THE USE OF ELECTROCHEMICAL DETECTORS FOR HIGH PRESSURE LIQUID CHROMATOGRAPHY

by

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ABSTRACT

The history and development of the Chromatopolarographic Method is traced through from the initial work of Kemula to the present day and covers a wide field of Electrochemical Detection Methods appropriate to flowing stream analysis.

In particular, A.C. and Pulse methods are described together with their applicability to detection in High Pressure Liquid Chromatography (H.P.L.C.).

A range of detector designs are described, incorporating the conventional D.M.E. and several modifications of it and a range of solid electrodes. Of the latter, glassy carbon is shown to be the most suitable electrode material especially when used in conjunction with the Wall Jet hydrodynamic principle.

A detector incorporating the Wall Jet Electrode is described together with its application to real chromatographic systems. A major part of the application section concerns reagent addition to the chromatographic eluent in order to add supporting electrolyte or to form derivatives of the eluting species. The method could equally well be used for colourimetric detection.

ACKNOWLEDGEMENTS

The work described in this thesis was carried out in the Chemistry Department of Imperial College between October, 1971 and September, 1973 and is entirely original except where due reference is made.

I would like to thank Professor T.S. West for placing at my disposal the facilities of the Analytical Chemistry Department and in particular, I am indebted to my immediate supervisor, Dr. B. Fleet, for his help, advice and guidance throughout the entire research programme and to Dr. R.D. Jee (School of Pharmacy) for valuable assistance with the design of the electronic circuits described herein. The initiation of the phase angle detector must be ascribed to him.

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INTRODUCTION

1.1 Chromatopolarography: an historical appraisal.

Since the discovery of "POLAROGRAPHY" by Heyrovsky in 1922 (1) the analytical applications of electrolysis at dropping mercury and solid electrodes has progressed to a state of eminence as a qualitative and quantitative analytical method.

Early in the development of this technique, Kemula appreciated the difficulties of working with mixtures of compounds that were reduced or oxidised at similar potentials. This prompted him to develop the "chromatopolarographic" approach which involved the separation of the components of the mixture by liquid chromatography followed by a polarographic detection of the eluant (2,3,4). The experimental set up is shown in Fig. 1.

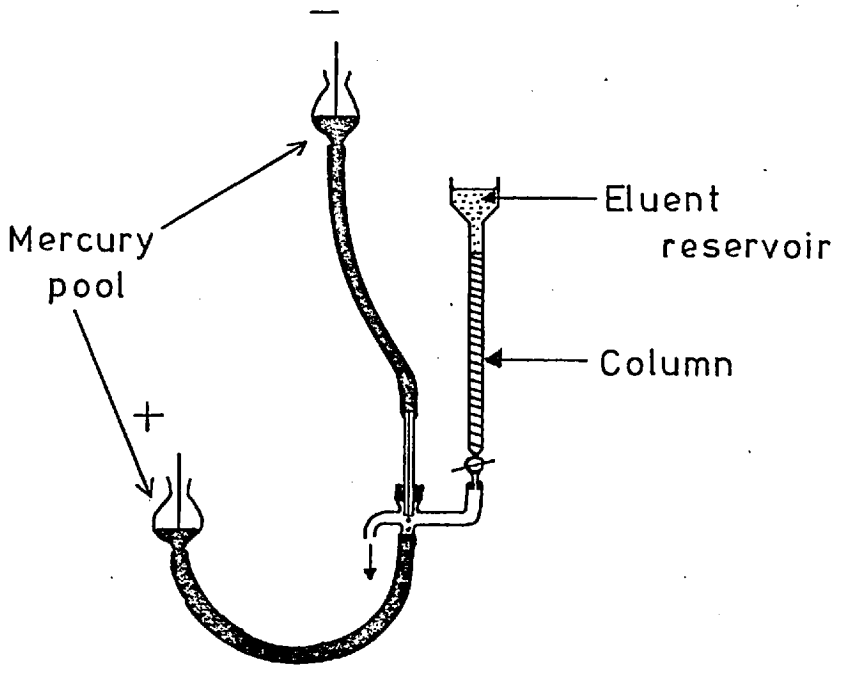


Figure 1. Chromatopolarographic apparatus as used by Kemula (3)

One of the requirements of the polarographic method is that the solution should be made electrically conducting by the addition of a polarographically inactive "supporting electrolyte". This is no problem in aqueous solution where several ionisable species have been used, preferably to form buffer solutions. For example, mineral acids, phosphate, citrate and borate buffers and strong alkalis give a useful pH range of about 0-14 in which many polarographic measurements can be made (5,6,15,16).

Table 1 indicates the various liquid chromatographic techniques which, theoretically, could be used with polarographic detection.

TABLE 1

Definition of the various liquid chromatographic procedures

Fixed Bed	Mobile Phase Composition	Type of Chromatography	Abbreviation
Solid	Aqueous and/or Organic	Liquid Solid	L.S.C.
Solid Gel	Aqueous and/or Organic	Gel Permeation	G.P.C.
Anion Exchanger	Usually aqueous	Ion Exchange	A.X.C.
Cation Exchanger	Usually aqueous	Ion Exchange	C.X.C.
Solid + Polar Liquid	Organic (non-polar)	Liquid Liquid	L.L.C.
Solid + Non Polar Liquid	Aqueous and/or Organic (polar)	Reversed phase Liquid Liquid	r.L.L.C.

In practice, however, those techniques employing aqueous and or polar organic mobile phases are the easiest to use and in conjunction with a d.c. polarographic detection system, form the bulk of Kemula's applications.

Three chromatographic procedures have been used extensively,

(a) Reversed phase liquid liquid chromatography (r.L.L.C.), (b) Ion exchange chromatography (particularly cation exchange chromatography), and (c) Liquid solid chromatography (L.S.C.).

(a) Reversed phase liquid liquid chromatography

In order to immobilise the non-polar organic solvents used as stationary phases in r.L.L.C., they are absorbed into powdered rubber (3,7,8). The eluent used invariably contains the supporting electrolyte, which it is claimed, improves the peak shapes resulting from the chromatographic separations.

Of particular interest, in this context, is the use of acidic and basic electrolytes to control the pH of the eluent (9), resulting in the successful separation of nitroanilines (10), chloronitrobenzenes (10), nitrophenols (11), nitrobenzoic acids (8), nitrofuraldoximes (12), and the isomers of D.D.T. (13).

The separation of the nitrobenzoic acids, Figure 2, presents a classic example of a separation which would benefit from one of the modern innovations in chromatography, "Gradient Elution" (14).

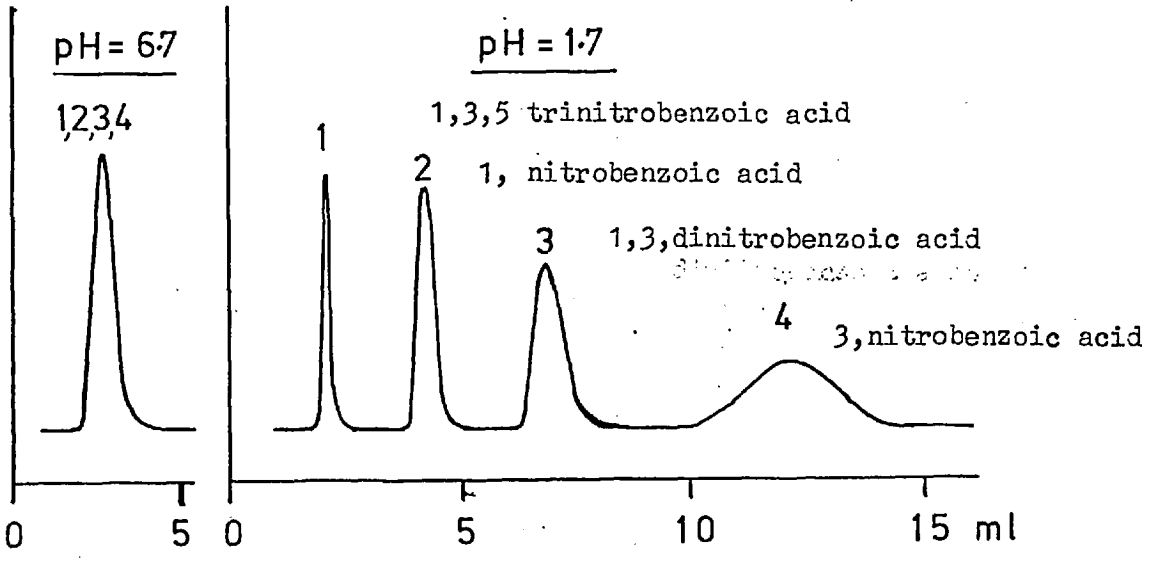


Figure 2. Effect of pH on the separation of nitrobenzoic acids by partition chromatography.

This is closely analogous to temperature programming in gas chromatography and is generally a better technique than either temperature or flow programming for controlling capacity ratios in liquid chromatography. Using this technique, a starting pH of about 1.7 would ensure complete separation of all four compounds shown. By gradually increasing the pH of the eluent, it should be possible to obtain complete separation, sharp peaks and short retention volumes.

(b) Ion Exchange Chromatography

This technique, again, enables the supporting electrolyte to be included in the mobile phase. Using various concentrations of ammonium sulphate (up to 3 Molar) in aqueous solution, successful separations of nitroalcohols, nitrobenzoic acids and nitroalkanes were monitored chromatopolarographically (11).

(c) Liquid Solid Chromatography

To overcome the problems of tailing with conventional solid supports such as silica and alumina, Kemula resorted to the use of clathrate-forming salts of the $Ni(SCN)_2$ (γ picoline)$_4$ type (7,8). These compounds are easy to prepare. For example the compound $Ni(SCN)_2$(4-methylpyridine)$_4$. (methyl pyridine)$_x$ is precipitated from $Ni(SCN)_4$ solution by the addition of excess 4-methylpyridine (17). When a mobile phase of NH_4SCN and 4-methylpyridine in water-acetone (or dimethylformamide) is used, adequate separation of the 1 and 2 methylnaphthalene isomers is obtained. Similar techniques result in the separation of the isomers of nitropropane and nitrobutane (18), and nitronaphthalenes (19), syn and anti furaldoximes (20), mononitrotoluenes (21,22), mononitroethylbenzenes (23) etc.

Kemula prefers this type of L.S.C. over L.L.C. for the separation of isomers and thus concurs with modern liquid chromatographic (L.C.) theory (24) which attributes this to the characteristic surface structure of the solid support which favours adsorption of those isomers whose molecular shape has the best fit for the geometry of the adsoprtion sites. Additionally, this characteristic feature of solid surfaces also makes one type of adsorbent better suited for a particular separation than another. Consequently it might be worthwhile reinvestigating the use of clathrate solid surfaces in the light of modern column technology.

Those techniques have been used successfully in the trace analysis of many of the compounds already mentioned (25,26) by adapting the detector for "DIFFERENTIAL" chromatopolarographic detection (Figure 3) (27). Impurities down to 10^{-6}M were readily detected (26) and it is interesting to note that at this level, Kemula was noticing the effects of his detector volume being too large. The solution of this problem forms a large part of this thesis.

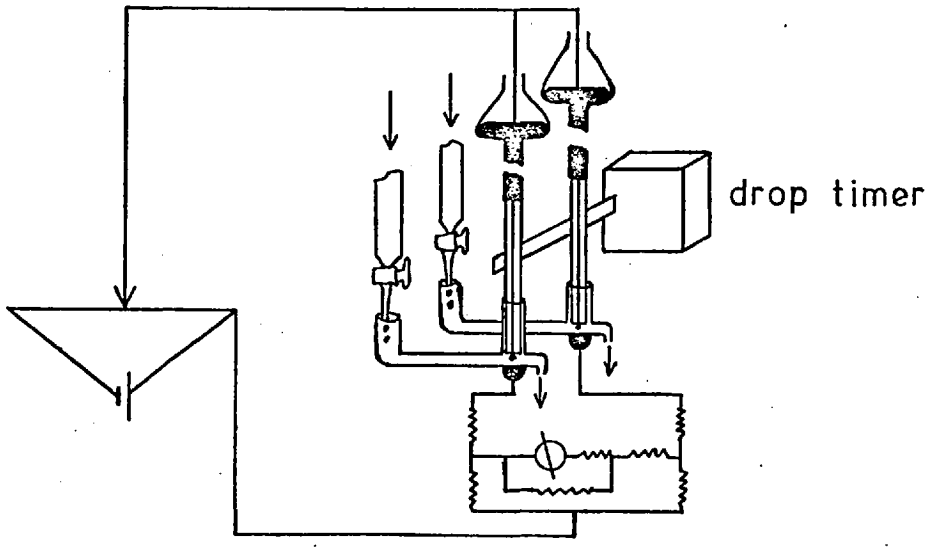


Figure 3. Scheme for differential detection of trace quantities (From refs. 3,27).

All of the preceding work refers to the analysis of compounds that are electrochemically active and capable of reduction at a dropping mercury electrode "D.M.E.". Two other categories exist (a) those compounds capable of oxidation at a solid electrode and (b) electroinactive compounds. No oxidative work has been done in the context of this chapter but will be discussed subsequently (Chapter 1.2). Experimental work in this field will also be included.

Electroinactive compounds can be analysed electrochemically in a number of ways. Kemula has successfully used two approaches namely derivative formation and maximum suppression.

Chemical reaction to form an electroactive derivative is a commonly used technique that has its counterparts in gas liquid chromatography (i.e. formation of volatile derivatives) and in u.v. spectroscopy (to form u.v. active compounds). The latter technique has been used in high resolution liquid chromatography where the only highly satisfactory, commercially available detector is the u.v. detector. Carbonyl compounds with low u.v. extinction coefficients, readily form highly u.v. active derivatives with 2,4-dinitrophenyl hydrazine (28,29) to enable trace analysis to be performed.

Electrochemically active derivatives open the field of polarography to a vast range of organic and biochemical compounds (30) as shown in the brief tabulation of the more important derivatives in general use (Table 2).

TABLE 2

Electrochemically active derivatives

Compounds	Derivatives	References
Aromatic hydrocarbons, phenols etc.	Nitration	31-35
2^{o} Amines, phenols, flavones	Nitrosation	36-38
Carbonyls, diamines	Condensation	39-45,51
Unsaturated compounds (C=C)	Addition	46-47
Diols, hydroxyacids, amino alcohols etc.	Oxidation	48
Amino acids	Complexation	26,49

It is in the case of complexation of amino acids with copper (26) that Kemula has used this technique. After the chromatographic column, a short column filled with solid copper phosphate was situated which served to prepare the derivative prior to chromatographic detection. In this manner the retrograde step, of adding the reagent to the eluent prior to chromatography, is avoided. This was not the case however when 2,4-dinitrophenylhydrazones were formed (51), the reagent, itself electroinactive, was added to the eluent. The reagent may have had little effect on the powdered rubber column used by Kemula but it is probable that a significant change in the chromatographic properties of modern, high resolution, columns would occur if such a procedure were used. Part of the experimental section of this thesis is concerned with this problem of adding reagents after the chromatographic separation.

D.C. polarograms are often complicated by the presence of maxima (5) which occur as sharp maxima on the rising part of the curve obtained from dilute solutions (maxima of the first kind) or as rounded maxima over the range of the limiting current obtained from concentrated solutions and at high Hg flow rates (maxima of the second kind). They arise from the effects of streaming of the electrolyte towards the electrode or in some cases from the mechanism of the electrode processes. In both cases, they are extremely sensitive to the presence of surface active species in the solution. Brucine and strychnine (50) are electroinactive towards reduction at the D.M.E. but both have a surface activity which has enabled their detection by the suppression of the polarographic maxima.

A different approach to the detection of surface active species is the use of the a.c. polarographic technique which responds strongly to reversible electrode reactions and also to processes which do not undergo electron transfer but only show adsorption-desorption phenomena (52) i.e. "Tensammetry". The a.c. polarogram is peak shaped, the maximum corresponding to the half-wave potential of the d.c. wave. With the detector set at the peak potential, detection of nitronaphthalenes (3,53,54) and methylnaphthalenes (17) was accomplished.

Bearing in mind the low resolution of the liquid chromatography used by Kemula et.al., the excellence of his work should not be underestimated. In fact this early work forms the groundwork on which the present investigation is based.

1.2 Electrochemical flow systems: their application to high pressure liquid chromatography.

The renaissance of liquid chromatography (L.C.) has stemmed mainly from advances in technique. The present situation is such that while column technology has reached a very advanced stage (24,25), there is still a great need for a detector of high sensitivity and wide applicability. Recent advances in the field of L.C. have been aimed at developing improved detector systems and in this respect electrochemical methods offer attractive possibilities.

Apart from the chromatopolarographic work of Kemula (3,4,17), electrochemical techniques have found widespread application as sensors for continuous analysis in flowing streams (56) but so far there are only a few examples of their use in flow cells of low dead volume suitable for high resolution liquid chromatography (H.R.L.C.).

1.2.1 Conductivity Detectors

Detectors based on the conductivity principle consist of a flow cell containing a pair of metal electrodes as shown in Figure 4 (57-59, 113, 116, 117). These electrodes are incorporated into an a.c. Wheatstone Bridge circuit for measurement. Normally an absolute measurement of conductance is made but with slight electrode modification (60), differential measurements are possible which can result in an increased sensitivity.

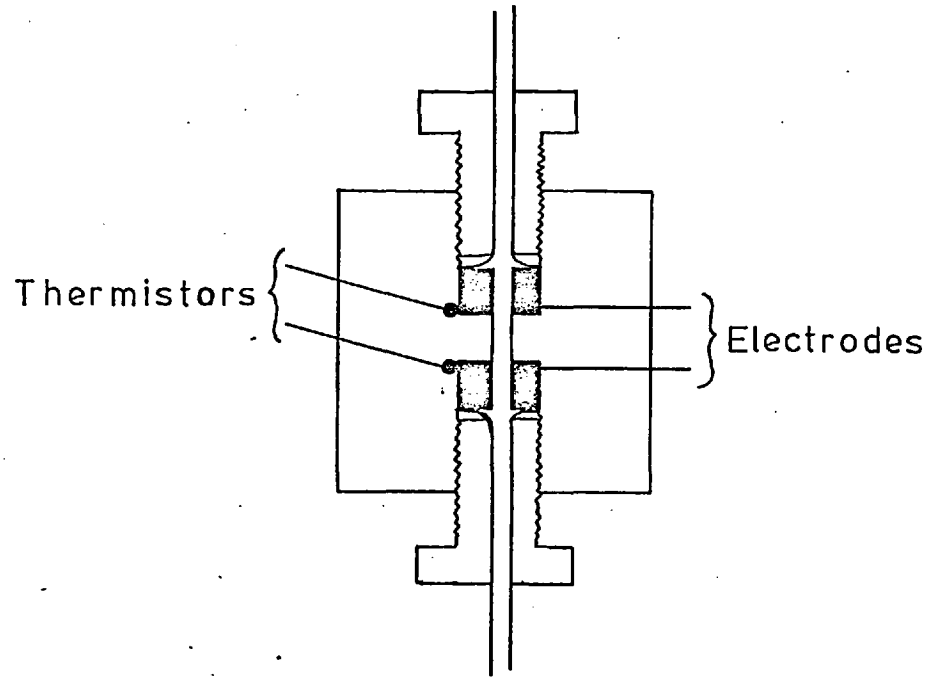


Figure 4. Conductivity Detector. Cross sectional view of Chromatronix MCC-75

The principle advantages of this detector are simplicity speed of response and low cost. The detector dead volume can be made very low. The Chromatronix detector has a volume of 1.5μl which makes it ideal for use with high resolution chromatographic systems. However its range of application is limited to ionic species as occurring in amino acid analysis, gel permeation and ion-exchange chromatography and the eluent must be electrically conducting, preferably aqueous. Under optimum conditions, the maximum sensitivity attainable corresponds to the determination of about 10^{-7}M NaCl (59).

1.2.2. Radiofrequency (R.F.) Conductivity Detectors

R.F. techniques (61-64) have the attractive feature that the electrodes, see Figure 5, are not in contact with the chromatographic eluent, which can have a low dielectric constant, such as benzene (65). The detector responds to a wider range of compounds

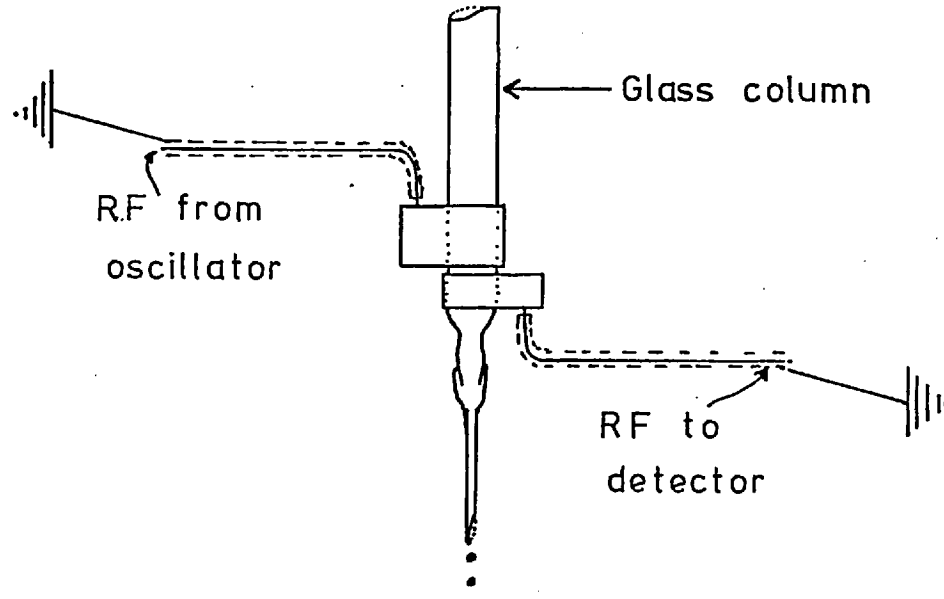


Figure 5. R.F. detector for liquid chromatography.

than the conductivity detector such as amino acids, proteins and methyl glucosides (65) but in general, the response is unstable and the reproducibility is poor. Both these factors have inhibited the development of this technique. To date, R.F. detection has only been applied to low resolution chromatographic columns, but there is no reason why it should not be used with modern high resolution columns. The detector dead volume could be made very small (1-2μl), but the prediction of sensitivity would be foolish. As electronic components have been transformed completely in recent years, with the perfection of integrated circuitry, it is arguable that this technique should be reinvestigated.

1.2.3. Permittivity Detectors

This type of detector, which is based on the measurement of changes in dielectric constant (66-68), has been applied to high resolution chromatographic systems and can be made with a very small dead volume (69). However, it is very susceptible to pressure changes resulting in a high frequency noise on the baseline recording. The performance has been compared with a conventional differential refractometer indicating marginal improvements in certain cases (69,114,115). While further developments in this field are expected it is unlikely that this principle will fulfill the requirements of a universal detector.

1.2.4. Coulometric Methods

Coulometric methods have not, as yet, found any application in liquid chromatography. Controlled current coulometry has been employed with gas liquid chromatography (G.L.C.) for the titration of eluted fatty acids (70) after absorption in a titration cell (Figure 6). Presumeably, a similar type of pH-stat could be employed for L.C. although the problems of keeping the dead volume minimal might be prohibitive.

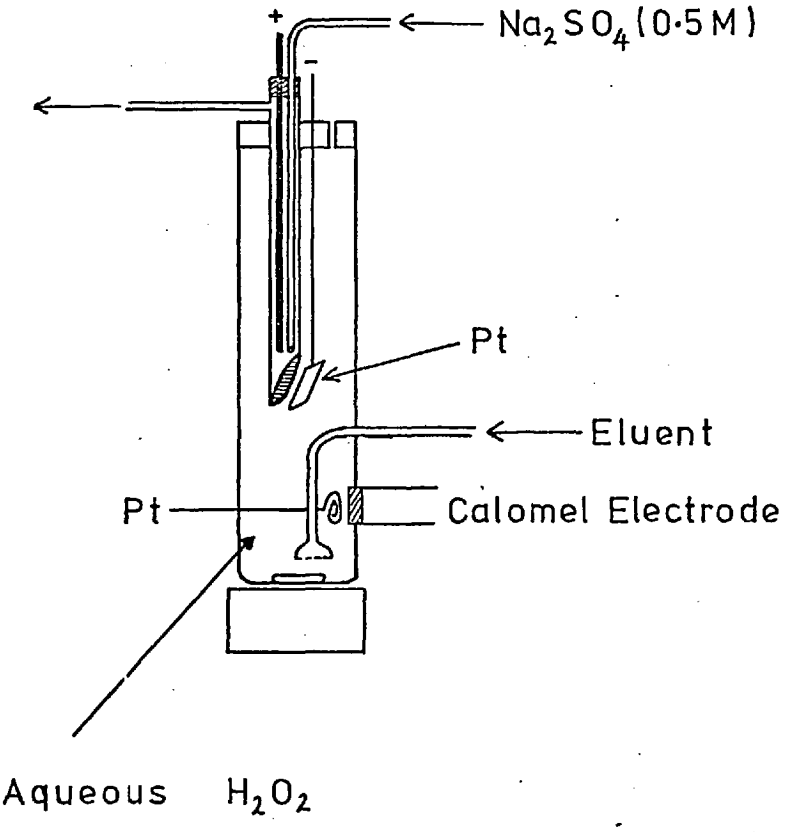


Figure 6. Coulometric detector for the G.L.C. detection of fatty acids.

Controlled potential coulometry is difficult to apply to organic systems due to problems in maintaining 100% current efficiency. Nevertheless numerous examples are available (71,72) of micro-coulometric techniques used in mechanistic studies and preparative electrochemistry.

Because of its inherent sensitivity and precision an investigation into its suitability as a method of detection for H.R.L.C. would be of interest (73).

1.2.5. Differential Amperometry

This approach (74) has to date only been used as an electrochemical flow cell which utilises the "hydrodynamic voltammetric" properties of forced convection at the electrode surface. This is analogous to the rotating electrode which results in similar increases in sensitivity and reduction of adsorption problems at the electrode surface. It differs from coulometry in that only a fraction of the electroactive species is reacted and that must be reacted rapidly. Consequently, the method offers a more likely approach to a L.C. detection system that does coulometry.

The cell is basically a 3-electrode voltammetric cell carrying a constant current between a massive, earthed, bipolar platinum working electrode and a platinum counter electrode, both of them situated in the flowing stream. The bipolar electrode is kept at a fixed potential the value of which controls the specificity of detection and is separated from the cell by a salt bridge of "thirsty" glass. It is connected to the summing point of an operational amplifier operating in a current to voltage configuration.

The current difference between the constant current input to the cell and the current demanded by the working electrode will be supplied or absorbed by the bipolar electrode circuit. Ordinarily the constant imput current is adjusted, initially, so as to bring the bipolar current to zero at the desired set-point concentration. Thus the sign and magnitude of the output voltage will follow the change in concentration of the electroactive species above or below the set point concentration. The method thereby enjoys

the advantages of specificity and lack of special end point detection characteristic of controlled potential electrolysis, together with the speed and simplicity of constant current electrolysis.

1.2.6. Electrochemiluminescence

When a low voltage a.c. potential is applied across two platinum electrodes immersed in a solution of aromatic (75) or substituted (80) aromatic compounds, free radical and charged species are formed which on combination form energetic species which subsequently luminesce.

This principle has been applied to a flow cell (see Figure 7) for use as a detector for H.R.L.C. (76) of polyaromatics.

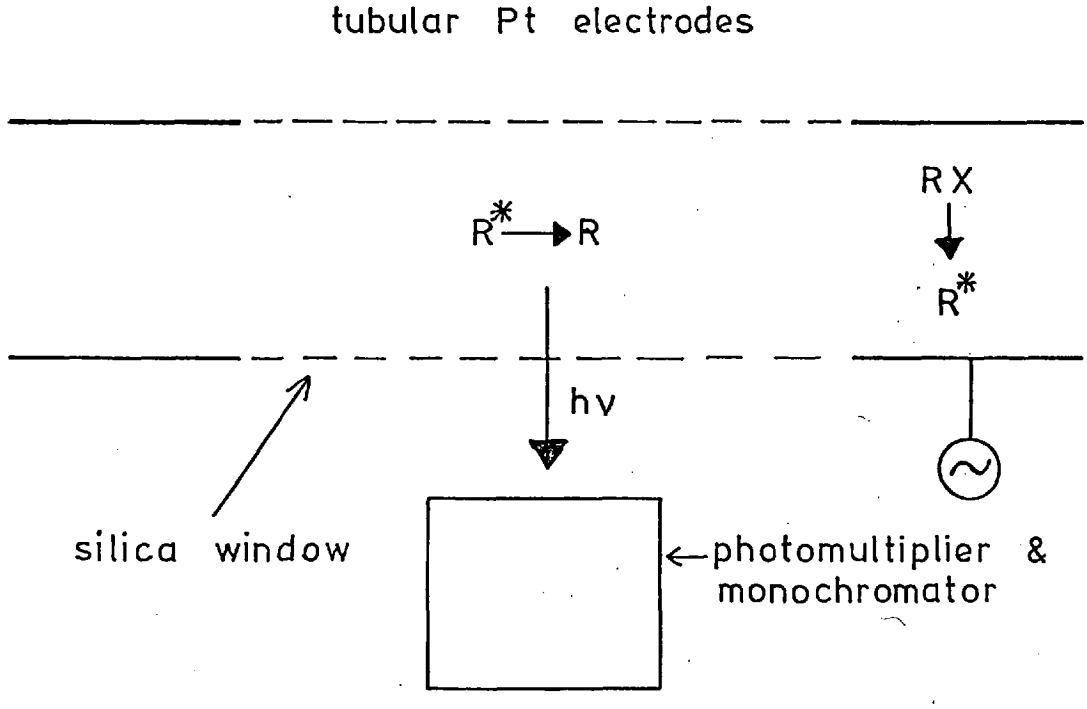


Figure 7. Diagrammatic form of Electrochemiluminescence Detector.

In use care has to be taken to optimise the flow rate so that the maximum luminescence occurs as the excited species pass the monochromator/photomultiplier position. Not only is it a specific detector for a small group of compounds but the choice of eluent is also restricted otherwise quenching of the luminescence is observed. Consequently it is not considered to be a worthwhile detection system for L.C. (unless a unique application crops up) as the only applications to date are better performed by the versatile and sensitive U.V. detector (55,77,78).

1.2.7. Pneumatopolarography

This is a comparatively recent modification of the polarographic method (79) and is well suited to measurement in flowing streams. A suggested design is shown in Figure 8/1

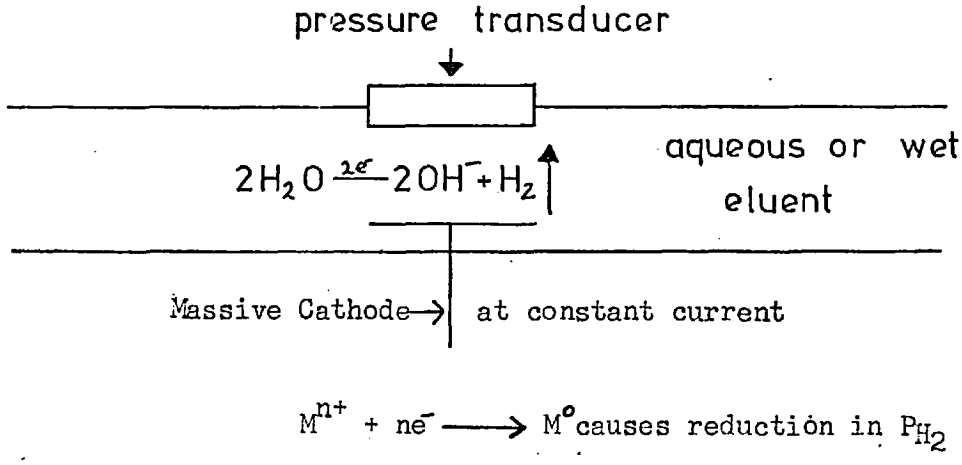


Figure 8/1 Pneumatopolarographic principle as could be applied to a L.C. detector

Aqueous, or semi-aqueous, solvent is hydrolysed at a constant

current at a solid electrode maintained at a potential in excess of the overpotential for oxygen or hydrogen, such that a constant partial pressure of oxygen or hydrogen is evolved. This pressure is monitored by a pressure sensitive transducer. If reducible or oxidisable species are added to the system, they compete for the current passed, thus reducing the partial pressure of oxygen or hydrogen which is thus observed and monitored as an indirect measure of the concentration of the species in the reaction vessel.

It should be possible to construct a low dead volume detector based on this principle which would be expected to be of wide application and particularly useful for the detection of those compounds which are reduced or oxidised near to the evolution potentials of oxygen or hydrogen. The gas formation effectively cleanses the electrode surface and prevents deposition or adsorption of surface active species.

1.2.8. Ion Selective Electrodes

Ion selective electrode (I.S.E.) potentiometry has been and remains as the most active and expanding area of electroanalytical chemistry (81-84). It is convenient to classify ion selective electrodes as follows:

(a) I.S.E. with solid membranes. In the electrode membrane, a net of a certain kind of ion is formed, the structure of which is constant in time. The membrane can either be homogeneous (81) [a single crystal, a crystalline substance or a glass] or heterogeneous (81,85), where a crystalline substance is built into a suitable polymer matrix or chalcogenide glass (86).

(b) I.S.E. with liquid membranes. The electrode membrane is represented by a water-immiscible liquid, in which is dissolved a substance capable of exchanging the ion in solution for which the electrode is selective. This substance is either an associate of this ion with an oppositely charged ion, soluble in the membrane, or it is a complex of the ion for which the electrode is selective, with an uncharged macrocyclic compound.

Both of these groups of electrodes are,by definition, ion sensitive. Consequently their application is virtually restricted to inorganic cations and anions. If such an electrode were to be used in conjunction with high resolution ion-exchange chromatography, probably the universal "Selectrode" of Ruzicka would be the most applicable (87,88). This is based on the use of hydrophobised graphite as the support material with an internal solid state contact. The active membrane is prepared by rubbing a small amount of the relevant ion exchanger onto the face of the electrode.

(c) Miscellaneous Electrodes. The immobilised enzyme electrode (89) can be made specific for certain organic compounds and as such could find a future application in the field of L.C.. Unfortunately the relatively slow response time of such electrodes would be a grave disadvantage in a flowing stream. Such an electrode that immediately springs to mind, in the context of H.R.L.C., is the amino acid selective electrode (90,91), which consists of the enzymes catalase and 1-amino acid oxidase, immobilised on a glass electrode, coated with an acrylamide film. When, biological samples are chromatographed on ion exchange columns (92) complex chromatograms are obtained which include the U.V. active carbohydrates, organic acids, amino acids etc.

If enzyme electrodes could be miniaturised and made to respond faster, this particular separation would benefit from such a selective detector.

1.2.9. Voltammetric Techniques

This approach stems mainly from the classical polarographic techniques which have recently found increasing application in organic analysis since the majority of organic compounds are either electroactive or show adsorption-desorption effects at the mercury electrode. Two great advantages of the method are the inherent high sensitivity and the selectivity that can be achieved by controlling the electrode potential. It is possible to envisage the electrode as a nucleophilic (cathode) or electrophilic (anode) reagent of widely varying strength. It is convenient to subdivide these techniques into solid electrode voltammetry and classical (D.M.E.) polarography.

1.2.9.1. Solid Electrode Voltammetry

A considerable literature has accumulated on the use of solid electrodes (93). This type of electrode can be operated in the diffusion controlled (stationary solution) or forced convection /stirred solution/ (93,94) modes. The latter approach, "hydrodynamic voltammetry", has been mostly used with solid electrodes where the high liquid flow rates employed are compatible with the requirements of L.C. detectors. The advantages are immediately obvious, namely the decreased detection limits and the possibility of removal of weakly adsorbed species from the electrode surface.

For L.C. detectors, the tubular electrode has great appeal. Its dead volume can be minimised and the geometry is ideal. Such electrodes have been made from platinum (95), gold (96) and wax impregnated graphite, coated with a mercury film (97).

Joynes and Maggs (98) have described a detector for use with H.R.L.C. based on a carbon impregnated silicone rubber membrane but neglect to point out the inherent disadvantages of carbon electrodes over mercury electrodes, namely the problems of adsorption and deactivation at solid electrodes. While solid electrodes undoubtably have important advantages, i.e. mechanical strength, improved anodic range etc., the problems of surface adsorption must be solved before these devices can be employed as routine detectors. None of the approaches used to date, mechanical abrasion (99-101) or intermittent polarisation (102) can be considered entirely satisfactory.

A wide variety of materials offer possibilities for use as solid electrodes (93,103) but it is probable that one of the newer forms of high density carbon (103-105) will be the most suitable in terms of reproducibility, freedom from deactivation and wide potential range from anodic to cathodic potentials.

1.2.9.2. Classical Polarography

The chromatopolarographic approach (section 1.1.) has already shown the wide applicability of this technique. To apply the technique to H.R.L.C. considerable miniaturisation of Kemula's detector was necessary (106). With this detector, Huber was able to monitor the separation of p.nitrophenol, parathion and methyl

parathion over the range 10^{-7} to 10^{-8}M.

This detection limit is some two orders of magnitude lower than the corresponding value for a stationary system. The limit of detection in classical polarography is mainly defined by the magnitude of the double layer charging current. Various related pulse techniques have been developed in order to overcome this limitation.

1.2.9.3. Choice of Technique

In the course of the experimental work, it will be shown that the direct comparison of a specific technique in static solutions with that in a flowing stream is not always predictable. But in general, the advantages of a technique in static solutions also apply to a flowing stream such as a L.C. detector system. Table - 3 compares the polarographic data for copper, determined by a variety of techniques (107) from which, it can be seen that pulse and phase sensitive a.c. techniques are superior. The detection limits should be viewed with some caution as they apply to an inorganic

Table 3

Comparison of polarographic techniques for Cu determination

No.	Method	Detection Limit M	Response shape
1	d.c.	2×10^{-6}	Conventional wave
2	Rapid d.c.	3×10^{-6}	Conventional wave
3	Tast d.c.	1×10^{-6}	Conventional wave
4	Derivative Tast d.c.		Peak
5	Pulse	5×10^{-7}	Conventional wave
6	Derivative Pulse	5×10^{-7}	Peak
7	Differential Pulse	1×10^{-7}	Peak
8	Phase sensitive a.c.	5×10^{-7}	Peak
9	Rapid sensitive a.c.	1×10^{-6}	Peak
10	Tast a.c.		Peak

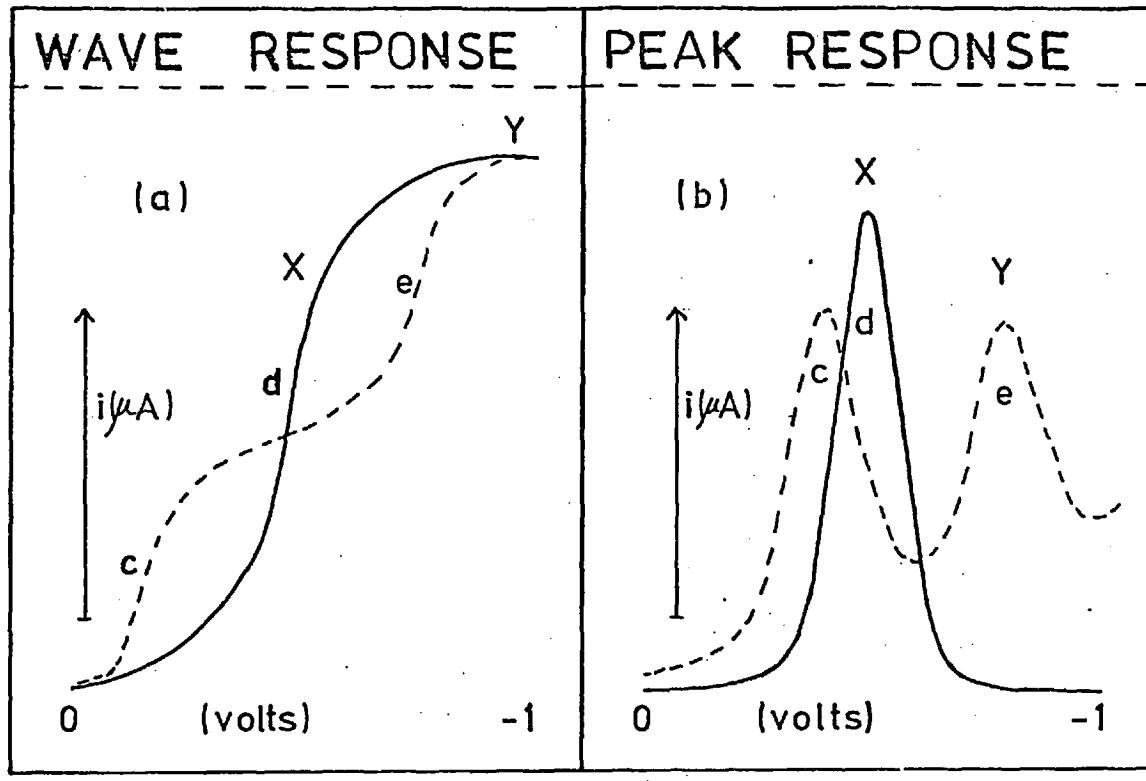
electrochemically reversible system. Many organic compounds react irreversibly or partially so and the detection limit is often higher by an order of magnitude. A comparison of a.c. techniques as applied to mesityl oxide (108) is shown in Table 7.

Choice of technique is also dependant upon the specificity of detection that is required. For a technique that gives a conventional wave shape, the applied potential is set negative or positive such that any compound that is reduced at a lower negative potential or any compound that is oxidised at a lower positive potential, is thereby detected.

For a technique that gives a peak response the applied potential should be set at the peak potential of the compound(s) of interest or the potential should be scanned over a given range. The peak potential imparts a greater measure of specificity into the determination, as peaks occurring at different potentials give diminished or zero response. (Table 3a)

Consequently for general monitoring of a chromatogram, techniques giving wave shaped responses are preferable, whereas techniques giving peak shaped responses are useful for detecting known compounds on a routine analytical basis and for situations where poor resolution occurs.

As mentioned in chapter 1.1. the majority of applications to chromatopolarography utilise classical d.c. techniques, only one being suitable for H.R.L.C. (106). Applications of pulsed techniques in chromatography have been confined to conventional a.c. (17) and square wave polarography (111), both to relatively low



Shape of polarographic responses.

(a) Wave e.g. D.C., Tast and Pulse polarography

(b) Peak e.g. a.c.*, Radiofrequency*, Square wave*, Differential Pulse and Fast scan D.C. polarography.

* 2nd, 3rd,(etc.) harmonic and intermodulation polarograms give multiple peaks some of which can be inverted when phase sensitive detection is used.

TABLE 3a

Comparison of use of Wave and Peak responses for use with detectors for H.P.L.C.

	WAVE	PEAK
Potential set at X	Detect c + d	Detect d + some c
Potential set at Y	Detect C + d + e	Detect e
Specificity	Some	More than wave
Universality	All detected	Not all detected unless potential scanned.
Sensitivity	Wave	peak
Ease of use	EASY	DIFFICULT

resolution systems at low detector sensitivity. Square wave, square wave intermodulation and radiofrequency polarography have been shown to be extremely sensitive (112), consequently the above application (111) has failed to demonstrate the full potential of the technique.

This array of techniques might at first sight seem unrealistic. However, it should be remembered that once a detector, (suitable for H.R.L.C.) has been designed, any one of the techniques mentioned and others to be discussed in the experimental section, could be applied to the detector. In addition, the column eluent can be stopped, trapping an eluate in the detector. The whole range of techniques could then be applied, including cyclic voltammetry thus yielding a lot of information about the compound which may be important if its identity is unknown.

1.2.10. Summary

The potential of electrochemical detection systems for H.R.L.C. is enormous, some applications being highly specific, others more general (Table 4). Of these, the voltammetric method is the most promising and in order to include the total resources of this technique I propose that the chromatopolarographic method should now be considered as part of the "CHROMATOVOLTAMMETRIC" method.

Table 4

Comparison of Electrochemical methods for detectors in liquid chromatography

No.	Electrochemical Method	Solute	Solvent	Volume (μl)	Possible Detection limits	Comments
1	Conductivity	Ionic	Electrically conducting	1.5	10^{-7}MNa^+	Limited application
2	R.F. Conductivity	Wider appl'n. than 1	Most	large[a]	-	Wider application than 1 but unreliable at present.
3	Permittivity	Varied	Most	10	10^{-7}M	Comparable to R.I. detector in certain cases
4	Coulometry	Electroactive	Conducting	large[a,b]	10^{-7}M	Difficult to assess potential. Probable low detection limit and high precision
5	Differential amperometry	Electroactive	Conducting	large[a]	10^{-7}M	Wide application. Worth investigating.
6	Electrochemi-luminescence	Mostly poly-aromatics	Non-quenching	10	—	Not viable proposition
7	Pneumato-polarography	Electroactive	Conducting	large[a]	10^{-2}M	Difficult to assess until miniaturisation has been attempted.
8	Ion Selective Electrodes	Electroactive & Enzyme specific	Conducting	large[a]	10^{-6}M	Enzyme electrodes might have highly specific application. Slow response.
9	Voltammetry	Electroactive & inactive	Conducting	10	10^{-7}M	Wide application and some specificity when required. V. low detection limits feasible ($\sim 10^{-9}$M).

(a) Capable of miniaturisation (b) G.L.C. application

1.3 Criteria for Detectors for use in Liquid Chromatography

The column is the heart of modern high resolution liquid chromatography (H.R.L.C.). It effects the desired separation by the combination of flow and distribution of the sample between the stationary bed and the moving fluid. The resolution "R" (Equation 1) of a mixture (Figure 9/1) depends upon the selectivity of the phase system and the mixing characteristics of the column, where the first term refers to the selectivity of the phase system (in

$$R_{BA} = \left[\frac{K_B^1}{K_A^1} - 1\right]\left[\frac{x_A}{1 + x_A}\right]\left[\frac{L}{HA}\right]^{\frac{1}{2}} \qquad \text{.....Equation 1}$$

liquid liquid chromatography), the second term (value between 0 and 1) describes the equilibrium distribution of A between the bed and the eluent (x being the capacity ratio) and the third term describes the mixing characteristics of the column (118) and incorporates "H" the "height equivalent to a theoretical plate" (119).

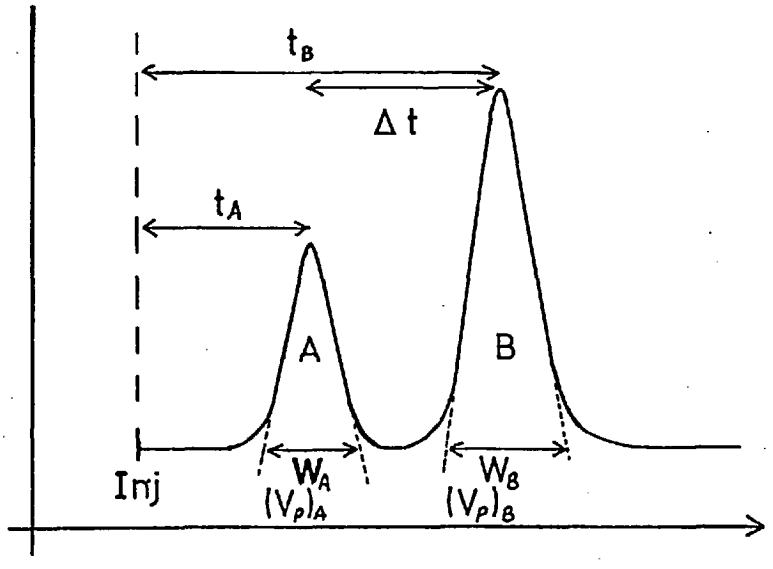


Figure 9/1 Chromatogram showing experimentally derived parameters from resolved components A and B

The mixing behaviour of a column can be characterised by the number of theoretical plates (N) produced by the column of length (L) and is related to H as in Equation 2.

$$H = \frac{L}{N} \qquad \text{..... Equation 2}$$

In practical chromatography, two more very useful equations [3] and [4] result from a simplification of the rigorous chromatographic theory (55,118,120,121).

$$N_A = 16\left[\frac{t_A}{W_A}\right]^2 \quad \text{or} \quad 16\left[\frac{V_A}{V_P}\right]^2 \qquad \text{.....Equation 3}$$

where V_A = elution volume of A and V_P = volume corresponding to the peak.

$$R_{BA} = 2\,\frac{\Delta t}{[W_B + W_A]} \qquad \text{.....Equation 4}$$

For maximum efficiency H tends to a minimum. Many parameters affect this and their optimisation has formed a major part of recent column development work, resulting in highly efficient separations at comparatively low elution volumes.

Now the effect of the detector volume on the eluate is to dilute it, resulting in a reduction of peak height and an increase in peak width. Both of these effects are undesirable. The effect of an increase in peak width is to decrease the resolution between peaks (see Equations 3 and 4). This becomes particularly serious when two peaks are only just or partially resolved. In this case, the peak spreading of both eluates combines to further increase the loss of resolution (Equation 4). It can be shown quite readily that when the detector volume $\ll$ peak volume a slight peak broadening is

of minimal importance. However as the detector volume approaches the peak volume, the loss in resolution becomes very serious. When resolution is vital, it is reasonable to minimise the loss of resolution to 2%, thus minimising the detector volume to 1% of the peak volume i.e. for a peak volume of 500μl, the detector volume should be 5μl.

The practical significance of the detector volume is clearly seen by looking at examples taken from the literature where chromatographic separation has been achieved on the best columns available to date, thus pointing to the future trends which must be allowed for in detector development.

Equation 3 was rearranged to give Equation 5.

$$V_p = \frac{4V_A}{\sqrt{N}} \qquad \text{..... Equation 5}$$

from which the peak volume was calculated. The data (Table 5) was calculated from published, reduced scale, chromatograms and consequently may not be very accurate but is sufficiently so to be useful.

Detector volume in L.S.C. (micro silica and silica microspheres) appears to be the least significant, as although resolution is high, so is peak volume, resulting from the high column capacity and high flow rates associated with the separations. Otherwise, it can be seen that a detector volume of less that 10μl is highly desirable. In fact, the peak volumes achieved with L.L.C. on ternary phase systems and Zipax supports suggests that a detector volume of less than 1μl might be necessary, particularly when high resolution is important.

