BEHAVIOUR OF K^+ SELECTIVE ELECTRODE IN THE PRESENCE OF HUMAN SERUM ALBUMIN

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ABSTRACT

Potentiometric measurements in protein containing solutions are affected by the presence of protein . Overall effects on the ISE, on the reference electrode and on the electrolyte solution have been reported for BSA (Bovine Serum Albumin) containing solutions using K^+ as a test cation (1-3).

In this work we present studies with Human Serum Albumin (HSA) as a further step to approaching physiological conditions.

Common features were found for both albumins, which are explained in terms of solution and interfacial chemistry.

Measurements in HSA of particularly high concentration (100 g dm^{-3}) show evolution in time that has not been accounted for in previous studies and do not find parallel at lower concentrations.

Explanation for these latest observations may be partly associated with the formation of HSA dimers.(4)

INTRODUCTION

Ion-selective electrodes are routinely used in clinical chemistry as well as in other applied fields despite the fact that some factors affecting the accuracy of measurements are still to be clarified.

This work follows previous studies (1-3) dealing with the influence of albumin and of reference electrode characteristics to the global cell potential. This, on its turn, determines the value of the concentration of the ion of interest.

Human serum albumin (HSA) is used in this work as a further step to approaching physiological conditions.

Portugaliæ Electrochimica Acta, 14 (1996) 217-221

MATERIALS AND METHODS

Potassium chloride - Merck, proanalysis; Valinomycin - Sigma V-0627; 2-Nitrophenyloctylether - Fluka, Selectophore; PVC - Fluka, pure; Potassium Tetrakys (4-chlorophenylborate) - Fluka, Selectophore; Tetrahydrophuran - Sigma T-5267; Human serum albumin - Sigma A-1887.

The solutions were prepared with conductivity water redistilled from distilled water, to which potassium permanganate and sodium hydroxide were added, under a current of nitrogen.

ELECTRODES

Reference electrodes used were:

Hypertonic - Commercial Saturated Calomel Electrode - SCE - (◆,✧)

Isotonic - Modified Calomel Electrode (0.15 mol dm^{-3} KCl was substituted for manufacturer's inner saturated KCl solution) with ceramic plug (●,○) - Mod. CE.

- Silver-Silver Chloride Electrodes - Ag/AgCl - prepared by the thermoelectrolytic method in our laboratory (5) as suggested by Bates (6). They were either used as an external (dipped in a 0.15 mol dm^{-3} KCl solution contained in a polycarbonate tube whose ending has an 1mm diameter orifice; to this ending a dialysis membrane is applied with an O-ring) (■,□) or as an internal reference electrode for the K^+SE (dipped in a 10^{-2} mol dm^{-3} KCl solution).

Selective electrodes:

Valynomicin based potassium ion-selective electrodes were prepared in this laboratory: 5 mg of valynomicin, 167.5 mg PVC, 330 mg of o-NPOE and 50% (mole % ionophore) of potassium tetrakis were dissolved in freshly distilled, under N_2, THF. The mixture was stirred overnight, put on a PTFE mould and allowed to dry. Several discs were cut from the same preparation and each one was applied to a polycarbonate stem with an O-ring. 10^{-2} mol dm^{-3} KCl solution and Ag/AgCl inner reference electrode were introduced in the stem.

METHODOLOGY

Potentiometric measurements were performed in cells of the type:

$$\text{Reference electrode} \left| \begin{array}{c} 10^{-2} \text{ mol dm}^{-3} \text{ KCl} \\ + \\ x \text{ g dm}^{-3} \text{ HSA} \\ (20 \le x \le 100) \end{array} \right| \text{K}^+\text{(I.S.E.)} \quad \text{(I)}$$

(◆, ■, ●)

◆- SCE ■- Ag/AgCl (Isotonic KCl) ●- Mod. CE (Isotonic KCl)

with a Hewlett Packard data acquisition/control unit, HP 3421A, interfaced with a HP-85 computer, in a water thermostated bath, at 25.0°C.

Measurements were taken in increasingly diluted - A (◆, ■, ●) and increasingly concentrated - B (✧,□,○) albumin (Human Serum Albumin (HSA))solutions.

Each albumin concentration solution (20, 40, 60, 80 g dm^{-3}) was prepared from direct dilution of a 100 g dm^{-3} stock solution with 10^{-2} mol dm^{-3} KCl. *Fig. 1, 2*.

RESULTS AND DISCUSSION

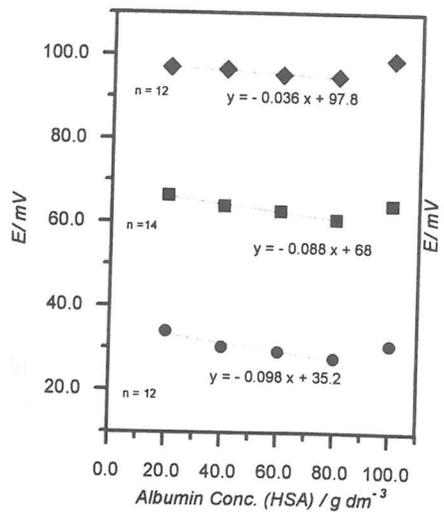

Fig. 1- Emf of cells I vs. HSA concentration for the dilution (A) methodology.

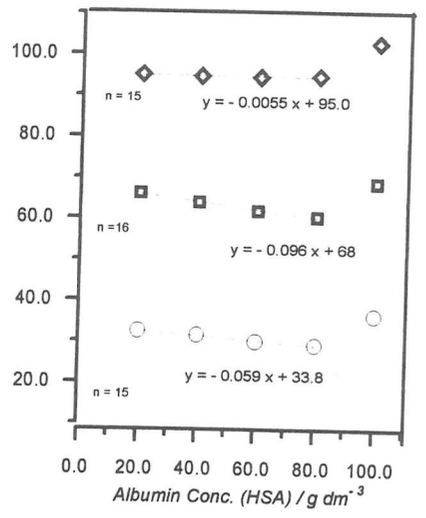

Fig. 2- Emf of cells I vs. HSA concentration for the concentration (B) methodology.

Various methodologies (3) for the preparation of solutions were investigated on their effects on the results. In this work we used the one that has shown the smallest variations in potential.

The results show negative correlations of emf values with albumin concentration, in the range 20-80 g dm^{-3}, in all cases. For both methodologies (A and B) the variation is larger for isotonic reference KCl electrolyte than for hypertonic KCl.

A new feature which was observed is the increase of potential for the highest albumin concentration (100 g dm^{-3}). This observation is in agreement with literature data, reporting on the dimerization of HSA (4). This increase becomes more pronounced with time. *Fig.3*.

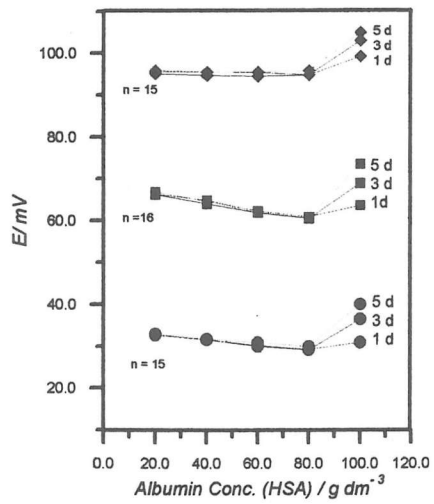

Fig.3 - Emf of cells I vs. HSA concentration for the (A) methodology with evidence to evolution with time for the highest concentration.

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