

ELECTROANALYSIS AT ACTIVATED ELECTRODES

**A THESIS SUBMITTED FOR THE
DOCTOR OF PHILOSOPHY
IN CHEMISTRY**

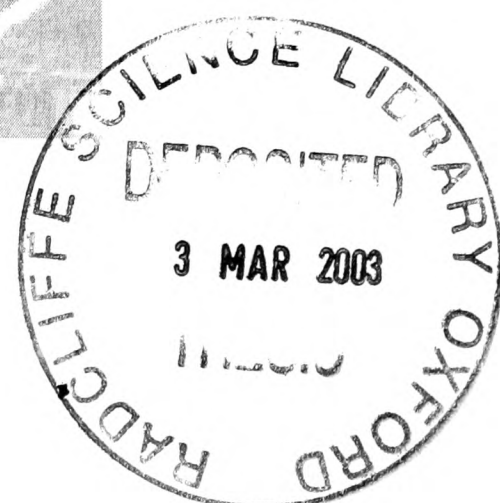
BY

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ST. JOHN'S COLLEGE

OXFORD UNIVERSITY

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ABSTRACT

ELECTROANALYSIS AT ACTIVATED ELECTRODES

A Thesis Submitted for the Degree of D. Phil. in Chemistry

by Matthew J. Moorcroft, St. John's College, Oxford University,

Michaelmas Term 2002.

This thesis details advances made within the field of electroanalytical chemistry through the use of working electrodes that have been activated through application of ultrasound, heat, geometry, chemical modification or composition.

Initially the thesis reports the enhanced analytical utility of chemically and compositionally modified working electrodes when directed towards the detection and determination of NO_3^- and NO_2^- anions in environmental samples. This has been achieved through the use of electrodes that have been a) modified with a Cu deposit and b) fashioned from a Cu-Ni alloy. Nitrate and nitrite anions have been successfully determined in a variety of passivating matrices, at analytically relevant detection limits of the order of 10^{-6} M with a dynamic linear range extending from 10 to 200 μM . The methods presented have been shown to surpass existing electrochemical techniques in terms of nitrate/nitrite speciation through separation of the voltammetric signals, where existing analyses have reported the interference of both species when present in the same solution. The use of ultrasound as a further enhancement to the sensitivity and versatility of the electrochemical detection of nitrate at a chemically modified electrode is then presented. The influence of ultrasound is shown to remove a portion of the deposited copper, but a significant catalytic layer remains, resulting in greater sensitivity during insonation.

The effect of temperature on electrochemical systems involving one- and two-electron redox reactions of $\text{K}_4\text{Fe}(\text{CN})_6$, $\text{Ru}(\text{NH}_3)_6\text{Cl}_3$, $\text{Fe}(\text{C}_5\text{H}_5)_2$, N,N,N',N'tetramethylphenylenediamine, N,N'dimethylphenylenediamine and tris(4-bromophenyl)amine have been studied under hydrothermal conditions using a novel hydrodynamic method based on a conventional channel flow cell where the working electrode is heated by radio frequency radiation. The diffusion activation parameters obtained with the radio frequency channel cell and computer simulation were compared

ABSTRACT

with independent data from microelectrode high temperature experiments. The application of the heated flow cell as a tool for mechanistic studies is discussed with the investigation of the well characterised ECE reaction of m-iodo-nitrobenzene in acetonitrile, giving a value of $80 \pm 5 \text{ kJ mol}^{-1}$ for the activation energy of the rate constant for the decomposition of the m-iodo-nitrobenzene radical anion. This represents the first observation of an ECE or mechanistically complex reaction at a locally heated electrode.

The work presented in the final two chapters of this thesis examines the enhanced activation achieved from modification of the electrode geometry, and in particular the application of microelectrodes to the development of electroanalytical techniques. The electrochemical reduction of the inhalation anaesthetic agent enflurane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether) is reported at a variety of microelectrode substrates (Au, Ag, Cu, Pt and glassy carbon) with electrode dimensions varying from 5 to 60 μm . The solvents water, dimethylsulfoxide and acetonitrile were investigated along with the supporting electrolytes potassium chloride, tetrabutylammonium hexafluorophosphate and various tetraalkylammonium perchlorates. The use of a gold microelectrode with dimethyl sulfoxide solvent and tetraethylammonium perchlorate as the supporting electrolyte was found to give well-defined voltammetry. Linear calibration curves were obtained between 0 and 2 % v/v (gaseous additions) or up to 135 mM (gravimetric additions), offering scope for the development of a rapid, inexpensive electrochemical gas sensor. The analytical utility of the system has been investigated in the presence of oxygen and nitrous oxide in DMSO solvent. The superoxide anion radical, formed from the electro-reduction of dissolved oxygen, is shown to react with enflurane complicating their simultaneous detection. The kinetics of the enflurane / superoxide reaction are found to be first order with respect to both superoxide and enflurane with a rate constant of $0.25 \text{ M}^{-1} \text{ s}^{-1}$ determined by three independent methods: steady-state voltammetry, digital simulation of cyclic voltammetric data and UV/Vis spectroscopic analysis.

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CONTENTS

Abstract	I
-----------------------	---

Acknowledgements	III
-------------------------------	-----

CHAPTER 1 – Introduction

1.1	General Electrochemistry.....	1
1.1.1	Equilibrium Redox Potentials.....	1
1.1.2	Thermodynamics.....	3
1.1.3	The Standard Hydrogen Electrode (SHE).....	5
1.1.4	Formal Potentials.....	6
1.1.5	Interfacial Phenomena.....	8
1.2	Dynamic Electrochemistry.....	9
1.2.1	Notation for Electrochemical Processes.....	10
1.2.2	Electron Transfer Kinetics.....	10
1.2.3	Mass Transport.....	15
1.2.3.1	Diffusion.....	15
1.2.3.2	Convection.....	16
1.2.3.3	Migration.....	17
1.2.4	Potential Sweep Methods.....	18
1.2.4.1	Linear Sweep Voltammetry.....	18
1.2.4.2	Cyclic Voltammetry.....	21
1.2.4.3	Square Wave Voltammetry.....	22
1.2.4.4	Potential Step Chronoamperometry.....	24
1.3	Hydrodynamic Voltammetry.....	26
1.3.1	Diffusion Layer.....	26
1.3.2	Rotating Disc Electrodes.....	28
1.3.3	Channel Flow Cells.....	30
1.3.4	Microelectrodes.....	35

1.4	Ultrasound.....	37
1.4.1	Physical Effects of Ultrasound.....	38
1.4.2	Sonochemistry.....	42
1.4.3	Sonoelectrochemistry.....	45
1.4.3.1	Sonoelectrochemical Cells.....	46
1.4.3.2	Mass Transport I - Acoustic Streaming.....	48
1.4.3.3	Mass Transport II - Microjetting.....	50
1.4.3.4	Surface Activation.....	52
1.5	Aims of the Work Reported in this Thesis.....	52
1.6	Chapter 1 References.....	54

CHAPTER 2 – Experimental Methods

2.1	Chemicals.....	60
2.1.1	Solution Preparation.....	61
2.2	Electrochemical Experiments.....	61
2.2.1	Control Electronics.....	61
2.2.2	Working Electrodes.....	61
2.2.3	Reference Electrodes.....	62
2.3	Sonoelectrochemical Apparatus.....	63
2.3.1	Sonoelectrochemical Cells.....	63
2.3.2	Power Calibration.....	64
2.4	RF-Heated Channel flow Cell.....	65
2.5	Heated Microelectrode Cell.....	66
2.6	Anaesthetic Gas Experiments.....	67
2.7	Scanning Electron Microscopy (SEM).....	68
2.8	Atomic Force Microscopy (AFM).....	69
2.9	Chapter 2 References.....	70

CHAPTER 3 – Detection of Nitrate and Nitrite: - a Review

3.1	Introduction.....	72
3.1.1	Background Perspective.....	74
3.2	Detection Methodologies.....	76
3.2.1	Spectroscopic Detection.....	78
3.2.2	Electrochemical Detection.....	84
3.2.3	Chromatography.....	88
3.2.4	Capillary Electrophoresis.....	90
3.3	Conclusions.....	91
3.4	Tables.....	92
3.5	Chapter 3 References.....	97

CHAPTER 4 – Electrochemical Detection of Nitrate and Nitrite at a Copper Modified Electrode

4.1	Introduction.....	107
4.2	Experimental.....	109
4.2.1	Deposition of Copper Film.....	110
4.2.2	Electroanalytical Procedure.....	110
4.2.3	Spectroscopic Assay of Sewage Outfall Water.....	111
4.3	Results and Discussion.....	112
4.3.1	Analytical Performance.....	116
4.4	Conclusions.....	119
4.5	Chapter 4 References.....	119

CHAPTER 5 – Electrochemical Detection of Nitrate and Nitrite at a Cu-Ni electrode

5.1	Introduction.....	121
5.2	Experimental.....	122

5.3	Results and Discussion.....	123
5.4	Conclusions.....	128
5.5	Chapter 5 References.....	128

CHAPTER 6 – Ultrasonically enhanced detection of Nitrate and Nitrite at a Copper Modified Electrode

6.1	Introduction.....	129
6.2	Experimental.....	131
6.2.1	Deposition of Copper Film.....	132
6.2.2	Sonoelectrochemical Measurements.....	132
6.2.3	Detection of Nitrate in Sewage Effluent.....	132
6.2.4	Spectroscopic Assay of Sewage Outfall Water.....	133
6.3	Results and Discussion.....	133
6.3.1	Analytical Performance.....	139
6.4	Conclusions.....	141
6.5	Chapter 6 References.....	141

CHAPTER 7 – High Temperature Electrochemical Studies Using a Channel Flow Cell Heated by Radio Frequency Radiation

7.1	Introduction.....	143
7.2	Experimental.....	144
7.3	Mass and Heat Transport.....	146
7.3.1	Radio Frequency Channel Cell.....	146
7.3.2	High Temperature Microelectrode Cell.....	148
7.4	Results and Discussion.....	148
7.5	Conclusions.....	157
7.6	Chapter 7 References.....	157

CHAPTER 8 – Non-Aqueous Electrochemical Studies at a High Temperature Channel Flow Cell Heated by Radio Frequency Radiation

8.1	Introduction.....	159
8.2	Experimental.....	160
8.3	Mass and Heat Transport.....	161
8.3.1	RF Channel Cell.....	161
8.3.2	Microelectrode Cell.....	163
8.4	Results and Discussion.....	164
8.4.1	Microelectrode Results.....	164
8.4.2	RFCC Results.....	166
8.4.3	ECE Reaction of m-Iodonitrobenzene.....	170
8.5	Conclusions.....	172
8.6	Chapter 8 References.....	173

CHAPTER 9 – Electrochemical Reduction of the Anaesthetic Gas Enflurane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether)

9.1	Introduction.....	174
9.2	Experimental.....	177
9.3	Results and Discussion.....	178
9.3.1	Reduction of Enflurane in DMSO at Au Microelectrodes.....	181
9.3.2	Solvent Effects.....	183
9.3.3	Role of the Electrode Surface.....	185
9.3.4	Role of the Supporting Electrolyte.....	190
9.4	Conclusions.....	190
9.5	Chapter 9 References.....	190

CHAPTER 10 – Electrochemical Studies of the Anaesthetic Gas Enflurane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether) in the Presence of Oxygen; Reaction with Electrogenerated Superoxide

10.1	Introduction.....	194
10.2	Experimental.....	197
10.3	Results and Discussion.....	199
10.3.1	Simultaneous Electrochemical Reduction of Oxygen and Enflurane at Au Microdisc Electrodes in DMSO.....	199
10.3.2	Determination of the Rate Constant for the Reaction of Superoxide with Enflurane in DMSO: UV/Vis Spectroscopic Study.....	204
10.3.3	Application of the Rate Constant for the Reaction of Superoxide with Enflurane in DMSO Analysis of Steady-State Voltammetry at Gold Microelectrodes.....	207
10.3.4	Determination of the Rate Constant for the Reaction of Superoxide with Enflurane in DMSO: Digital Simulation of Cyclic Voltammetry.....	210
10.3.5	Quantitative Sensing Strategy for Binary Mixtures of Enflurane and Oxygen.....	213
10.3.6	Electrochemical Reduction of Nitrous Oxide in the Presence of Oxygen.....	214
10.4	Conclusions.....	217
10.5	Chapter 10 References.....	218

APPENDIX A - FICK'S LAWS.....	221
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APPENDIX B - DETECTION LIMITS.....	224
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APPENDIX C - WILKE-CHANG.....	226
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CHAPTER 1 - INTRODUCTION

This thesis concerns itself with studies undertaken in the area of electroanalysis using electrodes that have been activated through the use of chemical modification, ultrasound, high temperature or electrode geometry. This chapter first aims to provide the necessary background in both the fundamentals of electrochemistry and the principles of electrochemical techniques to ensure a sound base for the experimental work presented herein. Initial attention is drawn to the area of equilibrium electrochemistry and then moves on to discuss the more related aspects of dynamic systems including fast mass transport effects observed through the use of hydrodynamic electrodes or through the use of ultrasound. The introduction presented in this thesis aims only to present an overview of the fundamental aspects of electrochemistry, and wider discussions can be readily found in the various texts [1-6].

1.1 GENERAL ELECTROCHEMISTRY

Electrochemistry can be defined as the study and the exploitation of charge transfer processes across an interface and through a solution. The storage of electricity in batteries, the production of nylon, electroplating, corrosion, nerve action and material transfer across biological membranes are all attributed to electrochemical phenomena. The field of quantitative electrochemistry was established in 1833 by Michael Faraday with the publication of the laws of electrochemical conversion and are widely accepted as the foundations on which modern electrochemistry is based.

1.1.1. EQUILIBRIUM REDOX POTENTIALS

Consideration of the situation where a metal is in contact with a solution of its ions, leads to the establishment of an equilibrium, as shown below.



The scheme shown in equation (1.1) predicts that as charge is passed between the metal and the solution phase, a potential difference that is a function of the position of the equilibrium, will result. This is schematically depicted in figure 1.1 showing the electron energetics at and prior to equilibrium.

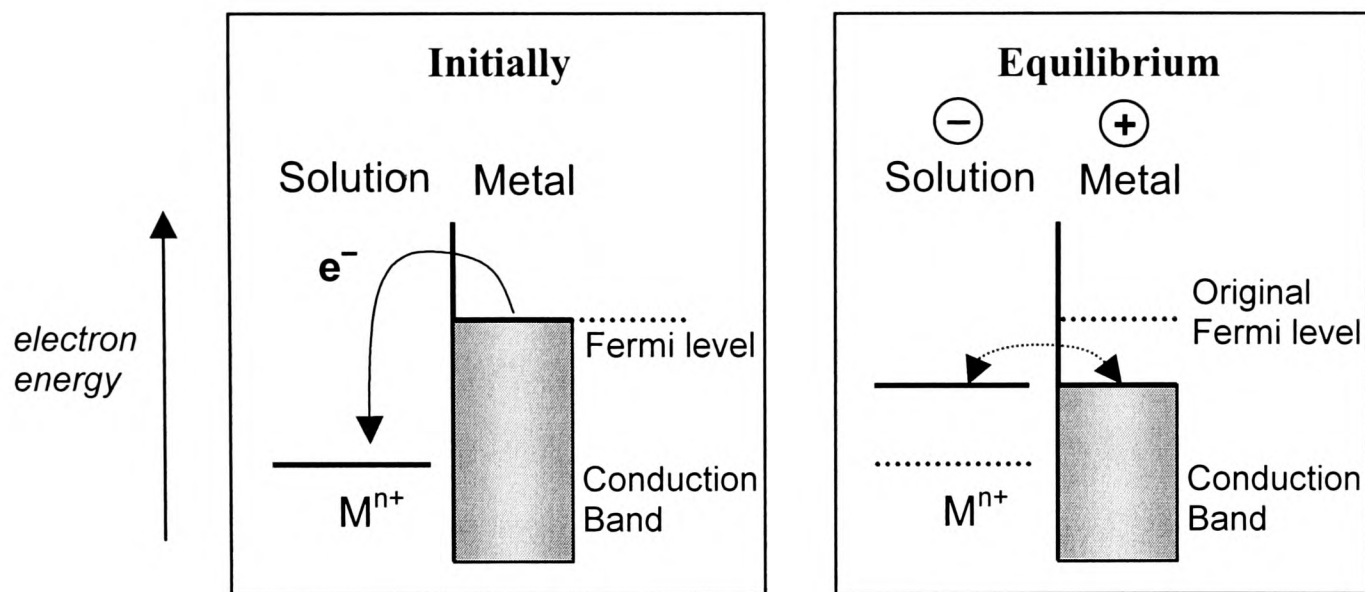


Figure 1.1 - Change in electron energies as a function of equilibrium. Adapted from reference 4.

If equilibrium is rapidly established, the potential difference (electrode potential) can be measured by means of a potentiometer device in comparison with another (reference) electrode. The electrode potential is defined as the work done by a coulomb of positive charge when moved from a position infinitely distant from the electrode and brought immediately close to it. The electrode potential and solution potential are denoted by the terms ϕ_M and ϕ_S respectively.

Considering the situation as represented in equation (1.1), as equilibrium is established the electrochemical potentials on either side must be equal, thus [3]

$$\overline{\mu}_{M^{n+}} + \overline{\mu}_e = \overline{\mu}_M \quad (1.2)$$

where $\overline{\mu}_{M^{n+}}$, $\overline{\mu}_e$ and $\overline{\mu}_M$ are the electrochemical potentials of the metal ions, electron and metal respectively. The electrochemical potential of each species 'i' ($\overline{\mu}_i$) in a given phase is equated by the standard chemical potential of the species (μ_i^0), the activity (a_i), the amount of charge (z_i) and the potential of each phase (ϕ)

$$\overline{\mu}_i = \mu_i^0 + RT \ln a_i + z_i F \phi \quad (1.3)$$

where R is the universal gas constant, F is the Faraday constant and T is the temperature. Substituting equation (1.3) for each phase into (1.1) and using the expression

$$\Delta\phi = \phi_{Electrode} - \phi_{Solution} \quad (1.4)$$

where $\Delta\phi$ represents the change in potential, gives the following relationship

$$\Delta\phi = \Delta\phi^O + \frac{RT}{nF} \ln a_{M^{n+}} \quad (1.5)$$

where the term $\Delta\phi^O$ represents the standard electrode potential. Equation (1.5) is familiar to electrochemists as the Nernst equation, and is usually expressed in a more general form using the notation



giving

$$E = E^O + \frac{RT}{nF} \ln \frac{(a_{Ox})^o}{(a_{Red})^r} \quad (1.7)$$

1.1.2 THERMODYNAMICS

Thermodynamic insights can be readily derived from electrochemical measurements [3,4]. Measurement of the electrode potential can quantify the change in free energy (ΔG) associated with a redox couple using the relationship outlined in equation (1.8)

$$\Delta G = -nFE \quad (1.8)$$

If the cell components are in their standard state and have unit activity, then the standard free energy change (ΔG^O) is given by

$$\Delta G^O = -nFE^O \quad (1.9)$$

Furthermore, in a closed system in the absence of non-expansive work and at constant composition

$$dG = V dP - S dT \quad (1.10)$$

thus

$$\left(\frac{\partial G}{\partial T}\right)_P = -S \quad \text{and hence} \quad \left(\frac{\partial \Delta G}{\partial T}\right)_P = -\Delta S \quad (1.11)$$

which shows how the Gibbs energy varies with the effect of temperature. Substitution of equation (1.11) into (1.8) and (1.9) yields

$$F\left(\frac{\partial E}{\partial T}\right)_P = \Delta S \quad \text{and} \quad F\left(\frac{\partial E^o}{\partial T}\right)_P = \Delta S^o \quad (1.12)$$

When combining the relevant terms in equation (1.12) with the expression for the change in enthalpy

$$\Delta H^o = \Delta G^o + T\Delta S^o \quad (1.13)$$

the relationship shown in equation (1.14) is derived.

$$\Delta H^o = -nFE^o + TF\left(\frac{\partial E^o}{\partial T}\right)_P \quad (1.14)$$

Equation (1.14) explains how the enthalpy and entropy of an electrochemical reaction at equilibrium can be readily obtained from the cell potential and its variation with temperature.

A second, more facile derivation of the Nernst equation (1.7) can be presented on thermodynamic grounds [3]. The free energy change for a chemical reaction can be written as the van't Hoff equation (1.15)

$$\Delta G = \Delta G^o + RT \ln \frac{\prod(a_{\text{products}})}{\prod(a_{\text{reactants}})} \quad (1.15)$$

Therefore, using the electrode reaction as written in equation (1.6) takes the form

$$\Delta G = \Delta G^{\circ} + RT \ln \frac{(a_{\text{Red}})^r}{(a_{\text{Ox}})^o} \quad (1.16)$$

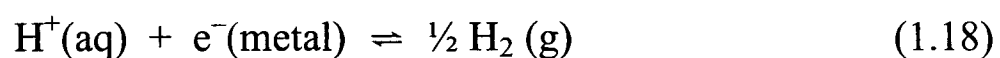
Substitution of equations (1.8) and (1.9) lead to the formation of equation (1.17)

$$nEF = nE^{\circ}F + RT \ln \frac{(a_{\text{Ox}})^o}{(a_{\text{Red}})^r} \quad (1.17)$$

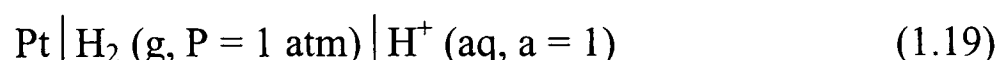
which, when divided by nF throughout, leads to the Nernst equation (1.7) obtained earlier through the consideration of electrochemical potentials.

1.1.3 THE STANDARD HYDROGEN ELECTRODE (SHE)

By convention, electrode potentials are quoted for the half-cell of interest against a chosen reference. A good reference must exhibit a potential that is stable as a function of time and temperature and which is not altered by the passage of a small current. The standard hydrogen electrode (SHE) or normal hydrogen electrode (NHE) is the reference of choice and has been used to define the standard electrode potential scale, readily quoted in most electrochemical literature. The electrode is based on the reaction shown in equation (1.18)



with the corresponding electrode half-cell reaction as ascribed in equation (1.19)

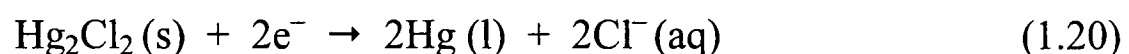


and operates under the following standardised conditions of 1 atmosphere pressure, unity proton activity ($a_{\text{H}^+} = 1$) and a constant cell temperature of 25.0 °C. Under these conditions, the SHE is defined to have a standard electrode potential (E°) equal to 0.00 V.

The reference is fabricated from a platinised platinum electrode (platinum black) to ensure fast electrode kinetics. Various procedures for platinisation exist and normally involve the deposition of platinum black from a solution of 3 % chloroplatinic acid

(H₂PtCl₆) containing a small quantity of lead acetate (0.005 %) to prolong electrode life. When such construction methods are employed, high reproducibility can be obtained, showing differences of only 10 μV between different hydrogen electrodes [2]. The purpose of depositing a fine layer of platinum black onto the electrode surface provides catalytic sites which ensure that the equilibrium shown in equation (1.18) is rapidly established. In contrast, using unmodified platinum electrodes result in sluggish electrode kinetics and the desired electrode potentials may not be achieved [4].

In practice, from an experimental standpoint at least, the use of the SHE is limited by its procedural inconvenience. The more widely used reference electrode by practical electrochemists is the saturated calomel electrode (SCE) and consists of the following electrochemical reaction and half-cell shown in equation (1.20) and (1.21) respectively.



with a standard electrode potential (relative to the SHE) equal to 0.242 V.

1.1.4 FORMAL POTENTIALS

The concentration of a species in solution can be related to its activity by equation (1.22) where a_i is the activity of species i , $[i]$ is the concentration and γ_i is the activity coefficient.

$$a_i = \gamma_i [i] \quad (1.22)$$

The interionic interactions that exist in relatively concentrated solutions are not present in dilute solutions, due to the large interionic distances involved. As a consequence of this, ion migration velocities (or ion momenta) are changed bringing about an increase or decrease in the activity. As a result activity coefficients are dependent upon the solution concentration. The use of activities is stipulated by the Nernst equation (1.7). However, in practice, this is usually an inconvenience as activity coefficients are often unknown [1].

The use of activity can be avoided through the use of concentrations and the term formal potential, $E^{\circ'}$, as shown in the example below using the $\text{Fe}^{3+} / \text{Fe}^{2+}$ redox couple.



The variation of cell potential with activities is stated by the Nernst equation as shown for the $\text{Fe}^{3+} / \text{Fe}^{2+}$ redox couple in equation (1.24) where $n = 1$.

$$E = E^{\circ} + \frac{RT}{F} \ln \frac{a_{\text{Fe}^{3+}}}{a_{\text{Fe}^{2+}}} \quad (1.24)$$

Substituting the relationship outlined in equation (1.22), this leads to

$$E = E^{\circ} + \frac{RT}{F} \ln \frac{\gamma_{\text{Fe}^{3+}} [\text{Fe}^{3+}]}{\gamma_{\text{Fe}^{2+}} [\text{Fe}^{2+}]} \quad (1.25)$$

By defining the relationship for the formal potential, $E^{\circ'}$, in terms of E°

$$E^{\circ'} = E^{\circ} + \frac{RT}{F} \ln \frac{\gamma_{\text{Fe}^{3+}}}{\gamma_{\text{Fe}^{2+}}} \quad (1.26)$$

equation (1.27) can be derived

$$E = E^{\circ'} + \frac{RT}{F} \ln \frac{[\text{Fe}^{3+}]}{[\text{Fe}^{2+}]} \quad (1.27)$$

The value of $E^{\circ'}$ will vary from medium to medium depending on the ionic strength. For example, the $E^{\circ'}$ values the $\text{Fe}^{3+} / \text{Fe}^{2+}$ redox couple vary from 0.70 V (vs. SHE) in 1 M HCl to 0.53 V in 10 M HCl [7].

The relation shown in equation (1.26) shows how standard electrode potential data can be readily obtained from plots of the formal potential with varying solution ionic strength, through extrapolation to zero ionic strength where the activity coefficients approach unity.

1.1.5 INTERFACIAL PHENOMENA - ELECTRIC DOUBLE LAYER

The interface between two conducting phases (e.g. the electrode and electrolyte) has a potential difference associated with it, that is a function of both the composition and nature of each phase. This potential difference is a result of the electrical double layer, arising from an excess of charges (either ions, electrons or oriented dipoles) residing at the interface. In the past hundred years, a number of scientists have taken on the challenge of devising a model of the electrode|electrolyte double layer to accurately explain observed experimental results. In 1879, Helmholtz proposed the first double layer model where positive and negative charges were ordered in a rigid fashion on the two sides of the interface, comparing the problem to that of a parallel plate capacitor [8]. Further modifications of this initial model were proposed by Gouy [9], Chapman [10], Stern [11], Grahame [12] and more recently in 1963, Bockris, Devanathan and Muller [13].

A schematic of the currently accepted model of the double layer by Bockris et al is shown in figure 1.2.

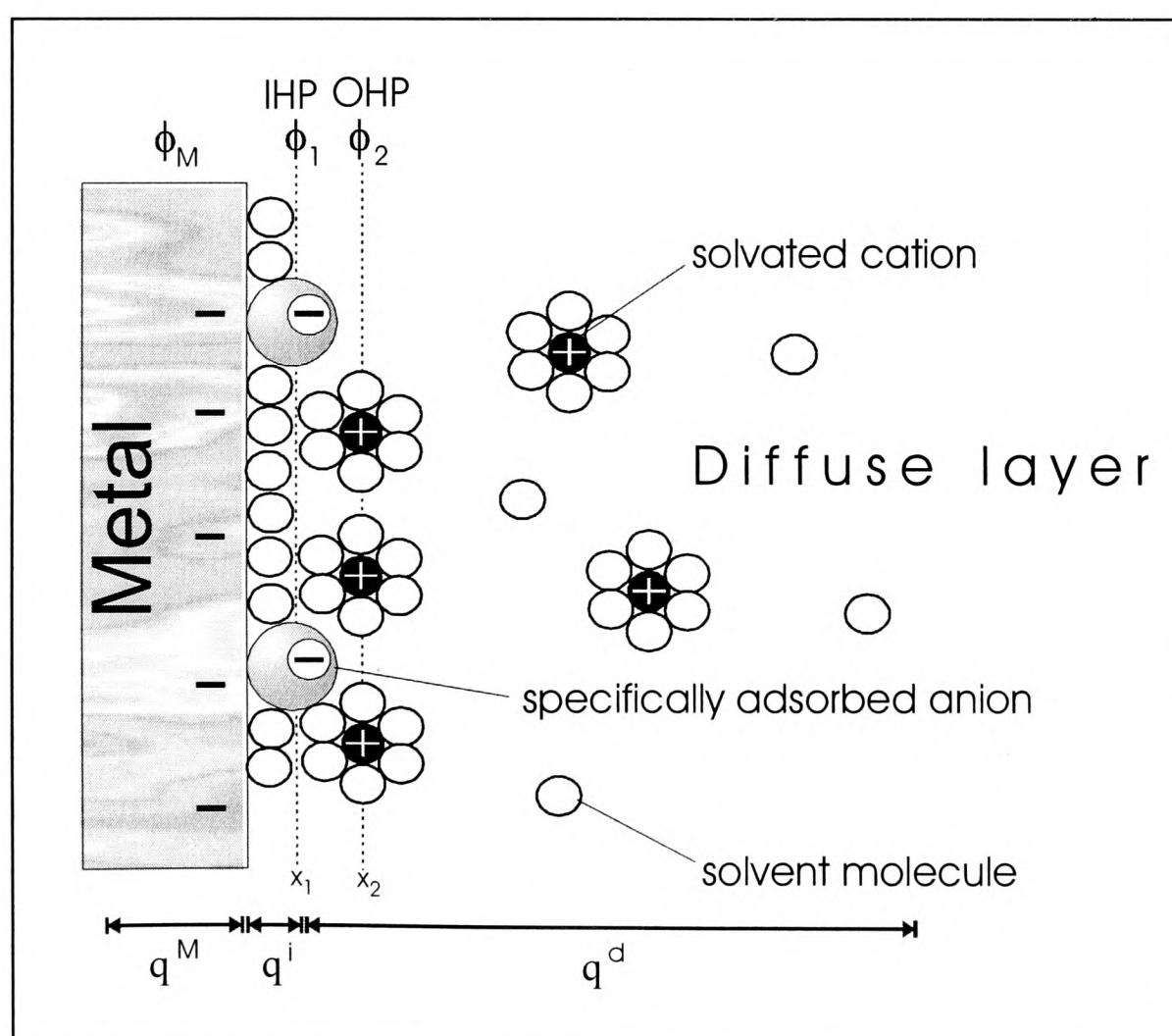


Fig. 1.2 - Proposed model of double layer region showing specifically adsorbed anions and solvated cations. Adapted from reference 1.

The solution component of the double layer is thought to consist of several, more discreet layers. The inner layer, known as the compact, Helmholtz or Stern layer is that closest to the electrode surface and consists of specifically absorbed species, such as solvent molecules or ions. The distance x_1 indicates the locus of the centres of the ions that are specifically absorbed and is referred to as the inner Helmholtz plane (IHP). In addition, x_2 corresponds to the closest distance of approach that can be made by the solvated ions and is known as the outer Helmholtz plane (OHP). The interaction of the solvated ions with the electrode surface involves only long-range electrostatic forces, independent of the chemical properties of the ions, and are thus said to be nonspecifically absorbed. These ions are distributed in a three-dimensional arrangement, extending from the OHP to bulk solution, referred to as the diffuse layer. The thickness of this diffuse layer is dependent on the total ionic concentration in solution; diffuse layer thicknesses of < 100 Å are reported for concentrations greater than 0.01 M [1].

From figure 1.2, the charge on the metal (q^M) represents an excess or deficiency of electrons and resides in a very thin layer (< 0.1 Å) on the metal surface. This quantity is said to be equal in magnitude, but opposite in sign, to the sum of charges accumulated between the region of the metal and IHP (q^i) and from the IHP extending to the diffuse layer (q^d).

1.2 DYNAMIC ELECTROCHEMISTRY

This chapter now moves on to discuss more relevant electrochemical systems that are not at equilibrium. In such systems a current is forced through the solution and electron transfer reactions occur and it is this theory that underpins most of the experimental work presented in this thesis. In this section, the kinetics of these electrode processes are first discussed followed by the overview of the varying techniques used for the interrogation of electrochemical systems. The final part to this chapter provides a discussion about the coupling of high mass transport with electroanalytical techniques in an attempt to not only develop novel methods of analysis, but to also provide mechanistic and kinetic insights into electrochemical processes.

1.2.1 NOTATION FOR ELECTROCHEMICAL PROCESSES

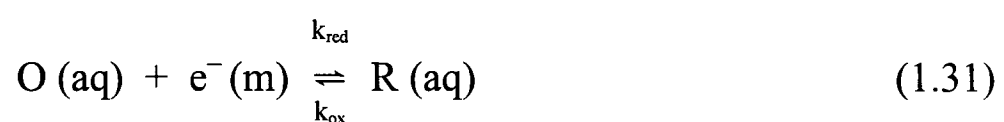
The E and C notation introduced by Reinmuth and Testa in 1961 is a convenient form of mechanistic nomenclature denoting (heterogeneous) electron transfer steps as 'E' and homogenous (chemical) steps as 'C' [14]. This is illustrated below in equations (1.28)-(1.30) denoting a general ECE mechanism.



In this example, species A undergoes an electron transfer at the electrode surface forming species B (E-step). This then chemically reacts homogeneously to form C (C-step), which can undergo a successive electron transfer at the electrode surface (E-step) forming the reaction products. It is this nomenclature that has been adopted throughout this thesis.

1.2.2 ELECTRON TRANSFER KINETICS

Consider the simple case outlined below in equation (1.31) where two chemical species are interconverted by a single electron transfer process at the electrode surface.



The current for the forward (reductive), I_c , and reverse (oxidative), I_a , processes can be written as

$$I_c = F A k_{\text{red}} [\text{O}]_o \quad (1.32)$$

$$I_a = F A k_{\text{ox}} [\text{R}]_o \quad (1.33)$$

where A is the electrode area, k_{red} and k_{ox} are the rate constants for the electron transfer and finally $[\text{O}]_o$ and $[\text{R}]_o$ are the concentrations of species O and R at the electrode surface. For the reaction, the net current flowing (I) is a sum of the anodic and cathodic contributions, hence

$$I = I_a + I_c \quad \text{thus} \quad I = FA(k_{ox}[R]_o - k_{red}[O]_o) \quad (1.34)$$

In an analogous fashion to chemical rate processes, we can consider the electron transfer reactions to behave in light of the Arrhenius equation and the transition state model, shown in figure 1.3.

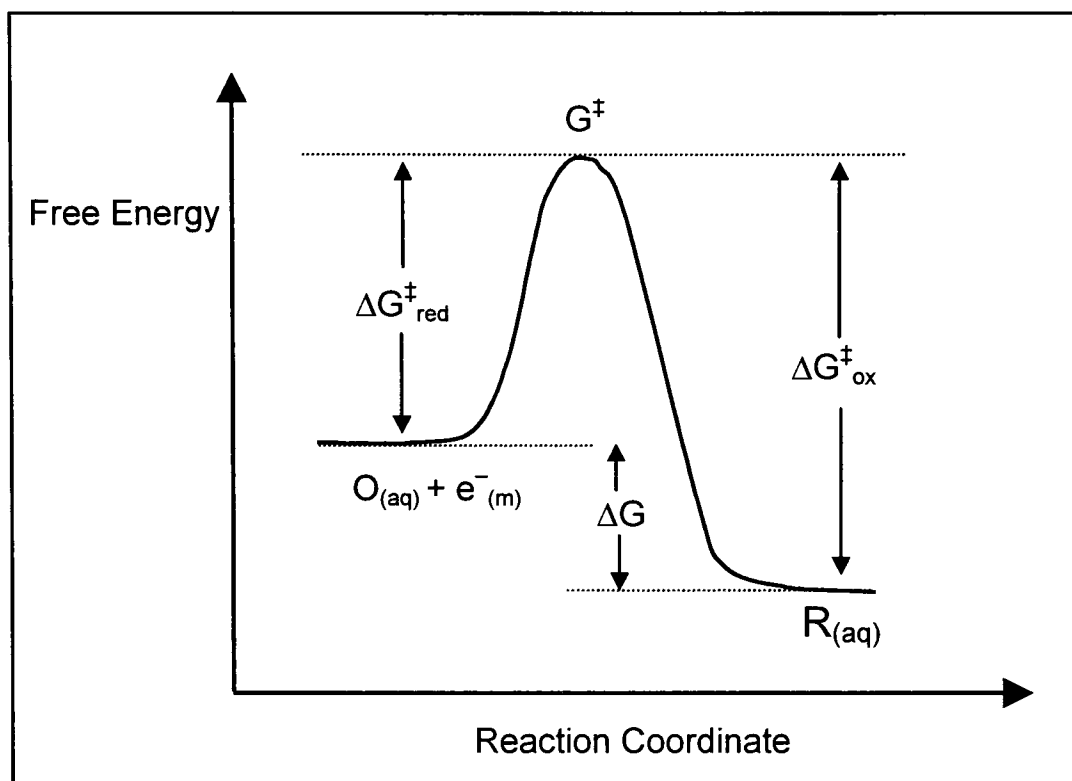


Fig 1.3 - Change in the free energy for one electron process illustrated in equation (1.31).

Using such a relation, the rate of reduction and oxidation (v_c and v_a respectively) as a function of temperature can be written as

$$v_c = k_{red}[O]_o e^{\frac{-\Delta G_{red}^\ddagger}{RT}} \quad \text{and} \quad v_a = k_{ox}[R]_o e^{\frac{-\Delta G_{ox}^\ddagger}{RT}} \quad (1.35)$$

where the quantity $k_{red}[O]_o$ or $k_{ox}[R]_o$ is the pre-exponential factor and accounts for the rate of collision between the species with the electrode surface. At equilibrium, the rate of reduction and oxidation are equal, hence

$$v_c = v_a \quad \text{and} \quad k_{red}[O]_o e^{\frac{-\Delta G_{red}^\ddagger}{RT}} = k_{ox}[R]_o e^{\frac{-\Delta G_{ox}^\ddagger}{RT}} \quad (1.36)$$

which rearranges to form

$$\frac{k_{red}[O]_o}{k_{ox}[R]_o} = \frac{e^{\frac{-\Delta G_{ox}^\ddagger}{RT}}}{e^{\frac{-\Delta G_{red}^\ddagger}{RT}}} \quad (1.37)$$

and taking logarithms of both sides

$$\ln \frac{k_{red}[O]_o}{k_{ox}[R]_o} = -\frac{\Delta G_{ox}^\ddagger}{RT} + \frac{\Delta G_{red}^\ddagger}{RT} \quad (1.38)$$

From figure 1.3, you can see that

$$\Delta G = \Delta G_{ox}^\ddagger - \Delta G_{red}^\ddagger \quad (1.39)$$

hence

$$\ln \frac{k_{red}[O]_o}{k_{ox}[R]_o} = -\frac{\Delta G}{RT} \quad (1.40)$$

Finally, substituting the relation $\Delta G = -nFE$ as illustrated in equation (1.8)

$$\ln \frac{k_{red}}{k_{ox}} - \ln \frac{[O]_o}{[R]_o} = \frac{nFE}{RT} \quad (1.41)$$

which can be rearranged to form

$$E = \frac{RT}{nF} \ln \frac{k_{red}}{k_{ox}} + \frac{RT}{nF} \ln \frac{[O]_o}{[R]_o} \quad (1.42)$$

and at equilibrium, this forms

$$E = E^o + \frac{RT}{nF} \ln \frac{[O]_o}{[R]_o} \quad (1.43)$$

Equation (1.43) will be familiar as the Nernst equation as previously discussed, however, this time derived using a kinetic consideration [3,5].

Attention is now directed towards the variation in electrode kinetics as a function of applied potential. In irreversible systems, no appreciable currents flow when the potential is set to E^o . Therefore to effect the flow of additional current from the reduction of $O_{(aq)}$, an over potential (η) must be applied where

$$\eta = E - E^o \quad (1.44)$$

In such cases, the magnitudes of $\Delta G_{red}^\ddagger$ and $\Delta G_{ox}^\ddagger$ are modified as a result (for $n = 1$), giving

$$\Delta G_{red}^\ddagger = (\Delta G_{red}^O)^\ddagger + \alpha F \eta \quad \text{and} \quad \Delta G_{ox}^\ddagger = (\Delta G_{ox}^O)^\ddagger - (1 - \alpha) F \eta \quad (1.45)$$

where α is known as the transfer coefficient and ΔG_{red}^O and ΔG_{ox}^O are the free energy changes when the applied potential is equal to E° . The effect of the applied η has two significant consequences; part of it is used to assist the cathodic process and the remainder is used to retard the anodic process. This can be shown schematically as increasing the free energy of the reactants, therefore increasing the magnitude of ΔG thereby promoting the formation of products as shown in figure 1.4.

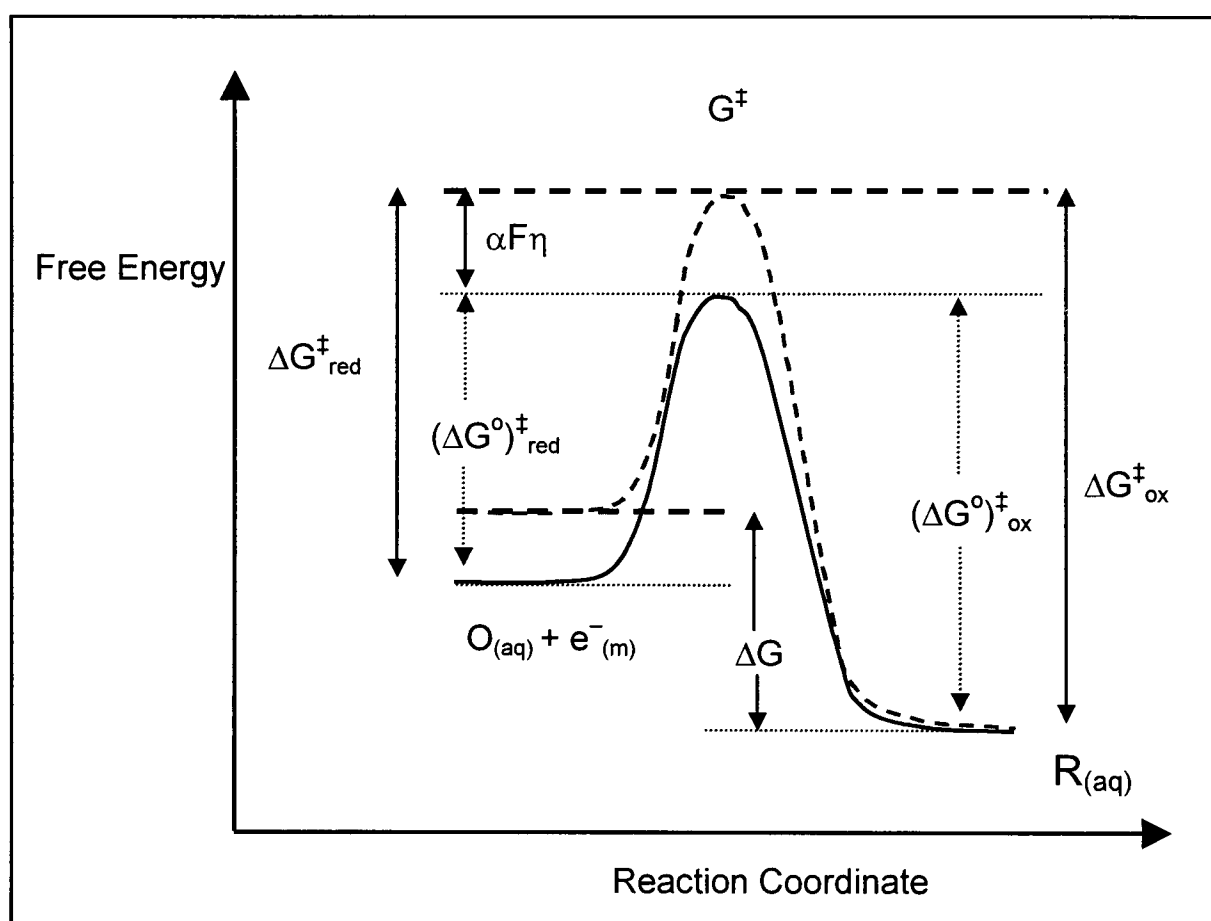


Fig 1.4 - Change in the free energy ($\Delta G_{red}^\ddagger$ and $\Delta G_{ox}^\ddagger$) through application of a cathodic over potential, η .

Adapted from reference 3.

The modified values for $\Delta G_{red}^\ddagger$ and $\Delta G_{ox}^\ddagger$ can be again written in terms of the Arrhenius expression, leading to equations (1.46) and (1.47).

$$v_c' = k_{red}[O]_o e^{\left[\frac{-(\Delta G_{red}^O)^\ddagger + \alpha F \eta}{RT} \right]} \quad (1.46)$$

$$v_a' = k_{ox}[R]_o e^{\left[\frac{(\Delta G_{ox}^0)^{\ddagger} - (1-\alpha)F\eta}{RT} \right]} \quad (1.47)$$

In this case, the rates of the cathodic and anodic reactions are not equal, we therefore have a net current flowing. The rate can be expressed in terms of current flow using the form

$$v_c' = \frac{I_c}{F} \quad \text{and} \quad v_a' = \frac{I_a}{F} \quad (1.48)$$

and substituting into equations (1.46) and (1.47)

$$I_c = Fk_{red}[O]_o e^{\left[\frac{(\Delta G_{red}^0)^{\ddagger} + \alpha F\eta}{RT} \right]} \quad (1.49)$$

$$I_a = Fk_{ox}[R]_o e^{\left[\frac{(\Delta G_{ox}^0)^{\ddagger} - (1-\alpha)F\eta}{RT} \right]} \quad (1.50)$$

and by expanding the exponent gives

$$I_c = Fk_{red}[O]_o e^{\left[\frac{(\Delta G_{red}^0)^{\ddagger}}{RT} \right]} e^{\left[\frac{\alpha F\eta}{RT} \right]} \quad (1.51)$$

$$I_a = Fk_{ox}[R]_o e^{\left[\frac{(\Delta G_{ox}^0)^{\ddagger}}{RT} \right]} e^{\left[\frac{(1-\alpha)F\eta}{RT} \right]} \quad (1.52)$$

Equations (1.51) and (1.52) can be simplified to

$$I_c = I_o e^{\left[\frac{\alpha F\eta}{RT} \right]} \quad \text{and} \quad I_a = I_o e^{\left[\frac{(1-\alpha)F\eta}{RT} \right]} \quad (1.53)$$

where I_o is known as the exchange current. For the reaction scheme posed in equation (1.31), when an over potential is applied, the net reductive current will be $I = I_c - I_a$ hence,

$$I = I_o \left\{ e^{\left[\frac{\alpha F\eta}{RT} \right]} - e^{\left[\frac{(1-\alpha)F\eta}{RT} \right]} \right\} \quad (1.54)$$

Equation (1.54) is known as the Butler-Volmer equation and shows the effect of potential on the rates of electrode processes [15,16]. The above equation is strictly valid for

processes involving the transfer of a single electron. In situations when more than one electron is involved it is normal to use the additional terms, α_c and α_a .

1.2.3 MASS TRANSPORT

From the previous chapter, it was shown that the electrode potential has a direct influence over the rate of electrode processes, in addition to the surface concentrations of O and R and the heterogeneous rate constants, k_{red} and k_{ox} . For these processes to occur, there must be a system that transports the electroactive species to the electrode from bulk solution, and vice-versa. The modes of mass transport that exist can be rationalised as diffusion, convection and migration and are presented in this section.

1.2.3.1 DIFFUSION

Diffusion is the natural movement of both neutral and charged species in solution that is not influenced by the presence of an electric field. It leads to an effective homogenization of the mixture that can be thought of as a "random walk" process [1]. The rate of diffusion is entirely dependent of the presence of a concentration gradient, as illustrated by Fick's laws of diffusion [17]. These laws are differential equations that describe the flux and concentration of a species as a function of position and time and have been derived on the basis of the random walk model. Fick's first law states that, for a planar geometry, the diffusional flux of a species is proportional to the concentration gradient

$$J_{d,O}(x,t) = -D_O \frac{\partial C_O(x,t)}{\partial x} \quad (1.55)$$

where $J_{d,O}$ is the diffusional flux of species O, $\partial C_O/\partial x$ is the concentration gradient in the x-direction and D_O is the diffusion coefficient. The diffusion coefficient is an intrinsic property of the species and can take values between 10^{-5} and 10^{-6} cm² s⁻¹ in aqueous solution.

The variation of the concentration of a substance as a function of time is of more interest to the electrochemist, and is obtained using Fick's second law.

$$\frac{\partial C_O(x,t)}{\partial t} = D_O \left(\frac{\partial^2 C_O(x,t)}{\partial x^2} \right) \quad (1.56)$$

The derivation of Fick's laws are included in Appendix A

1.2.3.2 CONVECTION

A convective component to mass transport occurs when a mechanical force acts on the solution, which can either be natural or forced in its origin. Natural convection can operate in any solution and arises due to a combination of thermal gradients and differences in density. Thermal gradients can be set up if the electrochemical reactions involve exo- or endothermic processes whereas density differences can occur if a species is produced at the electrode with a density different to that of bulk reagent. The effects of natural convection can normally be seen in electrolysis experiments after a few seconds when using electrodes no larger than millimeter size, and are undesirable since they are difficult to predict.