Table 5

Peak Volume data, calculated from published chromatograms

	Technique	Compounds Seperated	N	Elution Vol. V_A (μl).	Peak Vol. V_P (μl)	Principal Author	Ref.	Fig.
L.L.C.	Ternary liquid phase system on silanised Kieselguhr	p.Nitrophenol Me.Parathion Oxygen Et.Parathion	17.4 15.4 22.1 15.1	177 385 446 1077	41 100 81 286	Huber	106	9
L.L.C.	Permaphase O.D.S. Permaphase E.T.H.	9,10-Anthraquinone di-Me.Benzylalcohol	28.3 32.2	2655 3385	376 419	Kirkland	124	1 4
L.L.C.	C.S.P. support 'OPN/Zipax	NN'Diethylamine N Ethylaniline Diphenylamine N.Ph.2Naphthylamine	58.7 58.7 62.1 52.7	1600 1700 1900 2200	109 116 122 167	Majors	122	Tab. 2 Fig. 3
L.S.C.	(5-10) Silica	Me.Benzoate Carbazole Acetophenone 2,4-Dinitrobenzene	26.7 25.0 23.6 29.7	4400 8000 11400 16600	660 1280 2900 3350	Majors	123	2
L.S.C.	Zorbax Sil (8-9) Silica Microspheres	Peak 1 Peak 3 Peak 5 Peak 7	15.5 28.3 39.5 36.0	2763 7576 14090 40110	713 1070 1990 4460	Kirkland	24	4
C.X.C.	C.S.P. support	2 aminobenz-imidazole	16.8	5810	1384	Kirkland	125	10
A.X.C.	C.S.P. support	Fumaric Acid Maleic Acid	10.0 12.3	1023 3140	408 1021	Kirkland	125	11

As a general rule, it can be seen that the first peak to be eluted has the lower peak volume. This region is often the most likely to produce incomplete resolution, thus increasing the importance of low detector volume.

The situation can be expected to become more critical as better column packing techniques (123,126-128) enable smaller support particles to be used (129, 130). As efficiency of separation increases so does the general use of short columns (10-25 cm) yet another factor causing smaller peak volumes.

Much has been said about detector volume, the most important criterion in detector design, but there are others (106, 133-135) of significant importance which are listed in Table 6 and apply generally. Specific comments to electrochemical detectors are given in the subsequent chapters but it is relevant to comment on some of these criteria. When electroreductive techniques are used, oxygen must be removed from the eluent and for all techniques, the solvent must have a high dielectric constant either due to its own properties or those of an added supporting electrolyte, often at the 0.1M level.

Table 6

Important Criteria in Detector Design

Criterion	Comments
Detector Volume	Low as possible: $<10\mu l$
Detection Limit	Low as possible: 10^{-7}M to 10^{-8}M in order to compete with R.I. and U.V. detectors.
Application	Universal application highly desirable at present, but specificity, complementary to existing detectors would be very useful.
Temperature Sensitivity	Low: so that thermostat is unnecessary.
Solvent Limitations	None: also the detector should be insensitive to minor flow variations.
Gradient Elution	Changes in solvent composition should be possible - this technique is rapidly becoming more important.
Linearity	High linear dynamic range.
Detector Response	Instantaneous; such that detectable peak width is minimal.
Noise	Minimal: degas, if necessary to reduce gas bubble noise.
Use	Ease of use, without specialised knowledge is an advantage.

Electrochemical techniques are slightly temperature sensitive (131) but it is acceptable to keep the detector at room temperature. They are also destructive techniques. However, only a fraction of the solute is reacted so that fraction collection and further examination by other techniques is easily performed.

Unfortunately, a knowledge of electroanalytical chemistry would be advisable to use the technique to its full advantage although an introductory knowledge would be sufficient to use the equipment, providing recourse to fault finding was not necessary.

The detection limit isacriterion to which incorrect emphasis is often given. Although less sensitive than the ultra violet (U.V.) detector, by at least an order of magnitude, the refractive index detector (R.I.) is far more universal in application (113, 132-135) but is far less popular because of its extreme temperature sensitivity and incompatability with gradient elution. Where the column capacity is high as in L.S.C.,large injection volumes can effectively decrease the detection limit (106).

Low detection limits are becoming more important largely as a result of stricter government legislation concerning pharmaceuticals, pollution control and the extremely low level of solutes in biological materials.

ELECTROCHEMICAL TECHNIQUE

2.1. Development and Evaluation of a.c. Techniques

A brief survey of voltammetric techniques has been given (section 1.2.9.3.). It was decided to pursue the a.c. technique because, potentially, it has a very low detection limit ($<10^{-8}$M), gives a peak shaped response (not necessarily ideal for L.C. monitoring), and responds "tensammetrically" to electroinactive species (52). The a.c. polarographic technique is less sensitive to irreversible processes. The electroreduction of oxygen is an extremely irreversible process and consequently the a.c. signal is reduced with respect to the majority of organic electrode processes which are usually reversible or slightly so. In consequence, it is possible, especially at normal levels of concentration (10^{-2}M to 10^{-6}M) to dispense with the deoxygenation procedure (136). In practical terms, this is most useful, although at low levels of detection it is still advisable to deoxygenate.

Very little quantitative a.c. polarographic analysis of organic compounds has been reported, the most notable being the determination of a number of pharmaceuticals in acetonitrile solution (110). Consequently, it was decided to evaluate a range of a.c. techniques to assess their applicability for L.C. monitoring.

Diacetone alcohol [I] is a widely used solvent in the formulation of pesticides, many of which are sensitive to the presence of water. It is decomposed to mesityl oxide [II] and water [III] according to the following reaction.

$$\underset{\text{I}}{(CH_3)_2COH.CH_2COCH_3} \longrightarrow \underset{\text{II}}{(CH_3)_2C{:}CHCOCH_3} + \underset{\text{III}}{H_2O}$$

Consequently its analysis is important in formulation and quality control analysis. The most obvious analytical technique to use is gas liquid chromatography (G.L.C.). Unfortunately, unless low temperatures and carefully prepared columns are used, the only response obtained is that of mesityl oxide, complete decomposition having occurred on the column. Therefore the polarographic technique was investigated as an alternative with a view to ultimate determination by L.C. with polarographic detection.

Fortuitously, I is not reducible polarographically, facilitating easy recording of the mesityl oxide reduction which thus serves as a monitor for the decomposition of I. Furthermore, as polarography is a trace technique, it is suited to the type of analysis required.

A brief note (137) on the determination of mesityl oxide in I and in acetone has been published and is based on the conditions established by Pasternak (138). It will be shown subsequently that these conditions are far from optimal.

2.1.1 Experimental Conditions

D.C. polarograms were recorded on a Radelkis type OH-102 polarograph. A multipurpose modular instrument (139,140) constructed from well established integrated circuits was used in the study of related techniques, including the fast scan (a.c. and D.C.) and phase sensitive a.c. polarography.

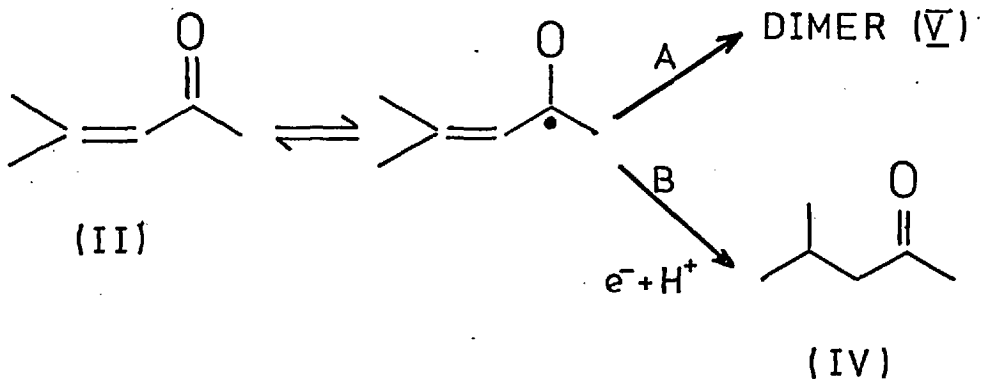
A three electrode cell was used, consisting of a Hg counter electrode, Ag/AgCl reference electrode separated from the solution by an agar salt bridge and a dropping mercury electrode "D.M.E." with a conically ground tip. This enabled the reference electrode to be placed much nearer to the Hg drop, thus minimising the I.R. drop and limiting the phase change due to the glass walls of a conventional electrode (141). Polarograms were recorded on a Bryans x - y plotter.

All reagents were of AnalaR grade. Buffer solutions (0.5M, unless stated otherwise) were prepared from (1) H_2SO_4 (pH = 1.23), (2) KCl + 0.05M HCl (pH = 2.27), (3) sodium acetate + 5M acetic acid (pH = 4.6), (4) NaH_2PO_4 (100 ml.) + NaOH (34ml.) (ph = 7.8), (5) NaH_2PO_4 (100 ml.) + NaOH (84 ml.) (pH = 9.4), (6) borate (pH = 11.0), (7) NaOH (pH = 13.1).

The solutions for polarographic analysis were prepared in 50% aqueous ethanol by mixing measured volumes of ethanol, buffer solution and stock solutions of depolariser. Prior to analysis all solutions were deoxygenated by bubbling nitrogen, presaturated with supporting electrolyte through the solutions.

2.1.2. Mechanism of the Electrode Reaction

This illustrates the more detailed information that can be obtained from polarographic detection, although it is appreciated that in most cases it would not be required. The mechanism has been studied previously and the following reaction scheme proposed. (30,138).



(II)

(IV)

Formation of the dimeric ketone [V] via pathway "A" is supported by coulometric evidence and identification of the products of the electrode reaction. Pathway "B", however, has been proposed as being analogous to that of the chalcones in basic media (138). The results obtained in this investigation support the existence of pathway "A", but no evidence of any contribution from pathway "B" could be observed.

In the context of L.C., these coulometric studies could be performed on the eluate in the detector, the solvent flow having been stopped. The electrolysis products could then be collected and subjected to further analysis, thus yielding information that would be useful in the elucidation of an unknown structure.

2.1.3. pH Dependence

Solutions of II (2×10^{-4}M) were made up in aqueous ethanol (50%) and buffered to the desired pH. Polarograms (D.C.) were recorded using the Radelkis polarograph in the two electrode mode using a Kalousek cell. The polarographic behaviour (Figure 8/2) and the dependence of $E_{\frac{1}{2}}$ and i_d on pH (Figure 9/2) clearly show the absence of any contribution from pathway "B". The optimum pH was

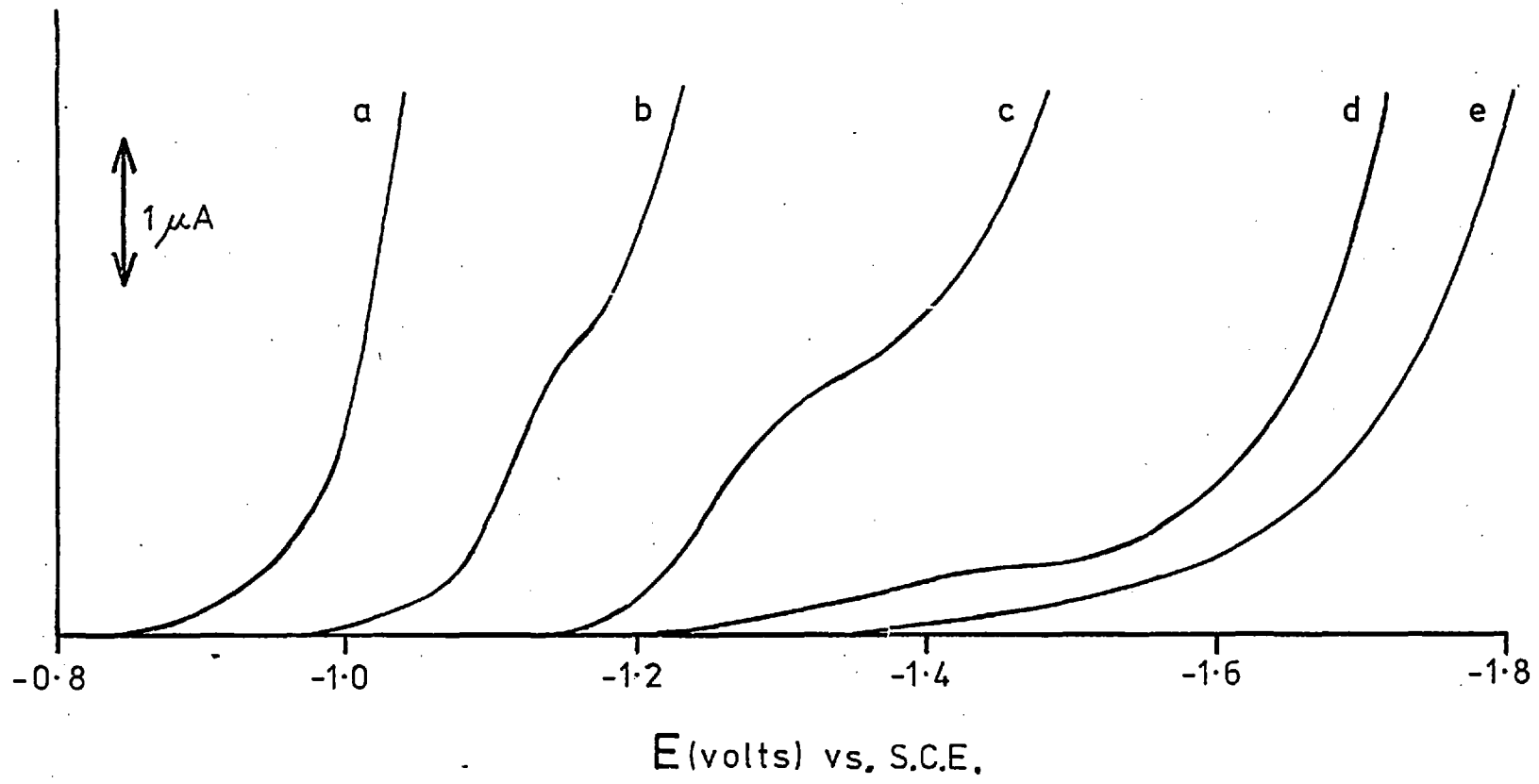
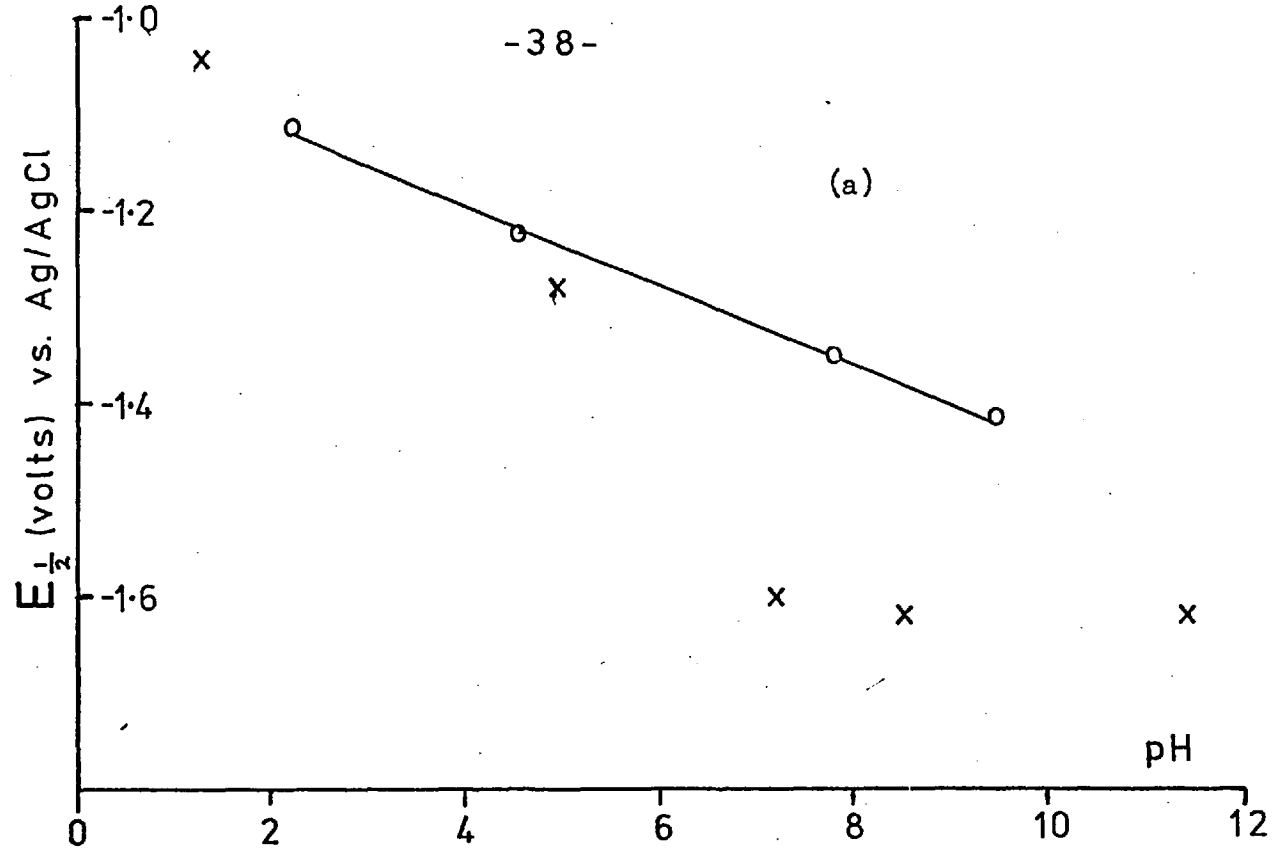


FIGURE 812: pH dependence of the polarograms of mesityl oxide at pH = (a) 1.23, (b) 2.27, (c) 4.6 (d) 7.8, (e) 11.0



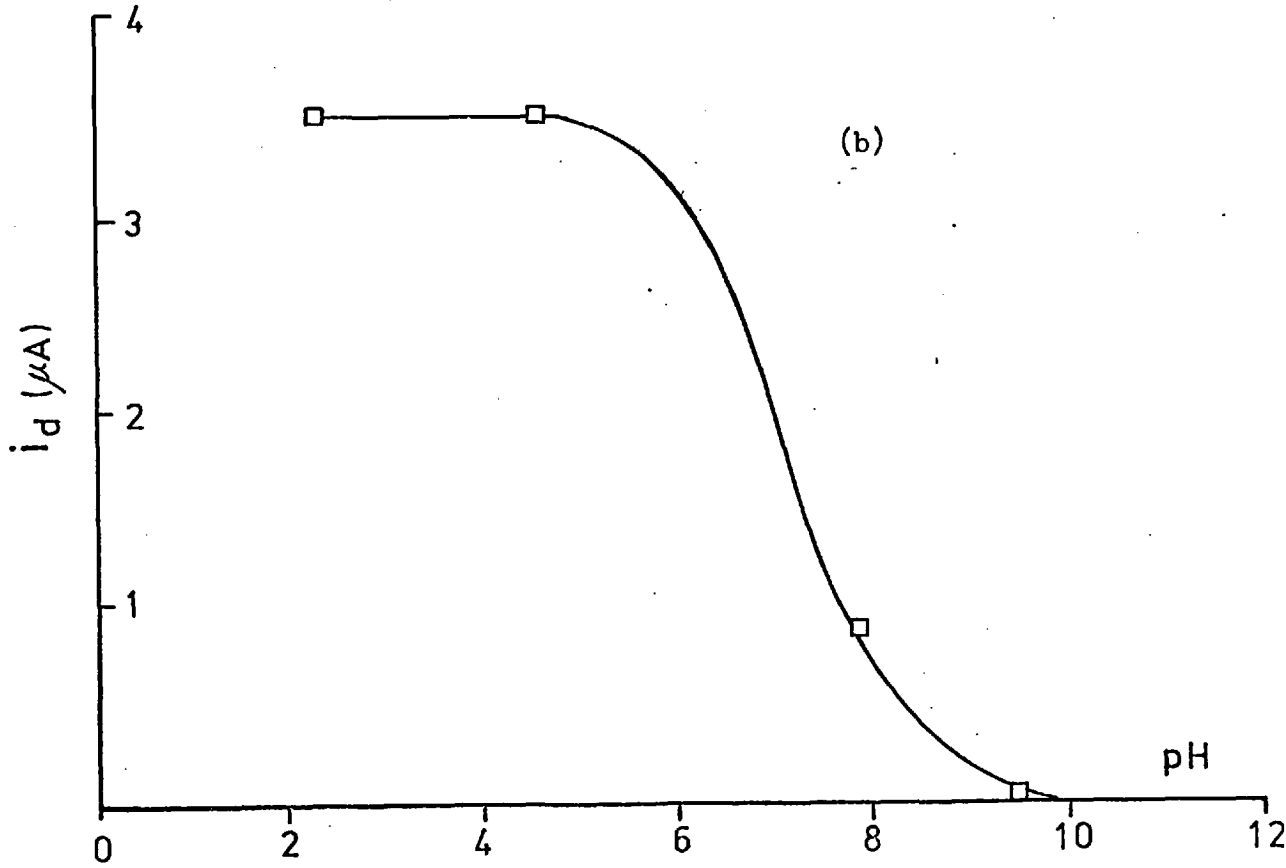


FIGURE 9/2: Variation in pH of a solution of mesityl oxide (2×10^{-7}M) Effect on (a) $E_{\frac{1}{2}}$ and (b) i_d. The values (X) in (a) are taken from Ref. 138.

found to be about 4.6 which was used in all subsequent work. $E_{\frac{1}{2}}$ values show a linear dependence on pH, typical of a reversible reaction involving protonation. The 1-electron step was confirmed by comparison with the wave height of benzaldehyde (2×10^{-4}M) and by controlled potential microcoulometry (72) at the DME which gave a value of n = 0.99 electrons.

2.1.4. Effect of solvent composition

The influence of ethanol concentration was studied at the optimum pH value. No influence on limiting current was observed over the range 0 - 80% v/v ethanol and only a minimal negative shift in $E_{\frac{1}{2}}$ (70 mV) was observed over the same range. Consequently, a 50% v/v aqueous solution was chosen for all subsequent work. This enabled standard buffer solutions to be used as supporting electrolytes, gave reasonable solubility of many organic species and afforded easy comparison with previously published work.

2.1.5. Quantitation by various techniques

A three electrode cell and the multipurpose instrument (139, 140) was used in the subsequent studies. The fast sweep determinations were based on the synchronised scanning of the D.C. and a.c. signals towards the end of the lifetime of each Hg drop. Time delay facilities were used to ensure that the total time of (delay + sweep) did not exceed the Hg droptime. This technique gives optimal discrimination between the FARADAIC and CAPACITANCE currents at the end of the drop life and is distinct from the RAPID SCAN method (107, 145-147) where a mechanically controlled drop time of short duration is used to enable moderately fast sweep rates to be used. This latter technique has the advantage of making it

possible to use tuned filters to remove drop oscillations, but from a theoretical and practical point of view, the sensitivity is limited and the charging current is large, producing a sloping baseline (148) which, however, can be compensated for.

The fast sweep technique used in this study overcomes the major limitation of a.c. polarography associated with the solution film within the electrode capillary (149). It also eliminates the noise problems which arise through the Hg drop hitting the reference pool.

A.C. and D.C. polarograms, determined by normal and fast sweeps (Figure 10) and the corresponding calibration curves (Figure 11) are shown. From these observations several conclusions can be drawn.

(i) Fast sweep rates are superior to normal sweep rates, resulting in well defined peaks of greater sensitivity and increased linear range.

(ii) As expected D.C. polarography is superior to the corresponding total harmonic a.c. technique and the fast sweep version has the lowest detection limit of 7×10^{-6}M under the conditions quoted.

(iii) The normal D.C. polarographic wave shows a sharp limiting value at about 10^{-3}M, indicative of an adsorption process which is further confirmed by a linear relationship of diffusion current (i_d) and height of the mercury reservoir (5, 150). Electrocapillary measurements, however, (Figure 12) are inconclusive.

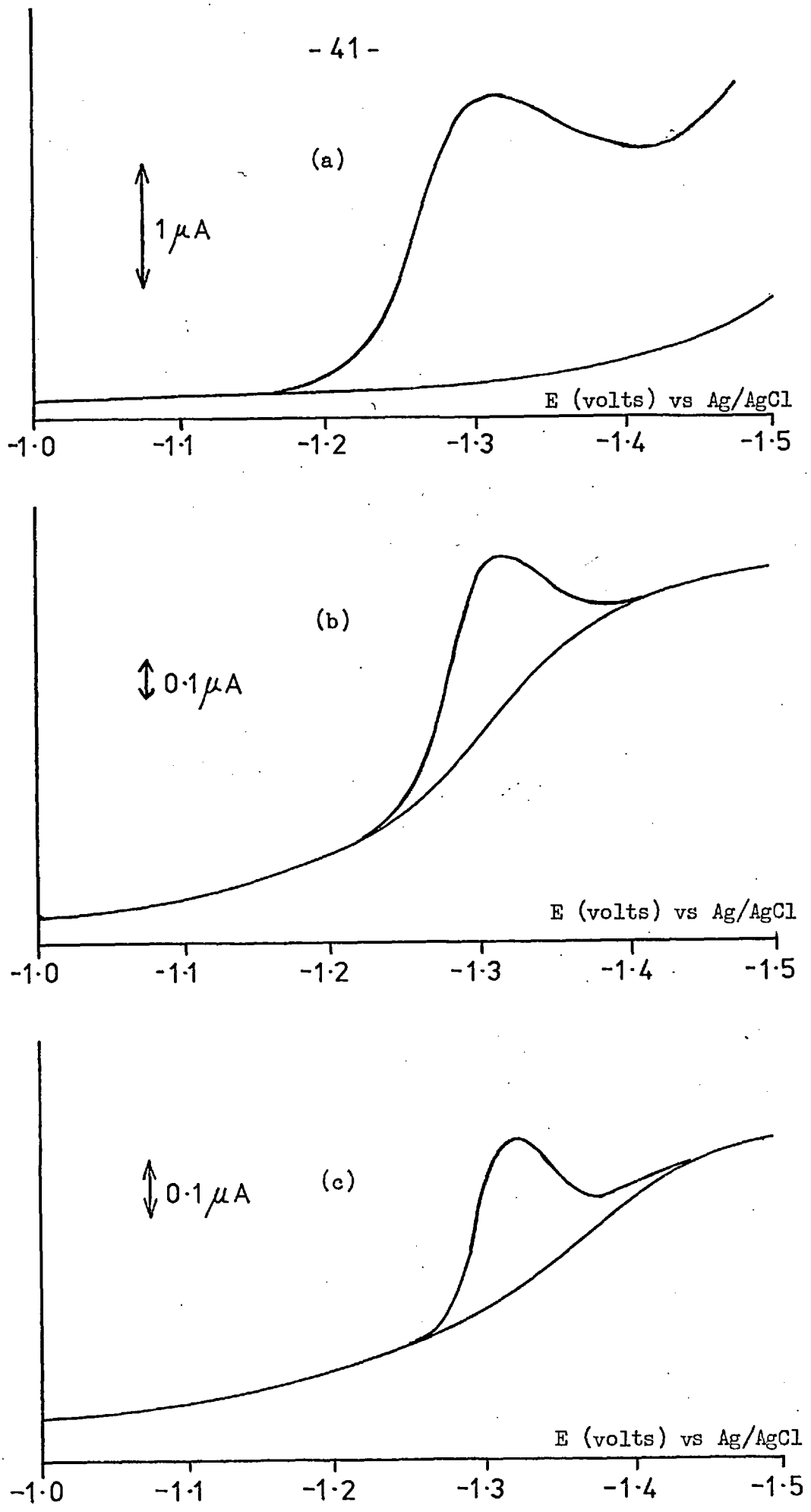


FIGURE 10: Comparison of polarograms for mesityl oxide (2×10^{-4}M): (a) D.C. (F), 500mV.sec^{-1} (b) a.c. (C) 2mV.sec^{-1}, E=50mV at 20Hz (c) a.c. (F) 500mV.sec^{-1}, E=50mV at 20Hz where F = fast scan and C = slow scan.

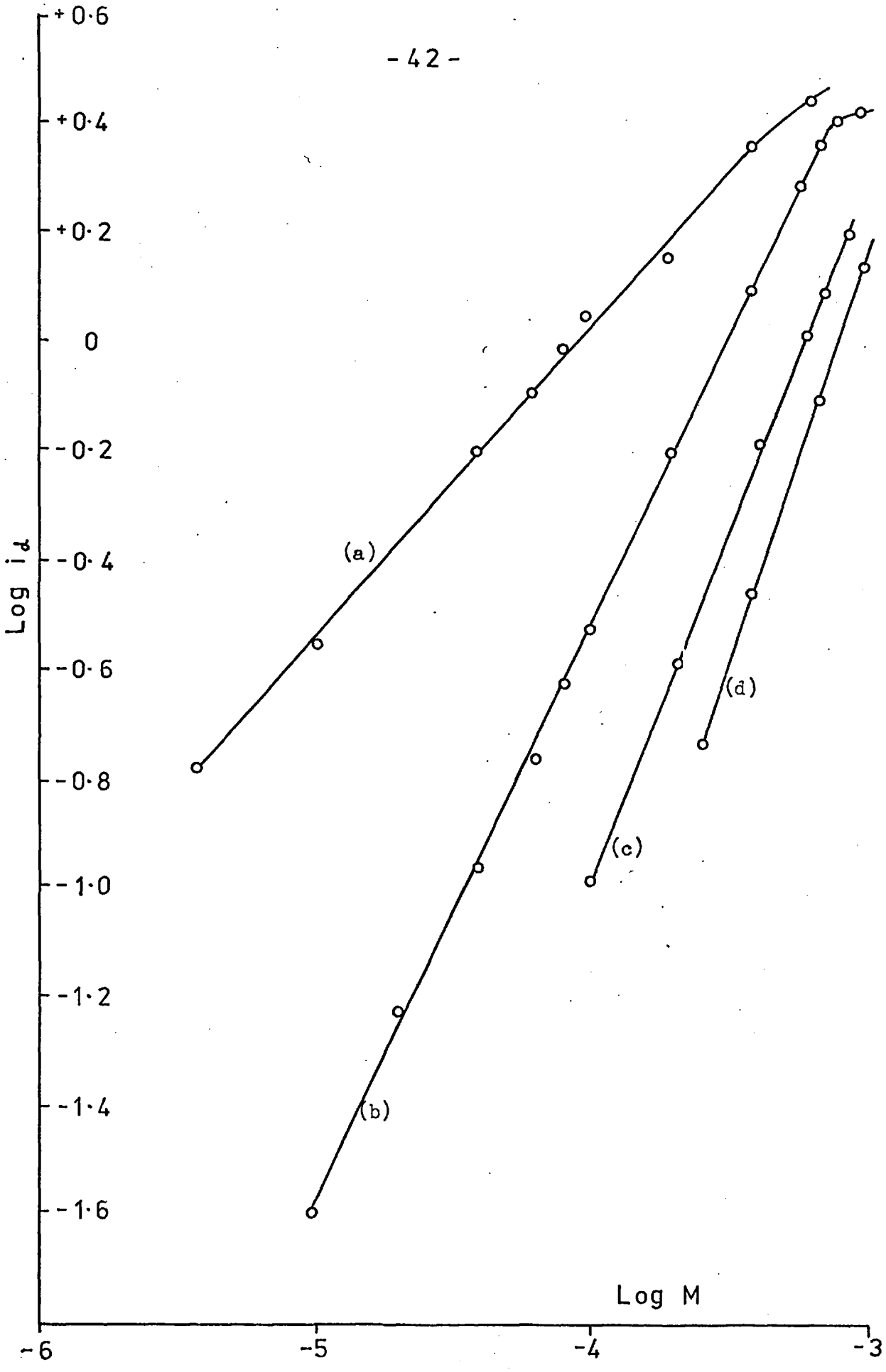


FIGURE 11: Calibration curves for mesityl oxide by various polarographic techniques: (a) D.C. (F), (b) D.C. (C), (c) a.c. (F) and (d) a.c. (C).

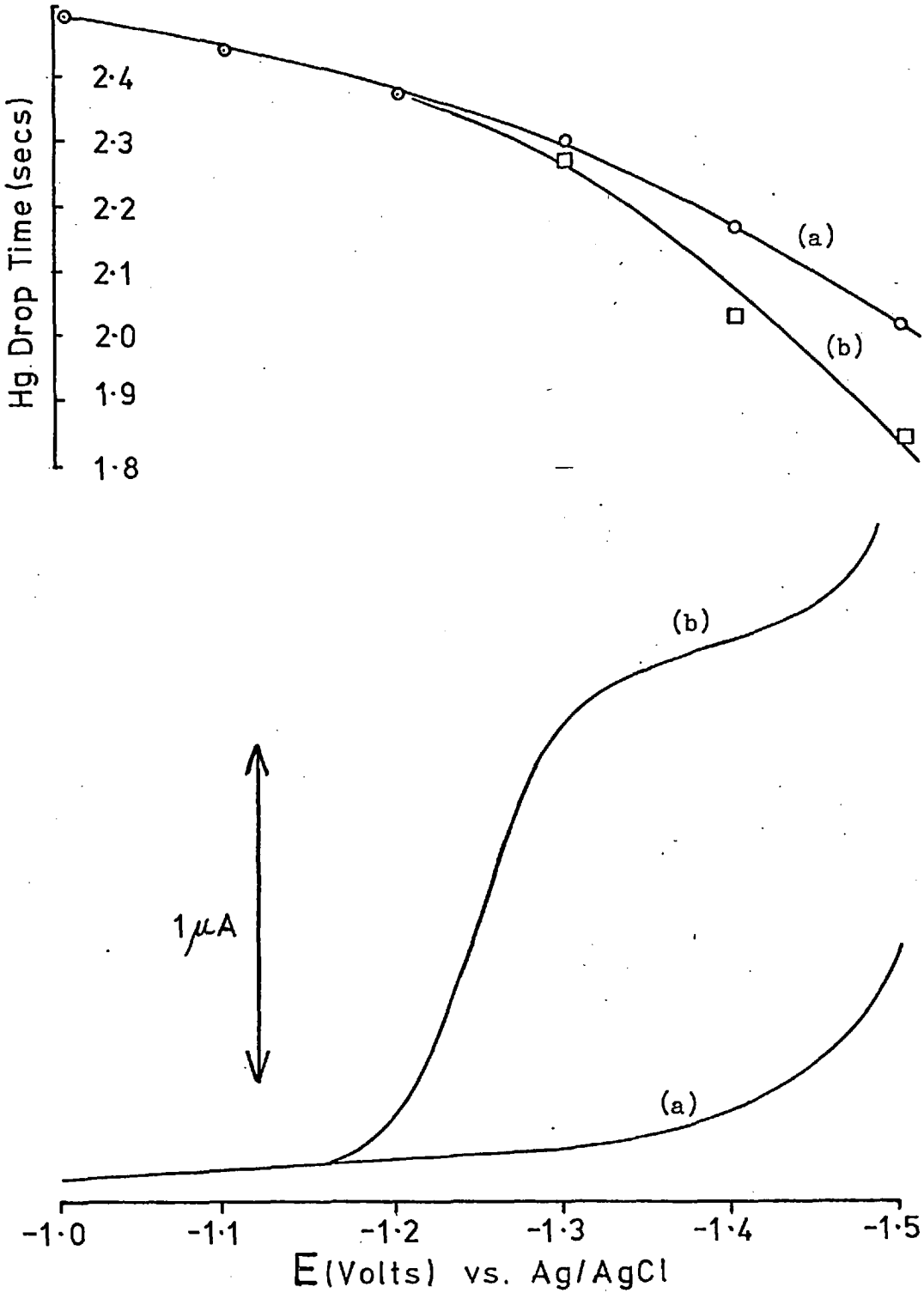


FIGURE 12: Electrocapillary curve for a mesityl oxide solution (2 x 10-4M) at pH = 4.6: (a) supporting electrolyte (b) after addition of mesityl oxide

There is a slight lowering of the electrocapillary curve after the addition of mesityl oxide to the supporting electrolyte but this occurrs after the D.C. wave has been recorded. Consequently it is probable that the mesityl oxide reduction is not governed by an adsorption process although it does appear that some adsorption process may be occurring after the reduction of mesityl oxide.

2.1.6. Phase Sensitive Detection

It has been demonstrated that the total harmonic a.c. polarography of mesityl oxide is less sensitive than D.C. polarography. This is attributed to the influence of the background charging of the electrical double layer which produces a large background response. The sensitivity can be improved by using the fundamental frequency, which at the time of doing the experiment was restricted by instrumentation to 318 Hz, whereas subsequent observations have indicated that the optimum frequency for this determination is about 25Hz.

Second harmonic a.c. polarography (109, 140, 151-154) produces a smaller response than the fundamental harmonic but its main analytical advantage lies in the fact that the background current is extremely small. The same criteria are characteristic of intermodulation polarography (112, 155). Both of these techniques, however, require tuned filters of the appropriate frequency.

"Phase Sensitive Detection" (52, 108, 109, 112) eliminates the charging current, hence considerable amplification of the Faradaic current is possible and this approach has been used in the present investigation. It should be noted, however, that a combination of phase sensitive detection with fundamental harmonic

a.c., second harmonic a.c. or intermodulation polarography would be a superior technique.

Total harmonic a.c. polarograms of mesityl oxide with phase sensitive detection are shown in Figure 13 and the corresponding calibration curve is shown in Figure 14. The analogous fast sweep technique gives a very similar response to that shown in Figure 13 and the corresponding calibration is included in Figure 14.

Both techniques show a wide linear range, the fast sweep being more sensitive by an order of magnitude. Unlike the conventional a.c. method the sensitivity of the fast sweep method is dependent upon the height of the Hg reservoir. Consequently, for very precise work, a constant height Hg reservoir should be used.

An additional, though minor, difference between them is illustrated by the region marked "C" on Figure 13. This corresponds to a lowering of the capacitance of the electrode and is attributed to adsorption. The effect is much more pronounced at the normal sweep rate as shown.

This observation is in accordance with the evidence of adsorption from Figure 12. Although it is necessary to use caution in interpreting such data when phase sensitive detection is used (156), it seems certain that adsorption is occurring after the reduction of the mesityl oxide from the fact that points "A" and "B" (Figure 13) show no baseline depression. The smaller baseline depression of region "C" by fast sweep methods points to the presence of a smaller concentration of adsorbing substance due to the shorter

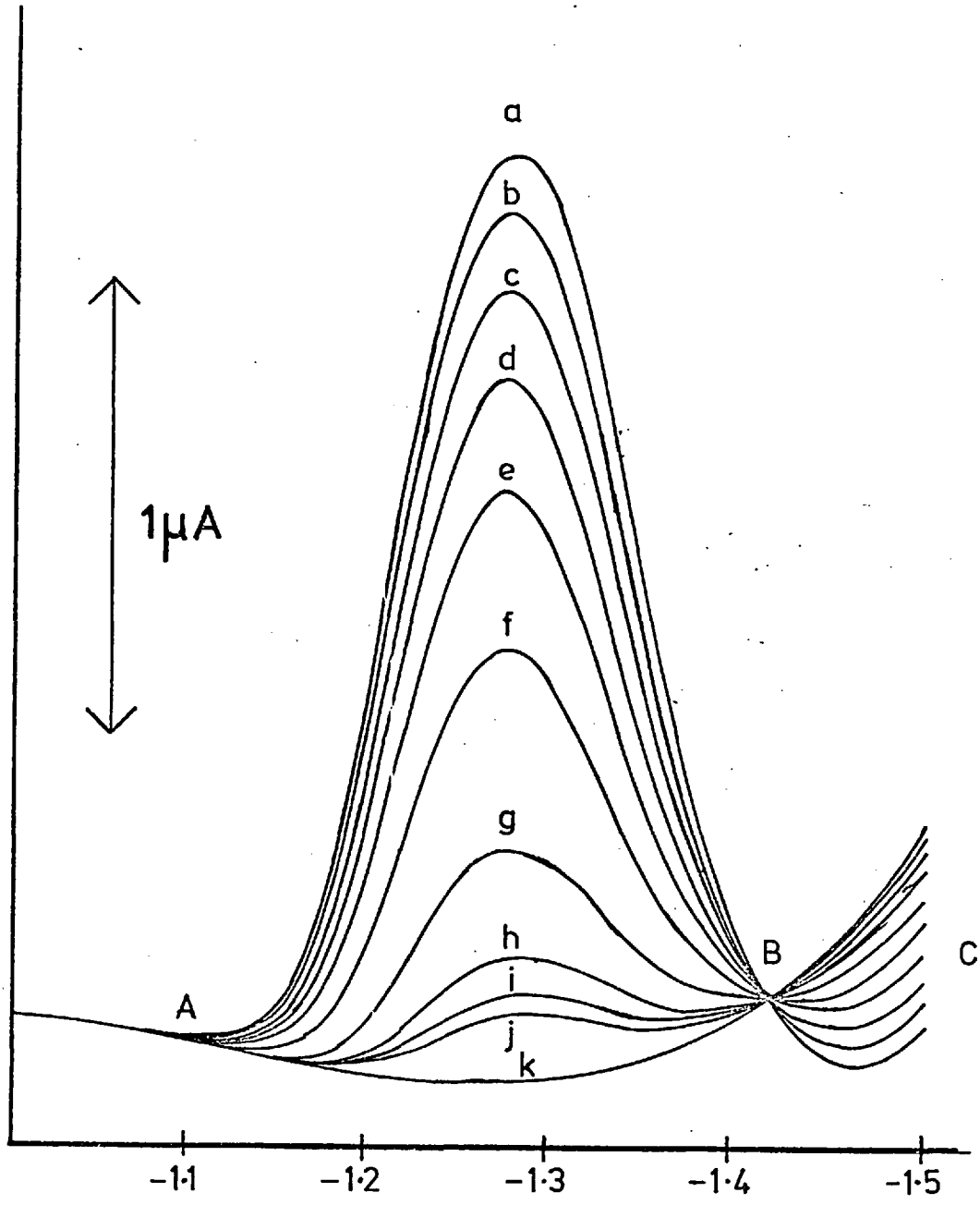


FIGURE 13

Phase sensitive A.C. polarography of MESITYL OXIDE at $20H_z$, $\phi \triangleq 270^\circ$, Δ E = 50mV.

(a) 1.5×10^{-3}M, (b) 1.2×10^{-3}M, (c) 1×10^{-3}M,

(d) 8×10^{-4}M, (e) 6×10^{-4}M, (f) 4×10^{-4}M,

(g) 2×10^{-4}M, (h) 1×10^{-4}M, (i) 7×10^{-5}M

(j) 5×10^{-5}M, (k) blank

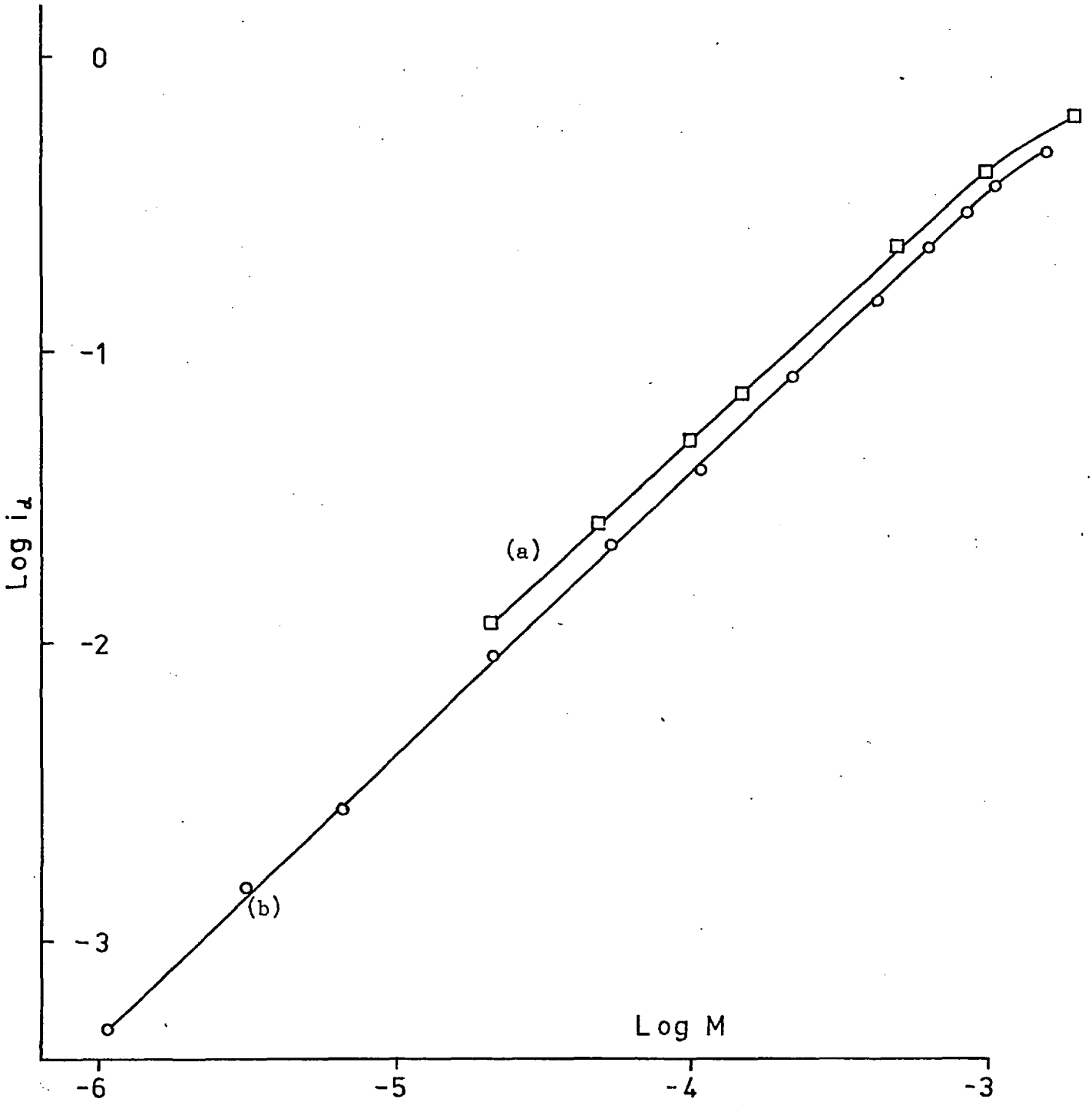


FIGURE 14: Calibration curve for mesityl oxide using a.c. polarography with phase sensitive detection (a) normal scan rate (b) fast scan rate.

time scale. This can only be explained in terms of adsorption of the reduction product, either the free radical intermediate or the dimeric ketone end product and hence it is probable that this adsorption is responsible for the linear id vs h curve. The results are summarised in Table 7.

Table 7

Summary of results for the determination of mesityl oxide by various techniques

METHOD	n	$E_{(volts)}$	L.D.(molarity)
D.C. (C)	0.93	-1.24	1×10^{-5}
D.C. (F)	0.90	-	7×10^{-6}
a.c. (C)	0.87	-1.31	2×10^{-4}
a.c. (F)	0.84	-1.33	7×10^{-5}
a.c. (P,C)	0.68	-1.28	1×10^{-5}
a.c. (P,F)	0.62	-1.26	1×10^{-6}

where C = slow scan; F = fast scan; P = phase sensitive detection; n = apparent n-value (5, 109, 110) and is defined in Equations 6 and 7.

$$n_{(D.C.)} = \frac{56}{E_{\frac{3}{4}} - E_{\frac{1}{4}}} \quad \text{..... Equation 6}$$

$$n_{(wt)} = \frac{90}{W_{\frac{1}{2}}} \quad \text{..... Equation 7}$$

$E_{\frac{3}{4}}$ and $E_{\frac{1}{4}}$ = three quarter and quarter wave potentials (mV)

$W_{\frac{1}{2}}$ = width of wave at half height (mV

L.D. = detection limit (approx.)

E = $E_{\frac{1}{2}}$ for D.C. technique and peak potential E_p for a.c. techniques.

For a reversible electrode reaction all the n and E values should be similar (5, 109). Within the limits of experimental error this would appear to be the case except for those relating to phase sensitive detection. However, as is subsequently shown, the $W_{\frac{1}{2}}$ values of the peaks is markedly increased due to overdamping of the signal and that when this parameter is properly adjusted, the n-value approximates to 1 as predicted for a reversible, diffusion controlled process. It should also be pointed out that the detection limit is lowered with optimum damping.

Detailed examination of the phase sensitive polarogram (109, 157) confirms this observation. Figure 15 shows the dependence of $E_{D.C.}$ vs $\log_{10} X$ where:

$$X = \left[\frac{I_p}{I}\right]^{\frac{1}{2}} \pm \left[I_p \frac{-}{I}\right]^{\frac{1}{2}} \quad \ldots\ldots \text{Equation 8}$$

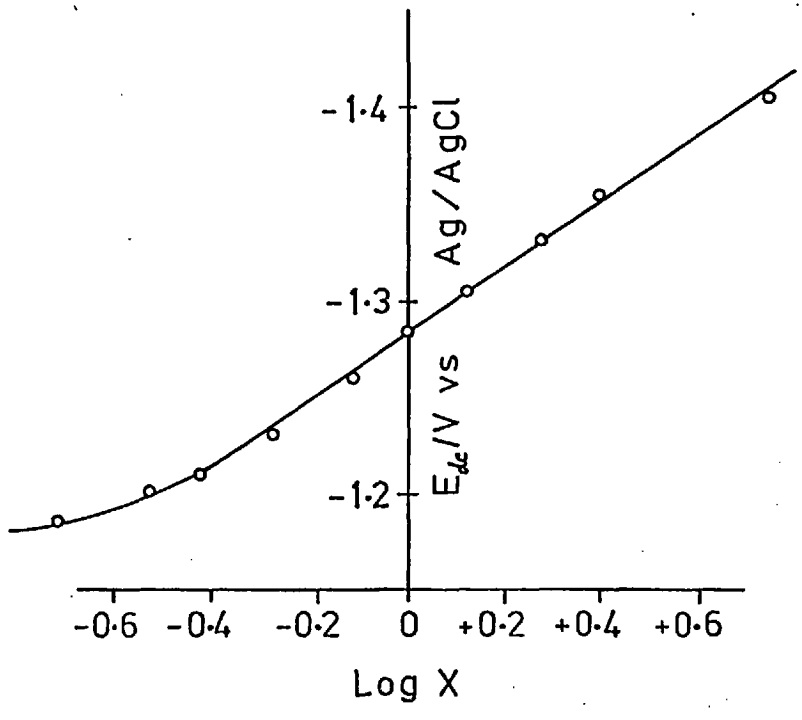


Figure 15 $E_{D.C.}$ vs $\log_{10} X$: a.c. polarography of mesityl oxide with phase sensitive detection.

The linearity is indicative of reversibility but the slope is larger than expected. For a reversible 1-electron reduction, it should be 118 mV instead of 177 mV. This can also be accounted for in terms of overdamping of the signal.

2.1.7. Effect of Low Pass Active Filter

The Effect of filtering on a solution (10^{-4}M) of mesityl oxide is clearly shown in Table 8 which indicates that a minimum damping is preferable to give maximum response and minimum distortion of the signal.

