THE APPLICATION OF DIRECT ELECTROANALYTICAL TECHNIQUES TO THE ANALYSIS OF ORGANIC SPECIES IN INDUSTRIAL AND BIOLOGICAL SAMPLES

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By

PETER JOSEPH ILES
B.App.Sc. A.R.A.C.I.

Department of Chemistry and Biology
Footscray Institute of Technology

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This thesis contains no material which has been accepted for the award of any degree or diploma in any tertiary educational institution, and to the best of my knowledge, no work published or written by another person or persons is included except where due reference is made in the text.

P. J. Iles

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ABSTRACT

This thesis has reviewed the electroanalytical chemistry of organic compounds. The theory and practical limitations of direct electrochemical techniques, used for the analysis of research, industrial and biological samples, have been introduced.

The hydrodynamic techniques of Pulsed Rotation Amperometry (PRA) and Flow Injection Analysis (FIA) with amperometric detection have been applied to the analysis of quinol and quinone in aqueous solution and tetrahydrofuran. PRA has been used to determine quinol residues in ion-selective electrode membranes manufactured by evaporation of tetrahydrofuran. A photochemical reaction product of quinone has been detected as an interferent in quinone analysis and has been detected in the electrode membranes.

PRA, at carbon paste electrodes, has been applied to the analysis of theophylline in cough mixtures and intravenous injections. The precision of these analyses has been found to be dependent on the geometry of the rotating electrode tip.

A statistical analysis of theophylline therapy has determined the required precision for safe and effective prescription of the drug based on the analytical results. An investigation of the applicability of FIA with amperometric detection to the direct determination of theophylline in saliva and blood samples has been carried out. The results of this investigation have been compared to alternative techniques with respect to sensitivity, selectivity, precision, speed and the cost of analysis.

CONTENTS

CHAPTER 1

Introduction

1.1 Organic Electrochemistry

An organic molecule can undergo reduction or oxidation via either heterogeneous or homogeneous electron transfer depending on the functional groups within the molecule and the nature of the electrical potential. Organic electrochemistry began in 1834 with Faraday's observation that ethane was produced by the electrolytic oxidation of aqueous acetate solutions.[1] The first study of an electroactive functional group was carried out by Kolbe in 1849. Starting with Faraday's observation he devised the well known 'Kolbe Synthesis' for the production of hydrocarbons from carboxylic acids.[2]

Although Faraday and Nernst laid down the foundations of electrochemistry in the mid nineteenth century the scope of organic electrochemistry was not realized until the turn of the century. The work by Haber[3] in 1898, on the importance of the electrode potential in controlling the course of a reaction and, in 1905 Tafel's description[4] of irreversible (non Nernstian) electron transfer (the behaviour exhibited by many organic molecules) led to the discovery of a wide range of controllable electrochemical reactions. Anthraquinone, benzidine and other molecules were electrochemically synthesized as intermediates in the German dye industry[5] in the early 1900's.

The invention of polarography by Heyrovsky[6] (1922) and the work of Ilkovic[7,8] laid the theoretical foundations for organic electroanalytical chemistry. In the 1950's and 60's several books and reviews containing large tables of data on the polarographic reduction of organic molecules were published.[9-18] The study and analytical use of solid electrodes, which allow an extension of the anodic potential range compared to the mercury electrodes employed

in polarography, led to similar tables being published for oxidations in the late 1960's.[18—20]

In the early 1970's an excellent monograph on synthetic organic electrochemistry containing a 390 page appendix of electrochemical data on organic molecules, edited by Weinburg, was published.[21] The compounds are divided into 27 functional groups plus 5 specialized topics. The solvent system, working electrode, reference electrode, half wave or peak potential and literature reference are reported for each compound. The wide variety of electroactive functional groups available for electroanalytical use can be appreciated from Table 1.1 where the divisions of Weinburg's appendix are tabulated. The specialized topics are: biologically important compounds, organometallics, heterocycles, dyes and a miscellaneous section containing data on commercial antioxidants, quaternary ammonium ions and insecticides. Although this information was compiled to aid synthetic chemistry it is also very useful as a starting point in the development of an electroanalytical procedure.

1.2 The Scope of Organic Electroanalytical Chemistry

The demand for sensitive environmental and clinical analyses increased rapidly in the 1970's.[22] Also at this time advances in electronics enabled the development of very sensitive and powerful electrochemical instrumentation.[23] These two factors produced a change of emphasis in the literature of organic electrochemistry from synthetic to analytical applications. For the purpose of review the literature can be divided into general application areas. These are:

TABLE 1.1 - Electroactive Functional Groups

FUNCTIONAL GROUP	FUNCTIONAL GROUP
1. Aliphatic Hydrocarbons	15. Esters
2. Olefins and Polyolefins	16. Peroxides, Peracids and Peresters
3. Acetylenes	17. Ethers
4. Aromatic Hydrocarbons	18. Carbohydrate Lactones
5. Aliphatic Aldehydes	19. Aliphatic Amines
6. Aromatic Aldehydes	20. Aromatic Amines
7. Aliphatic Ketones	21. Aliphatic Halides
8. Aromatic Ketones	22. Aromatic Halides
9. Alcohols, Polyols and Carbohydrates	23. Carbon-Nitrogen Functions
10. Phenols	24. Nitrogen-Nitrogen Functions
11. Quinones	25. Nitrogen-Oxygen Functions
12. Aliphatic Carboxylic Acids	26. Carbon-Phosphorus Functions
13. Aromatic Carboxylic Acids	27. Sulphur Functions
14. Anhydrides	

1. Food analyses
2. Environmental analyses
3. Clinical analyses

and 4. Industrial analyses

The reported methods provide very sensitive analyses and where the selectivity of the electrochemical technique is inadequate, chromatographic separations are used prior to quantitative measurement. High performance liquid chromatography (HPLC) with electro-

chemical detection is the most popular and successful technique since it allows the use of small sample volumes, provides good separations with high sensitivity and is amenable to automation where large sample throughputs are required.

Electrochemical analysis of foodstuffs for the determination of aflatoxins,[24] food dyes,[25-27] drug residues,[28-30] stabilizers and antioxidants,[31-32] pesticide residues,[33-36] vitamins[37-42] and contaminants[43] have been reported.

Environmental samples have been analyzed electrochemically for aromatic amine carcinogens,[44-47] anthraquinones,[48] hydrazines,[49-51] isocyanates,[52] pesticides,[53-55] phenolics[56-58] and surfactants.[59]

Clinical chemistry requires analyses of both endogenous and exogenous species in the complex matrices of body fluids and tissues. Most methods reported for clinical use employ HPLC with electrochemical detection. Procedures for acetaminophen and related compounds,[60-62] antibiotics,[63-67] benzodiazepines,[68-75] bile acids,[76-77] catecholamines,[78-85] chloramphenicol,[86] cytochrome,[87] nitroimidazoles,[88] NADH,[89-90] opiates,[91-93] purines,[94-96] oxalic acid,[97] steroids and hormones,[98-103] thioamides,[104-105] thiols,[106-110] tryptophans,[111-113] uric acid,[114-116] vitamins[39,80,117] and methylxanthines[118-119] have been reported.

Of the industrial analyses reported, pharmaceutical products form the largest single group. Unlike clinical analyses of drugs pharmaceutical analyses are not complicated by biological matrices and therefore electrochemical selectivity has been utilized in direct

measurement procedures for many products.

Where mixtures of compounds, with similar redox potentials, are present in samples, HPLC is used to achieve the necessary separations.

Procedures for antibiotics,[63-65] anaesthetics,[120-121] alkaloids,[93,122] butazones,[123] barbiturates,[124-125] benzodiazepines,[126-131] cacodylates,[132] chorhexidine,[133] contraceptives,[134-138] contaminants;[139] folic acid,[140] meptazinol,[141] opiates,[142-143] peroxides,[144] phenothiazines,[145-149] phenolic stimulants,[150-151] organic halides,[152] thioamides,[153] tinidazole,[154] tricyclic antidepressants[147] and vitamins[38,155-156] have been reported.

Other industrial analyses include butylated hydroxy toluene in transformer oils,[157] forensic analysis of explosives,[158-160] mineral flotation agents,[161-162] nitrohumic acids prepared from the oxidation of coal[163] and surfactant analysis.[59]

To take advantage of the large scope of organic electrochemistry in the development of sensitive analytical techniques, it is necessary to understand the basic principles of electroanalytical chemistry and the experimental modifications which aid the selectivity and/or sensitivity of organic analyses.

1.3 Electroanalytical Methods

Organic electroanalytical chemistry has two distinct fields, potentiometry and voltammetry. In potentiometry the developed potential difference between a sensing electrode and a reference is measured with the system at equilibrium. In voltammetry the current

flow at the sensing electrode is measured while the system is perturbed from equilibrium by changes in the applied potential.

1.3.1 Potentiometry

Organic species are potentiometrically determined by means of an electrode selective towards the analyte. These electrodes are either an enzyme coated ion-selective electrode[167] or a membrane electrode selective to the analyte.[164-166,168,169] The potential developed is related to the concentration of the analyte and is described by the following form of the Nernst equation.[170]

$$E = \text{Constant} + \frac{RT}{nF} \ln a_{0A} \qquad \text{Equation 1}$$

where a_{0A} = activity of the organic analyte

and E = the potential difference measured

n = charge of species causing developed potential

A limitation of organic potentiometry is that usually the analyte must be an ion to respond at the liquid membrane or be amenable to enzymatic reaction. An advantage for physiological analyses is that the electrodes respond only to the activity of the free species rather than total species concentration. In a biological matrix it is the activity of the free species that is physiologically relevant.

Since no net current flows through the system, measurements are made with a high impedance millivoltmeter. The technique is reasonably sensitive giving a log linear calibration usually from 10^{-1} to 10^{-5} molar concentrations.

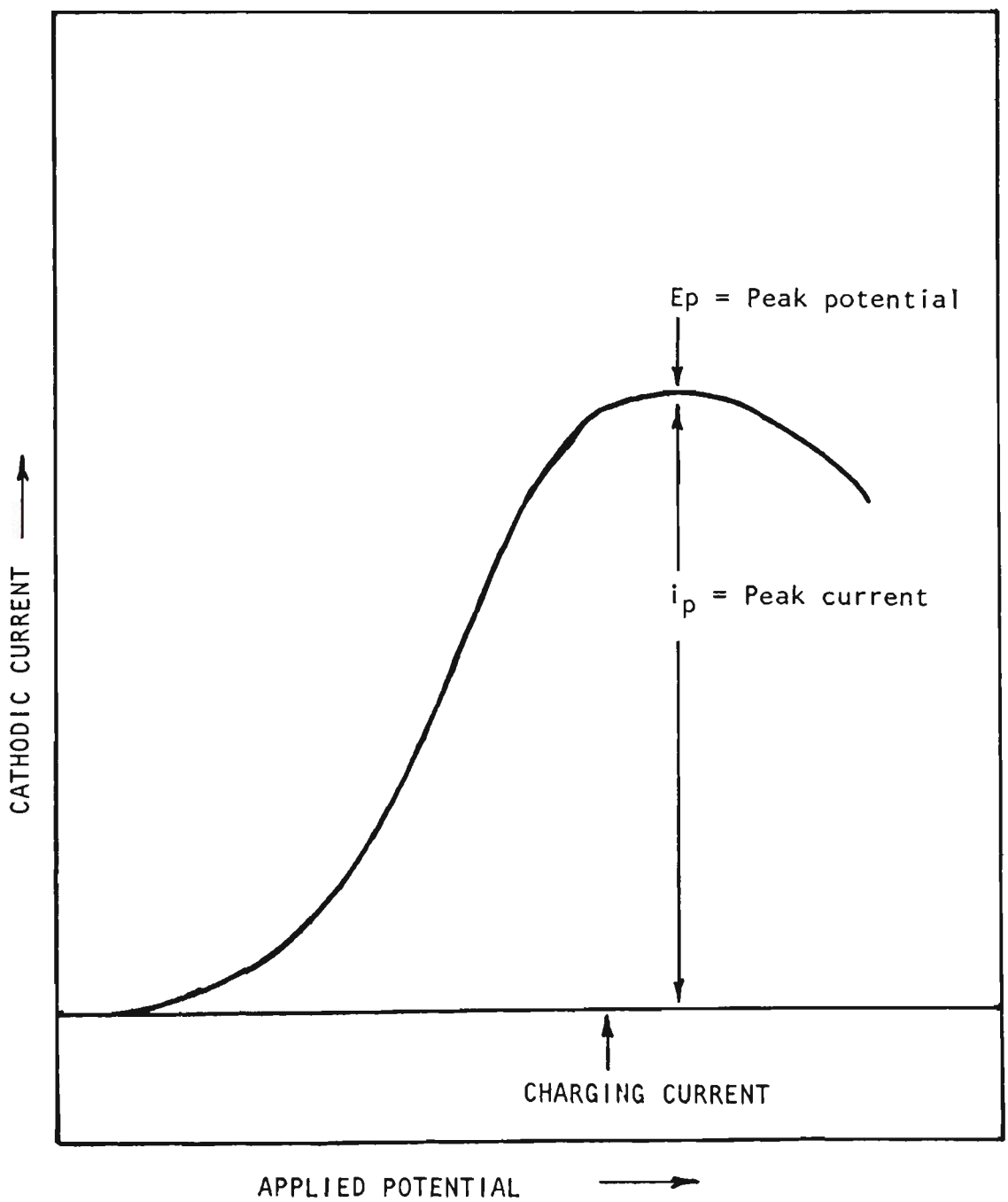
1.3.2 Voltammetry

Voltammetry may be divided into two forms; firstly polarography,[10] where a dropping mercury electrode is employed as the working electrode and secondly, solid electrode voltammetry where various conducting materials are used as working electrodes. Materials such as platinum, gold, carbon paste, glassy carbon and pyrolytic graphite are employed. Although polarography is useful for many organic analytes it has a limited anodic range due to the easy oxidation of the mercury electrode. On the other hand the solid electrodes have a wide anodic range which is usually limited by oxidation of the solvent and therefore enables the application of many organic oxidations to analysis.[20] In this thesis, all voltammetric measurements were made at solid electrodes.

1.3.3 DC Voltammetry

Voltammetric data provides both qualitative and quantitative information about the analyte. The current measured, as a function of the applied DC potential, is recorded as a current-voltage curve and these are called voltammograms. The current flow is caused by the oxidation or reduction of the analyte. A schematic diagram of a solid electrode DC voltammogram for a fast electron transfer reduction, with diffusion controlled transport, is shown in Fig. 1.1. The peak potential, Ep, is a qualitative property of the analyte. The current measured has two components. The charging (or background) current is the capacitative component. The current that flows when the analyte is oxidized or reduced is faradaic current. The peak current (faradaic) is measured as the difference between the charging current and the current at peak maximum (see Fig. 1.1). The peak current is proportional to the concentration of the analyte.

FIG. 1.1 - SCHEMATIC DIAGRAM OF SOLID ELECTRODE VOLTAMMOGRAM FOR DIFFUSION CONTROLLED MASS TRANSPORT

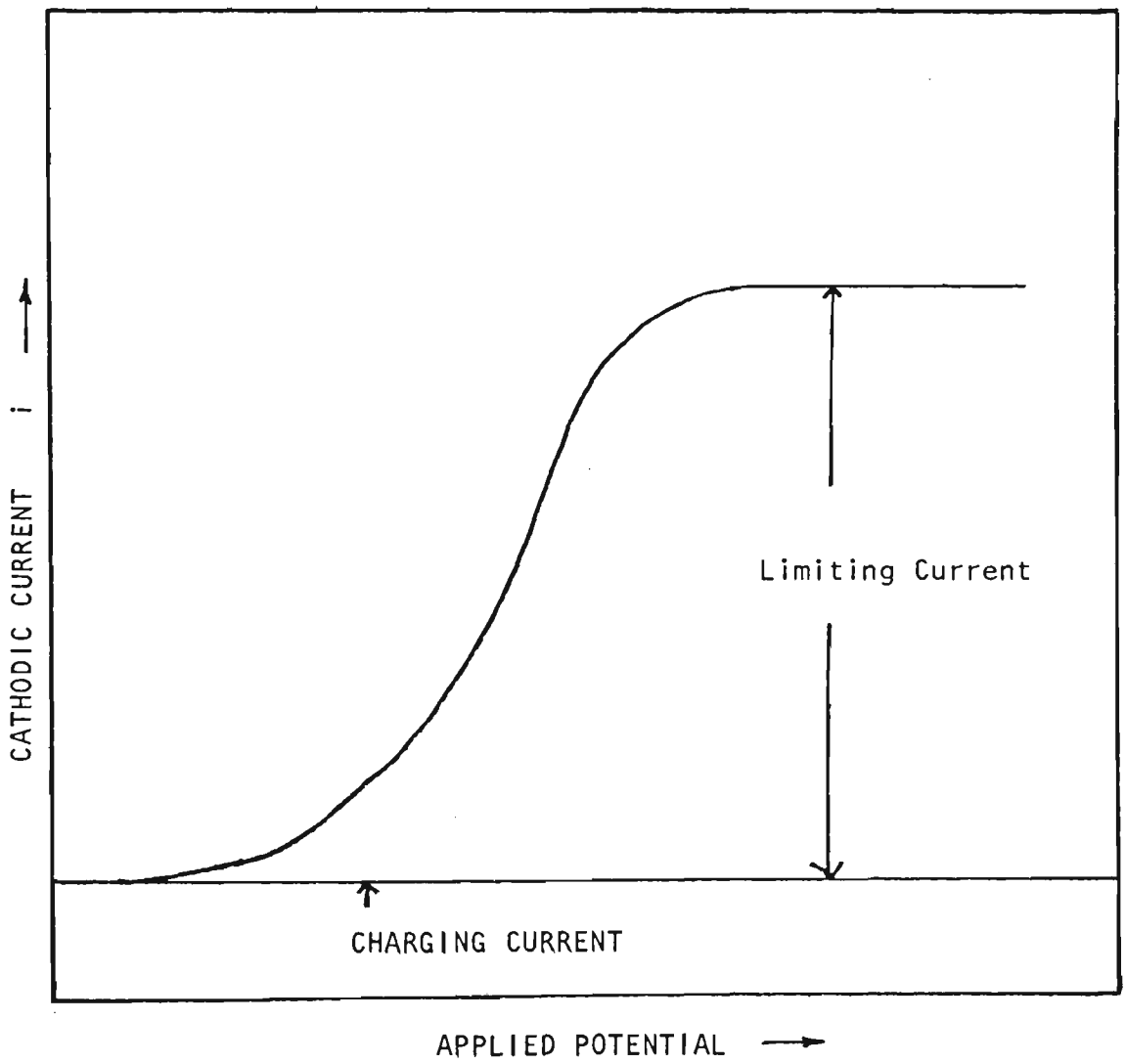


The peak shaped voltammogram at a solid electrode is due to the formation of a diffusion layer in the region of the electrode surface. The thickness of the layer increases with time since the layer is formed by depletion of the electroactive species at the electrode surface when current flows. Theoretical descriptions of solid electrode voltammetry are available in text books on electrochemical methods. The comprehensive books of Bard and Faulkner[23] and Bond[22] are particularly useful in aiding the understanding of this topic.

A wave shaped voltammogram is observed if the depleted region is replenished by convection. This is achieved by stirring the solution, rotating the electrode or employing a voltammetric flow cell. These situations are termed hydrodynamic voltammetry. An example of a hydrodynamic voltammogram is shown in Fig. 1.2. Theoretical descriptions for hydrodynamic methods are presented in section 1.8.

A peak shaped voltammogram is also observed under hydrodynamic conditions where the products of the electrochemical reaction are insoluble and accumulate at the electrode. In this case the peak shape is due to a depletion of the available electrode area. The work of Nicholson and Shain is the most commonly cited material for the theoretical treatment in this case.[171]

FIG. 1.2 - SCHEMATIC DIAGRAM FOR A HYDRODYNAMIC VOLTAMMOGRAM



1.3.4 Limitations of DC Voltammetry

In DC voltammetric methods, where the analyte concentration is low (ca 10^{-5}M) and consequently the faradaic current is small, the charging current becomes significant. In this situation it becomes difficult to distinguish between the large capacitative current and very small faradaic current and therefore the detection limit of DC methods is set by this characteristic.[172]

Modern voltammetric techniques overcome the limitation of the charging current by electronic, concentration at the electrode, or hydrodynamic means and therefore have lower detection limits. Most of the electronic techniques were developed for polarography but are applicable to voltammetry at solid electrodes. There are a variety of electronic modifications to the basic voltammetric experiment but the two most widely used are pulse techniques and A.C. methods. The other methods include square wave polarography,[173] radio frequency polarography,[174] chronopotentiometry,[175-176] controlled current techniques[177-179] and charge step polarography.[180-181]

Stripping voltammetry discriminates between charging and faradaic currents by maximizing the faradaic signal via concentration of either reduced or oxidized species on or in the electrode. Pre-electrolysis at a potential in the transport limited region accumulates the analyte and subsequent stripping of the accumulated material by either an anodic or cathodic voltammetric scan is used for quantitation. Anodic stripping is a very sensitive technique for metals and concentrations as low as 10^{-10}M silver have been determined.[182] Cathodic stripping is more applicable to organic analytes and inorganic anions, with 10^{-10}M levels of steroids being

detectable.[183]

Hydrodynamic techniques utilize changes in the rate of transport of the electroactive species to the electrode.[184]

Only those methods used in the following chapters will be discussed. The electronic methods are tast, differential pulse and AC voltammetry. The hydrodynamic techniques employed were pulsed rotation amperometry and flow injection analysis with amperometric detection.

1.4 Electronic Discrimination of Charging Current

1.4.1 Tast Voltammetry

Electronic modifications of the basic DC voltammetric experiment employed to enhance sensitivity in electroanalysis have been primarily developed for polarography. The tast modification is a current sampling technique utilizing the different time domain behaviour of the charging and faradaic currents during the life of a mercury drop. Since the charging current decreases steadily with time and the faradaic current increases as the size of the drop grows, then sampling the current towards the end of the drop life maximizes the ratio of the faradaic to charging current and therefore increases the sensitivity of the analytical method. Tast polarography has a detection limit of ca. 10^{-6}M, an order of magnitude over conventional DC polarography.[185]

At a solid electrode the faradaic current decays with time

since the electrode area remains constant. The rate of decay is slower than that of the charging current.[186] Therefore it is possible to use a tast type modification to increase sensitivity in this situation although the improvement will not be as substantial as that in tast polarography.

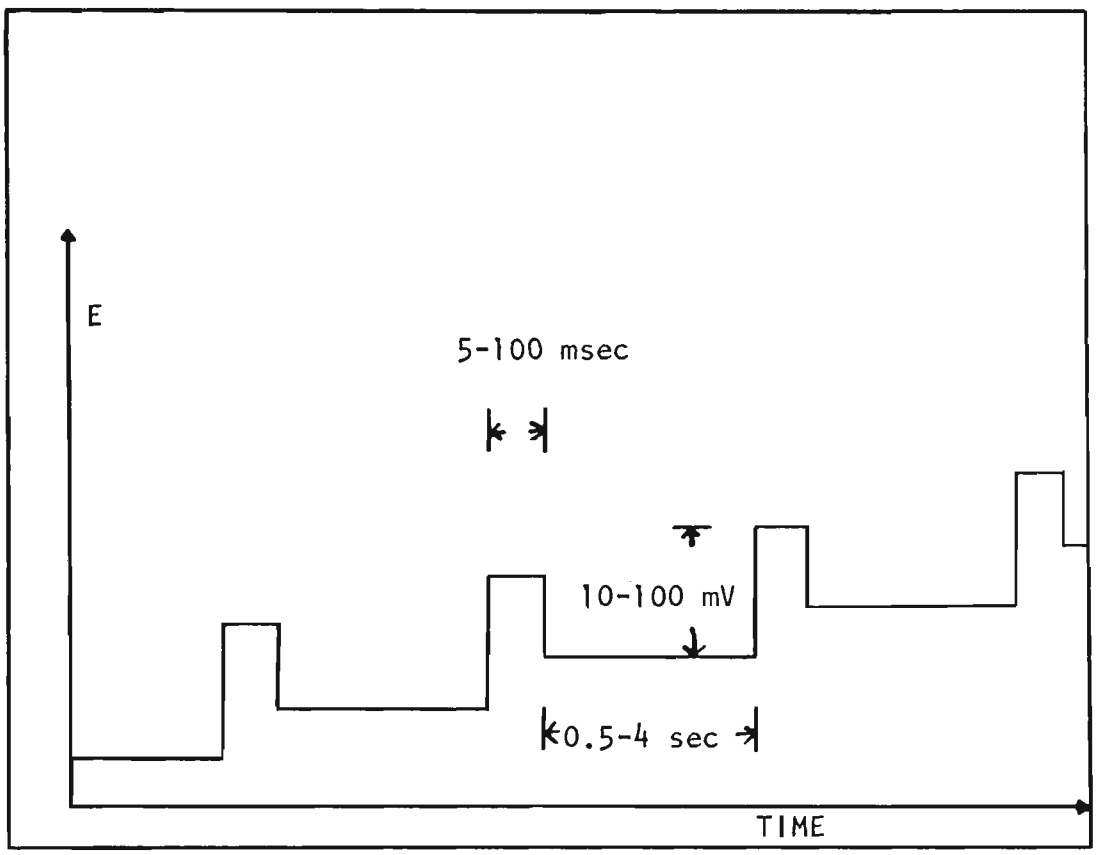
1.4.2 Differential Pulse Voltammetry

The differential pulse modification is another sensitivity enhancing technique primarily developed for polarography but is still quite useful for solid electrode voltammetry.[187] In this technique a small amplitude pulse (ca. 10 to 100 mV) is applied, in addition to the linear DC potential ramp, at set time intervals and the current is sampled prior to and towards the end of the pulse. The difference in these two currents is plotted as a function of the DC potential. In polarography the pulse is applied just prior to the end of the drop life to maximize the faradaic to charging current ratio. A plot of applied potential versus time (Fig. 1.3) demonstrates this.[188]

The resultant voltammogram is peak shaped rather than wave shaped and has the advantage of easier quantitation especially for multi-component analyses since the peaks occur along a common baseline rather than the additive result of DC waves.

High sensitivity is achieved by reducing the charging current contribution. Concentrations as low as 10^{-8} may be determined.[189] The peak height and width are dependent

FIG. 1.3 - POTENTIAL/TIME PLOT FOR DIFFERENTIAL PULSE EXPERIMENT



on the size of the pulse used. Large pulses give large but broad peaks whereas small pulses provide narrower but smaller peaks. Therefore selectivity may be enhanced by the use of small pulses, provided sensitivity is not a problem.[190]

Further increases in sensitivity may be achieved by coupling the differential pulse technique with stripping analysis[191] or rotating electrodes.[192]

An important point to consider at these very low concentrations is the choice of solvents and supporting electrolytes. Impurity levels in these materials will be significant in certain circumstances and great care must be exercised in their choice. Contamination from electrolytes may be reduced by lowering their concentrations from the customary 0.1-1.0M to 0.01-0.001M. The lower limit is fixed by the maximum cell resistance that can be tolerated.[193]

1.4.3 AC Voltammetry

Alternating current (AC) voltammetry encompasses a wide variety of periodic voltage waveforms applied to the electrochemical cell. The waveform may be sinusoidal, square wave, triangular or amplitude modulated. The most commonly used in analytical work is the sinusoidal waveform. Voltammograms are generated by superimposing a small amplitude sinusoidal alternating potential onto the usual potential ramp used in DC voltammetry and then filtering out the DC current component to record a plot of alternating current versus the DC potential.[194] Norm-

ally the first harmonic is used but higher harmonics may be utilized.[195]

The AC potential is of fixed frequency and of an amplitude usually in the range 10 to 50 millivolts. The AC current measured is of the same frequency as the AC potential. The AC current contains both faradaic and charging components and provides a peak shaped voltammogram.[195] The peak potential is closely related to the half wave potential and is characteristic of the electroactive species and the medium. The peak current is generally a linear function of concentration and is the basic parameter in analysis.

AC voltammetry also has the advantage of providing discrimination against charging current by utilizing a phase sensitive detector. It can be shown that if AC measurements are made at a phase angle of 0° or 180° relative to the applied AC voltage then the charging current component will be zero and the measured signal will consist solely of $\pm \sqrt{2}/2$ of the faradaic current for a reversible electrode process.[196] This is an ideal situation and in practice resistance effects lead to some charging current but significant discrimination is still achieved if three electrode, IR drop compensation voltammetric instrumentation is employed.[196] Detection limits using this technique approach 10^{-7}M.[197]

1.5 Hydrodynamic Methods

Electrochemical hydrodynamic methods are divided into two categories;

firstly where the electrode is rotated to achieve convective mass transport and secondly where the electrode is stationary and mass transport is achieved by employing a flowing stream. Both techniques exhibit good sensitivity due to the faradaic currents being transport limited rather than diffusion limited.

1.5.1 Rotating Electrode Systems

For rotated disc electrodes and a reversible electrode process, the following equation for the limiting current was derived by Levich.[198]

$$i_L = 0.62\, nFAD_O^{2/3}\, \omega^{1/2}\, \nu^{-1/6}\, C_O^*$$

where i_L = Limiting current
ω = Angular velocity of the disc
ν = Kinematic viscosity
C_O^* = Bulk concentration
D_O = Diffusion coefficient
A = Area of disc

and all other symbols are as previously used. The limiting current, i_L, is a linear function of concentration and proportional to the square root of the rotation rate. Therefore increases in rotation rate improve the sensitivity. The Levich equation holds for laminar flow and has lower and upper limits of rotation rate. At low rotation rates the hydrodynamic boundary layer becomes large and contribution from diffusion becomes significant. This can lead to peak shaped rather than wave shaped voltammograms (as discussed earlier) when the scan rate is fast compared to rotation rate. Typically values of N > 100 rpm are employed to avoid this condition.[199] The upper limit of rotation rate is set by

the start of turbulent flow. Theoretically the condition for non turbulent flow is $\omega < 2 \times 10^5 \nu/r^2$ where r = radius of the disc electrode. In practice, imperfect disc surfaces, bends or eccentricities in the rotating shaft and the cell design lower the upper limit of rotation rate. Also vortex formation around the electrode may occur. Generally rotation rates up to 10,000 rpm can be employed.[199]

Bruckenstein and Miller developed a technique called sinusoidal hydrodynamic modulation, which overcomes charging current limitations by utilising the change in current flow arising from the modulation of the electrode's rotation rate.[200] The modulation amplitude, $\Delta\omega$, is usually about 1% of ω and the amplitude, Δi, of the resultant modulated current is recorded. If the system follows the Levich equation then Δi is given by:

$$\Delta i = \left(\frac{\Delta\omega}{\omega}\right)^{\frac{1}{2}} i_\omega$$

where i_ω = the current for ω

Δi is free from factors that do not depend on the mass transfer rate and therefore the technique discriminates between the analyte current and (1) charging current, (2) currents due to the oxidation or reduction of the electrode or of adsorbed species. Also Δi is relatively insensitive to anodic and

cathodic background current enabling an extension of the working potential range normally limited by electrolysis of the solvent or supporting electrolyte. The technique is useful for the determination of sub-micromolar concentrations with rotating electrodes and for studies in the presence of surface complications.[201]

1.5.2 Pulsed Rotation Amperometry

Blaedel and Yim investigated the ferricyanide-ferrocyanide system by pulsed rotation voltammetry (PRV) at a glassy carbon disc. PRV is a square wave version of hydrodynamically modulated voltammetry employing large amplitude Δw. They report very low background currents enabling a detection limit of 10^{-8}M ferricyanide.[202]

Both pulsed rotation voltammetry and sinusoidal hydrodynamic modulation require more complicated instrumentation than conventional voltammetry. For the quantitative analysis of a known system only the magnitude of Δi in the transport limited region of potential is necessary. Therefore a simple amperometric technique employing manual changing of the rotation rate by a factor of ten (10) was developed for some of the analytical measurements reported in this thesis. The technique is called pulsed rotation amperometry (PRA). The conditions for the technique are set out in Chapter 2. Where the products of the electrochemical reactions are solvent soluble, triplicate measurements of Δi may be made in 15 s . Where the products of reactions are insoluble, measurement time is limited by renewal of clean electrode surfaces.

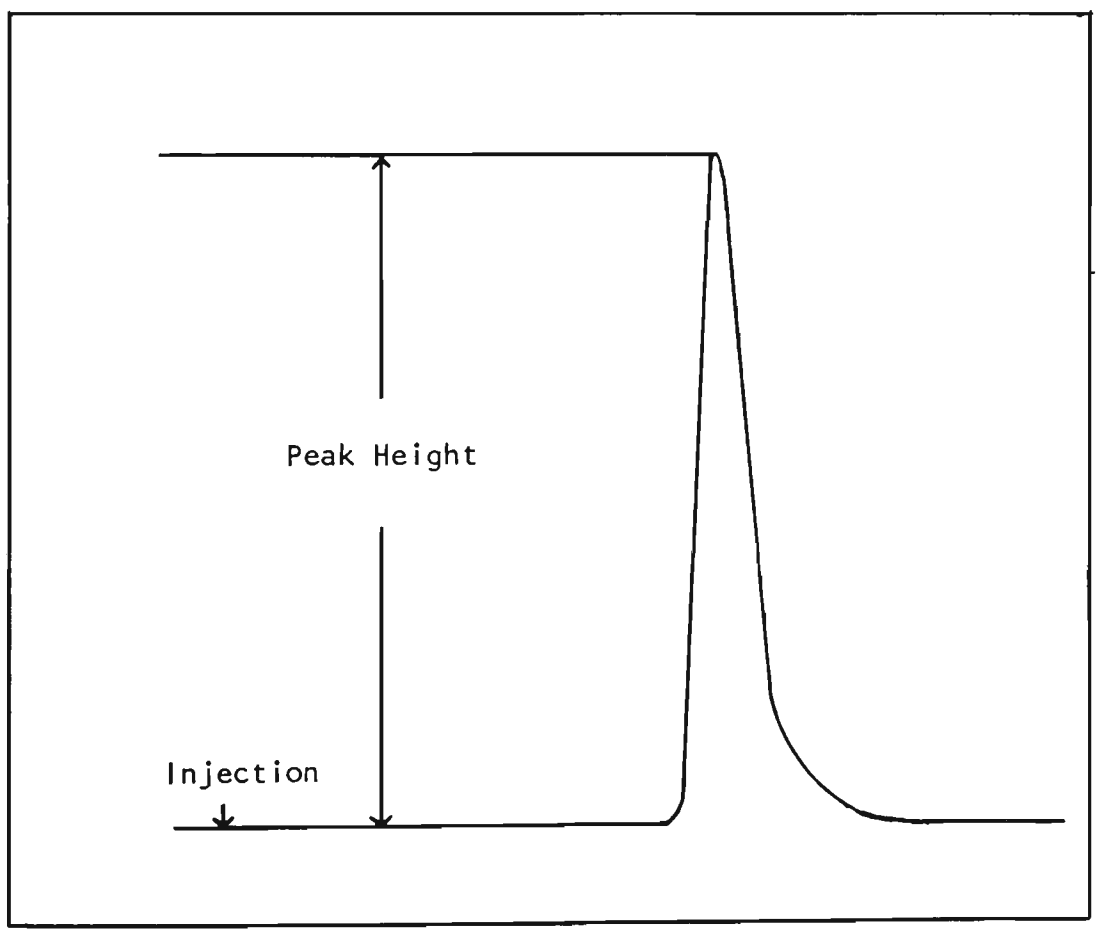
Pulsed rotation amperometry has all the discriminating advantages of hydrodynamically modulated voltammetry and provides analytical data more economically since measurement times are small and conventional instrumentation is used. Detection limits are comparable with differential pulse polarography but PRA has distinct advantages. Since the technique is insensitive to solvent oxidation and reduction, analytes electroactive in this potential region can be determined accurately. Secondly, with differential pulse polarography in certain sample matrices, it is difficult to estimate the background current accurately which leads to quantitative errors. For instance, Archer et al., report three different techniques for background estimation in the differential pulse polarographic determination of tertiary-butylhydroquinone in edible oils. Only one of these techniques provided acceptable accuracy.[32] With a PRA determination the background currents are eliminated when Δi is measured.

In the following chapters the sensitivity of PRA is demonstrated by sub parts per million concentrations of quinol and quinone being determined in small samples of ion selective electrode polymer membranes. The PRA determination of theophylline in pharmaceutical products provides an example of measurements made in the potential region where water oxidation is significant.

1.5.3 Flow Injection Analysis

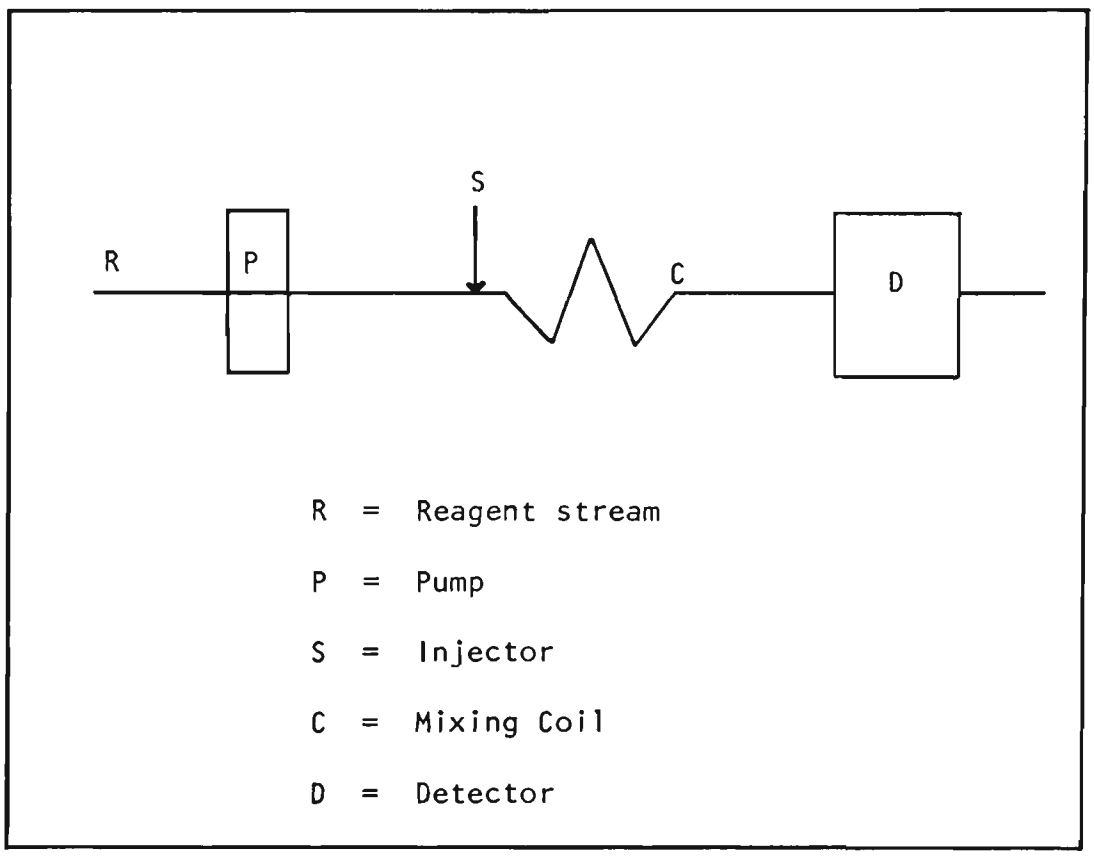
Flow injection analysis (FIA) is a measurement technique in which the sample solution is injected into a continuous carrier flowing stream. The carrier solution provides reagents and a reaction medium if necessary and transports the sample zone to the detector. The detection signal is recorded continuously providing a peak as the sample zone passes through the detector. (See Fig. 1.4). The height of the peak is proportional to the concentration of the analyte.[203]

FIG. 1.4 - TYPICAL FIA PEAK



The simplest FIA system consists of a pump (usually peristaltic since high pressures are not required), a thin tube (ca. 0.5 mm internal diameter) through which the carrier stream flows, an injection port enabling reproducible introduction of sample volumes between 1 and 200 micro litres (μL) and a detector. A schematic diagram of such a system is shown in Fig. 1.5.

FIG. 1.5 - SCHEMATIC DIAGRAM OF A SINGLE-LINE FIA MANIFOLD



A well designed FIA system has very rapid response with residence times in the system of 5 to 20 seconds enabling fast sample throughput. The use of thin tubing and small sample sizes provides low reagent consumption. FIA systems are also

very amenable to automation and therefore the technique has considerable economic advantages over conventional analysis methods.[204]

FIA is a very versatile technique since a variety of detectors may be employed. FIA analysis employing UV-VIS spectrophotometry, atomic adsorption spectroscopy, ion selective electrodes and amperometry have been reported.[205] By adjustment of injection volume, flow line length and diameter and flow rate, conditions suitable for many different chemistries may be obtained.[206] The major parameter altered by these adjustments is the dispersion of the sample zone in the carrier stream. The dispersion, D, is defined[207] as

$$D = \frac{C^{o}}{C^{max}} = \frac{H^{o}}{H} \frac{\text{Const.}'}{\text{Const.}''}$$

where C^{o} = original concentration of injected solution

C^{max} = maximum concentration of injected solution at the detector.

H^{o} = signal due to C^{o}

H = FIA peak height (signal due to C^{max})

For a linear signal-concentration relationship, const.' = const.'' and the ratio of the signals describes the dispersion of the system. The extent of dispersion is a measure of the dilution of the sample solution. For convenience dispersion has been classified into three categories.

1. Limited dispersion where D = 1 to 3
2. Medium dispersion where D = 3 to 10

3. Large dispersion where D > 10.[208]

It follows that to achieve maximum sensitivity limited dispersion must be employed. This is achieved by larger injection volumes and short line lengths between injector and detector.[208] Limited dispersion is used for rapid potentiometric measurements such as pH where no reaction is required. Spectrophotometric analyses requiring chromophore production by reaction in the stream usually require medium dispersion. This is achieved by either using smaller sample sizes, longer lines incorporating mixing coils or a combination of both. Large dispersion is employed in FIA titrations and is achieved by more extreme conditions than those employed for medium dispersion.

1.5.4 Amperometry in Flowing Streams

Amperometric measurements in flowing streams have two main applications. Electrochemical detection is employed in high pressure liquid chromatography (HPLC) and flow injection analysis (FIA). Both techniques provide very high sensitivity and require a small sample size. HPLC offers powerful separations but analysis time is limited by the retention time of the analyte. FIA offers rapid sample throughput and low reagent consumption where electrochemical selectivity is sufficient for the particular analysis.

There are four main electrochemical flow cell designs which may be employed for detection in either HPLC or FIA. These are thin-layer, wall-jet, polarographic and tubular detectors.[209 215] Wall-jet cells utilize increased mass transfer to increase sensitivity by impinging the solution through a jet nozzle onto the electrode at ninety degrees to the surface.[215] Polarographic detectors provide a wider cathodic range than the solid electrode materials such as platinum, gold and carbon,[213] used in thin-layer, wall-jet and tubular cells. Since small sample volumes are used, sensitivity for detectors is usually reported in amounts detected rather than concentrations. Picogram levels of catecholamines have been detected with HPLC incorporating electrochemical detection.[216]

For FIA, wall-jet detectors are recommended.[210] In this technique the emphasis is on rapid sample throughput and therefore FIA requires detectors with quick wash times. The wall-jet design not only provides increased mass transfer in the measurement of the analyte but also very effective cleaning of the electrode between sample injections.[205]

The FIA results reported in this thesis demonstrate the application of limited dispersion for amperometric measurement of quinol and quinone in tetrahydrofuran.

1.6 Electrode Materials

There are a variety of electrode materials available for voltammetric and amperometric measurements. This allows the choice of a suitable

material for a particular analyte, matrix or measurement technique. Polarography employs a dropping mercury electrode providing a clean surface for each drop and a wide cathodic range (limited by the production of hydrogen). Mercury suffers the disadvantage of easy oxidation and therefore has limited anodic applications. Many organic analytes of interest are anodically electroactive and in these cases solid electrode materials such as carbon, gold and platinum provide an extension of the anodic range (limited by the oxidation of water in most cases*).

Carbon paste and glassy carbon were employed for the work reported in this thesis. The use of glassy carbon in electroanalytical applications has been reviewed by Van Der Linden and Dieker.[217] They report on the material production, its structure, the chemical and electrochemical aspects of the electrode/solution interface, voltammetric applications including stripping techniques, its use in flow through detectors, the chemical modification of its surface and its pretreatment before use.