Forced convection is the deliberate movement of solution past the electrode and can be achieved through pumping and stirring. This has the effect of effectively swamping out the convective transport that is occurring naturally. Usually the forced convection is set up so that it operates with well-defined hydrodynamic behaviour. This facilitates a quantitative model of the solution flow, permitting the mass transport to the electrode to be accurately predicted. Mathematically, the one-dimensional convective flux is given by

$$J_{c,O}(x,t) = C_O(x,t) v_x \quad (1.57)$$

where $J_{c,O}$ is the convective flux and v is the velocity of solution.

The convective analogue of Fick's second law can be therefore written as

$$\frac{\partial C_O}{\partial t} = - \left(v_x \frac{\partial C_O}{\partial x} \right) \quad (1.58)$$

1.2.3.3 MIGRATION

The presence of an electric field, $d\phi/dx$, which exists between the electrode and solution interface, has the effect of exerting an electrostatic force on charged species. It is this force that induces movement of the charged species from the bulk solution to the electrode surface, and vice versa [18]. The magnitude of flux due to migration is directly proportional to the electric field gradient as illustrated below

$$J_{m,O}(x,t) = - \frac{zF}{RT} D_O C_O(x,t) \frac{\partial \phi}{\partial x} \quad (1.59)$$

where J_m is the migratory flux in the x-direction and z is the charge on the ion.

The coupling of migratory processes with electrolysis experiments leads to variation in the rates of mass transport and thus experimental interpretation becomes compromised. In order to simplify such problems, experiments are often conducted in conditions where migration can be neglected. This is achieved through the addition of an excess of 'background electrolyte' – a chemically and electrochemically inert substance, added in concentrations of 0.1 M and greater. Excess is broadly defined as the quantity of electrolyte that is required to ensure the migratory flux is 100 times smaller in magnitude than the diffusional flux, and values of 33-fold excesses are commonly stated [19]. The addition of background electrolyte to the solution helps maintain electroneutrality near the electrode surface as electron transfer processes occur at the electrode/solution interface, and as a result avoids the build up of an electric field. In this way, migratory mass transport effects as illustrated through equation (1.59) can be ignored.

The addition of background electrolyte has other consequences other than the migratory considerations. Firstly, added background electrolyte increases the conductivity of the solution allowing charge transfer processes to be limited by electrode kinetics rather than the solution's resistance to the passage of current. Secondly, the size of the double layer is reduced so that the change in potential ($\phi_m - \phi_s$) occurs in a region that is of the order of

10-20 Å. Not only does this ensure that the electric field is absent outside this region and therefore removing effects due to migration but also this distance is compatible with 'electron tunnelling' that occurs between the metal and the reactant in solution [5]. Thirdly, the ionic strength of the solution remains effectively constant due to the high concentration of electrolyte with respect to the reactants and products in solution. This has the effect to ensure the activity coefficients of reactants and products and therefore electrode potentials, predicted using the Nernst equation, and chemical reactivities are also kept constant.

1.2.4 POTENTIAL SWEEP METHODS

The current response as a function of time for a system when plotted at a series of potential steps yields the complete electrochemical behaviour for that system. However, such methods are extremely time-consuming and tedious in both the accumulation and analysis of data, and as a result other methods are preferred. A wealth of electrochemical information can be determined in a single experiment through sweeping the potential as a function of time and recording the resulting current, and in practice, it is these techniques that are normally at the forefront of an experimental electrochemist's toolbox.

1.2.4.1 LINEAR SWEEP VOLTAMMETRY

The potential is usually varied in a linear fashion with time, using sweep rates, v_s , ranging from 1 mV s^{-1} to about 1000 V s^{-1} at conventional electrodes, as shown below in figure 1.5(a).

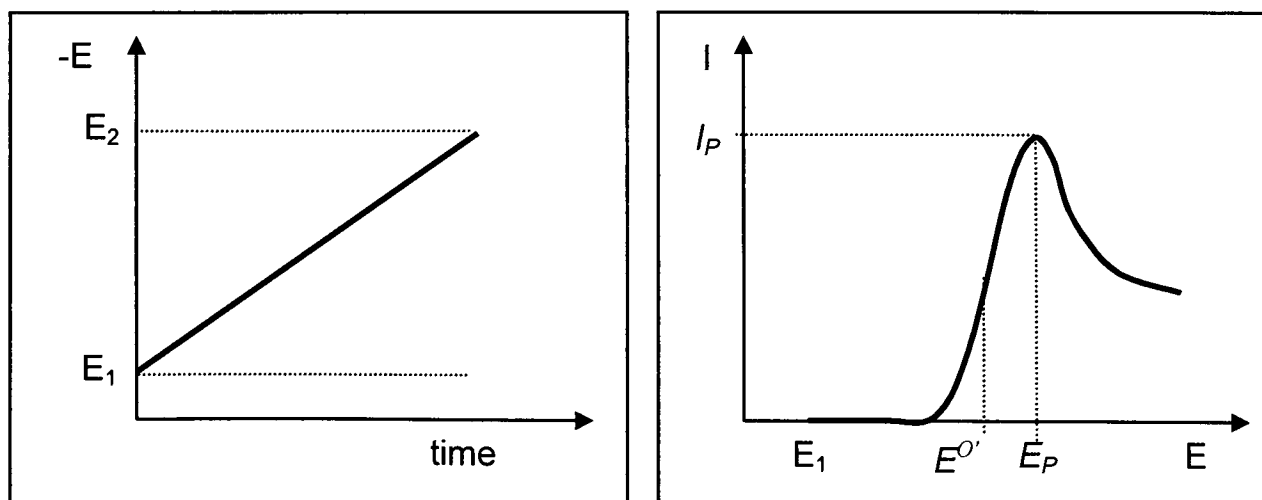


Fig. 1.5 - a) Linear potential sweep starting at E_1 . The gradient of the graph is equal to the scan rate, v_s .
b) Corresponding I-E response for a reversible one-electron system as outlined in equation (1.31).

where the potential is defined using the following relationship

$$E_t = E_1 - v_s t \quad (1.60)$$

During the experiment, the current is recorded as a function of potential (equivalent to recording the current versus time). This technique is traditionally known as linear potential sweep chronoamperometry but is more commonly referred to as linear sweep voltammetry (LSV) [1].

A typical LSV response for a reversible one-electron process is illustrated in figure 1.5(b). If the potential sweep is initiated at values more positive than $E^{\circ'}$ then any current flow is attributable to nonfaradaic processes. As the potential reaches the same magnitude as $E^{\circ'}$ then significant reductive currents begin to flow. As the potential is swept past $E^{\circ'}$, the surface concentration of the electroactive species begins to drop rapidly, increasing the flux of fresh material to the electrode surface. Here the current flow increases exponentially as predicted by the Butler-Volmer equation (1.54). Eventually the surface concentration drops effectively to zero, and therefore the mass transport to the electrode surface reaches a maximum rate. The flux of fresh material to the electrode surface is eventually compromised by gradual depletion of available species within the diffusion layer resulting in a decline in the observed current. These effects can explain the presence of a peak current response observed in the voltammetry (fig. 1.5b).

For a reversible system, the peak current, I_p , can be calculated using the following [1]

$$I_p = 0.4463 \left(\frac{F^3}{RT} \right)^{1/2} n^{3/2} A D_O^{1/2} C_{bulk} v_s^{1/2} \quad (1.61)$$

where A is the electrode area and C_{bulk} is the bulk concentration of the electroactive species. Similarly, the difference between the peak potential, E_p , and half-peak potential, $E_{p/2}$, (i.e. the potential at $I_{p/2}$) is derived using

$$|E_p - E_{p/2}| = 2.20 \frac{RT}{nF} = 56.5 / n \text{ mV at } 25^\circ \text{C} \quad (1.62)$$

Thus, for any reversible wave I_p is proportional to $v_s^{1/2}$, which is consistent for a process operating under diffusion control, and E_p is independent of scan rate. For potentials less than E_p the system is said to be under the direct influence of the electrode kinetics, however, at potentials greater than E_p , the processes are limited by the rate of diffusion of the electroactive species.

In the case of an irreversible system, as shown below in figure 1.6,

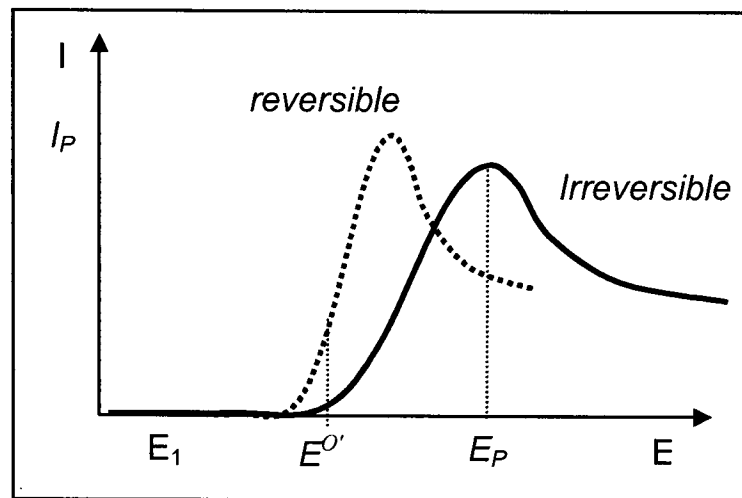


Fig. 1.6 - I-E curves for both reversible and irreversible processes.

the corresponding equations are

$$I_p = (2.99 \times 10^5) \alpha^{1/2} A D_O^{1/2} C_{bulk} v_s^{1/2} \quad (1.63)$$

$$|E_p - E_{p/2}| = 1.857 \frac{RT}{\alpha F} = 47.7 / \alpha \text{ mV at } 25^\circ \text{ C} \quad (1.64)$$

The above equations reflect the contrasting electrode kinetics of reversible and irreversible systems. In the case of a reversible process, at potentials near E° we observe large values for the heterogeneous rate constant k_{red} and k_{ox} . As a result, the concentrations of O and R at the electrode surface are effectively in equilibrium, with their ratio predicted by the Nernst equation, in comparison with irreversible systems, where electrolysis proceeds with negligible back reaction.

In both systems, however, it is seen that I_p increases linearly with $v_s^{1/2}$ and this can be interpreted as follows. At potentials close to E_p , the system is under the control of diffusion and Fick's first law states that I_p is a direct measure of the concentration

gradient of O near the electrode, which in turn is controlled by diffusion layer thickness. By sweeping the potential more swiftly, relatively less time is allowed for electrolysis and subsequently a thinner diffusion layer, and hence, a steeper concentration gradient results - giving rise to the larger currents observed.

1.2.4.2 CYCLIC VOLTAMMETRY

If the linear sweep voltammogram is extended back to the potential of origin, E_1 , a triangular potential cycle results (fig. 1.7a) and is referred to as cyclic voltammetry (CV).

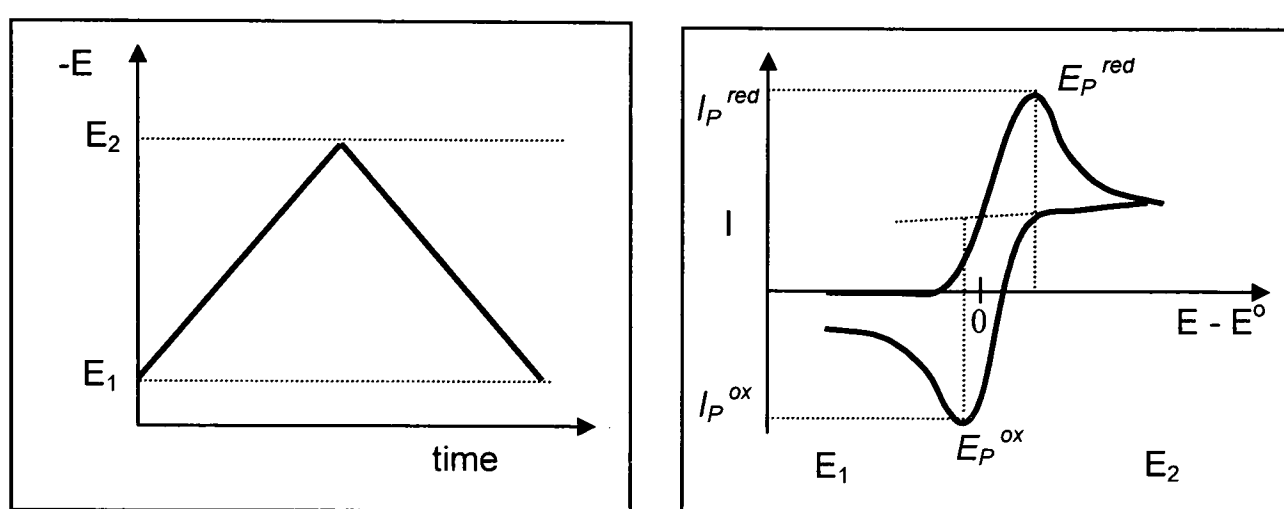


Fig. 1.7 - a) Cyclic potential sweep starting at E_1 . b) Corresponding I-E response for a reversible one-electron system as outlined in equation (1.31).

The resulting voltammogram is shown in figure 1.7(b) and is composed of two distinct processes. The forward scan is essentially identical to that obtained with LSV, and depends on the reversibility of the O/R redox couple. Once E_2 is attained, the potential is swept back resulting in the re-oxidation of species R formed from the forward scan. The current increases as the potential moves steadily past E^0 as seen previously, increasing the kinetics for the oxidation of R present in the diffusion layer. The current then drops to zero as the concentration of R depletes.

The two measured parameters of interest on a cyclic voltammogram are the ratio of peak currents (I_P^{ox} / I_P^{red}) and the separation of peak potentials ($E_P^{ox} - E_P^{red}$). Deviation of the ratio of peak currents from unity is indicative of homogeneous kinetics or other complications in the electrode process [20]. For a reversible system, equation (1.65) illustrates the relationship between the peak potential of the forward and reverse scan, when n electrons are transferred.

$$\left| E_p^{ox} - E_p^{red} \right| = 2.218 \frac{RT}{nF} \quad (1.65)$$

In such a system, at 25 °C, the peak potentials are separated by a potential of approximately 59/n mV and are independent of scan rate [21].

The case is also considered for both irreversible and quasi-irreversible systems and is shown in comparison to reversible electrode kinetics in figure 1.8.

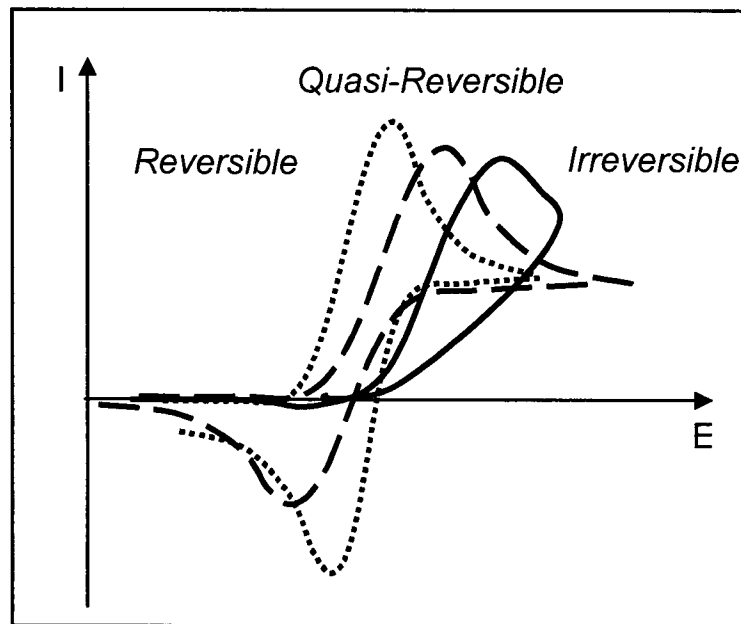


Fig. 1.8 - I-E curves for cyclic voltammetry at reversible, quasi-reversible and irreversible processes.

It is observed that under irreversible kinetics, an appreciable over potential is required to reduce species O and on the reverse scan the absence of any current due to the reoxidation of species R is noted. The size and position of the reverse peak relative to the forward peak, in fact, depends on the voltage sweep rate as seen in the case with LSV. This is in direct contrast with the reversible system, whereby the peak potentials are scan rate independent. The absolute magnitudes of the peak currents for both forward and reverse scans however, are dependent on scan rate regardless of the reversibility of the system.

1.2.4.3 SQUARE WAVE VOLTAMMETRY

Square wave voltammetry (SWV) offers exceptional versatility in the interrogation of electrochemical systems and was first pioneered in the 1950s by Barker [22] but was not widely adopted due to instrumentation limitations. The technique has been more recently

transformed into a powerful analytical tool through the work of Ramaley and Krause [23] and Osteryoung et al [24-26].

Square wave voltammetry is normally performed at a stationary electrode and involves the waveform and measurement scheme shown below in figure 1.9.

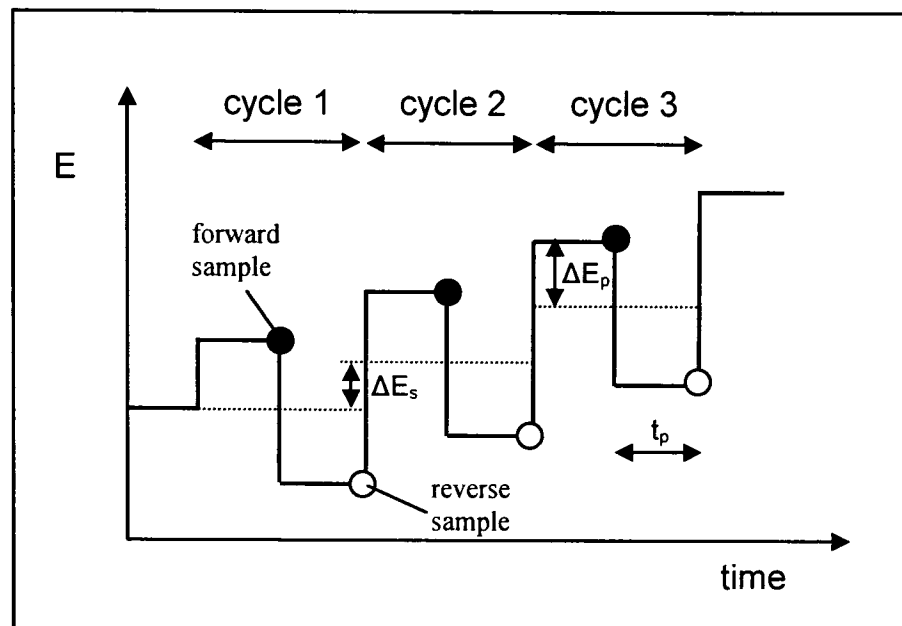


Fig. 1.9 - Waveform and measurement scheme for the application of SWV. Adapted from reference 1.

The key features of the square wave are defined by the pulse height, ΔE_p , measured with respect to the corresponding potential staircase (ΔE_s), and by the pulse width t_p . In turn, the pulse width is defined by the frequency of the square wave, f ,

$$f = \frac{1}{2t_p} \quad (1.66)$$

The staircase increases by ΔE_s at the start of each cycle and the scan rate, v , is therefore equal to

$$v = \frac{\Delta E_s}{2t_p} = f\Delta E_s \quad (1.67)$$

The current is monitored twice per cycle at the end of each pulse and is attributed to either the forward sample (I_f), which is the first pulse and in the direction of the staircase scan, and the reverse sample (I_r), which is the second pulse and in the opposite direction. The difference between the currents obtained at the forward and reverse samples, as shown in equation 1.66, is normally recorded as a function of potential.

$$\Delta I = I_f - I_r \quad (1.68)$$

A typical response of square wave voltammetry is shown in figure 1.10, where ΔI , I_f and I_r are plotted as a function of potential.

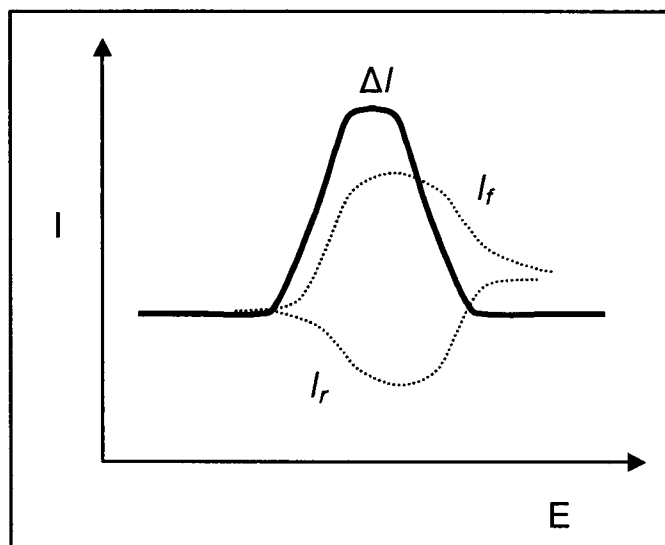


Fig. 1.10 - Typical current response following the application of SWV.

The strengths of SWV in comparison to CV manifest themselves in the ability to suppress background effects, in particular the current due to the reduction of dissolved oxygen, which in general permits the use of substantially lower concentrations of species [1,2]. Moreover, there is usually much less distortion of the response caused by the background, which allows for a greater degree of accuracy when fitting the data to theoretical models. Applications of SWV include the determination of the concentrations of individual species in multicomponent systems, such as antibiotics [27] and antioxidants in food [28].

1.2.4.4 POTENTIAL STEP CHRONOAMPEROMETRY

The diffusion-limited current as a function of time can be readily obtained by application of a potential step to a solution containing O and R as in equation (1.31), as illustrated in figure 1.11.

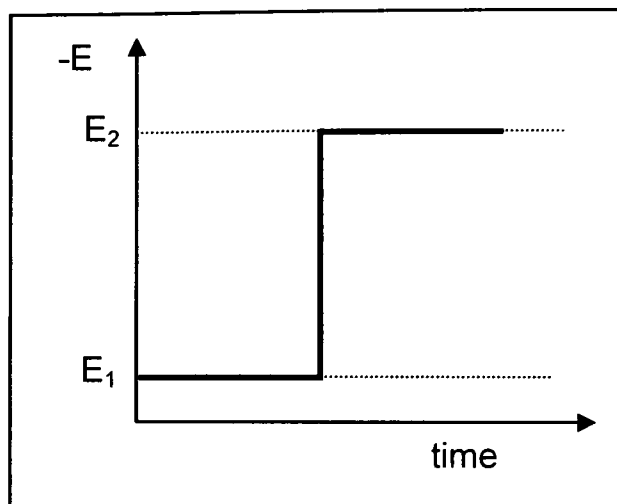


Fig. 1.11 - Application of a potential step to obtain diffusion-limited currents.

If the experiment is then taken from a potential where no electrolysis of the species occurs (E_1), to a potential where all the electroactive species that reach the electrode react to form products (E_2), the recorded current is diffusion-limited and varies with time. The variation of the diffusion-limited current as a function of time at a planar, uniformly accessible electrode is known as the Cottrell equation, shown below [29].

$$I(t) = \frac{nFAD^{1/2}[O]_{bulk}}{(\pi t)^{1/2}} \quad (1.69)$$

The Cottrell equation and the concentration profile of such a system at the electrode surface are illustrated below in figure 1.12.

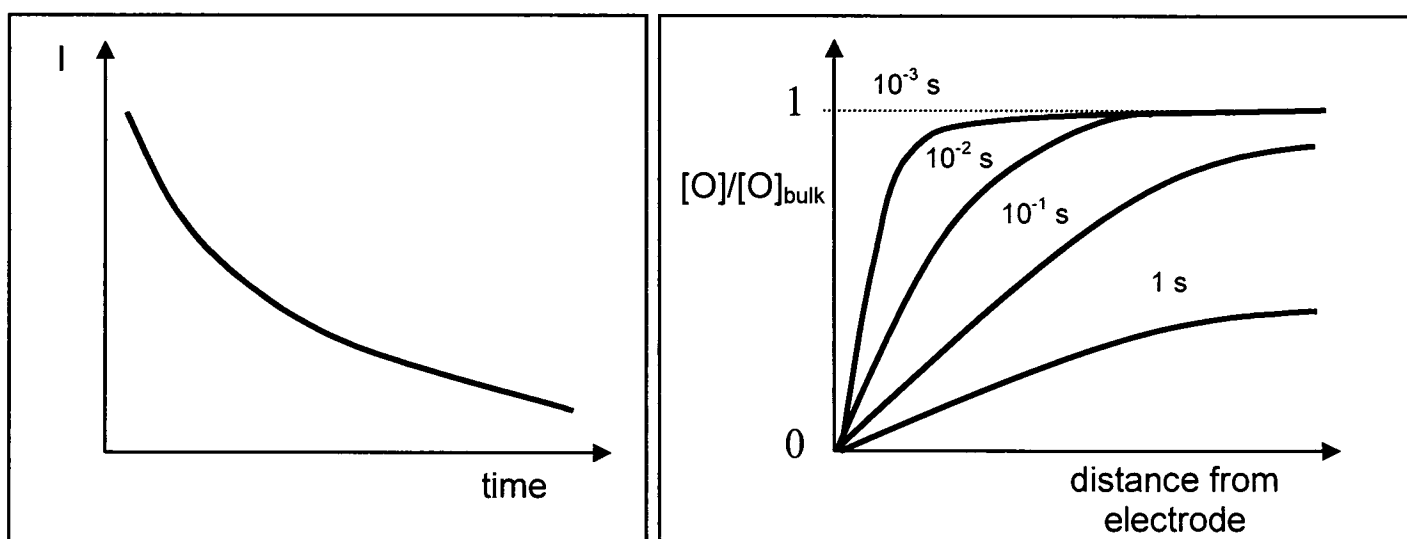


Fig. 1.12 - Electrochemistry following a potential step to E_2 at a planar electrode. a) Variation of the diffusion-limited current with time and b) corresponding concentration profile for various values of t .

Adapted from reference 2.

As the current is inversely proportional to $t^{1/2}$, after a certain time the effects of natural convection will have an influence over the experiment, perturbing concentration gradients and moving the system away from the diffusion-limited case.

1.3 HYDRODYNAMIC VOLTAMMETRY

Numerous techniques exist where mass transport is enhanced through the application of forced convection, first introduced in section 1.2.3.2. These techniques either fall into one of two subsets; the first is where the electrode itself is in motion (e.g. rotating disc electrode) and the second is where there is forced solution flow over a stationary electrode surface (e.g. channel electrode, microelectrode). Such techniques are known as hydrodynamic methods and the term hydrodynamic voltammetry is often used when referring to the measurement of limiting currents obtained with such systems. The benefits of such systems are due to the fact that the diffusion to the electrode occurs under controlled conditions and therefore measurements are made with high precision and reproducibility [30]. As steady-state is rapidly attained, measurements can also be made without the problem of double layer charging [31].

The use of forced convection is key to much of the work reported later in this thesis and a broad description of these hydrodynamic processes is now presented below. The use of ultrasound is another example of a system utilising forced convection, where the generated sound waves cause convective flow to the electrode surface (sonohydrodynamic systems) and is dealt with in section 1.4.

1.3.1 DIFFUSION LAYER

Prior to discussing hydrodynamic systems, it is useful to define the term diffusion layer. In non-hydrodynamic conditions, the diffusion layer is defined by the following relation

$$J_d = D_O \frac{[O]_{Bulk} - [O]_o}{\delta} \quad (1.70)$$

where δ is the diffusion layer thickness and was first estimated by Nernst [32]. This is achieved through the extrapolation of the concentration gradient at the electrode surface until the bulk concentration value is attained, as illustrated in figure 1.13.

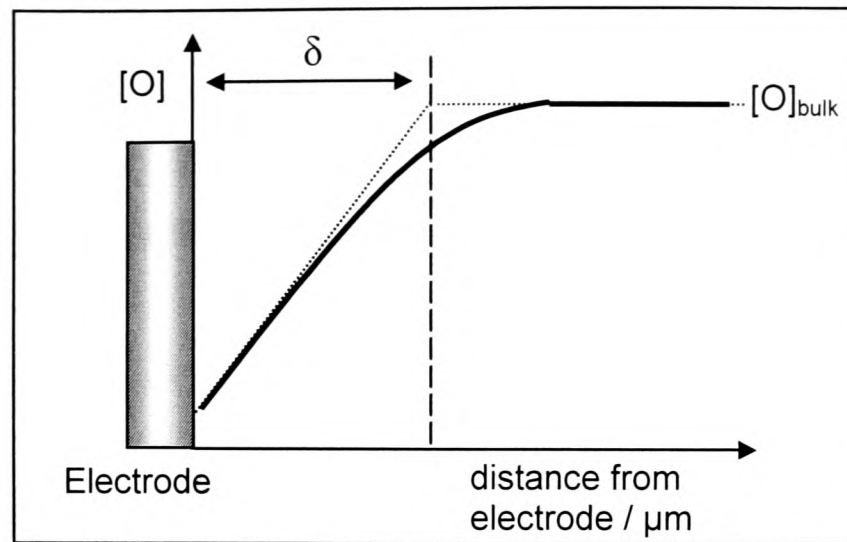


Fig. 1.13 - Definition of the diffusion layer thickness as estimated by Nernst.

Under the condition where the surface concentration of O is equal to zero, we get

$$J_d = D_O \frac{[O]_{Bulk}}{\delta} = k_d [O]_{bulk} \quad (1.71)$$

hence

$$k_d = \frac{D_O}{\delta} \quad (1.72)$$

where k_d is the mass transfer coefficient and has dimensions of a heterogeneous rate constant. δ can also be written in terms of the Cottrell equation and an expression for the diffusion layer thickness as a function of time can be deduced [2]. Equation (1.73) shows how the thickness of the diffusion layer grows as a function of time

$$\delta = (\pi D t)^{1/2} \quad (1.73)$$

In the case of electrodes operating under hydrodynamic conditions, we observe a comparative thinning of the diffusion layer from the convective flux of fresh material brought to the electrode surface, resulting in steeper concentration gradients and therefore higher currents.

1.3.2 ROTATING DISC ELECTRODE (RDE)

The rotating disc electrode (RDE) consists of electrode material that has been embedded in a shaft of insulating material, typically Teflon or epoxy resin, illustrated in figure 1.14.

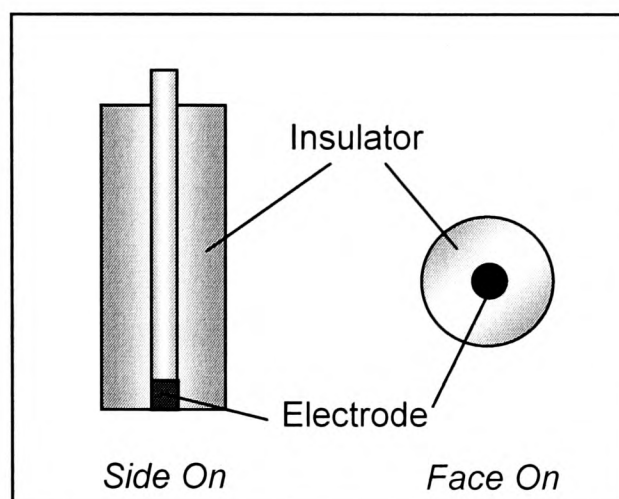


Fig. 1.14 - Schematic of the rotating disc electrode.

Generally the locus of the electrode material relative to the insulating material is well defined [33] and it is important to ensure no leakage of the solution occurs between the metal or the insulator. The shaft is driven by a motor and is rotated at a determined frequency, f (revolutions per second). It is more common to describe an angular velocity, ω , measured in rad s^{-1} where

$$\omega = 2\pi f \quad (1.74)$$

The use of RDEs in electrochemistry is made attractive by the fact that the mathematical descriptions covering hydrodynamic and convective-diffusion equations are well known for the steady state. In addition, the abundance of commercially available RDEs further add to their widespread use in hydrodynamic electrochemistry. Further details of the fabrication and application of RDEs can be found in a number of excellent reviews [33-37].

Through the solution of steady-state hydrodynamic equations, von Karman and Cochran first described the velocity profile of a fluid acting near a rotating disc [38] illustrated below in figure 1.15.

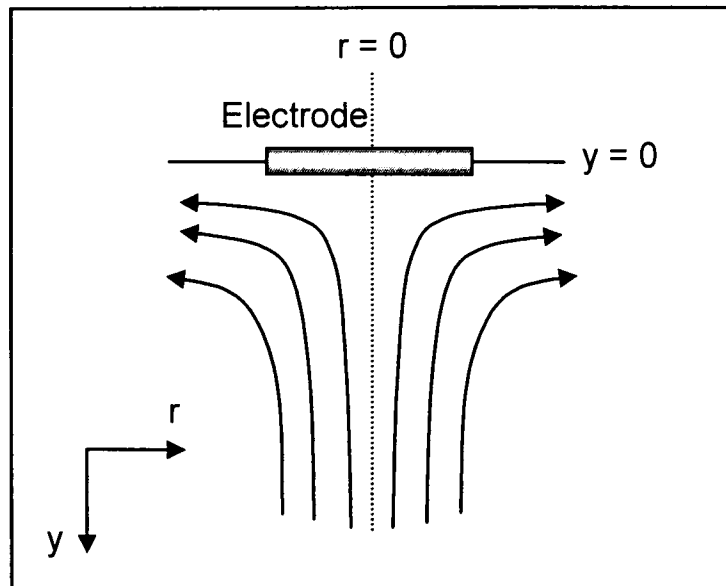


Fig. 1.15 - Flow velocities at a rotating disc electrode.

The velocity, v , of the flow normal to the electrode surface (y -direction) is given by

$$v = -C_y^2 \quad \text{where} \quad C_y = 0.510\omega^{3/2}\nu^{1/2}D^{-1/3} \quad (1.75)$$

where ν is the kinematic viscosity of the solution. After application of the above velocity profile to the convective-diffusion equations, we obtain a general solution for the transport limited current as a function of electrode rotation speed. This is known as the Levich equation, shown below

$$I_{Lim} = 0.62nFAD_O^{2/3}\omega^{1/2}\nu^{-1/6}[O]_{bulk} \quad (1.75)$$

and this equation predicts that the mass-transport limited current should vary linearly with $\omega^{1/2}$. Typical I-E plots obtained at a RDE as a function of rotation speed are presented below in figure 1.16.

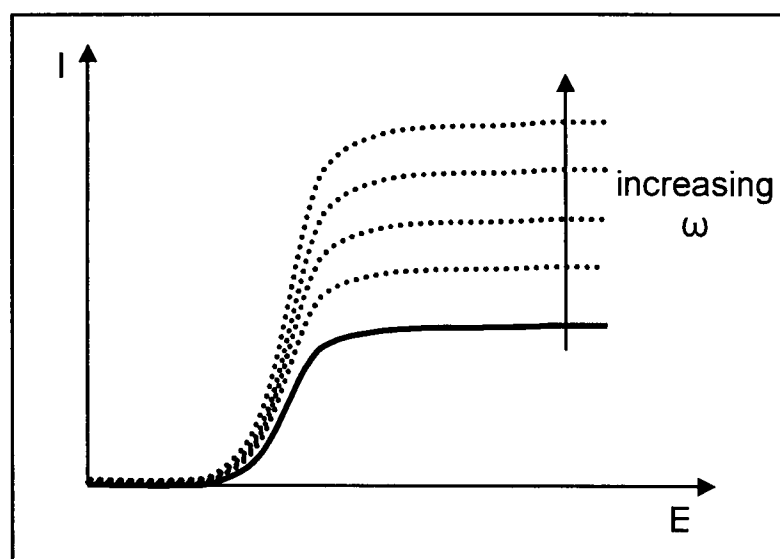


Fig. 1.16 - Effect of rotation speed on I_{Lim} .

The I-E curves obtained under hydrodynamic conditions are very different from those obtained under diffusion-only conditions in stagnant solutions due to the presence of a plateau or limiting current (I_{Lim}), predicted by equation (1.75), rather than a peak.

As with all hydrodynamic systems, the convective flux of fresh material has the effect of shrinking the diffusion layer, and in the case for the RDE this is quantitatively analysed by

$$\delta_O = 1.61 D_O^{1/3} \omega^{-1/2} \nu^{1/6} \quad (1.76)$$

where the diffusion layer thickness is inversely proportional to $\omega^{1/2}$ and is why greater currents are observed at higher rotation speed.

1.3.3 CHANNEL FLOW CELL

Another type of hydrodynamic electrode is the channel cell and operates through movement of a solution past a stationary electrode that has been smoothly embedded in a channel [39,40]. This type of electrode arrangement is shown below in figure 1.17.

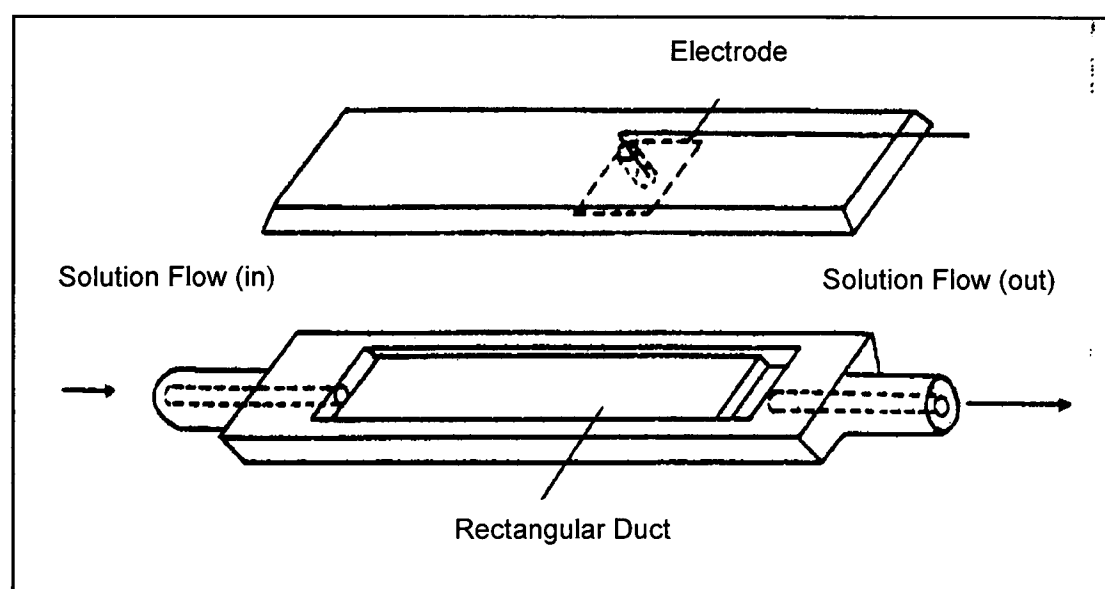


Fig. 1.17 - Schematic of a channel flow cell.

As with the rotating disc electrode, convection and diffusion both operate to ensure a fresh supply of electroactive material to the electrode surface. As a result of the enhanced mass transport to and from the electrode, steady-state I-E curves can be measured with well-defined plateaux corresponding to transport limited current (I_{Lim}) as seen in figure 1.16 with the RDE.

The nature of the flow of the solution through the channel is strongly dependent on the solution properties and flow rate. These terms can be expressed through use of the Reynold's number [41], which is given by

$$R_e = 2h \frac{v_0}{\nu} \quad (1.77)$$

where h is the channel half-height and v_0 is the centre line solution velocity. In order to mathematically describe the hydrodynamic and convection-diffusion equations, it is essential that the solution operates under conditions of laminar flow shown in the scheme below (figure 1.18).

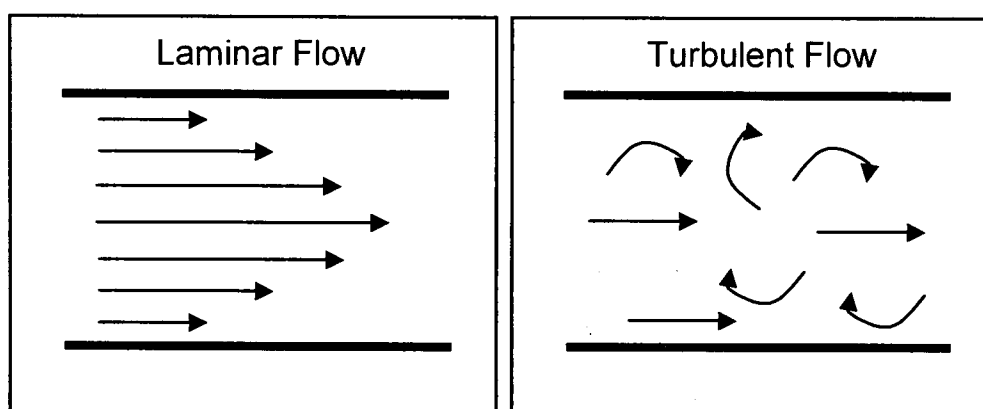


Fig. 1.18 - Schematic showing different types of fluid flow.

Two different types of flow are normally considered in hydrodynamic problems. When the flow is smooth and steady and occurs as if the separate layers of the fluid have steady and characteristic velocities, the flow is referred to as laminar. The flow velocity is understood to reach a maximum in the middle of the channel and decrease to zero along the walls (due to friction between the fluid and the wall) giving a typically parabolic flow profile. Turbulent flow, however, involves unsteady and chaotic fluid motion; only on average is there a net flow in a particular direction.

Laminar flow is achieved when R_e is typically below values of 2000 [42] and in most experimental conditions, R_e is generally found to be less than 10. Under such laminar conditions, the volume flow rate, V_f , can be related to v_0 through the expression [41]

$$v_0 = \frac{3V_f}{4hd} \quad (1.78)$$

where d is the width of the channel through which the solution is flowing.

The pattern of laminar flow can be quantitatively analysed as shown in figure 1.19,

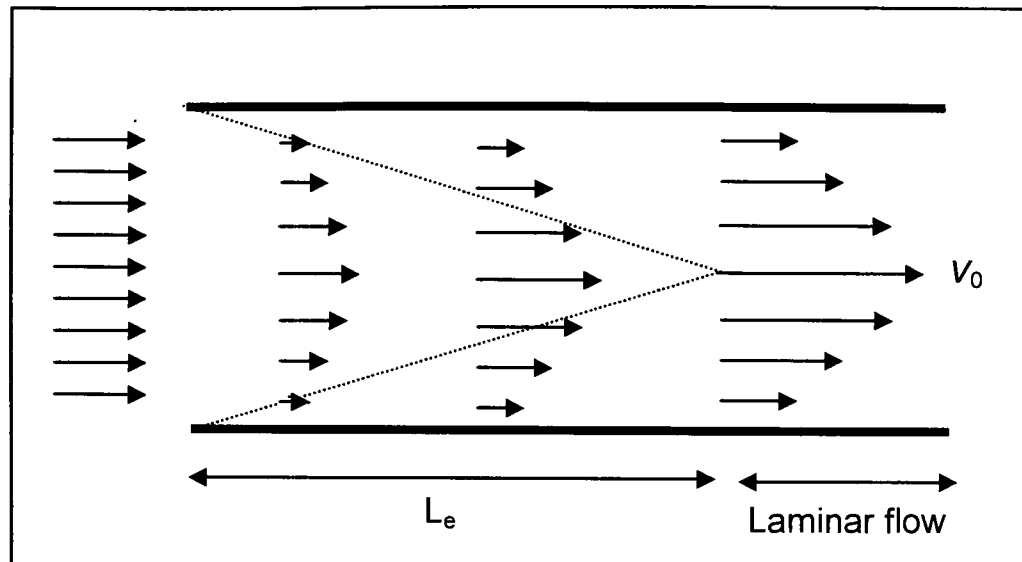


Fig. 1.19 - Development of laminar flow in a channel.

where solution entering the flow cell will ultimately achieve a parabolic flow profile after it has travelled the length of the lead-in section [43,44], L_e , given by

$$L_e = 0.1hR_e \quad (1.79)$$

The flow velocity parallel to v_0 can be described using the following relationship

$$v_x = v_0 \left(1 - \frac{(h-y)^2}{h^2} \right) \quad \text{and} \quad v_y = v_z = 0 \quad (1.80)$$

using the coordinate system as illustrated below in figure 1.20.

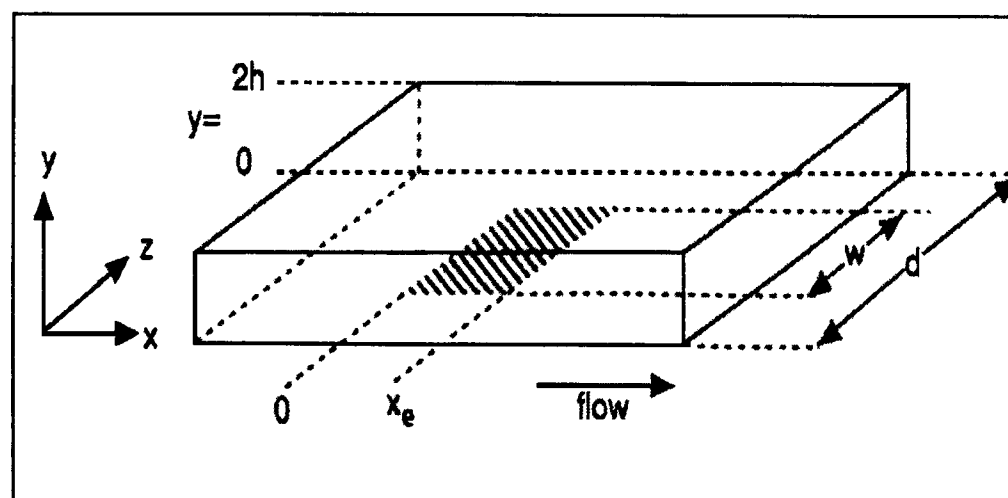


Fig. 1.20 - Schematic showing coordinate system of channel cell.

If the flow pattern in the channel cell is well know and there is sufficient background electrolyte present to suppress the effects of migration, then the convection-diffusion equation can be written as

$$\frac{\partial C_o}{\partial t} = D_o \nabla^2 C_o - \left(v_x \frac{\partial C_o}{\partial x} + v_y \frac{\partial C_o}{\partial y} + v_z \frac{\partial C_o}{\partial z} \right) \quad (1.81)$$

Equation (1.81) can be simplified using steady-state considerations, where $\partial C_o / \partial t = 0$, and using parabolic flow conditions, where $v_y = v_z = 0$. Using the assumption that the convective component to mass transport in the x -direction effectively swamps out any diffusive components in the x - and z -direction, valid for all cases except microelectrodes [45], hence

$$v_x \frac{\partial C_o}{\partial x} \gg D \left(\frac{\partial^2 C_o}{\partial x^2} + \frac{\partial^2 C_o}{\partial z^2} \right) \quad (1.82)$$

we can therefore re-write equation (1.81) as [46]

$$0 = D_o \frac{\partial^2 C_o}{\partial y^2} - v_x \frac{\partial C_o}{\partial x} \quad (1.83)$$

The only mass transport that operates in the channel cell is therefore diffusion from the y -direction and convection in the x -direction. Using these considerations, Levich derived an expression to equate the geometric cell parameters shown in figure 1.20 to the observed limiting currents [38] as shown below

$$I_{Lim} = 0.925 n F [O]_{bulk} w (x_e D)^{2/3} \left(\frac{V_f}{h^2 d} \right)^{1/3} \quad (1.84)$$

Returning to the concept of diffusion layers, we can write that the flux, J , of material reaching the electrode surface is

$$J \approx \frac{[O]_{bulk} - [O]_o}{\delta} \quad (1.85)$$

and if the electrode is held at a potential such that $[O]_o = 0$ and as J is proportional to the current, we see from equation (1.84)

$$\delta \propto x^{1/3} \quad (1.86)$$

which suggests that the diffusion layer varies as a function of distance along the electrode (x -axis).

The variation of diffusion layer thickness as a function of electrode length for a given flow rate is characteristic of non-uniform accessibility and makes the channel cell an ideal tool for mechanistic investigation. If we consider a simple ECE mechanism, as outlined in 1.2.1, the change in the effective number of electrons transferred (N_{Eff}) as a function of flow rate can be used to gauge the kinetics of the homogeneous chemical reaction. At fast flow rates (or fast rates of mass transport), only one electron is seen to be transferred as the intermediates B and C are swept downstream before a second heterogeneous electron transfer can occur. At slower flow rates, the rate of removal of the intermediates from the electrode is less and therefore species C can undergo a second electron transfer before it is swept away. Therefore, an ECE process performed at a channel cell electrode exhibits N_{Eff} values that vary between $1 < N_{\text{Eff}} < 2$.

Alternatively, the fluctuation in N_{Eff} as a function of flow rate can be considered in terms of diffusion layers, shown schematically in figure 1.21. Considering a constant flow rate, at the upstream edge of the channel electrode, the diffusion layer is thin and therefore the concentration gradients are steep, promoting the loss of B and C intermediates, once formed at the electrode surface, into bulk solution before a second electron transfer can occur. Further downstream, however, the diffusion layer is thicker and the concentration gradients shallower, therefore the rate of loss of B and C to bulk solution is slower and so a second electron transfer can occur in the timescales involved. Increasing the flow rate, however, has the effect on thinning the diffusion layer as shown below. This leads to steeper concentration gradients, forcing species B and C to diffuse away from the electrode before a second electron transfer can occur, as mentioned above.