Table 8

Effect of damping the analytical signal at 20H$_z$ and Δ E = 10mV

Filter	h(cm.)	$W_{\frac{1}{2}}$ (mV.)	n
2	-	-	N.D.
3	7.8	90	1.0
4	4.0	105	0.9
5	2.4	125	0.7
6	0.7	150	0.6

Where h = peak height
$W_{\frac{1}{2}}$ = peak width at half height
n = apparent n-value

2.1.8. Effect of amplitude " ΔE" of the applied a.c. voltage

This investigation was carried out on a solution (10^{-4}M) buffered at pH = 4.6, over the range ΔE = 2-100 mV and the results are shown in Figure 16. It is evident that the effect of Δ E on $W_{\frac{1}{2}}$ is negligible below Δ E = 50mV. Theoretical considerations predict optimum results at Δ E = 8mV (109) but this result, which agrees

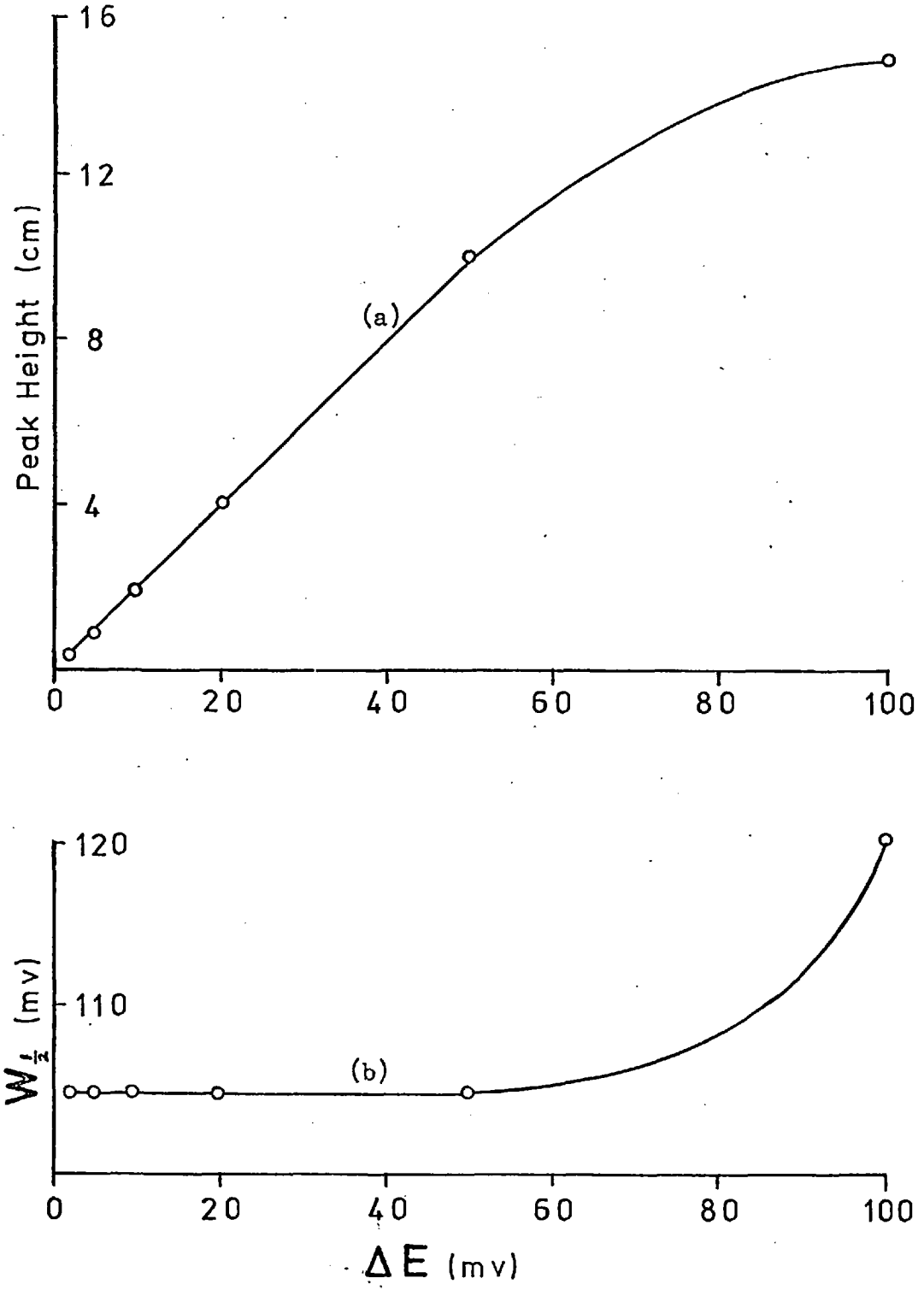


FIGURE 16: Effect of ΔE on the a.c. polarographic response of mesityl oxide (10^{-4}M : (a) peak height vs ΔE and (b) peak width at half height vs ΔE

with the findings of Bond (157) suggests that practically, higher values of ΔE are permissible in order to lower the detection limit. One argument against increasing ΔE is that the corresponding charging current is likewise affected by ΔE. However, as phase sensitive detection discriminates between these, the use of higher ΔE values is justified.

2.1.9. Effect of Frequency of the Applied Signal (157).

At $\Delta E = 10mV$ it was observed that mesityl oxide gave an a.c. response over a limited frequency range. The effect of $(wt)^{\frac{1}{2}}$ on the height (h), half width ($W_{\frac{1}{2}}$) and the peak potential (E_p) is shown in Figure 17. Figures 17(a) and (b) are complementary in that they show a maximum peak height at minimum peak width occurring at about $25H_z$, whereas E_p shows a linear relationship with $(wt)^{\frac{1}{2}}$. This limited range is unexpected and unfortunate and might indicate some kinetic control of the electrode processes. It also indicates the need for caution when using a.c. techniques for monitoring unknown liquid chromatograms, otherwise peaks might be missed out.

2.1.10 Effect of Sweep Time and Time Delay "t*" prior to scanning

Figure 18 shows the effect of sweep time on peak height, peak width and peak potential for time delay periods of one and two seconds. Figure 18 (i) indicates that measurement at the end of the drop life is preferable, as, under these conditions broadening of the peak at half width is minimised and in addition, sensitivity is greater [18(iii)]. However, although the use of longer sweep times gives increased sensitivity (iii), in practice the curvature

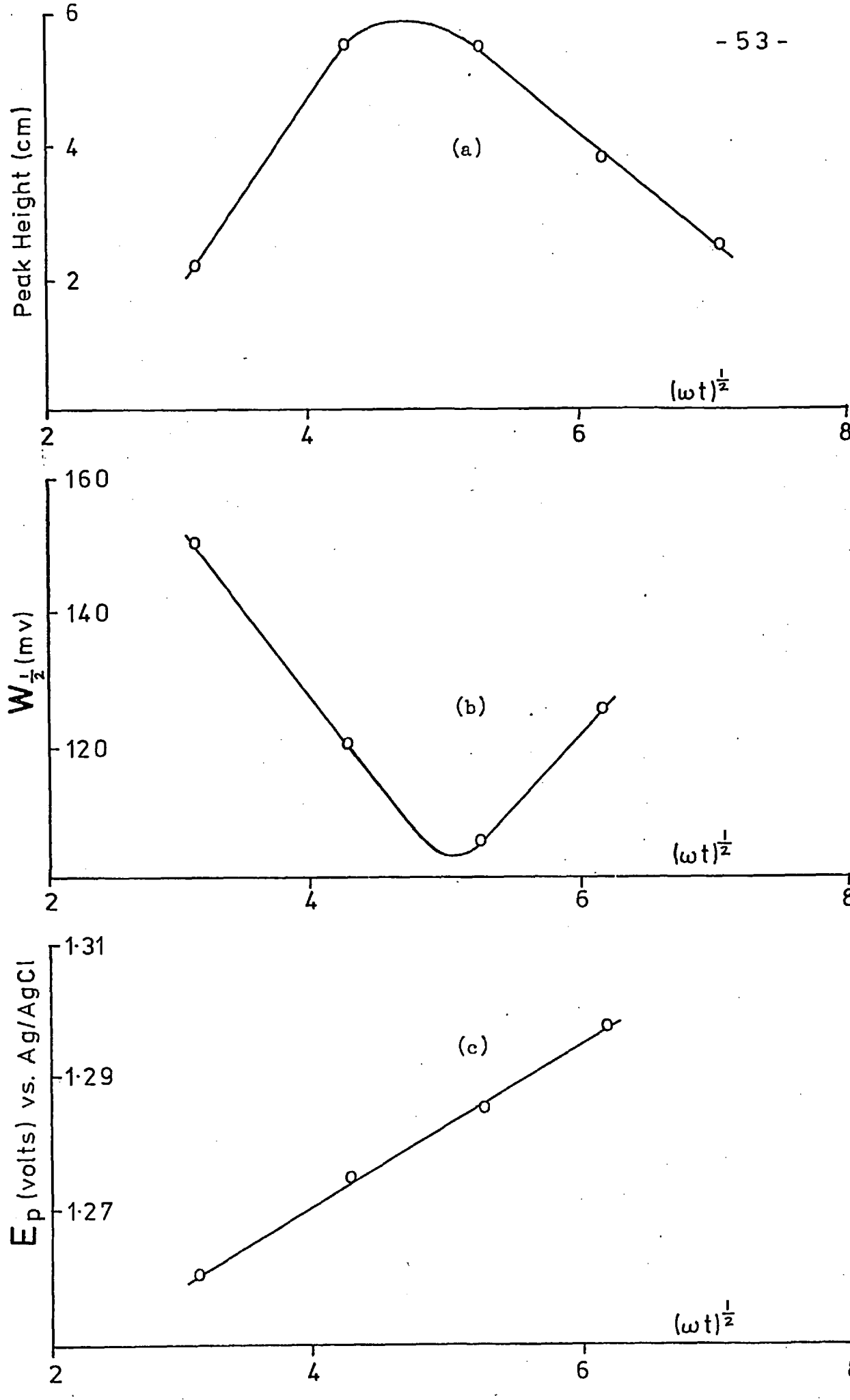


FIGURE 17: Effect of (Wt) on (a) peak height, (b) peak width and (c) peak potential of the a.c. polarogram of mesityl oxide (10^{-4}M)

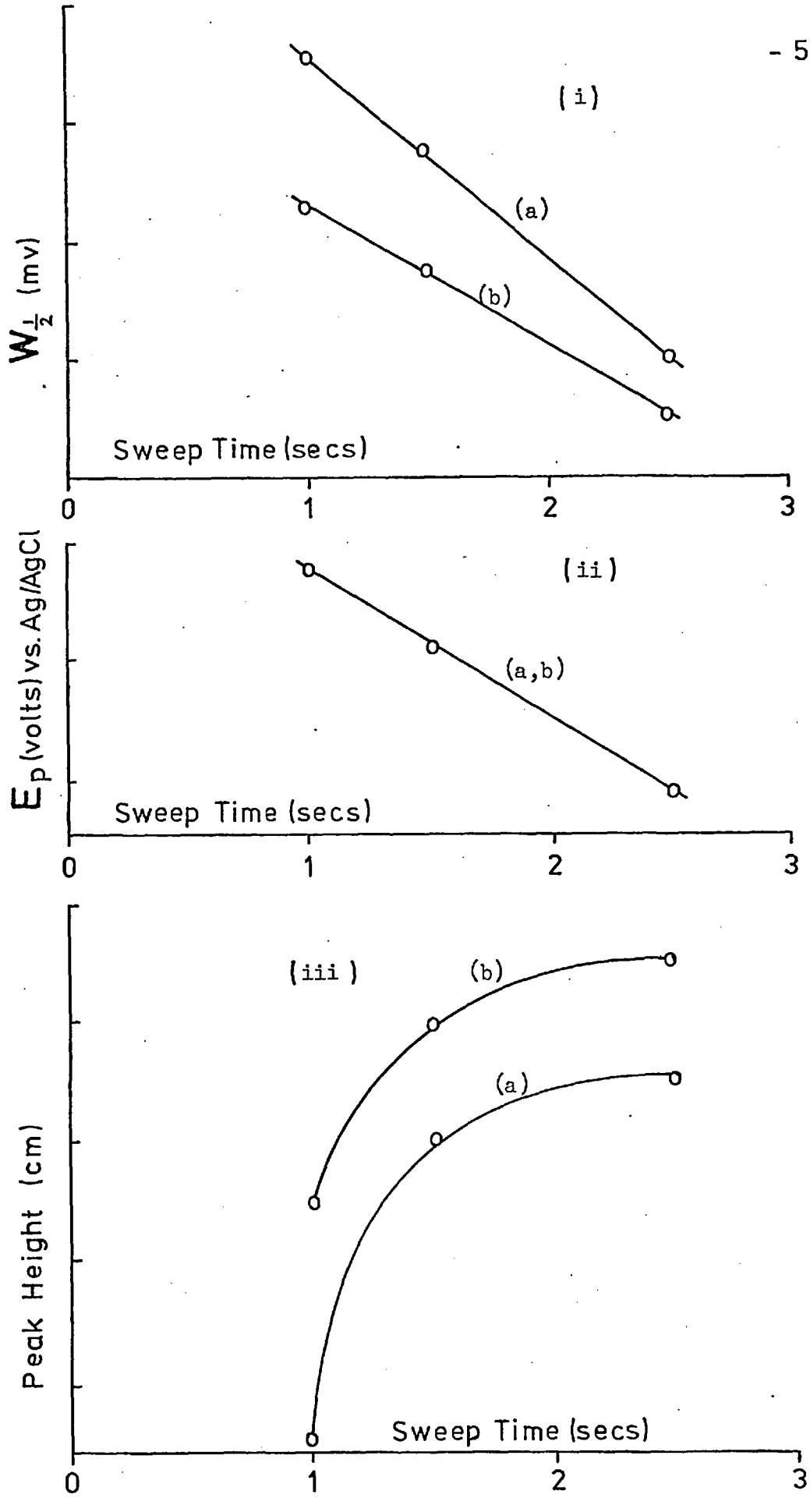


FIGURE 18: Effect of sweep time on i peak width, ii peak potential and iii peak height, where sweep (a) has a delay time of 1 sec. and (b) 2 secs.

of the baseline due to the phase change associated with the drop growth necessitates a compromise. In practice the lower sweep times are preferred due to the minimisation of the phase change during measurement. The loss in sensitivity is minimal and at the maximum values of t*, increase in $W_{\frac{1}{2}}$ is not great.

2.1.11 Quantitative Analysis

Using the fast sweep a.c. polarographic method (with phase sensitive detection) described previously, three samples of diacetone alcohol were analysed for mesityl oxide content. As the evidence indicated that the a.c. process was truly reversible and not quasi-reversible (157) then the effect of diacetone alcohol on the response was not expected to be significant. In order to confirm this, however, a calibration was carried out by two methods (a) direct reading from a calibration curve and (b) a standard addition procedure which allows for the presence of diacetone alcohol.

Diacetone alcohol (~1 ml.) was weighed into volumetric flasks (100 ml.) and made up to the mark with ethanol . Appropriate amounts, ~0.1 ml. for samples 1 and 2 and 1.0 ml. for sample 3 were made up to 10 ml. in the pH 4.6 buffer such that the solutions were 50% aqueous. The polarograms were recorded and the concentration of mesityl oxide calculated from the calibration curve (Figure 14). For the standard addition procedure, polarograms were recorded for the diacetone alcohol solution alone and then after the addition of a known amount of mesityl oxide. The solution concentration was then determined from the ratio of the peak heights. Care was taken to include the dilution factor obtained by diluting a fraction of

the weighed sample. This technique was found to be more accurate than weighing very small quantities of diacetone alcohol directly. The results obtained are given in Table 9.

Table 9

Percentage w/w of mesityl oxide in diacetone alcohol

Sample	Calibration Curve	Standard Addition
1	1.19, 1.20	1.20, 1.20
2	1.20, 1.21	1.21
3	0.046, 0.043, 0.046	0.044, 0.043

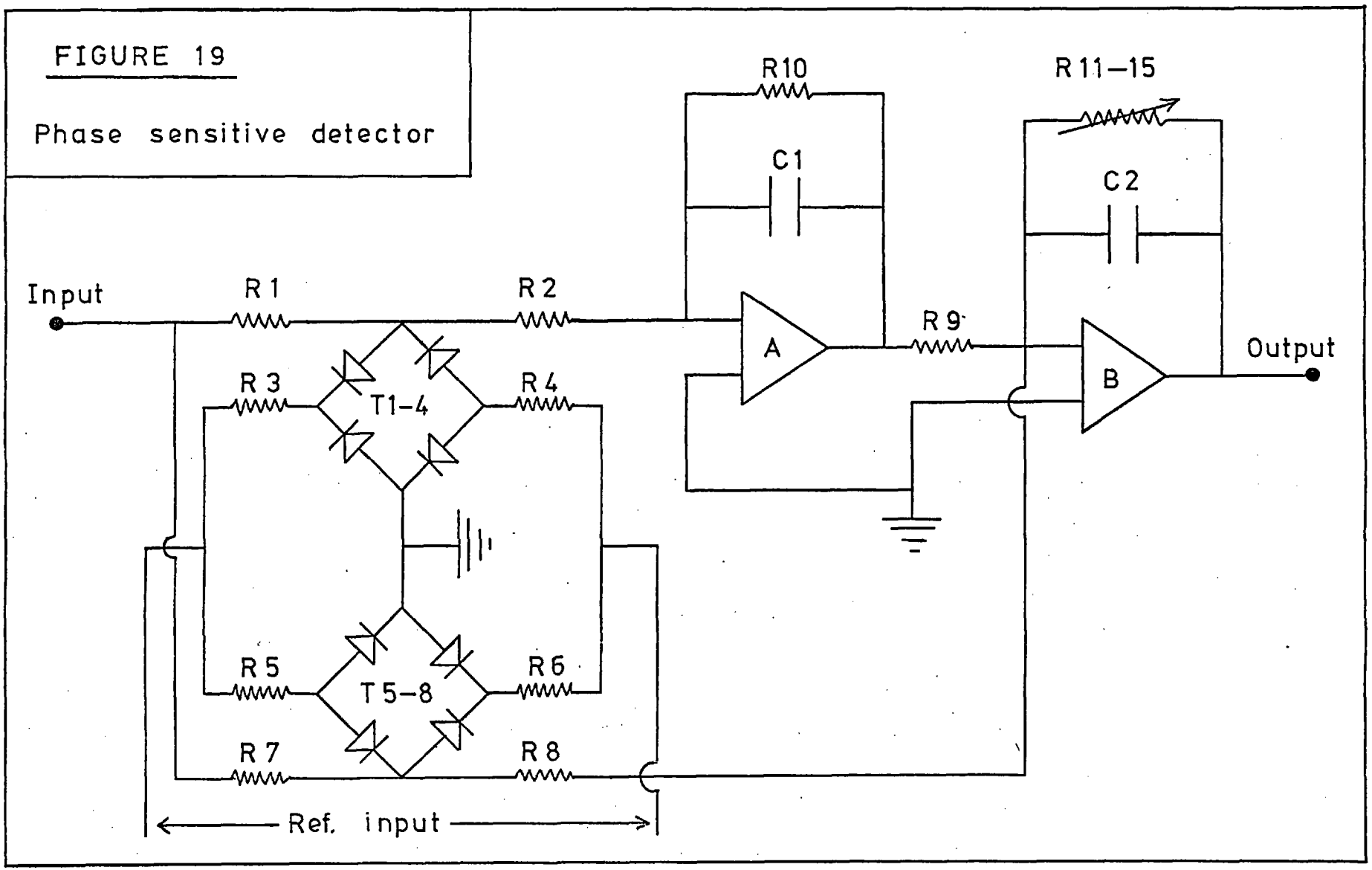
The standard addition results support the assumption concerning the effect of diacetone alcohol on the response. Both procedures would appear to be applicable. Some improvement of the precision would be expected if peak area integration were employed (158). This technique is well suited to the present phase sensitive detection method as the almost linear baseline is ideal for such measurements.

2.1.12 Improvements to the Electronic Circuitry

This study has shown that a.c. ploarography is probably suitable for L.C. monitoring providing phase sensitive detection is used. The circuitry of the phase sensitive detector (109) built for this purpose is shown in Figure 19. Although built by R.D. Jee (139), the circuits for the square wave reference signal and the phase shifter (109) are given in Figure 20 as they have not been published in detail.

FIGURE 19

Phase sensitive detector



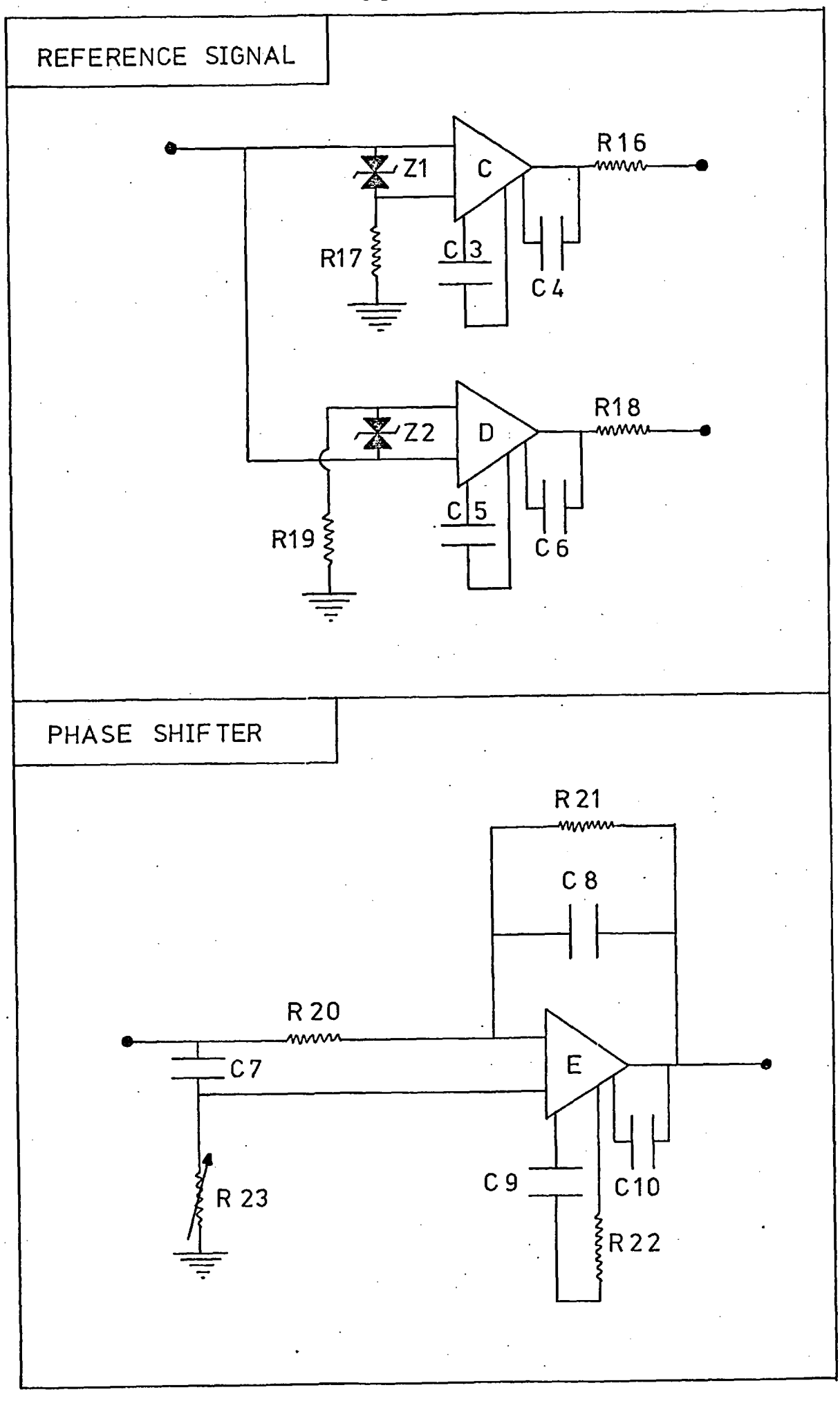
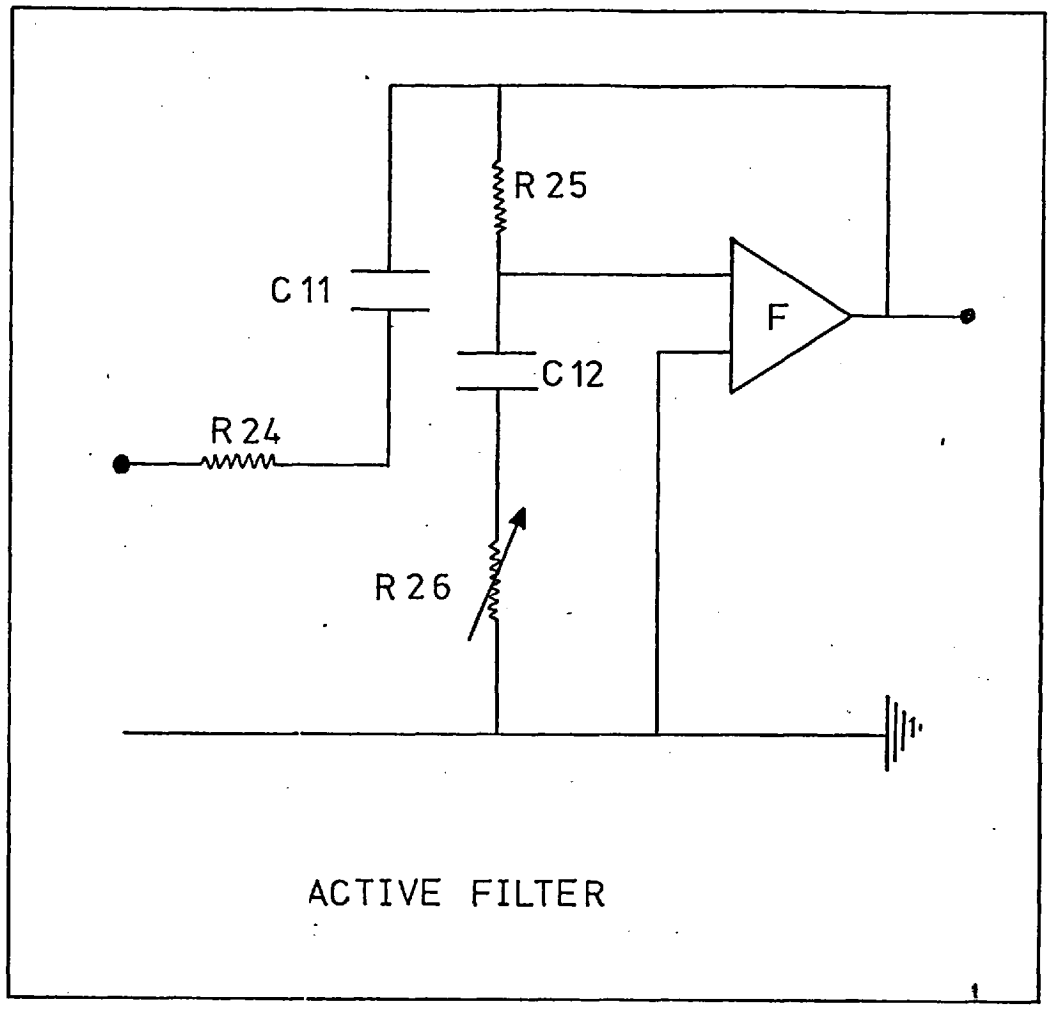


FIGURE 20 Circuitory for the REFERENCE SIGNAL and PHASE SHIFTER of the Phase Sensitive Detector.

So that a range of fundamental frequencies could be used an ACTIVE bandpass filter (257), Figure 21(a) was built. Thus increasing the scope of the phase sensitive detection method. It should be used as shown in the block diagram Figure 21 (b).

It has been stated that second harmonic signals reduce the effect of the non-Faradaic current and to utilise this, a frequency doubling circuit (258) was built, Figure 22(a). It should be used as shown in the block diagram Figure 22(b).



Choice of resistors R24 - R26 was made from the following expressions where $C = 0.1\mu F$, $\Delta = 10Hz$ and $G = 10$

RESISTANCE	FORMULA
R 24	$(2\pi\Delta GC)^{-1}$
R 25	$(\pi\Delta C)^{-1}$
R 26	$\left\|2\pi[(2f^2 \div \Delta) - \Delta G]\right\|^{-1/2}$

FIGURE 21 Active bandpass filter with adjustable centre frequency and constant bandwidth.

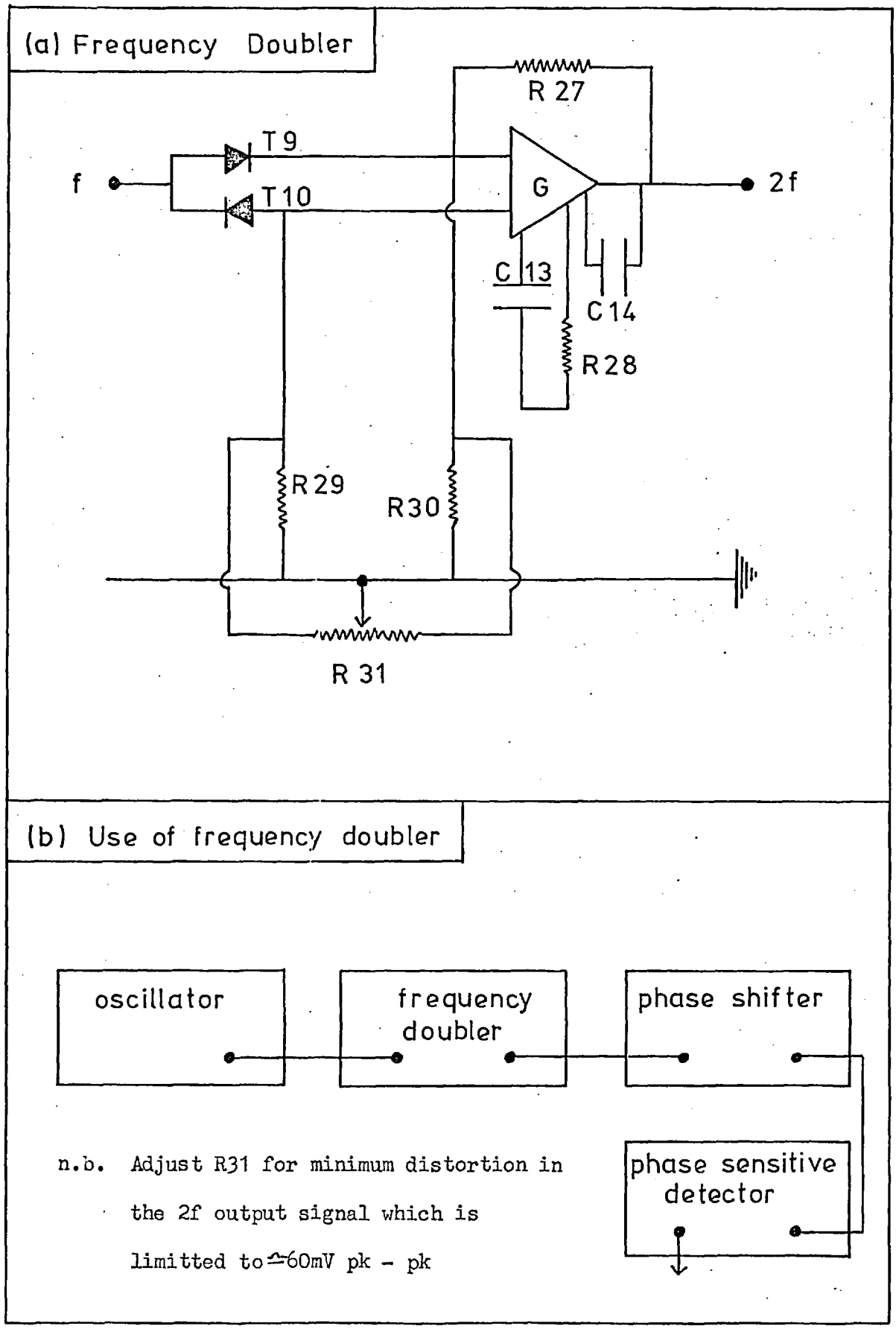


FIGURE 22 (a) Frequency doubling circuit and (b) How to incorporate (a) into the polarographic equipment.

2.2 Choice of Electrode

Historically, the mercury electrode has predominated in electroanalytical chemistry. This is undoubtedly due to the fundamental advantage of the dropping mercury electrode, namely its continually renewed surface. It also has a high hydrogen overvoltage which means that the majority of inorganic and organic species can be reduced before hydrogen is evolved from the reduction of water as illustrated in the following scheme.

$$H_2O + e^{-1} \longrightarrow OH^- + H$$

$$2H \longrightarrow H_2$$

This reaction, or the reduction of the supporting electrolyte, effectively limits the potential range of the electrode and consequently it limits the coupounds that can be determined at the electrode.

If a positive, instead of a negative, potential is applied to the working electrode, oxidation governs the electrode reactions. Unfortunately, mercury is readily oxidised and consequently cannot be used at more positive potentials than about +0.4 volts (vs. calomel reference electrode). In practice this is difficult to achieve.

In non-aqueous solvent systems the situation is somewhat different. If water is eliminated from the solvent (a difficult operation in practice) and if a supporting electrolyte such as tetrabutyl ammonium perchlorate, tetrafluoroborate or hexafluorophosphate is used, the useful potential range is increased considerably.

However, it has already been mentioned that electrooxidation at a mercury electrode is greatly restricted by oxidation of the mercury itself and to overcome this problem, much work has been done in recent years to evaluate and use solid electrodes (93,103).

The choice of electrode for L.C. detectors is thus an important criterion and depends on many factors that will be discussed as appropriate. A summary of some of the more important electrode materials is given in Table 10.

2.2.1 Dropping Mercury Electrode

The dropping mercury electrode (D.M.E.) consists of a glass capillary, about 0.5 cm. external diameter with a capillary bore of 0.01 - 0.1 mm. internal diameter, from which mercury flows in the form of drops. The capillary is connected to a mercury reservoir, the height of which affects the drop rate. (5).

The major advantages of such an electrode ensue from its high hydrogen overvoltage which enables very negative potentials to be reached before hydrogen evolution occurrs and the continuously renewed electrode surface which enables the recording of polarograms which would otherwise be difficult due to the adsorption of a variety of species on the electrode surface. Of significant importance is the minimisation of depolariser depletion in the solution resulting from the stirring effect of the mercury droplets falling through the solution.

However, its use is limitted to a potential range of +0.4 to -2.6V in aqueous solutions. These limits are not always attainable,

TABLE - 10 some electrode materials

Electrode Material	Overvoltage		Adsorption	Comments
	-ve	+ve		
Hg - DME / GDME	High	Low	———	Renewable surface
Hg - streaming	High	Low	———	High Hg consumption - constant, high, surface area
Hg - hanging drop	High	Low	√	Limited appl'n
Hg - film	High	Low	√	" "
Pt	Low	High	√	
Au	High	"	√	Underated
Cr	—	Med	√	Specific appl'n
Ni	—	"	√	" "
Co	—	"	√	" "
C - WIGE / paste	High	High	√	H_2O sol'n only
C - pyrolytic	"	"	Slight	Wide appl'n
C - vitreous	"	"	v. Slight	" " - material of choice
C - fibre	"	"	"	Promising
B_4C	"	"	"	Not reproducible - promising

depending on the solution composition. Although mercury is toxic and has a high vapour pressure, this has had little influence on its use over the last fifty years. Of greater industrial importance particularly in the context of its use with detectors for high pressure liquid chromatography is the difficulties of handling it. It is so easy to spill mercury which then forms minute droplets which are very difficult to pick up. It should only be used in the triply distilled form and is very expensive. Due to the growth and fall of the drop, current oscillations appear on the recording which can cause problems of damping when used for low level chromatographic detection.

2.2.1.1. Streaming Mercury Electrode

This modification of the D.M.E. (5,159-162) overcomes the problem due to current oscillations, is less prone to capillary blockages and gives larger electrolytic currents than the D.M.E.

For liquid chromatographic detection, however, the very high rate of mercury consumption is incompatible with the small volume of the detector.

2.2.1.2. Gas Pressure Operated Dropping Mercury Electrode

In an effort to reduce some of the practical difficulties of using a D.M.E. in an L.C. detector, the use of a gas pressure operated dropping mercury electrode (G.D.M.E.) was investigated (163) and its performance compared with the conventional D.M.E. controlled by a head of mercury.

The electrode design used is shown in Figure 23 and all measurements were made with a common capillary, mercury column and

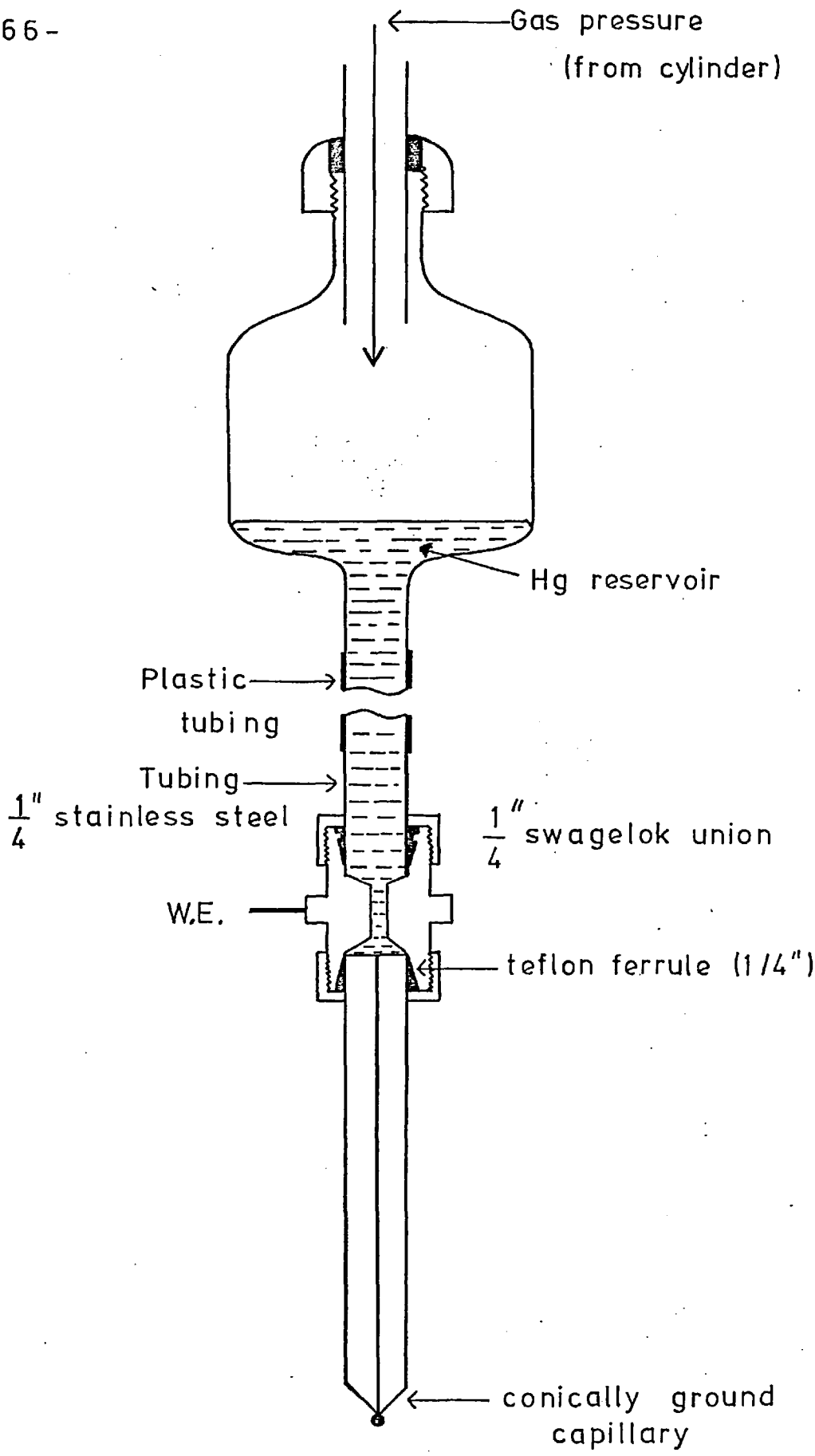


Figure 23 experimental arrangement for G.D.M.E.

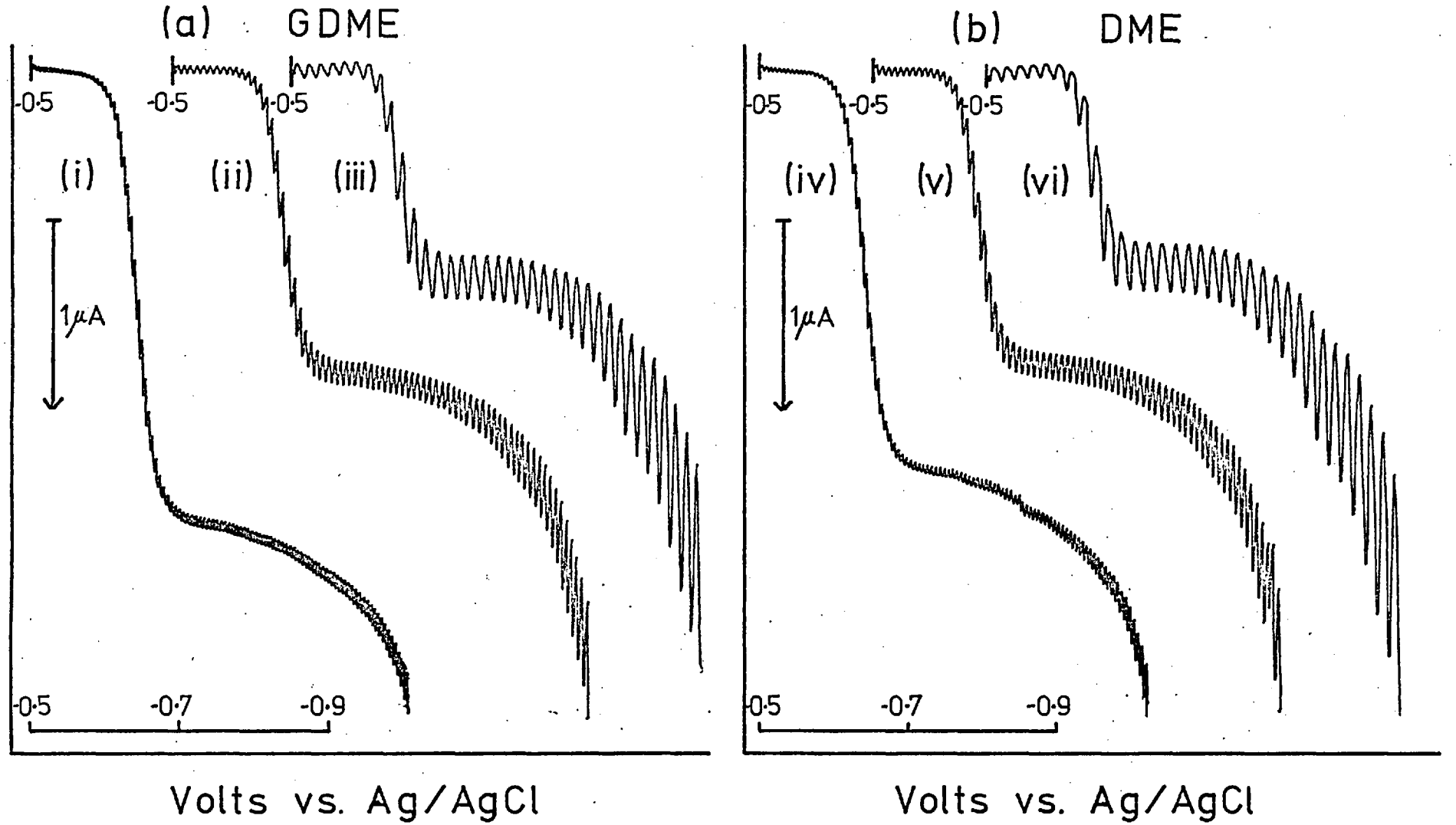


Figure 24: Polarograms for aqueous cadmium solution 1.10^{-4}M in 0.5M HCl at high (1) and (iv), medium (ii) and (v), and low (iii) and (vi) mercury outflow rates.

mercury reservoir. For normal use, the G.D.M.E. could be connected directly to the reservoir. The polarographic measurements were performed in a 3-electrode cell (139) as previously described. Solutions were degassed with solvent saturated nitrogen prior to analysis and the polarograms were recorded on a multipurpose instrument (139) operating in the D.C. mode.

Aqueous Solution. An aqueous solution of cadmium (10^{-4}M) in hydrochloric acid (0.5M) was used in this comparison. Figure 24 shows the polarograms obtained for high, medium and low mercury flow rates and indicates that they are sufficiently similar to justify the routine use of the G.D.M.E. in aqueous solution.

The limitting currents were plotted against their corresponding mercury heights or gas pressures (Figure 25) resulting in similar rectilinear relationships confirming that the electrode processes were unaltered by the change in electrode configuration.

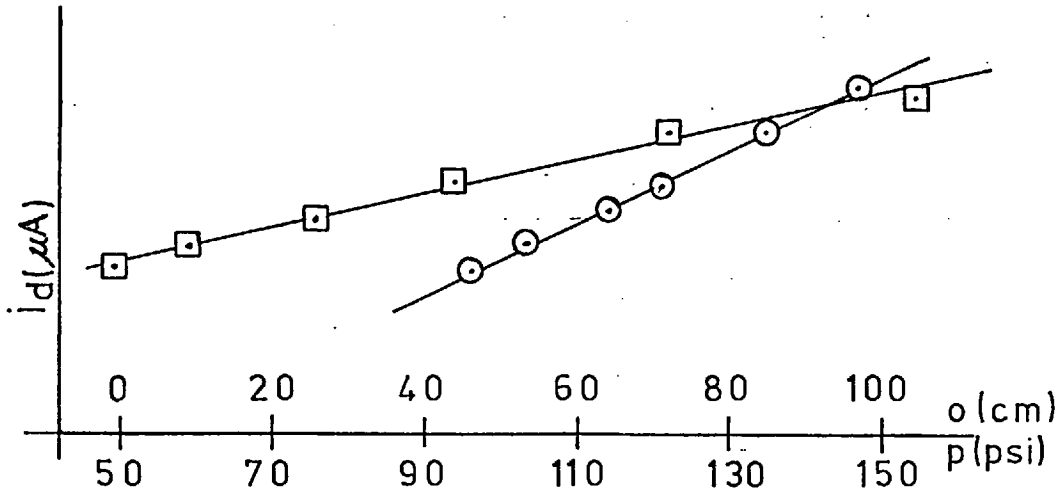


Figure 25 Limitting current relationship with height of the mercury column (O) or gas pressures (□)

The major difference between the electrodes was in the regularity of the drop rate (t) at high negative potentials as illustrated by the electrocapillary curves (Figure 26a). These show similar and reproducible "t" values up to -1.2 volts. At higher negative potentials the conventional D.M.E. behaved erratically whereas the G.D.M.E. maintained its reproducible drop rate.

Non-Aqueous Solution. A solution of anthraquinone (10^{-4}M) in acetonitrile (Spec. Pure) containing tetrabutylammonium perchlorate (T.B.A.P.) as depolariser (0.1M) gave similar polarograms and rectilinear current vs. mercury column height or pressure relationships.

As in aqueous solution, the major differences were observed in the regularity of the drop rate (Figure 26b). In the system examined the effect was more pronounced than in aqueous solution. Furthermore, the irregularity due to the conventional D.M.E. occurred only in the region involving the two reduction waves of anthraquinone corresponding to the formation of radical and charged species at the mercury drop (164). When premature drop fall occurs, solution can be seen to travel rapidly up the capillary to a height of 1-2 cms. This effect was not observed with the G.D.M.E.. At higher negative potentials the irregularity decreased until no distinction between the two electrodes (at -2.0V) occurred.

No disadvantages were experienced with the G.D.M.E. and apart from a more stable drop rate, other, minor advantages soon became evident.

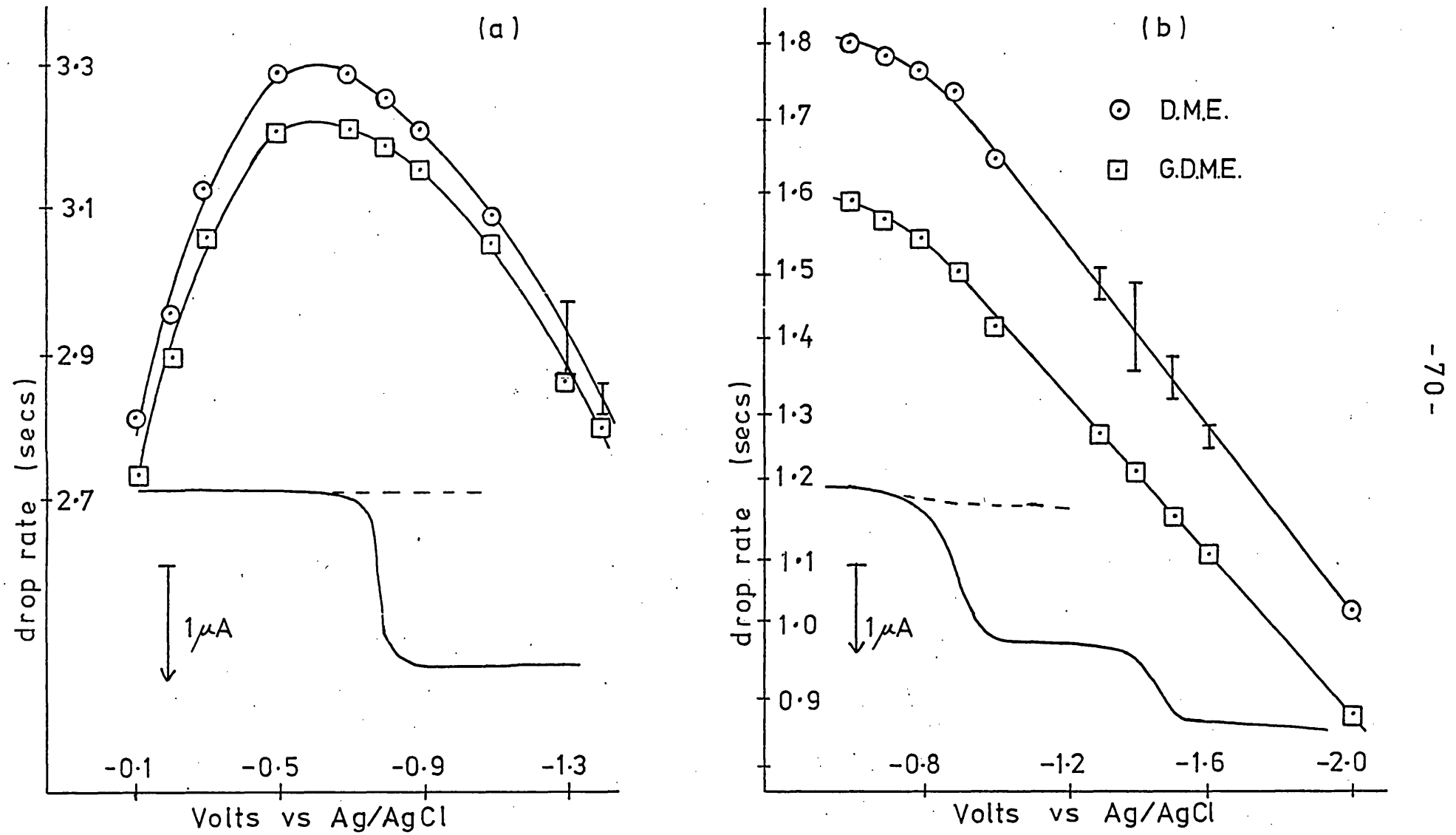


Figure 26: Electrocapillary curves for (a) 1.10^{-4} M Cd^{2+} in 0.5M HCl and (b) 1.10^{-4} M anthraquinone in acetonitrile containing 0.1M tetrabutylammonium perchlorate.

