

ELECTROANALYTICAL SENSORS AND INSTRUMENTATION FOR HYDROPONICS

A Thesis Submitted
for the
Degree of Doctor of Philosophy

by
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ABSTRACT

SENSORS AND INSTRUMENTATION FOR HYDROPONICS

The hydroponic technique is today well established all over the world. Improvements in the crop yield can be achieved if a better control of the plant nutrients are employed. In order to improve the nutrient control more information about the hydroponic system must be acquired.

This thesis describes the development of electro-analytical on-line sensors and instrumentation for data acquisition and control of a hydroponic greenhouse system.

A microcomputer controlled Ion Selective Electrode analyser (Autoanalyser) has been designed and developed, for the detection of K^+ , Na^+ , Ca^{2+} , Cl^- , NO_3^- and pH. This system has been tested and used successfully at the National Institute of Agricultural Engineering, Silsoe.

The Wall Jet Ring Disc Electrode has been automated for bromine microtitrations of biochemically interesting species such as proteins, aminoacids and peptides. This electrode finds its use in hydroponics as a root death detector. It has also been implemented as a detector for liquid column chromatography and flow injection analysis.

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CHAPTER 1

INTRODUCTION

1.1 Hydroponics

The word hydroponics was coined by W.F.Gericke (1) in 1930 , from the two Greek words hydro ("water") and ponos ("labour") - literally "water working". Hydroponics can be defined as the science of growing plants without the use of soil. The hanging gardens of Babylon , the floating gardens of the Aztecs of Mexico and those of the Chinese are the first examples of "hydroponic" cultures.

The earliest scientific approach to nutrient uptake from water was made in 1600 by the Belgian Jan van Helmont who showed that plants obtain minerals from water. In 1804 the Frenchman de Sassure proposed that plants were composed of chemical elements obtained from water , soil and air. His compatriot Boussingault proved this hypothesis in 1851 by growing plants under controlled conditions and analysing the chemical content of the dry plant matter.

Various research workers had by this time showed that plants could be grown in an inert medium moistened by a solution containing the necessary minerals. The next step was to eliminate the medium and grow the plants in a solution containing these minerals. This was accomplished by two German scientists Sachs and Knop in 1860. Their invention the "nutriculture" and similar techniques are still in use in plant physiology and plant nutrition studies today.

These early investigations showed that plants need certain elements in large quantities ,these "macronutrients" (macroelements) are; nitrogen (N) , phosphorus (P) , sulphur (S) , potassium (K) ,

calcium (C) and magnesium (Mg), other nutrients are required in much smaller quantities and are known as "micronutrients" (trace elements), these are ; iron (Fe), chloride (Cl), manganese (Mg), boron (B), zinc (Zn), copper (Cu) and molybdenum (Mo).

The hydroponic technique was not commercially explored until about 1925, when the glasshouse industry expressed interest in it. The first "nutriculture" greenhouses were built in the early 1930s, and the term hydroponics was coined. The man behind this work was Dr W.F. Giercke at the University of California. Giercke used hydroponic techniques to grow vegetables such as beet, potatoes, lettuce and tomatoes. The technique proved itself during the Second World War when it was used to grow fresh vegetables for troops stationed on non-arable islands in the Pacific.

After World War Two commercial hydroponics expanded throughout the world and by 1950 hydroponics were being used in Italy, Spain, France, Great Britain, Germany, Sweden, the USSR and Israel.

Over the forty year period for which the hydroponic technique has been used commercially it has proved to have many advantages compared to a soil culture, e.g. more efficient nutrient regulation and use of water and fertilizer, higher planting density, better yields and in addition it may be used in non-arable regions. Table 1 shows the comparative yields per acre for soil and soilless cultures. The hydroponic technique is well described and reviewed in the literature (2-12).

TABLE 1Comparative yield per acre in soil and soilless culture

<u>Crop</u>	<u>Soil</u>	<u>Soilless</u>
Soya	600 lb	1550 lb
Beans	5 tons	21 tons
Peas	1 ton	9 tons
Wheat	600 lb	4100 lb
Rice	1000 lb	5000 lb
Oats	1000 lb	2500 lb
Beets	4 tons	12 tons
Potatoes	8 tons	70 tons
Cabbage	13,000 lb	18,000 lb
Lettuce	9000 lb	21,000 lb
Tomatoes	5-10 tons	60-300 tons
Cucumbers	7000 lb	28,000 lb

1.2 The Nutrient Film Technique1.2.1 Introduction

Mass transfer by diffusion is a slow process in a stagnant solution , so as the roots consume the nutrients there is a risk that the solution around the roots will become depleted of nutrients. This may be overcome by stirring or bubbling air in the solution , but these expedients are expensive and difficult to perform in conventional hydroponic containers.

One answer to this problem is the Nutrient Film Technique (NFT). This is a water cultural technique in which plants are grown with their root systems in a gully through which a nutrient solution is circulated , thereby constantly supplying fresh aerated nutrient solution to the roots.

1.2.2 History of NFT

This technique was invented by Allan Cooper at the Glasshouse Crops Research Institute (GCRI) at Littlehampton in 1966 (13). Initially the technique was used to study the root growth of tomatoes in this circulating system (14), but soon Cooper realized the commercial potential of the method. Large scale trials on a commercial basis started in 1972 at GCRI, at Fisons Ltd research station in Fairfield and at a nursery in Sussex. By 1973 the NFT was attracting widespread attention, and several detailed descriptions had been published (15-17).

In 1978 16 ha (1ha=10000 m²) of NFT were in use in the UK. Two years later this figure had grown to 34 ha and last year (1982) 46 ha of NFT glasshouse were in production in this country.

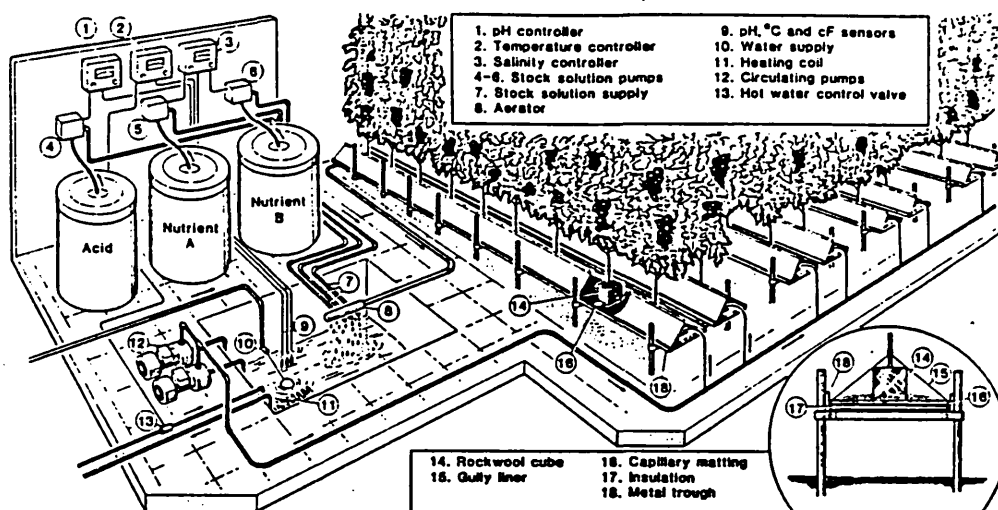


Fig 1.1 Typical layout of a NFT glasshouse system

1.2.3 Layout

A basic NFT system is shown in fig 1.1. The plants grow in gently sloping gullies. A pump feeds nutrient solution to the high end of the gully, from where it flows by gravity over the roots of the plants down to a catchment trough. This trough is also sloping in a direction which allows flow to the storage tank. It is this tank that supplies the pump with solution, thus closing the loop. A float valve regulates the liquid level in the storage tank, and the correct level of nutrients is maintained by a control system.

1.2.4 Control and Measurement

In its simplest form the control involves a manual measurement of pH and conductivity, and the grower then uses tables or his "green fingers" to assess the need for acid and nutrients. The GCRI recommends laboratory analysis of the macro nutrients every 1-2 weeks and trace elements at intervals of 2-4 weeks.

The automatic nutrient control system used by most growers today consists of two controllers , one for the pH and another for the conductivity. Both controllers work on a set-point basis - generally on a hourly basis , i.e if the sensor input is above or below a set point the controller reacts.

Conductivity depends on all ions in solution so it does not yield any information about the concentration of individual nutrients. The mains water which is used to make up the nutrient solution has a background conductivity , mainly from Ca^{2+} , Na^+ and Cl^- . So the desired conductivity must be set to $X + (\text{desired value})$ where X is the background conductivity. X is not a constant , as it will depend on how much mains water is fed in and the accumulation of the background species. A typical solution volume for a 1 ha greenhouse is 50,000 l and on a hot summer's day an equivalent amount of water may be lost by transpiration and evaporation. This leads to a fast build up of undesired chemicals in the system and a corresponding increase in X , with the consequence that the controller does not react when the plants need nutrients most. Some of the ions in the background may also be toxic at high levels , and the system must then be drained and filled with new solution.

Good pH control is essential for a healthy crop growth. The recommended range is pH 5.5-6.5. Growers favour a phosphoric-nitric acid mixture because of its nutrient content , unfortunately however this mixture has a very low buffer capacity in the desired pH range. Going outside the recommended pH range may have disastrous effects on the crop. Values below pH 5 can be very detrimental to the plants and , indeed , the circulating pump. At the other end of the scale the availability of many trace elements will decrease at

pH>6.5 because of complexation or precipitation. At pH>7 phosphates (monohydrogen phosphates) may precipitate , and such precipitates may coat the roots and prevent aeration , which if prolonged may lead to root death.

Many research workers have complained about the inadequacy of the present control systems (18,19) , and attempts have been made to develop better systems. At the Grassland Research Institute (GRI) , Hearley , a sophisticated control system was developed as far back as 1969 (20) (fig 1.2) , but it has only been used in research , probably because of the very high cost of the instrumentation.

Weaving and Hunter (21) assessed Ion Selective Electrodes for the monitoring of nutrients in solution. They found that , despite following the manufacturers recommendations , Ion Selective Electrodes were not suitable for on-line control due to instability

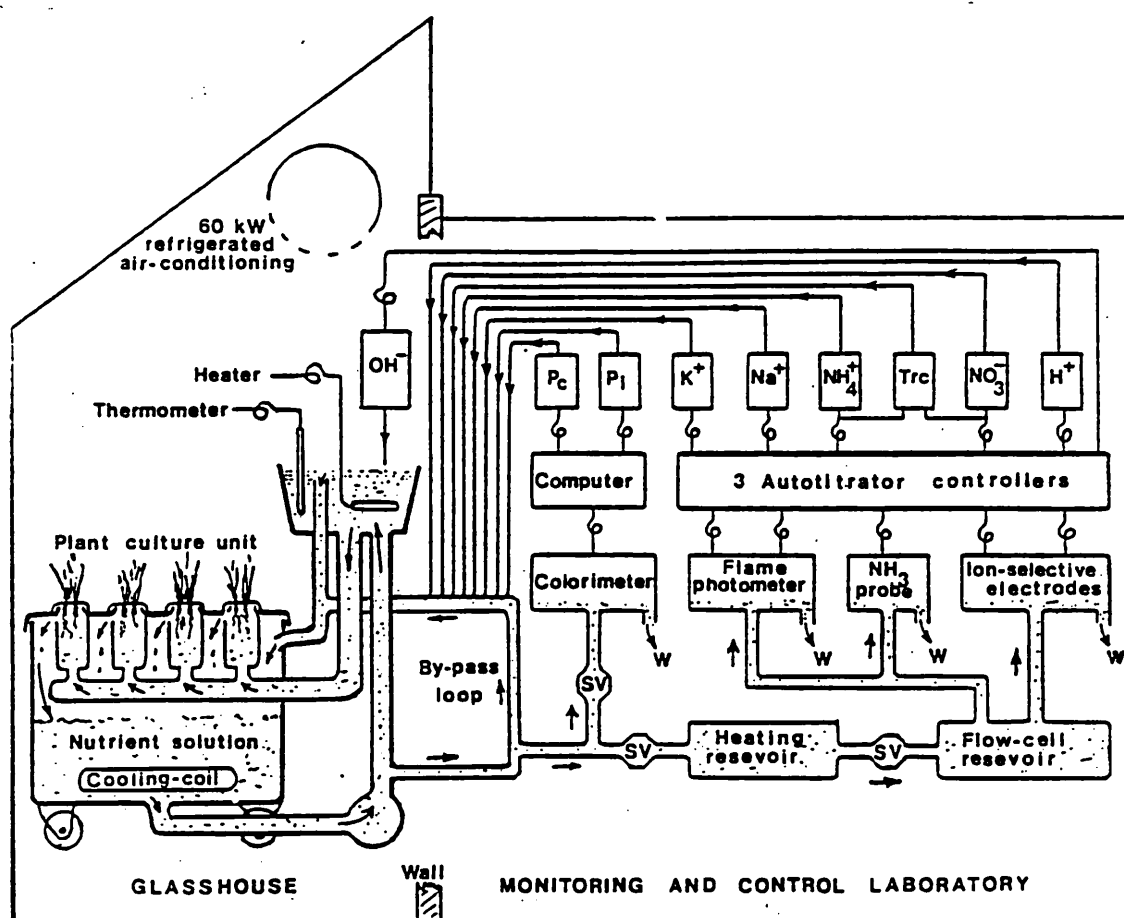


Fig 1.2 The NFT nutrient control system at GCRI

1.2.5 Advantages and Disadvantages of NFT

The NFT has many advantages over conventional systems of crop production (22). In particular it gives better possibilities for controlling the root environment. Watering is also simplified and supplied evenly over the crop, which yields a more uniform growth. Nutrient concentrations may also be matched to the varying needs of the plants throughout their lifetimes (23,24). The root temperature may be raised by heating the solution, which at times promotes growth (25,26). Heating the solution also leads to a saving in energy because the temperature in the greenhouse may be lowered,

but good growth is still maintained by the warm root environment. Pesticides may be added to the solution and spread evenly over the crop (27). Other important advantages include higher crop densities and consequently higher yields per m^2 (28,29) and a longer cropping period (March-December) .

A disadvantage of NFT is that it requires a certain expertise , mainly in chemistry. The initial capital cost is also higher compared to traditional methods , and the crop is more prone to the spread of disease because of the distribution of liquid throughout the glasshouse , but very few cases of mass failure have been reported.

1.3 ARC Research Project

The aim of this project was to design and develop a robust and reliable set of microprocessor controlled sensors for data acquisition and control of NFT.

Firstly an Ion Selective Electrode Autoanalyser was designed and developed. This instrument measures the concentration of; Na^+ , K^+ , Ca^{2+} , H^+ , NO_3^- and Cl^- . The Autoanalyser is very user friendly needing only a few commands to start it. It is also self-calibrating and provides the user with specific error messages.

An Autotitrator was designed and developed which detects any organic matter that reacts fairly fast with bromine ; typical species are proteins , amino acids , peptides , enzymes etc. This instrument has proved to be useful in the detection of root disease. When root cells die and the cell membranes disintegrate , the organic matter in the cell enters the solution , thus increasing the level of organics . The Autotitrator uses the Wall-Jet Ring-Disc

Electrode which is the best electrode for on-line measurements of this type , and may also be use as a detector in liquid chromatography.

Finally a Packed-Bed Wall-Jet Electrode (PBWJE) was designed and developed. This electrode has an upstream vitreous carbon packed bed electrode which generates bromine that is detected on the downstream Wall-Jet Electrode. The PBWJE has a higher theoretical sensitivity than the Wall-Jet Ring-Disc Electrode when it is used in the Autotitrator. This system needs further development to become a user-friendly sensor. It does however show great promise as a very sensitive detector.

CHAPTER 2

THEORY

2.1 ION SELECTIVE ELECTRODES

2.1.1 Introduction

The purpose of this theoretical outline is to make the reader familiar with the principles of operation of Ion Selective Electrodes (ISEs). For a deeper theoretical discussion the reader is referred to the abundant literature in this area (30-39).

2.1.2 The Nernst and Nicolsky-Eisenman Equations

The electrochemical potential, generated by the membrane, between the internal filling solution and the sample solution in figure 2.1.1, is described by the well known Nernst equation (40)

$$E_M = E_A^0 \pm \frac{RT}{n_A F} \ln (a_A) \quad [2.1.1]$$

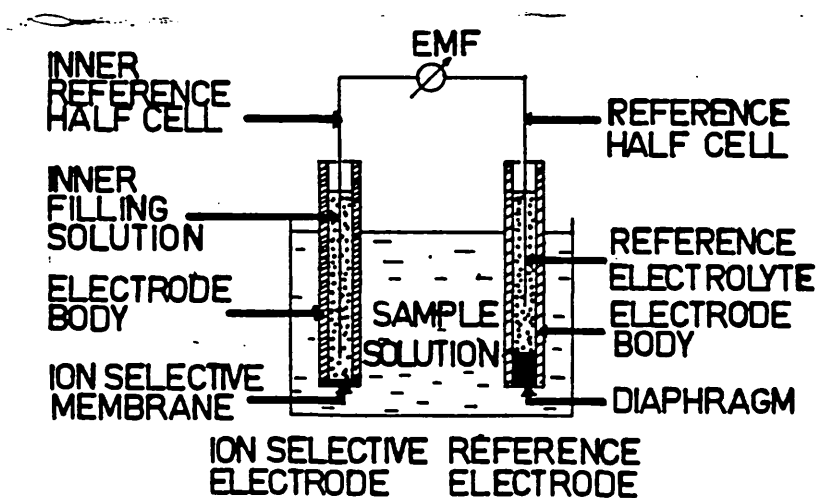


Fig 2.1.1 Schematic figure of membrane electrode cell

where

E_M = Potential difference between internal filling solution and sample solution

E_A^0 = Constant potential difference including the boundary potential difference between the internal filling solution and the membrane.

a_A = Activity of ion A in sample solution

R = Universal gas constant

T = Absolute temperature

F = Faraday's constant

n_A = Charge of ion A

The sign in equation [2.1.1] depends on n_A , i.e it is positive when A is a cation. The Nernst equation is applicable if the ISE only responds to the ion A, or, if the ion A is the only one present in the solution. Nicolsky (41) and Eisenman (42) independently derived a semi-empirical equation for the general case, when interfering ions are present. This equation is applicable to almost all ISEs.

$$E_M = E_A^0 + \frac{RT}{n_A F} \ln (a_A + \sum k_{AB} a_B^{n_A/n_B}) \quad [2.1.2]$$

where k_{AB} = Selectivity coefficient

a_B = Activity of interfering ion B

n_B = Charge of interfering ion B

k_{AB} is an empirical weighting factor for the interference of the different ions inside the summation on the right hand side of equation [2.1.2]. An ideal ISE has a $k_{AB}=0$. k_{AB} is not a constant for all activities of A and B, although it is sometimes a constant for a given ratio of the activities of A and B (43). The k_{AB} depends on the method by which it is determined. Many methods have been described (46), and several workers have tried to interpret

the meaning of the selectivity coefficient (44,45).

The electromotive force (EMF) from the ISE system in fig 2.1.1 for the ion A , when interfering ions are present , is accordingly written ;

$$E_A = E_A^0 \pm \frac{RT}{n_A F} \ln (a_A + \sum k_{AB} a_B^{n_A/n_B}) \quad [2.1.3]$$

where E_A^0 is subdivided into

$$E_A^0 = E_A^o + E_R + E_D \quad [2.1.4]$$

where E_R = Reference potential; the constant potential difference between the inner reference electrodes and their metallic conductors.

E_D = Liquid junction potential; the potential generated at the reference/sample interface by charge separation

From equation [2.1.3] we see that a 1% change in the activity of ion A corresponds to about $250 \mu V/n_A$. If the analyst wishes to measure the concentration of ion A , c_A , rather than the activity , he must correct his reading with, γ_A , the activity coefficient according to

$$c_A = a_A / \gamma_A \quad [2.1.5]$$

Debye-Huckel theory gives a fair approximation of the mean activity coefficient , $\gamma_{\pm}$, for an ion and its counter ion;

$$\log \gamma_{\pm} = \frac{A |n_+ n_-| \sqrt{I}}{1 + B a \sqrt{I}} \quad [2.1.6]$$

where I is the ionic strength;

$$I = 0.5 \sum c_m n_m^2 \quad [2.1.7]$$

$$\text{and } A = -0.509 \\ B = 0.328 \text{ @ } 25^\circ \text{C}$$

n_+, n_- = Charge of the ions

$a, C = \text{Constants}$

$C_m = \text{Concentration of ion } m$

$n_m = \text{Charge of ion } m$

Fig 2.1.2 shows the $\gamma_{\pm}$ relation to the logarithm of I for

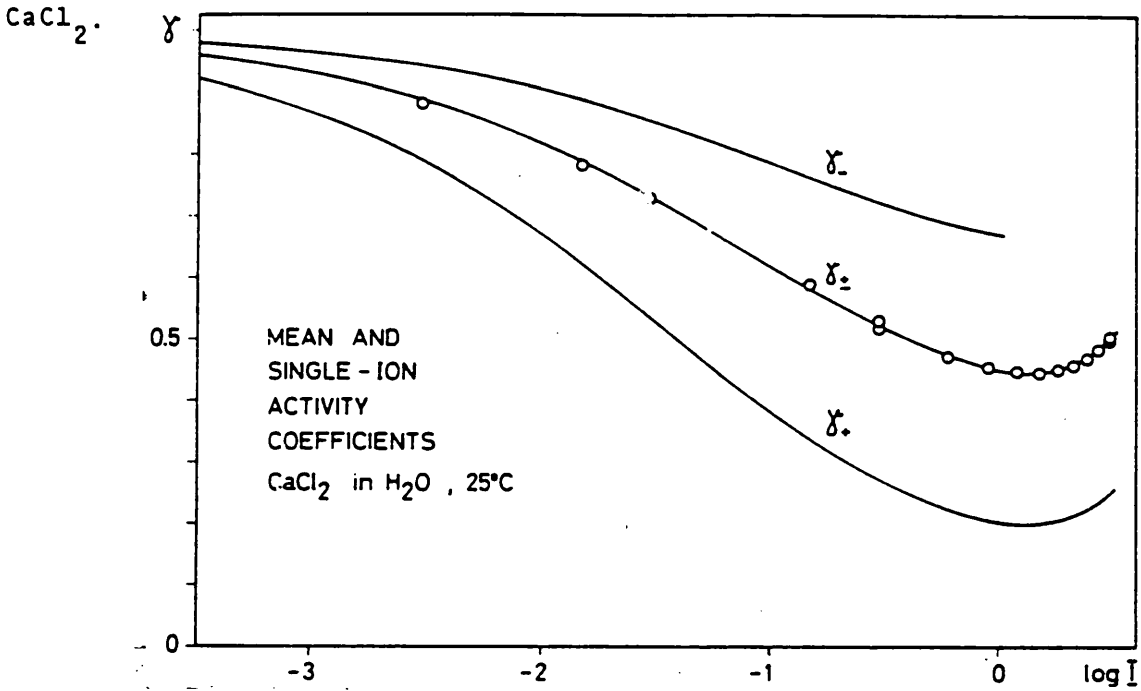


Fig 2.1.2 $\gamma_{\pm}$ plotted vs. $\log I$ for CaCl₂

The errors in concentration become unacceptable when I approaches a value of 0.001. Fig 2.1.2 also shows the single ion activities, which as such are immeasurable and are defined as (47,48)

$$\log \gamma_+ = \frac{|n_-|}{|n_+|} \log \gamma_{\pm} \quad [2.1.8]$$

The γ_- is obtained by inverting the $|n_+/n_-|$ quotient. Fig 2.1.2 also indicates that the Debye-Huckel theory is not applicable when the ions have different valency, e.g CaCl₂. Debye-Huckel and higher polynomials for γ calculation have been reported in the literature (49-54).

Most reference electrodes have a salt bridge between the refer-

ence electrolyte and the sample solution to avoid pollution of the former by the latter. A potential is generated at the salt bridge-sample solution interface which depends on both electrolytes. This potential derives from ion diffusion across the interface. Most ions have different mobilities and will therefore diffuse at different rates. A charge separation will occur depending on the difference in mobility between the cations and anions in the two solutions. This charge separation produces a potential across the junction which is called the liquid junction potential, and is described by the Henderson equation ;

$$E_D = \frac{-\sum_m u_m (c'_m - c''_m) \cdot \frac{RT}{F}}{\sum_m u_m (c'_m - c''_m) \cdot \frac{RT}{F}} \ln \left\{ \frac{\sum_m u_m^2 c'_m}{\sum_m u_m^2 c''_m} \right\} \quad [2.1.9]$$

where u_m = Absolute mobility of ion m

c'_m = Concentration of ion m

c''_m = Concentration of ion m in reference electrolyte

If we use a 1:1 electrolyte as the salt bridge solution and the sample solution is very dilute, equation [2.1.9] can be approximated to;

$$E_D = \text{const} - 59.16 \frac{(u_c'' - u_a'') \log(a_i)}{(u_c'' + u_a'')} \quad [2.1.10]$$

where u_c'' = Absolute mobility of cation in reference solution

u_a'' = Absolute mobility of anion in reference solution

The E_D is superimposed on the intrinsic ISE response, and may give rise to anomalous results if not taken into consideration.

Values of E_D are often difficult to calculate because of the lack of information about the absolute mobilities of the ions in question. We can see that E_D is reduced to a constant if $u_c'' = u_a''$.

In fig 2.1.3 the liquid junction potential is plotted against the logarithm of the activity of different primary ions and different concentrations of the potassium chloride bridge electrolyte. We observe a linear region at low activities followed by a non-linear region. The commencement of the non-linear region depends on the strength of the salt bridge electrolyte, stronger electrolytes give more linearity. The linear region is less inclined and the non-linear region is less curved if the cation and anion in the salt bridge have the same absolute mobility. The solution to the E_D problem is to use a strong salt bridge electrolyte, with ions of the same absolute mobility, for example a 3 M KCl solution.

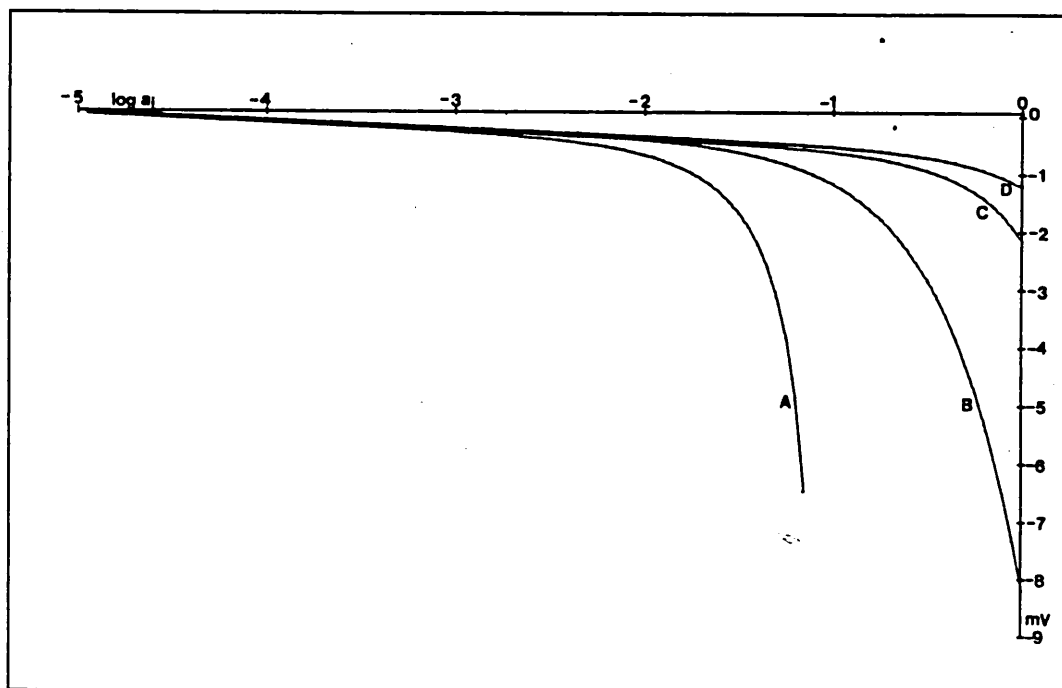


Fig 2.1.3 Liquid junction potential for different KCl salt bridge electrolyte concentrations plotted versus a variation in the primary ion. Curve A shows the E_D for $[KCl]=0.1$ M and $Ca(NO_3)_2$. Curve B shows the E_D for $[KCl]=0.1$ M and $NaNO_3$. Curve C shows the E_D for $[KCl]=3$ M and $Ca(NO_3)_2$. Curve D shows the E_D for $[KCl]=3$ M and $NaNO_3$.

2.1.3 Electrode Response

Fig 2.1.4 shows a typical EMF response curve for an ISE cell if the primary ion activity is changed in a constant background of interfering ions. We can deduce the k_{AB} and the detection limit for the ISE in that background electrolyte from such plots. The curvature at low $\log(a_A)$ comes from the summing term in the Nicolsky-Eisenman equation [2.1.2], which at low activities of primary ion starts to play a more dominating role. Ultimately the EMF response is almost entirely due to the interfering ions and thus yields a horizontal straight line, because their activities are constant.

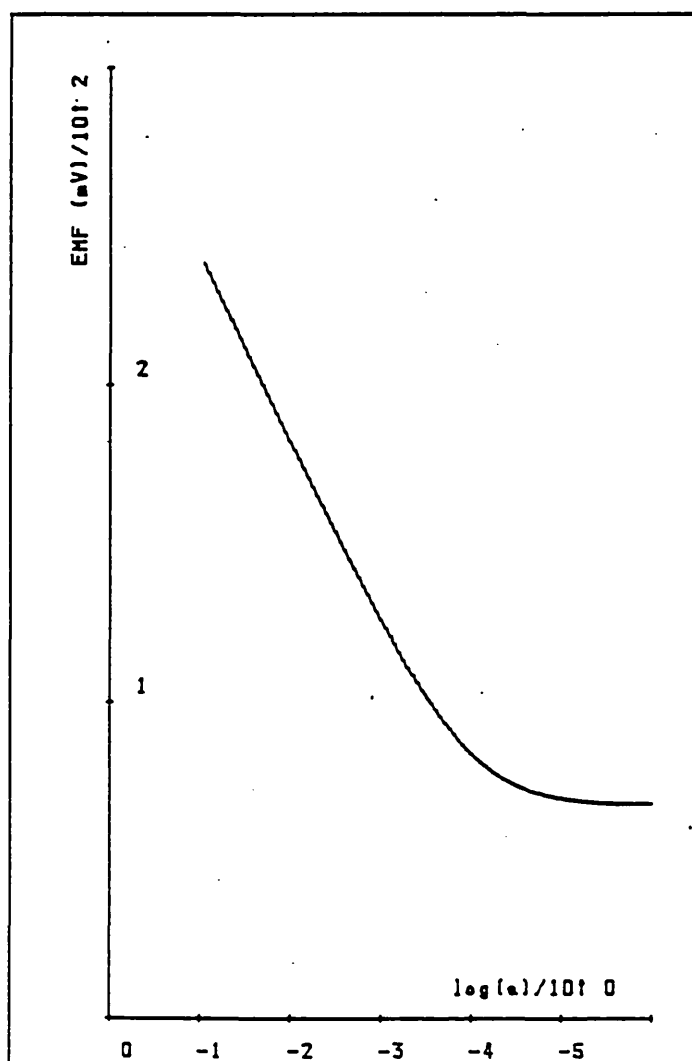


Fig 2.1.4 Typical EMF response curve for an ISE cell in an interfering background electrolyte

The k_{AB} may be calculated in several ways. The simplest one is to extrapolate the intersection of the two straight lines. At the point of intersection the ISE responds equally to both ions, i.e. $a_A = k_{AB} a_B^{n_A/n_B}$ from which k_{AB} is derived. Another method does not rely on the linearity of the interferent dominating region. Instead it derives a mathematical relationship at the point of intersection in the previous method. At this point we can write

$$E = E^0 + \frac{RT}{n_A F} \ln (a_A + k_{AB} a_B^{n_A/n_B}) = E_A^0 + \frac{RT}{n_A F} \ln (2 a_A) \quad [2.1.11]$$

if no ion B was present we write

$$E = E_A^0 + \frac{RT}{n_A F} \ln (a_A) \quad [2.1.12]$$

If we now subtract [2.1.11] from [2.1.12] we get

$$\Delta E = \frac{RT}{n_A F} (\ln (2a_A) - \ln (a_A)) = \frac{RT}{n_A F} \ln 2 \approx 18 \text{ mV} \quad [2.1.13]$$

By finding ΔE on the graph, where the difference between the experimental and the extrapolated line is equal to $18/n_A$ mV, the selectivity coefficient can be calculated. A least mean square curve fitting procedure for finding k_{AB} as well as E^0 has been suggested (55).

IUPAC's detection limit (56) is defined in the same way as k_{AB} was determined in the second method, i.e. the activity of A which corresponds to a deviation of $18/n_A$ mV from the extrapolated Nernstian slope.

2.1.4 Types of ISEs

ISEs can be categorised according to the nature of their membranes. The three types used in this work are shown in fig 2.1.5, i.e glass electrodes, liquid ion-exchange membrane electrodes and solid state membrane electrodes. These types of electrodes are described in the following section.

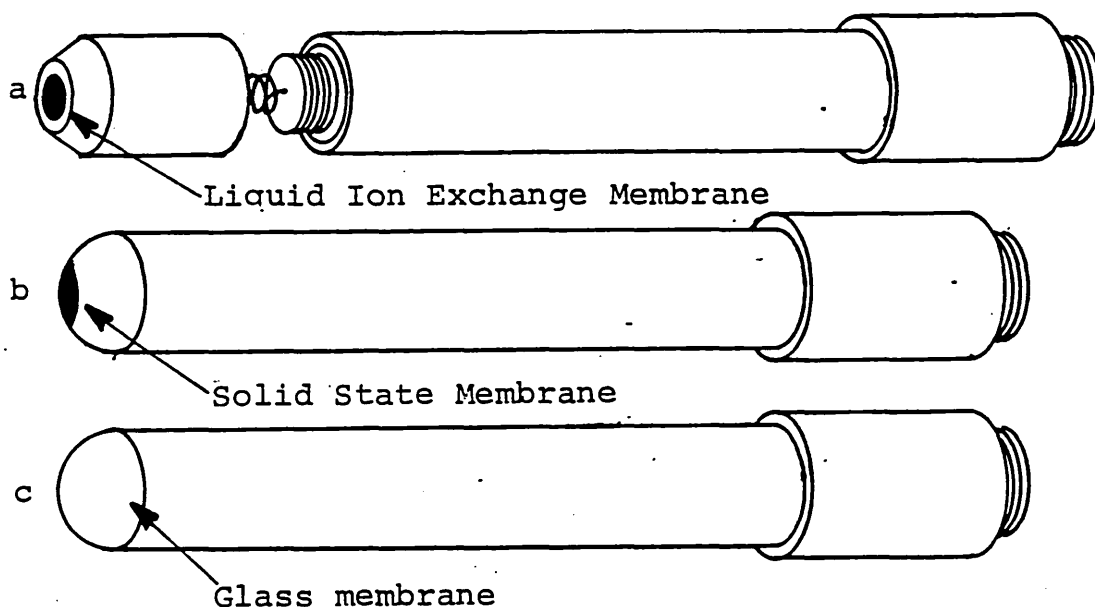


Fig 2.1.5 a) Ion Exchange Liquid membrane ISE b) Solid State membrane ISE c) Glass membrane ISE

2.1.4.1 Glass Electrodes

When a glass membrane is immersed in an aqueous solution it absorbs water and a hydrated layer is formed on the surface. The thickness of this layer, $10-10^{-2} \mu\text{m}$, depends on the glass and the electrolyte. Monovalent cations on the glass surface, usually Na^+ or Li^+ , are exchanged with the measured ion, thus forming part of an ion exchange equilibrium. The glass can be described as a cation exchanger with the number of sites determined by the structure of the hydrated layer. The potential at this boundary varies with the activity of the measured ion according to Nernst equation. When

there is competition for the ion exchange sites by different ions the response becomes more complicated. Eisenman (57) has developed a theory which has been successful in explaining the selectivity of a glass in terms of the ratio of mobilities of the competing ions within the glass and also the free energy changes which occur during the ion exchange process.

pH electrodes can usually span an activity range 10^{-10} to 10^{-14} M H^+ . The response limit for the best sodium glass membranes appear not to be limited by the membrane glass in the measurable range but by the purity of available water (58). It is very difficult to prepare water containing less than 5×10^{-8} M Na^+ (59). The pH range is usually restricted for Na^+ glass electrodes.

2.1.4.2 Liquid Ion Exchange Electrodes

The theoretical description of ion exchange electrodes is quite intricate. Eisenman (60) has derived theoretical expressions for the membrane potential and k_{AB} . Unfortunately these equations are only soluble in simple cases which do not correspond to real ISE situations.

Briefly, k_{AB} depends on the equilibrium between the primary ion A^+ and the interferent B^+ with the ion exchanger S^- in the membrane, and their distribution coefficients k_A^+ and k_B^+ . Schematically



and

$$K_{AB} = \frac{K_{BS} k_B^+}{K_{AS} k_A^+} \quad [2.1.15]$$

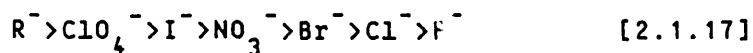
where

K_{AB}, K_{AS} = Stability constants for AB respectively AS

This treatment allows at least a ranking of the different interferences in the correct order. We expect large values for large lipophilic organic ions (R^-/R^+) and small values for ions strongly hydrated in the aqueous phase. For a cation-exchange membrane this sequence is usually found (61)



analogously for anion exchangers



The useful activity span for ion exchange type ISEs is limited by a number of factors. The upper limit is often determined by the concentration of the target ion in the filling solution, if the determinand activity is larger than this activity, an anomalous and non-Nernstian response may occur. The lower limit is often caused by the small solubility of the ligand into the aqueous phase, thus raising the activity of the primary ion in the sample solution. The lower pH limit is determined by the onset of protonation of the ligands. For cations the upper limit is the onset of complexation, e.g. $CaOH^+$, $MgOH^+$ etc, whereas for an anion ISE the upper limit is set by hydroxyl interference. Generally ion exchange electrodes are useful in the range $1-10^{-6}$ M in the applicable pH range.

2.1.4.3 Solid State Electrodes

Solid state electrodes detect ions in the insoluble salts forming the membrane, thus allowing detection of other ions interacting with the ionic sites.

When immersed in the sample solution interfacial potentials similar to those found in glass electrodes are developed which are associated with the ion-exchange processes on the membrane surface.

Bailey (62) gives a good account of this theory.

The selectivity of silver halide membranes ,e.g chloride ISEs , for interfering ion B^{n-} relative to the primary ion A^- is determined by the solubility products K_{AgA} and K_{AgB} (63,64) , i.e

$$K_{AB} = \frac{K_{AgA}}{(K_{AgB})^n}^{1/n} \quad [2.1.18]$$

Hence the selectivity sequence is the same for all membranes of this type ;



The lower response limit is set by the solubility of the membrane , since this leads to small activities of the membrane components. The upper limit is also set by the dissolution of the membrane. The silver-chloride electrode forms polychloroargentate(I) species , i.e $AgCl_2^-$, $AgCl_3^{2-}$, when immersed in strong Cl^- solutions. The pH range for silver-chloride ISEs is limited by hydroxide interference and at $pH < 2$ silver salt membranes are attacked by hydrogen ions. Generally the useful activity range is $1-10^{-5}$ M , in the pH range 2-11 , for silver halide electrodes.

2.2 Hydrodynamic Voltammetry

2.2.1 Introduction

Voltammetry is that part of electrochemistry which describes the voltage current relationships due to oxidation or reduction of ions or molecules in solution at electrode surfaces. The current produced is proportional to the rate of the reaction at the electrode surface.

The course of the reaction may be divided into three steps;

- 1) Transfer of the reactants from the bulk of solution to the electrode surface
- 2) The electrochemical reaction at the electrode surface
- 3) The formation of products and their deposition on the surface or their removal from the surface to the bulk of the solution

In electroanalytical chemistry it is desired to make the first step the rate limiting step. This enables us to control the reaction rate, and if the mass transfer rate is known the concentration of the reacting species may be deduced from the transport limited electrode current.

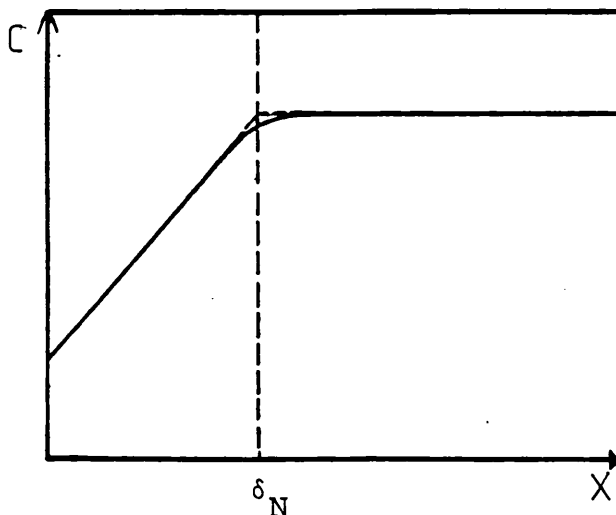


Fig 2.2.1 The concentration profile close to the electrode surface

Nernst suggested that there exists a thin layer of stationary solution close to the electrode surface, and within that layer diffusion controls the mass transfer. Outside this layer other processes dominate. Fig 2.2.1 illustrates the Nernst diffusion layer and the concentration gradient close to the electrode surface.

The flux of reactant to the electrode can be calculated from Fick's first law

$$j = D \left(\frac{\partial C(x)}{\partial x} \right)_{x=0} \quad [2.2.1]$$

If this is the rate limiting step, it is proportional to the current

$$i = jnFA = nFAD \left(\frac{\partial C(x)}{\partial x} \right)_{x=0} \quad [2.2.2]$$

where x = Normal distance from the electrode surface

D = Diffusion coefficient

n = Number of electrons involved in the reaction

A = Area of the electrode

F = Faraday's constant

Then, according to Nernst, the concentration gradient is linear inside the diffusion layer

$$\left(\frac{\partial C(x)}{\partial x} \right)_{x=0} = \frac{C - C_0}{\delta_N} \quad [2.2.3]$$

where C = Bulk concentration of reacting species

C_0 = Concentration of reacting species at electrode surface

If we combine [2.2.2] and [2.2.3] we get

$$i = \frac{nFADC}{\delta_N} \quad [2.2.4]$$

This equation forms the basis for voltametric analysis. The problem is to find an expression for the Nernstian diffusion

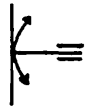
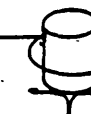
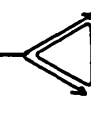
layer thickness δ_N .

2.2.2 Controlled Hydrodynamic Electrodes

The Nernstian diffusion layer thickness , δ_N , and the diffusion limiting current has been solved for a number of electrode systems . The most used ones are listed in Table 2.1.1.

Table 2.1.1

The most common hydrodynamic voltammetry electrode systems

Electrode	Geometry	Limiting current	Ref
Wall Jet		$1.73nFR^{3/4}a^{1/2}D^{2/3}u^{-5/12}V_f^{3/4}C$	64
Rotating Disc		$0.62nFAD^{2/3}u^{-1/6}\omega^{1/2}C$	62 63
Planar		$0.68nFAL^{-1/2}D^{2/3}u^{-1/6}V_f^{-1/2}C$	62
Conical		$0.77nFAL^{1/2}D^{2/3}u^{-1/6}V_f^{1/2}C$	65
Tubular		$2.01nFR^{2/3}L^{2/3}D^{2/3}V_f^{1/3}C$	66
Spherical		$4\pi r n F D C + k n r D^{2/3}f^{1/2}C$	67

n=number of electrons in the reaction;F=Faraday's constant;
A=Electrode area;D=Diffusion coeff.;u=Kinematic viscosity; ω =Rotation speed; V_f =Flow rate;L=Electrode length;R=Radius;a=Nozzle diameter;
k=Empirical constant;f=Stirring frequency

The rotating disc electrode (RDE) is a well established electroanalytical technique for batch analysis .The RDE's advantages are

- 1)Well behaved hydrodynamics
- 2)Uniform diffusion layer

3) Working electrode is easily maintained

4) Wide operating range using platinum or glassy carbon electrodes

Out of the hydrodynamic electrode systems in Table 2.2.1 we conclude that the wall-jet electrode (WJE) is the best suited for on-line analysis. We base our judgement on the following;

1) Ultra low dead volume, $< 1 \mu\text{l}$

2) Working electrode is easily maintained

3) High sensitivity

4) Wide operating range using platinum electrodes or glassy carbon electrodes

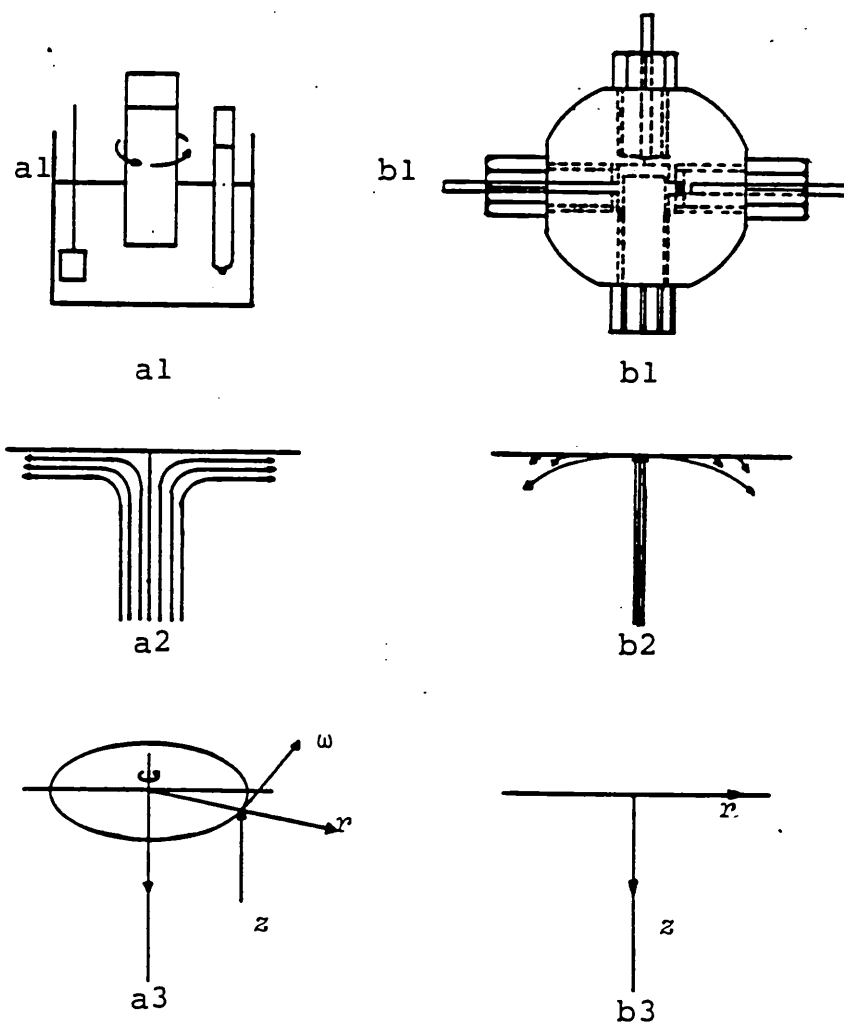


Fig 2.2.2 a) The RDE and WJE system b) Flow pattern b) Coordinates

Fig 2.2.2 illustrates the RDE and the WJD and their hydrodynamics . The major difference between the two electrodes is that the RDE has a uniform diffusion layer whereas the WJE has a parabolic shaped diffusion layer which is zero at the centre of the jet and then increases in proportion to $r^{5/4}$.

2.2.3 Ring-Disc Electrodes

A very useful extension to the WJE and the RDE is to add a concentric ring electrode with a thin gap between the electrodes. A typical ring disc electrode is illustrated in fig 2.2.3.

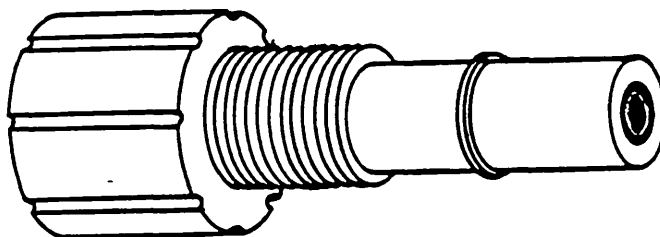


Fig 2.2.3 A typical Wall-Jet Ring-Disc Electrode

This electrode configuration allows us to study the electrochemical reactions at the disc on the downstream ring electrode . Albery and Brett (68) have developed a general ring disc theory , which they apply on the Wall-Jet Ring-Disc Electrode (WJRDE). They derive an analytical expression for the limiting current on the disc

$$i_{D,L} = 1.59 knFD^{2/3} u^{-5/12} v_f^{3/4} a^{-1/2} r_1^{3/4} C \quad [2.2.5]$$

They find that this current is related to the limiting ring current by a geometrical factor β

$$i_{R,L} = \beta^{2/3} i_{D,L} \quad [2.2.6]$$

This is the same expression as for the Rotating Ring Disc Electrode (RRDE). They move on to derive a formula for the collection efficiency and arrive at the same equation as for the RRDE (69) but with redefined α and β values , listed in Table 2.2.2

Table 2.2.2

RRDE and WJRDE α and β values

Electrode	α	β
RRDE	$(r_2/r_1)^3 - 1$	$(r_3/r_1)^3 - (r_2/r_1)^3$
WJRDE	$(r_2/r_1)^{9/8} - 1$	$(r_3/r_1)^{9/8} - (r_2/r_1)^{9/8}$

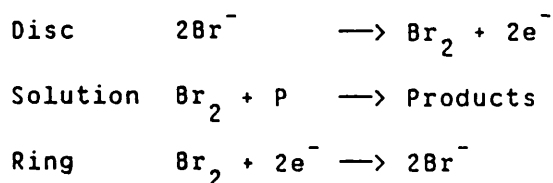
The collection efficiency, N_o , is then calculated from a common equation for RRDE's and WJDE's ;

$$N_o = 1 - F(\alpha/\beta) + \beta^{2/3} [1 - F(\alpha)] - (1 + \alpha + \beta) [1 - F\{(\alpha/\beta)(1 + \alpha + \beta)\}] \quad [2.2.7]$$

where

$$F(\theta) = \frac{3^{1/2}}{4\pi} \ln \left(\frac{1 + \theta^{1/3}}{1 + \theta} \right)^3 + \frac{3}{2\pi} \tan^{-1} \left(\frac{2\theta^{1/3} - 1}{3^{1/2}} \right) + 1 \quad [2.2.8]$$

Finally they derive a theory for diffusion layer microtitrations. In this technique Br_2 is generated on the disc and may react with brominatable species during its passage from the disc to the ring, thus diminishing the ring current. The reaction scheme is



The ring current as a function of the disc current is shown in fig 2.2.4. Initially no ring current is seen because all Br_2 is consumed by the reacting species P before it reaches the ring. Then the bromine dominated region spreads across the ring electrode giving rise to the curved trace. Finally the bromine dominated region covers the ring and the ring current becomes a linear function of the disc current. All the expressions derived for microtitrations on the RRDE hold for the WJRDE with the redefined α and β values. From their paper, the equation for the linear part of the

titration curve is

$$i_R = N_O |i_D| - \beta^{2/3} |i_P| \quad [2.2.9]$$

where i_P is the limiting current of P on the disc if it were electroactive. The linear region starts at the intersection of the line N' and equation [2.2.9]

$$N' = 1 - F\left(\frac{\alpha}{\beta}\right) - \frac{(1+\alpha)}{(1+\alpha+\beta)^{1/3}} \left[1 - F\left\{\frac{\alpha(1+\alpha+\beta)}{\beta}\right\} \right] \quad [2.2.10]$$

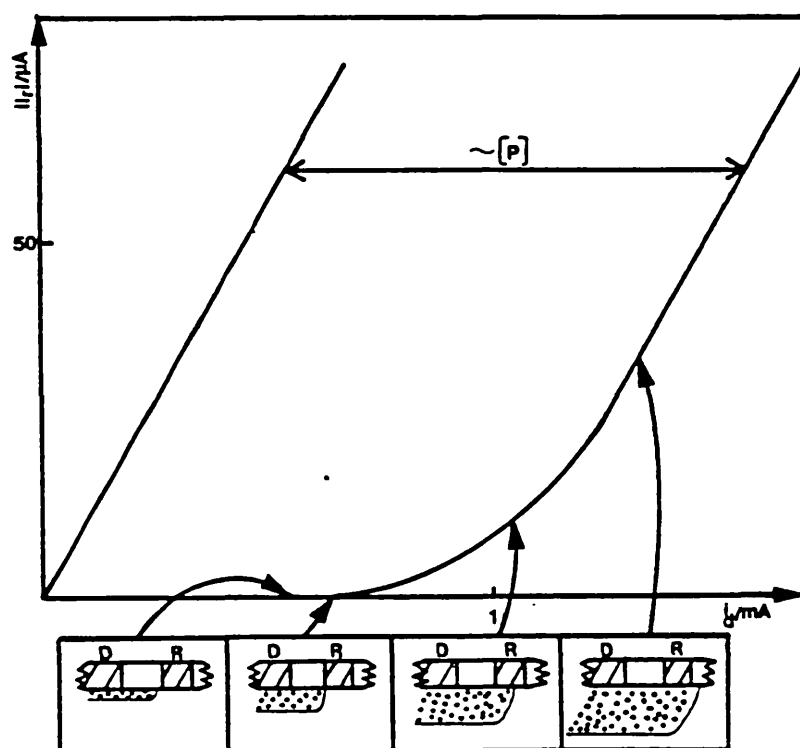


Fig 2.2.4 Ring-disc bromine titration curve. The insets show how the generated bromine spreads out over the electrode surface.

2.2.4 Extension of the Ring Disc Titration Theory

A subsequent paper by Alberly, Svanberg and Wood (70) presents an extension of the original titration theory (71). They derive a modified equation for the bromine ring current

$$|i_{R,B}| = N_O i_{D,B} - \beta^{2/3} i_{D,P} \quad [2.2.11]$$

where $i_{D,P} = 2mF\pi r_1^2 D_P [P] / \delta_P$

m =Number of moles of Br_2 consumed by P

D_p =Diffusion coefficient for P

$[P]$ =Conc of P

δ_p =Diffusion layer thickness

The titration current $i_{D,p}$ corresponds to the limiting current that would be observed if all the brominated groups on P were electroactive. Their argument shows that in the titration term, $i_{D,p}$, one must use the diffusion coefficient, D_p , for P in place of the bromine diffusion coefficient. This result is perhaps not surprising since it is the incoming flux of slow moving P that is being titrated by the bromine.

2.3 Control Theory

2.3.1 Introduction

A definition of engineering is (72) "Engineering is concerned with understanding and controlling the materials and forces of nature for the benefit of mankind". Control engineering falls fully under this definition ; in order to control a system we must understand its mechanism.

What is a system ? In control theory we define a system as a collection of interacting components separated from its environment by an imaginary boundary. These components will often be discrete physical elements of hardware but may also be functional parts thereof. There is an interaction between the system and its surrounding environment across the boundary. Signals going into the system are called inputs and conversely outgoing signals are called outputs. A control system may have many system inputs/outputs. A typical multi input-output system is a car and its driver. The boundary is the bodywork , inputs are traffic lights , police cars etc , outputs are the speed , direction etc.

2.3.2 System Analysis and Modelling

In order to understand the system we must analyse it. From the result of the analysis we can model the system and introduce control elements to achieve our aim ; to control the system.

Having defined the boundary of the system it is useful to think of the components in the system as "blocks". These blocks give a useful pictorial representation of the system , such a diagram is called a block diagram. Fig 2.3.1 illustrates the car-driver control system in a block diagram. The lines represent signal flow paths ,

and the arrows shows the direction of the signals. Signal subtraction/addition is indicated by a crossed circle with the sign of the operation in the corresponding quadrant.