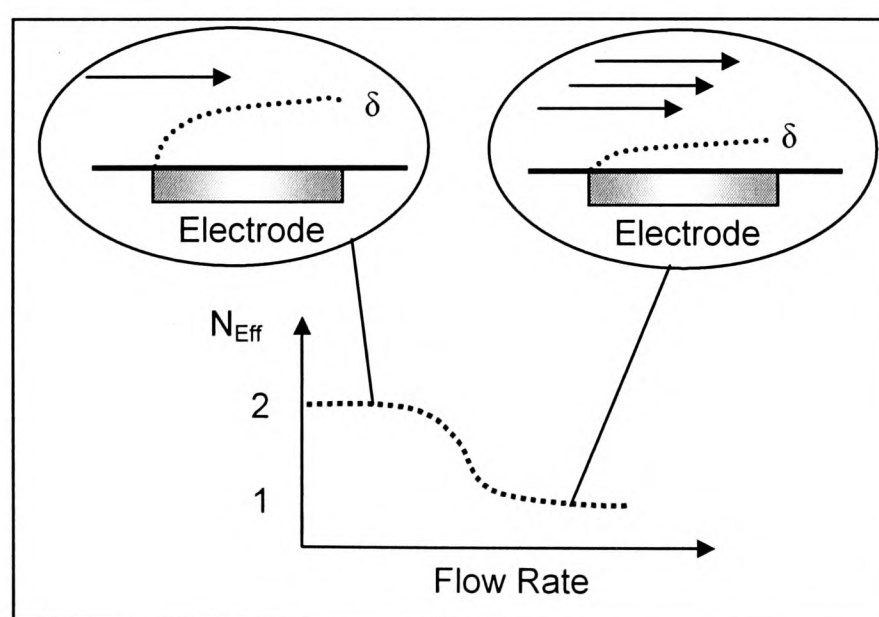


Figure 1.21 - Effect of diffusion layer and N_{Eff} as a function of flow rate at a channel electrode.

The variation of N_{Eff} with solution flow rate can provide kinetic insights about the electrode reactions [47].

1.3.4 MICROELECTRODES

Electrodes that have at least one dimension that is a function of size are referred to as microelectrodes (or ultramicroelectrodes) and are typically between 0.1 and 50 μm in size. The general definition for a microelectrode is that they are smaller than the scale of the diffusion layer developed in readily achievable experiments [1].

The most common of such electrodes are disc microelectrodes, but spherical, hemispherical and ring geometries are all commonly used in electrochemical experiments. First introduced into electrochemical research in the 1980s by independent work done by Wightman [48] and by Fleischmann et al [49], electrochemistry at microelectrodes has rapidly expanded into new areas of study [48-55].

Disc microelectrodes are routinely used in electrochemical experiments and theoretical treatment of such systems is made non-trivial through the fact that diffusion can occur in two dimensions [56,57]: radially with respect to the axis of symmetry and normal to the plane of the electrode. This is shown schematically below in figure 1.22.

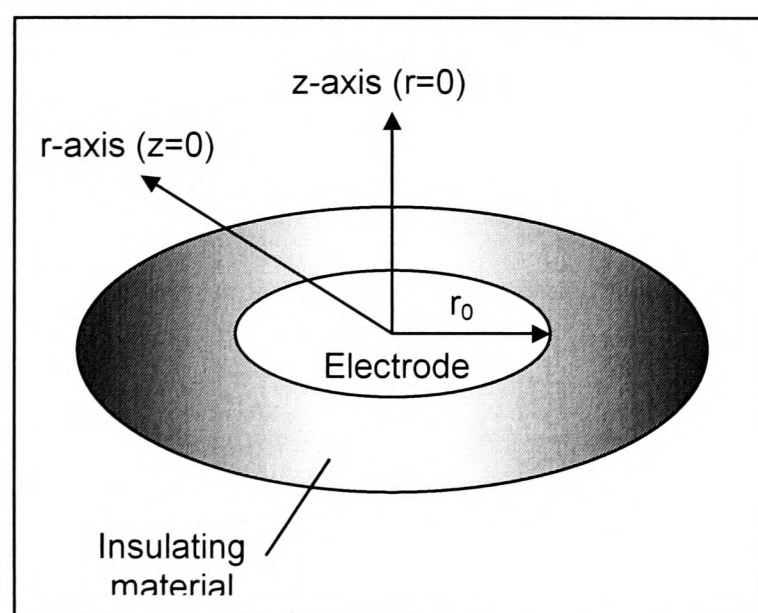


Fig. 1.21 – Geometry of a microelectrode.

A consequence of such a diffusional system means that the current density is not uniform over the electrode surface, but is greater at the edges. After solution of the diffusion

equations through applying relevant boundary conditions, the steady-state current can be expressed in terms of the electrode radius by

$$I_{Lim} = 4nFD_O[O]_{bulk}r_0 \quad (1.87)$$

where r_0 is equal to the radius of the electrode and is valid over longer time periods when a hemispherical diffusion layer has been allowed to develop at the electrode surface. In these situations, the electrode, as with the channel cell, is non-uniformly accessible since the edges are more available to the diffusing electroactive species, shown in figure 1.23.

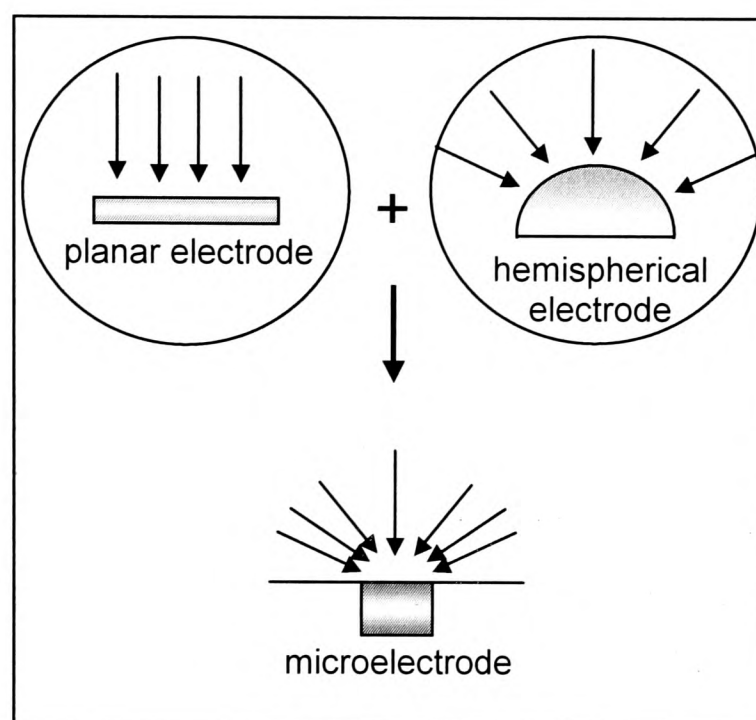


Fig. 1.23 - Schematic showing the uniform current density at planar and hemispherical electrodes in comparison to the non-uniform accessibility of the disc microelectrode.

It is this aspect that allows microelectrodes to be a suitable mechanistic probe for the investigation of the heterogeneous electron transfer at the electrode or the homogeneous kinetics of the chemical reaction between intermediates in solution.

The use of microelectrodes in electroanalytical applications is made attractive by their small size and can often be employed for *in vivo* analysis. Aside to this, since the electrode area is small, significantly lower currents are passed resulting in negligible electrolysis of the solution. This manifests itself in thin diffusion layers and therefore steep concentration gradients which enhance the rate of mass transport in comparison to macroelectrodes. In addition, the small passage of current has a reduced effect on the 'capacitive charging' and as a result electrolysis currents are less distorted allowing fast transient studies to be carried out [58]. The final benefit of microelectrodes comes in the

form of reduced 'ohmic drop' and this obviates the use of background electrolyte [59]. The requirement to use background electrolyte had previously limited electrochemical investigations to polar solvents and the use of microelectrodes therefore permits the use of non-polar solvents such as benzene or toluene.

1.4 ULTRASOUND

The origins of ultrasound generation go back to the 1880s with the discovery of the piezoelectric and inverse piezoelectric effect [60,61]. The inverse piezoelectric effect is still adopted by most present-day ultrasonic devices (via transducers), which operate through the application of a potential across a crystal face causing a change in the crystal dimensions. This has the effect of converting the electrical energy into mechanical energy, which manifests itself as sound. If the potential is oscillated at a high enough frequency, the result is the production of ultrasound.

The frequency range of the human ear varies from 16 Hz to 16 kHz. Ultrasound is classically defined as the frequencies of sound waves that cannot be heard by the human hearing (i.e. > 16 kHz) and extends to frequencies of 5 MHz for gases and 500 MHz for liquids and solids. Ultrasound is generally divided into two main areas; those that deal with high frequency or low power ultrasound (1-10 MHz) and those that deal with low frequency or high energy waves (20-100 kHz), known as power ultrasound. The spectrum of sonic frequencies is presented below in figure 1.24 [62].

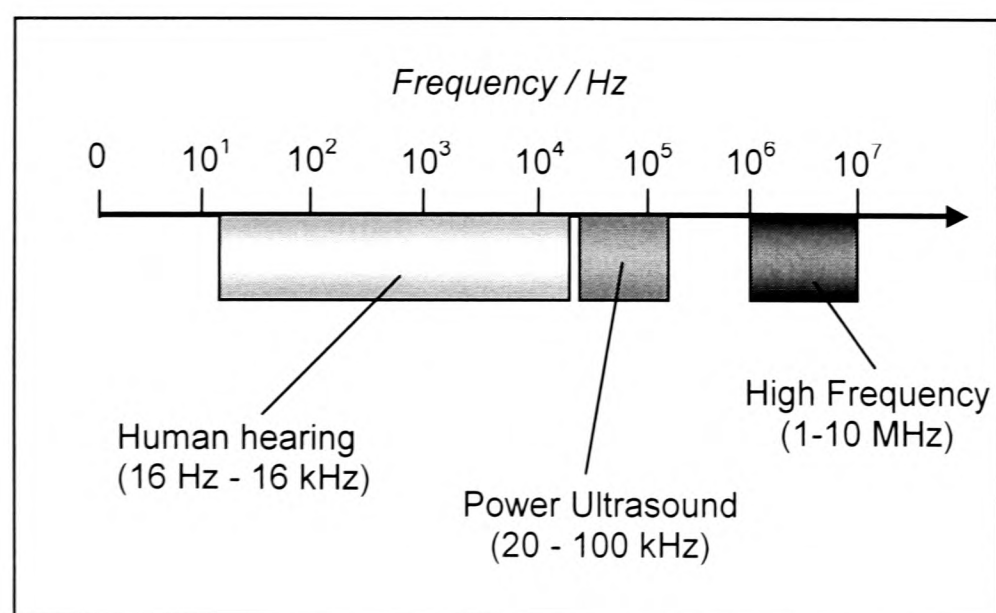


Fig. 1.24 - The sonic spectrum. Adapted from reference 62.

The use of ultrasound has proliferated in industry over the past 60 years, with applications varying from biology and biochemistry to plastics and polymers as outlined below in table 1.1.

Field	Application
Biology and Biochemistry	Cell rupture and homogenisation
Engineering	Drilling, grinding, cutting and welding
Dentistry	Cleaning and drilling
Geology	Pulse/echo techniques (e.g. SONAR)
Industrial	Mixing, cleaning, filtration and drying
Medicine	Fetal and surgical imaging and muscular physiotherapy
Plastics and Polymers	Welding, polymerisation initiation and polymer degradation

Table 1.1 - Industrial uses of ultrasound. Adapted from reference 62.

Ultrasound, more quantitatively, is a longitudinal wave that propagates through a medium in an identical manner to any other sound wave. Longitudinal waves are characterised by the fact that their motion is in the same direction as the oscillation of the particles, as opposed to transverse waves, the particles of which oscillate normal to the direction of motion. Considering the case where the medium is a liquid, the propagation of the sound wave causes the solution molecules to be continuously pulled apart, known as rarefaction, or pushed together, known as compression. The pressure experienced by the molecules at any point along the wave can be described by the sinusoidal equation (1.88)

$$P = P_0 \sin(2\pi ft) \quad (1.88)$$

where P_0 is the pressure applied by the transducer (acoustic pressure), f is the ultrasound frequency and t is time.

1.4.1 PHYSICAL EFFECTS OF ULTRASOUND

The propagation of longitudinal waves through a liquid causes rarefaction of the solution molecules as mentioned above. If the acoustic pressure (equation 1.88) is large enough to force the molecules to separate by a distance of approximately twice the Van der Waal's separation [63,64], the liquid breaks down to form a cavity or bubble. This

phenomenon is known as cavitation and the stability of these bubbles can either be long, surviving many cycles (stable cavitation) or short, collapsing only after a few cycles (transient cavitation). It is the collapse of these bubbles, however, that releases the large quantities of energy associated with them, either in terms of heat, causing a temperature rise of the solution, or as light, known as sonoluminescence [65].

It is possible for different forms of cavitation to occur in solution and generally can be described as either an empty cavity (true cavitation), a solvent vapour filled cavity, a dissolved gas filled cavity or a combination of a gas and vapour filled cavity. Taking the example of water, in order to generate true cavitation a critical separation of 4 Å must be achieved. This in turn requires a large amount of energy, approximate to an acoustic pressure of 4000 atm [63], which represents an ultrasound intensity of 5 MW cm⁻². This energy is substantially lowered by a factor of four if the cavity is filled with water vapour rather than existing as a true vacuum. Cavitation energies are further reduced through the existence of gases and particles that have been dissolved in the solution. This has the effect of creating 'weak spots' and enabling cavitation to occur at significantly lower pressures.

The cavitation nature of an insonated solution is directly dependent on various external factors, listed below.

- Frequency - Increasing the frequency of the ultrasonic wave has the effect of decreasing the time period of the rarefaction phase. As the production of a cavity requires a finite time to pull the molecules apart, shortening the rarefaction cycle makes cavitation difficult to achieve.
- Solvent Viscosity - The formation and collapse of cavities formed in solution creates large shearing forces to the liquid molecules. The greater the viscosity of the solution, the more resistance occurs to these shearing forces and hence the more difficult it is to produce cavitation.
- Solvent Surface Tension - As cavitation in liquids requires the formation of a liquid-gas interface, generally speaking, solvents with lower surface tensions facilitate cavitation events.

- Solvent Vapour Pressure - Cavitation events normally lead to the formation of bubbles into which vapour from the surrounding solution permeates. In solutions with a low vapour pressure, cavitation is extremely difficult as less vapour can enter the bubbles. Conversely, solutions with higher vapour pressures facilitate cavitation by producing vapour filled bubbles. This, however, has an unfortunate consequence that as the bubble collapses, the energy released is effectively cushioned by the surrounding vapour-filled bubbles and is therefore less energetic.
- Dissolved Gases - It is common practice to introduce a gas into a solution to maintain uniform cavitation. Using gases with larger specific heat ratios ($\gamma = C_p/C_v$) means that less energy can be absorbed by the gas during cavitation collapse ensuring more marked effects.
- External Pressure - As the external pressure on a liquid is increased, a greater rarefaction pressure is required to initiate cavitation. However, the increase in external pressure also brings about a concomitant increase in the intensity of cavitation collapse, which in turn can bring about greater sonochemical effects [66].
- Temperature - An increase in temperature to a solution raises the vapour pressure, which facilitates cavitation but leads to less violent collapse (mentioned above) [67]. In addition, a rise in temperature brings about a decrease in solution viscosity and surface tension, which also act to facilitate cavitation effects. If the temperature is taken too high, the cavitation events will become too great and the bubbles will form a barrier to the sound transmission, dampening the ultrasonic energy as it enters the liquid.
- Intensity - Sonic intensity is directly proportional to the square of the amplitude of vibration of the ultrasonic source. Generally speaking an increase in the intensity leads to an increase in the sonochemical effects on a system, with an upper and lower limit set by the ultrasonic source and solution respectively.

The introduction of ultrasound into a solution causes the sound waves to be dissipated via kinetic energy, resulting in a turbulent convective flow of solution that stems from the tip of the sonic horn and is directed away into bulk solution as illustrated in figure 1.25. This effect, known as acoustic streaming [68], is readily observed in solution with the naked eye and varies with the intensity of the ultrasound.

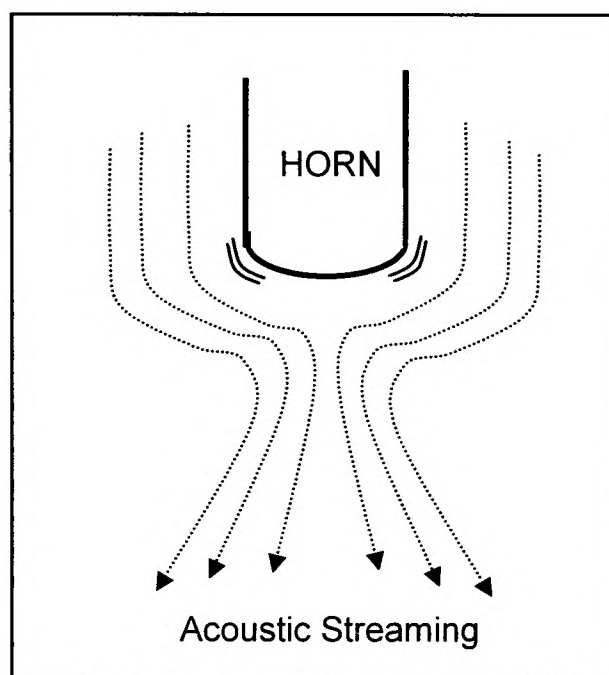


Fig. 1.25 - Schematic describing flow pattern generated by an ultrasonic horn in solution.

In addition to acoustic streaming, the convective flow of solution can be manipulated through the formation of microjets that occur in insonated solutions. The existence of microjets, or microstreaming, is a direct result of the asymmetric collapse of cavitation bubbles that have been formed in solution at the surface of the electrode. This is shown schematically in figure 1.26.

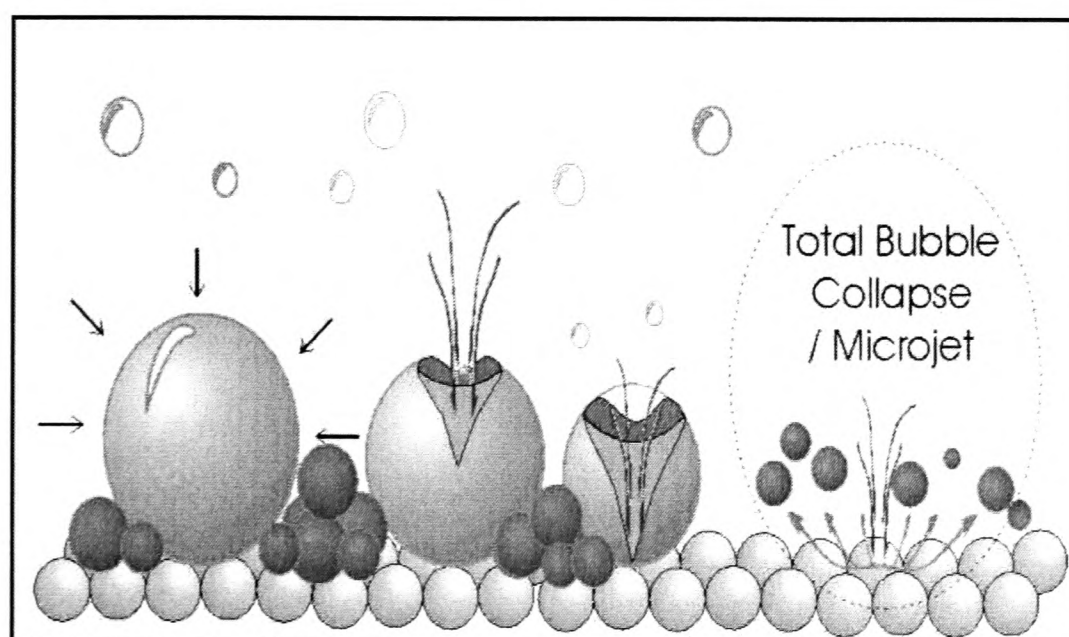


Fig. 1.26 - Asymmetric bubble collapse forming a microjet at the surface of an electrode.

Microstreaming can induce jets of liquid travelling at speeds of upto 100 m s^{-1} [69] and as a consequence can greatly increase mass transport and offer possibilities in surface activation / erosion.

1.4.2 SONOCHEMISTRY

The application of ultrasound to chemistry has been around since the 1940s, where it was introduced to polymer and chemical processes [70,71]. The use of ultrasonic baths for cleaning was widely implemented during the 1960s and lead to the introduction of ultrasound as a tool for enhancing chemical reactivity. Since then, ultrasound has steadily become entwined with various other chemical processes including synthesis, polymer chemistry and catalysis [72-79].

The chemical effects of ultrasound are dependent on the ultrasonic frequency and the composition of the solution (i.e. heterogeneous vs. heterogeneous). The effects of frequency on chemical behaviour are first considered.

The high pressures and temperatures associated with cavitation lead to the generation of radicals in solution [80,81] and as a consequence ultrasound is often used as a tool to initiate radical reactions [82]. In water, extreme pressures and temperatures of several hundred bars and several thousand degrees can be reached [83] resulting in the cleavage of water molecules, forming $\text{H}^\bullet$ and $\text{OH}^\bullet$ radicals, and molecular oxygen, forming O atoms as shown below



Transient $\text{OH}^\bullet$ radicals can either escape from the bubble and go on to react with other dissolved molecules in solution, or recombine to give



The effect of frequency on this reaction has been studied using aqueous KI solution to monitor the production of H_2O_2 and the oxidation of the iodide ion [84]. At high frequency (514 kHz) hydrogen peroxide is more efficiently produced whereas at lower

frequencies (20 kHz), a decrease in the production of hydrogen peroxide is observed. At higher frequencies, the bubble collapse is more rapid; thereby the $\text{OH}^\bullet$ radicals are given less time to react before leaving the bubble, resulting in the formation of H_2O_2 and I_3^- from reaction in solution. At lower frequencies, the radicals are given enough time to react in the bubble, forming other products such as H_2O and O_2 . The presence of oxygen serves to scavenge hydrogen atoms as shown below



and at low frequencies, the $\text{OH}^\bullet$ radical can be scavenged by the hydroperoxyl radical generated in (1.91) lowering the yield of H_2O_2 . Therefore at lower frequencies, H_2O_2 formation is more effective under argon. This is illustrated by figure 1.27.

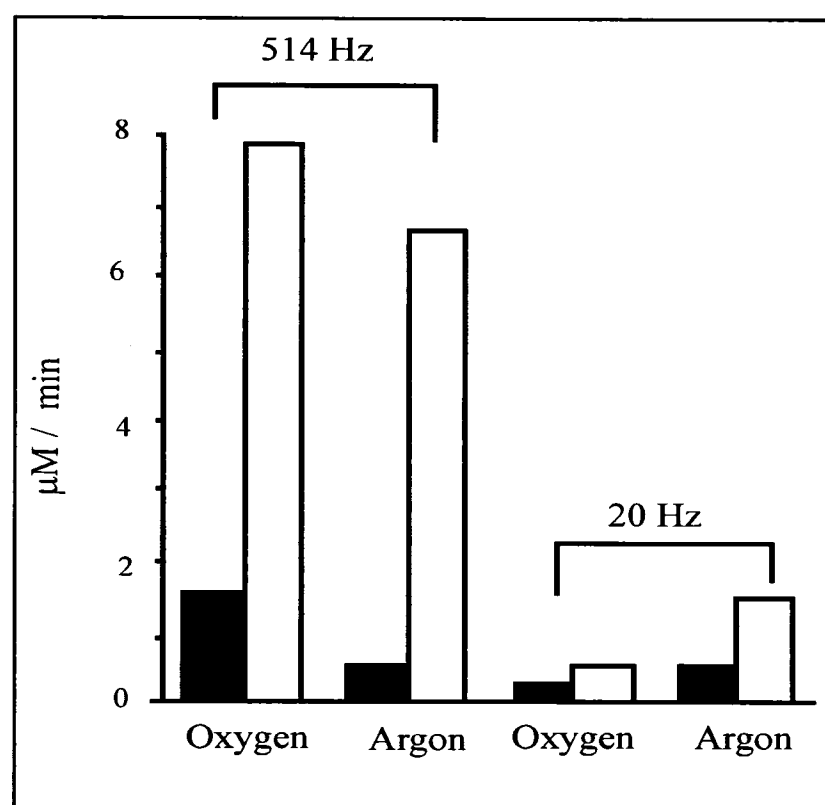


Fig. 1.27 – Graph showing the production of H_2O_2 (□) and I_3^- (■) as a function of frequency [84].

As mentioned above, the composition of the solution can also play a factor in influencing the chemical effects induced by ultrasound and, in addition to this, different reaction products can be formed under ultrasound in comparison to if the solution is mixed mechanically [85]. In homogeneous solutions, the course of the chemical reaction is modified by the presence of novel intermediates such as radicals that are formed from the collapse of cavitation bubbles.

With heterogeneous solutions, however, the influence of ultrasound is more complex. First, if the reaction involves surface catalysis, the rate of reaction is increased through the application of ultrasound via surface cleaning and enhanced mass transport. Considering the reaction between benzyl bromide and potassium cyanide with alumina, outlined below in figure 1.28,

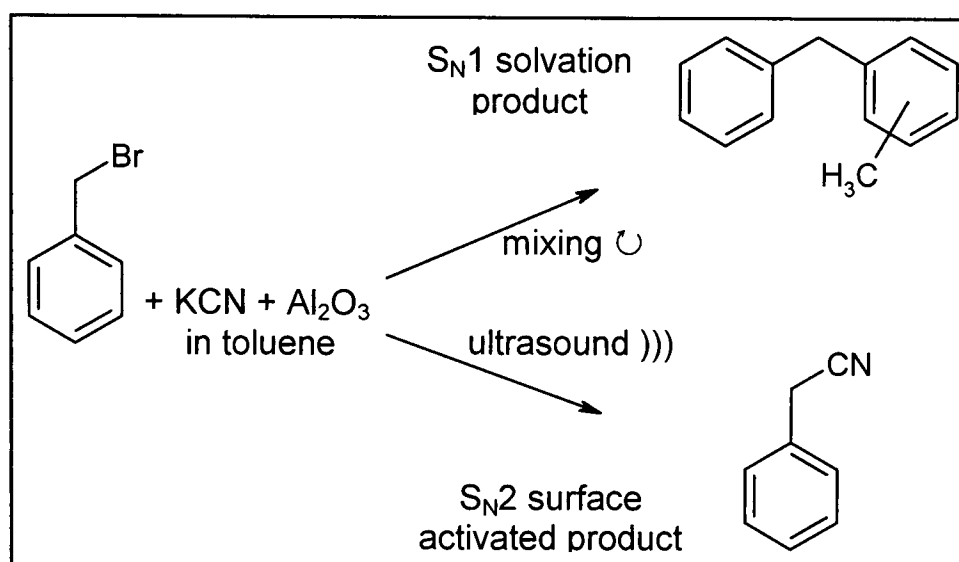


Fig. 1.28 – Surface activation via ultrasound.

the effect of ultrasound is thought to activate the alumina thereby promoting the reaction in favour of the S_N2 route. In the absence of ultrasound however, using conventional mixing, the S_N1 pathway is observed [86].

Secondly, the reaction pathway is promoted by the use of ultrasound if a radical intermediate or a single electron transfer (SET) is involved. The reaction scheme depicted below in figure 1.29 illustrates how ultrasound serves to promote the rate of the second SET and to generate radicals in solution that trap the intermediate following radical rearrangement.

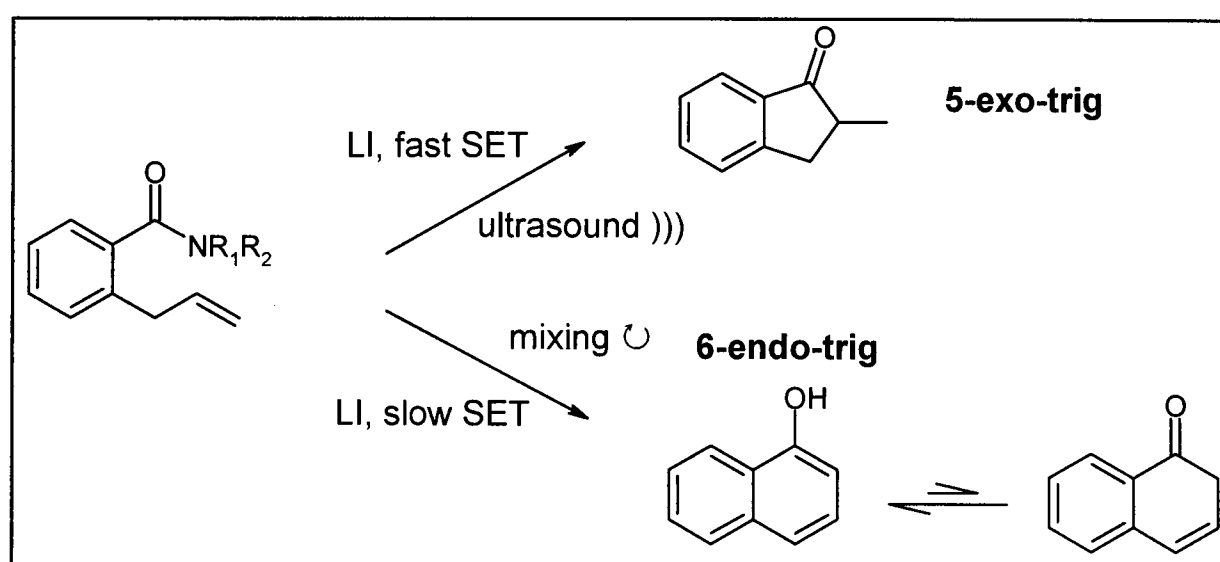


Fig. 1.29 – Radical promotion effect of ultrasound.

Under insonated conditions, the reaction favours the 5-membered ring kinetic product rather than with mechanically stirred solutions whereby the thermodynamic product of the 6-membered ring is formed.

Finally, ultrasound can facilitate chemical reactions in heterogeneous solutions if the inhomogeneity comes in the form of two immiscible liquids. The action of ultrasound enhances the mass transport across the phase boundary promoting mixing. An example of this is illustrated by the sono-emulsion Kolbe electrosynthesis [87].

1.4.3 SONOELECTROCHEMISTRY

The coupling of electrochemical techniques with ultrasound is often referred to as 'dual activation' and is an exciting and emerging area of research. This section aims to provide an overview of the fundamental sonoelectrochemical concepts [88,89].

The existence of cavitation and increased rates of mass transport via acoustic streaming and microstreaming from applying ultrasound to a solution gives sonoelectrochemistry numerous advantages in comparison to traditional, silent electrochemistry. These can be outlined as follows:

- The *in situ* cleaning effect observed through microjets formed near the electrode surface has the possibility of activating previously passivated or poisoned surfaces. This can be demonstrated through the example of Ni electrodes where under normal conditions, anodic sweeping passivates the electrode with a layer of an oxide rendering it inactive for electrochemical studies. Under the application of ultrasound, however, oxidations processes can be studied [90].
- The mass transport to the electrode surface under the application of ultrasound is considerably enhanced, through both acoustic streaming and microstreaming effects, when compared to silent or unstirred solutions [91].

- The formation of radicals and high-energy intermediates in solution from ultrasonic cavitation events can affect the route of an electrochemical reaction - leading to novel electrochemical syntheses [92,93].
- The degassing effect of ultrasound on a solution ensures that any gaseous product formed at the electrode surface is removed, ensuring regeneration of an active surface. This has been demonstrated in the production of ultrasonically enhanced yields of chlorine obtained through the electrolysis of hydrochloric acid [94].
- Ultrasound has also been shown to emulsify aqueous and non-aqueous phases, assist the dissolution of samples and more recently increase the leaching efficiency of analytes from dense solid materials [95,96].

In addition, there are a number of recent reviews detailing the advances in sonoelectrochemistry that have evolved over the past ten years [88,89,97-99].

1.4.3.1 SONOELECTROCHEMICAL CELLS

The application of ultrasound to an electrochemical system can be performed by a variety of different methods. The simplest of such methodologies is through the use of an ultrasonic bath, acting as either a container for the electrochemical apparatus, or as a solution vessel into which the experiments are performed [100]. These rather crude applications are prone to error in reproducibility as the electrode placement and cell geometry are crucial [101].

A more common approach, and is the method adopted by the work presented in this thesis, is through the use of a sonic horn that is attached to a transducer. The horn is then placed in the cell and can adopt essentially three possible configurations as illustrated in figure 1.30

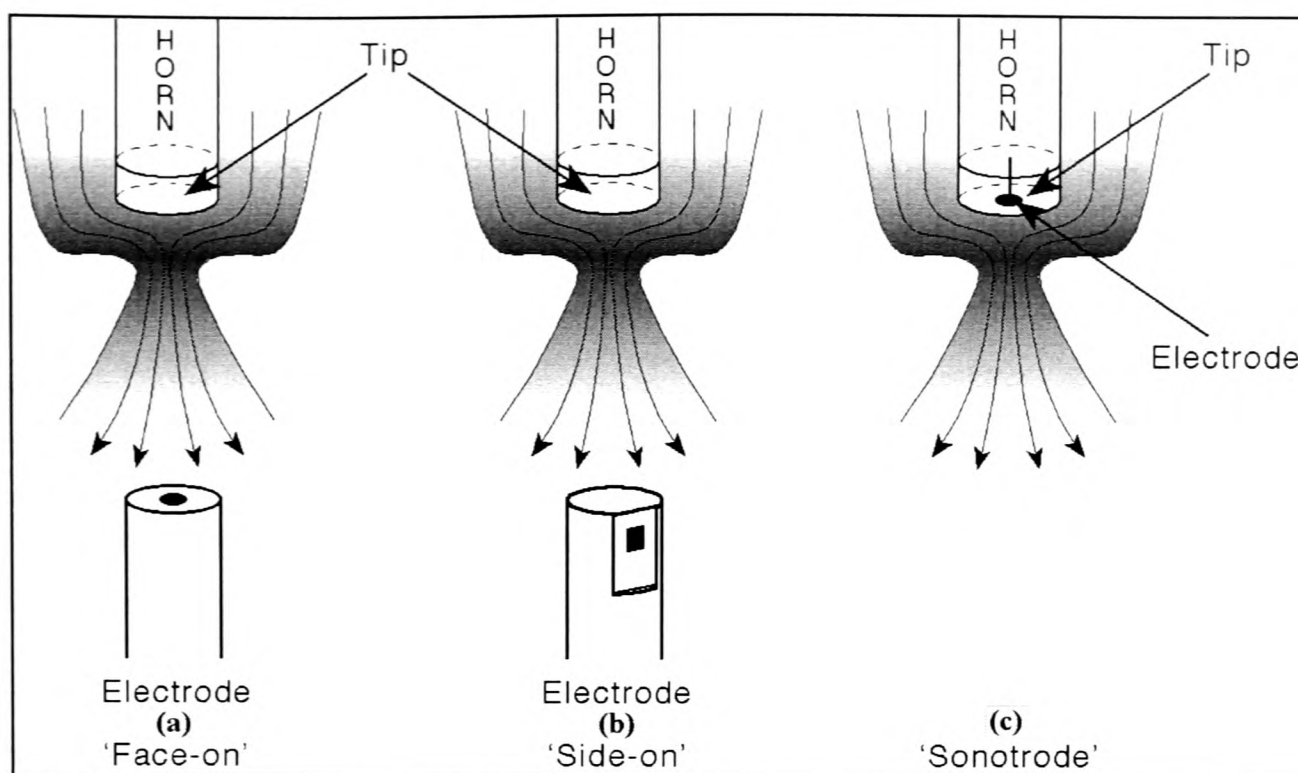


Fig. 1.30 - Schematic representing the three different sonoelectrochemical configurations achieved through use of an ultrasonic horn. a) face-on b) side-on and c) the sonotrode.

The more common configuration is shown in figure 1.30 (a) where the ultrasonic horn is placed directly opposite the electrode surface. This 'face-on' arrangement is exclusively dealt with in all the ultrasonic experiments presented later, as has been shown to be the more efficient in analytical applications [102]. This configuration is slightly modified to give the 'side-on' arrangement as shown in figure 1.30 (b) [103]. For both geometries, the electrode-horn separation can be closely regulated and as a result, a greater degree of reproducibility can be achieved.

The final geometry, shown in figure 1.30 (c) is known as the 'sonotrode' and offers the approach that the tip of the sonic horn further functions as an electrode [104,105]. This can be modified so that an electrode is housed in the horn tip, thereby giving a greater flexibility of the choice of electrode material [106]. In addition to the cavitation effects, as experienced by all three geometries, the sonotrode shows enhanced surface cleaning through vigorous shaking which can lead to removal of electrodeposited species [107], as highlighted through the production of fine metal powders for catalysis and metallurgy [105,108,109]. Diffusion layer thicknesses as low as 1 μm can also be obtained even at low ultrasound intensities, therefore providing a useful tool for the probing of fast electrode reactions.

1.4.3.2 MASS TRANSPORT I - ACOUSTIC STREAMING

The characterisation of ultrasonically enhanced mass transport has been the subject of scientific interest [91,110-112] and can be divided into two main areas - acoustic streaming and microjetting arising from cavitation collapse. These two modes of mass transport are dealt with in the following sections.

The effect of ultrasonically induced mass transport in comparison to silent voltammetry on the oxidation of ferrocene is shown in figure 1.31 [91].

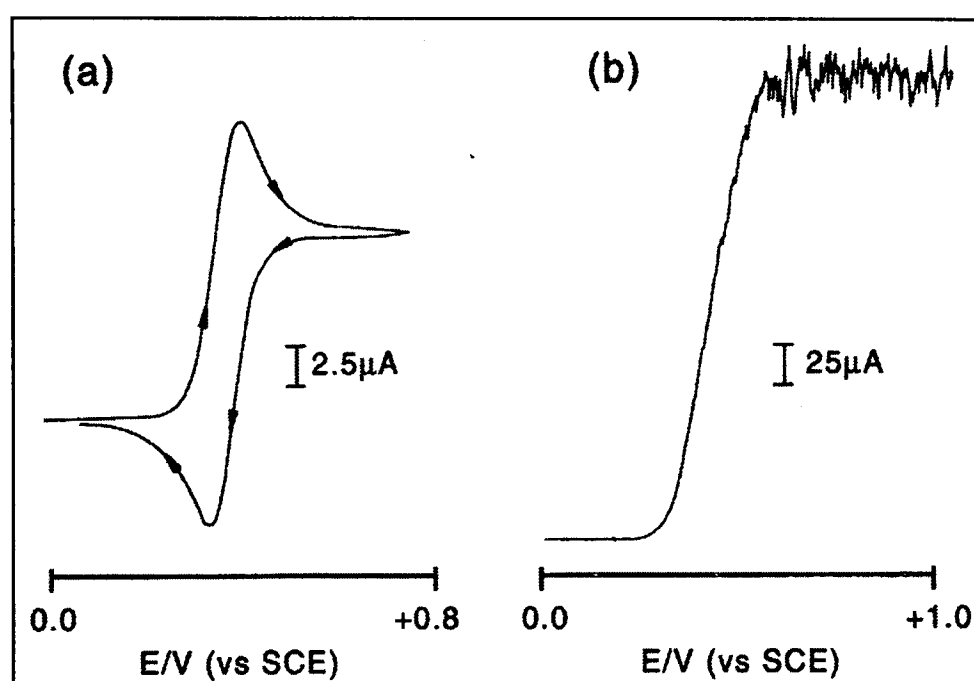


Fig. 1.31 - a) Cyclic voltammetry of 2 mM ferrocene in MeCN/TBAP at a 2 mm Pt disc and b) Equivalent voltammogram under 50 W cm^{-2} of 20 kHz ultrasound and an electrode-horn separation of 40 mm at 20 mV s^{-1} [91].

Under silent conditions, classical cyclic voltammetry is observed with well resolved peak heights operating under diffusional control. When ultrasound is applied, the shape of the voltammogram changes and a steady-state, limiting current is observed. The latter is consistent with voltammetry observed under conditions of forced convection with hydrodynamic systems such as the channel flow cell or rotating disc electrode. By analogy, the ultrasound operates to enhance the flux of electroactive species to and from the electrode, resulting in higher currents.

More quantitative analyses of the limiting currents under the influence of ultrasound have showed that the magnitude of the current, at low ultrasound powers, is directly proportional to the electrode area, bulk concentration of electroactive species and the diffusion coefficient [103]. Through the assumption of a Nernstian model for the mass

transport, assuming a linear concentration gradient in the diffusion layer as illustrated in figure 1.32, the limiting current can be related to the diffusion layer thickness, δ , via

$$I_{Lim} = \frac{nFAD_O[O]_{bulk}}{\delta} \quad (1.92)$$

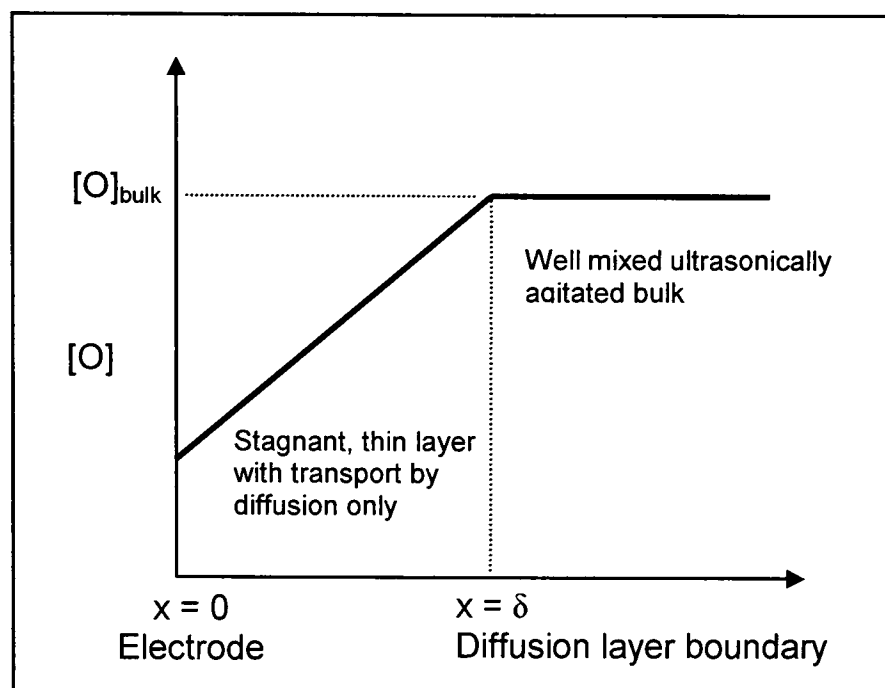


Fig. 1.32 - Simple diffusion layer model under ultrasonic conditions.

Values of δ obtained during the application of ultrasound, typically range from 1-3 μm . To achieve this using hydrodynamic voltammetry at the RDE, purely impractical rotation speeds between 5500 - 400 Hz would be required.

Application of the model proposed in figure 1.32 and equation (1.92) has been successful in the kinetic investigations of a number of sonoelectrochemical mechanisms [113-115]. The idea of a stagnant layer adjacent to the electrode has been applied to hydrodynamic electrodes such as the RDE, and it has been found that for hydrodynamic systems in general

$$I_{Lim} \propto D^{2/3} \quad \text{hence} \quad \delta \propto D^{1/3} \quad (1.93)$$

The comparison of equations (1.92) and (1.93) suggest the ultrasonic diffusion layer is dependent on both diffusion and convection. Further proof that sonoelectrochemical experiments are equivalent to hydrodynamic environments is illustrated through studies performed using the 'face-on' geometry in aqueous solutions [116]. These investigations have shown that

$$I_{Lim} \propto A[O]_{bulk} D^{2/3} \quad (1.94)$$

and such a relationship is characteristic of many hydrodynamic electrodes [2].

1.4.3.3 MASS TRANSPORT II - MICROJETTING

It is normally conceded that the majority of the mass transport within insonated solutions is carried by the effects of acoustic streaming [117,118]. The magnitude of the transport effects from microjetting formed from cavitation collapse have been studied and are illustrated in the reduction of $\text{Ru}(\text{NH}_3)_3^{3+}$ under high intensity ultrasound shown in figure 1.33.

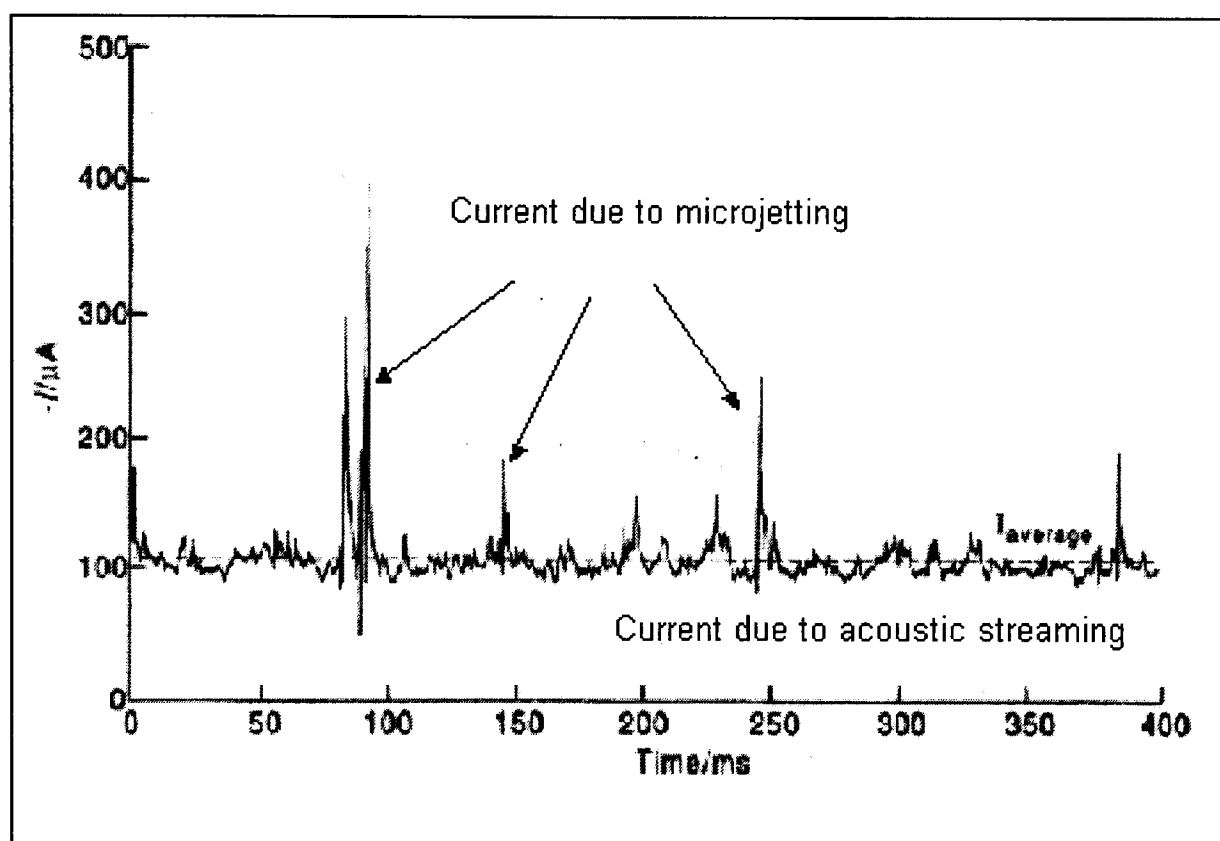


Fig. 1.33 - Reduction current of 1mM $\text{Ru}(\text{NH}_3)\text{Cl}_3$ (aq) as a function of time under insonation from 54 W cm^{-2} with a electrode-horn separation of 3 mm at a 2 mm copper coated mercury electrode [117].

From figure 1.33, the average current is maintained due to steady acoustic streaming effects and the current spikes is attributed to microjetting, from cavitation collapse.

Studies by Nonaka et al have reported the existence of a 'cavitation threshold' and refers to the minimum ultrasonic power required to induce cavitation events into solution [119] as illustrated in figure 1.34 below. Below this cavitation threshold, the mass transport is solely carried by acoustic streaming.

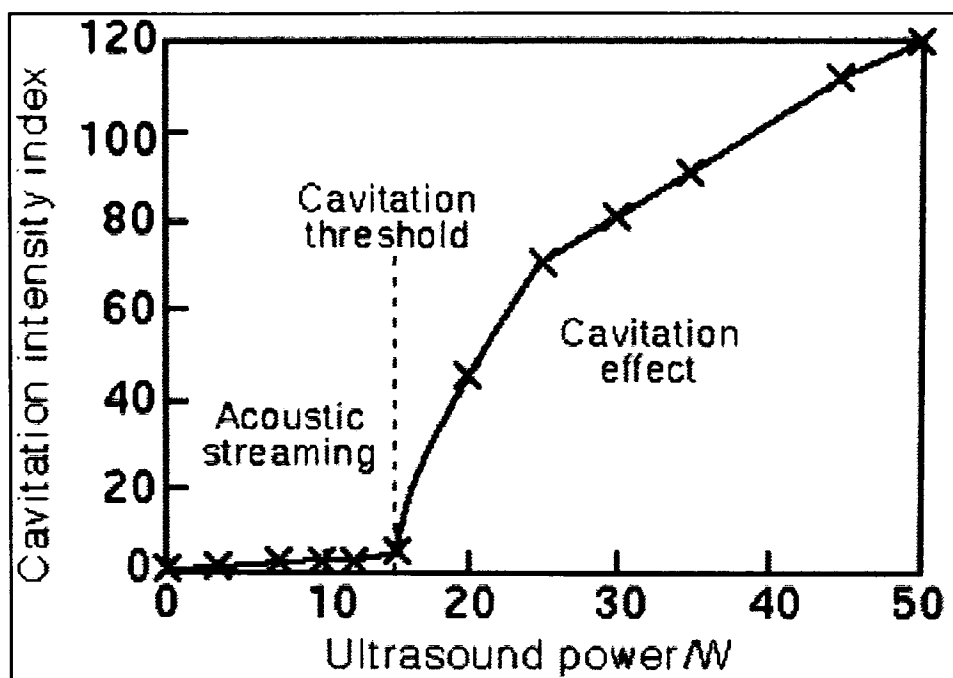


Fig. 1.34 - The onset of cavitation as a function of ultrasound power.
Measured using the reduction of *para*-methylbenzaldehyde [119].

