Flow injection amperometry and associated studies

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FLOW INJECTION AMPEROMETRY
AND ASSOCIATED STUDIES

by

AHMAD BADRUDDIN W. GHAWJI

A Doctoral Thesis
Submitted in partial fulfilment of the
requirements for the award of
Doctor of Philosophy
of the
Loughborough University of Technology

November 1986

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DEDICATION

This work and all it stands for is dedicated to my parents, my wife and my son Hassan.
I wish to express my gratitude to my supervisor, Dr A.G. Fogg, for his help, guidance, invaluable suggestions and friendly assistance during the period of my research.

I would like to thank Mr P. Scullion for reading part of the manuscript, and the technical staff of the Chemistry Department for their cooperation and expert advice.

I am most grateful to my parents and family members, especially Uncle Saad, for their moral and financial support and encouragement.

Last, but by no means least, I wish to express my special appreciation to my wife for her patience, understanding and encouragement during the last period of my study.
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A satisfactory method was obtained for the determination of phosphate, arsenate and germanium as heteropoly acids by flow injection amperometry at a sessile mercury drop electrode held at 0.22V, 0.23V and 0.18V vs SCE, respectively, without deoxygenation of the eluent or the sample solutions. Previous workers had shown that germanium could not be determined at a glassy carbon electrode owing to adsorption, and that arsenate could only be determined down to the $10^{-5}$M level owing to deterioration of the baseline.

The sessile mercury drop electrode was found to be particularly useful at low negative and low positive potentials where oxygen is not reduced and therefore does not interfere, or at more negative potentials in alkaline solutions, where sulphite can be added to remove oxygen traces. This was illustrated by the determination of nitrofurantoin at -0.7V in pH 7.5, with added sulphite.

The effect of electrochemical pretreatment of a glassy carbon electrode by holding it at about +2.0V for 2 min and then at about -1.0V for 2 min on the electrochemical oxidation of ascorbic acid, dopamine, dopac, HVA, artenerol and 5 HIAA was studied. The pretreatment of the electrode produced a substantial improvement in the shape of voltammograms, and in the reproducibility of both peak potential and peak current. Considerable shifts of peak potentials to less positive values, and substantial increase in peak heights were obtained by the electrode pretreatment. In the case of HVA different oxidation products were obtained at pretreated and untreated electrodes. Ascorbic acid and dopamine peaks were partially separated at the pretreated glassy carbon electrode. On-line determination of ascorbic acids, dopamine and dopac is improved with the use of a pretreated glassy carbon electrode. More reproducible and higher signals are obtained with a shift of the hydrodynamic voltammograms to less positive potentials, than when a newly polished untreated electrode is used.
Satisfactory methods were obtained for the construction of glassy carbon electrodes. The methods are simple, fast and inexpensive. The performance of the electrodes was at least as good as new commercial electrodes; however the laboratory constructed electrodes were more durable than the commercial electrodes.

A novel method of electrochemical pretreatment of glassy carbon electrodes in which the electrode was held at -3.0V for 1 min in 0.1M sulphuric acid was tested. This pretreatment was found to make the reduction of oxygen more difficult and to allow determinations at glassy carbon electrodes to be made up to about -0.65V without the need to deoxygenate the sample solutions. This was illustrated by the on-line determination of nitrofurantoin. The pretreatment was shown to improve the detection limit, to reduce the noise level and to make the deoxygenation of the sample solutions unnecessary. Similarly a flow injection amperometric method was obtained for the determination of vitamin K₃ in acetate buffer (pH 3.8) without the need to deoxygenate the sample solutions. However, the presence of methanol and ethanol was found to weaken the effect of this pretreatment.

Previous workers had developed on-line iodimetric methods of determining sulphite amperometrically using normal FIA and reverse FIA methods, but the dispersion studies with amperometry were anomalous. This anomaly has been solved here using the spectrophotometric determination of iodine at 352 nm. A spectrophotometric method for the determination of sulphite analogous to the amperometric method as well as a new FIA method was developed. In the first method a slightly alkaline iodate/iodide eluent is used. Iodine is formed in an injected bolus of dilute acid. When acidified sulphite samples are injected into the same eluent, a decreased iodine signal is obtained owing to the reaction of the sulphite injected with an equivalent amount of iodine. The decrease in signal size is proportional to the sulphite injected. In the second method, sulphite is used as eluent and solutions of preformed iodine of
constant concentration is injected into the eluent. The decreased iodine signals obtained when the eluent contains no sulphite and when it contains different concentrations of sulphite are proportional to the sulphite concentrations.
CHAPTER 1

INTRODUCTION TO VOLTAMMETRIC TECHNIQUES

INTRODUCTION

Voltammetry may be defined generally as the measurement of current-voltage relationships at an electrode immersed in a solution containing electroactive species\textsuperscript{1}. More specifically, it is the determination of the current at the working electrode, due to an electron-transfer reaction at the electrode surface, while a potential is applied. Polarography is a branch of voltammetry which employs a dropping mercury electrode as the working electrode.

Rapid development of voltammetry occurred after 1922 when Jaroslav Heyrovsky invented the technique\textsuperscript{2}. The technique, however, suffered from a number of shortcomings which made it less than ideal for routine analysis purposes until the late 1950's when modifications of the basic technique aimed at overcoming the various problems associated with it were introduced\textsuperscript{3}.

The decade 1955-1965 saw the development of a considerable number of new polarographic (voltammetric) techniques. Alternating current polarography, square wave polarography, normal and differential pulse polarography may be cited as examples in this respect.

Since the late 1960's a sudden and unexpected increase of interest in polarographic techniques has become clear. This is because of the demands of environmental scientists for a large number of heavy metal analyses, and because of the appearance of greatly improved commercially available instruments at a relatively low cost\textsuperscript{4}. In addition, because of the growing realization of its applicability in organic analysis, especially in the pharmaceutical industry, voltammetry has come to be more widely used again. The revival of the technique has mainly occurred in three areas:
1. Polarographic and related methods for use in biological systems especially the introduction of differential pulse polarography which offers greater sensitivity and resolution compared with conventional DC polarography. This technique has recently been applied to a vast number of problems involving the analysis of biological fluids.

2. The development of stripping voltammetric techniques which makes possible the determination of small amounts of both organic and inorganic substances at the $10^{-8}$M-$10^{-9}$M levels, and more recently the development of adsorptive stripping voltammetry which reduces the detection limit to about the $10^{-11}$M level.

3. The development of electrochemical detectors particularly for use with high performance liquid chromatography and flow-injection analysis.

POLAROGRAPHIC TECHNIQUES

DC Polarography

In classical direct current (DC) polarography the potential-time (E-t) waveform which is applied to a dropping mercury electrode (dme) is a linearly increasing dc potential ramp. The resulting current-voltage (i-E) curve obtained when an electroactive species is oxidized or reduced, takes the form of an S shape. When oxidation occurs, electrons are accepted by the dme and the current is anodic in nature; when reduction takes place, electrons are donated by the electrode and the current is cathodic. The basic principles of this technique have been well established by Heyrovsky and Kuta.

Simple electrochemical cells usually consist of two electrodes. In these cells the potential is applied across the entire cell, rather than across the working electrode-solution interface, and thus yields data which are considerably in error if solution resistances, and the resultant ohmic (IR) drop across the system is significant.
Modern instruments incorporate a potentiostat which controls the potential at the working electrode-solution interface, by making use of a three electrode system as shown in Figure 1.1.

The three electrode system minimizes the effect of solution resistance and provides greater flexibility in the location of the reference and working electrode. The electrodes used in a three-electrode control system in polarography are the working electrode, a reference, and an auxiliary electrode. The working electrode is the electrode at which the electrochemical phenomenon being investigated takes place. The reference electrode, known also as the unpolarized electrode, is the electrode whose potential is constant enough to be taken as the reference standard, against which the potential of the working electrode in the cell can be measured. The auxiliary electrode, known also as the counter electrode, is the electrode that serves as a source or sink for electrons, so that the cell current passes between the dme and it and no current passes through the reference electrode.

The dropping mercury electrode is a glass capillary of 0.05 to 0.08 mm internal diameter from which the mercury flows, forming drops at intervals of about 2 to 8 seconds, depending on the height of the mercury reservoir and thus the pressure of the mercury column. The dropping mercury electrode has several advantages over other types of electrode. The electrode surface is renewable which prevents the accumulation of the electrode reaction products. The reproducible surface area of the drop can be calculated at any time during the drop life. The overvoltage for the reduction of hydrogen ion is high which makes possible the electroreduction of many species at negative potentials unattainable on solid electrodes.

The electrode has however the disadvantage of being of limited use in the study of anodic processes because of the oxidation of mercury which occurs at approximately +0.40 volts versus a saturated calomel electrode.
FIGURE 1.1: CIRCUIT FOR CONTROLLED POTENTIAL OPERATION
The parameters of importance in polarography are the height of the diffusion limited current plateau $i_d$, which is directly proportional to the bulk concentration of diffusing species, and the half wave potential $E_{1/2}$ which is characteristic of the particular electroactive species that is being either oxidized or reduced, and which is defined as the potential at which the current is one-half of the value of the diffusion current.

The current observed is the total current which is the sum of the diffusion controlled current, or the Faradaic current, and the non-Faradaic current which flows because the electrode-solution interface exhibits a capacitive characteristic.

The capacitance or charging current will result from either a change of the potential of the electrode, or a change in the size of the electrode. Since the potential remains virtually constant over the lifetime of a single drop, the charging current is the result of the change in electrode area.

As the mercury drop grows a new mercury surface is exposed to the solution, and a current must flow to charge this new surface to the potential dictated by the electrode potential and solution conditions. This current can be either anodic or cathodic depending on the potential of the electrode, with respect to the potential of zero charge.

The charging current $I_C$ resulting from the growth of a mercury drop is directly proportional to the rate of change of drop area $dA/dt$:

$$I_C = C_F(e) \frac{dA}{dt} (E_m - E)$$

where $C_F(e)$ is the capacity of the double layer per unit area and $E_m$ is the potential of zero charge ($q = 0$).
From this equation it can be seen that $I_C$ is positive when $E$ is more negative than $E_m$, zero when $E = E_m$, and negative when $E$ is more positive than $E_m$.

The change in drop area is very large at the beginning of a new drop; therefore $I_C$ jumps at the beginning of the drop. As the drop grows, $dA/dt$ increases and $I_C$ decreases. The variation of $I_C$ with time is given by

$$ (I_C)_t = 0.00569 C_F' (E_m - E) m^{2/3} t^{-1/3} \quad 1.2 $$

where $(I_C)_t$ is the capacitance current after time $t$ seconds and $m$ is the rate of mercury flow in mg.s$^{-1}$.

The Faradaic current size depends on the rate at which the electroactive species being oxidized or reduced reaches the electrode surface under the sole influence of a diffusion force. However the movement of the electroactive species under the influence of the electric field gives rise to what is called migration current. Elimination of the migration current can be achieved by the presence of a large excess of supporting electrolyte that is not capable of oxidation or reduction at the potential used.

Unlike the capacitance current Faradaic current increases with time unless complicating phenomenon such as adsorption is present.

The diffusion controlled limiting current is given to a good approximation by the Ilkovic equation:

$$ i_d = 607 \text{ nc } d^{1/2} m^{2/3} t^{1/6} \quad 1.3 $$
where: \( i_d \) = mean limiting diffusion current (\( \mu A \))
\( n \) = number of electrons involved in the reduction or oxidation
\( c \) = the bulk concentration (m.mol cm\(^{-3}\))
\( D \) = the diffusion coefficient of electroactive species (cm\(^2\) s\(^{-1}\))
\( m \) = the flow rate of mercury (mg s\(^{-1}\))
\( t \) = drop time (s).

Changes of instantaneous current with time during the life of a single drop for (a) \( 10^{-4} \)M, (b) \( 10^{-5} \)M & (c) \( 10^{-6} \)M of solution are shown in Figure 1.2.

The charging current in conventional dc polarography masks the Faradaic current when the concentration of the electroactive species is in the \( 10^{-5}-10^{-6} \)M region, which makes quantitative determination at this level extremely difficult.

Conventional dc polarography also has the disadvantage of being a relatively slow technique because the time taken to record a polarogram is restricted by the slow rate at which the potential must be scanned.

**Current-Sampled DC Polarography:**

In this technique the current is measured for only a very short period of time just before the fall of each drop. Since Faradaic current increases with \( t^{1/6} \), but capacitance current decreases at \( t^{-1/3} \), there is a significant enhancement of the former over the latter at the end of the drop lifetime. In addition, the oscillations observed in conventional dc polarography, due to the continuous growing of the drop, are not observed. The increase in sensitivity of this technique over the conventional dc polarography is marginal.
FIGURE 1.2: CHANGES OF INSTANTANEOUS CURRENT WITH TIME DURING THE LIFE OF A SINGLE DROP.

$i_F$ Faradaic current, $i_C$ Charging current.

(a) $10^{-4} M$; (b) $10^{-5} M$, and (c) $10^{-6} M$ (schematic different current scale)
Normal Pulse Polarography:

Since sampled dc polarography actually records the current only during a very small time period late in a drop's life where the Faradaic current is maximum and the charging current is minimum, all the Faradaic current flow that occurs before the sampling period serves no useful purpose. Actually it even damages the sensitivity because it depletes the region near the electrode of the substance being measured and reduces its flux to the surface at the time of actual measurement. Normal pulse polarography is designed to eliminate this effect by blocking electrolysis prior to the measurement period. Figure 1.3 shows the way in which this is achieved.

The potential of the drop is held for most of the drop life at a base potential, $E_b$, at which negligible electrolysis occurs. After a fixed time from the birth of the drop a potential pulse moves the potential of the drop to a new potential, $E$, for a period of about 50 msec in duration. Then the potential returns to the base value $E_b$. The current is measured at a time $\tau$ near the end of the pulse. The pulses are of slowly increasing height, such that $E$ changes slowly just as the potential ramp does in classical dc polarography. The current is measured near the end of the pulse to allow the charging current, which flows due to the application of the pulse itself, to decay.

The current in normal pulse polarography is larger than in dc polarography, because the material at the electrode surface has not been depleted by reaction in the early part of the drop life. The increase has been calculated by Parry and Osteryoung as about 5 to 7 times that of dc polarography.

The limiting diffusion current obtained by the normal pulse polarography technique is described by the Cottrell equation:

$$i_d = nFAD^{1/2} c (\tau_m)^{-1/2} \quad (1.4)$$
where:  $F = \text{the Faraday (96500 coulombs/equivalent)}$

$A = \text{the electrode area (cm}^2\text{)}$

$t_m = \text{the time in seconds, measured from pulse application, at which the current is measured.}$

The other variables are as given in the Ilkovic equation.

The pulse technique was first developed by Barker$^{13}$. An extensive review of the theory and application was made by Osteryoung and Hasebe$^{14}$ and Bond$^{11}$.

Differential Pulse Polarography:

The approach to differential pulse polarography resembles normal pulse polarography, but several major differences exist:

a) the base potential applied for most of the drop's life time is not constant from drop to drop, but instead is increased steadily;

b) the pulse height is only 10 to 100 mV and is maintained at a constant level with respect to the base potential;

c) the current is sampled twice during each drop's lifetime, first at time $t'$ immediately before the pulse, and second at time $T$, late in the pulse;

d) the record of the experiment is a plot of the current difference, $i(T)-i(T')$, versus the base potential (see Figure 1.4).

The name of the method comes from this differential current measurement. The pulse width ($\sim 50$ msec) and the waiting period for drop growth, are both similar to them in NPP.

Differential pulse current-voltage curves have a peaked shape which looks very much like the derivative of a normal polarographic wave. This is because at potentials far less than $E_1$ for a given reducible compound, the concentration at the electrode surface will essentially be the bulk value at both of the points of measurement. The Faradaic
FIGURE 1.3: SAMPLING SCHEME FOR NORMAL PULSE POLAROGRAPHY
(a) Potential program, (b) and (c) current and potential during a single drop's lifetime.
FIGURE 1.4: THE POTENTIAL-TIME WAVEFORM FOR DIFFERENTIAL PULSE POLAROGRAPHY
current flow is zero at both of these times, so the difference is zero and the recorder response is flat. In the potential region where the normal current-voltage curve starts to rise, the current measured near the end of the pulse is larger than the current measured just before the pulse is applied. This is because when the potential is pulsed it moves into a region where the compound is reduced significantly faster than at the ramp potential before the pulse was applied. The difference between the current at the pulsed potential and the ramp potential will be a positive value, and the recorder response will be increasing as the voltage is scanned. At a ramp potential slightly greater than $E_{1/2}$, the concentration in the diffusion layer surrounding the mercury drop will be sufficiently depleted so that the pulse causes a diminishing increase in the current flow as a function of increasing ramp potential, so the difference in the two measurements becomes smaller, which causes the recorder response to go down. At ramp potentials much greater than $E_{1/2}$ of the compound, the recorder response is once again flat.

The theoretical relationship between the peak current, $i_p$, and pulse modulation amplitude, $\Delta E$, has been derived by Parry and Osteryoung$^{12}$. The maximum peak current when the pulse modulation amplitude is less than the value of $RT/nF$ for a reversible system is:

$$i_p = \frac{n^2 F^2 AC}{4 RT} \left( \frac{D}{\pi t} \right)^{1/2} \Delta E$$  \hspace{1cm} (1.5)

From equation 1.5 it can be seen that $i_p$ is directly related to $n^2$; therefore the current in DPP would be 9 times greater for a three-electron process than for a one-electron process when all other parameters are equal.

Another parameter of interest in DPP is the modulation amplitude, $\Delta E$, which is proportional to peak current over a narrow range. An increase in $\Delta E$ gives better sensitivity, but at larger pulse amplitudes the relative increase in current obtained by increasing
$\Delta E$ is less. In practice, the optimum pulse amplitude to maximise sensitivity lies in the range 50-100 mV. Larger pulse amplitudes are not useful because of the problem with residual capacitance currents and increased peak broadening with consequent loss of resolution.

The relationship between the peak current in DPP and NPP for a reversible electron transfer case is given by the equation:

$$i_{DPP} = i_{NPP} \frac{\sigma - 1}{\sigma + 1}$$

where:

- $i_{DPP}$ = peak current ($\mu$A)
- $i_{NPP}$ = limiting current in NPP ($\mu$A)
- $\sigma = \exp \frac{nF}{RT} \left(-\frac{\Delta E}{2}\right)$

The expression relating peak potential to half-wave potential is:

$$E_p = E_{1/2} - \frac{\Delta E}{2}$$

The ratio $\frac{\sigma - 1}{\sigma + 1}$ depends only on the number of electrons transferred and on pulse amplitude, and is always less than unity, therefore the value of $i_{DPP}$ is smaller than that of $i_{NPP}$. However the detection limit is about 10 times lower than NPP. This is because the charging current is much smaller in DPP than NPP, because only differential charging is observed. The peaked shape current-voltage curve makes the current measurement much easier especially for a low concentration of electroactive species and makes the resolution of compounds with close half-wave potentials possible.
VOLTAMMETRIC TECHNIQUES

Though the principles and instrumentation of polarography and voltammetry are similar, there are some differences between the two. Normally voltammetric work, including that done at a hanging mercury drop electrode (HMDE), is done on fast potential scan rate (5 to 500 mV sec\(^{-1}\)), whereas polarography with the dropping mercury electrode is done with very slowly changing applied potentials. Voltammetry is usually carried out with solid electrodes although the HMDE may well become more popular.

A variety of electrode materials have been employed as voltammetric electrodes and their utility is determined mainly by their potential limits considered as its useful potential range and by the size and reproducibility of their currents in the medium used in the experiment. The electrodes used in voltammetry can be divided into three types: mercury type electrodes, noble metal electrodes and carbon electrodes.

Mercury Electrodes:

The mercury electrode has advantages which no solid electrode has, but its range of application is restricted on the anodic side. Two types of mercury electrode have gained increasing importance for voltammetric determination. The first one in importance and popularity is the so-called hanging mercury drop electrode (HMDE) developed by Gerischer\(^{15}\) and Berzine and Delahay\(^{16}\). The other type of mercury electrode, the mercury thin-film electrode, is made of a thin film (1 to 100 µm) of mercury plated on some solid electrodes used as the support so that only the thin mercury film is exposed to the electrolyte. Platinum has been used for that purpose since the work of Marple and Rogers\(^{17}\).

Carbon electrodes have been preferred for the preparation of mercury thin film electrodes as the carbon surface does not interact with mercury to form amalgams, as in the case of metallic electrodes. The
great value of mercury thin film electrodes has been in stripping
voltammetry.

Noble Metal Electrodes:

Platinum is the noble metal most commonly used as a solid electrode. Its polarization range is broad mainly in the anodic direction. The main difficulty with the application of platinum electrodes is caused by surface phenomena such as adsorption, especially from hydrogen, and by the fact that platinum is not fully inert. In fact it may be oxidized, and a layer of platinum oxides formed on its surface.

Gold is recommended as the best choice for cathodic reactions, because it does not adsorb appreciable quantities of hydrogen and has a large overpotential for hydrogen evolution. Gold is also oxidizable, though to a lesser extent than platinum. Widespread application of the gold electrode is hindered by problems connected with sealing of metallic gold to glass; therefore the application of the gold electrode is more complicated than that of the platinum electrode.

Carbon Electrodes:

Carbon electrodes are very useful for both electrochemical oxidation and reduction. However, because of its wide range on the positive potentials, these electrodes have been mainly employed for anodic voltammetry which includes the oxidation of many important organic compounds.

Analytical interest in carbon rod electrodes was apparently initiated by the work of Lord and Rogers\textsuperscript{18}, who suggested that a new and reproducible surface could be obtained after each polarogram by simply breaking off the electrode tip. This offered a definite advantage over the platinum electrode. The utility of untreated graphite electrodes is limited by both poor sensitivity and, in some
cases, poor reproducibility\textsuperscript{18} and by the high background current associated with it.

Gaylor and co-workers\textsuperscript{19} described the use of a wax-impregnated graphite rod electrode and stated that absorption of wax into the electrode pores reduces the high residual current normally found with the plain graphite rod. They showed at the same time that the sensitivity was increased and the reproducibility improved. Several types of impregnating agents were considered beside the wax, including opal, ceresin and castor waxes.

Another type of graphite electrode, the pyrolytic graphite electrode has been produced in conditions of high temperature (1900-2500°C) by the decomposition of carbon-containing gases in an inert atmosphere\textsuperscript{20}. They were applied for the first time in aqueous solutions voltammetry by Miller and Zittel\textsuperscript{21,22} and Beilby et al\textsuperscript{23}. They are suitable for a potential range of +1.00V to -0.80V versus SCE in acidic chloride or nitrate media.

The so-called carbon paste electrode was proposed by Adams\textsuperscript{24} and has been applied to the study of many compounds by Olson and Adams\textsuperscript{25,26}, in aqueous medium. The carbon paste electrode is made by mixing powdered graphite with an organic liquid immiscible in water, such as Nujol and bromoform. After each measurement with this electrode, a small amount of the paste is removed, and a fresh surface replaces the previous one. This guarantees a satisfactory renewed surface and therefore better reversible voltammograms as Lindquist\textsuperscript{27} found in his study of seven different carbon paste electrodes. The residual current of the carbon paste electrode is small and the potential range extends from about -1.10 to +1.10V.

Yamada and Sato\textsuperscript{28} prepared glassy carbon in 1962, using a method of controlling the pyrolysis of phenolic resins. They prepared a gas impermeable carbon which they so designated as glassy carbon. This material presented several advantages over other types of carbon including a greater inertness to chemical attack. This property,
together with the very small pore-size, makes glassy carbon an attractive material for the preparation of inert electrodes. The glassy carbon structure has been extensively studied by Jenkins and Kawamura. They concluded that glassy carbon is made up from atomic ribbon molecules which are oriented randomly and are tangled in a complicated manner.

The physical and chemical properties of glassy carbon and the behaviour of glassy carbon as an electrode for voltammetry has been studied and described by many workers and reviewed by Van der Linden and Dieker. Zittel and Miller were the first to use a glassy carbon electrode in voltammetry. They found that it is suitable for use over the potential range from about +1.20 to -0.80V versus SCE in acid medium.

The behaviour of glassy carbon electrodes on anodic polarization and subsequent reduction in acidic medium was studied by Laser and Ariel. They concluded that the overall process in anodic polarization is the result of three processes or reactions: (a) formation of a redox couple caused by chemical adsorption of oxygen; (b) irreversible redox reactions of existing surface groups, and at sufficiently positive potentials; (c) evolution of oxygen. Possible surface groups formed on the oxidation are suggested to be carbonyl groups that subsequently can be reduced to hydroxyl groups at more negative electrode potentials. Also the possibility of the formation of a quinone-hydroquinone couple, as found on oxidized/reduced pyrolytic graphite cannot be excluded.

These results are supported by the pulse voltammetric experiments at glassy carbon electrodes by Dieker et al. They demonstrated that the large residual currents observed on scanning in either the positive or negative direction at glassy carbon electrodes (from various manufacturers) cannot be attributed to the presence of electroactive impurities in the solution or to the charging current. Therefore they suggested that oxidation as well as reduction of the glassy carbon surface might occur. Majer et al. earlier obtained
results that are somewhat at variance with the previous results. Their conclusion was that glassy carbon electrodes most closely resemble ideal inert redox electrodes.

Gunasingham and Fleet\textsuperscript{36} have recently made a comparative study of glassy carbon as an electrode material. They found that the electrochemical response of GC electrodes is significantly affected by the state of the carbon surface, in particular the occurrence of surface carbon functionalities. Mascini et al\textsuperscript{37} have reported the use of a polyethylene graphite electrode and found it comparable with the glassy carbon electrode and the pyrolytic graphite electrode. Anderson et al\textsuperscript{38} described the fabrication and the characterization of a Kel-F-graphite composition electrode for general voltammetric application. This electrode has been shown to be of outstanding value due to its resistance against organic solvents and chemicals.

Kaufman et al recently developed a graphite spray coating technique\textsuperscript{39} and a colloidal solution of graphite particles dispersed in methylmethacrylate polymer. This graphite-spray electrode has been applied to the anodic stripping voltammetric determination of bismuth\textsuperscript{39}.

Wang\textsuperscript{40} reviewed the use of epoxy-based graphite electrodes. These electrodes suffer from a large and irreproducible background current which limits their application. Very recently Henriques and Fogg\textsuperscript{41} described a new epoxy-based graphite electrode based on a new curing technique where 40\% m/m hydrofluoric acid was used as a hardener. They showed that this electrode is as good as commercially available well-polished glassy carbon electrode in terms of low background currents and the quality of the voltammograms. They showed also that smooth electrodes can be produced by this technique on surfaces of a variety of shapes and sizes\textsuperscript{42}.

Electrodes based on reticulated vitreous carbon are increasing in importance\textsuperscript{43}. The material is similar to glassy carbon. Its main characteristics as a versatile electrochemical carbon electrode have
been reviewed by Wang\textsuperscript{43}. This electrode has been applied to various electrochemical purposes in both static and flowing systems\textsuperscript{44,45,46}.

Increasing interest has also been shown in the use of carbon fibre as an electrode material. Initial studies\textsuperscript{47,48} concentrated on the use of these fibres for coulometric titrations as well as describing their response to pH. Lately the voltammetric behaviour of these electrodes has attracted more interest\textsuperscript{49,50}. Much of this interest has come from clinical research where the small size of the electrode has encouraged its use for in-vivo studies. Recently Edmonds\textsuperscript{51} has reviewed the analytical use of carbon fibre electrodes.

Voltammetric studies of solid electrodes are playing an increasing part in the recent interest of the chemical industry in organic oxidation-reduction processes. In studying the mechanism of electrode reactions, the use of stationary electrodes with a cyclic potential scan makes it possible to investigate the products of the electrode reaction and detect electroactive intermediates. Furthermore, the time scale for the method can be varied over an extremely wide range, and both relatively slow and fairly rapid reactions can be studied with a single technique.

The utility and application of solid electrode systems together with relevant theory are reviewed by Adams\textsuperscript{1}. A definitive treatment of linear sweep voltammetry is given by Nicholson and Shain\textsuperscript{52}. This study surveys both single-sweep and cyclic voltammetry for simple systems and those with various chemical reactions coupled to reversible and irreversible charge transfer.

\textbf{Single Sweep Voltammetry}

Due to the rapid scanning of the potential in linear sweep voltammetry, the shape of the current-voltage curve is in the form of a peak. This peak occurs because the diffusion process is too slow to supply electroactive material to the electrode at the rate sufficient to keep up with the rapidly increasing potential.
The diffusion problem to a plane electrode for a reversible reaction was first solved independently by Randles and Sevcik. The peak current is given by the following equation:

\[ i_p = 2.687 \times 10^5 n^{3/2} A^{1/2} C V^{1/2} \]

where:
- \( i_p \) = peak current (\( \mu \)A)
- \( n \) = number of electrons involved in the electrode process
- \( A \) = area of the electrode (cm\(^2\))
- \( D \) = diffusion coefficient (cm\(^2\) s\(^{-1}\))
- \( C \) = bulk concentration (mmol l\(^{-1}\))
- \( V \) = potential scan rate (V s\(^{-1}\))

The current thus depends on the area of the electrode, on the concentration of the electroactive species, and on its diffusion coefficient. The peak current \( i_p \) also shows a dependence on the number of transferred electrons \( n \) different from that observed on polarography. In DC polarography the diffusion current is directly proportional to \( n \), whereas in linear sweep voltammetry the peak current is proportional to \( n^{3/2} \). The essential difference between the current obtained by the two techniques is in the dependence of the peak current on the rate of scanning \( V \), which becomes an important variable, whereas in polarography diffusion currents are not dependent on \( V \).

Matsuda and Ayabe examined the relationship in peak voltammetry for reversible, quasi-reversible, and totally irreversible systems. For the reversible systems:

\[ E_p - E_{p/2} = \frac{0.057}{n} \text{ volts at } 25^\circ\text{C} \]

where \( E_p \) and \( E_{p/2} \) are the peak potential and the potential at which the current is one-half the peak value, respectively.
The relationship between $E_p$ and the half-wave potential $E_{1/2}$ at 25°C, is given by the equation

$$E_p = E_{1/2} - \frac{0.029}{n}$$

For irreversible systems, the corresponding equations are given by:

$$i_p = 2.985 \times 10^5 n(na)^{1/2} A D^{1/2} C v^{1/2}$$

and

$$E_p - E_{p/2} = \frac{0.048}{n_a} \text{ volts}$$

where: $\alpha$ = electron transfer coefficient
$n_a$ = number of electrons involved in the rate determining step

As $(n_a)$ decreases the peak voltammograms become more spread out and the peaks tend to be rounded. This situation is frequently met in organic oxidations. The peak current is significantly less than the reversible electrode process, and the whole curve is more drawn out as $n_a$ decreases.

$E_p$ and $E_{p/2}$ for irreversible processes vary with potential sweep rate. This differentiates reversible from irreversible processes, but the variation is only about $0.03/n_a$ volt per tenfold change in sweep rate.

**Cyclic Voltammetry**

Cyclic voltammetry was first practised by Sevcik$^{54}$. It is usually performed by varying the potential of the working electrode linearly with time until a switching potential is reached at which time the direction of potential sweep is reversed and the potential is reversed to the original value.
Up to the switching potential the current behaves just as in the linear sweep case, the surface concentration of ox, is near zero and the product of reduction, red, has built up in the solution surrounding the electrode. For the reversible case this species can be oxidized back to the original electroactive compound. When the reversed potential approaches the oxidation potential of red, Faradaic current flows, but in the opposite direction. The species red, diffuses to the electrode surface from the solution surrounding the electrode until it is depleted or its maximum rate or flux is reached. The position of the peak on the potential axis is related to the formal potential of the redox process, and the height and shape give information about the reactant concentration and the number of electrons in the half reaction.

For a completely reversible system, the magnitude of the anodic current peak \( i_{pa} \) is equal to the magnitude of the cathodic current peak, \( i_{pc} \). The potential difference between anodic and cathodic peaks for a reversible system is:

\[
E_{p,a} - E_{p,c} = \frac{0.058}{n} \text{ volt}
\]

A quasi-reversible system shows a greater separation in \( E_p \) values. The voltammograms are more drawn out and the peaks are more rounded.