To obtain reproducible results with glassy carbon, pre-treatment of the electrode surface is necessary. There are a large number of reported techniques, most of which are directed at the particular application of the work but in all cases the initial treatment involves polishing the surface. Various abrasives are used but the final surface is generally produced by polishing with alumina or chromium(III) oxide suspensions of particle size around 0.3 μm. This

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* *See Pulsed Rotation Amperometry, 1.8.2.*

produces a mirror finish and the surface is virtually free from functional groups. If the surface has to be kept free of oxygen containing groups, it must be washed after polishing in acids which have no oxidizing properties such as 50% HCl and the potential must be kept negative with respect to the SCE. When the solution contains strong oxidizing agents and/or the potential is made positive, carbonyl and even carboxyl groups can be formed. Cathodic scanning leads to the reduction of these functions to possibly hydroxyl groups. Electrochemical treatment cannot remove the oxygen functions which are only removed by repolishing the surface. Products or reactants adsorbed on the surface can often be removed by rinsing in organic solvents and/or wiping with a wet tissue. Where carboxyl groups are present it is possible to form permanent chemical bonds with nitrogen functions. It is also possible to irreversibly damage the material under anodic conditions with high current densities such as 20 mAmps per cm^2.

Electrochemical pre-treatment has been used to increase electrode response and therefore analytical sensitivity. One of the simplest and most successful techniques is that reported by Engstrom[218,219] where the electrode is pre-anodized at +1.75 V versus Ag/AgCl for 5 minutes followed by pre-cathodization for 10 seconds at -1.20 V. This treatment produces a marked enhancement in sensitivity and selectivity (via shifts in peak potential) in flow through detectors for several electrochemically irreversible oxidations at untreated glassy carbon. The treatment provides reproducible current levels over a whole day's continuous use.

Baldwin and Ravichandran investigated the electrochemical pre-treatment of carbon paste electrodes using Engstrom's technique.[220] They found that similar behaviour was observed but the enhanced sensitivity and shifts in peak potentials were not as long lived for carbon paste as they were for glassy carbon. In particular when carbon paste was treated and used in flow through detectors the enhanced sensitivity continuously decreased with time. This resulted in a return to current levels near that of the untreated carbon paste within 30 minutes.

The decrease in sensitivity with time was shown not to be due to the shift in peak potential (via cyclic voltammetry of untreated and treated electrodes) and was attributed to a change in the surface activation properties of the carbon paste.

Chapter 2

Experimental

2.1 Materials

2.1.1 Water

All solutions for analytical measurements were prepared from water distilled in glass which was passed through a Millipore "Milli Q" water deionizing system prior to use. The purified water had a resistance of greater than 1×10^7 ohms.

2.1.2 Inorganic Reagents

The quality and source of these reagents is shown in Table 2.1.1.

TABLE 2.1.1 - Inorganic Reagents

Reagent	Quality and Source
KH_2PO_4, K_2HPO_4	Ajax Chemicals "Analytical Reagent"
& $NH_4H_2PO_4$	May and Baker Aust. P/L "PRONALYS"
	BDH Chemicals Aust. P/L "AnalaR"
KOH and NaOH	May and Baker Aust. P/L "PRONALYS"
KCℓ	B.D.H. Chemicals Aust. P/L
$AgNO_3$	May and Baker Aust. P/L
NH_4NO_3	BDH Chemicals Aust. P/L "AnalaR"
K_2CrO_7	Ajax Chemicals "Laboratory Reagent"
H_2SO_4	Ajax Chemicals "Analytical Reagent"
	May and Baker Aust. P/L "PRONALYS"

2.1.3 Organic Reagents

Pure samples of theophylline, theobromine, caffeine and 3-methylxanthine were provided by the Royal Childrens Hospital, Melbourne and the Victorian College of Pharmacy. These materials were stored at less than 0°C and used without further purification.

Acetonitrile, methanol, hexane and tetrahydrofuran (THF) were obtained from B.D.H. Chemicals Australia Pty. Ltd. and were all "AnalaR" analytical reagent grade.

Quinol and Quinone were obtained from Merck and were "Puris" analytical reagent grade.

Ethylenediamine was obtained from B.D.H. Chemicals Australia Pty. Ltd. Laboratory reagent grade material was used without further purification.

Aliquat 336 was provided by R.W. Cattrall, Department of Inorganic and Analytical Chemistry, La Trobe University, Bundoora, and was used without further purification.

Samples of powdered polyvinylchloride (PVC) were obtained from I.C.I. Australia Pty. Ltd. and used without further purification. Infra-red spectroscopy was used to confirm the identity of the material.

2.1.4 Alumina

Two grades of alumina were used. Merck Alumina "For Column Chromatography" Activity IIIA was used for the removal of stabilizer from THF. The alumina was activated by heating to 300°C, under reduced pressure (ca. 10-20 mm/Hg), for 3 hours. Polishing alumina was used for the preparation of glassy carbon surfaces and it was provided with the Metrohm EA 276 glassy carbon electrode.

2.1.5 Nitrogen

High purity grade nitrogen, for deoxygenating analytical solutions prior to electrochemical measurement, was obtained from Commonwealth Industrial Gases, Preston.

2.1.6 Glassware

All glass apparatus used for analytical solutions was cleaned in chromic acid solutions.* The glassware was rinsed several times with tap water, followed by several rinsings with distilled water and finally rinsed three times with high purity distilled and deionized water. This procedure is especially necessary when working at the sub-parts per million level of analytes.

2.2 Instrumentation

2.2.1 Voltammetry

Direct current (DC), alternating current (AC) and differential-pulse (DP) voltammetry was performed with a Metrohm E506

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* *Prepared as outlined in A.I. Vogel, "A Textbook of Inorganic Analysis", 3rd. Edition, 1961, Longman, London.*

Polarecord and a Metrohm E506 Polarography stand. Cyclic voltammetry was performed with a potentiostat constructed at Footscray Institute of Technology, a Utah Instruments Model 1509 sweep generator and a Watenabe X-Y recorder.

2.2.2 Pulsed Rotation Amperometry (PRA)

A Beckman rotating electrode assembly, capable of rotation rates between 0.1 and 100 revolutions per second (r.p.s.), was employed for PRA. The system incorporates a manual control switch on the drive motor to change the rotation rate by a factor of ten (10). This enables currents to be recorded at two rotation rates and their difference (ΔI) to be measured. Detection potentials were maintained by the Metrohm E505 Polarecord and the currents recorded with the E505 in the current-time mode of the instrument. With water soluble reaction products, triplicate measurements of ΔI were obtained in 15 seconds with manual changing of rotation rate. Measurement time is limited by resurfacing of electrodes where reaction products accumulate at the surface.

2.2.3 U.V. Spectrophotometry

All UV spectrophotometric measurements were recorded on a Varian DMS90 Spectrophotometer. 1 cm quartz cells were employed for all measurements.

2.2.4 Infrared Spectroscopy

Infrared spectra were recorded with a Perkin-Elmer grating spectrometer. Model PE IR 720.

2.2.5 Flow Injection Analysis

A single line flow injection analysis (FIA) system with an electrochemical detector was constructed and used for all FIA measurements reported. A schematic diagram of the system is shown in Fig. 2.2.1. The reservoir, pump and sample injection parts of the system were modular to enable easy conversion from perstaltic pumping with loop injection to gas pressure pumping with septum injection.

FIA measurements for the determination of theophylline and quinol employed the carrier stream reservoir shown in Fig. 2.2.2, a Gilson "minipuls" peristaltic pump and a Pye sample loop injector, modified for small liquid samples. The Pye injector has an internal volume of 70 µL and was fitted with a 20 µL sample loop so that 90 µL of sample is injected into the carrier stream. The FIA tubing between injector and detector was 0.5 mm internal diameter teflon and kept at a minimum length (10 cm) to minimize dispersion of the sample bolus in the carrier stream and therefore maximize the sensitivity of the response. FIA measurements for the determination of quinone were performed with the Metrohm FIA Injection Stand. Gas pressure pumping and septum injection (10 µL sample size) were used, as is shown in Fig. 2.2.3. The minimum line length between injector and detector was 20 cm due to the geometry of the Metrohm system.

The electrochemical detector employed was a SGE wall-jet electrochemical cell incorporating a glassy carbon working electrode, a Ag/AgCℓ saturated KCℓ reference electrode

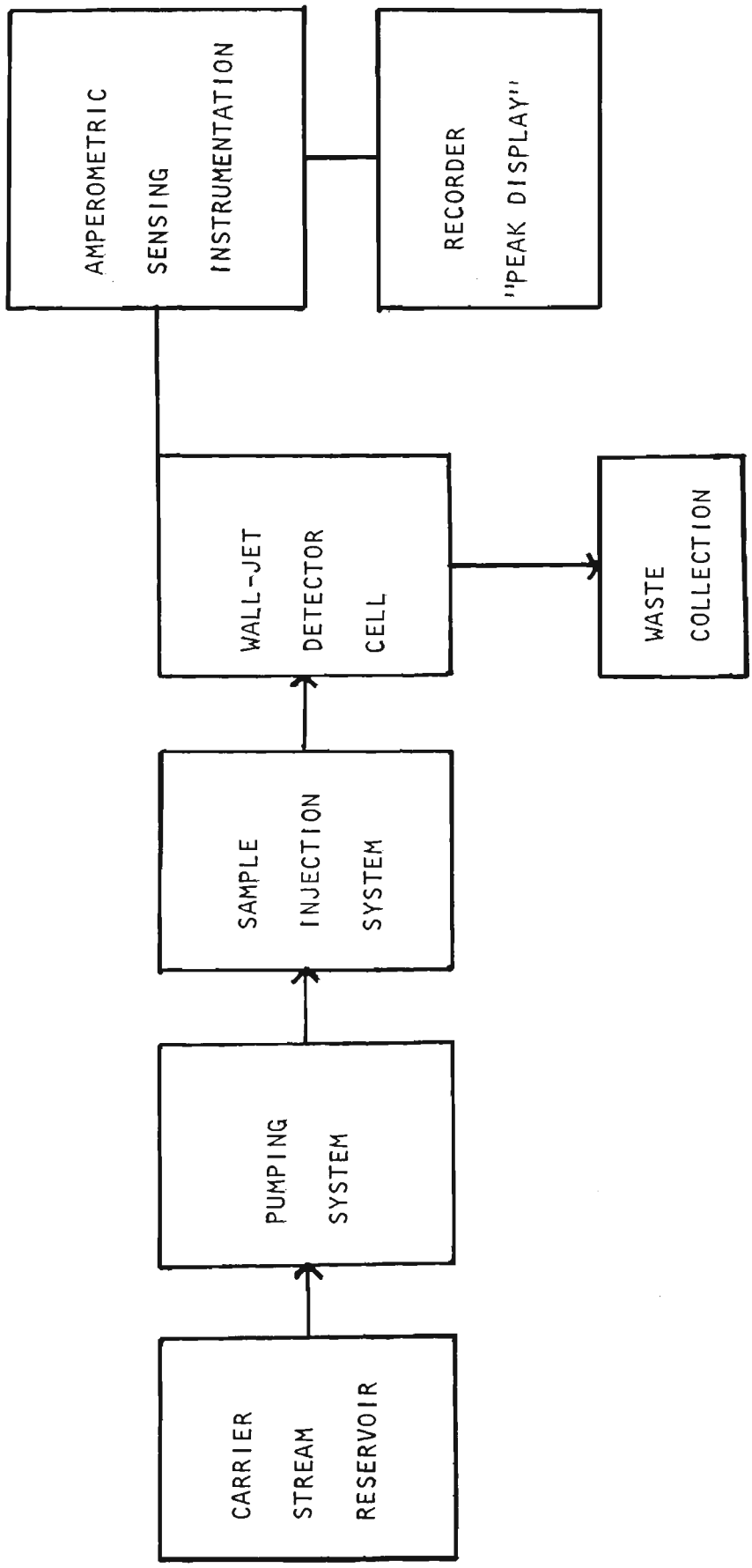
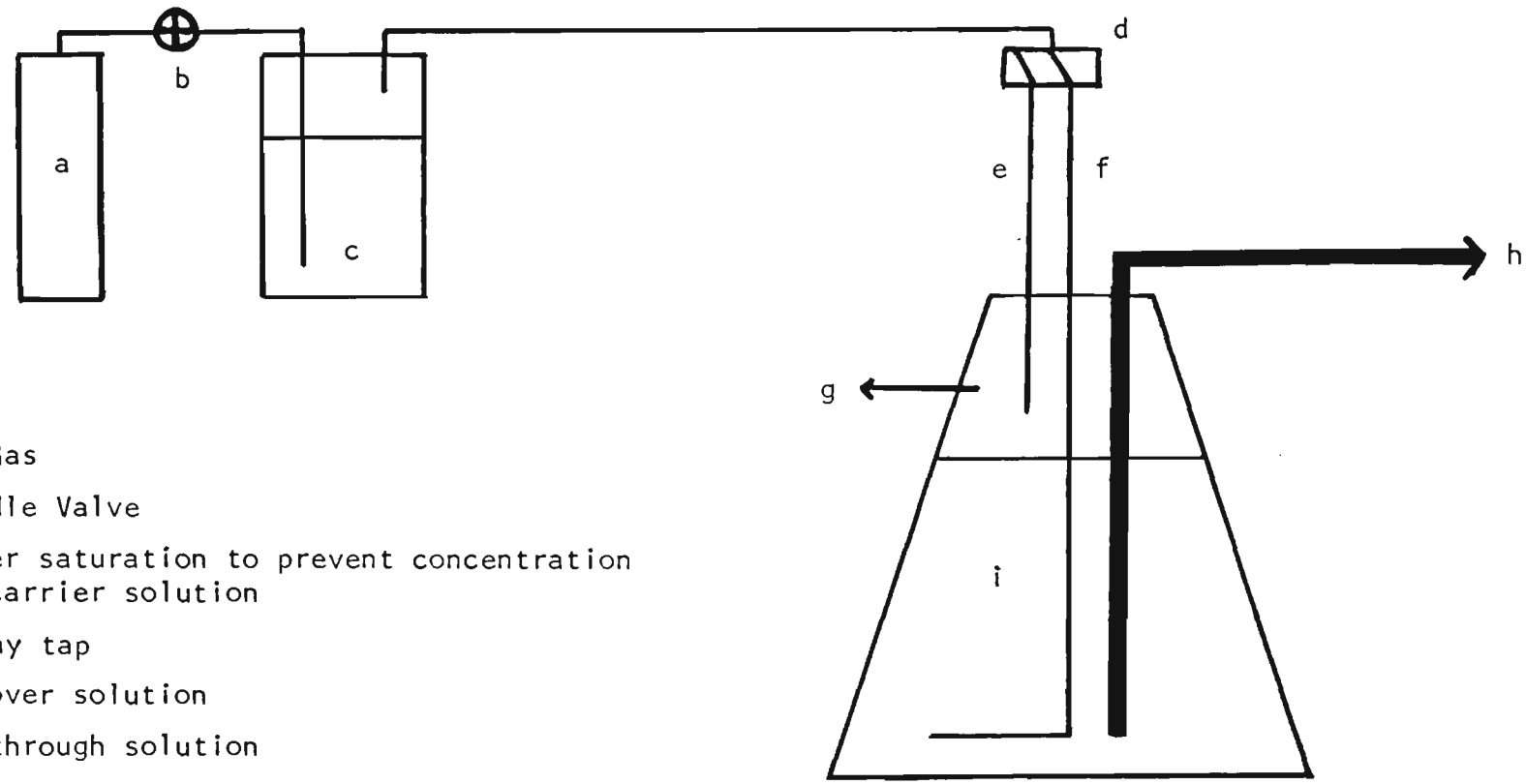


FIGURE 2.2.1 - SCHEMATIC REPRESENTATION OF F.I.A. SYSTEM

FIGURE 2.2.2 - F.I.A. CARRIER STREAM RESERVOIR WITH DEOXYGENATING SYSTEM



a N_2 Gas

b Needle Valve

c Water saturation to prevent concentration of carrier solution

d 2-way tap

e N_2 over solution

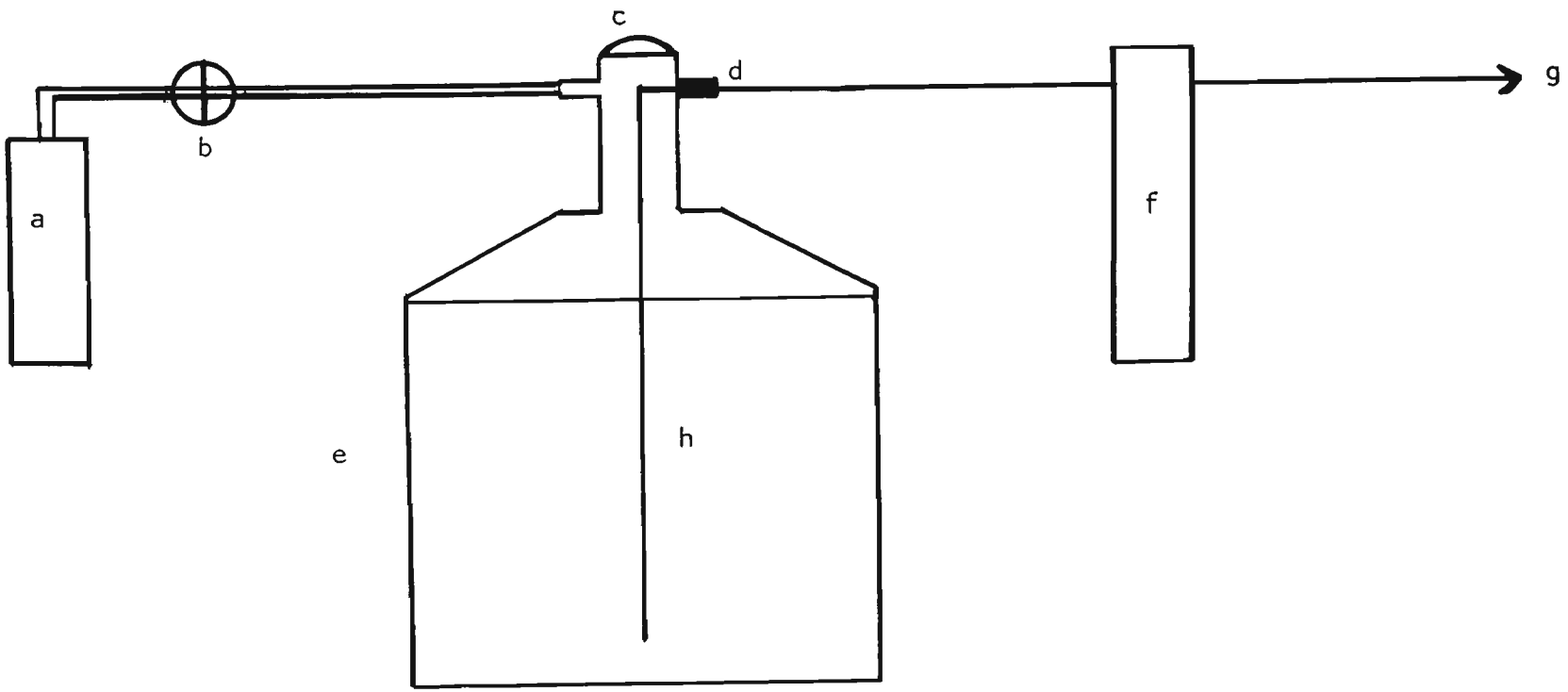
f N_2 through solution

g N_2 out

h to peristaltic pump

i carrier solution

FIGURE 2.2.3 - METROHM FIA INJECTION STAND (SCHEMATIC LAYOUT)



a N_2 Gas

b Needle Valve

c Blow out safety valve

d Carrier Stream Out

e Thick glass vessel

f Injector system with rubber septum

g To Detector

h Carrier Stream Solution

and a stainless steel exit line which served as the counter electrode. The geometry of the cell is shown in Fig. 2.2.4. The distance between the solution inlet nozzle and the glassy carbon disc is variable by means of a screw thread on the working electrode. The optimum distance, for maximum sensitivity and minimum electrical noise due to turbulence in the flow of the solution was found to be 1 mm.

Currents were measured by either the Metrohm E506 Polarecord or a circuit incorporating the "FIT" potentiostat, a Keithley 616 Digital Electrometer (used in the picoammeter mode) and a Y-t recorder. A schematic diagram of the circuit is shown in Fig. 2.2.5.

2.2.6 Potentiometry

pH measurements were made using an Orion 701 Ion Selective/pH digital millivoltmeter and a combined pH electrode. The system was calibrated with pH = 4.0 and pH = 9.2 buffers before use.

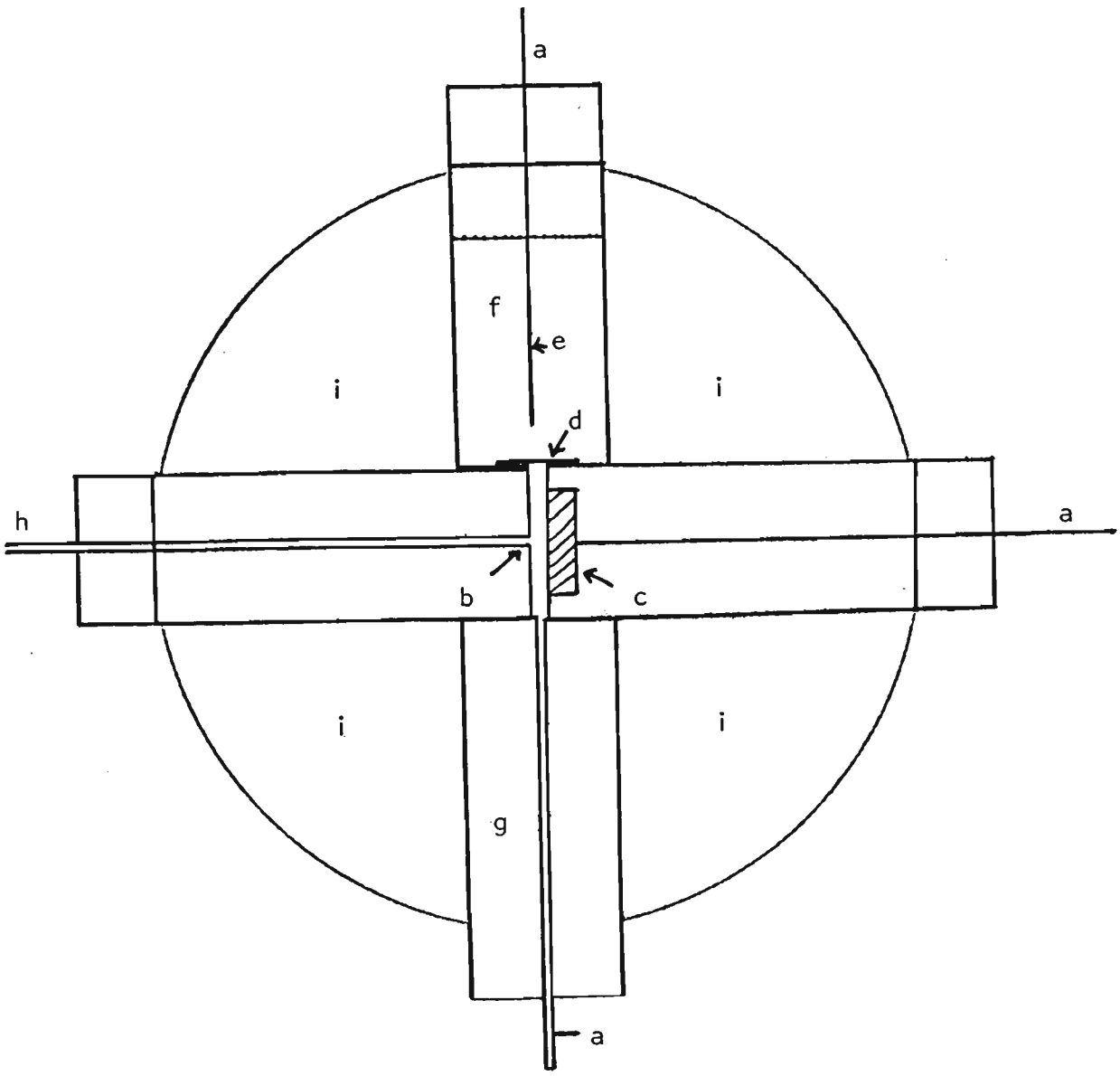
2.2.7 Computing

Computer calculations were performed with the following computer systems -

a) A SWTPC 6800 Computer System incorporating a dual 5¼ inch mini floppy disc drive.

b) A South West 6809 computer incorporating a dual 5¼ inch mini floppy disc drive.

FIGURE 2.2.4 - S.G.E. WALL JET CELL : CROSS SECTIONAL PERSPECTIVE



Legend:

a : Electrical contacts

b : Solution inlet jet nozzle

c : Glassy carbon disc - working electrode

d : Ceramic frit in reference electrode

e : Ag/AgCℓ wire

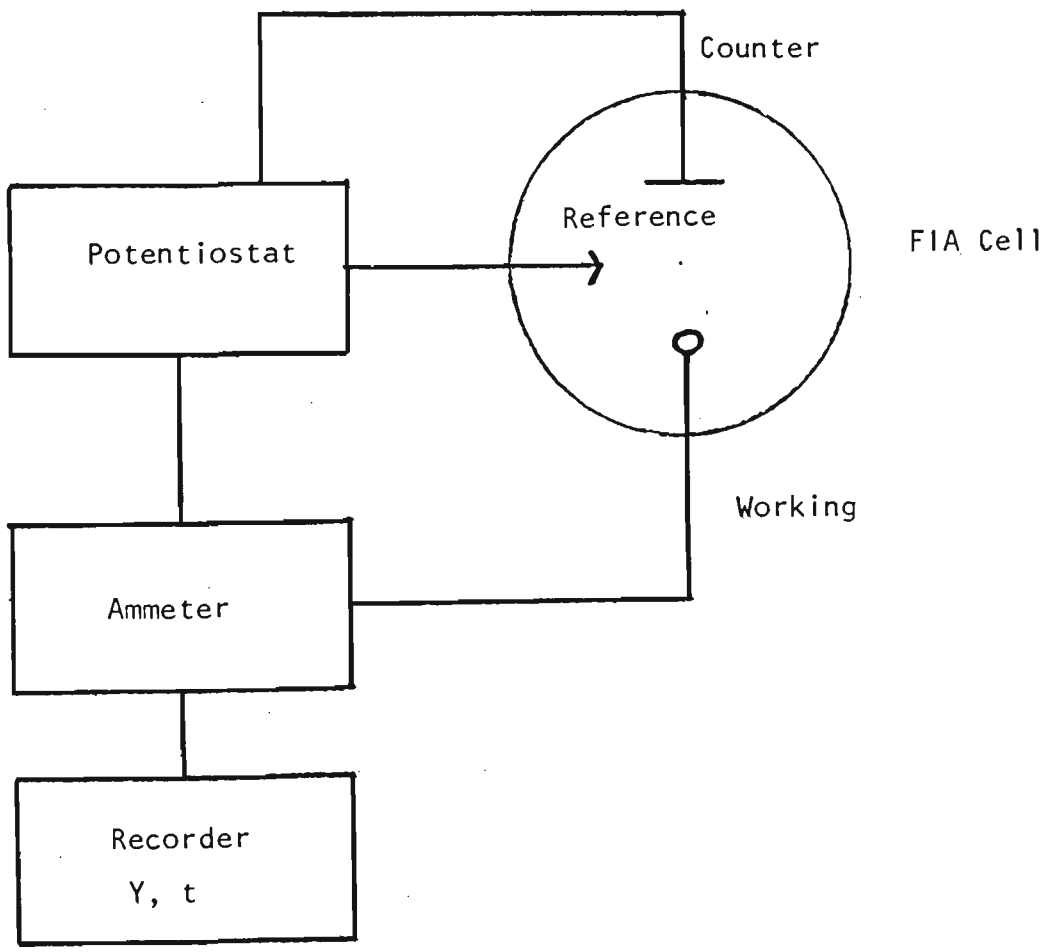
f : Saturated KCℓ internal solution for reference electrode

g : Stainless steel solution exit line - counter electrode

h : Solution inlet

i : Kel F cell body

FIG. 2.2.5 - SCHEMATIC DIAGRAM OF F.I.A. AMPEROMETRIC DETECTION CIRCUIT



c) An Apple II/e computer system incorporating a 5¼ inch mini floppy disc drive.

All programs were written in BASIC and are set out in appendices I to III.

2.3 Electrodes

2.3.1 Reference Electrodes

Reference electrodes employed in voltammetry and PRA were Ag/AgCℓ saturated KCℓ. In some instances a 10% KNO_3 salt bridge was employed; in these cases reference is made in the text. The junctions employed were all sintered glass frits. In flow injection analysis an Ag/AgCℓ saturated KCℓ reference supplied with the SGE flow cell was employed. This electrode has a ceramic frit junction.

2.3.2 Counter Electrodes

In voltammetry and PRA, platinum wire electrodes were employed. In flow injection analysis the stainless steel solution exit line was employed as the counter electrode.

2.3.3 Working Electrodes

In voltammetry both the Metrohm EA276 glassy carbon and the Metrohm EA267 carbon paste electrodes were employed. In PRA both glassy carbon and carbon paste electrode materials were employed. These electrodes are supplied as screw-on tips to the Beckmann rotating electrode apparatus. In flow injection analysis only the glassy carbon electrode supplied

with the S.G.E. wall jet detector was employed. The soft nature of carbon paste makes it unsuitable in wall jet detectors

2.4 Preparation of Electrodes

2.4.1 Reference Electrodes

All reference electrodes were thoroughly rinsed with high purity water and blotted dry with a tissue before use.

2.4.2 Counter Electrodes

Platinum wire electrodes were cleaned in chromic acid, rinsed thoroughly with high purity water and blotted dry with a tissue before use.

2.4.3 Working Electrodes

2.4.3.1 Glassy Carbon Electrodes (G.C.E.)

These electrodes were polished with a water/alumina paste (provided with the Metrohm EA276 Glassy Carbon Electrode) on a horizontal smooth surface until a mirror finish was obtained and no scratches were visible on the electrode surface. The electrodes were rinsed thoroughly with high purity water before use.

2.4.3.2 Carbon Paste Electrodes (C.P.E.)

The technical literature for the Metrohm EA267 Carbon Paste Electrode suggests smoothing off the carbon paste surface on fine filter paper to give a reproducible electrode surface area. Using Whatman No. 1 filter paper, this technique gave only fair results, enabling relative standard deviations

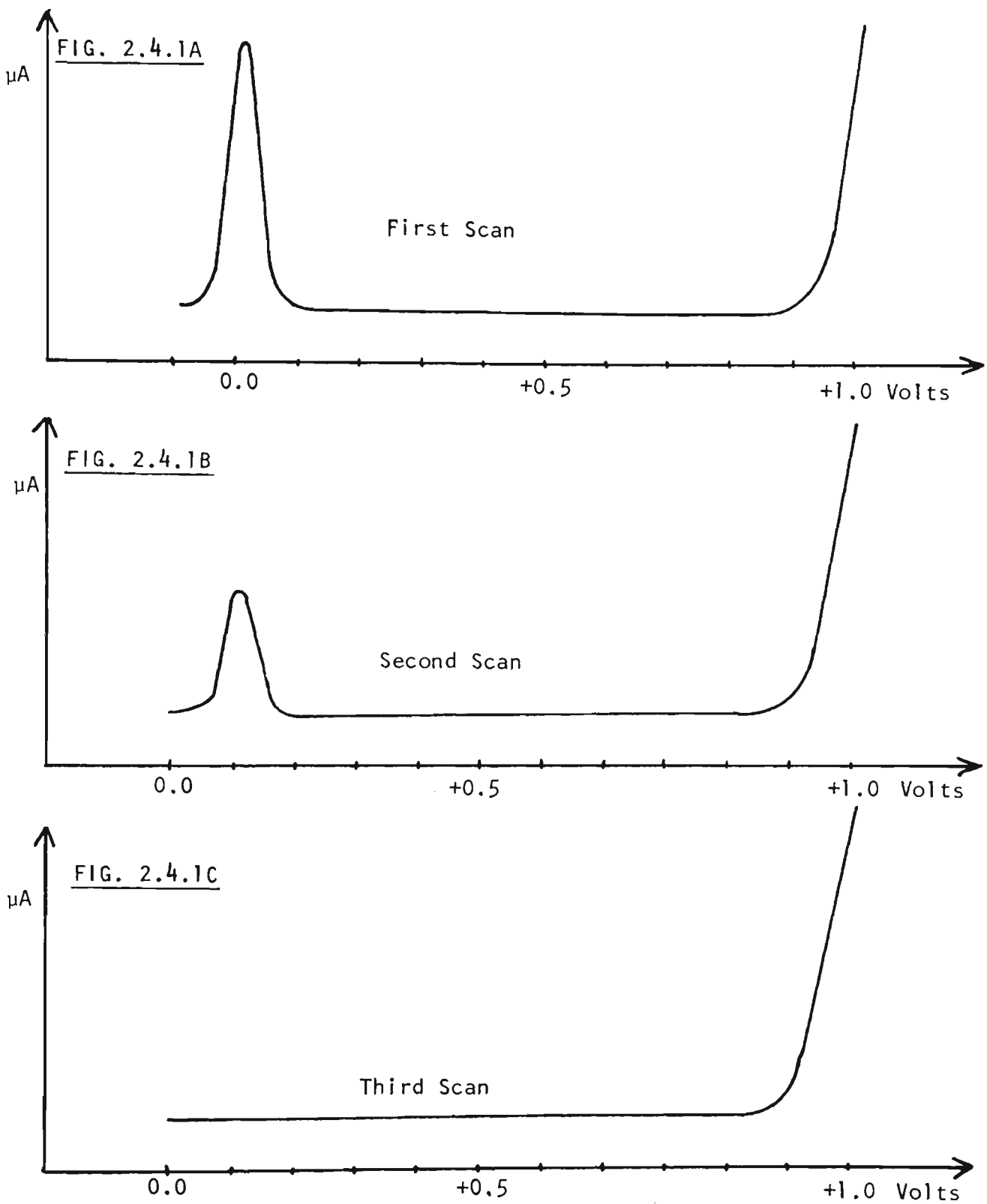
(RSD) of 4 to 5% to be achieved in voltammetric analysis. Using Whatman 'Benchkote' protective paper it was found that reproducibility (RSD) of 2% could be achieved. This material consists of fine paper coated on one side with a polyethylene film. The carbon paste was extruded out of the electrode (for Metrohm EA267) barrel to a distance of 2 mm and this material was wiped off onto the paper side of the 'Benchkote'. This gives a clean yet roughened surface which is then wiped on the polyethylene side to produce a very smooth, clean surface.

It was observed that in some cases surface contamination could occur. This resulted in a sharp peak being recorded on voltammograms as shown in Fig. 2.4.1A. This peak was verified as adsorption of an electroactive surface contaminant by repetitive voltammetric scans. After one or two scans, on the same surface, the peak disappears from the voltammograms. This indicates that the contaminant was adsorbed onto the surface, prior to insertion into the solution, and is removed by the oxidative scan. The effect is demonstrated in Fig. 2.4.1A, B & C.

2.4.4 Survey of electrode smoothing materials

A survey of various smoothing materials was carried out to locate the source of the adsorbing species. In each experiment new carbon paste surfaces were prepared and voltammograms recorded from 0.0V to +1.5V versus a Ag/AgCℓ saturated KCℓ electrode. The results of the survey are presented in Table 2.4.1. All materials were obtained from unopened packages unless otherwise stated.

FIG. 2.4.1 - CONTAMINANT ADSORPTION ON C.P.E. SURFACE



Conditions: Working Electrode - Carbon Paste (Metrohm)
Reference Electrode - Ag/AgCℓ sat. KCℓ
Counter Electrode - Platinum Wire
Phosphate Buffer - pH = 7
Scan Rate - 6 mV/sec.

TABLE 2.4.1 - A Survey of Smoothing Materials for Carbon Paste Electrode (CPE) Surfaces

Smoothing Material	Comment on Voltammogram	Suitability for CPE Preparation
Whatman No. 1 Filter Paper Paper Chosen at random Filter Paper from an old opened packet	 Adsorption peak observed at +0.11V Adsorption peak observed at +0.11V	Unsuitable
Whatman No. 40 Ashless Filter Paper	Adsorption peak observed at +0.18V	Unsuitable
Whatman No. 42 Ashless Filter Paper	Adsorption peak observed at +0.18V	Unsuitable
Whatman No. 43 Ashless Filter Paper (Old opened packet)	Adsorption peak observed at +0.18V	Unsuitable
Whatman No. 54 Hardened Filter Paper	Adsorption peak observed at +0.11V	Unsuitable
Whatman No. 541 Hardened Ashless Filter Paper	Adsorption peak observed at +0.11V	Unsuitalbe
Whatman No. 542 Hardened Ashless Filter Paper	Adsorption peak observed at +0.11V	Unsuitable
Toyo No. 1 Filter Paper	Adsorption peak observed at +0.11V	Unsuitable
M.N. Duren Nr. 1640m Filter Paper	Adsorption peak observed at +0.11V	Unsuitable

Table 2.4.1 (Cont'd)

M.N. Duren Nr. 1640d Filter Paper	No adsorption peak observed.	Suitable*
Bowscot Microwipes (Tissue Paper)	Large adsorption peak observed at +0.11V	Unsuitable
Whatman 'Benchkote' Paper Side only	Small adsorption peak observed at +0.11V	Unsuitable
Whatman 'Benchkote' Using both sides		
Test 1	No adsorption peak observed	Suitable
Test 2	No adsorption peak observed	
Test 3	No adsorption peak observed	
Test 4	No adsorption peak observed	
Test 5	No adsorption peak observed	
Computer Card Material	No adsorption peak observed	Suitable
Chromic Acid cleaned Glass Plate rinsed with water and dried with (a) soft facial tissue or (b) air dried.	(a) Adsorption peak observed at +0.11V	Unsuitable
	(b) No adsorption peak observed	Suitable*
Stainless Steel spatula cleaned and rinsed with water. Dried with (a) soft facial tissue (b) air dried	(a) Adsorption peak observed at +0.11V.	Unsuitable
	(b) No adsorption peak observed	Suitable*
Polyethylene Bag	No adsorption peak observed	Suitable*

Table 2.4.1 (Cont'd)

Soft Facial Tissue	Adsorption peak observed at + 0.11V	Unsuitable

* *Although these materials are marked as suitable materials, they are not ideal since they produce rough, cracked or irreproducible surfaces.*

2.4.5 Survey Conclusions

1. The contamination is due to filter and tissue paper products since adsorption is absent with materials kept free from contact with paper.

2. There are two compounds capable of adsorption since two peak potentials were observed. Most products contain the compound with an adsorption peak potential of +0.11V. The Whatman 'ashless' range (Whatman Nos. 40, 42 and 43) of papers were the only materials to show an adsorption peak at +0.18V.

3. The intermittent nature of the adsorption process with some materials is possibly due to the manual technique used to smooth the surface. For instance it could be possible to cover an area of paper with clean carbon paste while smoothing the surface. This would present a carbon paste surface rather than paper for the final smoothing process

resulting in an electrode with no contamination.

4. Polyethylene is ideal for surface preparation once the used carbon paste has been removed.

5. Whatman 'Benchkote' was found to be the most suitable material. It provides a surface to remove used carbon paste as well as a surface which gives smooth, clean and reproducible electrode areas.

2.4.6 Beckman Rotating Carbon Paste Electrode

The Beckman rotating carbon paste electrode was prepared in the same manner as the Metrohm electrode except with this electrode there is no facility for extruding paste from the electrode barrel. In this case the carbon paste cavity is emptied with a spatula, firmly refilled and smoothed on 'Benchkote'.

2.5 Synthesis of Quinone Photodimer

The quinone dimer, tricyclo[6,4,0^{0^2}]dodeca-4,10-diene-3,6,9,12-tetrone, was photochemically synthesized for the investigation of the interferent in the PRA analysis of quinone in ion-selective electrode polymer membranes. Gold and Ginsberg[221] report the synthesis of the quinone photodimer and the following procedure is an adaptation of their method.

A suspension of 5 grams of quinone in n-hexane was exposed to air and UV light from a low intensity mercury vapour lamp (10 watt) for 1 week. Periodically throughout the week small portions of n-hexane

were added to the suspension to replace that lost by evaporation. The reaction mixture was filtered and the solid residue washed with diethylether. Quinone is quite soluble in ether whereas the dimer is not. Therefore the ether washing was continued until the filtrate was colorless and only deep purple-red colored crystals of the dimer remained. The reaction yielded only 6% dimer but this was sufficient for the interference study.

The identity of the product was confirmed by melting point, solubility tests, infra-red and UV spectroscopy and comparison with the data reported by Gold and Ginsberg.

2.6 Purification of Tetrahydrofuran

To investigate the effect of the presence of tetrahydrofuran (THF) on the electrochemistry of quinol and quinone samples of pure, quinoid free, THF were required. Since unstabilized THF is expensive and forms dangerous peroxides it was decided to remove the stabilizer from small quantities of THF as necessary in the course of the investigation. This provides safe quantities which are free of peroxides.

About 50 ml of quinol stabilized THF is allowed to percolate through a dry activated alumina chromatography column (4 cm in diameter x 12 cm in length). The first 5 ml eluted is discarded and the next 25 ml is collected. The purification of the THF was confirmed by UV spectroscopy.

2.7 Analytical Procedures for Quinol in THF

2.7.1 Pulsed Rotation Amperometry (PRA)

THF samples (1.0 ml) were diluted one hundred-fold in pH = 7 phosphate buffer (0.1M K_2HPO_4 and 0.1M KH_2PO_4 and adjusted to pH = 7.0 with either KOH or H_3PO_4) and 30.0 ml was used as the test solution.

The PRA cell consisted of a rotating glassy carbon disc, a Ag/AgCℓ saturated KCℓ reference and a platinum counter electrode. The PRA step height, Δi, was measured in triplicate by changing the rotation rate between 2 and 20 rps and employing a DC detection potential of 0.36 Volts.

Quantitation was achieved by standard additions of 5 μg/ml quinol in phosphate buffer containing 1% purified THF. Δi was determined in triplicate after each addition.

2.7.2 Flow Injection Analysis

The test solution was prepared by one hundred-fold dilution of the THF samples (1.0 ml) in pH = 7 phosphate buffer.

A carrier stream flowing at 1 ml/minute and consisting of pH = 7 phosphate buffer which contained 1% purified THF was employed. The DC detection potential was +0.6 volts versus an Ag/AgCℓ saturated KCℓ reference electrode. Three, 90 μL, volumes of the test solution were injected and each corresponding peak height measured. Quantitation was achieved by comparison with a calibration graph. The calibration data were recorded just prior to sample injection and identical conditions were employed.

Peristaltic pumping and the loop injector were employed in these determinations.

2.7.3 U.V. Spectrophotometry

The test solution was prepared by dilution of the THF samples (1.0 ml) one hundred-fold in pH = 7 phosphate buffer.

The absorbance of the test solution is measured at 288 nm versus a reference cell containing 1% purified THF in pH = 7 phosphate buffer. 1 cm path length quartz cells were employed.

Quantitation was achieved by comparison with a calibration graph. The calibration data were obtained by measurement of the absorbance of quinol standard solutions prepared from pH = 7 phosphate buffer containing 1% purified THF.

2.8 Analytical Procedures for Quinone in THF

All test solutions were prepared by dilution of THF one hundred-fold in pH = 7 phosphate buffer.

2.8.1 Pulsed Rotation Amperometry

Quinone was determined by the same procedure as for quinol except that a detection potential of -0.15 Volts versus the Ag/AgCℓ saturated KCℓ reference was employed. The standard additions standard solution was 10 µg/ml quinone.

2.8.2 Flow Injection Analysis

Quinone was determined by the same procedure as for quinol except that a detection potential of -0.15 Volts versus the Ag/AgCℓ saturated KCℓ reference was employed. Also the test solution was introduced into the carrier stream by 10 µL syringe via the injection port of the Metrohm FIA stand. The Metrohm gas pressure pump was employed.

2.8.3 U.V. Spectrophotometry

Quinone was determined by the same procedure as for quinol except that absorbances were measured at 243 nm.

2.9 Analytical Procedures for Quinol and Quinone in Ion selective Electrode Polymer Membranes

2.9.1 Preparation of Fresh Membranes

Powdered PVC (0.35 g) and Aliquat 336 (0.65 g) were blended together in a minimum (3-5 ml) of quinol stabilized THF until a clear homogeneous and viscous solution was obtained. The solution was then poured onto a glass plate with a glass restraining ring. The THF was allowed to evaporate slowly through a filter paper cover placed over the ring to avoid contact with the mixture. After 2 days the polymer membrane was peeled off the glass plate and cut into segments for analysis.

