Chapter 7

Enantioselective, potentiometric membrane electrodes for the determination of L- and D-glyceric acids

7.1 Introduction

Inborn errors of metabolism disorders are rare genetic diseases. They can be diagnosed by assay of organic acids (e.g., L(D)-glyceric acids) in human body fluids. Glyceric acid (2,3-dihydroxypropionic acid, Figure 7.1) exists in two configurations, L- and D-enantiomers in mammalian metabolism.

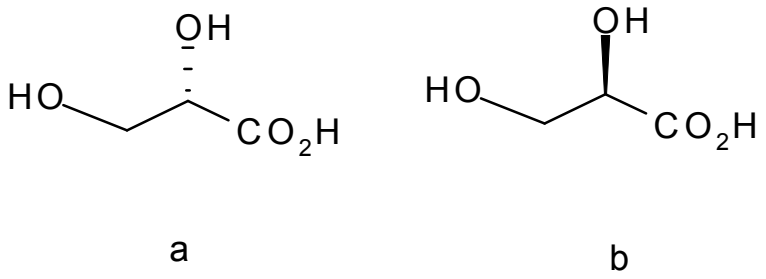

Figure 7.1 Glyceric acid enantiomers (a) L(-)-glyceric acid and (b) D-(+)-glyceric acid

The presence of one of these enantiomers in blood or urine in abnormal concentration caused a different type of illness. Enantiomers may originate from different metabolic pathway, due to the enzymes' deficiencies. D-glyceric dehydrogenase (D-GDH) catalyzes the interconversion of hydroxypyruvate to D-glycerate in the degradation pathway of serine metabolism [1]. D-GDH also has a glyoxylate-reductase (GR) activity, and it catalyzes the cytoplasmic reduction of glyoxylate to glycolate [2]. The genetic deficiency of D-GDH and GR causes a metabolic disorder named primary hyperoxaluria

type 2 (PH II) or L-glyceric acidurias/acidemia [3,4] where hydroxypyruvate is converted to L-glycerate by L-lactate dehydrogenase (LDH) in the presence of NADH (Figure 7.2) [5].

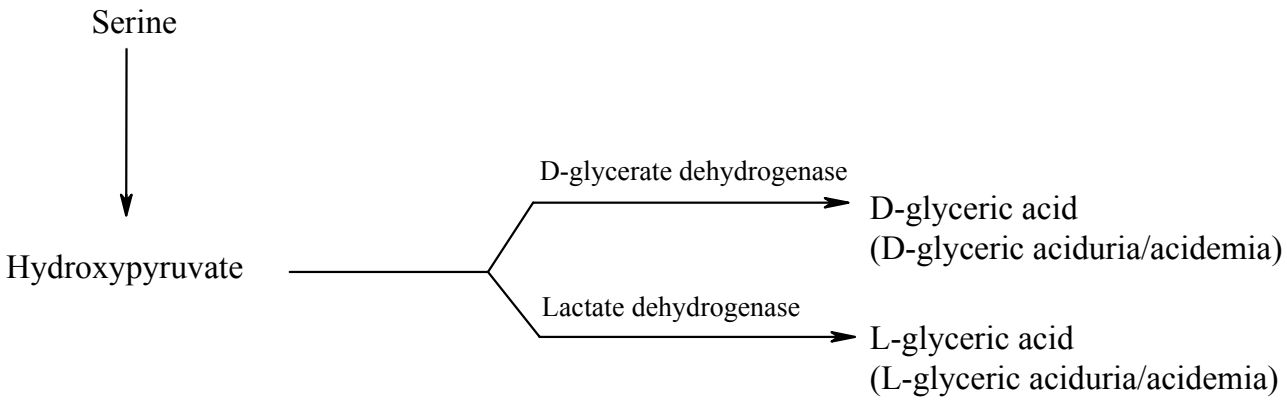

Figure 7.2 Metabolic pathways of L- and D-glyceric acid [5].

The metabolic disorder leads to excessive urinary oxalate and excretion of L-glycerate. PH II is characterized by urolithiasis or nephrocalcinosis with terminal renal failure [6]. Primary hyperoxaluria type 1 (PH I) is caused by deficiency of hepatic alanine: glyoxylate aminotransferase (AGT), which promotes the transamination of glyoxylate to oxalate. Deficiency of AGT caused oxidation of glyoxylate to oxalate and glycolate by glycolate oxidase and reductase, respectively [7,8]. PH I and PH II are described by marked increase of oxalate production associated with hyperglycolate or hyper-L-glycerate in blood and urine [9].

Another disease entity involving glyceric acid is D-glyceric acidemia/aciduira. This disease was first reported by Brandt, *et. al.* [10,11] showing ketotic hyperglycinemia with abnormal excretion of D-glyceric acid in urine and serum. D-glyceric acid is an

intermediate in the pathways of serine degradation and fructose metabolism [11,12]. This increase of excretion was explained by deficiency of D-glycerate kinase that is involved in the conversion of D-glyceric acid to D-2-phosphoglycerate [13-17]. D-glyceric acidemia/acidurias is associated with delayed psychomotor growth, mental retardation and seizures [11, 12, 14, 18]. The different syndrome entity of increased excretion of L and D-glyceric acid in urine and serum is a hallmark of two totally different inborn diseases, hyperoxaluria II and D-glyceric acidamia/aciduira, respectively. Accordingly, enantioanalysis of L- and D-GA is important for the diagnosis of patients with PH II and D-glyceric acidamia/aciduira.

Several methods have been reported for the configurational analysis of L- and D-GA based on capillary electrophoresis [19], colorimetry [20], polarimetry [15] and chromatographic methods [9, 21-26].

