ION-SELECTIVE SENSORS APPLIED TO THE ANALYSIS OF BLOOD ELECTROLYTES

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With

"Salutations to the Divine Mother who
dwells in all beings in the form of
intelligence. Salutations to Her.
Salutations to Her. Salutations to Her."

Sanskrit Hymn

या देवी सर्वभूतेषु बुद्धिरूपेण संस्थिता ।
नमस्तस्यै, नमस्तस्यै नमस्तस्यै नमो नमः ॥२०-२२॥

To

MA and PITA, my parents
for their love and sacrifices

to
Nandita and Sandeep, my children

"Let your light so shine before men,
that they may see your good works
and glorify your Father ...."

and to
Bipul, my husband

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A B S T R A C T

Direct potentiometric techniques are becoming an invaluable analytical resource in the clinical laboratory. One important aspect of their use is determining or monitoring the activities of ions present in physiological media such as blood, urine, cerebral fluid, serum etc. This work is based, mainly, on the measurement of the blood electrolytes sodium, potassium, calcium and pH.

Various automatic, multi-electrolyte, direct potentiometric, ion-selective analysers are available. The problem, to date, has been twofold - firstly different instruments give different results with the same sample and secondly, direct potentiometry senses the activity of the free (hydrated) ions - an entity unfamiliar to clinicians who have been used to flame photometric concentrations. To resolve these problems it is necessary to establish an operational pION scale by international consensus. This study was aimed at contributing towards achieving this end.

The first requirement for the establishment of a pION scale is a set of universally accepted standards. Multi-electrolyte calibration standards using "Good" buffers have been proposed. Analyte binding to these buffers have been evaluated using two non-linear least squares programs SCOGS2 and SUPERQUAD . Commercial and home made ion-selective electrodes and ion-selective field effect transistors were calibrated and tested in the calibration solutions by measuring transfer potentials.
A flow through rig was set up using several different ion-selective electrodes from manufacturers' instruments but with a common reference electrode and this system was tested with the aqueous calibration solutions, plasma and serum.

The second requirement for the establishment of a pION scale is resolution of the problem of residual liquid junction potentials. There are two aspects of this problem. The first aspect is the variations arising from the difference between the 'junction' potential with the

calibration solution and the junction potential with plasma. The second aspect is the 'inter' instrument differences arising because of choice of ions in the salt bridge solutions and different liquid junction geometries. To minimise the first aspect, the ionic composition of the calibration solutions were formulated to reflect the ionic composition of plasma. Residual liquid junction potentials of the calibration solutions against the NBS blood phosphate buffer were determined. The second aspect has been discussed.

Other aspects of standardisation such as sample pre-treatment and measurement protocol have been studied briefly. These include heparin binding and carbon-dioxide contamination. Reporting results in activities based on activity co-efficients calculated by Covington and Ferra (Pitzer and Hydration models) rather than the conventional flame photometric concentrations, has also been discussed.

CHAPTER ONE

INTRODUCTION

Imbalance of electrolytes in body fluids is indicative of various physiological disorders[1]. Monitoring of ions during surgery and intensive care is also very important. It is, therefore, necessary to evolve an accurate and reliable method of measuring electrolytes in body fluids.

1.1 PREVIOUS METHODS OF ANALYSIS

Tables 1.1(a-e)[2] summarise methods used for analysing some of the principal ions in body fluids. The tables are self-explanatory. Mention must be made of another parameter used to indicate electrolyte imbalance, the anion gap. The anion gap is calculated as

$$([Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-]) = + \text{Anion gap} \quad - 1.1$$

An increased anion gap may be indicative of decrease in unmeasured cations (calcium and magnesium, mainly) or an increase in unmeasured anions (mainly protein, phosphate, sulphate or organic acids). A decrease in the anion gap suggests the opposite possibility.

1.2 DIRECT POTENTIOMETRY

In recent years, there has been considerable growth in the use of direct potentiometric ion-selective electrodes or ion-selective field effect transistors for clinical measurements. Direct potentiometry, as the name implies, determines the activity of an ion directly in the untreated sample. A calibration curve is prepared by plotting the mV output from two or more concentration or activity standards versus the negative logarithm of the concentration or activity. The electrodes are then placed in the sample and the mV reading of the sample is converted to the corresponding concentration or activity via the calibration curve. The technique is described, in detail, in Chapter 2. The accuracy of the method can be $\pm$ 1.0%. An error of 1.0mV corresponds to approximately 4% relative error, in concentration with univalent ions and 8% for divalent ions.

The advantages of the direct potentiometric technique over the previous techniques are:-

(1) Sample pre-treatment is not required.

(2) Very small sample volumes are needed.

(3) It can be adapted for use at the bedside.

(4) The activity of the (hydrated) ion in their natural environment is measured. This is of particular significance in cases of hyperlipemia or hyperprotemia when flame photometers give erroneous value[3] because of dilution of the samples. This is discussed further in Chapter 3.

1.3 PRESENT STATE OF THE ART

Various automatic, multi-electrolyte, direct potentiometric ion-selective analysers are available. The problem, to date, has been two-fold - firstly, different instruments give different results with the same sample and secondly, direct potentiometry senses the activity of the free (hydrated) ion - an entity unfamiliar to clinicians who have been used to flame photometric concentrations. These fundamental issues are in the process of being resolved by various international bodies such as the European Working Group on Ion-Selective Electrodes (EWGISE) of the International Federation of Clinical Chemistry (IFCC), the National Bureau of Standards (NBS) and the US National Committee for Clinical Laboratory Standards (NCCLS)[4].

The factors contributing to this confusion are:-

(1) The problems in assigning activity co-efficients to ions in media of high ionic strength (0.1 mmol dm^{-3}).

(2) Non-zero residual liquid junction potentials arising from choice of ions in salt bridge solutions and varying liquid junction geometries.

(3) Use of different calibration solutions.

(4) The need for standardisation of other aspects of measurement such as levels of heparin to be used, sample collection and pre-treatment if required.

(5) The need for the introduction of acceptable correction procedures which estimate the relative contribution of the various effects to the overall measurement of the effective analyte concentration. This procedure could be based on a universal factor and bring results to a common basis or a factor introduced by individual manufacturers to yield results that are the same for normal samples in different instruments. However, the problem of abnormal samples may still remain (Chapter 3, Table 3).

1.4 AIMS OF THIS WORK

This work is based, mainly, on the measurement of the blood electrolytes sodium, potassium, calcium and pH. The principal ions in blood plasma are shown in Table 1.2[8]. The ionic strength of plasma is usually taken as 0.16 mol dm^{-3}. This refers to the water phase of plasma ignoring the protein zwitterions.

The aim of this work was to contribute towards standardising blood electrolyte measurements. To achieve this, the following subjects were studied.

In Chapter 2, the definitions and principles of ISEs and ISFETs are explained. The use of various electro-active materials for blood electrolyte measurements is discussed.

Emphasis is given to situations arising in clinical applications, in Chapter 3. Topics such as Direct versus Indirect Potentiometry, plasma water versus plasma volume, activity versus concentration and selectivity requirements are discussed. Clinical analysers available in the market are described.

In Chapter 4, the arguments for formulating a set of calibration solutions using the 'Good' buffers[9,10] are presented. The

relative merits and demerits of activity standards versus concentration standards are reviewed. Activity co-efficients for the calibration solutions calculated by Ferra[11], based on the hydration theory[12,14], and the Pitzer[15] equation are explained.

In the experimental chapter, Chapter 5, tests on the performance of ion-selective devices used in this work are described. The devices were used either as dip-type sensors or incorporated in a flow-through system. They were calibrated to check their condition. Some pH devices were tested for alkaline error. The performance of the electrodes was, finally, checked in the aqueous calibration solutions proposed and in plasma and serum.

In Chapter 6, the evaluation of the dissociation constants and formation constants (with Na^+, K^+ and Ca^{2+}) of the 'Good' buffers is described. Two non-linear least squares programs - SCOGS2[16] and SUPERQUAD[17] were used.

Other aspects of potentiometric measurements in blood, such as the residual liquid junction potential, effect of heparin and the effect of carbon dioxide on the calibration solutions are presented in Chapter 7. These topics need further investigations for a complete study.

In the concluding chapter, Chapter 8, the contributions made in this study to the direct potentiometric analysis of electrolytes in blood is discussed. An outline of future research possibilities is given.

TABLES

Table 1.1a Methods of calcium analysis

Method	Principle	Usage	Comments
1. Precipitation by oxalate and redox titration	Ca^{++} + Oxalate → Ca oxalate (ppt) Ca oxalate (ppt) + H_2SO_4 → Oxalate + $CaSO_4$ $KMn^{7+}O_4$ + Oxalate $\xrightarrow{70^\circ C}$ $Mn^{2+}SO_4$	Historical	Initial reference method
2. Precipitation by colored anions; spectrophotometric	Ca^{++} + Chloranilate → Ca-chloranilate (ppt) Ca-chloranilate (ppt) + EDTA $\xrightarrow{OH^-}$ Ca EDTA + Chloranilic acid (purple)	Historical	Labor intensive, imprecise, many other dyes available
3. Titration of fluorescent Ca^{++} complex	Ca^{++} + Calcein → Ca-calcein (fluorescent) EGTA + Ca-calcein → Ca^{+}-EGTA + Calcein (decreased fluorescence)	Stat. or small labs	Small sample size, dedicated instrument
4 Spectrophotometric measurement of Ca^{++} complexes			
a. Direct	Ca^{++} + *o*-Cresolphthalein $\xrightarrow{OH^-}$ Red complex (520 nm)	Most common	Early adapted to a variety of automated instruments; positive bias compared to atomic absorption
b. Dialysis	Ca^{++} complex + H^+ $\xrightarrow{\text{Dialysis}}$ Ca^{++} in recipient stream Ca^{++} detected as in (a)	On Technicon AutoAnalyzer	
5. Flame emission*	Ca^{++} $\xrightarrow[2e^-]{\text{Heat}}$ Ca^0 $\xrightarrow{\text{Heat}}$ Ca^* → Ca^0 + Photon	Historical	Poor sensitivity
6. Atomic absorption	Ca^{++} $\xrightarrow{2e^-}$ Ca^0 Photon + Ca^0 → Ca^*	Reference method	Excellent accuracy and sensitivity
7. Isotope-dilution mass spectrometry	Ca and known amount of Ca isotope; isolate Ca^{++} and record ratio of two isotopes on mass spectrometer	Definitive method	Available in reference centers only

*Ca^*, Calcium atom in excited state. Ca^0, Calcium atom in ground state; *EGTA*, ethylene glycol-bis(β-aminoethylether)*N,N'*-tetraacetic acid. *ppt*, precipitate

Table 1.1b Methods of sodium and potassium analysis

Method	Type of analysis	Principle	Usage	Comments
1. Flame atomic emission spectroscopy (FAES)	Quantitation of mass concentration	Excited atom emits photon	Serum, urine, CSF, other body fluids	Reference method as well as most commonly used; dilution error possible
2. Ion-selective electrode potentiometry (ISE)	Quantitation of chemical activity	Ion-selective electrode measures potentiometric change as function of ion concentration	Serum, urine, CSF	Dilution error possible by indirect procedure; urine analysis may have limited linear range
3. Atomic absorption	Quantitation of mass concentration	Ground state atoms absorb incident light from hollow cathode lamp	All biological fluids	Highest sensitivity, but not useful for routine analysis

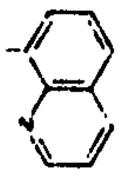
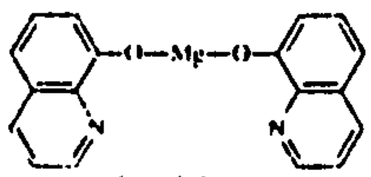
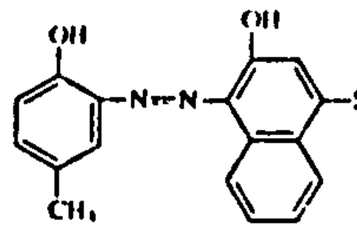
Table 1.1c Methods of lithium analysis

Method	Type of analysis	Principle	Usage
Flame emission spectroscopy	Quantitative	Emission of light at 670.8 nm by Li^0	Serum, plasma
Atomic absorption spectroscopy	Quantitative	Absorption of light at 670.8 nm by Li^0	Serum, plasma urine, red blood cells

Table 1.1d Methods of chloride measurement

Method	Type of analysis	Principle	Usage	Comments
1. Mercuric/ferric thiocyanate	Quantitative, end point	$2\ Cl^- + Hg(SCN)_2 \rightarrow HgCl_2 + 2\ (SCN)^-$ $3\ (SCN)^- + Fe^{+++} \rightarrow Fe(SCN)_3$ (red) A_{max}, 525 nm	Serum, plasma, urine; manual, automated	Most frequently used, good accuracy and precision
2 Coulometric titration	Quantitative titration, end point	$Ag^+ + Cl^- \rightarrow AgCl(\downarrow)$	Serum, plasma, urine, fluids, sweat; manual, automated	Reference method, highly accurate
3 Ion-selective electrode	Quantitative, potentiometric end point, or kinetic	Ag^+,AgCl(s) | Cl^- + AgCl, AgS Test solution | Reference electrode	Serum, plasma, urine, fluids, sweat; manual, automated	Increasingly used, good accuracy and precision
4. Mercuric nitrate	Quantitative titration	$2\ Cl^- + Hg(NO_3)_2 \rightarrow HgCl_2 + 2\ (NO_3)^-$ Excess Hg^{++} + Diphenylcarbazone → Mercuric diphenylcarbazone *(blue)*	Serum, plasma, urine; manual	Relatively uncommon, poor precision
5. Isotope dilution	Mass spectrometer	Dilution of stable isotope ^{36}Cl with ^{35}Cl	Research or reference laboratories	Definitive method

Table 1.1e Methods for analysis of magnesium

Method	Principle	Usage	Comments
1. Precipitation by ammonium phosphate with gravimetric analysis	Mg^{++} + Ammonium phosphate → Mg-ammonium phosphate (precipitate)	Historical	One of first methods of analysis
With subsequent analysis for phosphate	Fisk-Subbarow phosphorus analysis of precipitate	Historical	
2. Reaction with 8-hydroxyquinoline	$Mg^{++} + 2$  $+ 2\ NH_4OH \rightarrow$  (precipitate) $+ 2\ NH_4^+ + 2\ H_2O$	Historical	Labor intensive; supplanted by better methods
	Alternate analyses a. Quantitation with Folin's phenol reagent b. Blue-green color with ferric iron c. Bromination and titration of excess bromate		
3. Titration of fluorescent complex	a. Mg^{++} + Calcein → Mg-calcein (fluorescent)	Stat. or small laboratories	Difficulty with fluorescence background noise as well as quenching
	b. Mg^{++} + *o,o'*-Dihydroxyazobenzene → Fluorescent complex	Rarely used	
4. Flame emission	$Mg^{++} \xrightarrow{2e^-} Mg^0 \xrightarrow{Heat} Mg^* \rightarrow$ Photon + Mg^0 (370 nm, 383 nm)	Historical	Poor sensitivity
5. Calmagite	$Mg^{++} +$  $\xrightarrow{PVP}$ Complex at 532 nm (violet)	Labs without ACA, or atomic absorption widely used; adapted to many automated analyzers	Slight negative bias; positive shift caused by lipemia.
6. Methylthymol blue	Mg^{++} + Methylthymol blue → Complex at 510 and 600 nm	Du Pont ACA, most commonly reported method	Good correlation with atomic absorption
7. Titan yellow	Mg^{++} + 2NaOH + Titan yellow $\xrightarrow{PVP}$ $Mg(OH)_2$–Titan yellow (red lake) (colloidal ppt)	Infrequently used	Only one eighth sensitivity of Calmagite
8. Atomic absorption	$Mg^{++} \xrightarrow[+2e^-]{Heat} Mg^0 \xrightarrow[Photon]{} Mg^*$	Used in a number of routine laboratories	Excellent accuracy, reference method
9. Neutron activation isotope dilution	Uses magnesium isotope ^{27}Mg	Definitive method	

Species	Serum	
	Mean	95% Range
Na^+	144	138 - 151
K^+	4.3	3.40 - 5.20
Ca^{2+}	1.26	1.09 - 1.45
Mg^{2+}	0.83	0.75 - 0.9
Cl^-	106	101 - 111
HCO_3^-	24.3	21.3 - 28.3

Table 1.2 (after H.J. Marsoner)[8]

REFERENCES

R E F E R E N C E S

(1) Chapter 21 in "Clinical Chemistry - theory, analysis and correlation" ed, Kaplan, L.A., and Pesie, A.J., C.V. Mosley Co., Torento, (1984).

(2) Chapter 55 and Chapter 61 in reference 1.

(3) Maas, A.H.J., Siggaard-Andersen , O., Weisberg, H.F., and Zijlstra, W., 159, in reference 4.

(4) "Direct Potentiometric Measurements in Blood", Proceedings of the Workshop on Direct Potentiometric Workshop in Blood, sponsored by NCCLS and NBS, ed. Koch, W.F., Gaithersburg, Maryland (1983).

(5) "Ionised Calcium, Sodium and Potassium by Ion-Selective Electrodes". Proceedings of the first meeting of the EWG ISE, Volume 5, ed. Maas, A.H.J., Kofstad, J., Siggaard-Andersen, O. and Kokholm, G., Private Press Copenhagen, Oslo (1983).

(6) "Methodology and Clinical Applications of Ion-Selective Electrodes" Proceedings of the 4th meeting of the EWGISE, Volume 7, ed. Maas, A.H.J., Boink, A.B.T.J., Saris, N-E.L., Sprokholt, R. and Wimberley, P.D., Radiometer Copenhagen, Helsinki (1985).

(7) "Methodology and Clinical Applications of Ion-Selective Electrodes", Proceedings of etc. 8th meeting of the EWG ISE, Volume 8, ed. Maas, A.H.J., Buckley, B., Marsoner, H., Saris, N-E.L., and Sprokholt, R., Private Press, Copenhagen, Graz (1987).

(8) Marsoner, H.J., Biomed Tech, 30, 302-306 (1985).

(9) Good, N.E., Wingate, G.D., Winter, W., Connolly, T.N., Izawa, S. and Singh, R.H.M., Biochem. J., 5, 467-477 (1966).

(10) Good, N.E. and Izawa, S. in "Methods in Enzymology", ed. San Pietro, A., B24, 53-64, Academic Press, N.Y. (1972).

(11) Covington, A.K., and Ferra, M.I.A., p.239 in reference 6.

(12) Stokes, R.H., and Robinson, R.A., J. Amer, Chem.Soc., 70 1870 (1948).

(13) Siggaard-Andersen, O., Thode, J. and Fogh-Andersen, N., Scand, J., Clin. Lab. Invest. 43, Suppl. 165, 11 (1983).

(14) Bates, R.G., Staples, B.R., and Robinson, R.A. Anal. Chem, 42, 867 (1970).

(15) Pitzer, K.S., Chapter 7 in "Activity Co-efficients in Electrolyte Solutions", Vol. I, Ed. Pytkowicz, R.M., CRC Press (1979).

(16) Perrin, D.D., and Stunzi, H., in "Computational Methods for Determination of Formation Constants", ed. Leggett, D.J., Plenum, N.Y. 71-157 (1985).

(17) Gans, P., Sabatini, A., and Vacca, A., J. Chem. Soc., Dalton, 1195-1201 (1985).

CHAPTER TWO

ION-SELECTIVE DEVICES APPLIED TO THE ANALYSIS OF BLOOD ELECTROLYTES

INTRODUCTION

In the clinical laboratory, today, ion selective electrodes are gradually replacing flame photometers in the analysis of blood electrolytes. Considerable research has also been done on the clinical applications of ion-selective field effect transistors. In this chapter the principles of ISE's AND ISFET's will be discussed. Various electro-active materials used for sensing ions of clinical importance will be considered. Clinical applications of these sensors will also be reviewed.

2.1 THE ION-SELECTIVE ELECTRODE SYSTEM

A conventional ion-selective assembly (FIG. 2.1) comprises

(a) The ion-selective electrode (ISE) which responds more or less selectively to the activity of a particular ionic species in a mixture of electrolytes. The electroactive material is supported in an inert glass or teflon tube. The tube is fitted with an appropriate electrolyte solution (usually containing the chloride salt of the ionic species to which the ISE responds) and electrical contact is made via an inner reference electrode (usually Ag/AgCl).

(b) External Reference Electrode [1,2]

This should provide a stable, reproducible and reversible potential against which variations in the potential of the ion-selective electrode can be measured. External reference electrodes are usually anion reversible, such as Ag/AgCl for cells without liquid junction and calomel for cells with liquid junctions.

(c) Voltmeter interfaced with an Operational Amplifier

Ion-selective electrodes have high input impedance ($>10^{12}\Omega$). An impedance buffer is, therefore, required to match the impedance of the electrode to the DVM. An operational amplifier having an impedance of $10^{12}\Omega$ may be used [3,4].

2.2 NERNSTIAN RESPONSE OF ION-SELECTIVE ELECTRODES

The potential difference E, detected by the measuring system is

$$E = E_{ASS} + E_{IR} + E_{ER} + E_{LJ} + E_{INT} + E_{EXT} \quad - 2.1$$

E_{IR} and E_{ER} are of opposite sign and usually constant. By taking adequate precautions [5], E_{LJ} can be maintained constant.

Equation 2.1 can then be written as

$$E = E'_O + E_{ASS} + E_{INT} + E_{EXT} \quad , \quad - 2.2$$

where $E'_O = E_{IR} + E_{ER} + E_{LJ}$

In an ideal system, E_{ASS} is constant. E_{INT} and E_{EXT}, the two phase boundary (or Donnan) potentials are primarily dependent on the activity of the relevant ion in solution and on the temperature. E_{EXT} is dependent on the ion activity of the analyte and follows the Nernst relationship.

$$E_{EXT} = \text{CONSTANT} \pm 2.303\frac{RT}{zF} \log a_i \quad - 2.3$$

where R = gas constant

T = temperature in Kelvin

F = Faraday constant

z = charge of the relevant ion

E_{INT} is given by a similar Nernst relationship including the activity of the relevant ion in the electrodes inner filling solution. The value of E_{INT} is constant because the composition of the internal filling solution is constant. Applying these to equation 2.3 gives

$$E = E_o \pm 2.303 \frac{RT}{zF} \log a_i \quad \begin{matrix}(+ = \text{cations}) \\ (- = \text{anions})\end{matrix} \quad - 2.4$$

where $E_o = E_o + E_{ASS} + E_{INT}$

This works out to be

$$1mV \sim zx\ 4\%\ \text{change in}\ a_i \quad - 2.5$$

2.3 DEPARTURES FROM IDEAL BEHAVIOUR

In practice, the ideal behaviour of an ion selective electrode is limited by the following factors:-

2.3.1 Interferences from other ionic species present in the analyte:-

The Nicolsky-Eisenman[6] equation is an expression of the response of an ion-selective electrode to ions other than the primary ion.

$$E = E_o + 2.303 \frac{RT}{zf} \log \left[a_i + \sum_{j \neq i} K_{ij}^{POT} (a_j)^{Z_i/Z_j}\right] \quad - 2.6$$

where a_i = activity of the primary ion

a_j = activities of the interferents in the analyte

K_{ij}^{POT} = are weighting factors specifying the ion selectivity of the electro-active material and are termed selectivity coefficients.

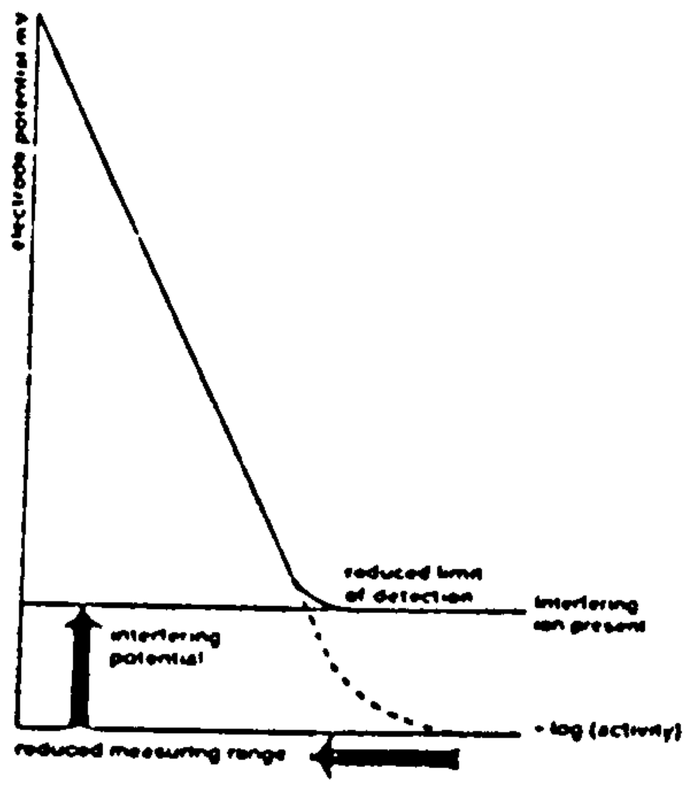
K_{ij}^{POT} can be defined as a ratio between the activities of the primary ion and the interferent ion that would give an identical electrode response when present alone in a solution of the ion. Thus

$$K_{ij}^{POT} = \frac{a_i}{a_j} \quad - 2.7$$

Interferences can be neglected if the product $K_{ij}^{POT} a_j^{z_i/z_j}$ is below the limit of detection. Methods for evaluation K_{ij}^{POT} are discussed elsewhere[7].

2.3.2 Limit of Detection

For the sake of convenience, a practical limit of detection has been recommended by I.U.P.A.C.[8]. This may be taken as the activity (or concentration) of the primary ion 'A' at the point of intersection of the extrapolated illustration.



Response limits depend both on the properties of the electrode and the conditions of measurement, such as temperature, age and contamination of the electro-active material and a high concentration of analyte. The limit of detection determines the measuring range of each electrode.

2.3.3 Response Time

The response time of an ion-selective electrode and a reference electrode is the time taken for the transition of the emf of the cell from the initial value at t=0 to the final value at $t \rightarrow \infty$. The steady state value as $t \rightarrow \infty$ has been defined as within 1mV of the final value [9] or within 90% (or 95%) of the final value [10]. Extensive discussions on response times can be found in references 9, 10, 11 and 12.

2.3.4 Liquid Junction Potentials

In equation 2.2, the liquid junction potential was assumed to be zero. In practical situations, liquid junction potentials are never non-existent. Various methods are used to reduce errors due to liquid junctions. These are discussed in greater detail in Section 7, Chapter 7.1.1. Ref. 1 gives an indepth discussion of the subject.

2.4 EXPLANATION OF AN ISFET VIA AN ISE

If the internal reference electrode and the reference solution in a conventional ion-selective electrode assembly are replaced by

direct contact to a conductor, the outcome is an "all solid state" device. This can be either coated wire electrodes, [13,14] Selectrodes [15] which make use of a carbon rod contact or electrodes formed by depositing a metal contact on to a previously formed membrane [16]. Elimination of the conventional internal reference system offers the first step towards miniaturisation of the system.

The next stage in this direction is deposition of the electro-active material directly on to a thick or thin film hybrid circuit substrate, on which metal contacts have previously been deposited. The electronics are also implemented on the same substrate and protected from the analyte by some form of encapsulation. These are termed 'hybrid micro-electronic sensors' [17, 18].

The ultimate stage in miniaturisation is deposition of the electro-active material on the insulator of an FET. The outcome is an ion-selective field-effect transistor. The process of miniaturisation has, thus, involved reduction in the size of the conductor between the electro-active material and the FET of the operational amplifier. Figure 2.2 illustrates the transition from an ISE to an ISFET.

2.5 PRINCIPLE OF ION-SELECTIVE FIELD-EFFECT TRANSISTORS (ISFETS)

The ISFET was evolved from the IGFET (Insulated Gate Field Effect Transistor) which in turn was conceived from the MIS (Metal-Insulator - Semi Conductor) system. (Fig. 2.3a, b and c). The theory and development of ISFETs are discussed extensively in references 19, 20 and 21. A brief resume from these references will be given here.

The bulk material of the IGFET is lightly doped p-type silicon. Heavily doped n-type silicon forms the source and the drain regions. Aluminium is generally used as the gate metal with SiO_2 or an SiO_2/Si_3N_4 composite for the gate and field insulation.

The ISFET is similar, except the gate metal is replaced by a chemically sensitive layer, or, for pH measurements, the SiO_2/Si_3N_4 surface, known to be pH responsive, is exposed directly to the solution. The rest of the device is protected by a suitable encapsulant.

2.5.1 The Inversion Layer and Threshold Voltage

In a semi-conductor, if a gate voltage positive with respect to the bulk is applied, holes in the underlying p-type silicon are repelled and a region of space charge consisting of the ionised acceptor atoms which are fixed in the lattice is formed; the semi-conductor is depleted of positive holes. If the gate voltage, Vg, is increased sufficiently, the surface potential will deviate substantially from the bulk potential and the surface will contain an excess of mobile electrons; the surface is said to invert. The surface inversion layer is separated from the bulk by a depleted region which is essentially charge free. If a negative gate voltage is applied, holes accumulate at the surface.

It is assumed that there exists a well defined threshold voltage V_T at which the formation of the surface inversion layer begins suddenly as the gate voltage is increased. In practice, the transition is continuous, but the approximation is good if the gate voltage exceeds the threshold voltage by 0.5 V[22].

2.5.2 Depletion and Enhancement Modes

Figures 2.4a and b show the n-channel enhancement mode. No channel exists and no conduction occurs until a positive voltage is applied to the gate. Then, the field induces an n-channel by repelling the holes of the p-type material. In the depletion mode, shown in figures 2.4c and d, a channel exists at 0 V. If the gate voltage is reduced below zero,

the channel can be reduced and completely cut off. If it is taken above zero, the channel is widened and conduction increases. The conductance is thus modulated by varying the gate voltage.

In an ISFET device, the gate voltage is controlled by the reference electrode - analyte - electro-active material potentials. These are relatively small. It is therefore desirable to operate the ISFET in the depletion mode when V_g=0. Conduction in the channel is enhanced when $Vg > 0$ and depleted when $V_g < 0$.

2.5.3 Saturation and Unsaturation

When the source and bulk of a semi-conductor are earthed and a positive bias is applied to the drain, a current flow is generated from the source to the drain. The relationship between the drain current I_D and drain voltage V_D, is linear according to Ohm's law. If Vg is maintained constant and V_D is increased, the potential difference between the gate and the inversion layer decreases. The effect is more evident towards the positively biased drain. The I_D vs V_D relationship starts to deviate from linearity. As V_D is increased further, the potential difference decreases even further until it falls below the voltage required to maintain an inversion layer (V_T). When this happens, the device is said to become "saturated". The drain voltage at which the channel just disappears at the drain end is denoted by $V_{D(SAT)}$. Current continues to flow beyond $V_{D(SAT)}$; but the conduction is due to electrons flowing down the inversion layer in the vicinity of the source to the depletion layer in the vicinity of the drain. The flow of current is now independent of V_D because the conversion layer terminates before the drain. Figures 2.5a and b and figures 2.6 a and b illustrate these effects.

The ISFET is operated in the unsaturated linear mode.

2.5.4 Modes of Operation

The operating mechanism in the ISFET system is the modulation of drain-source current by changes in the activity of the sensed ions in the analyte. Thermo- dynamic [20, 22] analysis of the electro-chemical system of an ISFET (Fg. 2.7) results in

$$V'_G = V_{Contact} + E_I - E_{ref} \qquad - 2.8$$

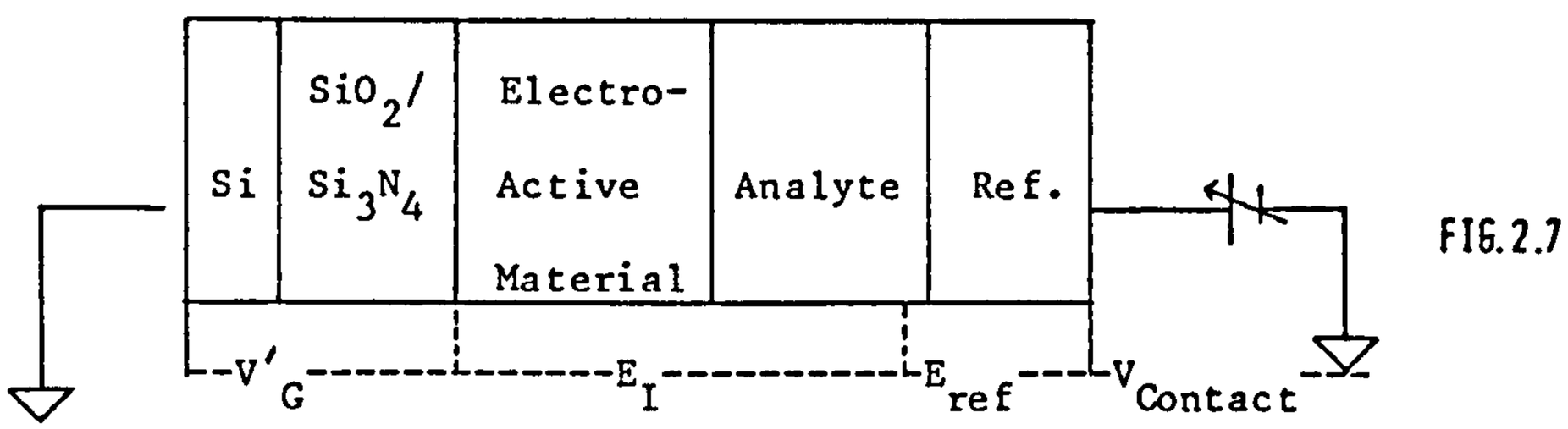


FIG. 2.7

V'_G = potential difference between the bulk of the semi-conductor and the gate material. This is analogous to the gate voltage in an IGFET.

$V_{Contact}$ = contact potential between the semi-conductor and reference electrode.

E_I = Electro-active material/analyte potential difference which is Nernstian.

E_{ref} = reference electrode potential.

a. Constant Gate Voltage Mode (Fig. 2.8a)

In this mode, all externally applied voltages are held constant and the change in drain current ΔI_D which reflects the change in ionic activity, is measured. In the circuit:-

$$V_{OUT} = I_D R_{SET} \qquad - 2.9$$

where R_{SET} is a calibration resistor.

b. Constant Drain Current Mode (Fig. 2.8b)

If the drain current is maintained constant by means of an operational amplifier which directly controls the applied gate potential using a negative feedback loop,

the output voltage varies with changes in activity of the sensed ion in a Nernstian manner. The readout is directly in milli-volts.

2.6 ELECTRO-ACTIVE MATERIALS FOR BLOOD ELECTROLYTES

2.6.1 Classification

The electro-active materials used can be categorised as:-

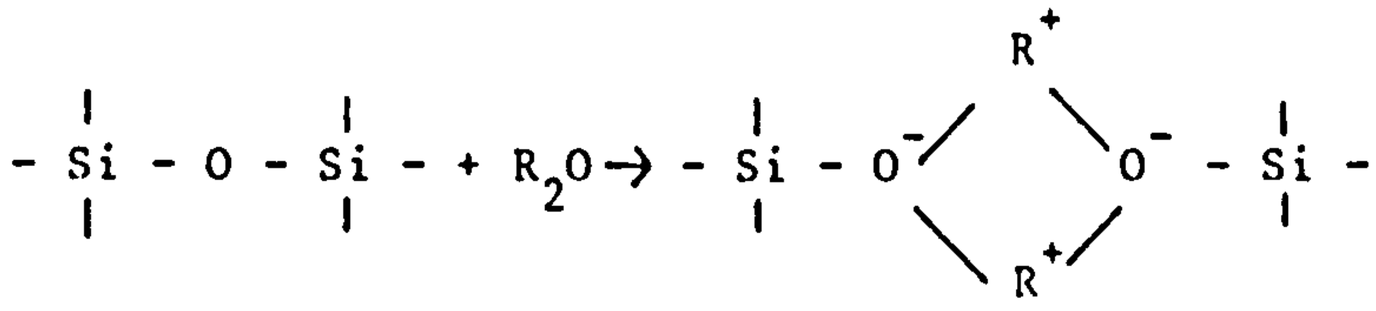
- **Electro-active Materials**
  - **Glass**
  - **Ion-Exchangers and Neutral Carriers**
    - **Liquid State** (the ion exchanger is dissolved in an organic solvent) e.g. calcium diakyl phosphate in dioctyl phenyl-phosphate
    - **Solid State** (the ionophore and plasticiser are dissolved in a solvent and incorporated in an inert matrix e.g. Valinomyin in PVC)
  - **Insoluble Inorganic Salts**
    - **Homogenous** (e.g. single crystal LaF_3)
    - **Heterogeneous** (active material supported in an inert material e.g. AgI in a silicone rubber matrix

The mechanism of ion-exchange and electrical conductance differ in the various categories, but, essentially, all ion-selective devices are expected to respond to the appropriate ion in a Nernstian manner as described in Section 2.1

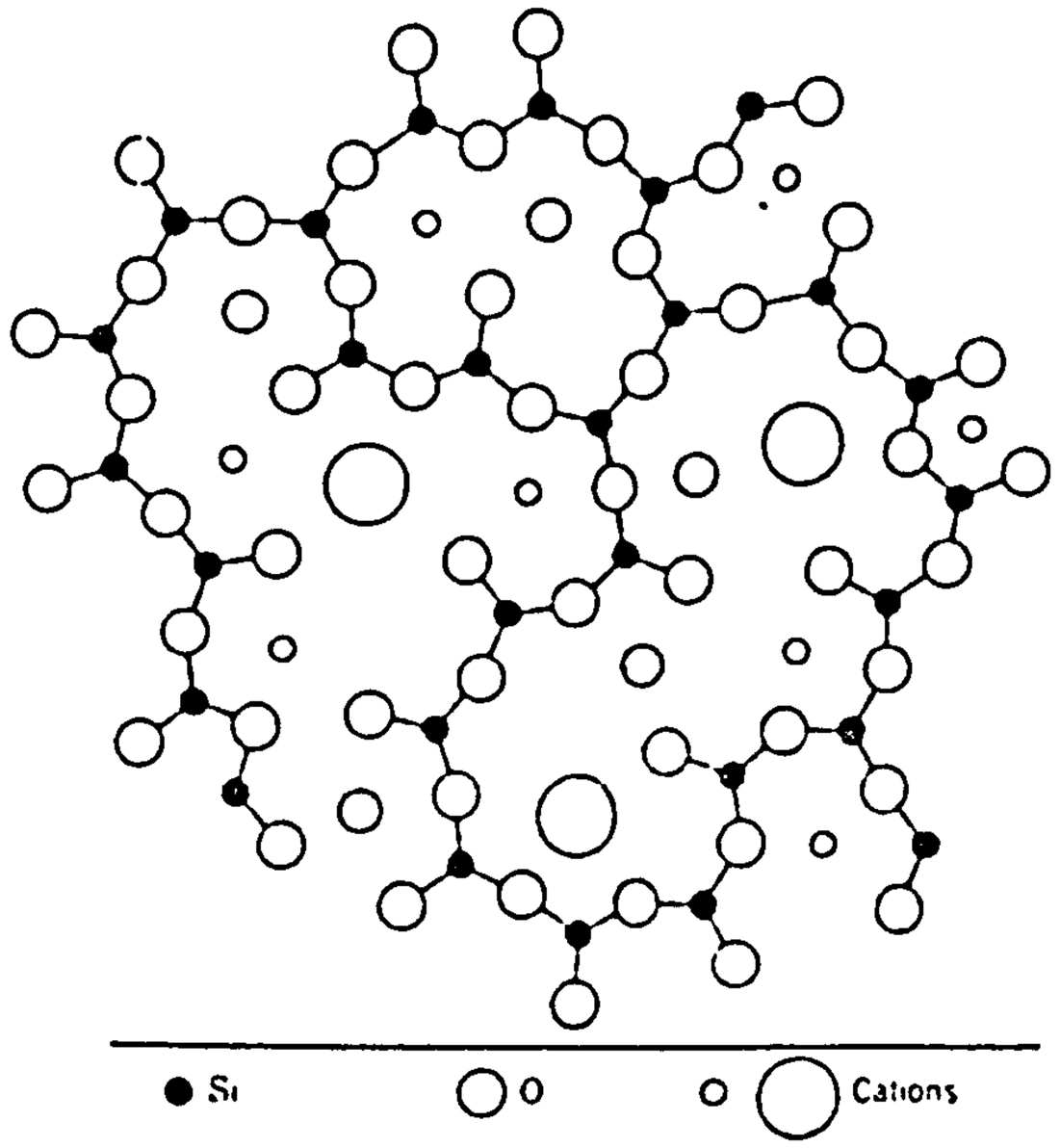
2.6.2 Glass

Electrode glasses usually have at least three constituents, SiO_2, R_2O and MO (or M_2O_3) where R is an alkali metal and M, a bivalent or trivalent metal (from the rare earth group).

X ray studies by Zachariasen [23] revealed that glass is comprised of irregular chains of silicon in tetrahedral co-ordination as an SiO_4 unit. Addition of an alkali oxide disrupts the silicon-oxide network.



The cations are held in the interstices of the SiO_4 network by the anionic electro-static attraction of the oxygen atom (Fig. 2.9).



Structure of silica glass (after Perley).

Fig. 2.9 (after Perley)[2.4]

By controlling the glass composition, increased pH responsive or increased alkali responsive glasses can be prepared.

Hydration of the glass surface controls the magnitude of specificity of a glass electrode. If the surface hygroscopicity of the glass layer is destroyed, the pH response can drop from 59mV per decade to 22mV per decade[24]. Etching the glass surface with 1:1 hydrofluoric acid restores its hygroscopicity. The role of this hydrated gel layer is not fully understood. One interpretation (Charles [25]) is that the first stage is hydrolysis of the salt of a weak acid.

(1) $-\overset{|}{\underset{|}{Si}}-O-Na + H_2O \rightarrow -\overset{|}{\underset{|}{Si}}-OH + Na^+ + OH^-$

The oxygen bridge in the silicate network is then broken by the OH^- ion.

(2) $-\overset{|}{\underset{|}{Si}}-O-\overset{|}{\underset{|}{Si}}- + OH^- \rightarrow -\overset{|}{\underset{|}{Si}}-OH + -\overset{|}{\underset{|}{SiO}}-$

(3) $-\overset{|}{\underset{|}{SiO^-}} + H_2O \rightarrow -\overset{|}{\underset{|}{Si}}-OH + OH^-$

The first stage dominates at pH $<$ 9 and the second stage at pH $>$ 10 and on prolonged hydration.

The anionic field strength of the silicate network is the principal factor in governing the sequence (and magnitude) of specificity of the electrode towards alkali metal cations and H^+. If a lattice breaking alkali cation, M, exists in an alkali silicate glass, it would cause an increase of electron density on the neighbouring silicon by induction:-

$-\overset{|}{\underset{|}{M}} \rightarrow O \rightarrow \overset{|}{\underset{|}{Si}}-$

The glass would then be less prone to attack by negative hydroxyl groups. This would thus reduce alkaline errors in high pH regions. If a triply charged ion such as Al^{3+} is introduced into the alkali silicate glass and as long as the M^+/Al^{3+} ratio exceeds 1, each Al^{3+} enters into tetrahederal co-ordination, restoring the silicate lattice and forcing one alkali cation from its "lattice breaking" position into an interestitial position. Eisenman[26] calculated electro-static forces of inter-action of Group 1A cations and H^+ in SiO^- sites in alkali silicate glasses and $(AlOSi)^-$ sites in alumino silicate glasses. He found that in the former the order of preference was

$H^+ \gg Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$

For the alumino silicate glasses, the order was exactly reversed.

Glasses can thus be optimised to act as pH sensors or alkali metal sensors.

The principal disadvantage of the glass electrode is a varying asymmetry potential which manifests itself as a time dependent drift. This effect can be eliminated by establishing the time dependence of the drift with the aid of a chart recorder and extrapolating the potential back to the point at which the composition was changed[27].

The alkaline error at pH>10 and acid error at pH<2 are other systematic errors that may hinder the performance of pH glass electrodes. Both types of error are time dependent. The alkaline error is temperature dependent, as well, and increases rapidly as the temperature rises above 30 ^{o}C. The alkaline error manifests itself as a positive departure from ideal behaviour and the acid error as a negative deviation. The origin of acid error is the penetration of small anions into the glass structure[25].

2.6.3 Organic Ion Exchangers and Neutral Carriers

These electro-active materials bind ions selectively at oppositely charged sites or at neutral sites. The term "liquid ion exchangers" is used if the material is incorporated in solution in an organic solvent. It is more convenient to prepare a plastic membrane by incorporating the ion exchanger and plasticiser (mediator) in an inert matrix such as PVC with the aid of a solvent (tetrahydrofuran or cyclohexanone). The solvent is allowed to evaporate leaving behind a flexible membrane with the ion exchanger in a PVC matrix.

2.6.4 Insoluble Inorganic Salts

No electrodes of this category have been used in the work except Ag/AgCl as internal reference. However, since inorganic salt based electrodes are available for clinically relevant anions such as F^- and Cl^-, it was considered appropriate to include a short discussion.

The electro-active material can be in a homogeneous or heterogenous form. In the former, the material is pressed or cut into a disc shape. In the latter, the electro-active material is supported in a minimum quantity of inert matrix material (PVC, polythene, silicone rubber) so that there is maximum contact between the sensor and analyte. The inner reference electrode can be Ag/AgCl with an inner filling solution containing chloride ions and the ion to which the membrane responds or a solid state internal contact may be used.

The third type of electrode in this category is based on electrodes of the second kind. A metal such as silver is coated with a metal salt by dipping in the molten salt or by electrolytic deposition e.g. Ag/AgCl, Ag/AgBr/Ag_2S. The electrodes may not be ion-selective in the strictest sense in that they respond to redox potentials if the analyte comes into contact with the bare metal through the porous membrane. In solutions where the appropriate redox system is absent, they behave satisfactorily as ion- selective electrodes.

2.7 pH SENSORS

Most modern pH glasses are lithia glasses. A typical pH glass composition can be

68.7% SiO_2, 14.3% Li_2O, 7% BaO, 1-2% Cs_2O and the rest La_2O_3.

pH glass micro electrodes of the spear type (Hinke-type), internal capillary type (Khuri-type) and recessed tip type (Thomas-type) have been widely used in clinical studies[28]. Commercial instruments such as Radiometer ICA1, ORION SS-20, AVL 980 use pH glass membranes. In vivo use can, however, present problems such as contamination by proteins, high membrane resistances due to miniaturisation and possible hazards by breakage of the delicate membrane.

Despite the high selectivity of pH glass electrodes, therefore, the neutral carrier tri-n-dodecylamine[29] (Fig. 2.10a) has been used by Schulthess et al.[30] and Ammann et al[31]. Initially, the membrane composition used was

(1.0% Tri-n-dodecylamine, 0.6% KTpClPB*, 65.6% DOS*, 32.8% PVC)

*See Fig. 2.11a and b.

Schulthess used the membrane in catheter electrodes of diameter about 1mm. It operated for several hours without deterioration, in contact with blood. This was claimed to be a major advance over glass electrodes which deteriorate rapidly probably because of protein deposition. Also, the resistance is lowered.

Ammann et al.[32] in a later publication recommend the use of ETH469 (Fig. 2.11c) as the plasticiser which further improves the life time and stability of the electrodes.

Membrane composition.

(1.0% Tri-n-dodecylamine, 0.5% KTpClPB, 65.6% ETH469, 32.9% PVC)

In pH ISFETs, the bare gate, or a H^+ responsive membrane cast over the bare gate, acts as a pH sensor. Schepel[33] et al. have used catheter tip-mounted Al_2O_3 gate, pH responsive ISFET's in vivo in dogs. The pH signal was comparable with conventional in vitro pH determinations of arterial blood samples. ISFET based pH sensors have been used by Kohama et al[35]. Ruzicka and Ramsing[36] have incorporated these devices in a miniaturised flow injection analysis (FIA) system. In this laboratory, Sibbald et al.[36] have used bare gate ISFETs in a four function ISFET array.

pH sensitive ISFETs have also been produced by deposition of electrode glasses on the gates by chemical vapour deposition (CVD) (Esashi and Matsuo[37]). Radio frequency sputtering techniques were used by various other workers among whom is Szonntagh[38]. Acceptable Nernstian slopes have been reported. No work with blood/serum has been done as yet.

2.8 SODIUM SENSORS

Na^+ glass sensors are made of either $NAS_{(11-18)}$ or $LAS_{(10.4-22.6)}$ glass compositions. The principal interferents for Na^+ glass sensors are H^+, K^+, Ag^+ and NH_4^+. Lesser interferents are Rb^+, Cs^+, Li^+, Tl^+, Ca^{2+} and Mg^{2+} which need to be present in large excess over the primary ion for significant interference. Of the blood electrolytes, therefore, only H^+ and K^+ need be considered. NAS_{11-18} glasses have higher selectivity coefficients than $LAS_{10.4-22.6}$ glasses; values of $K^{POT}_{Na/H}$ = 30-40 for NAS_{11-18} and 5-10 for $LAS_{10.4-22.6}$ have been quoted[39]. It has also been claimed that sodium can be determined only in media with $pH > (pNa + 3)$[39,40]. For potassium interference the $K^{POT}_{Na/K}$ values quoted are 10^{-2} - 5×10^{-3} for NAS_{11-18} and 10^{-3} - 10^{-4} for $LAS_{10.4-22.6}$ NaS_{11-18}[39] glasses exhibit faster responses compared with $LAS_{10.4-22.6}$ glasses.

Gotoh et al.[41] used an NAS_{11-18} glass electrode more than twenty years ago (1962) to measure Na^+ activity in blood, in vivo. Moore and Wilson[42] determined sodium in human serum by sodium electrodes and flame photometry. Khuri et al.[43, 44] used a glass micro-electrode, with 3M RbCl instead of KCl in the reference bridge, for in vitro determinations of Na^+ activity in serum. RbCl was preferred because NAS_{11-18} glass is less sensitive to Rb^+ than to K^+. Other work on Na^+ activity in serum includes that by Jacobson[45], Dahms[46] et al., Miyada[47] et al. and Trutnovsky[48].

Use for routine work became popular, however, only after automatic analysers became commercially available such as the ORION model SS-30, TECHNICON Stat/ION, NOVA 1, and the CORNING 902.

Neutral carriers used for Na^+ ISE include monensin (Fig. 2.10b), ETH 1097 (Fig. 2.10c), ETH 227 (Fig. 2.10d) and ETH 237 (Fig. 2.10e).

ETH 1097[49] is reported to have poor selectivity over K^+ but in view of the much higher concentration of Na^+ in blood, this does not hinder its use. The long term stability is affected by potential drifts[50]. Membrane composition recommended for blood is (2.4% ETH 1097, 67.0% DBE*, 30.6% PVC)

* Fig. 2.11d

ETH 227 and ETH 237[51] were found to compare well with glass electrodes. For blood/serum analysis, optimum membrane compositions reported are

[6] (1.0% ETH 227, 66.0% DNA, 33.0% PVC)

[33] (1.2% ETH 227, 66.3% ETH 469, 32.5% PVC)

[52] (4.95% ETH 237, 61.89% DOS*, 33.16% PVC)

* DOS = Fig. 2.11b

The antibiotic monensin[52] has been used in liquid micro-electrodes of composition (10% monensin, 90% nitrobenzene).

Na^+ membranes (ETH 227) have been used on ISFETs by Sibbald et al.[53]. The results, however, were not encouraging as neither stable response nor selectivity over potassium was found. Papacostas[12] has also reported sub-Nernstian behaviour and poor selectivity over K^+. Oesch[54] et al. used this neutral carrier in ISFETs and reported usable response in the range 10^{-1} mol dm^{-3} - 9×10^{-4} mol dm^{-3}. The sodium to potassium selectivity was of the order of 20 to 1.

Matsuo and Esashi[55] produced a Na^+ ISFET by chemical vapour deposition of alumino silicates which gave responses of the order of 55mV/decade but showed poor selectivity. Harbinson[56] reports an LAS, Na^+ sensitive glass, deposited by RF sputtering. It showed a high selectivity over K^+.

2.9 POTASSIUM SENSORS

Glass electrodes useful for the determination of potassium are based on KAS_{20-05} or NAS_{27-04} glasses. These electrodes, however, do not differentiate well between univalent cations (e.g. $K^{POT}_{K/Na}$ = 0.33-0.1). They are therefore unsuitable for use in blood/plasma/serum and will not be discussed further.

Several K^+ selective ionophores such as tetra-aryl borates and the crown compounds introduced by Pedersen[57] are available, but for blood plasma and serum measurements; the antibiotic valinomycin (Fig. 2.11f) is favoured. In non-polar solvents, valinomycin has a high selectivity for K^+ over Na^+ and H^+ ($\log K^{POT}_{K/Na} = -5.5$, $\log K^{POT}_{K/H} = -5.0$)[51]. It has a relatively low response time (< 1 min.) and membrane resistances are less than 10MΩ. These factors make valinomycin attractive for blood serum measurements. Membrane compositions recommended are:-

[51] (2.7% Valinomycin, 67.2% DNP*, 30.1% PVC) *Fig. 2.11e

[49] (1.3% Valinomycin, 68.3% ETH 469, 30.9% PVC)

[58] (1.0% Valinomycin, 66.0% DOS, 33.0% PVC)

Most clinical analysers use valinomycin-based membranes. Anderson[59] used the MICROLYTE and KNA1 analysers to examine factors influencing sodium and potassium results in whole blood, serum and plasma. Oswald et al.[60] have continuously monitored K^+ activity during open heart surgery. Fogt et al.[61] monitored K^+ in the whole blood of living dogs. Wuhrmann[62] reported that valinomycin-based membranes gave results comparable with flame photometric measurements in plasma. Band and Treasure[63], however, reported higher ISE readings compared with flame photometry. Linton and Band[64] used a valinomycin based catheter electrode for in vivo monitoring of plasma K^+ in humans and greyhounds. The results were compared with off-line, in vitro analysis and the differences were reported to be insignificant.

Micro-electrode membranes are usually based on potassium tetrakis - (p-chloro-phenyl) borate (KTpClPB, Fig.2.11a) in 2-3 dimethylnitrobenzene[58]. These have low electrical resistances and high selectivity over Na^+.

Among the crown ethers, the most successful contains dimethyl dibenzo-30 crown 10 (Fig. 2.10g) which, built into a PVC matrix has $K^{POT}_{K/Na} = 3 - 4 \times 10^{-3}$. This selectivity suffices for blood serum. However, compared with valinomycin, they have poor stability.

Moss et al.[65] constructed a potassium sensitive ISFET by casting the membrane from THF solutions on the gate and used it to analyse K^+ activity in horse serum. The device was found to operate satisfactorily in presence of proteins, but a negative drift due to protein deposition was observed following the serum analysis.

Sibbald et al.[36] have used valinomycin-based ISFETs for the on-line measurement of blood K^+ during massive blood transfusion and cardiac surgery. Harbinson[56] sputtered NAS(27-4) glasses on to ISFETs to produce K^+ sensitive devices. These gave a Nernstian response of 54mV/pK over the concentration range 10^{-1} mol dm^{-3} to 6.8×10^{-4} mol dm^{-3}; but selectivity over Na^+ was poor. This makes them unacceptable for clinical applications.

2.10 CALCIUM SENSORS

Calcium sensors usable for blood electrolyte determinations are of two types - those based on calcium salts of diakylphosphates as the electro-active material dissolved in an alkylphosphonate or those incorporating a neutral ionophore. The first sensor of the former type was proposed by Ross[66,67] in 1965. It comprised bis (di-n-decyl) phosphate (DDP; Fig.2.10h) dissolved in di-n-octyl phenyl phosphonate (DOPP; Fig. 2.11f) in a liquid form on a Millipore filter. Electrodes based on this type of membrane showed a fair selectivity over other blood electrolytes but their life times were short, they were clumsy to handle and their potential

stability was poor. In addition, their response to hydrogen ions made the electrodes useful only above pH 6, while below pH 5, a characteristic "dip" appeared in the potential difference pH diagram[68,69]. Moody et al.[75] improved on this situation by incorporating the ion-exchanger in a non-porous PVC membrane. This resulted in prolonged life times and reduced the awkwardness of electrode construction. A further improvement in selectivity over Na^+ and H^+ (which results in a shift of the "dips" in the potential difference vs pH curve towards the acid region) was achieved by Ruzicka et al.[71]. They modified the ion exchanger to di-(n-octylphenyl) phosphoric acid (HDOPP, Fig. 2.10i).

Neutral carrier ligands for calcium were introduced by Ammann et al.[58,51]. Membranes based on ETH 1001 (Fig. 2.10j) were reported to be highly selective to calcium over Mg^{2+}, H^+ and Zn^{2+} and selectivities over Na^+ and K^+ were adequate for blood electrolyte studies. Membrane compositions recommended are:-

(1.0% ETH 1001, 66.0% *oNOPE, 33.0% PVC)

(3.4% ETH 1001, 2.0% KTpClB, 62.9% DOS, 31.7% PVC)

*oNOPE = ortho nitrophenyl octyl ether (Fig. 2.11g)

Numerous work has been done with Ca^{2+} ISE's on whole blood, plasma and serum, some will be referred to here. Robertson[72] measured Ca^{2+} in plasma using spectrophotometry and potentiometry. Good agreement was reported between the two methods. Hattner et al.[73] found Ca^{2+} ISE measurements conformed with theoretical expectations. Raddle et al. studied the effects of exposure to air and addition of heparin on calcium ion activity in plasma[74]. Boinck et al.[75] compared four Ca^{2+} analysers (ICA1; Radiometer, ORION SS-20, Electrolyte Analyser 980; AVL, Microlyte; KONE) using whole blood. Larsson et al.[75] used the Radiometer ICA1 as a routine instrument for serum ionised calcium. Smith et al.[75] from serum measurements on the ICA1, concluded that the instrument is a convenient and precise method of measuring

calcium. Brauman et al.[75] investigated the effects of heparin on blood and plasma and compared two analysers (Orion SS-20, ICA1). Measurements during open heart surgery were performed by Vogel[75] et al. Further references can be found in 75, 76 and 77.

ISFET systems incorporating Ca^{2+} membranes have been used for in vivo continuous monitoring of ionised calcium in dogs by McKinley et al.[78]. Sibbald et al.[53] incorporated ETH 1001 as the Ca^{2+} sensor on the four function ISFET array used for the on-line measurement of blood

2.11 OTHER CLINICALLY SIGNIFICANT IONS

2.11.1 Lithium

The monitoring of Li^+ activity in blood is very important during the lithium therapy of patients with maniac depressive psychosis. Li^+ assay is performed by flame photmetry or atomic absorption spectophotometry. This is because sodium levels in blood serum are typically over 1400 times higher than lithium levels and lithium ISEs with $K^{POT}_{Li/Na}$ 10^{-3} are yet to be synthesised[79].

Recently, however, Metzger et al.[80] have reported a lipophilic neutral carrier (ETH 1810, Fig. 2.11k) with a selectivity coefficient of 5×10^{-3}.

2.11.2 Magnesium

Magnesium cannot be monitored, in serum, with ISE's because, so far, there is no electrode designed with sufficient selectivity for Mg^{2+} over Ca^{2}.

2.11.3 Chloride

Solid state chloride electrodes based on AgCl or $AgCl/Ag_2S$ in the homogeneous or heterogeneous forms are not very suitable for serum Cl^- determinations. They are prone to protein interferences. Publications on work with serum based on these include Dahms[81,82] and Cammann[83]. The

latter excluded protein interferences by using Cuprophane dialysis membranes to prevent protein contact with the silver chloride sensor. The Technicon Stat/Lyte chloride sensor uses a dialysis membrane.

Among sensors based on alkyl ammonium chlorides, which act as anion exchangers, methyltricapryl ammonium chloride ('Aliquat' 336,Fig. 2.10ℓ) is widely used[51]. The weakness with these sensors lies in insufficient selectivity over HCO_3^- and poor stability of potential measurements in blood serum.

Recently, Oesch et al.[84] have prepared compounds of the type R_3SnCl as chloride sensors (where $R = -C_4H_9$). They claim the tributyl compound meets the selectivity requirements for clinical sensors, but it shows poor potential stability.

Not much work has been done as yet on chloride sensitive ISFET's. Janata et al.[20] reported a chloride - ISFET based on AgCl. Harbinson[56] used tri-octyl propyl ammonium chloride in 10% V/V n-decanol incorporated in PVC over an ISFET gate. In the clinical range, reasonable selectivities over bicarbonate and monohydrogen phosphate were reported and the chloride response was almost linear.

2.11.4 Fluoride

LaF_3 single crystal doped with EuF_2 is the most widely used form of electro-active material for fluoride ion-selective electrodes. These sensors are very specific, OH^- being the only ion causing any interference effects. Fluoride ion monitoring is desirable, in the clinical laboratory, for certain medical conditions such as fluoride metabolism in renal insufficiency etc.[83] Routine analysis, however, is not performed probably because of the

low levels of fluoride is plasma. Fuchs et al.[85] developed a method based on the single known addition method and the electrode slope by dilution method to overcome this difficulty. Work on plasma fluoride determinations has also been done by Cowell et al.[86] and Venkateswarlu[87].

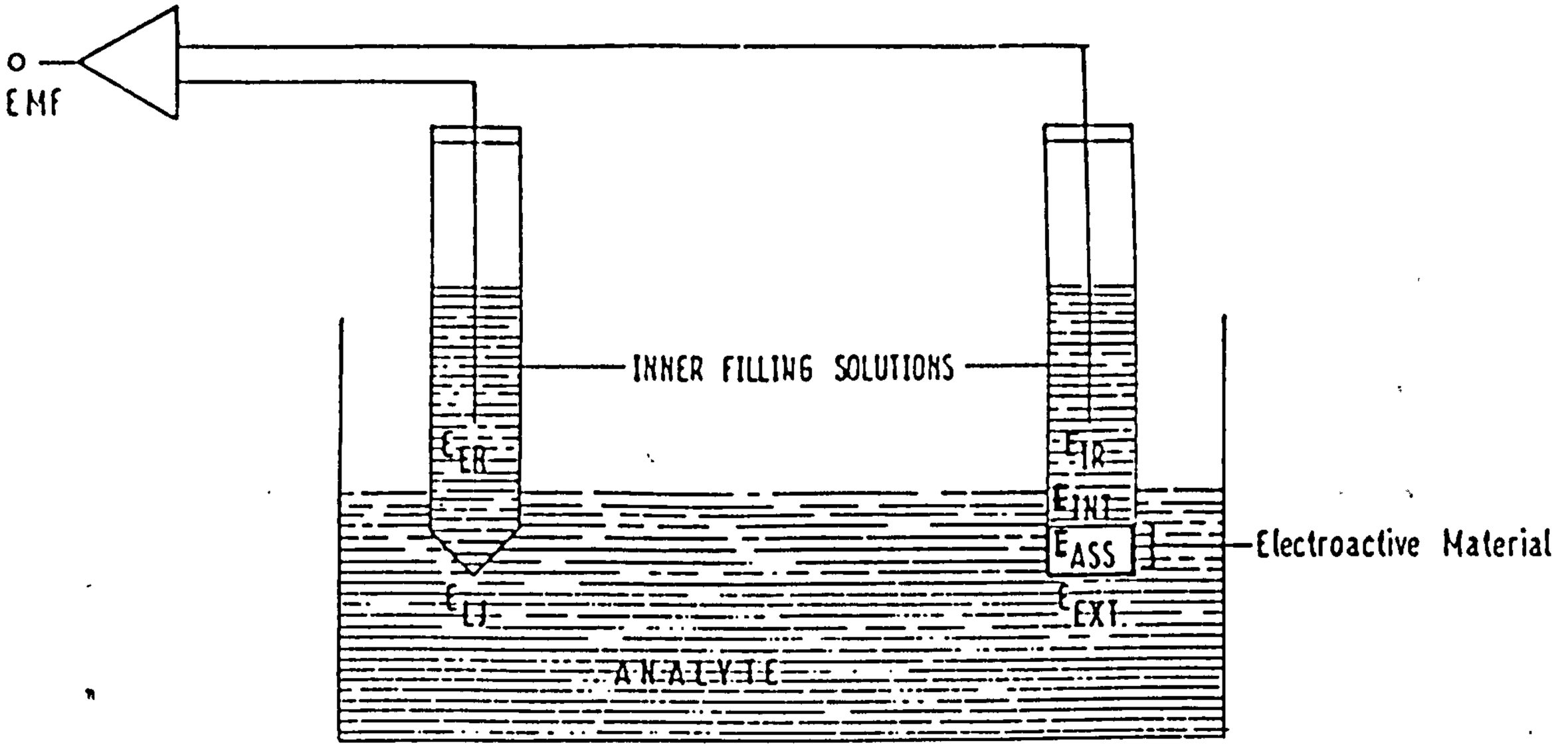
2.11.5 Bicarbonate

The usual method for determining bicarbonate levels in plasma is measuring pH and pCO_2 and using the Henderson-Hasselbalch equation.

$pH = 6.35 + \log [HCO_3^-]/[CO_2]_{dissolved}$.

The accuracy of these determinations is questionable[51]. Research into developing a satisfactory HCO_3^- electrode is being done. Among sensors proposed, membranes based on ethanolamine derivatives seem to be the most promising so far (e.g. ETH 595, Fig. 2.10m).

F I G U R E S



E_{ER} = potential of the external reference electrode

E_{LJ} = Liquid junction potential between the salt bridge and analyte

E_{EXT} = External phase boundary potential of the membrane

E_{INT} = Internal phase boundary potential of the membrane

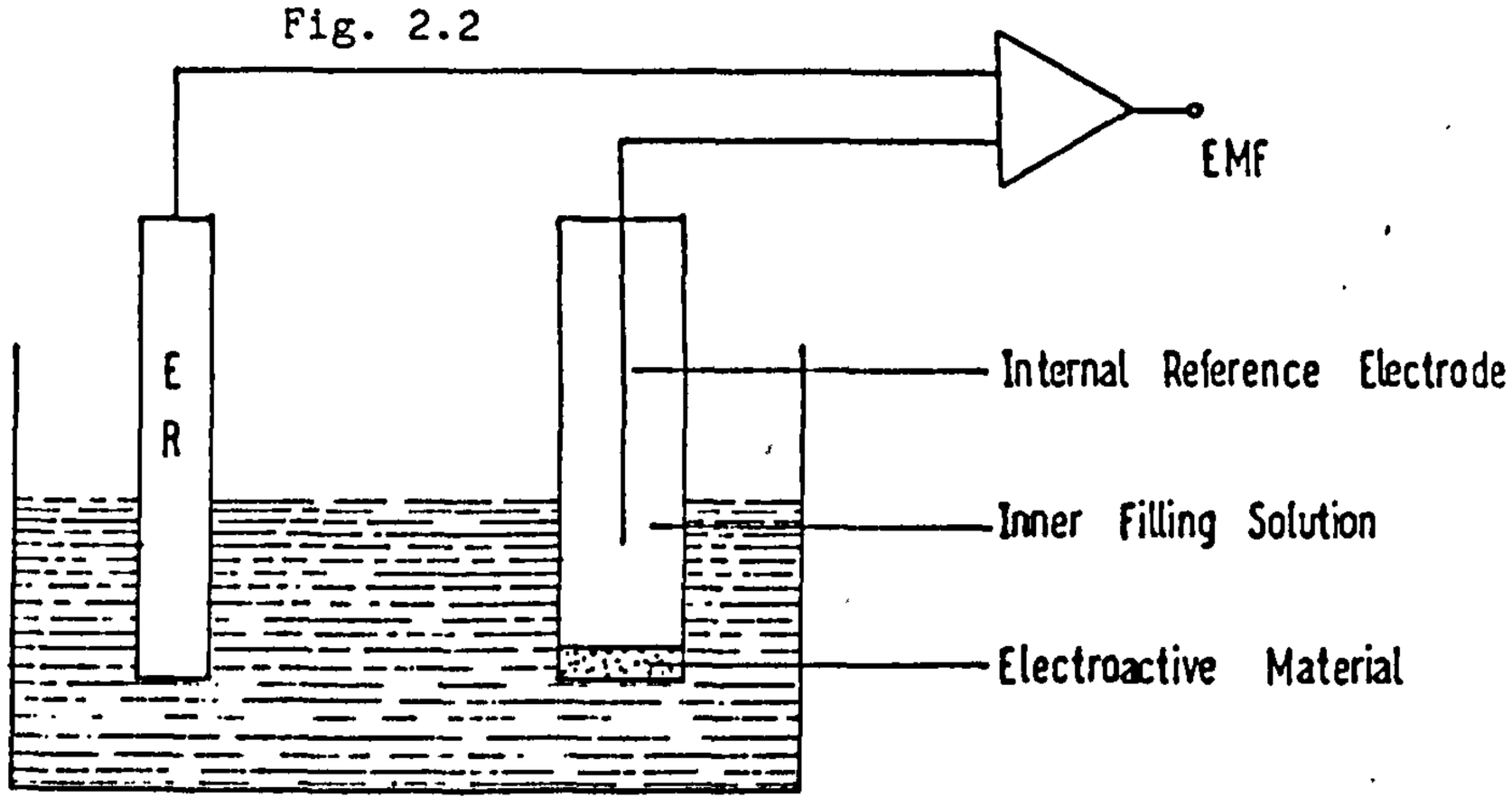
E_{ASS} = Assymetry potential within the membrane (also called diffusion potential)

E_{IR} = potential of the internal reference electrode

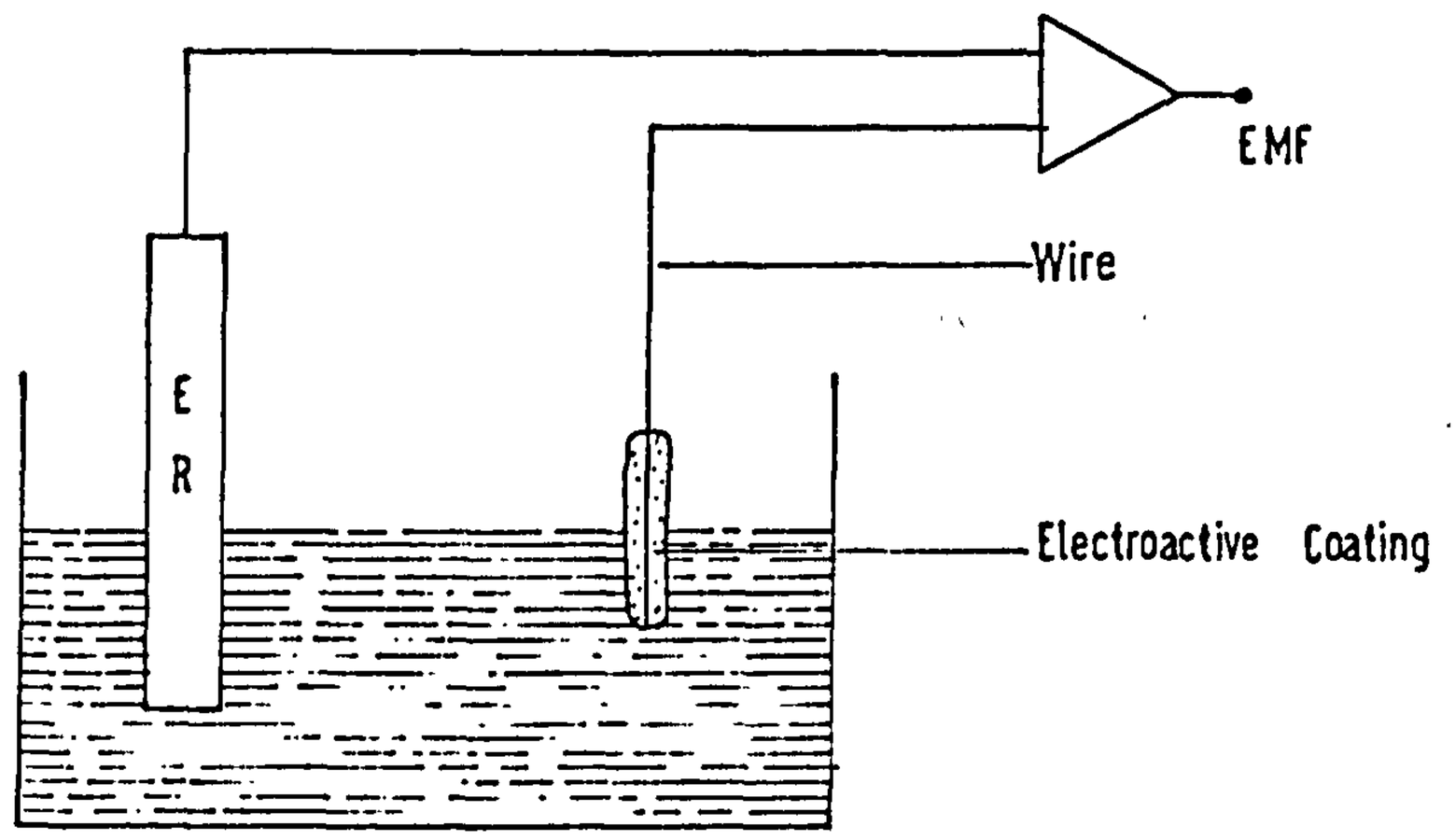
Fig. 2.1

Fig. 2.2

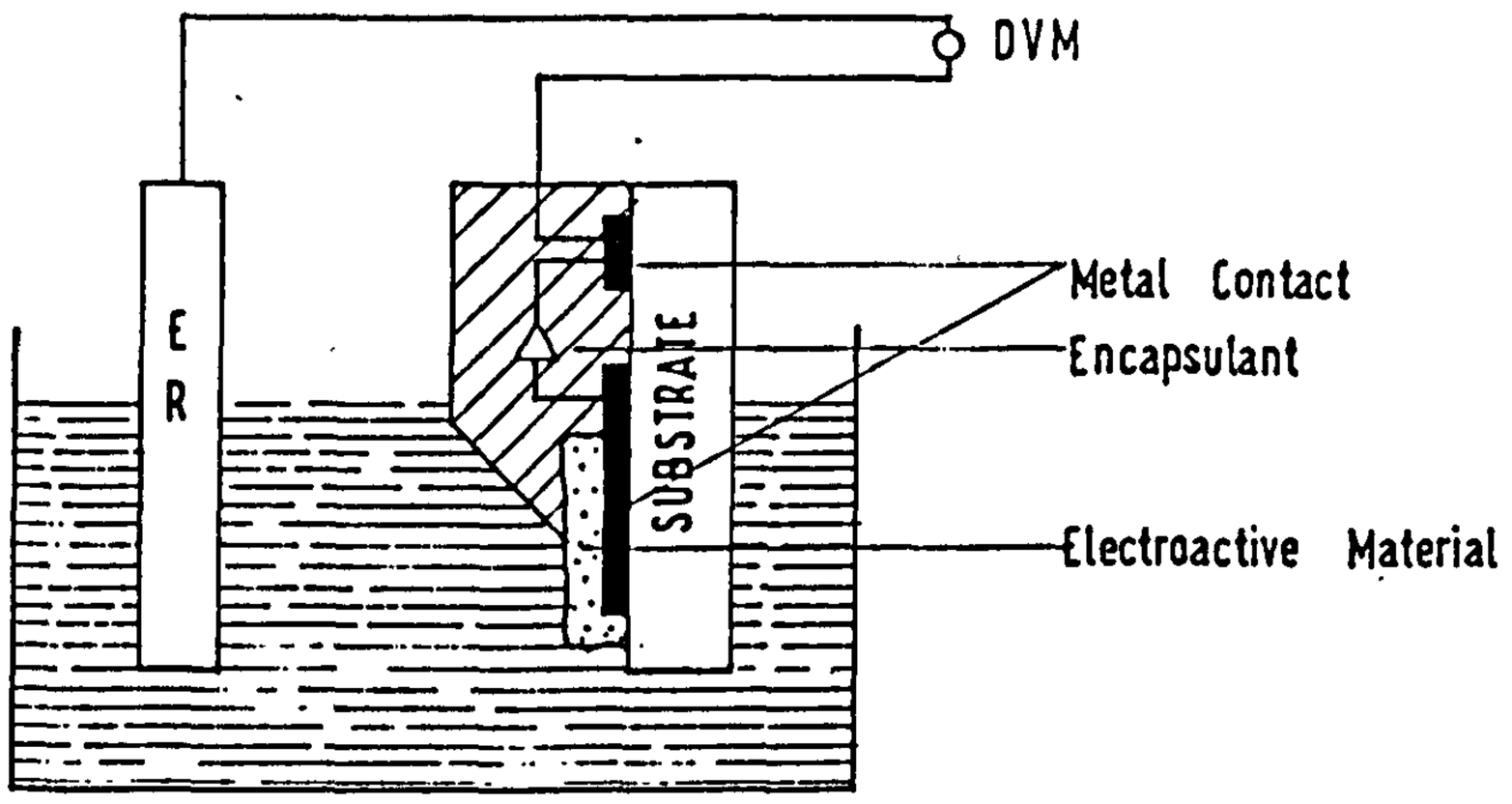
CONVENTIONAL ISE



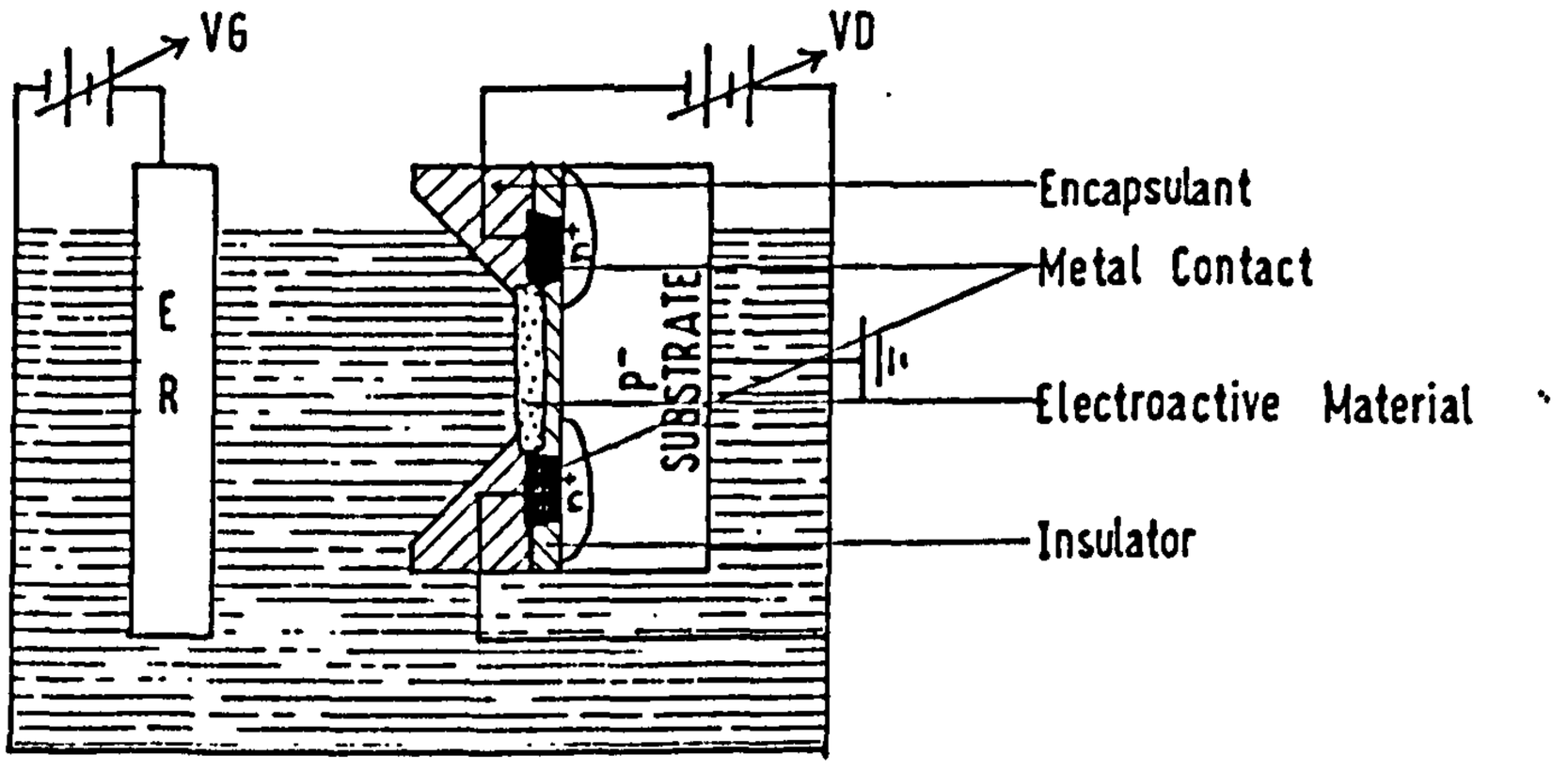
COATED WIRE ISE



HYBRID MICROELECTRONIC ISE



ISFET



THE MIS SYSTEM

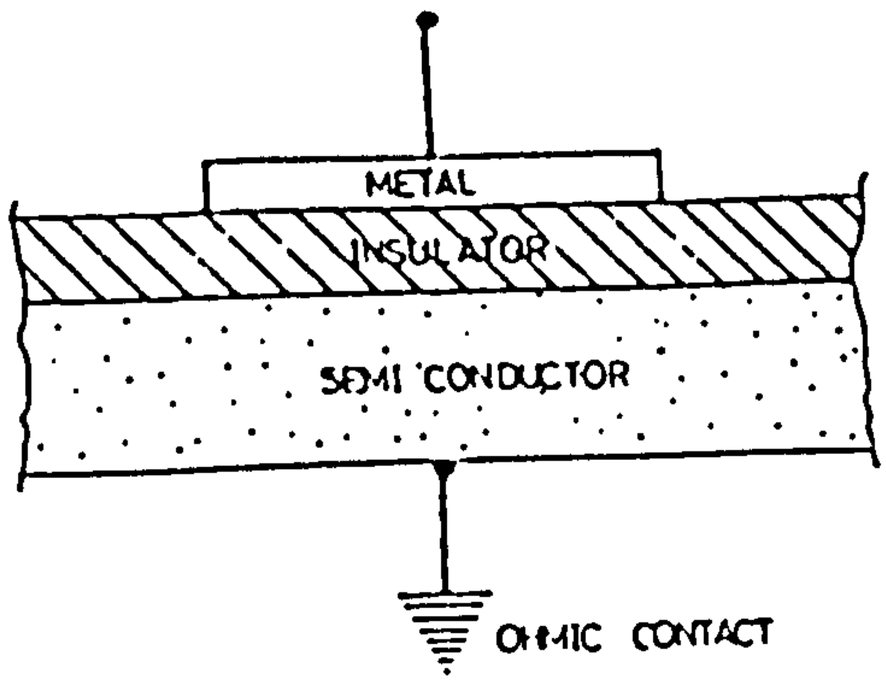


FIG 2.3a

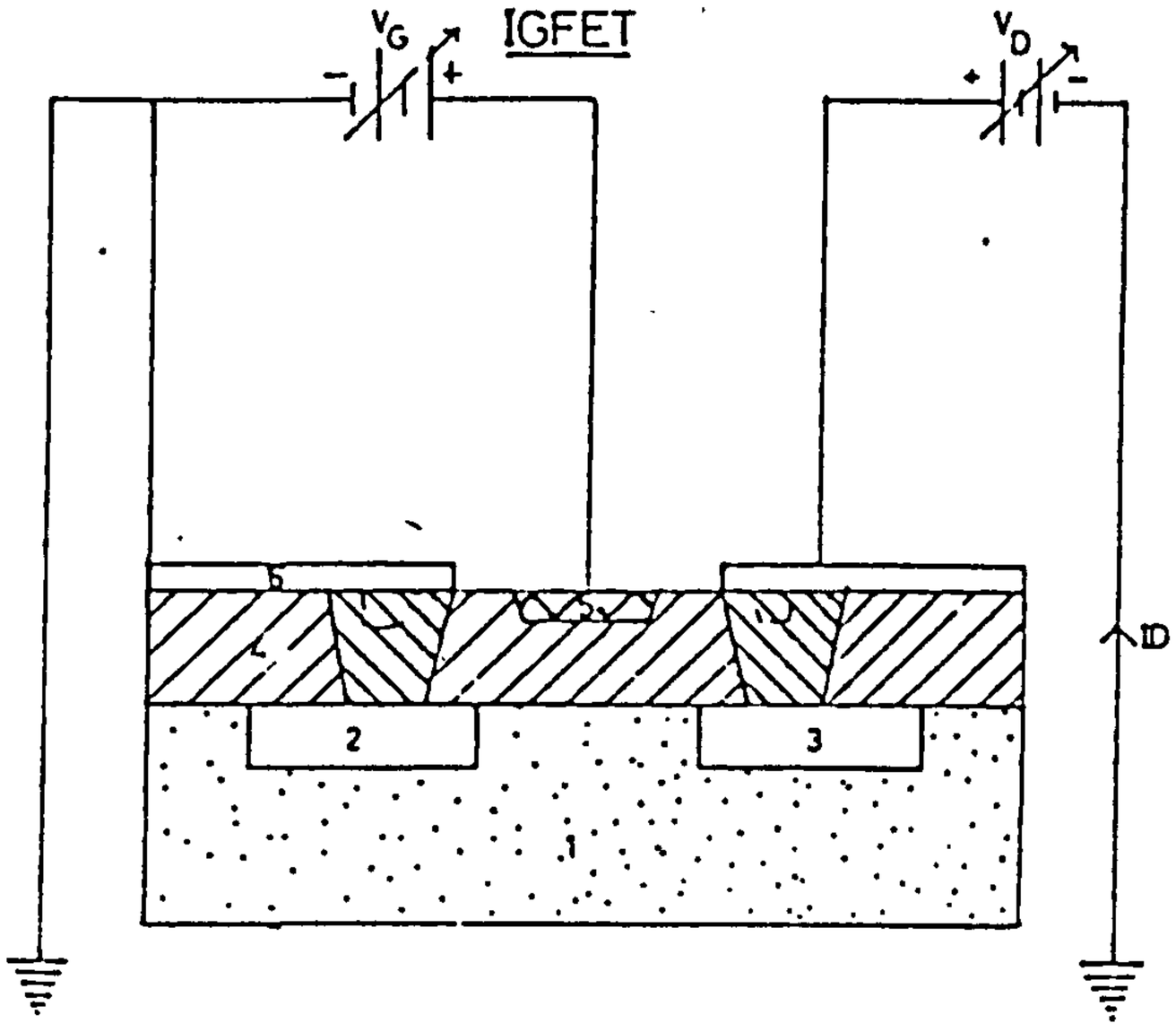


FIG 2.3b

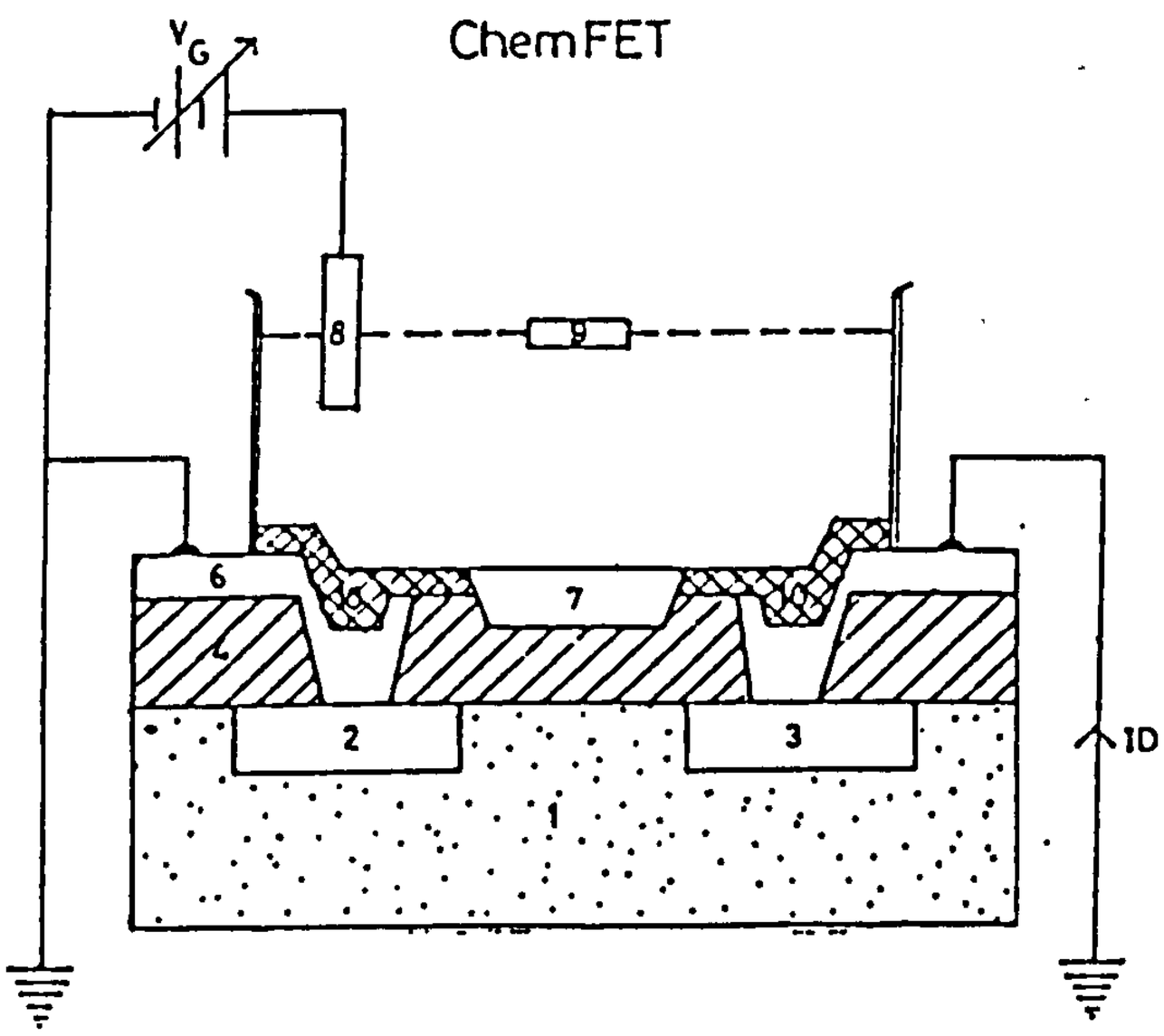
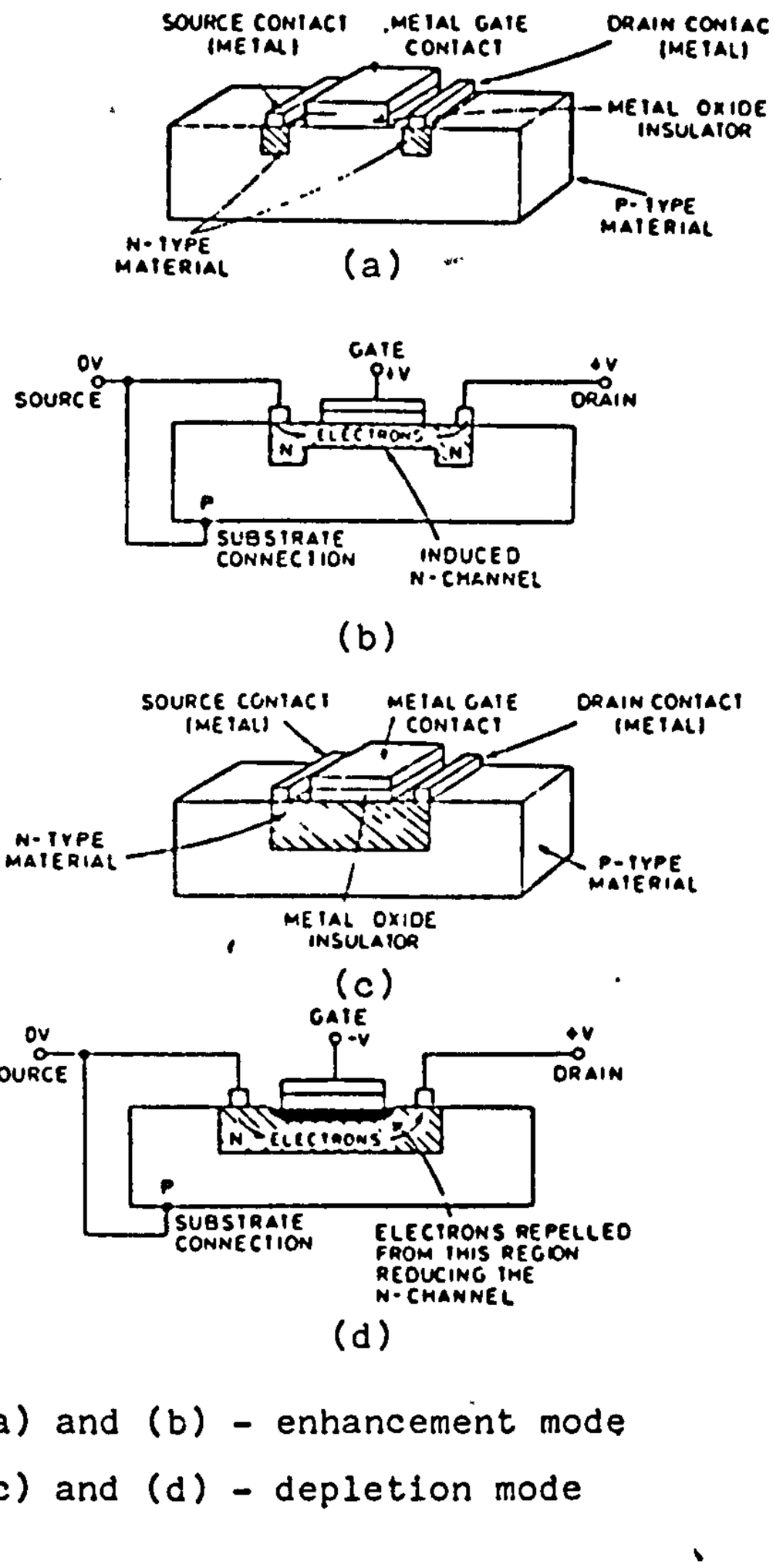


FIG 2.3c

Labelling of Fig 2.3b and Fig.2.3c

1. Lightly doped p-type Silicon substrate
2. Heavily doped n-type source
3. Heavily doped n-type drain
4. Insulator (SiO_2/Si_3N_4)
5. Gate
6. Metal contacts
7. Electroactive thin film (Bare Insulated Gate for H^+ sensitive (ISFET)
8. Reference Electrode
9. Solution
10. Encapsulation

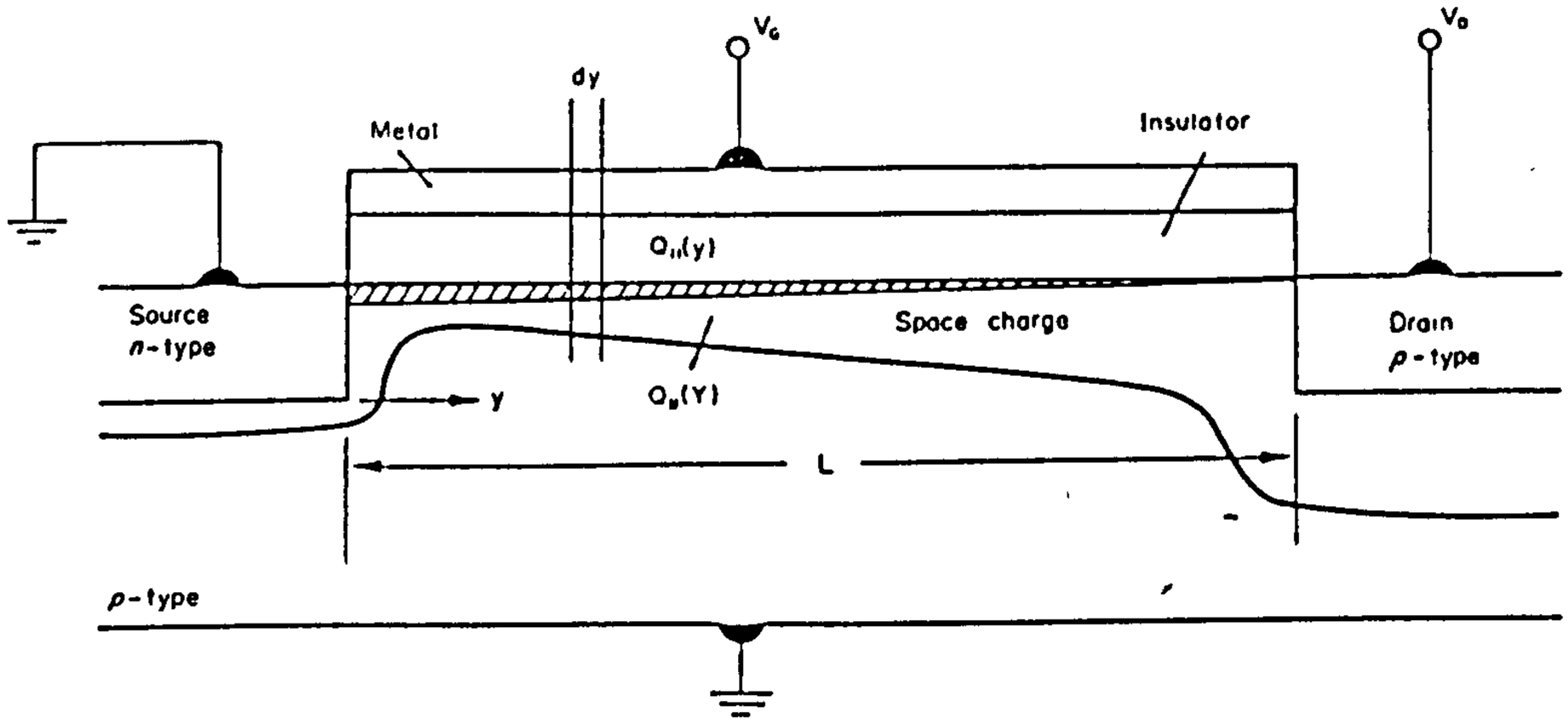


(a) and (b) - enhancement mode

(c) and (d) - depletion mode

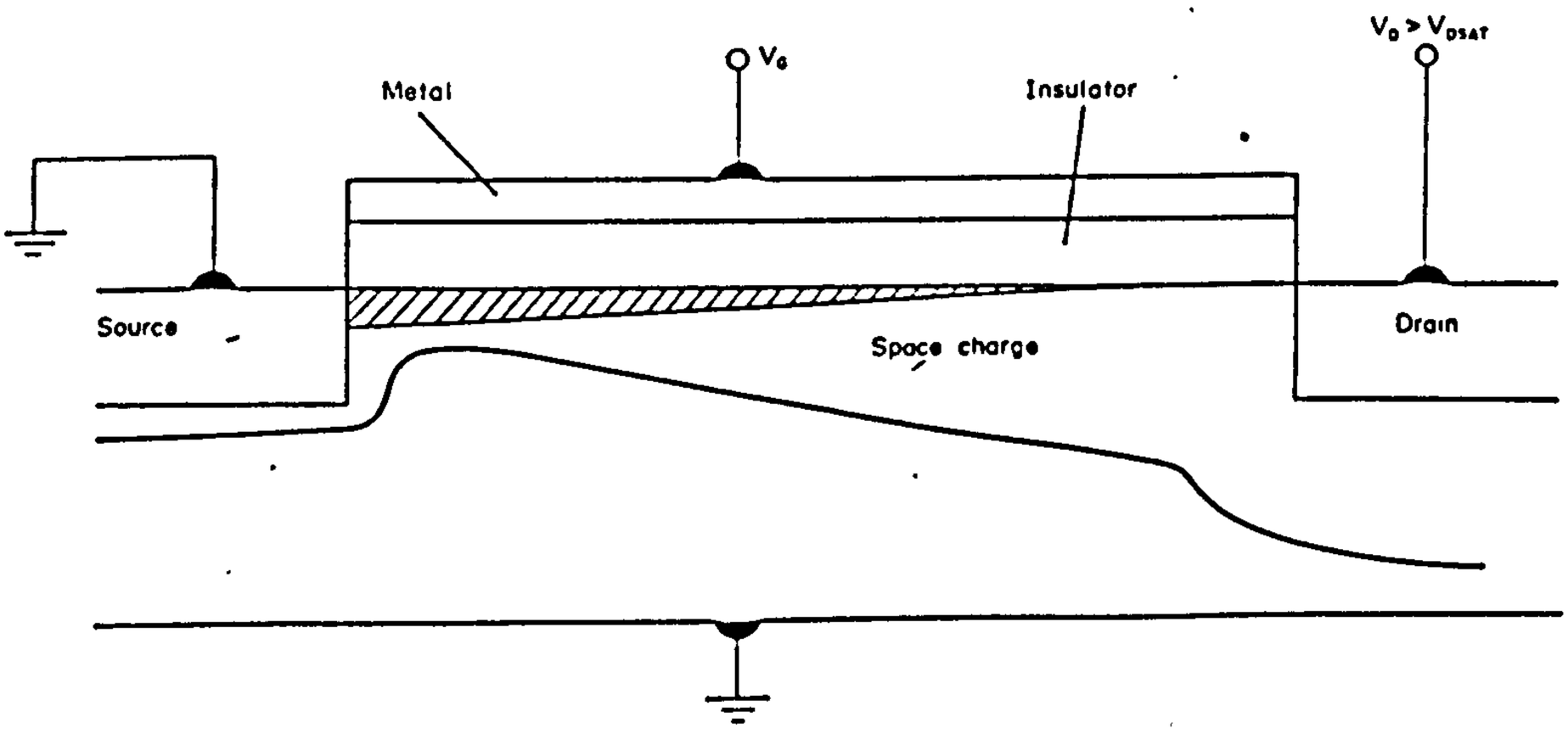
Fig. 2.4

[from "Beginners Guide to Electronics" by O. Bishop Newnes Technical Books (1985)]



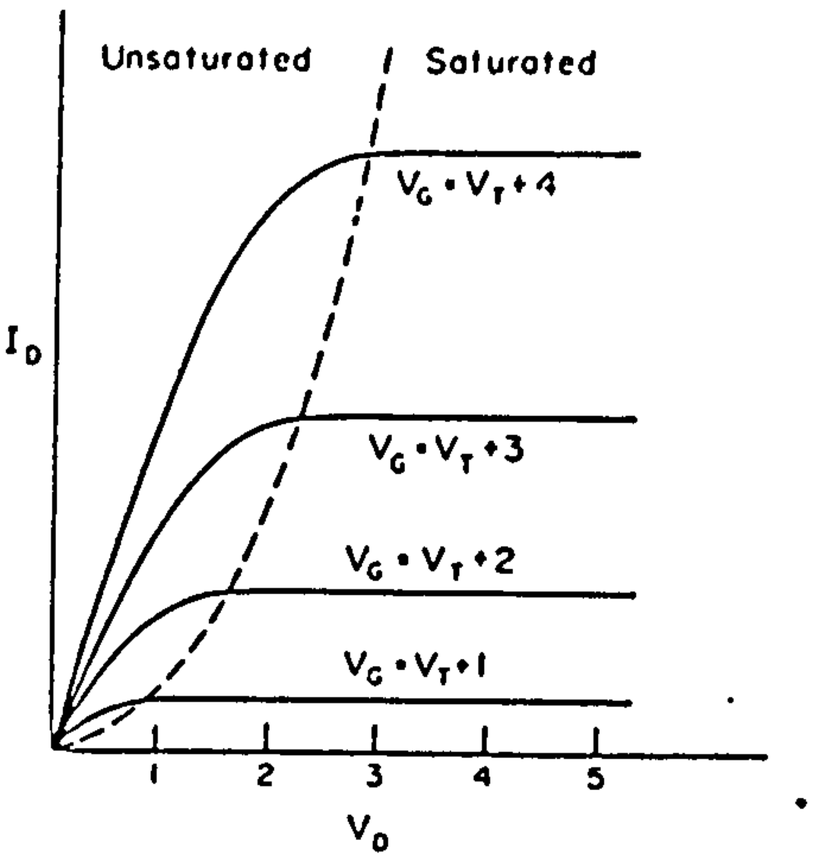
IGFET channel.