2.9.2 Sample Treatment

All membrane samples (10 to 20 mg) were dissolved in purified THF and made up to 25.0 ml. Aliquots of these solutions were diluted in the analysis medium just prior to measurement.

2.9.3 Pulsed Rotation Amperometry

a) Recovery of Quinol from Sample Matrix

To test the recovery of quinol from the membranes a known solution containing 10 µg/ml quinol, 0.35 mg/ml PVC and 0.65 mg/ml Aliquat 336 in 25.0 ml purified THF was employed Quinol was determined by standard additions under the following conditions.

Test solution: 20.0 ml pH = 7 phosphate buffer plus 0.5 ml of known THF solution.

PRA conditions: Rotating Glassy Carbon Disc at a detection potential of +0.36 V versus an Ag/AgCℓ saturated KCℓ reference. Platinum counter electrode. Δi determined by changing rotation rate between 5 and 50 r.p.s.

b) Analysis of Membranes for Quinol

The test solutions were 20.0 ml of pH = 7 phosphate buffer to which 2.0 ml of THF sample solution was added. Quinol was determined by standard additions. The standard solution was 5 µg/ml quinol in phosphate buffer (pH = 7) containing 9% purified THF.

A rotating glassy carbon disc at a detection potential of +0.36 Volts versus an Ag/AgCℓ saturated KCℓ reference and platinum counter electrode were used. Δi was measured by changing the rotation rate between 9 and 90 r.p.s.

Sample blanks were also determined by the same procedure.

c) Recovery of Quinone from Sample Matrix

A known solution containing 10 µg/ml quinone , 0.35 mg/ml PVC and 0.65 mg/ml Aliquat 336 in purified THF was investigated. The following conditions were employed.

Test solution: 20.0 ml pH = 7 phosphate buffer plus 0.5 ml of known THF solution.

PRA conditions: Rotating Glassy Carbon Disc at a detection potential of -0.15 Volts versus an Ag/AgCℓ saturated KCℓ reference. A platinum counter electrode. Δi was determined by changing the rotation rate between 9 and 90 r.p.s.

d) Analysis of Membranes for Quinone

The test solutions were 20.0 ml of pH = 7 phosphate buffer plus 0.5 ml of the THF sample solution. Quinone was determined by standard additions. The standard solution was 5 µg/ml quinone in phosphate buffer (pH = 7) containing 2.5% purified THF.

A rotating glassy carbon disc at a detection potential of -0.15 Volts versus an Ag/AgCℓ saturated KCℓ reference and a platinum counter electrode were employed. Δi was measured by changing the rotation rate between 9 and 90 r.p.s.

Sample blanks were also determined by the same procedure.

2.9.4 U.V. Spectrophotometry

Small aliquots of the THF sample solution (0.5 ml) were diluted tenfold in purified THF. Absorbance measurements of these solutions were taken at 288 nm for quinol and 243 nm for quinone. The reference cell contained a purified THF solution of PVC and Aliquat 336 in a ratio equivalent to the test solution.

Quantitation was achieved by comparison with calibration graphs. The calibration data were recorded employing identical conditions to the test solutions.

2.10 Analytical Procedures for Theophylline Determination by P.R.A.

Theophylline was determined in three pharmaceutical products by pulsed rotation amperometry.

2.10.1 Nuellin Cough Syrup

Samples of the syrup were diluted one hundred-fold in pH = 7 phosphate buffer and then 5.0 ml of this solution was added to 40.0 ml of buffer in the PRA cell. Duplicate measurements of the step height, Δi, were recorded using a new carbon paste surface for each measurement.

Quantitation was achieved by standard additions. The standard solution was 101 µg/ml theophylline in phosphate buffer (pH = 7). The following PRA conditions were employed.

A rotating bevelled tip carbon paste electrode at a DC detection potential of 1.16 Volts versus an Ag/AgCℓ saturated KCℓ/10% KNO_3 salt bridge reference and a platinum counter electrode were used. The step height Δi was produced by firstly applying the detection potential to the carbon paste electrode rotating at 5 r.p.s. and then exactly 20 seconds later changing the rotation rate to 50 r.p.s.

2.10.2 Elixophyllin Cough Syrup

Samples of the syrup were diluted two hundred-fold in pH = 7 phosphate buffer. This solution was analyzed by the same procedure as for Nuellin Syrup.

2.10.3 Aminophylline Injection

Sigma Pharmaceuticals Pty. Ltd. provided an analyzed quality control sample of an intravenous aminophylline injection. The sample was labelled as follows, "SIGMA-AMINOPHYLLINE B.P. 0.25 g in 10 ml for I.V. (B) AM120" and Sigma's assay value was

21.3 mg/ml theophylline by UV spectrophotometry.

One (1.0) ml of the sample was diluted to 200 ml with pH = 7 phosphate buffer and 5.0 ml of this solution was added to 40.0 ml of buffer in the PRA cell. Duplicate measurements of the step height, Δi, were recorded using a new carbon paste surface for each measurement.

Quantitation was achieved by standard additions. The standard solution was 101 µg/ml theophylline in phosphate buffer (pH = 7).

The PRA conditions were as follows. A rotating bevelled tip carbon paste electrode at a DC detection potential of +1.10 Volts versus an Ag/AgCℓ saturated KCℓ/10% KNO_3 salt bridge reference and a platinum counter electrode were employed.

The step height, Δi, was produced as in section 2.10.1.

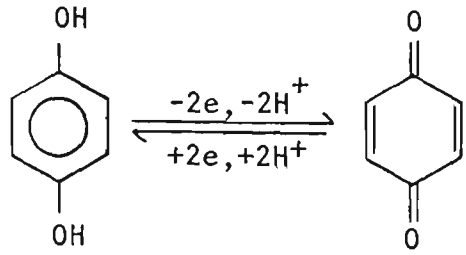
CHAPTER 3

The Electroanalytical Determination of Quinol and Quinone in Tetrahydrofuran and Ion-Selective Electrode Polymer Membranes

3.1 Introduction

The electrochemical behaviour of quinol has been utilized in the stabilization of many solvents and monomers,[222-223] photography[224] and pH sensitive electrodes.[225] Quinol is part of a reversible (fast electron transfer) electrochemical couple with quinone. (Fig. 3.1.1). The two components of the couple are water soluble and the redox reactions involve two electrons and two hydrogen ions.

FIG. 3.1.1 - THE STRUCTURES OF QUINOL AND QUINONE



QUINOL (*Hydroquinone*) *QUINONE* (*p-benzoquinone*)

Tetrahydrofuran (THF) is commonly stabilized with quinol to prevent formation of explosive peroxides by air oxidation.[222,226-228] Since quinol is easily oxidized it preferentially reacts with dissolved oxygen preventing oxidation of the solvent.

Ion-selective, polymer membrane, electrodes have been prepared by evaporation of THF.[229] In this process, when stabilized THF is used, quinol accumulates in the membranes. Investigations into the internal reference system of coated wire ion-selective electrodes required a knowledge of the residual quinol and quinone.

Therefore rapid, sensitive and reliable electrochemical methods for the determination of quinol and quinone in THF were developed. These served as preliminary studies for the analysis of the polymer membranes.

Modern electrochemical instrumentation and the range of electrode materials available enable the analyst to select from a wide variety of techniques. The following techniques were investigated at glassy carbon and carbon paste electrodes.

Voltammetry:	DC, AC and Differential Pulse
Amperometry:	Flow Injection Analysis (FIA) and Pulsed Rotation Amperometry (PRA)

The amperometric methods are faster than voltammetry and provide easier quantitation of the current signals. FIA uses small sample volumes. PRA provides the best sensitivity without loss in precision and is particularly useful for organic analytes in complex media.

3.2 Quinol and Quinone in Tetrahydrofuran

3.2.1 Voltammetric Behaviour in the Presence of THF

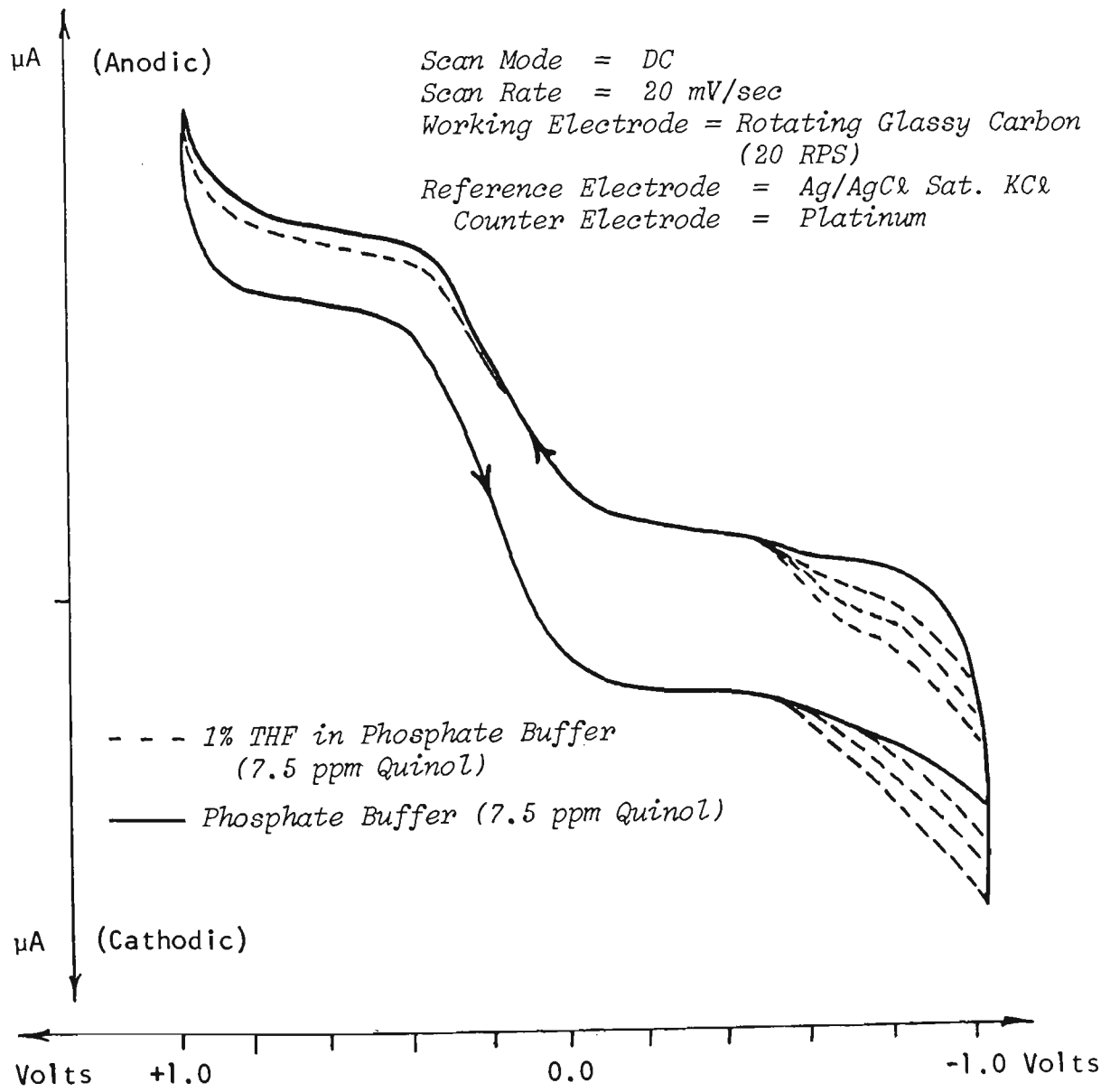
The effect of THF on the voltammetric behaviour of quinol and quinone in pH 7 phosphate buffer medium was investigated with cyclic voltammetry at a rotating glassy carbon disc electrode. Hydrodynamic conditions were employed so that the results would be applicable to PRA and FIA.

The concentration of THF and analytes used represent diluting stabilized THF one hundred-fold in buffer. The manufacturers label states that quinol is present at 0.1%.

Several voltammograms of quinol in buffer were recorded. The solution was adjusted to 1% THF by the addition of purified THF and the voltammograms recorded again. (See Fig. 3.2.1). The wave height for quinol was reduced in magnitude by 4% in the

presence of 1% THF after accounting for dilution. The half wave potential and potential region where the current is mass transport controlled were unaffected. Similar experiments were conducted with quinone and the same results were observed. Therefore close matching of sample and standard solutions is required with calibration curve quantitation. The method of standard additions provides an easier alternative, where calibration plots are linear and pass through the origin.

FIG. 3.2.1 - HYDRODYNAMIC CYCLIC VOLTAMMOGRAMS OF QUINOL WITH AND WITHOUT 1% THF



3.2.2 Survey of Voltammetric Analysis Techniques

Direct current (normal and test modes DC_N, DC_T), alternating current (AC) and differential pulse (DP) hydrodynamic voltammetry were surveyed to obtain current signal versus analyte concentration relationships. Each concentration was measured at two rotation speeds as a preliminary investigation into PRA. The hydrodynamic voltammetry also enables the selection of the detection potentials used in the amperometric techniques.

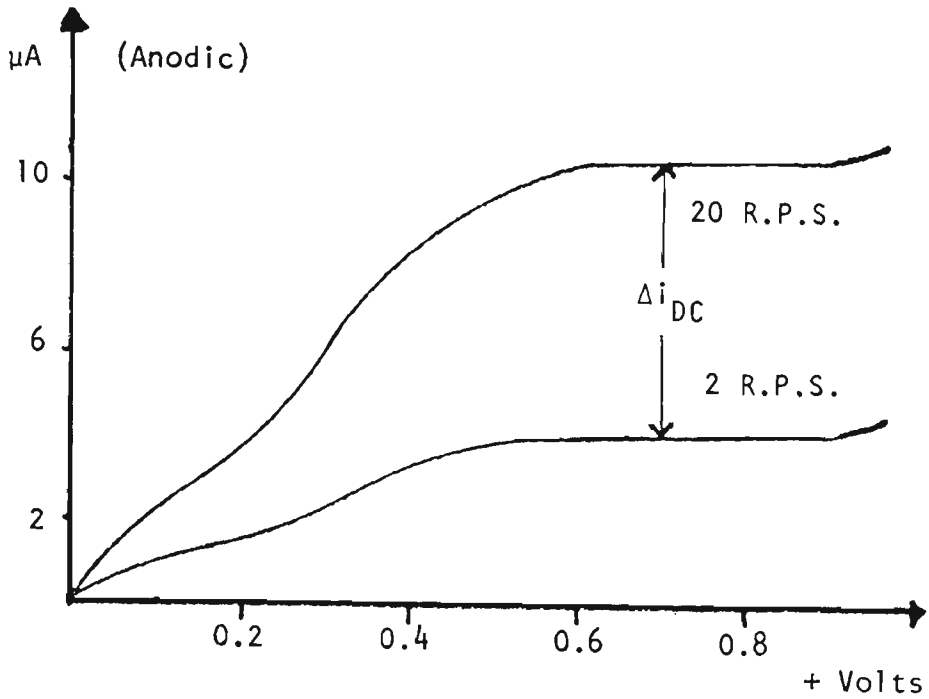
3.2.2.1 Rotating Glassy Carbon Electrode

The results are summarized in the diagrams labelled Fig. 3.2.2, Fig. 3.2.3, Fig. 3.2.4 and Fig. 3.2.5. The direct current techniques were the most precise. The difference in current (Δi_{DC}) at two rotation rates produced a linear response with the line passing through the origin, indicating PRA to be the optimum technique for analysis of quinol in THF, since standard additions can be used for quantitation. The amperometric technique has the fastest analysis time and best precision.

Differential Pulse and AC voltammetry at a rotating glassy carbon disc electrode used for the determination of quinol in THF have poor precision and offer no advantage over the DC methods.

In the differential pulse voltammetry experiments the current signal is larger for 2 R.P.S. than the signal for 20 R.P.S. The correlation coefficient is closer to unity for the data obtained at 20 r.p.s. than that obtained at 2 r.p.s.

FIG. 3.2.2 - DC_{NORMAL} ROTATING ELECTRODE VOLTAMMETRY



Amperometric Detection Potential + 0.6V

Conditions: Rotating Glassy Carbon Working Electrode
Ag/AgCl sat KCl Reference Electrode
Platinum Counter Electrode
[Quinol] = 3.1 ppm
Solvent = 1% THF in pH 7 Phosphate Buffer

Scan Mode : D.C. Normal
Scan Rate : 20 mV/sec.

DC_{NORMAL} VOLTAMMETRIC CALIBRATION CURVES

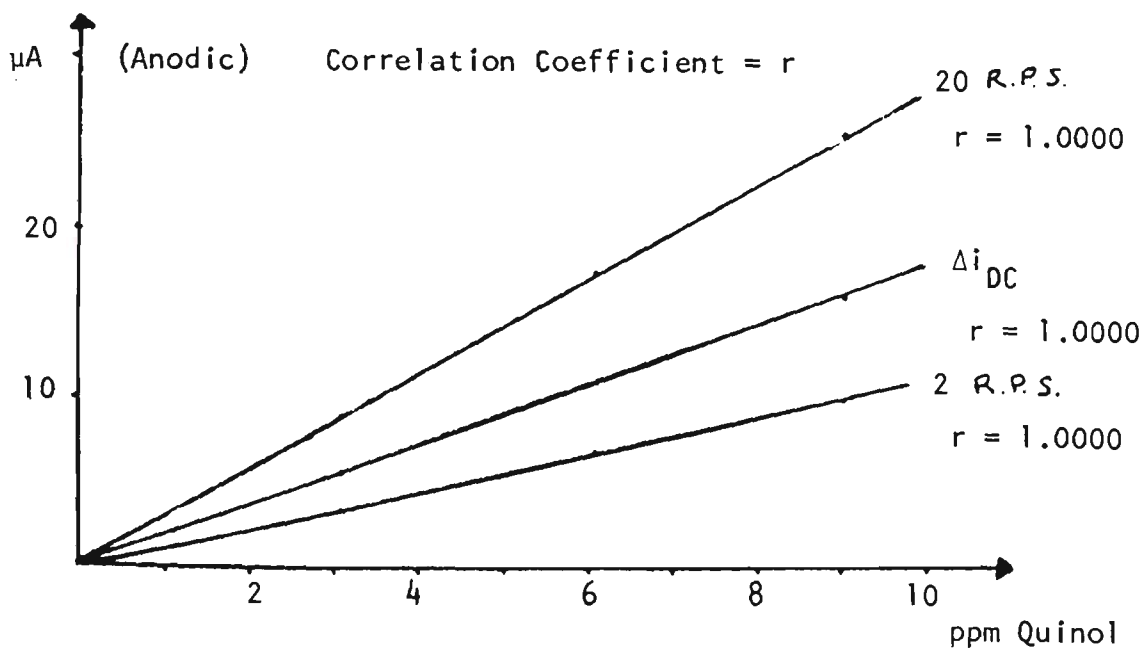


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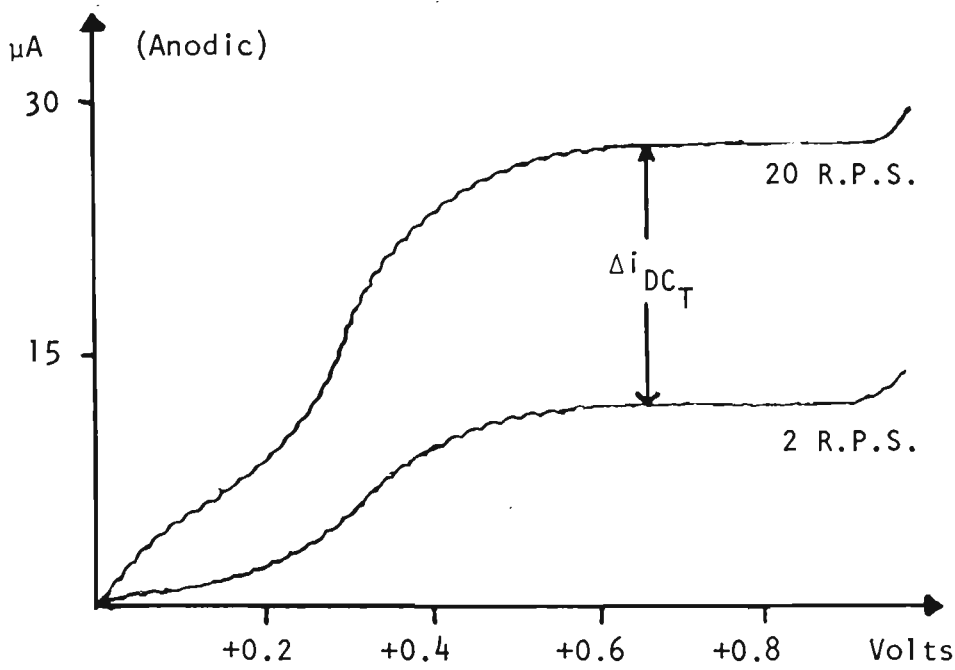
FIG. 3.2.3 - DC_{TAST} ROTATING ELECTRODE VOLTAMMETRY



Amperometric Detection Potential + 0.6V

Conditions: Rotating Glassy Carbon Working Electrode
Ag/AgCl sat KCl Reference Electrode
Platinum Counter Electrode
[Quinol] = 6.1 ppm
Solvent = 1% THF in pH 7 Phosphate Buffer
Scan Mode = DC_{TAST}
Scan Rate = 20 mV/sec.

DC_{TAST} VOLTAMMETRIC CALIBRATION CURVES

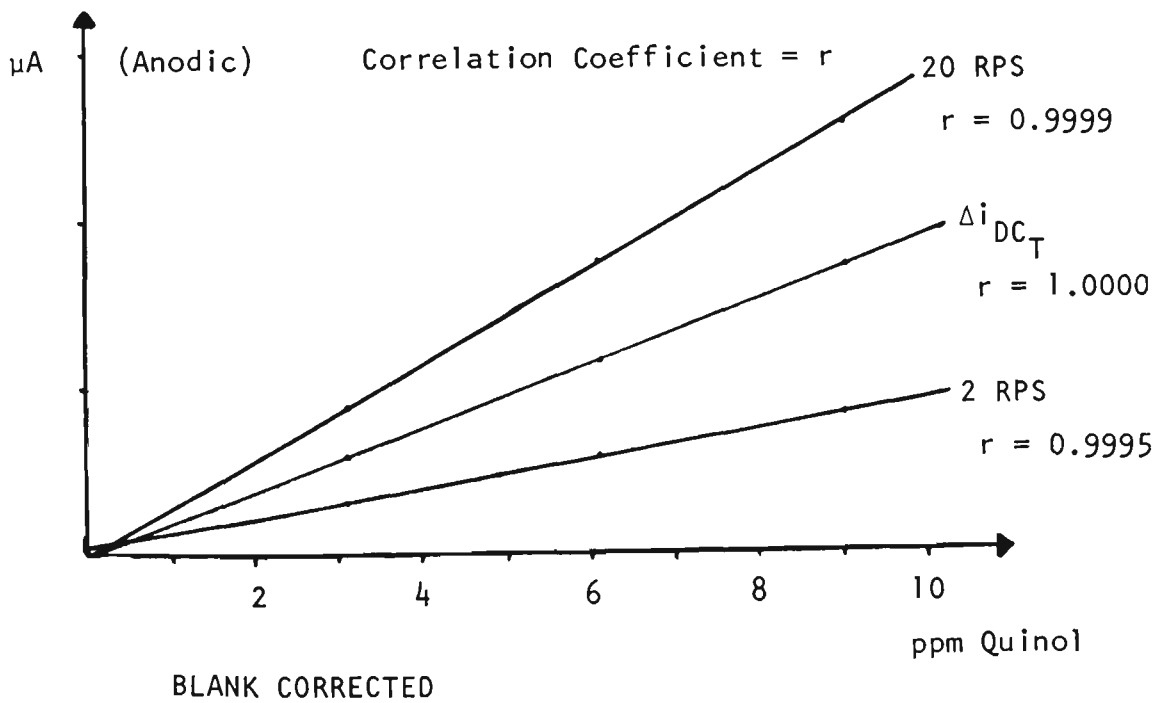


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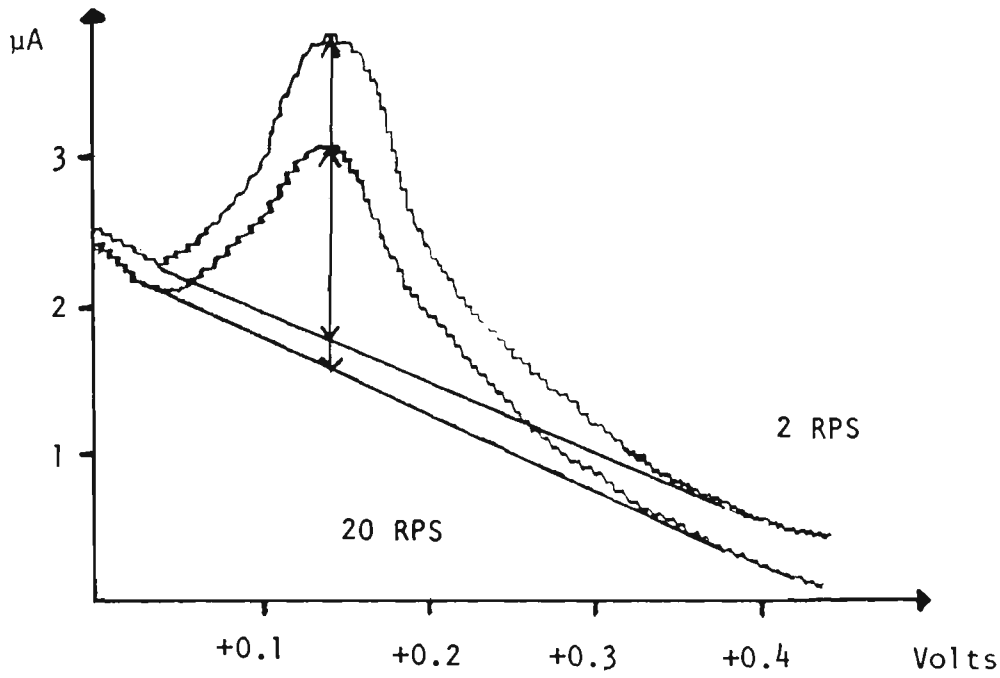
FIG. 3.2.4 - DIFFERENTIAL PULSE ROTATING ELECTRODE VOLTAMMETRY



Conditions: Rotating Glassy Carbon Working Electrode
Ag/AgCl sat KCl Reference Electrode
Platinum Counter Electrode
[Quinol] = 3.6 ppm
Solvent = 1% THF - pH 7 Phosphate Buffer
Scan Mode = Differential Pulse - +20 mV Pulse
Scan Rate = 20 mV/sec.

DIFFERENTIAL PULSE VOLTAMMETRIC CALIBRATION CURVES

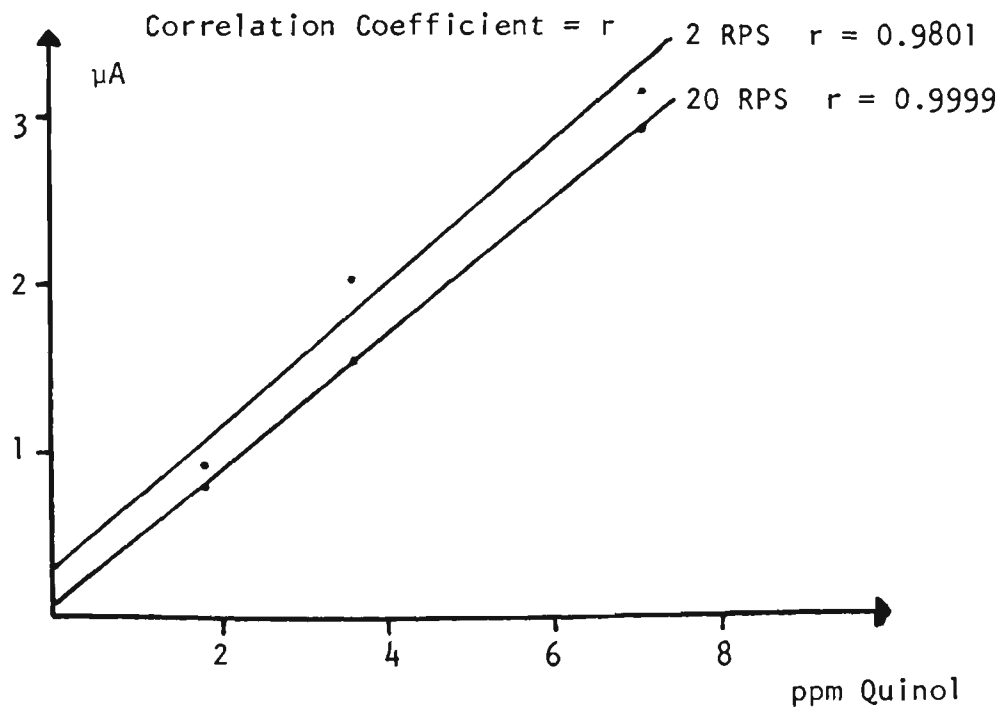
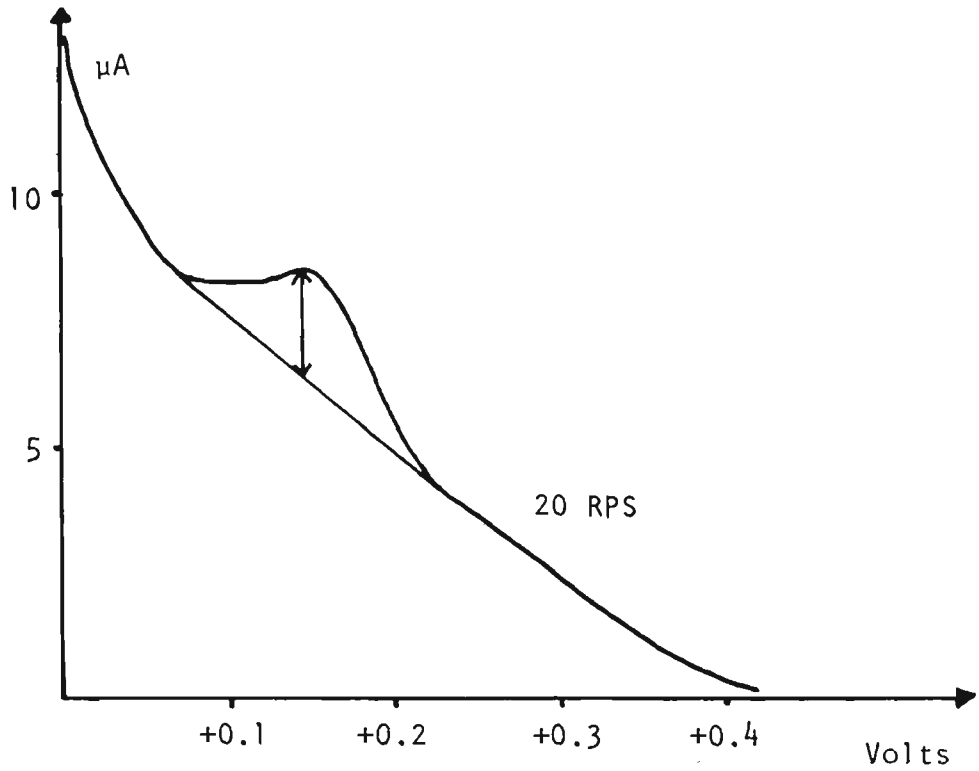


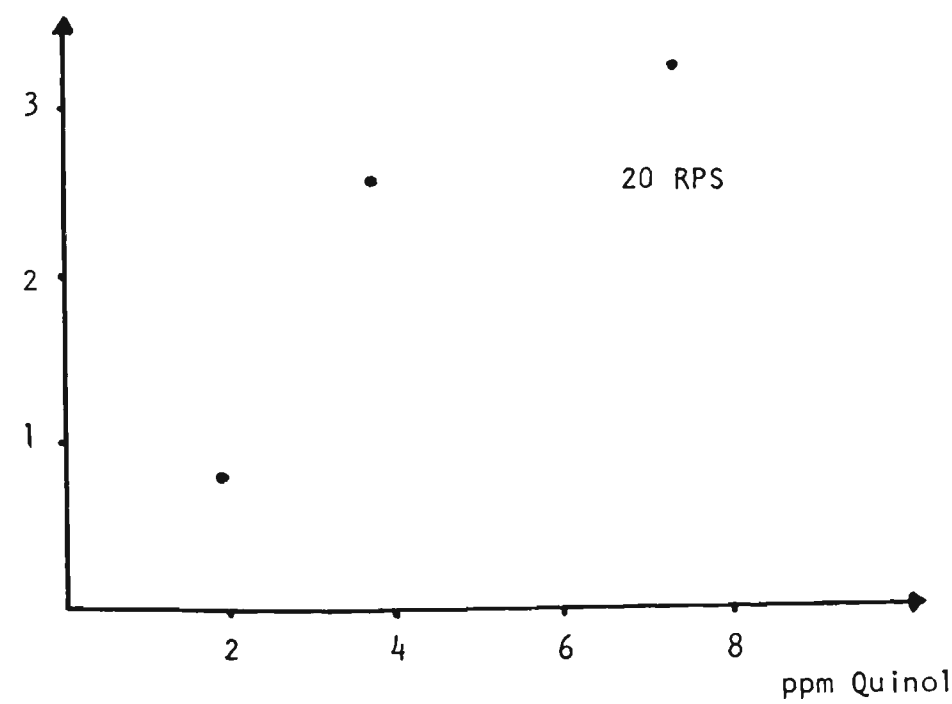
FIG. 3.2.5 - AC ROTATING ELECTRODE VOLTAMMETRY AT GCE



Conditions: Rotating Glassy Carbon Working Electrode
Ag/AgCl sat KCl Reference Electrode
Platinum Counter Electrode
[Quinol] = 3.6 ppm
Solvent = 1% THF - pH 7 Phosphate Buffer

Scan Mode = AC (1st. Harmonic - 5 mV AC)
Scan Rate = 20 mV/sec.

AC VOLTAMMETRIC CALIBRATION CURVES



In the AC voltammetric experiments insignificant change in signal was observed with changes in rotation rate. Also this technique gave the least analytically useful voltammograms.

3.2.2.2 THF and Carbon Paste

Carbon Paste is produced by blending paraffin oil with powdered carbon.[230] A 1% THF-phosphate buffer solution will slowly dissolve the paraffin in a carbon paste electrode. Therefore an experiment was carried out to determine whether the extent of this process would be significant during the time of analysis.

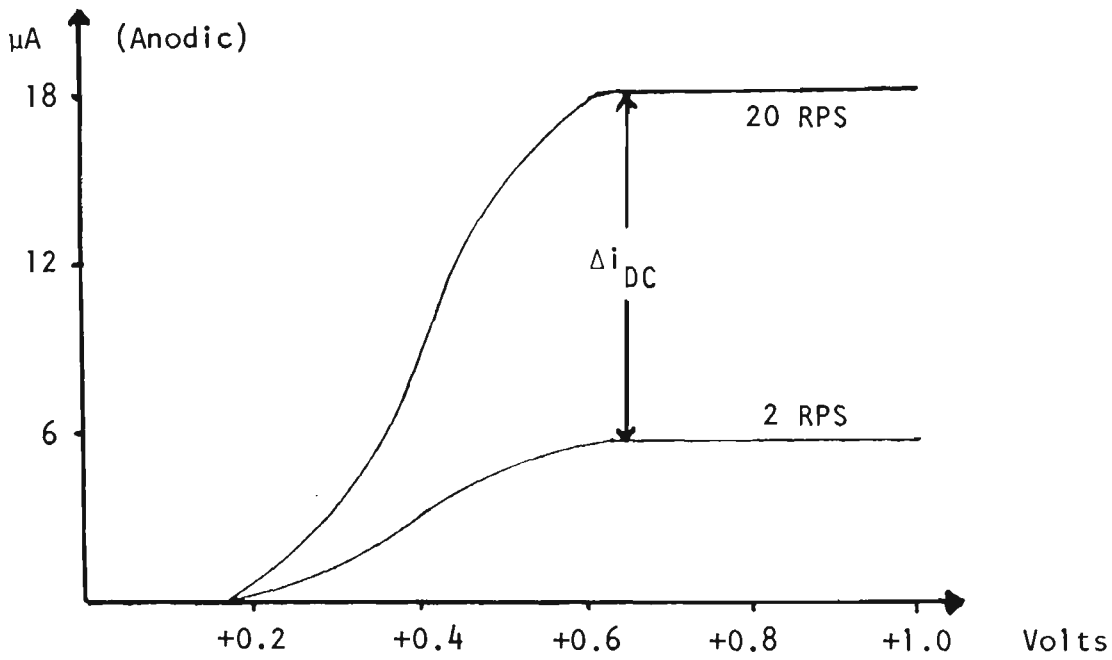
A DC hydrodynamic voltammogram of 7.1 ppm quinol in 1% THF phosphate buffer was recorded. Oxygen was prevented from entering the solution for 20 hours and the voltammogram was recorded again. Over this period there was less than 0.5% change in the wave height due to the oxidation of quinol. Therefore carbon paste electrodes are suitable for analysis in 1% THF solutions.

3.2.2.3 Rotating Carbon Paste Electrode

Direct current, differential pulse and alternating current voltammetry were investigated. The results are shown in the diagrams labelled Fig. 3.2.6, Fig. 3.2.7, Fig. 3.2.8 and Fig. 3.2.9.

All the techniques exhibit linear signal versus concentration

FIG. 3.2.6 - $DC_{(NORMAL)}$ ROTATING ELECTRODE VOLTAMMETRY AT CPE



Amperometric Detection Potential + 0.6V

Conditions: Rotating Carbon Paste Working Electrode
Ag/AgCl sat KCl Reference Electrode
Platinum Counter Electrode
[Quinol] = 5.5 ppm
Solvent = 1% THF - pH 7 Phosphate Buffer

Scan Mode = DC_{normal}
Scan Rate = 20 mV/sec

DC_{NORMAL} VOLTAMMETRIC CALIBRATION CURVES.

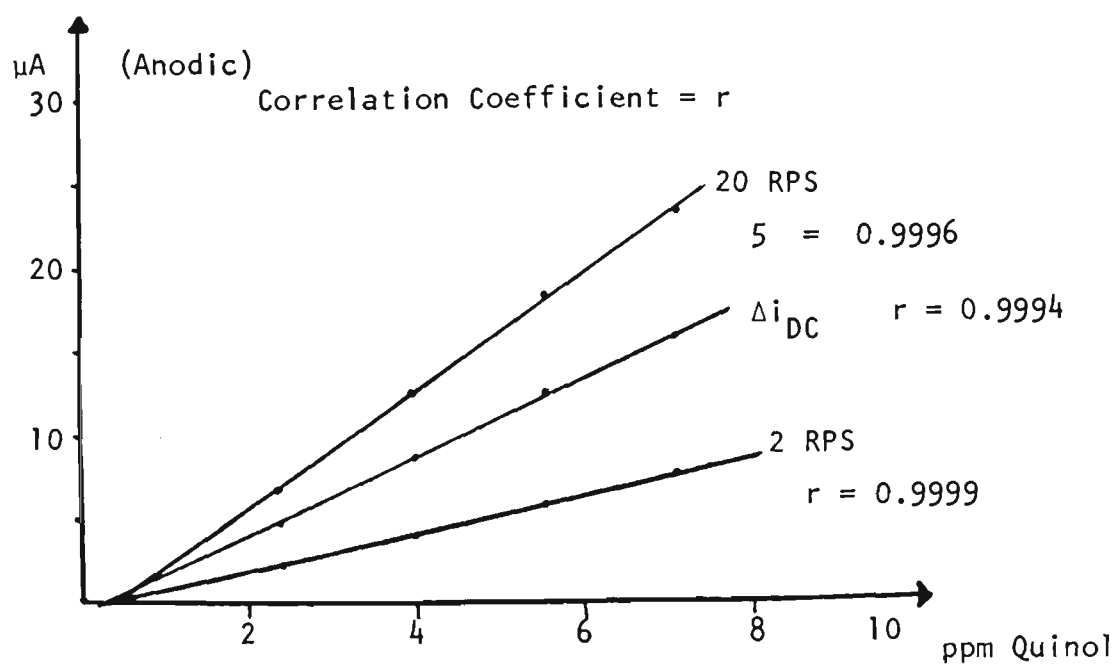
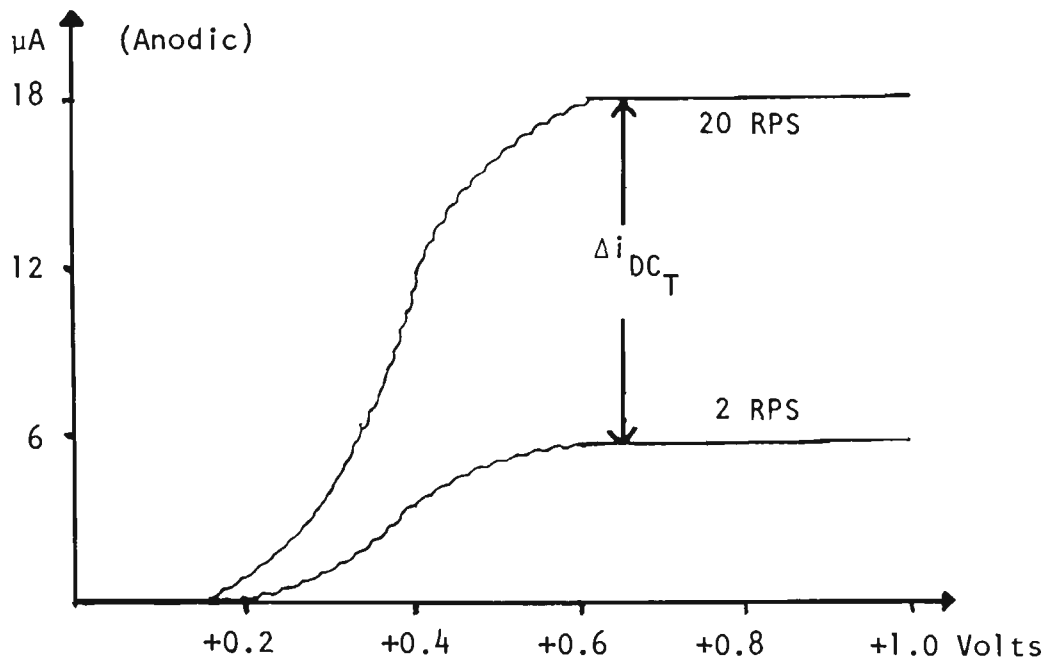


FIG. 3.2.7 - $DC_{(TAST)}$ ROTATING ELECTRODE VOLTAMMETRY AT CPE



AMPEROMETRIC DETECTION POTENTIAL = +0.6V

Conditions: Rotating Carbon Paste Working Electrode
Ag/AgCl sat KCl Reference Electrode
Platinum Counter Electrode
[Quinol] = 5.5 ppm
Solvent = 1% THF - pH 7 Phosphate Buffer
Scan Mode = $DC_{(TAST)}$
Scan Rate = 20 mV/sec.