Enantioanalysis of chiral substances of clinical importance is a vital subject for the biomedical applications and early discovery of illnesses. Enantioselective, potentiometric membrane electrodes (EPMEs) based on chiral selectors had been developed for enantiomeric assay. Cyclodextrins [27, 28], macrocyclic antibiotics [29], and maltodextrins [30] were used as chiral selectors in the design of EPMEs.

In this chapter, nine EPMEs based on different chiral selector have been designed for the enantioanalysis of L- and D-glyceric acids in serum and urine sample. EPMEs based on maltodextrin I, maltodextrin III, α-CD, γ-CD and vancomycin were used for the

determination of L-glyceric acid while EPMEs based on maltodextrin III and β-CD, 2-hydroxy-3-trimethylammoniopropyl-β-CD and teicoplanin were used for the analysis of D-glyceric acid. The proposed EPMEs were applied for the enantiomers analysis of L- and D-glyceric acids in biological fluid.

7.2 Reagents and materials

L- and D-glyceric acids, vancomycin and teicoplanin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Graphite powder (1-2μm, synthetic) and maltodextrins [DE 4.0-7.0 (I), 13.0-17.0 (II), and 16.5-19.5 (III)] were purchased from Aldrich (Milwaukee, WI, USA). Paraffin oil was purchased from Fluka (Buchs, Switzerland). α-, β-, γ-, and 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrins were supplied by Wacher-Chemie GmbH (Germany). Phosphate buffer (pH = 3.5) was supplied by Merck (Darmstadt, Germany).

De-ionized water from a Modulab system (Continental Water Systems, San Antonio, TX, USA) was used for all reagents and solutions preparation. 0.1mol/L stock solutions of L- and D-glyceric acids were buffered with phosphate buffer (pH 3.5). Solutions of L- and D-glyceric acid ($1x10^{-10}$ - $1x10^{-2}$ mol/L) were prepared by serial dilution from the stocks solutions of L- and D- glyceric acids and were buffered with phosphate buffer (pH 3.5).

10^{-3} mol/L solutions of each maltodextrin (I, II and III), and cyclodextrins (α-, β-, γ-, and 2-hydroxy-3-trimethylammoniopropyl-β-CD) were prepared. The solution of vancomycin ($2x10^{-3}$ mol/L) was prepared in phosphate buffer (pH 4.00). The solution of teicoplanin

($2x10^{-3}$ mol/L) was prepared using pH 6.00 phosphate buffer. The solution of teicoplanin ($2x10^{-3}$ mol/L) containing acetonitrile was prepared using pH 6.00 phosphate buffer containing 40% (v/v) of acetonitrile.

7.3 Enantioselective, potentiometric membrane electrodes based on maltodextrins

7.3.1 Apparatus

All chronopotentiometric (zero current) measurements were recorded using a Metrohm 663 VA stand (Metrohm, Herisau, Switzerland) connected to a PGSTAT 20 and a software version 4.9 (Eco Chemie, Utretch, The Netherlands). An Ag/AgCl (0.1 mol/L KCl) electrode was used as reference electrode in the cell.

7.3.2 Electrodes design

Plain carbon paste was prepared by thoroughly mixing 100 mg of graphite powder with 40 µL paraffin oil. Paraffin oil and graphite powder [1:4, (w/w)] were mixed well, followed by the addition of 10^{-3} mol/L aqueous solution of maltodextrin [DE 4.0-7.0 (I), 13.0-17.0 (II), or 16.5-19.5 (III)] (100 µL of each maltodextrin solution to 100 mg graphite powder). The plain 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 that contains the maltodextrin. The diameter of enantioselective, potentiometric membrane electrode was 3 mm. Electric contact was obtained by inserting a Ag/AgCl wire into the carbon paste. The internal electrolyte solution of EPMEs was 0.1 mol/L KCl. All the EPMEs tips were

gently rubbed on fine abrasive paper to produce a flat surface. The surface of the electrodes was wetted with de-ionized water and then polished with an alumina paper (polished strips 30144-011, Orion) before use for the analysis. When not in use, L- and D-GA electrodes were immersed in 10^{-3} mol/L L- or D-glyceric acid solution, respectively.

7.3.3 Recommended procedure

Direct potentiometric method was used for potential determination of each standard solution (10^{-10}-10^{-2} mol/L), utilizing two electrodes system. The electrodes were placed in stirred standard solutions. Calibration graphs were obtained by plotting E (mV) versus p(L-GA) or p(D-GA), respectively. The unknown concentrations of L- and D-glyceric acids were determined in serum and urine samples by interpolation of the potential measured into the calibration graphs (E (mV) versus p(L-GA) or E (mV) versus p(D-GA)).

7. 3.4 Results and discussion

7. 3.4.1 EPMEs response characteristics

The response characteristics exhibited by the EPMEs impregnated with maltodextrins were determined for enantiomers, L-glyceric and D-glyceric acids at pH=3.5 (phosphate buffer). The response obtained for L-glyceric acid was near-Nernstian only for maltodextrins I and III based EPMEs, while the response obtained for D-glyceric acid was near-Nernstian only for EPME based on maltodextrin II. The linear concentration ranges obtained are the following: for L-glyceric: 10^{-8}-10^{-6}mol/L and 10^{-6}-10^{-3}mol/L for

EPMEs based on maltodextrin I and III, respectively, and for D-glyceric acid 10^{-5}-10^{-3} mol/L. The equations of calibration and the corresponding correlation coefficients (r) are as follows:

