# Chapter 9

Enantioanalysis of L-vesamicol in serum samples using enantioselective, potentiometric membrane electrodes

9.1 Introduction

Human metabolism is involved in the production of both or only one of the enantiomers due to enzymatic deficiencies. Therefore, it is important to develop efficient methods for the enantioanalysis of chiral compounds of biological importance [1,2]. Biological activity differences of enantiomers arise due to differences between protein binding and transport.

L-vesamicol is a tertiary amine [L-(trans)-(-)-2-(4-phenyl-1-piperidinyl-cyclohexanol, Figure 9.1] and it is a potent, non-competitive inhibitor of acetylcholine uptake into the vesicles of cholinergic neurons [3-6]. It binds to an allosteric site on the specific vesicular acetylcholine transporter complex [7]. The transporter protein, which is responsible for the transfer of acetylcholine into synaptic vesicles, is located in cholinergic neurons. L-Vesamicol is potentially suitable for the study of presynaptic cholinergic mechanisms. In Alzheimer's disease, the degeneration of cholinergic neurons in brain is a significant neuropathological feature. The binding selectivity of L-vesamicol to the vesicular acetylcholine transporter could be used as a hallmark for diagnosis and follow up of degenerative disease [8]. L-Vesamicol was reported to exhibit high affinity, especially to σ-1 and σ-2 receptors, that are found in different parts of the central nervous system [9,10].

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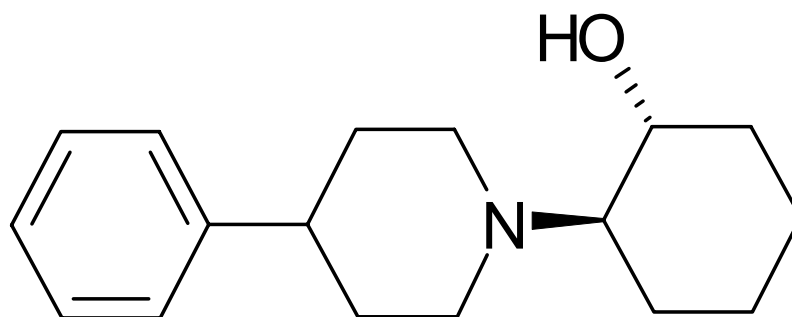
Figure 9.1 L-(-)-vesamicol

Acetylcholine, a neurotransmitter, stored by nerve terminals inside of synaptic vesicles is found in the cytoplasmic compartment. Stimulation of the nerve terminals effects excocytotic release of synaptic vesicle content into the synaptic gap and results in quantal transynaptic communication [11]. Cholinergic reduction in cerebral cortex and limbic brain may contribute to the cognitive impairments of patients with Alzheimer's disease and a disturbance in cholinergic neurotransmission [12,13], Parkinson's disease with dementia [14], Down's Syndrome [15] and olivopontocerebellar atrophy [16]. Since the vesicular storage of neurotransmitter is required for evoked release, factors that affect this storage are important to neurotransmission.

Very few methods have been used for the assay of L-vesamicol. Glidersleeve *et. al*. described direct resolution of vesamicol and its analogues by high performance liquid chromatography [17]. Zhou and Stewart determined vesamicol enantiomers in human serum by capillary electrophoresis using sulfated-β-cyclodextrin [18]. Hefnawy and Aboul-Enein described a validated liquid chromatographic method for the determination of vesamicol enantiomers in human plasma using vancomycin as chiral stationary phase [19].

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This chapter reports the applications of ten enantioselective, potentiometric membrane electrodes based on cyclodextrins (α-, β-, γ-, and 2-hydroxy-3-trimethylammoniopropyl-β-derivative-CD), maltodextrins I, II and III with different DE values [I (4.0-7.0), II (13.0-17.0) and III (16.5-19.5)], and macrocyclic antibiotics (vancomycin and teicoplanin) as chiral selectors for the determination of L-vesamicol in serum samples. The proposed EPMEs were applied for the assay of L-vesamicol in serum samples.

9.2 Reagents and materials

L-vesamicol, 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 = 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 preparations. A 0.1mol/L stock solution of L- vesamicol was buffered with phosphate buffer (pH 5). Solutions of L-vesamicol (1×10^{-10} - 1×10^{-2} mol/L) were prepared by serial dilutions from the stocks solution of L- vesamicol and were buffered with phosphate buffer (pH 5).

10^{-3} mol/L solutions of each maltodextrin (I, II and III), and each cyclodextrin (α-, β-, γ-, and 2-hydroxy-3-trimethylammoniopropyl-β-CD) were prepared. The solution of

vancomycin (2×10^{-3} mol/L) was prepared in phosphate buffer (pH 4.00). The solution of teicoplanin (2×10^{-3} mol/L) was prepared using pH 6.00 phosphate buffer. The solution of teicoplanin (2×10^{-3} mol/L) containing acetonitrile was prepared using pH 6.00 phosphate buffer containing 40% (v/v) of acetonitrile.

