Macro Cyclic Antibiotics as Chiral Supramolecular Receptors for Enantioslective Sensing in Biological Samples

Dr. Rooma Tyagi
Chemistry department, S.S.V. (P.G.) College, Hapur, U.P., India.

Enantioselective Potentiometric Membrane electrodes (EMPEs) based on macroyclic antibiotics derivative were proposed for determination of L-and D-enantiomers of glyceric acid in serum samples. The linear concentration ranges for the proposed sensor were in the F mol/L to P mol/L magnitude order. The sensor proved high reliability for the enantioanalysis of L and D glyeric acid in serum samples.

Keywords: Enantioselective potentiometric determination, Macro cyclic Antibiotics derivative and Biological samples.

1. INTRODUCTION

Macro cyclic antibiotics are chiral host molecules able of chiral discrimination even if they are not chemically modified.

Nevertheless, chemical modifications are often needed to improve their physic-chemical properties (i.e. Solubility) and also to graft on the macro cycle chemical function which could allow their covalent anchoring at surfaces. It is important to note that all these chemical modifications have to be done in a way that the chemical functions added do not intnibit the chiral inclusion properties of the macro cycle [1].

Potentiometric methods with ion- selective membranes electrodes can provide valuable and straight forward means of assaying L-and D-enantiomers of glyceric acid in pharmaceutical formulations because of the possibility to determine directly the active ions in the solution. Lowest cost, easy of use, low maintenance, simplicity, speed of assay procedure and the reliability of the analytical information make ISEs very attractive for the assay of biological samples [2-6].

2. EXPERIMENTAL

All chemicals were analytical grade and solutions were prepared with deionized water. Glyecric aeid was purchased from sigma (st Louis, Mo USA).

2.1. Electrodes Design

Paraffin oil and graphite powder were mixed in a ratio of 1:4 (w/w) followed by the addition of chiral selector (Macro cyclic antibiotics) to carbon paste. A certain quantity of

carbon paste free of chiralselector was prepared and it was placed into a plastic pipette peak leaving 3-4 mm empty in the top to be filled with the carbon paste that contains the chiral selector. The diameter of the potentiometric, enantio selective membrane electrode was 3 mm. Electric contact was obtained by inserting an Ag/Agcl wire in the carbon paste. 0.1 mol/L of KCl was used as internal solution. The surface of the electrodes was wetted with deionized water and polished with alumina paper before using them for each experiment. When it was not in use, the electrode was immersed in a 10^{-3}mol/L glyceric acid solution.

2.2. Recommended Procedure

Direct potentiometric method was used for the potential determination of each standard solution (10^{-10} -10^{-3} mol/L, pH=3.5). The electrodes were placed in the stirred solution and graphs of E (mV) versus P glyceric acid solution were plotted.

3. RESULT AND DISSCUASION

In born erross of metabolism disorders are rare gentic diseases. They can be diagnosed by assay of glyceric acid in human body fluids [7]. Glyceric acid (2, 3- dihydroxy propionic acid) exists in two configurations L- and D-enantiomers in mammalian metabolism as

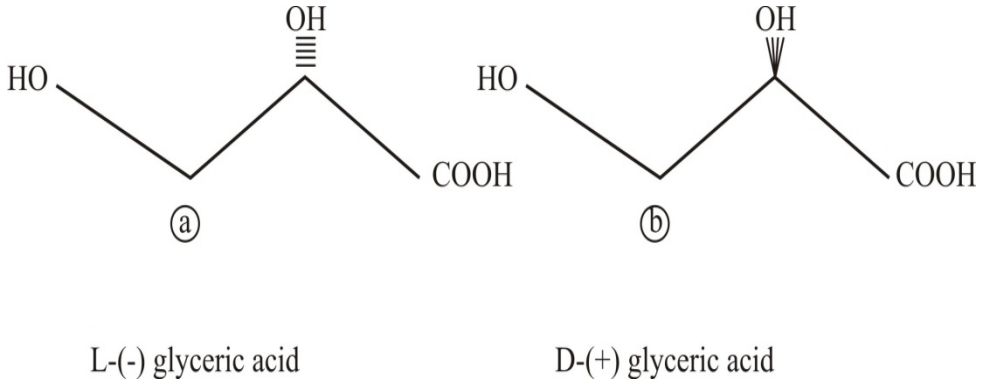

Fig. 1: Molecular structure of L and D glyceric acid.

3.1. Response Characteristics of the Electrodes

The response characteristics of EPMES were determined for both enantiomers, L-glyeric (L-GA) are D-glyeeric acid (D-GA) at pH=3.5 (phosphate Butter).

