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MEMORY EFFECTS AND OFFSET POTENTIALS IN FLOW-THROUGH, ION-SELECTIVE ELECTRODES

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ABSTRACT: As part of an ongoing project to develop a low-cost, portable blood electrolyte analyzer, the authors investigated the role of memory effects and offset potentials in limiting the performance of flow-through, ion-selective electrodes. These electrodes were used in a protoype portable analyzer to determine sodium, potassium, and chloride ion concentrtions in blood serum. A method of quantifying these effects and partially compensating for them was devised. Compensating for these effects significantly improved the performance of the prototype analyzer under laboratory conditions. The improved performance matched or exceeded that of one commercially available non-portable, automated system at the 90 percent confidence level. These effects and the method used to improve the performance of the prototype blood electrolyte analyzer are discussed in this paper.

KEYWORDS: Blood, electrodes, electrolyte, errors, flow-through, ion-selective, ISE, memory, offset potential, portable.

INTRODUCTION

As part of a project to develop a low-cost, portable blood electrolyte analyzer, the authors investigated methods to determine and compensate for errors caused by memory effects and offset potentials in flow-through, ion-selective electrodes. Offset potentials, particularly those arising from liquid junction potentials, have long been recognized as limiting the accuracy and precision of direct analyses using ion-selective electrodes (Skoog and West, 1988).

Memory effects are related to the immediate prior history of the electrode surface. It is difficult, if not impossible, to provide identical electrode surfaces to successive samples. Normally, the time required and the varying nature of serum samples makes cleaning alone insufficient to eliminate the memory effects. In addition to cleaning the electrode surfaces, using the potential produced by an intermediately measured reference solution can be used in the computation of sample concentration to help compensate for memory effects. This approach can best be understood by reference to equations [1] and [2] below.

$$C_x = C_r 10^{-[(E_x - E_r)/S]} \qquad [1]$$

$$S = (E_H - E_L)/(\log C_H - \log C_L) \qquad [2]$$

Equations [1] and [2] are the two basic expressions utilized in computing sample concentration (C_x) from measured potential (E_x). C_r and E_r are the concentration and potential of the reference solution, respectively. E_H and E_L are the potentials measured from high and low calibrating standards of concentrations C_H and C_L. (In equations [1] and [2], the activity coefficient differences are neglected at all ionic concentrations and compensated for by the

calibration procedure.) S is the slope as determined from [2] by measuring the potentials of a high and a low standard solution, which bracket the concentrations of interest. The calibrating standard solutions contain each of the analyte ions. A separate slope value is determined for each of the analyte ions. The value of this slope is different for each of the ions, and it remains constant between calibrations. In situations in which intermediate calculations are not used, the concentration and potential of one of the standards used in determining the slope is used in [1] to compute the sample concentration. In these cases, the values of C_L and E_L are used as C_r and E_r. When intermediate potentials are used, the potential of this same standard is measured just before each sample potential is measured, and this intermediate potential is used in [1] as E_r to compute the sample concentration.

Error-producing offset potentials are generated by asymmetry potentials with glass electrodes, liquid junction potentials, and interfering substances. Asymmetry potentials are variable, not susceptible to direct measurement, and not well understood. Liquid junction potentials are calculated by the Henderson equation (Byrne, 1988) and are related to the mobilities, concentrations, and charges of the ions at solution/interface boundaries. Such junction potentials always exist at the reference electrode/sample interface, and various methods to minimize these potentials are incorporated into common commercial electrolyte analyzers. The literature contains considerable discussion of the effect of proteins and other blood constituents on error potentials generated when using ion selective electrodes with blood serum (Reichenbach, 1986; Saris, 1987; Maas, 1988; Burnett, 1988). Maas (1988) concluded that observed error potentials are probably not due to the proteins per se but likely arise from their presence perturbing cation equilibria, from Donnan effects during sample preparation, or from semipermeable membranes, when they are part of the measuring system. A method of determining the factors which at least partially compensate for these offset error potentials (as long as the offset potentials are constant) will be introduced in this paper. The improved accuracy and precision resulting from utilizing these factors in the calculations associated with the prototype, portable, blood electrolyte system are presented.

