THE DEVELOPMENT OF AN ELECTROCHEMICAL DETECTOR FOR LIQUID CHROMATOGRAPHY

by

John Bland B.Sc. A.R.C.S.

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of the University of London.

Chemistry Department,
Imperial College of Science and Technology,
London, SW7 2AY.

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ABSTRACT

An introduction to the technique of liquid chromatography is given with particular reference to detection. A review of L.C. detectors is presented with special emphasis on electrochemical ones.

Use of a D.M.E. detector is described. An investigation into the residual current phenomenon at glassy carbon is presented along with a detailed account of the behavior of glassy carbon electrodes under the influence of potential steps and pulses.

Finally the construction of an electrochemical detector is described for use with a glassy carbon electrode. An investigation of wall jet cell parameters is shown along with a description of the pulse potentiostat design.

A series of applications is presented showing the usefulness of the pulse system, and a conclusion is given analysing the present state of electrochemical detection.

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PREFACE

From the first appearance of man on earth, his fascination for and interest in his surroundings has caused a gulf to appear between himself and the rest of the animal kingdom. The rise of the early civilisations of the fertile crescent indicate man's ability to modify his environment to his own advantage. The rise of mathematics in Egypt and astronomy in Chaldea, both had a part in the flowering of culture in classical Greece. It was the Greek's attitude to life that laid the foundations of modern scientific analysis. The philosophic schools of Plato and Aristotle started the whole world asking the question Why?. The development of these scientific principles can be traced through the middle ages to today. Usually the most rapid advances came in time of war, but as the state of scientific knowledge progressed, the theoretical schools flourished and broadened the horizons of scientific interest.

The 19th century was the age of the experimental chemist and the work carried out at this time has formed the basis of modern chemistry and physics. As the world moved into the industrial age, more and more money was made available for research and its importance in everyday life was emphasised. At this time chemical analysis began to assume an importance which has increased during this century to its position today. As time becomes more precious, the need for automation increases. The cost of manpower is such that modern analysis systems must be automated to be viable. On line monitoring is becoming more important and such techniques as atomic absorption, anodic stripping voltammetry, and ion selective electrodes have been adapted to it. Another technique which can be grouped with these is high performance liquid chromatography.

1.1 INTRODUCTION

Chromatography may be defined as follows " A differential migration process where the sample components are selectively retained by a stationary phase." It is basically a technique of the twentieth century, the first published work being that of Mikhail Tswett who in 1903 managed to separate some green leaf pigments on a chalk column (1,2). The various colours produced on the chalk gave rise to the term " Chromatography " or colour writing.

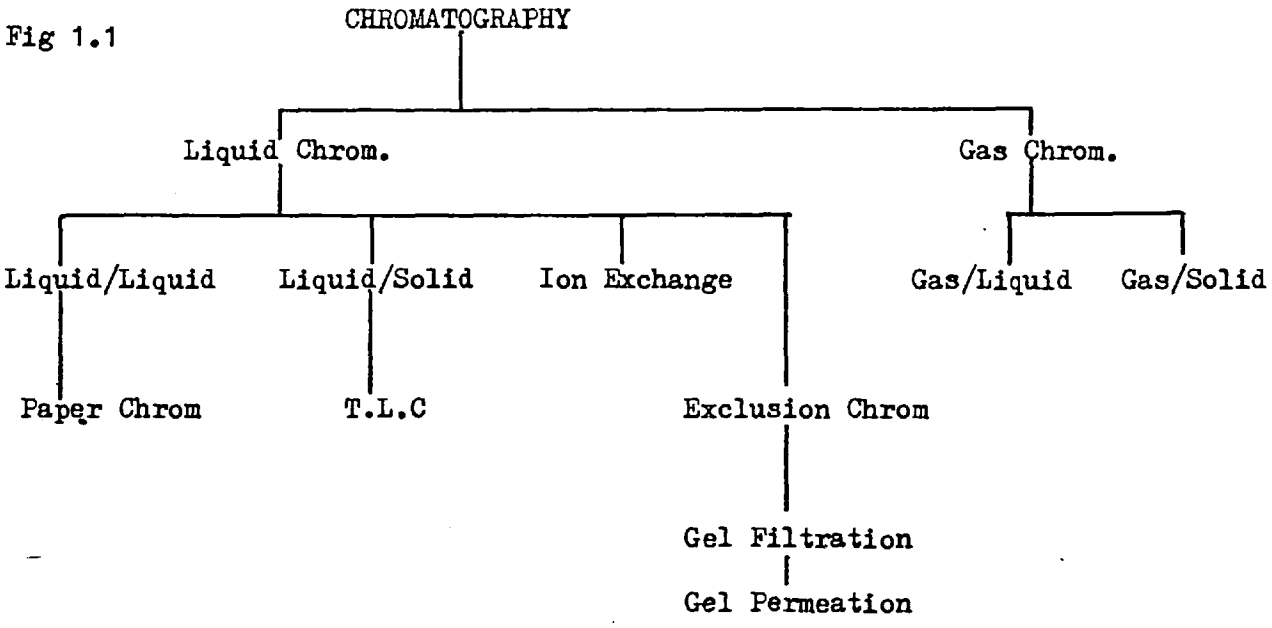
Chromatography has been slow to develop however, and not until the late thirties were any great advances made. Thin layer chromatography was born in 1938 through the work of Ismailov and Shraiber (3) and was later developed by Stahl(4,5). Work on liquid partition was done in the late forties by Martin and Synge (6) and this led to the foundation of gas and paper chromatography. Because of the long time scales involved in liquid column chromatography it received less attention than the other modes in the early years and was used primarily in its preparative mode. Advances in the last ten years, both in the field of column technology and detector design have led to renewed interest in its analytical applications.

Despite the numerous variations in technique applied in present day chromatography, they are all based on the same fundamental principles. The sample is carried by a mobile phase over a stationary phase, and the resulting interactions between the two phases and the sample cause selective retention of the sample components by the stationary phase, thus splitting the sample into its components

1.2 SEPARATION MODES

Chromatography can be divided into two main branches depending upon the state of the mobile phase. Each of these two branches can then be subdivided according to the nature of the stationary phase.

Fig 1.1



In Gas Chromatography the mobile phase is a gas and is not active in the separation process. It is usually either Nitrogen, Helium, or Argon and its sole purpose is to carry the sample over the stationary phase. In Liquid Chromatography the mobile phase is a liquid and it is the properties of both the mobile and the stationary phases which influence the separation. Because both phases are active in Liquid Chromatography, the technique is much more selective than Gas Chromatography. By altering the composition of the mobile phase it is possible to alter the appearence of the chromatogram completely, resolving different peaks, and even changing the order of elution.

1.2.1 Liquid Solid Chromatography (L.S.C.)

The separation mode in L.S.C. is one of adsorption. The sample and mobile phase (eluent) molecules are in competition for the active sites on the solid stationary phase. An equilibrium reaction can be written for the process as follows:-

$$Y + nE_a \rightleftharpoons Y_a + nE \qquad \text{—— Equation 1}$$

Y = Sample molecule

E = Mobile phase molecule

a = adsorbed state

n represents the number of solvent molecules equivalent to one molecule of Y in the adsorbed state.

Hence a distribution coefficient can be written :-

$$K = \frac{(Y_a)\,(E)}{(E_a)^n\,(Y)} \qquad \text{Equation 2}$$

Two of the most popular adsorbents are Alumina and Silica. Alumina $(Al_2O_3)x$ is slightly basic, while Silica$(SiO_2)x$ is slightly acidic. Both these compounds are polar and hence polar compounds are retained on them the longest. For example the following compounds are listed in order of increasing retention times :-

Hydrocarbons, Halogen compounds, Ethers, Esters, Ketones, Alcohols, Acids.

If two or more of these groups are present in one compound then generally the most polar group governs the adsorption characteristics.

L.S.C. is generally used to separate different classes of compound and is also used with multifunctional and isomeric compounds. The interaction of the hydrocarbon part of the solute molecule has little importance in L.S.C. as the competition of the solvent molecules for the active sites is too great for the interaction of the hydrocarbon to have much effect.

1.2.2 Liquid Liquid Chromatography (L.L.C.)

The separation mode in L.L.C. is by partition between two liquid phases. The stationary phase is evenly coated on either an inert support or an adsorbent such as Alumina or Silica. To ensure that the mobile phase doesn't wash off the stationary phase coating,it is important that the two liquids are immiscible. Furthermore the stationary phase should be a good solvent for the solute (sample).

A partition coefficient for each sample component can be written :-

$$K = \frac{C_s}{C_m} \qquad \text{Equation 3}$$

C_s = Concentration of sample in stationary phase.

C_m = " " " " mobile phase.

In normal L.L.C. the mobile phase is a non-polar liquid while the stationary phase is polar. The technique of reversed phase L.L.C. is often used however in which these polarities are reversed. Both these methods suffer

from several drawbacks.

a) The mobile and stationary phases must be in equilibrium. If this is not the case then the mobile phase will strip the stationary phase off the support

b) High flow rates cannot be used otherwise mechanical stripping will result

c) Even pairs of solvents with moderate mutual solubility must be avoided.

d) The liquid must be coated reproducibly and evenly onto the solid support to give good results.

e) Solvent programming cannot be used. Solvent programming is a method of reducing long retention times by gradually changing the composition of the mobile phase to move the strongly retained sample components off the column. The gradual change in composition means the two phases cannot maintain equilibrium. The technique is also known as gradient elution.

To overcome these problems Bonded Phase Chromatography (B.P.C.) has been developed. In this mode the liquid stationary phase is chemically bonded to the solid support. The stationary phase cannot now be washed off either mechanically or chemically (unless extreme non-chromatographic conditions are used), and gradient elution can be used. The resulting separation mode is probably a mixture of partition and adsorption the dominent mode depending upon the sample used.

Two types of bonded phase are at present in use and are made as follows:-

a) The surface hydroxyls of Silica are esterified with an alcohol to form an Si—O—C bond.

i.e. $\text{SILICA—OH} + \text{HO—R} \rightleftharpoons \text{SILICA—O—R} + H_2O$ ___Equation 4

These have been used in L.C. for the separation of Amines (7), Phenols (8), Benzodiazepines (9), Unfortunately these phases are thermally unstable and are decomposed by alcohols and water.

b) The surface hydroxyls of Silica are silylated to produce Si—O—Si bonds

which do not suffer from the weaknesses of the Si__O__C bonds. This second type of bonded phase is extremely useful and many commercial columns are available and are extremely popular. They compare very favourably with the conventionally coated stationary phases (10).

1.2.3. Ion Exchange Chromatography

In this method the stationary phase consists of a porous solid phase usually in the form of a resin onto which ionic groups are bonded. The eluent contains the counter ion and it is competition between the counter ion and the solute for the resin sites which determines the separation.

e.g.

$$M^+ + Na^{+-}O_3S\_\_R \rightleftharpoons M^{+-}O_3S\_\_R + Na^+$$

M^+ = Cation

Thus $$K = \frac{(Na)(M^{+-}O_3SR)}{(Na^{+-}O_3)(M^+)}$$ _____Equation 5

As K increases the interaction of solute with the resin increases and hence the retention time increases.

Some typical resins

Table 1

Type		Functional Group
Anion	Basic	$-CH_2N(CH_3)_3^+$
"	"	$NH(R_2)^+$
Cation	Acidic	SO_3^-
"	"	$-COO^-$

As might be expected the ionisation of these resins is pH dependent and hence pH can be used to control the K values and hence the separations.

Applications include Amino Acids, Sulphonic Acids, Nucleotides, Carbohyd--rates, and Barbiturates.

1.2.4. Steric Exclusion Chromatography (S.E.C.)

S.E.C. differs from the others in having an inert stationary phase. The stationary phase consists of a porous three dimensional matrix and the retention of solute depends upon its molecular size. A large molecule cannot permeate

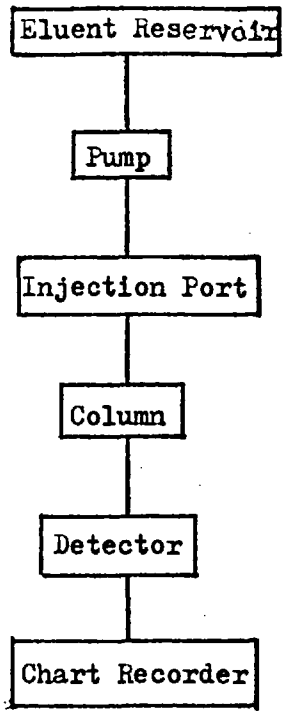
the smaller pores and hence will only be retained for a short time. The smaller molecules however will permeate the very small pores and thus be retained for longer periods of time. Usually any one column is packed with a material of a defined pore size and thus solutes with different radii will be retained for different periods.

This type of chromatography is usually used with high molecular weight compounds such as Sera Proteins, Crude Oils, and D.N.A.

1.3.1. CHROMATOGRAPHIC TECHNIQUE

The chromatographic system is shown below in block diagram form.

Fig 1.2



1.3.2. Eluent Reservoir

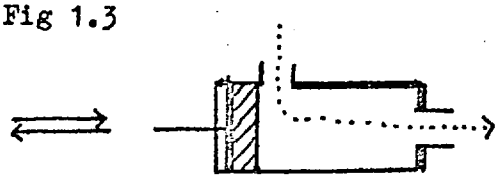
Since large amounts of eluent are needed, it is usually made up in bulk and placed in a container above the pump so that it can be gravity fed into it. Usually facilities are available for the solvent to be degassed, as if this is not done gas bubbles tend to be formed at the low pressure end of the column causing problems within the detector cell. These often give rise to spurious peaks.

1.3.3. Pump

Because the mobile phase in G.C. is a gas, it permeates the column relatively easily under its own cylinder pressure. In L.C. however, the mobile phase is a liquid and since it has to travel through perhaps a metre long column of finely packed powder, it is necessary to use a pump. There are two basic designs available.

a) Reciprocating Pumps.

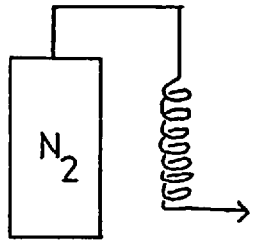
Fig 1.3



These pumps have an unlimited reservoir capacity but unfortunately are accompanied by pulsing. These pulses can cause periodic noise at the detector which can adversely affect the detector performance. Pulse damping systems have been employed which, although not perfect, do reduce the effect considerably.

b) Pressure Pumps

Fig 1.4



With these pumps, Nitrogen from a high pressure cylinder acts directly on a column of eluent and it is this Nitrogen pressure which causes the eluent to flow through the column. In order to stop the Nitrogen dissolving in the eluent, a small diaphram is placed before the eluent. These pumps have a limited capacity and it is not possible to use gradient elution with them. Although free from pulsing the flow rate may be slightly different with a full reservoir to that with an almost empty one.

1.3.4. Injection Port

Samples are normally introduced onto the column either dissolved in the eluent or a solvent compatible with it. This is to prevent any precipitation at the head of the column. The sample sizes are of the order of microlitres and can be introduced by means of a syringe. If the pressure at the head of the column is less than about 1200 p.s.i. then it is possible to inject the sample through a septum into the flowing stream. For pressures greater than this it is necessary to use the stop flow technique in which the pressure is released (and hence the flow stopped) to enable the top of the column to be removed so that the sample can be placed directly onto the head of the column.

The pressures needed to pump the eluent through the column vary from a few hundred p.s.i. to about 5000 p.s.i. depending upon the column parameters. The use of high pressures in the early days of the technique led to the use of

the term H.P.L.C. or high pressure liquid chromatography. Improved column technology has led to a reduction in pressures but the term H.P.L.C. remains although it now refers to high performance liquid chromatography.

1.3.5. Columns

Columns come in two types, high performance analytical, and preparative. Analytical columns are between 1 and 6 mm internal diameter and up to about 1 m. in length. In most cases preparative columns are longer, up to 20 feet long and 1 cm. in diameter.

The column is the heart of the chromatographic process for it is here that the separation takes place and the final results can only be as good as the column allows. Columns are packed with small particulate support materials which come in two basic types, porous and superficially porous.

The porous particles used are between 20 and 50 microns in diameter. If larger particles are used the deep pores that they contain lead to long path lengths and hence slow diffusion through the particles. This in turn spreads out the peaks and destroys resolution especially at high flow rates. Porous particles have a high sample capacity and since their surface area is about 300 $m^2/g.$, a 1m. column of i.d. 2 mm. can handle samples of 2-5 mg. without overloading. Usually regularly shaped particles can be dry packed down to 30 microns and irregular ones down to 40 microns. Below this they must be slurry packed.

The superficially porous packing materials were developed to reduce the length of the diffusion paths while keeping the particles large enough to be packed regularly. They consist of a small non-porous core surrounded by a porous outer shell. The mass transfer within the shell is rapid and thus high flow rates can be maintained. The sample capacity of these materials is much lower than the porous ones however, and a 1m. column i.d. 2mm. will overload with more than about 300$\mu g.$ of sample. This reduction in sample size is important and can be critical as it approaches the lower detection limit of the detector.

The actual column dimensions are all interelated and depend on the sample

capacity and resolution required. The column must be narrow enough not to allow radial flow and long enough to give good resolution and furthermore contain enough material to produce a reasonable sample capacity (determined by detector limitations and sample availability). It is also important to note that the pressure needed to maintain flow through a column is directly proportional to the column length and inversely proportional to the square of the particle diameter. As pumps are not unlimited in their pressure output, these factors are important in system design..

1.3.6. Detector

The detector in H.P.L.C. provides a means of following the concentration profile of the sample as it comes off the column and converting it into a signal which can be recorded via a chart recorder and later analysed. Various ways in which this has been done will be discussed later. There are several ideal criteria which all detectors should aim for however and these are as follows:-

a) The detector should have a large linear range. If the detector response is linear with concentration it is possible to carry out semi-quantitative analysis by eye straight off the recorder which saves a great deal of time. Furthermore if accurate data is required then linearity removes the necessity of using complex calibration curves which have to be plotted over the whole detector range. Most detectors deviate from linearity when large samples are used but usually this deviation occurs at a clearly defined point and does not cause too many problems. The lower detection limit is defined as the sample size which produces a detector response twice that of the prevailing noise. It is an advantage to have a low detection limit since if not, large samples have to be used which in turn mean large sample capacities, long columns and longer analysis times.

b) The detector sensitivity should be as large as possible. The larger the response for a given sample, then the more accurately the sample size can be determined. The sensitivity is given by the slope of a concentration versus peak height (peak area) plot, and is constant for a linear response detector.

c) The detector should give reproducible results. A consecutive series of sample injections of the same size should give responses of the same size within the limits of experimental error. i.e. The detector response should not vary with time, otherwise it is impossible to calibrate the system and accurate results cannot be obtained.

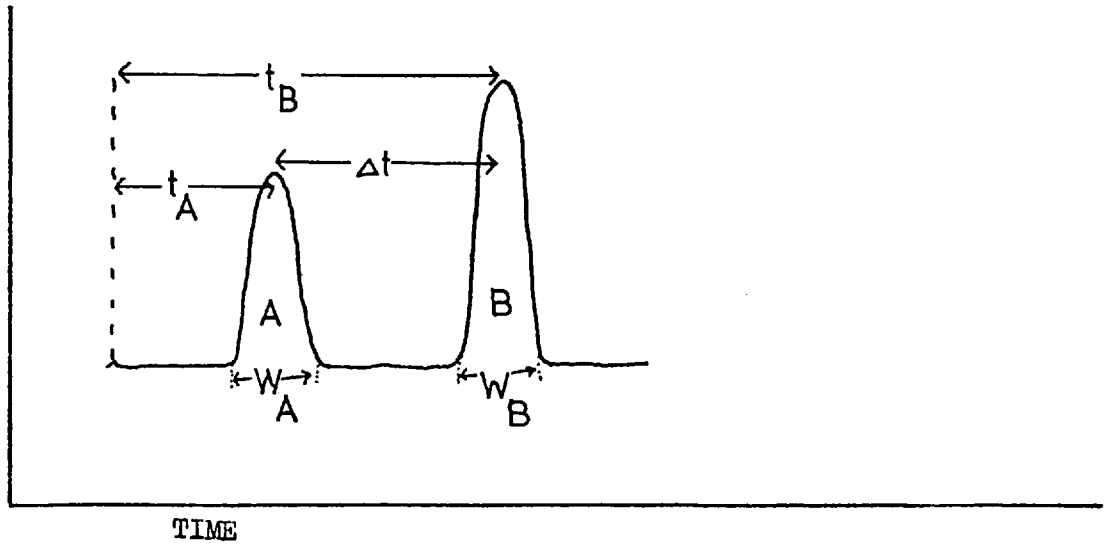
d) The detector should have as small a cell volume as possible to keep mixing down to a minimum. If the sample is allowed to mix after it comes off the column the resulting peaks will be broad and resolution will be lost. Most detectors have cell volumes less than 10μL and trends are towards volumes less than 1μL. Peak broadening due to the cell can be neglected if the cell volume is less than 1% of the peak volume(11). Thus as columns become more efficient cell volumes must decrease. Theoretically the column mixing can be characterised by the number of theoretical plates N produced by a column of length L.

$$H = \frac{L}{N} \qquad \text{Equation 6}$$

H = Height equivalent of a theoretical plate.

In practice two other equations are of importance. From Fig 1.5

Fig 1.5



V_A= Elution Volume of A

V_P= Volume of Peak

$$N_A = \frac{16(t_A)^2}{(W_A)^2} \quad \text{or} \quad N_A = \frac{16(V_A)^2}{(V_p)^2} \qquad \text{Equation 7}$$

and

$$R_{BA} = 2 \frac{\Delta t}{(W_B + W_A)} \qquad \text{Equation 8}$$

Where R is defined as the resolution of A and B.

For maximum efficiency H should be minimised and it is to these ends that column technology has been moving during the last decade.

e) Finally it is a great advantage if the detector is neither flow nor temperature dependent. The whole system can be thermostated but in general it is much more convenient to work on an open system. As regards flow, even non-pulsing pumps are subject to flow variations which will cause fluctuations in the base lines of flow sensitive detectors. These cause major problems if they are of short duration and occur at the same time as the sample elutes from the column.

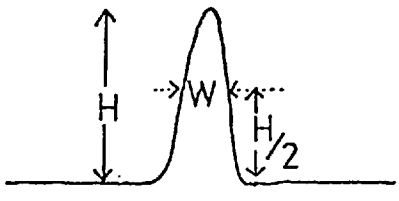
1.3.7. Detector Output

The detector response in chromatography is usually recorded on a strip chart recorder. This being the cheapest and most convenient method. Ideally the sample is recorded as a peak on a flat base line, and the height, or more accurately the area, is proportional to the sample size.

Peak heights can be measured with more precision than peak areas on a chart recorder however, and as long the operating conditions are carefully controlled and very great accuracy is not required, then they are acceptable.

There are several manual methods available for working out peak areas, all are based on assuming that the peaks approximate to triangles. The most popular being to multiply the width at half height by the height.

Fig 1.6



$A = \frac{1}{2}HW$

The technique should not be used for peaks on very sloping base lines and for non-symmetrical peaks.

Two instrumental methods are also available to measure peak areas.

The first is to use a digital integrator. The integrator is connected directly to the chromatograph and a voltage to frequency converter produces pulses at a rate proportional to the signal. To integrate the signal these pulses are fed into a digital counter and the total number of pulses during a peak is a measure of the peak area.

The second method is to use a computer (12). These can be used in both on line and off line modes depending upon the facilities available. Besides measuring peak areas the computer can do further calculations including data interpretation.

1.3.8. Data Interpretation

Once peak areas have been measured it is necessary to relate them to known standards in order to get some idea of the sample size they represent(13). Normally it is not possible to relate peak areas directly to the concentration as varying conditions change the size of the detector response to a given sample size. There are two methods of employing standards:-

One is to use an internal standard. Compounds do not produce the same magnitude response at any given detector for a given concentration; but it is possible to compute constants of proportionality to interelate various samples.

i.e. $C = f A$ ____Equation 9

A=Area f= Response Function C= Concentration

for any one compound and given conditions.

The response factors may then be related to a reference compound

$$f_x = \frac{A_R \, C_x \, f_R}{A_x \, C_R}$$ ____Equation 10

in a given set of conditions.

If an internal standard is mixed with the sample before injection the % x in the sample can be calculated as follows:-

$$\%x = \frac{A_x \; f_x \; W_{is}}{A_x \; f_{is} \; W_x} \cdot 100 \qquad \text{Equation 11}$$

W= Weight

The addition of an internal standard compensates for any errors in injection. There are several restrictions on the Internal Standard however;

a) It must be completely resolved

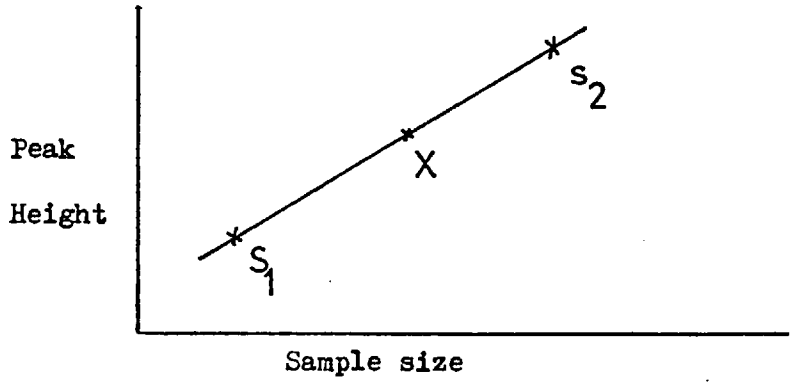
b) It must elute near the peaks of interest

c) It must be of similar concentration to the peaks of interest

d) It must be absent in the sample

The second method is to use an external standard. In this method the standard is of the same composition as the sample but of known concentration. Usually two standards are used which bracket the unknown.

Fig 1.7



If the detector response is linear the S_1 and S_2 can be a considerable distance appart and x calculated from the equation

$$A = K\,Wt \qquad \text{Equation 12}$$

K= Slope

To improve on the accuracy of the above methods the analyses are usually repeated several times and a system of standard deviations used to produce the final result.

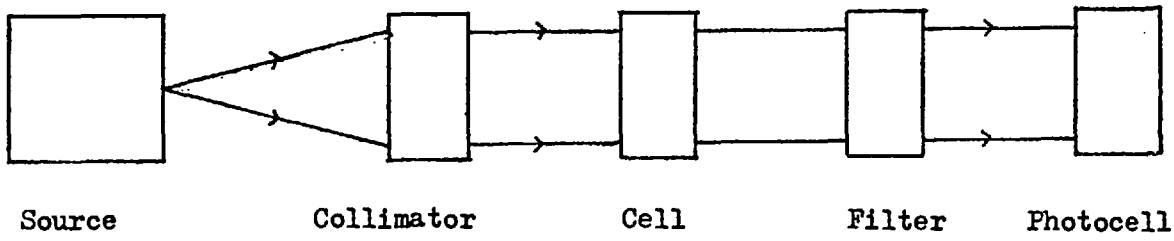
2.1. DETECTORS

During the last decade or so, great advances have been made in the field of column technology. These improvements have gradually put a greater and greater strain on the existing detector systems such that the detector has fast become the limiting factor in peak resolution. Historically the earliest chromatographic detector was the human eye and it is a spectroscopic development of this method which has proved to date to be the most popular.

2.2. The Ultra Violet Detector

Certain molecules, when irradiated with light in the U.V. range, can absorb it to undergo electronic transitions. This phenomenon is the basis of the U.V. detector.

Fig 2.1



U.V. light from a source is collimated and shone through a small volume cell through which the eluent passes. The transmitted light is then filtered and the intensity of a preselected wavelength measured by a photocell. The relationship between adsorption and concentration of adsorbing species follows from Beer's Law

$$\text{Log } \frac{I_o}{I} = \epsilon bc \qquad \text{Equation 13}$$

$$\frac{I_o}{I} = \frac{1}{T} \qquad \text{Equation 14}$$

$$A = \epsilon bc \qquad \text{Equation 15}$$

I_o = Incident Intensity

I = Transmitted "

ϵ = Molar Absorptivity

b = Path Length cms.

C = Concentration, Moles/ Litre.

A = Absorbance

T = Transmittance

The output used is usually absorbance as this is directly proportional to the concentration. In table 2.1. are listed some of the U.V. adsorbing groups along with their wavelengths of maximum absorption and their peak molar absorptivities.

Table 2.1.

Chromophore	System	λ Max	ϵ Max
Ether	-O-	185	1000
Amine	$-NH_2$	195	2800
Nitrile	-C≡N	160	
Acetylide	-C≡C-	180	6000
Azido	>C=N	190	5000
Ethylene	-C=C-	190	8000
Ketone	>C=O	195	1000
Esters	-COOR	205	50
Aldehyde	-CHO	210	strong
Carboxyl	-COOH	205	50-70
Sulphoxide	>S→O	210	1500
Nitro	$-NO_2$	210	strong
Nitroso	-N=O	302	100

Normally the U.V. light is of a fixed wavelength and is obtained either by using a continuous source and a monochromator or, as is generally the case, by using the strong resonance line of the low pressure Mercury lamp at 254 nm.

There are two main drawbacks to the U.V. method of detection:-

a) Certain important compounds do not absorb in the U.V. range. To overcome this these compounds have to be reacted with a chromophore after separation. This usually results in peak broadening through mixing. Furthermore in a mixture of compounds the variations in absorptivities can vary greatly for a given wavelength and hence the sensitivity of the detector can vary greatly from component to component in any separation.

b) It is necessary to use solvents which do not absorb in the U.V. otherwise the sensitivity of the technique will be substantially reduced. As can be seen from Table 2.2, if the 254 nm. line is used, many solvents are not compatible.

Table 2.2

Solvent	U.V. Cut Off. nm.
Isooctane	210
Pentane	210
Hexane	210
Diethyl ether	220
Carbontetrachloride	265
Chloroform	245
T.H.F.	280
Benzene	280
Acetone	330
Dioxane	220
Ethanol	210
Acetonitrile	210
Acetic acid	230
Methanol	210
Water	200

2.3 THE REFRACTIVE INDEX DETECTOR

The U.V. detector was an example of a specific detector i.e. one which responded to only a limited number of coumpounds. The Refractive Index detector is one which reponds to a much wider range of compounds and hence is known as a universal detector. This detector works on the principle that when a sample flows through the detector cell its refractive index is different from when only the eluent is present in the cell. Since all liquids have a refractive index the technique is applicable in theory to all samples. The problems arise in practice when the sample and eluent have very similar refractive indices, since then there is little difference between the refractive index of sample, and of sample and eluent. Thus the technique becomes less sensitive. There are two types of R.I. detector available. One senses the deflection of a light beam as it passes through a triangular cell, the other senses the change in the amount of light reflected or transmitted through a liquid glass interface.

The main problem with the R.I. detector is the dependence of refractive index on temperature. This necessitates very good temperature control for the system. Furthermore if gradient elution is used a sloping baseline usually results, the slope of which depends upon the difference in refractive index of the eluents at each end of the gradient. Typically the universal detector is less sensitive than the specific one. The U.V. detector is capable of measuring samples in the nanogram to microgram region while the R.I. detector is used normally in the microgram to milligram range (19).

2.4. MASS TRANSPORT DETECTOR

One of the most popular detectors in Gas Chromatography is the Flame Ionisation Detector. (20,21).

In recent years work has been done (22) to utilise this detector for L.C. A moving wire carries a small amount of the column effluent into an evaporator where the more volatile solvent is evaporated. From here it is carried into a pyrolysing oven where the sample is evaporated (or decomposed). The vapour produced is then carried in a stream of Argon or CO_2 (23) into a Flame Ionisation Detector (F.I.D). Although this detector can be used with most carbon containing compounds it is severely limited by the fact that the eluent used must be much more volatile than the sample. This not only reduces the number of solvents available but also means that salt buffers cannot be used. Gradient elution is possible however since in the case of volatile solvents, the solvent is removed before the detection process. On the purely instrumental side, reproducibility is not as good as the U.V. and R.I. detectors due to difficulty in obtaining a reproducible coating on the moving wire.

2.5. MICRO ADSORPTION DETECTOR

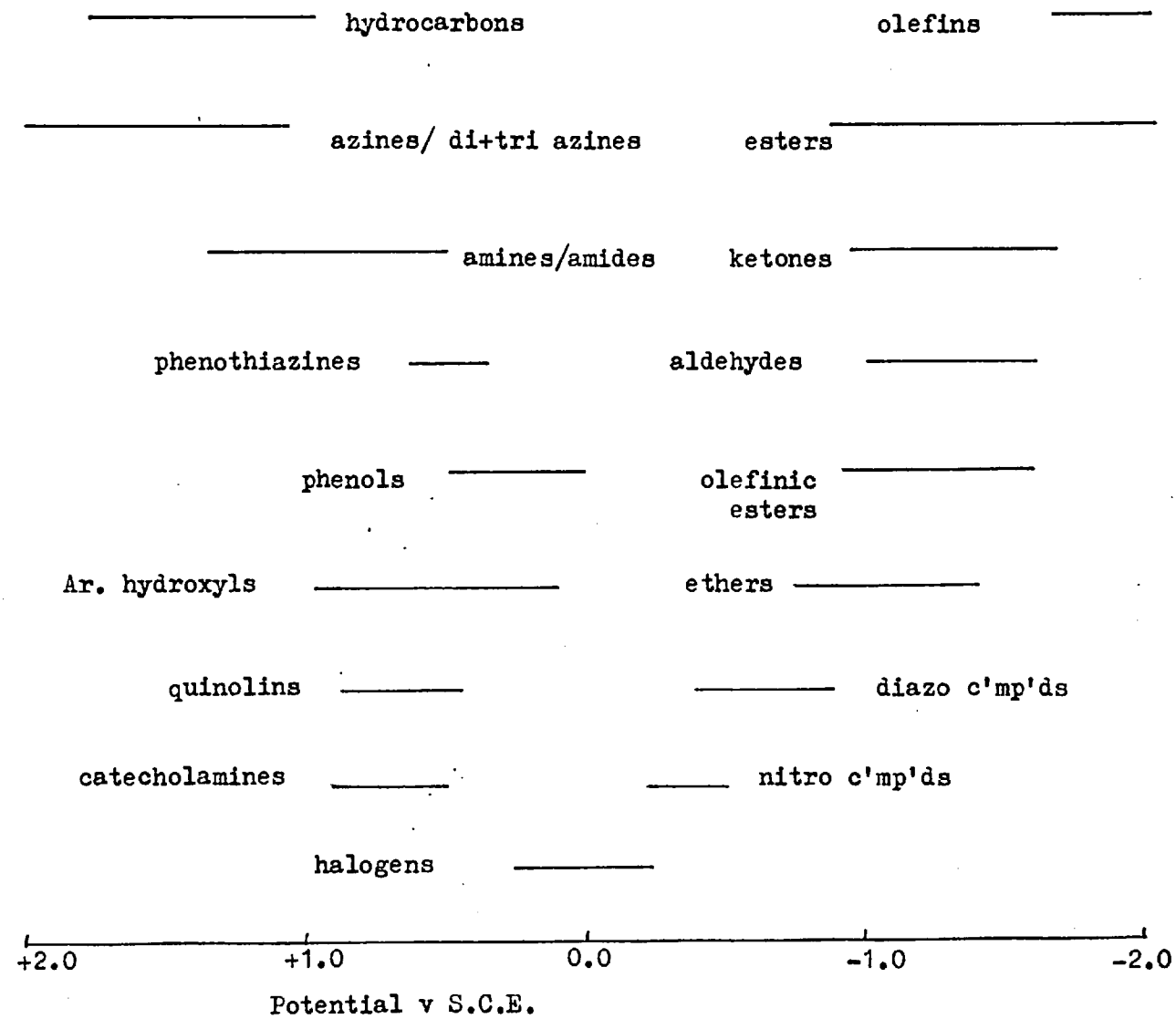
These are based on the principle that small amounts of heat are transferred whenever a sample is adsorbed on a suitable surface. The most sensitive of these detectors employs two thermistor probes situated in a cavity containing adsorber material (24). As the sample comes off the column the heat change is sensed, the magnitude of the response being proportional to the concentration.

2.6. ELECTROCHEMICAL DETECTOR

A large number of detection systems have been tried whose principle is based on the difference in electrochemical nature of the sample and the solvent. The most popular have been based on either a voltammetric or coulometric method. Most of the early work was done by Kemula (25,26,27,28), who first combined the techniques of Chromatography and Polarography, using a D.M.E. to detect the sample at the end of the column. This technique has been more thoroughly developed in recent years using solid electrodes instead of mercury and has resulted in several reports

of both Voltammetric(29,30,) and coulometric (31,32,33,34,35,) detectors capable of use on samples in the nanogram range. Although only a small number of compounds have been tried with these detectors these two techniques appear to present the best electrochemical approach to Chromatographic detection. The main problems using these electrochemical methods are found when using Organic solvents as it is necessary,especially in the voltammetric case to have a background electrolyte present at a concentration of about 0.1M to maintain a linear relationship between current output and concentration and maintain correct potentiostatic control. Both techniques can only be used on oxidisable or reducible species but since many of the samples of industrial interest are large multifunctional organics, this does not limit the detectors to any great extent.

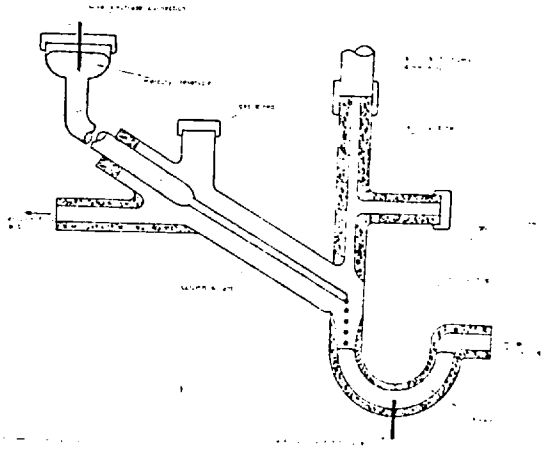
Fig 2.2



2.6.2 Voltammetric Detectors

The earliest electrochemical detector was built by Kemula (25) and involved the use of the D.M.E. at the end of the chromatography column. A constant voltage was applied between the D.M.E. and the reference electrode with the result that as samples eluted from the column, they were either oxidised or reduced. In the past few years several workers have developed Kemula's system and produced various working detectors. Joynes and Maggs (30) have reported a glass cell capable of use in L.C. detection, with a D.M.E.. See Fig.2.3

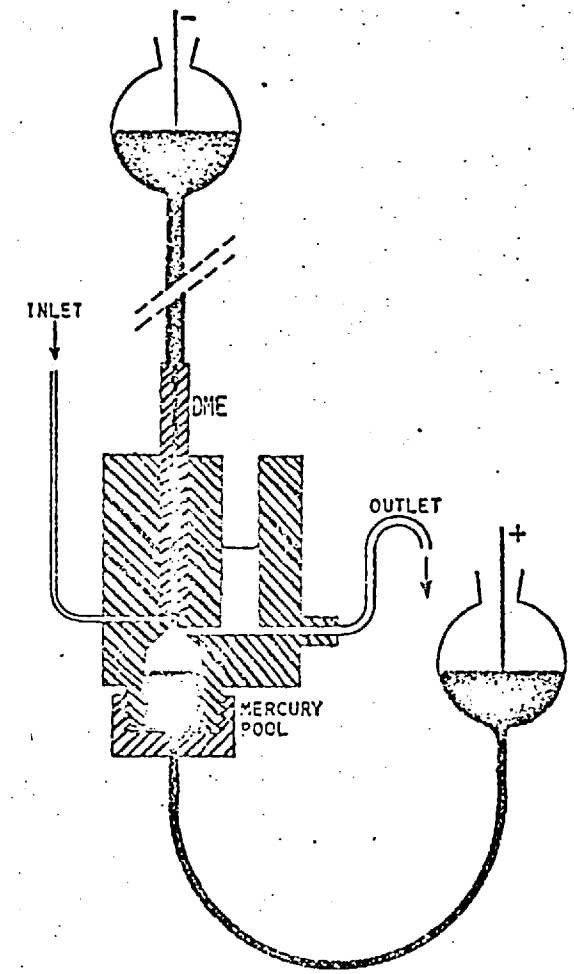
Fig 2.3



Cell Volume 20μl

The cell was tested using inorganic ion samples and was found to have a linear range from 10 to 100μA. with a sensitivity of 1.54×10^{-2} Al/M. The noise level being 1×10^{-8} A.(with a damping time constant of 0.5s.). The upper range of linearity was found to be 0.15μg/ml.. Huber has also reported a D.M.E. detector (29) but with a much smaller cell volume than that of Joynes. Huber's cell is shown in Fig 2.4

Fig 2.4



Again a mercury pool was used as the unpolarisable electrode. This cell has a small cell volume and the results produced with it were better than those of Joynes. Using parathion as a sample the following results were quoted:-

Sensitivity = 1.9×10^{-2} A.l/Mol.