The effect of microstreaming has been observed at both macro- and microelectrodes, and has been successfully modelled at the latter using a transient response [69,120-125] in approximation to a wall-tube electrode arrangement [126-128]. This has suggested the mechanism of an imploding cavitation bubble that compresses the diffusion layer but does not touch the electrode surface [123]. The compressed diffusion layer then relaxes back to steady state giving rise the observed transient behaviour. More recent work by Maisonhaute and co-workers has studied the mass transport effects attributed to the oscillation of ultrasonically created bubbles on the electrode surface using microelectrode arrays [98,99].

In conclusion, the application of ultrasound has shown to increase the rate of mass transport in two distinct ways. The majority of the mass transport is carried by acoustic streaming, which is particularly true at ultrasonic intensities below the cavitation threshold (figure 1.34), and has shown to be approximate to hydrodynamic behaviour. At progressively higher sonic intensities, the cavitation component comes into play, punctuating acoustic streaming effects with a certain element of microjetting. It is the interplay of these two phenomena that make the application of ultrasound extremely appealing to the analytical electrochemist.

1.4.3.4 SURFACE ACTIVATION

As previously mentioned, the presence of a collapsing bubble near the surface of an electrode can facilitate the eroding of adsorbed, chemically adhered or underlying electrode material. It is this action of cleaning that eventually leads to the cavitation damage common to electrodes that have been operating under insonated conditions [90]. The cleaning effect of the cavitation collapse has been harnessed to prevent electrode passivation by absorbing species; an example of such is the detection of copper ions in beer [129]. In silent conditions, no analytical signal is observed, whereas under ultrasound the removal of the adsorbed organic layer that has built up on the electrode surface permits a quantitative sonoelectroanalytical method.

In recent years through the work by Compton et al, this technique has been fully extended to encompass a library of other analytical protocols in challenging matrices. Examples of such include the quantitative detection of lead in wine [130] and petrol [131], nitrite in egg [132], nitrate in lettuce [133] and sewage [134], copper in blood [135] and fish gill mucus [136].

1.5 AIMS OF THE WORK REPORTED IN THIS THESIS

The overall aim for the work reported in this thesis was to develop new electroanalytical techniques that are enhanced through the activation of the working electrode. The research can be broadly divided into three areas:

- The first looks at the electroanalytical determination of nitrate and nitrite species through the activation of electrodes via chemical modification and the application of ultrasound. This 'dual activation' facilitated the analysis to encompass extremely challenging matrices such as sewage and lettuce.
- The second area of research looked at the modification of a channel flow cell using an electrode that was heated by rf-radiation. This achieved the fast local heating of the electrode surface in both aqueous and non-aqueous systems and was investigated with simple 1- and 2-electron oxidations and reductions as well as being extended to more complex ECE processes.

- The final area of research employed the use of microelectrodes with a high specificity for the target analyte and was employed in the electrochemical detection of anaesthetic gases. The possibility of the production of an electrochemical sensor was assessed through the study of compatibility with other important clinical gases required during anaesthesia.

1.6 CHAPTER 1 REFERENCES

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CHAPTER 2 - EXPERIMENTAL METHODS

This chapter aims to outline the experimental protocol and methodology used for much of the work presented in this thesis. More comprehensive reports of the specific experimental procedures are given in the relevant chapters.

2.1 CHEMICALS

Table 2.1 below details the chemicals used in the work presented throughout this thesis.

NAME	CHEMICAL FORMULA	SUPPLIER
1-Iodo-3-nitrobenzene	$\text{IC}_6\text{H}_4\text{NO}_2$	Aldrich
Acetonitrile	CH_3CN	Fisher
Argon	Ar	BOC
Dimethyl Sulfoxide	$(\text{CH}_3)_2\text{SO}$	BDH
Enflurane	$\text{ClCHFCF}_2\text{OCHF}_2$	Abbott
Ferrocene	$\text{Fe}(\text{C}_5\text{H}_5)_2$	Aldrich
Hexaammineruthenium (III) Chloride	$\text{Ru}(\text{NH}_3)_6\text{Cl}_3$	Aldrich
Hydrochloric Acid	HCl	Aldrich
Hydroquinone	$\text{C}_6\text{H}_4(\text{OH})_2$	Aldrich
N,N,N',N'-Tetramethylphenylenediamine	$\text{C}_6\text{H}_4[\text{N}(\text{CH}_3)_2]_2$	Aldrich
N,N'-Dimethylphenylenediamine	$\text{C}_6\text{H}_5\text{N}(\text{CH}_3)_2$	Aldrich
Nitrogen	N_2	BOC
Oxygen	O_2	BOC
Potassium Chloride	KCl	Fluka
Potassium Hexacyanoferrate (II)	$\text{K}_4\text{Fe}(\text{CN})_6$	Lancaster
Potassium Nitrate	KNO_3	Aldrich
Potassium Superoxide	KO_2	Aldrich
Sodium Nitrite	KNO_2	BDH
Sodium Sulfate Anhydrous	Na_2SO_4	Aldrich
Tetrabutylammonium Hexafluorophosphate	$[\text{CH}_3(\text{CH}_2)_3]_4\text{NPF}_6$	Fluka
Tetrabutylammonium Perchlorate	$[\text{CH}_3(\text{CH}_2)_3]_4\text{NClO}_4$	Fluka
Tetraethylammonium Perchlorate	$[\text{CH}_3\text{CH}_2]_4\text{NClO}_4$	Fluka
Tetrahexylammonium Perchlorate	$[\text{CH}_3(\text{CH}_2)_5]_4\text{NClO}_4$	Fluka
Tris(4-bromophenyl)amine	$(\text{BrC}_6\text{H}_4)_3\text{N}$	Aldrich

Table 2.1 - Chemicals used in the work presented in this thesis.

2.1.1 SOLUTION PREPARATION

Aqueous solutions were prepared using Elgastat (High Wycombe, Bucks) UHQ grade water with a resistivity of no less than 18 M Ω cm. Non-aqueous solutions were prepared using either acetonitrile or dimethyl sulfoxide (DMSO) solvents, purchased in the highest possible grade.

When preparing solutions from solids, masses were weighed with an Ohaus Explorer balance (model EORR80, Ohaus Corporation, NJ, USA) with an accuracy of ± 0.1 mg. Prior to experimentation, the solutions were purged of dissolved oxygen through either the bubbling of an inert gas, such as nitrogen or argon, for a period of 20 minutes or by placing the solution under a vacuum (approximately 10 mm Hg) and then allowing argon to gently bubble through the solution.

2.2 ELECTROCHEMICAL EXPERIMENTS

2.2.1 CONTROL ELECTRONICS

A computer-controlled potentiostat (μ -Autolab or PGSTAT 30 Autolab, Eco Chemie, Utrecht, Netherlands) operating through GPES PC software (version 4.7) was used to control the potential applied to the working electrode. For experiments conducted under ultrasound, the probe of the titanium sonic horn was insulated from the transducer through a thin TeflonTM disc connected via a screw thread made of DelrinTM. This prevented any unwanted electrolysis of the solution at the titanium probe and obviated the need for bipotentiostatic control of the sonic horn [1].

2.2.2 WORKING ELECTRODES

The working electrodes used were all fabricated in-house (PTCL Mechanical Workshop, Oxford University) and consisted of the electrode material sheathed in a PTFE support. The electrode area was measured to an accuracy of 0.01 mm using a travelling microscope. The microelectrodes employed varied from 5 to 60 μ m diameters in size and were either bought from a commercial supplier (Microglass Instruments, Greensborough, Australia) or made by Dr. B. A. Coles (PTCL, Oxford University). The

electrode diameters were carefully calibrated using a known concentration of ferrocyanide in aqueous solution and using the appropriate diffusion coefficient as reported in the literature [2]. The microelectrodes were thoroughly inspected using an optical microscope at the start and end of each experiment to ensure the electrode surface had not recessed into the electrode surface.

The working electrodes were polished prior to each electrochemical experiment using either alumina slurries or diamond pastes (Kemet, Kent, UK) of decreasing particle size ranging from 25 to 0.3 μm , in agreement with the procedure outlined by Bond et al [3].

2.2.3 REFERENCE ELECTRODES

In the aqueous and acetonitrile experiments, a saturated calomel electrode (SCE, Radiometer, Copenhagen) was employed as the reference electrode in conjunction with a spiral-wound Pt wire acting as the counter electrode. The SCE, shown below in figure 2.1, is based on the $\text{Hg}_2\text{Cl}_2 \mid \text{Hg}$ couple (as mentioned in section 1.1.3), the half-cell of which is represented by



with a corresponding E° value equal to +0.242 V.

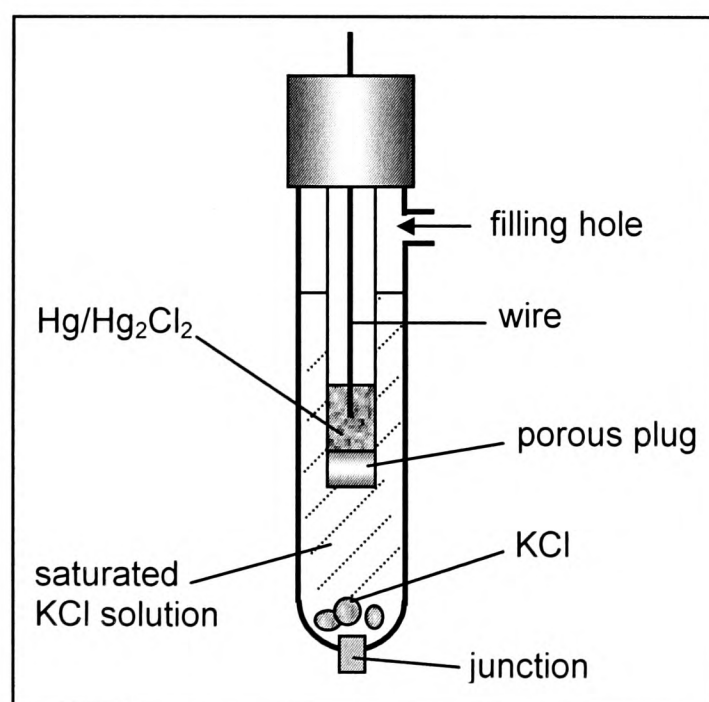


Fig. 2.1 - Schematic diagram of a SCE reference electrode.

The use of saturated KCl keeps the $[Cl^-]$ constant over long periods of time achieving high stability - a requirement of a good reference electrode. For the microelectrode experiments conducted in DMSO, a two-electrode arrangement was adopted using a pseudo-reference/counter electrode composed of a 0.5 mm thick Ag wire. This was measured to give a potential 220 mV more negative than compared with the SCE.

2.3 SONOELECTROCHEMICAL APPARATUS

2.3.1 SONOELECTROCHEMICAL CELLS

The electrochemical experiments performed under ultrasound presented in this thesis were used in the common three-electrode set-up [4], as illustrated in figure 2.2,

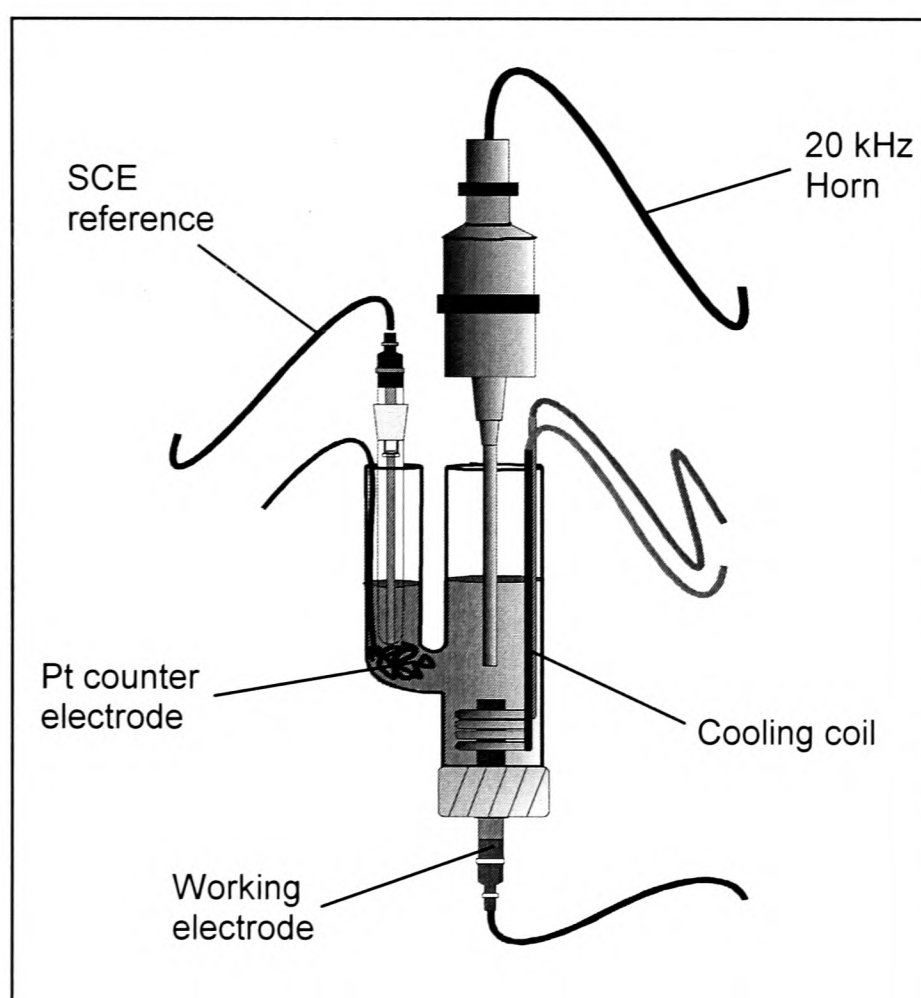


Fig. 2.2 - Sonoelectrochemical cell set-up employing a 20 kHz sonic horn.

using a cell volume of approximately 40 cm^3 and using a Sonics and Materials ultrasonic horn (model VCX 400) operating at 20 kHz. The horn tip was composed of a 3 mm diameter titanium alloy (90 % Ti, 6 % Al and 4 % V) so as to achieve a high fatigue strength, low acoustic loss and chemical inertness. The ultrasonic waves are generated via a transducer consisting of a crystal of a piezoelectric material [5-7], typically $BaTiO_3$ or $PbNb_2O_6$. Upon application of an oscillating potential, geometric distortions occur in

the crystal which are then translated into tiny vibrations creating an ultrasonic wave. The power is then focused into the horn tip through transducers which are multiples of $\lambda/2$ long. As a result, ultrasonic powers of upto 300 W cm^{-2} can be readily achieved at a 3 mm diameter horn tip.

A consequence of the vast power generated through ultrasonic waves is observed in the heating of the solution. Thermostating of the bulk solution is therefore often employed with the use of a water pump, forcing water through a cooling coil. This ensures that during insonation the surrounding solution is maintained at a constant temperature (± 2 °C).

2.3.1 POWER CALIBRATION

The intensity calibration of the sonic horn was performed using protocols outlined by Margulis [8] and Mason [9] over the range of 0-300 W cm^{-2} , and is related to the horn tip area and the power supplied through

$$\text{Intensity} = \frac{\text{Power}}{\text{Area}} \quad (2.2)$$

The power of the ultrasound can be calorimetrically calibrated through the monitoring of the temperature rise of the surrounding solution and using the relation shown below,

$$\text{Power} = C_p m \left(\frac{dT}{dt} \right)_{t=0} \quad (2.3)$$

where C_p is the specific heat capacity of water, m is the mass of liquid, T is the temperature and t is time. After activation of the horn, the temperature was recorded as a function of time and the initial slope of the graph was used to calculate the power. This procedure was repeated at different amplitudes and a calibration graph was determined allowing values of ultrasound intensities to be quoted.

2.4 Rf-HEATED CHANNEL FLOW CELL

Novel heating through the application of Radio frequency (Rf) is commonly used in industry [10] and is based on the principle of the induction of eddy currents in a metal slab. The metal is of a fixed resistance and therefore causes a concomitant rise in temperature, which can then be used to radiate heat. The application of 8 MHz Rf radiation is shown to cause effective heating of a 40 μm thick platinum foil mounted in a channel flow cell [11] which is then used as the working electrode of an electrochemical system. The experimental set up is illustrated in figure 2.3.

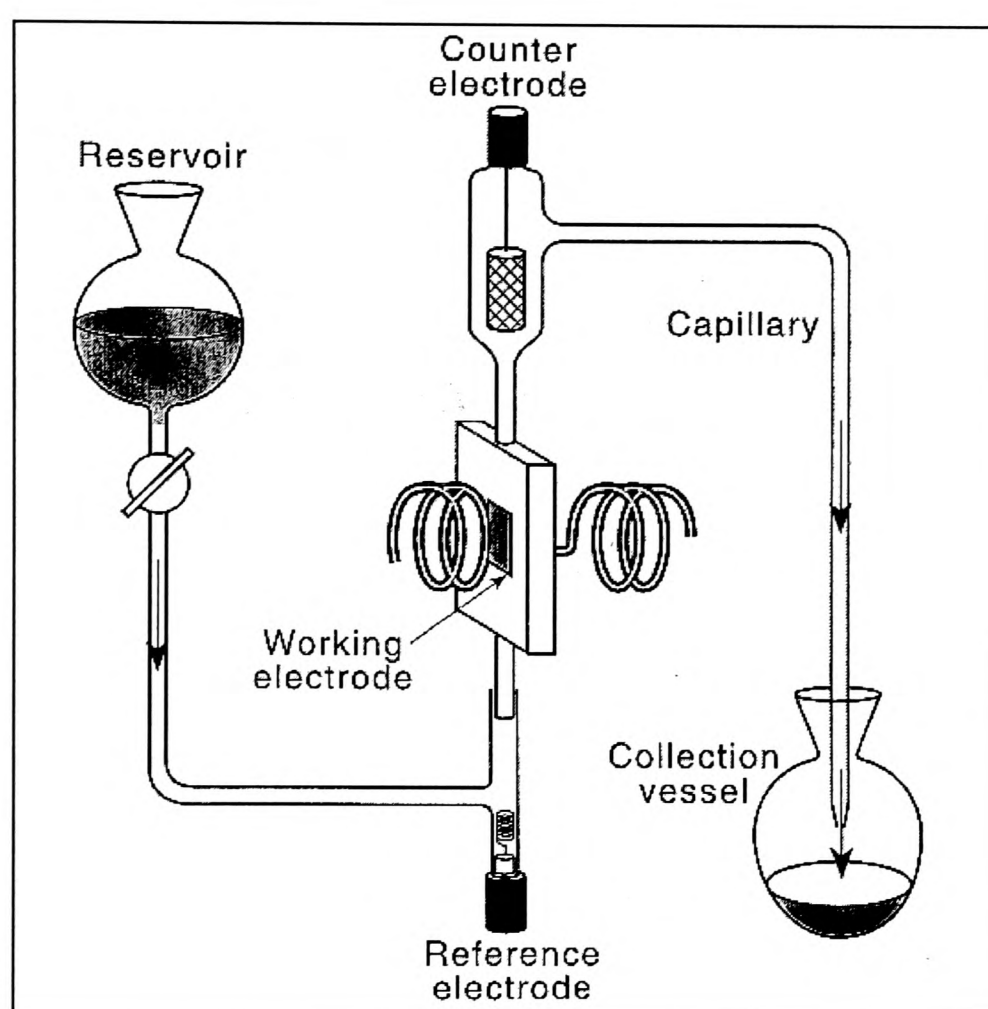


Fig. 2.3 - Rf-heated channel flow cell.

The design of the channel cell is discussed previously in section 1.3.3. The solution is passed through the channel by means of gravity-driven flow and collected at the end of the system. The flow rate was accurately determined through a capillary which was calibrated for both aqueous and acetonitrile solvents after the solution had been subsequently cooled to room temperature by passing through a stainless steel coil submerged in a beaker of water. The 8 MHz Rf field was applied to the channel cell through a water-cooled copper coil, which was placed perpendicular to the working electrode.

The electrochemical measurements were investigated at a 4.0 x 4.1 mm platinum foil working electrode of 40 μm thickness mounted in a channel cell of dimensions 6.0 mm length and 0.36 mm depth, in conjunction with a SCE reference electrode and a platinum mesh counter electrode. An approximate assessment of the electrode temperature was made through use of a 0.05 mm Pt/Rh Pt90/Rh10 thermocouple wire connected to the centre of the working electrode, whereas a more rigorous evaluation was performed using the half-wave potential shift of a reference system as a function of temperature. For aqueous and acetonitrile solvents, the reference systems used were ferrocyanide and ferrocene with a characterised half-wave potential shift of dE/dT equal to -1.53 and 0.53 mV K^{-1} respectively [2,12].

The potentiostat, thermocouple and Rf system were controlled via a PC and a safety shutdown option was used to prevent the electrode from overheating. The Rf generator was a Radyne C 12/B unit (Radyne Ltd., Wokingham, UK) modified by replacement of the single-phase power supply with a three-phase unit and with a driver circuit added to allow fast on-off control by the PC.

2.5 HEATED MICROELECTRODE CELL

The microelectrode high temperature experiments were carried out using a cell approximately 20 cm^3 in volume heated by hot air circulation within a small box of insulating material with a front glass wall. A Pt resistance thermometer was used to control the air temperature and a thermocouple in contact with the solution was used to read the temperature during the voltammetric scans. A 25 μm diameter platinum disc (99.99 %, Goodfellow Cambridge, UK) sealed in Pyrex glass was used as the working electrode, with a platinum coil counter electrode and a SCE maintained at room temperature was used as the reference electrode, shown in figure 2.4.

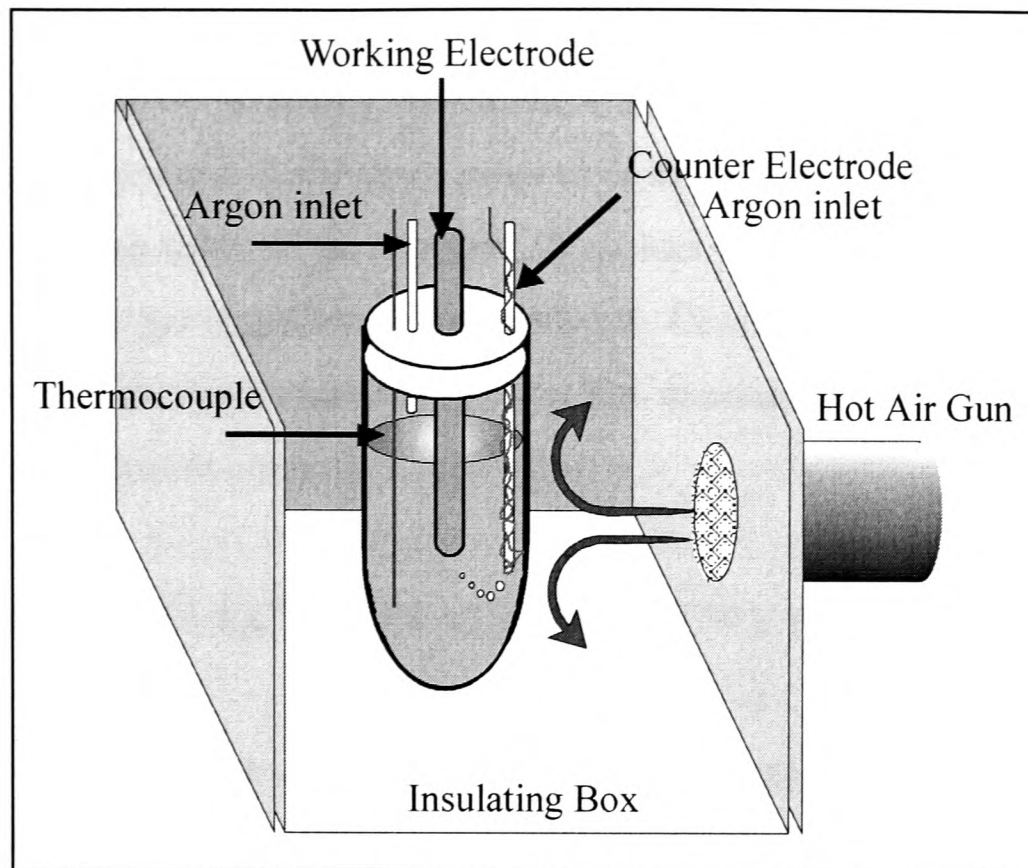


Fig. 2.4 - Electrochemical set up for the microelectrode high temperature experiments.

2.6 ANAESTHETIC GAS EXPERIMENTS

The electrochemical cell used throughout the experiments is shown in figure 2.5 and was thermostatted at 25 °C (± 0.5 °C) via a pump-driven water bath.

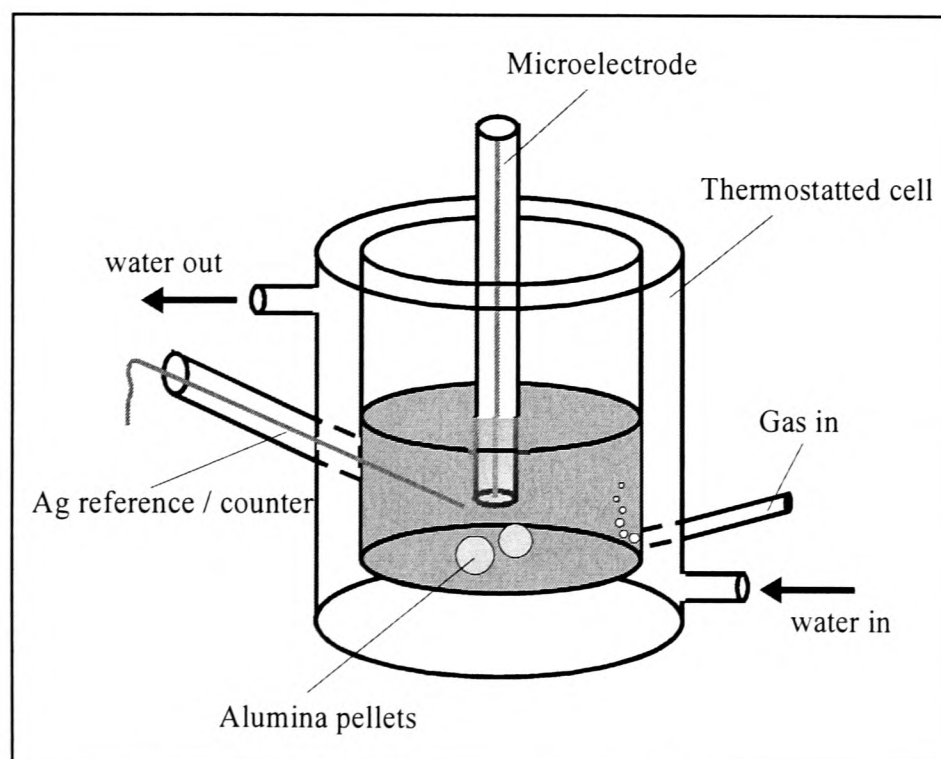


Fig. 2.5 - Electrochemical apparatus used in conjunction with the anaesthetic gases.

The enflurane anaesthetic agent was introduced into solution either as gravimetric aliquots in liquid form or via the gas, bubbled through the solution prior to each electrochemical sweep. In the latter case the enflurane gas was produced using a

commercial clinical vaporiser (PPV enflurane vaporiser, Penlon, Ltd.) and introduced into the cell as a v/v % dilution with pure N₂ gas (BOC). A respiratory gas monitor (Hewlett Packard model M1025A, Bracknell, U.K.) was used to accurately determine the concentration of the anaesthetic agent prior to dissolution into the solvent. The gas was bubbled through the solution for a period of no less than 20 minutes to ensure that full equilibration of the gas and solvent was achieved. Cessation of the gas supply during the voltammetric sweeps was important to ensure that measurements were taken in stationary solution, under diffusion-controlled conditions.

2.7 SCANNING ELECTRON MICROSCOPY (SEM)

Surface characterisation is widely performed under vacuum conditions using scanning electron microscopy (SEM) techniques [13] in which electrons rather than light are used to form an image. A schematic diagram of a typical SEM set up is shown in figure 2.6.

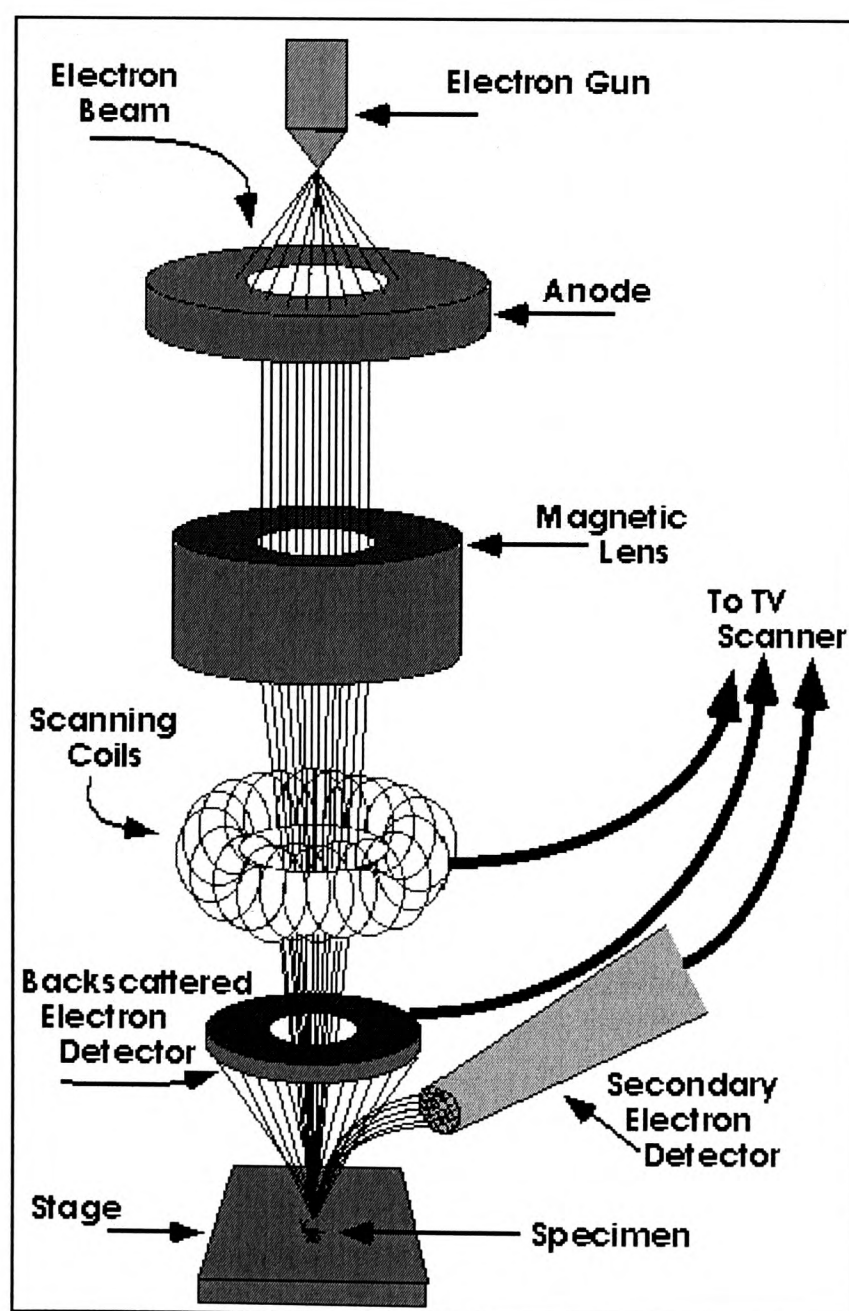


Fig. 2.6 - Schematic of a scanning electron microscope.

A heated metallic filament is employed for the production of a beam of electrons situated at the top of the microscope. Electromagnetic lenses are then used to focus and direct the beam towards the sample as it passes down through the microscope. On reaching the sample, other electrons (backscattered or secondary) are then ejected, which are then collected by detectors and converted to a signal and ultimately produced as an image.

Surface characterisation experiments presented in this thesis was carried out using a Cambridge Stereoscan scanning electron microscope at a magnification of 661x.

2.8 ATOMIC FORCE MICROSCOPY (AFM)

Surface morphology data used in this thesis was obtained using atomic force microscopy surface imaging performed using a Topometrix TMX 2010 Discoverer SPM (scanning probe microscope) system in contact mode (typically 3 Hz scan rate) with SFM probes (type 1520-00, Topometrix, CA, USA) and using Pyramidal silicon nitride tips (Topometrix AFM E-Chem probes 1750).

Deflection of the microscope cantilever during surface scanning was measured through a laser beam (wavelength 670 nm and power 3 mW) after it had been directed onto the back of the cantilever and reflected onto a position sensitive detector (PSD) consisting of two photoactive segments. The movement of the cantilever resulted in a movement of the reflected beam on the PSD, which, in turn, could be translated into a surface profile. The size of the scans ranged from 5 x 5 μm to 50 x 50 μm with a vertical range of ca. 10 μm . The basic operation of an AFM instrument is illustrated in figure 2.7.

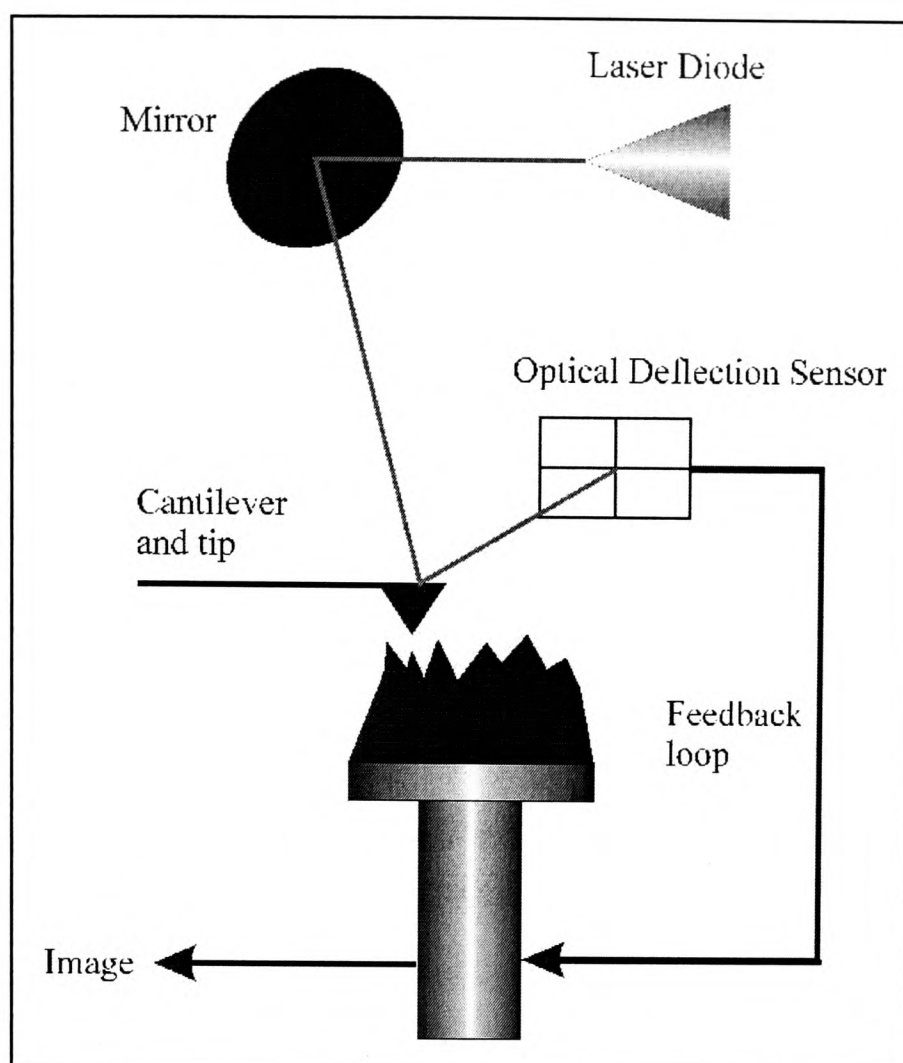


Fig. 2.7 - Schematic showing the operation of an AFM machine.

2.9 CHAPTER 2 REFERENCES

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CHAPTER 3

DETECTION AND DETERMINATION OF NITRATE AND NITRITE - A REVIEW

*The work presented in this chapter has been published in
Talanta 54 (2001) 785
and for the purpose of this thesis has been updated to incorporate
the recent advancements made over the past 2 years.*

In order to put the early work of this thesis into context (chapters 4-6), a review of the strategies employed to facilitate the detection, determination and monitoring of nitrate and/or nitrite is presented in this chapter. A concise survey of the literature covering 223 reports submitted over the past decade has been compiled and the relevant analytical parameters (methodology, matrix, detection limits, range etc.) tabulated. The various advantages / disadvantages and limitations of the various techniques have been exposed such that the applicability of a technique developed for one type of matrix can be meaningfully assessed before attempting to transfer the technology to another.

3.1 INTRODUCTION

Nitrate and nitrite are ubiquitous within environmental, food, industrial and physiological systems and while our understanding of their role within such matrices has increased, a substantial degree of uncertainty and speculation remains. These ions have been profitably exploited throughout the ages to further the development of our various societies but there is no doubt that our affection for them has waned in recent years. Our incessant use of and indeed reliance upon these versatile agents combined with revelations of their potential toxicity have raised numerous concerns [1,2]. These problems have been widely recognised and, as a consequence, statutory frameworks aimed at controlling their level within the wider environment and within food products have been imposed in most industrialised countries [3,4]. The restrictions placed upon the commercial utilisation of these ions have eased some of the apprehensions raised by the medical community but the establishment of adequate controls can only be achieved

by fully exposing their influence on the various pathways that govern environmental and physiological well being.

Our need and desire to monitor these ions is unquestionable yet their ubiquity can pose a significant challenge to the analytical community. Few techniques possess sufficient generic applicability to enable their detection amongst the huge number of potential interferences that can be encountered within environmental, food, industrial and physiological samples. As such, a large number of protocols, encompassing almost all major analytical methodologies, have been developed to overcome the peculiarities of the various matrices. The aim of this review has been to provide a representative survey of the scientific literature covering the detection of these important analytes and provide a concise appraisal of the relative merits of each approach. A number of excellent reviews have been compiled over recent years but each has narrowed their remit to specific matrices or techniques [5-11]. Through tabulating the various analytical parameters (detection limit, range, matrix, etc) of each system and exposing their advantages and limitations it was hoped that the specialism divides inherent within such a diverse subject such could be bridged.

The decision to cover both nitrate and nitrite was made on the basis that their chemistries are inextricably linked and as such one is rarely found without the other. Indeed, their inter-conversion, particularly the chemical reduction of nitrate to the more reactive nitrite, features strongly within many of the reports and is often the only way in which the relatively inert nitrate ion can be detected. The simultaneous detection and speciation of these ions has however gained increasing interest as the checks and balances that mediate many environmental processes have become clearer. Some of the more common reaction pathways that form the backbone of the detection strategies assessed herein are shown in figure 3.1.

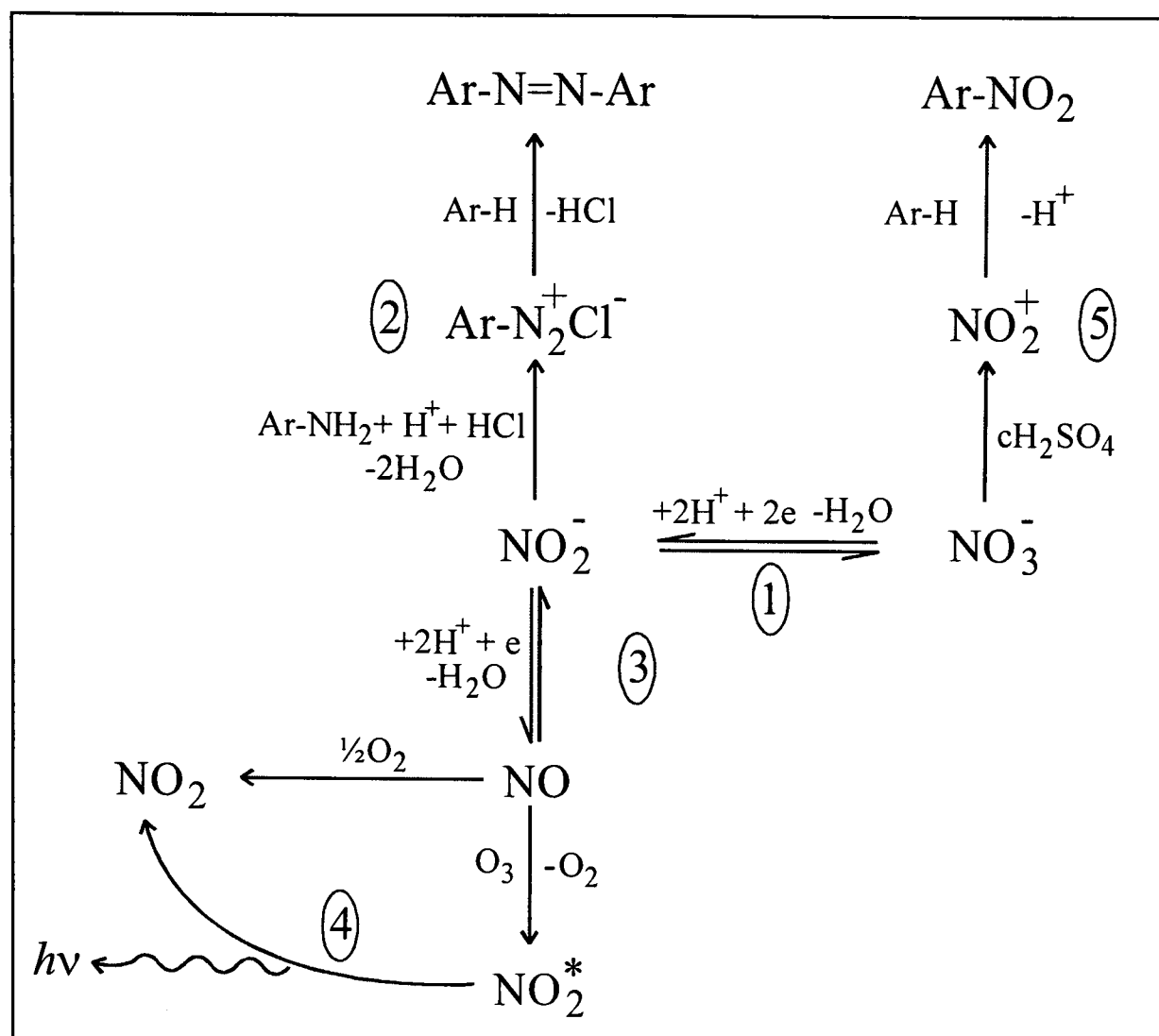


Fig. 3.1 - Common reaction pathways that form the basis of nitrate/nitrite detection strategies.

3.1.1 BACKGROUND PERSPECTIVE

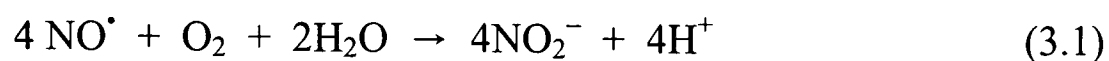
Nitrate and nitrite have become intertwined with domestic life and it is effectively impossible to engage in everyday activities without encountering these ions or the products of their use. The chemical versatility of these agents has ensured their utilisation within a multitude of industrial processes ranging from the manufacture of fireworks to the production of the latest dyes. Their anti-microbial action has been recognised for centuries and still used for the preservation of meat produce (E249, E250, E251 and E252) today [12]. Despite the huge number of products that are reliant upon these ions it is their association within environmental issues that have captured the interest of the public, and a significant proportion of the scientific community. Inputs of nitrate and nitrite to the environment can occur through industrial and domestic combustion processes with gaseous NO_x species converted to NO_3^- through photochemical conversion within the atmosphere [13-17]. The vast majority however arise from agricultural sources [18].

The misuse of inorganic fertilisers combined with the more general mismanagement of our natural resources have been suggested as resulting in the perturbation of both local and global nitrogen cycles [18,19]. The consequences of our environmental manipulations are as yet uncertain and therefore monitoring the ecological fate of nitrate and nitrite has gained increasing importance. The high solubility and mobility of these ions within the soil and our continued reliance on inorganic fertiliser has led to reports that “run off” is a continual hazard wherever agricultural processes are in close proximity to surface water [20]. Eutrophication of lakes and more recently coastal outfalls are thought to result in the generation of algal blooms that wreck havoc with local ecological systems [21,22]. The contamination of edible shellfish and the occurrence of “red tides” of potentially toxic algae near tourist resorts can also impart a degree of economic misery to the afflicted communities [23].

The potential contamination of groundwater through the percolation of nitrates through natural aquifers presents the most immediate risk to health [1,2] and as such, maximum permissible levels for these ions in drinking water supplies is often levied and currently stands at 50 mg L⁻¹ in the UK [3,4,24]. The two main threats to health that arise from the ingestion of these ions are reported as “blue baby” syndrome and gastric cancer [1,2,25,26]. In both cases the principal protagonist is nitrite obtained directly from contaminated water supplies or derived from the reduction of nitrate by the multifarious bacterial colonies that reside within the mouth. Passage of nitrite into the blood stream results in the irreversible conversion of hemoglobin to methemoglobin with oxygen uptake and transportation compromised [2]. This is particularly hazardous for infants given their limited physical stature and the susceptibility of their neural development to impeded oxygen transport. A more contentious health concern is the possible formation of carcinogenic nitroso-amines within the acidic conditions of the stomach and their subsequent implication in the pathology of gastric cancer [1,27]. Upon reaching the stomach, nitrite is converted to nitrous acid which can act as a powerful nitrosating agent. While nitrosoamines can be carcinogenic, conclusive evidence linking nitrite ingestion with the stomach cancer remains elusive [2].

The presence of nitrate and nitrite within physiological systems is often viewed with considerable and, as noted above, justifiable concern. Ingestion is not however the sole source by which these ions can arise within physiological systems. The endogenous

production of nitrate and nitrite within tissue can occur as a result of the activity of their more transient cousin, nitric oxide [28]. The reaction of nitric oxide with oxygen leads to the production of nitrite (equation 3.1) and it is through the greater stability of this ion that the action of NO can be detected [29].

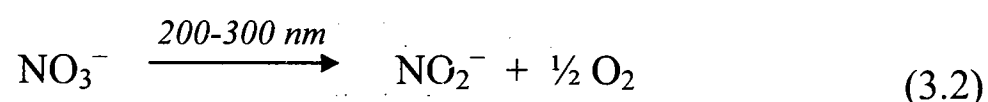


Nitric oxide has been shown to play an important role in many metabolic functions, including the regulation of vascular tone, inhibition of platelet aggregation, neurotransmitter, cytotoxic agent, thrombosis, inflammation and may also play a role in the immune system [30-32]. The measurement of nitrite can therefore provide a reliable measurement of NO action within the body and as such can be used as a biomarker that enables physicians to gauge the health of an individual [33]. This has particular importance when assessing inflammatory processes as the level of nitrite can be correlated to the degree of injury. Among the more common ailments to which the monitoring of nitrate and nitrite can be beneficial are sepsis [34], infectious gastroenteritis [35], meningitis [36], Parkinson's disease [27], minimal change nephritic syndrome in children [37], preeclampsia [38] and rheumatoid arthritis [39].

3.2 DETECTION METHODOLOGIES

The techniques that have been marshalled in our attempt to monitor nitrite and nitrate are compiled into tables 3.1 and 3.2 respectively. A third table is included which comprises reports that have dealt with the analysis of both analytes either simultaneously or sequentially. Simultaneous techniques include examples such as electrochemical and capillary electrophoresis, whereby the analytes are detected independent of one another in a single measurement. Sequential analysis is formed on the basis of detecting the more versatile nitrite anion initially, followed by bulk reduction of the sample (e.g. Cu/Cd column) to ensure that all the nitrate is converted to nitrite and repeating the nitrite analysis. Calculation of the nitrate concentration can then be obtained by difference. The characteristics of each strategy have been distilled and critically appraised according to the methodology applied and are discussed within the following sections.

It can be seen that there are nearly as many entries for nitrate as there are for nitrite. This observation must be clarified in that while the majority of the detection strategies for nitrate actually rely upon the detection of nitrite [40-47], the main impetus of the reports presented in table 3.2 was nitrate determination. Those techniques that utilise nitrite as an intermediate in the determination of nitrate have been highlighted in column 3 in table 3.2. The relatively inert nitrate ion is normally chemically reduced (route 1, figure 3.1) to the more reactive nitrite before initiating the detection sequence. A variety of reducing agents have been investigated to facilitate this conversion and include zinc [21,48], amalgamated cadmium [49], hydrazine–copper [43,45] and copperised cadmium [50]. Copperised cadmium columns [50] are the most common arrangements with efficiencies for nitrate to nitrite conversion approaching 100 %. A novel approach to nitrate-nitrite conversion has been the implementation of photo-induced reduction. Takeda et al have reported the use of UV irradiation using wavelengths between 200-300 nm, resulting in the formation of nitrite and oxygen as shown below [46]. This approach is particularly attractive as it obviates the use of toxic cadmium and provides a clean and efficient alternative.



This 'green' route is further extended by more recent reports, following advancements made in the field of microbiology where authors have advocated the use of Nitrate Reductase enzymes for the bulk reduction of nitrate to nitrite [47,51,52]. In addition, some methodologies, especially those based on chemiluminescence, require further reduction to nitric oxide (route 3, figure 3.1). A number of reducing agents have been reported to achieve this conversion, including Ti(III) [53,54], V(III) [53,55], Mo(VI) + Fe(II) and Cr(III) [53].