Fig. 2.5a



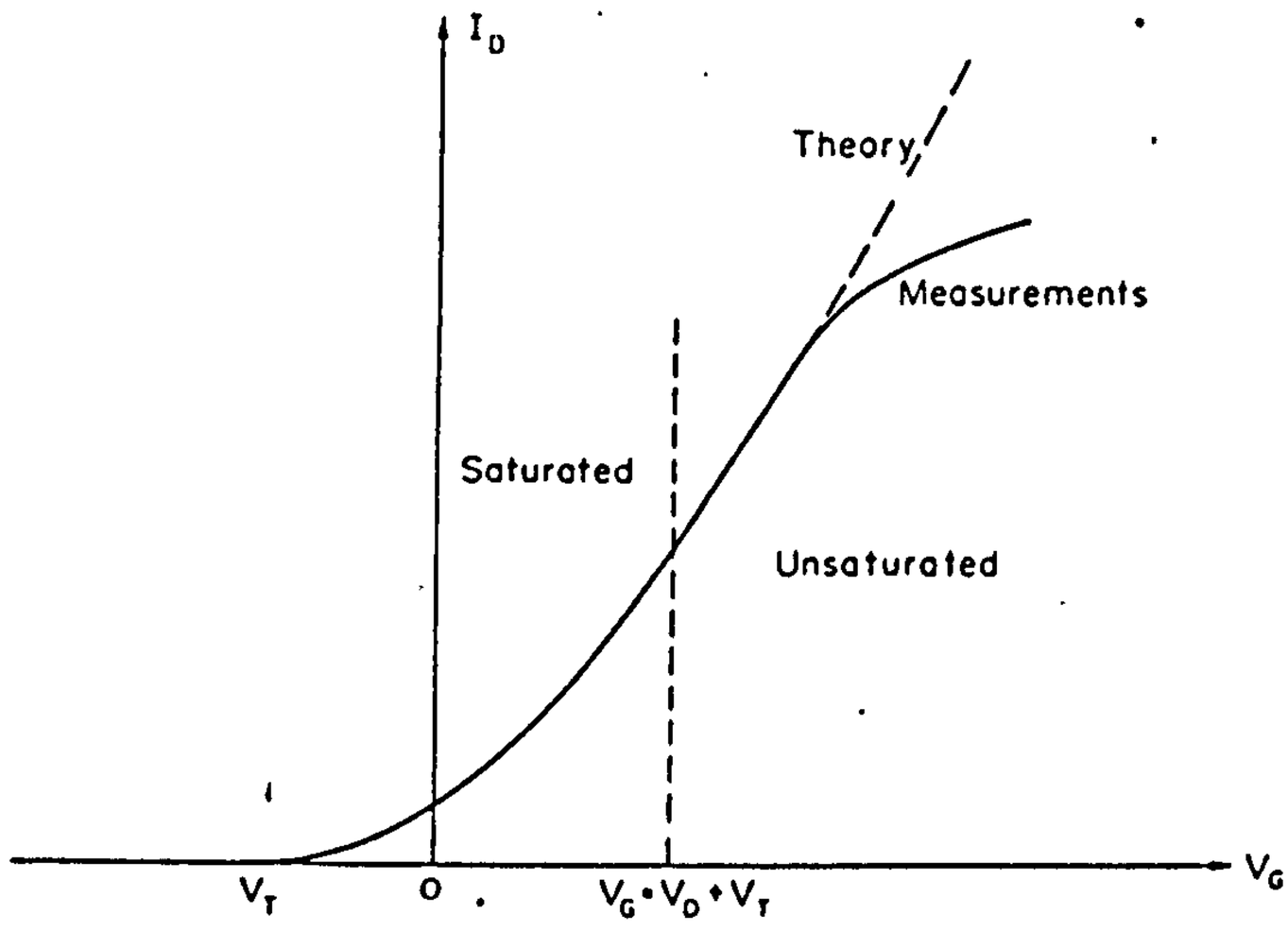
IGFET in saturation.

Fig. 2.5b



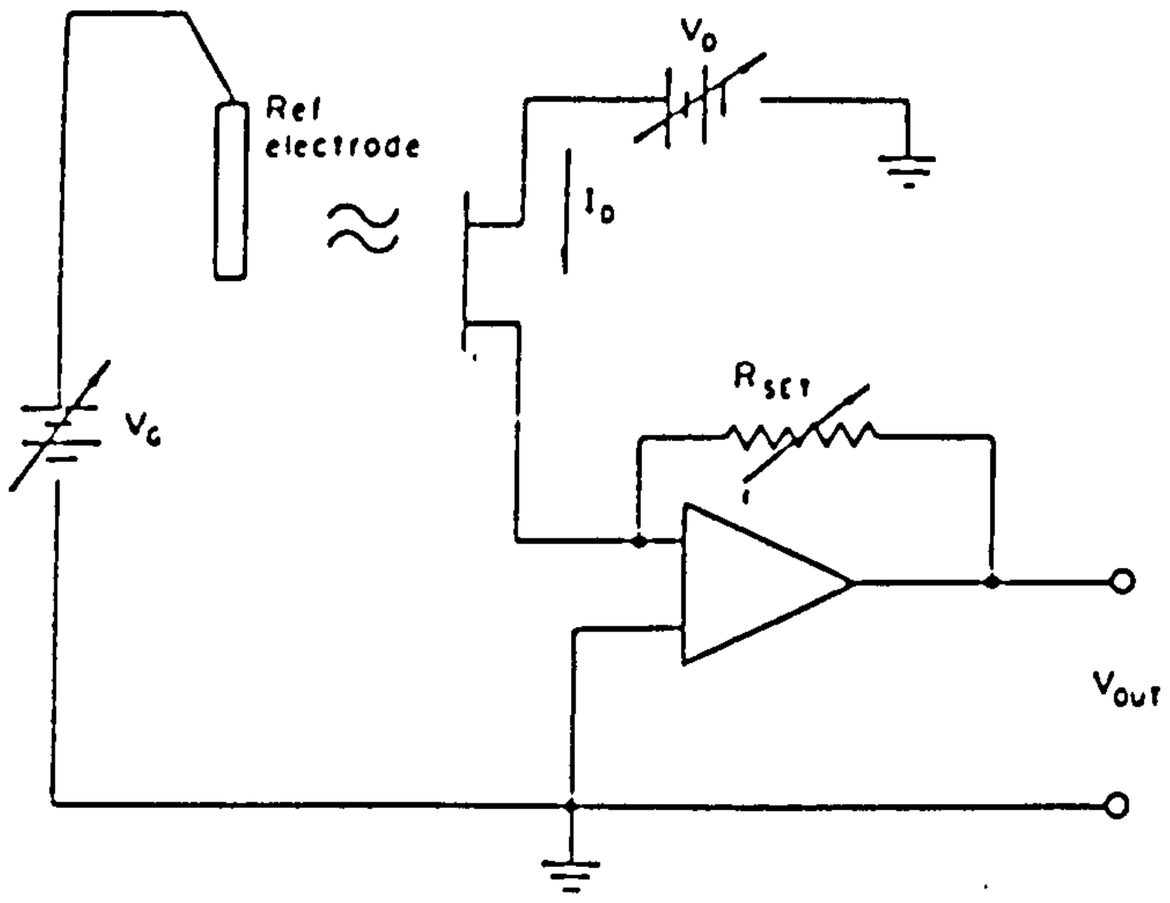
Change of drain current I_D with drain voltage V_D showing saturation and unsaturation.

Fig. 2.6a



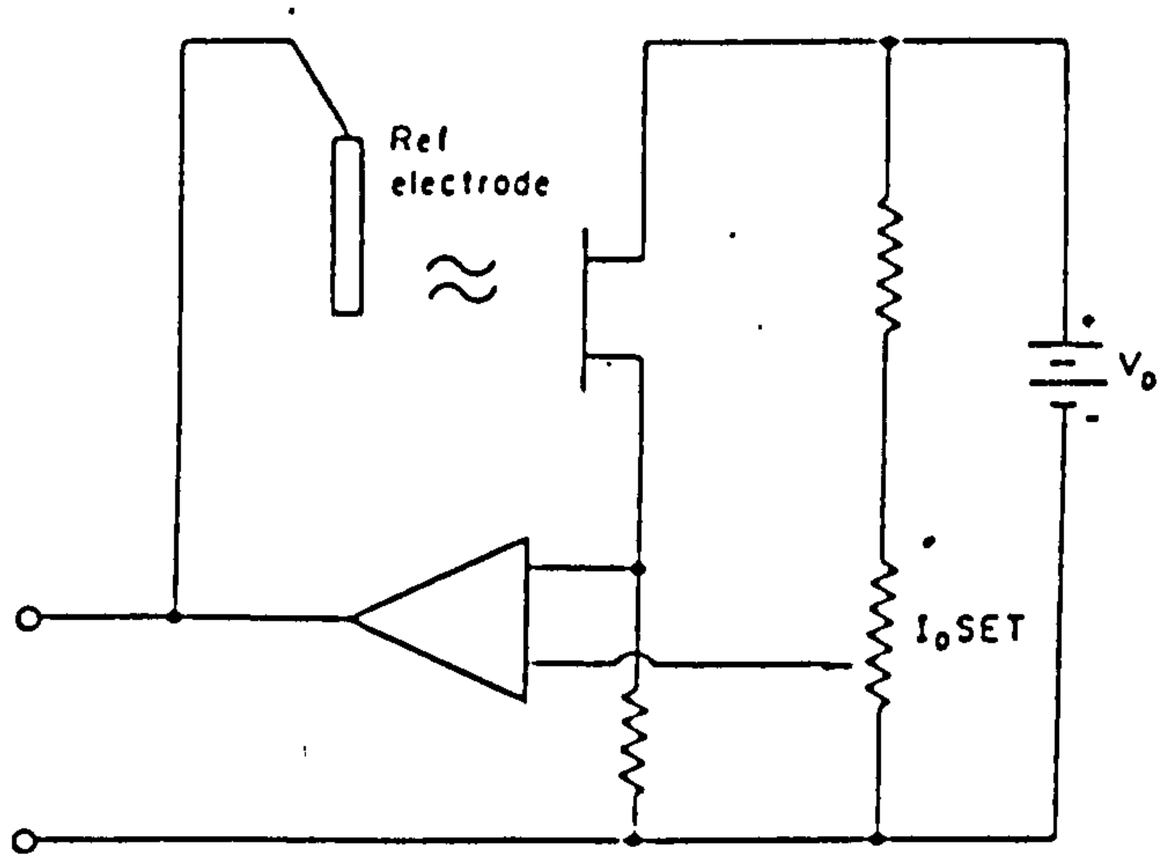
Change of drain current I_D with gate voltage V_G divided into saturated and unsaturated regions.

Fig. 2.6b



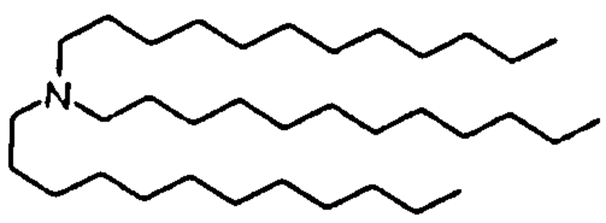
Schematic of circuit diagram for measurement of drain current at constant V_G.

Fig. 2.8a



Schematic of circuit diagram for measurement of ISFET response at constant I_D (feedback).

Fig. 2.8b



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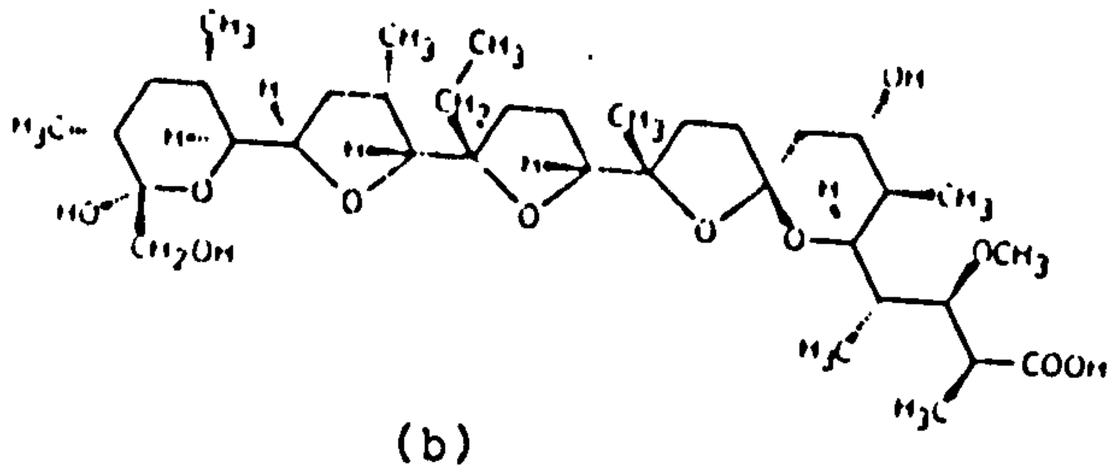

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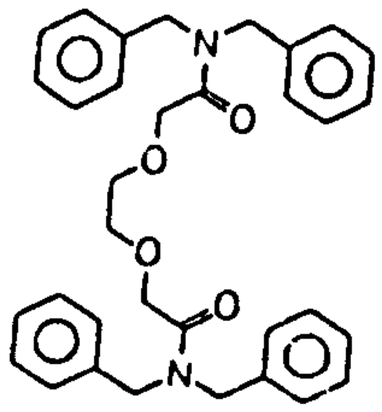

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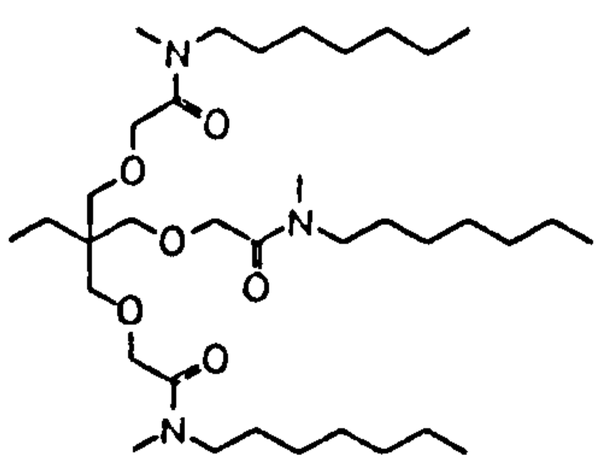

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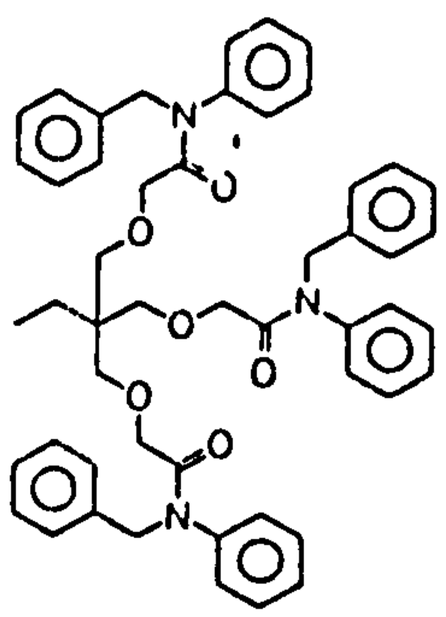

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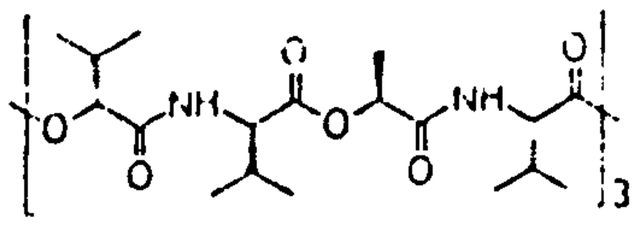

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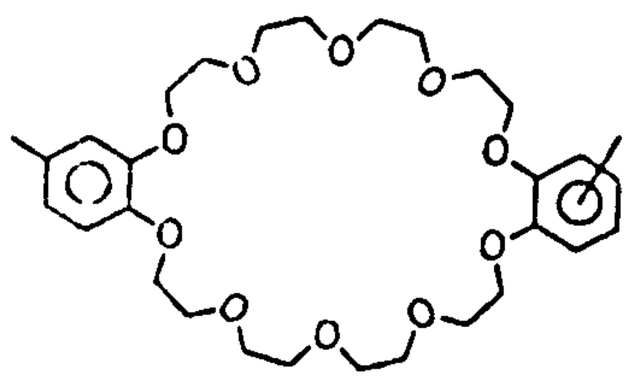

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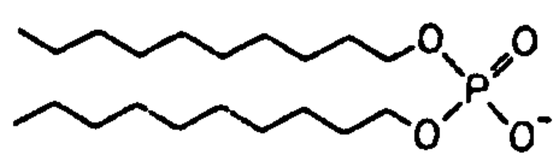

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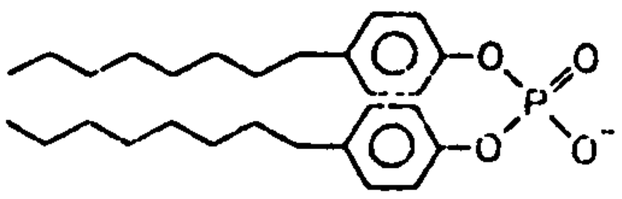

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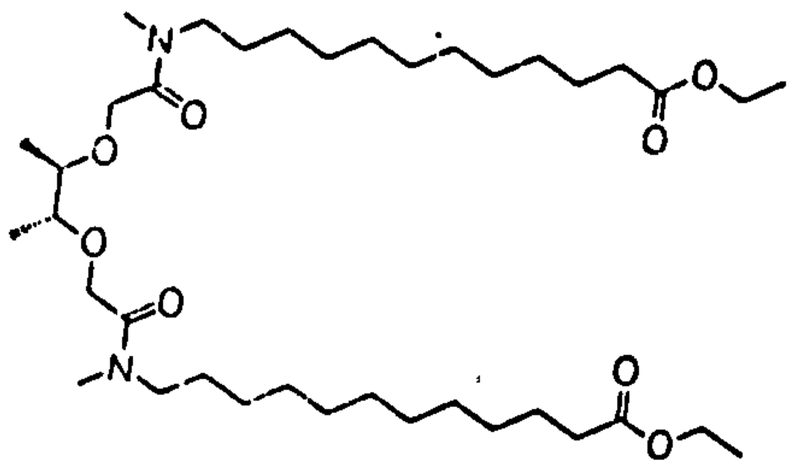

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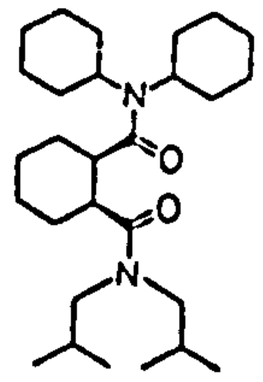

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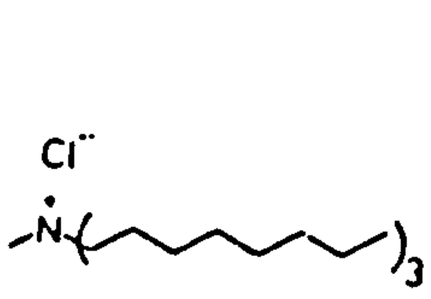

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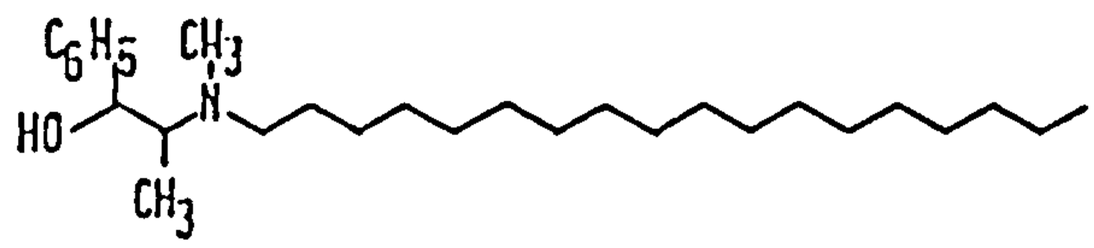
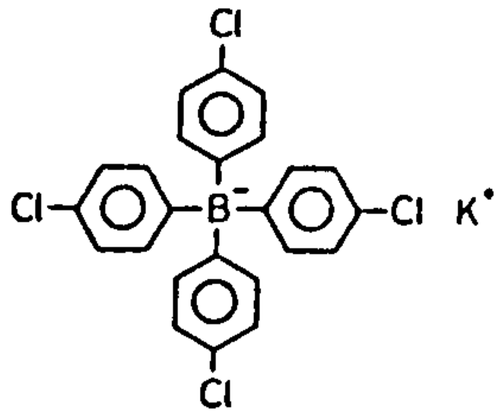


(m)

Fig. 2.10



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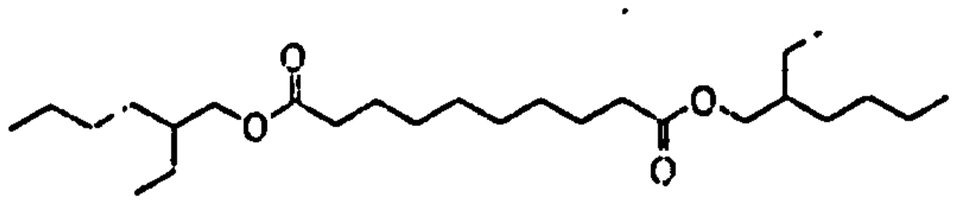

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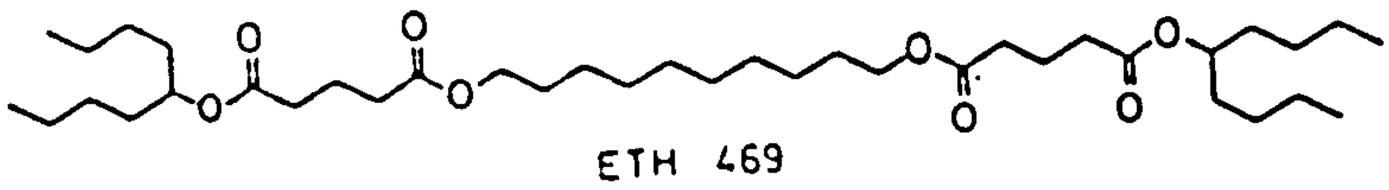

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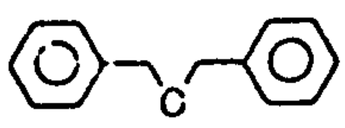

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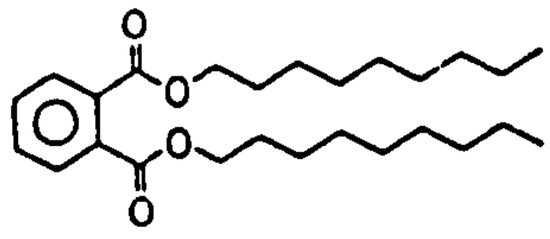

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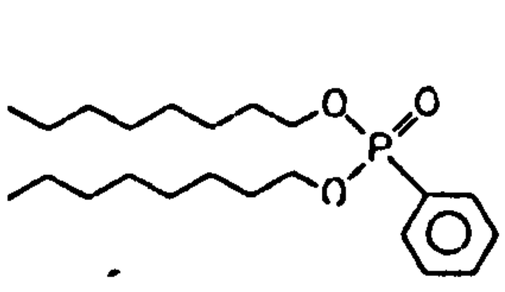

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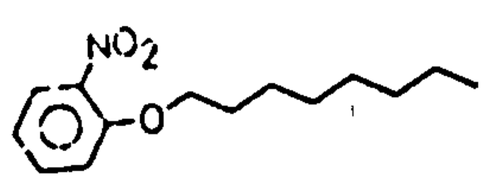


(g)

Fig. 2.11

REFERENCES

R E F E R E N C E S

(1) Covington A.K. and Rebelo M.J.F., Ion-Selective Electrode Rev, 5 93-128 (1983)

(2) Bailey P.L., "Analysis with Ion-Selective Electrodes", Chap. 2, Heyden, London (1976)

(3) Burton P.R., in "Ion-Selective Electrode Methodology" Vol. I, Chap. 2, ed. A.K. Covington, CRC Press, Florida (1979)

(4) Rebelo, M.J.F., Ph.D thesis, University of Newcastle upon Tyne (1981)

(5) Section 7.1, Chapter Seven, This Thesis.

(6) Eisenman, G., in "Ion-Selective Electrodes", Chap. 1, ed. R.A. Durst, Natl, Bur. of Standards Spec. Publ., No. 314, Washington D.C. (1969)

(7) Chap. 3 in reference 2.

(8) IUPAC Recommendation for Publishing Manuscripts on I.S.E.'s, Pure Appl. Chem, 1, 69 (1978)

(9) Pure and Appl. Chem, 48, 127 (1976)

(10) Pure and Appl. Chem, 58, 469 (1986)

(11) Morf W.E., in "The Principles of I.S.Es. and of Membrane Transport", Chap. 14, Elsevier, Amsterdam (1981)

(12) Papacostas S., Ph.D Thesis Chap. XI, University of Newcastle Upon Tyne (1986)

(13) Freiser H., J.C.S., Faraday, 1, 82, 1217-1211 (1986)

(14) Cattrall R.W., Chap. 8 in Reference 3

(15) Ruzicka J. and Lamm C.G., Anal Chim Acta, 54, 1 (1971)

(16) Cattrall R.W. and Hamilton I.C., Ion-Selective Electrode Rev., 6, 125 - 172 (1984)

(17) Kelly R.G. and Owen A.E., J.C.S., Faraday, 1, 82, 1195-1208 (1986)

(18) Fjeldly T.A., Nagy K. and Stark B., Sensors and Actuators, 3, 111-118 (1983)

(19) Grove A.S., "Physics and Technology of Semi-Conductor Devices" Wiley, N.Y. (1967)

(20) Janata J. and Huber R.J. in "Ion-Selective electrodes in Analytical Chemistry", Vol. 2, Chap. 3, ed. H. Freiser, Plenum, N.Y. (1980)

(21) Sibbald A., J. Molecular Electronics 1 (1986)

(22) Sibbald A., I.E.E. Proc., 130, Pt. 1, 5, (1983)

(23) Zachariasen W.H., J. Amer. Chem. Soc., 54, 3841 (1932)

(24) Bates R.G., "Determination of pH, Theory and Practice", Chap. 11, Wiley, N.Y. (1973)

(25) Schwabe, K., Dahms, H., Nguyen, W., and Hoffmann, G., Z Electrochem, 66, 304 (1962).

(26) Eisenman G., Biophys J.2, Part 2 Suppl. 259 (1962)

(27) Covington A.K. and Prue J.E., J. Chem. Soc., 3696, 3701 (1955)

(28) Hebert N.C., Chap. I (3) in "Ion-Selective Micro-electrodes" ed. Berman H.J., and Hebert N.C., Plenum, N.Y. (1974)

(29) Burgermeister W. and Winkler-Oswatitsch "Topics in Current Chemistry", 69, 91 (1977)

(30) Schulthess P., Shijo Y., Pham H.V., Pretsch E., Ammann D. and Simon W., Anal Chim Acta, 131, 111 (1981)

(31) Ammann D., Lanter F., Steiner R.A., Schulthess P., Shijo Y. and Simon W., Anal Chem., 53, 2267 (1981)

(32) Ammann D., Anker P., Metzger E., Oesch U. and Simon W., in "Ion Measurements in Physiology and Medicine", 102, Ed. M. Kessler et al, Springer-Verlag, Berlin Heidelberg (1985)

(33) Schepel S.J., De Rooji N.F., Koning G., Oeseburg B. and Zijlstra W.G., Med. Biol. Eng. Comput., 22, 6 (1984)

(34) Kohama A. et al, Crit. Care Med., 12, 940 (1984)

(35) Ruzicka J. and Ramsing A., Scand. J. Clin. Lab. Investig. Suppl. 161, 42, 35 (1982)

(36) Sibbald A., Covington A.K., Carter R.F., Clin. Chem. 30, 135 (1984)

(37) Esashi M. and Matsuo T., IEEE Trans. Biomed. Eng., 25, 184 (1978)

(38) Szonntagh E.L., J. Electrochem. Soc. 125, C357 (1978)

(39) Vesely J., et al, Chap. 4 in "Analysis with Ion-Selective Electrodes, ed. Chalmers R.A. and Masson M.R., Ellis Horwood Limited, Chichester, U.K. (1978)

(40) Eckfeldt E.L. and Proctor W.E., Anal. Chem. 43, 332 (1971)

(41) Gotoh F., Tzaki, Y., Hamagudi, K., and Meyer, J.S., J. Neurochem, 9, 81 (1962)

(42) Moore E.W., and Wilson D.W., J. Clin. Invest, 42, 293 (1963)

(43) Khuri R.N., Goldstein, D.A., Mairde, D.L., Edmonds, C., and Solomon, A.K., Amer, J. Physiol, 204, 743 (1963)

(44) Khuri R.N., Flanigan, W.J., and Oken, D.E., J Appl. Physiol, 21, 1568 (1966)

(45) Jacobson H., Anal Chem., 38, 1951-1954 (1966)

(46) Dahms H., Rock R., and Seligson D., Clin. Chem., 14, 859 (1968)

(47) Miyada D.S., et al; Clin. Chem., 17, 27-30 (1971)

(48) Trutnovsky H., Z. Klin Chem. Klin Biochem., 9, 341-345 (1971)

(49) Simon W., Ammann D., Oehme M., and Morf W.E., Ann, N.Y. Acad. Sci., 52, 307 (1978)

(50) Fielder U., Anal Chim. Acta. 89, 101 (1977)

(51) Meir P.C., Ammann D., Morf W.E., Simon W., Chap. 2 in "Medical and Biological Applications of Electro-Chemical Devices", ed. J. Koryta, Wiley (1980)

(52) Kraig R.P. and Nicholson Ch., Science, 194, 725 (1976)

(53) Sibbald A., Covington A.K. and Carter R.F., Clin. Chem. 30, 135 (1984)

(54) Oesch U., Caras S. and Janata J., Anal Chem., 53, 1983-1986 (1981)

(55) Matsuo T. and Esashi M., Sensors and Actuators, 7, 77 (1981)

(56) Harbinson T.R., Ph.D Thesis, University of Newcastle Upon Tyne (1986)

(57) Pedersen C.J., J. Amer. Chem. Sec., 89, 7017 (1967)

(58) Ammann D., Morf W.E., Anher P., Meier P.C., Pretsch E. and Simon W., Ion-Selective Electrode Rev., 5, 3-92 (1983)

(59) Andersen N.F., Clin. Chem., 30 (1984)

(60) Oswald H.F., Asper R., Dimai W. and Simon W. Clin. Chem., 25, 39-43 (1979)

(61) Fogt E.J., Eddy A.R., Clemons A.H., Fox J., Heath H., Clin. Chem., 26 (10), 1425-1429 (1980)

(62) Wuhrmann H.R., Biomed Technik, 21, 191 (1976)

(63) Band D.M. and Treasure T., Chap. 3 in "ION-SELECTIVE ELECTRODE METHODOLOGY", Vol. II, ed. A.K. Covington, C.R.C. Press (1979)

(64) Linton R.A.F. and Band D.M., Crit. Care Med., 10(5), 337 (1982)

(65) Moss S.D., Janata J. and Johnson C.C., Anal Chem. 47(13), 2238 (1975)

(66) Ross J.W., Science, 156, 1378 (1967)

(67) U.S. Patent 3 445 365 (10/8/1965)

(68) ORION Research, Instruction Manual, Calcium Ion Electrode Model 92-20 (1968)

(69) Bagg J. and Vinen R., Anal. Chem. 44 1773 (1972)

(70) Moody G.J., Oke R.B., and Thomas J.D.R., Analyst, 95, 910 (1970)

(71) Ruzicka J. et al., Anal. Chim. Acta., 67, 155-178 (1973)

(72) Robertson, W.C., Clin. Chim. Acta., 24, 149-157 (1969)

(73) Hathner R.S., Johnson J.W., Bernstein D.S., Wachman A. and Brackman J., Clin. Chim. Acta., 28, 67-75 (1970)

(74) Radde I.C., Hoffken B., Parkinson D.K., Sheepers J. and Luckham A., Clin. Chim. 17(10), 1002 (1971)

(75) Larsson, L., Finnstrom, O., Nilsson, B., and Ohman, S., 21, Scand J. Clin. Lab. Invest. Vol. 43 (165) (1983)

(76) "Ionised Calcium, Sodium and Potassium by Ion-Selective Electrodes", Vol. 5, ed. Maas A.H.J., et al. Private Press (1984)

(77) Moore E.W., Chapter 7 in reference 6.

(78) McKinley B.A., Crit. Care Med. 9, 333-339 (1981)

(79) Gadzekpo V.P.Y., Moody G.J. and Thomas J.D.R., Analyst, 111 567 (1986)

(80) Metzger E., Ammann D., Asper R. and Simon W., Anal. Chem. 58, 132 (1986)

(81) Dahms H., Clin. Chem., 13, 437-449 (1967)

(82) See reference 46

(83) Chapter 3 in reference 51

(84) Oesch U., Ammann D., Pham H.V., Wuther U., Zund R. and Simon W., J. Chem. Soc. Faraday 1, 82, 1179-1186 (1986)

(85) Fuchs C., Dorn D., McIntosh C. and Scheler F., Clin. Chim. Acta, 60, 157-167 (1975)

(86) Cowell D.C., Ann Clin. Biochem., 14, 275-278 (1977)

(87) Venkatesworlu P., Clin. Chim. Acta, 59, 277-282 (1975)

(88) Funck R.J.J., Morf W.E., Schulthess P., Ammann D., Simon W., Anal. Chem., 54, 423-429 (1982)

CHAPTER THREE

CLINICAL CONSIDERATIONS

CHAPTER THREE

In this chapter, additional considerations pertaining to the use of ion-selective devices in clinical media, particularly blood, plasma and serum will be outlined.

Ion-selective devices have been welcomed to the field of clinical analysis because of their relatively rapid response to the physiologically important parameter - the activity of the ions. Moreover, ion-selective devices, particularly ISFET's are easily miniaturised, economical to produce, compact and hence can be made into easily transportable units. However, there are some sources of errors and complications, a few of which are yet to be resolved.

3.1 Direct and Indirect Potentiometry

Potentiometric measurements on undiluted samples give the activity of the ion. Such measurements are termed direct potentiometry. When measurements are made on diluted samples and the original sample concentration is calculated, the operation is termed indirect potentiometry. If a species is present in different physiochemical forms, the two modes of operation can give rise to results of very different magnitudes. Calcium is present in blood as ionised calcium and as complexed calcium. Direct potentiometry measures the ionised fraction. When the sample is diluted for indirect potentiometry, the complex calcium dissociates and the quantity measured tends towards the total calcium. A further consequence of dilution is a result of the relatively high concentration of inert ion used in the diluent to raise the ionic strength. The activity coefficients of the ions present in the samples and calibrants are masked by this and the outcome is a reduction in any differences between the calibrant and sample liquid junction potentials.[18]

3.2 Plasma Water and Plasma Volume[1, 2, 18]

Blood is a heterogeneous mixture of

(a) Corpuscles, erythrocytes, leucocytes and platelets.

(b) A complex phase comprising proteins, lipids and lipoproteins.

(c) An aqueous phase called plasmatic water containing ions and small hydrophilic constituents such as glucose.

In direct potentiometry, an ion-selective device measures the activity of the ion in the plasma water; the associated concentration units being moles per litre of water (mmol kg^{-1} plasma water). In flame photometry, it is the concentration of ions per volume of plasma that is determined (mmol l^{-1} plasma). One part of a standard is diluted with 199 parts of a diluent and this calibrating solution is presented to the flame photometry. The plasma sample is diluted in the same way and presented to the flame photometer. In indirect potentiometry, with instruments such as Technicon SMAC and the Beckman Astra, the sample and calibrating solutions are diluted in a similar manner to flame photometry. Ionic activity is detected in plasma water and volume of diluent, the associated units are mmol l^{-1} plasma (solution).

The differences in plasma water and plasma volume values have been studied particularly with reference to sodium measurements. The effect is relatively small for normal plasma samples, but for a sample with abnormal lipids or proteins it can be considerable. This is illustrated in Fig. 3.1. In terms of absolute values, the difference between plasma water and plasma volume values is approximately 7% [19-21]. Fig. 3.2 shows the shift caused in the calibration line for sodium, due to this effect. Apple et al[19]. have reported that the effect is pH dependent; approximately 8% at pH 5-6 going to 3% at pH 8-9. This could be due to sodium binding to proteins. Bicarbonate binding; 2.7% for sodium and 3.1% for

potassium at 25 mmol l^{-1} bicarbonate concentration; reported by Coleman et al.[22], could account for some of the discrepancy. (See Chapter 7). Apple et al.[19] also suggest that the effective water content is greater than the measured water content due to binding of water by proteins.

In practical terms, in normal plasma samples, the difference between plasma water and plasma volume values, actually observed, for sodium and potassium measurements, is approximately 2.5%[18,23,24,25]. Czaban and Legg[1] suggest that the discrepancy could be due to calibration errors. Waugh[26] has proposed a formula for expressing results obtained from indirect analysis (flame photometry or indirect potentiometry) in terms of plasma water. The formula proposed is

Plasma water = 99.1 - 0.73 [PROTEINS] - 1.03 [LIPID] - 3.1

(ml/100ml serum) (g/100ml) (mg/100ml)

Using this calculation, in normal plasma samples, the plasma water volume works out to be approximately 93%. (5.4% proteins, 0.6% lipids and 0.9% soluble salts and sugars in normal plasma).

The percentage usually accepted for the volume occupied by proteins and lipids at normal plasma levels is seven. This value may have to be reconsidered in future, after further work is done in this field.

3.3 Activities vs Concentrations

At present, all knowledge of clinical diagnosis and therapy in electrolyte imbalance is based on flame photometric values i.e. concentrations per volume of plasma. Ion selective devices, in direct potentiometry, on the other hand, measure activities in plasmatic water. Until clinicians become more adapted to activities rather than concentrations, this point will remain a matter of controversy.

Maas[4] et al. have recommended that measured activities of Na^+ and K^+ should be multiplied by an appropriate constant to obtain values that are the same as flame photometry values with normal plasma. For sodium, this factor has been given as 1.25 mol dm^{-3} (= mass concentration of water/activity coefficient = 0.933/0.747). An example of the differences obtained by flame photometry and direct ISE and the use of the factor 1.25 mol dm^{-3} is shown in TABLE 3.1[5]. Large differences are seen only in severe hyperlipemia (or hyper- and hypo-proteinemia) when there is a large change in the mass concentration of water. In such cases, the ISE values are the true values[5,2].

3.4 Selectivity

The practical selectivity of an ion-selective device depends on two factors:-

(a) the value of the selectivity coefficient for the interfering ions.

(b) the effective concentration of the interfering ion in the environment.

Blood electrolytes do not cause mutual interferences at the concentrations in which they occur[6]. The minimum selectivity coefficient[7] permissible for physiological activity determinations can be written as -

$$K_{ij}^{POT} = \frac{a_i low}{(a_j high)^{Zi/Zj}} \quad (P_{ij}/100) \qquad - 3.2$$

where

$a_i low$ = lowest value of the physiological activity range of the ion I^{Zi} to be measured.

$a_j high$ = highest value of the physiological activity range of the interferring ion J^{Zj}.

P_{ij} = percentage of maximum tolerated error caused by the ion J.

In table 3.2, K_{ij}^{POT} for the worst possible cases have been calculated setting P_{ij} = 1%.

Ion selective devices used in clinical work have selectivities that are sufficient to avoid corrections for interferring ions present within the physiological range.

3.5 Liquid - Junction Potentials

This topic will be discussed in detail in Chapter 7. Briefly, the aspects of liquid junction effects that have to be considered are erythrocyte effects, varying geometries of the liquid junction and varying compositions of bridge solutions and calibration solutions.

3.6 Other Aspects

(1) Since the slope of the electrode depends on temperature, the measuring cell should be thermostated at 37 ^{o}C.

(2) The high resistance of the ion-selective devices, particularly micro-electrodes, requires that the cell is adequately shielded.

(3) Pollution of the electro-active material by drugs, sterilising agents and protein deposits may occur.

(4) If glass micro-electrodes are used in vivo, breakage of the device is a potential hazard.

(5) PVC membranes may lift off the devices and cause leakage problems.

(6) In intracellular use, the tip potential[7] of micro-electrodes is a source of uncertainty. The tip potential depends on the tip diameter, type of glass used in the capillary and the concentration and composition of the solution in the capillary and sample. It can be minimised with a capillary having the largest tip diameter feasible and calibrating the electrode in a solution similar to the sample.

(7) Streaming potentials[7] may change the liquid junction potentials by variations in the flow rate of the sample. The

highest values of streaming potentials, in flow through systems, are predicted for the combination of small channel diamter, high flow velocity, low electrical conductivity of the sample and large distance between the ISE and reference electrode[17].

(8) In intracellular experiments, measurements in the vicinity of the ion-selective micro-electrode may not be representative of the entire cell because of heterogeneous distribution of ions caused by various factors such as piercing the cell and existence of unstirred layers.

(9) Membrane electrodes may generate potentials due to pulsatile pressures, liquid junctions are also affected to some extent.

(10) Differences in viscosities of the calibrant and sample may also contribute to error.

(11) In in-vitro measurements; the sampling method is very important. Sample pre-treatment should be standardised. Intermittent sampling could give rise to hysterisis effects.

(12) Ex vivo, on-line monitoring of whole blood[8] seems to circumvent some of the problems. Technical problems associated with in-vivo use are eliminated. Sterilization, encapsulation and breakage hazards are less severe. The need for a reference electrode - micro-electrode pair is eliminated.

3.7 Some Clinical Instruments

Tables 3.3a and b present details of some clinical analysers available. Important features of a few of these analysers will be discussed.

3.7.1 Radiometer KNA 1[9] (Figs. 3.3 a-e)

(1) The instrument may be used for sodium and potassium determinations in whole blood, plasma, serum or diluted urine. The sodium sensor is a glass membrane and a valinomycin in PVC membrane is used for potassium. The latter is covered by a cellophane membrane to protect is against protein contamination.

(2) The reference electrode is a calomel electrode. A 4.6 mol dm^{-3} sodium formate solution is used in the salt bridge. The liquid junction is open and static. These features will be discussed in detail in Chapter 7.

(3) The activities of plasma water measured by the analyser are converted to concentration per volume of plasma using the equations:-

$$cK^{+}_{Read} = 1.0074\ cK^{+}_{Meas} - 0.1612 \qquad - 3.3$$

$$CNa^{+}_{Read} = 0.92981\ cNa^{+}_{Meas} + 5.385 \qquad - 3.4$$

These linear functions compensate for protein content and for systematic measuring error due to carry over from calibration and rinse solutions.

(4) Calibration solutions used are (concentrations in mmol/Kg):-

CAL 1 - 4.0 KCl, 153.0 NaCl, 4.6 TRIS, Germicide and $NaHCO_3$

CAL 2 - 81.0 KCl, 40.5 NaCl, 12.1 $CaCl_2$, 4.6 TRIS

(5) The sample handling time in the plasma mode is one minute. Sample volume is 125μl. Measurements are performed at 37 ^{o}C.

(6) Samples can be received from capillaries, test tubes or syringes. For whole blood and plasma samples in syringes or test tubes, addition of lithium or sodium based heparin in concentrations not greater than 10 IU/ml is recommended.

Capillaries are available which are specially coated with lithium based heparin so that the concentration in blood becomes 50 IU/ml.

(7) <u>Status and Sensitivity</u>

These are an indication of the electrodes condition.

The STATUS is the voltage (A) actually measured by the

KNA1 when the solution CAL1 is introduced compared to the actual calibration line permanently stored in the computer memory.

$$\text{STATUS} = C_{CAL} \cdot 10^{A/61.54}$$

$$A = 61.54 \log \frac{\text{STATUS}}{C_{CAL}} \qquad - 3.5$$

where C_{CAL} = concentration of sodium/potassium in CAL1.

The measured mV difference between CAL1 and CAL2 divided by the theoretical mV difference gives the sensitivity of the electrode.

3.7.2 [10] Radiometer ICA1 (Figs. 3.4, a-c)

(1) This instrument is used for ionised calcium and pH measurements in whole blood, plasma and serum. The concentration of ionised calcium increases with a decrease in pH because the calcium binding capacity of plasma proteins decrease when pH decreases. A simultaneous pH and ionised calcium read out is, therefore, desirable.

(2) The calcium SELECTRODE is a PVC membrane containing DOPP covered by a cellophane membrane to protect it against protein contamination. The pH sensor is a glass membrane. The reference electrode unit is similar to that of the KNA1.

(3) Calibration solutions are (concentrations in mmol/kg).

CAL 1 - 1.4 $CaCl_2$, 106 NaCl, 83.7 TES

pH = 7.381 $\pm$ 0.010 at 37 ^{o}C.

CAL 2 - 2.8 $CaCl_2$, 102.2 NaCl, 107.9 BES

pH = 6.840 $\pm$ 0.010 at 37 ^{o}C.

(4) The Radiometer anticoagulant for calcium measurements in sodium heparinate with calcium

chloride added to prevent errors due to calcium binding by heparin (more details are given in Chapter 7).

(5) The corrected concentration of calcium at pH 7.4 may be calculated using -

$$cCa^{2+}\ (7.4) = cCa^{2+}_{Meas}\ [1-0.53\ (7.4-pH_{meas})] \quad - 3.6$$

(6) The sensitivity and status tests are similar to that for the KNA1.

The principles of operation of both the KNA1 and ICA1 are the same (Fig. 3.5). When the 'READY' lamp is lit, the measuring chambers are filled with rinse solution. The sample can be introduced through the inlet flap. It passes through the heat exchanger and reaches the first sample sensor SS_1. The 'READY' lamp goes out and the 'BUSY' lamp lights. The rinse solution is transported out and the sample goes to the measuring unit where it remains for 35s to allow equilibration. Enough sample is allowed to enter so that the leading edge of the flowing sample passes through SS_2. Approximately one minute after the inlet flap is closed, the liquid junction is established. Both the salt bridge and rinse solutions are preheated to 37 ^{o}C before entering the measuring chamber. After the measurements are made, the results are fed into the computer and displayed. The rinse programme starts again, automatically.

3.7.3 [11] AVL 982 (Figs. 3.6 a-c)

(1) The AVL 982 Electrolyte Analyser can measure sodium and potassium ions in blood, plasma and serum by direct potentiometry and in urine after dilution.

(2) The electro-active material for sodium is a sodium sensitive glass capillary and for potassium it is a PVC based valinomycin membrane.

(3) The calibration solutions are (concentrations in mmol dm^{-3}).

STANDARD A - 150 NaCl, 5KCl, 1 $CaCl_2$, 1.5 Triethanolamine, in distilled water; pH is adjusted with HCl to 7.45.

STANDARD B - 50 NaCl, 1.8 KCl, 10 $CaCl_2$, 10 Mg $(CH_3COO)_2$, 1.5 Triethanolamine in distilled water; pH adjusted to 7.45 with HCl.

(4) The reference electrode is a calomel with 1.2 mol dm^{-3} KCl as the filling solution. The junction is open and static.

(5) The results are displayed in mol dm^{-3}. There is provision for 'flame correction' values. Details of the method used were not available.

3.7.4 [12]Ektachem (400) Kodak (Figs. 3.7, a-c)

The KODAK analyser has four discrete, dry, thin-film, disposable I.S.E 'slides' for the analysis of sodium, potassium, chloride and carbon dioxide. The sample (10μl) and reference fluid (10μl) are applied simultaneously through the drop holes. A stable liquid junction is formed across a cellulose acetate strip. After three minutes, the potential difference between the two half cells is measured. The results are corrected for about 6% difference with flame photometer values and are reported as mmol dm^{-3} of normal serum. 'Serum based' calibration solutions are used, the commercial formulation is not specified.

3.7.5 [13]Corning Analysers (Fig. 3.8, a and b)

Figures 3.6 a and b show the designs of the electrodes and electrode block of the CORNING 614 and 634 analysers used in this work. A TRIS buffer is used for the calibration solutions. The mode of operation and exact formulations of the calibrants and correction algorithms were not available.

3.7.6 [14] Beckman Astra (Automated Stat Routine Analyser System) 8 (Fig. 3.9 a-e)

The Beckman sodium/potassium electrodes are used in other Beckman systems besides the Astra 8, namely the Electrolyte 2 Analyser and the Electrolyte 4 Analyser. The sodium potassium module described here is used as an optional sub-system operating in conjunction with the Beckman Astra™ 8. Salient features are -

(1) The electro-active material in the sodium ISE is a glass membrane, valinomycin in PVC is used in the potassium ISE.

(2) Sodium and potassium measurements are carried out by indirect potentiometry. 50μl of sample are used per determination and 1.3 ml of constant ionic strength buffer is used to dilute the sample. The composition of the buffer and calibration solutions are not specified.

(3) Levels of heparin recommended for plasma/blood are sodium heparin 30 IU/ml or lithium heparin 35 IU/ml or ammonium heparin 30 IU/ml.

(4) Liquid junction effects are cancelled out by simultaneous measurements with two ISEs in conjunction with a calomel reference electrode.

TABLES

Table 3.1

Calculated Examples

Specimen	Flame c_{tNa} mmol/l	m_{tNa} mmol/kg	ISE a_{Na^+}		"c" $_{tNa}$ mmol/l	Difference c_{tNa} - "c" $_{tNa}$ mmol/l
Normal plasma Na Y = 0.747 ρ = 0.933 kg/l I = 0.160 mol/kg	140	150	112	1.25 →	140	0.0
Hypernatremia Na Y = 0.737 ρ = 0.933 kg/l I = 0.190 mol/kg	170	182.2	134.3	1.25 →	167.7	+2.3
Hyponatremia Na Y = 0.760 ρ = 0.933 kg/l I = 0.130 mol/kg	110	117.9	89.6	1.25 →	111.9	-1.9
Hyperlipemia Na Y = 0.747 ρ = 0.800 kg/l I = 0.160 mol/kg	120	150	112	1.25 →	140	-20.0

(after A.H.J. Maas et al.[5])

TABLE 3.2

I.S.E.	Maximum Value of Log K_{ij}^{POT} Acceptable					Electroactive Materials	Reported Log K_{ij}^{POT} values				
	Na^+	K^+	Ca^{2+}	Mg^{2+}	H^+		Na^+	K^+	Ca^{2+}	Mg^{2+}	H^+
SODIUM C_{Na} = 135 - 150 (mmol dm^{-3})	x	- 0.6	0.1	0.2	3.4	a. Glass NAS_{11-18}	x	- 2.0	x	x	- 1.5
						a. Glass $LAS_{10.4-12.6}$	x	- 3.0	x	x	0.7
						b. 24% ETH 1097, 67% DBE, 30.6% PVC (SSM)	x	- 0.7	- 2.3	- 3.5	0
						b. 0.6% ETH 227. 64.3% O-NPOE, 35.1% PVC (SSM)	x	- 2.4	- 0.5	- 2.8	- 0.7
						b. 4.95% ETH 237, 61.89% DOS, 33.16% PVC (SSM)	x	- 0.9	- 1.1	- 3.0	- 0.7
						b. 10% MONENSIN, 90% NITROBENZENE (FIM)	x	- 1.1	- 2.2	- 1.1	- 0.8
POTASSIUM C_K = 3.5 - 5.0 (mmol dm^{-3})	- 3.6	x	- 1.5	- 1.4	1.8	b. 2.7% VAL, 67.2% DNP, 30.1% PVC (SSM)	- 5.5	x	- 4.6	- 5.3	- 5.0
CALCIUM C_{Ca} = 10-1.2 (mmol dm^{-3})	- 6.4	- 3.4	x	4.6	- 1.9	b. 6.5% Ca complex of DDP, 64.5% DOPP, 29% PVC (FIM)	- 4.2	- 4.5	x	- 1.6	x
						b. 6.5% Ca complex of HDOPP, 65% DOPP, 28.5% PVC (SSM)	- 5.2	- 5.7	x	- 3.6	x
						b. 10% ETH 1001, 66.0% O-NPOE, 33.0% PVC (FIM)	- 4.6	- 4.6	x	- 5.2	1.2
HYDROGEN C_H = 4.3 E-5 - 5.6 E-5 (mmol dm^{-3})	- 7.5	- 6.0	- 5.4	- 5.3	x	Glass	<13	<13	x	x	x
						c. 1.0% TRI-N DODECYLAMINE, 0.6% KT_pClPB, 65.6% DOS, 32.9% PVC (FIM)	-10.4	- 9.8	<-11.1	x	x

FOOTNOTES:

a = Reference 8
b = Reference 6
c = Reference 15
FIM = Fixed Interference Method
SSM = Separate Solutions Method
C_{Mg} = 0.45 - 0.8 (mmol dm^{-3})

TABLE 3.3a DIRECT POTENTIOMETRIC SODIUM/POTASSIUM ISE ANALYSERS[16]

MANUFACTURER/ INSTRUMENT	SENSING ELECTRODE SYSTEM	REFERENCE ELECTRODE/ JUNCTION		CALIBRANT SYSTEM
AVL 980	K - VAL Na - GLASS	OPEN STATIC 1.2 M KCl	] CALOMEL	TRIETHANOLAMINE
CORNING M902	K - VAL Na - GLASS	MEMBRANE 4M KCl	] Ag/AgCl	TRIS
IL 502	K - VAL Na - GLASS	OPEN STATIC 3M KCL	] Ag/AgCl	ACETATE
KONE MICROLYTE	K - VAL Na - NEUTRAL CARRIER	PASSIVE, POROUS PLUG 3M KCl	] Ag/AgCl	TRIS/ACETATE
NOVA 1	K - VAL Na - GLASS	OPEN FLOWING 2M KCl	] Ag/AgCl	ACETATE
ORION SS30	K - VAL Na - GLASS	OPEN FLOWING 2M KCl	] Ag/AgCl	PHOSPHATE
ORION 1020	K - VAL Na - GLASS	MEMBRANE 2M KCl	] Ag/AgCl	PHOSPHATE
RADIOMETER KNA 1	K - VAL Na - GLASS	OPEN STATIC 4.6M Na FORMATE	] CALOMEL	TRIS

VAL = VALINOMYCIN PVC POTASSIUM ELECTRODE

TABLE 3.3b IONISED CALCIUM ISE ANALYSERS[16]

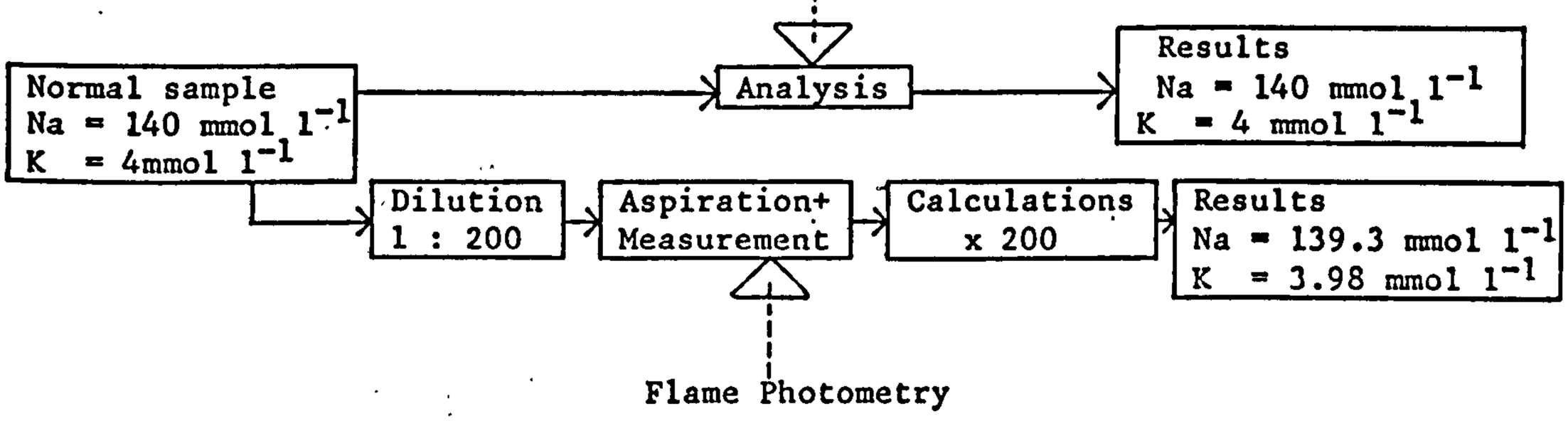
MANUFACTURER/ INSTRUMENT	SENSING ELECTRODE SYSTEM		REFERENCE ELECTRODE/ JUNCTION		CALIBRANT SYSTEM
KONE MICROLYTE	Na] K] Ca]	ALL NEUTRAL CARRIERS/PVC	PASSIVE, POROUS PLUG] 3M KCl]	Ag/AgCl	TRIS/ ACETATE
NOVA 2	Ca -	LIQUID ION EXCHANGER Ca^{2+} ALKYLPHOSPHATE/PVC	OPEN FLOWING 2M KCl	] Ag/AgCl	$CaCl_2$/ NaCl
ORION SS20	Ca -	LIQUID ION EXCHANGER Ca^{2+} ALKYLPHOSPHATE/PVC	OPEN FLOWING 2M KCl	] Ag/AgCl	TRIS
RADIOMETER ICA 1	Ca -	Ca^{2+} BIS (DI-N-OCTYPHENYL) PHOSPHATE/PVC	OPEN STATIC 20%	] CALOMEL	BES/TES
	pH -	GLASS	(OR 4.6M Na FORMATE)		

Tables 3.3a and b

F I G U R E S

Effect of dilution on normal and abnormal sera.

	Aqueaus Calibrant	Normal Serum	Serum with abnormal Lipids/Proteins
Plasma water volume (µl)	100	99.5	95
Lipid/protein volume (µl)	0	0.5	5
Total sample volume (µl)	100	100	100
Diluent volume (µl)	19,900	19,900	19,900
Total diluted volume (µl)	20,000	20,000	20,000
Dilution ratios	1 : 200	1 : 201	1 : 210

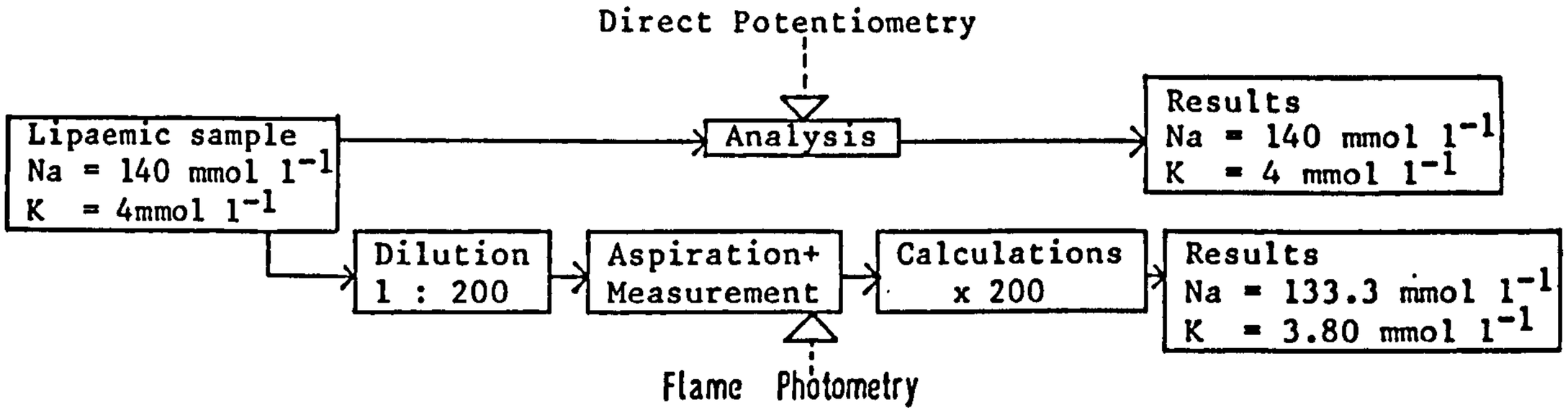


Plasma water dilution rate = 1 : 201
Conc. in diluted plasma water

$Na = \frac{140}{201} = 0.6965$ mmol 1^{-1} Na : (0.6965 mmol 1^{-1}) (200) = 139.3 mmol 1^{-1}

$K = \frac{4}{201} = 0.0199$ mmol 1^{-1} K : (0.0199 mmol 1^{-1}) (200) = 3.98 mmol 1^{-1}

CONTD. —



Plasma water dilution = 1 : 210
Conc. in diluted plasma water

$Na = \frac{140}{210} = 0.6667$ mmol l^{-1} Na : (0.6667 mmol l^{-1}) (200) = 133.3 mmol l^{-1}

$K = \frac{4}{210} = 0.0190$ mmol l^{-1} K : (0.0190 mmol l^{-1}) (200) = 3.80 mmol l^{-1}

Fig. 3.1 (after D.C. Cowell et al.; ref. 18)

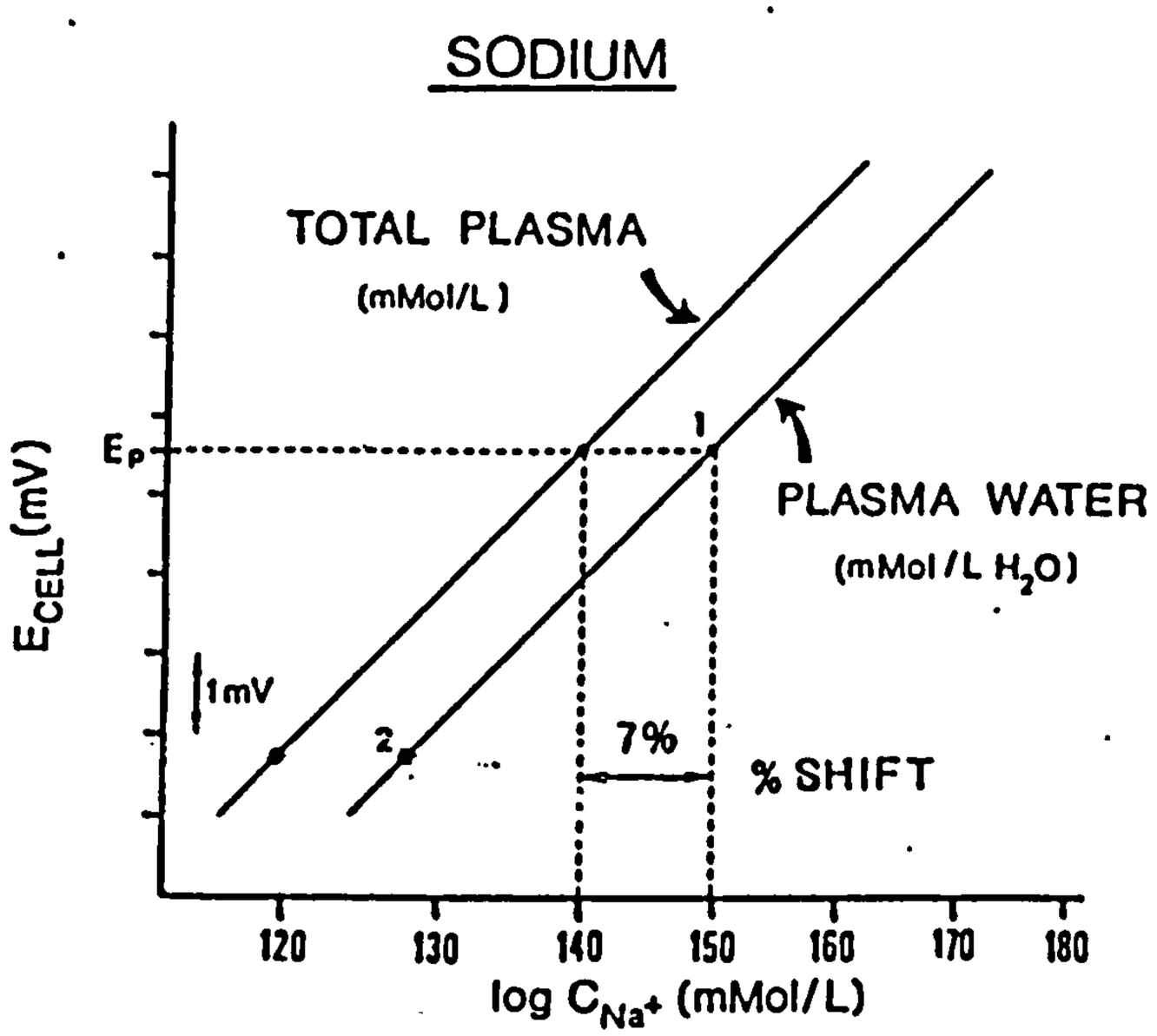

SODIUM
TOTAL PLASMA
(mMol/L)
PLASMA WATER
(mMol/L H2O)
Ep
1
2
1mV
7%
% SHIFT
ECELL(mV)
120
130
140
150
160
170
180
log CNa+ (mMol/L)

Fig. 3.2 (Ref. 1)

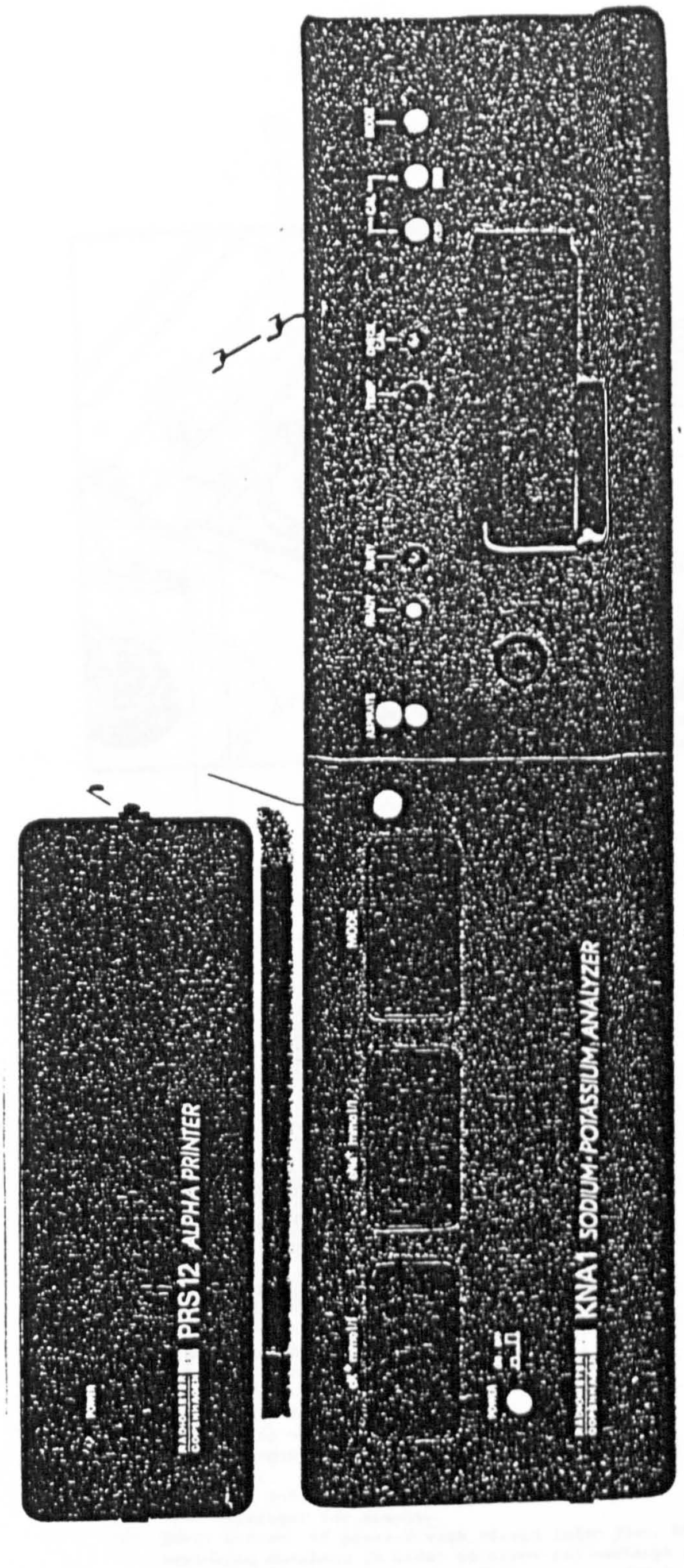
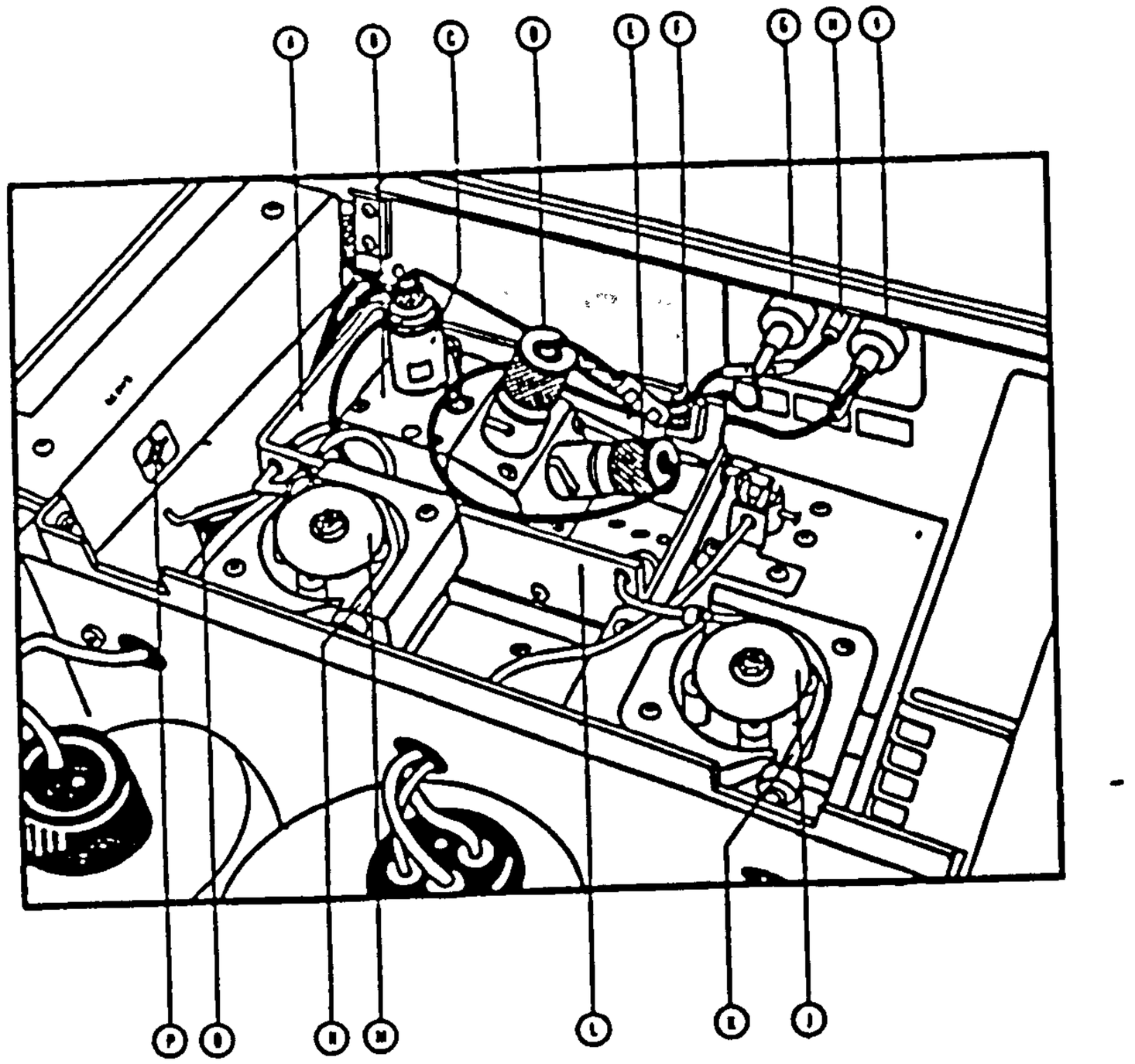


Fig. 3.3a



Wet Section and Measuring Chambers.

(A) Thermostatted section (with the cover removed).
(B) Measuring Unit.
(C) K1702 Sealed Calomel Reference Electrode.
(D) F2211Na SELECTRODE®.
(E) F2311K SELECTRODE®.
(F) Holder for the three electrode leads.
(G) Receptacle for F2311K plug.
(H) Receptacle for K1702 plug.
(I) Receptacle for F2211Na plug.
(J) Salt Bridge Solution Pump.
(K) Pump Tubing with connectors (842-091)
(L) Heat Exhanger for S4512 and S3641 Solutions (behind partition).
(M) Main Pump.
(N) Main Pump Tubing with connectors (842-091).
(O) Heat Exchanger for Sample.
(P) EMPTY button. If pressed with closed inlet flap, KNA1 empties the measuring chambers in order to allow for exchange of electrodes. If pressed with open inlet flap, KNA1 is ready for a special protein remover programme.