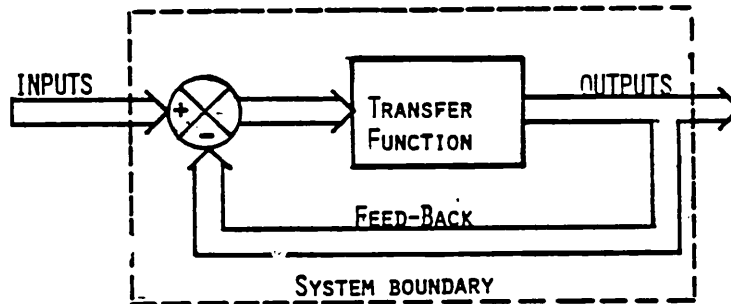


Fig 2.3.1 Block diagram of the car-driver system

Block diagrams show only the inter-relationships between the parts of the system, but for analysis we must add some sort of mathematical description of the blocks. The assignment of this mathematical description is called modelling. If the system is dynamic the equations relating the outputs to the inputs of the blocks will in general be differential equations. Take the capacitor resistor network in fig 2.3.2 for example;

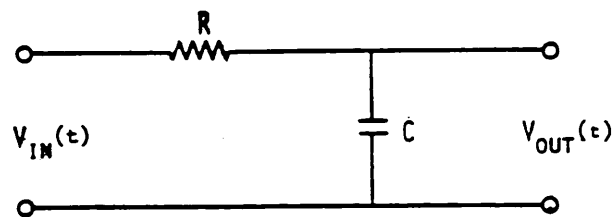


Fig 2.3.2 Input-output relationship of resistor capacitor network

Kirchoff's law gives

$$I(t) = \frac{V_i(t) - V_o(t)}{R} = C \frac{dV_o}{dt} \quad [3.2.1]$$

rearranged

$$V_i(t) = RC \frac{dV_o}{dt} + V_o(t) \quad [3.2.2]$$

This is an input (V_i) , output (V_o) relationship. For ideal systems the mathematical model is often linear , and if it is possible to represent the system by linear differential equations the analysis is very much simplified.

A system is linear if it obeys the principle of superposition. This requires that the independent inputs $I_1(t)$ and $I_2(t)$ produce outputs $O_1(t)$ and $O_2(t)$, and the simultaneous application of $I_1(t)+I_2(t)$ will produce $O_1(t)+O_2(t)$. Although no real system is linear there is often a linear operating range , or if the system is inherently non-linear but the perturbations from the chosen set-point are small it may be treated as a linear system using linear approximations.

2.3.3 The LaPlace Transform Technique

The LaPlace transform technique is very useful for solving differential equations. The LaPlace transform theory is outlined in Appendix I.

In the LaPlace technique the equation is transferred to LaPlace space by changing the time variable t for the complex variable $s=\sigma+j\omega$, the LaPlace operator. The transform gives an algebraic equation , which may be solved in terms of s . Finally the time domain solution is achieved by inverting the LaPlace transform. Large tables exist for LaPlace transformation to and from LaPlace space.

The transformation of a linear system is the ratio of the transformed input to the transformed output when all initial conditions are zero. Conventionally the symbol $G(s)$ is used for the system transform. Re-examining the example in fig 2.3.2 , the linear

input/output differential equation is

$$V_i(t) = RC \frac{dV_o(t)}{dt} + V_o(t) \quad [2.3.2]$$

Table 1 in Appendix I gives

$$V_i(s) = RCsV_o(s) + V_o(s) \quad [2.3.3]$$

Thus the transfer function is

$$G(s) = \frac{V_o(s)}{V_i(s)} = \frac{1}{1 + RCs} \quad [2.3.4]$$

It is seen that the transfer function is a property of the system and describes its dynamic characteristics, but is not influenced by the state of the system. The resulting transfer function for the general case takes the form;

$$G(s) = \frac{P(s)}{Q(s)} \quad [2.3.5]$$

where $P(s)$ and $Q(s)$ are polynomials in s . A physically realizable system must have the order of the denominator larger than the numerator. The equation obtained as $Q(s) = 0$ is called the characteristic equation, whose roots are referred to as poles because $G(s) = \infty$ at these values. The number of roots, i.e. the order of $Q(s)$ is termed the order of the system. The roots of $P(s)$ are called zeros because $G(s) = 0$ at these values. This notation simplifies the mathematical treatment of block diagrams. Consider the resistor capacitor network in fig 2.3.3 which we can break down to a block diagram in fig 2.3.4

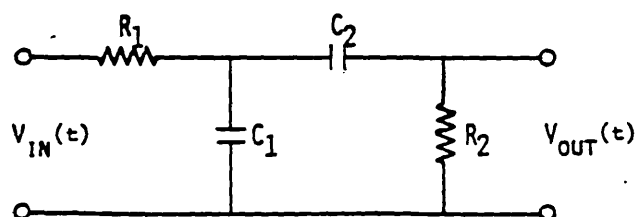


Fig 2.3.3 Double capacitor resistor network

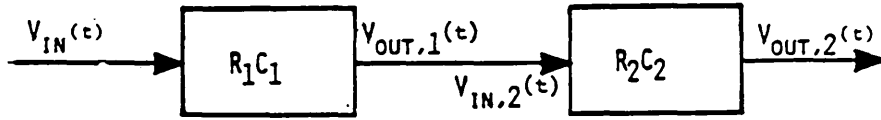


Fig 2.3.4 Block diagram of resistor capacitor network

Taking the LaPlace transform of the blocks in fig 2.3.4 gives the result shown in fig 2.3.5

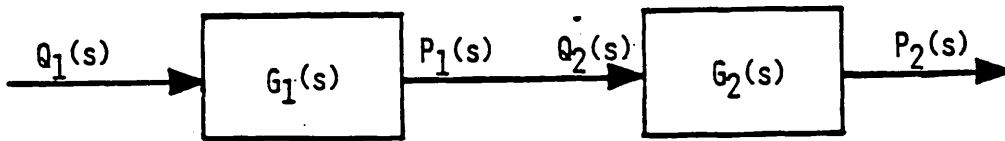


Fig 2.3.5 LaPlace transformed block diagram

where $G_1(s) = (1 + R_1 C_1 s)^{-1}$ from equation [2.3.4] and $G_2(s) = R_2 C_2 (1 + R_2 C_2 s)$. We notice that $P_1(s) = Q_2$, thus we may write

$$G_2(s) = \frac{P_2(s)}{P_1(s)} \quad [2.3.7]$$

and

$$G_1(s) = \frac{P_1(s)}{Q_1(s)} \quad [2.3.8]$$

multiplying [2.3.7] by [2.3.8] gives ;

$$G_1(s) * G_2(s) = \frac{P_2(s)}{Q_1(s)} \quad [2.3.9]$$

$Q_1(s)$ describes the input to the system and $P_2(s)$ describes the output of the system, thus we can write

$$G(s) = G_1(s) * G_2(s) = \frac{R_2 C_2 s}{(1 + R_1 C_1)(1 + R_2 C_2)} = P_2(s) \quad [2.3.10]$$

The block diagram is therefore simplified to just one block, fig 2.3.6.

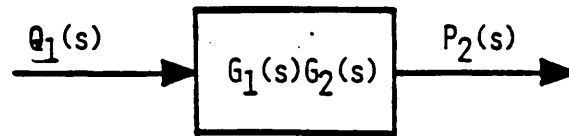


Fig 2.3.6 Simplified block diagram

This simplification applies to all linear transfer functions and justifies their great usage in modelling of complex systems.

2.3.4 Feedback Control Systems

In order to control the system ,i.e to make a certain input give the desired output, we feed a proportion of the output signal back to the input ,where it is subtracted from the input signal. This operation is called negative feed-back control. Fig 2.3.7 is a schematic illustration of a feed-back control system

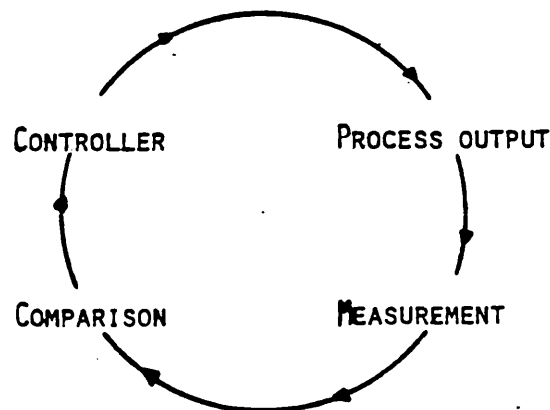


Fig 2.3.7 A schematic closed loop feed-back system

The resulting signal from the feed-back is called the error-signal $E(s)$. The closed loop system , when performing correctly ,operates so that the error will be reduced to a minimum. Fig 2.3.8 illustrates a general negative feed-back system ,

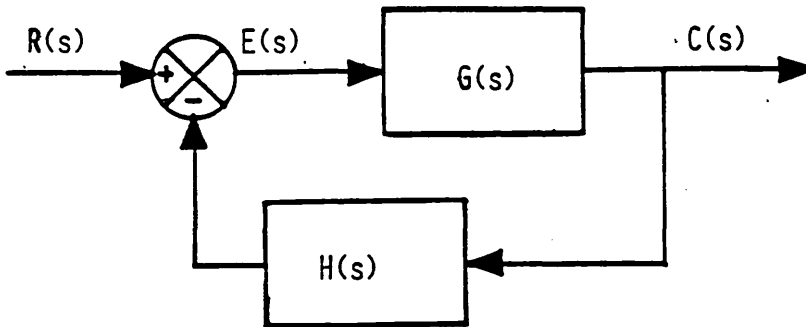


Fig 2.3.8 A general negative feed-back system

where $R(s)$ is the chosen value ; the set point. $E(s)$ is the error signal and $G(s)$ is the transfer function of the open loop path , $H(s)$ is the negative feed-back transfer function and $C(s)$ is the output. The output from the feed-back system is;

$$C(s) = E(s)G(s) \quad [2.3.11]$$

but

$$E(s) = R(s) - H(s)C(s) \quad [2.3.12]$$

inserting [2.3.12] in [2.3.11] gives ;

$$C(s) = G(s)\{R(s) - H(s)C(s)\} \quad [2.3.13]$$

rearranging [2.3.13] gives ;

$$C(s) = \frac{G(s)R(s)}{1 + G(s)H(s)} \quad [2.3.14]$$

The activating error signal is

$$E(s) = \frac{R(s)}{1 + G(s)H(s)} \quad [2.3.15]$$

It is now clear that the magnitude of $1 + G(s)H(s)$ must be larger than unity in order to reduce the error.

One of the greatest advantages of feed-back is its ability to reduce the system's sensitivity to unwanted changes in system parameters . Recalling the transfer function for the closed loop, equa-

tion [2.3.14], and assuming $G(s)H(s) \gg 1$ for all frequencies of interest, we obtain

$$C(s) = \frac{R(s)}{H(s)} \quad [2.3.15]$$

i.e the output $C(s)$ is only affected by the feed-back transfer function $H(s)$. Variations in the process parameters $G(s)$, which are more likely to change, are suppressed by introducing negative feed-back. A typical negative feed-back system is an inverting operational amplifier, fig 2.3.9.

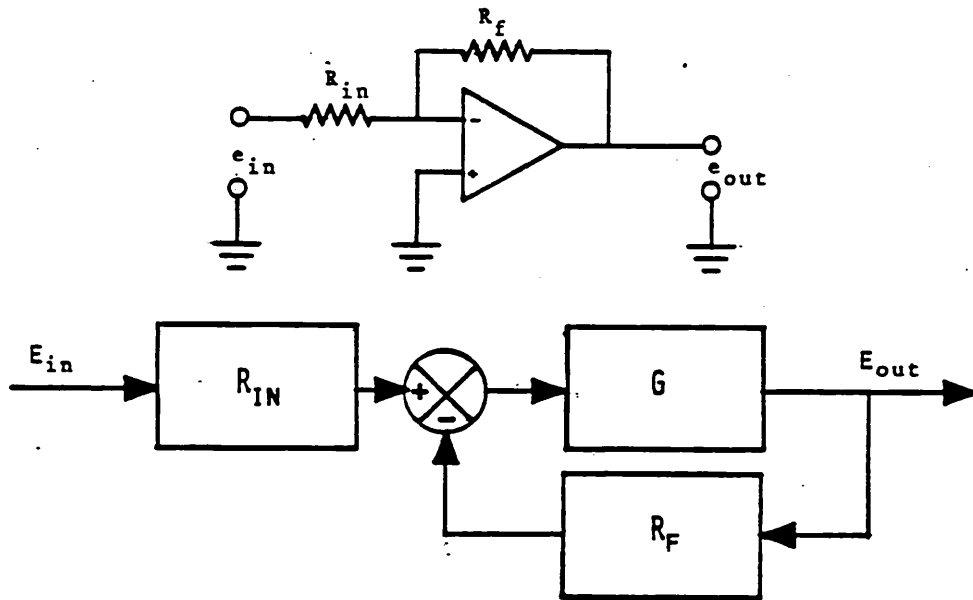


Fig 2.3.9 a) Inverting operational amplifier b) Block diagram of the amplifier circuit

An operational amplifier has an open-loop gain of about 10^6 , and an input impedance of about $10^{10} \Omega$ so no current will flow into the amplifier. Using the feed-back theory and Kirchhoff's second law at the summing point S we obtain

$$\frac{e_{in} - e_s}{R_i} + \frac{e_{out} - e_s}{R_f} = 0 \quad [2.3.16]$$

$$\text{but } e_{out} = e_s G \Rightarrow e_s = e_{out} / G \quad [2.3.17]$$

inserting [2.3.17] in [2.3.16]

$$\frac{e_{in} - e_{out}}{R_i} + \frac{e_{out} - e_{out}}{R_f} = 0 \quad [2.3.18]$$

Rearranging gives

$$\frac{e_{out}}{e_{in}} = \frac{G}{1 - G(R_i/R_f)} = \frac{G(s)}{1 + G(s)H(s)} \quad [2.3.19]$$

Thus the open-loop gain G is the process transfer function $G(s)$ and R_f/R_i the feed-back transfer function $H(s)$. The sensitivity of the system transfer function $T(s)=C(s)/R(s)$ to changes in $G(s)$ can be expressed as

$$S = \frac{\partial T/T}{\partial G/G} = \frac{1}{(1 + G(s)H(s))^2} \frac{G(s)}{G(s)/(1 + G(s)H(s))} = \frac{1}{1 + G(s)H(s)} \quad [2.3.20]$$

Applying equation [2.3.20] in [2.3.19]

$$S = \frac{1}{1 - G(R_i/R_f)} = \frac{1}{1 - 10^{-5}} \quad [2.3.21]$$

A typical value for R_i is 1 k Ω and for R_f is 10 k Ω , which gives the magnitude of the sensitivity $S=10^{-5}$. Since for an open-loop system the sensitivity S is 1, the feed-back has improved the performance 100,000 times.

Another important benefit from feed-back is its immunity to noise. Consider the system in fig 2.3.10, illustrating a pH-stat

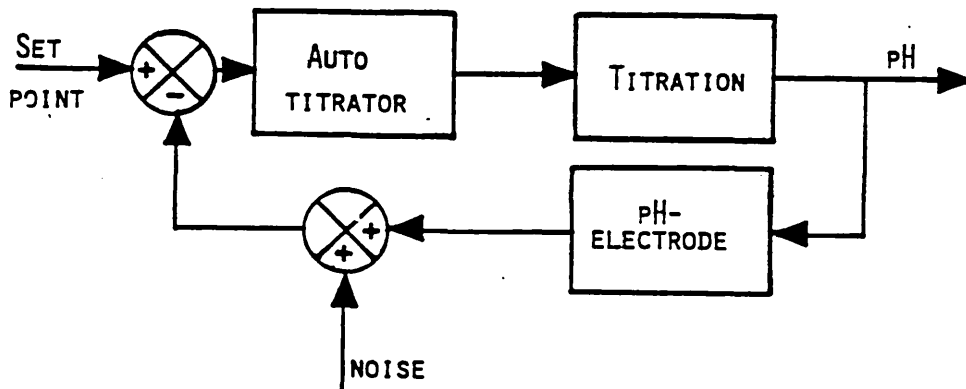


Fig 2.3.10 Block diagram of a noisy pH-stat

The pH-electrode is exposed to noise (electrical , chemical etc) $N(s)$. $P(s)$ is the pH set-point. The transformation to transfer functions is shown in fig 2.3.11

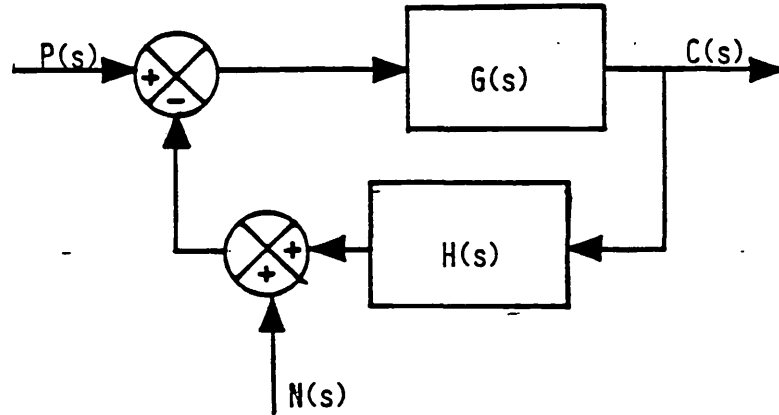


Fig 2.3.11 Transfer function block diagram of the pH-stat

The effect of the noise $N(s)$ on the output is

$$C(s) = \frac{[P(s) - (H(s) - N(s))]G(s)}{1 + [P(s) - (H(s) - N(s))]G(s)H(s)} \cdot N(s) \quad [2.3.22]$$

which is approximately

$$C(s) = \frac{N(s)}{H(s)} \quad [2.3.23]$$

Therefore the designer must obtain a maximum value of $H(s)$ to maximize the signal to noise ratio.

2.3.5 Stability and Transient Response

The cost of introducing feed-back is the possibility of instability. A stable system is defined as a system with a bounded response, that is , if the system inputs are bounded the outputs will also be bounded in magnitude.

The stability concept can be illustrated , as in fig 2.3.12 , by considering a right circular cone on a plane surface.

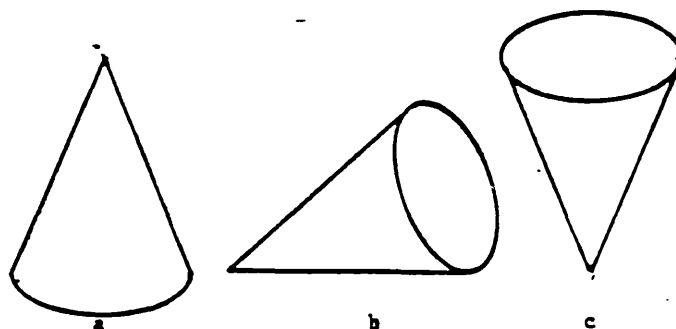


Fig 2.3.12 a)Stable b)Neutral stability c)Unstable

If the cone is resting on its base , as in fig 2.3.12a ,and is tipped slightly it will return to its original position. This position and response is called stable. If the cone rests on its side and is displaced slightly it will roll but not leave its position on its side. This response is called neutral stability. If the cone is placed on its tip and released , it will fall on its side , which is an unstable response.

The transient response of a system reveals if it is stable and how soon it will reach stability. Two very important transient test functions are the unit step function and the unit impulse function , shown in fig 2.3.13

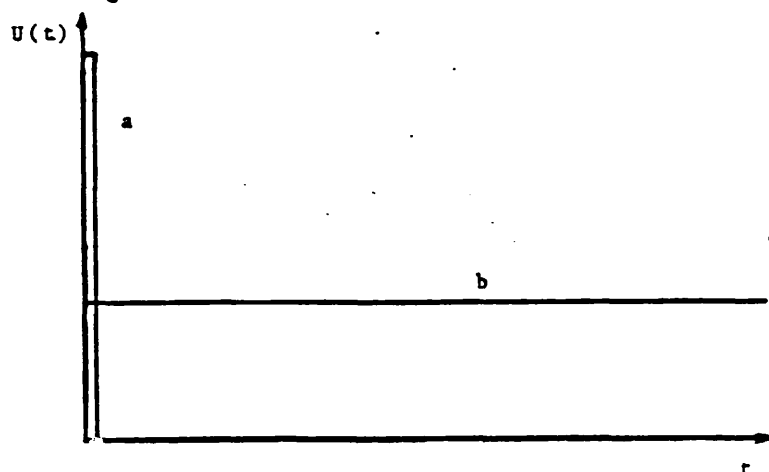


Fig 2.3.13 a)Unit impulse function b)Unit step function

The unit step function is a sudden change in the input normalized to unit magnitude , occurring at an arbitrarily chosen time

t=0. The step function change is the most severe disturbance possible and is a type of change which frequently occurs in practice. The unit impulse function is the limiting case of a pulse of unit area , where the pulse duration tends to zero. The LaPlace transforms of these and some other useful functions are given in Table 1.

Table 2.3.1
LaPlace transforms of some test functions

F(t)	F(s)
Unit step	s^{-1}
Unit impulse	1
Unit ramp	s^{-2}
$\sin(x)$	$x(s^2 + x^2)^{-1}$

Depending on the response to a unit step we can define some very common response types. Table 3.2.2 lists some of the most common types of systems.

If we once again look at the resistor capacitor network in fig 2.3.2 and recall the system transfer function

$$G(s)=\frac{C(s)}{P(s)}= \frac{1}{1+RCs} \tag{2.3.4}$$

and the input is a unit step function

$$P(s)=s^{-1} \tag{2.3.25}$$

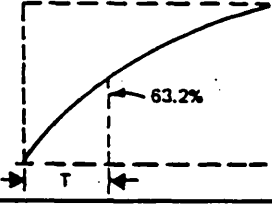
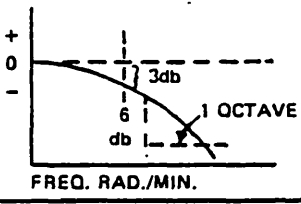
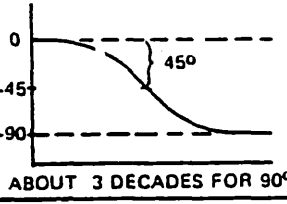
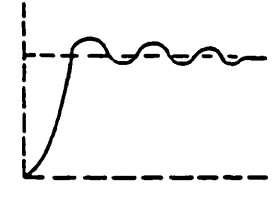
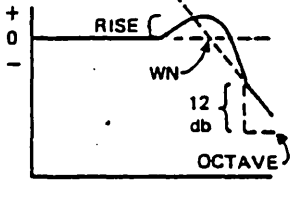
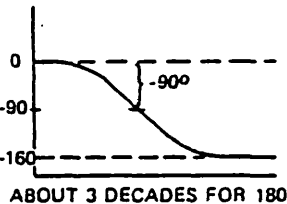
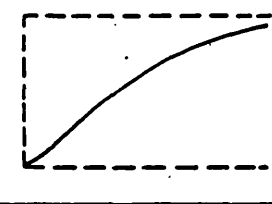
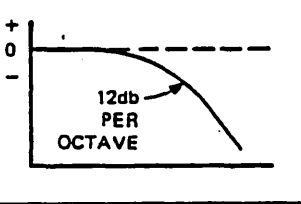
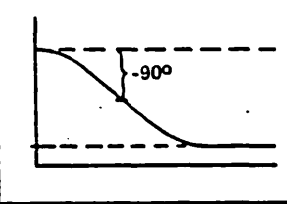
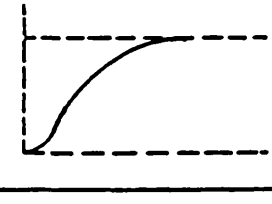
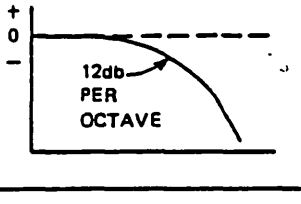
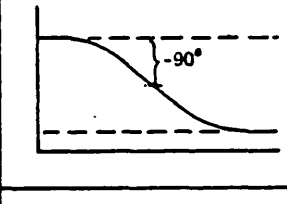
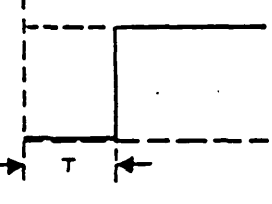
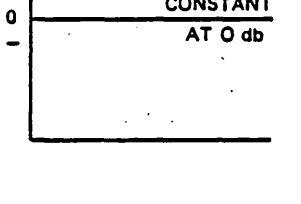
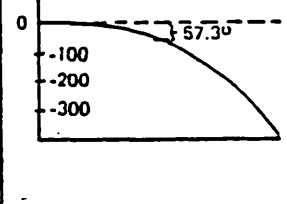
solving for the output

$$C(s)=P(s)G(s)= \frac{1}{s(1+RCs)} \tag{2.3.26}$$

In order to find the response in the time domain , we invert the LaPlace transform

$$c(t)=1-e^{-RC/t} \tag{2.3.27}$$

Table 2.3.2
Definition of some common systems

	TRANSIENT	GAIN	PHASE
FIRST ORDER LAG			
SECOND ORDER LAG UNDER-DAMPED			
SECOND ORDER OVER-DAMPED			
SECOND ORDER CRITICALLY DAMPED			
DEAD TIME			

The time response is shown in fig 2.3.14. Comparison with table 2.3.2 classifies this response as a first order lag , which is inherently limited , i.e stable.

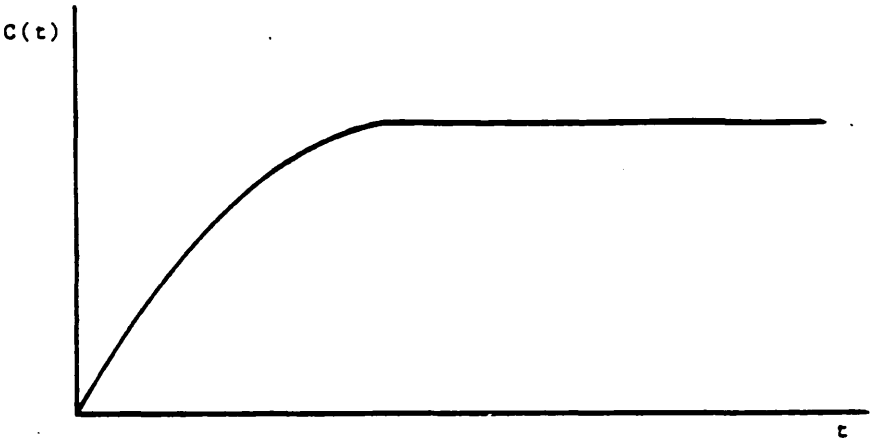


Fig 2.3.14 Unit step time response

The locations of the poles in the s -plane (remember $s=\sigma+j\omega$) indicate the resulting transient response. The poles on the left hand side result in decaying response, whereas poles on the $j\omega$ -axis or the right hand side result in either a neutral or an increasing response. Typical responses to unit steps are shown for different pole locations in the s -plane in fig 2.3.15.

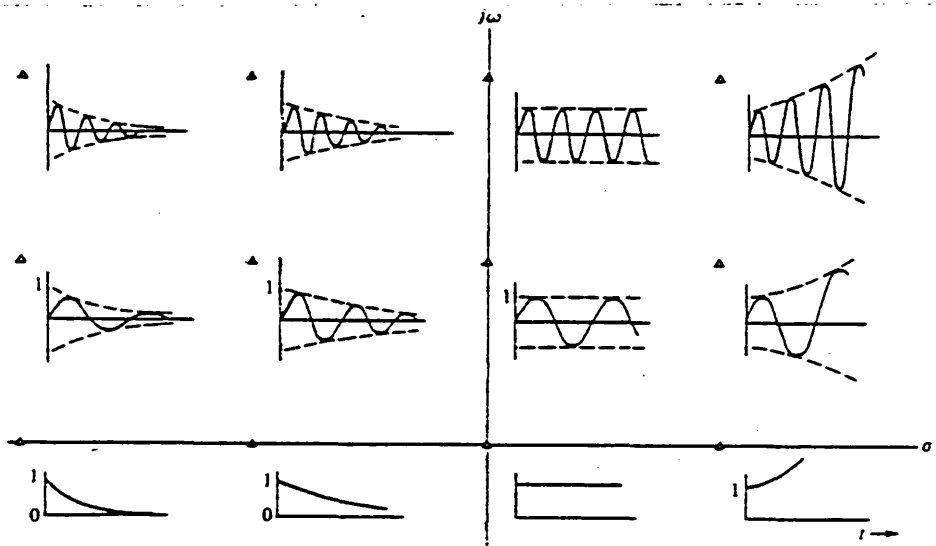


Fig 2.3.15 Typical responses for pole locations in the s -plane

Clearly the poles of a desirable system must lie in the left hand part of the s -plane. Thus a necessary and sufficient condition that a negative feed-back system is stable is that all poles of the system have negative real parts.

A number of mathematical stability criteria exist , e.g Routh-Hurwitz's , and those of Nyquist and Bode'. The reader is referred to the literature for a stringent derivation of these criteria (72-74). The simplest stability criterion to use is the Routh-Hurwitz method , which determines if a linear equation of the complex variable s has any positive real parts. Consider the general form of the characteristic equation

$$1+G(s)H(s)=a_n s^n+a_{n-1} s^{n-1}+.....+a_0=0 \tag{2.3.28}$$

If there are no roots with positive real parts there is a necessary but not sufficient condition that all coefficients have the same sign and that none are zero. If this is true the Hurwitz determinants of the polynomial must be positive , where the determinants are given by

$$D_1=a_{n-1} \quad D_2=\begin{vmatrix} a_{n-1} & a_{n-3} \\ a_n & a_{n-2} \end{vmatrix} \quad D_3=\begin{vmatrix} a_{n-1} & a_{n-3} & a_{n-5} \\ a_n & a_{n-2} & a_{n-4} \\ 0 & a_{n-1} & a_{n-3} \end{vmatrix}$$

Routh simplified the Hurwitz determinant evaluation by deriving an array of the following form

s^n	$a_{(n)}$	$a_{(n-2)}$	$a_{(n-4)}$	$a_{(n-6)}$	
s^{n-1}	$a_{(n-1)}$	$a_{(n-3)}$	$a_{(n-5)}$	$a_{(n-7)}$	
s^{n-2}	$b_{(1)}$	$b_{(2)}$	$b_{(3)}$	$b_{(4)}$	
s^{n-3}	$c_{(1)}$	$c_{(2)}$	$c_{(3)}$	$c_{(4)}$	
.	.	.	.	.	
.	.	.	.	.	
s^0	.	.	.	.	

The $b_{(n)}, c_{(n)}, \dots$ etc values are calculated according to the following pattern

$$b_{(1)} = \frac{a}{(n-1)} - \frac{a}{(n-2)} - \frac{a}{a_{(n-1)}} \frac{a}{(n-3)} \quad b_{(2)} = \frac{a}{(n-1)} - \frac{a}{(n-4)} - \frac{a}{a_{(n-1)}} \frac{a}{(n-5)}$$

$$c_{(1)} = \frac{b_{(1)} a}{(n-2)} - \frac{a}{b_{(1)}} \frac{b_{(2)}}{(n-1)} \quad c_2 = \frac{b_{(1)} a}{(n-5)} - \frac{a}{b_{(1)}} \frac{b_{(3)}}{(n-3)}$$

etc.

Every change in sign in the first column signifies the presence of a root with a positive real part. For stability therefore, all values in the first column must be positive.

As shown above the stability and transient responses are directly related to the locations of the poles. These two parameters are the most important ones in designing a control system. So if we could optimise the locations of the roots, we would be optimising the performance of the system.

The Root-Locus method was introduced in 1948 by Evans (75) and has been developed and used extensively in control engineering (76,77). This is a graphic method for drawing the locus of roots in the s-plane as a parameter is varied. The final trace provides the designer with a measure of the sensitivity of the pole locations. Evans outlined eight steps for obtaining the necessary information for drawing the locus.

- 1) Write characteristic equation in pole-zero form so the parameter of interest, K, appears as $1 + KF(s) = 0$
- 2) Locate the roots and poles of F(s) in the s-plane
- 3) Locate the segments of the real axis which are root-loci
- 4) Determine the number of separate loci
- 5) Locate the angles of the asymptotes and the centre of the asymptotes

- 6) Determine the break-away point on the real axis (if any)
- 7) By utilising the Routh-Hurwitz criterion, determine where the locus crosses the $j\omega$ -axis
- 8) Estimate the angle of locus departure from complex poles and the angle of locus arrival at complex zeros

The reader is referred to the literature for the details of point 1-8 (76,77). A typical Root-Locus plot for a feed-back system is shown in fig 2.3.16

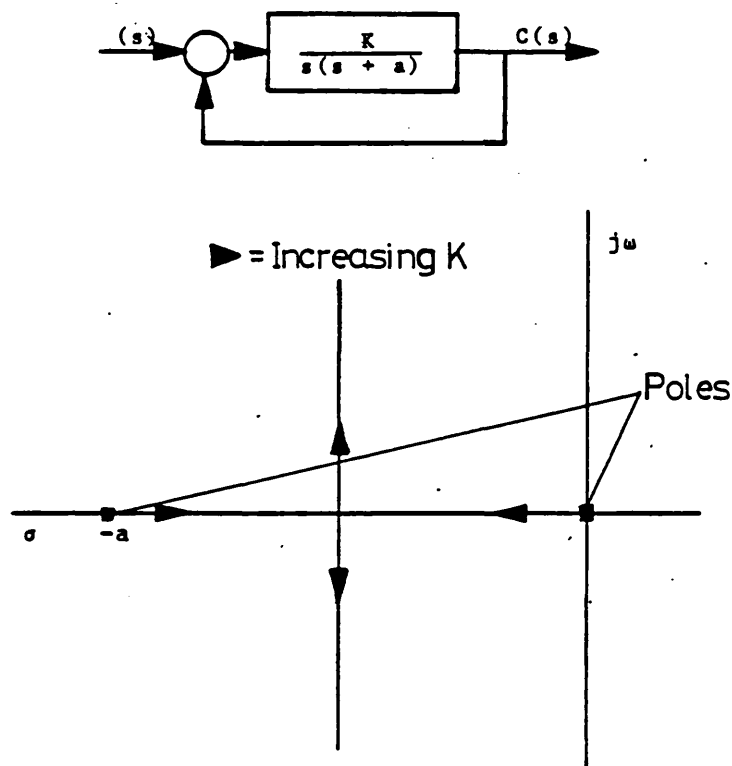


Fig 2.3.16 A root-locus plot for a second order system

2.3.6 Control Algorithms

Very often a closed loop system cannot perform up to the desired result without an active controlling device, a controller, being introduced into the system. This unit is programmed to compensate for the lack of self adjustment in the feed-back. Depending on the system the designer introduces proportional, integral or derivative control, or a mixture of these. Consider the feed-back

system in fig 2.3.17

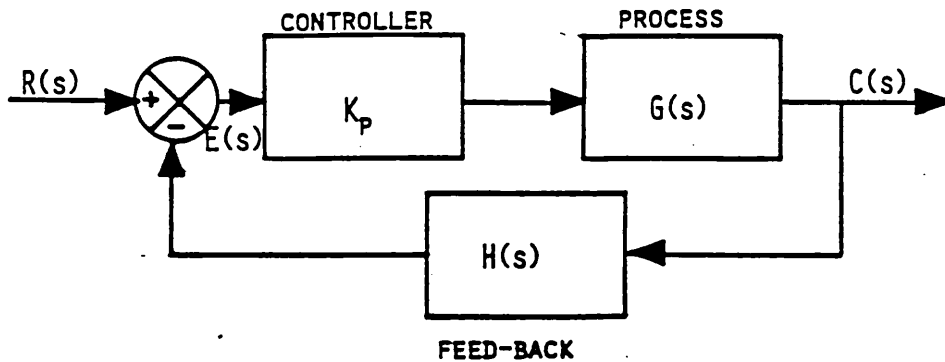


Fig 2.3.17 Feed-back system with proportional controller

The proportional controller multiplies the error signal $E(s)$ by a factor K_p , thus the process input is $K_p E(s)$. The numeric value of K_p determines the corrective effort which is applied to the system. Fig 2.3.18 shows the response $K_p e(t)$ of a step change in $R(t)$.

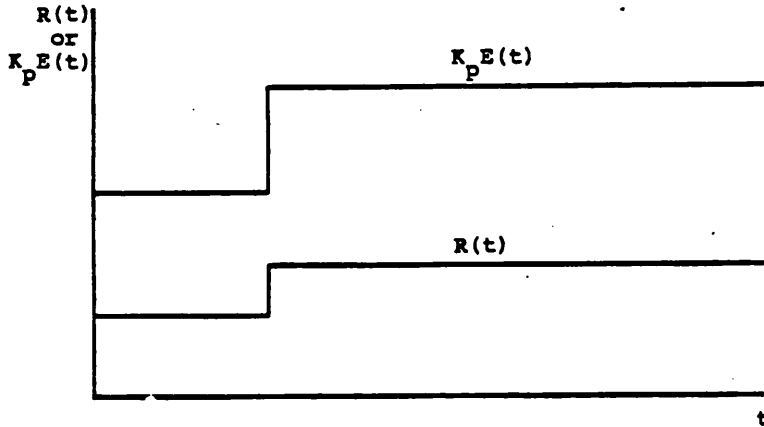


Fig 2.3.18 Error signal response to a step change in input

But if we look at the steady state of $E(s)$ after a step change

$$E(s) = R(s) - H(s) = R(s) - K_p E(s) G(s) \quad [2.3.28]$$

rearranged

$$E(s) = \frac{R(s)}{1 + K_p G(s)} \quad [2.3.29]$$

In order to find the steady state value we use the final value theorem, see Appendix I

$$\lim_{t \rightarrow \infty} e(t) = \lim_{s \rightarrow 0} sE(s) \quad [2.3.30]$$

and if $R(s) = s^{-1}$ and e_{ss} = Steady State error

$$e_{ss} = \lim_{s \rightarrow 0} \frac{1}{1 + K_p G(s)} = \frac{1}{1 + K} \quad [2.3.31]$$

Proportional control has a steady state error depending on the parameters in the feedback loop. A prime requirement of a control system is a zero steady state error.

Integration of $e(t)$ would eliminate a steady state error, because even the smallest error would produce a corrective signal of sufficient magnitude to actuate the system eliminating the error. Therefore the system would only come to rest when $p(t) = 0$. An integral feed-back control system block diagram is shown in fig 2.3.19. The integral $k \int e(t) dt$ has the LaPlace transform K/s . Fig 2.3.20 shows the integral controller's response to an input step change.

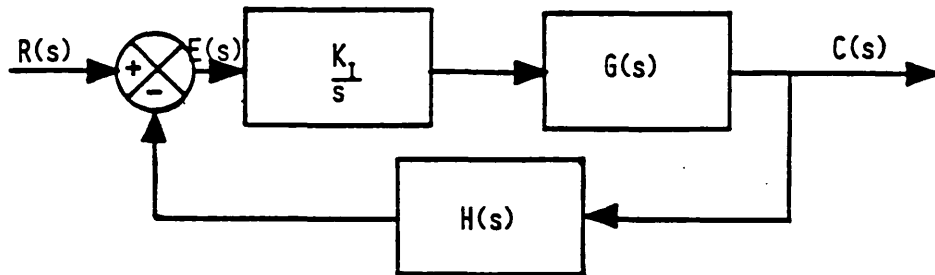


Fig 2.3.19 Integral control feed-back loop

From fig 2.3.19 we see that the error from the integral controller is

$$E(s) = R(s) - H(s) = R(s) - E(s)K_i G(s)s^{-1} \quad [2.3.32]$$

rearranging [2.3.32] gives ;

$$E(s) = \frac{R(s)}{1 + K_i G(s)s^{-1}} \quad [2.3.33]$$

if the input is a step $R(s) = s^{-1}$ and using the final value theorem we can calculate the steady state error

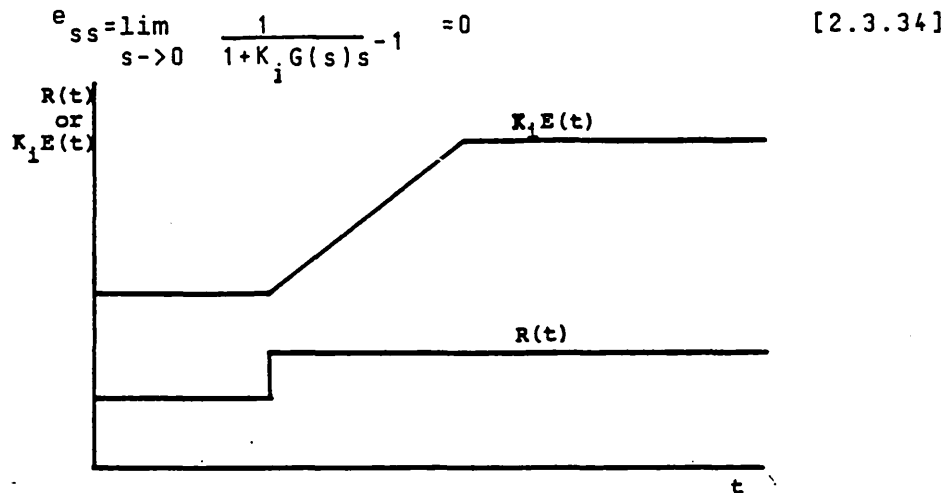


Fig 2.3.20 Integral control response to input step change

The integral control will slow down the response because of the inherently slow process of integration. A form of control action which can increase the response is derivative control. The derivative action is a derivative of the error $K_d de(t)/dt$, i.e. this term senses the rate of change in the error. Derivative control is not used by itself but in conjunction with proportional or proportional-integral control, in the latter case forming a so called three term controller or PID controller. Fig 2.3.21 shows a PID controller block diagram. The controller may be P (proportional) PI (proportional integral) or PID (proportional integral derivative). Fig 2.3.22 shows the response of these controller combinations for a step in set point.

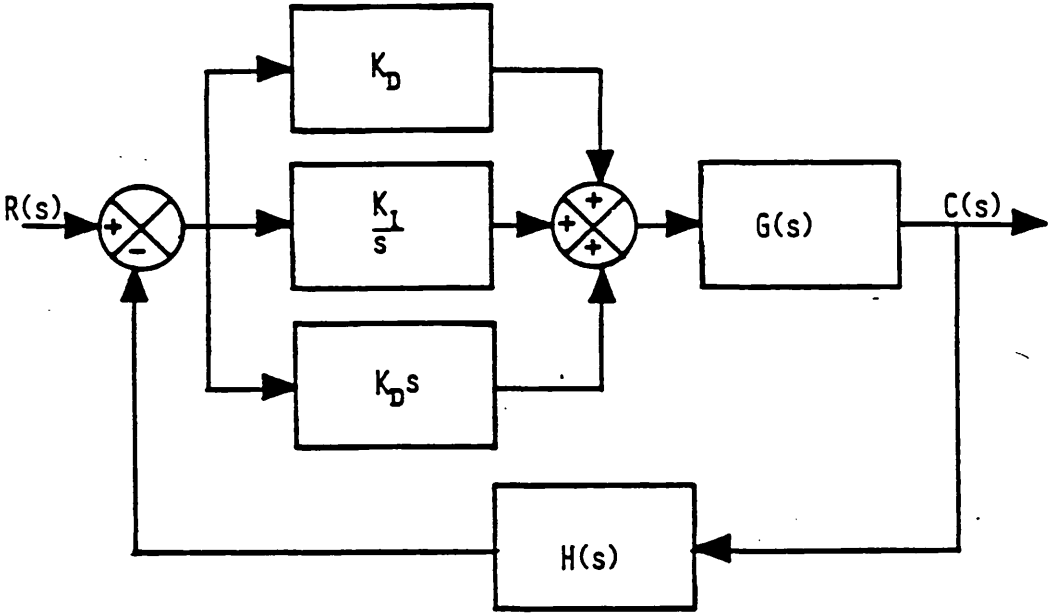


Fig 2.3.21 PID controller

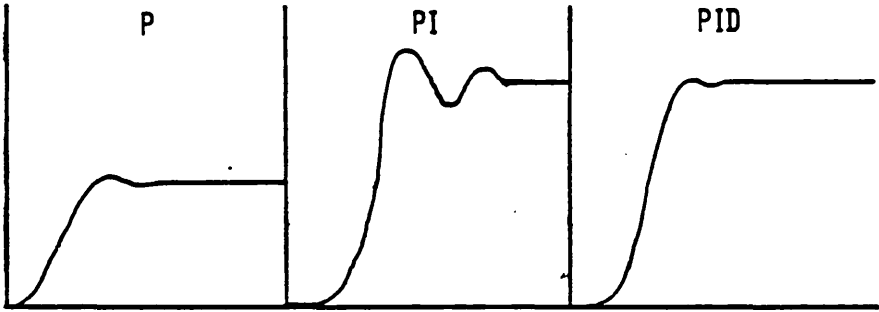


Fig 2.3.22 a) P controller response b) PI controller response
c) PID controller response to a step change

2.3.7 Digital Control

Analog control theory may be directly applied in design of digital control systems. The additional knowledge required is the conversion of the controller's continuous transfer function to a discrete transfer function. Digitizing the analog controller creates various new phenomena not encountered in continuous design. The major difficulty in the digital design is the fidelity of the impulse and frequency response of the analog controller. The fidelity depends on the sampling rate and on the particular method of discretization.

The same conditions for linearity, time-invariance and impulse response applies to the digital system. The digital system S is illustrated in fig 2.3.23

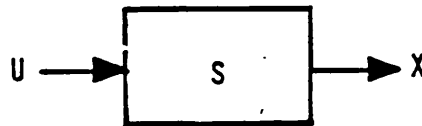


Fig 2.3.23 The discrete system S

The input is a string of numbers $u = \{u_0, u_1, \dots, u_i\}$ and the output is also a string of numbers $x = \{x_1, x_2, \dots, x_i\}$. Generally

$$x = S(u) \quad [2.3.34]$$

and if the system is linear then

$$x = S(au + bu') = aS(u) + bS(u') \quad [2.3.35]$$

and is time invariant if i and k represent time instances and $k \geq 0$

$$S(u_{i-k}) = x_{i-k} \quad [2.3.36]$$

and $u_{i-k} = 0$ for $i = 0, 1, 2, 3, \dots, k-1$.

The system S is described by a discrete equation, rather than a linear differential equation in the analog case. Equation [2.3.37] defines the discrete equation.

$$b_0 x_{i+n} + b_1 x_{i+n-1} + \dots + b_{n-1} x_{i+1} + b_n x_i = u_i \quad [2.3.37]$$

where n is the order of the system and i represents independent time instances. For example, a second order discrete equation is of the form

$$b_0 x_{i+2} + b_1 x_{i+1} + b_2 x_i = u_i \quad [2.3.38]$$

The discrete equation is usually solved by a z -transform method, in the same way that a linear differential equation is solved by Laplace transform methods. The theory and application of the z -transform is described in Appendix I.

For example to find the z -transform of the second order discrete equation

$$x_{i+2} - 3x_{i+1} + 2x_i = 4^i \quad [2.3.39]$$

one transforms the equation term by term, either by looking up tables or by using the z -transform definition, and then adds up the result

$$Z(x_{i+2}) = z^2 x(z) - zx(1) - zx(0)$$

$$Z(x_{i+1}) = zx(z) - x(0)$$

$$Z(x_i) = x(z)$$

$$Z(4^i) = z(z-4)^{-1}$$

added up

$$(z^2 - 3z + 2)x(z) - z^2 x(0) - zx(1) + 3zx(0) = z(z-4)^{-1}$$

A continuous linear differential equation or its transfer function may be converted into a discrete form by adding a sampler in the flow path, shown in fig 2.3.24. The sampler signifies the inherent digitization of output and input signals in a digital system, that takes place at regular intervals T . The continuous transfer function may be written as

$$H(s) = \frac{C(s)}{R(s)} = \frac{A_1}{s+a_1} + \frac{A_2}{s+a_2} + \dots + \frac{A_n}{s+a_n} \quad [2.3.40]$$

in the time domain

$$c(t) = r(t) \{ A_1 e^{-a_1 t} + A_2 e^{-a_2 t} + \dots + A_n e^{-a_n t} \} \quad [2.3.41]$$

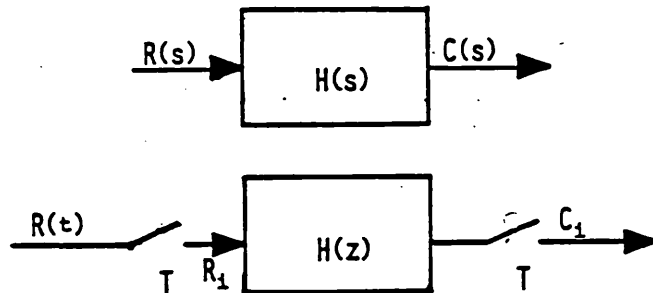


Fig 2.3.24 Conversion of continuous transfer function to a discrete transfer function by introducing samplers

By sampling $h(t)$ at T intervals the output $c(t)$ is a train of impulses, i.e. the solution after time $i \cdot T$ is

$$c(iT) = \sum_{k=0}^i r(k) (A_1 e^{-a_1(i-k)T} + \dots + A_n e^{-a_n(i-k)T}) \quad [2.3.42]$$

the z-transform of [2.3.42] is

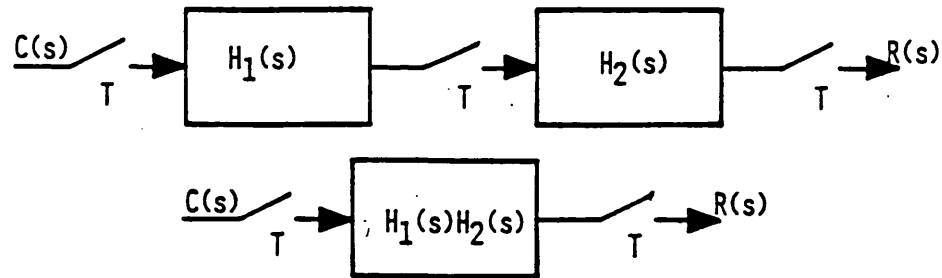
$$C(z) = \frac{A_1}{1 + e^{-a_1 T} z^{-1}} + \dots + \frac{A_n}{1 + e^{-a_n T} z^{-1}} R(z) \quad [2.3.43]$$

$$C(z) = H(z) R(z) \quad [2.3.44]$$

$H(s)$ may often be transformed to $H(z)$ by looking up tables of z-transforms, but complicated $H(s)$ may require solution by partial fractions.

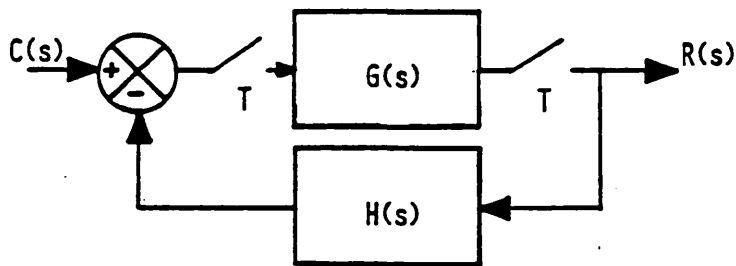
The z-transformed block diagram does not obey the same rules as the Laplace transformed one. Firstly we must introduce a sampler in the flowpath in order to treat the transfer functions as products. Fig 2.3.25 illustrates a block diagram with sampler. Secondly there

are also samplers in the feed-back loop in fig 2.3.25



$$Z[H_1(s)]Z[H_2(s)] = Z[H_1(s)H_2(s)]$$

Fig 2.3.25 Block diagram simplification with samplers



$$\frac{C(z)}{R(z)} = \frac{H(z)}{1 + H(z)G(z)}$$

Fig 2.3.26 Digital feed-back loop with samplers

The digital controller also introduces a time delay in the loop , due to the finite time , T ,it takes to convert the analog signal and to compute the new output . This is represented by a so called Zero Order Hold (ZOH) , which holds the output constant during the interval T . A ZOH's action is shown in fig 2.3.27.

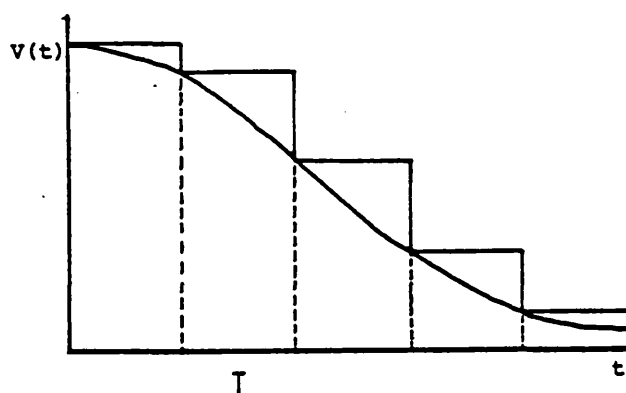


Fig 2.3.27 Zero Order Hold action

If we now insert a ZOH in the block diagram in fig 2.3.28 we get

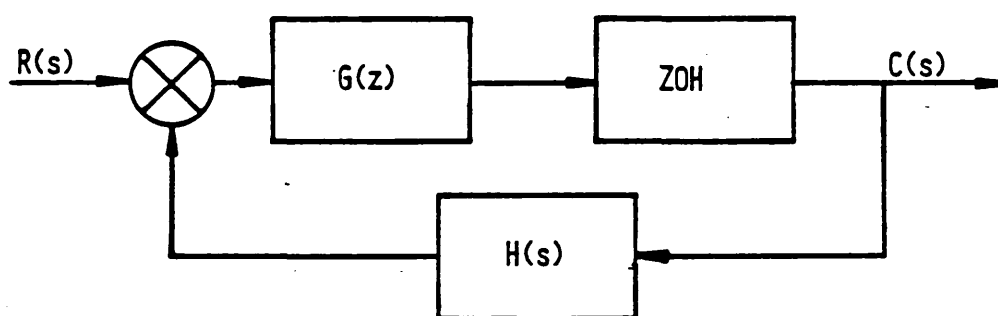


Fig 2.3.28 Feed-back loop with Zero Order Hold

The transfer function for the ZOH is given by

$$ZOH(s) = \frac{1 - e^{-sT}}{s} \quad [2.3.45]$$

The ZOH and sampler must be designed carefully so the controller's response is not distorted. If the sampling frequency $f = T^{-1}$ is less than twice the maximum frequency of the sampled signal the frequency response is severely distorted. This distortion is called aliasing, because frequencies that were not part of the original signal are created. This is illustrated in fig 2.3.29 where a signal is sampled at two different frequencies.

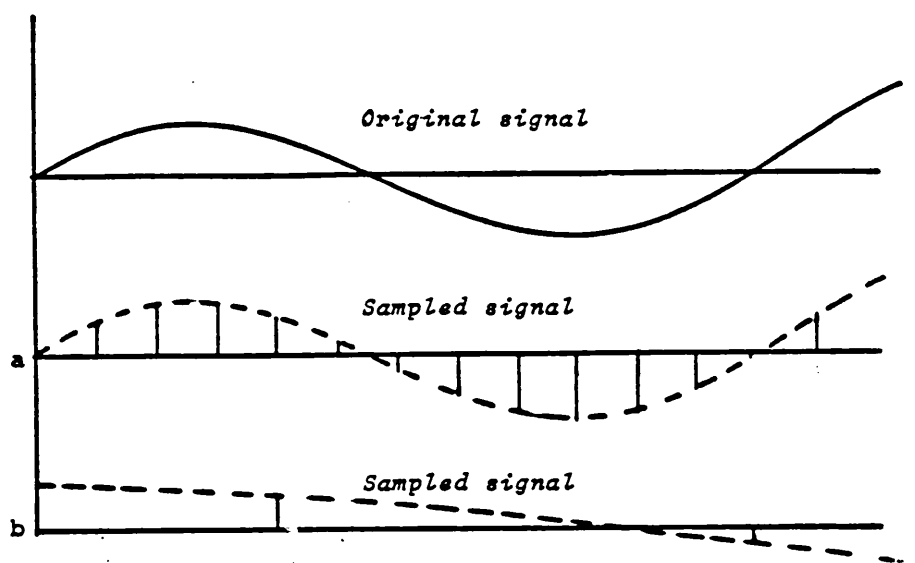


Fig 2.3.29 a) High sampling frequency , no distortion
b) Low sampling frequency , aliasing

It is seen that a distorted signal is reconstructed at the low sampling frequency. This can be avoided by limiting the bandwidth of the sampled signal by a pre-filter or by selecting an appropriate sampling frequency. The problem is that we cannot sample faster than the delay of the ZOH. This inherently limits the use of a digital controller.

