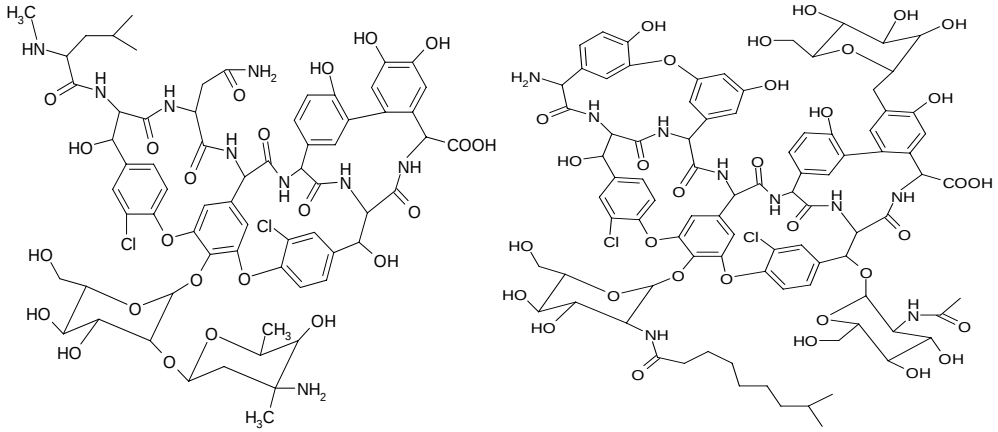
Chapter 5

Enantioselective, potentiometric membrane electrodes based on antibiotics

5.1 Macrocyclic antibiotics as chiral selectors for EPMEs design

Enantiorecognition of several classes of pharmaceutical drugs and molecules of biological importance has been successfully achieved by using macrocyclic antibiotics as chiral selectors. Macrocyclic antibiotics have several functional groups that are responsible for multiple stereoselective interaction.

Many macrocyclic antibiotics exhibit similar physico-chemical properties, on the hand showing different stereoselective power [1]. The most commonly used macrocyclic antibiotics are vancomycin and teicoplanin [2-4].



(a) (b)

Figure 5.1 Chemical structures of (a) Vancomycin and (b) Teicoplanin

Vancomycin is “basket” shaped (Figure 5.1a) having three fused macrocyclic rings and two side chains, a carbohydrate dimmer and a N-methyl leucine moiety [4]. There are 18 asymmetric centers and several and several functional groups such as carboxylic, hydroxyl, amino, amido, and aromatic rings [1].

It is very soluble in water and can dimerize in aqueous solutions depending on vancomycin concentration [5]. Vancomycin solutions are very stable at low temperatures and in buffered solutions (pH 3.0-6.0) [5,6].

Teicoplanin (Figure 5.1b) is obtained from fermentation by Actinoplanes teichomyceticus. It is structurally related to the antibiotics vancomycin, and ristocetin A, but differs from these antibiotics in several ways [7], it contains the carbohydrates D-glucoseamine and D-mannose, and the amino group of the glucoseamine is substituted with a long fatty acid chain that contains 10 or 11 carbons, and is more hydrophobic than vancomycin and it aggregates in aqueous solution [8].

Teicoplanin contains one free amine and one free carboxylic acid group. However it contains 23 stereogenic centers, four phenolic groups and seven aromatic groups and is obtained as a mixture of five analogous compounds containing different fatty acid chains (C_{10}-C_{11}) attached to the amine of 2-amino-2-deoxy-β-D-glucopyranozyl groups. Self-association of teicoplanin with micellization favoured by lower pH [8] is caused by this hydrophobic tail.

The most important functional groups for amino acid recognition are the $–NH_2$ and the –COOH groups ionised over pH 3.5-8.0 range [9]. Teicoplanin is soluble in water, slightly soluble in methanol and ethanol and insoluble in non-polar organic solvents.

5.2 Enantioanalysis of S-ibuprofen using enantioselective, potentiometric membrane electrodes based on antibiotics

Two enantioselective, potentiometric membrane electrodes based on vancomycin and teicoplanine modified with acetonitrile are proposed for the enantioanalysis of S-ibuprofen [10].