Fig. 3.3b

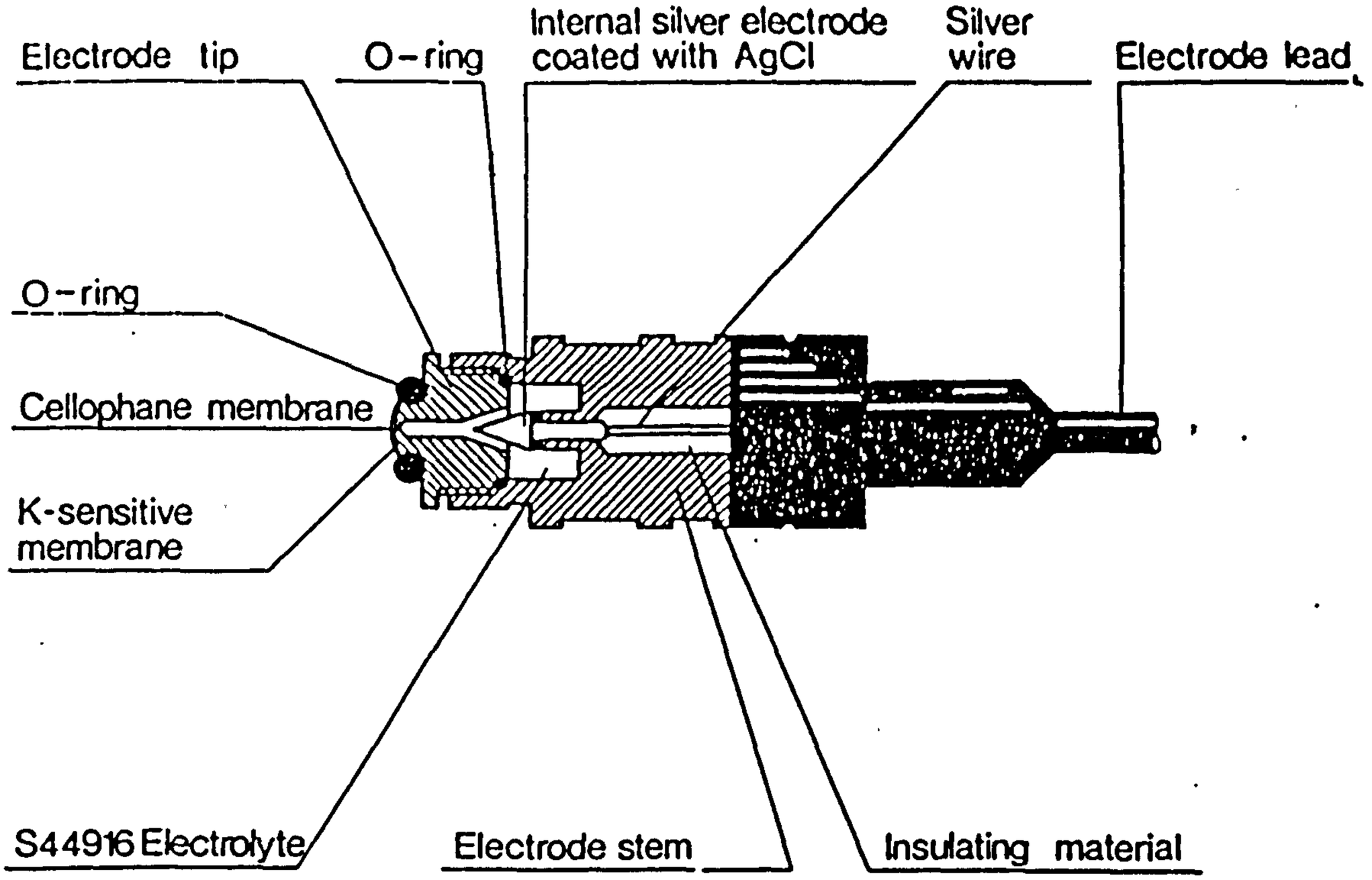
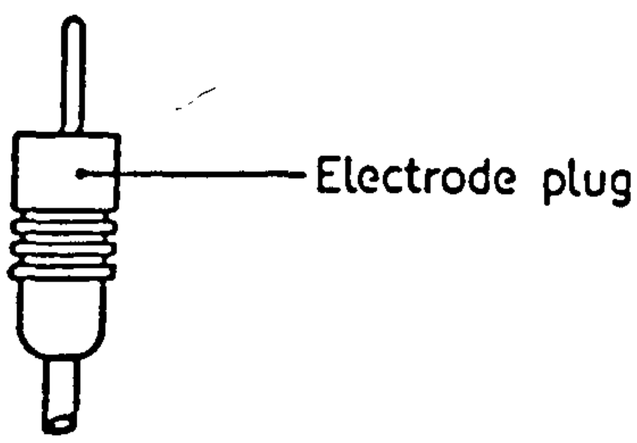


Fig. 3.3c
(F2311 K, K^+ electrode)



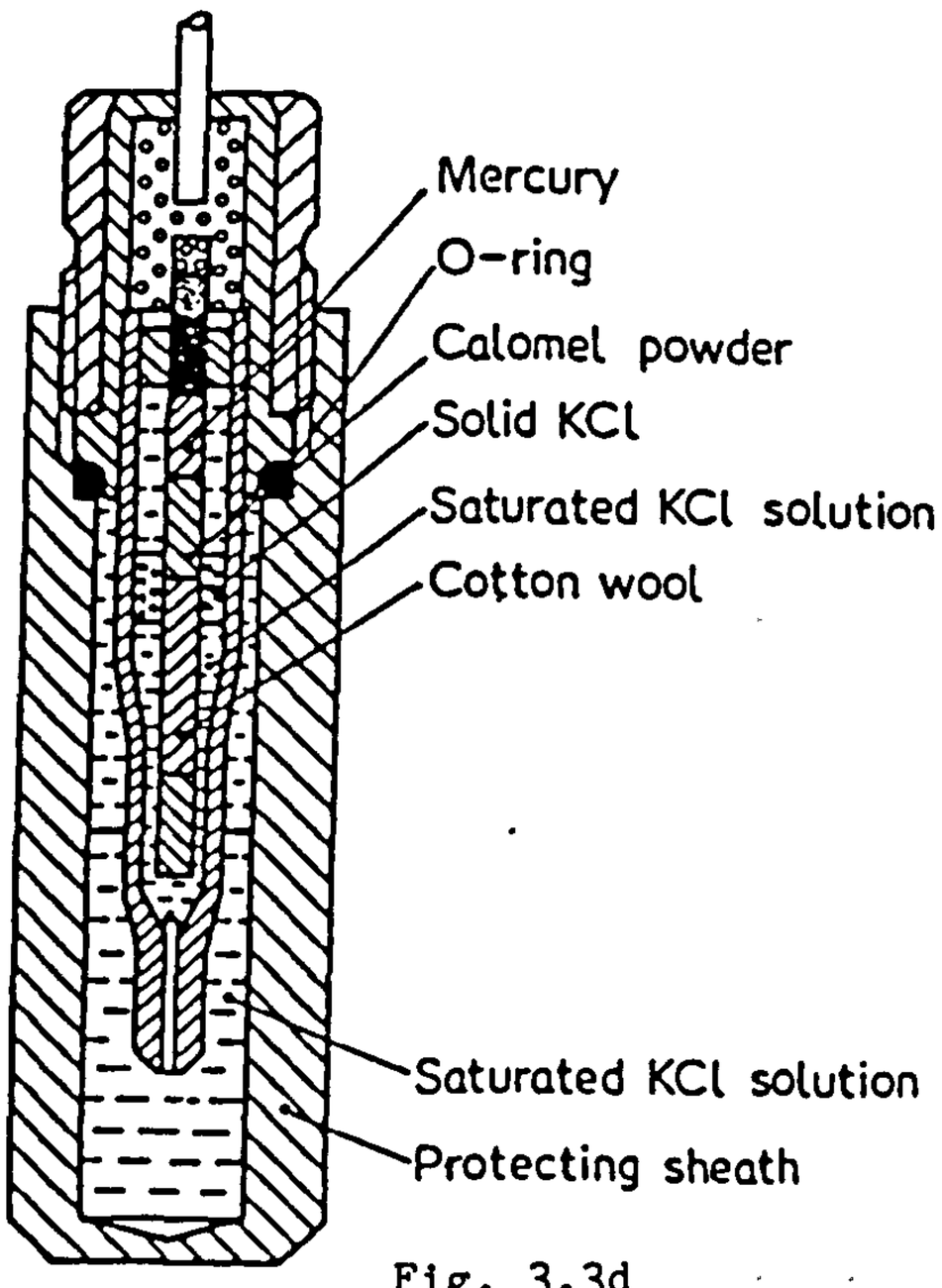


Fig. 3.3d
(F2211 Na Na^+ electrode)

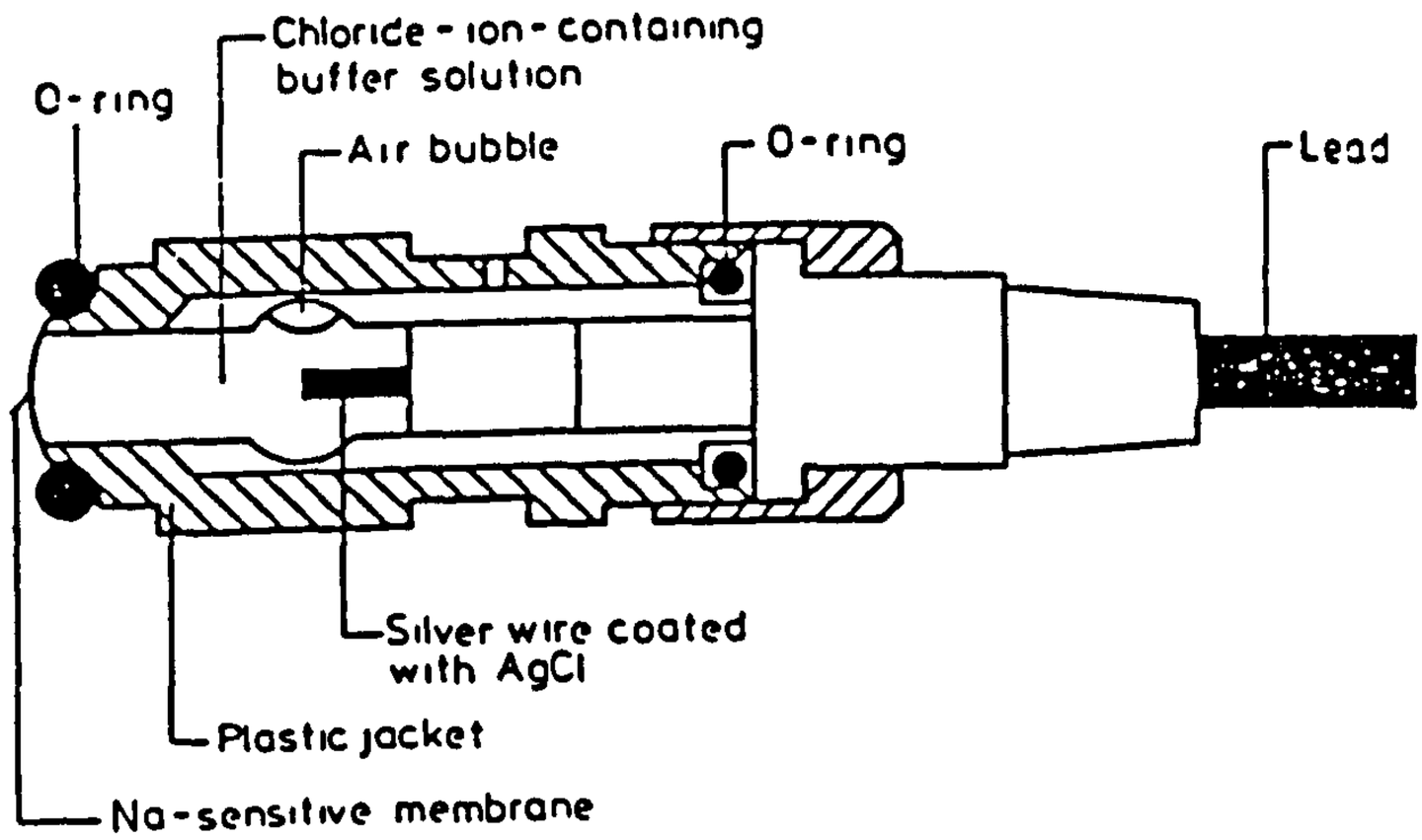


Fig. 3.3e
(K1702 Calomel reference electrode)

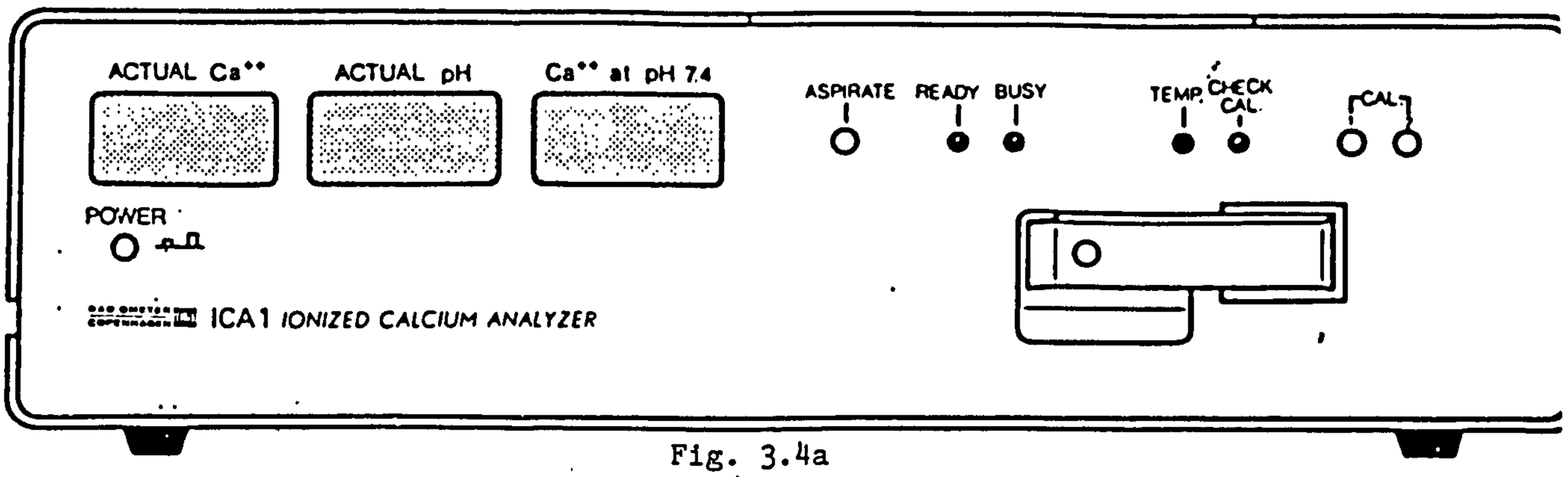


Fig. 3.4a

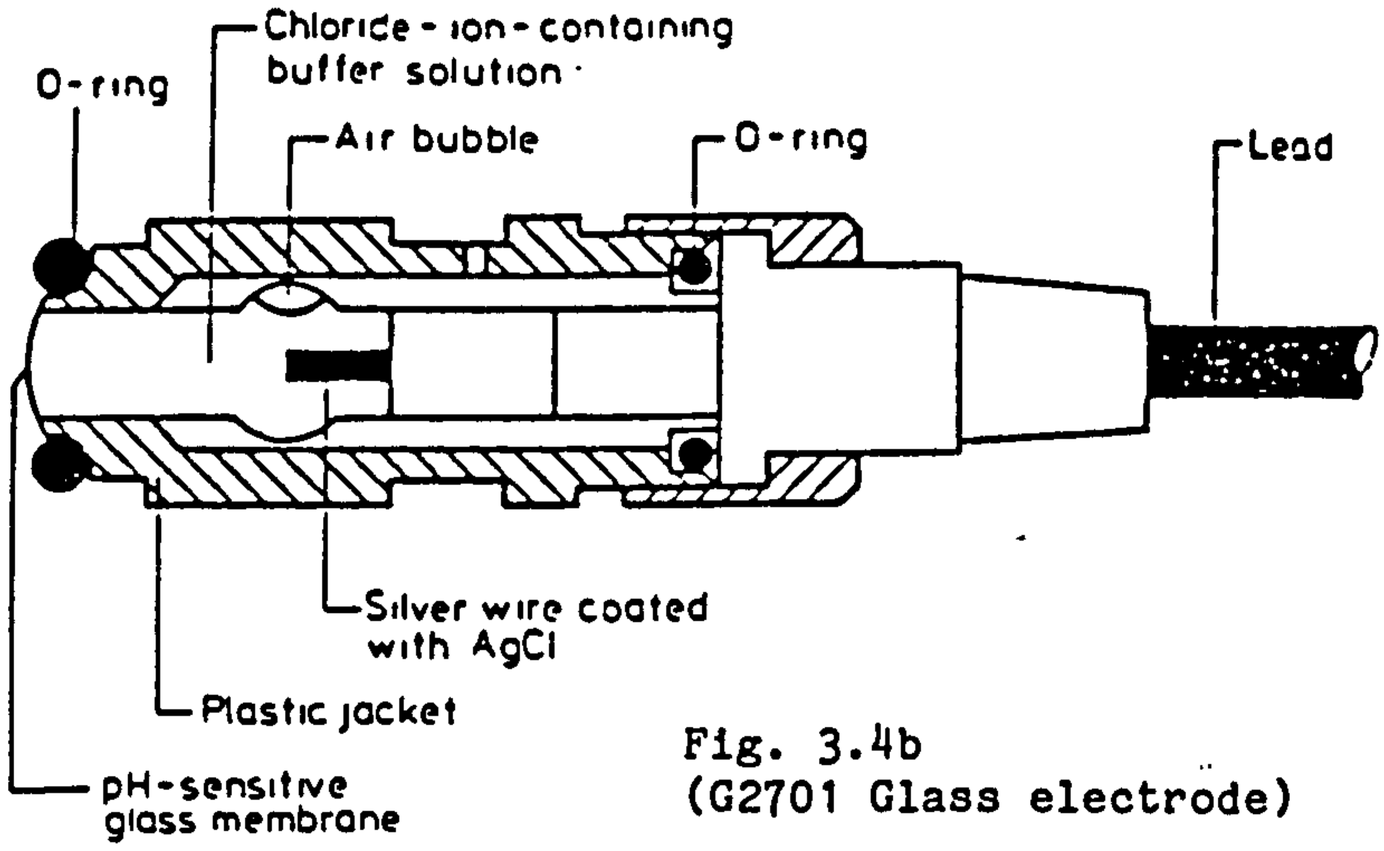


Fig. 3.4b
(G2701 Glass electrode)

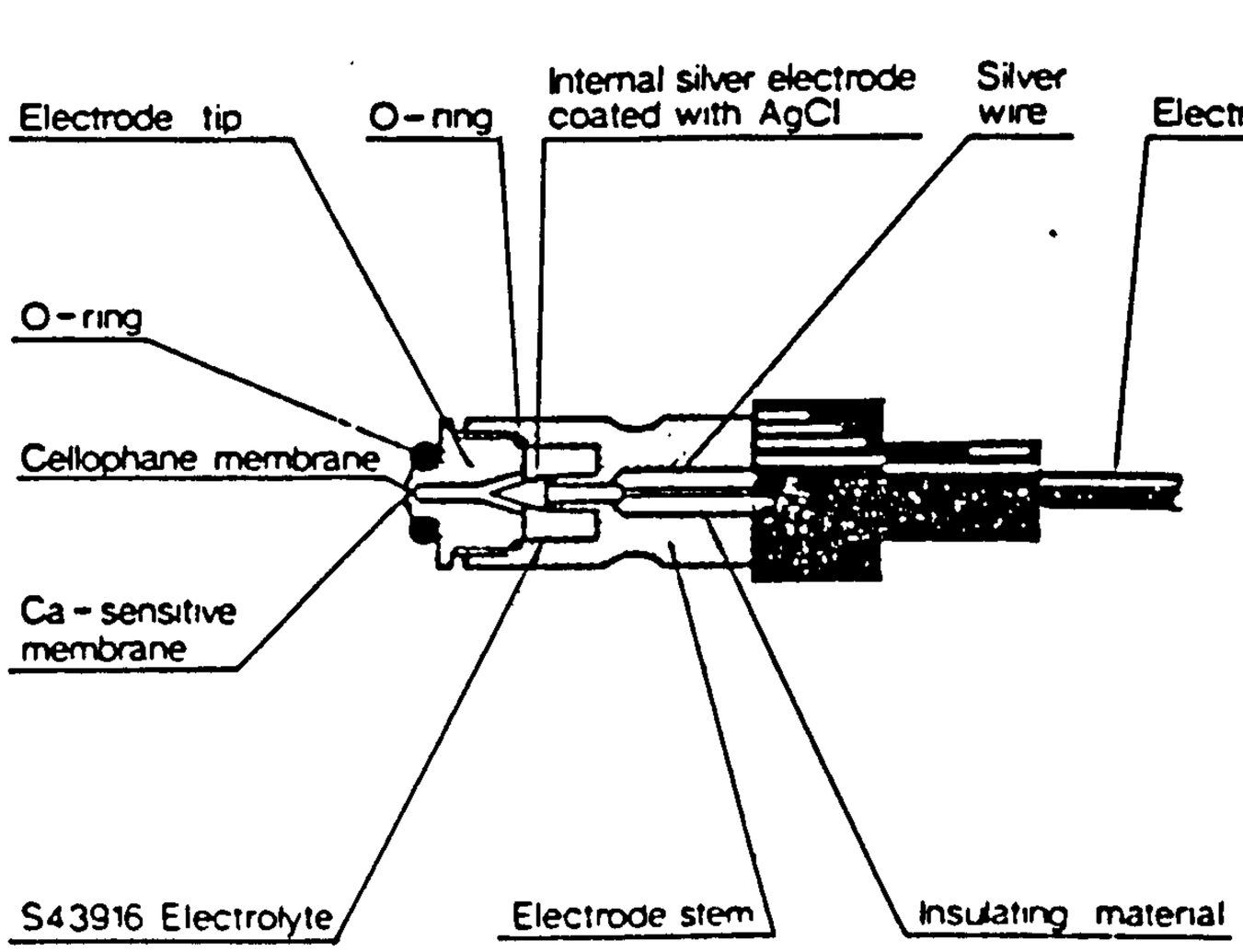


Fig. 3.4c
(F2111 Ca Ca^{2+} electrode)

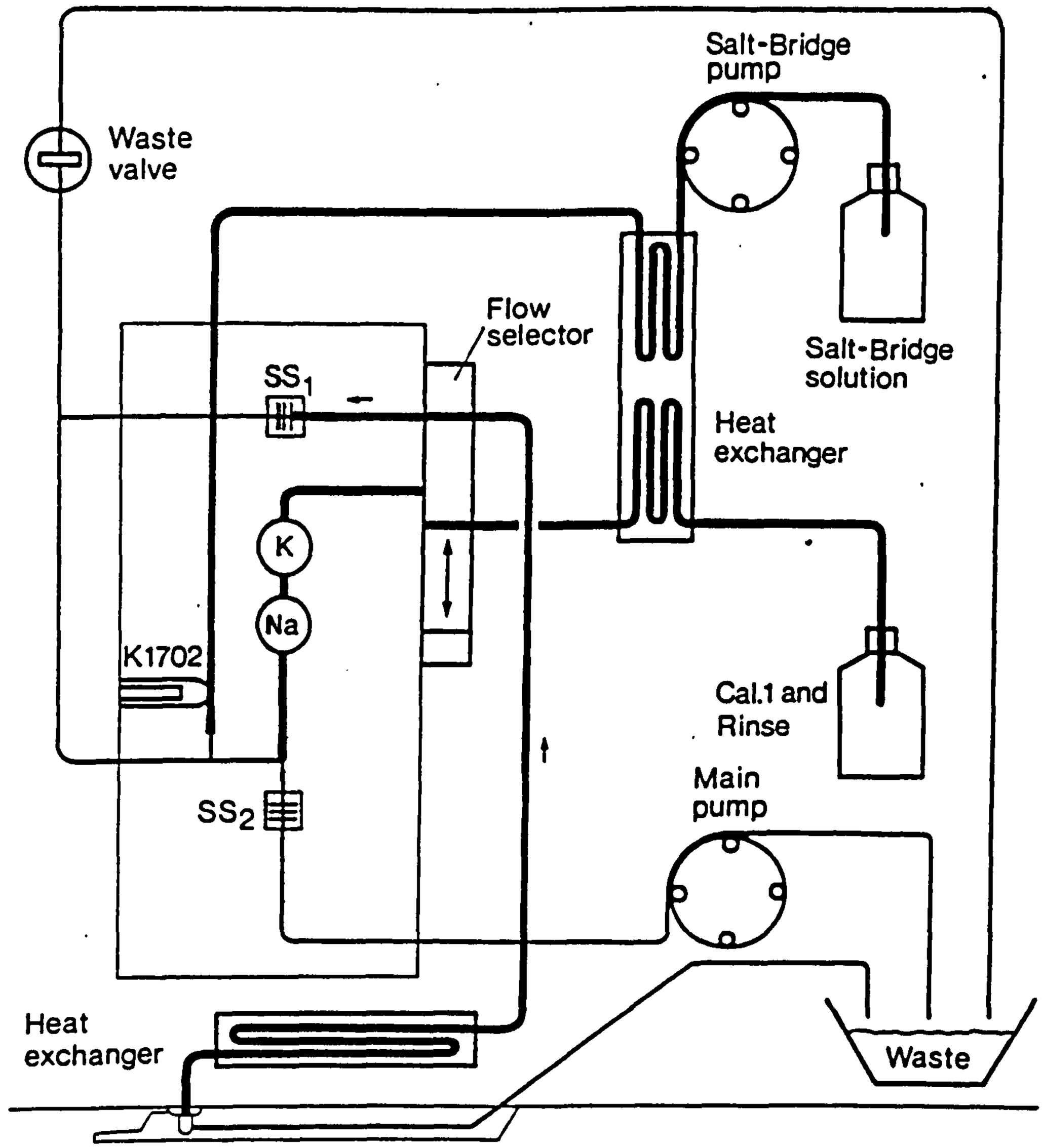


Fig. 3.5
(mode of operation of Radiometer KNA1 + ICA1)

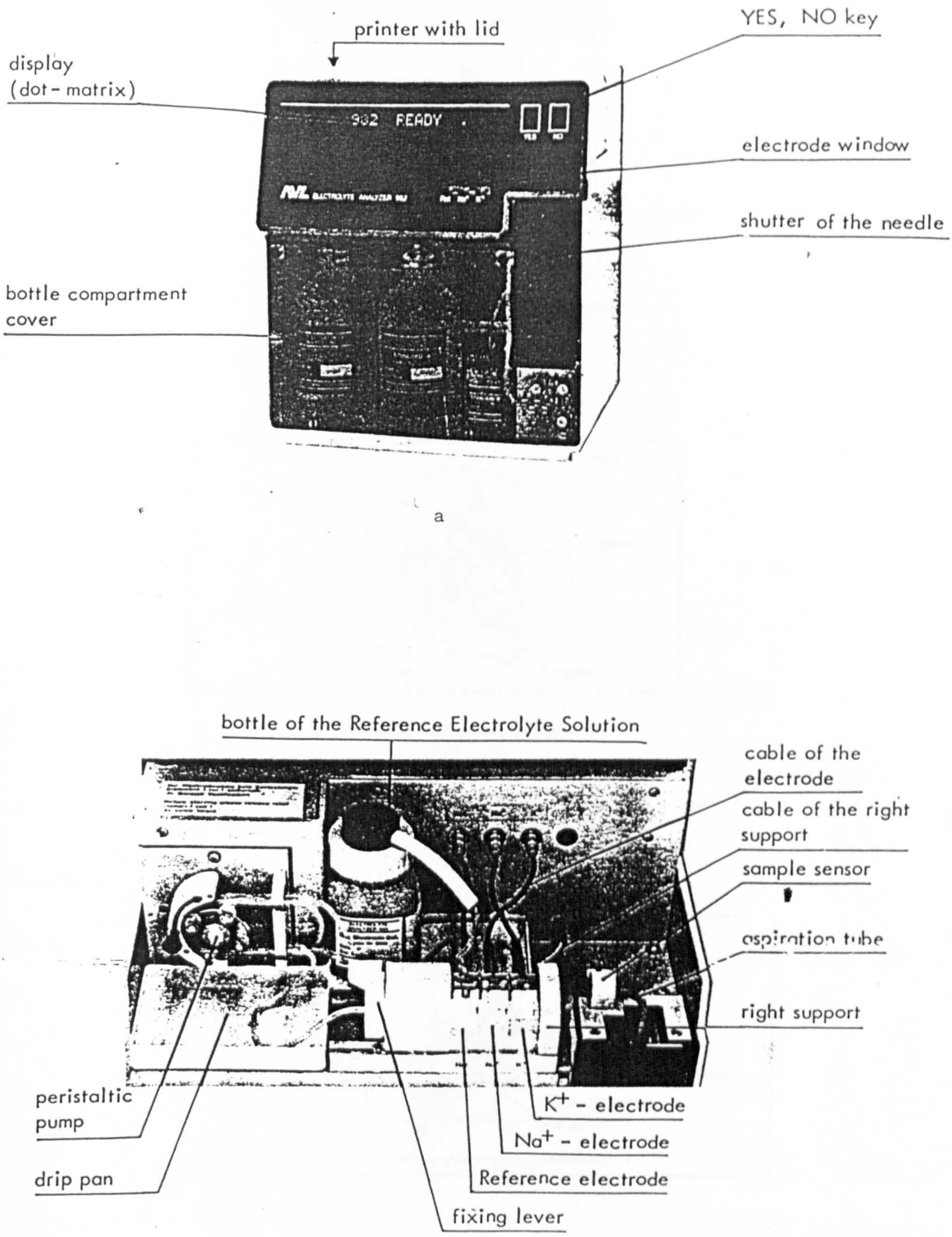
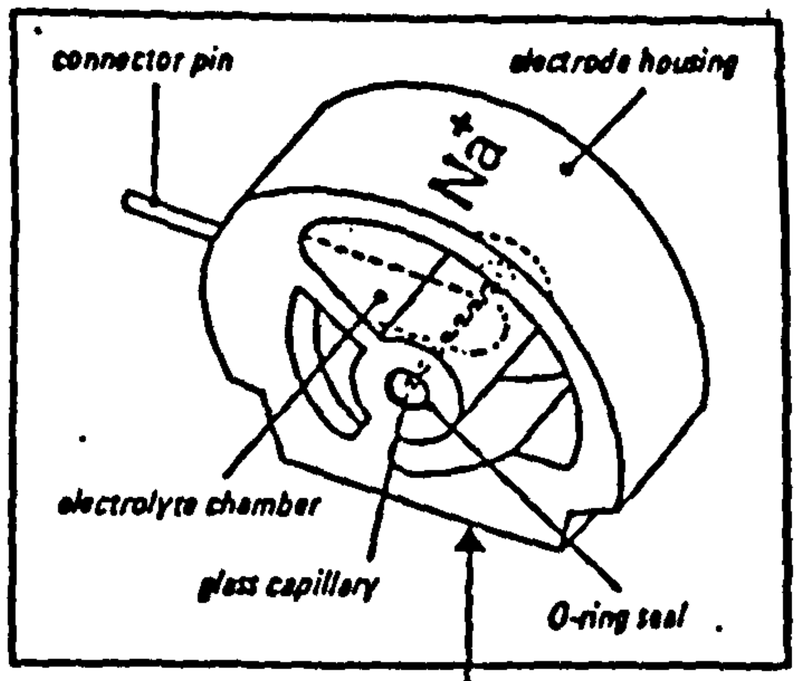


Fig. 3.6 (AVL 982)



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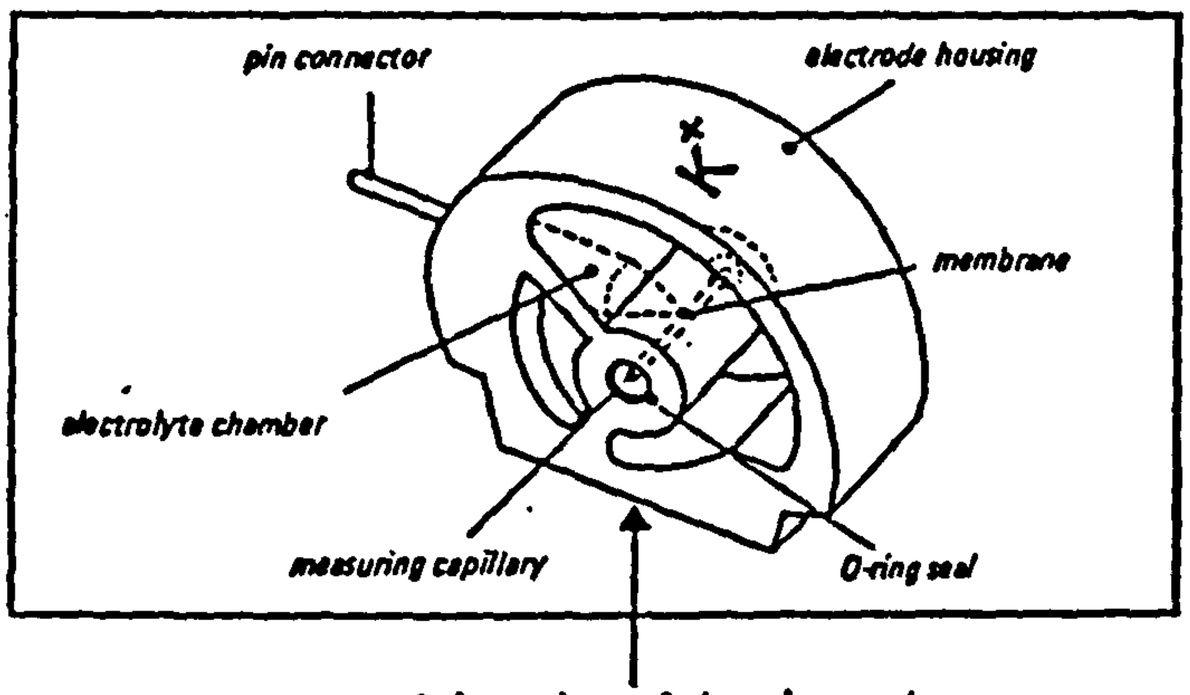

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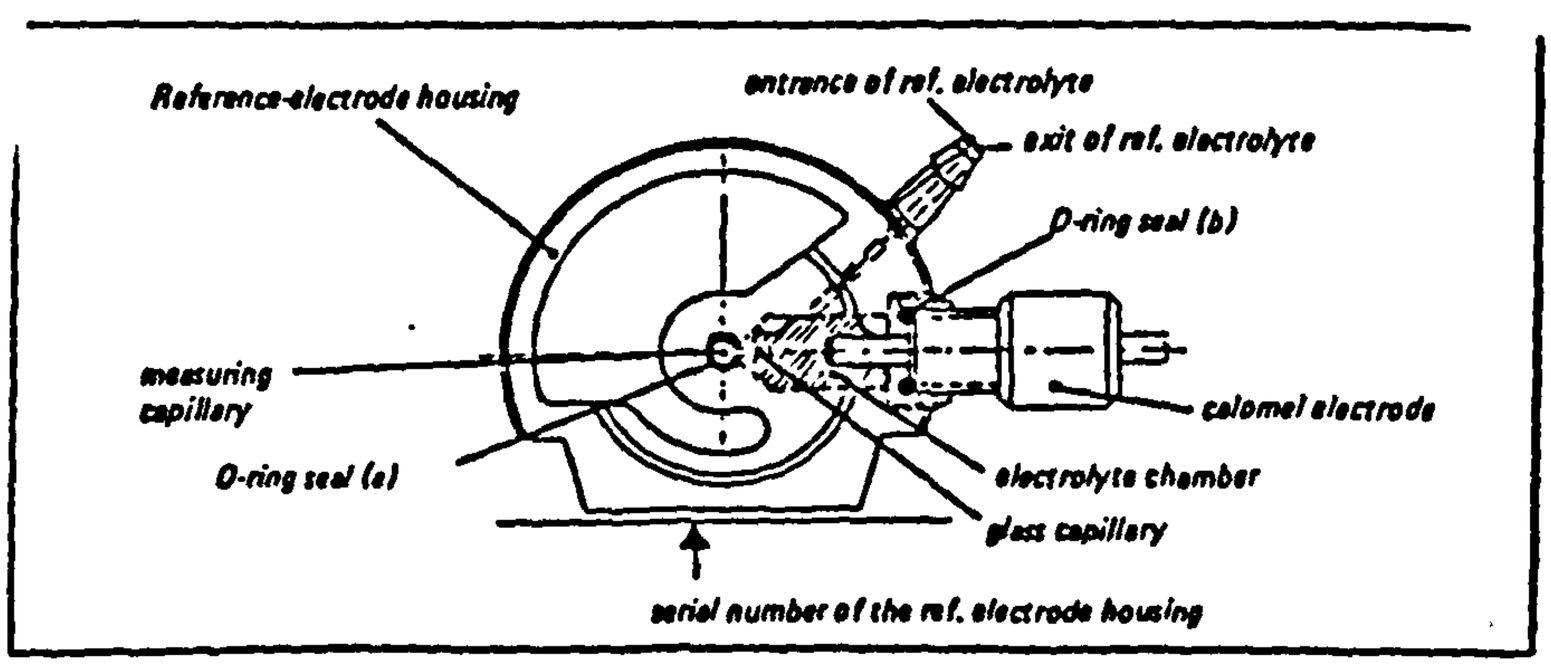
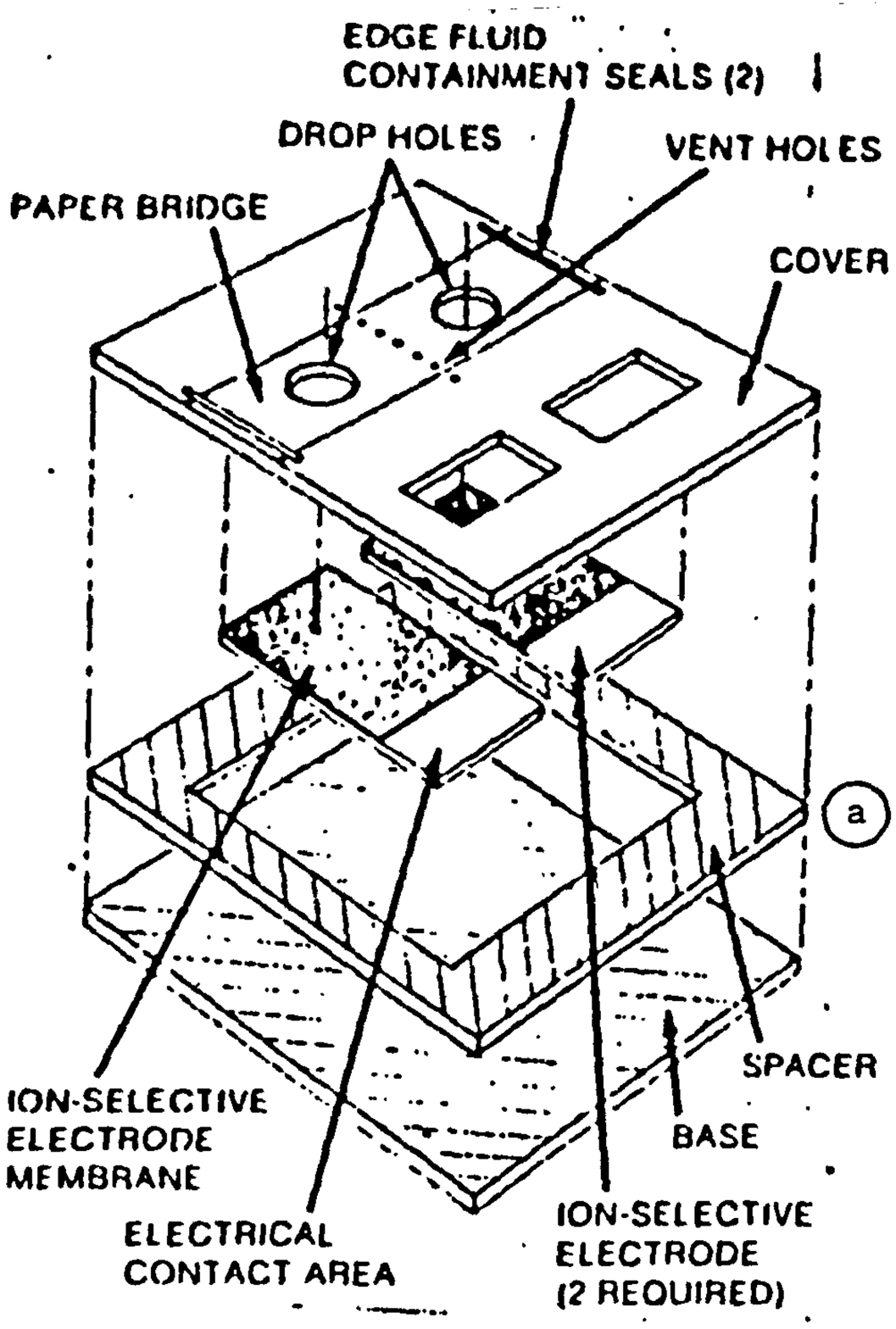
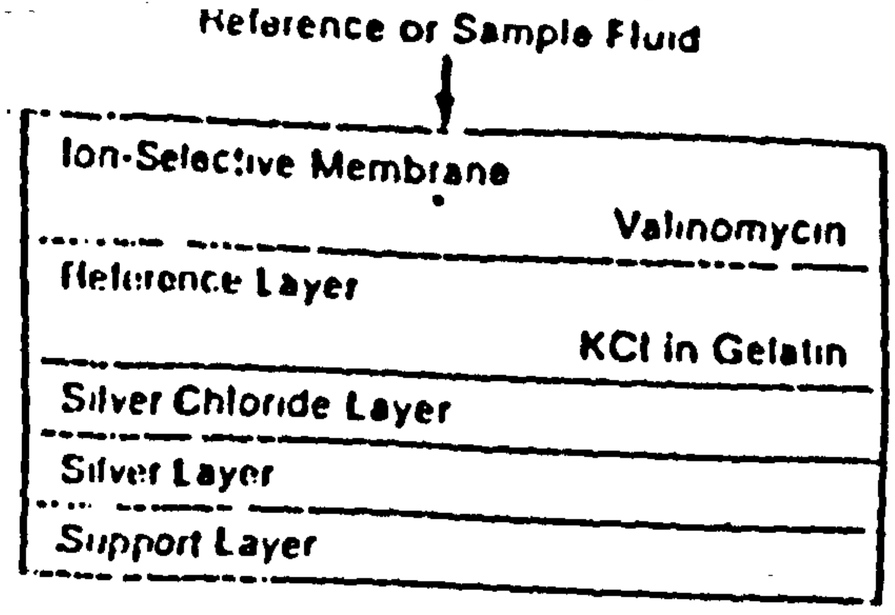


e

Fig. 3.6 (AVL 982)

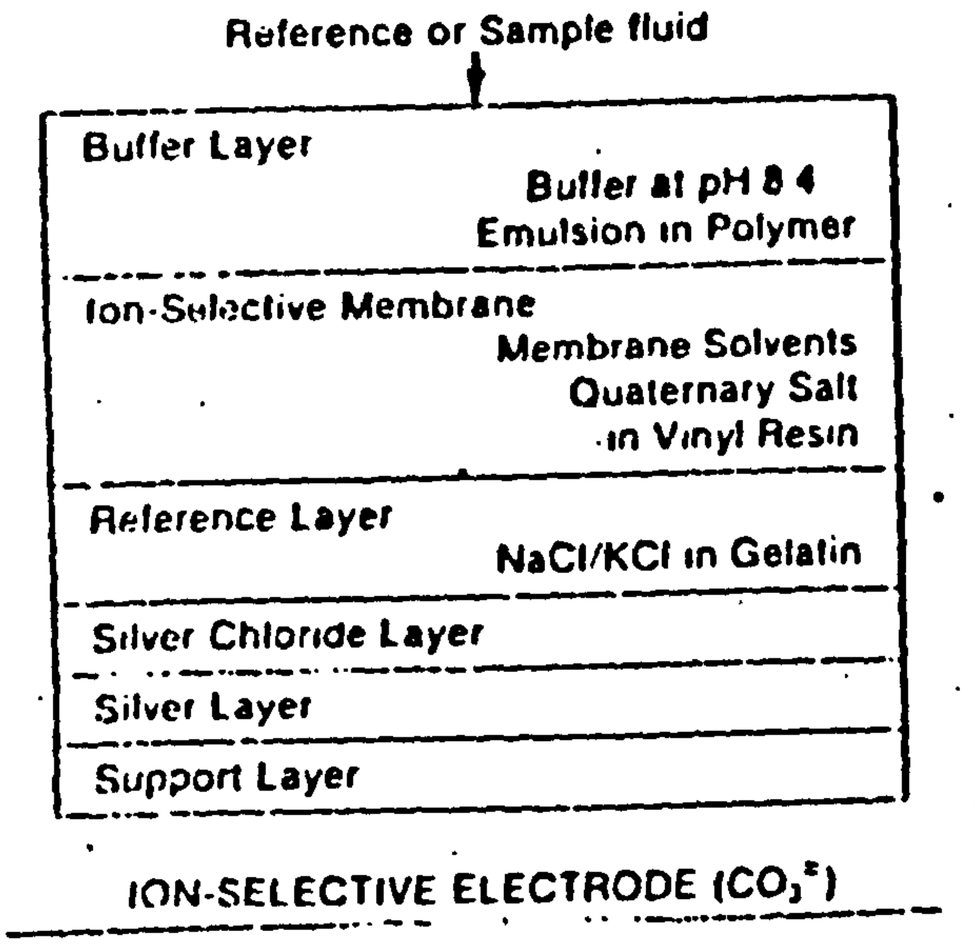


(a)



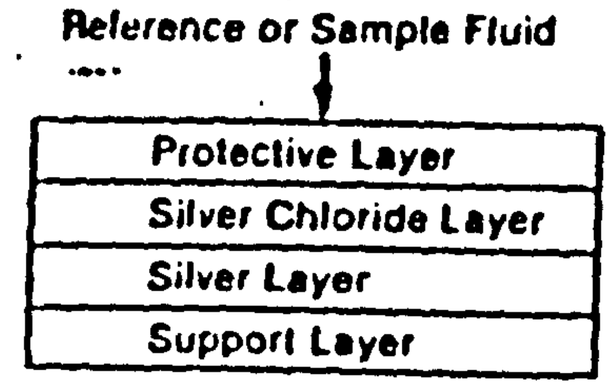
ION-SELECTIVE ELECTRODE (K^+)

(b)



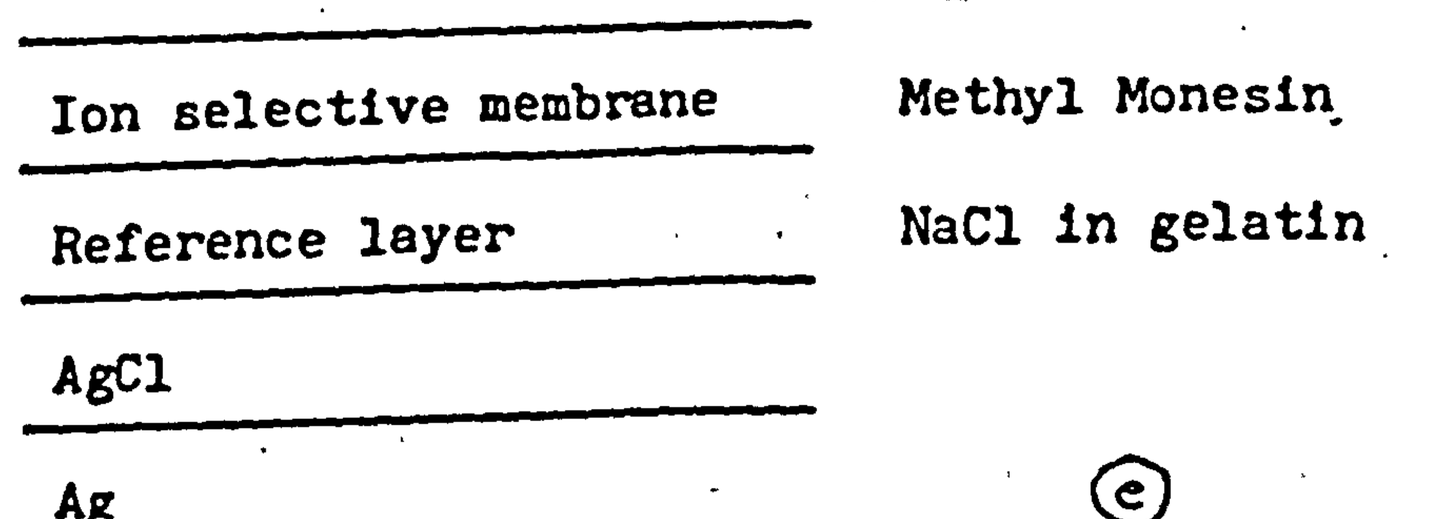
ION-SELECTIVE ELECTRODE ($CO_3^=$)

(c)



ION-SELECTIVE ELECTRODE (Cl^-)

(d)



ION-SELECTIVE ELECTRODE, Na^+

(e)

Figs. 3.7
Ektachem (400) Kodak

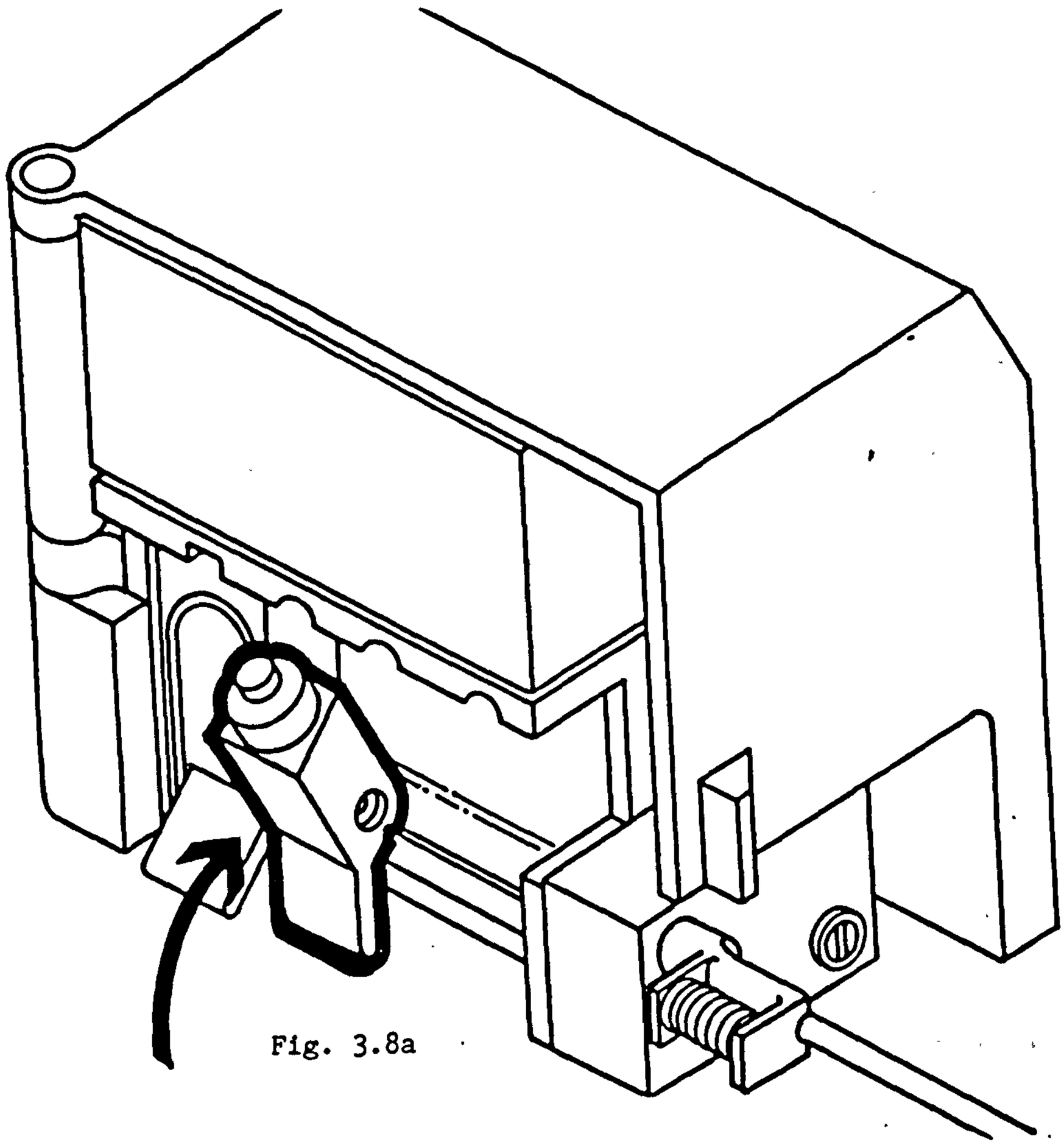


Fig. 3.8a

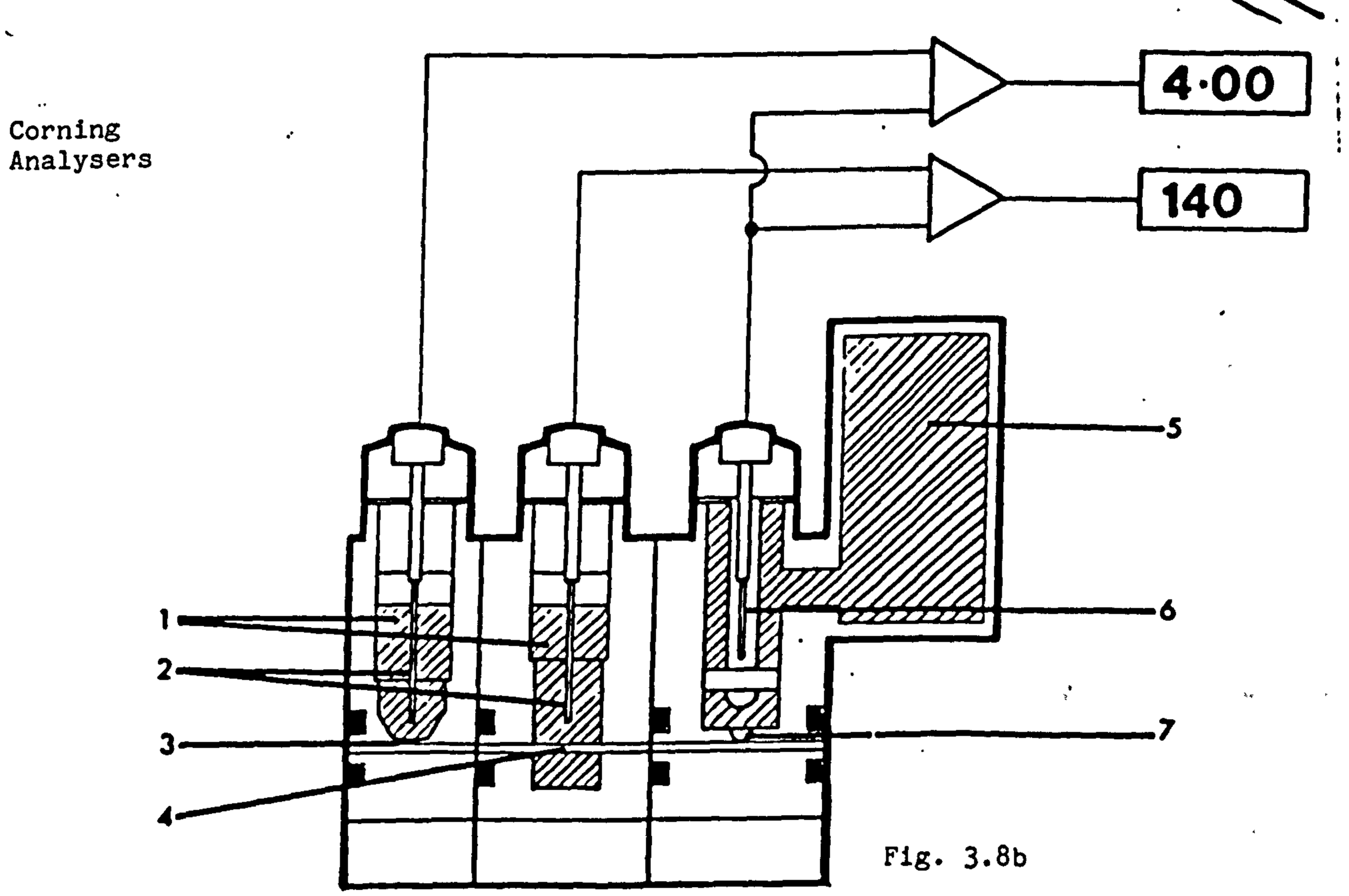
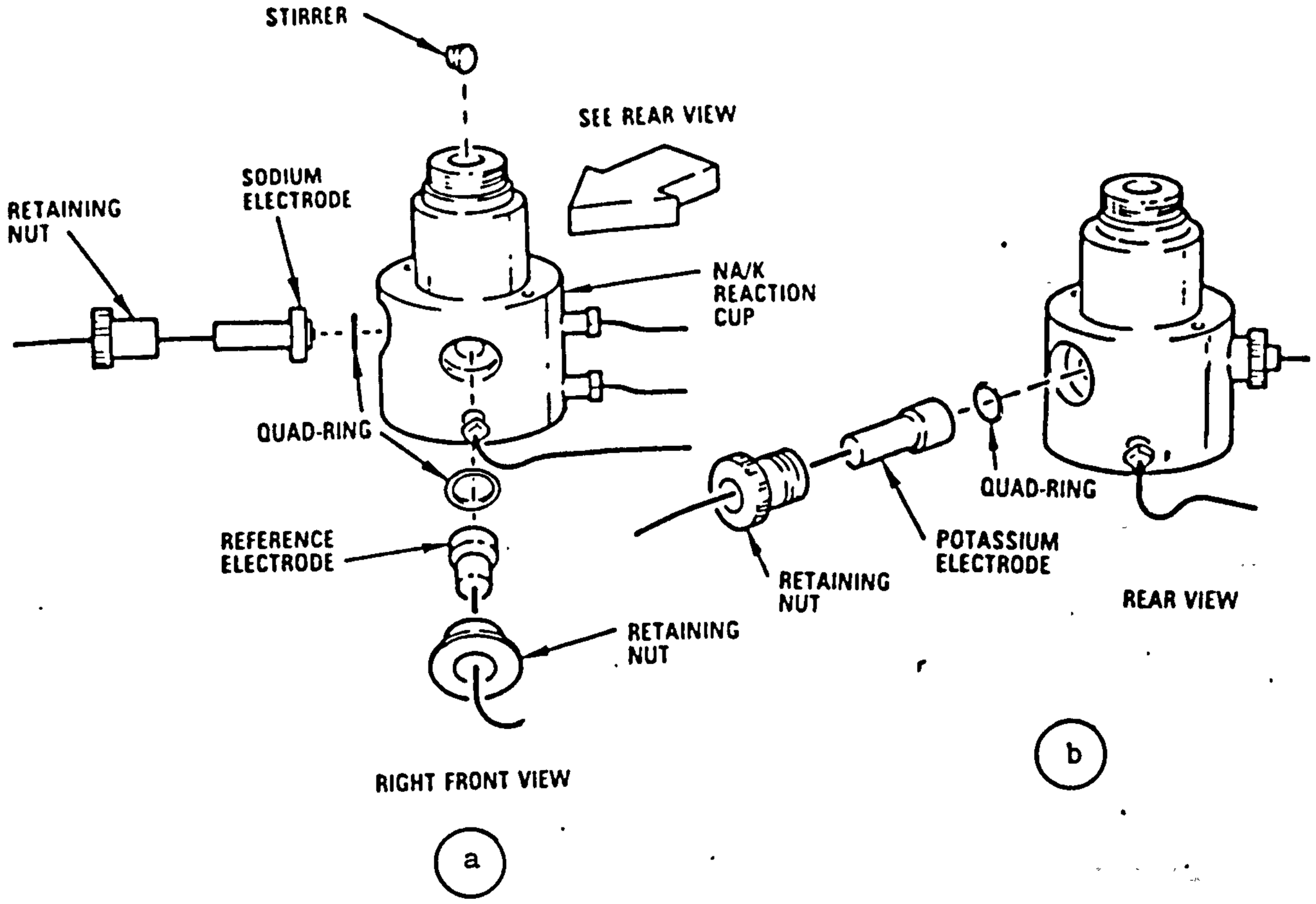
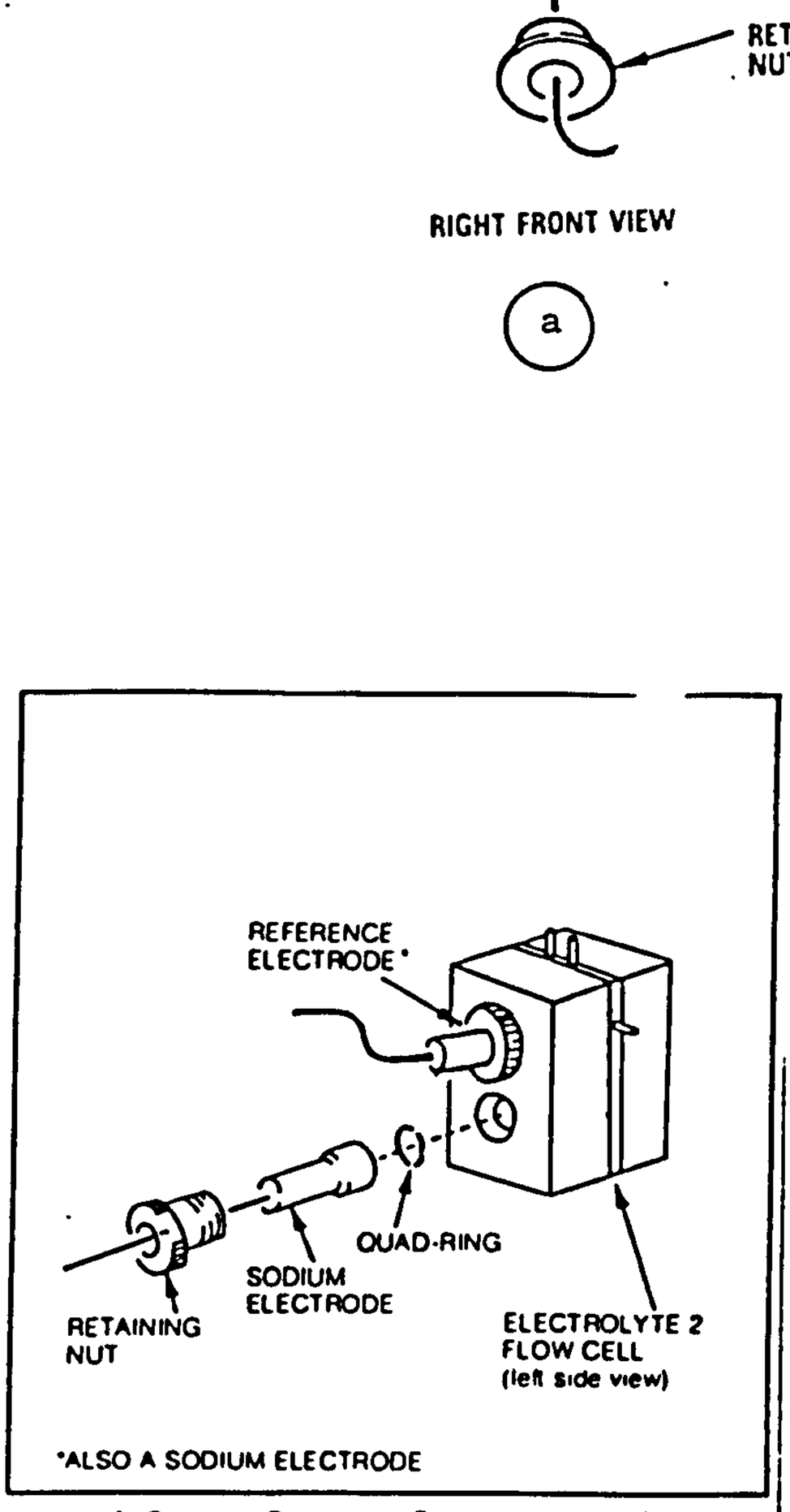


Fig. 3.8b

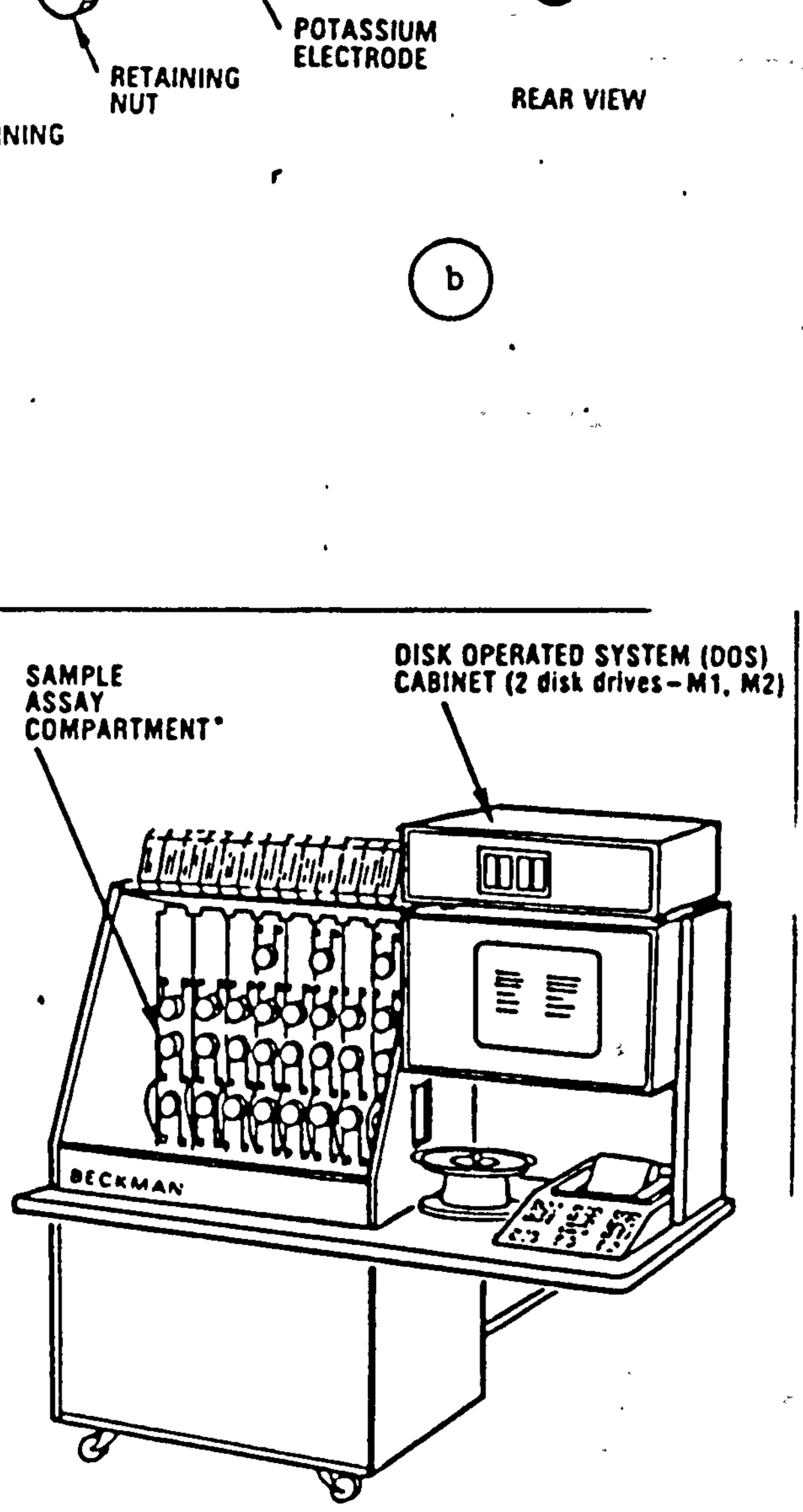
1. Na^+/K^+ electrode fill solution.
2. Silver chloride coated silver wire.
3. Potassium sensitive P.V.C. membrane.
4. Sodium sensitive glass capillary.
5. Reference electrode fill solution.
6. Nafion™ inner.
7. Reference membrane.





Sodium Electrode Exploded View Showing Installation with Electrolyte 2 Analyzer.

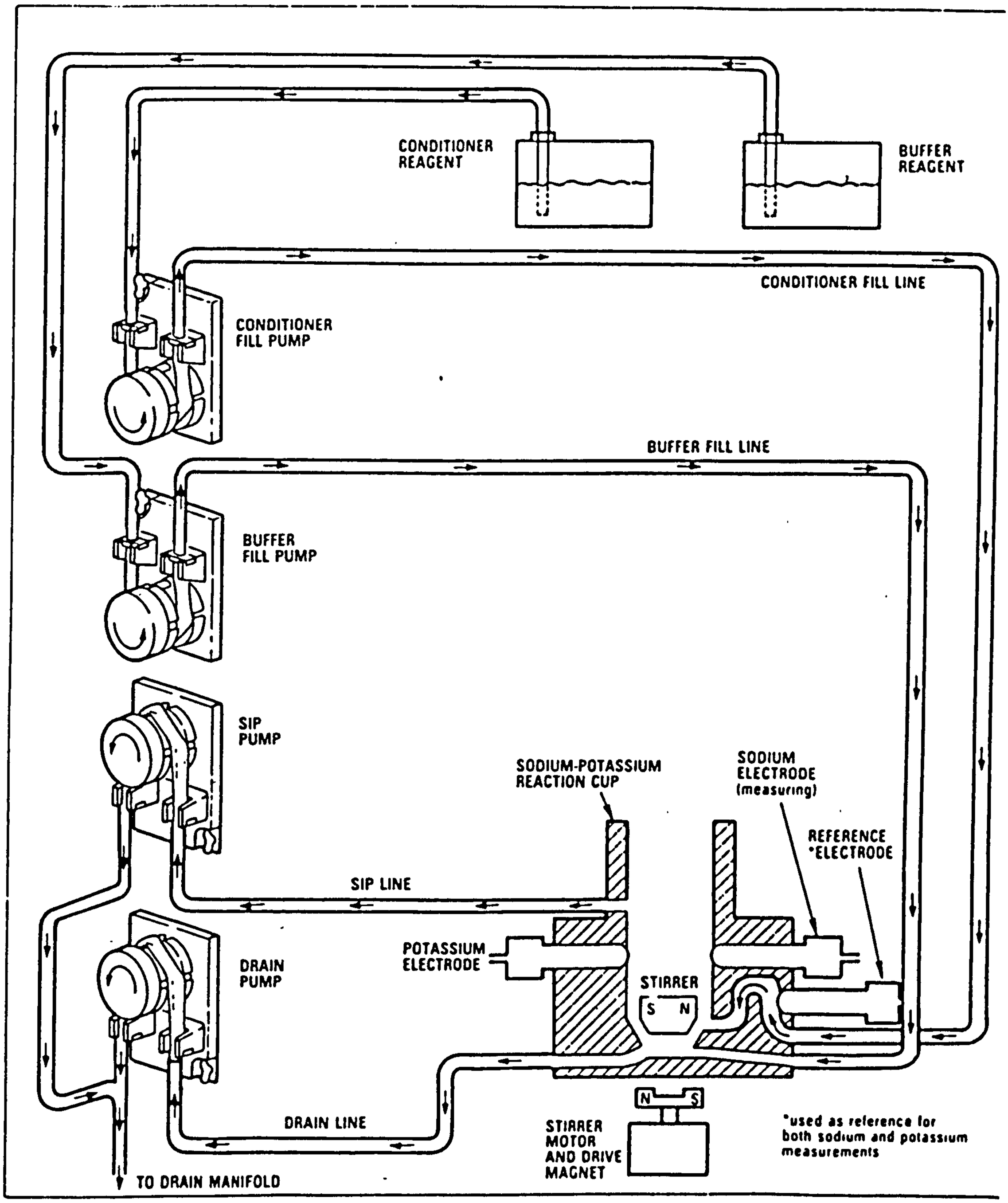
c



Typical Installation of Chemistry Modules in an ASTRA® System Sample Assay Compartment

d

Fig. 3.9 (Beckman)



. Sodium-Potassium Reaction Cup and Reagent Flow Diagram

Fig. 3.9e

REFERENCES

R E F E R E N C E S

(1) Czaban, J.D. and Legg, K.D. in "Proceedings of the Workshop on Direct Potentiometric Measurements in Blood"., ed. Koch, W.F., Gaithersburg, 63-80 (1983)

(2) Sachs, Ch, and Truchaud, A, in reference 1 (182-184)

(3) Waugh, W.H., Metabolism, 18, 706-712 (1969)

(4) Mass, A.H.J., Siggard-Andersen, O., Weisberg, H.F. and Zijlstra, W.G., Clin. Chem, 31/3, 482-485 (1985)

(5) Maas, A.H.J., Siggard-Andersen, O., Weisberg, H.F., and Zijlstra, W.G., 159-165, Reference 1

(6) Meir, P.C., Ammann, D., Morf, W.E., Simon W., Chap. 2 in "Medical and Biological Applications of Electrochemical Devices", ed. J. Koryta, Wiley (1980)

(7) Hebert N.C., Chap. I(3), in "Ion Selective Micro-Electrodes" ed. Berman H.J., and Herbert N.C., Plenum, N.Y. and London (1974)

(8) Vesely J. et al., Chap. 4, in "Analysis with Ion-Selective Electrode", ed. Chalmers R.A. and Masson M.R., Ellis Harwood Limited, Chichester, U.K. (1978)

(9) KNAl Sodium-Potassium Analyser Handbook, Radiometer, Cophenhagen (1981)

(10) ICAl Ionised Calcium Analyser Handbook, Radiometer, Cophenhagen (1981)

(11) Electrolyte Analyser 902 Operating Manual Parts A and B, Issue 1 (1985)

(12) Curme H.G. et al. "Single-use I.S.E.s. General Concepts", presented at the American Association for Clinical Chemistry meeting, New Orleans (1979)

(13) Corning (private Communication Russell, L. to Covington A.K., 1986)

(14) Beckman Astra™ Manual 015-555588

(15) Ammann D., Morf. W.E., Anker P., Meier P.C., Pretsch E., and Simon W., Ion-Selective Electrode Rev. 5, 3-92 (1983)

(16) Russell L.J. in "Ionised Calcium, Sodium and Potassium by Ion-Selective Electrodes", ed. Maas A.H.J., Kofstad J., Siggard-Andersen O., and Kokholm G., Chap. IV, 187, Proceedings of the first meeting of the European Working Group on ISEs, Oslo (1983)

(17) Van den Winkel P., Mertins J., Massart D.L., Anal Chem., 46, 1765-1768 (1974)

(18) Cowell D.C., Browning D.M., Clarke S., Kilshaw D., Randell J. and Singer R., Med. Lab. Sci., 42, 252-261 (1985)

(19) Apple F.S., Koch D.D., Graves S., Ladenson J.H., Clin. Chem. 28, 1931/1935 (1982)

(20) Ladenson J.H., J. Clin. Lab. Med., 90, 654-665 (1977)

(21) Shyr C., Young C.C., Clin. Chem., 26, 517 (1980)

(22) Coleman R.L., Young C.C., Sidoni L., Clin. Chem., 26, 1922-1923 (1980)

(23) Treasure T., Band D.M., Proc. Analyt. Div. Chem. Soc., 14, 334 (1977)

(24) Annan W., Kirwan N.A., Robertson W.S., Clin. Chem., 25, 643 (1979)

(25) Langhoff E., Steiness I., Clin. Chem., 28, 170 (1982)

(26) Waugh W.H., Metabolism, 18, 706-712 (1969)

CHAPTER FOUR

CALIBRATION SOLUTIONS

CHAPTER FOUR

(CALIBRATION SOLUTIONS)

4.1 INTRODUCTION

Direct potentionmetry seems to be the logical, ultimate choice for the analysis of electrolytes in blood. The problem now, is the establishment of a universally recognised calibration convention to eliminate biases observed among different direct potentiometric analysers.

The chaotic situation arising from manufacturers producing specific standards and conversion factors adapted to their particular instruments has been reported by several workers. Truchaud et al.[1] studied 24 ISE instruments of ten different brands. They concluded that while most ISE instruments met the modern clinical laboratory requirements, instrumental internal corrections gave rise to differences even when a common reference solution (bovine serum) was used. Boinck[2] et al. compared four calcium analysers and showed that while satisfactory results were obtained when the manufacturers' specific standard solutions were used with their particular instruments, discrepancies as large as 20% were observed when the calibration solutions were interchanged. A common calibration solution also gave diverse results. This problem has also been discussed by Russell[3], Bowers[4], Covington et al.[5] and Czaban and Legg[6].

The primary causes of these discrepancies seem to be:

(1) Widely variant calibration solutions

(2) Different residual liquid junction potentials due to:

  (a) different geometrics

  (b) different electrolyte compositions

(3) If whole blood or plasma is used, various sources and levels of heparin used affect the measurements.

To introduce inter-laboratory compatibility, there is need for:

(1) Well defined calibration solutions.

(2) Standardisation of the electro-chemical cell with its ISEs/ISFETs, reference electrodes and liquid junctions.

(3) Exploratory studies to judge the repeatability of measurements.

In this chapter, the first aspect of these problems; establishing a satisfactory calibration solution, will be discussed.

To date, several proposals for suitable calibration solutions have been made. Mohan and Bates[7] suggested several synthetic electrolyte mixtures simulating serum at ionic strengths ranging from 120-200 mmol l^{-1} and using TRIS as the pH buffer. TRIS, however, has disadvantages as a biological buffer because of its reactivity as a primary amine, its appreciable solubility in organic solvents and its limited buffer capacity in the pH range 7.0-7.4 because of its high pK value.

Osswald and Wuhrman[8] proposed two multi-electrolyte standard solutions at pH 7.385 and 6.839 (37 ^{o}C). The ionic strength of both solutions was made up to 160 mmol l^{-1} using magnesium chloride and sulphate and sodium azide was added to inhibit biological growth and to stabilise the solutions. The Good[9,10] buffer, TES was used as a pH buffer substance. ISE manufacturers supply their own calibration solutions, several of which use Good buffers to fix the pH. Serum based[11] calibration solutions or aqueous solutions with human serum protein added[12] have been suggested, but the financial viability and adequate screening procedures for such solutions do not justify their use.

4.2 CALIBRATION PROCEDURES

ISE and ISFET devices measure the activity or concentration of an ion relative to that of the same species in a reference (calibration) solution of known or assigned activity or concentration. A typical electro-chemical cell representation is:-

ISE | Calibration Solution or Plasma || Salt Bridge | Reference Electrode

The double line denotes a liquid/liquid junction potential (E_j).

$$E_{cell} = E_{left} - E_{right} = E_{ISE} - (E_{ref} + E_j) \quad - 4.1$$

$$E_{ISE} = E^{\circ}_{ISE} + S \log a \quad - 4.3$$

where E°_{ISE} = standard electrode potential

S = $2.303 \frac{RT}{nF}$, the slope factor

a = the activity of the ion being measured

$$E_{ISE} = E^{\circ}_{ISE} + S \log C + S \log \gamma \quad - 4.4$$

where $a = C\gamma$, C and γ being the concentration and activity coefficient of the ion, respectively.

$$\therefore E_{cell} = E^{\circ}_{ISE} + S \log C + S \log \gamma - E_{ref} - E_j \quad - 4.5$$

The difference in cell potential between a plasma sample (p) and a calibration solution (c) can be expressed as

$$\Delta E\ (p\text{-}c) = S \log \frac{C_p}{C_c} + S \log \frac{\gamma_p}{\gamma_c} - \Delta E_j\ (p\text{-}c) \quad - 4.6$$

The ion selective device can be calibrated either with solutions of known concentration or activity.

Concentration standards are relatively easy to prepare. Error may rise due to mismatch of slope in an aqueous calibrant and a plasma matrix or by differences in liquid junction potentials and activity coefficients between sample and calibrant. Czaban and Legg[6] have shown that slope errors between a plasma and an aqueous matrix, for sodium measurements, are negligible over the entire physiological range. By maintaining ionic strengths constant and by devising calibration solutions with ionic concentrations that resemble those in plasma, errors due to activity coefficients and liquid junctions can be minimised. The ionic activity which the electrode senses is

then proportional to the ionic concentration and ideally, equation 4.6 can be written as:

$$C_p = C_c \exp_{10}\left\{\frac{\Delta E(p-c)}{S}\right\} \qquad - 4.7$$

Preparation of an activity standard requires a knowledge of the activity coefficients of the ions involved. Single ion activities can be defined on an operational basis only, through the use of arbitrary assumptions. In clinical media where the ionic strength is greater than 0.1, the theory involved becomes complex. Some of these theories are discussed in Section 4.8. A major source of error, in activity standards, is the residual liquid junction potential. If calibration and sample solutions are well matched this error is reduced and the equation involved can be written as:

$$a_p = a_c \frac{(\gamma c)}{(\gamma p)} \exp_{10}\left\{\frac{(\Delta E\ (p-c)}{S}\right\} \qquad - 4.8$$

4.3 CRITERIA FOR BLOOD ELECTROLYTE CALIBRATION SOLUTIONS

The criteria for devising the calibration solutions for blood electrolytes were:-

(1) The calibration solutions should have compositions closely paralleling the composition of blood serum to compensate for liquid junction and ionic strength effects.

(2) Multi-purpose standards for ions detectable by ISE and ISFET s are preferable in view of future multi-function analysers. At present, calibration solutions proposed tend to be for sodium plus potassium and calcium plus pH, separately.

(3) Variation of ionic concentrations between calibration solutions should reflect the normal range of ions in blood, but, at the same time, the millivolt swing should be adequate for reliable measurements. Table 4.1 shows the normal range of ions measured in this work and the millivolt swing at 37 ^{o}C.

4.4 CONSEQUENCES OF THE CHOSEN CRITERIA

(1) A constant ionic strength of 160 mmol dm^{-3} was chosen.

(2) Zwitterionic Good buffers (see next section) were used.

(3) Calibration solutions were devised so that one had ionic concentrations at the centre of the normal range and one upper and lower slope solutions, not necessarily the same solutions for each ion due to restricted degrees of freedom of choice.

(4) If necessary, additional calibration solutions for extreme conditions such as hypernatremia requiring 200 mmol dm^{-3} sodium may be provided.

(5) The binding of metal ions to the chosen buffers were determined so that corrections could be made to obtain free ion concentrations in the calibration solutions.

(6) Residual liquid junction potentials of the calibration solutions against blood pH phosphate solutions should be determined.

(7) Materials that are readily available and did not require tedious preparation were used. Thus, Good buffers whose sodium forms are commercially available were used. Volumetric standard solutions of calcium chloride were used avoiding the need for calcium carbonate dissolved in HCl.

(8) The addition of ions not normally present in blood to make up the ionic strength was avoided.

4.5 THE GOOD BUFFERS

Until 1966, when the Good buffers[9,10] were introduced, substances suitable for use as buffers in the physiologically important pH range 6-8 were inadequate. The NBS phosphate buffer has poor buffering capacity above pH 7.5 and it forms complexes with most polyvalent cations. A mixture of 1:3 TRIS:TRIS HCl (tris (hydroxymethyl) amino methane) has a pH of 7.382 at 37 ^{o}C [13]. It has been a major biological buffer but its reactivity as a primary amine and its poor buffer capacity below pH 7.5 are drawbacks.

The Good buffers, used in this work, are zwitterionic amino sulphonic acids. These buffers were especially designed for use as suitable biological buffers. The criteria[9] that make these buffers desirable as blood buffers are:-

(1) Their pKa's are between 6 and 8.

(2) They pass through biological membranes with difficulty (low membrane penetration).

(3) Salt effects are minimal i.e. the buffers are not adversely affected by salts.

(4) Complexation with cations is low and if complexes are formed they are soluble.

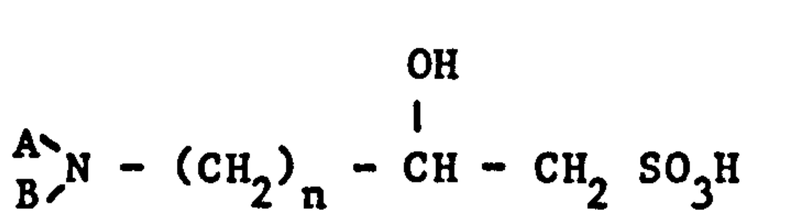
(5) There is minimum influence of buffer concentration, temperature, and ionic composition of the medium on the dissociation of the buffers.

(6) The buffers are stable.

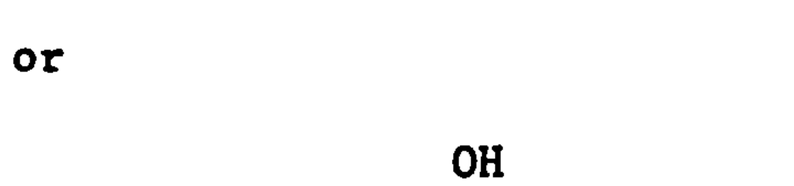
(7) They are easy to prepare the purify.

Of the range of buffers, the four used in this work are shown in Table 4.2 (I, II, III and IV).

Buffers from another range proposed by Ferguson[13] were also tried at one stage. This range was synthesized to extend the range of biological buffers available, particularly buffers having increased water solubility and slightly lower pKa values. They are amino hydroxy alkene sulphonic acid zwitterions and can be represented by the general formulae -



or



where A and B are a hydrogen atom, an aliphatic, cyclo- aliphatic or hydroxy aliphatic group.

n is 1 or 2.

R = N - is a cyclic radical comprising one or two ring nitrogen atoms, zero or one ring oxygen atoms, the remainder of the ring being carbon atoms and water soluble salts of acids.

The buffers tried are shown in Table 4.2, V and VI. After initial studies, these buffers were not used in further work because they are relatively more expensive and did not appear to have any special advantage over the Good buffers.

4.6 INITIAL STUDIES

4.6.1 Preparation of the solutions - the various permutations of calibration solutions studied initially are given in Table 4.3. Solutions 8 and 9 were used only for residual liquid junction potential studies (Chapter 7).

The materials used were -

NaCl - Analytical grade, BDH (Analar Grade)

KCl - Analytical grade, BDH (Analar Grade)

$CaCl_2$ - Stock solution (1 mol dm^{-3}, BDH Volumetric standard solutions)

Buffers - Sigma and Research Organic Inc. and their sodium salts

Water - Distilled and CO_2 free

NaCl and KCl were dried before use.

The buffers and their sodium salts were recrystallised from 80% alcohol and dried at 80 ^{o}C for five hours in a vacuum oven.

$CaCl_2$ solution was assayed by EDTA titrations and the percentage purity found to be 99.8 $\pm$ 0.1.

4.6.2 Tests in ISE/Flame Photometer Analysers

Solutions 1, 3, 4, 5, 6 and 7 were tested in various ISE analysers and by flame photometry. The results are shown in Table 4.4. The flame photometer, AVL, ASTRA and Radiometer ICA1 values were obtained from Dr. A.B.T.J. Boink (Department of Cardiac Surgery and Cardiology, State University Hospital, Utrecht, The Netherlands).

The instruments were calibrated with solutions supplied by the manufacturers. This could be one of the reasons for the variability in the results. Different liquid junction geometries and compositions of the electrolyte are other probable causes.

4.6.3 Comparative Tests in ISE s and ISFET s

Various ISE and ISFET devices were transferred between solutions 1, 2 and 3. Theoretical potential differences at 37 °C were calculated using the formula

$$E = S \log \frac{C^1}{C^2} \qquad - 4.9$$

where S = slope at 37 °C (61.54 mV, monovalent; 30.77 divalent) and C^1 and C^2 are the concentrations of the appropriate ions in the solutions between which the device is transferred. Comparisons between these theoretical differences, and actual differences were made.

The transfers were performed in a thermostated glass beaker. The reference electrode in all cases was a Russell pH Ltd. Type CRR calomel electrode. The electrodes were connected to a Sinclair Multimeter DM450, via a buffer amplifier. Electrode drifts were followed on a LINSEIS; 0480L, Y/T chart recorder and readings were taken after equilibrium. The chart recorder was fitted with a capacitor and resistance in series to reduce noise due to external interference. All solutions were stirred with a magnetic stirrer.

The various ISE and ISFET devices used were:-

pH — Russell pH Ltd. (Glass)

Bare-gate ISFET

Na^+ — Orion Research Inc. Type 9411A (ISE1) Kent EIL Ltd. (ISE2)

	ISFET; electro-active material : commercially available CORNING ELECTRO-ACTIVE
K^+	Corning Medical (from 902 K^+ analyser electro-active analyser)
	ISFET; electro-active material: valinomycin
Ca^{2+}	Radiometer AG (ISE1)
	Corning; electro-active material based on ETH ligand 1001 (ISE2)
	ISFET; electro-active material : commercially available PYE (Philips)

Table 4.5 shows the differences between theoretical and actual values for transfer of the devices between solutions. This presents a comparison of ISEs with ISFETs. There is also a possibility of complexing of ions by the Good buffers. At this stage, it was thought that further investigations were necessary.

4.7 THE CALIBRATION SOLUTIONS PROPOSED

A significant aspect related to the amount of buffer material which should be used to set and maintain the pH of the calibration solutions had, hitherto, been neglected; the possible binding effects of the buffers. In the calibration solutions finally proposed, a low concentration of buffers was chosen to minimise these effects.

Table 4.6 shows the composition of the calibration solutions. The solutions have to be related to an activity or concentration scale. This is done through a series of intermediate reference solutions which are related to the calibration solutions in that they are not buffered. The intermediate reference solutions are related again to primary reference solutions of single electrolytes, only. For all these primary reference solutions, mean activities are known and by convention, single ion activities

can be assigned by experiments on cells without liquid junctions. Based on theories of mixed electrolytes, the mean activities for the reference solutions and by convention single ion activities can be assigned.

4.8 ACTIVITY - COEFFICIENT CALCULATIONS

4.8.1. Introduction

The partial molal free energy or the chemical potential of an ion of species 'i' in an electrolyte may be written as

$$\mu_i = \left(\frac{\partial G}{\partial n_i}\right)_{n_j,\ n_s\ T,\ P} \qquad - 4.10$$

where 'j' refers to all ionic species other than i and 's' to the solvent. Equation 4.10, however, has no true meaning because ions of a single species 'i' cannot be added to the solution. Also, the equation ignores the fact that addition of ions of one species only will build up a charge in the solution. The consequence is that experimental methods of measuring the chemical potential lead to values for an electrically neutral assemblage of ions, or else to differences between the chemical potentials of electrically equivalent amounts of ions of the same sign. Theoretical treatments, on the other hand, can lead to calculated values for the chemical potentials of single ionic species.

4.8.2 Relation of Activities and Activity Coefficients to the Chemical Potential

The activity a_i of the ionic species 'i' is related to the chemical potential as

$$\mu_i = \mu_i^o + RT \ln a_i/a_i^o \qquad - 4.11$$

where the subscript 'o' denotes an arbitrarily chosen standard state for the component 'i'. If the standard activity a_i^o is chosen as unity then

$$\mu_i = \mu_i + RT \ln a_i \qquad - 4.12$$

Activities are related to concentrations by $a_i = \gamma_i\, m_i$ on the molal scale where γ_i is the molal activity coefficient and m_i the molality of the solution. Thus equation 4.12 can be written as

$$\mu_i = \mu_i^o + RT \ln m_i + RT \ln \gamma_i \qquad - 4.13$$

$RT \ln \gamma_i$ represents the non-ideal part of the chemical potential; $\mu_i^o + RT \ln m_i$ being the ideal value.

On the molar scale the equivalent expression is

$$\mu_i = \mu_i^o + RT \ln C_i + RT \ln f_i \qquad - 4.14$$

where 'f_i' is the molar activity coefficient and 'C_i' the concentration in molarity. 'f_i' and 'γ_i' are related by the equation

$$\gamma_i = \frac{C_i}{m_i\, d_o} f_i \qquad - 4.15$$

where d_o = density of the pure solvent.

The chemical potential of a salt is expressed as a sum of the chemical potentials of its ions and a corresponding mean activity. For a salt of stoichiometry $M_{\nu_+}^{z+}\, A_{\nu_-}^{z-}$

$$\mu_s = \nu_+ \mu_+ + \nu_- \mu_-$$

$$= \mu_o^s + RT \ln \left(a_+^{\nu_+}\, a_-^{\nu_-}\right)$$

$$= \mu_o^s + RT \ln \left(m_+^{\nu_+}\, m_-^{\nu_-}\right) + RT \ln \left(\gamma_+^{\nu_+} \gamma_-^{\nu_-}\right) \qquad - 4.16$$

The mean molality and mean activity coefficients are defined as -

$$m\pm = \left(m_+^{\nu_+}\ \ m_-^{\nu_-}\right)^{1/\nu} \qquad - 4.17$$

$$\gamma\pm = \left(\gamma_+^{\nu_+}\, \gamma_-^{\nu_-}\right)^{1/\nu} \qquad - 4.18$$

where $\nu = \nu_+ + \nu_-$

so equation 4.16 becomes

$$\mu_s = \mu_o^{\circ} + \nu RT \ln (m \pm \nu\pm) \qquad - 4.19$$

4.8.3 Osmotic Coefficient of Solvents

From the Gibbs-Duhem equation, written in terms of the chemical potential for a binary solute-solvent solution.

$$\sum_i n_i d\mu_i = 0 \qquad - 4.20$$

$$d\mu_s = -\frac{n_j}{n_s} d\mu_j \qquad - 4.21$$

where 's' refers to solvent and 'j' to solute species, it can be seen that non-ideality with respect to solute behaviour is reflected in solvent properties.

For the solvent s,

$$\mu_s = \mu_s^{o} + RT \ln a_s$$

where the standard state is, by convention the pure solvent at the same temperature and pressure as the solution. Hence the activity of the pure solvent is unity. In dilute solutions of electrolytes, the activity and even the activity coefficient of the solvent are very little different from unity, so that reporting of solvent properties in these terms requires a large number of significant figures. To overcome this problem and to simplify calculations[14], the osmotic coefficient was introduced. This is defined by

$$\phi = -10001n\ a_s/(M_s \sum_j \nu_j m_j) \qquad - 4.22$$

is used. m_s is the molar mass of solvent, a_s the activity of the solvent, and $\sum_j \nu_j m_j$ the summation of the product of the number of moles and molality of all the solute species present in solution. The mean activity coefficient of the solute is related to the osmotic coefficient of the solvent by the equation

$$\phi = 1 + \frac{1}{m}\int_o^m m\ d\ \ln \gamma_{\pm} \qquad - 4.23$$

4.8.4 Theoretical Treatments of Activity - Coefficient Calculations used in this Work

In this section, the theoretical treatments of activity coefficient calculations of relevance in this work will be outlined, briefly:

(a) Debye-Huckel Treatment

Debye and Huckel[15] considered the effects of long range interionic forces tending to orient the ions in an ordered structure and kinetic collisions tending to destroy this order, in proposing their theory applicable to dilute solutions. The Poisson equation $\nabla^2\psi = -4\pi\rho_r/\varepsilon$, where ψ is the electric potential at a distance r, calculated from the Boltzmann equation applied to the distribution of cations and anions was used. This model gave the expression

$$\ln \gamma_{\pm} = -A|z_+ z_-| I^{1/2} / 1 + B a I^{1/2} \qquad - \ 4.24$$

where 'a' is the distance of closest approach of ions,

$$A = (2\pi N/1000)^{1/2} \ e^3/2.303k^{3/2} \ \frac{1}{(\varepsilon T)^{3/2}} =$$

$$(1.8246 \times 10^6)/(\varepsilon T)^{3/2}$$

and

$$B = (8\pi Ne^2/1000k)^{1/2} \ \frac{1}{(\varepsilon T)^{1/2}} =$$

$$(50.29 \times 10^8)/(\varepsilon T)^{1/2}$$

(ε = dielectric constant of the solvent and

T = absolute temperature).

Equation 4.24 often holds up to an ionic strength of $I \approx$ 0.1. The term $-A|z_+ z_-| I^{1/2}$ gives the effect of the long range Coulomb forces, while the denominator $(1 + Ba\ I^{1/2})$ accounts for short range inter-actions between ions represented as rigid spheres of equal radii. Short range interactions between ions and solvent molecules and interactions arising between ions which, in a real

situation, cannot be represented as rigid spheres, are more pronounced at higher concentrations. An empirical term + CI was added to equation 4.24 to allow for these effects. Davies[16] uses a value of C = $0.1|z_+ z_-|$ for aqueous solutions at 25 °C.

(b) Hydration Model

Stokes and Robinson[17] pointed out that the activity coefficient predicted by the Debye-Huckel treatment is actually the mean ionic activity coefficient of the hydrated ions whereas in computing activity coefficients from experimental data the concentration of the solution is expressed in terms of number of moles of anhydrous solute. In the molarity scale this does not affect the figure expressing the concentration of the solution but in the molality and mole fraction scales the figure will be different if the solute is considered as a solvated species. The chemical potentials of the solvated and unsolvated species will differ as well. But the total free energy of a fixed amount of solution is obviously fixed regardless of the method for expressing its composition. Stokes and Robinson considered two equivalent states of the solution; one of the unsolvated ions + S solvent molecules and the other of solvated ions; h moles of solvent combined with ν moles of ions leaving (S-h) free solvent molecules. The result expressing the activity coefficient of the ions for these two equivalent systems for an aqueous solution of a single electrolyte is

$$\ln \gamma_\pm = |Z_+ Z_-| \ln f_{DH} - h/\nu \ln a_w - \ln [1+0.018(\nu-h)m] \quad - \quad 4.25$$

where

h = number of moles of water bound to one mole of electrolyte, irrespective of how it is distributed among cations and ions.

a_w = activity of water

f_{DH} = Debye Huckel expression for hydrated ions.