9.3 Enantioselective, potentiometric membrane electrodes based on maltodextrins

9.3.1 Equipments and apparatus

 All direct potentiometric measurements were recoded using a Metrohm 663 VA stand (Metrohm, Herisau, Switzerland) connected to a PGSTAT 100 computer-controlled potentiostat (Eco Chemie, Ultrech, The Netherlands) and software version 4.9. The electrochemical cell is a two-electrode compartment, consists of enantioselective, potentiometric membrane electrode (carbon paste electrode modified with the maltodextrins) and reference electrode (Ag/AgCl electrode).

9.3.1 Electrodes design

20 μL paraffin oil and 100 mg graphite powder were mixed followed by the addition of 100 μL of aqueous solution of maltodextrin {DE 4.0-7.0 (I), 13.0-17.0 (II), and 16.5-19.5 (III)}, (100 μL of chiral selector/100 mg carbon paste). Plain carbon paste was prepared by mixing graphite powder and paraffin oil in a ratio 4:1 (w/w). A certain quantity of carbon paste, free of chiral selector, was filled in a plastic pipette peak, leaving 3-4 mm in the top to be filled with the modified carbon paste. The diameter of all the EPMEs was 3 mm. Electric contact was obtained by inserting

silver wires into the carbon paste. The internal solution was 0.1 mol/L KCl. The entire electrode surface was gently rubbed on fine abrasive paper to produce a flat surface. The surface of the electrode was wetted with de-ionized water, refreshed with modified carbon paste and then polished with an alumina paper (polished strips 30144-011, Orion) before use for the analysis. When not in use, EPMEs were immersed in 10^{-3} mol/L of L-vesamicol solution.

9.3.3 Recommended procedure

Direct potentiometric method was employed for the measurement of potential variation, E (mV), over different standard solution concentrations (10^{-10} -10^{-2} mol/L) of L-vesamicol. The electrodes were immersed in the stirred standard solutions for assay. Calibration graphs were drawn by plotting E (mV) versus p(L-vesamicol). The same technique is used to record the produced potential for the spiked serum samples. Unknown concentrations of L-vesamicol in serum samples were determined from the calibration graphs.

9.3.4 Results and discussion

9.3.4.1 EPMEs response characteristics

The equations of calibration and correlation coefficients (r) obtained for L-vesamicol using maltodextrin (I), maltodextrin (II) and maltodextrin (III) based electrodes, are as follows:

Maltodextrin I: E(mV) = 206.8 -55.00 p (L-vesamicol)   r = 0.9834

Maltodextrin II: E(mV) = 294.7 -55.60 p (L-vesamicol)   r = 0.9913

Maltodextrin III: E(mV) = 483.33 -51.00 p (L-vesamicol)   r = 0.9999

The response characteristics exhibited by the three EPMEs impregnated with maltodextrins were determined for L-vesamicol at pH 5.0 (phosphate buffer). All the response characteristics are summarized in Table 9.1. All EPMEs exhibited near-Nernestian slope (in the range 51 to 55.6 mV/decade of concentration of L-vesamicol). EPME based on maltodextrin (III) exhibited the lowest detection limit of 10^{-10} mol/L magnitude order.

**Table 9.1** Response characteristics of EPMEs for the assay of L-vesamicol [a]

Chiral selector	Parameters			
	Slope (mV/decade)	Intercept, E^o (mV)	Linear concentration range (mol/L)	Detection limit (mol/L)
Maltodextrin I	-55.00 mV/p(L-ves)	206.80	10^{-4}-10^{-2}	1.34×10^{-5}
Maltodextrin II	-55.60 mV/p(L-ves)	294.70	10^{-5}-10^{-3}	5.00×10^{-6}
Maltodextrin III	-51.00 mV p(L-ves)	483.33	10^{-9}-10^{-7}	3.33×10^{-10}

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

The best response time was recorded for the maltodextrin I and II based EPMEs: less than 1 min for the concentration range 10^{-5}-10^{-2} mol/L and higher than 1 minute for the concentration range 10^{-10}-10^{-6} mol/L. The response time for maltodextrin III was higher than 2 min in the concentration range 10^{-9}-10^{-7} mol/L and 2 min in the concentration range 10^{-6}-10^{-2} mol/L.