EXPERIMENTAL

Figure 1 shows the experimental setup for the prototype portable system in which the sodium, potassium, and chloride ion-selective electrodes (ISEs) are contained in a plastic housing along with a silver/silver chloride reference electrode. The electrodes and housing were all obtained from the Medica Corporation (Bedford, Massachusetts) and were the same as used in their automated EasyLyte Plus instrument. Shielded cables were used to connect each electrode to a switch box containing a rotary switch. The switch box was connected to a Fisher 950 pH/ion meter. The rotary switch enabled the user to manually select a particular ISE and measure the potential versus the reference electrode. The RS-232 output of the pH/ion meter was fed directly to a computer; a locally developed, macro-driven *Lotus 123* program transferred the data directly into a spreadsheet for analysis via the serial port on the computer.

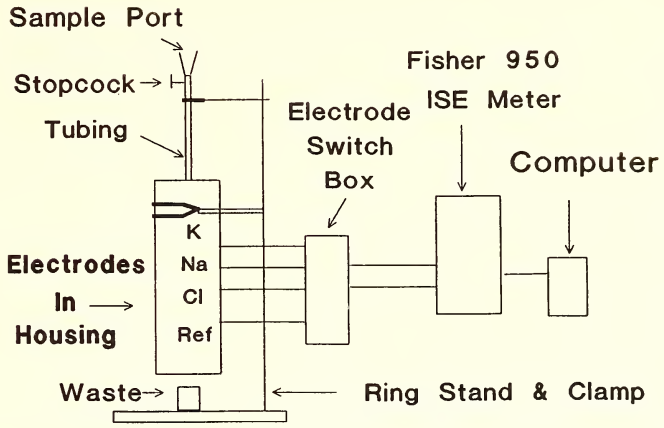

Figure 1. System configuration for the prototype portable blood electrolyte analyzer.

The initial goal of this study was to improve the electrochemical performance of the system. The portable system was calibrated with pure salt standards using reagent grade sodium chloride, potassium chloride, and sodium acetate. A high standard containing 160 mm/l sodium ions, 6.0 mm/l potassium ions, and 130 mm/l chloride ions and a low standard containing 120 mm/l sodium ions, 2.0 mm/l potassium ions, and 85 mm/l chloride ions were used to calibrate the portable system. Each of the calibrating solutions contained 0.01% thimerosal as a preservative and 0.01% Triton X as a wetting agent. Calibrations were carried out both before and after a series of trials. Periodically, a calibration was carried out during a series of trials. Blood serum samples were analyzed on an EktaChem 700XR by hospital laboratory technicians at Marion General Hospital, Marion, Indiana. These samples were refrigerated until used, and all experiments were carried out on these samples on the same day they had been analyzed at the hospital.

A protocol for electrode treatment between potential measurements was developed to provide an optimum electrode surface for each sample measurement. This protocol consisted of the following steps: 1) four 0.2 ml aliquots of the low calibrating standard solution were passed through the cells as rinses; 2) the serum sample was injected and allowed to equilibrate for about 45 seconds; and 3) the potential of each elecrode was read, and the sample was ejected.

Table 1 shows the efficacy of performance resulting from using four rinses as compared to only one rinse. The protocol used for the 1/29/93 experiment was to rinse the electrodes once with a 0.2 ml aliquot of standard solution (the low concentration standard used in the electrode calibration procedure). The potential of this standard solution was recorded after it equilibrated. Then, the electrodes were rinsed once with a 0.2 ml aliquot of the serum to be measured. This rinse was followed by another 0.2 ml aliquot of serum from which the potentials were recorded. The absolute percent error and standard deviation of the standard rinse solution for a series of six serum samples is shown for each of the electrolytes analyzed. The potential of the standard rinse solution was also recorded before it was allowed to discharge from the electrode column. Eleven such potentials for each indicator electrode were recorded throughout the series of measurements

Table 1. Constancy of intermediately measured potentials from standard solutions (PE = absolute percent error; SD = standard deviation).