For the calculation of single ions activity coefficients in a mixture of electrolytes; the following expressions can be used[18] for a cation C.

$$\ln \gamma_{\pm} = z_+^2 \ln f_{DH} - h_c \ln a_w - \ln [1+0.018 \sum_i (1-h_i)m_i] \quad - \quad 4.26$$

and for an anion A.

$$\ln \gamma_- = z_-^2 \ln f_{DH} - h_A \ln a_w - \ln [1+0.018 \sum_i (1-h_i)m_i] \quad - \quad 4.27$$

where h_c and h_A are the hydration numbers of the cation and anion respectively and m_i and h_i are the molality and hydration number of each ion in the solution.

By combining equations 4.26 and 4.27, the mean activity coefficient can be expressed as

$$\ln \gamma_{\pm} = |z_+ z_-| \ln f_{DH} - \frac{h}{\nu} \ln a_w - \ln [1+0.018 \sum_i (1-h_i)m_i] \quad - \quad 4.28$$

since

$$\nu \ln \gamma_{\pm} = \nu_c \ln \gamma_+ + \nu_A \ln \gamma_-$$

and

$$h = \nu_c h_c + \nu_A h_A$$

where ν_c and ν_A are the number of moles of cations and anions per mole of electrolyte.

Bates, Staples and Robinson[19] calculated activity coefficients of alkali and alkaline earth chlorides, taking the hydration number of the chloride ion as zero, using the expressions -

$$\ln \gamma_{M^+} = \ln \gamma_{\pm} - h/_2 \ln a_w \quad - \quad 4.29$$

$$\ln \gamma_{Cl^-} = \ln \gamma_{\pm} + h/_2 \ln a_w \quad - \quad 4.30$$

$$\ln \gamma_{M^{2+}} = 2\ln \gamma_{\pm} - h/_3 \ln a_w + \ln [1+0.018(3-h)m] \quad - \quad 4.31$$

$$2\ln \gamma_{Cl^-} = \ln \gamma_{\pm} + h/_3 \ln a_w - \ln [1+0.018 (3-h)m] \quad - \quad 4.32$$

(c) Pitzer Model

Pitzer's specific inter-action model was developed from Bronsted[20] and Guggenheim[21] postulates. Bronsted

proposed that there were two types of interactions one the non-specific inter-actions of ions due to long range coulombic forces and the second due to specific interactions between oppositely charged ions at short ranges. Guggenheim used these principles in conjunction with those of Debye-Huckel. However, neither of these theories was satisfactory.

Pitzer[22-25] has developed a specific interaction model which includes short range interactions between ions of like charge as well as opposite charge. Ternary interactions are also considered. The model has been extended to mixed electrolyte solutions as well. The equation for the calculation of the mean activity coefficient of a single electrolyte M is

$$\ln \gamma_{MX} = |z_M z_X| f^{\gamma} + m\,(2\upsilon_m \upsilon_x/\upsilon)\, B^{\gamma}_{MX} + m^2 \left[2(\upsilon_m \upsilon_x)^{3/2}/\upsilon\right] C^{\gamma}_{MX} \qquad - 4.33$$

where

m is the molality of the electrolyte

υ_m and υ_x are number of ions of M and X per molecule.

z_M and z_X are the respective electric charges.

υ is the total number of ions per molecule.

f^{γ} is the modified Debye-Huckel term giving long range coulombic interactions given by

$$f^{\gamma} = - A_{\phi} \left[I^{1/2}/(1 + bI^{1/2}) + 2/b \ln (1 + bI^{1/2})\right] \qquad - 4.34$$

$$B^{\gamma}_{MX} = 2\beta^{o}_{MX} + 2\beta^{1}_{MX}/\alpha^2 I\left[1-(1+\alpha I^{1/2} - \tfrac{1}{2}\alpha^2 I) \exp(-\alpha I^{1/2})\right] \qquad - 4.35$$

$$C^{\gamma}_{MX} = 3/2\, C^{\phi}_{MX} \qquad - 4.36$$

I is the molal ionic strength. A_{ϕ} is the Debye Huckel coefficient for the osmotic function $A_{\phi} = (2.303/3) A_{\gamma} = 0.392$ at $25^{o}C$ for water, b and α are two adjustable parameters and the values b = 1.2 and α = 2.0 were selected for all 1:1, 1:2 and 2:1 electrolytes[26]. The two parameters $\beta^{(0)}$ and $\beta^{(1)}$ define the second

virial coefficient whereas C^{ϕ} defines the third virial coefficient; this is usually very small. These values have been tabulated for strong electrolytes in reference 23.

The mean activity coefficient of the solute MX in a mixture of electrolytes is given by:-

$$\ln\gamma_{mx} = |z_m z_x| f^{\gamma} + (2\nu_m/\nu)\sum_a m_a [B_{ma} + (\Sigma mz)C_{ma} + (\nu_x/\nu_m)\theta_{xa}] +$$
$$(2\nu_x/\nu)\sum_c m_c [B_{cx} + (\Sigma mz)C_{cz} + (\nu_m/\nu_x)\theta_{mx}] +$$
$$\sum\sum_{ca} m_c m_a \{|z_m z_x| B'_{ca} + \nu^{-1}[2\nu_m z_m C_{ca} + \nu_m \Psi_{m_{ca}}) + \nu_x \Psi_{c_{ax}}]\} +$$
$$\frac{1}{2}\sum\sum_{cc'} m_c m_{c'} [(\nu_x/\nu)\Psi_{c_{c'x}} + |z_m z_x| \theta'_{cc'}] +$$
$$\frac{1}{2}\sum\sum_{aa'} m_a m_{a'} [(\nu_m/\nu)\Psi_{m_{aa'}} + |z_m z_x| \theta'_{aa'}] \qquad 4.37$$

where

$$B_{mx} = \beta^0_{mx} + 2\beta^{(1)}_{mx}[1-(1+\alpha I^{1/2})\exp(-\alpha I^{1/2})] \qquad 4.38$$

$$C_{mx} = C^{\phi}_{mx}/2|z_m z_x|^{1/2} \qquad 4.39$$

$$B'_{mx} = (2\beta^{(1)}_{mx}/\alpha^2 I^2)[-1+(1+\alpha I^{1/2}+1/2\alpha^2 I)\exp(-\alpha I^{1/2})] \qquad 4.40$$

Indices C and C' cover all the cations while a and a' cover all anions. Parameters θ and Ψ arise from additional combinations of the individual second and third virial coefficients. They have been tabulated in reference 24. The terms including θ' are omitted since they involve triply or higher charged ions.

The equations for calculating the activity coefficients of single ions is given by

$$\ln\gamma_m = z_m^2 f^{\gamma} + 2\sum_a m_a [B_{ma} + (\Sigma mz C_{ma}] + 2\sum_c m_c \theta_{mc} + \sum\sum_{ca} m_c m_a [z^2 B'_{ca} + z_m C_{ca} + \psi_{m_{ca}}] +$$
$$1/2\sum\sum_{aa'} m_a m_{a'} [z_m^2 \theta'_{aa'} + \psi_{m_{aa'}}]$$

for a cation M - 4.41

The corresponding expression for an anion X is obtained by interchanging X for M, a for c and c for a throughout.

4.8.5 Calculations of Activity Coefficient of the Proposed Calibration Solutions

The mean and single ion activity coefficients of NaCl, KCl and $CaCl_2$ in solutions 1.1-1.3 and 2.1-2.5 were calculated by Isabel M. Ferra[26] using both the Stokes and Robinson's hydration theory and the Pitzer equations. The values obtained are shown in Tables 4.7a - c.

TABLES

Physiological Range, Normal Values and ISE Millivolt Ranges in Blood

Sodium ion

	2.92 mV		2.56 mV	
130	----------	145	----------	159.6 mmol/L

Potassium ion

	11.42 mV		17.94 mV	
3.0	----------	4.6	----------	9.0 mmol/L

Calcium ion

	6.83 mV		6.28 mV	
0.75	----------	1.24	----------	2.0 mmol/L

Hydrogen ion

	20.92 mV		12.31 mV	
6.86	----------	7.20	----------	7.40 pH

Table 4.1

TABLE 4.2

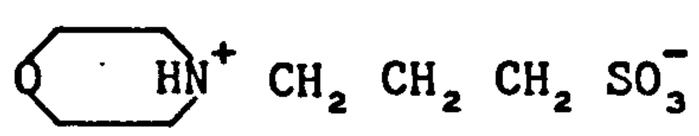
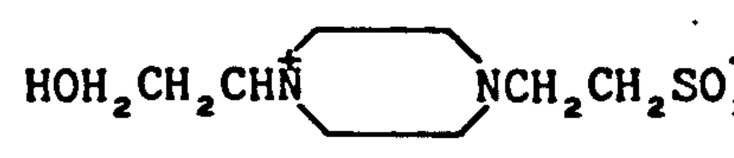
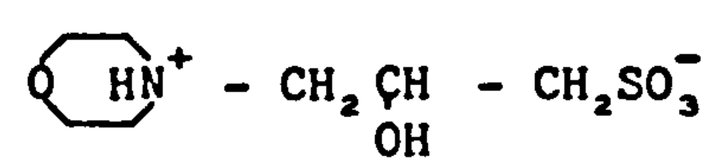
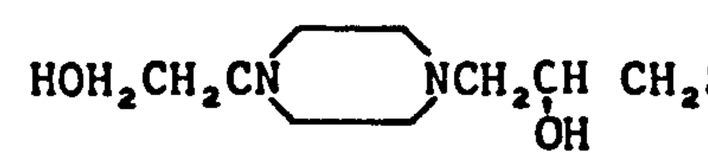
No.	Name	Structure	mol wt	pKa at 20° C	pKa/°C	pKa at 37° C
I	3 - (N - morpholino) propane sulphonic acid (MOPS)		209.3	7.15	-0.013	6.93
II	N, N - BIS (2 - Hydroxy ethyl) - 2 - Aminoethane sulphonic acid (BES)		213	7.15	-0.016	
III	N - TRIS (hydroxymethyl) methylamino ethane sulphonic acid (TES)		229.18	7.50	-0.020	7.16
IV	N_1- 2 - hydroxyethyl piperazine - N^1 - ethane sulphonic acid (HEPES)		238.30	7.55	-0.014	7.31
V	3 (N - morpholino) 2 hydroxy propane sulphonic acid (MOPSO)		225.30	6.95		6.75
VI	N - 2 - hydroxyethyl piperazine N'2 hydroxy propane sulphonic acid (HEPPSO)		268.30	7.90		7.73

TABLE 4.3

Soln No.	pH at 37° C	Buffer	COMPONENTS (conc in mmol dm^{-3}, I = 160 mmol dm^{-3})			
			$[Ca^{2+}]$	$[Na^{+}]$	$[K^{+}]$	$[Cl^{-}]$
1	6.8	80.75 MOPS + 60.00 NaMOPS	0.75	94.75	3.00	99.25
2	7.2	45.62 TES + 50.00 NaTES	1.67	100.00	5.00	108.34
3	7.4	40.65 HEPES + 50.00 NaHEPES	4.00	90.00	8.00	106.00
4	6.8	80.75 MOPS + 60.00 NaMOPS	4.00	85.00	3.00	97.00
5	7.4	40.65 HEPES + 50 NaHEPES	0.75	99.75	8.00	110.00
6	6.8	80.75 MOPS + 60.00 Na-MOPS	-	-	-	-
7	7.4	40.65 HEPES + 50.00 Na-HEPES	-	-	-	-
8	6.8	53.48 MOPSO + 60.00 Na-MOPSO	0.75	94.75	3.00	99.25
9	7.4	106.90 HEPPSO + 50.00 NaHEPPSO	4.00	90.00	8.00	106.00

Table 4.4

Solution No.	MOPS 1	HEPES 3	MOPS 4	HEPES 5	6	7
Na^+						
Stated	155.25	140.0	145.0	150.5	155.25	140.0
* Flame IL-50_2	155.7	140.2	146.0	150.3	156.2	140.9
* AVL 980	147.7	136.1	139.0	144.7	150.9	141.3
* ASTRA - 8	152.0	137.0	142.0	146.0	154.0	139.0
Corning 902	153.0	136.0	140.0	141.0	155.0	137.0
Corning 905	156.0	138.0	-	-	-	-
K^+						
Stated	3.00	8.00	3.00	8.00	3.00	8.00
* Flame IL-502	3.09	8.01	3.10	8.03	3.06	8.07
* AVL 980	3.15	7.34	3.19	7.35	3.11	7.61
* ASTRA - 8	2.90	7.70	2.95	7.80	2.90	7.95
Corning 902	2.94	7.75	2.97	7.79	2.95	7.65
Corning 905	3.20	7.50	-	-	-	-
Ca^{2+}						
Stated	0.75	4.00	4.00	0.75	0.75	4.00
* AVL 980	0.72	3.08	3.17	0.66	0.79	3.68
* Radiometer ICA-1	0.75	3.60	3.75	0.70	0.79	3.99

Corning 902, 905, AVL-980, Radiometer ICA-1 - direct potentiometry
Beckman Astr8-8 - indirect potentiometry
* - Work done by Dr. A.B.T.J. Boink

Table 4.5

		pH			Ca^{2+}				Na^{+}				K^{+}		
		Th	Glass	FET	Th	ISE1	ISE2	FET	Th	ISE1	ISE2	FET	Th	ISE	FET
	A.	24.7	24.6	27.0	10.8	11.4	11.6	11.0	0.8	1.3	1.3	1.5	13.7	12.0	13.0
	B.	35.2	36.3	38.0	22.5	20.8	21.3	22.0	2.7	3.5	3.8	3.5	26.3	23.9	25.0

Th = Theoretical values

A. Changes in potential on transfer from Solution 1 to Solution 2 (mV)

B. Changes in potential on transfer from Solution 1 to Solution 3 (mV)

(Ref. Table 4.3)

Table 4.6

(Concentration are in mmol dm^{-3})

PRIMARY SOLUTION	INTERMEDIATE REFERENCE SOLUTION	WORKING SOLUTION
1.1 160 NaCl	2.1 159.6 NaCl 0.1 KCl 0.1 $CaCl_2$	3.1 - 3.5H Contains 5 Na HEPES 4.06 HEPES with NaCl reduced by 5 pH = 7.40
1.2 160 KCl	2.2 130.0 NaCl 9.0 KCl 7.0 $CaCl_2$	3.1 - 3.5M contains 5 NaMOPS 6.73 MOPS with NaCl reduced by 5 pH = 6.86
1.3 $160/_3$ $CaCl_2$	2.3 145.0 NaCl 9.0 KCl 2.0 $CaCl_2$	3.1 - 3.5T Contains 5 NaTES 4.78 TES with NaCl reduced by 5 pH = 7.20
	2.4 154.75 NaCl 3.0 KCl 0.75 $CaCl_2$	
	2.5 151.75 NaCl 4.6 KCl 1.25 $CaCl_2$	

To calibrate for sodium requires 3.1, 3.2, 3.3

To calibrate for potassium/calcium requires 3.3, 3.4 and 3.5

To calibrate for pH requires any combination of H, T and M

Table 4.7a - Mean and Single Ion Activity Co-efficients for NaCl (a = 3.97A°)

Solution	Pitzer Formula $\gamma+$	γNa	γCl	Hydration Model $\gamma\pm$	γNa	γCl	$\gamma\pm$	γNa	γCl
1.1	0.7461	0.7461	0.7461	0.7442	0.7512	0.7372	-0.0019	0.0051	-0.0089
2.1	0.7461	0.7460	0.7461	0.7442	0.7512	0.7372	-0.0019	0.0052	-0.0089
2.2	0.7470	0.7431	0.7509	0.7439	0.7504	0.7375	-0.0031	0.0073	-0.0134
2.3	0.7460	0.7453	0.7469	0.7440	0.7508	0.7372	-0.0020	0.0055	-0.0097
2.4	0.7461	0.7457	0.7464	0.7441	0.7510	0.7372	-0.0020	0.0053	-0.0092
2.5	0.7461	0.7455	0.7467	0.7441	0.7510	0.7372	-0.0020	0.0055	-0.0095

Table 4.7b - Mean and Single Ion Activity Co-efficients for KCl (a = 3.63A°)

Solution	Pitzer Formula $\gamma\pm$	γK	γCl	Hydration Model $\gamma\pm$	γK	γCl	$\gamma\pm$	γK	γCl
1.2	0.7326	0.7326	0.7326	0.7307	0.7344	0.7270	-0.0019	0.0018	-0.0056
2.1	0.7374	0.7288	0.7461	0.7341	0.7378	0.7304	-0.0033	0.0090	-0.0157
2.2	0.7388	0.7266	0.7509	0.7341	0.7375	0.7306	-0.0047	0.0109	-0.0203
2.3	0.7376	0.7284	0.7469	0.7340	0.7376	0.7303	-0.0036	0.0092	-0.0166
2.4	0.7375	0.7287	0.7464	0.7341	0.7378	0.7304	-0.0034	0.0091	-0.0160
2.5	0.7376	0.7286	0.7467	0.7340	0.7377	0.7304	-0.0036	0.0091	-0.0163

Table 4.7c - Mean and Single Ion Activity Co-efficients for NaCl (a = 4.73A°)

Solution	Pitzer Formula $\gamma\pm$	γCa	γCl	Hydration Model $\gamma\pm$	γCa	γCl	$\gamma\pm$	γCa	γCl
1.3	0.5717	0.2984	0.7913	0.5702	0.3255	0.7681	-0.0015	0.0271	-0.0232
2.1	0.5770	0.3450	0.7461	0.5743	0.3355	0.7514	-0.0027	0.0095	-0.0053
2.2	0.5755	0.3381	0.7509	0.5736	0.3341	0.7517	-0.0019	0.0040	-0.0008
2.3	0.5762	0.3430	0.7469	0.5740	0.3351	0.7514	-0.0022	0.0079	-0.0045
2.4	0.5767	0.3441	0.7464	0.5742	0.3354	0.7514	-0.0025	0.0087	-0.0050
2.5	0.5766	0.3438	0.7467	0.5742	0.3353	0.7514	-0.0024	0.0085	-0.0047

REFERENCES

R E F E R E N C E S

1. Truchaud, A., Gourmelin, Y., Boigne, J.M., Sachs, C., Proc. of the Workshop on Direct Potentiometric Measurements in Blood, Ed. W.F. Koch, Gaithersburg, 47-54 (1983)

2. Boink, A.B.T.J., Gimpel, J.A., Maas, A.H.J., Scand, J., Clin. Lab. Invest., 43 (165) 17-19 (1983)

3. Russell, L.J., "Ionised Calcium, Sodium and Potassium by Ion-Selective Electrodes", Vol. 5, Ed. A.H.J. Maas, J. Kofstad, O. Siggard-Andersen and G. Kokholm, I.F.C.C. Workshop, Oslo, 187-196 (1983)

4. Bowers, G.N., 81-103, in reference 3.

5. Covington, A.K., Boink, A.B.T.J. and Maas, A.H.J., 229-238, in reference 3.

6. Czaban, J.D. and Legg, K.D. (63-80) in reference 1.

7. Mohan, M.S. and Bates, R.G., Clin. Chem. 21, 864 (1975).

8. Osswald, H.F. and Wuhrman, H.R., "Progress in Enzyme and Ion-Selective Electrodes, Ed. D.W.Lubbers, H. Acker, R.P. Buck, G. Eisenman, M. Kessler, W. Simon, Springer-Verlag, Berlin, 74 (1981).

9. Good, N.E., Winget, G.D., Winter, W., Connolly, T.N., Izawa, A., Singh, R.M.M., Biochem 5(2) 467-477 (1966).

10. Good, N.E., Isawa, S., "Methods in Enzymology", Ed. S.P. Colowick, N.O. Kaplan, N.Y. Academic Press, B24 (1972).

11. Toffaletti, J. and Lee, K.M., Clin. Chem. 31(8), 1349-1352 (1985).

12. Toffaletti J., Bird, C., Berg. C., and Abrams, B., Clin. Chem. 32(8), 1548-1550 (1986).

13. Ferguson, N.J., U.S. Patent, 4246, 194, 20th January (1981).

14. Robinson, R.A. and Stokes R.H., "Electrolyte Solution", Second Edition (Revised), p.29, Butterworth and Co. (1970).

15. Debye, P. and Huckel, E., Phys.Z , 24, 185 (1923).

16. Davies, C.W., J. Chem. Soc., 2093 (1938).

17. Stokes, R.H., and Robinson, R.A., J. Amer, Chem. Soc., 70, 1870 (1948).

18. Siggaard-Andersen, O., Thode, J. and Fogh-Andersen, N., Scand. J., Clin. Lab. Invest. 43, Suppl. 165, 11 (1983).

19. Bates, R.G., Staples, B.R., and Robinson, R.A., Anal. Chem., 42, 867 (1970).

20. Bronsted, J.N., J. Am. Chem. Soc., 44, 877-898 (1922).

21. Guggenheim, E.A., Phil. Mag. 19, 588-643 (1935).

22. Pitzer, K.S., J. Phys. Chem., 77, 263 (1973).

23. Pitzer, K.S. and Mayorga, G., J. Phys. Chem. 77, 2300-2308 (1973).

24. Pitzer, K.S., Chapter 7 in "Activity Coefficients in Electrolyte Solutions", Vol. I, Ed. R.M. Pytkowicz, CRC Press (1979).

25. Pitzer, K.S. and Kim J.J., J. Phys. Chem. 96, 5701-5707 (1974).

26. Covington, A.K., and Ferra, M.I.A., 239, in "Methodology and Clinical Applications of ion-selective electrodes" eds. Maas, A.H.J., Boink, A.B.T.J., Saris, N-EL, Sprokholt, R., and Wimberley, P.D., Vol. 7, Radiometer, Copenhagen (1986)

CHAPTER FIVE

EXPERIMENTAL

CHAPTER FIVE

EXPERIMENTAL

INTRODUCTION

The ion-selective devices in this work were used either as dip-type sensors or were incorporated in a flow-through system. The devices were subjected to various tests. They were calibrated either by a constant dilution technique[3] or by serial dilution. Their performance was stringently tested using transfer potentials[16-19]. The alkaline error of pH sensitive devices was checked. The performance of the devices, incorporated in a flow-through rig, in presence of plasma and serum was also tested. These experiments are described in this chapter.

5.1 CALIBRATION OF ION-SELECTIVE DEVICES USED IN THIS WORK

5.1.1 Ion-Selective Electrodes Used

The ion-selective electrodes used were:-

Table 5.1

Make	Electro-Active Material	Type
Sodium		
AVL (U5833 345)	Glass	Flow-through
CORNING	Glass	Flow-through
BECKMAN (668295)	Glass	Flow-through
RUSSELL (944119)	Glass	Dip-Type
CORNING (003070082)	Glass	Dip-Type
EIL (1048-4)	Glass	Dip-Type
PYE (PHILIPS)	ETH 227	Dip-Type
Potassium		
AVL (B7568355)	Valinomycin	Flow-through
CORNING	Valinomycin	Flow-through
PYE (PHILIPS I5561-K)	Valinomycin	Dip-Type
HOME MADE ISE	Valinomycin	Dip-Type
Calcium		
AVL (C4070385)		Flow-through
CORNING	ETH 1001	Flow-through
RADIOMETER	ETH 1001	Dip-Type
PYE (PHILIPS)	ETH 1001	Dip-Type
pH		
CORNING	Glass	Flow-through
RADIOMETER	Glass	Dip-Type
RUSSELL	Glass	Dip-Type

5.1.2 Ion Selective Field Effect Transistors Used

The following devices designed by Doctor A. Sibbald were used:-

Eμ145 These are similar to the Utah UU03 devices[2]. The chip size is 1.25mm x 2.00mm. They are comprised of two ISFET's and two IGFET's, each with channels of dimensions 20μm x 400μm. Each IGFET/ISFET pair share a common drain connection (fig. 5.1a).

Eμ146 The chip and channel dimensions are similar to the Eμ145 device. Each device comprises three ISFET's and one IGFET with folded gates. A common drain connection for all devices minimises the number of lead out wires (fig. 5.1b).

The semi-conductor devices were used as dip-type sensors. They were stuck on to p.c.b.'s of dimensions 0.9 x 10cm by a thermally cured expoxy (EPOTEK, H54, Alpha Metals Limited). The p.c.b.'s have an array of nine conducting tracks attached to a 10 way 2.5mm pitch edge connector. The devices were bonded to the conducting tracks with an ultrasonic wire bonder (Kulficker and Soffa) using 25μm gold wire. The sensors were then encapsulated so that only the gates of the devices were exposed to the analyte. EPOTEK H54 was used as the encapsulant. It was applied with a syringe and syringe needle and cured at 95 ^{o}C for 60 minutes. The temperature was subsequently raised to 115 ^{o}C for 30 minutes. The devices were allowed to cool slowly. The gates were cleaned ultrasonically in 5% Decon-90 (60 s), distilled water (60 s) and finally in iso-propyl alcohol (120s). Electro-active membrane material, dissolved in THF, was deposited on the gates by syringe. Three layers were deposited with an interval of

about two hours between each deposition to allow for evaporation of THF. Fig. 5.2 shows a dip-type sensor.

The devices were operated in the constant current mode with the drain current maintained at 100μA.

All devices were conditioned in 10^{-3} mol dm^{-3} solutions of the appropriate chloride salts for at least 24h.

5.1.3. Methods of Calibration

The ISE s and ISFET s were calibrated either by a constant dilution technique introduced by Horvai et al.[3] or by serial dilution.

In the constant dilution technique, C_o, the concentration of an ion A in a constant volume, stirred, flow-through cell, is diluted by a diluent not containing the ion A. The concentration of A in the cell is given by:-

$$C = C_o e^{-V/V_r} \quad - 5.1$$

$$\text{or } C = C_o e^{-wt/V_r} \quad - 5.2$$

$$\text{or } t = V_r \ln (C/C_o)/-w \quad - 5.3$$

where

V_r = constant volume of the cell.

V = volume of solution passing through the cell.

w = flow rate (in minutes).

t = time elapsed from the beginning of the dilution period (in minutes).

If an electrode sensitive to A is immersed in the cell or placed in its outlet flow, the potential of the electrode with respect to a reference electrode will change continuously with 'V' or 't'. The variation of potential with 't' can be monitored on a Y/t recorder and the calibration curve is simply obtained by rescaling the t axis as log c equivalent to

$1 \text{ min.} = hV_r/2.303w$ mm.

5.1.4 Experimental

In this work, the constant volume cell shown in fig. 5.3b was used for macro electrodes. It was made from a water jacketed glass tube with B19 ground glass joint. Drilled PTFE stoppers were used for fitting the electrodes. The electrodes were sealed in position with paraffin wax. The micro electrodes in flow-through systems were placed in the outlet of the constant volume cell.

The ISE and reference electrodes were connected to a digital multimeter (SINCLAIR) via a buffer amplifier. A flat-bed LINSEIS chart recorder, provided with back off facilities, was used for monitoring potential difference changes. A suitable capacitance was connected across the input of the chart recorder to smooth out residual noise. To further minimise electrical interferences, the constant volume cell and reference cell were placed in a Faraday cage. The reference electrode was a porous plug, saturated calomel electrode (RUSSELL pH PLC). (Fig. 5.3a).

5.1.5 Results and Discussions

The calibration curves for various ISE s are shown in Figs. 5.4-5.19. The clinical ranges are shown in shaded boxes. The ISE s showed reasonable responses in the clinical range. Fig. 5.4, the AVL calcium calibration curve shows a curvature when the calibration is performed with 10^{-1} mol dm^{-3} $CaCl_2$ solutions but the curvature disappears when a 0.15 mol dm^{-3} NaCl background is used. The CORNING sodium electrode (fig. 5.12) was unstable beyond 10^{-3} mol dm^{-3}, but operable in the clinical range.

Calibration curves for K^+, Ca^{2+} and pH responsive ISFET's are shown in figs. 5.20-5.22. The sodium devices showed very poor response. The ETH 227 sensor (PYE, PHILIPS) was used. The results were not reproducible.

Figs. 5.23 - 5.25 show the results obtained when a triple function ISFET was calibrated. This device was prepared using an Eμ/146 chip which has three ISFET gates (figs. 1b). Each gate was isolated from its neighbour by bridges of epoxy (EPOTEK, H54, Alpha Metals Limited) applied carefully using a syringe. Commercially available PYE (PHILIPS) electro-active potassium and calcium sensors were deposited on two gates in the method described in section 5.1.2. The third gate was left bare to function as a pH sensor. The device was conditioned in a solution containing 10^{-3} mol dm^{-3} each of NaCl, KCl and HCl. Fig. 5.23 is the pH calibration curve (K and Ca constant) obtained using buffer solutions B_4, B_5, B_6, B_7 and B_9 (Table 5.2) devised by Covington and Ferra.[4] These buffers are explained in section 5.2.2. A slope of 46 mV per decade was obtained at 19 ^{o}C. Fig. 5.24 is the calibration curve for calcium. This was obtained by serial dilution of a 10^{-1} mol dm^{-3} solution of $CaCl_2$ (made from BDH Analar 1mol dm^{-3} volumetric solution) in a background of 10^{-4} mol dm^{-3} KCl and 0.14 mol dm^{-3} NaCl. pH was maintained constant using B_7. A slope of 24 mV per decade was obtained at 19 ^{o}C. Fig. 5.25 is the calibration curve for potassium, obtained similarly using a 10^{-1} mol dm^{-3} KCl solution in a background of 10^{-4} mol dm^{-3} $CaCl_2$ and 0.14 mol dm^{-3} NaCl. The slope obtained was 43.8 mV per decade at 19 ^{o}C. The slopes obtained were low, but the experiment showed that such devices are feasible.

5.2 COMPARATIVE STUDY OF THE ALKALINE ERROR OF A pH GLASS ELECTRODE, A UUO3 (UTAH) ISFET AND A EμI/145 ISFET

5.2.1 Method

The sodium error (which is positive i.e. falls below the ideal Nernstian behaviour) was investigated using tests devised by Covington and Ferra.[4] An indirect comparison of the potentials of the pH devices with those of a hydrogen electrode are made using Ag/AgCl reference electrodes without liquid junctions. Specially prepared buffer solutions (listed in table 5.2) were used. The pH response was studied by using alkali - metal ion free buffers and the alkaline error by adding alkali ions to the buffers.

The principle is simple. For an ideal pH responsive device, the emf of the cell

$Pt, H_2 \,|\, solution \,|\, pH\ responsive\ device$ (I)

should be constant and independent of the composition of solutions. Departure from ideal behaviour can be seen by differences between emf values obtained with two different solutions.

The cells G and H, corresponding to the cell I, in which an identical reference solution R is used, are set up:-

$pH\ responsive\ device \,|\, Solution\ R \,|\, AgCl/Ag$ (G)

$Pt, H_2 \,|\, Solution\ R \,|\, AgCl/Ag$ (H)

Then,

$$E_I^R = E_H^R - E_G^R \qquad - 5.4$$

where

E_I^R = emf of CELL I

E_H^R = emf of CELL H

E_G^R = emf of CELL G

If the solution R is replaced by another solution T, then

$$E_I^T = E_H^T - E_G^T \qquad - 5.5$$

and

$$\Delta E = E_I^T - E_I^R$$

$$= (E_H^T - E_G^T) - (E_H^R - E_G^R)$$

$$= (E_H^T - E_H^R) - (E_G^T - E_G^R) \qquad - 5.6$$

ΔE is thus a measure of the error of the pH responsive device and can be obtained by subtracting the difference between the two values obtained from Cell G from the corresponding two values for Cell H.

5.2.2 Experimental

The buffer solutions listed on Table 5.2 were prepared in double distilled water purged with nitrogen to remove dissolved carbon dioxide. Buffers having pH values > 7 were stored under nitrogen. The solutions are designated B_i indicating pH lies between i and (i + 1). Buffer solutions containing sodium ions are written as B_iNaj (j = pNa). The reference solution is chosen to be a neutral pH buffer, pH 6.58, designated as B_6.

The reagents used were:-

(a) Hydrochloric Acid - stock solution of 1 mol dm^{-3} prepared from CVS-BDH ampoules

(b) BIS-TRIS - 2, 2 Bis (Hydroxy ethyl) imino tris (hydroxy methyl) methane, (MW-209.25), BDH Analar

(c) TRIS - Tris (hydroxymethyl) amino ethane (MW 121.05) BDH, Analar

(d) Sodium Perchlorate $NaClO_4.H_2O$, BDH Analar

(e) Tetramethyl Ammonium Hydroxide (TMAH) BDH, available as 25% $\pm$ 1.5% W/W aqueous solution. TMAH is prone to carbon-dioxide contamination. To determine the exact molarity of the solutions used, they were titrated against 10^{-1} mol dm^{-3} hydrochloric acid. The determined molarity was then divided by its assumed molarity (25% = 2.792 mol dm^{-3}) and this factor was used to adjust the weights given in Table 5.2.

(f) Sodium Hydroxide - 4 mol dm^{-3} and 1 mol dm^{-3} AVL-BDH analytical volumetric solutions.

(g) Succinic acid - BDH Analar, dried at 80 ^{o}C.

(h) Sodium chloride - BDH Analar dried at 110 ^{o}C.

(i) Ethanolamine - BDH Analar.

The ion-selective devices and Ag/AgCl reference electrode were transferred between the reference solution B_6 and the buffer solutions. They were allowed to come to equilibrium at 25 ^{o}C in thermostated beakers before voltage readings were taken on a Hewlett Packard 9815A micro-computer which was programmed to print the average of ten voltage readings every two minutes.

5.2.3 Results and Discussion

The results are tabulated in Tables 5.3 and 5.4 and plotted in Fig. 5.29. pH responsive FETs with inorganic thin film gates have been widely studied. Originally, an SiO_2 gate dielectric was used which was assumed to behave in a manner similar to the glass electrode.[6] (Figs. 2.2 and 2.3; Chapter 2). The devices were originally reported as sodium

responsive sensors and were subsequently found to exhibit a pH response as well. However, the practical value of a pH ISFET with an SiO_2 gate is doubtful. Their response is poor compared to glass electrodes, they exhibit considerable alkaline error and have limited lift times[7,8] A response of 47mV/pH at pH 7.5 and 10^{-2} mol dm^{-3} NaCl and 37mV/pH at pH 7.5 and 10^{-1} mol dm^{-3} NaCl was reported by Schenck[8]. Sub-Nernstian response was assumed to be due to gradual hydration of the SiO_2 layer which would affect its insulating properties and/or to the existence of pinholes in the SiO_2 gate dielectric causing leakage currents.[6,7,9] Considerable improvement is achieved when a silicon nitride layer is deposited over the oxide film. Silicon nitride masks the diffusion of impurity ions such as sodium.[29] The silicon nitride layers are generally deposited by a vapour deposition process in which NH_3 is reacted with either SiH_4 or $SiCl_4$ in the temperature range 700° - 1000 °C. Auger-Ar^+ sputtering studies of Si_3N_4 [10,11,12] and ESCA-Ar^+ sputtering studies[12] indicate the presence of a spontaneously formed thin film (40Å) of silicon oxynitride [$Si_3N_4(O)$] at the surface of the Si_3N_4 film which might cause pH sensitivity exhibited by Si_3N_4 gate dielectrics. Responses comparable to the glass electrode, between 50-60 mV/pH have been reported from different laboratories.[2]

In this work, the sensitivity and alkaline error of an Eμ145 and a UUO3 ISFET device were compared with a Russell glass electrode in the pH range 12-14. The results are tabulated in Tables 5.3 and 5.4 and plotted in Figs. 5.29 and 5.30. From these results it is evident that the order of closeness to the theoretical response is

Eμ145 > UU03 > Russell Glass Electrode.

The response with the ISFET devices was unexpectedly good. A possible explanation for this behaviour is the condition of deposition of the Si_3N_4 film. Stein and Wells[13] and Vlasov et al.[14] have reported that charge trapping can occur in the SiO_2/Si_3N_4 interface resulting in chemically bound H to N and Si. With increase in deposition temperature (> 800 °C) there is a decrease in Si-H bonding. Thus there is more complete Si-N bonding resulting in a decrease in available H-bonding sites. In the Eμ145 chips, the deposition temperature was 800 °C[1] whereas in the UU03 chips it was 900 °C.[15] This could be one of the reasons for the improved response of the Eμ145 chip. Another possibility is the etching of the gel layer on the [Si_3N_4(O)] film by the highly alkaline tetramethyl ammonium hydroxide solutions used. This could result in a continuous renewal of the surface layer responsible for pH response. The results observed could therefore be merely an outcome of a continuous etching process. More work should be done in this field to clarify the results.

5.3 TESTS OF THE PERFORMANCE OF ION-SELECTIVE DEVICES IN THE PROPOSED CALIBRATION SOLUTIONS USING TRANSFER POTENTIALS

5.3.1 Introduction

Some of the ion-selective devices used in this work were subjected to a stringent test in the calibration solutions based on a method adopted by Covington and Prue[16-18] for glass electrodes and later extended by Briggs and Lilley to calcium electrodes.[19]

The measuring system of an I.S.E. may be represented as

$$\underbrace{Ag\,|\,AgCl\,|\,XCl_n(m_r)\,\|}_{\text{I.S.E.}}\;XCl_n(m_x)\,|\,AgCl\,|\,Ag$$

where m_r and m_x are the molalities of the aqueous solution of the chloride salt of X (= K, Na or Ca) in the I.S.E. and the beaker, respectively. $\|$ represents the electroactive membrane. If $m_r > m_x$ then the observed cell potential is

$$E_M = E_{MX} - E_{MR} = \frac{RT}{nF} \ln\left\{ a(X^{n+})_R \Big/ a(X^{n+})_x \right\} + E_a \qquad - 5.7$$

where E_{MX} and E_{MR} are potentials on either side of the membrane and E_a is the asymmetry potential. The response of the Ag/AgCl electrodes to the Cl^- ion gives a potential difference E_E given by

$$E_E = E_{EX} - E_{ER} = \frac{RT}{nF} \ln\left\{ a(Cl)_R / a(Cl)_x \right\} \qquad - 5.8$$

where E_{EX} and E_{ER} are the potentials from the Ag AgCl electrodes in $XCl_n\ (m_x)$ and $XCl_n\ (m_r)$ respectively.

The overall cell potential is

$$E = E_M + E_R = \frac{RT}{nF} \ln\left[\left\{a(X^{n+})_R\ a(Cl^-)_R^n\right\} \Big/ \left\{a(X^{n+})_X\ a(Cl^-)_X^n\right\}\right] + Ea \qquad -5.9$$

If the I.S.E. is transferred to a second solution, the difference between the second cell potential E_2 and the first E, is referred to as the transfer potential E_T and is given by

$$E_T = E_2 - E_1 = RT/_{nf} \ln\left[\left\{a(X^{n+})_1\, a(Cl^-)_1^n\right\} \Big/ \left\{a(X^{n+})_2\ a(Cl^-)_2^n\right\}\right]$$

$$= k \log\left[\left\{a(X^{n+})_1 / a(X^{n+})_2\right\}\left\{a(Cl^-) / a(Cl^-)_2\right\}^n\right] \qquad - 5.10$$

where k = slope of the electrode.

In terms of molality and activity co-efficients the equation becomes

$$E_T = k \log\left[\left\{m(X^{n+})_1 \gamma(X^{n+})_1 / m(X^{n+})_2 \gamma(X^{n+})_2\right\}\left\{m(Cl^-)_1 \gamma(Cl^-)_1 / m(Cl^-)_2 \gamma(Cl^-)_2\right\}^n\right] \quad - \quad 5.1$$

5.3.2 Experimental

Transfer studies were carried out by transferring the I.S.E. and Ag/AgCl reference electrode between the primary calibration solutions (1.X) intermediate (2.X) and working solutions (3.X).

Silver - silver chloride electrodes were freshly prepared according to the method described by Bates[20]. The electrode base was a B-19 cone sealed with 10 cm of Pt-wire. A helix was formed on the wire using a syringe needle (Fig. 5.24). Silver oxide was prepared by the dropwise addition of sodium hydroxide to a vigorously stirred silver nitrate solution. The precipitate was washed several times with distilled water to ensure complete removal of soluble electrolytes. A thick paste of silver oxide was applied to the platinum helix and allowed to dry partially before being reduced in a furnace at 450 °C. Subsequent layers of silver oxide were added until a smooth sphere was formed. The electrodes were electrolysed in 1 mol l^{-1} HCl for 45 minutes at a current of 5mA electrode. Silver formed the positive electrode and a Pt-wire the negative electrode. The Ag/AgCl electrodes were placed in a 0.05 mol l^{-1} solution of HCl, overnight. Electrodes with bias potentials greater than 100μV were rejected.

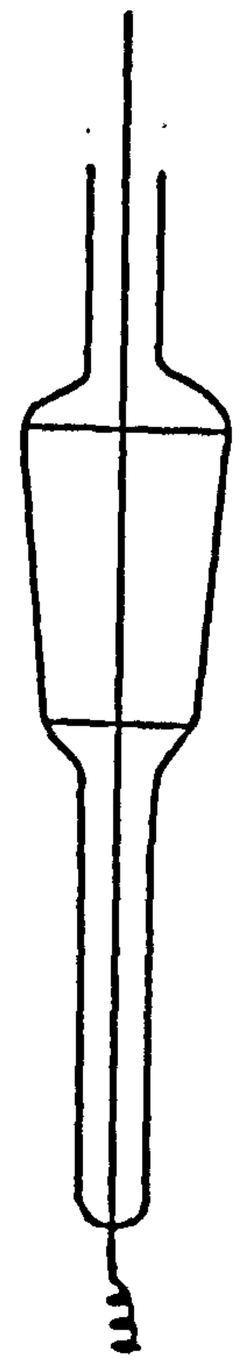


Fig. 5.24

Measurements were made in blackened, thermostated beakers at 37 ^{o}C under a blanket of nitrogen. The dip-type ion selective electrodes, after calibration, were transferred, with the Ag/AgCl reference electrode between the primary solutions and the intermediate and working solutions. The electrodes were washed with distilled water and solution in which they were immersed prior to each transfer. They were connected to a digital multimeter (SINCLAIR) via a buffer amplifier. A flat bed LINSEIS chart recorder, provided with back-off facilities, was used for monitoring potential difference changes. A suitable capacitance was connected across the input of the chart recorder to smooth out residual noise.

Transfer studies were also carried out in a flow-through system similar to the one illustrated in Photograph 5.1. A Beckman sodium electrode AVL 982 sodium potassium and calcium electrodes and CORNING 614/634 sodium, potassium, calcium and pH electrodes (described in Chapter 3) were inter-connected by P.V.C. tubing. A special clamp was made to hold the CORNING electrodes together. All the electrodes were connected to a multiple switch. An Ag/AgCl reference electrode was used in the reference cell. Solution was drawn through the system at a suitable rate using a peristaltic pump. It was essential to ensure that the system was free from air bubbles prior to measurement.

Measurements were carried out at ambient temperature in a manner similar to that described previously.

Theoretical transfer potentials were calculated using activity co-efficients obtained both by the Pitzer and Hydration models in the equation

$$E_T = (E_{N.X} - E_{1.X}) = RT/_{ZF}[(C_{MX}/C_{M1})^{1/2} (\gamma MX/\gamma M1)^{1/2}(C_{Cl_x^-}/C_{Cl_1^-}) (\gamma Cl_x^-/\gamma Cl_1^-)] \quad 5.12$$

(See Chapter Four).

These were compared with the experimental transfer potentials.

5.3.3 Results and Discussions

Tables 5.5a-5.5c compare the experimental and theoretical transfer potentials. In the last column, $E_T(3.X)-E_T(2.X)$ was calculated to ascertain whether any systematic trend between solutions containing the buffers (the working solutions) and solutions without the buffers (the intermediate reference solutions) could be detected. The mean results of these electrode tests are summarised in Table 5.6. E_T(exp-Th) should be zero if the activity co-efficients are correct. Non-zero values of E_T (3.X) - E_T(2.X) shows any medium effect of the buffer addition.

In Tables 5.7a-5.7c, the transfer potentials were converted to concentrations using

$$\text{Antilog}\,\Delta E_T \quad (k/z) = C_{exp}/C_{Th} \qquad - 5.13$$

where ΔE_T is the difference between the experimental and theoretical transfer potentials, k is the slope, z the charge of the ion, C_{exp} the experimental concentration and C_{Th} the calculated concentration. The concentration differences were compared using

$$[(C_{N.X})_{exp} - (C_{N.X})_{Th}]/(C_{N.X})_{Th} \times 100 \qquad - 5.14$$

It should be noted that the ΔE_T values are well within $\pm$ 1 mV which in concentration norms is equivalent to $\pm$ 4% for univalent and $\pm$ 8% for divalent ions.

In Table 5.8, data from experiments using the proposed calibration solutions, performed by Dr. D.C. Cowell[21] were converted to values comparable to those obtained by equation 5.14 and tabulated. For the flame photometry and indirect I.S.E. systems, Dr. Cowell used his own standards for calibrating the instruments. He did not specify their composition. The IL502 values were expressed twice, the

"flame equivalent" value has a -7% correction applied to the "plasma water" (P.W.) value by the instrument. The 23/39 instrument was designed by Dr. Cowell using valinomycin and Simon's sodium sensor. The results of Dr. Cowell's experiments and the experiments performed in this laboratory seem to indicate that the concentration differences obtained reflect the condition of the electroactive material, the environment in which the measurements are performed and the method of calculation used. These results stress the need for standardising the methods of measurement of ions in solution.

5.4 MEASUREMENT OF CALCIUM, SODIUM, POTASSIUM AND pH IN PLASMA AND SERUM

5.4.1 Method

Calcium, sodium, potassium and pH in plasma and serum were determined in the flow-through system described in Section 5.3.2 and shown in Photograph 5.1. The reference electrode in this experiment was a single ceramic plug KCl electrode. The reference cell contained the experimental fluid. The electrodes were calibrated using working standards that were appropriate to the ion measured. This is explained in Table 4.6, Chapter Four. All measurements were carried out at ambient temperature.

Sodium and potassium were also determined on three commercial instruments (Radiometer KNA1, IL502 and Beckman Astra 8), calibrated with the manufacturer's standards. The results were expressed as mmol dm^{-3} plasma water. For the Astra and KNA1, this involved correction of the results using the mass concentration of water (kg water dm^{-3} plasma) in the samples. Dr. C.T.G. Flear (Department of Clinical Biochemistry and Metabolic Medicine, University of Newcastle Upon Tyne) conducted this portion of the work.

Blood plasma samples from six healthy volunteers, after addition of 15IU of heparin per ml of whole blood and centrifugation were separated into three portions.

Portion I - 25 mmol dm^{-3} of dried NaCl was added.

Portion II - 25 mmol dm^{-3} of dried $NaHCO_3$ was added.

Portion III - left unadulterated.

These were analysed. Two serum samples were also analysed.

5.4.2 Results and Discussion

The concentrations of sodium, potassium and calcium and the pH of the six plasma samples and two serum samples analysed in the flow-through system are tàbulated in Table 5.9. The concentrations were calculated using the formula -

$$E_{Sample} - E_{Standard} = k \log C_{Sample}/C_{Standard} \quad - 5.15$$

where k is the theoretical slope at ambient temperature.

The activities were calculated using

$$E_{Sample} - E_{Standard} = k \log a_{Sample}/a_{Standard} \quad - 5.16$$

where $a = C\gamma$, γ being the ion activity co-efficients.

These are tabulated in Table 5.10.

In Table 5.9, the figures in parenthesis in I and II represent the under or over estimation of the measured ion calculated using the expression

$$P = \left(\frac{M-E}{E}\right) \times 100 \quad - 5.17$$

where P = percentage under or over estimation

M = measured concentration

E = expected concentration

For Serum (IV), the figures in parenthesis indicate the percentage difference between the serum and plasma concentrations of the ions.

The following observations can be made from the experiments.

(1) There is a reasonably good agreement between the I.S.E. values in the flow system. This could be because the

electrodes were calibrated with the same calibration solutions and a common reference electrode was used.

(2) For Plasma + 25 mmol dm^{-3} NaCl (I), there is an over-estimation of Na^+ by approximately 4% and of K^+ and Ca^{2+} by 5% and 15% respectively. This could be due to the activity co-efficient effect of the media which is dependent on the ionic strength : at a higher ionic strength the activity co-efficient ($C \rightarrow 0, \gamma \rightarrow 1$) decreases giving higher concentrations. This effect is probably compensated, to some extent, by the opposing liquid junction effect.[22]

(3) For Plasma and 25 mmol dm^{-3} $NaHCO_3$, III, there is an under-estimation of Na^+ by approximately 26% and of Ca^{2+} by approximately 12%.

The effect of bicarbonate on the measurement of sodium and potassium by direct potentiometry has been studied by several workers (23-28). There is a controversy over the interpretation of the bicarbonate induced decrease in concentrations observed. Some authors ascribe the decrease to liquid-junction potential and activity co-efficient effects and others to ion-pair formation. There is no report on the effect of bicarbonate on calcium measurements. In this study, no attempt was made at distinguishing liquid junction potential, activity co-efficient and ion-pair formation effects. It is, therefore, not possible to analyse the contribution of each effect to the observed under-estimation.

(4) For Serum (IV), K^+ concentrations are about 3.5% and Ca^{2+} concentrations about 12% higher than the corresponding plasma values. This is probably due to heparin binding (see Chapter, 7, Section 7.2).

(5) The pH Values are about 1.0 higher than expected. This could be due to the loss of CO_2 from the samples.

Dr. C.T.G. Flear's comments on the analysis with the commercial instruments Radiometer KNA1, IL502 and Beckman Astra 8, were:-

(i) Systematic differences exist between commercial instruments

(ii) Increased NaCl causes over-estimation of Na by approximately 30% and of K by approximately 6% on the IL502, over-estimation of Na by approximately 6% on the KNA1 and Astra.

(iii) Increased $NaHCO_3$ causes under-estimation of the expected increase in Na by approximately 39% (IL502) and 11% (KNA1).

In Figs. 5.26, 5.27 and 5.28, the effects of the addition of NaCl and $NaHCO_3$ to Plasma and the serum concentrations of the relevant ions are illustrated. $\Delta E = E(X) - E(S)$ values (where E(S) is the potential of the standard with lowest concentration of the relevant ion and E(X) is the potential of the solution) are plotted against log [M] values (where [M] is the concentration of K, Na or Ca). These figures clearly illustrate the similarity of results obtained with the Corning and AVL electrodes. The bicarbonate and heparin effects discussed in points (3) and (4) on the previous page are also evident.

TABLES

Table 5.2

	Buffer	Components	Volume
(1)	B_4	4.724 of succinic acid 5.844g NaCl 20 ml 1mol dm^{-3} NaOH	1 litre
(2)	B_5	4.724g succinic acid 5.844g NaCl 60 ml 1mol dm^{-3} NaOH	1 litre
(3)	B_6	20.924 g Bis Tris 50 ml 1mol dm^{-3} HCl	1 litre
(4)	B_7	18.171g Tris 100 ml 1mol dm^{-3} HCl	1 litre
(5)	B_9	3.054g ethanol amine 30 ml 1mol dm^{-3} HCl	1 litre
(6)	B_{12}	100 ml 1mol dm^{-3} HCl * 54.7g 25% T.M.A.H.	1 litre
(7)	B_{13}	100 ml 1mol dm^{-3} HCl *105.73g 25% T.M.A.H.	1 litre
(8)	B_{14}	100 ml 1mol dm^{-3} HCl *328.1g 25% T.M.A.H.	1 litre
(9)	$B_{12}NaO$	30 ml 1mol dm^{-3} NaOH 20 ml 1mol dm^{-3} HCl 23.877g $NaClO_4$	200 ml
(10)	$B_{13}NaO$	25 ml 1mol dm^{-3} HCl 75 ml 1mol dm^{-3} NaOH	250 ml
(11)	$B_{14}NaO$	50 ml 1mol dm^{-3} HCl 125 ml 4mol dm^{-3} NaOH	500 ml

Table 5.3

Errors for the pH sensitive devices transferred between sodium free buffers. Readings were taken as $B_6 \rightarrow B_{12}$, $B_6 \rightarrow B_{13}$ and $B_6 \rightarrow B_{14}$, at 25°C.

Buffers	E_{Bi}/mV			$(E_{Bi}-E_{B6})$/mV			$(E_{Bi}-E_{B6})_H$	ΔE		
	Eμ/145	UU03	Glass	Eμ/145	UU03	Glass		Eμ/145	UU03	Glass
B_6	1953.6	50.2	-34.8	339.2	334.8	335.0	*340.8	1.6	6.0	5.8
B_{12}	2292.8	385.0	300.2							
B_6	1954.0	41.4	-34.1	378.9	374.2	368.9	+381.2	2.3	7.0	12.3
B_{13}	2332.9	430.2	334.8							
B_6	1953.5	41.5	-34.5	426.9	414.1	414.7	*434.5	7.6	20.4	19.8
B_{14}	2380.4	455.6	380.2							

* Reference 4
+ Reference 5

$(E_{Bi} - E_{B6})_H$ are the emf differences for a perfect glass electrode i.e. a S.H.E.

Table 5.4

Alkaline errors for the pH sensitive devices studied. Readings were taken as: $B_6 \rightarrow B_{12} \rightarrow B_{12}NaO$, $B_6 \rightarrow B_{13} \rightarrow B_{13}NaO$, $B_6 \rightarrow B_{14} \rightarrow B_{14}NaO$; at 25°C.

Buffers	$E_{Bi}/E_{Bi}NaO$			$(E_{Bi}NaO-E_{Bi})$			$(E_{Bi}NaO-E_{Bi})_H$	ΔE		
	Eμ/145	UU03	Glass	Eμ/145	UU03	Glass		Eμ/145	UU03	Glass
B_{12}	2292.8	345.4	301.0	-3.8	-5.4	-8.6	* -3.0	-0.8	-2.4	-5.6
$B_{12}NaO$	2289.0	340.0	292.4							
B_{13}	2332.9	371.5	333.6	-6.1	-7.2	-7.5	+ -6.2	-0.1	-1.0	-1.3
$B_{13}NaO$	2326.8	364.3	326.1							
B_{14}	2380.4	415.0	376.2	-19.1	-20.7	-20.6	*-17.5	+1.6	+3.2	+3.1
$B_{14}NaO$	2361.3	394.3	355.6							

* Reference 4
\+ Reference 5

$(E_{Bi}NaO - E_{Bi})_H$ are the emf differences for an S.H.E.

Table 5.5(a) - Transfer Potentials of the Calcium Devices (mV)

DIP TYPE ELECTRODES

TRANSFERS	E_T/Th		E_T/exp		E_T (exp - Th)				$E_{T\,(3.x)} - E_{T\,(2.x)}$			
					Radiometer		Pye		Radiometer		Pye	
	Pitzer	Hyd	Radio	Pye	Pit	Hyd	Pit	Hyd	Pit	Hyd	Pit	Hyd
1.3 2.3	-33.09	-33.62	-34.00	-33.08	-0.91	-0.38	+0.01	+0.54				
1.3 3.3	-33.95	-34.47	-34.95H	-32.89H	-1.00H	-0.48H	+1.06H	+1.58H	-0.09H	-0.10H	+1.05H	+1.05H
T = 37°C			-34.60T	-33.83T	-0.65T	-0.13T	+0.12T	+0.64T	+0.26T	+0.25T	+0.11T	+0.11T
			-34.90M	-33.43M	-0.95M	-0.43M	+0.52M	+1.04M	-0.04M	-0.05M	+0.53M	+0.53M
1.3 2.4	-45.97	-46.50	-46.05	-45.90	-0.08	+0.45	+0.07	+0.60				
1.3 3.4	-46.83	-47.36	-46.99H	-47.71H	-0.16H	+0.37H	-0.88H	-0.35H	-0.08H	-0.08H	-0.95H	-0.95H
T = 37°C			-46.60T	-47.00T	+0.23T	+0.76T	-0.17T	+0.36T	+0.31T	+0.31T	-0.24T	-0.24T
			-47.21M	-46.96M	-0.38M	+0.15M	-0.13M	+0.40M	-0.30M	-0.30M	-0.20M	-0.20M
1.3 2.5	-39.23	-39.76	-39.45	-39.45	-0.22	+0.31	-0.22	+0.31				
1.3 3.5	-40.08	-40.62	-40.55H	-40.86H	-0.47H	+0.07H	-0.78H	-0.24H	-0.25H	-0.24H	-0.56H	-0.55H
T = 37°C			-40.00T	-40.30T	+0.08T	+0.62T	-0.22T	+0.32T	+0.30T	+0.31T	0.00T	+0.01T
			-40.55M	-39.92M	-0.47M	+0.07M	+0.16M	+0.70M	-0.25M	-0.24M	+0.38M	+0.39M
FLOW-THROUGH					AVL		CORNING		AVL		CORNING	
			AVL	CORNING	Pit	Hyd	Pit	Hyd	Pit	Hyd	Pit	Hyd
1.3 2.3	-31.56	-32.06	-32.18	-31.68	-0.62	-0.12	-0.12	+0.38				
1.3 3.3	-32.38	-32.88	-33.22H	-31.90H	-0.84H	-0.34H	+0.46H	+0.98H	-0.22H	-0.22H	+0.58H	+0.58H
T = 23°C			-32.87T	-32.47T	-0.49T	-0.01T	-0.11T	+0.41T	+0.13T	+0.13T	+0.01T	+0.01T
			-33.33M	-33.07M	-0.95M	-0.45M	-0.71M	-0.19M	-0.33M	-0.33M	-0.59M	-0.59M
1.3 2.4	-43.40	-43.91	-42.76	-43.96	+0.64	+1.15	-0.06	+0.45				
1.3 3.4	-44.21	-43.91	-44.77H	-44.31H	-0.56H	-0.05H	-0.10H	+0.41H	-1.20H	-1.20H	-0.04H	-0.04H
T = 20°C			-44.29T	-44.05T	-0.08T	+0.43T	+0.16T	+0.67T	-0.72T	-0.72T	+0.22T	+0.22T
			-43.24M	-44.35M	+0.47M	+0.98M	-0.17M	+0.37M	-0.17M	-0.17M	-0.11M	-0.08M
1.3 2.5	-36.79	-37.29	-36.40	-36.81	+0.39	+0.89	-0.02	+0.48				
1.3 3.5	-37.59	-38.09	-36.72H	-37.72H	+0.87H	+1.37H	-0.13H	+0.37H	+0.48H	+0.48H	-0.11H	-0.11H
T = 18°C			-37.20T	-37.76T	+0.39T	+0.89T	-0.17T	+0.33T	0.00T	0.00T	-0.15T	-0.15T
			-37.17M	-38.09M	+0.42M	+0.92M	-0.50M	0.00M	+0.03M	+0.03M	-0.48M	-0.48M

Table 5.5(b) - Transfer potentials of the Potassium Devices (mV)

DIP-TYPE ELECTRODES

TRANSFERS	E_T/Th		E_T/exp		E_T (exp - Th)				E_T (3.x) - E_T (2.x)			
	Pitzer	Hyd	VAL	PYE	PIT VAL	HYD	PIT PYE	HYD	PIT VAL	HYD	PIT PYE	HYD
1.2 2.3	- 76.96	- 77.09	- 76.96	- 76.64	0.00	+0.13	+0.32	+0.45				
1.2 3.3	- 77.82	- 77.95	- 78.31H	- 77.86H	-0.49H	-0.36H	-0.04H	+0.09H	-0.49H	-0.49H	-0.36H	-0.36H
T = 37°C			- 78.03T	- 77.13T	-0.21T	-0.08T	+0.69T	+0.82T	-0.21T	-0.21T	+0.37T	+0.37T
			- 77.99M	- 78.22M	-0.17M	-0.04M	-0.40M	-0.27M	-0.17M	-0.17M	-0.72M	-0.72M
1.2 2.4	-106.15	-106.26	-106.83	-106.87	-0.68	-0.57	-0.72	-0.61				
1.2 3.4	-107.00	-107.11	-107.84H	-107.53H	-0.84H	-0.73H	-0.53H	-0.42H	-0.16H	-0.16H	+0.19H	+0.19H
T = 37°C			-107.98T	-107.47T	-0.98T	-0.87T	-0.47T	-0.36T	-0.30T	-0.30T	+0.25T	+0.25T
			-107.88M	-107.55M	-0.88M	-0.77M	-0.55M	-0.44M	-0.20M	-0.20M	+0.17M	+0.17M
1.2 2.5	- 95.38	- 95.50	- 95.35	- 94.83	+0.03	+0.15	+0.55	+0.67				
1.2 3.5	- 96.24	- 96.35	- 97.23H	- 95.92H	-0.99H	-0.88H	+0.32H	+0.43H	-1.02H	-1.03H	-0.23H	-0.24H
T = 37°C			- 99.24T	- 95.91T	-1.00T	-0.89T	+0.33T	+0.44T	-1.03T	-1.04T	-0.22T	-0.23T
			- 96.67M	- 96.14M	-0.43M	-0.32M	+0.10M	+0.21M	-0.46M	-0.47M	-0.45M	-0.46M
FLOW THROUGH			AVL	CORNING	PIT AVL	HYD	PIT CORNING	HYD	PIT AVL	HYD	PIT CORNING	HYD
1.2 2.3	- 73.41	- 73.54	- 73.02	- 73.21	+0.39	+0.52	+0.20	+0.33				
1.2 3.3	- 74.23	- 74.36	- 74.95H	- 74.76H	-0.72H	-0.59H	-0.53H	-0.40H	-1.11H	-1.11H	-0.73H	-0.73H
T = 23°C			- 74.65T	- 74.71T	-0.42T	-0.29T	-0.48T	-0.35T	-0.81T	-0.81T	-0.68T	-0.68T
			- 75.35M	- 75.07M	-1.12M	-0.99M	-0.84M	-0.71M	-1.51M	-1.51M	-1.04M	-1.04M
1.2 2.4	-100.23	-100.33	- 99.94	- 99.81	+0.29	+0.39	+0.42	+0.52				
1.2 3.4	-101.03	-101.13	-102.04H	-102.35H	-1.01H	-0.91H	-1.32H	-1.22J	-1.30H	-1.30H	-1.74H	-1.74H
T = 20°C			-101.72T	-101.54T	-0.69T	-0.59T	-0.51T	-0.41T	-0.98T	-0.98T	-0.93T	-0.93T
			-101.68M	-101.13M	-0.65M	-0.55M	-0.10M	0.00M	-0.94M	-0.94M	-0.52M	-0.52M
1.2 2.5	- 89.44	- 89.56	- 89.23	- 89.30	+0.21	+0.33	+0.14	+0.26				
1.2 3.5	- 90.25	-90.37	- 89.43H	- 90.44H	+0.82H	+0.94H	-0.19H	-0.07H	+0.61H	+0.61H	-0.33H	-0.33H
T = 18°C			- 90.08T	- 90.94T	+0.17T	+0.29T	-0.69T	-0.57T	-0.04T	-0.04T	-0.83T	-0.83T
			- 90.07M	- 90.92M	+0.18M	+0.30M	-0.67M	-0.55M	-0.03M	-0.03M	-0.81M	-0.81M

Table 5.5(c) - Transfer potentials of the Sodium Devices (mV)

DIP-TYPE ELECTRODES

TRANSFERS	E_T/Th		E_T/exp		E_T (exp - Th)				E_T(3.x) - E_T (2.x)			
					CORNING		EIL		CORNING		EIL	
	Pitzer	Hyd	CORNING	EIL	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD
1.1 2.1	-0.08	-0.08	-0.06	+0.11	+0.02	+0.02	+0.19	+0.19				
1.1 3.1	-0.93	-0.93	-1.15H	-1.02H	-0.22H	-0.22H	-0.09H	-0.09H	-0.24H	-0.24H	-0.28H	-0.28H
T = 37°C			-1.28T	-1.28T	-0.35T	-0.35T	-0.35T	-0.35T	-0.37T	-0.37T	-0.54T	-0.54T
			-1.51M	-1.29M	-0.58M	-0.58M	-0.36M	-0.36M	-0.60M	-0.60M	-0.55M	-0.55M
1.1 2.2	-6.69	-6.77	-6.55	-6.53	+0.14	+0.22	+0.16	+0.24				
1.1 3.2	-7.57	-7.66	-6.91H	-6.84H	+0.66H	+0.75H	+0.73H	+0.82H	+0.52H	+0.53H	+0.57H	+0.58H
T = 37°C			-7.17T	-7.14T	+0.40T	+0.49T	+0.43T	+0.52T	+0.26T	+0.27T	+0.27T	+0.28T
			-6.75M	-7.20M	+0.82M	+0.91M	+0.37M	+0.46M	+0.68M	+0.69M	+0.21M	+0.22M
1.1 2.3	-2.97	-2.98	-2.47	-2.73	+0.50	+0.51	+0.24	+0.25				
1.1 3.3	-3.83	-3.84	-3.80H	-3.72H	+0.75H	+0.76H	+0.11H	+0.12H	+0.25H	+0.25H	-0.13H	-0.13H
T = 37°C			-3.22T	-3.90T	+0.61T	+0.62T	-0.07T	-0.08T	+0.11T	+0.11T	-0.31T	-0.31T
			-2.93M	-3.72M	+0.90M	+0.91M	+0.11M	+0.12M	+0.40M	+0.40M	-0.13M	-0.13M
			RUSSELL		RUSSELL				RUSSELL			
					PIT		HYD		PIT		HYD	
1.1 2.1	-0.08	-0.08	-0.10		-0.02		-0.02					
1.1 3.1	-0.87	-0.88	-1.15H		-0.28H		-0.27H		-0.26H		-0.25H	
T = 18°C			-1.10T		-0.23T		-0.22T		-0.21T		-0.20T	
			-0.99M		-0.12M		-0.11M		-0.10M		-0.09M	
1.1 2.2	-6.27	-6.35	-6.28		+0.01		+0.07					
1.1 3.2	-7.10	-7.18	-6.80H		+0.30H		+0.38H		+0.29H		+0.31H	
T = 18°C			-6.98T		+0.12T		+0.20T		+0.11T		+0.13T	
			-7.05M		+0.05M		+0.13M		+0.04M		+0.06M	
1.1 2.3	-2.83	-2.82	-2.65		+0.18		+0.17					
1.1 3.3	-3.65	-3.63	-3.43H		+0.22H		+0.20H		+0.04H		+0.03H	
T = 23°C			-3.51T		+0.14T		+0.12T		-0.04T		-0.05T	
			-3.57M		+0.08M		+0.08M		-0.10M		-0.09M	

Table 5.5(c) (Continued)

FLOW THROUGH ELECTRODES

TRANSFERS	E_T/Th		E_T/exp		E_T(exp - Th)				E_T(3.x) - E_T(2.x)			
					AVL		CORNING		AVL		CORNING	
	Pitzer	Hyd	AVL	CORNING	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD
1.1 2.1	-0.08	-0.08	-0.05	-0.12	+0.03	+0.03	-0.04	-0.04				
1.1 3.1	-0.87	-0.88	-1.07H	-1.02H	-0.20H	-0.19H	-0.15H	-0.14H	-0.23H	-0.22H	-0.11H	-0.10H
T = 18°C			-1.25T	-1.31T	-0.38T	-0.37T	-0.44T	-0.43T	-0.41T	-0.40T	-0.40T	-0.39T
			-1.28M	-1.33M	-0.41M	-0.40M	-0.48M	-0.47M	-0.44M	-0.43M	-0.44M	-0.43M
1.1 2.2	-6.27	-6.35	-6.07	-6.28	+0.20	+0.28	-0.01	+0.07				
1.1 3.2	-7.10	-7.18	-7.71H	-7.10H	-0.61H	-0.53H	0.00H	+0.08H	-0.81H	-0.81H	+0.01H	+0.01H
T = 18°C			-7.47T	-7.12T	-0.37T	-0.29T	-0.02T	+0.06T	-0.57T	-0.57T	-0.01T	-0.01T
			-8.80M	-7.00M	+0.30M	+0.38M	+0.10M	+0.18M	+0.10M	+0.10M	+0.11M	+0.11M
1.1 2.3	-2. 83	-2.82	-2.55	-2.72	+0.28	+0.27	+0.11	+0.10				
1.1 3.3	-3.65	-3.63	-3.40H	-3.17H	+0.25H	+0.23H	+0.48H	+0.46H	-0.03H	-0.04H	+0.37H	+0.36H
T = 23°C			-2.96T	-3.68T	+0.65T	+0.63T	-0.03T	-0.05T	+0.37T	+0.36T	-0.14T	-0.15T
			-3.24M	-3.63M	+0.41M	+0.39M	+0.02M	0.00M	+0.13M	+0.12M	-0.09M	-0.10M

		BECKMAN	BECKMAN		BECKMAN	
			PIT	HYD	PIT	HYD
1.1 2.1	-0.08	-0.08	-0.11	-0.03	-0.03	
1.1 3.1	-0.87	-0.88	-1.10H	-0.23H	-0.22H	-0.20H -0.19H
T = 18°C			-1.43T	-0.56T	-0.55T	-0.53T -0.52T
			-1.44M	-0.57M	-0.56M	-0.54M -0.53M
1.1 2.2	-6.27	-6.35	-6.95	-0.68	-0.60	
1.1 3.2	-7.10	-7.18	-8.40H	-1.30H	-1.22H	-0.62H -0.62H
T = 18°C			-7.59T	-0.49T	-0.41T	+0.19T +0.19T
			-7.94M	-0.84M	-0.76M	-0.16M -0.16M
1.1 2.3	-2.83	-2.82	-2.28	+0.55	+0.54	
1.1 3.3	-3.65	-3.63	-3.47H	+0.18H	+0.16H	-0.37H -0.38H
T = 23°C			-2.94T	+0.71T	+0.69T	+0.16T +0.15T
			-3.06M	+0.56M	+0.54M	+0.01M 0.00M

TABLE 5.6

Mean Results of Electode Tests

Electrode	E_T (exp - Th) PIT	HYD	$E_T(3.X)-E_T(2.X)$
Ca (RAD)D	-0.41	+0.12	+0.05
Ca (PYE)D	-0.04	+0.49	+0.01
Ca (AVL)F	-0.04	+0.47	-0.22
Ca (CORN)F	-0.12	+0.39	-0.07
K (VAL)D	-0.55	-0.44	-0.45
K (PYE)D	-0.03	+0.08	-0.11
K (AVL)F	-0.21	-0.10	-0.67
K (CORN)F	-0.37	-0.26	-0.84
Na (CORN)D	+0.30	+0.34	+0.11
Na (EIL)D	+0.12	+0.15	-0.10
Na (RUS)D	+0.04	+0.04	-0.01
Na (AVL)F	+0.01	+0.04	-0.21
Na (CORN)F	-0.04	-0.01	-0.08
Na (BECK)F	-0.23	-0.20	-0.23

D = Dip type
F = flow-through

Table 5.7(a) - Transfer Potentials of the Calcium devices converted to % concentration differences

	$(C_{N.x})$ exp								$[(C_{N.x})exp - (C_{N.x})_{Th}]/(C_{N.x})_{Th}$ x 100							
	Radiometer		PYE		AVL		Corning		Radiometer		PYE		AVL		CORNING	
	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD
2.3	1.87	1.94	2.00	2.08	1.91	1.98	1.98	2.06	-6.5	-3.0	0.0	+ 4.0	-4.5	-1.0	-1.0	+3.0
3.3H	1.86	1.93	2.16	2.25	1.88	1.95	2.07	2.15	-7.0	-3.5	+8.0	+12.5	-6.0	-2.5	+3.5	+7.5
3.3T	1.90	1.98	2.02	2.10	1.93	2.00	2.02	2.06	-5.0	-1.0	+1.0	+ 5.0	-3.5	0.0	+1.0	+3.0
3.3M	1.86	1.94	2.08	2.16	1.86	1.93	1.90	1.97	-7.0	-3.0	+4.0	+ 8.0	-7.0	-3.5	-5.0	-1.5
$(C_{N.x})_{Th}$ = 2.0 mmol dm^{-3}																
2.4	0.75	0.78	0.75	0.78	0.79	0.82	0.75	0.78	0.0	+0.4	0.0	+ 4.0	+5.3	+9.3	0.0	+4.0
3.4H	0.74	0.77	0.70	0.72	0.70	0.75	0.74	0.77	-1.3	+2.7	-6.7	- 4.0	-6.7	0.0	-1.3	+2.7
3.4T	0.76	0.79	0.74	0.77	0.75	0.77	0.76	0.79	+1.3	+5.3	-1.3	+ 2.7	0.0	+2.7	+1.3	+5.3.
3.4M	0.73	0.76	0.74	0.77	0.72	0.81	0.74	0.77	-2.7	+1.3	-1.3	+ 2.7	-4.0	+8.0	-1.3	+2.7
$(C_{N.x})_{Th}$ = 0.75 mmol dm^{-3}																
2.5	1.23	1.28	1.23	1.28	1.29	1.34	1.25	1.30	-1.6	+2.4	-1.6	+ 2.4	+3.2	+7.2	0.0	+4.0
3.5H	1.21	1.26	1.18	1.23	1.33	1.38	1.24	1.29	-3.2	+0.8	-5.6	- 1.6	+6.4	+10.4	-0.8	+3.2
3.5T	1.26	1.31	1.23	1.28	1.29	1.34	1.23	1.28	+0.8	+4.8	-1.6	+ 2.4	+3.2	+7.2	-1.6	+2.4
3.5M	1.21	1.26	1.26	1.32	1.29	1.34	1.20	1.25	-3.2	+0.8	+0.8	+ 5.6	+3.2	+7.2	-4.0	0.0
$(C_{N.x})_{Th}$ =1.25 mmol dm^{-3}																

Table 5.7(b) - Transfer Potentials of the Potassium devices converted to % concentration differences

SOLUTIONS	$(C_{N.x})_{exp}$								$[(C_{N.x})_{exp} - (C_{N.x})_{Th}]/(C_{N.x})_{Th}] \times 100$							
	Valinomycin		PYE		AVL		CORNING		Valinomycin		PYE		AVL		CORNING	
	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD
2.3	9.00	9.04	9.11	9.15	9.14	9.19	9.07	9.12	+0.0	+0.04	+1.2	+1.7	+1.6	+2.1	+0.8	+1.3
3.3H	8.83	8.88	8.99	9.03	8.75	8.79	8.82	8.86	-1.9	-1.3	-0.1	+0.3	-2.8	-2.3	-2.0	-1.6
3.3T	8.93	8.97	9.24	9.28	8.85	8.90	8.83	8.88	-0.8	-0.3	+2.7	+3.1	-1.7	-1.1	-1.9	-1.3
3.3M	8.95	8.99	8.87	8.91	8.61	8.67	8.71	8.75	-0.6	-0.1	-1.4	-1.0	-4.3	-3.7	-3.2	-2.8
$(C_{N.x})_{Th}$ = 9.0 mmol dm^{-3}																
2.4	2.92	2.94	2.92	2.93	3.03	3.05	3.05	3.06	-2.7	-2.0	-2.7	-2.3	+1.0	+1.7	+1.7	+2.0
3.4H	2.91	2.92	2.94	2.95	2.88	2.89	2.85	2.86	-3.0	-2.7	-2.0	-1.7	-4.0	-3.7	-5.0	-4.7
3.4T	2.89	2.90	2.95	2.96	2.92	2.93	2.94	2.95	-3.7	-3.3	-1.7	-1.3	-2.7	-2.3	-2.0	-1.7
3.4M	2.90	2.91	2.94	2.95	2.92	2.94	2.99	3.00	-3.3	-3.0	-2.0	-1.7	-2.7	-2.0	-0.3	0.0
$(C_{N.x})_{Th}$ = 3.0 mmol dm^{-3}																
2.5	4.51	4.53	4.59	4.61	4.54	4.56	4.53	4.55	+0.2	+0.7	+2.0	+2.4	+0.9	+1.3	+0.7	+1.1
3.5H	4.34	4.35	4.55	4.57	4.65	4.67	4.47	4.49	-3.6	-3.3	+1.1	+1.6	+3.3	+3.8	-0.7	-0.2
3.5T	4.33	4.35	4.56	4.57	4.53	4.55	4.38	4.40	-3.8	-3.3	+1.3	+1.6	+0.7	+1.1	-2.7	-2.2
3.5M	4.22	4.45	4.52	4.54	4.53	4.55	4.38	4.40	-6.2	-1.1	+0.4	+0.9	+0.7	+1.1	-2.7	-2.2
$(C_{N.x})_{Th}$ = 4.5 mmol dm^{-3}																

Table 5.7(c) - Transfer potentials of the sodium devices converted to % concentration differences

SOLUTIONS	$(C_{N.x})$exp						$[(C_{N.x})$exp $- (C_{N.x})_{Th}/(C_{N.x})_{Th} \times 100$					
	CORNING (DIP TYPE)		EIL		RUSSEL		CORNING (DIP TYPE)		EIL		RUSSEL	
	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD
2.1	159.7	159.7	160.8	160.8	159.5	159.5	+0.1	+0.1	+0.8	+0.8	-0.1	-0.1
3.1H	158.3	158.3	159.1	159.1	157.8	157.9	-0.8	-0.8	-0.3	-0.3	-1.1	-1.1
3.1T	157.5	157.5	157.5	157.5	158.2	158.3	-1.3	-1.3	-1.3	-1.3	-0.9	-0.8
3.1M	156.2	156.2	157.5	157.5	158.9	158.9	-2.1	-2.1	-1.3	-1.3	-0.4	-0.4
$(C_{N.x})_{Th}$ = 159.6 mmol dm^{-3}												
2.2	130.7	131.1	130.8	131.2	130.1	130.4	+0.5	+0.8	+0.6	+0.9	+0.1	+0.3
3.2H	133.3	133.7	133.6	134.0	131.6	132.0	+2.5	+2.8	+2.8	+3.1	+1.2	+1.5
3.2T	132.0	132.4	132.1	132.6	130.6	131.0	+1.5	+1.8	+1.6	+2.0	+0.5	+0.8
3.2M	134.1	134.5	131.8	132.3	130.3	130.7	+3.2	+3.5	+1.4	+1.8	+0.2	+0.5
$(C_{N.x})_{Th}$ = 130 mmol dm^{-3}												
2.3	147.7	147.8	146.3	146.4	146.0	146.0	+1.9	+1.9	+0.9	+1.0	+0.7	+0.7
3.3H	149.1	149.2	145.6	146.7	146.3	146.1	+2.8	+2.9	+0.4	+0.5	+0.9	+0.8
3.3T	148.3	148.4	144.6	144.6	145.8	145.7	+2.3	+2.3	-0.3	-0.3	+0.5	+0.5
3.3M	149.9	150.0	145.6	145.7	145.5	145.5	+3.4	+3.4	+0.4	+0.5	+0.3	+0.3
$(C_{N.x})_{Th}$ = 145 mmol dm^{-3}												

Table 5.7(c) (Continued) - Transfer potentials of the sodium devices converted to % concentration differences

	$(C_{N.x})$exp						$[(C_{N.x})$exp $- (C_{N.x})_{Th}]/(C_{N.x})_{Th} \times 100$					
	AVL		CORNING (FLOW THROUGH)		BECKMAN		AVL		CORNING (FLOW THORUGH)		BECKMAN	
	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD
2.1	159.8	159.8	159.3	159.3	159.4	159.4	+0.1	+0.1	-0.2	-0.2	-0.1	-0.1
3.1H	158.3	158.4	158.6	158.7	158.1	158.2	-0.8	-0.8	-0.6	-0.6	-0.9	-0.9
3.1T	157.2	157.3	156.8	156.9	156.1	156.1	-1.5	-1.4	-1.8	-1.7	-2.2	-2.2
3.1M	157.0	157.1	156.6	156.6	156.0	156.1	-1.6	-1.6	-1.9	-1.9	-2.3	-2.2
$(C_{N.x})_{Th}$ = 159.6 mmol dm^{-3}												
2.2	131.0	131.5	129.9	130.4	126.5	126.9	+0.8	+1.2	-0.1	+0.3	-2.7	-2.4
3.2H	126.9	127.3	130.0	130.4	123.4	123.8	-2.4	-2.1	0.0	+0.3	-5.1	-4.8
3.2T	128.1	128.5	129.9	130.3	127.5	127.9	-1.5	-1.2	-0.1	+0.2	-1.9	-1.6
3.2M	131.6	132.0	130.5	130.9	125.7	126.1	+1.2	+1.5	+0.4	+0.7	-3.3	-3.0
$(C_{N.x})_{Th}$ = 130 mmol dm^{-3}												
2.3	146.6	146.5	145.6	145.6	148.2	148.1	+1.1	+1.0	+0.4	+0.4	+2.2	+2.1
3.3H	146.4	146.3	147.8	147.6	146.0	145.9	+1.0	+0.9	+1.9	+1.8	+0.7	+0.6
3.3T	148.7	148.6	144.8	144.7	149.1	149.0	+2.6	+2.5	-0.1	-0.2	+2.8	+2.8
3.3M	147.3	147.2	145.1	145.0	148.2	148.1	+1.6	+1.5	+0.1	0.0	+2.2	+2.1
$(C_{N.x})_{Th}$ = 145 mmol dm^{-3}												

Table 5.8 (Experimental Value - Target Value)/(Target Value) x 100

obtained by Dr. D.C. Cowell

K^+ Sensors	Flame IL543	Flame Il1143	Indirect ISE SMAC	Corning 902	IL502 Flame Eq	IL502 P.W.	23/39
Solutions							
2.3	-1.1	0.0	0.0	+8.9	-3.3	+3.3	+1.1
3.3H	-1.1	-1.1	+7.8	-2.2	-4.4	+4.4	-1.1
3.3M	0.0	-1.1	+7.8	-2.2	-5.6	+3.3	0.0
2.4	0.0	+3.3	-3.3	-10.0	-6.7	0.0	0.0
3.4H	0.0	-3.3	0.0	-6.7	-6.7	0.0	0.0
3.4M	0.0	-3.3	0.0	-6.7	-6.7	0.0	0.0
2.5	0.0	+2.2	-4.4	-2.2	-4.4	+2.2	0.0
3.5H	0.0	-2.2	-4.4	-4.4	-6.6	0.0	0.0
3.5M	0.0	0.0	-4.4	-2.2	-6.6	+2.2	0.0
Na^+ Sensors							
Solutions							
2.1	0.0	-0.6	+2.5	-1.3	-4.4	+3.1	-1.3
3.1H	-0.6	-1.3	+1.9	-3.1	-5.0	+1.9	-4.4
3.1M	-1.9	-1.3	+1.3	-1.3	-5.0	+2.5	-2.5
2.2	-0.8	-0.8	-0.8	-0.8	-3.8	+3.1	+1.5
3.2H	-0.8	-0.8	-1.5	-1.5	-4.6	+2.3	+6.3
3.2M	-0.8	-0.8	-1.5	-2.3	-4.6	+2.3	+2.3
2.3	0.0	-0.7	-1.4	-1.4	-4.1	+2.8	0.0
3.3H	-0.7	-0.7	+0.7	-2.1	-4.8	+2.8	-2.1
3.3M	-0.7	-0.7	+0.7	-0.7	-4.1	+4.1	-0.7

Table 5.9 - Concentrations of Sodium, Potassium and Calcium and pH of the Plasma and Serum Samples (mmol dm^{-3})

		SODIUM						POTASSIUM				CALCIUM				pH
		CORNING		AVL		BECKM		CORNING		AVL		CORNING		AVL		CORNING
lasma +	I	175.21	(- 3.3)	176.11	(- 1.56)	-		3.30	(+2.5)	3.38	(+3.4)	0.92	(+ 8.2)	0.92	(+ 9.5)	8.45
5 mmoldm^{-3}	II	181.93	(- 0.8)	185.46	(+ 8.4)	-		3.33	(+6.0)	3.38	(+5.3)	1.30	(+11.1)	1.36	(+13.3)	8.46
aCl	III	179.59	(+ 6.8)	181.30	(+ 4.7)	182.30	(+ 4.7)	3.58	(+5.9)	3.61	(+6.8)	1.65	(+11.5)	1.68	(+13.5)	8.60
	IV	178.62	(+ 1.0)	178.91	ns	178.84	ns	3.88	(+3.7)	3.92	(+3.7)	1.12	(+21.7)	1.07	(+18.9)	8.40
	V	171.77	(+ 2.9)	170.10	(+ 4.1)	171.75	(+ 3.8)	4.14	(+6.4)	3.96	(+3.9)	1.15	(+18.6)	1.12	(+19.1)	8.60
	VI	181.76	(+ 9.8)	180.67	(+ 9.2)	179.94	ns	3.31	(+4.7)	3.38	(+5.3)	1.33	(+14.7)	1.33	(+14.7)	8.53
lasma	I	168.35	(-30.7)	169.34	(-28.6)	-		3.12	(- 3.1)	3.26	ns	0.71	(-24.1)	0.69	(-17.8)	8.64
5 mmoldm^{-3}	II	174.12	(-32.0)	178.54	(-19.3)	-		3.18	(+ 1.3)	3.27	(+1.9)	1.15	(- 1.7)	1.09	(- 9.2)	8.66
aHCO_3	III	173.12	(-19.1)	173.90	(-24.9)	175.04	(-24.3)	3.53	(+ 4.4)	3.54	(+4.7)	1.36	(- 8.1)	1.38	(- 9.4)	8.71
	IV	171.65	(-26.9)	172.13	(-27.9)	171.52	(-28.4)	3.82	(+ 2.1)	3.73	(-1.3)	0.88	(- 4.3)	0.86	(- 4.4)	8.71
	V	166.18	(-19.5)	165.35	(-14.9)	166.80	(-16.0)	3.99	(+ 2.6)	3.83	ns	0.84	(-13.4)	0.78	(-17.0)	8.44
I	VI	175.81	(-14.0)	171.21	(-28.7)	173.21	(-26.4)	3.12	(- 1.3)	3.19	ns	0.99	(-14.7)	1.00	(-13.8)	8.90
lasma	I	151.03		151.50		-		3.22		3.27		0.85		0.84		8.48
	II	157.13		158.36		-		3.14		3.21		1.17		1.20		8.51
	III	152.89		155.13		156.12		3.38		3.38		1.48		1.48		8.61
	IV	153.37		154.11		153.62		3.74		3.78		0.92		0.90		8.61
	V	146.05		144.08		145.81		3.89		3.81		0.97		0.94		8.67
II	VI	154.30		153.38		154.81		3.16		3.21		1.16		1.16		8.71
erum	IV	154.17	ns	155.59	ns	154.42	ns	3.93	(+5.1)	3.94	(+4.2)	1.06	(+15.2)	1.02	(+13.3)	8.57
V	VI	154.99	ns	155.36	(+ 1.3)	154.00	ns	3.20	(+1.3)	3.30	(+2.8)	1.28	(+10.3)	1.28	(+10.3)	8.61

and II Figures in parenthesis indicate the under (-) or over (+) estimation of the measured ion as a percentage.

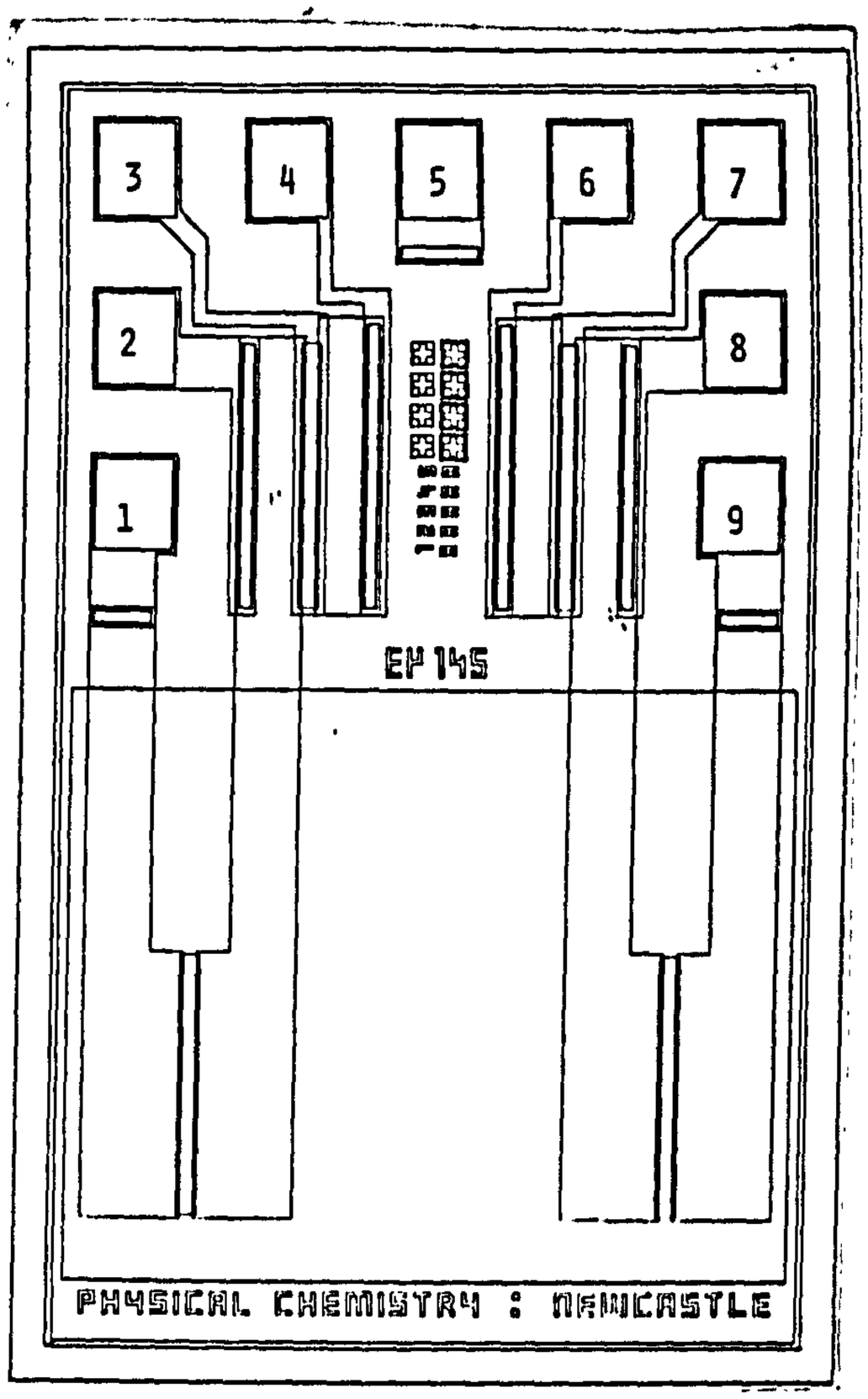
Figures in parenthesis indicate the percentage difference between the measured ions in serum and plasma.

not significant for values less than 1.