For an irreversible system, a complete separation of anodic and cathodic peak potentials is observed.

Cyclic voltammetry is a very useful technique for studying charge transfer rates\(^\text{56}\) and for investigating the overall processes which may occur in a complex electrode reaction\(^\text{52}\).
Hydrodynamic Voltammetry

Hydrodynamic voltammetry is the voltammetric technique where the mass transfer to the electrode surface occurs by forced convection rather than solely by diffusion. This is generally achieved by moving the electrode through the solution or by letting the sample solution flow past a stationary electrode which works as an electrochemical detector. The recording of voltammograms under these conditions is referred to as hydrodynamic voltammetry. The use of hydrodynamic voltammetry and more specifically hydrodynamic amperometry in continuously flowing solutions has seen a remarkable increase in interest largely due to the widespread use of electrochemical detectors in high performance liquid chromatography and flow injection analysis. Flow-through solid electrodes based on various configurations have been introduced in recent years. The most popular cells are those in which the solution flows through a thin-layer channel, through an open tubular electrode or onto a wall-jet electrode.

Theoretical treatment of hydrodynamic voltammetry with various electrode configurations have been reported by Levich, while the principles of current distribution and mass transport in flowing systems have been reviewed by Newman. Recently Pungor et al. published a comprehensive review on the theory and practical application of hydrodynamic voltammetry. Hydrodynamic voltammetry offers a better sensitivity under steady-state conditions than does classical voltammetry.
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CHAPTER 2

FLOW INJECTION ANALYSIS

INTRODUCTION

One of the significant analytical developments in the last two decades has been the spread of continuous analysis for the automation of wet chemical analysis. The application of continuous flow analysis for the automation of wet chemical methods of analysis did not become practical until the work of Skeggs\textsuperscript{1} who introduced an air bubble into the analytical stream to portion the sample into segments to preserve its identity. The air bubble has the advantages of reducing carry-over between the samples, limiting the dispersion of the sample in the stream, and aiding in mixing the sample with reagents by generating turbulent flow in the segments. This technique is called segmented continuous flow analysis.

Since Skeggs' work, it had been assumed that air segmentation of the stream was a necessary prerequisite for performing continuous flow analysis. It was assumed that without the air bubble, sample dispersion would be excessive resulting in excessive sample dilution, carry-over and cross-contamination between the samples. This technique however, suffered from several drawbacks associated with air segmentation:

1. because of the air bubbles, the stream tends to pulsate rather than flow regularly;
2. streams have to be debubbled before they enter the flow cell;
3. reagent consumption is generally higher than for discrete analysis;
4. typical analysis output is about 40 samples per hour. Higher sampling rates lead to a significant carry-over, and the precision and accuracy of the determination deteriorate.
With these disadvantages in mind, Ruzicka and Hansen\textsuperscript{2} and Stewart et al.\textsuperscript{3,4} have performed independently similar experiments of injecting the sample directly into the carrier stream (reagent) and have proved that analysis without air segmentation is not only possible, but advantageous. Ruzicka and Hansen have termed it flow injection analysis.

In this new technique a sample solution is introduced directly into an unsegmented carrier stream of a reagent solution, forming a well-defined sample zone. During the passage to the detector, the sample zone is dispersed in the carrier, which may contain a reagent, and may be merged with other reagent-containing streams. Finally, the reaction product is measured by a flow-through detector.

Flow injection analysis has challenged the idea that air segmentation is necessary to prevent carry-over from one sample zone to another. It was demonstrated that carry-over was not a problem and furthermore that the dispersion could be controlled easily without the need for air segmentation because there is no need to ensure complete mixing of sample and reagent, to produce the maximum concentration of the product, as the extent of mixing is reproducible due to the use of a precise injection valve and a constant flow rate.

According to the comprehensive reviews by Ruzicka and Hansen\textsuperscript{5}, Betteridge\textsuperscript{6} and recently by Ruzicka and Hansen\textsuperscript{7,8}, Karlberg\textsuperscript{9} and Landis\textsuperscript{10}, this technique has many advantages over the well-known air segmented continuous flow method and can be applied easily to the rapid analysis of various samples from agricultural, clinical and environmental sources.

In this technique the absence of air segmentation leads to a higher sample throughput, while the sample consumption is reduced. The reproducibility is good and there is no sample carry-over. The response curves do not reach the steady state plateau, but have the form of very sharp peaks. The requisite apparatus can be easily assembled, easily manufactured or purchased as a complete unit.
PRINCIPLES OF FLOW INJECTION ANALYSIS

The simplest form of a flow injection analysis system is illustrated in Figure 2.1.

![Diagram of a single channel flow injection system]

**FIGURE 2.1: SINGLE CHANNEL FLOW INJECTION SYSTEM**

It consists of a suitable pump, an injection valve, a reaction delay coil, a flow through detector cell, and a recording device.

The technique is based on three main principles:

1. **Sample Injection**
2. reproducible timing, and
3. controlled dispersion.

1. **Sample Injection**

The purpose of sample injection is to insert a well-defined sample zone into a continuously moving stream in such a way that the movement of the stream is not disturbed. The amount of sample, although it does not need to be accurately known, has to be injected with great precision, so that the volume and length of the sample zone at the point of injection is reproducible. The reproducibility of peak height is largely dependent on the injection technique, which requires
practice to achieve the required reproducibility; The sample is introduced to the system by means of an injection valve. Different types of injection valves have been used but the most suitable one is the rotary valve\textsuperscript{11,12}, similar to that used in high performance liquid chromatography.

2. Reproducible Timing

The reproducibility of the residence time of the sample inside the delay coil is essential because the signal is no longer to be read on the flat part of the curve but on the peak. Therefore a good pump which can produce a pulse-free constant flow of carrier and reagent is required. The most widely used pump is the peristaltic pump. This is a multi-channel pump. Variable flow rates can be obtained for each channel by varying the inside diameter of the pump tubes used, or by varying the pump speed. The pressurized gas pump\textsuperscript{13} has been used but less widely. This pump is constructed by placing the reagent or carrier in a bottle with two tubes. The content of the bottle is pressured by attaching one tube to a pressure source such as a nitrogen gas cylinder. The other tube is connected to the system.

The peristaltic pump has many advantages over the pressurized gas pump. It is more precise\textsuperscript{14,15}, less sensitive to changes in sample viscosity\textsuperscript{15}, and it maintains an appropriate flow rate even with a long length of tubing\textsuperscript{15}.

3. Controlled Dispersion

A sample injected into a carrier stream flowing through a narrow bore straight section of tube initially exists as a well defined plug. As the plug travels downstream it disperses and mixes with the carrier stream. A well defined concentration gradient is formed. If the sample dispersion is due to convection, the flow profile is characterized by a parabolic head and tail. This type of flow is called laminar flow, and is characteristic of most flow injection systems. If this were the only mass transport process operating, the
peak would have an infinitely long tail, as the velocity at the walls
of the conduit is zero. Two additional mass transport processes are
operational though; molecular diffusion in the longitudinal
direction (parallel to the direction of flow) and molecular diffusion
in the radial direction (perpendicular to the direction of flow).
The longitudinal diffusion is small compared with the dispersion due
to the flow velocity and can be ignored under the conditions of most
flow injection experiments. Radial diffusion moves sample molecules
to and from conduit walls where the flow velocity profile is zero
towards the centre of the tube, where the flow velocity is at a
maximum.

The net result of radial diffusion is that a peak with a finite peak
width is obtained. Radial diffusion becomes more important as the
residence time of the sample increases. At long residence times, the
dispersion process is controlled primarily by the diffusion process
and the peak shape assumes a symmetrical Gaussian shape.

The degree of dispersion of the sample zone depends on the tube
length and radius, the flow rate, the sample volume injected, and the
molecular diffusion coefficient of the species concerned.

By changing the flow parameter, the dispersion can be easily
manipulated to suit the requirements of a particular analytical
procedure so that optimum response is obtained at minimum time and
reagent expense. As the dispersion increases, the degree of dilution
of the sample with the carrier increases. For dispersion of 1 there
is no dilution; for dispersion of 2 the dilution is 1:1, and for
dispersion of 10 the dilution of the sample is 1:10.

The dispersion types fall into three categories: limited, medium and
large. The peak shape varies from sharp and asymmetric for limited
dispersion, Gaussian for medium dispersion and broad with exponential
peak shape for large dispersion.
a) **Limited dispersion:**

Systems with dispersion 1-2 are classified as limited dispersion. Limited dispersion systems are designed to minimise the dilution of the sample bolus with the carrier. Ideally, the readout is obtained at the centre of the sample zone on undiluted samples. This is obtained by injection of a large sample volume into the shortest possible delay coil. This design maximises sensitivity and minimises analysis time. The flow rate should be kept at a minimum in order to minimise dispersion. Limited dispersion is employed when the sample requires no modification before reaching the detector, and the original sample integrity is to remain intact. Examples of applications of limited dispersion are pH measurements\(^\text{16}\) and atomic absorption for metals\(^\text{17}\).

b) **Medium dispersion:**

FIA systems with dispersion from 2-10 have been classified as medium dispersion systems. In these systems, one or more reagents are mixed with the sample to transform the analyte into a form suitable for detection. The centre of the sample zone must be efficiently mixed with the reagents for maximum sensitivity. These systems make up the majority of FIA applications and are the most useful from the analytical point of view.

Broad areas of application are possible. Fast and slow chemical reactions can be employed. For slow reactions, there is a trade off between extent of mixing, reaction time (residence time) and peak broadening. As the residence time of the sample increases, the extent of the reaction increases, which tends to increase sensitivity. This effect is countered by the increase in sample dilution and peak broadening as the residence time increases, which tends to decrease sensitivity and reduce sample throughput. Many examples of FIA medium dispersion systems are reported\(^\text{17,18,19}\).
c) Large dispersion:

Large dispersion systems are defined as those with dispersion $> 10$. These systems are constructed by placing a mixing chamber between the injector and the detector. The function of the mixing chamber is to ensure complete mixing between the sample and the reagent and to produce a reproducible concentration gradient. In these systems the large dispersion reduces the sensitivity and sample throughput. Also a great deal of reagent is consumed per sample.

Large dispersion systems may be useful if the analyte concentration is too large to be accommodated by the chemistry or the readout or if the sample zone is to be stretched along the time coordinate in the form of a well-defined concentration profile. An example of the use of large dispersions is in flow injection titrations.$^{20-23}$

Several types of detection techniques have been used in flow injection analysis. These include amperometric, atomic absorption, chemiluminescence, coulometric, fluorometry, potentiometric, spectrophotometric and voltammetric detectors. Practically any detector described for use in high performance liquid chromatography can be utilized in a flow injection analysis system. The main criteria for a flow injection analysis detector is that the response time of the detector is fast and the volume of the detector is small. Since the peak width of most FIA systems is but a few seconds, the detector and its associated electronics should have a response time of less than 1 second. A slower response time can affect peak shape. The volume of the detector is also an important parameter that affects peak shape. As the detector volume increases, the dispersion of the sample increases in the flow cell and the sensitivity and throughput decrease.
FLOW INJECTION AMPEROMETRY

Amperometric detectors have rarely been used for flow injection analysis, but in recent years these detectors have gained popularity because they are sensitive, their response is generally rapid, and they can be applied to a relatively broad range of organic and inorganic compounds. The theoretical and practical aspects of amperometric detectors used in flow injection systems have been discussed by Pungor et al. Kissinger has listed several electrochemical liquid chromatography detectors that could be adapted for flow injection analysis. The construction of amperometric detectors has been discussed by Stulik and Pacakova, Lankelma and Poppe, and Fleet and Little. Recently reviews of electrochemical detection in flowing systems, including voltammetric, polarographic and amperometric detectors have been published by Hanekamp et al., Rucki, Brunt, and Stulik and Pacakova. Burmicz has listed and compared commercially available electrochemical sensors.

The measuring technique in flow injection amperometry involves monitoring the limiting current at a constant electrode potential. This technique has many advantages when used in flowing systems if compared with the linear sweep voltammetric technique when used in static systems. These advantages are:

1. the current is increased because of increased mass transport to the electrode, the diffusion layer being thinner in flowing streams;
2. the background current is decreased because at constant potential no current is needed to charge the double layer and the oxidation states of the functional groups in the carbon electrode surface are in equilibrium.

The requirements of the measuring cell in a flowing system are that its dead volume is as small as possible with hydrodynamic conditions permitting the highest reproducibility and sensitivity, and the impedance between the electrodes is minimal.
The most popular flow through cells which have been used as amperometric detectors in flowing systems are tubular, wall-jet and thin-layer flow cells. The wall-jet in particular offers the advantages of very high sensitivity, extremely small dead volume and relative freedom from surface adsorption. In this configuration, which has been used throughout this project, a jet of fluid strikes a wall (electrode surface) perpendicularly and then spreads radially over the surface of the wall. Based on Glauert's findings, Yamada and Matsuda described the limiting diffusion current equation in terms of the hydrodynamic parameters of the flowing solution. The equation they derived defines the performance of the wall-jet electrode in terms of the volume flow rate, diameter of the nozzle, and radius of the electrode.

\[
\text{i}_{\text{lim}} = 1.38 n F C D^{2/3} v^{-5/12} a^{-1/2} R^{3/4}
\]

where:
- \( n \) = number of electrons involved in the electrode reaction
- \( F \) = Faraday constant (96500 coulombs/equivalent)
- \( C \) = bulk concentration of electroactive species (mole cm\(^{-3}\))
- \( D \) = diffusion coefficient of the electroactive species (cm\(^2\) sec\(^{-1}\))
- \( v \) = kinematic viscosity (Stoke)
- \( v \) = flow rate (ml sec\(^{-1}\))
- \( a \) = inlet diameter (mm)
- \( R \) = radius of the disk electrode (mm)

For most other electrode geometries the limiting current depends on 1/3-1/2 power of the flow-rate, but in the wall-jet case, the dependence is to the power 3/4, which is a distinct advantage over other cell geometries.

The use of different polarographic and voltammetric techniques in flowing systems has been reported by several authors. The
important aspects that have been examined are response time, linearity, limit of detection, and selectivity. Some attention has also been paid to the influence of adsorption phenomena at the electrode surface. All the authors, except MacDonald and Duke\textsuperscript{40} have found that the DC mode is the most favourable, that square-wave, normal and differential pulse measurements do not improve the sensitivity of the measurements and that the detection limit is usually higher than that obtained using constant electrode potential. Moreover it has been found\textsuperscript{41,46-48} that the background current and noise are higher in normal and differential pulse measurements than in the DC method. However some advantages of pulse techniques have been reported. Dieker et al\textsuperscript{47} found that using the normal pulse technique decreases the adsorption of substances on the electrode surface. However, this effect is not general and is not always encountered. Another advantage is an improvement in selectivity compared with DC measurement of square-wave\textsuperscript{42,44} and differential pulse\textsuperscript{48-51}, but not normal pulse voltammetry\textsuperscript{47}. The improvement in the selectivity depends on the choice of the pulse amplitude, which must be a compromise with respect to the signal to noise ratio and the selectivity, as the selectivity improves with decreasing amplitude while both the signal and the noise decrease\textsuperscript{49}.

**ANALYTICAL APPLICATIONS OF FLOW-INJECTION AMPEROMETRY**

The first use of electroanalysis in a forced convection flowing stream was reported in 1947 by Muller\textsuperscript{52}. With a platinum microelectrode sealed in the constricted portion of a glass tube, the limiting current was found to be a linear function of the bulk concentration.

After the work of Kemula\textsuperscript{53} and the introduction of chromatography, several designs and applications of amperometric flow-through cells were reported\textsuperscript{54,55}. However, with the resurgence of interest in high performance liquid chromatography more than a decade ago, the search for selective detectors intensified. As electrochemical techniques possess considerable sensitivity, combination of the resolution of high performance liquid chromatography with the sensitivity of
Electrochemical detection yields a powerful analytical technique. Stulik and Pacakova have reviewed the theory and applications of amperometric detectors in HPLC.

The first paper dealing with amperometric detectors in flow injection analysis was published in 1978. Since then the number of publications has been continuously increasing and covers a wide range of applications.

Several types of electrodes have been used in these studies. Mercury based electrodes were the first to be used in flow injection amperometry but their use was limited by the presence of oxygen, which reduces at the mercury electrode at negative potentials, and therefore gives rise to a high noise level.

Eglli and Asper described an electrochemical double cell for the detection of cystine in aqueous liquid systems. A column electrode of amalgamated silver powder was used to reduce cystine quantitatively to cysteine, which was detected amperometrically at a mercury pool electrode. The potential of the column electrode was adjusted to -1.1 volts versus SCE, and the potential of the mercury pool electrode in the flow cell was kept at +50 mV versus SCE. Baltensperger and Eggli have used a renewable stationary mercury electrode to determine 1,4-benzoquinone in the concentration range 0.5 ng to 260 ng. For avoiding the interference of dissolved oxygen at mercury electrodes Maitoza and Johnson have used reverse pulse amperometry to monitor electroactive metal ions in a flow injection system using a static or hanging mercury electrode, without the need of deoxygenation. The reverse pulse amperometry technique is based on the application of an unsymmetrical square-wave with a large negative potential for the deposition of the metal ion, followed by a positive potential pulse for anodic stripping of that metal. The analytical signal is measured during the stripping process at a potential where dissolved oxygen is not reduced. The detection limit was 13 ng for the static mercury and 2 ng for the hanging mercury drop electrode. Person and Rosen used dropping mercury and hanging
mercury electrodes for determining isosorbide dinitrate in the concentration range 0.05-1 mM. To avoid deoxygenation they added 0.01M of sodium sulphite to the eluent which also contained 0.025M of sodium tetraborate. Lyle and Saleh used dropping mercury electrodes for the determination of nitrophenol and nitrobenzene at a concentration range 41 ng - 25 µg and 27 ng - 20 µg respectively and copper and zinc at 0.3 ng - 16 µg and 1.6 ng - 16 µg respectively. They used stainless steel tubing in their flow injection system to reduce the concentration of oxygen after deoxygenation, as Teflon tubes are permeable to oxygen. Forsman and Karlson determined penicilloic acid at a concentration of $2 \times 10^{-6} - 1 \times 10^{-4}$M content in penicillin preparations at DME held at +0.04 volts versus SCE. They used 0.05% Triton X-100 to prevent dissolved oxygen interference, as Triton X-100 displaced the oxygen wave by 400 mV in borate buffer. Recently Fogg and Summan described a sessile mercury drop electrode which was inserted in a laboratory-made detector cell of wall-jet configuration. They used that detector cell in the determination of synthetic food colouring matters. Their limit of detection in neutral or slightly alkaline media where sodium sulphite was added was 0.1 ppm and in acidic media was about 1 ppm using a 120 µl injection loop. The same detector cell was used for the determination of nitroprusside in pH 8 Britton-Robinson buffer. The method was not satisfactory because of the detector noise at the negative potential used (-1.25V). The detection limit was found to be about $10^{-5}$M. More promising results were obtained for the determination of ascorbic acid and dopamine in pH 5.5 acetate buffer at +0.19 volts and +0.26 volts versus SCE, respectively. The calibration graphs were rectilinear in the range of 0.1-60 ppm for both ascorbic acid and dopamine.

The use of solid electrodes in flow injection amperometry has been reported by many workers. Fogg and co-workers are a major group applying flow injection amperometry for a large number of species. They used first a Metrohm EA 1069/2 electrochemical detector and later a laboratory made detector cell, both having the wall-jet configuration with a glassy carbon electrode. Their first publication
was the determination of the phenolic analgesic maptazinol at a concentration range 0.1 to 10 \( \mu \text{g/ml} \), with 10 \( \mu \text{l} \) sample injections in an eluent of 0.05M sodium acetate, 0.1M acetic acid in 98% ethanol\(^6^5\). Later Fogg and Bhanot\(^6^6\) compared the behaviour of carbon paste and glassy carbon electrodes in flow injection amperometry in the determination of some synthetic food colours. They found that sensitivity was slightly higher for the carbon paste electrode, but the precision at intermediate and lower concentrations was better with the glassy carbon electrode. Determination of nitrite has been reported by injecting a nitrite sample directly into an eluent of acidic bromide solution and the reduction current of the nitrosylbromide produced inside the delay coil was measured at a glassy carbon electrode held at +0.3 volts versus SCE\(^6^7\). This method has been used for the determination of some aromatic amines at a concentration level of \( 10^{-8} - 10^{-4} \text{M} \), and nitrate at \( 10^{-6} - 10^{-4} \text{M} \) by determining the excess nitrite which had remained after the diazotization of the amine\(^6^8\), and nitrate by determining the nitrite produced by reduction of the nitrate by means of a cadmium sponge column, where nitrate sample solutions pass continuously through it, and then through the flow injection system. Determinations were also made by injection of nitrate sample solutions directly into an acidic bromide eluent and reducing the nitrate with cadmium wire\(^6^9\). An on-line bromimetric method for the determination of phenol, aniline, aspirin and isoniazid has been described. In this method the compounds in an acidic solution injected into a bromate-bromide eluent, and the difference in the bromide signal before and after the bromination or oxidation of these compounds is noted, at a glassy carbon electrode held at +0.40 volts versus SCE\(^7^0\). Hypochlorite and hypobromite were determined as bromide at a concentration of 0.08 - 2 \( \times 10^{-3} \text{M} \). Ammonia and hydrazine in the range 0.05 - 0.7 \( \times 10^{-3} \text{M} \) were also determined by reaction with hypobromite in phosphate buffer solution and determining the excess of hypobromite\(^7^1\). Procedures have been given by Fogg and Bsebsu\(^7^2,7^3\) for the determination of phosphate, silicate, arsenate and germanate by injecting heteropoly acids preformed in various aqueous acetone and aqueous ethanolic reagents into eluents consisting of reagent blanks. Silicate and
phosphate can be determined at $10^{-7}$ and $10^{-6}$M levels respectively. Arsenate has only been determined at the $10^{-5}$M level, and the precise determination of germanate is difficult due to adsorption at the glassy carbon electrode.

Ivaska and Ryan\textsuperscript{74} have used glassy carbon electrodes in a new detector based on the wall-jet configuration. They used DC and normal pulse voltammetry for their determination of paracetamol and concluded that DC was more sensitive, but normal pulse gave more reproducible results, especially at high concentration. Wang and Dewald\textsuperscript{75} have used glassy carbon electrodes in a wall-jet configuration detector cell to determine compounds which oxidized at high potentials where background and noise levels are high in flow injection systems, by reverse pulse amperometry which they claimed gives better sensitivity than DC amperometric detection. A detection limit of 57 nM for chloropromazine was obtained using that technique.

Blaedel and Wang\textsuperscript{76} have described the use of two thin-layer carbon graphite electrodes in a pulse flow detector using a flow injection system. They applied this to the determination of ascorbic acid and dopamine down to the nanomolar concentration level. Wang and Dewald\textsuperscript{37} described an electrochemical flow detector cell based on a jet of solution directed at a thin porous carbon electrode. They claimed that their detector exhibits better sensitivity and detectability than a wall-jet detector. They tested their cell by determining dopamine and ferrocyanide. The detection limit was of nanomolar concentration. Sharma et al have described a tubular graphite electrode, and used it for the determination of many pharmaceuticals including phenothiazines, sulpha drugs, purines, phenolic acids, local anaesthetics and antifertility compounds\textsuperscript{77-79}. Strohl and Curran\textsuperscript{80} used a reticulated vitreous carbon flow-through detector in a flow injection system and applied it to the reduction of ferricyanide and the oxidation of ascorbic acid, epinephrine and L-dopa. The detection limits were in the range of a few tenths of a nanogram and the linear working range covered about a thousand fold concentration range.
The use of a platinum wire electrode in flow injection systems was reported by Koile et al. They studied the use of a platinum wire electrode in the determination of As(III) and iodide. They observed that at a potential higher than +0.6V the growth rate of the oxide film on the platinum electrode in acidic media increases with the result that the current decreases at a rate of 0.7% per hour for concentration of $8.2 \times 10^{-5}$M, As(III) at a potential of +1.00V versus SCE. To maintain a uniform electrode activity, Scott et al used a triple pulse potential waveform at a platinum electrode in a flow injection system. They determined simple alcohols at a concentration of 0.17 to 2.4 $\times 10^{-3}$M and carbohydrates at a concentration of 0.1 to 1 mM using a 250 µL injection loop. Pratt and Johnson used a vibrating platinum wire electrode for amperometric detection in flow injection analysis. A concentration of $5 \times 10^{-10}$M of iodide was detected with a linear dynamic range extending to over 6.3 decades. Morrison et al. have used a tubular nickel oxide electrode to determine ethanol at a concentration range of 0.005-4 m.mole. Later Ben Hui and Huber used the same type of detector cell to determine propyl or butylamine, isopropylamine and glycine at the concentration ranges of $10^{-5}$ - $10^{-2}$M, $10^{-3}$ - $10^{-1}$M and $5 \times 10^{-6}$ - $10^{-3}$M respectively. Alexander and Akapongkul used a copper amalgam working electrode to determine copper, cadmium and zinc in a flow injection system using an ammonical buffer eluent. After deoxygenation of the eluent and the sample solutions, detection limits of 2-3 ng were obtained for copper, cadmium and zinc.

The use of modified electrodes, especially enzyme electrodes as amperometric detectors in flow injection analysis, is expanding rapidly. Several enzyme electrodes have been fabricated, resulting in relatively selective sensors for a variety of organic substrates. Thevanot et al. designed a trace glucose analyser consisting of a differential device that includes a platinum electrode covered by a β-D-glucose oxidase collagen membrane, a compensating electrode mounted with a nonenzymatic membrane, a platinum wire auxiliary electrode, and a silver/silver chloride reference electrode. In the presence of β-D-glucose oxidase, glucose is oxidized by dissolved
oxygen, resulting in gluconic acid and hydrogen peroxide. The hydrogen peroxide is detected amperometrically. The current outputs of both collagen membrane electrodes are subtracted and differentiated. A linear dynamic range of detection of $10^{-7} - 2 \times 10^{-3}$ mole/litre glucose was reported. Sternberg et al. tested this glucose detection system in clinical, food and environmental analysis with success. Blaedel and Engstrom have used an enzyme electrode for the determination of ethanol, lactate, and malate by constraining a dehydrogenase enzyme and nicotinamide adenine dinucleotide ($\text{NAD}^+$) onto the surface of a platinum electrode. Electrochemical regeneration of $\text{NAD}^+$ from the enzymatically produced NADH provided a current that was dependent on the substrate concentration. The platinum working electrode was covered with an acetylated dialysis membrane that exhibited low permeability to $\text{NAD}^+$ but fairly unrestricted permeability to the substrates. The acylated dialysis membrane constrained both enzyme and $\text{NAD}^+$ to the working electrode. Using this electrode in flow injection systems showed promising results. The linear range of the electrodes is about two orders of magnitude and the lower limit of detection appears to be in the concentration range of a few μM substrates. Macholan et al. have used a platinum electrode modified chemically with different enzymes for the amperometric determination of phenol, ascorbic acid, lysine and glucose in a flow injection system. Bradbury and Adams determined ascorbic acid at a thin-layer glassy carbon flow-through cell held at +0.8V versus SCE, where ascorbic acid oxidase has been immobilized on. A detection limit of $10^{-8}$M was obtained, with no interference from catecholamines. Very recently Wang and Hutchins described a surface modified glassy carbon electrode with a hydrolyzed cellulose acetate film. This electrode permits selective flow injection measurements of a single analyte in the presence of large electroactive species as they described in the measurement of $2 \times 10^{-5}$M phenol in the presence of nitro- and di-nitro phenols which do not affect the phenol peaks at +0.9 volts versus SCE.
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CHAPTER 3

GENERAL INSTRUMENTATION AND REAGENTS

INSTRUMENTATION

Model 174A Princeton Applied Research Polarographic Analyser

The Model 174A Princeton Applied Research (PAR) Polarographic Analyser was used for all electrochemical experiments in this project. It is capable of performing:

- DC polarography
- Sampled DC polarography
- Normal pulse polarography
- Differential pulse polarography
- Various voltammetric modes (linear sweep, pulse techniques).

In this model, the output (ramp, pulses) is summed with the initial potential and with a feedback signal taken from a reference electrode positioned as close as possible to the working electrode. The potentiostat in turn applies a potential to the auxiliary electrode and drives it to whatever potential is required to make the reference electrode equal to (but of opposite polarity to) the sum of the output and initial potentials. Any current that flows through the working electrode is then converted to a voltage. This voltage is further amplified and applied to the vertical or "Y" axis of the X-Y recorder. The "X" axis output is driven by a signal derived from the scan-potential generator. In the sampled DC and pulse mode the current is sampled during the last 16.7 ms of the timing period, the measured current value is stored in a memory and held until the next sample is taken. Four different time constants: 0, 0.3, 1.0 and 3.0 are available. They are generally used to smooth over signal fluctuations.

The model 174A is supplied with a drop timer that precisely dislodges the mercury drop with minimal perturbation of the solution, allowing
polarograms to be run with a variety of preselected drop times, often far more rapidly than is possible with naturally falling mercury drops.

Spectrophotometer

An HP 8451 Diode Array Spectrophotometer was used. This spectrophotometer is a single beam, microprocessor-controlled, UV-visible spectrophotometer which operates at high speed. Absorbance spectra over the full 190 to 820 nm wavelength range can be obtained in 0.1 second with 0.7 second repetition rate. Values at up to 25 wavelengths can be obtained every 0.1 second.

The HP 8451 includes the operating system of the HP 85A Personal Computer including a built-in CRT and printer/plotter. The built-in functional keyboard allows easy entry of measurement parameters and data processing instructions. An alphanumeric keyboard accessory provides the extended capability and versatility of BASIC programming. A Hellma flow-through cell of 30 μl volume was used with it for flow injection studies.

Ismatec Mini-S Peristaltic Pump

An Ismatec Mini-S Peristaltic Pump was employed to propel the eluent in the flow injection system. The barrel has eight rollers, and can accommodate three pump tubes providing they are all of the same diameter. Different flow rates can be achieved either by changing the diameter of the pump tubes or by tightening the screw of the clamp on the pump tube. This pump generates amplitude pulsing which produces a low level of signal noise at the detector.

Rheodyne Injection Valve

The injection valve used in the flow injection system was a low pressure Teflon rotary valve Rheodyne 5020. It is a convenient assembly with easily replaceable components. All parts of the valve
FIGURE 3.1: DETAILS OF CONSTRUCTION OF DETECTOR CELL.

(a) Part holding eluent entry port
(b) Part holding electrode
which are in contact with the eluent are of Teflon construction, the remainder are stainless steel and polypropylene. The valve has six ports, two ports to form the loop, one port for injection of the sample, one for excess of injected sample and the last two are for the inlet of the eluent and the other the outlet of the eluent towards the detector cell.

The Detector Cell

The construction of this detector cell has been described in detail by Fogg and Summan\textsuperscript{1}. Details of the construction of the detector cell from a PTFE rod of 2.5 cm diameter are given in Figure 3.1. The turning and recessing necessary were carried out before the machining of the side of the cell. The detector cell is designed to accommodate a Metrohm glassy carbon electrode EA 286 which is cylindrical and of 7 mm outer diameter. The eluent inlet block was drilled as follows. The first 1 cm depth was drilled threaded (1/4 in x 28 threads per inch UNF) and flat-bottomed for convenient butting of a flanged tube. The next 1 cm depth was drilled straight with a No 60 drill (1 mm diameter) and the remaining small depth (approximately 1 mm) was drilled with a fine No. 80 drill (0.35 mm). The depth and width of X-channel which allows separation of the eluent exit port and glassy carbon electrode and allows escape of eluent into the bulk electrolyte, were 0.25 and 2.5 mm respectively.

