bought from Aldrich (Milwaukee, WI, USA).

Deionised water was obtained using a Modulab system (Continental Water Systems, San Antonio, TX, USA). 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 solutions were buffered with phosphate buffer (pH 2.40, 0.1mol/L) from Merck (Darmstadt, Germany) (1:1, v/v, buffer: deionised water).

2.2. Apparatus

The potentiometric measurements were done using a system comprising a 663 VA stand (Metrohm, Herisau, Switzerland), a PGSTAT 20 (Eco Chemie,

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Utretch, Netherlands), and a software version 4.9. Ag/AgCl (0.1 mol/L KCl) was used as reference electrode in the cell.

2.3. Solid Membranes and Electrodes Design

Paraffin oil was added to graphite powder in a ratio of 1:4 (w/w). A solution of maltodextrin (DE 4.0-7.0 (I), 13.0-17.0 (II), or 16.5-19.5 (III); 10^{-3}mol/L) was added to the paste in a ratio 1:1 (μL:mg). The diameter of the active area of the potentiometric, enantioselective membrane electrode was 3 mm. Ag/AgCl was used as electric contact. 0.1 mol/L KCl solution was used as internal solution.

2.4. Recommended Procedures

Direct potentiometric method was used for all measurements using solution with concentrations between 10^{-10} and 10^{-2} mol/L. The working and reference electrodes were placed in stirred standard solutions. Graphs of E(mv) versus pL-Cys were plotted and unknown concentrations were determined from the graphs.

2.4.1. 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 potentiometric method was used to determine L-cysteine in urine samples.

3. RESULTS AND DISCUSSION

3.1. Electrodes Response

The response characteristics of the electrodes were determined using potentiometric method (Table **1**). All calibration equations had correlation coefficients of 0.9999. The limits of detection are very low – of 10^{-11} and 10^{-12}mol/L magnitude order. The response of the proposed electrodes was between 58.00 and 60.00 mV/decade of concentration, very closed with the Nernstian value. The electrodes did not respond for D-cysteine assay; 3-5mV/decade of concentration was obtained when the three sensors were used.

The proposed electrodes were highly stable and reproducible over 6 months test period (RSD value for the slopes did not exceeded 1%). The response time was 20s for concentration range 10^{-5} – 10^{-3} mol/L and 1min for concentrations lower than 10^{-5} 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 for solutions of 10^{-5} mol/L L-Cys at different pH values (pH 1-12). These solutions were prepared by addition of small volumes of HCl and/or NaOH solution (0.1-1 mol/L of each) to a L-Cys solution.

The plots of E (mV) versus pH (Figure **1**) show that the response of the electrodes is not depending on pH, in the following ranges 2.0-5.0, 2.0-7.0, and 2.0-6.0 for the EPMEs based on maltodextrins I, II, and III, respectively.

3.3. The Selectivity of the Electrodes

Mixed solution method was used for the study of the selectivity of the proposed electrodes versus D-Cys, polyvinylpyrolidone (PVP), creatine, creatinine, Na^+, K^+, and Ca^{2+}. The concentration of the interfering ions and L-Cys were 10^{-4} and 10^{-5} mol/L, respectively. The EPMEs based on maltodextrins were selective over PVP, creatine and creatinine and enantioselective (Table **2**). Potentiometic selectivity coefficients were calculated using the equation:

$$K_{i,j}^{pot} = \left(10^{\frac{\Delta E}{S}} - 1\right) \times \frac{a_i}{a_j^{z_i/z_j}}$$

Table 1: Response Characteristics of Enantioselective, Potentiometric Membrane Electrodes for the Assay of L-Cysteine

EPME based on maltodextrin	Slope (mV/decade of conc.)	Intercept, E° (mV)	Linear conc. range (mol/L)	Detection limit (mol/L)
I	58.5±0.2	587.5±12.2	10^{-10}-10^{-3}	9.0x10^{-12}
II	59.0±0.1	659.8±11.3	10^{-10}-10^{-3}	5.2x10^{-12}
III	59.2±0.2	600.7±12.5	10^{-10}-10^{-3}	7.1x10^{-11}