Table 5.10 - Activities of Sodium, Potassium and Calcium in Plasma and Serum using Pitzer and Hydration Model Values of Activity Co-efficients

		SODIUM						POTASSIUM				CALCIUM			
		CORNING		AVL		BECKM		CORNING		AVL		CORNING		AVL	
		PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD	PIT	HYD
Plasma	I	112.56	113.40	112.92	113.75	-	-	2.34	2.38	2.39	2.41	0.29	0.29	0.29	0.28
	II	117.11	117.98	118.03	118.90	-	-	2.29	2.31	2.34	2.37	0.40	0.39	0.41	0.40
	III	114.01	115.13	115.28	116.41	116.01	117.15	2.46	2.49	2.47	2.49	0.51	0.50	0.51	0.50
	IV	114.42	115.21	114.96	115.76	114.60	115.40	2.73	2.76	2.75	2.79	0.32	0.31	0.31	0.30
	V	108.52	109.59	107.06	108.12	108.35	109.42	2.83	2.87	2.78	2.81	0.33	0.33	0.32	0.32
	VI	115.11	115.92	115.48	115.29	115.48	116.29	2.30	2.33	2.34	2.36	0.40	0.39	0.40	0.39
Plasma + 25 mmoldm^{-3} NaCl	I	130.59	131.55	131.26	132.23	-	-	2.41	2.44	2.47	2.50	0.32	0.31	0.32	0.31
	II	135.59	136.60	138.22	139.25	-	-	2.43	2.46	2.47	2.49	0.45	0.44	0.47	0.46
	III	133.45	134.76	134.72	136.04	135.46	136.80	2.62	2.64	2.63	2.66	0.57	0.55	0.58	0.56
	IV	133.25	134.18	133.46	134.39	133.41	134.34	2.83	2.87	2.86	2.89	0.39	0.37	0.37	0.36
	V	127.64	128.89	126.40	127.61	127.62	128.84	3.01	3.05	2.88	2.92	0.40	0.39	0.39	0.38
	VI	135.59	136.54	134.78	135.72	134.24	135.17	2.72	2.44	2.46	2.49	0.46	0.45	0.46	0.45
Plasma 22 mmoldm^{-3} $NaHCO_3$	I	125.47	126.40	126.21	127.15	-	-	2.28	2.30	2.37	2.40	0.24	0.24	0.27	0.23
	II	129.77	130.73	133.07	134.05	-	-	2.32	2.34	2.39	2.41	0.39	0.39	0.37	0.36
	III	128.64	129.91	129.20	130.47	130.07	131.35	2.57	2.60	2.58	2.61	0.47	0.46	0.48	0.47
	IV	128.05	128.95	128.41	129.30	127.95	128.84	2.78	2.82	2.72	2.75	0.30	0.29	0.30	0.29
	V	123.48	124.70	122.87	124.05	123.95	125.15	2.91	2.94	2.79	2.82	0.29	0.29	0.27	0.26
	VI	131.14	132.06	127.72	128.61	129.22	130.11	2.27	2.31	2.32	2.35	0.34	0.33	0.35	0.34
Serum	IV	115.00	115.81	116.07	116.88	115.19	116.00	2.86	2.90	2.87	2.91	0.36	0.35	0.35	0.34
	VI	115.62	116.43	115.90	116.71	114.88	115.68	2.33	2.36	2.41	2.43	0.44	0.43	0.44	0.43

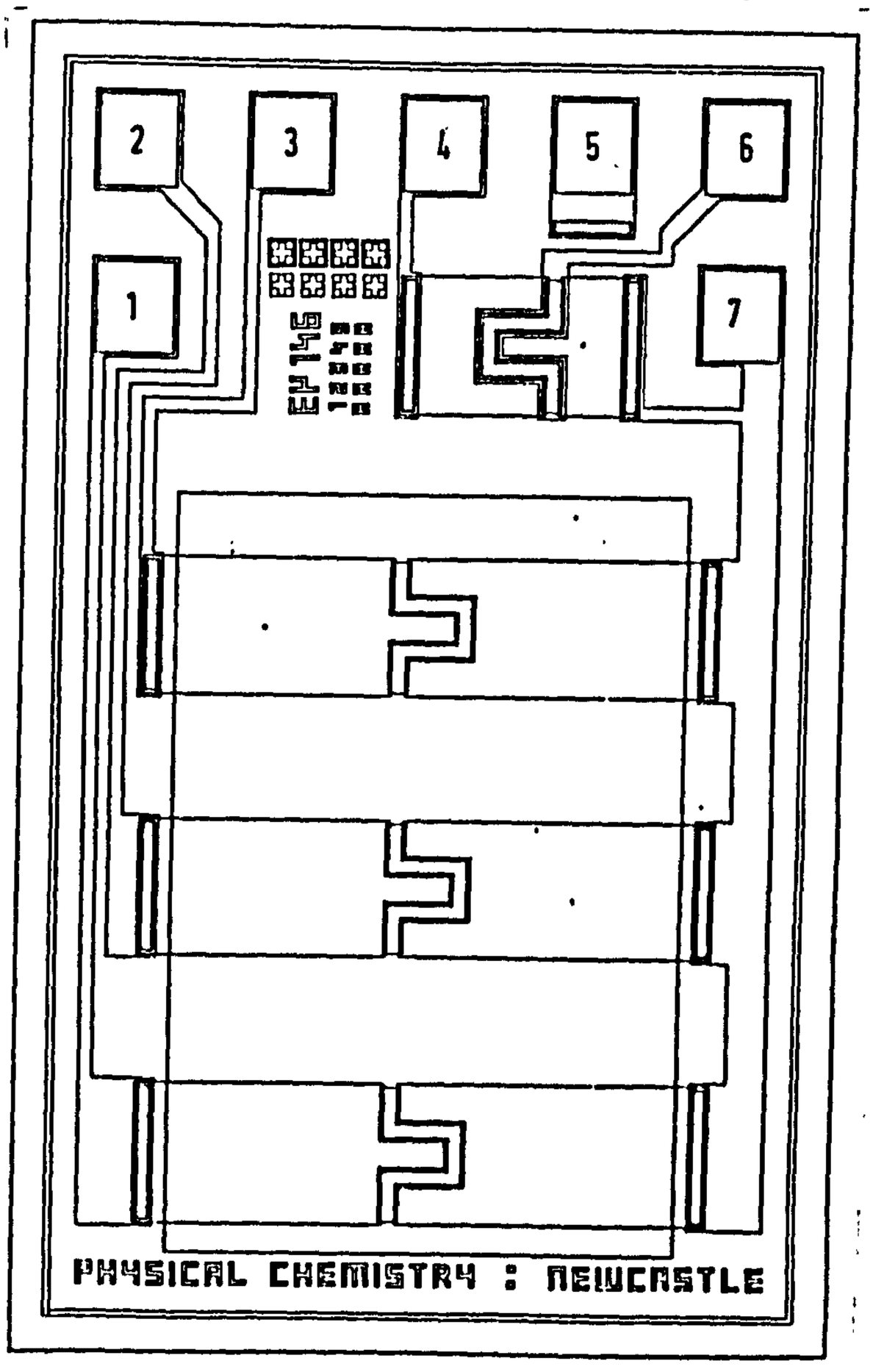
F I G U R E S



Eμ145 ISFET I.C. CHIP

1. ISFET A source
2. Common Drain A
3. IGFET A gate
4. IGFET A source
5. Bulk connection
6. IGFET B source
7. IGFET B gate
8. Common Drain B
9. ISFET B source

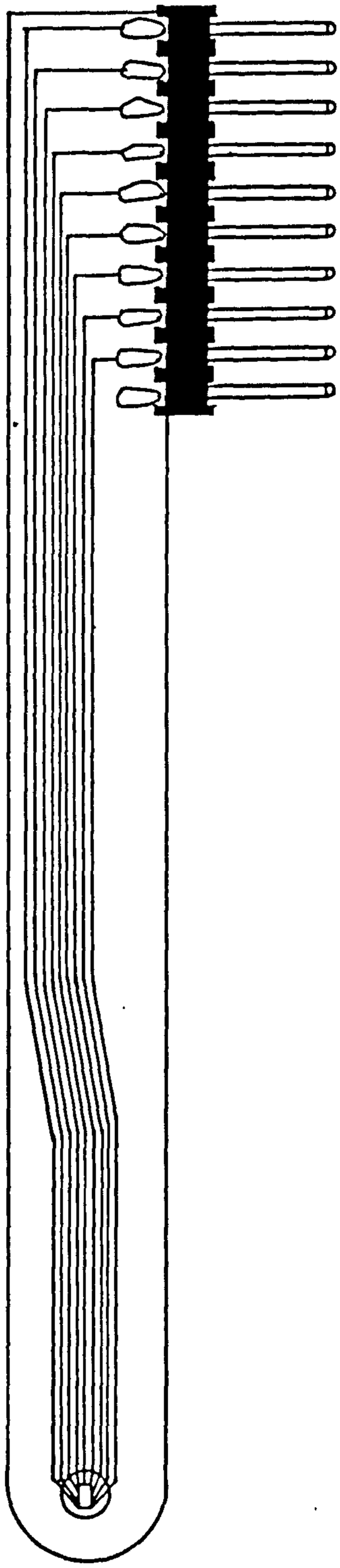
Fig. 5.1a



Eμ146 ISFET I.C. CHIP

1. ISFET A source
2. ISFET B source
3. ISFET C source
4. IGFET source
5. Bulk Connection
6. IGFET gate
7. Common Drain

Fig. 5.1b



N.C. = Not connected

EM146 Dip Type ISFET

Fig. 5.2

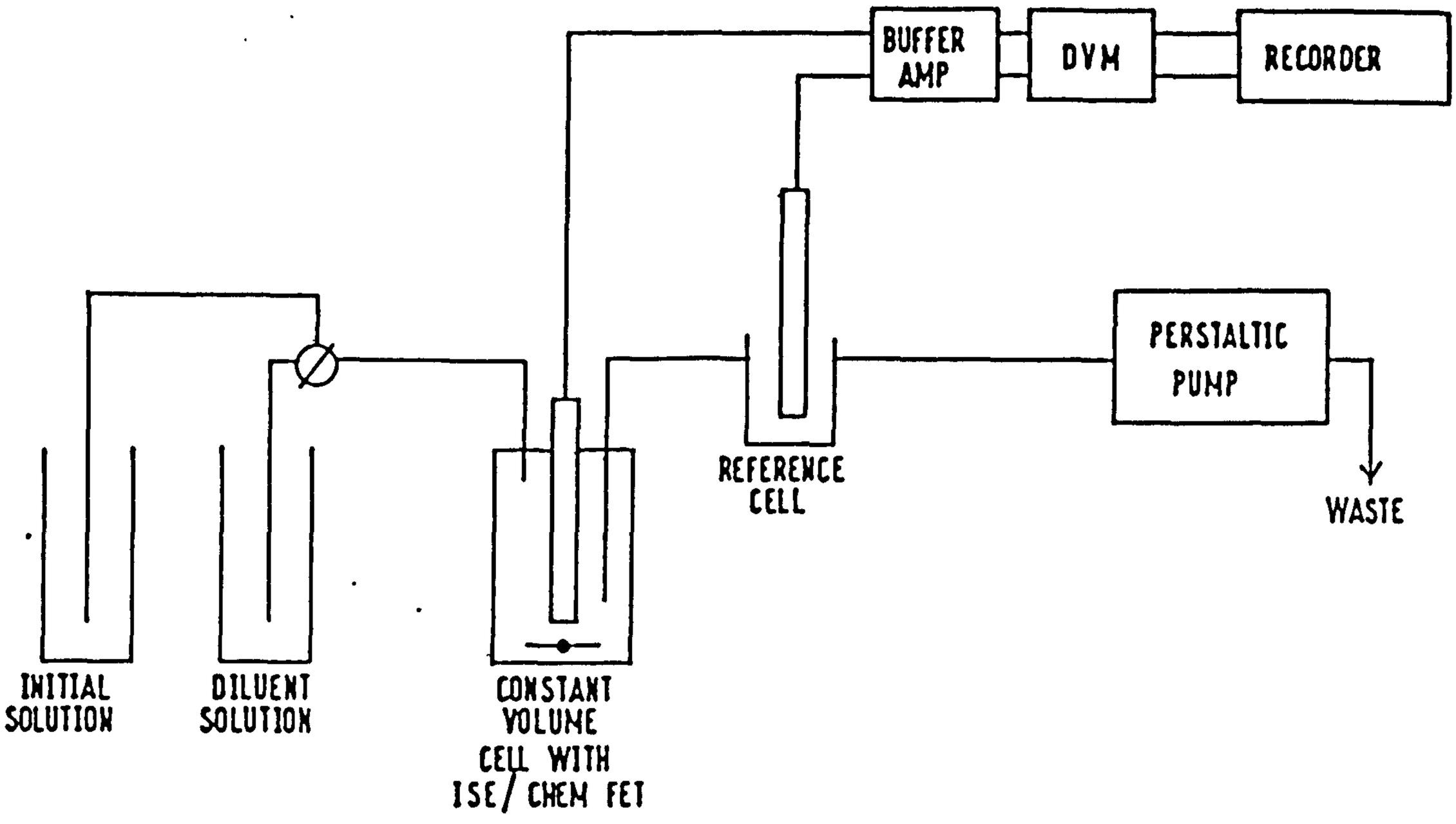


Fig. 5.3a

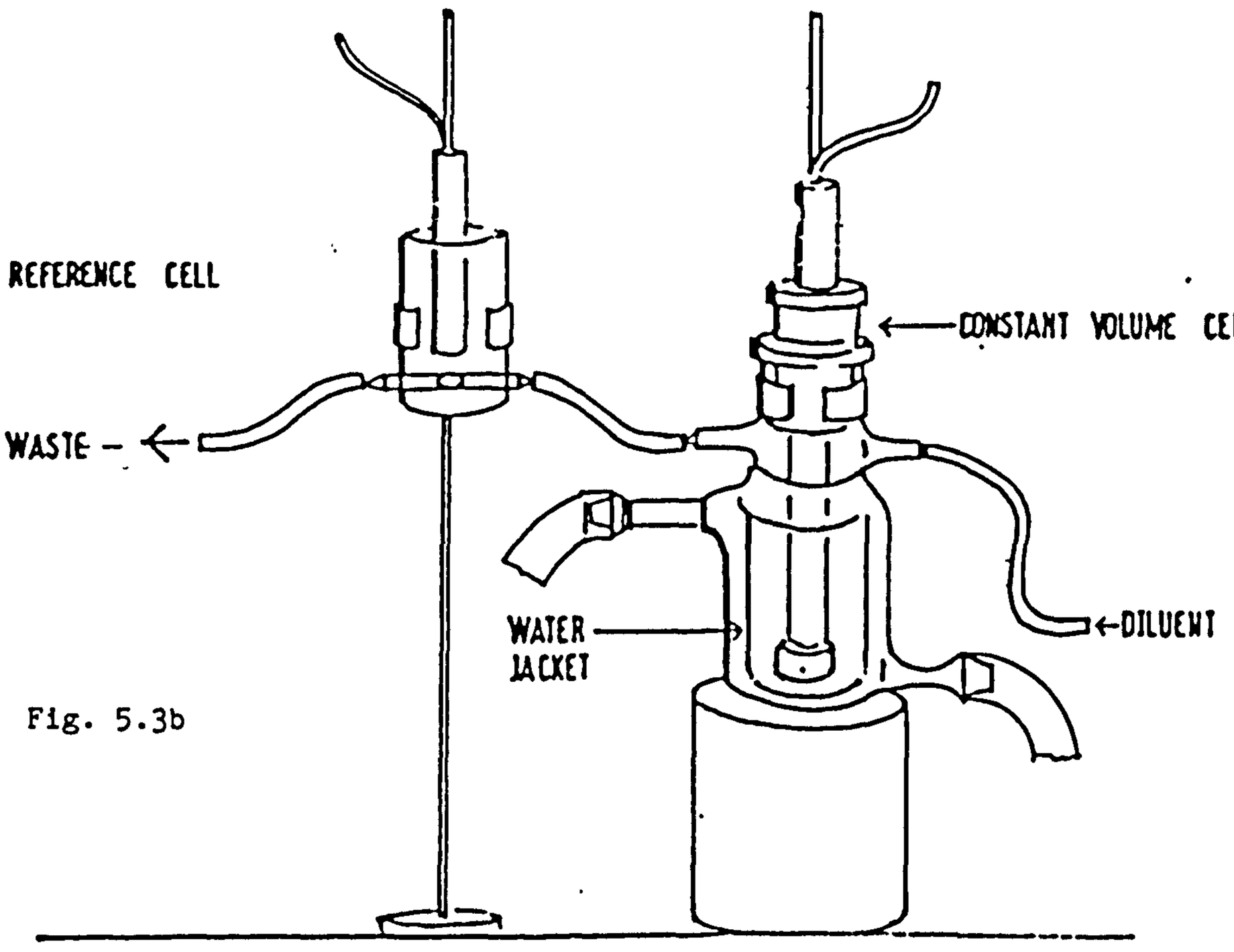


Fig. 5.3b

55/0-10/3T

AVL CALCIUM ISE

500 mVFSD
200 mm/hr.
T=21.5°C

AVL CALCIUM ISE (FLOW THROUGH)

W = 1.76 mL/min
Vt = 15.8 mL
DISTANCE FOR 1 DECADE CHANGE = 6.9 cm
SLOPE AT CLINICAL RANGE = 27.5 mV APPROX.

500 mV FSD
200 mm/hr
T = 21.5 °C
Curve I = 0.1 mol dm^{-3} $CaCl_2$ + 0.1 mol dm^{-3} NaCl
Curve II = 0.1 mol dm^{-3} $CaCl_2$.

0.1M $CaCl_2$ in 0.1M NaCl background

0.1M $CaCl_2$

FIG. 5.4

AVL SODIUM ISE (FLOW THROUGH)

W = 2.82 mL/min
Vt = 15.8 mL
DISTANCE FOR 1 DECADE CHANGE = 4.3 cm
SLOPE AT CLINICAL RANGE = 55.7 mV

500 mV FSD
200 mm/hr
T = 19.5 °C

50mV

FIG. 5.5

AVL POTASSIUM ISE (FLOW THROUGH)

W = 3.13 ml/min
Vr = 15.8 ml
DISTANCE FOR 1 DECADE CHANGE = 3.88 cm
SLOPE AT CLINICAL RANGE = 58 mV APPROX.

500 mV FSD
200 mm/hr
T = 17 °C

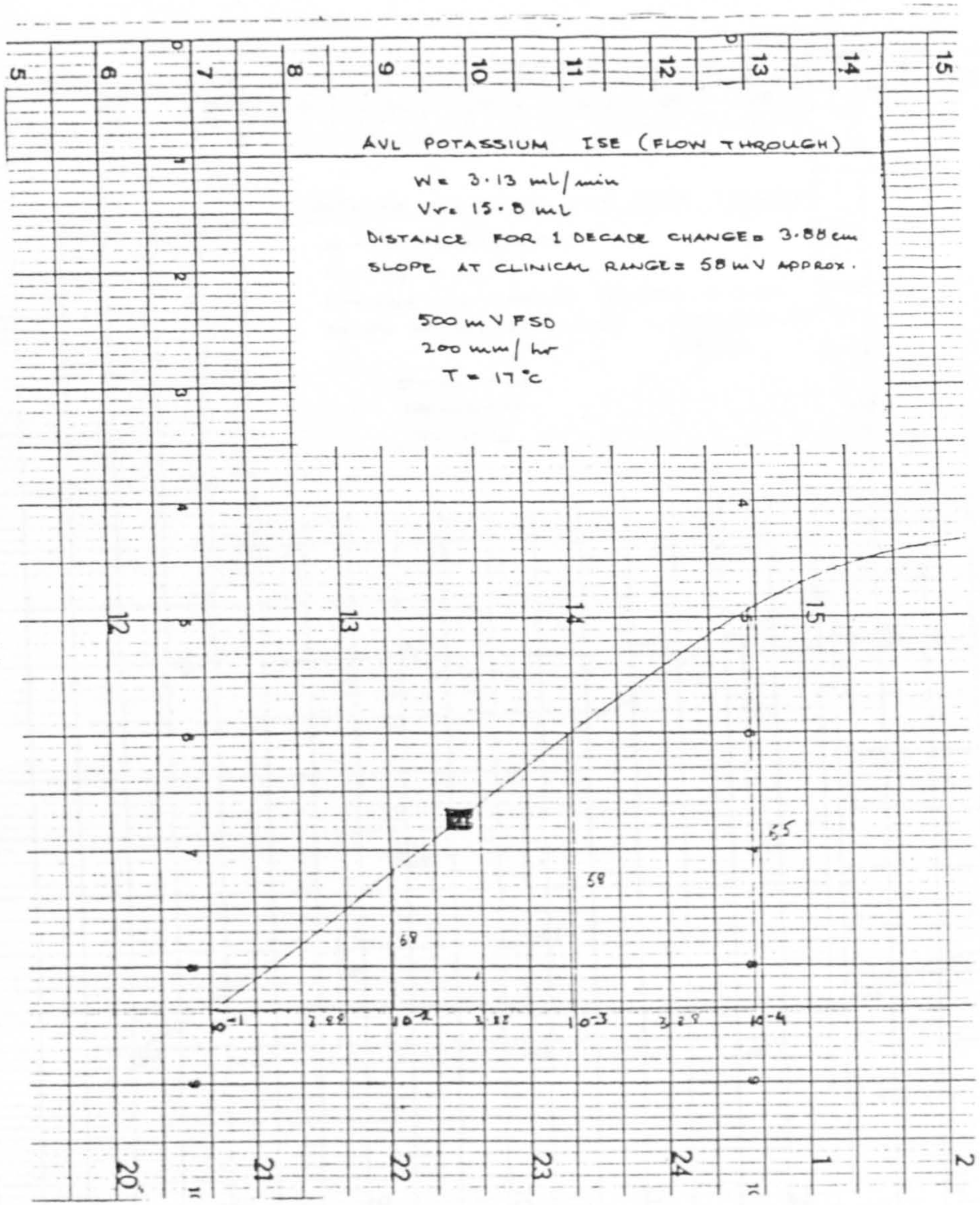


FIG. 5.6

BECKMAN SODIUM ISE (FLOW THROUGH)

W = 4.17 ml/min
Vr = 15.8 ml
DISTANCE FOR 1 DECADE CHANGE = 2.9 cm
SLOPE AT THE CLINICAL RANGE = 59.2 mV APPROX.

500 mV FSD
200 mm/hr
T = 19 °C

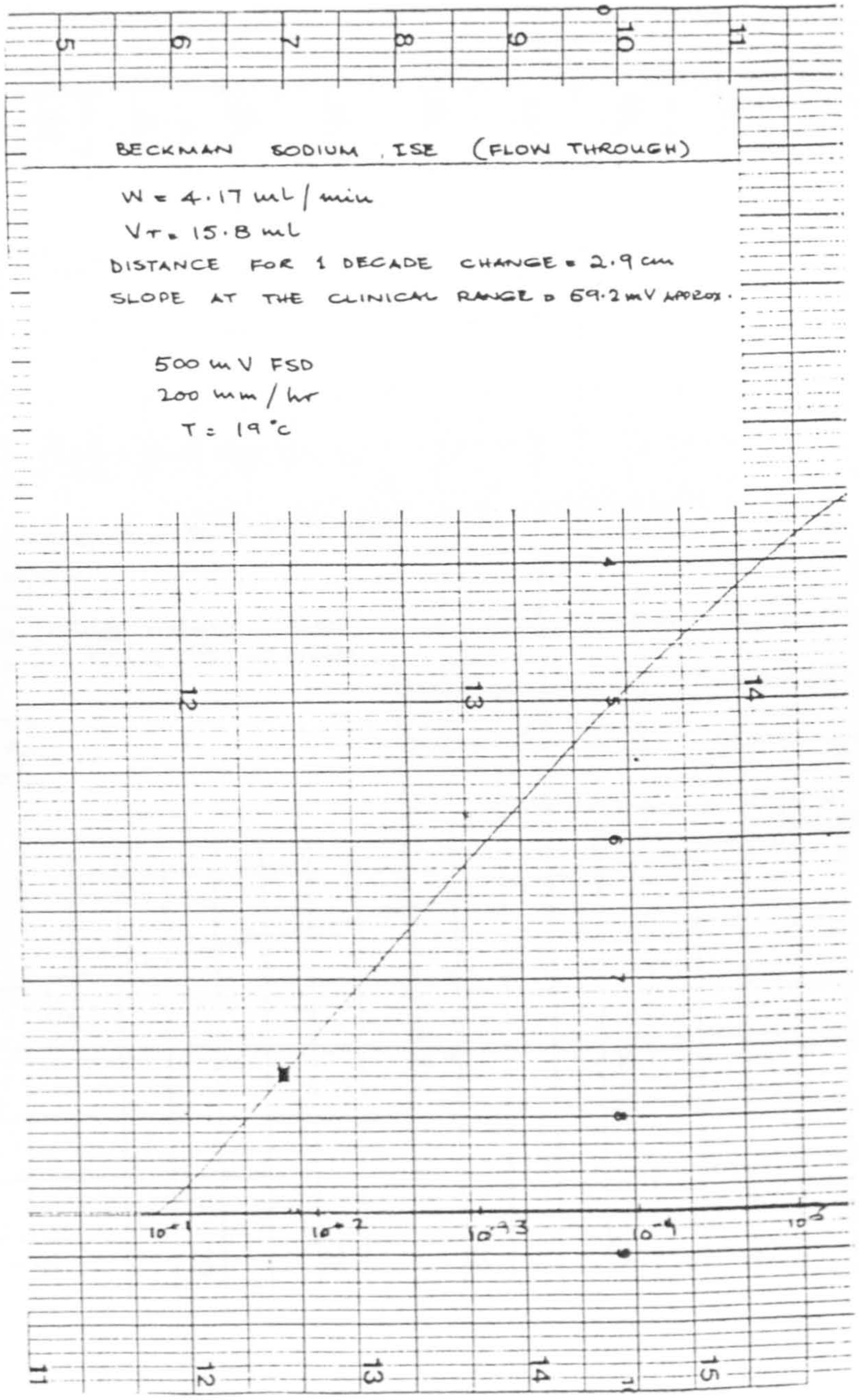


FIG. 5.7

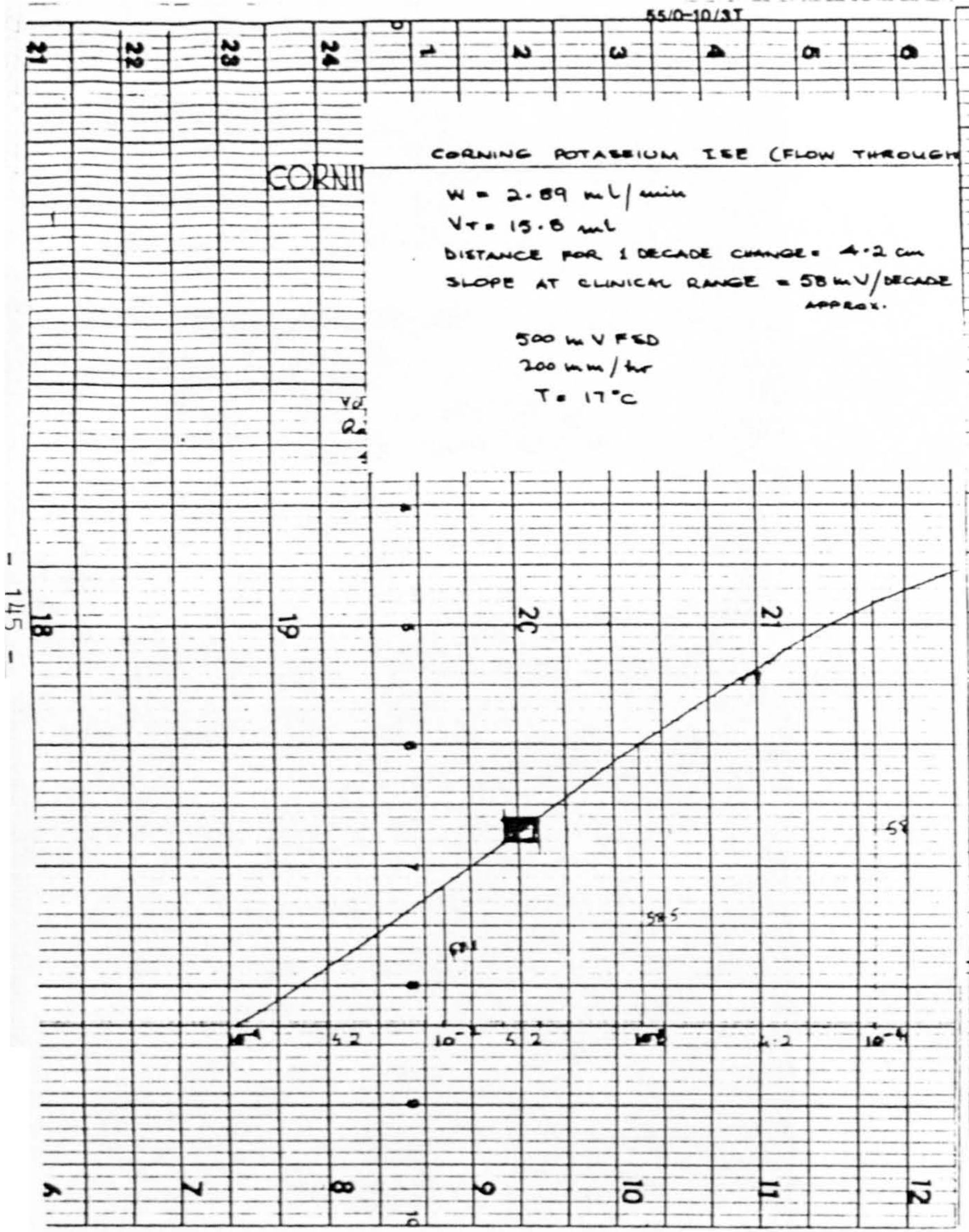

55/0-10/3T
CORNII
CORNING POTASSIUM ISE (FLOW THROUGH
W = 2·89 ml/min
Vt = 15·8 ml
DISTANCE FOR 1 DECADE CHANGE = 4·2 cm
SLOPE AT CLINICAL RANGE = 58 mV/DECADE APPROX.
500 mV FSD
200 mm/hr
T = 17°C
58
58·5
4·2
5·2

FIG. 5.8

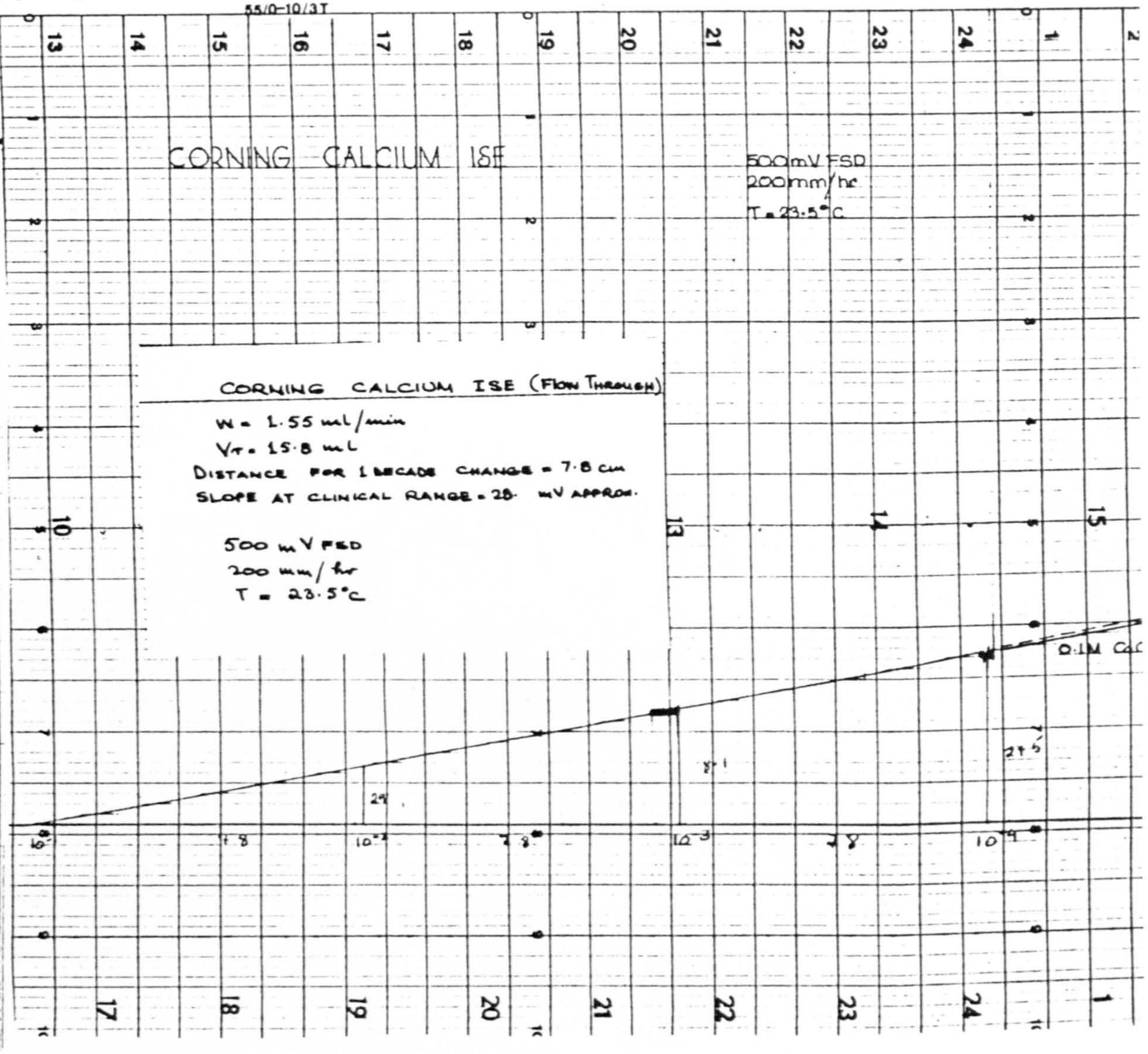

55/0-10/3T
CORNING CALCIUM ISE
500 mV FSD
200 mm/hr
T = 23·5°C
CORNING CALCIUM ISE (FLOW THROUGH)
W = 1·55 ml/min
Vt = 15·8 ml
DISTANCE FOR 1 DECADE CHANGE = 7·8 cm
SLOPE AT CLINICAL RANGE = 28· mV APPROX.
500 mV FSD
200 mm/hr
T = 23·5°C
0·1M Ca
29
7·8

FIG. 5.9

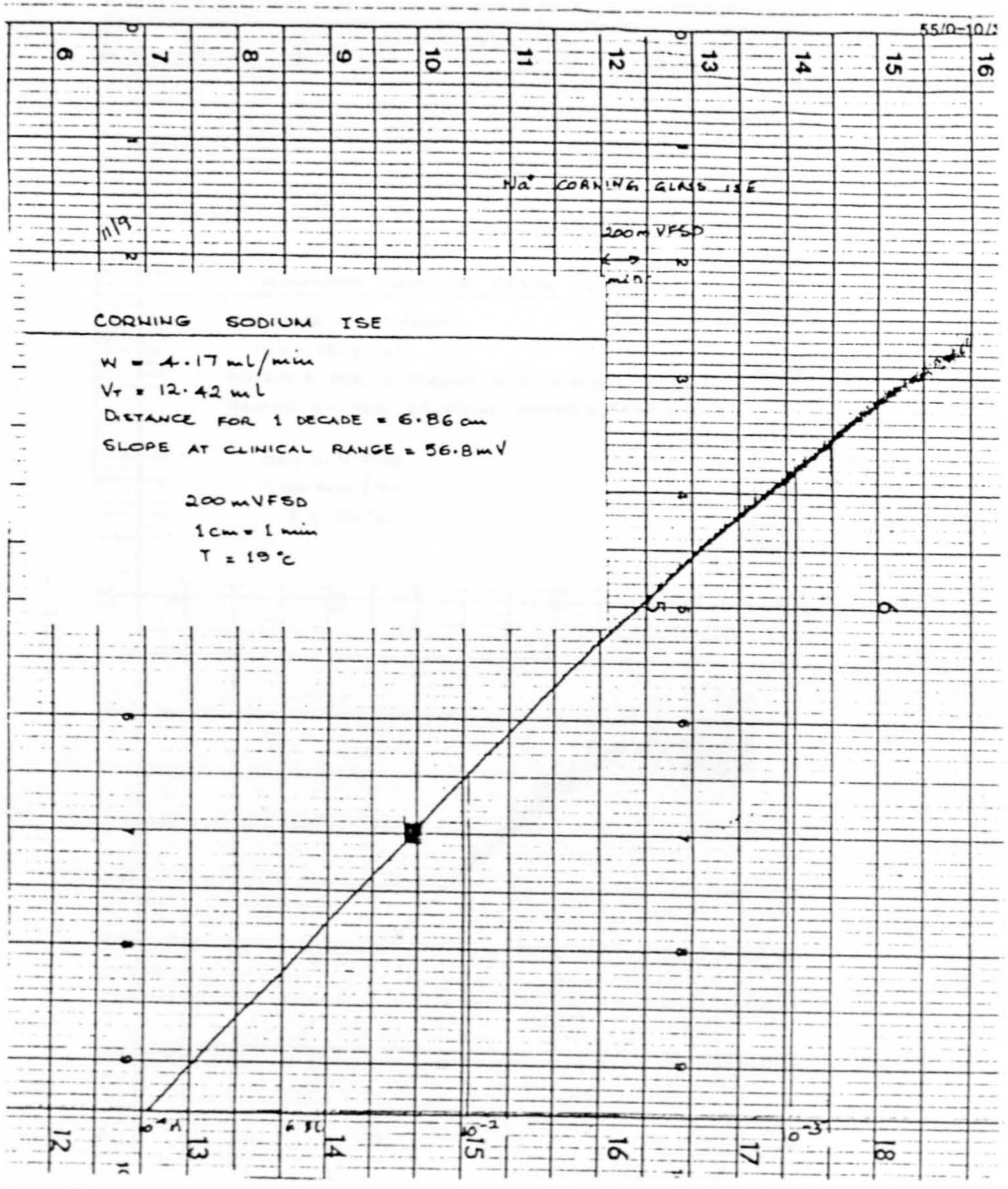


FIG. 5.10

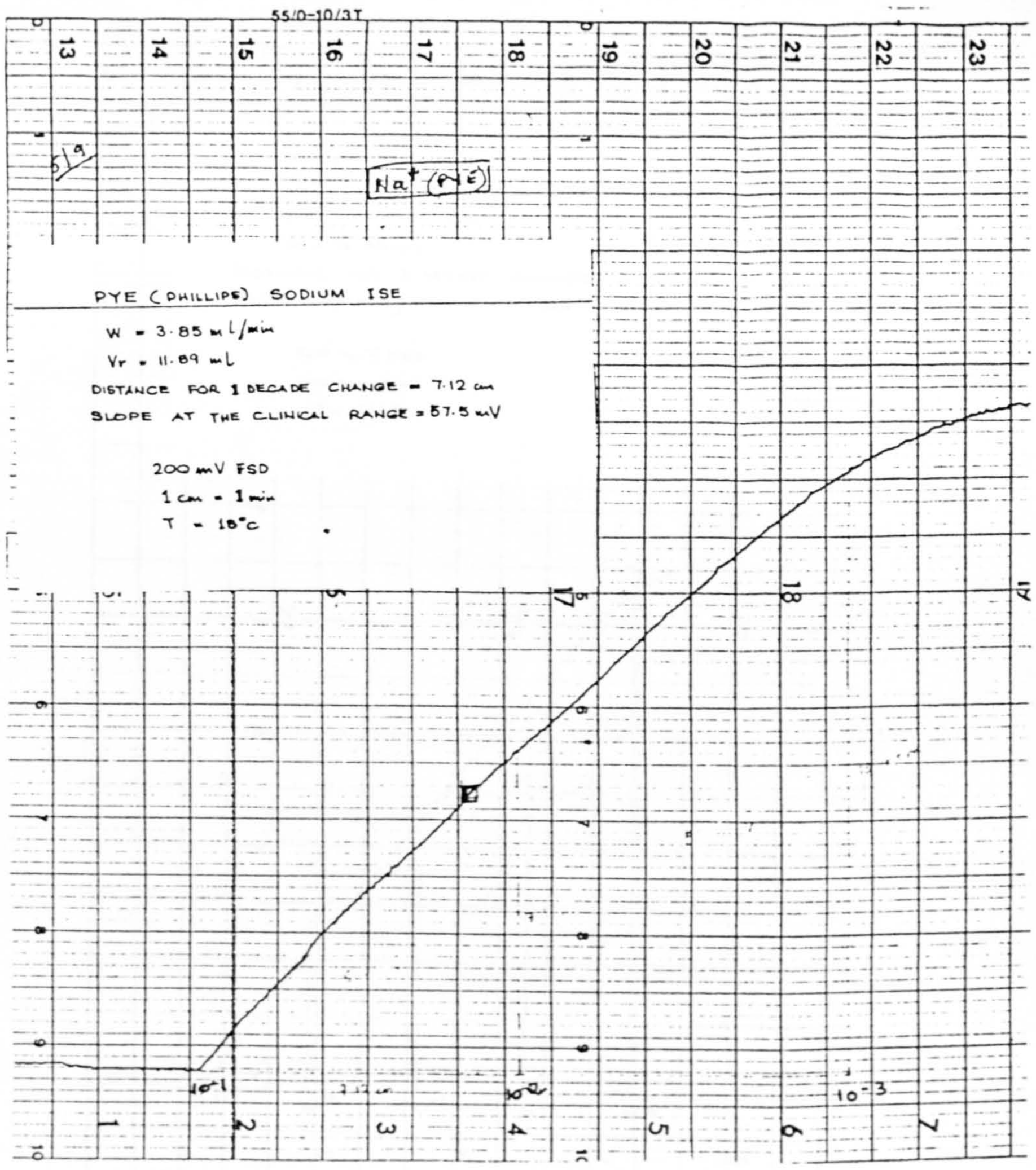


FIG. 5.11

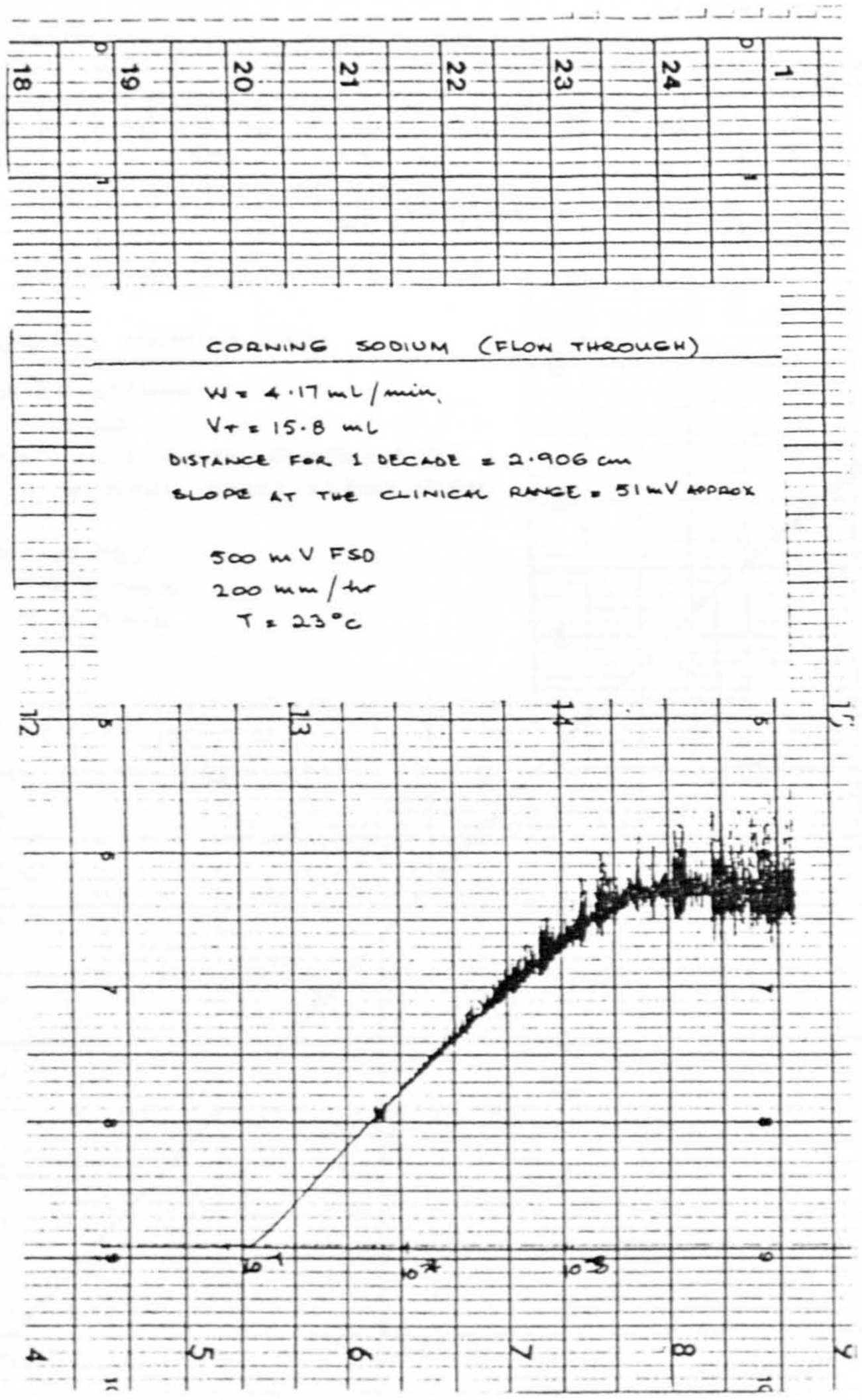


FIG. 5.12

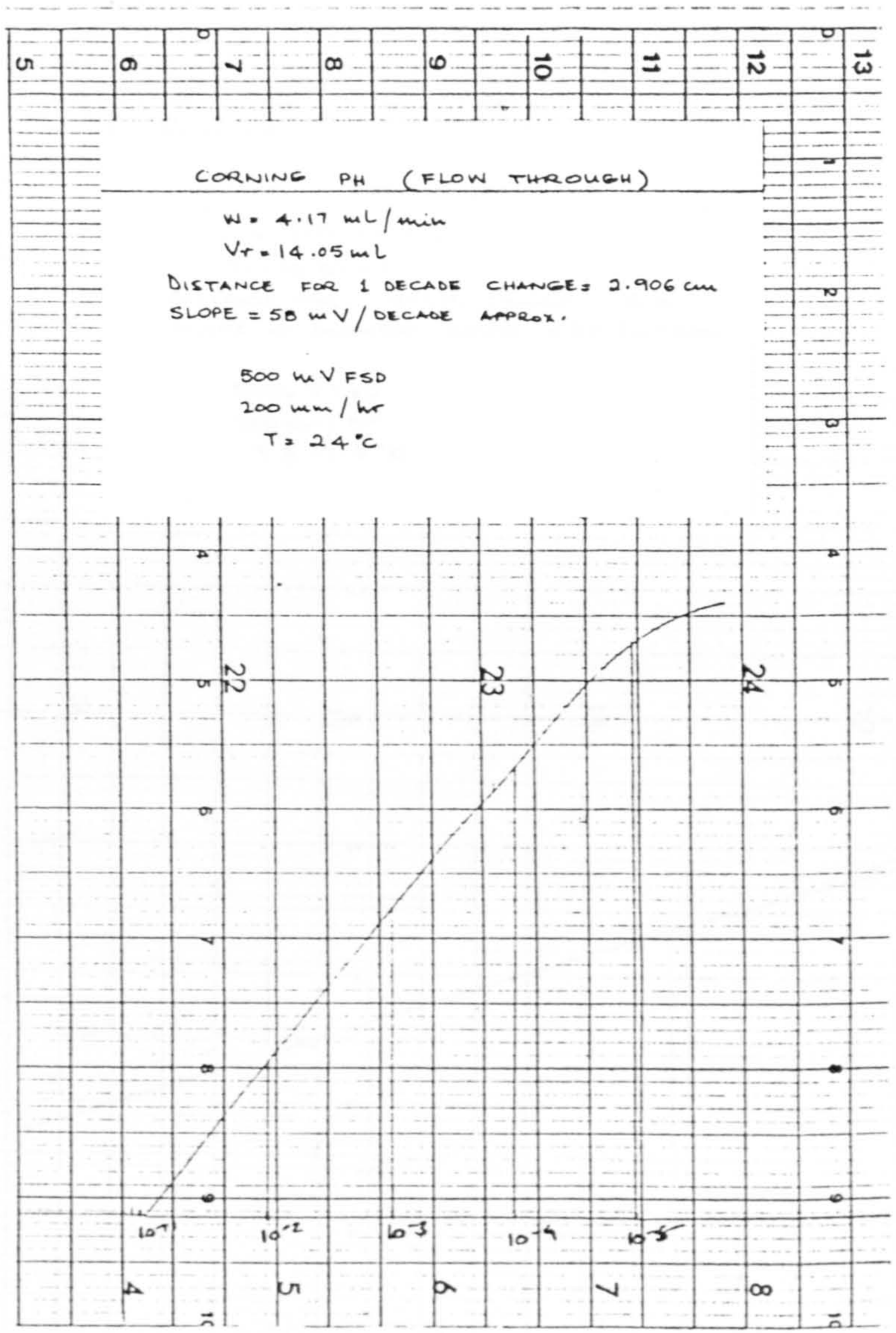
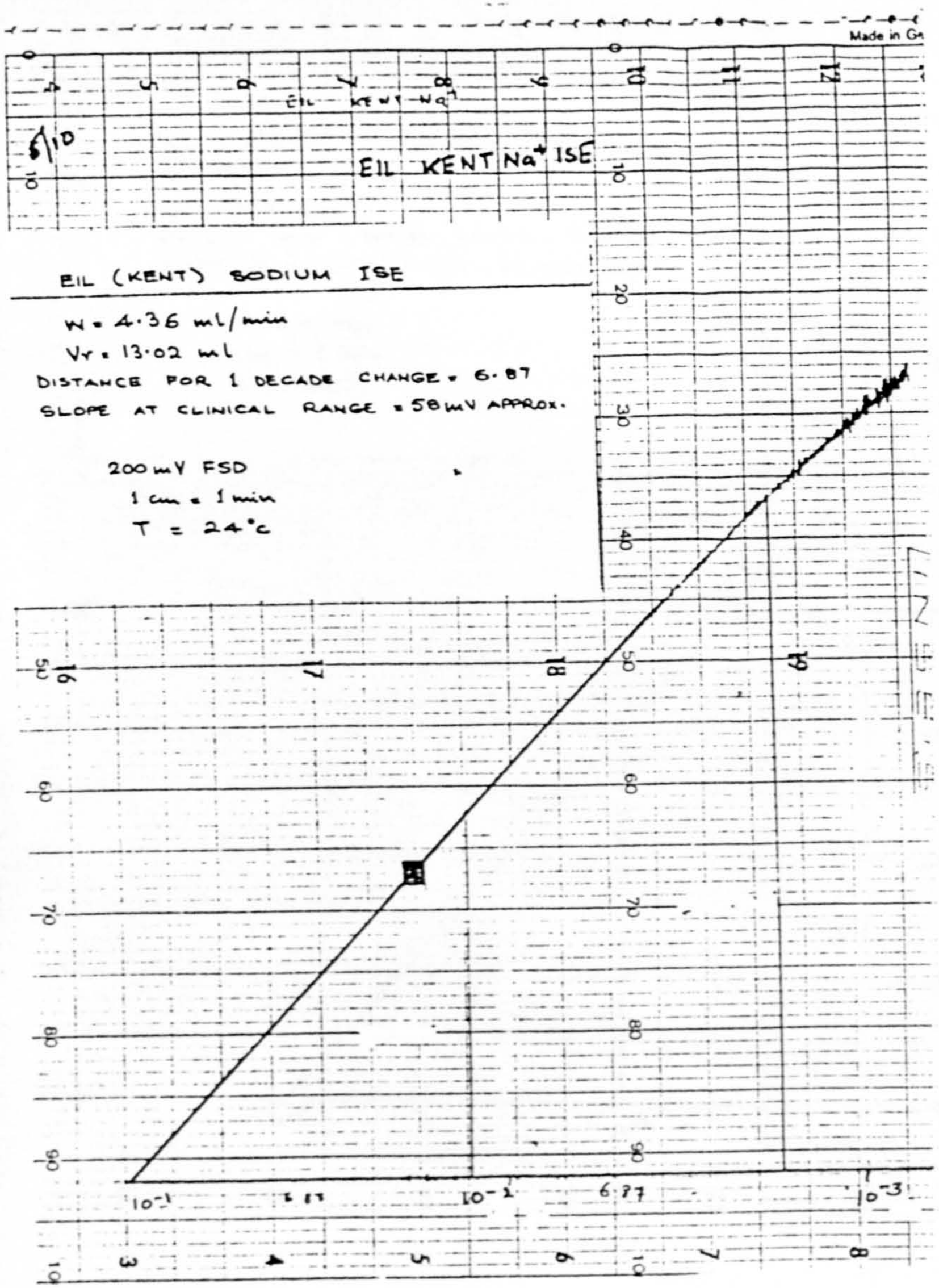
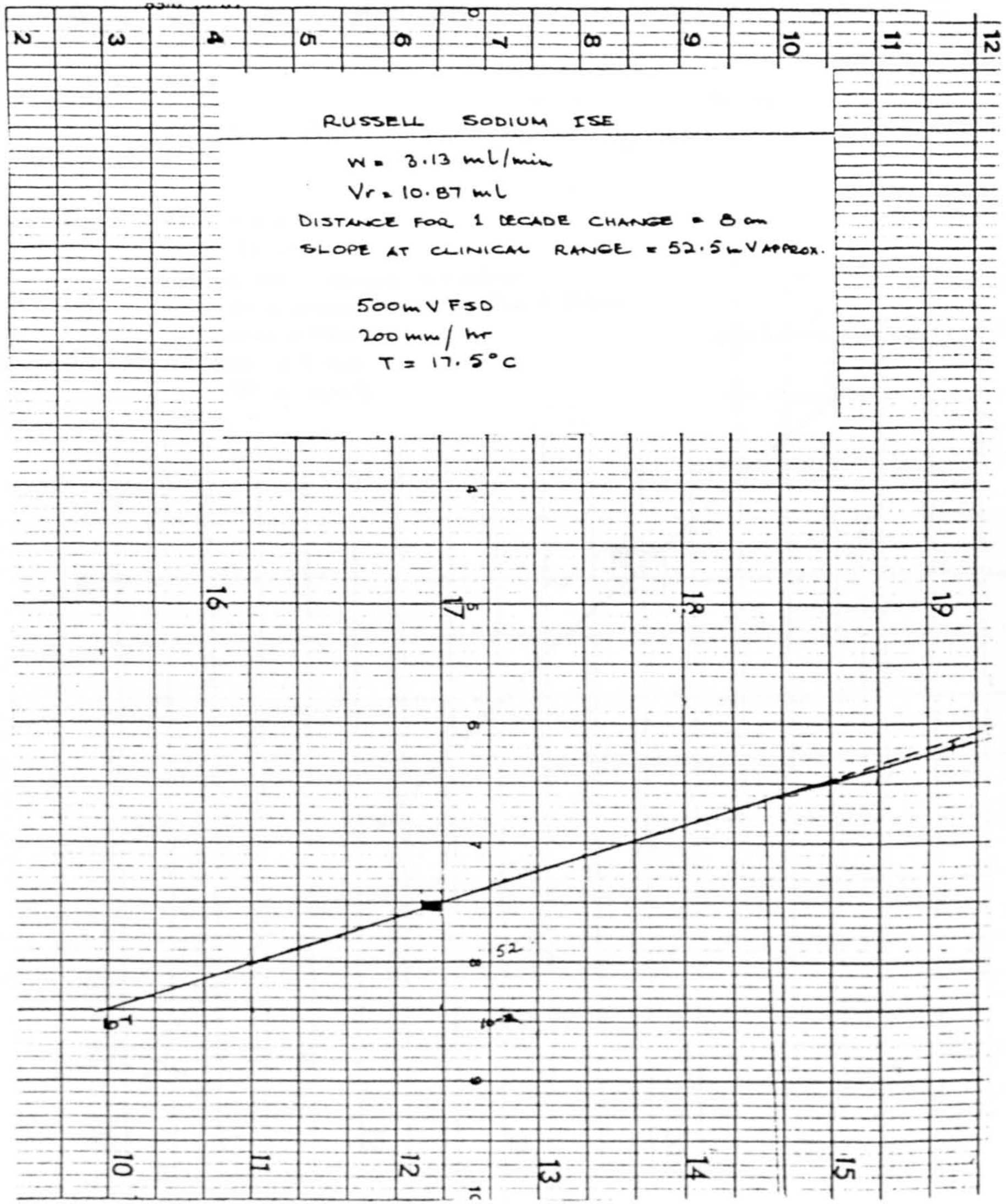


FIG. 5.13


EIL KENT Na+ ISE
EIL (KENT) SODIUM ISE
W = 4.36 ml/min
Vr = 13.02 ml
DISTANCE FOR 1 DECADE CHANGE = 6.87
SLOPE AT CLINICAL RANGE = 58mV APPROX.
200 mV FSD
1 cm = 1 min
T = 24°C


RUSSELL SODIUM ISE
W = 3.13 ml/min
Vr = 10.87 ml
DISTANCE FOR 1 DECADE CHANGE = 8 cm
SLOPE AT CLINICAL RANGE = 52.5 mV APPROX.
500mV FSD
200 mm/hr
T = 17.5°C

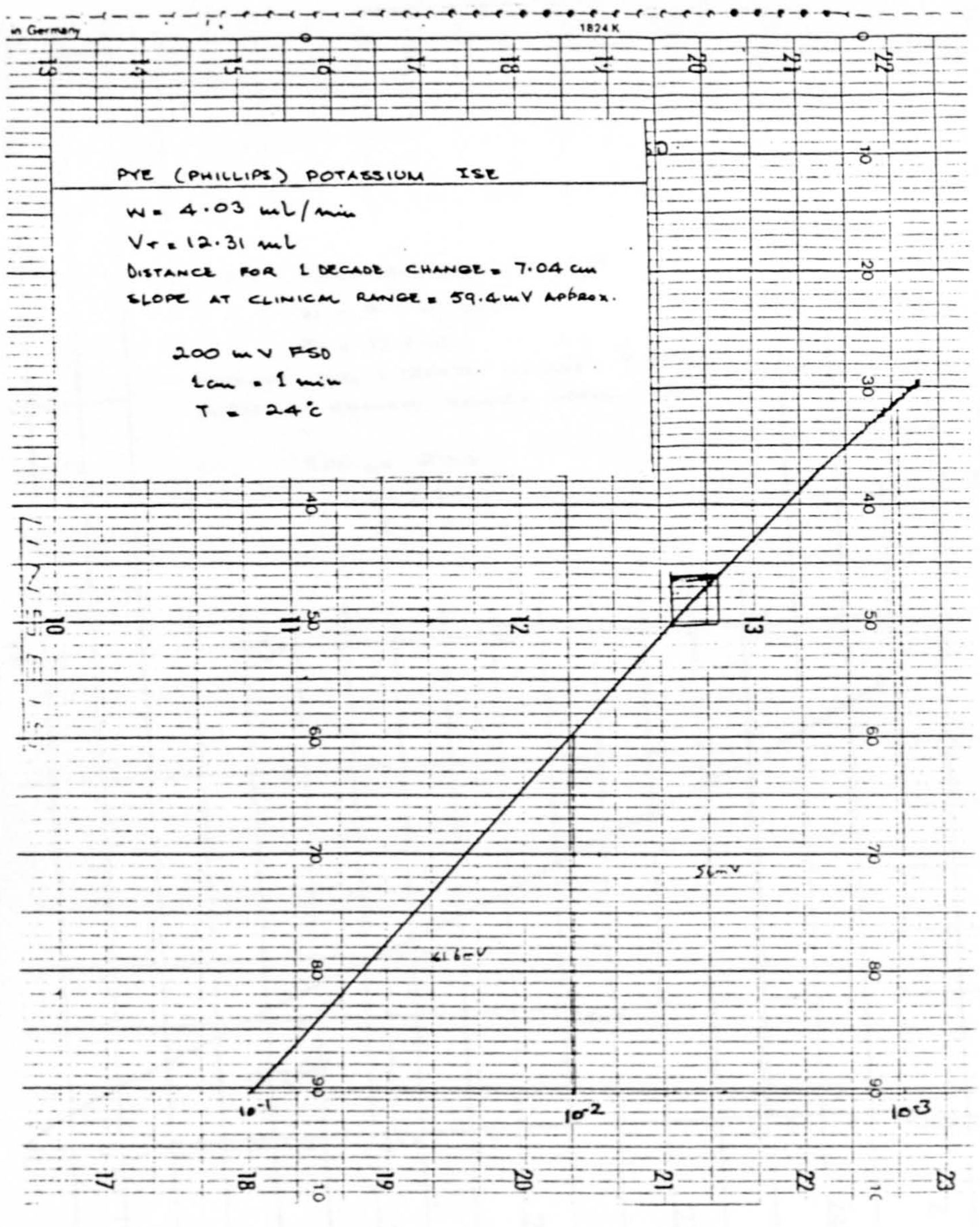


FIG. 5.16

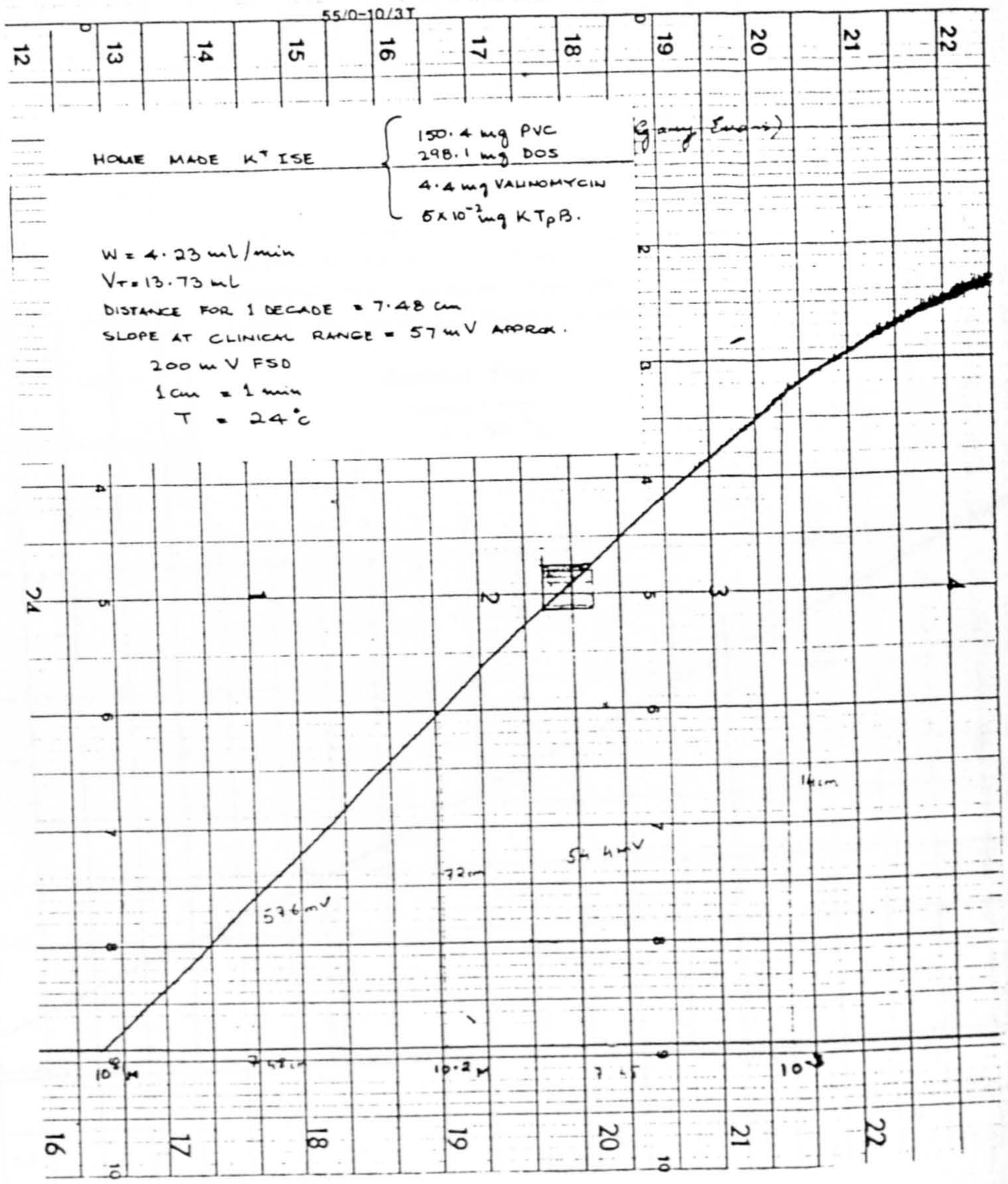


FIG. 5.17

RADIOMETER CALCIUM ISE

RATE OF FLOW = 4.11 ml/min

VOLUME OF CELL = 14.9 ml

DISTANCE FOR 1 DECADE CHANGE = 8.35 cm

SLOPE AT CLINICAL RANGE = APPROX. 32 mV

200 mV FSD

1 cm = 1 min

T = 37 °C

PYE (PHILLIPS) CALCIUM ISE

W = 4.11 ml/min

V_T = 13.2 ml

DISTANCE FOR 1 DECADE CHANGE = 7.41 cm

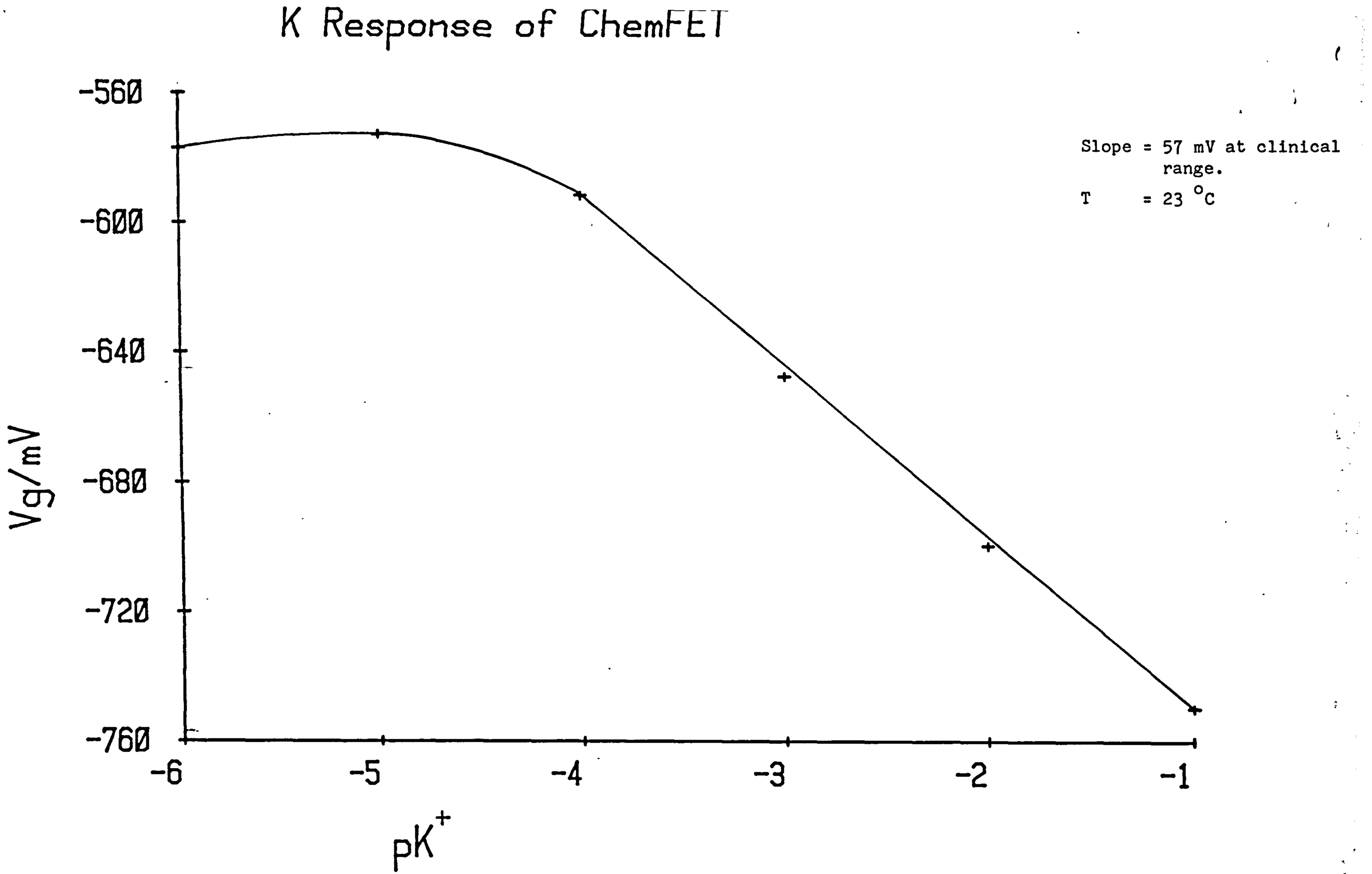
SLOPE AT CLINICAL RANGE = APPROX. 28 mV

200 mV FSD

1 cm = 1 min

T = 20 °C

K Response of ChemFET



Slope = 57 mV at clinical range.

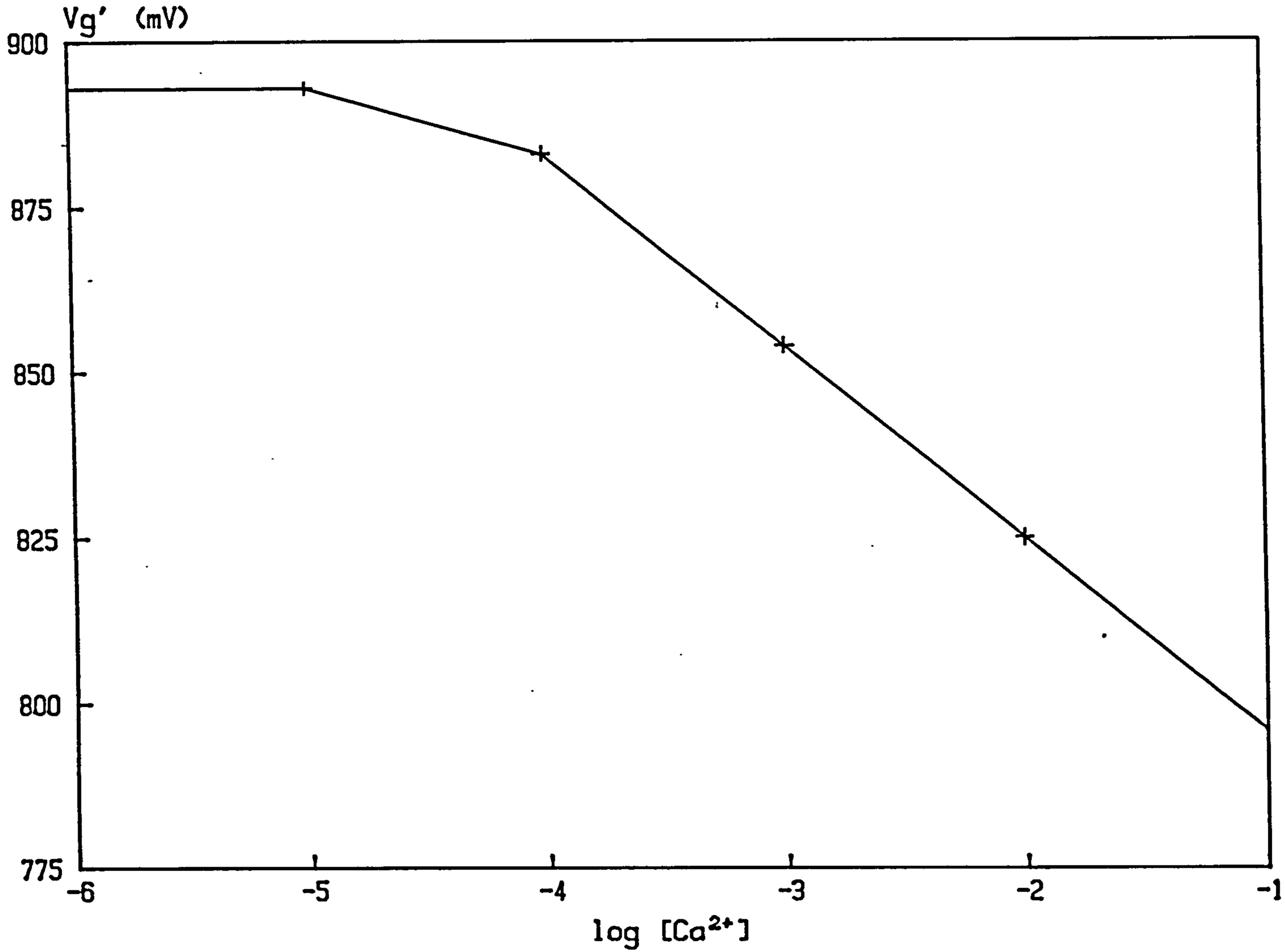
T = 23 °C

FIG. 5.20

CALCIUM-RESPONSIVE ChemFET

Eμ146: Vds = +1 V; Id = 100 μA

Slope - 28.5 mV at Clinical Range
Temp - 22 °C



pH-RESPONSIVE ChemFET

Eμ146: Vds = +1 V; Id = 100 μA

Slope = 53.6 mV
T = 22 °C

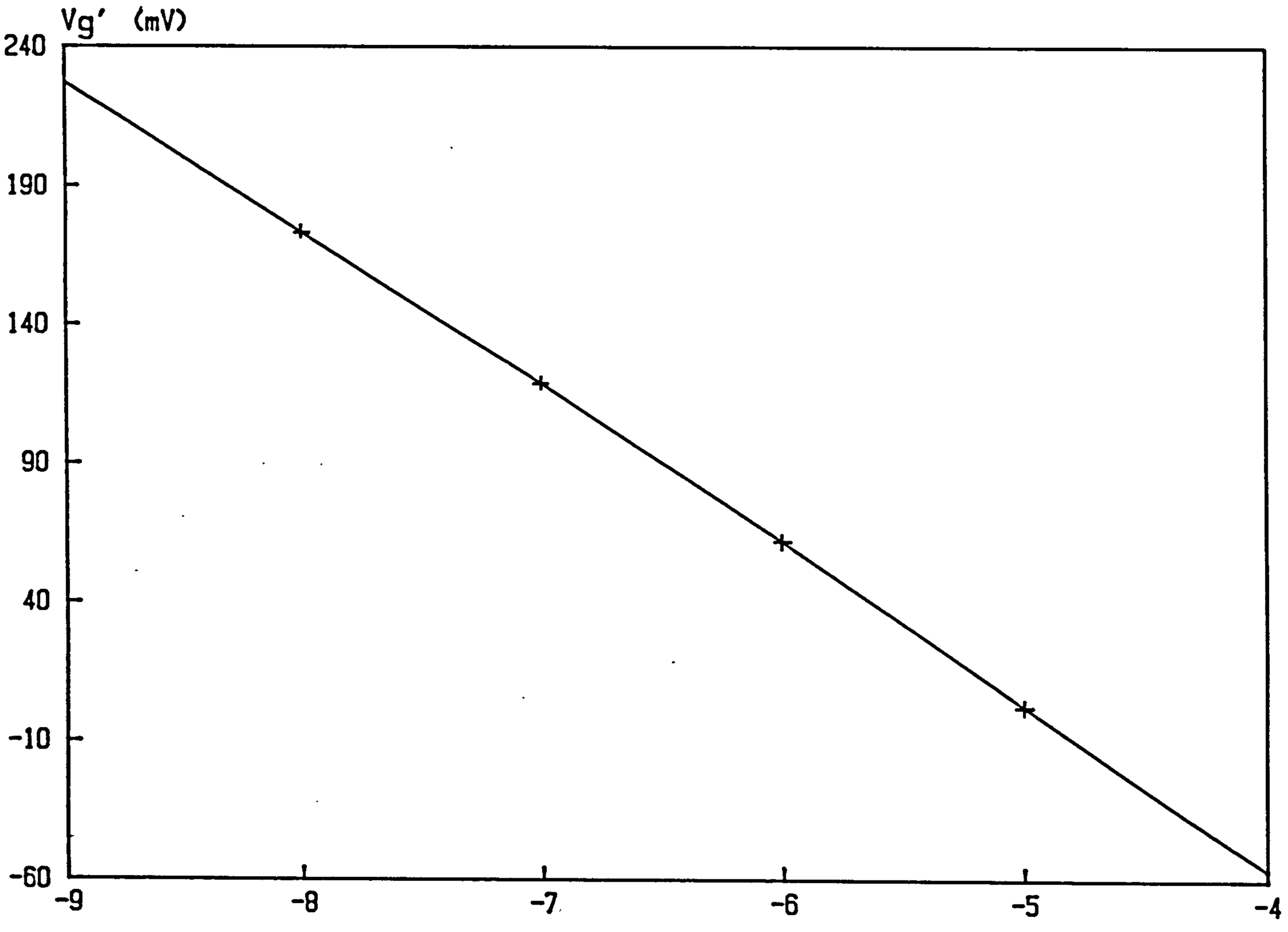


Fig. 5.22

TRIPLE-FUNCTION ChemFET

Eu146 DEVICE

Slope = 46 mV
Temp = 19 °C

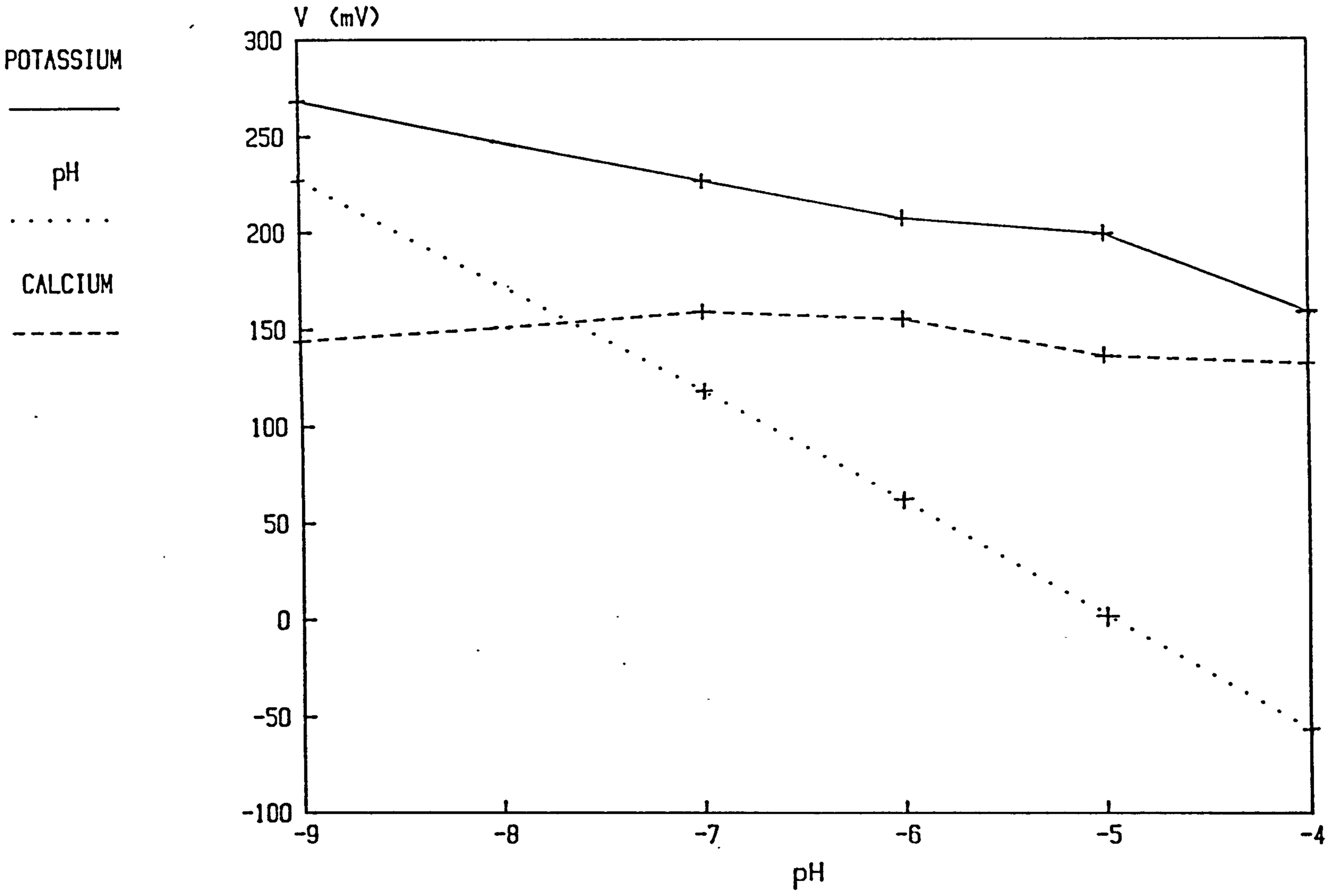


Fig. 5.23

TRIPLE-FUNCTION ChemFET

Eu146 DEVICE

Slope = 24 mV
Temp = 19 °C

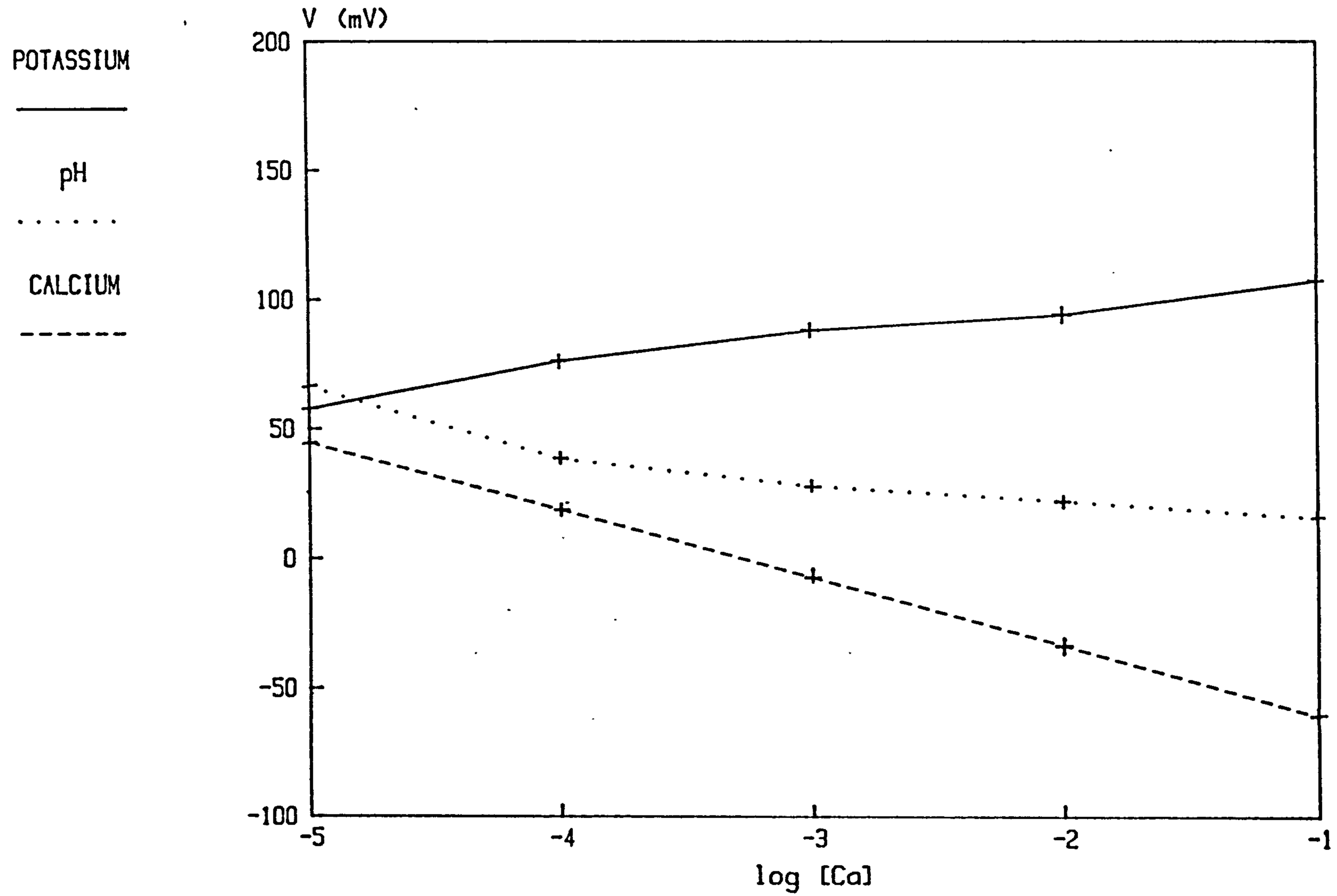


Fig. 5.24

TRIPLE-FUNCTION ChemFET

Eu146 DEVICE

Slope = 43.8 mV
Temp = 19 °C

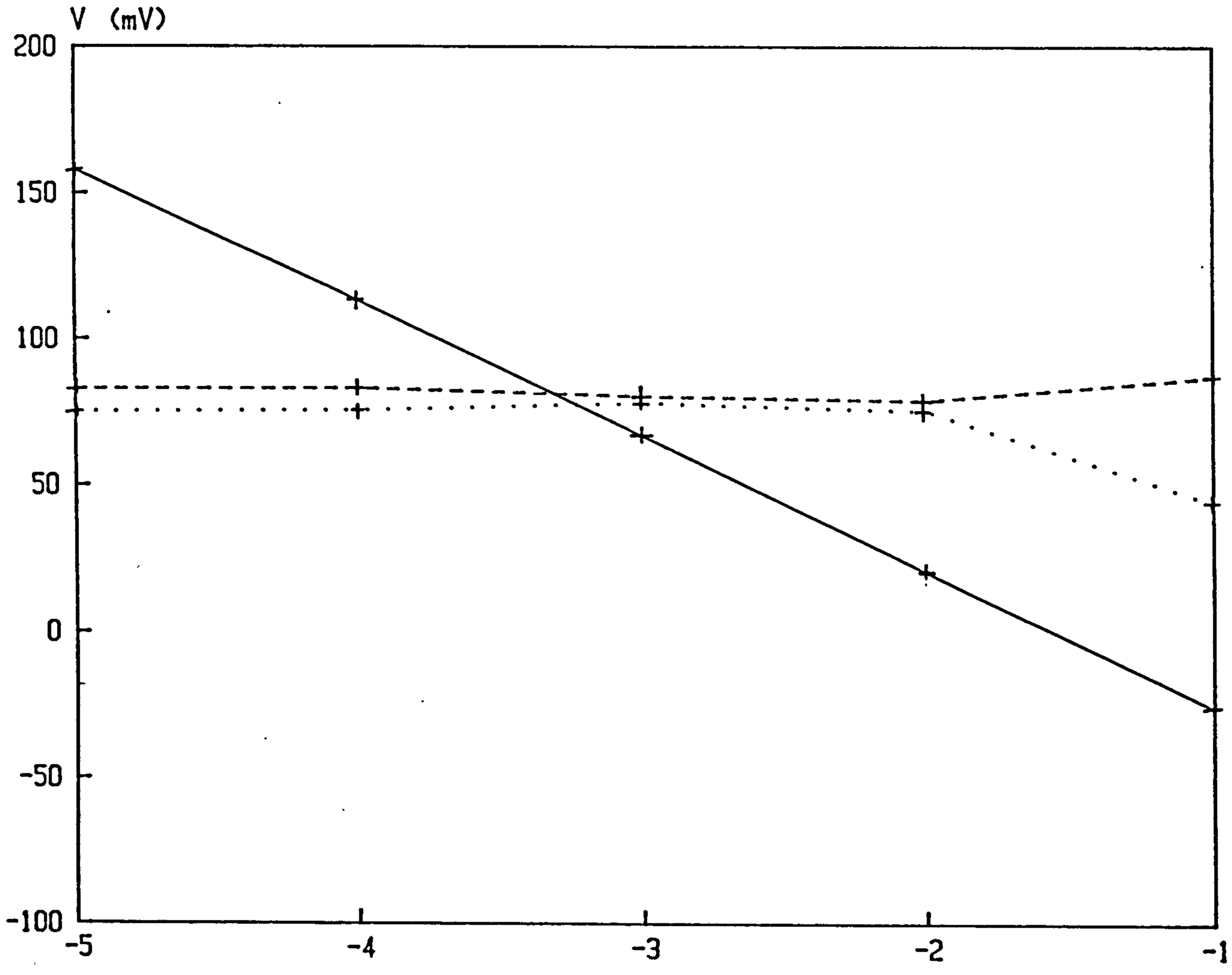


Fig. 5.25

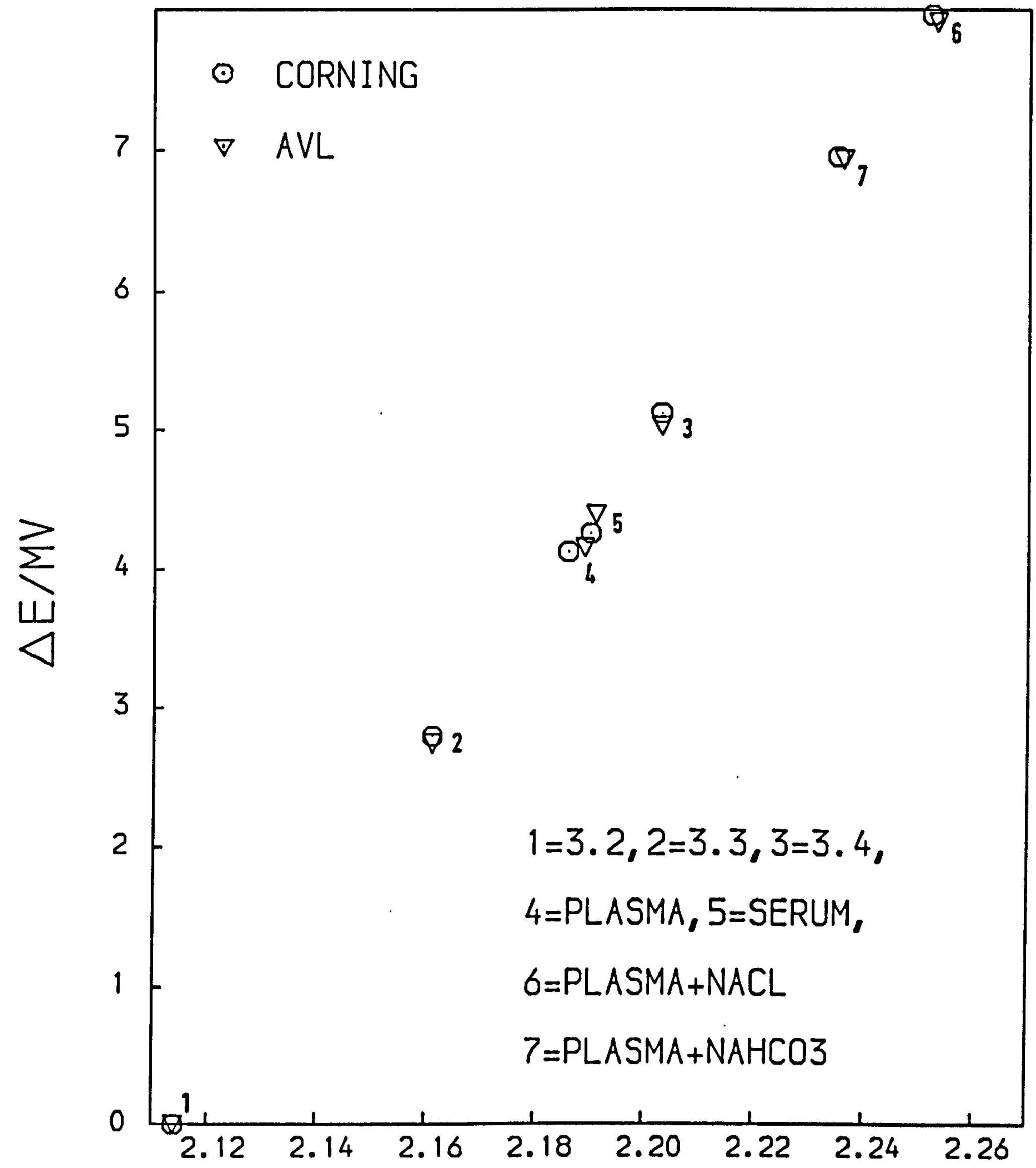
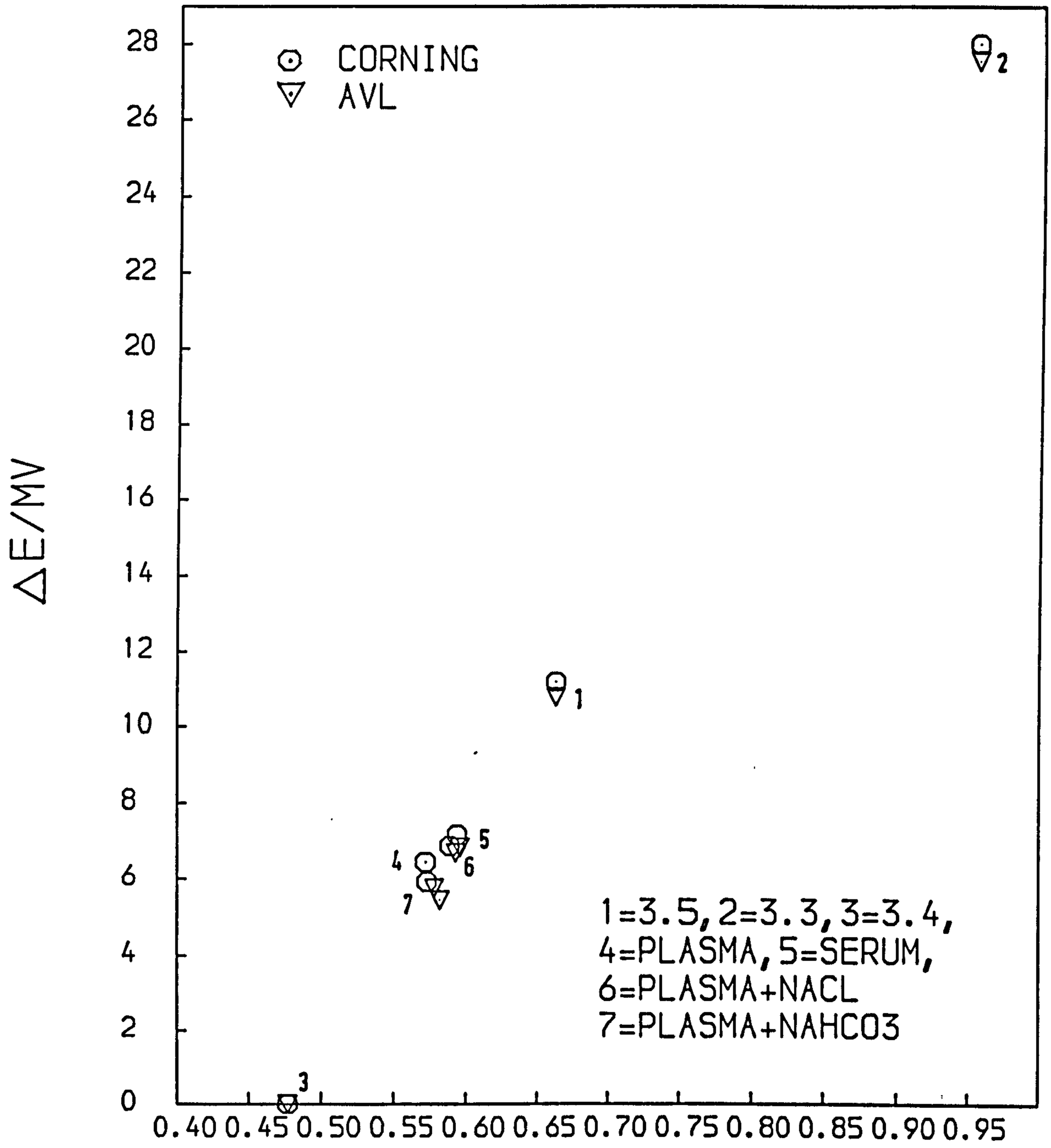

