

Applications and Characteristics of Polymer Membrane
Ion-Selective Electrodes

A thesis presented for the degree of Master of
Science.

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This work is an accurate record of work carried out
by Francis Regan during the period October 1988 to
August 1989.

School of Chemical Sciences, Dublin City University,
August 1990.

Foreword

Francis Regan completed one year of full-time research at the School of Chemical Sciences, Dublin City University, and would undoubtedly have continued to a Ph.D. had he not died tragically in October 1989 after a long and courageous battle against leukemia. It was typical of him to choose a research topic which was mainly concerned with developing and improving sensors for blood analysis, so that his work might contribute to more efficient monitoring methods.

During the course of his research, he completed two major projects, both of which have been published, and had started another two, both of which are still being pursued at the time of writing.

Chapter 1 is a reprint of work carried out on the use of d.c. resistance measurements of ISE membranes for diagnostic purposes and which was published in the journal *Electroanalysis* (volume 2 (1990) 113-117).

Francis was also involved in attempting to apply a novel sodium electrode for the purpose of analysing sodium in human plasma. He combined regular check-ups at St James Hospital with the collection of plasma samples for his experiments, and together with his co-worker, Martin Telting-Diaz, showed that these devices could successfully be used for plasma sodium determination. This work is presented in chapter 2, and has been accepted for publication in the *Journal of Pharmaceutical and Biomedical Analysis*.

He was additionally involved in two other projects, one being an attempt to automatically calibrate ISEs using a constant dilution cell, and the other a collaborative study with Rory Boland and Jim Dowling of the School of Electronic Engineering into the use of ISE arrays. Unfortunately, neither of these projects reached a stage where they could be published, but he has provided a useful basis for fellow researchers who are continuing this work, notably Aodhmar Cadogan and Eamonn McEnroe (ISE characterisation) and Robert Forster (sensor arrays).

It is fitting that his work should be collated into a thesis, as the areas he worked in were innovative, and will serve as a useful guide to others who will follow in his footsteps. In addition, it will be a permanent record of the achievements of a person we will all remember fondly as a friend and colleague.

A handwritten signature in cursive script that reads "Dermot Diamond". The signature is written in dark ink and is positioned above the typed name.

Dermot Diamond, Dublin City University, August 1990.

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Chapter 1. Resistance Measurements as a Simple Diagnostic Tool for Ion-Selective Electrode Performance.

1.1 Introduction

Since the late 1960's, much experimentation with neutral-carrier substances has resulted in more selective and more stable ionophores being developed [5,6]. The use of these electrically-neutral metal-ion complexing agents in liquid-membrane electrodes has provided greater ion-selectivity and quite acceptable working lifetimes [7]. The major requirement for any neutral-carrier is its selective chelation of the target ion. The configuration or spatial arrangement of atoms in the ionophore governs to a large extent the resulting ionic selectivity [8]. The concentration of charge-carriers in the membrane is important in the provision of an adequate trans-membrane ionic transport system. Studies have shown that, in order to maintain the selective properties only a small percentage of available carrier ligands should be engaged in ion-complex formation [9], and at any instant in time, the bulk of charge-carriers should exist in the free or uncomplexed form. This situation is analogous to other exchange reactions, for example in chromatography, where only a small

proportion of the active sites in a column should be populated by sample species at a given time.

In a potentiometric circuit, the total circuit resistance will include contributions from the measuring circuitry, the reference electrode, the ion-selective electrode (ISE) components excluding the ion-sensitive membrane, the sample solution and the ISE membrane. Large variations in cell resistance can only arise from the ISE or the external reference electrode. With reference electrodes (such as the saturated calomel or silver/silver chloride electrodes), the most common cause of resistance fluctuations will be blockage of the junction between the internal bridge electrolyte and the sample solution arising from the formation of bubbles or solid material. In the latter case, this can happen due to precipitation of sample components on contact with the diffusing reference electrode solution. Resistance fluctuations can be easily checked by combining the suspect reference with a good reference electrode and measuring the resistance or potential. With the ISE, large variations in resistance are indicative of the state of the ion-selective membrane. For PVC/liquid-membrane electrodes of the type used in this study, resistances are typically of the order of $0.5\text{M}\Omega$ to $10\text{M}\Omega$ for freshly made electrodes, and the resistance

will gradually rise in use as the active components are slowly leached out [10]. These values are at least an order of magnitude higher than all other contributions to the total cell resistance, meaning that the overall cell resistance is dominated by the ISE membrane resistance.

The modification of microelectrode selectivity and sensitivity on inclusion of a lipophilic anion (in neutral-carrier based membranes) has been widely reported [9,11]. An accompanying reduction (typically 10-fold) in membrane resistance is thought to be due to anion participation in cation transfer across the aquo-membrane boundary. A low membrane resistance, and hence low overall circuit resistance will obviously help to minimize the effect of any electrical noise arising within the circuit. This work investigates the effect of loss of membrane sensing components, with time, on selectivity, sensitivity and membrane resistance. We also examine the possibility of using d.c. cell resistance measurements for frequent assessment of sensor performance and rapid indication of electrode malfunction.

1.2 Experimental

1.2.1 Reagents

Aqueous standards were prepared using Analar grade Chloride salts obtained from Riedel-de Haen with distilled, de-ionised water. The membrane components poly (vinyl chloride)(PVC), potassium tetrakis (para-chloro phenyl)borate (KTPClPB), and 2-nitrophenyl octyl ether (2-NPOE) were obtained from Fluka Chemicals. Details of the ionophore synthesis have been published previously [5].

1.2.2 PVC membrane electrode fabrication.

Mini-electrodes were constructed using PVC tubing (0.8mm i.d.) containing a chloridised silver wire as internal reference and a 0.1M NaCl filling solution, after the design of Linton et al[12]. A 4mm long ceramic frit was inserted in the end of the PVC tube and sealed using Tetrahydrofuran (THF). Membranes were prepared using THF as solvent (see Table 1 for original membrane composition) and dip-coated onto the ceramic support. Allowed setting time was 24 hours with a 30 minute membrane 'soaking' period in 0.1M NaCl prior to performance testing. The method of 'infinite dilution' involved repeated dilution of

the PVC membrane components using a PVC:NPOE diluent (1:2 w/w). Five mini-electrodes were constructed at each dilution stage and performance tested.

1.2.3 Experimental methods and measurements

The potentials of PVC membrane electrodes were measured in a series of aqueous NaCl standards in the range 10^{-4} M to 10^{-1} M sodium against a free-flowing saturated calomel electrode using a Corning 240 digital pH meter as a high impedance voltmeter (input impedance = 10^{12} Ω). Potentials were noted after 1 minute equilibration in stirred solutions. Selectivity coefficients ($K^{pot}_{i,j}$), as defined by the well known Nikolsky-Eisenman equation [11], were determined using the Separate Solution method [13] in 0.1M chloride salt solutions of some common ions. Resistance measurements were obtained using a Fluke 8060A digital multimeter. Membrane resistances were estimated by the following procedure;

1. Measure the resistance of a normal (i.e. membrane coated) ISE and reference cell (R_1);

2. Measure the resistance of a cell identical to that above except in that the ISE has no membrane coating (R_2);

The contribution of the membrane to the cell resistance (R_m) is given by (figure 2);

$$R_m = R_1 - R_2 \quad (1)$$

1.3 Results

Initially, the effect of the individual constituents on variation in membrane d.c. resistance was explored (Table 2). Blank plasticised membranes (item 1, table 2) were found to have very high resistances ($>300M\Omega$), while membranes containing the ionophore had resistances in the range $10M\Omega$ to $20M\Omega$ (item 2, table 2). Inclusion of the exchanger reduced the resistance further as expected to between $2M\Omega$ to $4M\Omega$.

