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Comparison of a calixarene-based ion-selective electrode with two automated analyzers for the clinical determination of sodium in blood plasma*

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Abstract: Neutral-carrier ion-selective electrodes based on methyl *p*-t-butylcalix[4]aryl acetate have been prepared that are responsive to sodium ions. The miniaturized catheter-type electrodes were obtained by dip-coating their porcelain tips in a PVC membrane cocktail. Examination of the general performance of the electrodes revealed excellent characteristics in terms of Nernstian response, selectivity, stability, reproducibility and response time. The results from the indirect potentiometric assessment of a large number of plasma samples with the electrodes showed a good correlation with the results from two automated analyzers (Technicon Smac 3, Hitachi 704) and with flame photometric data. Although inconsistencies were observed in the measurement of some plasma samples, the variance seemed to be method-dependent, and the overall performance of the electrodes showed promise as an alternative to the sodium glass electrode. Some factors influencing the standard potential of the measuring cell are discussed as a source of error.

Keywords: *Sodium analysis; steady state potentiometry; ISE (ion-selective electrodes).*

Introduction

Ion-selective electrodes (ISEs) have proven to be reliable sensors for the determination of target ions in chemical analyses [1, 2]. With respect to clinical analysis, ISEs incorporated into laboratory analyzers perform the vast majority of routine measurements of the important physiological ions [3, 4]. Currently, most automated analyzers that measure sodium levels utilize the sodium glass electrode. The problems associated with sodium-selective glass membranes are well documented with regard to their high electrical resistance [5], poor hydrogen selectivity [6], propensity toward adsorption of biological components (e.g. proteins) on the glass surface [7], and the technical difficulty of incorporating glass membrane electrodes in miniaturized flow cells. This latter problem is compounded by the fact that PVC ion-selective membranes are used for the determination of plasma potassium and calcium. The use of a sodium selective PVC membrane would enable a single coherent ISE block to be manufactured, which would greatly reduce the engineering problems associated with the hybrid blocks used in many systems at present. The fact that PVC membranes based on neutral-carriers can overcome the problems listed above has been widely recognized [8].

In search of alternative membranes to glass electrodes, chemists have focused on the development of solvent polymeric membranes, and extensive work has been devoted to the synthesis of compounds with suitable host–guest properties. Reports on the characteristics of sodium-selective PVC membranes based on acyclic structures [9, 10], the naturally occurring antibiotic ionophore monensin [11], and crown ethers [12] have been published.

Calixarenes are a relatively new group of macrocyclic compounds which possess a cup-like configuration into which a cation of suitable dimensions can fit to form a complex. By chemically modifying *p*-t-butylcalix[4]arenes (i.e. by attaching various groups to the phenolic functions of these tetramers), a high degree of phase transfer affinity for sodium ions can be attained [13]. Furthermore, PVC membranes that incorporate the methyl acetate tetramer (Fig. 1) have been shown to display a near-Nernstian potentiometric response to sodium, and exhibit excellent

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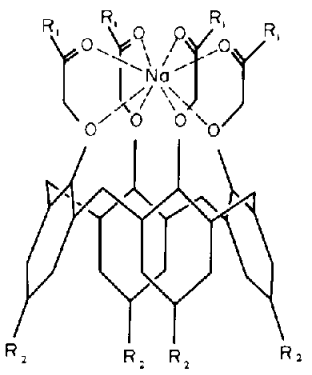

Figure 1
Structure of calixarene methyl *p*-t-butylcalix[4]aryl acetate encapsulating the sodium ion, R_1 = methoxy groups, R_2 = butyl groups.

selectivity against other clinically important cations [14]. The present work reports on the performance of ISEs that incorporate the methyl acetate tetramer in the determination of sodium in plasma samples.