The practical selection of sampling rate depends on the word-length (i.e the number of bits in a digital word) of the micro-processor. With a high sampling rate the difference between two consecutive samples is going to be small, therefore we need a long computational word to be able to resolve this small difference. After selecting a microprocessor with the right word-length the choice of sampling rate is based upon the required transient characteristics of the controller. This is translated into a closed loop bandwidth , then a sampling rate at least ten times the closed loop bandwidth is chosen , if this is possible.

Finally the digital controller transfer function must be translated into a numerical algorithm, which is simply the corresponding discrete equation. We may think of $G(z)$ as a ratio of two polynomials

$$G(z) = \frac{R(z)}{C(z)} = \frac{\sum_{j=0}^n a_j z^{-j}}{1 + \sum_{j=1}^n b_j z^{-j}} \quad [2.3.46]$$

To realize [2.3.46] into software, we consider the difference equation based upon this equation.

$$c_i = \sum_{j=0}^n a_j r_{i-j} - \sum_{j=1}^n b_j c_{i-j} \quad [2.3.47]$$

For an example let us consider a linear ramp, $g(t) = kt$, which has the Laplace transform

$$G(s) = \frac{1}{s} \quad [2.3.48]$$

which we first z-transform

$$G(z) = \frac{z^{-1}}{(1+z^{-1})^2} = \frac{z^{-1}}{1+2z^{-1}+z^{-2}} \quad [2.3.49]$$

then turn into a discrete equation using [2.3.47]

$$c_i = r_{i-1} - 2c_{i-1} - c_{i-2} \quad [2.3.50]$$

where c_i is the new output, c_{i-1} and c_{i-2} are old outputs and r_{i-1} is the previous input.

2.3.8 Digital Control Algorithms

The implementation of PID control is a straightforward programming task. For the proportional control the output is proportional to the error, which has the transfer function

$$H(z) = K_p \quad [2.3.51]$$

which gives the discrete equation

$$c_i = K_p r_i \quad [2.3.52]$$

We recall from the analog controller that the integral controller had a transfer function

$$H(s) = K_i s^{-1} \quad [2.3.53]$$

equation [2.3.53] is z-transformed to

$$H(z) = \frac{K_i}{1 - z^{-1}} \quad [2.3.54]$$

once again using equation [2.3.46] and [2.3.47]

$$c_i = c_{i-1} + K_i r_i \quad [2.3.55]$$

Finally for the derivative term

$$H(s) = K_d s \quad [2.3.56]$$

z-transforms to

$$H(z) = K_d (1 - z^{-1}) \quad [2.3.57]$$

using [2.3.46] and [2.3.47] gives

$$c_i = K_d (r_i - r_{i-1}) \quad [2.3.58]$$

Adding up [2.3.52], [2.3.55] and [2.3.58] we get the three term or PID control algorithm

$$c_i = K_p r_i + c_{i-1} + K_i r_i + K_d (r_i - r_{i-1}) \quad [2.3.59]$$

This algorithm is easily programmed in a high level language ,
e.g BASIC

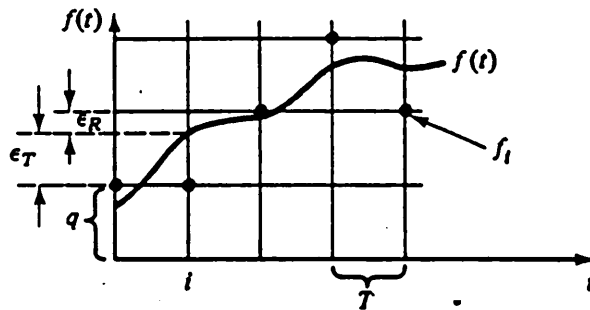
```

.
.
90 INPUT=ADC:REM input from analog/digital converter
100 ERROR=SETPOINT-INPUT :REM calculate error
110 INTEGRAL=INTEGRAL+ICONST*ERROR:REM integral term
120 PROP=PCONST*ERROR:REM proportional term
130 DERIV=DCONST*(OLDERROR-ERROR):REM derivativ term
140 PID=INTEGRAL+PROP+DERIV:REM PID algorithm
150 OLDERROR=ERROR:REM update error
160 OUTPUT=PID:REM output to digital/analog converter
.
.

```

The digital control algorithm suffers from the problem of finite wordlength, which may cause severe errors. There are three main sources of error

1) Errors in analog to digital converters



T=Sampling interval
 q=Quantization level
 ϵ =Error due to quantization
 ϵ_R =Round-off error

The probability density function for round-off errors in fixed point notation is shown in fig 2.3.30

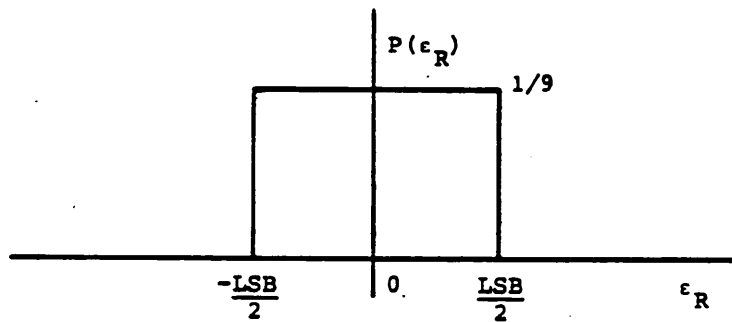


Fig 2.3.30 Probability density function for round-off errors from which we can see that the mean error

$$\epsilon_R = 0 \quad [2.3.60]$$

and the variance is

$$\sigma_R^2 = \int_{-LSB/2}^{LSB/2} \epsilon^2 d\epsilon = \frac{q^2}{12} \quad [2.3.61]$$

The variance may be thought of as random noise on the signal. For a binary system (base 2) with the word-length L, the maximum obtainable signal to noise ratio (S/N) for a maximum analog voltage

V is

$$(S/N) = \frac{E^2}{\epsilon^2} = \frac{12 \cdot E^2}{q^2} = 12 \cdot 2^L \quad [2.3.62]$$

e.g for a 12 bit analog to digital converter

$$(S/N) = 12 \cdot 2^{24} = 2 \cdot 10^8 = 83 \text{ dB}$$

This Signal to Noise ratio is sufficient for electrochemical control applications.

2) Round-off errors in arithmetic operations

Multiplication generates these errors , e.g in decimal arithmetics with a 4 digit word length

$$0.140 \cdot 0.140 = 0.0196$$

rounded off to 0.020 , $\Rightarrow \epsilon_R = 0.0004$.These errors are generally insignificant when high level languages with high precision are used.

3) Parameter and coefficient storage errors

The time constant for the integral term was calculated to 0.24635 with a calculator but using an eight bit microprocessor it is stored as 0.246 , $\Rightarrow \epsilon_s = 0.00035$

The above described errors may propagate through the controller either amplified or attenuated since the algorithm is a linear time invariant system. For example let us look at a first order system

$$\frac{C(s)}{R(s)} = \frac{1}{s + b} \quad [2.3.63]$$

Equation [2.3.63] is z-transformed to

$$\frac{C(z)}{R(z)} = \frac{1}{1 - e^{-bT}} \frac{1}{z} \quad [2.3.64]$$

next we use the final value theorem on [2.3.64]

$$c = \epsilon \lim_{z \rightarrow 1} z \frac{1}{1 - e^{-bT}} \frac{1}{z} = \frac{\epsilon}{1 - e^{-bT}} \quad [2.3.65]$$

If T is fast , i.e $T \ll 1$ then c is going to amplify the error ,e.g $T=0.001 \text{ s} \Rightarrow c=1000 \cdot e$. The obvious explanation is that with fast sampling , the difference between two successive samples is very small.

Despite the limitations of digital control it is often favoured because of greater flexibility and very often better stability than an analog counter part , but great care must be taken in the digital design.

CHAPTER 3

MICROCOMPUTER SYSTEM

3.1 Introduction

In the dawn of the microelectronic age even the analytical chemist has seen "the light". Today there is almost no valid excuse for not using microprocessors in the automation of instrumentation. In fact almost all automatic analytical instruments launched in 1983 used microprocessors.

The analyst's problem in designing microprocessor controlled instrumentation is a lack of knowledge. He is confronted with a thick forest of jargon, which seems impenetrable at a first glance, but with a little effort he begins to see the wood rather than the trees.

This chapter tries to take the reader through "the forest of jargon" and outlines the analytical requirements on microprocessor automation. Finally a description of the equipment used in this work is given.

3.2 Microcomputer Development System

3.2.1 Preamble

The first problem in an instrument design is to specify the required performance of the microcomputer system. Our experience is that the microcomputer system cannot be separated from the rest of the system; it must be an integral part of the overall specification of the instrument. This systematic approach is described in a "design flow-chart" in fig 3.1.

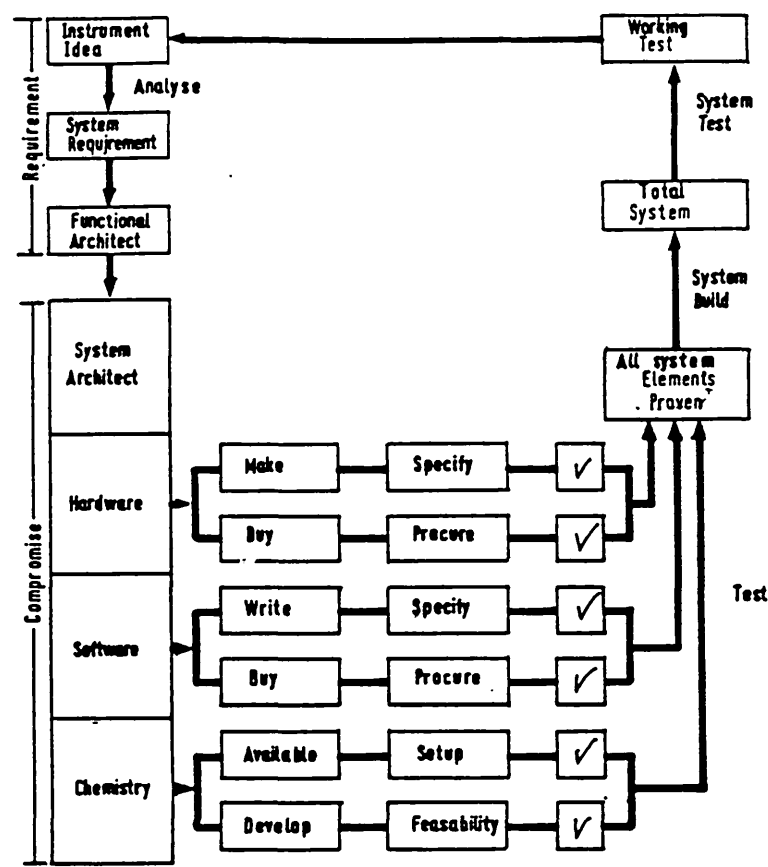


Fig 3.1 Design flow-chart

The solution is a compromise between economic and scientific requirements. A good piece of advice is to try to buy hardware and software whenever it is possible. Too many research workers spend too much time "fault finding" and "reinventing the wheel".

3.2.2 Microelectronics

In order to specify requirements on the microcomputer hardware, some knowledge of microelectronics is essential. Fig 3.2 is a map of a typical microcomputer system. It consists of the microprocessor with associated buffers for driving the address bus, the data bus and the control bus. The address bus is a set of parallel wires carrying a memory location address. The data bus is another set of parallel wires carrying data to and from the microprocessor. Similarly the control bus carries control signals to and from the micro-

processor. The clock controls the timing of events in the system. The memory contains the program and the data it has acquired or created. There exist two types of memory, firstly volatile memory which is called RAM (Random Access Memory) and can be thought of as a scratchpad which is erased when the power is turned off, secondly non-volatile memory called ROM (Read Only Memory) which holds programs permanently. The interface logic is a set of microelectronic chips controlling the interface under the command of the microprocessor.

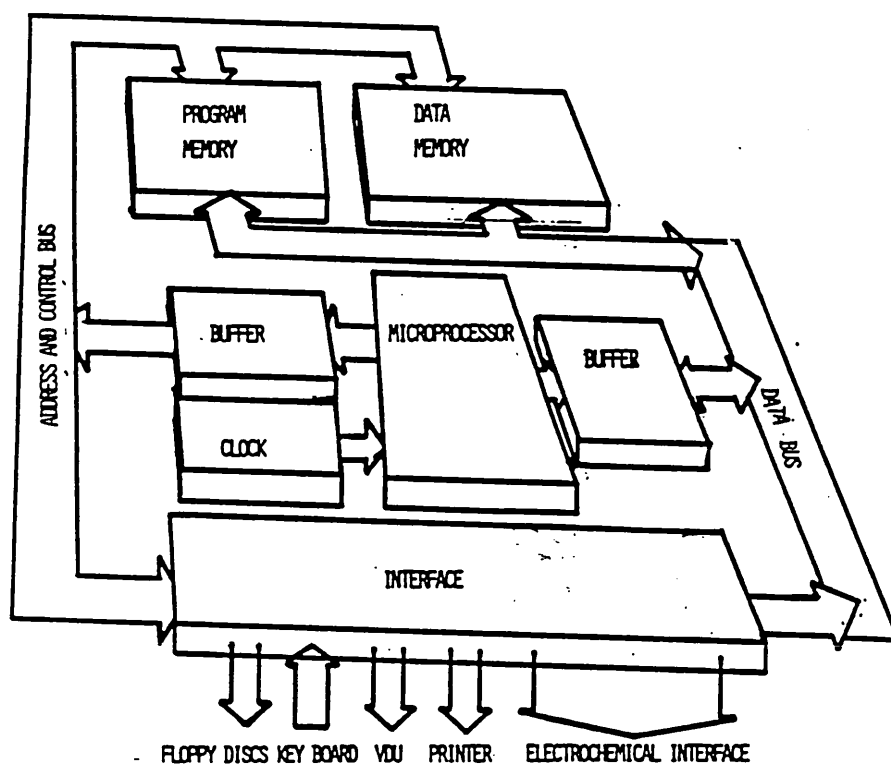


Fig 3.2 Topological map of a microcomputer system

The most important part of the system is the microprocessor. The choice of microprocessor is going to determine the overall performance of the microcomputer system. Points to take into consideration when choosing a microprocessor are;

- 1)Wordsize
- 2)Speed
- 3)Software

4) Interfacing

5) Cost

6) Service

These points are explained in the following section

3.2.3 Wordsize

The microprocessor wordsize determines the accuracy and speed of alphanumeric operations. For "number crunching", e.g Fast Fourier Transforms, one prefers a 16-bit (one bit is a digital digit) microprocessor, but for control a 4-bit system might be enough. The wordsize also limits the memory. A 4-bit microprocessor can address $2^8=256$ memory locations, whereas an 8-bit one can address $2^{16}=65,536$ memory locations. For large programs and high data volumes the need for memory must be assessed carefully.

3.2.4 Speed

The microprocessor speed determines how fast a program can run. The actual execution speed is a compromise between the programming language used and the microprocessor speed. For high speed operation, e.g transient recording, a fast microprocessor and a fast programming language (assembler) is required, but for low speed applications a slow microprocessor and a slow language (interpreted BASIC) is sufficient.

3.2.5 Software

A very important factor is the amount of available software for the hardware. The major cost in a project is the development of the software. Popular microprocessors have a multitude of software rang-

ing from Space Invaders to Fast Fourier Transforms , whereas new , less widely used microprocessors usually lack strong software back-up.

3.2.6 Interfacing

Interfacing is the major hardware problem for the designer. A system well prepared for this is preferred. There exists a number of "standard interfaces". The most popular ones are the S-100 , RS-232 and IEEE-488. If the microcomputer system can be made to accept a standard interface, the interfacing will be simple. Commercial units may then be connected and will be ready to use. Sometimes these standard interfaces do not meet the requirements of the designer and other solutions must be found.

3.2.7 Cost

The cost is inevitably an important factor. The cost of the microcomputer itself is only a fraction of the total. If we take £1000 as the cost of the microcomputer, the peripherals ,e.g printers , Video Display Unit etc , might easily cost £2000 and the chemical interface a similar sum.

3.2.8 Service

Swift and competent service is appreciated by the development system user when he inevitably runs into problems. Furthermore if the system has good information back-up , problems may be avoided or easily solved.

3.3 The Research Machines Ltd's 380 Z

Since 1975 Research Machines Ltd , Oxford , have produced a

microcomputer system ,the 380 Z , which is used extensively in secondary schools , and by the Microcomputer Centre and the Chemistry Microprocessor Unit at this college. It has proved to be a rugged and reliable system with a large user community. The 380 Z system uses a Zilog Z 80 8-bit microprocessor, running at 8 MHz. This is one of the most used microprocessors in the world. The Z80 operating system CP/M (Digital Research Inc,Cal) is almost an industrial standard , and has a very good software support.

The 380 Z system is supplied with 56 kbyte (1 byte=8 bits , 1 kbyte=1024 bytes) of memory , a 50 way bus for interfacing purposes, an RS 232 interface , a twin 8" disc drive for permanent program storage , a line printer and a Video Display Unit. Fig 3.3 shows the complete 380 Z development system with its peripherals. We used a Research Machines extended BASIC interpreter for the software development.

3.4 Electrochemical Interface

3.4.1 Requirements

Our requirements on the electrochemical interface are summarised in the list below:

- 1)Flexible
- 2)Simple
- 3)Complete
- 4)Robust
- 5)Accurate

Our aim is to automate many different electroanalytical techniques , therefore we need an interface that is easily changed from

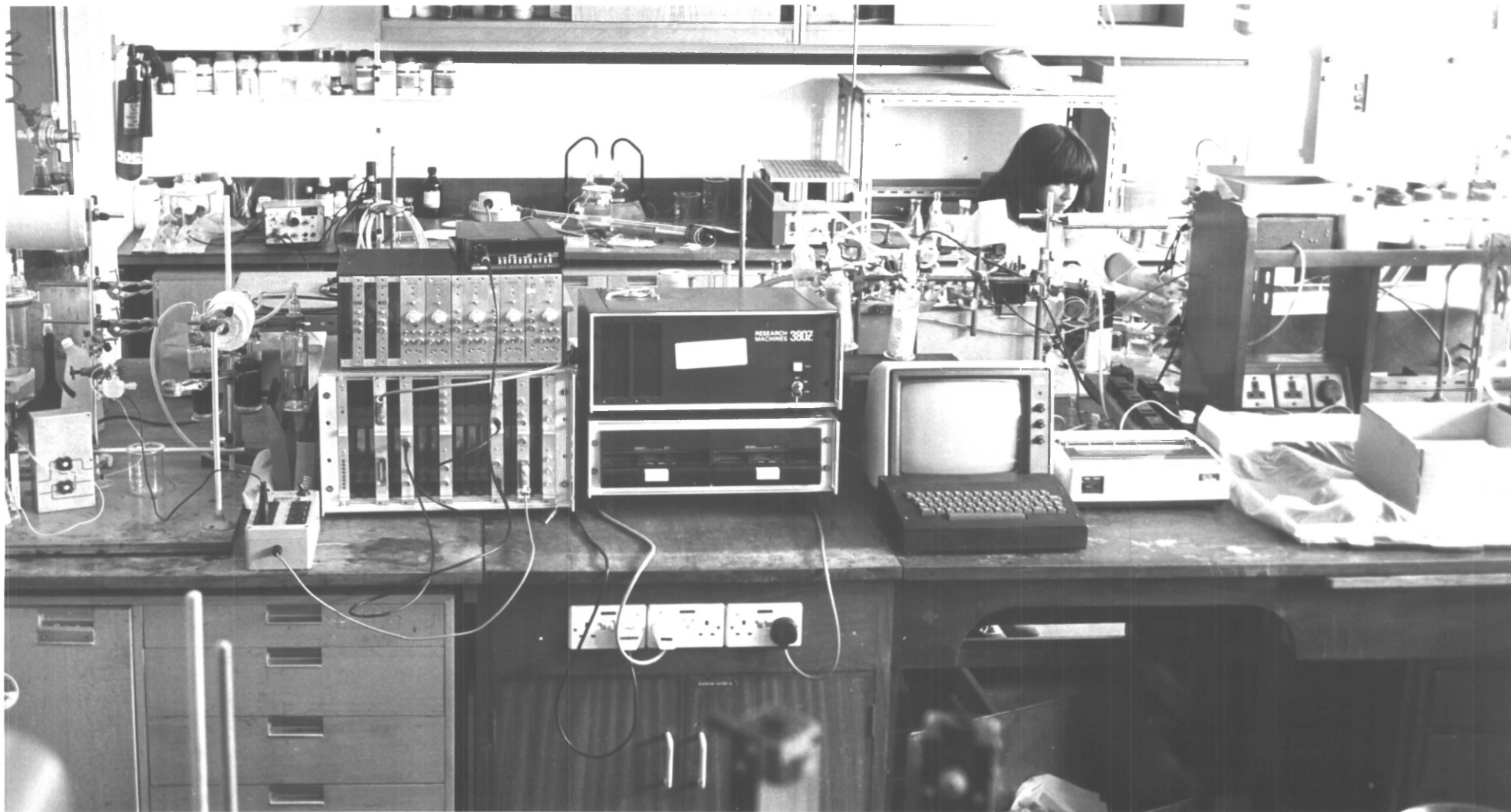


Fig 3.3 The Microcomputer development system

one experiment to another. This interface must also be able to perform all the different types of experiments we wish to do. It must also work satisfactorily under harsh conditions , e.g in a glasshouse.

3.4.2 The Chemistry Microprocessor Unit Interface

Our interface was designed by Dr. N.J.Goddard and built by the Chemistry Microprocessor Unit (CMU) at Imperial College. Two types of interfaces were used;

- 1)The Standard Rack;this interface unit gives the user 16 slots for function cards, e.g ADC card
- 2)The Power Rack;this is a smaller version of the Standard Rack which can house up to four power switching modules

3.4.2 The Standard Rack

The Standard Interface Rack , illustrated in fig 3.4 ,is based on a standard 19" high sub-rack mounted in a steel case. The power supply is housed in the rear of the case, and is not physically mounted on the sub-rack. The rack is designed to house 16 double-eurocard size printed circuit boards.

3.4.4 The Power Rack

The power rack is based on a standard 19" sub-rack in a steel instrument case. The power supply is mounted on the rear of the case , along with bus connectors and mains inlet. The rack is designed to accept four power switching modules , each containing a standard eurocard.

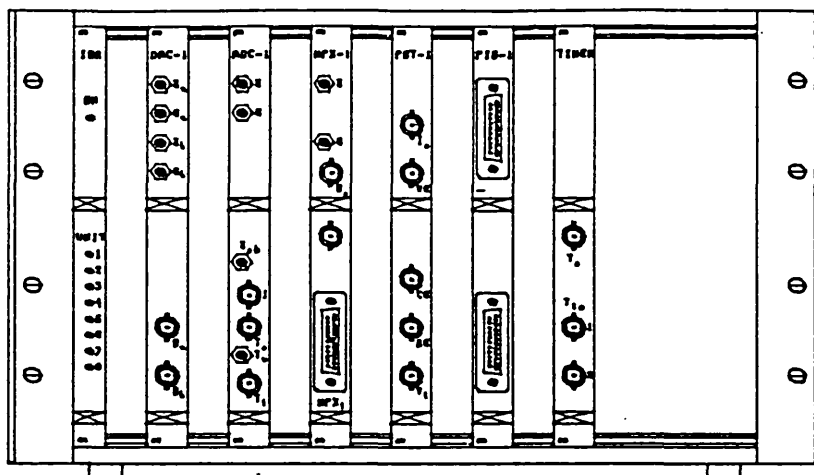


Fig 3.4 The CMU interface rack

3.4.5 System Interconnections

The 380 Z 50-way bus is taken in parallel to all the units in the system, which guarantee that they receive the control signals they need to operate. To ensure that no rack overlap with another in the address space of the system, there is another set of bus signals that are carried in a daisy-chain fashion around the system, starting at the first and finishing at the last unit. This is the unit priority daisy-chain which is used to determine the order in which units appear in the address space of the 380 Z. Up to eight units may be linked in this chain. The system interconnections are shown in fig 3.5. The software has been designed so the user need only know what number a particular function board has, the software finds the exact address location for this board. This simplifies the use tremendously. No "hard-wiring" is necessary between experiments and this also obviates the need for complicated memory maps.

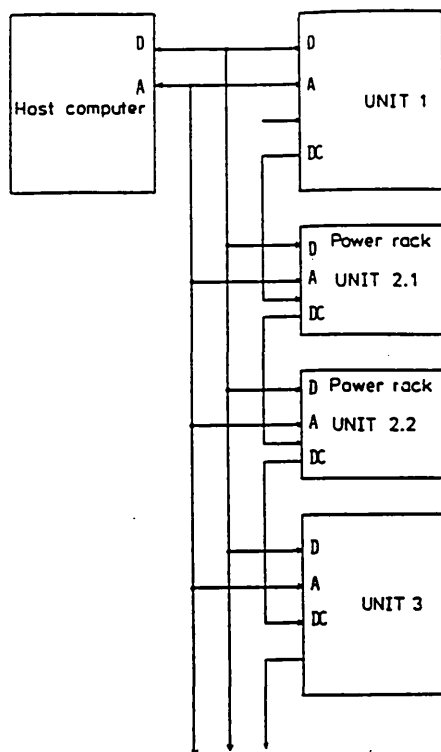


Fig 3.5 System interconnections

Another possibility is to run the interface rack or a daisy-chain as a stand-alone system. After the software has been developed and put into ROM the system may run with a Z80 microprocessor in the rack. This card is then in full control of the whole system and the 380 Z is made redundant. This is the idea of a development system;

- 1) Develop software
- 2) Put software in ROM
- 3) Divorce development system from interface
- 4) Run stand alone system

3.4.6 Function Boards

The function boards for the standard interface are based on the double eurocard size printed circuit board with two 32 way edge connectors. To fix these cards into position and to carry out any external connections, an aluminium panel is attached to each board.

The power rack modules are based on the single eurocard size

cards with a single 32 way edge connector for logic signals and a 24 way edge connector for power supply. Fig 3.6 shows the lay out of the module.

Each board contains up to eight distinct input/output (I/O) ports. Of these , up to five may be used for functions specific to that board and the remaining three are reserved for functions common to all boards. These three common ports handle the board identification and control functions.

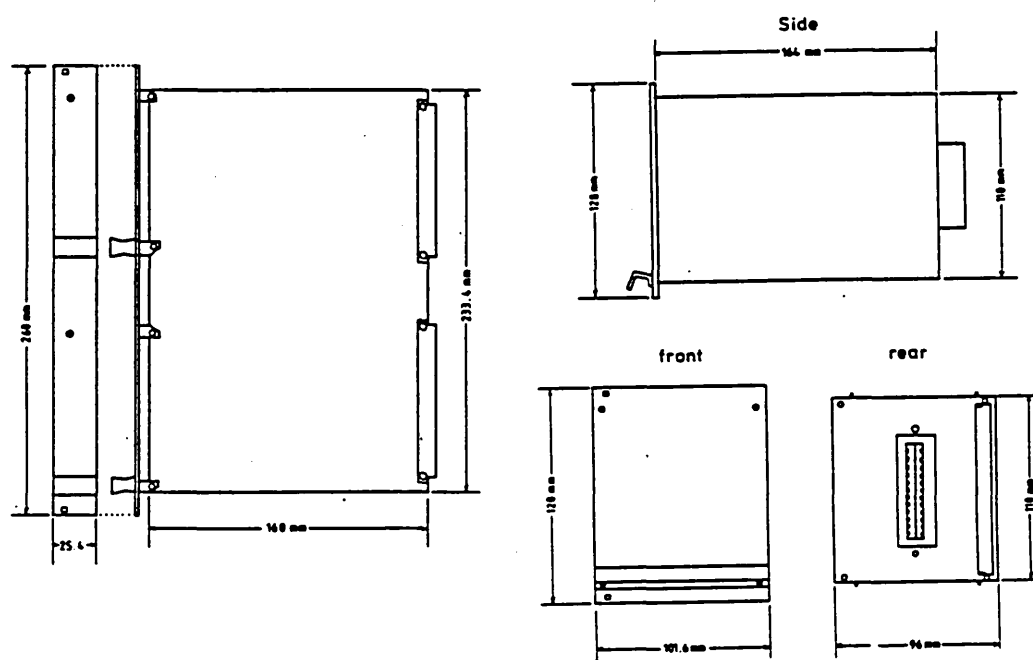


Fig 3.6 a) The Standard Rack function board b)The Power Rack function module

3.4.6.1 The Digital to Analog Converter Board

The digital to analog converter (DAC) board contains two 12-bit DACs , which may be set up to give voltage spans in the ranges ± 10 V , ± 5 V , ± 2.5 V or $+10$, $+5$ V. Important characteristics in DAC performance are as follows

- 1)Resolution
- 2)Offset error

- 3)Gain error
- 4)Settling time
- 5)Linearity
- 6)Stability

These points are explained below

1)Resolution ; determines the accuracy and dynamic range of the analog output. The resolution is defined as the maximum voltage span divided by the maximum number of digital states. The dynamic range is the number of digital states . For an 8-bit DAC with a ± 10 V range the resolution is $20/2^8=0.078$ V/bit , whereas for a 12-bit DAC the resolution is $20/2^{12}=0.005$ V/bit . This is a 25 fold improvement , but increases the cost.

2)Offset error ; is the output from the DAC with zero input code. Offset error can be nulled with an external trim circuit, but offset error drift cannot be eliminated. Offset error is illustrated in fig 3.7a.

3)Gain error ; is the departure from the design output voltage for a given input code. This error is due to inaccuracies in the manufacturing of the silicon chip , and may be eliminated by trimming. Gain error is illustrated in fig 3.7.b.

4)Settling time ; is important for high speed applications. It takes a finite time before the analog output has stabilized after a change in the digital code. Typical settling times for 12-bit converters are in the order of $0.5 \mu s$, which is much faster than the time scale of electrochemical experiments.

5)Non-linearity ; is the most difficult error to compensate for because it cannot be eliminated by adjustment. Differential non-linearity is the difference between the actual voltage and the ideal

for a one bit change.

5) Stability ; is governed mainly by the DAC's inner reference voltage and the temperature stability of its active and passive components. The absolute value of the output of a DAC depends on the stability of the external power supply unit , which must be well regulated and free from noise.

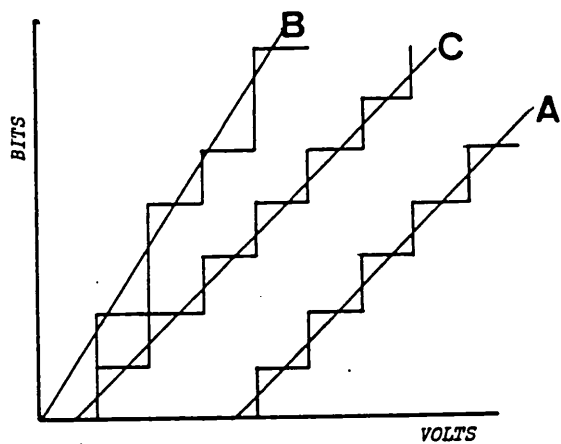


Fig 3.7 DAC errors . a)offset error b)Gain error c)Ideal

TABLE 3.1
Error budget for 8 and 12 bit DACs

Error	8-bit	12-bit
Differential non-linearity	0.2	0.012
Linearity temp.coeff.	0.002	0.002
Gain temp.coeff	0.02	0.02
Offset temp.coeff.	0.005	0.005
Root Mean Square Error (RMS)	0.201	0.024

Table 3.1 shows representative error budgets for a 12 and an 8-bit DAC, assuming a 10⁰ C temperature variation. The 8-bit DAC error is too large for electrochemical precision work. The CMU DAC-card

contains two 12-bit DACs , Hybrid Systems DAC 336C-12 whose characteristics are listed in Table 3.2.

TABLE 3.2
Error budget for the 336C-12 DAC

Differential Non linearity	0.012 %	0.012 %
Linearity temp.coeff.	±2ppm/C ⁰	0.006 %
Gain temp.coeff.	±20ppm/C ⁰	0.06 %
Offset temp.coeff.	±20ppm/C ⁰	0.06 %
Settling time	5 μs	
Total error RMS		0.086

$\Delta T=30^{\circ}$

3.4.6.2 Analog to Digital Converter Board

There exist many types of analog to digital converters (ADCs), and the buyer must become familiar with the various types in order to get the right converter for his application and budget. Table 3.3 lists the different types and their characteristics.

TABLE 3.3
ADC types and characteristics

DAC type	Noise Rejection	Speed	Reso- lution	Price
Successive approximation	No	High	High	High
Single/Dual Slope Integration	Yes	Low	High	Medium
Voltage comparator Counter loop	No	Medium	Medium	Low
Multiple comparator	No	High	Low	High
Voltage to frequency conversion	Yes	Low	High	Low

The reader is referred to the literature for a fuller description (78,79).

The following terms describe the performance and characteristics of an ADC, and should form the basis for decision making.

- 1)Resolution
- 2)Conversion time
- 3)Linearity
- 4)Offset error
- 5)Gain error
- 6)Noise rejection

These parameters are discussed below

1)Resolution ; is a measure of the smallest amplitude value as a percentage of full scale (FS) to which a quantity can be determined. The resolution depends on the conversion time and the dynamic range, which is 2^n where n is the number of bits in the ADC word. Each discrete level, called Least Significant Bit (LSB), will have a spacing of the FS voltage, V_{FS} , divided by the number of spacings, $2^n - 1$. Hence

$$LSB = V_{FS} / (2^n - 1) \quad [3.1]$$

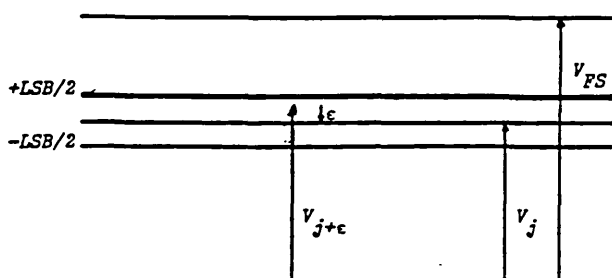


Fig 3.8 ADC quantizing

The level 2^j corresponds to a voltage

$$V_j = V_{FS} / 2^j \quad [3.2]$$

and from fig 3.8 we see that the spacing around V_j is $\pm \text{LSB}/2$. A signal will to be encoded with a quantization error of ϵV , ranging upto $\pm \text{LSB}/2$, which is unreducible noise added to the signal. This noise has a normal probability density function centred on the quantization level and is similar to Gaussian noise. The quantization error is calculated as a root mean square (RMS) error, E , in equation [3.3]

$$E = \frac{1}{\text{LSB}} \int_{-\text{LSB}/2}^{\text{LSB}/2} \epsilon^2 d\epsilon = \frac{\text{LSB}}{2\sqrt{3}} V \quad [3.3]$$

2) Conversion time ;if the sampled signal changes during the conversion there will be an error in the quatization , illustrated in fig 3.9.

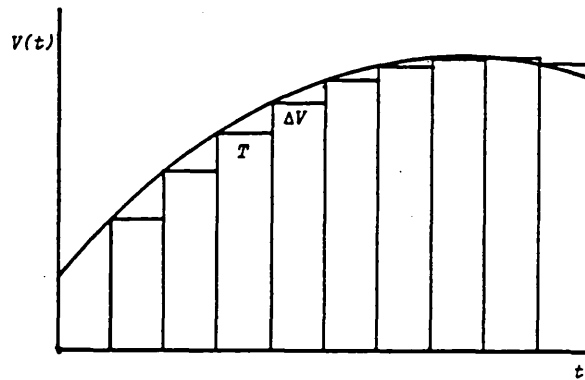


Fig 3.9 Conversion error

If we evaluate this error, ΔV for a sinusoidal signal we arrive at

$$\Delta V = \frac{T dv_s}{dt} = T \frac{d[V_s \sin(\frac{2\pi t}{w})]}{dt} = T\pi w^{-1} V_s \quad [3.5]$$

This equation gives us a relationship between the LSB and the conversion time. For example if we wish to sample a signal to with an bandwidth of 10 Hz with a full 12-bit accuracy ,equation [3.5] gives

$$\Delta V = \text{LSB} = V_{FS} \cdot 2^{-n} = T \pi \omega^{-1} V_s \quad [3.6]$$

now $\omega = 2\pi f$ and $V_s = V_{FS}$, rearranging gives

$$T = 2^{-n} \cdot 2f = 2^{-12} \cdot 20 = 0.005 \text{ s} \quad [3.7]$$

4) Linearity ; is defined as the maximum deviation from the best straight line drawn through the staircase waveform obtained by stepping through all its states. The maximum allowable deviation must be less than $\pm 1/2$ LSB.

5) Offset error ; is the digital output when the analog input is zero. This error may be cancelled with an external trim-circuit , but it will be temperature dependent. See fig 3.10a.

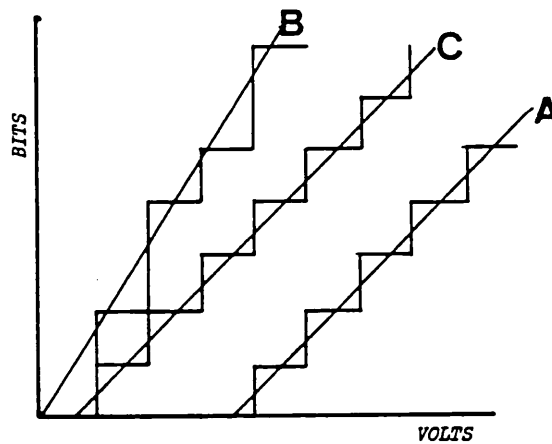


Fig 3.10 a) Offset error b) Gain error c) ideal response

6) Gain error ; is the slope deviation from the ideal for the straight line through the staircase waveform , see fig 3.10b

7) Noise reduction ; applies to integrating and voltage to frequency converters. Ben-Yaakov et al. (80) have shown that the signal to noise ratio for these converters for sinusoidal noise is

$$\frac{(S/N)_{in}}{(S/N)_{out}} = \frac{\pi(T/T_n)}{|\sin(\pi T/T_n)|} \quad [3.8]$$

where T is the integration time and T_n is the period of the noise.

Equation [3.8] is plotted in fig 3.11.

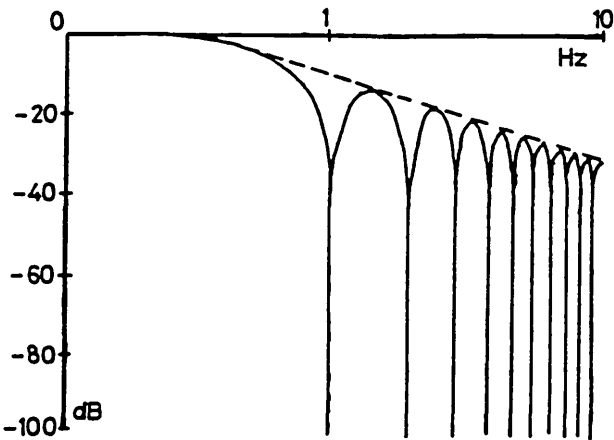


Fig 3.11 Frequency response of an integrating ADC

We can see that harmonics of the integration time are suppressed. By selecting an integration time which is a harmonic of the predominant noise frequency , canceles out this noise , e.g mains noise at 50 Hz is cancelled out by integrating over a multiple of 20 ms.

7)Stability is very important as it is going to determine the over all performance of the ADC under the working conditions. In a green-house one might expect a temperature variation of 30 °C.

The error budget for the used ADC-HS12B , which is a 12-bit successive approximation ADC by DATEL Inc , under glasshouse conditions is given in table 3.4.

TABLE 3.4
Error budget for the ADC-HS12B ADC

Quantization error	0.012
Differential linearity	0.012
Gain temp.coefficient	0.040
Offset temp.coefficient	0.020
Error RMS	0.047 %

$\Delta T=30C^{\circ}$

3.4.6.3 Multiplexer Card

A multiplexer (MPX) is a device that selects , controlled by a digital code , one among many available signals. The MPX can be arranged in in several ways , illustrated in fig 3.12.

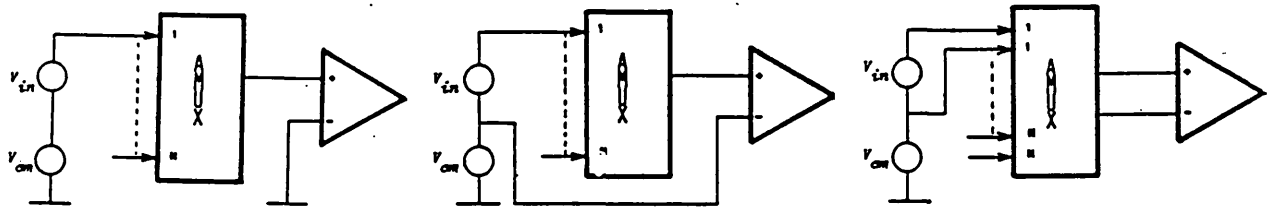


Fig 3.12 a)Single ended MPX b)Differential MPX
c)Pseudo-differential MPX

There exist three basic types of MPX devices , Reed relays (electro mechanical) , CMOS (semiconductor) and JFET (semiconductor). For signal levels below about 10 mV mechanical switching is still recommended , because of offset problems with the semiconductor types. CMOS-type MPX have become dominant in computer applications because of their unfailing turn-off and their ability to accommodate signals up to the supply voltage. A typical error budget for an MPX is given in table 3.5

TABLE 3.5
Error budget for different MPX types

Error	Reed Relay	CMOS
Transfer error	0.01 %	0.01 %
Cross talk	0.001 %	0.001 %
Leakage error	-	0.001 %
Thermal offset	0.001 %	-
Error RMS	0.01 %	0.01 %

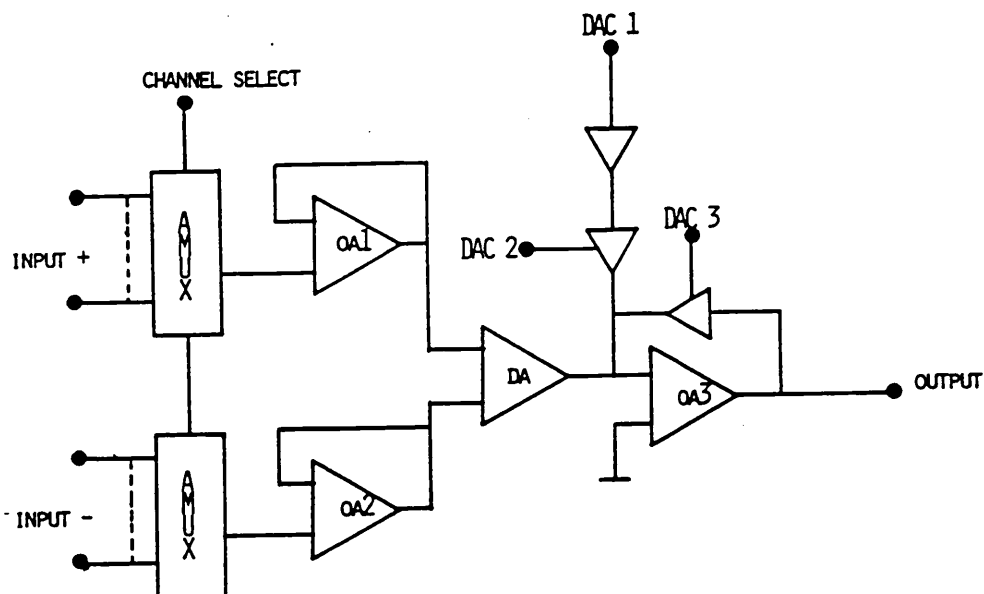


Fig 3.13 The CMU MPX card circuit

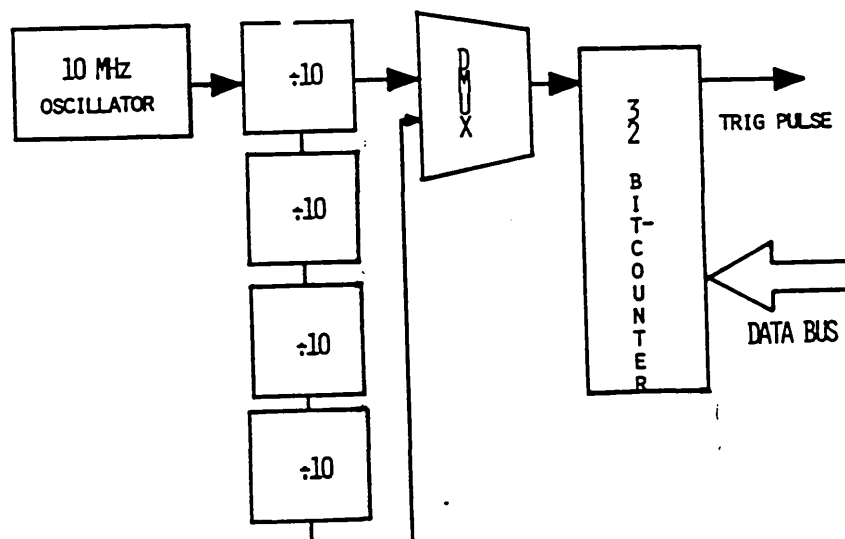
The MPX card for the CMU interface uses two eight channel CMOS MPX in a fully differential mode. The MPX card is also equipped with two buffer amplifiers to eliminate loading of the MPX , and a programmable amplifier with programmable offset , the full MPX card circuit is shown in fig 3.13. This MPX was initially used in the Ion Selective Electrode experiments , but was found to be too noisy. The noise comes from the digital electronics in the CMU interface and is conveyed onto the analog electronics .

3.4.6.4 The Timer Board

A clock and counter is necessary for the timing of the events in the interface , for example , the ADC must receive a trigger signal to start the conversion .

0.1 μ s timing pulses are generated by a very stable crystal oscillator. These pulses are scaled up to 1 μ s and 1 ms pulses by four decade dividers. A digital multiplexer selects the pulse time and feeds the signal to a 32-bit counter. This counter can be set up to count to a specific number, thus enabling us to set up any delay

between 1 μ s and 72 min. The timer circuit is shown schematically in fig 3.14



3.5 The Software

3.5.1 Preamble

Microcomputing is a fairly recent event in electrochemistry and the user community is still small , consequently little software is commercially available. The analyst is therefore often faced with software development. Without a sensible approach to this problem he will spend too much time on this task.

3.5.2 Software Development Strategy

A rational approach to the software development divides this into seven tasks, listed below

- | | |
|-----------------------|-------|
| 1) Problem definition | (10%) |
| 2) Choice of solution | (15%) |
| 3) Program design | (40%) |
| 4) Program building | (15%) |

- 5) Testing and verification (15%)
- 6) Documentation (5%)
- 7) Use

The percentages in the brackets indicate a sensible fraction of the total time to spend on that task. These points are discussed below.

1) Problem definition ; is very important. With a diffuse problem definition we are bound to end up with a diffuse program , but with a clear goal ahead we know when we have reached it.

2) Choice of solution ; there is often a risk for programmers to "re-invent the wheel". If there is software available or similar problems have been solved and published a lot of time can be saved by plagiarism.

3) Program design ; most of the time ought to be spent on the program design. This will pay off in the following "program building" and "program testing and verification" tasks.

A good design approach is so called "structured programming" , which means dividing the program into blocks with only one way in and one way out. Each block is developed from successive elaborations around the block problem definition , and can be error checked (debugged) separately. The advantages of this technique are clarity and simpler debugging. Consider a program consisting of N blocks , each containing P_i paths. Debugging the whole program as an unstructured monolith involves

$$P_1 * P_2 * P_3 * \dots * P_N \quad [3.13]$$

tests to check all paths , whereas a structured validation involves

$$P_1 + P_2 + P_3 + \dots + P_N \quad [3.14]$$

tests to check all paths.

4) Program building ; as little time as possible should be spent coding and typing in the program. A good program design lead to easy coding. The actual programming language is a matter of availability and preference.

5) Testing and verification ; the law of diminishing returns applies to the debugging task. A professional programmer gave me the advice - "stop debugging when you can't be bothered to find any more errors". The program must be tested both for the expected range of inputs and for "crazy inputs".

6) Documentation ; a good program is almost self-explanatory. Typically 30-50 % of a program should be should consist of helpful comments. All variables should be given intelligible names and be listed .

3.5 CMU Interface Software

A substantial amount of software is necessary for controlling the CMU interface and must be written in low level language (assembler) in order to keep up with the speed of the events in the interface. Dr N.J.Goddard at the CMU has written a library of Z 80 assembler subroutines for interface control , which were patched into the BASIC interpreter. The interface routines are called by a simple BASIC command viz.

```
CALL "USERSUB",A,B,VARADR(C),VARADR(D)
```

which executes the assembler subroutine with the variables A,B and returns the result in variables C,D. The BASIC subroutines are listed in appendix II

3.6 Conclusions

The 380 Z microcomputer together with the CMU interface forms a

very flexible and user friendly development sytstem. This system has been used for the design and development for many successful instruments in this laboratory.

CHAPTER 4

THE AUTOANALYSER

4.1 Development

Preliminary work at this lab by Dr G.Pu with ISEs in NTF solution showed promise, he concluded that Na^+ , K^+ , pH, Ca^{2+} , Cl^- and NO_3^- ISEs can be used in NFT if reasonable care is taken in their application (81). A computer controlled ISE analyser (Auto-analyser) was therefore investigated for NFT analysis and control.

Initially the ISEs were connected via a distribution box to the MPX-card in the CMU interface, but this proved to be unsatisfactory for two reasons; Firstly the input impedance of the MPX-card is $10^{10} \Omega$, which is about ten times less than some of the ISEs. For low error measurement the input impedance should be at least 1000 times larger than the impedance of the sensor. Secondly the signal was polluted by noise from the computer, which amounted to about 60 mV p-p. This forced us to abandon the MPX-card and to design a separate Ion Selective Electrode Interface Unit (ISEIU). The ISEIU was developed over three prototypes. This chapter describes ISE-U III, which was designed and developed after a visit to Professor W.Simon's group at ETH Zurich.

4.2 Experimental

4.2.1 Ion Selective Electrodes

All the ISEs used in this work were manufactured and supplied by Russel pH, Auchtermuchty, Scotland. Table 4.1 lists the elec-

trodes , electrode type , expected lifetime , selectivity coefficient and price in Aug 1983.

TABLE 4.1
ISE characteristics

ISE	Type	Life time	Selectivity coefficients	Price
NO_3^-	LIEM	1-2 months	$\text{Cl}^-: 9.4 \times 10^{-3}$, $\text{PO}_4^{3-}: 10^{-6}$	169:40
Na^+	glass	years	$\text{K}^+: 3 \times 10^{-3}$, $\text{NH}_4^+: 2.10$ H^+ if $\text{pH} > \text{pNA} + 3$	60:50
K^+	LIEM	1-2 months	$\text{Na}^+: 2.6 \times 10^{-3}$, $\text{NH}_4^+: 0.3$ $\text{Ca}^{2+}: 2.5 \times 10^{-3}$, $\text{Mg}^{2+}: 2 \times 10^{-3}$	187:55
Ca^{2+}	LIEM	1-2 months	$\text{Mg}^{2+}: 2.5 \times 10^{-4}$ $\text{Na}^+: 1.46 \times 10^{-4}$, $\text{K}^+: 10^{-6}$	175:45
NO_3^-	LIEM	1-2 months	$\text{Cl}^-: 9.4 \times 10^{-3}$ $\text{PO}_4^{3-}: 10^{-6}$	169:40
Cl^-	Solid State	>2 years	-	145:20

LIEM; Liquid Ion Exchange Membrane , Selectivity coefficients according to the manufacturer, price excluding VAT

The PVC membrane electrodes have the membrane mounted on a screw cap , so that it may be replaced when exhausted. Fig 4.1 shows the different types of electrodes. All electrodes were tested and had logarithmic concentration responses in accordance with the manufacturers claims. The ISE's responses in NFT solution were tested in an artificial nutrient solution containing maximum concentrations of interfering ions. Figures 4.2-4.7 show the results , the vertical lines represent the maximum and minimum concentrations of the analysed ion in NFT. These plots show that no correction is necessary for measurements in NFT solution.

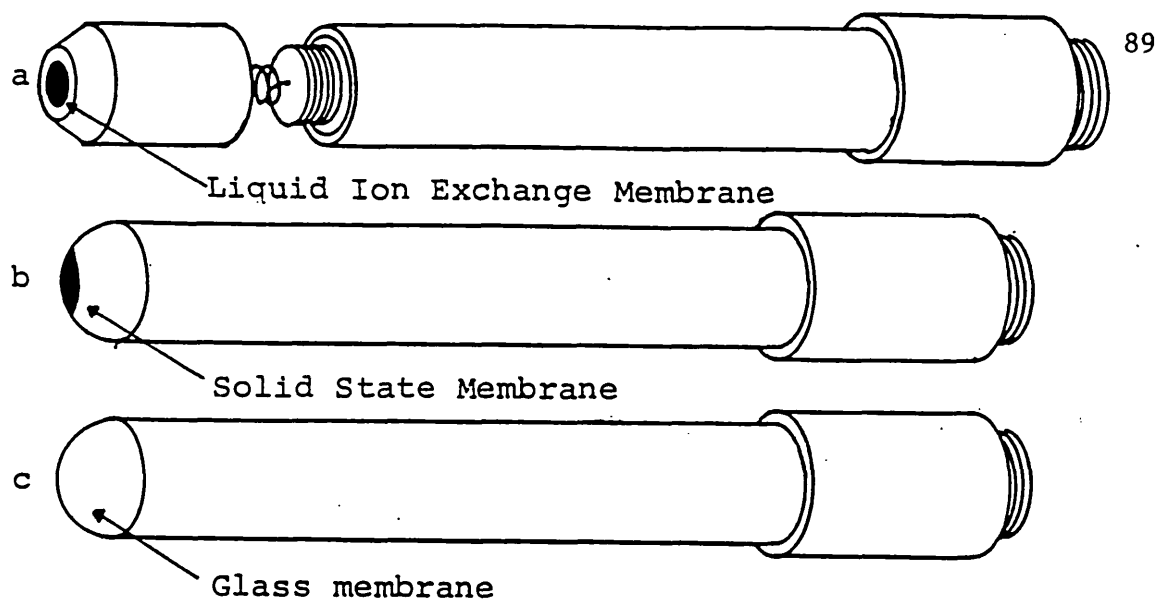
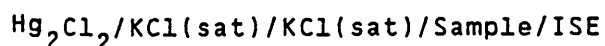


Fig 4.1 a) Liquid Ion Exchange ISE b) Solid state ISE c) Glass ISE

4.2.2 Reference electrodes

Two types of reference electrodes were used. The first was a double junction sulphate electrode (Russel pH , CRR/DJ/S) (which was smashed to smithereens by our carelessness) and later a double junction calomel electrode (Russel pH , CRR/DJ) was used. All results are reported with respect to this electrode



4.2.3 Chemicals and water

All water used in solutions and final rinses was deionized and processed in a Millipore Milli-Q water purifier. The manufacturer claims this water is ultra pure. This water is referred to as MQW.

All glassware was cleaned in DECON 90 solutions in an ultrasonic bath, thoroughly rinsed with MQW.

All chemicals were of AnalaR or AVS grade.