DC_{TAST} VOLTAMMETRIC CALIBRATION CURVES

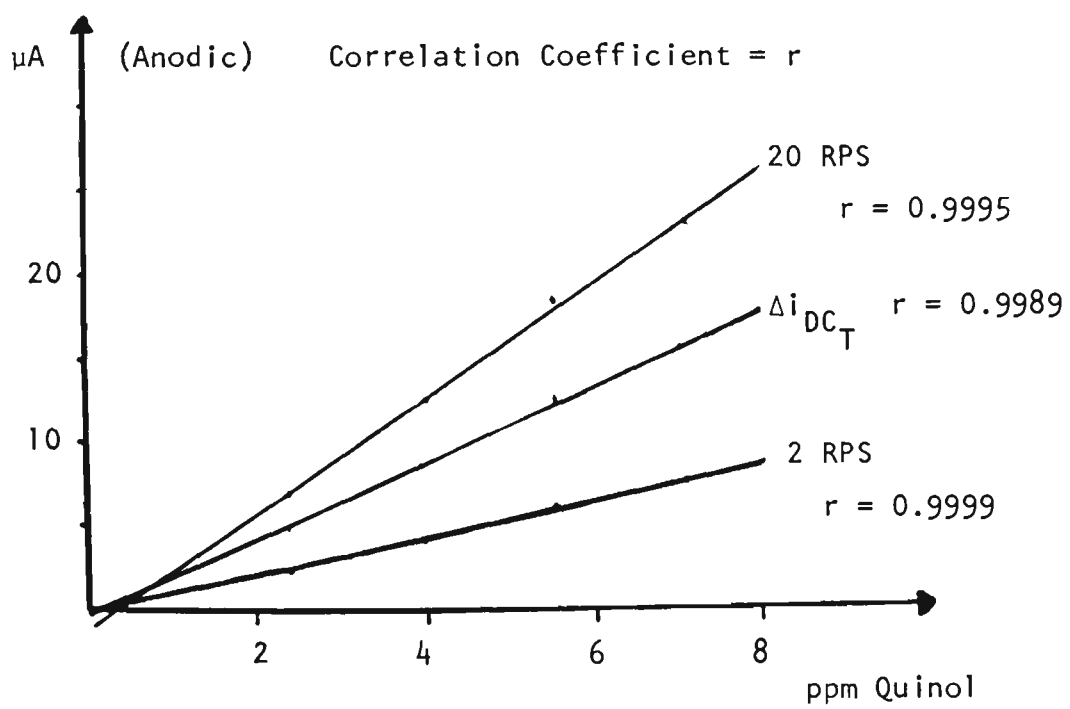
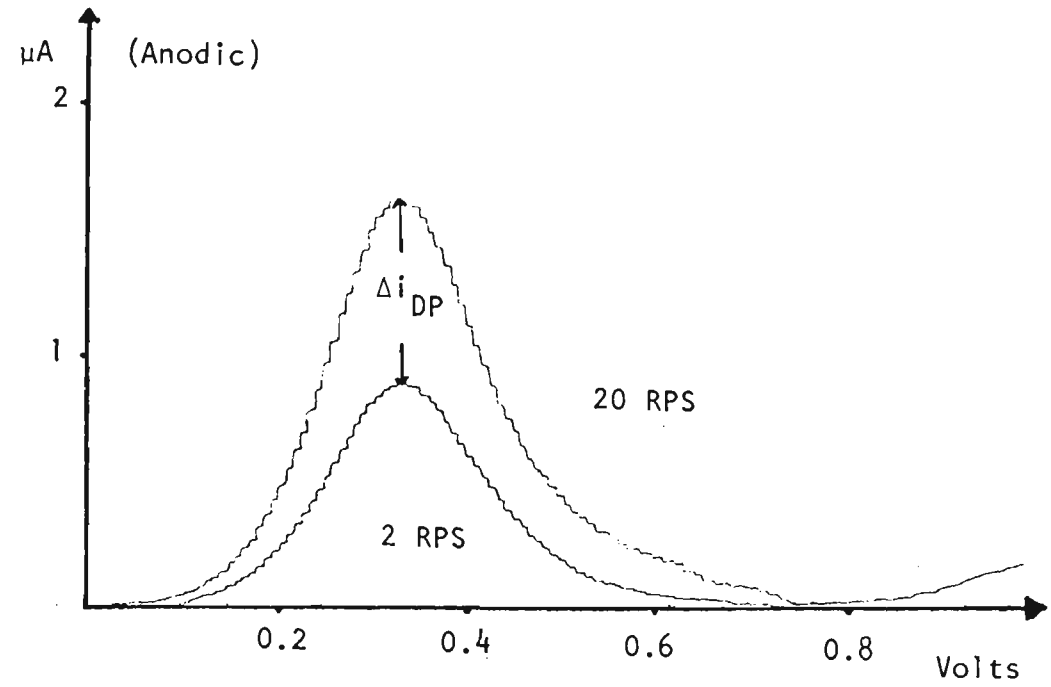


FIG. 3.2.8 - DIFFERENTIAL PULSE ROTATING ELECTRODE VOLAMMETRY AT CPE



AMPEROMETRIC DETECTION POTENTIAL + 0.36V

Conditions:

Rotating Carbon Paste Electrode
Ag/AgCl sat KCl Reference Electrode
Platinum Counter Electrode
[Quinol] = 2.4 ppm
Solvent = 1% THF - pH 7 Phosphate Buffer
Scan Mode = Differential Pulse (DP) - (+40 mV Pulse)
Scan Rate = 20 mV/sec.

DIFFERENTIAL PULSE VOLTAMMETRIC CALIBRATION CURVES

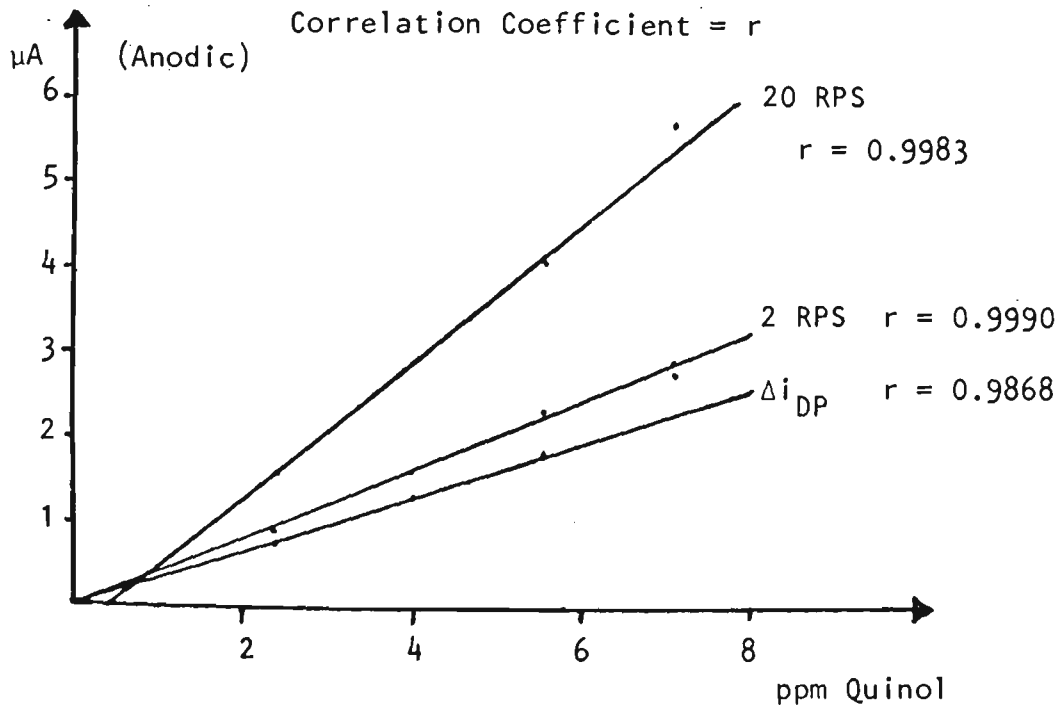
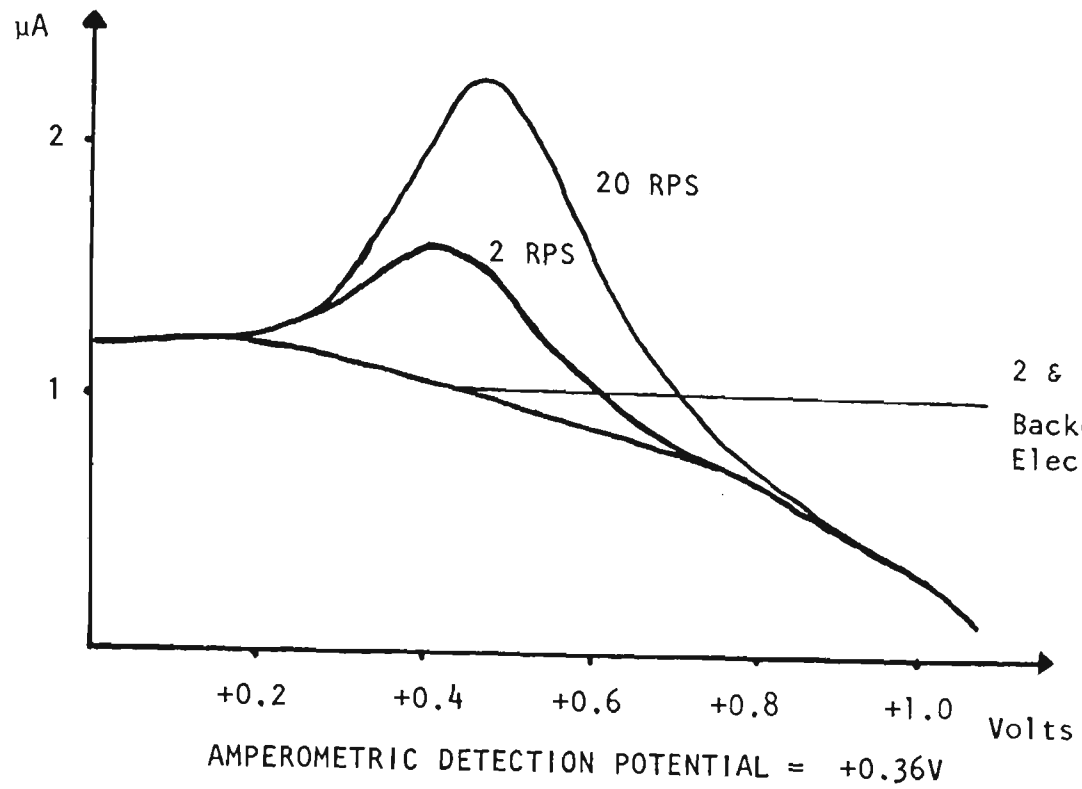
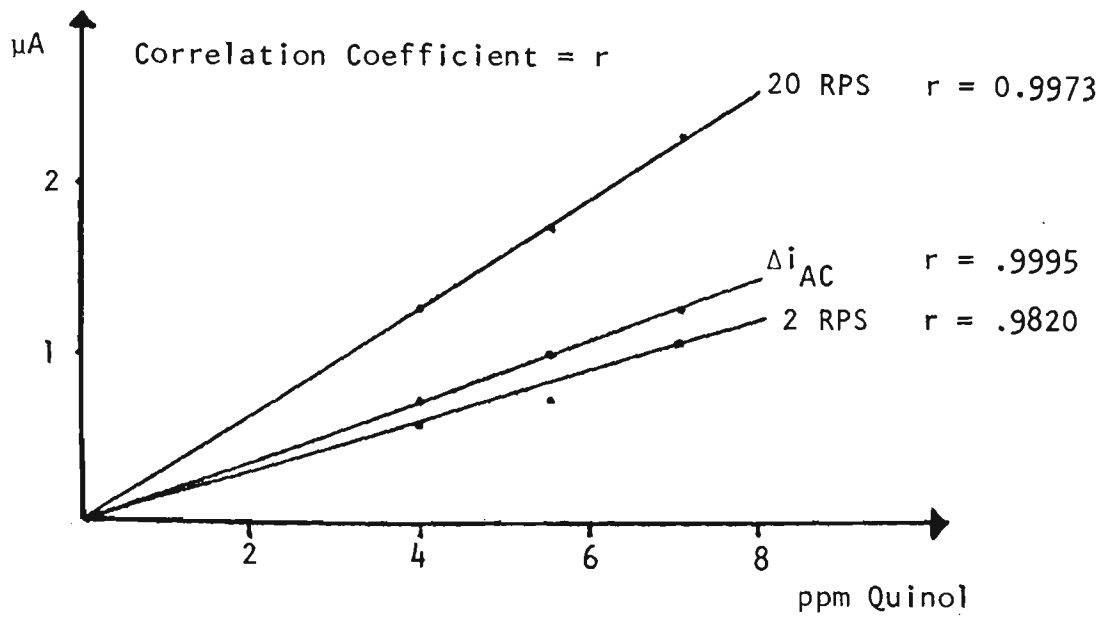


FIG. 3.2.9 - AC ROTATING ELECTRODE VOLTAMMETRY AT CPE



CONDITIONS: Rotating Carbon Paste Working Electrode
Ag/AgCl sat KCl Reference Electrode
Platinum Counter Electrode
[Quinol] = 4.0 ppm
Solvent = 1% THF - pH 7 Phosphate Buffer
Scan Mode = AC - (15 mV AC)
Scan Rate = 20 mV/sec.

AC VOLTAMMETRIC CALIBRATION CURVES.



plots. As was observed at the glassy carbon electrode, the direct current techniques gave the best correlation. The AC and Differential Pulse techniques were greatly improved at carbon paste with the peak current increasing with rotation rate which was not observed at glassy carbon. This behaviour highlights the different catalytic properties of the two carbon surfaces. The good AC results indicate a high exchange current density (i.e., fast kinetics) for quinol at carbon paste and that at glassy carbon the kinetics are slow.

The difference current, Δi, gave linear plots passing through the origin indicating PRA to be the optimum technique for quinol analysis. Therefore PRA was investigated for the determination of quinol in 1% THF-phosphate buffer at both carbon paste and glassy carbon electrodes.

3.2.3 PRA of Quinol

The PRA analysis of quinol was investigated at the CPE and the GCE using DC (for both electrodes), AC and DP (for CPE only) detection modes at the detection potentials determined by the voltammetry in section 3.2.2.3. Typical amperometric results for each detection mode are shown in Fig. 3.2.10 and Fig. 3.2.11. Least squares analysis and correlation data are presented in Table 3.2.1. The DC techniques at both electrodes offer the best precision. Correlation coefficients are optimum with the glassy carbon electrode while carbon paste offers the highest sensitivity. Employing the maximum rotation rates, provided by the rotating electrode assembly (10 → 100 RPS), the PRA detection limit for quinol was found to be 0.4 ± 0.1 parts per billion (ng/ml). The increase in sensitivity was found to be in agreement with the

Levich equation since the current increased as a function of the square root of the rotation rate.

To minimize the chance of an unknown compound in the sample matrix interfering in the analysis of real samples, a detection potential of +0.36 V was investigated. The results are presented in Table 3.2.2.

Although this potential is not in the region where the current is limited by transport, it is positive enough to control the current and good PRA results were obtained. This practice must be exercized with caution because processes that affect the kinetics of the reaction, such as adsorption, will alter the PRA results. Voltammetry provides a means of checking for such processes and sample voltammograms must be recorded and inspected before using PRA for quantitation. Where non transport limited detection potentials were used in the following analyses, sample voltammograms were checked for such behaviour prior to PRA analysis.

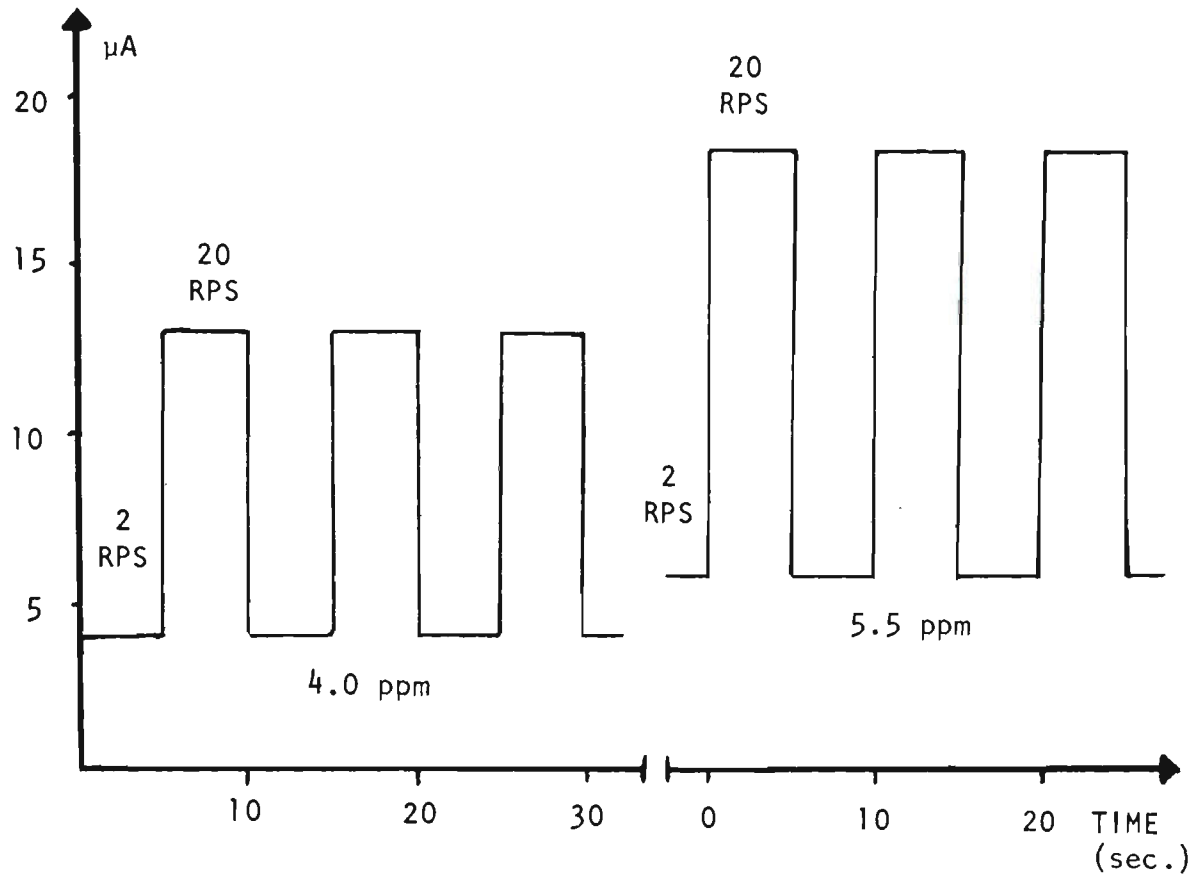
PRA with DC detection is rapid, precise, sensitive and as selective as other electrochemical methods. Since calibration curves pass through the origin, standard additions may be used for quantitation.

3.2.4 Quinol in THF by PRA

Two bottles of AR grade, BDH, Tetrahydrofuran were chosen as samples and their quinol level determined by PRA and UV spectrophotometry. A glassy carbon disc and a potential of +0.36 V were employed.

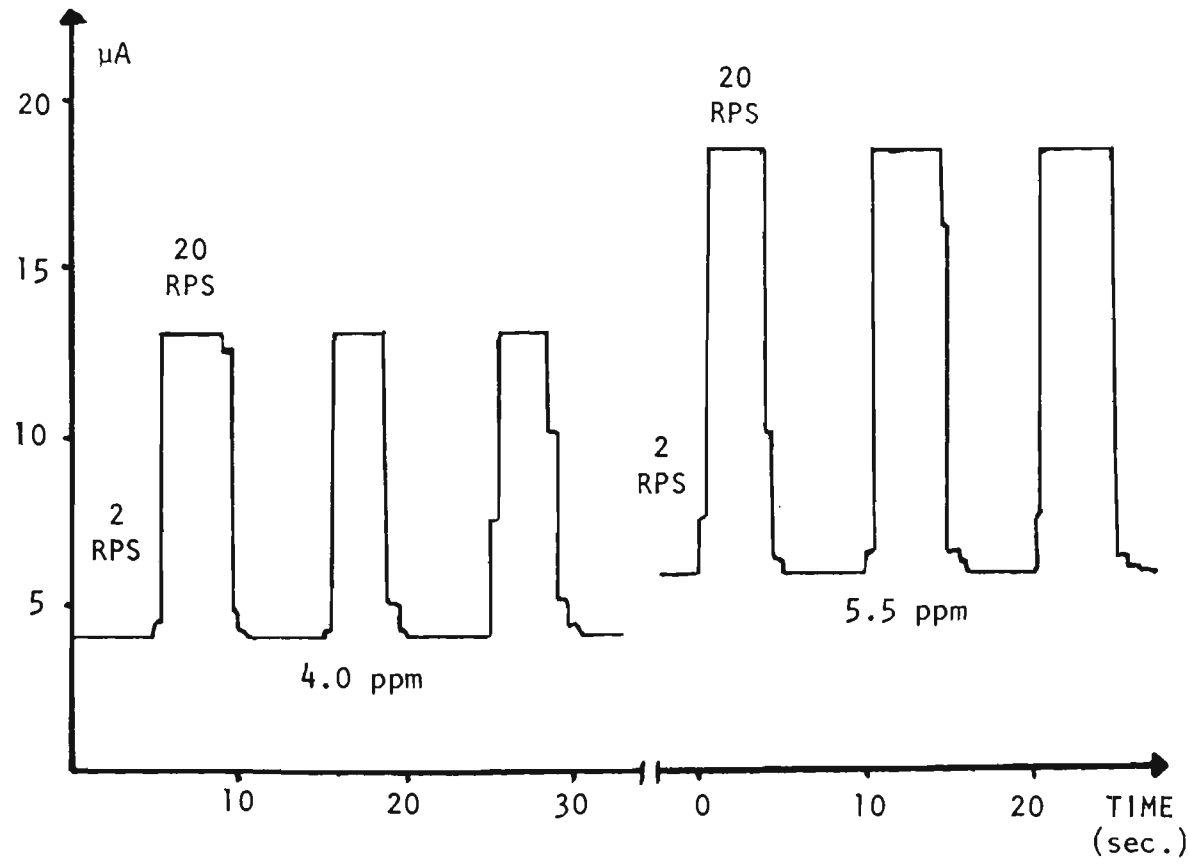
The results are shown in Table 3.2.3.

FIG. 3.2.10 - PRA OF QUINOL: DC_{NORMAL}, DC_{TAST} AMPEROMETRIC RESULTS



DETECTION: DC_{NORMAL} +0.6V vs. Ag/AgCl sat KCl

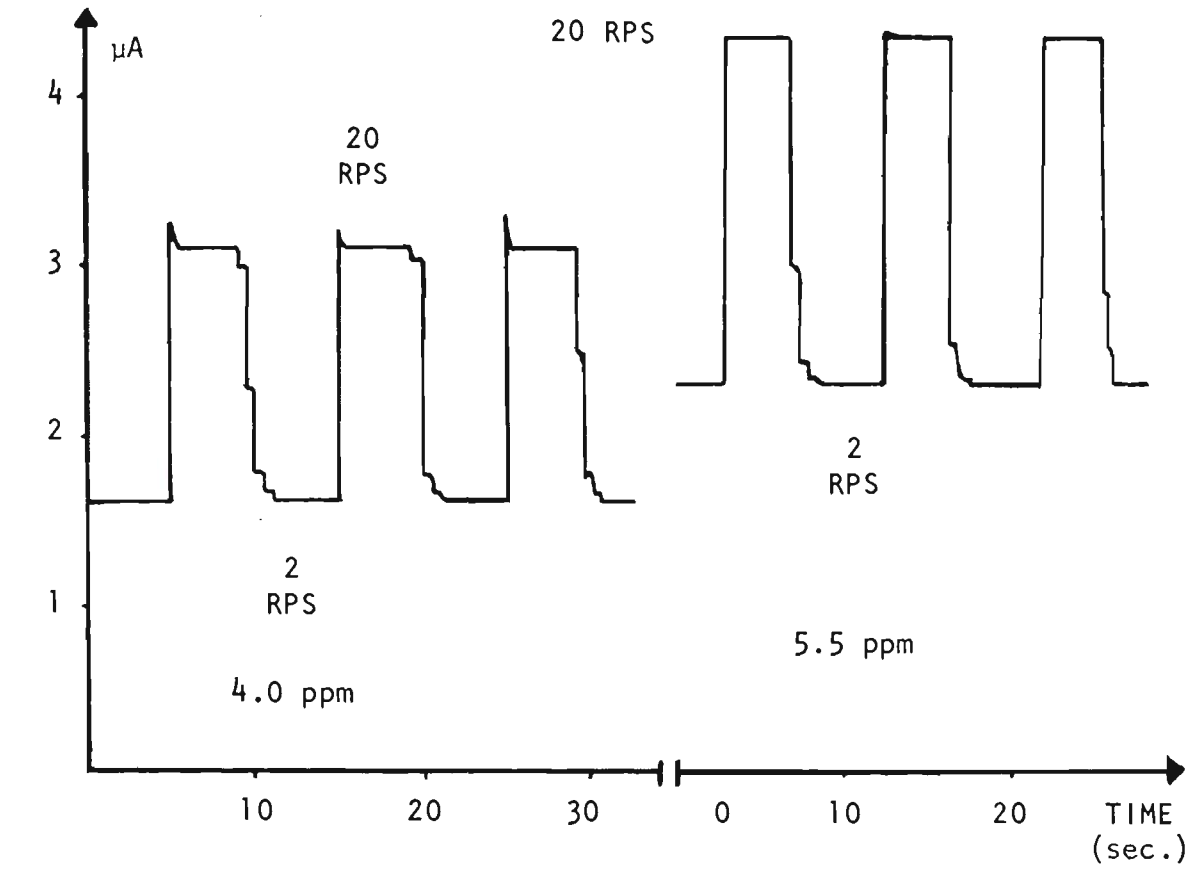
1% THF - pH 7 Phosphate Buffer



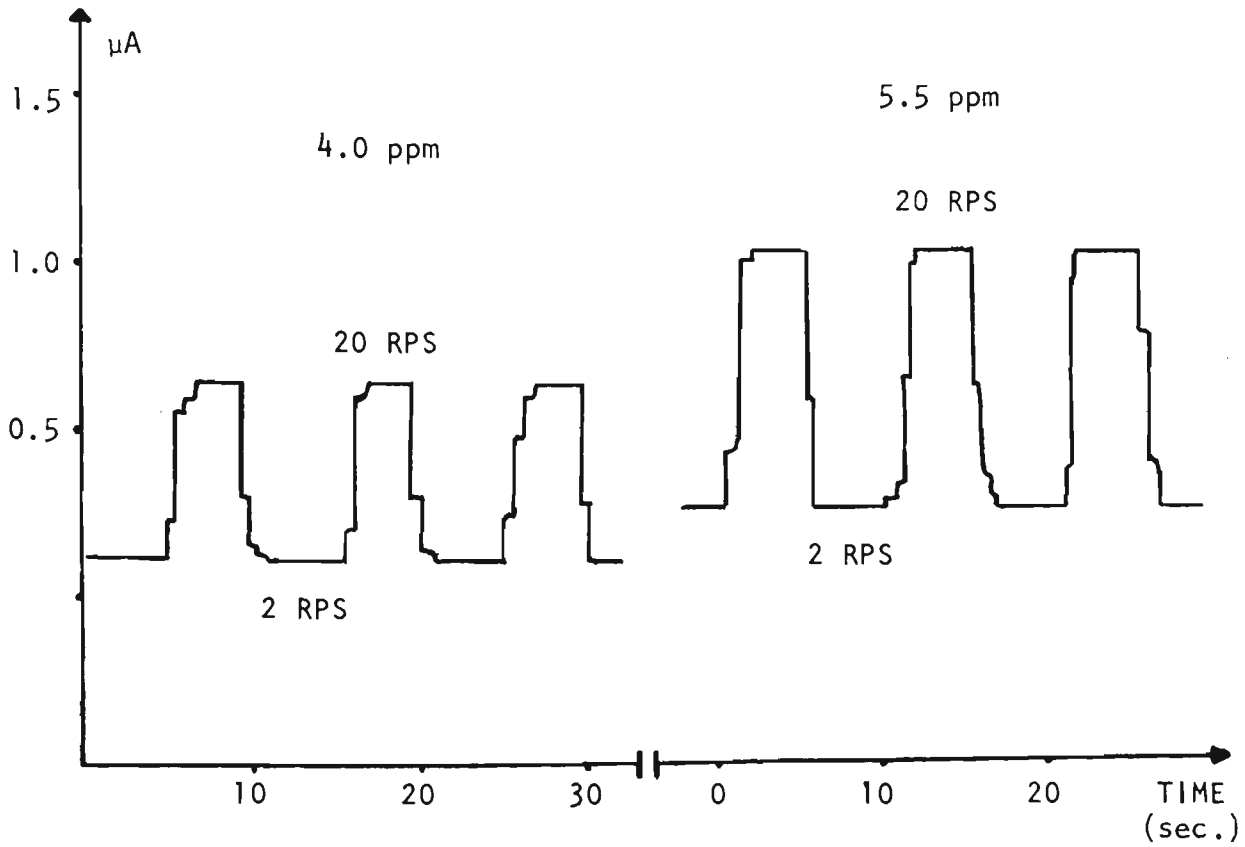
DETECTION: DC_{TAST} +0.6V vs. Ag/AgCl sat KCl

1% THF - pH 7 Phosphate Buffer

FIG. 3.2.11 - PRA OF QUINOL: DP, AC AMPEROMETRIC RESULTS



DETECTION: DP (+40 mV Pulse) +0.36V
1% THF - pH 7 Phosphate Buffer



DETECTION: AC (15mV AC) +0.36V
1% THF - pH 7 Phosphate Buffer

TABLE 3.2.1 - Statistical Analysis of the Relationship between P.R.A. Current and Quinol Concentration

P.R.A. DETECTION MODE AND POTENTIAL vs. Ag/AgCℓ SAT. KCℓ	LEAST SQUARES ANALYSIS Current (I) = S x Concentration (C)ppm (μA) + B	CORRELATION COEFFICIENT r	PRECISION OF P.R.A. STEP HEIGHT R.S.D.
ROTATING CARBON PASTE ELECTRODE			
DC_{NORMAL} + 0.60 V	I = 2.40 C + 0	r = 0.9995	0.1%
DC_{TAST} + 0.60 V	I = 2.38 C + 0	r = 0.9994	0.1%
DP(+ 40 mV Pulse) + 0.36 V	I = 0.34 C + 0	r = 0.9961	1.0%
AC(15 mV AC) + 0.36 V	I = 0.09 C + 0.2	r = 0.9837	2.0%
ROTATING GLASSY CARBON ELECTRODE			
DC_{NORMAL} + 0.60 V	I = 1.86 C + 0	r = 1.000	0.1%
DC_{TAST} + 0.60 V	I = 1.85 C + 0	r = 1.000	0.1%

TABLE 3.2.2 - Quinol P.R.A. Results with Detection at +0.36 Volts

ELECTRODE MATERIAL	P.R.A. DETECTION MODE	LEAST SQUARES ANALYSIS Signal (I) = S x Conc. (C) + B (mm) (ppm)	CORRELATION COEFFICIENT r	PRECISION P.R.A. STEP HEIGHT R.S.D
CARBON PASTE	DC_{NORMAL}	I = 7.97 C + 0	r = 0.9999	0.1%
	DC_{TAST}	I = 7.89 C + 0	r = 0.9999	0.1%
GLASSY CARBON	DC_{NORMAL}	I = 8.66 C + 0	r = 0.9999	0.1%
	DC_{TAST}	I = 8.62 C + 0	r = 0.9998	0.1%

CONDITIONS:
P.R.A. 2 → 20 RPS
1% THF - pH 7 PHOSPHATE BUFFER
QUINOL CONCENTRATION RANGE 1-10 ppm
Ag/AgCℓ SAT. KCℓ REFERENCE ELECTRODE

TABLE 3.2.3 - PRA and UV Results for Quinol in THF

A.R. BDH Tetrahydrofuran Bottle No. 6615870			
Quantitation Technique	Detection Mode		
	PRA DC_{NORMAL}	PRA DC_{TAST}	UV
Computer Calculation ppm in THF	263 ± 7	263 ± 8	257 ± 12
Graphical Calculation ppm in THF	255	256	-
Manual Calculation ppm in THF	257	260	-
A.R. BDH Tetrahydrofuran Bottle No. 6216180			
Quantitation Technique	Detection Mode		
	PRA DC_{NORMAL}	PRA DC_{TAST}	UV
Computer Calculation ppm in THF	216.2 ± 0.2	215 ± 1	221 ± 12
Graphical Calculation ppm in THF	215	215	-
Manual Calculation ppm in THF	216	216	-

Quantitation was achieved by standard additions and computer calculation of results. As a check on the validity of the calculation program, the quinol levels were determined graphically and by a calculation described in the Metrohm E506 Polarecord manual. (see Appendix 4).

Good agreement was obtained between PRA and UV spectrophotometry. All the calculation techniques investigated are valid means of quantitation.

The difference in results between the two samples corresponds to the time of exposure to air, with the sample containing 216 ppm being opened a few weeks prior to the other.

The difference in precision observed for the PRA results of the two samples was coincidental. The RSD for PRA analyses of quinol was found to be <3% in all samples whereas the RSD for the UV technique was found to be 5%.

3.2.5 Quinol in THF by FIA

FIA offers advantage over conventional analysis techniques due to its speed, small sample volumes and low reagent consumption.[231] Where fast, accurate, inexpensive and reliable determinations of stabilizer in THF are required, FIA is an ideal technique. Therefore a FIA method with electrochemical detection was developed and its results compared with UV spectrophotometry. This required investigating and optimizing the following parameters:

(a) the detection potential,
(b) the dependence of FIA peak height on concentration of quinol,
(c) the sensitivity of the FIA system,

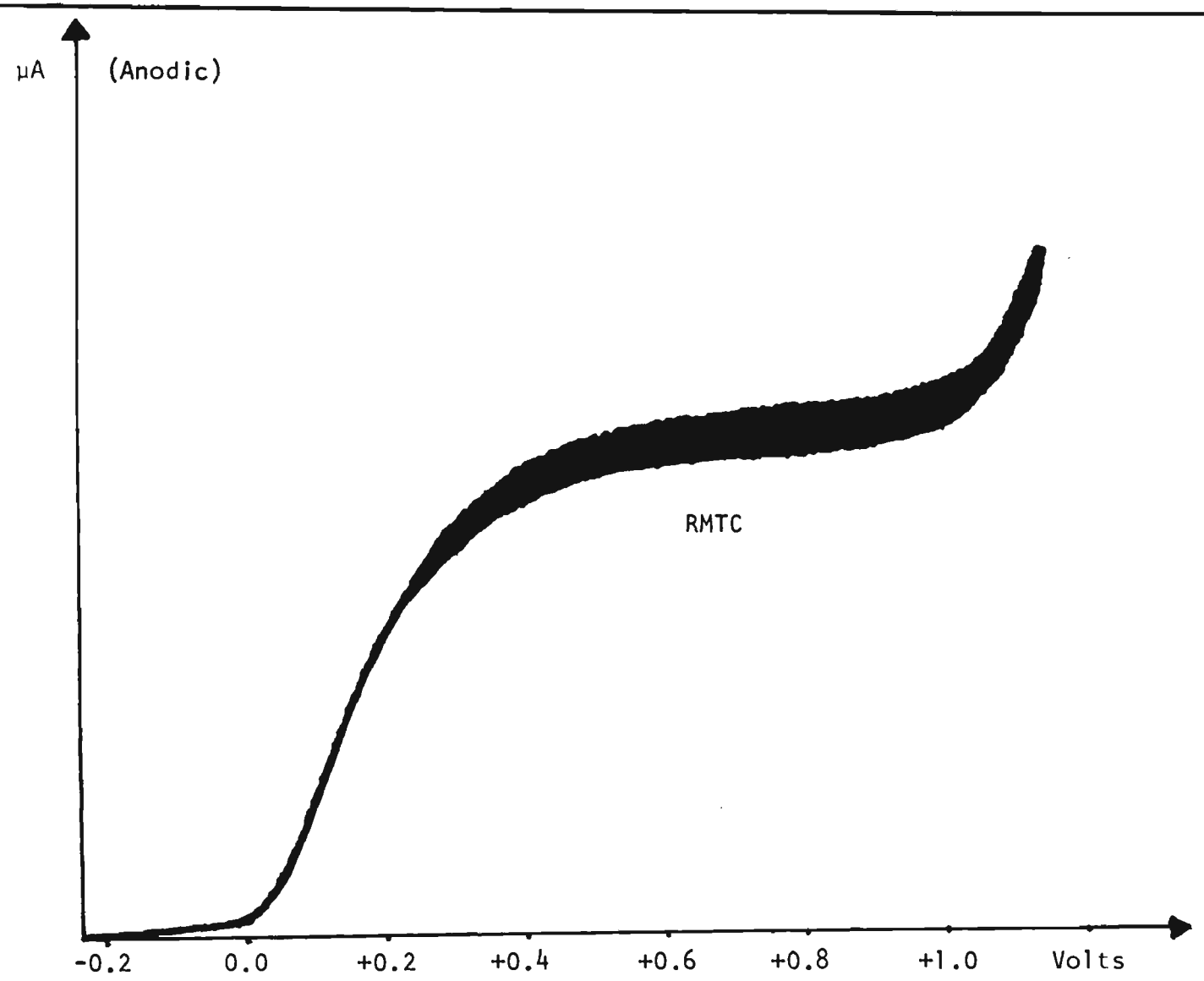


FIG. 3.2.12 - HYDRODYNAMIC VOLTAMMETRY OF QUINOL AT A GLASSY CARBON ELECTRODE

Conditions:

Flow through electrochemical detector - Wall Jet configuration.
Glassy Carbon working electrode.
Ag/AgCl sat KCl - reference electrode.
Stainless Steel - counter electrode.

Flow Rate = 1.0 ml/min
Scan Rate = 20 mV/sec
Background Electrolyte
pH 7 Phosphate Buffer
Deoxygenated with N_2

[QUINOL] = 10 ppm

RMTC = Region of Mass Transport Control

(d) the precision of measurements, and
(e) the accuracy of the technique,

before the analysis of THF samples.

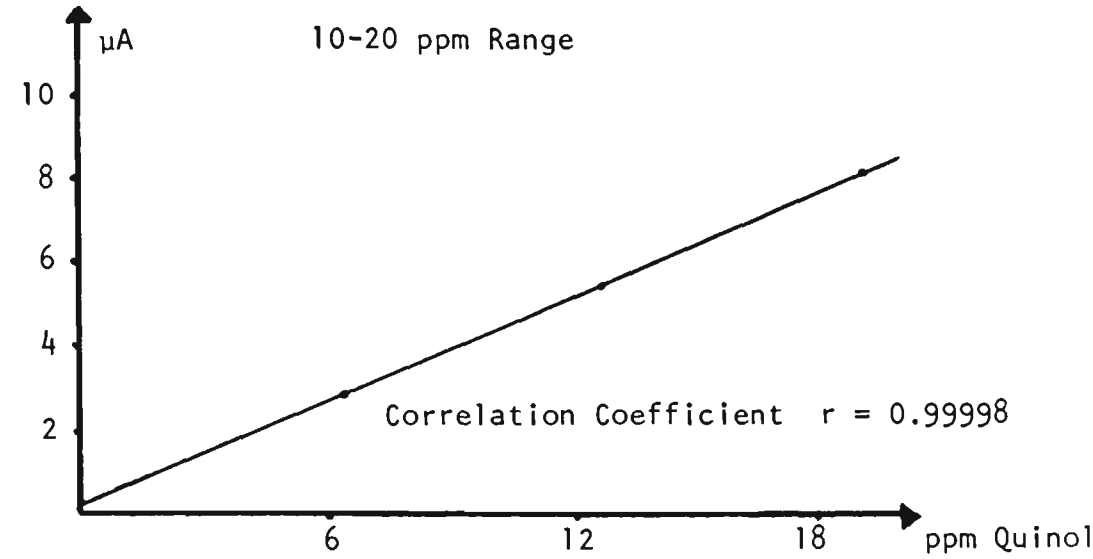
3.2.5.1 Detection Potential

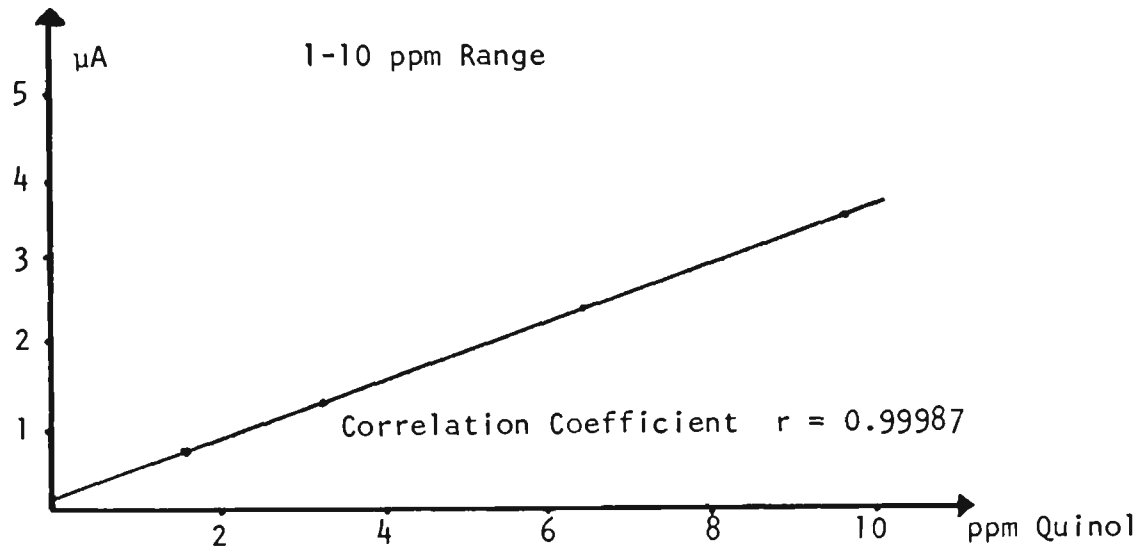
A detection potential of 0.62 volts versus an Ag/AgCl saturated KCl reference was selected after investigation of the hydrodynamic voltammogram recorded at a glassy carbon electrode in the wall jet electrochemical flow cell. The voltammogram is shown in Fig. 3.2.12. The potential chosen (=0.62V) is at the least positive end of the potential region where the current is mass transport controlled. This potential provides the best selectivity without a reduction in sensitivity. The choice of more anodic potentials would not provide extra sensitivity due to transport limitations. It would, however, allow detection of compounds with oxidation potentials in the range greater than quinol's and less than or equal to the potential chosen.

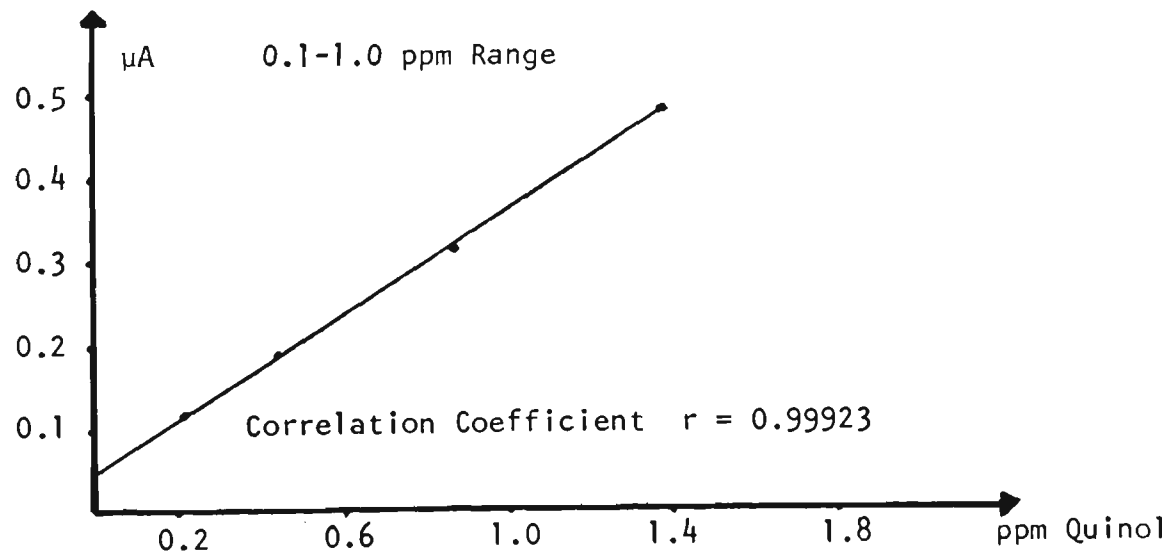
3.2.5.2 FIA Behaviour of Quinol

The FIA peak height (anodic current) is linearly dependent on quinol concentration with calibration curves exhibiting a positive intercept. (Fig. 3.2.13). The source of the intercept is unknown but it may be due to changes in the charging current (baseline in FIA with amperometric detection) between the carrier stream and the FIA sample bolus. Quantitation by calibration curve is necessary because the intercept excludes the use of standard additions.