5.2.1 Reagents and materials

Graphite powder (1-2μm, synthetic) was purchased from Aldrich. Paraffin oil was purchased from Fluka (Buchs, Switzerland). Vancomycin and teicoplanin were purchased from Sigma-Aldrich. (S)-(+)-Ibuprofen was purchased from Sigma-Aldrich. Phosphate buffer (pH 4.00) was obtained from Merck (Darmstadt, Germany). Deionized water from a Modulab system (Continental Water Systems, San Antonio, TX, USA) was used for all solutions preparations.

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) containing acetonitrile was prepared using pH 6.00 phosphate buffer containing 40% (v/v) of acetonitrile. All standard and diluted

solutions were buffered with phosphate buffer pH 4.00 using the ratio buffer:distilled water 1:1 (v/v).

Myprodol capsules (200mg (S)-(+)-ibuprofen per capsule) and Nurofen tablets (200mg (S)-(+)-ibuprofen per tablet) were obtained from Nutrent (Sandton, South Africa). The Myprodol capsules and Nurofen tablets contain (S)-(+)-ibuprofen as an active compound, paracetamol and usual additives such as starch.

5.2.2 Apparatus

A 663 VA Stand (Metrohm, Herisau, Switzerland) in combination with a μAutolab and Ecochemie (Utrech, The Nertherlands) Software version 4.9 were used for all potentiometric measurements. An Ag/AgCl (0.1 mol l^{-1} KCl) electrode served as reference electrode in the cell.

5.2.3 Electrode design

The paraffin oil and graphite powder were mixed in a ratio of 1:4 (w/w) followed by the addition of solution of vancomycin or teicoplanin modified with acetonitrile (100μl of chiral selector solution to 100mg of carbon paste). A certain quantity of carbon paste free from chiral selector was prepared and placed in a plastic pipette peak, leaving 3-4mm empty in the top to be filled with the modified carbon paste. The diameter of the electrode was 3 mm. Electric contact was made by inserting an Ag/AgCl wire into the carbon paste. The internal solution was 0.1mol l^{-1} KCl. Before each set of measurement the surface of the electrode was "refreshed" with a new portion of carbon paste,

containing the chiral selector and then polished with alumina paper (polishing strips 30144-001 Orion). The carbon paste prevents the leaching of the antibiotic from the membrane into the solution.

5.2.4 Recommended procedures

5.2.4.1 Direct potentiometry

The potentiometric method was used for potential determination of each standard solution (10^{-10}-10^{-4}mol/L). The electrodes were placed into stirred standard solutions and graphs of E (mV) versus pS-Ibuprofin were plotted. The unknown concentrations were determined from the calibration graphs.

5.2.4.2 Content uniform assay of Ibuprofen capsules and tablets

Each of the five capsules and five tablets (200mg (S)-(+)-ibuprofen per capsule and per tablet) were placed into 100ml calibrated flask, dissolved and diluted to the mark using a phosphate buffer (pH 5.34):deionized water = 1:1 (v/v). The unknown concentration of (S)-(+)-ibuprofen was determined using the direct potentiometric method.

5.2.5 Results and discussion

5.2.5.1 Electrodes response

The responses of the electrodes were determined for both enantiomers S- and R-ibuprofen, at pH = 4.0 (phosphate buffer) using potentiomatric method. The responses obtained for R-ibuprofen were not linear and non-Nernstian. That proved that the

electrodes cannot be used for the assay of R-ibuprofen. The equations of calibration obtained for S-ibuprofen are as follows:

(I) E =235.0 - 58.5 pS-ibuprofen r = 0.9997

(II) E = 370.0 - 57.0 pS-ibuprofen r = 0.9999

where E(mV) is the cell potential, pS-ibuprofen = -log[S-ibuprofen], and (I), (II) correspond to the EPMEs based on vancomycin and teicoplanine modified with acetonitrile. The linear concentration ranges were 10^{-4} to 10^{-10} and 10^{-8} to 10^{-10} mol l^{-1} for electrodes based on vancomycin and acetonitrile modified teicoplanin, respectively, with detection limits of 9.6 x 10^{-5} and 3.2 x 10^{-7} mol/L, respectively. The responses of the electrodes showed good stability and reproducibility for all the performed tests for 6 months, when they are used daily for measurements (RSD<1.0%).