EPME based on maltodextrin I:	E(mV) = 58.00 p(L-GA) + 517.67	r = 0.9681
EPME based on maltodextrin III:	E(mV) = 59.07 p(L-GA) - 198.00	r = 0.9994
EPME based on maltodextrin II:	E(mV) = 59.00 p(D-GA) - 156.67	r = 0.9999

where E (mV) is the cell potential, p(L-GA) = -log [L-GA] and p(D-GA) = -log [D-GA]. The limits of detection obtained for the assay of L-glyceric acid are 1.19 x 10^{-9} and 1.00 x 10^{-7} mol/L when maltodextrin I and III based EPME, respectively, are used while for the assay of D-glyceric acid the limit of detection is 1.00x10^{-6}mol/L when the EPME based on maltodextrin II is used. The electrode based on maltodextrin I exhibited the lowest limit of detection. The electrodes responses have a good stability and reproducibility for the tests performed for 1 month, when used daily for measurements (RSD<0.1%). The best stability was recorded for EPME based on maltodextrin I, because maltodextrin I has got a more rigid conformation compared to maltodextrin II and III [31].

The best response time was recorded for the maltodextrin II, where the electrode potential was recorded in 1min for the concentration range 10^{-5}-10^{-3}mol/L. The response time recorded for the assay of L-glyceric acid is 2min for maltodextrin I based EPME in the concentration range 10^{-8}-10^{-6}mol/L and 90s for maltodextrin III based EPME in the concentration range 10^{-6}-10^{-3}mol/L.

7. 3.4.2 Effect of pH on the responses of the electrodes

Influence of pH on the response of the electrodes was studied by recording the emf of the cell containing solutions of L- or D-glyceric acid at different pH values (pH range 1-10). For L-glyceric, measurements were performed for a concentration of 10^{-6} mol/L, whereas for D-glyceric measurements were investigated for a concentration of 10^{-4}mol/L. These solutions were prepared by adding very small volumes of HCl or NaOH solution (10^{-1} mol/L or 1 mol/L of each) to L- or D-glyceric acid solutions, respectively.

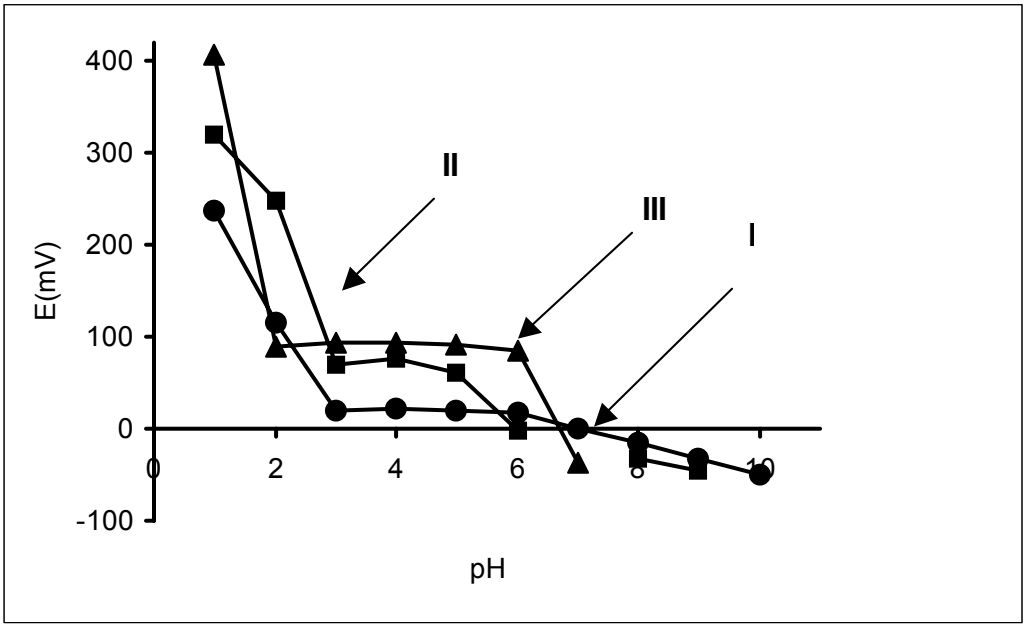

Figure 7.3 Influence of pH variation on the response of EPMEs based on maltodextrins (I) and (III) to solutions of L-glyceric acid (10^{-6} mol/L L-GA) and of EPME based on maltodextrin (II) to solutions of D-glyceric acid (10^{-4}mol/L).

The plots of E(mV) versus pH show the influence of pH variation on the responses of EPMEs (Figure 7.3). For the assay of L-glyceric acid, the responses of EPMEs based on maltodextrins I and III are pH-independent in the pH ranges 3.0-6.0 and 2.0-6.0 while for

the assay of D-glyceric acid the response of EPME based on maltodextrins II is pH-independent in the pH range 3.0-5.0.

7. 3.4.3 Selectivity of the electrodes

The selectivity of the electrodes was investigated using the mixed solutions method [32]. The selectivity of the proposed electrodes was checked over L- or D-GA, creatine and creatinine. A ratio of 1:10 was used between the concentrations (mol/L) of analyte and possible interferent. The potentiometric selectivity coefficients, K_{sel}^{pot}, for maltodextrin (I) and (III) based EPMEs proved that D-GA, creatine and creainine do not interfere in the determination of L-GA; as well, K_{sel}^{pot} values of maltodextrin (II) based EPME proved that L-GA, creatine and creatinine do not interference in the assay of D-GA (Table 7.1). The interference of inorganic cations such a Na^{+}, K^{+}, and Ca^{2+} was verified for all proposed EPMEs; the values of K_{sel}^{pot} (all lower than 10^{-3}) shown that these ions do not interfere in the analysis of L- and D-GA.