The capillary can be connected directly to the reservoir, thus eliminating the need for a large mercury reservoir and flexible connecting tube which is subject to movement and temperature fluctuations.

The G.D.M.E. is ideally suited to fast sweep and pulsed polarographic techniques where drop synchronisation is very important. If fine bore capillary tubing is used, only short lengths (3-5 cm.) are necessary and in this configuration we are using the G.D.M.E. embedded into the body of a detector for H.P.L.C. (165). We have used a similar system for controlling the mercury flow through a teflon capillary as prepared by Bond (166). Further investigation is obviously needed to understand the mode of action of the G.D.M.E.

2.2.2. Solid Electrodes

Solid electrodes (93) are ideally suited to the construction of detectors for use with high pressure liquid chromatography and the resulting detector is far easier to use in practice. No mercury reservoirs are needed, no capillary problems occur and because the area of the electrode is constant, the noise levels are greatly decreased. As indicated in Table 10, solid electrodes, apart from a few such as platinum, have a wider application than mercury in terms of over potential restrictions. Gold in particular has a wide anodic and cathodic range but has been used very little due to the popularity of the various carbons.

There is one aspect of solid electrode voltammetry that

imposes severe restrictions on the use of solid electrodes, namely ADSORPTION of a variety of species onto the surface of the electrode which occurs by physical adsorption chemisorption,reaction of the electrode surface (e.g. oxide formation), coverage of the surface by polymer films, the accumulation of the primary products of electrolysis and others, some of which have yet to be characterised. as the science of adsorption is,as yet,still young. To complicate matters, different electrode materials have different adsorption characteristics which can be attributed to the differences in surface structure. Catalytic activity is not uncommon.

Various attempts have been made to eliminate or reduce the effects of adsorption such as subjecting the electrode to hydrodynamic conditions or continuously grinding the electrode surface to renew the surface (99,100,102) or potential cycling techniques (102).

It has already been stated (section 1.2.9.1.) that the hydrodynamic approach is more readily adaptable to the requirements of a detector for H.P.L.C. The name implies tha t the solution around the electrode is moving relative to the electrode and this movement can result in the removal of adsorbed species from the electrode. Various methods of achieving hydrodynamic conditions have been tried and the most efficient or convenient methods in normal usage are not necessarily the most convenient when applied to flowing streams. For example, vibrating electrodes (167-171), rotating electrodes (172-180) and in particular, rotating disc electrodes (181-186) have contributed significantly to solid electrode voltammetry, the latter is particularly useful in mechanistic studies (93), but their incorporation into small volume detectors poses many engineering problems.

Stationary electrodes in a variety of geometries (93) have been used in flowing streams and although their analytical applications are few compared with the rotating disc electrode, the principle is exactly that which exists in a liquid chromatographic detector.

Finally, it should be recognised that the electrode reactions of many organic molecules at solid electrodes are irreversible. This adversely affects the sensitivity of several techniques such as D.C. and a.c. polarography. However, pulse polarography is reputedly applicable to irreversible processes and consequently much of the latter work in this thesis has been performed by that technique.

2.3. Carbon Electrodes

Of the solid electrodes shown in Table 10, the carbons show the most promise as electrode materials. In general, they can be used over a wide range of potentials, both negative and positive and with a few exceptions (such as carbon paste), can be used in non-aqueous solvents.

2.3.1. Graphite Rod/Paste

Graphite has a comparatively high permeability (10^{-3} to 10^{-6} $m^2.sec^{-1}$) (104) which results in rapid adsorption of various species onto the electrode surface and into the bulk of the electrode. Two approaches have been used to fill these pores of the graphite with inert material. Firstly impregnation with molten wax (187,188) to form the Wax Impregnated Graphite Electrode (W.I.G.E.). These electrodes give excellent anodic and cathodic ranges but can be subject to anomalous high residual currents which are apparently due to variations in impregnation procedures. Secondly by preparing a

graphite paste (93,189) by mixing graphite powder with an organic solvent, such as nujol, that is immiscible with the solution. Both of these electrode forms are unsuitable for electrolysis in organic solvents.

The carbon paste electrode has virtually zero residual current and can be used over a wide anodic and cathodic range (e.g. -1.4 volts in 1M NaOH to +1.3 volts in 0.1M H_2SO_4). It is easy to use in practice and has been used successfully as the working electrode of a low volume detector for high pressure liquid chromatography (190) using aqueous perchloric acid (0.1M) as eluent.

2.3.2. Pyrolytic Graphite

Pyrolytic Graphite Electrodes (P.G.E.) are not really graphite at all but should properly be called "pyrolytic carbon" electrodes and consist of crystallites, each composed of a group of imperfect hexagonally bonded carbon sheets which are parallel but not necessarily in stacking registry with each other. This structure is termed "Turbostratic" (104,191).

There are two distinct ways of producing pyrolytic graphite (104). A gaseous hydrocarbon, (methane or propane) which is usually mixed with an inert carrier gas is passed over a large heated substrate which is to be coated with pyrolitic graphite. The graphite is deposited, often under reduced pressure, and forms highly ordered carbon crystallites in layers, parallel to the substrate surface. Secondly, a more controllable technique entails making a fluidized bed of substrate particles in a hot zone. The hydrocarbon gas

maintain s the fluidized bed within which there is excellent heat transfer. A combination of these methods utilises the best features of both techniques by suspending a large object in the fluidized bed. The pyrolytic graphite so formed depends on the nature of the hydrocarbon gas, the temperature of pyrolysis, the contact time which is related to gas flow rate and the ratio of surface area of substrate to volume of pyrolysing gas. By altering the composition of the gas, it is possible to "alloy" the graphite, for instance with boron (192) to obtain special properties. Similarly if titanium is added during deposition (193), regions of zone crystalline graphite are nucleated and gradually grow at the expense of the turbostratic material. It is likely, that some of these carbon alloys will be of considerable interest for electrode materials in the future.

Formed in this manner, pyrolytic graphite is "anisotropic", being highly impervious to liquids only if the crystal planes are correctly orientated. (i.e. minimum permeability $\sim 10^{-14}$ $m^2.sec^{-1}$). For this reason, it has been used extensively in recent years as an electrode material (105,194-196). Care must be taken to allign the planes correctly and seal the edges with epoxy resin (e.g. Araldite) to prevent adsorption and absorption into the bulk of the electrode. Although not readily available commercially, pyrolytic graphite of low anisotropy (197) can be prepared and entirely non-porous pyrolytic graphite can be made if annealing processes are used after deposition. (198-200). This material should certainly be investigated as a future electrode material.

It has been shown (105) that the method of preparation of the electrode surface is vital in voltammetry. Grinding the graphite produces a shiny, flat surface but produces a high surface area electrode that is superficially porous. This is attributed to the macroscopic layered structure extending to the microscopic structure (201) and produces sharper more reversible peaks by cyclic voltammetric measurements (105). However, the surface retains electroactive species, is not reproducible on resurfacing and produces a relatively large residual current. If the graphite is cleaved (195) along the macroscopic layer structure, coarser surfaces are produced which give lower peak currents, the electrode processes are more irreversible, but lower residual currents are obtained. This procedure is the preferred technique and until high grade pytolytic graphite can be readily obtained and chracterised, glassy carbon would appear to be more useful in solid electrode voltammetry.

Several reasons are proposed (105) for the adsorption phenomena that alter the nature of graphite electrode surfaces.

(1) Chemisorbed oxygen may exist by itself or may serve as an attractive site for water molecules.

(2) Tenaciously held water which limits the use of the electrode in non-aqueous solvents.

(3) Carbon-oxygen reaction sites that may cause complexes to be localised at certain stress regions on the surface.

(4) Carbon-oxygen surface compounds such as quinoids, quinhydrones, phenols and carbonyls. Also sulphate, hydrogen bonded

interlayer groupings and free radicals may be involved (202,203).

(5) Mellitic acid derivatives such as pyromellitic acid, chloroquinone, pentacarboxychlorobenzene and similar compounds which could be produced under mild oxidising conditions (204,205).

(6) Lammellar compounds or their residues such as bisulphates or other species that are difficult to oxidise or reduce and which are formed when graphite electrodes are subjected to strong oxidising conditions.

(7) Surface films which constitute a broad field of polymers and could encompass any of the previously listed effects as well as other unknown effects peculiar to the dense structure of pyrolytic graphite.

The formation of some of these surface effects can be visualised by considering the result of grinding pyrolytic graphite surfaces in air. Bonds are broken between adjacent atoms and between planes. The latter is particularly serious for pyrolytic graphite, the energy released is partially dissipated as heat. However, the surface area and surface energy is vastly increased which provides an ideal environment for oxidation of the carbon by atmospheric oxygen, to form the types of C-O compounds postulated by Boehm (206). These occur on adjacent carbon atoms on the edges of the hexagonal planes of the graphite and can lead to hydrogen bonding when hydroxyl groups are present. These compounds then interfere with the reduction or oxidation of other species when used in voltammetric studies.

2.3.3. Vitreous Carbon

"Vitreous" or "glassy" carbon is very different to conventional graphites and carbons. It has a low porosity and permeability ($\sim 10^{-15}$ $m^2.sec.^{-1}$), is isotropic and therefore does not suffer from the limitations of pyrolytic graphite in having to be properly orientated. It is more inert to a wide range of environments and has a low electrical resisitivity (3 to $8x10^{-5}$ ohm.m. compared to 150 to $250x10^{-5}$ ohm.m. for pyrolytic graphite).

It is formed from non-graphitising carbons produced by charring polymers having some degree of cross-linking which inhibits the reorientation of the graphitic nuclei into crystallites during subsequent heat treatments.

By pyrolysing these polymers under closely controlled conditions, glassy or vitreous carbon is produced (207).

The expansion coefficient of vitreous carbon is so close to that of some borosilicate glasses that a satisfactory carbon/glass seal can be made. This could be useful in electrode manufacture, although araldite seals have proved to be quite successful. Because the carbon is so hard and brittle, machining and drilling can only be carried out with diamond or ultrasonic techniques.

Being isotropic, glassy carbon does not suffer from the same problems of grinding associated with the preparation of pyrolytic graphite electrodes. Grinding with 600 mesh SiC paper produces a flat surface which, under magnification, reveals microfine scratches

over the entire surface (105). These were found to be responsible for the very high residual currents in voltammetry. For example, electrodes treated with 600 mesh SiC paper gave a residual current of 70μA which after lapping with 0.5μ alumina produced residual currents of only 0.5μA.

As with cleaved pyrolytic graphite it is advisable not to use surfactants such as Triton X-100 with glassy carbon as it drastically suppresses electroactivity, due to rapid adsorption onto the electrode surface. This appears to be more significant in dimethylformamide solutions where Triton X-100 causes reduced peak height, shifts the anodic potential in a positive direction and the cathodic potential in a negative direction and causes rounding of the peaks obtained in cyclic voltammetry. Resurfacing is readily accomplished by immersing the electrode in a nitric-sulphuric acid mixture (6).

Both glassy carbon and pyrolytic carbon electrodes function well in non-aqueous solutions and appear to give higher currents in solvents of low viscosity as diffusion to the electrode is then more rapid. Acetonitrile is one of the best organic solvents, being of low viscosity and producing the most reversible voltammograms on all solid electrode materials. This may not be such an important criterion for detectors used in hydrodynamic voltammetry or high pressure liquid chromatography as the forced convection conditions prevailing aid diffusion to the electrode and remove adsorbed species. Under similar conditions glassy carbon produces better defined voltammograms and is less prone to adsorption.

2.3.4. Carbon Fibres and Whiskers

Graphitic Whiskers are produced (208) by forming a d.c. arc between carbon electrodes in an inert gas atmosphere at pressures of about 90 atmospheres. They are a few microns in diameter and up to 3 cm. long and consist of graphite sheets, several hundred μM thick and rolled up into a tight scroll. Although very strong, their application as electrode materials is probably not as important as that of Carbon Fibres.

Carbon Fibres are made by a complicated pyrolysis procedure that inhibits the cross-linking of the fibres (209-210) that exists in glassy carbon. Some fibres clsely resemble glassy carbon and therefore offer very good properties for electrode material.

Very little work has been attempted to utilise carbon fibres electrochemically, but it is known that electrochemical oxidation of the fibre can occur (211) as in other carbons. Its most notable application to date is under hydrodynamic conditions (212-215) where a flow-through electrode consists of a bundle of fibres arranged such that a liquid flow is directed along the fibres. Such an electrode has a high surface area and comparatively high residual current but this is compensated for by large hydrogen overvoltages, rapidity of overall electrode reaction and ease of quantitation. It is expected to find wide application in kinetic studies and could prove to be a useful electrode material for L.C. detector design.

2.3.5 Intercalation Graphite Compounds

Graphite can absorb many metal atoms or radicals between the carbon sheets. When the proportion is approximately stoichimetric, the product is called an intercalation or crystal compound (216,217). Many species can be introduced into the carbon framework such as nitrate (218), sodium and potassium (219,220), bromine and chlorine (221-223) not only into single crystal graphite but also pyrolytic graphite, carbon fibre (224) and vitreous carbon (225). Carbon alloys with boron (192) and titanium (193) have already been mentioned in the preparation and modification of pyrolitic graphite. In fact, impure boron carbide has found some use as an electrode material (226,227), where it appears to be chemically inert with a wide potential range and low residual current. It has not achieved much prominence due to difficulties in mounting the electrode and in reproducibility of the samples. The alloying technique of manufacture might solve this problem.

It is difficult to assess the potential of such carbon compounds as they are not readily available and electrochemical data is scanty. Apart from the possibilities of boron alloys, it is thought that they may have interesting catalytic properties and thus form more specific electrode systems (228).

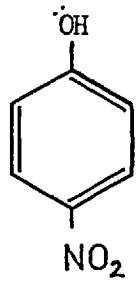
2.3.6. Conclusions

The best solid electrode material to date is probably vitreous carbon. Its permeability, resistance to chemical attack and to adsorption, and low residual current are all desirable properties which outweigh the disadvantages. The latter are not too serious

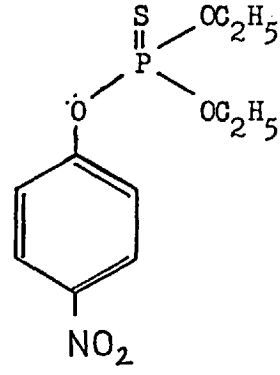
but the hardness and brittleness of the material make it necessary to use special techniques to grind or drill the material. Like all the other carbons, it does suffer from some adsorption problems and it will be shown in chapter three that the most serious problems we have found are associated with carbonyl containing compounds. Providing hydrodynamic conditions prevail, we have found the least trouble to occur when operating under anodic conditions.

2.4. Voltammetry at Vitreous Carbon Electrodes

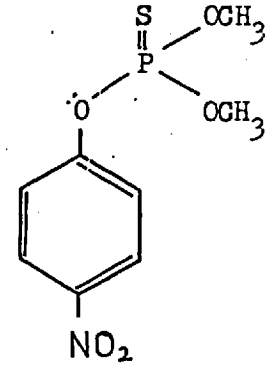
In order to justify to use of vitreous (glassy) carbon as an electrode material suitable for use in liquid chromatographic detectors, the voltammetry of paranitrophenol (VI), parathion (VII), and methyl parathion (VIII) was studied in both still and stirred solutions.



(VI)



(VII)



(VIII)

These organophosphorous compounds were chosen because they give well defined polarographic waves (229-231) (Figure 27), they have been used by Huber (106) to evaluate his detector and therefore offer a good comparison and they are representative of a large group of very important organophosphorous insecticides. All the voltammetric determinations were carried out in an acetate buffer

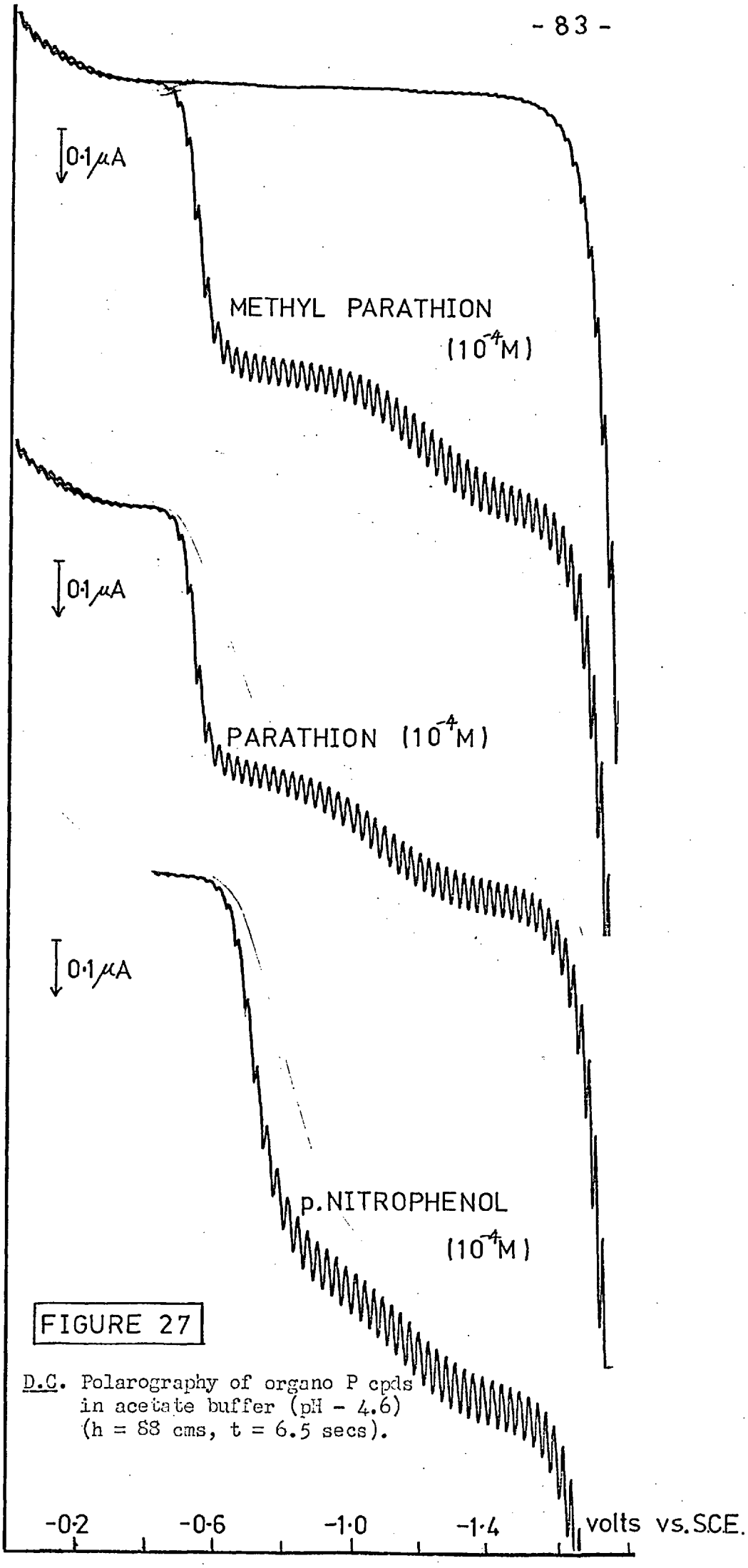


FIGURE 27

D.C. Polarography of organo P cpds in acetate buffer (pH - 4.6) (h = 88 cms, t = 6.5 secs).

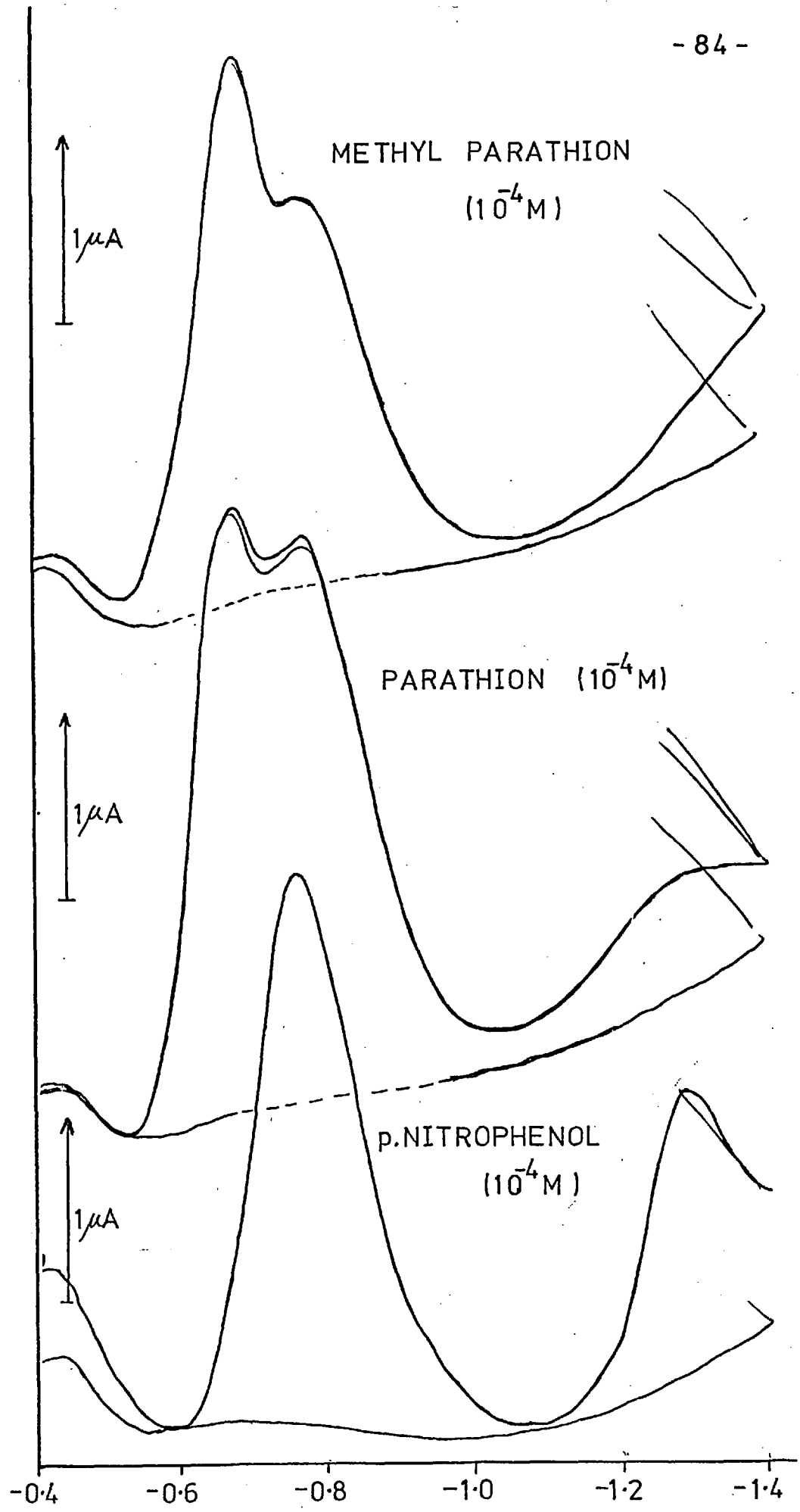


FIGURE 28: A.C. Polarography of organo P cpds. in acetate buffer (pH = 4.6). Electrodes = DME (h = 88 cms, t = 6.5 secs), Scan = 1 sec., ΔE = 10mV, ϕ = 180° v = 318Hz

saturated with iso-octane as this forms the basis of a proven solvent, stationary phase system that will separate the compounds by liquid chromatography (106).

The emphasis throughout this thesis is towards the exploitation of the a.c. polarographic technique wher ever possible and to evaluate its potential as the monitoring technique for chromatographic detection. Consequently it was necessary to compare the a.c. polarograms of these compounds (Figure 28) with their corresponding d.c. polarograms (Figure 27).

The a.c. polarograms were obtained using the fast scan technique and a dropping mercury electrode and show some deviation from the expected results. Paranitrophenol shows only one peak at an intermediate potential whereas the d.c. polarogram indicates at least two waves. Two peaks were observed for the reduction of parathion and methyl parathion but the potentials are much closer together than the corresponding $E_{\frac{1}{2}}$ values and resolution between the two compounds is lost. The values of E_p and $E_{\frac{1}{2}}$ are summarised in Table 11.

At the vitreous (glassy) carbon electrode several differences from the results at the d.m.e. were immediately obvious. All voltammograms both d.c. and a.c. gave a single wave or peak at different potentials to those observed at a d.m.e. and the responses were liable to be dependent upon stirring of the solution. This is certainly due, in part, to a depletion effect but some adsorption effects cannot be ruled out as a contributory factor.

D.c. voltammetry at the glassy carbon electrode is illustrated in Figure 29. Five consecutive scans (Figure 29a) were

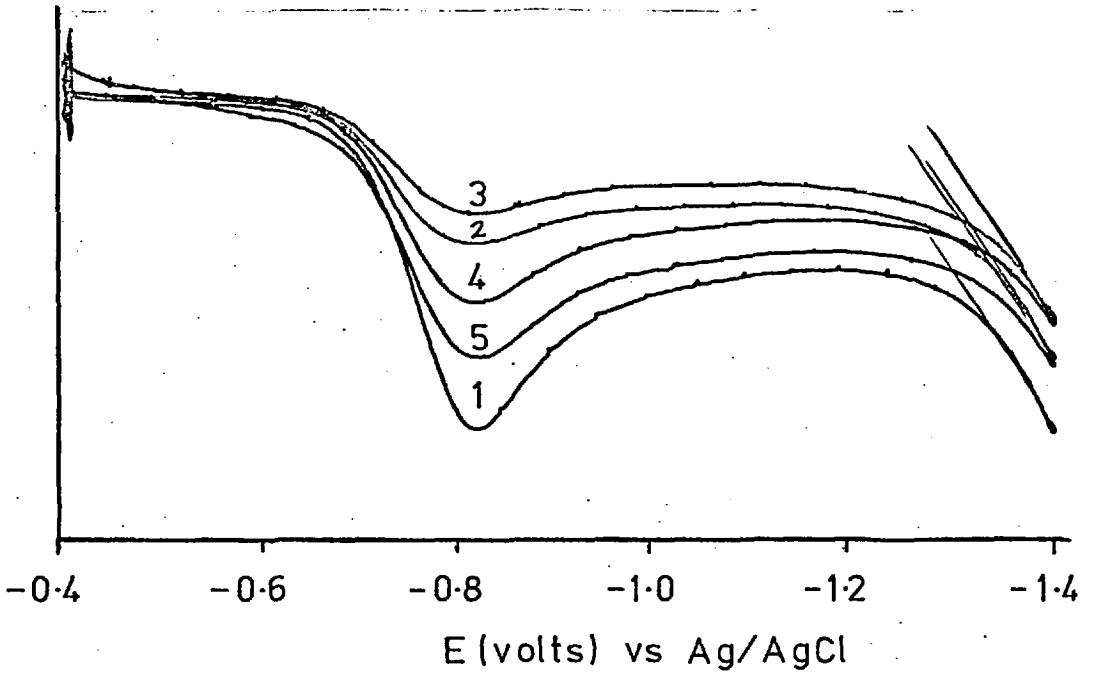


Figure 29 a: Fast scan D.C. polarography of METHYL PARATHION (10^{-4}M) at a glassy carbon electrode in a still solution

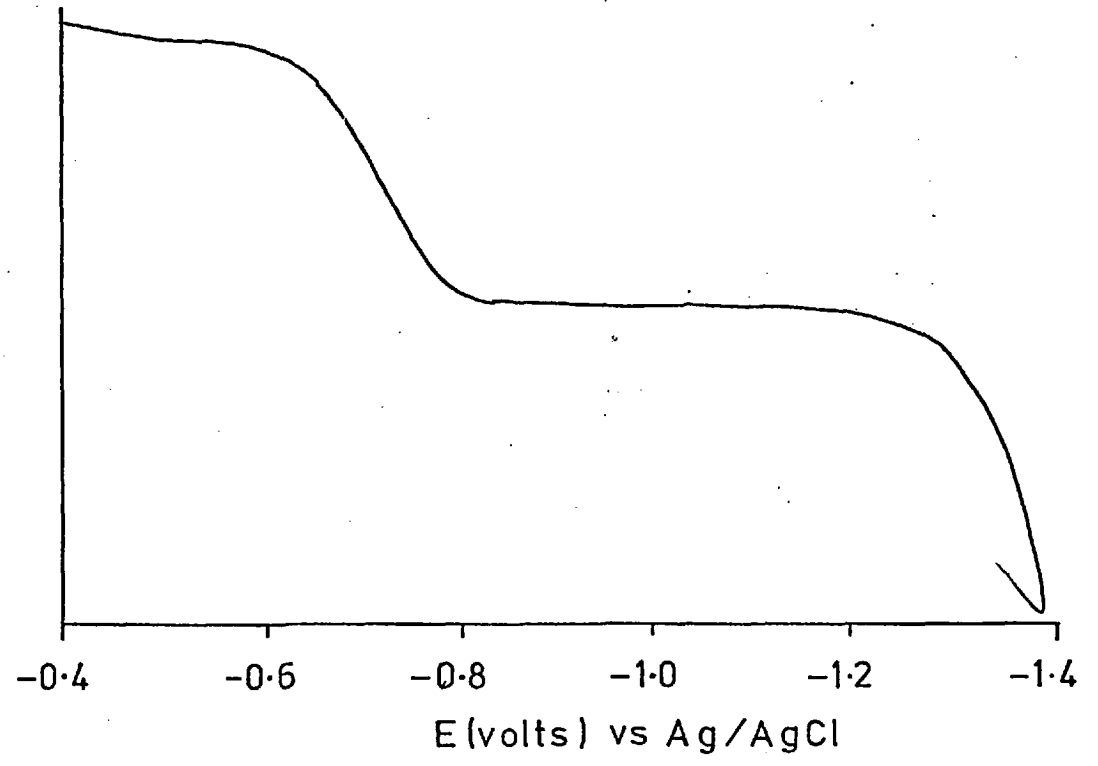


Figure 29 b: D.C. polarography of METHYL PARATHION (10^{-4}M) at a glassy carbon electrode in a stirred solution.

recorded by fast linear scan voltammetry of methyl parathion. Scans 1-3 show the pronounced depletion effect in a still solution. When solution stirring commenced the response was increased (scans 4-5) until a constant maximal, response was obtained. Figure 29b shows the voltammogram obtained at a conventional scan rate under stirred conditions and beautifully illustrates the absence of the second wave that is present for reduction at the d.m.e. and indicates a different reaction mechanism for reduction at the carbonelectrode. Even at conventional electronic scan rates the filtering required to eliminate noise is minimal, thus permitting the recording of undistorted voltammograms. No serious disadvantages were observed concerning the use of the glassy carbon electrode for these determinations and their comparative ease of use is a great advantage over the d.m.e.

A.c. "polarography" (voltammetry) has been applied very little to solid electrode systems (154,186), but it was no surprise to find that like the d.c. methods, only one reduction peak was observed. Figure 30 shows some typical successive a.c. voltammograms for parathion (similarly for methyl parathion), in Figure 30a and b shows that parathion and methyl parathion are relatively unaffected by the absence of a stirred solution, this is contrary to their d.c. voltammetric behaviour. Para-nitrophenol in unstirred solution (Figure 30c) shows a gradual reduction in peak height on successive scans. Again, in stirred solution, the depletion effect is far less noticeable and points to the necessity of using solid electrodes in a hydrodynamic system such as exists for rotating electrodes or for electrodes in flowing streams as in liquid chromatographic detectors. The scan numbers are indicated by the side of the appropriate peaks.

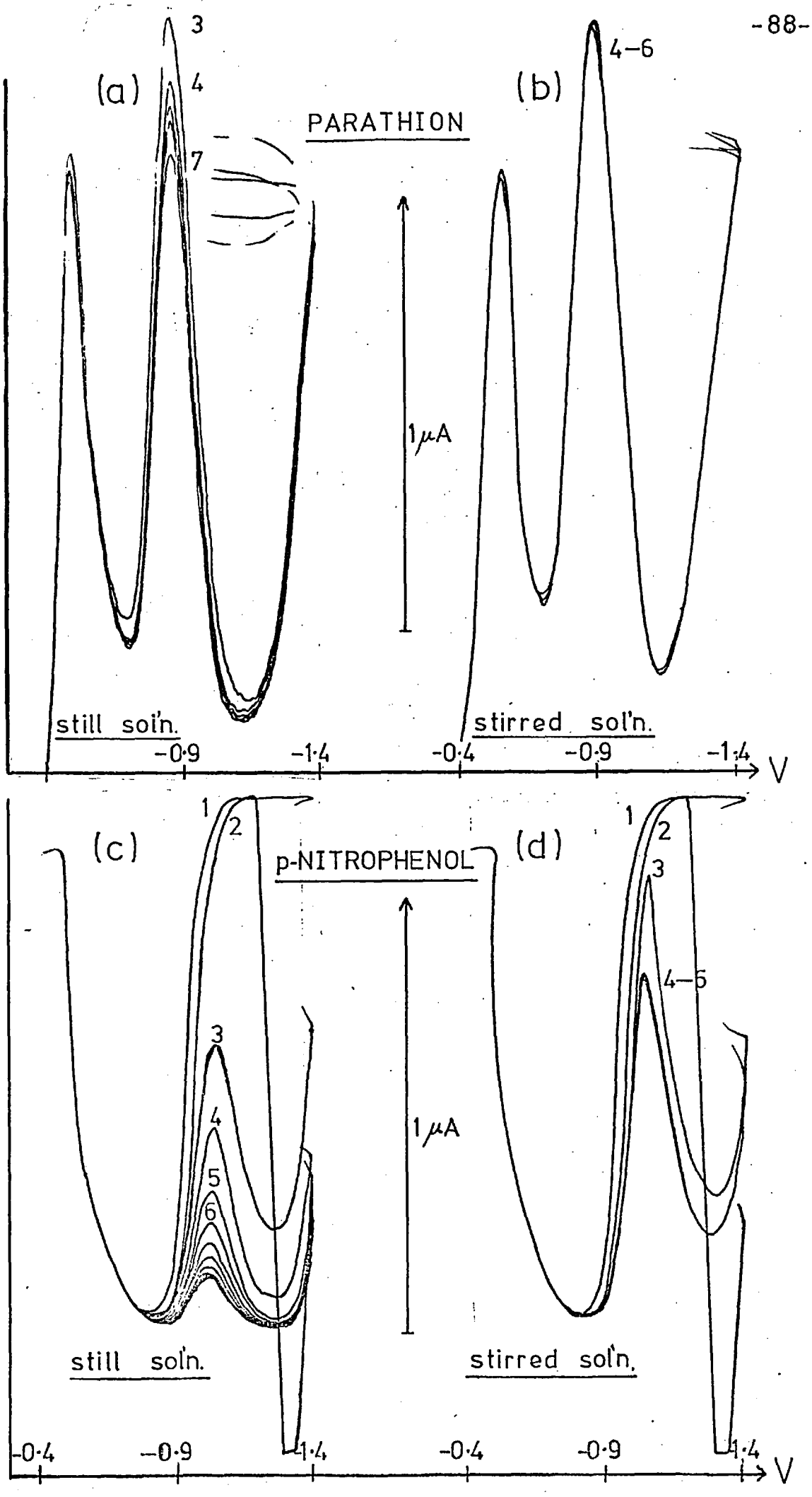


FIGURE 30: A.C. voltammetry at glassy carbon electrode

So far nothing has been said about scan numbers 1 and 2. These occurred also for parathion and methyl parathion but were omitted from the recorded traces and appear to be indicative of some alteration of the electrode surface during the electrochemical reduction processes. This "Initial Current Phenomena" has been said to be connected with the method of polishing or preparation of the electrode surface (105) but a precise explanation of its significance is yet to be forthcoming. All electrodes used in this study were ground with successively fine grades of emery cloth and a final mirror finish was obtained by polishing with "Brasso".

Along with the data for reduction at a d.m.e., the corresponding data for reduction at a glassy carbon electrode is included in Table 11.

Table 11

Half wave and Peak Potential data for VI, VII and VIII at a d.m.e. and glassy carbon electrode

Compound	d.c. voltammetry at		a.c. voltammetry at	
	d.m.e. ($E_{\frac{1}{2}}$)volts	glassy C (E_p)volts	d.m.e. ($E_{\frac{1}{2}}$)volts	glassy C (E_p)volts
p-nitrophenol	-0.634,-1.188	S.W.	-0.75	-1.07
MethylParathion	-0.567,-1.134	-0.73	-0.67,-0.76	-0.90
Parathion	-0.520,-1.055	S.W.	-0.67,-0.77	-0.88

S.W. = S ingle wave at intermediate potentials (exact figures not recorded

2.4.1. Optimisation of a.c. conditions

The a.c. technique used thoughout these studies was found to be optimal under certain conditions. All measurements were performed using the phase sensitive detector previously described at a phase angle setting of 180°. This applied for measurements at the d.m.e. and glassy carbon electrodes. The applied a.c. signal was at the fundamental frequency of 318Hz and its amplitude was 50mV unless stated otherwise. An active low pass filter setting of 4 was sufficient to eliminate all noise without imposing too much distortion on the voltammograms.

Scan Rate

The affect of scan rate on the response is shown in Figure 31.

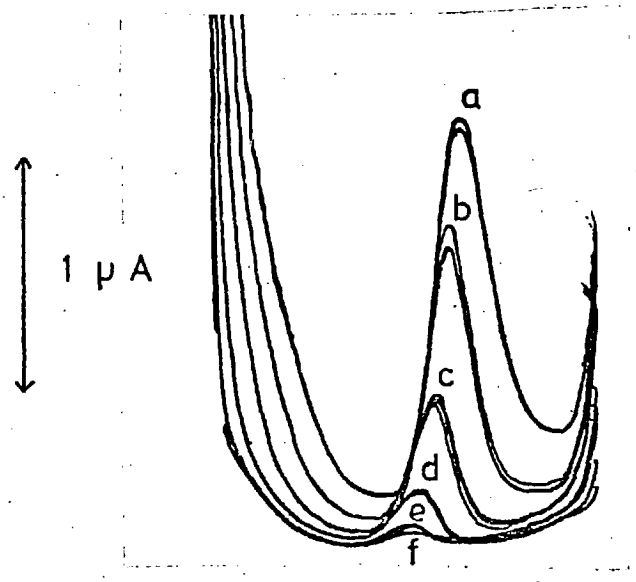


Figure 31 Effect of scan rate on a.c. voltammetry of p-nitrophenol (10^{-4} M) at a polished glassy carbon electrode. Voltage scanned between -0.4V to - 1.4V in (a) 1 sec. (b) 1.5 sec. (c) 2.5 secs. (d) 5 secs. (e) 10 secs and (f) 15 secs.

Data taken from these voltammograms is shown in Table 12 and Figure 31 and shows that a greater response is achieved at higher scan rates. High scan rates is also associated with a shift of peak potential to more negative potentials. Peak width appears to be constant, the high value for the 1 second scan is probably due to distortion caused by the writing speed of the recorder being too slow.

Table 12

Effect of scan rate on a.c. voltammetry of p.nitrophenol at a polished glassy carbon electrode.

Scan Rate (secs)	Peak Height (mm)	Peak Potential (volts vs Ag/AgCl)	Peak Width at half height (mV)
1	49	-1.05	165*
1.5	37	-1.02	130
2.5	17	-0.98	125
5	6	-0.93	125
10	3	-0.91	125
15	2	-0.90	125

*Probably some lateral distortion due to insufficient writing speed of the recorder (Bryans XY plotter, Model No

Amplitude

The amplitude of the a.c. signal has been shown to be of some importance for the analysis of mesityl oxide. A.c. polarography of organophosphorous compounds at the d.m.e. and glassy carbon electrodes is in direct contrast to previous results and is summarised in Table 13.

Table 13

Effect of a.c. amplitude (Δ E) on the polarography of para-nitrophenol and parathion.

Δ E(mV)	paranitrophenol at d.m.e.			parathion at C. electrode		
	E_p(v)	$W_{\frac{1}{2}}$(mV)	h(mm)	E_p(v)	$W_{\frac{1}{2}}$(mV)	h(mm)
100	-0.81	160	16	-0.91	200	42
50	-0.80	160	31	-0.91	200	46
20	-0.76	160	41	-0.88	200	51
10	-0.74	150	46	-0.86	200	54
5	-0.73	130	53	-0.85	160	59
2	-0.73	120	66	-0.85	120	65
1	-0.73	120	70	-0.85	120	127

In this case it can be seen that maximum response is obtained at minimum values of ΔE, although this also corresponds to maximum baseline noise and curvature. The latter is of particular importance if a scanning method is ever to be used as a monitoring technique for liquid chromatography. As expected, the higher the amplitude the more the peak potential is shifted to negative potentials and the greater the peak width at half height becomes.

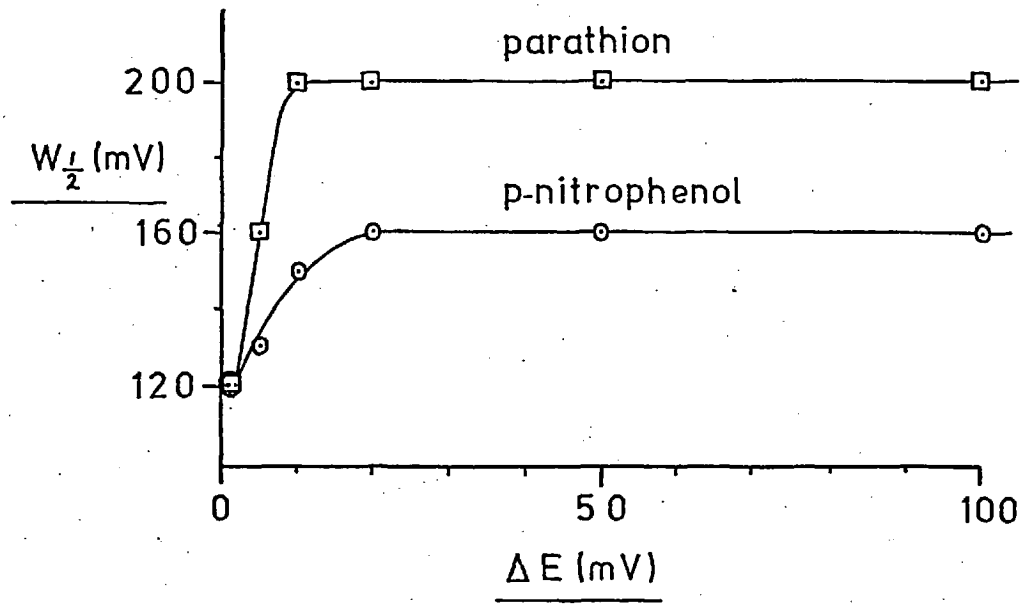


Figure 33 Variation of peak width with increasing amplitude of the a.c. signal.

Substitution of $W_{\frac{1}{2}}$ in Equation 7 shows that $n < 1$ and hence the electrode reaction is probably irreversible. The electrode reaction appears to be more irreversible at the carbon electrode than at the d.m.e.

The effect on peak height is unusual in that the response decreases as the amplitude increases (Figure 34).

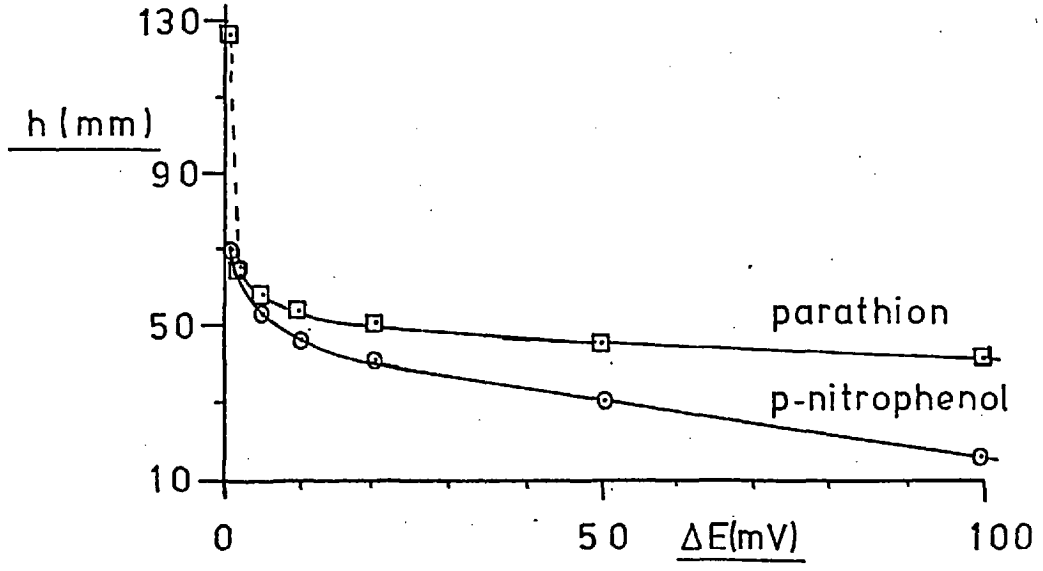


Figure 34 Variation of peak height with amplitude of the a.c. signal.

This information is more useful if plotted logarithmicly (Figure 35) andshows a linear relationship for all except extreme values of Δ E. Again, the greatest differences occur at the glassy carbon electrode, emphasising the decrease in reversibility associated with solid electrodes and the need to establish optimum conditions for analysis.

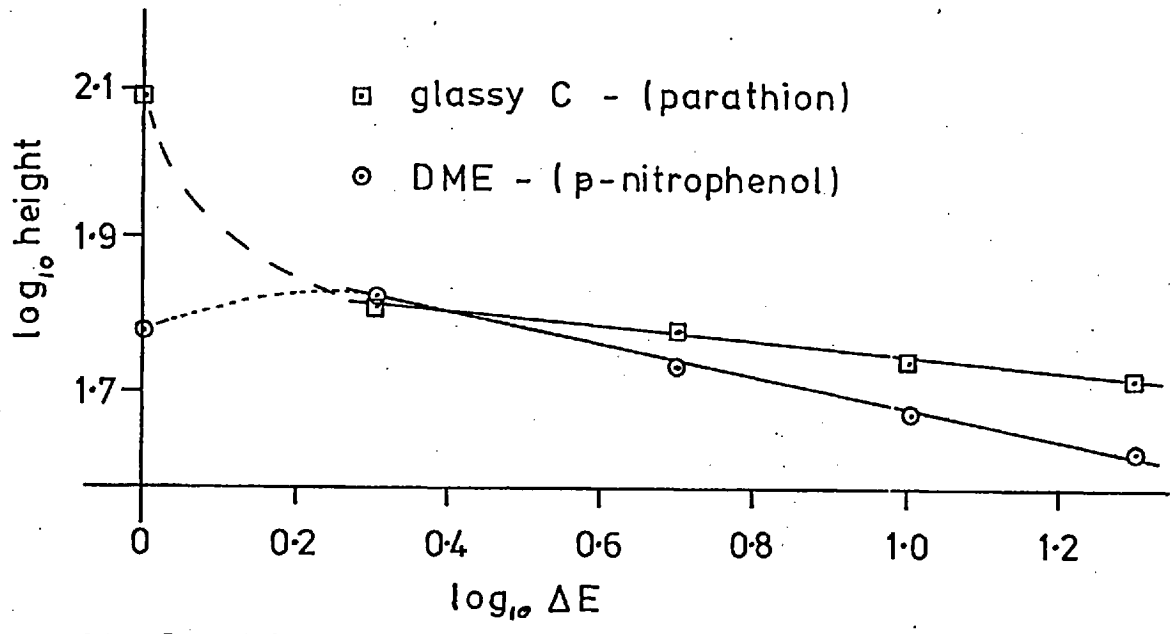


Figure 35 Logarithmic variation of peak height with amplitude of the a.c. signal.

Finally, if log X is plotted against $E_{d.c.}$ (see page 49 of this thesis) (Figure 36) a non-linear relationship is obtained and has an estimated slope far in excess of that expected for a reversible mechanism.

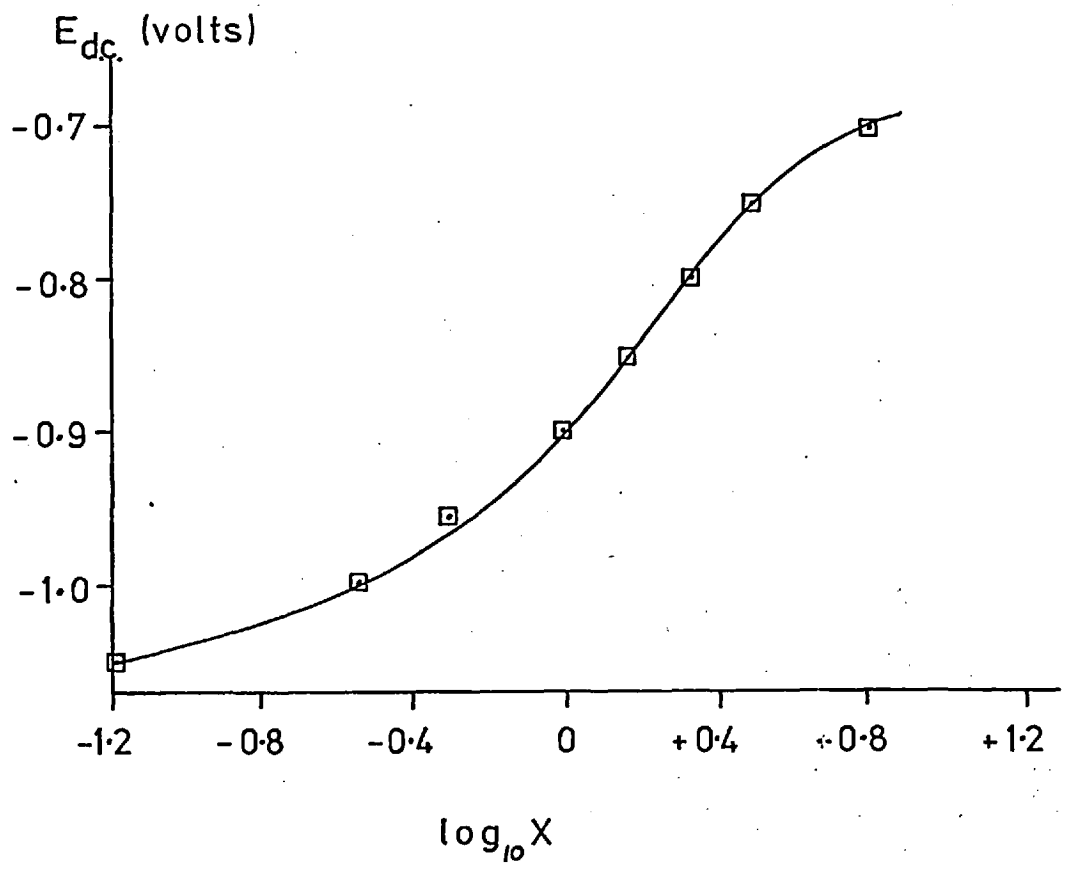


Figure 36 log X vs $E_{d.c.}$ for the phase sensitive a.c. polarogram of para-nitrophenol at a glassy carbon electrode.