It is clear from the tables that a significant proportion of the reports employ some form of flow injection analysis/automation (FIA). Such assemblies are particularly amenable to the sequential analysis of both ions through the in-line incorporation of a reductor column. A schematic of a typical FIA system is shown in figure 3.2, for the indirect electrochemical detection of nitrate/nitrite (discussed in a later section) however the set-up can be easily adapted to suit the need of those protocols that require some form of derivatisation/conversion prior to conducting the actual determination. FIA techniques

are highlighted in the three tables with the appropriate sample throughput, the remainder of the protocols refer to batch analysis.

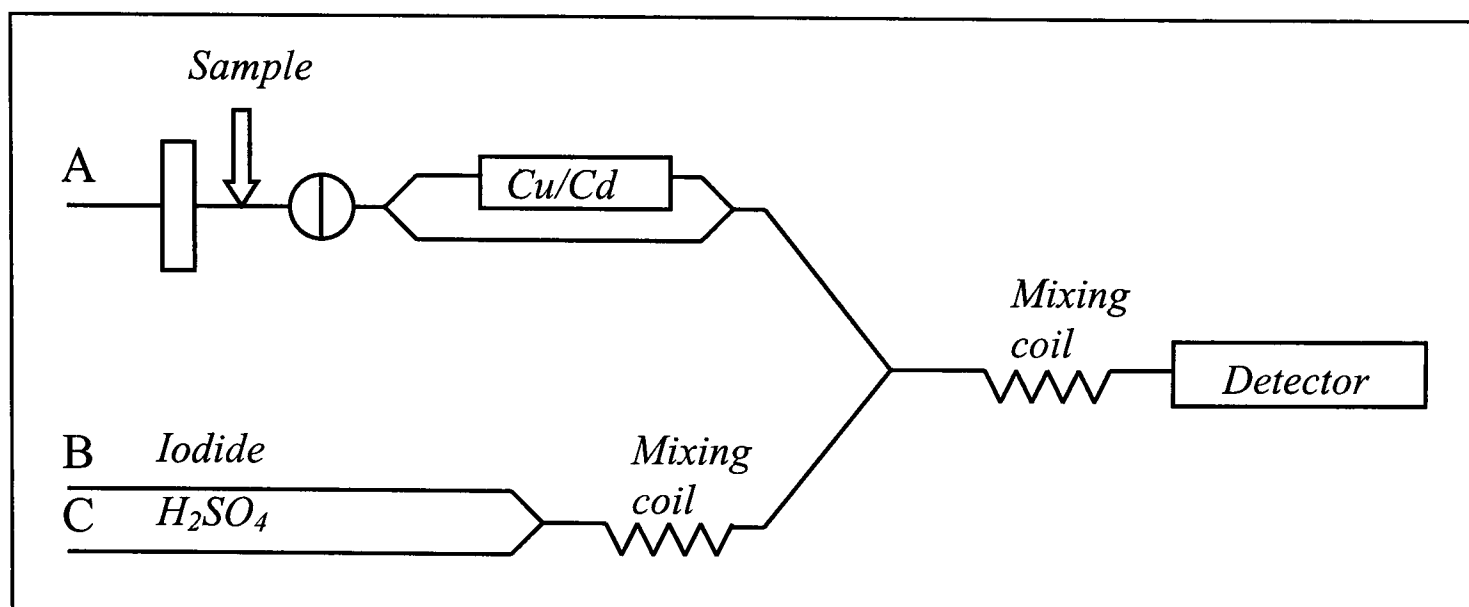


Fig. 3.2 - FIA manifold of the flow system used in the electrochemical determination of nitrate and nitrite after reaction with iodide [147].

The diversity of samples that have been investigated is almost matched by the protocols and techniques applied. Nevertheless, there are a number of issues that are common to all which should be highlighted before considering the vagaries of the actual analysis. While the nitrate ion is relatively inert, long-term storage of the sample prior to analysis should be discouraged through the propensity for bacterial spoilage. This is also true for nitrite but a number of additional precautions must be considered. Nitrite is stable in neutral or alkaline solutions but will decompose on standing in acidic conditions with the process considerably exacerbated when the solutions are heated. The redox properties of nitrite are such that many matrix constituents will act to either reduce it (e.g. ascorbic acid, sulfamic acid, urea, iodide) or oxidise it (e.g. Cr(III), Fe(III), Cu(II), MnO_4^- , CrO_4^{2-} , BrO_3^- and Ce(IV)) resulting in poor recoveries. A number of reviews have covered the effects of such interferences within food and environmental matrices [56,57].

3.2.1 SPECTROSCOPIC DETECTION

Spectroscopic methods are by far the most widely used for nitrate/nitrite determination due to the excellent limits of detection obtained and facile assay type protocols. A broad range of techniques have been evaluated, including UV/Vis [40,41-47,51,52,58-100], chemiluminescence [53,54,101,102], fluorimetric [45,58,103-121], IR [122], Raman [123-125] and molecular cavity emission [62,126,127] protocols. While the

compositional complexities offered by real world media often precludes the direct UV determination of either nitrate or nitrite, such an approach does serve as the principal detection systems in a host of chromatographic and electrophoretic systems which are discussed in a later section. The most common approach to the detection of nitrite however is undoubtedly the Griess Assay. First developed in 1879 [128], it has found numerous applications (cf. tables 3.1-3.3) for both nitrate and nitrite analysis. The assay typically relies on the diazotisation of a suitable aromatic amine by acidified nitrite with the subsequent coupling reaction providing a highly coloured azo chromophore from which the concentration of nitrite can be assessed (route 2, figure 3.1).

The absorption maximum for the azo product is generally in the range 500-600 nm (depending on the selected reagents) and can be detected using conventional visible spectrometry. The most common arrangement utilises sulphanilamide and N-(1-naphthyl)ethylenediamine [40,41,43,45,46,68,72-75,129] as the target amine and coupler respectively with the product of the reaction detected at 540 nm. Numerous adjustments have been made to the basic procedure with assay conditions, reagents and final detection methodology being manipulated to suit given matrices. Sulphanilic acid, nitroaniline and p-aminoacetophenone have been used as the target amine with phenol, 1-naphthol, 1-naphthol-4-sulphonate, 1-amino naphthalene and 1,3-diaminobenzene investigated as potential coupling agents. The reaction can be used directly for the determination of nitrite but nitrate requires the incorporation of a reduction step prior to commencing the assay.

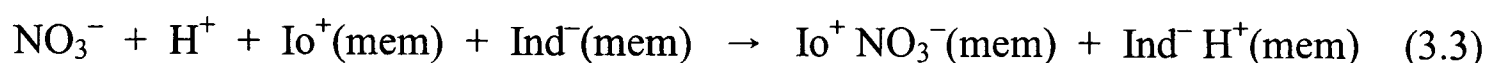
Detection limits for the Greiss assay tend to range between 0.02 and 2 μM according to the specific reagents chosen with linearity typically spanning 2 orders of magnitude (1-100 μM). The technique offers a simple and effective method of detecting nitrite in a variety of matrices but the approach can suffer in complex media such as food. Antioxidants (particularly ascorbate and sulphydryl thiols) can effectively destroy the nitrous acid before it has time to react with the indicating aromatic amine and lead to a reduction in the recovery of nitrite. The various problems that these matrices can introduce and methods to alleviate them have been reviewed previously [37]. Other problems that arise are in general those which afflict most spectroscopic determinations (i.e. highly coloured and heterogeneous matrices). HPLC and FIA have also been coupled with the Greiss protocol, to extend the diversity of samples and enable the

analysis of nitrate in particularly complex matrices such as biological fluids and food samples [71-73,75,93-95].

A number of other indicating species have also given rise to coloured products. The reaction of nitrite with proflavin (3,6-diaminoacridine) in acidic conditions has been found to form a stable violet compound (λ_{max} 328 nm) with low limits of detection (2 nM) but the position of the absorption maximum can however increase the susceptibility to coloured interferences with Fe(III) providing significant interference at concentrations exceeding 1 mg L⁻¹ [70]. Similar problems have been encountered when the nitrosation of activated phenolics (phenol, resorcinol, phloroglucinol) was used as the indicating reaction [5]. The absorption maximum could be attenuated through exploiting the ability of the resulting 1,2-hydroxy-nitroso species to coordinate with metal ions (notably Zr [130] and Cu [5]). The absorption maximum was shown to shift from 312 nm to 348 nm in the case of phloroglucinol / Cu. Rendering the solution basic after completion of the nitrosation steps was found to promote the greatest shift in the absorption maxima (400 nm) through the formation of the nitrosophenolate anion [5]. Devi et al have also reported the use of anion-exchange coupled spectrophotometric detection, in which thiocyanate is displaced from a column and is subsequently reacted with Fe(III) to form a red complex which can be monitored at 480 nm, achieving detection limits of 50 nM [60].

While the majority of the UV/Vis systems employ simple assay procedures, kinetic spectroscopic protocols have also been investigated. The oxidation of gallocyanine by bromate under acidic conditions [64] occurs very slowly but is dramatically increased by the presence of nitrite. The technique relies upon measuring the decrease in the absorbance of the dye at 530 nm. Selectivity remains poor however as the procedure is significantly influenced by a large number of ions, particularly Fe(II), Fe(III), Ag(I), SO₃²⁻, Br⁻ and I⁻ ions. Under the same theme, Pettas et al have reported the use of nitrite-catalysed thymol blue oxidation by bromate under acidic conditions. Subsequent monitoring at 543 nm has achieved detection limits lower than 0.1 μ M [76]. The use of kinetic spectroscopy for the detection of nitrite remains an active area of research, and in recent years the library of dyes that can be catalytically oxidised by the NO₂⁻ anion has since been extended [77,84,85,87,88,92,97,98,100].

An optical sensor comprising a poly(vinylchloride) (PVC) membrane, impregnated with a nitrate-selective ionophore and a proton-selective (chromo)-ionophore has been developed for the continuous monitoring of nitrate in aquatic environments [59]. The potential that would arise from the complexation of nitrate ions by the ionophore (Io) in the membrane is compensated by the co-extraction of protons by a second (chromo)-ionophore. This process is illustrated below:



The nitrate concentration in the solution is correlated with the amount of protons in the membrane and can be detected spectroscopically by the protonation of the indicator anion (Ind^-). In the absence of nitrate ions, the positively charged ionophore and the water-soluble indicator anion form an ion pair ($\text{Io}^+ \text{Ind}^-$) and remain in the hydrophobic membrane. When freshly prepared, the membrane shows an intense blue colour with an absorbance maximum (λ_{max}) at 612 nm. If nitrate and protons enter the membrane, the protonation of the indicator leads to a decrease in the absorption band at 612 nm. This process is reversible and can be restored to the initial absorbance by washing with buffer solution. A detection limit of 20 μM is achieved with a linear range from 300 μM to 4 mM. Despite the high detection limit, this technique has a number of advantages over potentiometric sensors in that no reference element is required, there is minimal electrical interference and baseline variation can be eliminated using multi-wavelength calibration. As in the previous situation, selectivity remains an issue in that nitrite, chloride and sulphate are potential interferences.

Fluorimetric protocols have explored a number of avenues of varying procedural complexity. Fluorimetric determination of Ce(III) resulting from the oxidation of nitrite with Ce(IV) presents one of the more simpler processes but can be susceptible to the influence of other redox species [104]. More selective approaches have utilised the chemical versatility of acidified nitrite. The nitrosation of 4-hydroxycoumarin in acidic medium, followed by reduction results in the production of fluorescent 3-amino-4-hydroxycoumarin [103]. Lapat et al have reported the use of two fluorimetric methods that can detect nitrite when reacted with either 2-amino-4-chloro-1-hydroxybenzene-6-sulphonic acid or 4-aminofluorescein to form the diazonium salt shown in figure 3.3.

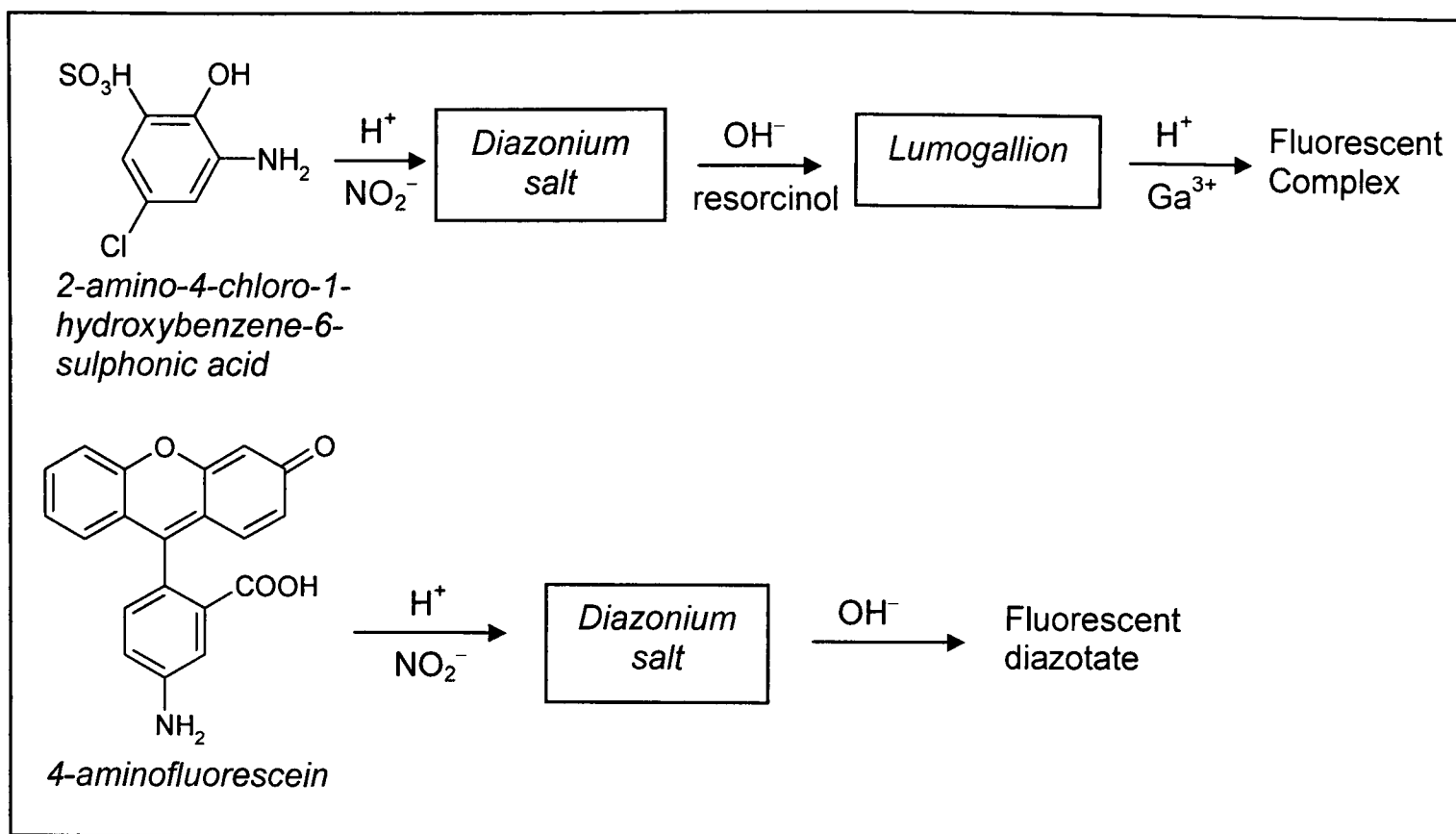


Fig. 3.3 - Fluorimetric methods for the detection of nitrite [58].

The resulting solutions can be easily manipulated to produce highly fluorescent complexes [58]. Detection can be achieved at 608 nm (495 nm excitation) and 518 nm (492 nm excitation) for the respective compounds and has been successfully applied as a method of detecting the explosive 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX) after its decomposition to nitrite.

Addressing the issue of selectivity is vital when attempting an analysis on a sample that has received little pre-treatment. In the majority of the cases presented thus far, the interferences are minimised by utilising the chemical specificity of the nitrite ion to undergo nitrosation/diazotisation. This goes some way to explain the remarkable popularity of the Griess assay. However, an alternative route which also utilises the chemical versatility of nitrite has also come to the fore.

Gas-phase chemiluminescence can offer greater scope for the elimination of matrix effects in that it is based upon monitoring the reaction between gaseous NO and ozone [53,54,101,131]. Conversion of nitrite through reduction with acidified KI liberates gaseous NO from the matrix (route 3, figure 3.1). This subsequently reacts with ozone to give nitrogen dioxide in an excited state (NO_2^*) plus molecular oxygen. The excited state of NO_2^* then decays to give a weak infrared chemiluminescence above 600 nm (route 4, figure 3.1). This route can also be used for the analysis of nitrate but requires

the application of stronger reductants (typically Ti(III) [53,54]). The nitrate concentration can then be calculated by difference. The fusion of chemiluminescence with FIA assemblies can lead to significantly improved detection limits (10 nM [54,101]) over conventional UV/Vis systems can also facilitate high throughput sample analysis with the quantitative determination of nitrate, nitrite and ammonia requiring less than 3 minutes. A significant drawback to the protocol lies in the increased technical complexity of the system and the requirement of high combustion temperatures (600 °C). This is due to the reaction of trace oxygen with NO forming NO₂, resulting in a decrease of the chemiluminescence intensity. At high temperatures (600 °C), the re-conversion of NO₂ to NO increases, compensating for the interference caused by oxygen.

Molecular emission cavity analysis (MECA) continues along a similar vein with particular applicability to the analysis of highly coloured solutions that would prove intractable for standard spectroscopic techniques. Nitrite is again reduced to NO and swept into the flame by nitrogen carrier gas. The resulting emission is then monitored at 640 nm. Spectral interferences from carbon dioxide and hydrogen sulphide, which have been found to emit within the cavity, can be problematic although the latter could be alleviated through precipitation as a suitable metallic sulphide.

The remainder of the spectroscopic systems tend to deviate from mainstream detection and have been used largely for a number of specialised tasks but have been included for the sake of completeness. Fourier-transform infrared spectroscopy (FT-IR) and Raman spectroscopy have been utilised for the spectroscopic determination of the nitrate ion in aerosols [14,122-125]. The former measures the absorbance bands at 1384 and 2430 cm⁻¹ which are attributed to the nitrate ion [14]. Morphology-dependent stimulated Raman scattering (MDSRS) is a non-linear spectroscopy that uses the set of ‘macroscopic’ natural electromagnetic modes of oscillation characteristics of μm-sized axisymmetric particles to enhance Raman scattering from molecules contained within the particle. The modes of oscillation are commonly called morphology-dependent resonances (MDRs) because their spatial and spectral positions are determined by particle size, shape and refractive index. The detection limits are of the order of low μM with the dynamic range extending to 3 mM [123].

Gallego et al have reported the indirect determination of nitrate and nitrite using atomic absorption spectroscopy coupled with FIA (FIA-AAS) [132]. Nitrite and nitrate form ion-pairs with copper(I)-neocuproine chelate, which are then extracted into methyl-isobutyl-ketone, where the atomic-absorption signal of copper from the organic phase is proportional to the nitrate or nitrite concentration. Reported detection limits are 8.7 and 0.65 μM for nitrite and nitrate respectively, at a sample throughput of 35 per hour. Interferences are evident from thiocyanate, perchlorate and chlorate ions. Wennmalm et al have reported the use of nitrite detection using electron paramagnetic resonance spectrometry (EPR) [133]. The system is based on measuring the paramagnetic properties of NO bound to heme-containing proteins, which can be used for quantitative determination in the nanomolar range.

3.2.2 ELECTROCHEMICAL DETECTION

The electrochemical detection of nitrate and nitrite can be divided into a number of categories. Fortunately, these can be broadly grouped within the distinctions of voltammetric and potentiometric systems. Voltammetric techniques have been employed from the beginning of the 1900s whereby copper electrodes were used to electrochemically reduce the nitrate ion [134-141]. A wide variety of electrode substrates have since been investigated and include: nickel [142,143], copper-nickel alloys [144], cadmium [135,145,146], platinum [147,148], glassy carbon [149,150], gold [151,152], lead [142,153], silver [140,154,155] and more recently boron doped diamond [156-158]. The profusion of electrode materials effectively betrays the fact that the electroanalytical determination of nitrate and nitrite at bare electrodes is far from facile. Despite the thermodynamic feasibility of the reduction, the kinetics of the charge-transfer are slow [159] and as such direct reduction of nitrate has been characterised by poor sensitivity and often marked irreproducibility through cumulative electrode passivation effects. The need to apply large overpotentials can also affect the selectivity of the approach.

Nitrite would appear to be rather more fortunate in that this ion can be oxidised or reduced at a glassy carbon electrode [160-163]. Unfortunately neither option is particularly favourable in terms of direct electroanalysis, succumbing to similar problems that afflict nitrate analysis [159,162-164]. A number of routes, outlined below, have

been pursued in an attempt to counter these problems of which electrode modification appears to offer the most benefits in terms sensitivity and selectivity. Immobilised electrocatalysts can offer marked improvements in selectivity and sensitivity (particularly those based on biorecognition). Surface modifications are often perceived as increasing the technical and procedural complexity of the protocol and while this is certainly true when considering the immobilisation of enzymes, much simpler options exist for enhancing the reduction of both nitrate and nitrite.

Increasing the sensitivity of the electrode response can be achieved through maintaining a large and highly active surface area. This has traditionally been attempted [139,141,146,165] through the deposition of a fresh electrode layer prior to each analysis. This can be done simply by the introduction of the appropriate metal salt(s) into the sample medium. The analysis is performed by sweeping a potential range in the cathodic direction. The metal ion (typically Cu [139] or Cu/Cd [146,165] mixtures) is electrolytically plated onto the electrode and effectively provides a fresh surface from which nitrate or nitrite reduction is induced as the potential scan progresses. A significant advantage of this approach is that the analysis is relatively independent of the base electrode material as the nitrate/nitrite reduction occurs at the freshly deposited metal layer. This has been exploited in the development of disposable capillary fill devices in which screen printed carbon electrodes were used in conjunction with cupric sulphate to provide a response to nitrate [141].

An alternative approach is to prepare the electrode *ex-situ* whereby the electrode surface is conditioned in a plating solution of defined composition and then transferred to the analysis solution [166-168]. This approach offers greater control over the morphological features of the deposit which, in the case of a cupric ion/sulphate/chloride mixture, was found to lead to a particularly active granular deposit. Whilst this approach will still fall foul of cumulative passivation effects it was found to retain substantial activity over a 24-hour period of continued use. More recently, electrode cleaning and activation has been achieved through the application of 20 kHz ultrasound, extending the sample diversity of nitrate/nitrite detection to highly passivating matrices such as whole egg [149] or sewage [169]. Sono-electroanalytical techniques provide a novel on-site method of analysis precluding the requirement for sample clean-up.

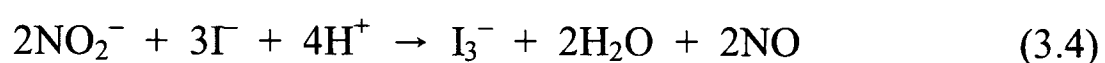
More complicated surface modifications have also tended to make use of metal ions though usually bound within some form of complex [152,170-173]. Electrocatalysts used for the reduction of nitrate and nitrite include: alternating layers of μ -meso (tetrapyrridyl)porphyrinate Co(III) tetrakis[bis(bipyridine)(chloro) Ru(II)] and meso-tetra(4-sulphonatephenyl)porphyrinate Zn(II) [174], polypyrrole films doped with tungstodiphosphate anion (P₂W₁₈O₆₂6⁻) [175], P-Mo-V heteropolyacid [176] or iron-substituted heteropolytungstates [177] and Nafion coated mercury film electrodes incorporating Yb³⁺ or UO₂²⁺ [178]. The ability to electrochemically oxidise nitrite has not gone unnoticed and a number of electrode assemblies based upon metal ions (typically Os [44], Ru [179-181] or Ir [182]) coordinated to or electrostatically entrapped within polyvinyl pyridine supports have also been investigated. Other techniques utilise the reduction of nitrite to NO which can then be detected amperometrically at modified Au electrodes covered with a PTFE film [183].

The more sophisticated approaches to the electrochemical detection of nitrate and nitrite probably lie with the use of biological catalysts. Reductase enzymes [184,185] can significantly enhance both electrode sensitivity and selectivity towards the reduction of nitrate and nitrite respectively. Reshetilov et al has proposed the use of a biosensor based on the oxidation of nitrite by bacteria based on the *N. vulgaris* strain [186]. The nitrite oxidation is then followed using amperometric methods for oxygen content measurement, which is released as a by-product of the reaction with nitrite. Despite these advantages, the high reagent expense combined with the fragility and complexity of the sensing layer has, as yet, tended to preclude the widespread acceptance of this route.

A large number of assay type protocols for the detection of nitrate and more commonly nitrite have arisen as consequence of the difficulties experienced in the direct electroanalysis of these ions. As with the previous spectroscopic assays, nitrate determination is usually accomplished through its reduction to nitrite using a Cu/Cd column. There are however a number of procedures that do in fact utilise the chemical properties of nitrate – principally its ability to nitrate aromatics such as benzoic acid, salicylic acid, isoquinoline and thiophene-2-carboxylic acid (route 5, figure 3.1). The resulting nitro derivative is determined reductively at a glassy carbon electrode, using linear sweep voltammetry (LSV) between 0 and -0.5 V. Of the compounds studied, thiophene-2-carboxylic acid gave the best electrochemical response [150]. A similar

approach has been developed in conjunction with HPLC techniques whereby the nitration of phenol is the principal indicating reaction with the analytical signal provided through the reduction of o-nitrophenol at -0.47 V [187]. In comparison with the thiophene-2-carboxylic acid nitration, 1000-fold higher detection limits are achieved (0.1 μ M) with fewer problems reported from interferences, such as dissolved oxygen [150]. Nitrate has also been shown to oxidise uranyl ions which can then be reduced catalytically at an electrode surface and used as an indirect method of detection with detection limits of 2 μ M [188].

Indirect methods for nitrite analysis are slightly more diverse given the greater versatility of this ion. An electrochemical variation/adaptation of the Griess assay has been attempted with the reduction of the diazonium salts resulting from the reaction of nitrous acid with substituted phenylene diamines providing the analytical signal. The nitrosation of activated phenolic compounds can open up another route with the reduction of the nitroso group to the corresponding amine providing a sensitive response within a region (~ -0.2 V) where there are few electroactive interferences [5,189,190]. The iodine-iodide couple is a reversible electrochemical system on both gold and platinum electrodes in sulphuric acid media [191], which can be exploited for indirect analysis of nitrite. The technique is based on the reaction of nitrite with iodide in acidic medium to form triiodide, which is then amperometrically monitored via triiodide reduction between $+0.2$ to $+0.3$ V (vs. SCE).



This approach has been studied at Au and Pt macroelectrodes [192] and also at a Pt microelectrode [147]. This technique is relatively simple, quick and easy and can be combined with Flow Injection Analysis (FIA) protocol – giving analytical throughput in the region of 60 samples hr^{-1} . When coupled with FIA (figure 3.2), detection limits as low as 70 nM nitrite have been established [147]. Biamperometric detection of the iodine/triiodide system has also been employed in a flow-through cell using two teflonized graphite or platinum electrodes. This set-up offers advantages in experimental simplicity due to lower reduction potentials required (100 mV) [148]. The main problem

with using the reaction of nitrite with iodide is interference by oxygen, which can oxidise both Γ^- and NO, and therefore stringent use of degassed solutions is required.

In addition to the numerous amperometric and voltammetric detection protocols previously mentioned, potentiometric methods for nitrate/nitrite analysis are also routinely available, further extending the diversity of electrochemical analytical methods. The most common approach is the use of commercially available ion-selective electrodes (ISEs) whereby the selective passage of charged species from one phase to another, typically from solution to membrane, gives rise to a potential difference which varies with Nernstian fashion with the activity of the ionic species, in this case NO_3^- , so that a calibration graph of potential with nitrate concentration can be obtained. Several authors acknowledge the widespread use of a PVC membrane [193-198] used in conjunction with an ion-exchanger and plasticizer. In modern times, the use of both tetradodecylammonium bromide (TDABr) [193] and tetradodecylammonium nitrate (TDAN) [195,196] as ion-exchangers and the use of dibutylphthalate (DBP) [193,195] and o-nitrophenyl octyl ether (o-NPOE) [194,196,197,199] as plasticizers have all been reported. Detection limits for nitrate offer mM resolution coupled with good dynamic ranges spanning from 10^{-5} to 10^{-2} M. Direct detection of nitrite has been reported when coupled with ion chromatographic separation methods [193]. ISEs offer attractive features as nitrate sensors, including the ease to which they can be coupled with continuous flow systems or in flow systems with sample injection, such as FIA, enhancing sample diversity. Excellent longevity is commonly observed, with in-situ operation lasting in excess of 15 months [196]. Interference from other anions is often minimal by virtue of the low selectivity coefficients (10^{-3}) achieved.

3.2.3 CHROMATOGRAPHY

Most branches of chromatography have been utilised in the search for technologies capable of detecting nitrate and nitrite. Derivatisation protocols are a necessity for many (particularly gas chromatography) but direct sample introduction can be accomplished with relative ease in most HPLC and ion chromatographic systems. In most cases, some form of sample pre-treatment is required whether it be simple filtration or derivatisation and it is these stages that tend to remove the attractiveness of the technique. One of the more common derivatisation procedures is the conversion of nitrate to the electrophilic

nitrosonium ion (NO_2^+) with the subsequent nitration of an appropriate activated aromatic (2,4-dimethylphenol [200] and 1,3,5-trimethoxybenzene [30]) providing the indicating species (route 5, figure 3.1) [201,202]. Nitrite can be similarly determined through prior oxidation to nitrate with weak hydrogen peroxide. An alternative derivatisation protocol for nitrite that also involves the formation of a nitro species is by nucleophilic substitution with an aliphatic halide. Pentafluorobenzyl bromide (PFB) is particularly adept at scavenging nitrite ions to form the corresponding nitro derivative as shown in figure 3.4.

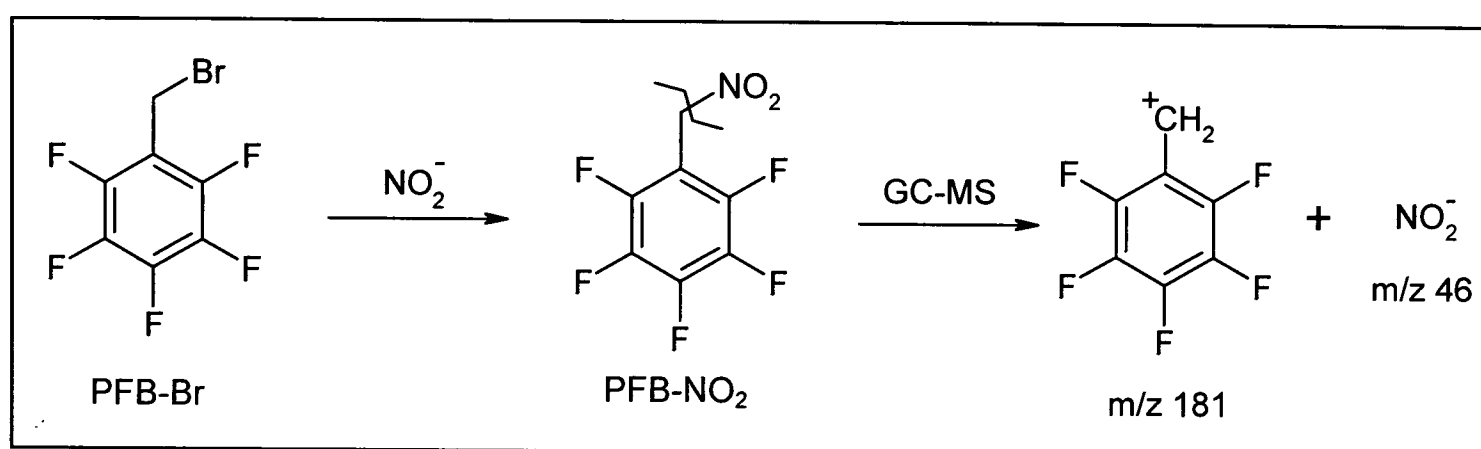


Fig. 3.4 - GC-MS detection of nitrite via nucleophilic substitution with pentafluorobenzyl bromide (PFB-Br) [203].

Analysis by GC-MS results in the weak methylene-nitro bond being fragmented and the NO_2^- ion detected unambiguously [203-206]. This technique can also be extended to incorporate ^{15}N detection, after reaction of the aromatic with labelled nitrate/nitrite. An alternative derivatisation agent for nitrite is the use of N-acetyl-L-cysteine with the production of S-nitroso-N-acetyl-L-cysteine [65]. The exploitation of such a route is quite ironic given that sulphydryl thiols are often the source of poor nitrite recoveries in food matrices [56].

End column detection systems include simple UV [30,61,65,69,96,207], fluorimetric [104,113], electron capture [208], electrochemical [66,67,187,209], conductivity [210-212] and mass spectroscopic [9,203-206] analysis of the eluent. The popularity of UV detection lies in the simplicity of the approach and the ability to attenuate the detection wavelength. The low absorbance of most inorganic ions helps to maintain a low background from which nitrite and nitrate can be resolved. However, the aromatic eluents typically used in anion exchange tend to give rise to high background UV absorbance, complicating the direct detection of nitrate/nitrite. Alkanesulphonates, which are eluents with low background UV absorbance, have proven to be useful

alternatives in this respect [207]. In contrast, Grosjean has studied the use of ‘negative’ UV detection whereby a highly absorbing eluent is used (e.g. aromatic acid) in the HPLC [213]. In this way, analytes that absorb only weakly are recorded as ‘negative’ peaks. This ‘indirect photometry’ allows for substantially better detection limits than that normally achieved for direct methods for weakly absorbing anions.

Electrochemical detection is normally reserved for nitrite through oxidation at glassy carbon electrodes (typically poised between +0.7 and +0.9 V) [66,67]. Nitrate can also be detected amperometrically but requires exposure to UV radiation prior to reaching the electrochemical detector. The photolysis product has been shown to be capable of oxidation in a manner analogous to that of nitrite. Excellent detection limits of 0.9 and 4.4 nM were obtained for nitrite and nitrate respectively [66].

3.2.4 CAPILLARY ELECTROPHORESIS

Capillary electrophoresis (CE) is a powerful separation technique first reviewed for clinical use in 1995 [214], but which has rapidly been transformed for more general analytical purposes [6,7,215-217]. The main advantages of this technique lie in the ability for the fast simultaneous detection of a wide variety of anions. Other attractive features of CE are small sample requirements (nL) and low buffer consumption, in comparison with HPLC. In addition the instrumentation has automated sample loading, requires little maintenance and is arguably more cost-effective than other methods. A more recent development of CE is the use of capillary zone electrophoresis (CZE), which has increased the sensitivity of the technique by a factor of ten, giving 0.1 μ M limits of detection [218-222]. Detection of nitrate and nitrite is normally performed by UV detection at 214 nm. While CE can be directly applied to a number of matrices, a number of preparative/separation stages are usually required to remove proteinaceous material that would otherwise exert a detrimental influence on both the analytical signal and the actual equipment through adsorption on the wall of the capillary. The addition of deproteinization steps can dramatically increase the lifetime of the system to 250-300 determinations without significant change in retention times [7].

3.3 CONCLUSIONS

The aim of this chapter was to explore the vast diversity of methods currently available to the reader for the detection and analysis of nitrate and/or nitrite in a variety of sample matrices. Awareness has been drawn to the benefits and drawbacks of each technique in an attempt to provide a general evaluation of this broad scientific area of study. Where possible the chapter has focussed on the more contemporary techniques of the past decade in an attempt to offer a more modern and up-to-date account of nitrate/nitrite analytical strategies. In summary, the use of spectroscopic analysis appears to be the preferred method of choice due to the simplicity of the protocols and the wide availability of the instrumentation involved. The increasing demand for rapid on-site analysis will ensure the continued development of both spectroscopic and electrochemical methods, which are more applicable to miniaturisation and remote operation. It could be envisaged that while chemiluminescent and chromatographic techniques offer superior performance, the instrumental complexity required for their successful implementation will limit their operation to a laboratory based environment. The following chapters (4-6) aim to build on the work presented in this chapter, detailing the research performed into electrochemical methods in the detection and analysis of the nitrate/nitrite species.

3.4 TABLES

Technique	Matrix	Detection Limit / μM	Detection Range / μM	RSD / %	FIA Sample Throughput / hour	Reference
ED	Saliva / Urine / River Water	6	4000-80000	5.0	N/A	[5]
Fluorescence	Aqueous	N/A	0.045-15	3.0	N/A	[58]
Fluorescence	Aqueous	N/A	0.023-15	3.0	N/A	[58]
Greiss	Aqueous	N/A	2-30	3.0	N/A	[58]
Visible	Aqueous	0.098	0.11-540	2.2	N/A	[76]
Visible	Water / Food / Soil	0.15	0.87-300	N/A	N/A	[77]
Visible	Water, food and soil	0.15	0.87-304	N/A	N/A	[77]
Greiss	Water	0.29	0.29-3.5	4.0	15	[81]
Greiss	Water	0.018	0.018-0.43	4.0	3	[81]
Visible	Well / Waste Water	0.065	0.32-16	3.0	N/A	[82]
Visible	Aqueous	0.20	1-100	2.6	N/A	[83]
Visible	Water samples	0.13	0.22-3.0	N/A	N/A	[84]
Visible	Water and brake fluid	0.024	1-180	N/A	N/A	[85]
Greiss	Water samples	0.0038	0.0087-0.52	0.36	N/A	[86]
Visible	Water	0.20	0.22-8.7	N/A	N/A	[87]
Visible	Water	0.0017	0.0087-0.52	N/A	N/A	[88]
Greiss	Rain and tap samples	0.010	N/A	4	12	[89]
Greiss	Aqueous	0.26	0.30-5.2	N/A	N/A	[90]
Greiss	Rain water and saliva	0.26	1.2-18	0.85	N/A	[91]
Visible	Waster and seawater	0.13	0.22-1.7	0.8	15	[92]
Greiss	Food samples	0.1	0.27-70	2.8	15	[93]
Chemiluminescence	Water	N/A	0.25-65	1	N/A	[102]
ED	Water	N/A	0.1-50	N/A	N/A	[102]
Fluorescence	Saliva	0.043	N/A	2.8	40	[103]
Fluorescence	Spiked Water	0.16	0.16-8.7	N/A	N/A	[105]
Fluorescence	Water	0.030	0.215-14	3	N/A	[106]
Fluorescence	Tap / Lake Water	0.002	0.017-2.4	4.9	N/A	[107]
Fluorescence	Aqueous	0.12	0.22-2.6	N/A	N/A	[108]
Fluorescence	Synthetic mixtures	0.12	0.22-2.6	N/A	N/A	[108]
Fluorescence	Water / Food	0.054	0.22-13	N/A	N/A	[109]
Fluorescence	Fish	1.1	5.0-100	N/A	N/A	[110]
Fluorescence	Meat and fish	1.1	5.0-100.0	N/A	N/A	[110]
Fluorescence	Soil / Water	N/A	0-8.7	3	N/A	[111]
Fluorescence	Aqueous	0.059	1.7-28	N/A	N/A	[112]
Fluorescence	Seawater	0.007	0.021-0.840	3.2	N/A	[117]
Fluorescence	Waste, tap and well water	0.059	0.13-1.9	N/A	N/A	[118]
ED	Egg / Water	1	10-100	4.0	N/A	[149]
ED	Saliva	0.005	5-10000	0.82	N/A	[170]
ED	Saliva	0.0005	5-10000	0.82	N/A	[170]
ED	Aqueous	0.05	0.1-2000	N/A	N/A	[171]
ED	Aqueous	1	1-250000	N/A	N/A	[173]
ED	Water	0.1	0.2-0.8	1	N/A	[174]
ED	Aqueous	1	5-20000	3.8	N/A	[175]

ED	Aqueous	1.0	5.0-10000	10.5	N/A	[176]
ED	Water	N/A	50-30000	N/A	N/A	[177]
ED	Water	0.03	0.05-500	2.9	N/A	[179]
ED	Aqueous	0.043	21-210	N/A	N/A	[180]
ED	Aqueous	0.25	1-30	0.42	10	[183]
ED	Biological Fluids	10	10-10000	N/A	N/A	[186]
ED	Water	0.13	0.43-13	1.15	N/A	[189]
ED	Natural water	0.0652	0.109-10.9	N/A	N/A	[190]
ED	Water	0.01	500-5000	N/A	N/A	[192]
ED	Aqueous	2.2	5.0-10000	N/A	N/A	[198]
CZE	Aqueous solutions	1	1-250	N/A	N/A	[222]
ED	Snow and water	0.17	0.33-7.6	N/A	N/A	[223]

Table 3.1 - Parameters for nitrite detection.

Technique	Matrix	Direct Detection of NO ₃ ⁻	Detection Limit / μM	Detection Range / μM	RSD / %	FIA Sample Throughput / hour	Reference
CE	Biological Fluids	Yes	5	5-194	5.7	N/A	[6]
Greiss	Seawater	No - via NO ₂ ⁻	0.1	0-20	2	N/A	[40]
Greiss	Seawater	No – via NO ₂ ⁻	0.45	0-150	5	45	[41]
Visible	Natural Water	No – via NO ₂ ⁻	0.02	0-5	1	40	[42]
Greiss	Lake water	No – via NO ₂ ⁻	0.2	0.2-10	3	240	[43]
ED	Water	No – via NO ₂ ⁻	4	7-13600	0.91	100	[44]
UV	Water	Yes	29	29-2100	1.7	N/A	[44]
Greiss	Rain Water	No – via NO ₂ ⁻	16	16-160	3	40	[45]
Greiss	Sea Water	No - via NO ₂ ⁻	0.05	1-100	3	10	[46]
Griess	Aqueous	No - via NO ₂ ⁻	N/A	20-900	6	N/A	[47]
UV	Natural water	No - via NO ₂ ⁻	1.8	5-100	4.20	N/A	[51]
UV	Biological samples	No - via NO ₂ ⁻	0.2	1-100	N/A	N/A	[52]
Visible	Drinking Water	Yes	20	300-4000	N/A	N/A	[59]
Visible	Tap Water	Yes	0.05	50-600	1	N/A	[60]
UV	Lake Water	Yes	0.1	5-50	1	N/A	[61]
Visible	Aqueous	No – via NH ₃	70	700-28500	3.5	N/A	[62]
Greiss	Aqueous	No - via NO ₂ ⁻	0.42	0.5-20	8.3	N/A	[94]
Chemiluminescence	Atmospheric	No – via NO	0.016	0.016-16	N/A	20	[101]
Fluorescence	River water	No - via NH ₃	0.16	0.81-40	2.56	N/A	[119]
IR	Aqueous	Yes	N/A	100-1000	0.5	N/A	[122]
Raman	Aerosols	Yes	5	50-3000	N/A	N/A	[123]
Raman	Aqueous	Yes	4200	N/A	N/A	N/A	[124]
ED	Aqueous	No - via nitration	100	500-5000	4.8	N/A	[150]
ED	Rain / river water	Yes	3.53	10-5000	N/A	N/A	[152]
ED	Natural and waste water	Yes	121	121-4840	N/A	N/A	[155]
ED	Water	Yes	2.8	2.8-80	N/A	N/A	[167]
ED	Water	Yes	0.1	1-2100	9.1	N/A	[168]
ED	Sewage / Water	Yes	10	10-200	4	N/A	[169]
ED	Aqueous	Yes	2	2-400	3	N/A	[178]
ED	Aqueous	Yes	0.4	1-35	4	N/A	[185]
ED	Drinking / River Water	Yes	2	5-60	1	60	[188]
ED	Drinking Water	Yes	2	25-10000	N/A	N/A	[194]
ED	Water	Yes	10	10-10000	2.0	N/A	[195]
ED	Ground / Tap Water	Yes	1.5	20-10000	N/A	N/A	[196]
ED	Carbon Black	Yes	N/A	1-50	0.17	120	[197]
ED	Aqueous	Yes	0.34	5-100000	N/A	N/A	[199]
GC	Waste Water / Plants	No – via nitration	1.6	16-1600	N/A	N/A	[200]
GC	Rat urine	No – via nitration	0.5	1-1000	6.5	N/A	[201]
GC	Body Fluids / Serum	No – via nitration	1	1-200	N/A	N/A	[202]
GC	Rat Urine	No – via nitration	1	1-1000	N/A	N/A	[202]
GC	Whole Blood	Yes	10	20-1000	10	N/A	[204]
GC	Cheese / Meat Samples	No –via nitration	0.1	0.8-16	4.5	N/A	[208]

Table 3.2 - Parameters for nitrate detection.

Technique	Matrix	NO ₂ ⁻ Detection Limit / μM	NO ₂ ⁻ Detection Range / μM	RSD / %	NO ₃ ⁻ Detection Limit / μM	NO ₃ ⁻ Detection Range / μM	RSD / %	FIA Sample Throughput / hour	Ref
CE	Biological Fluids	1	2-20	3.3	1	2-20	3.3	N/A	[7]
ED	Water / Food / Saliva	1.8	2.50 - 1000	1.75	1.7	2.50 - 1000	0.83	60	[48]
Chemilum.	Pig and Dog Plasma	N/A	0.4 - 2	N/A	N/A	0.4- 2	N/A	N/A	[53]
Chemilum.	Water	0.01	0.01-10	0.6	0.1	0.1-100	6.7	20	[54]
UV	Water / Human Serum	2	1-260	N/A	3	3-400	N/A	10	[63]
Visible	Food / Water	0.02	0.2-54	1.70	0.03	0.3-56	1.75	20	[64]
UV	Human Plasma	0.05	0-50	6.3	N/A	0-100	2.5	N/A	[65]
UV	Rat Brain Tissue	0.0009	1-1000	3.4	0.0044	1-1000	3.4	N/A	[66]
UV	Food / Water	1.1	0.12-240	N/A	0.81	0.083-170	N/A	N/A	[67]
ED	Food / Water	1.1	0.12-24	N/A	0.81	0.83-17	N/A	N/A	[67]
Greiss	Water	0.11	0.14-2.86	0.42	0.86	0.11-2.14	0.42	N/A	[68]
Greiss	Water	0.57	0.71-18.86	0.49	0.43	0.54-14.00	0.49	N/A	[68]
Greiss	Water	0.14	0.21-7.21	0.32	0.1	0.16-5.36	0.32	N/A	[68]
UV	Biological Fluids	0.1	0.2-100	5	N/A	0.2-100	5	N/A	[69]
Visible	Water Samples	1.30	1.30-86.9	0.5	1.21	1.21-161	0.76	37 / 26	[70]
Greiss	Food / Water / Soil	0.02	0.22-48	2	0.16	1.6-56	2	30	[71]
Greiss	Biological Fluids	1	1-300	3	1	1-300	3	30	[72]
Greiss	Biological Fluids	0.025	0.025 - 20	N/A	0.025	0.025-20	N/A	60	[73]
Greiss	Brain Tissue	0.5	1-5	1.6	0.5	1-5	1.6	40 / 25	[74]
Greiss	Mouse Brain	0.5	1-5	1.6	0.5	1-5	1.6	40 / 25	[75]
Greiss	Cell Cultures	0.3	N/A	N/A	0.5	N/A	N/A	N/A	[78]
Greiss	Water	0.054	N/A	2.0	0.19	N/A	2.0	35	[79]
Griess	Water	0.11	0-3.0	1	0.65	0-8.7	1	N/A	[80]
UV	Rat nerve tissue	0.2	1-25	14	0.5	1-25	14	N/A	[96]
Visible	Food samples	0.011	0.022-65	N/A	0.048	0.081-55	N/A	20	[97]
Visible	Food samples	0.022	0.065-43	N/A	0.16	0.48-32	N/A	20	[98]
UV	Water samples	8.7	N/A	2	3.2	N/A	2	60	[99]
Visible	Fish and water samples	0.22	1.1-25	N/A	0.40	0.97-26	N/A	25	[100]
Fluorescence	Water	0.028	1 - 100	5.0	0.052	1 - 100	5.0	N/A	[104]
Fluorescence	Biological Samples	0.010	0.013-2.0	N/A	0.010	0.013-2.0	N/A	N/A	[113]
Fluorescence	Seawater	0.0046	N/A	N/A	0.0069	N/A	N/A	18	[114]
Fluorescence	Rain water	0.1	N/A	N/A	N/A	N/A	1	10 / 8	[120]
Fluorescence	Rain water	0.1	2-50	N/A	1	2-50	N/A	8	[121]
Raman	Waste water	14	7-3500	N/A	14	7-3500	N/A	N/A	[125]
Visible	Meat / Water	2.2	22-150	3.9	1.6	16-110	4.3	N/A	[126]
Visible	Aqueous	36	71-21400	3	36	71-7100	10	N/A	[127]
Visible	Aqueous	71	71-14300	5	143	143-14300	5	N/A	[127]
AAS	Aqueous	8.7	11-220	4.7	0.65	1.6-35	3.7	35	[132]
ED	Sewage / Water	5	16-200	N/A	11	16-200	N/A	N/A	[144]
ED	Human Saliva	0.07	0.1-10	1.9	0.25	0.5-10	1.3	60	[147]
ED	Soil	0.4	2 - 1000	3	0.4	2 - 800	3	12	[148]

ED	Sewage / Lettuce / Water	12	12-200	4	10	10-200	4	N/A	[166]
ED	Water, saliva and meat	0.1	1-1000	1.5	0.1	1-1000	1.5	50	[172]
ED	Fertiliser	0.5	1 - 1000	0.42	5	10-10000	2.0	720 / 60	[181]
ED	Water	0.11	0.22-22	N/A	0.081	0.16-16	N/A	N/A	[187]
GC	Human plasma	5	10-1000	10.4	5	10-1000	10.4	N/A	[205]
ED	Human Saliva	0.33	N/A	N/A	0.54	N/A	N/A	N/A	[209]
Conductivity	Hard Water	2.2	N/A	N/A	1.6	N/A	N/A	N/A	[210]
Conductivity	Saliva	0.33	N/A	N/A	0.54	N/A	N/A	N/A	[211]
Conductivity	Wine	2.2	2.2-435	N/A	8.1	8.1-1290	N/A	N/A	[212]
CE	Aqueous	0.73	N/A	10	1.13	N/A	10	N/A	[215]
CE	Vegetables	6.7	2.2-170	8.52	5.2	1.6-160	3.83	N/A	[216]
CE	Vegetables	0.74	2.2-54	4.21	0.6	1.6-26	7.37	N/A	[216]
CE	Cells	3.6	10-200	N/A	2.6	10-200	N/A	N/A	[217]
CZE	Water	0.276	N/A	3.6	0.145	N/A	6.5	N/A	[219]
CZE	Water	0.084	N/A	3.6	0.048	N/A	6.5	N/A	[219]
CZE	Water / Urine	11	22-220	1	8.1	16-160	1	N/A	[220]
CZE	Seawater	1.4	N/A	1.6	0.53	N/A	2.1	N/A	[221]

Table 3.3 - Parameters for the dual detection of nitrite and nitrate.

