Hypertonic versus isotonic salt bridges, ion strength effects and albumin influence in ion selective electrode measurements - a further insight.

Maria J.F. Rebelo

Cecul - Departamento de Química, FCUL

Rua da Escola Politécnica, 58, 1294 Lisboa Codex

Abstract

Careful experiments were done with isotonic and hypertonic electrolytes in reference electrodes as well as with different forms of accurately making the liquid junction (open junction, dialysis membrane and frit restricted junctions) in cell assemblies involving ion selective electrodes. The ionic strength of test solutions was varied adding NaCl at concentrations which limit the physiological range. The experiments show that dialysis membrane restricted liquid junctions follow the same trend as open junction when NaCl concentration varies, contrary to what had been found in experiments made with modified commercial analysers by other authors.

Solutions with and without added albumin were studied

Clear evidence that protein influences the liquid junction potential and thus measurements with ion selective electrodes is presented.

Key words: Hypertonic and isotonic salt bridge, calcium, potassium ion selective electrodes, blood electrolytes, protein effect on ion selective electrode measurements, liquid junction potential.

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Introduction

The advantages of using ion selective electrodes to measure blood electrolyte activities is well recognized and the large number of commercial apparatus which use this technique evidence this fact. However, in an attempt to minimize some unresolved problems, manufacturers offer different forms of the liquid-liquid junction between the reference electrode and the test solution, as well as different electrolyte reference solutions, among other things, while a worldwide agreement is not reached.

The influence of protein on the final result is one of the problems involved and its effect on the liquid junction potential is the "most poorly understood and the most variable feature among commercial analyzers" (1).

Some authors claim there is an influence of protein in measurements of blood electrolytes with ion selective electrodes (2-6) while others say there is not (7-9).

Payne has been making a longstanding effort to clarify this situation and has tried to introduce isotonic bridge electrolytes, claiming that they minimize the protein effect (10-13). However the residual liquid junction potential is larger when concentration of equitransferent salts decreases as it happens when changing from hypertonic KCl to isotonic KCl and even larger if isotonic NaCl is used. This has been pointed out by D'Orazio, who criticizes Payne's point of view that isotonic bridges should be used and sustains that hypertonic KCl must continue to be used (1).

Materials and Methods

Potassium chloride; Sodium chloride; Calcium chloride - were Merck, proanalysis; Valinomycin - Sigma VO627; 2-Nitrophenyloctyl ether - Fluka, Selectophore; PVC - Fluka, pure; Potassium Tetrakis (4-chlorophenylborate) - Fluka, Selectophore; Tetrahydrofuran - Sigma T5267; Albumin - chicken egg Grade V, A5503, Sigma

The solutions were prepared with conductivity water, redistilled from distilled water, to which potassium permanganate and sodium hydroxide were added under a current of N_2.

Electrodes

The silver, silver chloride electrodes were prepared by the thermal electrolytic method (14). Several electrodes were prepared at the same time and only those which showed bias potentials < 0.05 mV were used.

The dialysis membrane restricted electrode was prepared applying the membrane to a polycarbonate stem with an O ring. A silver, silver chloride electrode and 0.15 mol/L KCl or NaCl were then put inside the stem.

A Russell saturated calomel electrode CRL/DWG 1213 (Fife, UK) was used as supplied.

The potassium ion sensitive membrane was made dissolving 5mg of valinomycin, 167.5mg PVC, 330mg NPOE (nitrophenylocthyl ether) and 50 mole % with respect to valinomycin of potassium tetrakis in freshly distilled THF (tetrahydrofuran). The mixture was stirred overnight, cast onto a PTFE (polytetrafluoroethylene) mould and allowed to dry. Membrane discs were cut from this larger one and applied to polycarbonate stems.

The calcium ion selective electrode was from Radiometer F2002 (Copenhagen, Denmark).