Nitrogen Gas

White spot nitrogen supplied by British Oxygen Corporation was used for deoxygenation when it was required. This nitrogen grade is virtually free of oxygen. However, to remove final traces, or to act as a safeguard, a vanadium (II) scrubbing solution was used.
Nitrogen Gas Scrubbing Solution

The scrubber solution was prepared by boiling 2g of ammonium metavanadate with 25 ml of concentrated hydrochloric acid, diluting to 200 ml, and shaking with a few grams of heavily amalgamated zinc, adding a little more acid when precipitate or turbidity formed. The partly-reduced solution was divided between two 250 ml gas washing bottles with coarse-porosity sintered glass gas-dispersion cylinders or glass bulbs with a number of holes. Each bottle contained 25 gm of heavily amalgamated zinc to reduce V(III) that formed in the presence of oxygen. Prior to use, nitrogen gas was usually bubbled through until the blue colouration changed to violet. The vanadium solutions tend to lose their violet colour and some precipitation may be formed after some time. They were easily restored, however, by adding a little HCl. The two scrubbing bottles containing V(II) solution were used in series with a third containing water. The purpose of the third container was to limit carry over of acid fumes or scrubbing solution in the gas stream.

SOLUTIONS

Britton-Robinson Buffer (0.04M)

Britton-Robinson buffer was prepared by dissolving 2.47g of boric acid in 500 ml of distilled water containing 2.3 ml of glacial acetic acid, and then adding 2.7 ml of orthophosphoric acid and diluting to 1 litre with distilled water. This solution has a pH of approximately 2. The pH of the buffer was adjusted as required by means of 2 or 4M sodium hydroxide solution.

Phosphate Buffer (0.1M)

a) Sodium dihydrogen orthophosphate:

27.60g of sodium dihydrogen orthophosphate was dissolved in distilled water and diluted to 1 litre in a calibrated flask
b) Disodium hydrogen orthophosphate:

28.39g of disodium hydrogen orthophosphate was dissolved in distilled water and diluted to 1 litre in a calibrated flask.

The phosphate buffer (pH 7.4) was prepared by making up 9.5 ml of solution (a) to 50 ml with solution (b), then diluting to 100 ml by distilled water. The pH was further checked by a pH meter.

Sulphuric acid solution 1M

Concentrated sulphuric acid (55 ml) was diluted to one litre with distilled water.

References

CHAPTER 4

FLOW INJECTION AMPEROMETRIC DETERMINATION OF NITROFURANTOIN AND ACETAZOLAMIDE AT A SESSILE MERCURY DROP ELECTRODE

INTRODUCTION

Nitrofurantoin, 1-[(5-nitrofurfurylidene)amino] hydantoin; 2,4-Imidazolidinbedione, 1-[[5-nitro-2-furanyl)methylene]-amino], I, is used as an antibacterial drug for the urinary tract of both man and animals.

\[\text{I} \]

Due to its importance, various methods have been described for its determination. The commonly used method is a colorimetric method\(^1\)-\(^3\), based on extraction of the drug from urine into nitromethane followed by the formation of the nitrofurantoin-hyamine hydroxide [p-(diisobutylresoxyethoxyethyl) dimethylbenzylammonium hydroxide] complex which absorbs at 400 nm.

Summa\(^{16}\) has described a polarographic method for nitrofurantoin determination in nitrofurantoin oral suspension. Nitrofurantoin was found to produce a polarographic wave having a half-wave potential of -0.384 volts in an equimolar solution of 1M ammonium chloride and 1M ammonium hydroxide solution, versus SCE. The calibration graph was rectilinear over the range 8 x 10\(^{-5}\) - 4.8 x 10\(^{-4}\)M. This method was used for nitrofurantoin assay in USPXX\(^9\).

Mason et al\(^4\) have described a method for the reduction of nitrofurantoin at a rotating platinum electrode, with the objective of
developing a simple and rapid method for the determination of this drug in urine. They claimed that their method is faster and more efficient with precision and accuracy comparable to those of the colorimetric methods. When the reduction was carried out in 0.5M sodium borate buffer (pH 12.1), the calibration graph was rectilinear over the range 0.5 - 1.5 µg ml⁻¹.

Burmicz et al.⁵ have investigated the reduction of nitrofurantoin over the pH range 2-12 and proposed reduction mechanisms at the various pH ranges. Nitrofurantoin gives rise to four waves iₐ, iₐ, iₐ, and iₐ in acid media (pH 2-5). At pH 5, wave iₐ and iₐ coalesce (iₐ + iₐ) and iₐ disappears. At pH 10, iₐ + iₐ does not occur and there is only one wave iₐ in the pH range 10-12. The proposed reduction mechanism is illustrated in Figure 4.1.

Recently Morales et al.⁶ have investigated the polarographic behaviour of nitrofurantoin and chloramphenicol in pyridine-formic acid buffer and tetramethylammonium chloride solution at pH 4.3. Simultaneous determination of both drugs was performed in a concentration range suitable for application to pharmaceutical formulation and body fluids. This method has the advantage over previous electrochemical methods of not requiring prior separation of the drugs as the supporting electrolyte permits high selectivity.

Very recently Ebel et al.⁷,⁸ have used spectrophotometry and differential pulse polarography in Britton-Robinson buffer (pH 5) to determine nitrofurantoin in urine and blood serum. The use of these two techniques eliminated systematic errors caused by metabolites. Detection limits were 0.2 µg ml⁻¹ and 1.0 µg ml⁻¹ by the polarographic and spectrophotometric methods, respectively.

Acetacolamide, acetamide, N-[5-(aminosulfonyl)-1,3,4-thiadiazol-2-Yl], II, is a carbonic anhydrase inhibiting drug which is used for the treatment of certain types of glaucoma. Methods for the quantitation of this drug in biological fluids include measurement of the carbonic anhydrase inhibition⁹,¹⁰, colorimetry¹²,¹³, gas chromatography¹⁴ and
FIGURE 4.1: THE REDUCTION MECHANISM OF NITROFURANTOIN PROPOSED
However, the GLC and HPLC procedures require extensive and
time consuming extraction and evaporation steps. The colorimetric
method is only partially successful and the enzymatic assay lacks sufficient precision.

A single publication has described a polarographic method for
acetazolamide determination in its tablets. Acetazolamide produces a
polarographic wave having a half wave potential at -0.518V in 0.1N
hydrochloric acid solution. The calibration graph is rectilinear in
the range $8 \times 10^{-5} \text{M} - 4 \times 10^{-4}\text{M}$.

In polarographic analysis, dissolved oxygen must usually be removed
from the sample solution before the measurement, because dissolved
oxygen gives two reduction waves which correspond to the reduction of
oxygen to hydrogen peroxide and its reduction to water or hydroxyl
ion.

Purging of samples with an inert gas, usually nitrogen, is currently
the most popular method of oxygen removal. This method is quite
effective and is usually suitable for static solution analysis.

The use of chemical reductants that are capable of reducing or
otherwise removing dissolved oxygen have been reported. These
include sodium sulphite, enzymes such as glucose oxidase and
catalase, ascorbic acid and sodium dithionate. However each
chemical is useful in a narrow pH range only. For example ascorbic
acid is useful for removing dissolved oxygen over a range of pH 5-8.
Sodium sulphite is useful over the range of 6-9.

The effect of dissolved oxygen interference becomes more serious in
flow systems. When an amperometric flow through cell is used for
reductive work in flow injection analysis or high performance liquid
chromatography usually with a mercury based electrode, removal of
oxygen is necessary for both sample and eluent. The difficulty in
removing final trace amounts of oxygen causes relatively high
background current and has retarded development in using the
reductive mode at negative potentials. Nevertheless, Lloyd\textsuperscript{24-26} has shown recently that very low detection limits can be obtained in the determination of organic explosives by careful removal of oxygen from eluent and sample solutions with nitrogen gas. Fogg and Summan\textsuperscript{27} have successfully determined some food colouring matters down to 0.1 ppm levels at a sessile mercury drop electrode held at -0.82 by careful deoxygenation of both eluent and sample with nitrogen gas.

This chapter describes the development of flow injection amperometric procedures for the determination of nitrofurantoin and acetazolamide at a sessile mercury drop electrode held at negative potentials. Procedures for the determination of nitrofurantoin and acetazolamide in their tablets on-line are also described.

EXPERIMENTAL

Apparatus for Polarography

The polarography was carried out using a Princeton Applied Research PAR 174A polarographic analyser to which a Gould HR 2000 recorder was connected. Three-electrode operation was employed with the polarograph using a dropping mercury electrode, a platinum counter electrode and a reference SCE. The polarographic cell was a double-walled glass vessel with a perspex top cover. The top had an O-ring fitted tightly onto the cell. There were four holes on the perspex top to enable the three electrodes and the deoxygenation train to be placed in the solution in the cell. The temperature of the solution in the cell could be regulated by pumping water between the walls of the cell. The nitrogen gas used for deoxygenation was purified as described in Chapter 3. A two-way tap was connected at the end of the deoxygenation train to keep the nitrogen gas over the solution throughout the duration of the polarographic analysis. The mercury height was kept constant at 65 cm throughout the work.
Apparatus for Flow Injection Amperometry

The flow of eluent was produced with an Ismatec Mini-S peristaltic pump. Injections of 100 μl of solution were made with a Rheodyne 5020 injection valve. The injection valve was connected to a laboratory-built detector cell incorporating a sessile mercury drop electrode by means of a 50 cm length of 0.58 mm bore size Teflon tubing. The detector cell with the platinum counter electrode was partially immersed in an electrolyte similar to that of the eluent. The saturated calomel reference electrode was also immersed in the electrolyte and was situated as close to the working electrode as possible. The potential of the sessile mercury drop (SMDE) was controlled by means of a PAR 174A polarographic analyser. Current peaks were monitored on a Linseis L650 recorder. The eluent reservoir was a 2 litre flask covered by a tight glass cover in which the deoxygenation train was placed. A two-way tap was connected at the end of the deoxygenation train to make possible either bubbling nitrogen into the eluent or maintaining a nitrogen atmosphere over the eluent. Sample solutions were also deoxygenated.

Details of the Sessile Mercury Drop Electrode

The method used to construct the SMDE was similar to the one described by Fogg and Summan. Its construction was as follows: a length of 6 cm capillary tube having 2 mm i.d. and 7 mm o.d. was joined to a length of 8 mm o.d. walled tubing. A disk of platinum foil, approximately 4 mm in diameter was lightly tacked on to the end of the capillary tubing sealing the bore. Another length of capillary tubing was butt-jointed over the platinum disc, care being taken to maintain the capillary bore. A U-bond was formed in the 8 mm tubing and the capillary tube was cut back to approximately 2 mm from the platinum disk. To make electrical contact with the platinum disk small pieces of small diameter solder were passed down the 8 mm tube and shaken down the capillary to the platinum disc. A length of single-strand copper wire was inserted into the tube and pushed round the U-bend. The solder was heated until softening occurred and the
wire was pushed down to ensure good connections subsequently between the copper wire, the hardened solder and the platinum disk. Mercury was placed in the well of 2 mm diameter whilst the platinum contact was still dry. An indication of the amount used in relation to the size of the well is given in Figure 4.2. The position of the mercury electrode in the detector cell is also shown in Figure 4.3.

Construction of a Pulse Dampener

The pulse dampener was made as described by Bergamin et al\textsuperscript{28}, from glass and had an inner volume of 15 cm\textsuperscript{3}. During operation the bulb remained partly filled with air which acts as the dampener. Earthing the eluent between the peristaltic pump and the injection valve was done by placing a platinum wire in the pulse dampener so that one side of the wire was in contact with the eluent and the other side was connected to an earth source. This eliminated the static electricity pulses generated by the peristaltic pump. Figure 4.4 is a schematic representation of the pulse dampener device.

REAGENTS

All chemicals were of analytical reagent grade.

Standard nitrofurantoin solution (250 \( \mu \text{g ml}^{-1} \))

Weigh 25 mg of nitrofurantoin and transfer it to a 100 ml calibrated flask. Dissolve and dilute to 100 ml with the supporting electrolyte (pH 7.5 Britton-Robinson buffer). Each ml of this solution contains 250 \( \mu \text{g} \) nitrofurantoin. More dilute solutions are prepared by dilution of this solution with pH 7.5 Britton-Robinson buffer.

Standard acetazolamide solution (250 \( \mu \text{g ml}^{-1} \))

Weigh 25 mg of acetazolamide, transfer to a 100 ml volumetric flask. Add about 40 ml of boiling water and heat on a steam bath for about 15 minutes. Cool to room temperature, add 10 ml of 1M hydrochloric acid, dilute with distilled water to volume and mix.
Mercury in the well

Approx. 19 cm

Approx. 7 cm

FIGURE 4.2: SESSILE MERCURY DROP ELECTRODE HOLDER
(a) General construction
(b) Detail of the well
Figure 4.2(b)

Well of mercury
Platinum disk
Copper wire in solder
Capillary tube

Mercury in well
One part of the detector cell

Figure 4.4: SCHEMATIC REPRESENTATION OF THE PULSE DAMPENER DEVICE
FIGURE 4.3: LABORATORY BUILT ELECTROCHEMICAL DETECTOR CELL WHEN USED WITH A SESSILE MERCURY DROP ELECTRODE
Nitrofurantoin tablets solution

Weigh and finely powder 10 nitrofurantoin tablets. Weigh accurately a portion of the powder equivalent to about 20 mg of nitrofurantoin and transfer it to a 100 ml calibrated flask, dissolve with supporting electrolyte and dilute to volume with the same electrolyte. Filter the solution through a dry filter paper. Discard the first 10 ml of filtrate and then pipette a 10 ml aliquot of the filtrate accurately measured to a 100 ml calibrated flask, dilute to volume with deoxygenated Britton-Robinson buffer (pH 7.5) and mix.

Acetazolamide tablets solution

Weigh and finely powder 10 acetazolamide tablets. Weigh accurately a portion of the powder equivalent to about 40 mg of acetazolamide, transfer it to a 100 ml calibrated flask, and dissolve it in about 40 ml of boiling water. Heat the mixture on a steam bath for 15 minutes, cool to room temperature, add water to volume and mix. Filter a portion of this solution, discarding the first 10 ml of filtrate, and then pipette a 10 ml aliquot of the filtrate, accurately measured to a 100 ml calibrated flask. Add 10 ml of 1N hydrochloric acid, dilute to volume with deoxygenated water and mix.

Britton-Robinson buffer

Prepare Britton-Robinson buffer solution as described in Chapter 3. Adjust the pH using 4M sodium hydroxide.

Hydrochloric acid 1M

Dilute 45.5 ml of concentrated hydrochloric acid to 500 ml in a volumetric flask.
Procedure for the Determination of Nitrofurantoin

Place 35 ml of a 20 ppm nitrofurantoin solution in pH 7.5 Britton-Robinson buffer solution in a polarographic cell. Deoxygenate the solution for 10 min. Record the DC or DP polarogram between 0.0V to -1.0V versus SCE. In this work a forced drop time of 1 second was used with a pulse modulation amplitude of 50 mV and a potential scan rate of 10 mV sec\(^{-1}\).

Procedure for the Determination of Acetazolamide

Prepare five solutions of 20 ppm acetazolamide concentration in 0.1M HCl, and Britton-Robinson buffer at pH 2, 4.7, 5.5 and 6.5. Place each solution in a polarographic cell. Deoxygenate for 10 min. Record the DC or DP polarogram between 0.0V to -1.1V versus SCE. In this work a forced drop time of 1 second was used with a pulse modulation amplitude of 50 mV, and a potential scan rate of 10 mV sec\(^{-1}\).

Procedure for the Determination of Nitrofurantoin and Acetazolamide by Flow Injection Amperometry

Set the detector potential at -0.70V for nitrofurantoin and -0.85V for acetazolamide. Let the deoxygenated supporting electrolytes which consist of pH 7.5 Britton-Robinson buffer containing 1g l\(^{-1}\) sodium sulphide for nitrofurantoin, and 0.1M HCl for acetazolamide, flow for a few minutes at 7 ml/min. Deoxygenate and inject 100 \(\mu\)l aliquots solutions containing 1-50 ppm of nitrofurantoin or 10-70 ppm of acetazolamide. Record the reduction current resulting from each injection using a y-t recorder.

RESULTS AND DISCUSSION

Performance of the Detector Cell with a Sessile Mercury Drop Electrode

The sessile mercury drop detector is used mainly for reductive amperometric determinations. However it can be used for oxidative
amperometric determinations if the oxidation potentials are lower than the potential at which the oxidation of mercury appears to begin.

Figure 4.5 summarises in graphic form the various parameters useful in evaluating detector performance in an amperometric flow injection system. These are signal current, background current and noise level. Detection limits refer to the amount of analyte required to give a signal x times greater than the noise (usually x = 2).

The main contribution to the current response is the Faradaic current, due to redox processes either from the analyte or solvent impurities. The charging current is not an issue since the detector is operated at fixed potential. The background current is principally Faradaic current arising from the oxidation or reduction of electroactive impurities in the eluent. A common source of background current at reductive potentials is oxygen reduction.

Noise is the random or periodic pattern superimposed on the steady-state background signal. Usually measured from peak to peak, the noise represents the summation of spurious contributions from pump pulsation, flow-cell hydrodynamics, surface reactions, static electricity, power frequency pick-up, electrical pulses produced by other instruments received via the power cords or other connections, and electronic amplification. As with most other quantitative measurements, the noise with an amperometric detector is dependent on the magnitude of the background signal. Generally the greater the background the greater the noise and the ratio of the noise to the background current stays approximately the same. As the detection limit is dependent on the noise level, when the noise level rises, so necessarily must the detection limit. Since noise level is dependent on potential, it follows that the smallest minimum detectable quantities will be capable of being determined in cases where the substances of interest are easily reduced.
FIGURE 4.5: GRAPHICAL REPRESENTATION OF VARIOUS PARAMETERS USEFUL IN EVALUATING DETECTOR PERFORMANCE IN AN AMPEROMETRIC SYSTEM
The parameter most useful for analytical comparison is the signal-to-noise ratio (SNR). An extremely responsive electrode may be equally noisy whereas a less noisy baseline of another electrode may be due to passivation. Therefore the situation may not be evaluated by comparing just the signal or just the noise. For this reason the SNR is most pertinent.

The variation of the noise level at the sessile mercury drop electrode with applied potential is shown in Table 4.1. The eluent used was pH 7 Britton-Robinson buffer which had been deoxygenated for 30 min before the measurements.

**TABLE 4.1: Effect of applied potential at the sessile mercury drop electrode on the noise level**

<table>
<thead>
<tr>
<th>Eluent: pH 7 Britton-Robinson buffer</th>
<th>Flow rate: 7 ml min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low pass filter: 3 seconds</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Potential/V</th>
<th>0.00</th>
<th>-0.40</th>
<th>-0.80</th>
<th>-1.20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noise level/nA</td>
<td>1.5</td>
<td>25</td>
<td>95</td>
<td>310</td>
</tr>
</tbody>
</table>

The background levels obtained at various potentials with the flow injection system in which a deoxygenated Britton-Robinson buffer (pH 7) was used as eluent are shown in Table 4.2. The effect of adding sulphite (1g/l) to the eluent on the background currents is also shown.
TABLE 4.2: Effect of applied potential at the sessile mercury drop electrode on the background current
Parameters as in Table 4.1

<table>
<thead>
<tr>
<th>Potential/V</th>
<th>Background current/μA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No sulphite added</td>
</tr>
<tr>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>-0.20</td>
<td>0.03</td>
</tr>
<tr>
<td>-0.40</td>
<td>0.08</td>
</tr>
<tr>
<td>-0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>-0.80</td>
<td>3.11</td>
</tr>
<tr>
<td>-1.00</td>
<td>6.23</td>
</tr>
<tr>
<td>-1.20</td>
<td>10.22</td>
</tr>
</tbody>
</table>

Polarographic Behaviour of Nitrofurantoin and Acetazolamide

A typical differential pulse polarogram for nitrofurantoin in pH 7.5 Britton-Robinson buffer is shown in Figure 4.6. The polarogram shows a peak at -0.36 volts versus SCE and a smaller peak at -1.26 volts versus SCE. Britton-Robinson buffer at pH 7.5 was used so that sodium sulphite could be added in order to remove dissolved oxygen traces. Addition of sulphite up to 1 g/l showed no effect on the reduction of nitrofurantoin under these conditions.

The polarographic behaviour of acetazolamide was investigated over the pH range 1-12. In acidic media of 0.1M HCl acetazolamide reduction at the dropping mercury electrode produced a well-defined wave having a half-wave potential of -0.52 volts and a poorly defined wave having a half-wave potential of -1.03 volts versus SCE. As the pH was increased the half-wave potential was found to be shifted to more negative potentials whilst the first wave started to disappear. At pH 5.5 the first wave disappeared completely while the second wave shifted to a half-wave potential of -1.44 volts versus SCE. Figures 4.7, 4.8, 4.9 and 4.10 show some typical polarograms at four pH values.
FIGURE 4.6: DIFFERENTIAL PULSE POLAROGRAM OF NITROFURANTOIN IN pH 7.5 BRITTON-ROBINSON BUFFER. NITROFURANTOIN CONCENTRATION $2 \times 10^{-5}$M; scan rate 10 mV s$^{-1}$
FIGURE 4.7: DIFFERENTIAL PULSE POLAROGRAM OF ACETAZOLAMIDE IN 0.1M HYDROCHLORIC ACID. ACETAZOLAMIDE CONCENTRATION 10 µg ml⁻¹; SCAN RATE 10 mV s⁻¹
Figure 4.8: Differential pulse polarogram of acetazolamide in pH 2 Britton-Robinson buffer. Acetazolamide concentration 10 μg ml⁻¹; scan rate 10 mV s⁻¹
FIGURE 4.9: DIFFERENTIAL PULSE POLAROGRAM OF ACETAZOLAMIDE IN pH 4.7 BRITTON-ROBINSON BUFFER. ACETAZOLAMIDE CONCENTRATION 10 μg ml⁻¹, SCAN RATE 10 mV s⁻¹
FIGURE 4.10: DIFFERENTIAL PULSE POLAROGRAM OF ACETAZOLAMIDE IN pH 6.5 BRITTON-ROBINSON BUFFER. ACETAZOLAMIDE CONCENTRATION 10 μg ml⁻¹, SCAN RATE 10 mV s⁻¹
On-line Amperometric Determination of Nitrofurantoin and Acetazolamide at a Sessile Mercury Drop Electrode

The use of flow injection analysis using the sessile mercury drop electrode for the determination of these drugs was investigated. A short delay coil of 50 cm was used to minimise the dispersion effect on the drug samples. A constant flow rate of 7 ml/min was used and the eluents used were pH 7.5 BR buffer for nitrofurantoin and 0.1M hydrochloric acid for acetazolamide.

The eluents were deoxygenated by means of nitrogen gas for 30 min. When Britton-Robinson buffer was used as eluent, sulphite was added after the 30 min deoxygenation. Drug samples were prepared by diluting the standard sample solution to the desired concentrations by deoxygenated supporting electrolytes. Nitrofurantoin sample solutions were prepared using deoxygenated pH 7.5 Britton-Robinson buffer containing sulphite. Sample solutions were deoxygenated further before they were injected. Usually three injections were made of each sample solution.

To determine the optimum applied potential at the sessile mercury drop electrode in this flow injection system, 100 µl aliquots of each drug were injected into the solution stream. The resulting potential and current values are shown in Figures 4.11 and 4.12. Potentials of -0.70 volts and -0.85 volts versus SCE were chosen for the determination of nitrofurantoin and acetazolamide respectively.

Calibration graphs of peak current against concentration of drug in the injected samples were rectilinear for nitrofurantoin over the range 1-50 ppm and for acetazolamide over the range 10-70 ppm. Fluctuations in the base line were measured. The detection limit was measured as double the size of these fluctuations and was 0.3 ppm for nitrofurantoin, and 7 ppm for acetazolamide. Recorder traces of the peak current response of nitrofurantoin over 10-40 µg ml⁻¹ are shown in Figure 4.13. Similarly, calibration signals for the determination of acetazolamide at the concentration range of 20-60 µg ml⁻¹ are
FIGURE 4.11: HYDRODYNAMIC VOLTAMMOGRAM OF 10^{-4} M NITROFURANTOIN IN pH 7.5 BRITTON-ROBINSON BUFFER. SAMPLE INJECTED 100 μL
FIGURE 4.12: HYDRODYNAMIC VOLTAMMOGRAM OF $10^{-4}$ M ACETAZOLAMIDE IN 0.1 M HYDROCHLORIC ACID. SAMPLED INJECTED 100 µl
FIGURE 4.13: TYPICAL SIGNALS OBTAINED FOR THE DETERMINATION OF ACETAZOLAMIDE.
A, 0; B, 20; C, 40; D, 60 and E, 70 μg ml⁻¹. Flow rate 7 ml min⁻¹. $E_{\text{app.}} = -0.85V$

FIGURE 4.14: TYPICAL SIGNALS OBTAINED FOR THE DETERMINATION OF NITROFURANTOIN
A, 0; B, 10 μg ml⁻¹; C, 20 μg ml⁻¹; D, 30 μg ml⁻¹ and E 40 μg ml⁻¹. Flow rate 7 ml min⁻¹. $E = -0.70V$
shown in Figure 4.14.

On-line Determination of Nitrofurantoin and Acetazolamide in their Tablets

Excipient material present in the commercially available preparations did not interfere with the polarographic analysis and, therefore, no attempt was made to separate them prior to analysis. The average volume occupied by the undissolved tablet excipients was approximately 0.05 ml, so the error introduced by leaving the tablet excipient in the solution is very small.

Ten tablets of each drug were weighed, finely powdered and mixed. From each drug tablets, 3 portions were taken, weighed accurately, and then dissolved and diluted as described earlier. The final solutions contained about 20 µg ml⁻¹ of nitrofurantoin and about 40 µg ml⁻¹ of acetazolamide. For the solutions containing nitrofurantoin, 3 injections of 100 µl of each sample solution were made into pH 7.5 Britton-Robinson buffer eluent, and the peak currents were recorded at -0.70V versus SCE. Three injections of 100 µl of each sample solution containing acetazolamide were made into 0.1M hydrochloric acid eluent. The potential of the sessile mercury drop electrode was set at -0.85V versus SCE. The peak currents corresponding to the concentration of the drug in the injected sample solutions were recorded. The peak currents obtained for both drugs were compared with those obtained for the standards.

The amount of each drug in its tablet formulation was determined by using the recommended procedure and this was compared with the nominal amount that should be present. The average nitrofurantoin content per tablet was found to be 98.4 mg (mean of three determinations, coefficient of variation was less than 1%) compared with the nominal amount of 100 mg. The average acetazolamide content per tablet was found to be 245.2 mg (mean of three determinations, coefficient of variation was less than 1%) compared with the nominal amount of 250 mg.
The results obtained here for nitrofurantoin and acetazolamide tablets show greater precision than those obtained by the USPXX method, which make the on-line method quite satisfactory.

CONCLUSION

Procedures are given for the on-line determination of nitrofurantoin and acetazolamide using flow injection analysis with amperometric detection by means of a sessile mercury drop electrode held at -0.7V and -0.85V versus SCE. Nitrofurantoin down to the 0.3 ppm level can be determined by direct injection of the sample. A higher detection limit for acetazolamide was caused by the difficulty of removing trace amounts of dissolved oxygen from the acidic eluent. The method is simple and rapid with a coefficient of variation of 1.9% and 2.3% at 10 ppm concentration, for nitrofurantoin and acetazolamide respectively.

On-line methods for the assay and determination of nitrofurantoin and acetazolamide in their tablets without the need for prior separation of the drug compound from tablets, are given. Precision of these methods make it suitable for use in routine analysis.
References

15. W.F. Bayne, G. Rogers and N. Crisologo, ibid, 64 (1975) 402.
CHAPTER 5

FLOW INJECTION AMPEROMETRIC DETERMINATION OF PHOSPHATE, ARSENATE
AND GERMANATE AS PREFORMED HETEROPOLYACIDS AT A
SESSILE MERCURY DROP ELECTRODE

INTRODUCTION

In recent years, considerable interest has been shown in the
heteropoly compounds of molybdenum, not only in the structure and
colorization of such compounds, but also in those aspects of
their chemistry that have made them of importance to industrial
applications.1

The subject of heteropoly compounds has been covered in many recent
reviews2-5. This literature has primarily dealt with the structural
chemistry and electronic properties of these complexes and has
emphasised not only the heteropoly anions of molybdenum but also
those of tungsten and vanadium.

The heteropoly molybdic acids formed when excess molybdate reacts in
acidic solution with phosphate, silicate, arsenate, germanate and
other oxy-anions are made the basis of sensitive methods for the
determination of the corresponding elements. The heteropoly anion
formed in the common series of the 12-molybdoheteropoly acids is of
the type:

\[ \text{[PMo}_{12}\text{O}_{40}]^{-3} \text{ or } [P(Mo}_{3}\text{O}_{10}]_{4}^{-3} \]

The central atom in the anion is P, to which the Mo atoms are
coordinated through O atoms. In the 12-heteropoly-molybdic acids
there are 12 Mo atoms arranged around the P, or other central atom.
Stoichiometric studies$^6$ indicated that in nitric acid solution the reaction between phosphate and molybdate can be represented by

$$H_3PO_4 + 6[Mo(VI)] \rightleftharpoons PO_4^{3-} (MoO_3)_{12} + 9H^+$$

12-molybdo-phosphate

The kinetics of this reaction have been investigated$^7,8$. Phosphate and a Mo(VI) dimer react initially and the polymerization to 12-Mo-P heteropolyacid follows.

12-Heteropolyacids exist in two isomeric forms, the α and β structures. Their existence was postulated by Strickland$^9$. At high acidity and $[H^+]/[MoO_4^{2-}]$ ratio, they indicated that the unstable β form is produced, although the significance of the $[H^+]/[MoO_4^{2-}]$ ratio has been challenged recently. It decays spontaneously into the stable α-form which is obtained at low acidity and $[H^+]/[MoO_4^{2-}]$ ratio. The existence of the α- and β-forms of heteromolybdates has been reported and a structure for the β-form has been proposed by Chalmers and Sinclair$^{10,11}$. The unstable β-forms can be stabilised by addition of comparatively large amounts of polar organic solvents, especially acetone.

The assumption that the formation of the α- and β-forms were fundamentally dependent on the acid to molybdate mole ratio has gone unchallenged until recently. Truesdale and Smith$^{12,13}$ have rejected the above condition and instead have proposed a mechanism that involves only pH and molybdate concentration as the primary factors that determine which form of molybdosilicic acid is produced when molybdate and silicate species react. Their investigation showed that solutions of constant acid to molybdate ratio could span a wide range of pH values. Thus either form of molybdosilicic acid or a mixture of both forms could be formed in solutions with the same acid to molybdate ratio. They concluded that the form produced does not depend on the acid to molybdate ratio but on the pH and on the concentration of molybdate. They found that the optimum pH values for
the preparation of the $\beta$-isomer were 1.0-1.8 and 3.8-4.8 for the $\alpha$-isomer. The mechanism of the formation of the $\alpha$-form is suggested by Truesdale and Smith\textsuperscript{12} to be via the $\beta$-form. However they found that formation via the $\beta$-form is slower than by direct formation. The conversion of the $\beta$-isomer to the $\alpha$-form is a simple first order reaction with respect to the concentration of the $\beta$-form. A solution of $\beta$-molybdosilicate at pH 1.8 is buffered at pH 4.2 with acetate to form $\alpha$-molybdosilicate.

Polarography of heteropolyacids at the DME has been applied to the determination of phosphate, arsenate and germanate\textsuperscript{14-16}. Polarographic methods of analysis have been reviewed by Alimarin et al\textsuperscript{5}.

In this laboratory a procedure has been developed for the differential pulse polarographic determination of nanogram amounts of orthophosphate based on the reduction of molybdenum blue formed from chemical reduction of 12-molybdophosphate\textsuperscript{17}. The molybdenum blue is extracted into iso-amyl alcohol from acid solution, and the extract is then washed free of excess molybdate with dilute sulphuric acid. Finally, the molybdenum blue is back-extracted into a tartrate buffer and is pulse polarographed. Precise determination was made on 10 ng ml$^{-1}$ of PO$_4^{3-}$ in the polarographic solution.

