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Research Article

Enantioselective Potentiometric Membrane Electrodes Based on Antibiotics for the Determination of L- and D-Glyceric Acids

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Glyceric acid (GA) is a human metabolite existing in L- and D-configurations, which are considered the markers for the diseases L- and D-glyceric aciduria/academia, respectively. Enantioselective, potentiometric membrane electrodes based on carbon paste modified with antibiotics as chiral selectors, vancomycin, and teicoplanin were designed for the assay of L- and D-GA, respectively, in the concentration ranges of 10^{-9}–10^{-7} and 10^{-4}–10^{-2} moL/L with very low detection limits (1.5×10^{-10} moL/L for L-GA and 1.6×10^{-4} moL/L for D-GA, resp.). The surface of the electrodes can be regenerated simply by polishing in order to obtain a fresh surface ready to be used in a new assay. The proposed electrodes can be successfully applied for the enantioanalysis of L- and D-glyceric acids in serum samples.

1. Introduction

The enantiomers of the urinary organic acids are important markers for inborn errors of metabolism. Accordingly, there is a growing demand for determining the metabolic products in human blood (academia) and urine (aciduria). Different enantiomers may originate from separate metabolic pathway, due to enzyme deficiency.

Glyceric acid (2,3-dihydroxypropionic acid, GA) is a human metabolite existing in L- and D-configurations. These two enantiomers are vital biological markers for the diagnosis of two different metabolic diseases, primary hyperoxaluria type II (L-glyceric aciduria, PH2) and D-glyceric aciduria [1–6]. Therefore, enantioselective analysis of glyceric acid is necessary to differentiate between the two inherited metabolic diseases.

Up to date, the assay of GA was done using capillary gas chromatography [7–10], liquid chromatography [11], high-performance liquid chromatography [12], capillary electrophoresis [13], polarimetry [14], and colorimetric methods [15].

Enantioselective, potentiometric membrane electrodes (EPMEs) proved to be very reliable for the enantioanalysis of pharmaceutical compounds as well as of compounds of clinical importance [16]. Macrocyclic antibiotics represent a new class of chiral selectors used in the design of EPME, offering a high selectivity and enantioselectivity [17]. The macrocyclic antibiotics contain stereogenic centers and functional groups, which allow them to interact with chiral molecules by hydrophobic, dipole-dipole, π-π interactions, hydrogen bonding, steric repulsion [18, 19], and charge-to-charge repulsions [20–22].

This paper describes the design, response characteristics, (enantio) selectivity, and applications of two EPMEs based on vancomycin and teicoplanin for the enantioanalysis of GA.

Table 1: Response characteristics of enantioselective, potentiometric membrane electrodes for L- and D-glyceric acids[a].

EPME based on	Parameters			
	Slope (mV/decade of concentration)	Intercept, E^o (mV)	Linear range (moL/L)	Detection limit (moL/L)
Vancomycin	58.6	574.6	10^{-9}–10^{-7}	1.56×10^{-10}
Teicoplanin	50.0	206.0	10^{-4}–10^{-2}	7.60×10^{-5}

[a] All measurements were made at 25°C; all values are the average of ten determinations.

2. Experimental

2.1. Electrode Design. Paraffin oil and graphite powder were mixed in a ratio of 1 : 4 (w/w) to form the carbon paste. The modified carbon pastes were obtained by the addition of the aqueous solutions of vancomycin (pH = 4) or teicoplanin (pH = 6) (10^{-3} moL/L) (100 μL chiral selector solution to 100 mg carbon paste) to the carbon paste. The unmodified carbon paste was filled into a plastic pipette peak leaving a space of 3-4 mm into the top to be filled with the modified carbon paste.

The diameter of the proposed EPMEs was 3 mm. Electric contact was obtained by inserting an Ag/AgCl wire into the carbon paste. 0.1 moL/L KCl was used as internal solution. All the sensors tips were gently rubbed on fine abrasive paper to produce a flat surface. The surface of the sensors was wetted with deionized water and then polished with an alumina paper (polished strips 30144-011, Orion) before use. When not in use, the electrodes were immersed in 10^{-3} moL/L of L- or D-glyceric acid solution, respectively.

2.2. Apparatus. A 663 VA Stand (Metrohm, Herisau, Switzerland) connected to a PGSTAT 100 and software (Eco Chemie version 4.9) was used for all potentiometric measurements. An Ag/AgCl (0.1 moL/L KCl) electrode was used as reference electrode in the cell.