9.3.4.2 Effect of pH on the responses of the electrodes

L-vesamicol solutions of different pH values were prepared by adding small aliquots of HCl (0.1 mol/L) or NaOH solution (0.1 mol/L) to a L-vesamicol solution. The effect of pH variation on the recorded potentials of EPMEs is shown in the Figure 9.2. The responses of EPMEs of L-vesamicol are pH-independent in the pH ranges 3.0-6.0 for maltodextrin I-based electrode, 6.0-10.0 for maltodextrin II-based electrode and 3.0-5.0 for maltodextrin III-based electrode.
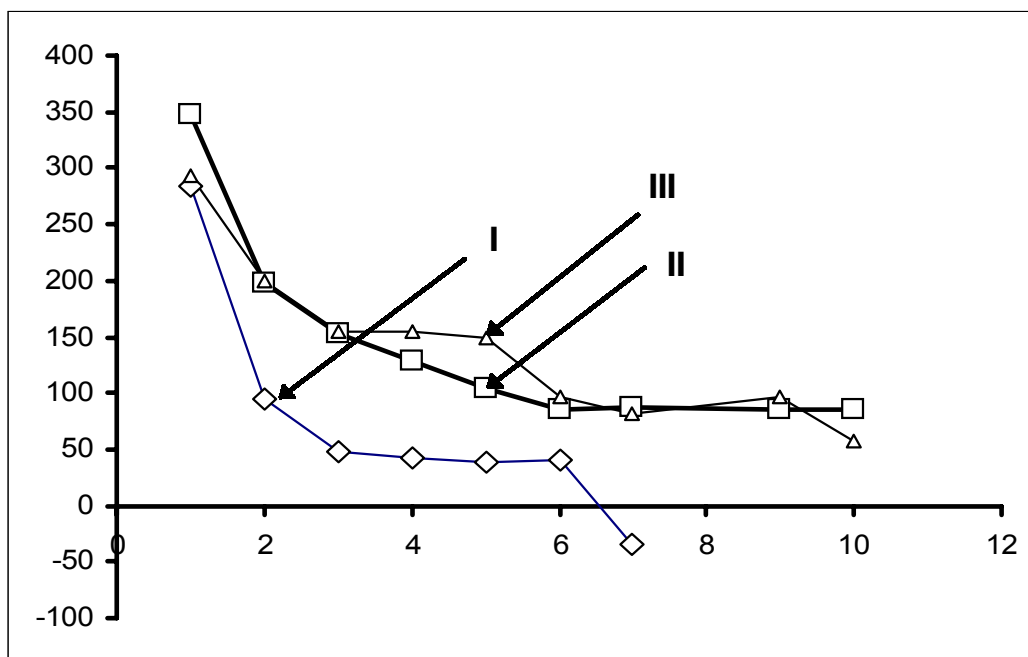
Figure 9.2 Influence of pH variation on the response of: I, EPME based on maltodextrin (I) (10^{-4} mol/L L-vesamicol); II, EPME based on maltodextrins (II) (10^{-4} mol/L L-vesamicol); and III, EPME based on maltodextrins (III) (10^{-8} mol/L L-vesamicol).

9.3.4.3 Selectivity of the electrode

The selectivity of the three electrodes have investigated using the mixed solutions method proposed by Ren [20]. The selectivity of L-vesamicol assay was tested over creatine, creatinine and some inorganic ions. The ratio between the concentrations of L-vesamicol and the imposed interfering ions was 1:10. The values for potentiometric selectivity coefficients, K_{sel}^{pot}, of maltodextrins I-, II- and III-based electrodes proved that creatine and creatinine do not interfere in the determination of L-vesamicol (Table 9.2). Inorganic cations such a Na^+, K^+, and Ca^{2+} do not interfere in the analysis of L- vesamicol.

Table 9.2 Potentiometric selectivity coefficients of the enantioselective, potentiometric membrane electrodes [a]

Chiral selector	pK_{sel}^{pot}	
	Interference species (J)	
	Creatine	Creatinine
Maltodextrin I	2.37	2.06
Maltodextrin II	2.10	2.347
Maltodextrin III	2.34	2.025

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

9.3.4.4 Analytical applications

Ten serum samples were donated form healthy volunteers and stored at -20°C. Samples are spiked with L-vesamicol. The results obtained for the analysis of L– vesamicol in serum sample using EPMEs based on maltodextrins I, II, and III are summarized in Table 9.3. The results obtained using the proposed EPMEs are in good concordance with the spiked values of L-vesamicol in serum samples.

Table 9.3 Recovery of L-vesamicol in serum samples using EPMEs based on maltodextrin I, II and III [a]

Sample no.	Concentration of L-vesamicol (ng/L)	% Recovery, L-vesamicol Maltodextrin III	Concentration of L-vesamicol (µg/L)	% Recovery, L-vesamicol	
				Maltodextrin I	Maltodextrin II
1	15.54	99.23±0.01	1.554	99.22±0.02	99.72±0.01
2	20.72	98.50±0.01	2.072	99.13±0.03	99.22±0.04
3	25.90	99.83±0.01	2.590	99.44±0.05	99.82±0.01
4	31.08	99.67±0.01	3.108	99.54±0.01	99.44±0.01
5	36.26	99.27±0.02	3.626	99.50±0.03	99.28±0.01

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

9.4 Cyclodextrins based enantioselective, potentiometric membrane electrodes

9.4.1 Apparatus

All measurements were carried out using a Metrohm 663 VA Stand (Metrohm, Herisau, Switzerland) connected to a PGSTAT 100 computer-controlled potentiostat (Eco Chemie, Ultrech, The Netherlands) and software version 4.9. The electrochemical cell consisted of enantioselective, potentiometric membrane electrode (carbon paste electrode modified with the cyclodextrin) and a reference electrode (Ag/AgCl electrode).