Test		Na PE/SD	K PE/SD	C1 PE/SD
1 Rinse				
1/29/93	IC[a]	5.2/1.3	7.4/1.9	1.4/0.8
4 Rinses				
6/25/93	NIC[b]	0.3/0.4	0.6/0.5	0.7/0.7
7/6/93	NIC	0.4/0.5	0.6/0.6	1.1/1.0
7/13/93	IC	3.0/1.4	0.9/1.0	1.8/0.8
7/13/93	IC & IS[c]	1.0/0.9	1.4/0.8	1.5/0.7
7/16/93	IC & IS	0.9/0.7	1.0/1.2	1.3/1.3

[a] IC = Intermediately measured potentials used in calculations.
[b] NIC = No use of intermediate potentials.
[c] IS = Intermediately measured slopes used in calculations.

of six serum samples and five controls. Each of those potentials was used to compute the concentration of each of the electrolytes in the known standard used as a rinse. The absolute percent error shown reflects the deviation from the known values, and the standard deviation reflects the variation in these percent errors.

The results of an improved protocol in which four 0.2 ml aliquots of standard were passed through the electrodes as rinses between each serum sample are shown in the lower portion of Table 1. The absolute percent errors and standard deviations are considerably smaller for both the sodium and potassium electrodes. The chloride electrode appears to be relatively unaffected. Eight serum samples intermixed with control samples were analyzed. The potential used in the computations shown was that of the fourth standard solution rinse. These data suggest that the surfaces of the sodium and potassium electrodes are more reproducible by using the four rinses than by using one rinse. Additional experiments using five rinses showed no significant improvement over the protocol using four rinses. The improvement, resulting from four rinses, justifies the slight amount of additional time required for these rinses (less than one minute per sample) and the additional volume of standard solution required. This procedure was followed in all subsequent analyses. The designation *IC* indicates that intermediate calculations were performed; *NIC* indicates that no intermediate calculations were performed; and the designation *IS* means that the electrodes were recalibrated during the series of runs and that the newly computed intermediate slopes were used in all subsequent computations.

To investigate the effect of electrode surface condition further, several series of analyses were carried out in which, in addition to rinsing the electrodes with standard four times between each serum sample analysis, each serum sample was run three times in succession with no rinsing between these successive runs. Table 2 shows the results from these experiments on two separate days, 6/25 and 7/6. On both of these days, a marked increase in absolute percent error and standard deviation appeared after the first run for both sodium and potassium. The chloride electrode appeared to be relatively unaffected. As a rough comparison,

Table 2. Absolute percent error (PE) and standard deviation (SD) after each of three sequential runs with no rinsing of electrodes between runs.

	Na PE/SD			K PE/SD			Cl[a] PE/SD		
	R1	R2	R3	R1	R2	R3	R1	R2	R3
6/25	2.6/0.7	3.4/0.9	3.5/1.0	2.0/0.9	4.3/2.4	4.8/2.8	0.9/0.8	1.0/0.6	1.2/0.6
7/6	2.6/1.0	3.3/1.2	3.3/1.5	1.9/1.5	3.4/1.6	3.6/1.3	1.6/1.1	1.1/0.7	0.8/0.6
1/29		3.7/2.3			4.4/2.3			0.9/1.1	

[a] Cl Offset = 1.5 mV.

the data from serum samples run earlier on 1/29 are also shown. Since the electrodes on 1/29 were only rinsed once with standard between different serum samples (rather than four times) and once with the sample, the comparison is not exact. However, the conditions most nearly correspond to the second run of the serum in the series shown for 6/25 and 7/6. These data suggest that the most reproducible measurements for the sodium and potassium ions can be obtained from electrode surfaces which have not been rinsed with the sample to be analyzed. The significance of the chloride offset shown in Table 2 will be discussed subsequently.