4.2.4 Calibration solutions

The ISE calibration solutions were designed to bracket the NFT solution's minimum and maximum concentrations. The preparation of

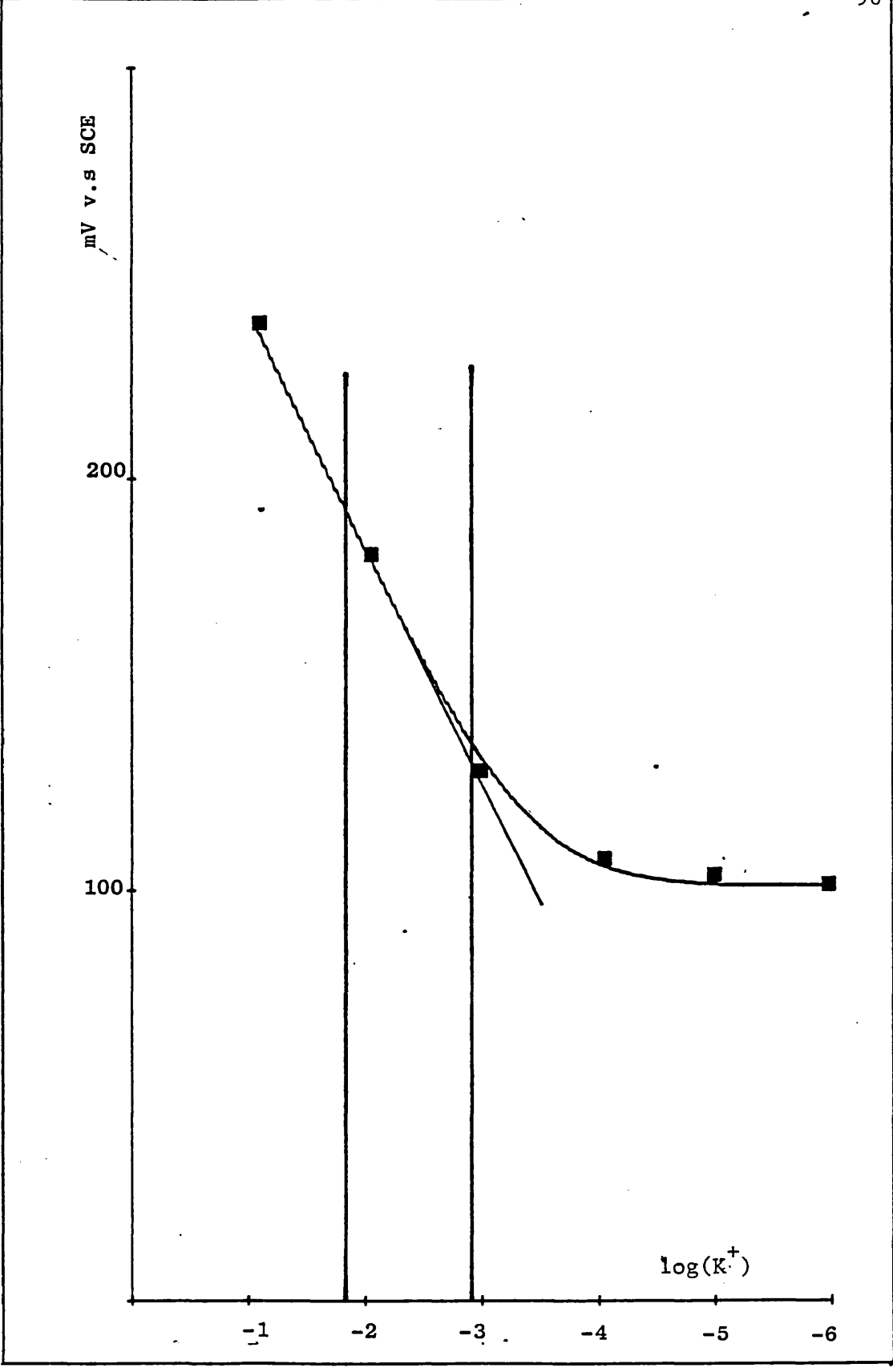


Fig 4.2 The Russel pH potassium ISE calibration curve

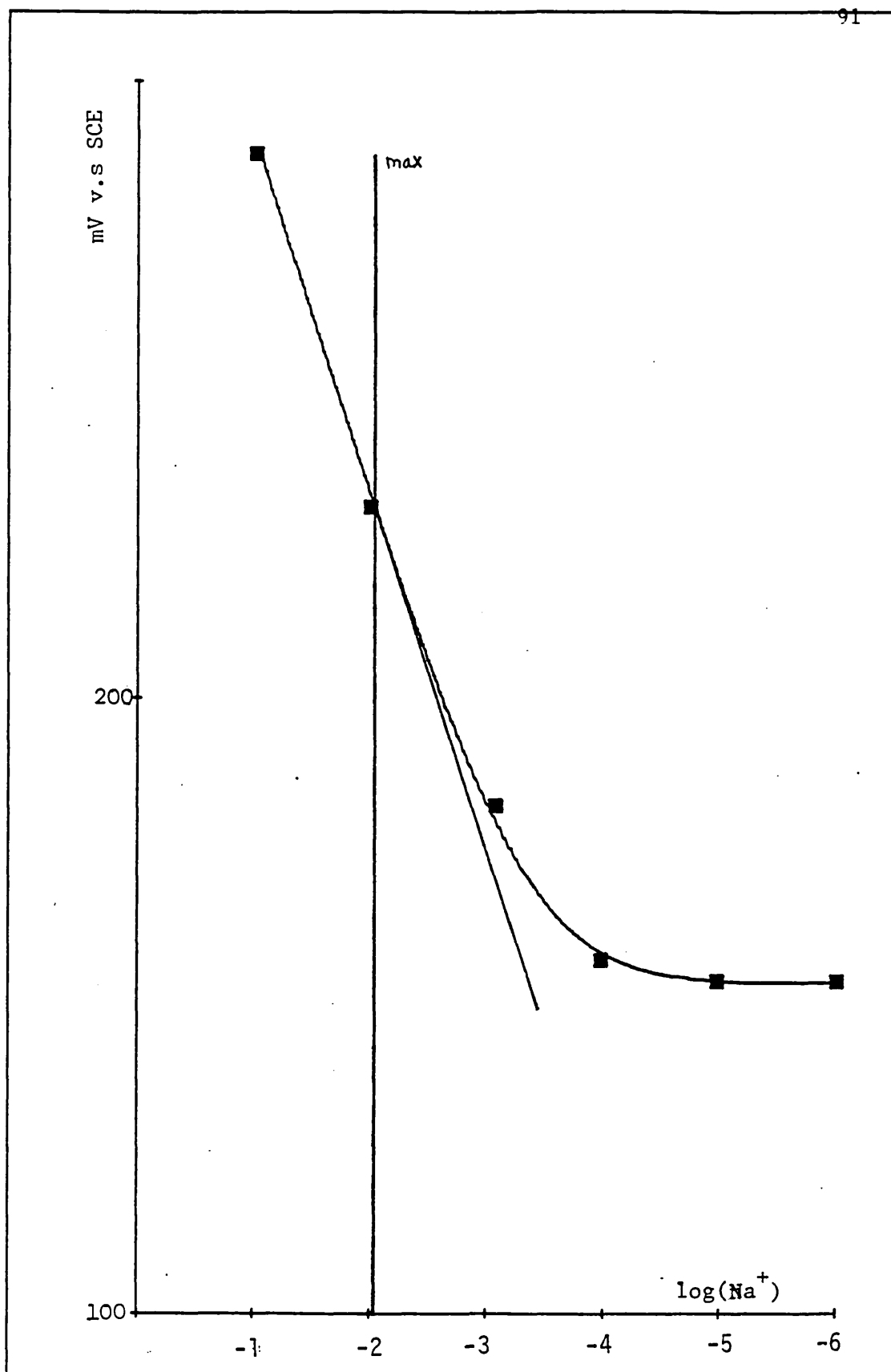


Fig 4.3 The Russel pH sodium ISE calibration curve

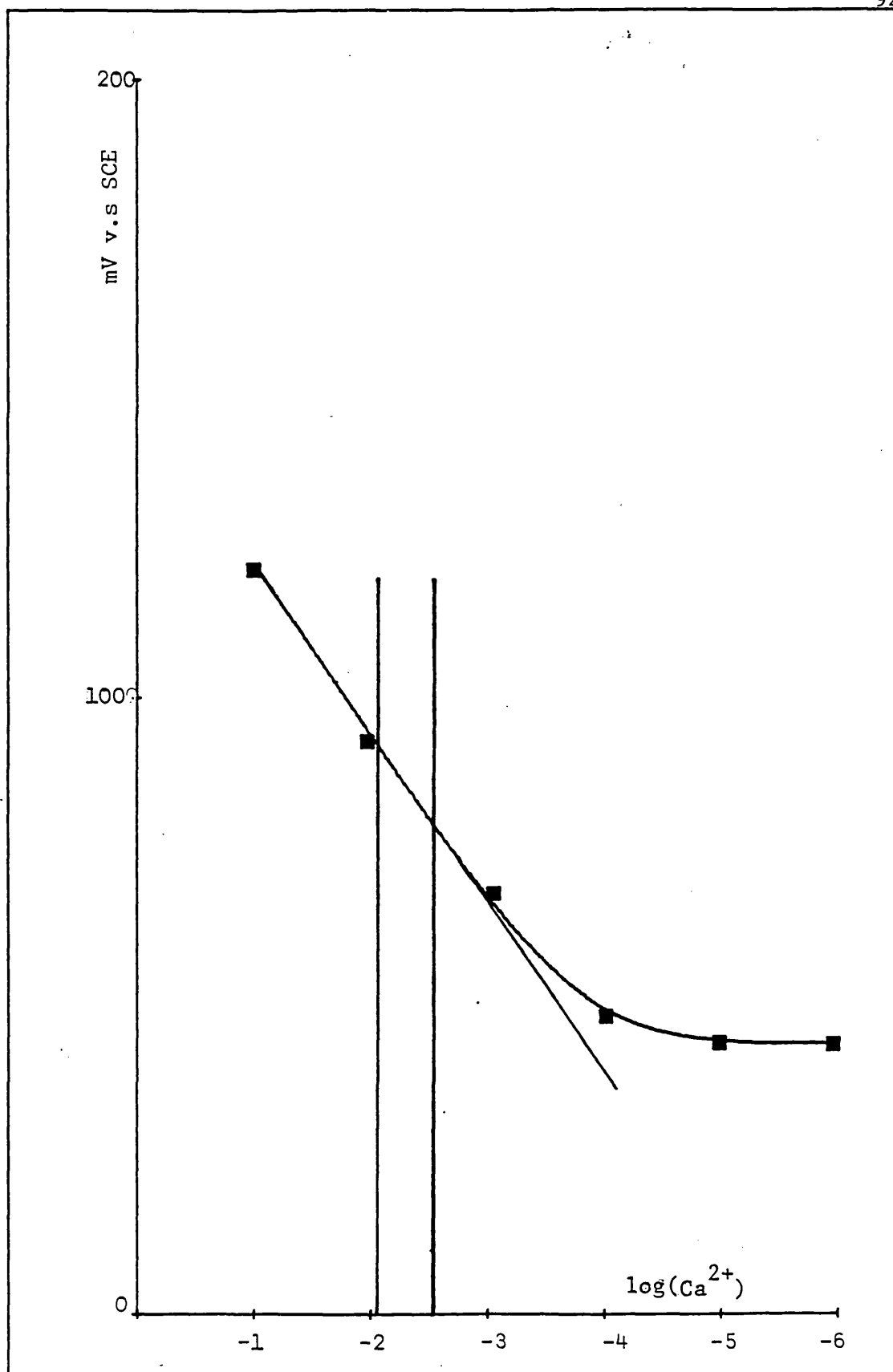


Fig 4.4 The Russel pH calcium ISE calibration curve

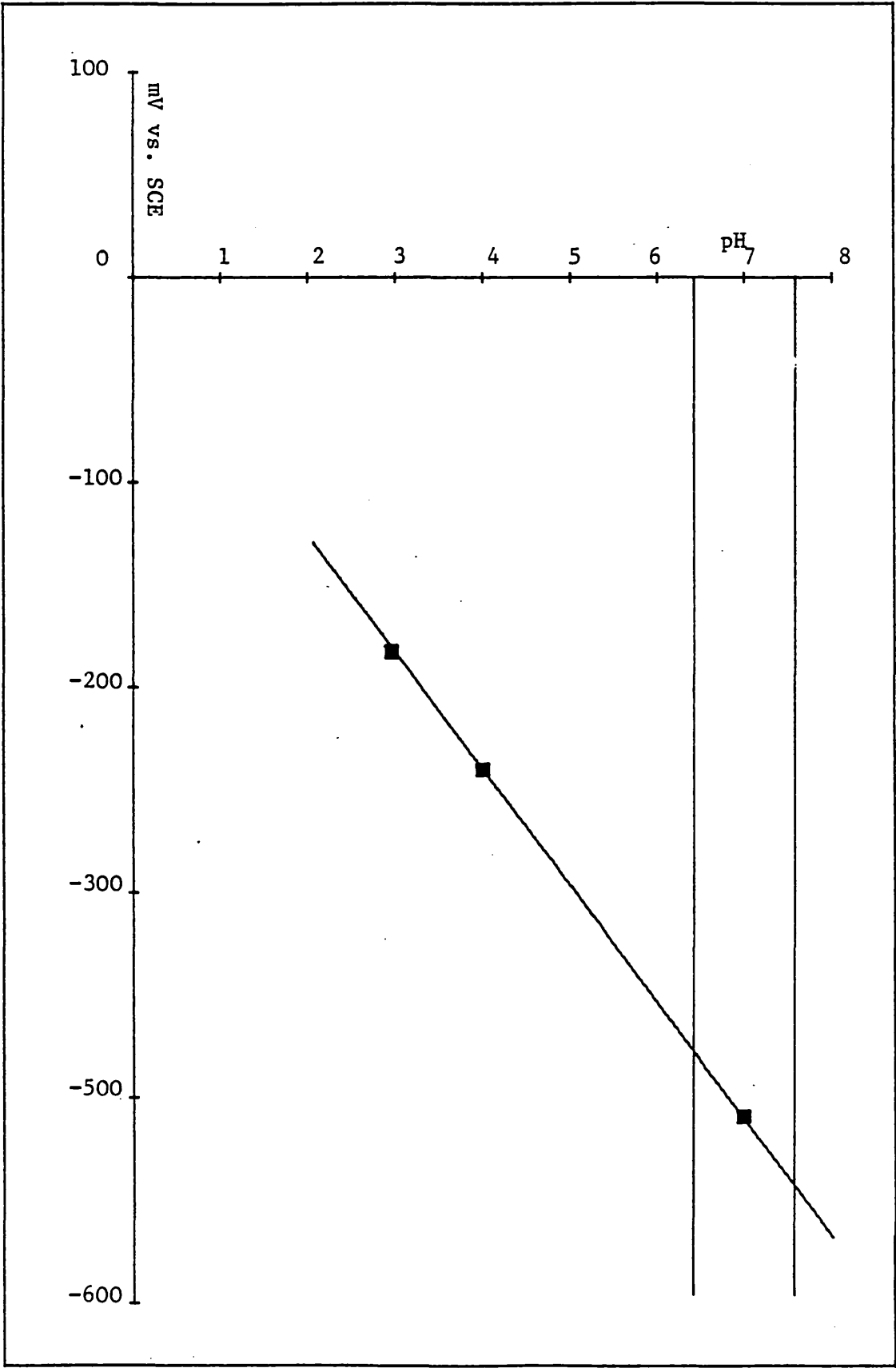


Fig 4.5 The Russel pH Ltd pH electrode calibration curve

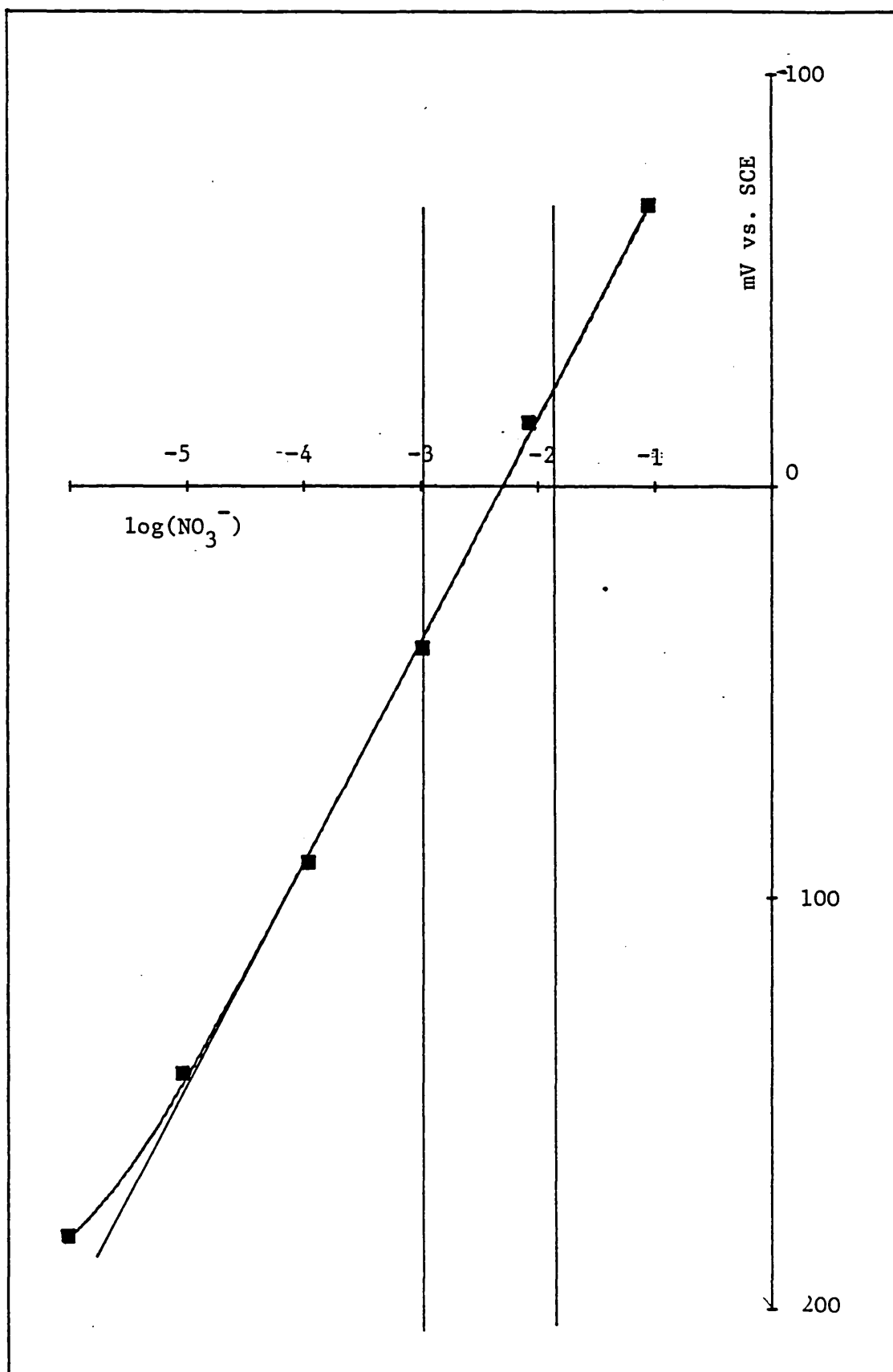


Fig 4.6 The Russel pH Ltd Nitrate ISE calibration curve

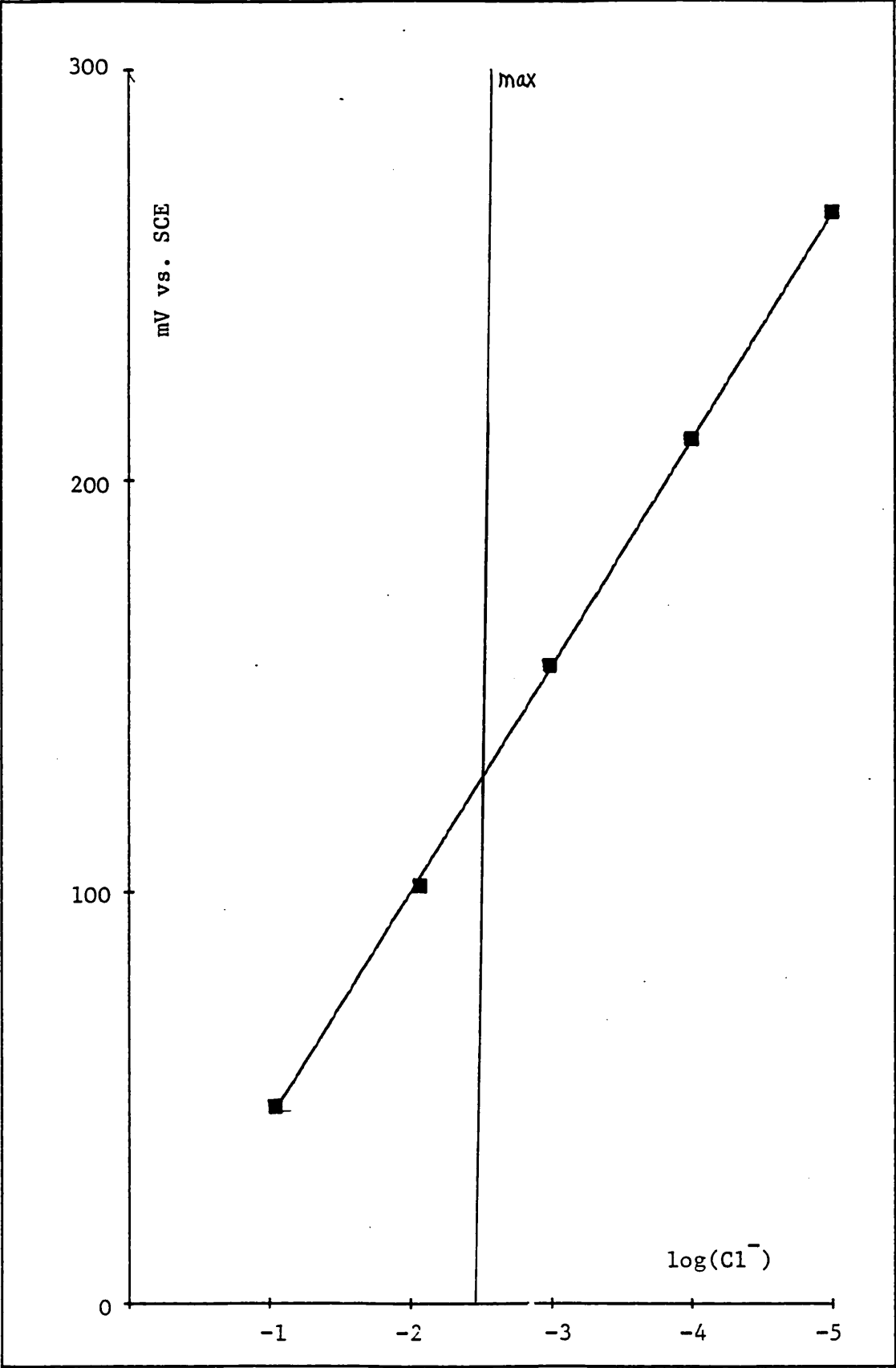


Fig 4.7 The Russel pH Ltd Chloride ISE calibration curve

these calibration solutions , called HI and LO , was simplified by dilution from a stock solution , called STOCK. Table 4.2 gives the contents of these solutions and the associated ionic activity coefficients.

TABLE 4.2
Stock and calibration solutions

Ion	STOCK	HI	γ	LO	γ
Na ⁺	0.10	0.010	0.85	0.0010	0.95
K ⁺	0.10	0.010	0.85	0.0010	0.95
Ca ²⁺	0.10	0.010	0.57	0.0010	0.91
pH	n.r	4.4	0.90	5.4	0.96
HA ^c	0.12	0.012	n.r	0.0012	n.r
SO ₄ ²⁻	-	0.006	n.r	-	-
NO ₃ ⁻	0.20	0.020	0.85	0.0020	0.95
Cl ⁻	0.10	0.010	0.85	0.0010	0.95

n.r=not relevant -=not in solution

The HI solution was made up by diluting 100.00 ml of STOCK and 3.00 ml of 2.00 N H₂SO₄ to 1000.00 ml with MQW , similarly the LO solution was made up by diluting 10.00 ml STOCK to 1000.00 ml with MQW. The acetate was used for buffer , adjusted to the right pH by the addition of H₂SO₄.

4.3 Flow System

4.3.1 ISE Flow-Cell

The ISEs were adapted for in-line measurements with a flow-cell. Many flow-cells for ISEs have been described in the literature (83-87), and they divide into the four generic types illustrated in

fig 4.8.

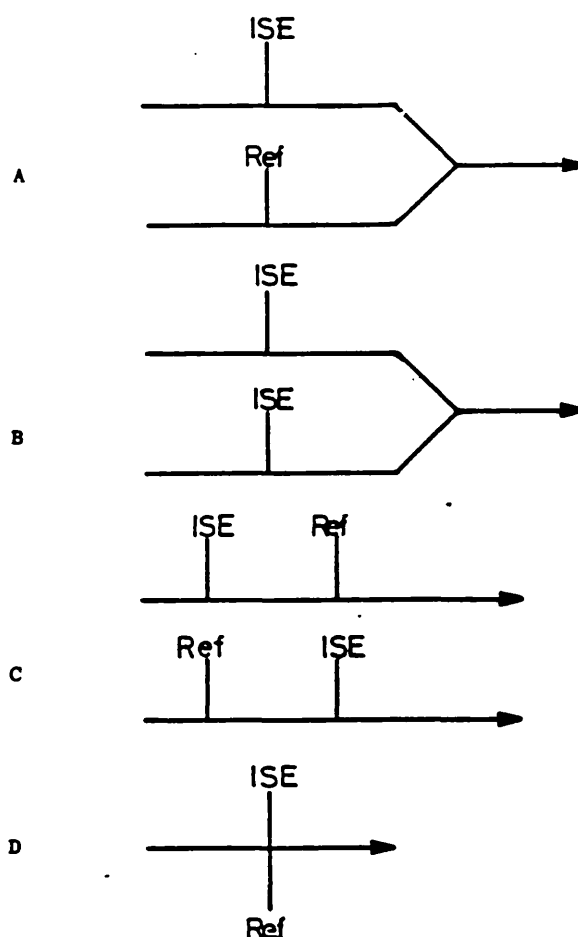


Fig 4.8 Flow cell types

Type 4.8a is generally used with a free flowing liquid junction and a differential measurement technique. Very good results have been reported with this flow-cell (88). Type 4.8b measures the EMF between two identical electrodes, one in a standard solution stream and the other in a sample solution stream. This method compensates for activity corrections (89). Type 4.8c is the most common and least complicated of the four, the sensing surface protrudes into the duct, and causes some turbulence which improves the response time. Type 4.8d is less common, with the ISE juxtaposed opposite the reference electrode (90).

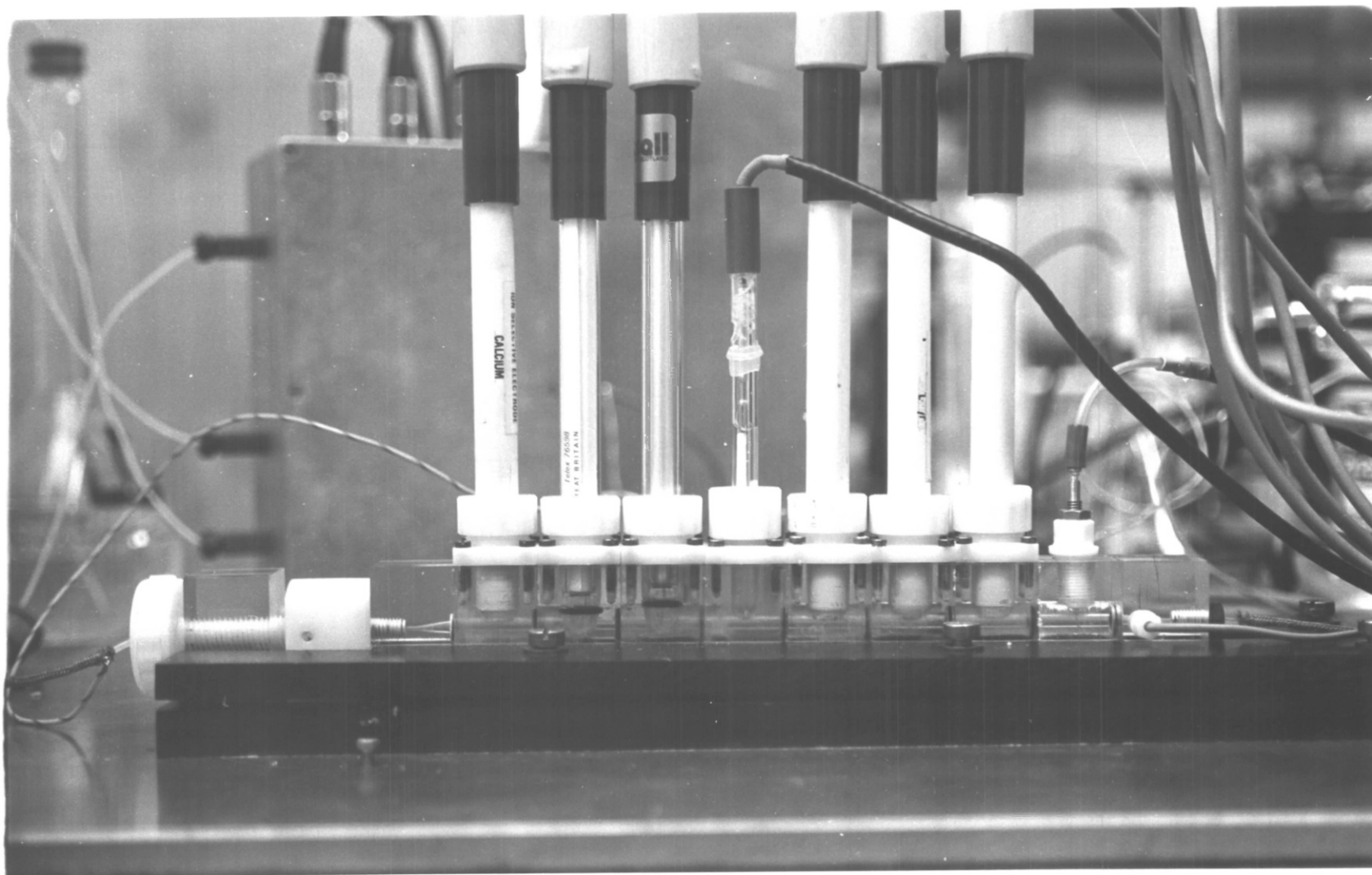


Fig 4.9 The ISE flow cell

Our requirements for the flow-cell were;

- 1) Uses commercially available electrodes
- 2) As simple as possible
- 3) Flexible
- 4) Small dead volume
- 5) Fast response time
- 6) Easy maintenance

This led us to choose type 4.8c with some added features. Flexibility was achieved by designing the flow-cell on a modular basis , with each electrode occupying one module. The modules are clamped together face to face on a slotted base , which minimizes the dead volume and simplifies the maintenance. The first design had a zig-zag duct with the electrodes at the highest point. The idea behind this was to improve the response by letting the solution impinge on the sensing surface in order to displace old solution more efficiently. An unforeseen problem in this design was air bubbles. An air saturated solution will produce air bubbles when pumped through narrow bore tubing , they will naturally find their way to the highest accessible point , which in this design most unfortunately was the electrode surface. The design was abandoned after many unsuccessful attempts to get rid of the air bubbles by degassing and using debubblers. This experience of the zig-zag design led to a fairly large bore straight channel design , shown in fig 4.9. A temperature sensor was mounted in the inlet module , separated from the duct by a very thin perspex wall. This flow-cell has been used successfully throughout the laboratory and field trials. It proved to be reliable and inexperienced users found it easy to use and maintain.

4.3.2 Pump system

The pump system supplies the flow-cell with sample and calibration solution. It consists of a computer controlled solenoid valve unit and a computer controlled peristaltic pump. The solenoid valve unit was controlled by the power interface and consists of three LIF 1200 118H (Lee company , Harrow) solenoid valves in a "tree configuration" , illustrated in fig 4.10 .

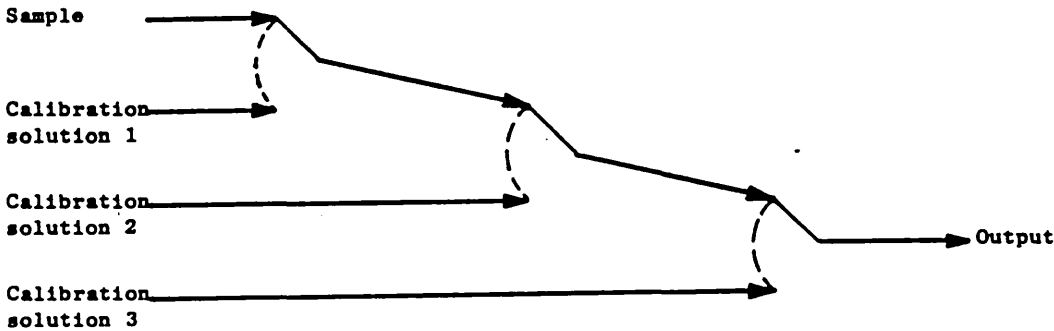


Fig 4.10 Solenoid valve unit configuration

A Pharmacia P 3 (Pharmacia Fine Chemicals , Uppsala , Sweden) peristaltic pump was modified for computer control , shown in fig 4.11. The interface supplies the controlling voltage to point A in the circuit diagram. The pump shows a good linear relationship between digital output and flowrate.

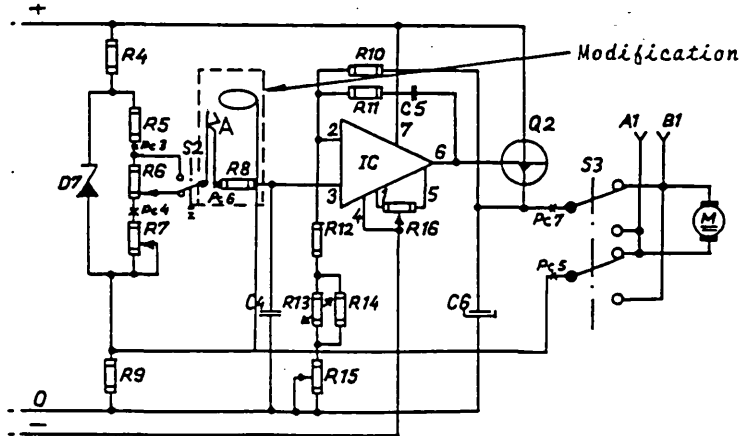


Fig 4.11 The peristaltic pump modification

Initially a pulse damping system was used to mitigate the pulsation from the pump , but nevertheless a residual ripple was present , which caused the plastic membranes to flex and thereby generate a spurious potential. Eventually our pragmatic solution to this problem was to carry out the measurements in a still solution. The pump was simply switched off by the computer before the measurements. An advantage of this simple solution is that we can abandon the pulse damping system , which cuts down the dead volume by a factor of almost two , thus saving calibration solution and improving the response time.

4.3.3 Flow System Noise

A number of noise preventing measures must be taken in a flow system design. The major noise culprits are static electricity , Radio Frequency Interference (RFI) and induced noise.

Static electricity is generated by the peristaltic pump , which is conveyed to the flowcell by the solution and the tubing. This problem is overcome by earthing the solution and covering the tubing with a metal braid connected to earth. The peristaltic pump must also be carefully earthed. In order to completely avoid static charging all units in the flow system were placed on a large aluminium sheet (connected to earth) , and electrically connected to one central point on this earth plane.

The proximity of the microcomputer caused pick-up of digital switching RFI. This noise was eliminated by screening the flow-cell in a Faradaic cage , and by the dense copper braid on the tubing.

Induced noise arises from changes in the electric or magnetic field , caused typically by power lines or moving material with

magnetic permeability, e.g human beings. These currents are very small, but the high impedance of the ISEs, ranging from 10-500 MΩ, amplifies this noise. An induced current of 10^{-9} A, i.e waving a hand, produces a voltage according to Ohm's law of

$$U = 5 \times 10^8 \times 10^{-9} = 0.5 \text{ V}$$

This nuisance was circumvented by converting the high impedances of the ISEs to less than one ohm. Impedance conversion and active screening (driving the screen at the same potential as the conductor) made the cables from the ISEs very immune to noise.

4.4 The ISEIU III

The experience from ISEIU I and ISEIU II led us to the formulation of the following design requirements

- 1) Overall error < 1 %
- 2) Supports external impedance converters
- 3) Temperature compensation
- 4) High Common Mode Noise Rejection
- 5) Precision low-pass filtering
- 6) Mains derived trigger pulses
- 7) Separate high quality power supply
- 8) Large dynamic range
- 9) As little computer contact as possible
- 10) At least 6 ISE inputs

The best way to analyse the overall error is a Root Mean Square (RMS) error analysis. If we assume that the errors e_x , e_y , etc are uncorrelated, which is a reasonable assumption, then they may be combined as in equation [4.1] (91)

$$\begin{aligned}
 e_{\text{tot}}^2 &= (e_x + e_y + e_z + \dots)^2 = \\
 &= (e_x^2 + 2e_{xy} + e_y^2 + 2e_{xz} + e_z^2 + 2e_{yz} + \dots) = \\
 &= e_x^2 + e_y^2 + e_z^2 + \dots
 \end{aligned}
 \quad [4.1]$$

The cross product terms are zero because they are uncorrelated. The error from a transfer function $H = f(x, y, z, \dots)$, due to uncorrelated errors $e_x, e_y, e_z, \dots$ is given by equation [4.2] (92)

$$e_H^2 = \left(\frac{\partial H}{\partial x} e_x \right)^2 + \left(\frac{\partial H}{\partial y} e_y \right)^2 + \left(\frac{\partial H}{\partial z} e_z \right)^2 + \dots \quad [4.2]$$

Both equations [4.1] and [4.2] demonstrate that large errors are going to dominate the error budget, and indicate where improvements are productive.

These requirements led us to a design in which the ISEIU is split into a signal amplification part and a signal conditioning part with multiplexing in between, shown in fig 4.12

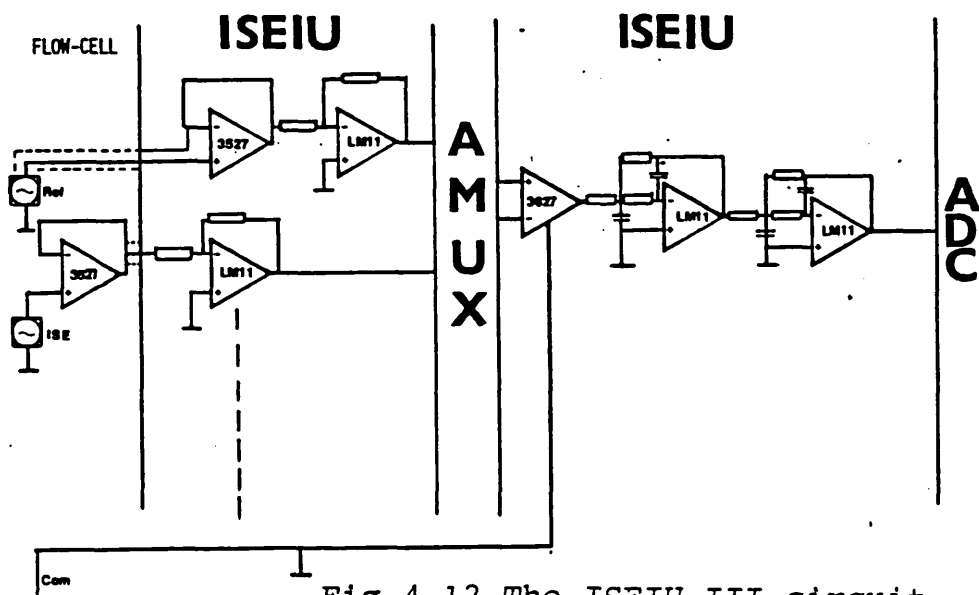


Fig 4.12 The ISEIU III circuit

4.4.1 Impedance converters

The previously described impedance converter consists of a voltage follower with an active screen, screwed on to a connector on the top of the ISE. A split view of a converter is shown in fig

4.13. We used an FET input operational amplifier (op-amp) , 3527 (Burr-Brown International Ltd ,Watford) , which is laser trimmed at the factory and does not require any external offset trimming. The error budget for the converter is given in Table 4.3.

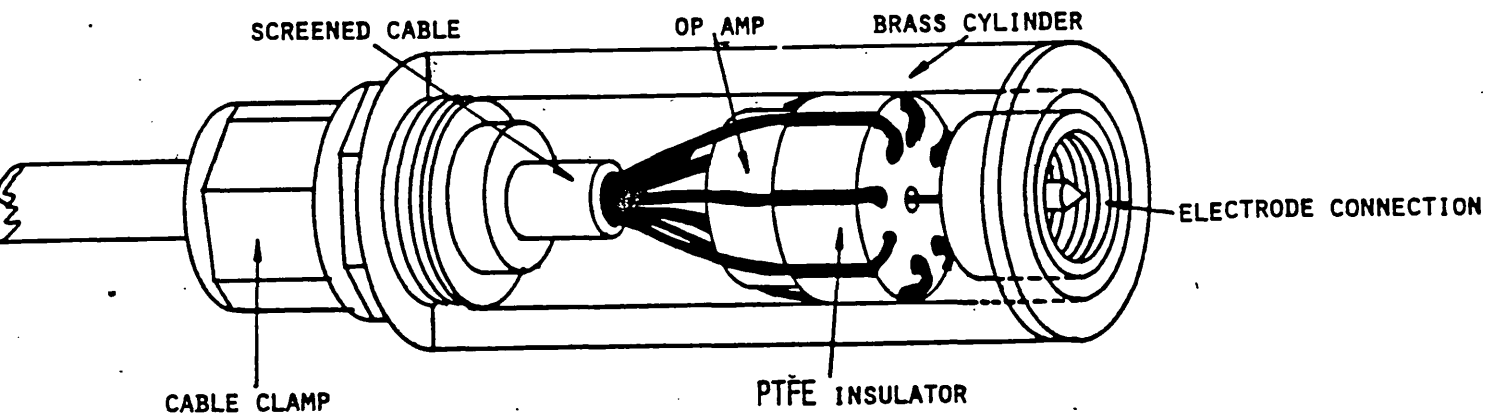


Fig 4.13 A split view of an impedance converter

TABLE 4.3
Impedance converter error budget

V_{os}	100 μ V	100 μ V
$dV_{os}/dT \cdot \Delta T$	2 μ V/ C^0	60 μ V
$I_{os} \cdot R_s$	0.7 pA	7 μ V
$dI_{os}/dT \cdot \Delta T$	$2 \cdot I_{os} / 10 C^0$	21 μ V
V_n	2.6 μ V	3 μ V
Error RMS		118 μ V

Table legend: Assumptions $\Delta T=30 C^0$ source resistance
 $R_s=10 M\Omega$; V_{os} =Offset voltage ; I_{os} =Offset current
 V_n^s =Noise voltage

4.4.2 Precision amplifiers

The signals from the impedance converters are fed into precision amplifiers with a ten fold gain , showed in fig 4.14. National Semiconductor's op-amp LM11 was selected for this purpose because it is a state-of-the-art op-amp with extremely good performance. In

order to maintain a good gain accuracy , high precision resistors with a 0.1 % error and a 20 ppm temperature coefficient were used.

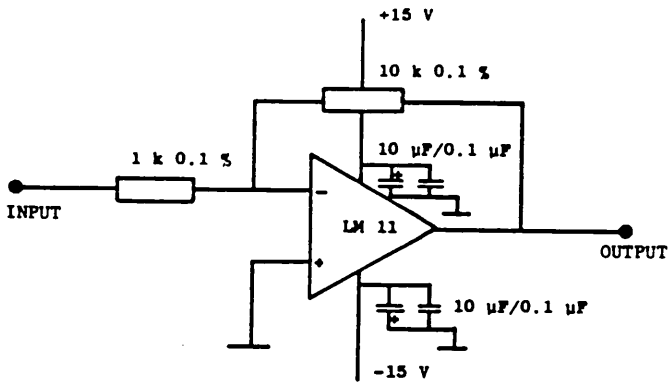


Fig 4.14 The precision amplifier circuit

The amplifier error budget is given in table 4.4 and 4.5

TABLE 4.4
The precision amplifier error budget

V_{os}	100 μ V	100 μ V
$I_{os} \cdot R_s$	0.5 pA	50 nV
$dI_{os}/dt \cdot \Delta T$	20 fA/C ⁰	0.6 pV
$dV_{os}/dt \cdot \Delta T$	1 μ V/C ⁰	30 μ V
V_n	0.2 μ V	0.2 μ
Error RMS		104 μ V

Table legend: Assumptions $\Delta T=30$ C⁰ source resistance $R_s=0.01$ Ω ; V_{os} =Offset voltage ; I_{os} =Offset current V_n^s =Noise voltage

TABLE 4.5
The precision amplifier gain error budget

Resistance accuracy	0.1 %	0.14 %
Temperature coefficient	10 ppm/C ⁰	0.03 %
Long term drift	0.05 %	0.07 %
Error RMS		0.16 %

$\Delta T=30$ C⁰

4.4.3 Multiplexer

The output from the amplifier section is fed out from the ISEIU in a screened multiway cable to the multiplexer. The CMU multiplexer board uses CMOS multiplexers, which were found to be unreliable and noisy. Consequently a Reed Relay MPX board was built for the CMU interface for the ISEIU multiplexing, shown in fig 4.15. It consists of a data bus buffer (25LS2518), a latch (74LS8304) that holds the selected channel number, an octal Darlington driver (ULN 2803A) that supplies current to the selected relay, eight double pole dry Reed Relays and some miscellaneous digital electronics for interface control.

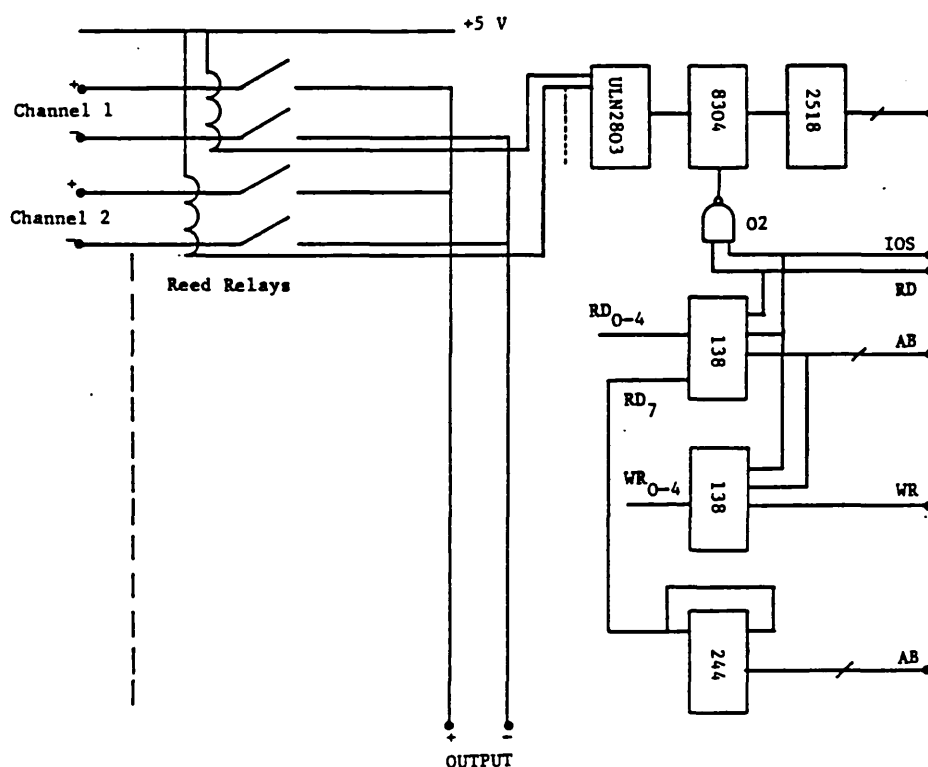


Fig 4.15 The ISEIU MPX circuit

When one of the eight data lines in the data bus goes to a logic high, i.e. from 0 V to +5 V, a corresponding dual Reed Relay is

energized. This eliminates the need for new software because a standard command can be used for MPX control. The multiplexer error budget is given in table 4.6.

TABLE 4.6
The MPX error budget

Noise from the interface	10 mV
Transfer error	0.01 %
Cross talk error	0.001 %
Thermal offset	0.001 %

4.4.4 Signal Conditioning

Table 4.6 shows that the contact with digital electronics has polluted the previously pure signal. The signal was therefore cleaned up with a signal conditioning stage.

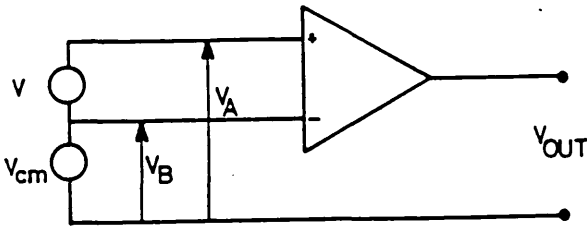


Fig 4.16 Common mode noise

The differential signal from the multiplexer is fed back in a screened dual core cable to the ISEIU where the signal is conditioned. The first stage is a differential amplifier , which rejects common mode noise , defined as the difference of potential V , fig 4.16 , between the zero signal reference point and the signal input points A and B. Thus $V_A = V_S + V_{CM}$ and $V_B = V_{CM}$ so that the differential amplifier gives an output

$$V_{out} = V_A - V_B = V_S + V_{CM} - V_{CM} = V_S \qquad [4.3]$$

The effectiveness of the differential amplifier in eliminating common mode noise , which can be several orders of magnitude larger than the original signal , is described by the Common Mode Rejection Ratio (CMRR) given by

$$\text{CMRR} = \frac{V_{\text{CM}}}{\text{Unwanted noise pick-up at input}} \quad [4.4]$$

The zero reference point must be chosen carefully to correspond to the right EMF. In our system the solution is taken as the zero reference point by connecting it to earth with a platinum wire. The differential amplifier in the ISEIU is a Burr-Brown 3627 , which has a very high CMRR and excellent overall performance.

The next signal conditioning stage is a four pole Butterworth filter with a cut-off frequency of 1 Hz , and roll-off of -80dB/decade. This filter rejects mains 50 Hz noise by -120 dB. The signal conditioning circuit is shown in fig 4.17.

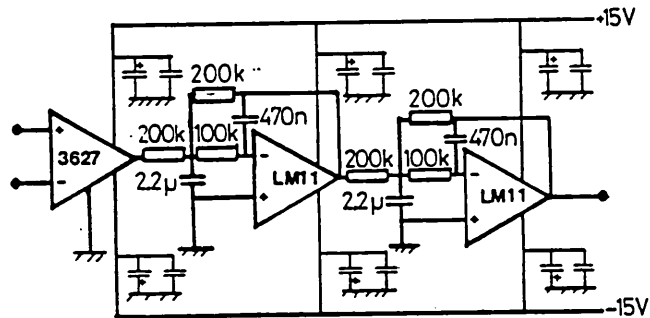


Fig 4.17 The signal conditioning circuit

The signal improvement after the signal conditioning stage is expressed as a signal-to-noise ratio (S/N). The improved (S/N) is described by equations [4.5-10] (91)

$$(S/N)_{\text{input}} = (V_{\text{diff}}/V_{\text{CM}})^2 \quad [4.5]$$

$$(S/N)_{\text{diffamp}} = (S/N)_{\text{input}} * \text{CMRR}^2 \quad [4.6]$$

$$(S/N)_{\text{filter,random}} = \frac{(S/N)_{\text{diffamp}} * K * \text{amp} * f_{\text{hi}}}{f_{\text{filter}} * f_c} \quad [4.7]$$

$$(S/N)_{\text{filter, coherent}} = \frac{(S/N)_{\text{diffamp}}}{(\text{filter attenuation})^2} \quad [4.8]$$

$$e_{\text{RMS}} = (e_{\text{diffamp}}^2 - e_{(S/N)\text{diffamp}}^2 + e_{\text{filter}}^2 + e_{(S/N)\text{random}}^2 + e_{(S/N)\text{coherent}}^2 + e_{\text{MPX}}^2 + e_{\text{amp}}^2 + e_{\text{converter}}^2)^{0.5} \quad [4.9]$$

$$P(\Delta a, a) = \text{erf}\left\{\frac{1}{2} \frac{\Delta a}{a} \sqrt{(S/N)}\right\} \quad [4.10]$$

The K factor in equation [4.7] is the filter efficiency and is defined as

$$K = \frac{(S/N)_{\text{filter}}}{(S/N)_{\text{diffamp}}} \frac{f_{\text{co}}}{f_{\text{hi}}} \quad [4.11]$$

where f_{hi} is the frequency when the amplifier gain is down by -3 dB.

Fig 4.18 shows the K factor's relationship to $(S/N)_{\text{diffamp}}$.

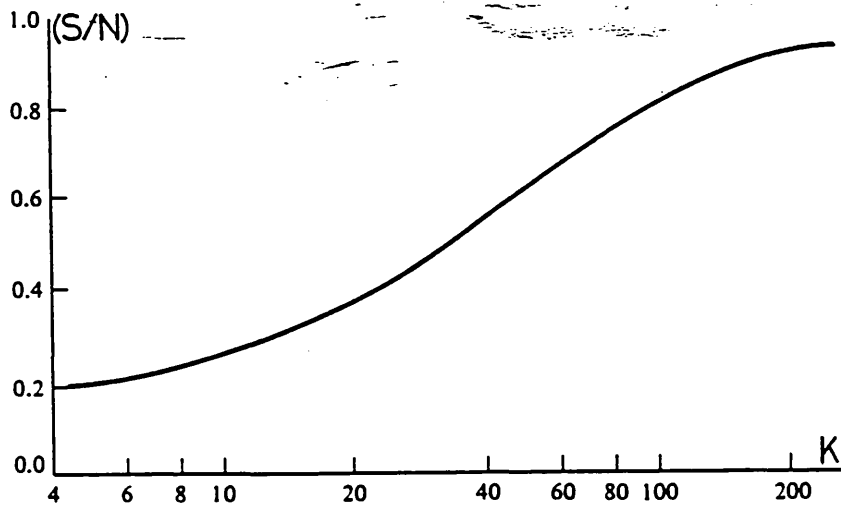


Fig 4.18 The K factors dependence of $(S/N)_{\text{diffamp}}$

Under coherent noise conditions, e.g mains 50 Hz noise, additional signal quality improvement is a function of filter attenuation in relation to the interfering frequency, as expressed in equation [4.8].

One easily confused relationship is that between the error budget of a signal conditioning unit and its output signal quality. The amplitude quality of the signal output is determined by ele-

ments that upgrade this quality and these are independent of component errors such as those contributed by the electronics. Therefore the RMS combination of both component and signal quality errors determines the overall measurement accuracy, where the former is usually dominant. This is expressed in equation [4.9] which gives the total error of the ISEIU.

Equation [4.10] gives the probability that a signal corrupted by interference is within a specified region Δa centred about the true value a . Table 4.7 presents a tabulation of equation [4.10] for 1σ (68 %) and 2σ (95 %) confidence limits, which is a good confidence limit for this application.

TABLE 4.7
The S/N confidence relationship

(S/N)/dB	$e_{FS}(1\sigma)/\%FS$	$e_{FS}(2\sigma)/\%FS$
10	44.0	88.0
20	14.0	24.0
30	4.4	8.8
40	1.4	2.8
50	0.44	0.88
60	0.14	0.28
70	0.044	0.088
80	0.014	0.024
90	0.0044	0.0088
100	0.0014	0.0028

Using equations [4.5-10] enables us to calculate the signal improvement and the total error in the ISEIU. Let us first determine the input (S/N) using equation [4.5]. We assume that the ISE EMF=100 mV and the common mode noise V_{CM} =500 mV, after the amplification V_{diff} =1000 mV.

$$(S/N)_{input} = \frac{1000}{500}^2 = 4$$

The differential amplifier 3627 has a CMRR=106 dB @ 50 Hz , inserted in equation [4.6]

$$(S/N)_{\text{diffamp}} = 4*(2*10^5)^2 = 1.5*10^{11}$$

The impedance converter and amplifier have very large frequency bandwidths. The frequency response for cascaded amplifiers is given by equation [4.12] (91)

$$BW_{\text{cascaded}} = \frac{0.35}{1.1[(0.35/f_{hi,1})^2 + \dots + (0.35/f_{hi,N})^2]^{0.5}} \quad [4.12]$$

The amplifier f_{hi} is 100 kHz and the converter f_{hi} is 1 MHz , equation [4.12] yields

$$BW = \frac{0.35}{1.1[(0.35/10^5)^2 + (0.35/10^6)^2]^{0.5}} = 90 \text{ kHz}$$

The K factor for $(S/N)=1.6*10^{11}$ is read from fig 4.18 to be 0.9. Insertion of these numbers into equation [4.7] gives

$$(S/N)_{\text{random}} = \frac{1.6*10^{11} * 0.9*9*10^4}{1} = 1.3*10^{16}$$

and for 50 Hz coherent noise equation [4.8] gives with a filter rejection of -120 dB

$$(S/N)_{\text{filter, 50 Hz}} = \frac{1.6*10^{11}}{10^{-6}} = 1.6*10^{17}$$

Using equation [4.10] for a 2σ (95 %) confidence limit with the above calculated results gives

$$P(0.95) = \text{erf}\left\{\frac{1}{2} \frac{\Delta a}{a} \sqrt{(S/N)}\right\} = 1.39$$

This enables us to calculate the (S/N) terms in equation [4.9]

$$e_{(S/N)} = \frac{2*1.39}{\sqrt{(S/N)}} \quad [4.13]$$

thus for the differential amplifier

$$e_{(S/N)\text{diffamp}} = \frac{2.78}{\sqrt{1.6*10^{11}}} = 7*10^{-6}$$

the coherent noise then yields

$$e_{(S/N)coherent} = \frac{2.78}{\sqrt{1.3 \times 10^{16}}} = 2.4 \times 10^{-8}$$

and finally we calculate the random noise factor as

$$e_{(S/N)random} = \frac{2.78}{\sqrt{1.6 \times 10^{15}}} = 7 \times 10^{-8}$$

In order to use equation [4.9] for the calculation of the total error we must calculate the amplitude error of the differential amplifier $e_{diffamp}$ and the filter e_{filter} . The error budget for the differential amplifier is given in table 4.8 and table 4.9 .