CORNING
AVL
ΔE/MV
7
6
5
4
3
2
1
0
2.12
2.14
2.16
2.18
2.20
2.22
2.24
2.26
1
2
3
4
5
6
7
1=3.2, 2=3.3, 3=3.4,
4=PLASMA, 5=SERUM,
6=PLASMA+NACL
7=PLASMA+NAHCO3
LOG[NA]
PLASMAIV SODIUM

FIG. 5.26



LOG[K]
PLASMAIV POTASSIUM

FIG. 5.27

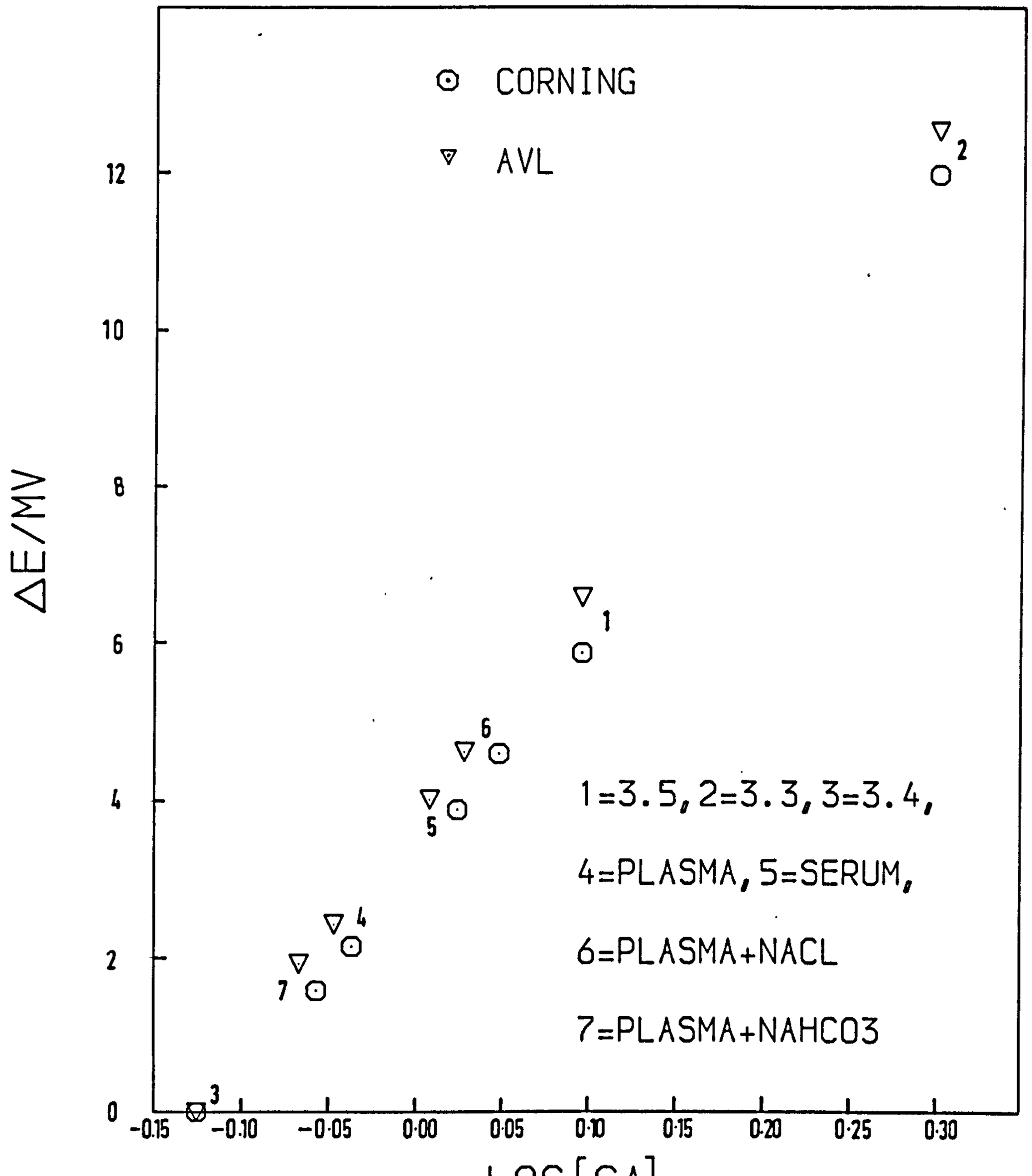
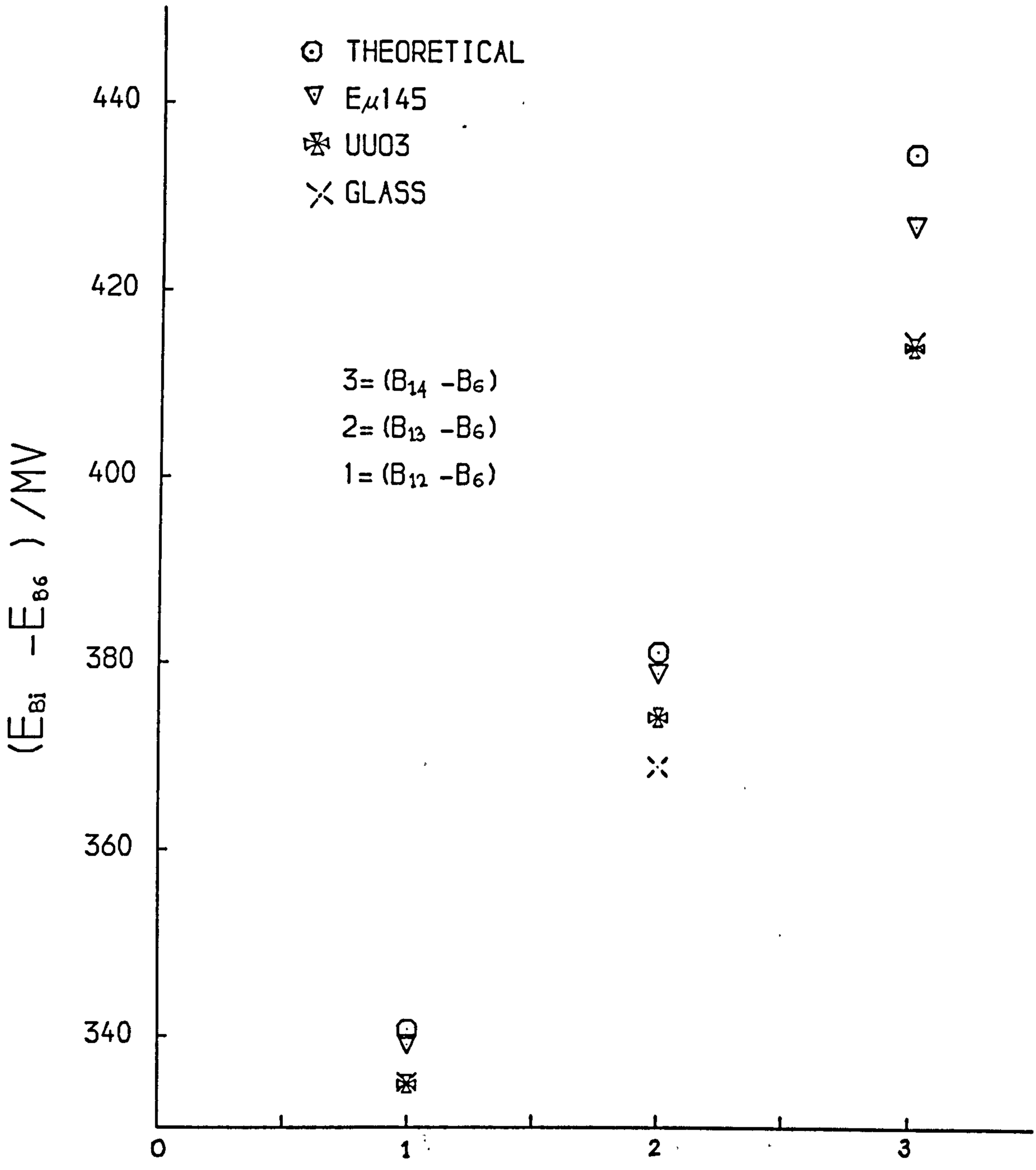

CORNING
AVL
ΔE/MV
12
10
8
6
4
2
0
−0.15
−0.10
−0·05
0·00
0·05
0·10
0·15
0·20
0·25
0·30
1
2
3
4
5
6
7
1=3.5, 2=3.3, 3=3.4,
4=PLASMA, 5=SERUM,
6=PLASMA+NACL
7=PLASMA+NAHCO3
LOG[CA]
PLASMAIV CALCIUM

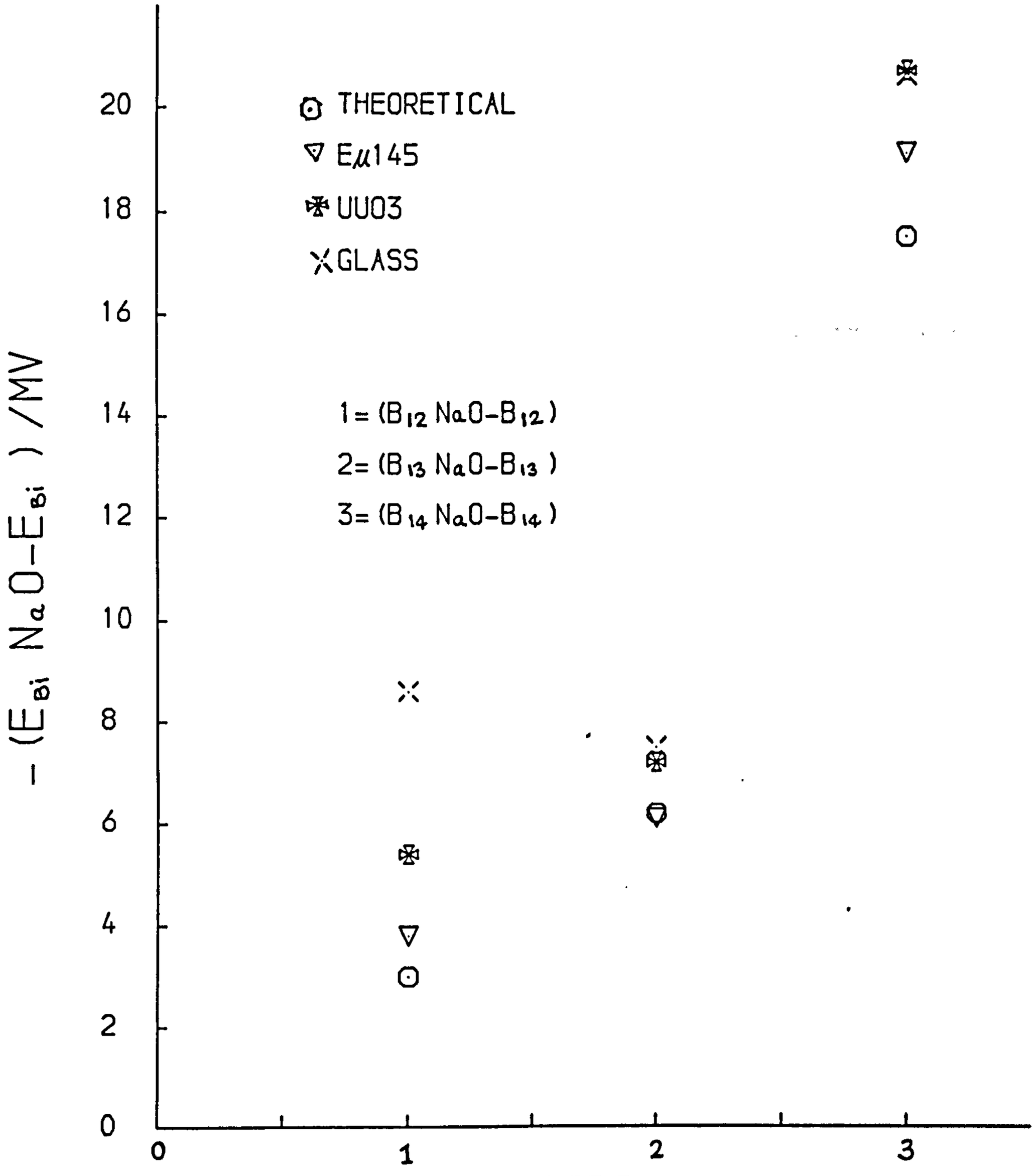
FIG. 5.28



TRANSFERS

H-ERRORS

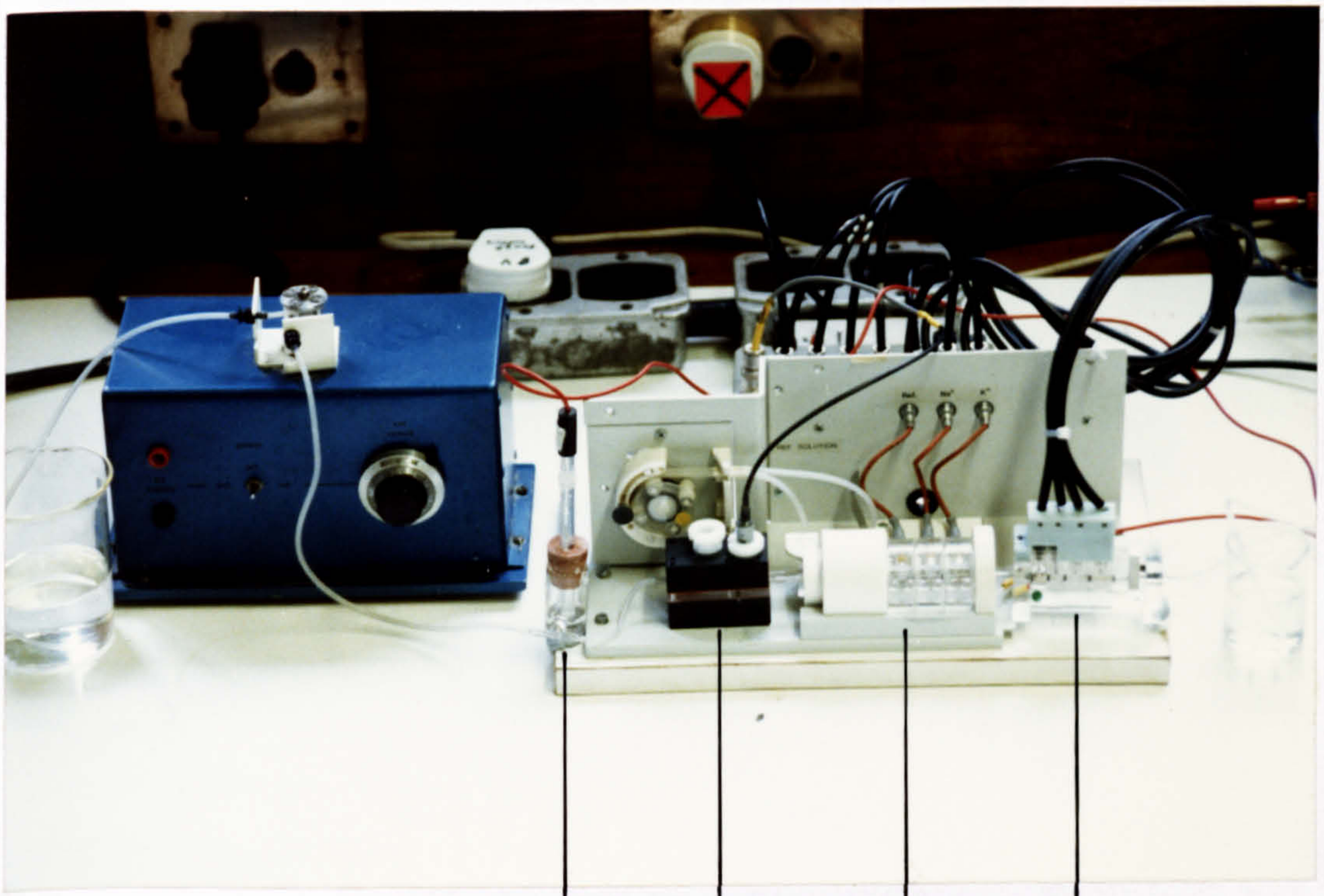
FIG. 5.29



TRANSFERS

ALKALINE ERRORS

FIG. 5.30



PHOTOGRAPH 5.1

REFERENCES

CHAPTER FIVE

R E F E R E N C E S

1. Sibbald, A., PhD. Thesis, University of Newcastle Upon Tyne (1982).

2. Janata, J. and Huber, R.J., in "I.S.E.'s in Analytical Chemistry" Vol. 2, Chap. 3, ed. H. Freiser, Plenum, N.Y. and London (1980).

3. Horvai, G., Toth, K., and Pungor, E., Anal. Chim. Acta., 82, 45 (1976)

4. Covington, A.K., Ferra, M.I.A., Anal. Chem. 49, 1363 (1977).

5. Rebelo, M.J.F., Ph.D. Thesis, University of Newcastle-Upon-Tyne, 184 (1981).

6. Bergveld, P., IEEE Trans. on Biomed. Eng., BME 19, 5, 342-351 (1972).

7. Schenck, J.F., "Workshop on Theory, Design and Biomedical Applications of Solid State Chemical Devices" ed. Chung, Fleming, Ko, Neumann, CRC Press, Cleveland, Ohio (1978).

8. Schenck, J.F., J. Coll. INt. Sci., 61, 569 (1977).

9. Esashi, M., and Matsuo, T., Proc. of the 6th Conference on Solid State Devices, Tokyo (1974) J. Jpn. Soc. Appli. Phys., 44, 339 (1975).

10. Johannessen, J.S., Spuer, W.E. and Strausser, Y.E., Thin Solid Films, 32, 311 (1976).

11. Maguiri, H.G., and Augustus P.D., J. Electroclem Soc., 110, 791 (1972).

12. "Ion Selective Electrodes in Analytical Chemistry, Vol. 2, ed. Fruser, H., Plenum Press, N.Y. (1980).

13. Stein, H.J., and Wells V.A., J. Electrochem, Soc. Extended Abstracts (ABS NO113), 77-1, 303-305 (1977).

14. Vlasov Y.G., Bratov, A.V., and Letavin, V.P., 3rd Symposium on I.S.E.'s, Matra Fired, ed. Pungor, E. and Buzas, I., 387-397, Elsevier (1981)

15. Personal Communication (Janata, J. to Sibbald, A.).

16. Covington, A.K., Prue, J.E., J. Chem. Soc. 3696 (1955).

17. Covington, A.K., J. Chem. Soc., 4441 (1960).

18. Covington, A.K., J. Chem. Soc., 4906 (1965).

19. Briggs, C.C., Lilley, T.H., J. Chem. Thermodynamics, 6, 599-607 (1974).

20. Bates, R.G., "Determination of pH - Theory and Practice", Wiley and Sons, N.Y. (1973).

21. Private Communication, Cowell, D.C., Royal Liverpool Hospital to Covington, A.K., Oct. (1984).

22. Czaban, J.D. and Legg, K.D., "Proceedings of the Workshop on Direct Potentiometric Measurements in Blood"; ed. Koch, W.F., Gaithersburg, 63 (1983).

23. Coleman, R.L., and Young, C.C., Clin. Chem., 27 (1937-1939) (1981).

24. Covington, A.K., Flear, C.T.G. and Lockie, R. in "Ionised Calcium, Sodium and Potassium by I.S.E.'s", 5, ed. A.H.J. Maas et al., Private Press, Copenhagen (1984).

25. Czaban, J.D., Cormier, A.D., Legg, K.D., Clin. Chem., 28, 1703-1705 (1982).

26. Czaban, J.D., Cormier, A.D., Legg, K.D., Clin. Chem., 28, 1936-1945 (1982).

27. Wimberley, P.D., Siggard-Andersen, O., Fogh-Andersen, N., Boink, A.B.T.J., Scand J. Clin. Lab. Invest. 43/165 (1983).

28. Boink, A.B.T.J., Bijster, P., Vink, K.L.J. and Maas, A.H.J., Clin. Chem., 31, 523-526 (1985).

29. Deal, B.E., MacKenna, E.L., and Castro, P.L., J. Electrochem. Soc. 997 (1969).

CHAPTER SIX

EVALUATION OF THE DISSOCIATION CONSTANTS AND FORMATION CONSTANTS OF THE "GOOD" BUFFERS USED

6.1 INTRODUCTION

The acid dissociation constants and the complexation properties of the'Good' buffers were evaluated by computer controlled, pH glass electrode, alkalimetric, titrations at 37 °C. A knowledge of the percentage metal binding to the buffers can be used to add additional quantities of salts, or to introduce a correction factor, to compensate for the decrease of metal ion concentration, if any, resulting from complexation.

Dissociation constants for the 'Good' buffers, in aqueous media, were evaluated, initially, by N.E. Good et al. [1] using a Radiometer automatic titrator - titrigraph. Subsequently, R.G. Bates and co-workers [2 3] and R.N. Roy et al.[4], used cells without liquid junction, of the type

Pt; H_2 (g,1 atm)$\left|\right.$ $Z^{\pm}(m_1)$, NaZ (m_2), NaBr $(m_3)$$\left|\right.$ AgBr/Ag

where $Z^{\pm}$ represents the zwitterionic buffer and NaZ its sodium salt, to calculate conventional pa_H values of a few 'Good' buffers. This latter method is similar to the procedure on which the NBS pH scale is based, the only difference being the use of an Ag/AgBr electrode instead of an Ag/AgCl electrode because of the lower solubility of AgBr in amine bases. The pertinent values at 37 °C are shown in TABLE 6.11.

N.E. Good et al.[1] deduced approximate values for metal buffer binding at 20°C by the displacement of the pH titration curve in the presence of an equivalent of the chloride of the metal. They assumed that the amine nitrogen of the buffer forms a co-ordinate bond with the metal. The equations used were:-

$$[A] = [N] + [NH^+] + [NM^+] \qquad - 6.1$$

where [A] = total buffer concentration

[N] = concentration of free amine

$[NH^+]$ = concentration of protonated amine

$[NM^+]$ = concentration of metal buffer complex

$[M] = [M^{2+}] + [NM^+]$ - 6.2

$Ka = [N]\ [H^+]/[NH^+]$ - 6.3

$K_M = [NM^+]/[N]\ [M^{2+}]$ - 6.4

$$K_M = \frac{[A] - K_a[NH^+]/[H^+] - [NH^+]}{(K_a[NH^+]/[H^+])([M] - [A] + K_a[NH^+]/[H^+] + [NH^+])} \quad - 6.5$$

Initially, all the buffer is protonated, therefore half is protonated at the mid-point of the titration curve i.e. $[NH^+] = [A]/_2$.

In the experiments, equivalent concentrations of buffer and metal ion were taken, so [A] = [M]. For the mid-point of the titration curve, equation 6.5 can, therefore, be written as

$$K_M = 2([H^+]/K_a - 1)/[A](K_a/[H^+] + 1) \quad - 6.6$$

$[H^+]$, K_a and [A] are provided by the titrations curves in presence and absence of the metal; K_M can, thus, be obtained.

For the buffers investigated in this work, negligible calcium binding was reported [1]. However, these data are not reliable; to quote N.E. Good "The scope and accuracy of these data are not such at to commend our work to physical chemists".[1]

More recently, the complexation properties of a few "Good" buffers were investigated by G.N. Bowers and co-workers. [6,7,8] It was reported [8] that calcium binding increased in the order

HEPES < TES < BES

Only the data for the calcium binding of HEPES are available in detail [6,7]. Initially, this was reported as 1.4% for 10 mmol/l HEPES [6] at a total ionic strength of 160 mmol/l and pH 7.39. Measurements were done with calcium ion-selective electrode analysers ICA1 (Radiometer) and a NOVA8 (NOVA, Biomedical).

In a later publication [7], the 1.4% decrease attributed to Ca^{2+} ion association was reported as a combination of two effects - the calcium + HEPES association and a change in residual liquid

junction potential due to differences in mobilities between the HEPES test solution and the manufacturer's calibrating solutions. The experiments were repeated using cells with calcium and sodium ion-selective electrodes connected to a common reference electrode so as to avoid the effects of liquid junction. The calcium concentrations were obtained from differences in emfs between solutions containing HEPES and calcium ions (B) and solutions containing calcium ions alone (A), at a total ionic strength of 303 mmol/l, T = 37 ^{o}C and pH = 7.4.

$$\triangle E = (E_{Ca}2+ - E_{Na}+)_B - (E_{Ca}2+ - E_{Na}+)_A \quad - 6.7$$

The association constant K(1:1) was calculated from the equation.

$$K(1:1) = \frac{[Ca\text{-}HEPES]}{[Ca^{2+}]\ [HEPES]} = \frac{10^{\Delta E/k} - 1}{[HEPES]} \quad - 6.8$$

These results are shown in Table 6.1.

TABLE 6.1

$[Ca^{2+}]$ = 1 mmol/l

Solution	[HEPES] mmol/l	ΔE	% Ca^{2+} bound	K(1:1) mmol/l
A	0	0	0	-
B	137	1.07±0.16	7.7±1.1	0.61±0.09
C	274	2.27±0.25	15.6±1.6	0.68±0.08
D	548	4.98±0.25	31.1±1.3	0.82±0.05

In calculating K(1:1) the activity coefficients have not been taken into account. These could be significant at such high ionic strengths.

G.N. Bowers and co-workers [7] also measured the decrease in Ca^{2+} ions on the Radiometer ICA1 and the NOVA 8 Ca^{2+} analyzers using reference solutions containing 1 and 110 mmol/l HEPES and Ca^{2+} at 0.50, 1.25 and 2.00 mmol/l. The instruments were calibrated with the manufacturer's calibrating solutions. Their results for [1.25 mmol/l Ca^{2+} + 110 mmol/l HEPES] - [1.25 mmol/l Ca^{2+} + 1 mmol/l HEPES] are summarised in Table 6.2.

TABLE 6:2

	% difference	Radiometer	NOVA 8
1.	Observed	10.6	11.1
2. a*	Calculated due to binding	6.2	6.2
3. b*	Calculated due to ΔE_j	5.0	6.5
4.	2 + 3	11.2	12.7
5. c*	Discrepancy (4.1)	0.6	1.6

a* - using K(1:1) = 0.6

b* - ΔE_j, the residual liquid junction potential, between the manufacturer's calibrating solutions and the solutions used, were calculated using the Henderson equation. It is known that Henderson's equation yields higher values of ΔE_j than observed values [23,24]. To quote from 23, "Useful accuracy is obtained only in dilute solutions, where the assumption of ideal behaviour is approximately satisfied".

c* - These were attributed to the manufacturer's calibration solutions being adjusted, presumably, to account for complexation and liquid junction effects.

6.2 MATERIALS AND METHOD

6.2.1 Reagents

MOPS, TES, BES and HEPES, obtained from Sigma Chemical Co., were recrystallised from 80% ethanol and dried overnight in a vacuum oven (T$\sim$40 ^{o}C), over phosphorus pentoxide.NaCl, KCl, LiCl and KNO_3, obtained from BDH (Analar Grade), were also dried in the vacuum oven. All the chemicals were stored in desiccators over phosphorus pentoxide. Volumetric standard solutions of 1 mol dm^{-3} $CaCl_2$, obtained from BDH, were assayed by EDTA titrations [9]; the percentage purity was found to be 99.8 $\pm$ 0.1. KOH and NaOH and HCl solutions were prepared from BDH ampoules ('CONVOL' concentrate). The NBS standards, KHPh (0.05 moldm^{-3}) and

KH_2PO_4 (0.008695 moldm^{-3}) + Na_2HPO_4 (0.03043 moldm^{-3}), with pH(s) of 4.028 and 7.385 at 37 ^{o}C, respectively, were prepared from BDH samples as recommended (10). These were used for calibrating the glass electrode.

6.2.2 Apparatus and Instrumentation

The titration system used is shown in Fig. 6.1. The titration cell was a double walled beaker, thermostated at 37 ^{o}C. It was fitted with a Teflon cap with five apertures for the glass electrode, reference electrode, burette tube, thermometer and nitrogen bubbler. The stirring was by a magnetic stirrer. An automatic burette (Mettler DV 11) was used. The emf was measured with a Radiometer glass electrode (type 202C) in conjunction with a Russell (type CRR) calomel reference electrode.
A Rockwell, AIM65 microprocessor via a serial interface was used for recording data and controlling the burette. The input - output from the CPU was through a VIA (Versatile Interface Adaptor) which contained two 16 bit counters and two 8 bit bi-directional input/output ports connected as shown in Appendix 6.1. The burette functions (volume increments, total volume delivered and time interval allowed for equilibration between each reading) and data acquisition were controlled by the use of BASIC and ASSEMBLER software stored on a cassette tape. The data output was displayed on the integral 20 digit alpha-numeric display, printed on the thermal printer and simultaneously transferred via a second serial interface to the MTS (Michigan Terminal System) mainframe. These data were, subsequently, analysed by two non-linear least squares programs SCOGS 2[10,11] and SUPERQUAD [12]. The BASIC and ASSEMBLER programs are listed in Appendices 6.2 and 6.3.

6.2.3 Titration Procedure

The titrand was solutions of HEPES, MOPS, BES and TES protonated with HCl in a background of 0.15 mol dm^{-3} KNO_3, KCl, NaCl or LiCl, for the determination of dissociation constants. For evaluating the formation constants, metal ion (Ca^{2+}/K^{+}): Buffer were taken in the ratios 1:1 or 1:2 in the same ionic strength backgrounds. The titrant was 1 mol dm^{-3} or 0.5 mol dm^{-3} KOH (NaOH for K^{+} association determinations). The titrant was added in 0.02 or 0.05 ml increments. After each addition, 2 to 3 minutes were allowed for equilibration before the readings were taken.

The pH glass electrode was calibrated with the NBS phthalate and phosphate buffers before each titration.

6.3 THE PRINCIPLE OF NON-LINEAR LEAST SQUARES [13,14,15,16,17]

If there are 'n' experimental observations Y_1, Y_2 ... Y_n, each having an associated random error e_1, e_2 ... e_n and each depending non-linearly on a set of $m \leq n$ parameters, then:

$$Y_i = f_i (x_1, x_2 .... x_m) + e_i \quad - 6.9$$

If the initial estimates of the parameters x_1, x_2 ... x_m are x_1^{o}, x_2^{o} ... x_m^{o}, then the observations Y_i can be expanded using the Taylor Series. Neglecting terms higher than the first order, equation (6.9) can be written as:

$$Y_i = f_i (x_1^{o}, x_2^{o} ... x_m^{o}) + \left(\frac{\delta fi}{\delta xi}\right)_o (x_1 - x_1^{o}) + (\delta f_i/\delta x_m)_o (x_m - x_m^{o}) + e_i \quad - 6.10$$

$$\text{or } Y_i - f_i (x^{o}, x_2^{o} ... x_m^{o}) = \sum_{j=1}^{m} (\delta f_i/\delta x_j)_o \Delta x_j + e_i$$

$$\text{or } \Delta f_i = \sum_{j=1}^{m} (\delta f_i/\delta x_j)_o \Delta x_j + e_i \quad - 6.11$$

The problem is thus reduced to a linear form. To write equation (6.11) in matrix notation, let

F = vector containing Δf_i terms

E = vector containing e_i terms

A = matrix containing the $(\frac{\delta fi}{\delta xj})_o$ terms

X = matrix containing Δx_j terms

$$F = AX + E \qquad - 6.12$$

If a vector $\vec{X}$ represents the 'best fit' values of X, then a vector of residuals V can be defined as:

$$V = F - A\vec{X} \qquad - 6.13$$

The quantity to be minimised is the sum of the square of the residuals S:

$$S = V^T V \qquad - 6.14$$

where V^T is the transpose of V

If the variances of the residuals are not equal

$$S = V^T W V \qquad - 6.15$$

where W is a weighting matrix; usually a diagonal matrix with the variances (6_i^2) as diagonal element

Substituting (6.13) in (6.14) and noting that

$$(A\vec{X})^T = A^T\vec{X}^T$$

$$S = F^T WF + \vec{X}^T A^T WA\vec{X} - 2\vec{X}^T A^T WF \qquad - 6.16$$

To minimise S, (6.16) is differentiated with respect to each of the parameters $\vec{X}$ in turn and equated to zero.

$$\delta s = 2(\delta\vec{x}^T)(A^T WA\vec{X} - A^T WF) = 0 \qquad - 6.17$$

$$A^T WA\vec{X} = A^T WF \qquad - 6.18$$

$$\text{Or } \vec{X} = (A^T WA)^{-1} A^T WF \qquad - 6.19$$

$\vec{X}$ obtained from equation (6.19) gives the least squares estimates of the parameters in one iteration, which produces a lower sum of squares of the residuals. This is used as initial estimates for the next calculation. This iterative procedure continues until the process converges in a finite number of iterations to specified convergence limits. The variance of the parameters is given by [14]

$$6^2 = \frac{V^T WV}{(n-m)} \qquad - 6.20$$

where (n - m) is the number of degrees of freedom of σ^2;

n = number of observations;

m = number of parameters to be determined.

In the Gauss-Newton method of minimisation of a non-linear function, the function is expanded as a Taylor series and truncated at the first order term. In the Newton-Raphson approach, the second derivatives are taken into account to improve convergence properties.

To protect against divergence and to accelerate convergence, several non-linear least squares programs, including SUPERQUAD, use the Marquardt technique [18]. To explain this briefly, a concept of the steepest descent method of minimisation is needed. The parameters are improved from the current trial values by adjustments in proportion to the derivatives at the particular point, along a descent line. This method converges rapidly, initially. However, when the minimum is approached, derivatives have smaller magnitudes and convergence becomes very slow. The Marquardt algorithm uses the steepest descent algorithm away from the minimum while the Gauss-Newton iteration is used as the minimum is approached.

6.4 APPLICATION TO THE REFINEMENT OF STABILITY CONSTANTS

6.4.1 General

The formation constant can be represented by the equation

$$\beta_{mlh} = [M_m L_l H_h]/[M]^m [L]^l A_H^h \quad - 6.21$$

Where m and l are positive integers or zero and h is positive for protonated species, negative for hydroxo complexes or zero.

$[M_m L_l H_h]$ is the concentration of the complex species

[M] and [L] are concentrations of the uncomplexed reactant species.

A_H^h is the measured proton activity.

For each experimental point of the titration curve, the following mass balance equations are valid:

$$T_M = [M] + \sum_{m=1}^{j} \beta_{mlh} [M]^m [L]^l [H]^h \quad - 6.22$$

$$T_L = [L] + \sum_{l=1}^{j} \beta_{mlh} [M]^m [L]^l [H]^h \quad - 6.23$$

$$T_H = [H] + \sum_{h=1}^{j} h \beta_{mlh} [M]^m [L]^l [H]^h + A_H/f - K_W/A_H^h) \quad - 6.24$$

where T_M, T_L and T_H are the analytical concentrations of metal, ligand and proton respectively and are known from the quantities used to make up the test solutions. f is the activity coefficient for H^+ and K_W the dissociation constant of water A_H is obtained from the potentiometric titrations.

The unknown parameters are [M] and [L] for each point and the stability constant β_{mlh}.

From equations 6.22 and 6.23, [M] and [L] can be calculated by the Newton-Raphson method. β_{mlh} is estimated. The calculated titration volume or the calculated emf, Y^K_{Calc}, can be obtained from

$$Y^K_{Calc} = EQ - \frac{EQ}{n_1 [L]} \left(\sum_{h=1}^{j} \beta_{mlh} [M]^m [L]^l [H]^h + A_H/\gamma - K_W/A_H\right) + E_x \quad - 6.25$$

where EQ is the equivalence point volume/emf.

n_1 is the number of protons of the ligand that can be titrated.

E_x is the volume/emf corresponding to the excess of strong acid.

The error square sum is thus given by:

$$S_i = \sum_j (Y^K_{exp} - Y^K_{Calc})^2 \quad - 6.26$$

Y^K_{exp} is the experimentally observed volume/emf. To

obtain the 'best' fit, the minimum of the error square sum is sought by solving

$\delta Si/\delta \log \beta_{mlh} = 0$ (SCOGS) or $(\delta Si/\delta \beta_{mlh} = 0$ (SUPERQUAD).

The correction vector $\overrightarrow{X}$ thus obtained is used to estimate the new values of the formation constants which are better approximations to the final value and are used for the next refinement cycle. The iterative procedure continues until the best values of β are obtained.

6.4.2 Applied to Good Buffers

The 'Good' buffers used, MOPS, TES, BES and HEPES are zwitterionic. They were considered as $LH^{\pm}$ in the unprotonated form (h = 1), the equilibrium is therefore:

$$LH_2^+ \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} LH^{\pm} \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} L^-$$

Considering equation 6.21 and noting that m = 0, l = 1 and h = 1 or 2, the formation constants can be written as:

$$K_{a_2} = \frac{[LH^{\pm}]}{[L^-]A_H+} (= \beta_{0,1,1}) : L^- + H^+ \rightleftharpoons LH^{\pm} \quad - 6.27$$

$$K_{a_1} = \frac{[LH_2^+]}{[LH^{\pm}]A_H+} (= \beta_{0,1,2} = K_{a_1} \times K_{a_2}) : LH^{\pm} + H^+ \rightleftharpoons LH_2^+ \quad - 6.28$$

Metal complexes of the 1:1 type were formed with the zwitterionic and protonated forms of the buffers. The corresponding formation constants are therefore given by the equations:

$$LH^{\pm} + M \rightleftharpoons MLH^{\pm}$$

$$K_{MLH^{\pm}} = [MLH^{\pm}]/[M]\ [LH^{\pm}] \quad - 6.29$$

$$\beta_{1,1,1} = K_{a_2}\ K_{MLH^{\pm}} \quad - 6.30$$

$$LH_2^+ + M \rightleftharpoons MLH_2^+$$

$$K_{MLH_2^+} = [MLH_2^+]/[M][LH_2^+] \quad - 6.31$$

$$\beta_{1,1,2} = K_{a_1} \times K_{MLH_2^+} \quad - 6.32$$

6.5 SCOGS 2 AND SUPERQUAD

SCOGS 2 [11] is the latest version of the program "Stability Constants of General Species". In a critical examination of the numerical manipulation of titration data, Field and McBryde [19]

state that "experience with simulated titration data has revealed that the SCOGS program consistently achieves good recovery of the starting equilibrium constants and also the smallest values of standard deviations in pM and pL. In view of this performance, the authors, Field and McBryde, have come to place most reliance on this program to indicate 'correct' equilibrium constants, derived from real titrations ....". A typical input data file for a SCOGS 2 evaluation of formation constants is shown in Appendix 6.4.

SUPERQUAD [12] is a recent program. It was designed as an improvement to a previous program, MINIQUAD [22], thus it SUPERsedes miniQUAD. A typical input data file for SUPERQUAD is shown in Appendix 6.5.

The following is a comparison of the two programs.

SCOGS 2	SUPERQUAD
1. The residual minimised is the titre volume.	1. The residual minimised is pH/emf. This means that the pH/emf is the dependent variable, whereas the titre volume is the independent variable and so is presumed error free. The pH/emf are assumed to have a Gaussian distribution of errors.
2. Since the experimental error in titre volume is similar over all points, no weighting of the readings has been applied.	2. Since electrode readings near the end point are more error prone, because of larger values of dpH/dV, a weight is assigned to each titration point, inversely proportional to the variance at that point.

SCOGS 2	SUPERQUAD
3. The residuals are minimised using the Newton-Raphson method	3. The Gauss-Newton method of minimisation protected against divergence by the Marquardt (Fletcher) algorithm is used.
4. The refinement parameter is $\log \beta$	4. β is preferred as the refinement parameter [21].
5. Allows for variations in ionic strength by inputing the mean activity coefficient of H^+ and OH^-(f).	5. Activity coefficients have been ignored.
6. Liquid junction effects have been ignored.	6. Liquid junction potentials were accounted for in earlier versions of the program, but were found to be of limited utility.
7. Numerical derivatives are used.	7. This program uses implicit (analytical) differentiation. The advantage of analytical derivatives is higher speed of calculation and better convergence [20,33].
8. There is no provision for impure substances.	8. There is provision for treating data relating to substances which are not available in a high state of purity, by treating the corresponding parameters as variables.
9. The diagnostics generated are, for each experimental	9. The diagnostics available are relative errors, sums of squares,

SCOGS 2	SUPERQUAD
point, the residual (volume of titrant added: calculated - experimental), the total concentration of each metal and ligand, the concentrations of each free metal and ligand and the concentrations of each complex species. Finally, the standard deviation and a scatter plot are printed.	sample standard deviation, standard deviations of each constant, ψ^2 statistics, plots of residuals and species distributions. ψ^2 is a goodness of fit statistic. The residuals have a Gaussian distribution and are divided into eight classes defined by the limits $-\alpha$, -1.15σ, -0.675σ, -0.319σ, 0.0 $+0.319\sigma$, $+0.675\sigma$, $+1.15\sigma$ and α. The degrees of freedom (k) are (n-1) =6 . ψ is defined as $\psi = kS^2/\sigma^2$ where S^2 = sum of the squares of residuals and σ^2 = variance. Values of ψ^2 for various confidence intervals can be found in tables. For k = 6 and for 95% confidence $\psi^2 <$ 12.60.
10. SCOGS 2 does not have any automatic model selection for different species and each model must be independently tested by the user[33]	In SUPERQUAD, the best model is taken as the one with the lowest sample standard deviation and no ill defined formation constants (i.e. positive values with standard deviations less than 33% of their value). If after refinement a formation constant

is found to be ill-defined, a new model is automatically generated from which the ill-defined species is rejected. Negative constants are rejected at the end of the refinement. Each successive model uses as initial estimates the best constants stored for the previous model before a new refinement is started[33].

6.6 RESULTS AND DISCUSSIONS

The titrations were performed with 0.5 mol dm^{-3}/1.0 mol dm^{-3} base in 0.15 mol dm^{-3} KNO_3, KCl, NaCl on LiCl as supporting electrolyte. A saturated calomel electrode was used as the reference electrode. The correct equation for pH measurements in such a system would be

$$p[H] = pH - \log F \quad - 6.33$$

where [H] = concentration of hydrogen ions

and

$$\log F = \log f - \Delta Ej + \Delta pH \quad - 6.34$$

f = activity coefficient of hydrogen ions

ΔEj = residual liquid junction potential

ΔpH = small systematic errors which may arise

Neither SCOGS2 nor SUPERQUAD offer any means of corrections for liquid junction effects. P. Gans [12] states such corrections are negligble in pH range 3 - 11. In SCOGS2 there is provision for activity coefficient corrections. This will be discussed in the next paragraph.

It is normally possible to calculate the free hydrogen ion concentration by titrating the standardised acid in the appropriate background. If V_o ml of acid of concentration C_o mol dm^{-3} is titrated with V_t ml of base of concentration H_t mol dm^{-3}, the charge balance at any point of titration will be

$$[H^+] + [B^+] = [A^-] + [OH^-] \qquad -6.35$$

substituting $[OH^-]$, $[A^-]$ and B^+ by their actual values and making $V_{TOT} = V_o + V_T$ gives

$$[H^+] + (H_tV_t/V_{TOT}) = (C_oV_o/V_{TOT}) + (K_W/[H^+]) \qquad -6.36$$

Rearrangement gives

$$[H^+]\ calc = \left\{\left(C_oV_o - H_tV_t\right) + \left[(C_oV_o - H_tV_t)^2 + 4K_W\ V_{TOT}^2\right]^{\frac{1}{2}}\right\}/2V_{TOT} - \quad 6.37$$

The program MINIGLASS [30] has this provision. In this situation, however, the ligand is zwitterionic and is a source of protons, hence this procedure cannot be adopted. To find an alternative, initially, a value of f = 0.81 which was used by previous workers[25,26], for 0.15 mol dm^{-3} KNO_3 background, was tried. This was found to cause a systematic error in the acidic regions (FIG 6.11 a and b). Subsequently, the variation of the standard deviations obtained with values of f, $0.75 \leq f \leq 1.00$ were examined in the various backgrounds (FIG. 6.12). The minimum was found to be towards f = 1. This value was, therefore, used in the analysis of data. It seems logical to assume that the activity coefficient (and liquid junction) effects are probably negligible in this system. A similar procedure was adopted by Childs and Perrin [26] for metal ion-amino acid systems. The uncertainty in the value of f seems to affect pK_{a1} and $\log\beta_{MH_2L^+}$ values in the acid region. The dissociation constants and formation (binding) constants evaluated at 37° C for MOPS, TES, BES and HEPES are given in detail in Tables 6.3 - 6.10. A summary of the results, together with other published values, is given in Table 6.11.

First derivative plots of the titrations were plotted using the GHOST facilities of MTS. The programs used for calculating the first derivatives and plotting the graphs are listed in Appendices 6.6 and 6.7 respectively. Some first derivative plots are shown in FIGS. 6.2 - 6.10. These show clearly that there is one predominant equivalence point in both the pH titrations in absence of a metal ion and in presence of a metal ion thus supporting the conclusions that follow.

Both SCOGS2 and SUPERQUAD give excellent agreement in the values of dissociation and binding constants. The models seem to be fulfil the criteria for acceptability with both programs (SCOGS:$\sigma < 3 \times 10^{-3}$) (SUPERQUAD:$\psi < 12.6, \sigma < 3$, SD of formation constant $<$ 33% of its value) Examination of the data for dissociation constants does not seem to reveal any obvious media trends. This can probably be attributed to the fact that K^+ binding is negligble. Na^+ and Li^+ binding are probably negligible as well. Also, liquid junction effects must be minimal in the pH regions of interest. The pK_{a2} values are comparable with the values obtained by Bates [2,3] (Table 6.11) Except for HEPES ($pK_{a1} = 3.09$) the pK_{a1} values are found from the region pH < 2, which is too low for them to be meaningful.

The formation constant data support only 1:1 models; the 1:2 or 2:1 models were rejected as excessive by SUPERQUAD. The percentage of metal iron bound to the buffer was calculated using the $K_{MLH}^{\pm}$ constants. The $K_{MLH_2}^{+}$ constants were ignored because, as seen by the points D in the plots in FIG. 6.13, they are not of interest in the pH range of physiological interest

The mass balance equation is

$$T_M = [M] + [MLH^{\pm}]$$

where T_M = total metal concentration

$[M]$ = concentration of uncomplexed metal

$[MLH^{\pm}]$ = concentration of the $MLH^{\pm}$ species.

If α = fraction of free ion, then

$$\alpha = \frac{M}{T_M} = \frac{\cdots [M] \cdots}{[M] + [MLH^{\pm}]} \quad - 6.39$$

or $\alpha = ([M] + [MLH^{\pm}]/[M])^{-1}$

$= (1 + [MLH^{\pm}]/[M])^{-1}$

but $K_{MLH}{}^{\pm} = [MLH^{\pm}]/[M]\ [LH^{\pm}]$

therefore

$$\alpha = (1 + K_{MLH^{\pm}}\ [LH^{\pm}])^{-1} \quad - 6.40$$

$$\% \text{ of metal bound} = 100\ (1 - \alpha) \quad - 6.41$$

The percentage of calcium bound versus ligand concentration is plotted in FIG. 6.14. The order of binding was

MOPS < HEPES < TES < BES

The percentage of potassium bound was too low to give meaningful plots. The order of binding is the same. From the point of view of calcium and potassium complexation, therefore, it appears that MOPS is the preferable of the four buffers.

The binding of sodium to HEPES was investigated in this laboratory by a project student[32]. His results are reproduced here:

Temp. = 37 °C.
Conc of HEPES = 0.02 mol dm^{-3}
Conc of SODIUM = 0.02 mol dm^{-3}
Conc of HCl = 0.02 mol dm^{-3}
Conc of KOH = 0.05 mol dm^{-3}
Initial Volume = 50 ml

SCOGS2

$\left.\begin{aligned} \text{Log}\beta_{NaL} &= 6.0249 \\ \text{Log}\beta_{NaL_2} &= 8.8983 \end{aligned}\right\}$ S.d. = 4.093×10^{-3} in ml titrant

SUPERQUAD

$\left.\begin{aligned} \text{Log}\beta_{NaL} &= 6.02305, \text{ S.d.} = 9.97 \times 10^{-4} \\ \text{Log}\beta_{NaL_2} &= 8.89704, \text{ S.d.} = 9.35 \times 10^{-4} \end{aligned}\right\}$ $\Psi^2 = 8.79$

Thus

$K_{MLH} = \log\beta_{NaL} - \log K(HL) = -1.3$ (approx.)

showing that no complexation occurs.

The binding of sodium to TES, BES and MOPS has not been investigated.

6.7 FUTURE-WORK

Programs such as ESTA [27] and MIQUV [28], allow for liquid junction potential corrections. In ESTA, this is done using

$$E_K^{LJ} = E_K^{LJ'} - E_K^{LJ_o} \qquad 6.42$$

where $E_K^{LJ_o}$ is the liquid junction potential, calculated from Henderson equation [29], between the bridge solution and a test solution containing the bridge ions only at pH = 7 and at the reference ionic strength.

$E_K^{LJ'}$ is the liquid junction potential between the actual solution and the bridge solution. This results in E_K^{LJ} values tending to zero as the pH tends to 7. Hence, in the pH region of interest, liquid junction effects will be small.

ESTA also allows for activity coefficient corrections using the Davies extension of the Debye-Huckel formula and for electrode selectivity corrections. It also provides for interconversion of data into input files for other programs such as MINIQUAD, SUPERQUAD, SCOGS2 etc., making intercomparisons easier. Another facility provided by this program is that plots of the formation and of the deprotonation functions can be obtained. This gives useful information on the nature of the main species. This is well illustrated by Casassas et al. in reference 34. It would be of interest to analyse the present data using ESTA.

The program STBLTY (Avdeef)[31], has provisions for corrections of dilution effects introduced by the addition of titrant and for changes in ionic strength due to the presence of species other than the supporting electrolyte. The Davies modified Debye-Huckel equation is used for the latter. The present data could be analysed using this program as well.

A further possibility of extending this work is to evaluate the binding constants using metal ion selective electrodes and ion selective field effect transistors instead of the conventional glass electrode.

TABLES

Table 6.3

DISOCIATION CONSTANTS OF MOPS (CONC IN MOL/L)

[MOPS]	[HCL]	[KOH]	MEDIA	NO OF PTS	pKa1	pKa2	SCOGS (1E-03) SIGMA	SUPERQUAD CHI	SUPERQUAD SIGMA	SUPERQUAD (1E-03) pka1 S.D.	SUPERQUAD (1E-03) pka2 S.D.
.02	.04	1.00	.15KCl	55	1.190	6.971	1.0	18.5	1.0	0.6	0.7
.01	.02	1.00	.15KNO3	28	1.104	6.991	1.4	5.1	1.4	2.1	3.5
.01	.02	1.00	.15KNO3	27	1.004	7.002	1.0	2.3	1.0	1.6	2.7
.01	.02	1.00	.15KNO3	28							
.01	.02	1.00	.15KNO3	27	1.056	6.996	4.8	12.6	4.7	5.4	9.0
.02	.02	1.00	.15KNO3	30	0.998	7.000	1.4	11.1	1.3	0.9	1.9
.02	.04	0.50	.15KNO3	54	1.250	6.976	1.0	10.9	1.0	0.3	0.4
.01	.02	1.00	.15NaCl	26	1.005	6.999	1.0	9.1	1.5	2.3	4.7
.02	.04	0.50	.15NaCl	89	1.000	7.000	1.1	11.4	1.1	0.3	0.4
.02	.04	1.00	.15LiCl	54	1.002	7.001	1.2	2.0	1.4	0.8	1.9
.01	.04	1.00	.15LiCl	52	1.001	6.997	0.9	4.3	1.0	0.6	0.8
.02	.04	1.00	.15LiCl	54							
.02	.04	1.00	.15LiCl	52	1.001	6.999	1.2	10.2	1.2	0.5	0.7

Table 6.4

DISOCIATION CONSTANTS OF TES (CONC IN MOL/L)

[TES]	[HCL]	[KOH]	MEDIA	NO OF PTS	pKa1	pKa2	SCOGS (1E-03) S.D.	SUPERQUAD CHI	SUPERQUAD SIGMA	SUPERQUAD (1E-03) pKa1 S.D.	SUPERQUAD (1E-03) pKa2 S.D.
.01	.02	1.00	.15KCl	30	1.620	7.273	0.9	11.1	1.4	2.6	2.1
.01	.02	1.00	.15KCl	29	1.711	7.263	0.8	9.9	1.4	2.6	2.1
.02	.02	1.00	.15KCl	36	1.599	7.270	0.6	6.7	0.7	0.5	0.4
.01	.02	1.00	.15KNO3	30	1.626	7.264	0.8	10.0	1.1	2.1	1.7
.005	.01	1.00	.15KNO3	15	1.637	7.253	0.8	21.8	2.4	10.0	13.8
.005	.02	1.00	.15NaCl	62	1.707	7.260	0.6	4.3	0.7	2.2	1.9
.01	.02	1.00	.15NaCl	37	1.619	7.269	0.8	14.7	0.9	1.6	1.2
.01	.02	1.00	.15NaCl	30	1.633	7.262	0.9	14.3	0.9	1.6	1.3
.02	.02	1.00	.15LiCl	37	1.620	7.273	0.6	3.4	0.7	0.6	0.4
.01	.02	1.00	.15LiCl	30	1.621	7.270	0.6	5.2	0.9	1.7	1.3
.01	.02	1.00	.15KCl	30							
.01	.02	1.00	.15KCl	29	1.663	7.268	8.1	14.6	8.1	10.5	8.4
.01	.02	1.00	.15NaCl	37							
.01	.02	1.00	.15NaCl	30	1.625	7.266	1.5	13.4	1.6	1.9	1.5

Table 6.5

DISOCIATION CONSTANTS OF BES (CONC IN MOL/L)

[BES]	[HCL]	[KOH]	MEDIA	NO OF PTS	pKa1	pKa2	SCOGS (1E-03)	SUPERQUAD CHI	SUPERQUAD SIGMA	SUPERQUAD (1E-03) pKa1	SUPERQUAD (1E-03) pKa2
.01	.02	0.50	.15KCl	60	0.880	7.010	0.5	8.8	0.5	0.6	0.3
.02	.02	0.50	.15KCl	79	0.890	7.000	0.6	10.8	0.6	0.4	0.1
.01	.02	0.50	.15KNO3	58	0.971	7.009	0.7	10.4	0.7	0.7	0.4
.01	.02	0.50	.15KNO3	75	0.920	7.010	0.6	3.8	0.6	0.3	0.1
.01	.02	0.50	.15NaCl	58	0.910	7.011	0.5	8.2	0.5	0.6	0.3
.02	.02	0.50	.15NaCl	75	0.963	7.010	0.5	9.8	0.5	0.3	0.1
.02	.02	0.50	.15NaCl	73	0.965	7.013	0.5	5.6	0.5	0.3	0.1
.02	.02	0.50	.15NaCl	75							
.02	.02	0.50	.15NaCl	73	0.964	7.012	1.1	10.3	1.1	0.4	0.1
.01	.02	0.50	.15LiCl	60	0.970	7.011	0.5	8.8	0.5	0.5	0.3
.02	.02	0.50	.15LiCl	75	0.980	7.009	0.5	4.5	0.6	0.3	0.1

Table 6.6

DISOCIATION CONSTANTS OF HEPES (CONC IN MOL/L)

[HEPES]	[HCL]	[KOH]	MEDIA	NO OF PTS	pKa1	pKa2	SCOGS (1E-03)	SUPERQUAD CHI	SUPERQUAD SIGMA	SUPERQUAD (1E-03) pKa1	SUPERQUAD (1E-03) pKa2
.02	.02	1	.15KCl	40	3.030	7.426	1.9	5.6	2.0	1.0	1.0
.02	.04	1	.15KCl	47	3.239	7.425	2.4	4.9	2.5	1.0	2.0
.01	.02	1	.15KNO3	23	3.147	7.435	3.2	3.8	3.0	5.0	6.0
.02	.04	1	.15KNO3	41							
.02	.04	1	.15KNO3	29	3.179	7.403	5.6	5.7	7.7	3.0	3.0
.02	.04	1	.15KNO3	41	3.169	7.402	3.4	3.4	11.5	1.9	2.5
.02	.04	1	.15KNO3	29	3.194	7.407	3.3	3.6	7.7	2.7	3.6
.02	.02	1	.15KNO3	37	2.841	7.420	0.9	1.7	0.9	0.5	0.6
.01	.01	.50	.15KNO3	28	3.106	7.423	2.1	8.6	2.2	1.0	1.0
.02	.02	.55	.15NaCl	29	3.125	7.416	2.0	4.4	2.0	0.7	0.9
.01	.02	.55	.15NaCl	23	3.078	7.420	2.2	5.2	2.4	2.0	3.0
.02	.02	.55	.15LiCl	65	2.923	7.419	2.1	5.3	2.0	0.5	0.6
.01	.02	.55	.15LiCl	49	3.041	7.412	2.2	11.2	2.2	2.0	2.0

Table 6.7

CALCIUM BINDING CONSTANTS OF MOPS (CONC IN MOL/L) T=37 °C

[MOPS]	[M]	[HCl]	[KOH]	MEDIA	NO OF PTS	LOG Kmh1	LOG Kmh21+	SCOGS S.D (1E-03	SCOGS CHI	SUPERQUAD SIGMA	SUPERQUAD S.D. (1E-03) log Bmh1	SUPERQUAD S.D. (1E-03) log Bmh21
.02	.02	.02	1.0	.15KCl	39	-0.48	1.23	0.58	12.1	0.7	63.4	3.6
.02	.01	.02	1.0	.15KCl	37	-0.44	1.24	0.49	9.1	0.6	76.2	5.2
.02	.02	.02	1.0	.15KNO3	37	-0.44	1.24	0.61	13.8	0.7	59.6	3.6
.02	.02	.02	1.0	.15KNO3	37	-0.45	1.22	0.77	6.0	0.8	64.2	4.0
.02	.02	.02	1.0	.15KNO3	37							
.02	.02	.02	1.0	.15KNO3	37	-0.45	1.23	0.75	8.0	0.9	45.8	2.9
.02	.01	.02	1.0	.15KNO3	35	-0.35	1.20	0.45	14.6	0.5	67.1	5.2
.02	.02	.02	1.0	.15NaCl	38	-0.36	1.25	0.60	16.7	0.6	38.9	2.9
.02	.02	.02	1.0	.15NaCl	39	-0.51	1.24	0.67	16.2	0.7	66.5	3.5
.02	.02	.02	1.0	.15NaCl	38							
.02	.02	.02	1.0	.15NaCl	39	-0.43	1.25	0.69	16.6	0.7	38.0	2.4
.02	.01	.02	1.0	.15NaCl	37	-0.39	1.24	0.71	9.6	0.9	134.8	8.4
.02	02	.02	1.0	.15LiCl	38	-0.40	1.23	0.92	9.2	1.0	74.1	5.0
.02	.01	.02	1.0	.15LiCl	38	-0.43	1.22	0.77	4.3	0.9	148.3	8.5

POTASSIUM BINDING CONSTANTS OF MOPS

[MOPS]	[M]	[HCl]	[NaOH]	MEDIA	NO OF PTS	LOG Kmh1	LOG Kmh21+	SCOGS S.D (1E-03	SCOGS CHI	SUPERQUAD SIGMA	SUPERQUAD S.D. (1E-03) log Bmh1	SUPERQUAD S.D. (1E-03) log Bmh21
.02	.02	.02	.50	.15NaCl	75	-1.34	0.20	0.5	11.1	0.5	123	7.9
.02	.02	.02	.50	.15NaCl	74	-1.33	0.13	0.7	3.4	0.7	*ex	11.7
.01	.01	.01	.50	.15NaCl	39	-1.35	0.13	0.6	6.4	0.6	*ex	108.7

*ex=excessive

Table 6.8

CALCIUM BINDING CONSTANTS OF TES (CONC IN MOL/L) T=37 °C

[TES]	[M]	[HCl]	[KOH]	MEDIA	NO OF PTS	LOG Kmhl	SCOGS S.D (1E-03)	CHI	SUPERQUAD SIGMA	SUPERQUAD S.D (1E-03) log Kmhl
.02	.02	.02	0.5	.15KCl	75	0.14	0.6	5.5	0.6	3.3
.02	.02	.02	0.5	.15KCl	73	0.14	0.7	9.1	0.7	3.9
.02	.02	.02	0.5	.15KCl	75					
.02	.02	.02	0.5	.15KCl	73	0.14	0.6	5.4	0.6	2.6
.02	.01	.02	0.5	.15KCl	69	0.15	0.6	6.3	0.6	6.8
.02	.02	.02	0.5	.15KNO3	74	0.14	0.6	5.8	0.6	3.5
.02	.01	.02	0.5	.15KNO3	72	0.14	0.6	6.2	0.6	6.9
.02	.02	.02	0.5	.15NaCl	72	0.15	0.7	14.4	0.7	4.1
.02	.01	.02	0.5	.15NaCl	72	0.14	0.6	8.7	0.6	6.4
.02	.02	.02	0.5	.15LiCl	74	0.14	0.6	4.3	0.6	3.4
.02	.01	.02	0.5	.15LiCl	72	0.13	0.5	8.2	0.5	5.8

POTASSIUM BINDING CONSTANTS OF TES

		[NaOH]								
.02	.02	.02	.50	.15NaCl	75	-0.68	0.6	6.8	0.6	22.5
.01	.01	.01	.50	.15NaCl	39	-0.68	0.4	14.5	0.5	114.6

Table 6.9

CALCIUM BINDING CONSTANTS OF BES (CONC IN MOL/L) T=37 °C

[BES]	[M]	[HCl]	[KOH]	MEDIA	NO OF PTS	LOG Bmhl	LOG Bmh2l+	SCOGS S.D (1E-03)	SCOGS CHI	SUPERQUAD SIGMA	SUPERQUAD S.D. (1E-03) log Bmhl	SUPERQUAD S.D. (1E-03) log Bmh2l
.02	.02	.02	0.50	.15KNO3	77	0.23	0.65	0.7	9.3	0.7	4.2	4.4
.01	.01	.01	0.50	.15KNO3	40	0.24	0.63	0.4	4.8	0.4	14.1	25.7
.02	.02	.02	0.50	.15KCl	78	0.23	0.66	0.5	6.3	0.5	3.0	3.12
.02	.01	.02	0.50	.15KCl	40	0.25	0.63	0.5	8.4	0.5	16.9	31.7
.01	.01	.01	0.50	.15NaCl	39	0.24	0.60	0.4	6.7	0.5	14.8	29.2
.01	.01	.01	0.50	.15NaCl	40	0.24	0.53	0.4	8.0	0.4	14.8	33.9
.02	.02	.02	0.50	.15NaCl	75	0.23	0.66	0.6	5.8	0.6	3.8	3.9
.01	.01	.01	0.50	.15LiCl	40	0.26	0.68	0.5	4.8	0.5	17.2	28.4
.02	.02	.02	0.50	.15LiCl	78	0.24	0.68	1.0	9.2	1.0	5.9	6.1
.01	.01	.01	0.50	.15NaCl	39							
.01	.01	.01	0.50	.15NaCl	40	0.24	0.57	0.4	3.3	0.5	10.5	22.0

POTASSIUM BINDING CONSTANTS OF BES

[BES]	[M]	[HCl]	[NaOH]	MEDIA	NO OF PTS	LOG Bmhl	LOG Bmh2l+	SCOGS S.D (1E-03)	SCOGS CHI	SUPERQUAD SIGMA	SUPERQUAD S.D. (1E-03) log Bmhl	SUPERQUAD S.D. (1E-03) log Bmh2l
.02	.02	.02	.50	.15NaCl	74	-0.17	0.06	0.5	17.2	0.5	7.8	12.8
.02	.02	.02	.50	.15NaCl	74	-0.18	0.07	0.6	6.0	0.6	9.2	13.9
.01	.01	.01	.50	.15NaCl	37	-0.17	0.16	0.5	8.2	0.4	47.3	99.9

Table 6.10

CALCIUM BINDING CONSTANTS OF HEPES (CONC IN MOL/L) T=37 °C

[HEPES]	[M]	[HCl]	[KOH]	MEDIA	NO OF PTS	LOG Kmh1	LOG Kmh21+	SCOGS S.D (1E-03)	SCOGS CHI	SUPERQUAD SIGMA	SUPERQUAD S.D. (1E-03) log Bmh1	SUPERQUAD S.D. (1E-03) log Bmh21
.02	.02	.02	.55	.15KNO3	66	0.09	1.48	1.3	10.4	1.4	14.4	1.7
.02	.02	.02	.55	.15KNO3	66	0.10	1.34	1.2	3.3	1.2	11.7	1.5
.01	.01	.02	.55	.15KNO3	44	0.07	1.16	1.7	6.9	1.8	116.9	12.7
.02	.02	.02	1	.15KCl	35	0.09	1.17	1.7	7.7	1.9	49.9	7.5
.02	.01	.02	.50	.15KCl	73	0.11	1.17	1.3	6.9	1.3	21.2	3.3
.02	.02	.02	.50	.15NaCl	35	0.05	1.21	3.8	4.5	3.8	56.1	7.3
.02	.01	.02	.50	.15NaCl	35	0.09	1.18	1.0	6.8	1.1	27.8	3.9
.02	.02	.02	.50	.15LiCl	38	0.08	1.18	0.9	8.7	0.9	11.0	1.7
.02	.01	.02	.50	.15LiCl	36	0.08	1.18	0.9	3.1	0.9	24.1	3.3
.02	.02	.02	.55	.15KNO3	66							
.02	.02	.02	.55	.15KNO3	66	0.09	1.36	3.3	7.4	3.2	23.3	2.8

POTASSIUM BINDING CONSTANTS OF HEPES

[HEPES]	[M]	[HCl]	[NaOH]	MEDIA	NO OF PTS	LOG Kmh1	LOG Kmh21+	SCOGS S.D (1E-03)	SCOGS CHI	SUPERQUAD SIGMA	SUPERQUAD S.D. (1E-03) log Bmh1	SUPERQUAD S.D. (1E-03) log Bmh21
.02	.02	.02	.50	.15NaCl	69	-0.43	0.51	0.6	9.0	0.6	18.0	2.9
.02	.02	.02	.50	.15NaCl	74	-0.43	0.479	0.6	13.4	0.6	16.8	3.0
.01	.01	.01	.50	.15NaCl	37	-0.31	0.515	0.4	8.6	0.4	45.5	9.0

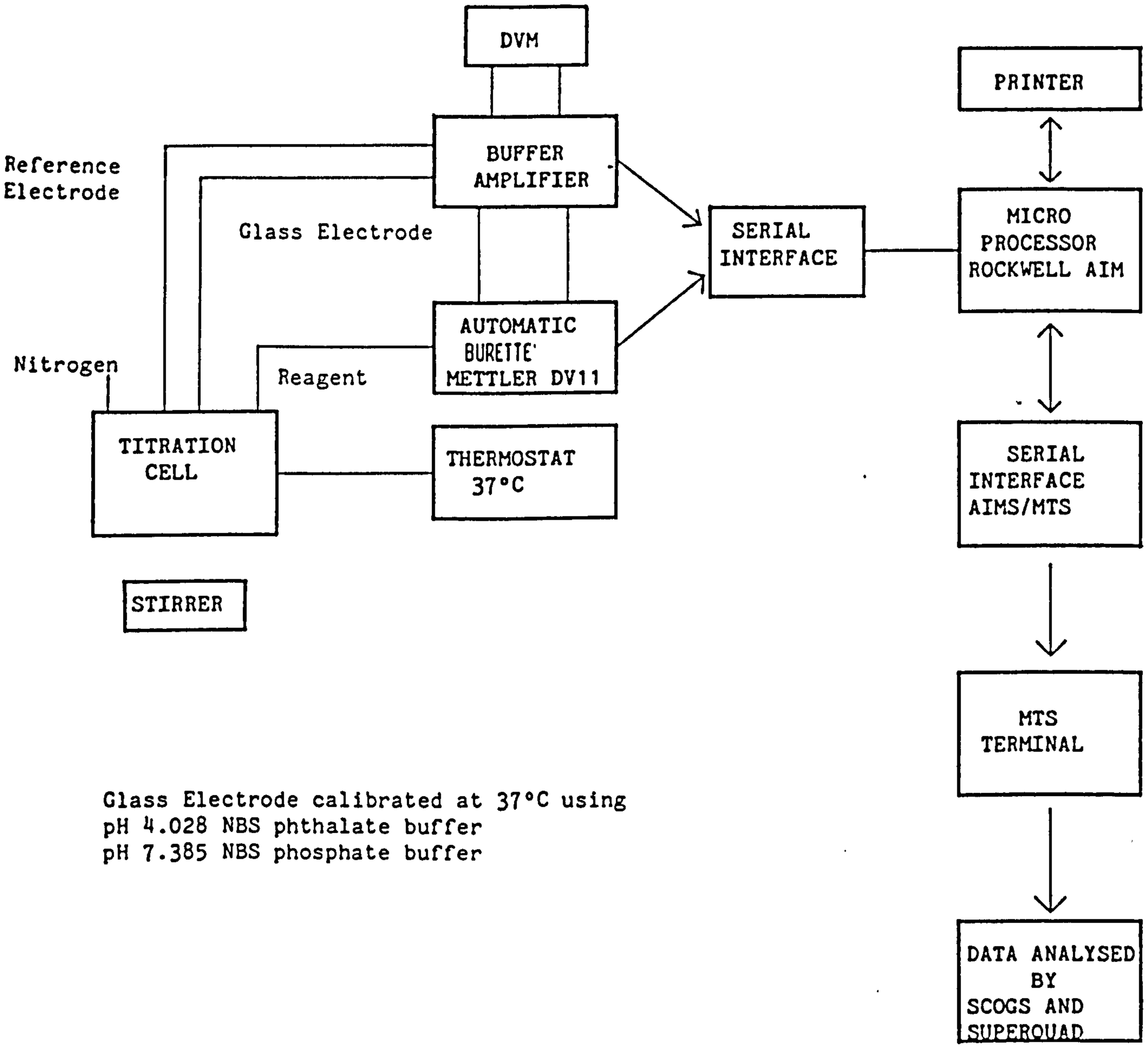
Table 6.11

Summary of dissociation constants & binding constants T-37°C

Buffer	pKa1	pKa2	pKa2(Good)	pKa2(Bates)	CALCIUM Kmh1	POTASSIUM Kmh1	%Binding [L]-100mmol/l CALCIUM	POTASSIUM
MOPS	1.06(.09)	6.99(.01)	6.93	7.04	0.37(.05)	0.05(.008)	3.57	0.50
TES	1.64(.34)	7.27(.006)	7.16	7.31	1.38(.006)	0.21(0)	12.13	2.06
BES	0.94(.35)	7.01(.003)	6.89	7.02	1.73(.01)	0.67(.006)	14.75	6.28
HEPES	3.09(.12)	7.42(.009)	7.31	7.43	1.22(.002)	0.41(.007)	10.87	3.94

Figures in parenthesis represent standard deviations

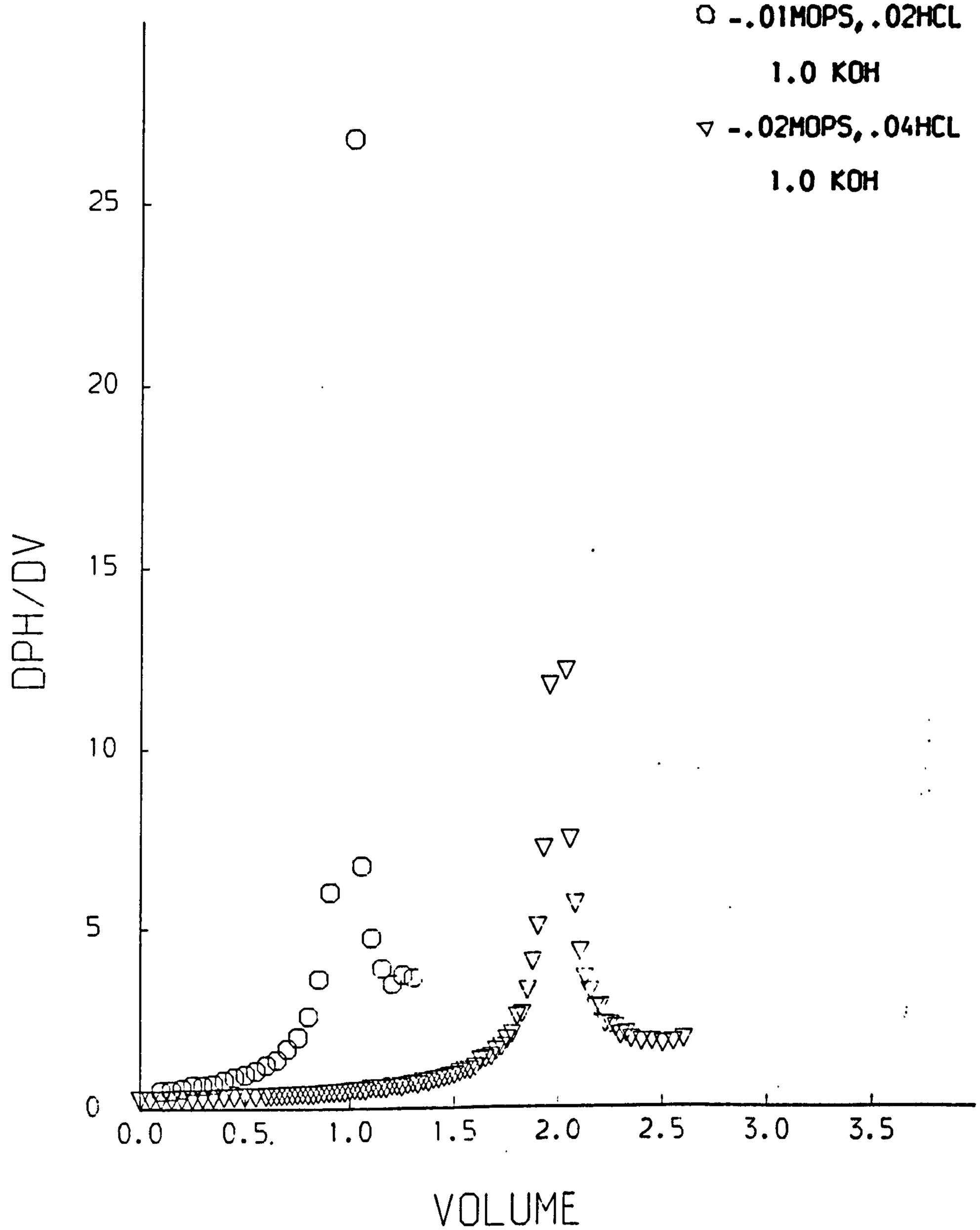
F I G U R E S



Glass Electrode calibrated at 37°C using
pH 4.028 NBS phthalate buffer
pH 7.385 NBS phosphate buffer