Experimental

Materials and samples

All chemicals used for electrolyte solutions (NaCl, LiCl, KCl, $CaCl_2$, $MgCl_2$) were of analytical reagent grade. Salts of Tris buffer were obtained from Sigma; D-glucose (anhydrous) was obtained from BDH, and urea from Riedel-de Haen. The synthesis of the calixarene ionophore has been described earlier [13]. The plasticiser mediator 2-nitrophenyl octyl ether (2-NPOE), the lipophilic additive potassium *p*-tetrakis-chlorophenyl borate (KpTClPB), poly(vinyl chloride) (PVC) and tetrahydrofuran (THF) were all supplied by Fluka.

Plasma samples were obtained from St. James and Beaumont hospitals (Dublin, Ireland). Each provided their respective results for the sodium analysis. The St. James results were obtained with a Technicon Smac 3 analyzer, while those from Beaumont hospital were obtained with the Hitachi 704 analyzer. Some flame photometric data were also obtained from Beaumont hospital.

Membrane and electrode fabrication

The construction of catheter type mini-electrodes has been described elsewhere [14]. Basically, the porcelain tips of the PVC tubing, which act as the electrode body, were dipped several times in a membrane solution containing the calixarene complexing agent. When the polymeric film was formed on the tip, the electrodes were left to dry at room temperature for 24 h. The coating membrane used in this study consisted of 2.08 mg of ionophore, 202.69 mg of 2-NPOE, 0.52 mg KpTClPB, and 101.06 mg of PVC. The internal filling solution was 0.1 M NaCl. The electrodes were soaked for 1 h in 0.1 M NaCl before use.

Measurements

All measurements were made at 21 ± 1°C, except when the standard addition technique was used, during which the temperature was kept at 25°C using a thermostatted cell. A Corning 240 pH/millivoltmeter coupled to a Fluka 8060A digital multimeter with 0.01 mV resolution was used. The external reference was a saturated calomel electrode (SCE) with a free flowing tip (Metrohm ref. 60.705.000). The electrochemical cell could be represented by:

$$Hg,Hg_2Cl_2/KCl(satd)/\text{measured sample/PVC membrane/0.1 M NaCl/AgCl,Ag.}$$

The response curves were obtained from measurements taken in NaCl solutions ranging from 10^{-6} to 10^{-1} M prepared by serial dilution. Curves of pH versus e.m.f. were obtained by adjusting 10^{-2} and 10^{-3} M NaCl solutions to pH 10.5 with ammonia solution (ca. 1 M), and then lowering the pH to the desired value with concentrated HCl.

Plasma samples were stored at 4°C. At least 1 h after removal from storage was allowed for temperature equilibration to occur before measurements were taken. Samples and standards were stirred slowly during measurement. Results were obtained both from a calibration curve and by the standard addition method. Regression analysis was carried out using Deming's method [15].

The calibration solutions consisted of five artificial plasma samples containing 0.100, 0.125, 0.140, 0.155 and 0.180 M NaCl, respectively, with a fixed background of K^+ (4.4 mM) Ca^{2+} (2.4 mM), Mg^{2+} (0.8 mM), glucose (4.7 mM), and urea (2.5 mM). An identical procedure was used for the calibration solutions and the plasma samples: 1-ml aliquots of the calibrant solutions and plasma samples were diluted 10-fold by adding 9 ml of a diluent (pH 7.4 Tris buffer in 0.11 M LiCl). The fixed excess of 0.11 M LiCl in the solution maintained the ionic strength such that a relatively

constant sodium activity coefficient (ranging from 0.758 to 0.754 in the calibrants) was obtained. This enabled the ISE to be calibrated in terms of sodium concentration rather than activity. From previous work [14], the ISE was known to be very selective against Li^+ ions, and at this level, the LiCl did not affect the ISE response to sodium. Unless stated otherwise, the measurements were taken after 2 min.