2.3. Reagents and Materials. L- and D-glyceric acids, vancomycin, and teicoplanin were purchased from Sigma-Aldrich (USA). Graphite powder (1-2 μm) was purchased from Aldrich (Milwaukee, WI, USA); paraffin oil was purchased from Fluka (Buchs, Switzerland), and phosphate buffer (pH = 3.5) from Merck (Darmstadt, Germany).

Deionized water from a Modulab system (Continental Water Systems, San Antonio, Tex, USA) was used for all solutions preparation. L- and D-glyceric acid solutions were prepared from standard L- and D-GA solutions (1×10^{-1} moL/L) by serial dilutions. Serum and urine samples were buffered with phosphate buffer (pH = 3.5), sample : buffer = 1 : 1.

2.4. Recommended Procedure. Direct potentiometry was used for potential determination of each standard solution (10^{-10}–10^{-2} moL/L). All measurements were performed at 25°C. The electrodes were placed in stirred standard solutions. Calibration graphs were obtained by plotting E(mV) versus pL-GA or pD-GA, respectively. The unknown concentrations were determined from the calibration graphs.

3. Results and Discussion

3.1. EPMEs Response Characteristics. The response characteristics of the EPMEs were determined at pH = 3.5 (phosphate buffer) using the potentiometric method. The response obtained for L-GA was linear and near-Nernstian only for the EPME based on vancomycin, while the response obtained for D-GA was linear and near-Nernstian only for the EPME based on teicoplanin. The following are the equations of calibration for the EPMEs based on vancomycin and teicoplanin:

$$\begin{aligned} &\text{L-GA: } E = 574.6 - 58.6\ \text{pL-GA}, \quad r = 0.9957, \\ &\text{D-GA: } E = 206.0 - 50.0\ \text{pD-GA}, \quad r = 0.9988, \end{aligned} \tag{1}$$

where E (mV) is the potential of the electrochemical cell, pL-GA $= -\log[\text{L-GA}]$, pD-GA $= -\log[\text{D-GA}]$, and r is the correlation coefficient. The response characteristics of the EPMEs are shown in Table 1. A very low detection limit was recorded for the assay of L-GA: 10^{-10} moL/L magnitude order. The electrodes responses displayed a good stability and reproducibility for the tests performed for 3 months, when daily used for measurements (RSD < 1.0%).

The response time recorded for the assay of the D-enantiomer was 2 min while the response time recorded for the assay of the L-enantiomer was 30 s.

3.2. The Influence of pH on the Responses of the Electrodes. The effect of pH on the response of the electrodes was determined by recording the emf of the cell containing solutions of L- or D-GA of different pH values. The pHs of the solutions of the enantiomers were adjusted using small volumes of HCl (0.1 moL/L) or NaOH (0.1 moL/L) solutions. E (mV) versus pH plots (Figure 1) show that the emf is not depending on the pH in the ranges of 4–9 and 3–8 for vancomycin- and teicoplanin-based EPME, respectively.

3.3. Selectivity of the Electrode. The selectivity of both electrodes was checked using the mixed solutions method proposed by Ren [23], over L- or D-GA, creatine, and creatinine. The ratios between the concentrations of analyte and interferent were 1 : 10. The potentiometric selectivity coefficients (Table 2) obtained for EPMEs proved their

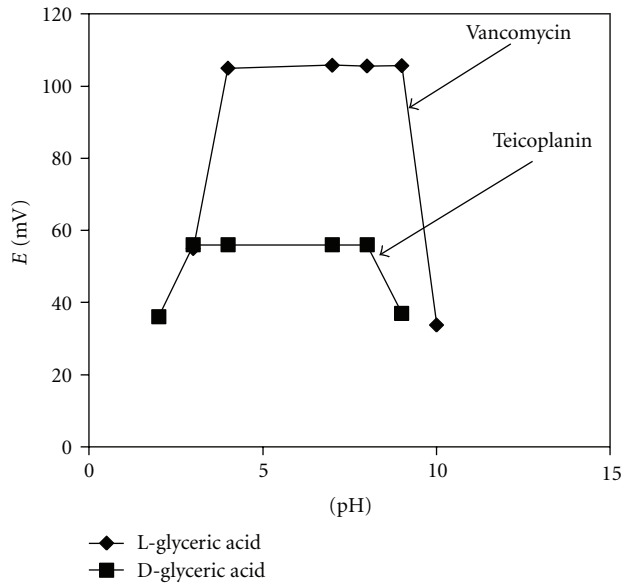

Figure 1: Effect of pH on the response of the EPMEs to L-glyceric acid (10^{-8} moL/L L-GA) and D-glyceric acid (10^{-3} moL/L) solutions. (I) Vancomycin-based EPME; (II) teicoplanin-based EPME.