Noise Level = 7.2×10^{-9} Mol/l equivalent

Linear Dynamic range = 140×10^{3}

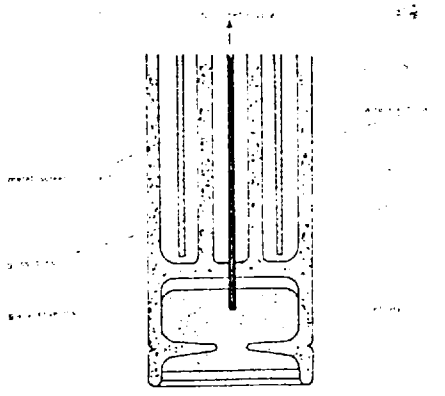
The detection limit was found to be about 10^{-5}M/l and for routine analysis a standard deviation of 2% was claimed.

Stillman et. al. (106) have produced a detector using a silver/ silver chloride reference. A detection limit of $10^{-2}\mu g.$ for parathion was claimed at a noise level of 10^{-8} A.. Buchanan and Bacon (117) have reported the application of pulse polarography to the D.M.E. detector using a three electrode system (D.M.E., calomel reference, platinum counter). They detected a mixture of metal ions from an ion exchange column but did not report any values of detection limits or sensitivities.

Generally the D.M.E. detectors reported are only capable of optimum performance for a very limited number of compounds and need considerable skill on the part of the operator to achieve this performance. Their major drawback lies in the large amount of damping required and their lack of response to fast peaks.

To extend the oxidative side of voltammetric detection the use of solid electrodes has been tried by several groups. Joynes and Maggs have used a carbon inpregnated silicone rubber electrode (30) Fig.2.5

Fig. 2.5



Reference = Platinum Wire.

The cell produced had a volume of $10\mu l.$ and gave a detection limit of 1.9×10^{-8} Mol./l for the inorganics used, and 1.6×10^{-9} Mol./l. for the organics used.(2.6 dinitrophenol).

Using a carbon paste electrode as working electrode, Kissinger has also reported an L.C. detector (32). He employed a three electrode system (silver/silver chloride wire reference, platinum counter). With a cell volume of 0.7μl he managed to perform routine determinations of catecholamines at the 10ng. level. The linear range was tested using dopamine and found to be good for between 5 and 200 ng. of injected sample. Peaks were shown derived from samples of 100pg. and a detection limit of 10pg. is claimed. A current efficiency of 4.5% is stated.

The main advantages of using solid electrodes over the D.M.E. are outlined below:-

a) Better positive range.

b) Lower noise levels.

c) Ease of use.

d) Much less damping required.

e) Much faster peaks can be seen.

The carbon paste electrode of Kissinger does however suffer from poor reproducibility of the surface which adversely affects the peak reproducibility and results also in short electrode lifetime. Platinum electrodes have also been evaluated in flow streams . Work done by MacDonald and Duke (107) indicated that the application of pulses produced improvements over normal D.C. methods including increased sensitivity, reduction in noise and greater electrode stability. Very little has been reported in the literature in connection with the adsorption problems known to exist at solid electrodes.

2.6.3 Coulometric Detection

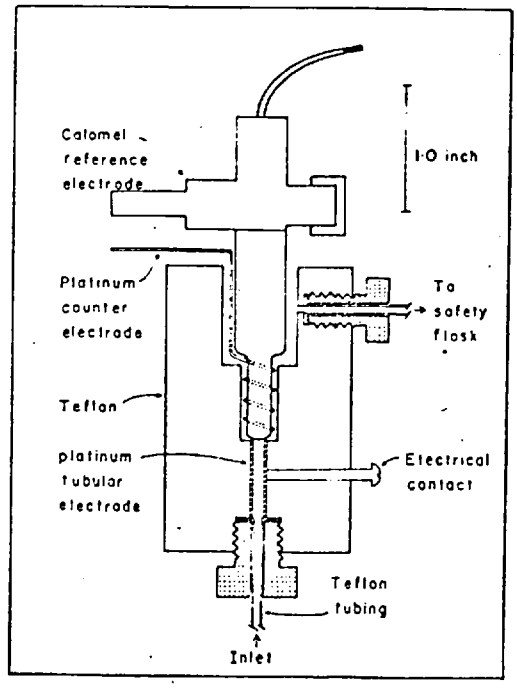
The coulometric method involves the controlled potential electrolysis of column effluent. It can be applied to electrolytes, non electrolyte solutes in electrolyte solvents and to non electrolytes which form ionised complexes.

If all the electroactive material passing the electrode is reacted

then 100% efficiency is produced and the area under the current time curve can be related to the quantity of electroactive material via Faraday's laws of electrolysis. If not 100% efficient, calibration graphs have to be drawn. As flow rates increase, the system becomes less efficient and deviations from 100% efficiency are found. To overcome this problem the electrode area has to be increased to maintain 100% efficiency. Most coulometric cells employ a working electrode of packed particulate material to ensure a large enough surface area whilst maintaining a small cell volume. In these cells the convective mass transport is reduced to that produced by the flowing eluent stream. Various packing materials and uses have been reported. Molner (108) used platinum chips to monitor heavy metals in solution, Blaedal (109) reported the electrodeposition of trace metals onto granular glassy carbon followed by anodic stripping. Strohl has also used granular glassy carbon for inorganic and organic determinations (110,111), and Roe (112) has used a granular mercury electrode formed by amalgamising nickel chips.

Johnson and Larochelle (33) have described an L.C. detector with a platinum tube working electrode, platinum wire counter electrode and S.C.E. reference. See Fig 2.6

Fig 2.6



The separation of iron(II) and copper (II) is shown and a lower detection limit of 1ng. is given for sodium iodide. Johnson (113) has further used this detector to determine antimony in alloys and in human hair at the nanogram level.

Takata and Muto have described a cell (31) which is capable of giving 99.5% efficiency at flow rates up to 6ml/min. The working electrode material used was variable and included carbon cloth, platinum gauze and silver wire netting. See Fig 2.7

Fig 2.7

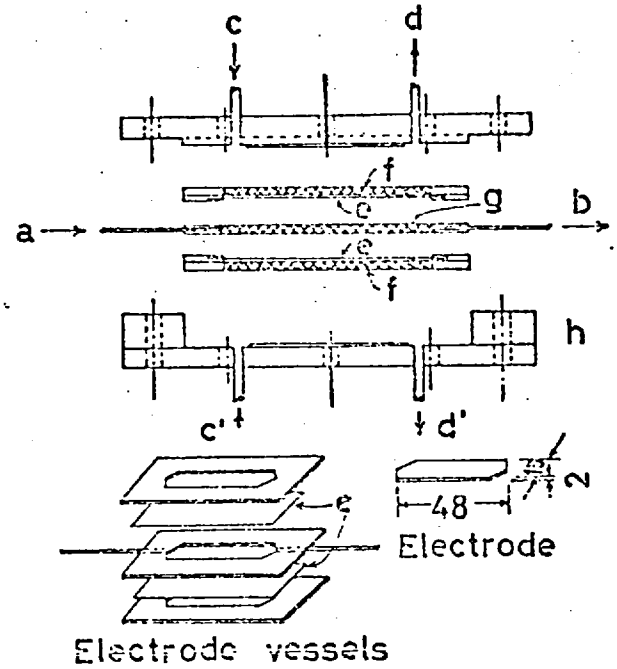


Figure 2. Flow coulometric detector cell

a: Inlet of the cell; *b*: outlet of the cell, *c*, *c'*: electrolyte inlet, *d*, *d'*: electrolyte outlet, *e*: ion exchange membrane, *f*: auxiliary electrode, *g*: working electrode, *h*: case of the electrode vessels

A large number of applications were given many of which involved the reaction of the sample with an electrochemically reactive substance to make it compatible with the form of detection. Compounds detected included metal ions, inorganic anions, organic acids, phenols and sugars. In favourable cases the detection of 5×10^{-10} Mole of sample was claimed, but generally the detection limit was orders of magnitude lower than this. The linear dynamic range was not indicated nor was

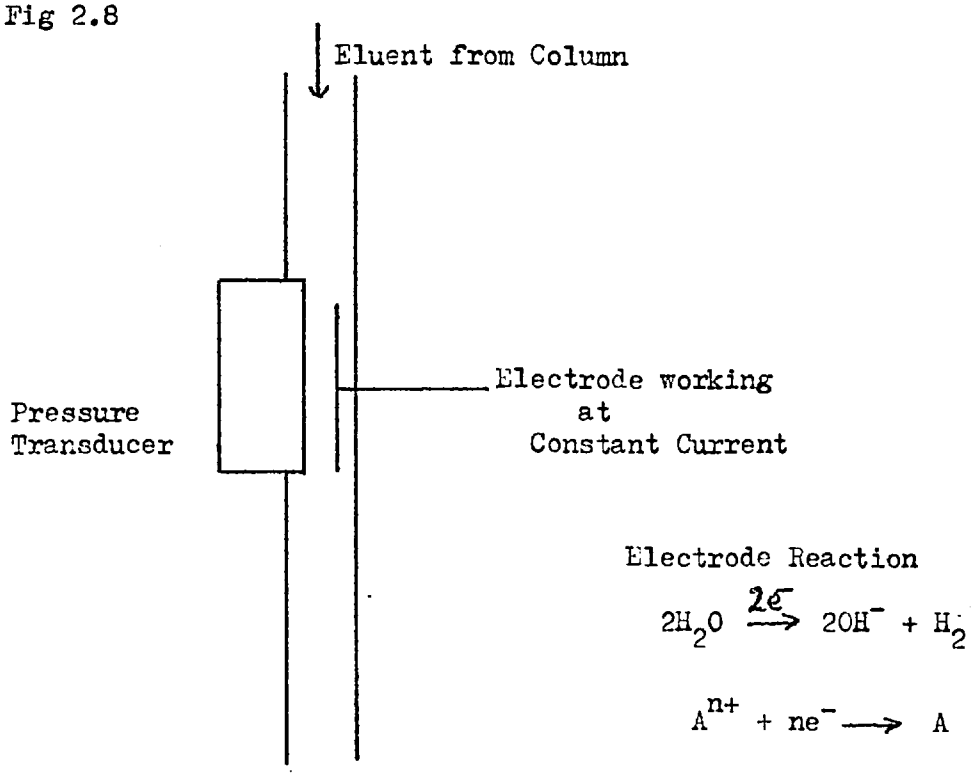
the reproducibility of the system. Adsorption problems were not discussed.

As in the case of voltammetric cells, only the most favourable results are usually quoted by the authors, and very little discussion of adsorption is given.

2.6.4 Pneumatopolarography Detection

The application of Pneumatopolarography to L.C. detection has not yet been tried to date but offers a possible area of research. The principle of the method is to hydrolyse the aqueous eluent at a constant potential well in excess of the hydrogen and oxygen overpotentials. If this is done, a constant gas pressure is produced. When a sample comes off the column, its competition for the available current produces a variation in the gas pressure which can be seen by use of a pressure sensitive transducer. The advantages of this method lie in its sympathy with small volume cells, and the fact that the formation of gas at the electrode surface should prevent the deactivation of the electrode by adsorption.

Fig 2.8



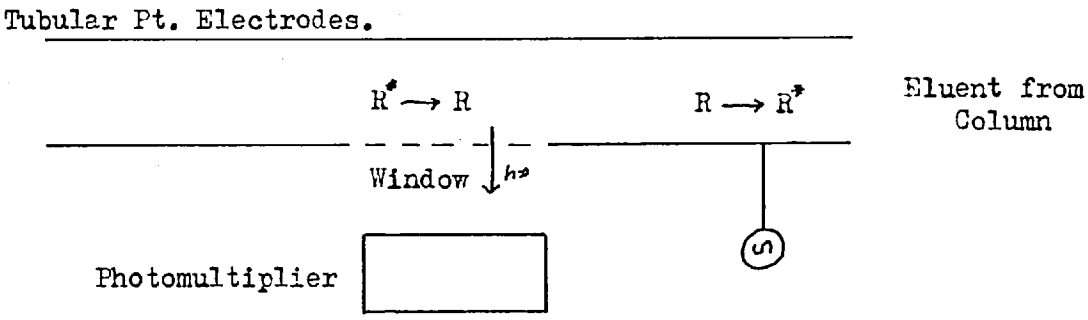
2.6.5 Electrochemiluminescence Detection

It has been reported that an A.C. signal applied to a solution of an aromatic or substituted aromatic causes the formation of free radical and charged species, which can then lose energy by luminescence. The phenomenon is termed electrochemiluminescence (114,115).

A detector based on this principle has been reported (116). Although no detection limits have been made available the practical problems have been investigated. Importance has been attached to the optimisation of the flow rate to achieve maximum luminescence at the photomultiplier window.

The detector is however severly limited in its application to a small group of compounds and to eluents which do not quench the luminescence.

Fig 2.9



Design for Electrochemiluminescence Detector.

2.6.6 Capacitance Detectors

Capacitance measurements have been applied(19) to L.C. detection

to produce cells in which the difference in capacitance is noted with and without sample. The sensitivities reported (36) are comparable with R.I. methods with the frequency tuned to give optimum performance.

2.6.7 Conductivity Detection

Conductivity detectors have been produced based on one of two techniques:-

a) Low Frequency Method

This can be applied to electrolytes, non electrolyte forming ionic complexes and non electrolyte solutes in conducting solvents. An A.C. wheatstone bridge network is used to measure the conductance (37,38), and the technique can be used in cells of very small volume i.e. 1μl.(39).

b) High Frequency Method.

This can be used for dielectric (non conducting) materials providing they are dipolar or can be made so, and has the added advantage that the electrodes are not in contact with the solution (40,41).

In both cases the detectors have only been used on low resolution columns. Their main drawback is their extreme sensitivity to temperature fluctuations, which result in an extremely unsteady base line.

Fig 2.10 shows a comparison of detectors taken from a discussion lecture (19).

Fig 2.10

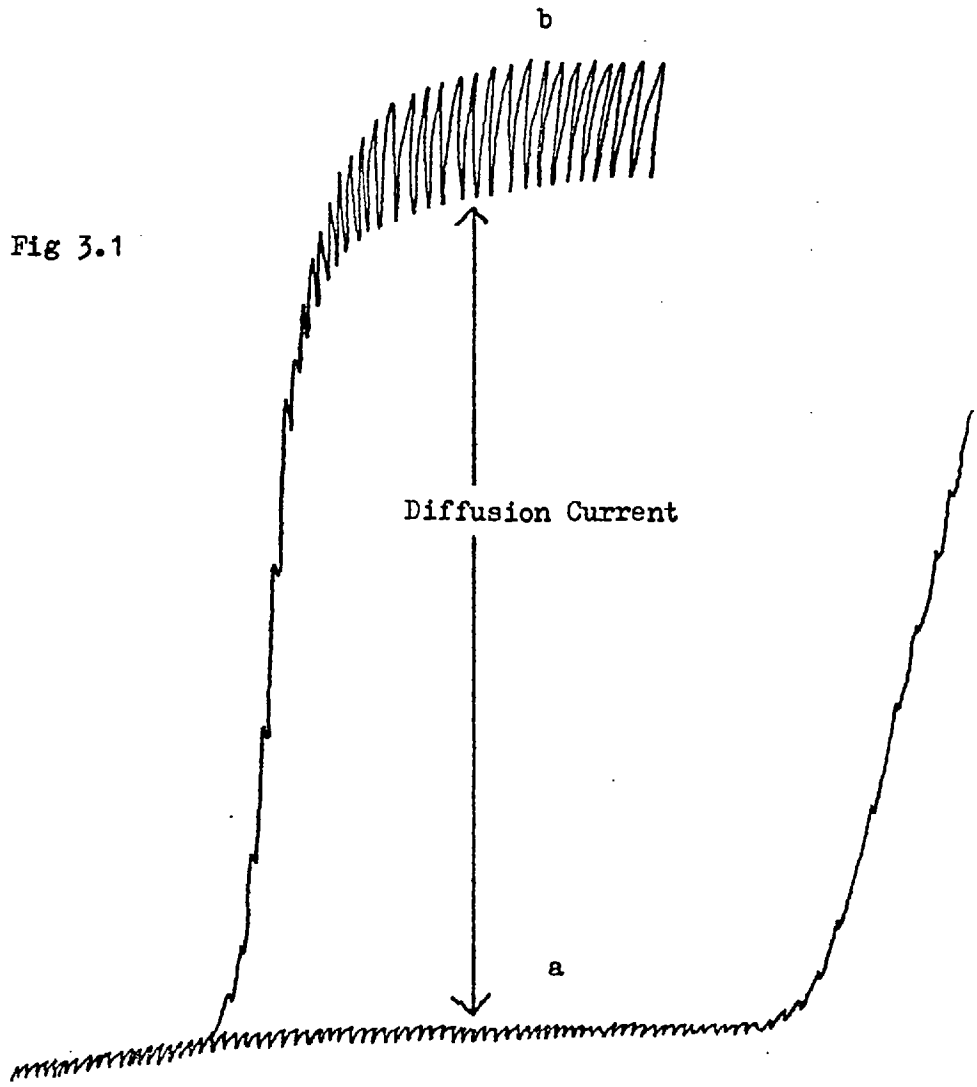
Detector	Fundamental Noise Level	Volume V V=2Noise	Detectable Amount	Sample Size = 2 Noise
U.V. 254nm.	2.5×10^{-5} A.U.	5×10^{-10}	4×10^{-11} g/s.	2×10^{-10}
Variable U.V.	10^{-4} A.U.	2×10^{-9}	2×10^{-10}	10^{-9}
Voltammetry Carbon Paste	Variable	3×10^{-10}	2×10^{-10}	10^{-10}
Refractive Index	5×10^{-8} R.I.U.	5×10^{-7}	4×10^{-8}	2×10^{-7}
Wire F.I.D.	5×10^{-8}			
Fluorescence	ca. 10 times lower than variable U.V.			

3.1.1 PRINCIPLES OF VOLTAMMETRIC DETECTION

Many organic and inorganic compounds are capable of undergoing electro oxidation and reduction. The earliest work done by Heyrovsky (42) in the 1920's laid the foundations of Polarography, a specialised form of voltammetry in which the working electrode is a Dropping Mercury Electrode (D.M.E.). Voltammetry in its widest possible sense consists of applying a potential between two (sometimes three) electrodes and noting the current that flows between them. When this is done at several different potentials (usually by scanning over a potential range) a current voltage plot is obtained (voltammogram) whose shape depends upon the composition of the solution analysed.

Fig 3.1 shows a typical polarogram for the reduction of cadmium.

Fig 3.1



a is the residual current in the absence of any depolariser. This current is made up of various components ranging from I.R. drop , to currents due to trace impurities and to charging current.

b is the current due to the reduction of the Cadmium ions

$$Cd^{2+} + 1e^- \rightleftharpoons Cd^0 \qquad \text{Equation 16}$$

The plateau region is the most important for here the current is independent of the potential and under ideal conditions diffusion controlled. It is in this region that the current is proportional to the concentration of the depolariser. The limiting current (plateau current) is defined by the Ilkovic equation

$$i_d = 607.0\ n\ D^{\frac{1}{2}}\ C\ m^{\frac{2}{3}} t^{1/6} \qquad \text{Equation 17}$$

i_d= Average Diffusion Current C= Concentration t= Drop Time

m= Flow Rate D= Diffusion Coefficient

If the electrode assembly used to produce the above wave was placed at the end of a Chromatographic column and the potential of the working electrode set on the plateau region (- 0.9V) then anything coming off the column would be automatically reduced and the currents produced, being proportional to the concentration, would follow the concentration profile exactly. If a mixture of compounds is to be detected it is only necessary to set the potential far enough negative to be in the plateau region for all the components and all the compounds will be detected. Conversely a much more selective detector can be made by suitable choice of detector potential. In this way if two peaks are poorly resolved it is possible to detect just one of them if the detector can be set at a potential which is not on the plateau region of the other compound.

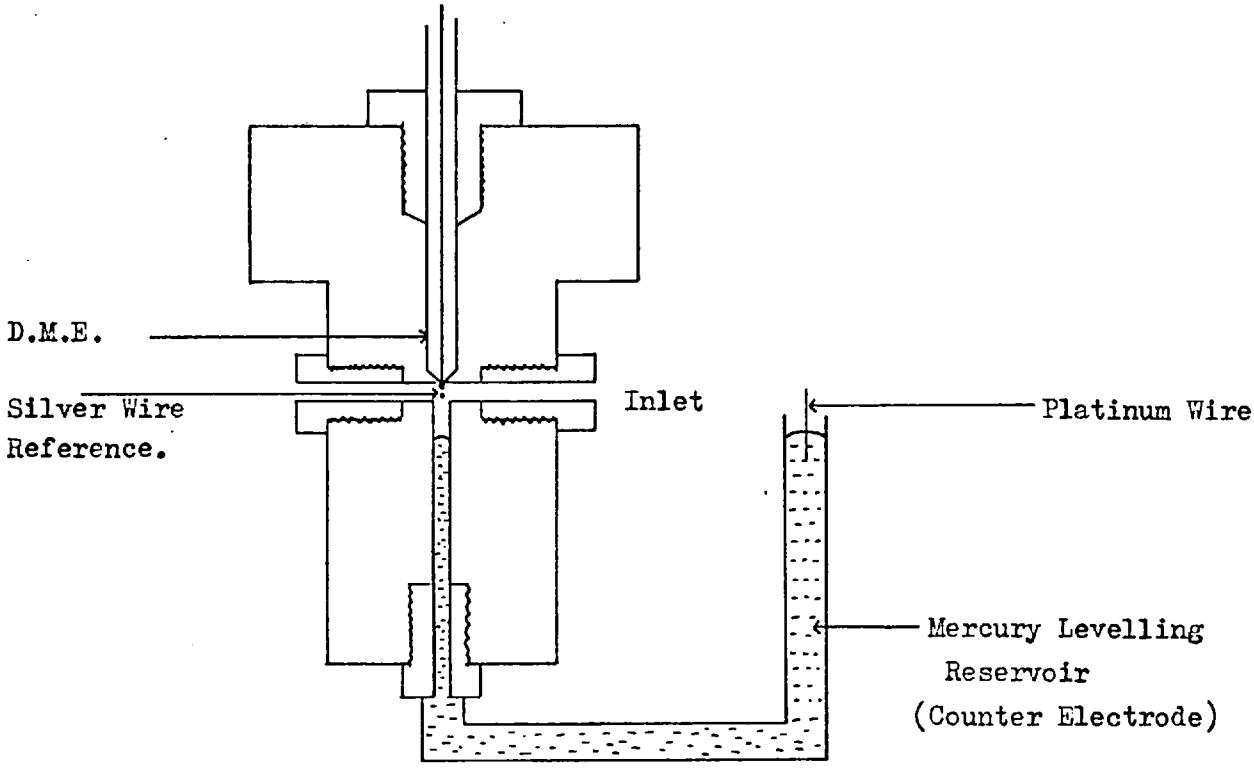
The advantage of this technique over coulometry can be seen at this stage. In coulometry the need is for 100% efficiency which means a large electrode area and a fairly slow flow. This results in total destruction of the sample and the possibility of peak broadening. The voltammetric method destroys only a fraction of the sample because of the concentration

diffusion current relationship and hence is compatible with small electrodes, small volume flow cells and a high range of flow rates.

3.2.1. DETECTOR DESIGN

The cell shown in Fig 3.2 was machined out of a perspex block. Although perspex is not the ideal material for a working cell since it is not compatible with many organic solvents, it has the useful property of being transparent which is ideal for research purposes.

Fig 3.2



The main object of the design was to keep the cell volume as small as possible to reduce mixing, and to keep the three electrodes as close together as possible to reduce the effect of I.R. drop.

Because the paths inside the cell are so narrow I.R. drop in them is

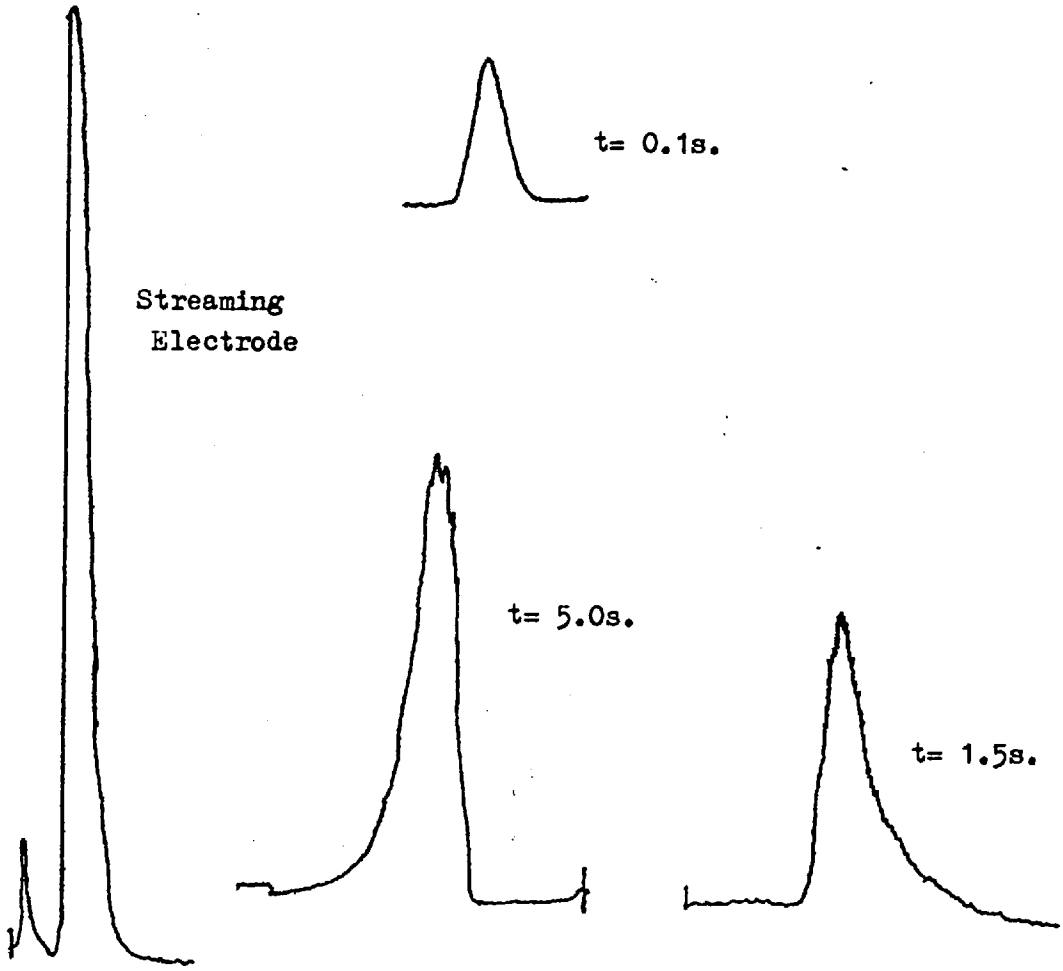
considerable and so besides keeping the electrodes as close together as possible, it is necessary to use the three electrode system. If this is not done, the indeterminate I.R. drop results in very poor potential control. The counter electrode used was a platinum tube and the reference, a Silver / Silver Chloride wire. Because the potential is set somewhere on a wave plateau and not at an exact potential it is possible to relax restrictions on the quality of the reference electrode and sacrifice perfect stability for convenience. To ensure good flow around the Mercury drop it has been found advantageous to use a conically ground capillary. It is important however that a small piece of flat glass is left around the nozzle otherwise the grinding process produces small cracks in the capillary which in turn leads to poor drop reproducibility. The conical D.M.E. also prevents the drop from being knocked off by the flowing stream as would happen if it were knocked against a large flat capillary end. Finally the conical shape allows a smaller cell chamber to be used.

The main advantage of using a D.M.E. electrochemically is the fact that it produces a new surface with each drop and thus it has no memory effects. The very fact that drops are produced however means that an extra parameter has been formed. The optimum drop time has to be determined. The larger the drops are, the more surface is available for reaction, and hence the greater are the currents produced. This leads to a greater sensitivity in the detector. Unfortunately the larger the drops, the slower is the drop time. In normal electrochemical work the drop time for D.C. analysis is of the order of 4 seconds. This time scale is much too slow for

chromatographic studies as peaks can be eluted with modern columns in considerably less time. Therefore a compromise has to be achieved between small fast drops with reduced sensitivity and large slow ones with the possibility of poor peak definition.

Some relative peak sizes and drop times are shown in Fig 3.3

Fig. 3.3



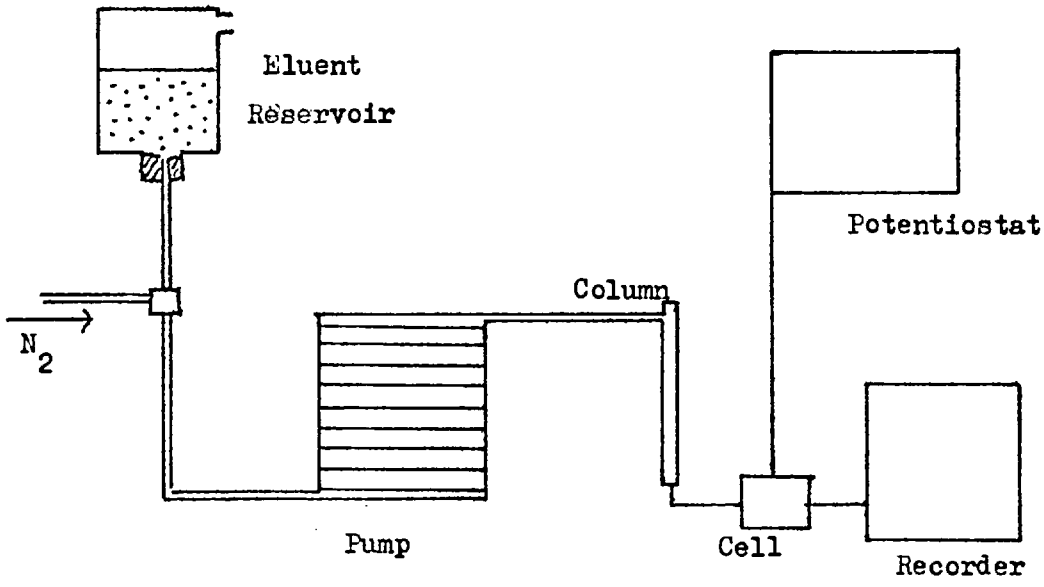
The most sensitive method is to use a streaming mercury electrode. This consists of a wide bore capillary through which mercury is forced so that it comes out in a continuous stream. After about 1 cm.(depending upon the pressure), this stream breaks up. Tha resulting electrode is a cylinder whose surface area can be carefully controlled. The improvement over the standard D.M.E. is about 1 to 2 orders of magnitude. The main drawback to this method is its difficulty in use and its enormous consumption of mercury. It would not be practical to use a

streaming mercury electrode on a commercial detector and it is really only of use on specialised research projects.

The most useful drop time was found to be about 1 second and this, normally produced by a drop knocker, was used in all the following D.M.E. work. The cell was initially tested out by carrying out D.C. scans on Cd^{2+} solution both in a flowing stream and also in a static stop flow mode. Pumping for this system was carried out by a Technicon Auto-analyser pump. This was done because the D.C. performance of Cd^{2+} is well known and any deviation from the normal could then be attributed to faults in the cell. Once good D.C. polarograms had been produced by the cell it was then used on the end of a chromatographic column. The main problems in the flow stream were the mercury drops catching on the silver wire reference, and the introduction of air bubbles into the cell. Air bubbles cause the drops to be released prematurely and also lead to very high resistances in the cell which affect the output considerably.

The cell was finally tested with very simple apparatus.

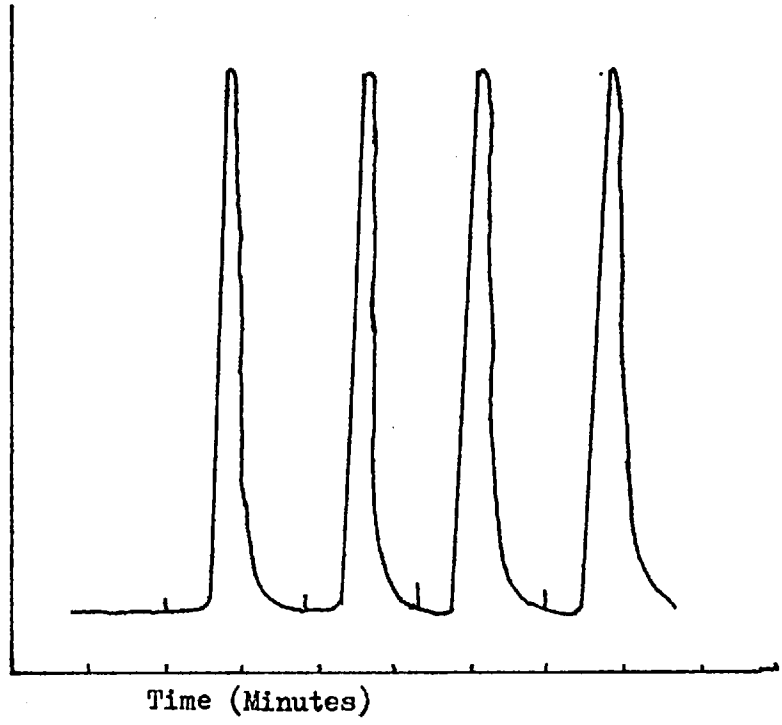
Fig 3.4



The column was simply packed with glass beads which, although they could not separate any compounds, made a very good dummy column. Because the glass beads require very little column pressure there is no need to degas, since air bubbles are not formed at the bottom of the column. Therefore it is only necessary to deoxygenate the eluent before use. The pump used was a gravity fed pump giving no pulsing.

Fig (3.5) shows the result of consequentive injections of Cd^{2+}

Fig 3.5



Eluent:- 0.1M KCl Potential:- -1.0V v S.C.E.

The reproducibility was very good, within 1% and the approach to chromatographic detection looked very promising. Work has also been done applying differential pulse and A.C. techniques to the D.M.E. (43) but before continuing on this line of approach it is useful to take another look at the drawbacks using a D.M.E. as opposed to the promising results it produced.

3.2.2. PROBLEMS WITH THE D.M.E.

a) The D.M.E. can only be used for reductive detection. Mercury itself begins to oxidise at about + 0.3 V. versus a S.C.E. (exact potential depends upon the solution in question) and so its use is limited to a potential range from say + 0.1 V to the negative breakdown potential of the electrolyte, (The base line is usually very bad at potentials more positive than about + 0.1 V.). Since many of the chromatographically interesting compounds are large organic molecules with very often only oxidisable groups on them, the D.M.E. is useless as a detector for them.

b) The selection of the drop time as used so far in this work is far from ideal. Peaks have been reported in the literature which come off the column in less than 1 second. With a drop time of 2 seconds it is necessary for the peaks to be greater than 10 seconds long i.e. 5 sampled points before any accurate information can be obtained from them. Accurate measurements of peak heights cannot be obtained on faster peaks. This is a serious drawback as column technology improves.

c) Although the cell volume is small i.e. less than 1μl. when the drop is at its largest. While the drop is growing the cell volume is much larger. This large cell volume, coupled with the fact that the growing drop will cause turbulent flow in the cell, results in considerable mixing and this will become very apparent especially at concentrations approaching the detection limit. As the growing drop is fundamental to the D.M.E. there is no way around this problem.

d) The periodic current growth as the drops grow, characteristic of the D.M.E. causes great difficulty as the faradaic currents become smaller. Often at low levels the current variation throughout the drop life is greater than the chromatographic peak height. When this is the

case it is very difficult to see the chromatographic peak. There are two possible ways around this. Either the system can be heavily damped using an R.C. filter, or a sample hold technique can be used. An R.C. filter capable of blocking (or at least reducing) signals of $\frac{1}{2}$Hz. i.e. 1 drop every two seconds, will also block out most fast chromatographic peaks probably up to about 10 seconds and will reduce greatly the detector response to other slightly slower ones. If on the other hand a sample hold method is used which samples the current at the end of the drop life and puts this value out until the next sampling takes place, only peaks faster than 2 seconds will be completely missed. However the growth of drops in a flowing stream is not always very regular and a sampler synchronised to a drop knocker will not always find the drops in the same condition. This leads to a very noisy base line which is not periodic but random. In fact this lack of stability of the drop in a flow stream causes problems even on the ordinary non sampled system.

e) When the drop is at full size it has very little clearance at the cell walls. This means that the cell is very sensitive to physical vibrations. Any slight vibration of the chromatographic system or the bench it stands on tends to knock the drops off. In a commercial detector this is a great drawback especially for routine analysis in a large laboratory.

f) Finally, a great amount of expertise is needed to use a D.M.E. correctly. When organics are present it is easy to block up the capillary. Furthermore, mercury is a very unpleasant element and its use in a routine analysis in a commercial detector should be avoided if possible.

On reflection, although quite promising on large samples, the D.M.E. detector has many theoretical and practical limitations when applied to real systems and realistic sample sizes. The noise produced by both drop

oscillations and random fluctations raises the detection limit to too high a value for routine chromatographic work. Thus it is unlikely that the D.M.E. detector will find much use in routine industrial analysis. However it does have a place in specialised research work where more time and skill can be applied to it and where it could be of use in the solution of specialised problems.

It was this search for a detector which could be used generally that led to the investigation of the available solid electrode materials to try and solve or avoid the problems associated with the D.M.E.

3.3.1. SOLID ELECTRODES

Because of the poor positive range of the D.M.E., various solid electrodes have been used in the last few years to study the oxidation of organic compounds. When deciding which electrode material to use it is necessary to look at two characteristics of the electrode. First its potential range in the solvent of interest and secondly its inertness in the potential region of interest. The potential range can be defined as the distance between the onset of the cathodic electrolyte breakdown and the anodic one. In aqueous solution this corresponds to the reduction of hydrogen ions and the oxidation of water.

$$2H^{+} + 2e \longrightarrow H_2 \qquad \text{Equation 18}$$

$$2H_2O \longrightarrow O_2 + 4H^{+} + 4e \qquad \text{Equation 19}$$

The potentials of these reactions are given by the Nernst equation :-

$$E = E^{0}_{H_2/H^{+}} - 0.059pH \qquad \text{Equation 20}$$

and

$$E = 1.23 - 0.059pH \qquad \text{Equation 21}$$

If the pressures of O_2 and H_2 are assumed to be 1 atmosphere, Equation 20 reduces to

$$E = -0.059\ pH \qquad \text{Equation 22}$$

Plots of these two equations give the reversible break down limits for aqueous solution at various pH's, and the theoretical curves are independant of electrode material. Practical variations to these lines are known as overpotential effects and are characteristic among other things of the electrode type.

3.3.2. PLATINUM

Platinum has been used more than any other solid electrode to date, probably because of the widely held view that it is inert. It has the advantage that its anodic range exceeds the theoretical values considerably because of the high oxygen overpotential.

Its cathodic range is not very good however as hydrogen has a negligible overpotential. Fig 3.6. shows some of the potential limits for platinum quoted by Adams (44).

Fig 3.6

Electrolyte	Anodic	Cathodic
0.1M HCl	+1.1	-0.3
Acetate pH 4	+0.9	-0.5
Phosphate pH 7	+0.94	-0.7
0.1M NaOH pH 12.9	+0.72	-0.91

Two other phenomena can also be seen at platinum electrodes. Firstly, if the potential is kept just positive of the hydrogen evolution potential for any length of time a layer of sorbed hydrogen is formed as a film. When the potential is then moved away from this point a large anodic current starts to flow which is extremely irreproducible and which results from the dissolution of this hydrogen film (45-50). The second problem arises from the fact that platinum is not an inert metal and at positive potentials an oxide film is formed on the electrode surface. This oxide film can be seen when a negative scan is made as it is shown up as a peak corresponding to a Pt / Pt(OH) couple.