Conclusions

Glassy carbon produces irreversible polarograms for the organophosphorous compounds investigated. This, in itself, is no great disadvantage analytically. Of greater importance are the depletion and adsorption effects at the electrode surface. Fast scan a.c. polarography appears to be the least affected by these phenomena and is improved, as a technique, if performed under hydrodynamic conditions. It is unfortunate that the baseline can have a considerable curvature which is insignificant for electroanalytical purposes as it can be adjusted to suit the analysis by alteration of the phase angle. However, if a scanning technique were to be used to L.C.

monitoring, the expected baseline noise would nullify the advantages of the technique. This will subsequently be shown to be the case.

At this stage of development, therefore, the technique should be used at a fixed potential for L.C. monitoring and consequently another technique such as d.c. or pulse polarography would probably be a more suitable and generally applicable technique to use.

Recent evidence (232) suggests that if positive feed back techniques are used, the baseline curvature can be greatly diminished and it is to be hoped that further work along these lines will allow potential scanning techniques to be used for L.C. monitoring.

DESIGN OF ELECTROCHEMICAL DETECTORS FOR H.P.L.C.

3. Introduction

The importance of minimal dead volume has already been stressed and the achieving of this objective has been paramount in the following detector designs.

Although much has been said in favour of solid electrodes, the conventional D.M.E. with its continually, renewable surface has an important role to play, at least until all the problems of adsorption at solid electrodes can be adequately dealt with. Consequently much effort has been put into the design and use of a low volume detector, incorporating a D.M.E., for use in H.P.L.C..

3.1. Multielectrode Detectors

Whereever a D.M.E. is used it is a relatively easy matter to substitute for the D.M.E. a similarly shaped solid electrode. Hence any detector designed for use with a D.M.E. has the potential of being used with a wide range of electrode materials, simply by exchanging the working electrode.

Hubers design (106) was used as a starting block (Figure 37) which in turn was based on the miniaturisation of the classical work of Kemula (2-4).

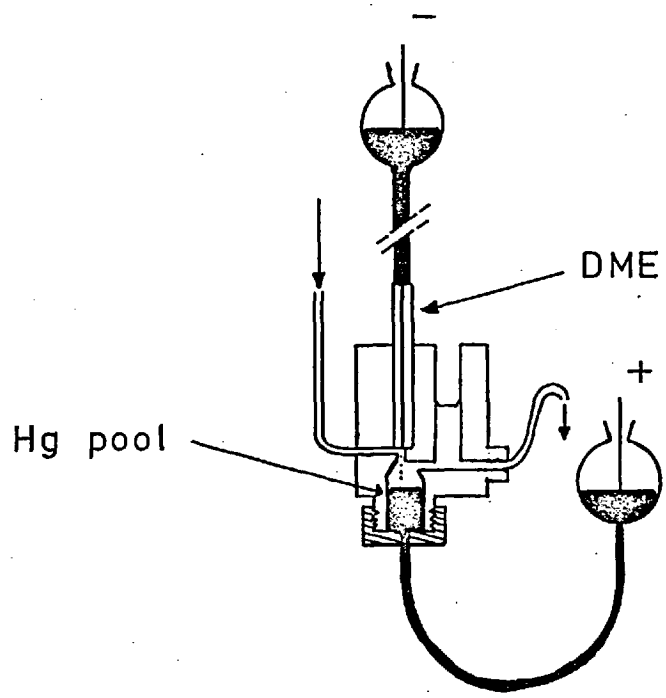


Figure 37 Polarographic detector (Huber et. al.)

Several aspects of this design were found to be unsatisfactory. The most significant practical difficulty was associated with collecting the mercury from the working electrode. The external levelling reservoir, connected to the auxilliary electrode via a flexible plastic tube was found to require skill in use and constant attention to make sure that the auxilliary mercury pool surface was kept at a constant level. This became a critical factor when thinking of long term developments concerning the use of non-aqueous solvents. This would entail making the detector out of a plastic such as Teflon or Kel-F to prevent dissolution of the detector body. The initial material was Perspex, a transparent material, soluble in many solvents, but which enables critical control of the mercury level by sighting the surface. With an opaque material such as Teflon

or Kel-F, it would be very much a trial and error situation when optimising the detector characteristics. Such a levelling device is subject to temperature change and is very sensitive to vibrations and knocks.

A siphon arrangement has been suggested as an alternative (98), but we have found this to be totally unsatisfactory. A wide range of siphon shapes constructed from a wide range of capillary bores has been investigated and in each case there is a great tendency for the mercury pool to be completely siphoned out of the detector, causing a break in electrical continuity.

Again, looking to the development of detectors for use in non-aqueous or semi-aqueous solutions of low electrical conductivity, it was decided that a 3-electrode cell was vital for making maximum use of the electrochemical techniques available. This means that the reference electrode must be situated as close to the working electrode as possible.

Bearing all these points in mind, we developed the detectors as shown in Figures 38 and 39 which incorporate the following special features.

(a) Conical or pointed electrodes. This is particularly important when phase sensitive techniques are used as the glass surrounding the tip of the capillary causes phase change to occur, severely limits the effective surface area of the mercury drop in contact with the solution and hence restricts sensitivity and acts as a trap for minute gas bubbles which result in noise, especially at high sensitivities.

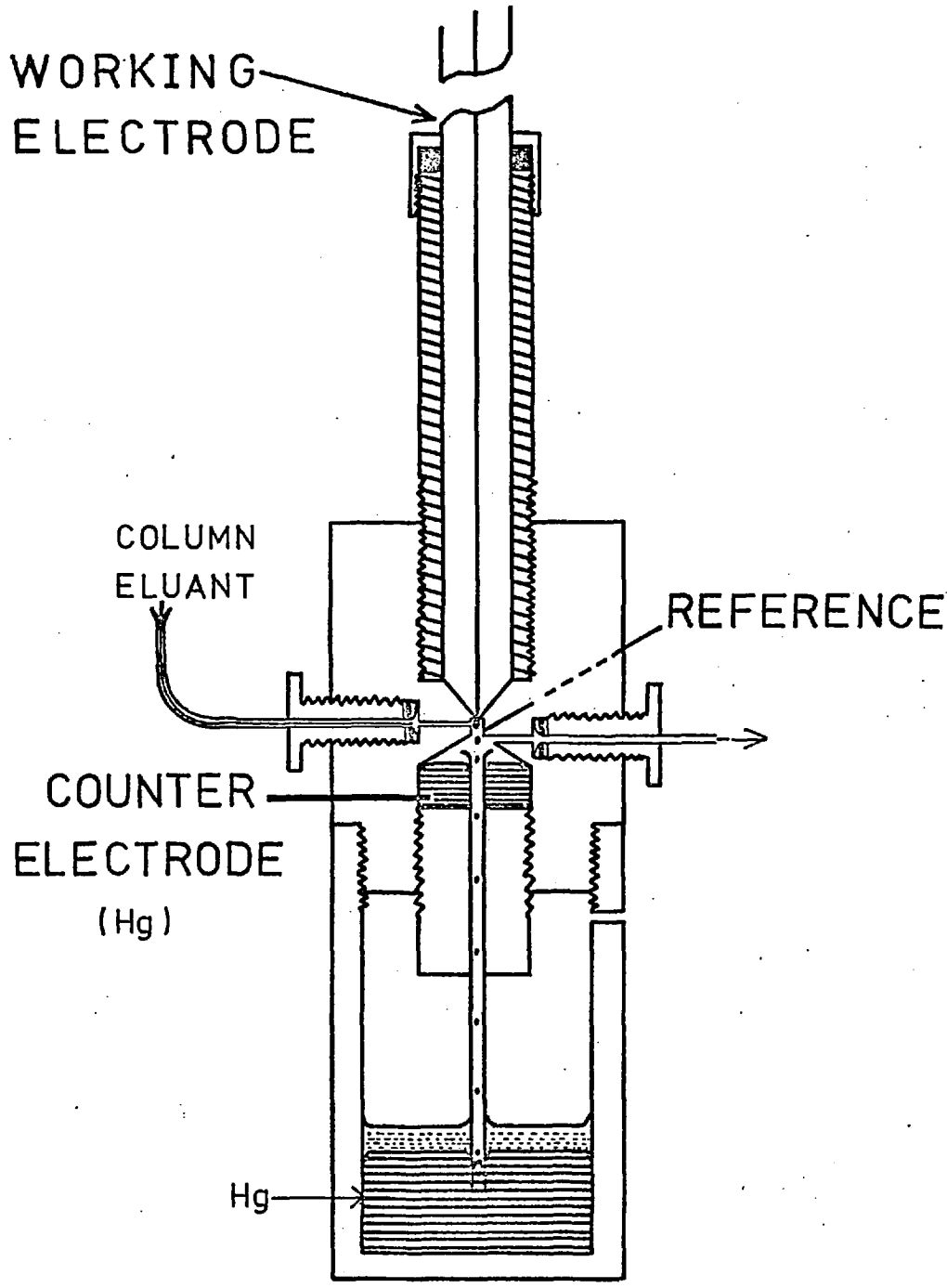


FIGURE 38: Multielectrode Detector for H.P.L.C. for use with conically shaped electrodes like the D.M.E. shown above. (To scale)

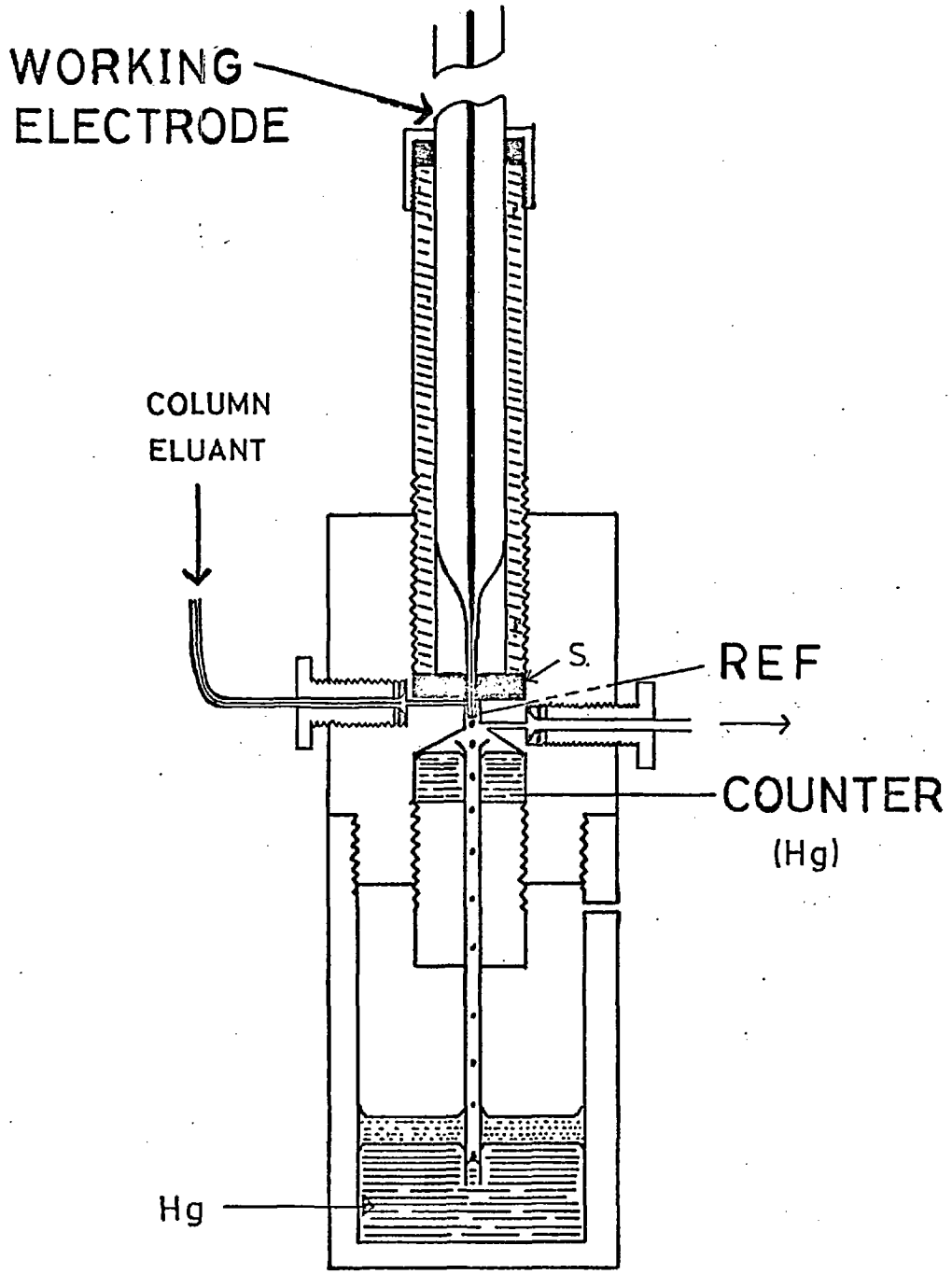


Figure 39 Multielectrode Detector for H.P.L.C. for use with very narrow electrodes like the drawn out D.M.E. shown above. (To scale)

Conical electrodes are robust and relatively easy to prepare by grinding under a stream of water. This causes water to penetrate the capillary but can be removed by baking the capillary in an oven at about 200°C. If this is deemed to be unsatisfactory, then a continual flow of mercury through the capillary will prevent any ingress of water during grinding. However, the method is tedious and care must be taken to collect the spent mercury. The major difficulty encountered with this electrode was in mating the conical electrode tip with the inside of the detector. When the shapes are as near as can be obtained, a smear of quick drying silicone rubber will act as an effective seal. Care must be taken not to chip the glass near to the capillary opening, otherwise erratic drop formation will result.

Narrow, drawn out electrodes (Figure 39) are very easy to prepare by pulling out wide bore capillary tubing or thin walled tubing (241) after heating in an oxygen-hydrocarbon gas flame. The capillary tip is ideal for use in polarography but has the misfortune of being fragile. It is easily inserted into the detector cell through a silicone rubber septum , as shown, but because of its fragility, is not recommended for general use.

A further point in favour of the conical capillary concerns the lesser practical problems of drilling several very fine holes into the detector cell, as close to the capillary tip as possible.

(b) Reference Electrode. A silver wire reference electrode is inserted, as shown, such that the working surface is extremely close to the working electrode (d.m.e.). Such a reference electrode

is not ideal if precise $E_{\frac{1}{2}}$ and peak potentials are to be quoted as it is not immersed in a standard electrolyte such as chloride ion. The electrolytic nature of the eluant is subject to some change such as variation in flow rate and the effect of dissolved solutes and is consequently difficult to define. However, in practice it acts extremely efficiently and can be inserted into a very small volume. If necessary, a liquid junction such as asbestos fibre, could be inserted into the fine bore reference inlet and the outlet side could be connected to an external standard reference electrode such as silver/silver chloride or the saturated calomel electrode.

(c) Annular auxilliary electrode. A tube, about 2 mm. internal diameter, (glass or teflon) protrudes through the mercury pool auxilliary electrode. The upper tip of this tube is funnel shaped and serves to catch the mercury from the d.m.e. and channels it down to the secondary mercury pool reservoir. Consequently the mercury pool auxilliary electrode is annular in shape, static and of constant surface area. It suffers no surface disturbance due to mercury drops hitting the surface and as such is subject to less noise at high sensitivity (139). The apparently large liquid volume after the d.m.e. is not detrimental to resolution as measurement of the eluant is completed after the solution has passed the mercury drop connected to the d.m.e..

(d) Effective detector volume. The bore of the detector cell is 1 mm. internal diameter from which the maximum detector volume can be calculated. It is unlikely that a mercury drop with a drop time of less than 2 seconds in a flowing stream will ever reach

1.0 mm. in diameter. The total volume of a 1.0 mm. section of the detector bore has a value of about 0.8μ l. This is without taking the volume of the mercury drop itself into consideration, which would decrease the practical dead volume of such a detector to much less than 0.8μl.

(e) Multielectrode design. The combination of the two designs shown in Figures 38 and 39 enables the interchange of a variety of electrodes. It has already been stated that solid electrodes have many advantages (93,103) and they can be incorporated in designs to fit these detectors (Figure 40). It is worth reiterating that the

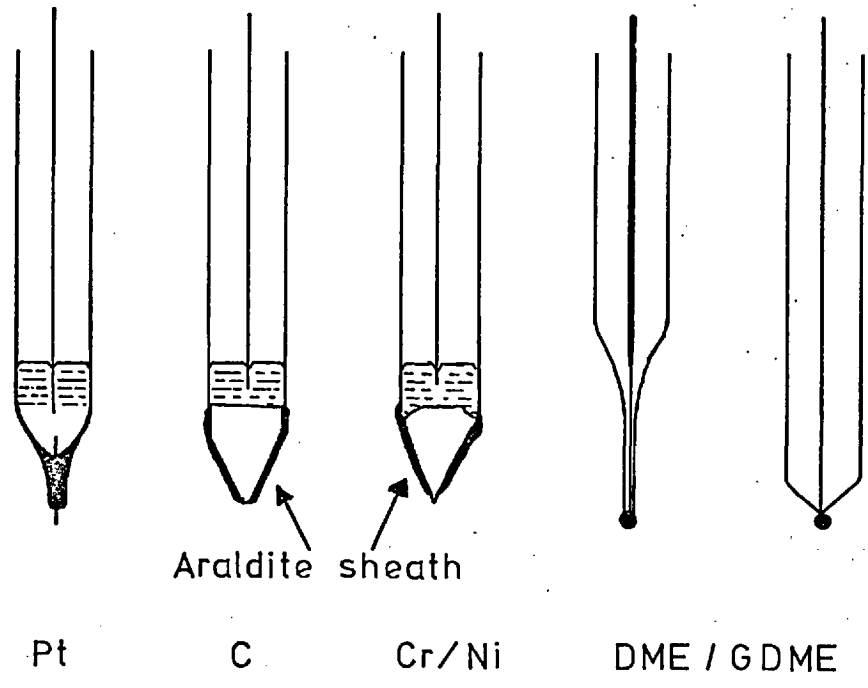


Figure 40 Some electrode designs for use with the Multielectrode Detector.

gas pressure operated dropping mercury electrode (GDME) produces a more reliable drop rate and hence the detector characteristics are more reproducible.

(f) Experimental set up. This is shown in Figure 41 and consists of a high pressure liquid pump of the Haskell type. This is gas pressure operated and incorporates a pressure enhancement due to variable sized pistons. Gas pressure (from a cylinder) actuates a large diameter piston which in turn actuates a small diameter piston which forces liquid out of a stainless steel reservoir. The pressure enhancement is approximately 20:1 so that if a gas pressure of 10 p.s.i. is used, the pressure of the liquid at the outlet side of the pump would be 200 p.s.i.. Consequently the pump is capable of very high pressure operation under pulse-free conditions. Its major disadvantage lies in the fact that it is not refilled continuously. Thus for a 100ml. capacity pump being operated at an eluent flow of 1 ml. per minute, the pump must be refilled every 100 minutes. In practice, this is not a great hardship and the pulse free flow is very important for obtaining the optimum response from the detector.

The diagram also illustrates how deoxygenation takes place, both of the eluant and the pump itself. This is important when electro-reductive techniques are to be used, but can be dispensed with when electrooxidative methods are used. It is a popular practice in high pressure liquid chromatography to degas the eluant prior to use either by refluxing to boil out dissolved gases or even by subjecting the eluant reservoir to a vacuum. In practice, this is not always necessary unless very high pressures are used resulting in the dissolution of large quantities of gas which then comes out of solution at the detector side (low pressure) of the column and causes a very noisy response. The trend is towards short, wider bore columns of fine particles ($<$ 10 microns) which have very high efficiency and

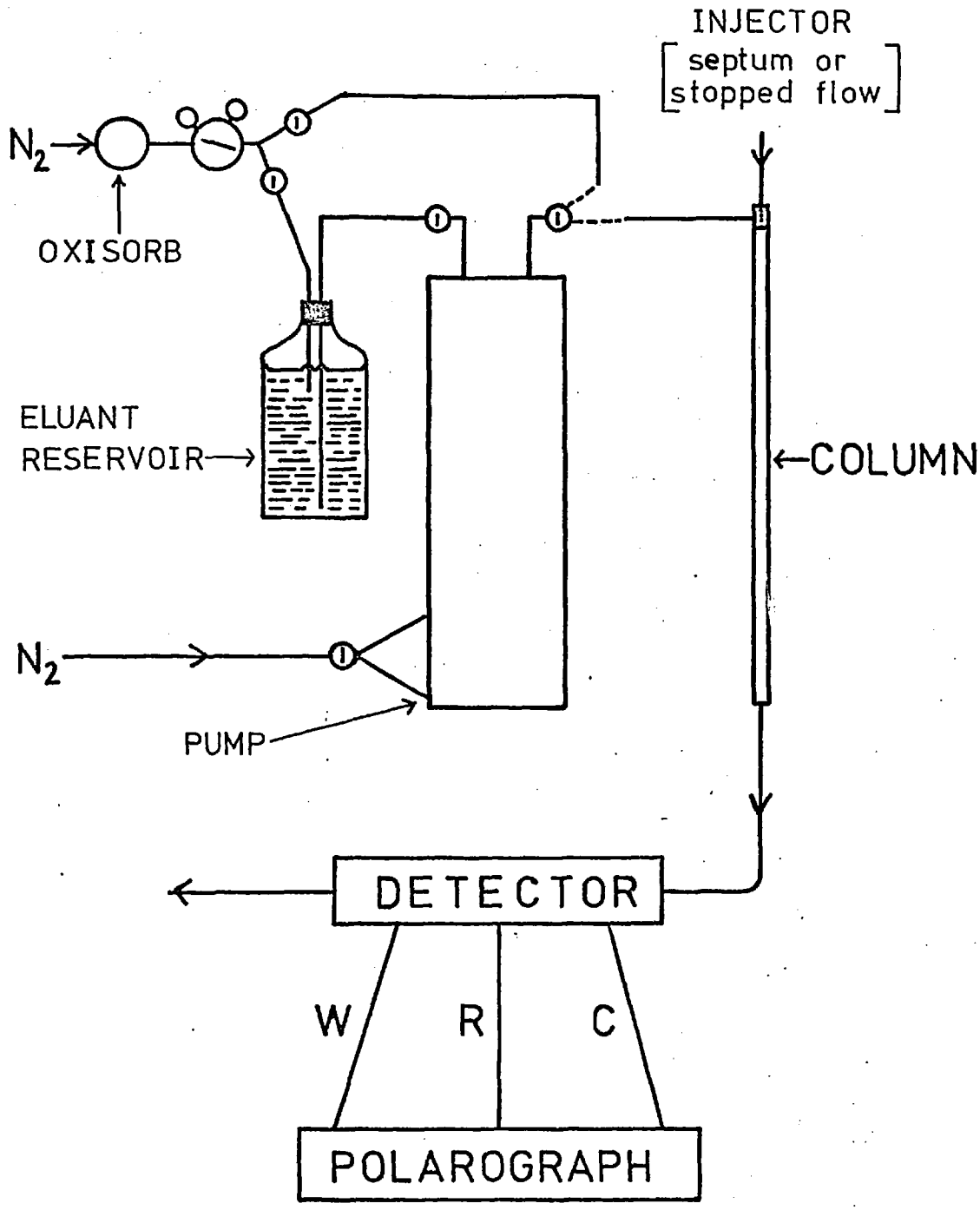


Figure 41 Experimental arrangement for chromatovoltammetry. For Electro-oxidative détection, the deoxygenating apparatus is unnecessary.

relatively little solvent hold up. Consequently relatively low pressures are required. Most liquid chromatography can now be done at pressures below 2000 p.s.i..

(g) Column Packing. This is a very important aspect of chromatography that has been neglected until quite recently. It is still more of an art than a science, but some recognised procedures are developing such as various methods of dry packing with (127,234), and without (122,126,127,235,236) tamping and slurry packing, particularly balanced density slurry packing (123,126,237,238). The later technique is particularly important for the efficient packing of fine particles ($<$ 10 microns) especially when they are irregularly shaped like alumina or silica. Packing the newer synthetic, micro-spherical particles appears to require a modified technique due to the very fine flow of the particles over each other. Apart from purchased columns such as Permaphase-ODS (Dupont), all columns prepared throughout the work of this thesis were packed by a modification of the method recommended for packing spherical alumina (233). The column (stainless steel, 3-4 mm. I.D.) is cleaned, dried and coated with teflon from an aerosol spray. A retaining plug of porous teflon is inserted in the bottom of the column as shown in Figure 42.

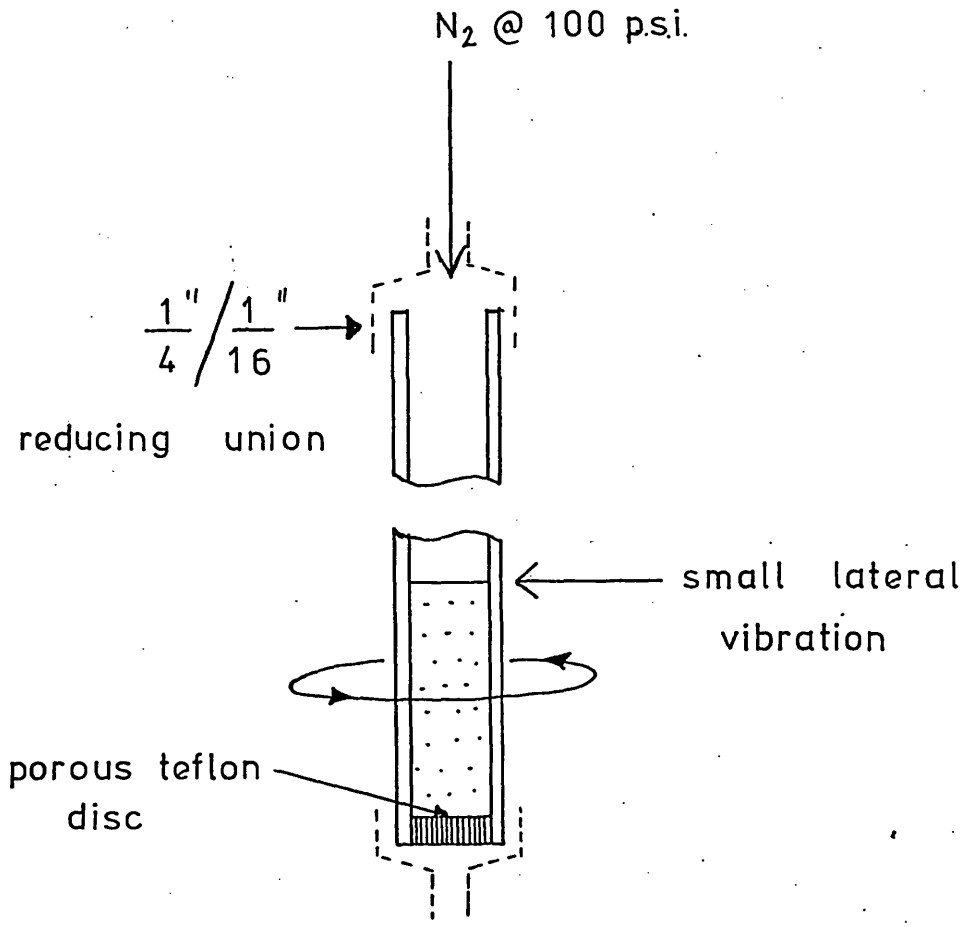


Figure 42. Experimental arrangement for column packing

A plug of glass wool is tamped down to the bottom of the column to prevent fine particles of Harwell alumina passing through the teflon frit. Support material is then fed into the column through a funnel and compacted by gently rotating the column and vibrating it laterally with a vibrotool. When the column appears to be full and no further compaction occurrs, the top end of the column is connected to a nitrogen gas cylinder, via Swagelok fittings and nitrogen is passed though the column at 100 p.s.i.. The column is vibrated (laterally using a vibrotool) under these conditions and inspected to see if any further compaction has occurred. If so, more support is added to the column and the process repeated. This procedure is repeated until no further compaction occurrs. The top of the

column is then sealed with porous teflon and connected to the set up as shown.

(h) Construction of the Multielectrode Detector . At this stage of the thesis it is probably important to include details of the construction of the detectors already shown. Figure 43/1 shows the appropriate drill, tap and die sizes for constructing the detector. If perspex rod is to be machined, all cutting edges should be liberally bathed in oil to prevent overheating with subsequent melting and distortion of the perspex. The leading cutting edge should also be ground away so that it does not chip the very brittle perspex. Of the plastic materials available, Kel-F is to be preferred as it is harder than Teflon and much easier to machine. When putting threads onto Teflon, extreme care should be taken to prevent stripping the thread.

Most of the construction is self evident from the diagram. However, it should be emphasised that perspex (or Kel-F) rod is easily put onto a lathe and all centrally placed holes and threads can be machined on the lathe. This ensures proper alignment. The drilling into the side must, however, be done with a vertical drill. The fine holes for reference and eluant inlets can be made either with a number 80 drill bit or for Teflon materials, a sharpened length of syringe plunger (Hamilton 10μl) will suffice. The latter has the advantage of being longer than the drill bit and is easier to direct into the detector block.

The conical electrode housing is shaped by using a drill bit ground to the required angle.

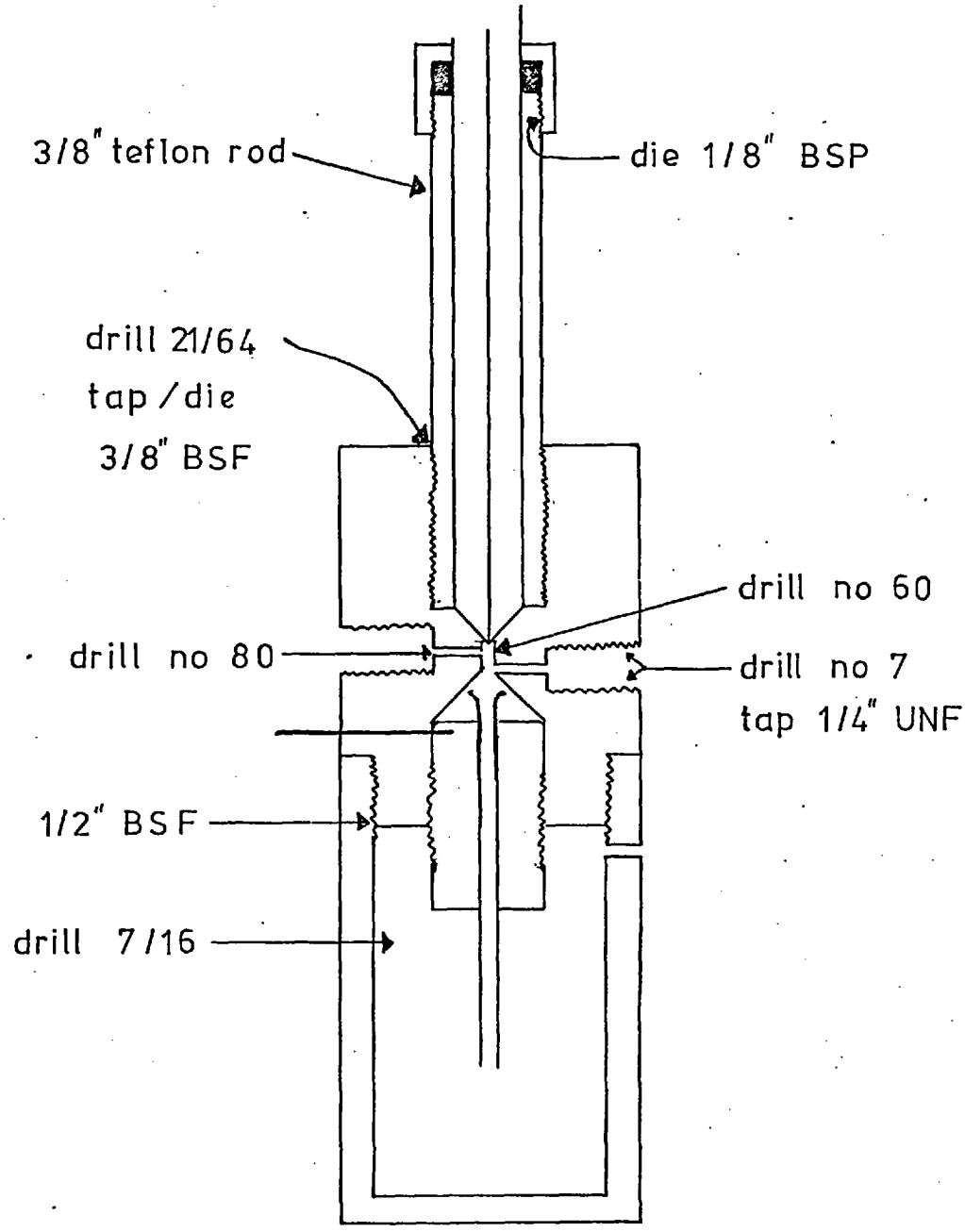


FIGURE 43/1 Construction of Detector

3.1.1. Results

Much of the detector development was carried out by injecting single component mixtures onto glass bead columns packed as mentioned. When using this techniq ue, care must be taken not to work at too low a concentration when using electroreductive methods, otherwise confusion will occur due to reduction of dissolved oxygen. For low level determinations, a column, efficient enough to separate the dissolved oxygen from the solute is required. In this case, two peaks are observed (Figure 43) one of which is due to oxygen. It could be argued that this is a possible method for determining dissolved oxygen. The effect is least pronounced when using a.c. techniques as the oxygen reduction is highly irreversible. When using pulse techniques, the effect is highly pronounced and great care must be taken to separate the oxygen.

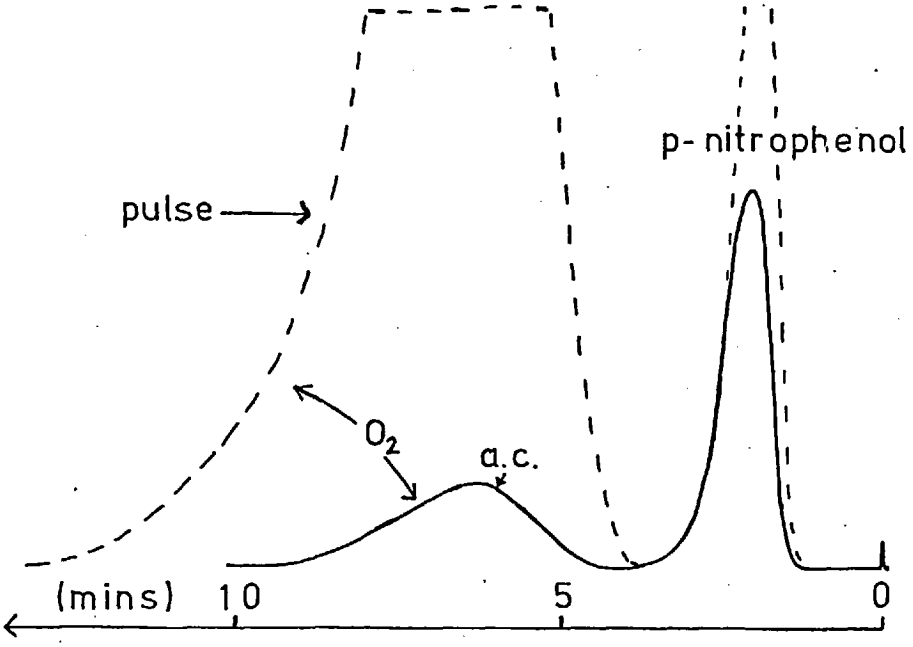


Figure 43. Chromatograms showing potential interference of oxygen when using reduction methods. Support, Silica (36-45 microns). Stationary phase - isooctane, Eluent Acetate buffer (Ref.106) saturated with iso octane, flowrate 1 ml/min., Column stainless steel (10 cm. long 4 mm. I.D.) coated with Teflon.

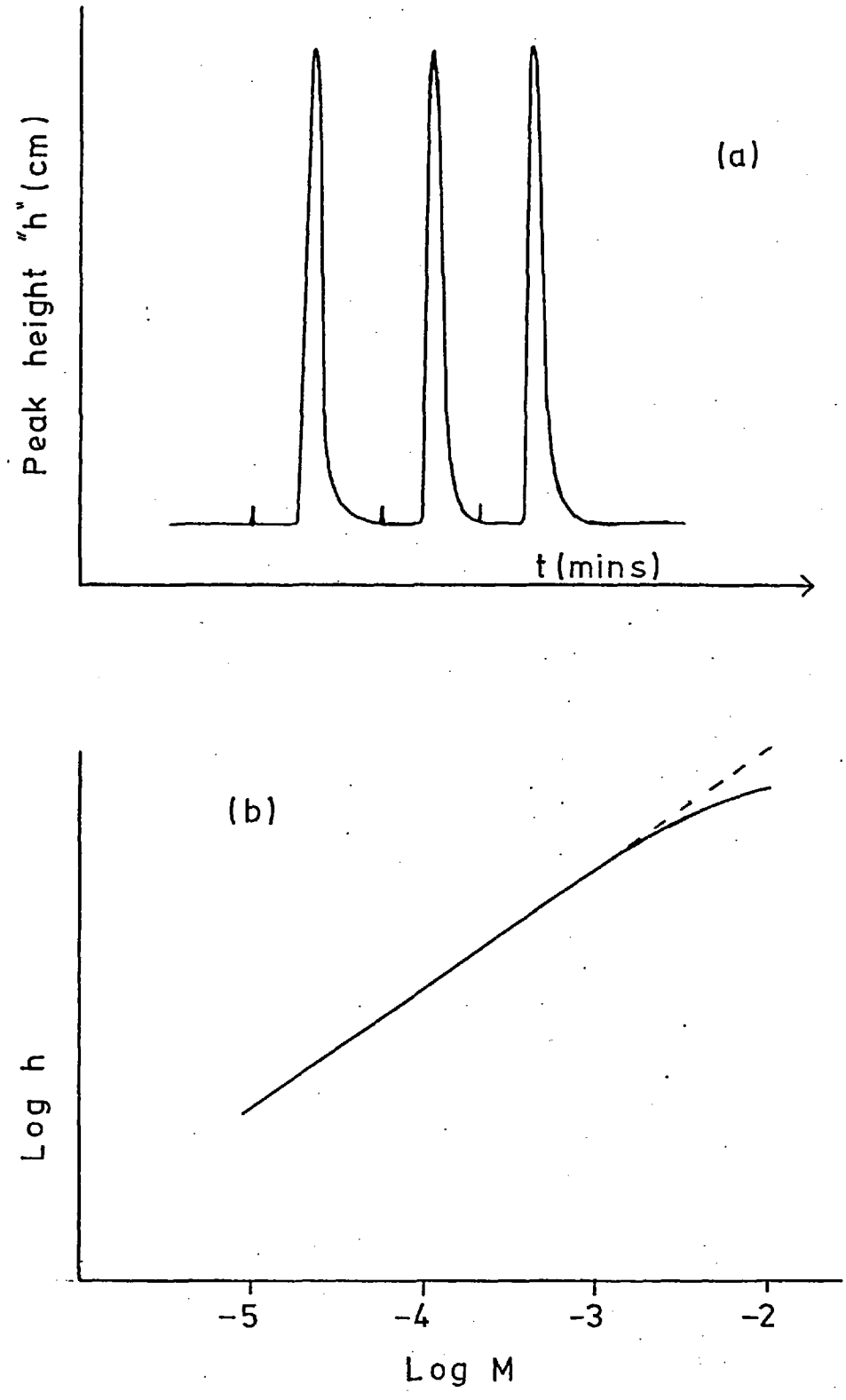


FIGURE 44 Chromatopolarographic detection of p.nitrophenol, using the PULSE POLAROGRAPHIC TECHNIQUE, showing repeat injections (a) and the calibration (b) over a wide concentration range.

Column: $\frac{1}{2}$m Zipax HCP

Eluent: Acetate buffer (50% EtOH)

Potential: fixed at -1.2 volts.

Detector: d.m.e. - drop time of 2 seconds, measuring technique was d.c. polarography at a fixed potential of -0.8 volts vs Ag/Ag^{+}.

Using the above separation procedure for paranitrophenol the pulse technique was used to construct a calibration curve and to test the detector for reproducibility (Figure 44). It can be seen that reproducibility is good and although not shown by the illustration, long term drift is negligible. A wide linear range is obtained(for a logarithmic scale) and the lower limit can easily be extended down to 10^{-7}M - 10^{-8}M.

The use of the pulse technique exagarates the limitations of the detector because a sample and hold electronic measuring system is used. To be successful, this requires a perfectly reproducible mercury drop and although a drop knocker was used, this was inadequate at high sensitivity and a very noisy baseline was obtained. The method was subsequently modified to use the gas pressure operated d.m.e. resulting in the expected decrease in detection limit.

Drop Time.

It is important to adjust the drop time currently for L.C. detection (Figure 45). Conventional drop times of about 5 seconds are not suitable for several reasons. Essentially this would mean taking a reading every 5 seconds. The precise measurement of peak maxima would be difficult under these conditions (Figure 45a) and because the drop formation involves a large change in surface area, with consequent current oscillations, considerable electronic damping would be required to continuously record the chromatogram. As

chromatographic column technology improves, one direct result is to produce very narrow peaks eluted from the column. It is possible to produce chromatographic peaks which are eluted over a period of time which is less than the drop rate of a conventional d.m.e.. This could result in non-detection of some solutes on a very random basis.

Figure 45(b) indicates a compromise situation with drop times of about 1 second. This would suffice for the vast majority of current chromatographic applications and would not be subject to too much loss of sensitivity. This last aspect is a consequence of faster drop times, smaller drops and lower surface areas.

Figure 45(c) shows the effect of going to really fast drop rates (say of the order of 0.1 seconds). Mercury drops are so small, that current oscillations can be adequately damped with a minimum of filtering e.g. with an active low pass filter. Unfortunately the loss of sensitivity is quite pronounced. It is an ideal arrangement for the detection of concentrated solutions but all these variants begin to impose an increasing knowledge of electrochemistry upon the chromatographer. We therefore consider this to be the wrong approach to the problem.

The extreme example is shown in Figure 45(d) where a streaming mercury electrode is used. This consists of a wide bore capillary through which the mercury literally streams out in a continuous thread of mercury. After a given distance (say 1 cm.) the thread begins to break up, so the electrode shape is cylindrical. Its length and hence its surface area can be carefully controlled and because its area is large, the sensitivity is increased by at least

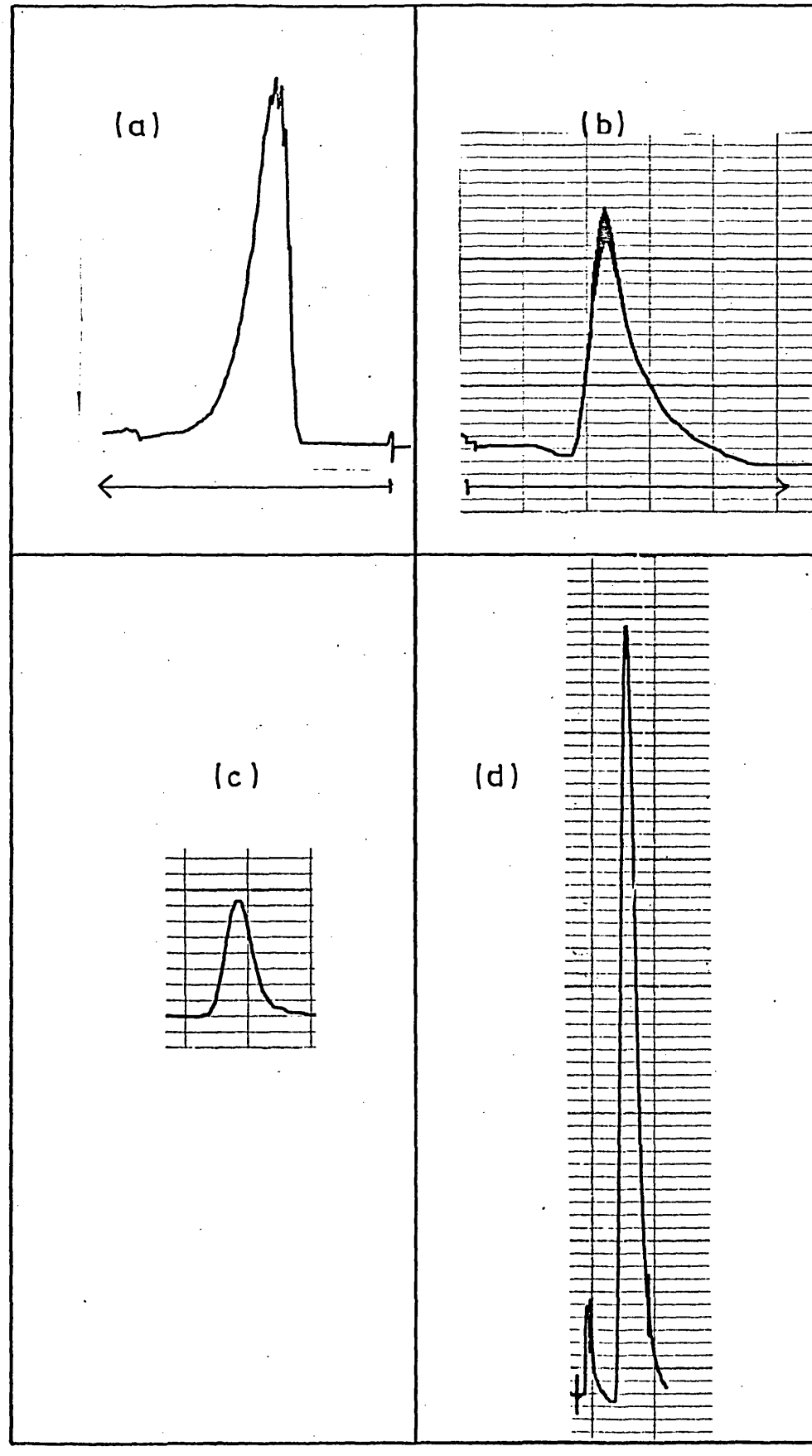


Figure 45 Chromatopolarographic detection at various mercury flow rates (a) Drop time = 5 secs (b) Drop time = 1.5 secs (c) Drop time = 0.1 secs (d) Streaming Hg Electrode.

1 order of magnitude and possibly 2 orders. The electrode area is constant, so there is no problem of damping out current oscillations but it suffers from one enormous drawback, the rate of mercury consumption is very high indeed. The current design does not cope with this consumption very readily and so it can only be recommended for very special analyses where optimum sensitivity is of paramount importance.

3.1.2. a.c. polarographic detection

a.c. polarography has already been shown to be a viable analytical method and it is one of the best methods available for mechanistic studies. However, the response is peak shaped and the associated difficulties of using such a technique have already been stressed.

Phase sensitive a.c. detection at a fixed peak potential is the easiest way of using this technique and imposes a small level of specificity on the detection. In practice, it was found that a response was obtained over a wider potential range than was expected (Figure 46). This means that the method would be less specific than envisaged but would have a more useful general application for liquid chromatographic monitoring.

The maximum information from the technique would be obtained if the d.c. potential was scanned over as wide a potential range as possible. The theoretical chromatogram would then appear as in Figure 47(a). With the appropriate peak following circuitory this could be recorded in the conventional fashion Figure 47(b).

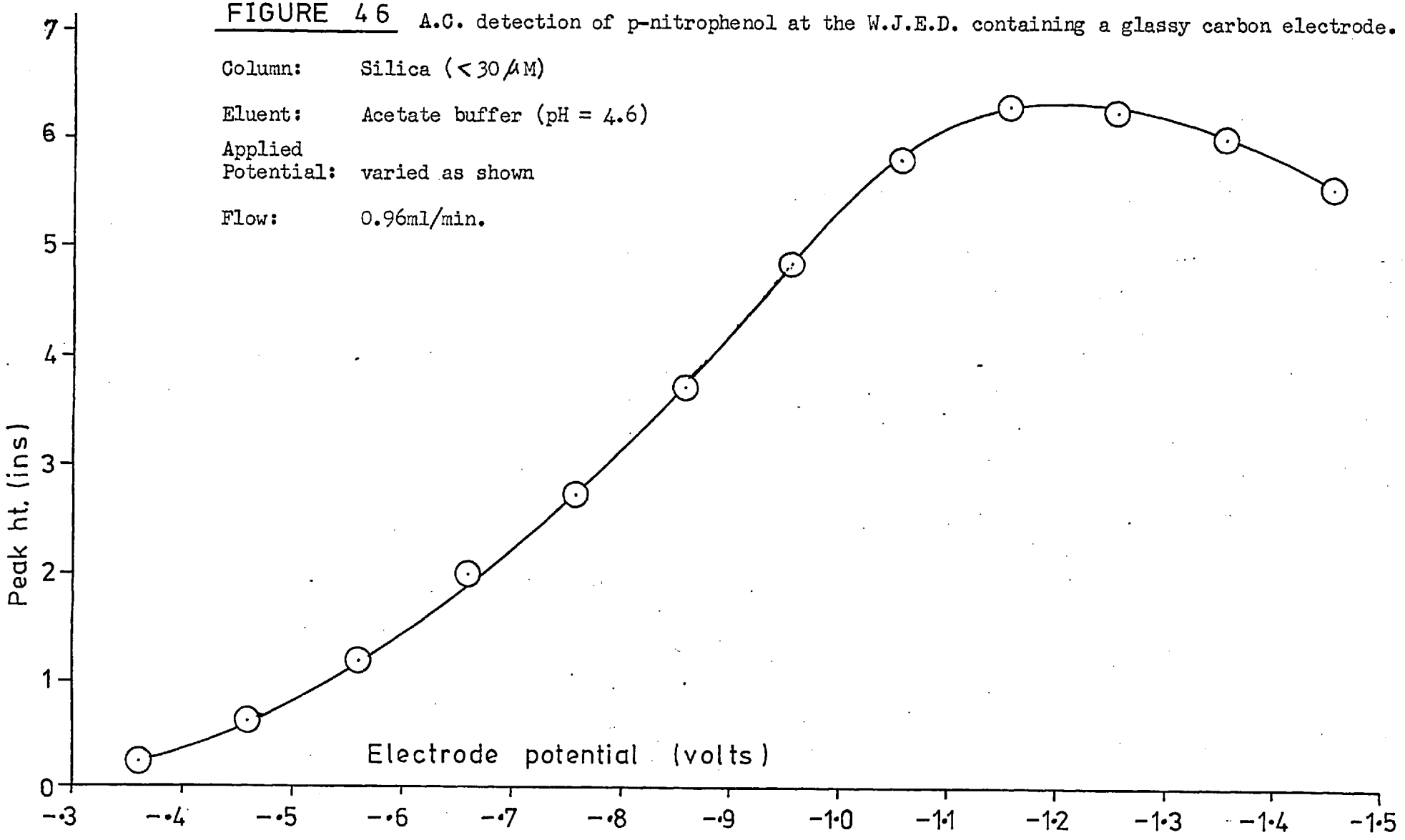
FIGURE 46 A.C. detection of p-nitrophenol at the W.J.E.D. containing a glassy carbon electrode.

Column: Silica (< 30 μM)

Eluent: Acetate buffer (pH = 4.6)

Applied Potential: varied as shown

Flow: 0.96ml/min.