A Metrohom saturated calomel electrode 60701.100, (Herisau, Switzerland) with 0.15 mol/L KCl substituted for its internal saturated potassium chloride solution was used after enough time had passed to get steady values (variations of potential were less than 0.05 mV in 0.5h).

Method

1. Solutions without albumin; Ionic Strength Effects

In order to .clarify the situation with modified commercial analyzers, careful experiments were done with isotonic and hypertonic electrolytes in reference electrodes as well as with different forms of accurately making the liquid junction. The open junction was formed in a capillary tube with cylindrical geometry using a cell vessel which gives very stable and reproducible results (15-19), a dialysis membrane was used in a stem which contained silver, silver chloride electrodes of the thermal electrolytic type (14) and the isotonic bridge electrolyte. A Russell Saturated Calomel Electrode which has the liquid junction formed in a ceramic plug was also used. (These geometries of the junctions are of the same type as used in the instruments Payne modified).

Either 0.15mol/L KCL or NaCl were used as bridge electrolytes. Calcium chloride solutions at fixed concentrations, with 100 or 150 mmol/L added NaCl were the test solutions.

The emf of cells

Ref. Electrode	0.15 mol/L MCl	x mol /L NaCl 10^{-3} mol /L $CaCl_2$	Ca(ISE)	I at 25°C

where M = Na or K and x = 0.10 or 0.15, were measured with a 3421A Hewlett Packard Data Acquisition control unit (Loveland, CO) interfaced with a HP 85 computer (Corvallis, OR). An operational amplifier was interposed between the electrodes and the measuring system. The system was thermostated in a water bath thermostat at 25.0°C.

The results obtained are shown on Table 1. Each value is the average of three measurements, the error being always less than 0.2 mV.

TABLE 1

Emf of cells (mV)

Ag | AgCl | 0.15 mol /L MCl | x mol /L NaCl, 10^{-3} mol /L $CaCl_2$ (J) | Ca(ISE) at 25°C

MCl	X	c.l.j.	d.m. (J)
NaCl	0.10	17.0	17.2
	0.15	18.4	18.4
KCl	0.10	25.0	27.0
	0.15	24.3	25.9

c.l.j. - cylindrical liquid junction; d.m. - dialysis membrane

and cells

SCE | x mol /L NaCl, 10^{-3} mol /L $CaCl_2$ | Ca(ISE)

X	emf/ mV
0.10	58.5
0.15	56.3

2. Solutions with albumin; Protein Effect

Taking into account the fact that Ca binds to albumin whereas K does not significantly do it (20-21) and that ultrafiltration is argued to give rise to a Donnan distribution of the ions (8), an experiment that avoids these complications was designed. Thus: albumin concentration was varied by dilution of a concentrated one and potassium selective electrodes were used together with potassium chloride solutions in combination with different geometries of the junction of the reference electrode as well as different concentration of the bridge electrolytes (isotonic and hypertonic).

Five potassium ion-selective electrodes were prepared from the same initial membrane and only those which showed good Nernstian response to K^+ and a negligible drift were used. The emf of cells

Ref. electrode	0.01 mol/L KCl x g/L Albumin	K(ISE)	II at 25.0ºC

were measured with a Hewlett Packard Data Acquision control unit interfaced with a HP 85 computer. An operational amplifier was interposed between the electrodes and the measuring system. Measurements were first taken in albumin free solutions and then in the most concentrated albumin solution which was subsequently diluted with 0.01 mol/L KCl. The system was thermostated in a water bath thermostat at 25.0ºC.

Three different kinds of reference electrodes were used. One of them was a silver silver chloride electrode, prepared in this laboratory, dipped in a 0.15 mol/L KCl solution contained in a polycarbonate stem onto which a dialysis membrane was applied. The other two are the previously referred commercial electrodes - Metrohom calomel electrode 60701.100 with 0.15 mol/L KCl and a Russel saturated calomel electrode CRL/DWG 121B.