The concentration of the ionophore at each step in the infinite dilution of the liquid membrane is given in table 3. Batches of five electrodes were prepared at each stage and the effect on membrane sensitivity and selectivity determined. Overall electrode performance remained relatively unimpaired until step five. At this point, electrode sensitivity (figure 3) and selectivity (figure 4) completely collapsed. In a similar manner, d.c.

resistance readings for electrodes increased slowly for the first four dilution steps. At step five, a massive increase in resistance occurred coinciding with the complete collapse of selectivity and sensitivity described above.

In tandem with this work, two batches of five electrodes (0.66% w/w ionophore) were constructed and stored in 0.1M NaCl, and distilled water respectively. At regular intervals, sensitivity, selectivity and membrane resistance measurements were made in aqueous standards. After 150 days storage, only minor variations in initial resistance and response characteristics were observed (figure 6), these changes corresponding to a slight loss of active ingredients with time according to figure 5.

1.4 Discussion

A distinct reduction in membrane d.c. resistance was observed on inclusion of the lipophilic ion-exchanger in the membrane cocktail, which confirmed previous studies [9,14]. The high resistance readings obtained for the blank plasticised membranes were indicative of inefficient ion transport across the membrane due to lack of charge-carriers. Assuming a similar rate of loss of

ligand/exchanger on dilution, reduction in the concentration of sensing components is seen to affect the sensitivity and the ion-selective nature of the liquid membrane only when the concentration falls to a critical level (see figures 3 and 4). Figure 5 shows that below this critical concentration i.e. around 9.0×10^{-4} mole of ionophore in the plasticiser, the membrane d.c. resistance rises steeply. It therefore follows that electrodes with resistances below the critical resistance should perform satisfactorily, in contrast to those with resistances above it. This provides a simple diagnostic test for satisfactory electrode performance, and an approximate indicator of useful device lifetime (around two years for these electrodes), assuming that the rate of increase in electrode resistance remains constant.

A possible interpretation of the hyperbolic form of figure 5 is that below the critical concentration, most of the ionophore molecules are involved in ion-complexation, and the condition of excess ionophores required for manifestation of electrode selectivity and sensitivity mentioned earlier is not met. Above this level, excess free ionophores are available, and the anticipated selectivity and sensitivity is obtained. It is interesting to note that this

critical ionophore level is similar in magnitude to that reported previously with other ionophores [7].

Analogies can be drawn to the effect of doping charge carriers into semiconductors, where initially, as small amounts of the tri- or penta-valent impurities are added, the resistance of the material decreases sharply, until eventually, the material becomes saturated. At this stage, further additions of charge carriers has relatively little effect on the resistance [15].

This work adds to the evidence presented by other workers [7], that total electrode failure occurs when ionophore concentration falls below a critical level. Resistance measurements can be used to give a rough measure of the concentration of ionophore in the membrane, and hence provide warning of impending malfunction. In addition, sudden low resistances are indicative of major physical damage, such as membrane rupture or tearing, which allow a low resistance pathway between the ISE internal electrolyte and the sample solution. Sudden high resistances may be caused by faults in the external measuring circuit, or by the formation of impervious coatings on the ISE membrane.

Resistance measurements are easy to make and the circuitry required is cheap and reliable. The hardware can be incorporated into conventional potentiometric measurement circuits and the resistance of electrodes regularly checked. For automated systems using arrays of electrodes, this provides a simple method which can give advance warning of a deteriorating or damaged electrode independently of information obtained by means of routine calibration.

1.5 References

1. V.P.Y. Gadzekpo, G.J. Moody and J.D.R. Thomas, Anal. Proc., 23 (1986) 62.
2. W. Davison and T.R. Harbinson, Analyst, 113 (1988) 1537.
3. C.W. Beswick, G.J. Moody and J.D.R. Thomas, Anal. Proc., 26 (1989) 2.
4. M.A. Arnold and R.L. Solsky, Anal. Chem., 58 (1986) 84R.
5. M.A. McKervey, E.M. Seward, G. Ferguson, B. Ruhl and S.J. Harris, J. Chem. Soc., Chem. Commun., (1985) 388.
6. G.J. Moody, B.B. Saad and J.D.R. Thomas, Analyst, 144 (1989) 15.
7. U. Oesch and W. Simon, Anal. Chem., 52 (1980) 692.
8. W.E. Morf and W. Simon, Helv. Chim. Acta., 54 (1971) 2683.
9. D. Ammann, W.E. Morf, P. Anker, P.C. Meier, E. Pretsch and W. Simon, Ion-Selective Electrode Rev., 5 (1983) 3.
10. G.J. Moody and J.D.R. Thomas: Organic Sensor Materials in Entangled and Polymer-Bound Matrices for Ion-Selective Electrodes, in T.E. Edmonds (ed.): "Chemical Sensors", Blackie, Glasgow and London (1988), p. 111.
11. D. Diamond, G. Svehla, E.M. Seward and M.A. McKervey, Anal. Chim. Acta., 204 (1988) 223.
12. A.F. Linton, M. Lim and D.M. Band, Crit. Care Medicine, 10 (1982) 337.
13. G.J. Moody and J.D.R. Thomas, "Selective Ion-Sensitive Electrodes", Merrow, Watford (1971), Ch. 2, pp 10-20.
14. D. Ammann, E. Pretsch, W. Simon, E. Lindner, A. Bezegh and E. Pungor, Anal. Chim. Acta., 171 (1985) 119.
15. S.M. Sze, "Semiconductor Devices, Physics and Technology", Wiley, New York (1985), Ch. 2.

1.6 Figures

Figure 1: Structure of the Sodium-selective ionophore.

oxygen atom large circle
carbon atom standard circle

Hydrogen atoms not included for clarity.