9.4.2 Electrodes Design

Paraffin oil and graphite powder were mixed thoroughly in the ratio 1:4, followed by the addition of aqueous solution of α-, β-, γ- or 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrins, (100 μl of chiral selector/100 mg carbon paste). Plain carbon paste was prepared by mixing 100mg of graphite powder with 40 μl paraffin oil. The plain carbon paste was filled into a plastic pipette peak leaving a space of 3-4 mm into the top of the peak to be filled with the modified carbon paste that contains the cyclodextrin. The diameter of the enantioselective, potentiometric membrane electrodes was 3 mm. Electric contact was obtained by inserting a silver/silver chloride wire into the carbon paste. The internal electrolyte solution was 0.1 mol/L KCl. The surface electrode was gently rubbed on fine abrasive paper to produce a flat surface. The surface of the electrode was also wetted with deionized water, refreshed with modified carbon paste and then polished with an alumina paper

(polished strips 30144-011, Orion) before each use. When not in use, EPMEs were immersed in 10^{-3} mol/L of L-vesamicol solution.

9.4.3 Recommended procedure

Direct potentiometry was employed for the measurement of potentials variation for different standard solution of L-vesamicol (10^{-10} -10^{-2} mol/L , pH 5.0) and samples. Calibration graphs were obtained by plotting E (mV) versus p[L-vesamicol]. The potentiometric technique was used for the analysis of L-vesamicol in the serum samples. The unknown concentrations of L-vesamicol in serum samples were calculated from the calibration graphs.

9.4.4 Results and discussion

9.4.4.1 EPMEs response characteristics

The response characteristics exhibited by the EPMEs impregnated with cyclodextrins were determined for L-vesamicol at pH = 5.0. The response obtained for L-vesamicol was near-Nernstian and linear for α-, β-, 2-hydroxy-3-trimethylammoniopropyl-β- and γ-cyclodextrins based EPMEs with correlation coefficients (r) 0.9694, 0.9990, 0.9844 and 0.9985, respectively. The linear concentration ranges obtained are 10^{-9}-10^{-7} mol/L for EPMEs based on α-, β- and 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrins and 10^{-5}-10^{-2} mol/L for γ-cyclodextrin based EPME. The equations of calibration are as follows:

EPME based on α-cyclodextrin: E(mV) = 529.33 -57.00 p (L-vesamicol)

 EPME based on β-cyclodextrin: E(mV) = 562.50 -58.50 p (L-vesamicol)

EPME based on γ-cyclodextrin: E(mV) = 268.00 -52.50 p (L-vesamicol)

EPME based on 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrins -cyclodextrin:

$$E(mV) = 512.67 - 55.00 \ p \ (L\text{-vesamicol})$$

where E (mV) is the cell potential, p(L-vesamicol) = -log[L-vesamicol]. The limits of detection obtained for the analysis of L-vesamicol are 5.70×10^{-10}, 2.42×10^{-10}, 4.77×10^{-10} and 7.86×10^{-6} mol/L when α-, β-, 2-hydroxy-3-trimethylammoniopropyl-β- and γ-cyclodextrins based EPMEs were used. The electrode based on β-cyclodextrin exhibited the lowest limit of detection, while electrode based on γ-cyclodextrin has the highest limit of detection. This may be explained by the excellent recognition power of β-cyclodextrin for chiral molecules containing a substituted aromatic ring [21]. The electrodes responses displayed a good stability and reproducibility for the tests performed for 3 weeks, when daily used for measurements (RSD<0.1%).

The best response time was recorded for β-cyclodextrin based EPME where the electrode potential was recorded in 26 seconds for the concentration range 10^{-9}-10^{-7} mol/L, while the response time recorded for the assay of L-vesamicol, EPME based on β-derivative-cyclodextrin, was one minute for the same concentration range. The response time for α- and γ-cyclodextrins based electrodes was 2 min in the concentration range 10^{-9}-10^{-7} mol/L and 10^{-5}-10^{-2} mol/L, respectively.