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CHAPTER 4

ELECTROCHEMICAL DETECTION OF NITRATE AND NITRITE AT A COPPER MODIFIED ELECTRODE

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The work presented in this chapter deals with the electroanalytical utility of an electrode that has been activated via chemical modification and applied to the development of a reagentless electrode system for the determination of nitrate. The approach is based upon the deposition of a macroporous copper deposit which shows marked selectivity for nitrate ion under mildly acidic conditions (pH 3) with a linear range extending from 10 to 200 μM nitrate. Fabrication of the layer is both inexpensive and simple and is relatively substrate independent (Cu, Au, Glassy Carbon) as the response is attributed to the electrodeposited support. The influence of potential interferents such as chloride, nitrite and complexing agents such as citric acid were examined. In particular, the reduction of nitrite was found to occur at potentials significantly less negative than those required for nitrate reduction and as a result clear resolution of both voltammetric peaks is obtainable. The lack of direct interference between the two ions combined with wide peak separation (200 mV) provides a system that may offer some potential in speciation studies. The analytical applicability of the proposed system was demonstrated through the standard addition determination of nitrate in a number of authentic samples with the nitrate concentrations independently corroborated using established Griess assay spectroscopic protocols in collaboration with Dr. M. F. Cardosi, Department of Biological Sciences, Paisley University. SEM imaging experiments were performed in collaboration with Dr. S. J. Wilkins, PTCL, Oxford University.

4.1 INTRODUCTION

The electrochemistry of nitrate has been studied for the best part of a century [1,2] but it is only recently that significant effort has been expended in its quantitative determination. The need to monitor this ion in both environmental and physiological

contexts has been identified in chapter 3 and is recognised by various regulatory bodies worldwide and, as such, legislation relating to maximum permissible concentrations in both drinking water and food produce is often levied [3,4]. A large number of strategies have been employed in the study of nitrate reduction with various reactive metal substrates (copper [1,2,5-10], cadmium [2,11,12], lead [13,14] and silver [9,15,16]), boron doped diamond electrodes [17-19], surface or solution based catalysts (multivalent cations [15], metal macrocycles [14,20-22]) and, more recently, biocatalysts such as nitrate reductase proteins [23-26] being assessed. However, the majority of these investigations have often focused on the fundamental processes associated with the reduction process rather than on the analytical utility of the technique in authentic samples.

The use of bare unmodified electrodes for direct nitrate analysis is undoubtedly the most attractive option but there are a number of problems. Low sensitivity and reproducibility associated with cumulative passivation effects have served to strengthen the interest in the use of immobilised electrocatalysts. These can offer marked improvements in selectivity and sensitivity (particularly those based on biorecognition) however the complexities inherent in their fabrication and use have tended to limit their applicability. In addition, higher reagent expense and lifetimes which are not significantly better than those exhibited by their bare electrode counterparts have contributed to the low acceptance of electrochemical techniques.

The work reported herein details the characterisation of an electrodeposited layer that shows significant sensitivity towards nitrate within a concentration range (10-200 μM nitrate) that is relevant to a number of analytical applications. The immobilised layer consists of a macro-porous copper structure deposited on a suitable electrode substrate through simple fixed potential electrolysis of an acidified cupric sulphate solution. While copper electrodes have been shown to facilitate nitrate reduction [2,5-10] the approach taken in this instance provides a number of procedural advantages and simplifications which make it more accessible for routine analytical applications. Moreover, the reduction of nitrite was found to occur at potentials significantly less negative than nitrate with no direct interference between the two. The clarity of peak resolution in both instances offers the possibility of nitrate/nitrite speciation.

The analytical applicability of the system was investigated in two different sample matrices (lettuce, sewage treatment discharge water) for which statutory concentration limits are imposed [3,4]. The amount of nitrate present in the samples was assessed through a standard addition technique and the results independently corroborated using traditional (Cu/Cd Reduction – Griess Assay) colorimetric techniques.

4.2 EXPERIMENTAL

All reagents were of the highest grade available and used without further purification. Standardised hydrochloric acid (0.974 M), anhydrous sodium sulphate and potassium nitrate were obtained from Aldrich. Nitrite standard solutions (BDH Ion Chromatographic Grade) were prepared daily and refrigerated when not in use. All solutions and subsequent dilutions were prepared using deionised water from an Elgastat (Elga, UK) UHQ grade water system with a resistivity of 18 M Ω cm. Samples of lettuce were obtained locally and analysed on the day of purchase. Water samples from two different sewage treatment discharge outfalls were obtained from Windsor Scientific (Slough, UK) and refrigerated upon receipt.

Electrochemical measurements were conducted using a μ Autolab computer controlled potentiostat (Eco-Chemie, Utrecht, Netherlands) with a standard three electrode configuration. The dimensions and electrode arrangement within the cell have been previously described [27]. The typical working volume was 18 cm³. Copper wire (2 mm diameter) or gold wire (1 mm diameter) mounted in PTFE, or glassy carbon (3 mm diameter, BAS Technicol, UK) served as the working electrode with spiral wound platinum wire as the counter electrode and a saturated calomel (Radiometer, Copenhagen) reference electrode. Both copper and carbon electrodes were polished between experiments with 1 μ m alumina powder (Buehler Micropolish II, USA) and sonicated in an ultrasound bath immediately prior to use. All electrochemical measurements were conducted at scan rate of 10 mV s⁻¹.

Surface characterisation of the copper modified electrodes was carried out using a Cambridge Stereoscan scanning electron microscope with the images obtained at a magnification factor of 661. Spectroscopic investigations were conducted using an LKB Ultraspec spectrophotometer using 1 cm path length cuvettes.

4.2.1 DEPOSITION OF THE COPPER FILM

The copper film was prepared by fixed potential electrolysis in an unstirred solution. The solution consisted of 0.05 M CuSO_4 and 0.1 M Na_2SO_4 which was adjusted to pH 3 with 0.1 M HCl . The solution was degassed using argon and the electrolysis conducted by fixing the potential at -1 V for 20 minutes. The electrode was then removed and washed with deionised water. The majority of the work reported herein was obtained using a copper electrode but similar responses were obtained at gold and glassy carbon electrodes. Care must be taken with the deposit formed on planar electrodes after long electrolysis periods as the shearing motion generated within the solution when emptying the cell and with subsequent rinses can detach large loosely held deposits from the electrode surface. The surface of the electrode was examined using scanning electron microscopy and a micrograph detailing the macroporous structure of the deposit is shown in figure 4.1.

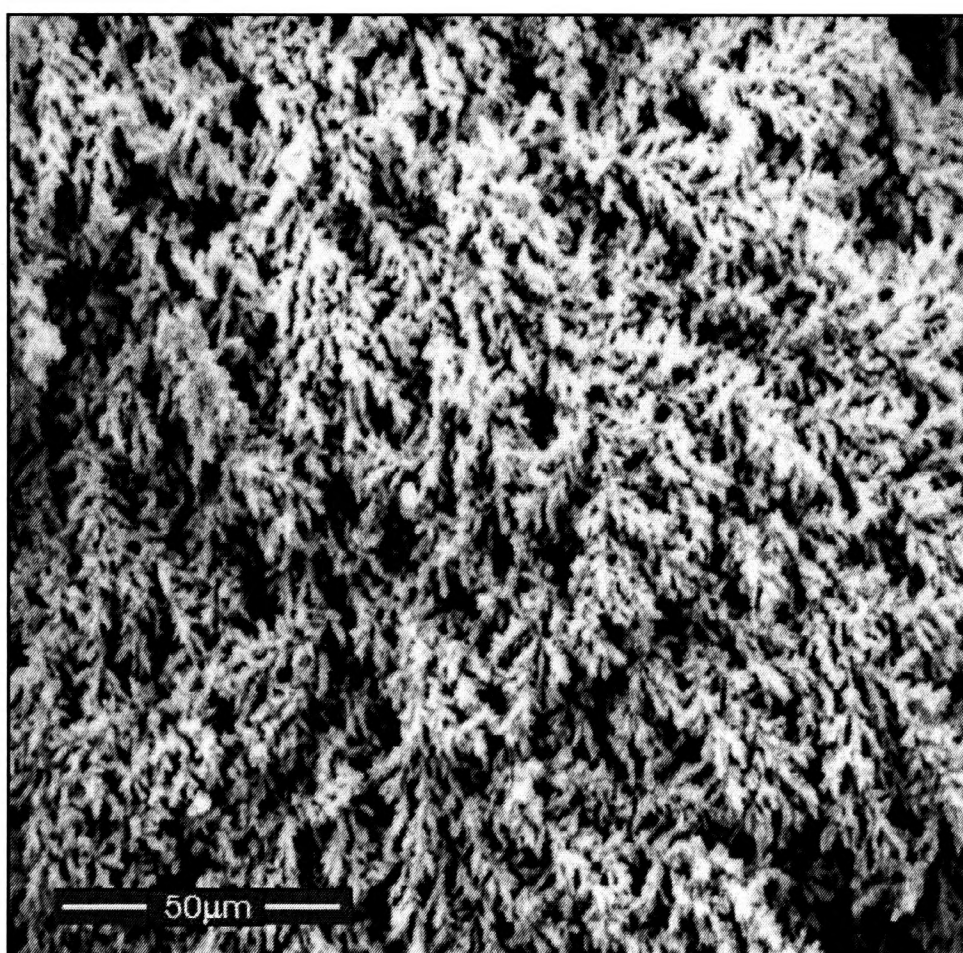


Figure 4.1 - SEM micrograph detailing the porous nature of the copper plated surface.

4.2.2 ELECTROANALYTICAL PROCEDURE

The electrode was placed in a solution (typically 18 cm^3) containing 0.1 M Na_2SO_4 that had been previously adjusted to pH 3 with 0.1 M HCl . Aliquots of the appropriate test

reagent (nitrate, nitrite, chloride, cupric ion, citric acid) were then added and the solution gently mixed by means of argon bubbling. All electrochemical measurements were conducted under a blanket of argon. The electrochemical scan was performed using linear sweep voltammetry scanning from 0 V to -0.65 V at 10 mV s^{-1} . Standard addition protocols comprised of adding an aliquot of the given sample (typically 1 mL of either lettuce homogenate or discharge water) to 18 cm^3 of electrolyte (described above) followed by four or five additions of a standard nitrate solution ($25 \text{ }\mu\text{L}$, 0.025 M).

4.2.3 SPECTROSCOPIC ASSAY OF SEWAGE OUTFALL WATER

The samples of treated sewage discharge were filtered through $0.2 \text{ }\mu\text{m}$ bacteriological filters into sterile plastic universal bottles (20 mL of each sample) and stored at $4 \text{ }^{\circ}\text{C}$ until required. The amount of nitrate in each sample was evaluated through the combination of a copperised-cadmium reduction column and spectroscopic detection of the resulting nitrite ion using an established Griess protocol. Nitrite standards (0.625 , 1.25 , 2.5 , 5 and $10 \text{ }\mu\text{g mL}^{-1}$) were prepared by serial dilution from the stock nitrite (IC grade) solution with distilled water. The nitrate standard used to determine the column reduction efficiency was prepared by dilution of a standard solution of NaNO_3 (1.0 g L^{-1}) with distilled water giving a final concentration of $117.6 \text{ }\mu\text{M}$. Griess reagent was freshly prepared by mixing equal volumes of two separate indicator solutions. The first comprised *a*-naphthylamine (0.1 g) dissolved in 100 mL deionised water with the second solution containing 1 g of sulphanilamide (1.0 g) and phosphoric acid (5.9 mL , $85 \text{ }\%$ w/v) made up to 100 mL with deionised water.

The Cu/Cd reduction column was prepared by treating the column with HCl (0.1 M) for 10 seconds, rinsing with deionised water followed by activation with a CuSO_4 ($1 \text{ }\%$ w/v) solution. This process was repeated for each sample with the reduction efficiency assessed after each run with the standard nitrate solution. Each water sample was diluted ($1 + 1$, $1 + 4$ and $1 + 9$) with deionised water and the reduction process allowed to proceed for 10 minutes.

Aliquots of the reduced sample (0.1 mL diluted to 0.5 mL with deionised water) were combined with 0.5 mL of Griess reagent. The resultant solution was mixed by vortexing and left to react for 30 minutes at $37 \text{ }^{\circ}\text{C}$. Each determination was carried out in triplicate

through measuring the absorbance at 543 nm (1 cm path length) and the blank response (0.5 mL of deionised water and 0.5 mL of Griess reagent) subtracted. The two water samples were also tested independently for the presence of endogenous nitrite and the final nitrate results adjusted accordingly.

4.3 RESULTS AND DISCUSSION

The electroreduction of nitrate at a bare, freshly polished, copper electrode and at the copper deposit modified electrode (CD) was examined under identical conditions and the responses obtained to increasing nitrate additions (25 μL , 0.01 M) compared in figure 4.2.

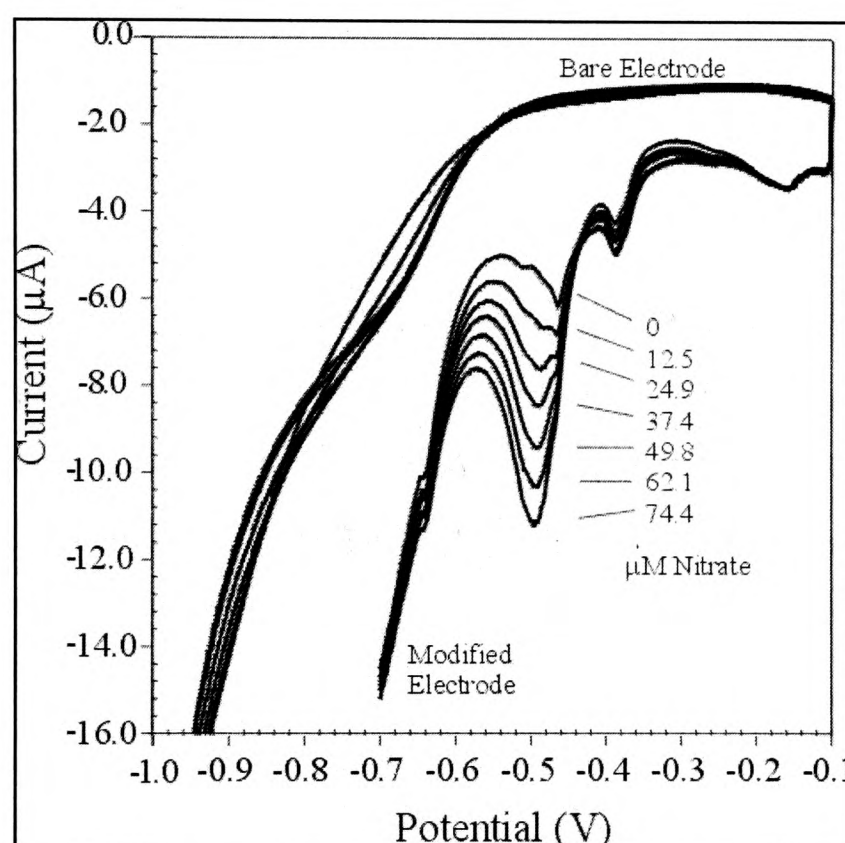


Fig. 4.2 - Linear sweep voltammograms recorded at a bare and a copper modified electrode at a scan rate of 10 mV s^{-1} after the addition of nitrate aliquots (25 μL , 0.01 M).

While there is very little change in the voltammetric response at the bare electrode, a consistent peaked response was observed around -0.5 V using the CD electrode. The peak increased in height with subsequent additions of nitrate with the linear range extending from 10-200 μM nitrate (Regression Data : $I_p / \text{A} = 0.085 [\text{NO}_3^- / \text{mol dm}^{-3}] + 1.14 \times 10^{-7}$, $R^2 = 0.998$, $N = 7$). The peak height in this instance and in proceeding examples was typically measured by subtracting the peak current recorded at -0.5 V in the presence of the analyte from that obtained in the blank supporting electrolyte.

In each case the linear scan was initiated at 0 V which immediately induces the stripping of copper from the surface. As the scan progresses in the negative direction, the majority of the stripped copper is re-reduced at -0.05 V. This part of the linear sweep has been omitted in figure 4.2 for the sake of clarity as the stripping / plating process effectively dwarfs the other features of the voltammetric trace.

The presence of chloride ion in the electrolyte solution serves to scavenge some of the stripped copper and as a result of the complexation the re-reduction of the chloro-copper species occurs at more negative potentials and in two consecutive processes $\text{Cu(II)} + \text{e}^- \rightarrow \text{Cu(I)}$ observed at -0.4 V followed by $\text{Cu(I)} + \text{e}^- \rightarrow \text{Cu(0)}$ at -0.5 V. This behaviour is consistent with previous reports [5,8,10] and can be seen clearly on the CD response recorded in the absence of nitrate (first scan, figure 4.2). The presence of chloride serves to stabilise the Cu(I) state and hence two one electron transfers are seen rather than the single two electron process normally associated with the re-deposition of the bulk copper ion. The reduction of nitrate at copper electrodes is known to be dependent upon chloride ion concentration [5,8,10] and this is also observed with the CD electrode. Fresh electrolyte solution containing $70 \mu\text{M}$ nitrate was prepared but in this instance the solution was adjusted to pH 3 with sulphuric acid in order to exclude chloride ion. This permitted the recording of linear sweep voltammograms before and after the addition of potassium chloride aliquots ($25 \mu\text{L}$, 1 M) with the resulting voltammograms compared in figure 4.3.

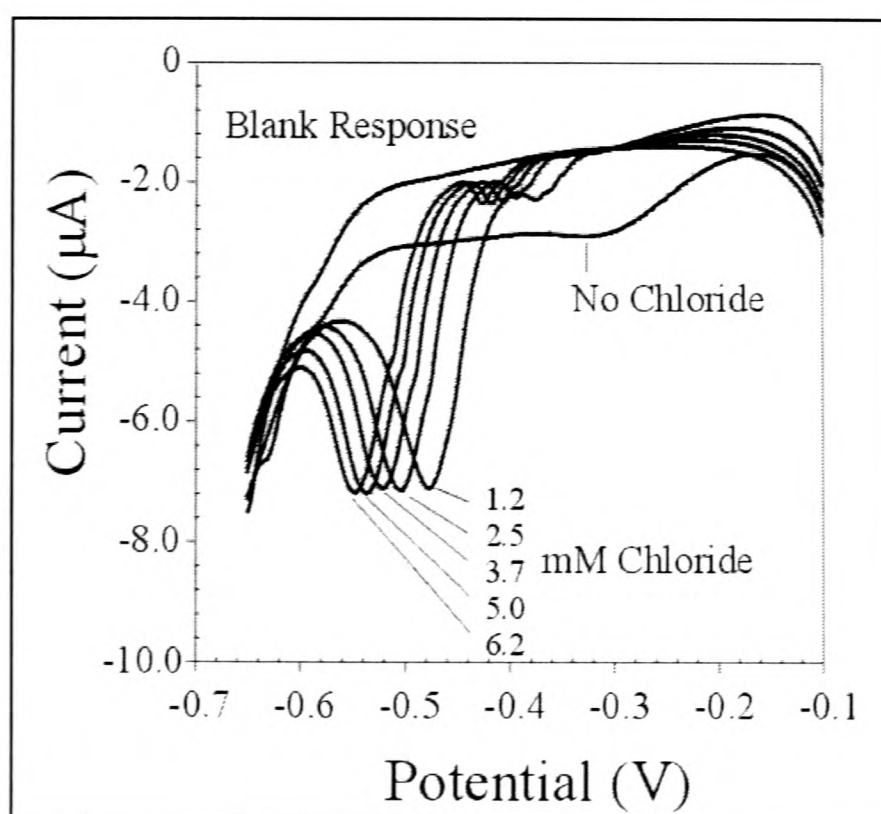


Fig. 4.3 - Linear sweep voltammograms detailing the response towards the reduction of $70 \mu\text{M}$ nitrate at a copper modified electrode before and after the addition of potassium chloride aliquots ($25 \mu\text{L}$, 1 M). Scan rate: 10 mV s^{-1} .

It can be seen that although chloride serves to enhance the nitrate reduction peak it also shifts it towards more negative potentials. Increasing the chloride concentration beyond 1.2 mM has little effect on the magnitude of the nitrate reduction peak. The introduction of chloride into the electrolyte solution at a concentration of 1.2 mM ensures that a maximum response is obtained. Any further increments in chloride ion concentration, originating from the introduction of an authentic sample in the standard addition experiments for example, will therefore only serve to shift the peak position. The choice of electrolyte pH was selected on the basis of previous work at freshly plated copper electrodes where it had been established that low pH favours the reduction process [5,8,10]. An electrolyte solution adjusted to pH 3 was selected in accordance with the system used in previous analytical studies for nitrate detection at a rotating copper disk and which in this instance was found to give excellent peak resolution. Shifting to lower pH has the effect of making the potential at which hydrogen evolution occurs less negative and hence obscures the nitrate reduction.

The mechanism of nitrate reduction at solid electrodes remains unresolved and would appear to be dependent upon the experimental conditions employed. However, in most cases the final product of the electroreduction reaction, and particularly at copper, is usually ammonia [2,5,8]. The scope of this work is limited to the analytical attributes of the modified electrode and the mechanism involved in the enhanced response is still speculative. However, the influence of some potential interferences in the determination of nitrate can also serve to shed some light on some aspects of the reduction process. The electrode response to 140 μM nitrate before and after the addition of 140 μM citric acid is shown in figure 4.4.

Upon the addition of citric acid, the nitrate reduction current is significantly reduced. This is not inconsistent with the enhanced nitrate reduction signal being attributable to a solution based copper species whose removal through complexation with the citric acid results in a diminution of the reduction current. Replacing the solution with fresh electrolyte containing 140 μM nitrate provides an electrode response that is essentially identical to that recorded before the addition of the citric acid (dashed line, figure 4.4). Thus, the retention of nitrate sensitivity after exposure to and subsequent removal of the complexing agent would support solution based catalysis rather than a direct surface interaction; however further work is required to explore these mechanistic issues. Given

that the nitrate reduction current arises at a similar potential to that observed for the reduction of the Cu(I) chloro species to the metal atom, then it could be postulated that nitrate serves to chemically re-oxidise the Cu atom. Re-reduction of this ion back to the metal may effectively set up a catalytic cycle that serves as the analytical response.

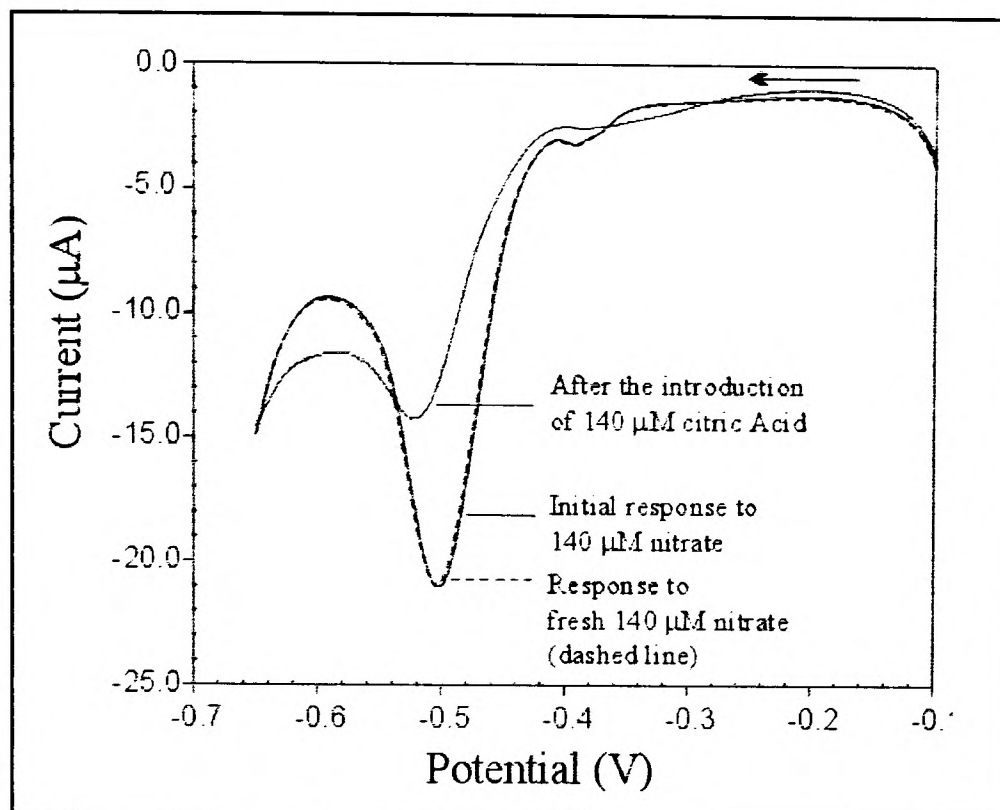


Fig. 4.4 - Influence of 140 μM citric acid on the response of a copper modified electrode to 140 μM nitrate. The subsequent response to 140 μM nitrate in fresh electrolyte is also included (dashed line). Scan rate: 10 mV s^{-1} .

The electrode response towards 70 μM nitrate in the presence of increasing nitrite aliquots (100 μL , 0.01M) is shown in figure 4.5.

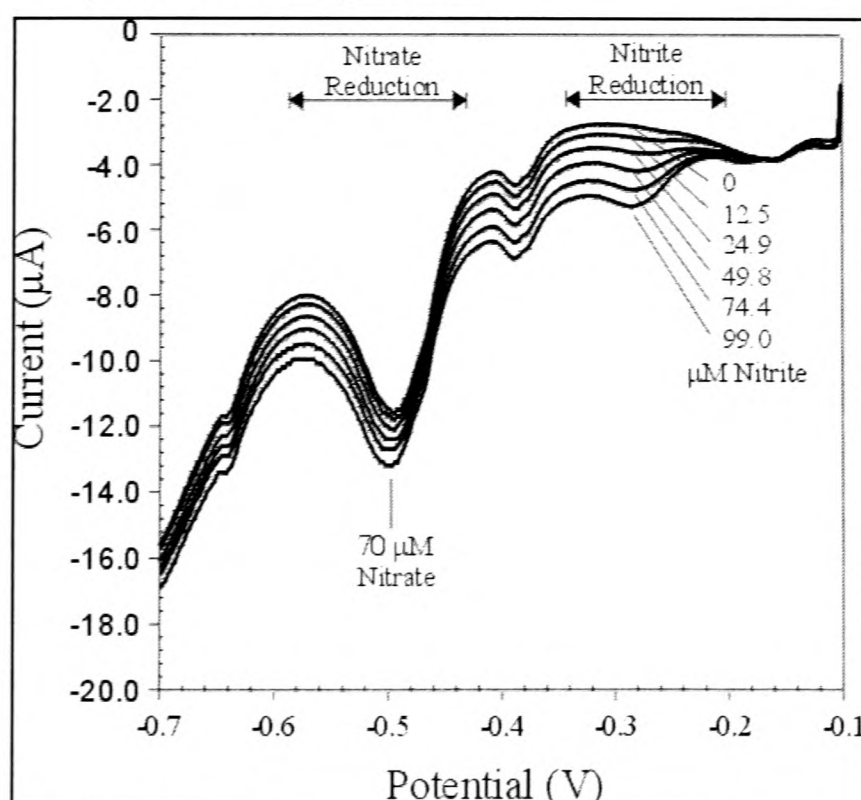


Fig. 4.5 - Linear sweep voltammograms detailing the response of a copper modified electrode to 70 μM nitrate in the presence of increasing nitrite concentration (12-100 μM). Scan rate: 10 mV s^{-1} .

Nitrite reduction was observed at -0.3 V with a linear response observed over 12 to 200 μM nitrite (Regression Data : $I_p / \text{A} = 0.024 [\text{NO}_2^- / \text{mol dm}^{-3}] + 8.36 \times 10^{-8}$, $R^2 = 0.999$, $N = 8$). The reduction of nitrite at -0.3 V contrasts previous studies of nitrite reduction at copper electrodes where the reduction potential occurs at similar or more negative potentials to those observed for nitrate reduction [5,8]. Increasing the concentration of nitrite was found to increase the nitrate peak current. However, closer examination of the voltammetric traces reveals that the height of the actual nitrate reduction peak remains essentially unchanged with the presence of nitrite increasing the baseline rather than substantially influencing nitrate reduction. The height of the nitrate peak (estimated by subtracting the current recorded immediately prior to nitrate reduction, -0.39 V, from the subsequent peak current, -0.5 V) was found to decrease by 8 % after the introduction of 0.1 mM nitrite.

The reduction of nitrite was found to occur irrespective of the presence of complexing agents such as citrate (not shown) and would suggest a different reduction mechanism to that attributed to nitrate reduction. The potential separation and clear resolution in the response to the two species combined with the absence of direct interference provides an option for speciation studies which is generally unavailable at single electrode configurations.

4.3.1 ANALYTICAL PERFORMANCE

The applicability of the CD electrode for routine analytical applications was assessed through the determination of nitrate levels in a number of authentic samples (lettuce and treated sewage discharge water). The electrodes were prepared as before and the pH 3 electrolyte used as the analytical solution. The nitrate levels in the given sample were assessed using a standard addition protocol in which a known quantity of the sample (typically 1 mL) is added to the electrolyte solution followed by a series of standard nitrate aliquots. With the exception of the lettuce, which required homogenisation, no further sample manipulation was undertaken prior to conducting the analysis. Linear sweep voltammetry was used as the detection methodology. Initial studies examined the determination of nitrate in lettuce as green vegetables are known to contain significant amounts of nitrate [4] and, as such, it was of interest to determine how the electrode

would function in a real matrix that contained fine particulate matter. The electrode responses to the blank electrolyte, added lettuce homogenate and four consecutive nitrate additions (25 μL , 0.025 M) are presented in figure 4.6 with the resulting standard addition plot is shown in the inset diagram. It must be noted that the shape and the peak positions of the various redox processes observed after the introduction of an aliquot of an authentic sample may vary from those recorded in blank electrolyte as a consequence of the matrix composition (chloride concentration, complexing agents, pH etc).

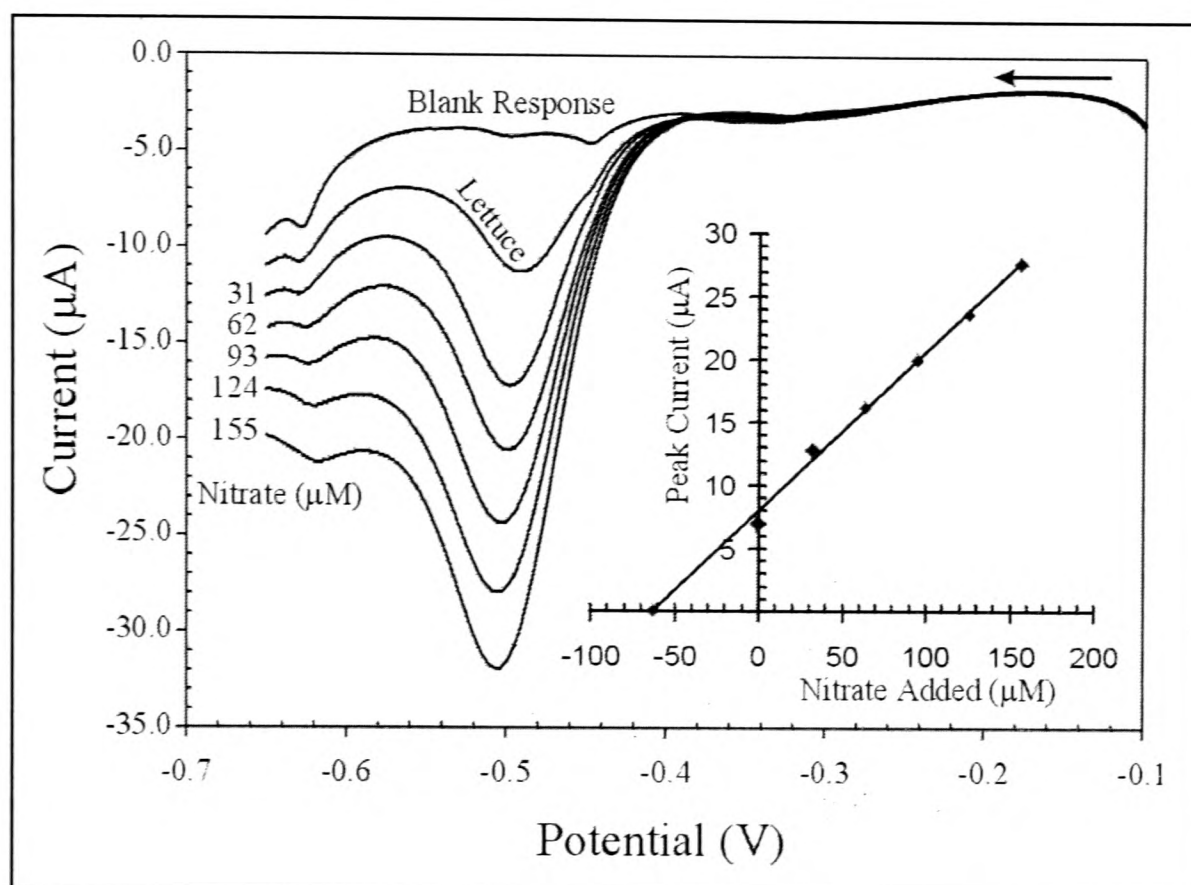


Fig. 4.6 - Linear sweep voltammograms detailing the standard addition determination of nitrate in lettuce.

Inset: The corresponding standard addition plot. Scan rate: 10 mV s^{-1} .

Once dilution factors are taken in account the nitrate concentration was calculated as 1.7 $\text{g NO}_3^- \text{ kg}^{-1}$ lettuce which is below the statutory maximum level [4] for nitrate in lettuce and spinach (3.5 g kg^{-1}). The ability of the electrode to function in the presence of such particulates is important given the desire to retain procedural simplicity and conduct the analysis in what is essentially “raw” media (albeit after dilution). The mesh like structure of the deposit (figure 4.1) may additionally serve to preserve electrode functionality in such instances by providing a physical barrier against fouling by large particulates such as leaf fragments/homogenate. However, it must be noted that the electrode will still be susceptible to the adsorption of small molecular sized species and particularly those capable of forming insoluble chelates with copper ion.

A more detailed study was conducted in the case of the sewage discharge samples as these present the electrode with a significantly lower nitrate concentration range with the two samples liable to contain differing anion (nitrate, nitrite, chloride, phosphate, bicarbonate) constituents. Given that no sample manipulation was conducted on the samples prior to conducting the electrochemical assay, the presence of particulates, algae, humic acids and other “natural” complexing species provide a genuine challenge to this approach as they possess the ability to influence nitrate reduction through a similar mechanism exhibited by citric acid (figure 4.4). The detection protocol remained unchanged from the initial lettuce experiments and the electrode response obtained were similar to those detailed in figure 4.6. The results obtained for each water sample (five replicate standard addition measurements) are detailed in table 4.1.

Water Sample	CD Modified Electrode [NO ₃ ⁻]/μM	% RSD (n=5)	Cu/Cd - Griess Assay [NO ₃ ⁻]/μM	% RSD (n=3)
Outfall A	481	3.7	479	4.4
Outfall B	574	2.7	552	3.1
Determination of Nitrate in Sewage Discharge				

Table 4.1 - Determination of nitrate in water obtained from two different sewage treatment outfalls. Comparison of the CD electrode results with an independent spectroscopic analysis.

The concentration of nitrate in both samples was independently verified by using the spectroscopic Griess procedure detailed previously and the results obtained are compared in table 4.1. It can be seen that the values obtained for the CD electrode are within 4 % of those obtained with the spectroscopic assay. Five independent standard addition determinations (each constructed from four additions of a standard nitrate solution) were conducted for each effluent sample. The percentage relative standard deviation (% RSD) was found to be less than 4 % for each effluent batch (table 4.1) confirming the utility of the electrochemical protocol. Moreover, the analysis can be accomplished far quicker, with less expense and without the additional need for calibrating a copperised cadmium column.

The same electrode could be used for multiple analyses but a gradual decrease in the peak response was observed as copper is gradually lost into solution. The electrode half-

life (time required for the nitrate peak current to decay by half in a solution containing 140 μM nitrate) is approximately 9 hours if stored at pH 3 under argon. While the electrode is essentially effective for only one working day, the ease with which the system can be regenerated combined with the procedural simplicity involved in standard addition determinations serves to offset much of the inconvenience associated with limited operating life.

4.4 CONCLUSIONS

A simple, inexpensive method for determining nitrate (and / or nitrite) in authentic sample matrices has been demonstrated. The technique is tolerant of a range of potential interferences (chloride and nitrite) that can significantly influence rival techniques whether amperometric or potentiometric based. The linear range (10 μM - 200 μM nitrate, 12 μM – 200 μM nitrite) is suitable for a number of analytically relevant applications and particularly those requiring water quality measurements. The following chapter continues the theme of modified electrode substrates and investigates the utility of a Cu-Ni alloy electrode in nitrate/nitrite analysis.

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CHAPTER 5

ENHANCED ELECTROCHEMICAL DETECTION OF NITRITE AND NITRATE AT A CU-30NI ALLOY ELECTRODE

*The work presented in this chapter has been published in
Analytical Letters 33 (2000) 3127.*

In an extension to the work presented in chapter 4, the electrochemical reduction of nitrate and nitrite was investigated at electrodes fabricated from an activated alloy. Significant catalytic activity towards the reduction of nitrite at unmodified Cu - 30 % Ni alloy electrodes was found within the concentration ranges 16 – 200 μM nitrite. The reduction of nitrite was found to occur at potentials significantly less negative than the reduction of nitrate thereby providing clear resolution and the possibility of reliable nitrite / nitrate speciation. The results are contrasted with those found at a bare copper electrode where the spatial resolution of the nitrate and nitrite reduction processes is severely limited with significant overlap of the reduction processes. The procedure represents an inexpensive and facile method for nitrite determination in a number of applications.

5.1 INTRODUCTION

In chapter 4, a reagentless electrode system for the determination of nitrate and nitrite was reported based upon the deposition of a macroporous copper deposit, which showed marked selectivity for these ions under mildly acidic conditions (pH 3) [1]. The reduction of nitrite was found to occur at potentials significantly less negative than those required for nitrate reduction and as a result clear resolution of both voltammetric peaks was obtainable. However, the complexities involved in the preparation and use of such modified electrodes often mitigates against their acceptance as a viable, routine analytical tool. The application of bare, unmodified electrodes for direct nitrite / nitrate analysis is undoubtedly a more attractive option but, unfortunately, in most cases low sensitivity and reproducibility associated with cumulative passivation effects have been apparent. The use of copper-nickel (CuNi) alloy electrodes have been previously used to good effect in

the electrochemical detection of carbohydrates [2] and dissolved oxygen [3-5] where passivation effects have proven problematic for traditional electrode substrates. It was therefore of interest to assess the potential applicability of a similar, unmodified Cu – 30 % Ni alloy electrode for nitrite / nitrate analysis.

5.2 EXPERIMENTAL

All reagents were of the highest grade available and used without further purification. Standardised hydrochloric acid (0.992 M) and anhydrous sodium sulphate were obtained from Aldrich. Sodium nitrite and potassium nitrate standards (BDH) were prepared daily and refrigerated when not in use. All solutions and subsequent dilutions were prepared using deionised water from an Elgastat (Elga, UK) UHQ grade water at a resistivity of 18 M Ω cm. Samples of treated sewage effluent were obtained from Windsor Scientific Ltd (Slough, UK).

Electrochemical measurements were conducted using a μ -Autolab computer-controlled potentiostat (Eco-Chemie, Utrecht, Netherlands) with a standard three-electrode configuration and a typical working cell volume of 15 cm³. Copper wire (1.9 mm diameter) and copper-nickel alloy (3.5 mm diameter) were mounted into PTFE and served as the working electrodes. Spiral-wound platinum wire served as the counter electrode and a saturated calomel (Radiometer, Copenhagen) as the reference electrode. Both copper and copper-nickel electrodes were polished using 1 μ m alumina powder (Buehler Micropolish II, USA) and cleaned in an ultrasonic bath prior to use. An acidic electrolyte was used throughout these investigations and was prepared by the neutralisation of sufficient sulphuric acid to provide a solution comprising 0.1 M sulphate (pH 3). The electrochemical methodology employed was linear sweep voltammetry (LSV) conducted from 0.0 to –1.0 V at a scan rate of 10 mV s⁻¹. In all cases the electrode responses have been converted to current density to aid direct comparison given the slight difference in the area of each electrode.

5.3 RESULTS AND DISCUSSION

The electrochemical reduction of nitrite and nitrate was investigated at electrodes fashioned from the CuNi alloy, pure copper and pure nickel. The latter did not provide any analytically useful response to either analyte and therefore the following discussion focuses upon a comparison of the responses obtained at the CuNi and pure copper electrodes. Voltammograms detailing the responses obtained at the CuNi electrode to increasing concentrations of nitrite (25 μL aliquots, 10 mM NaNO_2) are shown in figure 5.1.

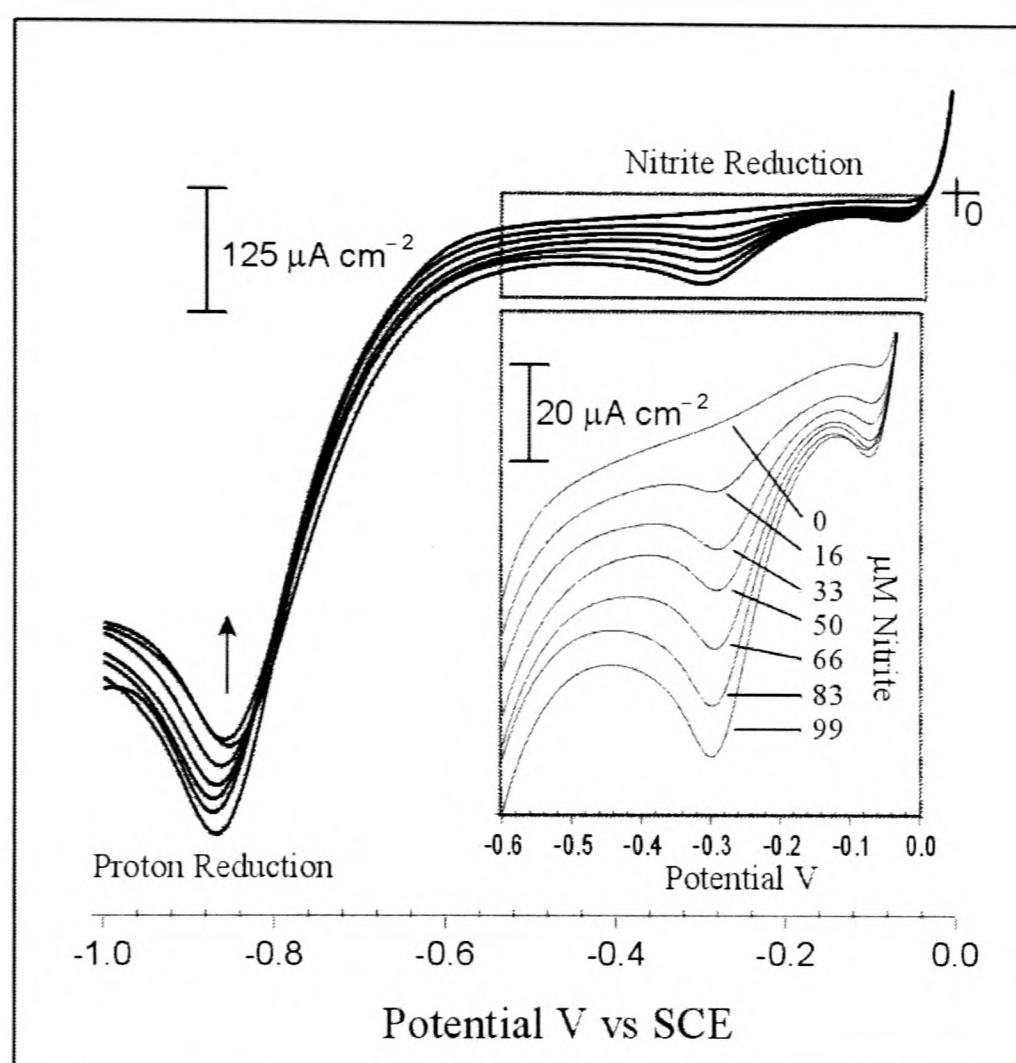


Fig. 5.1 - Linear sweep voltammograms recorded at a CuNi alloy electrode in the presence of nitrite (pH 3).

Two principal voltammetric features can be clearly observed. The first, reduction of nitrite, was found to occur at -0.3 V and is shown in greater detail in the inset diagram. The reduction of nitrite at the CuNi electrode provides a well-defined peak with the response found to be linear from 16 μM up to 0.1 mM. This is within an analytically relevant range for a number of sensing applications and is comparable with some recent developments in the electroanalytical detection of nitrite [6,7].

The second redox process, observed at -0.9 V, can be assigned to the reduction of protons / evolution of hydrogen in the weakly acidic electrolyte. This peak was also found to vary according to the concentration of added nitrite or nitrate and it can be seen in figure 5.1 that the magnitude of the peak decreases with increasing nitrite. This decrease can be accounted for through the localised depletion of protons as a consequence of their participation in the electroreduction of nitrite (and nitrate). The monitoring of this peak is of little analytical utility for the detection of either nitrite or nitrate given its potential susceptibility to the action of buffering agents. However, it may be of value in more fundamental studies where the unambiguous elucidation of the complex mechanisms attributed to the reduction of these analytes at solid electrodes has yet to be achieved.

The response of the CuNi electrode to nitrite can be better appreciated through comparing the response obtained at a bare copper electrode, figure 5.2.

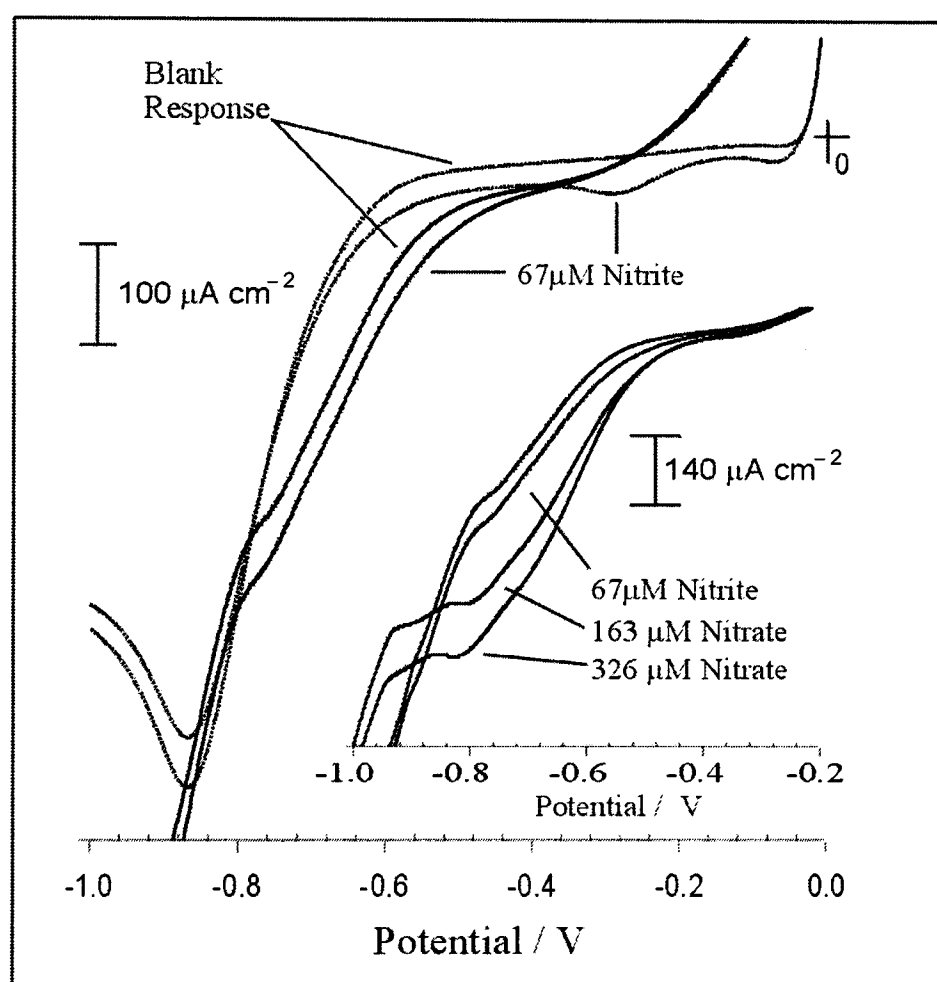


Fig. 5.2 - Response of a pure copper electrode to 67 μM nitrite (solid lines) compared with that obtained at a CuNi electrode (dashed lines). Inset: Response of the Cu electrode after the introduction of aliquots of nitrate.