TABLE 4.8
The differential amplifier error budget

V_{OS}	100 μV	100 μV
$dV_{OS}/dT \cdot \Delta T$	10 $\mu V/C^0$	300 μV
V_n	2 μV	2 μV
Error RMS		316 μV

Table legend: Assumptions $\Delta T=30 C^0$ V_{OS} =Offset voltage
 I_{OS} =Offset current V_n =Noise voltage

TABLE 4.9
The differential amplifier gain error budget

Gain error	0.005 %	0.005 %
Gain nonlinearity	0.0005 %	0.0005 %
Gain temperaturecoefficient	0.0002 %/C ⁰	0.006 %
Error RMS		0.008 %

$\Delta T=30C^0$

The filter section uses a 747 dual operational amplifier and 0.1 % precision resistors , its error budget is given in table 4.10 and table 4.11 .

TABLE 4.10
The filter error budget

$dV_{OS}/dT*\Delta T$	$3 \mu V/C^0$	$90 \mu V$
$I_{OS}*R_s$	20 nA	0.2 nV
$dI_{OS}/dT*R_s*\Delta T$	$0.5 \text{ nA}/C^0$	0.2 nV
V_n	$10 \mu V$	$10 \mu V$
Error RMS	$91 \mu V$	

Table legend: Assumptions $\Delta T=30 \text{ } C^0$ source resistance $R_s=0.01 \text{ } \Omega$; V_{OS} =Offset voltage ; I_{OS} =Offset current
 V_n^s =Noise voltage

TABLE 4.11
The filter gain error budget

Resistance accuracy	0.1%	0.2%
Temperature coefficient	$10\text{pm}/C^0$	0.06%
Long term drift	0.05%	0.1%
Error RMS	0.23%	

$\Delta T=30C^0$

As seen throughout the error analysis two types of errors occur , dynamic relative voltage errors , and static absolute gain errors. The gain errors come from the precision amplifiers , differential amplifier and the filter. The voltage errors come from all units and are relative to the input EMF , expressed in equation [4.9]. These two errors can be RMS summed and converted to a concentration error by differentiating the Nernst equation

$$\Delta E = \frac{RT}{nF} \ln(1 + \frac{\Delta c}{c})$$

[4.14]

and the RMS voltage error is

$$\Delta E = \sqrt{0.0028^2 * E^2 + 0.18^2}$$

[4.15]

combining [4.14] and [4.15]

$$\frac{\Delta c}{c} = \exp\left(\frac{nF}{RT} \sqrt{0.0028 \cdot E^2 + 0.18^2}\right) - 1 \quad [4.16]$$

Equation [4.16] is plotted for typical ISE EMFs in figure 4.19, which shows that the error is less than 2% within the whole range, and the minimum error is 0.3% for monovalent ions (it doubles for divalent ions). This performance is within our specification for the ISEIU.

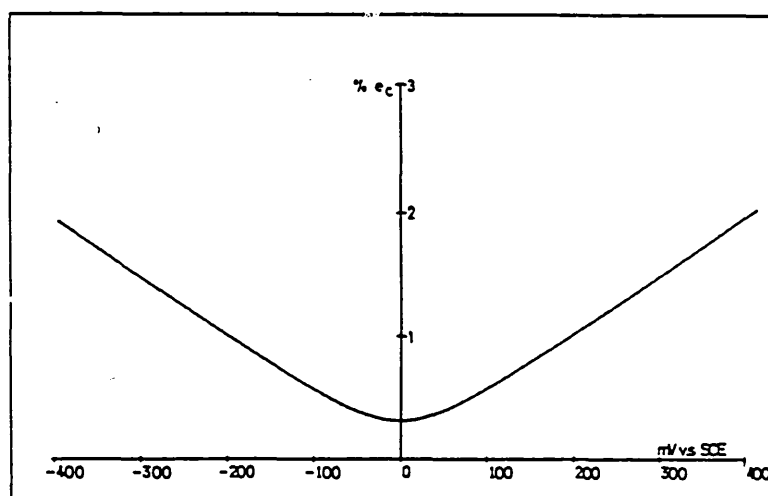


Fig 4.19 The relative concentration error for a monovalent ion plotted against the ISE EMF

4.4.5 Temperature compensation

As the temperature may vary widely in the greenhouse, temperature compensation is necessary to maintain the accuracy of the measurements. An RS 590 (Radio Spares Ltd, London) semiconductor temperature sensor was fitted in the flowcell. The RS 590 is a high impedance constant current generator passing $1 \mu\text{A/K}^\circ$ and is factory trimmed to high accuracy and linearity. The associated electronics, fig 4.20, were housed in the ISEIU so the temperature sensor could be multiplexed. The current to voltage converter, op-amp 2 in fig 4.20, has a gain of 10^4 , thus giving a resolution of 10 mV/K° .

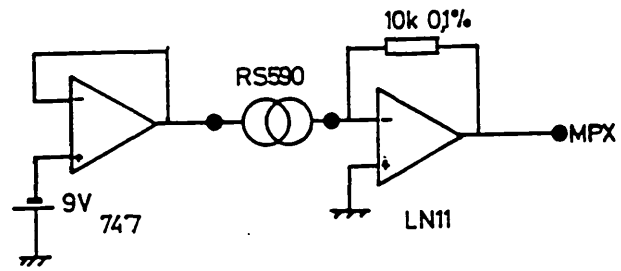


Fig 4.20 Temperature sensor electronics circuit diagram

4.4.6 50 Hz trigger

If an integrating ADC is used , as described in chapter 3 , and is set to integrate over a multiple of the mains period , extremely good mains noise rejection is achieved. The ISEIU contains a unit that sends out pulses at the mains zero crossing (fig 4.21) , consisting of an opto-coupled zero crossing detector , IC 1 , and associated logic chips for pulse generation.

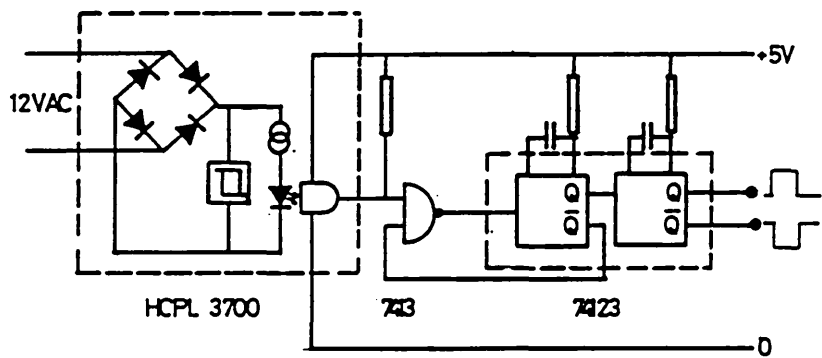


Fig 4.21 The 50 Hz trigger unit

4.4.7 Power supply

The ISEIU uses a high quality power supply unit. The voltage is regulated by two monolithic semiconductor fixed voltage regulators , the 7915 and 7815 (Radio Spares Ltd , London) providing ± 15 V with good line and load regulation. It also boasts good ripple rejection and low noise voltages.

4.5 The ADC

The conditioned ISE EMF must be converted to a digital signal with preserved accuracy and signal to noise ratio. The standard ADC board for the CMU interface uses a 12-bit converter with a ± 10 V range. This means that the maximum resolution of the digital signal is $20000/2^{12}=4.88$ mV/bit. The ISEIU gain is 10 so the overall resolution is 0.488 mV/bit. This corresponds to an accuracy of about 2 % in concentration. Our aim was to design a system with an overall error and accuracy much better than the sensor accuracy of about 2 %. Therefore a new ADC had to be constructed.

Voltage to frequency converters (VFCs) have some attractive features for this design. Referring to table 3.3 we summarise

- 1) High resolution
- 2) Large dynamic range
- 3) Good noise rejection

A VFC board was built around a Burr-Brown VFC 32 and two counters, fig 4.22, one counting the pulses from the VFC and the other counting the pulses from the mains zero crossing unit for the integration timing. Fifty pulses from the zero-crossing unit are counted, giving a one second integration time. This means in effect that the VFC is a low pass averaging filter with a cut-off frequency of 1 Hz, which also improves the signal quality. An extra off-set voltage was necessary for bipolar operation, supplied by a very stable band-gap diode. The extra logic chips deal with the interface control. Hence with a one second integration time and a ± 5 V range, together with a maximum pulse frequency of 50 kHz we obtain a resolution of $10000\text{mV}/50000$ pulses = 0.2 mV/pulse. The ISEIU gain is

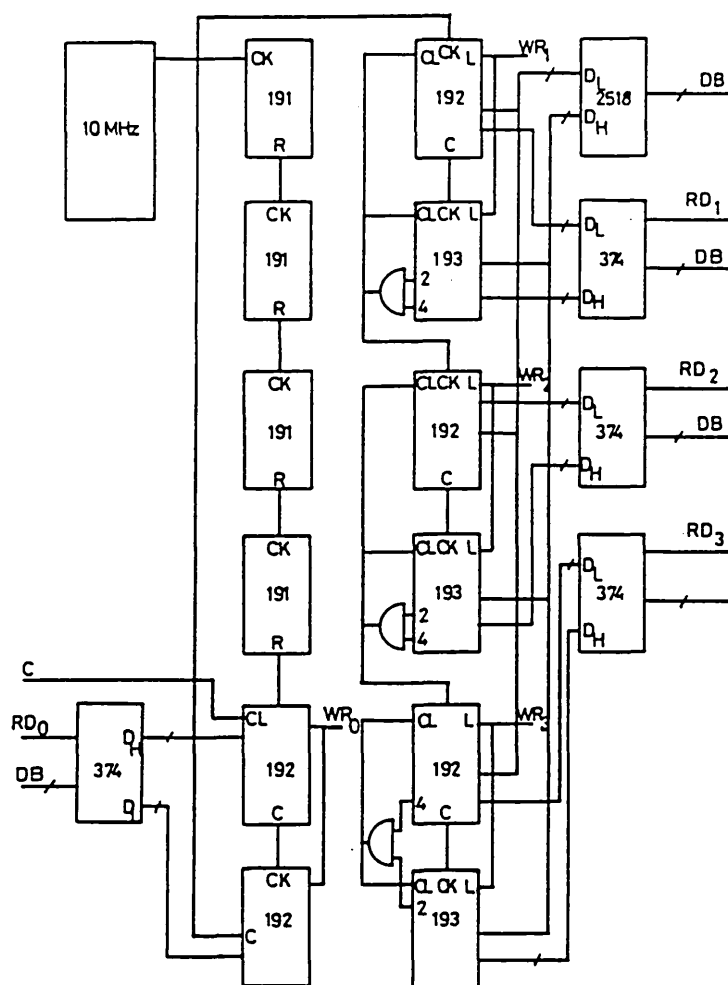


Fig 4.23 The real time clock circuit

4.7 Mains conditioning

Power line disturbances are a major cause of malfunctions in computers and other sensitive equipment. Two IBM research workers measured power line conditions at 49 different locations over several years. Using pre-established thresholds, which corresponded to levels at which interference in most computers would be noted they found the average monthly occurrence rate to be 128.3 times. 89 % of these disturbances were decaying oscillatory transients and spikes. These two phenomena derive from noise on the power line, and may occur either as common mode noise or as transverse mode noise, fig 4.24, depending on the source of the noise and the

transmission method. Common mode noise occurs between the earth and both the live and neutral conductors whereas transverse mode noise occurs between the live and the neutral conductors

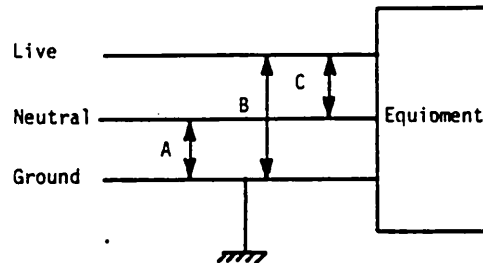


Fig 4.24 Power line noise types

Common mode noise creates serious problems for computer equipment which uses the earth as a reference point for its logic circuitry. Therefore protection against this noise is essential for trouble free operation.

We used a 1000 VA isolation transformer giving a 100-140 dB rejection of common mode noise and 30-40 dB rejection of transverse mode noise in addition to a high overload capacity. The isolation transformer was followed by a mains voltage regulator capable of $\pm 3\%$ voltage regulation, 30 dB of common mode noise rejection and 50 dB of transverse mode noise rejection. These precautions have ensured a trouble free operation of the system in the greenhouse environment.

4.8 Software

4.8.1 Aim

The Autoanalyser will be used by personnel unfamiliar with both electroanalytical and computer techniques. This demands a very user friendly system , i.e as little user interaction as possible. The operator should merely have to start up the system , after that it should run automatically with little or no maintenance. In the data acquisition mode , the data should be able to be analysed during a run , and be displayed easily.

4.8.2 Problem Definition

The user needs a program that ;

- 1)Converts the EMF from 1 up to 7 ISEs to ppm
- 2)Compensates for temperature changes
- 3)Quality controls the data
- 4)Calibrates the ISEs
- 5)Checks the condition of the ISEs
- 6)Acquires the data in real time
- 7)Runs automatically

4.8.3 Choice of Solution

The program consists of three parts (fig 4.25) . The first part sets up parameters and goes through a check list with the user. The main program controls the overall timing , and calls the appropriate subroutines. Finally the subroutine part provides the main program with the requested service.

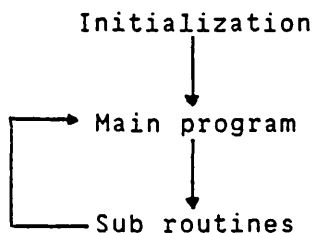


Fig 4.25 Autoanalyser software structure

4.8.4 Program Building

The program was written in Research Machines Extended BASIC , to which we have patched in the CMU interface machine code routines. The program was built in blocks which were tested separately , then merged into the final monolith. The final program is 26 kbyte , together with 26 kbyte BASIC interpreter and 5 kbyte 380 Z operating system , we have actually exceeded the available 56 kbyte of memory. This was overcome by running the program through a compressing program , SQUASH.BAS (81), which compressed the program to 15 kbyte , leaving us with 10 kbyte of scratch-pad memory

4.8.5 Documentation

The program was written to be self-documenting , so that a person with minimal knowledge of BASIC should be able to read and understand it. An autotitrator manual was written for the personnel at NIAE.

4.9 The Autoanalyser Program

The complete autotitrator program is listed in appendix II. The program , which follows the structure outlined in 4.8.3 , is described in this section.

4.9.1 Initialization

The user enters his name , date and time (starts up the real time clock) for the protocol , and selects whether to store the results on disc , hardcopy , or both. He then goes through a check-list under control of the computer , which tells him what to do in reply to his answers. The following items are checked;

<u>User question</u>	<u>Computer response</u>
Volume of calibration solution?	When more is required
Soleniod valves OK ?	Check connections
Tubing OK?	Check with instructions
Pump OK?	Check connections
Flowcell filled ?	Fill up cell
Flow from glasshouse OK ?	Check tubing and filter
Discs and discdrives OK ?	Available disc space
Electrode configuration ?	Enter new configuration
Calibration solutions ?	Enter new solutions
Error limits ?	Enter new error limits
Minimal response slope ?	Enter new minimum

After the check list a calibration follows in order to set all the system parameters before the first measurement. A typical start up protocol is shown in fig 4.26.

LRS ISE DATA ACQUISITION PROGRAM	
DATE: 28 / 7 / 83	TIME: 17 : 27
OPERATOR: ROB S	DATAFILE: JULY28.DAT
CHECK LIST	
LO CALIBRATION SOLUTION FOR 30 CALIBRATIONS OR 1 DAYS HI CALIBRATION SOLUTION FOR 25 CALIBRATIONS OR 12 DAYS SOLENOID VALVES OK TUBING OK PUMP OK FLOW CELL OK FLOW FROM GREEN HOUSE OK DATA DISCS IN DRIVES, WITH 857 KBYTE OF FREE MEMORY DISC SPACE FOR 6856 MEASUREMENTS OR 285 DAYS PRINTER PAPER OK ELECTRODE POSITIONS: Ca(1), pH(2), Na(3), NO3(4), Cl(5), K(6) STANDARD CALIBRATION SOLUTIONS STANDARD ERROR LIMITS	
CALIBRATION STARTED: 28 / 7 / 83 AT 17 : 29	
ISE	HIGH EMF(mV)
TEMP	302.118
Ca	-293.719
pH	-147.33
Na	-114.471
NO3	-84.4555
Cl	-18.2176
K	-98.727
ISE	LOW EMF(mV)
TEMP	301.259
Ca	-354.316
pH	-87.4778
Na	-65.4513
NO3	-144.456

MEASUREMENT STARTED 28 / 7 / 83 AT 21 : 16	
ION	ppm
Ca	114
pH	5.87
Na	181
NO3	470
Cl	127
K	56
MEASUREMENT STARTED 28 / 7 / 83 AT 22 : 16	
ION	ppm
Ca	188
pH	5.86
Na	183
NO3	475
Cl	123
K	55
MEASUREMENT STARTED 28 / 7 / 83 AT 23 : 16	
ION	ppm
Ca	294
pH	5.86
Na	181
NO3	478
Cl	182
K	53
ERROR REPORT Ca CALIBRATION ERROR, CALIBRATION OUTSIDE ERROR LIMIT Ca EB= 323.191 mV AVERAGE=-349.844 mV	
MEASUREMENT STARTED 29 / 7 / 83 AT 8 : 16	

Fig 4.26 a) Start up protocol b) Measurement print out

4.9.2 Main Program

The main program, whose flow chart is shown in fig 4.27, controls the timing of the measurements and the calibrations. Calibrations take place at 6.00 and 18.00 daily, and have the highest priority, i.e. any measurements that should take place then are abandoned in favour of a calibration.

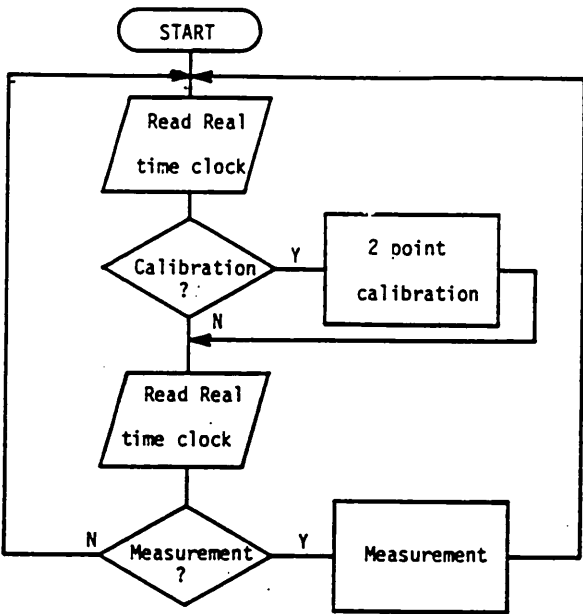


Fig 4.27 The timing flow chart of the main program

4.9.3 Subroutines

There are two head subroutines ; CALIBRATION and MEASUREMENT , which call a number of common subroutines ,illustrated in fig 4.28.

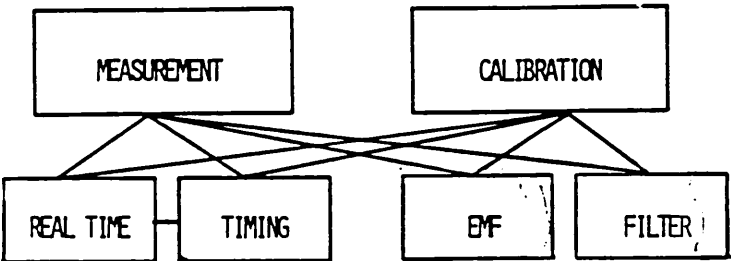


Fig 4.28 Subroutine structure

4.9.3.1 CALIBRATION Subroutine

The flow chart for the CALIBRATION subroutine is shown in fig 4.29a. The measured EMFs , temperatures , calibration solution concentrations and activities are stored in arrays and are put sequentially into the temperature compensated Nicolsky-Eisenmann equation. The resulting slopes and E^0 's are quality controlled in a subroutine called FILTER and stored in an array. If an ISE calibration fails

the tests in FILTER an error report is printed and the calibration is repeated. If the ISE fails the second calibration another error report is printed , and the program proceeds to the measurements. The user is made aware that the electrode is malfunctioning in the printed error report.

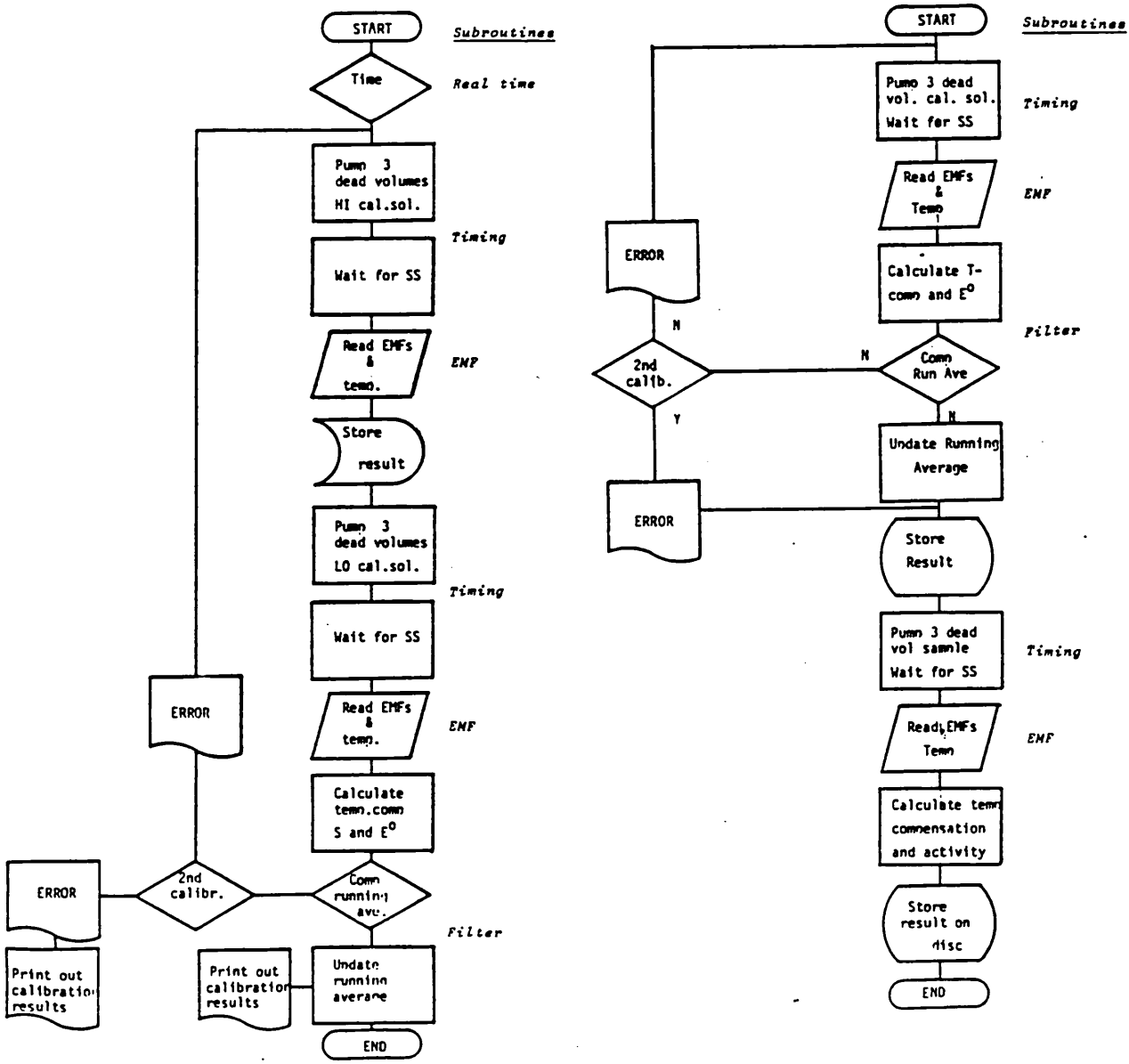


Fig 4.29 a)The CALIBRATION subroutine flow chart
b)The MEASUREMENT subroutine flow chart

4.9.3.2 MEASUREMENT Subroutine

The flow chart of the MEASUREMENT is shown in fig 4.29b. A 1-point calibration is made before each measurement in order to establish E^0 . Our experience is that the response slope is adequately stable over a 12 h period, but the E^0 drifts significantly over short periods. The temperature compensated E^0 is quality controlled in FILTER and if it fails another 1-point calibration is made, and an error report is printed. In the measuring section the ISE's EMFs and the temperature are measured and put in the Nicolsky-Eisenmann equation. The concentrations are converted to ppm, and the results stored on disc and/or printed out.

4.9.3.3 The Timing Subroutine

This program controls the pumping time, so with a controlled flowrate, a known volume of solution is pumped to the flow-cell. Three dead volumes are pumped in order to flush out all old solution in the flow-cell. The measurement takes place in a still solution in order to avoid all pump interference. The responses of the liquid ion exchange membrane electrodes are sluggish, up to 10 min are necessary for these electrodes to reach a steady state in a still solution. Therefore, a ten minute delay is timed by the routine before each measurement.

4.9.3.4 The EMF Routine

This program section deals with the analog-to-digital conversion. The count from the Voltage-to-Frequency converter is read and is put in this transfer function;

$$EMF = (COUNT - OFFSET) / 51.87 \quad [4.17]$$

The offset voltage (for bipolar operation) comes from a precision

voltage source , but due to the wide temperature variation the offset is calibrated before each measurement. Channel number 8 has a dummy cell connected to the input , and the resulting count from this channel is used for the OFFSET. Almost all offset errors are therefore eliminated , if make the reasonable assumption that the other channels are similar to channel 8. The VFC response slope remains adequately stable over the temperature range.

4.9.3.5 The FILTER Subroutine

This subroutine performs the quality control of the data and checks the state of the electrodes , its flow chart is shown in fig 4.30. Three checks are made;

- 1) E^0 v.s running average
- 2) Slope v.s running average
- 3) Slope v.s minimum acceptable slope

Our initial approach was a 3-point running average , from which a 3σ limit was calculated. If the result was outside this 3σ window it failed and the system was recalibrated. A problem with this dynamic error window was that it narrowed down to very small values if three similar values were acquired. In one particular case it narrowed down to 0.1 mV which produced error reports for all subsequent data. One may either update the running average with the erroneous data , which could completely corrupt the running average, or use a static error window. We settled for the latter , whose value was found by looking at the data produced by 100 calibrations. A 10 mV window was chosen for the slope and a 100 mV window for the E^0 . Finally , the actual response slope value is checked against a minimum acceptable value. Some of the electrodes work satisfactorily

under sub-Nernstian conditions , but slopes less than 40 mV/dec were not considered to be of any use.

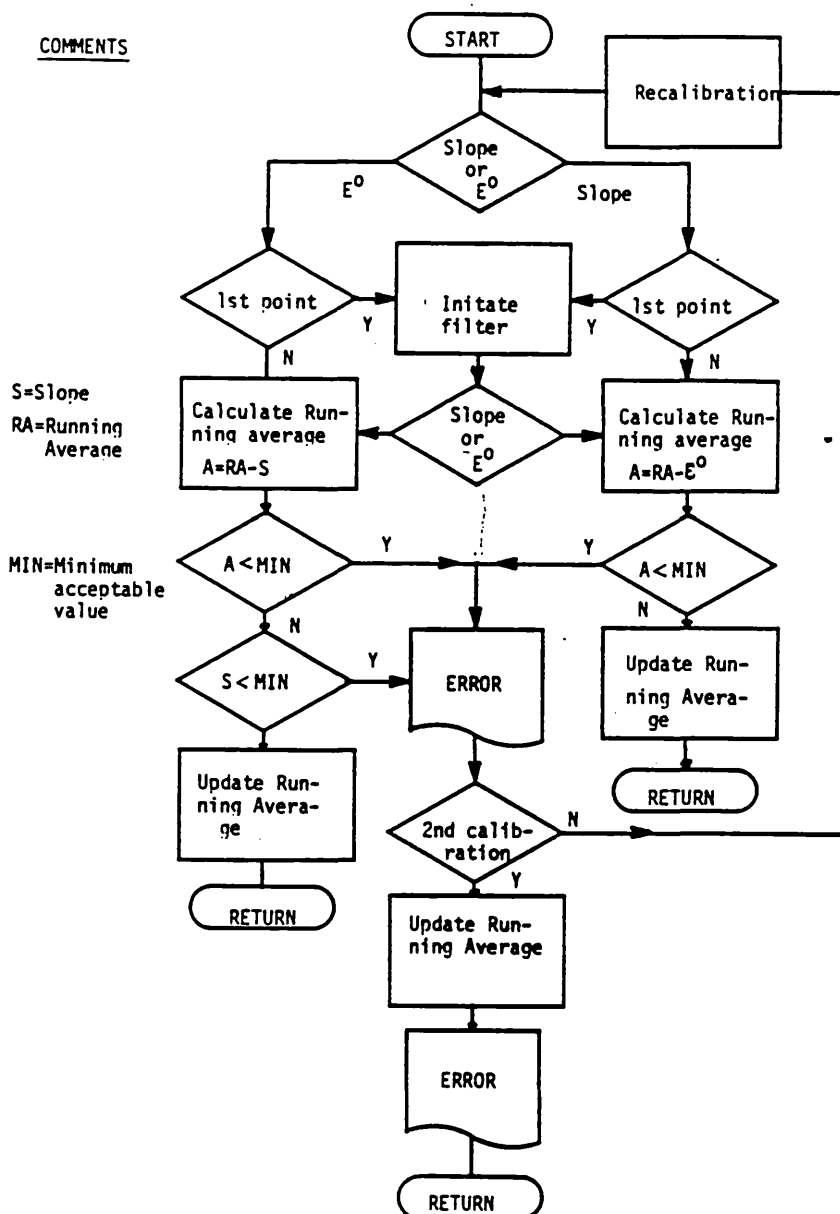


Fig 4.30 The FILTER subroutine flowchart

4.10 Results

4.10.1 Laboratory Tests

The ISEIU III was tested with an ISE dummy cell , illustrated in fig 4.31. The output was recorded on a Kontron W+W 316 chart recorder for 9 hours , the recorded drift was less than 0.25 mV and

the noise less than 0.1 mV over that period. These figures are within the specification of the recorder so the noise and the drift could just as well be attributed to the chart recorder. These ISEIU III noise and drift figures are within our specifications.

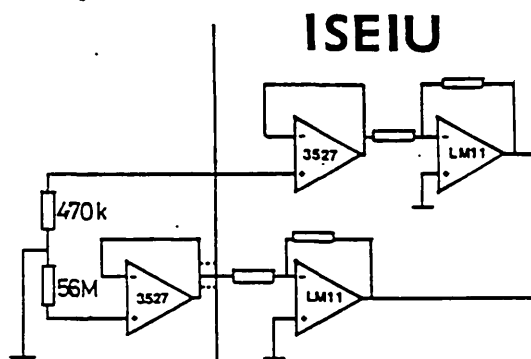


Fig 4.31 The ISE dummy cell

4.10.2 ISE Flowcell Test

The total performance of the system was bench tested by running the Autoanalyser for nine hours with the HI calibration solution as the sample solution. The result is summarised in table 4.12

TABLE 4.12
ISEIU III bench test results

ISE	a/mM	[HI]/mM	γ	a_{HI}/mM	Rel.err/%
Ca ²⁺	6.8±1.2	10.0±0.5	0.57±0.03	5.7±0.04	16.2±1.2
Na ⁺	9.2±0.23	10.0±0.5	0.85±0.04	8.5±0.6	7.7±0.6
NO ₃ ⁻	18.7±2.2	20.0±1	0.853±0.04	17.06±1.17	8.8±1.2
Cl ⁻	8.93±0.24	10.0±0.5	0.853±0.04	8.53±0.58	4.5±0.63
K ⁺	10.2±1.4	10±0.05	0.85±0.04	8.5±0.6	16.7±2.6
pH	4.49±0.03	4.50±0.02	0.90±0.05	4.55±0.02	1.30±0.04

γ values from manufacturers manual

The errors observed are somewhat higher than expected, but the auto analyser is still sufficiently accurate for adequate nutrient data acquisition and control. The errors come mainly from inaccuracy

cies in calibration solutions and the uncertainties in the activity coefficients.

4.10.3 Field Trials

The Autoanalyser was field tested at the National Institute of Agricultural Engineering (NAIE) , Silsoe , Bedfordshire on an NFT glasshouse tomato crop which is used for experimental purposes.

The first trial took place in March 1983 , and was an initial "debugging" trial in which we hoped to find as many system flaws and errors as possible. The outcome was very successful from that point of view , as a result the following amendments were made;

- 1) Zig-zag flowcell was abandoned in favour of a straight channel one
- 2) Software rewritten with a more user-friendly approach
- 3) New multiplexer and ADC
- 4) Mains voltage stabilization
- 5) Quality control routines

The next trial took place in July 1983 , but had to be abandoned at an early stage because the reference electrode was broken and no substitute was available. Trials were resumed after a replacement was bought in August 1983. The Autoanalyser was initially placed in the glasshouse , but as temperatures up to +45 C⁰ were recorded in the glasshouse the disc drive ceased to work properly. The disc drive unit is the weakest link in the Autoanalyser chain , and unfortunately only very expensive units have wide operating temperature ranges. Since the disc drives are not used when the Autoanalyser is employed for control , it may be relocated to the glasshouse for this purpose. The failing disc drives forced us to

move to a laboratory in the vicinity of the glasshouse. NFT solution from the glasshouse was pumped to the laboratory via a by-pass loop, which introduced a delay in the measurement, but this delay can be calculated and compensated for. The flow rate from the glasshouse was $V_f = 3$ l/min, and the holding volume of the sample tank in the laboratory was $V_t = 12$ l and that of the pipeline was $V_p = 2$ l. The transport dead time in the pipeline is then $t_p = 2/3 = 0.66$ min and the delay time in the tank can be modelled as in fig 4.32.

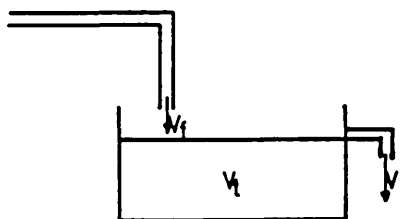


Fig 4.32 Model of the sample tank time lag

The change in concentration in the tank can then be written as

$$\frac{dC}{dt} = \frac{V_f}{V_t} C \quad [4.18]$$

where C is the concentration of any species in the tank. Separating and integrating gives

$$C = C_0 e^{V_f/V_t (t-t_0)} \quad [4.19]$$

where C_0 is the concentration at $t=t_0$ and C is the concentration at $t=t$. Equation [4.19] is the same as for an exponential decay or transient with a response time of $V_t/V_f = 4$ min. This response time together with the transport dead time is insignificant in the control or data acquisition as concentration changes take place on an hourly time scale.

The first data acquisition run took place between 1 and 4 August 1983 just after an old NFT solution had been drained away and replaced with new solution. In the first run the pH was controlled

but no control of nutrients was executed. The results for Na^+ , K^+ , Ca^{2+} , pH, Cl^- and NO_3^- are plotted in fig 4.33-38. It is interesting to note that the sodium level follows the glasshouse temperature. Fig 4.39 shows the sodium concentration and the temperature plotted on the same time scale, where the vertical lines indicate day and night. This pattern is expected since the plants transpire more when the temperature goes up, and more mains water is pumped in to compensate for this loss. The mains water contains sodium which is accumulated in the system. This excess sodium is taken up by the plants during the night. Figure 4.34 shows clearly that potassium control is very important. The potassium level has decayed to almost zero in about 60 hours. In contrast to sodium, potassium does not show any temperature dependence because its concentration in the mains water is very small. Figure 4.35 shows how calcium builds up in the system. This is also a result of the mains water input. Figure 4.36 shows the efficiency of the pH controller. It is capable of controlling the pH within one pH unit.

The next set of data was acquired over a 7 day period stretching from 8th to 15th of August 1983. The results are plotted in fig 4.40-45. The first thing we notice in fig 4.40 is that the sodium level has gone up from about 5 ppm to about 90 ppm. These measurements took place during a very hot period in August 1983, the glasshouse temperature is recorded in fig 4.46, which means that large amounts of mains water had to be pumped in in order to compensate for the water loss by transpiration and evaporation. The mains water contains about 6 ppm sodium, 50 ppm chloride and has a $\text{pH} \approx 6.3$. Therefore a chloride and sodium accumulation is expected, and this is seen in figures 4.40 and 4.44.

An interesting phenomenon is the peaks and troughs in the nitrate , chloride , pH and sodium concentrations. Fig 4.33 , 4.36 , 4.40 and 4.43 indicate an interaction between sodium and pH; the proton concentration increases to a maximum , at that point the sodium concentration quickly builds up to a peak. At the same time the nitrate goes to a minimum (fig 4.38) , followed by a chloride peak (fig 4.37) and a re-establishment of the previous concentrations. This indicates that the plants reject protons and sodium under certain conditions. The nitrate and chloride peaks suggest a rejection of chloride when nitrate uptake is fast. However these are preliminary conclusions and more work is required before definitive conclusions can be drawn about the consumption of nutrients in the NFT system. Further studies are in progress at NIAE .

Meanwhile these results show that the Autoanalyser can automatically monitor six different ions that are important in plant nutrition studies , NFT system modelling and control.

4.11 Conclusions

The results from our tests at NIAE shows that nutrient control is very important in NFT; some nutrients are consumed very fast and some species , mainly from the mains water , build up quickly and may harm the plants. Since this work was completed the Autoanalyser has been used routinely over a matter of weeks at NIAE, and reliable results have been obtained for NFT analysis and control.

The Autoanalyser therefore opens up a new field in plant nutrition studies. The plant physiologists may now acquire information from six different nutrients simultaneously and continuously.

The results from these studies will lead to the formulation of better control algorithms for NFT growing and a better understanding of the plants responses to changes in the environment. Such studies are currently being carried out at NIAE with the Autoanalyser constructed in this work.

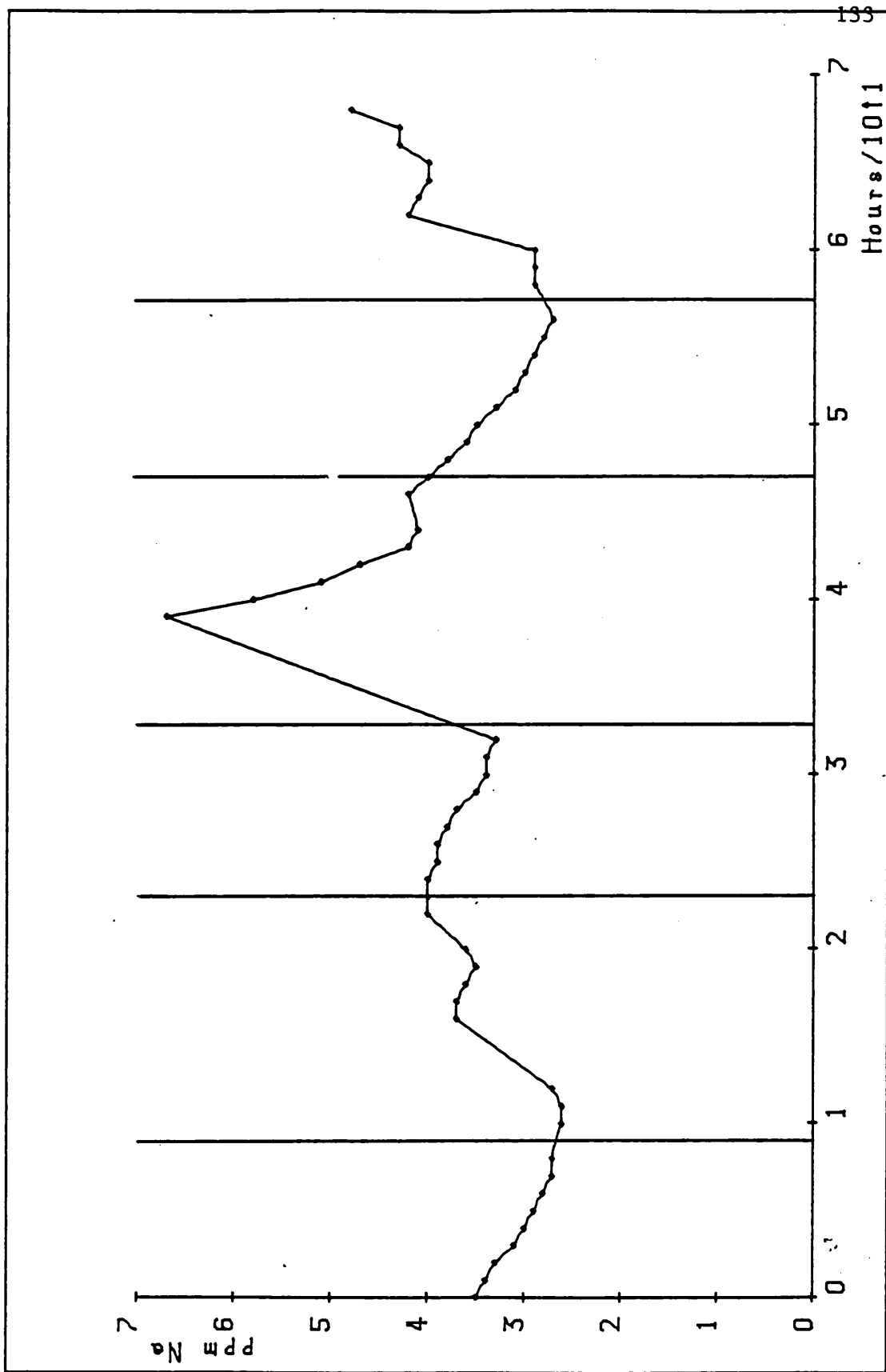


Fig 4.33 The Sodium variation 1-4 Aug 1983 (no nutrient control)

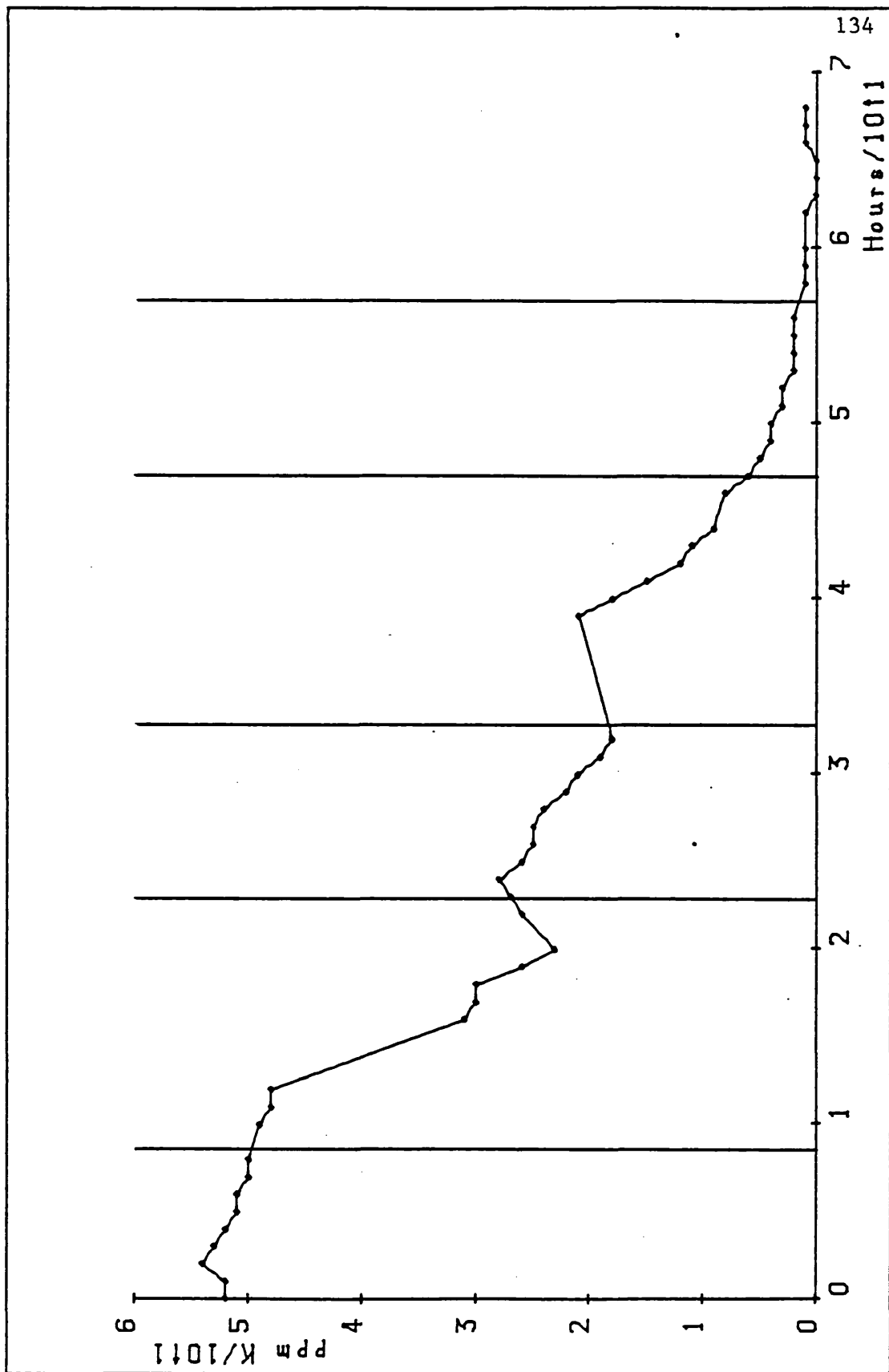


Fig 4.34 The Potassium variation 1-4 Aug 1983 (no nutrient control)

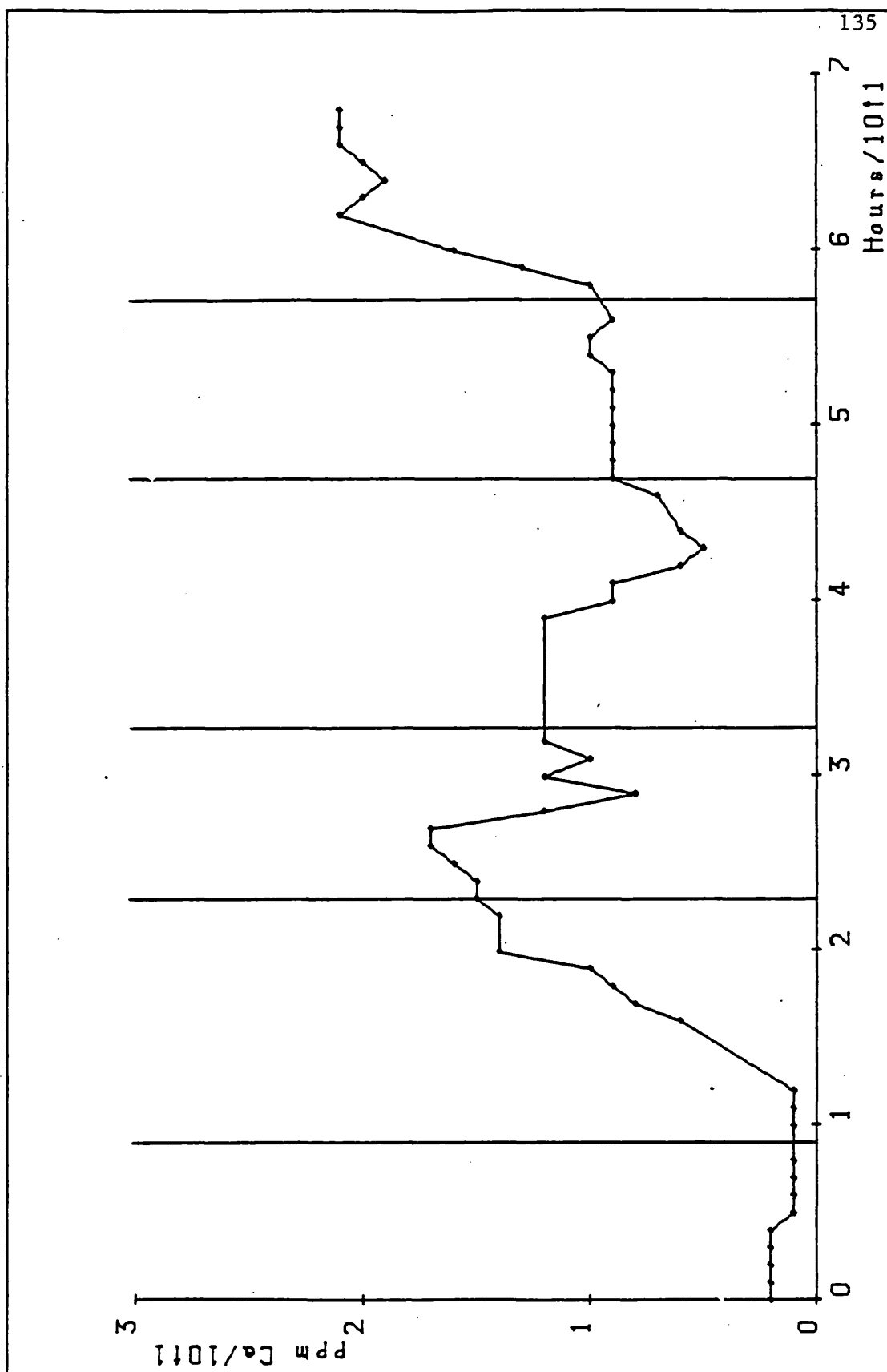


Fig4.35 The Calcium variation 1-4 Aug 1983 (no nutrient control)

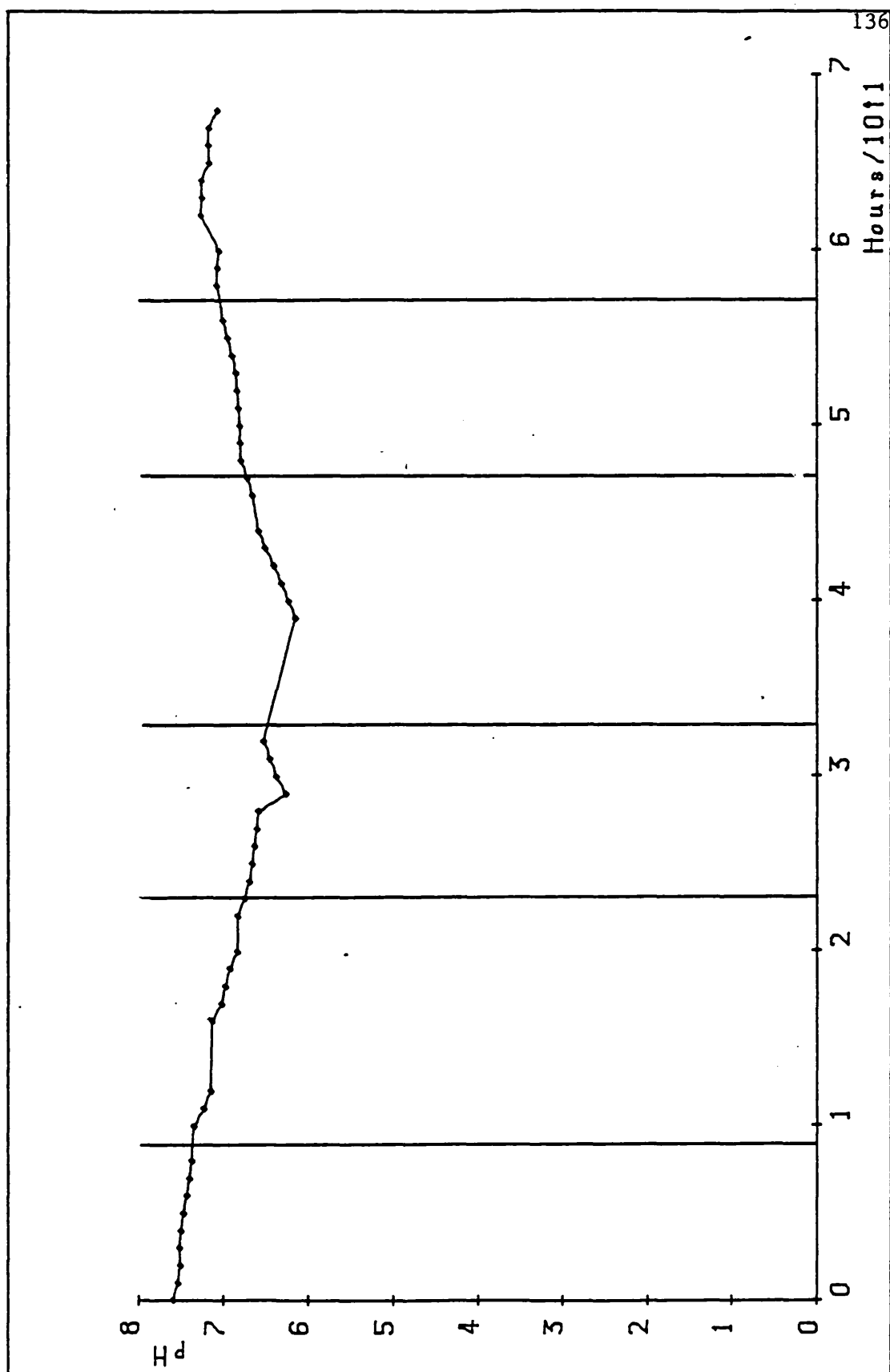


Fig 4.36 The pH variation 1-4 Aug 1983 (pH controlled)

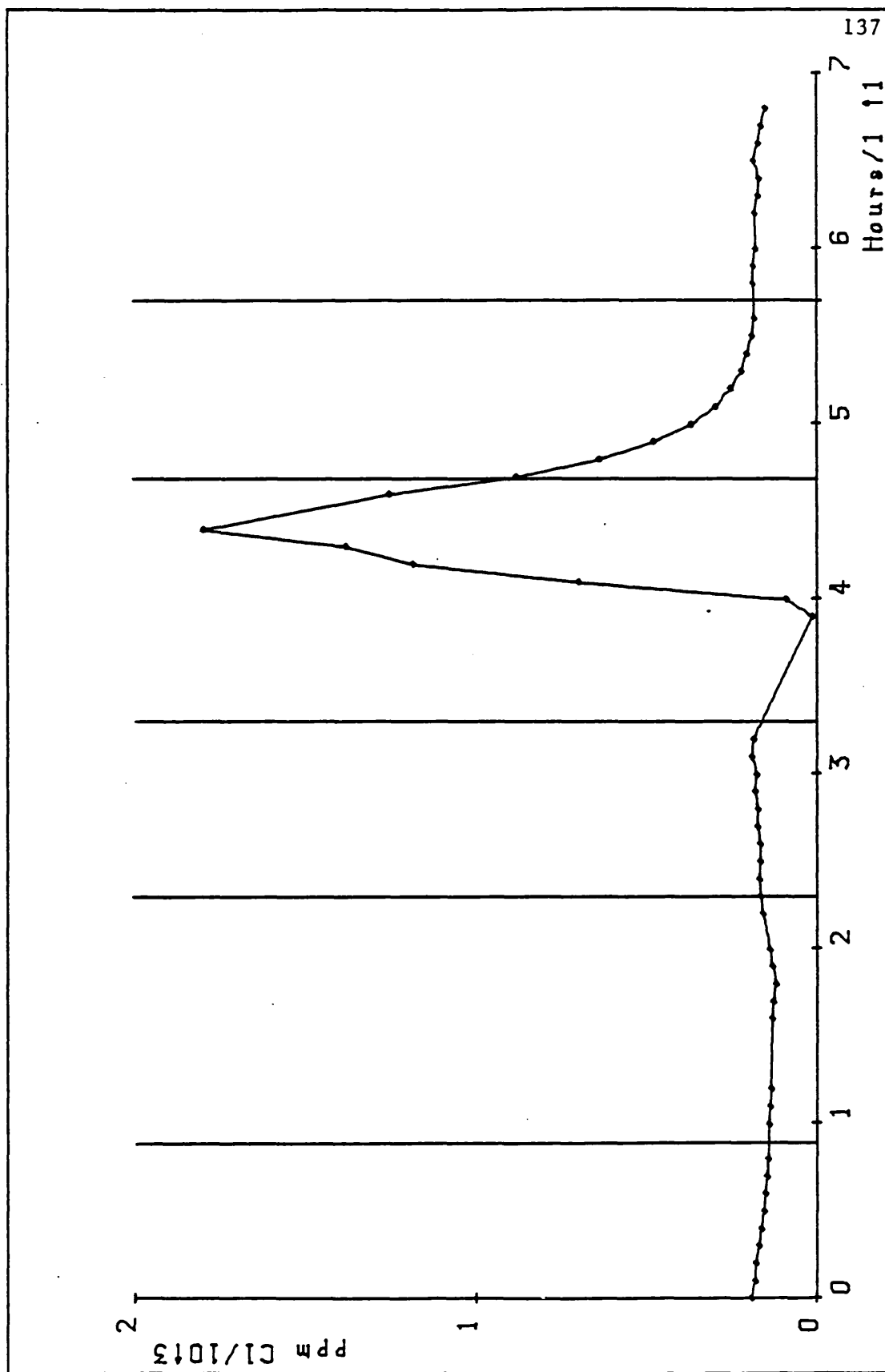


Fig 4.37 The Chloride variation 1-4 Aug 1983 (no nutrient control)