9.4.4.2 Influence of pH on the responses of the electrodes

Influence of pH on the response of the electrodes was tested by recording the emf of the cells, containing solutions of L-vesamicol at different pH values varied for 1 to 10. These solutions were prepared by adding small volumes of HCl or NaOH solution (10^{-1} mol/L or 1 mol/L of each) to a L-vesamicol solutions. The influence of pH

variation on the responses of EPMEs is shown in Figure 9.3. The responses of the electrode over L-vesamicol are pH-independent in the pH ranges 4.0-9.0, 5.0-8.0, 3.0-6.0 and 3.0-10.0 for electrodes based on α-, γ-, β-, and 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrins, respectively.
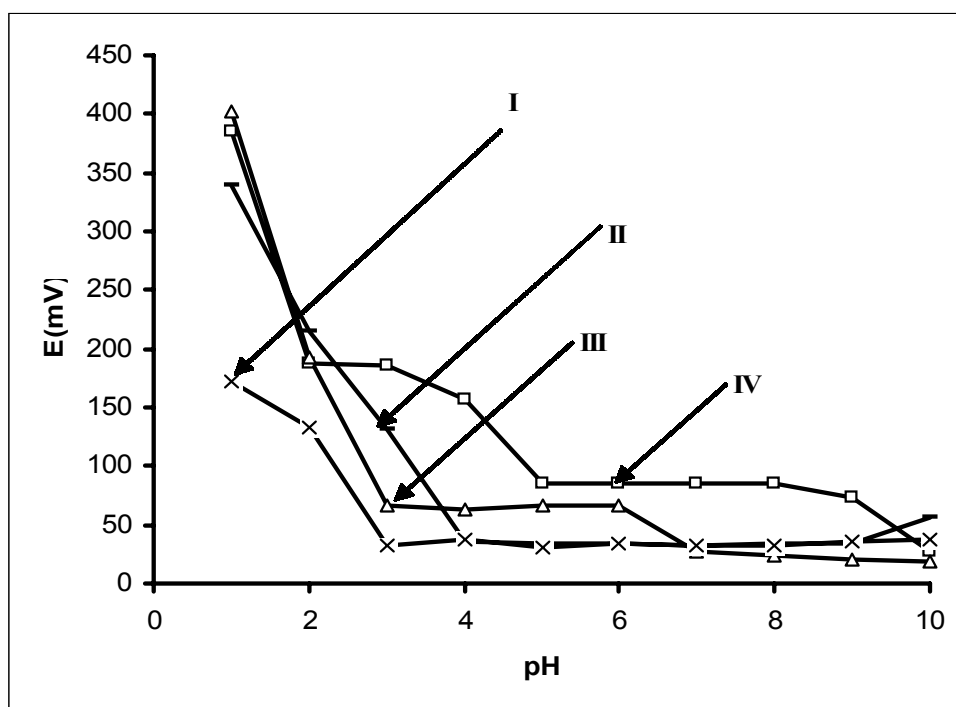
Figure 9.3 The influence of pH on functioning of the electrodes based on (I) 2-hydroxy-3-trimethylammoniopropyl-β- (II) α-cyclodextrin, (III) β-cyclodextrin ($C_{L-vesamicol} = 10^{-8}$ mol/) and (IV) γ-cyclodextrin ($C_{L-vesamicol} = 10^{-4}$ mol/l).

9.4.4.3 Selectivity of the electrode

The selectivity of the four electrodes was investigated using the mixed solutions method [32]. The selectivity of the proposed EPMEs was checked over creatine, creatinine, and some inorganic ions. The ratio between concentrations of L-vesamicol and interfering species was 1:10. The values of potentiometric selectivity coefficients, K_{sel}^{pot}, (Table 9.4) determined for electrodes based on α-, γ-, β-, and 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrins proved that creatine and creatinine do not interfere in the determination of L-vesamicol. The interference of inorganic cations

was checked for all proposed EPMEs, all K_{sel}^{pot} values are lower than 10^{-3} shown that

these ions do not interfere in the analysis of L- vesamicol.

Table 9.4 Selectivity coefficients K_{sel}^{pot}, of the enantioselective, potentiometric membrane electrodes.[a]

EPME based on	K_{sel}^{pot}	
	Interfering species (j)	
	Creatine	Creatinine
α-cyclodextrin	4.28×10^{-3}	4.10×10^{-3}
β-cyclodextrin	4.02×10^{-3}	3.86×10^{-3}
2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrins	3.96×10^{-3}	4.12×10^{-3}
γ cyclodextrin	4.48×10^{-3}	4.29×10^{-3}

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

9.4.4.5 Analytical applications

Table 9.5 Determination of L-vesamicol in serum samples using EPMEs based on α-, β- 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin [a]

Sample no.	Concentration of L-vesamicol (ng/L)	% Recovery, L-vesamicol		
		α-cyclodextrin	β- cyclodextrin	2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrin
1	8.88	99.50±0.02	99.86±0.02	99.22±0.02
2	11.84	99.41±0.01	99.93±0.01	99.13±0.01
3	14.80	99.36±0.01	99.90±0.01	99.44±0.04
4	17.76	99.74±0.01	99.72±0.02	99.54±0.02
5	20.72	99.22±0.01	99.89±0.01	99.50±0.02

Sample no.	Concentration of L-vesamicol (mg/L)	% Recovery, L-vesamicol
		γ-cyclodextrin
6	8.88	99.72±0.03
7	11.84	98.56±0.02
8	14.80	99.42±0.02
9	17.76	99.57±0.02
10	20.72	99.51±0.02

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

Ten human serum samples were donated form different healthy volunteers and stored at -20°C. The serum samples were spiked with L- vesamicol. The results obtained for the analysis of L–vesamicol in serum samples using EPMEs based on α-, β-, and 2-hydroxy-3-trimethylammoniopropyl-β-cyclodextrins,   and   γ-cyclodextrin   are summarized in Table 9.5. The concentration of L-vesamicol calculated from results obtained by using the proposed EPMEs are in good concordance with the quantities of L-vesamicol in real samples, and proved the suitability of the electrodes for the proposed electrodes for the determination of L-vesamicol in serum samples.