Table 7.1 Selectivity coefficients of enantioselective, potentiometric membrane electrodes.

Interfering species (J)	K_{sel}^{pot}		
	EPME based on		
	Maltodextrin I	Maltodextrin III	Maltodextrin II
L-glyceric acid	-	-	3.98×10^{-3}
D-glyceric acid	3.89×10^{-3}	3.98×10^{-3}	-
Creatine	4.05×10^{-3}	7.50×10^{-3}	7.51×10^{-3}
Creatinine	8.26×10^{-3}	3.82×10^{-3}	3.83×10^{-3}

All measurements were made at room temperature; all values are the average of ten determinations.

7. 3.4.4 Analytical applications

The suitability of the proposed EPMEs for the enantioanalysis of GA was investigated by recovery of each enantiomer in the presence of its antipode. Therefore, solutions containing different ratios between L:D or D:L (2:1 to 1:99.99) enantiomers of glyceric acid were prepared. The recovery tests demonstrated the suitability of the enantioselective, potentiometric membrane electrodes for the analysis of the enantiopurity of GA (Tables 7.2 and 7.3). 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 7.2 The results obtained for the determination of L-glyceric acid in the presence of D-glyceric acid.

L:D (mol/mol)	EPMEs based on % L-GA, Recovery	
	Maltodextrin I	Maltodextrin III
2:1	99.28±0.01	99.46±0.01
1:1	99.71±0.01	99.42±0.02
1:2	99.69±0.01	99.51±0.03
1:4	99.24±0.02	99.31±0.01
1:9	99.72±0.01	99.84±0.01

All measurements were made at room temperature; all values are the average of ten determinations.

Table 7.3 The results obtained for the determination of D-glyceric acid in the presence of L-glyceric acid.

D:L (mol/mol)	EPME based on maltodextrin II % D-GA, Recovery
2:1	99.87±0.03
1:1	99.80±0.02
1:2	99.48±0.01
1:4	99.27±0.01
1:9	99.63±0.03

All measurements were made at room temperature; all values are the average of ten determinations.

Healthy volunteers donated three serum and five urine human samples. These samples were stored at -20^{o}C before use. Serum and urine samples were spiked with L- or D-glyceric acid to make the final concentrations as shown in Tables 7.4 and 7.5. Serum (1-3) and urine (4-8) samples were spiked with different aliquots of L-GA. On the other hand, serum (9-11) and urine (12-16) samples were spiked with aliquots of D-GA. These samples were used for the recovery of L-GA and D-GA in the real human matrices. The results obtained for the analysis of L–GA and D-GA in serum and urine samples are shown in Tables 7.4 and 7.5, respectively. The results obtained by using the proposed EPMEs are in good concordance with the quantities of the acids added to the real samples, showing the suitability of the electrodes for the diagnosis of the associated diseases.

Table 7.4 Recovery of L-glyceric acid in serum and urine samples.

Type of sample	Sample no.	Spiked L-GA final concentrations (mg/L)	EPMEs based on % L-GA, Recovery	
			Maltodextrin I	Maltodextrin III
Serum	1	0.0212	99.59±0.02	99.89±0.03
	2	0.2120	98.89±0.05	99.90±0.02
	3	0.6360	99.40±0.07	99.27±0.02
Urine	4	0.0424	99.29±0.02	99.35±0.01
	5	0.0848	99.39±0.01	99.27±0.01
	6	0.1060	99.50±0.03	99.18±0.01
	7	0.4240	99.14±0.08	99.15±0.03
	8	0.7420	99.44±0.04	99.18±0.02

All measurements were made at room temperature; all values are the average of ten determinations.

Table 7.5 Recovery of D-glyceric acid in serum and urine samples.

Type of sample	Sample no.	Spiked D-GA final concentrations (mg/L)	EPME based on Maltodextrin II
			% D-GA, Recovery
Serum	9	2.120	99.30±0.02
	10	21.20	99.76±0.05
	11	63.60	99.76±0.03
Urine	12	4.240	99.64±0.08
	13	8.480	99.94±0.08
	14	10.60	99.39±0.02
	15	42.40	99.78±0.01
	16	74.20	99.82±0.01

All measurements were made at room temperature; all values are the average of ten determinations.

7.4 Enantioselective, potentiometric membrane electrodes based on cyclodextrins

7.4.1 Apparatus

The direct potentiometric measurements was recorded using a Metrohm 663 VA stand (Metrohm, Herisau, Switzerland) connected to a PGSTAT 20 and a software version 4.9 (Eco Chemie, Utretch, The Netherlands). An Ag/AgCl (0.1 mol/l KCl) electrode was used as reference electrode in the cell.

7.4.2 Electrodes design

Graphite powder and paraffin oil were mixed with in a ratio 1:4 (w/v) followed by the addition of solution containing $1x10^{-3}$mol L^{-1} of α-, β-, γ-, and 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrins (100μL chiral selector solution to 100 mg carbon paste). A plain carbon paste was also prepared by thoroughly mixing 100 mg of

graphite powder with 40µL paraffin oil. The plain carbon paste was filled into a plastic pipette peak leaving a space of 3-4 mm into the top to be filled with the carbon paste that contains the chiral selector. The diameter of enantioselective, potentiometric membrane sensor was 3 mm. Electric contact was obtained by inserting Ag/AgCl wire into the carbon paste. The internal electrolyte solution of EPMEs was 0.1mol L^{-1} KCl. All the EPMEs tips were gently rubbed on fine abrasive paper to produce a flat surface. The surface of the electrodes was wetted with de-ionized water and then polished with an alumina paper (polished strips 30144-011, Orion) before use for the analysis. When not in use, L- and D-GA electrodes were immersed in a 10^{-3} mol/L solution of L- or D-glyceric acid, respectively.