For the standard addition method, the following routine was employed: 1 ml of plasma was diluted 10-fold and the e.m.f. of the cell was recorded (E_1). After addition of a standard solution of known sodium concentration, a second e.m.f. value was obtained (E_2). The concentration of the analyte (C_a) was calculated using equation (1) [16]:

$$C_a = \frac{V C_s}{V_o \left\{\left[\text{antilog}\left(\frac{E_2 - E_1}{S}\right)\right]\left(\frac{V}{V_o + V_r} + 1\right) - 1\right\}}, \quad (1)$$

where V_o is the known volume of plasma sample containing an unknown sodium concentration C_a, C_s is the concentration of a standard sodium solution of volume V added to the sample, V_r is the total volume of reagents added to the sample (diluent buffer) and S is the calibration slope of the electrode. After addition of a diluent volume V_d (as for V_r), the e.m.f. (E_3) was obtained and the slope (S) was calculated according to equation (2) [16]:

$$E_2 - E_3 = S \log\left(\frac{V_o + V + V_d}{V_o + V}\right). \quad (2)$$

Results

An average Nernstian slope of 59.6 mV decade^{-1} (SD ± 0.17 mV) was obtained for five electrodes when the response to the concentration range of 10^{-4} to 10^{-1} M NaCl was measured. The linear range for clinical application has been previously examined over the 50–500 mM range [17]. The mean resistance of the electrodes in 0.1 M NaCl was 1.03 ± 0.04 MΩ. Stability was examined by immersing two of the electrodes in stirred 10^{-2} M NaCl and monitoring the output of each versus a saturated calomel reference electrode (SCE) over a 24-h period. In both cases, the drift was found to be +1.7 ± 0.1 mV. However, results from five electrodes showed a drift of +0.5 ± 0.1 mV over a 1-h period. Continuous recalibration is therefore advisable unless correction is applied while performing sample run experiments. A drift magnitude of about 0.3 mV was observed when recalibrating the electrodes. The reproducibility of the electrode response when transferred from a 10^{-3} M to a 10^{-2} M NaCl solution was ±0.08 mV, and ±0.22 mV when transferred in the opposite direction (average of five measurements in each case, with every sample measured after 2 min). The electrodes exhibited fast response times (t_{90} <10 s) to transient shifts involving a change in concentration from 10^{-4} to 10^{-3} M NaCl (Fig. 2).

In selectivity investigations, the electrodes displayed excellent discrimination against Li^+, K^+ and H^+ ions, with the respective selectivity coefficients (log K^{pot}_{NaJ}) being −2.86, −2.59 and −1.98, respectively (values determined in each solution at a concentration of 0.1 M). Variation in pH had little effect on the electrode response, with <1.0 mV variation between pH 9.5 and 5.5 in either 10^{-3} or 10^{-2} M NaCl solutions, and virtually no change (<0.1 mV) over pH values of interest for plasma determinations (7.0–7.6).

One electrode was selected for use in the plasma tests and was used throughout the rest of the experimental work. Figure 3 and Table 1 show a comparison of results obtained with a Hitachi plasma analyzer and the ISE using a calibration curve. In the first set of 61 samples, the electrode was calibrated before and after 20 samples, and the readings were taken after 1 min equilibration time. The correlation coefficient for this set (r = 0.820) showed no difference with a second set of 59 samples in which the electrode was recalibrated after five samples and the e.m.f. was obtained after 2 min (r = 0.816). Although inconsistency was found with the values for the slope, intercept and standard deviation of the residuals, an approximate correlation between the ISE results and the Hitachi results is evident. Further results (Fig. 2) of 18 samples with the