RESULTS

Offset potentials are generated both from liquid junctions and from interfering species. Equation [1] along with equation [3] below show how offset potentials are related to the expression for computing concentrations from observed potentials.

$$E_x = E_T + E_j + E_i \qquad [3]$$

The observed potential, E_x, is really comprised of several components. E_T is the desired true potential, E_j is the contribution of junction potentials, and E_i is the contribution of interfering species. This relationship suggests that a factor, E_{os}, exists which, if chosen with the appropriate sign and magnitude, could cancel out the effects of the E_j and E_i terms as shown in equation [4].

$$E_x = E_T + (E_j + E_i) \pm E_{OS} \qquad [4]$$

The theoretical effect of junction potentials on percent error has been shown (Czaban,1982) to be

$$\%\text{Error} = \left| [10^{(-\Delta E_j/S)(\gamma_s/\gamma_c)} - 1] \right| \times 100 \qquad [5]$$

where γ_s and γ_c are activity coefficients of the sample and calibrant solution, respectively. ΔE_j is the difference between the junction potential of the calibrating solution and the sample solution. A plot of this equation is shown in Figure 2 with the activity coefficients taken as unity. This plot suggests that for small offsets, such as would be expected from ISEs under the conditions of this study, the percent error increases rather linearly on either side of zero. The procedure for determining an offset correction (E_{OS}) is to first analyze a series of serum samples in which the concentrations of sodium, potassium, and chloride ions are

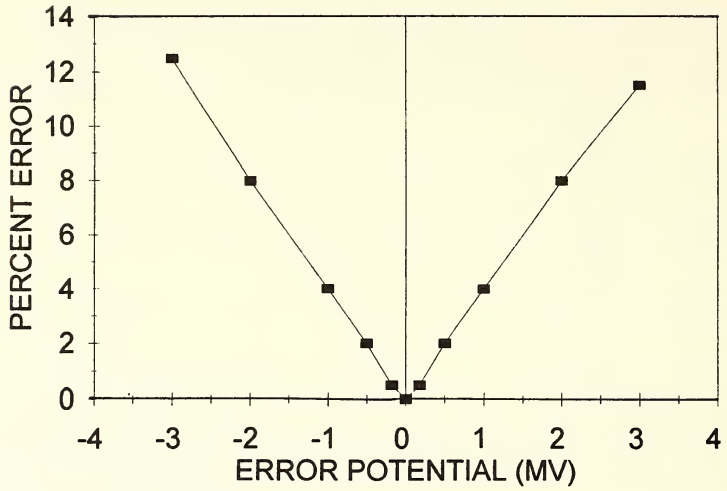

Figure 2. Theoretical effects of junction potential on absolute percent error.

known. Second, one iteratively substitutes a sequence of values for E_{OS} into equation [4] and computes corresponding concentrations from equation [1]. The resulting concentration can be used to compute an absolute percent error associated with each value of E_{OS} substituted. Third, these absolute percent errors are plotted versus the associated E_{OS}. Where that plot goes through a minimum, the factor which best cancels out the total offset is indicated.

Figure 3 shows the results of employing the approach just described with a set of eight serum samples run on 6/25/93 using data obtained from the first sequential rinse with serum sample in the chloride electrode. Since one desires to minimize both the absolute percent error and the standard deviation, both PE and SD, as well as the sum of the two, are shown. The minimum occurs at 1.4 mV. A similar procedure applied to the data obtained from the second sequential run with serum yielded a larger offset of 1.7 mV. This suggests that a coating of species that creates an offset may be left on the electrode surface after the first rinse. The offset using data from the third rinse shows little change. The offset

Table 3. Absolute percent error (PE) and standard deviation (SD) resulting from using intermediately measured potentials in calculations for serum samples analyzed for Na, K, and C1 as well as using offset in the choloride calculations (N = 8 for each day).

	Na PE/SD	K PE/SD	C1[a] PE/SD
6/25	2.8/0.5	23/1.2	1.0/0.9
7/6	2.4/0.7	1.6/1.4	0.9/0.7
7/13	4.0/1.6	3.1/2.2	1.5/0.8
7/16	2.6/1.5	2.1/1.7	2.3/1.4
Average (7/6 - 7/16)	3.0/1.2	2.3/1.7	2.3/1.0

[a] C1 Offset = 1.4 mV.