3.3.3. GOLD

Another of the so called "inert" noble metals, gold suffers from the same type of problems as does platinum. Cathodically gold is much better than platinum as the hydrogen overpotential is some 0.4 to 0.5 V.. Furthermore the absence of any anodic currents indicates that sorbed films of hydrogen are not formed on gold. On the anodic side however, gold electrodes are extremely complex in their behavior. In certain buffers such as acid chloride media, gold will react to form metal chloride complexes. Of greater importance however is the oxidation of gold to gold hydroxide. This couple is indicated by the presence of a peak in the cathodic scan.

The formation of oxide and adsorbed hydrogen films on platinum and of complexation and oxide films on gold means that the residual currents for both electrodes are extremely irreproducible. The currents formed by these processes can be of the order of microamps and since chromatographic peaks will probably be in the hundred nanoamp range or less, these base line variations will be too large for use in the chromatographic detector. This unsuitability of gold and platinum led to the investigation of the many carbon electrodes now becoming so popular.

3.3.4. GRAPHITE

The first carbon electrodes used were made out of this material. Although they have a wide potential range their molecular structure is such that solvent molecules can diffuse into the electrode material causing strange current effects (graphite permeability being 10^{-3} to 10^{-6} m^2 $Sec.^{-1}$ (51)). In order to overcome this problem two approaches have been used. The first is to impregnate the graphite with wax (52,53) to form the Wax Impregnated Graphite Electrode (W.I.G.E.).

The potential range of these electrodes is excellent.See Fig 3.7

Fig 3.7

Electrolyte	Anodic	Cathodic
1M $HClO_4$	+1.5	-0.2
Acetate pH 4		-0.88
0.1 M NaOH pH 12.9		-1.28

Their main problems are their rather large residual currents which are anomalous and probably due to variations in impregnation procedure. Besides this they are prone to adsorbing organic compounds and the mechanical cleaning needed to remove them tends to wear down the electrode surface resulting in a short electrode lifetime.

The second method is to mix powdered graphite with an organic liquid such as Nujol to form a Carbon Paste Electrode (54,55). These electrodes have much lower residualcurrents than their W.I.G.E. counterparts but are

again prone to organic adsorption. The mechanical properties of the paste are much worse than the W.I.G.E.s and the electrode lifetime is severely reduced by cleaning.

3.3.5. PYROLYTIC GRAPHITE

Pyrolytic Graphite really isn't graphite at all but consists of a collection of crystallites, each composed of groups of imperfect hexagonally bonded sheets of carbon atoms. This structure is known as " Terbostratic" (56,51). It is produced by one of two methods. The first is to pass a mixture of a gaseous hydrocarbon (methane or propane) and an inert carrier gas over a heated substrate. The graphite is then deposited under reduced pressure to form a series of layered carbon crystallites. The second method is to form a fluidising bed of substrate particles in a hot zone. The structure of the pyrolytic graphite depends upon the temperature, the ratio of the surface area of substrate to pyrolising gas and the type of hydrocarbon gas used. Alloyed graphite can also be made with various elements such as Boron (57) and Titanium (58).

Pyrolytic graphite is anisotropic and as such its permeability depends upon the direction of alignment of its surface crystal planes. The minimum permeability being of the order of $10^{-14} m^2 Sec^{-1}$. When used as an electrode it is necessary to chose properly alligned planes for the surface and to seal the edges in an epoxy resin to prevent adsorption into the bulk of the electrode. Work done in recent years (59-62) indicates a potential range from +1.0 V. to -0.8 V. in an acidic chloride or nitrate media.

The main problem with pyrolytic graphite electrodes lies in the fact that when polished the planes tend to fracture along stress lines. This makes it very difficult to machine the graphite and maintain an unbroken surface. Because of this pyrolytic graphite is prone to surface adsoption effects. Several reasons have been proposed for the adsorption phenomenon including these listed below :-

a) Chemisorbed oxygen may exist on the surface thus attracting water

molecules.

b) Carbon- oxygen reaction sites exist causing localised complexes to be formed.

c) Carbon- oxygen groups may be formed on the electrode surface such as quinoids, quinhydrones, phenols and carbonyls. These are probably formed in the grinding process when the free energy released in polishing is great enough to make these reactions go. These in turn give rise to redox couples when a potential is applied to the electrode thus producing high residual currents.

The majority of the adsorption takes place in the deep cracks which are characteristic of pyrolytic graphite surfaces and thus is very difficult to remove except by grinding away the whole of the surface. Although this is certainly possible in physico-chemical studies it is a major obstacle to the use of pyrolytic graphite in continuous on line monitoring.

3.3.6. VITREOUS CARBON

Vitreous or glassy carbon is fundamentally different from the carbons so far described. It is an isotropic substance and as such has a general low permeability regardless of orientation $\approx 10^{-5}$ m^2Sec^{-1}. It is formed from non-graphitising polymers. The polymers must be cross linked in order to prevent the reorientation of the nuclei into crystallites. The pyrolysis of these polymers produces glassy carbon(63). The resulting carbon has a much lower electrical resistivity than pyrolytic graphite $\approx 8 \times 10^{-5}$ ohm.m. compared with $\approx 200 \times 10^{-5}$ ohm.m.. Glassy carbon is an extremely hard and brittle substance and as such it must be machined with a diamond or ultrasonic drill. If these are available however, machining can be carried out with considerable accuracy. Being very hard it is possible to get a very smooth surface on glassy carbon which is extremely important when solution is passing over it and hydrodynamic conditions are necessary. Furthermore it does not contain the deep cracks which are a feature of pyrolytic graphite and thus any adsorbed material can be mechanically rubbed off the surface

without removing too much of the surface. This means that the electrode itself has a long lifetime and that adsorbed products can be removed much more easily. As will be shown later glassy carbon has far fewer surface oxide groups than the other carbons. Adsorption however is known to take place at the surface but to a lesser extent than on the other carbons.

Although the use of glassy carbon is not well documented in the literature it certainly presents the best qualities of all the carbons when looked upon as an electrode material for continuous monitoring. This, and the obvious problems that are known to exist with the noble metal electrodes, led to glassy carbon being chosen as an initial electrode material. Its main attractive features being its inertness and ability to produce a highly polished surface.

4.1.1. VOLTAMMETRY AT SOLID ELECTRODES

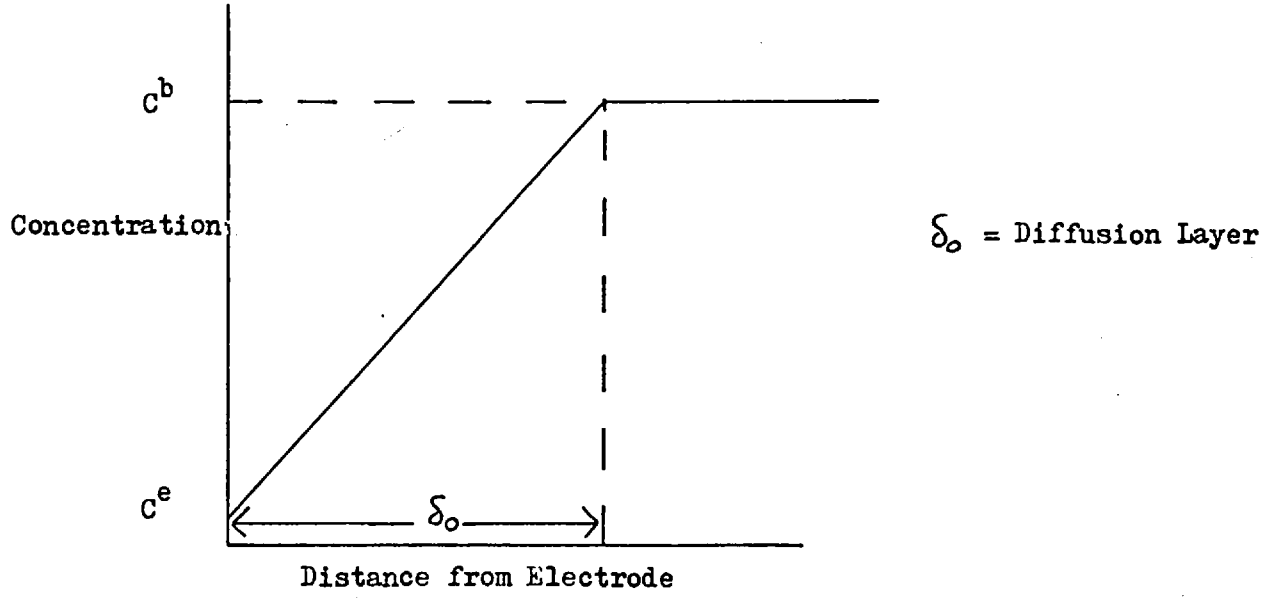
If an electrode is placed in a solution containing a redox couple the electrode tries to maintain a certain ratio of oxidised to reduced species at its surface in accordance with the Nernst equation.

i.e. if $Ox + ne^- \rightleftharpoons Red$ _____ Equation 23

$$E = E_{Ox/Red} + \frac{RT}{nF} \ln \frac{Ox}{Red}$$ _____ Equation 24

If the bulk concentrations of oxidant and reductant are not in the correct ratio as determined by the electrode potential, a current flows to produce the correct ratio of concentrations at the electrode surface. This in turn produces a concentration gradient between the electrode surface and the bulk solution down which can flow reactant species to the electrode surface. The same gradient in reverse removes product from the electrode surface. Eventually a steady state is set up in which the rate of diffusion of the reactant species down the gradient produces a limiting current. Nernst (64) proposed the presence of a diffusion layer close to the electrode in which most of the concentration gradient appeared and within which mass transport was by diffusion alone. Outside this layer mass transport was mainly by convection but within the layer the solution was static.

Fig 4.1



C^b= Bulk Concentration C^e= Concentration at Electrode Surface

4.1.2. FORCED CONVECTION SYSTEMS

The diffusion layer concept is an extremely useful model although it is no longer accepted without reservation. The main criticism levelled at it lies in its assumption that the diffusion layer is stationary. When this theory is applied to flowing solutions it has to be adapted considerably. Whenever a solution flows past a solid surface there is a distribution of velocities spreading out into the solution. At the solid surface the velocity is zero. At some distance away the velocity is that characteristic of the bulk solution. In between lies an area of wide variations in velocity due to the viscosity of the liquid. This region of high velocity gradients is known as the Boundary Layer or Hydrodynamic Boundary Layer. There is now the possibility of two different mass transport paths to the electrode surface, one by diffusion, and the other by the physically moving liquid, i.e. convection. As one moves towards the electrode diffusion plays a gradually increasing role in the process. Theoretical treatments have been developed by applying hydrodynamics to electrochemical models (65, 66). Normally they have to be altered to accommodate different electrode geometries but simple models have been developed for tubular electrodes (67) and Rotating Disc electrodes (68).

4.1.3 ROTATING DISC ELECTRODE

The rotating disc electrode (R.D.E.) is one of the few forced convection systems where a completely rigorous treatment has been applied. Levich (68) set up the mass transport equations in 1942 and these were later developed by the Russian school. The theoretical treatment is applied by assuming that the R.D.E. is a thin plane surface whose area is so large that the edges may be neglected when compared to the total surface. This surface is then rotated at a constant angular velocity. The solution at the electrode surface assumes the angular velocity of the disc and thus develops a radial velocity out from the disc causing flow to occur across the surface of the disc. To maintain solution

balance, liquid must then flow towards the disc in an axial direction. The result being an increase in mass transport to the electrode surface due to forced convection and a corresponding increase in limiting current. The hydrodynamic boundary layer is given by:-

$$\delta_o = \left[\frac{3(\nu)}{(\omega)}\right]^{\frac{1}{2}}$$

At distances less than from the electrode surface, the radial velocity decreases as the distance (y) increases, while above this value only axial flow towards the electrode is thought to exist. When the concentration gradients produced by the electrode potential are considered, the diffusion problem can be solved using the normal boundary conditions.

$C=C^b$ as $y \rightarrow \infty$ and $C=0$ at $y=0$

The resulting limiting current equation for a reversible system is:-

$$i_L = 0.62\ n\ F\ A\ C^b\ D^{2/3}\ \nu^{-1/6}\ \omega^{\frac{1}{2}}$$

ω = angular velocity of disc. ν = kinematic viscosity C^b = bulk concentration. i_L = limiting current.

4.1.3. WALL JET ELECTRODE

The term " Wall Jet " was first used by Glauert (69) to describe the fluid flow when a jet impinges on a surface normal to it. The fluid then spreads along the surface, radiating out from the point of impact. All the solution outside the jet being at rest. This concept was developed by Matsuda (70) who applied fluid mechanics to the system and managed to solve the corresponding boundary equation.

Matsuda's cell is shown in Fig 4.2

The limiting current equation for this system is:-

$$I_d = (1.60k)\ n\ F\ C_0\ D^{\frac{2}{3}}\ \nu^{\frac{-5}{12}}\ V^{\frac{3}{4}}\ a^{-\frac{1}{2}}\ R^{\frac{3}{4}} \qquad \text{Equation 25}$$

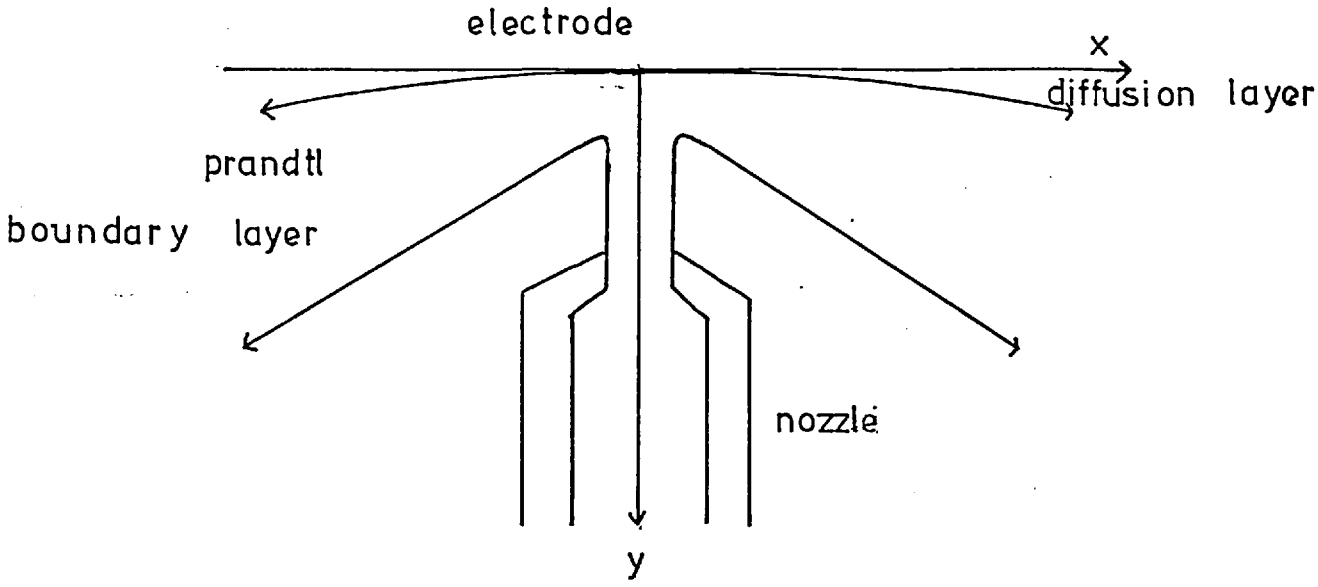
ν = Kinematic Viscosity V = Volume Flow Rate a = Nozzle Diameter

R= Electrode Radius C_0 = Bulk Concentration D = Diffusion Coefficient

h = Proportionality Constant

The rest of the symbols have their usual meaning.

Fig 4.2



The equation was experimentally verified by Matsuda. The idea of a wall jet cell as a possible cell design for an H.P.L.C. detector is promising for several reasons:-

a) The current produced is proportional to the concentration

b) Because of the hydrodynamic properties of the cell, the bulk concentration is maintained very close to the electrode surface. This means that the concentration gradient in the diffusion layer is very large and this in turn increases the cell current thus improving the sensitivity.

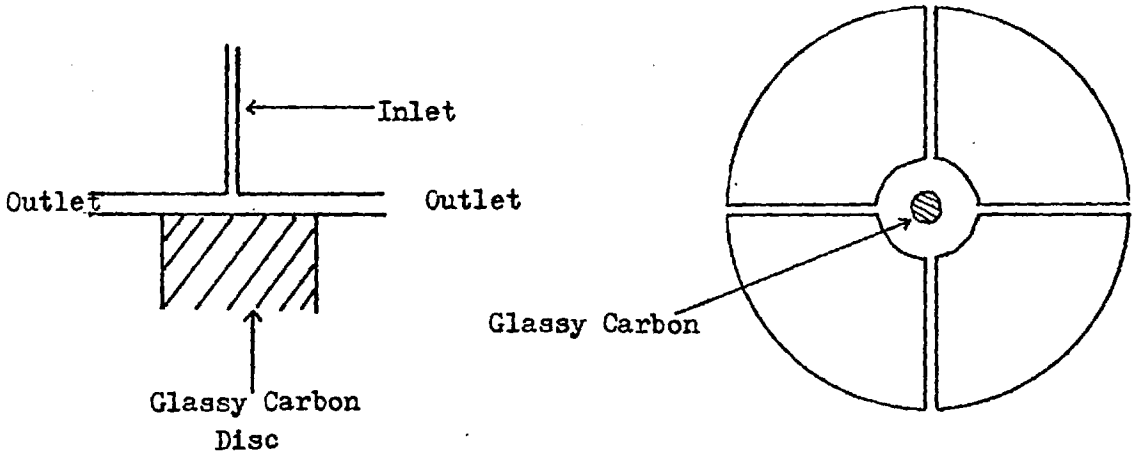
c) The efficiency of the mass transport means that a small electrode can be used thus reducing the cell volume to a minimum. This reduces mixing inside the cell.

d) The high radial flow rates mean that the solution can be moved away from the electrode very quickly.

4.2.1. CELL DESIGN

Once the desired hydrodynamic conditions had been chosen it was necessary to make a cell which could produce them. The first cell produced consisted of a glassy carbon working electrode disc fixed in a teflon rod. This could be screwed into a perspex block into which a small diameter hole had been drilled normal to the glassy carbon disc. In an attempt to facilitate radial flow four outlets were supplied. See Fig 4.3

Fig 4.3

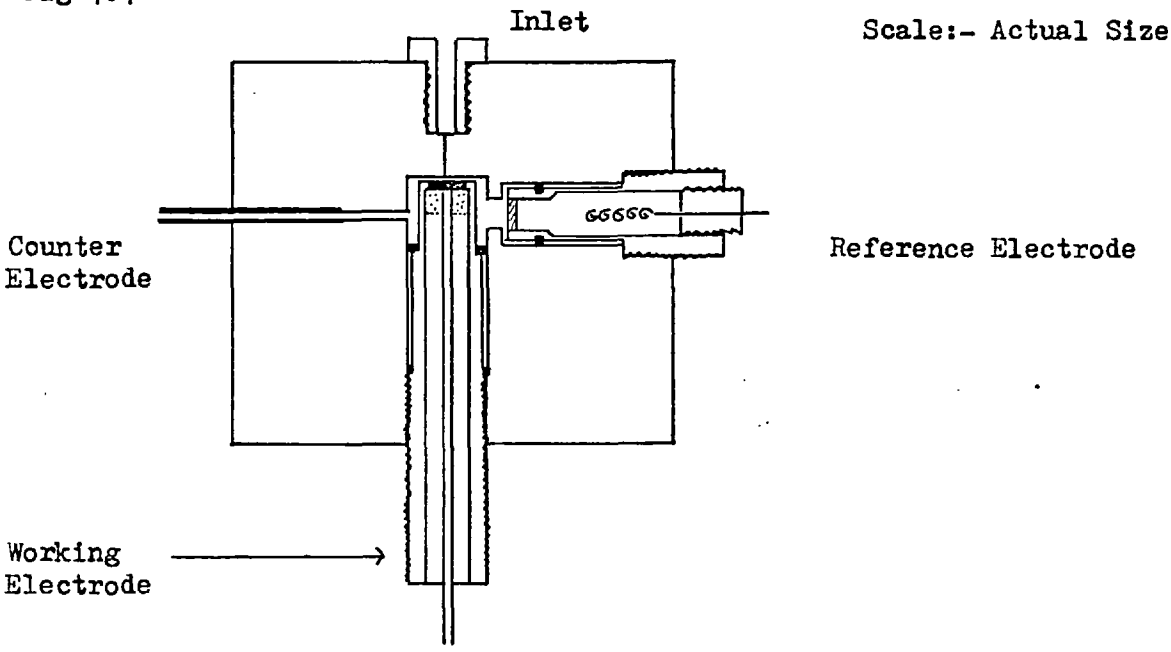


In one outlet arm was positioned a reference electrode which consisted of a compartment drilled into the perspex block and filled with Saturated Potassium Chloride. The reference electrode being a Silver wire. Contact with the eluent was made via a Vycor glass plug. In the opposite arm was placed a Platinum tube counter electrode. Unfortunately, to ensure that the I.R. drop between the three electrodes was not too high it was necessary to make the outlet tubes with a fairly large diameter (about 2mm.). Compared with the inlet path of 0.1mm. these were very large, and it was impossible to maintain flow out of all the tubes. The result was that flow took place out of only one of them,and very often air was sucked in the others.

The next cell designed had just two outlets but once again flow could only be maintained out of one of them. This cell was then modified by allowing the solution to flow along the reference outlet until electrical contact had been made and then the tube was blocked off downstream of the

reference. The only solution outlet left was therefore the path through the counter electrode. Work later done with this cell proved very satisfactory, and it was shown that even with only one outlet the jet produced at the inlet was enough to maintain good radial flow, certainly until well after the active part of the working electrode had been passed. This fact led to the design of the final cell below:-

Fig 4.4



A feature of this cell is that both reference and working electrodes are removable from the cell body. In the early stages of the work all the cells were made in perspex because it is transparent and as such allowed the electrode assembly to be seen. While the cells were being perfected faults in cell design often led to the trapping of air bubbles in the system. If these broke electrical contact between the working and the reference electrodes, the potential control of the working electoode was lost and the working electrode could take up any potential. If it went to a very positive potential the electrode surface could be destroyed and made useless for voltammetric work. If this happened the electrode had to be ground down and repolished. Therefore a perspex cell was used so that air bubbles could be seen and removed from the system before the electrodes were

connected to the potentiostat. Later cells were made of "Delrin", an opaque material which is much more resistant to solvent attack.

4.2.2. THE REFERENCE ELECTRODE

The reference electrode consists of a partially threaded cylinder which can be screwed into the cell body. The electrode itself is a Silver or Silver/Silver Chloride wire in contact with a solution of saturated Potassium Chloride. The bridge between the internal filling solution and the eluent is made via a ceramic frit. The frit should be as thin as possible to reduce I.R. drop to a minimum. A seal between the electrode body and the cell is made by the use of a rubber "O" ring. This is to prevent solution being forced out of the cell up the side of the reference electrode. The positioning of the reference electrode alongside the body of the working electrode means that the long solution paths between reference and working electrodes characteristic of previous cells have been abolished thus giving a more precise knowledge of the potential applied. If the detector is to be used in a selective mode and a very precise potential has to be set and maintained it is possible to replace the Silver wire reference with a salt bridge leading to an external S.C.E.. However in most cases the Silver wire is adequate.

4.2.3. THE COUNTER ELECTRODE

The Counter electrode consists of a platinum tube which also serves as the outlet. A tube is the most convenient way of producing a large electrode without increasing the cell volume. A large counter electrode is needed to ensure that most of the current is carried between this electrode and the working electrode, otherwise current will flow through the reference electrode thus changing its potential. The tube is sealed directly into the cell body using an epoxy resin (Araldite). Because the counter electrode also serves as the outlet for the cell it was placed on the opposite side to the reference so that any air bubbles introduced into the cell would be carried away from the reference and therefore not short out the working-reference path.

4.2.4. THE WORKING ELECTRODE

The working electrode consists of a glassy carbon disc sealed in the end of a partially threaded perspex cylinder. The seal is made of epoxy resin and electrical contact on the reverse side of the glassy carbon disc is made through a wad of Silver loaded epoxy onto a Copper wire. A solution tight seal is made between the working electrode and the cell body by means of a rubber "O" ring. The working surface of the glassy carbon has to be in the form of a flat i.e. level, mirror like finish. The safest method of doing this from the point of view of eliminating errors which could destroy the electrode is to grind the electrode by hand using gradually decreasing grades of Emery Paper and finally polishing with a diamond polishing compound. A Carborundum wheel can be used instead of the Emery Paper but precise control of the angle at which the disc is then ground is then difficult and although much faster than by hand the flat surface produced is often at an angle other than the required right angle to the main electrode axis. Continued efforts to correct this usually result in too much of the electrode being ground away. This means that when the electrode is finally screwed into position in the cell its own "O" ring seals off the cell above the reference electrode. The result being that a new electrode has to be made.

4.2.5. GENERAL FEATURES

The cell inlet is via a general purpose Chromatronix fitting. The actual jet is produced not by a nozzle, as in Matsuda's cell, but by a hole in a flat surface. As a result of this the cell behavior is modified as discussed later. To ensure good flow throughout the cell the internal walls should be as smooth as possible and good machining is essential to prevent stress cracks and give good walls. To provide a good jet the inlet nozzle should be as small as possible but size should not be sacrificed for quality as a smooth surface especially at the lip of the inlet is imperative.

4.2.6. PREPARATION OF THE CELL

The cell is extremely easy to use. The reference electrode should be filled with KCl and then screwed into place. The same should be done with

the working electrode. To ensure that all the air is removed from the cell the flow should be started with the counter electrode vertical so that the air is displaced towards the outlet. Thereafter the cell is best used with the reference electrode vertical so that solution contact is maintained on the inside of the reference frit.

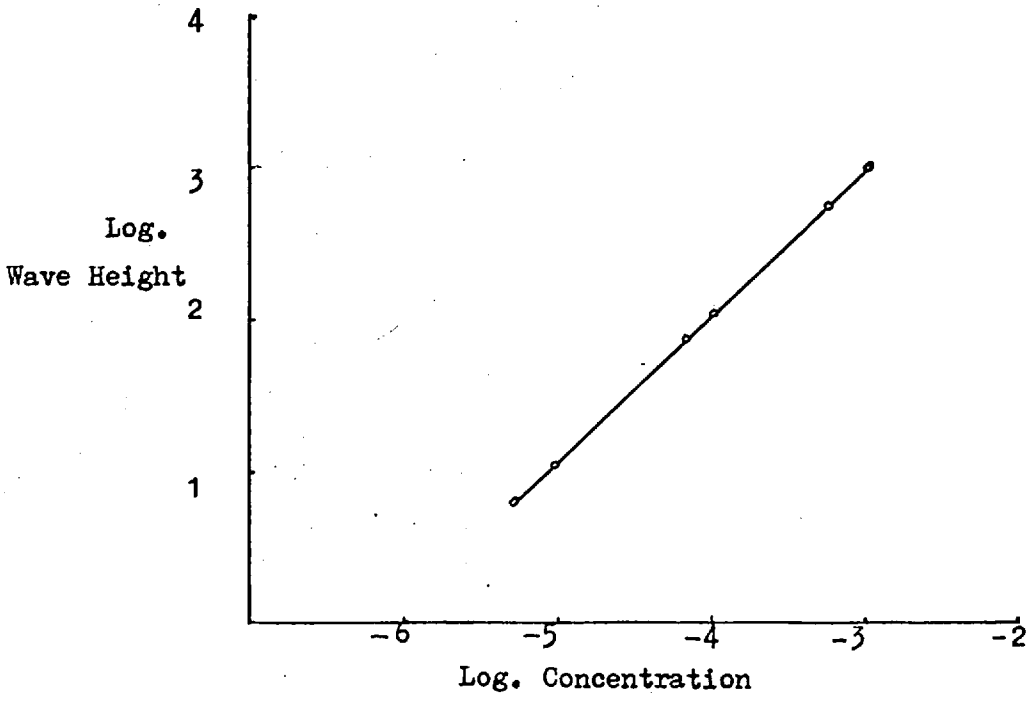
4.3.1. INVESTIGATION OF CELL PARAMETERS

Because the cell differed from the design of Matsuda , beforeit could be used as a detector its properties had to be investigated. It was most important to find out if the current produced was still proportional to the concentration and then to find the combination of cell parameters which gave the maximum current response for a given sample size while maintaining a relatively small cell volume.

4.3.2. CONCENTRATION DEPENDENCE

It is a general feature of voltammetricprocesses that despite variations in flow patterns and electrode geometries the current dependence upon concentration always appears to be linear. However to make sure that this was the case in this cell, an investigation was made into the dependence of the wave height for the oxidation of potassium ferrocyanide on concentration. The wave height was plotted against concentration and the results are shown in Fig 4.5

Fig 4.5



Flow Rate = 1ml./min. Background Electrolyte = 0.1M KNO_3

It should be noted that if consecutive scans are carried out on any one particular concentration , the $E_{\frac{1}{2}}$ value moves more positive as the scan number increases even though the final wave height remains the same. This results in a stretching out of the wave and indicates that the reaction is becoming less reversible. In order to avoid having to mechanically clean the electrode between samples it was necessary to measure the wave heights at a potential well positive of the $E_{\frac{1}{2}}$ value to ensure that the true final current was always taken. For this reason the current was noted when the potential reached +0.8 V. which was far enough away from the $E_{\frac{1}{2}}$ value (+0.29) but still not so far positive to produce problems from electrolyte break down. It is also interesting to note that the flow stream produced waves and not peaks due to more efficient mass transport to the electrode surface.

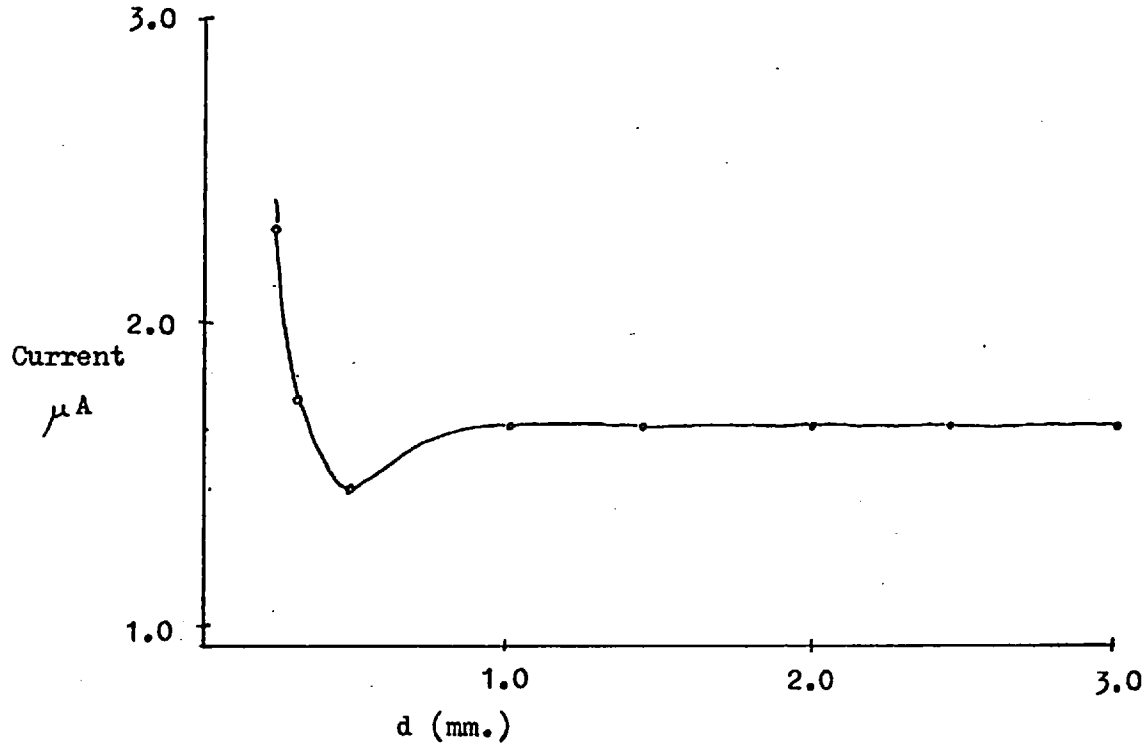
The lower concentration limit was fixed at this value because pulsing from the pump prevented accurate current readings below this.

However it can be seen that the limiting current is proportional to the concentration.

4.3.3. INLET TO DISC DISTANCE (d)

To keep the cell volume as small as possible it is best to keep this distance between the inlet and the working electrode surface as short as possible. However it was necessary to see if a very small value here had an adverse affect on the electrode response. d could be varied by screwing the electrode in as far as it would go and then turning it back by varying amounts. By measuring the thread on the electrode body the distance d for one turn back could be calculated accurately. The currents produced for various values of d are shown in Fig 4.6. The flow rate was fixed at 1 ml./min. as this is a typical chromatographic rate.

Fig 4.6



Flow = 1mm./min. 10^{-3} $Fe(CN)_6$ in 0.1M KNO_3

The shape of this curve can be explained by the proposition of the following model.

The flat part of the curve corresponds to smooth lamina flow over the surface of the electrode. When d is decreased, the whole of the cell volume is decreased and therefore to maintain a fixed volume flow rate the solutions radial velocity must increase. This effect becomes very apparent at about d = 0.7mm. At this point the radial velocity must be such that time for electroactive material to be carried to the edge of the electrode must be comparable to the time it takes to travel through the diffusion layer. The result being that electroactive material is swept off the electrode before it has time to react. This becomes more apparent as the radial flow increases i.e. d decreases, producing a reduction in current. At about d = 0.5mm. this effect is overshadowed by a change from lamina to turbulent flow conditions brought about by the high radial flow rates

and the proximity of the electrode surface to the cell body. Turbulent flow delivers much more material to the electrode surface than does lamina flow and thus a large increase in current is found.

All subsequent work with this cell was done with d = 0.2mm.. This produced a cell volume of 1.415μl., and meant that the cell was completely washed out 12 times per second at a volume flow rate of 1ml./min.. This indicated an ideal cell design.

4.3.4. VOLUME FLOW RATE

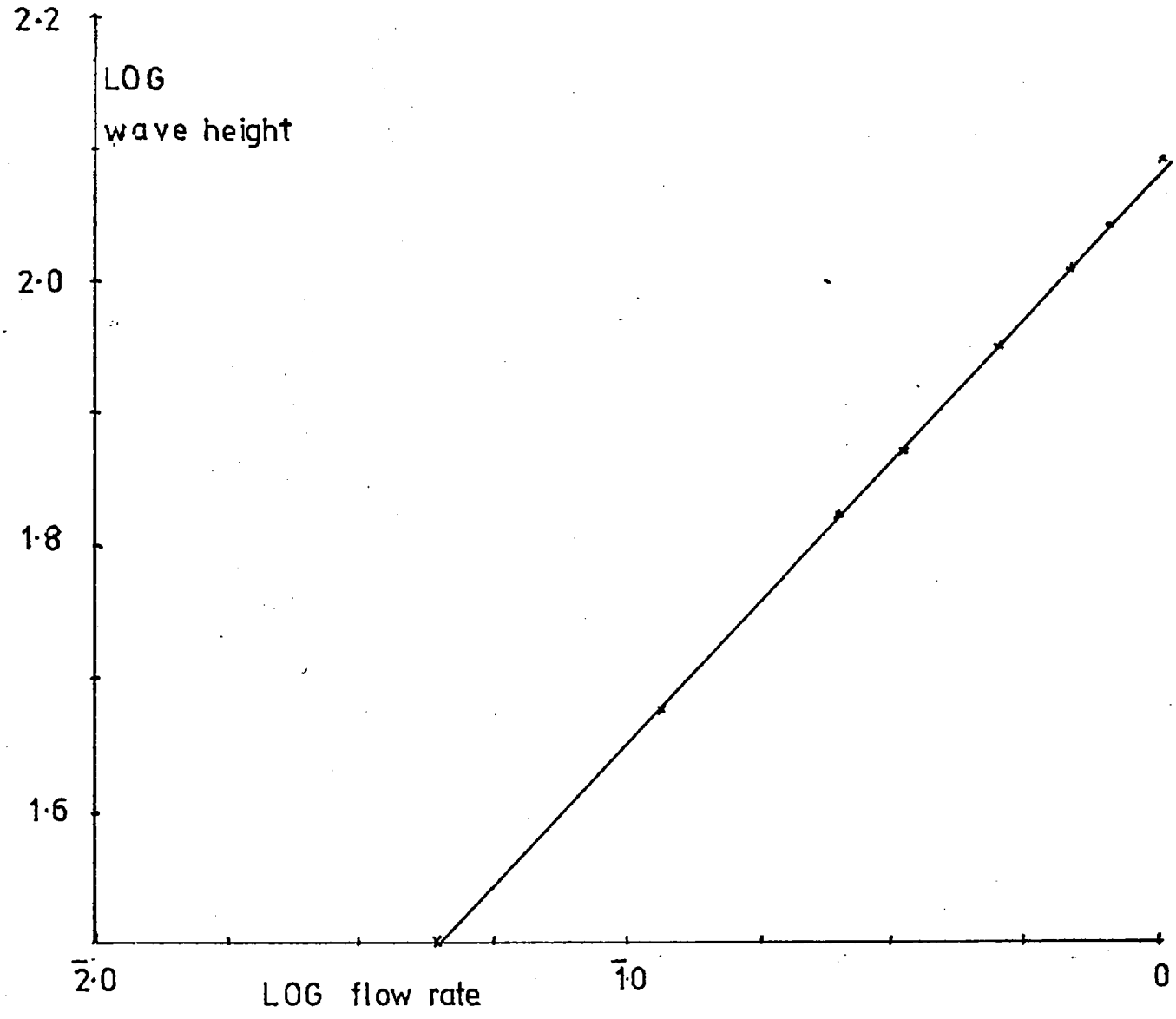
Matsuda stated that in his cell "wall jet" flow produced a current proportional to $V^{\frac{3}{4}}$. Because of the difference in cell design the volume flow rate dependence was investigated. The results are shown in Table 4.1.

Table 4.1

Wave Height mV.	Flow Rate ml/min.
125	1.0
116	0.825
102	0.695
89	0.47
74	0.342
67	0.26
47	0.115
33	0.0927

From the Log. Log. plot of Fig 4.7 it can be seen that the current is proportional to $V^{0.43}$ which does not correspond to Matsurda's wall jet flow but to more usual hydrodynamic conditions where the dependence lies between the $\frac{1}{3}$ and $\frac{1}{2}$ powers.