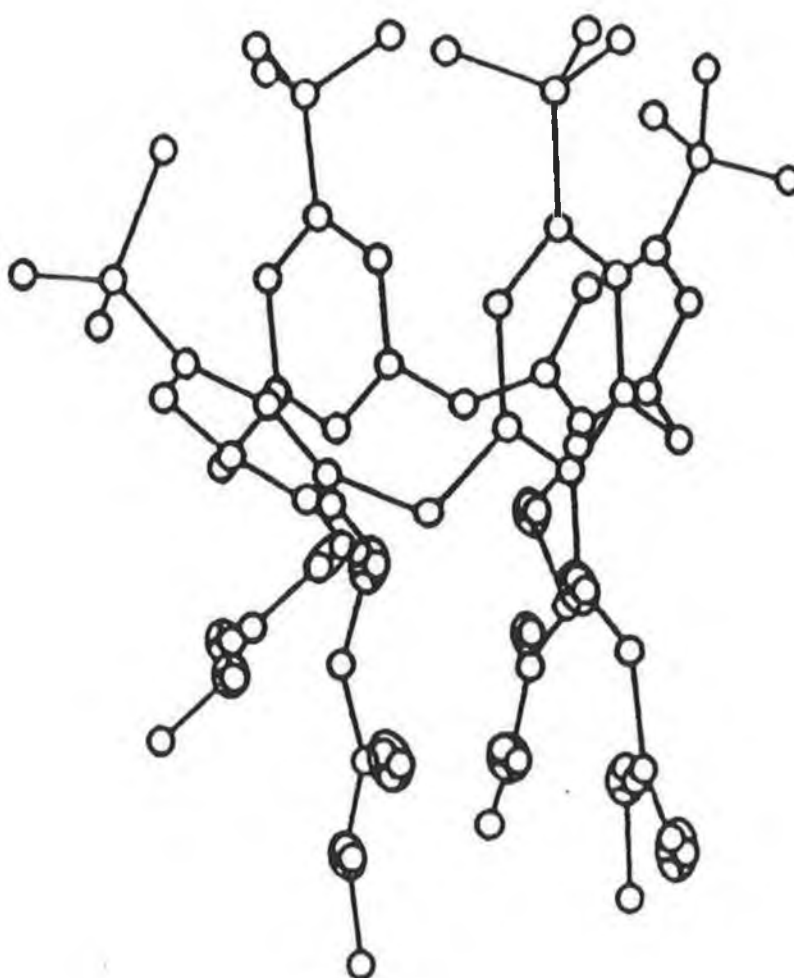
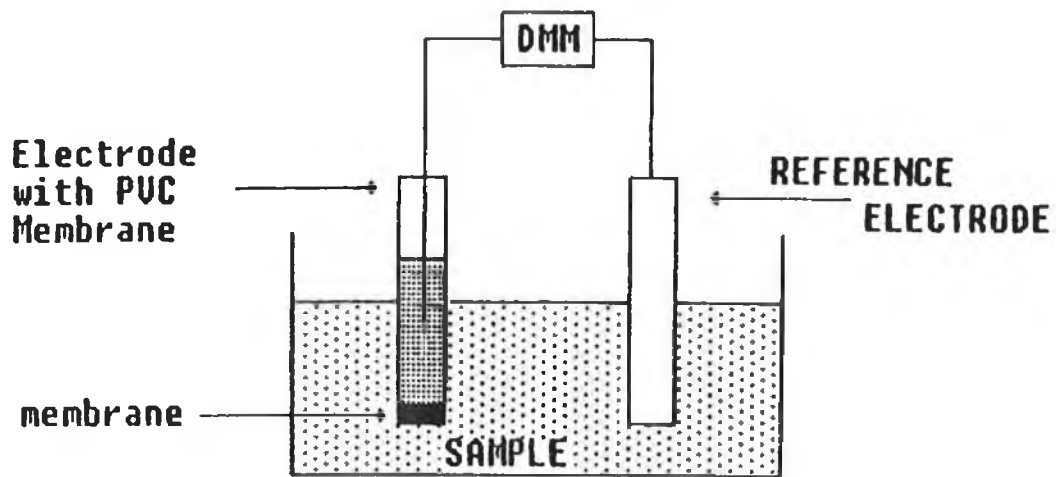
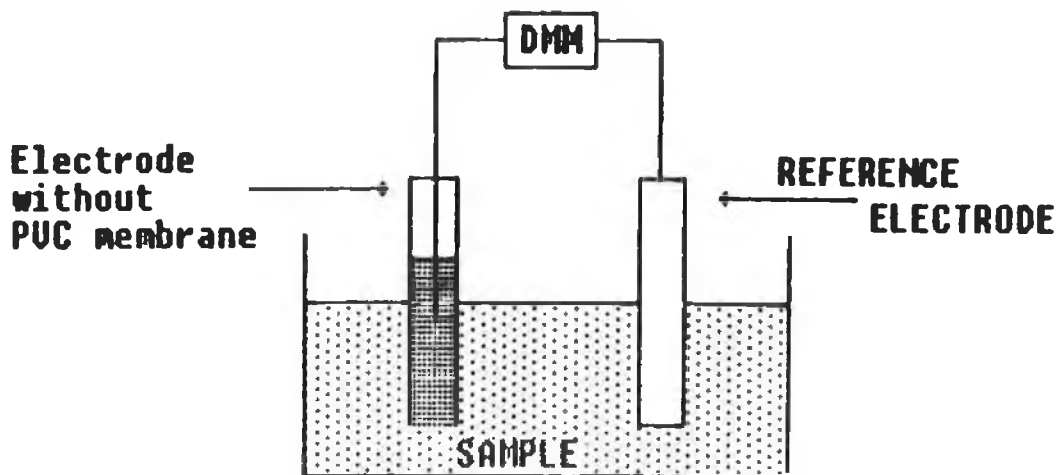


Figure 2: Method of measuring membrane resistance.



Stage 1: Resistance of Cell with PUC-coated ISE (R_1)



Stage 2: Resistance of cell with uncoated ISE (R_2)

$$\text{Membrane resistance } (R_m) = R_1 - R_2$$

Figure 3: Variation in sensitivity on dilution of the membrane components.