The response obtained for L-GA was Liner and near Nernst ion only when vancomycin was used as chiral selector for the design of EPMES, while response obtained for D-GA was liner and near Nernst ion only when teicoplanin based EPMES was used. The response characteristics of the two EPMES are shown in Table 1.

Table 1: Response characteristics of EPMES for the determinations of D - and, L – glyceric acids.

Analyte	Parameters			
	Slope [mV/decode)	**Intercept E^o [mol/L]**	**Liner range [mol/L]**	**Detection limit [mol/L]**
L-glyeric acid	58.6	574.6	10^{-9}-10^{-7}	$1.56x10^{-10}$
D-glyceric	50	206	10^{-4}-10^{-2}	$7.60x10^{-5}$

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.

3.2. Effect of pH on the Response of Electrodes

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) glceric acids having defferent pHs were prepared by adding small volumes of HCl (0.1 mol/L). Plots of E (mV) vs. pH shown in Figure 2 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.

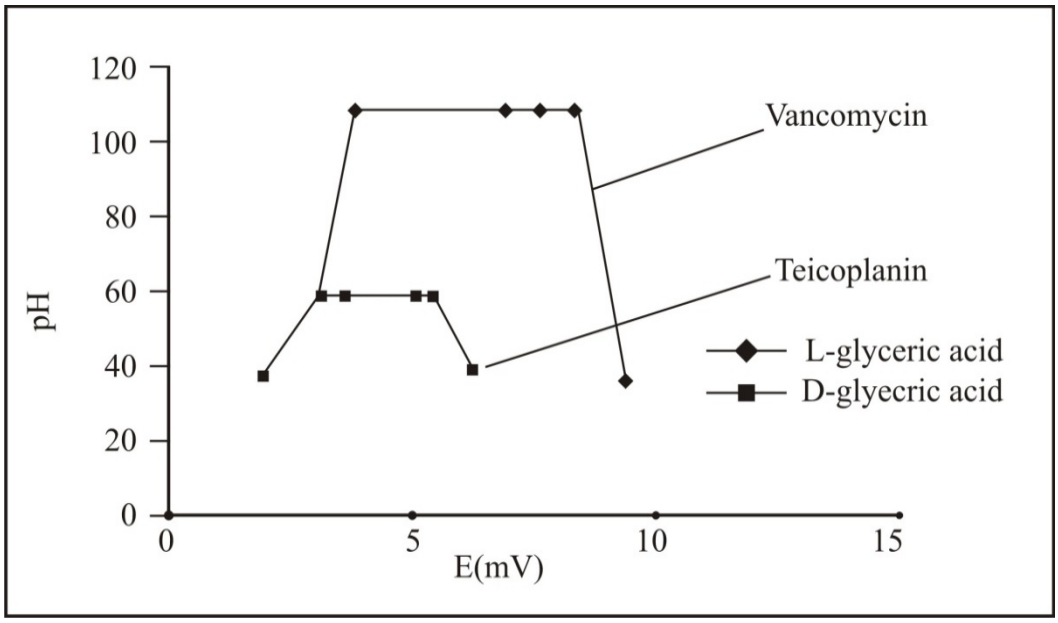

Fig 2: Effect of pH on the response of the EPMES to L-glyeric acid (10^{-8} mol/L) and D-glyceric acid (10^{-3} mol/L) solutions.

3.3. Selectivity of the EPMES

The selectivity of both electrodes was checked using the mixed solutions method 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 as shown in Table 2 for vancomycin and teicoplanin based EPMES proved that L and D-GA, creatine and creatinine do not interfere in the determination of L-and D-GA demonstrating the enantioselective property of the EPMES. Inorganic cations such a Na^{+}, K^{+} and Ca^{++} do not interfere in the analysis of L-and D-GA.

Table 2: Selectivity coefficients for the response of the enantioselecitive membrane electrodes used L and D glyceric acid assay.

Interfering species (J)	$K^{pot}_{l.j.}$	
	Vancomyein based EPME	**Tecoplanin based EPME**
L-glyceric acid	-	2.39
D-glyceric acid	2.415	-
Creative	2.086	2.08
Creatinine	2.41	2.39

3.4. Analytical Application

Solutions containing L-and D-GA in different ratio 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 analysis of each enantiomer in the presence of it antipode proved the suitability of the electrodes. No significant differences in the recovery values were recorded for the ratios b/w L:D or D:L enantiomors varying from 1:9 to 1:99.99 as shown in Table 3.

Table 3: The results obtained for the determination of L-glyceric acid in the presence of D-glyceric acid and vice-versa.

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
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

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