FIG. 3.2.13 - FIA PEAK HEIGHT vs. QUINOL CONCENTRATION



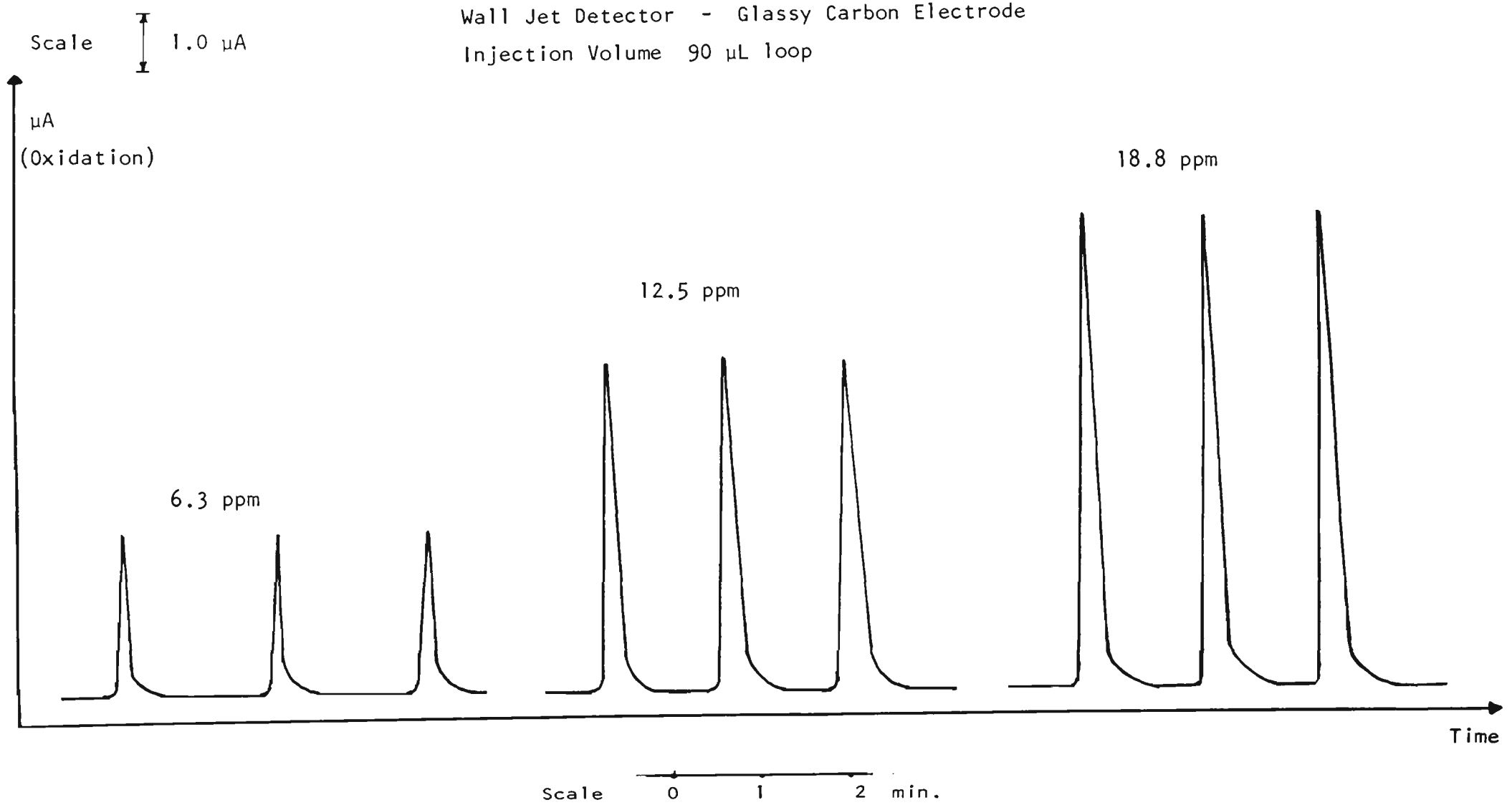




FIA Conditions: Flow Rate 1.0 ml/min - pH Phosphate Buffer
Detection Potential + 0.62V vs Ag/AgCl sat KCl
Glassy Carbon Electrode - Wall Jet
Buffer Deaerated with Nitrogen

FIG. 3.2.14 - TYPICAL FIA PEAKS—QUINOL

Conditions: Flow Rate 1.0 ml/min pH 7 Phosphate Buffer
Amperometric Detection Potential = 0.62V vs Ag/AgCl sat KCl
Wall Jet Detector - Glassy Carbon Electrode
Injection Volume 90 µL loop



Typical FIA peaks are shown in Fig. 3.2.14. The precision of the results decreases with decreasing concentration as shown in Table 3.2.4.

TABLE 3.2.4 - The Variation of Precision with Concentration

Uncertainty (RSD %)	Concentration (p.p.m.)
0.5	25.0
1.6	10.0
2.1	1.0
3.1	0.2
10-20*	0.02#

** At this concentration precision varies from day to day*

 # 0.02 ppm is the detection limit for quinol with a flow rate of 1.0 ml/min.

A detection limit of 5 ppb was obtained when conditions of maximum sensitivity were employed. This is achieved by minimizing dispersion of the sample bolus in the flowing stream and thus avoiding dilution. The dispersion is kept low by employing a combination of a short line length between injector and detector (5 cm) and a large injection volume (90 µL). Also the carrier stream flow rate was at a maximum (4.5 ml/min.) since the current is proportional to the rate of mass transfer to the electrode surface, especially with a wall jet detector.

3.2.5.3 Comparison of FIA with UV Spectrophotometry

The accuracy of the FIA technique was confirmed by comparison with UV spectrophotometry. Phosphate buffer samples of unknown quinol concentration were analyzed by both techniques and the results are presented in Table 3.2.5.

TABLE 3.2.5 - FIA and UV Analysis of Quinol

FIA Sample Concentration (p.p.m.)	UV Sample concentration (p.p.m.)
0.63	0.59
7.81	7.90

The analysis time for FIA was 5 minutes while that for UV spectrophotometry was 15 minutes. This difference is due to the time taken for the rinsing of the UV cells between measurements whereas in FIA the flowing stream rinses the electrode in situ. The analysis time for UV analyses would be comparable if an FIA technique with UV detection was employed.

3.2.5.4 THF Samples

Three samples of THF were analyzed for quinol by FIA after dilution one hundred-fold in pH 7 phosphate buffer. To eliminate the effect of THF in the injected samples, a carrier stream of phosphate buffer containing 1% purified THF was employed. The THF samples were also analyzed by UV spectrophotometry and the results of both techniques are shown in Table 3.2.6.

Similar precision to the PRA analysis was obtained. The RSD = 3% for FIA and RSD = 5% for UV spectrophotometry.

3.2.6 Quinone in THF

The label on the THF bottles states that 0.1% (1000 ppm) quinol is added as stabilizer. The amounts of quinol detected

TABLE 3.2.6 - FIA and UV Results for Quinol in THF

THF Sample	FIA [Quinol] p.p.m.	UV [Quinol] p.p.m.
A	281 ± 5	290 ± 13
B	265 ± 6	261 ± 12
C	210 ± 6	215 ± 12

A = Unopened bottle of THF.

B = Bottle opened for 1 week.

C = Bottle opened for 3 weeks.

(ca. 200-300 ppm) in the THF samples suggest that comparable levels of quinone would be present. Although quinone levels in THF are not important in preventing the oxidation of THF, the analysis of ISE membranes required the detection of quinone in the presence of THF. Therefore quinone was determined in THF by FIA and PRA to test the suitability of the techniques for the analysis of the electrode membranes.

3.2.6.1 Quinone FIA

Hydrodynamic voltammetry at a glassy carbon disc incorporated in a wall jet detector cell, was used to determine the FIA detection potential. In pH 7 phosphate buffer, a plateau region, where the current is mass transport controlled, was observed between -0.15 and -0.60 volts. In 1% THF-phosphate buffer the region was between -0.15 and -0.25 volts. To maximize the selectivity, -0.15 volts was chosen for detection. The decrease in the potential range of the plateau region with

THF present is due to the reduction of either unknown impurities in THF or THF itself.

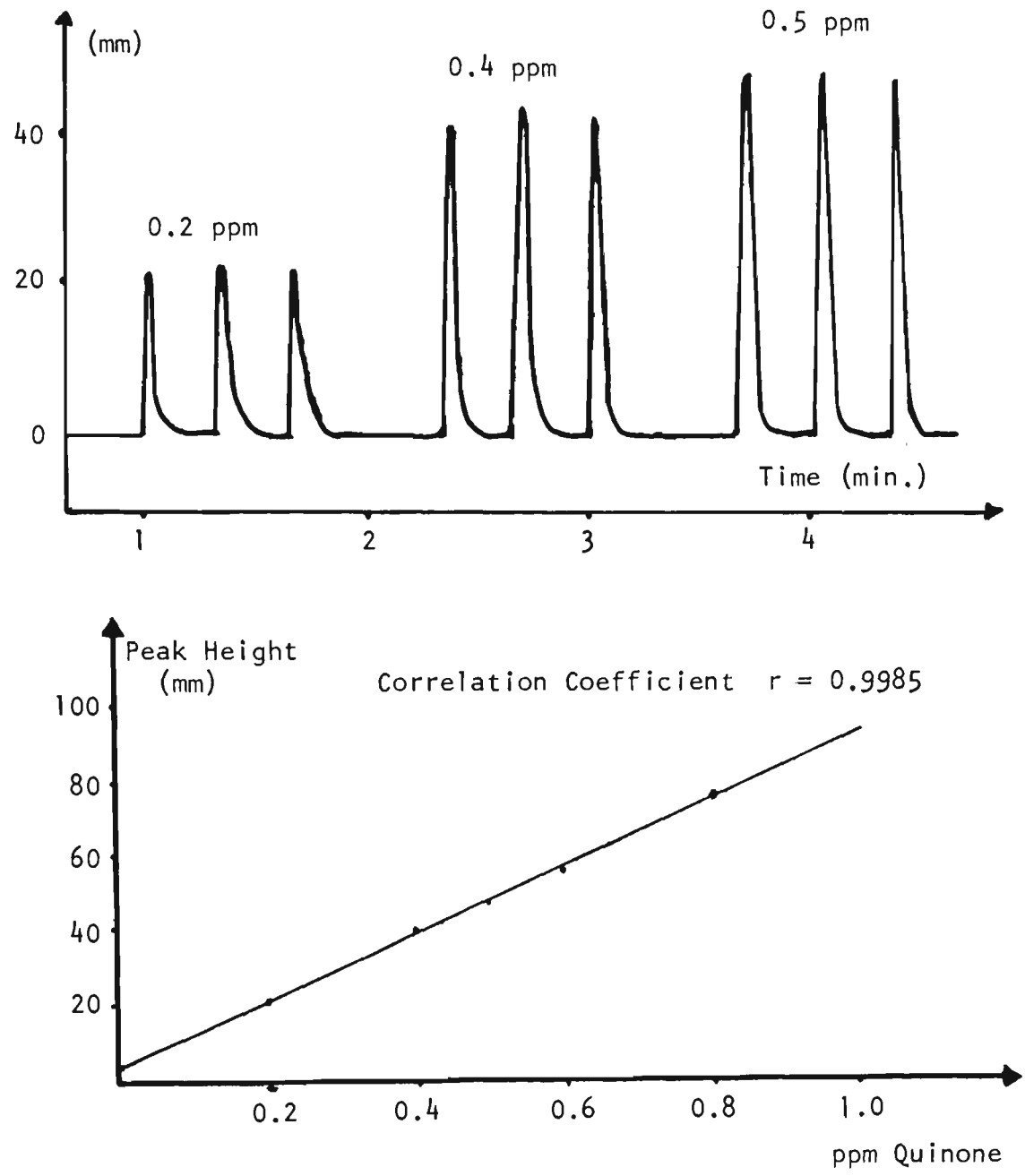
Reproducible detection of cathodic currents in FIA requires stringent control over oxygen penetration into the system.[232] Preliminary experiments, in which oxygen could enter the FIA system, yielded RSD's between 10 and 30% at sub-ppm levels of quinone. Therefore two different pumping and reservoir arrangements, both with stringent control of oxygen penetration, were compared to determine the optimum precision achievable in quinone FIA. Typical FIA peaks and calibration graph for both arrangements are shown in Fig. 3.2.15 and Fig. 3.2.16.

The Metrohm gas pressure arrangement gave the most precise results (peak height RSD = 3%) compared to the peristaltic system (peak height RSD = 5%). Although the Metrohm system offers slightly better precision it is limited to short term uninterrupted use because it delivers a constant flow for only about 30 minutes at 2 ml/min. The peristaltic system offers the advantage of constant flow for a few hours at comparable flow rates.

3.2.6.2 THF Samples

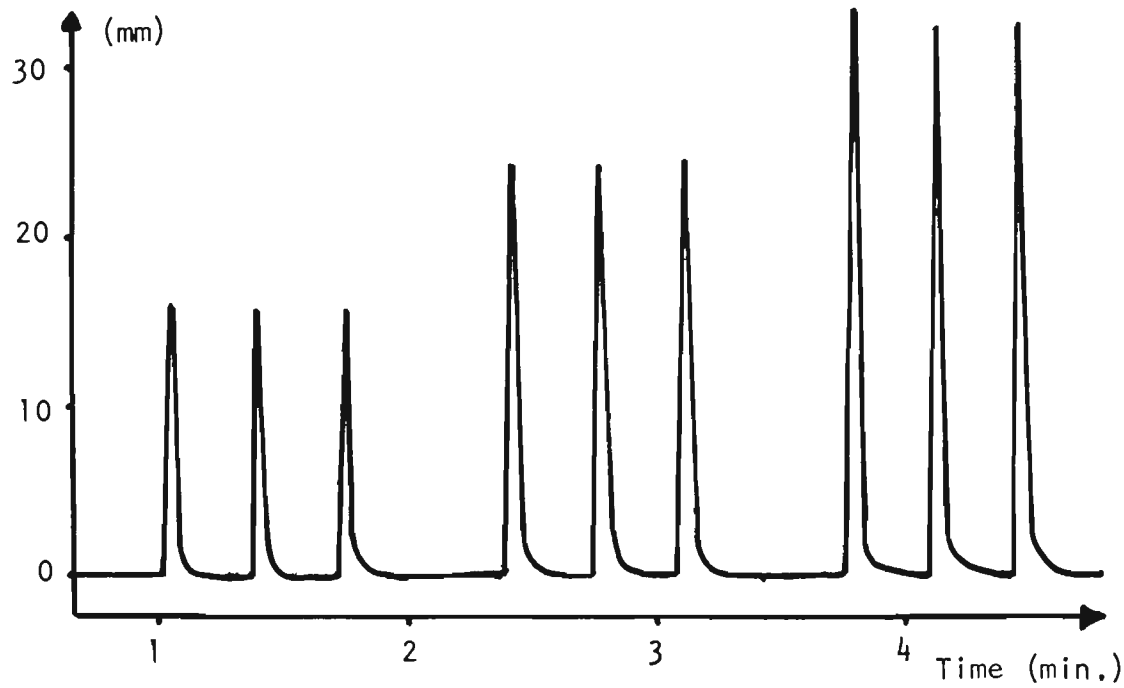
A sample of stabilized THF was diluted one hundred-fold in pH 7 phosphate buffer and analyzed using the Metrohm FIA assembly. A carrier stream of 1% purified THF in phosphate buffer was employed. Quantitation was achieved by calibration graph and the result was compared with a UV spectrophotometric

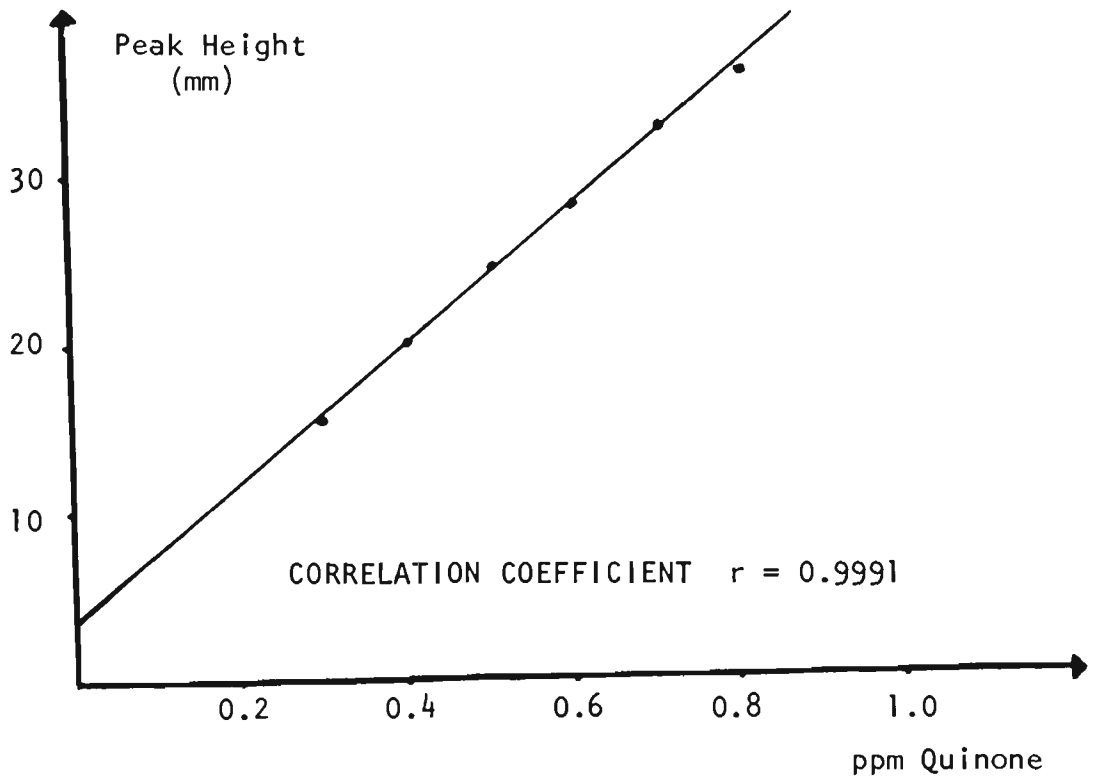
FIG. 3.2.15 - QUINONE FIA WITH PERISTALTIC PUMP



Conditions: Peristaltic Pump
Continuous N_2 Purge of Reservoir
Glassy Carbon Electrode
Wall Jet Detector - DC Mode
Detection Potential = -0.15 Volts
versus Ag/AgCl sat KCl reference
Flow Rate = 2 ml/min, pH 7 Phosphate Buffer
Injection Volume = 10 µL
Samples Deoxygenated with N_2

FIG. 3.2.16 - QUINONE FIA WITH METROHM GAS PRESSURE PUMP





CONDITIONS: Metrohm Gas Pressure Pump
Glassy Carbon Electrode
Wall Jet Detector - DC Mode
Detection Potential = -0.15 Volts
Versus Ag/AgCl sat KCl Reference
Flow Rate = 2 ml/min pH 7 Phosphate Buffer
Injection Volume = 10 µL
Samples Deoxygenated with N_2

assay. (Table 3.2.7).

TABLE 3.2.7 - FIA and UV Results for Quinone in THF

FIA	UV
[Quinone] p.p.m. 18 ± 0.6	[Quinone] p.p.m. 16 ± 0.8
RSD = 3.3%	RSD = 4.7%

3.2.6.3 PRA of Quinone in THF Samples

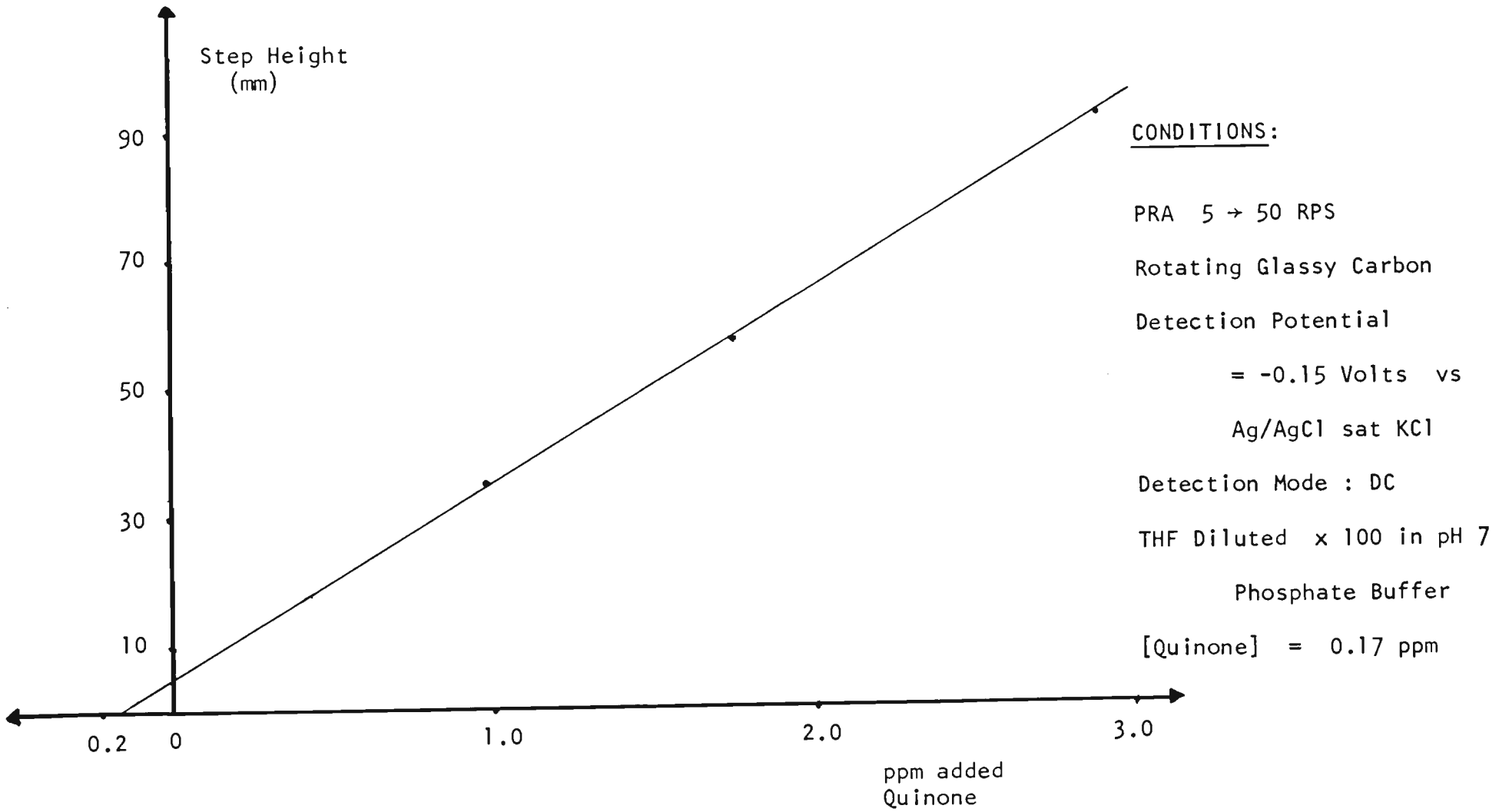
The PRA calibration graph for quinone was linear in the concentration range 0.1-4 ppm and passed through the origin enabling standard additions to be used for quantitation. The data had a correlation coefficient of 0.9997.

The same sample tested by FIA was analyzed and the result compared with a UV assay. The standard addition graph is shown in Fig. 3.2.17 and the analyses results are shown in Table 3.2.8.

TABLE 3.2.8 - PRA and UV results for Quinone in THF

	PRA	UV
[Quinone] in THF (p.p.m.)	17	15
RSD	1.8%	4.7%

FIG. 3.2.14 - STANDARD ADDITIONS QUANTITATION OF QUINONE IN THF BY PRA



Both electrochemical techniques proved to be more precise than the UV technique yet they gave a slightly higher level of quinone. When purified THF was spiked with known amounts of quinone and the techniques were compared agreement was achieved. This indicated that a small amount of electroactive interferent was present in the quinol stabilized THF samples.

3.3 Quinol and Quinone in Ion Selective Electrode Polymer Membranes

Since PRA is rapid, precise, sensitive and is particularly useful where results are complicated by adsorption or interference from slow electron transfer processes, it was decided to test the utility of PRA for the analysis of quinol and quinone in the polymer membrane.

The membranes are prepared by dissolution of the components in THF and subsequent slow evaporation to form a polymer film on the metal contact of the electrode.[229] In this study chloride sensitive membranes fabricated from 35% w/w polyvinylchloride (PVC) and 65% w/w Aliquat 336 were investigated.

3.3.1 Sample Matrix

The PRA analysis of the membranes required the release of the analytes from the PVC/Aliquat 336 matrix into a buffered solution. Since THF is an excellent solvent for the membrane material, is electro-inactive under the conditions of the PRA determination of quinol and quinone, and reasonably miscible with pH 7 phosphate buffer, THF was used to effect this release.

It was found that dissolution of quinol or quinone, PVC and Aliquat 336 in purified THF and subsequent addition to phosphate

buffer (up to 9% v/v THF) yielded complete recovery of the quinol or quinone upon PRA analysis using standard additions for quantitation. A glassy carbon electrode was used rather than carbon paste to avoid problems arising from the higher percentage of THF in the test solutions.

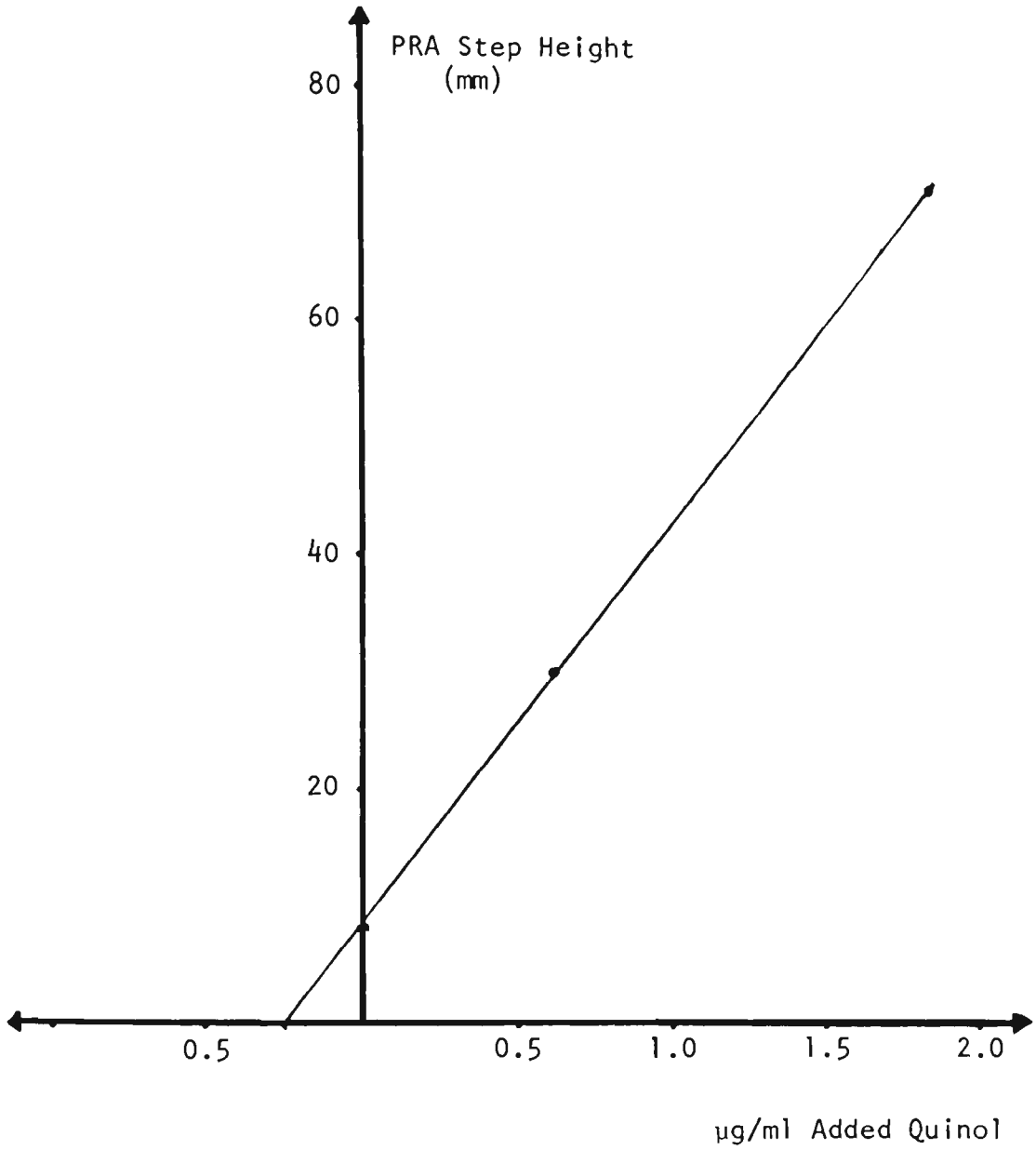
The analysis of quinol in electrode membrane samples by PRA proved suitable, being fast, simple and exhibiting good agreement with a UV spectrophotometric analysis.

The analysis of quinone in membranes by PRA was found to be unreliable, the results being consistently higher by PRA than for the UV spectrophotometric analysis. This discrepancy was found to be due to an interferent arising from the photochemical reaction of quinone to produce a photodimer. The dimer was found to be electroactive under the conditions of PRA analysis and to have a UV absorption wavelength maximum of 222 nm, 21 nm lower than that of quinone at 243 nm. Therefore the PRA measurement included a contribution from the dimer whereas the dimer's different UV absorption behaviour enabled a selective UV analysis of quinone.

3.3.2 The Behaviour of Quinol in THF/PVC/Aliquat 336/Phosphate Buffer

Hydrodynamic voltammetry was used to investigate the effects of PVC and Aliquat 336 on the PRA analysis of quinol. Both materials were found to be electro-inactive in the applicable potential range. To test the recovery of the THF sample dissolution technique, a THF solution, of known quinol concentration and containing PVC and Aliquat 336 in a ratio equivalent to an electrode membrane, was analyzed by PRA.

FIG. 3.3.1 - PRA STANDARD ADDITIONS ANALYSIS OF QUINOL IN PHOSPHATE BUFFER/THF/PVC/ALIQUAT 336 SAMPLE MATRIX SOLUTION



GRAPHICAL RESULT = 0.25 µg/ml

COMPUTER RESULT = 0.23 µg/ml

The graphical standard addition result is shown in Fig. 3.3.1. After dilution of the THF in phosphate buffer the known quinol concentration was 0.24 µg/ml. The standard addition results of 0.23 ± 0.01 µg/ml by computer least squares calculation and 0.25 µg/ml by graphical means indicate complete recovery of quinol from the sample matrix. Therefore PRA was used to determine quinol in electrode samples.

3.3.3 Quinol in Electrode Membranes

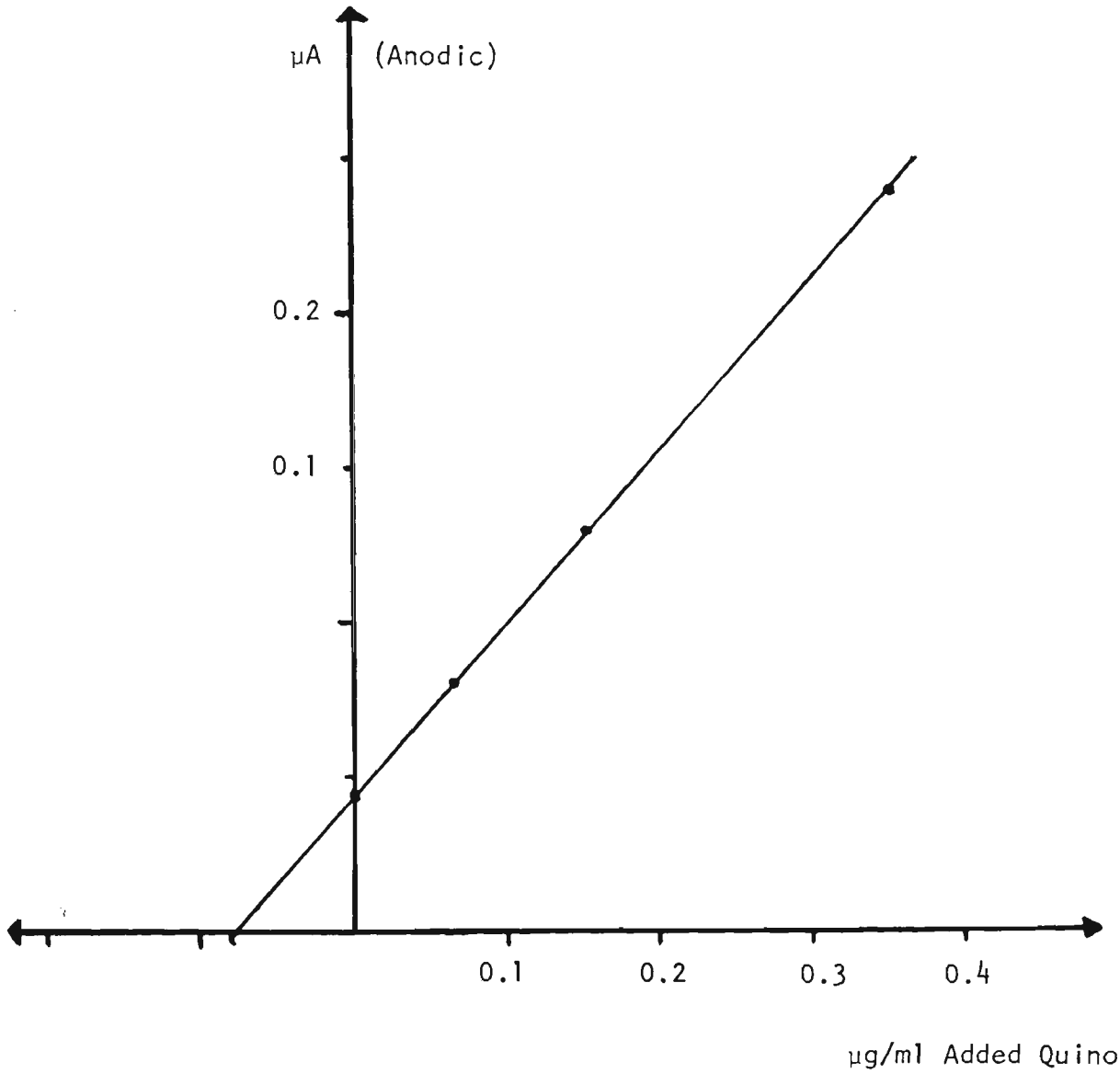
Two sample types of electrode membrane were analyzed. Firstly membranes that were freshly prepared from quinol stabilized THF and secondly, membranes that had been exposed to air and natural light for a few months prior to analysis were tested.

The rotation rates were changed between 9 and 90 RPS and 2.0 ml of THF solution (10-15 mg of sample/25 ml) was added to 20.0 ml of phosphate buffer (i.e., 9% THF). Also a detection potential of + 0.304 V versus a Ag/AgCl sat. KCl reference was employed to minimize the possibility of an interferent being detected.

Sample blanks prepared from purified THF, PVC and Aliquat 336 were tested under the conditions of membrane analysis. No signal was detected.

Standard additions were used for quantitation and the results were compared with a UV spectrophotometric assay. Fig. 3.3.2 and Fig. 3.3.3 show the standard additions graphs and experimental conditions for the "fresh" and "old" membranes respectively. Table 3.3.1 shows the comparison of PRA and UV analysis results.

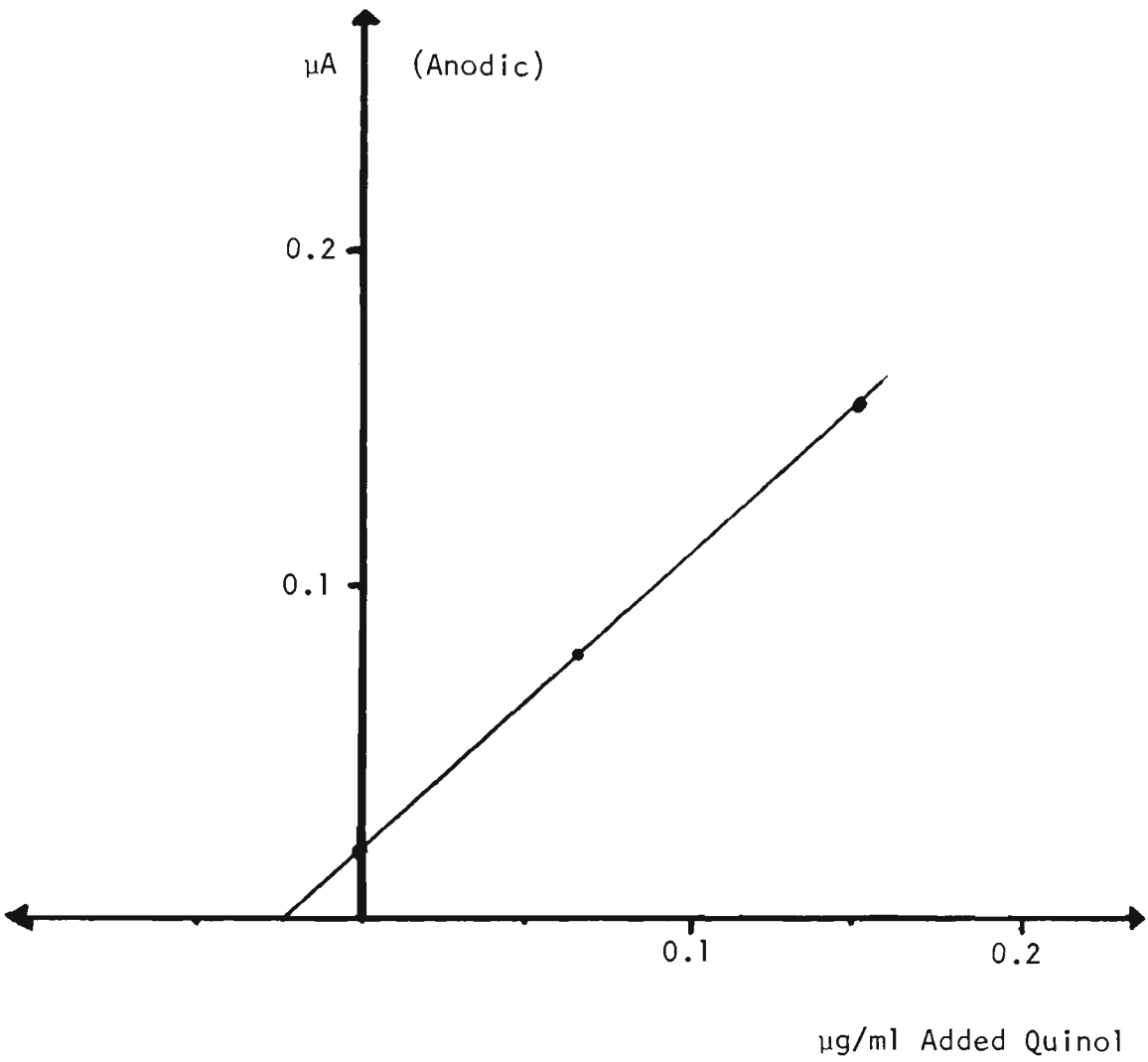
FIG. 3.3.2 - PRA STANDARD ADDITIONS ANALYSIS OF QUINOL IN "FRESH" POLYMER MEMBRANE



GRAPHICAL RESULT = 0.08 µg/ml

COMPUTER RESULT = 0.078 µg/ml

FIG. 3.3.3 - PRA STANDARD ADDITIONS ANALYSIS OF QUINOL IN "OLD" POLYMER MEMBRANE



GRAPHICAL RESULT = 0.024 µg/ml

COMPUTER RESULT = 0.024 µg/ml

TABLE 3.3.1 - PRA and UV Analysis Results for Polymer Membranes

MEMBRANE	PRA [QUINOL]		UV [QUINOL]	
	Test Solution µg/ml	Membrane µg/mg	Test Solution µg/ml	Membrane µg/mg
"FRESH" Clear-Pale Yellow Colour (13.4 mg)	0.078	1.60	0.073	1.50
"OLD" Opaque-Purple/ Brown Colour (10.5 mg)	0.024	0.63	0.025	0.65
DETECTION LIMIT 10 mg sample	0.001	0.028	0.006	0.165
RSD	3.7%		3.6%	

As expected the amount of quinol present in the membranes decreases with increasing exposure to air and light. The colour change of the membranes is attributed to the formation of the quinone photodimer and other degradation products (See sections 3.3.4 and 3.3.5).

3.3.4 The Behaviour of Quinone in THF/PVC/Aliquat 336/Phosphate Buffer

A PRA calibration curve for quinone, in the presence of purified THF/PVC/Aliquat 336 at a ratio equivalent to membrane analysis, was found to be linear for the 0.005-0.50 µg/ml concentration range. The sample matrix without quinone exhibited a PRA step height of 0.01 µA, equivalent to 2.6 ng/ml quinone in the test

solution. After subtraction of this blank value, a least squares analysis showed the line to pass through the origin. The graph is shown in Fig. 3.3.4.

A standard additions analysis of a sample THF solution containing 1.9 µg/ml quinone gave 2.0 µg/ml by computer calculation, demonstrating complete recovery of the analyte. Therefore PRA was used to determine quinone in electrode membranes.

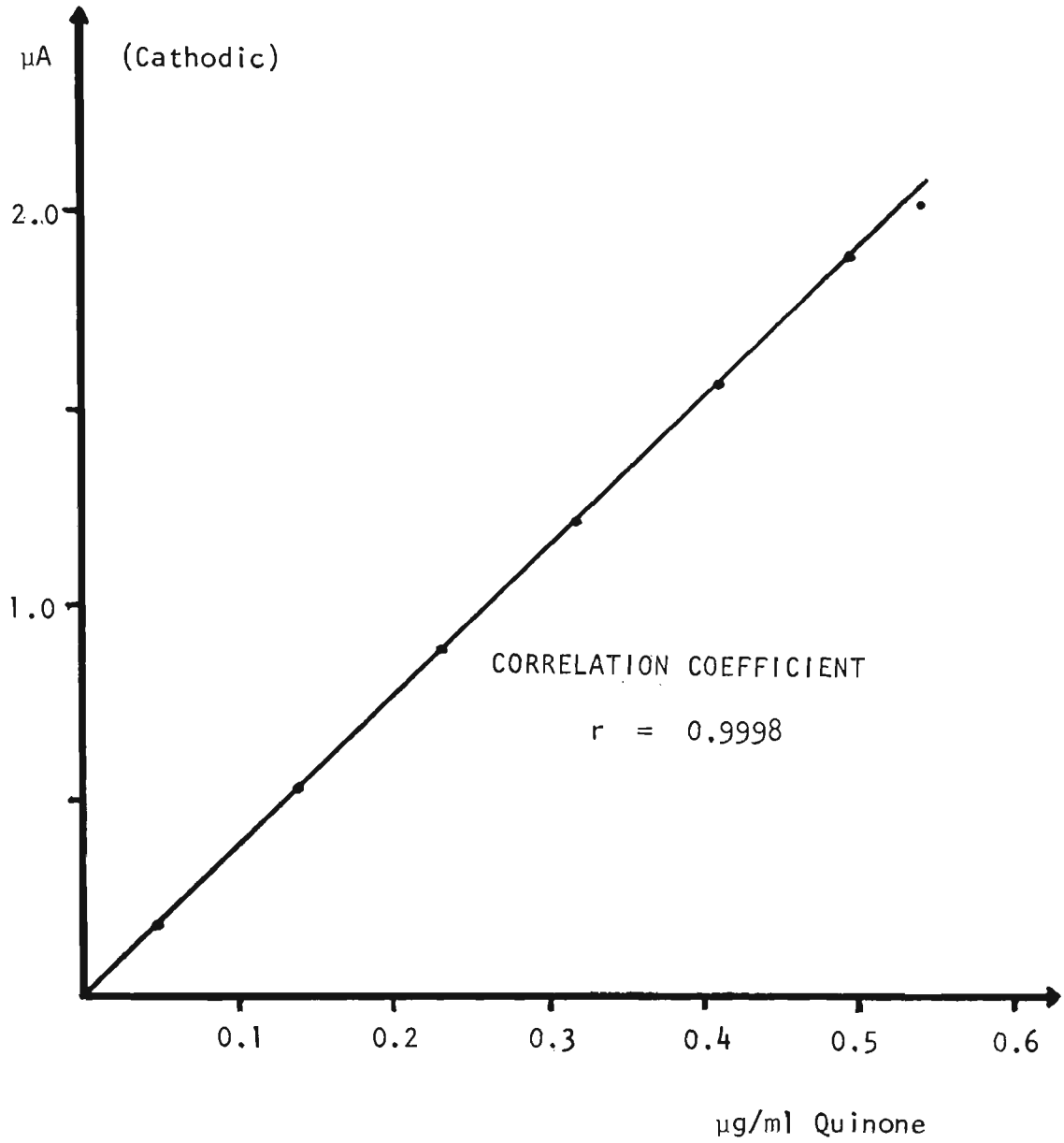
3.3.5 Quinone in Electrode Membranes

The same membranes previously analyzed for quinol were analyzed for quinone by PRA. The results differed with a UV spectrophotometric determination, with PRA giving consistently higher results. Table 3.3.2 shows the comparison.