9.5 Enantioselective, potentiometric membrane electrodes based antibiotics

9.5.1 Apparatus

A 663 VA stand (Metrohm, Herisau, Switzerland) connected to a PGSTAT 100 computer-controlled potentiostat (Eco Chemie, Ultrech, The Netherlands) and software 4.9 was used for all measurements. An Ag/AgCl electrode was used as reference electrode in the cell.

9.5.2 EPMEs Design

Plain carbon paste was prepared by mixing graphite powder and paraffin oil in a ratio 4:1 (w/w). Modified carbon pastes were prepared by the addition of 100 μl of 10^{-3} mol/L solution of each antibiotic (vancomycin or teicoplanin or teicoplanin with acetonitrile), to 100 mg of the plain carbon paste. A certain quantity of carbon paste, free of chiral selector, was filled in a plastic pipette peak, leaving 3-4 mm in the top to be filled with the modified carbon paste containing the chiral selector. The diameter

of all the EPMEs was 3 mm. Electric contact was obtained by inserting silver wires into the carbon paste. The internal solution was 0.1 mol/L KCl.

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

9.5.3 Recommended procedure

Direct potentiometric method was employed for the measurement of potential (E (mV)) of each L-vesamicol standard solution (10^{-10} -10^{-2} mol/L) and serum sample. Calibration graphs were obtained by plotting E (mV) versus p(L-vesamicol). Unknown concentrations of L-vesamicol in serum samples were determined from the calibration graphs.

9.5.4 Results and discussion

9.5.4.1 The response characteristics of EPMEs

 The presence of several functional groups in the glycopeptides antibiotics contribute to enantiorecognition through hydrogen bonding, charge-charge, hydrophobic and steric interactions [22]. The calibration equations obtained for L-vesamicol are:

$$E(mV) \quad = 386.83 – 55.50 \text{ p(L-vesamicol),} \quad r = 0.9929 \qquad (A)$$

$$E(mV) \quad = 402.00 - 57.30 \text{ p(L-vesamicol),} \quad r = 0.9881 \qquad (B)$$

$$E(mV) \quad = 399.58 – 53.66 \text{ p(L-vesamicol),} \quad r = 0.9870 \qquad (C)$$

where E(mV) is the potential, p(L-vesamicol) = -log[L-vesamicol], r is the correlation coefficient, and A, B, and C are indicating the EPMEs based on vancomycin, teicoplanin and teicoplanin modified with acetonitrile. The response characteristics exhibited by the EPMEs based on different types of antibiotics (vancomycin, teicoplanin and teicoplanin & acetonitrile) for the determination of L-vesamicol are summarized in Table 9.6. Proposed EPMEs exhibited linear and near-Nernstian responses between 53.0-58.0 mV per decade of L-vesamicol concentration with correlation coefficients of 0.9929, 0.9881 and 0.9870 for vancomycin, teicoplanin and teicoplanin modified with acetonitrile, respectively. The EPME based on teicoplanin modified with acetonitrile exhibited the wider linear concentration range and the lowest detection limit. These results may explained as organic modifiers are altering and/or inhibiting the aggregation of the teicoplanin monomers making more teicoplanin molecules available to interact with the enantiomer (L-vesamicol) [22].

**Table 9.6** Response characteristics of EPMEs based on antibiotics for L-vesamicol [a]

EPME based on	Parameters			
	Slope (mV/decade of conc.)	Intercept, E^o (mV)	Linear conc. range (mol/L)	Detection limit (mol/L)
Vancomycin	-55.50	386.83	10^{-6}-10^{-4}	1.07×10^{-7}
Teicoplanin	-57.30	402.0	10^{-6}-10^{-3}	9.60×10^{-8}
Teicoplanin modified with acetonitrile	-53.66	399.58	10^{-7}-10^{-2}	3.60×10^{-8}

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

For all the proposed EPMEs, the response time was less than 1 minute for the concentration range 10^{-6}-10^{-2} mol/L and more than 1 minute for 10^{-10}-10^{-7} mol/L**.** The response show good stability and reproducibility for tests performed for more than 1 month, when used daily (RSD < 1%).