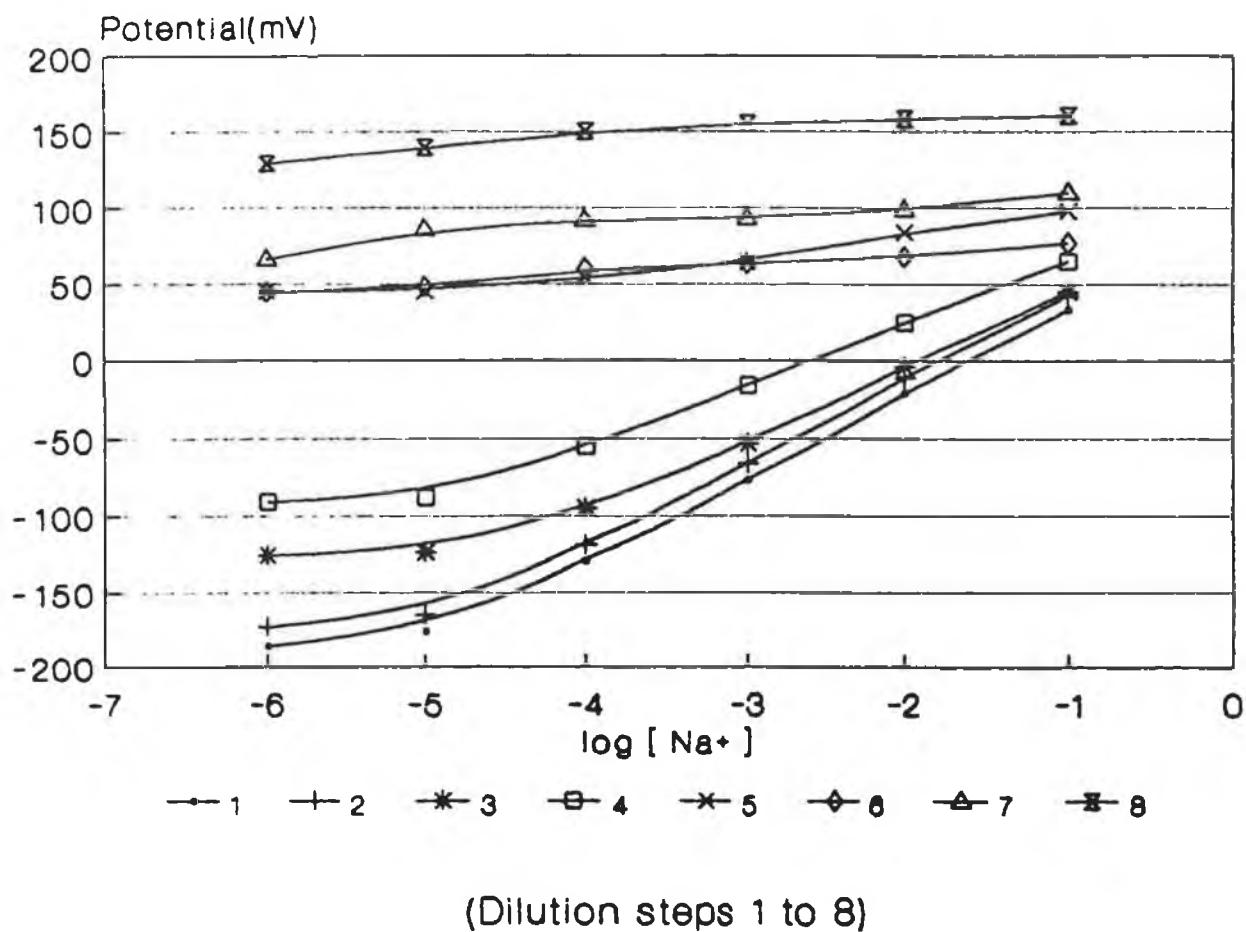


Figure 4: Effects on electrode selectivity (K^{pot}_{ij}) with dilution of the membrane components. i = primary ion (Na^+) ; j = interfering ions (Li^+ , K^+ , NH_4^+ , Ca^{2+} , H^+)

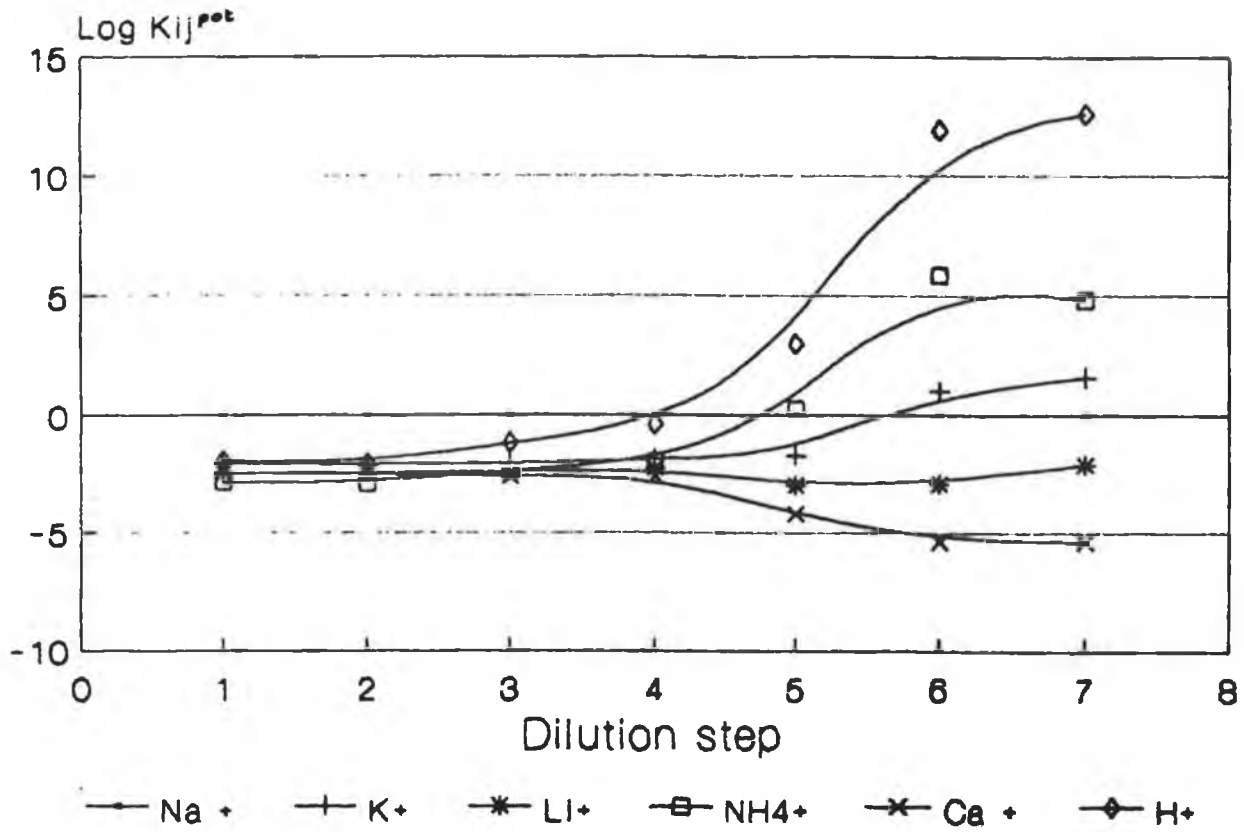


Figure 5: Variation in electrode d.c. resistance on dilution of membrane sensing components.