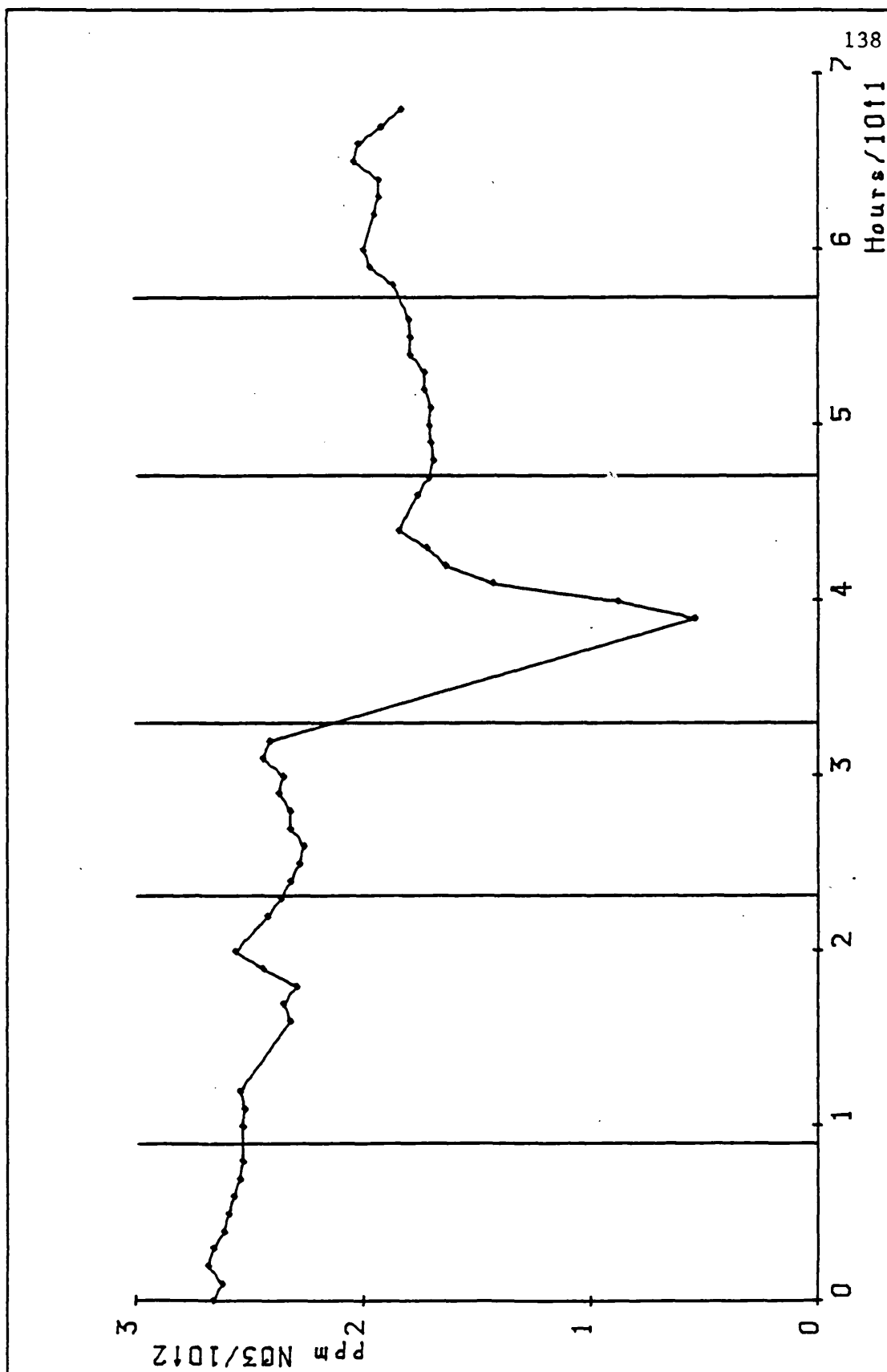


Fig 4.38 The Nitrate variation 1-4 Aug 1983 (no nutrient control)

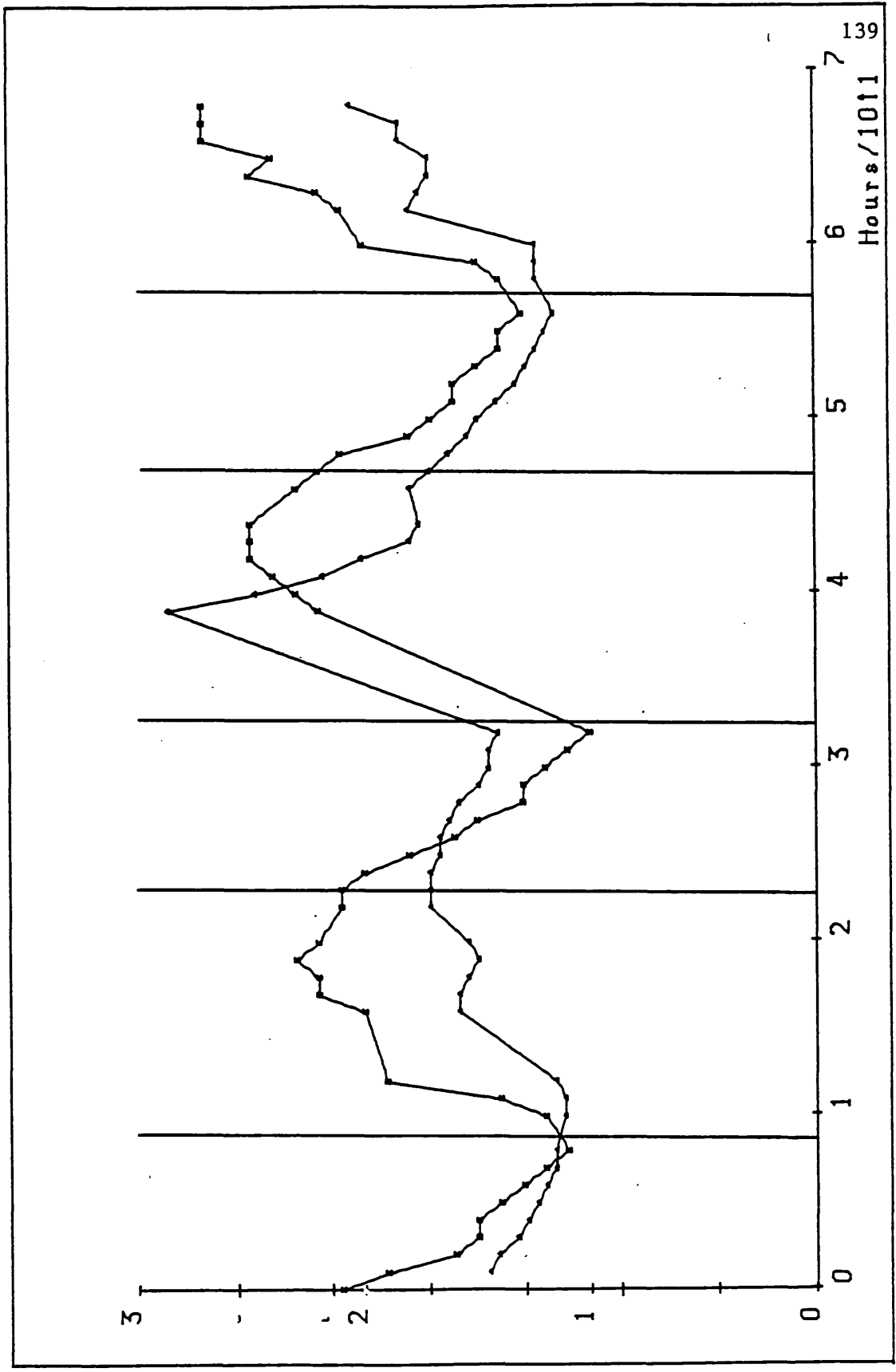


Fig 4.39 The temperature and Sodium variation 1-4 Aug 1983

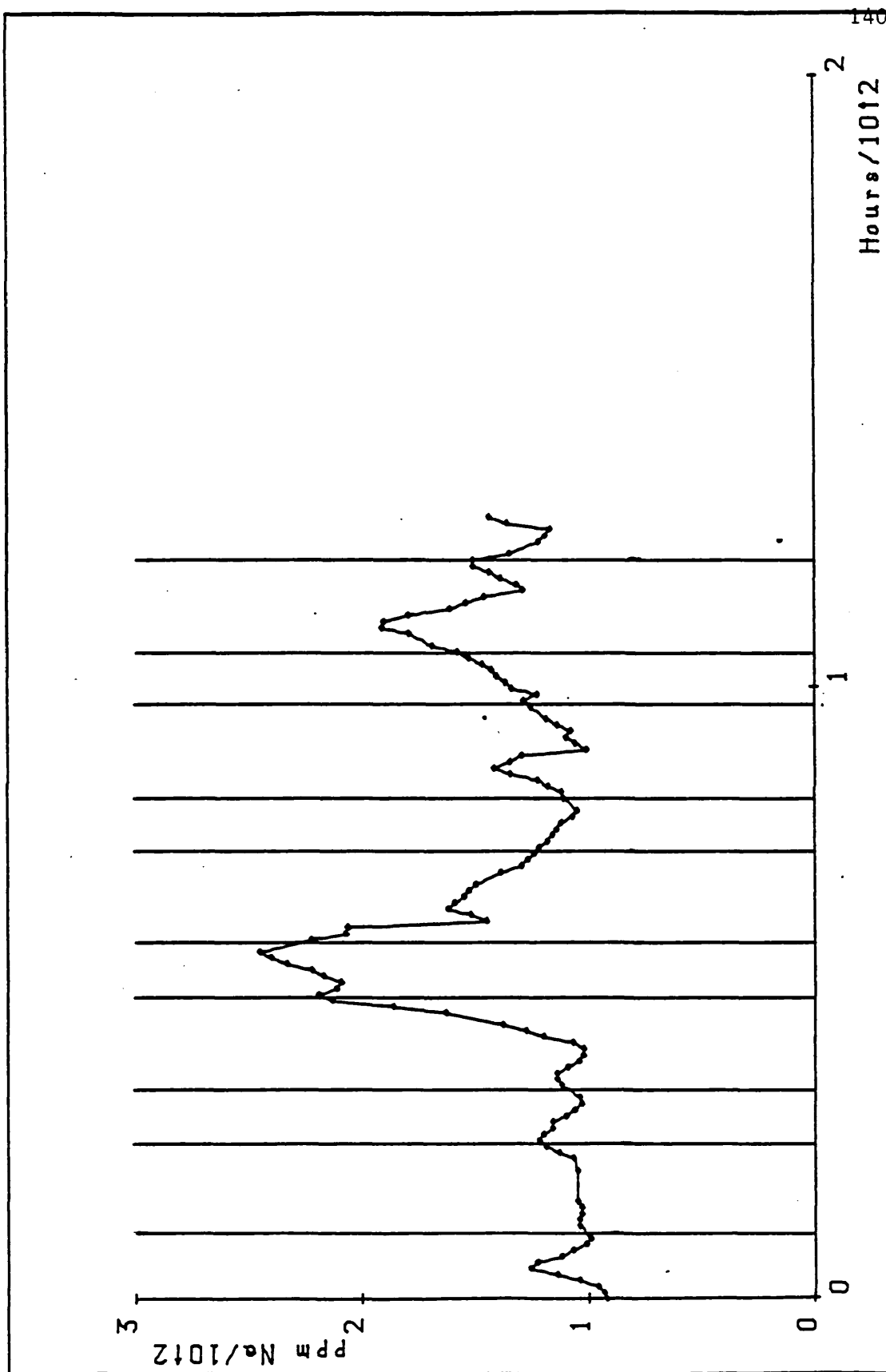


Fig 4.40 The Sodium variation 8-15 Aug 1983

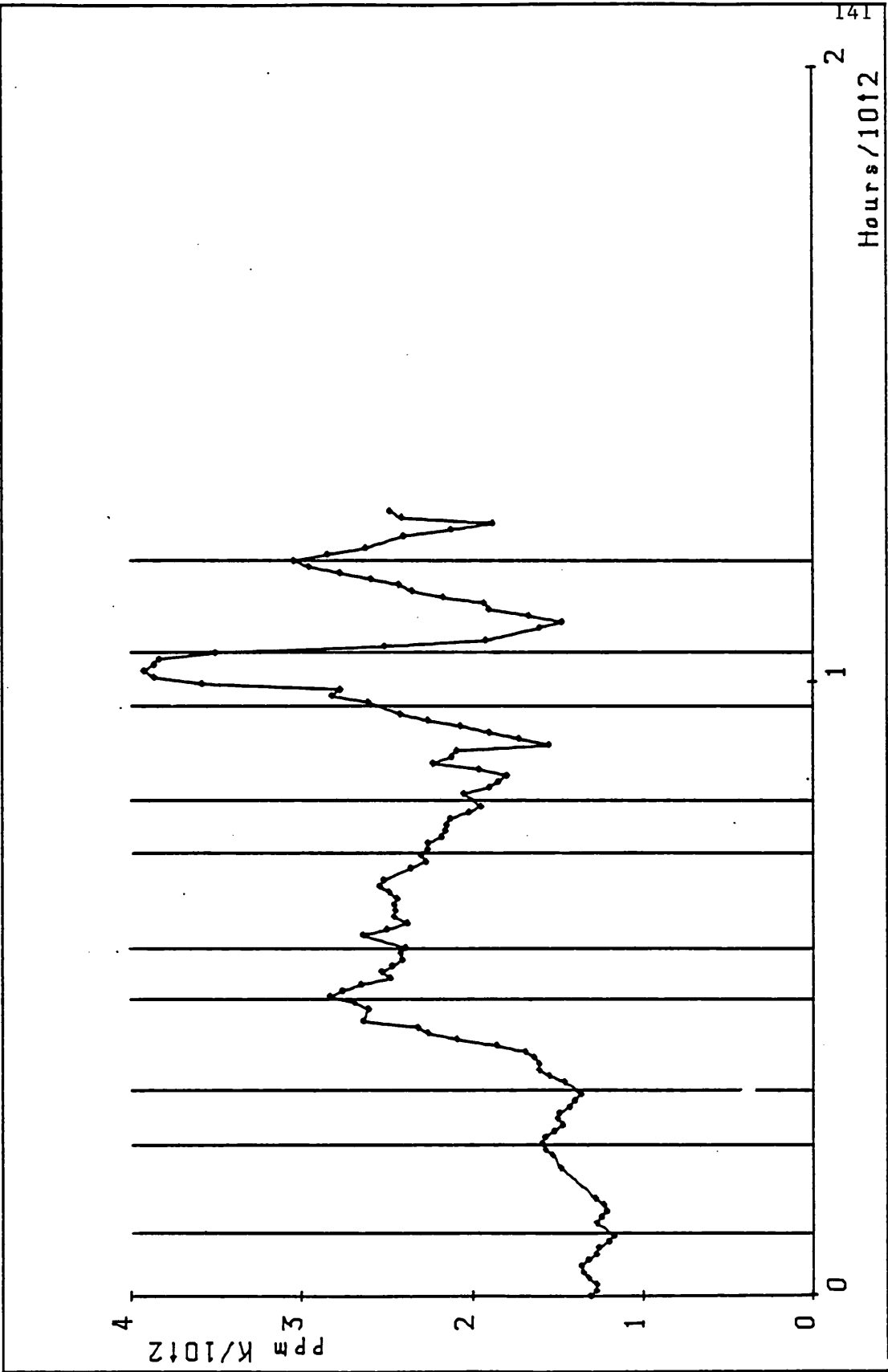


Fig 4.41 The Potassium variation 8-15 Aug 1983

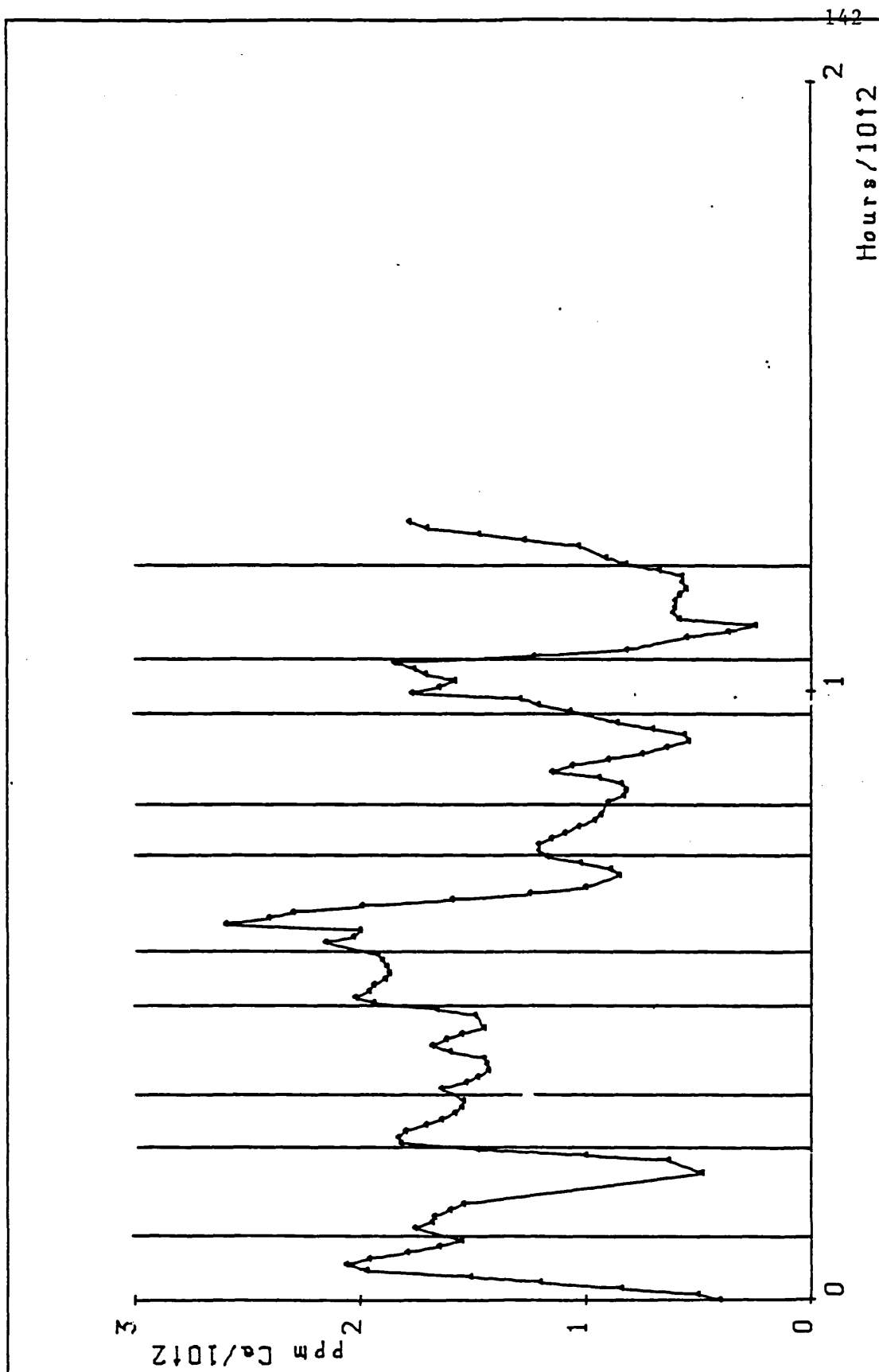


Fig 4.42 The Calcium variation 8-15 Aug 1983

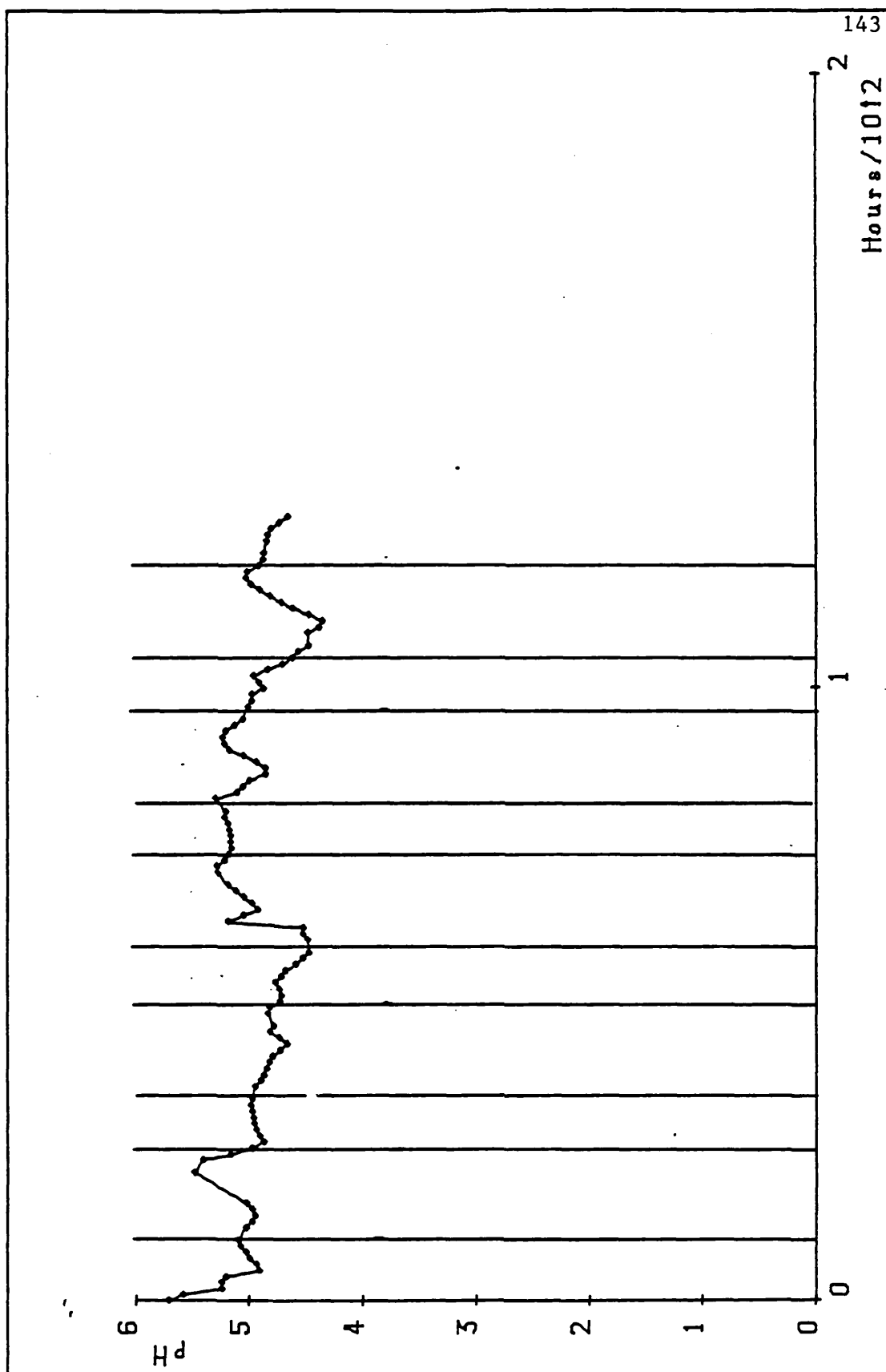


Fig 4.43 The pH variation 8-15 Aug 1983

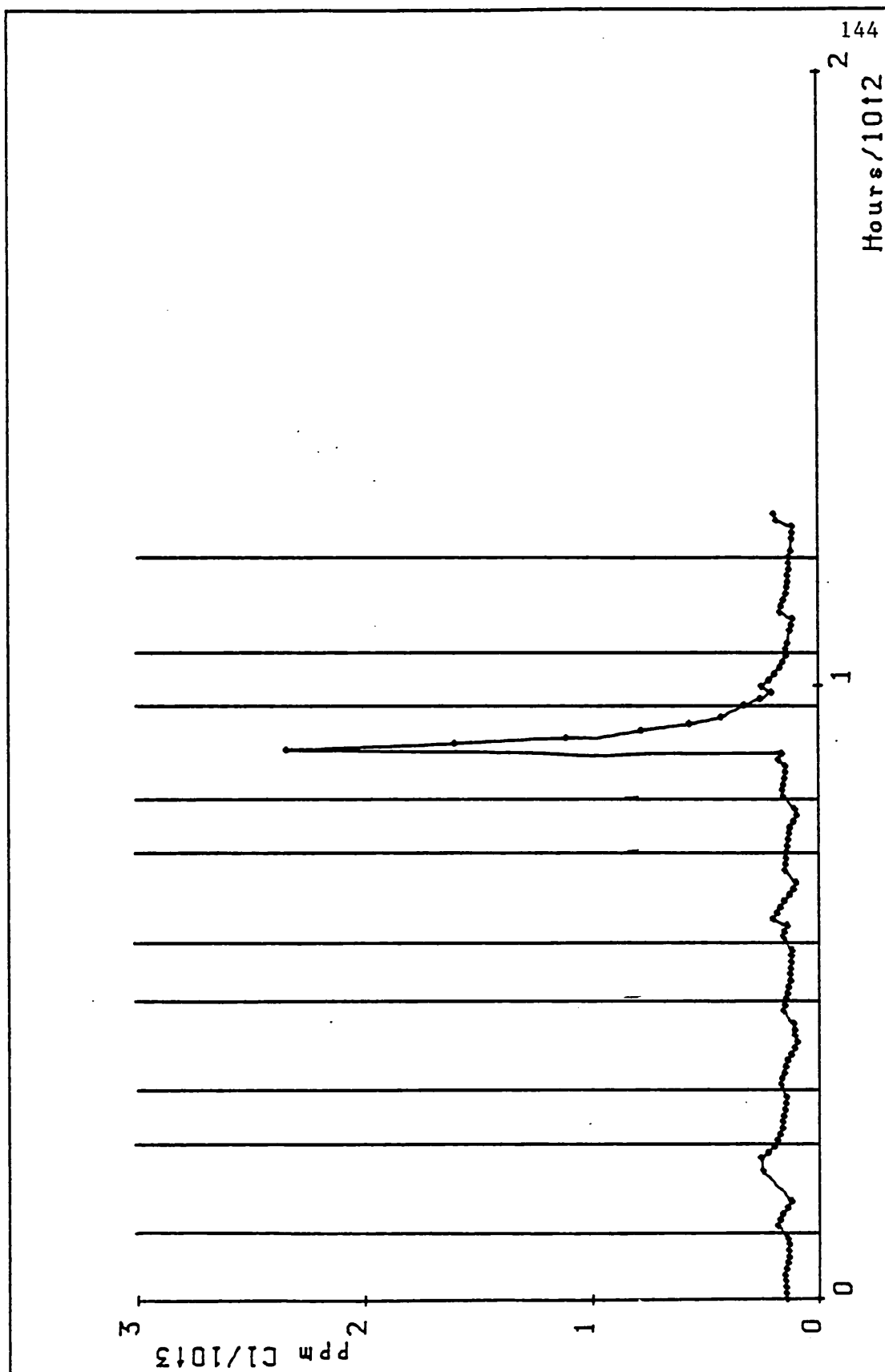


Fig 4.44 The Chloride variation 8-15 Aug 1983

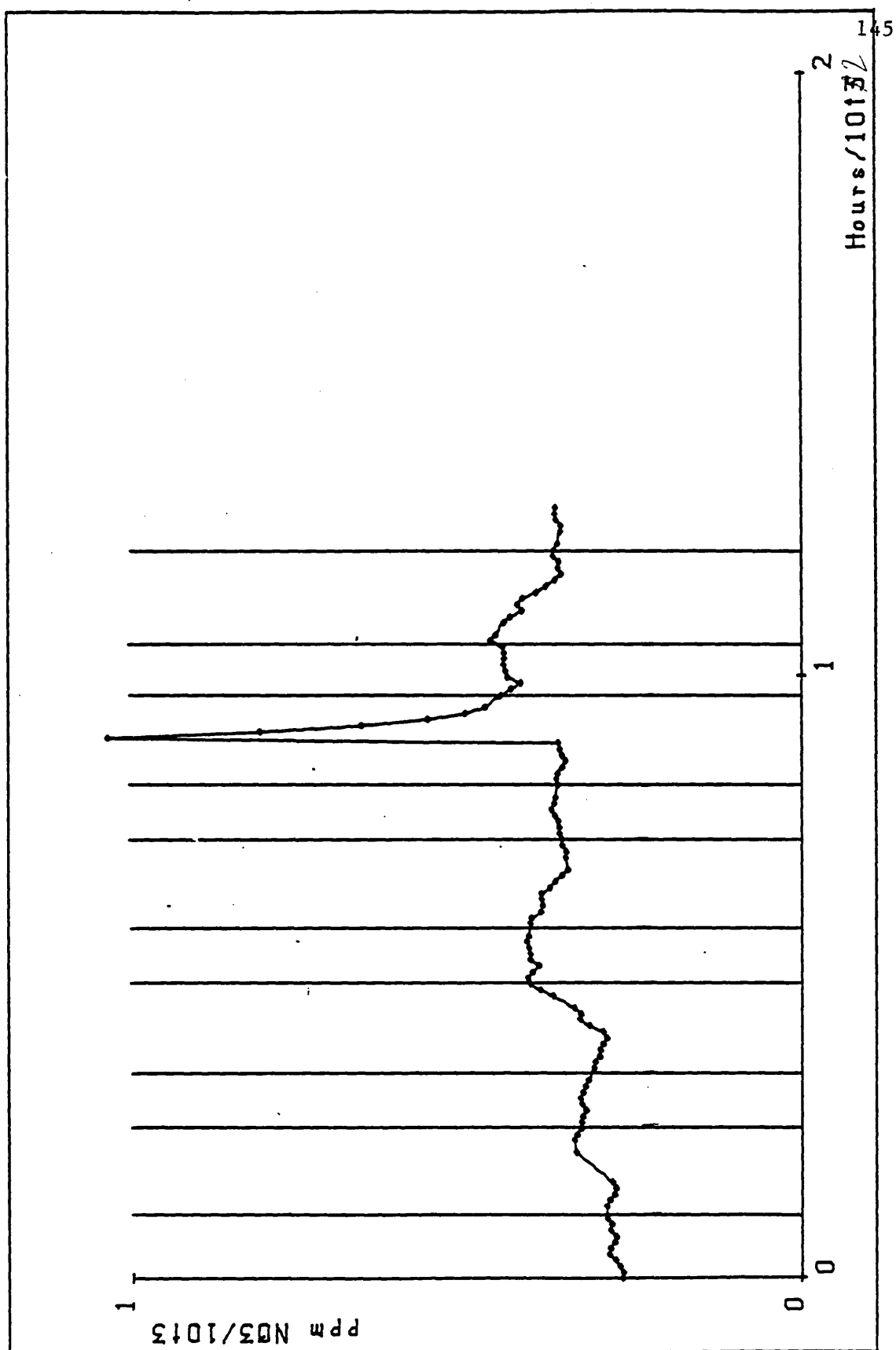


Fig 4.45 The Nitrate variation 8-15 Aug 1983

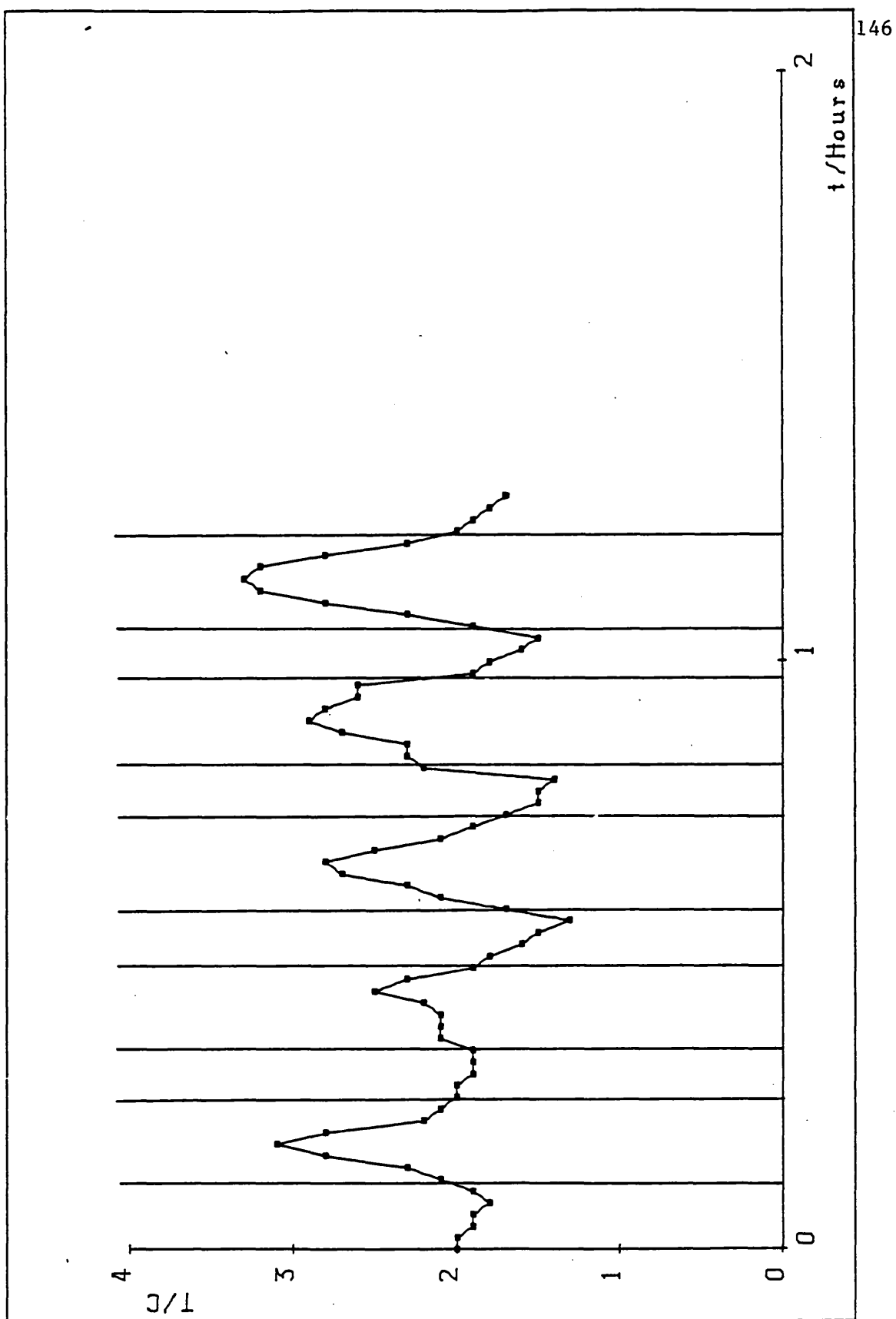


Fig 4.46 The temperature variation 8-15 Aug 1983

CHAPTER 5

AUTOTITRATOR

5.1 Introduction

Root death is a problem in NFT , and cucumber crops are especially vulnerable to this disease (93). In most cases it is caused by a pytoxin in the solution (94). Root death can be stopped , if it is discovered at an early stage , by adding bactericides to the NFT solution.

When roots die and disintegrate organics from the plant cells enter the solution. If this rise in organics in the solution could be detected an early warning could be sent to the grower , who would then be able to save his crop by taking the appropriate action.

5.2 The microtitration technique

The RRDE microtitration technique has been described by several authors (95,96). Albery and Brett (97) describe the microtitration of As(III) on a WJRDE. They establish the same relationships for the WJRDE as for the RRDE. The WJRDE microtitration technique is extended to proteins , aminoacids and peptides in a paper by Albery , Svanberg and Wood (98). They show results for 14 proteins , 5 nucleotides , 4 peptides and 6 aminoacids. Preliminary work at this lab by Mrs M.Neto showed that WJRDE microtitrations can detect root death (99).

5.3 Experimental

5.3.1 The Wall Jet Electrode

The Wall-Jet Ring-Disc Electrode (WJRDE) used in this work is developed from the design by Fleet and Little (100). At the start of this project there was a lack of compatability between the variuos Wall-Jet units used in our laboratory. Together with our laboratory technician Mr M.Pritchard an improved standardised version was designed. This system is now the group standard and has been used successfully by many of my co-workers. This design features;

- 1) Standardised threads in all fittings to the cell body
- 2) All units made of Kel-F
- 3) Replaceable nozzle for easy change of nozzle size
- 4) Improved electrical contacts
- 5) New flow-through reference electrode
- 6) Simplified clamping arrangement
- 7) Standardized library of subunits , i.e reference electrodes , counter electrodes etc..
- 8) Improved sealing
- 9) "Collar" for the ring-disc electrode for maintaining a fixed nozzle electrode distance

Figure 5.1 illustrates the improved WJRDE with subunits. A ring-disc electrode for a Wall-Jet cell is illustrated in fig. 5.2 and table 5.1 lists the electrodes , their dimensions and collection efficiencies.

TABLE 5.1
Wall Jet Ring Disc electrode characteristics

Electrode	r_1/cm	r_2/cm	r_3/cm	N_o
RDE 1	0.166	0.175	0.189	0.124
RDE 2	0.165	0.176	0.189	0.118

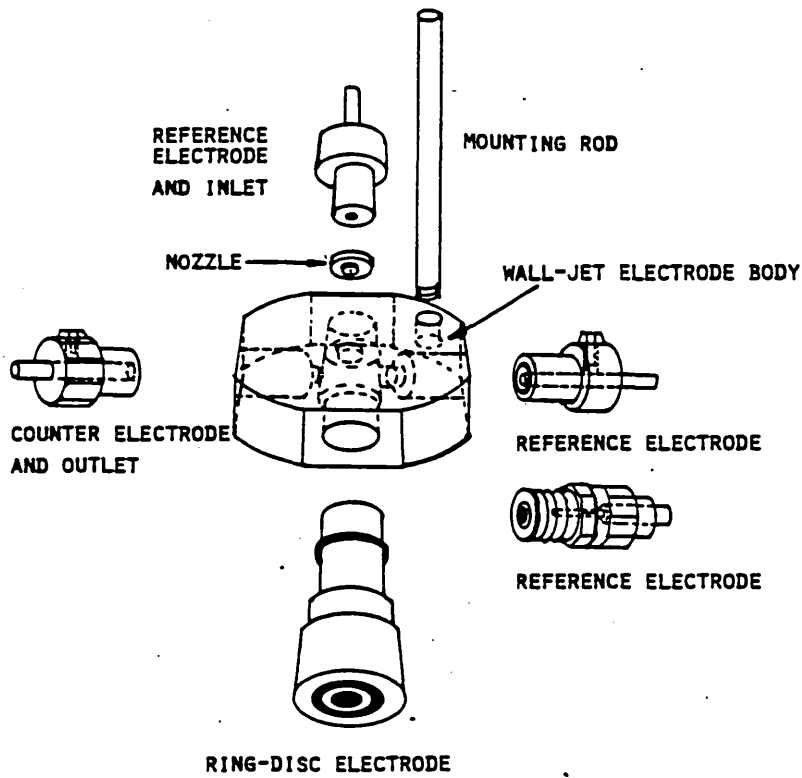


Fig 5.1 The WJRDE electrode system with subunits

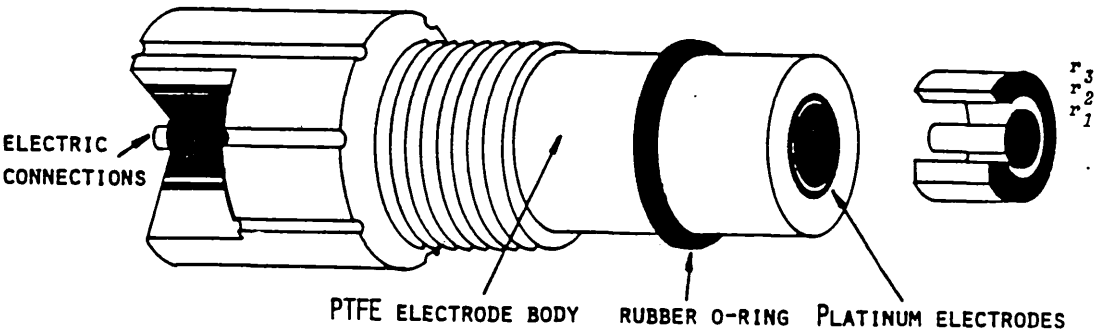


Fig 5.2 A typical ring-disc electrode for the Wall-Jet cell

A novel reference electrode was designed , which consists of a silver tube i.d=1 mm , l=20 mm which was anodised in potassium bromide according to Janz (101) . This electrode has no dead volume as it forms part of the inlet tube , and was cleaned and re-anodised in situ every week. All results in chapter 5 are reported with respect to this electrode;



A similar electrode has been described by Freed and Asmus (102).

5.3.2 Background solution

The background electrolyte question was investigated by my predecessor Dr P.Wood (103). He found that a high bromide concentration is necessary for a 100 % current efficiency , since the solvent decomposition current interferes at low bromide concentrations. In the microtitration technique , described in chapter 2 , we find unprotonated aminogroups the most active site. Most aminogroups have a $\text{pK}_a \approx 8$, thus a suitable background electrolyte buffers at $\text{pH} \approx 9$. The background electrolyte was therefore chosen to be a 0.5 M KBr , 0.025 M Borax solution buffering at $\text{pH}=9.2$. This solution was prepared by dissolving AnalaR KBr and AnalaR borax in MQW. The KBr contains up to 0.001 % nitrogen impurities , which were not removed for these experiments though two techniques for removal have been described by Wood (103). A ring^{and}/disc voltammogram of this electrolyte is shown in figure 5.3 , from which we can see that a suitable bromine reduction potential for the ring is about 300 mV.

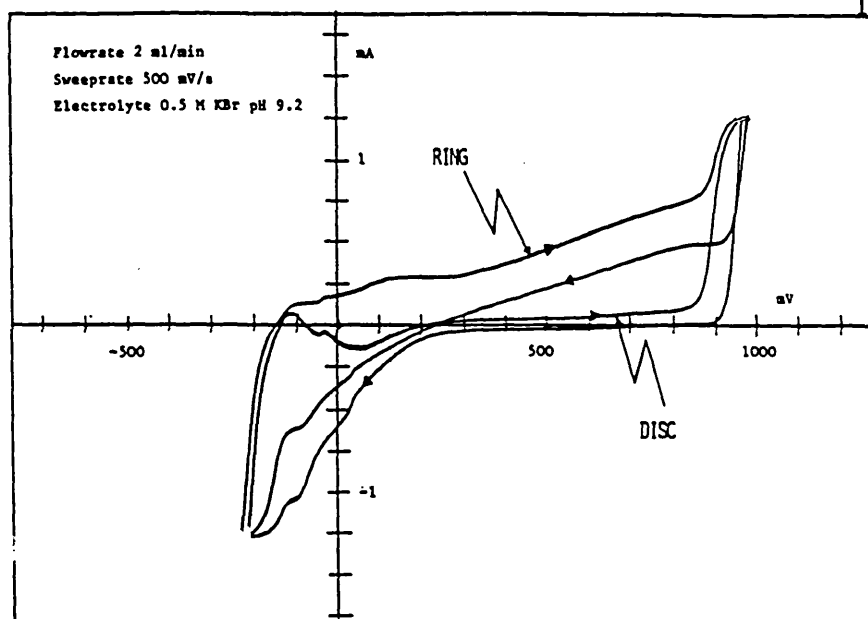


Fig 5.3 Voltammogram of the background solution on a platinum wall-jet ring disc electrode

5.3.3 Electrode polishing

The electrodes were initially polished on a polishing table with 25 μm alumina followed by 6 μm and 3 μm diamond lapping compound. A mirror like surface were obtained by hand polishing with a 1 μm and a 0.3 μm alumina slurry. The used electrode was hand polished before every experiment with 0.3 μm alumina to ensure a reproducible platinum surface.

5.3.4 Electrode pretreatment

The Br^-/Br_2 reaction depends on the state of the platinum surface (104). Voltammograms of the background electrolyte (fig 5.3) do not show any platinum oxide peaks. The highest current efficiency is achieved when the platinum surface is oxidized (105). The oxidation must therefore be carried out in a different medium. Figure 5.4 is a voltammogram of the platinum disc and ring in 2 M H_2SO_4 , where the platinum oxidation is clearly visible at $E \approx 700$ mV

and the reduction at $E \approx 150$ mV. We decided to use the pretreatment recommended by Rubinstein (106), which involves following steps;

- 1) Wash in concentrated HNO_3 for 10 minutes
- 2) Step between following potentials for 10 min
 - i) 0.03 V reducing for 10 seconds
 - ii) 1.07 V remove oxygen for 10 seconds
 - iii) 1.72 V oxidizing for 10 seconds

The final step is oxidizing, so the electrode is left in its most active state. A program was written for the platinum pretreatment, which automated the procedure. This pretreatment followed the polishing before every experimentation session.

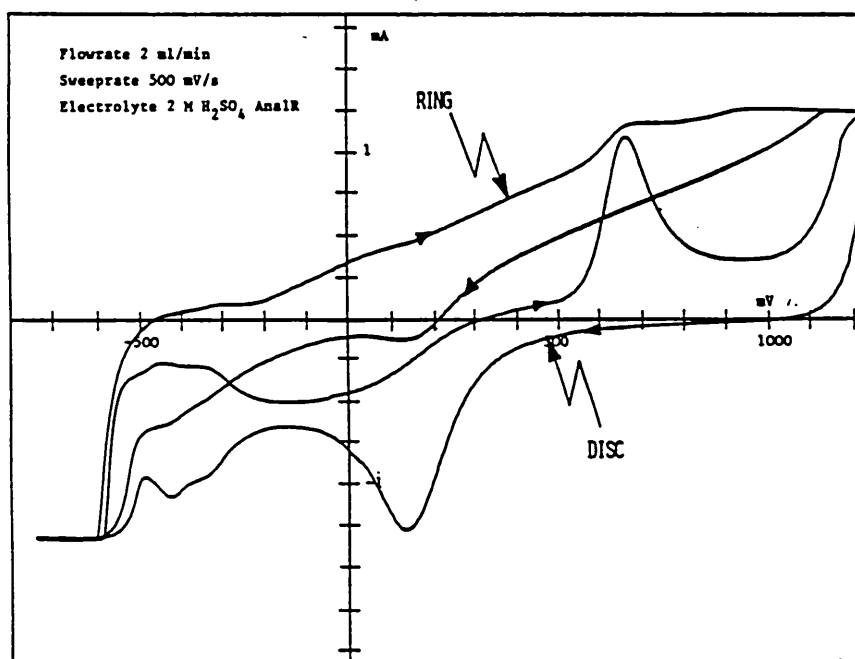


Fig 5.4 Voltammogram of 2 M H_2SO_4 on a platinum wall-jet ring disc electrode

5.3.5 Electrode nozzle distance

Albery and Brett have reported the dependence of both the limiting current and the collection efficiency on the distance between the nozzle and the electrode (97). These effects have been

described quantitatively by Fleet and Gunasingham (107) , who pointed out that the nozzle must not be inside the hydrodynamic boundary layer. A limiting current v.s nozzle distance plot for the WJRDE used in this work is shown in fig 5.5 , from which we can see that a distance larger than 4 mm is required for ideal behaviour. This distance was maintained either by using a "collar" on the ring-disc electrode so it is screwed in to the right position or by screwing in the ring-disc electrode fully and then backing off the required number of turns , calculated from the thread pitch.

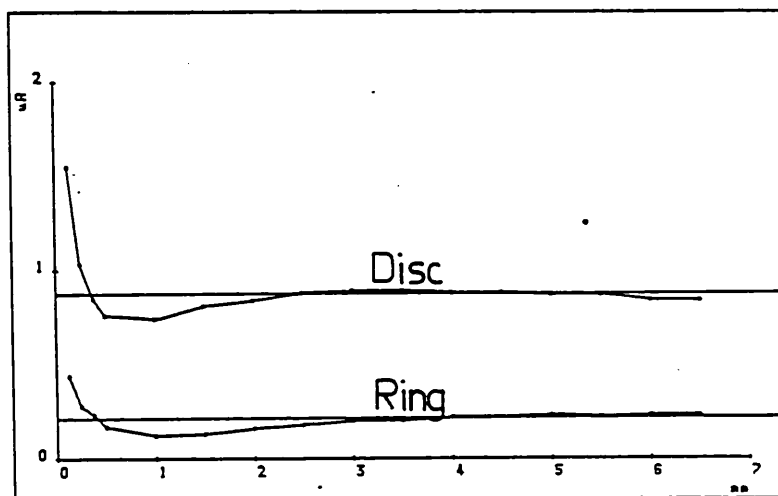


Fig 5.5 The limiting ring and disc current v.s the electrode nozzle distance

5.3.6 Hydrodynamic region

The useful flowrate region was determined by plotting the $(\text{flowrate})^{3/4}$ v.s limiting current in a Levich type plot , fig 5.6 . This plot shows that the limiting current deviates from the ideal at flowrates lower than ~ 1 ml/min. The non ideal intercept is caused by residual background currents.

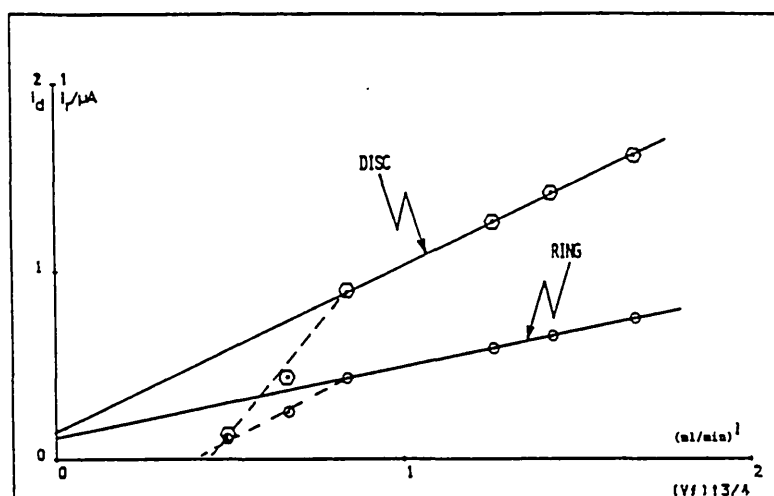


Fig 5.6 The limiting current v.s the (flowrate)^{3/4} for 5 mM potassium bromide

5.3.7 Pulse damping

The Pharmacia P3 peristaltic pump's flow is stable within $\pm 10\%$, but a ripple is present on the flow. This ripple comes from the pinching action of the pump rollers on the pump tubing.

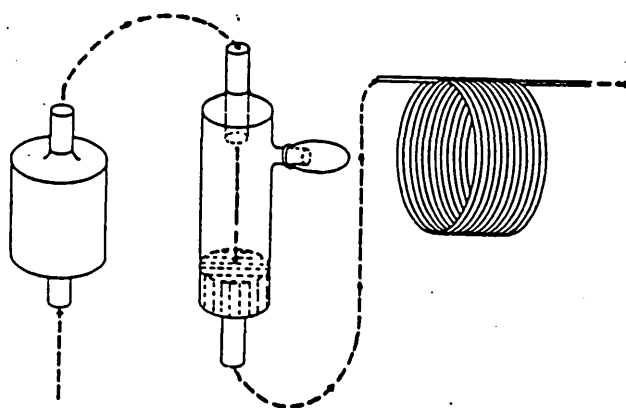


Fig 5.7 The pulse damping system

A pulse damping system was designed to mitigate this flow pulsation, which otherwise would show up on the titration curves. The pulse damping system, illustrated in fig 5.7, consists of a glass cylinder and a coil. The glass cylinder has a rubber teat on a side arm for improved pulse damping. The coil is about 3 m long with

an inner diameter of 0.5 mm. Fig 5.8 shows the improved signal with the pulse damping system .

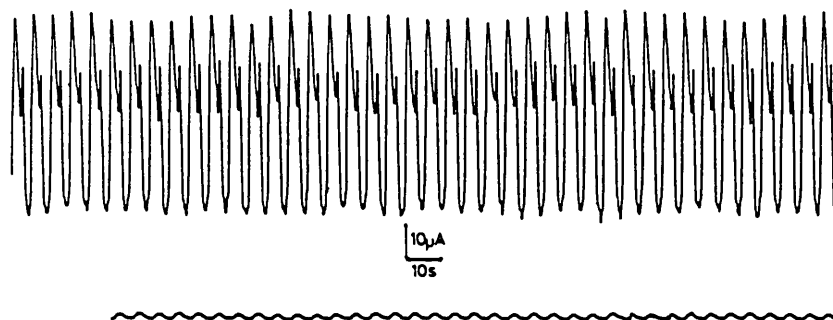


Fig 5.8 a)Signal without the pulse damping system
b)Signal with the pulse damping system

5.4 Associated electronics

In the microtitration technique we use a novel potentiostat/galvanostat unit (fig 5.9) designed and built at Imperial College to galvanostat the disc while the ring is controlled potentiostatically.

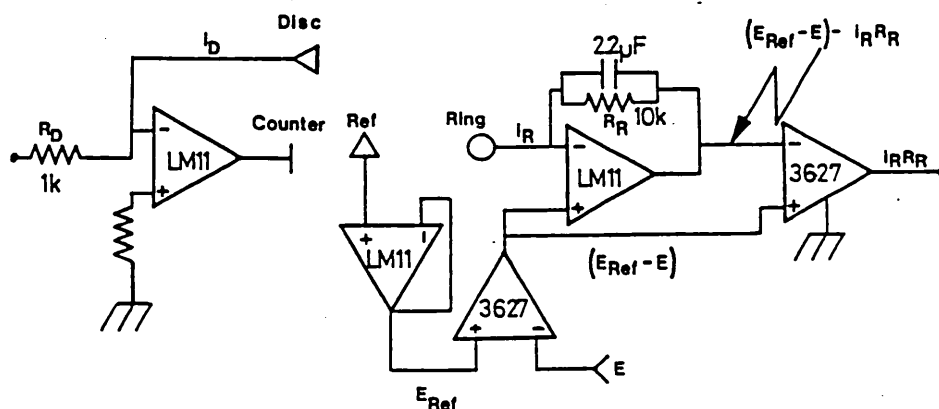


Fig 5.9 The new potentiostat and galvanostat circuit

The 3627 from Burr-Brown is a unity gain summer/subtractor which has a laser trimmed resistor network in the silicon chip which more or less cancels the off-set and gain errors. The LM11 operational amplifier from National Semiconductor also has a laser trimmed resistor network in the silicon chip which compensates for off-set

errors and obviates the need for trimming potentiometers. The LM 11 has an offset current less than 0.5 pA , thus current measurements down to 50 pA is feasible with an error less than 0.1 % . Unconditional stability of the current to voltage converter is ensured by the stabilising capacitor C in the feed-back loop. The cable screening is made active because the potentiostat is floating i.e it has no fixed ground point , it also reduces cable capacitance and improves the noise shielding . Other features of the design are ;

- 1) All op-amps are mounted on PTFE stand offs. This minimises the leaking and creeping currents. A normal printed circuit board limits the detection limit to about 1 nA
- 2) All components mounted on a ground plane which improves the screening , and prevents earth-loops.
- 3 Good power supply decoupling. This practise has been overlooked in previous designs , but good decoupling can significantly improve the op-amp performance

An error analysis of the potentiostat gives an rms error of less than 0.1 % for currents larger than 50 pA. The galvanostat is similar to older versions but a better op-amp and precision resistor are used in this design. The galvanostat generates current down to 25 pA with a 0.1 % accuracy.