Recently Fogg and Bsebsu\textsuperscript{18} have determined phosphate, silicate, arsenate and germanate as $\beta$-12-heteropolyoxo-molybdate under the solution conditions developed by Chalmers and Sinclair\textsuperscript{10,11} i.e. the stabilisation of the $\beta$-heteropolyoxo-molybdate with acetone to prevent its conversion into the corresponding $\alpha$-heteropolyoxo-molybdate, at a glassy carbon electrode. They also described a simple flow injection amperometric procedure for the determination of phosphate, silicate, arsenate and germanate by the injection of heteropoly acid pre-formed in various eluents at a glassy carbon electrode held at +0.22, +0.18, +0.24 and +0.15V respectively\textsuperscript{19}. Silicate and phosphate were determined down to the 10$^{-7}$M and 10$^{-6}$M levels respectively, but arsenate and germanate could only be determined down to the 10$^{-5}$M
level owing to deterioration of the baseline of the reagents and eluents used. Loss of rectilinearity occurred for all four determinations above the $0.5 \times 10^{-4} \text{M}$ level and loss of signal owing to adsorption was clear in the case of germanate. The pre-formed procedures were used to determine total phosphate and soluble silicate in commercial washing powders\textsuperscript{20}.

Clearly as reduction of the heteropolytungstate at a glassy carbon electrode in a flow injection systems has occurred at low positive potentials, it is expected to be possible to monitor these reductions at a sessile mercury drop electrode held at a potential between 0.0 and $+0.3 \text{V}$ where mercury is not oxidised and dissolved oxygen is not reduced.

The present chapter describes a simple flow-injection amperometric procedure for the determination of phosphate, arsenic and germanium by the injection of heteropolyacids pre-formed in acidic eluents at a sessile mercury drop electrode without deoxygenation of the eluent or the sample solutions. Solution conditions developed by Chalmers and Sinclair\textsuperscript{10} and used later by Fogg and Bsebsu\textsuperscript{19} have been adapted here.

**EXPERIMENTAL**

A single channel flow injection system was used. Eluent was pumped through the system by means of an Ismatec Mini-S pump. Injections were made with a Rheodyne low pressure sample injection valve (5020) fitted with a 75 $\mu\text{L}$ sample loop and connected to the laboratory built amperometric detector\textsuperscript{21} by means of 50 cm of 0.58 mm bore PTFE tubing. The amperometric detector was fitted with the sessile mercury drop holder, the sessile mercury drop electrode being held in the wall-jet configuration. The detector was used partly immersed in electrolyte of the same composition as the eluent and the platinum counter and saturated calomel reference electrodes were placed in this electrolyte to complete the three electrode system. The potential of the sessile mercury drop electrode was maintained at the
required potential by means of a PAR 174A polarographic analyser (Princeton Applied Research). Signals were recorded on a Linseis L650 y-t recorder.

REAGENTS AND SOLUTIONS

Standard Orthophosphate Solution, $10^{-3}$ M (94.99 µg ml$^{-1}$ of PO$_4$$^{3-}$)

Dissolve 0.136 g of analytical reagent grade potassium dihydrogen orthophosphate in distilled water and dilute to 1 litre in a calibrated flask. This solution is $10^{-3}$ M in orthophosphate. Prepare less concentrated standard solutions by dilution.

Standard arsenic(V) solution, 111.6 µg ml$^{-1}$

Accurately weigh 0.93 g of disodium arsenate, dissolve in distilled water and dilute to 1 litre in a calibrated flask.

Standard germanium solution, 113.82 µg ml$^{-1}$

Accurately weigh 0.164 g of germanium (IV) oxide. Dissolve in dilute ammonia solution (silica free), neutralise with dilute sulphuric acid and dilute to 1 litre in a calibrated flask.

Acid molybdate solution 2% m/v

Add 35 ml of analytical-reagent grade concentrated sulphuric acid to 200 ml of distilled water. Dissolve 5 g of ammonium molybdate in the resulting solution and dilute when cold to 250 ml with water. Store in a polyethylene bottle.

Ammonium molybdate solution 8% m/v

Dissolve 8 g of ammonium molybdate tetrahydrate in distilled water. Dilute to 100 ml in a calibrated flask and store in a polyethylene bottle.
**Ammonium molybdate-sulphuric acid solution**

Dissolve 40g of ammonium paramolybdate heptahydrate in 500 ml of water in a polyethylene beaker and add 500 ml of 1M sulphuric acid solution. Mix and store in a polyethylene bottle.

**PROCEDURES FOR FORMATION OF HETEROPOLYACIDS**

**Procedure for Phosphate**

Transfer by pipette aliquots of standard orthophosphate solution containing 0.05-1.5 mg of orthophosphate into a series of 50 ml calibrated flasks. To each add 5 ml of acidic molybdate solution and dilute to about 20 ml with distilled water. After allowing to stand for 15 min, dilute to 50 ml with distilled water.

**Procedure for arsenic**

Pipette an aliquot of sample solution containing 0.3 μg-1.2 mg of arsenic (IV) into a 50 ml calibrated flask, add 7 ml of 4M sulphuric acid, 7 ml of 8% ammonium molybdate solution and add 16 ml of acetone, dilute to volume with water and mix. Leave for 30 min.

**Procedure for germanium**

In a 50 ml calibrated flask place 6 ml of ammonium molybdate solution, 10 ml of acetone and 3 ml of 1M sulphuric acid. Add an aliquot of sample solution containing 0.3 μg-1.2 mg of germanium, dilute to volume with water, mix and allow to stand for 5 min.

**Assessment of Eluents**

Eluents having the same composition as the blank were used initially, but these produced a high and noisy background. Therefore, an aqueous acetone eluent was used for arsenic and germanium determinations (the ratio of acetone to water is the same as in the sample solutions), whilst a dilute sulphuric acid (0.25M) eluent was used in the
determination of phosphate. The eluents were degassed for 10 min before use.

RESULTS AND DISCUSSION

Graphs of current obtained at the detector cell for the injection of 100 μl of 2 \times 10^{-4} \text{M} solutions of arsenate and germanate, and 100 μl solutions of 4 \times 10^{-4} \text{M} solutions of phosphate at potentials between 0.0V and 0.35V into eluents of 0.25M sulphuric acid for phosphate and aqueous acetone for germanate and arsenate are shown in Figure 5.1. These graphs can be used to select suitable potentials to apply to the sessile mercury drop electrode when making determinations in a particular eluent. Potentials of 0.22V, 0.23V and 0.18V versus SCE were chosen for phosphate, arsenate and germanate respectively.

Calibration graphs of peak current against concentration of heteropolyacids in the injected samples were rectilinear for phosphate over the range 0.5 - 4 \times 10^{-5} \text{M}, and for arsenate and germanate over the range 2 \times 10^{-7} - 8 \times 10^{-4} \text{M} and 1 \times 10^{-6} \text{M} - 2 \times 10^{-4} \text{M} respectively.

The reproducibility of the heteropolyacid signals was checked by injecting over 50 injections of 100 μl of 1 \times 10^{-5} \text{M} solutions. No decrease in signals was observed at concentrations lower than 5 \times 10^{-5} \text{M} phosphate, 8 \times 10^{-4} \text{M} arsenate, and 2 \times 10^{-4} \text{M} germanate. The coefficient of variation was less than 1% for well over 50 injections (Table 5.1).
FIGURE 5.1: CURRENTS OBTAINED AT VARIOUS POTENTIALS FOR FLOW INJECTION OF 100 μL OF PREFORMED HETEROPOLYACIDS INTO AQUEOUS ACETONE ELUENT.
A) $2 \times 10^{-4}$M arsenate; B) $2 \times 10^{-4}$M germanate
FIGURE 5.1(c) CURRENTS OBTAINED AT VARIOUS POTENTIALS FOR FLOW INJECTION OF 100 µl OF PHOSPHOMOLYBDIC ACID (4 x 10^{-4}M) INTO 0.25M SULPHURIC ACID ELUENT
TABLE 5.1: Variation of the values of coefficient of variation of 50 injections of heteropolyacid solutions of phosphate, arsename and germanate (1 x 10^{-5}M)

<table>
<thead>
<tr>
<th>Number of Injections*</th>
<th>Coefficient of Variation %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phosphate</td>
</tr>
<tr>
<td>5</td>
<td>0.36</td>
</tr>
<tr>
<td>10</td>
<td>0.59</td>
</tr>
<tr>
<td>20</td>
<td>0.61</td>
</tr>
<tr>
<td>30</td>
<td>0.72</td>
</tr>
<tr>
<td>40</td>
<td>0.84</td>
</tr>
<tr>
<td>50</td>
<td>0.98</td>
</tr>
</tbody>
</table>

* Number of injections is cumulative i.e. a total of 50 injections were made.

Typical signals obtained for producing calibration graphs for phosphate, arsename and germanate are shown in Figures 5.2, 5.3 and 5.4. Comparing the results obtained for arsenic and germanium at the sessile mercury drop electrode, and that obtained previously at a glassy carbon electrode\textsuperscript{19} shows that their determination at the sessile mercury drop electrode has several advantages. These include longer linearity range, better reproducibility and higher sensitivity. Calibration graphs at a glassy carbon electrode were rectilinear in the range of 0.5 - 4 x 10^{-5}M for both arsename and germanate, with loss of signals due to adsorption in the case of germanate. High and noisy backgrounds prevented determinations at concentrations lower than 10^{-5}M. However in the determination of phosphate at a glassy carbon electrode, the calibration graph was rectilinear over the range 0.5 x 10^{-6} - 4 x 10^{-5}M phosphate, compared with the range of 0.5 x 10^{-5} - 4 x 10^{-5}M at the sessile mercury drop electrode.
FIGURE 5.2: FLOW INJECTION SIGNALS FOR THE DETERMINATION OF PHOSPHATE AT VARIOUS CONCENTRATIONS. A, 0; B, 1; C, 2 and D, $4 \times 10^{-5}$M. Glassy carbon electrode held at +0.22V.
FIGURE 5.3: FLOW INJECTION SIGNALS FOR DETERMINATION OF ARSENATE AT VARIOUS CONCENTRATIONS
A, 0; B, 1; C, 2; D, 4; & E $8 \times 10^{-6}$ M. Glassy carbon electrode held at 0.23V
FIGURE 5.4: FLOW INJECTION SIGNALS FOR DETERMINATION OF GERMANATE AT VARIOUS CONCENTRATIONS
FLOW INJECTION AMPEROMETRIC DETERMINATION OF PHOSPHATE BY MEASURING
THE CATALYTIC REDUCTION OF HYDROGEN PEROXIDE IN THE PRESENCE OF
Mo(VI) AT A SESSILE MERCURY DROP ELECTRODE

Introduction

Various electrochemical methods have been reported for the
determination of phosphate, after its conversion into 12-
molybdophosphoric acid. The best sensitivity achieved was by a
differential pulse polarographic method where a detection limit of 0.1 mg l⁻¹ was obtained

The sensitivity can be enhanced by measurement of the polarographic
waves or peaks resulting from the catalytic reduction of some
oxidants, such as perchlorate or nitrate in the presence of Mo(VI).
Hight et al. proposed the use of nitrate and evaluation of the
catalytic wave by differential pulse polarography. A detection limit
of 9 μg l⁻¹ was reported. Prado et al. have used a similar approach
with hydrogen peroxide as the oxidant, which also undergoes
polarographic reduction catalysed by Mo(VI). A detection limit of
2.3 μg l⁻¹ was reported using DPP. The method involves several steps:

1. formation of 12-phosphomolybdic acid in the aqueous phase
2. extraction with an organic solvent
3. determination of the Mo(VI) by means of the indicator reaction.

For formation of 12-phosphomolybdic acid an acidic medium and an
excess of Mo(VI) are required. This excess of molybdate is the
reason for separation of the 12-phosphomolybdic acid being necessary
so that only the molybdate present in the 12-phosphomolybdic acid is
determined. The heteropoly acid is dissociated into aqueous solution
with sodium hydroxide solution to extract Mo(VI).

The mechanism of the reaction between Mo(VI) and hydrogen peroxide
was proposed by Kolthoff and Parry and can be briefly described as:

\[
\text{MoO}_4^{2-} + \text{H}_2\text{O}_2 \xrightarrow{\text{H}_2\text{O}} \text{MoO}_5^{2-} + 2\text{H}^+ + 2\text{e}^- \xrightarrow{} \text{MoO}_4^{2-} + \text{H}_2\text{O} + \text{H}_2\text{O}_2
\]
The catalytic current was measured by Pardo et al.\textsuperscript{23} at 0.20V versus SCE in an acidic medium of 0.5M sulphuric acid, and therefore deoxygenation of the solutions was not necessary.

A method is proposed here for the on-line determination of phosphate, based on the measurement of the catalytic reduction of hydrogen peroxide in the presence of molybdenum (VI) at a sessile mercury drop electrode. Solution conditions developed by Pardo et al.\textsuperscript{23} for differential pulse polarography have been used for the on-line measurements.

**Experimental**

The flow injection system was used as described previously for the determination of phosphate, arsenic and germanium as 12-heteropolyacids at a sessile mercury drop electrode. The eluent used for this method was 1M sulphuric acid solution. Neither the eluent nor the sample solutions were deoxygenated or degassed.

**REAGENTS**

**Standard Phosphate Solution**

Dissolve 7.099g of dried sodium dihydrogen orthophosphate in 1 litre of distilled water and store in polythene. This solution is 5.00 \times 10^{-2}\text{M}. Prepare less concentrated standard solutions by dilution.

**Standard Molybdate Solution**

Dissolve 12.36g of ammonium paramolybdate tetrahydrate in 1 litre of distilled water and store in polythene. This solution is 1.00 \times 10^{-2}\text{M}. Prepare less concentrated solutions by dilution.

**Molybdate Solution 2%**

Prepare and store in polyethylene.

All reagents were of analytical grade.
PROCEDURE

Transfer a known volume of sample solution into a separating funnel. Add 2 ml of 2% molybdate solution and dilute with distilled water to 25 ml. Add 1 ml of 5M sulphuric acid and after 15 min, 1 ml more of the acid and 15 ml of ethyl acetate. Shake the mixture for a few minutes and then separate the organic phase. Wash the organic phase with 10 ml portions of 0.8M sulphuric acid and then strip the molybdate with 25 ml of 1M sodium hydroxide. Acidify this aqueous extract with 0.8M sulphuric acid to about pH 2.5. Add 4 ml of 5M sulphuric acid and 10 ml of 0.2M hydrogen peroxide and dilute to volume in a 100 ml calibrated flask. Prepare solutions of different phosphate concentrations using the same procedure.

RESULTS AND DISCUSSION

Typical hydrodynamic voltammogram obtained by injecting 100 μl of 100 ng ml⁻¹ phosphate solution into 1M sulphuric acid eluent and measuring the signals obtained at various potentials are given in Figure 5.5. A potential of 0.20V was chosen for the on-line determination of phosphate by this method.

A slight decrease in signals was obtained with successive injections of sample solutions. At 0.1 μg ml⁻¹ concentration level, the decrease in signals was 3.19% after 10 successive injections. Table 5.2 shows loss of signals with successive injections.
### TABLE 5.2 Decrease in signals in 30 successive injections of sample solution (0.1 μg ml⁻¹)

<table>
<thead>
<tr>
<th>Number of Injection</th>
<th>Peak Current/μA of the Injection</th>
<th>Decrease in Signals %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.94</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0.93</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>0.92</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>0.91</td>
<td>3</td>
</tr>
<tr>
<td>20</td>
<td>0.87</td>
<td>7</td>
</tr>
<tr>
<td>30</td>
<td>0.83</td>
<td>12</td>
</tr>
</tbody>
</table>

Calibration graphs of the reduction current against the amount of phosphate were rectilinear over the range 5-100 ng ml⁻¹, (approximately 5.2 x 10⁻⁸ - 1.0 x 10⁻⁶ M). The signals shown in Figure 5.6 are typical of those obtained when producing a calibration graph for the determination of phosphate.

The efficiency of various organic solvents for extraction of 12-phosphomolybdic acid has been discussed by several authors²⁵-²⁷. The main conclusion was that 12-phosphomolybdic acid is best extracted with oxygen-containing solvent. Ethyl acetate was used in this work because it provided the highest extraction coefficient of 94.6%.

### CONCLUSION

Flow injection amperometric procedures have been described for the determination of phosphate, arsenate and germanate as 2-12-heteropoly acids at a sessile mercury drop electrode held at 0.22V, 0.23V and 0.18V respectively. The determinations were made by injecting the preformed heteropoly molybdate into an eluent. Previously¹⁹ determination of these species at a glassy carbon electrode was described. Determination of both arsenate and germanate was
unsatisfactory due to high background, and adsorption at the electrode surface of the product in the case of germanate. However, more successful results were obtained for phosphate determination.

In the present work more sensitive and reproducible results were obtained for arsenate and germanate at a sessile mercury drop electrode, whilst less successful results were obtained for phosphate due to loss of rectilinearity below the $0.5 \times 10^{-5}$ level. For phosphate determination at concentrations below $5 \times 10^{-6}$M, a method developed by Pardo et al.\textsuperscript{23} was adapted here. The method is based on forming the phosphomolybdic acid, extracting it by organic solvent and then dissociating phosphomolybdic acid by sodium hydroxide, to strip MO(VI) off. The catalytic reduction of hydrogen peroxide in the presence of MO(VI) was measured. This reduction current is proportional to phosphate concentration used to form phosphomolybdic acid. On-line determination of phosphate by this method was found to be very sensitive, with a useful signal at the $5 \times 10^{-8}$M level.
FIGURE 5.5 CURRENTS OBTAINED AT VARIOUS POTENTIALS FOR
(A) FLOW INJECTION OF 100 µl OF 100 ng ml\(^{-1}\) PHOSPHATE INTO 1M SULPHURIC ACID ELUENT
(B) FLOW INJECTION OF 100 µl OF 0.02M HYDROGEN PEROXIDE INTO 1M SULPHURIC ACID
FIGURE 5.6: TYPICAL SIGNALS OBTAINED FOR THE DETERMINATION OF PHOSPHATE.
Phosphate concentration, A, 0; B, 25; C, 50; and D, 100 ng ml⁻¹. Glassy carbon electrode held at +0.20V
References

13. Idem, ibid, 100 (1975) 797.
CHAPTER 6

CONTRIBUTION TO THE VOLTAMMETRIC DETERMINATION OF SOME CATECHOLAMINES AND ASCORBIC ACID AT AN ELECTROCHEMICALLY PRETREATED GLASSY CARBON ELECTRODE

INTRODUCTION

The catecholamines, dopamine (DA) and norepinephrine (NE), are essential participants in the neurotransmission process. Accordingly, they are frequently implicated in neurological diseases of the brain. Advances in the treatment of such diseases have resulted from the study of these amines and their metabolites in the central nervous system. Homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) are the principal metabolites of DA. 5-Hydroxyindole acetic acid (5-HIAA) is the metabolite of 5-hydroxytryptamine (5-HT) or serotonin, which is a chemically and anatomically distinct transmitter. Table 6.1 shows the structures of these compounds.

The first attempts to use electroanalytical techniques for monitoring neurotransmitters in cerebral tissue in vivo were made by Adams' and Lane's groups in the early 1970's. Since the catecholamines are easily oxidizable, they have been extensively studied. The major difficulty was the presence in brain tissue of high levels of ascorbic acid (AA) which oxidizes on untreated carbon electrodes at the same potential as the catecholamines. The ascorbic acid problem was first approached by Lane et al. using a chemically modified platinum electrode. This involved exposing the surface of a platinum electrode to iodide solutions, resulting in the formation of a chemisorbed layer capable of preventing deposition of oxidized substances at the electrode surface which otherwise inhibited the electron transfer process. Such electrodes were capable of producing two distinct peaks from a mixture of ascorbic acid and dopamine when used with DPV but proved too unstable for use in vivo.
TABLE 6.1: Structure of some neurotransmitters

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine (DA)</td>
<td><img src="image1" alt="Structure of Dopamine" /></td>
</tr>
<tr>
<td>Norepinephrine (NE)</td>
<td><img src="image2" alt="Structure of Norepinephrine" /></td>
</tr>
<tr>
<td>5-hydroxytryptamine (5-HT) (Serotonin)</td>
<td><img src="image3" alt="Structure of 5-Hydroxytryptamine" /></td>
</tr>
<tr>
<td>Homovanillic acid (HVA)</td>
<td><img src="image4" alt="Structure of Homovanillic acid" /></td>
</tr>
<tr>
<td>Dihydroxyphenylacetic acid (DOPAC)</td>
<td><img src="image5" alt="Structure of Dihydroxyphenylacetic acid" /></td>
</tr>
<tr>
<td>5-Hydroxyindole acetic acid (5-HIAA)</td>
<td><img src="image6" alt="Structure of 5-Hydroxyindole acetic acid" /></td>
</tr>
</tbody>
</table>
More encouraging results have been obtained using another approach to electrode surface modification. Gonon et al.\textsuperscript{13,14} have obtained clear separation of ascorbic acid and dopamine/DOPAC oxidation potentials using electrochemically pretreated carbon fibre microelectrodes. The active surface of these electrodes consists of a 0.5 mm exposed length of pyrolytic carbon fibre (8 \( \mu \)m diameter) supported in a pulled glass capillary. Pretreatment of the electrode was carried out in phosphate-buffered saline and involved exposure of the electrode to a triangular wave potential, followed by various continuous potentials for several seconds.

The use of differential pulse voltammetry with electrochemically pretreated carbon fibre electrodes to monitor in vivo changes in the extracellular levels of either ascorbic acid and DOPAC\textsuperscript{5,13-15} or 5-hydroxyindoleacetic acid (5-HIAA)\textsuperscript{16-19} is now well reported\textsuperscript{20,21}. The electrodes used were specifically and differently electrochemically pretreated to record separate peaks for ascorbic acid and DOPAC or 5-HIAA.

The major limitation of the carbon fibre electrode is its short working life in vivo when recording the ascorbic acid and dopamine/DOPAC peaks. Furthermore it is more difficult to fabricate and implant than the robust carbon paste or graphite-epoxy electrodes\textsuperscript{22}.

Falat and Cheng\textsuperscript{23} have used an electrochemically pretreated graphite epoxy electrode for voltammetric differentiation of ascorbic acid and dopamine. These electrodes require a severe treatment of -5V to +7V square-wave signal, 35 Hz for 5 min, in order to obtain two separate peaks for ascorbic acid and dopamine. The long-term stability of these electrodes in vivo remains to be evaluated\textsuperscript{22}.

Another approach to the problem of selectivity has involved immobilizing enzymes onto the surface of the electrode which selectively inhibit the oxidation of compounds at that surface. For example, the enzyme ascorbate oxidase converts ascorbic acid into
the electroactive inert dehydroascorbate. The enzyme has been immobilized on the surface of graphite-epoxy electrodes producing an electrode that is essentially insensitive to ascorbic acid since it is unable to reach the electrode surface in an oxidizable form.

Although some success has been achieved in the previous voltammetric methods, especially in selectivity, there is still a need for electrodes with improved selectivity, sensitivity and stability.

Glassy carbon electrodes have found common usage as the working electrodes in numerous electroanalytical applications. The design of electrocatalytic glassy carbon surfaces has been the object of many investigations. Specific procedures have been shown to increase the rate of various redox reactions at the glassy carbon electrode. These include activation by radio frequency plasma, dispersion of metal oxide particles, e.g. α-alumina, heating the glassy carbon to 500°C and a variety of chemical and electrochemical pretreatments.

Electrochemical pretreatment procedures appear to be a very promising means of achieving surface modification, in terms of the overpotential decrease, stability and convenience (time and instrumentation). For this purpose the carbon electrode potential has been scanned or stepped between positive and negative values at different rates or frequencies over different time periods. In particular, a simple DC electrochemical treatment of the surface (5 min at +1.75V vs SCE, 10s at -1.2V) has been evaluated in detail. Engstrom attributed the lowering of the overpotential to the introduction of quinone functionalities that serve as mediators in the redox reaction of interest. Engstrom and Strasser attributed it to cleaning the surface of impurities from the polishing step.

Another electrochemical pretreatment has been reported, in which an alternating current (AC) waveform was used. Blaedel and Mabbott have illustrated that when glassy carbon surfaces are subjected to a severe treatment, ±4V at 30-70 Hz for 4-6 min, its catalytic ability
towards the hexacyanoferrate(II) hexacyanoferrate(III) system was improved.

Wang and Hutchins\textsuperscript{35} have described a detailed examination of the effect of alternating current pretreatment on a variety of redox reactions at the glassy carbon electrode. All compounds tested (uric acid, dopamine, hexacyanoferrate(II), ascorbic acid, oxalic acid and benzoquinone) exhibited lowering of the overpotential by 110-300 mV, compared to highly polished bare glassy carbon electrodes.

The purpose of this chapter is to examine the effect of electrochemical pretreatment of the glassy carbon electrode on the oxidation of ascorbic acid, dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindole-3-acetic acid (5-HIAA) and 3,4-dihydroxyphenylethanolamine (noradrenaline or norepinephrine), and to examine the possibility of voltammetric differentiation of either ascorbic acid and dopamine or DOPAC. The effect of electrochemically pretreating glassy carbon electrodes on-line on a flow injection system for on-line amperometric determination of ascorbic acid, dopamine and DOPAC is studied. The effect on the background currents and on the hydrodynamic voltammograms, is examined, and the analytical capability of an untreated and pretreated electrode in a flowing system is investigated.

**EXPERIMENTAL**

**Apparatus for Linear Sweep Voltammetry**

Voltammograms were obtained using a PAR 174A polarographic analyser (Princeton Applied Research Corp) and recorded using an X-Y Gould HR2000 recorder. The voltammetric cell comprised a working glassy carbon electrode, a platinum counter electrode and a saturated calomel reference electrode. The glassy carbon electrode used was the Metrohm 6.0805.010 having a nominal surface area of 0.07 cm\textsuperscript{2}. An EDT glassy carbon electrode (0.5 mm diameter) was used for HVA determination only.
Apparatus for Flow Injection Amperometry

The flow of eluent was produced with an Ismatec Mini-S peristaltic pump. Injections of 100 µl were made with a Rheodyne (5020) injection valve. The injection valve was connected to a laboratory built detector cell, incorporating a glassy carbon electrode (Metrohm 6.0805.010) as seen in Figure 6.1, by means of a 50 cm length of 0.58 mm bore size Teflon tubing. The detector cell with the platinum electrode and the saturated calomel reference electrode were partially immersed in an electrolyte similar to that of the eluent which consisted of 0.1M phosphate buffer solution. The potential of the glassy carbon electrode was controlled by means of the PAR 174A polarographic analyser. Current peaks were monitored on a Linseis L650 recorder.

REAGENTS

Standard solutions of $10^{-2}$M of DA, AA, DOPAC, NE, 5-HIAA and HVA

Dissolve separately 0.1896g of dopamine hydrochloride (Sigma Chemicals), 0.1761g of ascorbic acid (Fisons), 0.1682g of DOPAC, 0.1692g of norepinephrine, 0.1912 of 5-HIAA and 0.1822g of HVA (all from Sigma Chemicals) in 0.1M phosphate buffer (pH 7.4), and dilute each solution to 100 ml in a calibrated flask with phosphate buffer (pH 7.4). Keep these solutions in the refrigerator and use within one day of their preparation. Make more dilute solutions by further dilution with 0.1M phosphate buffer (pH 7.4).

0.1M phosphate buffer (pH 7.4)

Mix 40.5 ml of 0.2M of disodium hydrogen orthophosphate solution with 9.5 ml of 0.2M sodium dihydrogen orthophosphate, and dilute to 100 ml in a calibrated flask with distilled water. Check the pH with a pH meter.
FIGURE 6.1: DETECTOR CONFIGURATION WHEN USED WITH GLASSY CARBON ELECTRODE
0.1M sulphuric acid

Dilute 5.6 ml of concentrated sulphuric acid to 1 litre with distilled water in a calibrated flask.

Procedure

The glassy carbon electrode was polished with a series of silicon carbide papers (NP 9088 Scotch 3M) of decreasing roughness, followed by alumina (0.3 μm and 0.015 μm) slurries. To eliminate the possibility of alumina catalysis as reported recently by Zak and Kuwana\textsuperscript{27}, the electrode was thoroughly sonicated in distilled water. An electrode prepared by this procedure will be referred to and considered as an untreated glassy carbon electrode. The electrode was usually reconditioned in pH 7.4 phosphate buffer before it was used. The electrochemical pretreatment was performed by applying a DC positive potential of 1.5-2.0V versus SCE for two minutes followed by a negative potential of -0.3 - -1.0V vs SCE for another 2 minutes. The electrode was immersed in pH 7.4 phosphate buffer or 0.1M sulphuric acid during this potential sequence. For flow injection experiments, the pretreatment was carried out on-line; the electrode was inserted in the detector cell, and an eluent of pH 7.4 phosphate buffer or 0.1M sulphuric acid, was pumped at a flow rate of 6 ml min\textsuperscript{-1}.

During the electrochemical pretreatment, when the applied potential was switched to 2.0V, a current spike resulted, and gas bubbles were produced at the electrode surface which were considered to be oxygen produced from the oxidation of water\textsuperscript{33}.

The electrode was polished with alumina (0.3 and 0.015 μm) slurries between different pretreatments.
RESULTS AND DISCUSSION

Optimisation of the Electrochemical Pretreatment

The optimum electrochemical pretreatment conditions for each one of the compounds studied were investigated. That involved the application of different anodic and cathodic potentials for different periods of time, and the results obtained were interpreted from the cyclic and linear sweep voltammograms. The pretreatment was carried out either in pH 7.4 phosphate buffer or in 0.1M sulphuric acid solutions.

It was observed that the peaks obtained were dependent on the potential at which the electrode was held, the length of time the electrode was held at that potential and the medium in which the pretreatment had been carried out. The results obtained are summarised in Table 6.2.