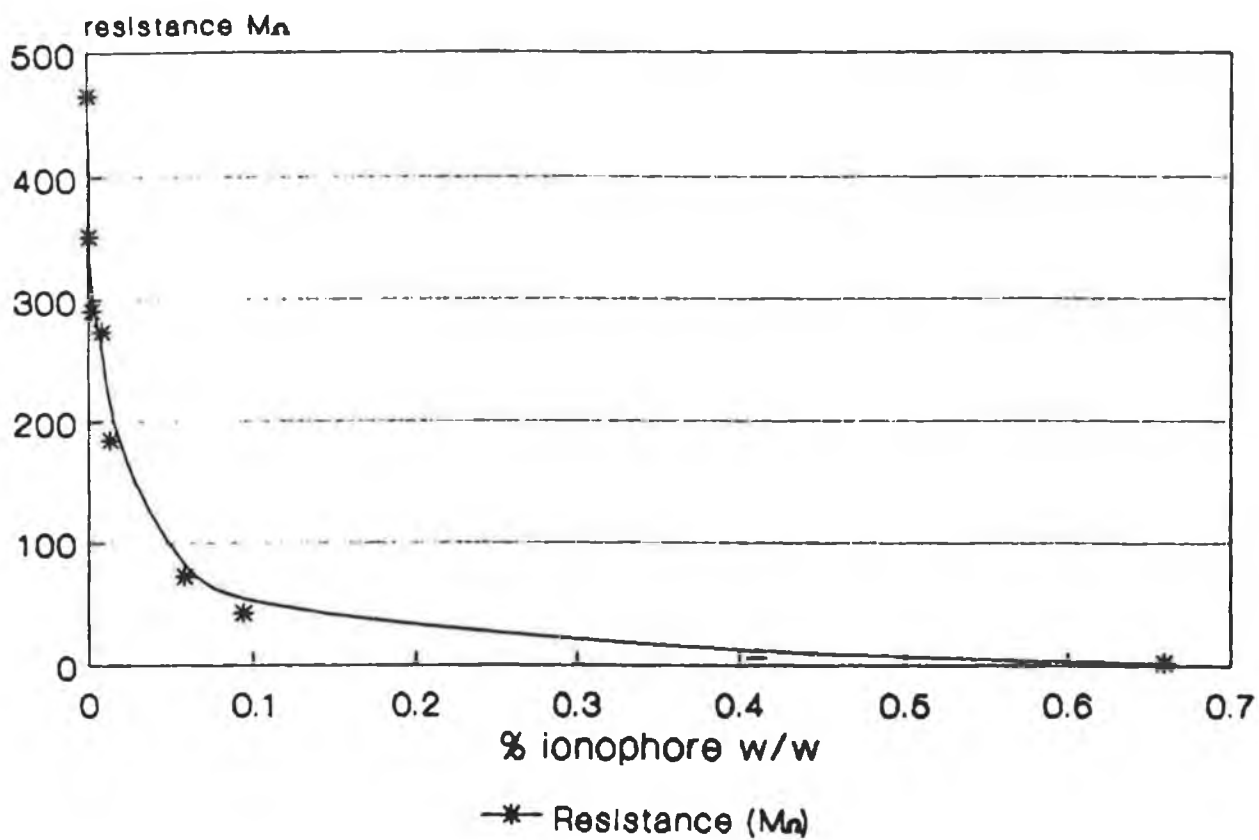
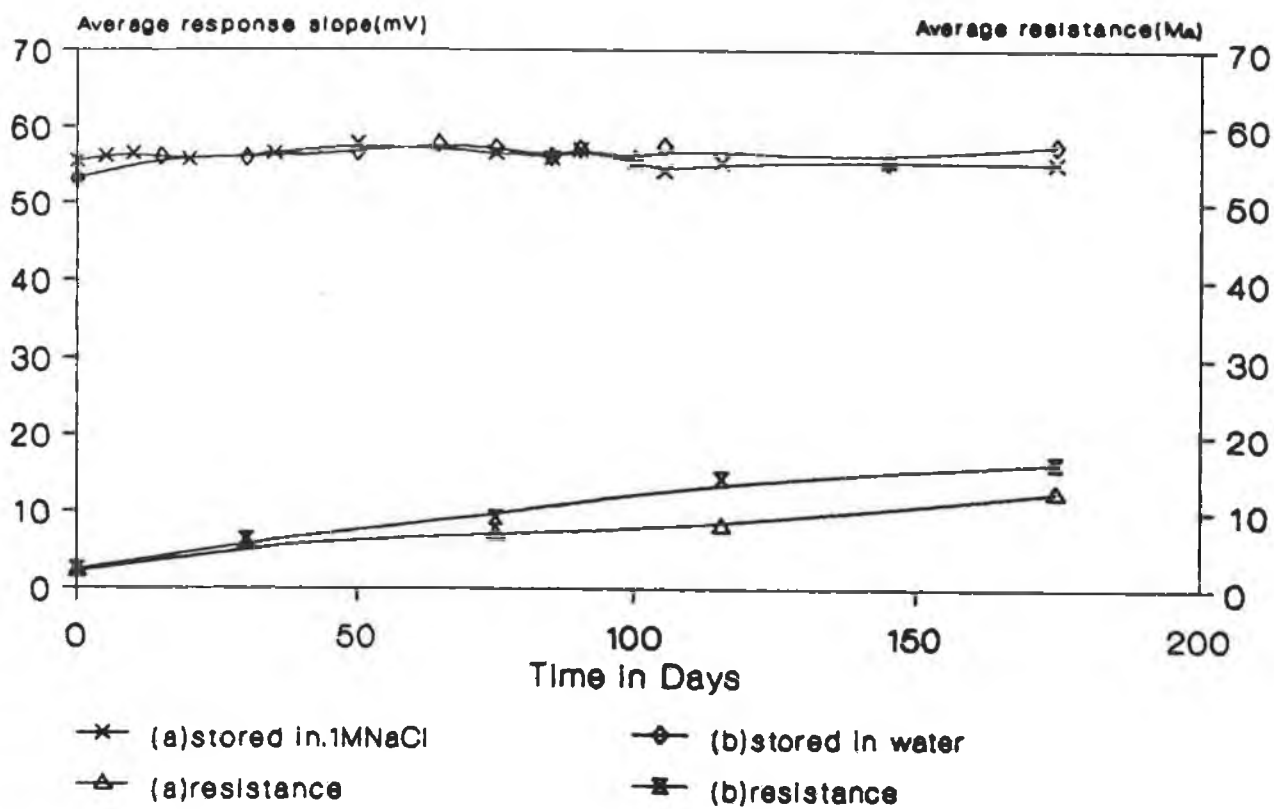


Figure 6: Ageing profile of electrodes represented by changes in sensitivity and d.c. resistance.



1.7 Tables

Table 1: Initial membrane composition

Component	%w/w
Plasticising solvent (2-NPOE)	66.10
Ion-exchanger (KTPClPB)	0.16
Poly (vinyl chloride) (PVC)	33.10
Ionophore	0.66

Table 2: d.c. resistance data for electrodes of varying membrane composition

Membrane Composition (w/w%)

No.	PVC	Plasticiser	Ligand	Exchanger R	(M Ω)
1	66.5	33.5	0.00	0.00	>300
2	66.2	33.1	0.66	0.00	10-20
3	66.1	33.1	0.66	0.16	2-4

Table 3: Steps taken in the dilution of the liquid membrane. (Tabulated response slope and resistance values reflect the average values from five electrodes at each stage).

Dilution step.	Log [ionophore]	%(w/w) ionophore	Response slope(mV)	Resistance (M Ω)
1.	-1.658	0.66	56.5	2.23
2.	-1.854	0.41	55.8	6.97
3.	-2.509	0.094	48.6	42.3
4.	-2.721	0.059	41.6	72.2
5.	-3.366	0.013	15.7	185.0
6.	-3.575	0.008	5.8	274.0
7.	-4.176	0.002	6.2	291.0
8.	-5.000	0.0003	3.4	352.0

Chapter 2. The Application of Calixarene-based ISEs to the Analysis of Sodium in Blood Plasma

2.1 Introduction

Ion-selective electrodes (ISEs) have proven to be reliable sensors for the determination of target ions in chemical analysis[1,2]. With respect to clinical analysis, ion-selective electrodes incorporated into laboratory analyzers perform the vast majority of routine measurements of the important physiological ions[3,4]. Most automated analyzers presently performing measurements for sodium incorporate the sodium glass electrode. The problems associated with sodium-selective glass membranes are well documented in relation to high electrical resistance[5], poor hydrogen selectivity[6], deposition of biological components (e.g. proteins) on the glass surface [7], and the technical difficulty of incorporating glass membrane electrodes in miniaturised 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 such problems has been widely recognised[8]. In search of alternative membranes to glass electrodes, chemists have focussed 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 be drawn to form a complex. By chemically modifying p-t-butylcalix[4]arenes (that is, 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, it has been shown that PVC membranes incorporating the methyl acetate tetramer (Fig. 1) display a near-Nernstian potentiometric response to sodium and exhibit excellent selectivity against other clinically important cations[14]. The present work reports on the performance of ISEs incorporating the methyl

acetate tetramer in the determination of sodium in plasma samples.

2.2 Experimental

2.2.1 Materials and samples

All chemicals used for electrolyte solutions (NaCl, LiCl, KCl, CaCl₂, MgCl₂) 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 one provided their respective results for the sodium analysis. The St. James results were obtained with a Technicon Smac 3 analyser, while those from Beaumont hospital were obtained with the Hitachi 704 analyser. Some flame photometric data were also obtained from Beaumont hospital.

2.2.2 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 acts 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 hours. 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 0.1M NaCl. The electrodes were soaked for one hour in a 0.1M sodium chloride solution before use.