9.5.4.2 Effect of the pH on the response of EPMEs
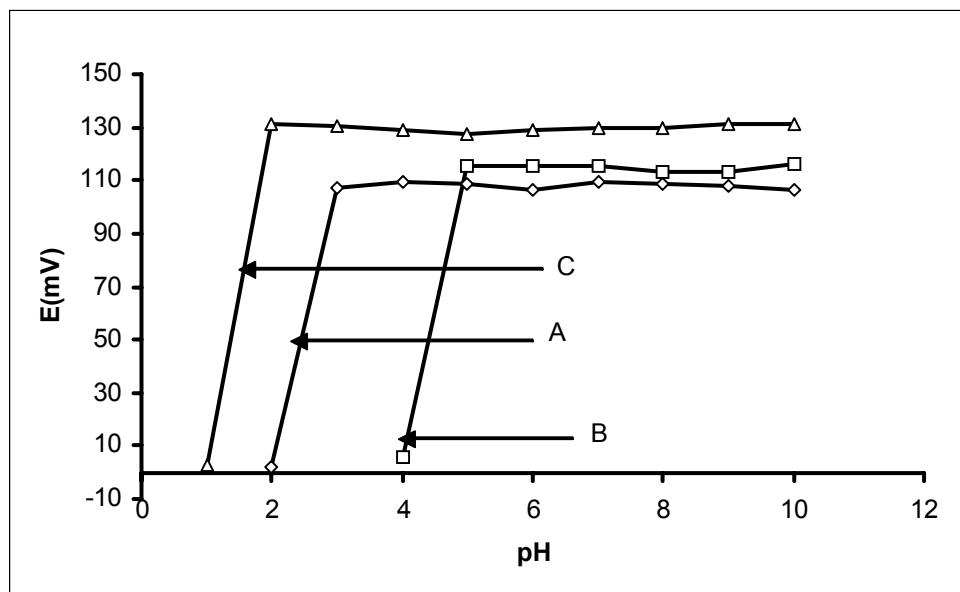
Figure 9.4 The influence of pH on the response of EPMEs for the determination of L-vesamicol (10^{-5} mol/L). (A) EPME based on vancomycin, (B) EPME based on teicoplanin and (C) EPME based on teicoplanin modified with acetonitrile.

The effect of pH variations on the response of the propped electrodes was checked for the assay of L-vesamicol in a pH range 1-10. The L-vesamicol concentration was 10^{-5} mol/L. In figure 9.4, the plot shows the variation of E(mV) with the pH. The response of EPME based on teicoplanin modified with acetonitrile does not depend on the pH value on the pH range 2.0-10.0, while the response of EPMEs based on teicoplanin and vancomycin are independent on pH on the pH ranges 5.0-10.0 and 3.0-10.0.

9.5.4.3 Selectivity of the Electrodes

The selectivity of all EPMEs was investigated using the mixed solution method. The concentrations of L-vesamicol and interfering ions were 10^{-5} and 10^{-4} mol/L, respectively. The selectivity was investigated over creatine, creatinine and inorganic cations such as Na^+, K^+ and Ca^{2+}. The selectivity coefficients for the enantioselective, potentiometric membrane electrodes, K_{sel}^{pot}, obtained are summarized at Table 9.7. The

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K_{sel}^{pot} values proved that the constructed EPMEs are selective over creatine and creatinine. D-vesamicol is also not interfering proving the enantioselectivity property of the electrodes (K_{sel}^{pot} <10^{-4}). For Na^+, K^+ and Ca^{2+}, the K_{sel}^{pot} is less than 10^{-4}, accordingly they do not interfere in the assay of L-vesamicol.

Table 9.7 Selectivity coefficients of EPMEs.[a]

Interference species (J)	PK_{sel}^{pot}		
	Vancomycin	Teicoplanin	Teicoplanin modified with acetonitrile
Creatine	4.06×10^{-3}	3.94×10^{-3}	4.38×10^{-3}
Creatinine	4.24×10^{-3}	4.10×10^{-3}	4.20×10^{-3}

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

9.5.4.4 Analytical Applications

The electrodes proved to be useful for the assay of L-vesamicol in serum samples. The results recorded for the assay of L-vesamicol in serum samples are shown in Table 9.8. These results are in good concordance with those obtained using the method proposed by Gildersleeve, *et al* [29], proving the suitability of these EPMEs for the enantioanalysis of L-vesamicol in serum sample. .