The results obtained are shown on Table 2.

TABLE 2

Study of the albumin and liquid junction effect on ise measurements

Emf(mV) of cell: Ref. el. | 0.01 mol/L KCl
x g/L Albumin | K(ISE) 25.0ºC

Reference Electrode	x =			
	0	40.1	59.6	101.3
Ag/AgCl 0.15 mol/L KCl (Dialysis membrane)	65.7	59.0	57.8	55.3
Cal. 0.15 mol/L KCl (ceramic plug	51.9	45.9	45.1	43.0
SCE (ceramic plug)	93.6	92.0	90.7	89.0

Results and Discussion

1. Solutions without albumin: ionic strength effects

It was observed that there was an increase in the emf of cells containing 0.15 mol/L NaCl as bridge electrolyte both with the junction with cylindrical symmetry and the dialysis membrane when NaCl added to the $CaCl_2$ solution increased from 100 to 150 mmol/L. On the other hand, both geometries of the junction gave an emf value which decreased when added NaCl increased as before, when 0.15 mol/L KCl was used as bridge electrolyte. Saturated KCl and a ceramic plug also followed the same trend as the latter referred case.

These results are consistent with what can be theoretically predicted when taking into account the effect of added NaCl on the activity coefficient of Ca^{2+} and on the liquid junction potential, as shown on Table 3.

TABLE 3

Variation of liquid junction potential and activity coefficient contributions to the global cell emf when sample electrolyte varies

from : 1mmol/L $CaCl_2$ + + 100mmol/L NaCl to : 1mmol/L $CaCl_2$ + +150mmol/L NaCl at 25.0°C

	Calculated (mV)			Experimental (mV)		
Bridge electrolyte	$\Delta Ej(150\text{-}100)$ (A)	$\frac{RT}{2F} \ln \frac{\gamma Ca(150\ NaCl)}{\gamma Ca(100\ NaCl)}$ (B)	A+B	c.p.	c.l.j.	d.m.
Sat KCl	0.27	-2.02	-1.75	-2.2		
0.150 mol /L NaCl	2.12	-2.02	+0.12		+1.4	+1.2
0.150 mol /L KCl	1.08	-2.02	-0.94		-0.7	-1.1

c.p. - ceramic plug; c.l.j. - cylindrical liquid junction; d.m. - dialysis membrane

The expression used for the determination of the activity coefficient of Ca^{2+} was the extension of the Debye-Hückel equation

$$\log f = \frac{-A z^2 \sqrt{I}}{1 + \sqrt{I}} + 0.2\ I$$

due to Davies as referred by Robinson and Stokes(22).The equation used for the liquid junction potential was the Henderson equation. It can be seen that the agreement between this work experimental values and the calculated ones is very good, despite the limitations of the equations used. (Henderson equation considers a linear concentration profile of the limiting solutions in their mixing region and concentrations instead of activities).

Results with sodium chloride bridge solutions show larger variatios between calculated values and experimental ones, then potassium chloride does. Despite this

fact, which deserves further studies, it is important to notice that the results with the dialysis membrane restricted liquid junction follow the same trend as the open junction. That is, both cells which include those junctions and 0.15 mol/L NaCl as bridge electrolyte, have a global emf which increases when NaCl added to $CaCl_2$ solutions increases from 100 to 150mmol/L. This is contrary to what happened when commercial analyzers where modified (12) to study the same effect. As a matter of fact, looking at Payne's results - as shown on fig. 1 - page 236 of ref.(12): "Relations between reported ionized calcium activities and the sodium chloride concentrations of aqueous solutions containing the same amount of calcium. Nova NaCl, Corning NaCl and Corning KCl indicate analysers that have been modified by substituting 150 mmol/L NaCl or KCl for the manufacturer's reference electrode solutions" - a discrepancy between the behaviour of the modified instruments Nova NaCl and Corning NaCl (the first one shows an increase of calcium ion activity when NaCl added to calcium chloride solution increases from 100 to 150mmol/l, while the second one shows a decrease) seems to be strange. Note that Nova instrument has a reference electrode with an open junction and Corning has a dialysis membrane junction.