5.6 Autoanalysis

The microtitration technique must be automated in order to make it an on-line technique. We have reported two types of microtitration autotitrators (108), whose basic principle is shown in fig 5.10 .

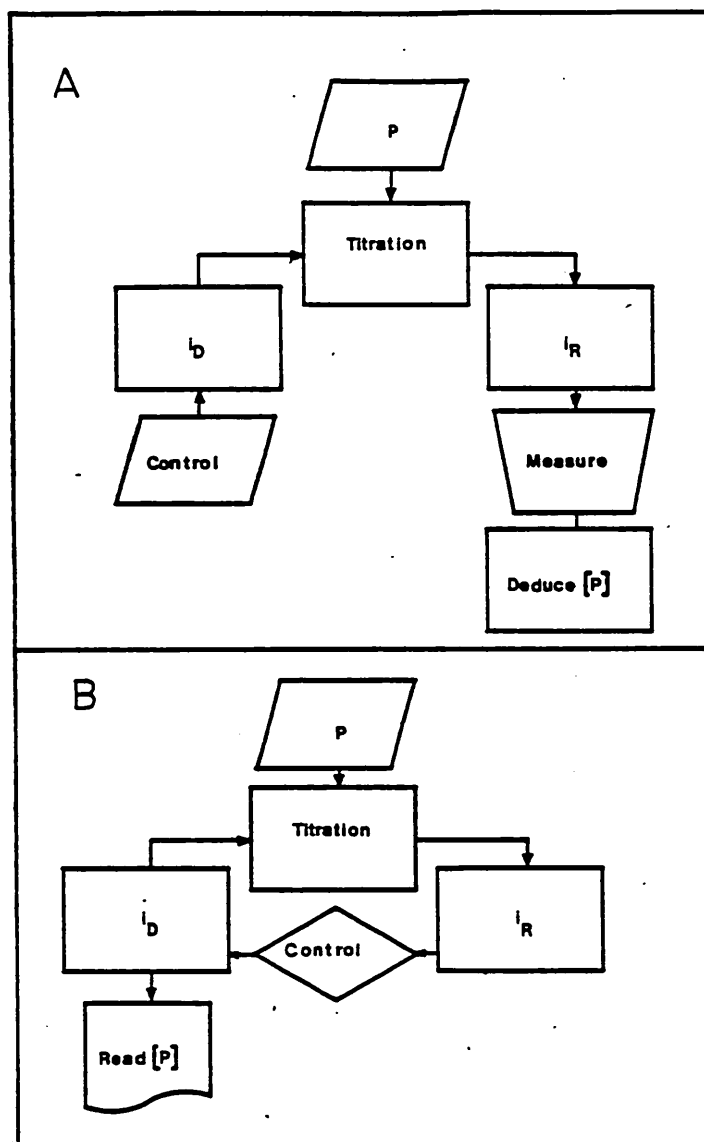


Fig 5.10 a) Manual titration b) The Autotitrator principle

Fig 5.11.b illustrates the principle of an autotitrator designed by Dr P.Wood in 1979 (103). An electronic circuit maintains the ring/disc current on the line N' and the controlled disc current is proportional to the concentration of the titrand. This technique suffers from problems at low disc currents where anomalous readings and instability occur. Non ideal titration curves could not be controlled.

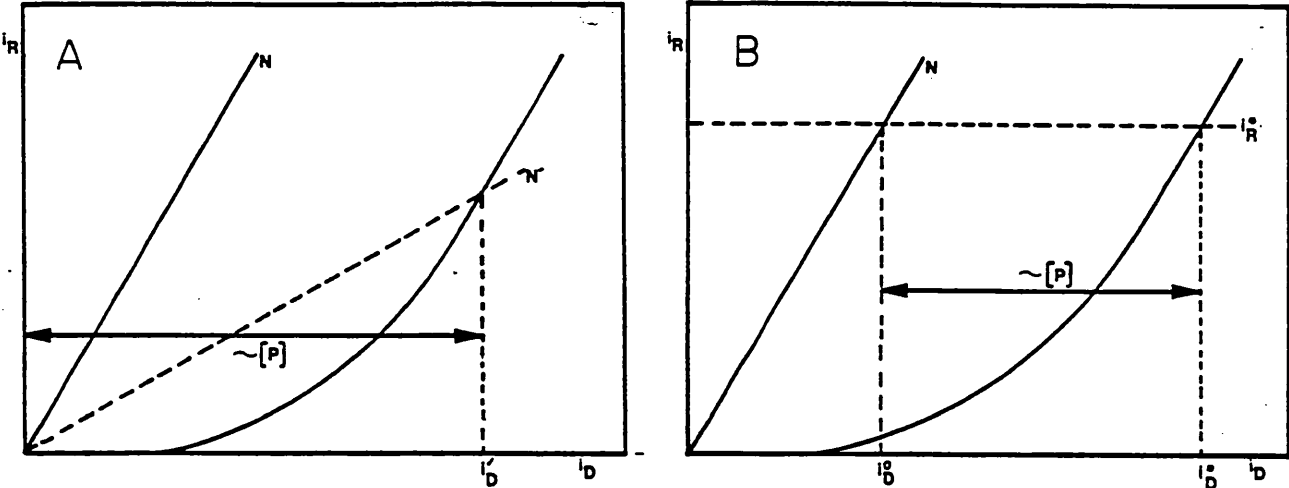


Fig 5.11 a)Autotitrator principle invented by P.Wood
b)The new Autotitrator principle

Our new approach ,illustrated in fig 5.11.b , is to clamp the ring current to a fixed value and read the disc current that is produced in order to maintain this ring current. The disc current in this technique is the sum of the collection efficiency current and a current due to titration , i_t

$$i_d = i_r/N_o + i_t \quad [5.1]$$

This means that there will be a constant off-set on the signal , amounting to $i_{r,off-set}/N_o$. This current can easily be backed-off before it is recorded , but can also be used to check the collection efficiency .

5.6.1 System modelling

In order to implement an active control in the Autotitrator it is essential to model the system. The open-loop system consist of the galvanostat , the WJRDE and the potentiostat. This system is modelled in fig 5.12 , together with a model closed loop feed-back control system.

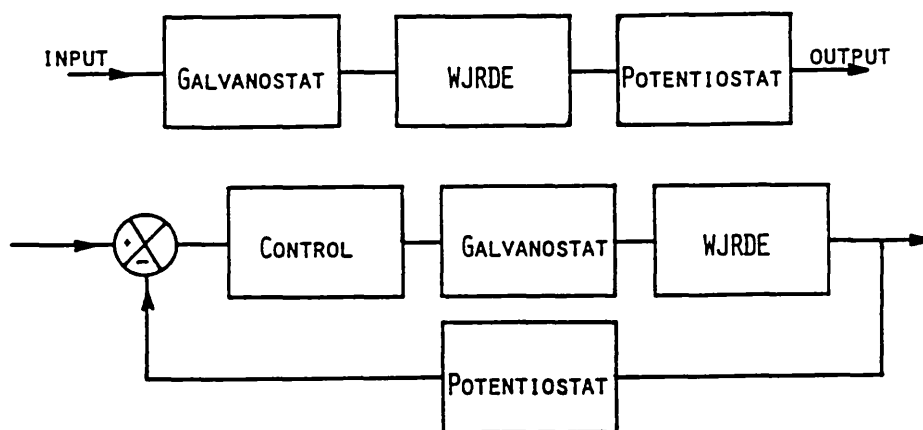


Fig 5.12 The microtitration open-loop and closed loop system:

Open loop system transients were recorded which showed that it is a second order system with a dead time. A typical open loop system transient is shown in fig 5.13.

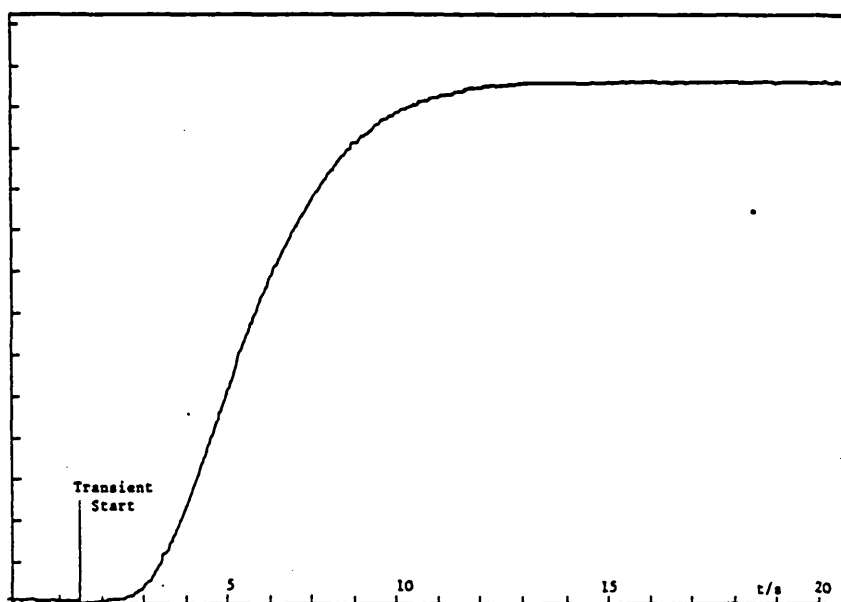


Fig 5.13 A typical open loop system transient recorded at a flowrate of 0.052 ml/s

The frequency and phase response were recorded with a Solartron 1250 frequency analyser. A Bode' diagram for a typical flow rate is shown in fig 4.14. This plot shows that there is a pole around 0.5 Hz , because the phase shift is 45° , and the gain is down to -3 dB in this region. The steep drop at about -40 dB indicates the presence

of another pole , which is verified by a phase shift of 45° at 5 Hz.

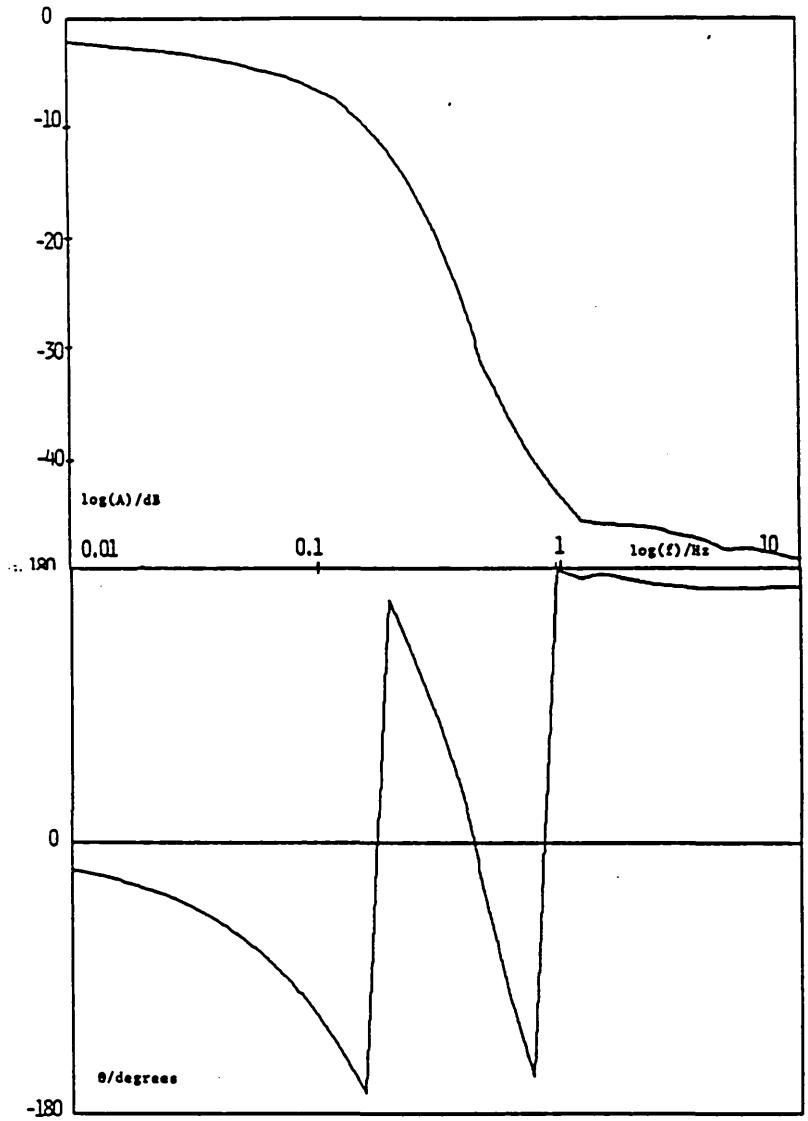


Fig 5.14 The recorded frequency and phase response for the open loop system

If we once again look at the block diagram in figure 4.11 , we may now assign a transfer function to each block. The galvanostat is merely a gain function , determined by the input resistor R_D in fig 5.9. The transfer function is then

$$G_{\text{galv}}(s) = 10^{-3} \quad [5.2]$$

The WJRDE can be modelled as a transport dead time and a first order lag. The transfer function for such a system is

$$G(s) = \frac{K * e^{-T_1 s}}{(1 + T_2 * s)} \quad [5.3]$$

where K is a constant and T_1 is the dead time and T_2 is the first order time constant. If $T_1 \ll T_2$ then we may neglect the dead time without introducing a significant error. Examining the transients show that this is the case, which simplifies the WJRDE transfer function to

$$G_{WJRDE} = \frac{K_2}{(1 + T_1 * s)} \quad [5.4]$$

where K_2 is the collection efficiency $N_0 = 0.12$ and T_1 is the time constant for the first order lag. T_1 has been shown to be a function of the $(\text{flowrate})^{-1}$ (109), so it is possible to compensate for different flowrates. A typical value for T_1 is 2 seconds.

The potentiostat (fig 5.9) has a stabilising capacitor in parallel with the feedback resistor, R_R . This RC-network is a first order lag with the time constant $T_2 = (2\pi R_R C)^{-1}$. The gain, K_3 , is determined by the feedback resistor R_R . The transfer function may then be written

$$G_{\text{pot}} = \frac{K_3}{(1 + T_2 * s)} \quad [5.5]$$

and from fig 5.9 $K_3 = R_R = 10 \text{ k}\Omega$ and $T_2 = (2\pi * 2 * 10^{-6} * 10^4)^{-1} = 0.13$ inserted in [5.5]

$$G_{\text{pot}} = \frac{10^4}{(1 + 0.13s)} \quad [5.6]$$

The total transfer function, G_{tot} , is the linear combination of $G_{\text{gal}}(s) * G_{WJRDE}(s) * G_{\text{pot}}(s) = G_{\text{tot}}$

$$G_{\text{tot}} = \frac{1.2}{(1+2s)(1+0.13s)} \quad [5.7]$$

If we now examine the Bode' plot in fig 4.14 and identify the two time constants at -45° we find $T_1 = 1.61 \text{ s}$ and $T_2 = 0.1 \text{ s}$ which is

well in accordance with the suggested model.

5.7 Analog Autotitrator

If the system model is applied in a feed-back control system we arrive at the block diagram model in fig 5.15

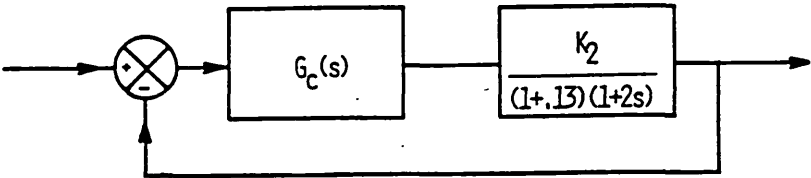


Fig 5.15 Block diagram of the analog Autotitrator

Integral control is used for controlling second order time lags . The integral gain term K_i determines the stability and the performance of the system. The most convenient way to assess K_i is a Root Locus plot.

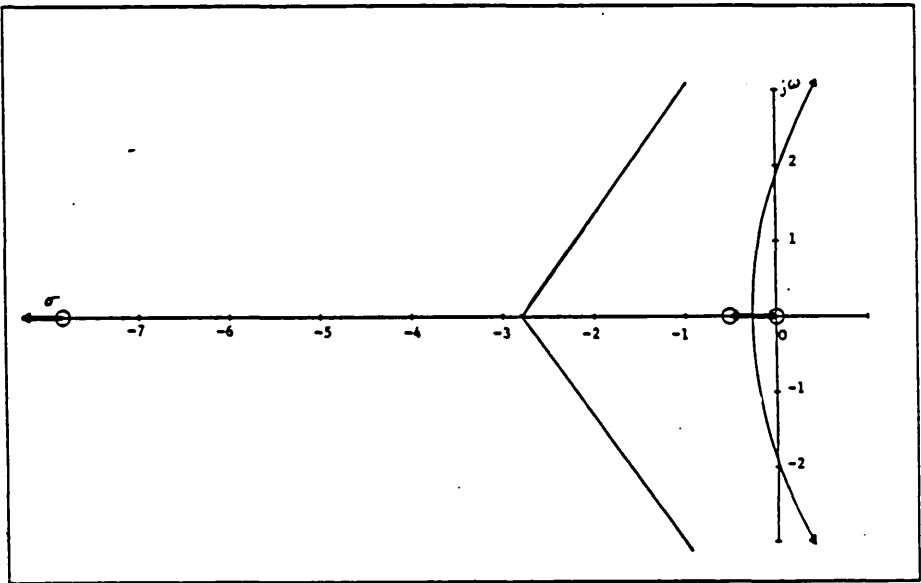


Fig 5.16 The root locus plot for the Autotitrator

This locus is drawn in figure 5.16. We desire to have the roots of the characteristic equation on the σ -axis and as far away as possible from the origin. In this case we find $\sigma = -0.35$ a suitable position , which corresponds to $s = -0.35$. Insertion in the character-

istic equation yields

$$K = \frac{0.35(1 - 0.0460)(1 - 0.7)}{1.2} = 0.5 \quad [5.8]$$

The hardware for this control algorithm consists of subtractor and an integrator. A very good subtractor, the 3627, is available from Burr-Brown. The integrator was built around an LM 11 op-amp from National Semiconductors. The LM 11 has very low input bias current and high input resistance, this makes the LM 11 well suited for integration because it will lose very little of the integrated current stored in the integrating capacitor. The analog Autotitrator circuit diagram is shown in fig 5.17.

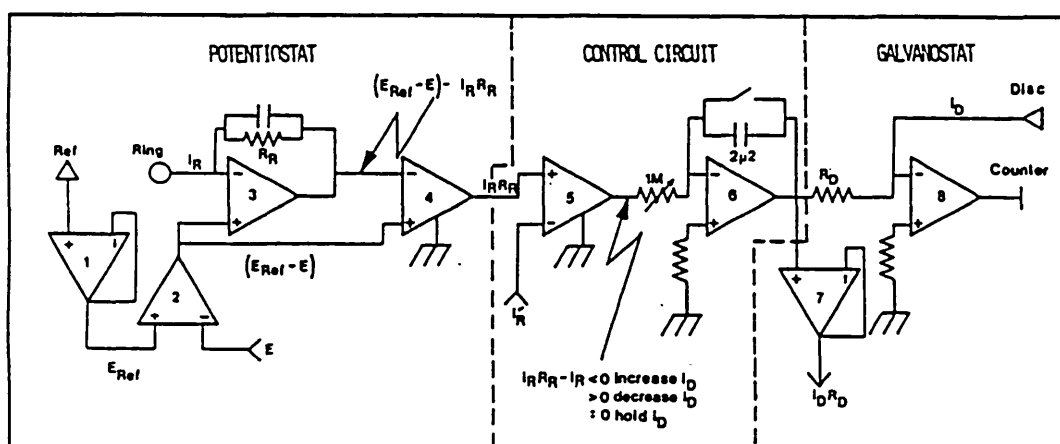


Fig 5.17 The analog Autotitrator circuit diagram

The transfer function for the analog integrator is

$$e_o = \frac{1}{RC} \int_0^t e_i dt \quad [5.9]$$

the LaPlace transformed equation [5.9] is

$$G(s) = \frac{e_o(s)}{e_i(s)} = \frac{1}{RC * s} \quad [5.10]$$

Identifying with the LaPlace transformed integrating control element $G(s) = Ks^{-1}$ with $K = (RC)^{-1}$ shows that K equals $(RC)^{-1}$.

5.8 Digital Autotitrator

5.8.1 Introduction

The autotitration technique is easily implemented on a micro-computer. The microcomputer offers many advantages over the analog system ;

- 1) It can send out warning signals to the grower if the level of organics exceeds a maximum value
- 2) Quality control of the data
- 3) Flexible control algorithms , e.g flowrate dependent integration constant for maximal performance
- 4) Real time controlled titrations
- 5) Peak integration in Liquid Chromatography
- 6) Peak separation in Liquid Chromatography

5.8.2 Digital control

We may use the same system model that we derived for the analog Autotitrator in the digital design , if it is stable when a delay is inserted in the control loop. This delay is called a hold and is inherent in computer control due to the finite time it takes for the computer to acquire and convert an input, calculate a new output value and convert and output it. A good approximation of this process is the Zero Order Hold (ZOH) , which is generally used in digital design, and is illustrated in figure 5.18.

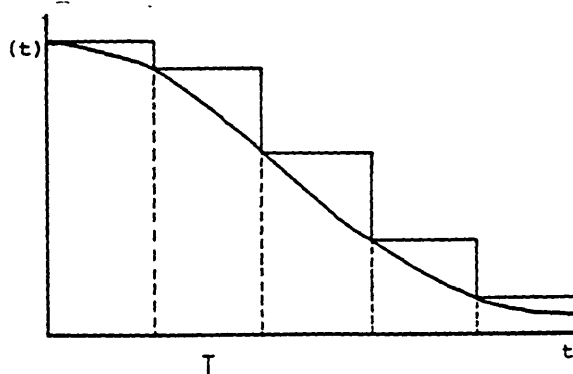


Fig. 5.18 The Zero Order Hold process

The ZOH is approximated to $T/(2+2sT)$ where T is the sampling time. We wished to use the Autotitrator as a detector in Liquid Chromatography (LC). The characteristics of the LC signal include such parameters as a signal bandwidth 0.005 to 5 Hz and a dynamic range of 10^6 (110). The potentiostat bandwidth is about 8 Hz so alias filtering is not necessary. The Nyquist sampling theorem states that a sampling frequency at least twice the bandwidth eliminates aliasing. Accordingly a sampling rate of 10 Hz (100 ms) was chosen. This sampling rate rejects 50 Hz because it is an even multiple, furthermore it is adequate for the recovery of at least 99.99 % of a Gaussian peak 1 sec wide at half height point. Thus a 10 Hz sampling rate can account for all but the most extreme peaks. The digital ZOH approximation is then $ZOH \approx 0.1(1+0.05s)$. Fig 5.19 shows the complete block diagram of the digital Autotitrator.

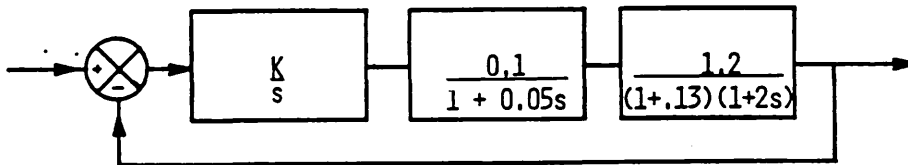


Fig 5.19 Block diagram of the digital Autotitrator

The Autotitrator transfer function $T(s)$ is then

$$T(s) = \frac{K \cdot 0.12}{s(1+0.05s)(1+0.13s)(1+2s)} \quad [5.11]$$

$$1 + \frac{K \cdot 0.12}{s(1+0.05s)(1+0.13s)(1+2s)}$$

with the characteristic equation

$$1 + \frac{K \cdot 0.12}{s(1+0.05s)(1+0.13s)(1+2s)} = 0 \quad [5.12]$$

A root locus plot of equation [5.12] is drawn in figure 5.20, from which we can see that the system is unstable for $K > 47, w = -1.63$, but is otherwise stable. We chose a value of $w = -0.4$ corresponding to

$K=0.62$.

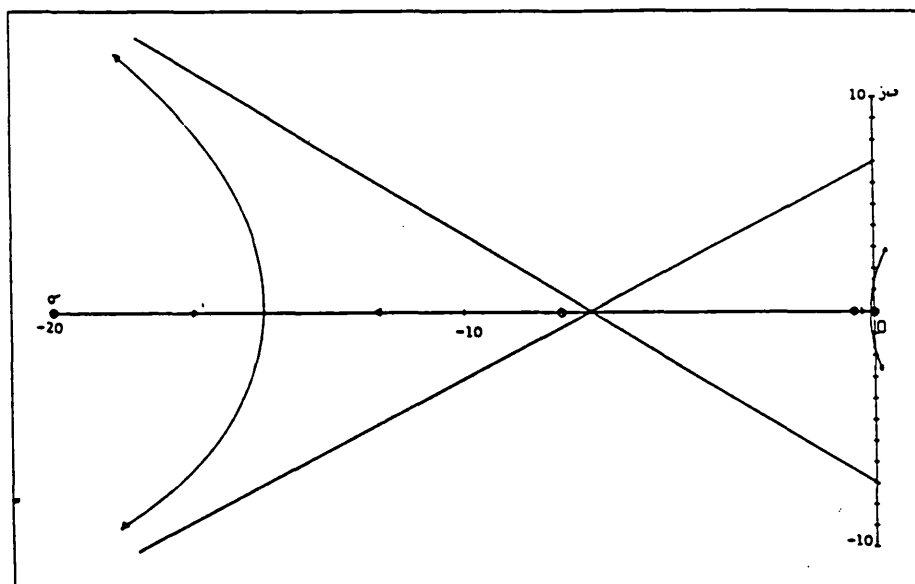


Fig 5.20 Root locus for the digital Autotitrator

The z-transform of the integral control term is

$$G(z) = ZG(s) = Z(Ks^{-1}) = K(1-z^{-1})^{-1} \quad [5.13]$$

whose numerical computer algorithm is

$$e_i = SP - i_i \quad [5.14]$$

$$o_i = o_{i-1} + Ke_i \quad [5.15]$$

where

i_i = the new input

SP = the set point

e_i = the new error

o_i = the new output

o_{i-1} = the previous output

This algorithm is easily translated into a few lines of BASIC

```

:
100 CALL "INADC",1,VARADR(INPUT):REM Read ADC,store result in INPUT
110 ERROR=SETPOINT-INPUT:REM Calculate the error
120 NEWOUT=OLDOUT+K*ERROR:REM The control algorithm
130 CALL "WRIT16",1,1,0,NEWOUT:REM Output new value to DAC
140 OLDOUT=NEWOUT:REM Update OLDOUT
150 GOTO 100:REM Close the loop
:

```

This program sequence's execution time was measured to 40 ms , which means that it cannot interfere with the external sampling time of 100 ms.

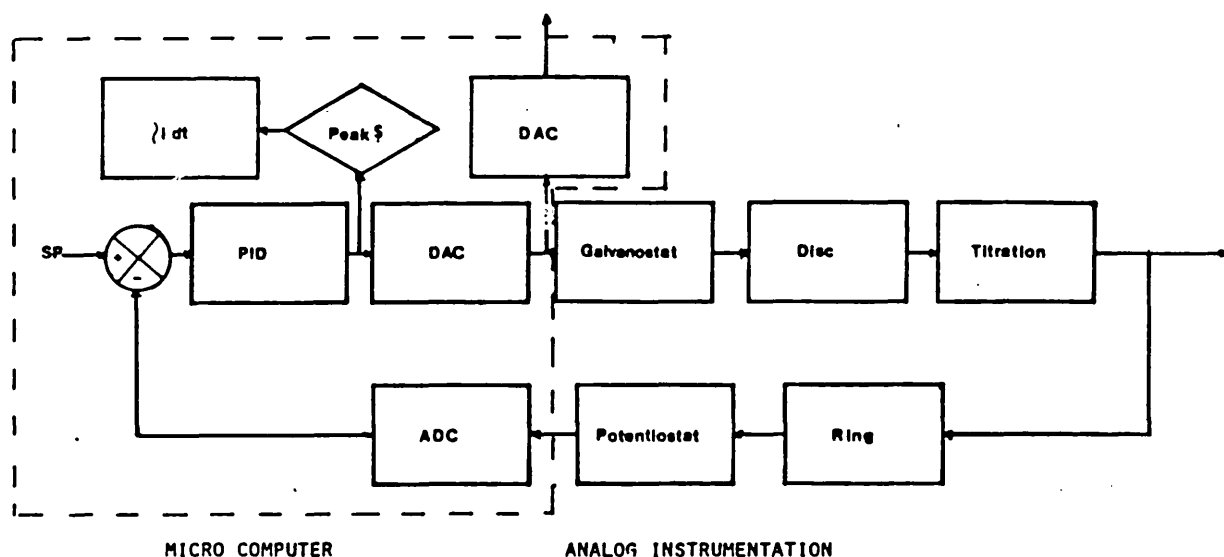


Fig 5.21 The digital Autoanalyser hardware

The hardware for the Autoanalyser , illustrated in fig 5.21 , consists of the previously described potentiostat/galvanostat unit and the 380 Z development system. For the interfacing , the ADC board was used to read the ring current from the potentiostat and the DAC board used to control the disc current from galvanostat. The timing was controlled by the timer board , which was set up to give a 10 Hz sampling rate. A second DAC board was used to control the flowrate from the pump and the potential of the ring.

5.8.4 Peak Integration

The computer enables us to not only control the titration , but also simultaneously analyse and display the obtained data.

Equation [5. 35] in section 5.9.1 can be used for a direct

concentration measurement (if the protein is known). The integration is approximated to summation over the peak. Knowing the sample rate and the value of the LSB allows us to calculate the charge under the peaks as

$$Q = \text{LSB} \cdot N \cdot T \sum_{i=0}^N \text{Output}_i \quad [5.16]$$

The commencement of the peak is detected by a four point running average of the outputs; integration begins if the difference between the new control output and the running average is larger than 0.1. The summation finishes when the signal has returned to the baseline, and the result is printed out. Two error routines check the existence of the peak in order to avoid ghost peaks created by injection valve switching and noise. The Autoanalyser software is listed in appendix III.

5.8.5 Dynamic Control

The characteristics of the WJRDE depends on the flowrate, therefore a dynamic control algorithm was designed in order to compensate for changes in the flowrate. The time constant, τ , for the first order lag of the WJRDE has been shown to be inversely proportional to the flowrate (109), and we found

$$\tau = (92V_f - 0.78)^{-1} \quad [5.17]$$

Recalling the characteristic equation of the system, [5.12], and inserting τ

$$1 + \frac{K \cdot 0.12}{s(1+0.05s)(1+0.13s)(1+\tau s)} = 0$$

The root locus plot in fig 5.20 shows that τ is responsible for the locus between τ and the origin, which determines the stability of the system.

The best response is achieved s-values on the σ -axis. We used an σ value equal to -0.4 , which gives

$$K = 0.37(1 - 0.4\tau) \quad [5.18]$$

substituting for τ gives

$$K = 0.37[1 - 0.4/(92V_f - 0.78)] \quad [5.19]$$

The flowrate was controlled by the same technique as in the Autoanalyser chapter , section 4.3.2. This algorithm was tested and worked to our satisfaction.

5.9 Packed-Bed Wall-Jet Electrode

This electrode was designed in order to improve the sensitivity of the microtitration technique. The sensitivity limiting factor is the magnitude of the detected current when titration takes place. The ring current, i_r , in the WJRDE is

$$i_r = N_o (i_d - 8[T]n_{Br}) \quad [5.16]$$

where

N_o = the collection efficiency

8 = Constant

$[T]$ = Titrand concentration

n_{Br} = Bromine number

In order to increase *the sensitivity* we must increase the second term in equation [5.16]. One may try to increase N_o by for instance reducing the gap thickness, and this is mechanically very difficult. Therefore one must increase n_{Br} , which depends on the reaction rate between the analysed species and the bromine. Bromination reactions are pH dependent, but pH=9.2 has proved to be the best pH. The conclusion is that the WJRDE is working under optimum sensitivity conditions.

At this point it was natural to look at other but similar coulometric techniques. A conceivable system was a coulometric flow-through reaction chamber followed by a downstream detector. This system was realised as an upstream platinum tube electrode and

a downstream Wall-Jet disc electrode illustrated in figure 5.21.

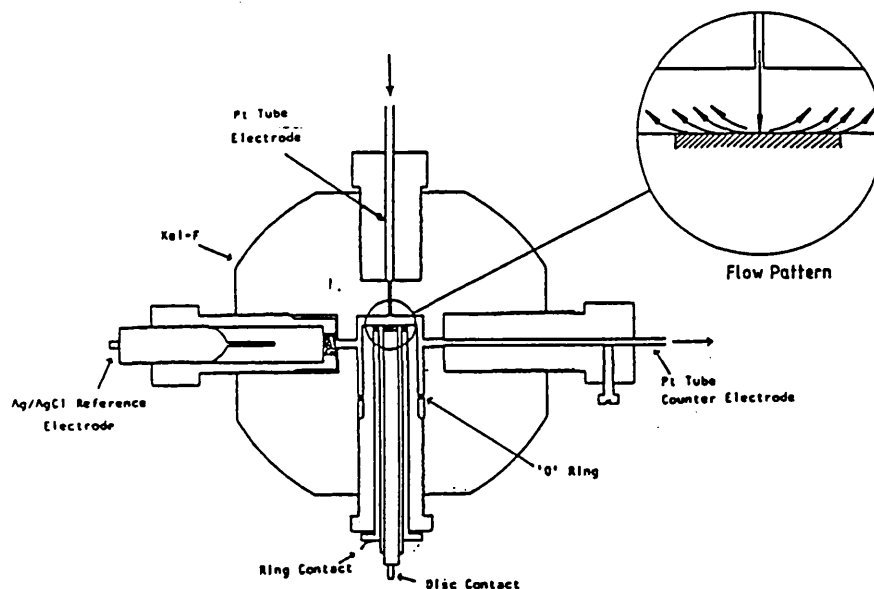


Fig 5.21 The Tube Wall Jet electrode

The equation for the generated current is

$$i_t = nFV_f C_{Br} \quad [5.17]$$

where

V_f = the flow rate

C_{Br} = the concentration of the generated bromine

and the current from the detecting WJE is

$$i_d = 1.38nFD^{2/3} u^{-5/12} a^{-1/2} R^{3/4} C V_f^{3/4} \quad [5.18]$$

where D = the diffusion coefficient

u = the kinematic viscosity

a = the nozzle area

R = the disc radius

Let us define the collection efficiency, N , similarly to the ring-disc case as

$$N = \frac{i_d}{i_t} = 1.38D^{2/3} u^{-5/12} a^{-1/2} R^{3/4} V_f^{-1/4} \quad [5.19]$$

thus N is not a constant but depends on $V_f^{-1/4}$. N is typically 2 % for bromine at normal flowrates.

If we compare i_d from this system with i_r from the WJRDE system when titration takes place we arrive at

$$i_d = N(i_t - nFV_f[T]n_{Br}) \quad [5.20]$$

but from equations [5.16] and [5.20] we find

$$B = nFV_f N \quad [5.21]$$

inserted in [5.20]

$$i_d = Ni_t - B[T]n_{Br} \quad [5.22]$$

compared to [5.16]

$$i_r = N_o i_d - N_o B[T]n_{Br} \quad [5.16]$$

Typical numbers are $N_o = 0.10$ and $N = 0.02$. Thus the "titrand current" ($B[T]n_{Br}$) is 10 times larger for the Tube Wall-Jet Electrode and as N is about 5 times smaller than N_o the total sensitivity improvement is about 50 times. The extended reaction time may also lead to a larger N_{Br} , that is more bromine molecules may find time to react with brominatable sites.

Initial experiments with the TWJE were not satisfactory. Collection efficiency curves were recorded with values much lower than the theory predicts. This abnormal behaviour is probably due to a "streaming" effect ; the bromine generated at the wall of the tube travels down the tube into the nozzle close to the wall. Looking at the stream function of a wall jet shows that it is mainly the central "core" of the jet that is going to hit the electrode surface. The Reynolds number for the tube and the nozzle were calculated to

$$Re_{tube} = \frac{2V_f}{\pi R U} = 6.35 \quad [5.24]$$

$$Re_{nozzle} = 12.7$$

According to experimental findings, a three dimensional jet remains

laminar up to critical Reynolds number Re_{Cr} , where $25 < Re_{Cr} < 1000$ (111). This means that the laminar flow is maintained throughout the tube-nozzle system and little mixing will occur in the flow. This situation is illustrated in figure 5.22. Attempts to "stuff" the tube in order to improve the mixing were not successful.

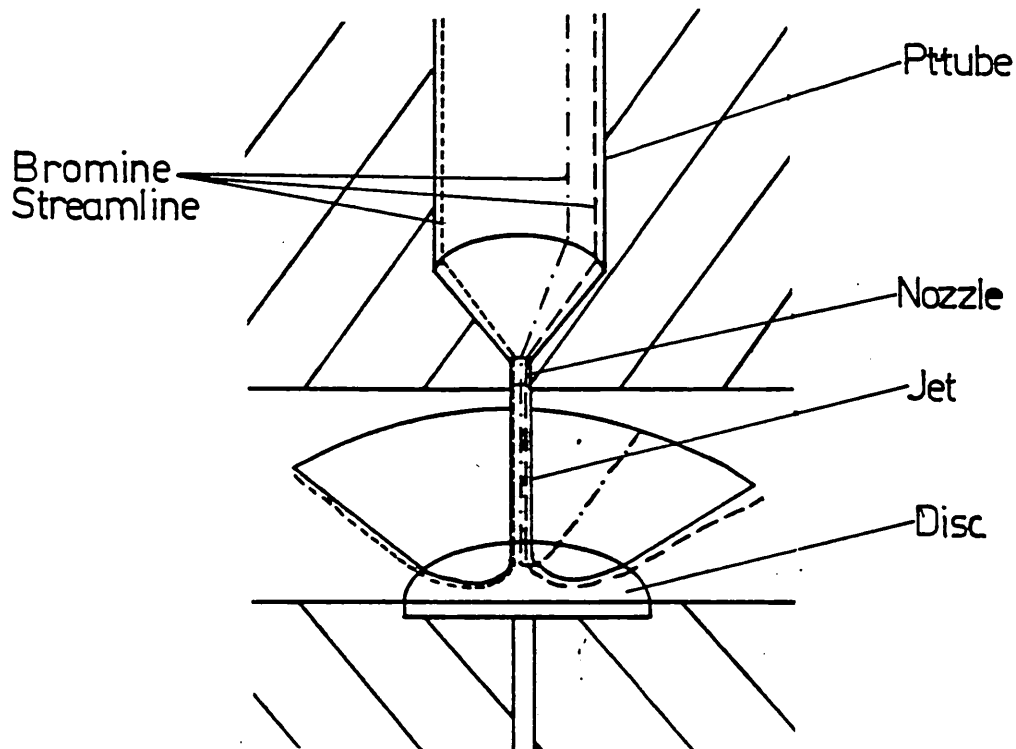


Fig 5.22 The tube electrode "streaming" effect. The dotted lines represent streamlines close to the tube wall travelling through the nozzle and finally impinging on the disc.

Our attention was then turned to a new electrode material introduced on the market. This material is called Reticulated Vitreous Carbon (RVC), and is a very porous vitreous graphite. We assumed this electrode material would improve the mixing as the reaction now takes place in a more "homogenous" fashion. The bromide/bromine system was investigated on this material and showed quite good kinetics. Platinisation of the carbon did not improve matters significantly, so experiments were carried out using the RVC in its original state. An RVC packed-bed electrode was constructed to fit

the inlet thread of the WJE. This electrode is illustrated in figure 5.23a.

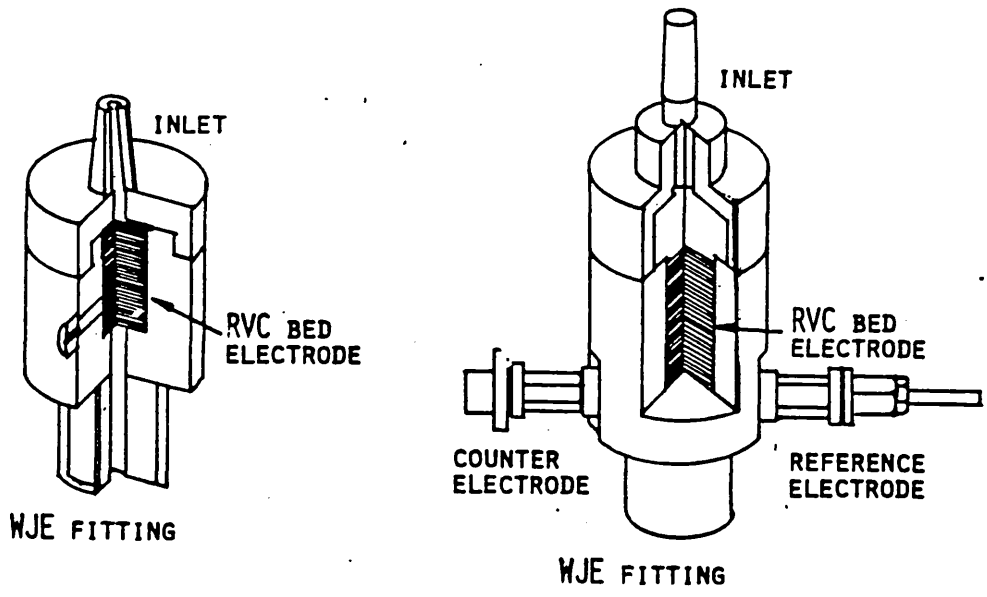


Fig 5.23 a)The old vitreous carbon packed bed electrode
b)The new vitreous carbon packed bed electrode

An unforeseen problem occurred with this electrode , namely the electrical resistance across the nozzle and operating range of the galvanostat. The used galvanostat is illustated in figure 5.24

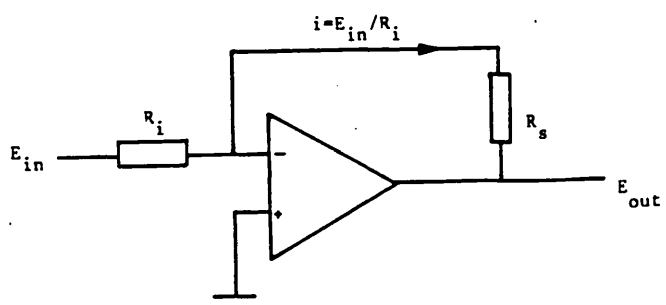


Fig 5.24 Circuit diagram of the galvanostat and the packed-bed electrode

It works as follows ; in order to maintain a virtual earth potential at the summing junction S in fig. 5.24 , the amplifier outputs a current in the feed-back loop over the solution resistance , R_s , that equals the input current $i_{in} = E/R_{in}$. The maximum output voltage the LM 11 can produce is ± 14 V , and the maximum input voltage is

±10 V. Balancing the input and the output currents we arrive at

$$\frac{E_{in}}{R_{in}} = \frac{E_{out}}{R_s} \quad [5.25]$$

which yields

$$R_{s,max} = \frac{E_{out}}{E_{in}} * R_{in} \quad [5.26]$$

This means that the galvanostat will cease to operate if $R_s > 1.4 * R_{in}$.

A simple DC resistance measurement across the packed bed electrode and the counter electrode gave a reading of 1.26 MΩ. This solution

resistance limits the current to the packed bed electrode at 11 μA

using an LM 11 op-amp. The LM 11 has an offset current of $4 * 10^{-11}$ A

, thus for a 0.1 % accuracy the minimum current is $10^3 * 4 * 10^{-11} = 4 * 10^{-8}$

A. The dynamic range of this system is then $11 * 10^{-6} / 4 * 10^{-8} = 275$.

This is a very poor performance, ideally one wishes to have a

dynamic range of 10^6 for a chromatographic detector. The dynamic

range may be expanded by using a more powerful galvanostat, but

this leads to another problem; the temperature rise in the cell as

we pass large currents. The produced power is

$$P = U * I = R * I^2 = W/t \quad [5.27]$$

and the quantity of heat

$$W = C_p * m * \Delta T \quad [5.28]$$

where C_p = specific heat

m = the mass

T = the absolute temperature

If we now combine [5.27] and [5.28] together with $m = \rho * V$ and $V = V_f * t$

we get

$$\Delta T = \frac{R * I^2}{C_p * \rho * V_f} \quad [5.29]$$

Assuming we wish to increase the current to 10 mA, and using

typical values for the other variables we find the temperature change ΔT is

$$\Delta T = \frac{1.26 \times 10^6 \times (10^{-2})^2}{4.18 \times 10^3 \times 0.9988 \times 4 \times 10^{-4}} = 77 \text{ K}^\circ$$

This led us to abandon the idea of passing a current through the nozzle. The next design ,illustrated in fig 5.23b , was a separate upstream electrode system. The counter electrode was placed behind a glass sinter in a plastic tube to allow the produced hydrogen gas to diffuse into the atmosphere.

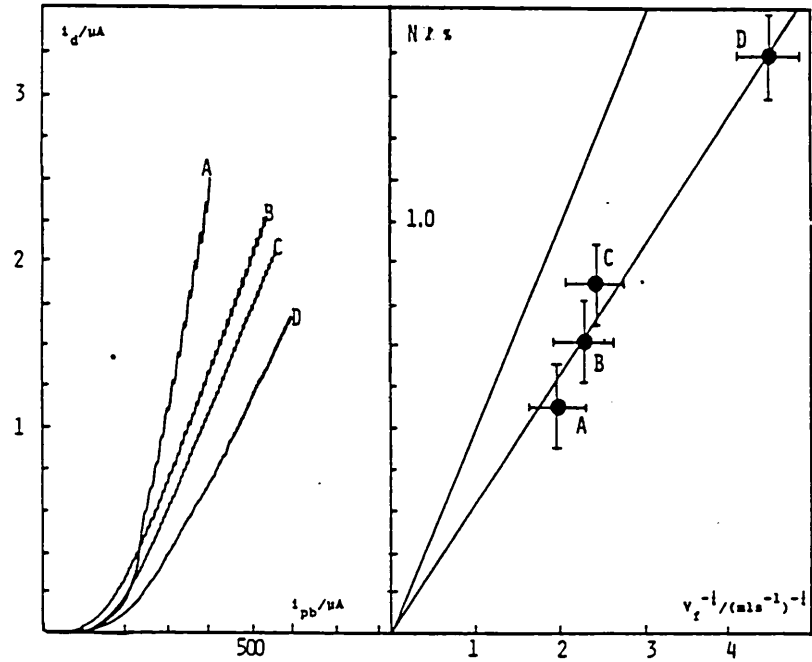


Fig 5.25 The left hand part shows collection efficiency curves recorded at different flowrates at a sweep rate of $2 \mu A/s$. The measured collection efficiency is plotted versus the $(\text{flowrate})^{-1/4}$ in the right hand part. The theoretical line is also shown.

Fig 5.25 shows collection efficiency curves at different flowrates and the collection efficiency plotted versus the $(\text{flowrate})^{-1/4}$. The initially curved shape is a typical response of a first order lag to a linear ramp when the sweep rate is faster than the time lag. A linear ramp's , $g(t)=kt$, LaPlace transform is

$G(s)=ks^{-2}$, combined with a first order lag $C(s)=(1+Ts)^{-1}$ yields the total transfer function

$$R(s) = G(s)*C(s) = ks^{-2}(1 + Ts)^{-1} \quad [5.30]$$

The time response $r(t)$ can be obtained by LaPlace inversion after separation into partial fractions, i.e

$$R(s) = k(s^{-2} - Ts^{-1} + T^2(1 + Ts)^{-1}) \quad [5.31]$$

and hence from table 1 in appendix I

$$r(t) = k(t - T + Te^{-t/T}) \quad [5.32]$$

The transient response is $ke^{t/T}$ which decays to less than 2% of T in $4T$, and the steady state response is $k(t-T)$, i.e for large values of time t the output lags the input by a constant value kT . This lag can be compensated for in the Autotitrator technique by changing the control algorithm.

The result in the right hand part of fig 5.25 shows that the experimental collection efficiency is lower than the theoretical. This suggests that the bed is not 100 % efficient in producing bromine or some of the produced bromine is not detected at the disc. Voltammogram of the bromine system on the packed bed electrode shows good kinetics with little solvent decomposition interference. In the latter case it might again be a streaming effect , or the case of some bromine escaping to the counter electrode compartment ,and being reduced at the counter electrode.

An inconvenience in this technique is the lag between generator and detector. This lag consists of two parts , firstly a transport dead time and secondly a first order lag (typical of packed bed reactors). The dead time t_d ,depends linearly on the flow-rate, V_f ,the distance between the electrodes , d , and the nozzle area , A .

$$t_d = \frac{d \cdot A}{V_f} \quad [5.33]$$

The first order lag depends on the volume of the packed bed , V , and the flowrate , V_f .

$$C = C_o (1 - e^{-V_f t / V}) \quad [5.34]$$

For a 0.1 % error in concentration we get

$$\frac{C}{C_o} = 1 - e^{-V_f t_1 / V} = 0.999 \quad [5.35]$$

which yields $t_1 = 6.91(V/V_f)$ [5.36]

The total time lag , t , is then the sum of [5.33] and [5.36]

$$t = (d \cdot A + 6.91 \cdot V) / V_f \quad [5.37]$$

With typical values equation [5.37] gives

$$t = \frac{0.07 \cdot 0.002}{0.01} + \frac{6.91 \cdot 0.03}{0.01} = 0.14 + 20.7 = 20.87 \text{ sec}$$

Clearly it is the first order lag that is the most important lag factor. A conflicting aspect is the collection efficiency (which depends on $V_f^{-1/4}$) versus the response time (which depends on V_f^{-1}). Figure 5.26 shows the collection efficiency and response time plotted versus the flowrate.

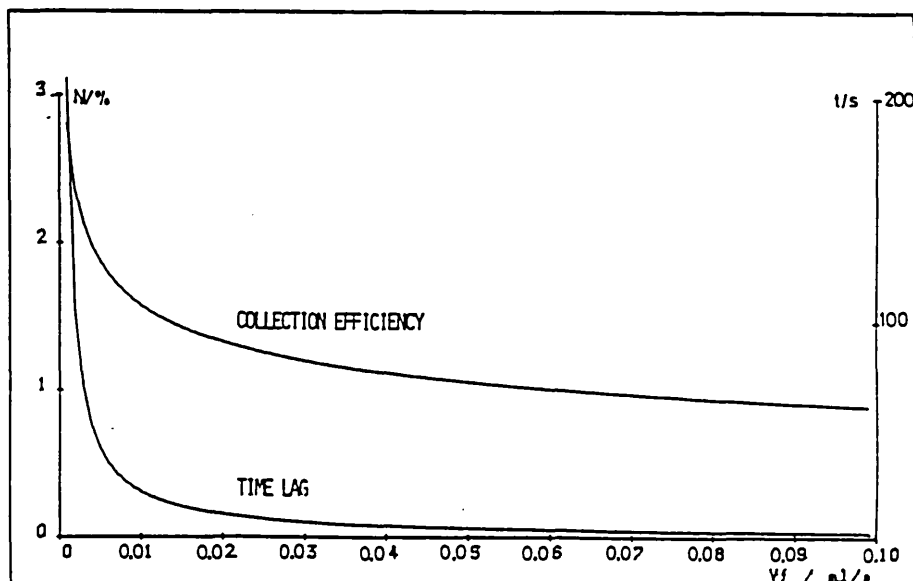


Fig 5.26 Collection efficiency and response time plotted against flowrate

Further investigations on this technique are continuing in this laboratory.

5.9 Results

5.9.1 Analog Autotitrator

The analog Autotitrator was first used as a detector in protein liquid chromatography. It was run in parallel with a Pharmacia UV-2 uv-detector, the two detector outputs were recorded on a dual channel Y-t recorder. A typical LC set up is illustrated in figure 5.27

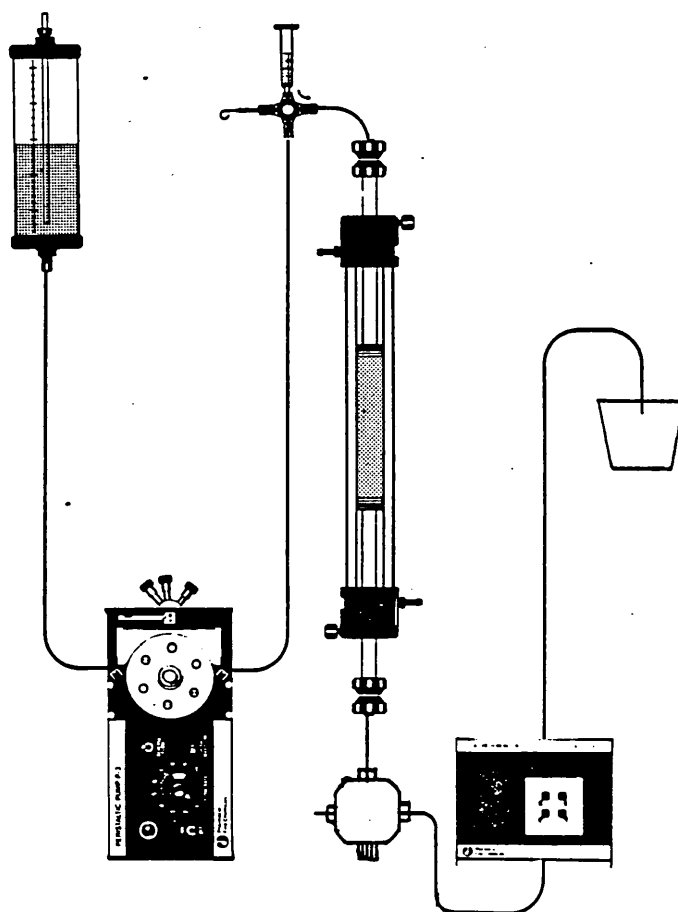


Fig. 5.27 A typical chromatography experiment

The columns were Pharmacia C10/10 or C10/40 packed with Sepha-cryl 200 Superfine. Typical results for the separation and detection of a mixture of catalase and ribonuclease at different flowrates are

shown in fig 5.28. The phase shift between the uv and Autotitrator peaks is due to the serial arrangement of the detectors in the experiment.