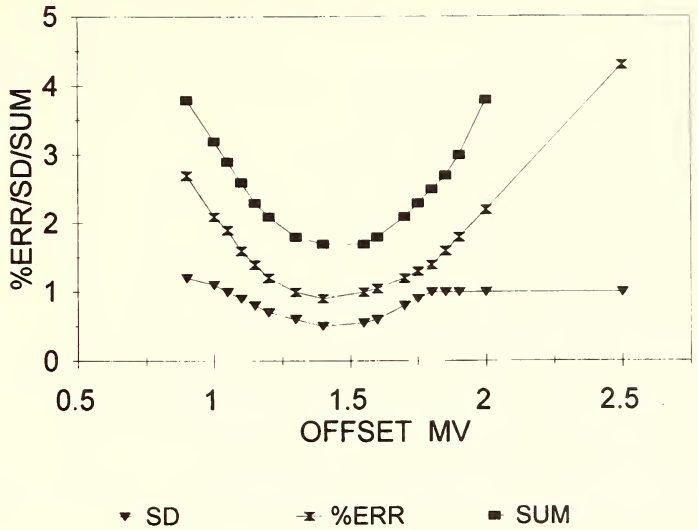

Figure 3. Experimental determination of offset potential for a new chloride electrode using data obtained after rinsing the electrode four times with the low calibrating standard and then once with the serum sample being analyzed.

correction is related to the rinsing protocol and the particular electrode used. When the 1.4 mV offset was used to correct chloride electrode data acquired on three other days, 7/6, 7/13, and 7/16, the results shown in Table 3 were obtained. The average percent error and standard deviation for the chloride electrode for those three days is seen to be similar to the results obtained from the 6/25 data. The data shown for 6/25 are those resulting after the absolute percent error and standard deviation were minimized in the process of determining the 1.4 mV offset correction. This comparison shows that similar absolute percent errors and standard deviations are obtained when that same offset correction is used on subsequent sample sets run on different days.

Table 4 shows a statistical comparison of the data taken with the portable analyzer (shown in Table 3) with the data taken with the automated EasyLyte

Table 4. Comparison of absolute percentage error (PE) and standard deviations (SD) obtained from 32 serum sample analyses using the EasyLyte Plus (ELP) and the prototype portable analyzer.

	ELP ('93) PE/SD	PROTOTYPE ('93)[a] PE/SD	*F*	*t*
Na	2.3/1.1	3.0/1.2	1.2	2.4
K	2.1/1.5	2.3/1.7	1.3	0.5
Cl	3.1/1.1	1.4/1.0	1.2	-6.4
N	32	32		

Critical Values: $F = 1.79$; 95% $df = 31/31$
$t = 1.96$; 95% $df = 62$

[a] Using intermediately measured potentials in calculations and a chloride offset of 1.4 mV.

Table 5. Optimum offsets for the sodium and potassium electrodes determined after each of three sequential runs with no electrode rinsing between runs but using our rinses with the low calibrating standard prior to running each sequence.

	SERUM (N = 8)			CONTROL (N=10)		
	R1	R2	R3	R1	R2	R3
SODIUM						
6/25	-0.7	-0.9	-0.9	- -	-0.4	- - -
7/6	-0.7	-0.8	-0.8	0.2	-0.3	-0.3
7/13	-0.9	-1.1	-1.0	-0.3	-0.5	-0.8
7/16	-0.6	-0.8	-0.9	-0.1	-0.3	-0.4
POTASSIUM						
6/25	-0.3	-1.0	-1.2	- - -	-0.3	- - -
7/6	-0.4	-0.9	-0.9	+0.4	-0.3	-0.4
7/13	-0.8	-1.6	-1.5	+0.2	-0.7	-0.8
7/16	-0.3	-0.9	-0.9	+0.6	-0.1	-0.2

Plus system on the same samples on the same days. The percent errors (PEs) shown in this table are the absolute percent errors computed using ion concentration values from an EktaChem 700XR (Marion General Hospital, Marion, Indiana) as reference values for the electrolytes. The values of *F* show that, at the 95% confidence level, no significant difference in standard deviations for any one of the three analytes exists between the two instruments, since all of the values are less than the critical *F* value at the 95% confidence level. The values of *t* show that the automated EasyLyte Plus instrument was significantly better than the portable at the 95% confidence level for the sodium analyses, that there was no significant difference for the potassium analyses, and that there was a very significant difference which favored the portable system for the chloride analyses. This difference arises because the commercial system corrects for the chloride offset merely by subtracting a constant amount from each chloride result rather than using the method proposed in these studies.