TABLE 3.3.2 - PRA and UV Results for Quinone in Membranes

MEMBRANE	PRA [QUINONE]		UV [QUINONE]	
	Test Solution µg/ml	Membrane µg/mg	Test Solution µg/ml	Membrane µg/mg
"FRESH" Clear-Pale Yellow Colour (10.1 mg)	0.0055	0.23	0.0079	0.08
"OLD" Opaque-Purple Brown Colour (10.1 mg)	0.0082	0.34	0.0089	0.09
RSD	5%		5%	

FIG. 3.3.4 - CALIBRATION CURVE FOR QUINONE IN THF/PVC/ALIQUAT 336/ PHOSPHATE BUFFER



LEAST SQUARES ANALYSIS

$$I = 3.79 \times Q + 0$$

where I = Current (µA)

Q = Concentration of quinone (µg/ml)

3.3.6 Identification of Interferent

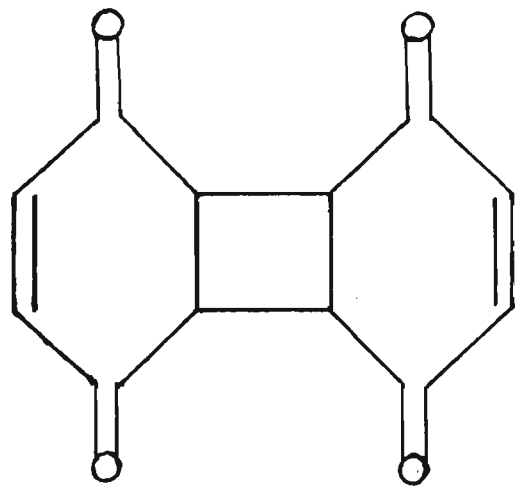
The positive interference in the PRA measurement and the membrane colour change, which is also observed in aqueous quinol and quinone solutions, suggested that the interference may have been due to an electro active material generated by photochemical reaction. The slight interference observed in the amperometric analyses of quinone in THF was most likely due to the same reaction. The process occurs to a lesser extent in THF since it is supplied in dark glass containers.

Quinones are known to react photochemically.[233 234,221] Gold and Ginsberg[221] synthesized the photodimer of quinone and report its UV absorption maximum as 222 nm. The structure is shown in Fig. 3.3.5. Therefore aqueous PVC/THF/quinol solutions exposed to UV light from a 14 Watt low intensity mercury vapor lamp and air were monitored by UV spectroscopy at intervals over one week. The spectra are shown in Fig. 3.3.6. Initially only an absorption peak is observed for quinol, then as air enters the solution there is a corresponding increase in the absorption peak for quinone. Also there is increased absorption in the region of the photodimer and after one week a substantial peak is observed at 222 nm.

These results demonstrate that the photodimer is likely to be present in the membranes. Therefore the dimer was synthesized and its voltammetric behaviour was investigated under the conditions of quinone analysis.

The photodimer exhibits two cathodic waves at a rotating glassy carbon electrode in phosphate media. The voltammogram is shown

FIG. 3.3.5 - QUINONE PHOTODIMER



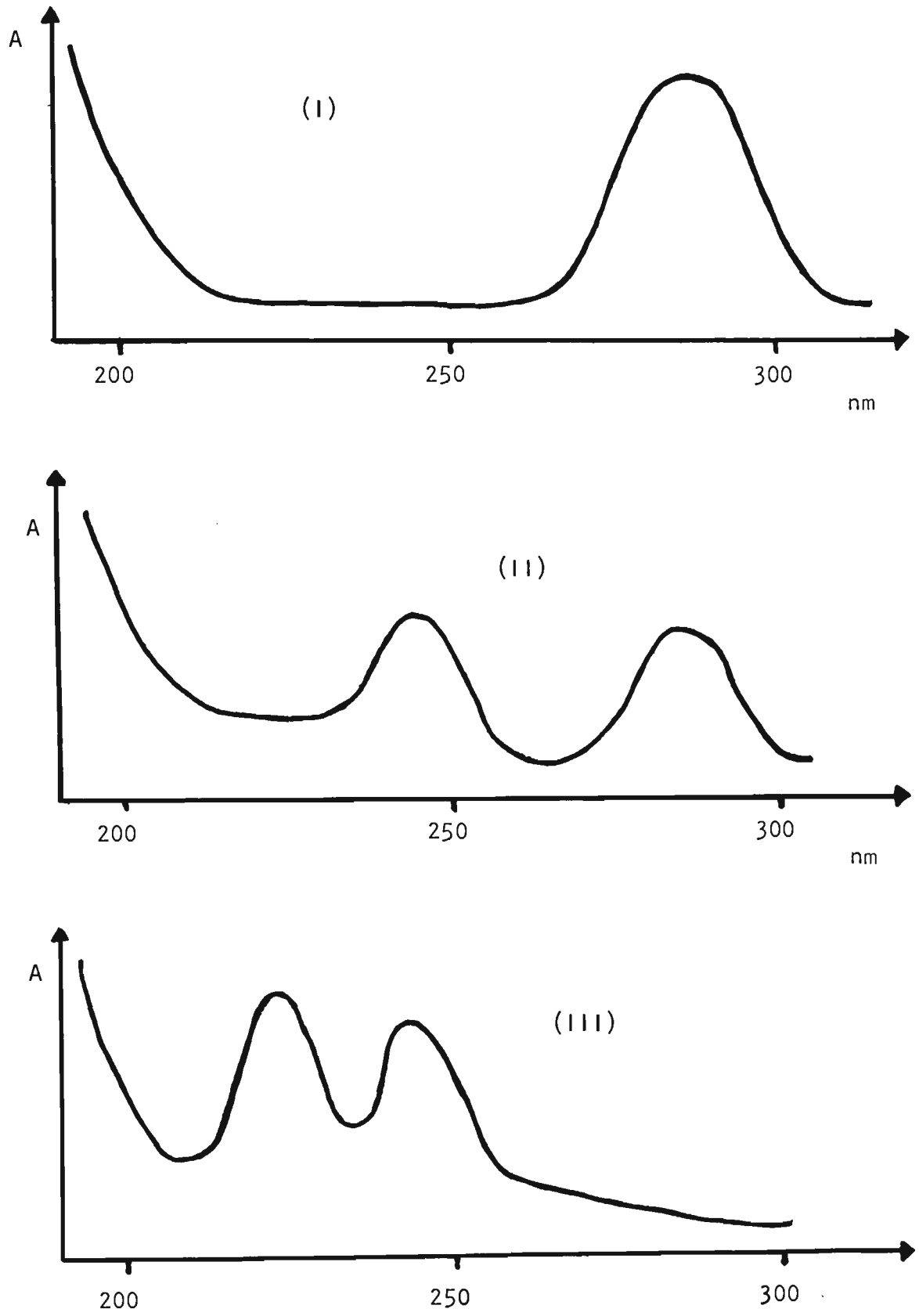
tricyclo[6,4,0,$0^{2,7}$]dodeca-4,10-diene-3,6,9,12-tetrone

in Fig. 337 superimposed on a voltammogram of quinone under the same conditions. Although the drawn out wave shape indicates slow electron transfer for the dimer, significant current is observed at -0.15 V, the detection potential for the PRA analysis. Therefore it was concluded that the photodimer was responsible for the positive interference in the PRA quinone analyses.

3.3.7 The Fate of Quinol in Membranes

The results of these investigations and reports that quinone compounds can also react photochemically via the ketone double bond,[235] allow the following reaction pathway to be proposed for the fate of quinol in the electrode membranes. (Fig. 3.3.8). Quinol is air oxidized to quinone which in turn can undergo photochemical reaction via two mechanisms. The production of tricyclo[6,4,0,$0^{2,7}$]dodeca-4,10-diene-3,6,9,12-tetrone occurs via a Π-Π^* transition and the material has a UV absorption maximum

FIG. 3.3.6 - THE UV ABSORPTION BEHAVIOUR OF AQUEOUS/PVC/THF/QUINOL SOLUTIONS



(I) INITIAL AQUEOUS QUINOL SOLUTION

(II) SOLUTION EXPOSED TO AIR AND UV LIGHT (3 Days)

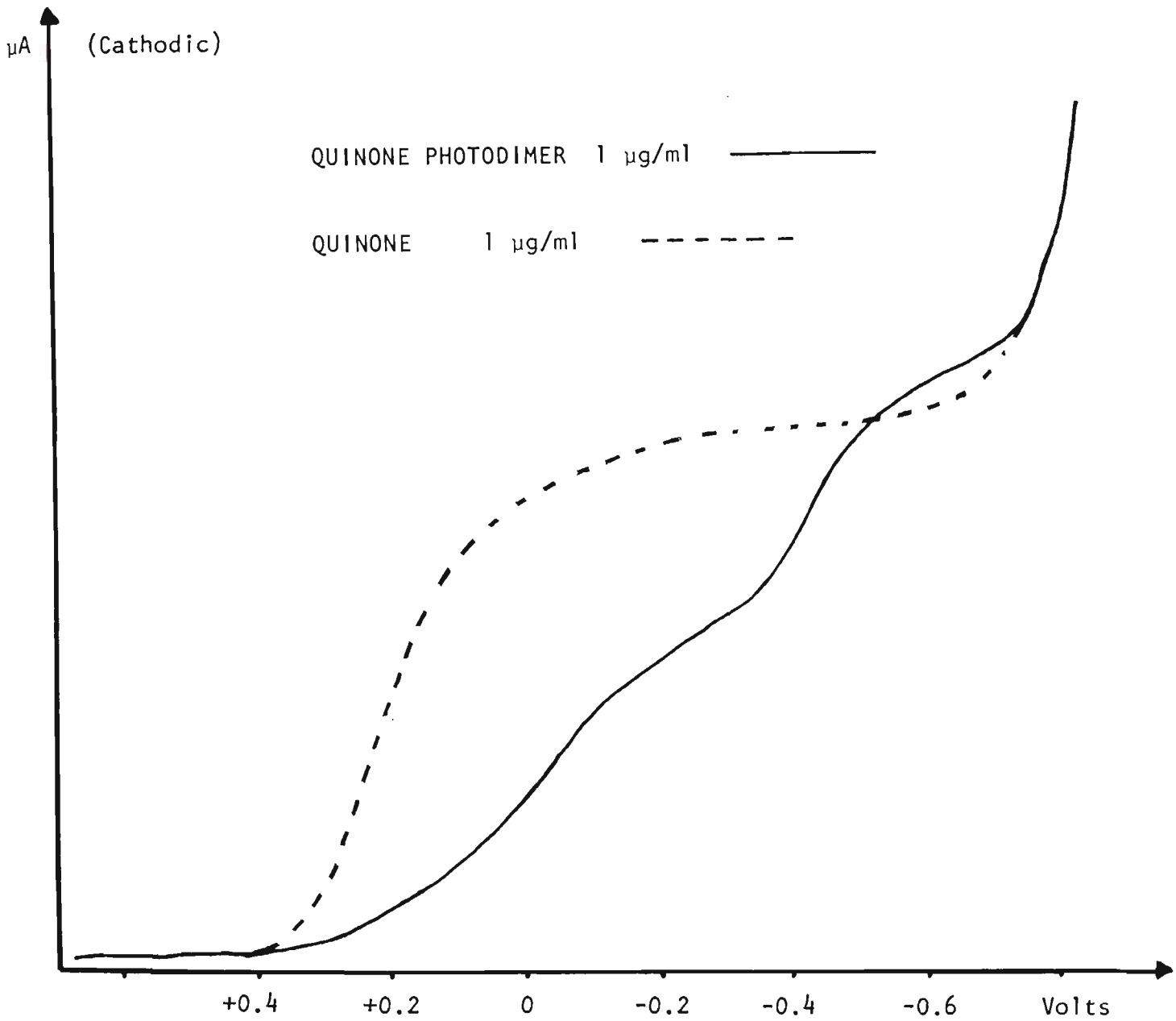
(III) SOLUTION EXPOSED TO AIR AND UV LIGHT (7 Days)

at 222 nm corresponding to an α, β unsaturated ketone without further conjugation.[221] The other photochemical reaction proceeds via a n-Π* transition at the carbonyl group resulting in an α, β unsaturated ketone with an extra double bond in conjugation. Such a molecule would have an absorption maximum in the region of quinone's maximum.[236] Therefore, from the results of the UV measurements, the Π-Π* transition is proposed as the major photochemical pathway. The product of the n-Π* transition can undergo further addition, via either transition, to result in a mixture of high molecular weight materials and it is possible that these would be present in the electrode membranes.

In conclusion it has been demonstrated that quinol, quinone and electroactive photochemical reaction products are all present in the membranes. The presence of these electrochemical couples will contribute to the internal reference potential of coated wire ion selective electrodes. The change in membrane composition with exposure to air and light will contribute to changes in the electrode's internal reference potential since the potential is dependent on the concentrations of the oxidized and reduced forms of the couples.[237]

Future research could be undertaken to elucidate the complete electrochemistry of the quinone photodimer.

FIG. 3.3.7 - HYDRODYNAMIC VOLTAMMETRY OF THE QUINONE PHOTODIMER



CONDITIONS: Rotating Glassy Carbon Electrode 90 RPS

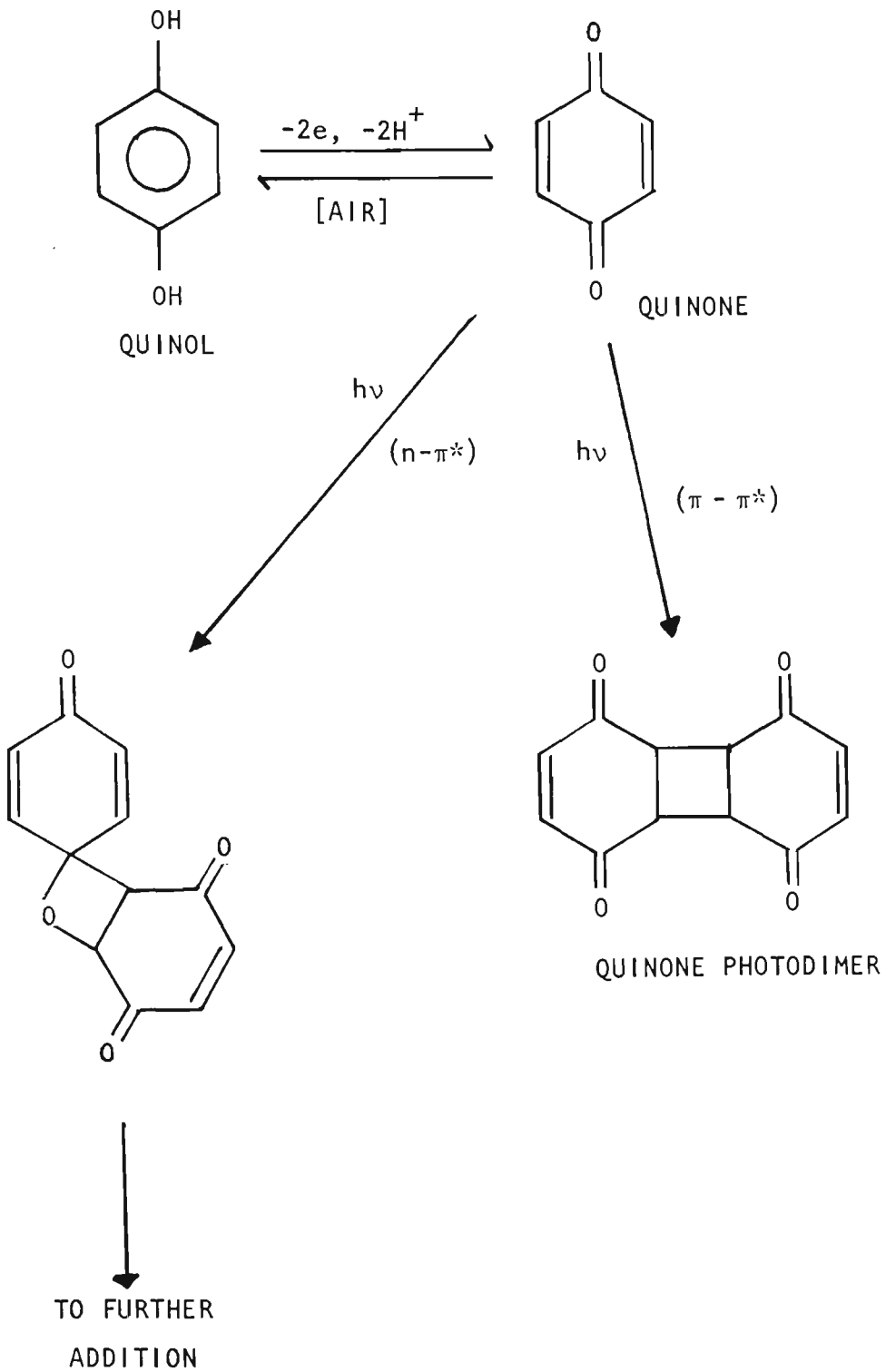
Ag/AgCl sat KCl Reference

5% THF in pH 7 Phosphate Buffer

Scan Mode DC

Scan Rate 20 mV/sec.

FIG. 3.3.8 - THE FATE OF QUINOL IN ELECTRODE MEMBRANES



CHAPTER 4

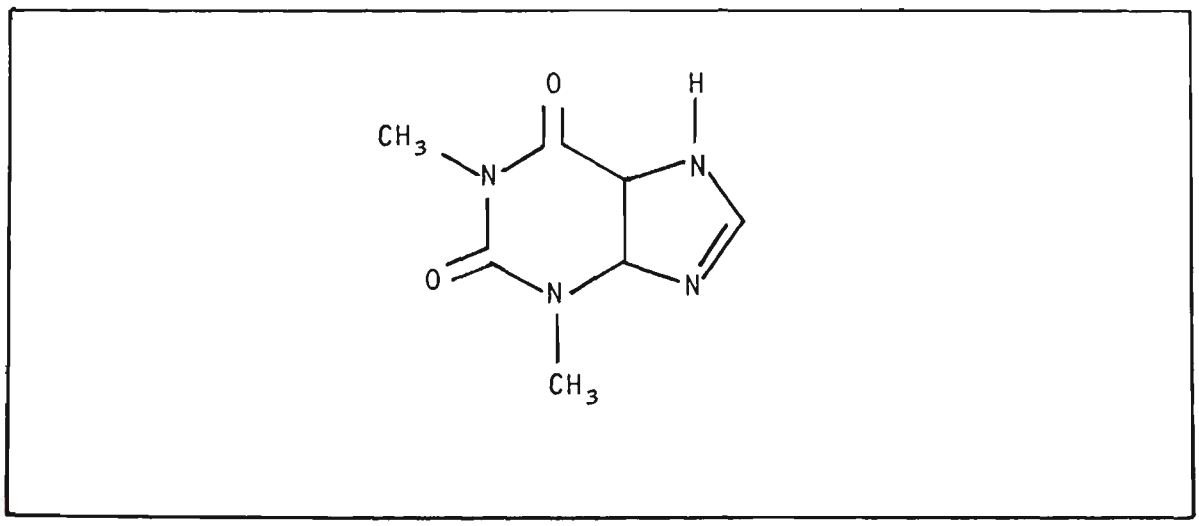
The Electroanalytical Determination of Theophylline in Pharmaceutical Samples

4.1 Introduction

Theophylline, 1,3-dimethylxanthine (structure shown Fig. 4.1.1), is a bronchodilator drug employed in the treatment of asthma in adults and children. The generally accepted therapeutical plasma concentration range for theophylline is 10-20 µg/ml for children. Above these levels toxic effects are encountered and below these levels the drug is of little therapeutic use.[238 242]

When theophylline is introduced into the human body it is metabolized to different degrees by different individuals. Also the sensitivity of individuals towards the drug varies widely. These differences give rise to the problem of prescribing a dose that will be effective therapeutically and yet not cause toxic effects. Patients undergoing theophylline therapy should be monitored clinically for their initial treatment so that their theophylline absorption data can be acquired.[243]

FIGURE 4.1.1 - THE STRUCTURE OF THEOPHYLLINE



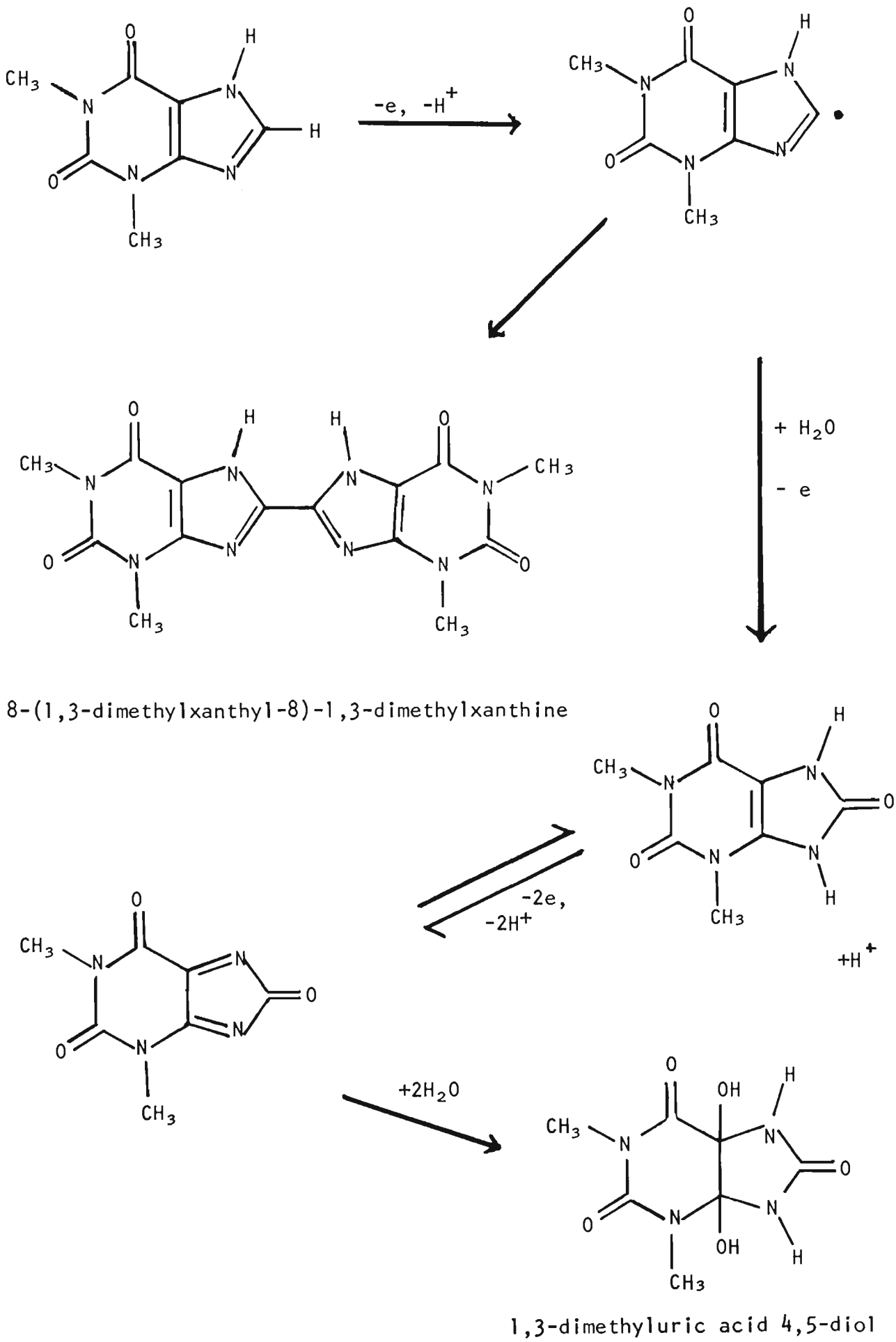
This requires fast and reliable analyses of samples. Pharmaceutical quality control techniques are required to monitor products containing theophylline such as tablets, elixirs and intravenous injections.

The reported methods for theophylline vary in analysis time, expense, sensitivity and selectivity. For the purpose of this work the methods can be divided into electrochemical techniques and other techniques. The non-electrochemical techniques include the following. Ultraviolet (U.V.) spectrophotometry;[244-248] gas chromatography;[249-252] gas chromatography/mass spectrometry;[253] radioimmunoassay;[254-256] fluorimetry[257-258] and high performance liquid chromatography (H.P.L.C.) with U.V. detection.[259-271] The electrochemical techniques are a direct voltammetric procedure, reported by Munson and Abdine[272] and a H.P.L.C. technique using electrochemical detection reported by Lewis and Johnson.[273]

The electrochemistry of theophylline and the methylxanthines, in general, was first studied by Dryhurst in the early 1970's.[274-276] The oxidation of the xanthines was studied at pyrolytic graphite electrodes with conventional direct current voltammetry. Using infra red (I.R.), U.V. and mass spectroscopy, fast scan cyclic voltammetry and polarography, Dryhurst postulated mechanisms for these oxidations and compared them to biological oxidations. The primary electrode processes in the oxidation of theophylline at pyrolytic graphite is shown in Fig. 4.1.2[276-277] After the initial removal of an electron to produce a free radical the reaction proceeds via two pathways to produce a dimer and 1,3-dimethyluricacid-4,5-diol.

The 4,5 diol is unstable undergoing secondary reaction to eventually produce a parabanic acid, dimethyl urea and carbon dioxide. Dryhurst reports the oxidation of theophylline to be first order with respect to hydrogen ions signifying the need for buffered analyte solutions. Two anodic waves are observed at pyrolytic graphite electrodes. The first wave is attributed, by Dryhurst, to the adsorption of

FIGURE 4.1.2 - PATHWAYS FOR THEOPHYLLINE OXIDATION AT A CARBON ELECTRODE.



8-(1,3-dimethylxanthyl-8)-1,3-dimethylxanthine, the second wave being due to the faradiac process. Munson and Abdine[272] report the use of a carbon paste electrode (CPE) for the differential pulse voltammetric analysis of theophylline in plasma. They do not report an adsorption wave with this electrode. Therefore the voltammetric oxidation of theophylline at a CPE was investigated.

4.2 Voltammetry of Theophylline at the Carbon Paste Electrode

At the CPE in pH = 7 phosphate buffer, theophylline exhibits an anodic peak at +1.1 Volts versus an Ag/AgCℓ saturated KCℓ/10% KNO_3 reference electrode. This potential corresponds to the faradaic wave observed by Dryhurst after correction for different reference electrodes. Successive anodic voltammograms (as shown in Fig. 4.2.1) on the same surface show a decrease in peak height. This is due to the dimeric oxidation product accumulating and diminishing the available electrode surface area. Dryhurst has isolated the dimer and shown that it is very insoluble in aqueous solution and it accumulates at the electrode surface.

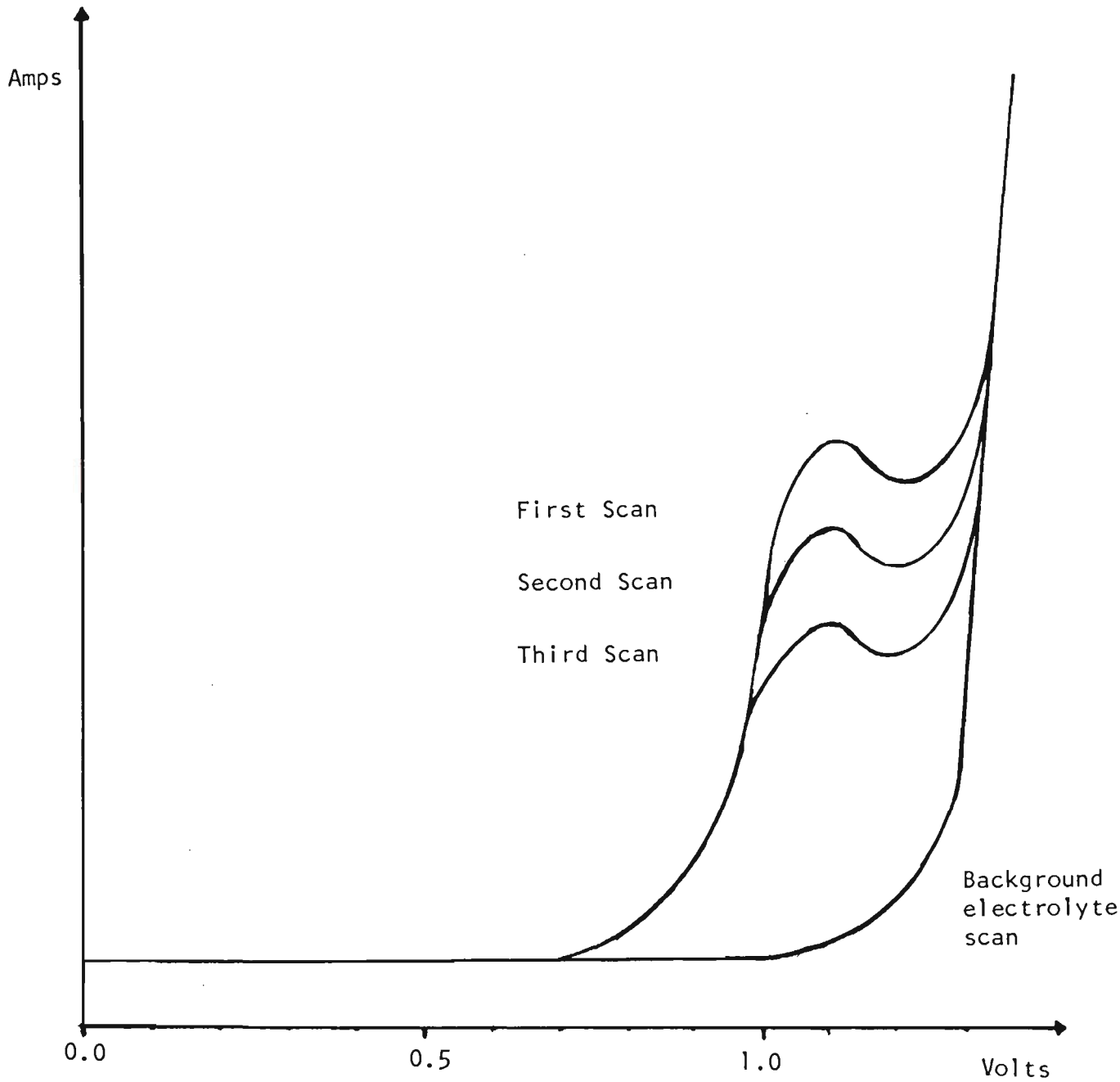
When fresh carbon paste surfaces are used for each voltammogram constant peak heights are observed for each concentration. Also using this technique the peak height is linearly proportional to the theophylline concentration. The calibration graph for the differential pulse voltammetric data has a large positive intercept. The correlation coefficient was found to be 0.9999 and a least squares analysis yielded the following equation:

$$I = (4.16 \pm 0.03) \times C_T + 0.40 \pm 0.02$$

where I = Peak height in μ Amps

and C_T = Theophylline Concentration (moles/litre x 10^{-5})

FIGURE 4.2.1 - SUCCESSIVE SCANS USING SAME CARBON PASTE SURFACE



CONDITIONS: CARBON PASTE WORKING ELECTRODE - METROHM

PLATINUM AUXILIARY ELECTRODE

Ag/AgCl saturated KCl/10% KNO_3 REFERENCE ELECTRODE

SCAN RATE = 5 mV/sec

SCAN MODE - D.C.

[THEOPHYLLINE] = 10 µg/ml

pH = 7 PHOSPHATE BUFFER

Phosphate buffer solutions containing theophylline were analysed with a relative standard deviation (RSD) of 3.5%.

4.3.1 Hydrodynamic Voltammetry of Theophylline at Rotating Carbon Paste Disc Electrodes in Phosphate Buffer

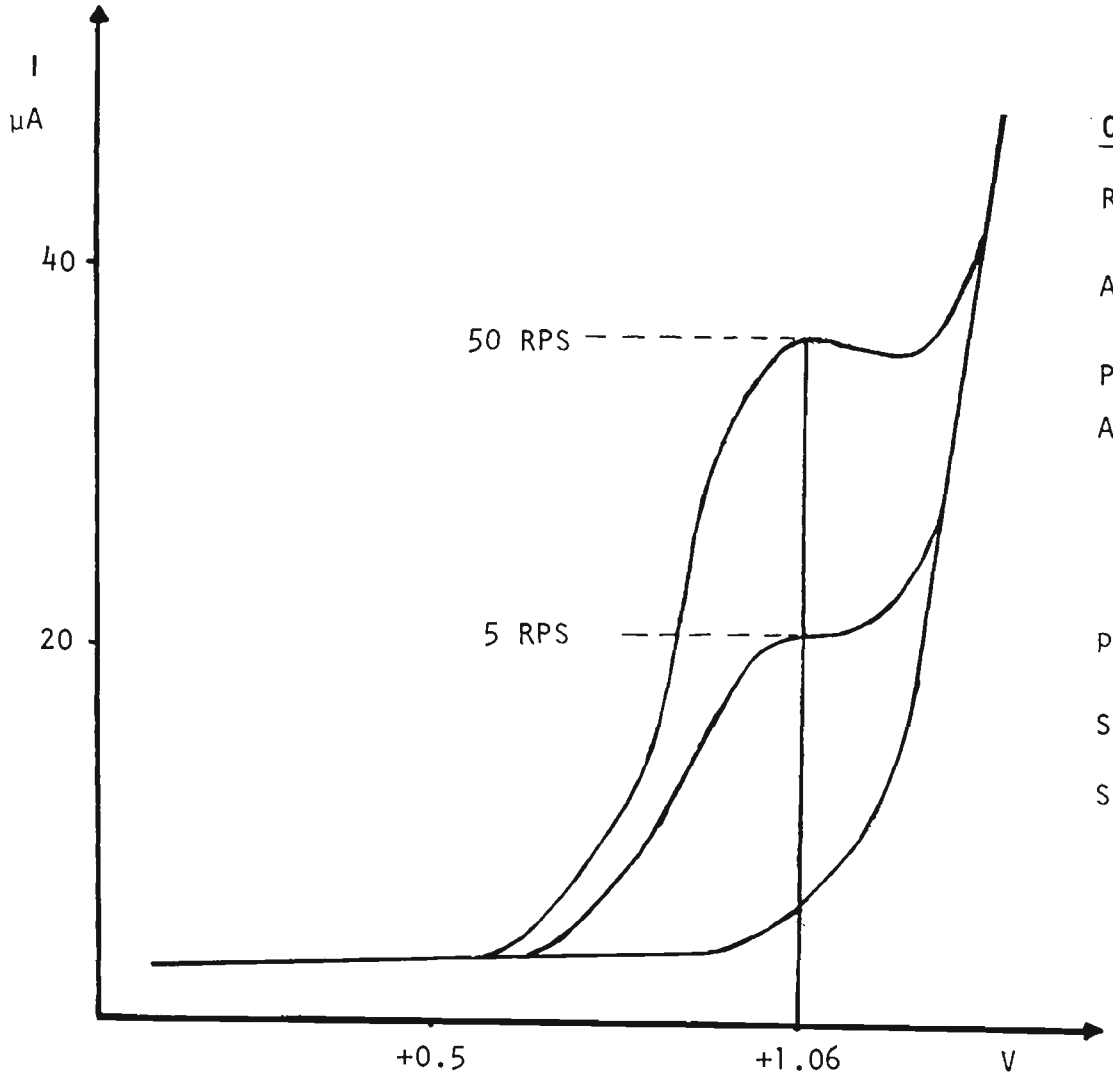
Hydrodynamic voltammetry was used to determine the best region of applied potential for PRA. Fig. 4.3.1 shows voltammograms at two rotation rates for theophylline in phosphate buffer. The peak potential was found to be +1.06 V versus an Ag/AgCℓ sat. KCℓ/10% KNO_3 reference. A peak is observed in these hydrodynamic voltammograms because of product accumulation at the electrode surface. This potential was considered to be the start of the region where the faradaic current from theophylline oxidation is mass transport controlled and not potential dependent. Therefore the potentials used for PRA detection were more positive than +1.06 V.

4.3.2 The Pulsed Rotation Amperometric Determination of Theophylline

An analytical procedure, using pulsed rotation amperometry (PRA) at a carbon paste disc electrode, was developed to determine theophylline in various materials. Calibration curves are linear and pass through the origin enabling the use of standard additions for quantitation. (See Figure 4.3.2). The results compare well with U.V. spectrophotometry.

PRA offers advantages over conventional voltammetric techniques as it provides good discrimination between mass-transport dependent and potential dependent components of current. It is particularly useful where background currents are high and

FIG. 4.3.1 - HYDRODYNAMIC VOLTAMMETRY OF THEOPHYLLINE AT CARBON PASTE ELECTRODE



Conditions:

Rotating Carbon Paste Disc - Working Electrode

Ag/AgCl Sat KCl/10% KNO_3 - Reference Electrode

Platinum Wire - Auxiliary Electrode

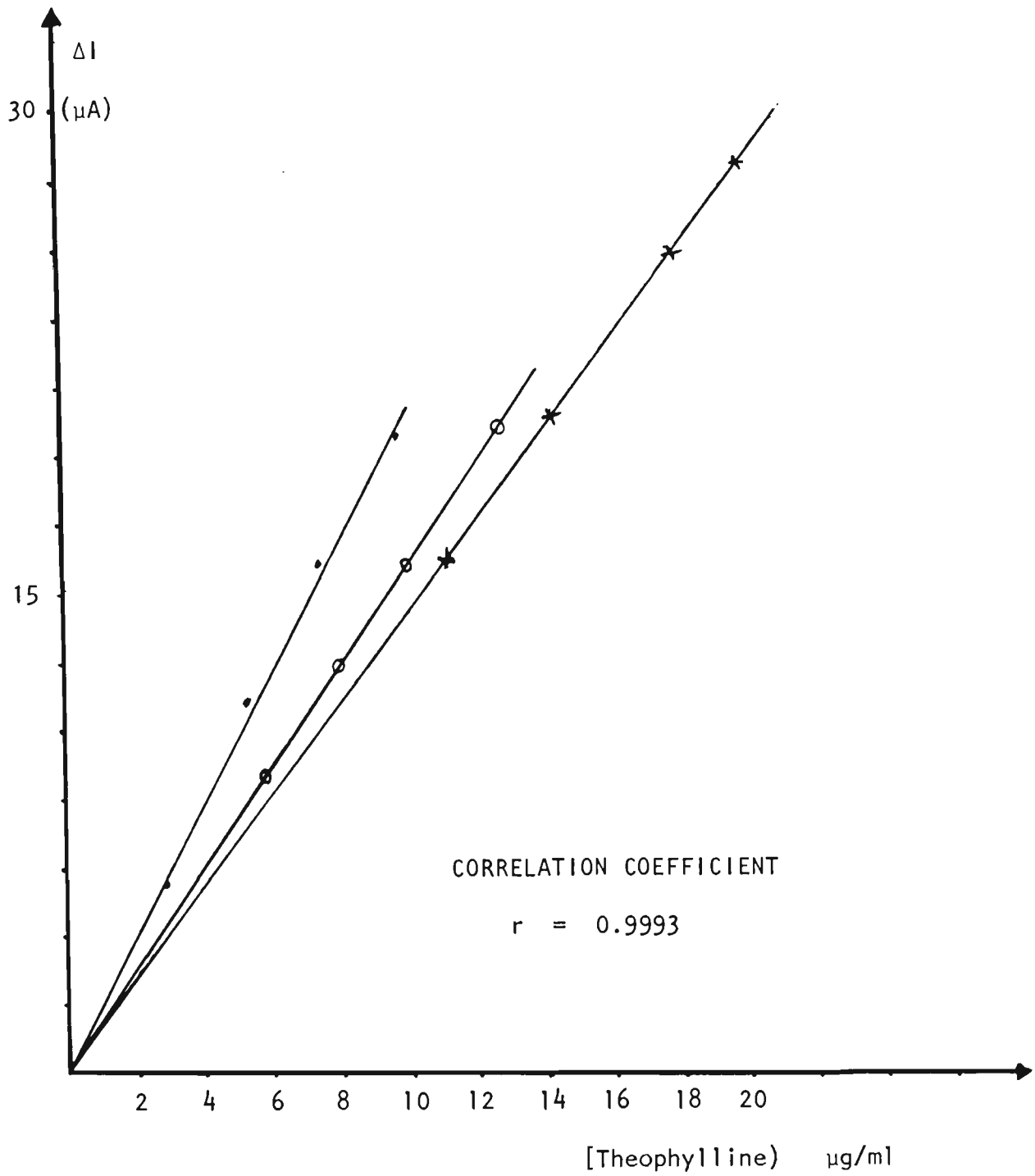
Analyte - 11.7 µg/ml Theophylline

pH 7 PHOSPHATE BUFFER

Scan mode - DC normal

Scan rate - 7.5 mV/sec.

FIG. 4.3.2 - P.R.A. CURRENT (ΔI) vs. THEOPHYLLINE CONCENTRATION FOR VARIOUS THEOPHYLLINE CONTAINING MATERIALS



All samples diluted with pH 7 phosphate buffer.

results are complicated by adsorption.[278-279]

The oxidation of theophylline occurs in a potential region where the background current is high due to significant oxidation of the aqueous buffer.

The developed procedure provides a good demonstration of the utility of the PRA technique under these conditions.

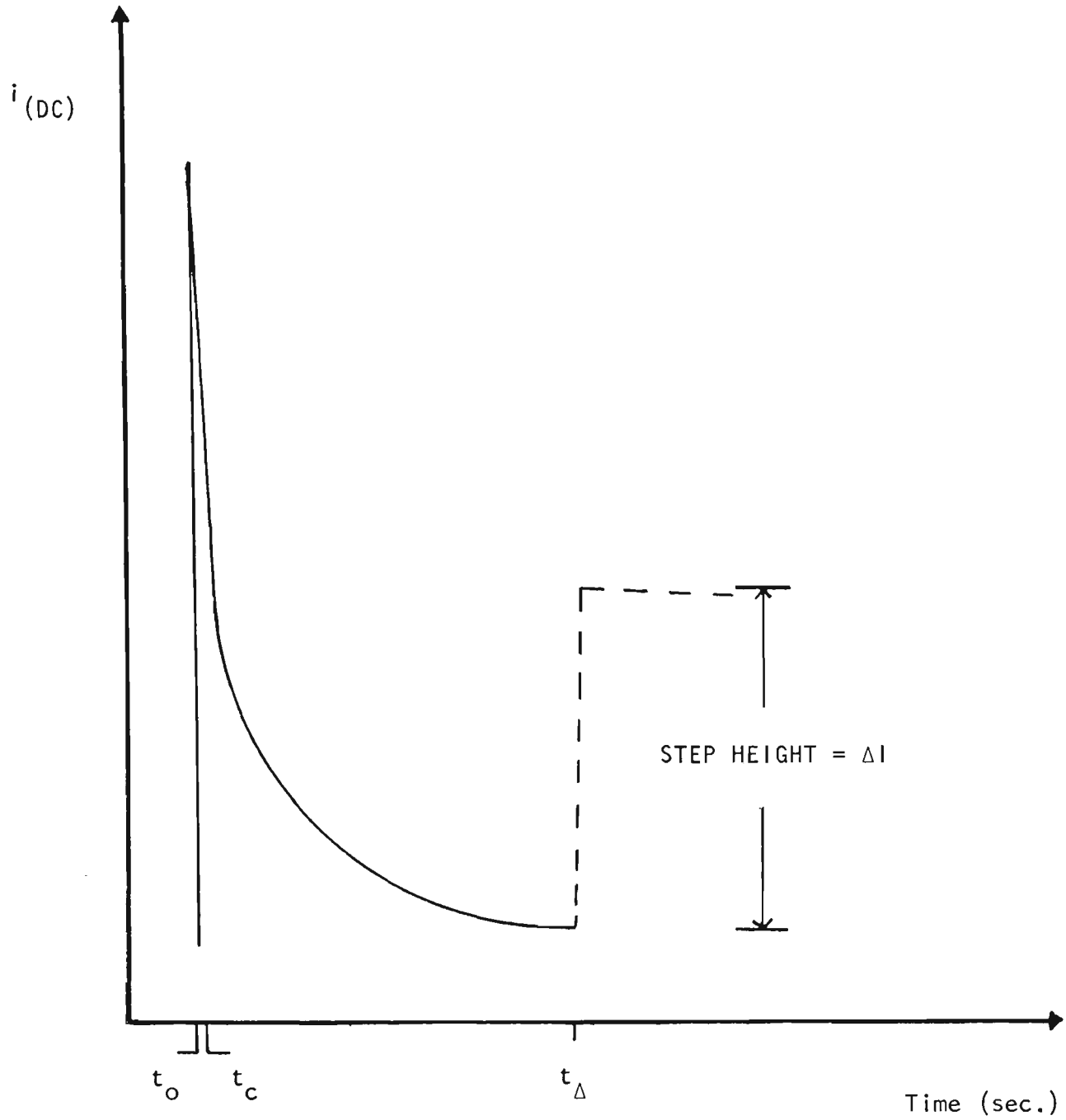
4.3.3 Optimization of P.R.A. Conditions for Theophylline

For theophylline to be determined accurately and readily using this technique it was necessary to optimize the following parameters: the reproducibility of the carbon paste electrode area, (a fresh electrode surface is required for each measurement due to product accumulation at the electrode surface) the concentration range used for measurement and the time taken between applying the detection potential and changing the rotation rate of the electrode (t_{Δ}) (See Fig. 4.3.3).