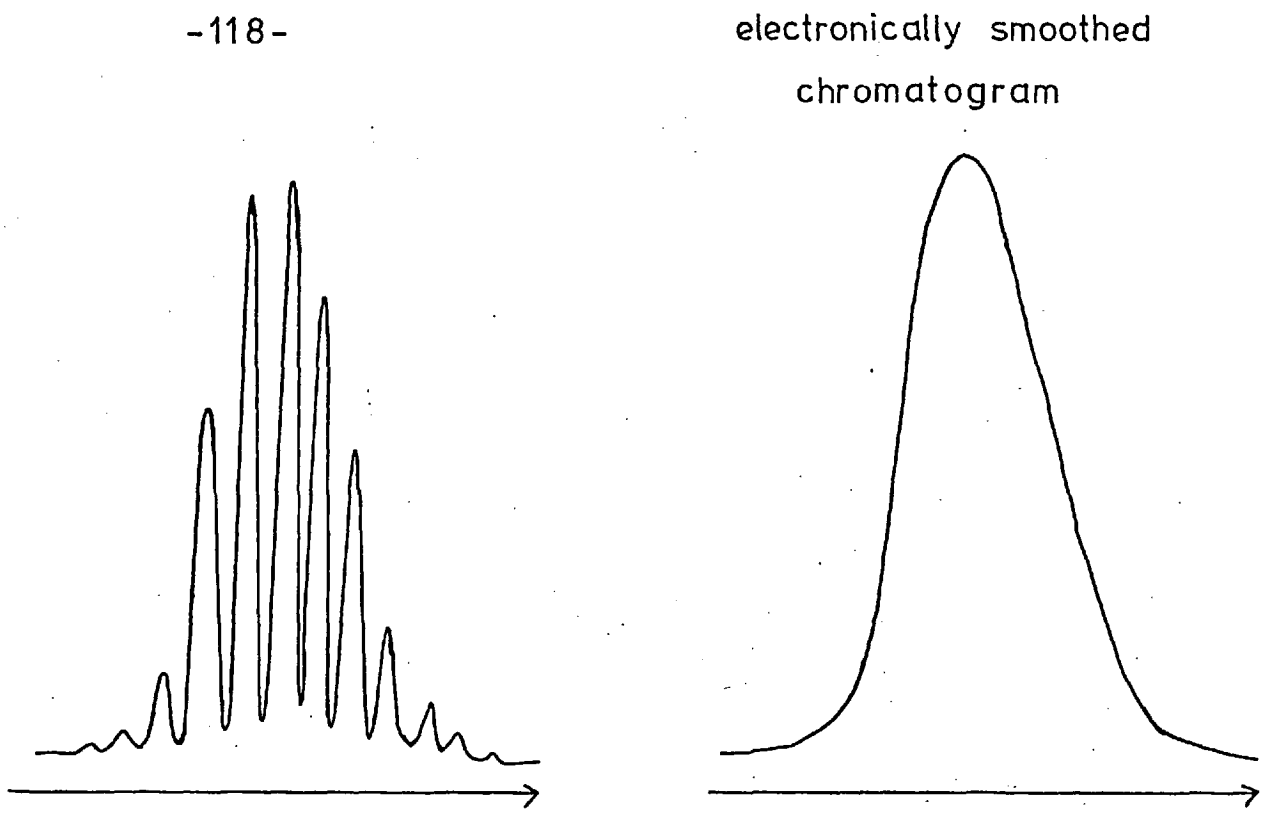


Figure 47 Theoretical and smoothed out chromatogram for scanning a.c. polarographic detection methods,

As already mentioned, the practical a.c. polarograms of most organic solutes has a curved baseline. This results in the very noisy recording as shown in Figure 48. The recording becomes noisier the larger the potential range scanned. This is unfortunate because not only would the potential scanning method be of more universal application , it is also a more sensitive technique. It is to be hoped that the positive feedback approach to Dr. Jee will enable a partial solution to this problem.

3.1.3. Gas Bubbles

One of the major disadvantages of the detectors as described concerns the presence of gas bubbles in the eluant. Gas bubbles are readily purged from the 1 mm. I.D. detector cell but are prone to accumulate in the cone shaped part just above the mercury auxilliary

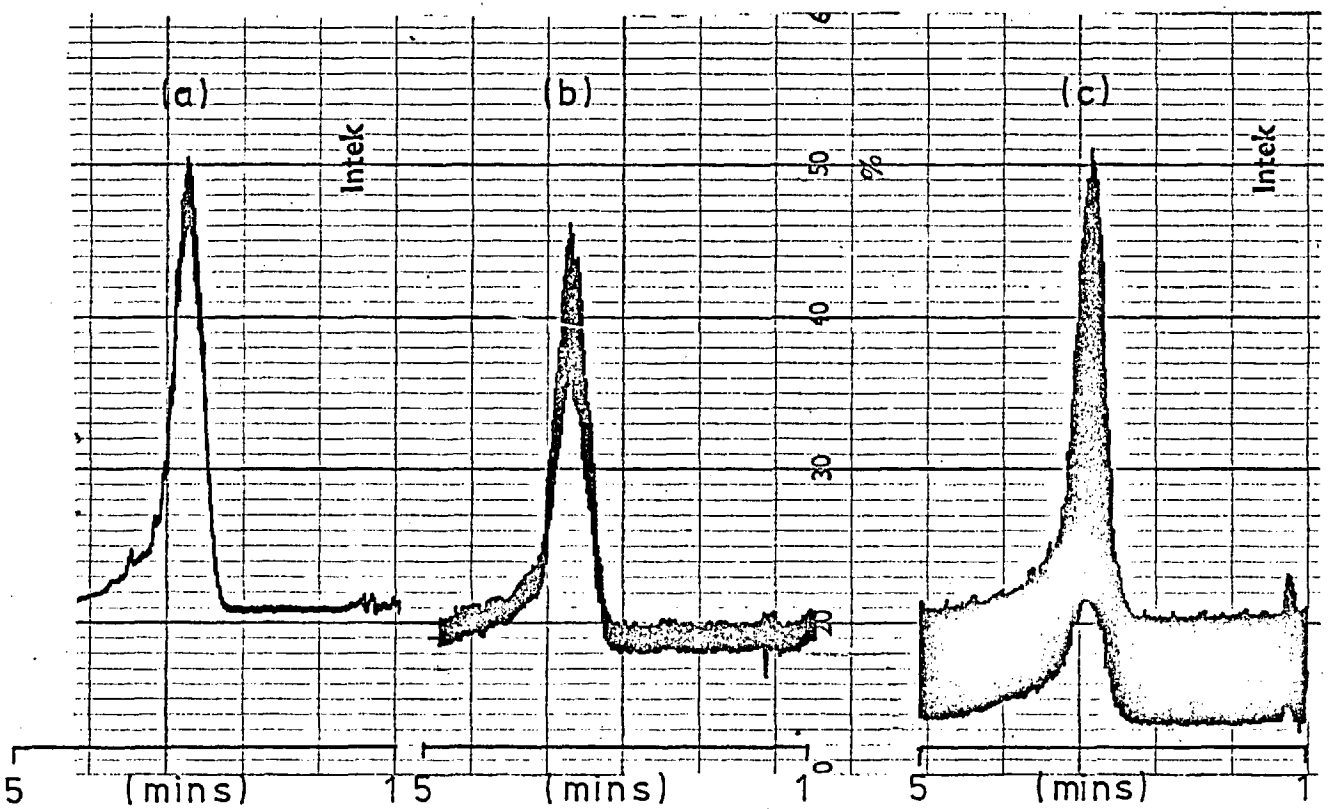


FIGURE 48 Chromatovoltammetric detection of parathion (10^{-3}M) at a solid electrode (glassy carbon) by a repetetive scanning A.C. technique. (a) Potential scanned from -0.9V to -1.0V, (b) Potential scanned from -0.8V to 1 .0V, (c) Potential scanned from -0.5V to -1.0V. A.C. conditions were ΔE = 5mV, ϕ 80°, V = 318Hz and each scan was performed in 1 sec.

electrode. If this happens the practical electrode surface area is variable, resulting in noise. More seriously, large gas bubbles can result in a high resistance between the working and auxilliary electrodes which is manifest by a neglible detector response.

Consequently, a new detector design was developed (Figure 49) which eliminated the need for a mercury pool auxilliary electrode and substituted it with a platinum wire auxilliary electrode. This design has been subjected to preliminary trials and promises to be a great improvement over the model used for the majority of the thesis work. Because the mercury is no longer being used as an auxilliary electrode, the method of collection can revert to the external reservoir connected via a flexible tube. Although not ideal, this method is the easiest to use in practice, a vital argument if the detector is to be used routinely. Secondly, as all the electrodes necessary for 3-electrode operation are clustered around the eluant inlet, it is not necessary to be able to see what is happening further downstream. The detector can be made out of an opaque plastic such as Teflon or Kel-F and is therefore capable of being used with such non-aqueous solvents as propylene carbonate, dimethyl sulphoxide, chloroform, hexane, acetonitrile etc., all of them common electrochemical and chromatographic solvents. Gas bubbles can be released from the exit with ease. If desired, the exit can be situated further downstream and because the tubing is narrow, the gas bubbles readily pass along with the flow. There is very little tendency for gas bubbles to remain in the cell. This is particularly important for aqueous solutions which tend not to wet the detector material very well. Such a detector is better if a streaming mercury electrode is to be used.

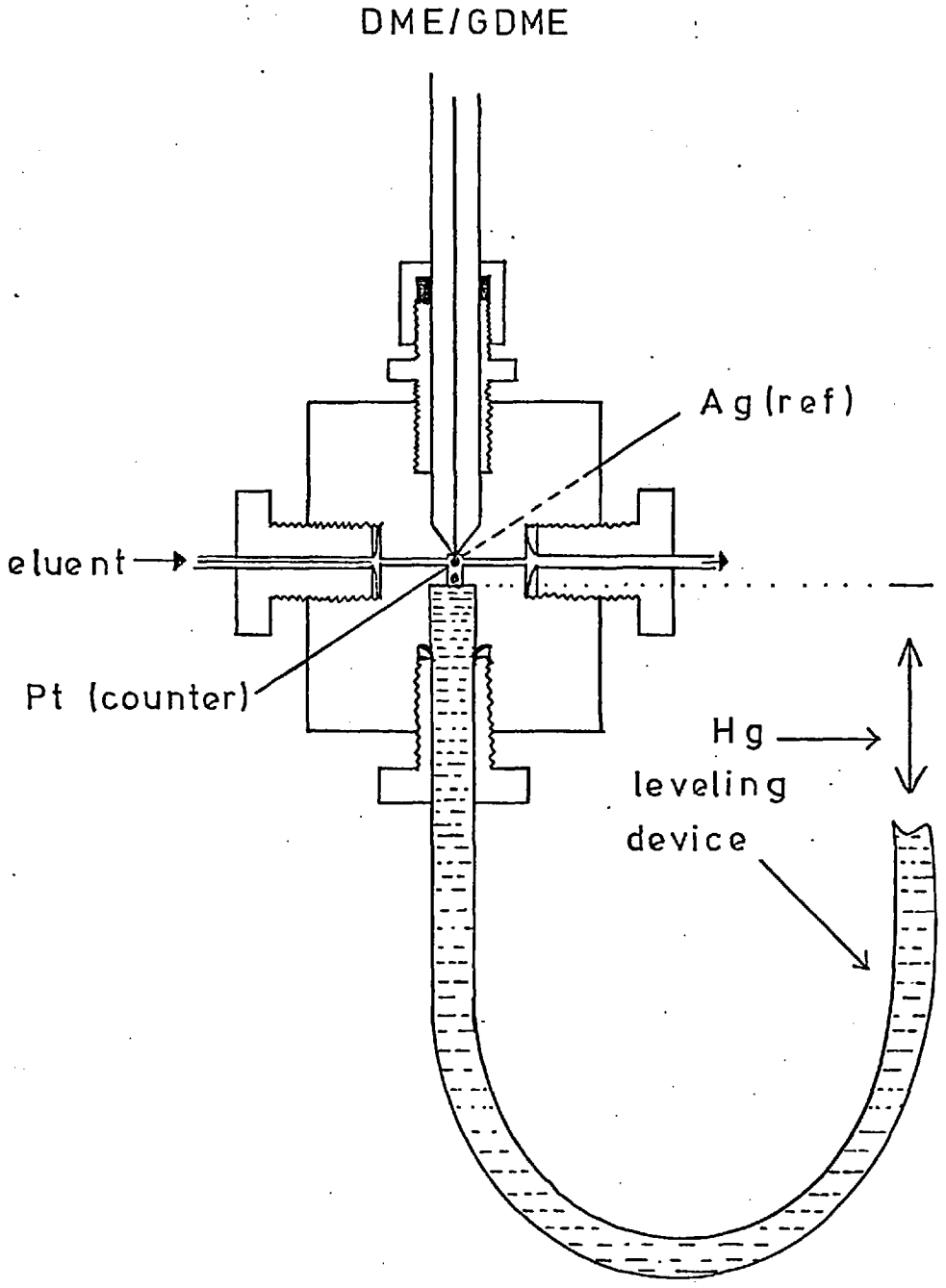


FIGURE 45 Multielectrode detector

Another design which has only been tried out and shown to work consists of a machined Teflon detector incorporating a teflon capilliary (Figure 50). This design has several attractive features. In particular, the problem of creating a leak-proof seal between the d.m.e. and the detector no longer exists.

The built-in Teflon capillary was made by the method of Bond (166) and involved blasting a fine capillary through the Teflon by means of a high voltage electrical discharge from a Tessler coil. Control of the mercury drop-rate is obtained by incorporating a length of glass capillary as shown.

3.1.4. Phase Angle Detector

If an a.c. signal is applied across two electrodes in a flowing stream and is monitored by phase sensitive equipment it should be possible to adjust the phase angle such that the detector output is zero. When a solute enters the detector it is adsorbed and then, probably, desorbed from the electrodes, resulting in a phase change. The result should be an increase in detector output which is monitored as a typical chromatogram. Such a system should be completely universal in application.

A detector (Figure 51) was made according to these plans and connected up to phase sensitive monitors as shown. The chromatographic eluant passed through a U.V. detector and then into the phase angle detector in order to give a comparative result (Figure 52).

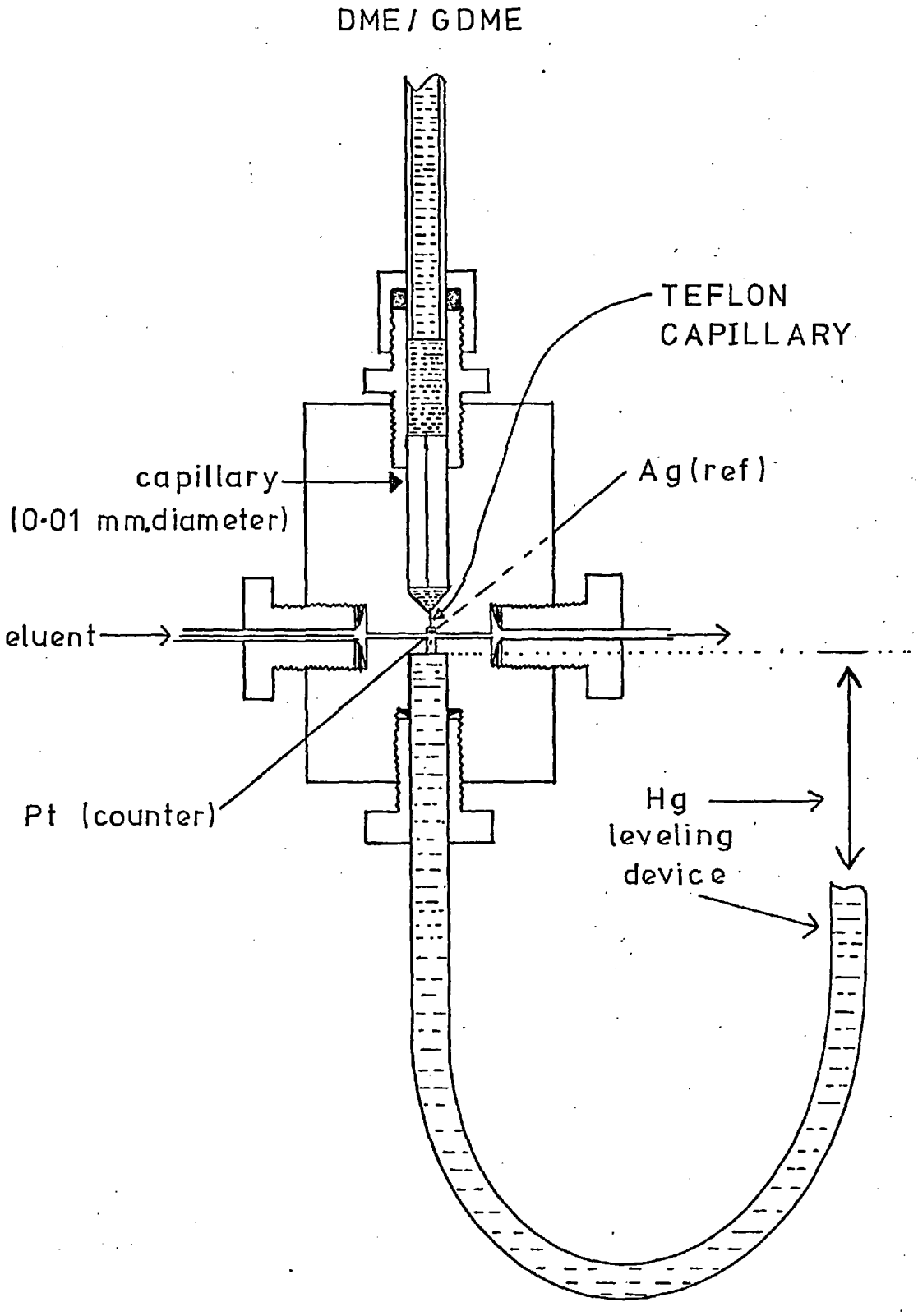
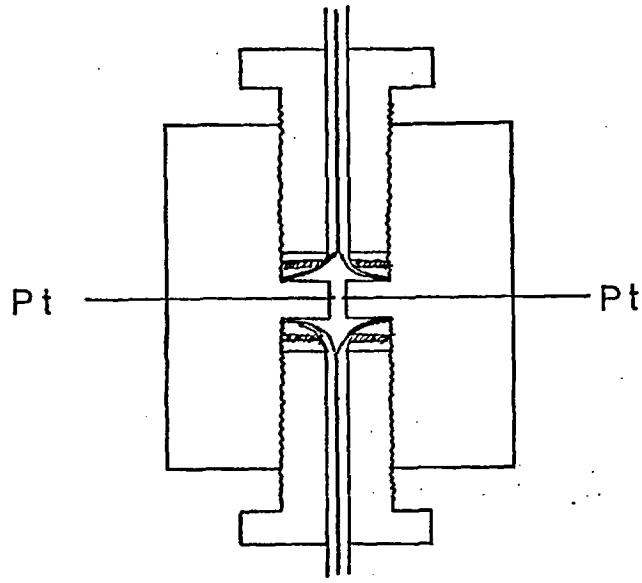


FIGURE 49 Detector with TEFLON capillary



Phase angle detector

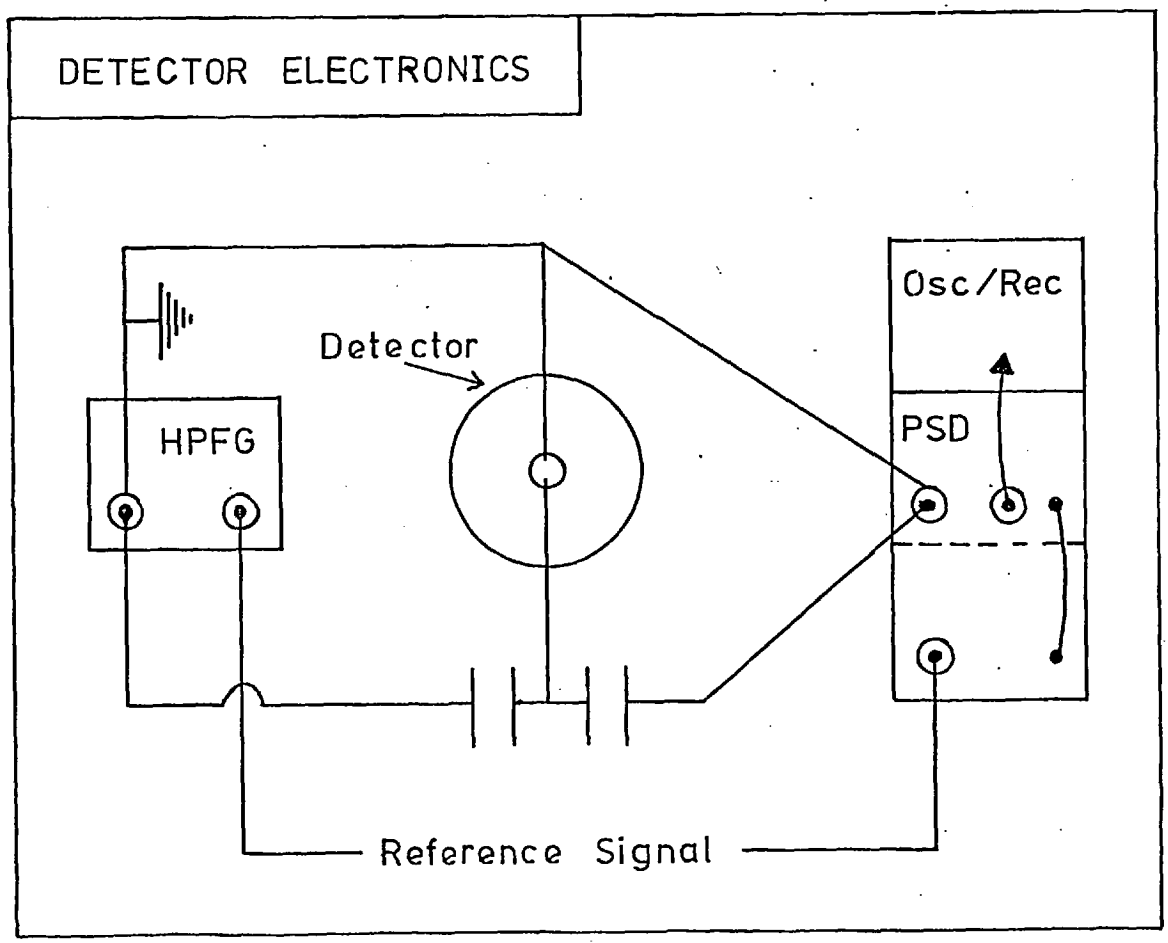


FIGURE 51: Phase angle detector and associated electronics

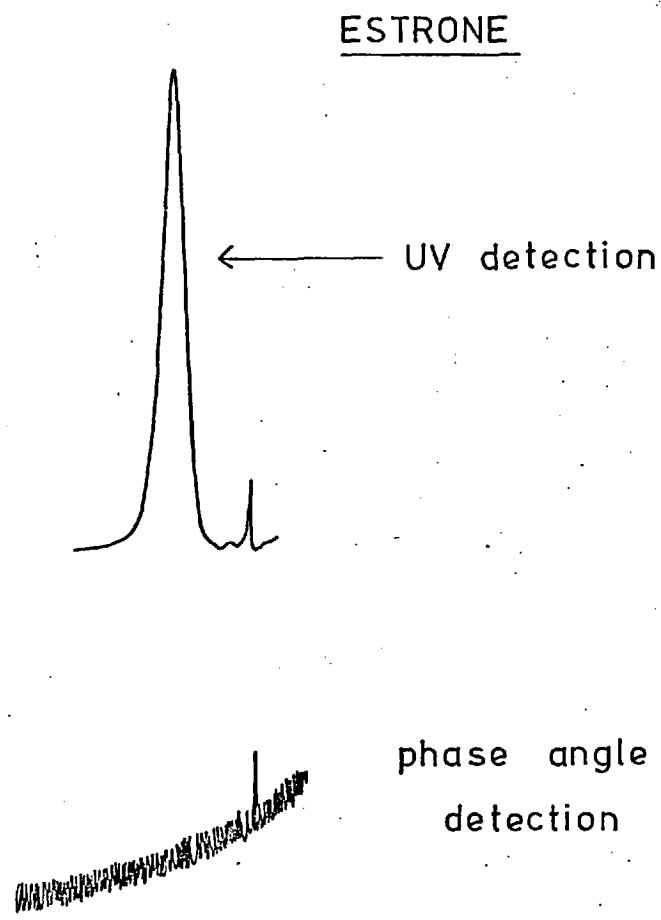


Figure 52 Phase angle detection of chromatographic eluant

The recording was conspicuous by its absence, only the U.V. giving a response. More work could be tried by varying the condenser values, in order to optimise the resistance but the initial results were extremely disappointing. It may be possible to improve matters by applying a d.c. ramp as well as the a.c. signal to the electrodes but this must be the subject of further research.

3.1.5. Conclusions

Several detector designs are described for use with a d.m.e.. Multielectrode capabilities are shown to be possible and the detector is shown to be suitable for use with high pressure liquid chromatography.

3.2. Tubular Detectors

As already mentioned, solid electrodes readily lend themselves to detector geometries ideally suited by hydrodynamic voltammetric measurements. Perhaps the most obvious design is the tubular electrode (240). A detector based on this principle was made out of Teflon (Figure 53). Tubular electrodes made from graphite, impregnated with wax (97) have been used for stripping voltammetry and tubular platinum and gold electrodes have been used for determinations of inorganic species (96). We have committed ourselves to the use of vitreous "glassy" carbon and were thus faced with daunting problems of drilling a tubular hole through the carbon and polishing the interior working surface.

3.2.1. Drilling the Glassy Carbon

Glassy carbon rods, obtained from the Allan Clarke research centre of Plesseys, could only be drilled with diamond tipped drills or by ultrasonic methods. Diamond tipped drills were not available for the bore size we required, so we were forced to use ultrasonic methods. High speed twist drills were embedded in small brass attachments designed to fit the ultrasonic drill. The ends of the drills were ground flat such that the length of drill protruding from the brass holder was 0.6 cm. This was necessary, as too long a drill would bend and produce poor drillings. The assembly was then fitted to the drill.

Drilling was performed by placing the drill tip onto the centre of a piece of carbon rod, firmly embedded in a piece of perspex. The ultrasonic power was increased to initiate drilling. The drill

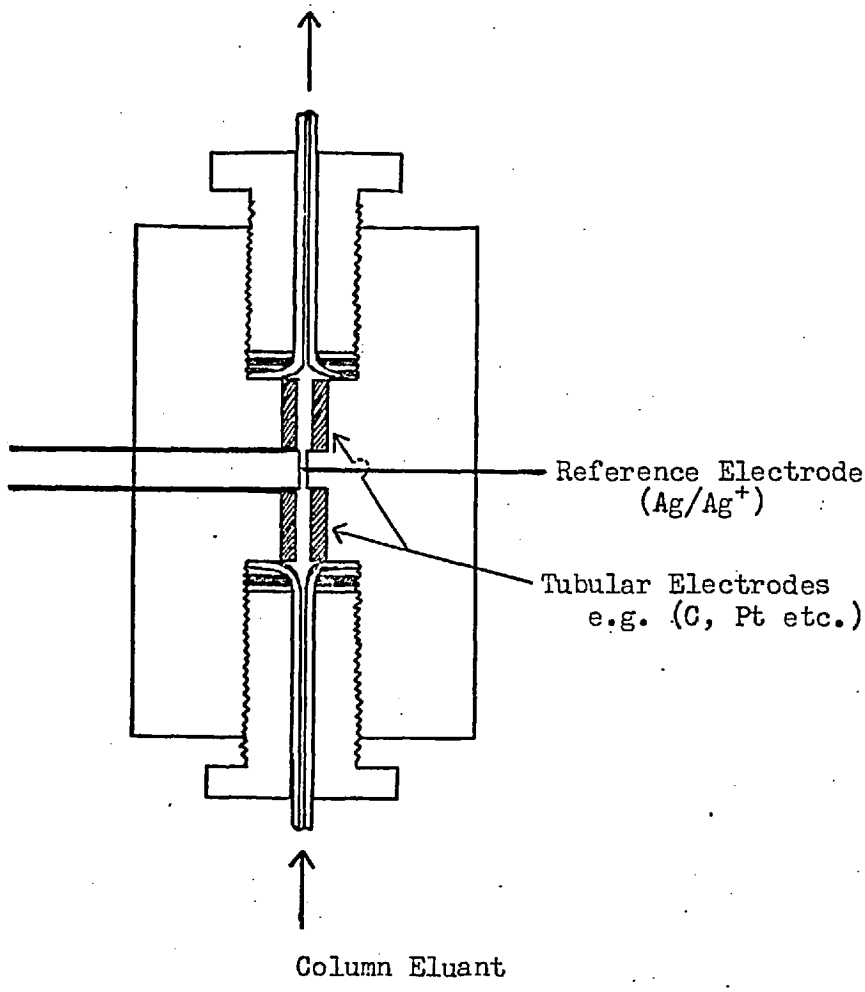


Figure 53 TUBULAR ELECTRODE detector for H.P.L.C.

TABLE 14

Dead volume data for a TUBULAR ELECTRODE*

BORE (mm)	VOLUME (μl)	AREA(cm)^2
1.5	8.9	0.24
1.0	3.9	0.16
0.5	2.8	0.08

* Electrode length of 5 mm.

tip and the carbon rod were lubricated with fine abrasive powder, dispersed in water and the ultrasonic power was increased to a maximum. After about 15 minutes, the drilling was complete and a tubular carbon electrode was ready for polishing.

This proved to be very difficult and was never performed adequately. The surface was always too rough and after producing a few voltammograms, became completely deactivated. It is possible that the energy dissipated during drilling caused considerable oxidation of the carbon surface, which needed much better polishing techniques to regenerate the active surface.

A possible solution to this problem is to have the tubular carbon made as such by depositing the carbon on a polished metal former.

Table 14 indicates that the detector dead volume is very satisfactory and could conceivably be reduced to about 1μl by using shorter electrodes. This would reduce the sensitivity considerably and is a poor compromise especially as diffusion from the centre of the tube to the electrode surface is so slow, that much of the solute never comes into contact with it.

3.3. Wall Jet Electrode Detector

As it is comparatively easy to polish to a mirror finish, the end of a piece of glassy carbon rod, it was decided to incorporate such an electrode into the detector as shown (Figure 54).

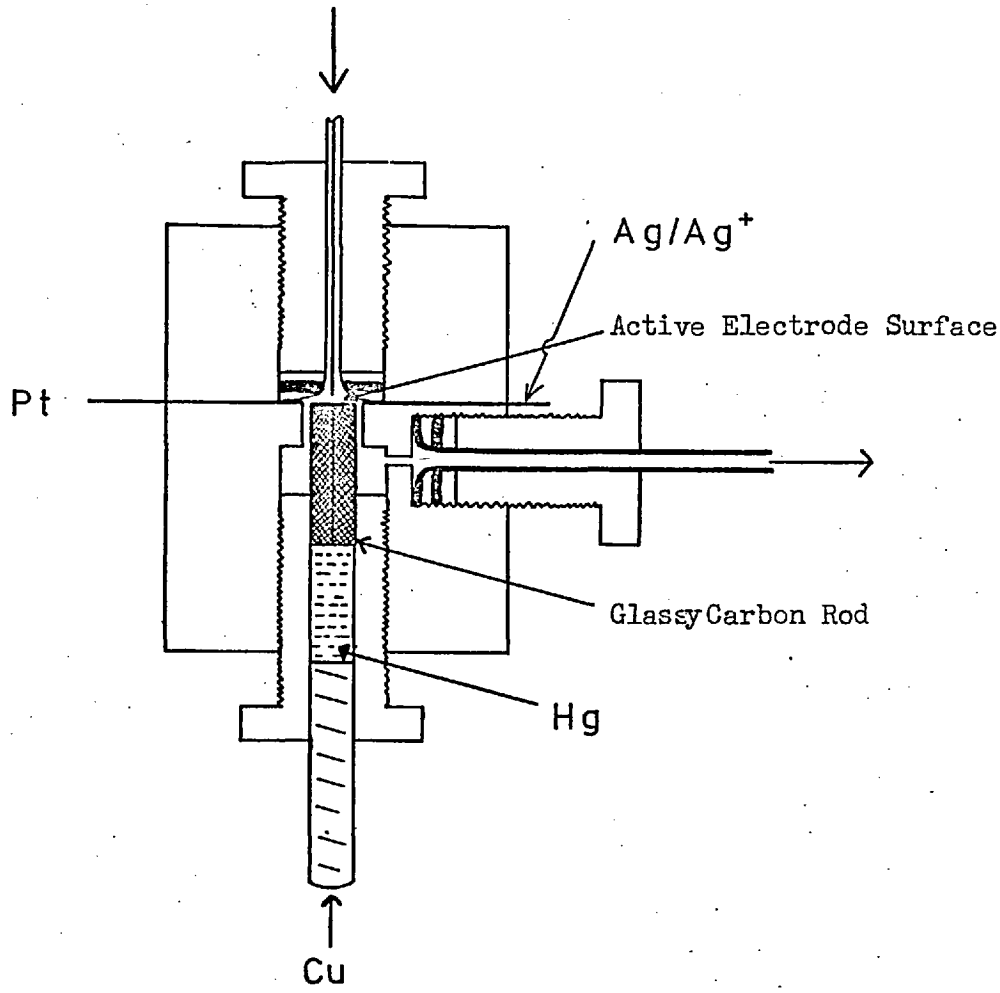


Figure 54 WALL JET Electrode Detector for H.P.L.C.

TABLE 15

Dead volume data for a WALL JET ELECTRODE*

RADIUS (mm)	VOLUME (μl)	AREA $(cm)^2$
3	2·8	0·28
2	1·3	0·13
1	0·3	0·03

* based on separation between inlet and electrode surface of 0.1 mm.

The eluant strikes the centre of the working electrode in a fine jet and spreads out over the electrode surface and escapes round the sides of the electrode to the exit.

The geometry of the detector is such that dead volume can be restricted to an absolute minimum (Table 15). Detector volumes of about 0.1 μl could be prepared with careful machining.

The outer surface of the carbon, not required for electrode activity was coated with a thin film of epoxy cement.

3.3.1. Fundamentals of the Wall Jet Electrode

The limiting diffusion current (I_d) for the wall jet electrode (239) is given in Equation 9.

$$I_d = (1.60k)nFC^{o}D^{\frac{2}{3}}v^{-5/12}V^{\frac{3}{4}}a^{-\frac{1}{2}}R^{\frac{3}{4}} \quad \text{..... Equation 9}$$

where
- v = kinematic viscosity of the eluent
- V = volume flow rate of the solution issuing from the circular nozzle of the inlet tubing.
- a = internal diameter of the circular nozzle.
- n = number of electrons involved in the electrode reaction
- F = the Faraday.
- C^{o} = bulk concentration of depolarizer
- D = diffusion coefficient of depolariser.
- R = radius of the disc electrode.
- k = proportionality factor.

Although equation 9 does not contain any parameter concerning the distance, d, between the electrode surface and the tip of the nozzle,

it does have a significant effect on the electrode performance.

If d is less than 0.2 mm., decreased electrode performance is obtained (239) probably because a back pressure is built up restricting the outflow of the eluent. Above 0.2 mm. I_d rapidly reaches a maximum value and then decreases gradually with increasing values of d. The working electrode of the detector should therefore be adjusted to give an optimum response.

For quantitative work it is most fortunate that the limiting diffusion current is directly proportional to the bulk concentration of solute in the eluant, providing all other experimental conditions remain the same.

As the flow rate increases, so does the value of I_d. Directly related to this effect is the nozzle diameter. The smaller the value of a, the greater the sensitivity.

Sensitivity can be increased if the radius of the electrode is increased. A minimum value of 1.5 mm. is recommended.

It can be seen that the inevitable compromise is necessary in order to optimise both electrochemical requirements and chromatographic requirements.

Maximum sensitivity can only be attained by increasing R and choosing an intermediate value for d. This has the adverse effect of increasing detector volume. The dead volume is so small, however that no great disadvantage is met. In fact, in the cases where low detector volumes are necessary peak volumes are minute and have greater responses due to minimal dilution effects.

Both the increase in flow rate and minimal nozzle diameter are compatible with chromatographic requirements.

The detector used in the following illustrations of hydrodynamic voltammetry had the following characteristics: d = 0.1-0.2mm., V = 0.5-2 ml./min., a = 0.01 inches, R = 2 mm.. The detector volume was therefore 1-2 μl. These and other important geometrical values are indicated in Figure 55.

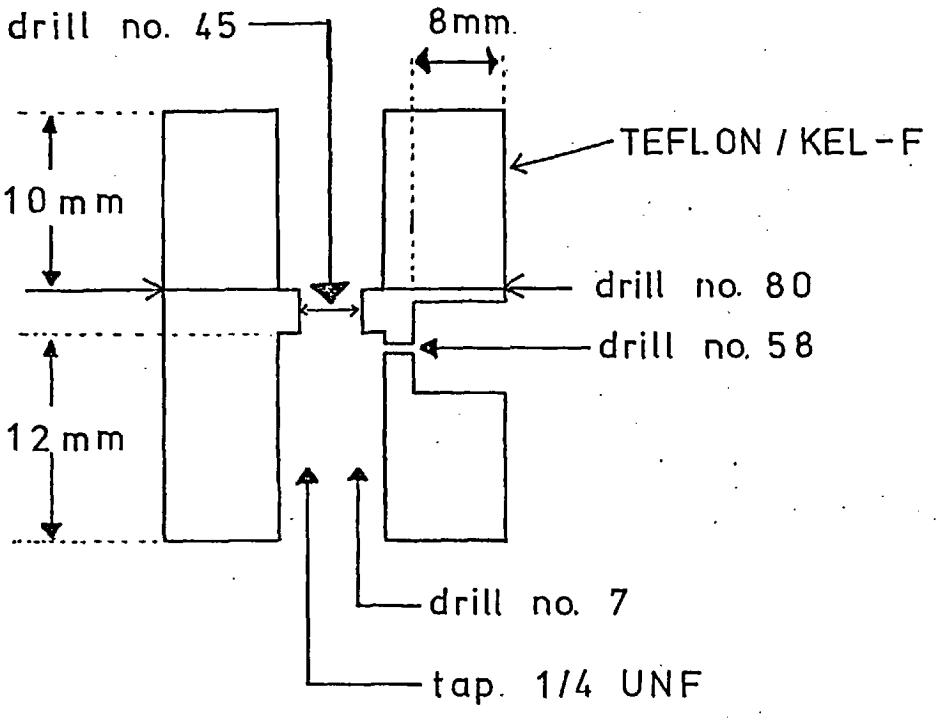


Figure 55 Construction details for Wall Jet Electrode Detector (WJED)

3.3.2. Evaluation of the W.J.E.D.

The detector was evaluated under hydrodynamic conditions. A series of cobalt complexes obtained from the University of Kent (Dr. L.S. Dollimore) were examined at the dropping mercury electrode by d.c. and pulse polarography. The most probable electronic transitions

observed were $Co^{3+} \longrightarrow Co^{2+}$ and $Co^{2+} \longrightarrow Co^{\circ}$. The $E_{\frac{1}{2}}$ values vs Ag/AgCl are given in Table 16.

Table 16

Compound	$E_{\frac{1}{2}}$: volts vs Ag/AgCl	
	$Co^{3+} \rightarrow Co^{2+}$	$Co^{2+} \rightarrow Co^{\circ}$
1. $[Co(en)_3]Cl_3$	-0.32	-1.14
2. $[Co(en)_2phen]Br_3$	+0.06	-0.94
3. $[Co(en)_2bipy](ClO_4)_2Cl$	-0.08	-1.02
4. $K[Co(EDTA)]$	-0.20	-1.68
5. $[Co(en)_2sal]Cl_2 2H_2O$	-0.17	-1.16
6. $[Co(en)_2pic]Cl_2$	-0.12*	-0.36*
7. $[Co(NH_3)_6]Cl_3$	-0.38	-1.22
8. $[Co(gly\text{-}o)_3]$ α isomer	-0.04	-1.24
9. $[Co(phen)_3](ClO_4)_3.3H_2O$	+0.12	-0.78
10. $[Co(dipy)_3](ClO_4)_3.3H_2O$	-	-1.02

* $Co^{3+} \longrightarrow Co^{1+}$ and $Co^{1+} \longrightarrow Co^{\circ}$

en = ethylene diamine

phen= 1,10 phenanthroline

bipy= 2,2' dipyridyl

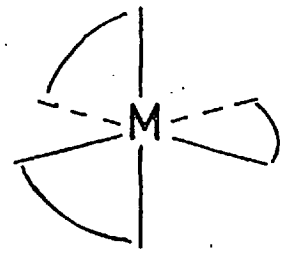
sal = salicylate dianion

pic = picolinate

gly-o= glycinato

All (with the exception of No. 7) are tris-chelated complexes

i.e.



Two of these complexes, numbers 1 and 8 were studied at the glassy carbon electrode. The immediate effect of hydrodynamic conditions is shown (Figure 56) by repetetive fast scan d.c. polarography of $[Co(en)_3]Cl_3$ in flowing and static streams.

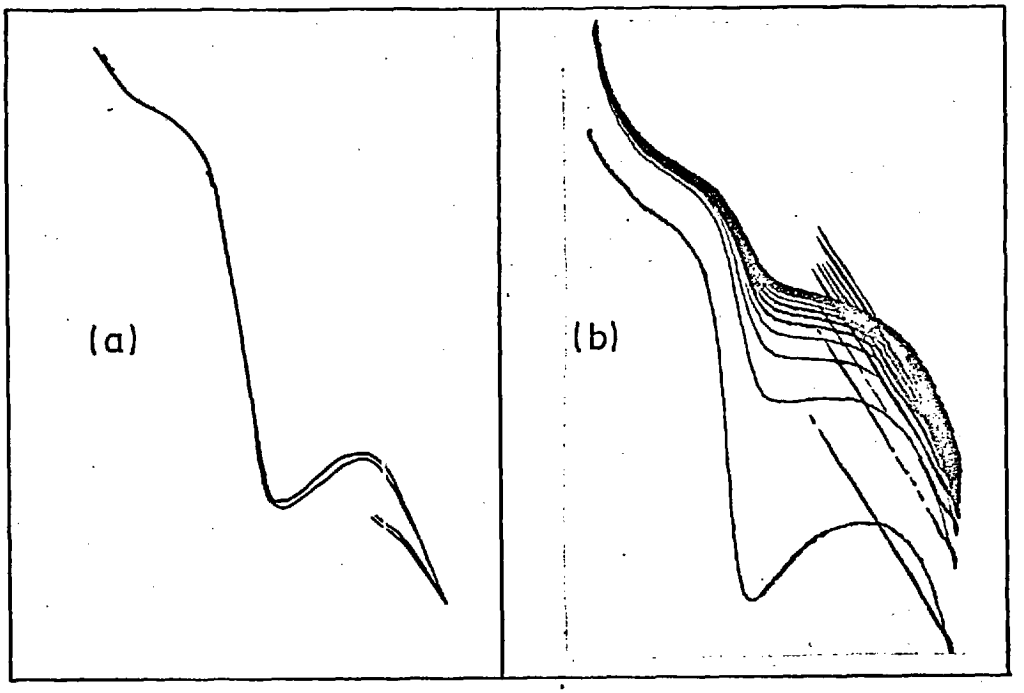


Figure 56 Fast sweep d.c. voltammetry of $[Co(en)_3]Cl_3$ at a glassy carbon electrode (a) Flowing stream - 1 ml/min.
(b) Static stream

Under hydrodynamic conditions repetetive scans are of maximum response and reproducible. When the flow is stopped the fast scan voltammogram diminishes and the characteristic peak shape changes to the conventional d.c. polarographic shape. A limiting value is reached where no further loss of sensitivity occurrs. There is no apparent change in $E_{\frac{1}{2}}$ associated with this depletion effect.

The a.c. voltammetric behaviour is somewhat different (Figure 57). No decrease in peak height is observed when the solution

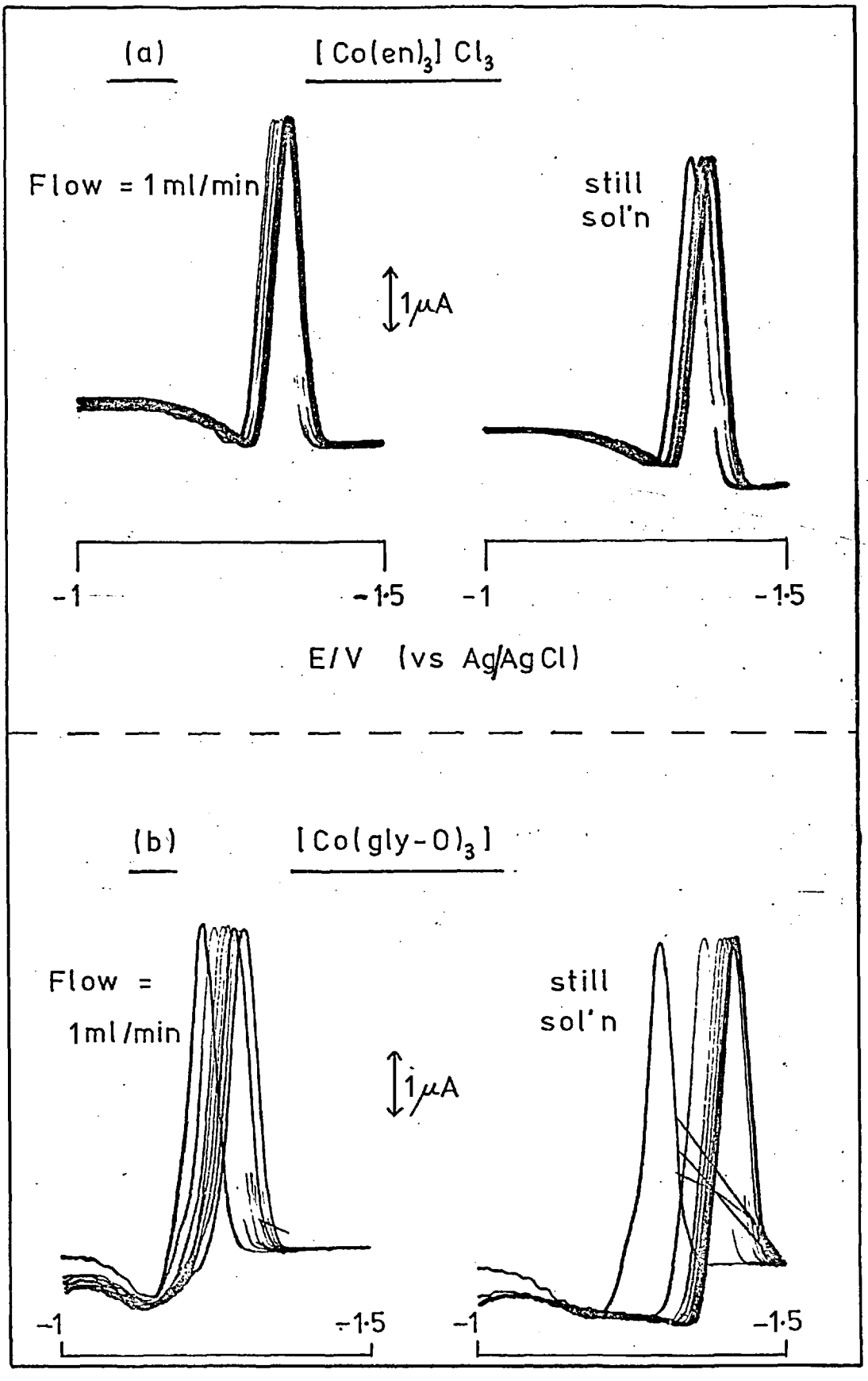


FIGURE 57: A.C. polarography of Co complexes at a glassy (vitreous) carbon electrode (W.J.E.D.)

ΔE = 5mV, $\phi = 90^{\circ}$, V = 318H_z (phase sensitive detection)

flow is stopped. This applies to both $[Co(en)_3]Cl_3$ (Figure 57(a)) and to $[Co(gly\text{-}O)_3]$ (Figure 57(b)). What is observed, however, is a change in peak potential to more negative values. This effect is more pronounced for $[Co(gly\text{-}O)_3]$ and becomes more pronounced in static solutions . This would indicate that for conventional electrochemical measurements, the a.c. technique has considerable advantages when applied under hydrodynamic conditions to solid electrodes. For monitoring L.C. eluants, the d.c. or pulse method is still preferable for the reasons that have already been mentioned.

The effect of scan rate on the a.c. signal is quite interesting (Figure 58). It appears that a fast scan is essential at a glassy carbon electrode, otherwise the signal is grossly diminished This could possibly be due to phase change effects or the building up of a surface layer of adsorbed species.

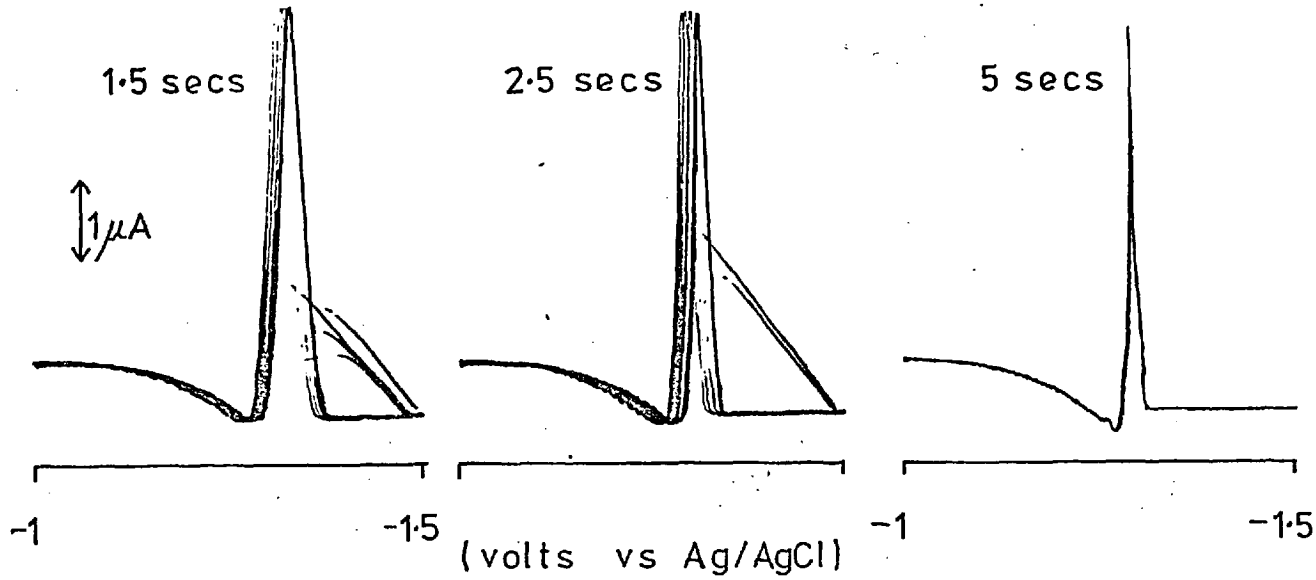
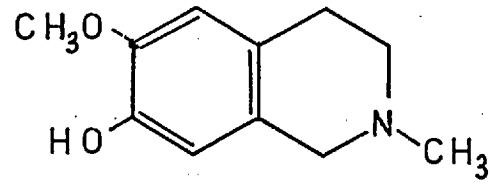


Figure 58 Effect of scan rate on a.c. voltammetry of $[Co(en)_3]$ at a glassy carbon electrode. $\Delta E = 5$ mV, $\phi \sim 90°$, ν 318 H_z (phase sens itive detection). Potential scanned from -1 to -1.5 volts in the times indicated.