2.2.3 Measurements

All measurements were made at $21 \pm 1^\circ\text{C}$, except for the standard addition technique, 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 millivolt resolution was used. The external reference was a saturated calomel electrode (SCE) with a free

flowing tip (Metrohm ref. 60.705.000). Overall, the electrochemical cell can be represented by:

Hg,Hg₂Cl₂/KCl(satd)/measured sample/PVC membrane/
0.1M NaCl /AgCl,Ag

The response curves were obtained from measurements taken in NaCl solutions ranging from 10^{-6} to 10^{-1} mol l⁻¹ made up by serial dilution. Curves of pH vs emf were obtained by adjusting 10^{-2} mol l⁻¹ and 10^{-3} mol l⁻¹ NaCl solutions to pH 10.5 with ammonia solution (ca. 1M) and then lowering the pH to the desired value with concentrated HCl. Plasma samples were stored at 4°C. At least one hour was allowed after removal from storage for temperature equilibration to occur before measurements were taken. Samples and standards were stirred slowly during measurements. 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 0.180M NaCl respectively, with a fixed background of K⁺ (4.4mmol l⁻¹), Ca²⁺ (2.4 mmol l⁻¹), Mg²⁺ (0.8 mmol l⁻¹), glucose (4.7 mmol l⁻¹), and urea (2.5 mmol l⁻¹).

An identical procedure was adopted for the calibration solutions and the plasma samples. The procedure was as follows:

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 mol l⁻¹ LiCl). The fixed excess of 0.11 mol l⁻¹ LiCl in the solution dominates the ionic strength, and results in a relatively constant sodium activity coefficient (ranging from 0.758 to 0.754 in the calibrants). This enables the ISE to be calibrated in terms of sodium concentration rather than activity. From previous work[14], it is known that the ISE is very selective against Li⁺ ions, and at this level, it did not affect the ISE response to sodium. Unless stated otherwise the measurements were taken after two minutes.

For the standard addition method, the following routine was employed:

1 ml of plasma was diluted and the emf of the cell was recorded (E_1). After addition of a standard solution of known sodium concentration, a second emf value was obtained (E_2). The concentration of the analyte (C_2) can then be calculated using equation (1), below[16]:

$$C_a = \frac{V C_s}{V_0 \left[\text{antilog} \left(\frac{E_2 - E_1}{S} \right) \left(\frac{V}{V_0 + V_r} + 1 \right) \right]^{-1}} \quad (1)$$

where V_0 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 emf (E_3) was obtained and the slope (S) calculated according to equation (2) below[16]:

$$E_2 - E_3 = S \log \left[\frac{V_0 + V + V_d}{V_0 + V} \right] \quad (2)$$

2.3 Results

An average Nernstian slope of 59.6 mV decade⁻¹ (S.D. \pm 0.17 mV) was obtained for the five electrodes when measured in the activity range 10^{-4} to 10^{-1} mol l⁻¹ NaCl. The linear range for clinical application has been previously examined over the 50 to 500 mmol l⁻¹ range[17]. The mean resistance of the electrodes in 0.1 mol l⁻¹ NaCl was 1.03M Ω \pm 0.04 M Ω . Stability was examined by immersing two of the electrodes in stirred 10^{-2} mol l⁻¹ NaCl and

monitoring the output of each vs a saturated calomel reference electrode (SCE) over a 24 hour period. In both cases, the drift was found to be $+1.7 \pm 0.1$ mV. Results on 5 electrodes however, showed a drift of only $+0.5 \pm 0.1$ mV over a 1 hour period. Continuous recalibration is therefore advisable unless correction is applied while performing sample run experiments. In this respect, a drift magnitude of about 0.3 mV was observed when recalibrating the electrodes. The reproducibility of the electrode response on transfer from a 10^{-3} mol l^{-1} to a 10^{-2} mol l^{-1} 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 being sequentially measured after two minutes). The electrodes exhibited fast response times ($t_{90} < 10$ s) to transient shifts involving a change in concentration from 10^{-4} to 10^{-3} mol l^{-1} NaCl (Fig. 2). In selectivity investigations, the electrodes displayed excellent discrimination against Li^+ , K^+ and H^+ ions, with the respective selectivity coefficients ($\log K_{pot}$) being -2.86 , -2.59 and -1.98 respectively (values determined by the separate solution method in 0.1 mol l^{-1} solution). Variation in pH had little effect on the electrode response, with less than 1.0 mV variation between pH 9.5 and 5.5 in either 10^{-3} and 10^{-2} mol l^{-1} NaCl solutions, and virtually no change (< 0.1 mV) over pH values of

interest for plasma determination (7.0 to 7.6). One electrode was selected for use in the plasma tests and 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 minute equilibration time. The regression coefficient for this set ($r = 0.820$) showed no difference with a second set of 59 samples in which the electrode was recalibrated after 5 samples and the emf obtained after two minutes ($r = 0.816$). Although inconsistency was found with the values for slope, intercept and standard deviation of the residuals, a reasonable correlation between the ISE results and the Hitachi results is evident. Further results (Table 2) of 18 samples with the Technicon Smac analyzer showed a similar regression 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 more reduced number of samples was assayed. The standard addition technique did not improve the correlation with the Hitachi analyzer and a rather 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.

2.4 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, this means paying close attention to matching the sample/standard matrices, maintaining a constant temperature, and following an identical procedure for each measurement. 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 obstruction of the junction by proteins 'salted out' by the bridge electrolyte (saturated KCl), or by slight differences in the plasma/standards matrices. Problems arising from protein deposition can be minimised 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 mmol l⁻¹), and is equivalent to a theoretical ISE response change of around 1.9 mV. This being the case, it is not surprising that good precision and accuracy is difficult to achieve by means of simple 'dip' methods.

2.5 Conclusions

The results demonstrate that the ion-selective electrodes incorporating 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 when using flow methods. In this respect, dynamic characteristics are improved since, with suitable cell design, the rate of transport processes is improved. Chemical and mechanical interference associated with the electrode operation (washing, cleaning, changing solution, etc.) are

significantly smaller in flow systems compared to the steady-state technique. However, it is worth noting 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.

2.6 References

1. G.J. Moody and J.D.R. Thomas in "Ion Selective Electrodes in Analytical Chemistry", (H. Freiser, ed.), Vol. 1, Plenum, New York (1978) pp 339-433.
2. G.J. Moody and J.D.R. Thomas, Talanta, 19 (1972) 623-639.
3. R.L. Solsky, Anal. Chem. 60 (1988) 106R-113R.
4. J.A. Lutsgarten, R.E. Wenk, C. Byrd and B. Hall, Clin. Chem., 20 (1974) 1217-1221.
5. R.G. Bates, "Determination of pH, Theory and Practice", John Wiley & Sons, New York (1973) p. 351.
6. D. Ammann, R. Bissig and Z. Cimerman in "Ion and Enzyme Electrodes in Biology and Medicine" (M. Kessler, L.C. Clark Jr. and D.W. Lubbers, eds.), Urban & Schwarzenberg, Munchen-Berlin-Wien (1976) p. 2.
7. W. Simon, D. Ammann, P. Anker, U. Oesch and D.M. Band, Ann. NY. Acad. Sci., 428 (1984) 279-285.
8. U. Oesch, D. Ammann and W. Simon, Clin. Chem. 32 (1986) 1448-1459.
9. U. Fiedler, Anal. Chim. Acta 89 (1977) 101-109.
10. P. Anker, HB. Jenny, U. Wuthier, R. Asper, D. Ammann and W. Simon, Clin. Chem., 29 (1983) 1508-1512.
11. M. Kessler, J. Hoepfer and B.A. Krumme, Anesthesiology, 45 (1976) 184-197.
12. G.J. Moody, B.B. Saad and J.D.R. Thomas, Analyst, 114 (1989) 15-20.
13. M.A. McKervey, E.M. Seward, G. Ferguson, B. Ruhl and S. Harris, J. Chem. Soc. Chem. Commun. (1985) 388-390.
14. D. Diamond, G. Svehla, E.M. Seward and M.A. McKervey, Anal. Chim. Acta., 204 (1988) 223-231.