The response time was 30s for the concentration range between 10^{-7} and 10^{-4} mol/L, and 1min for the concentration range between 10^{-8} and 10^{-10} mol/L for both electrodes.

5.2.5.2 Effect of pH on the response of the electrodes

Potentiometry was used to determine the effect of pH on the response of the proposed electrodes. The solution used for measurements were containing (S)-(+)-ibuprofen (C = 10^{-7} mol l^{-1}) at different pH values. The plots of E (mV) versus pH (fig. 5.2) indicate, that the response of the electrodes does not depend on the pH changes in the following pH ranges: 3.0-8.0 for vancomycin based electrode and 4.0-10.0 for teicoplanin, modified by acetonitrile based electrode.

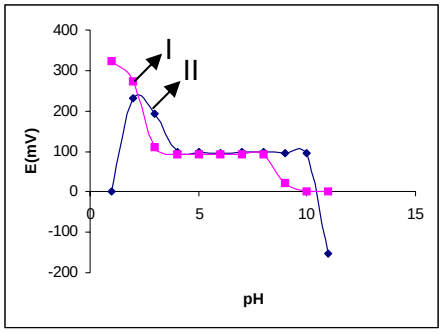


Figure 5.2 Effect of pH on the response of the potentiometric enantioselective membrane electrodes for (S)-(+)-ibuprofen (10^{-7} mol l^{-1}) (I) vancomycin based electrode; (II) teicoplanin, modified with acetonitrile based electrode.

5.2.5.3 Selectivity of the electrodes

The selectivity of the electrodes was investigated using the mixed solution method. The ratios between the concentrations of (S)-(+)-ibuprofen and the concentration of interferent were 1:10. As it follows from the values of the potentiometric selectivity coefficients that are shown in table 5.1, the electrodes are selective over all possible interfering compounds.

Also they are enantioselective because the values of the potentiometric selectivity coefficients over R-ibuprofen were lower than 10^{-4}. These results proves that the two electrodes can be used for (S)-(+)-ibuprofen enantioanalysis.

Table 5.1. Selectivity coefficients (K_{sel}^{pot}) for the enatioselective, potentiometric membrane electrodes.

Interfering species	K_{sel}^{pot}	
	EPME based on	
	Vancomycin	Teicoplanin
PVP	1.7×10^{-3}	6.2×10^{-3}
Creatine	$<< 10^{-4}$	5.0×10^{-4}
Creatinine	2.0×10^{-3}	4.0×10^{-4}
Paracetamol	$<< 10^{-4}$	3.9×10^{-4}

All measurements were made at room temperature; all values are average of 10 determinations.

5.2.5.4 Analytical applications

The response characteristics and the selectivity and enantioselectivity of the proposed electrodes indicate that the electrodes are suitable for the enantiopurity tests of (S)-(+)-ibuprofen raw materials and for uniformity content of Myprodol capsules and Nurofen tablets. The uniformity content test for Myprodol capsules and Nurofen tablets shows that (S)-(+)-ibuprofen can be reliably assayed in the tablets with average recoveries of 98.79 ± 0.10 and 98.96 ± 0.12% for Myprodol capsules and Nurofen tablets respectively when vancomycin based electrode was used, and 98.77 ± 0.12 and 98.45 ± 0.13% for Myprodol capsules and Nurofen tablets when teicoplanin, modified with acetonitrile based electrode was used. These results show that the tested pharmaceutical compounds contain (S)-(+)-ibuprofen as main component. They are also in good agreement with the results obtained using a HPLC method: 98.80% and 98.50% (S)-(+)-ibuprofen in Myprodol capsules and Nurofen tablets, respectively.

5.3 Conclusions

The proposed enantioselective, potentiometric membrane electrodes based on macrocyclic antibiotics vancomycin and teicoplanin have attractive features in enantioselective analysis. The construction of the electrodes is simple, fast and reproducible with reliable response characteristics for the proposed enatioselective membrane electrodes. The electrodes can be successfully used for enantiopurity tests of pharmaceutical formulations of (S)-(+)-ibuprofen.

5.4 References

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