The electrochemical pretreatment of the glassy carbon electrode which produced the highest peak current was chosen as the recommended electrochemical pretreatment.
TABLE 6.2: Effect of electrochemical pretreatment of the glassy carbon electrode on the anodic current of 1 mM of AA, DA, DOPAC, NE, 5-HIAA and HVA in pH 7.4 phosphate buffer

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Potential/V</th>
<th>Time Interval/ min</th>
<th>Pretreatment Medium</th>
<th>Peak Current μA</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>+1.5</td>
<td>2</td>
<td>P</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>+1.7</td>
<td>2</td>
<td>P</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td>+1.7</td>
<td>5</td>
<td>P</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>+1.7</td>
<td>10</td>
<td>P</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>+1.9</td>
<td>2</td>
<td>P</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>+1.9</td>
<td>5</td>
<td>P</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>+2.0</td>
<td>2</td>
<td>P</td>
<td>10.8</td>
</tr>
<tr>
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<td>+1.5</td>
<td>5</td>
<td>P</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>-0.5</td>
<td>1</td>
<td>P</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>+1.7</td>
<td>5</td>
<td>P</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>-1.0</td>
<td>1</td>
<td>P</td>
<td>13.9</td>
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<tr>
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<td>+2.0</td>
<td>2</td>
<td>P</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>-0.5</td>
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<td>P</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>+2.0</td>
<td>5</td>
<td>P</td>
<td>15.3</td>
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<tr>
<td></td>
<td>-1.0</td>
<td>2</td>
<td>A</td>
<td>15.3</td>
</tr>
<tr>
<td></td>
<td>-0.5</td>
<td>2</td>
<td>A</td>
<td>15.3</td>
</tr>
<tr>
<td></td>
<td>-1.0</td>
<td>2</td>
<td>A</td>
<td>15.3</td>
</tr>
<tr>
<td>DA</td>
<td>+2.0</td>
<td>2</td>
<td>P</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>+2.0</td>
<td>5</td>
<td>P</td>
<td>12.3</td>
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<tr>
<td></td>
<td>+2.0</td>
<td>2</td>
<td>P</td>
<td>12.3</td>
</tr>
<tr>
<td></td>
<td>-0.3</td>
<td>2</td>
<td>P</td>
<td>14.7</td>
</tr>
<tr>
<td></td>
<td>+2.0</td>
<td>2</td>
<td>P</td>
<td>16.8</td>
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<td></td>
<td>-0.5</td>
<td>2</td>
<td>P</td>
<td>16.7</td>
</tr>
<tr>
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<td>+2.0</td>
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<td>P</td>
<td>15.2</td>
</tr>
<tr>
<td></td>
<td>-0.5</td>
<td>2</td>
<td>P</td>
<td>15.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>-------</td>
<td>-------</td>
<td>-----</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>+2.0</td>
<td>2</td>
<td>A</td>
<td>30.1</td>
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</tr>
<tr>
<td>-0.5</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+2.0</td>
<td>2</td>
<td>A</td>
<td>30.0</td>
<td></td>
</tr>
<tr>
<td>-1.0</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DOPAC**

|       |       |     |   
|-------|-------|-----|-----|
| +1.8  | 2     | P   | 4.6 |
| +2.0  | 2     | P   | 6.1 |
| +2.0  | 2     | P   | 6.3 |
| -0.3  | 2     | P   |     |
| +2.0  | 2     | P   | 6.8 |
| -0.5  | 2     | P   |     |
| +2.0  | 2     | P   | 7.2 |
| -1.0  | 2     | P   | 7.3 |
| +2.2  | 2     |     |     |
| -1.0  | 2     | P   |     |
| +2.0  | 2     | A   | 7.7 |
| -0.5  | 2     |     |     |
| +2.0  | 2     | A   | 8.0 |
| -1.0  | 2     |     |     |

**NE**

|       |       |     |   
|-------|-------|-----|-----|
| +1.8  | 2     | P   | 7.1 |
| +1.9  | 2     | P   | 11.8|
| +2.0  | 2     | P   | 12.3|
| +2.0  | 5     | P   | 14.0|
| +2.0  | 2     | P   | 18.2|
| -0.5  | 2     |     |     |
| +2.0  | 2     | P   | 18.1|
| -1.0  | 2     |     |     |
| +2.0  | 2     | A   | 19.3|
| -0.5  | 2     |     |     |
| +2.0  | 2     | A   | 19.1|
| -1.0  | 2     |     |     |

**5-HIAA**

|       |       |     |   
|-------|-------|-----|-----|
| +1.8  | 2     | P   | 4.9 |
| +2.0  | 2     | P   | 6.2 |
| +2.0  | 2     | P   | 7.1 |
| -0.5  | 2     | P   | 8.0 |
| +2.0  | 2     | P   | 8.4 |
| -0.8  | 2     |     |     |

**TABLE 6.2 (continued)**
where: $P =$ phosphate buffer (pH 7.4)  
$A =$ 0.1M sulphuric acid

<table>
<thead>
<tr>
<th>P</th>
<th>A</th>
<th>P</th>
<th>A</th>
<th>P</th>
<th>A</th>
<th>P</th>
<th>A</th>
<th>P</th>
<th>A</th>
<th>P</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
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<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>23.9</td>
<td>23.3</td>
<td>23.2</td>
<td>22.9</td>
<td>22.8</td>
<td>21.5</td>
<td>19.2</td>
<td>18.7</td>
<td>18.3</td>
<td>17.4</td>
<td>16.2</td>
<td>9.4</td>
</tr>
</tbody>
</table>

### TABLE 6.2
EFFECT OF THE ELECTROCHEMICAL PRETREATMENT OF THE GLASSY CARBON ELECTRODE ON THE VOLTAMMETRY OF DA, DOPAC, AA, 5-HIAA, HVA AND NE

As has been shown, electrochemical pretreatment causes the half-wave or the peak potentials of several anodic reactions to shift to less anodic values than those observed at freshly polished electrodes. The effect of electrochemical pretreatment on the peak potentials of the compounds studied here is shown in Table 6.3, and the cyclic voltammograms obtained before and after pretreatment of the glassy carbon electrode for these compounds are shown in Figures 6.2-6.7.

### TABLE 6.3: Effect of electrochemical pretreatment on peak potentials of various electroactive species at the glassy carbon electrode

<table>
<thead>
<tr>
<th>Species</th>
<th>Peak potential (V versus SCE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated electrode</td>
</tr>
<tr>
<td>DA</td>
<td>0.41</td>
</tr>
<tr>
<td>DOPAC</td>
<td>0.46</td>
</tr>
<tr>
<td>AA</td>
<td>0.56</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>0.66*</td>
</tr>
<tr>
<td>HVA</td>
<td>0.60*</td>
</tr>
<tr>
<td>NE</td>
<td>0.48</td>
</tr>
</tbody>
</table>

* Poorly defined peak (broad peak)
† Peak potential of the first scan

Upon pretreatment, the peak potential for all the species was shifted to less extreme values (less positive values); however the magnitude of the shift depended to a great degree on the electroactive species. For example, the peak potential for the oxidation of ascorbic acid shifted by 570 mV upon pretreatment, whereas the peak potential for the oxidation of dopamine shifted by 210 mV only. The degree of pretreatment activation of the compounds in terms of peak potential
shift decreases in the order ascorbic acid > 5-HIAA > DOPAC > NE > HVA > DA. The lowering of the overpotential obtained in the present work was much greater than that reported for AC electrochemical pretreatment\textsuperscript{35} where the shifts on peak potential of ascorbic acid and dopamine were only 257 and 176 mV respectively.

Another feature of the pretreated glassy carbon electrode is the excellent reproducibility in the peak potentials compared with those obtained at an untreated glassy carbon electrode. Table 6.4 summarises the coefficient of variation in peak potentials obtained for five compounds at both untreated and pretreated glassy carbon electrodes. The coefficient of variation is less than 1\% for the five species. However for HVA the peak potential was less reproducible, although the reproducibility was improved by electrochemical pretreatment of the electrode. On pretreatment, a new peak appears at 0.17V on the second and subsequent scans and this increases in size with successive scans, while the original peak decreases in size and is shifted to more positive potentials (Figure 6.7). This new peak was not observed when several scans were applied to an untreated electrode. This must mean that different oxidation products are produced at a pretreated and untreated electrode.

**TABLE 6.4:** Effect of electrochemical pretreatment of the glassy carbon electrode on peak potential reproducibility. Pretreatment was performed in pH 7.4 phosphate buffer

<table>
<thead>
<tr>
<th>Species</th>
<th>Coefficient of variation (%) in 5 experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated electrode</td>
</tr>
<tr>
<td>DA</td>
<td>3.2</td>
</tr>
<tr>
<td>AA</td>
<td>6.2</td>
</tr>
<tr>
<td>DOPAC</td>
<td>4.4</td>
</tr>
<tr>
<td>NE</td>
<td>9.5</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>8.5</td>
</tr>
</tbody>
</table>
FIGURE 6.2: CYCLIC VOLTAMMOGRAMS OF 1 mM DA AT
(a) newly polished electrode
(b) a pretreated electrode
FIGURE 6.3: CYCLIC VOLTAMMOGRAM OF 1 mM DOPAC
(a) newly polished electrode; (b) pretreated electrode
FIGURE 6.4: CYCLIC VOLTAMMGRAMS OF 1 mM ASCORBIC ACID AT (a) AN UNTREATED ELECTRODE; (b) A PRETREATED ELECTRODE
FIGURE 6.5: CYCLIC VOLTAMMOGRAMS OF 1 mM NE
(a) Newly polished electrode
(b) Pretreated electrode
FIGURE 6.6: CYCLIC VOLTAMMGRAMS OF 1 mM 5 HIAA
(a) untreated electrode
(b) pretreated electrode

Potential (V)

+0.6

-0.2

5 μA
FIGURE 6.6(b): LINEAR SWEEP VOLTAMMOGRAMS OF 1 mM 5 HIAA
(i) before pretreating the electrode
(ii) after pretreating the electrode
(---) first scan
(---) fifth scan
FIGURE 6.7(a) CYCLIC VOLTAMMOGRAM OF 1 mM HVA AT FRESHLY POLISHED ELECTRODE
FIGURE 6.7(b) CYCLIC VOLTAMMOGRAM OF 1 mM HVA AFTER ELECTROCHEMICAL PRETREATMENT OF THE ELECTRODE
The effect of electrochemical pretreatment on the peak shape and electrode reaction reversibility was remarkable. At a freshly polished glassy carbon electrode poorly defined irreversible cyclic voltammograms were obtained especially with HVA and 5-HIAA. With the pretreated glassy carbon surface better shaped peaks and more electrochemical reversible voltammograms were obtained as shown in Figures 6.2-6.7. This improvement is due to the faster rates of electron transfer which yield well defined voltammetric behaviour and possess various advantages for quantitative measurements.

Increase of the peak current also occurred with a pretreated electrode. The peak currents were nearly doubled for AA, 5-HIAA, HVA after pretreating the electrode on either pH 7.4 phosphate buffer or 0.1M sulphuric acid; for DOPAC and NE nearly three times peak currents were obtained after pretreatment. The highest increase in peak current was obtained for DA where the increase in peak current was 4.4 times when the electrode was pre-anodized for 2 minutes at +2.0V, then pre-cathodized for 2 minutes at -0.5V in 0.1M sulphuric acid. Reproducibility of the voltammograms has also improved with pretreating the glassy carbon electrode. For example, the variation in peak currents in 5 successive measurements for 5-HIAA is less than 1% after pretreatment, where it was about 80% before pretreatment. This is illustrated in Figure 6.6b.

As a result of the enhancement of the electrochemical kinetics following the electrochemical pretreatment, an increase in the background current also occurred. This was reported by Bjelica et al.\textsuperscript{36} who reported that the differential capacity of glassy carbon increased by more than an order of magnitude upon anodic preoxidation resulting in increased background current. Similar effects were noted for glassy carbon electrodes that had been oxidized chemically\textsuperscript{29}. Figure 6.8 shows cyclic voltammograms of the glassy carbon electrode taken in pH 7.4 phosphate buffer. Curve (a) shows the voltammogram obtained at a freshly polished electrode. Curve (b) is the background voltammogram obtained at an electrode pretreated
for 2 minutes at +2.0V followed by 2 minutes at -1.0V in pH 7.4 phosphate buffer. Curve (c) is the background response after 3 days of use, where the electrode was pretreated twice a day; the electrode was polished with alumina (0.3 and 0.015 μm) slurries between experiments. The high background obtained after 3 days, was because polishing with alumina was not sufficient to remove the catalytic activity of the pretreatment. Only polishing with silicon carbide papers, followed by alumina slurries yielded a response characteristic of untreated electrodes. In view of this, background scans were obtained after every electrochemical pretreatment, and a vigorous polishing procedure was applied when the background increased significantly (higher than 2 μA at 0.6V).

Although the background current had increased upon pretreatment of the glassy carbon electrode, the detection limits had slightly decreased. The rectilinearity of calibration graphs obtained with both treated and untreated electrodes is indicated in Table 6.5.

### Table 6.5: Rectilinearity ranges of calibration graphs obtained at both untreated and pretreated glassy carbon electrodes.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Untreated electrode</th>
<th>Pretreated electrode</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA</td>
<td>2 x 10^{-5} - 5 x 10^{-4}M</td>
<td>1 x 10^{-5} - 5 x 10^{-4}M</td>
</tr>
<tr>
<td>DOPAC</td>
<td>1 x 10^{-5} - 1 x 10^{-3}M</td>
<td>8 x 10^{-6} - 1 x 10^{-3}M</td>
</tr>
<tr>
<td>DA</td>
<td>2 x 10^{-5} - 1 x 10^{-3}M</td>
<td>9 x 10^{-6} - 1 x 10^{-3}M</td>
</tr>
<tr>
<td>NE</td>
<td>2 x 10^{-5} - 1 x 10^{-3}M</td>
<td>1 x 10^{-5} - 1 x 10^{-3}M</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>2 x 10^{-5} - 1 x 10^{-3}M</td>
<td>1 x 10^{-5} - 1 x 10^{-3}M</td>
</tr>
<tr>
<td>HVA</td>
<td>2 x 10^{-5} - 5 x 10^{-4}M</td>
<td>1 x 10^{-5} - 5 x 10^{-4}M</td>
</tr>
</tbody>
</table>

The electrochemical pretreatment of the glassy carbon electrode used in the present work has significant effect on the electrochemistry of the DA/AA and DOPAC/AA at the glassy carbon surface. At an untreated electrode voltammograms obtained for AA and DA or AA and DOPAC
solutions were a poorly defined peak with no separation between either AA and DA peaks or AA and DOPAC peaks. After pretreating the electrode by preanodized it at +2.0V for 2 minutes and then precathodized at -1.0V for 2 minutes, two separate peaks for either ascorbic acid and dopamine or ascorbic acids and DOPAC were obtained, as shown in Figure 6.9. The separation between the oxidation peak potentials of AA and DA was 144 mV where the separation in peak potentials of AA and DOPAC was 160 mV. The relative sensitivity of AA/DA and AA/DOPAC with the pretreated electrode was 38.1 for AA/DA and 10:1 for AA/DOPAC. This is a very significant factor for in vivo applications since the ascorbic acid content in brain tissues is 10 to 50 times more than that of the catecholamines. The peaks potentials and heights were stable over 20 successive scans.

The catalytic activity was observed to decrease slightly with time. Table 6.6 shows the loss in lowering of overpotential and increasing of peak height for dopamine over different periods of time. Scans are reproducible at the times given. The electrode dried between scans and was only washed with distilled water before each successive scan.

**TABLE 6.6: The effect of time on the loss of catalytic activity (lowering of overpotential and increasing the peak current) for 1 mM DA in pH 7.4 phosphate buffer. The electrode was pretreated in 0.1M sulphuric acid. Pretreatment conditions: two minutes at +2.0V followed by two minutes at -0.5V.**

<table>
<thead>
<tr>
<th>Time/h</th>
<th>Potential/V</th>
<th>Peak Current/μA</th>
<th>% Decrease in Increased Peak Current</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.200</td>
<td>30.10</td>
<td>0.00</td>
</tr>
<tr>
<td>0.5</td>
<td>0.199</td>
<td>29.91</td>
<td>0.62</td>
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<td>1.0</td>
<td>0.198</td>
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<td>0.85</td>
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<td>2.0</td>
<td>0.198</td>
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<td>0.96</td>
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<tr>
<td>6.0</td>
<td>0.193</td>
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<td>2.82</td>
</tr>
<tr>
<td>18.0</td>
<td>0.183</td>
<td>28.30</td>
<td>5.98</td>
</tr>
<tr>
<td>24.0</td>
<td>0.178</td>
<td>27.87</td>
<td>7.41</td>
</tr>
</tbody>
</table>
FIGURE 6.8: CYCLIC VOLTAMMOGRAM BACKGROUND SCANS:
(a) untreated electrode, (b) freshly treated electrode (c) after treating the electrode for 3 days. Supporting electrolyte 0.1M phosphate buffer (pH 7.4). Scan rate 10 mV s\(^{-1}\).
FIGURE 6.9: LINEAR SWEEP VOLTAMMOGRAMS OF (A) A MIXTURE OF AA (9.5 × 10⁻⁴) AND DA (2.5 × 10⁻⁵ M) AT A NEWLY POLISHED GLASSY CARBON ELECTRODE; (B) THE SAME MIXTURE AT A PRETREATED ELECTRODE; (C) A MIXTURE OF DA (9 × 10⁻⁴ M) AND DOPAC (10⁻⁴ M) AT A NEWLY POLISHED ELECTRODE; (D) THE SAME MIXTURE AS IN (C) AT A PRETREATED ELECTRODE
As the loss of catalytic activity was about 1% after 3 hours from pretreating the electrode in this work the electrode was pretreated approximately every 3 hours.

**EFFECT OF THE ELECTROCHEMICAL PRETREATMENT OF THE GLASSY CARBON ELECTRODE ON THE FLOW INJECTION AMPEROMETRIC DETERMINATION OF DA, DOPAC AND AA**

In view of the encouraging performance of the electrochemically pretreated glassy carbon electrode in decreasing overvoltages, improving the peak shape and increasing the peak current, the same electrode pretreatment was applied in a flow injection system to test the effectiveness of that pretreatment on the oxidation of AA, DA and DOPAC. The eluent employed was pH 7.4 phosphate buffer (0.1M).

The hydrodynamic voltammograms for oxidation of DA, DOPAC and AA at both pretreated and untreated glassy carbon electrodes are shown in Figures 6.10-6.12. The pretreatment procedures used were to anodize the electrode at +2.0V versus SCE for 2 minutes and then to cathodize the electrode at -1.0V versus SCE for another 2 minutes. As expected, shifts of the oxidation processes to less anodic potentials after pretreating of the electrode were observed, and sharper voltammograms were also obtained. In the hydrodynamic voltammograms obtained at the pretreated glassy carbon electrode, plateaus starting at about 0.2, 0.4 and 0.2V were obtained for DA, DOPAC and AA respectively. With untreated electrodes the plateaus started at about 0.6, 0.7 and 0.6V for DA, DOPAC and AA respectively. The importance of the oxidation potentials shift to less anodic potentials in the flow injection systems, is that the interference from noise and background currents will be less at lower anodic potentials.

The size of the anodic current signals of DA, DOPAC and AA at the plateaus of the hydrodynamic voltammograms were increased after pretreating the electrode. However the background current was increased also. Figure 6.13 shows the background currents obtained before and after pretreating the electrode by anodizing it at +2.0V
Figure 6.10: Hydrodynamic voltammogram of $10^{-4}$ M DA at (a) freshly polished electrode, and (b) pretreated electrode.
FIGURE 6.11: HYDRODYNAMIC VOLTAMMOGRAM OF $10^{-4}$ M DOPAC AT (a) FRESHLY POLISHED ELECTRODE, AND (b) PRETREATED ELECTRODE
FIGURE 6.12: HYDRODYNAMIC VOLTAMMOGRAM OF ASCORBIC ACID AT (a) FRESHLY POLISHED ELECTRODE, AND (b) PRE-TREATED ELECTRODE
FIGURE 6.13: BACKGROUND CURRENTS OBTAINED AT VARIOUS POTENTIALS AT (a) FRESHLY POLISHED ELECTRODE AND (b) PRETREATED ELECTRODE
for 2 minutes followed by cathodizing it at -1.0V for 2 minutes in 0.1M phosphate buffer (pH 7.4). In order to understand the variation of both the current signal and the background current, calculation of the ratio of the former current over the latter current was made at various potentials and for both untreated and pretreated electrodes. Table 6.6 summarises the values obtained for the ratio of the signal to background current (which has been named "R" value here) obtained at various potentials for DA, DOPAC and AA. The highest R values at the pretreated electrode were 98.5, 15.0 and 55.0 for DA, DOPAC and AA, respectively. At the potentials where these values were obtained, an increase in R values by 8.6, 12.0 and 55 compared with those obtained at an untreated electrode at the same potentials, were obtained for DA, DOPAC and AA respectively. From the hydrodynamic voltammograms and the R values obtained at different potentials, the potentials chosen for flow injection amperometric determination of DA, DOPAC and AA at a pretreated glassy carbon electrode were 0.22, 0.45 and 0.25V versus SCE respectively.

The detection limits obtained at a pretreated glassy carbon electrode were about 5 x 10^{-9} M for the three compounds, compared with 3 x 10^{-8}, 2 x 10^{-8} and 3 x 10^{-8} M for DA, DOPAC and AA respectively at an untreated electrode. This is explained by the high R values obtained at the pretreated electrode. Calibration graphs were rectilinear in the ranges 2 x 10^{-8} - 10^{-4} M, 8 x 10^{-8} - 10^{-4} M and 4 x 10^{-8} - 10^{-4} M for DA, DOPAC and AA, respectively, using the recommended technique including the recommended electrochemical pretreatment of the electrode. Calibration graphs obtained with an untreated electrode were rectilinear in the ranges 6 x 10^{-7} - 10^{-4} M, 10^{-6} - 10^{-4} M and 2 x 10^{-7} - 10^{-4} M for DA, DOPAC and AA respectively. Figure 6.14 illustrates signals of 10^{-5} M ascorbic acid obtained at untreated and pretreated glassy carbon electrodes at 0.25V versus SCE. Typical signals obtained with the pretreated electrode for DA, DOPAC and AA are shown in Figures 6.15-6.17. The coefficient of variations at the 10^{-6} M level were 0.95%, 1.1% and 1.25% for DA, DOPAC and AA respectively (10 injections) compared to 2.0%, 1.90% and 3.2% at an
TABLE 6.7: Effect of the on-line electrochemical pretreatment of the glassy carbon electrode on the peak current and background current with changing the potential for 100 μl injections of 10^{-4}M solutions of DA, DOPAC and AA in phosphate buffer (pH 7.4) eluent

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<th>Compound</th>
<th>Potential /V</th>
<th>Peak Current/μA</th>
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<th>Background Current/μA</th>
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<td></td>
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<td>After pre-treat-</td>
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<td>0.74</td>
<td>0.84</td>
<td>0.09</td>
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</table>

where \( R \) = ratio of the current signal to the background current.
FIGURE 6.14: COMPARISON OF SIGNALS FOR ASCORBIC ACID AT (a) UNTREATED ELECTRODE, AND (b) PRETREATED ELECTRODE; ASCORBIC ACID CONCENTRATION $10^{-5}$M, $E_{\text{app.}} = +0.25$V
FIGURE 6.15: SIGNALS OBTAINED FOR CONSTRUCTING A CALIBRATION GRAPH FOR THE DETERMINATION OF DA AT A PRETREATED GLASSY CARBON ELECTRODE. DA CONCENTRATION: A, 1; B, 2; C, 4; D, 8; and E, 10 x 10^{-6} M
FIGURE 6.16: SIGNALS OBTAINED FOR CONSTRUCTING A CALIBRATION GRAPH FOR THE DETERMINATION OF DOPAC AT A PRE-TREATED GLASSY CARBON ELECTRODE. DOPAC CONCENTRATION: A, 0; B, 1; C, 2; D, 4; and E, $8 \times 10^{-6}$ M.
FIGURE 6.17: SIGNALS OBTAINED FOR CONSTRUCTING A CALIBRATION GRAPH FOR THE DETERMINATION OF AA AT A PRETREATED GLASSY CARBON ELECTRODE. AA CONCENTRATION: A, 0; B, 1; C, 2; D, 4; and E, $8 \times 10^{-6}$
untreated electrode. The decrease in catalytic activity (decrease in signals) was about 2.4% in six hours of continuous work.

CONCLUSION

The effect of the electrochemical pretreatment of the glassy carbon electrode on the oxidation of some neurotransmitters, and ascorbic acid was investigated. Electrochemical pretreatment has offered many advantages, these include: shift of the oxidation potentials of all the compounds studied to less anodic values allowing the use of lower potentials to monitor the maximum anodic current, better shaped and more reproducible voltammograms, more reversible electrode reaction and better sensitivity. That has made the voltammetric differentiation of AA and DA, and AA and DOPAC possible, as separate peaks for either AA and DA or AA and DOPAC were obtained at a pretreated glassy carbon electrode.

Similar advantages were noticed when DA, DOPAC and AA were determined on-line, however the increase in sensitivity was much higher which is because lower background currents were obtained. It is expected that the electrochemical pretreatment of the glassy carbon electrode will affect the redox signals of NE, 5-HIAA and HVA in a similar way when determined in a flow injection system. Electrochemical pretreatment should be borne in mind when developing flow injection amperometric methods and other voltammetric methods.
References

17. F. Crespi and M. Jouvet, Brain Research, 272 (1983) 263.
CHAPTER 7

SIMPLE TECHNIQUES FOR THE PREPARATION OF GLASSY CARBON ELECTRODES

INTRODUCTION

Glassy carbon has found common usage as the material of the indicator electrode in voltammetric applications. Its popularity comes from the fact that it has comparatively good electrocatalytic properties and is, moreover, less susceptible to surface poisoning than other materials such as platinum and gold. A full review of the analytical applications and the physical and chemical properties of glassy carbon electrodes was made by Van der Linden and Dieker.

Commercial glassy carbon electrodes frequently consist of a short cylindrical rod of glassy carbon glued into plastic or glass tubes, or pushed into a tightly fitting Teflon or plastic tube. Most commercially available glassy carbon electrodes do not offer completely satisfactory performance with respect to the fitting of the glassy carbon rod into the holder material. Fissures resulting from unsatisfactory fitting are inevitably filled with grains from abrasive powder during the polishing procedure. This can create a long lasting source of contamination during analysis. Unsatisfactory fitting also causes high background currents; most commercially available glassy carbon electrodes show this after being used for some time. The deterioration in the electrode performance with time, which is reflected in more noise and higher background currents, is known as "ageing" and is of extreme importance for low level determinations. It is believed that electrode fabrication plays an important role in ageing.

Several methods have been reported for the fabrication of home-made glassy carbon electrodes. Zittel and Miller were the first to report a method of fabricating a glassy carbon disk electrode by cutting a
piece of the material and sealing it into the end of a cylinder of epoxy resin. A small amount of mercury was poured into the cylinder and electrical contact was made by means of a copper wire inserted into the mercury.

Laser and Ariel\textsuperscript{8} prepared electrodes by pressing glassy carbon disks into a tightly fitting housing. This method has been used by many workers\textsuperscript{9-11}. However, the use of sealants to seal the glassy carbon rod into the electrode holder is more common.

Panzer and Elving\textsuperscript{12} sealed the glassy carbon rod into Pyrex tubing using melted polyethylene. Dieker et al\textsuperscript{13} sealed the electrode into a glass tube with UHU-plus adhesive, but the most commonly used sealant has been the epoxy resins. Engstrom and Strasser\textsuperscript{10} have epoxided the glassy carbon rod into cast acrylic housings, whilst Blaedel and Jenkins\textsuperscript{14} have cast glassy carbon rod (0.3 cm) in the end of a Plexiglas sleeve (1.27 cm o.d., 0.63 c.m. i.d) with a low-temperature setting epoxy. The sleeve was then fitted to a 23 cm length of borosilicate tubing using the aforementioned epoxy. Nagaoka et al\textsuperscript{15} have also epoxided a glassy carbon rod into Pyrex glass tubing. Epoxy resins are also used in the preparation of some commercial glassy carbon electrodes.

Levy and Farina\textsuperscript{16} described a different technique to obtain a good seal between glassy carbon and glass. They coated the glassy carbon with a thin layer of silicon, by placing it inside a Pyrex vessel where a stream of silane (SiH$_4$), diluted with hydrogen and argon is passed through the vessel. The glassy carbon was heated for a few seconds. Silane, upon contacting the hot carbon, decomposes and deposits a thin film of silicon on the rod. A glass tubing was then slipped over the glassy carbon and sealed to it by evenly heating to the softening point in a glass lathe.

Recently Hoogvliet et al\textsuperscript{6} claimed that these electrodes showed very low detection limits when used directly after fabrication but with time their electrochemical behaviour gradually deteriorated and
cracks in the glass holders can be noticed. They showed that initial electrode characteristics cannot be recovered by repolishing and that the solvent can penetrate in the thin void between the glassy carbon and glass. They also observed similar ageing behaviour of glassy carbon electrodes press-fitted or glued to Kel-F. Alternatively they suggested a complicated method to obtain leak-tight electrodes sealed in glass, by coating the glassy carbon disk with silicon in a chemical vapour deposition process at 1250°C.

Very recently Henriques and Fogg17 reported a new hardening technique, where unloaded and graphite loaded epoxy resins were cured by addition of 40% m/m hydrofluoric acid solution. They showed that epoxy resins hardened by hydrofluoric acid have many advantages over resins hardened by conventional hardeners. These include higher resistance to absorb organic compounds or to be attacked by organic solvents, lower tendency to form air bubbles when the hardener is added to the epoxy resin, and better fluid characteristics which allow it to be smoothly applied before it hardens. They also described a method of reclaiming used commercial glassy carbon disk electrodes exhibiting high background currents, by using the unloaded epoxy resin cured by 40% m/m hydrofluoric acid solution as a sealant.

This chapter describes simple procedures for the preparation of glassy carbon electrodes in glass and Teflon. The electrochemical behaviour of these electrodes is compared with commercial glassy carbon electrodes.

EXPERIMENTAL

Graphite-loaded Epoxy Base and Resin

The graphite loaded epoxy base was prepared by mixing 0.56g of Specpure graphite powder pelletable grade 1 (Johnson Matthey Chemicals Ltd) with 0.44g of the contents of the adhesive tube of a two-tube Araldite pack (Ciba Geigy). This mixture can be stored indefinitely in a suitable glass or plastic container. Immediately
before use one drop (0.1 ml) of 40% m/m hydrofluoric acid solution was mixed thoroughly into 0.5g of the graphite-loaded epoxy base. This mixture is electrically conducting and can be used to produce an electrically conducting connection.

**Unloaded Epoxy Resin**

This was prepared similarly before use by mixing one drop (0.1 ml) of 40% m/m hydrofluoric acid solution into 0.5g of the contents of the adhesive tube of a two-tube Araldite pack.

**Caution**

Extreme precautions and care must be taken when using hydrofluoric acid. Work should be carried out in a fume cupboard using the relevant safety equipment. Care must also be taken not to directly handle epoxy surfaces that may have retained hydrofluoric acid.

**Glass Bond Adhesive**

This is a single component adhesive which cures when exposed to ultraviolet light. It contains methacrylate ester and minor amounts of acrylic acid.

**Glassy Carbon Electrode Fabrication**

The glassy carbon was obtained from Le Carbone (France) and Ciba Geigy (Switzerland) as 3 mm diameter rods. The rods were cut with a glass grinder wheel in about 5-8 mm length pieces.

Several methods have been used to fabricate the electrodes:

1. A cut piece of glassy carbon was coated with a thin layer of epoxy containing 40% m/m hydrofluoric acid solution and then allowed to harden for about 1 hr over a hot-plate (50-60°C). Care was taken to ensure that there were no air bubbles trapped. A copper wire was connected to the glassy carbon rod with an
amount of graphite loaded epoxy containing 40% m/m hydrofluoric acid solution. This was left to harden at room temperature.

A Teflon sheath of about 3 mm i.d. (7 mm o.d) was heated to about 100°C, and the glassy carbon rod was then pushed into it leaving about 1 mm of the glassy carbon cylinder outside. Electrodes prepared by this method will be named GC1.

2. A piece of a glassy carbon rod was covered with epoxy containing 40% hydrofluoric acid solution and then inserted into a glass tube of about 3.1 mm i.d. of which the sides of the tip were covered with epoxy containing hydrofluoric acid solution. Care was taken to ensure that no epoxy reached the rear side of the glassy carbon cylinder where the electrical contact was placed. The assembled electrode was left to harden over a hot-plate or at room temperature. When the epoxy remained tacky, the electrode was suspended in the vapour chamber17 over 40% m/m hydrofluoric acid solution for 2 min and was then removed and held at 50-60°C over a hot-plate for 5 mins. Any air bubbles formed were released before hardening completed.

Electrical contact was made by placing a few drops of mercury into the glass tube, and a copper wire was then inserted into the mercury. Electrodes prepared in this way will be named GC2.

3. A piece of glassy carbon cylinder was coated with a thin layer of epoxy containing 40% m/m hydrofluoric acid solution and then left to harden. The rod was inserted into a glass holder of about 3.1 mm i.d. and the gap between the glassy carbon rod and the inner wall of the glass tube was carefully filled with a glass glue (Glass Bond, Loctite UK) avoiding air inclusion. The assembly was left to be cured by sunlight. Electrical contact was made as in method (2). Electrodes prepared by this method will be named GC3.
4. Another piece of glassy carbon rod was covered with Glass Bond glue, and then inserted into a glass tube of about 3.1 mm i.d. of which its tip was covered with Glass Bond glue to ensure that the gap between the inner wall of the glass tube and the glassy carbon rod was filled with the glue, but not the rear side of the glassy carbon rod. The assembly was then left to cure by exposing it to sunlight for a few days. Electrical contact was made as in method (2). Electrodes prepared in this way will be named GC4.

The rear side of all the electrodes was sealed with epoxy containing 40% m/m hydrofluoric acid solution.