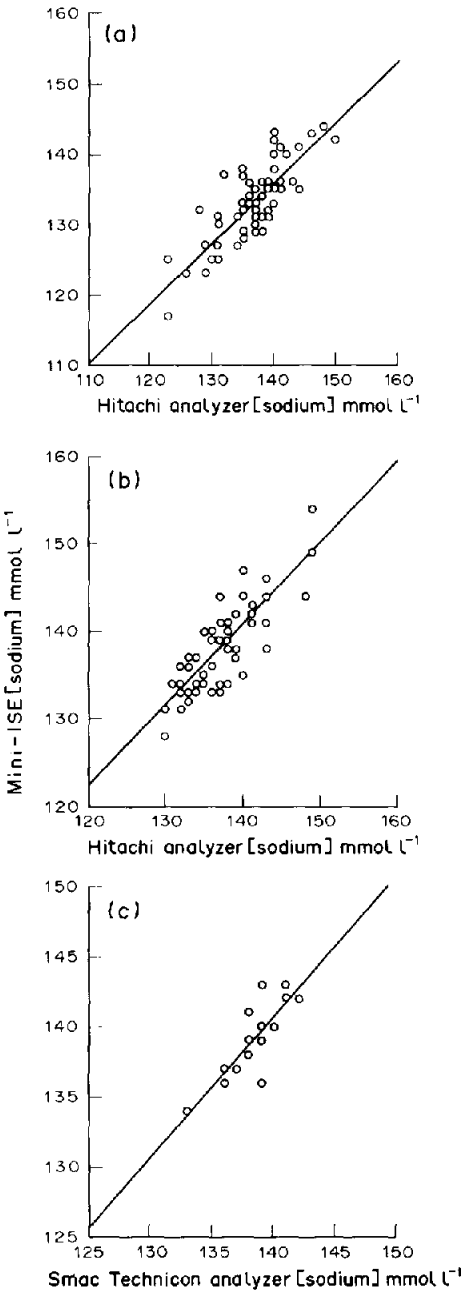

Figure 2
Correlation for Na^+ measurements between mini-electrodes based on the calixarene ligand and Hitachi analyzer: (a) reading taken at 1 min; (b) readings at 2 min; and Smac Technicon analyzer (c) taken from calibration curves. Equations: (a) $y = 0.93x + 5.86$, $r = 0.820$; (b) $y = 1.01x - 0.37$, $r = 0.816$ (c) $y = 1.11x - 15.03$, $r = 0.827$.

Technicon Smac analyzer showed a similar correlation coefficient ($r = 0.827$). In another batch of samples, the results from the ISE (Table 2) showed improved agreement when compared with flame photometry, although a smaller number of samples was assayed. The

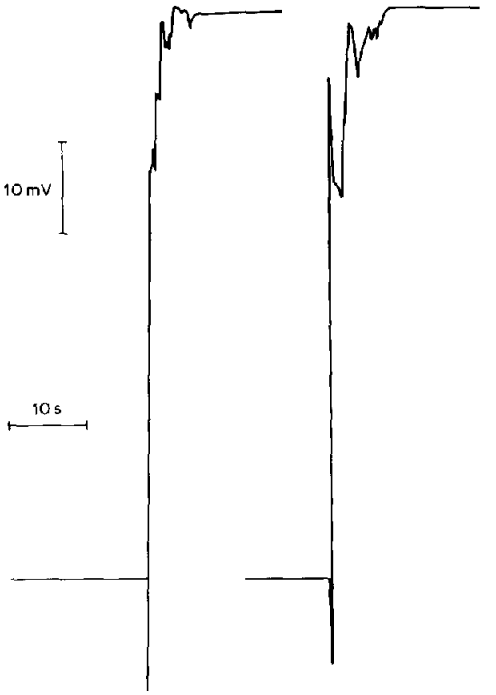

Figure 3
Dynamic response of mini-ISE to injections of 500 µl of 0.1 M NaCl into 40 ml of 10^{-4} M NaCl.

standard addition technique did not improve the correlation with the Hitachi analyzer, and a higher residual standard deviation of y on x was obtained. The results of 17 samples as depicted in Fig. 4 gave a correlation coefficient of 0.79.