All the investigations to date have been concerned with the application of a negative potential to the electrode and that the species being determined is electrochemically reduced. One of the major features of carbon electrodes, in particular glassy carbon electrodes, is the wide positive potential range over which they can be used. This results in electrooxidation of the species being determined.

Corypalline (Compound IX) is an isoquinoline derivative that has been the subject of intensive electrooxidative studies. (99-101). It is known to adsorb onto the electrode surface and was therefore considered to be a good test of the hydrodynamic approach to anodic voltammetry.



IX

Previous voltammetric studies have used the d.c. polarographic approach and attempts to reactivate the electrode surface have involved potential cycling techniques and mechanical abrasion of the electrode surface. These were considered to be superior to rotating electrode methods.

Corypalline solutions (10^{-3}M) in borate buffer cause an increasing deactivation of the glassy carbon electrode on repetetive scanning if the flow through the W.J.E.D. is stopped (Figure 59(a)) the scan numbers are indicated on the respective voltammograms recorded

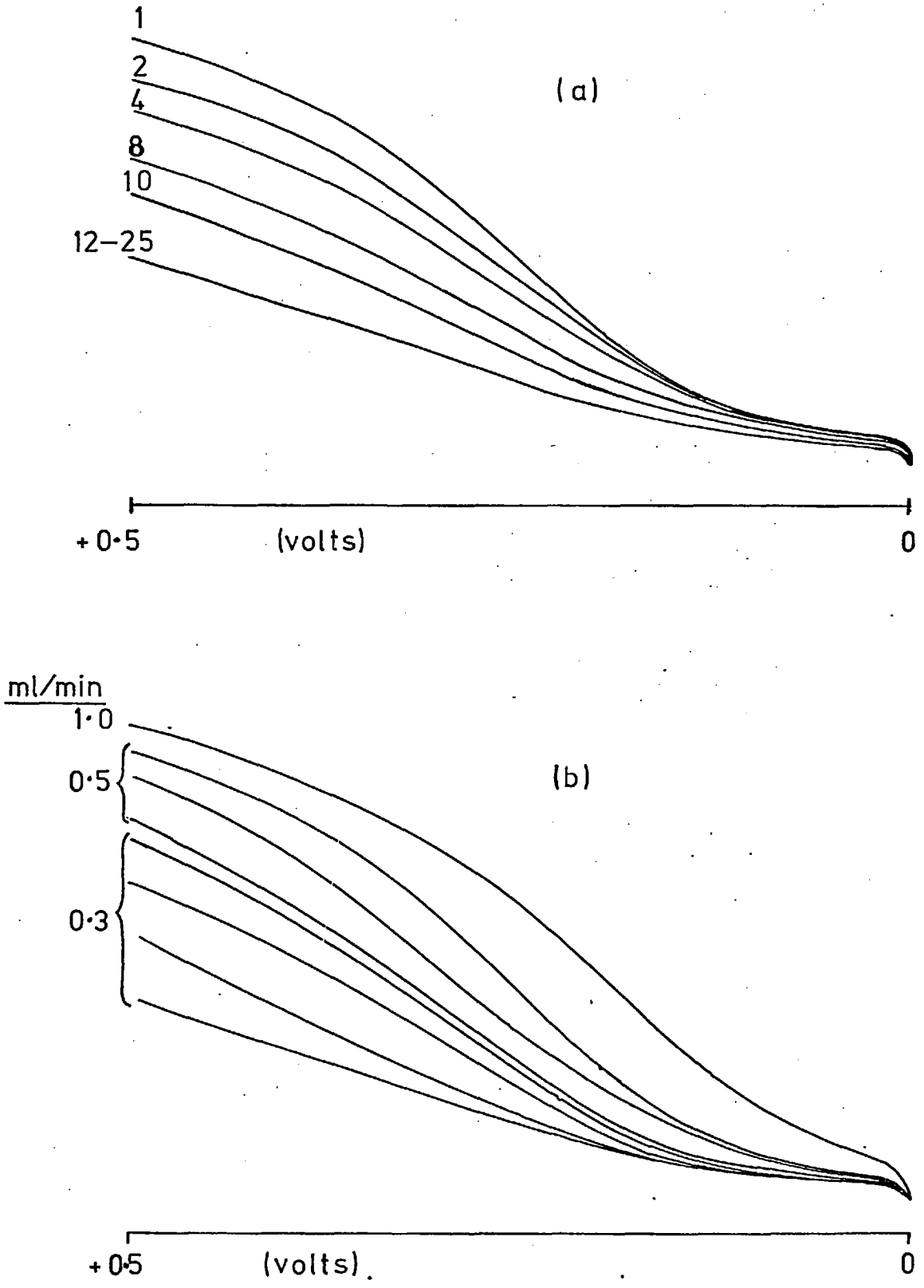


Figure 59 Hydrodynamic voltammetry (at the W.J.E.D.) of Corypalline (10^{-3}M) in borate buffer, at a glassy carbon electrode showing (a) Successive deactivation of the electrode in static solution and (b) successive reactivation of the electrode as the flow rate is increased.

after scanning from 0 to +0.5 volts vs Ag/Ag^{+} reference electrode.

As soon as the solution is made to flow through the detector "REACTIVATION" of the electrode surface occurrs progressively. This process can be speeded up by increasing the hydrodynamic flow through the detector. At a flow of 1 ml. per minute through the detector, rapid reactivation occurrs (Figure 59(b)) and anodic voltammograms of increased limiting diffusion current are obtained. These are realistic flow rates for liquid chromatography and therefore indicates the suitability of such a detector for monitoring L.C. columns. It has now been shown to be a viable detector for both cathodic and anodic monitoring which effectively means its potential application is enormous. For hydrodynamic voltammetric purposes only, it is interesting to see that the response increases as the scan rate increases (Figure 60).

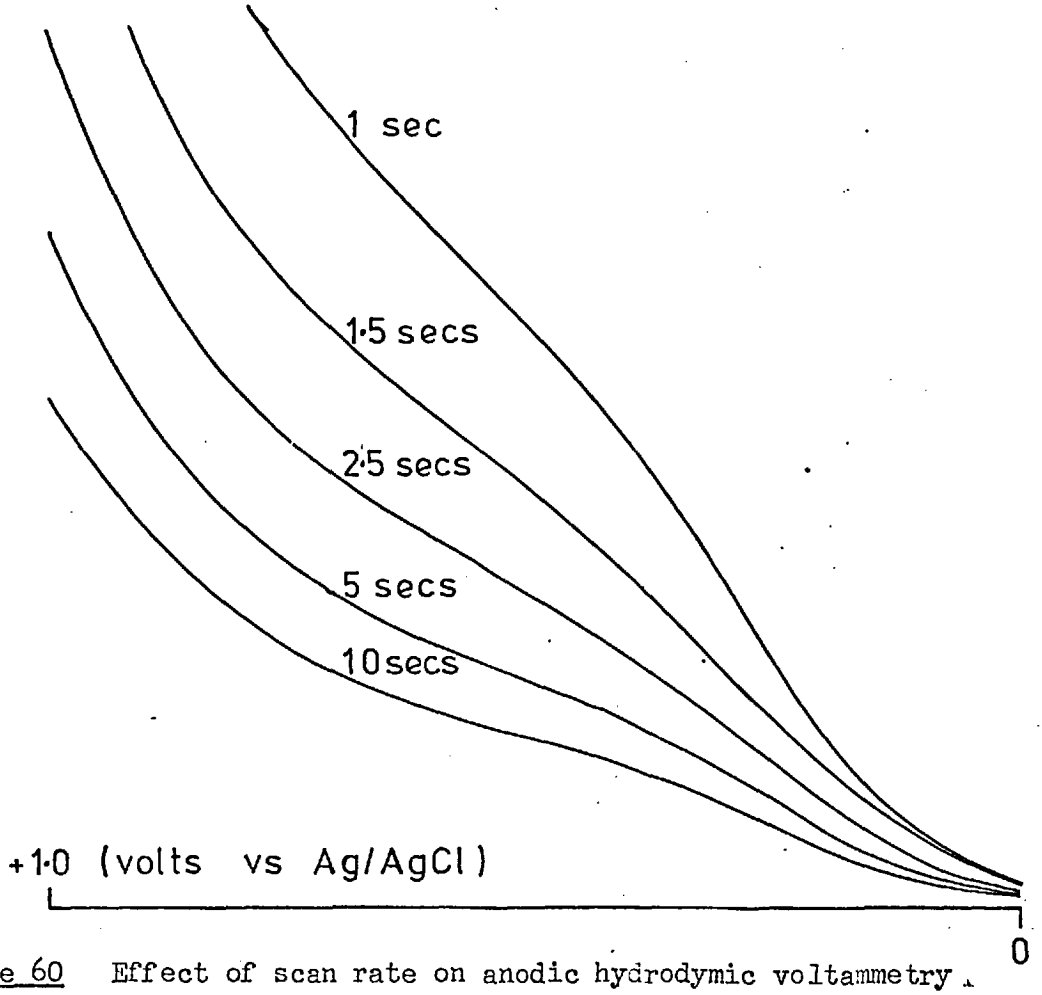


Figure 60 Effect of scan rate on anodic hydrodymic voltammetry. Fast scan D.C. voltammetry at the W.J.E.D.. Solution flow = 1 ml/min.

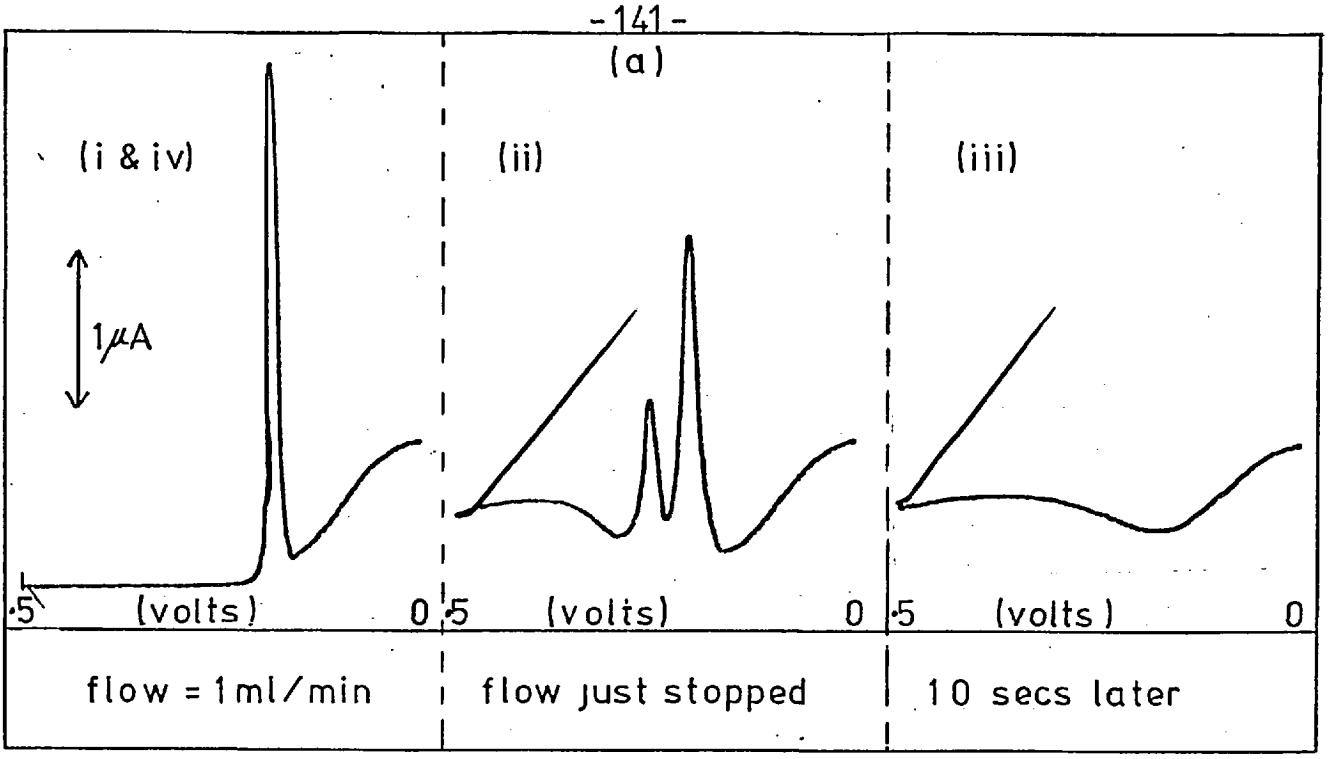
Furthermore the potential scan from 0 to 1.0 volts indicates tha irreversible electrode deactivation does not occur under these conditions.

All the d.c. voltammograms shown are poorly defined when compared with typical cathodic polarograms and obviously represent highly irreversible electrode mechanisms. They can be used qualitatively but not quantitatively. This arguement does not apply for chromato-voltammetric determinations at fixed potential because the peak shaped chromatographic response is easily measured.

For quantitative anodic hydrodynamic voltammetry by scanning methods, techniques which produce peak shaped responses are to be recommended, such as a.c., differential pulse, square wave and radiofrequency techniques.

The a.c. polarography of corypalline at a glassy carbon electrode under hydrodynamic conditions is shown ih Figure 61 and illustrates a further important aspect, namely the preparation of the electrode surface. If the glassy carbon is ground down on successively finer emery cloths a reasonably shiny surface is obtained which produces adequate a.c. voltammograms under hydrodynamic conditions (Figure 61,a,(i). As soon as the flow of 1 ml. per minute is stopped, adsorption occurrs immediately. Figure 61,a(ii) shows the onset of this and 5 seconds later, complete deactivation has occurred. (Figure 61,a,(iii). The depression of the baseline is indicative of adsorption at the electrode surface which can be completely removed if the flow is restarted (Figure 61,a,(iv).

If the electrode is highly polished either by using brasso,



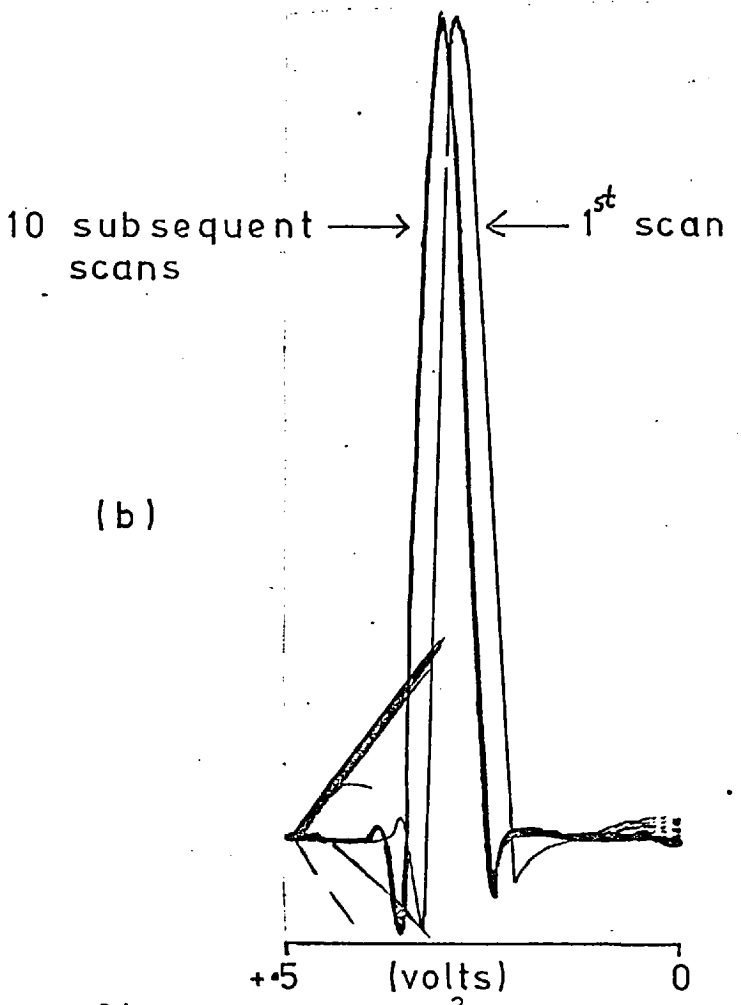


Figure 61 Corypalline (10^{-3}M), a.c. polarography (fast scan) at a glassy carbon electrode (a) ground, (b) highly polished in borate buffer (0.1M).
ΔE = 10mV, $\phi \simeq 75^{o}$, w = 318H_z, scan rate of 500mV in (a) 5 secs and (b) 1 sec.

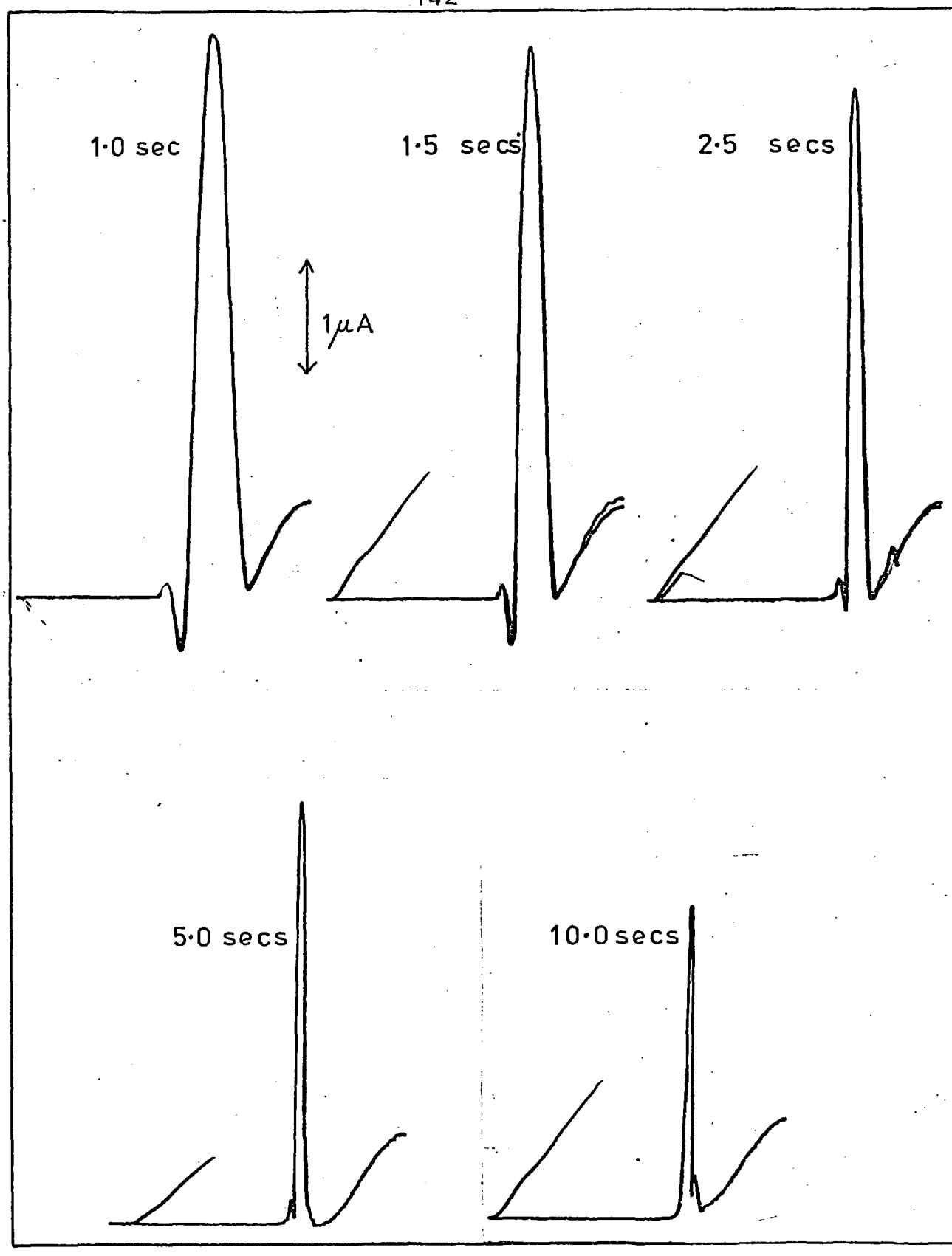


FIGURE 62 Corypalline (10^{-3}M). Effect of scan rate on A.C. polarography at a glassy (vitreous) carbon electrode, E = 5mV, $\phi = 75^{0}$, scan rates indicated (in seconds) for a scan of 500mV.

diamond polishihg paste, or silica (< 20 microns), the resulting a.c. voltammogram is apparently insensitive to flow rate and produces reproducible peaks in static solutions (Figure 61,b). This is similar to the behaviour of the cobalt complexes under cathodic conditions and suggests that the highly polished electrode surface minimised or even eliminates the adsorbtive processes and is predominantly subject to depletion effects. It also shows that the first scan occurrs at a less positive potential than the final scans. This again, is similar tothe cathodic behaviour of glassy carbon electrodes and may be a consequence of depletion.

The effect of scan rate (Figure 62) is similar to the effect on the reduction of Cobalt complexes at the glassy carbon electrode. It appears to be a feature of a.c. polarography at glassy carbon electrodes and probably other solid electrodes as well.

3.3.3. CONCLUSION

It has been shown that the wall jet is an ideal system for using glassy carbon (and other solid electrodes) under hydrodynamic conditions. Optimum conditions for hydrodynamic voltammetry are not necessarily optimum for chromatographic detection, but the differences are of minor importance only. Its application to cathodic and anodic processes is of great importance, the limits of the potential range being determined by solvent and electrolyte properties.

Although not measured experimentally, it is reasonable to assume that far more of the solute is reduced or oxidised than would be the case for a tubular or rotating electrode and consequently,

further experimentation should be able to verify the advantages of such a detector for coulometric measurements and for preparative electrochemistry. It is conceivable that recycling of the solution can be carried out until complete formation of the desired product has occurrdd.

3.4. Other Designs

For solid electrodes there is some scope for using other electrode geometrics. There is also scope for using other electrochemical measuring methods such as differential amperometry. Further research is required to optimise these parameters, but what is clear is that the basic technique is well founded.

3.4.1. Carbon Paste Detector

Recently Adams et. al. have described such a detector for use with H.P.L.C. (190). The electronic circuitory is very simple and could form the basis of a cheap model. It is however, very limited in scope. The application cited, namely the anodic oxidation of catecholamines in perchloric acid media, is obviously one of the best for this detector, but it is more prone to adsorption effects than the glassy carbon electrode and can only be used in aqueous media. Its volume ($<1\mu l$) is quite compatible with modern H.P.L.C. methods.

3.4.2. Flow Coulometric Detector

Flow coulometry (212,214,242) utilises a packed tube electrode. Packings that have been used consist of silver grains (243-245), amalgamated nickel particles (246), amalgamated platinum(247), porous platinum grid (248,249), graphite powder (250,251), glassy carbon grains

(252,-255) and carbon fibre (214,215).

Recently this principle has been transplanted into a detector for H.P.L.C. (256) using a column packed with glassy carbon grains and applied to the ion exchange chromatography of metal ions, halide ions, amino acids, carboxylic acids, phenols and monosaccharides.

Its main advantages lie in the vastly increased electrode surface area which results in almost complete oxidation or reduction of the solute, thus increasing considerably the sensitivity. It is also hardly affected by changes in flow rate and temperature. As a detector for L.C. its main disadvantage lies in the fact that it is totally destructive, thus preventing collection of separated components for further study.

However, the idea is a good one even though the sensitivity quoted is no greater than with the wall jet electrode. The flow of liquid over the electrode surface is probably slower than in the wall jet design and is therefore more prone to adsorption problems. Tubes packed with carbon fibres might be the next obvious stage of development as they produce very fine channels along which the eluent can pass, thus minimising band-spread of eluted solutes.

The quantitative oxidation or reduction of the eluted solute is an important aspect to consider for electrosynthetic purposes.

APPLICATIONS TO REAL SYSTEMS

4. Introduction

All the following applications apply to the use of the W.J.E.D. unless otherwise stated and only touch the fringe of potential applications. Figure 63 attempts to give some idea of the potential applications for direct detection. Many derivatives can be formed to increase this range still further.

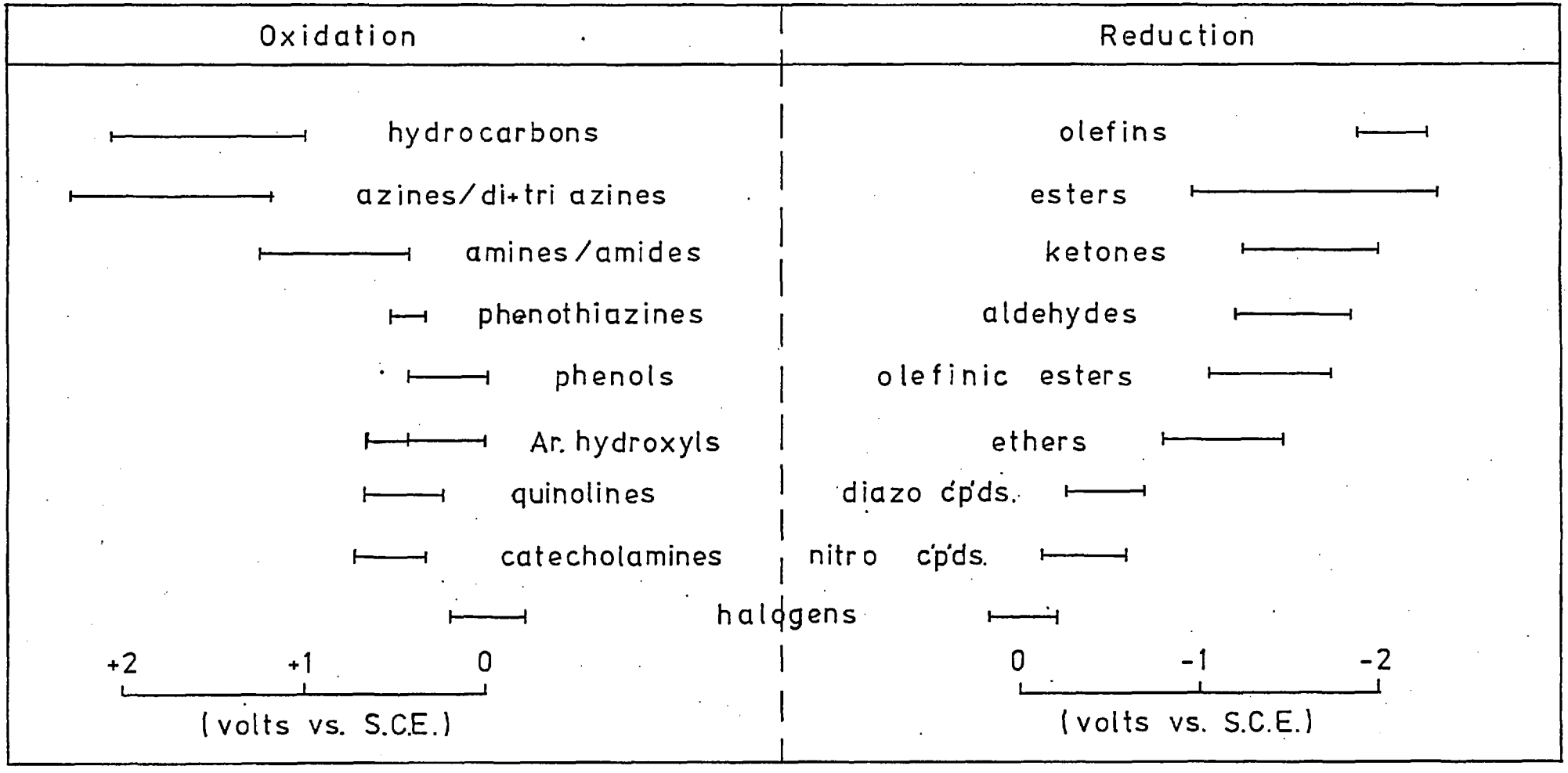
4.1. Reagent Addition

The question of determining derivatives raises some problems from the chromatographic point of view. Many chromatographic systems are already tried and tested procedures. The chromatography of their derivatives might very well be more or less successful. The addition of reagent to the eluant is not a practice that should be considered unless no other method is available, as the reagent will almost certainly modify the support and alter the chromatography. In the long run, some better chromatographic systems might emerge but many more will be far worse. This is particularly so when considering the question of adding supporting electrolyte to the eluent. Such common electrolytes as tetrabutylammonium perchlorate, hexafluorophosphate etc. are necessary for L.C. monitoring in non-aqueous eluents. Their effect on the chromatography would be expected to be significant at the required concentrations of say 0.1M.

The obvious way of solving this problem is to add the reagent after the chromatographic separation and just prior to detection. In turn, this can have adverse effects on the separation

Figure-63

Some Electroactive Groups



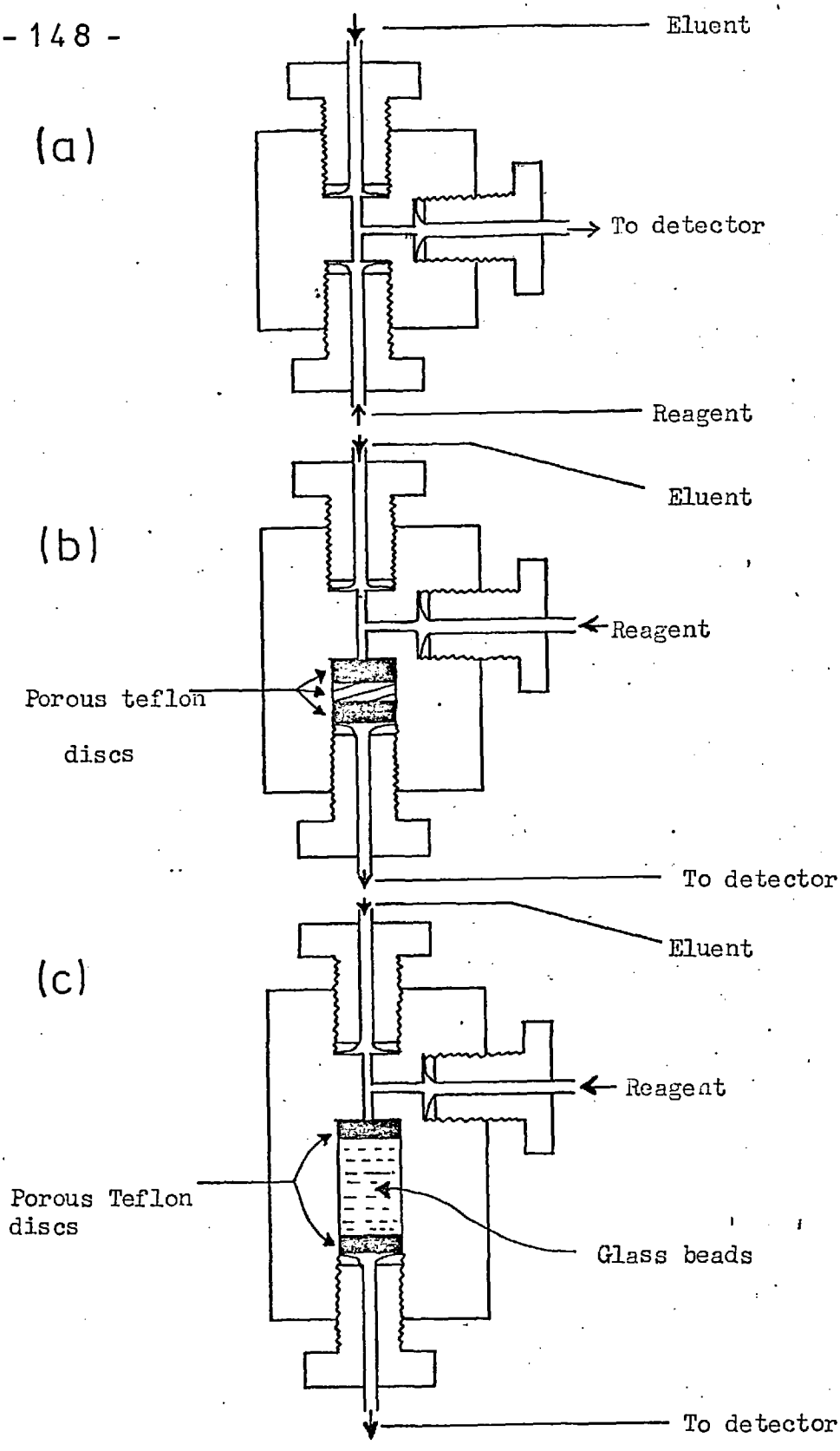


FIGURE 64 Three ways of adding reagent to chromatographic eluents. (a) Simple T-piece, (b) Porous teflon mixer, (c) Glass bead mixer.

by increasing the dead volume of the extra-column fittings. Obviously such methods would be avoided if possible, but when needed the following techniques and equipment keep the dead volume to aceptible minimum.

Figure 64 shows three ways of adding the reagent after the column, The T-piece was constructed out of nylon rod (1" diameter) and had an effective dead volume of 1-2μl. In conjunction with the low dead volume W.J.E.D. this was reasonably acceptable. Unfortunately the reagent, added from the side, did not mix very well, leading to very poor reproducibility. This was overcome by increasing the bore of the T-piece as shown (Figure 64b,c) and packing it with porous teflon and glass beads. These caused efficient mixing of the eluent and reagent as shown in Figure 65. The column can either be screwed directly into the T-piece or via a low dead volume connection as shown.

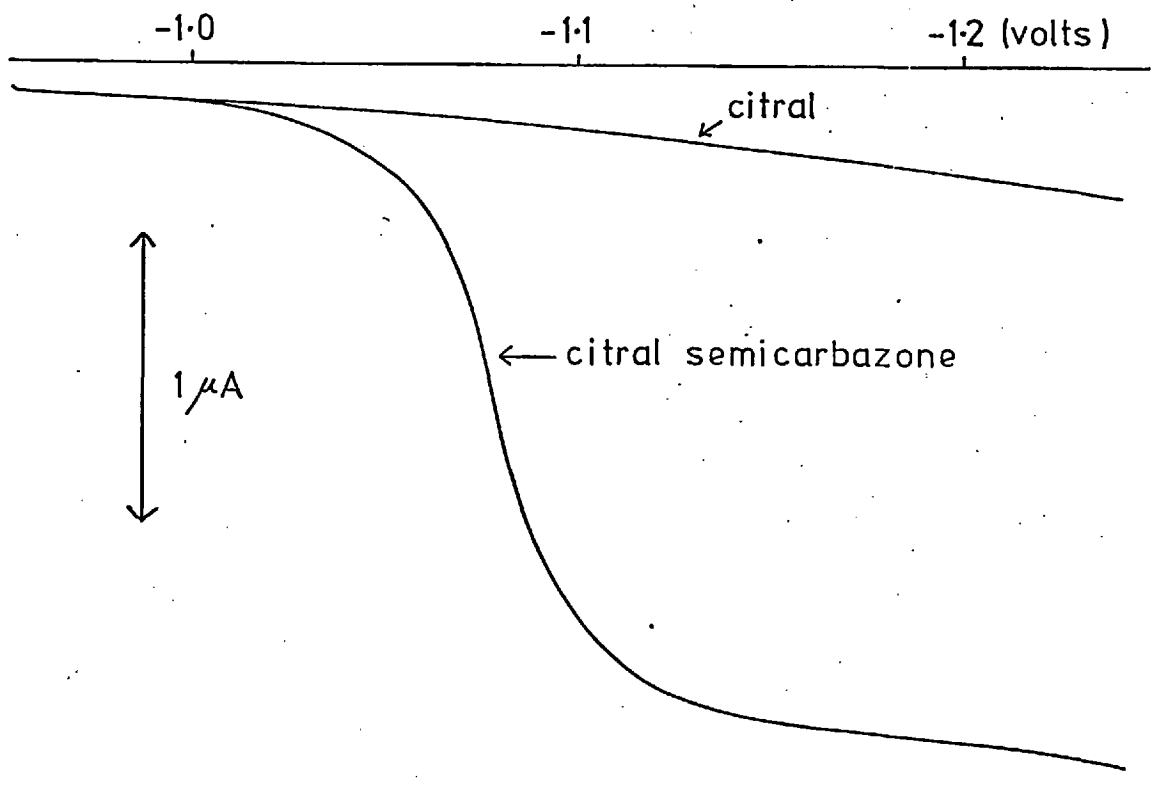


Figure 65 d.c. voltammograms for Citral (10^{-4}M) and Citral semicarbazone in acetate buffer (pH=4.6), flow rate = 1ml. per minute.

The voltammograms were obtained by pumping a Citral solution (10^{-4}M) through the detector. With the reagent inlet blanked off, no response was achieved for the reduction of Citral over the potential range -0.5 to -1.5 volts (vs Av/Ag^{+}). When a solution of semicarbazide hydrochloride was pumped in through the reagent inlet, a voltammetric response for the reduction of the semicarbazone was obtained. Such behaviour has proved typical of carbonyl compounds at glassy carbon electrodes. No reduction waves were observed for Citral, isophorone mesityl oxide, diacetone alcohol, acetyl acetone and the steroids estrone and chlormadizone, all of which, except diacetone alcohol and acetyl acetone are readily reduced at a dropping mercury electrode(15). The polarographic behaviour of mesityl oxide has been dealt with in chapter 2.1. Examples of the polarographic behaviour of citral and isophorone are given in Figures 66 and 67 respectively. The most probable explanation of this phenomenon is chemisorbtion of the carbonyl bond to the glassy carbon surface. Reduction of the citral semicarbazone at the glassy carbon electrode collaborates this explanation as the formation of the semicarbazone destroys the carbonyl bond (Figure 68) and the resulting imine is not chemisorbed and remains available for reduction.

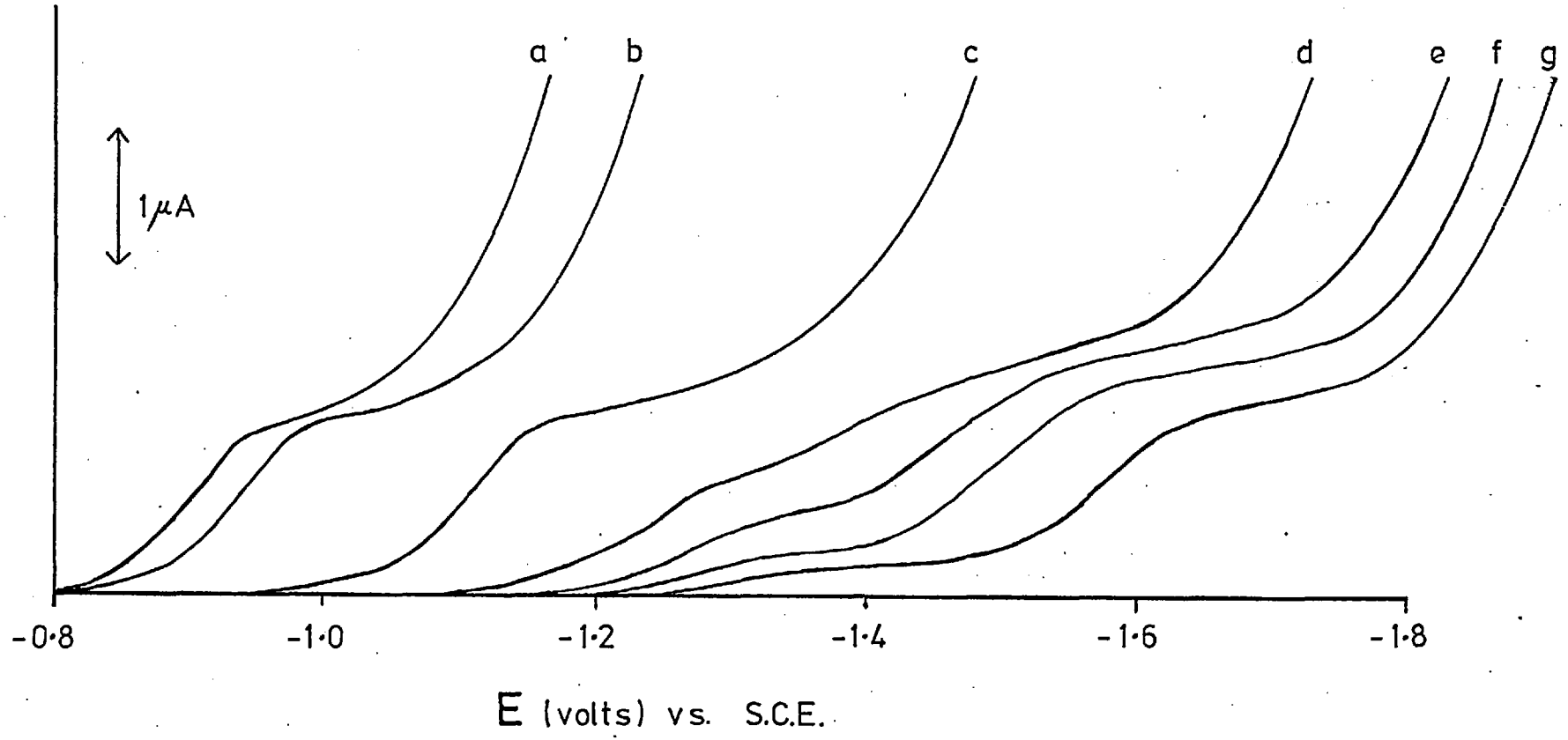


Figure 66: pH dependence of the polarography of CITRAL (2×10^{-4}M) at pH = (a) 1.23, (b) 2.27, (c) 4.6, (d) 7.8, (e) 9.4, (f) 10.1 and (g) 13.1

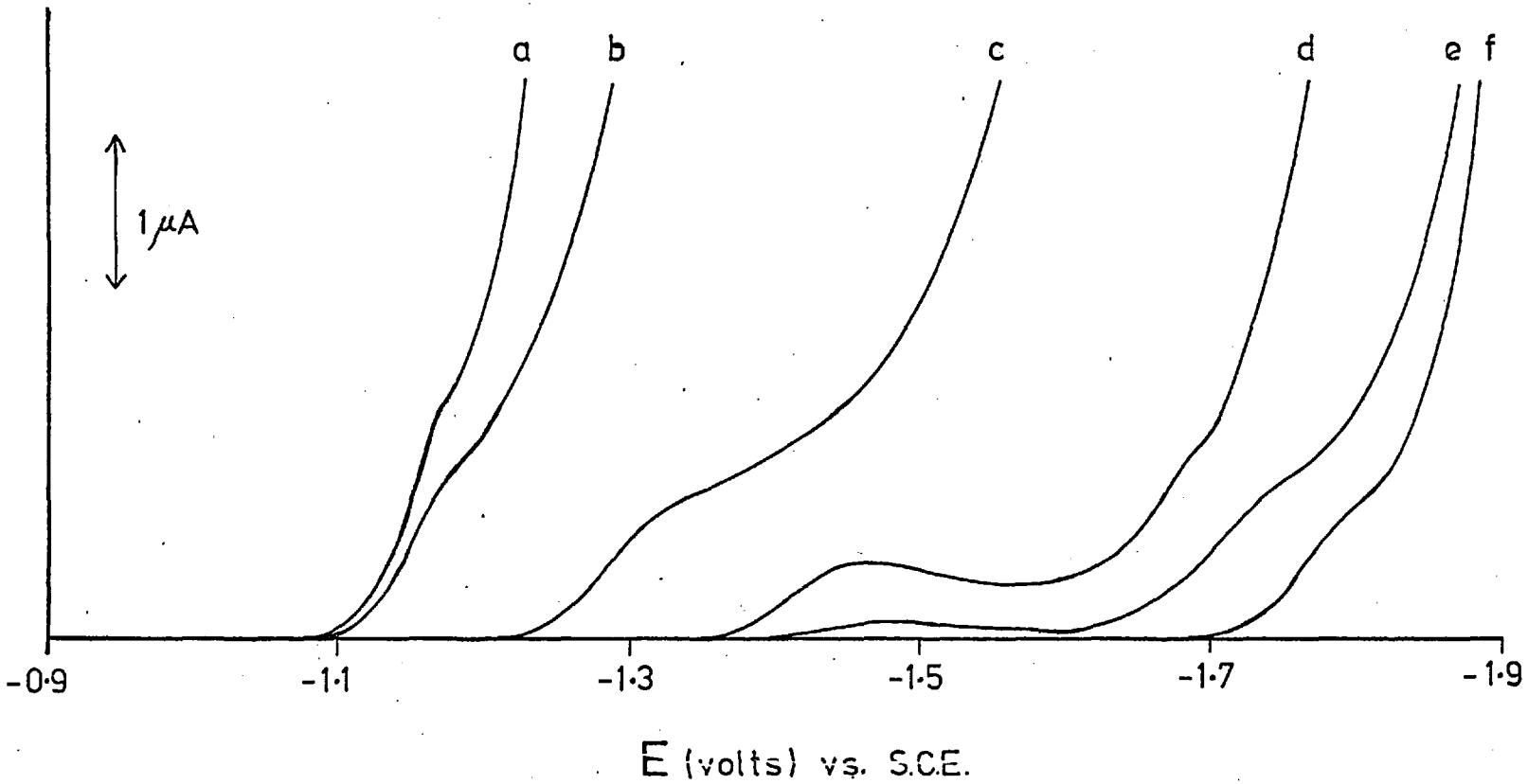
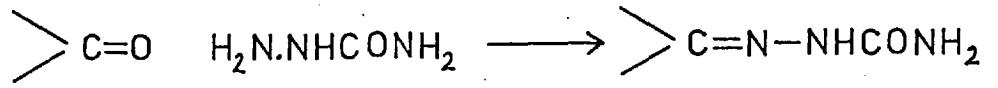
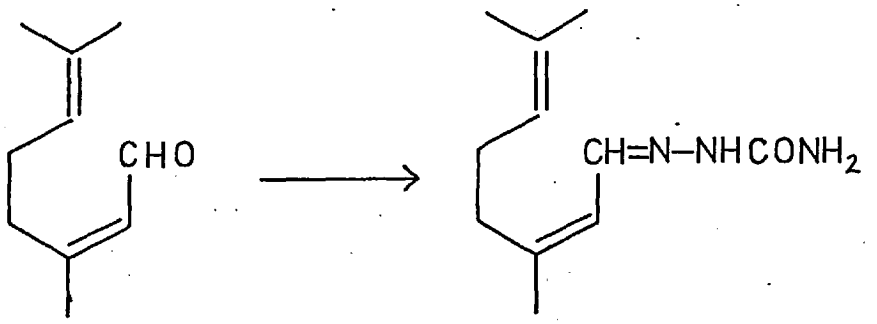


Figure 67: pH dependence of the polarography of ISOPHORONE (2×10^{-4}M) at pH = (a) 1.23, (b) 2.27, (c) 4.6, (d) 7.8, (e) 9.4 and (f) 13.1.



carbonyl semicarbazide semicarbazone

e.g.



citral citral semicarbazone

Figure 68 Conversion of a carbonyl grouping to an imine grouping by reaction with semicarbazide hydrochloride.

Theoretically, all carbonyl compounds should form semicarbazone derivatives and should therefore be capable of being detected from a chromatographic column using the W.J.E.D.

But, for the reagent addition to be effective, immediate reaction must take place. Benzaldehyde and mesityl oxide (Figure 69) citral and isophorone (Figure 70), diacetone alcohol and acetyl acetone (Figure 71) all form semicarbazone derivatives which can be reduced at a d.m.e. and glassy carbon electrode. This is important because diacetone alcohol and acetyl acetone would not be detected otherwise. Unfortunately, the semicarbazone formation is slow and only a very small response is obtained from the W.J.E.D.

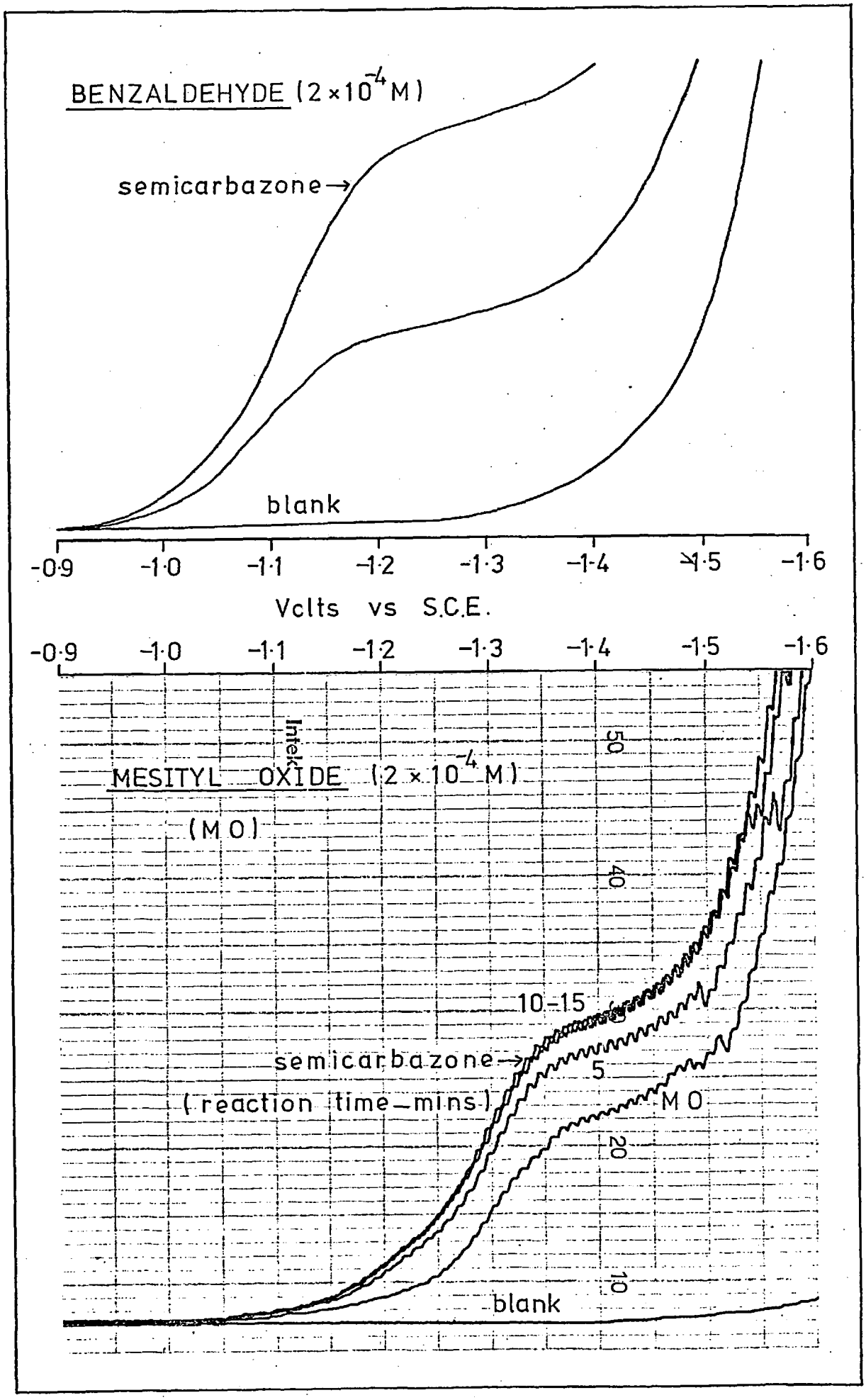


FIGURE 69 Polarography of BENZALDEHYDE and MESITYL OXIDE and their semicarbazone derivatives.