After construction of the glassy carbon electrodes, its surface was prepared by polishing on a series of silicon carbide papers (NP 9088 Scotch 3M) of decreasing roughness. Fresh abrasive papers were used at each stage of the grinding process with a light covering of distilled water to lubricate the carbon/paper interface. This grinding was continued first until all the excessive glue had been removed and the bare glassy carbon surface had appeared, and second until the glassy carbon was levelled with the Teflon or glass body. Abrasion with the fine silicon carbide paper had the effect of reducing the coarseness and scratches made by the rough abrasive to the point where cloth-polishing could be applied. The next step in polishing is the fine polishing with diamond paste and fine alumina. A diamond paste of 10-15 μm (Winter Diaplast, W. Germany) was applied on an appropriate polishing pad and the electrodes were polished; this removed the grinding silicon carbide particles of the abrasive paper used in the previous abrasion step. The fine polishing was accomplished by polishing the electrodes with aqueous alumina slurries containing aluminium oxide particles 0.3 and 0.015 μm in diameter. This produced glass-like mirror finish surfaces. Electrodes were finally cleaned by distilled water in an ultrasonic bath.
Testing the Electrodes

The electrodes were tested in both static mode and by flow injection analysis. In both modes, the three electrode system consisted of the test electrode, a calomel reference electrode and a platinum counter electrode. Voltammograms were obtained using a PAR 174A polarographic analyser (Princeton Applied Research Corporation) and recorded using an X-Y Gould HR2000 recorder.

For flow injection experiments, the flow of eluent was produced with an Ismatec Mini-S peristaltic pump. Injections of 100 μl were made with a Rheodyne (5020) injection valve. The injection valve was connected to a laboratory built detector cell, incorporating the test electrode, by means of a 50 cm length of 0.58 mm bore Teflon tubing. The detector cell with the platinum counter electrode and the saturated calomel reference electrode were partially immersed in an electrolyte similar to that of the eluent. The potential of the test electrode was controlled by means of a PAR 174A polarographic analyser. Current peaks were monitored on a Linseis L650 y-t recorder.

The background currents associated with the glassy carbon electrodes constructed in the laboratory were compared with those associated with commercially available electrodes (Metrohm and EDT) in both static and flow injection systems. Electrodes were tested further by obtaining linear sweep voltammograms in 10^{-3}M potassium ferrocyanide in 10^{-1}M potassium chloride solution. Hydrodynamic voltammograms of the injection of 100 μl of 10^{-4}M in potassium ferrocyanide in pH 6.5 Britton-Robinson buffer were obtained also at the laboratory-made and the commercially available electrodes. Detection limits and linearity of the calibration graphs for potassium ferrocyanide when determined in flow injection systems at both home-made and commercially available electrodes, were obtained and compared with each other.
Results and Discussion

Twelve electrodes were constructed and tested, three by each method of construction. The electrodes constructed in the same way behaved similarly. The cyclic voltammograms obtained in pH 2 Britton-Robinson buffer for four electrodes constructed in four different ways are shown in Figure 7.1. For comparison, the cyclic voltammograms for some commercially available glassy carbon electrodes are also shown in Figure 7.1. Clearly all the home-made electrodes have produced featureless voltammograms with low background currents, whereas higher background currents were obtained for the commercially available electrodes.

The background currents obtained at various potentials on a flow injection system when an eluent of pH 6.5 Britton-Robinson buffer was pumped through the system at a flow-rate of 6 ml min⁻¹, are shown in Figure 7.2 for electrodes GC1, GC3 and Metrohm electrode.

Cyclic voltammograms for the GC1 and Metrohm electrodes in 10⁻³M potassium ferrocyanide in 10⁻¹M potassium chloride solution are shown in Figure 7.3. Cyclic voltammograms for GC2, GC3 and GC4 in the same electrolyte were very similar to that obtained for GC1. The voltammograms obtained with the home-made electrodes are better shaped and more reversible. The separation between anodic and cathodic peaks in the cyclic voltammograms of potassium ferrocyanide was 140 mV at GC1 and 220 mV at Metrohm electrode. This indicates a more reversible electrode reaction and a higher rate of electron transfer at the GC1 surface compared with the Metrohm electrode.

Hydrodynamic voltammograms for the injection of 100 µl of 10⁻⁴M in potassium ferrocyanide in pH 6.5 Britton-Robinson buffer were obtained at the GC1 and Metrohm electrodes. The eluent used was pH 6.5 Britton-Robinson buffer. These are shown in Figure 7.4. The hydrodynamic voltammogram obtained at GC2 was better shaped than that obtained at the Metrohm electrode. The detection limit at 0.65V versus SCE was 5 x 10⁻⁹M of potassium ferrocyanide at GC2, compared
FIGURE 7.1: CYCLIC VOLTAMMOGRAMS BACKGROUND SCANS OF DIFFERENT ELECTRODES: A, USED EDT ELECTRODE (5 mm DIAMETER); B, GC2 ELECTRODE; C, GC3 ELECTRODE; D, GC4 ELECTRODE; E, GC1 ELECTRODE; AND F, NEW UNPOLISHED ELECTRODE (0.3 mm DIAMETER).

SCAN RATE: 10 mV s⁻¹
FIGURE 7.2: BACKGROUND CURRENTS OBTAINED AT VARIOUS POTENTIALS AT (A) METROHM ELECTRODE; (B) GC3 ELECTRODE, AND (C) GC1 ELECTRODE. ELUENT pH 6 BRITTON-ROBINSON BUFFER. FLOW RATE 6 ml min$^{-1}$
FIGURE 7.3: CYCLIC VOLTAMMOGRAM OF $10^{-3}$ M POTASSIUM FERROCYANIDE IN 0.1M POTASSIUM CHLORIDE AT (A) GC1 ELECTRODE, AND (B) NEW UNPOLISHED METROHM ELECTRODE
FIGURE 7.4: HYDRODYNAMIC VOLTAMMOGRAMS OF $10^{-4}$ M POTASSIUM FERROCYANIDE AT (a) GC2 ELECTRODE, AND (b) METROHM ELECTRODE. ELUENT pH 6.5 BRITTON-ROBINSON BUFFER
with $1 \times 10^{-8}$M at the Metrohm electrode. Calibration graphs were rectilinear over the range $5 \times 10^{-7} - 5 \times 10^{-5}$M at GC2 and over the range $1 \times 10^{-6} - 5 \times 10^{-5}$M at the Metrohm electrode.

The performance of the electrodes constructed by the four different methods described before has been examined over a long time of use. Except for GC4, the electrodes showed satisfactory performance over a long time of use. An electrode constructed by method (1) has been used for about a year for different voltammetric applications including electrochemical pretreatment at high potentials; its performance was good and its original performance could be recovered by polishing. However, the performance of GC4 has shown deterioration with time. This was because the adherence of Glass Bond to glassy carbon was not as good as its adherence to epoxy, or the adherence of epoxy to glassy carbon or glass, which could allow the leakage of electrolyte into a thin void between the glassy carbon and the glass.

CONCLUSION

Glassy carbon electrodes prepared in this work have been shown to be very satisfactory in terms of low background currents and the quality of linear sweep and cyclic voltammograms as well as hydrodynamic voltammograms in flow injection analysis systems. The performance of most commercial glassy carbon electrodes deteriorate with use, that is due to permeation of electrolyte between the electrode body and the glassy carbon disk. The methods applied here for producing leak-tight electrodes have been shown to be highly satisfactory in preventing electrolyte leakage. However, one method was less successful than the others.

The preparation of these electrodes is simple, fast, and more important, inexpensive. Their performance is at least as good as a new commercial glassy carbon electrode; however they have less ageing effect than the commercial electrodes.
References

INTRODUCTION

Increasing attention is being paid to the advantages of electrochemically pretreating glassy carbon electrodes used for amperometric detection in high performance liquid chromatography and in flow injection analysis. The studies reported to date have been made to improve the performance of glassy carbon electrodes used for monitoring oxidation processes at positive potentials. In many irreversible oxidation processes electrochemical pretreatment first at a high positive potential and then at about -1.0V reduces the overpotential for oxidation of the determinand such that an improved hydrodynamic voltammogram is obtained. Oxidation occurs more completely and at a less positive potential such that a higher and more reproducible signal is obtained. At any particular potential the background signal is also increased, but this does not detract significantly from the technique.

Taylor and Humffray during their studies on oxygen reduction at various pH values have reported that pretreating the glassy carbon electrode by pre-cathodising it at -1.0V for 20h, in dilute sulphuric acid, decreased the limiting current for oxygen reduction waves compared to the values recorded before the pretreatment. They also reported that anodic pretreatment of the electrode at +2.2V caused a decrease in the cathodic potential at which the reduction of oxygen occurred.

Although most attention has been paid to the applicability of glassy carbon in the more positive potential range, a growing interest in monitoring reduction processes at negative potentials has been
reported, especially for high performance liquid chromatography and flow injection analysis applications. An important HPLC method that involves reductive amperometric detection at a glassy carbon electrode held at negative potentials is the determination of vitamin K and its analogues. Hart et al. determined vitamin K in a 95% methanol eluent that was 0.05M in a pH3 sodium acetate-acetic acid electrolyte holding the potential of the glassy carbon electrode at -1.0V; the eluent was deoxygenated with nitrogen and an all-metal solvent delivery system was used to prevent the re-entry of oxygen. Calibration graphs were obtained by injecting 1-10 ng of vitamin K. Adsorbed product on the electrode was removed periodically by holding the electrode at +0.7V, which re-oxidised the product as the reduction process is quasi-reversible. The rigorous exclusion of oxygen was important in this method.

Wang and Dewald have used glassy carbon electrode, mercury-coated glassy carbon and carbon paste electrodes for the determination of lead, cadmium, bismuth and zinc in a flow injection system. They subtracted the background current due to the reduction of oxygen from the response of the reduction of these metal ions to get a net signal from the analyte, free from background effects. The subtractive responses were obtained by using a computer.

In this chapter a study of the possibility of improving signals for reductive processes at glassy carbon electrodes held at negative potentials by applying electrochemical pretreatment is reported.

**EXPERIMENTAL**

Flow injection analysis was carried out in a single-channel system that has been described previously. Eluent flow was produced by means of an Ismatec Mini-S peristaltic pump. A sample (approximately 100 µl) was injected with a Rheodyne 5020 low-pressure injection valve connected to a laboratory-built detector cell by means of 50 cm of 0.58 mm bore PTFE tubing. The detector cell holds the glassy carbon electrode only, eluent being presented to it in a wall-jet
configuration. The cell is used partially immersed in an electrolyte having the same composition as the eluent. A counter platinum and a conventional potentiometric calomel reference electrode are placed in the electrolyte to obtain electrical contact with the working electrode. The glassy carbon disk electrode (3 mm diameter) was constructed from Le Carbone glassy carbon and was mounted in PTFE as described in Chapter 7. An eluent flow rate of 6.5 ml min$^{-1}$ was used. The potential of the glassy carbon electrode was controlled by means of a PAR 174 polarographic analyser and current signals were monitored on a Linseis L650 y-t recorder. Linear sweep voltammetry was carried out at a sweep rate of 10 mV s$^{-1}$ using the same working, counter and reference electrodes immersed in the appropriate measuring solution.

**REAGENTS**

All chemicals were of analytical reagent grade.

**Britton-Robinson buffer**

Dissolve 2.47g of boric acid in 500 ml of distilled water containing 2.3 ml of glacial acetic acid. Add 2.7 ml of orthophosphoric acid and dilute to 1 litre with distilled water. Adjust the pH using 2 or 4M sodium hydroxide solution.

**Sulphuric acid solution 0.1M**

Dilute 5.5 ml of concentrated sulphuric acid to 1 litre with distilled water.

**Standard nitrofurantoin solution (10$^{-3}$M)**

Weigh 23.82 mg of nitrofurantoin and transfer it to a 100 ml calibrated flask. Dissolve and dilute to 100 ml with pH 7 Britton-Robinson buffer. Prepare more dilute solutions by dilution of this solution with pH 7 Britton-Robinson buffer.
Standard cephalonium solution 250 µg ml⁻¹

Weigh 25 mg of cephalonium and transfer it to a 100 ml calibrated flask. Dissolve and dilute to 100 ml with 0.1M sulphuric acid solution. Prepare more dilute solutions by dilution of this solution with 0.1M sulphuric acid solution.

PROCEDURE

The glassy carbon electrode was polished with a series of silicon carbide papers (NP 9088 Scotch 3M) of decreasing roughness, followed by alumina (0.3 µm and 0.015 µm) slurries, and then sonicated in distilled water. An electrode prepared by this procedure will be referred to as an untreated electrode.

The electrochemical pretreatment was performed by applying a DC negative potential of -2.7 or -3.0V for 1 min. The electrode was immersed in 0.1M sulphuric acid solution. On-line pretreatment was carried out using an eluent of 0.1M sulphuric acid at flow rate of 2 ml min⁻¹.

RESULTS AND DISCUSSION

Preliminary Linear Sweep Experiments in a Static System

During studies of the effect of positive- and negative-potential electrochemical pretreatment of glassy carbon electrodes on oxidation processes at low positive potentials, a question about the effect of electrochemical pretreatment in reduction processes at negative potentials arose. It was noticed that electrochemical pretreatment at high positive potentials caused higher background currents due to enhancing the oxygen reduction at the electrode surface. However, electrochemical pretreatment at high negative potentials was found to be effective in making smaller the background currents obtained at negative potentials. This is clearly illustrated in Figure 8.1, in which baseline linear sweep voltammograms obtained with a static
electrode system in 0.01M sulphuric acid before and after electrochemical pretreatment are shown; the electrode was electrochemically pretreated in 0.1M sulphuric acid solution. Pretreatment at -3V in the static mode is seen to remove the oxygen reduction wave most effectively. Further, electrochemical pretreatment was shown to be effective only when carried out in dilute sulphuric acid; attempts to effect pretreatment in Britton-Robinson buffer solution of pH between 2 and 8 were unsuccessful. The pretreatment that had been effected in dilute sulphuric acid, however, was also as effective when the electrode was used in these buffer solutions.

Linear sweep voltammograms obtained for the reduction of nitrofurantoin, which occurs at about -0.58V in pH7 Britton-Robinson buffer, are shown in Figure 8.2. When the electrode is newly polished a hump due to the reduction of dissolved molecular oxygen is apparent as a post-peak. After pretreating the electrode at -3V this hump is no longer apparent. An illustration of the oxygen reduction process occurring before that of a determinand is shown in Figure 8.3, in which linear sweep voltammograms for the reduction of the cephalosporin, cephalonium, are shown. Here also electrochemical pretreatment at -3V removes visible signs of the oxygen reduction process. Polarographic methods are available for the determination of nitrofurantoin\textsuperscript{17} and cephalonium\textsuperscript{18}.

**EFFECT OF ELECTROCHEMICAL PRETREATMENT AT HIGH NEGATIVE POTENTIALS IN FLOW INJECTION ANALYSIS**

In using a glassy carbon electrode for amperometric detection in HPLC or flow injection analysis, two characteristics of the system should be considered before the quality of the signal obtained with the determinand is studied. These are the background current associated with the eluent and the blank signal obtained when a control blank is injected. When the eluent is used as the control blank, clearly eluent and sample are the same and no signal should be observed when the control blank is injected, except at high sensitivities owing to
FIGURE 8.1: BLANK LINEAR SWEEP VOLTAMMOGRAMS IN 0.01M SULPHURIC ACID WITHOUT DEOXYGENATING THE SOLUTION. A AND A', FIRST AND SECOND SCANS AT A NEWLY POLISHED GLASSY CARBON ELECTRODE; B, SCAN AFTER PRETREATMENT AT -2.5V FOR 1 MIN IN 0.1M SULPHURIC ACID; AND C, SCAN AFTER PRETREATMENT AT -3V FOR 1 MIN IN 0.1M SULPHURIC ACID.
Figure 8.2: Linear sweep voltammograms of nitrofurantoin (2 x 10^{-4} M) in unddeoxygenated pH 7 Britton-Robinson buffer. (a) at a newly polished glassy carbon electrode, and (b) at an electrode pretreated at -3V for 1 min. The blank linear sweep voltammograms are given as broken lines in both instances.
disturbance to the flow of eluent caused by the process of injecting the eluent. In determinations made at potentials where oxygen reduction occurs, however, a finite blank signal will be observed if the oxygen contents of the eluent and the blank sample solution differ. Clearly analytical determinations become very unreliable when the level of interference in the solvent system and sample solution have to be balanced, and this is particularly so with dissolved molecular oxygen. In general, with increasing background current the detection limit attainable is increased.

The results reported here for electrochemically pretreated electrodes were obtained with electrodes pretreated either at -2.7V for 1 min in a static system before being inserted into the detector cell, or at -3V for 1 min on-line in 0.1M sulphuric acid at a flow rate of 2 ml min⁻¹. These were found to be the optimum off-line and on-line electrochemical pretreatment conditions. This latter process was readily effected by switching eluents before the pump. The use of higher pretreatment potentials than those recommended led to higher background noise.

The background current levels obtained at various potentials with the flow injection system in which pH 7 Britton-Robinson buffer was used as the eluent are shown in Figure 8.4. These were obtained for a newly polished electrode and for pretreated electrodes in all instances with and without deoxygenation of the eluent with nitrogen (it should be borne in mind that the term "deoxygenation" which is used extensively by polarographers, is misleading in that the oxygen concentration is reduced only to a particular level that is determined by the effectiveness of the "deoxygenation" process and also by the effectiveness of preventing oxygen from re-entering the eluent before the measurement is made).

It is clear from Figure 8.4 that electrochemical pretreatment extends the useful range of the electrode to more negative potentials both when the eluent is deoxygenated and when it is not, and that the static electrochemical pretreatment process is more effective than
FIGURE 8.3: LINEAR SWEEP VOLTAMMOGRAMS OF CEPHALONIUM (100 μg ml⁻¹) IN 0.1M SULPHURIC ACID. A, WITHOUT DEOXYGENATING THE SOLUTION AT A NEWLY POLISHED GLASSY CARBON ELECTRODE; B, WITHOUT DEOXYGENATING THE SOLUTION AT AN ELECTRODE PRETREATED AT -3V FOR 1 MIN; AND C, AFTER DEOXYGENATING THE SOLUTION AT A NEWLY POLISHED GLASSY CARBON ELECTRODE.
FIGURE 8.4: BACKGROUND CURRENTS OBTAINED WITH FLOW INJECTION AMPEROMETRY USING pH7 BRITTON-ROBINSON BUFFER AS ELUENT. A, WITHOUT DEOXGENATING THE SOLUTION AT A NEWLY POLISHED ELECTRODE; B, WITHOUT DEOXGENATING THE SOLUTION AT AN ELECTRODE PRETREATED AT -3V FOR 1 MIN ON-LINE; C, AFTER DEOXGENATING THE SOLUTION AT A NEWLY POLISHED ELECTRODE; D, AFTER DEOXGENATING THE SOLUTION AT AN ELECTRODE PRETREATED AT -3V FOR 1 MIN ON-LINE; AND E, AFTER DEOXGENATING THE SOLUTION AT AN ELECTRODE PRETREATED AT -2.7V FOR 1 MIN (DIC SYSTEM)
the on-line pretreatment. In effect, on pretreatment the reduction of oxygen is being made more difficult by the pretreatment process.

Perhaps not surprisingly, electrochemical pretreatment has a more significant effect on the useful range of the electrode when the oxygen content of the eluent has been reduced to a lower level by deoxygenation. Nevertheless, deoxygenation of eluent and sample solutions is a time-consuming task and there is a distinct advantage to be gained in avoiding the necessity of having to carry it out. Compounds which can be determined at potentials less negative than -0.5V can be determined at low levels even with an untreated electrode without having to deoxygenate the eluent and sample solutions. Nevertheless, even in these instances, the background current is reduced and the detection limit should be lowered by using a pretreated electrode. Electrochemical pretreatment produces a slight extension of the useful range of the electrode in an eluent that has not been deoxygenated and this should allow other compounds to be determined without the need to deoxygenate the eluent or sample solutions, particularly if determinations are to be made at high concentrations.

The extension of the useful range on pretreating the glassy carbon electrode, however, is much greater for the deoxygenated eluent. From Figure 8.4 it can be seen that the potential at which a background current of 1 µA is obtained is moved from -0.72 to -1.05V on pretreating the electrode at -2.7V in the static mode. Hence electrochemical pretreatment should make amperometric detection possible for compounds that are reduced at these more negative potentials. Again, the added advantage that lower background currents are obtained in determining compounds at lower negative potentials should not be overlooked.

Deoxygenation of eluent in a flow injection system by means of nitrogen is readily carried out and nitrogen can be bubbled continuously through the eluent in the eluent reservoir during determinations with no great inconvenience or loss of time once the
initial deoxygenation has been effected. Deoxygenation of every sample solution is extremely time consuming, however, and the need to do this should be avoided if at all possible. The size of signals obtained at various potentials on injecting pH7 Britton-Robinson buffer that had not been deoxygenated into deoxygenated eluent of the same composition is illustrated in Figure 8.5. These results were obtained with both newly polished and electrochemically pretreated electrodes. The marked effect of electrochemical pretreatment on the size of the signal obtained can be clearly seen. The potential at which the blank signal due to oxygen in the blank sample reaches 1 μA, is moved from -0.77 to -0.95V on pretreating the electrode at -2.7V for 1 min in the static mode.

In Figures 8.6 and 8.7 are shown hydrodynamic voltammograms of nitrofurantoin in pH7 Britton-Robinson buffer using newly polished and pretreated electrodes respectively. In both instances the hydrodynamic voltammograms that are shown were obtained using deoxygenated eluent. The effect of deoxygenating the sample on the hydrodynamic voltammograms obtained is also clear. Blank hydrodynamic voltammograms in which undeoxygenated eluent was injected into deoxygenated eluent are also shown. The beneficial effect of the electrochemical pretreatment in making smaller the size of the oxygen signal can be clearly seen in Figure 8.7. At the current sensitivity used in obtaining the hydrodynamic voltammograms shown in Figure 8.7 there is no difference in the signal at -0.7V on deoxygenating the sample solution. Hence it is clear that at these levels of determinand there is no need to deoxygenate the sample solutions.

It should be noted that the signals due to the reduction of nitrofurantoin are made smaller by the pretreatment process. Clearly the reduction of nitrofurantoin is also being inhibited, although not to the same extent as the reduction of oxygen. Table 8.1 illustrates the effect of electrochemical pretreatment on nitrofurantoin signals and blank signals obtained at different potentials compared to the signals obtained at a freshly polished electrode. Both nitrofurantoin
FIGURE 8.5: BLANK HYDRODYNAMIC VOLTAMMOGRAMS REPRESENTING THE SIZE OF SIGNALS OBTAINED ON INJECTING UNDEOXYGENATED ELUENT INTO DEOXYGENATED ELUENT (pH 7 BRITTON-ROBINSON BUFFER) (a) AT A NEWLY POLISHED ELECTRODE; (B) AT AN ELECTRODE PRETREATED ON-LINE IN 0.1M SULPHURIC ACID AT -3V FOR 1 MIN; AND (C) AT AN ELECTRODE PRETREATED IN THE STATIC MODE AT -2.7V FOR 1 MIN
FIGURE 8.6: HYDRODYNAMIC VOLTAMMOGRAMS OBTAINED AT A NEWLY POLISHED ELECTRODE FOR INJECTION OF NITROFURANTON (2 x 10^-4M) INTO DEOXYGENATED pH 7 BRITTON-ROBINSON BUFFER. (A) SAMPLE SOLUTION UNDEOXYGENATED; (B) SAMPLE SOLUTION DEOXYGENATED; AND (C) UNDEOXYGENATED BLANK INJECTION.
FIGURE 8.7: HYDRODYNAMIC VOLTAMMOGRAMS OBTAINED AT AN ELECTRODE PRETREATED IN THE STATIC MODE AT -2.7V FOR 1 MIN FOR INJECTION OF NITROFURANTOIN (2 x 10^{-4}M) INTO DEOXYGENATED pH 7 BRITTON-ROBINSON BUFFER. (A) SAMPLE SOLUTION UNDEOXYGENATED; (B) SAMPLE SOLUTION DEOXYGENATED; AND (C) UNDEOXYGENATED BLANK INJECTION
TABLE 8.1: Effect of electrochemical pretreatment of the glassy carbon electrode on the current signal of nitrofurantoin and blank injections at different potentials.
Nitrofurantoin concentration $2 \times 10^{-4}$M

<table>
<thead>
<tr>
<th>Potential /V</th>
<th>Undeoxynogenated blank peak current/μA</th>
<th>Undeoxynogenated nitrofurantoin peak current/μA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated Electrode</td>
<td>Pretreated Electrode</td>
</tr>
<tr>
<td>-0.50</td>
<td>0.10</td>
<td>0.00</td>
</tr>
<tr>
<td>-0.55</td>
<td>0.15</td>
<td>0.00</td>
</tr>
<tr>
<td>-0.60</td>
<td>0.22</td>
<td>0.00</td>
</tr>
<tr>
<td>-0.65</td>
<td>0.35</td>
<td>0.01</td>
</tr>
<tr>
<td>-0.70</td>
<td>0.60</td>
<td>0.05</td>
</tr>
<tr>
<td>-0.75</td>
<td>0.90</td>
<td>0.10</td>
</tr>
<tr>
<td>-0.80</td>
<td>1.35</td>
<td>0.12</td>
</tr>
<tr>
<td>-0.85</td>
<td>2.50</td>
<td>0.17</td>
</tr>
<tr>
<td>-0.90</td>
<td>4.00</td>
<td>0.26</td>
</tr>
</tbody>
</table>
and blank solutions were undeoxegenated and injected into
deoxegenated eluent of pH7 Britton-Robinscn buffer. The electrode was
pretreated in the static mode at -2.7V for 1 min.

It can be seen from Table 8.1 that the blank signal obtained at the
electrochemically pretreated electrode was made smaller by about 35
times, while the nitrofurantoin signal was made smaller by only 32% from its value at newly polished electrode at -0.65V. Figure 8.8
shows signals obtained near the determination limit both with a newly
polished electrode and a pretreated electrode. The measurement
potential used here was -0.65V to reduce the oxygen blank to an
acceptable level for this concentration of determinand. The large
scale removal of background noise on electrochemically pretreating
the electrode can be seen clearly. The extensive reduction in the
signal from the oxygen dissolved in the sample solution on
electrochemical pretreatment is also apparent; this blank is
equivalent to 6 x 10^{-8}M nitrofurantoin. At significantly higher
concentrations determinations would normally be made at -0.7V where
the blank signal is equivalent to 2 x 10^{-7}M nitrofurantoin. At
levels of nitrofurantoin above 5 x 10^{-6}M coefficients of variation
for five injections at the same concentration were typically less
than 1%.

Polishing the electrochemically pretreated electrode with alumina
slurries was found to be sufficient to remove the effect of
pretreatment and to yield a response characteristic of the untreated
electrode.

CONCLUSION

Electrochemical pretreatment at high negative potentials in dilute
sulphuric acid is effective in inhibiting the reduction of dissolved
molecular oxygen at glassy carbon electrodes and therefore lowers the
background currents caused by reduction of dissolved oxygen when such
electrodes are used at negative potentials in flow injection analysis
and, by extrapolation, in HPLC applications. With nitrofurantoin a
FIGURE 8.8: SIGNALS OBTAINED AT NEAR THE DETERMINATION LIMIT OF NITROFURANTOIN AT (a) A NEWLY POLISHED ELECTRODE AND (b) AT AN ELECTRODE PRETREATED AT -2.7V IN THE STATIC MODE FOR 1 MIN. MEASUREMENT POTENTIAL = -0.65V. NITROFURANTOIN CONCENTRATION = A, 0; B, 2 x 10^{-7} M; C, 5 x 10^{-7} M; AND D AND D', 10 x 10^{-7} M. ELUENT AND SAMPLE SOLUTION D', DEOXYGENATED; SAMPLE SOLUTIONS, A-D, UNDEOXYGENATED
slight loss of determinand signal also occurs, but without loss of precision. It is expected that detection limits even for compounds that are determined at low negative potentials, where oxygen is not a major interferent at high determinand concentrations, will be lowered.

In flow injection applications using PTFE transmission tubing it is possible to deoxygenate eluents to a sufficiently low level to enable compounds that are reduced at potentials up to about -0.7V to be determined at a pretreated electrode without the need to deoxygenate the sample solutions.
References

10. Idem, ibid, 64 (1975) 85.
11. Idem, ibid, 64 (1975) 95.
INTRODUCTION

The biological importance of vitamins K in relation to blood coagulation processes is well reported \(^1\). Phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone) (vitamin K\(_1\)) is known to be involved in the formation of both blood clotting factors\(^2\) and a small protein, called osteocalcin, which is found in bone\(^3\). Vitamin K\(_1\) and K\(_3\) (2-methyl-1,4-naphthoquinone) (menadione) are structurally similar. Both vitamins contain the 1,4-naphthoquinone moiety. In protic media (i.e. containing H\(^+\)) the electrochemical reduction of this moiety at a mercury electrode involves the addition of two electrons and two protons to produce the corresponding hydroquinones:

\[
\begin{align*}
\text{R} & = \text{H} \quad \text{in vitamin K}_3 \\
\text{R} & = \text{CH}_2 - \text{CH} = \text{C} - \text{CH}_2 - \text{CH}_2 - \text{CH} - \text{CH}_2 + \text{H} \quad \text{in vitamin K}_1
\end{align*}
\]

where R = H in vitamin K\(_3\)
Vire and Patriarche\textsuperscript{4} have studied the electrochemical behaviour of vitamin K\textsubscript{1} using DC, AC and DP polarography in solutions containing methanolic acetate buffers. One well-defined wave, or peak, was obtained in acid or neutral solutions, but at pH 9.6 AC and DP polarograms exhibited two peaks. The appearance of the second peak was said to be due to adsorption of the vitamin K\textsubscript{1} molecule at the mercury surface and was pH dependent.

Hart and Catterall\textsuperscript{5} using cyclic voltammetry at a hanging mercury drop electrode confirmed that adsorption occurred with both the quinone and the hydroquinone at the mercury surface. They also confirmed that the reduction of vitamin K\textsubscript{1} to the corresponding hydroquinone was a reversible reaction in 90\% EtOH -0.05M acetate buffer at pH 6.0.

The electrochemical characteristics of vitamin K\textsubscript{3} were studied by Patriarche and Lingane\textsuperscript{6} in aqueous methanolic acetate buffers using a combination of electrochemical techniques. These authors indicated that the reduction was not perfectly reversible; cyclic voltammetry demonstrated the quasi-reversibility of the electrode process.

Mechanistic data were obtained for the reduction of vitamins K\textsubscript{1} and K\textsubscript{3} at carbon electrodes\textsuperscript{7} using thin-layer voltammetry. These authors reported that vitamin K\textsubscript{3} was reduced in a reversible two-electron, two-proton process to the hydroquinone. However, vitamin K\textsubscript{1} was reduced first to the semiquinone by addition of one electron and one proton; then at more negative potentials a further electron and proton were added to the semiquinone to produce the hydroquinone.

Lindquist and Farroha\textsuperscript{8} have investigated the differential pulse polarographic behaviour of vitamin K\textsubscript{1} and K\textsubscript{3}, and recommended supporting electrolytes containing 75\% propan-2-ol -0.06M ammonium chloride and 0.1M acetate buffer of pH 5.0 containing 25\% of methanol for vitamin K\textsubscript{1} and K\textsubscript{3} respectively. The limits of determination were about 0.5 \textmu g ml\textsuperscript{-1} for vitamin K\textsubscript{1} and 0.02 \textmu g ml\textsuperscript{-1} for vitamin K\textsubscript{3}.\textsuperscript{8}
Similar behaviour of vitamin K₃ was reported by Hart and Catterall⁹ who used a supporting electrolyte containing 90% ethanol -0.05M acetate buffer at pH 6.0.

The optimum conditions for the differential pulse polarographic determination of vitamin K₁ at submicromolar concentrations were found by varying the ionic strength and the concentration of the substituents of ethanolic acetate buffer over the pH range 3-6⁹. In this study it was shown that the peak current measured at -0.36V versus SCE, was proportional to concentration over the range 2.6 x 10⁻⁷ - 1.5 x 10⁻⁶M when the supporting electrolyte consisted of 90% ethanol 0.05M acetate buffer at pH 6.0. Later Hart and Catteral⁵ achieved better sensitivity by using differential pulse voltammetry at a hanging mercury drop electrode; concentrations down to 10 ng/ml of vitamin K₁ could be measured by using a medium containing 60% methanol -0.05M acetate buffer at pH 6.0.