Fig. 6.1

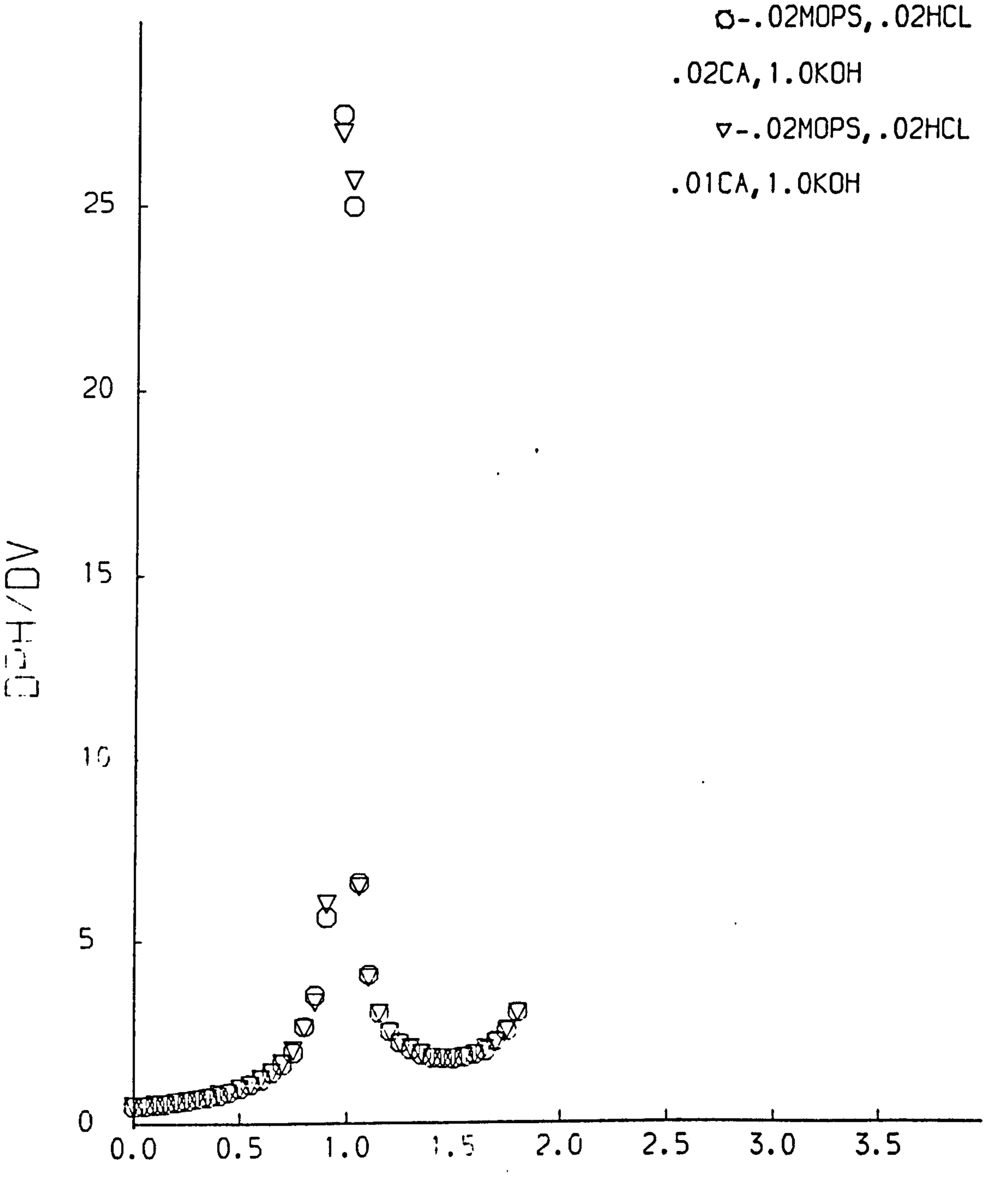
Fig. 6.2



1ST DERIV PLOT OF PKA OF MOPS

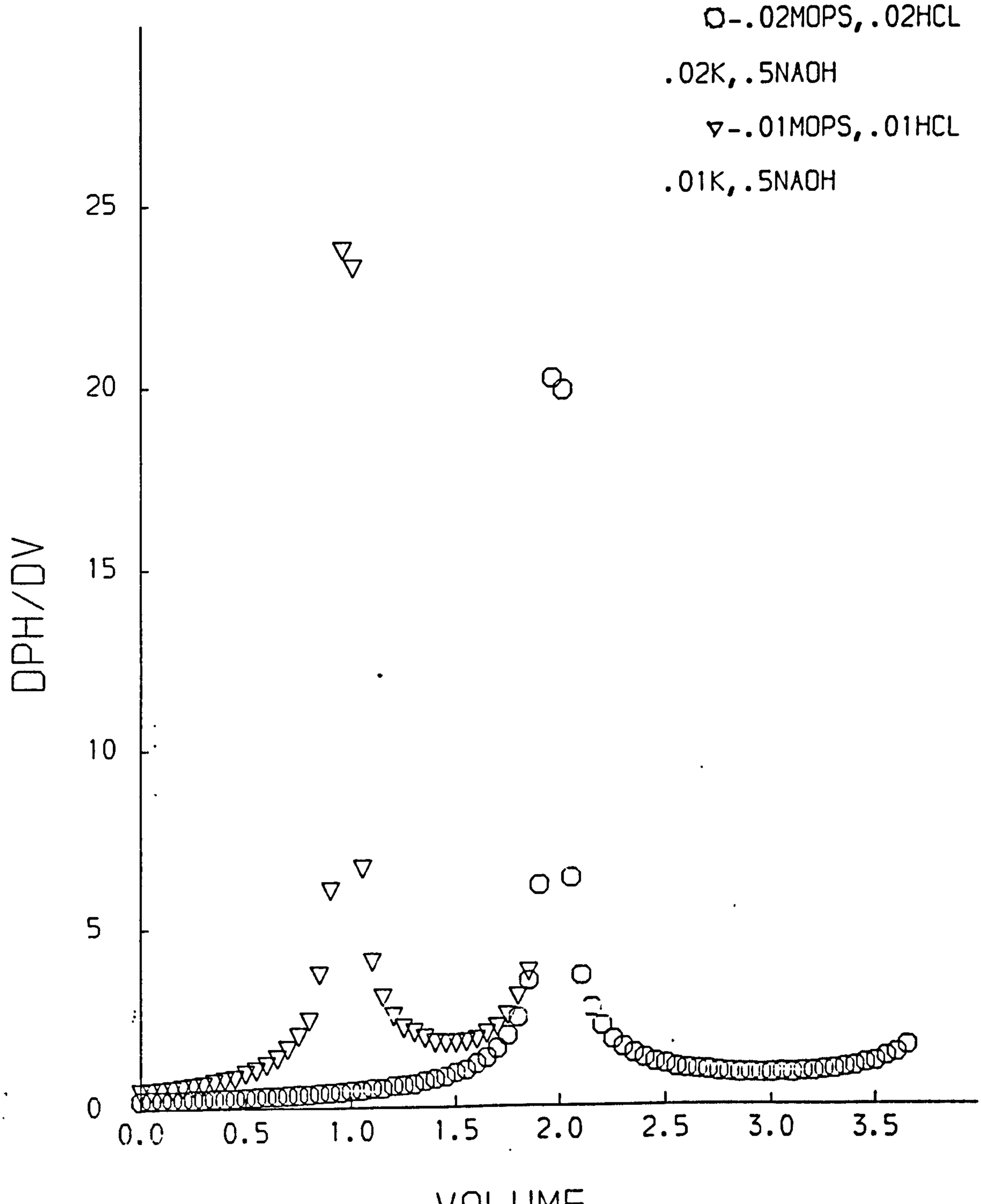
I=.15NACL

Fig. 6.3

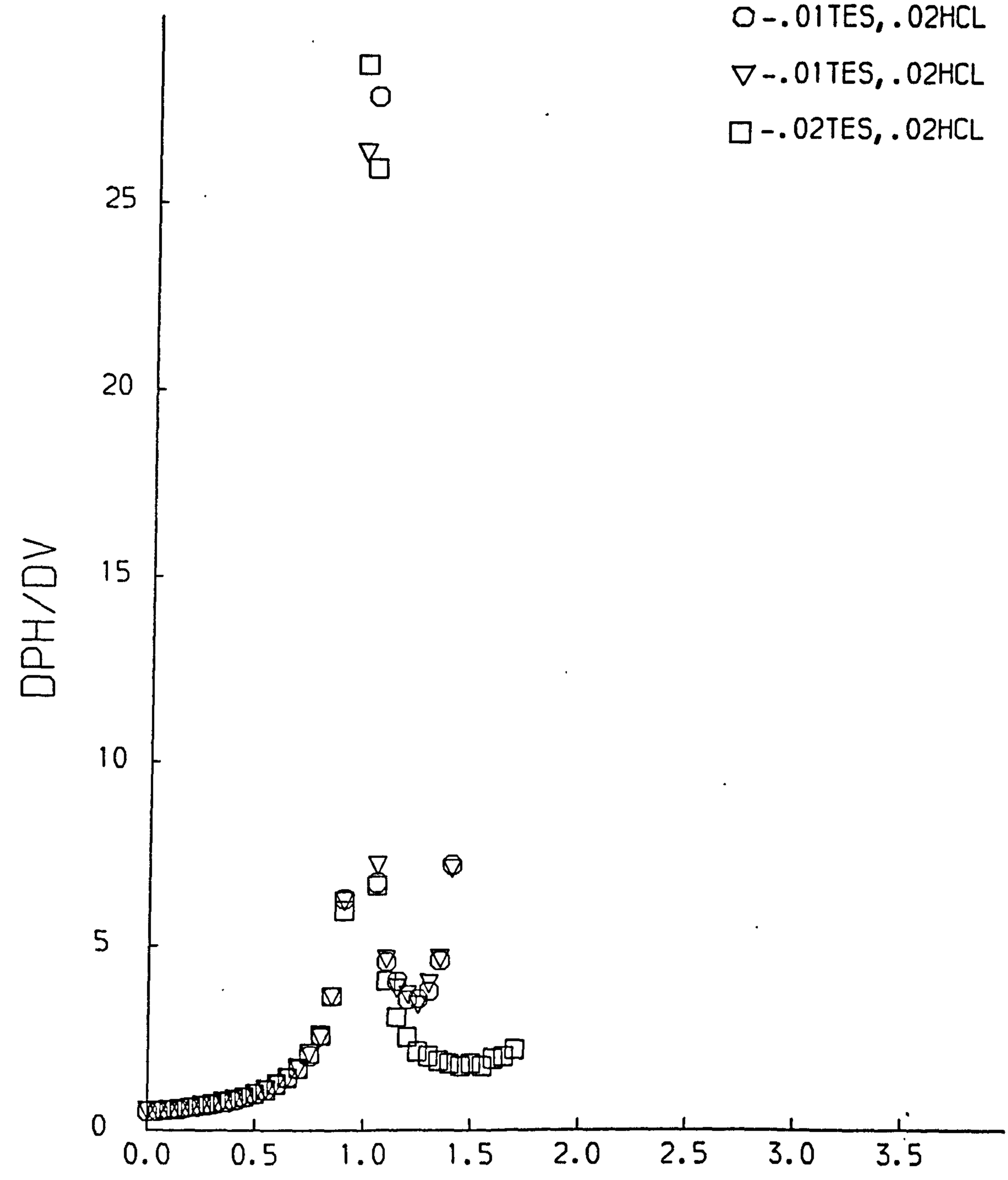


pH TITRATIONS OF MOPS + Ca
I = 0.15 NaCl

Fig. 6.4



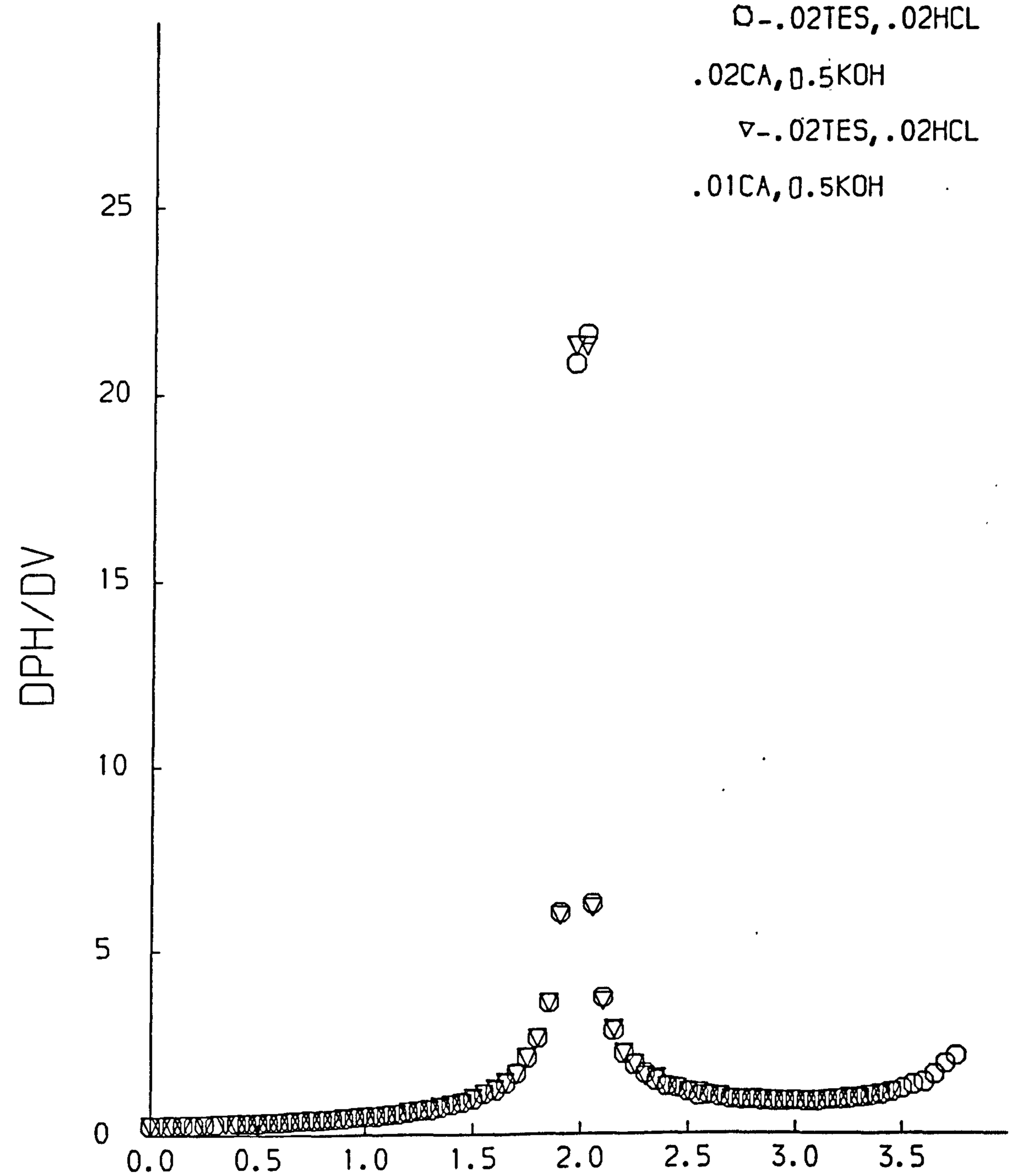
pH TITRATIONS OF MOPS + K
I = 0.15 NaCl



VOLUME

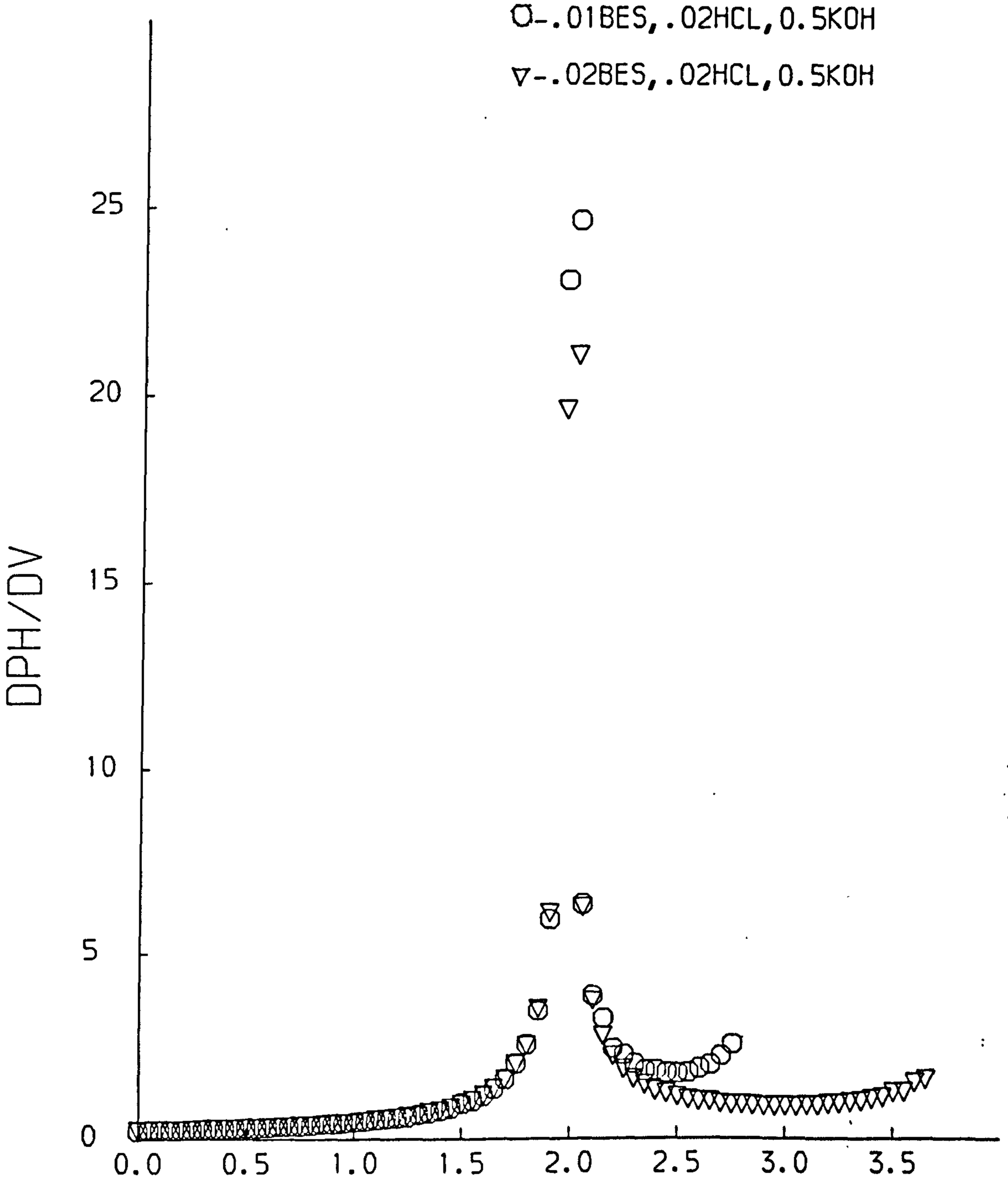
1ST DERIV PLOT OF PKA OF TES

I=.15KCL



pH TITRATIONS OF TES + Ca
I = 0.15 KCl

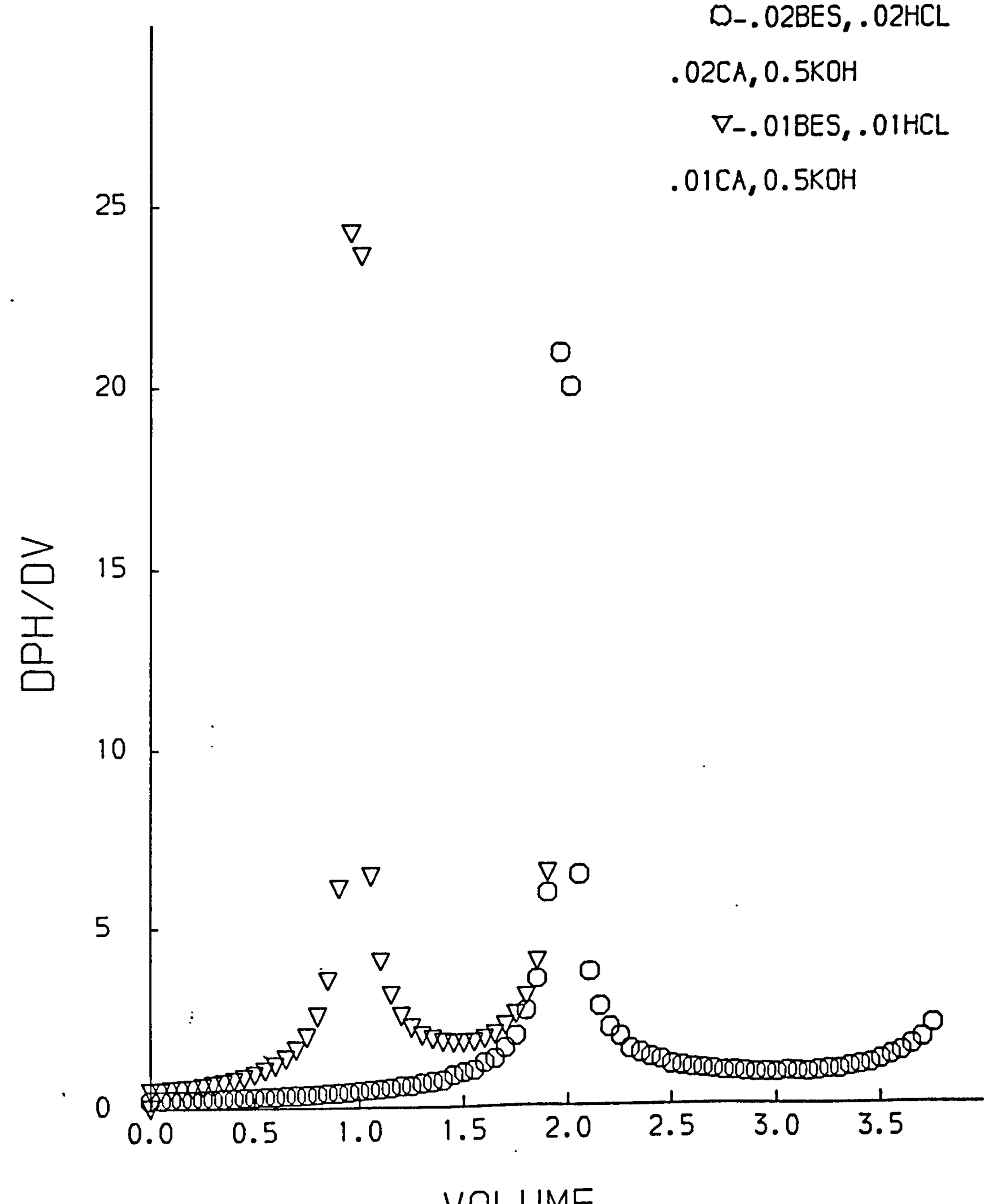
Fig. 6.7



1ST DERIV PLOT OF PKA OF BES

I=.15KNO3

Fig. 6.8



pH TITRATIONS OF BES + Ca
I = 0.15 KNO_3

Fig. 6.9

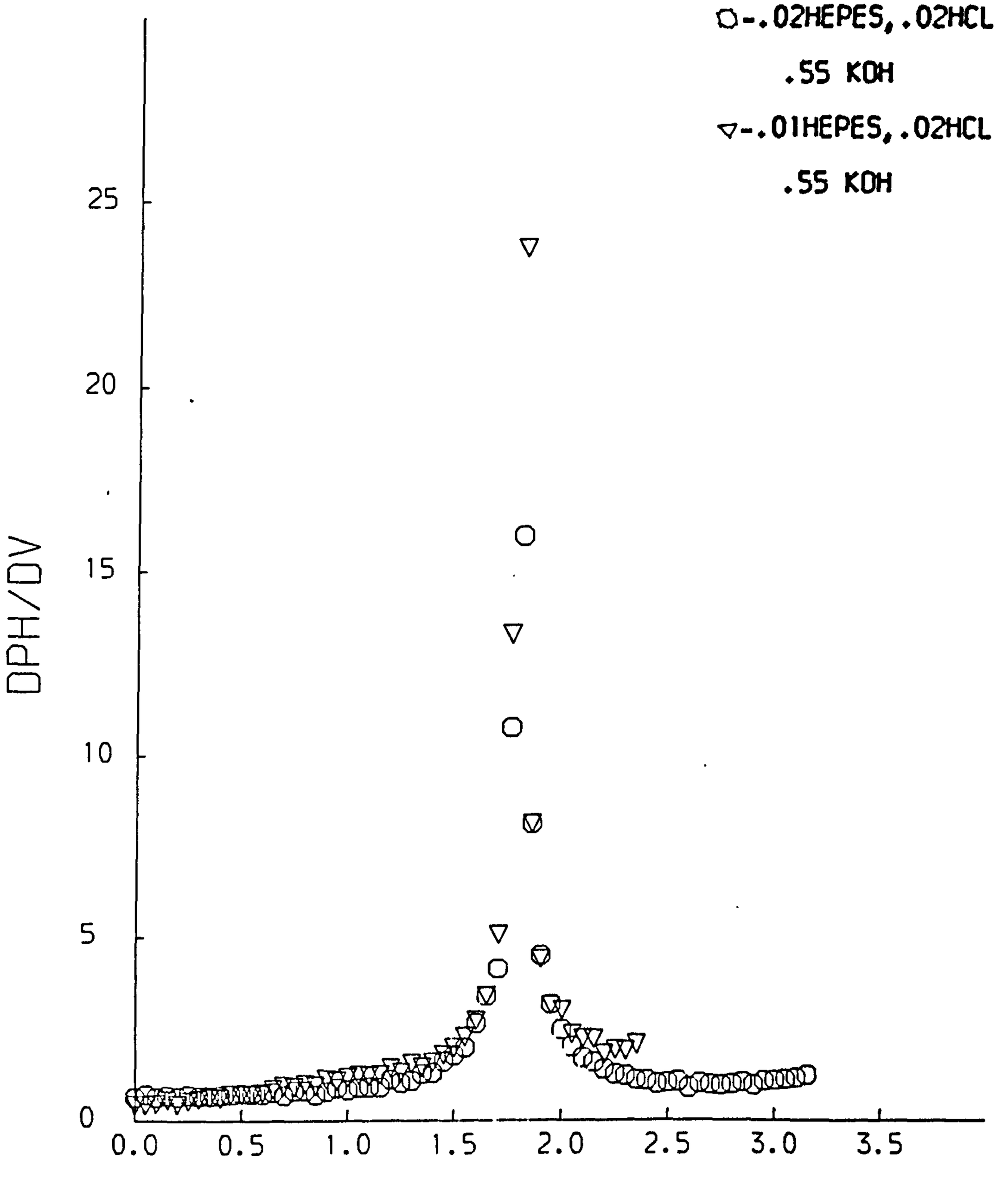
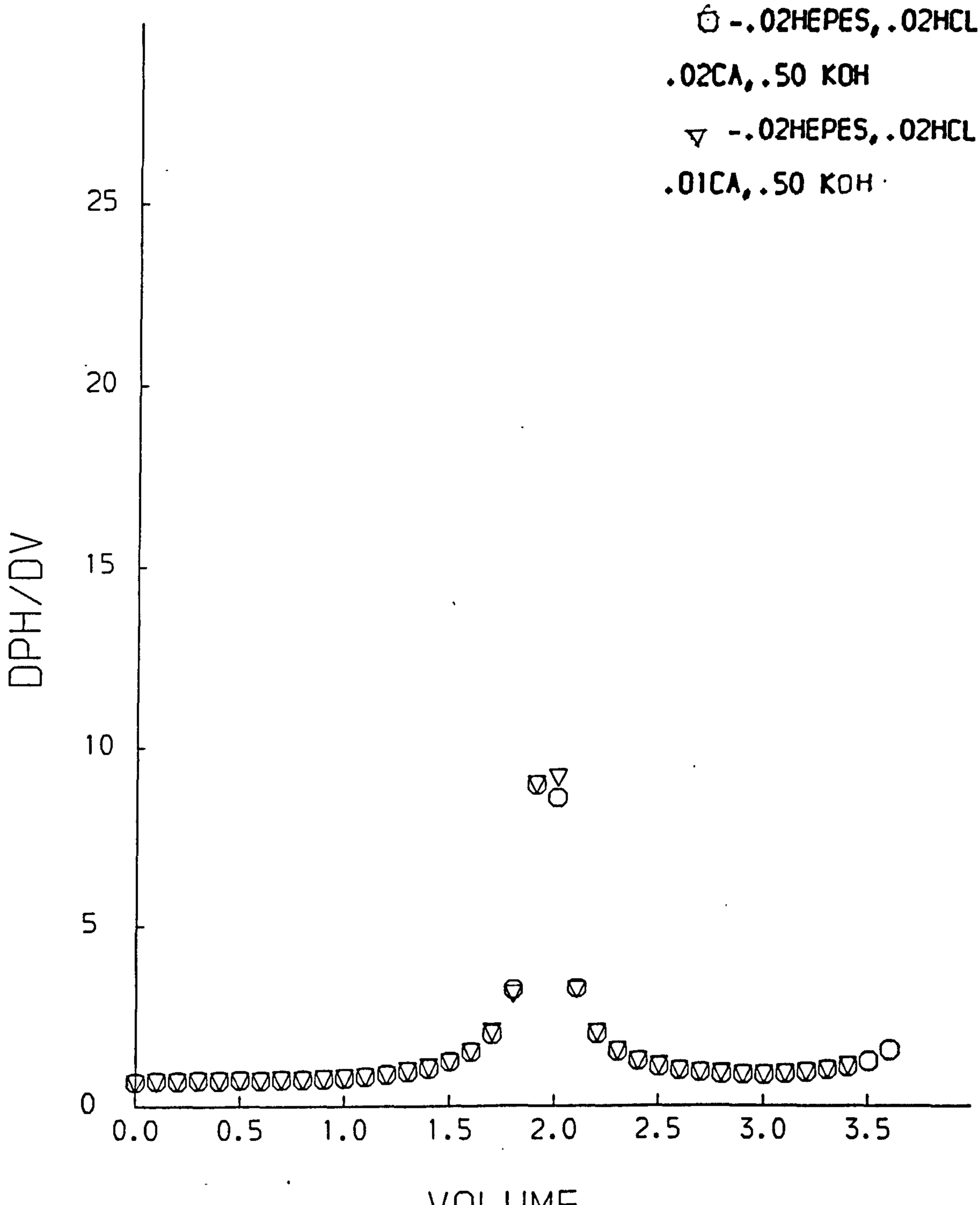

○-.02HEPES,.02HCL
.55 KOH
▽-.01HEPES,.02HCL
.55 KOH
DPH/DV
25
20
15
10
5
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
VOLUME
1ST DERIV PLOT OF PKA OF HEPES
I=.15LICL

Fig. 6.10



pH TITRATIONS OF HEPES + Ca
I = 0.15 LiCl

Fig. 6.11a

f = 1.00

TITRATION NUMBER 1 TITLE: .02MOPS,.02CaCl2,.02HCl/1KOH I=.15KNO3

COMPOSITION OF SOLUTIONS USED IN EACH TITRATION

TITRATION NUMBER	METAL 1	METAL 2	LIGAND 1	LIGAND 2	ACID	BASE	INITIAL VOLUME	PH CORRECTION	NO. OF POINTS
	MOLES PER LITER								
1	2.0000E-02	0.0	2.0000E-02	0.0	0 0	1.000000	50.000	0 0	37

BURETTE CORRECTION FACTOR : 1.00000

1 METAL IONS 1 LIGANDS 6 COMPLEXES

2 H

	M1	M2	L1	L2	H	LOG.BETA
1	1	0	0	0	-1	-11.5700
2	1	0	0	0	-2	-24.1700
3	0	0	1	0	1	7.0000
4	0	0	1	0	2	8 0000
5	1	0	1	0	1	6 7100
6	1	0	1	0	2	9 2400

-PKW-13 620 F= 1.00

NUMBER OF PARAMETERS 6 TOTAL NUMBER OF READINGS 37

STANDARD DEVIATION IN ML TITRANT WITH THE INPUT CONSTANTS : 7.579E-04

STANDARD DEVIATION IN ML TITRANT : 6.117E-04

5	1 0 1 0 1	6.5806	+- 0 0340	SHIFT=-0.1294
6	1 0 1 0 2	9.2393	+- 0 0031	SHIFT=-0.0007

1 CYCLES CALCULATED

STANDARD DEVIATION IN ML TITRANT : 6 086E-04

5	1 0 1 0 1	6.5576	+- 0.0453	SHIFT=-0 0230
6	1 0 1 0 2	9 2393	+- 2 v030	SHIFT=-0 0000

2 CYCLES CALCULATED

STANDARD DEVIATION IN ML TITRANT : 6 086E-04

5	1 0 1 0 1	6.5570	+- 0.0478	SHIFT=-0 0006
6	1 0 1 0 2	9.2393	+- 0.0030	SHIFT= 0 0000

3 CYCLES CALCULATED

STD DEV. = 0.00061

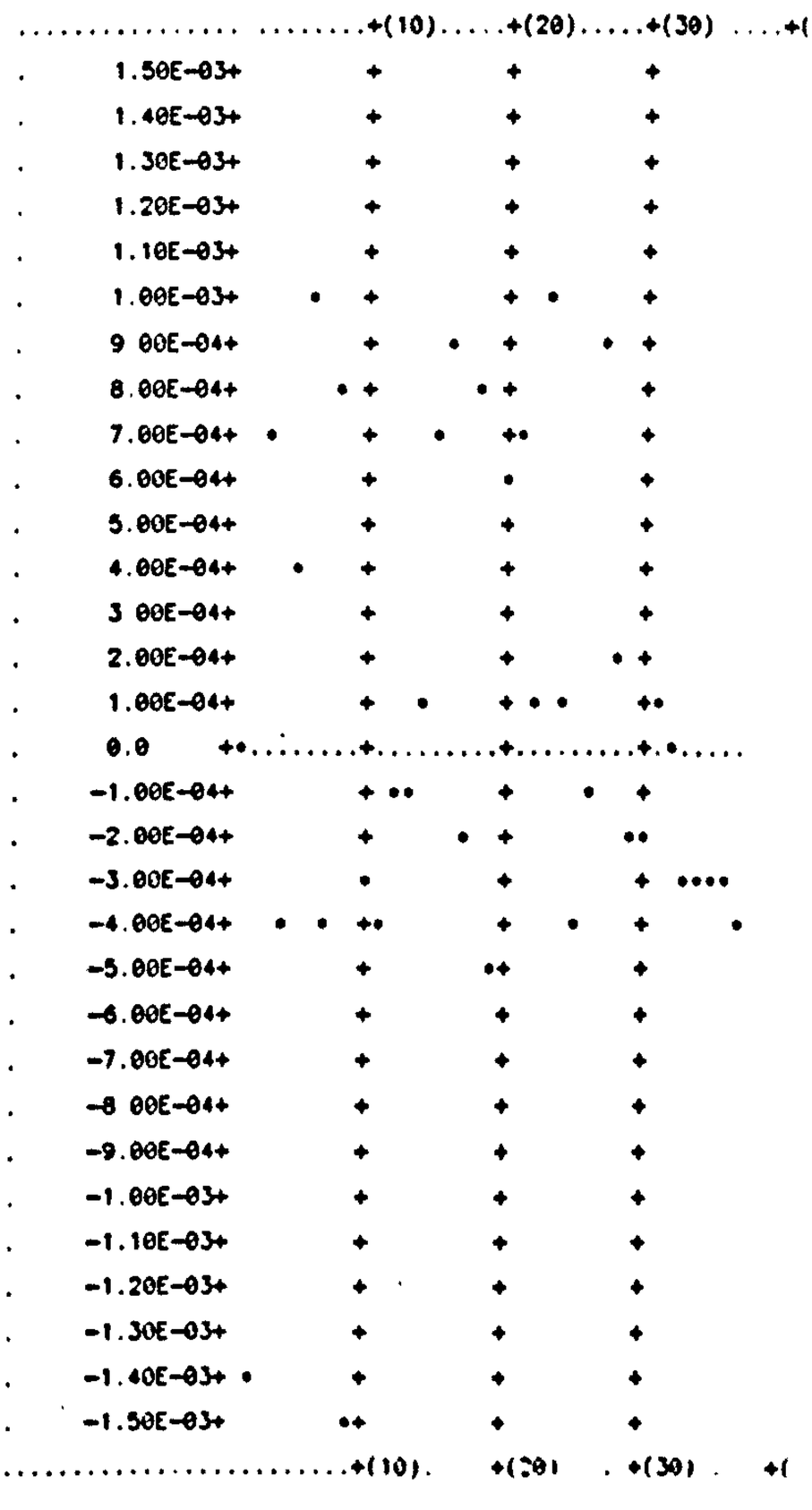


Fig. 6.11(b)

f = 0.81

TITRATION NUMBER 1 TITLE .02MOPS..02CaCl2,.02HCl/1KOH I= 15KNO3

COMPOSITION OF SOLUTIONS USED IN EACH TITRATION

TITRATION NUMBER	METAL 1	METAL 2	LIGAND 1	LIGAND 2	ACID	BASE	INITIAL VOLUME	PH CORRECTION	NO. OF POINTS
	MOLES PER LITER								
1	2.0000E-02	0.0	2 0000E-02	0.0	0.0	1.000000	50.000	0.0	37

BURETTE CORRECTION FACTOR : 1.00000

1 METAL IONS 1 LIGANDS 6 COMPLEXES

2 H

	M1	M2	L1	L2	H	LOG.BETA
1	1	0	0	0	-1	-11.5700
2	1	0	0	0	-2	-24 1700
3	0	0	1	0	1	7.0000
4	0	0	1	0	2	8 0000
5	1	0	1	0	1	6.7100
6	1	0	1	0	2	9 2400

-PKW-13.620 F= 0 81

NUMBER OF PARAMETERS 6 TOTAL NUMBER OF READINGS 37

EXTREME OVERSHIFT, CONSTANT NO. 6 SHIFT(I)= -2.164

STANDARD DEVIATION IN ML TITRANT WITH THE INPUT CONSTANTS · 8.641E-02

STANDARD DEVIATION IN ML TITRANT : 7 016E-02

5 1 0 1 0 1 6.5793 ← 3 8991 SHIFT=-0.1307

6 1 0 1 0 2 8.2600 ← 0.3516 SHIFT=-0 9800

1 CYCLES CALCULATED

EXTREME OVERSHIFT, CONSTANT NO 6 SHIFT(I)= -14 799

STANDARD DEVIATION IN ML TITRANT 6 834E-02

5 1 0 1 0 1 6 5545 ← 5 1044 SHIFT=-0 0198

6 1 0 1 0 2 7.2800 ← 2 6885 SHIFT=-0 9800

2 CYCLES CALCULATED

EXTREME OVERSHIFT, CONSTANT NO. 6 SHIFT(I)=-135.689

STANDARD DEVIATION IN ML TITRANT : 6.814E-02

5 1 0 1 0 1 6.5588 ← 5.3241 SHIFT=-0.0007

* CONTD.

* CONTD.

6 1 0 1 0 2 6.3000 ← 25 0417 SHIFT=-0.9800

3 CYCLES CALCULATED

EXTREME OVERSHIFT, CONSTANT NO. 6 SHIFT(I)=••••••••

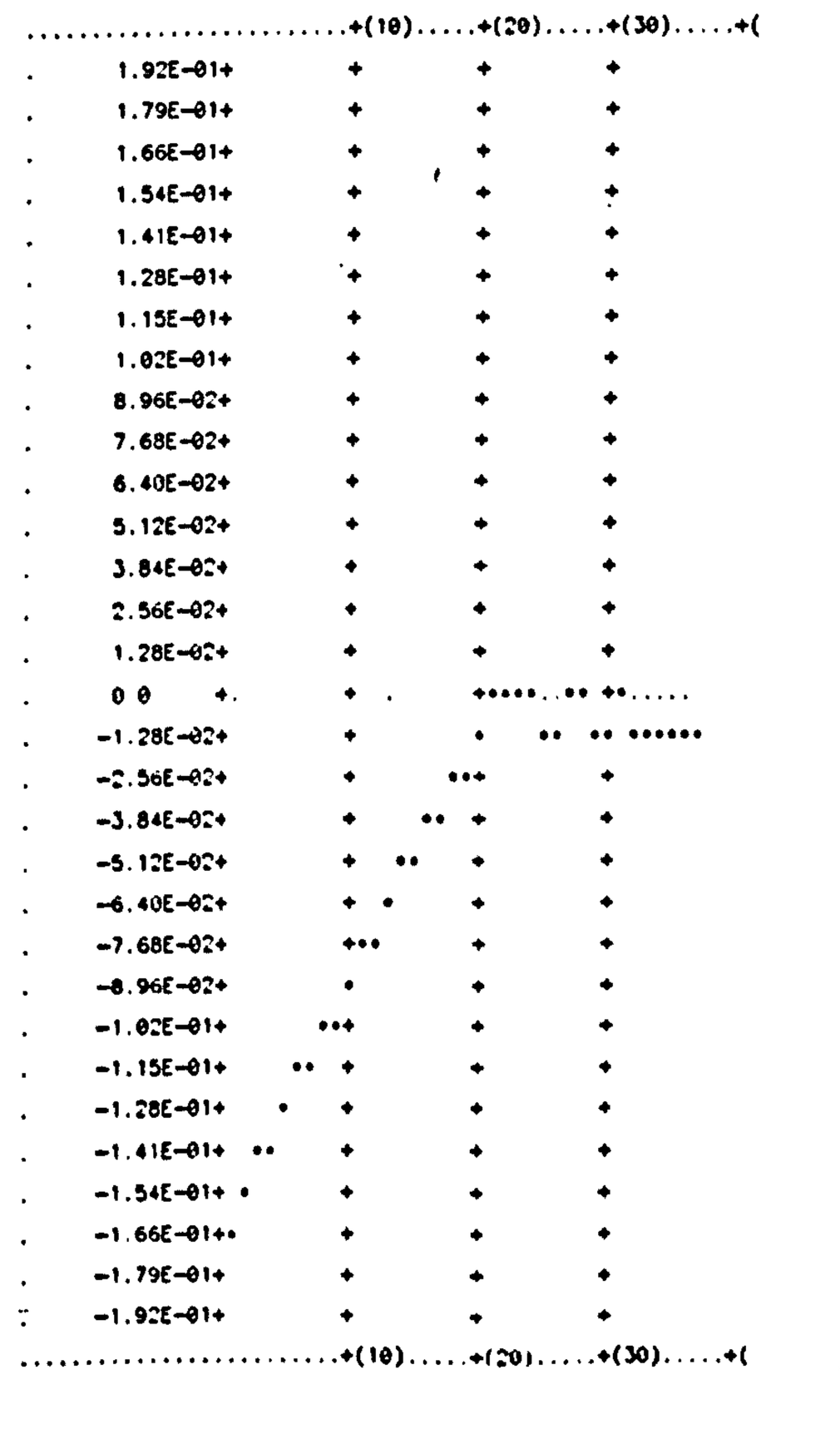
STANDARD DEVIATION IN ML TITRANT 6 811E-02

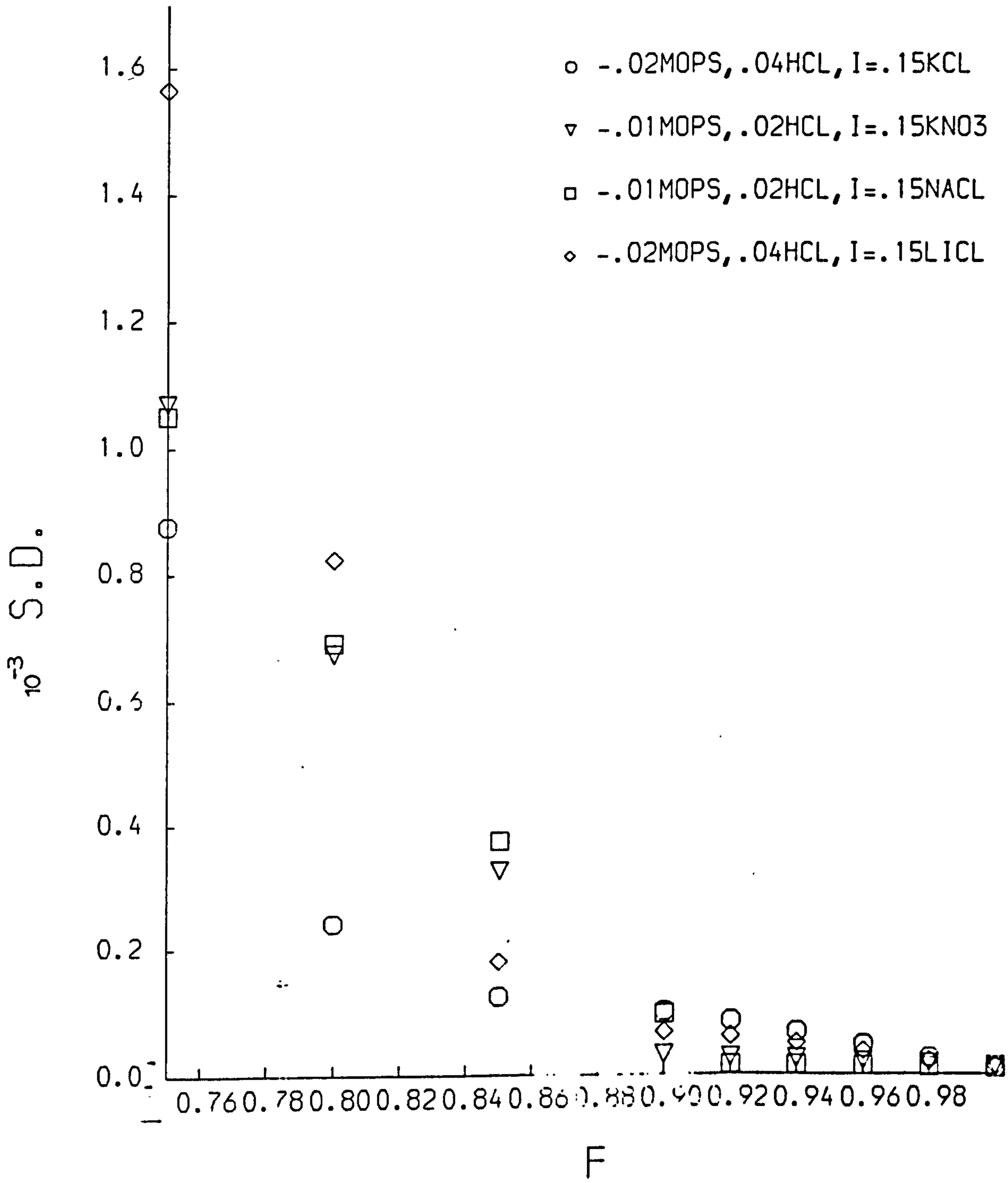
5 1 0 1 0 1 6.5670 ← 5 3296 SHIFT= 0.0082

6 1 0 1 0 2 5.3200 ←238 3167 SHIFT=-0 9800

99 CYCLES CALCULATED

STD. DEV. = 0.06811

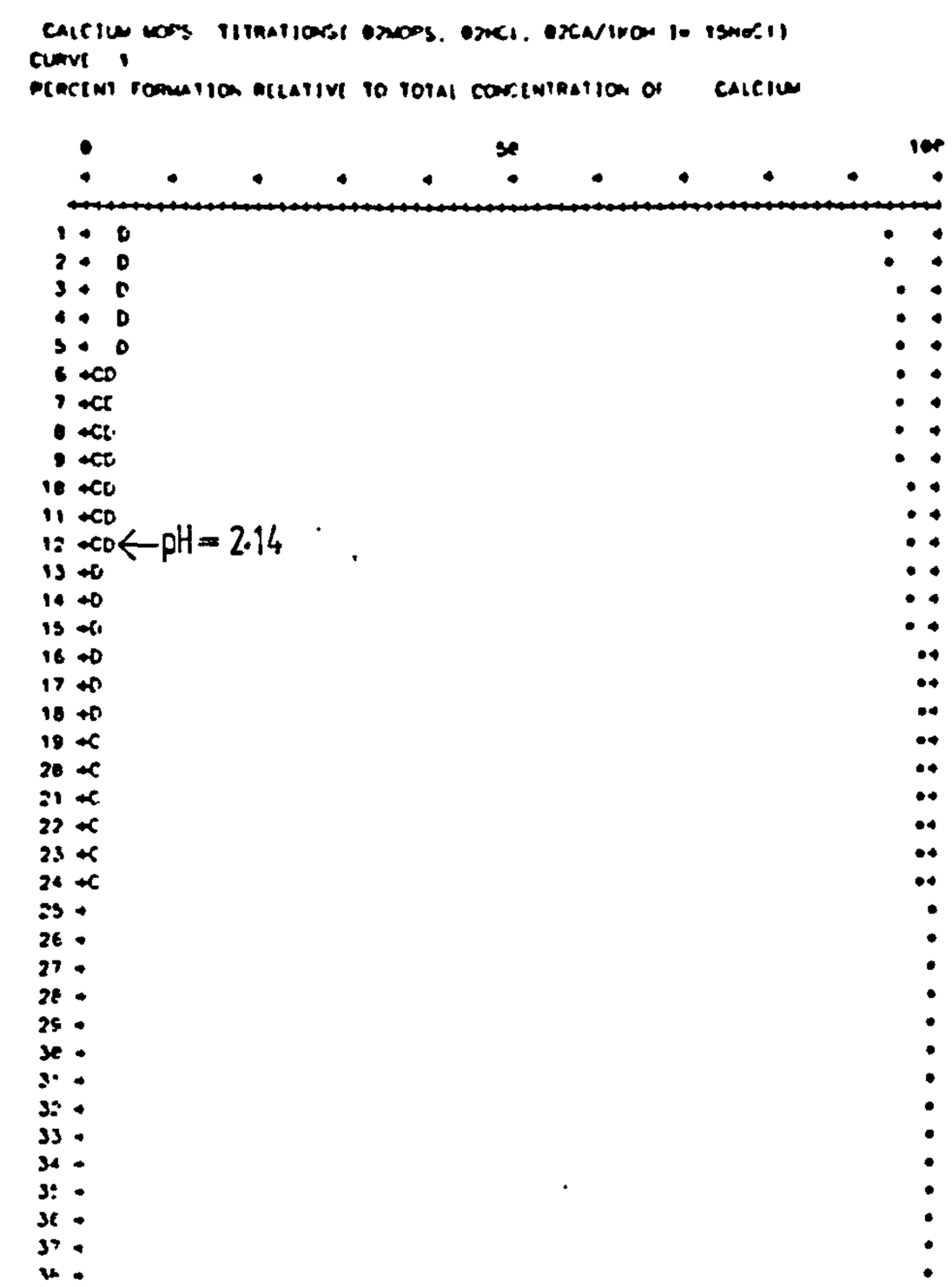




ACTIVITY COEFF EFFECTS

Fig. 6.12

Fig. 6.13


CALCIUM MOPS TITRATIONS(02MOPS, 02HCl, 02CA/1KOH I= 15NaCl)
CURVE 1
PERCENT FORMATION RELATIVE TO TOTAL CONCENTRATION OF CALCIUM
0
50
100
pH = 2.14

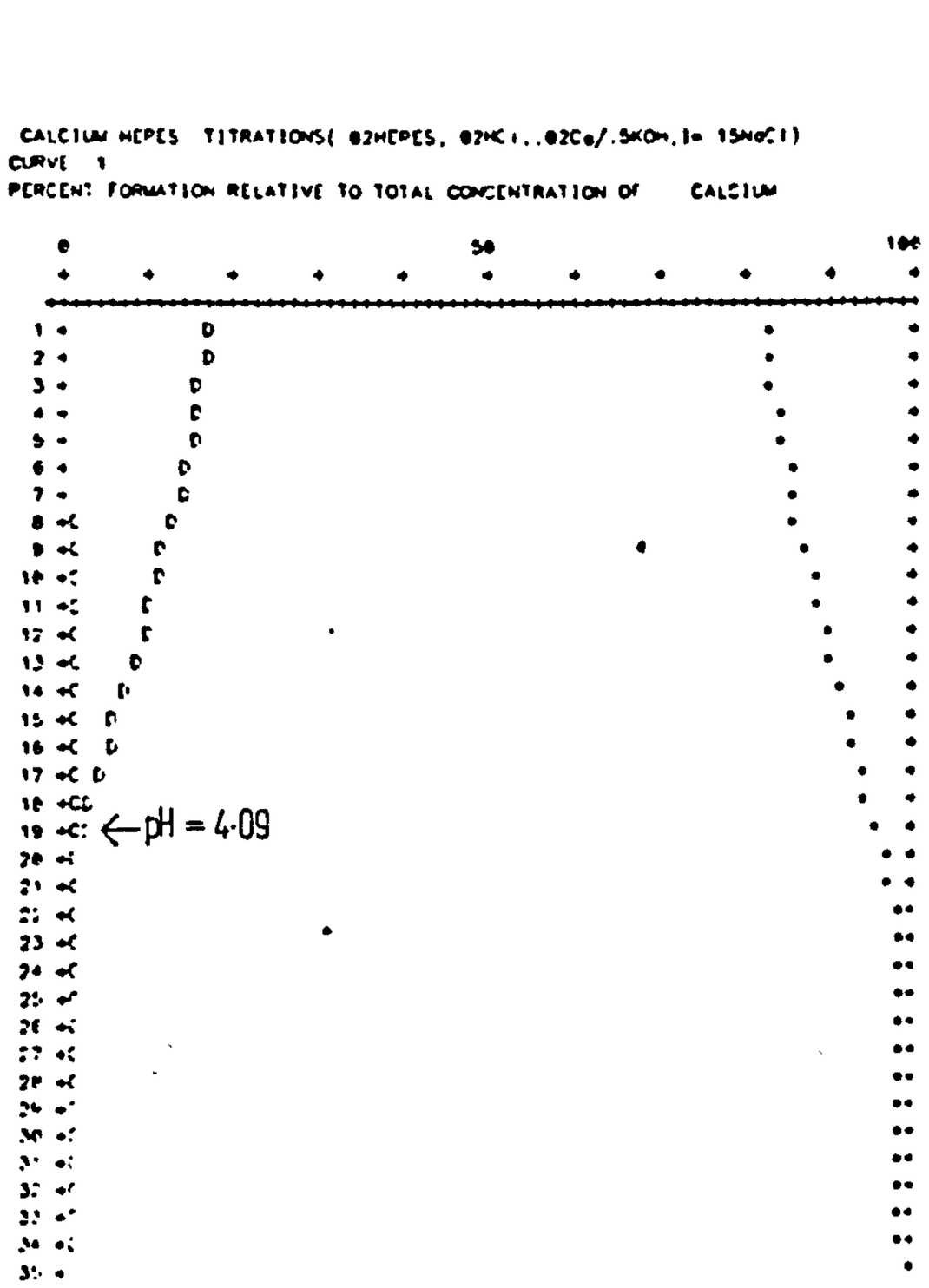
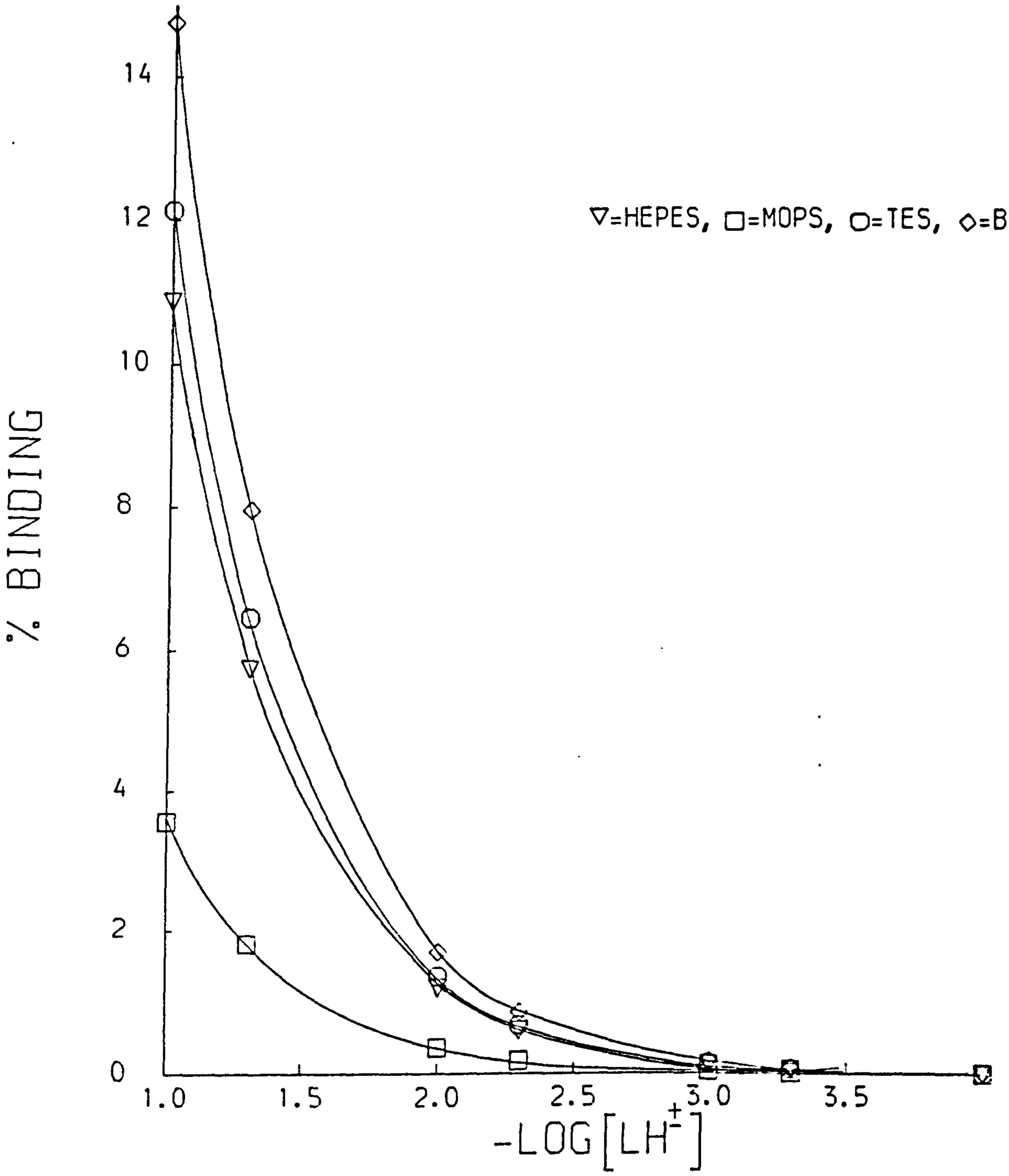

CALCIUM HEPES TITRATIONS(02HEPES, 02HCl, 02Ca/.5KOH, I= 15NaCl)
CURVE 1
PERCENT FORMATION RELATIVE TO TOTAL CONCENTRATION OF CALCIUM
0
50
100
pH = 4.09

Fig. 6.14



BINDING OF BUFFERS TO CALCIUM

REFERENCES

REFERENCES

1. GOOD, N.E., WINGET, D.G., WINTER, W. CONNOLLY, T.N., Biochemistry, 5, 467 (1966)

2. SANKAR, M. and BATES, R.G., Anal Chem, 50, 1922 (1973)

3. VEGA, C.A. and BATES, R.G., Anal Chem, 48, 1293 (1976)

4. ROY, R.N., SWENSSON, E.E., LA CROSS, G. JR., and KRUEGER, C.W., Anal Chem, 47, 8 (1975)

5. BATES, R.G. "Determination of pH", 2nd ed., Wiley, N.Y., Chapter 4 (1973)

6. CROWELL, J.A. and BOWERS, G.N., CLIN CHEM, 31, 267 (1985)

7. SENA, S.F., BURNETT, R.W., BOWERS, JR. G.N.; Private Comm.

8. BOWERS, G.N., Proc. of the Workshop on Direct Potentiometric Measurements in Blood, KOCH, W.F., ed., NBS, Gaithsburg, Maryland, 167, (1983)

9. VOGEL, A.I., "Textbook of Quantitative Inorganic Analysis", 4th ed., Longmans, London and N.Y., p 320 (1978)

10. SAYCE, I.G., Talanta, 15, 1397 (1968)

11. PERRIN, D.D. and STUNZI, M. "Computational Methods for the Determination of Binding Constants", Ed. Leggett D.J., Plenum Press, N.Y. and London, Chapter 4 (1985)

12. GANS, P., SABATINI, A., VACCA, A., J. Chem. Soc. Dalton Trans., 1195 (1985)

13. DEMING, W.E., "Statistical Adjustment of Data", Wiley, N.Y. (1943)

14. HAMILTON, W.C., "Statistics in Physical Science", The Ronald Press Company, N.Y. (1964)

15. WENTWORTH, W.E., J. Chem. Ed., 42, 96 (1965)

16. ALCOCK, R.M. et al., J. Chem. Soc. Dalton Trans, 115 (1978)

17. SABATINI, A, VACCA, A., J. Chem. Soc. Dalton Trans, 1693 (1972)

18. MARQUARDT, D.W., J. Soc. Indust. Appl. Math 11, 431 (1963)

19. FIELD, T.B. and McBRYDE, W.A.E., Can J. Chem. 56, 1202 - 1211 (1978)

20. NAGYPAL, I. and PAKA, I., Talanta, 25, 549 - 550 (1978)

21. GANS, P. and VACCA, A., Talanta 21, 45 - 51 (1974)

22. SABATINI, A, VACCA, A. and GANS, P., Talanta, 21, 53 - 77 (1974)

23. HARPER, H.W., J. Phys. Chem., 89, 1659 (1985)

24. MacINNES, D.A. and LONGSWORTH, L.G., Cold Spring Harbour Symposium Quant. Biol, 4, 18 (1936)

25. STUNZI, H., PERRIN, D.D., TEITEI T. and HARRIS, L.N., Aust. J. Chem. 33, 2207 - 2220 (1980)

26. CHILDS, C.W. and PERRIN, D.D., J. Chem. Soc. A, 1039 (1969)

27. MAY, P.M., MURRAY, K. WILLIAMS, D.R., Talanta, 32, 483 - 489 (1985)

28. Chap. 5 in Ref. 11

29. BATES, R.G. "Determination of pH - Theory and Practice", Chap. 3, Wiley, N.Y. (1973)

30. IZQUIERDO, A. and BELTRAN, J.L., Anal Chim Acta, 81, 87-96 (1986)

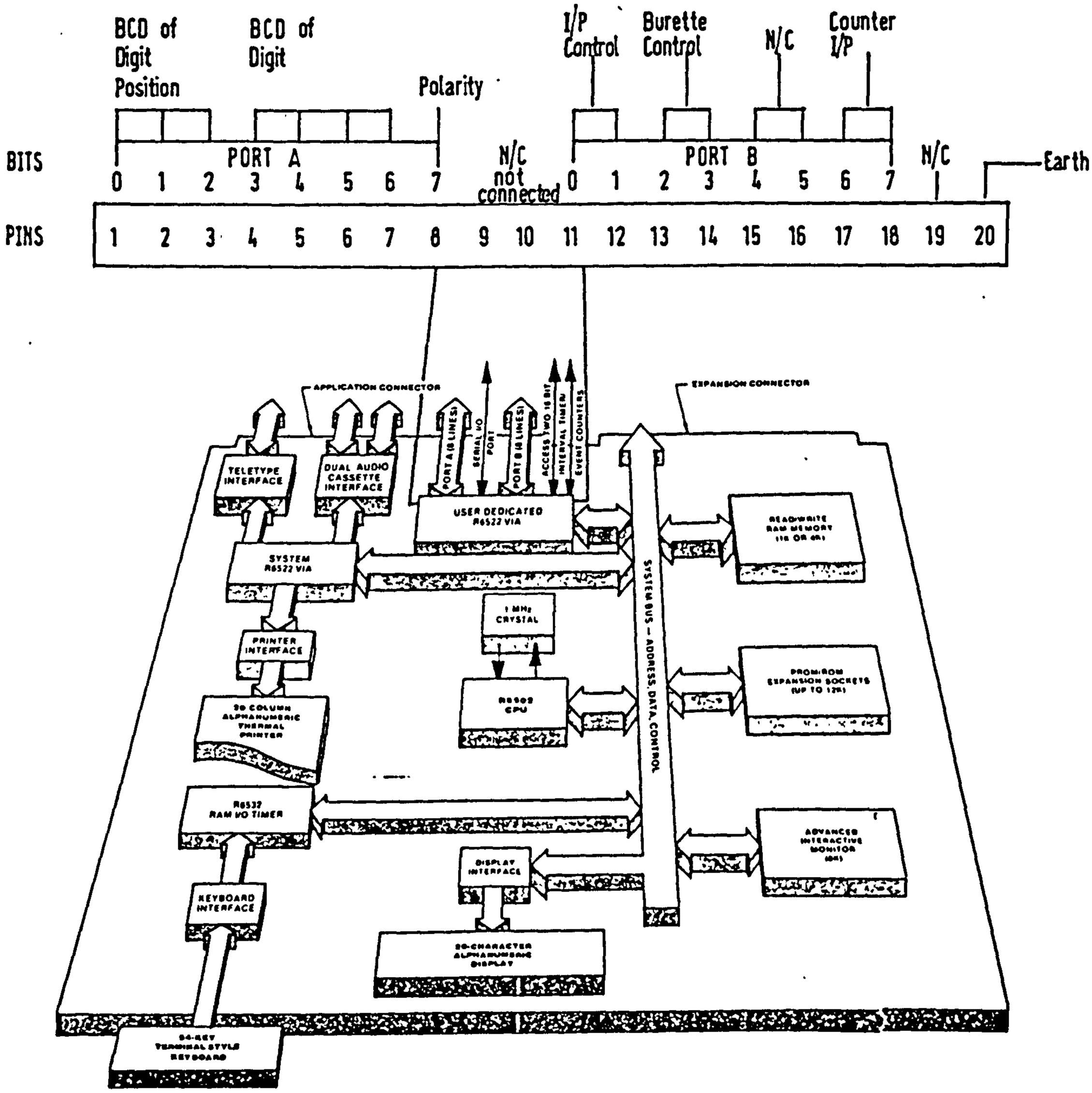
31. AVDEFF, A., in "Computational Methods for the Determination of Formation Constants", ed Leggett, D.J., Chap. 9, Plenum Press, N.Y. and London (1985)

32. AUSTIN, R., Project Report University of Newcastle Upon Tyne (December 1986)

33. CASASSAS, E., TAULER, R. and FILELLA, M., Anal Chim Acta, 191, 399-411 (1986)

34. CASASSAS, E., FILELLA, M. and TAULER, R., Anal Chim Acta, 191, 413-423 (1986)

A P P E N D I X



AIM 65 Block Diagram

Appendix 6.1

Appendix 6.2

Basic Program

```
10  REM "CALIBRATION
OF ELECTRODES"
20  PRINT "PUT ELECT
RODES IN BUFFER 1"
30  INPUT P1
40  PRINT "PH(1)=", P1
50  POKE 5,31
60  POKE 4,0
70  W=USR(0)
80  E1=0
85  GOSUB 585
90  FOR N=1 TO 5
100 POKE 4,27
110 W=USR(0)
120 E0=W/10
130 E1=E1+E0
140 NEXT N
150 E1=E1/5
155 PRINT "E1=", E1
160 PRINT "PUT ELEC
TRODES IN BUFFER 2"
170 INPUT P2
180 PRINT "PH2=",P2
190 E2=0
200 GOSUB 585
210 FOR N=1 TO 5
220 POKE 4,27
230 W=USR(0)
240 E3=W/10
250 E2=E2+E3
250 PRINT "E2=",E2
300 K=-(E2-E1)/(P2-P1)
310 PRINT "SLOPE K=",K
320 REM..TITRATION.
321 PRINT "SET UP EXPT"
323 PRINT "PH OF S="
324 INPUT PS
325 PRINT "E OF S="
326 INPUT ES
327 PRINT "SLOPE="
328 INPUT KS
330 PRINT "VOL", TAB
(5),"EMF",TAB(10),"PH"
340 DIM A(200,3)
350 G1=0
355 V0=0
360 G2=0
370 I=1
375 GOSUB 480
380 I=I+1
390 POKE 4,197
```

Remarks

Entry Points from Basic to M/C

FORMAT	POKE 4 POKE 5 W=USR( )
1. Initialisation	POKE 4,0 POKE 5,31 W=USR(0)
2. A.D.C.	POKE 4,27 POKE 5,31 W=USR(0)
3. Counter	POKE 4,187 POKE 5,31 W=USR(0)(-ve because counts down)
4. Burette on	POKE 4,197 POKE 5,31 W=USR(0)
5. Burette off	POKE 4,203 POKE 5,31 W=USR(0)
6. Time Delay (IS)	POKE 4,236 POKE 5,31 W=USR(0)

10,000 pulses { Vol of Burette / 1V

```
400 W=USR(0)
410 POKE 4,187
420 G=-USR(0)
430 G1=G
440 IF G1-G2<25 THEN GOTO 420
450 G2=G1
460 POKE 4,203
470 W=USR(0)
475 GOSUB 585
476 GOSUB 480
477 GOTO 550
480 Z=G2/500
481 A(I,1)=INT(Z*10^3+.5)/10^3
490 POKE 4,27
500 W=USR(0)
510 Q=W/18
511 A(1,2)=INT(Q+10^3+.5/10^3
520 Y=((ES-8)/KS)+PS
525 A(I,3)=INT (Y*10^4+5)/10^4
526 L=1
530 PRINT A(I,1),TA
B(5),A(I,2),TAB(18);
A(I,3)
540 RETURN
542 STOP
550 IF (A(I,1)-V0)< (5ml burette)
5 THEN GOTO 380
560 PRINT "REFILL BURETTE"
570 INPUT A
580 IF A=0 THEN GOTO 390
581 GOTO 590
585 FOR M=1 TO 30 -  Time delay (30s)
586 POKE 4,236
587 W=USR(0)
588 NEXT X
589 RETURN
590 STOP
600 FOR I=1 TO L -  Sends data to MTS
610 T$=STR$(A(I,1))
620 S$=STR$(A(I,3))
630 U$="  "
640 V$=T$+U$+S$
650 P=LEN(v$)
660 POKE 4,209
670 FOR J=1 TO P
680 POKE 256, ASC(MID $ (V$,J,1))
690 W=USR(0)
700 NEXT J
710 POKE 256,13
720 W=USR(0)
730 POKE 4,216
740 W=USR(0)
750 NEXT 1
760 END
```

Appendix 6.3
Assembler Program

```
<K>*=1F00
/99
 1F00  A9  LDA  #00
 1F02  8D  STA  A003
 1F05  8D  STA  A000
 1F08  8D  STA  A009
 1F0B  A9  LDA  #0F
 1F0D  8D  STA  A002
 1F10  A9  LDA  #20
 1F12  8D  STA  A00B
 1F15  A9  LDA  #0C
 1F17  8D  STA  A00A
 1F1A  60  RTS
 1F1B  A9  LDA  #00
 1F1D  85  STA  20
 1F1F  A0  LDA  #01
 1F22  29  AND  #07
 1F24  C9  CMP  #00
 1F26  F0  BEQ  1F51
 1F28  A0  LDA  #07
 1F2B  29  AND  #07
 1F2D  C5  CMP  20
 1F2F  D0  BNE  1F30
 1F31  A2  LDX  #00
 1F33  CE  DEX
 1F34  D0  BNE  1F31
 1F36  A0  LDA  #01
 1F39  A6  LDX  20
 1F3B  85  STA  2E
 1F3D  29  AND  #07
 1F3F  C5  CMP  20
 1F41  F0  BEQ  1F20
 1F43  A5  LDA  2E
 1F45  29  AND  #07
 1F47  4A  LSR  A
 1F48  4A  LSR  A
 1F49  4A  LSR  A
 1F4A  95  STA  24,X
 1F4C  CA  DEX
 1F4D  86  STX  20
 1F4F  D0  BNE  1F20
 1F51  A9  LDA  #00
 1F53  85  STA  31
 1F55  18  CLC
 1F56  A6  LDX  20
 1F58  86  STX  30
 1F5A  A5  LDA  24,X
 1F5C  F0  BEQ  1F67
 1F5E  A5  LDA  30
 1F60  69  ADC  #06
 1F62  CA  DEX
 1F63  D0  BNE  1F60
 1F65  85  STA  30
 1F67  A6  LDX  20
 1F69  F0  BEQ  1F7A
 1F6B  A5  LDA  30
 1F6D  69  ADC  #04
 1F6F  85  STA  30
 1F71  A5  LDA  31
 1F73  69  ADC  #00
 1F75  85  STA  31
 1F77  CA  DEX
 1F78  D0  BNE  1F6B
 1F7A  A6  LDX  20
 1F7C  F0  BEQ  1F8D
 1F7E  A5  LDA  30
 1F80  69  ADC  #30
```

```
1F82  85  STA  30
1F84  A5  LDA  31
1F86  69  ADC  #03
1F88  85  STA  31
1F8A  CA  DEX
1F8B  D0  BNE  1F7E
1F8D  A6  LDX  29
1F8F  F0  BEQ  1FA0
1F91  A5  LDA  30
1F93  69  ADC  #10
1F95  85  STA  30
1F97  A5  LDA  31
1F99  69  ADC  #27
1F9B  85  STA  31
1F9D  CA  DEX
1F9E  D0  BNE  1F91
1FA0  A5  LDA  21
1FA2  20  ROL  . 0
1FA3  90  BCC  1FA0
1FA5  A4  LDY  30
1FA7  A5  LDA  31
1FA9  20  JSR  C0D1
1FAC  60  RTS
1FAD  38  SEC
1FAE  A9  LDA  #00
1FB0  E5  SBC  30
1FB2  A8  TAY
1FB3  A9  LDA  #00
1FB5  E5  SBC  31
1FB7  20  JSR  C0D1
1FBA  60  RTS
1FBB  AC  LDY  A000
1FBE  AD  LDA  A003
1FC1  20  JSR  C0D1
<K>*=1FC1
/30
 1FC1  20  JSR  C0D1
 1FC4  60  RTS
 1FC5  A9  LDA  #00
 1FC7  8D  STA  A000
 1FCA  60  RTS
 1FCB  A9  LDA  #0C
 1FCD  8D  STA  A000
 1FD0  60  RTS
 1FD1  AD  LDA  0100
 1FD4  20  JSR  EEA8
 1FD7  60  RTS
 1FD8  20  JSR  E8D6
 1FDB  C9  CMP  #25
 1FDD  D0  BNE  1FD8
 1FDF  60  RTS
1FE0  20  JSR  E844
1FE3  20  JSR  E910
1FE6  20  JSR  EEA8
1FE9  4C  JMP  1FE2
1FEC  A9  LDA  #21
1FEE  A2  LDX  #95
1FF0  A0  LDY  #EA
1FF2  CA  DEX
1FF3  D0  BNE  1FF2
1FF5  88  DEY
1FF6  D0  BNE  1FF2
1FF8  38  SEC
1FF9  E9  SBC  #01
1FFB  D0  BNE  1FEE
1FFD  60  RTS
```

Appendix 6.4
Input File for SCOGS

```
1       SCOGSH
2       1
3       1,1,6
4       1,0,0,0,-1,-11.57
5       1,0,0,0,-2,-24.17
6       1,0,1,0,1,6.6
7       1,0,1,0,2,9.24
8       0,0,1,0,1,7.00
9       0,0,1,0,2,8.00
10      2,0
11      1
12      .02MOPS,.02HCL,.02Ca/1KOH I=.15KCL
13      .02,0,.02,0,0.0,1,50,0
14      0.0         1.7844
15       .05        1.8077
16       .1         1.8320
17       .15        1.8581
18       .2         1.8857
19       .25        1.9144
20       .3         1.9454
21       .35        1.9792
22       .4         2.0148
23       .45        2.0538
24       .5         2.0971
25       .55        2.1436
26       .6         2.195
27       .65        2.2545
28       .7         2.3222
29       .75        2.4025
30       .8         2.4998
31       .85        2.6292
32       .9         2.8085
33       .95        3.1113
34       1.0        4.4802
35       1.05       5.7328
36       1.1        6.0471
37       1.15       6.2477
38       1.2        6.4015
39       1.25       6.5242
40       1.3        6.6348
41       1.35       6.7329
42       1.4        6.8273
43       1.45       6.9146
44       1.5        7.0035
45       1.55       7.0891
46       1.6        7.1795
47       1.65       7.2707
48       1.7        7.3699
49       1.75       7.4802
50       1.8        7.6061
51       1.85       7.7578
52       1.9       -7.9628
53      -13.62,0.81
54      2,3,4
55      0 . .
```

Appendix 6.5
Input File for SUPERQUAD

```
CALCIUM HEPES  TITRATIONS(.02HEPES,.02Ca,.02HCl/.55KOH,I=.15KNO3)
 100  005    3    0
  CALCIUM
  HEPES
  PROTON
 37.00000
-11.57       1    0   -1    0
-24.17       1    0   -2    0
  7.8        1    1    1    1
  11.6       1    1    2    1
  7.42       0    1    1    0
  10.420     0    1    2    0
-13.62       0    0   -1    0

 1 1    1.0000   0.00000    0    0
   2    1.0000   0.00000    0    0
   3    2.0000  -0.55000    0    0

      50.00000   -.001

   3   0         0.00003    0

 0       2.4545
.05      2.4939
.1       2.5314
.15      2.5731
.2       2.6128
.25      2.6524
.3       2.6933
.35      2.7331
.4       2.7723
.45      2.8146
.5       2.8553
.55      2.8969
.6       2.9375
.65      2.977
.7       3.0185
.75      3.0612
.8       3.1029
.85      3.1449
.9       3.1873
.95      3.2312
1.00     3.2751
1.05     3.3203
1.1      3.3678
1.15     3.414
1.2      3.4654
1.25     3.5179
1.3      3.5713
1.35     3.6328
1.4      3.6974
1.45     3.7649
1.5      3.8462
1.55     3.9335
1.6      4.0380
1.65     4.163
1.7      4.327
1.75     4.579
```

CONTD –

```
1.8      5.0127
1.85     5.7156
1.9      6.1022
1.95     6.3211
2.00     6.472
2.05     6.5996
2.1      6.6909
2.15     6.7767
2.2      6.8571
2.25     6.9261
2.3      6.9851
2.35     7.0452
2.4      7.103
2.45     7.1586
2.5      7.2079
2.55     7.2588
2.6      7.3081
2.65     7.3535
2.7      7.4025
2.75     7.4537
2.8      7.5003
2.85     7.5485
2.9      7.5968
2.95     7.6473
3.00     7.6982
3.05     7.7533
3.1      7.8072
3.15     7.8686
3.2      7.9265
3.25     7.9943
blank line
    "
    "
    "
```

Appendix 6.6

Program for Calculating the First Derivative

```
1             DIMENSION  X(250),Y(250),A(250),B(250),C(250),D(250)
2             NX=44
3             DO 2 I=1,NX
4             READ(5,*) X(I),Y(I)
6           2 CONTINUE
7             NM=NX-1
8             DO 4 I=1,NM
9             E=X(I+1)-X(I)
10            A(I)=X(I)+E/2
11            B(I)=(Y(I+1)-Y(I))/E
12            WRITE(6,* ) X(I),B(I)
13          4 CONTINUE
33            STOP
34            END
```

```
      DIMENSION   X(250),Y(250),A(250),B(250),C(250),D(250)
      NX=39
      DO  2  I=1,NX
      READ(5,*) X(I),Y(I)
    2 CONTINUE
      NM=NX-1
      DO  4  I=1,NM
      E=X(I+1)-X(I)
      A(I)=X(I)+E/2
      B(I)=(Y(I+1)-Y(I))/E
      WRITE(6,* ) X(I),B(I)
    4 CONTINUE
      STOP
      END
      DIMENSION  X(250),Y(250)
      NX=75
      DO  2  I=1,NX
      READ(5,*) X(I),Y(I)
    2 CONTINUE
      CALL  PAPER(1)
      CALL  PSPACE  (0.2,0.75,0.25,0.95)
      CALL  BORDER
      CALL  MAP(0.0,4.0,0.0,30.0)
      CALL  CTRSET(4)
      CALL  PTPLOT(X,Y,1,37,54)
      CALL  CTRSET(1)
      CALL  CTRSET(4)
      CALL  PTPLOT(X,Y,38,75,51)
      CALL  CTRSET(1)
      CALL  AXES
      CALL  PLOTCS(1.45,-3.0,'VOLUME',6)
      CALL  CTRORI(1.0)
      CALL  PLOTCS(-0.5,12.0,'dpH/dV',6)
      CALL  CTRORI(0.0)
      CALL  CTRMAG(25)
      CALL  THICK  (2)
      CALL  PLOTCS(-0.70,-5.0,'CALCIUM  TITRATIONS  OF  MOPS',26)
      CALL  PLOTCS(1.0,-7.5,'I=.15LiCl',9)
      CALL  CTRMAG(12)
      CALL  THICK(1)
      CALL  PLOTCS(2.5,30.0,'       -.02MOPS,.02HCl',19)
      CALL  PLOTCS(2.5,28.5,'.02Ca,1.0KOH',12)
      CALL  PLOTCS(2.5,27.0,'       -.02MOPS,.02HCl',19)
      CALL  PLOTCS(2.5,25.5,'.01Ca,1.0KOH',12)
      CALL  FRAME
      CALL  GREND
      STOP
      END
```

Appendix 6.7
Ghost Program for Plotting First Derivatives

CHAPTER SEVEN

OTHER ASPECTS OF POTENTIOMETRIC MEASUREMENTS IN BLOOD

CHAPTER SEVEN

Other Aspects of Potentiometric Measurements in Blood:

(1) Liquid Junction Effects

(2) Heparin Binding of Electrolytes

(3) Carbon Dioxide Contamination of the Calibration Solutions

7.1 LIQUID JUNCTION POTENTIALS AND RESIDUAL LIQUID JUNCTION POTENTIALS

7.1.1 Introduction

Liquid junction potentials arise when two electrolyte solutions of different compositions are brought into contact. The ions diffuse at different rates resulting in a potential difference between the two solutions. In the cell

$Hg \mid Hg_2Cl_2 \mid KCl \parallel$ Test Solution, X $\mid$ I.S.E.

the double line represents the liquid - liquid junction between the test solution X and and bridge solution KCl and E_{JX} the corresponding liquid junction potential. This individual liquid junction potential cannot be determined. However, if a similar cell represented by

$Hg \mid Hg_2Cl_2 \mid KCl \parallel$ Standard Solution, S $\mid$ I.S.E.

is set up, where E_{JS} is the liquid junction potential between KCl and a standard solution, the residual liquid junction potential ($E_{JX} - E_{JS}$) can be experimentally determined.

7.1.2 Liquid Junction Potentials in Direct Potentiometric Measurements in Blood

Liquid junction potentials are a significant source of error in blood electrolyte measurements and cause considerable variation in measurements with instruments from different manufacturers. [1, 2, 3, 4] The various aspects of liquid junction that cause these discrepancies are:

1. Liquid Junction Configuration:-

Reference to Tables 3.3a and b (Chapter 3) shows the various designs of liquid junctions used by manufacturers. The criterion for choosing a particular geometry should be the establishment of a well defined reproducable liquid junction, ensuring emf stability. A constrained diffusion junction based upon a porous plug is unsuitable because it is highly susceptible to protein precipitation in the plug.[5] If a membrane is used instead of a porous plug and provided the salt bridge solution is renewed between each sample, a satisfactory junction is established. If, however, the bridge solution is not renewed, there may be a 'memory effect' in the liquid junction.[1] Flowing liquid junctions fulfill the requirement of renewal between each sample, but they are less stable than the open static junctions.[6] Open free diffusion functions are reproducible and static during measurements but are difficult to incorporate in commercial analysers.

2. Residual Liquid Junction Potentials:-

Residual liquid junction potential arises because of mismatch of the calibration solution and the sample. The error can be minimised by matching the ionic composition of the calibration solution and the sample. In the measurement of plasma, however, the solution is not so simple because the protein and bicarbonate anions present in plasma have much lower mobilities compared with the chloride anion from the potassium chloride bridge solution. The error can be reduced by optimising the calibration solutions so that the residual liquid junction potential difference at the salt bridge/

calibration solution and the salt bridge/plasma interfaces is minimised.[33] Alternatively, it is possible to calculate the residual liquid junction potential and introduce a correction factor. The residual liquid junction potential is, most commonly, calculated by the Henderson equation[7]:-

$$E_j = \{-\Sigma z_n u_n (C'_n - C''_n) / \Sigma z_n^2 (C'_n - C''_n)\} \cdot \{RT/F \ln \Sigma z_n^2 u_n c'_n / z_n^2 u_n c''_n\} \quad -7.1$$

Z_n = charge of the n^{th} species

U_n = mobility of the n^{th} species

C'_n = concentration or activity of the n^{th} species in the sample solution

C''_n = concentration or activity of the n^{th} species in the bridge solution

Henderson's equation was derived for a "continuous linear mixture" junction with solutions of constant mobility and activity coefficients. Since plasma differs widely in ionic strength and composition from saturated KCl, the conditions of constant ionic mobility and activity coefficients will be invalid. The equation is, therefore, only a rough estimate of E_j [2,4]. Hefter[2,8] has proposed the use of a constant ionic medium salt bridge (0.168 mol dm^{-3} NaCl) with low leakage plugs (vycor) to circumvent the problem of calculating liquid junction potential in this manner. Sena et al[9] suggest that, although the Henderson equation may not be vigorously correct for determining the absolute value of E_j, it is a fairly reliable estimate of the relative differences in E_j. Christiansen[1] has claimed that by incorporating an

anion of low single ion conductance, such as the HEPES anion (λ_o = 33 S. Cm^2mol^{-1}) in calibration solutions instead of phosphate buffers, it is possible to obtain solutions of the same calculated liquid junction potential as that of normal plasma.

3. The Erythrocyte Effect

The presence of erythrocytes in whole blood results in a liquid junction potential at the salt bridge/sample interface which is different from that or the corresponding plasma sample[10,11]. Siggaard-Andersen et al.[11] explains the effect as crenation of the red cells caused by the concentrated salt solution which results in precipitation of proteins of the cell membrane and exposure of the precipitated hemoglobin. The hemoglobin acts as a poly-electrolyte ion exchanger which affects the diffusion of the ions. In the case of KCl, the Cl^- is bound more strongly than K^+ creating a positive diffusion potential while in the case of NaCl, Na^+ is bound more strongly causing a negative diffusion potential. The bias depends on the type of liquid junction used and the concentration of the electrolytes[11,34]

Methods suggested for compensating this effect are (a) using a 4mol dm^{-3} sodium formate salt bridge solution [12] (b) using a dynamic flowing junction [13] (c) using a mixture of salts of opposite effects (i.e. with biases of opposite signs)[11] (d) by interposing a bridge of plasma originating from the blood sample being measured [14].

4. The Liquid Junction Temperature

Siggaard-Andersen[6] has shown that the pH of plasma is 0.01 too high if the temperature of the liquid junction is 10^o C lower than that of the electrode. This is due to differences in ion activity and ion mobility at different

temperatures. To avoid this effect, the whole measuring system should be thermostated at 37 ^{o}C [14]

7.1.3 Determiantion of Residual Liquid Junction Potentials of some 'Good' Buffers

(a) Method

Experiments were carried out to determine the presence of any appreciable residual liquid junction potential between NBS 1:1 phosphate buffer and the calibration solutions used initially (Table 4.3, Chapter 4). The method used was similar to that used by Rebelo [15].

Emf measurements were made on the operational cell represented by

$$Pt, H_2 \left| \text{Standard (S)} \right\| \text{3.5 mol dm}^{-3}\text{KCl} \left\| \text{Calibration Soln (x)} \right| H_2, Pt \quad \text{- I}$$

The pH of (x) is given by

$$pH(x) = pH(S) - [(E_x - E_s)/k + (E_{JX} - E_{JS})/k]$$

where pH(x) = pH of the Calibration Solution

pH(S) = pH of the 1:1 NBS phosphate buffer

(0.025 mol dm^3 KH_2PO_{4+} + 0.025 mol dm^{-3} Na_2HPO_4

pH = 6.841 at 37 ^{o}C)

k = Nernstian slope = 61.54 mV at 37 ^{o}C

The term within the square brackets is the emf of the cell and includes the residual liquid junction potential term $\Delta E_j = (E_{JX} - E_{JS}/k$

Emf measurements were also made in cells without liquid junctions represented by

$$Pt, H_2 \left| \text{Calibration Solution (X)} \right| AgCl \left| Ag \right. \quad \text{-II}$$

From the emf of this cell and using the Nernst equation,

$$E = E^{o} - k \log a_H a_{Cl} \quad \text{- 7.2}$$

$$\text{or } (E - E^{o})/k = -\log m_H \gamma_H \, m_{Cl} \gamma_{Cl} \quad \text{- 7.3}$$

(a = activity term, m = concentration terms,

γ = activity coefficient terms)

or $(E - E^{o})/k + \log m_{Cl} = - \log m_{H} \gamma_{H} \gamma_{Cl} =$

$P_{(a_H \gamma_{Cl})}$ - 7.4

where $P_{(a_H \gamma_{Cl})}$ is the acidity function[16]

(E^{o} for the Ag/Agcl electrodes at 37 ^{o}C is 214.2 mV [18])

Pa_H values can be obtained by the expression

$Pa_H = P(a_H \gamma_{Cl} + \log \gamma_{Cl}$ - 7.5

The single ion activity coefficient for the chloride ion was calculated using the Pitzer equation[31]. (Chapter 4).

$$\ln \gamma_{Cl} = -0.36208 + 2.56061 m_{Ca} + 0.471361 m_{Na}$$
$$+0.3502892 m_{K} - 1.4952833 m_{Ca} m_{Cl}$$
$$0.2448407\ m_{Na} m_{Cl} - 0.1978044\ m_{K} m_{Cl}$$
$$-0.015 m_{Ca} m_{K} - 0.0018 m_{Na} m_{K} \quad \text{- 7.6}$$

For solutions which do not contain the chloride ion, values of $pa_H \gamma_{Cl}$ are obtained at various chloride concentrations and the value of the function at zero chloride concentration is obtained by extrapolation. The operational cell,[15,17] shown in Fig. 7.1 was used with hydrogen electrodes for determining pH values. The liquid junctions were formed within the capillary tubes. The cell was cleaned with CCl_4, chromic acid and distilled water and dried in an oven. A light film of grease was applied to the top and bottom of the Y tap. Arm 1 was filled with 3.5 mmol dm^{-3} KCl solution and the tap was carefully rotated to allow solution half way up the capillary tubes. Arms 2 and 3 were filled with the phosphate standard and Good buffer respectively. These solutions were then slowly forced into the capillaries with the aid of a syringe fitted with a needle (the point of the needle was flattened).

Great care was taken to avoid the two solutions from mixing thereby destroying the liquid junction. Freshly plated hydrogen electrodes were made from previously used ones. The base of these electrodes is a 1 cm^2 plate of Pt-foil spot welded on to 10cm of S.W.G. 26 Pt wire and sealed into a B.19 cone (Fig. 7.2). These were cleansed in 50% aqua-regia to remove the Pt-black surface, then washed in concentrated nitric acid and distilled water. They were then platinised using chloroplatinic acid at a current of 10 mA per electrode for 3 minutes. Hydrogen was supplied at a steady rate through needle valves (Edwards High Vaccuum Limited) and copper tubing. Presaturators filled with the solution in the cell, were used to saturate the hydrogen gas with water vapour.

Pa_H values were obtained using the Harned cell shown in Fig. 7.3 in conjunction with a hydrogen electrode and a silver/silver chloride electrode (Section 5.3.2, Chapter 5). The dimension of the capillary was 0.5 mm. This construction is necessary to prevent silver ions reaching the hydrogen electrode and being reduced. An inverted test tube was placed over the hydrogen waste tube to prevent solution contamination by atmospheric oxygen or carbon dioxide.

The temperature, in the cells, was maintained at 37° C in a well-lagged water bath with a Techne (Type TU8) temperature control unit. The emf of the cells was read on a Data Precision 3500 DVM, connected to a Hewlett Packard HP-9815A microcomputer programmed to read the average of five emf values every five minutes.

(b) Results and Discussion

The results are shown in Table 7.1a and b. The operational pH values were obtained using equation 7.1. The Harned cell, pa_H values were obtained using equations 7.4, 7.5 and 7.6.

From the residual liquid junction potential values, it is apparent that the solutions with added ions have a lower error than those with only the buffer. The highest error observed with the former is +0.008 pH which is equivalent to -0.5 mV decade $^{-1}$; a small error well within the limits of experimental precision. The error would be reduced even further if the ionic strength of the phosphate standard (0.1 mol dm^{-3}) was matched with that of the experimental solution (0.16 mol dm^{-3}).

Further work using plasma and the calibration solutions is required for a thorough investigation of residual liquid junction errors and means of reducing any significant error.

7.2 EFFECT OF HEPARIN

7.2.1. Introduction

When whole blood or plasma is analysed, heparin is often used as an anticoagulant. Heparin is a polyelectrolyte of repeating disaccharide units (Fig. 7.4). Since its discovery in 1916 by McLean[19], neither its role in whole blood not its mechanism as a potent anticoagulant is fully understood.[20] When it is brought into contact with solutions containing counter ions there is a competition for the binding sites. The most satisfactory theoretical framework to analyse the interactions of single micro ions (inorganic ions, as opposed to macro ions such as proteins) with heparin seems to be Manning's condensation theory[22]. An alternative approach is a site binding theory involving chemical equilibrium[21,23,24].

Herwats et al.[21] and Delville and Lazlo[23] have applied both these approaches to study the interaction between micro ions and heparin and have arrived at similar conclusions using either theory. The complexing aptitude of heparin towards various cations seems to depend on the valence of the ion and follows the sequence

$Ca^{2+} > Mg^{2+} > K^{+} > Na^{+} > Li^{+}$

Analysis of the distribution of counter ions, surrounding heparin, in a mixed counter ion system such as the physiological environment is yet to be performed. Öbrink et al.[25] have studied the sedimentation and diffusion coefficients of heparin sulphate from human aorta in buffers of physiological ionic strength containing either NaCl or $CaCl_2$. The results indicate that the molecule contracts in the presence of calcium, presumably due to an increased binding of counter ions. In the physiological ratio of Na:Ca, however, the polysaccharide sedimented as the sodium salt due to the low calcium concentration. Lages and Stivala[26] have also indicated that calcium at low concentrations does not cause any major changes in heparin configuration.

The interference from heparin in the measurement of sodium, potassium and calcium has been reported by several workers. Brauman[27] et al. tested the effect of two different kinds of heparin at the recommended concentration of 1 ml of blood to 10μl of heparin from either Radiometer (8 IU heparin) or Roche (50 IU heparin). The latter showed an average of 3.2% lower calcium results. Mann and Green[28] noticed an interference from heparin in commercial heparinised tubes in the measurement of plasma sodium by I.S.E's They studied the effect by adding increasing quantities of mucus heparin to serum to produce a range of lithium heparin from 100 to 1000 IU/ml. Sodium and

potassium were determined. These results are shown as Fig. 7.5. They also studied the effect of small sample volumes collected in large sized commercial heparinised tubes and concluded that this caused the lowering of sodium values. They recommend the use of 2ml size pre-heparinised tubes. Narayanan[29] examined the effect of two concentrations of heparin salts (sodium, lithium and ammonium heparin) on sodium and potassium measurements by indirect and direct potentiometry. The levels of heparin used were 14.3 IU/ml and 28.6 IU/ml representing the level of heparin in fully filled and half filled VACUTAINER Brand Tubes. He concludes that although some statistically significant differences were seen at the higher concentration level, these differences are not clinically significant. Another aspect of variations that may be caused by non-standard addition of heparin is the fact that heparin from various origins differ in concentrations and anticoagulant activity[30]. To avoid such variations, the use of heparinised tubes and syringes should be standardised.

7.2.2 Experimental

A few preliminary experiments were performed to investigate the effect of Na-Heparin on potassium and calcium devices. The heparin used was PULARIN containing 0.15% w/v chlorocreosol as preservative (DUNCAN FLOCKHART and Co.). Pularin is supplied in 5 ml vials; 5 mls being equivalent to 125000 IU of heparin (1 mg = 155 IU).

The K devices used were

1. An Eμ 145 FET with a potassium sensitive electroactive membrane. The mix was

300 mg DL-n-Butyl Phthalate

5 mg Valinomycin

165 mg PVC

0.5 mg. Tetra-phenyl-borate

dissolved in 5 ml THF.

2. A CORNING 902 electrode.

3. A PHILIPS (PYE) electrode.

The calcium devices were:-

1. Radiometer

2. CORNING 903

3. E μ 145 with a PHILIPS (PYE) electroactive membrane.

Fig. 7.6 shows the results of adding various concentrations of heparin to a 10^{-3} mol dm^{-3} KCl solution using a E μ 145 device. There appears to be a rapid change initially, gradually falling off at about 64IU/ml.

Fig. 7.7 shows the calibration curves for an E μ 145 device with 0, 2, 20 and 200 IU/ml of heparin. The average slopes/decade were

0 IU/ml - 58.3 mV

2 IU/ml - 59.5 mV

20 IU/ml 61.3 mV

200 IU/ml 62.7 mV

Figs. 7.8 - 7.11 show the results of titrating 10^{-3} mol dm^{-3} $KCl/CaCl_2$ with Heparin. The titrations were peformed at ambient temperature. Pularin was diluted 50 times and appropriate volumes added with a burette. In Figs. 7.8 and 7.10 ($E-E_o$) values are plotted against the heparin concentration. In Figs. 7.9 and 7.11 dE/dV values are plotted against heparin concentrations. Fig. 7.12 was plotted to compare the effects of heparin on K and Ca devices. As expected, the effect is far greater on Ca devices than on K

devices. Even small concentrations of heparin seem to cause significant changes. This could cause a problem when multi-functioned sensors are used. For measuring individual ions a Ca-, K-or Na-titrated heparin, as appropriate, can be used. It is necessary to agree on the use of a particular make of heparin, evaluate the percentage binding for each ion and introduce arithmetical corrections if necessary.

7.3 A Preliminary Study of the Effect of Carbon Dioxide on Aqueous Solutions of some GOOD buffers

Carbon dioxide dissolves in aqeueous solutions and is present as $CO_2(aq), H_2CO_3, H_3C_2O_6^-, HCO_3^-$ or CO_3^{2-} depending on the pH of the solution[32]. The species distribution curve is shown in Fig. 7.17. The species $H_3C_2O_6^-$ (HCO_3^- H^+ HCO_3^-) was reported at pH 6-8.

Titrations were carried out with the automatic titrator apparatus described in Chapter 6 Section 6.2.2, to investigate the effect of dissolution of Co_2 in the buffer solutions. A solution of carbon dioxide was prepared in a Sparklets (BOC) cylinder. This was diluted with degassed water in the proportion 1:1. To 10 ml of this solution 30 ml of 0.05 mol dm^{-3} Ba $(OH)_2$ was titrated with 0.05 mol dm^{-3} HCl using phenolphthalin as indicator. The concentration of CO_2 in the solution was thus calculated. Titrations of the buffers with this solution were then performed with the automatic titrator. The buffers were prepared as follows:-

1. HEPES : 0.025 mol dm^{-3} pH = 7.4 at 37 °C
   Na HEPES : 0.0306 mol dm^{-3}

2. TES : 0.025 mol dm^{-3} pH = 7.2 at 37 °C
   NaTES : 0.0274 mol dm^{-3}

3. MOPS : 0.025 mol dm^{-3} pH = 6.8 at 37 °C

NaMOPS : 0.0186 mol dm^{-3}

4. BES : 0.025 mol dm^{-3} pH = 6.8 at 37 °C

NaBES : 0.0199 mol dm^{-3}

The titre values are shown in Appendix 7.1. The results are plotted out in Figs. 7.13, 7.14 7.15 and 7.16. HEPES appears to be the most affected by CO_2 showing a change of 0.07 pH per 1.0 mmol dm^{-3} of titrant. An attempt was made at analysing the data using SUPERQUAD[35]. This was unsuccessful, however, possibly due to ill-defined pKs.

It would seem, from this preliminary investigation, that the buffering capacity of the Good buffers is sufficient for buffering against contamination from atmospheric carbon dioxide. This titration seems unsatisfactory for quantitative results as it involves a weak acid. A possible means of carrying out more satisfactory investigations is, perhaps, to tonometer the buffer solutions with varying concentrations of CO_2 and then measure the pH change.