The precision of the measurement was improved by using more dilute concentrations of theophylline and using longer times between the application of detection potential and changing the rate of transport to the electrode. The following tables demonstrate this point. (Table 4.3.1 and Table 4.3.2).

The trend observed for the variation of precision with concentration is opposite to that observed in general with analytical

FIG. 4.3.3 - P.R.A. RESULT FOR THEOPHYLLINE AT CARBON PASTE DISC ELECTRODE



t_o = Time at which detection potential is applied.

t_Δ = Time at which transport rate is changed.

t_c = Time at which charging current is a maximum

——— Current measured at 5 R.P.S.

----- Current measured at 50 R.P.S.

CONDITIONS: t_Δ = 20 sec.

Detection potential + 1.15 V vs Ag/AgCl sat KCl/10% KNO_3

TABLE 4.3.1 - Variation in Precision with Theophylline Concentration

Theophylline Concentration µg/ml	Uncertainty in Measurement R.S.D.
0.42	1.0%
1.04	2.5%
2.07	7.0%

TABLE 4.3.2 - Variation in Precision with t_{Δ}

Uncertainty in Current R.S.D.	t_{Δ} (sec.)
7.5%	4
6.2%	10
4.6%	20
4.5%	60

techniques where the precision decreases as the concentration decreases. This difference is due to product accumulation at the electrode surface. With more dilute solutions the accumulation is less and therefore its effect on precision is less.

The trend observed with the variation of precision with t_{Δ} can be explained by a visual study of the current versus time curve of the PRA result. (See Fig. 4.3.3). As t_{Δ} increases the current at 5 R.P.S. is becoming more constant and the curve

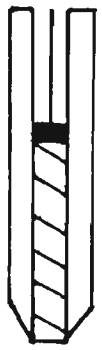
approaches a line parallel with the time axis. This allows easier and more precise measurement of the step height (ΔI) than is the case where t_Δ is small and the angle between the lines at the change in rotation rate is more acute. The otpimum time for t_Δ was found to be 20 s , since further increases showed insignificant improvement in precision.

Both these parameters were studied using the conventional electrode preparation technique (see experimental section 2.4.3) with a Beckman carbon paste rotating electrode. The R.S.D. achieved with this electrode was found to be unacceptably high compared to the Metrohm stationary carbon paste electrode.

In the work where a Metrohm stationary carbon paste electrode was used a R.S.D. of 1-2% was achieved for several measurements at the same concentration. Therefore the two electrodes were compared and one major difference was found. The Beckman rotating electrode was cylindrical to the tip while the Metrohm electrode had a bevelled tip. (See Fig. 4.3.4). This bevelling results in the annulus of plastic electrode body surrounding the carbon paste disc being much thinner than is the case with the unbevelled electrode. Since the carbon paste surface is prepared by smoothing on suitable material, a thin layer of paste is smeared over the plastic annulus.

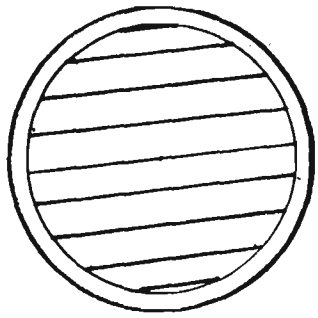
The amount of the thin film over the annulus that is in

FIG. 4.3.4 - METROHM AND BECKMAN C.P.E.'s



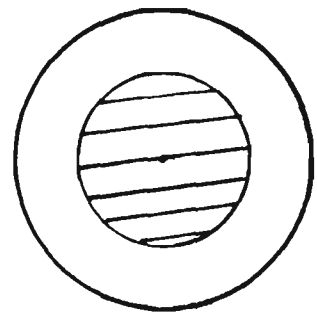
METROHM

BECKMAN



DISC AREA = 16π mm^2
ANNULUS AREA = 4.25π mm^2

Annulus is 26.5% of the area of disc.



DISC AREA = 9π mm^2
ANNULUS AREA = 11.25π mm^2

Annulus is 125% of the area of disc.

METAL ELECTRICAL CONTACT

PLASTIC ELECTRODE BODY

CARBON PASTE

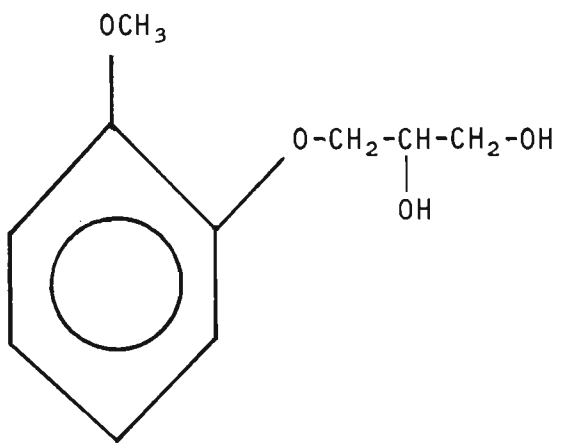
electrical contact with the carbon paste disc is variable and hence the overall electrode area is variable. For a constant electrode area from run to run the annulus must be kept at a minimum. It was found that for the Beckman electrode the area of the annulus was 125% of the area of its carbon paste disc. Therefore the Beckman electrode was machined to give a bevelled tip with an annulus similar to the Metrohm electrode. When this new electrode geometry was evaluated the precision was found to be 1.5% R.S.D. for several measurements at one concentration and between 2 and 3% R.S.D. for a 3 additions standard addition analysis. This is comparable with that found for voltammetric determination of theophylline at the Metrohm stationary carbon paste disc.

Also this degree of precision was found over a wider concentration range than was initially indicated by the results of the variation in precision with concentration experiment (Table 4.3.1). The analyses can be carried out in solutions ranging in concentration from 0.1 µg/ml to 30 µg/ml theophylline without reducing the precision. At concentrations above 30 µg/ml the R.S.D. increases due to more severe fouling of the electrode surface. Also curvature of the calibration plot is observed.

4.3.4 The Determination of Theophylline in Pharmaceuticals

Theophylline was determined in a range of pharmaceutical products by PRA. Each product was investigated by hydrodynamic voltammetry to detect the presence of any electroactive interferent. Of the products studied, only those that contained guiaiphenin (See Fig. 4.3.5 for the structure) showed any interference.

FIG. 4.3.5 - THE STRUCTURE OF GUIAIPHENIN



GUIAIPHENIN 3-(2-methoxyphenoxy)-propane-1,2 diol

Guiaphenin was found to have an oxidation peak potential of +0.83V versus an Ag/AgCℓ sat. KCℓ/10% KNO_3 reference. Fig. 4.3.6 shows the hydrodynamic voltammogram for the product 'Quibron' in phosphate buffer and demonstrates the interference due to guiaiphenin under the conditions of theophylline PRA analysis. Theophylline could be determined in this mixture by PRA if two detection potentials (+0.83 and +1.06 Volts) were employed. The difference in current signals would be proportional to the theophylline concentration. This procedure was not attempted because of the following disadvantages. The increase in analysis time would increase the cost per analysis. The extra measurement step would decrease the precision of the analysis.

4.3.5 Cough Syrups

The following four commercial cough syrups containing theophylline were investigated; Neullin Syrup, Elixophyllin, Brondecon and Quibron. Of these Brondecon and Quibron were found to contain guiaiphenin and therefore were not analyzed by PRA.

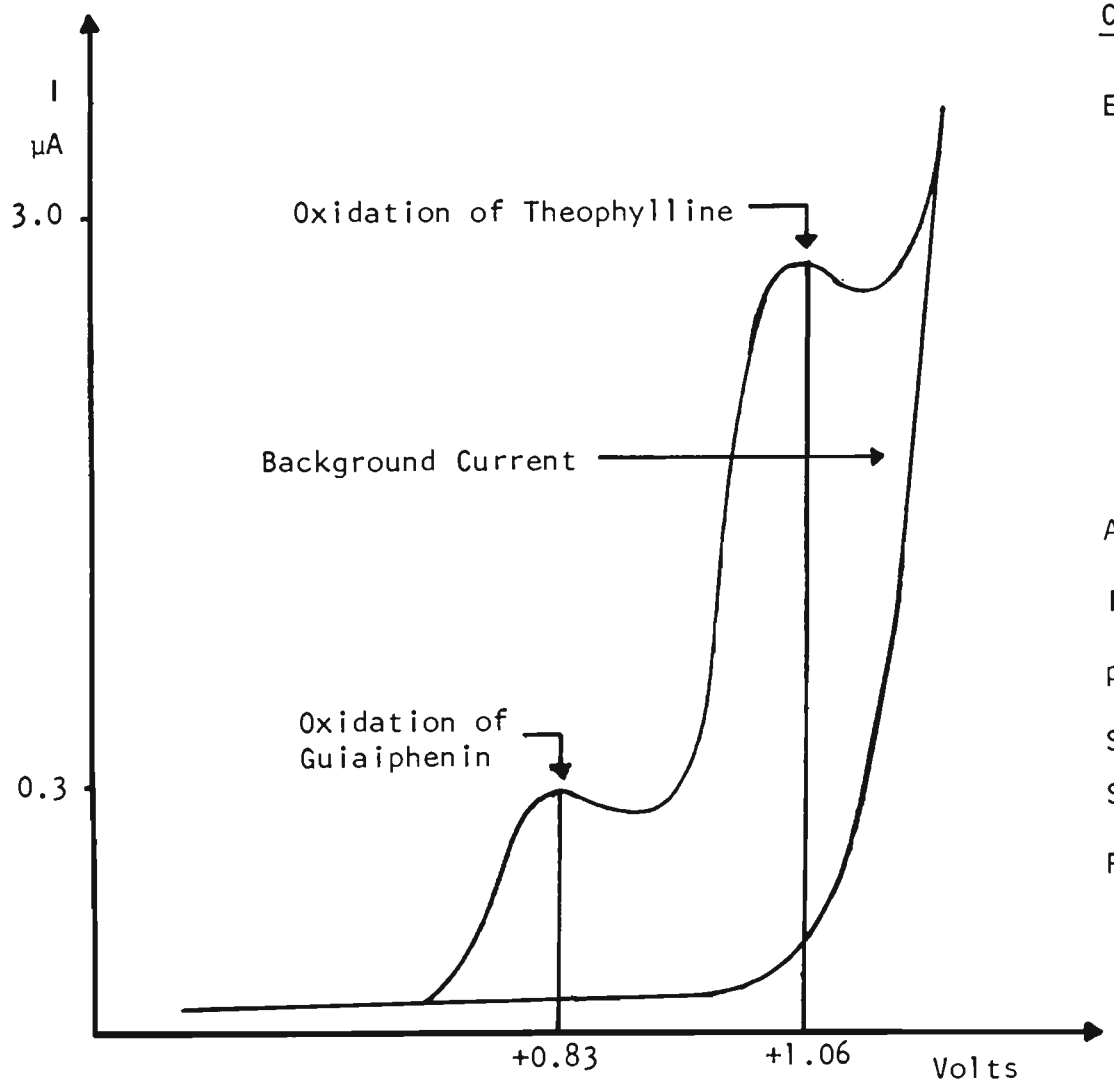
Neullin Syrup and Elixophyllin were analyzed with ease, requiring only dilution in phosphate buffer at pH 7 before measurement and quantitation by standard additions.

4.3.5.1 Nuellin Syrup

A sample of the cough syrup which had been assayed by UV spectrophotometry was analyzed by PRA. The results for the standard additions analysis are shown in Table 4.3.3.

The initial concentration was determined graphically as well as by computer calculation. The graphical result is shown

FIG. 4.3.6 - HYDRODYNAMIC VOLTAMMETRY OF QUIBRON



Conditions:

Electrodes: Working - Rotating
Carbon
Paste Disc.

Reference - Ag/AgCl
Sat. KCl/10% KNO_3

Auxiliary - Platinum
Wire

Analyte - Theophylline

Interference - Guiaphenin

pH 7 PHOSPHATE BUFFER

Scan Mode - DC normal

Scan Speed - 20 mV/sec.

PHARMACEUTICAL - QUIBRON

in Fig. 4.3.7. The plot is linear and gives good agreement with the computer calculation.

The PRA result compares well with the UV spectrophotometric result of 5.33 mg/ml theophylline and therefore PRA provides a suitable alternative quality control analysis of Neullin Syrup.

TABLE 4.3.3 - Standard Additions Result for Nuellin Syrup

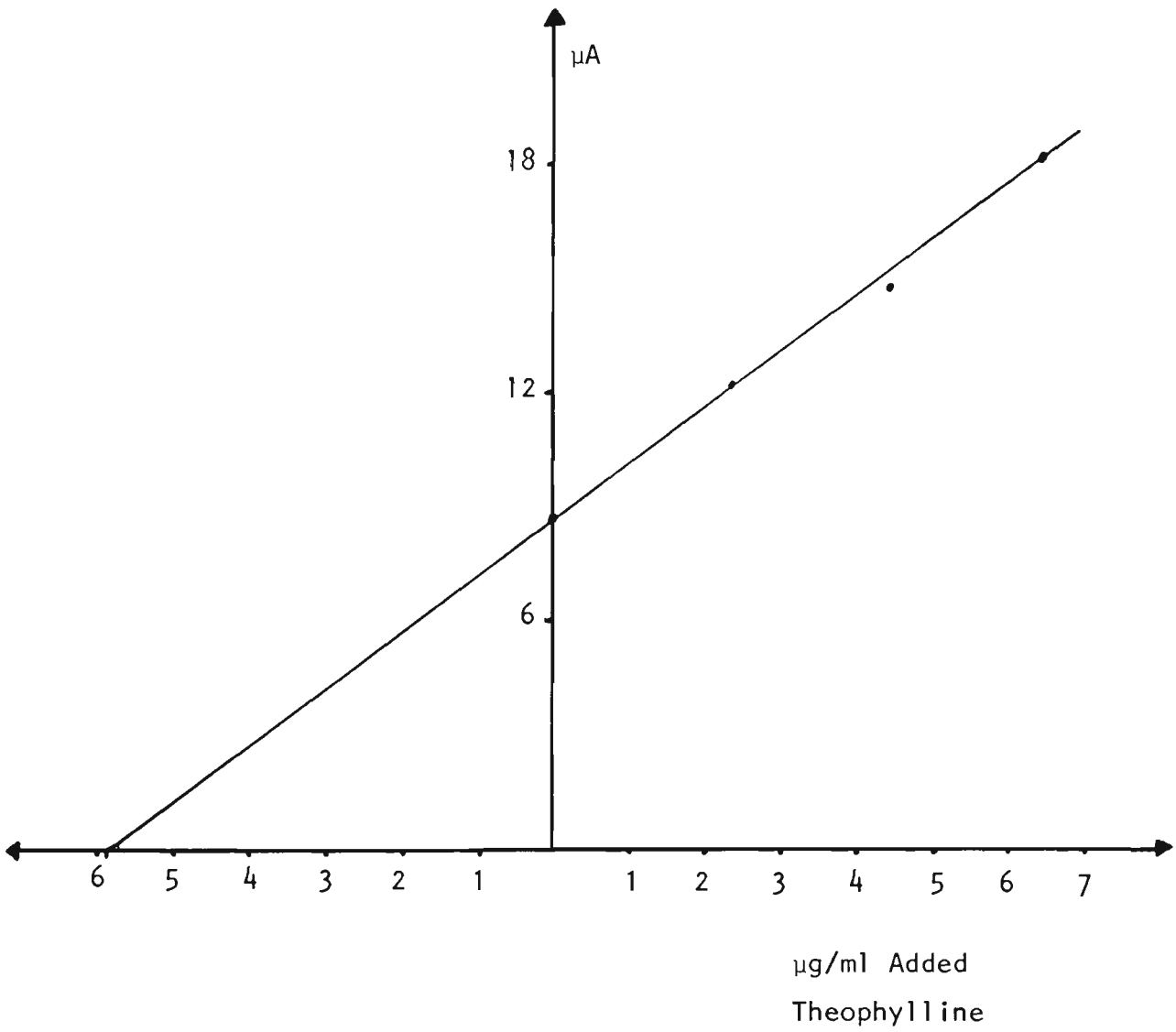
Concentration Added of theophylline µg/ml	Step Height, ΔI µA
0.00	8.98
2.20	12.18
4.30	14.73
6.31	18.20
Initial concentration by computer least squares standard additions calculation = 5.84 ± 0.2 5.84 ± 0.2 µg/ml ≡ 5.27 ± 0.2 mg/ml of theophylline in syrup	

4.3.5.2 Elixophyllin

A sample of this cough syrup was analysed by PRA under the same conditions as Neullin Syrup. The results for the standard additions analysis are shown in Table 4.7.4.

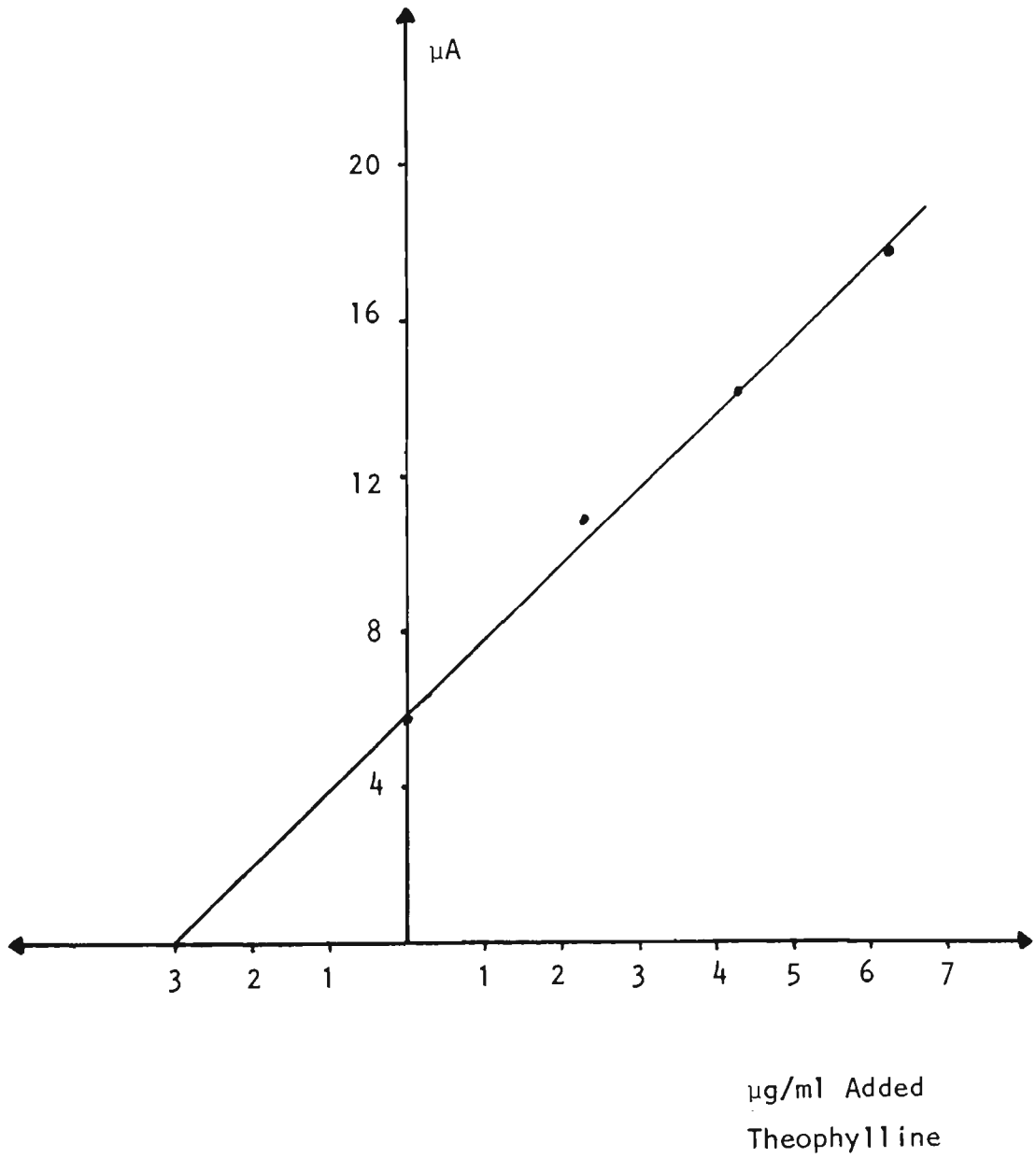
The concentration was also determined graphically. The standard additions plot is shown in Fig. 4.3.8.

FIG. 4.3.7 - GRAPHICAL STANDARD ADDITIONS ANALYSIS OF THEOPHYLLINE IN NUELLIN*SYRUP



Initial Concentration = 5.9 µg/ml

FIG. 4.3.8 - GRAPHICAL STANDARD ADDITIONS ANALYSIS OF THEOPHYLLINE IN ELIXOPHYLLIN



INITIAL CONCENTRATION = 3.0 µg/ml

The PRA result, compared with the UV spectrophotometric result of 5.38 mg/ml theophylline, shows that PRA can be used for quality control analyses of Elixophyllin cough syrup.

TABLE 4.3.4 - Standard Additions Result for Elixophyllin

Concentration Added of Theophylline µg/ml	Step Height, ΔI µA
0.00	5.64
2.20	11.09
4.30	14.79
6.3.	18.24
Initial concentration by computer least squares standard additions calculation = 3.02 ± 0.2 µg/ml theophylline 3.02 ± 0.2 µg/ml ≡ 5.44 ± 0.4 mg/ml of theophylline in Elixophyllin	

4.3.5.3 Intravenous Injection Solution

As an independent check on the accuracy of PRA, a product sample containing theophylline was sought from an industrial quality control laboratory. Sigma Pharmaceuticals Ltd. provided an analyzed sample of an aminophylline injection. The product was labelled as follows: 'SIGMA - AMINOPHYLLINE B.P. 0.25 g in 10 ml for I.V. (B) AM 120'. Quality control UV spectrophotometric analysis by Sigma found the sample to contain 21.3 mg/ml theophylline. Since aminophylline is a 2:1 theophylline:ethylenediamine mixture, the 21.3 mg/ml

assay result is equivalent to 24.9 mg/ml aminophylline.

Hydrodynamic voltammetry was used to test for the presence of electroactive interferents in the sample. Theophylline was the only electroactive compound observed in the voltammograms. Ethylenediamine is possibly electroactive but under the conditions of the PRA analysis it is more difficult to oxidize than theophylline and therefore presents no interference.

The aminophylline was diluted with pH 7 phosphate buffer and determined by PRA. The results for the standard additions analysis are shown in Table 4.3.5.

TABLE 4.3.5 - Standard Additions Result for Aminophylline I.V. Injections

Concentration Added Theophylline µg/ml	Step Height, ΔI µA
0.00	15.96
2.20	19.09
6.31	23.71
8.25	37.29

Initial concentration by computer least squares standard additions calculation = 11.8 ± 0.2 µg/ml theophylline.

11.8 ± 0.2 µg/ml ≡ 21.2 ± 0.4 mg/ml Theophylline in Aminophylline Injection

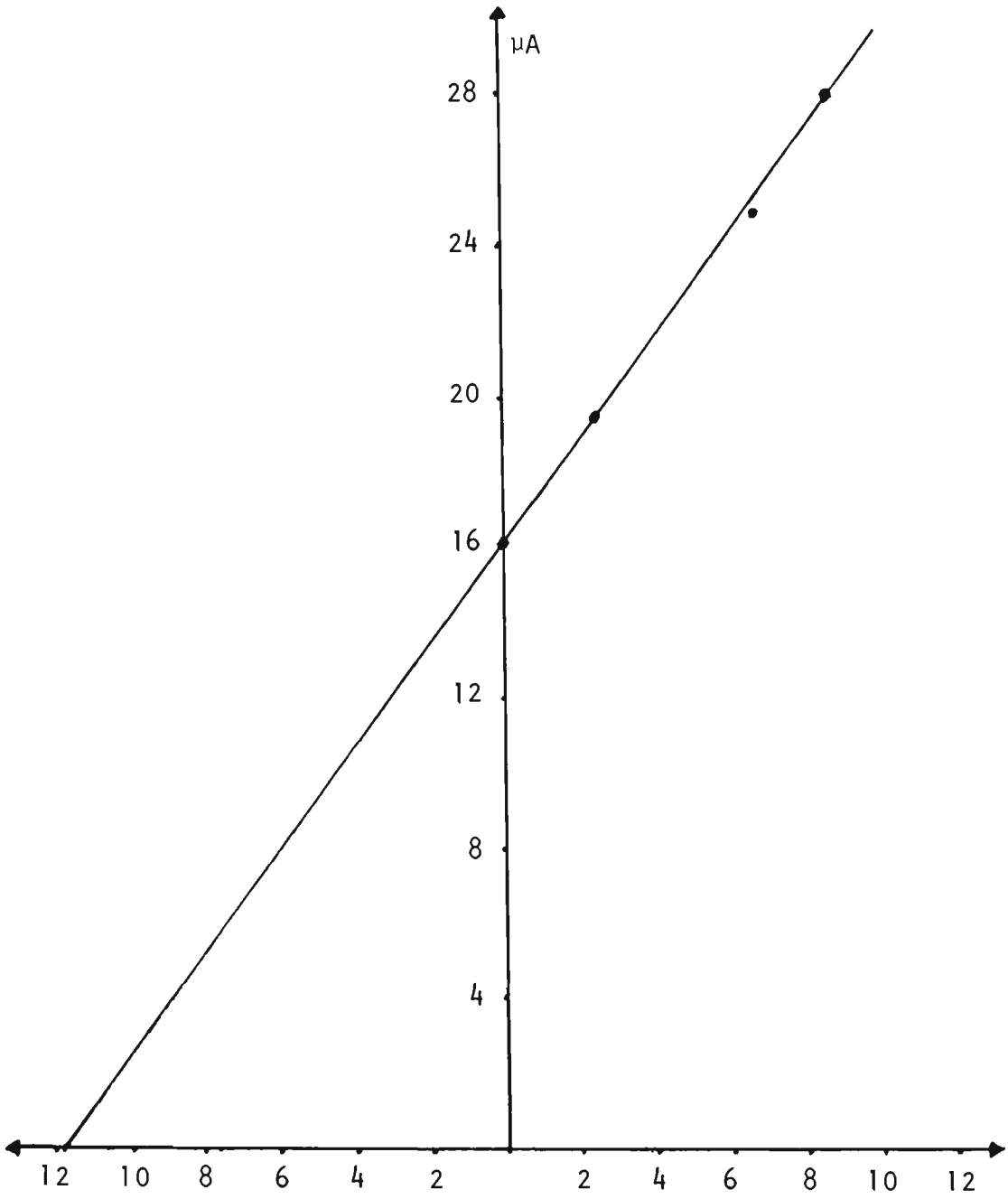
The concentration was also determined graphically. The result is shown in Fig. 4.3.9.

The PRA result of 21.2 mg/ml theophylline agrees very well with the independent assay of 21.3 mg/ml. This demonstrates that PRA can also be applied to quality control analyses of aminophylline injections.

4.3.6 Conclusion

The pulsed rotation amperometric determination of theophylline provides a fast, reliable and accurate quality control procedure for a variety of pharmaceutical products. Analysis time is in the order of 15 minutes per sample using computer calculation of results. The relative standard deviation of the analyses is 3% and the results all agree with UV spectrophotometric determinations.

FIG. 4.3.9 - GRAPHICAL STANDARD ADDITIONS ANALYSIS OF THEOPHYLLINE IN AMINO PHYLLINE I.V. INJECTION SOLUTION



INITIAL CONCENTRATION = 11.8 µg/ml

CHAPTER 5

Investigation of a Direct Electroanalytical Technique for the Determination of Theophylline in Clinical Samples

5.1 Introduction - Statistical Analysis

The development of an analytical method requires awareness of the accuracy and precision needed for effective use to be made of the analytical data. Statistical investigation provides a means of determining allowable tolerances in analytical results. The tolerances aid the development of a protocol with respect to 1) the number of measurements required per sample, 2) the sampling techniques and 3) the pretreatment procedures.

A statistical analysis of theophylline therapy was carried out prior to the investigation of direct electrochemical techniques for the monitoring of theophylline serum levels. The statistical analysis determined how many measurements per sample are required so that safe and effective prescription, based on the analytical data, may be achieved with a certainty of 95%.

5.1.1 The Theophylline Therapeutic Range

The generally accepted theophylline therapeutic range for the treatment of asthma symptoms is 10-20 μg/ml in serum. This level, determined initially by Jenne and co-workers,[238] is based on clinical trials relating to the effect and toxicity of theophylline. The results of these trials are based on a statistically small population (7 patients) but were proved correct by subsequent workers.[239-242] Although the population is small, Jenne et al. results are used in this analysis because they also conducted a clinical trial to prove the validity of adjusting theophylline doses using a single measurement of theophylline serum concentration. This provided a means of verifying the results of this statistical analysis.

Since the population is small, only an approximate estimate is achieved by this analysis but the procedures used provide a technique that would give more accurate results when based on a statistically reliable population size.

5.1.2 The Standard Deviation of Therapeutic Range

The therapeutic range of 10-20 μg/ml is assumed to be of normal distribution and as such has a mean, $\bar{x}$, and a variation associated with it equal to Ks, where K is a tolerance and confidence limit factor and s is the standard deviation.[280]

$$\text{i.e., THERAPEUTIC RANGE} = \bar{x} \pm Ks$$

The factor K is such that the probability is P that a certain proportion, F, of the distribution will be included between $\bar{x} \pm Ks$, where $\bar{x}$ and s are estimates of the mean and standard deviation computed from a sample population N.[281]

Since the range is known and the distribution is assumed symmetrical then the range can be expressed as,

$$15 \pm 5 \text{ μg/ml} \equiv 10\text{-}20 \text{ μg/ml}$$

Using this expression for the range, the product Ks is 5.

$$\text{i.e., } Ks = 5$$

K is dependent on the number of the population, N, the fraction, F, and probability, P, (or certainty) and these dependents are known, so the appropriate value of K, for this case, can be determined and therefore so can s be determined.

Using J.W. Jenne's et al. results, where N = 7, F = 0.85 and the certainty required, i.e., 95% (P = .95), K was calculated from standard statistical tables.[282] In this case K = 3.033 and therefore the standard deviation, s, for the therapeutic range is

$$s = 5/3.033$$
$$= 1.65 \text{ µg/ml}$$

Using the mean of 15 µg/ml the standard deviation, ± 1.65 µg/ml, can be expressed as a percentage, i.e., 11%. This standard deviation is the maximum error allowable to have 85% of the cases fall in the therapeutic range and have 95% confidence in the range. When prescribing the actual dose required to give the therapeutic level, based on laboratory analysis of theophylline, then the maximum allowable standard deviation in the total laboratory analysis must not exceed the standard deviation in the range.

5.1.3 Calculation of Required Number of Measurements

It is known that the total variance of an analysis, S_T, is equal to the sum of the component variances.[283]

$$\text{i.e., } (S_T)^2 = (S_A)^2 + (S_S)^2 + (S_p)^2$$

where S_A = the standard deviation of the actual measuring stage. This includes instrumental, voltammetric and mechanical errors.

S_S = The standard deviation in the actual representativeness of the blood sample compared to the whole body's blood system.

and S_p = The standard deviation due to any pretreatment of the sample such as storage, freezing or centrifugation.

Since S_T must be less than or equal to the standard deviation for the therapeutic range, s, then the condition for safe and effective prescription is

$$\sqrt{(S_A)^2 + (S_S)^2 + (S_p)^2} \leq 1.65 \text{ µg/ml or } 11\%$$

The literature reveals[284] that, based on a comparison between venous and capillary samples, S_S is in the order of 0.1%. Also storage studies on theophylline serum levels show that S_p is in the order of 0.3%.[285] A comparison of S_p and S_S with the S_A for a number of measurement techniques (see Table 5.1) shows that S_S and S_p are negligible compared to S_A and the actual measurement step limits the precision of the total analysis.

TABLE 5.1 S_A for Various Techniques

Measurement Technique	S_A (RSD %)
Fluorimetry[286]	6.0%
Spectrophotometry[238]	4.0%
HPLC[287]	1.8%
Voltammetry*	3.5%

* *Experimental result - see Section 4.2*

The condition for safe and effective prescription becomes,

$$S_A \leqq 11\%$$

and the number of measurements per sample required to meet this condition can be calculated.

Where a reliable estimate of standard deviation, σ, (such as reported RSD's) is available, the following expression for confidence limits can be used.[288]

$$\bar{x} \pm CL = \bar{x} \pm \frac{Z\sigma}{\sqrt{N}}$$

where CL = Confidence limit

$\bar{x}$ = Mean

σ = Reliable estimate of standard deviation

N = Number of readings or measurements

Z = A factor related to the level of confidence required.

The allowable confidence limit may be expressed as a percentage of the mean.

$$\text{i.e.,} \quad CL = \frac{y}{100} \cdot \bar{x}$$

where y = the actual percentage of the mean allowable, in this case 11%.

Therefore

$$\frac{y}{100} \cdot \bar{x} = \frac{Z\sigma}{\sqrt{N}}$$

When using RSD, σ can be expressed as

$$\sigma = \frac{\alpha}{100} \cdot \bar{x}$$

where α = the RSD percentage value.

Combination of these equations results in the following expression for N.

$$N = \left(\frac{Z\alpha}{y}\right)^2$$

The factor Z (obtained from standard tables.[280]) for 95% certainty is 1.96 and therefore N was calculated for the analytical techniques. The results are presented in Table 5.2

TABLE 5.2 Values of N for Analytical Techniques

Technique	σ	N
Voltammetric	3.5%	0.4
Fluorometric	6 %	1.1
Spectrophotometric	4 %	0.5
H.P.L.C.	1.8%	0.1

and show that only one (1) measurement is required for safe and effective prescription at these levels of measurement precision. This agrees with the clinical trial conducted by Jenne et al. where one measurement of theophylline serum concentration was found to be adequate to adjust serum levels to the therapeutic range.

5.2 Introduction - Direct Electrochemical Analysis of Theophylline

Munson and Abdine reported[272] the direct determination of theophylline in plasma by oxidation at the stationary carbon paste electrode using differential pulse voltammetry. The theophylline metabolite 3-methylxanthine significantly interferes in the analysis of samples from patients who have undergone prolonged theophylline therapy. Therefore they state that the method is unsuitable for routine clinical application but may be of use in overdose cases.

In cases of asthma in neonates Munson and Abdine's method would be applicable for the initial monitoring of theophylline absorption, since 3-methylxanthine would be absent from the samples. The electrochemical method would provide faster results than the conventional, large sample batch, radioimmuno-assay techniques,[254-256] which require a few hours to obtain results; but not as selectively as HPLC.[259-271] The method would require modification to account for the smaller sample sizes (ca. 100 µL) and the lower therapeutic range (1-10 µg/ml).

Non invasive methods, based on saliva levels of theophylline have been reported.[289-290]

The saliva and serum concentrations are of the same order of magnitude and therefore within the detection capabilities of the electrochemical methods.

To apply a direct electrochemical technique to the initial monitoring of theophylline in neonates the methods must be rapid, be capable of using small sample volumes of serum or saliva, be

sensitive enough to detect the lowest concentration in the therapeutic range, be used under selective conditions, meet the reliability criterion set out in the statistical analysis of theophylline therapy and compare well with other analytical techniques.

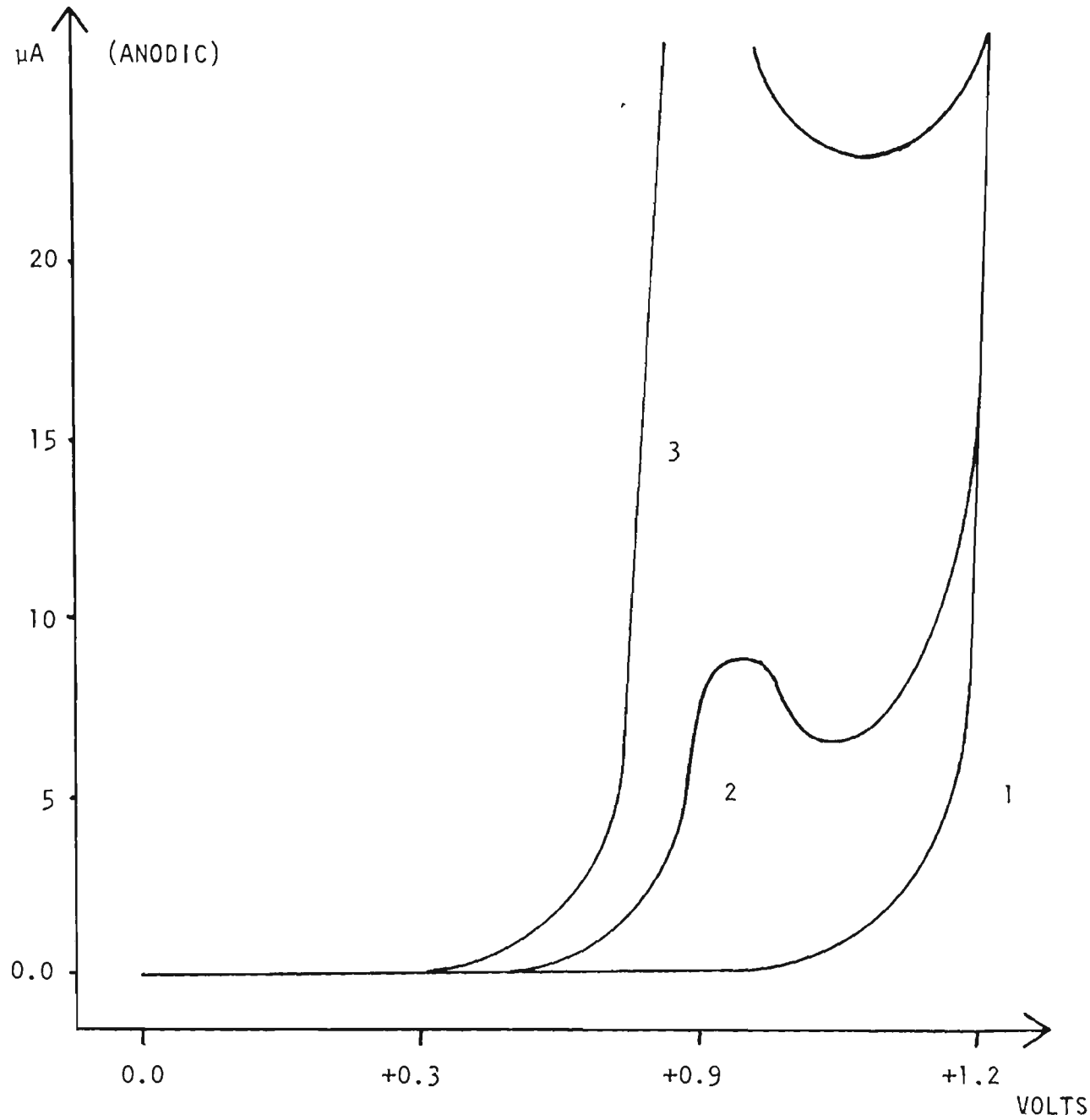
Of the direct electroanalytical techniques available for this investigation, Flow Injection Analysis with electrochemical detection was the most favourable since it is rapid, sensitive, requires small sample volumes, is as selective as other direct electroanalytical methods and is precise.[291]

Therefore the voltammetric behaviour of theophylline in a saliva matrix was studied in an attempt to utilize a non invasive method and the optimum FIA conditions for theophylline determination were investigated.

5.2.1 Voltammetry in Saliva

Saliva diluted twofold in pH = 7 phosphate buffer exhibits a large anodic peak in the region of theophylline oxidation (see Fig. 5.1) at a carbon paste electrode. Comparison of the magnitude of the saliva peak with that of a 10 µg/ml theophylline peak is also shown in Fig. 5.1. The result clearly demonstrates that the electroactive component in saliva makes the determination of theophylline in this medium impossible by a direct electrochemical technique.

FIGURE 5.1 - VOLTAMMETRY OF 1:1 SALIVA/PHOSPHATE BUFFER



CONDITIONS: STATIONARY CARBON PASTE ELECTRODE
PLATINUM COUNTER ELECTRODE
Ag/AgCl saturated KCl/10% KNO_3 REFERENCE ELECTRODE
SCAN RATE : 5 mV/sec. SCAN MODE : DIFFERENTIAL PULSE

LEGEND: 1 - Phosphate buffer pH 7
2 - Phosphate buffer pH 7 + [Theophylline] = 10 μg/ml
3 - 1:1 Saliva/Phosphate buffer

5.3 Investigation of F.I.A. Optimum Conditions for Theophylline Determination

Since carbon paste is unsuitable for wall jet electrochemical detectors the voltammetric behaviour of theophylline was investigated at a glassy carbon electrode. Anodic voltammograms show a steady decrease in the height of the theophylline peak at +1.0 Volts with consecutive voltammetric scans, due to product accumulation, as was observed with carbon paste. (Section 4.2). Also an FIA system using a glassy carbon electrode was tested with theophylline and the corresponding decrease in FIA peak height was observed with consecutive injections. (See Fig. 5.2). For FIA to be applied to theophylline determinations an electrode cleaning technique that gives reproducible glassy carbon electrode areas was required. Therefore the following factors were investigaged with respect to their effect on product accumulation at glassy carbon electrodes under hydrodynamic conditions.

Cathodic Cleaning

Solvent Composition of FIA Carrier Stream

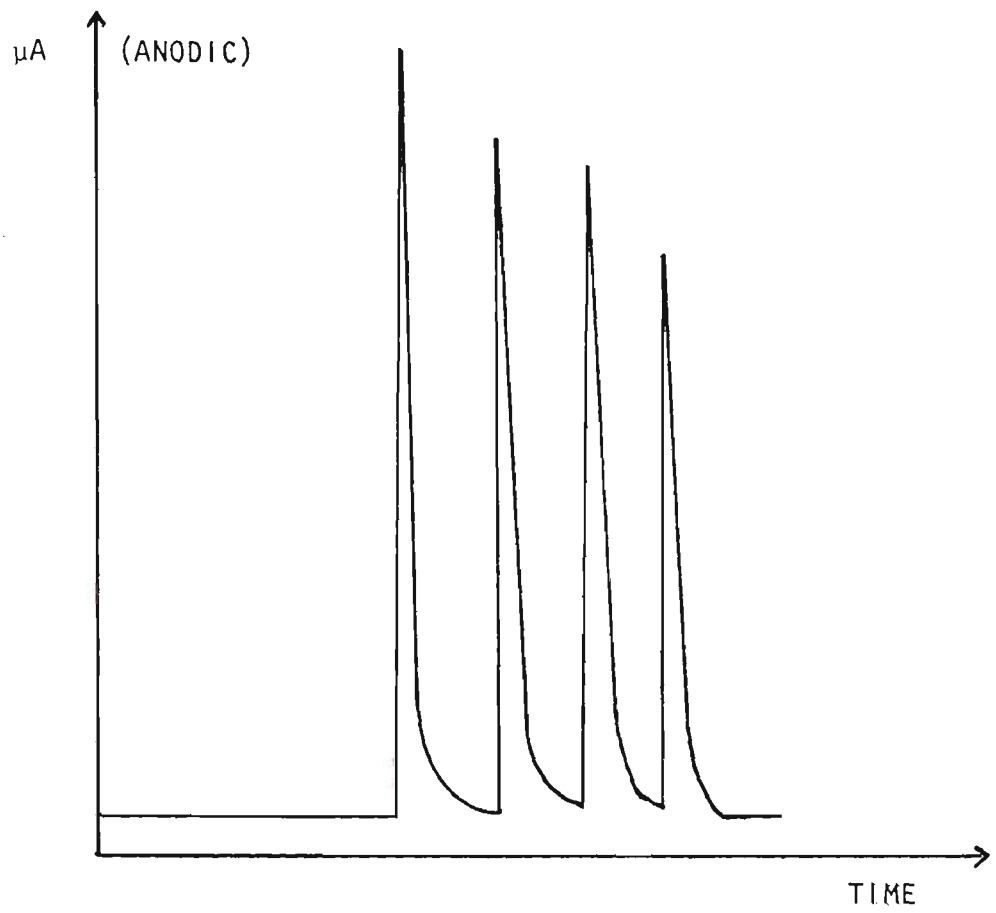
Pure Methanol Cleaning

Potassium Hydroxide Cleaning Injections.