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

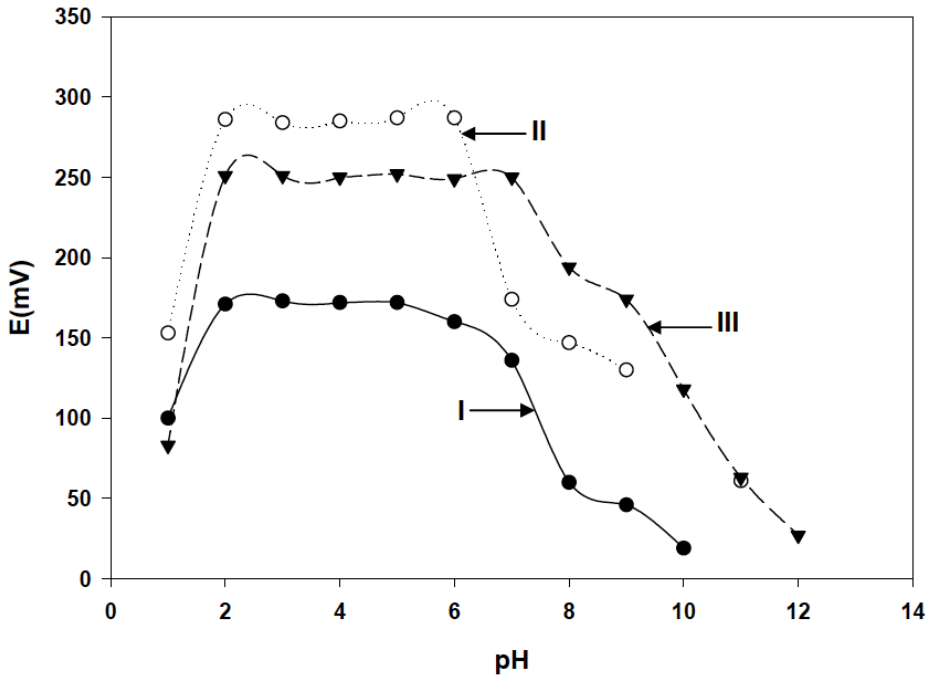

Figure 1: The influence of pH on the response of the enantioselective, potentiometric membrane electrodes ($C_{L\text{-}Cys}$=10^{-5}mol/L); I - MD-I, II - MD-III and III - MD-II based EPMEs.

where ΔE is the difference between the potential recorded for mixed solution ($E_{i,j}$) and for the solution that contains only the main ion (E_i), $\Delta E = E_{i,j} - E_I$ (all recorded in mV); S is the slope of the electrode deducted from the equation of calibration (mV/decade of concentration); a_i is the activity of the main ion, i, a_j is the activity of interfering species, j; z_i is the charge of the main ion, i, z_j is the charge of interfering species, j.

Inorganic ions such as Na^+, K^+, and Ca^{2+} did not interfere with the analysis of L-Cys, because the potentiometric selectivity coefficients calculated were less than 10^{-4}.

3.4. Analytical Applications

Recovery tests were performed first for the assay of L-cys in the presence of D-cys; different ratios between L- and D-Cys concentrations were used. The results obtained (Table **3**) demonstrated that D-Cys did not interfere in the assay of L-Cys.

L-cysteine was reliable determined in urine samples; results are shown in Table **4**.

The average recovery of L-cysteine in urine samples was higher that 99% from the amount of L-cysteine determined using a standard method - HPLC [34]. These values were taken as reference for the validation of the proposed method.

4. CONCLUSION

Maltodextrins were excellent chiral selectors for the design of enantioselective membranes. The construction of the membranes was simple, fast, and

Table 2: Potentiometric, Selectivity Coefficients of the Electrodes Used for the Enantioanalysis of L-Cysteine

Interfering species (J)	$K_{i,j}^{pot}$		
	EPME based on		
	MD-I	MD-II	MD-III
D-Cys	6.0×10^{-3}	8.1×10^{-4}	4.0×10^{-4}
PVP	3.2×10^{-3}	3.2×10^{-3}	8.1×10^{-4}
Creatine	4.0×10^{-4}	3.3×10^{-3}	4.0×10^{-4}
Creatinine	$<< 1.0\times10^{-4}$	4.0×10^{-4}	8.1×10^{-4}

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

Table 3: Determination of L-Cysteine in the Presence of D-Cysteine

L:D mol/mol	L-cys, Recovery, %		
	EPME based on MD		
	I	II	III
2:1	99.63±0.01	99.72±0.02	99.98±0.02
1:1	99.95±0.02	99.99±0.01	99.60±0.02
1:2	99.81±0.02	100.00±0.01	99.24±0.01
1:4	99.90±0.01	99.97±0.02	100.00±0.01
1:9	99.54±0.02	99.99±0.01	99.99±0.02

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

Table 4: Determination of L-Cysteine in Urine Samples

Urine sample	L-cys, Recovery			
	EPME based on MD, % from the amount determined using HPLC			HPLC [34], mol/L
	I	II	III	
1	99.42±0.20	99.80±0.15	99.99±0.25	$2.3x10^{-6}$
2	99.50±0.15	99.58±0.18	99.59±0.20	$2.7x10^{-7}$
3	99.09±0.20	99.86±0.21	99.49±0.10	$3.2x10^{-7}$
4	100.01±0.21	100.00±0.20	99.99±0.21	$4.3x10^{-7}$
5	99.87±0.10	99.85±0.12	99.57±0.21	$4.0x10^{-7}$
6	99.39±0.15	99.66±0.20	99.19±0.18	$3.9x10^{-7}$

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

reproducible. The electrodes' selectivity and enantioselectivity made them suitable for enantioanalysis of L-Cysteine in urine samples. The best maltodextrin based electrode for the enantioanalysis of L-cysteine proved to be the one based on maltodextrin III because it exhibited the best selectivity and enantioselectivity as well as the highest slope. Accordingly, this electrode is the electrode of choice for the enantioanalysis of L-cysteine.

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