Fig 4.7



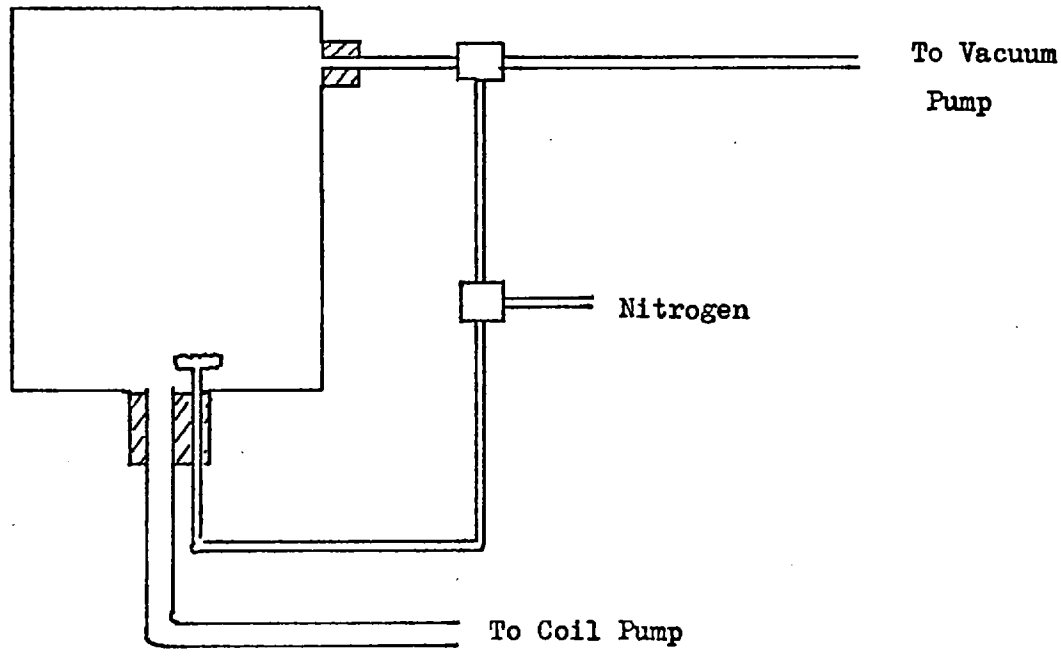
4.3.5. ELECTRODE DIAMETER

Work done by T.A.Berger with this cell has shown qualitatively that the current is not proportional to the electrode area as in Matsurda's cell. Using an electroactive species which produced a precipitate on reaction, the precipitate was found to occupy only a small area in the centre of the electrode. The current produced with a 7mm. diameter electrode was no greater than that produced with a 5mm. one, and only fractionally greater than that produced with a 3mm. one. Therefore to keep the cell volume to a minimum a 3mm. electrode was used in the detector.

4.4.1. APPLICATIONS TO REAL SYSTEMS

Initially, the Wall Jet cell was tried out on a glass bead column in the same way as the D.M.E. cell. The results are not shown here but they indicated that the cell was functioning correctly. It was necessary to modify the previous chromatographic system when using real columns in order to provide facilities to degas and deoxygenate when using reductive detection. The following system was used:-

Fig 4.8



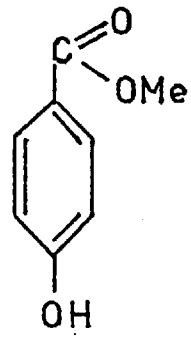
When using oxidative detection the solution was degassed by a vacuum pump and then air allowed back into the reservoir to even up the pressure so that the eluent could be gravity fed into the coil pump. The time it took the air to redissolve in the eluent was so long that once degassed the eluent remained so even if left in contact with the air for several days.

When working on the reductive side however the degassing procedure was not good enough to remove the trace amounts of oxygen necessary to obscure the reduction peaks. It was therefore necessary to deoxygenate with nitrogen first and then degas with the vacuum pump. The pressure

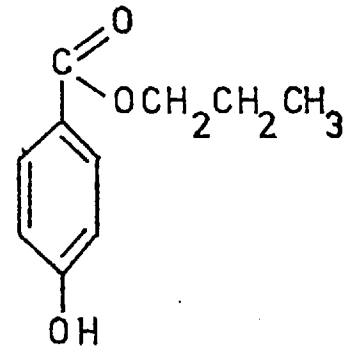
then had to be evened up with nitrogen, since if air was used the trace amounts of oxygen would redissolve very quickly.

4.4.2. DETECTION OF BENZOATES

The first attemptedseparation was carried out on a "Permaphase" O.D.S. column. This was a chemically bonded phase column (Permaphase is a trademark of E.I.Dupont) commercially available which was suited to use with aqueous eluents. The compounds separated were the following two Benzoates:-



Methyl 4 OH Benzoate

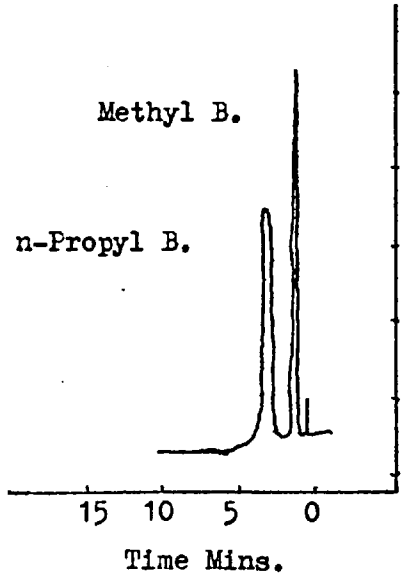


n-propyl 4 OH Benzoate

Detection was accomplished by the oxidation of the aromatic hydroxyl group.

The resulting separation is shown in Fig 4.9

Fig 4.9



Eluent:- 10% MeOH
90% H_2O
0.1M KNO_3

Sample size 5µl.
5µg.

Flow Rate 1ml./min.

Detection Potential:- +0.8 V.

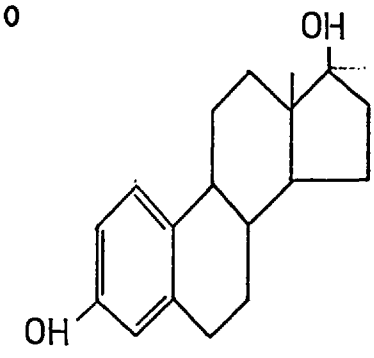
These results were very promising and led to a more intensive investigation of several much more important compounds.

4.4.3. DETECTION OF STEROIDS

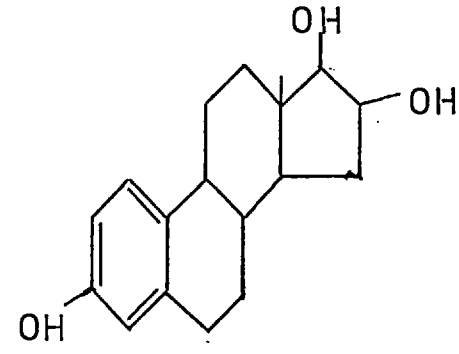
In recent years the analysis of steroids has become extremely important in both pharmacutical and biological analysis(71,72). Hormones are present in such small quantaties that a low level of detection is necessary even for routine analysis. Until recently the U.V. detector has been the only one with a low enough detection limit to be useful, but a large number of steroids have in fact a very low U.V. extinction coefficient. This being the case, an electrochemical detector with a low detection limit could be usefully employed in routine analysis.

The steroids chosen were a series of closely related Oestrone derivatives.

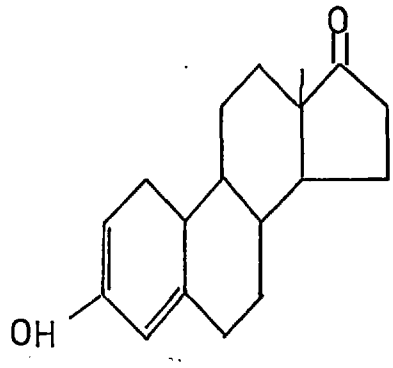
Fig 4.10



OESTRA-1.3,5(10)-Trien-3,17 Diol



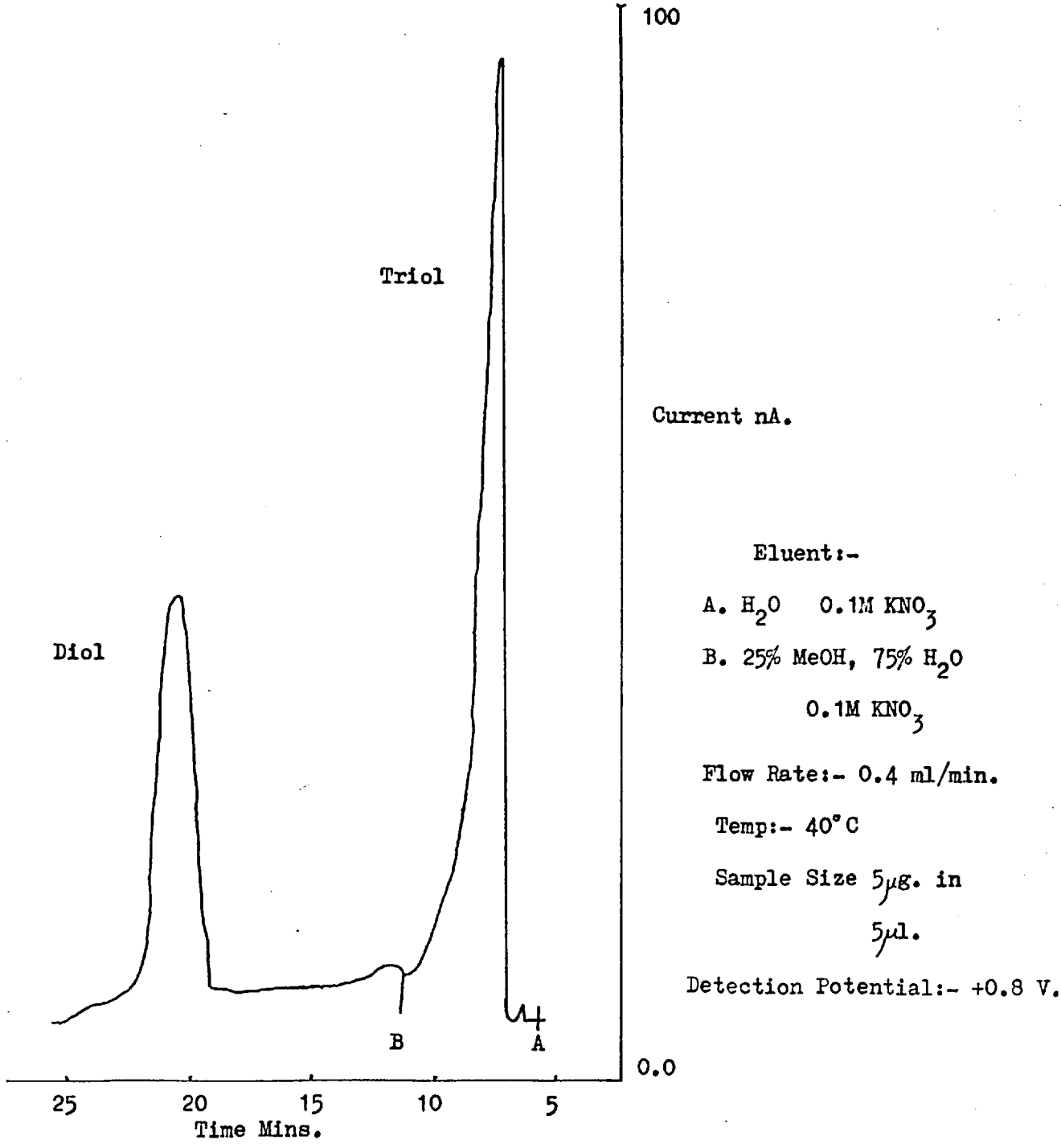
OESTRA-3,16 ,17 Triol



OESTRONE

Fig 4.12 shows the separation of two of the samples using a Permaphase O.D.S. column and a water/methanol eluent. The temperature was maintained at 40° C using a small oven to heat the column. Permaphase columns can be used at any temperature up to 70° C, and although not as effective as in G.C., temperature does have a slight effect on the column's efficiency of separation.

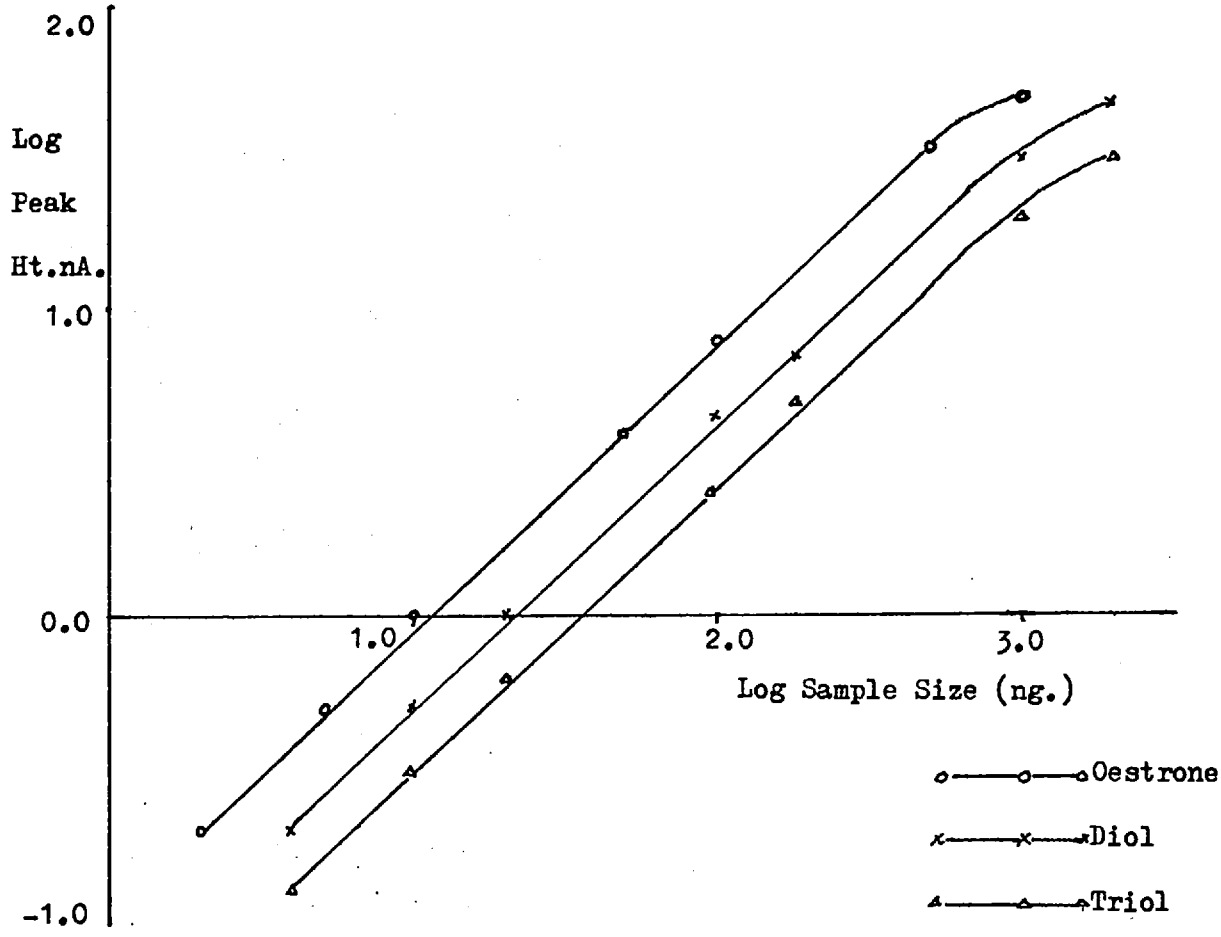
Fig 4.12



In all three cases the steroids were detected by oxidation of the aromatic hydroxyl by setting the detector at +0.8V v S,C.E.. The eluents used were different methanol water mixtures, all 10^{-1}M in potassium nitrate. This was used instead of potassium chloride because it can be left in the column for long periods of time whereas the chloride will attack the stainless steel columns if left too long. The steroids were separated on a Permaphase column.

A calibration graph was drawn for each of the samples Fig 4.11 over a range from the normal working levels in the microgram region down to the lower detection limit.

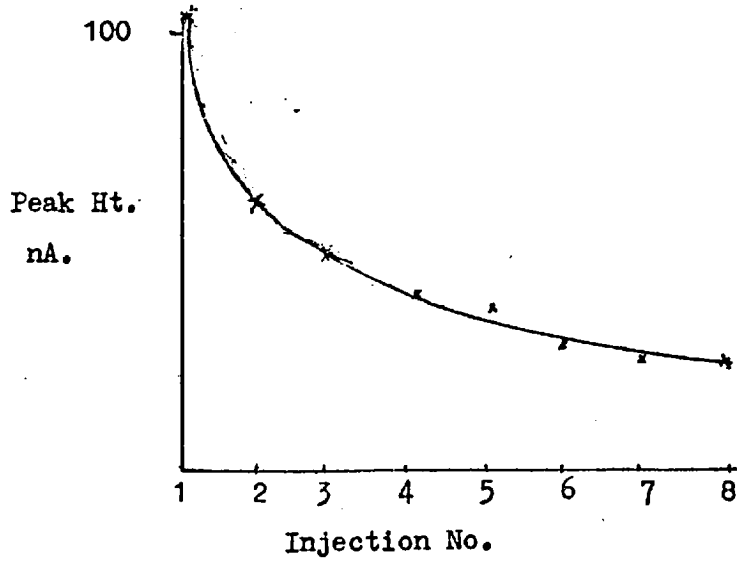
Fig 4.11



The lower detection limit was defined as the point where the signal from the sample was twice the noise level. It was noted that the noise was in fact electrical pick up and not noise produced by the flowing stream.

Various conclusions can be drawn from Fig 4.12. The detector appears to have a very good linear range from the microgram region down to the lower detection limit in the nanogram region. This compares well with the U.V. detector and in certain cases is better than it. The main problem with this detector is not very apparent from the graph. It can be seen however that as the concentration of sample increases the graph begins to deviate from linearity slightly and the detector becomes less sensitive. This is not due to any fundamental change in the response of the detector to high concentrations, but is due to the adsorption of the product of the electrode reaction onto the electrode surface thus blocking the active sites where the reaction occurs. Fig 4.12 was produced by initially injecting small samples and then gradually increasing the sample size. This reduced the effect of the site blocking to a minimum. If on the other hand the production of the curve had been attempted from the other end i.e. high concentration samples injected first, then the graph would have a much more curved appearance. This is because a large sample blocks more sites in a more effective way than a small sample and hence the response to the next sample is reduced considerably. The result of this adsorption can be seen more clearly in Fig 4.13 which shows the result of a series of injections of the same sample size of oestrone.

Fig 4.13



As can be seen, the second peak is much smaller than the first and thereafter there is a tendency for the response to decrease with increasing sample number. The response does not however disappear completely. This phenomenon has far reaching consequences when looked upon in the light of continuous chromatographic detection.

If a series of peaks are produced from one injection, only the first peak resolved will be of the correct size. Furthermore, because the height of the following peaks will depend to some extent on the amount or type of adsorption from the first peak, it is impossible to calibrate them by external or internal standards. This is especially apparent during the analysis of real samples when impurities cause unknown amounts of adsorption. A method therefore had to be devised by which the adsorbed product could be removed before the following peaks came off.

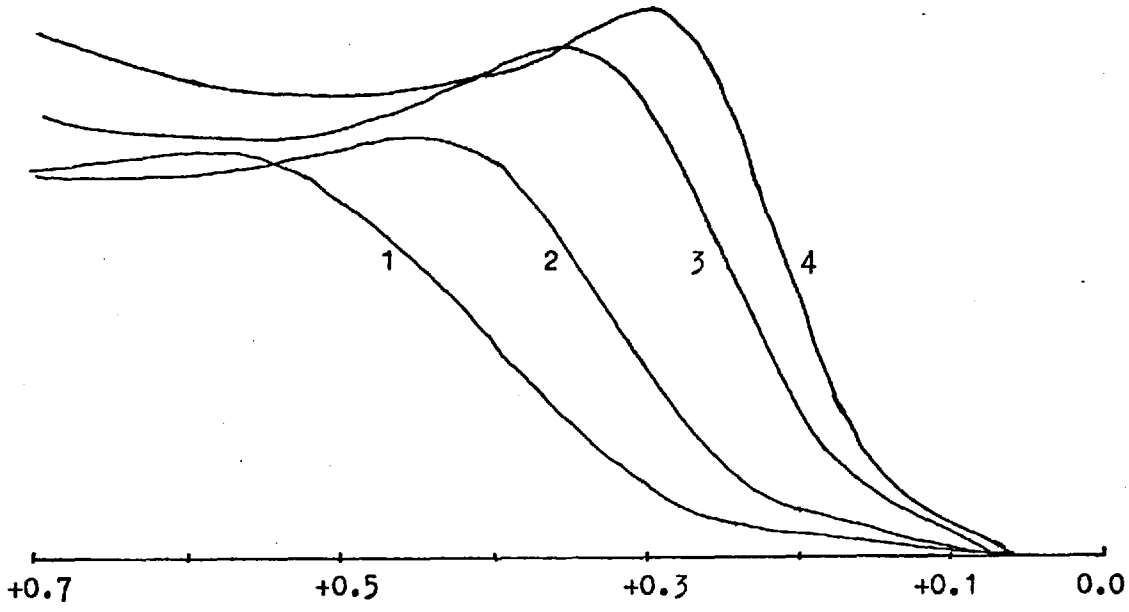
The most straightforward method of removing adsorbed material was to mechanically clean the electrode. In routine analysis this would be at best inconvenient, producing long analysis times, and at worst, impossible, especially on peaks very close together or only partially resolved. Stopping the flow to allow the electrode to be removed from the cell causes mixing of the components on the column, thus reducing resolution. Furthermore, whenever an electrode is cleaned mechanically, the surface is left in an unknown state which may differ from the one produced by the previous mechanical cleaning, and hence it is again impossible to relate peaks produced on this surface to those produced by a standard on a different surface.

Therefore a method had to be devised to remove adsorbed species without resorting to mechanical cleaning. After several attempts to do this electrochemically it became obvious that a greater understanding of the behavior of glassy carbon surfaces was required. For this reason an intensive investigation into the electrochemical properties of glassy carbon was undertaken.

5.1.1. ELECTROCHEMISTRY AT GLASSY CARBON

Before any detailed work could be carried out on glassy carbon at low current levels it was necessary to place the glassy carbon surface in a fairly reproducible state. Initially the oxidation wave of the $Fe(CN)_6^{2-}/Fe(CN)_6^{3-}$ couple was used as a standard. The electrode was prepared as described in Chapter 4 and then various final cleaning methods were tried. Anodic scans were made after each cleaning process and the results are shown in Fig 5.1

Fig5.1



Potential v. S.C.E.

Solution:- 10^{-3}M $K_3Fe(CN)_6$

0.1M KNO_3

Scan Rate 10 mV./s

1. This curve was produced straight after polishing with a diamond paste compound. Its poor shape is due to traces of polish blocking the active sites of the electrode surface. This means that the electrode has to be taken to more positive potentials for the reaction to occur. The result being a very stretched out wave.

2. Further mechanical cleaning with a clean cloth produced this curve which is not much better than curve 1.

3. This was produced after rubbing the electrode with a cloth soaked in methanol.

4. This final curve, which corresponds closely to the theoretical wave for a reversable system, was produced after washing in chloroform.

The chloroform obviously removed all trace of polish from the electrode and produced a surface capable of giving a well shaped $Fe(CN)_6^{3-}$ oxidation wave. In all the following work the electrode, after being polished with diamond paste, was washed in methanol and then chloroform. The surface thus produced was fairly reproducible, at least at the μA. current level produced by 10^{-3}M. Ferrocyanide oxidation.

Once the electrode surface had been prepared mechanically, it was necessary to devise some sort of electrochemical method of keeping the surface clean during the on line monitoring. If it is the product of the electrode reaction which blocks the surface, as seems likely, the reduction of that product should clear the surface and allow further reaction to take place. There are two possible methods of doing this. One is to use an A.C. signal, the other is to use a square wave.

5.2 ALTERNATING SIGNALS

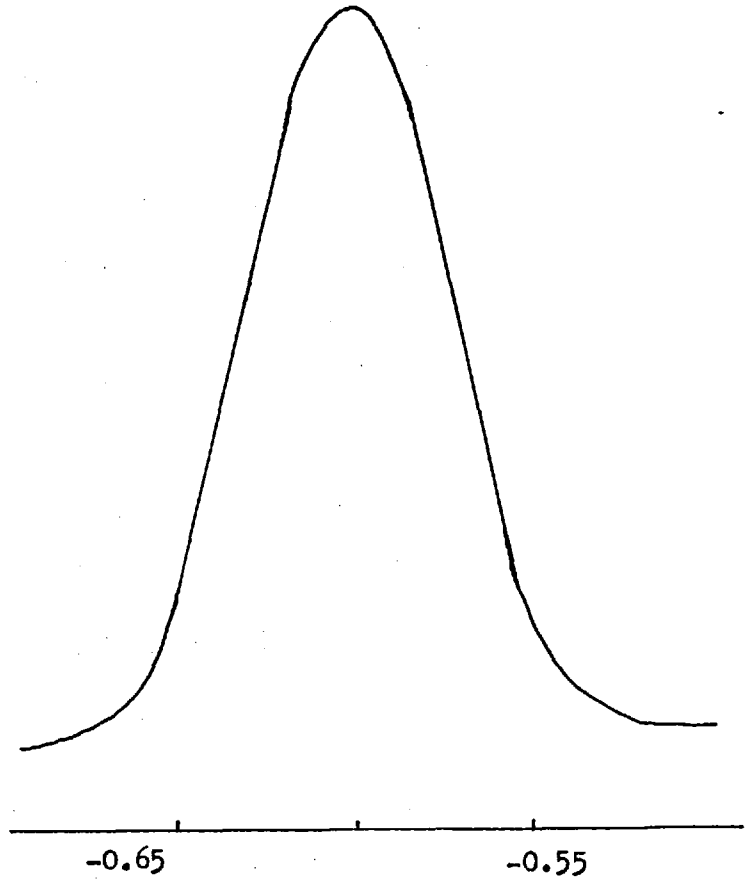
The first application of an alternating potential to an electrode was made by Kohlraush (73) but it wasn't until 1938 that Muller (74) applied a small sinusoidal voltage under polarographic conditions. Great advances were made during the 1940's due to the physiochemical studies of Breyer and Gutmann(75-77) , Graham (78) and Randles (79) and the technique emerged in the 1960's as a powerful method of investigating electrode reaction kinetics. The theory of the A.C. process has been recently expanded and is presented in three recent reviews (80-82).

In fundamental A.C. polarography, a small amplitude sinusoidal voltage is superimposed on a D.C. ramp. When the D.C. potential reaches the point where a redox reaction occurs, the superimposed alternating voltage produces periodic concentration changes of oxidised and reduced forms at the electrode surface. These in turn give rise to periodic diffusion to the electrode surface, and thus an alternating current flows. As the current produced is due to a chemical reaction it is known as the Faradaic current. Because the redox reaction is a non-linear element (due to the rate constant dependence upon potential) the A.C. voltage is partially rectified and thus higher harmonic currents are observed as well as the fundamental one.

In practice the A.C. response can be looked upon as the derivative of the D.C. process (this is an approximation, and does not hold for theoretical considerations or kinetic studies), and the response is peak shaped as in Fig 5.2. Fundamental A.C. signals are always accompanied by a large alternating current due to the charging of the electrical double layer. The most effective method of separating the charging current from the faradaic current is to use phase sensitive detection (83-89). The charging current component is 90° out of phase with the applied voltage, whereas the faradaic current is

usually less than 45° out of phase. By tuning to a particular phase current the contribution from the charging current can almost be eliminated. Total resolution is not possible because ohmic resistance in the cell means that the charging current is not exactly 90° out of phase. Typical Second Harmonic response is shown in Fig 5.3a and the phase sensitive equivalent in Fig 5.3b.

Fig 5.2



Typical fundamental alternating current response.

Fig 5.3a

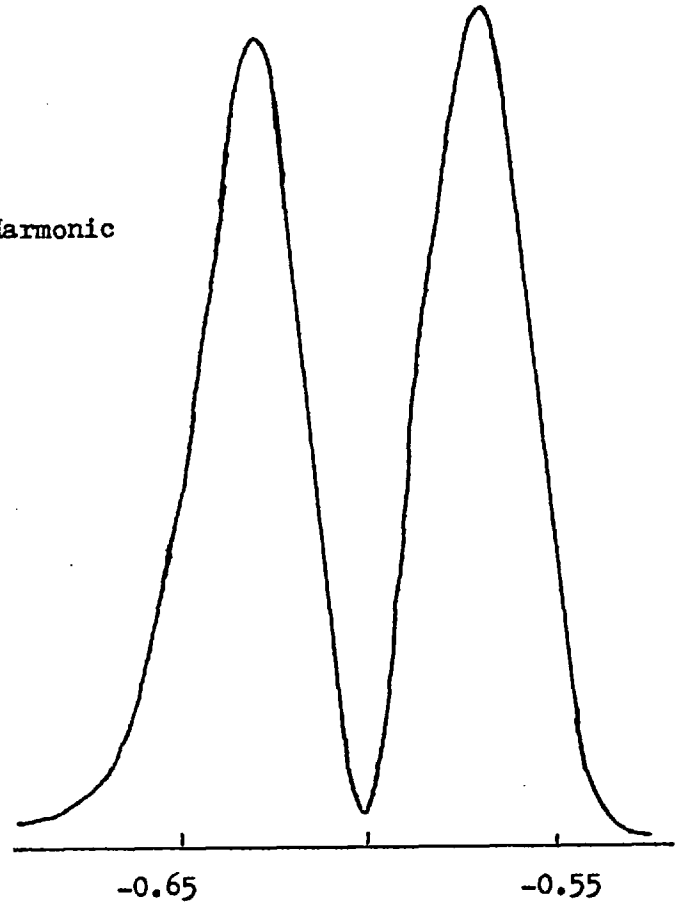
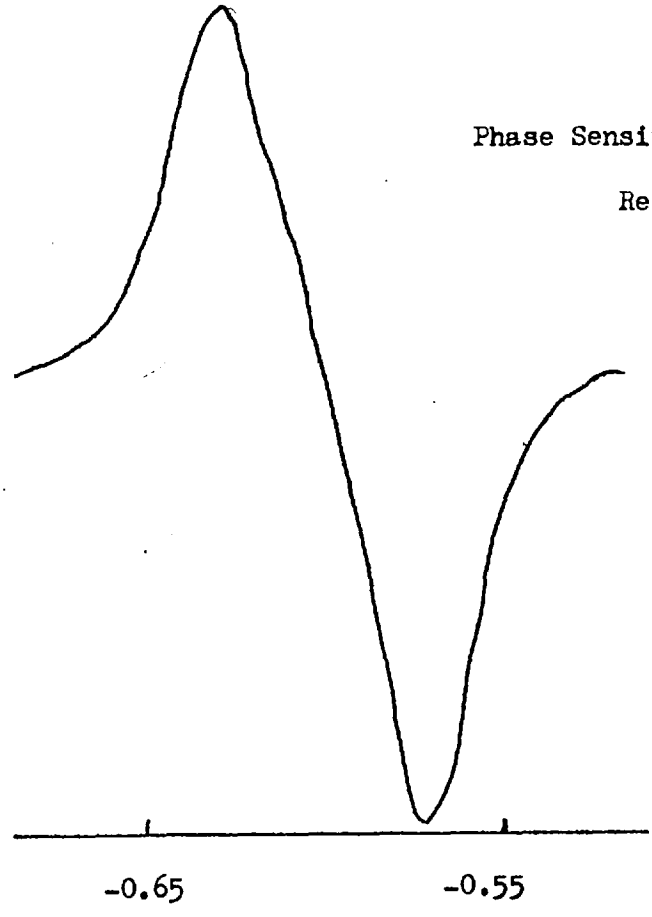


Fig 5.3b

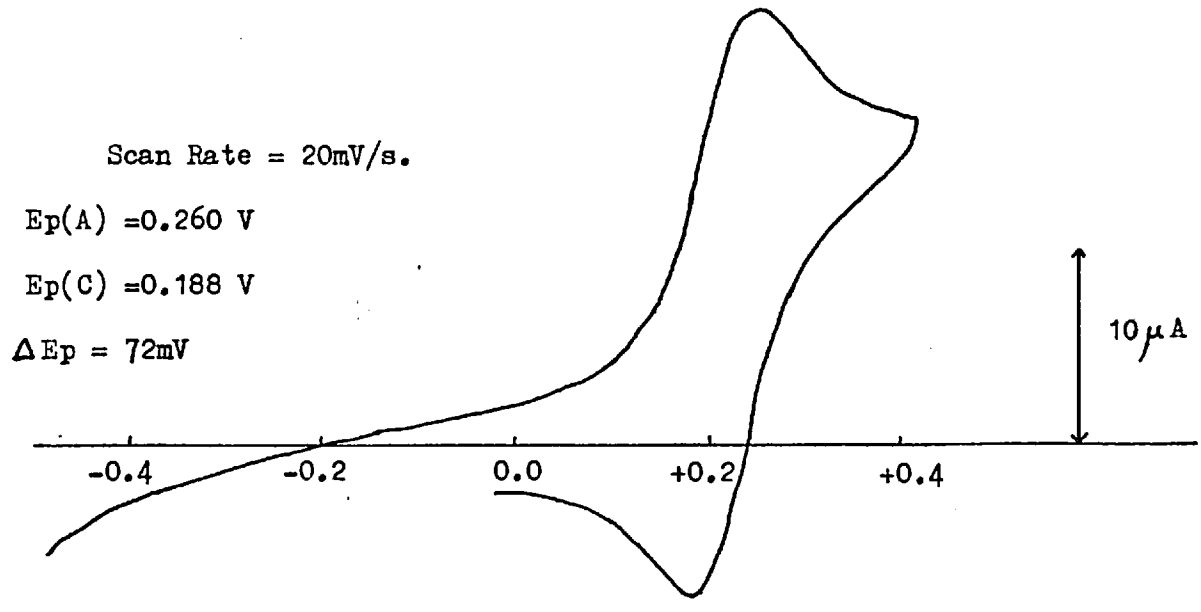


For chromatographic detection the alternating signal must be applied at the redox potential of one of the sample components. The sucœssive oxidation and reduction at the electrode surface should prevent or at least reduce the amount of adsorption on the electrode. Normally the applied alternating voltage amplitude is small (about 10mV.) which would produce a very selective detector indeed. However, since kinetic studies are not being made, it is possible to relax this restriction and apply a much larger amplitude signal. If a 100mV. signal is used a slightly less selective detector is produced. Even in this case however the technique is not as universal as the D.C. mode. Any A.C. technique could be used in the detector i.e. Fundamental or Second Harmonic, but the safest is fundamental, as it is easier to tune to the broader response produced by this mode than risk the possibility of drifting into the null point of the second harmonic response. Phase sensitive detection is not so important in the chromatographic detector for although the charging current is still present, the current from the sample is produced only by the faradaic process and thus is either absent or present depending upon the sample concentration. Furthermore its presence is seen on a flat base line and not a sloping one, thus it is much easier to see.

The use of A.C. in a chromatographic detector looks at first sight to be promising despite its selective nature, however the popularity of A.C. polarography today is a result of the great influence of electrode kinetics on the shape of the A.C. wave. Basically this means that the magnitude of the A.C. response is reduced as the system becomes less reversible. At mercury a great many systems have been studied by A.C. methods, but very little has been reported for glassy carbon. Before the A.C. technique was tried in a chromatographic detector the reversibility of some reactions at glassy carbon were investigated.

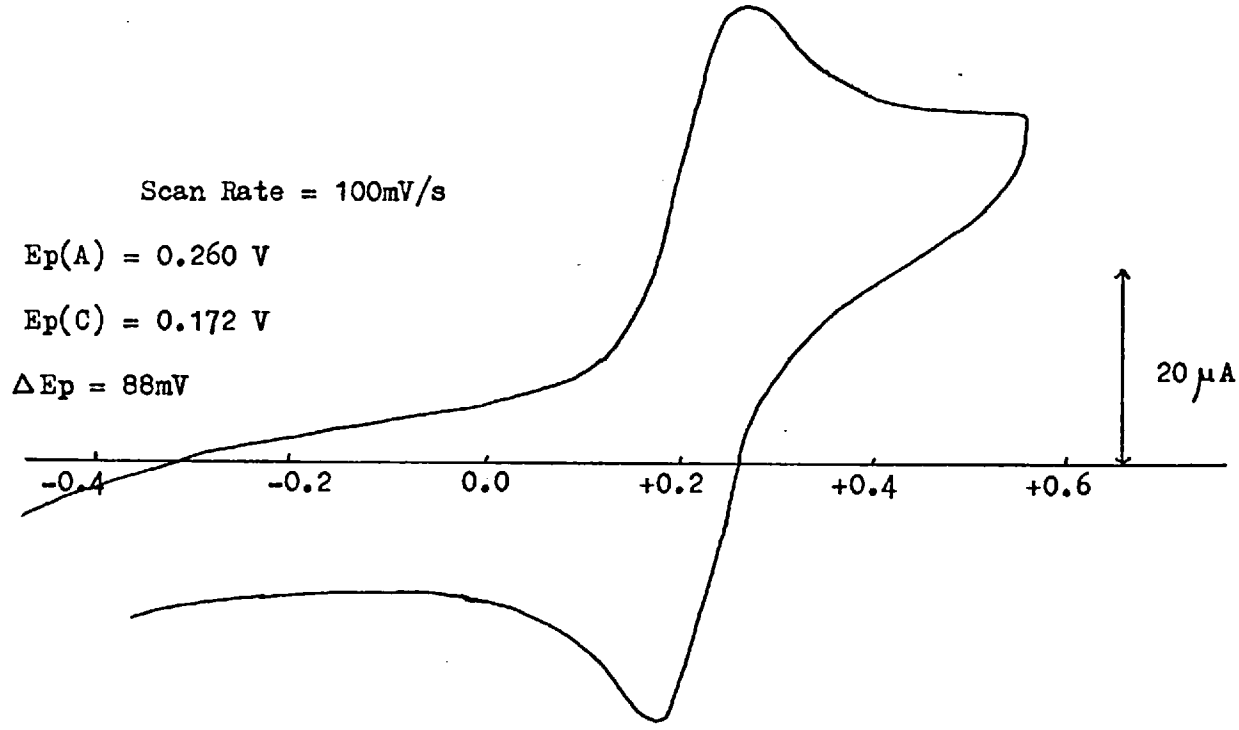
The system investigated was again the Ferro/Ferri cyanide couple and the technique used was cyclic voltammetry. Cyclic voltammetry was first used by Sevčik (90) and this was later developed by Kemula to study electrode mechanisms (91,92). The technique consists of scanning a D.C. potential range, first in one direction and then switching the direction and scanning back again. From the resulting wave shapes a great deal can be said about the reversibility of the electrode reaction. Recently Shain produced two exceptional papers in which theoretical models were produced which fitted the observed results (93,94). Of particular note are the effects of adsorption and following and preceeding chemical reactions on the shape of the cyclic voltammograms. In Fig 5.4 are shown the cyclic voltammograms produced by the ferro/ferri cyanide couple at various scan rates.