TABLES

TABLE 7.1a

Solutions	Operational Cell		Harned Cell				Residual LJP
	Average emf of 3 sets of readings	pH	Average emf of 3 sets of readings	PaHγCl	-log γCl	PaH	(pH - paH)
0.75 $CaCl_2$ 94.75 NaCl 3.00 KCl 60.00 NaMOPS 80.75 MOPS	- 0.80	6.854	705.0	6.9720	.1260	6.8760	+ 0.008
1.67 $CaCl_2$ 100.00 NaCl 5.00 KCl 50.00 NaTES 45.62 TES	-24.58	7.240	726.7	7.3626	.1258	7.2368	+ 0.003
4.00 $CaCl_2$ 90.00 NaCl 8.00 KCl 50.00 NaHEPES 40.65 HEPES	-35.85	7.424	738.5	7.5449	.1246	7.4203	+ 0.004
6.75 $CaCl_2$ 94.75 NaCl 3.00 KCl 60.00 NaMOPSO 53.48 MOPSO	7.53	6.719	696.7	6.8371	.1260	6.7117	+ 0.008
4.00 $CaCl_2$ 90.00 NaCl 8.00 KCl 50.00 NaHEPPSO 106.90 HEPPSO	34.67	7.405	737.4	7.5270	.1246	7.4024	+ 0.003

TABLE 7.1b

Solutions	Operational Cell		Harned Cell						Residual LJP
			Buffer + ·01NaCl		Buffer +0.005 NaCl				
	Average emf of 3 sets of readings in mV	pH	Average E/mV	PaHγCl	Average E/mV	PaHγCl	P(aHγCl)°	PaH	pH - paH
60.00 NaMOPS 80.75 MOPS	- 0.13	6.843	764.00	6.9340	782.30	6.9304	6.9268	6.8334	+ 0.010
50.00 NaHEPES 40.65 HEPES	-37.12	7.444	799.19	7.5060	817.70	7.5056	7.5054	7.4181	+ 0.026

F I G U R E S

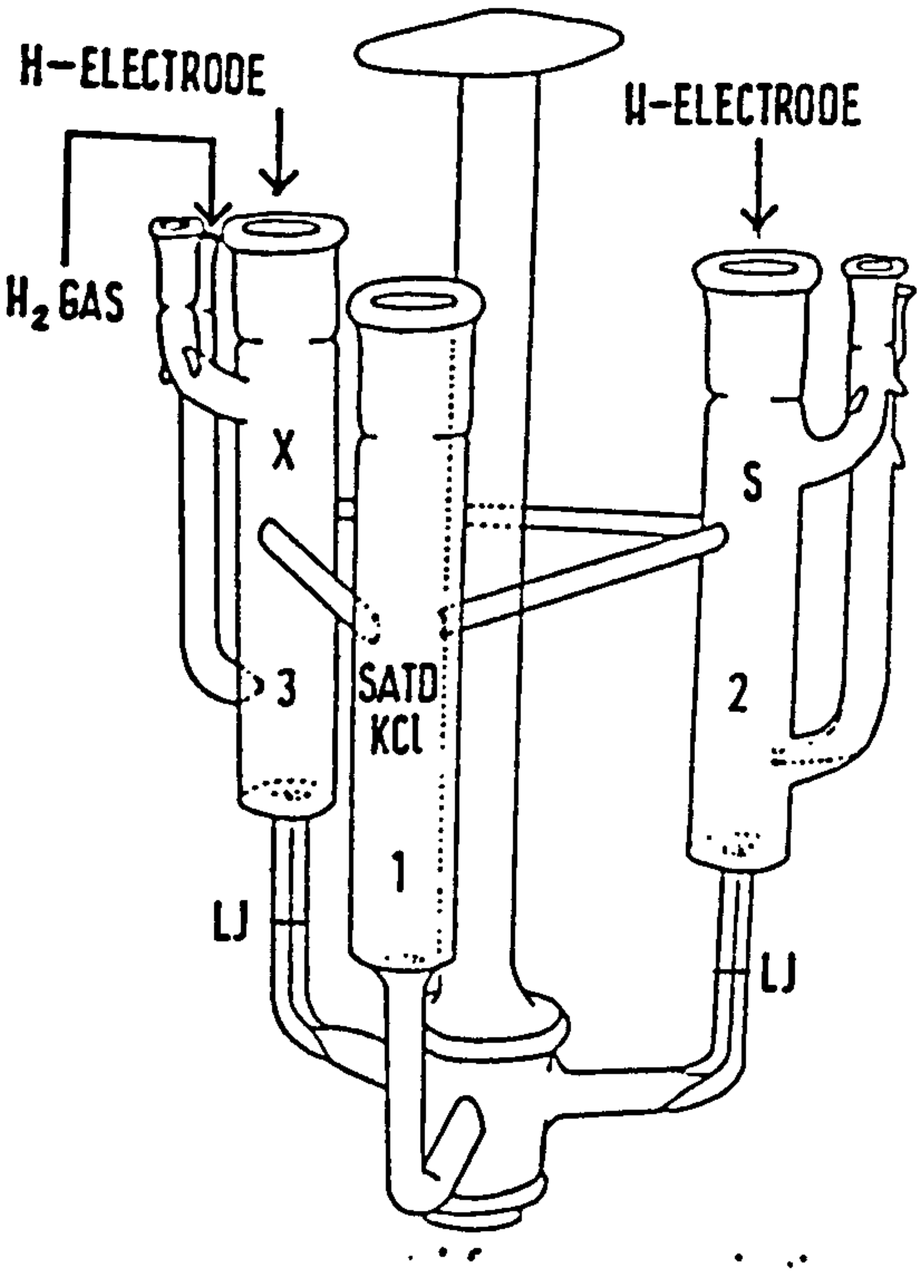


FIG 7.1 – OPERATIONAL CELL

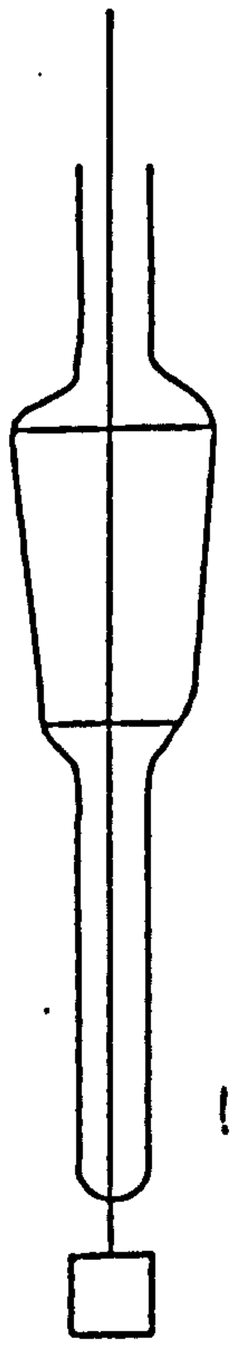


FIG 7.2
BASE FOR H-ELECTRODES

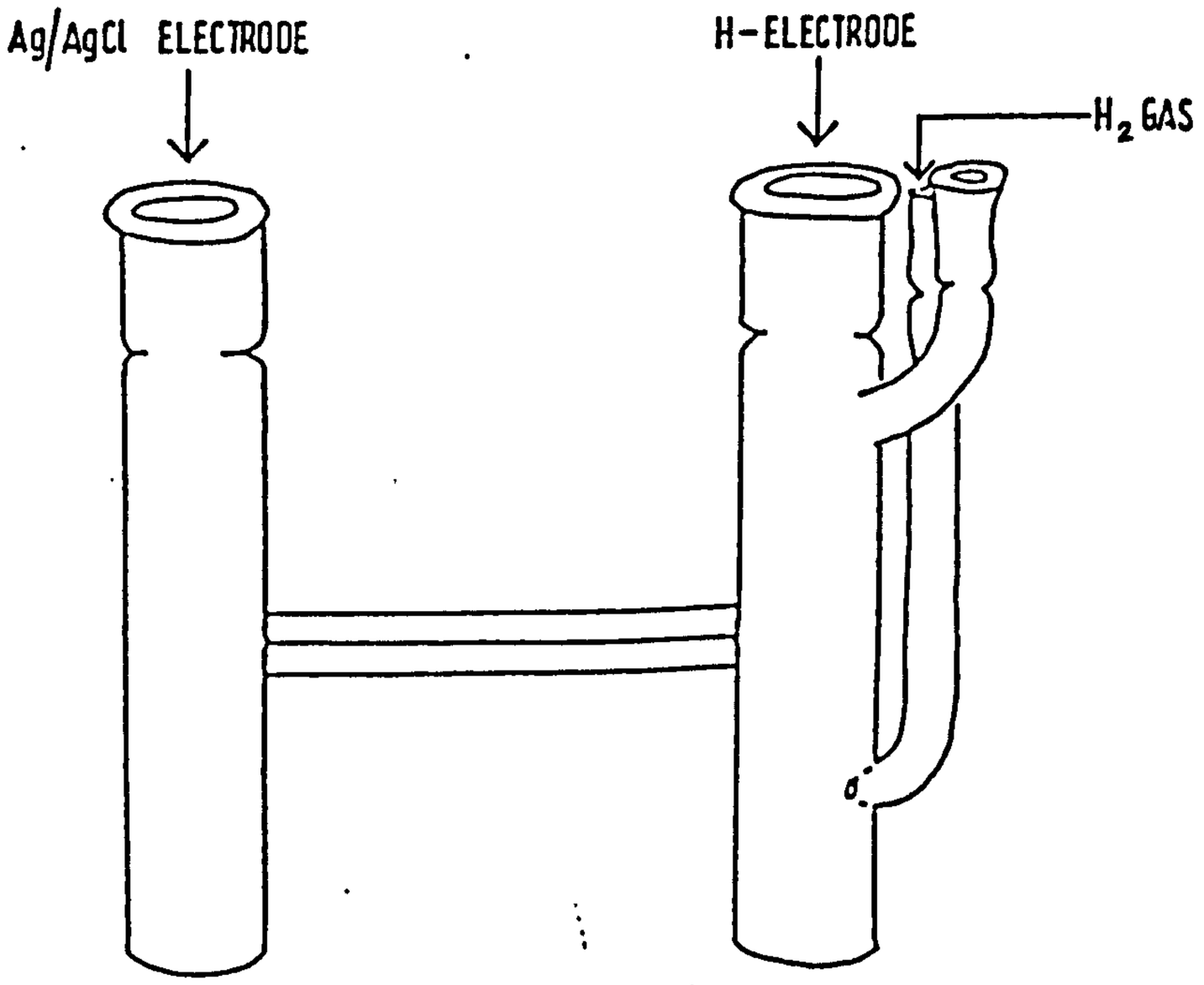


FIG 7.3 HARNED CELL

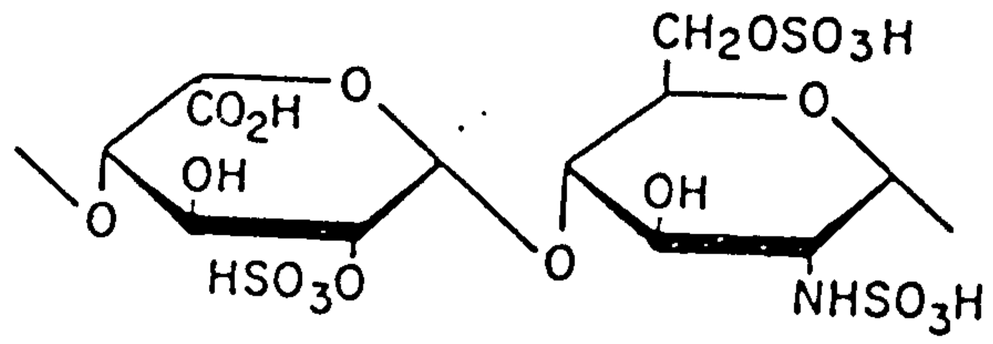


Fig.7.4 Repeating unit of heparin.

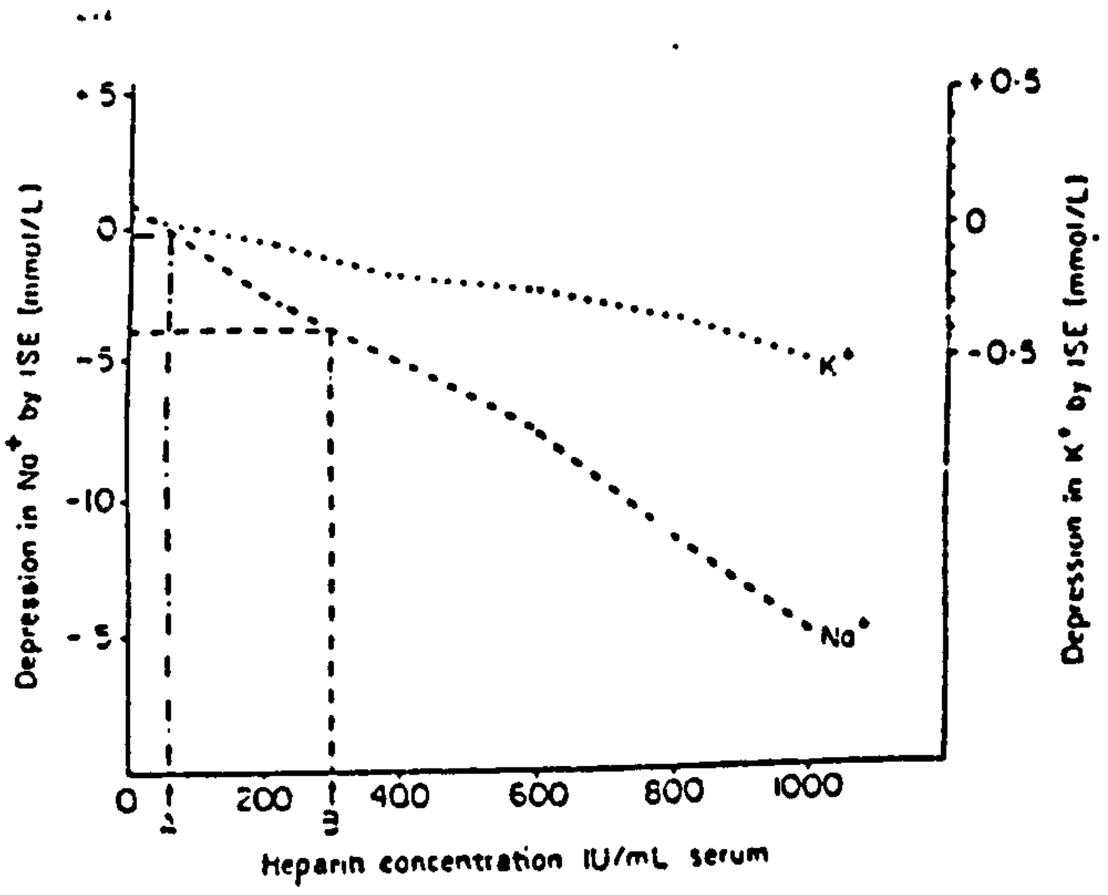
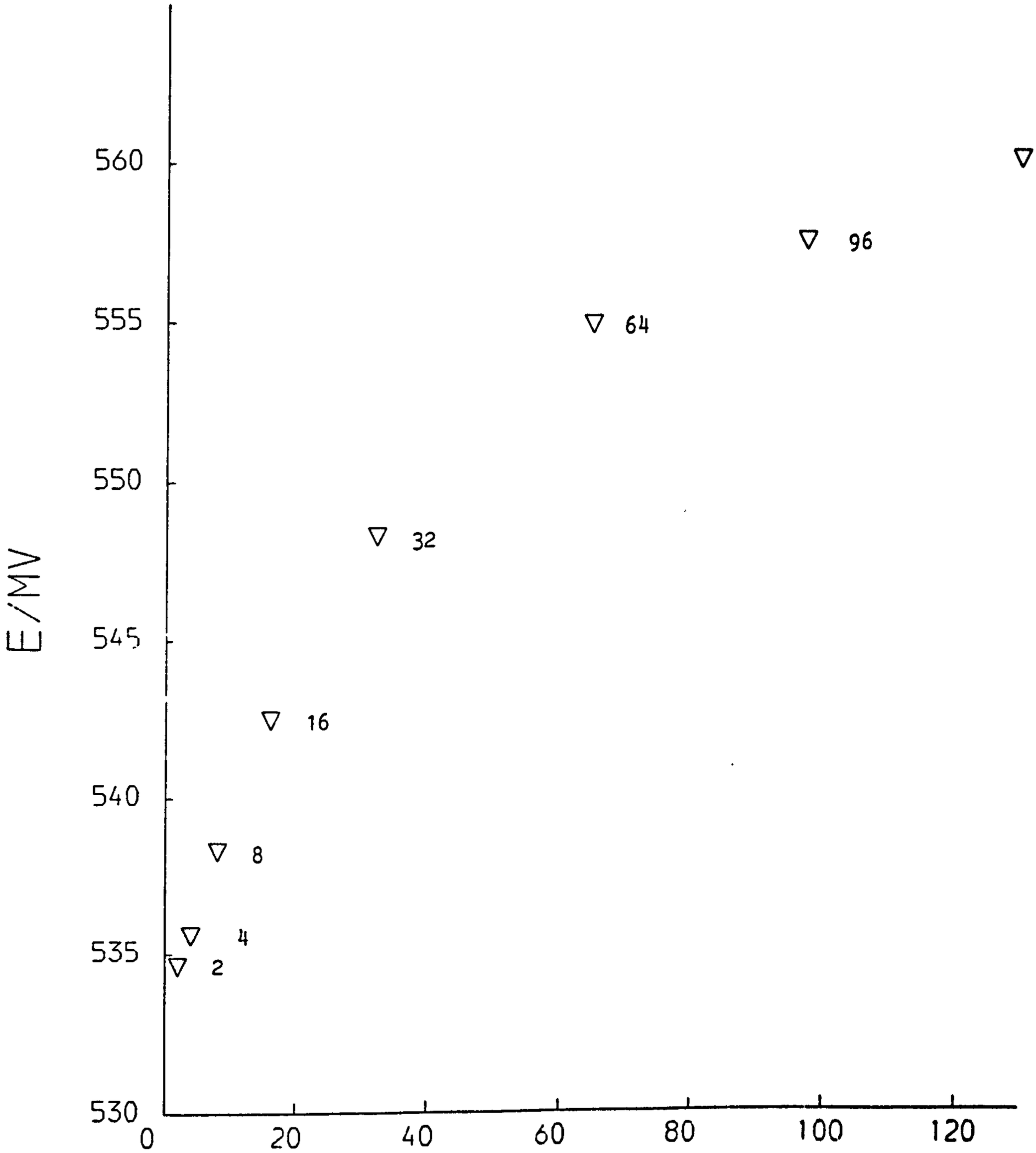
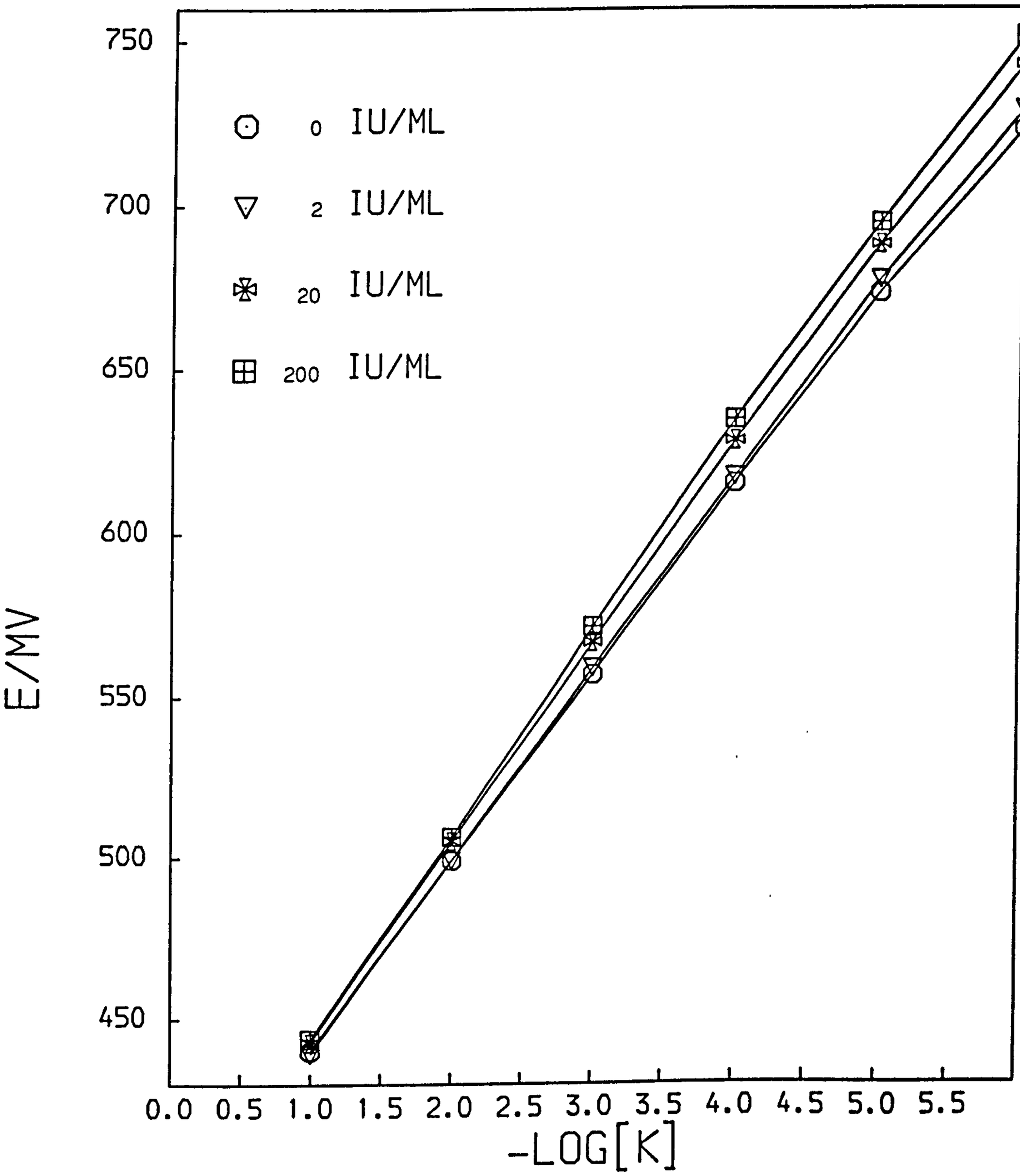


Fig7.5he depression in observed sodium and potassium values produced by adding increasing quantities of heparin (5000 IU/mL) to aliquots from a serum pool. A: 2 mL tube: B: 10 mL tube.



EFFECT OF HEPARIN (K)

Fig. 7.6



EFFECT OF HEPARIN (K)

Fig. 7.7

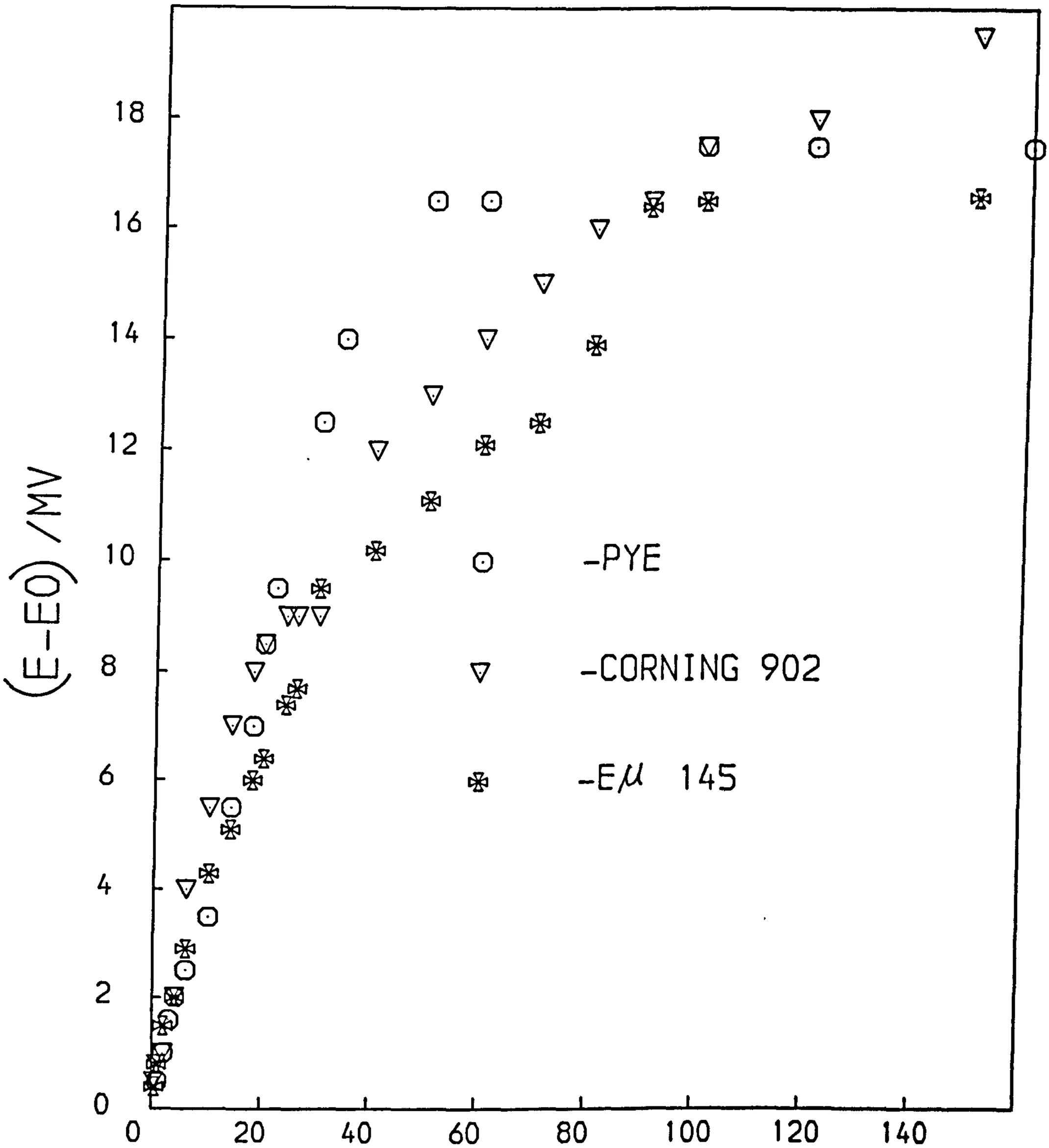
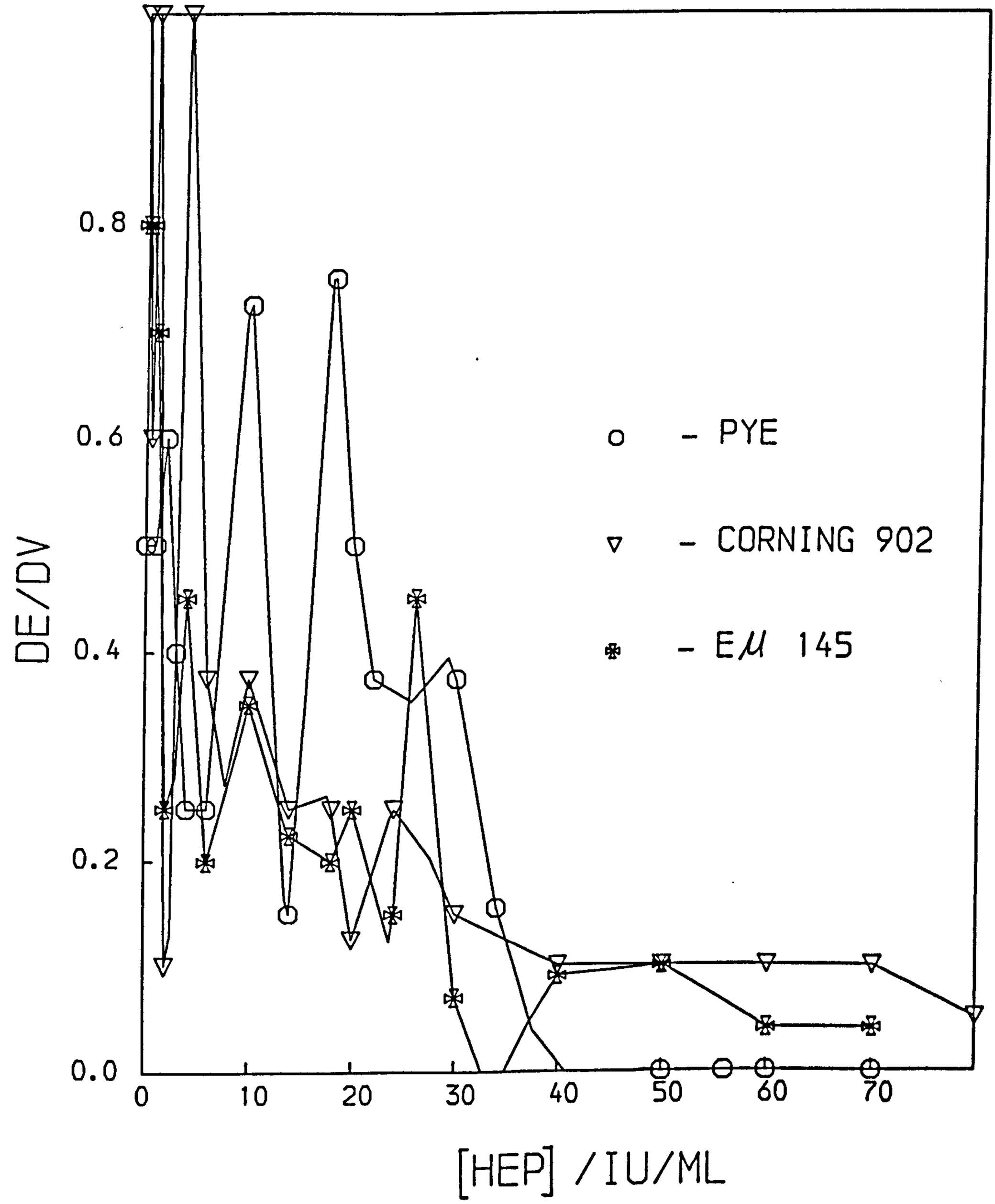

(E-E0) /MV
18
16
14
12
10
8
6
4
2
0
0
20
40
60
80
100
120
140
-PYE
-CORNING 902
-Eμ 145
[HEP] /IU/ML
EFFECT OF HEPARIN (K) 1

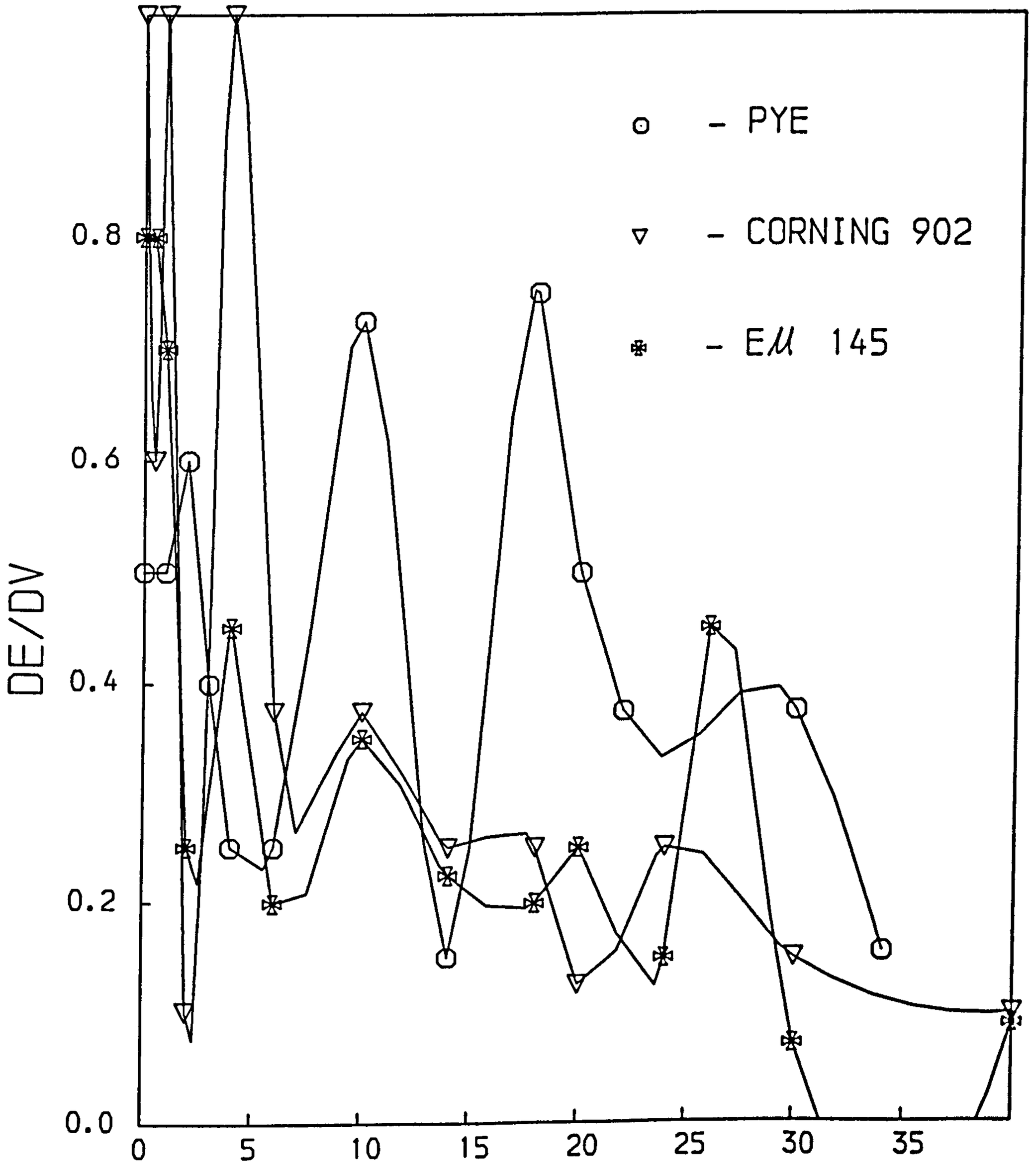
Fig. 7.8



[HEP] /IU/ML

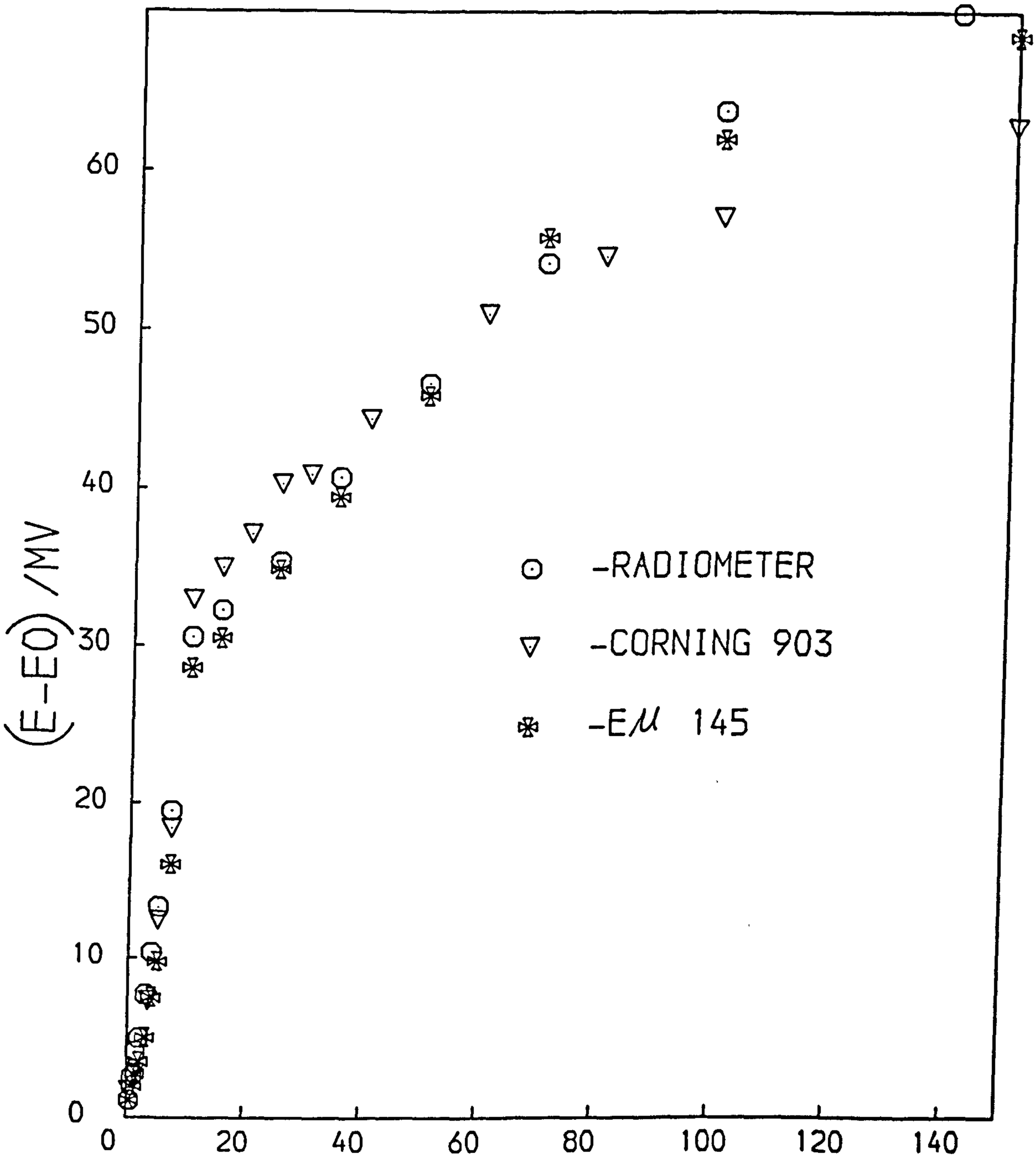
EFFECT OF HEPARIN (K) 2

Fig. 7.9a



EFFECT OF HEPARIN (K) 2

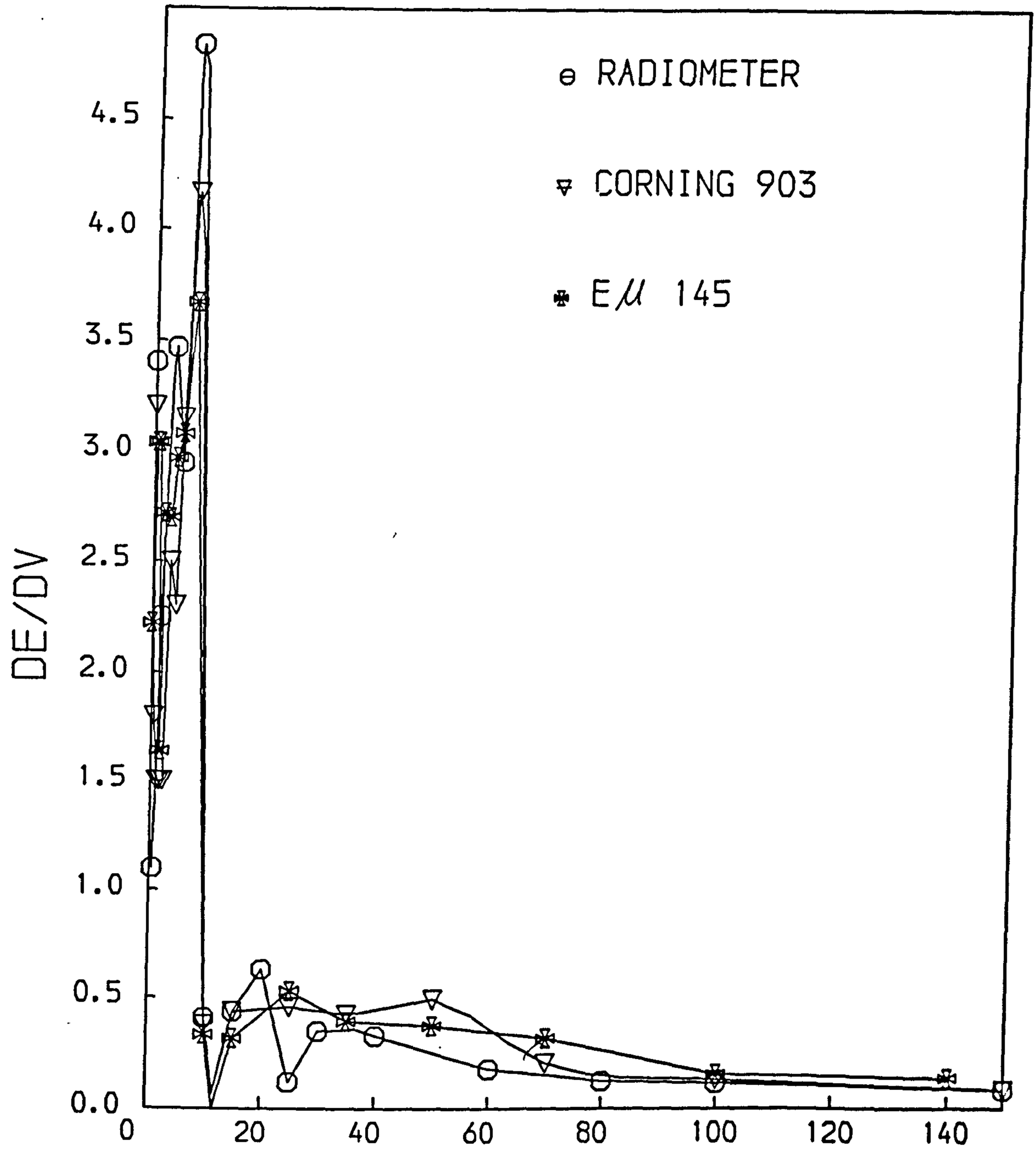
Fig. 7.9b



[HEP] /IU/ML

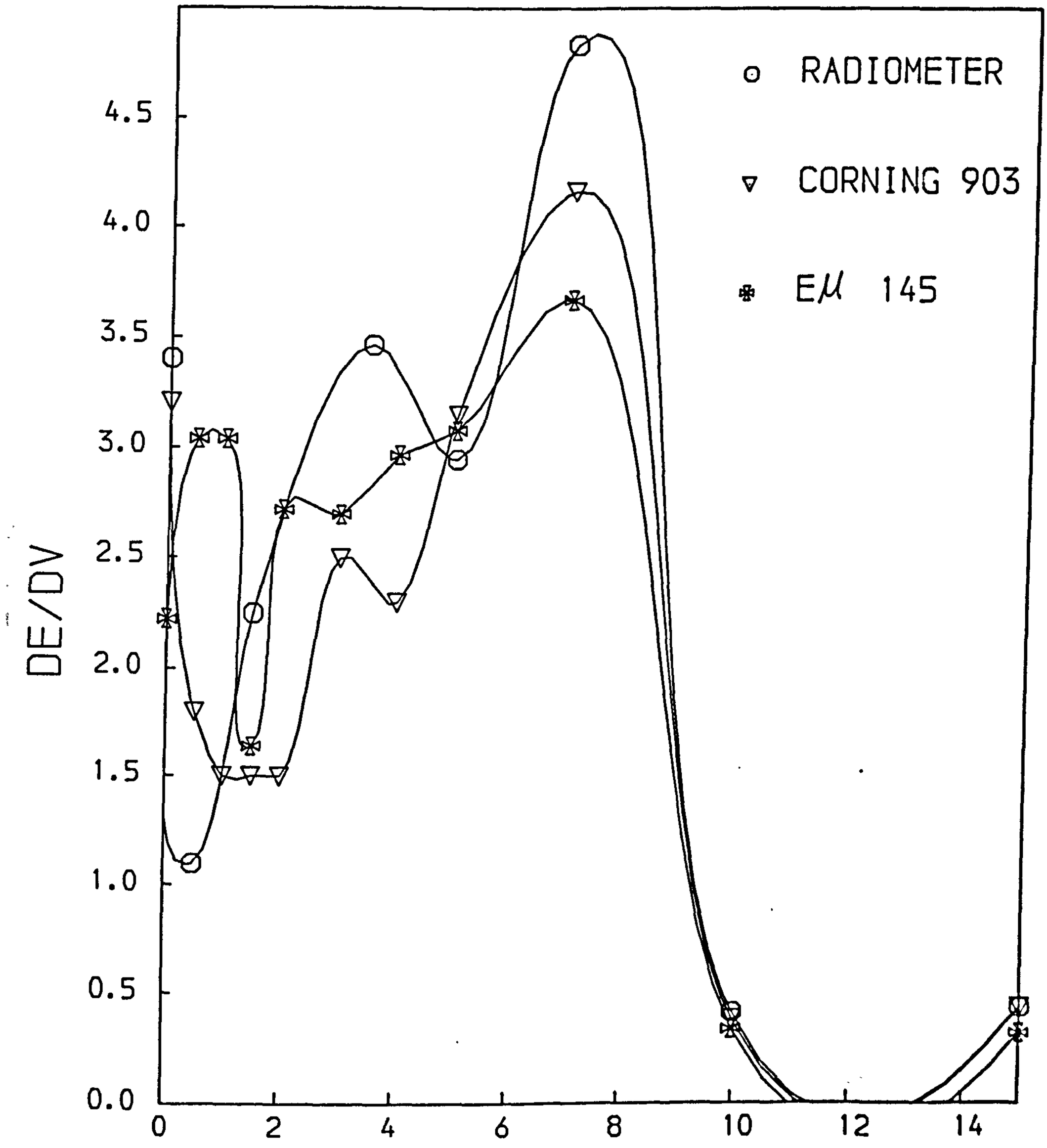
EFFECT OF HEPARIN (CA) 1

Fig. 7.10



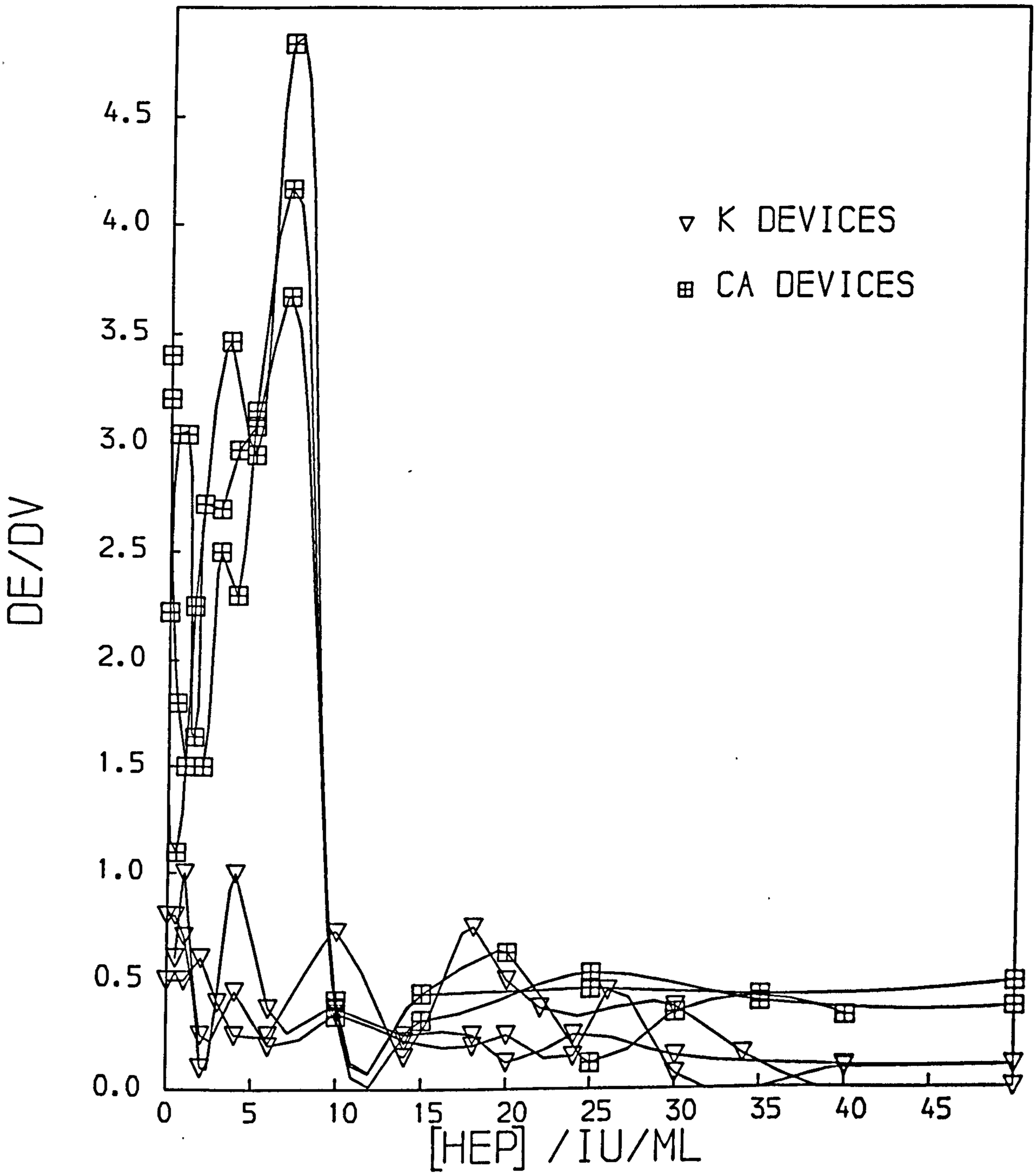
EFFECT OF HEPARIN (CA) 2

Fig. 7.11a



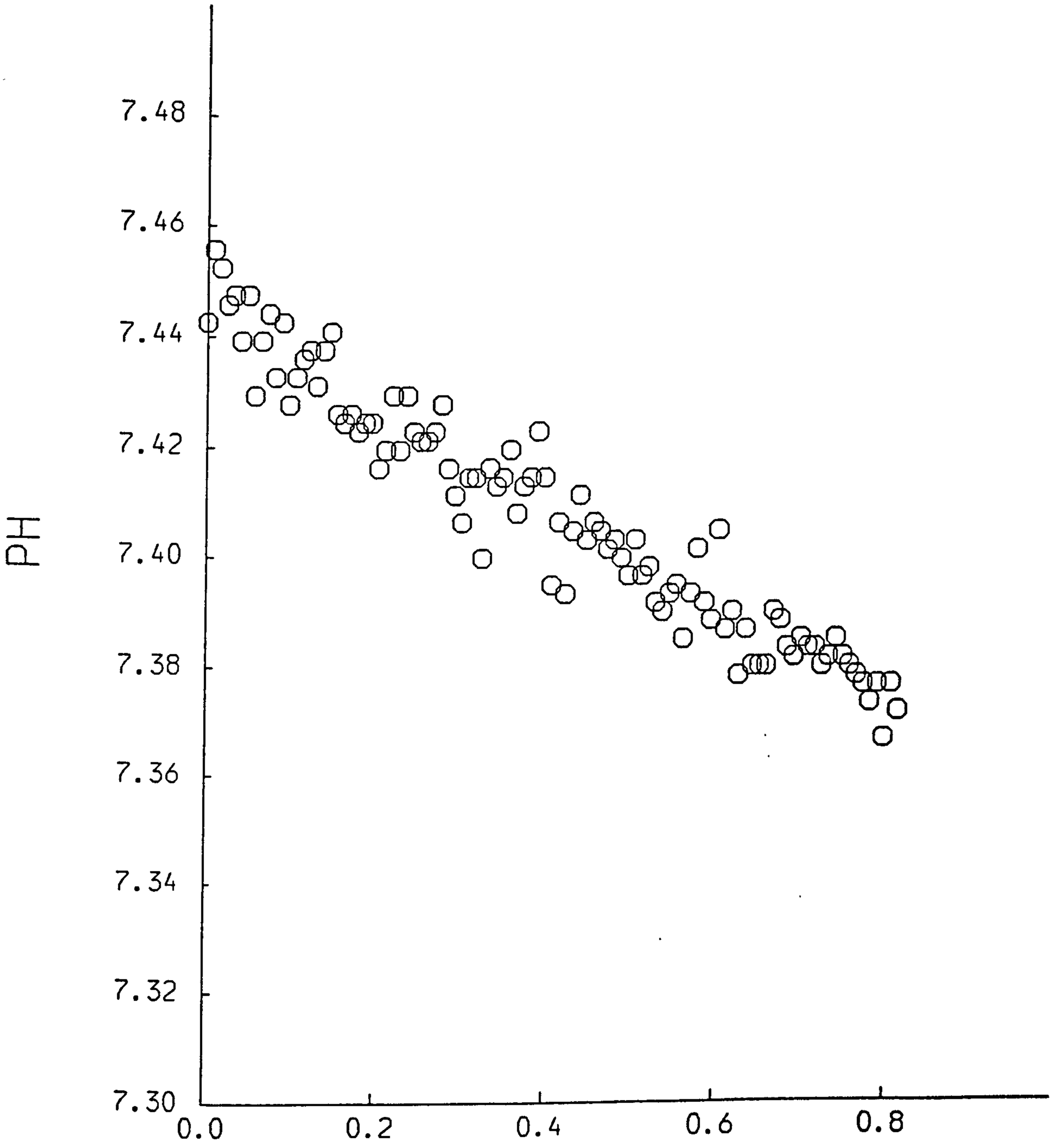
EFFECT OF HEPARIN (CA) 2

Fig. 7.11b



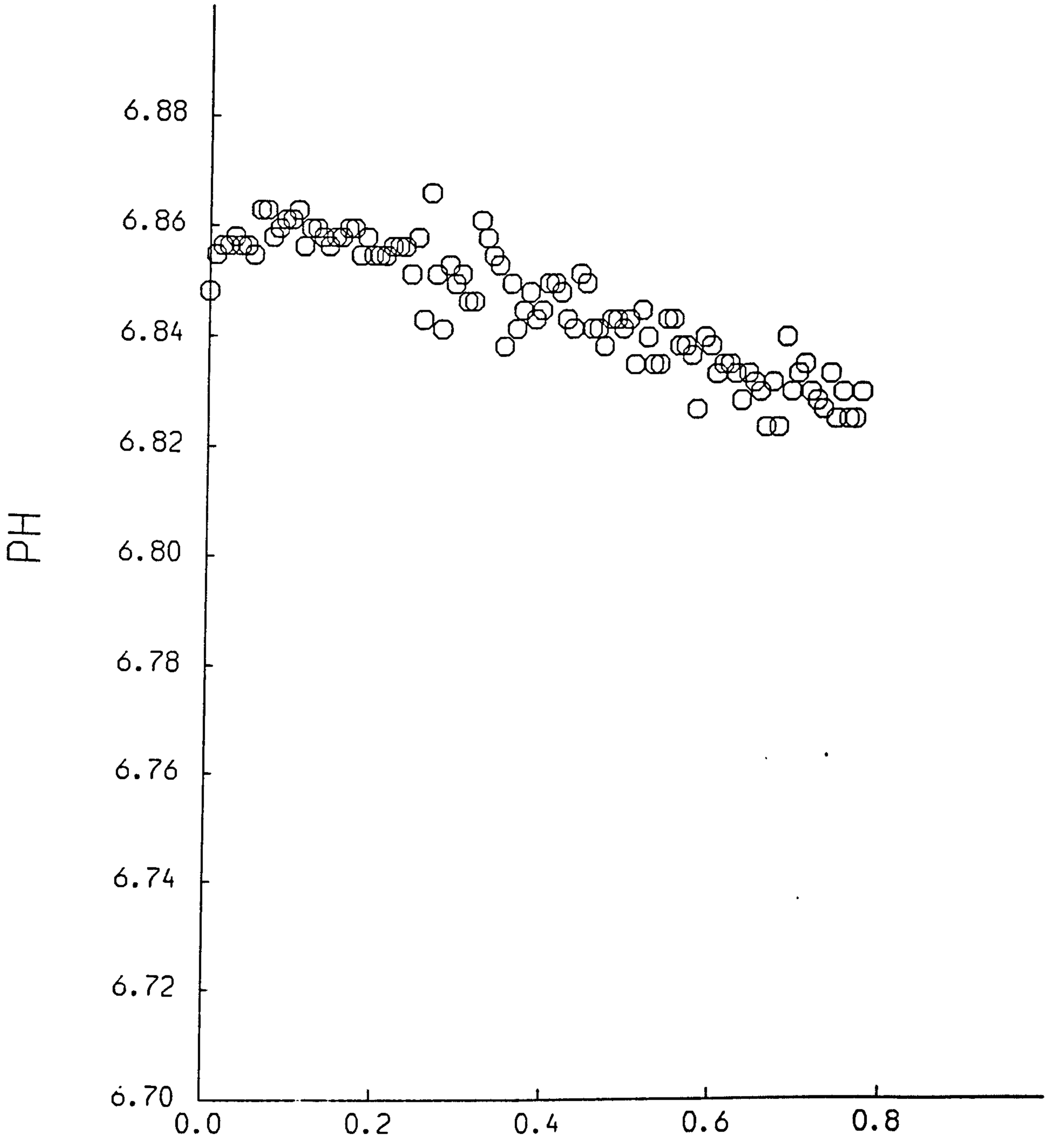
EFFECT OF HEPARIN ON K AND CA

Fig. 7.12



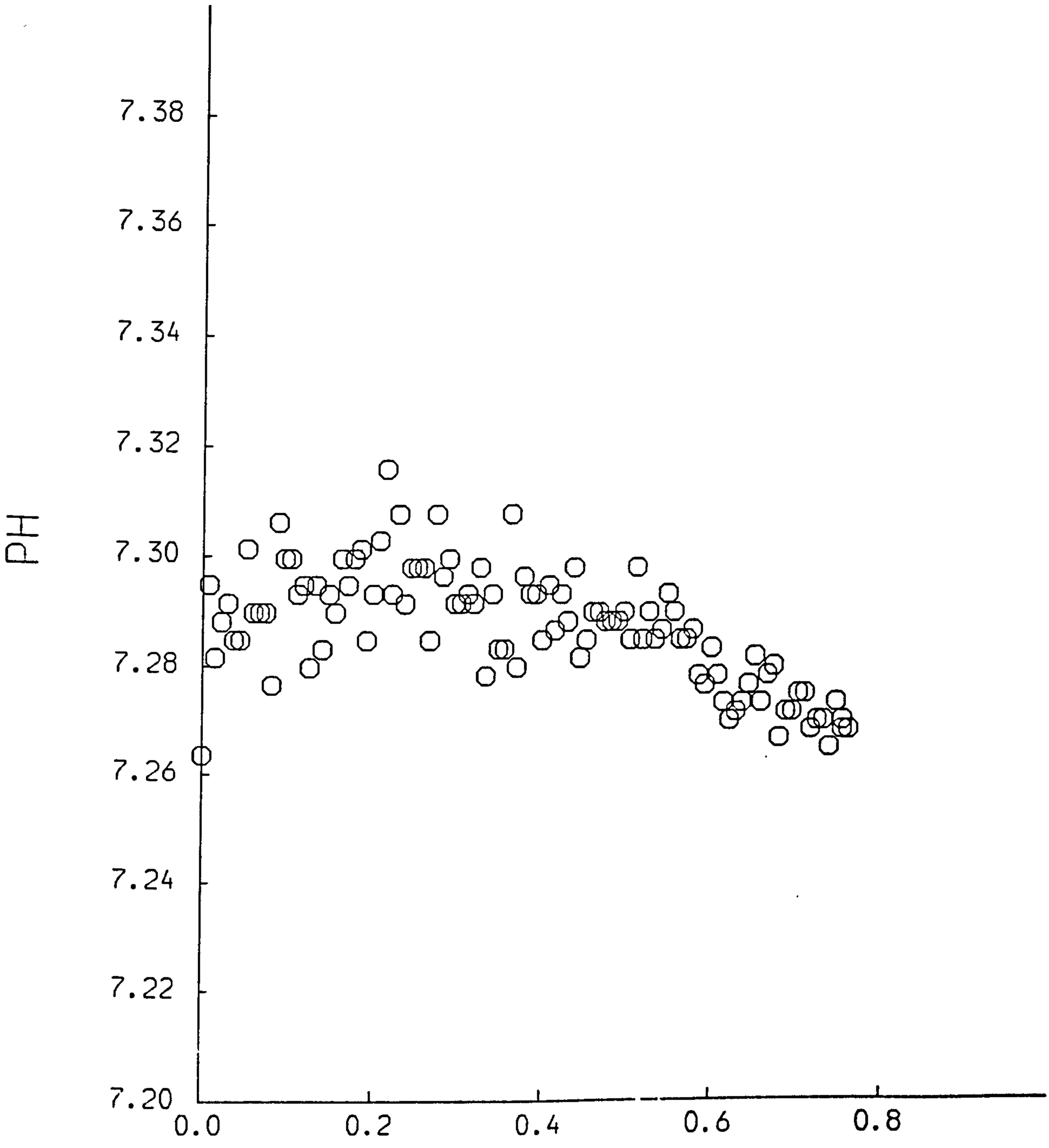
EFFECT OF CO2 ON HEPES

Fig. 7.13



EFFECT OF CO2 ON MOPS

Fig. 7.14



EFFECT OF CO2 ON TES

Fig. 7.15

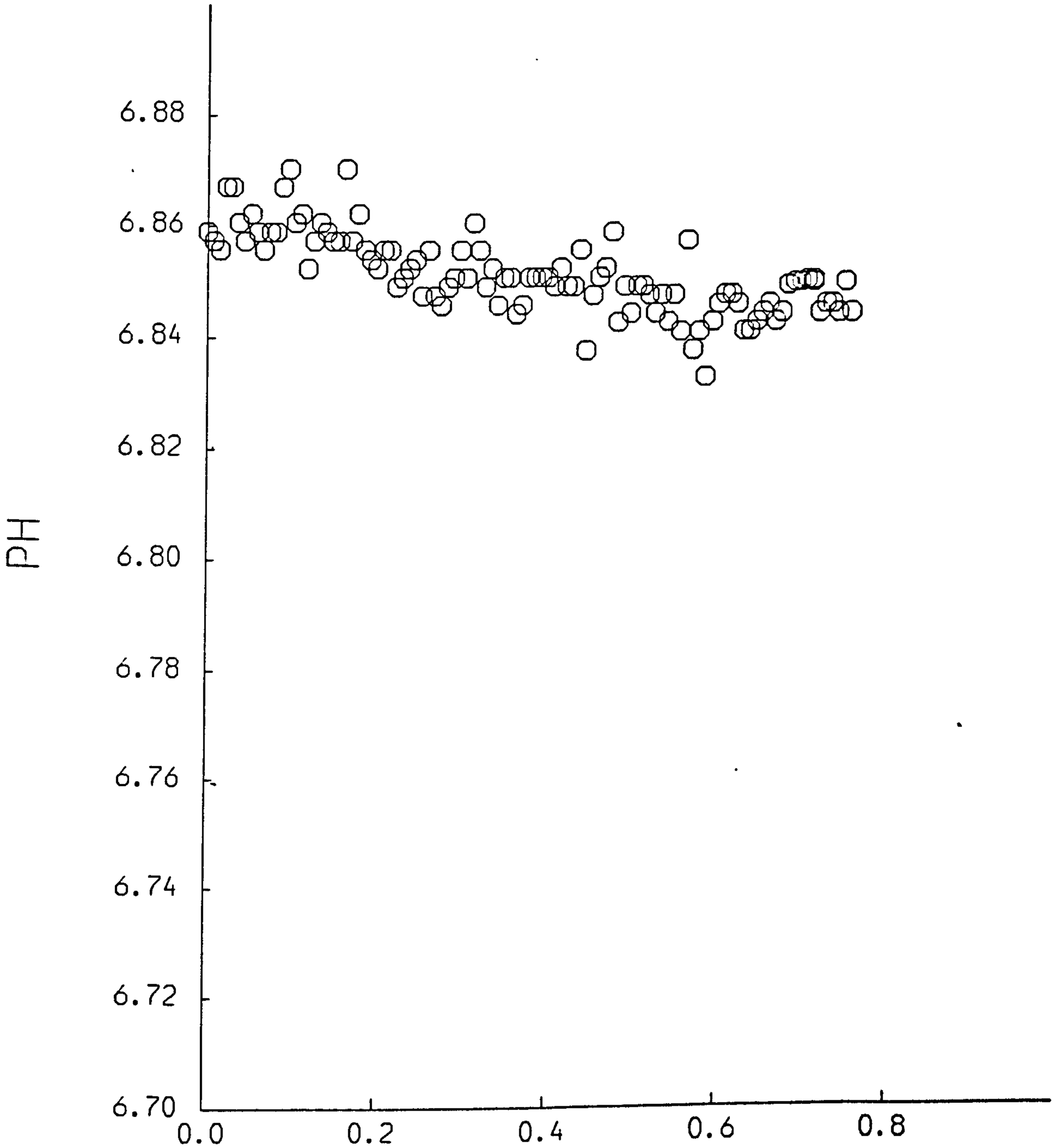
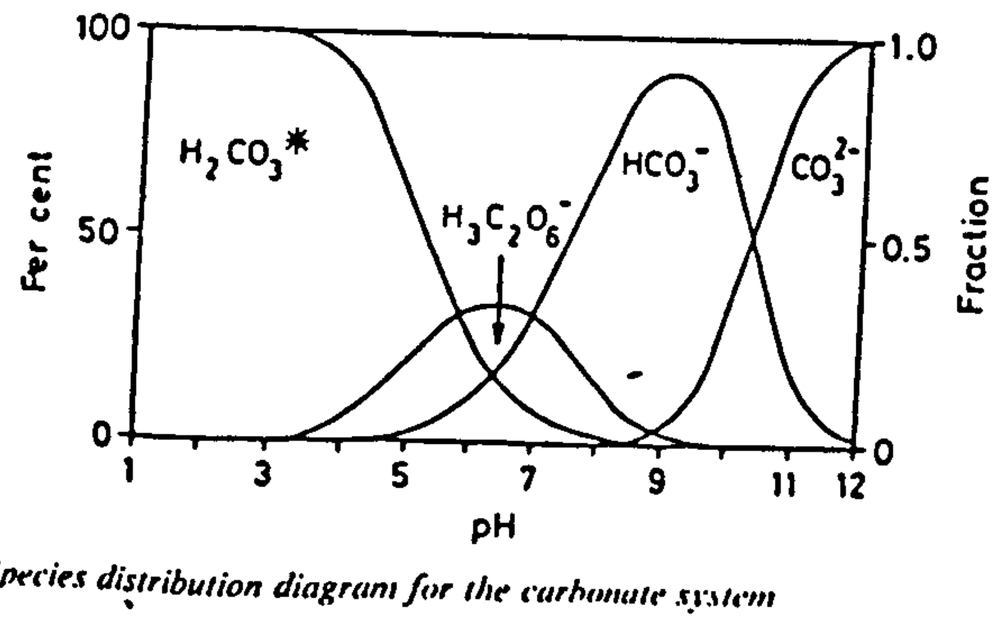

PH
6.88
6.86
6.84
6.82
6.80
6.78
6.76
6.74
6.72
6.70
0.0
0.2
0.4
0.6
0.8
MMOL/L OF CO2
EFFECT OF CO2 ON BES

Fig. 7.16



Species distribution diagram for the carbonate system

Fig. 7.17

REFERENCES

R-E-F-E-R-E-N-C-E-S

1. Christiansen, T.F., IEEE Trans BME, BME-33 No. 2, 79-82, (1986)

2. Hefter, G.T., in "Proceedings of the Workshop on Direct Potentiometric Measurements in Blood", ed. Koch, W.F. Gaithensburg (55-61), (1983).

3. Covington, A.K., Anal. Chim. Acta, 127, 1 (1981).

4. Bates, R.G., Crit. Rev. Anal. Chem., 10, 247 (1981).

5. Bradley, A.F. and Severinghaus, J.W., Fed. Proc., 17, 18 (1958).

6. Siggaard-Andersen, O., Scand, J., Clin. Lab. Invest., 13, 205-211, 1961.

7. Henderson, P.Z., Phys. Chem., 59, 118 (1907) and 63, 325 (1908).

8. Hefter, G.T., Anal. Chem., 54, 2518 (1982).

9. Burnett, R.W., Send, S.F., and Bower, G.N., in "Methodology and Clinical Applications of Ion-Selective Electrodes", Vol. 8, ed. Maas, A.H.J. et al., IFCC Workshop, Graz, Elinkwjik, Utrecht, 27, (1986).

10. Salling, N., Siggaard-Andersen, O., Scand, J., Clin. Lab. Invest., 28, 33-40 (1971).

11. Siggaard-Andersen, O., Fogh-Andersen, N., Thode, J., and Falch Christiansen, T., in "Ionised Calcium, Sodium and Potassium by Ion-Selective Electrodes", Vol. 5, ed. Maas, A.H.J., Kofstad, J., Siggaard-Andersen, O., and Kokholm, G., 149-154, Private Press, Copenhagen (1984).

12. Eisenharcht, A.R., and Jensen, O.J., Euro-Patent Appl. 0 094 677 (1983).

13. Cormier, A.D., Czaban, J.D. and Fejes, A.M., in reference 11, 139-149.

14. Boink, A.B.T.J., Buckley, B.M., Christiansen, T.F., Covington, A.K., Maas, A.H.J., Mueller-Plathe, O., Sachs, Ch., Siggaard-Andersen, O., in reference 9, 39-61.

15. Rebelo, M.J.F., Ph. Thesis, University of Newcastle Upon Tyne (1981).

16. Chapter Three in "Determination of pH - Theory and Practice", R.G. Bates, ed. Wiley, N.Y. (1973).

17. Guggenheim, E.A., J. Amer. Chem. Soc., 52, 1315 (1930).

18. Bates, R.G. and Bower, G., J. Res. N.B.S. 53 (5) (1954).

19. McLean, J. Amer. J. Physiol, 41, 250 (1916).

20. Heparin (and related Polysaccharides) : Structural and Functional Properties, Comper, W.D., Gordon and Breach Science Publishers, Inc. N.Y. (1981).

21. Herwats, L., Laszlo, P., Genard, P., Nouveau. J. Chimie, 1 173-176 (1977).

22. Manning, G.S., Acc. Chem. Res., 12, 443 (1979).

23. Delville, A., and Laszlo, P., Biosphys. Chem., 2-18 (1983).

24. Spegt, P. and Weill, G., Biophys. Chem., 143 (1976).

25. Obrink, B., Pertoft, H., Iverius, O.H., and Laurent, T.C., Connect-Tiss. Res. 3, 187 (1975).

26. Lages, B. and Stivala, S.S., Biopolymers, 12, 127 (1973).

27. Brauman, J., Devigne, Ch., Deconinck, I. and Willems, D., Scan. J. Clin. Lab. Invest, 165 43 (1983).

28. Mann, S.W., and Green, A., Ann Clin. Biochem, 23, 355 (1986).

29. Narayanan, S., in reference 9, 199 (1986).

30. Jaques, L.B., in "Advances in Experimental Medicine and Biology", ed. Bradshaw, T.A. and Weisler, S., Vol. 52, 139, (1975).

31. Covington, A.K. and Ferra, M.I.A., in "Methodology and Clinical Applications of Ion-Selective Electrodes", Vol. 7, Maas, A.H.J., Boinck, A.B.T.J., Saris, N.E., Sprokholt, R., and Wimberley, P.D., (eds.), 239-248, Radiometer, Copenhagen (1986).

32. Covington, A.K., Chem. Soc. Rev. 14, 265-281 (1985).

33. Czaban, J.D., and Legg, K.D., 63-80 in reference 2.

34. Manzoni, A., and Botton, M., 191, in reference 9.

35. Gans, P., Sabatini, A., Vacca, A., J. Chem. Soc. Dalton Trans. 1195 (1985).

A P P E N D I X

HEPES/CO_2 TITRATIONS

0.0	7.442500
0.8098494E-02	7.455600
0.1619698E-01	7.452300
0.2429548E-01	7.445700
0.3239397E-01	7.447400
0.4049248E-01	7.439200
0.4859097E-01	7.447400
0.5668944E-01	7.429300
0.6478792E-01	7.439200
0.7288641E-01	7.444100
0.8098495E-01	7.432600
0.8908343E-01	7.442500
0.9718186E-01	7.427700
0.1052804	7.432600
0.1133788	7.435900
0.1214774	7.437500
0.1295759	7.431000
0.1376743	7.437500
0.1457729	7.440800
0.1538713	7.426000
0.1619699	7.424400
0.1700684	7.426000
0.1781668	7.422800
0.1862654	7.424400
0.1943638	7.424400
0.2024624	7.416200
0.2105609	7.419500
0.2186593	7.429300
0.2267579	7.419500
0.2348563	7.429300
0.2429549	7.422800
0.2510533	7.421100
0.2591516	7.421100
0.2672502	7.422800
0.2753487	7.427700
0.2834473	7.416200
0.2915459	7.411300
0.2996442	7.406300
0.3077427	7.414500
0.3158413	7.414500
0.3239398	7.399800
0.3320381	7.416200
0.3401366	7.412900
0.3482352	7.414500
0.3563336	7.419500
0.3644321	7.408000
0.3725306	7.412900
0.3806292	7.414500
0.3887277	7.422800
0.3968260	7.414500
0.4049246	7.394800
0.4130231	7.406300
0.4211217	7.393200
0.4292203	7.404700
0.4373186	7.411300
0.4454171	7.403100
0.4535156	7.406300
0.4616142	7.404700
0.4697127	7.401400
0.4778110	7.403100
0.4859096	7.399800
0.4940082	7.396500
0.5021065	7.403100
0.5102050	7.396500
0.5183036	7.398100
0.5264021	7.391600
0.5345007	7.389900
0.5425990	7.393200
0.5506975	7.394800
0.5587960	7.385000
0.5668947	7.393200
0.5749932	7.401400
0.5830915	7.391600
0.5911900	7.388300
0.5992886	7.404700
0.6073871	7.386600
0.6154857	7.389900
0.6235840	7.378400
0.6316826	7.386600
0.6397804	7.380100
0.6478797	7.380100
0.6559782	7.380100
0.6640760	7.389900
0.6721746	7.388300
0.6802731	7.383400
0.6883717	7.381700
0.6964703	7.385000
0.7045686	7.383400
0.7126671	7.383400
0.7207657	7.380100
0.7288642	7.381700
0.7369627	7.385000
0.7450613	7.381700
0.7531598	7.380100
0.7612584	7.378400
0.7693569	7.376800
0.7774555	7.373500
0.7855538	7.376800
0.7936524	7.366900
0.8017504	7.376800
0.8098494	7.371900
0.8179480	7.381700

TES/CO_2 TITRATIONS

0.0	7.263500
0.7355150E-02	7.294700
0.1471030E-01	7.281600
0.2206545E-01	7.288100
0.2942061E-01	7.291400
0.3677576E-01	7.284800
0.4413091E-01	7.284800
0.5148606E-01	7.301300
0.5884121E-01	7.289800
0.6619632E-01	7.289800
0.7355148E-01	7.289800
0.8090663E-01	7.276600
0.8826178E-01	7.306200
0.9561694E-01	7.299600
0.1029721	7.299600
0.1103272	7.293100
0.1176824	7.294700
0.1250376	7.279900
0.1323927	7.294700
0.1397479	7.283200
0.1471030	7.293100
0.1544582	7.289800
0.1618133	7.299600
0.1691685	7.294700
0.1765236	7.299600
0.1838788	7.301300
0.1912339	7.284800
0.1985891	7.293100
0.2059442	7.302900
0.2132994	7.316000
0.2206545	7.293100
0.2280097	7.307800
0.2353649	7.291400
0.2427200	7.298000
0.2500750	7.298000
0.2574303	7.298000
0.2647855	7.284800
0.2721406	7.307800
0.2794957	7.296300
0.2868508	7.299600
0.2942060	7.291400
0.3015611	7.291400
0.3089164	7.293100
0.3162715	7.291400
0.3236266	7.298000
0.3309818	7.278300
0.3383369	7.293100
0.3456920	7.283200
0.3530471	7.283200
0.3604022	7.307800
0.3677576	7.279900
0.3751127	7.296300
0.3824679	7.293100
0.3898230	7.293100
0.3971781	7.284800
0.4045332	7.294700
0.4118885	7.286500
0.4192437	7.293100
0.4265988	7.288100
0.4339539	7.298000
0.4413090	7.281600
0.4486641	7.284800
0.4560193	7.289800
0.4633746	7.289800
0.4707298	7.288100
0.4780849	7.288100
0.4854400	7.288100
0.4927951	7.289800
0.5001502	7.284800
0.5075054	7.298000
0.5148607	7.284800
0.5222158	7.289800
0.5295709	7.284800
0.5369261	7.286500
0.5442812	7.293100
0.5516363	7.289800
0.5589917	7.284800
0.5663468	7.284800
0.5737019	7.286500
0.5810570	7.278300
0.5884121	7.276600
0.5957673	7.283200
0.6031224	7.278300
0.6104775	7.273400
0.6178328	7.270100
0.6251880	7.271700
0.6325428	7.273400
0.6398980	7.276600
0.6472533	7.281600
0.6546080	7.273400
0.6619633	7.278300
0.6693187	7.279900
0.6766738	7.266800
0.6840287	7.271700
0.6913838	7.271700
0.6987392	7.275000
0.7060945	7.275000
0.7134492	7.268400
0.7208045	7.270100
0.7281599	7.270100
0.7355150	7.265100
0.7428703	7.273400
0.7502250	7.270100
0.7502250	7.268400
0.7575803	7.268400

MOPS/CO_2 TITRATIONS

0.0	6.848100
0.7355150E-02	6.854700
0.1471030E-01	6.856300
0.2206545E-01	6.856300
0.2942061E-01	6.858000
0.3677576E-01	6.856300
0.4413091E-01	6.856300
0.5148606E-01	6.854700
0.5884121E-01	6.862900
0.6619632E-01	6.862900
0.7355148E-01	6.858000
0.8090663E-01	6.859600
0.8826178E-01	6.861200
0.9561694E-01	6.861200
0.1029721	6.862900
0.1103272	6.856300
0.1176824	6.859600
0.1250376	6.859600
0.1323927	6.858000
0.1397479	6.856300
0.1471030	6.858000
0.1544582	6.858000
0.1618133	6.859600
0.1691685	6.859600
0.1765236	6.854700
0.1838788	6.858000
0.1912339	6.854700
0.1985891	6.854700
0.2059442	6.854700
0.2132994	6.856300
0.2206545	6.856300
0.2280097	6.856300
0.2353649	6.851400
0.2427200	6.858000
0.2500750	6.843200
0.2574303	6.866200
0.2647855	6.851400
0.2721406	6.841500
0.2794957	6.853000
0.2868508	6.849700
0.2942060	6.851400
0.3015611	6.846500
0.3089164	6.846500
0.3162715	6.861200
0.3236266	6.858000
0.3309818	6.854700
0.3383369	6.853000
0.3456920	6.838300
0.3530471	6.849700
0.3604022	6.841500
0.3677576	6.844800
0.3751127	6.848100
0.3824679	6.843200
0.3898230	6.844800
0.3971781	6.849700
0.4045332	6.849700
0.4118885	6.848100
0.4192437	6.843200
0.4265988	6.841500
0.4339539	6.851400
0.4413090	6.849700
0.4486641	6.841500
0.4560193	6.841500
0.4633746	6.838300
0.4707298	6.843200
0.4780849	6.843200
0.4854400	6.841500
0.4927951	6.843200
0.5001502	6.835000
0.5075054	6.844800
0.5148607	6.839900
0.5222158	6.835000
0.5295709	6.835000
0.5369261	6.843200
0.5442812	6.843200
0.5516363	6.838300
0.5589917	6.838300
0.5663468	6.836600
0.5737019	6.826800
0.5810570	6.839900
0.5884121	6.838300
0.5957673	6.833300
0.6031224	6.835000
0.6104775	6.835000
0.6178328	6.833300
0.6251880	6.828400
0.6325428	6.833300
0.6398980	6.831700
0.6472533	6.830000
0.6546080	6.823500
0.6619633	6.831700
0.6693187	6.823500
0.6766738	6.839900
0.6840287	6.830000
0.6913838	6.833300
0.6987392	6.835000
0.7060945	6.830000
0.7134492	6.828400
0.7208045	6.826800
0.7281599	6.833300
0.7355150	6.825100
0.7428703	6.830000
0.7502250	6.825100
0.7575803	6.825100
0.7649357	6.830000

BES/CO_2 TITRATIONS

0.0	6.858900
0.0	6.858900
0.7355150E-02	6.857300
0.1471030E-01	6.855700
0.2206545E-01	6.867100
0.2942061E-01	6.867100
0.3677576E-01	6.860600
0.4413091E-01	6.857300
0.5148606E-01	6.862200
0.5884121E-01	6.858900
0.6619632E-01	6.855700
0.7355148E-01	6.858900
0.8090663E-01	6.858900
0.8826178E-01	6.867100
0.9561694E-01	6.870400
0.1029721	6.860600
0.1103272	6.862200
0.1176824	6.852400
0.1250376	6.857300
0.1323927	6.860600
0.1397479	6.858900
0.1471030	6.857300
0.1544582	6.857300
0.1618133	6.870400
0.1691685	6.857300
0.1765236	6.862200
0.1838788	6.855700
0.1912339	6.854000
0.1985891	6.852400
0.2059442	6.855700
0.2132994	6.855700
0.2206545	6.849100
0.2280097	6.850700
0.2353649	6.852400
0.2427200	6.854000
0.2500750	6.847500
0.2574303	6.855700
0.2647855	6.847500
0.2721406	6.845800
0.2794957	6.849100
0.2868508	6.850700
0.2942060	6.855700
0.3015611	6.850700
0.3089164	6.860600
0.3162715	6.855700
0.3236266	6.849100
0.3309818	6.852400
0.3383369	6.845800
0.3456920	6.850700
0.3530471	6.850700
0.3604022	6.844200
0.3677576	6.845800
0.3751127	6.850700
0.3824679	6.850700
0.3898230	6.850700
0.3971781	6.850700
0.4045332	6.849100
0.4118885	6.852400
0.4192437	6.849100
0.4265988	6.849100
0.4339539	6.855700
0.4413090	6.837600
0.4486641	6.847500
0.4560193	6.850700
0.4633746	6.852400
0.4707298	6.858900
0.4780849	6.842600
0.4854400	6.849100
0.4927951	6.844200
0.5001502	6.849100
0.5075054	6.849100
0.5148607	6.847500
0.5222158	6.844200
0.5295709	6.847500
0.5369261	6.842600
0.5442812	6.847500
0.5516363	6.840900
0.5589917	6.857300
0.5663468	6.837600
0.5737019	6.840900
0.5810570	6.832700
0.5884121	6.842600
0.5957673	6.845800
0.6031224	6.847500
0.6104775	6.847500
0.6178328	6.845800
0.6251880	6.840900
0.6325428	6.840900
0.6398980	6.842600
0.6472533	6.844200
0.6546080	6.845800
0.6619633	6.842600
0.6693187	6.844200
0.6766738	6.849100
0.6840287	6.849700
0.6913838	6.849700
0.6987392	6.850100
0.7060945	6.849700
0.7060945	6.850100
0.7134492	6.844200
0.7208045	6.845800
0.7281599	6.845800
0.7355150	6.844200
0.7428703	6.849700
0.7502250	6.844200

CHAPTER EIGHT

CONCLUSIONS

C O N C L U S I O N

In this concluding chapter, the aims of the work, achievement of the aims and possibilities for future research will be discussed.

8.1 REVIEW OF THE MAIN AIMS OF THIS WORK

The main objectives of this work were:-

(1) To contribute towards the establishment of a universally recognised operational pION scale for blood electrolytes related to the operational pH scale -

$$pION(X) = p(ION)S - (E_X - E_S)k \quad - 8.1$$

where

pION(X) = negative logarithm of the ionic activity or concentration in the sample

pION(S) = negative logarithm of the ionic activity or concentration in the standard

$E_{(X)}$ = potential of the cell containing the sample

$E_{(S)}$ = potential of the cell containing the standard

k = electrode slope at 37 ^{o}C

This requires -

(a) An established set of standards

(b) Elimination of or correction for the residual liquid junction potential

(c) Standardisation of sample collection and pre-treatment

(d) Standardisation of a measurement protocol - temperature control, flushing the electrode system, sequence of calibration and measurement.

(2) To test the performance of a varied range of ion selective devices as ideal tools for the direct potentiometric analysis of blood electrolytes.

8.2 ACHIEVEMENT OF THE AIMS

8.2.1 Calibration Solutions

Multi-electrolyte calibration solutions were proposed with a view to future multi-electrolyte analysis. (Present day, commercial analysers are Na^+/K^+ analysers or Ca^{2+}/pH analysers). These standards appear to be a convenient basis for the establishment of universally accepted standards. Some important features and controversial aspects are:

(i) The standards were formulated to reflect the range of ionic concentrations of Na^+, K^+, Ca^{2+} and of pH in normal blood. Ideally, the concentrations of the calibration solutions should provide a span of at least 5mV[1]. The lowest span is approximately 3mV for sodium (Table 4.1, Chapter 4). If the performance of the ion-selective device is Nernstian, a span of 3mV appears to be adequate to obtain a calibration line. This is illustrated in Fig. 5.26, Chapter 5.

(ii) There is no doubt that the "Good" buffers are the ideal physiological buffers. The dissociation constants and the binding constants of HEPES, MOPS, TES AND BES were evaluated using carefully monitored titration procedures and two well-established non-linear least squares programs SCOGS2[3] and SUPERQUAD[4]. There was excellent agreement in the results using both programs. The dissociation constants are close to the physiological pH range and the binding of the buffers to calcium, sodium and potassium was very low. (Chapter 6). Low concentrations of the buffers were used because it was considered sufficient for buffering against atmospheric carbon dioxide contaminations.

(iii) Concentration standards seem to be more practical in the present circumstances. Diagnosis is based on the concept of concentrations in mmol dm^{-3}. Moreover concentration standards require only that the activity co-efficients of the ions in the calibration solution and the sample be well matched. Activity standards, on the other hand, require accurate calculations of the activity co-efficient of the ions in the calibration solution so that their activities can be defined. Theoretical calculations based on the Pitzer equation and the Hydration model have been used by Covington and Ferra[5]. These were used in the calculation of activities. It is not simple to verify the accuracy of one method over the other. Reference to Tables 5.7 a-c, indicate that calculations based on the Pitzer equation give lower values of the percentage concentration differences compared with calculations based on the Hydration theory. The mean values of the percentage concentration differences calculated from Tables 5.7 a-c are:

	PIT	HYD
Ca^{2+}	-1.2	+2.8
K^{+}	-1.2	-0.8
Na^{+}	+0.1	+0.2

(iv) The composition of the calibration solutions were formulated also, to overcome the selectivity limitations of most ISE's by meeting the requirement:

$$\frac{K_{ij}\ C_j^{z_i/z_j}}{C_i} < 0.01$$

where K_{ij} is the selectivity co-efficient, C is the concentration (mmols dm^{-3}), z is the valency of the ion, 'i' is the sensor ion and 'j' is the interferring

ion. The error arising in potential difference from the presence of interferring ions will then not exceed 0.3mV for Na^+ and K^+ ISE's and 0.13mV for Ca^{2+} ISE's. This is calculated as follows:-

Without any interfering ions

$$E = E^o + k \log C_i \qquad A$$

In the presence of interfering ions

$$E = E^o + k \log [C_i + \lesssim_j K_{ij} C_j^{z_i/z_j}] \qquad B$$

A-B gives

$$\text{Antilog } \frac{\Delta E}{k} = 1 + \lesssim_j \frac{K_{ij} C_j^{z_i/z_j}}{C_i} \qquad - 8.2$$

$$\text{i.e. } \Delta E < (\log 1.01) \times k \qquad - 8.3$$

$$< 0.13 \text{ for } Ca^{2+} \ (k \sim 30\text{mV per decade})$$

$$< 0.25 \text{ for } K^+ \text{ and } Na^+ \ (k \sim 60\text{mV per decade})$$

8.2.2 Residual Liquid Junction Potentials

The second criteria to be considered is the residual liquid junction potential. It is impossible to eliminate completely the residual liquid junction potential i.e. the difference between the junction potential with the calibration solution and the junction potential with plasma. Bias can be minimised by matching the ionic composition of the calibration solutions as closely as possible to the ionic composition of plasma; the calibrations solutions proposed were formulated to reflect the ionic composition of plasma. Results from the determination of the residual liquid junction potentials of the initial calibration solutions against the NBS blood phosphate buffer are encouraging (Table 7.1, Chapter 7). Further work is required in this field, particularly in determining the residual liquid junction potentials of the calibration solutions against plasma.

International consensus on the geometry of the liquid junction is yet to be achieved. It would appear that a free diffusion junction would be most suitable. Boink et al.[1] suggest a free diffusion type junction arranged in such a way that a higher density salt bridge solution is below the test solution and a capillary tube less than 0.4mm in diameter dips at least 5mm into the salt bridge solution with a distance of at least 10mm to the calomel reference electrode to avoid contamination. In the flow through system (Chapter 5, photograph 5.1) a porous, ceramic plug constrained diffusion junction was used with serum and plasma sample. There was no evidence of noisy signals or drift of electrode potentials attributable to the reference electrode system, leading to the conclusion that these junctions are suitable for serum/plasma samples. No work with whole blood samples was done, so the effect of erythrocytes has not been studied. Methods suggested for eliminating or compensating for this effect include changing the potassium chloride salt bridge solution to 4 mol dm^{-3} sodium formate salt bridge[6], using a dynamic flowing junction[7] or avoiding the presence of erythrocytes by interposing a bridge of plasma originating from the blood sample being measured[1].

8.2.3 Other Aspects

It is very important that the level and source of heparin to be used in measurements with plasma and whole blood is standardised. Significant changes were observed even with small levels of heparin, particularly in calcium measurements (Fig. 7.12, Chapter 7). For measuring individual ions, a Ca, K or Na titrated heparin may be used to circumvent the problem. For multi-functional analysers, however, it will be essential to agree on a particular make of heparin, to evaluate the percentage binding for each ion and introduce a correction factor.

Reference to Table 5.9 in Chapter 5, shows that the pH values obtained for the plasma and serum samples were very high. This could be due to loss of CO_2 absorbed by plastic tubing.[8] To avoid pH changes anaerobic sampling is recommended[8].

Recommendations for specimen collection and processing for quality control purposes and a protocol for sample measurement have been made by the European Working Group on Ion Selective Electrodes (EWGISE) and can be found in references 1, 8 and 9.

8.2.4 Performance of the Ion-Selective Devices Used

In Table 8.1, the sensitivity of the electrodes used in this work were calculated using

$$S = (k/_{ko}) - 1 \qquad - 8.2$$

where S = sensitivity

k = practical slope of the electrode

k^o = ideal slope of the electrode

For an ideal electrode $k/_{ko} = 1$

The slopes of the electrodes were taken from the calibration curves in Figs. 5.4 - 5.22, Chapter 5. The sensitivities of most devices are within +0.02 and -0.06. The exceptions are the sodium devices using the neutral carrier ETH 227 and a pH FET.

In Chapter 3, Section 3.4 and Table 3.2, the selectivity of the ion selective devices used in the measurement of blood electrolytes was discussed. Selectivity co-efficients were not determined because blood electrolytes do not cause mutual interference[11]. However, it has been reported by Vadgama[12] et al. that calcium ISE's show an increased sensitivity towards sodium and magnesium upon ageing. It would be worthwhile, therefore, to check the selectivities of the devices prior to use.

Evaluation of the transfer potentials of various ion selective electrodes was a sensitive test of the devices. Most electrodes deviated less than 0.5mV from the theoretical transfer potentials which is within the experimental error. It was observed that when activity co-efficients were calculated using the Hydration theory, higher (i.e. more positive) values were obtained for the transfer potentials E_T (exp-Th) than if the Pitzer model was used. This is not sufficient, however, to establish the superiority of one theory over the other as the differences did not necessarily imply that the theoretical values obtained by a particular method of calculation were closer to the experimental values (ref. Table 5.6, Chapter 5).

The ISE's incorporated in the flow-through rig (photograph 5.1, Chapter 5) were stable and their response was satisfactory in presence of plasma and serum. The calomel electrode in the reference cell with a porous ceramic plug was also stable indicating that the plug was not contaminated during the period of contact with plasma/serum. Only the pH values with the pH Corning ISE showed unusal values. This is probably because special precautions were not taken to avoid loss of CO_2 from the samples.

8.3 FUTURE WORK

There is scope for future work in the following areas:-

(1) The calibration standards can be extended to include other ions of clinical importance (e.g. HCO_3^-). Special standards could be introduced for "abnormal" plasma samples.

(2) ISE's of other makes, ISE's for sensing clinically relevant ions other than Na^+, K^+, Ca^{++} and H^+ and ISFET's could be incorporated in the flow-through system. The system could be adapted for analysis with whole blood, a suitable liquid junction would have

to be used and the effect of whole blood on the response of the electro-active materials could be tested. The system should be thermostated at 37 °C.

(3) Inter-laboratory tests should be carried out using calibration standards supplied from a single source, to minimise variations that could arise from the preparation of solutions.

(4) The percentage binding of sodium, potassium and calcium to a particular source of heparin must be evaluated.

From this study, it is apparent that direct potentiometry using ISE's and ISFET is well suited for clinical analysis. The problems of standardisation, precision and reproducibility of results can be resolved by using the suggested calibration solutions and by reaching an international consensus on the design of the liquid junction and composition of the salt bridge solution.

TABLES

TABLE 8.1

Ion Selective Device	Sensitivity $S = (k/k_o - 1)$ (eqn. 8.2)
AVL Calcium (F)	- 0.059
AVL Sodium (F)	- 0 042
AVL Potassium (F)	+ 0.009
Beckman Sodium (F)	+ 0.021
Corning Potassium (F)	+0.009
Corn Calcium (F)	-0.048
Corn Calcium (F)	-0.132
Corn pH (F)	-0.016
Corn Sodium (D)	-0.020
Pye Sodium (D)	-0.005
Pye Potassium (D)	+0.007
Pye Calcium (D)	-0.037
EIL Sodium (D)	-0.016
Russell Sodium (D)	-0.088
Homemade Potassium (D)	-0.033
Radiometer Calcium (D)	+0.039
Potassium FET (D)	-0.030
Calcium FET (D)	-0.027
pH FET (D)	-0.085

(F) = flow through

(D) = dip type

REFERENCES

REFERENCES

(1) Boink, A.B.T.J., Buckley, B.M., Christiansen, T.F., Covington, A.K., Maas, A.H.J., Muether-Plathe, O., Sachs, Ch., Siggaard-Andersen, O., in "Methodology and Clinical Applications of Ion-Selective Electrodes", Vol. 8, ed. Maas, A.H.J., Buckley, B., Marsoner, H., Saris, N-E, L., and Sprokholt, R., 137, Elinkwijk, Utricht, (1986)

(2) Good, N.E., and Izawa, S. in "Methods in Enzymology" ed. San Pietro, A., B24, 53-64, Academic Press, N.Y. (1972).

(3) Perrin, D.D., and Stunzi, H., in "Computational Methods for Determination of Formation Constants", ed. Leggett, D.J., Plenum, N.Y., 71-157 (1985).

(4) Gans, P., Sabatini, A. and Vacca, A., J. Chem. Soc., Dalton, 1195-1201 (1985).

(5) Covington, A.K., and Ferra, M.I.A., 239 in "Methodology and Clinical Applications of Ion-Selective Electrodes" eds. Maas, A.H.J., Boink, A.B.T.J., Saris, N-E.L., Sprokholt, R. and Wimberley, P.D., Vol. 7, Radiometer, Copenhagen (1986).

(6) Siggaard-Andersen, O., Fogh-Andersen, N., Thode, J., and Falch-Christiansen, T., in "Ionised Calcium, Sodium and Potassium by Ion-Selective Electrodes", Vol. 5, ed. Maas, A.H.J., Kofstad, J., Siggaard-Andersen, O., and Kokholm, G., 149-154. Private Press, Copenhagen (1984).

(7) Cormier, A.D., Czaban, J.D., and Fejes, A.M., 139-149 in reference 5.

(8) Same authors in reference 1, 39

(9) Same authors in reference 1, 81

(10) Meir, P.C., Ammann, D., Morf, W.E., Simon, W., Chapter 2 in "Medical and Biological Applications of Electro-chemical Devices", ed. Koryta, J., Wiley (1980).

(11) Vadgama, P., Mitchison, J., Covington, A.K., and Alberti, K.G.M.M., Clin. Chim-Acta 119, 249-256 (1982).

"I know a planet where there is a certain redfaced gentleman. He has never smelt a flower. He has never looked at a star. He has never loved and has never done anything in his life but add up figures. And all day, he says over and over, just like you: "I am busy with matters of consequence!" And that makes him swell up with pride. But he is not a man - he is a mushroom!"

- Antoine De Saint - Exupery

in

"The Little Prince"