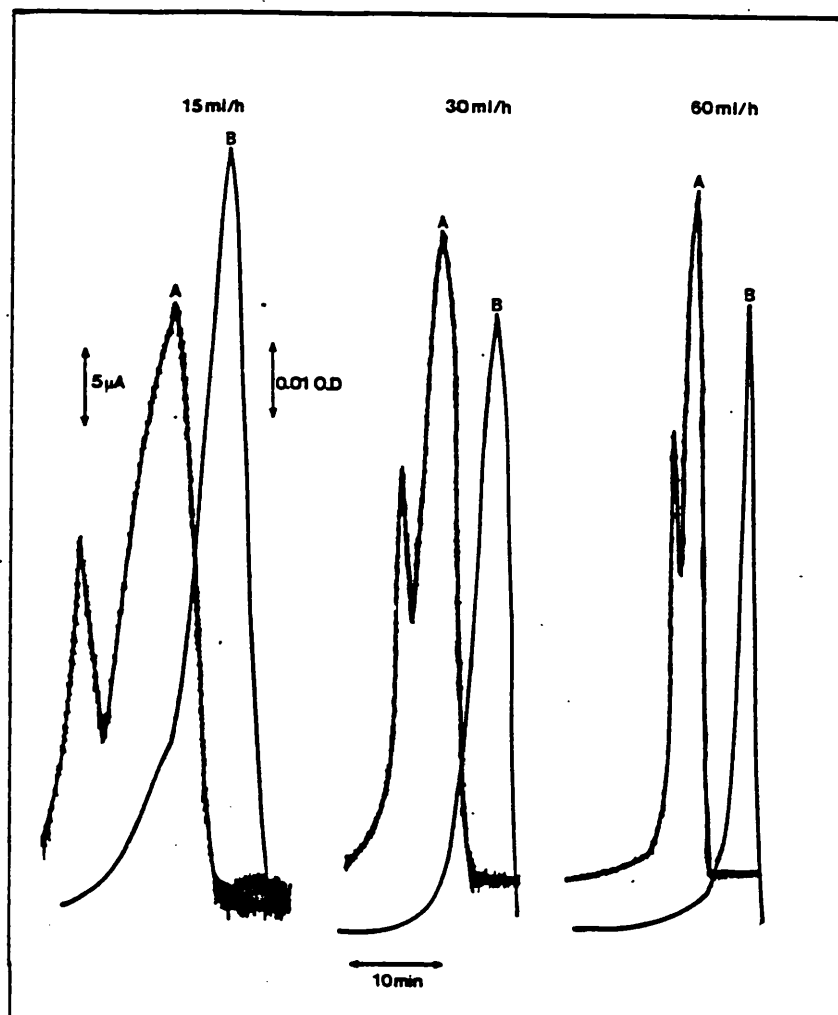


Fig 5.28 Typical results: for the separation of catalase and ribonuclease on a Pharmacia C10/13 column packed with Sephacryl 200 Super fine. Curve A is the Autotitrator and curve B is the uv-detector

It is interesting to note that the resolution seems higher for the Autotitrator than for the uv-detector. The actual resolution is determined by the chromatographic system and is the same for the two detectors. The apparent resolution is determined by the sensitivity for the minor component. This is simulated in figure 5.29 where the minor component is plotted for two different sensitivities. The

Autotitrator brominates electrophilic active sites in proteins ,typical groups are ;

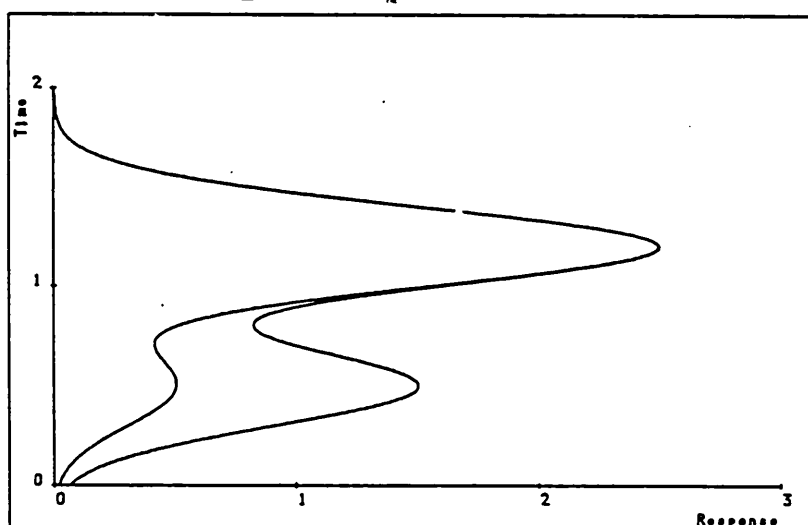
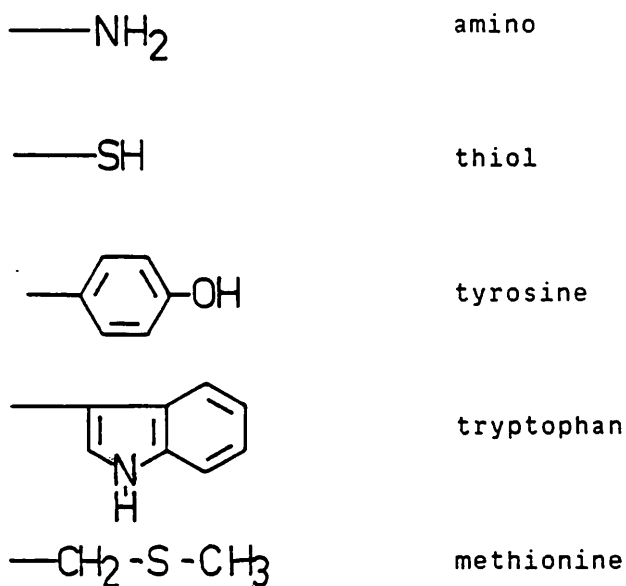


Fig 5.29 Computer simulation of two overlapping gaussian peaks with the relative peak heights 1:3 for the minor peak

In contrast ,the uv-detector relies on the chromophoric groups at 280 nm , which are mainly tyrosinyl and tryptophanyl groups. The autotitrator senses , in most cases , more groups in a protein than absorption at 280 nm does , and therefore has a higher intrinsic sensitivity .

The area under the peaks from the Autotitrator are proportional to the concentration of the analysed species. We have derived a formula for the protein concentration from the peak area (108).

$$[P] = \frac{V_f^{1/4}}{f B m'} \int_0^{\infty} i_{d,p} dt \quad [5.35]$$

where f = the fraction of the eluted solution that passes through the WJRDE

$$B = 2.76 \beta^{2/3} N_0^{-1} F D^{2/3} u^{-5/12} a^{-1/2} r_1^{3/4}$$

$$\beta = (r_3/r_1)^{9/8} - (r_2/r_1)^{9/8}$$

u = the kinematic viscosity

a = the diameter of the jet

$$m' = m(D_p/D_{As})^{2/3}$$

$$D = D_{As} = 0.98 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1} \text{ @ } 25^\circ \text{C}$$

m' is the "observed bromine number", i.e. the number of the molecules of bromine that have reacted with the protein. This number is the product of the "true stoichiometric bromine number" and the (diffusion coefficients)^{2/3}. Note that $[P]$ can be measured without calibrating the column and the detector with a standard solution of pure protein. We have tested this procedure for the catalase system. When 0.2 nmol of catalase were injected onto the column at a flowrate of $15 \text{ cm}^3 \text{ h}^{-1}$, we found that $[P]$ calculated from eqn [5.35] was 0.18 nmol. This shows that reasonable estimations of quantities of protein may be obtained from peak integration. Note also the sensitivity of the technique.

An interesting development is to record the response of the Autotitrator versus that of the uv-detector. This yields not only a more reliable quantitative measurement but also some qualitative information. Most proteins have different responses to uv and auto-

titration , therefore a "mapping" of the different proteins would enable an identification. Figure 5.30 shows a typical result when optical density (OD) is plotted against the Autotitrator. The cigar shape comes from the time lag between the two detectors as they were connected in series. The different proteins give "cigars" of different sizes and at different angles. A parallel detector arrangement gets rid of the "cigars" but also half the sensitivity because the eluent stream is split in two.

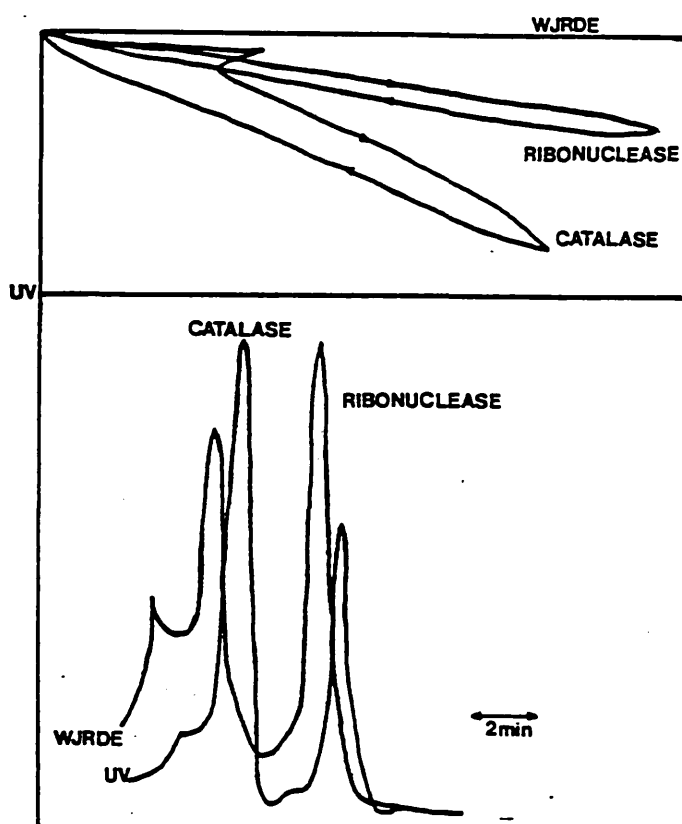


Fig 5.31 Comparison of the output of the Autotitrator and the uv detector for a mixture of ribonuclease and catalase eluting from a Pharmacia C10/40 column packed with Sepha-cryl 200 Superfine. The upper diagram shows the output of the uv-detector plotted against the Autotitrator , and the lower is the y-t recorder plot.

5.9.2 Digital Autotitrator

The digital Autotitrator was assessed in a Flow Injection Analysis (FIA) system, illustrated in fig 5.32, which employed a Rehodyne injection valve with a 10 μ l sample loop and a 3 m 0.5 mm I.D PTFE tubing coil. The flow from the peristaltic pump was pulse damped before entering the FIA system. The coil length was determined empirically and gave well shaped FIA peaks.

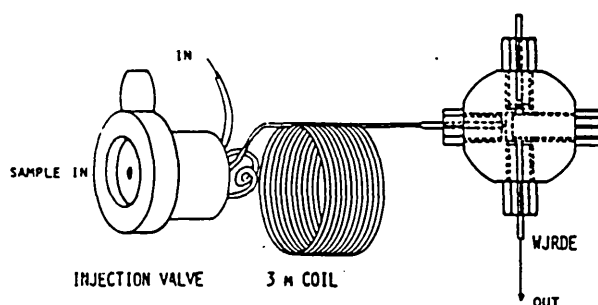


Fig 5.31 The FIA system

Initial autotitration experiments with the protein cytochrome C showed poor sensitivity and resolution. These factors are determined by the DAC which controls the disc current (which is proportional to the concentration of the protein). The CMU interface uses a 12-bit DAC with a ± 10 V range, described in chapter 3. This DAC gives a LSB resolution of

$$\text{LSB} = 20000/2^{12} = 4.88 \text{ mV/bit}$$

which the galvanostat converts to

$$\text{LSB} = 4.88/10 \text{ k}\Omega = 4.88 \text{ }\mu\text{A/bit}$$

Recalling the equation for the protein current, i_p , [2.2.11]

$$i_p = 1.6knFD^{-2/3}v^{-5/12}v_f^{3/4}a^{-1/2}r_1^{3/4} [P]$$

with typical values for cytochrome c equation [2.2.11] yields

$$i_p = 3.9[P]$$

The constant's unit is $\mu\text{M}/\mu\text{A}$, which gives the detection limit for

cytochrome c

$$[P]_{\min} = 4.88/3.9 = 1.2 \mu\text{M}$$

This protein was easily detected at much lower levels with the analog Autotitrator. In order to improve the resolution following amendments were made;

- i) The DAC and ADC were changed to cover the range 0 to 10000mV
- ii) The galvanostat and potentiostat gains were changed by a factor of 10

This improved the LSB resolution to 0.244 $\mu\text{A/bit}$, which corresponds to a detection limit for cytochrome c of about 60 nM. The detection limit depends on the molecular weight, the diffusion coefficient and the bromine number of the analysed species, but will in general deteriorate with increasing molecular weight. Nevertheless experiments were carried out with the digital Autoanalyser and gave reasonable results, which shows that it can be used as a root death detector in NFT. Typical results for the proteins cytochrome c (MW=12348) and protease (MW not known) are shown in fig 5.32 and fig 5.33.

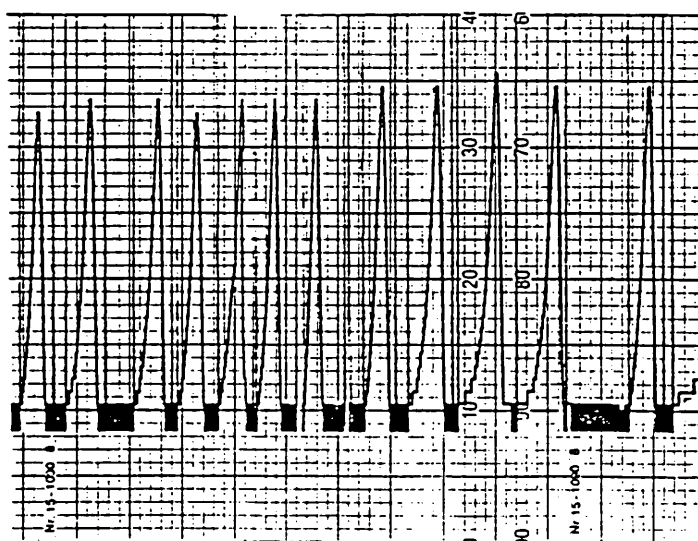


Fig 5.32 Typical results for Autotitration of cytochrome c

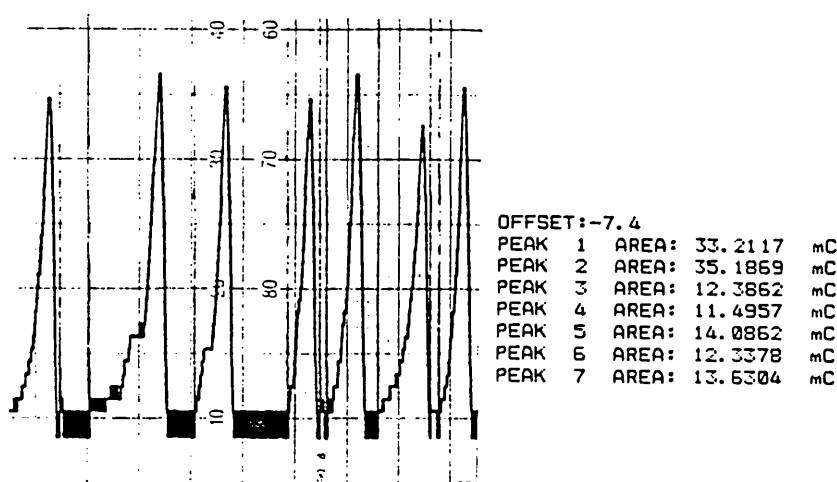


Fig 5.33 Typical results for Autotitration of protease.

24 μ g protease was injected in the FIA system

Another disadvantage in the digital implementation is the small dynamic range; The set-point in the control algorithm must be in the linear range of the titration curve. The commencement of the linear region for a titration curve is found by combining equations [2.2.10] and [2.2.11], which yields an expression which depends on the protease current i_p

$$i_{r,\text{set-point}} = \frac{i_p \beta^{2/3}}{N_0 N' - 1} \quad [5.36]$$

If we anticipate a high protein concentration in the peak a high $i_{r,\text{setpoint}}$ is required. This means that a fraction of the DAC range will be used by the control condition, which at high set-point might cut down the DAC's dynamic range to less than 2000 which is unsatisfactory in chromatography but probably useful in NFT.

These findings show that that an analog Autotitrator with a microcomputer for the data analysis is the best combination.

5.11 Conclusions

In this section we summarise the findings of the work described in this chapter.

We have designed and built a novel microtitration Autotitrator, which can be used successfully as a detector in liquid column chromatography of biochemically interesting species such as proteins, amino acids and peptides, and enables a direct concentration reading from the peak area without calibration. It can also yield qualitative information if it is used together with a conventional uv-detector.

We have found, despite the current trend to computerise everything, that the analog Autotitrator performs better than its digital counterpart. This is mainly due to the limited dynamic range of Analog to Digital and Digital to Analog converters. Nevertheless the digital Autotitrator works satisfactorily for hydroponic work and performs automatic peak integration and has a dynamic control algorithm. The best combination is an analog Autotitrator with computer controlled data acquisition and conversion.

A packed bed wall jet electrode has been designed and built. This electrode system has a higher intrinsic sensitivity than the wall jet ring disc electrode, but has a slow response and experimental result does not agree with theory.

APPENDIX I

The LaPlace transform of a function of time $f(t)$ is written as $F(s)$ and is defined as

$$F(s) = [f(t)] = \int_0^{\infty} f(t)e^{-st} dt \quad [I.1]$$

where $s = \sigma + j\omega$ is an arbitrary complex variable, . The complex variable s must be sufficiently large to ensure that the integral converges. Table I.1 list the LaPlace transform pairs of most importance in control work. Some important theorms are often used in control. The final value theorem ;

$$\lim_{t \rightarrow \infty} f(t) = \lim_{s \rightarrow 0} sF(s) \quad [I.2]$$

and the shifting theorem

$$\text{If } [f(t)] = F(s) \text{ then } [f(t-T)] = e^{-sT}F(s) \quad [I.3]$$

Inverse LaPlace transformation, $^{-1}[F(s)] = f(t)$, is required to obtain the time response. Transformed solutions will in general be a ratio of two polynomials:

$$F(s) = \frac{a_m s^m + a_{m-1} s^{m-1} + \dots a_0}{s^n + b_{n-1} s^{n-1} + \dots b_0} \quad [I.4]$$

When this is not in a form which can be found in the available tables it must be split by partial fraction expansion into a number of functions which are listed in the tables;

$$F(s) = F_1(s) + F_2(s) + \dots \quad [I.5]$$

therefore

$$f(t) = ^{-1}[F_1(s)] + ^{-1}[F_2(s)] + \dots \quad [I.6]$$

The basic z-transform may be defined as;

$$Z[f_i] = F(z) = \sum_{i=0}^{\infty} f_i z^{-i} \quad [1.7]$$

and f_i must be defined for all values $i \geq 0$ and be zero for $i < 0$. The property of convergence must be satisfied, this states that

$$Z[f_i] \text{ exist if } \lim_{k \rightarrow \infty} \sum_{i=0}^k f_i z^{-i} \text{ exist}$$

An important theorem is the shift theorem

$$Z[f_{i+k}] = z^k F(z) - \sum_{i=0}^{k-1} f_i z^{k-i-1}$$

The z-transform is usually solved by partial fraction expansion and tables. Table I.1 list the most used z-transforms

Table I.1
Table of z-transforms and s-transforms

$f(t)$	$F(s)$	$F(z)$
1	$1/s$	$1/(1 - z^{-1})$
t	$1/s^2$	$T(z^{-1} - z^{-2})^{-2}$
e^{-at}	$1/(s + a)$	$1/(1 - e^{-aT} z^{-1})$
te^{-at}	$(s + a)^{-2}$	$Te^{-aT} z^{-1} (1 - e^{-aT} z^{-1})^{-2}$
$1 - e^{-at}$	$a(s^2 + sa)^{-1}$	$\frac{(1 - e^{-aT})z^{-1}}{(1 - z^{-1})(1 - e^{-aT} z^{-1})}$

APPENDIX II

PROGRAMS

This is a listing of the Autoanalyser program NAIE. The extended BASIC interface commands are explained in the next section.

```

1 CALL"IDENT",0,0:REM Initiate interface
3 CALL"WRIT16",12,1,0,0:REM Select millisecond timer
4 CALL"WRITE8",12,1,2,2
5 CALL"WRITE8",12,1,3,0
6 CALL"SETTR",12,1,0,0:REM Select external trigger
8 CHANNEL=6:OFFSET=0:REM Initial values
10 DATA B,C,D:SIDE$="A":REM Data for disc side control
12 ON BREAK GOTO 11000:REM Jump out subroutine
14 CALL"IDENT",0,0
18 LNULL 10,127
20 NULL 10,32:REM Offset screen 10 columns
22 PUT 27,"=OJ":?CHR$(31)
24 PLOT10,45,"*****"
26 PLOT10,42,"*"
28 PLOT 10,39,"* Welcome to LRS Ion Sel- *"
30 PLOT10,36,"* ective Electrode Data log- *"
32 PLOT10,33,"* ging program. *"
34 PLOT10,30,"*"
36 PLOT10,27,"*****"
38 CALL"WAIT",500:REM &&&&&&&& Wait 10 sec.
40 ?CHR$(31):REM &&&&&&&& Clear screen
42 PLOT 10,45,"*****"
44 PLOT 10,42,"*"
46 PLOT 10,39,"* This program measures the *"
48 PLOT 10,36,"* activity of the nutrient *"
50 PLOT 10,33,"* solution every hour.The *"
52 PLOT 10,30,"* data is stored on disc or *"
54 PLOT 10,27,"* hard copy. *"
56 PLOT 10,24,"* The system is calibrated *"
58 PLOT 10,21,"* 6.00 a.m and 6.00 p.m *"
60 PLOT 10,18,"*"
62 PLOT 10,15,"*****"
64 CALL"WAIT",500
66 ?CHR$(31)
68 PLOT 10,45,"*****"
70 PLOT 10,42,"*"
72 PLOT 10,39,"* If you wish to switch off *"
74 PLOT 10,36,"* the system,remove the dis- *"
76 PLOT 10,33,"* cs first , then you may *"
78 PLOT 10,30,"* switch off the mains. *"
80 PLOT 10,27,"*"
82 PLOT 10,24,"*****"
84 CALL"WAIT",500:?CHR$(31)
86 PLOT 10,39,"*****"
88 PLOT 10,36,"*"

```

```

90 PLOT 10,33,"* Lets proceed to the sta-  *"
92 PLOT 10,30,"* rt up procedure.          *"
94 PLOT 10,27,"*                           *"
96 PLOT 10,24,"*****"
98 CALL "WAIT",250: ?CHR$(31):
100 PUT 27,"=1J":NULL 20,32: ?CHR$(31)
102 INPUTLINE "OPERATOR:",OPRATR$
104 IF LEN(OPRATR$)=0 THEN ?"NO CHEATING,TYPE IN YOUR NAME":GOTO 102
106 ?::?
108 ?"DATE ENTRY USING NUMBERS"
110 INPUT "YEAR:",YEAR
112 INPUT "MONTH:",MNTH
114 INPUT "DAY:",DAY
116 ?::?
118 ?"TIME ENTRY USING THE 24 HOUR CLOCK"
120 INPUT "HOUR:",HOUR
122 IF HOUR<0 OR HOUR>24 THEN ?"REMEMBER IT IS 24 HOURS PER DAY,TRY
    AGAIN!":GOTO 120
124 INPUT "MINUTE:",MINUTE
126 IF MIN<0 OR MIN>60 THEN ?"REMEMBER IT IS 60 MIN PER HOUR,TRY
    AGAIN!":GOTO124
128 INPUT "SECOND:",SECND:GOSUB 2000:REM Start clock
130 ?::?
132 ?CHR$(31);"                HOW DO YOU LIKE TO STORE THE RESULTS ?"
134 ?::?:INPUT LINE"ENTER:DISC , HARDCOPY OR BOTH :",KEEP$
136 IF KEEP$<>"DISC" AND KEEP$<>"HARDCOPY" AND KEEP$<>"BOTH" THEN
    ?"ILLEGAL ENTRY,TRY AGAIN !":GOTO 134
138 IF KEEP$="HARDCOPY" GOTO 148
140 INPUTLINE"ENTER FILENAME FOR DATAFILE:",FLNME$
142 IF LOOKUP(FLNME$)=0 GOTO 148
144 ?FLNME$" EXIST , TRY AGAIN !":GOTO 140
148 PRINTER 4,4:LNUL 10,127:REM &&&&&&&&& Set indent to 10
150 LPRINT
152LPRINT "-----"
    "-----"
154LPRINT"|
    |"
156LPRINT"|                LRS ISE DATA ACQUISITION PROGRAM
    |"
158LPRINT"|                =====
    |"
160LPRINT "|+++++|
    +++++|"
162LPRINT "|
    |"
164LPRINT"|                DATE:"DAY"/"MNTH"/"YEAR"
    TIME:"HOUR": "MIN;TAB(68);"|"
166LPRINT"|
    |"
168 IF KEEP$="HARDCOPY" GOTO 174
170 LPRINT "|
    OPERATOR:"OPRATR$;TAB(40);"DATAFILE:"FLNME$;TAB(68);"|"
172 GOTO 176
174 LPRINT "|                OPERATOR:"OPRATR$                ;TAB(68);"|"
176 LPRINT"|
    |"

```

```

178LPRINT " | ++++++|
+++++|"
180LPRINT " |
|"
182 LPRINT " | CHECK LIST
|"
184 LPRINT " | =====
|"
186 ?CHR$(31)
188 ?::?
190 PUT 27,"=OJ":REM &&&&&&&&& 40 char screen
192 ?CHR$(31)
194 PLOT 10,45,"*****"
196 PLOT 10,42,"* "
198 PLOT 10,39,"* We now proceed with the * "
200 PLOT 10,36,"* check list.Answer every * "
202 PLOT 10,33,"* question and take the * "
204 PLOT 10,30,"* necessary action. * "
206 PLOT 10,27,"* "
208 PLOT 10,24,"*****"
210 LPRINT" |-----| "
212 CALL"WAIT",250
214 PUT 27,"=IJ":?CHR$(31):NULL 5,32:REM 40 char screen, clear and
5 indentation
216 ?::?:?
218 INPUT "ESTIMATE THE VOLUME (in ml) OF LO CALIBRATION SOLUTION:",VOL
220 ?::?" THIS WILL LAST "INT(VOL/20)" MEASUREMENTS OR
"INT(VOL/480)" DAYS"
222 ?::?
224 LPRINT" |";TAB(5);"LO CALIBRATION SOLUTION FOR "INT(VOL/20)"
CALIBRATIONS OR "INT(VOL/480)" DAYS";TAB(68);" |"
226 INPUT "ESTIMATE THE VOLUME (in ml) OF HI CALIBRATION SOLUTION:",VOL
228 ?::?" THIS WILL LAST "INT(VOL/20)" CALIBRATIONS OR
"INT(VOL/40)" DAYS"
232 LPRINT " |";TAB(5);"HI CALIBRATION SOLUTION FOR "INT(VOL/20)"
CALIBRATIONS OR "INT(VOL/40)" DAYS";TAB(68);" |"
234 ?::?
236 ?"IF MORE SOLUTION IS NEEDED PLEASE SEE MANUAL FOR INSTRUCTIONS"
238 CALL"WAIT",300: ?CHR$(31)
240 ?::?
242 ?"CAN YOU HEAR THREE CLICKING NOISES FROM THE SOLENOID VALVE BOX ?"
244 FOR N=0 TO 2
246 CALL"WRITE8",64,1,0,2^N+0.1:REM Output to valves
248 ZZ$=GET$(30)
250 IF ZZ$="Y" THEN GOTO 264
252 IF ZZ$="N" THEN GOTO 258
254 NEXT N
256 GOTO 244
258 ?"CHECK CONNECTIONS AGAIN !"
260 ?::?"IS IT OK NOW (PRESS Y FOR YES/N FOR NO) ?"
262 GOTO244
264 CALL"WRITE8",64,1,0,0:REM Switch off valves
266 LPRINT" |";TAB(10);"SOLENOID VALVES OK";TAB(68);" |"
268 ?" GOOD"
270 CALL"WAIT",200
272 ?CHR$(31)

```

```

274 ? : ?
276 ? "CHECK TUBING FROM CALIBRATION SOLUTIONS TO THE FLOW CELL"
277 ? "CHECK ALSO THAT THE VALVE IS IN THE RIGHT POSITION"
278 INPUTLINE "          OK (YES/NO) ? ", A$
280 IF A$ <> "NO" AND A$ <> "YES" THEN ? "ILLEGAL ENTRY, TYPE YES
    OR NO": GOTO 278
282 IF A$ = "NO" THEN ? "CHECK WITH THE MANUAL FOR CORRECT
    CONNECTIONS": GOTO 278
284 LPRINT "|"; TAB(10); "TUBING OK"; TAB(68); "|"
286 ? CHR$(31): ? : ?
288 CALL "WRITE8", 64, 1, 0, 4: REM &&&&&&&&& Switch to nutrient solution
290 CALL "WRIT16", 1, 1, 0, 300: REM &&&&&&&&& Switch on pump
292 ? "IS THE PUMP WORKING NOW (YES/NO) ? "
293 INPUTLINE "(CHECK ALSO IF THE FLOW FROM THE GREEN HOUSE IS OK)", A$
294 IF A$ <> "NO" AND A$ <> "YES" THEN ? "ILLEGAL ENTRY, TYPE YES
    OR NO": GOTO 292
296 IF A$ = "NO" THEN ? "CHECK 1) MAINS 2) SWITCH ON PUMP 3) COMPUTER
    CONNECTION (socket 0a on board DAC-1)": GOTO 292
298 ? "          WELL DONE !": CALL "WAIT", 200
300 LPRINT "|"; TAB(10); "PUMP OK"; TAB(68); "|"
302 CALL "WRIT16", 1, 1, 0, 0: REM &&&&&&&&& Switch off pump
304 ? CHR$(31): ? : ?
306 INPUTLINE "IS THE FLOW CELL FILLED WITH SOLUTION (YES/NO) ? : ", A$
308 IF A$ <> "NO" AND A$ <> "YES" THEN ? "ILLEGAL ENTRY, TYPE YES
    OR NO": GOTO 306
310 IF A$ = "YES" GOTO 324
312 ? "THE PUMP IS NOW PUMPING , PLEASE PRESS THE RETURN KEY WHEN"
314 ? "THE FLOW CELL IS FILLED"
316 CALL "WRIT16", 1, 1, 0, 500: REM &&&&&&&&& Switch on pump
318 ZZ = GET()
320 CALL "WRIT16", 1, 1, 0, 0: REM &&&&&&&&& Turn off pump
322 ? "          WELL DONE !": CALL "WAIT", 100
324 LPRINT "|"; TAB(10); "FLOW CELL OK"; TAB(68); "|"
326 ? CHR$(31): ? : ?
328 INPUTLINE "IS THE FLOW FROM THE GREEN-HOUSE OK (YES/NO) ? : ", A$
330 IF A$ <> "NO" AND A$ <> "YES" THEN ? "ILLEGAL ENTRY, TYPE YES
    OR NO": GOTO 328
332 IF A$ = "NO" THEN ? "CHECK 1) PUMP IN GREENHOUSE 2) FILTER": GOTO 328
334 LPRINT "|"; TAB(10); "FLOW FROM GREEN HOUSE OK"; TAB(68); "|"
336 ? CHR$(31): ? : ?
338 IF KEEP$ = "HARDCOPY" GOTO 360
340 INPUTLINE "HAVE YOU REMOVED THE PROGRAM DISC AND INSERTED THE DATA
    DISCS ? ", A$
342 ? "DON'T FORGET TO CLOSE THE DOORS ON THE DISC DRIVES"
344 IF A$ <> "NO" AND A$ <> "YES" THEN ? "ILLEGAL ENTRY, TYPE YES
    OR NO": GOTO 340
346 IF A$ = "NO" THEN ? : ? : ? "THE DATA DISCS MUST RESIDE IN THE
    DRIVES !": GOTO 340
348 ZZ = SIZE("A:") + SIZE("B:") + SIZE("C:") + SIZE("D:")
350 ? "THERE IS "ZZ" KBYTE OF DISCSpace AVAILABLE"
352 ? "THIS WILL LAST "ZZ*8" MEASUREMENTS OR "INT(ZZ*8/24)" DAYS"
354 LPRINT "|"; TAB(10); "DATA DISCS IN DRIVES , WITH "ZZ" KBYTE OF FREE
    MEMORY"; TAB(68); "|"
356 LPRINT "|"; TAB(10); "DISC SPACE FOR "ZZ*8" MEASUREMENTS
    OR "INT(ZZ*8/24)" DAYS"; TAB(68); "|"
357 RESET: CREATE #10, FLNME$: QUOTE #10, 34

```

[illegible]

[illegible]

[illegible]

```

1390 CALL "WRIT16",1,1,0,0:REM &&&&&&& Swich off pump
1400 TFLAG$="WAITING FOR ELECTRODE EQUILIBRIUM"
1410 GOSUB 4000:REM &&&&&&& Goto wait sub
1420 LPRINT";";TAB(18);";";TAB(20);" ISE";TAB(29);";";TAB(31);"LOW
    EMF(mV)";TAB(43);";";TAB(68);";"
1430 LPRINT";";TAB(18);";|-----|-----|";TAB(68);";"
1440 FOR N=0 TO CHANNEL
1450 CALL "WRITE8",8,1,0,2~N+.5:REM &&&&&&& Select channel
1460 GOSUB 10000:REM &&&&&&& Read EMF
1470 CAL(N,1)=EMF:REM Store result in matrix CAL(N,1) row 1
1480 LPRINT";";TAB(18);";";TAB(20);ISE$(N);TAB(29);";";TAB(31);EMF;
    TAB(43);";";TAB(68);";"
1490 LPRINT";";TAB(18);";|-----|-----|";TAB(68);";"
1500 NEXT N
1510 LPRINT"|-----|
    -----|"
1520 LPRINT";";TAB(20);"CALIBRATION RESULT";TAB(68);";"
1530 LPRINT"|-----|
    -----|"
1540 LPRINT";";TAB(12);";";TAB(14);"ISE";TAB(23);";";TAB(25);"
    SLOPE ";TAB(34);";";TAB(36);" EO ";TAB(45);";";TAB(68);";"
1550 LPRINT";";TAB(12);";|-----|-----|-----|";TAB(68);";"
1560 FOR N=1 TO CHANNEL
1570 CAL(N,2)=CAL(0,1)*(CAL(N,1)-CAL(N,0))*LOG(10)/(CAL(0,0)*LOG(LO(N,0)*
    LO(N,1)/(HI(N,0)*HI(N,1))))
1580 REM &&&&&&& Line 410 calculates the response slope
1590 REM &&&&&&& The formulae is
1600 REM &&&&&&& Slope=T1o*(Elo-Ehi)*log(10)/Thi*log(gammalo*C1o/
    gammahi*Chi)
1610 CAL(N,3)=CAL(N,1)-CAL(N,2)*LOG(LO(N,1)*LO(N,0))/LOG(10)
1620 REM &&&&&&& Line 430 calculates the EO
1630 REM &&&&&&& The formulae is
1640 REM &&&&&&& EO=E-Slope*log(a)/log(10)
1650 RESULT=CAL(N,2):OS=3:REM &&&&&&& Transfer slope to filter sub
1660 GOSUB 6000:REM &&&&&&& Goto filter sub
1670 IF FLAG=1 THEN WARNING=1:REM Unsuccesful calibration if FLAG=1
1690 RESULT=CAL(N,3):OS=0:REM &&&&&&& Transfer EO to filter sub
1700 GOSUB 6000:REM &&&&&&& Goto filter
1710 IF FLAG=1 THEN WARNING=1
1730 LPRINT";";TAB(12);";";TAB(14);ISE$(N);TAB(23);";";TAB(25);CAL(N,2);
    TAB(34);";";TAB(36);CAL(N,3);TAB(45);";";TAB(68);";"
1740 LPRINT";";TAB(12);";|-----|-----|-----|";TAB(68);";"
1750 NEXT N
1760 IF WARNING=1 AND ATTEMPT=1 THEN ATTEMPT=2:GOTO 1830
1770 IF WARNING=1 AND ATTEMPT=2 THEN GOTO 1800:REM Only 2 cal attempts
1780 LPRINT"|*****
    *****|"
1790 RETURN
1800 LPRINT"|*****
    *****|"
1810 LPRINT";";TAB(20);"RECALIBRATION UNSUCCESSFUL";TAB(68)"|"
1820 RETURN
1830 LPRINT"I|*****
    *****|"
1840 GOSUB 3000:REM &&&&&&& Read clock
1850 LPRINT";";TAB(10);"RECALIBRATION STARTED "DAY"/"MNTH"/"YEAR;

```

[illegible]

```

3270 IF DAY=31 AND MNTH=11 THEN GOSUB 3300
3280 IF DAY=32 THEN GOSUB 3300
3290 RETURN
3300 REM***** Update month and year
3310 MNTH=MNTH+1
3320 DAY=1
3330 IF MNTH>12 THEN GOTO 3350
3340 RETURN
3350 YEAR=YEAR+1
3360 MNTH=1
3370 RETURN
4000 REM *****-WAIT ROUTINE-*****
4010 REM & This routine looks for the flag TFLAG$ which &
4020 REM & tells what sort of routine it is going to be. &
4030 REM & Then TIME is calculated as the sum of the &
4040 REM & DELAY and the real time MIN. &
4050 REM *****
4060 ?CHR$(31)
4070 IF TFLAG$="PUMPING CALIBRATION SOLUTION" THEN DELAY=3
4080 IF TFLAG$="PUMPING SAMPLE SOLUTION" THEN DELAY=6
4090 IF TFLAG$="WAITING FOR ELECTRODE EQUILIBRIUM" THEN DELAY=10
4095 PLOT 45,30,TFLAG$
4097 PLOT 59,25,RIGHT$(STR$(DELAY),2)+" MIN TO GO"
4100 GOSUB 3000 :REM ***** Read real time clock
4110 TIME=MIN
4120 GOSUB 3000:REM Read clock
4130 IF TIME= MIN THEN GOTO 4120
4140 TIME=MIN:DELAY=DELAY-1:REM DELAY is decremented every minute
4150 IF DELAY=0 THEN GOTO 4180:REM Timing is finished when DELAY=0
4160 PLOT 59,25,RIGHT$(STR$(DELAY),2)+" MIN TO GO"
4170 GOTO 4120
4180 ?CHR$(31):PLOT 55,25,"TIMING SEQUENCE FINISHED"
4190 RETURN
5000 REM ***** MEASUREMENT PROGRAM *****
5010 REM & This program calibrates the system before &
5020 REM & the measurement.All temperatures are com- &
5030 REM & compensated for the temperature at &
5040 REM & calibration L0.The activity is filtered &
5050 REM & in a 1st order recursive filter in a sub- &
5060 REM & routine .If the E0 value is outside the &
5070 REM & 3-sigma value of the running average &
5080 REM & filter,it will recalibrate,and if that is &
5090 REM & unsuccessful an error report is printed. &
5100 REM*****
5120 REM***** Start 1-point calibration *****
5130 CALL"WRITE8",64,1,0,2:REM ***** Select L0 cal soln
5140 CALL"WRITE16",1,1,0,500:REM ***** Swich on pump
5150 TFLAG$="PUMPING CALIBRATION SOLUTION"
5160 GOSUB 4000:REM ***** Goto wait sub
5170 CALL"WRITE16",1,1,0,0:REM ***** Swich off pump
5180 TFLAG$="WAITING FOR ELECTRODE EQUILIBRIUM"
5190 GOSUB 4000:REM ***** Goto wait sub
5200 FOR N=0 TO CHANNEL
5210 CALL"WRITE8",8,1,0,2^N+.5:REM ***** Select channel
5220 CALL"WAIT",10
5230 GOSUB 10000:REM ***** Read EMF

```

[illegible]


```
6250 LPRINT"|";TAB(10);ISE$(N)" CALIBRATION ERROR.CALIBRATION  
OUTSIDE ERROR LIMIT";TAB(68);""|"  
6260 LPRINT"|";TAB(10);ISE$(N)" EO="RESULT" mV";TAB(68);""|"  
6270 LPRINT"|";TAB(10);"AVERAGE="AVERAGE" mV";TAB(68);""|"  
6275 LPRINT"|------  
-----|" "  
6280 IF OS=0 GOTO 6360  
6290 IF ABS(RESULT)>SMIN(N) GOTO 6360  
6300 FLAG=1  
6310 LPRINT"|------  
-----|" "  
6320 LPRINT"|";TAB(25);"ERROR REPORT";TAB(68);""|"  
6330 LPRINT"|";TAB(10);ISE$(N)" ERROR.RESPONSE-SLOPE LESS THAN  
MINIMUM.";TAB(68);""|"  
6340 LPRINT"|";TAB(10);"RESPONSE-SLOPE="ABS(RESULT)"mV/dec.  
MINIMUM="SMIN(N)"mV/dec";TAB(68);""|"  
6345 LPRINT"|------  
-----|" "  
6360 FLTR(N,OS+2)=FLTR(N,OS+1):FLTR(N,OS+1)=FLTR(N,OS+0):  
FLTR(N,OS+0)=RESULT  
6370 RETURN  
6380 REM &&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&  
6390 FLTR(N,0+OS)=RESULT*1.2:FLTR(N,1+OS)=RESULT*0.8:FLTR(N,2+OS)=RESULT  
6395 LPRINT"|------  
-----|" "  
6400 IF OS=0 THEN LPRINT"|";TAB(12);ISE$(N)" EO-FILTER RUNNING  
";TAB(68);""|"  
6410 IF OS=3 THEN LPRINT"|";TAB(12);ISE$(N)" RESPONSE SLOPE  
FILTER RUNNING ";TAB(68);""|"  
6420 IF OS<>3 AND OS<>0 THEN LPRINT"|";TAB(10);ISE$(N)" FILTER  
OFFSET ERROR ";TAB(68);""|"  
6421 IF OS=0 THEN LPRINT"|------  
-----|"":RETURN  
6422 IF ABS(RESULT)>SMIN(N) GOTO 6428  
6423 FLAG=1  
6424 LPRINT"|------  
-----|" "  
6425 LPRINT"|";TAB(25);"ERROR REPORT";TAB(68);""|"  
6426 LPRINT"|";TAB(10);ISE$(N)" ERROR.RESPONSE-SLOPE LESS  
THAN MINIMUM.";TAB(68);""|"  
6427 LPRINT"|";TAB(10);"RESPONSE-SLOPE="RESULT"mV/dec.  
MINIMUM="SMIN(N)"mV/dec";TAB(68);""|"  
6428 LPRINT"|------  
-----|" "  
6430 RETURN  
10000 REM &&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&  
10010 REM & This subroutine converts the reading from&  
10020 REM & the V-f converter to a voltage in mV &  
10022 REM & The system is calibrated prior a reading &  
10030 REM &&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&&  
10031 CALL "WRITE8",8,1,0,128:REM Select channel 7  
10032 CALL "SETTR",3,1,0,1:REM Start counting  
10033 CALL "WAIT",300:REM Wait until updated  
10034 CALL "SETTR",3,1,0,0:REM Stop counting  
10035 CALL "READ16",3,1,0,VARADR(COUNT):REM Read count for offset  
10036 IF SGN(COUNT)=-1 THEN COUNT=COUNT+65536
```

```

10037 IF OFFSET=0 THEN OFFSET=COUNT:REM Starts filter
10038 OFFSET=OFFSET+(COUNT-OFFSET)/3:REM Recursive filter
10039 CALL"SETTR",3,1,0,1:REM Restore counting
10040 CALL"WRITE8",8,1,0,2^N+0.5:REM Select channel
10045 CALL"WAIT",300:REM Make sure V-f is updated
10050 CALL"SETTR",3,1,0,0:REM Prevents updating
10060 CALL"READ16",3,1,0,VARADR(COUNT):REM Read V-F
10065 IF SGN(COUNT)=-1 THEN COUNT=COUNT+65536:REM Convert to
      unsigned binary
10070 EMF=(COUNT-OFFSET)/51.8738
10080 CALL"SETTR",3,1,0,1:REM Restore counting
10090 RETURN
11000 ?CHR$(31)
11010 INPUT LINE"DO YOU WISH TO FINISH OR STOP TEMPORARILY
      (FINISH/STOP)";A$
11020 IF A$<>"STOP" AND A$<>"FINISH" THEN ?"ENTER FINISH OR
      STOP !":GOTO11010
11030 IF A$="FINISH" GOTO 11060
11040 ?"PRESS THE RETURN KEY WHEN YOU ARE READY,THE PROGRAM
      RETURNS TO THE NEXT MEASUREMENT"
11050 A$=GET$():RESET:?CHR$(31)
11055 ON BREAK GOTO 11000:GOTO 532
11060 CLOSE#10
11070 GOSUB 3000
11080 LPRINT"|-----"
      -----|"
11090 LPRINT"|";TAB(10);"DATA ACQUISITON FINISHED "DAY"/"MNTH"/"YEAR"
      AT "HOUR": "MIN";TAB(68);"|"
11100 LPRINT"|-----"
      -----|"

```

INTERFACE SOFTWARE

For the Chemistry Microprocessor Unit Interface System

Software for the interface system can be divided into two main classes:

- (1) General purpose routines
- (ii) Special purpose routines

The general routines are those which can be used with more than one type of board, or even more generally, with any type of board, and the special routines can be used only with one type of board or a specific combination of boards.

1 General purpose routines

The general purpose routines can be further sub-divided into two classes:

1.1 Universal Routines

These can operate on any board, regardless of its type.

1.2 Restricted Routines

These can only operate on a specified sub-range of types of board. Normally, these would be routine to control the interrupt system on boards that are capable of generating interrupts.

We will now give a basic set of general-purpose routines that can be used to assemble more complex special-purpose routines.

1.3 General-purpose Subroutine Descriptions

A number of formal representations of parameters are used in this section.

These are:

- (a) T = Type number (0 - 255)
- (b) N = Number of type (1 - 128)

- (c) P = Port number (0 - 7)
- (d) B = Bit number (0 - 15)
- (e) D = 8/16 Bit data

(f) X = Variable for returned data (8/16 bit)

1.3.1 IDENT

Format: IDENT,A1,A2

This routine performs a number of useful functions:

(a) It identifies all the boards in all the racks on the VDU and/or the printer, if these output devices are enabled by the arguments A1 and A2.

(b) It initialises the interruptsystem, disabling all board interrupts and the CPU maskable interrupt.

(c) It creates tables in memory which are used by the interrupt software to identify which board an interrupt originated from. The arguments A1 and A2 are used to set flags which enable the output from any of the user subroutines (including IDENT) to be sent to the VDU and/or the printer. Argument A1 is used to enable/disable the VDU output stream, and argument A2 is used to enable/disable the printer output. A zero argument disables the output stream to that device and a non-zero argument enables output.

1.3.2 READ8

Format: READ8,T,N,P,X

This routine is used to read an eight-bit byte from the address given by T,N,P. Data is returned as an unsigned integer in the range 0 to 255.

1.3.3 READ16

Format: READ16,T,N,P,X

This routine behaves in the same way as READ8, except that it returns a signed 16-bit integer result, formed with the lower 8 bits from address T,N,P and the higher 8 bits from T,N,P+1. This format is the same as that used in the Z80 for the storage of 16 bit numbers.

1.3.4 WRITE8

Format: WRITE8,T,N,P,D

This routine writes the 8-bit data represented by D to the address specified by T,N,P. The data can be signed or unsigned, 8-bit or 16-bit. Only the lower 8 bits of the data word are written.

1.3.5 WRIT16

FORMAT:WRIT16,T,N,P,D

WRIT16 acts in exactly the same way as WRITE8, but transfers a 16-bit integer to two successive ports. The lower 8 bits are sent to address T,N,P and the higher 8 bits to T,N,P+1.

1.3.6 STROBE

Format: STROBE,T,N

The STROBE function is used to write a zero to the address given by T,N,4. This is used to give start signals to timers and data conversion devices such as analogue-to-digital converters. It is slightly faster to use this command than to use an explicit WRITE8 command, since no port or data need be specified with STROBE.

1.3.7 SETTR

Format: SETTR,T,N,TR,CB

This call sets the interrupt trigger source and spare control bit to the values in TR and CB. The TR field is a two bit field

which specifies one of four trigger sources (0 to 3) and the CB field is a single bit that is used as a spare control bit. Only interrupt generating boards use this call. The exact values of the parameters TR and CB will depend on the type of board.

1.4 Limitations on General Purpose Routines

Because of the division of ports on the function boards into a board-dependent part (ports 0 to 4) and a board-independent part (ports 5 to 7), it is possible to write a set of general purpose routines that control the interrupt status of the board (ports 5, 6 and 7) and another set that control the board-dependent functions. To prevent the use of the board-dependent routines from affecting the interrupt status of any board, there are limitations on the range of values that the "port" part of the address can adopt. This will only affect the routines that have the port location in their parameter list. The table below gives a list of these routines and the limitation on the port range:

1.4.1 Specific Limits on Port Range

Routine	Port Range
READ8	0 to 4
READ16	0 to 3 (high byte read from port 4)
WRITE8	0 to 4
WRITE16	0 to 3 (high byte written into port 4)

2 Special Purpose Routines

The special purpose routines are those where the type of board or boards accessed by the command is inherent in the nature of the command. In these commands it is therefore not necessary to specify the "type" part of the board address; the type is supplied automatically by the routine. An example would be a command to

trigger and then read an analogue-to-digital converter. A special purpose routine called, say, INADC might be used in this case:

```
in BASIC      1000 CALL"INADC",N,VARADR(X)
```

Which would result in the conversion from ADC number N being placed in the variable X. This special purpose command could also be performed by using two general purpose commands:

```
in BASIC      1000 CALL"STROBE",4,N
               1010 CALL"READ16",4,N,0,VARADR(X)
```

The STROBE command starts the ADC and the READ16 command reads a 16-bit conversion from board type 4 (the ADC type), number N, port 0. It can be seen that the special purpose command is considerably more compact, as it eliminates the need to know the type number and the internal port location on the ADC board. However, it should be stressed that all the special purpose routines are really made up from combinations of general purpose routines with some of the variable parameters of the general routines replaced with constants that depend on the nature of the special routine.

The only special purpose routine used in this work is a routine called GETADC , which returns a value from the ADC as per above.

APPENDIX III

This appendix list the digital Autotitrator program and the analog Autotitrator data acquisition and peak integration program.

```

10 PRINTER 4,4:PN=0:T=0:SUM=0
20 CALL"IDENT",0,0
30 ?CHR$(31):NULL 10,32:?:?:?:?
40 INPUT"Ring potential (mV):",ER
50 CALL"WRIT16",1,2,0,ER*4096/20000
55 ??:?:?
60 INPUT"Flowrate (ml/h):",VF
70 CALL"WRIT16",1,2,2,VF*1.25+85.5
73 ??:?:?
74 KI=0.015/(91.7*(VF/3600)-0.8)
75 INPUT "Set point (uA):",SP
76 SP=INT(SP/.0244)
77 UI=SP*.1
80 CALL"TIMER",1,100
90 CALL"SETTR",12,1,0,0
100 CALL"SETTR",4,1,0,0
110 ??:?:?:?
120 ?"Ramping to steady state"
130 CALL"WRIT16",1,1,0,-2047
140 CALL"WAIT",200
150 CALL"STROBE",12,1,4
160 CALL"SETTR",63,1,1,0
170 FOR N=1 TO 20
180 CALL"INADC",1,VARADR(IR):SUM=SUM+IR
190 NEXT N
200 OS=SUM/20:LPRINT"OFFSET:"OS*0.0244" uA"
210 CALL"INADC",1,VARADR(IR)
220 UITGRL=UITGRL+KI*(SP-IR-OS)
230 AV=(UI+A1+A2+A3)/4
240 IF ABS(AV-UI)<.001 THEN GOTO 290
250 A3=A2:A2=A1:A1=UI
260 CALL"WRIT16",1,1,0,UI*2-2047
270 CALL"WRIT16",1,1,2,UI
280 GOTO 210
290 ?CHR$(31):?:?:?:?"Controlling titration"
300 CALL"INADC",1,VARADR(IR)
310 UI=UI+KI*(SP-IR-OS)
320 AV=(UI+A1+A2+A3)/4
330 IF UI-AV<.06 THEN 380
340 A3=A2:A2=A1:A1=UI
350 CALL"WRIT16",1,1,0,UI*2-2047
360 CALL"WRIT16",1,1,2,UI
370 GOTO 300
380 BL=A3:PN=PN+1
390 ?CHR$(31):?:?:?:?"Integrating peak"
400 CALL"INADC",1,VARADR(IR)

```

```

410 UI=UI+KI*(SP-IR-OS)
420 AR=AR+INT(UI-BL):T=T+1
430 CALL"WRIT16",1,1,0,UI*2-2047
440 CALL"WRIT16",1,1,2,UI-BL
450 IF UI<=BL AND T<30 THEN AR=0:T=0:PN=PN-1:GOTO 290
460 IF UI<=BL AND AR<0 THEN AR=0:T=0:PN=PN-1:GOTO 290
470 IF UI<=BL THEN LPRINT"PEAK "PN" AREA:"AREA*T*2.44E-7" mC":
    AREA=0:T=0:GOTO 210
480 ON BREAK GOTO 500
490 GOTO 400
500 AR=0:T=0:PN=PN-1:GOTO 210

```

This is the analog Autotitrator data acquisition program , with baseline and peak intergration routines. The electrode is pretreated before the analysis is started.

```

10 ?CHR$(31):O=0
20 NULL 20,32
30 INPUTLINE"Do you wish to pretreat the electrode (Y/N)?",A$
40 IF A$="N" OR A$="n" THEN GOTO 390
50 ?CHR$(31):?::?:?
60 ?"Electrode pretreatment"
70 ?::?:?::?
80 ?"Connect counter to ground and disc to potentiostat"
90 CALL"IDENT",0,0
100 CALL"WAIT",300
110 CALL"WRIT16",1,2,2,950*4096/20000:~CHR$(31)
120 ?::?:?::?:?~"Oxidizing at +950 mV"
130 CALL"WAIT",1000
140 CALL"WRIT16",1,2,2,300*4096/20000:~CHR$(31)
150 ?::?:?::?:?~"Removing oxygen at +300 mV"
160 CALL"WAIT",2500
170 CALL"WRIT16",1,2,2,-150*4096/20000:~CHR$(31)
180 ?::?:?::?:?~"Reducing at -150 mV"
190 CALL"WAIT",2500
200 ?CHR$(31):?::?:?::?:?~"Sweeping"
210 FOR M=1 TO 10
220 FOR N=195 TO -30 STEP -1
230 CALL"WRIT16",1,2,2,N
240 CALL"WAIT",1
250 NEXT N
260 FOR N=-30 TO 195
270 CALL"WRIT16",1,2,2,N
280 CALL"WAIT",1
290 NEXT N
300 NEXT M
310 ?CHR$(31):?::?:?::?:?~"Final oxidation at +1000 mV"
320 CALL"WRIT16",1,2,2,1000*4096/20000
330 CALL"WAIT",3000
340 IF O=2 GOTO 380
350 ?CHR$(31):?::?:?::?:?~"Connect ring to potentiostat"
360 ?::?:?::?:?~PUT18,"Press the return key to start",20:A=GET()
370 O=2:GOTO 110
380 ?CHR$(31):?::?:?::?:?~"Electrode pretreatment finished"
390 ?CHR$(31):?::?:?~"Connect the ring and disc for autotitration"
400 ?::?:?

```

```

410 PUT18,"Press the return key to start the control program",20
420 A=GET()
430 ?CHR$(31):?::?::?::?::?::?::?
440 PUT 18,"Ring potential (mV):",20
450 INPUT"",ER
460 CALL"WRIT16",1,1,2,ER*4096/20000
470 ?::?::?::?
480 PUT18,"Flowrate (ml/h ):",VF,20
490 INPUT"",VF
500 CALL"WRIT16",1,2,2,VF*1.25+85.5
510 ?::?::?::?
520 PUT18,"Set point (uA):",20
530 INPUT"",SP
540 CALL"WRIT16",1,2,0,SP*4096/2000
545 CALL"WRIT16",1,1,0,-2047
550 PRINTER 4,4:PN=0:T=0:SUM=0
560 CALL"TIMER",1,100
570 CALL"SETTR",12,1,1,0
580 CALL"SETTR",4,1,0,0
590 ?CHR$(31):?::?::?::?::?::?::?"Swich off integrator for offset measurement"
600 ?::?::?::?::?::?::?PUT18,"Press the reurn key to start the measurement",20
610 A=GET()
620 FOR N=1 TO 20
630 CALL"INADC",1,VARADR(IR):SUM=SUM+IR
640 NEXT N
650 OS=INT(SUM/20):LPRINT"OFFSET:"OS*0.0244" uA"
660 ?CHR$(31):?::?::?::?::?::?::?"Swich on the integrator "
670 ?::?::?::?::?::?::?PUT18,"Press the return key to start the
    control program",20
680 A=GET()
700 PUT31:~::~~::~~::~~::~~::~~::~?"Autotitrating"
710 FOR N=1 TO 7
720 CALL"INADC",1,VARADR(IR):IR=IR-OS
730 AV=(IR+A1+A2+A3)/4
740 A3=A2:A2=A1:A1=IR
750 NEXT N
760 CALL"INADC",1,VARADR(IR):IR=IR-OS
770 AV=(IR+A1+A2+A3)/4
780 IF ABS(AV-IR)=>.1 THENGOTO 820
790 A3=A2:A2=A1:A1=IR
800 CALL"WRIT16",1,1,0,-2047
810 GOTO 760
820 BL=AV:PN=PN+1
830 ?CHR$(31):~::~~::~~::~~::~~::~~::~?"Integrating peak"
840 CALL"INADC",1,VARADR(IR):IR=IR-OS
850 AR=AR+INT(IR-BL):T=T+1
855 IF IR-AV<=2 THENGOTO870
860 CALL"WRIT16",1,1,0,(IR-BL)*2-2047
870 IF IR<=BL AND T<30 THEN AR=0:T=0:PN=PN-1:GOTO 700
880 IF IR<=BL AND AR<0 THEN AR=0:T=0:PN=PN-1:GOTO 700
890 IF IR<=BL THEN LPRINT"PEAK "PN" AREA:"AREA*T*2.44E-7" mC":
    AREA=0:T=0:GOTO 700
900 ON BREAK GOTO 920
910 GOTO 840
920 AR=0:T=0:PN=PN-1:GOTO 700

```

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