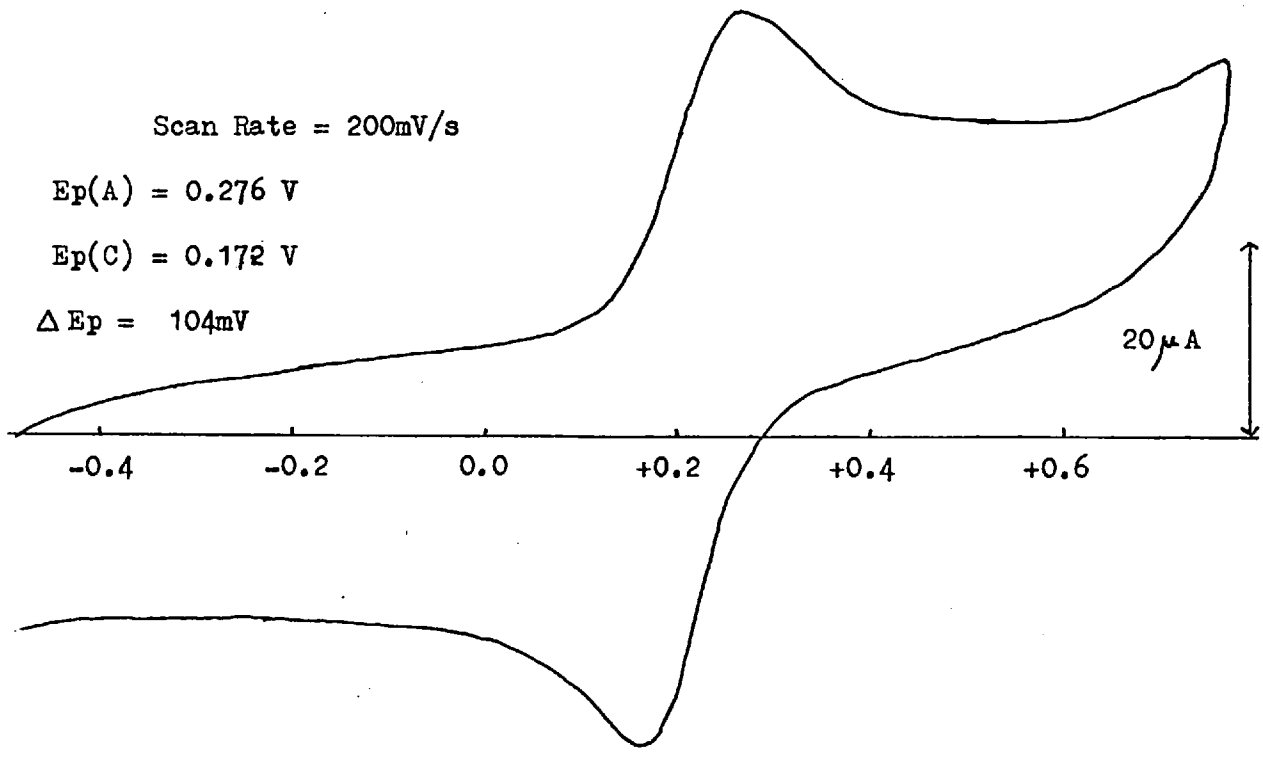
Fig 5.4a

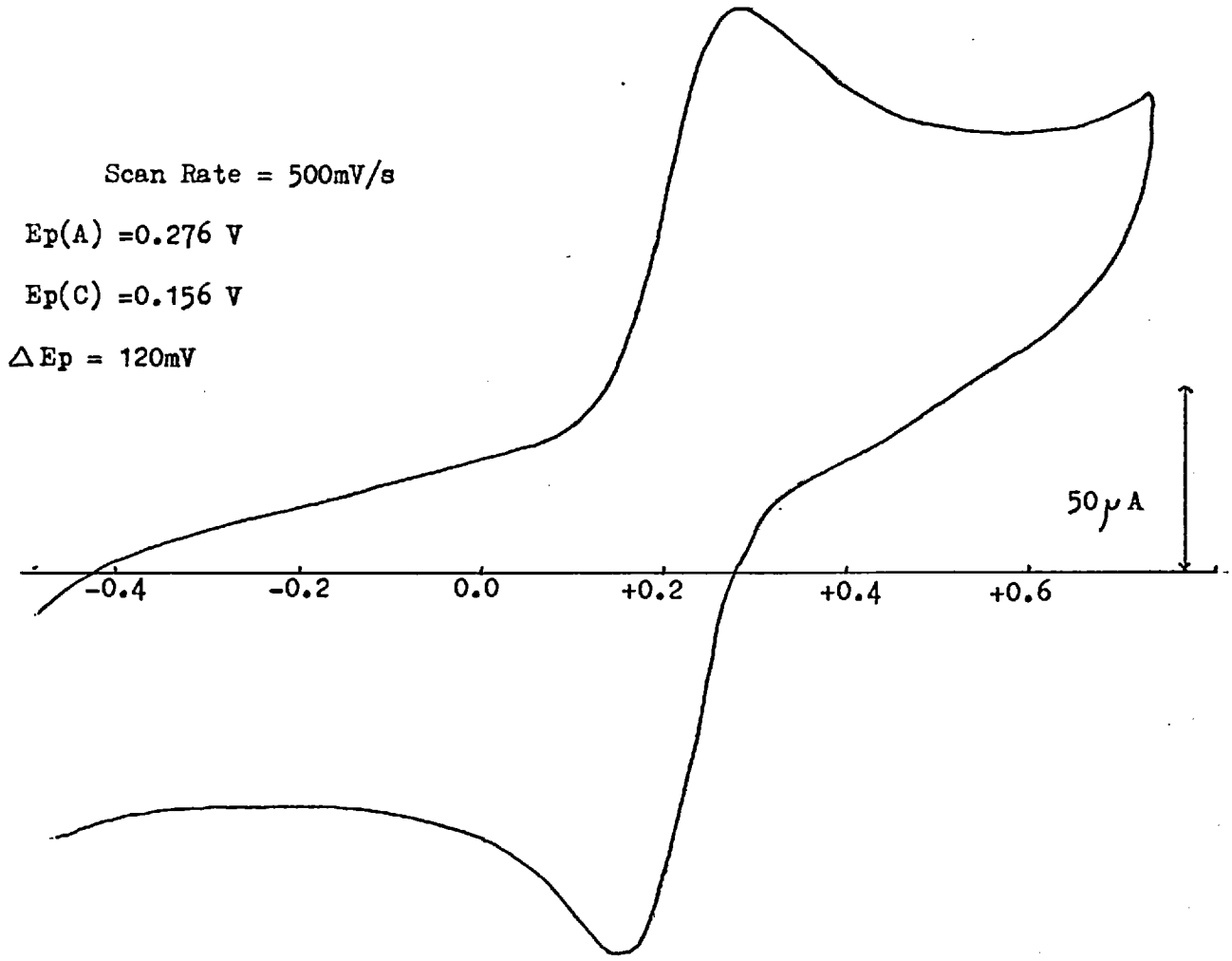


10^{-3}M. Potassium Ferrocyanide

1M. Potassium Chloride


Scan Rate = 100mV/s
Ep(A) = 0.260 V
Ep(C) = 0.172 V
ΔEp = 88mV
20 μA
-0.4
-0.2
0.0
+0.2
+0.4
+0.6


Scan Rate = 200mV/s
Ep(A) = 0.276 V
Ep(C) = 0.172 V
ΔEp = 104mV
20 μA
-0.4
-0.2
0.0
+0.2
+0.4
+0.6



All the voltammograms produced in this series were obtained in a static solution using a platinum foil counter electrode and a S.C.E. reference electrode.

Matsuda has reported (95) that steady state cyclic voltammograms differ little from the first scan for a reversible system, but differ greatly for an irreversible one. Since adsorption interferes greatly at glassy carbon, only the first scans were of use.

A totally reversible system should produce a peak separation of $\frac{0.058}{n}$ Volts, where n is the number of electrons involved in the electron transfer process. In fact the totally reversible case was never obtained, but at a scan rate of 20mV/s the system appeared almost reversible. As the scan rate was increased the kinetics of the electron transfer process became slow compared to the scan rate and the peak separation

increased.

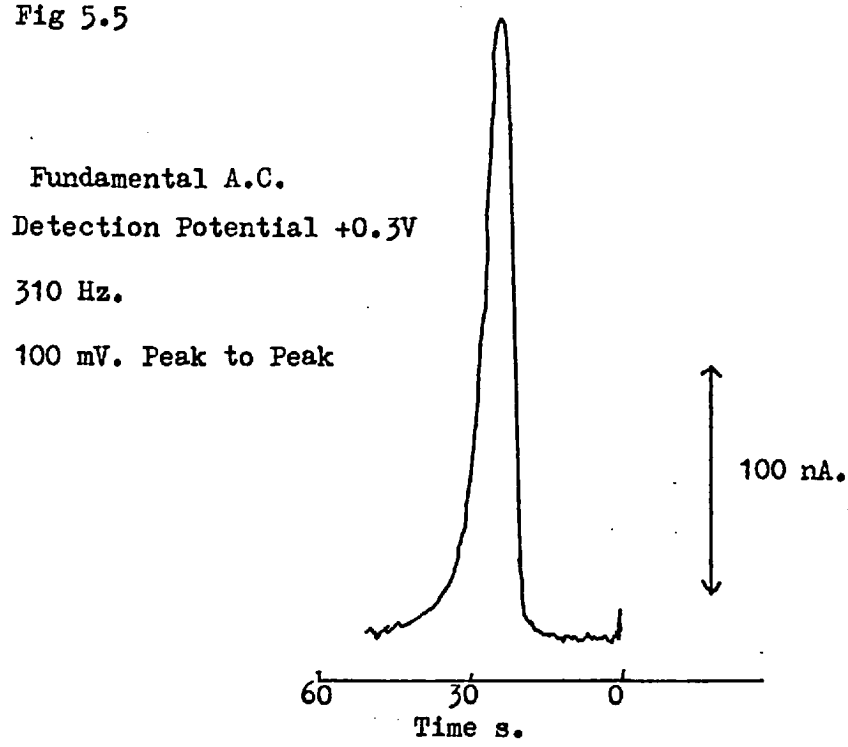
Of greater importance however are the results shown in Table 5.1 . This lists the peak separations for the system in various electrolytes and at different electrolyte concentrations.

Table 5.1

Scan Rate.	Electrolyte ΔE_p		
	1M KNO_3	0.1M KNO_3	1M KCl
20mV/s	88	144	104
100mV/s	96	206	136
200mV/s	136	264	219
500mV/s	158	317	296

As can be seen, the reversibility of the electrode reaction at glassy carbon is extremely sensitive to changes in concentration and composition of the background electrolyte. It is here that the weakness of the A.C. method for chromatographic detection lies, for the A.C. method needs a reversible reaction to produce a useful signal but the background conditions cannot be determined by the electrochemistry but must be determined by the chromatography. Thus a great deal of luck is required for the chromatographic conditions to correspond to the ideal electrochemical ones. It has been reported by Smith (82) that a detectable response is obtained even for an irreversible system, but this response is so small that it is too insensitive to be of use in a chromatographic detector. The A.C. mode of detection was however tested out on a chromatographic system. The results are shown in Fig 5.5

Fig 5.5



Column:- 6" glass beads. Sample Size 5 l 200 g.

Eluent:- Water, 0.1M KNO_3

Flow Rate:- 0.8 ml/min.

Table 5.2

Detection Potential	Peak Height
+0.1 V	300 nA
+0.2 V	275 nA
+0.3 V	300 nA
+0.4 V	250 nA

D.C. Response:- 16.5μA.

As can be seen, the A.C. response is some two orders of magnitude less than the corresponding D.C. one. Furthermore a very large sample had to be used to see any A.C. response at all. The great breadth of the A.C. peak is indicated by the wide range of possible potential settings which produced a response. It is interesting to note that there is much more reproducibility in the peak heights obtained from sucessive sample injections in the A.C. mode than the D.C. one. This does not mean however that the electrode is being cleaned, for if this was the case a much larger A.C. response would be obtained corresponding to the

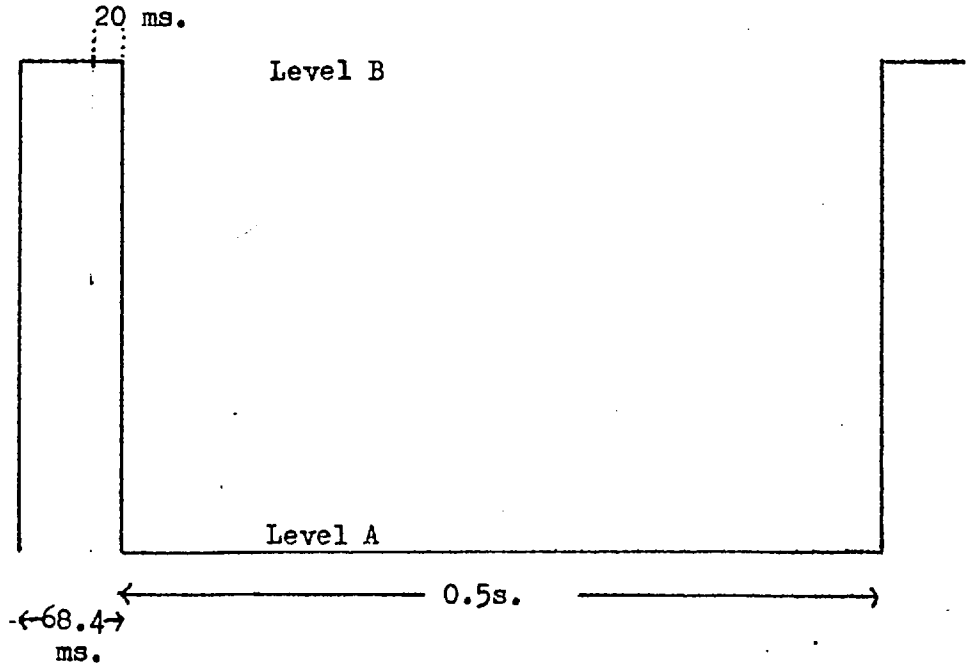
reversible system. These results indicate that A.C. voltammetry is too insensitive in the majority of cases at glassy carbon to be of use in a chromatographic detector.

5.3.1. PULSE METHODS

The second approach to reducing (or oxidising) off adsorbed species, was to apply a square wave or pulse signal. The basic idea was to apply a potentiostatically controlled wave form to the electrode utilising one part of the pulse as a measuring level, and the other part as a "cleaning" level. i.e. The potential at the top of the pulse was set on the wave plateau of the sample, and the potential at the foot of the pulse was set off the wave. The great advantage of this method lay in the fact that the size of the cleaning pulse could be increased if the adsorbed species proved difficult to remove, or if the reversibility of the reaction was such that the anodic/cathodic peak separation was large. i.e. A fairly irreversible process. Originally a commercial instrument was used to create the pulse train. The one used was the P.A.R. 174 which was a multipurpose instrument capable of a variety of polarographic techniques including pulse and differential pulse polarography. The pulse cycle applied is shown in Fig 5.6

Fig 5.6

P.A.R. 174 Pulse Train



Level A was used as the cleaning pulse and was dialled up on the instrument as the initial potential. Level B, the measuring pulse, was produced by setting the mode switch to Pulse and then initiating the scan at a known scan rate for a given length of time. When the correct amount of time had elapsed for the potential at A to have reached the desired level, the hold button was pressed, thus allowing the correct waveform to be applied continuously. The P.A.R. was designed specifically for use in the pulse mode with a D.M.E.. For this reason the current was only sampled in a small window at the end of the measuring pulse. This was to allow the charging current at mercury to die away before any measurments were made. Before this system was used with a chromatographic column it was necessary to see if the time scale of the pulses was compatible with the size and decay time of the charging current at glassy carbon. To investigate this, different size pulses were applied to the electrode in base electrolyte (0.1M KNO_3) using a three electrode system. (Platinum foil counter, S.C.E. Reference). The results showed that even for very small pulses (of the order of 100 mV.) a large charging current spike was produced which overloaded the Current to Voltage converter of the P.A.R. 174. With pulses greater than 250 mV. the Current to Voltage converter remained overloaded throughout the cycle, while below this value, the Current to Voltage converter was not overloaded during the measuring window. However, it was impossible to tell whether the current measured during the measuring window was the true current or an apparent one resulting from a relatively slow recovery of the amplifier from overload. In which case the output could bear no resemblence to the true current value. For this reason, added to the fact that continuous overload was obtained with pulses greater than 250 mV., the P.A.R. 174 could not be used in the pulse mode for chromatographic detection. Thus before work could be carried out on a real system, a specialised potentiostatically controlled pulse generator had to be built.

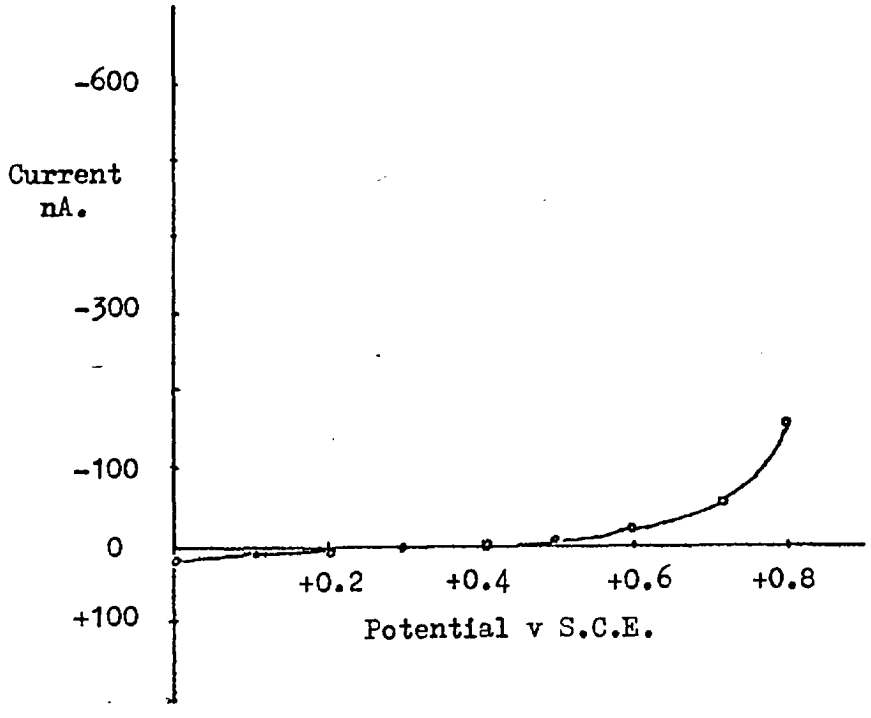
The design of this controller depended upon two characteristics of glassy carbon. One was the nature and size of the residual currents and the second was the decay characteristics of the charging current.

5.3.2. RESIDUAL CURRENT

The mechanical cleaning process described in section 5.1 was always applied to an electrode that had been left in an unknown state for some time, and its resulting effect on a large faradaic process has already been reported. The effect on the residual current was investigated using a three electrode system and a static 0.1M potassium nitrate solution. When a freshly cleaned electrode was placed in this solution the residual current took a long time (about 20 mins.) to reach a steady value. Thereafter the response of the electrode to step changes in potential took about 5 minutes, the actual time depending upon the size of the potential step. By waiting 10 minutes between potential steps an absolute residual current versus potential plot was produced which contained neither a charging current component nor any other short term current component.

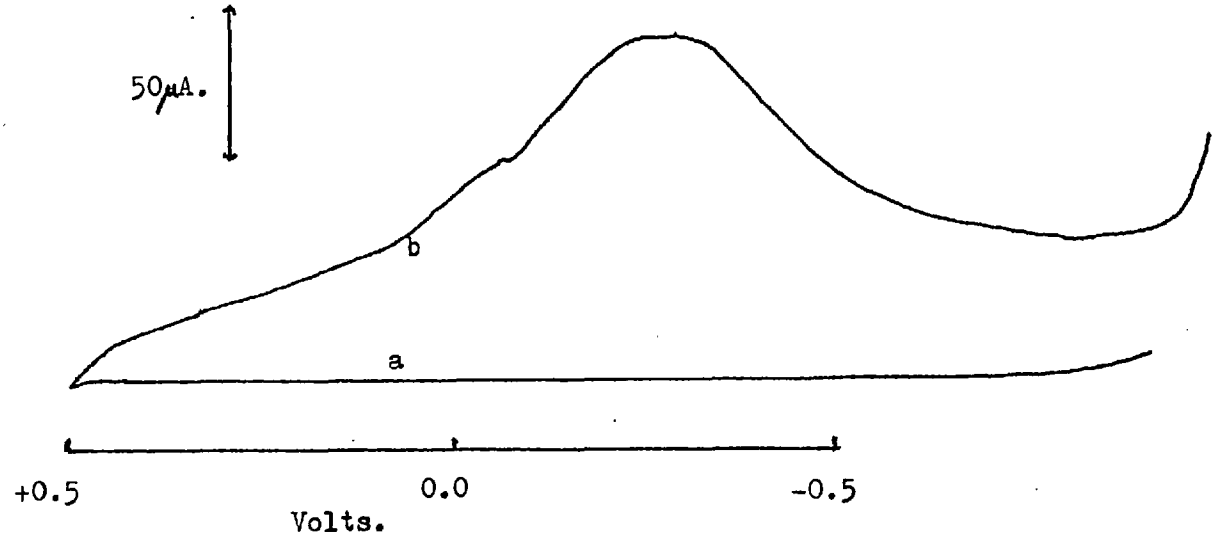
Fig 5.7

Residual Current at Glassy Carbon



A similar method to this has been used by Blaedal to produce steady state voltammograms (96).

The curve shown in Fig 5.7 was found to be fairly reproducible over a short time (about one day) but over a longer period , although the slope remained the same, the potential of zero current flow moved around slightly, ± 50 mV.. This was most apparent when the electrode had been left out of solution for a few days. The overall picture produced by these results indicates that the glassy carbon electrode exhibits a very strong "history" effect i.e. its rate of attainment of equilibrium at any applied potential depends very much on the recent history of the electrode surface. To overcome this problem Blaedal (96) suggested a potential cycling process before the electrode was used in order to put the electrode surface in a reproducible state. During his pretreatment technique the electrode was placed at +1.35 V. versus a S.S.C.E.. If however the electrode is then scanned in a negative direction a wave appears at -0.4 V in the residual current voltammogram. This potential corresponds very closely to the quinone/hydroquinone couple.



a:- Residual current from untreated electrode

b:- Residual current after sitting at +1.5 V. for 2mins.

The size of the peak was found to depend upon the time spent at the positive potential.

The presence of this wave was not reported by Blaedal. Its appearance is probably due to the fact that oxidation of the glassy carbon surface occurs when large positive potentials are applied to the electrode. This oxidation causes the formation of carbon-oxygen groups on the surface such as carbonyls, quinoids etc. When the electrode is then scanned cathodically, a peak is produced corresponding to the reduction of these surface groups. The presence of these groups on the surface appears to aid electron transfer to electroactive material with a corresponding increase in faradaic current. This is a result of the delocalised π-systems of these surface groups. Unfortunately these groups also have extremely good electron donating properties and as such can form very good donor complexes with both reactant and product. The result being that although enhancement of the faradaic current may at first be apparent, there is also a great increase in adsorption on the electrode surface, especially of product. This leads to fast deactivation of the electrode surface. Again Blaedal did not report this since he only carried out a single steady state voltammogram. It has been reported (97) that a freshly broken piece of glassy carbon has very few surface carbon-oxygen bonds. The action of grinding the electrode surface however produces enough energy to allow these surface groups to be formed (98,99). The application of a positive potential serves to produce many more of these groups on the surface. A similar effect can be seen if the electrode surface is treated with a concentrated nitric acid, sulphuric acid mixture (1:1) for a few seconds. Obviously a similar oxidation of the surface occurs.

When glassy carbon is to be used in a chromatographic detector any oxidation of the surface should be avoided. Although an increase in sensitivity may occur, the increase in adsorption is much more important and must be avoided. Therefore it is imperative that the applied potential should not exceed +1.2 V v S.C.E. Great care should

be taken also to remove all air bubbles from the system before connecting up the electrodes, since an open circuit caused by the air bubbles between the working electrode and the reference electrode could make the working electrode assume a very positive potential, thus oxidising the electrode surface.

5.3.3. CHARGING CURRENT DECAY

The residual current values produced in the previous section gave a reference value to which all succeeding currents could be compared. The problem with using the pulse train generated by the P.A.R. 174 had been the appearance of the charging current during the measuring window. To overcome this problem it was necessary to design a pulse generator which allowed the charging current to decay before a measurement was made. It was necessary therefore to look at the behavior of glassy carbon when under the influence of a pulse train, in order to produce the best generator design.

During the following studies two electrodes were used. The first was the one which gave the residual currents already quoted. It was new and had been polished only once to a mirror finish. The second electrode was one that had been in constant use for about six months. It had been subjected to oxidation by both chemical and electrochemical methods, and had been ground and polished many times. The first difference noted in the two electrodes was the much higher residual currents produced by the second electrode hereafter referred to as electrode No 2. Initially the current profile produced on the application of a single potential step was studied.

5.3.4. SINGLE POTENTIAL STEP

Application of a potential step was accomplished by use of the P.A.R. 174. A three electrode system was used (platinum counter, S.C.E. reference) in a solution of aqueous potassium nitrate (0.1 M). A potential was dialled up on the P.A.R. and the electrode allowed to come to

equilibrium. The system was then open circuited and the potential changed. Finally the circuit was closed at the new potential and the current response recorded on a strip chart recorder.

Fig 5.9 shows the results produced when stepping from +0.8 to +0.6 to +0.4 to +0.2 V using electrode No 2.

One fact is very apparent from all these steps. The time taken for the electrode to reach a steady value is of the order of minutes. Calculations done on charging current decay times indicated that the charging current at glassy carbon should decay within 100 ms.(96). Therefore it must be concluded that this slow decay to a steady state is not caused by conventional charging current decay. Close inspection of the +0.8 to +0.6 step shows that the current does not approach its steady value from the charging current spike but does in fact overshoot the final value slightly and approaches from the other direction. This feature is even more apparent in the +0.6 V to +0.4 V step where the time at open circuit was reduced considerably. In this case the charging current spike is very small and a large current can be seen to flow in the opposite direction. The step from +0.4 V to +0.2 V crosses the point of zero current and both the charging current and this new current now flow in the same direction and cannot be separated. From these results it appears that the standard charging current at glassy carbon does in fact decay quite quickly (though less so than at mercury) but that another current is produced on stepping which has a much slower decay time and whose direction of flow is determined by parameters different from those which determine the direction of flow of the charging current. One other fact that can be determined from the results is that when the system is open circuited the double layer breaks up very quickly. This must be so since the charging current spike during the step from +0.6 V to +0.4 V goes in the opposite direction to the one that would be expected from an instantaneous step, even though the time at open circuit was only 5 seconds

Fig 5.9a

Current Time Plot

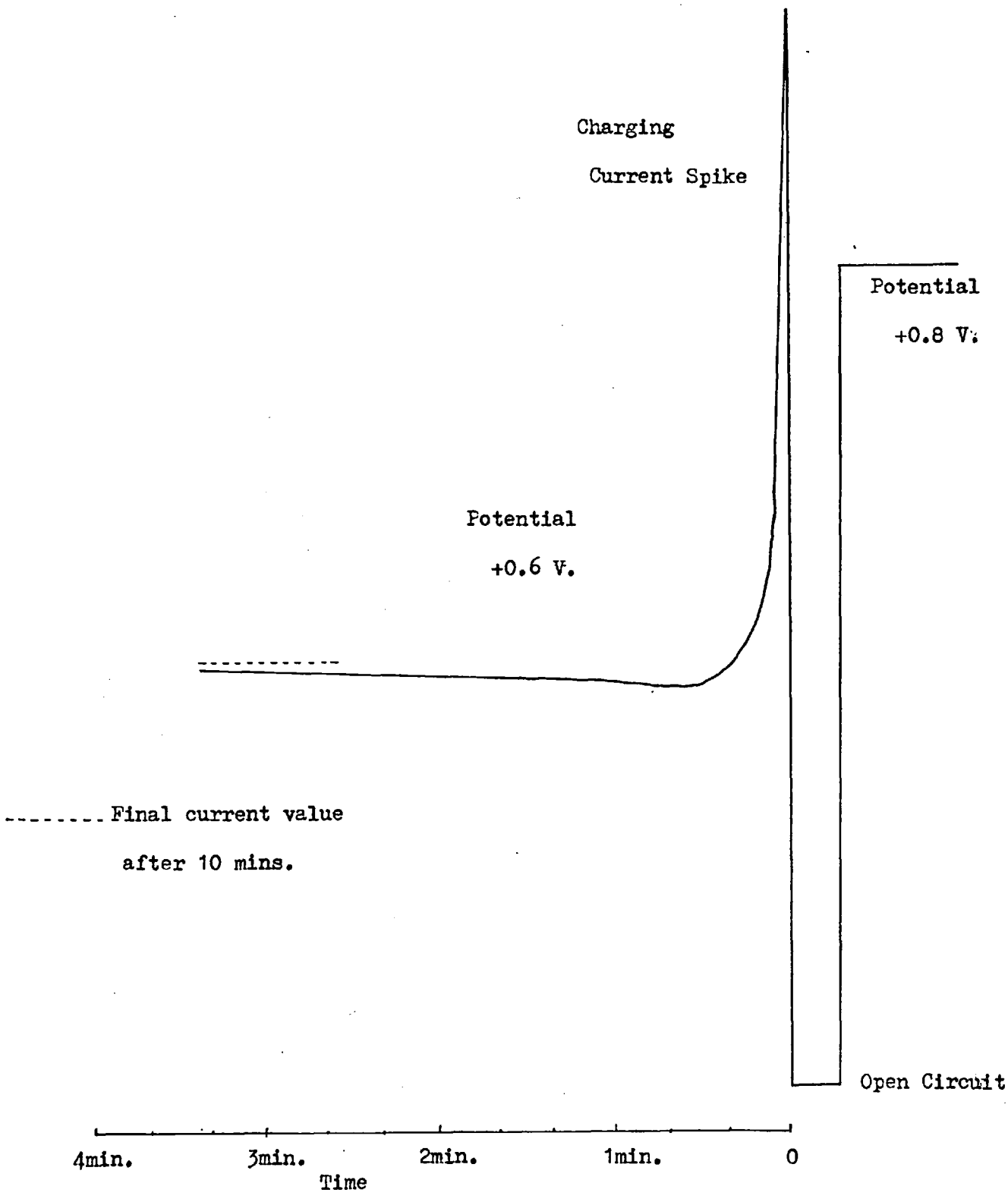


Fig 5.9b

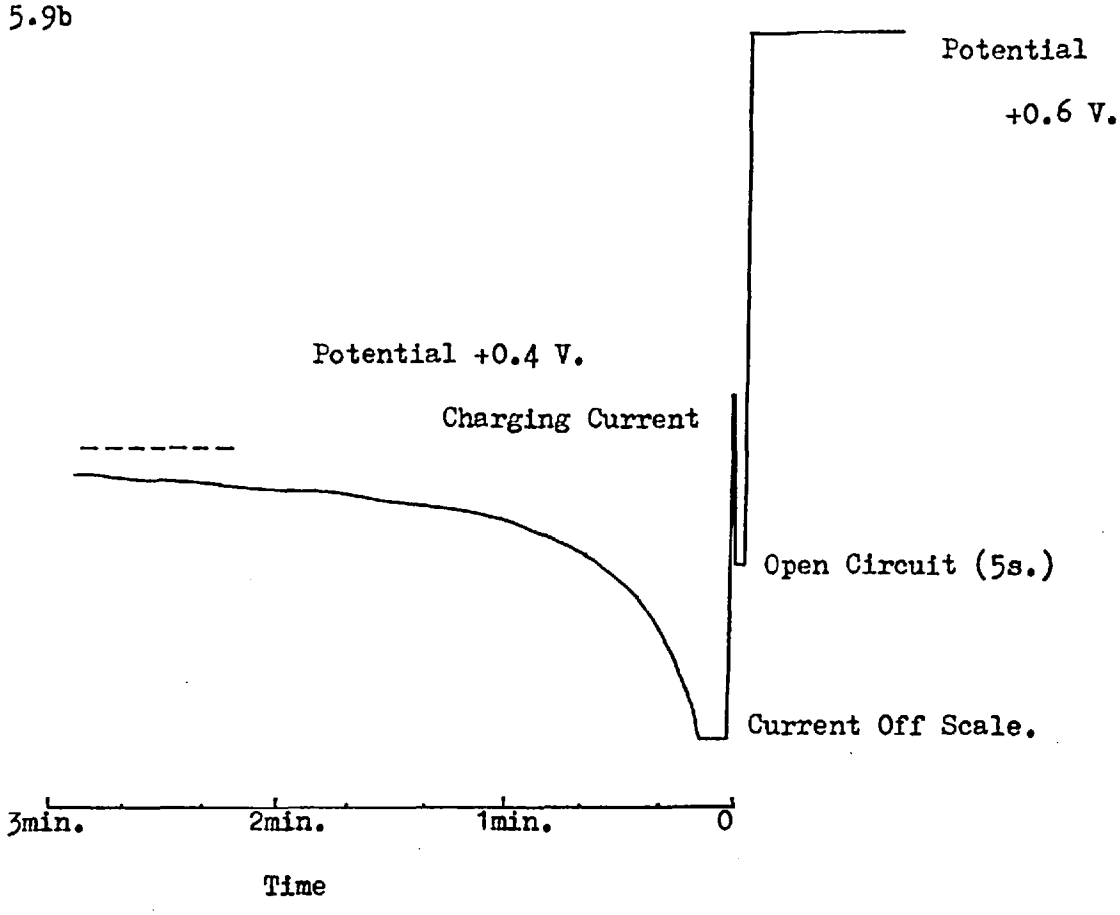
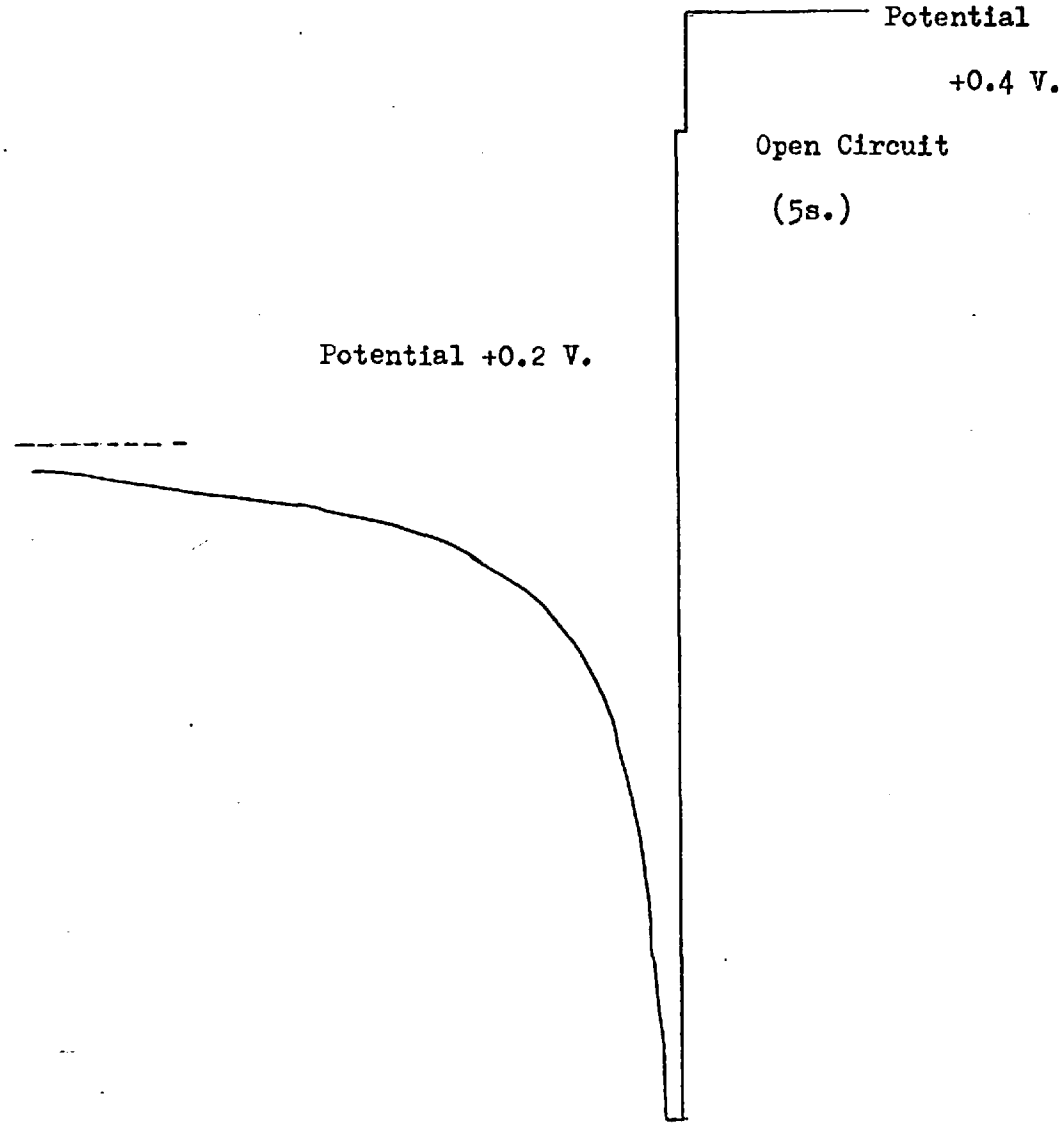


Fig 5.9c



It became apparent from later work that when the system was open circuited, the electrode tended very quickly to approach the condition it would be in at the potential of zero net current flow. A further series of curves was produced by stepping from +0.7 V to +0.5 V while varying the time at open circuit. See Fig 5.10 .

To explain these results it is necessary at this point to propose the following theory. The surface of the glassy carbon electrode is known to contain many carbon-oxygen groups. When a potential is applied to the electrode these groups slowly come to redox equilibria. Their response to potential changes is very slow because the reactions are solid state ones and may involve conformational changes in the solid at the electrode surface. Thus when a potential step is applied the current that flows to charge up the electrical double layer decays very quickly, however the current produced by the approach to redox equilibrium of these surface groups decays much more slowly. Using this model the curves of Fig 5.10 can be explained.