The response of a copper electrode to 67 μM nitrite (solid line) is compared with that obtained at the CuNi electrode (dashed line). The reduction of nitrite at the copper electrode was found to occur at significantly more negative potentials (-0.6 V) to that

observed with the CuNi electrode (-0.3 V). In addition, the response appears as a poorly defined shoulder just short of where hydrogen evolution commences which can be problematic when attempting to accurately assess nitrite concentration.

The potentials required for nitrite reduction have particularly important consequences when considering that nitrate reduction can also occur at these electrodes and will often be present in significantly higher concentrations than nitrite. The response of the bare copper electrode to nitrate is shown in the inset diagram in figure 5.2. It can be seen clearly that nitrate reduction occurs at similar potentials to that of nitrite and hence would provide an intolerable interferent in the determination of nitrite.

The selectivity of the CuNi electrode for nitrite in the presence of excess nitrate was also examined. The electrode response to 50 μM nitrite after the addition of aliquots of a standard nitrate solution (25 μL , 0.1 M KNO_3) was assessed and voltammograms for the first three additions are shown in figure 5.3.

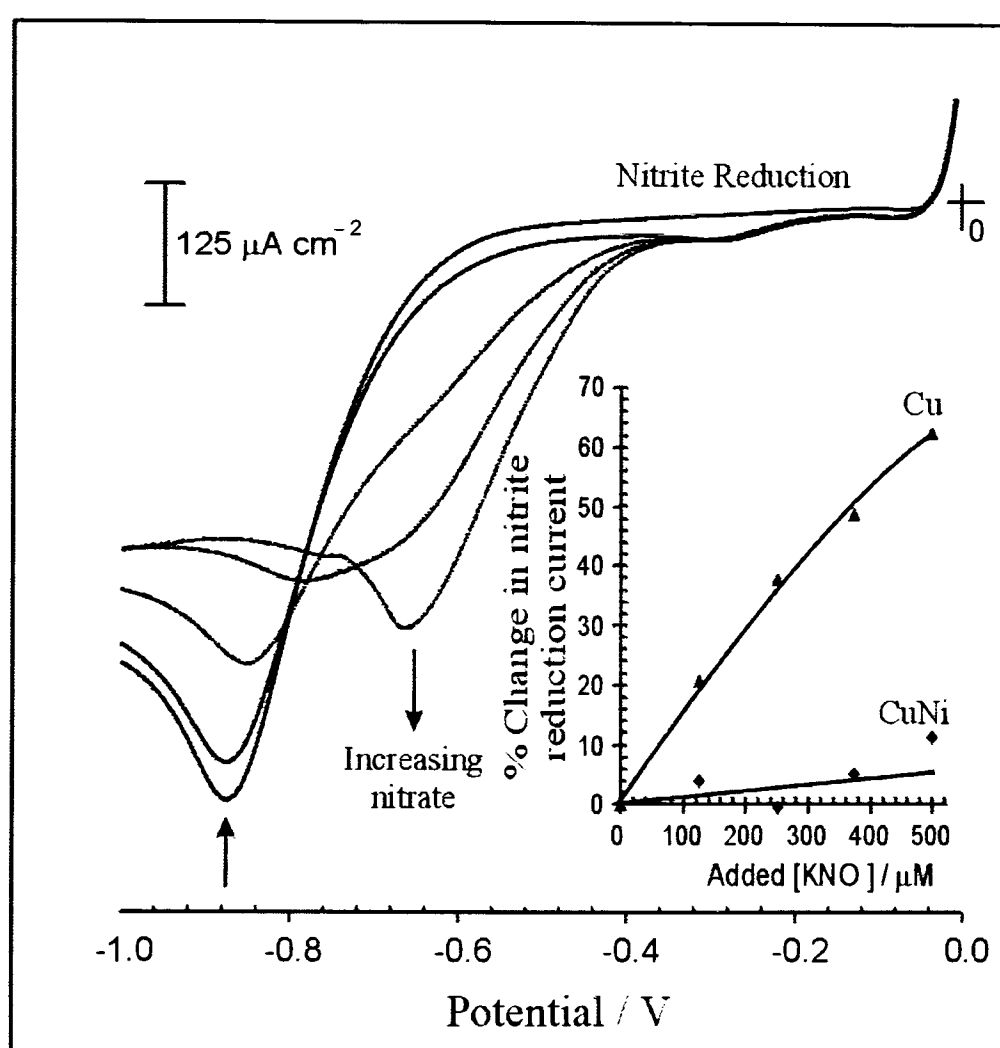


Fig. 5.3 - Influence of increasing nitrate concentration (dashed lines) on the response of a CuNi electrode to 50 μM nitrite (solid line). Inset: Comparative assessment of the influence of increasing nitrate concentration on the current response to 50 μM nitrite recorded at the CuNi and pure copper electrodes.

It can be seen that in contrast to the copper electrode, nitrite reduction is generally unaffected by the increasing nitrate concentration. A more quantitative comparison is provided in the inset graph. The change in the current, at the potential where nitrite reduction is observed for each respective system, was monitored as a function of increasing nitrate concentration. There is little change in the magnitude of the nitrite current for the CuNi electrode until exceptionally large concentrations are reached. However, there is a substantial increase in the current recorded at the pure copper electrode even at low concentrations of nitrate. The electrode response of the bare Cu and the CuNi electrode to increasing aliquots of nitrate and nitrite covering the range 16 – 200 μM was assessed and the regression data obtained from the current density-concentration plots presented in table 5.1.

	Response to Nitrite		Response to Nitrate	
	CuNi	Cu	CuNi	Cu
Potential / V	-0.3	-0.6	-0.7	-0.6
Gradient / mol dm^{-3}	-0.70	-0.25	-0.7	-0.98
Intercept	-1.1×10^{-6}	-2.6×10^{-6}	3.4×10^{-6}	-1.4×10^{-5}
R^2	0.998	0.990	0.998	0.943
Standard Error	1.2×10^{-6}	2.0×10^{-6}	2.6×10^{-6}	1.9×10^{-5}
Observations	7	7	7	7
LOD / mol dm^{-3}	5×10^{-6}	2.4×10^{-5}	1.1×10^{-5}	5.8×10^{-5}

Table 5.1 - Calibration data relating current density to increasing concentrations of the target analyte (16 - 200 μM) with the resulting straight line taking the form $j / \text{A cm}^{-2} = x [\text{NO}_2^-] \text{ or } [\text{NO}_3^-] / \text{mol dm}^{-3} + c$ to enable direct comparison.

The calculated limits of detection, based on $3s_B$ (Appendix B), have been included for comparison. The high sensitivity of the CuNi electrode for nitrite, highlighted in figure 5.1, is clearly evident when comparing the gradients of the current/concentration plots detailed in table 5.1 with the lower detection limit and operating potential confirming the effectiveness of the alloy electrode approach.

The influence of chloride ion on the shape and magnitude of the nitrite reduction peak was also assessed. Linear sweep voltammograms recorded at the CuNi electrode in the presence of 164 μM nitrite and increasing concentrations of chloride (0-4 mM KCl) are shown in figure 5.4.

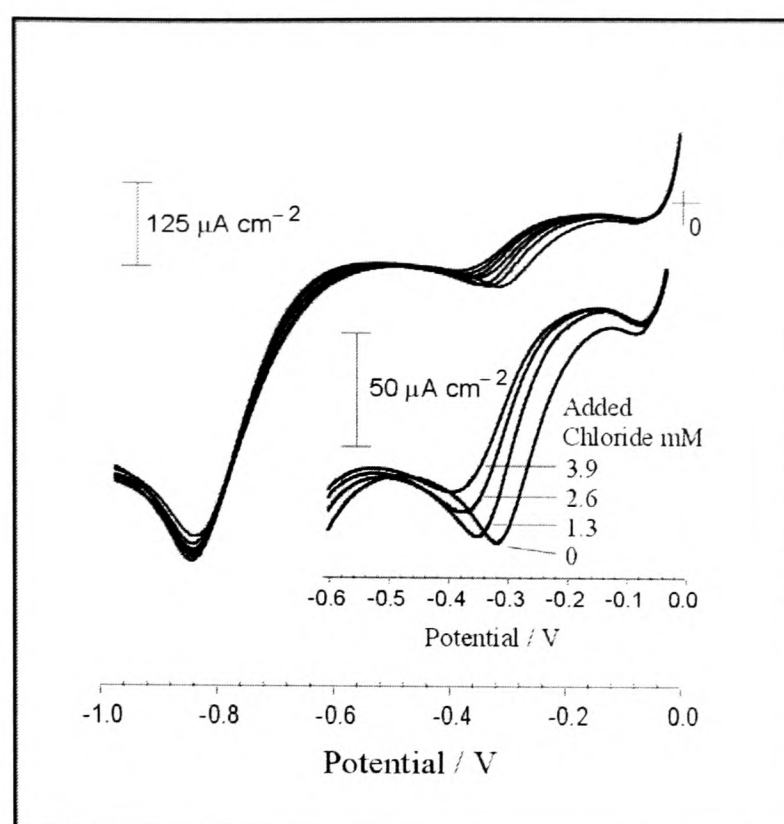


Fig. 5.4 - Influence of increasing chloride additions on the CuNi electrode response to 164 μM nitrite.

The potential at which nitrite reduction commences was found to shift to more negative potentials with increasing chloride concentration. In addition, the height of the peak was found to decrease with a fall of 8 % observed after the introduction of 3.9 mM Chloride. This behaviour mirrors that observed for a number of systems involving copper substrates [1,8]. Given the influence of chloride on the nitrite peak it was necessary to briefly assess the analytical performance of the CuNi electrode in an authentic matrix. Samples of treated sewage effluent were selected for this purpose. While the endogenous concentration of nitrite was found to be below the limit of detection for this system, the samples were known to contain substantial concentrations of nitrate ($\sim 480 \mu\text{M}$) [1]. The samples were spiked with 39 μM nitrite and a series of recovery experiments conducted. The recovery of nitrite was found to be 96 % and confirms that despite high nitrate concentration and a multitude of other matrix constituents, the selectivity of the CuNi electrode is retained in complex media.

5.4 CONCLUSIONS

This chapter highlights the attractiveness of CuNi alloy electrodes for electrochemical nitrite sensing applications in light of the electrochemical methodology employed with the modified electrodes reported in chapter 4. This initial report has highlighted the response to nitrite but clear voltammetric resolution of both anions is possible and provides considerable potential for the use of CuNi system in speciation studies. While the adaptation of the methodology to long-term measurements in compositionally diverse samples has yet to be addressed, the potential utility of the CuNi electrode has been clearly demonstrated. Attention is now directed towards the activation of the electrode through the application of ultrasound and is presented in chapter 6.

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CHAPTER 6

ELECTROCHEMICAL DETECTION OF NITRATE AT A COPPER MODIFIED ELECTRODE UNDER THE INFLUENCE OF ULTRASOUND

*The work presented in this chapter has been published in
Electroanalysis 12 (2000) 1363.*

The electrochemical protocol outlined in chapter 4 has been adapted to incorporate the use of ultrasound at a copper modified electrode and this chapter details the influence ultrasound (20 kHz) exerts on the structure of the deposit and on the response to nitrate. The deposit was substantially removed by the acoustic streaming and cavitation processes common to 20kHz ultrasound even at relatively mild power outputs and large horn electrode separations. However, bombardment with 20 kHz ultrasound does not remove the deposit entirely with a compact copper layer remaining. This portion of the deposit was found to retain significant nitrate sensitivity compared with the bare, unmodified electrode. The retention of the compact catalytic layer under the influence of ultrasound enabled the use of sono-linear sweep voltammetry which was found to provide signal enhancement significantly greater than the response observed under silent conditions. The analytical utility of the technique was assessed through the determination of nitrate in treated sewage outfall water. The silent and sonochemical results were found to be within 5 % of the value obtained from an independent spectroscopic analysis performed in collaboration with Dr. M. F. Cardosi, Department of Biological Sciences, Paisley University. SEM and AFM imaging experiments were performed in collaboration with Dr. S. J. Wilkins, PTCL, Oxford University.

6.1 INTRODUCTION

Sonoelectrochemistry has been successfully applied to the detection of numerous analytes with the acoustic streaming and cavitation processes resulting from the induced sonic field providing significant advantages over traditional techniques [1-6]. The greatly enhanced mass transport and in-situ cleaning effects generated by the asymmetric collapse of bubbles at the electrode solution interface have tended to favour

applications where the target analyte requires accumulation at the electrode surface prior to analysis [1,2] or where highly fouling matrices are encountered and in-situ cleaning required [1-6]. These two factors have also been combined to good effect where the electrochemical analysis is conducted during insonation [4-6]. The resulting sonovoltammograms benefit from both the increase in mass transport and continual electrode activation through cavitation cleaning. This direct application of ultrasound has been successfully demonstrated for the determination of ascorbic acid in fruit drinks [4,5] and nitrite in homogenised egg [6].

While the erosive capability of the cavitation process is an invaluable asset in many circumstances, there are a number of situations where it may prove to be a liability. One situation in particular is where the electrode response relies on the selectivity of an immobilised catalyst. The enhanced mass transport processes accruing from the introduction of ultrasound offer considerable improvements in the sensitivity but any advantage may be offset through the gradual ablation of the electrode surface [1,7-9]. Ultrasound has been shown to degrade solid electrodes (metallic and carbon) and numerous coatings (polymeric and inorganic) and may in fact serve to remove the catalyst responsible for the improved sensitivity.

This chapter aims to examine the influence ultrasound exerts when a catalytic layer is employed to enhance the electrode sensitivity towards nitrate reduction as previously reported in chapter 4. The necessity for nitrate monitoring is recognised by most health authorities world wide with legislation often levied on permissible levels in drinking water and food produce [10]. As such, strategies for its detection are continually evolving as the demand for increasingly sensitive techniques grows. The response to nitrate under conventional, ultrasonically silent, conditions has been described in chapter 4, based primarily upon an electrodeposited copper layer that can provide a linear response over 10-200 μM nitrate [11]. While this range is acceptable for a number of applications it is inadequate when considering the analysis of physiological nitrate concentrations [12]. The possibility of surface fouling whether in the latter situation or indeed in environmental conditions where dirty matrices are typically encountered can also severely restrict the applicability of the system [13].

The potential increases in sensitivity offered by the ultrasonically induced increased mass transport could extend the applicability of the modified electrode. Provided cavitation erosion of the catalytic layer can be tempered, the introduction of ultrasound may also facilitate electrode reactivation in difficult matrices. The stability of the electrodeposited copper layer is examined under the influence of fairly mild sonication and the effect on nitrate sensitivity assessed. The morphology of the catalytic layer is examined using scanning electron microscopy and atomic force microscopy and the electrochemical response to nitrate assessed by both linear sweep voltammetry and sonovoltammetry.

6.2 EXPERIMENTAL

All reagents were of the highest grade available and used without further purification. Standardised hydrochloric acid (0.974 M), anhydrous sodium sulphate and potassium nitrate were obtained from Aldrich. All solutions and subsequent dilutions were prepared using deionised water from an Elgastat (Elga, UK) UHQ grade water system with a resistivity of 18 M Ω cm. Electrochemical measurements were conducted using a mAutolab computer controlled potentiostat (Eco-Chemie, Utrecht, Netherlands) with a standard three electrode configuration. The dimensions and electrode arrangement within the cell have been previously described [3]. Copper wire (2 mm diameter) mounted flush in a PTFE coating or wound into a short coil (0.25 mm diameter, 3 cm length) served as the working electrode with spiral wound platinum wire as the counter electrode and a saturated calomel (Radiometer, Copenhagen) reference electrode. The planar electrodes were polished between experiments with 1 μ m alumina powder (Buehler Micropolish II, USA) whereas coil electrodes were immersed in 5 M HNO₃ for 10 seconds. The electrodes were thoroughly rinsed and sonicated in an ultrasound bath immediately prior to use. All electrochemical measurements were conducted at scan rate of 10 mV s⁻¹.

Surface characterisation was carried out using a Cambridge Stereoscan scanning electron microscope at a magnification of 661x. Surface morphology comparisons were obtained using a Topometrix TMX 2010 Discoverer atomic force microscope operating in contact mode with SFM probes (type 1520-00) and a 75 mm scanner (type 5590-00).

6.2.1 DEPOSITION OF THE COPPER FILM

The copper film was prepared by fixed potential electrolysis of an unstirred solution containing CuSO_4 (50 mM) and Na_2SO_4 (0.1 M) which was adjusted to pH 3 through the dropwise addition of HCl. The solution was degassed with argon and the electrolysis conducted by fixing the potential at -1 V for 20 minutes. The solution was then carefully removed and the cell rinsed with deionised water. Where possible, the electrodes should be stored wet (preferably under argon) as copper oxide quickly forms on the electrode if left dry for any extended period of time and has the effect of reducing nitrate sensitivity.

6.2.2 SONOELECTROCHEMICAL MEASUREMENTS

Sonoelectrochemical measurements were conducted using a 20 kHz transducer with a stepped 3 mm titanium horn tip placed 3 cm vertically above the working electrode. The analysis solution consisted of 0.1 M Na_2SO_4 (adjusted to pH 3 with HCl) which was degassed with argon prior to commencing the electrochemical measurement. A transducer power setting of 10 % was applied throughout with a stainless steel cooling coil employed to minimise localised heating effects resulting from the applied sonic field and maintain solution temperature at $22\text{ }^\circ\text{C}$ ($\pm 2\text{ }^\circ\text{C}$).

6.2.3 DETERMINATION OF NITRATE IN SEWAGE EFFLUENT

The electrode was placed in a solution (typically 18 cm^3) containing 0.1 M Na_2SO_4 that had been previously adjusted to pH 3 with 0.1 M HCl. An aliquot of the effluent sample (typically 1 mL) was added and the voltammetric trace recorded (0 V to -0.65 V at 10 mV s^{-1}) under silent conditions and during the application of ultrasound. This process was repeated for four or five additions of a standard nitrate solution ($25\text{ }\mu\text{L}$, 0.025 M). In the case of the sonoelectrochemical sweep an electrode conditioning procedure was initiated prior to commencing the measurement scan. This involved poisoning the electrode potential at -0.1 V for 30 s under the influence of ultrasound with the aim of re-depositing lost copper ion (detailed in the following sections) and stabilising the subsequent measurement trace.

The amount of nitrate contained within the original sample was assessed through a conventional standard addition plot. The current responses were taken at the reduction peak maximum of -0.5 V in the case of silent measurements and the average limiting current at -0.65 V for the sonochemical measurements. Spectroscopic analysis of the sewage effluent was carried out by reducing the nitrate to nitrite using a Cu/Cd reductor column.

6.2.4 SPECTROSCOPIC ASSAY OF SEWAGE OUTFALL WATER

The amount of nitrate in each sample was evaluated through the combination of a copperised cadmium reduction column and spectroscopic detection of the resulting nitrite ion using an established Griess protocol [11]. Nitrite standards (0.625 , 1.25 , 2.5 , 5 and 10 mg mL⁻¹) were prepared by serial dilution from the stock nitrite (IC grade) solution with distilled water. Griess reagent was freshly prepared by mixing equal volumes of two separate indicator solutions. The first comprised α -naphthylamine (0.1 g) dissolved in 100 mL deionised water with the second solution containing 1 g of sulphanilamide (1.0 g) and phosphoric acid (5.9 ml, 85 % w/v) made up to 100 mL with deionised water. Each water sample was diluted ($1 + 1$, $1 + 4$ and $1 + 9$) with deionised water and the column reduction process allowed to proceed for 10 minutes.

Aliquots of the reduced sample (0.1 mL diluted to 0.5 ml with deionised water) were combined with 0.5 mL of Griess reagent. Each determination was carried out in triplicate through measuring the absorbance at 543 nm (1 cm path length) and the blank response (0.5 mL of deionised water and 0.5 mL of Griess reagent) subtracted. The two water samples were also tested independently for the presence of endogenous nitrite and the final nitrate results adjusted accordingly.

6.3 RESULTS AND DISCUSSION

The morphology of the copper deposit was examined by scanning electron microscopy and a typical micrograph obtained after deposition of the copper layer onto a copper electrode is shown in figure 6.1. A porous, mesh like structure is clearly observable. However, the structure is more complex than the fern-like coating indicates and essentially consists of two distinct layers.

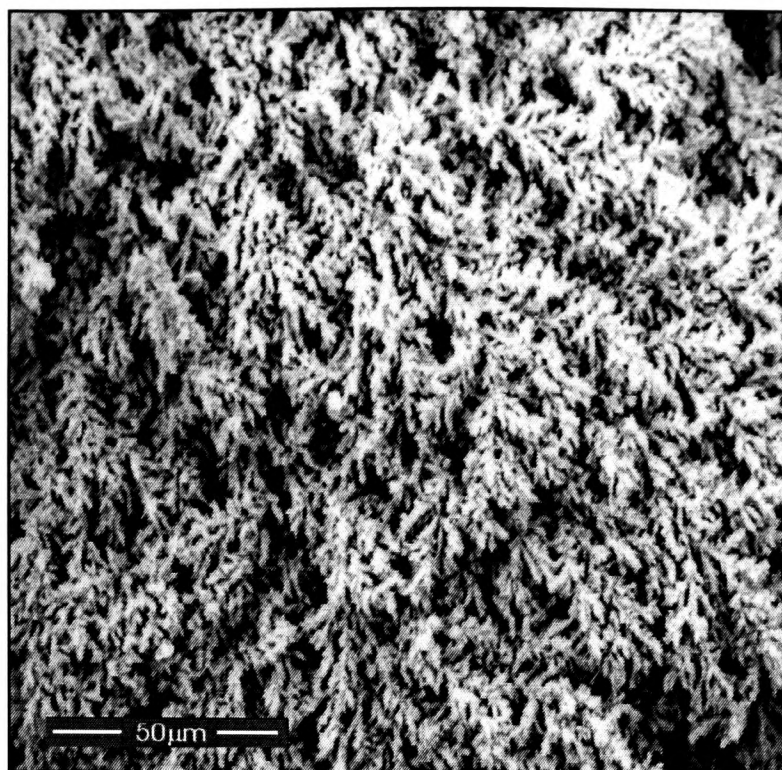


Fig. 6.1 - SEM micrograph of the surface of the copper electrode after the electrodeposition of the copper.

The initial deposition of the copper results in a dense compact layer from which the porous network grows. This behaviour was observed by taking a freshly polished copper electrode and covering one half with adhesive tape. Copper was deposited on the exposed side and after removal of the shielding tape, the electrode was examined using scanning electron microscopy. The resulting micrograph, shown in figure 6.2, highlights the interface between the bare and coated side of the electrode. A dense, compact layer can be seen on the right hand side of the electrode where the copper was deposited with fibrous mesh-like strands beginning to nucleate throughout the compact layer.

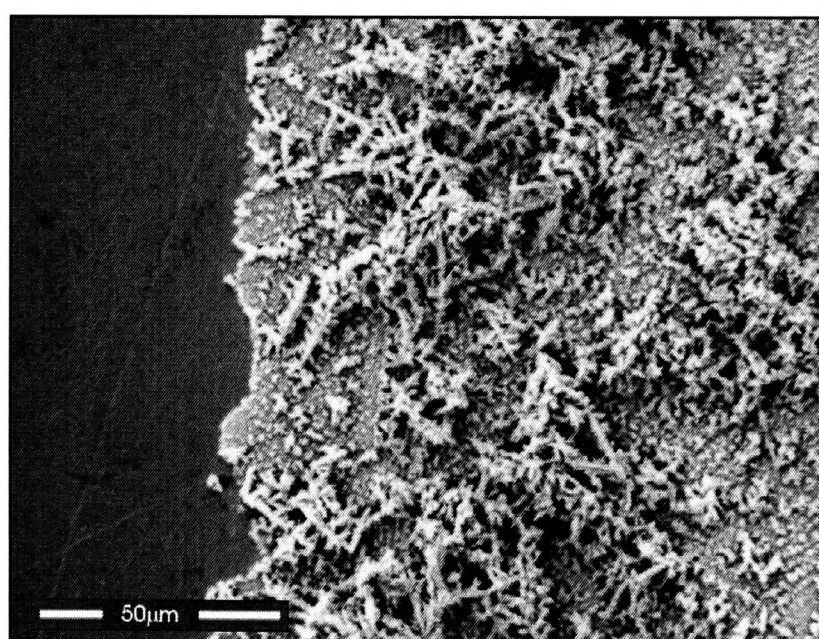


Fig. 6.2 - SEM micrograph highlighting the two distinct layers (compact and macro-porous) resulting from the electrodeposition of copper. The bare copper electrode substrate is shown on the left hand side of the image.

The stability of the electrodeposited structure under the influence of ultrasound was examined using a low power setting (10 %) and large horn to electrode separation (3 cm). These settings represent fairly mild conditions compared with the configurations used in the majority of sonoelectrochemical applications [1-6] and were selected to minimise cavitation erosion of the surface. The copper electrode was placed in fresh buffer solution and 150 μM nitrate added to the solution. Linear sweep voltammograms were recorded under silent conditions before and after the application of a 20 second period of ultrasound. The resulting voltammetric traces are compared in figure 6.3 along with the response obtained at a bare, freshly polished, electrode.

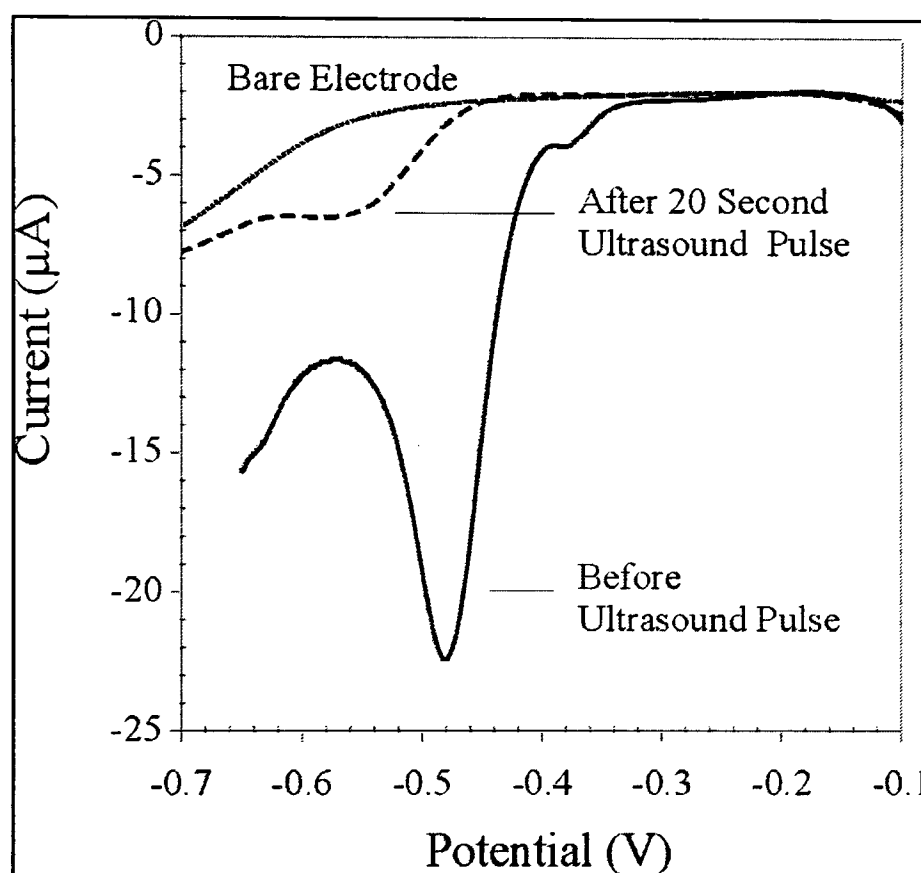


Fig. 6.3 - Linear sweep voltammograms of the electrode response towards 150 μM nitrate before (solid line) and after (dashed line) exposure to 20 seconds of ultrasound. The response obtained at a freshly polished electrode (dotted line) is included for comparison.

At first glance the application of ultrasound would appear to destroy the film and hence the sensitivity towards nitrate reduction. The sharp reduction peak observed at -0.48 V before the introduction of ultrasound decreases markedly in height after the 20 second insonation period. However, nitrate reduction remains significantly above the response obtained at a bare electrode.

The introduction of ultrasound essentially removes the fern like growth from the electrode but the fact that the nitrate response is still greater than that observed at the bare electrode suggests that some catalytic portion remains. The dense compact layer shown in figure 6.2 is not immediately removed by the ultrasound and is responsible for the nitrate response, albeit at a significantly lower sensitivity. The increase in sensitivity provided by this layer over the bare electrode response can be appreciated through comparing the respective surface morphologies. This was achieved by atomic force microscopy and the resulting scans for the bare, polished, copper metal and the compact layer resulting from the copper deposition are compared in figure 6.4.

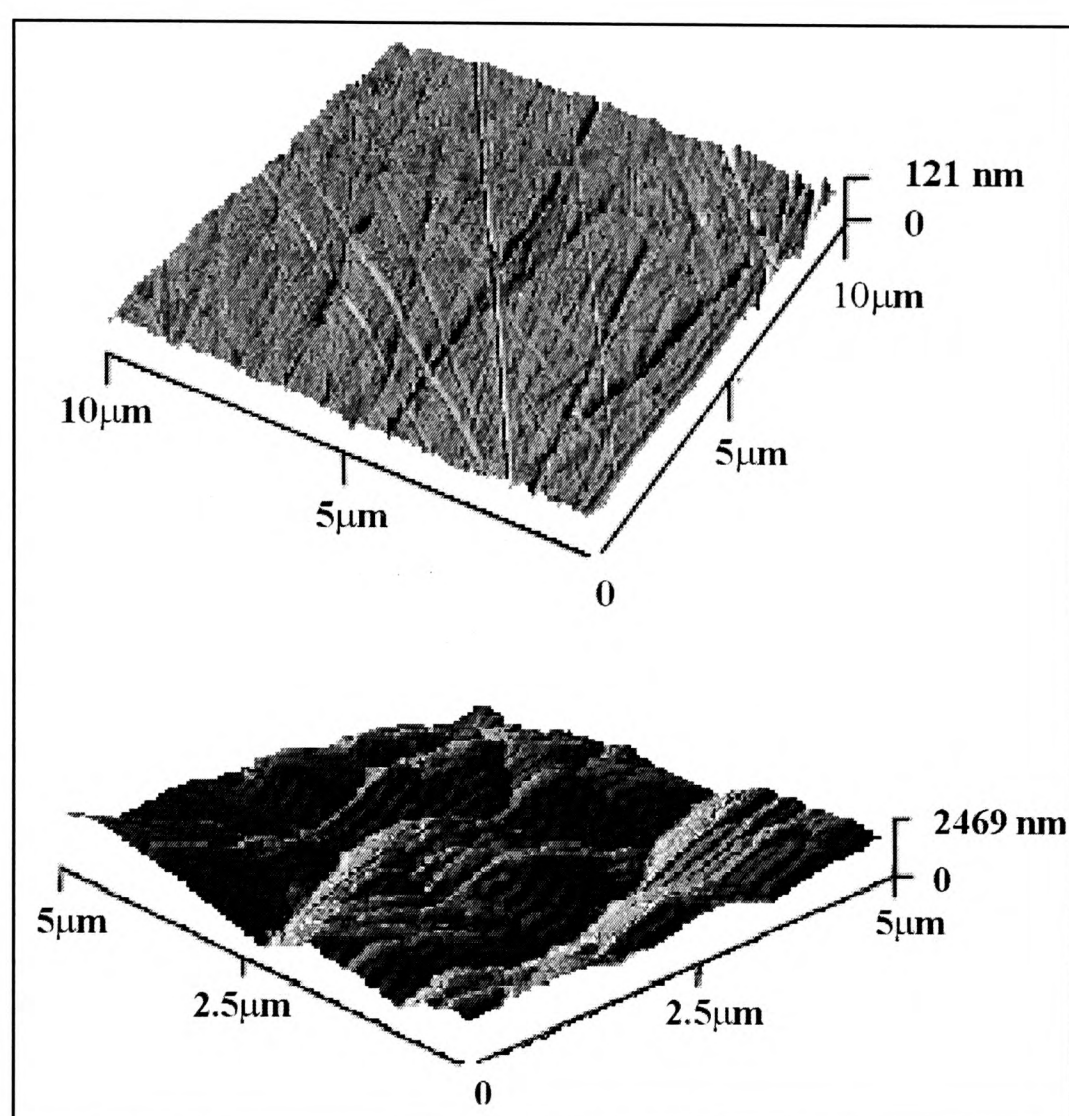


Fig. 6.4 - AFM image of a freshly polished copper metal disc (top) and of the compact layer resulting from the electrodeposition of copper where the porous mesh has been removed (bottom).

The compact layer formed through the electrodeposition process has a far rougher texture with typical depth profiles of up to several microns compared with the hundred nanometre profiles observed with the bare copper disc. The rougher texture of the copper deposit presents a far greater surface area than the bare electrode but it must also be noted that as the deposit was formed under anaerobic conditions it will be more active

than the polished surface. Oxides generated through the combined action of surface abrasion and air exposure will lead to a degree of surface deactivation and contribute to the poor response obtained at bare electrodes.

The compact layer was found to be stable to repeated pulses of ultrasound but as such this does not represent any significant advance on previous results as the sensitivity remains lower than that observed at an intact copper modified electrode under silent conditions. However, having ascertained that the compact layer is retained under the influence of ultrasound a series of sonovoltametric investigations were carried out. This involved scanning the potential range, as in previous studies, but in this instance ultrasound is applied during the actual measurement. A comparison between the conventional linear sweep voltammograms and the sonovoltammograms recorded at the copper modified electrode in the presence of 70 μM and 140 μM nitrate is shown in figure 6.5.

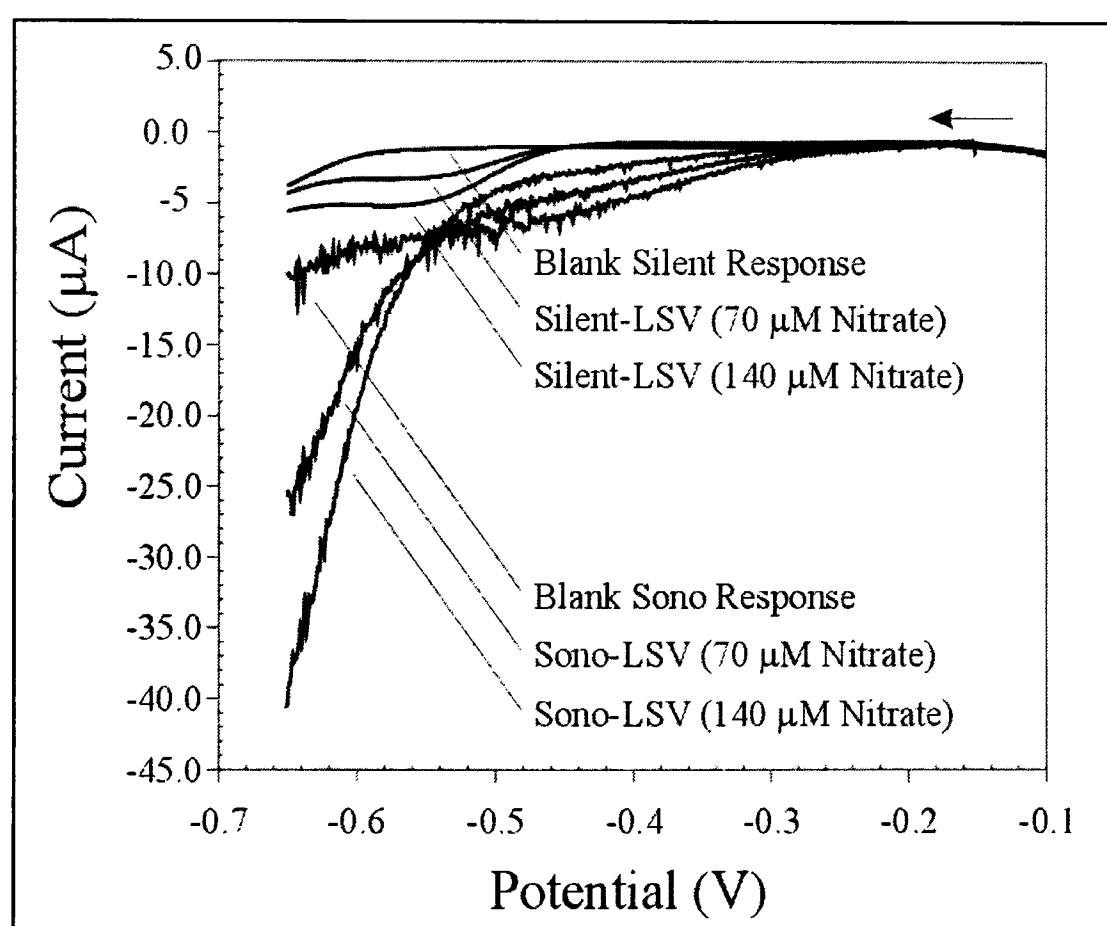


Fig. 6.5 - Linear sweep voltammograms comparing the response of the compact catalytic layer to 70 μM and 140 μM nitrate under silent conditions and during the application of ultrasound.

The limiting currents observed with the sonovoltammograms are skewed rather than the level plateau normally associated with sonoelectrochemical enhanced mass transport [1]. This can be attributed in part to the close proximity of the nitrate reduction peak to the onset of hydrogen evolution. The skewed response makes interpretation of the response

slightly more complicated (discussed below) but a consistent approach over the selection of the measurement potential (-0.7 V) can be used to gauge the relative sensitivities of the silent and insonated techniques.

A quantitative comparison of the relative sensitivities of the silent and sonochemical response is provided in figure 6.6. The response obtained at an intact copper modified electrode has also been included for comparison.

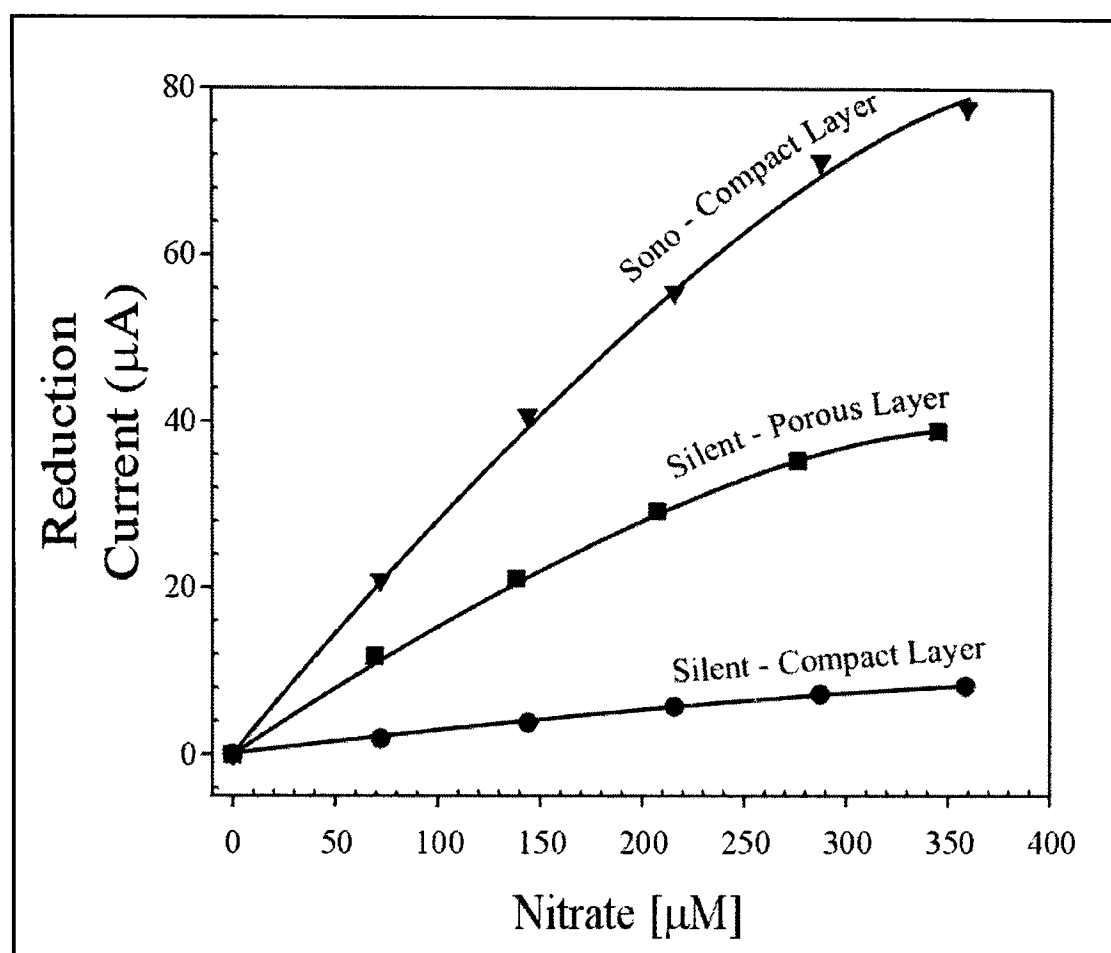


Fig. 6.6 - A quantitative comparison of the compact deposit modified electrode response to increasing nitrate concentration under silent and sonicated conditions. The response obtained at an intact, porous, copper modified electrode under silent conditions is also included.

The introduction of ultrasound provides a dramatic improvement in the sensitivity of the electrode towards nitrate over the silent response obtained with the compact copper layer. The sonochemical response is also significantly greater than that obtained with the intact, macroporous, system and hence may be worthy of consideration for a number of analytical applications. The fragility of the macroporous system is a definite drawback to the utility of that system and hence the ability to use the more robust underlying layer as the sensing system without compromising the sensitivity is undoubtedly a major advantage.

The electrode lifetime remains problematic as, in addition to the expected cavitation erosion of the surface, the analytical procedure requires sweeping the potential range from 0V to -0.8 V. Initiating the scan at 0 V has been found to substantially benefit the reproducibility of the linear sweep in both silent and insonated cases but at this potential copper is actively stripped from the electrode surface. Under silent conditions the majority of the copper is redeposited as the scan progresses in the negative direction but the turbulence created as a consequence of ultrasonically induced acoustic streaming will effectively sweep a significant portion of the copper ion out into the bulk solution. Stabilising the compact copper layer under such conditions is currently under investigation with the most promising option involving the introduction of a small quantity of cupric ion into the solution during analysis. There have been a number of reports where copper salts are added to the solution such that a new surface is effectively generated for each analytical scan [14,15]. The presence of cupric ions in this instance could similarly serve as a source of copper that can be redeposited during the initial stages of the scan thereby combating the loss through both stripping and cavitation erosion.

6.3.1 ANALYTICAL PERFORMANCE

The analytical utility of the sonochemical approach was briefly assessed through the evaluation of nitrate in treated sewage effluent. The level of nitrate was determined through a standard addition protocol (described previously) under silent conditions and during insonation. A typical set of sonovoltammograms recorded during the standard addition determination of nitrate in the sewage effluent is detailed in figure 6.7.

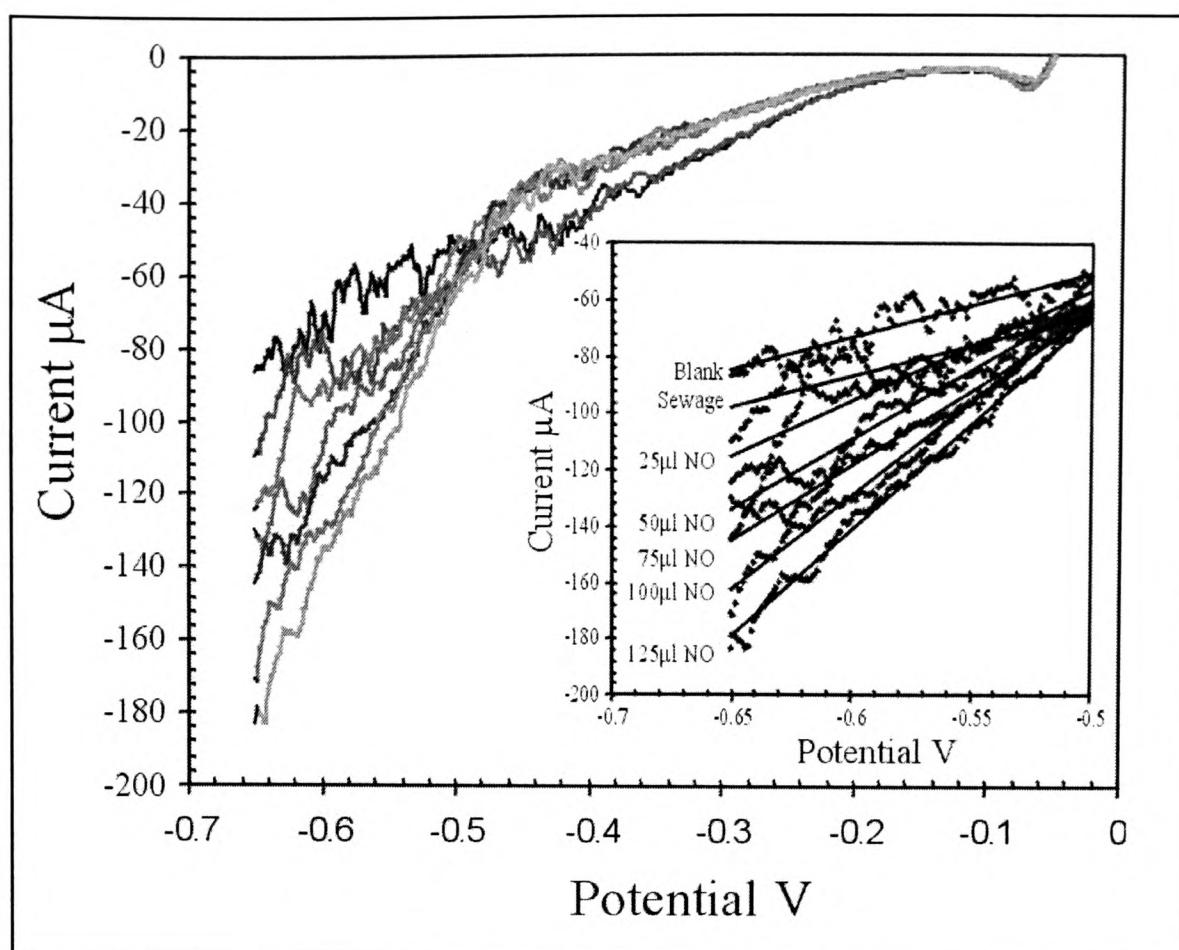


Fig. 6.7 - Sonovoltammograms detailing the standard addition determination of nitrate in sewage effluent. Inset: Application of linear regression methods to provide an estimate of the “limiting current” at -0.65 V.

These correspond to the blank, the introduction of the sewage sample and five additions of standard nitrate ($25 \mu\text{L}$, 0.025 M). The results were found to be $481 \mu\text{M}$ and $469 \mu\text{M}$ for the silent and sonochemical analysis respectively with the relative standard deviation in both cases found to be less than 4 % (three replicate measurements). This compared favourably with an independent spectroscopic analysis based on Cu-Cd Reduction / Griess Assay [11] whereby the level was evaluated at $479 \mu\text{M}$.

The analysis of the sonoelectrochemical data proved slightly more complex than with the data obtained from silent conditions. This was due to the lower signal to noise ratio, a consequence from the insonation, whereby the limiting current could not be as easily defined. While the current could be estimated through simply averaging a small number of data points near end of the each sweep, signal averaging over a larger potential range (-0.5 to -0.65 V) and using the resulting linear regression to estimate the “limiting current” at -0.65 V was found to give consistently reproducible results. The process is shown in the inset in figure 6.7. However, irrespective of which approach is taken, it is necessary to ensure that the response to increasing nitrate is linear within the anticipated

concentration range to be studied such that the integrity of the standard addition protocol is retained.

6.4 CONCLUSIONS

In an extension to the work presented in chapter 4, the electrode activation through the use of ultrasound has been analytically investigated. While the application of ultrasound has been shown to remove the fragile / porous deposit, the retention of a compact sub-layer with an appreciable sensitivity towards nitrate reduction can be achieved. This catalytic layer possesses a number of features that may in certain applications be of more value than the macroporous system. Providing long term stability can be achieved, it is conceivable that such a system may be suitable for nitrate analysis in oil based products where the application of ultrasound could be used to both generate the microemulsion [5] needed to release the analyte and provide the enhanced detection. Electrode activation is now investigated through the application of heat to a channel flow cell and is discussed in chapter 7.

6.5 CHAPTER 6 REFERENCES

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CHAPTER 7

HIGH TEMPERATURE ELECTROCHEMICAL STUDIES USING A CHANNEL FLOW CELL HEATED BY RADIO FREQUENCY RADIATION

*The work presented in this chapter has been published in
Journal of Electroanalytical Chemistry 506 (2001) 28.*

The work presented in the following two chapters moves on from the effects of ultrasound and electrode chemical modification and deals with electrochemical processes operating under 'activation' from the effect of high temperature. Systems involving one and two electron redox reactions were studied under hydrothermal conditions using a novel hydrodynamic method based on a conventional channel flow cell where the working electrode was heated by radio frequency radiation. The diffusion activation parameters obtained with the RFCC (Radio Frequency Channel Cell) were compared with independent data from microelectrode high temperature experiments and analysed in terms of the Stokes-Einstein equation. The FIDAPTM computer simulations presented in this chapter were performed in collaboration with Dr. B. A. Coles, PTCL, Oxford University.