7.4.3 Recommended procedure

The direct potentiometric method was used for the potential determination of each standard solution (10^{-10}-10^{-2} mol/L, pH 3.50) of L- and D-glyceric acids. Calibration graphs were obtained by plotting E (mV) versus pL-GA and pD-GA respectively. The unknown concentrations of L- and D-glyceric acid were determined in serum and urine samples by interpolation of the potential measured, into the calibration plots.

7. 4.4 Results and discussion

7. 4.4.1 EPMEs response characteristics

The truncated cone shape of cyclodextrins and its relative hydrophobic cavity able to host analytes by means of inclusion-complexation is the reason of interaction beween enantiomer and chiral selectors. Weak bonds between substituent groups on the

asymmetric center of analytes and secondary and/or primary hydroxyl groups of the CD are mainly responsible for chiral recognition [33]. From the proposed EPMEs, only those based on α-CD and γ-CD worked for the assay of L-glyceric acid, while β-CD and 2-hydroxy-3-trimethylammoniopropyl-β-CD based EPME worked for the asay of D-glyceric acid. The calibration equations obtained for the L- and D-glyceric acid are:

$$E(mV) = -59.00\ pL\text{-}GA + 639.00 \qquad (\alpha\text{-CD based EPME})$$

$$E(mV) = 52.80\ pL\text{-}GA - 64.30 \qquad (\gamma\text{-CD based EPME})$$

$$E(mV) = 58.00\ pD\text{-}GA + 100.33 \qquad (\beta\text{-CD based EPME})$$

$$E(mV) = 59.00\ pD\text{-}GA - 160.50 \qquad (\beta\text{-derivative-CD based EPME})$$

where E (mV) is the cell potential and pL-GA = -log [L-GA] and pD-GA = -log [D-GA]. All the response characteristics of the electrodes are shown in Table 7.6. EPMEs displayed a good stability and reproducibility over the tests performed for 2 months, when they were used daily for measurements.

The response times were lower than 1 min and 1min for the EPMEs designed for the assay of L-glyceric acid, respectively based on α- and γ-CD in the concentration ranges (10^{-9}-10^{-7} and 10^{-5}-10^{-2} mol/L). The response times for the determination of D-glyceric acid was higher than 1min when EPME based on β-CD was used in the range 10^{-5}-10^{-3} mol/L and lower than 1min when EPME based on β-derivative-CD was ued in the concentration range 10^{-6}-10^{-3} mol/L.

Table 7.6 Response characteristics of EPMEs for the determinations of D- and L-glyceric acids.

Analyte	Chiral selector	EPMEs characteristics			
		Slope [mV/p(C)]	Intercept, E^o [mV]	Linear range [mol/L]	Detection limit [mol/L]
L-GA	α-CD	59.00	139.00	10^{-9}-10^{-7}	1.48×10^{-11}
	γ-CD	52.80	-64.30	10^{-5}-10^{-2}	1.00×10^{-6}
D-GA	β-CD	58.00	-100.33	10^{-5}-10^{-3}	1.00×10^{-6}
	β-derivative-CD	59.00	-160.50	10^{-6}-10^{-3}	1.00×10^{-7}

[a]All measurements were made at room temperature; all values are the average of ten determinations.

7.4.2.2 Effect of pH on the responses of the electrodes

The influence of the pH on the response of the proposed EPMEs was checked by measuring the potential of the potentiometric cells at pHs between 1 and 10. Solutions of pHs between 1 and 10 of L- and D-glyceric acid were prepared by adding different volumes of HCL (0.1 mol/L) or NaOH solutions (0.1 mol/L) to their standard solutions. Plots showing the variation of E (mV) with pH values are shown in Figure 7.4a and 7.4b. For L-glyceric acid, the responses of EPMEs are pH-independent in the pH ranges 2.0-5.0 (α-CD based EPME) and 2.0-6.0 (γ-CD based EPME), while for D-glyceric acid the repones of EPMEs are not depending on pH in the ranges 3.0-5.0 (β-CD based EPME) and 4.0-7.0 (β-derivative-CD based EPME).

7. 4.2.3 Selectivity of the electrodes

The selectivity of electrodes has been investigated using the mixed solutions method proposed by Ren [32] and it was checked against L- and D-glyceric acid, creatine, creatinine, Na^+, K^+ and Ca^{+2}. The ratio between the concentration of the main analyte and interfering ion was 1:10. The potentiometric selectivity coefficients, K_{sel}^{pot} Table 7.7, proved that L (D)-glyceric acid, creatine and creatinine do not interfere in the

determination of L- and D-glyceric acid, and that the proposed EPMEs are enantioselective. Also, inorganic cations such a Na^{+}, K^{+}, and Ca^{2+} do not interfere in the analysis of L- and D-GA, as the values of K_{sel}^{pot} obtained were lower than 10^{-3}.