Fig 5.10a

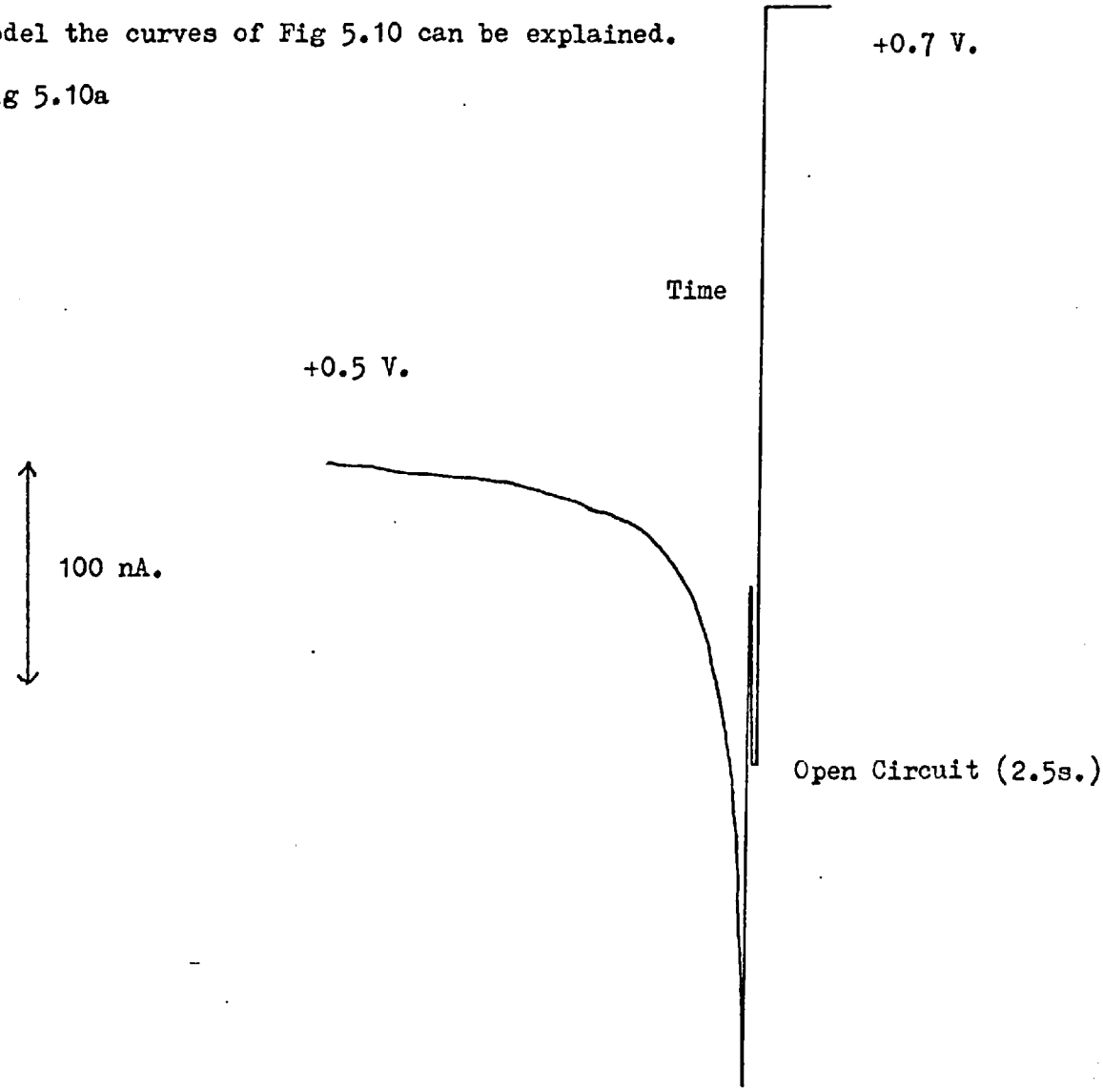


Fig 5.10b

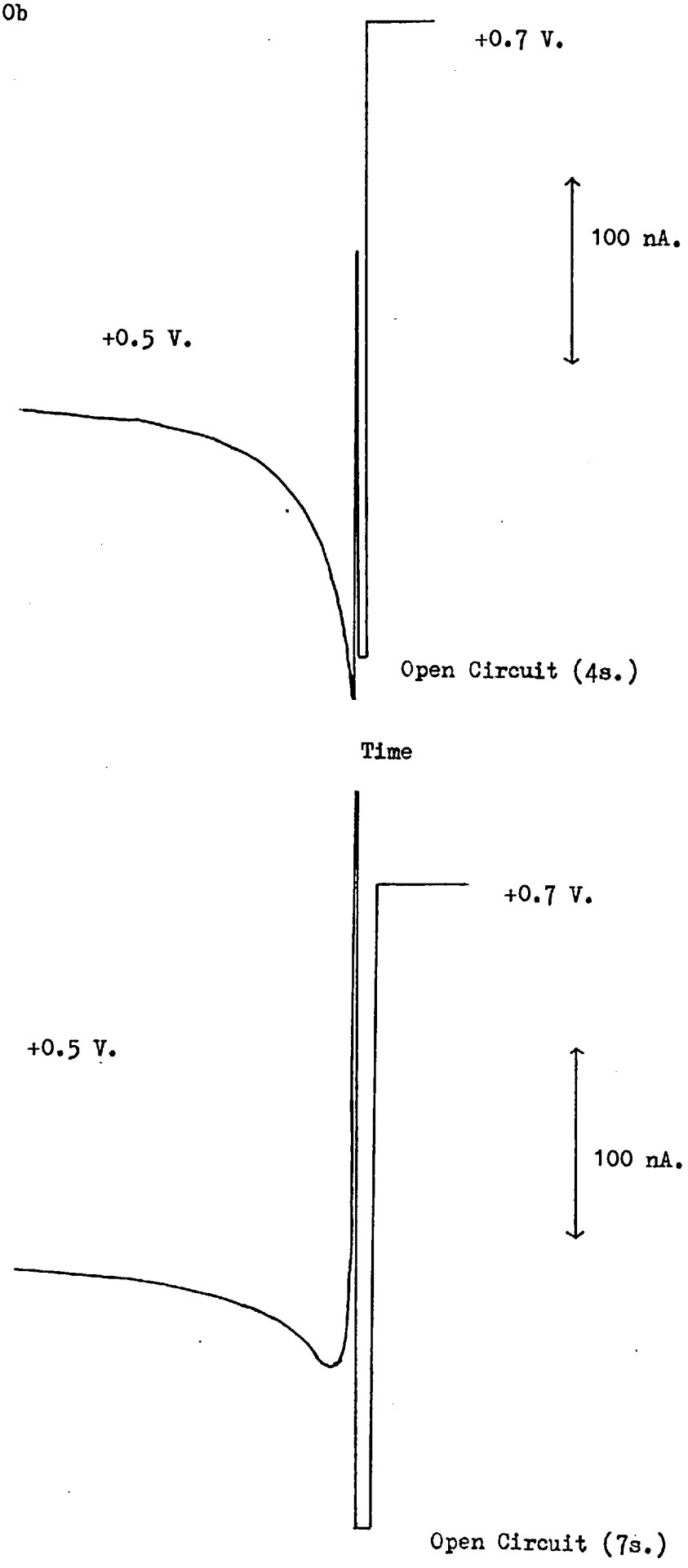
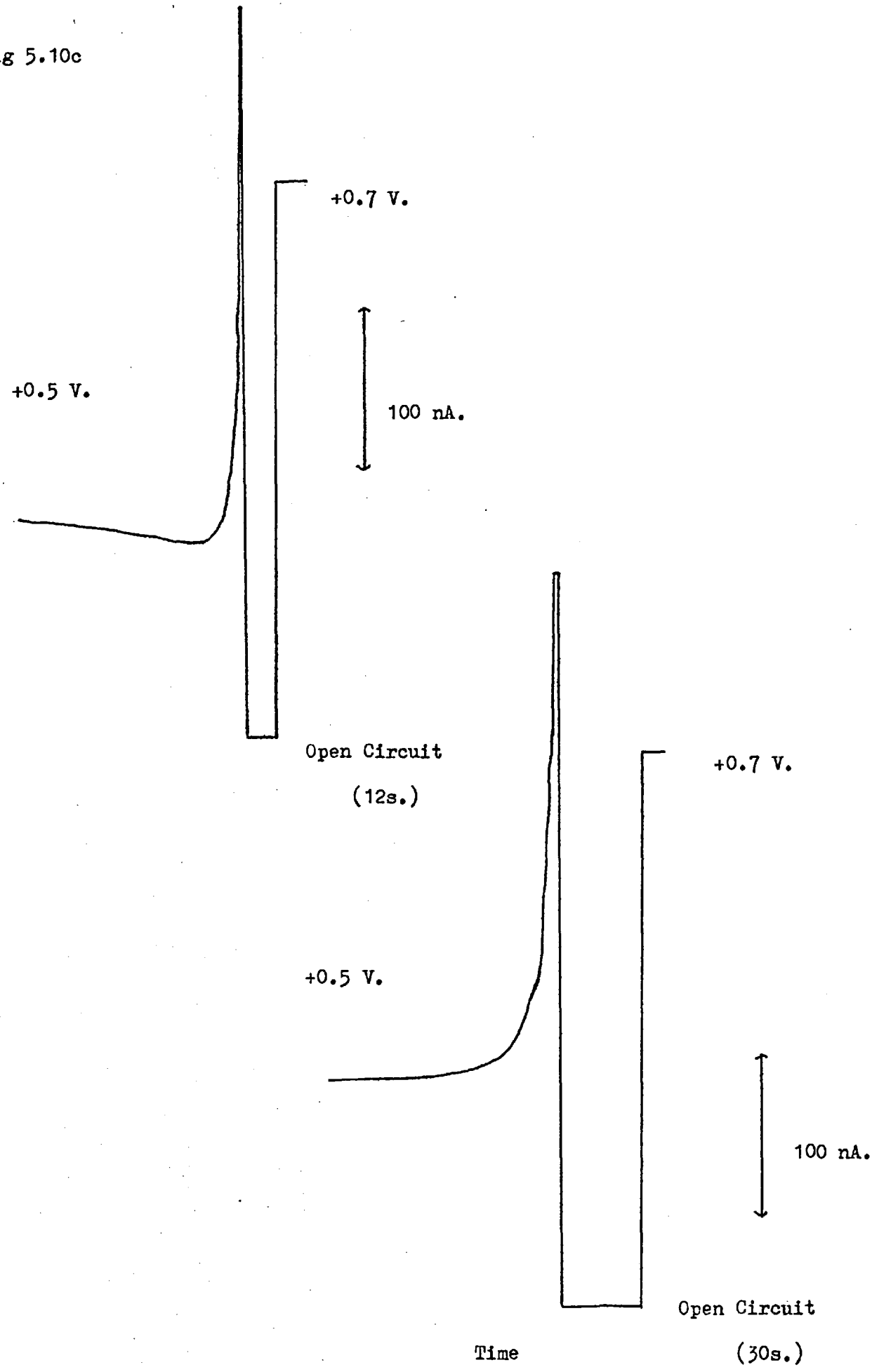
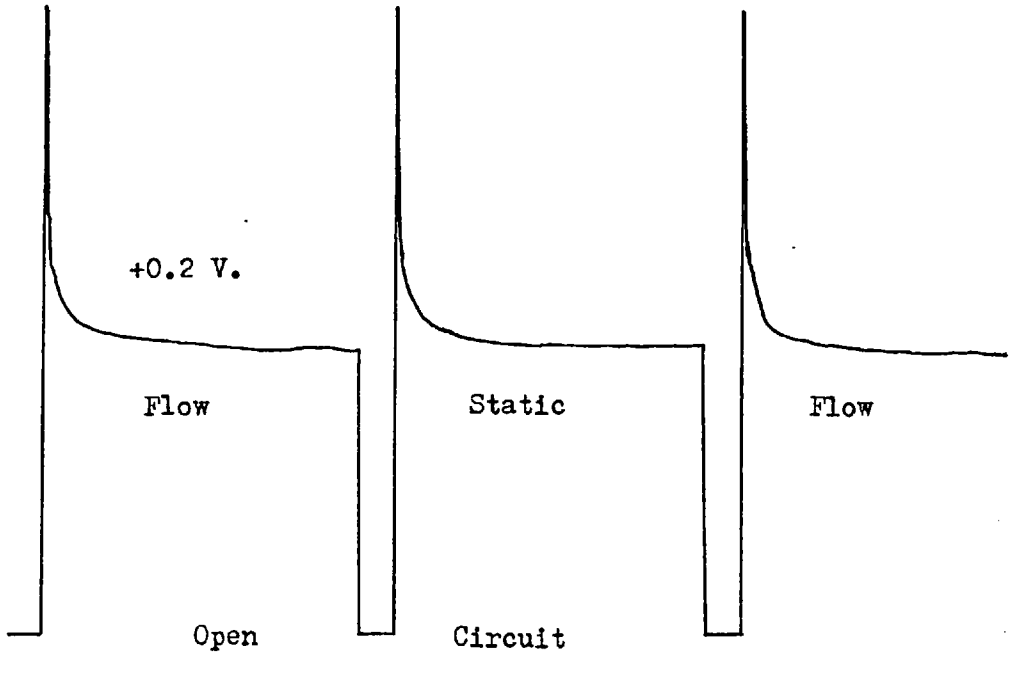

+0.7 V.
100 nA.
+0.5 V.
Open Circuit (4s.)
Time
+0.7 V.
+0.5 V.
100 nA.
Open Circuit (7s.)

Fig 5.10c


+0.7 V.
+0.5 V.
100 nA.
Open Circuit
(12s.)
+0.7 V.
+0.5 V.
100 nA.
Open Circuit
(30s.)
Time

When the system is open circuited the double layer breaks up very quickly, thus even after a short period at open circuit (2.5s.) the charging current always flows in the positive direction (towards the top of the page) when the circuit is closed again at +0.5 V.. The surface groups on the other hand try to reach the oxidation state that they would be in at the potential of zero current flow.(this corresponds to a potential of about +0.4 V.) when the system is open circuited. If the time at open circuit is short then the surface groups do not have time to relax into this state and hence a large current is seen to flow on closing the circuit. As the time at open circuit is increased, this current is reduced, since the surface has had more time to relax and hence the algebraic sum of the two currents (charging current and surface group current) is made up mainly of the charging current and thus the charging current spike is seen to increase. By the time the open circuit period has reached 30s., the surface groups have attained a state which corresponds to a potential less positive than +0.5 V. and thus when the circuit is then closed at +0.5 V. both currents are seen to flow in the same direction. i.e. positive. Further evidence that these effects are surface ones and not normal charging current ones can be seen in Fig 5.11, which shows that a flowing stream has no effect on this slow decay current. The same shaped curves being produced both in a static solution and in a flow stream.

Fig 5.11



Examination of electrode 1 by application of similar steps produced apparently different results. Fig 5.12

Fig 5.12a

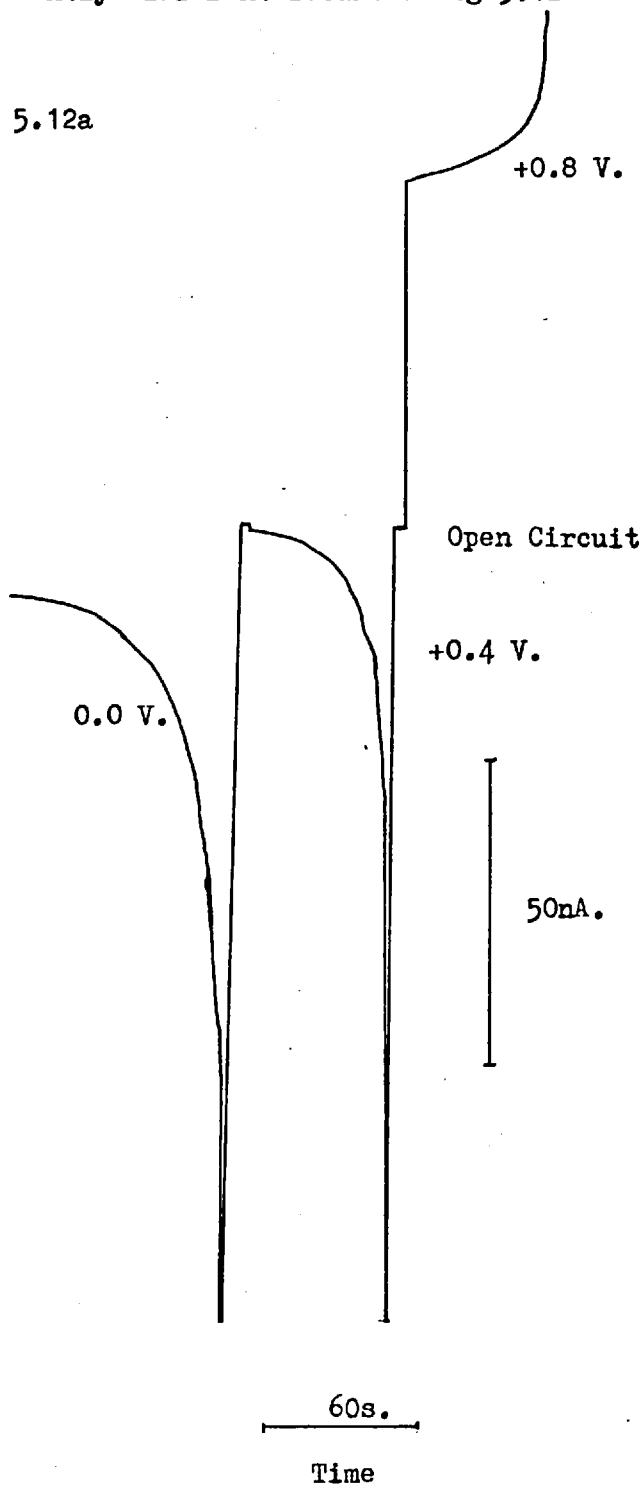
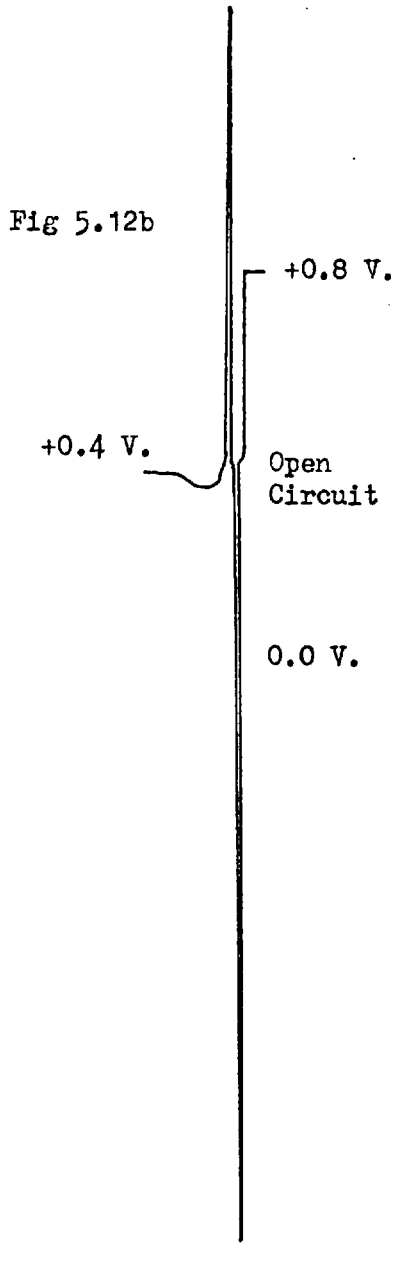


Fig 5.12b



The residual currents in this case are much smaller than the previous one, and the approach to equilibrium is much faster. Because +0.4 V. lies on the opposite side of the zero current potential (+0.42 V) to +0.8 V., the charging current for this step is negative,i.e. the same direction as the charging current for an instantaneous step change. In order to see if the slow surface group current was present for this step it was necessary to make the charging current flow in the opposite direction as +0.4 V. was approached. To do this the following potential programming was carried out. From +0.8 V. the potential was switched very quickly via open circuit to 0.0 V.. This meant that the 0.0 V. double layer was set up immediately while the slow surface group current from +0.8 V. was just beginning to flow. Immediately the potential was then switched to +0.4 V.. This produced a charging current spike in the positive direction due to the switch from 0.0 V. to +0.4 V. which was in the opposite direction to the slowly responding surface group current from +0.8 V.. Because of the slow response of this current the time spent at 0.0 V., although enough to change the direction of the charging current, was too short to have much influence upon it. The presence of this current can be seen in its separated form in Fig 5.12b.

From this work the following conclusions can be drawn.

a) The size of the residual current at glassy carbon depends upon the number of surface carbon-oxygen groups. The greater the number, the greater is likely to be the residual current. Hence the recent history of the surface is important, especially with regard to its exposure to oxidising conditions (electrochemical, chemical and mechanical).

b) The charging current at glassy carbon decays relatively quickly, probably within 100ms..

c) When the potential applied to the surface of glassy carbon is changed the carbon-oxygen groups respond very slowly to this change thus producing a slowly decaying current whose magnitude depends upon the

number of these groups on the surface.

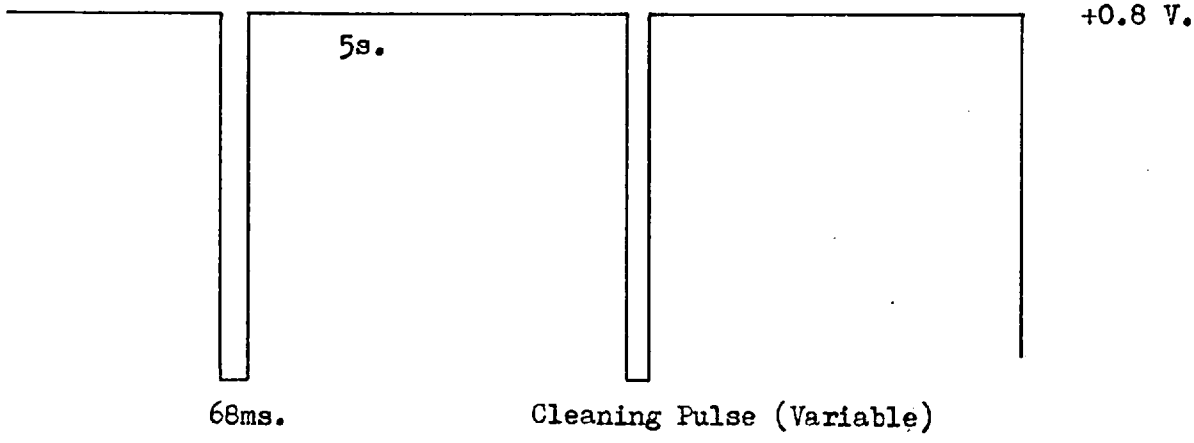
d) The potential of zero net current flow was found to vary from electrode to electrode. Again this was probably due to the number and nature of the carbon-oxygen groups.

Once the behavior of glassy carbon under the influence of a single potential step had been investigated, it was necessary to look at the behavior under the influence of a continuous pulse train.

5.3.4 CONTINUOUS PULSING

The P.A.R. 174 was used to produce the pulse train. The pulse shape applied was as follows.

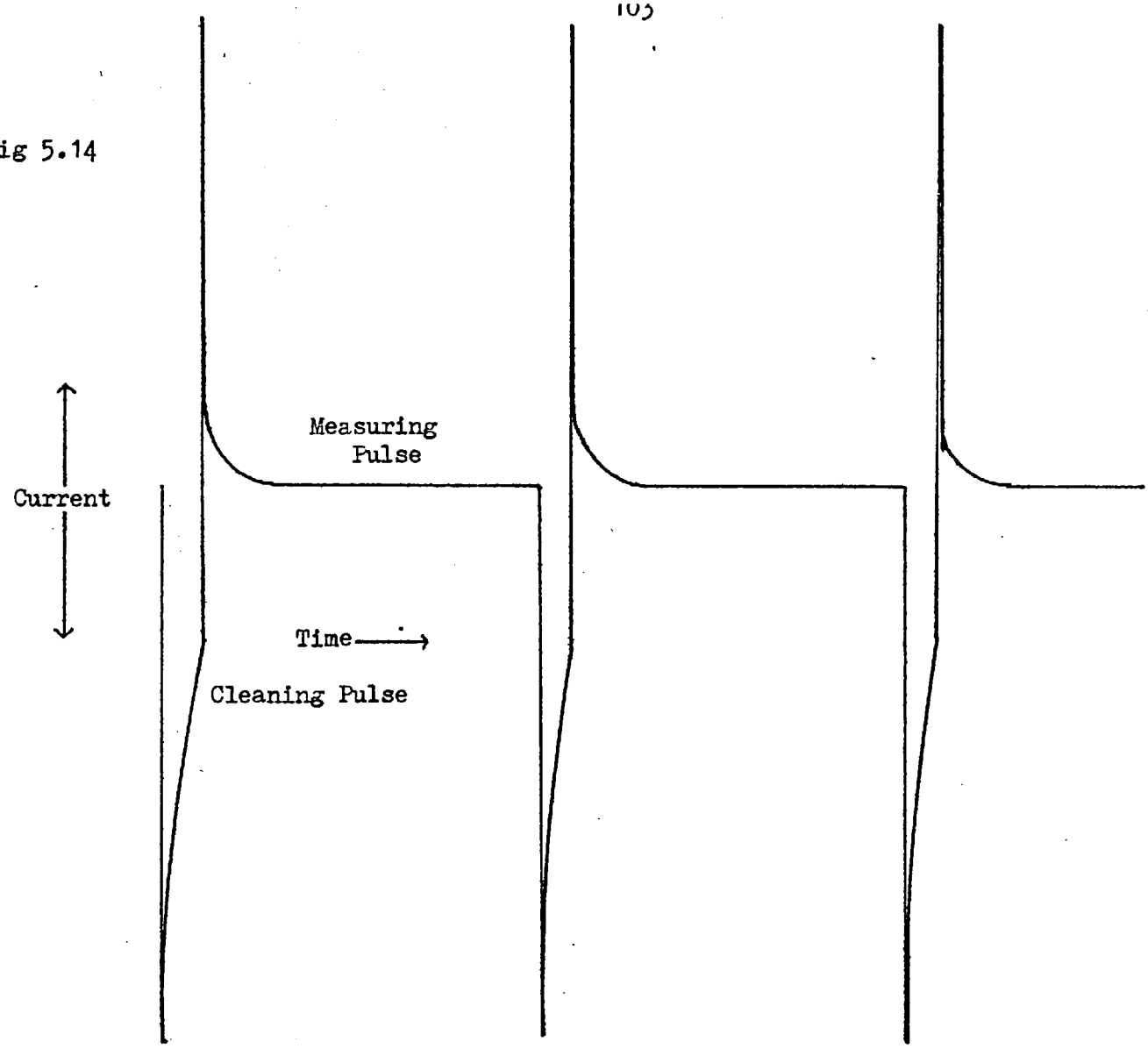
Fig 5.13



The position of the window on this pulse train in fact appeared in the cleaning pulse, but this did not matter since a chart recorder was not used. Instead the output of the Current to Voltage converter was displayed on an oscilloscope in order to produce a fast response system.

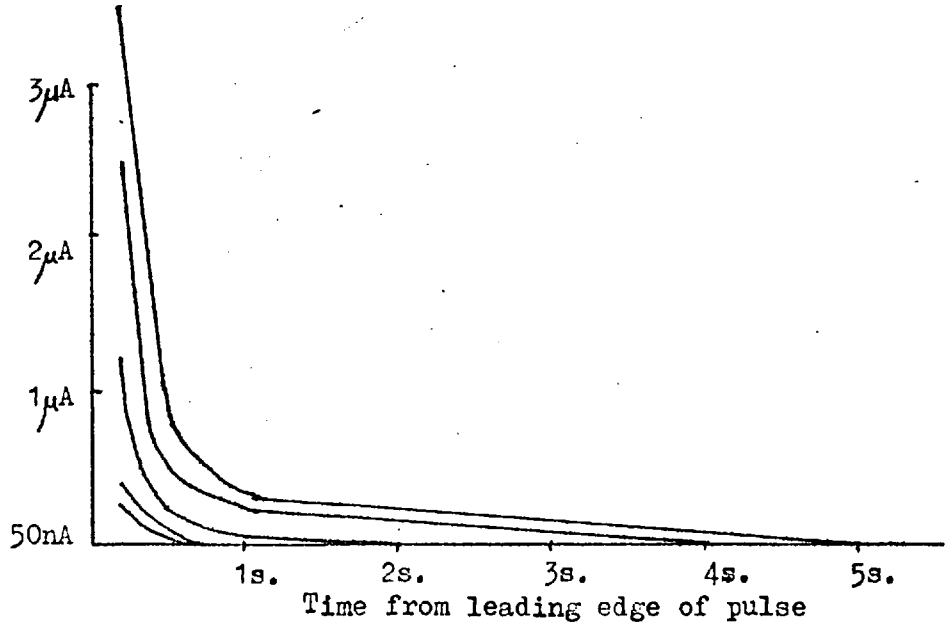
The current output on application of the above pulse train was as follows:-

Fig 5.14



The residual current at +0.8 V. was initially found to be -50 nA. in a non-pulsing system.

The decay currents for the step from cleaning pulse to measuring pulse are shown in Fig 5.15 for various size cleaning pulses.



A long measuring pulse was used to allow the currents to decay to as close to the residual current as possible before applying the next pulse.

It is important to note that the currents all decayed to the residual current value in less than 4s. and that even the large pulses decayed to relatively small values within 0.5s. (i.e. less than 1μA). This is contrary to what would be expected from the previous results which would indicate a much slower decay. The reason for the fast decay is that since the cleaning pulse was so short, the surface carbon-oxygen groups did not have time to respond to this potential before the measuring potential was reapplied, and hence they remained in a state very close to that which they were in at +0.8 V.. This meant that the slow decay currents were kept to a minimum and a steady state was set up. After the application of a few pulses the decay currents became very reproducible and any sampled point on one of them coinsided with a similar point on a succeeding pulse (within 0.1nA.).

The fact that a steady state was set up was extremely important since it indicated that if sampled points were taken at the end of each measuring pulse and fed to the output, a steady background base line could be produced which should vary by less than 0.1nA. (this does not take into account flow variations etc.).

Thus the results indicated that it would be possible to apply a sampled pulse train whose period could be of the order of 0.5s.. This would be compatible with a liquid chromatographic detector for all but the fastest peaks. It was also thought possible that even quicker pulses could be used if slightly larger background currents could be accommodated.

6.1.0 THE H.P.L.C. DETECTOR

Due to the special effects of current decay found at glassy carbon and reported in chapter 5, it was necessary to build a potentiostatically controlled pulse generator with a specialised timing system. The use of the P.A.R. 174 was severely restricted due to the very short duration of its measuring pulse. The detector reported in this chapter is the final model and was the result of various steps. The various earlier models have not been described but the final circuit diagram is shown and parts of the circuit whose design was critical have been discussed in detail. It was necessary to produce a controller capable of coping with the specialised problems produced by H.P.L.C. detection and glassy carbon electrodes. As more information was gained in these respects the detector design was improved. The actual detector was built by my colleague Mr. T.A.Berger, with assistance on special aspects of circuit design from Mr. D. Alger of the electronics workshop of Imperial College (chemistry department).

6.2.0 GENERAL FEATURES

The electrical circuits were confined as far as possible to two boards. The boards used were two $\frac{1}{10}^{th}$ inch pitch "Vero" boards which were already divided into copper pads built to mount D.I.L. (dool-in-line) integrated circuit packages. External connections were made via 32 pin edge connectors which had gold plated contacts to ensure low resistances. The edge connectors were mounted in a bent aluminium rack fixed to one end of the box. The box itself was of mild steel to provide a good faraday cage and measured $10\frac{1}{2}$" by $6\frac{1}{2}$" by $7\frac{1}{2}$". All the logic operations and the switching were confined to the logic board. The logic was based on a counter timer running off a 50 Hz. signal. These signals were used to operate the direct set and clear inputs of J.K. flip-flops and the output of these flip-flops was then used to controle F.E.T. switches (103) See Fig. 6.11.

The analog parts of the controller were all placed on the second board. It was found to be necessary to separate the current to voltage converter as far as possible from the other components. If this was not done the high impedence of the operational amplifier and the high resistance values of the associated scale resistors produced pick up of the switching spikes from the logic board.

All the operational amplifiers were bought from R.S. Components Ltd. London and all the F.E.T.s from Henry's Radio Ltd. The resistors used were all 1% precision or better and when sets were used they were hand matched to produce accurate ratio values(103). The capacitor used in the sample hold/integrator circuit was a polystyrene one. This was used because of its very low leakage current.

The controller was powered by two power supplies, one of 15 V., the other of 5 V.. The 15 V. supply was obtained from Coultron Electronics Ltd.. This supply was floated with respect to mains and grounded where it and the 5 V. supply entered the chassis. The 5 V. supply (R.S. Components Ltd) was grounded to mains. All the other components that had to be grounded were grounded directly to this point to eliminate the formation of ground loops.

External connections from the controller to the cell were accomplished via shielded B.N.C. connectors, the shield being grounded to the chassis. The Servoscribe recorder used in combination with the controller, was floated, its negative input being grounded to the chassis through a B.N.C. shield.

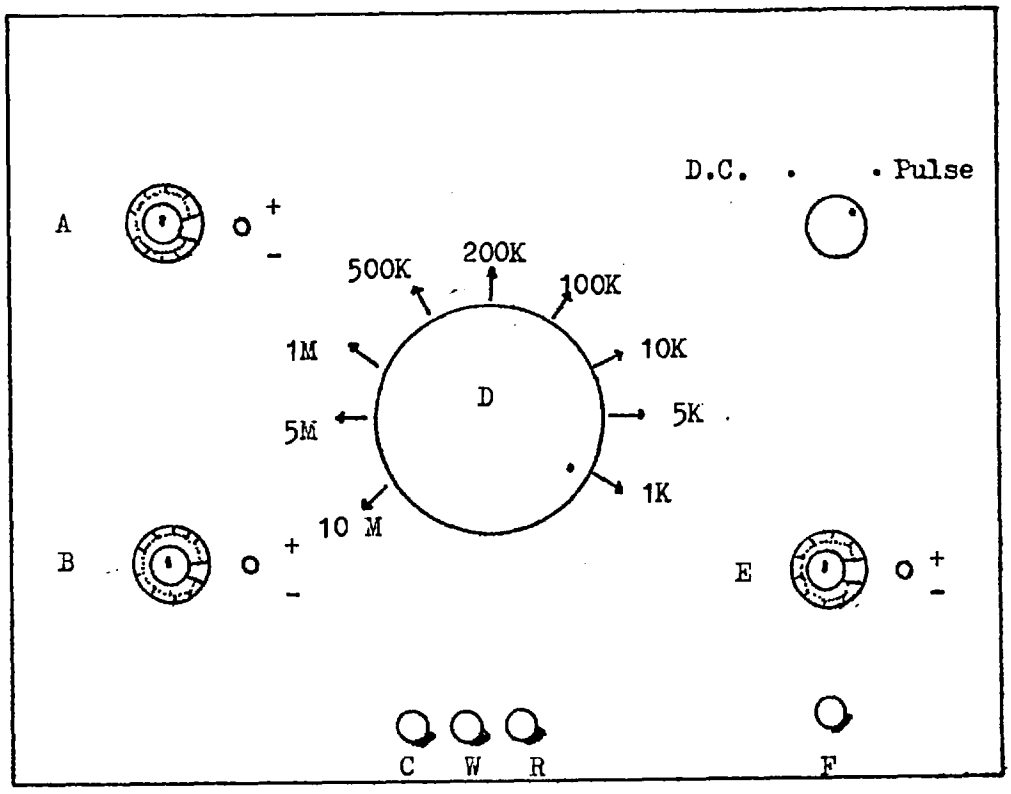
6.3.0 FRONT PANEL

All the manual controls were placed on the front panel of the box. The measuring pulse levels and cleaning pulse levels were set by means of two 10 turn 1KΩ precision potentiometers. See Fig 6.1 A and B. The sensitivity scale was selected by a 9 position switch D. Backing off was accomplished by means of another 10 turn potentiometer E. In all the cases A,E, and B the polarity was determined by toggle switches. C. R, W,

denote the external connections to the cell (counter, reference and working), and the B.N.C. socket F was used to connect to the recorder.

Fig 6.1

The Front Panel

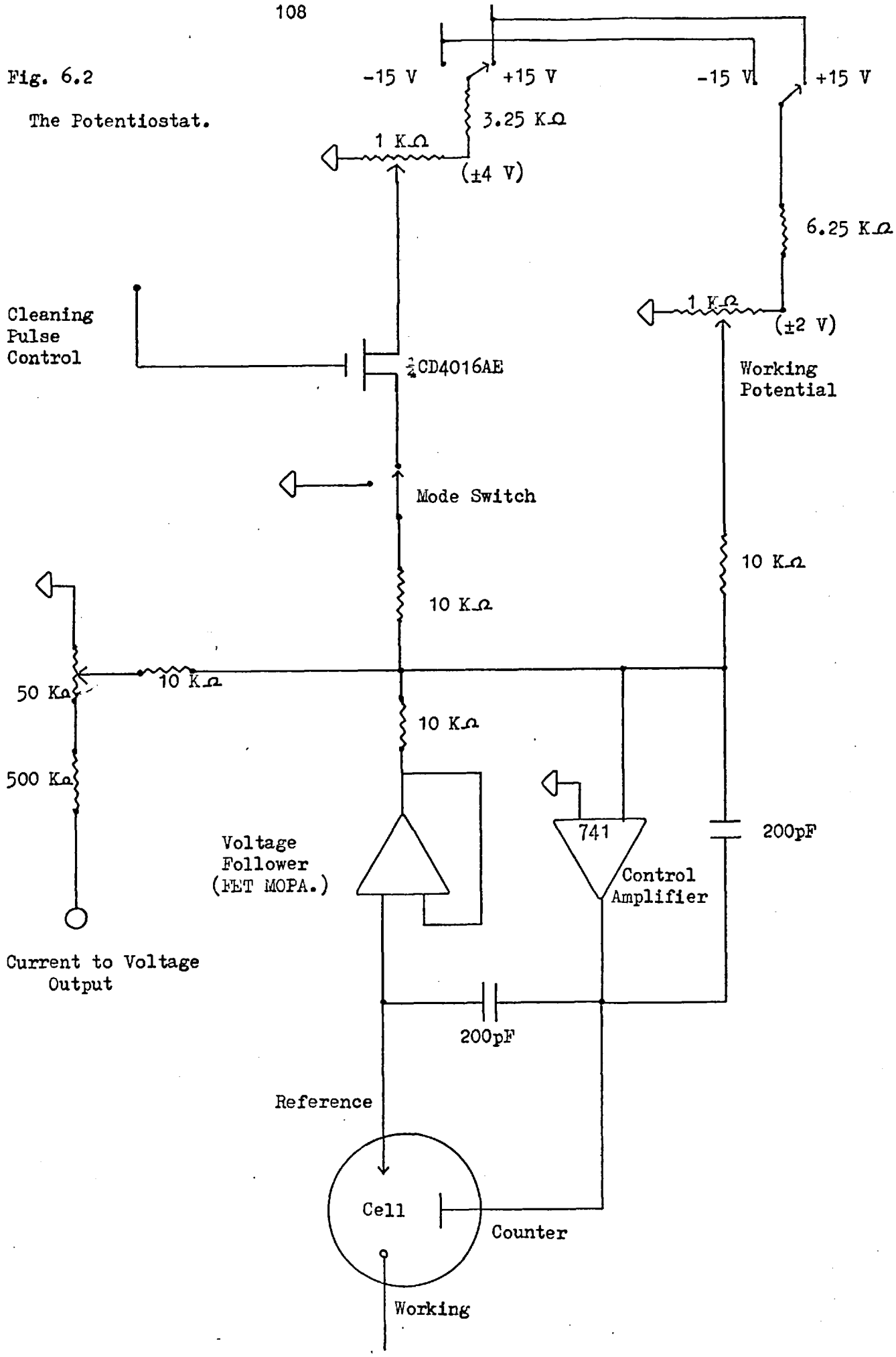


6.4.0 PULSE GENERATOR AND POTENTIOSTAT

The pulse generator and potentiostat are shown in Fig 6.2. The potentiostat was based on that of Booman (104) who produced the first published operational amplifier potentiostat. The potentiostat contains a F.E.T. M.O.P.A. operational amplifier used as a voltage follower using a high inpedence non-inverting input. The control amplifier was a standard R.S. 741. The working potential was produced by the ± 15 V. power lines and a 1K 10 turn precision potentiometer. A series resistance

Fig. 6.2

The Potentiostat.



produced a ± 2 V. drop across the potentiometer which allowed the working electrode to be set at any potential between +2 V. and -2 V.. The pulse train was introduced by means of a ¼ CD4016AE switch controlled from the logic board. When the switch is open, a very high impedence path is presented to the cleaning potential, and thus the normal working potential is applied. When the switch is closed however this impedence is very much reduced and the cleaning potential is applied in preference to the working potential. The switch has a total swing of 15 V. i.e. should work off a supply of ±7.5 V.. These power supplies are very difficult to produce however and therefore the ±5 V. supply was used. A 1KΩ10 turn potentiometer was used to produce a cleaning pulse between +4 V. and -4 V. with the aid of the series resistance of 3.25 KΩ. A mode switch was introduced so that the controller could be used in the pulse mode or straight D.C. mode. When used in the D.C. mode, the working potential was dialled up on potentiometer A, potentiometer B having no effect.

Facilities for positive feedback were introduced to allow compensation for I.R. drop (105). The potentiostat maintained the working electrode at virtual ground and applied the dialled potential to the counter electrode. An electrochemical reaction occurred when the free energy change for the whole cell was favourable. To maintain good potential control the I.R. drop between reference and working electrodes should be as small as possible. In normal systems this is done by placing these two electrodes as close togeather as possible but in the H.P.L.C. detector this was not convenient. The effect of the I.R. drop can be minimised by the use of positive feedback and this may prove to be very useful when solvents are used which cannot sustain support electrolyte at the 0.1 M. level.