A differential pulse polarographic method for the determination of vitamin K₁ in plasma has been reported¹⁰. A solvent extraction procedure involving methanol, CHCl₃ and H₂O was used to separate vitamin K₁ from plasma; the solvent was evaporated to dryness and the residue dissolved in 90% ethanol -0.05M acetate buffer at pH 6. Differential pulse polarography was performed on the extracts and a well-defined peak was obtained at -0.58V versus the SCE; recoveries of vitamin K₁ added to plasma in the concentration range 0.2-3 μg ml⁻¹ of plasma were 72.2%. The coefficient of variation was 3% at a concentration of 2.75 μg ml⁻¹ of plasma.

Ikenoya et al⁰¹ developed a high-performance liquid chromatographic method with electrochemical detection for the determination of vitamin K₁ in plasma. Detection was made at a potential of -3.0V versus Ag/AgCl, and the detection limit was 100 pg. Recently Hart et al¹² have described an HPLC method that involves reductive amperometric detection at a glassy carbon electrode for the determination of vitamin K₁. They determined vitamin K₁ in a 95% methanol eluent that was 0.05M in pH3 sodium acetate-acetic acid
electrolyte holding the potential of the glassy carbon electrode at -1.0V; the eluent was deoxygenated with nitrogen and an all-metal solvent delivery system was used to prevent the re-entry of oxygen. Calibration graphs were obtained by injecting 1-10 ng of vitamin K₁. Adsorbed products on the electrode were removed periodically by holding the electrode at +0.7V which re-oxidised the product as the reduction is quasi-reversible.

Previously in Chapter 8 it had been demonstrated that electrochemical pretreatment at high negative potentials in dilute sulphuric acid was effective in inhibiting the reduction of dissolved molecular oxygen at glassy carbon electrodes and therefore had lowered the background currents caused by reduction of dissolved oxygen when such electrodes were used at negative potentials in flow injection analysis. Compounds reduced at -0.65V could be determined at a pretreated electrode without the need to deoxygenate the sample solutions.

In this chapter a method has been described for the flow injection amperometric determination of vitamins K₁ and K₃. A glassy carbon electrode pretreated at high negative potentials was used in order to inhibit the reduction of dissolved molecular oxygen and therefore to improve the sensitivity of the method.

EXPERIMENTAL

A single-channel flow injection system was used. Eluent was pumped through the system by means of an Ismatec Mini-S pump. Injections were made with a Rheodyne low pressure sample injection valve (5020) fitted with a 100 µl sample loop and connected to a laboratory-built amperometric detector by means of 50 cm of 0.58 mm bore PTFE tubing. The amperometric detector¹³ which holds a glassy carbon electrode in the wall-jet configuration is used partially immersed in electrolyte of the same composition as the eluent. The platinum counter and saturated calomel reference electrodes were placed in this electrolyte to complete the three-electrode system. The potential of the glassy carbon electrode was maintained at the required potential
by means of a PAR 174 polarographic analyser (Princeton Applied Research). Signals were recorded on a Linseis L650 y-t recorder. The glassy carbon disk electrode (3 mm diameter) was constructed from Le Carbo ne glassy carbon and was mounted in PTFE as described in Chapter 7. The eluent reservoir was a 2 litre flask covered by a tight glass cover in which the deoxygenation train was placed. A two-way tap was connected at the end of the deoxygenation train to make possible either bubbling nitrogen into the eluent or maintaining a nitrogen atmosphere over the eluent.

REAGENTS

All chemicals were of analytical-reagent grade. Vitamin K₁ and K₃ were obtained from Sigma Chemicals Company.

Methanolic Acetate Buffer Solution

a) Acetic acid, 5M
   Add 28.7 ml of glacial acetic acid to about 50 ml of water and dilute to 100 ml in a calibrated flask.

b) Sodium acetate, 5M
   Dissolve 41.02g of sodium acetate in about 50 ml of water and dilute to 100 ml in a calibrated flask.

The methanolic acetate buffers were prepared by adding 1.25 ml of sodium acetate solution to 8.75 ml of acetic acid solution to prepare a 5M sodium acetate-acetic acid buffer (pH 3.8), then by mixing a 5M sodium acetate-acetic acid buffer, methanol and distilled water to give a final electrolyte concentration of 0.05M.

Vitamin K₁ Standard Solution, 10⁻²M

Vitamin K₁ standard solutions were prepared by dissolving 450.7 mg of vitamin K₁ (Sigma Chemicals) in methanol and diluting to 100 ml in a calibrated flask. Aliquots of these solutions were diluted with
methanol and acetate buffer (pH 3.8) to give the desired methanol concentration and an electrolyte concentration of 0.05M. All glassware containing solutions of vitamin K₁ were protected from light during the course of the studies by covering it with aluminium foil. The standard solutions were stored in the refrigerator.

**Vitamin K₃ Standard Solution, 5 x 10⁻² M**

Vitamin K₃ standard solutions were prepared by dissolving 860.9 mg of vitamin K₃ (Sigma Chemicals) in methanol, and diluting to 100 ml in a calibrated flask with methanol. Aliquots of these solutions were diluted with methanol and acetate buffer (pH 3.8) to give an acetate buffer concentration of 0.05M, the desired concentration of methanol and the desired concentration of vitamin K₃. The standard solutions were stored in the refrigerator.

**PROCEDURE**

The glassy carbon electrode was pretreated as described in Chapter 8.

**RESULTS AND DISCUSSION**

Because of the low solubility of vitamins K, especially vitamin K₁ in water, stock solutions of vitamins K₁ and K₃ were prepared in methanol and aliquots of these solutions were diluted with methanol and acetate buffer. Methanol was chosen here because it is cheaper than ethanol.

In order to investigate the effect of methanol content of the electrolyte on hydrodynamic voltammograms and current signals of vitamins K₁ and K₃, eluents of acetate buffer (pH 3.8) containing 1% to 90% methanol were used. Sample solutions were also prepared to contain 1% to 90% methanolic acetate buffer (pH 3.8). However, for vitamin K₁ sample solutions, the lowest methanol percentage that could be used in the electrolyte was about 50% due to the low solubility of vitamin K₁ in water.
Figure 9.1 shows the hydrodynamic voltammograms obtained for vitamin K₃ when acetate buffer (pH 3.8) was used as eluent, and vitamin K₃ sample solutions were prepared in 1% methanolic acetate buffer and when 90% methanolic acetate buffer was used for both eluent and sample solutions. Figure 9.2 shows the hydrodynamic voltammograms obtained for vitamin K₁ when 50% and 90% methanolic acetate buffer electrolytes were used in both eluent and sample solutions. It is clear from Figures 9.1 and 9.2 that the hydrodynamic voltammograms were shifted to more negative potentials as the percentage of methanol increased.

The effect of the concentration of methanol on the peak current and the background current is shown in Tables 9.1 and 9.2. It is clear that signals decreased with the increase in concentration of methanol. A decrease in the background current with the increase in concentration of methanol can also be seen. However, the calculation of the peak current/background current ratio which gives a better way of understanding the effect of methanol concentration indicates that the ratio decreases with the increase in percentage of methanol for vitamin K₃, while the ratio increases with the increase in percentage of methanol for vitamin K₁. Therefore, a 90% methanolic acetate buffer was used in both eluent and vitamin K₁ sample solutions whereas vitamin K₃ solutions were prepared in 1% methanolic acetate buffer and the eluent used was acetate buffer (pH 3.8) solution.

EFFECT OF ELECTROCHEMICAL PRETREATMENT OF GLASSY CARBON ELECTRODES AT HIGH NEGATIVE POTENTIALS ON THE FLOW INJECTION AMPEROMETRIC DETERMINATION OF VITAMIN K₃ AND K₁

It was found in Chapter 8 that electrochemical pretreatment at high negative potentials in dilute sulphuric acid inhibits the reduction of oxygen at glassy carbon electrodes and therefore lowers the background currents caused by reduction of dissolved oxygen which will lower the detection limit. When nitrofurantoin was determined reductively at a glassy carbon electrode pretreated either at -2.7V
**TABLE 9.1:** Effect of methanol concentration on the current signal of vitamin K$_1$ ($5 \times 10^{-6}$M) and background current, at -0.7V
Flow rate: 6 ml min$^{-1}$

<table>
<thead>
<tr>
<th>% of Me</th>
<th>Signal/µA</th>
<th>Background Current/µA</th>
<th>Signal to Background Current Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.84</td>
<td>0.29</td>
<td>2.9</td>
</tr>
<tr>
<td>60</td>
<td>0.75</td>
<td>0.26</td>
<td>2.9</td>
</tr>
<tr>
<td>70</td>
<td>0.69</td>
<td>0.23</td>
<td>3.0</td>
</tr>
<tr>
<td>80</td>
<td>0.65</td>
<td>0.21</td>
<td>3.1</td>
</tr>
<tr>
<td>90</td>
<td>0.46</td>
<td>0.15</td>
<td>3.1</td>
</tr>
</tbody>
</table>

**TABLE 9.2:** Effect of methanol concentration on the current signal of vitamin K$_3$ ($5 \times 10^{-6}$M) and background current at -0.7V
Flow rate: 6 ml min$^{-1}$

<table>
<thead>
<tr>
<th>% of Me</th>
<th>Signal/µA</th>
<th>Background Current/µA</th>
<th>Signal to Background Current Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.1</td>
<td>0.36</td>
<td>8.6</td>
</tr>
<tr>
<td>10</td>
<td>3.0</td>
<td>0.35</td>
<td>8.6</td>
</tr>
<tr>
<td>20</td>
<td>2.85</td>
<td>0.34</td>
<td>8.4</td>
</tr>
<tr>
<td>30</td>
<td>2.75</td>
<td>0.33</td>
<td>8.3</td>
</tr>
<tr>
<td>40</td>
<td>2.6</td>
<td>0.31</td>
<td>8.4</td>
</tr>
<tr>
<td>50</td>
<td>2.4</td>
<td>0.29</td>
<td>8.3</td>
</tr>
<tr>
<td>60</td>
<td>2.13</td>
<td>0.26</td>
<td>8.2</td>
</tr>
<tr>
<td>70</td>
<td>1.8</td>
<td>0.23</td>
<td>7.8</td>
</tr>
<tr>
<td>80</td>
<td>1.5</td>
<td>0.20</td>
<td>7.5</td>
</tr>
<tr>
<td>90</td>
<td>1.1</td>
<td>0.15</td>
<td>7.3</td>
</tr>
</tbody>
</table>
for 1 min in a static system before being inserted into the detector cell or at -3V for 1 min on-line in 0.1M sulphuric acid at a flow rate of 2 ml min⁻¹, it was found that at -0.65V no interference from oxygen was observed when unddeoxygenated nitrofurantoin sample solutions were injected into a deoxygenated eluent of pH7 Britton-Robinson buffer, and therefore the detection limit was lowered by about ten times.

In this work a similar effect of pretreatment was observed when determining vitamin K₃ in 1% methanolic acetate buffer and the eluent is acetate buffer (pH 3.8). Figure 9.3 shows the hydrodynamic voltammogram obtained at a pretreated glassy carbon electrode for vitamin K₃. A slight shift of the hydrodynamic voltammogram to more cathodic potential can be seen. Table 9.3 shows the effect of electrochemically pretreating the electrode on the current signals of vitamin K₃, the background currents and the signals to background currents ratios.

It is clear from Table 9.3 that the signal to background current ratio has been increased by pretreating the electrode. At a potential of -0.6V the current signal to background current ratio has increased by about 5 times by pretreating the electrode. At the same time at this potential the signals obtained at a pretreated electrode when unddeoxygenated eluent were injected into deoxygenated eluent were equivalent to about 5 x 10⁻⁷M. Figure 9.4 shows signals obtained near the determination limit both with a newly polished electrode and a pretreated electrode. The measurement potential used was -0.6V. The large scale removal of background noise on electrochemically pretreating the electrode can be seen clearly. The extensive reduction in the signal from the oxygen dissolved in the sample solution on electrochemical pretreatment is also apparent. Calibration graphs of the reduction current against the concentration of vitamin K₃ at a pretreated electrode were rectilinear over the range 7 x 10⁻⁷ - 1 x 10⁻⁴M compared with the concentration range of 3 x 10⁻⁶ - 1 x 10⁻⁴M at a newly polished electrode where both eluent and sample solutions were deoxygenated. Figure 9.5 shows some signals
FIGURE 9.1: HYDRODYNAMIC VOLTAMMOGRAMS OF VITAMIN K3 (5 x 10^{-4}M) AT A NEWLY POLISHED GLASSY CARBON ELECTRODE; A, ELUENT IS pH 3.8 ACETATE BUFFER AND SAMPLE SOLUTION IS IN 1% METHANOLIC ACETATE BUFFER AND B, BOTH ELUENT AND SAMPLE SOLUTIONS ARE IN 90% METHANOLIC ACETATE BUFFER
FIGURE 9.2: HYDRODYNAMIC VOLTAMMOGRAMS OF VITAMIN K1 (5 x 10^{-4} M) AT A NEWLY POLISHED GLASSY CARBON ELECTRODE. (A) ELUENT AND SAMPLE SOLUTIONS ARE IN 50% METHANOLIC ACETATE BUFFER; (B) ELUENT AND SAMPLE SOLUTIONS ARE IN 90% METHANOLIC ACETATE BUFFER; C AND D, THE BACKGROUND CURRENTS
FIGURE 9.3: HYDRODYNAMIC VOLTAMMOGRAM OF VITAMIN K3 AT A PRETREATED GLASSY CARBON ELECTRODE. ELUENT, pH 3.8 ACETATE BUFFER; SAMPLE SOLUTIONS ARE IN 1% METHANOLIC ACETATE BUFFER. THE BACKGROUND HYDRODYNAMIC VOLTAMMOGRAM IS GIVEN AS BROKEN LINES.
obtained when producing a calibration graph for the determination of vitamin K$_3$ at an electrochemically pretreated electrode.

When the effect of electrochemical pretreatment at high negative potentials on the reduction of vitamin K$_1$ in 90% methanol -0.05M acetate buffer (pH 3.8) was investigated, the results obtained were very different from that obtained with vitamin K$_3$ in 1% methanol -0.05M acetate buffer, or with that obtained before in Chapter 8 with nitrofurantoin in pH7 Britton-Robinson buffer. In fact, pretreating the electrode for 1 min at -2.7V in a static mode in 0.1M sulphuric acid had no effect on either background noise or undeoxygenated blank signal when this electrode was used at negative potentials in a flow injection system. The reason for that is thought to be the presence of methanol in the eluent and sample solutions. Therefore the effect of methanol concentration on the electrochemical pretreatment was investigated.

Table 9.4 illustrates the effect of methanol concentration on the signals obtained when undeoxygenated blank solutions of different concentration of methanol were injected into a deoxygenated eluent of 0.05M acetate buffer (pH 3.8). Five injections were made for each blank solution.

It is clear from Table 9.4 that the presence of methanol weakens the effect of pretreatment on making the reduction of oxygen more difficult at the electrode surface. In fact, an injection of methanol was found to remove any effect of the electrochemical pretreatment, as the signal obtained was equivalent to that obtained at a newly polished electrode. Ethanol was found to have a very similar effect on electrochemical pretreatment. Therefore vitamin K$_1$ was determined at -0.7V in 90% methanolic acetate buffer at a newly polished electrode. Both eluent and sample solutions were deoxygenated. The calibration graph was rectilinear over the range 8 x 10$^{-6}$ - 5 x 10$^{-5}$M. Figure 9.6 shows some signals obtained when producing a calibration graph for the determination of vitamin K$_1$. 
TABLE 9.3: Effect of electrochemical pretreatment of the glassy carbon electrode on the peak current of vitamin K₃, background current and peak current to background current ratio. Vitamin K₃ concentration 5 x 10⁻⁶M

<table>
<thead>
<tr>
<th>Potential/V</th>
<th>Peak Current/μA</th>
<th>Background Current/μA</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before pre-</td>
<td>After pre-</td>
<td>Before</td>
</tr>
<tr>
<td></td>
<td>treatment</td>
<td>treatment</td>
<td>treatment</td>
</tr>
<tr>
<td>-0.6</td>
<td>3.10</td>
<td>1.75</td>
<td>0.17</td>
</tr>
<tr>
<td>-0.7</td>
<td>3.10</td>
<td>2.20</td>
<td>0.36</td>
</tr>
<tr>
<td>-0.8</td>
<td>2.95</td>
<td>2.22</td>
<td>0.92</td>
</tr>
<tr>
<td>-0.9</td>
<td>2.65</td>
<td>2.25</td>
<td>1.65</td>
</tr>
</tbody>
</table>

R = peak current to background current ratio

TABLE 9.4: The effect of methanol concentration on the blank signal at a pretreated electrode
Eluent: 0.05M acetate buffer (pH 3.8)
Potential: -0.7V
Flow rate: 5 ml min⁻¹

<table>
<thead>
<tr>
<th>% of methanol in the blank injectate*</th>
<th>Blank signal/μA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st Injection</td>
</tr>
<tr>
<td>0</td>
<td>0.03</td>
</tr>
<tr>
<td>10</td>
<td>0.08</td>
</tr>
<tr>
<td>20</td>
<td>0.09</td>
</tr>
<tr>
<td>40</td>
<td>0.18</td>
</tr>
<tr>
<td>60</td>
<td>0.29</td>
</tr>
<tr>
<td>80</td>
<td>0.43</td>
</tr>
<tr>
<td>90</td>
<td>0.45</td>
</tr>
<tr>
<td>100</td>
<td>0.50</td>
</tr>
</tbody>
</table>

* The injectate consisted of the % methanol mentioned made up with 0.05M aqueous acetate buffer
FIGURE 9.4: SIGNALS OBTAINED NEAR THE DETERMINATION LIMIT FOR VITAMIN K₃ AT (a) A NEWLY POLISHED ELECTRODE AND (b) AT A PRETREATED ELECTRODE. MEASUREMENT POTENTIAL = -0.6V VITAMIN K₃ CONCENTRATION: A, 0; B and B' 2 x 10⁻⁶M; C, 2 x 10⁻⁶M; and D, 4 x 10⁻⁶M. ELUENT AND SAMPLE SOLUTION C DEOXYGENATED; SAMPLE SOLUTIONS, A-D, UNDEOXYGENATED.
FIGURE 9.5: SIGNALS OBTAINED FOR CONSTRUCTING A CALIBRATION GRAPH FOR THE DETERMINATION OF VITAMIN K3 AT A PRETREATED GLASSY CARBON ELECTRODE. VITAMIN K3 CONCENTRATION A, 0; B, 1; C, 2; D, 4; AND E, 8 x 10^{-5} M
FIGURE 9.6: SIGNALS OBTAINED FOR CONSTRUCTING A CALIBRATION GRAPH FOR THE DETERMINATION OF VITAMIN K1 AT A GLASSY CARBON ELECTRODE. VITAMIN K1 CONCENTRATION: A, 0; B, 1; C, 2, AND D, 4 x 10^{-5}M
CONCLUSION

The effect of electrochemical pretreatment of glassy carbon electrodes at high negative potentials in dilute sulphuric acid on the on-line determination of vitamins K1 and K3 were studied. By pretreating the electrode at -2.7V for 1 min in a static system before being inserted into the detector cell, vitamin K3 was determined at -0.6V without the need to deoxygenate the sample solution. The eluent used was 0.05M acetate buffer (pH 3.8) where the sample solutions were in 1% methanol -0.05M acetate buffer (pH 3.8). The determination limit was improved by about 5 times at a pretreated electrode than at a newly polished electrode. However the effect of electrochemical pretreatment was found to be weakened by the presence of methanol or ethanol in either the eluent or the sample solutions, and therefore, in determining vitamin K1 in 90% methanol -0.05M acetate buffer (pH 3.8) the electrochemical pretreatment was not effective, i.e. the size of signals obtained on injecting undeoxygenated eluent into deoxygenated eluent at a pretreated electrode when 90% methanolic acetate buffer was used, was almost equivalent to that obtained at a newly polished electrode.
References

CHAPTER 10

IODIMETRIC FLOW INJECTION METHODS OF DETERMINING
SULPHITE USING SPECTROPHOTOMETRIC DETECTION

INTRODUCTION

Sulphite is widely used in foods and pharmaceutical preparations as a preservative due to its action as an antioxidant. It is also used in the production of paper pulp, as an oxygen scavenger in the treatment of water used for steam generation and is used in the metallurgy of sulphide ores. Sulphite is one of the predominant sulphur species in combustion derived particulates and as sulphur dioxide, in gaseous emissions. As a result of its technological importance and its environmental impact, there are a wide range of methods for its determination.

Titrimetric methods have been used extensively. They depend on the reducing properties of sulphite and therefore, most of the titrants used are oxidising reagents although aqueous solutions of sulphite can also be titrated alkalimetrically. The iodimetric determination of sulphite is based on the reaction:

\[ \text{SO}_3^{2-} + I_2 + H_2O \rightarrow \text{SO}_4^{2-} + 2I^- + 2H^+ \]

Since the iodine/iodide system has a higher oxidation potential than the sulphate/sulphite system, direct titration is possible, but it is not preferred because of the difficulty of preparing standard solutions of iodine, and usually yields inaccurate results.

Much controversy exists in the literature regarding iodimetric titration of sulphite in relation to the source of error. Air
oxidation of sulphite which is especially rapid in alkaline medium is the principal source of error\textsuperscript{6}, and to reduce it Ruff and Jeroch\textsuperscript{9} suggested the use of mannitol which acts as a negative catalyst. According to Rasching, however, accurate results are obtained only when the sulphite solution is determined by an inverse titration method where it is allowed to flow from a pipette into an excess of standard iodine solution and the excess is back-titrated with thiosulphate\textsuperscript{10}.

Although numerous titrimetric methods using a wide range of titrants have been reported for the determination of sulphite\textsuperscript{6}, iodimetric methods are still used extensively, because of their sensitivity. Besada et al\textsuperscript{11} have extended their utility by increasing the sensitivity using amplification reactions. This method involves the oxidation of sulphite by iodine in a bicarbonate medium, extraction of the excess iodine with chloroform, oxidation of the resulting iodide with bromine water followed by iodometric titration of the iodate produced. The method gives a six-fold amplification as compared with the direct iodimetric method.

West and Gaeke\textsuperscript{12} noting that the iodometric methods for sulphite determination suffer several disadvantages, mainly volatilisation of iodine and poor selectivity, have developed a colorimetric method for sulphite determination. The method depends on the reaction of sulphite with mercury(II) solutions to form the disulphitomercurate(II) ion which is known to be very stable\textsuperscript{13}. The procedure uses the method developed by Steigmann\textsuperscript{14}, modified and used earlier by Urone and Boggs\textsuperscript{14}, where sulphite stabilised as disulphitomercurate is added to a solution of acidic p-rosaniline and formaldehyde to form a red-violet complex whose absorption at 560 nm gives a measure of the sulphite concentration.

Okutani and Utsumi\textsuperscript{15} developed a new indirect spectrophotometric method for sulphite in which a definite amount of mercuric nitrate is mixed with potassium bromide and an ethanolic solution of diphenylcarbazone. When this is added to a sulphite solution kept at
pH7, sulphite reacts with the mercuric reagent and the excess of the reagent forms a reddish-violet complex with diphenylcarbazone which is extracted into a benzene organic phase. Sulphite is determined by measuring the absorbance of the complex at 562 nm which decreases as sulphite concentration increases giving a calibration graph with a negative slope. The calibration graph is rectilinear over the concentration range 0.1-2.5 ppm sulphite. Studies of the effect of pH, temperature and chloride ion concentration show that the best sensitivity is obtained at pH 2.5 but at this pH, chloride ion, present in many samples of interest, interferes with the determination. The analysis is therefore done at pH7 where chloride does not interfere up to 100 ppm but there is a loss in sensitivity. Increasing the temperature lowers the absorbance but the effect is very small. The mercury(II) sulphite complex is very stable\textsuperscript{13} and the absorbance remains constant even after two hours from the addition of the mercuric reagent. The application of various metal chloranilates to the spectrophotometric determination of a number of anions using either UV absorption at 330 nm or the visible peak at 525 nm is well known\textsuperscript{16}. Humphrey and Hinze\textsuperscript{16} found that the sulphite ion could be determined by reaction with mercuric chloranilate in 5% aqueous ethanol to release the chloranilate ion and presumably form the soluble, non-dissociated mercuric sulphite. By measuring the absorbance of the chloranilated ion at 330 nm or 525 nm quantitative determination can be achieved. The calibration graphs are rectilinear in the range 5-100 µg/ml at 525 nm and 0.5-8.0 µg/ml at 330 nm. Using a similar approach Hinze et al\textsuperscript{17} developed another spectrophotometric method for sulphite. They investigated the reaction of sulphite with mercuric thiocyanate which is known to react with certain anions to form less dissociated or insoluble mercuric compounds to release the thiocyanate ion\textsuperscript{17}. The thiocyanate ion is complexed with ferric ion and the resulting complex absorbs in the visible region. No reaction between sulphite and mercuric thiocyanate occurs in water alone but the thiocyanate ion is released in aqueous ethanol allowing sulphite determination in this solvent by measuring the absorbance of the ferric thiocyanate complex obtained on adding a ferric salt to the reaction vessel. Calibrations obtained at 470 nm in the 75% aqueous
ethanol as a solvent are rectilinear in the range 1-20 ppm sulphite but show negative deviation from Beer's law at higher sulphite concentrations. Better sensitivities are obtained using ferric nitrate in perchloric acid medium because the ferric cyanide complex has a higher absorption in perchloric acid medium. The visible absorption of FeSCN$^{2+}$ complex is considerably higher than that of chloranilate making this method more sensitive than that of Humphrey and Hinze$^{16}$. However the absorbance tends to decrease slowly with time and readings are best made soon after addition of the ferric solution. Fading is less in 50% aqueous ethanol but the sensitivity is lower in this solvent than in the 75% aqueous ethanol solvent.

Recently Yamada et al$^{2}$ noted that the available methods for sulphite determination have some drawbacks such as lack of sensitivity, selectivity or simplicity, have developed a method which combined flow injection analysis and chemiluminescence detection. The method depends on the reaction between sulphite and acidic permanganate which produces a weak chemiluminescence. To increase the emission they used a sensitisier (fluorescer). Riboflavine phosphate and brilliant sulphaflavine are the recommended sensitisers as they markedly enhance the emission intensity. The detection limit for sulphite by this method is 0.9 ng SO$^{2-}_3$ when riboflavine phosphate is used as sensitisier and 1.8 ng SO$^{2-}_3$ when sulphaflavine is used.

As the flow injection analysis method offers rapid analysis with high reproducibility, many methods have been developed for on-line determination of sulphite. Marshall and Midgley$^{18}$ have determined sulphite using mercuric sulphide/mercurous chloride ion selective electrode in a flow injection system. They noted that the use of this electrode for direct determination of sulphite in aqueous samples is limited by interfering ions, notably chloride which is commonly present in real samples such as food and beverages.

Fogg et al$^{5}$ have developed a direct oxidative flow injection amperometric method of determining sulphite at an electrochemically pre-treated glassy carbon electrode, but they found that EDTA which
is frequently included in formulations with sulphite, was shown to interfere as it is also oxidized to some extent at the electrochemically pre-treated electrode at the measurement potential used.

Very recently Fogg et al\textsuperscript{19} described a flow-injection amperometric method for the iodimetric determination of sulphite. Both the formation of iodine and its reaction with sulphite are carried out on-line. Iodine is formed reproducibly in the reverse FIA manner in an injected bolus of dilute acid using a slightly alkaline potassium iodate eluent containing an excess of potassium iodide and is monitored at a glassy carbon electrode held at -0.2 volts versus SCE, without interference from dissolved molecular oxygen. When acidified sulphite standards or samples are injected into the same eluent a decreased iodine signal is obtained owing to the reaction of the sulphite injected with an equivalent amount of iodine. The decrease in signal size is rectilinear with the sulphite concentration of the injected solution in the range $0.1 \times 10^{-4}$ - $4 \times 10^{-4}$M when injection is made into an eluent $3 \times 10^{-5}$M in potassium iodate and 0.1M in potassium iodide. Methods of determining sulphite in which the reaction between iodine or bromine and sulphite is carried out off-line and the excess of iodine or bromine is determined amperometrically by injecting the reacted solution into an inert iodide or bromide eluent, or in which a slightly alkaline sample solution containing iodate/iodide is injected into an acid eluent, have been developed also.

In this chapter on-line spectrophotometric methods of determining sulphite are described. The methods are based on similar principles used by Fogg et al\textsuperscript{19} for flow injection amperometric determination of sulphite.

**EXPERIMENTAL**

A spectrum of a solution $10^{-5}$M in iodine ($I_2$) and 0.4% in potassium iodide solution was recorded in order to find $\lambda_{\text{max}}$ for iodine in 0.4%
potassium iodide solution. The spectrum showed a maximum absorbance of iodine at a wavelength of 352 nm. This wavelength has been used throughout this work.

An Ismatec Mini-S pump was used to maintain the eluent flow. Sample injections were made with a low-pressure Rheodyne 5020 injection valve, and 0.71 mm bore PTFE transmission tubing was used between the valve and the detector cell.

Single and dual channels flow injection manifolds were used. In the dual channels manifold, two Ismatec Mini-S pumps were used to propel the iodate/iodide eluent. The two lines of tubing were connected to a confluence point, and the sample solution injections were made prior to the confluence point as shown in Figure 10.1.

A Helma flow through cell of 30 μl volume, equipped with screw fittings which allow connections with a minimum dead volume, was used. The flow cell is shown in Figure 10.2.

An HP 8451 Diode Array spectrophotometer which is a single beam, microcomputer-controlled UV-visible spectrophotometer which operates at high speed, was used.

The flow cell position was adjusted in accordance with the instrument instructions in order to obtain maximum throughput of light. The cell position was kept fixed throughout the remainder of this work.

REAGENTS

Stock Standard Potassium Iodate Solution, 1 x 10⁻²M
Dissolve 2.14g of potassium iodate in distilled water and dilute to 1 litre in a calibrated flask.

Potassium Iodide Solution, 2M
Dissolve 166g of potassium iodide in water, add 1 ml of 2M sodium hydroxide solution and dilute to 500 ml with water.
Preparation of About 0.05M Iodine Solution
Dissolve 2.0g of potassium iodide in 3 to 4 ml of distilled water in a glass-stoppered 100 ml calibrated flask. Weigh out 1.27g of iodine (A.R. grade) and transfer by means of a small dry funnel into the concentrated potassium iodide solution. Insert the glass stopper into the flask, and shake in the cold until all the iodine has dissolved. Allow the solution to reach room temperature and make it up to the mark with distilled water. The iodine solution is best preserved in small glass-stoppered bottles, kept in a cool, dark place. Make more dilute solutions by diluting this stock solution with 0.4% w/v potassium iodide.

Standardisation of Iodine with Thiosulphate
Transfer 25 ml of the iodine solution to a 250 ml conical flask, dilute to 100 ml and add the standard thiosulphate solution from a burette until the solution has a pale-yellow colour. Add 2 ml of starch solution and continue the addition of the thiosulphate solution slowly until the solution is just colourless.

EDTA Solution, 0.1M
Dissolve 9.31g of disodium ethylenediaminetetraacetic acid in distilled water and dilute to 250 ml.

Stock Standard Sodium Sulphite Solution, 1 x 10⁻²M
Transfer 0.315g of anhydrous sodium sulphite (assay not less than 97%) to a 250 ml calibrated flask containing 25 ml of 0.1M EDTA solution. Agitate the solution to dissolve the solid, and then dilute the solution to 250 ml with distilled water. This solution should be prepared fresh daily.

Britton-Robinson Buffer
Dissolve 2.47g of boric acid in 500 l of distilled water containing 2.3 ml of acetic acid. Add 2.7 ml of orthophosphoric acid and dilute to 1 litre. The pH was adjusted to pH 2.5 using sodium hydroxide solution.
FIGURE 10.1: DUAL-CHANNELS FLOW INJECTION MANIFOLD

FIGURE 10.2: FLOW CELL USED IN THIS WORK. A, FRONT SIDE AND B, REAR SIDE
Hydrochloric Acid, 4.0M
Dilute 182.2 ml of concentrated hydrochloric acid to 500 ml in a volumetric flask.