2. Solutions with albumin; Protein Effect

Most commercial ion-selective electrodes analyzers have concentrated electrolyte bridge solutions in an attempt to decrease the liquid junction potential.

Thus, Kone Microlyte uses 3.0 mol/L KCl; Ciba Corning-Saturated KCl at 37°C, Radiometer - 4.6 mol/kg sodium formate, Nova 2.0 mol/L KCl, etc. Table 4. The problems associated with the unavoidable leakage of the salt of the bridge electrolyte to the sample and interference with protein and erythrocytes led some people to

choose isotonic bridge solutions with respect to physiological fluids when measurements are to be taken in these media.

TABLE 4

Comparison of some Ca^{2+} analyzers

Model	Output	Ionophore	Reference Electrode	Reference Electrolyte	Liquid Junction
AVL 980	Ca^{2+}	ETH 1001	Calomel	1.2 mol/L KCl	Open Static
Corning 634	Ca^{2+}, pH Ca^{2+} (7.4)	ETH 1001	Ag/AgCl	Sat. KCl	Dialysis Membrane
Kone Microlyte	Ca^{2+}, K^+ Na^+	ETH 1001	Ag/AgCl	3mol/LKCl	Ceramic Pl ug
NOVA 8	Ca^{2+}, pH Ca^{2+} (7.4)	ETH 1001	Ag/AgCl	2mol/L KCl	Flowing
Radiometer ICA 1	Ca^{2+}, pH Ca^{2+} (7.4)	ETH 1001	Calomel	4.6 mol/kg CHOONa	Open Static

However the advantages of having an isotonic rather than concentrated bridge solution are not well clarified and the procedure has not found acceptance yet (11-12, 23-24).

Payne (12) made measurements of ionized calcium with several commercial instruments with their concentrated bridge solutions and with substituted, isotonic bridge solutions. He used a calcium chloride solution with bovine serum albumin and increased the concentration of this solution by ultrafiltration. Payne found that the measured Ca^{2+} activity increased with increasing protein concentration when analysers with their concentrated bridge solutions were used. The isotonic systems did not show a significant change of Ca^{2+} activity with protein concentration.

However, Payne himself is puzzled with the interpretation of this results. He says *"The difference between hypertonic and isotonic junctions is readily explained if it is due to protein denaturation at hypertonic reference electrode liquid junctions. On the other hand, if the increase in Ca^{2+} activity recorded with hypertonic junctions is due to a Donnan equilibrium, then when isotonic junctions are used the true increase in Ca^{2+} activity must be fortuitously closely matched by a change of the same magnitude but opposite sign in the residual liquid junction potential caused by protein".*

In order to clarify the protein effect we studied a system which used potassium ion selective electrodes and potassium chloride solutions containing albumin, because potassium ion is recognized not to bind significantly to albumin (21) which does not interfere with the K^+ selective membrane either (25). The concentrated albumin solution was subsequently diluted with potassium chloride solution of the same concentration as the initial one.

We found a decrease of the overall cell potential when albumin increases. The variation is larger when isotonic reference solutions are used then when hypertonic are, which is what should be expected taking into consideration a liquid junction potential effect. .