6.5 CURRENT FOLLOWER

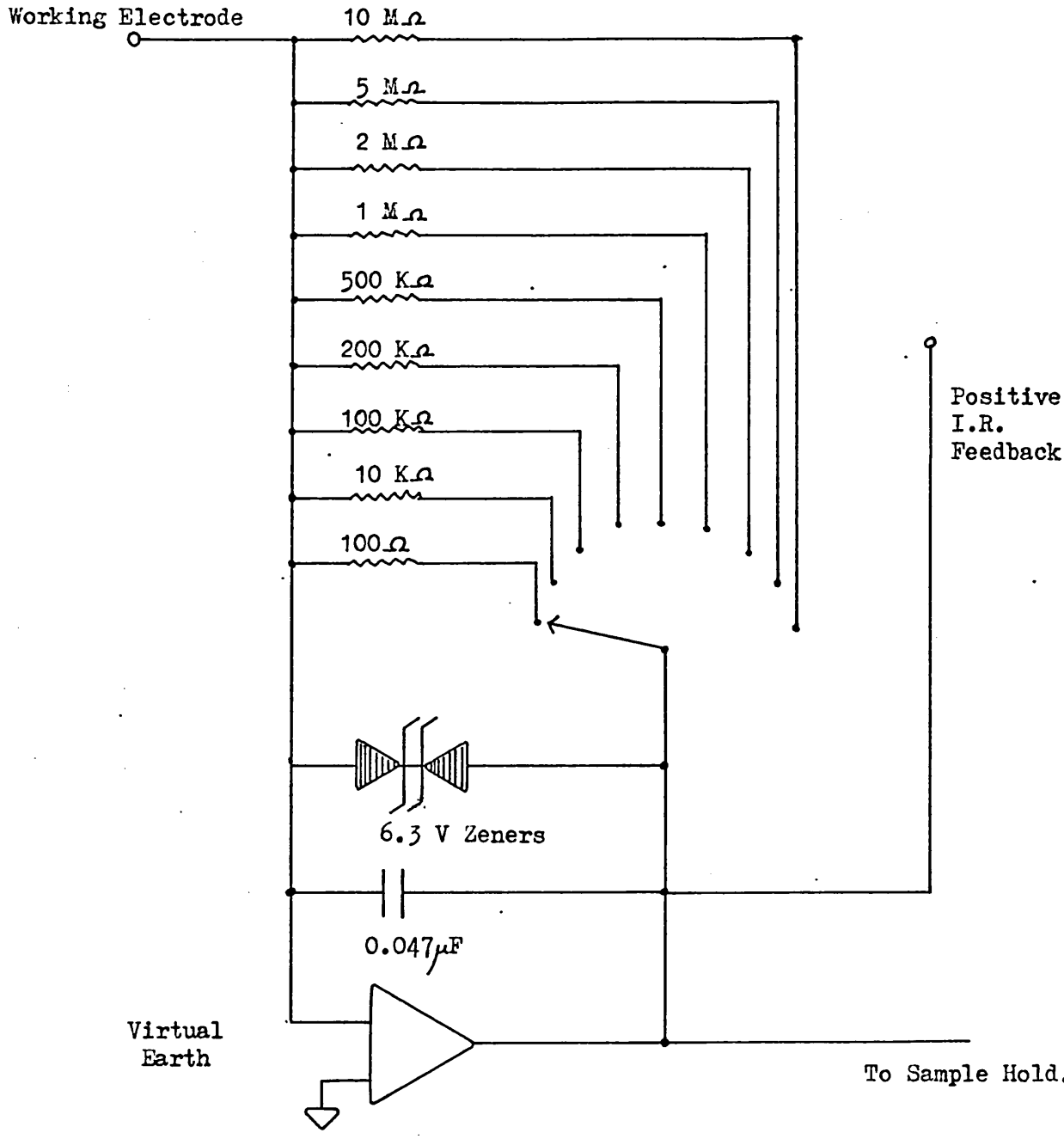
The current follower provides a means of converting the current flowing through the working electrode into a voltage which can then be amplified and displayed. Because of its function it is also known as the current to voltage converter. Fig 6.3

The working electrode and the input to the current follower are both at virtual earth. Thus when a current flows through the working electrode, the amplifier must match this current with an equal and opposite one. To provide this current the amplifier must provide a voltage accross the scale resistors. The voltage produced being proportional to the current.

$$E_{out} = I_{in} \cdot R_f \qquad \text{Equation 26}$$

When a small value feedback resistor was used (insensitive scale) the voltage needed to produce a given current was quite small. As the feedback resistor was increased (increased sensitivity) the voltage required increased. When the current flowing necessitated a voltage in excess of ±10 V. the amplifier overloaded. If the current flowing through the working electrode had been fairly constant, then all that would have been necessary would have been to keep the scale resistor small enough to stop the amplifier overloading. Unfortunately in the pulse mode the current was not constant due to the presence of charging current. The charging current spike was three orders of magnitude or more larger than the current produced by the chromatographic peaks and hence on a scale sensitive enough to see the chromatographic peak, the charging current spike overloaded the amplifier. Once again the problem was that seen with the P.A.R. 174, in which even if the amplifier recovered from overload by the time the current measurement was made, it was impossible to know whether or not the overload had affected the current obtained. In order to protect the amplifier from the charging

Fig 6.3 Current to Voltage Converter.



current spike (or for that matter from too high a scale setting for the current in the window) a double zener diode system was used.

The current characteristics of a zener diode are shown in Fig 6.4

Fig 6.4a

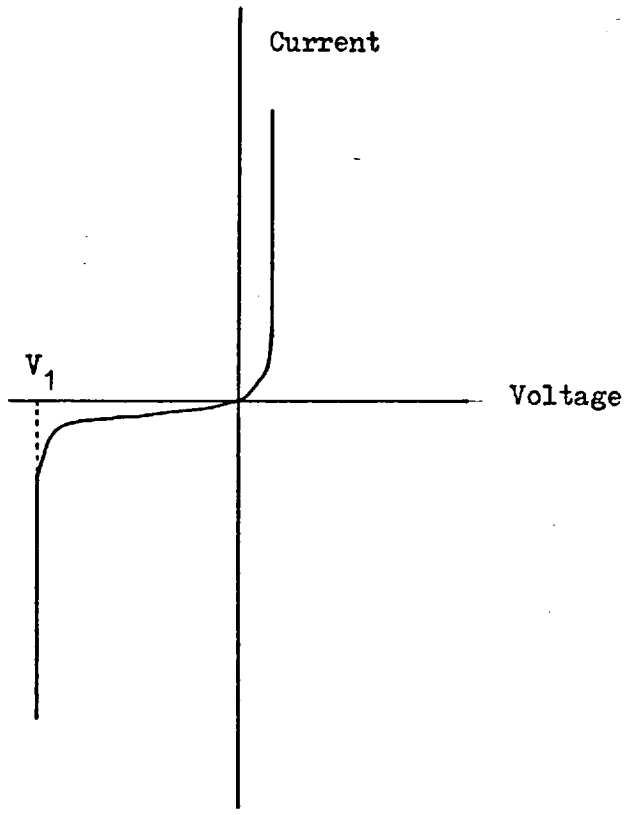
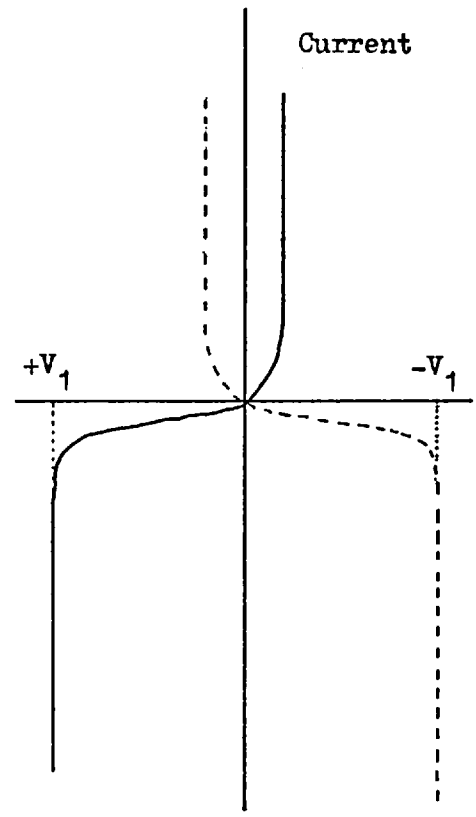


Fig 6.4b



The zener diode allows current to flow in one direction with very little resistance. In the other direction however, the zener presents a very high impedence until its break down voltage is reached, after this voltage the resistance drops to a very small value and current can once again flow. If two of these zeners are placed back to back, the current characteristics obtained are shown in Fig 6.4b. In this case , if the voltage applied is less than $\pm V_1$ volts, no current can flow. If these voltages are exceeded however, the resistance is reduced to almost nothing and current can flow.

The current follower amplifier had a range of ± 10 V. and thus two zeners were used in the feedback circuit whose break down voltage was 6.3 V. This meant that when the current flowing through the working electrode was such that the amplifier was required to put out more than 6.3 V. the scale resistor circuit was shorted out via the zener circuit and a current was produced to match the working electrode current at a potential from the amplifier of 6.3 V. i.e. well below the 10 V. overload of the amplifier.

The introduction of the zener diodes solved the main problem associated with the pulse mode. It did however produce another one. The metal chromatographic column acted as a very good antenna and picked up mains frequency very effectively (50 Hz.). When using a sensitive scale on the current to voltage converter, this noise was large enough to break down the zeners and produce a steady output of 6.3 V. except on a very insensitive scale. Two methods were devised to overcome this. One was to place the whole system in a faraday cage, and the second was to ground the column. Because the first method was impracticle, the second was investigated initially. To ensure that an earth loop was not formed, the column was grounded with a single wire to the earth input of the 5 V. power supply. The main objection to grounding the column was that it would then be at the same potential as the working electrode and thus might function as a massive working electrode itself. To see if this was in fact the case, the controller was used in the D.C. mode on a system involving very large samples so that a scale resistor could be used which was small enough for the pick up to be negligible compared to the signal. It was found that the peaks produced with the column grounded were the same size as those with the column floating. Thus the column did not appear to act as a working electrode. The reason for this is probably due to the fact that the inlet to the cell is very narrow and quite long. This results in a large I.R. drop between the reference electrode and the column, and this I.R.

drop serves to deactivate the column as a working electrode. As a result of earthing the column the noise can be kept below the level where it overloads the current to voltage converter on the scale resistors used i.e. up to 10 MΩ.

6.6.0 THE SAMPLE HOLD

The output from the current to voltage converter was then fed into a sample hold system. The current was only sampled at the end of the measuring pulse in a small window 22ms. long. The current during this window was averaged and this value was put out to the output amplifier until the next sampling took place whereupon this new value was put out and so on. A sample hold technique was used because the variation in output from the current to voltage converter (Fig 6.5) produced by the charging current was very large (±6.3 V.) compared to the increase in voltage produced by the faradaic current from the sample, and hence it was impossible to see the effects of the faradaic current when the whole current response was fed to the output. Instead, only the current in a small window at the end of the measuring pulse was measured, the variation across this window being small compared to the faradaic current. (Fig 6.6).

Fig 6.5

Output of the current to voltage converter

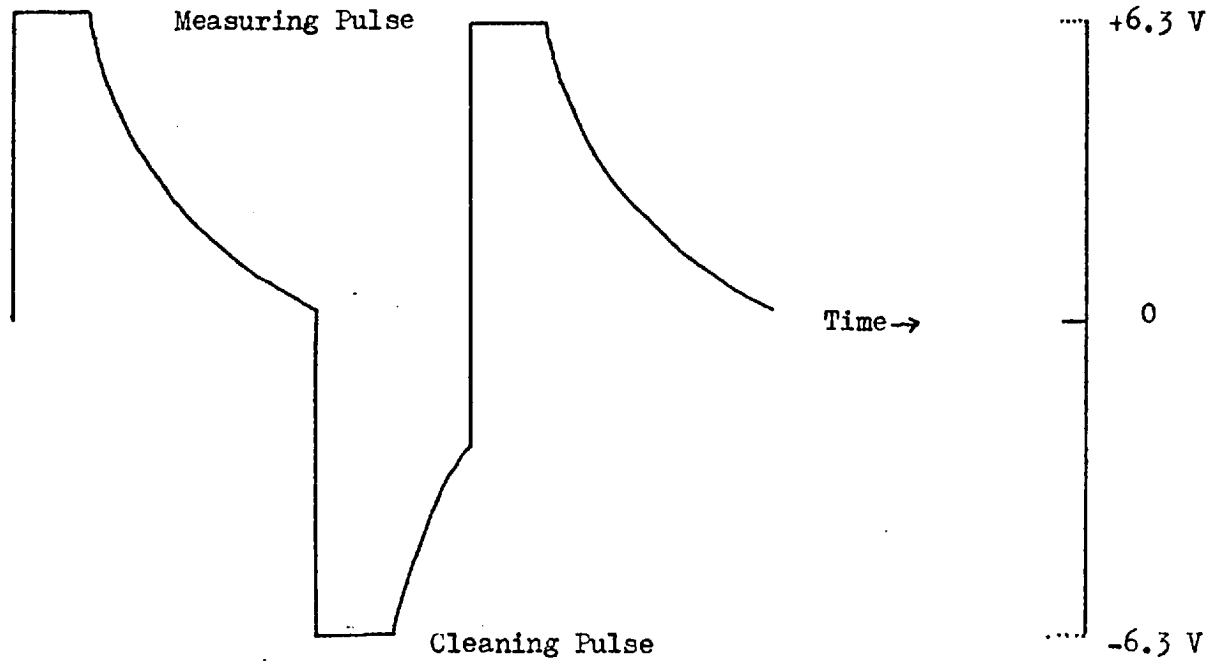
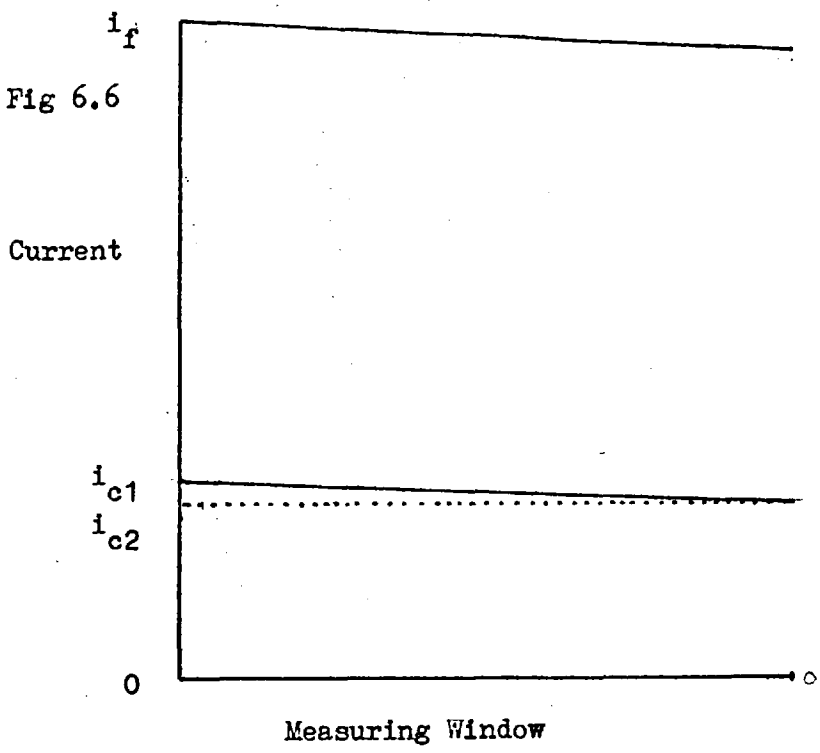


Fig 6.6



i_f= Faradaic Current

i_{c1}= Charging Current at begining of window

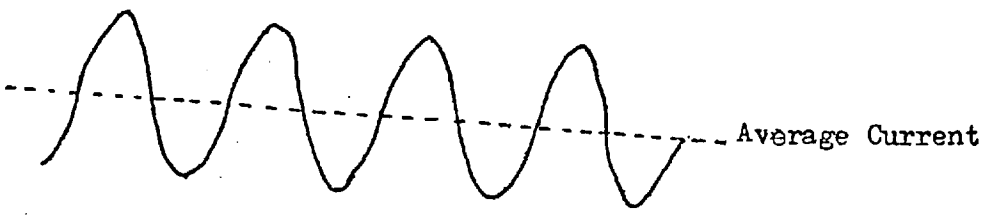
i_{c2}= Charging Current at end of window

$\delta i_c = i_{c1} - i_{c2}$

$\delta i_c \ll\ll i_f$

The further the window could be placed from the leading edge of the pulse the better, since this reduced i to a minimum. It also reduced the avarage value of i_c in the window and hence made backing off easier. The sample hold amplifier worked as an integrator and as such had a low bias current with a high impedence non-inverting input. Because of its integrating properties the correct choise of sample window width increased sensitivity considerably. The output from the current to voltage converter is shown in magnified form in Fig 6.7.

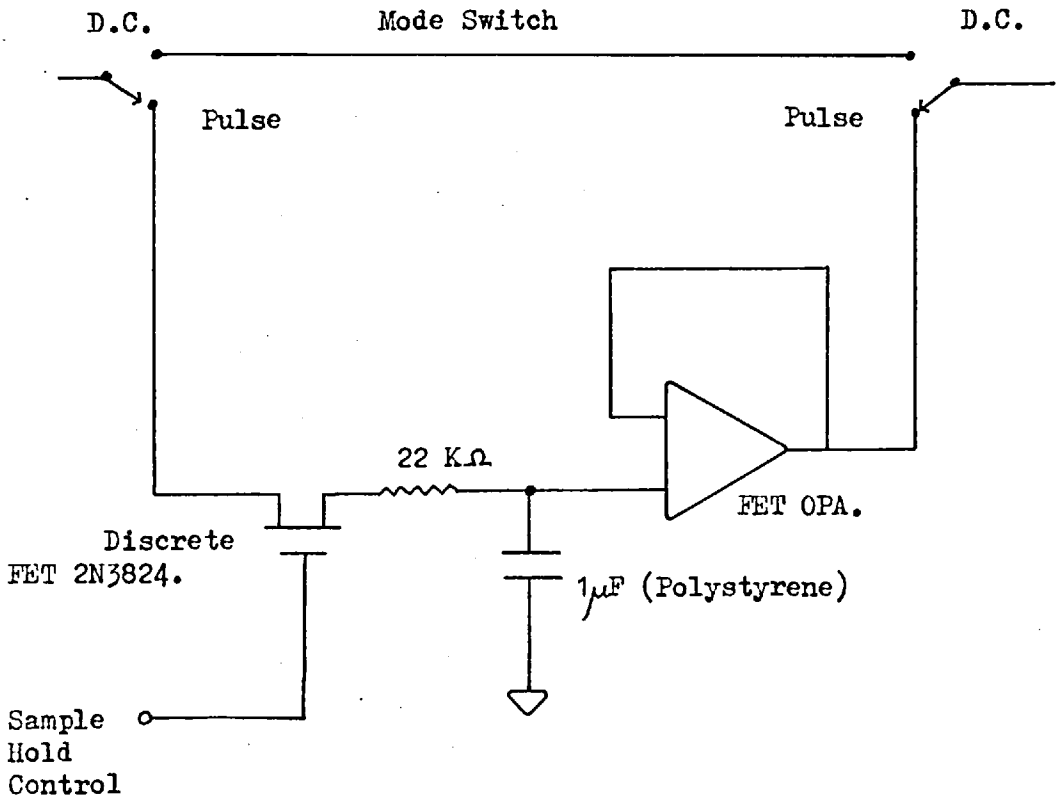
Fig 6.7



The predominant noise frequency was found to be 50 Hz.. When the time constant of the sample hold was set equal to one cycle of mains the integration of the noise resulted in its disappearance, (the noise being symetrical about the real current). This resulted in an elimination of line frequency pick up up to this point in the circuit, and a severe reduction in other higher frequencies. For this reason the sample window was set at 22ms..

The actual sampling was carried out by a discrete F.E.T. 2N3824 switch worked off the logic board. When the switch was open i.e. outside the sample window, the voltage produced by the current to voltage converter went to ground, when closed it was fed into the sample hold. Fig 6.8.

Fig 6.8 The Sample Hold.



6.7.0 OUTPUT AMPLIFIER

The output from the sample hold was taken directly to the output amplifier. Connected to this amplifier was a second gain and the back off system. Chromatographic peaks were found to be typically between 0.5 and 100 nA. high, while the background current during the window was anything between 0 and 5μA. depending upon the size of the pulses and the specific potentials pulsed between. The sensitivity of the current to voltage converter was determined by the total current, hence often it was found that a small faradaic current had to be detected on top of a large background on a fairly insensitive scale. To overcome this a back off system was devised which could offset the maximum output of every current to voltage converter scale.

Fig 6.9 The Output Amplifier.

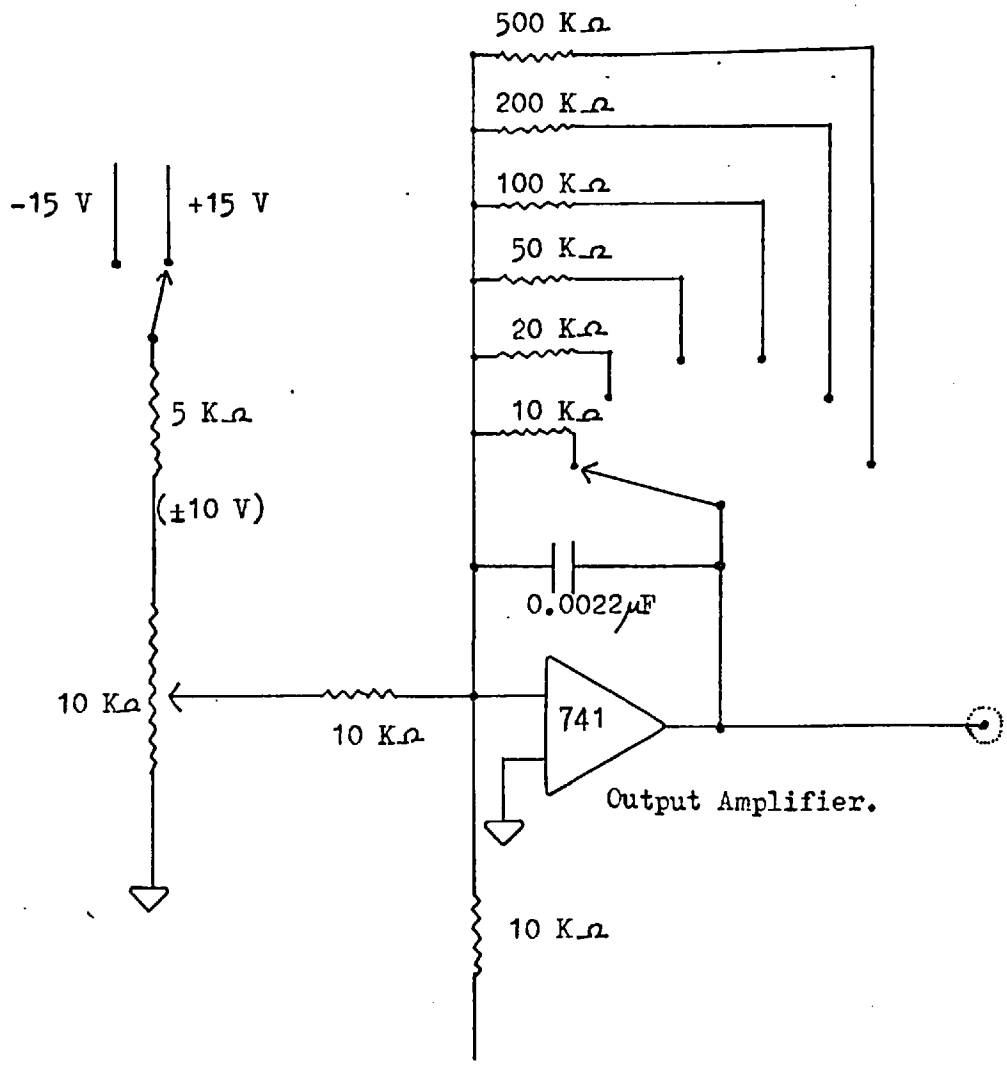


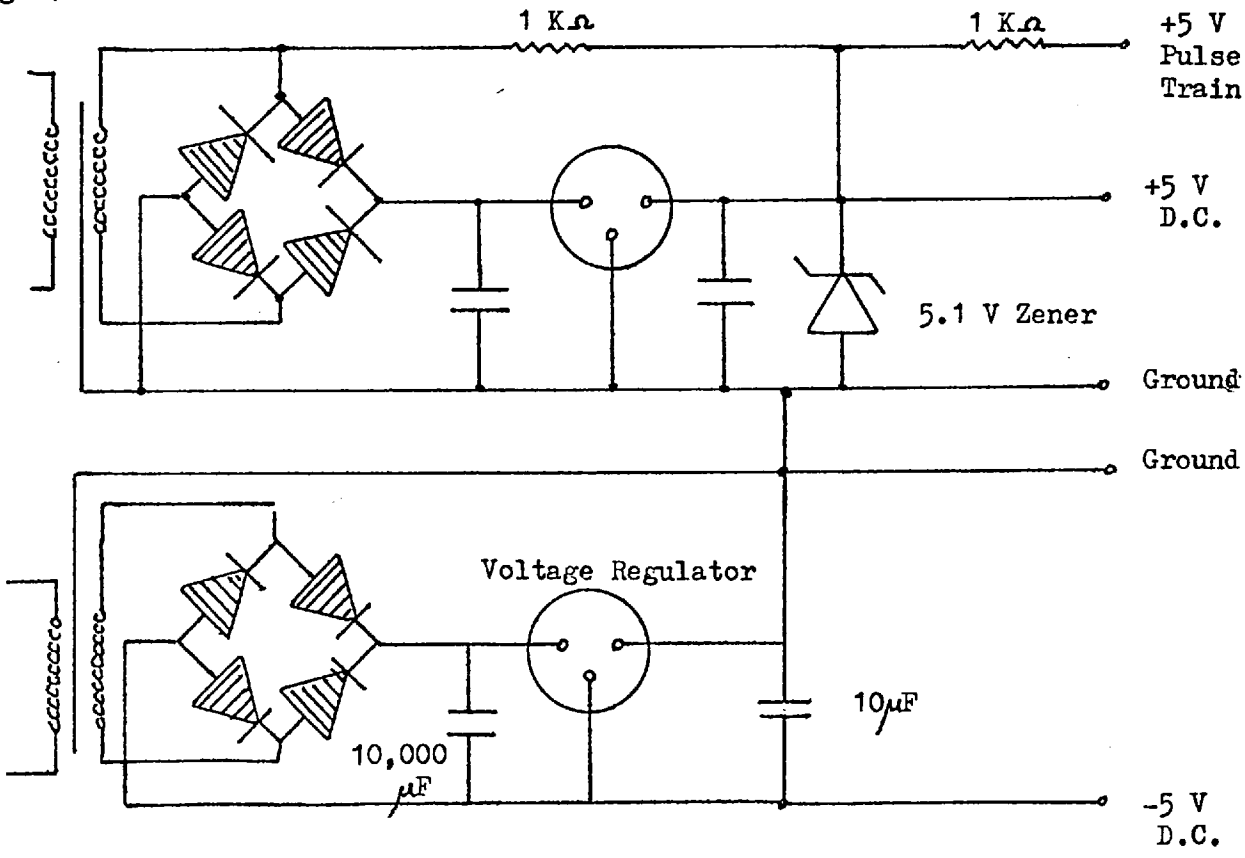
Fig 6.9 shows the system used which was capable of giving ± 10 V. back off for each sensitivity scale. In practice this meant setting the current to voltage converter on the least sensitive scale and backing off to zero. The scale on the current to voltage converter was then gradually increased to the maximum that could be allowed without overloading it while continually offsetting to zero. If the maximum scale was too small for accurate measurement of the chromatographic peaks, the second gain on the output amplifier was used. Where possible however it was found to be best to use the maximum gain on the current to voltage converter instead of using this second gain.

The output from the controller was displayed on a Servoscribe chart recorder.

6.8.0. POWER SUPPLY

The circuit diagram of the 5 V. supply is shown in Fig 6.10.

Fig 6.10



6.9.0 LOGIC

The logic circuits are shown in Fig 6.11. The output of the power supply was fed into a Schmitt Trigger to produce a series of very narrow spikes. This output was fed into a decade counter and a four line output A, B, C, D. produced. This was then fed into a Binary Coded Decimal to Decimal decoder where it was decoded. It was the negative going edges of this output which activated the flip-flops (103). These in turn operated two switches, one which introduced the cleaning pulse (CD4016AE F.E.T. SW), and the other which worked the sample hold (F.E.T. SW 2W3824.).

The various stage outputs are shown in Fig 6.12.

Fig 6.11 Logic

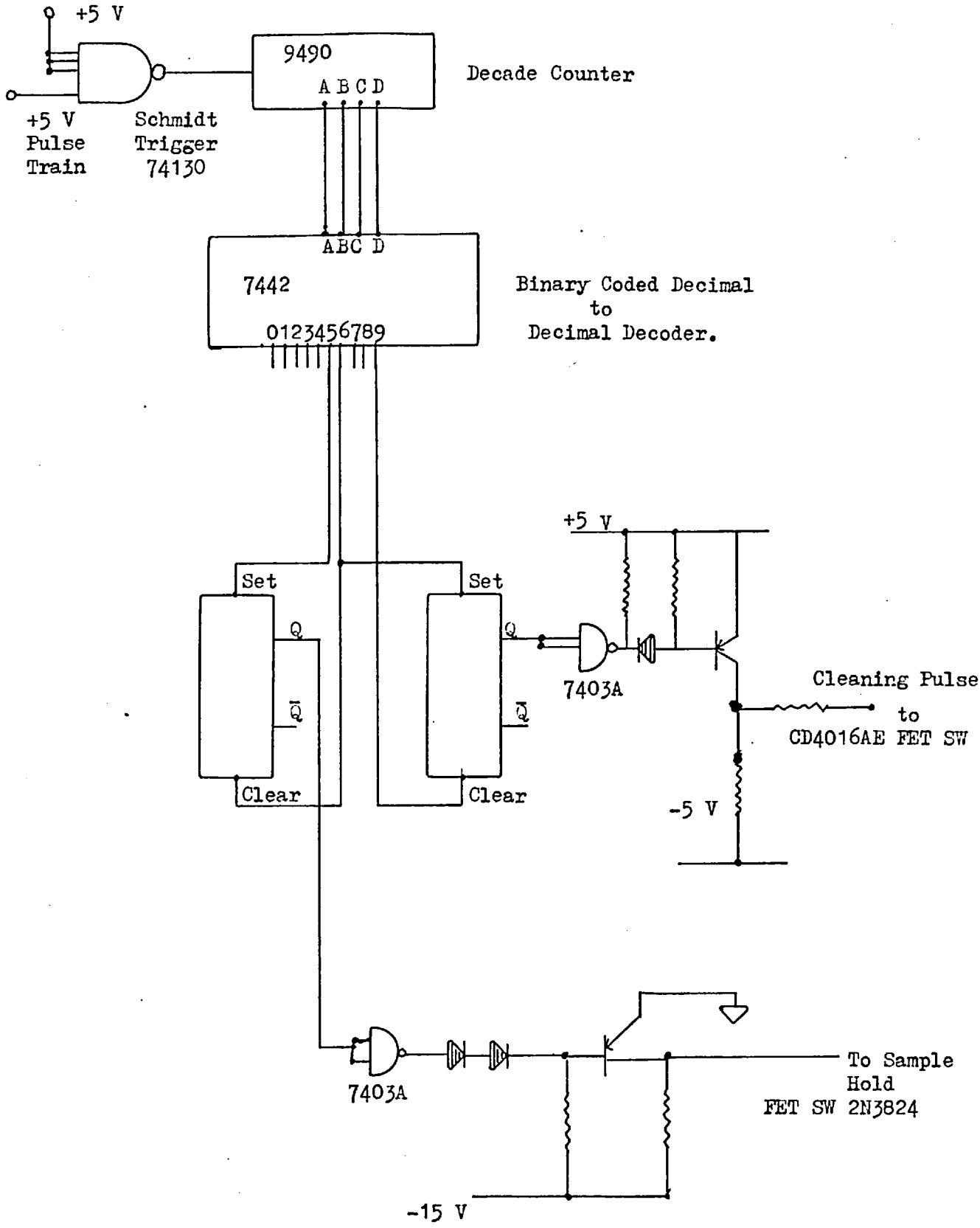
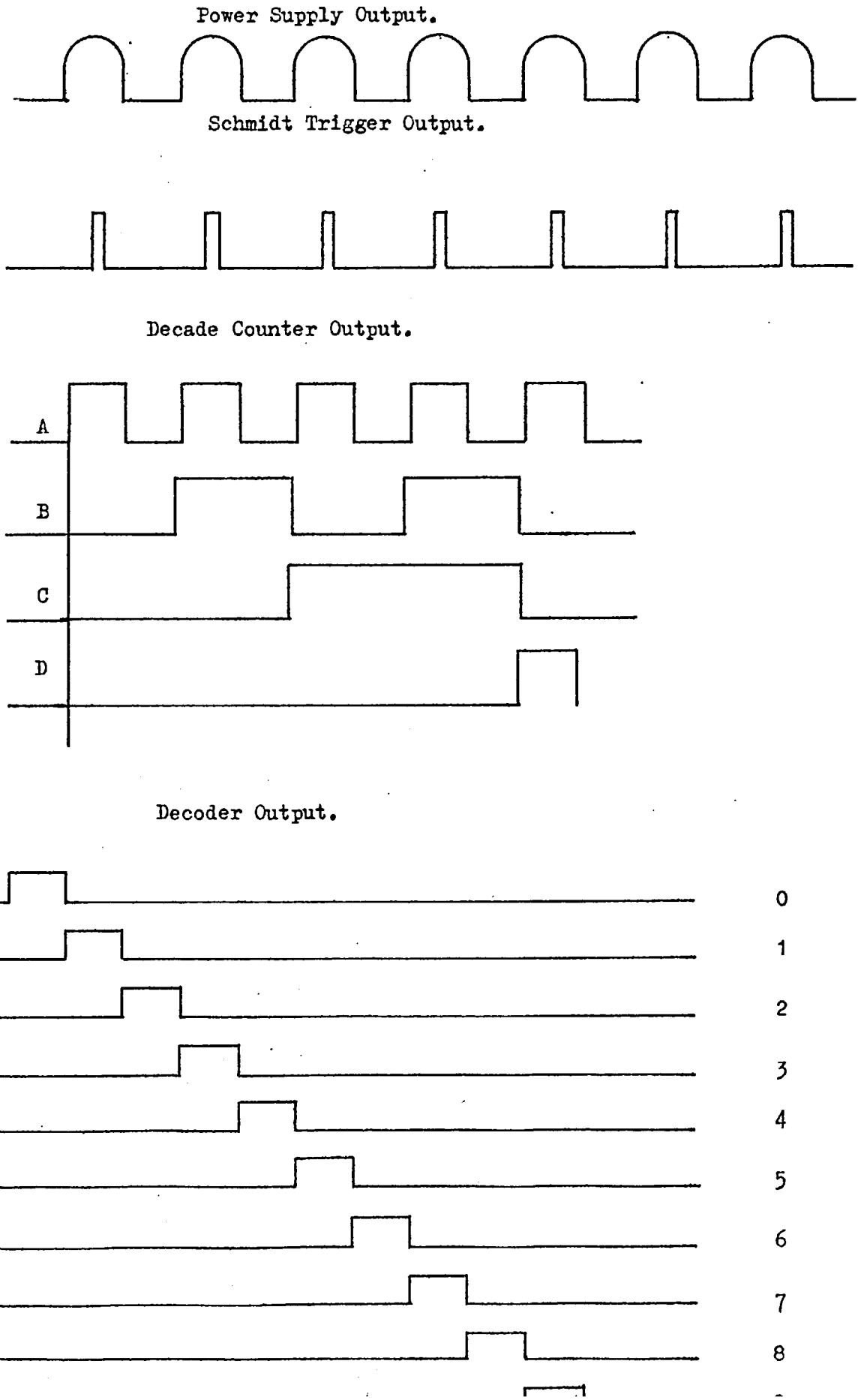


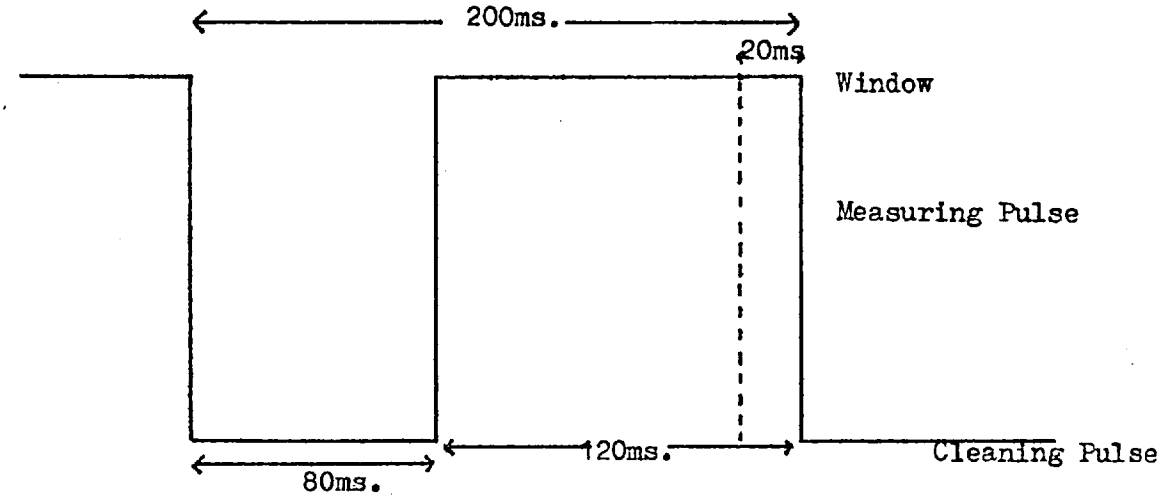
Fig 6.12 Timing



6.10.0 PULSE SHAPE

The circuits described in the previous section were found to be capable of pulse generating and sampling up to the standard necessary for chromatographic detection. Because of the importance of sampling over an integral number of line frequency cycles, the basic pulse train had the following shape.

Fig 6.13

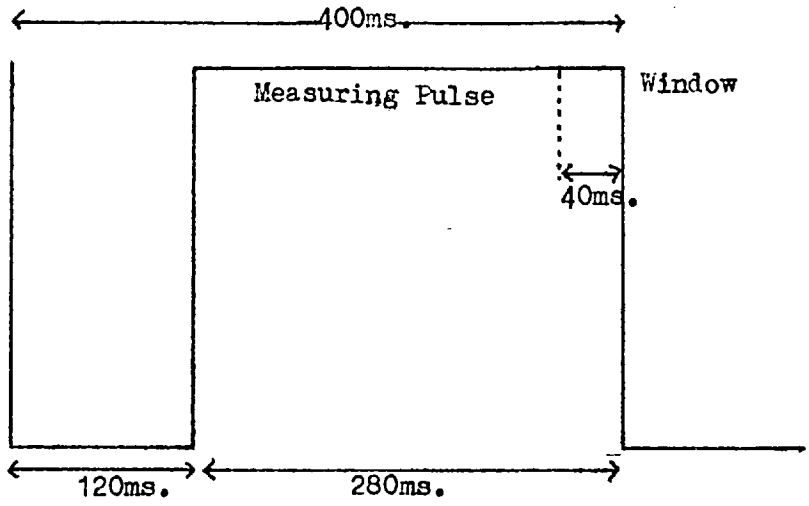


Applied Pulse Train.

The pulse frequency was 10 /s. giving a total wavelength of 200ms. The cleaning pulse was of 80ms. duration which allowed 100ms. for the current to decay before the measuring window. This cycle was origonally used on a poor quality electrode and the background currents during the window were found to be too large to back off sucessfully. The timing of the pulse train was therefore altered to that shown in Fig 6.14

Fig 6.14

Fig 6.14



Applied Pulse Train.

A relatively short cleaning pulse of large dimensions was found to be more compatible with this system than a long shallow one. This was so because the application of short pulses did not disturb the equilibrium of the surface groups as much as the long ones did, thus reducing the steady state current when a continuous pulse was applied. To determine the magnitude of the steady state currents the pulse controller was applied to a chromatographic cell with only eluent flowing and the background currents noted for various pulse heights. The results are shown in Fig 6.15

Pulse	Current
+0.8 to +0.6	-180 nA
+0.8 to +0.4	-100 nA
+0.8 to +0.2	-100 nA
+0.8 to +0.0	-80 nA

Fig 6.15

Eluent:- 0.1 M. KNO_3

The decrease in residual current as the pulse size increased did not indicate that there was a fault with the system. Indeed, the difference in residual currents between +0.8 V. and the succeeding steps did in fact increase as the steps got larger. The important factor however was that the currents measured were absolute currents, all measured with respect to the zero current level (equivalent to a potential of about +0.42 V.). Thus as the cleaning pulse became more negative, the steady state current gradually moved towards the zero current line and thus got smaller.

It can be seen from the results that the size of the steady state current depends not only upon the size of the potential step, but also upon the actual potentials pulsed between. For oxidative detection in aqueous solution a measuring pulse of +0.8 V. is probably the best, and the low currents produced with this potential means that the detector is extremely useful in this range.

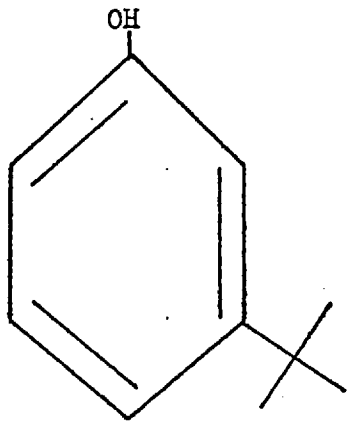
7.1 APPLICATIONS

The detector described in the previous section was tested on a variety of compounds known to adsorb at the electrode surface under their chromatographic conditions. The compounds chosen were all of pharmaceutical interest and were chosen because they were all U.V. active to some extent and as such a comparison could be made.