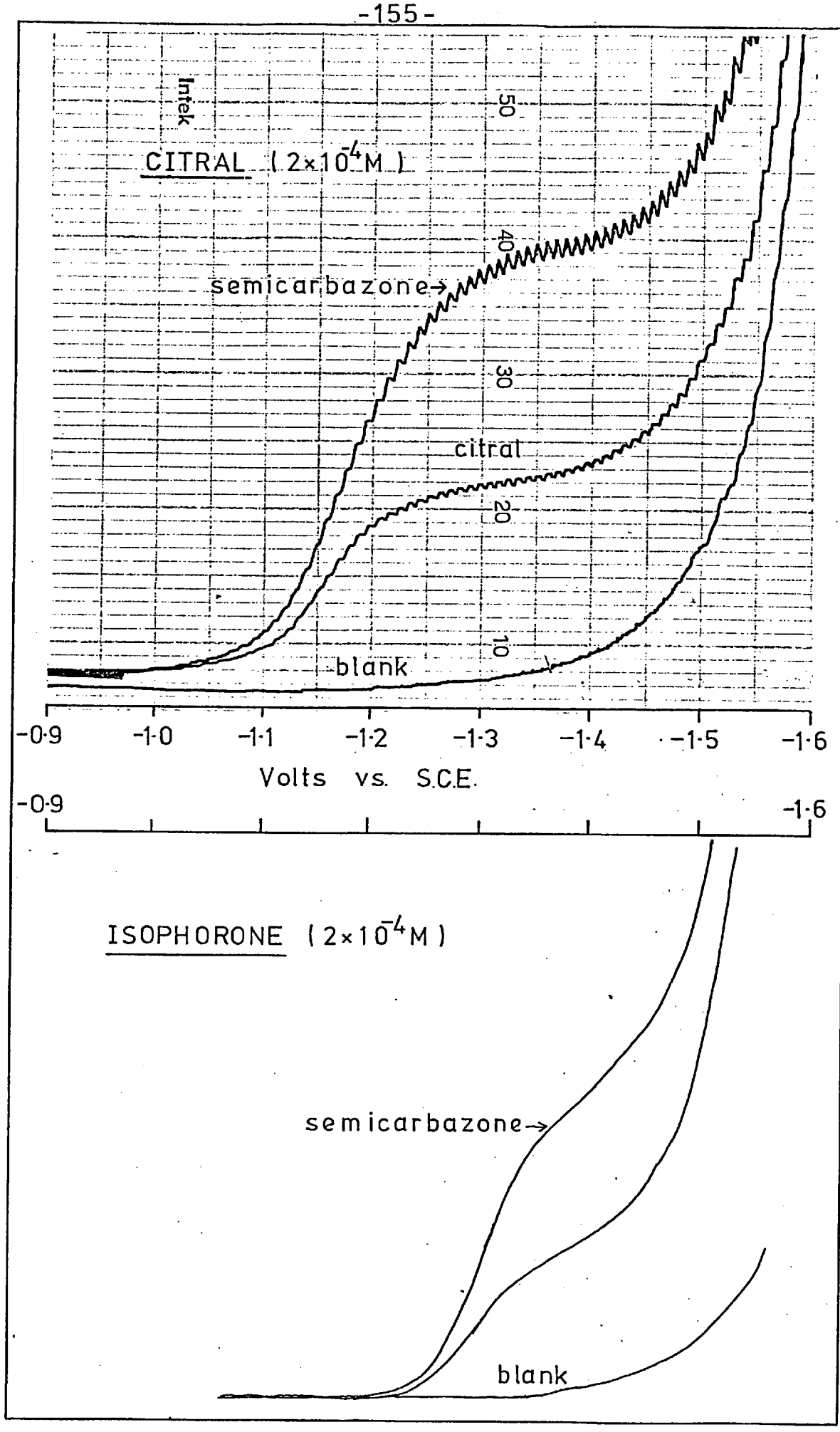


FIGURE 70 Polarography of CITRAL and ISOPHORONE and their semicarbazone derivatives

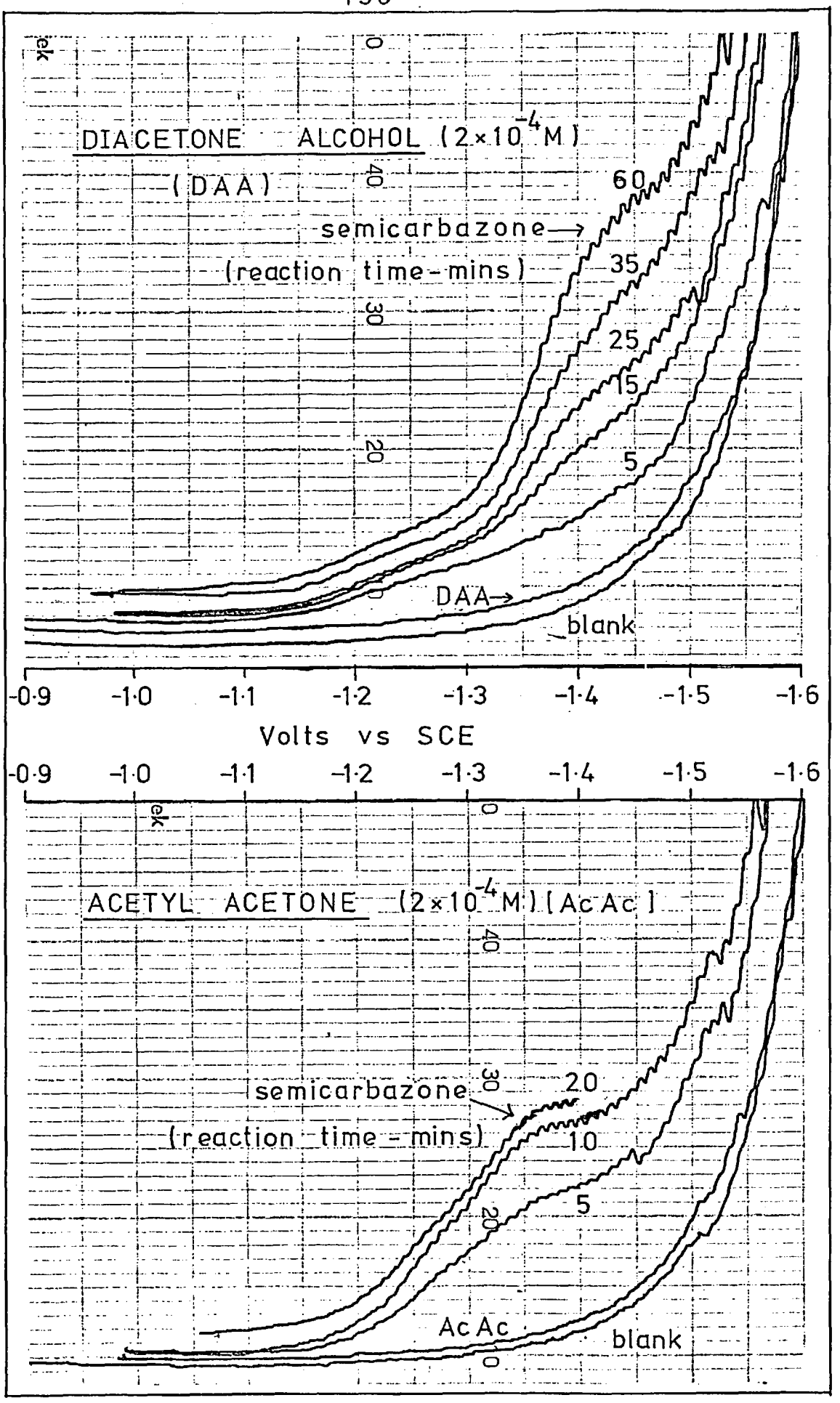


FIGURE 71 Polarography of DIACETONE ALCOHOL and ACETYL ACETONE and their semicarbazone derivatives

If acetate buffer is pumped through the detector, via a chromatographic column (1 ml. per minute), no response is obtained for injections of citral (The detector being set at -1.4 volts). When semicarbazide hydrochloride solution (10^{-3}M) is pumped through the reagent inlet (1 ml. per minute), the detector monitors successfully, the elution of citral (Figure 72).

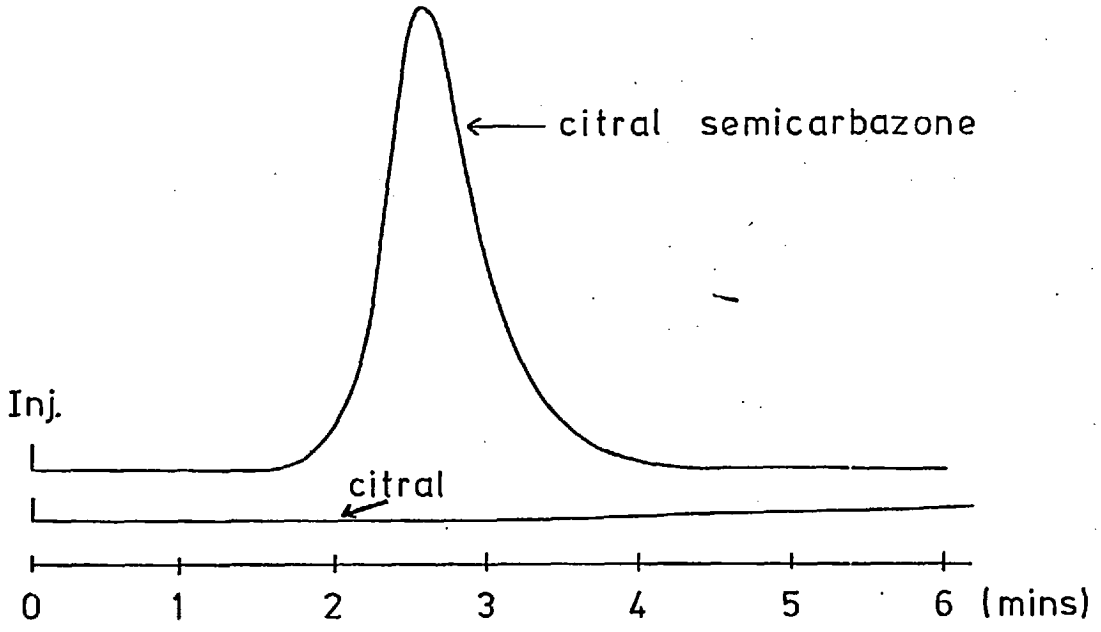


Figure 72 Detection of citral as the semicarbazone after reagent addition to the chromatographic eluent.

This lack of response for carbonyl compounds is not restricted to d.c. voltammetry at the glassy carbon electrode, a.c. techniques are affected similarly. The a.c. voltammetry of mesityl oxide at the W.J.E.D. gave no response for mesityl oxide.

When chromatography is performed in non aqueous solvents the reagent inlet works equally satisfactorily for the introduction of supporting electrolyte. Tetrabutyl ammonium perchlorate (T.B.A.P.) was successfully added to methylene chloride, chloroform, propylene

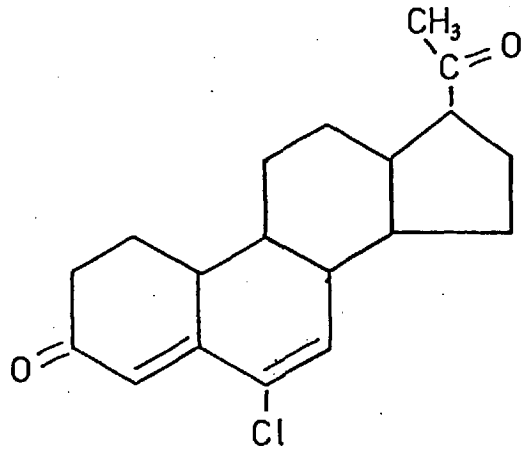
carbonate, acetonitrile and dimethyl sulphoxide and alcohol, all of which are potential chromatographic solvents.

Hydrocarbon solvents remain as the stumbling block for electrochemical detection. Hexane/acetonitrile (9:1) plus glacial acetic acid (0.1M) was non-conducting as the acetic acid was non-ionised under these conditions. Addition of T.B.A.P. to Hexane/acetonitrile mixtures was also a failure as the T.B.A.P. preferentially saturated the acetonitrile, which then separated out as an immiscible liquid. The only way to get substantial quantities of T.B.A.P. into solution with hexane was to add up to 50% of benzonitrile or nitrobenzene. No chromatographic experiments were tried with the latter two. It is conceivable that if a non-saturated solution of hexane/acetonitrile + T.B.A.P. is used in conjunction with a pulse as monitoring technique, that a response will be obtained as the pulse method requires only a very dilute solution of supporting electrolyte. No experiments along this line have been tried, but it could be worthwhile. This is another point in favour of the pulse technique for monitoring L.C. columns.

4.2. Detection of steroids

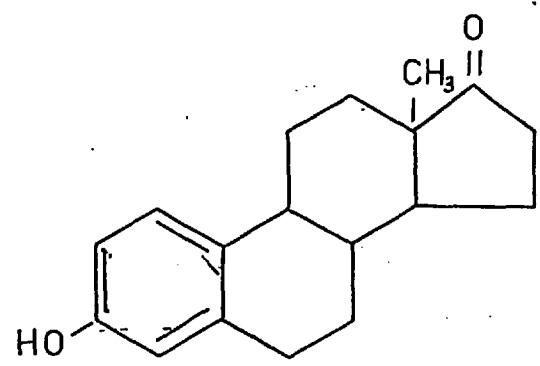
The chromatography of steroids is an important field of H.P.L.C. (55,259). Steroids are becoming increasingly used in the pharmaceutical field. Because of their potency, only very small qunatities are used which necessitates sensitive detection procedures. Many have quite low U.V. extinction coefficients which means that a successful detection by the W.J.E.D. is a valuable asset to the armoury of the liquid chromatographer. As has been mentioned before, keto steroids (e.g. chlormadizone X) are not detected at the

glassy carbon electrode unless a derivative is formed. Fortunately, many steroids such as estrone (XI) contain hydroxyl groups which are readily oxidised.



X Chlormadizone

(E_1' ~ 300-500)



XI Estrone

(E_1' - low)

A direct comparison of U.V.&wall jet electrode detectors (Figure 73) was made for the separation of estrone and chlormadizone on a Permaphose O.D.S. column ($\frac{1}{2}$ metre) using an eluent of methanol/water (25:75) plus KCl (0.1M) as supporting electrolyte. A flow of 1 ml. per minute was used under a pressure of 700 p.s.i.. The column eluent was led directly to a Cecil U.V. monitor set at 285 nm and 0.1 absorbance units (F.S.D.). The eluant from the U.V. detector was led directly to the W.J.E.D. and monitored at +0.8 volts. The comparison shows no loss in resolution due to the electrochemical detector although only the one component (XI) is monitored. Both could be monitored reductively as the semicarbazone or Girard derivatives. However, the oxidative detection method is to be recommended where possible as there is no need to deoxygenate the eluent.

Figure 73 (ii) shows comparative traces for estrone at

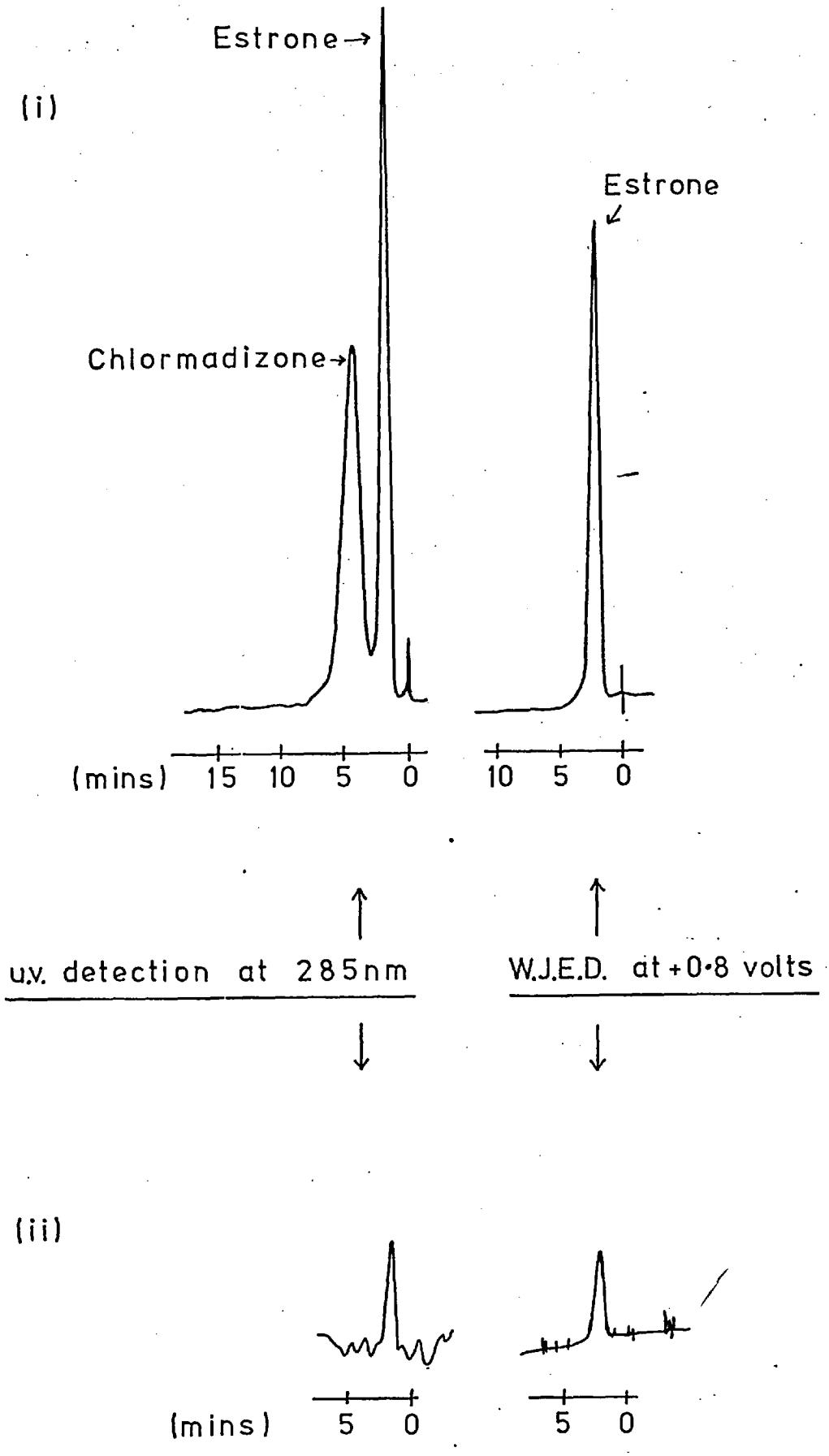


Figure 73 Comparison of U.V. and electrochemical detection (i) Mixture of chlormadizone and estrone, (ii) Detection limits for Estrone.

the limits of detection for each method. U.V. detection is limited by a low frequency noise. Since that trace was made, the electronic noise was greatly reduced as a faulty earth connection and incorrect adjustment of the W.J.E.D. parameters had not been diagnosed. Comparative detection limits for these detectors are given in Table 17.

Table 17

Detection limits for Estrone

Detector	Detection Limit
U.V. at 285 nm	10 ng.
W.J.E.D. at +0.8v.	0.2 ng.

* Detection limit of twice the noise level was used. Again, this is in favour of the W.J.E.D., as it is easier to measure a small peak on top of high frequency noise than on the low frequency noise of the U.V. detector.

4.3. Detection of Amino Acids

Amino acid analysis is of great importance diagnostically and is performed by ion exchange chromatography followed by visible spectrophotometric determination of the ninhydrin complex. This requires reagent addition after the chromatography. Recently, fluorimetric methods have been attempted to determine the amino acids in the eluent directly. Now it is to be expected that amino acids are capable of anodic oxidation. In fact, a recent paper shows such a separation, followed by direct detection with a coulometric detector (256). We have made another direct comparison with U.V. detection methods (Figure 74) that shows the vast superiority of the W.J.E.D. over the U.V. detector for this determination.

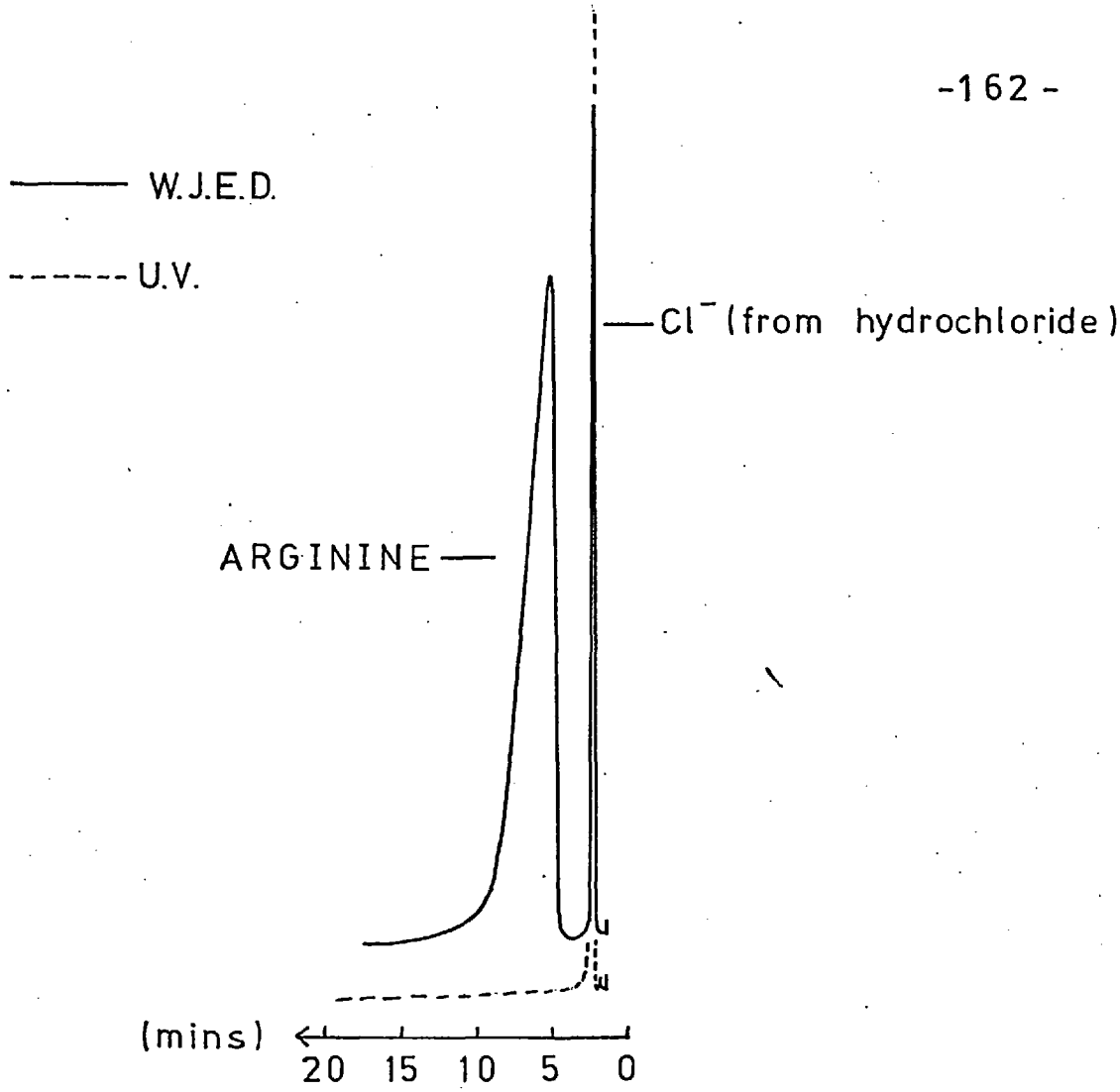


Figure 74 Chromatography of amino acids - Arginine (1 mg/ml).

Direct comparison of U.V. with W.J.E.D.

Column;	½ metre SCX
Eluant;	MeOH/Borate buffer (0.1M) (1:9)
Potential of W.J.E.D. ;	+0.8 volts
U.V. set at 223 nm.	

4.4. Detection of isoquinoline type compounds

Corypalline (IX), isocorypalline, tyramine (XIII) and 7-hydroxytetrahydroisoquinoline (XIV) samples (obtained from Professor J. Stock) were dissolved in borate (0.1M) buffer and subjected to

anion exchange chromatography using the buffer as eluent. The W.J.E.D. was used in the pulse polarographic mode (PAR 174) and set at a fixed potential of +0.8 volts. These compounds were ideally suited to this form of detection and gave the lowest detection limits to date (Table 18)

Table 18

Detection limits of isoquinoline type compounds

Compound	Detection limit (p.g.)
Corypalline	20
IsoCorypalline	20
Tyramine	50
7-Hydroxytetrahydro-isoquinoline	200

With care, these detection limits could be lowered by at least 1 order of magnitude, probably more and indicates the tremendous potential of the W.J.E.D. for monitoring L.C. columns.

REFERENCES

1. Heyrovsky, J., Chem. Listy., 16, 256 (1922).
2. Kemula, W., Roczniki Chem., 26, 281 (1952).
3. Kemula W., Chem. Anal., 13, 1013, (1968).
4. Kemula, W., Pure and Applied Chem., 25 (4), 763, (1971).
5. Heyrovsky, J., and Kuta, J., Principles of Polarography, Academic Press, 1966.
6. Meites, L., Polarographic Techniques (2nd Ed.), Interscience, N.Y., 1965.
7. Kemula, W., Sybilska, D., and Geisler, J., Roczniki Chem., 29, 643, (1955).
8. Kemula, W., and Sybilska, D., Chem. Anal., 4, 123 (1959).
9. Kemula, W., Butkiewicz, K., Geisler, J., and Sybilska, D., Chem. Anal., 1, 158 (1956).
10. Kemula, W., Sybilska, D., and Geisler, J., Chem. Anal., 1, 144, (1956).
11. Kemula, W., and Brzozowski, S., Roczniki Chem., 35, 703, (1961).
12. Kemula, W., Sybilska, D. and Chlebicka, K., Roczniki Chem., 39, 1499, (1965).
13. Kemula, W., and Krzeminska, A., Chem. Anal., 5, 611 (1960).
14. Snyder, L.R. in Chromatographic Reviews, Vol. 7, Lederer, M. (Ed.)., Elsevier, N.Y., 1965.
15. Brezina, M., and Zuman, P., Polarography in Medicine, Biochemistry and Pharmacy, Interscience, N.Y., 1958.
16. Zuman, P., Organic Polarographic Analysis, Pergamon Press, 1964.
17. Borkowska, Z., Sybilska, D. and Behr, B., Roczniki Chem., 45, 269, (1971).

18. Kemula, W., and Sybilska, D., Acta Chimica Ac. Sci. Hungarica , 27, 137, (1961).

19. Kemula, W., Sybilska, D., and Duszczyk, K., Microchem. J., 11, 296, (1966).

20. Kemula, W., Sybilska, D. and Chlebicka, K., Revue de Chimie, Academic de la Republique Populaire Ronmaine, 7, 1003, (1962).

21. Kemula, W., and Butkiewicz, K., Roczniki Chem., 39, 73, (1965).

22. Kemula, W., Sybilska, D. and Kwiecinska, A., Roczniki Chem., 39, 1101, (1965).

23. Kemula, W., and Sybilska, D., Anal. Chim. Acta., 38, 97, (1969).

24. Leitch, R.E. and Destefano, J.J., J. Chromatog. Sci., 11, 105 (1973).

25. Kemula, W., Kurjan, A. and Kwiecinska, A., Chem. Anal., 12, 869, (1967).

26. Kemula, W. and Witwicki, J., Roczniki Chem., 29, 1153, (1955).

27. Kemula, W., Brzozowski, S. and Butkiewicz, K., Chem. Anal., 3, 489, (1958).

28. Carey, M.A. and Persinger, H.E., J. Chromatog. Sci., 10, 537, (1972).

29. Papa, L.J. and Turner, L.P., J. Chromatog. Sci., 10, 747, (1972).

30. Zuman, P., in Organic Polarography Ed., Zuman and C.L. Perrin, Interscience Reprint (from originals), N.Y.

31. Srbova, J., Anal. Chem., 24, 917, (1952).

32. Matsumoto, K., J. Pharm. Soc. Japan., 73, 1375, (1953).

33. Monnier, D. and Guerne, R., Anal. Chim. Acta., 19, 90, (1958).

34. Wenger, P.E., Monnier, D. and Vogel, J., Microchim. Acta., 3-4, 406, (1957).

35. Monnier, D., Vogel, J. and Wenger, P.E., Anal. Chim. Acta., 22, 369, (1960).

36. English, F.L., Anal. Chem., 23, 344, (1951).

37. Davidek, J. and Manousek, O., Cesk, Farm., 7, 399, (1958).

38. Davidek, J., and Nanousek, O., Cesk. Farm., 7, 73, (1958).

39. Fleet, B., Anal. Chim. Acta., 36, 304, (1966).

40. Fleet, B. and Jee, R.D., J. Electroanal. Chem., 25, 289, (1970).

41. Booth, M.D. and Fleet, B., Analyst, 94, 844, (1969).

42. Booth, M.D. and Fleet, B., Analyst, 95, 649 (1970).

43. Manousek, O. and Zuman, P., J. Electroanal. Chem., 1, 324, (1960).

44. Zuman, P., Zumananova, R. and Soucek, B., Coll. Czech. Chem. Comm., 18, 632, (1953).

45. Brand, M.J.D. and Fleet, B., Analyst, 95, 905, (1970).

46. Fleet, B. and Jee, R.D., Talanta, 16, 1561, (1969).

47. Fleet, B. and Jee, R.D., J. Electroanal. Chem., 25, 397, (1970).

48. Zuman, P. and Krupicka, J., Coll. Czech. Chem. Comm., 23, 598, (1958).

49. Blaedel, W.J. and Todd, J.W., Anal. Chem., 32, 1018, (1960).

50. Kemula, W., and Stachurski, Z., Roczniki Chem., 30, 1285, (1956).

51. Kemula, W., Butkiewicz, K. and Sybilska, D., Rev. Polarog. (Japan), 11, 37, (1963).

52. Breyer, B. and Bauer, H.H.,"Alternating current Polarography and Tensammetry". Interscience, 1963.

53. Kemula, W., Behr, B., Borkowska, Z. and Doylido, J. Coll. Czech. Chem. Comms., 30, 4050, (1965).

54. Kemula, W., Behr, B., Chlebicka, K. and Sybilska, D., Roczniki Chem., 39, 1315, (1965).

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

56. Fleet, B. and Ho, A.Y.W., Ion Selective Electrode Symposium, 1972, p. 17-35 (Eng.) (published in 1973 by Akademiai Kiado, Budapest, Editor, E. Pungor.

57. Veening, H., J. Chem. Ed., 47, (11), A749, (1970).

58. Pecsok, R.L. and Saunders, D.L., Anal. Chem., 40, 1756, (1968).

59. Chromatronix Bulletin CHR 1270.

60. Lab. Data Control, inc., Bulletin 708-6.

61. Stigmark, L. and Johansson, G., Mikrochim. Acta., p. 131, (1963).

62. Ishii, K., Hayashi, S. and Fujawara, S., Anal. Chem., 31, 1587, (1959).

63. Johansson, G., Karrman, K.J. and Norman, A., Anal. Chem., 30, 1397, (1958).

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

65. Jackson, A., J. Chem., Ed., 42, 447, (1965).

66. Haderka, S., J. Chromatog., 52, 213, (1970).

67. Haderka, S., J. Chromatog., 54, 357, (1971).

68. Haderka, S., J. Chromatog., 57, 181, (1971).

69. Poppe, H. and Kuysten, J., J. Chromatog. Sci., 10, 16A, (1972).

70. Liberti, A., Anal. Chim. Acta., 17, 247, (1957).

71. Jeftic, L., and Adams, R.N., J. Am. Chem. Soc., 92, 1332, (1970).

72. Fleet, B. and Zuman, P., Coll. Czech, Chem. Comms., 32, 2066, (1967).

73. Martin, A.J.P., Pure and Applied Chem., 34, (1), 83, (1973).

74. Kendall, D.R. and Freund, H., J. Electroanal. Chem., 34, 253, (1972).

75. Fleet, B., Keliher, P.N., Kirkbright, G.F. and Pickford, C.J., Analyst. 94, 847, (1969).

76. Eldridge, P. and Fleet, B., unpublished results.

77. Schmit, J.A. Henry, R.A., Williams, R.C. and Dieckman J.F., J. Chromatog. Sci., 8, 645, (1971).

78. Bombaugh, K.J., Levangie, R.F., King, R.N. and Abrahams, L., J. Chromatog. Sci., 8, 657, (1970).

79. Tenygl, J., Coll. Czech. Chem. Comms., 33, 4141, (1968).

80. Matsumoto, T., Musanori, S. and Hirayama, S., Bull. Chem. Soc. (Japan), 46, 369, (1973).

81. Durst, R.A., (ed) Ion-Selective Electrodes, Proc. Symp at Nat. Bureau - Standards, Maryland, 1969, N.B.S., Pub. 314, 1969.

82. Koryta, J., Anal. Chim. Acta., 61, 329, (1972).

83. Buck, R.P., Anal. Chem., 44, 270R, (1972).

84. Moody, G.J. and Thomas, J.D.R., "Selective Ion Sensitive Electrodes", Merrov, 1971.

85. Pungor, E., Anal. Chem., 39, 28A, (1967).

86. Baker, C.T. and Trachtenberg, I., J. Electrochem. Soc., 118, 571, (1971).

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

88. Ruzicka, J., Lamm., C.G. and Tjell, J.C., Anal. Chim. Acta., 62, 15, (1972).

89. Guilbault, G.G. and Das, J., Anal. Biochem., 33, 341, (1970).

90. Guilbault, G.G. and Hrabankova, E., Anal. Letters, 3, 53, (1970).

91. Guilbault, G.G. and Hrabankova, E., Anal. Chem., 42, 1779, (1970).

92. Scott, C.D., Chilcote, D.D., Katz, S. and Pitt, W.W., J. Chromatog., Sci., 11, 96, (1973).

93. Adams, R.N., Electrochemistry at solid electrodes, Dekker, N.Y., 1969.

94. Nicholson, R.S., Anal. Chem., 44, 478R, (1972).

95. Blaedel, W.J. and Boyer, S.L., Anal. Chem., 43, 1538, (1971).

96. Blaedel, W.J. and Boyer, S.L., Anal. Chem., 45, 258, (1973).

97. Seitz, W.R., Jones, R., Klatt, L.N. and Mason, W.D., Anal. Chem., 45, 840, (1973).

98. Joynes, P.L. and Maggs, R.J., J. Chromatog. Sci., 8, 427, (1970).

99. Stock, J.T., Microchem., J., 15, 564, (1970).

100. Stock, J.T., Anal. Chem., 43, 289, (1971).

101. Kirkbright, G.F., Stock, J.T., Pugleise, R.D. and Bobbitt, J.M., J. Electrochem. Soc., 116, 219, (1969).

102. Stock, J.T. and Pugleise, R.D., Anal. Chem., 41, 198, (1969).

103. Alder, J.F., Fleet, B and Kane, P.O., J. Electroanal. Chem., 30, 427, (1971).

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

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

106. Koen, J.G., Huber, J.F.K., Poppe, H. and den Boef, G., J. Chromatog. Sci., 8, 192, (1970).

107. Bond, A.M. and Canterford, D.R., Anal. Chem., 44, 721, (1972).

108. Fleet, B., Jee, R.D., and Little, C.J., J. Electroanal. Chem., 43, 349, (1973).

109. Smith, D.E., "Electroanalytical Chemistry" (Ed. Bard), Vol 1, Dekker, N.Y., (1966).

110. Woodson, A.L. and Smith, D.E., Anal. Chem., 42, 242, (1970).

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

112. Barker, G.C., Gardner, A.W. and Williams, M.J., J. Electroanal. Chem., 42, App 21, (1973).

113. Polesuk, J. and Howery, D.G., J. Chromatog. Sci., 11, 226, (1973).

114. Krejci, M. and Pospisilova, N., J. Chromatog., 73, 105, (1972).

115. Yespalec, R. and Hana, K., J. Chromatog., 65, 53, (1972).

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

117. Kambara, T. and Tachikaiva, T., J. Chromatog., 32, 728, (1968).

118. Huber, J.F.K., "Comprehensive Analytical Chemistry," Ed. Wilson, Wilson., Vol II, p. 28.

119. Martin, A.J.P., and Synge, R.L., Biochem., J., 35, 1358, (1941).

120. Giddings, J.C., "Dynamics of Chromatography I", principles and theory, Arnold (Lond/Dekker (N.Y.), 1965.

121. Snyder, L.R., "Principles of Adsorption Chromatography", Arnold/(Lond)/Dekker (N.Y.), 1968.

122. Majors, R.E., J. Chromatog. Sci., 8, 338, (1970).

123. Majors, R.E., Anal. Chem., 44, 1722, (1972).

124. Du Pont, Product Bulletin, August, 1971, No. 820PB4.

125. Kirkland, J.J., J. Chromatog. Sci., 7, 361, (1969).

126. Sie, S.T., and van den Hoed, J., J. Chromatog. Sci., 7, 257, (1969).

127. Kirkland, J.J., J. Chromatog. Sci., 10, 129, (1972).

128. Waters Associates, "Column Packing Procedures for Polystyrene Column Packing".

129. Snyder, L.R., J. Chromatog. Sci., 7, 352, (1969).

130. Piel, E.V., Anal. Chem., 38, 670, (1966).

131. Zuman, P. and Perrin, C.L., Organic polarography, Interscience Reprint, p. 134.

132. Deininger, G. and Halasz, I., J. Chromatog. Sci., 8, 499, (1970).

133. Huber, J.F.K., J. Chromatog. Sci., 7, 172, (1969).

134. Keiller, R.A., J. Chromatog. Sci., 11, 223, (1973).

135. Mirk, M.N., J. Chromatog. Sci., 8, 491, (1970).

136. Bond, A.M. (submitted to Talanta).

137. Sobine, N.A., and Bezuglyi, V.D., Zavodskaya Lab., 30, 1212, (1964).

138. Pasternak, R., Helv. Chim. Acta., 31, 753, (1948).

139. Jee, R.D., Ph.D. Tehsis, University of London, 1971.

140. Jee, R.D., Z. Anal. Chem., 264, 143, (1973).

141. Gardner, A.W., Polarography (1964), Proc. 3rd Int. Conf., Southampton, Hills, G.J. (Ed), Vol 1, p. 187.

142. P.A.R. Corp., P.O. Box 2565, Princeton, N.J. 08540, Application note AN-108, "Why Outgassing - And How".

143. Bond, A.M., Anal. Chim. Acta., 62, 415, (1972).

144. Bond, A.M. and Canterford, J.H., Anal. Chem., 43, 228, (1971).

145. Bond, A.M. and Canterford, D.R., Anal. Chem., 44, 1803, (1972).

146. Bond, A.M., J. Electrochem. Soc., 118, 1588, (1971).

147. Belen, W.L., Fischer, D.J., Jones, H.C. and Kelley, M.T., Anal. Chem., 41, 779, (1969).

148. Cover, R.E. and Connery, J.G., Anal. Chem., 41, 918, (1969).

149. Barker, G.C., Proc. 2nd Int. Polarog. Congress, Cambridge (1959), Vol.1, p. 144.

150. Brdicka, R., Coll. Czech. Chem. Comms., 12, 522, (1947).

151. Paynter, J. and Reinmuth, W.H., Anal. Chem., 34, 1335, (1962).

152. Smith. D.E., and McCord, T.G., J. Electroanal. Chem., 26, 61, (1970).

153. Smith, D.E., and McCord, T.G., Anal. Chem., 41, 1423, (1969).

154. Stulikova, M., and Vydra, F., J. Electroanal. Chem., 42, 127, (1973).

155. Neeb, R., Z. Anal. Chem., 208, 168, (1965).

156. Hayes, J.W. and Bauer, H.H., J. Electroanal. Chem., 3, 336, (1962).

157. Bond, A.M., Anal. Chem., 44, 315, (1972).

158. Fleet, B., and Jee, R.D., J. Appl. Electrochem., 1, 269, (1971).

159. Heyrovsky, J., Chem. Listy., 40, 222, (1946).

160. Heyrovsky, J., Sorm, F. and Forejt, J., Coll. Czech. Chem. Comms., 12, 11, (1947)

161. Koryta, J., Chem. Listy, 46, 204, (1952).

162. Koryta, J., Coll. Czech. Chem. Comms, 20, 1125, (1955).

163. Fleet, B., Little, C.J., and Bristow, P., In press.

164. Edsberg, R.L., Eichlin, D. and Garis, J.J., Anal. Chem., 25, 298, (1953).

165. Fleet, B. and Little, C.J., In Preparation.

166. Bond, A.M., J. Electroanal. Chem., 39, 137, (1972).

167. Berman, D.A., Saunders, P.R. and Winzler, R.J., Anal. Chem., 23, 1040, (1951).

168. Harris, E.D. and Lindsay, A.J., Nature, 162, 413, (1948).

169. Lindsey, A.J., J. Phys. Chem., 56, 439, (1952).

170. Harris, E.D. and Lindsay, A.J., Analyst. 76, 647 and 650, (1951).

171. Roberts, E.R. and Meek, J.S., Analyst, 77, 43, (1952).

172. Stricks, W. and Kolthoff, I.M., J. Am. Chem. Soc. 78, 2085, (1956).

173. Nerust, W. and Merriam, E.S., Z. Physik. Chem., 53, 235, (1905).

174. Eisenberg, M., Tobias, C.W. and Wilke, C.R., J. Electrochem. Soc., 101, 306, (1954).

175. Laitinen, H.A., and Kolthoff, I,M., J. Phys. Chem., 45, 1079, (1941).

176. Ferrett, D.J. and Phillips, C.S.G., Trans. Faraday. Soc., 51, 390, (1955).

177. Nightingale, E.R., Anal. Chim. Acta., 16, 493, (1959).

178. Jordan, J. Anal. Chem., 27, 1708, (1955).

179. Jordan, J. and Javick, R.A., Electrochem. Acta., 6, 23, (1962).

180. Arvia, A.J. and Carrozza, J.S.W., Electrochim Acta., 7, 65, (1962).

181. Galus, Z. and Adams, R.N., J. Phys. Chem., 67, 866, (1963).

182. Newson, J.D. and Riddiford, A.C., J. Electrochem. Soc., 108, 695, (1961).

183. Johnson, G.R. and Rurner, D.R., J. Electrochem. Soc., 109, 918, (1962).

184. Azim, S. and Riddiford, A.C., Anal. Chem., 34, 1023, (1962).

185. Swofford, H.S., and Carman, R.L., Anal. Chem., 38, 966, (1966).

186. Vydra, F., Stulikova, M. and Petak, P., J. Electroanal. Chem., 40, 99 (1972).

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

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

189. Adams, R.N., Rev. Polarog., (Japan), 11, 71, (1960).

190. Kissinger, P.T., Refshange, C., Dreiling, R. and Adams, R.N., Anal. Letters, 6, 465, (1973).

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

192. Katz, R.N., and Gazzara, C.P., J. Materials, Sci., 3, 61, (1968).

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

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

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

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

197. Bockross, J.C., Chemistry and Physics of Carbon (Ed. P.L. Walker), p. 5, Dekker. N.Y., 1969.

198. Blackman, L.C.F., Ubbelohde, A.R. and Young, D.A., Proc. Royal. Soc., A.266, 20 (1962).

199. Moore, A.W., Ubbelohde, A.R. and Young, D.A., Proc. Royal Soc., A280, 153, (1964).

200. Ubbelohde, A.R., Endeavour, 63, (1965).

201. Coy, W.J., J. Am. Ceram. Soc., 45, 223, (1962).

202. Hennig, G.R., J. Chim. Phys. 58, 12, (1961).

203. Hennig, G.R., Proc. Conf. Carbon, Penn. State, (1961), 5, 143, (1962).

204. Heller, H.H., Trans Electrochem. Soc., 87, 501, (1945).

205. Wawzonek, S. and Eftax, D.S.P., J. Electrochem. Soc., 104, 494, (1967).

206. Boehm, N.P., Diehl, E., Heck, W. and Sappock, R., Angew. Chem. 76, 742, (1964).

207. Cowlard, F.C. and Lewis, J.C., J. Materials, Sci., 2, 507, (1967).

208. Bacon, R., J. Appl. Phys., 31, 283, (1960).

209. Tang, M.M. and Bacon, R., Carbon, 2, 211 and 221, (1964).

210. Badami, D.V., Campbell, C., Davy, A.D. and Lindsay, M.J., Proc. 2nd Conf. on Ind. Carbon and Graphite. (London, Soc. Chem. Ind., 1960).

211. Weinberg, N.L. and Reddy, T.B., J. Appl. Electrochem., 3, 73, (1973).

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

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

214. Kihara, S., Yamamoto, T., Motojima, K. and Fujinaga, T., Bunseki Kagaku, 21, 496 (1972).

215. Kihara, S., Motojima, K. and Fujinega, T. Bunseki Kagaku, 21, 883, (1972).

216. Ubbelohde, A.R. and Lewis, F.A., Graphite and its crystal compounds, (Clarendon Press, Oxford, 1960).

217. French Carbon Study Group., Les Carbonnes, 2 (Masson, Paris, 1965).

218. Nixon, D.E., Parry, G.S. and Ubbelohde, A.R., Proc. Royal Soc. A291, 499, (1963).

219. Parry, G.S. and Nixon, D.E., Nature, 216, 909, (1967).

220. Nixon, D.E. and Parry, G.S., Brit. Appl. Phys. ser 2, 1, 291, (1968).

221. Saunders, G.A., Ubbelohde, A.R., and Young, D.A., Proc. Roy. Soc., A271, 499, (1963).

222. Eeles, W.T., and Turnbull, J.A., Proc. Roy. Soc., A283, 179,(1965).

223. Brocklehurst, J.E. and Martin, W.H., J. Nucl. Materials, 23, 341, (1967).

224. Herinckx, C., Perret, R. and Ruland, W., Nature, 220, 63, (1968).

225. Halpin, M.K., and Jenkins, G.M., Nature, 218, 950, (1968).

226. Mueller, T.R., Olson, C. and Adams, R.N., Proc. 2nd Inter. Polarog. Congr., Cambridge, 1959, p. 198.

227. Mueller, T.R. and Adams, R.N., Anal. Chim. Acta., 23, 467, (1960).

228. Ottmers, D.M. and Rase, H.F., Ind. and Eng. Chem. Fundamentals, 5, 302, (1966).

229. Anastasi, A., Mecarelli, E. and Novacic, L., Mikrochemie, 40 113, (1952).

230. Szyszko, E., Roczniki Panstwowego Zakladi Hig. 16, 445, (1965).

231. Gajan, R.J., J. Assoc. Offic. Agr. Chem., 46, 216, (1963).

232. Jee, R.D., Personal Communication.

233. Phase Separations. Technical Data Sheet for using Harwell Alumina or Silica.

234. Huber, J.F.K., Chimia, Suppl. 24, (1970).

235. McNair, H.M. and Chandler, C.D., Anal. Chem., 45, 1117, (1973).

236. Kennedy, G.J. and Knox, J.H., J. Chromatog. Sci., 10, 549, (1972).

237. Majors, R.E., Anal. Chem., 45m 755, (1973).

238. Kirkland, J.J., J. Chromatog. Sci., 7, 7, (1969).

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

240. Bockris, J. O'M. and Conway, B.E. (Eds), Modern Aspects of Electrochemistry No. 6, Chapter 3 by Arvia A.J. and Marchiano, S.L.

241. Takemori, Y. and Honda, M., Rev. of Polarography (Japan), 16, 96, (1970).

242. Fujinaga, T., Pure and Applied Chem., 25, 709, (1971).

243. Fujinaga, T., Nagai, S., Okazaki, S. and Takagi, C., Nippon, Kagaku Zasshi, 84, 941, (1963).

244. Fujinaga, T., Takagi, C and Okazaki, S., Kogyo Kagaku Zasshi, 67, 1798, (1964).

245. Eckfeldt, E.L. and Shaffer, E.W., Anal. Chem., 36, 2008, (1964).

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

247. Blaedel, W.J. and Strohl, J.H., Anal. Chem., 37, 64, (1965).

248. Sioda, R.E., Electrochim. Acta. 13, 375, (1968).

249. Sioda, R.E., Electrochem. Acta., 15, 783, (1970).

250. Blaedel, W.J., Strohl, J.H., Anal. Chem., 36, 1245, (1964).

251. Sioda, R.E., Electrochim. Acta., 15, 1559, (1970).

252. Funjinaga, T., Izutsu, K., Okazaki, S., Rev. Polarog. (Kyoto), 14, 164, (1967).

253. Funjinaga, T., Izutsu, K., Koyama, M., Okazaki, S. and Tsuji, K., Nippon Kagaku Zasshi, 89, 673, (1968).

254. Kihara, S. Yamanoto, T., Motojima, K. and Funjimaga, T., Talanta, 19, 329, (1972).

255. Kihara, S., Yamamoto, T., Motojimo, K. and Fujimaga T., Talanta, 19, 657, (1972).

256. Takata, Y. and Muto, G., Anal. Chem., 45, 1864, (1973).

257. Electronic Circuit Design Handbook (Eds. of EEE magazine) p. 81; Patent Office HD80

258. Webb, R.E., Wireless World, p. 540, November, 1972.

259. Bailey, F., and Brittain, P.N., J. Chromatog. 83, 431, 1973.

PUBLICATIONS

1. A polarographic study of Mesityl Oxide and its determination in DIACETONE ALCOHOL by Fast Scan, Phase Sensitive, A.C. polarography.

   B.Fleet, R.D. Jee and C.J. Little, J. Electroanal. Chem. 43, 349, (1973).

2. Gas Pressure Operated Dropping Mercury Electrode.

   B. Fleet, C.J. Little and P. Bristow. Submitted to J. Appl. Electrochem. 1974.

3. Electrochemical Detectors for H.P.L.C.

   B. Fleet and C.J. Little. Submitted to Anal. Chem. 1974

4. A.C. Voltammetry at glassy carbon electrodes.

   B. Fleet and C.J. Little. In preparation.

5. A review of Electrochemical Detectors for H.P.L.C.

   B. Fleet and C.J. Little. In preparation.

6. Lectures given at

   (i) Analysis 73

   (ii) Salford University; S.A.C. Meeting, 1973.