Since using offsets as described in this study improves the chloride electrode results as significantly as indicated in Table 4, similar offsets were determined for the sodium and potassium electrodes. The results of these determinations are shown in Table 5. Eight different analyzed blood serum samples were used for each day listed. Looking first at the offsets determined for the sodium analyses with serum samples, one notes that all of the values are negative (rather than positive as with the chloride electrode), and, in general, their magnitudes are significantly smaller than the chloride offsets. Theoretically, the sign change is expected, since cations have opposite potentials compared to anions, and the offsets generated change accordingly. Minimal variation between days and different sets of blood serum samples occurred. Some increase of offset occurred after successive rinses with the serum sample. This increase is expected from the model described above, which pictures the electrode surface becoming coated with serum from previous rinses in the sequence. This trend is distinctly present between the first and second rinses. Similar results are observed for potassium offsets with the striking difference that the magnitudes of the offsets for the first rinse are considerably smaller than for sodium except for the data from 7/13.

Table 6. Comparison of absolute percent errors (PE) and standard deviations (SD) obtained from serum analyses using the EasyLyte Plus and the prototype protable system and utilizing intermediately measured potentials as well as offsets in the portable system calculations for all three electrodes.

	ELP ('93) PE/SD	PROTOTYPE ('93) PE/SD	*F*	*t*
Na	2.3/1.1	1.8/1.1	1.0	-1.8
K	2.1/1.5	2.0/1.4	1.1	-0.3
Cl	3.1/1.1	1.4/1.0	1.2	-6.4
N	32	32		
Critical Values:	F = 1.79; 95% *df* = 31/31			
	t = 1.67; 95% *df* = 62			
Offset mV				
Na = -0.7				
K = -0.3				
Cl = 1.4				

The offsets shown in the Control section of Table 5 for the sodium ion are, in general, considerably smaller than the corresponding values for sodium in serum. This suggests that the controls do not simulate the matrix of serum as well as might be desired and that the offsets should be determined with actual blood serum. Controls from three different suppliers were employed, and the results combined. Three of the controls used were based on lyophilized human blood serum. This group of controls was comprised of a high and low control from Instrumentation Laboratories (SeraChem Red and SeraChem Blue) and an abnormally high control from Sigma Company. A second group of two controls, comprised of a normal and an abnormally high control, was supplied by the Medica Corporation. These controls were bovine based. Each of the controls was run twice to provide a total of 10 control analyses on each day.

Using the offset value from the first serum rinse on 6/25 for both sodium (-0.7 mV) and potassium (-0.3 mV), the recalculated results for the sodium and potassium analyses that were performed on 7/6, 7/13, and 7/16 were compared to those determined using the EasyLyte Plus system (Table 6). The negative *t* values that are associated with the portable system at the 95% confidence level indicate superior performance by the portable system. These data show that a significant difference favoring the portable system exists for the sodium analyses but that no significant difference exists between systems for the potassium analyses (although the results of the portable system were improved slightly by the use of the offset). A significant difference favoring the portable system for the chloride analyses also existed, as would be expected, since the same offsets were used for chloride in both Tables 4 and 6.

CONCLUSION

The results of this study demonstrate that an appropriate electrode-rinsing protocol between samples can improve the accuracy obtained using ion-selective electrodes in a portable system. Offset potential corrections for sodium, potassium, and chloride ion-selective electrodes manufactured by the Medica

Corporation can be determined experimentally on one set of blood serum samples. These results can then be used to significantly improve the accuracy of subsequent blood serum electrolyte measurements in a prototype portable blood electrolyte analyzer under laboratory conditions.

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