15. P.J. Corbleet and N. Gochman, Clin. Chem., 25 (1979) 432-438.
16. D. Midgley, Analyst, 112 (1987) 557-572.
17. M. Telting-Diaz, D. Diamond, M.R. Smyth, E. Seward, G. Svehla and M.A. McKervey, Anal. Proc., 26 (1989) 29-31.
18. R.E. Dohner, D. Wegmann, W.E. Morf and W. Simon, Anal. Chem., 58 (1986) 2585-2589.
19. E. Pungor, K. Toth and A. Haberczy-Pall, Trends Anal. Chem., 3 (1984) 28-30.

2.7 Figures

Figure 1. Structure of the Calixarene methyl p-t-butylcalix[4]arene acetate illustrating the position of a sodium ion within the polar cavity, R = methyl groups.

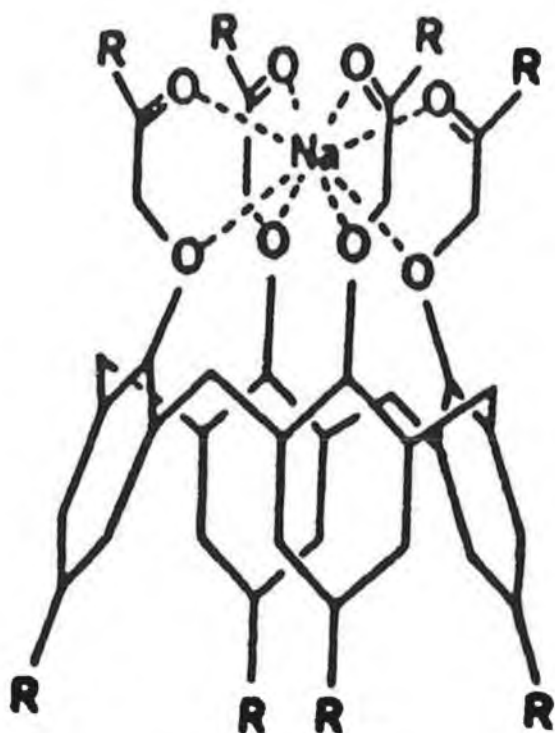


Figure 2. Dynamic response of mini-ISE to injections of 500 μ l of 0.1M NaCl into 40 ml of 10^{-4} M NaCl.

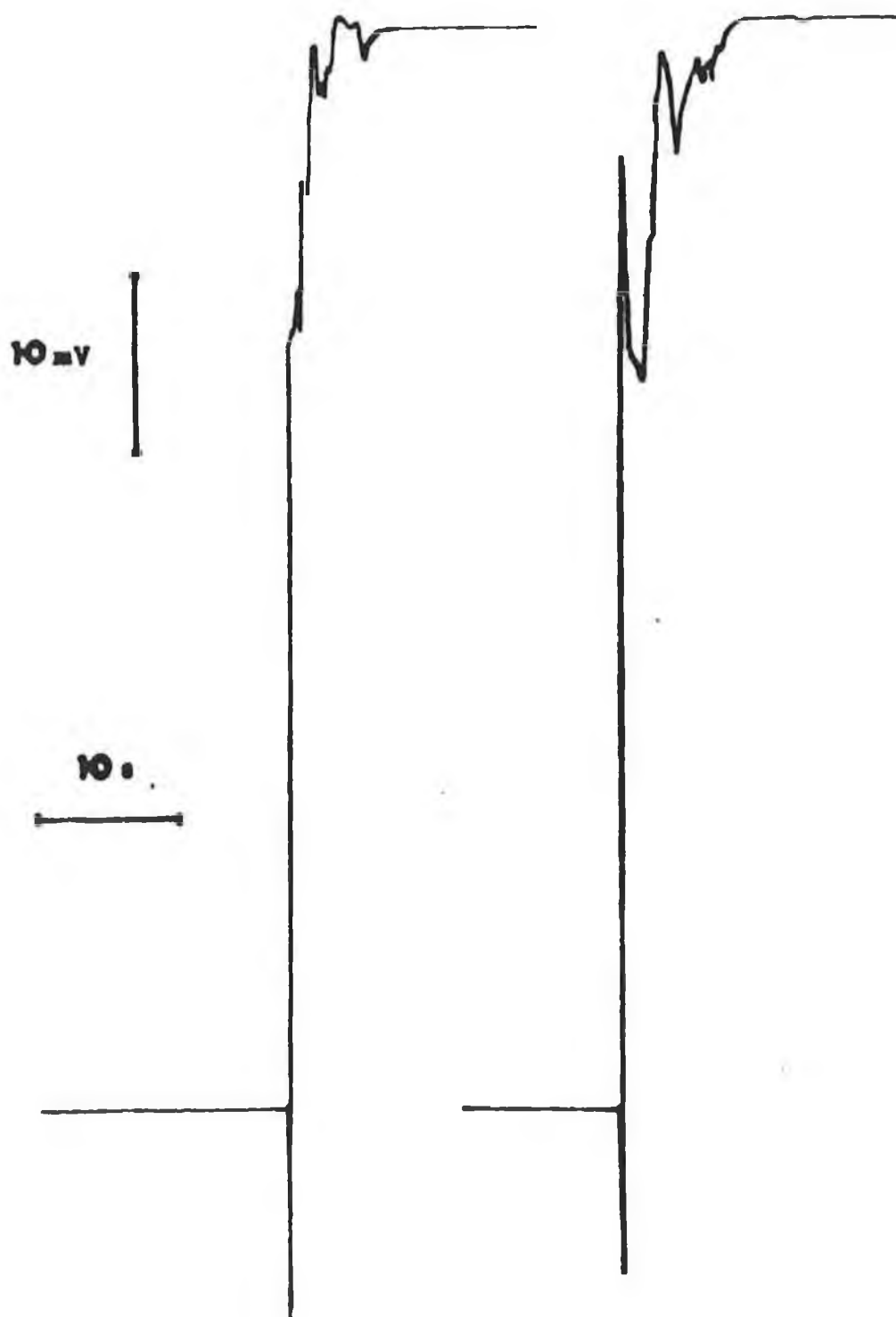


Figure 3. Correlation between mini-ISE and Hitachi Analyser measurements for sodium in split human plasma samples with the ISE reading taken at;

(a) 1 min and

(b) 2 min

after immersion in the plasma sample.

(c) Comparison of mini-ISE and Technicon SMAC analyser (see overleaf).

Regression Equations:

(a) $Y = 0.93X + 5.86, R = 0.820;$

(b) $Y = 1.01X - 0.37, R = 0.816;$

(c) $Y = 1.11X - 15.03, R = 0.827.$

Note: ISE values obtained from calibration curve.