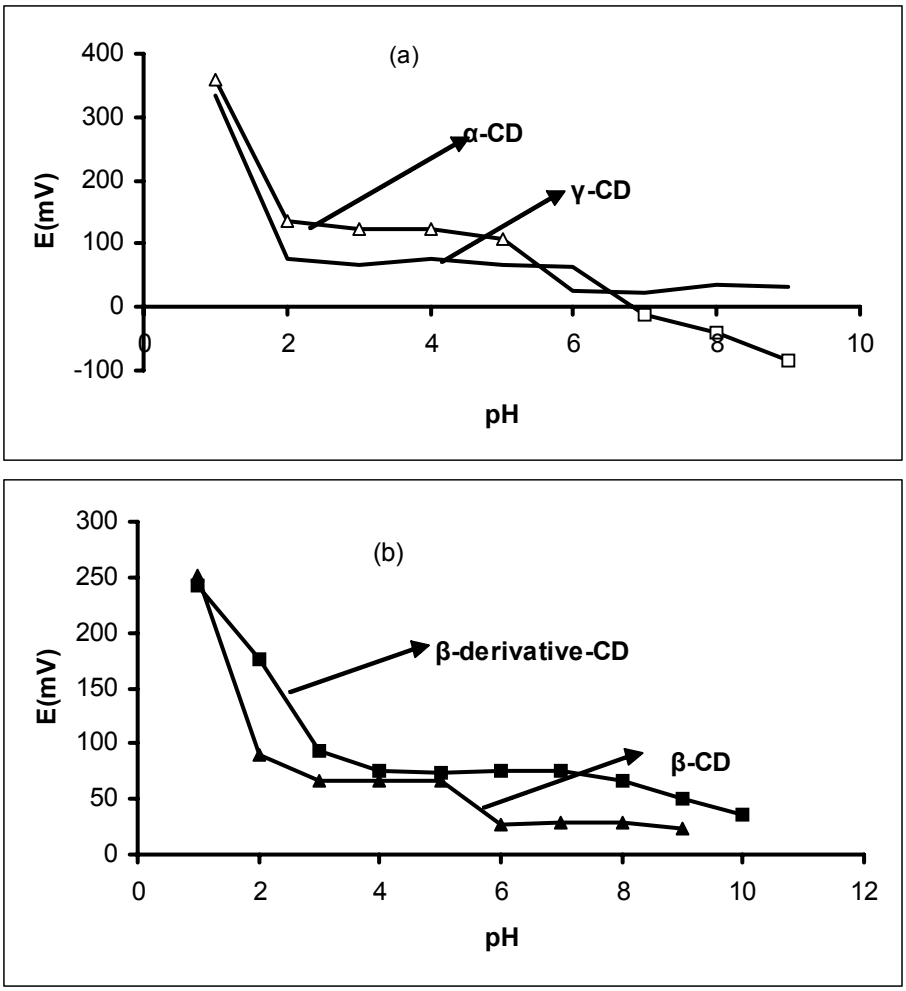

Figure 7.4 (a) Influence of pH variation on the response of EPMEs based on α- and γ-cyclodextrins, respectively, for the assay of L-glyceric acid, (b) Influence of pH variation on the response of EPMEs based on β-cyclodextrin and 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin (as chloride salt) (β-CD-derivative) for the assay of D-glyceric acid.

Table 7.7 Selectivity coefficients for the response of EPMEs for the determination of L- and D-glyceric acid.[a]

Interfering species (j)	K_{sel}^{pot} of EPME based on			
	α-CD	γ-CD	β-CD	B-derivative-CD
L-glyceric acid	-	-	3.89×10^{-3}	3.98×10^{-3}
D-glyceric acid	3.98×10^{-3}	4.46×10^{-3}	-	-
Creatine	3.83×10^{-3}	4.27×10^{-3}	4.05×10^{-3}	7.51×10^{-3}
Creatinine	7.51×10^{-3}	8.35×10^{-3}	7.63×10^{-3}	3.83×10^{-3}

[a]All measurements were made at room temperature; all values are the average of ten determinations.

7. 4.2.4 Analytical applications

Table 7.8 The recovery results obtained for the analysis of L-glyceric acid in the presence of D-glyceric acid[a]

L:D (mol/mol)	% L-GA, Recovery	
	EPMEs based on	
	α-CD	γ-CD
2:1	99.88±0.01	99.38±0.01
1:1	99.60±0.03	99.69±0.01
1:2	99.71±0.02	99.94±0.02
1:4	99.65±0.01	99.68±0.02
1:9	99.73±0.01	99.52±0.01
D:L (mol/mol)	% D-GA, Recovery	
	EPMEs based on	
	β-CD	β-derivative-CD
2:1	99.17±0.04	99.99±0.01
1:1	99.52±0.01	99.97±0.01
1:2	99.78±0.01	99.98±0.01
1:4	99.17±0.02	99.98±0.02
1:9	99.99±0.03	99.96±0.01

[a] All measurements were made at room temperature; all values are the average of ten determinations.

The suitability of EPMEs was investigated for the recovery of L- and D-GA in the solutions containing the antipode of the enantiomer assayed. Solutions containing L:D or

D:L of glyceric acid were prepared in different ratios (2:1 to 1:99.99) to check the recovery for L- and D-GA, respectively.

The recovery tests demonstrated the suitability of the enantioselective, potentiometric membrane electrode for the enantioanalysis of L- and D-GA (Table 7.8). 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 7.9 Recovery of L-glyceric acid in serum and urine samples[a].

Type of sample	Sample no.	% L-GA, recovery		
		Chromatographic method [19]	EPMEs based on	
			α-CD	γ-CD
Serum	1	99.36	99.44±0.01	99.09±0.04
	2	99.68	98.65±0.02	99.72±0.02
	3	99.39	98.37±0.03	99.03±0.03
Urine	4	99.75	99.76±0.02	99.73±0.03
	5	99.57	99.54±0.02	99.59±0.01
	6	99.60	99.52±0.01	99.69±0.01
	7	66.57	99.60±0.03	99.50±0.02
	8	99.80	99.81±0.01	99.72±0.01
	9	99.63	99.62±0.01	99.65±0.04

[a]All measurements were made at room temperature; all values are the average of ten determinations.