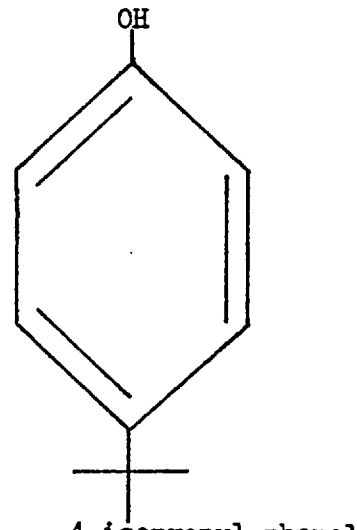
7.2 DETECTION OF ISOPROPYL PHENOLS

Initially the separation of 3-isopropyl phenol and 4-isopropyl phenol was attempted.

Fig 7.1



3-isopropyl phenol

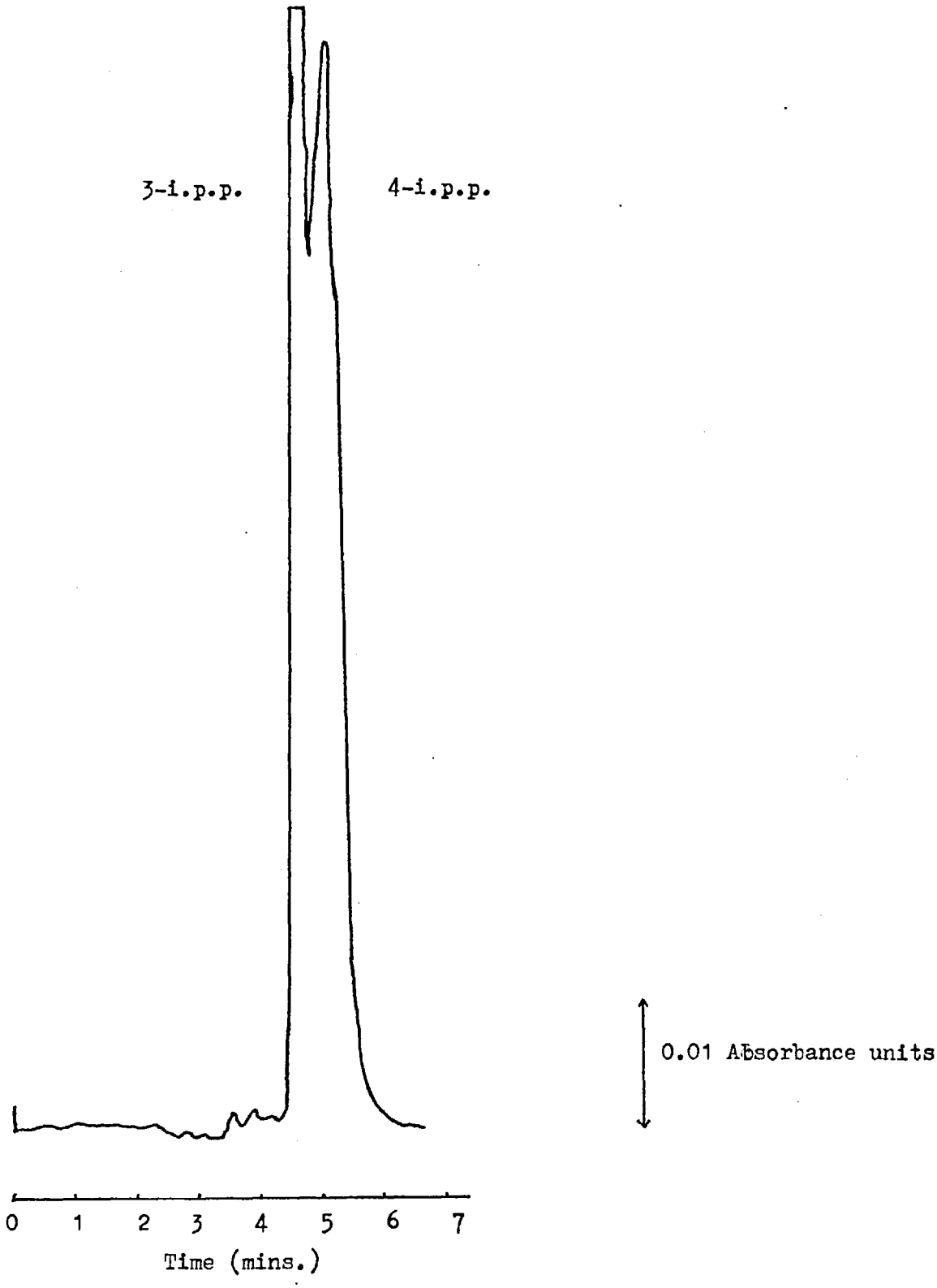


4-isopropyl phenol

A system of Bonded phase chromatography was used, employing a ½m. C-18 Merkosorb column which had been treated with T.C.M.S.. The eluent used was 70% Methanol, 30% Water with a base electrolyte of 0.1 M KNO_3.

The response of a U.V. detector is shown in Fig7.2 and the corresponding response from the electrochemical detector is shown in Fig 7.3.

Fig 7.2 U.V. Detection

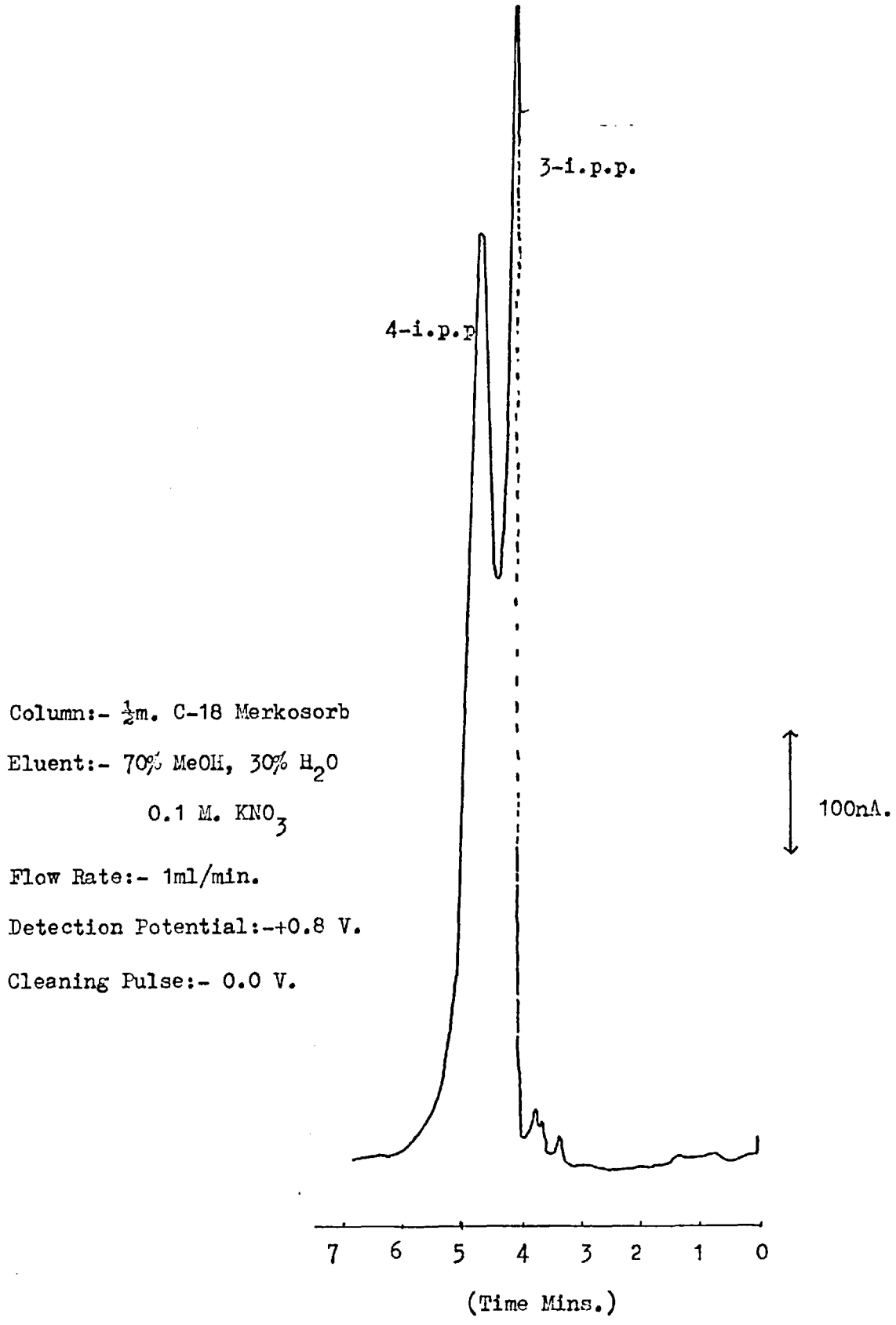


Column ½m. C-18 Merkosorb

Eluent:- 70% MeOH, 30% H_2O, 0.1 M KNO_3.

Flow Rate:- 1ml/min.

Fig 7.3 Electrochemical Detection.



Comparison of the two chromatograms shows a slightly better resolution of the impurity peaks preceeding the main peaks by the electrochemical detector. The sampled points of the pulse mode can be clearly seen on the leading edge of the 3-isopropyl phenol peak, and it is obvious that for this system the sampling rate is sufficiently fast. The sensitivity used on both methods indicates a similar detection limit. i.e. about 150 times less than the actual sample size in both cases. The size of base line noise is similar for both methods.

The main purpose of applying the pulses was to produce reproducible peaks for consecutive injections of the same size sample. This was tested by a series of injections of the phenol mixture. The results are shown in Fig 7.4

The variation in peak heights is due mainly to errors in injection. Although not exactly the same height, a complete absence of any gradual reduction in peak sizes can be seen, even for the small impurity peaks. These results indicate that for this series of compounds the pulse method appears to clean the electrode and produce a system capable of giving reproducible peaks under chromatographic conditions.

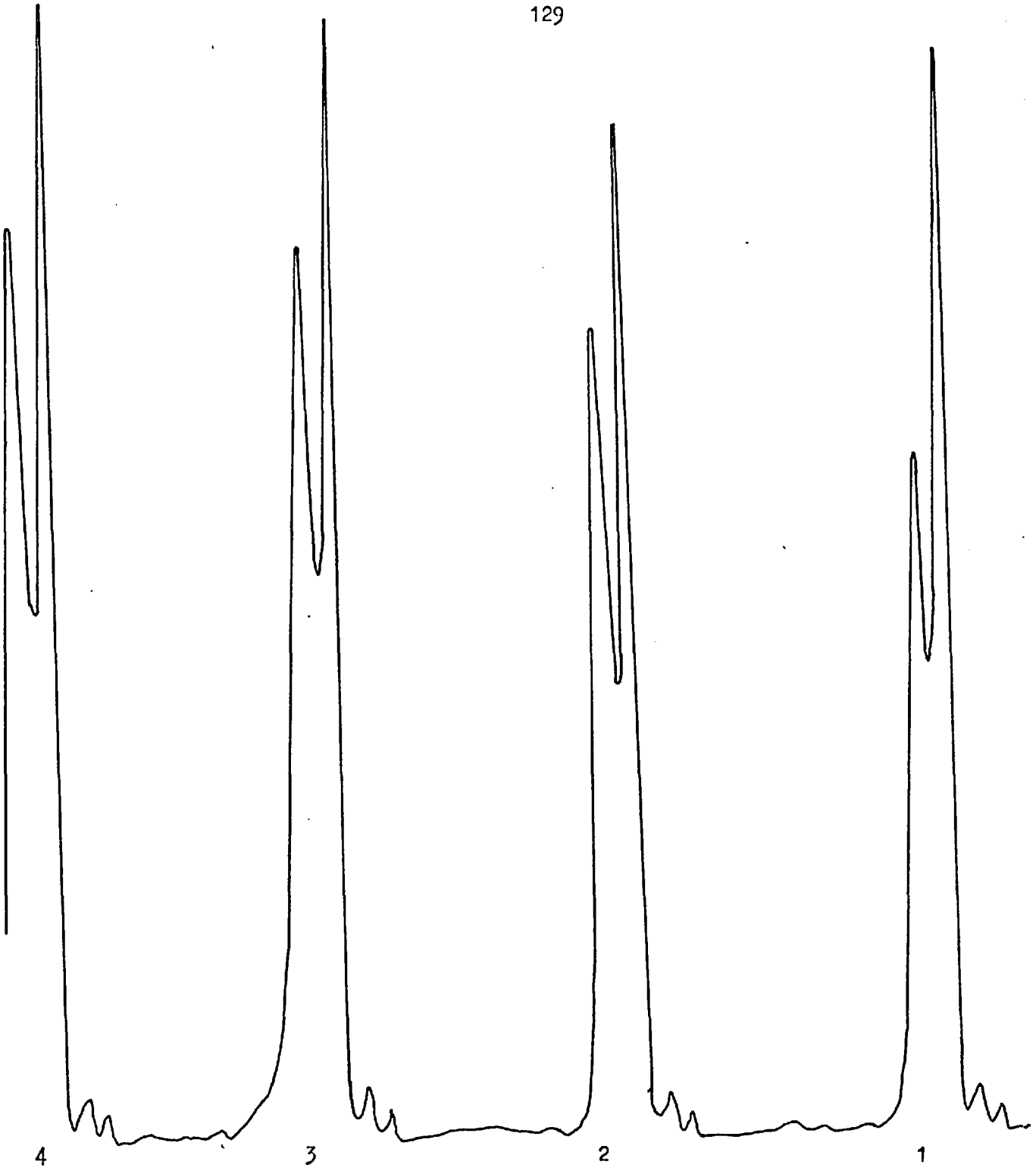


Fig 7.4

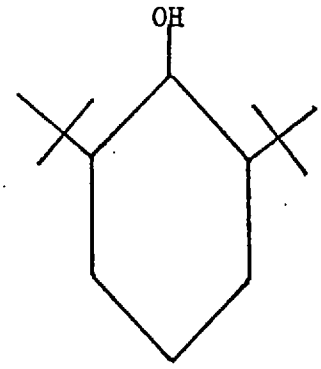
Consecutive injections of a mixture of 3 and 4 isopropyl phenol.

The chromatographic conditions are given in Fig 7.3

7.3 ANALYSIS OF 2,6-DI-ISOPROPYL PHENOL

One of the main uses of liquid chromatography is in detecting low levels of impurities in pharmaceuticals. This application is illustrated in the following separation. Impure 2.6.diisopropyl phenol was chromatographed under the conditions of the previous separation.

Fig 7.5



2.6-diisopropyl phenol

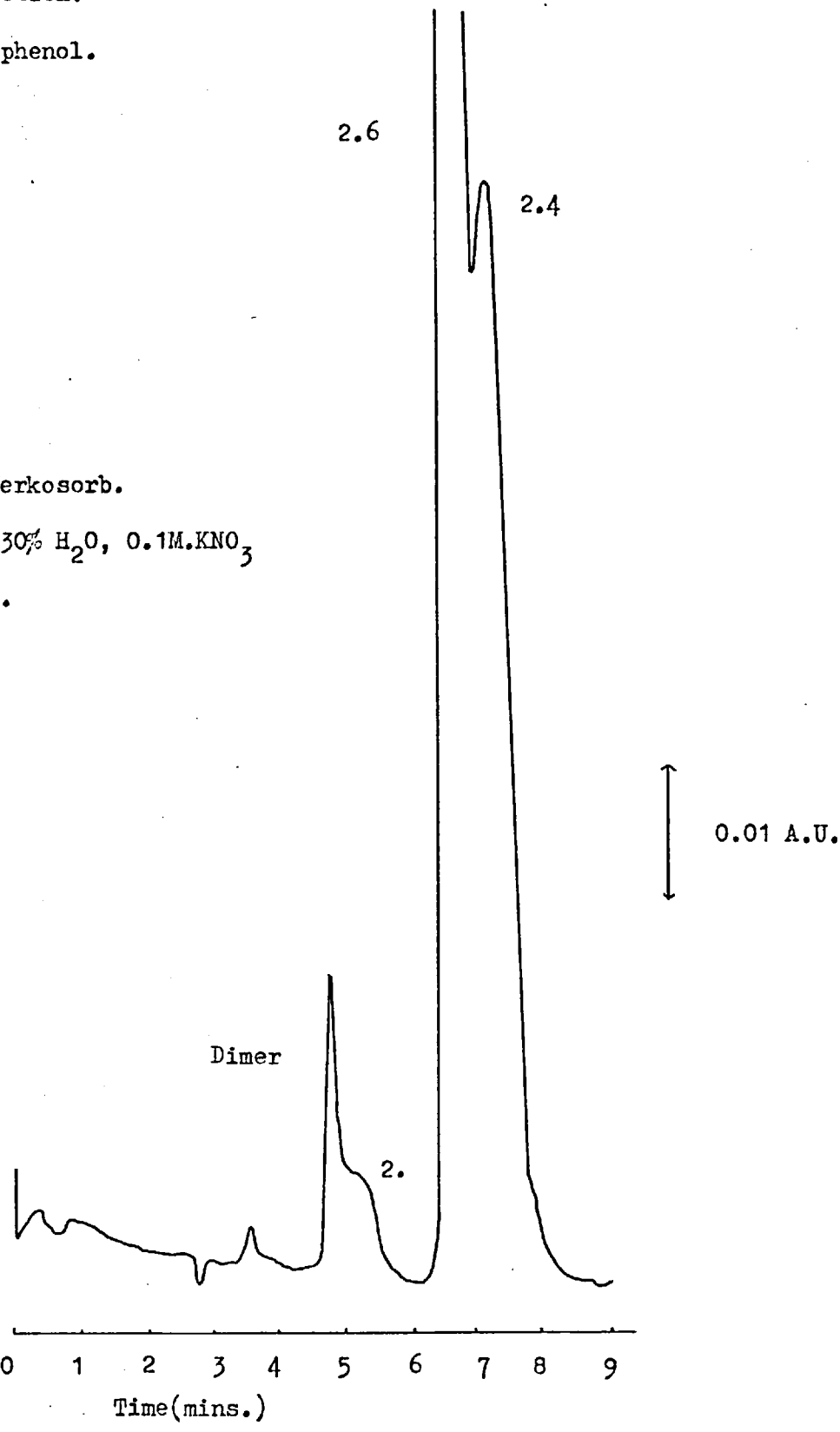
The U.V. chromatogram was included again for comparison. See Fig 7.6 and Fig 7.7.

Again comparable signals were obtained. Many of the impurity peaks were better resolved in the electrochemical case, especially those peaks corresponding to the dimer and the 2.4 isomer.

In this case, the electrochemical detector appears to be slightly superior to the U.V. one.

Fig 7.6 U.V. Detection.

2.6-isopropyl phenol.



Column:- ½m. C-18 Merkosorb.

Eluent:- 70% MeOH, 30% H_2O, 0.1M.KNO_3

Flow Rate:- 1ml/min.

Sample Size:- 5µl

Fig 7.7

Electrochemical Detection.

2.6-diisopropyl phenol.

Column:- ½m. C-18 Merkosorb.

Eluent:- 70% MeCH, 30% H_2O,

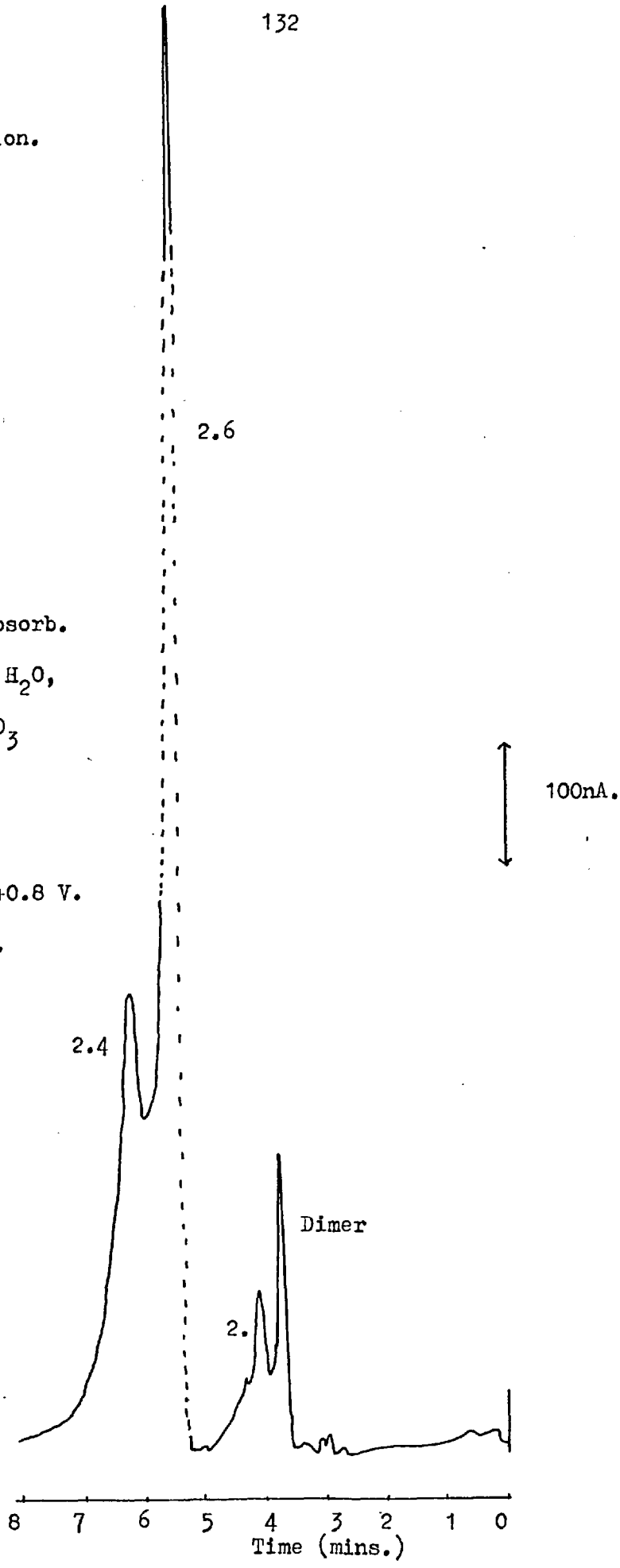
0.1 M. KNO_3

Flow Rate:- 1ml/min.

Sample Size:- 5 l.

Detection Potential:- +0.8 V.

Cleaning Pulse:- 0.0 V.



8.1 POTENTIAL LIMITS

The use of the voltammetric method for chromatographic detection is limited to those eluents which can accommodate a supporting electrolyte at a concentration of around 0.1 M.. This means that the technique is most useful in the realms of reversed phase and bonded phase chromatography where the eluents tend to be aqueous or aqueous mixtures. The potential range of the electrode in these mixtures is of importance since the breakdown of base electrolyte could interfere with the faradaic process from the sample and hence obscure the sample peaks. Setting the working potential either too far positive or too far negative on these break down curves will produce a large background current which could prove difficult to back off.

In Fig 8.1 are shown the potential limits for various aqueous alcohol mixtures.

Eluent	Potential Cutoff +ve.	-ve.
Water	+1.0	-0.98
90% Water, 10% Methanol	+1.05	-0.98
70% Water 30% Methanol	+1.05	-0.98
50% Water 50% Methanol	+1.05	-0.98
90% Water 10% Ethanol	+1.00	-0.95
70% Water 30% Ethanol	+1.04	-1.00
50% Water 50% Ethanol	+1.05	-1.08
90% Water 10% Isopropanol	+1.00	-0.95
70% Water 30% Isopropanol	+1.00	-1.00
50% Water 50% Isopropanol	+1.00	-1.05

Electrolyte= 0.1M KNO_3 , Pt counter electrode, S.C.E. reference.

These were produced by scanning in both the positive and negative directions from 0.0 V. v. S.C.E. in a deoxygenated solution. The cutoff voltage was taken as the potential where the residual current became larger than 1μA..

If the detector is always used within these limits, then the residual current should always be small enough for the back off system to prove adequate.

Another very common solvent for liquid chromatography is acetonitrile. It is possible to detect electrochemically in this solvent by adding tetra-ethyl-ammonium perchlorate as base electrolyte. The electrochemical range in this solvent is much better than in aqueous media, allowing a wider range of compounds to be detected. The reported ranges at the platinum electrode are +1.8 to -1.5 V. in sodium perchlorate (100), and +2.5 to -3.0 V. in lithium perchlorate (101). The proposed anodic reaction is the formation of perchlorate radicals followed by reaction with the solvent.

$$ClO_4^- \longrightarrow .ClO_4 + e^-$$

$$.ClO_4 + CH_3CN \longrightarrow H^+ + ClO_4^- + .CH_2CN$$

$$2.CH_2CN \longrightarrow NCCH_2\text{—}CH_2CN$$

The range reported for carbon paste was +1.1 to -0.7 V. v. S.C.E.(103). In all these case the acetonitrile was water free, as the presence of water affects the anodic cutoff. To obtain the completely water free eluent it is necessary to distil from phosphorous pentoxide.

As only commercially available acetonitrile is used in chromatography the range at glassy carbon was determined using this spectroscopic grade. The results are shown in Fig 8.2.

Eluent	Potential Cutoff	
	+ve.	-ve.
Acetonitrile	+1.5	-1.45
70% Acetonitrile 30% Water	+1.05	-1.42
50% Acetonitrile 50% Water	+1.05	-1.40
37% Acetonitrile 23% Water	+1.05	-1.38

The same conditions for determining the cutoff were used as before.

Base electrolyte= 0.1M T.E.A.P.

Silver wire reference

Platinum counter.

9.1 CONCLUSION

The results of the last chapter indicate that the application of pulses is to some extent sucessful in keeping the glassy carbon surface free from adsorbed material during the continuous monitoring of chromatographic eluent. It cannot be said at this time however that the technique has solved all the problems associated with the voltammetric approach to liquid chromatographic detection. A complete understanding of the processes involved in adsorption at electrode surfaces is still far from being realised. In systems where the product of the electrode reaction is itself electroactive, the pulse mode should have a reasonable chance of being sucessful. On the other hand, for reactions which are totally irreversible due to the product being electroinactive or even non-conducting, the controller will have a much more limited application. The fact that non-conducting films are formed on electrode surfaces has been acknowledged in the last few years. In general an electrochemical method would appear to have no chance of sucess in removing them. However, the films are unlikely to cover the whole electrode surface and it is possible that by applying a very large cleaning pulse such that background electrolyte breakdown is produced, the gaseous products formed by this reaction would disrupt the films on the surface and allow them to be washed away by the "wall jet". This is one area of electrode behavior that will have to be further investigated. It is possible that the background noise level produced by this method will raise the detection limit, but if the cleaning pulse is of short duration, the flowing stream should have time to remove the products of this pulse (gas and film) before the measuring window, and thus keep the noise to a minimum. When doing oxidative detection a large negative cleaning pulse should not harm the electrode in any way, on the other hand the positive cleaning pulse required for reductive detection may oxidise the electrode surface and thus mean that the technique is less useful in this range. It has

been reported that a flowing stream itself is capable of washing off adsorbed product (43). This has not been found to be the case with this cell design and detector. Furthermore, the necessity of mechanically rubbing with chloroform to clean the electrode indicates that this is highly unlikely with any cell design.

The wall jet cell probably delivers as much electroactive material to the surface of the electrode as any other method at present known. The optimisation of the cell parameters has taken the detection limit of the system down to the point where this limit is determined by the electrical noise as opposed to cell noise. Any great decrease in the detection limit will only come about once this electrical noise has been reduced. The two main problems associated with the use of pulses at glassy carbon (high residual currents and charging current decay) have been overcome by suitable choice of electrode and electronic technique. to such an extent that they no longer affect the detection limits of the system. The known problems with the existing system have been overcome or bypassed, and the detector is now at the stage where it can be applied easily to real systems.

The unsuitability of the D.M.E. and A.C. techniques has been established from the work of chapters 3 and 5, due mainly to the high detection limits associated with both methods. The use of differential pulse has also been suggested (43), but because of the small amplitude of the pulses involved, this mode is accompanied by similar adsorption to the D.C. process. The A.C. method appears to have no place in chromatographic detection at all, but use of the D.M.E. may be considered for specialised projects especially where non-conducting films are formed which cannot be removed by the use of the pulse mode. There will however be a corresponding increase in the detection limit its use should not be considered for routine analysis.

Although many more applications need to be done before the detector in the pulse mode can be said to be completely sucessful, the indications

are that the improvements produced to date are such that the detector, at least for a number of compounds, is capable of use in routine analysis. Its lower detection limit is comparable with that of the most favourable U.V. cases (nanogram) and it has the added advantage that its sensitivity does not vary so much from sample to sample. Its degree of reproducibility has not been investigated thoroughly enough as yet for any definite claims to be made in this area and to this end, further research should be carried out.

If however the trends reported in this work are continued, the voltammetric detector should prove to be a necessary complement to the U.V. detector.

REFERENCES

1. Twsett M., Ber. Deut. Botan. Ges. 24, 316, (1906).
2. Twsett M., Ber. Deut. Botan. Ges. 24, 384, (1906).
3. Izmailov N. A., Shraiber M.S., Farmatsiya 3, (1938).
4. Stahl E., Pharmazie 11, 633, (1956).
5. Stahl E., Chemiker Ztg., 82, 323, (1958).
6. Martin A.J.P., Synge R.L.M., Biochem. J. 35, 91, 1358, (1941).
7. Snyder L.R., J. Chrom. Sci. 8, 338, (1970).
8. Bombaugh K.J., et. al. J. Chrom. Sci. 8, 657, (1970).
9. Scott C.G., Bommer P., J. Chrom. Sci. 8, 446, (1970).
10. Horgan D.F., Little J.N., J. Chrom. Sci. 10(2), 76, (1972).
11. Knejci M., Pospisilova N., J. Chrom. [illegible] 73(1), 105, (1972).
12. Bonnelycke B.E., J. Chrom. 61(2), 322, (1971).
13. Chilcote D.D., Scott C.D., Anal. Chem. 45(4), 721, (1973).
14. Willard H.H., Merrill L.L., Dean J.A.,
    Instrumental Methods of Analysis. 4th Ed. D.Van Nostrand Co,Inc.,(1965).
15. Huber J.F.K., J. Chrom. Sci. 7, 172, (1969).
16. Conlon R.D., Anal. Chem. 41, 107A, (1969).
17. Munk M.N., J. Chrom Sci. 8, 491, (1970).
18. Veening H., J. Chem. Ed. 47, A549, A675, A749, (1970).
19. Knox J., Lecture Gas Chromatography, Discussion Group Dec. 1974.
20. Young T.E., Maggs R.J., Anal. Chem. Acta., 38, 105, (1967).
21. Karmen A., Anal. Chem. 38, 286, (1966).
22. Maggs R.J., Chromatographia 1, 43, (1968).
23. Scott R.P.W., Lawrence J.G., J. Chrom. Sci. 8, 65, (1970).
24. Hupe K.P., Bayer E., J. Gas. Chrom. 5, 197, (1967).
25. Kemula W., Roczniki Chem. 26, 281, (1952).
26. Kemula W., Chem. Anal. 13, 1013, (1968).
27. Kemula W., Pure and Applied Chem. 25,(4), 763, (1971).
28. Borkowska Z., Sybilska D., Bahr B., Roczniki Chem. 45, 269, (1971).

29. Koen J.G., Huber J.F.K., Poppe H., den Boef G.
J. Chrom. Sci. 8, 192, (1970).

30. Joynes P.L., Maggs R.J., J. Chrom. Sci. 8, 427, (1970).

31. Takata Y., Muto G., Anal. Chem. 45, 11, 1864, (1973).

32. Kissinger P.T., Refshauge C., Dreiling R., Adams R.N.,
Anal. Letters. 6(5), 465, (1973).

33. Johnson D.C., Larochelle J., Talanta 20, 959, (1973).

34. Kihara S., J. Electroanal. Chem. 45, 31, (1973).

35. Kihara S., J. Electroanal. Chem. 45, 45, (1973).

36. Yespalec R., Hana D., J. Chrom. 32, 728, (1968).

37. O'Laughlin J.W., Banks C.V., Anal. Chem. 36, 1222, (1964).

38. Kambara T., Tachikaiva T., J. Chrom. 32, 728, (1968).

39. Chromatronix Bulletin C.H.R. 1270.

40. Baumann F., Blaedel W.J., Anal. Chem. 28, 2, (1956).

41. Polesuk J., Howery D.G., J. Chrom. Sci. 11(5), 226, (1973).

42. Heyrovsky J., Chem. Listy., 16, 256, (1922).

43. Little C.J., Ph.D. Thesis 1974.

44. Adams R.N., Electrochemistry at Solid Electrodes.
M. Dekker Inc. New York.

45. Rogers L.B., Miller H.H., Goodrich R.B., Stahney A.F.
Anal. Chem. 21, 777, (1949).

46. Kolthoff I.M., Jordan J., J. Amer. Chem. Soc. 74, 382, (1952).

47. Tsukamoto T., Kambara T., Tachi I.,
Proc. 1st Polarog. Congr. Prague. 1951 p.540.

48. Lord S.S., Rogers L.B., Anal. Chem. 26, 284, (1954).

49. Muller O.H., J. Amer. Chem. Soc. 69, 2992, (1947).

50. Baumann F., Shain I., Anal. Chem. 29, 303, (1957).

51. Cahn R.W., Harris B., Nature 221, 132, (1969).

52. Morris J.B., Schempf J.M., Anal. Chem. 31, 286, (1959).

53. Elving P.J., Smith D.L., Anal, Chem. 32, 1849, (1960)

54. Lindquist P.J., J. Electroanal. Chem. 45, 1006 (1973).

55. Lindquist P.J., J. Electroanal. Chem. 52, 37, (1974).

56. Biscoe J., Warren B.E., J. Appl. Phys. 13, 364, (1942).

57. Katz R.N., Gazzara C.P., J. Material Science. 3, 61, (1968).

58. Thomas J.M., Roscoe C.
Proc. 2nd Conf. on Industrial Carbon and Graphite. 249
(Land. Soc. Chem. Ind. 1966).

59. Panzer R.E., Elving P.J., J. Electrochem. Soc. 119, 864, (1972).

60. Beilby A.L., Brooks W., Lawrence G.L., Anal. Chem. 36, 22, (1964).

61. Miller F.J., ZiHel H.E., Anal. Chem. 35, 1866, (1963).

62. Chuang L., Fried I., Elving P.J., Anal. Chem. 36, 2426, (1964).

63. Cowlard F.C., Lewis J.C., J. Material Sci. 2, 507, (1967).

64. Nernst W., Z. Physik. Chem. 47, 52, (1904).

65. Newman J., Advances in Electrochemistry and Electrochemical
Engineering. Ed. C.W. Tobias. 1963.

66. Newman J., Electrochemical Systems.
Prentice Hall Englewood Cliffs, N.J. 1973

67. Bazan J.C., Arvia A.J., Electrochim Acta. 9, 17, 667, (1964).

68. Levich B., Acta. Physicochim. U.R.S.S. 17, 257, (1942).

69. Glauert M.B., J. Fluid Mechanics. 1, 625, (1956).

70. Matsuda H., Yamada J., Electroanal. and Interfacial Chem. 44, 189, (1973).

71. Kirkland J.J., (Ed). Modern Practice of Liquid Chromatography.
Wiley Interscience N.Y. 1971.

72. Bailey F., Brittain P.N., J. Chrom. 83, 431,(1973)

73. Kohlraush F., Ann. Physikic. Chemie. Series 5, 28, (Poggendorff's Ann.)
58, 37, (1873).

74. Muller R.H., German R.L., Droz M.E., Petras J.,
Ind. Eng. Chem. (Anal. Ed.) 10, 399, (1938).

75. Breyer B., Gutmann F., Trans. Faraday Soc. 42, 645, (1946).

76. Breyer B., Gutmann F., Trans. Faraday Soc. 43, 785, (1947).

77. Breyer B., Gutmann F., Discussions Faraday Soc. 1, 19, (1947).

78. Grahame D.C., J. Amer. Chem. Soc. 63, 1207, (1941).

79. Randles J.E.B., Discussions Faraday Soc. 1, 11, 47, (1947).

80. Reinmuth W.H., Anal. Chem. 36, 211R, (1964).

81. Breyer B., Bauer H.H., Alternating Current Polarography and Tensammetry. Chemical Analysis Series, 13. Interscience N.Y. 1963.

82. Smith D.E., Electroanalytical Chemistry, Vol 1. Bard A.J. Ed. M. Dekker Inc. N.Y. 1966 Chapter 1.

83. Smith D.E., Bauer H.H., CRC.Reviews in Analytical Chemistry. 247. (1971).

84. Delahay P., Advances in Electrochemistry and Electrochemical Engineering. 1, Chapter 5, Wiley Interscience N.Y. 1961.

85. Smith D.E. Anal. Chem. 35, 1811, (1963).

86. Barker G.C., Anal. Chim. Acta. 18, 118, (1958).

87. Oldham K.B., J. Electrochem. Soc. 107, 766, (1960).

88. Matsuda H., Delahay P., J. Amer. Chem. Soc. 82, 1547, (1960).

89. Senda M., Delahay P., J. Amer. Chem. Soc. 83, 3763, (1961).

90. Sevcik A., Collection Czeck. Chem. Commum. 13, 349, (1948).

91. Kemula W., Kublik Z., Nature 182, 793, (1958).

92. Kemula W., Advan. Polarog. Proc. Intern. Polarog. Congr. 2nd Cambridge. Engl. 1959, 1, 105, (1960).

93. Wopschall R.H., Shain I., Anal.Chem. 39, 13, 1514, (1967).

94. Nicholson R.S., Shain I., Anal. Chem. 36, 4, 706, (1964).

95. Matsuda H., Z. Electrochem. 61 489, (1957).

96. Blaedel W.J., Jenkins R.A., Anal. Chem. 46, 13, 1952, (1974).

97. Panzer R.E., Elving P.J., Electrochemica Acta. 20, 635, (1975).

98. Epstein B.D., Dalle-Molle E., Mattson J.A., Carbon 9, 609, (1971).

99. Panzer R.E., Elving P.J., J. Electrochem. Soc. 7, 119, 864, (1972).

100. Kolthoff I.M., Coetzee J.F., J. Amer. Chem. Soc. 79, 870, (1957).

101. Popov A.I., Geshe D.H., J. Amer Chem. Soc. 80, 1340, (1958).

102. Schmidt H., Noack J., Anorg. Allgem. Chem. 296, 262, (1958).

103. Berger T.A., Ph.D. Thesis 1975.

104. Booman G.L., Holbrook W.B., Anal. Chem. 35, 1793, (1963).

105. Brown E.R., Smith D.E., Booman G.L., Anal. Chem. 40, 1411, (1968).

106. Stilman R., Ma T.S., Mikrochimica Acta. 4-6 491 (1973).

107. MacDonald A., Duke P.D., J. Chrom. 83, 331, (1973).

108. Molnar J., Magy. Kem. Foly. 68, 504, (1962).

109. Blaedal W.J., Strohl J.H., Anal. Chem. 36, 2008,(1964).

110. Strohl J.H., Polutanovich T.A., Anal. Letters 2, 423, (1969).

111. Bamberger R.J., Strohl J.H., Anal. Chem. 41, 1450, (1969).

112. Roe D.K., Anal. Chem. 36, 2371, (1964).

113. Taylor L.R., Johnson D.C. Anal. Chem. 46, 262, (1974).

114. Fleet B., Keliher P.N., Kirkbright G.F., Pickford C.J., Analyst 94, 847, (1968).

115. Matsumoto T., Musanori S., Hirayama S., Bull. Chem. Soc. (Japan) 46, 369, (1969).

116. Eldridge P., Fleet B., Unpublished

117. Buchanan E.B., Bacon J.R., Anal. Chem. 39, 615, (1967).

118. Knox J., Lecture Gas Chromatography, Discussion Group, Dec. 1974.