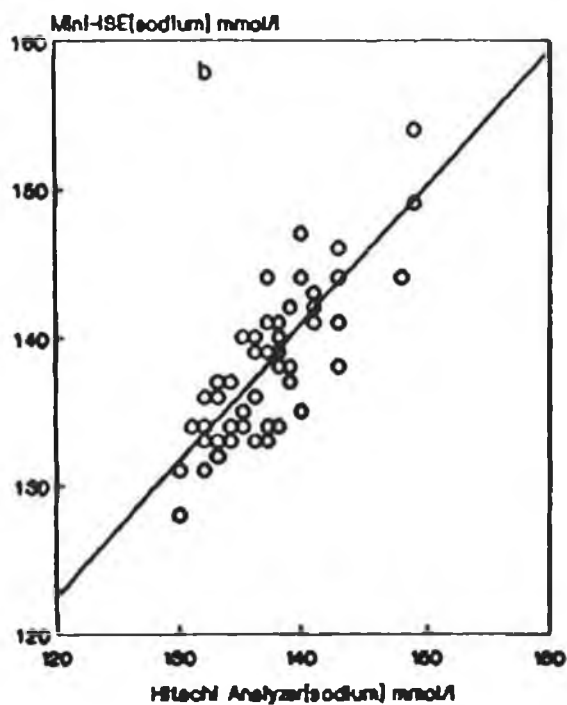
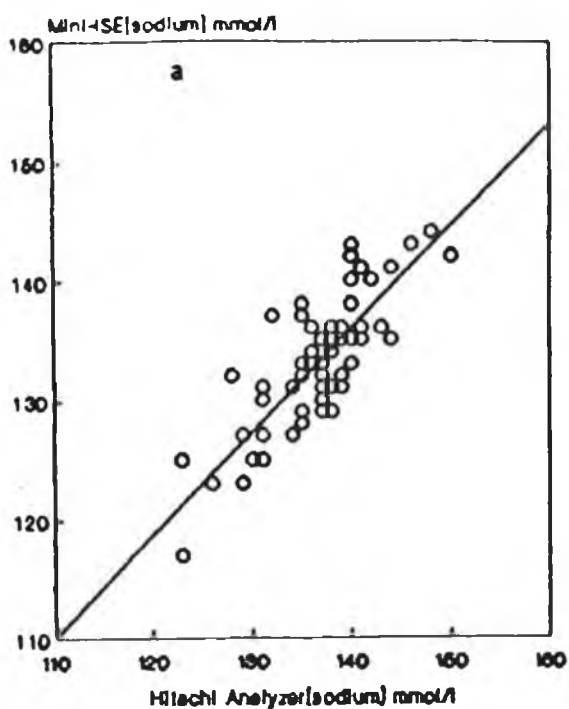


Figure 3 (continued)

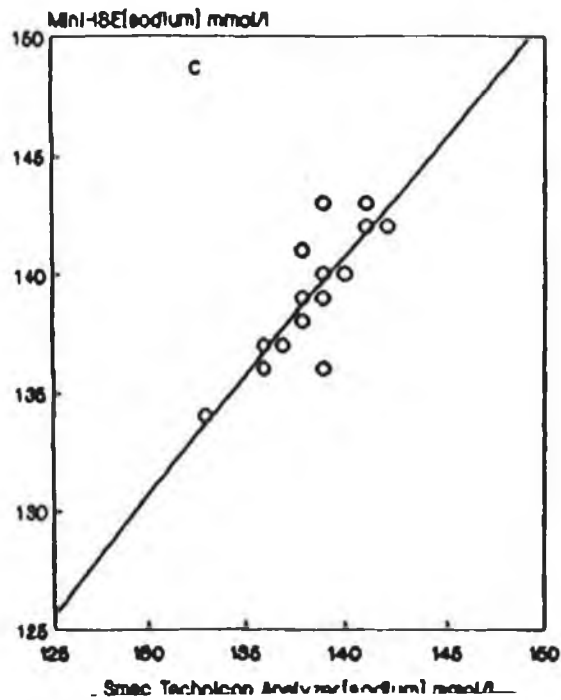
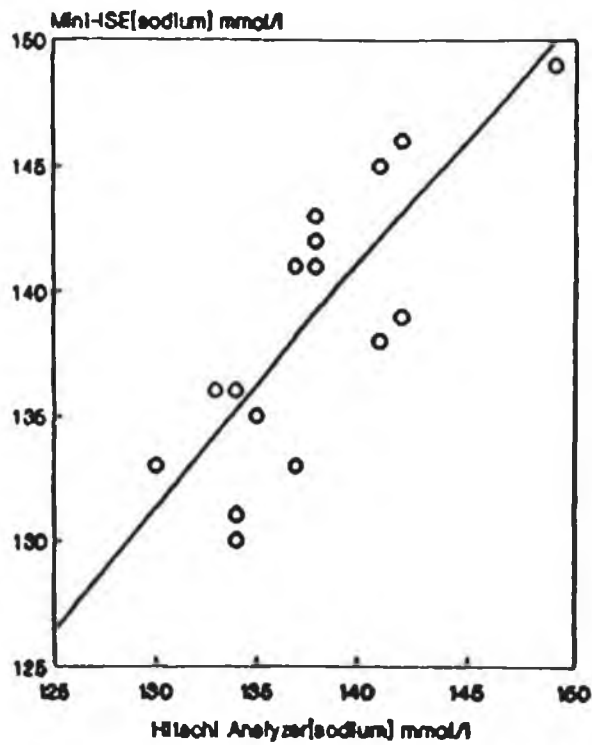


Figure 4. Correlation of duplicated measurements of sodium in human plasma by mini-ISE using the standard addition method, and an Hitachi Analyser employing the glass membrane sodium ISE in a flow system ($R = 0.79$). Average slope determined for each analysed sample was 57.8 ± 0.9 mV decade⁻¹.



2.8 Tables

n	assay	slope	intercept	r	SD
61 ^a	Hitachi	0.93	5.85	0.820	+3.0
59 ^a	Hitachi	1.01	-0.37	0.816	+2.6
18 ^a	Technicon Smac	1.11	-15.03	0.827	+1.3
17 ^b	Hitachi	1.02	-1.95	0.792	+5.3
7 ^a	Flame Photometry	1.000	-1.14	0.904	+1.4

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

^a mini-ISE results obtained using a calibration curve.

^b performed by standard addition method.

sample	Mini-ISE ^a	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

Regression Coefficients of Paired Methods

Mini-ISE vs Hitachi	Mini-ISE vs Flame Photometry	Flame Photometry vs Hitachi
0.932	0.905	0.979

Table 2: Sodium concentration (mmol l⁻¹) and correlation coefficient measured for plasma samples by two different ISEs and flame photometry.

^aresults obtained from calibration curve.

Publications and Presentations

1. d.c. Resistance Measurements - A simple Diagnostic Tool for Indication of Membrane Failure in Chemical Sensors, D.Diamond and F.Regan, *Electroanalysis* 2 (1990) 113.
2. The Characteristics and Performance of Calixarene-based PVC Membrane Electrodes, Aodhmar Cadogan, Francis Regan and Dermot Diamond, *Irish Chemical News*, Autumn 1990, 25-28.
3. Calixarenes as Active Agents for Chemical Sensors, Robert Forster, Francis Regan and Dermot Diamond, *Sensors and Actuators* (in preparation).
4. Comparison of a Calixarene-Based Ion-Selective Electrode with Two Automated Analysers for the Clinical Determination of Sodium in Blood Plasma, M. Telting Diaz, F.Regan, D.Diamond and M.R.Smyth, *J. Pharm. Biomed. Anal.*, 1990 (in Press).
5. The Production, Development and Characterisation of Miniature Ion-selective PVC Membrane Electrodes, D.Diamond and F.Regan, a poster presented at the 26th R&D Topics meeting of the Analytical Division, the Royal Society of Chemistry, DCU, March 1989.