Healthy volunteers donated serum and urine human samples. These samples were stored at -20^{o}C. Serum and urine samples were spiked with L- and D-glyceric acid These samples were used for the recovery of L-GA and D-GA in the real matrices and to show the suitability of the EPMEs for the enantioanalysis of L- and D-GA in serum and urine samples. The results obtained for the analysis of L–glyceric and D-glyceric acid in serum and urine samples (Table 7.9 and 7.10) were compared with those obtained using a

chromatographic method of analysis [19]. The results obtained using the proposed EPMEs are in good concordance with those obtained using the chromatographic method [19] showing the suitability of the proposed EPMEs for diagnosis of hyperoxaluria type 2 (PH II) and D-glyceric acidemia/aciduria.

Table 7.10 Recovery of D-glyceric acid in serum and urine samples[a]

Type of sample	Sample no.	% D-GA, Recovery		
		Chromatographic method [19]	EPME based on	
			β-CD	β-derivative-CD
Serum	1	99.44	99.08±0.02	99.55±0.01
	2	99.79	99.27±0.01	99.98±0.02
	3	99.51	99.36±0.01	99.87±0.02
Urine	4	99.47	99.57±0.02	99.33±0.02
	5	99.68	99.75±0.01	99.63±0.01
	6	99.87	99.99±0.01	99.52±0.02
	7	99.76	99.93±0.02	99.66±0.01
	8	99.78	99.91±0.02	99.59±0.02
	9	99.72	99.89±0.01	99.67±0.01

[a] All measurements were made at room temperature; all values are the average of ten determinations.

7.5 Enantioselective, potentiometric membrane electrodes based on macrocyclic antibiotics

7.5.1 Apparatus

A 663 VA Stand (Metrohm, Herisau, Switzerland) connected to a PGSTAT 100 and software (Eco Chemie version 4.9) was used for all chronopotentiometric (zero current) measurements. An Ag/AgCl (0.1 mol/l KCl) electrode was used as reference electrode in the cell.

7.5.2 Electrodes design

Paraffin oil and graphite powder were mixed in a ratio 1:4 (w/w) followed by the addition of the aqueous solutions of vancomycin (pH=4) and teicoplanin (pH=6) (10^{-3} mol/l) (100 µl chiral selector solution to 100 mg carbon paste to form the modified carbon paste. A plastic pipette peak was filled with carbon paste, a space of 3-4 mm was left into its top to be filled with the modified carbon paste. The diameter of enantioselective, potentiometric membrane electrodes was 3 mm. Electric contact was obtained by inserting Ag/AgCl wire into the carbon paste. The internal solution used for EPME was 0.1 mol/L KCl. All the sensors tips were gently rubbed on fine abrasive paper to produce a flat surface. The surface of the sensors was wetted with de-ionized water and then polished with an alumina paper (polished strips 30144-011, Orion) before use for the analysis. When not in use, the electrodes were immersed in a 10^{-3} mol/l L- or D-glyceric acid solution, respectively.

7.5.3 Recommended procedure

Direct potentiometric method was used for potential determination of each solution (10^{-10}-10^{-2} mol/l). Calibration graphs were obtained by plotting E(mV) versus p(L-GA) or p(D-GA), respectively. The unknown concentrations were determined from the calibration graphs.

7.5.4 Results and discussion

7.5.4.1 Response characteristics of EPMEs

The response characteristics of EPMEs were determined for both enantiomers, L-glyceric (L-GA) and D-glyceric acid (D-GA) at pH=3.5 (phosphate buffer). The response obtained for L-GA was linear and near-Nernstian only when vancomycin was used as chiral selector for the design of EPME, while response obtained for D-GA was linear and near-Nernstian only when teicoplanin based EPME was used. The equations of calibration obtained for L-GA and D-GA when vancomycin and teicoplanin based EPME were used, respectively, are as follows:

L-glyceric acid: $E = 574.6\text{-}58.6\ p$ (L-GA) $\quad r = 0.9957$

D-glyceric acid: $E = 206 - 50\ p$ (D-GA) $\quad r = 0.9988$

where E (mV) is the cell potential, p (L-GA) = -log [L-GA] and p (D-GA) = -log [D-GA]. The response characteristics of the two EPMEs are shown in Table 7.11. The limits of detection are very low of 10^{-10} and 10^{-5} mol/L orders of magnitude for L-glyceric acid and D-glyceric acid, respectively. 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 L-glyceric acid is higher than 2 min in the 10^{-9} – 10^{-7} mol/L concentration range, while for D-glyceric acid is lower than 2 min in the 10^{-4}-10^{-2} mol/L concentration range.

Table7.11 Response characteristics of enantioselective, potentiometric membrane electrodes for L-and D-glyceric acid [a]

Analyte	Parameters			
	Slope (mV/decade)	Intercept, E^o (mV)	Linear range (mol/L)	Detection limit (mol/L)
L-glyceric acid	58.6(mV/*p*L-GA)	574.6	10^{-9}-10^{-7}	$1.56x10^{-10}$
D-glyceric acid	50.00(mV/*p*L-GA)	206	10^{-4}-10^{-2}	$7.60x10^{-5}$

[a] All measurements were made at room temperature; all values are the average of ten determinations.