5.3.1 Cathodic Cleaning of F.I.A. Glassy Carbon Electrode

The glassy carbon electrode used in the FIA of theophylline in the previous section was found to be cleaned by hydrodynamic continuous cyclic voltammetry. Cyclic voltammograms were recorded from -0.5 to +1.0V vs. a Ag/AgCl sat. KCl reference electrode by continuous cycling of the potential while having a flow rate of 1.5 ml/minute through the cell. After 15 to 20

FIGURE 5.2 - THEOPHYLLINE F.I.A. AT GLASSY CARBON ELECTRODE WITHOUT SURFACE CLEANING



DETECTION POTENTIAL + 1.0V vs. Ag/AgCl sat KCl

FLOW RATE 1.0 ml/min

ANALYTE 10^{-5}M THEOPHYLLINE 90 μL LOOP INJECTIONS

SGE WALL JET ELECTROCHEMICAL DETECTOR.

pH 7 PHOSPHATE BUFFER ELECTROLYTE

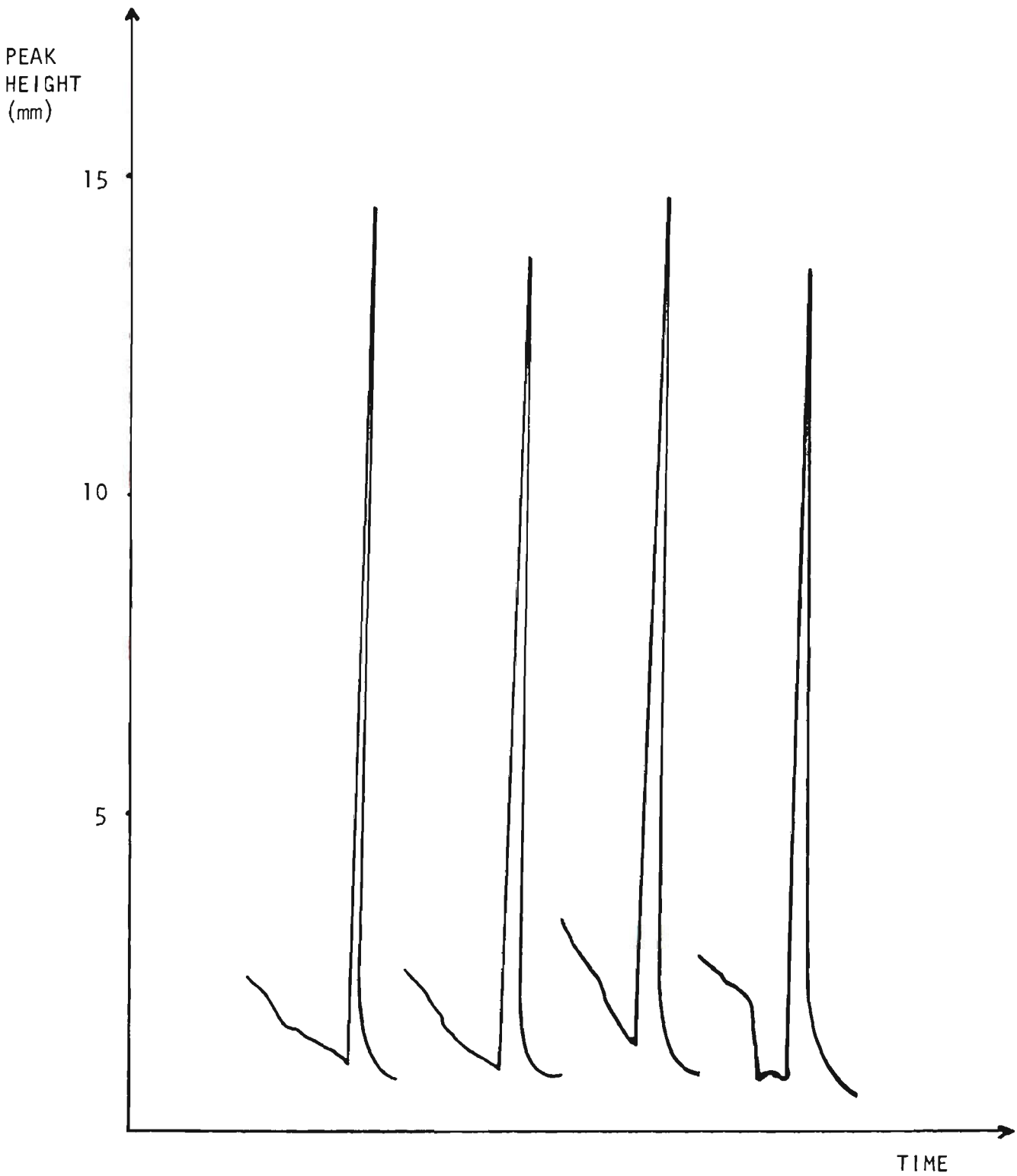
minutes of this process the electrode surface is reproducible.

Therefore cathodic cleaning in between theophylline injection was investigated. A potential of -0.5V was applied to the electrode for 20 seconds with electrolyte flowing through the cell, then the potential was returned to +1.0 Volts and when the capacitive current decayed to a steady baseline a theophylline injection was made.

The results of 4 consecutive injections using this technique are shown in Fig. 5.3.

These peaks have a mean and standard deviation of 134 ± 5 mm corresponding to a RSD = 4%. The time required for the recorder pen to reach a useable baseline after the 20 second cathodic clean was about 3 minutes. To achieve a flat baseline requires between 10 and 15 minutes. Other cleaning conditions were investigated, such as increasing cleaning time and using less negative cleaning potentials, but the results were not as good as those reported here. The disadvantage of this technique is that after a number of cleanings and injections the detector cell becomes susceptible to extraneous capacitive influences giving rise to random electrical noise problems similar in behaviour to that of gas bubbles being trapped in the flow cell. Theory[292] predicts that a potential of -0.6 Volts versus a Ag/AgCl reference is necessary to produce hydrogen gas at pH = 7 and therefore it was thought unlikely that H_2 bubbles were produced during the cathodic cleaning at -0.5 Volts.

FIGURE 5.3 - F.I.A. WITH CATHODIC CLEANING BETWEEN INJECTIONS



FLOW RATE = 1.5 ml/min

BACKGROUND ELECTROLYTE = pH 7 PHOSPHATE BUFFER

[THEOPHYLLINE] = 3.3 µg/ml - 90 µL LOOP INJECTIONS

APPLIED POTENTIAL = +1.0V vs. Ag/AgCl sat. KCl

S.G.E. WALL JET CONFIGURATION WITH GLASSY CARBON ELECTRODE

CLEANING CONDITIONS : 20 SECONDS AT -0.5 V.

These random noise problems introduce unacceptable uncertainty into the technique and therefore other cleaning procedures were investigated.

5.3.1.1 The Investigation of Solvent Composition on the Deposition of Theophylline Oxidation Products Under Hydrodynamic Conditions

For convenience this investigation was carried out using a rotating glassy carbon electrode rather than the electrochemical flow cell.

5.3.1.2 The effect of 20% methanol 80% phosphate buffer

The influence of 20% methanol on the solubility of the oxidation products was investigated by the following procedure. Three consecutive rotating electrode differential pulse voltammograms of theophylline in 20% Methanol 80% Phosphate were run and the peak heights recorded. The electrode was left at the solution open circuit potential and with a rotation rate of 20 r.p.s. for 10 minutes and three more consecutive voltammograms were run. The results of these experiments are shown in Table 5.3.

These results indicate that the oxidation products are slightly soluble in this solvent composition but even under hydrodynamic conditions the electrode area is still reducing with consecutive sweeps. Therefore this solvent composition is unsuitable for FIA of theophylline.

TABLE 5.3 20% Methanol/80% Phosphate Buffer Voltammetric Results

Run	Theophylline Peak Heights (mm)
1	160
2	150
3	130
10 Minute Pause	
4	140
5	125
6	115

CONDITIONS: ROTATING GLASSY CARBON ELECTRODE
Ag/AgCl sat KCl REFERENCE ELECTRODE
PLATINUM COUNTER ELECTRODE
ROTATION RATE 20 r.p.s.
VOLTAMMETRIC MODE - DIFFERENTIAL PULSE
+20 mV PULSE
SENSITIVITY 4×10^{-8} A/mm.
SCAN RANGE 0.0 → +1.5V
ELECTROLYTE = 80% 0.2% $NH_4H_2PO_4$ (pH = 7.5)/
20% METHANOL
AVERAGE RUN TO RUN REDUCTION IN PEAK HEIGHT = 9.5%

5.3.1.3 The effect of Methanol/Acetonitrile/Phosphate buffer solvent

The following solvent compositions were investigated by the same technique as the Methanol/Buffer system (5.3.1.2). Each solvent system basically showed the same results as the previous work. The average reduction in peak height from run to run is reported in Table 5.4.

TABLE 5.4

System	% Peak Height Reduction	Composition
1	16.5%	19% METHANOL 1% ACETONITRILE 80% PHOSPHATE BUFFER
2	16.0%	18% METHANOL 9% ACETONITRILE 73% PHOSPHATE BUFFER
3	11.5%	20% ACETONITRILE 80% PHOSPHATE BUFFER
4	12.5%	38.5% ACETONITRILE 61.5% PHOSPHATE BUFFER

These solvent compositions offer no improvement in the reproducibility of the electrode surface area. Also the 20% methanol/buffer composition gives the smallest reduction in average peak height (9.5%). Therefore higher percentages of methanol in the solvent were investigated.

5.3.1.4 60% Methanol/Phosphate Buffer Solvent

The glassy carbon electrode was cathodically cleaned at -0.6V versus a Ag/AgCl sat KCl reference electrode in the methanol/buffer mixture. A differential pulse voltammogram of the background electrolyte was recorded followed by seven consecutive voltammograms of the electrolyte solution containing 1.46 µg/ml of theophylline. The electrode was

allowed to stand without rotation in the solution for 2 hours and then another voltammogram was recorded. These results are shown in Table 5.5.

TABLE 5.5 Peak Heights for Theophylline in 60% Methanol Phosphate Buffer

Concentration	Consecutive Voltammetric Run Number	Peak Height mm
BACKGROUND ELECTROLYTE	0	0
1.46 µg/ml Theophylline	1	52
	2	45
	3	44
	4	41
	5	41
	6	41
	7	41
1.46 µg/ml	8	53

These results show that after cathodic cleaning the available electrode area is large as was observed for the other solvent systems, then it decreases as oxidation products are deposited. But in this case after 4 runs the rate of deposition equals the rate of cleaning and therefore the recorded peak heights become constant. Run number 8 shows that as the cleaning rate increases past the deposition rate the available electrode area increases.

TABLE 5.6 Peak Heights for Theophylline in 60% Methanol 40% Phosphate Buffer at various concentrations

Concentration µg/ml	Consecutive Voltammetric Run Number	Peak Heights (mm)	Mean ± Standard Deviation of Peak Heights (mm & R.S.D.)
0	-	0	---
1.46	1	48.0	45.7 ± 2.0
	2	44.5	
	3	44.5	i.e., 4.4%
2.92	1	64.5	61.8 ± 1.9
	2	62.0	
	3	61.0	i.e., 3.1%
	4	60.0	
4.38	1	83.0	81.8 ± 1.3
	2	82.0	
	3	82.0	i.e., 1.5%
	4	80	
5.84	1	98.0	92.3 ± 2.2
	2	96.0	
	3	93.5	i.e., 2,4%
	4	93.5	
7.30	1	108.5	99.1 ± 8.5
	2	103.0	
	3	96.0	i.e., 8.5%
	4	89.0	

The differential pulse voltammogram peak heights for a series of theophylline solutions were recorded. Several consecutive voltammograms were run for each concentration. The only cleaning process taking place was that achieved by convection of the solvent to the electrode between and during each voltammogram. The results are presented in Table 5.6.

The same trend is observed for each concentration as was exhibited by the previous results except that as the concentration of theophylline increases the problem of electrode area depletion becomes more severe. Two calibration curves were plotted from these results, the first run peak heights were used and the mean of each set of voltammetric runs were used. The calibration curves are shown in Fig. 5.4 and Fig. 5.5.

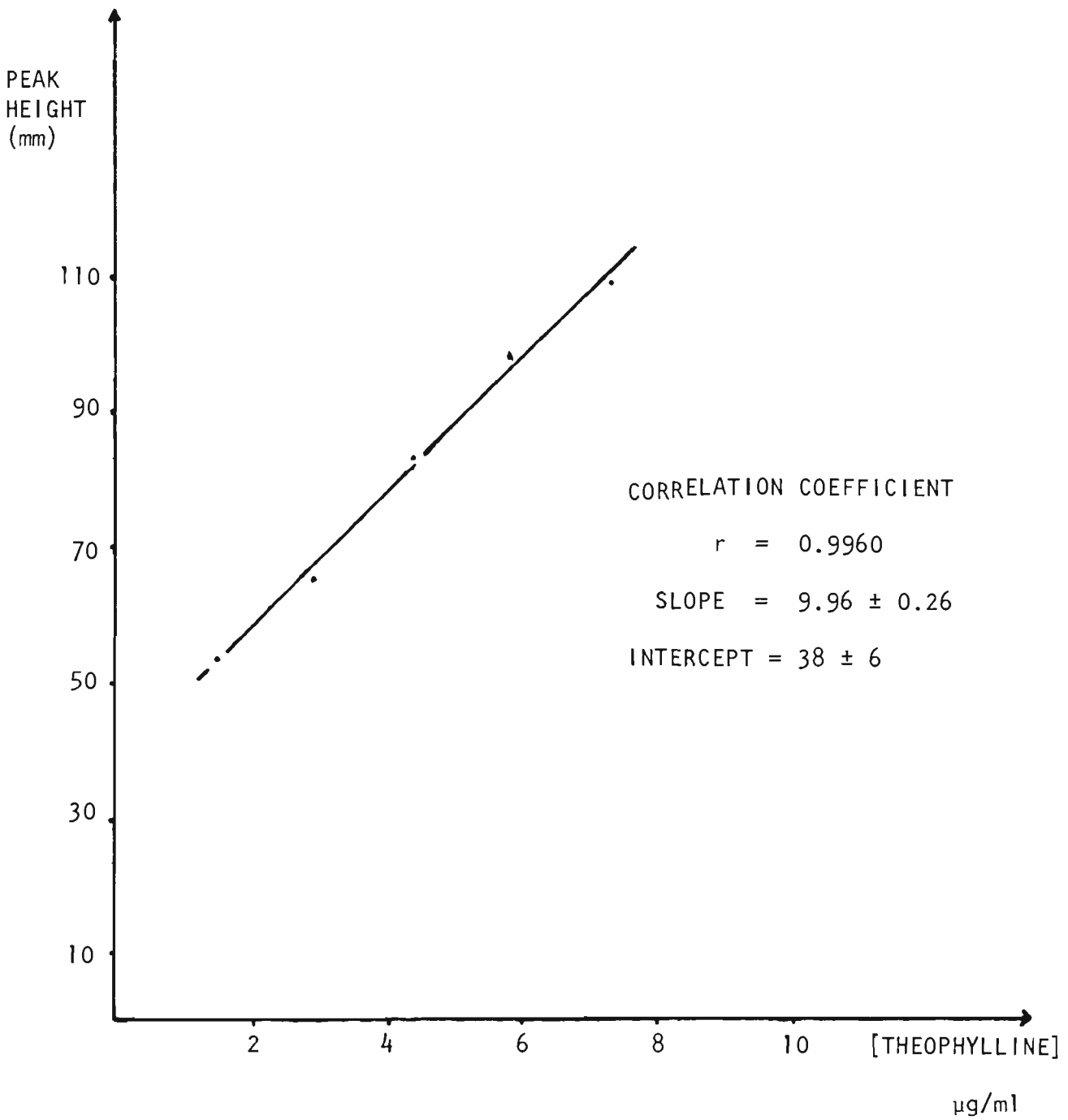
These results show that linear calibration curves can be achieved for theophylline at a glassy carbon electrode provided that precise cleaning procedures are used. Also the concentration of theophylline must be kept below approximately 6 µg/ml for these conditions to avoid curvature of the calibration plot.

In these measurements the cleaning time is in the order of 5 minutes between product depositions.

5.3.2 Methanol electrode cleaning between measurements

The data from the previous experiments indicates that to improve analysis time and the precision of FIA peak heights a more vigorous cleaning technique must be used. Cleaning of the electrode with methanol was investigated by the rotating electrode technique for preliminary studies. FIA was attempted with pure methanol cleaning injections between measurements.

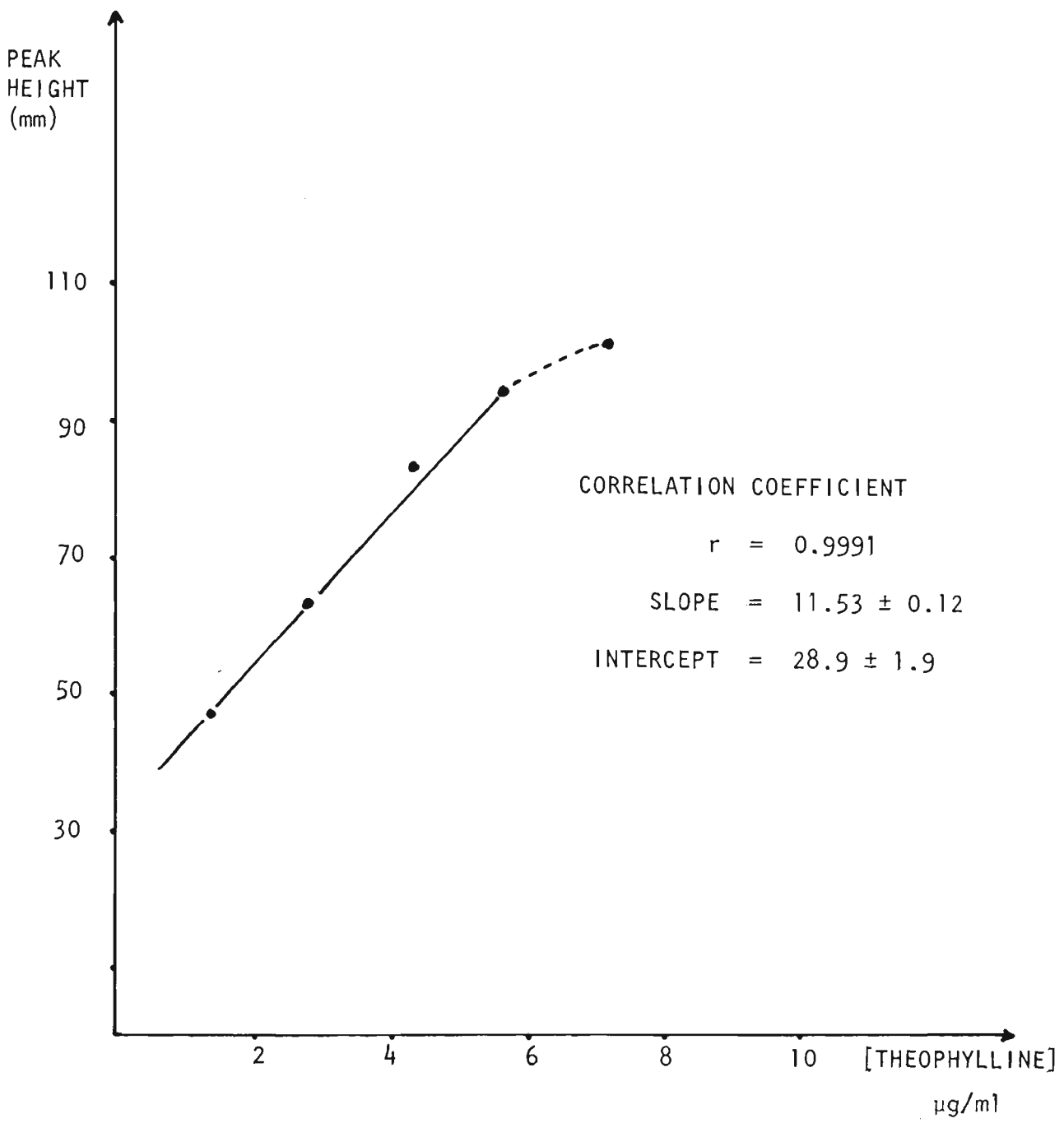
FIGURE 5.4 - FIRST RUN PEAK HEIGHTS vs. THEOPHYLLINE CONCENTRATION



60% METHANOL/40% PHOSPHATE BUFFER

GLASSY CARBON ROTATING ELECTRODE (20 R.P.S.)

FIGURE 5.5 - AVERAGE PEAK HEIGHTS vs. THEOPHYLLINE CONCENTRATION



60% METHANOL/40% PHOSPHATE BUFFER

GLASSY CARBON ROTATING ELECTRODE (20 R.P.S.)

5.3.2.1 Electrode Cleaning by Soaking in Methanol

The rotating glassy carbon electrode was soaked in pure methanol for 30 seconds in between measurements. 30 seconds is the approximate residence time of a sample bolus in the electrochemical F.I.A. cell and this procedure was used to approximate the F.I.A. conditions where a cleaning injection of methanol would be used between each theophylline injection.

The results of this cleaning technique, for three theophylline solutions are shown in Table 5.7.

TABLE 5.7 The Effect of 30 second Methanol Cleaning on Peak Heights

Theophylline Concentration (µg/ml)	Peak Heights (mm)	Mean Peak Height $\bar{x} \pm \sigma$ RSD, %
1.6	53.0 55.0 52.5 50.0	52.8 ± 1.9 3.5%
6.4	159.0 150.0 152.0 155.0	154.0 ± 3.9 2.5%
9.6	205 194 188 164	187.8 ± 17.3 9.2%

CONDITIONS: DIFFERENTIAL PULSE VOLTAMMETRY: +20 mV pulse
60% METHANOL/40% PHOSPHATE BUFFER
ROTATING GLASSY CARBON ELECTRODE: 20 R.P.S.

This technique exhibits no improvement in precision or linearity. The obvious curvature at higher concentrations suggests that the cleaning system is still not vigorous enough and therefore wiping the electrode with a methanol soaked tissue was investigated.

5.3.2.2 Mechanical and Methanol Cleaning

The rotating glassy carbon electrode was wiped thoroughly with a tissue soaked in pure methanol between measurements. The results for a series of theophylline solutions are shown in Table 5.8.

TABLE 5.8 The Effect of Methanol soaked tissue cleaning on Peak Heights

Theophylline Concentration (µg/ml)	Peak Heights (mm)	Mean Peak Heights $\bar{x} \pm \sigma$ (mm) (µA) (RSD %)
0.57	125.5 121.0 122.0	122.8 ± 2.4 mm ≡ 0.74 µA 1.9%
1.13	192.0 185.0 185.5	187.5 ± 3.9 mm ≡ 1.13 µA 2.1%
1.70	164.0 162.0 165.0	163.7 ± 1.5 mm ≡ 1.64 µA 0.9%
2.27	125.0 125.5 128.5	126.3 ± 1.9 mm ≡ 1.90 µA 1.5%
5.02	109.0 111.0 114.0	111.3 ± 2.5 mm ≡ 2.78 µA 2.3%

CONDITIONS: DIFFERENTIAL PULSE VOLTAMMETRY: +40 mV pulse
ROTATING GLASSY CARBON ELECTRODE: 20 R.P.S.
PHOSPHATE BUFFER

FIGURE 5.6 - MECHANICAL AND METHANOL CLEANING OF ELECTRODE SURFACE

- CALIBRATION

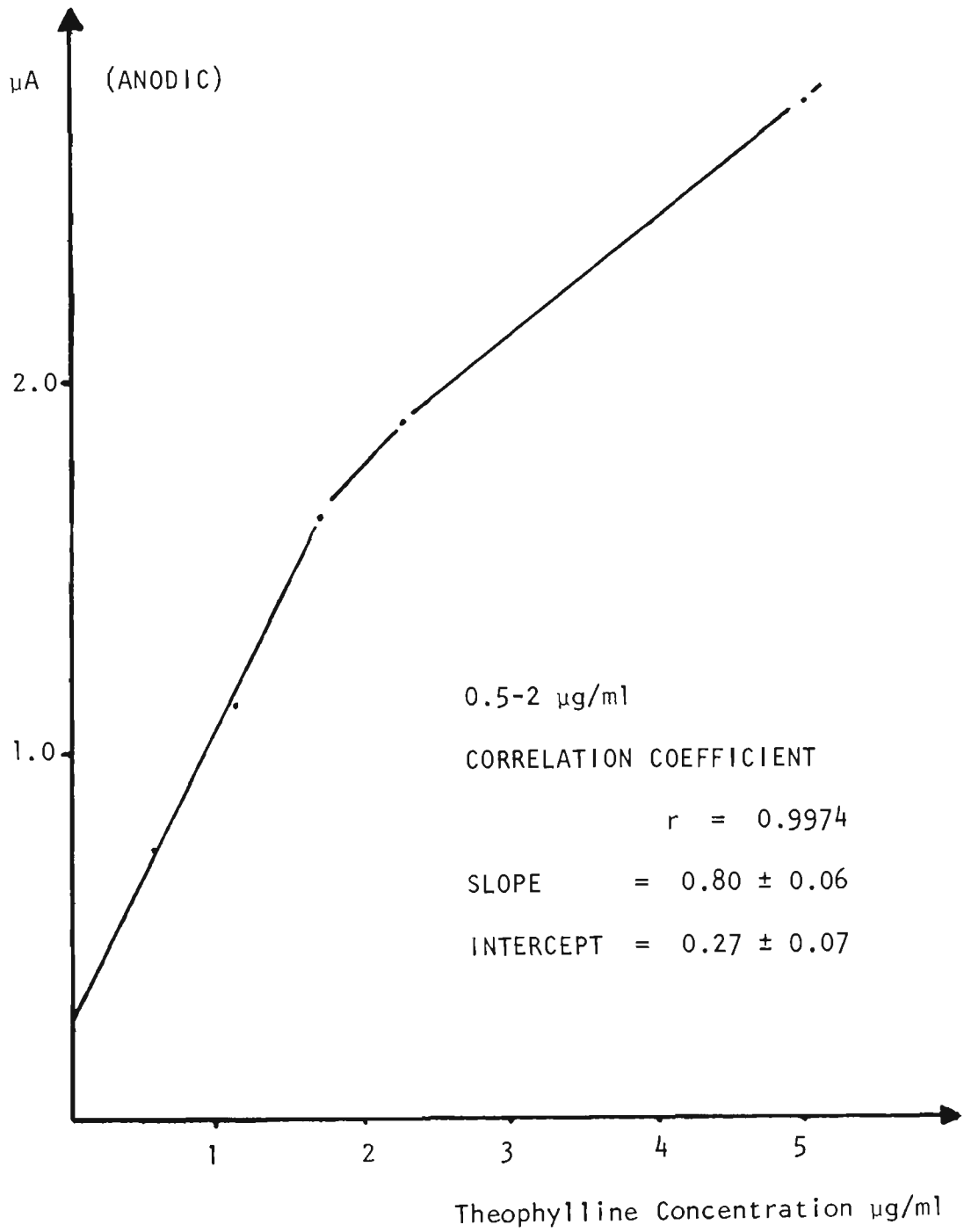


Fig. 5.6 shows a plot of mean peak height versus theophylline concentration for this cleaning technique. In this case the plot is linear up to ~2 μg/ml theophylline, which is lower than observed for the other cleaning techniques.

5.3.2.3 F.I.A. with methanol cleaning injections

Since the best precision (RSD < 2% for each concentration) was achieved with the combination of methanol and mechanical action, methanol cleaning injections in the FIA system were investigated. To optimize cleaning and minimize precipitation problems (due to methanol/buffer interaction) in the FIA solvent line, a solvent composition of 60% methanol/40% phosphate buffer was used. The results for 4 injections of theophylline separated by 4 methanol injections are shown in Table 5.9.

TABLE 5.9 F.I.A. Peak Heights with Methanol Cleaning Injections

Injection No.	Peak Height (mm)	$\bar{x} \pm \sigma$ R.S.D.
1	73.0)	73.1 ± 0.6
2	72.5)	
3	73.0)	0.8%
4	74.0)	

[THEOPHYLLINE] = 4.2 μg/ml
FLOW RATE = 1.0 ml/min
DETECTION POTENTIAL = +1.0V versus Ag/AgCl sat KCl
DETECTION MODE = D.C.

This technique gives very good precision but unfortunately requires more than 5 minutes between injections to allow for the capacitive decay of the background current caused by the methanol injection. This technique's calibration plots also exhibit curvature indicating incomplete cleaning of the surface.

5.3.2.4 F.I.A. with Potassium Hydroxide Injections

1.0×10^{-4}M KOH in 60% Methanol/40% Water was investigated as a cleaning reagent since this solution contains a more vigorous cleaning compound. Also the higher ionic strength than pure methanol enables the time of the capacitive decay observed after cleaning injections to be reduced.

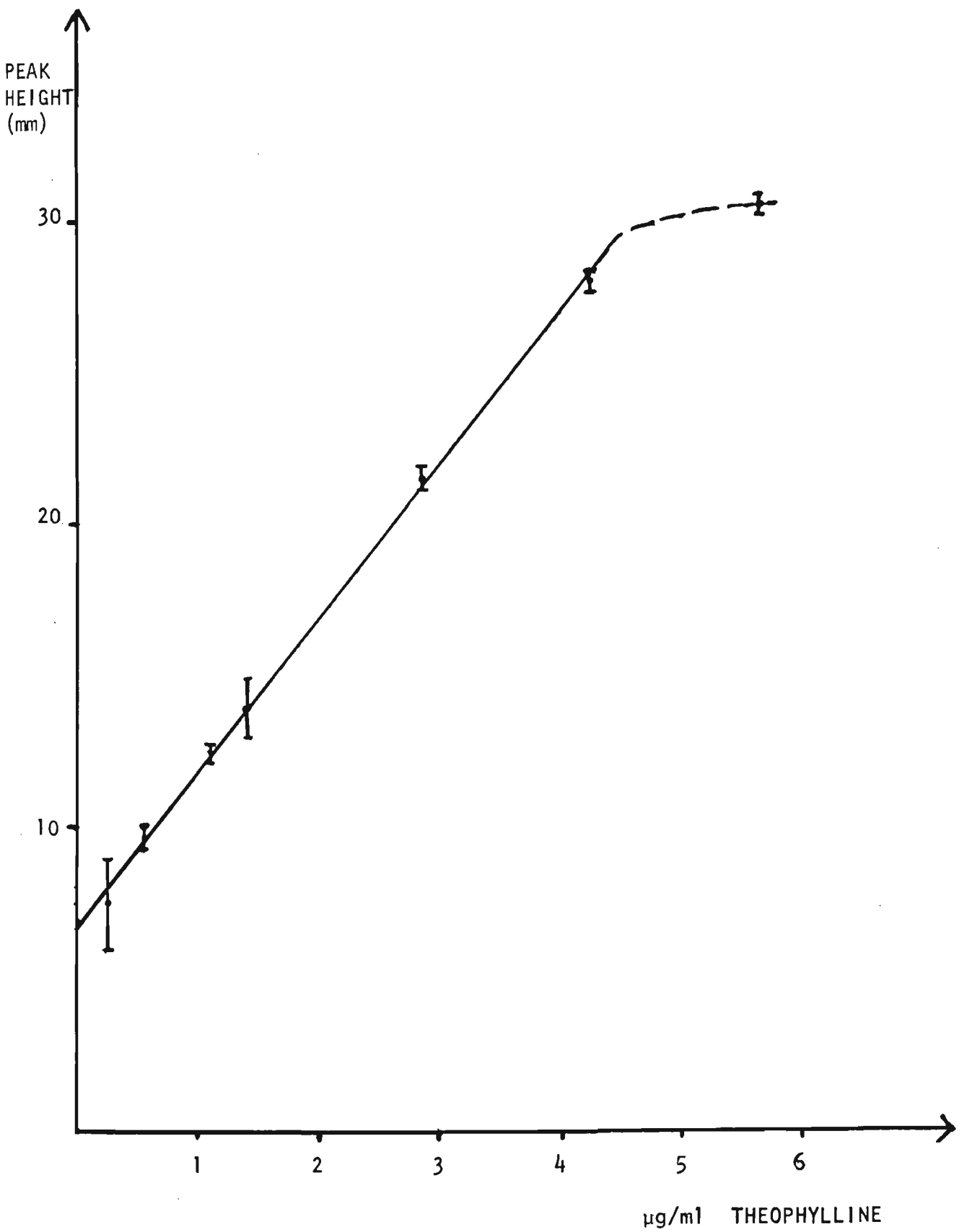
The results for a series of theophylline concentrations using this FIA cleaning technique are shown in Table 5.10.

TABLE 5.10

Theophylline Concentration µg/ml	Peak Height (mm) $\bar{x} \pm \sigma$
0.28	7.5 ± 1.5
0.57	9.7 ± 0.3
1.14	12.5 ± 0.4
1.42	14.0 ± 1.0
2.84	21.6 ± 0.4
4.26	28.2 ± 0.3
5.68	30.8 ± 0.3

A plot of concentration versus peak height is shown in Fig. 5.7. The plot is linear from 0.1 to 4.0 µg/ml

FIGURE 5.7 - KOH CLEANING SYSTEM F.I.A. PEAK HEIGHT vs. THEOPHYLLINE CONCENTRATION



theophylline and fits the following least squares analysis equation:

$$I = 5.2 \pm 0.1 \times C + 6.5 \pm 0.3$$

where I = Peak Height (mm)

C = Concentration (µg/ml)

The correlation coefficient is 0.9992.

These results were the best obtained for any of the cleaning techniques investigated. About 3 minutes is required to obtain a useable base line after each KOH injection.

Sample measurements need to be made directly after calibration since the electrodes response is dependent on the experimental history of the electrode surface.

5.3.3 Summary of Cleaning procedures for F.I.A. of Theophylline

1. Cathodic cleaning provides precise results (RSD = 4%) in an acceptable time (3 minutes per theophylline injection) but suffers the uncertainty of random noise problems.

2. The optimum solvent composition was found to be 60% methanol and 40% pH = 7 phosphate buffer (prepared from $NH_4H_2PO_4$ to aid solubility in methanol). Linear calibrations with acceptable precision are possible but require greater than 5 minutes per measurement.

3. Methanol cleaning injections provide precise results (RSD ≅ 1%) but the technique requires greater than 5 minutes per measurement and exhibits curved calibrations.

4. Potassium Hydroxide cleaning injections, combined with the optimum solvent, provide the best technique for theophylline FIA. The results are precise (RSD < 2%) and rapid (3 minutes per measurement resulting in a total analysis time of about 15 minutes). Calibrations are linear in a useable concentration range and the technique is selective enough to be used for initial neonatal monitoring or overdose cases.

5.4 Conclusion

A protocol for the direct determination of theophylline in serum by FIA was not developed since the method would not significantly outperform (with respect to speed, precision, sensitivity, selectivity and cost) the alternative analytical techniques. HPLC is the most favourable and versatile method for theophylline since it requires small sample volumes, it has good precision (RSD ≅ 2%), is relatively fast (analysis time 20-30 min.) and offers the advantage of separating theophylline from interferents. The only drawback of HPLC compared to the other techniques is the expense of the equipment.

The spectrophotometric and fluorimetric methods are fast (10-20 minutes) and precise enough to permit the use of one measurement per sample, (RSD = 4% and 6% respectively). Both techniques may be adapted to account for small sample sizes. Since these techniques are direct procedures they have the disadvantage of a high number of possible interferents and cannot be used in all circumstances. The

cost of instrumentation for these techniques is comparable to the cost of electrochemical instrumentation.

The results of this investigation provide a good example of the limitations of direct electroanalytical techniques for organic compounds. Although the methods are rapid (120 injections per hour for quinol FIA), precise (RSD = 0.1% PRA for quinol) and sensitive (0.4 ppb quinol detection limit by PRA), they are limited to situations where the electrochemical selectivity is adequate. Insoluble products which accumulate at the electrode surface are produced with many organic analytes and this condition necessitates either the preparation of new surfaces (such as with carbon paste) or cleaning techniques such as those reported here for glassy carbon electrodes. These procedures lengthen analysis time (and therefore the cost per analysis) and introduce more uncertainty in the measurements by increasing the number of steps in the analytical method.

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APPENDIX 1

COMPUTER PROGRAM FOR LEAST SQUARES ANALYSIS OF DATA

(Program written by I.C. Hamilton, Footscray Institute of Technology)

```
 10   REM***LEASQ5*** A LINEAR LEAST SQUARES PROG.
 20   PRINT "LEAST SQUARES CALC. OF STRAIGHT LINE FROM EXPT. DATA"
 30   PRINT "TREATMENT ASSUMES NEGLIGIBLE ERROR IN 'X' VALUES"
100   DIMX(21),Y(21),Z(21),F(21)
110   PRINT "UP TO 2Ø DATA POINTS MAY BE USED"
112   PRINT "TO STOP PROGRAMME TYPE 'Ø' FOR NUMBER OF DATA POINTS"
120   INPUT "NUMBER OF DATA POINTS", N
122   IF N=Ø GOTO 999
125   PRINT "TYPE DATA POINTS 'X, Y' AFTER EACH '?'"
126   FOR I=1 TON
128   INPUT X(I), Y(I)
130   NEXT I
140   LET A=X(1)
150   FOR I=1 TO N-1
160   LET A=A+X(I+1)
170   NEXT I
180   B=Y(1)
190   FOR I=1 TO N-1
200   LET B=B+Y(I+1)
210   NEXT I
220   LET C=X(1)*X(1)
230   FOR I=1 TO N-1
240   LET C=C+(X(I+1)*X(I+1))
250   NEXT I
260   LET D=X(1)*Y(1)
270   FOR I=1 TO N-1
```

Appendix 1 (Cont'd)

```
280   LET D=D+(X(I+1)*Y(I+1))
290   NEXT I
300   LET S=(N*D-(A*B))/(N*C-(A*A))
310   LET M=(B-(S*A))/N
320   LET E=Y(1)*Y(1)
330   FOR I=1 TO N-1
340   LET E=E+Y(I+1)*Y(I+1)
342   NEXT I
390   PRINT "SLOPE=";S;TAB(15);"INTERCEPT=";M
405   PRINT
406   PRINT"X";TAB(15);"OBSERVED";TAB(30);"CALCULATED";TAB(45);
        "DIFFERENCE"
407   FOR I=1 TO N
408   LET Z(I)=M+(S*X(I))
410   LET F(I)=Y(I)-Z(I)
420   PRINTX(I);TAB(15);Y(I);TAB(30);Z(I);TAB(45);F(I)
430   NEXT I
435   PRINT
436   LET V1=(N*E-B*B-S*S*(N*C-(A*A)))/(N*(N-2))
437   LET V2=C*V1/(N*C-(A*A))
438   LET V3=N*V1/(N*C-(A*A))
439   PRINT "VAR. SLOPE= ";V3;"VAR.INTERCEPT=";V2
440   PRINT "TOTAL VARIANCE OF Y VALUES="; V1
450   GOTO 120
999   END
```

APPENDIX 2

COMPUTER PROGRAM FOR CALCULATION OF CORRELATION COEFFICIENT

```
 10   PRINT "CORRELATION COEFFICIENT r"
 20   DIM X(2Ø),Y(2Ø)
 30   INPUT "NO. OF POINTS FOR CORRELATION";N
 32   SE=Ø
 34   SY=Ø
 36   SD=Ø
 37   SX=Ø
 38   SC=Ø
 40   FOR I=1 TO N
 50   PRINT "X";I;", Y";I;
 60   INPUT X(I);Y(I)
 80   SY=SY+Y(I)
 90   SX=SX+X(I)
100   SC=SC+X(I)*Y(I)
110   SD=SD+X(I)*X(I)
120   SE=SE+Y(I)*Y(I)
125   NEXT I
130   A=(N*SC)-(SX*SY)
140   B=SQR(ABS(((N*SD)-(SX*SX))*((N*SE)-(SY*SY))))
150   R=A/B
160   PRINT "CORRELATION COEFFICIENT, r=";R
170   PRINT
180   INPUT "ANOTHER RUN (Y OR N)";P$
190   PRINT
200   IF P$="Y" THEN 3Ø
204   PRINT
210   END
```

APPENDIX 3

COMPUTER PROGRAM FOR CALCULATION OF UNKNOWN CONCENTRATION BY THE METHOD OF STANDARD ADDITIONS

(Program written by I.C. Hamilton, Footscray Institute of Technology)

```
 10   REM STANADD.BAS
 20   PRINT "LEAST SQUARES CALC. OF CONCENTRATION BY STANDARD ADDITIONS"
 30   PRINT "TREATMENT ASSUMES NEGLIGIBLE ERROR IN 'X' VALUES"
 40   PRINT "'X' VALUES ARE THE 'CONCENTRATION ADDED'"
100   DIM X(2Ø), Y(2Ø), Z(2Ø), F(2Ø)
110   PRINT "UP TO 2Ø DATA POINTS MAY BE USED"
112   PRINT "TO STOP PROGRAMME TYPE '0' FOR NUMBER OF DATA POINTS"
120   INPUT "NUMBER OF DATA POINTS", N
122   IF N=Ø GOTO 999
125   PRINT "TYPE DATA POINTS 'X, Y' AFTER EACH '?'"
126   FOR I=1 TON
128   INPUT X(I), Y(I)
130   NEXT I
140   LET A=X(1)
150   FOR I=1 TO N
160   LET A=A+X(I+1)
170   NEXT I
180   B=Y(1)
190   FOR I=1 TO N
200   LET B=B+Y(I+1)
210   next i
220   LET C=X(1)*X(1)
230   FOR I=1 TO N
240   LET C=C+(X(I+1)*X(I+1))
250   NEXT I
260   LET D=X(1)*Y(1)
```

Appendix 3 (Cont'd)

```
270   FOR I=1 TO N
280   LET D=D+(X(I+1)*Y(I+1))
290   NEXT I
300   LET S=(N*D-(A*B))/(N*C-(A*A))
310   LET M=(B-(S*A))/N
320   LET E=Y(1)*Y(1)
330   FOR I=1 TO N
340   LET E=E+Y(I+1)*Y(I+1)
342   NEXT I
390   PRINT "SLOPE=";S;TAB(15);"INTERCEPT=";M
405   PRINT
406   PRINT "X";TAB(15);"OBSERVED";TAB(30);"CALCULATED";TAB(45);
        "DIFFERENCE"
407   FOR I=1 TO N
408   LET Z(I)=M+(S*X(I))
410   LET F(I)=Y(I)-Z(I)
420   PRINT X(I);TAB(15);Y(I);TAB(30);Z(I);TAB(45);F(I)
430   NEXT I
435   PRINT
436   LET V1=(N*E-B*B-S*S*(N*C-(A*A)))/(N*(N-2))
437   LET V2=C*V1/(N*C-(A*A))
438   LET V3=N*V1/(N*C-(A*A))
439   PRINT "VAR. SLOPE=";V3;"VAR.INTERCEPT=";V2
440   PRINT "TOTAL VARIANCE OF Y VALUES=";V1
460   LET G=M/S
463   LET V1=(N*E-B*B-S*S*(N*C-(A*A)))/(N*(N-2))
470   PRINT "CONCENTRATION IN ORIGINAL SAMPLE =";G
480   GOTO 120
      END
```

APPENDIX 4

MANUAL STANDARD ADDITIONS METHOD

This standard additions example shows the manual calculation of an unknown quinol concentration.

Conditions: Sample solution volume = 60.0 ml

Standard solution concentration = 610 µg/ml

Measurement Number	Analyte Solution	Signal Magnitude (mm)	Volume Correction (mm)	Change in Signal (mm)
1	60.0 ml Sample	31.60	31.60	14.85
2	Sample + 0.1 ml	46.37	46.45	22.20
3	Sample + 0.25 ml	68.37	68.65	44.78
4	Sample + 0.55 ml	112.4	113.4	

Calculation:

0.1 ml of standard contains 61 µg of quinol

The mean change in signal size per 0.1 ml added = Signal magnitude per 61 µg of quinol

$$= \frac{14.85 + 22.20 + 44.78}{5.5}$$

$$= 14.878 \text{ mm}$$

Appendix 4 (Cont'd)

$\therefore$ 60.0 ml of sample solution contains $\frac{31.60 \times 61}{14.878}$ µg

$= 129.6$ µg

and the concentration of quinol in the sample solution $= 129.6/60.0$

$= 2.16$ µg/ml