Moreover the variations observed when transferring the electrodes from 0.01 mol/L KCl without albumin to the albumin containing KCl solution evidence that there is an effect of protein on the liquid junction potential. As a matter of fact, subtracting the appropriate differences between emf of cells containing albumin solutions from those ones without albumin, the protein effect on the liquid junction potential is evidenced. Taking values from Table 3, if we consider $E_{i,j}$ as the emf of celis II, where i refers to the reference electrode and j to the albumin concentration in the KCl solutions, with

i Ref. Electrode

1 Ag/AgCl/ 0.15mol/L KCl (dialysis membrane)

2 Cal. El./0.15 mol/L Kcl (ceramic plug)

3 SCE

and

j =0 40.1 59.6 101.3 g/L albumin in 0.01mol/L KCl

we have, for instance,:

$$(E_{3,0} - E_{2,0}) - (E_{3,101.3} - E_{2,101.3}) = (E_{K3,0} - E_{SCE} + E_{j3,0} - E_{K2,0} + E_{Ref2} - E_{j2,0}) - (E_{K3,101.3} - E_{SCE} + E_{j3,101.3} - E_{K2,101.3} + E_{Ref2} - E_{j2,101.3}).$$

Since $E_{K3,0} = E_{K2,0}$ and $E_{K3,101.3} = E_{K2,101,3}$ (because these terms refer the response of the K ion selective electrode in solutions of 0.01mol/L KCl with 0 and 101.3 g/L added albumin respectively), they cancel each other and so do the terms related to the reference electrode itself (excluding the liquid junction potential which is usually included in the reference electrode potential). Thus , the above mentioned difference is reduced to the liquid junction potential terms, that is , in this case, =

$$(E_{j3,0} - E_{j2,0}) - (E_{j3,101.3} - E_{j2,101.3}) = -4.3 \text{ mV}$$

The same type of calculation yields -5.8mV when SCE and Ag/AgCl 0.15mol/L KCl (dialysis membrane) are used. If there was no influence of albumin on the liquid junction potential the differences between the liquid junction potential terms, as shown above, should cancel out and the final differnce should be zero, which is not the case for all the albumin concentrations studied, which include the normal physiological value.

It is, thus, clearly demonstrated that albumin influences the liquid junction potential. Experiments in progress with other types of albumin also confirm that influence. A deeper insight into this problem is being pursued.

It was also observed (results not shown) that the substituted calomel electrode although having a steady potential response during the time that an experiment lasted, must be continuously changing (bias potential with repect to SCE was different from day to day) and the actual KCl concentration at the moment of the experiment must have been different from the initial solution

Conclusions

Emf of cells consisting of a calcium selective electrode and reference electrodes containing isotonic reference electrolytes (0.15mol/LKCl or 0.15 mol/L NaCl) follow the same trend when the ionic strength of the sample solution varies whether an open junction or a dialysis membrane restricted junction is used., that is, it decreases when NaCl added to a fixed concentration of $CaCl_2$ varies from 100 to 150 mmol/L if the bridge electrolyte is 0.15 mol/L KCl and increases when it is 0.15 mol/L NaCl. Both forms of liquid junction follow the same trend. Experiments with modified instruments(12), which give different patterns of variation when open junction and dialysis membrane restricted one are used, must have added an extra contribution to the global emf which was not accounted for.

It was evidenced that albumin influences the liquid junction potential.

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Effect of Water - Organic Solvent Mixtures on Pitting Corrosion of Mild Steel

By

S.T.ARAB

Girls College of Education, General Presidency of Education, Jeddah, Saudi Arabia.

ABSTRACT

The pitting corrosion of mild steel was studied in 0.1 M NaCl + 0.015 M NaOH in water-glycerol, water-ethylene glycol, water-ethanol and water-dioxane solutions with various compositions (from 0.0 to 60 %v/v of the organic solvent component). The results obtained from the potentiodynamic and potentiostatic measurements show that the pitting corrosion is inhibited by the organic components in the medium. The inhibition percentage was increased with the increasing of the concentration of the organic solvent in the medium and reached about 80%.

It is suggested that the organic solvents inhibited the pitting corrosion of mild steel by (i) increasing the viscosity of the medium, which leads to a decrease in the diffusion coefficient of the corrosion products. It also decreases the dielectric constant of the medium, which lowers the basicity of the solution, and (ii) adsorption of solvent molecules on steel surface.

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