Table 2: Potentiometric selectivity coefficients for the electrodes proposed for the assay of L- and D-glyceric acids[a].

Interference species (J)	pK_{sel}^{pot} EPME based on	
	Vancomycin	Teicoplanin
L-GA	—	2.39
D-GA	2.41	—
Creatine	2.09	2.08
Creatinine	2.41	2.39

[a]All measurements were made at 25°C; all values are the average of ten determinations.

Table 3: The results obtained for the determination of L-glyceric acid in the presence of D-glyceric acid[a].

L : D (moL/moL)	Recovery, %
2 : 1	99.25 ± 0.01
1 : 1	99.75 ± 0.02
1 : 2	99.26 ± 0.06
1 : 4	99.30 ± 0.04
1 : 9	99.67 ± 0.06

[a]All measurements were made at 25°C; all values are the average of ten determinations.

enantioselectivity as well as their selectivity over creatine and creatinine. Inorganic cations such Na^+, K^+, and Ca^{2+} do not interfere in the analysis of L- and D-GA.

3.4. Analytical Applications. Solutions containing L- and D-GA in different ratios were prepared to test the recovery for each enantiomer in the presence of its antipode and the suitability of the EPMEs for the enantioanalysis of L- and D-GA in serum and urine samples. The recovery tests (Tables 3 and 4) obtained for each enantiomer proved the suitability of the electrodes for enantioanalysis. No significant differences in the recovery values were recorded for the ratios between L : D or D : L enantiomers varying from 1 : 9 to 1 : 99.99.

Table 4: The results obtained for the determination of D-glyceric acid in the presence of L-glyceric acid[a].

D : L (moL/moL)	Recovery, %
2 : 1	99.96 ± 0.04
1 : 1	99.57 ± 0.03
1 : 2	99.99 ± 0.03
1 : 4	99.95 ± 0.02
1 : 9	99.93 ± 0.03

[a]All measurements were made at 25°C; all values are the average of ten determinations.

Table 5: Recovery of L-glyceric acid in serum and urine samples, (%)[a].

Type of sample	Sample no.	% Recovery, L-GA	
		Standard method [24]	EPMEs
Serum samples	1	98.47	98.52 ± 0.04
	2	98.15	98.08 ± 0.08
	3	98.02	98.00 ± 0.06
Urine samples	4	99.30	99.25 ± 0.02
	5	99.50	99.49 ± 0.03
	6	99.45	99.50 ± 0.03
	7	99.86	99.87 ± 0.02
	8	99.12	99.13 ± 0.01
	9	99.89	99.99 ± 0.02

[a]All measurements were made at 25∘C; all values are the average of ten determinations.

Table 6: Recovery of D-glyceric acid in serum and urine samples, (%)[a].

Type of sample	Sample no.	% Recovery, D-GA	
		Standard method [24]	EPMEs
Serum samples	10	97.20	97.23 ± 0.02
	11	96.70	96.65 ± 0.03
	12	97.70	97.21 ± 0.08
Urine samples	13	99.20	99.18 ± 0.02
	14	99.50	99.48 ± 0.01
	15	99.93	100.00 ± 0.02
	16	99.43	99.40 ± 0.03
	17	99.15	99.12 ± 0.02
	18	99.11	99.13 ± 0.02

[a]All measurements were made at 25°C; all values are the average of ten determinations.

The results obtained for the analysis of L-glyceric and D-glyceric acid in serum and urine samples are shown in Tables 5 and 6, respectively. Different serum samples and urine samples were collected from different patients suspected of L-glyceric academia (1–3) or aciduria (4–9) and D-glyceric academia (10–12) or aciduria (13–18) for the recovery of L- and D-glyceric acid. All the serum and urine samples were buffered with phosphate buffer pH = 3.5. The results obtained using the proposed EPMEs are in good concordance with those obtained using the standard method, which is an HPLC technique [24]. The advantage of the proposed method over the standard one was the high reliability measured through low values of RSD (%), short time of analysis, and low cost of the enantioanalysis.

4. Conclusions

The macrocyclic antibiotics vancomycin and teicoplanin proved to be viable chiral selectors for the design of EPMEs. The enantioselective, potentiometric membranes electrodes proposed can be reliably used for the enantioselective analyses of L- and D-glyceric acids in serum and urine samples. Accordingly, they can be used for the fast and reliable diagnosis of L- or D-glyceric academia/aciduria. The construction of the electrodes is simple, fast, and reproducible. The serum and urine samples need only to be buffered with phosphate buffer of pH of 3.5 before L- and D-glyceric acids were determined.

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