RESULTS AND DISCUSSION
Preliminary Studies

Preliminary studies were carried out to measure the values of dispersion in the flow injection system used in this work. Particular attention was paid to the measurement of dispersion values in a reverse flow injection analysis system. The calculation of dispersion was based on the definition given by Ruzicka and Hansen as the ratio of the original analyte concentration, $C_0$, to the concentration of the analyte in that element of fluid which corresponds to the maximum of the peak $C_{\text{max}}$

$$D = \frac{C_0}{C_{\text{max}}}$$

Dispersion is also frequently used to describe the ratio in which the sample has been mixed with the reagent in the carrier stream. Therefore, for $D = 1$, there is no mixing, while for $D = 4$, a dilution of 3.1 by the reagent carrier stream has occurred to the sample.

In these studies measuring dispersion in a normal flow injection system was carried out by monitoring the absorbance when 75 μl of $10^{-5}$M I$_2$ solution in 0.4% potassium iodide was injected into 0.4% potassium iodide eluent, and then the steady state absorbance of $10^{-5}$M iodine in 0.4% potassium iodide by pumping it continuously. For the reverse flow injection analysis system, the measurements were made by monitoring the steady state absorbance of $10^{-5}$M iodine in 0.4% potassium iodide, and then the loss of absorbance (i.e. negative signal) occurred when 75 μl of 0.4% potassium iodide was injected into $10^{-5}$M iodine in 0.4% potassium iodide eluent. The absorbance of
iodine was measured at a wavelength of 352 nm where maximum absorbance was found.

The values of dispersion obtained at different delay coil lengths, flow rates and tubing bore sizes for the normal flow injection experiments are shown in Tables 10.1-10.3.

### TABLE 10.1: Effect of delay coil length

<table>
<thead>
<tr>
<th>Delay coil length/metre</th>
<th>C&lt;sub&gt;max&lt;/sub&gt;</th>
<th>C&lt;sub&gt;O&lt;/sub&gt;</th>
<th>D</th>
<th>% sample (injectate) at the detector</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.123</td>
<td>0.273</td>
<td>2.219</td>
<td>45.1</td>
</tr>
<tr>
<td>1.0</td>
<td>0.091</td>
<td>0.275</td>
<td>3.022</td>
<td>33.1</td>
</tr>
<tr>
<td>2.0</td>
<td>0.059</td>
<td>0.274</td>
<td>4.644</td>
<td>21.5</td>
</tr>
<tr>
<td>3.0</td>
<td>0.043</td>
<td>0.274</td>
<td>6.372</td>
<td>15.7</td>
</tr>
<tr>
<td>4.0</td>
<td>0.037</td>
<td>0.273</td>
<td>7.378</td>
<td>13.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flow rate/ml/min</th>
<th>C&lt;sub&gt;max&lt;/sub&gt;</th>
<th>C&lt;sub&gt;O&lt;/sub&gt;</th>
<th>D</th>
<th>% sample (injectate) at the detector</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.080</td>
<td>0.166</td>
<td>2.084</td>
<td>48.0</td>
</tr>
<tr>
<td>2</td>
<td>0.085</td>
<td>0.207</td>
<td>2.444</td>
<td>40.9</td>
</tr>
<tr>
<td>3</td>
<td>0.088</td>
<td>0.243</td>
<td>2.766</td>
<td>36.1</td>
</tr>
<tr>
<td>4</td>
<td>0.090</td>
<td>0.260</td>
<td>2.891</td>
<td>34.6</td>
</tr>
<tr>
<td>5</td>
<td>0.092</td>
<td>0.275</td>
<td>2.990</td>
<td>33.4</td>
</tr>
<tr>
<td>6</td>
<td>0.096</td>
<td>0.290</td>
<td>3.060</td>
<td>32.7</td>
</tr>
<tr>
<td>7</td>
<td>0.099</td>
<td>0.307</td>
<td>3.110</td>
<td>32.1</td>
</tr>
<tr>
<td>8</td>
<td>0.101</td>
<td>0.319</td>
<td>3.158</td>
<td>31.7</td>
</tr>
</tbody>
</table>
TABLE 10.3: Effect of tubing diameter
Flow rate: 5 ml/min
Delay coil length: 1.0 metre
Sample volume: 75 µl

<table>
<thead>
<tr>
<th>Tubing diameter (mm)</th>
<th>C^max</th>
<th>C_o</th>
<th>D</th>
<th>% sample (injectate) at the detector</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.71</td>
<td>0.090</td>
<td>0.273</td>
<td>3.033</td>
<td>33.0</td>
</tr>
<tr>
<td>0.89</td>
<td>0.093</td>
<td>0.296</td>
<td>3.180</td>
<td>31.4</td>
</tr>
<tr>
<td>1.01</td>
<td>0.096</td>
<td>0.321</td>
<td>3.343</td>
<td>30.0</td>
</tr>
</tbody>
</table>

Determination of the values of dispersion of the reagent becomes important when a chemical reaction takes place between the sample and reagent in the carrier stream. Reagent dispersion can be defined in exactly the same way as sample dispersion $D^R = (C_o/C^{\text{max}})_R$. The relationship between $D^R$ and $D$ is

$$D^R = D/(D-1)$$

This equation can be used to select an appropriate reagent concentration in relation to the sample concentration. If, for example, a ten-fold excess of reagent is required for complete reaction, i.e. it is required that $(C^{\text{max}})_R = 10 C^{\text{max}}$, and if a dispersion of 5 is selected, then at the peak maximum the original sample concentration will have decreased to a fifth of its original value, but the reagent concentration will have decreased to four-fifths of the original value. So, the original reagent concentration needs only to be 2.5 times more concentrated than the sample to achieve a ten-fold excess at the peak maximum for a dispersion of 5. The relationship can be summarised as follows:
\[
\frac{(C_{\text{max}})^R}{C_{\text{max}}} = \frac{R_{R/a}}{D-1}
\]

where \( R_{R/a} \) is the ratio of reagent to analyte concentration required at the peak maximum.

In reverse FIA, the positions of the sample and reagent solutions are reversed; the reagent solution is injected into the sample solution which is now the eluent\(^2^1\). The reagent and sample mix in exactly the same manner as in normal FIA, so that the fundamental principles of flow injection analysis (reproducible timing, injection and dispersion) apply to reverse flow injection analysis as well\(^2^2\). However, the sample concentration in normal FIA is at a maximum at the time of injection and it then decreases. On the other hand, the initial sample concentration in the zone of the reagent is zero in reverse FIA. However, it increases with time until it reaches the value in the carrier stream. Therefore, values of dispersion of the sample into the reagent in reverse FIA should be equal to the values of dispersion of the reagent into the sample in normal FIA, and vice versa. This has been confirmed here by the results obtained for reverse FIA. Table 10.4 shows the values of dispersion obtained at different delay coil lengths when \(10^{-5}\)M iodine in 0.4% potassium iodide was used as eluent, and 75 \(\mu\)l of 0.4% potassium iodide was injected. The maximum loss of absorbance caused by injecting potassium iodide was measured as \(C_{\text{max}}\). Some peaks obtained are shown in Figure 10.3.
FIGURE 10.3: SIGNALS OBTAINED FOR DETERMINING DISPERSION VALUES IN A SINGLE CHANNEL FLOW INJECTION SYSTEM. (A) IODINE INJECTED INTO POTASSIUM IODIDE ELUENT; (B) IODINE PUMPED CONTINUOUSLY, AND (C) POTASSIUM IODIDE INJECTED INTO IODINE ELUENT.
TABLE 10.4: Effect of delay coil length on dispersion in reverse FIA
Flow rate: 5 ml min\(^{-1}\)
Tubing diameter: 0.71 mm
Sample volume: 75 \(\mu\)l

<table>
<thead>
<tr>
<th>Delay coil length/m</th>
<th>(C_{\text{max}}^{*})</th>
<th>(C_0)</th>
<th>D</th>
<th>% injectate at the detector</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.124</td>
<td>0.273</td>
<td>2.20</td>
<td>45.4</td>
</tr>
<tr>
<td>1.0</td>
<td>0.092</td>
<td>0.275</td>
<td>2.99</td>
<td>33.5</td>
</tr>
<tr>
<td>2.0</td>
<td>0.058</td>
<td>0.274</td>
<td>4.72</td>
<td>21.2</td>
</tr>
<tr>
<td>3.0</td>
<td>0.043</td>
<td>0.274</td>
<td>6.37</td>
<td>15.7</td>
</tr>
<tr>
<td>4.0</td>
<td>0.037</td>
<td>0.273</td>
<td>7.38</td>
<td>13.6</td>
</tr>
</tbody>
</table>

\(^*\) The loss of absorbance

ON-LINE DETERMINATION OF SULPHITE USING IODATE/IODIDE ELUENT

In this method sulphite was determined by injecting an acidic sample solution into a slightly alkaline iodate/iodide eluent and monitoring the absorbance of iodine formed in excess of that required to react with the sulphite injected. The difference between this signal and the larger signal obtained, in which an acidic blank was injected into eluent, was expected to be proportional to the concentration of sulphite injected. Previously\(^{19}\) studies were carried out to discover the level of acidity required in the injected solution to maximize formation of iodine at various levels of iodate in the eluent. It was found that a very high acidity is required in order for rectilinearity with iodate concentration to be extended to higher iodate concentrations, but at iodate concentrations of less than 5 \(\times\) \(10^{-5}\)M the size of the signal is less dependent on acidity providing that the acidity is at pH lower than 3.5.

As hydrogen ion is a product of the reaction of iodine and sulphite as shown in the equation:
increased acidity might be expected to inhibit their reaction. The results obtained previously\textsuperscript{19}, however, indicated that this effect if present was not particularly significant as there is little change in the recorded loss of signal from iodine in the presence of sulphite on changing the acidity within the range studied. Therefore it was recommended that the control and sulphite solutions injected were 0.04M in hydrochloric acid, or were preformed in pH 2.5 Britton-Robinson buffer.

The amount of iodine reaching the detector will depend on the flow parameters, and also on the on-line reaction between sulphite and iodine. Both of these are expected to depend on the extent of dispersion which in turn depends on the eluent flow rate, diameter and length of delay coil, and sample volume. In the present study, the effect of flow rate, diameter and length of delay coil were investigated. The sample volume injected was kept constant (75 µl).

The effect of transmission tubing bore size and length, and of the flow rate on the iodine absorbance and on the loss of absorbance on injecting sulphite are shown in Tables 10.5-10.7.
TABLE 10.5: Effect of transmission tubing bore size on signals

Eluent: \(3 \times 10^{-5}\) M in potassium iodate, 0.1 M in potassium iodide
Flow rate: \(8 \text{ ml min}^{-1}\)
Transmission tubing length: 3 m
Acidic blank: pH 2.5 Britton-Robinson buffer
Sulphite concentration: Control, 0; Sample: \(2 \times 10^{-4}\) M

<table>
<thead>
<tr>
<th>Transmission tubing bore size/mm</th>
<th>Control Signal</th>
<th>Sample Signal</th>
<th>Loss of Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.71</td>
<td>2.09</td>
<td>1.63</td>
<td>0.45</td>
</tr>
<tr>
<td>0.89</td>
<td>2.11</td>
<td>1.86</td>
<td>0.25</td>
</tr>
<tr>
<td>1.01</td>
<td>2.12</td>
<td>1.98</td>
<td>0.14</td>
</tr>
</tbody>
</table>

TABLE 10.6: Effect of transmission tube length on signals

Transmission tube bore size: 0.71
Other parameters as in Table 10.5

<table>
<thead>
<tr>
<th>Length of Transmission tube/m</th>
<th>Control Signal</th>
<th>Sample Signal</th>
<th>Loss of Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>1.54*</td>
<td>0.83*</td>
<td>0.71</td>
</tr>
<tr>
<td>2.0</td>
<td>1.80*</td>
<td>1.22*</td>
<td>0.58</td>
</tr>
<tr>
<td>3.0</td>
<td>2.09</td>
<td>1.63</td>
<td>0.46</td>
</tr>
<tr>
<td>4.0</td>
<td>2.10</td>
<td>1.80</td>
<td>0.30</td>
</tr>
</tbody>
</table>

* Double peaks; measurement made at top of higher peak
TABLE 10.7: Effect of eluent flow rate on signals
Length of transmission tube: 3m
Other parameters as in Table 10.5

<table>
<thead>
<tr>
<th>Eluent flow rate ml min⁻¹</th>
<th>Control Signal</th>
<th>Sample Signal</th>
<th>Loss of Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>**</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>**</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>**</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>2.05*</td>
<td>1.54*</td>
<td>0.51</td>
</tr>
<tr>
<td>5</td>
<td>2.08*</td>
<td>1.60*</td>
<td>0.48</td>
</tr>
<tr>
<td>6</td>
<td>2.1</td>
<td>1.64</td>
<td>0.46</td>
</tr>
<tr>
<td>7</td>
<td>2.09</td>
<td>1.63</td>
<td>0.46</td>
</tr>
<tr>
<td>8</td>
<td>2.09</td>
<td>1.63</td>
<td>0.46</td>
</tr>
</tbody>
</table>

* Double peak, measurement made at top of highest peak
** Double peak, in this case the peaks were very well separated.

The FIA method used for this experiment has rather unusual features as the species monitored - the monitorand - is neither the original reagent nor the determinand. The monitorand (iodine) is being formed in the reverse FIA manner because the reagent of limiting concentration (the iodate) i.e. the reagent whose concentration limits the amount of monitorand formed, is in the eluent and is dispersing - with an excess of a second reagent (iodide) also present in the eluent - into the bolus which contains a third reagent of high concentration (the acid). With increasing dispersion, the concentration of the monitorand in the bolus increases to a limiting value equal to the full equivalent of the limiting reagent concentration in the eluent. The determinand (sulphite) is in the bolus and is dispersing into the eluent in the normal FIA manner. Thus the equivalent concentration of determinand in the bolus decreases with the increasing dispersion. This will explain the unusual nature of these results when they are compared with those
obtained for normal FIA. However, Table 10.6 shows that the size of iodine signal increases with transmission tube length up to about 3m. That is in accord with its reverse FIA mode of formation. At longer delay coil lengths than 4m, however, the iodine signal is constant. The loss of iodine absorbance on adding sulphite decreases with increasing tube length in accord with the normal FIA mode of dispersion of the sulphite.

With increasing tube bore size the iodine signal increases, but becomes constant above a given bore size, in accord with dispersion theory, as the iodine concentration in the bolus reaches the concentration equivalent to the iodate concentration in the eluent, whilst the loss of signal on injecting sulphite decreases continuously.

The size of iodine signal was affected only slightly by changes in flow rate between 4 and 8 ml min\(^{-1}\).

Double peaks were obtained at low flow rate values (lower than 5.8 ml min\(^{-1}\), when 3m transmission tubing of 0.71 mm bore size was used) and at short transmission tubing length (shorter than 3m, when 0.71 mm bore size tubing, and a flow rate of 5 ml min\(^{-1}\) were used). This is possibly because the iodine is formed at the edges of the acid bolus, and due to insufficient dispersion, the concentration of iodine is small at the centre of the acid bolus causing a 'dip' in the signal.

Formation of double peaks is more commonly observed in reverse FIA than in normal FIA\(^{23}\). Here we expected that the iodine signal would be double peaks when dispersion was insufficient due to low flow rate or short and narrow transmission tubing. On increasing dispersion we expected hollow-shaped peaks, and then a flat topped peak at sufficient dispersion\(^{19}\). The presence of determinand dispersing in the normal FIA manner which decreases the size of this signal would be expected to stress the hollow-topped, or double-peaked character of the resulting signal. This was the case in this work.
Figure 10.4 shows the shapes of different iodine peaks at different dispersion values compared to a signal obtained in normal FIA, by using the printer at a fast speed. However by using the printer at a slow speed the peaks appear to be shaped like conventional normal FIA peaks. According to the results presented earlier in this chapter the conditions for maximum formation of iodine within the bolus and maximum loss of iodine signal on addition of sulphite under the parameter range tested were found to be obtained using 3m of 0.71 mm bore size transmission tubing with a flow rate of 5.8 ml min\(^{-1}\) or higher. The eluent is \(3.10^{-5}\)M in iodate and 0.1M in iodide. The acidic solution used for both control and standard sulphite solutions injected is pH 2.5 Britton-Robinson buffer.

Under the above mentioned flow parameters the dispersion is about 6.55, so in a flow injection system the segment of solution giving maximum signal will consist of 15.3% injected solution and 84.7% eluent. Thus in determining sulphite by the present method using a system of dispersion 6.55 the concentration of sulphite in the injected sample can be calculated from equation 10.3 as needing to be 5.5 times greater than that of iodine equivalent of the iodate in the eluent for no iodine or sulphite to be present at the centre of the bolus - that is, for an equivalent point to be reached - when the bolus reaches the detector.

Examples of the type of signals obtained in this study for a calibration graph for the determination of sulphite using the recommended procedure are shown in Figure 10.5. A typical calibration graph obtained using the recommended conditions is shown in Figure 10.6.

The discrepancy in the actual and calculated ratio of signal sizes in the equivalent point may arise from two reasons:
1. Iodine is not fully formed due to the iodate/iodide and acid reaction kinetics
2. Incomplete reaction between the iodine formed on-line and the sulphite solution injected, due to the dispersion features.
FIGURE 10.4: SIGNALS SHAPE OBTAINED FOR INJECTION OF pH 2.5 BRITTON-ROBINSON BUFFER INTO IODATE/IODIDE ELUENT AT DIFFERENT DISPERSION (D) VALUES USING FAST SPEED RECORDER: (A), 2.1; B, 4.2; C, 5.3; AND D, 6.6; E, TYPICAL SIGNAL OBTAINED IN NORMAL FIA MANNER (INJECTION OF IODINE INTO POTASSIUM IODIDE ELUENT)
FIGURE 10.4
FIGURE 10.5: SIGNALS OBTAINED FOR CONSTRUCTING A CALIBRATION
GRAPH FOR THE DETERMINATION OF SULPHITE USING
IODATE/IODIDE ELUENT. SULPHITE CONCENTRATION:
A, 0; B, 1; C, 2; D, 3; E, 4; F, 5; G, 6; AND
H, 7 x 10^{-4} M
FIGURE 10.6: TYPICAL CALIBRATION GRAPH FOR THE DETERMINATION OF SULPHITE USING IODATE/IODIDE ELUENT IN A SINGLE CHANNEL FLOW INJECTION SYSTEM
Although complete formation of iodine is desirable, as the sensitivity will increase, because of the reproducible nature of the pumping and injection processes, it is not necessary that a complete reaction is achieved in a flow injection technique.

Concerning the second reason suggested, different flow parameters were applied. The results are discussed in the next experiment.

**ON-LINE DETERMINATION OF SULPHITE USING IODATE/IODIDE ELUENT IN A DUAL CHANNELS FLOW INJECTION MANIFOLD**

The flow injection analysis manifold used in this experiment was shown in Figure 10.1. It is expected that the presence of a confluence point will produce a different mixing pattern to the diffusion-convection mechanism of the single line and thus the dispersion values will differ. Indeed dispersion values obtained using this manifold were different from those obtained earlier using a single channel FIA system. This is shown in Table 10.8 for different transmission tubing lengths.

In fact a better condition of mixing between sample and reagent occurs at the confluence point. This is confirmed by the results obtained here.

The conditions of maximum formation of iodine and maximum loss of signal were very similar to those found using the single channel FIA system. The significant difference is that by using the dual channels manifold, the results obtained were closer to the expected results than those obtained previously when a single channel FIA system was used, i.e. the difference between the actual and calculated ratio of signal sizes at the equivalent point was less when a dual channels FIA manifold was used. This is because of the better mixing provided by this FIA manifold.
The peak's shape obtained when a dual channels FIA manifold was used was better than that obtained when a single channel system was used, which allowed lower flow rates to be used.

The conditions for maximum formation of iodine within the bolus and maximum loss of iodine signal on addition of sulphite were found to be obtained using 3 m of 0.71 mm bore size transmission tubing with a flow rate of 4.2 ml min\(^{-1}\) or higher. Typical signals obtained for a calibration graph for the determination of sulphite using this method are shown in Figure 10.7. The calibration graph is shown in Figure 10.8.

<table>
<thead>
<tr>
<th>Transmission tubing length/m</th>
<th>D</th>
<th>D'</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.0</td>
<td>4.8</td>
</tr>
<tr>
<td>2</td>
<td>4.6</td>
<td>6.7</td>
</tr>
<tr>
<td>3</td>
<td>6.4</td>
<td>8.9</td>
</tr>
<tr>
<td>4</td>
<td>7.4</td>
<td>9.8</td>
</tr>
</tbody>
</table>

D = dispersion values obtained by using single channel FIA system
D' = dispersion values obtained by using dual channels FIA manifold

**DETERMINATION OF SULPHITE BY ON-LINE MONITORING OF THE EXCESS OF IODINE INJECTED, BY USING SODIUM SULPHITE ELUENT**

In this method iodine was formed off-line from a known iodate concentration in excess iodide and acid, and then injected into an eluent of sodium sulphite solution. The iodine concentration injected was constant, whereas the sulphite concentration was varied.
FIGURE 10.7: SIGNALS OBTAINED FOR CONSTRUCTING A CALIBRATION GRAPH FOR THE DETERMINATION OF SULPHITE USING IODATE/IODIDE ELUENT IN A DUAL-CHANNELS FLOW INJECTION MANIFOLD: SULPHITE CONCENTRATION A, 0; B, 4; AND C, 8 x 10^-4 M
FIGURE 10.8: TYPICAL CALIBRATION GRAPH OBTAINED FOR THE DETERMINATION OF SULPHITE USING IODATE/IODIDE ELUENT IN A DUAL-CHANNELS FLOW INJECTION MANIFOLD.
The loss of iodine signal when injected into sulphite is expected to be proportional to the concentration of sulphite in the eluent.

In contrast to classical titrimetry where the effect of oxygen is usually not taken seriously, this method requires deoxygenation of the reagents. This is so because iodine is formed photolytically from iodide in the presence of excess acid and dissolved oxygen. Therefore all reagents were sparged with nitrogen gas.

The detector response to iodine was investigated. At flow rate of 5 ml min\(^{-1}\) with 1m of 0.71 mm bore transmission tubing, the response of the detector to iodine was linear in the range 3 \(\times 10^{-5}\) - 4.5 \(\times 10^{-4}\)M \(I_2\). The results obtained are reported in Table 10.9.

<table>
<thead>
<tr>
<th>Iodate Concentration x (10^{-5})M</th>
<th>Peak Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.27</td>
</tr>
<tr>
<td>2.0</td>
<td>0.55</td>
</tr>
<tr>
<td>3.0</td>
<td>0.81</td>
</tr>
<tr>
<td>4.0</td>
<td>1.10</td>
</tr>
<tr>
<td>6.0</td>
<td>1.65</td>
</tr>
<tr>
<td>8.0</td>
<td>2.20</td>
</tr>
<tr>
<td>10.0</td>
<td>2.75</td>
</tr>
<tr>
<td>12.0</td>
<td>3.31</td>
</tr>
<tr>
<td>15.0</td>
<td>4.15</td>
</tr>
<tr>
<td>20.0</td>
<td>5.12</td>
</tr>
</tbody>
</table>
The dependence of the control iodine signal on the flow parameters is shown in Tables 10.10, 10.11 and 10.12.

**TABLE 10.10: Effect of transmission tube bore size on iodine signal**

<table>
<thead>
<tr>
<th>Transmission Tube Bore Size/mm</th>
<th>Peak Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.71</td>
<td>2.76</td>
</tr>
<tr>
<td>0.89</td>
<td>2.54</td>
</tr>
<tr>
<td>1.01</td>
<td>2.31</td>
</tr>
</tbody>
</table>

Flow rate: 5 ml/min  
Transmission tube length: 1 m  
Injected solution: 10^-4M in iodate, 0.2M in iodide and 0.8M in hydrochloric acid  
Eluent: distilled water

**TABLE 10.11: Effect of transmission tube length**

<table>
<thead>
<tr>
<th>Transmission Tube Length/m</th>
<th>Peak Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.76</td>
</tr>
<tr>
<td>2</td>
<td>1.75</td>
</tr>
<tr>
<td>3</td>
<td>1.30</td>
</tr>
<tr>
<td>4</td>
<td>1.12</td>
</tr>
</tbody>
</table>

Flow rate: 5 ml min^-1  
Transmission tube bore size: 0.71 mm  
Other parameters as in Table 10.10
TABLE 10.12: Effect of flow rate
Transmission tube: bore 0.71 mm, length 1.0m
Other parameters as in Table 10.10

<table>
<thead>
<tr>
<th>Flow rate/ml min(^{-1})</th>
<th>Peak Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.97</td>
</tr>
<tr>
<td>2</td>
<td>2.90</td>
</tr>
<tr>
<td>3</td>
<td>2.84</td>
</tr>
<tr>
<td>4</td>
<td>2.79</td>
</tr>
<tr>
<td>5</td>
<td>2.75</td>
</tr>
<tr>
<td>6</td>
<td>2.69</td>
</tr>
</tbody>
</table>

The results in Tables 10.10-10.12 show that the size of the iodine signal decreases as diameter and length of transmission tube and flow rate increase. This is due to dispersion which increases with length and diameter of transmission tubing and with flow rate. This is in contrast with the method in which iodine is formed on-line in the reverse FIA manner where peaks increased with transmission tubing length and bore size.

A recommended procedure is as follows: Pipette 10 ml of deoxygenated stock iodate solution into 100 ml calibrated flask and add 10 ml of deoxygenated 2M potassium iodide solution. Dilute to about 50 ml and add 20 ml of deoxygenated 4M HCl solution, dilute to volume with deoxygenated distilled water, mix, and inject 75 μl into an eluent consisting of distilled water using 1m of 0.71 mm bore transmission tubing with a flow rate of 2-5 ml min\(^{-1}\). Repeat this procedure with sodium sulphite eluent in the concentration range of 0-2 x 10\(^{-4}\)M.

Typical signals obtained for a calibration graph for the determination of sulphite are shown in Figure 10.9 for a flow rate of 2 ml/min. A typical calibration graph obtained using the recommended conditions is shown in Figure 10.10.
FIGURE 10.9: SIGNALS OBTAINED FOR CONSTRUCTING A CALIBRATION GRAPH FOR THE DETERMINATION OF SULPHITE USING SULPHITE AS ELUENT; SULPHITE CONCENTRATION A, 0; B, 1; AND C, $1.5 \times 10^{-4}$ M
FIGURE 10.10: TYPICAL CALIBRATION GRAPH FOR THE DETERMINATION OF SULPHITE USING SULPHITE AS ELUENT
CONCLUSION

Dispersion values were obtained for different flow parameters for methods in which the monitorand is dispersing in the normal and in the reverse flow injection analysis manners.

Two methods have been developed here by which sulphite can be determined iodimetrically on-line. In the first method acidic sulphite solutions were injected into an iodate/iodide eluent. In the second method off-line preformed iodine solutions of constant concentration were injected into an eluent of sodium sulphite. In the first method iodine is formed in the reverse FIA manner which makes the optimising of the method to obtain well-shaped peaks more difficult. The second method has the advantages that a lower detection limit can be detected, and that, by changing the iodate concentration in the preformed iodine solution, the calibration graphs can be extended.

The methods described here are used to determine sulphite at levels below those which are normally titrated and are therefore more versatile than the titrimetric method. A difficulty that becomes more apparent when working with lower concentrations is the photolytic oxidation of iodide to iodine by dissolved oxygen especially in acidic solutions. The first method has the advantage that the iodine is formed on-line and monitored within seconds which minimizes this problem.
References

4. Idem, ibid, 9 (1975) 969.
Previously, a simple wall jet detector cell which can be used either with a solid electrode or with a sessile mercury drop electrode was constructed in this laboratory. The performance of the detector cell when used with sessile mercury drop electrodes was investigated here. It was found to be particularly useful at low negative and low positive potentials where oxygen is not reduced and therefore does not interfere, or at more negative potentials in solutions of pH 6-9 where sulphite can be added to remove oxygen traces. This was illustrated by the determination of nitrofurantoin at -0.7V in pH 7.5 with added sulphite. The calibration graph was rectilinear over the range 5-50 µg ml⁻¹ and the detection limit was 0.3 µg ml⁻¹. However, in determining acetazolamide at -0.85V in acidic medium where sulphite has no effect on oxygen, the detection limit obtained was 7 µg ml⁻¹. Phosphate, arsenate and germanate were determined as heteropoly acids at a sessile mercury drop electrode held at +0.22V, +0.23V and +0.18V respectively, without deoxygenation of the eluent or the sample solutions. They can be determined at 5 x 10⁻⁶, 2 x 10⁻⁷ and 1 x 10⁻⁶M respectively. Previous workers had shown that germanium could not be determined at a glassy carbon electrode owing to adsorption and that arsenate could only be determined down to 10⁻⁵M level owing to deterioration of the baseline. Phosphate was also determined by monitoring the catalytic reduction of hydrogen peroxide in the presence of Mo(VI) which came from the dissociation of phosphomolybdic acid. The calibration graph was rectilinear over the range 5-100 ng ml⁻¹, but a loss of signal was observed.

Catecholamines and ascorbic acid when oxidized at a newly polished glassy carbon electrode showed poorly-defined and irreproducible voltammograms. Electrochemical pretreatment of a glassy carbon electrode by holding it at about +2.0V for 2 mins and then at about -1.0V for 2 mins was found to improve the shape of the voltammograms and give more reproducible and higher peaks for the determination of
ascorbic acid, dopamine, dopac, HVA, NE and 5 HIAA at positive potentials. A remarkable shift of the peaks potential to less positive values and more reversible cyclic voltammograms were also obtained with a pretreated electrode. The voltammetric differentiation of AA and DA or AA and DOPAC was possible as separate peaks were obtained for either AA and DA, or AA and DOPAC at a pretreated glassy carbon electrode. In the determination of HVA at a pretreated electrode a new peak had appeared on the second and subsequent scans. This new peak was not observed when several scans were applied to an untreated electrode. This means that different oxidation products are produced at a pretreated and untreated electrode.

On-line determination of ascorbic acid, dopamine and dopac is improved with the use of a pretreated glassy carbon electrode. Higher and more reproducible signals were obtained, and a shift of the hydrodynamic voltammograms to less positive potentials than with a newly polished electrode was also obtained. However, the increase in sensitivity with pretreating the electrode was higher in flow injection experiments than in static mode experiments.

As the behaviour of commercial glassy carbon electrodes deteriorate with use due to the leakage of electrolytes into the thin void between the glassy carbon and the electrode housing, several methods have been described for the construction of leak-tight electrodes. The methods are simple and inexpensive. The performance of the electrodes prepared was satisfactory and favourably comparable to the commercially available electrodes tested; however, the laboratory constructed electrodes were more durable than the commercial electrodes.

A novel method was used for the electrochemical pretreatment of glassy carbon electrodes in which the electrode was held at -3V for 1 min in 0.1M sulphuric acid. This pretreatment was found to inhibit the reduction of oxygen to some extent and to allow determinations at glassy carbon electrodes to be made at about -0.65V without the need
to deoxygenate the sample solutions. This was illustrated by the on-line determination of nitrofurantoin. The pretreatment was shown to improve the detection limit, to reduce the noise level and to make the deoxygenation of the sample solutions unnecessary. This pretreatment is particularly useful for HPLC and FIA where deoxygenation of the sample solutions is time consuming and difficult. A flow injection amperometric method was obtained for the determination of vitamin K₃ in pH 3.8 acetate buffer at -0.6V without the need to deoxygenate the sample solutions. However, an attempt to determine vitamin K₁ in 90% methanolic acetate buffer at a pretreated electrode was unsatisfactory because of the presence of methanol which was found to weaken the effect of the cathodic pretreatment. Further work is needed to investigate the effect of cathodic pretreatment on the electrode surface, and in applying this pretreatment to reductive HPLC determinations.

Dispersion values were obtained for different flow parameters for methods in which the monitorand is dispersing in the normal and in the reverse FIA manners spectrophotometrically by monitoring iodine at 352 nm. Spectrophotometric methods for the determination of sulphite were described also. In the first method acidic sulphite solutions were injected into an iodate/iodide eluent. In the second method off-line preformed iodine solutions of constant concentrations were injected into an eluent of sodium sulphite. The flow parameters were optimized to obtain the optimum conditions for each method.