7.5.4.2 The influence of pH on the responses of the EPMEs

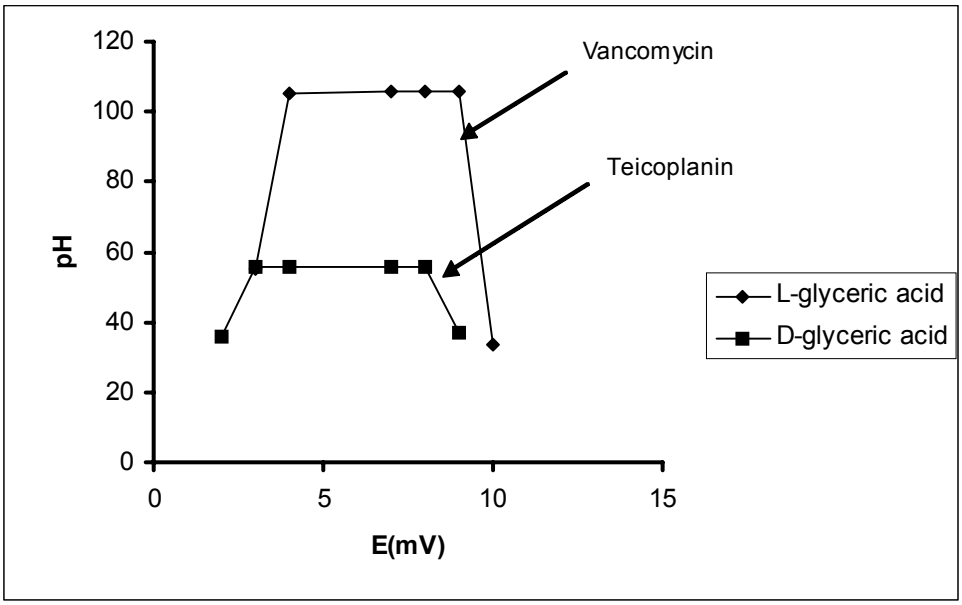

Figure 7.5 Effect of pH on the response of the EPMEs to L-glyceric acid (10^{-8} mol/l L-GA), and D-glyceric acid (10^{-3}mol/l) solutions. (I) Vancomycin based EPME; (II) Teicoplanin based EPME

The effect of pH on the response of the electrodes was checked by recording the emf of the cell. Solutions of L-(10^{-8} mol/L) and D-(10^{-3}mol/L) glyceric acids having different pHs were prepared by adding small volumes of HCl (0.1 mol/L) or NaOH solution (0.1 mol/L). Plots of E (mV) vs. pH (Figure 7.5) show that the emf does not depend on pH in the ranges of 4.0-9.0 for vancomycin and 3.0-8.0 teicoplanin based EPMEs, respectively.

7.5.4.3 Selectivity of the EPMEs

The selectivity of both electrodes was checked using the mixed solutions method proposed by Ren [32], over L-(or D)-glyceric acid, creatine, creatinine and some inorganic ions. The ratio between the concentrations of interfering ion and enantiomer was 10:1. The potentiometric selectivity coefficients (Table 7.12) for vancomycin and teicoplanin based EPMEs proved that L(D)-GA, creatine and creatinine do not interfere in the determination of L- and D-GA demonstrating the enantioselectivity property of the EPMEs. Inorganic cations such a Na^+, K^+, and Ca^{2+} do not interfere in the analysis of L- and D-GA.

Table 7.12 Selectivity coefficients for the response of the enantioselective membrane electrodes used for L- and D-glyceric acid assay.[a]

Interference species (J)	pK^{pot}_{sel}	
	Vancomycin based EPME	Teicoplanin based EPME
L-glyceric acid	-	2.39
D-glyceric acid	2.415	-
Creatine	2.086	2.08
Creatinine	2.41	2.39

[a]All measurements were made at room temperature; all values are the average of ten determinations.

7.5.4.4 Analytical applications

Solutions containing L- and D-GA in different ratios were prepared to test the recovery for each enantiomer and the suitability of the EPMEs for the enantioanalysis of L- and D-GA in serum and urine samples. The results of recovery for enantioanalysis of each enantiomer in the presence if it antipode (Tables 7.13 and 7.14) proved the suitability of the electrodes. 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 7.13 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 room temperature; all values are the average of ten determinations.

Table 7.14 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 room temperature; all values are the average of ten determinations.

Table 7.15 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 room temperature; 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 7.15 and 7.16, respectively. Serum and urine samples were

collected from patients suspected of L-glyceric academia (1-3) or aciduria (4-9) and D-glyceric acidemia (10-12) or aciduria (13-18). The results obtained using the proposed EPMEs is in good concordance with those obtained using the standard method [34]

Table 7.16 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	97.7.0	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 room temperature; all values are the average of ten determinations.

7.6 Conclusion

The proposed enantioselective, potentiometric membranes electrodes have excellent features in the real time enantioselective analyses of glyceric acid in biological fluids, e.g., serum and urine samples. The construction of the electrodes is simple, fast and reproducible. One of the main advantages of the proposed method is that the serum and urine samples need only a minimum of pre-treatment before the assay of any of the enantiomers that makes the method simple, fast and economical.

EPMEs based on maltodextrin I, maltodextrin III, α-CD, γ-CD and vancomycin were applicable for the determination of L-glyceric acid while EPMEs based on maltodextrin

II, β-CD, 2-hydroxy-3-trimethylammoniopropyl-β-CD and teicoplanin were used for the analysis of D-glyceric acid. These EPMEs describe a reliable direct method for the analysis of L- and D-glyceric acids in real human fluids (serum and urine). EPMEs exhibited a good enantioselectivity over D- or L-glyceric acid, creatine , creatinine and inorganic ions.

For L-glyceric acid determination, α-CD based EPME has the best slope and the lower limit of detection, while 2-hydyroxy-3-trimethylammoniopropyl-β-CD has the best slope and the lower limit of detection. All the proposed EPMEs can be used successfully for fast and reliable diagnosis of L- or D-glyceric acidemia/aciduria. Miniaturization of the electrodes will make possible *in vivo* diagnosis of hyperoxaluria type 2 (PH II) and D-glyceric acidemia/acidurias

7.7 References

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