Discussion

When making steady-state potentiometric measurements, one must be careful to ensure that the standard cell potential remains constant throughout the duration of the experimental work. In general, the sample/standard matrix must be matched, a constant temperature must be maintained, and an identical procedure for each measurement must be followed.

Variation in standard cell potential arises mainly from changes in the ISE external boundary potential or the reference electrode junction potential. In plasma samples, the ISE membrane external boundary is affected both by protein coating and by extraction of marginally lipophilic membrane components into the plasma. Variation in the reference electrode junction potential can also arise due to

Table 1
Comparison of linear regression data for plasma sodium determination by different methods versus mini-ISE based on methyl *p*-t-butylcalix[4]aryl acetate

n	Assay	Slope	Intercept	*r*	Residual SD (mM)
61*	Hitachi	0.93	5.86	0.820	±3.0
59*	Hitachi	1.01	−0.37	0.816	±2.6
18*	Technicon Smac	1.11	−15.03	0.827	±1.3
17†	Hitachi	1.02	−1.95	0.792	±3.2
7*	Flame photometry	1.00	−1.14	0.904	±1.4

*Mini-ISE results obtained using a calibration curve.
†Performed by standard addition method.

Table 2
Sodium concentration (mM) and correlation coefficient measured for plasma samples by two different ISEs and flame photometry

Sample	Mini-ISE*	Flame photometry	Hitachi analyzer (glass electrode)
1	138	140	139
2	138	138	137
3	130	133	133
4	140	140	139
5	140	142	141
6	136	135	136
7	136	138	137

Correlation coefficient		
Mini-ISE versus Hitachi	Mini-ISE versus flame photometry	Flame photometry versus Hitachi
0.932	0.904	0.979

*Results obtained from calibration curve.

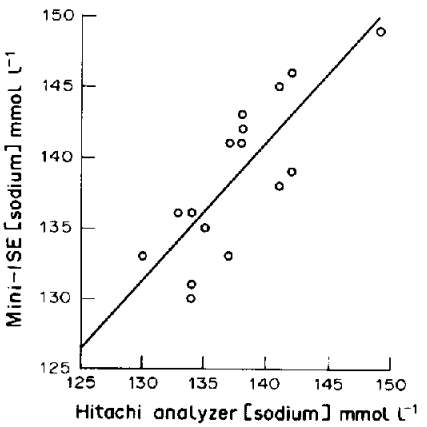

Figure 4
Correlation of duplicated measurements of Na^+ by standard addition using mini-ISEs versus Hitachi analyzer employing glass membrane sodium ISE, $r = 0.79$. Average slope determined for each analysed sample 57.8 ± 0.9 mV decade^{-1}.

obstruction of the junction by proteins "salted out" by the bridge electrolyte (saturated KCl), or by slight differences in the plasma/standard matrices. Problems arising from protein deposition can be minimized by using a micro-capillary free-diffusion junction [18] of the type used in this study.

In most applications, these minor fluctuations are of little consequence. However, in the determination of plasma sodium, the normal range is relatively small (135 to 145 mM), and is equivalent to a theoretical ISE response change of approximately 1.9 mV. Thus, good precision and accuracy is difficult to achieve by means of simple "dip" methods.

Conclusions

The results demonstrate that the ISEs that incorporate the macrocyclic carrier studied can be used in the clinical determination of sodium ions. An important advantage, which has been found particularly attractive for this electrode. is the selectivity pattern exhibited against LI^+, K^+ and H^+ ions [14]. Although measurements by the classical dipping technique pose limitations in terms of best precision and accuracy, improvements can be expected if flow methods are used. In this respect, dynamic character-

istics are improved since, with suitable cell design, the rate of transport processes is improved. Chemical and mechanical interferences associated with the electrode operation (washing, cleaning, changing solution, etc.) are significantly smaller in flow systems compared with the steady-state technique. Important to note is that ISEs can be easily adapted to flow cells, as the signal is basically independent of the solution flow rate [19]. Work is currently in progress in this area.

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