Table 9.8 Recovery of L-vesamicol in serum samples [a]

Sample no.	Electrophoresis method [29] (µg/L)	Recovery, % L-vesamicol		
		EPME based on		
		Vancomycin	Teicoplanin	Teicoplanin modified with acetonitrile
1	15.54	99.41±0.04	99.62±0.02	99.49±0.02
2	20.72	99.97±0.05	99.66±0.02	98.73±0.03
3	25.90	99.68±0.02	99.72±0.03	99.80±0.01
4	31.08	99.84±0.02	99.78±0.03	99.78±0.01
5	36.26	99.61±0.06	99.66±0.02	99.83±0.01

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

9.6 Conclusions

Utilization of EPMEs have several advantages over chromatographic methods such as low cost, no or very simple sample preparation, short time of analysis and simplicity of analysis. The proposed enantioselective, potentiometric membrane electrodes based on maltodextrins (I, II and III) showed excellent features for enantioselective analysis of L-vesamicol. The maltodextrin (III)-based electrode exhibited the lowest detection limits (3.33×10^{-10} mol/L) compared to maltodextrin (I)- and (II)-based electrodes. The proposed electrodes showed good selectivity and proved to be reliable for the direct determination of L-vesamicol in serum samples, without any special pre-treatment before the analysis.

The proposed enantioselective, potentiometric membrane electrodes based on cyclodextrins were successfully applied for the determination of L-vesamicol in human serum samples. These electrodes proved to be reliable for the direct determination of L-vesamicol in real human serum samples.

The proposed enantioselective, potentiometric membrane electrodes based on antibiotics can be successfully used for the enantioselective analysis of L-vesamicol. EMPE also has some advantages over other techniques, such as high precision, rapidity, and low cost of analysis. The construction of these electrodes is simple, fast and reproducible. If miniaturized, these electrodes can be applied for *in vivo* diagnosis patients suffering of Alzheimer and Parkinson diseases.

9.7 References

1. S. Fanali, P. Catarcini, C. Presutti, M. Quaglia, and P. G. Righetti, Electrophoresis, 24, (2003), 904.

2. R. I. Stefan, J. F. van Staden and H .Y. Aboul-Enien, Electrochemical Sensors in Bioanalysis, Marcel Dekker, New York, 2001.

3. B. A. Baher and S. M. Parsons, Proc. Natl. Acad. Sci. U.S.A., 83, (1986), 2267.

4. G. A. Rodgers, S. M. Parsons, D. C. Anderson, L. M. Nilsson, B. A. Bahr, W. D. Kaufman, B. S. Jacobs and B. J. Kirtman, Med. Chem., 32, (1989), 1217.

5. I. G. Marshal, S. M. Parsons and C. Alter, C. Natl. Acad. Sci. U.S.A., 84, (1987), 876.

6. C. A. Altar and M. R. Marien, Synapse, 2, (1988), 486.

7. B. A. Bahr, E. D. Clarkson, G. A. Rogers, K. J. Noremberg and S. M. Parsons, Biochemistry, 31, (1992), 5752.

8. M. J. Scheunemann, D. Sorger, B. Wenzel, K. Heinitz, R. Schliebs, M. Klingner, O. Sabri, and J. Steinbach, Bioorg. Med. Chem., 12, (2004), 1459.

9. G. A. Rogers, S. Stone-Elander, M. Ingvar, L. Eriksson, S. M. Parsons and L. Widen, Nucl. Med. Biol., 21, (1994), 219.

10. R. H. Mach, M. L. Voytko, R. L. E. Ehrenkaufer, M. A. Nader, J. R, Tobin, S. M. N. Efange, S. M. Parsons, H. D. Gage, C. R. Smith, and T. E. Morton, Synapse, 25, (1997), 368.

11. L. F. Reichard and R. B. Kelly, Anu. Rev. Biochem., 52, (1983), 871.

12. P. Etrenne, Y. Robitaille, P. Wood, S. Gauthier, N. P. V. Nair and R. Quirion, Neuroscience, 19, 1986, 1279.

13. S. J. Kish, L. M. Distefano, S. Dozič, Y. Robitaille, A. Rajput, J. H. N. Deck and O. Hornykiewicz, Neuroscience Letters, 117, (1990), 347.

14. E. K. Perry, R. H. Perry, C. J. Smith, D. J. Dick, J. M. Candy, J. A. Edwardson, A. Fairbairn and G. Blessed, Psychiat., 50, (1987), 806.

15. C. M. Yates, G. Fink, J. G. Bennie, A. Gordon, J. Simpson and R. L. Eskay, J. Neurol. Sci., 67, (1985), 327.

16. S. J. Kish, L. Schut, J. Simmons, J. Glibert, L. J. Chang and M. Rebbetoy, Psychiat., 51, (1988), 544.

17. D. L. Gildersleeve, Y. W. Jung and D. M. Wieland, J. Chromatogr. A, 667, (1994), 183.

18. M. Zhou and J. T. Stewart, J. Pharm. Biomed. Anal., 30, (2002), 443.

19. M. M. Hefnawy and H. Y. Aboul-Enein, J. Pharm. Biomed. Anal., 35, (2004), 535.

20. K. Ren, Fresenius J, Anal. Chem., 365, (1999), 389

21. T. De Boer, R. A. De Zeeuw, G. J. De Jong and K Ensing, Electrophoresis, 20 (1999), 2989.

22. K. L. Rundlett, M. P. Gasper, E. Y, Zhou, and D. W. Armstrong, Chirality, 8, (1996), 88.