7.1 INTRODUCTION

In a previous publication [1] a novel design of a high temperature channel flow cell has been presented. This thermo-electrochemical apparatus utilises a working electrode of 40 mm thick platinum foil, which is heated by eddy currents induced by 8 MHz radio frequency (Rf) radiation, as illustrated in figure 7.1. Some advantages of such a system include the very fast local heating and the short residence time of the solution at the high temperature region. This offers the possibility of novel study at temperatures close to the boiling point of the solvent under normal pressure without phase separation. Also, it allows study of electrochemical systems that undergo thermal decomposition when exposed to high temperatures for long periods of time. Since the Rf-channel cell operates under "non-isothermal" conditions, the relation between the limiting current and the volume flow rate cannot be described by an analytical equation. The mass and heat

flow must be calculated by computer simulation in order to describe the voltammetric experiments taking into account the changes in viscosity and diffusivity in the electrochemical system. The necessary simulations were described in reference [1].

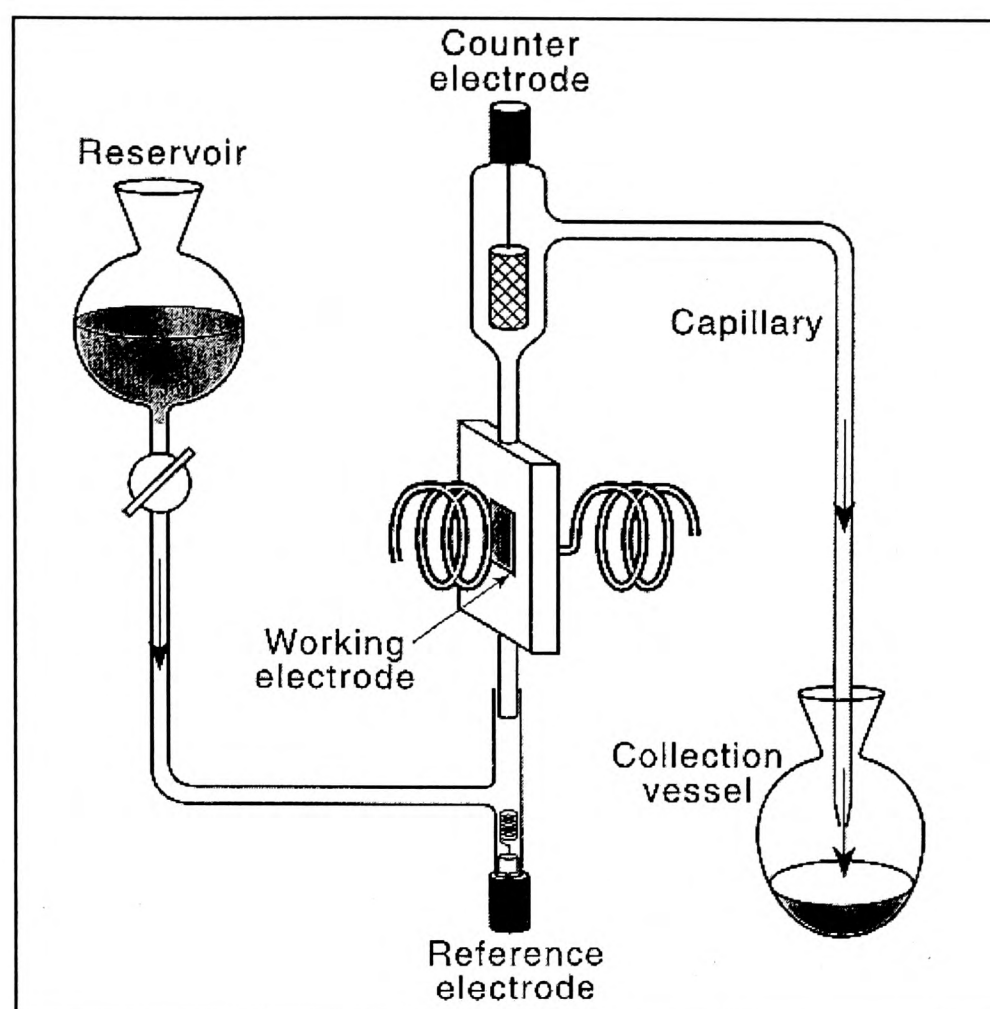


Fig. 7.1 - Rf-heated channel cell.

In this chapter, a systematic study of one and two electron redox reactions in a wide range of temperatures and flow rate conditions was carried out, resulting in a complete characterisation of the RFCC. The diffusion activation parameters obtained are in agreement with independent “isothermal” experiment data carried out at heated microelectrodes.

7.2 EXPERIMENTAL

All reagents were of the highest grade available and used without further purification, KCl (Fluka 99.5 %), $\text{Ru}(\text{NH}_3)_6\text{Cl}_3$ (Aldrich 98 %), $\text{K}_4\text{Fe}(\text{CN})_6$ (Lancaster 98 %), N,N,N',N'-tetramethylphenylenediamine (TMPD) (Aldrich, 98 % purity), hydroquinone (H_2Q) (Aldrich 99 %) and N,N'-dimethylphenylenediamine (DMPD) (Aldrich 97 %). All solutions and subsequent dilutions were prepared using deionised water from an Elgastat (Elga, UK) UHQ grade water system with a resistivity of 18 $\text{M}\Omega\text{ cm}$. Solutions were

deoxygenated prior to use and this was achieved by bubbling with argon in the microelectrode cell or with argon at a reduced pressure (ca. 10 mm Hg) for 10 minutes when working with the radio frequency channel cell (RFCC). In both cases the solutions were maintained under argon during the experiments. The RFCC experimental set-up was the same as described previously [1], with the minor change that a potassium chloride saturated calomel electrode (SCE) was employed as reference electrode instead of Ag/AgCl. The working electrode comprised of 40 μm thick platinum foil (99.99 %, Goodfellow Cambridge, UK) of 4.0 x 4.1 mm and platinum mesh was used as a counter electrode.

The potentiostat, thermocouple and the RF system were controlled via a PC. The RF generator was a Radyne C 12/B unit (Radyne Ltd., Wokingham, England) modified by replacement of the single-phase power supply with a three-phase unit and with a driver circuit added to allow fast on-off control by the PC. FIDAPTM (version 8.52 Fluent Europe Ltd., Sheffield, UK, <http://www.fluent.com>), a commercial finite element fluid dynamics software package executed on a Silicon Graphics Origin 2000 was used for the numerical simulations. A detailed description of the simulation procedure has been given by Qiu et al [1].

The microelectrode high temperature experiments were carried out using a cell approximately 20 cm^3 in volume heated by hot air circulation within a small box of insulating material with a front glass wall. A Pt resistance thermometer was used to control the air temperature and a thermocouple in contact with the solution was used to read the temperature during the voltammetric scans. A 25 μm diameter platinum disc (99.99 %, Goodfellow Cambridge, UK) sealed in Pyrex glass was used as the working electrode, with a platinum coil counter electrode and a SCE maintained at room temperature was used as the reference electrode, shown in figure 7.2.

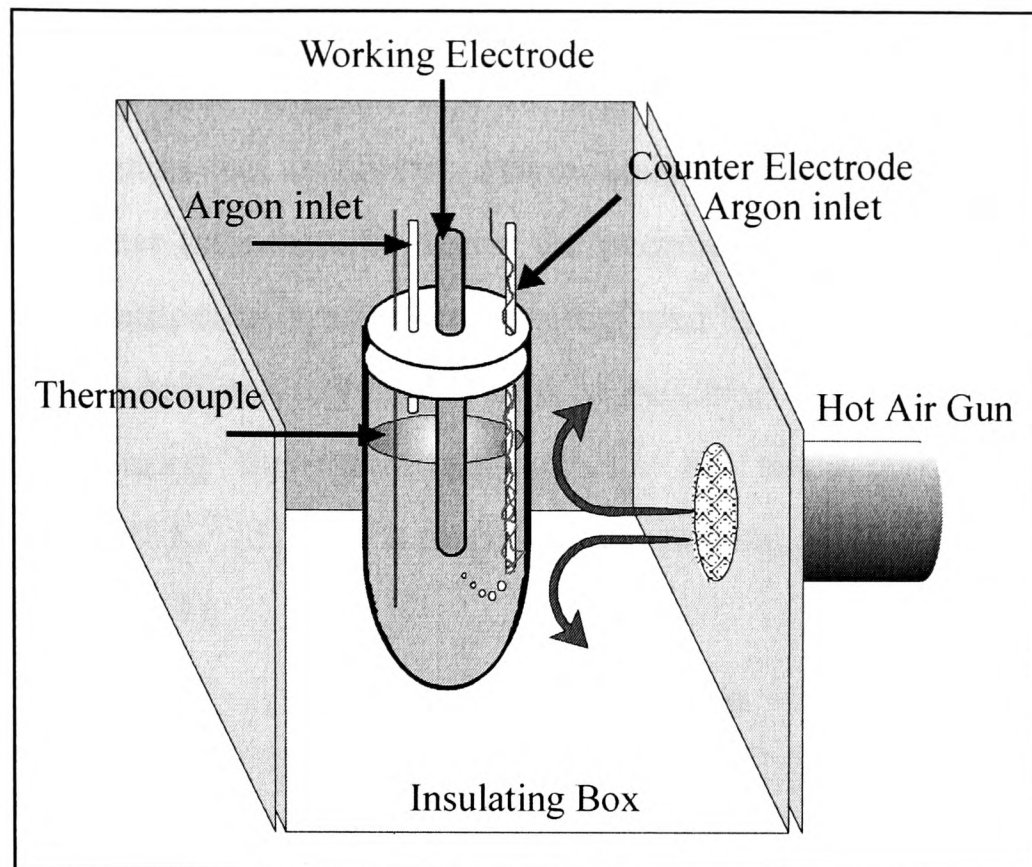


Fig. 7.2 - Experimental set-up for the high temperature verification of RFCC data using a microelectrode heated via a hot air stream.

7.3 MASS AND HEAT TRANSPORT

7.3.1 RADIO FREQUENCY CHANNEL CELL

For the RFCC, under laminar hydrodynamic regime and constant temperature the limiting current can be calculated by using the Levich equation for channel flow [2]

$$I_{lim} = 0.925 n F C (D x_e)^{2/3} w_e (v_f / d h^2)^{1/3} \quad (7.1)$$

where n , F , C and D are the number of electrons, the Faraday constant, the bulk concentration and the diffusion coefficient of the electroactive species respectively. The other parameters represent the length, x_e , and width, w_e , of the electrode, the channel width, d , the channel height, $2h$, and the volume flow rate, v_f .

In the absence of heating, a parabolic velocity profile and limiting currents (I_{lim}) consistent with equation (7.1) were obtained from the simulation [1]. When RF-heating is applied, the electrode is cooled by the solution flow so that its temperature is relatively low at the leading (upstream) edge and consequently increases along the length of the

electrode as the solution becomes heated. It was found from experimentation that the assumption [1] of a uniform heat density (W cm^{-2}) over the electrode was not sufficiently accurate for the electrode thickness and the frequency employed, as measurements on a similar sized platinum foil using two thermocouples indicated transverse temperature gradients with higher temperatures nearer the periphery of the foil. In the simulations, the thermocouple temperature was therefore replaced by an “effective” temperature, T_{eff} , calculated from the half wave potential shift of $\text{Fe}(\text{CN})_6^{4-/3-}$ observed in the experiments with the RFCC, using the factor reported for the same system under isothermal conditions, -1.53 mV K^{-1} [3]. For a thermocouple reading of 50°C , T_{eff} is approximately 25°C higher (Table 7.1).

v_f / $\text{cm}^3 \text{ s}^{-1}$	Thermocouple (centre) / $^\circ\text{C}$	$E_{1/2}$ / V	T_{eff} / $^\circ\text{C}$
0.020	20	0.1884	20
0.066	20	0.2010	20
0.121	20	0.2041	20
0.176	20	0.2063	20
0.245	20	0.2063	20
0.020	50	0.1081	72
0.066	50	0.1135	77
0.121	50	0.1200	75
0.176	50	0.1185	77
0.245	50	0.1162	79

Table 7.1 - “Effective” temperatures as a function of the volume flow rate.

For the same flow rates and thermocouple temperatures, the $\text{Fe}(\text{CN})_6^{4-/3-}$ effective temperature values were used to estimate the actual temperatures for the other redox systems. Published values for the temperature-dependent viscosity and density of a 0.1 M KCl solution [4] along with thermal conductivity and specific heat values of water [5] were used for the FIDAP™ simulation. When a steady state thermal gradient is established and the “effective” temperature calculated, the two-dimensional model simulation indicates that the relationship between I_{lim} and $v_f^{1/3}$ is linear (but not direct) to a good approximation.

7.3.2 HIGH TEMPERATURE MICROELECTRODE CELL

The diffusion coefficients were calculated from the steady-state limiting currents by using the following equation for a microdisc electrode of radius r_0 [2] after correction for changes in the solution density and, hence, corresponding changes in solution volume [4]

$$I_{lim} = 4 n F D C r_0 \quad (7.2)$$

The use of microelectrodes under isothermal conditions, however, was limited to temperatures below 70-75 °C. The formation of bubbles at the electrode surface, observed at higher temperatures, gave unsuitable voltammograms with poorly defined limiting plateaux.

7.4 RESULTS AND DISCUSSION

As in previous work [1], the oxidation of $\text{Fe}(\text{CN})_6^{4-}$ was adopted as a reference system and used to evaluate the “two-dimensional” simulation model based on the assumption of an effective temperature for a given volume flow rate. The experimental steady-state limiting currents under defined flow rate conditions were accurately reproduced by adopting $E_A = 13.0 \text{ kJ mol}^{-1}$ and $D_{(20 \text{ }^\circ\text{C})} = 6.64 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ (see figure 7.3) in the simulation. This data was in reasonable agreement with 13.8 kJ mol^{-1} and $D_{(25 \text{ }^\circ\text{C})} = 6.88 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ obtained under isothermal conditions with the microelectrode cell for the same system between 25-71 °C and also compares closely to literature values (Table 7.3). A modification of the microelectrode cell set-up was made to allow the incorporation of a SCE reference electrode. This allowed greater precision when measuring the half wave potential shift of $\text{Fe}(\text{CN})_6^{4-}$ using the microelectrode cell, agreeing closely with the value of -1.53 mV K^{-1} reported in the literature[3].

Consequently this model was applied to analyse the experimental RFCC data over a wide range of volume flow rates and temperatures for the reduction of $1 \text{ mM Ru}(\text{NH}_3)_6^{3+}$ and oxidations of 1 mM TMPD , H_2Q and DMPD in aqueous 0.1 M KCl . This model also permits the calculation of the diffusion activation energy of these species.

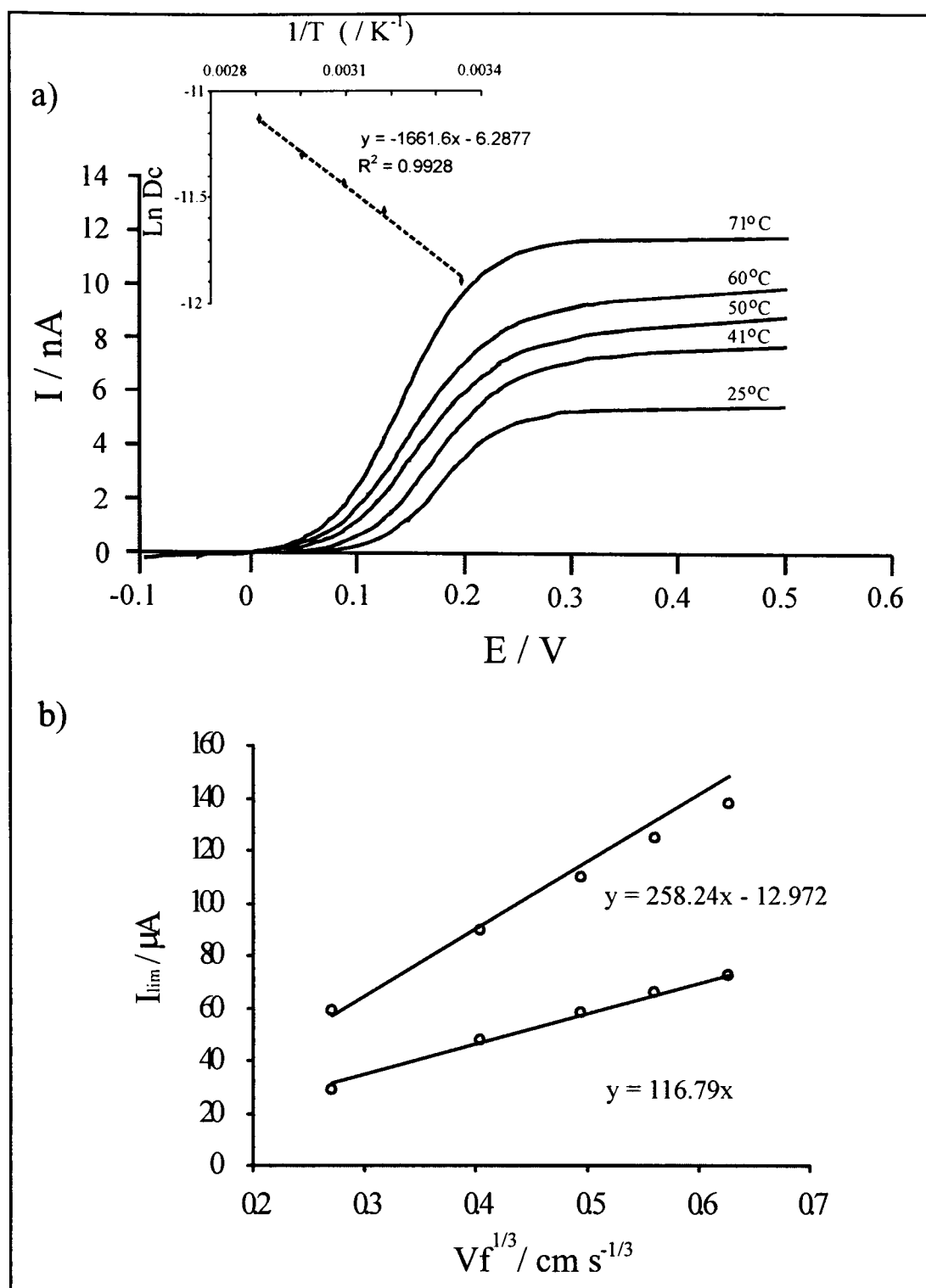
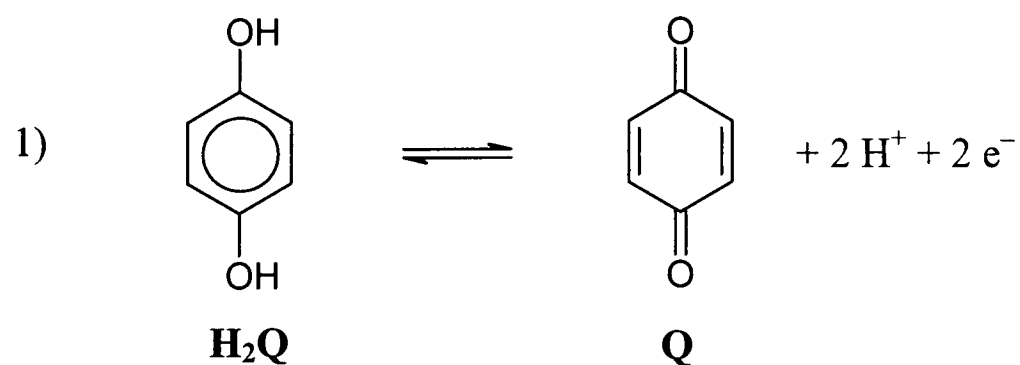
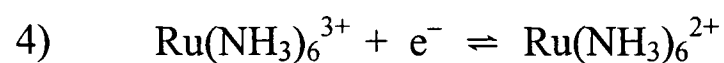
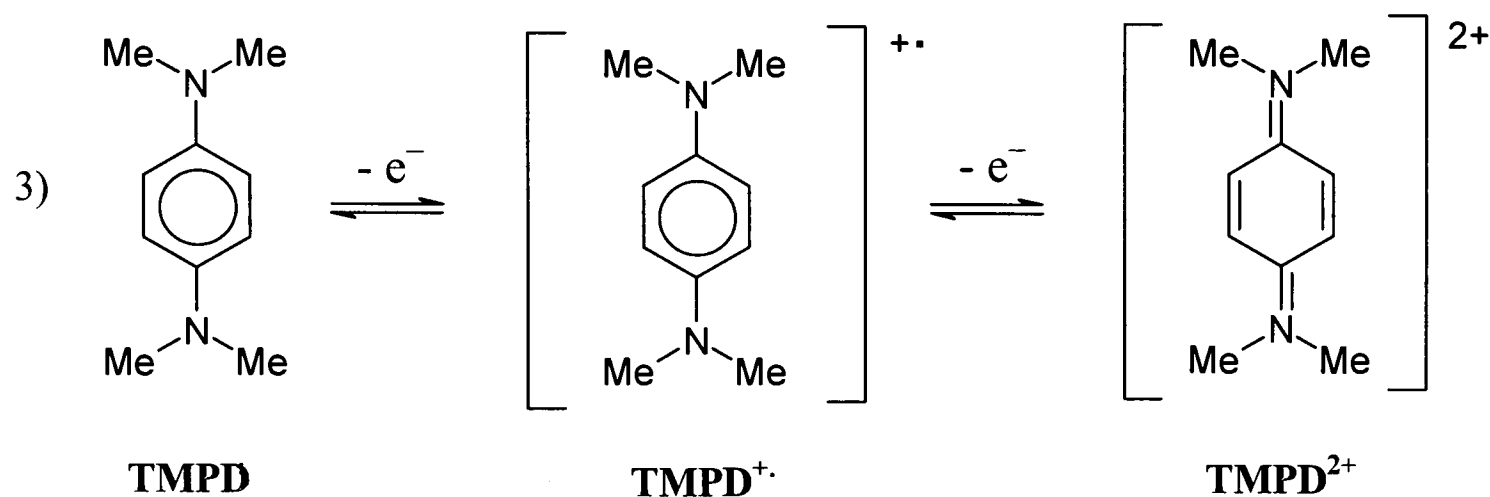
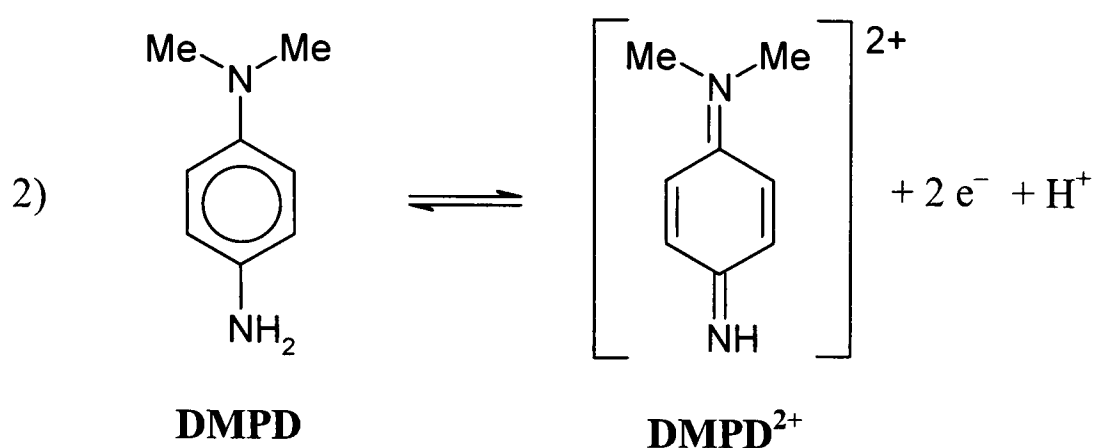


Fig. 7.3 - a) RFCC experimental (empty bullets) and simulated (lines) limiting currents as a function of the volume flow rate (0.020 to $0.245 \text{ cm}^3 \text{ s}^{-1}$) for temperatures (T_{eff}) of 20 and 75°C for the oxidation of $1.1 \text{ mM Fe(CN)}_6^{4-}$ in aqueous 0.1 M KCl . b) Verification of data using a microelectrode cell over a similar temperature range.

The relevant redox equations for the electrochemical systems are:





Data for TMPD were obtained from the first oxidation step (formation of the cation radical) as the plateau for the 2nd electron transfer lies at ca. 1.0 V, which can be problematic due to the formation of platinum oxide and solvent oxidation. Typical linear sweep steady state voltammograms for the reduction of $\text{Ru}(\text{NH}_3)_6^{3+}$ and the oxidation of TMPD at 75 °C at variable flow rate (0.020 to 0.245 cm³ s⁻¹) are shown in figure 7.4 (a) and 7.4 (b), while in figure 7.5 (a) and 7.5 (b) the effect of temperature on the voltammetric response at a constant flow rate of 0.020 cm³ s⁻¹ are shown.

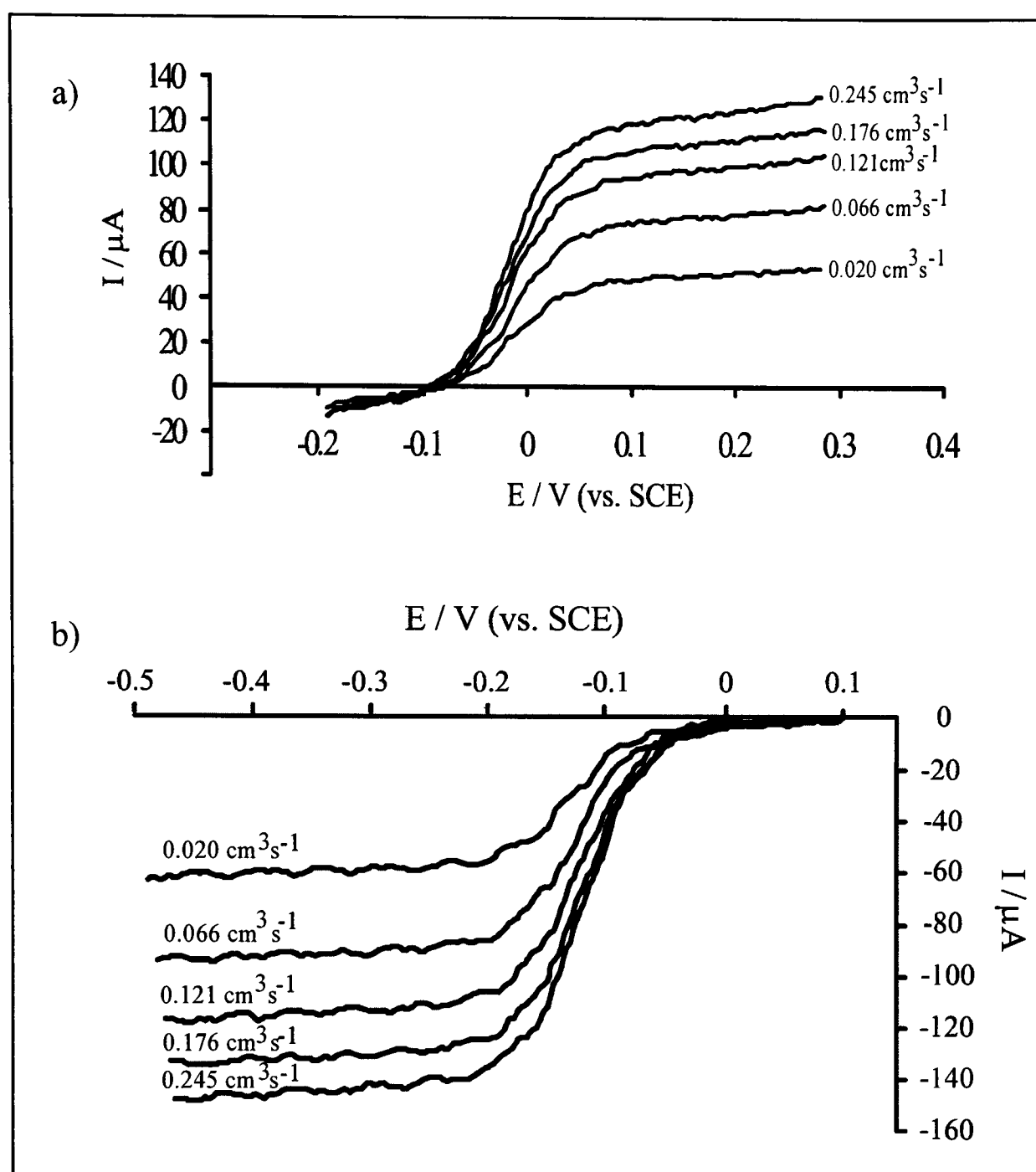


Fig. 7.4 - Voltammograms obtained under variable flow conditions (0.020 to $0.245 \text{ cm}^3 \text{s}^{-1}$) for the a) oxidation of 0.996 mM TMPD and b) the reduction of 1.000 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ in aqueous 0.1 M KCl at 75°C .

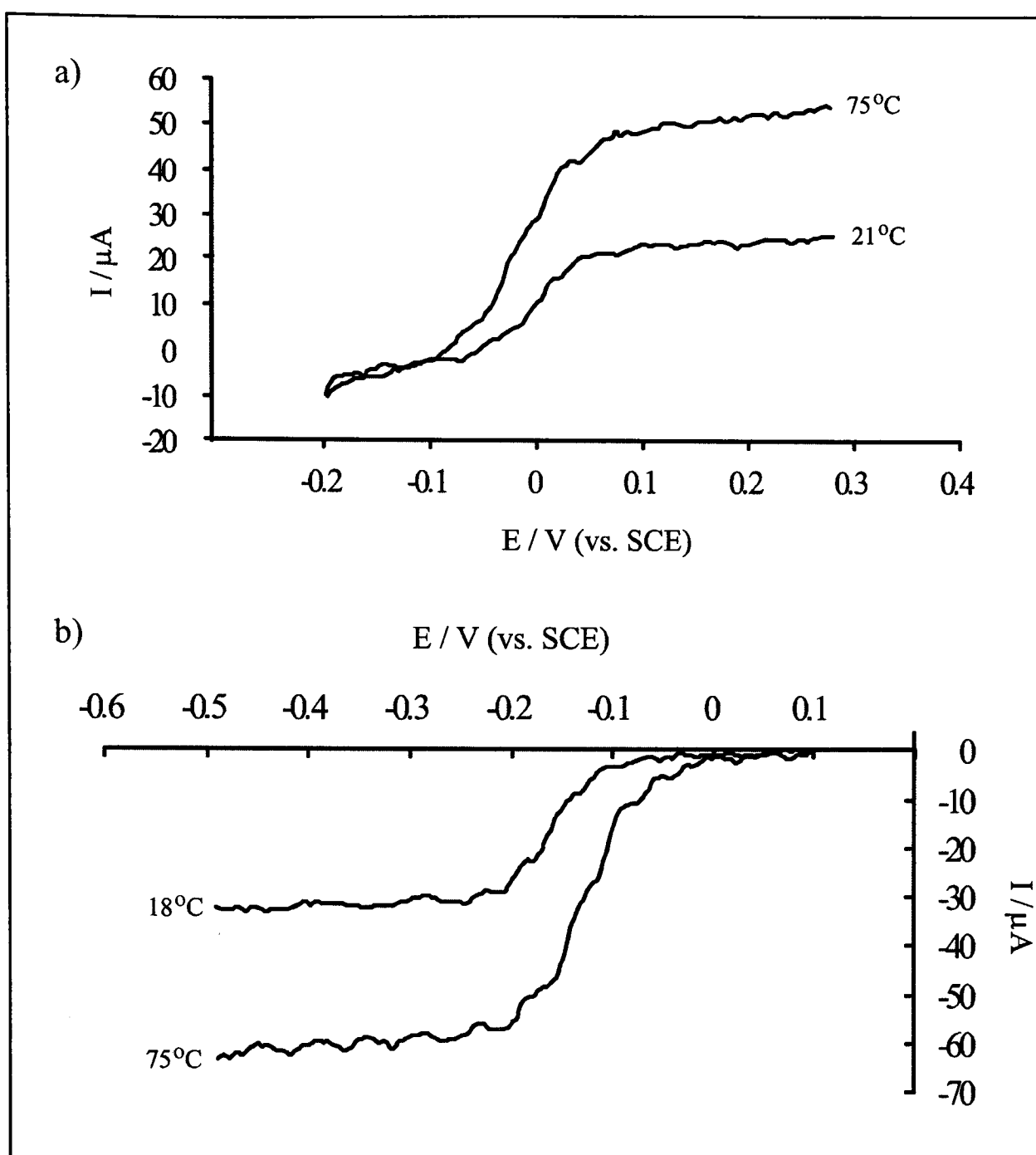


Fig. 7.5 - Voltammograms obtained under flow conditions ($0.020 \text{ cm}^3 \text{ s}^{-1}$) for the a) oxidation of 0.996 mM TMPD and b) reduction of 1.000 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ in aqueous 0.1 M KCl at temperatures (T_{eff}) upto 75 °C.

A plot of experimental and calculated (simulation) limiting currents for the oxidation of H_2Q , TMPD and DMPD and the reduction of $\text{Ru}(\text{NH}_3)_6^{3+}$ as a function of the flow rate and the temperature can be seen in figure 7.6. A good agreement between the experimental (points) and simulated limiting current (lines) up to 75 °C is achieved when the RF power is adjusted to give similar temperature rises (T_{eff}) at different flow rates.

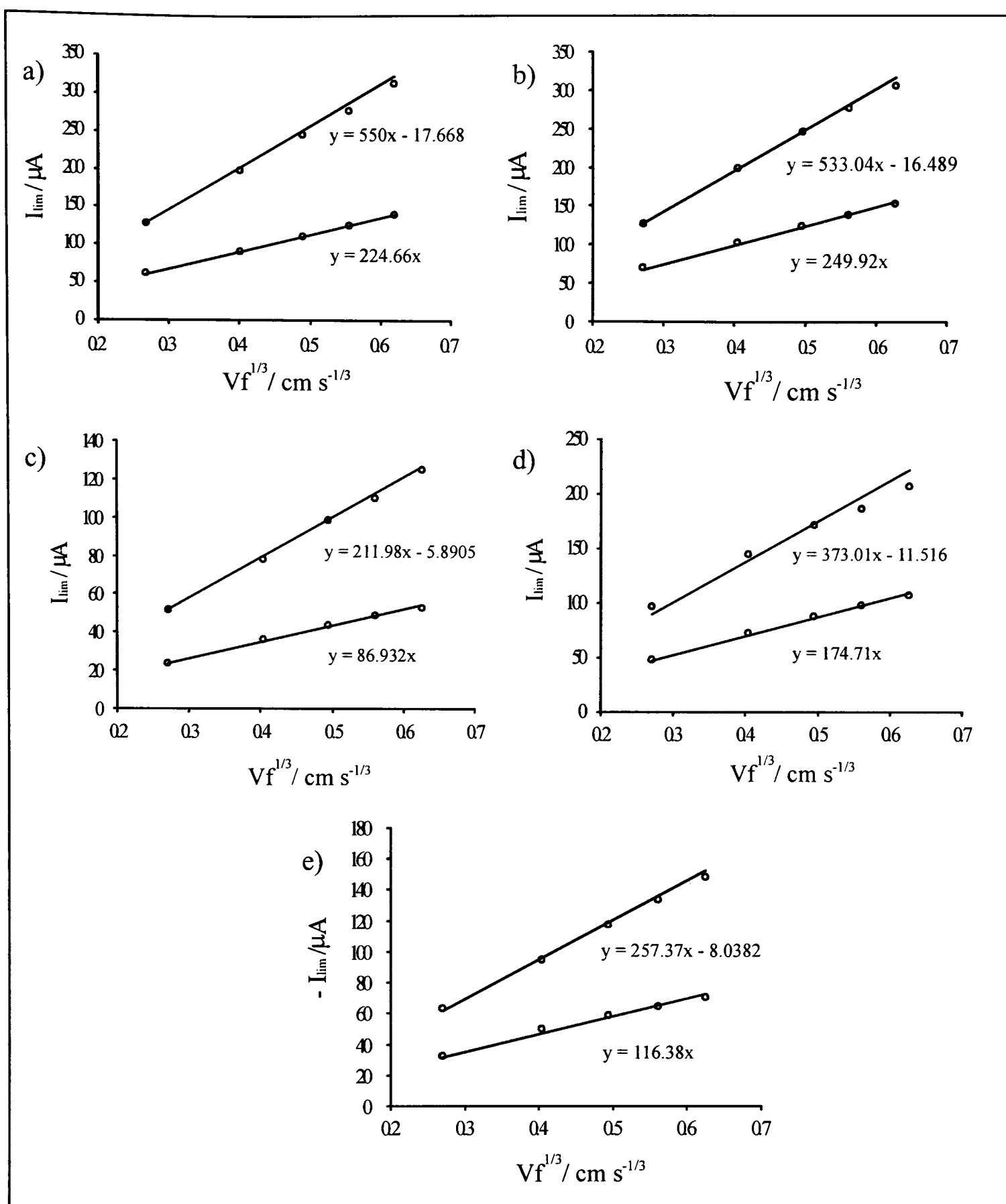


Fig. 7.6 - RFCC experimental (empty bullets) and simulated (lines) limiting currents for the oxidation of a) H₂Q (pH 1.7), b) H₂Q (pH 6.6), c) TMPD, d) DMPD and the reduction of e) Ru(NH₃)₆³⁺ in aqueous 0.1 M KCl as a function of flow rate and temperature.

The results shown in figure 7.6 were used to determine diffusion coefficients, D_{RT} , under isothermal conditions (room temperature experiments) using equation 7.1 and diffusion activation energies, E_A , by minimising the deviation between simulated and experimental limiting current data over a range of different flow rates. The results are summarised in table 7.2.

System	RF-Channel Cell			Microelectrode Cell		
	$D_0 \cdot 10^3$ / $\text{cm}^2 \text{s}^{-1}$	E_A / kJ mol^{-1}	$D_{(RT)} \cdot 10^6$ / $\text{cm}^2 \text{s}^{-1}$	$D_0 \cdot 10^3$ / $\text{cm}^2 \text{s}^{-1}$	E_A / kJ mol^{-1}	$D_{(RT)} \cdot 10^6$ / $\text{cm}^2 \text{s}^{-1}$
Fe(CN)_6^{4-}	1.37	13.0	6.64 (20°C)	1.86	13.8	6.88 (25°C)
$\text{Ru(NH}_3)_6^{3+}$	2.03	13.5	7.55 (17°C)	2.46	13.8	8.48 (20°C)
$\text{H}_2\text{Q (pH 1.7)}$	5.43	16.0	7.15 (17°C)	8.50	17.1	7.66 (20°C)
$\text{H}_2\text{Q (pH 6.6)}$	2.62	14.0	8.65 (22°C)	3.43	14.9	7.91 (22°C)
TMPD	7.91	18.0	4.91 (20°C)	4.23	16.1	6.07 (24°C)
DMPD	1.16	13.0	5.39 (18°C)	5.57	16.2	7.80 (23°C)

Table 7.2 - Diffusion activation parameters of Fe(CN)_6^{4-} , $\text{Ru(NH}_3)_6^{3+}$, H_2Q , TMPD and DMPD in aqueous 0.1M KCl solution from RFCC and microelectrode high temperature experiments.

Diffusion activation parameters were also determined under isothermal conditions with the microelectrode high temperature cell, where the dependence with temperature of the diffusion coefficients was analysed in terms of the Arrhenius equation:

$$D = D_0 \exp (- E_A / RT) \quad (7.3)$$

Plots of $\ln D$ vs $1/T$ yield straight lines, the slopes of which lead to the activation energies listed in table 7.2. As it can be seen, good agreement between both methods was achieved despite the fact that the RFCC operates under “non-isothermal” conditions and an “effective” temperature must be adopted to simulate the experimental results.

A set of available high temperature literature data for the same species has been collected in table 7.3, but it should be noted that they were measured in supporting electrolyte of different composition and should be used for tentative agreement only [6-13]. The temperature dependence of the diffusion coefficient was analysed with the Stokes-Einstein relationship:

$$D = k_B T / \pi n \eta r \quad (7.4)$$

where k_B is the Boltzman constant, η is the macroscopic dynamic viscosity of the solution, r , defines the molecular “effective” radius and n is a constant that can adopt two

values, either 6, on the assumption that the fluid “sticks” to the particle surface (frictional coefficient infinite) or 4, for the “slip” condition of zero surface friction.

System	Method	Supporting Electrolyte	ΔT / °C	$D_0 \cdot 10^3$ / $\text{cm}^2 \cdot \text{s}^{-1}$	E_A / $\text{kJ} \cdot \text{mol}^{-1}$	$D_{(RT)} \cdot 10^6$ / $\text{cm}^2 \cdot \text{s}^{-1}$	Ref.
$\text{Fe}(\text{CN})_6^{4-}$	RDE	0.5 M NaOH	25-42	8.4	18.0	6.16 _(25°C)	[6]
$\text{Fe}(\text{CN})_6^{4-}$	RDE	2 M NaOH	25-41	11.0	19.6	4.25 _(25°C)	[6]
$\text{Fe}(\text{CN})_6^{4-}$	RDE	1 M NaOH	30-90	2.1	15.0	5.60 _(30°C)	[7]
$\text{Fe}(\text{CN})_6^{4-}$	WTE	0.2 M KNO_3 (pH 10)	25-45	2.4	20.5	6.10 _(25°C)	[8]
$\text{Fe}(\text{CN})_6^{4-}$	Microwave	1 M KCl	60-150	----	14.3	----	[3]
$\text{Fe}(\text{CN})_6^{4-}$	Rotating Fluid	1 M KNO_3	----	----	13.2	6.30 _(25°C)	[9]
H_2Q	Cottrell	0.2 M NaHSO_4	25-200	3.3	15.0	7.4 _(25°C)	[10]
H_2Q	WTE	0.2 M Na_2SO_4 (pH 2)	25-45	5.0	16.0	7.1 _(25°C)	[11]
H_2Q	RDE	0.2 M Na_2SO_4 (pH 2)	25-40	2.7	14.8	7.2 _(25°C)	[8]
H_2Q	Impedance	1 M KNO_3 (pH 5.6)	25	----	----	9.5 _(25°C)	[11]
TMPD	Microelectrode	0.1 M KCl	25-80	11.9	19.0	6.32 _(30°C)	[12]
$\text{Ru}(\text{NH}_3)_6^{3+}$	Hot Wire	0.1 M KCl	25-75	----	13.0	6.40 _(25°C)	[13]

Table 7.3 - Comparative diffusion activation parameters of $\text{Fe}(\text{CN})_6^{4-}$, $\text{Ru}(\text{NH}_3)_6^{3+}$, H_2Q and TMPD in the literature.

For neutral species such as H_2Q , TMPD and DMPD excellent linear plots of D against T/η were obtained from using both RFCC and microelectrode data, shown in Figure 7.7. In comparison, data from $\text{Fe}(\text{CN})_6^{4-}$ and $\text{Ru}(\text{NH}_3)_6^{3+}$ systems gave rise to discrepancies which are not surprising since equation (7.4) neglects ion solvation and dielectric friction effects [14-19]. The effective radius, r , of H_2Q , TMPD and DMPD calculated from the best linear regression of the slopes in figure 7.7 and slip boundary condition are: 4.58, 6.39 and 5.75 Å respectively. In contrast the effective radius of $\text{Fe}(\text{CN})_6^{4-}$ and $\text{Ru}(\text{NH}_3)_6^{3+}$ calculated from room temperature diffusion coefficients: 4.99 and 4.05 Å (slip condition) are consistent with values in the literature reported for $\text{Fe}(\text{CN})_6^{4-}$ and $\text{Co}(\text{NH}_3)_6^{3+}$ under slip boundary conditions from ion equivalent conductance data: 5.00 and 4.07 Å respectively [20].

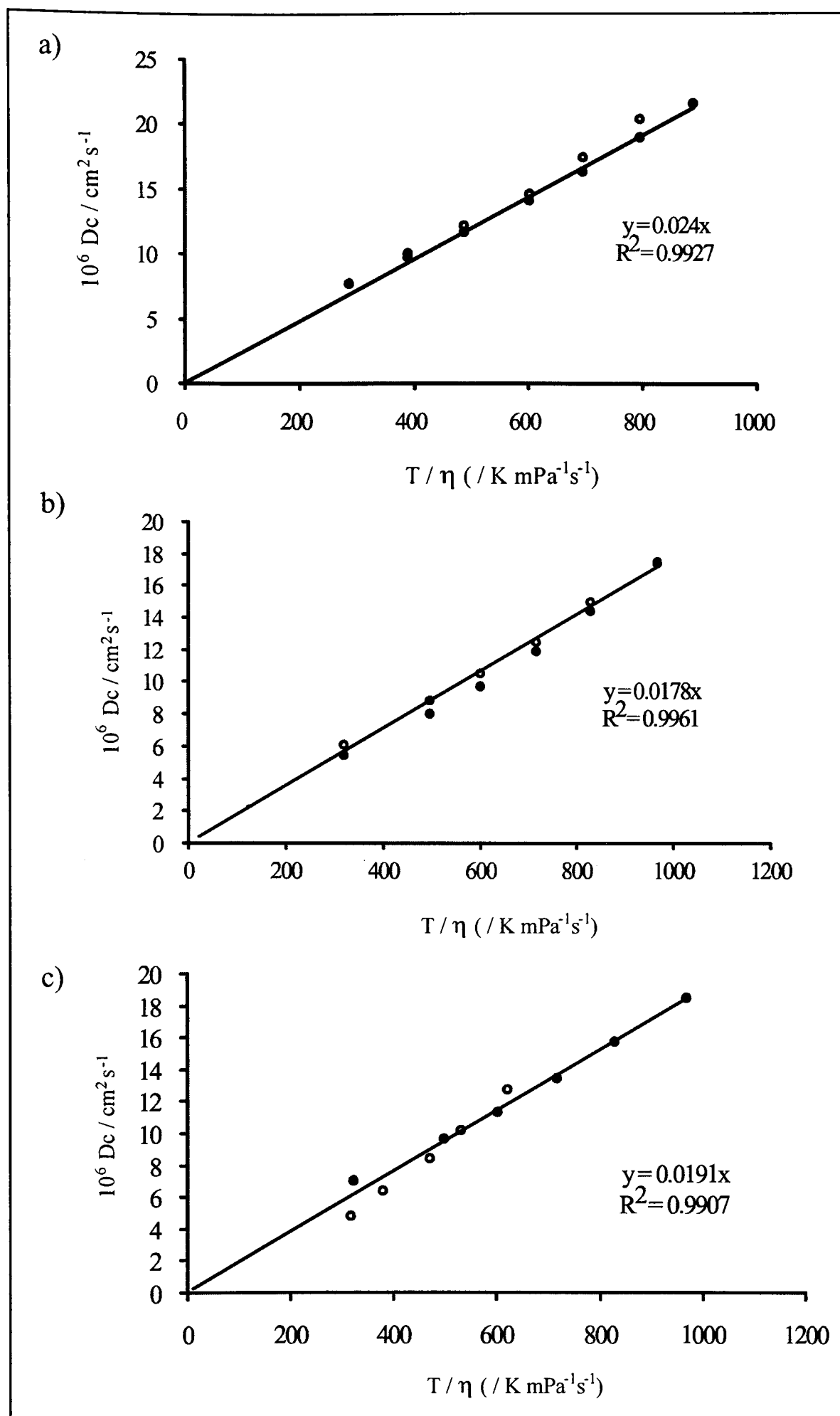


Fig. 7.7 - Stokes-Einstein plots (D vs. T/η) of a) H_2Q (pH 1.7), b) TMPD and c) DMPD in 0.1 M KCl aqueous solution. Data from simulation of RFCC experiments (filled bullets), data from microelectrode (empty bullets) and best fit linear regressions with zero ordinate (lines).

7.5 CONCLUSIONS

In this work, a more comprehensive characterisation of the RF-heated channel cell electrode was obtained. It was shown that while the mass transfer and heat distribution is a three-dimensional problem, the experimental results could be successfully described by a two-dimensional model by using an “effective” temperature determined from the response of a known reference system. Diffusion activation energies obtained from simulation using experimentally-derived limiting currents as a function of solution volume flow rate and temperature were in excellent agreement with independent microelectrode results. Aside from the limitation of the Stokes-Einstein model to quantitatively describe the temperature dependence of the diffusion coefficient with temperature when dealing with highly charged species, the results obtained for the transport parameters are physically meaningful. The work presented in the following chapter extends this technique to encompass non-aqueous systems and investigates the versatility of the heated channel flow cell to the study of a mechanistically complex ECE process.

7.6 CHAPTER 7 REFERENCES

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CHAPTER 8

NON-AQUEOUS ELECTROCHEMICAL STUDIES AT A HIGH TEMPERATURE CHANNEL FLOW CELL HEATED BY RADIO FREQUENCY RADIATION

*The work presented in this chapter has been published in
Journal of Electroanalytical Chemistry 513 (2001) 87.*

In an extension to the work presented in chapter 7, the effect of applied temperature was studied on various non-aqueous 1- and 2-electron systems of ferrocene, tris(4-bromophenyl)amine and N,N,N',N'-tetramethyl-1,4-phenylenediamine in acetonitrile solvent at both radio frequency channel cell (RFCC) and microdisk electrode apparatus. Experimentally obtained diffusion coefficients (D_c) and activation energies (E_A) for both RFCC and microelectrode experiments are in excellent agreement with each other and published data. Analysis of the data in terms of the Stokes-Einstein equation is also presented. This expands on the previous work reported in chapter 7, to incorporate not only solvent-based systems in addition to simple aqueous electrochemistry, but also more mechanistically complex electrode processes with coupled homogeneous kinetics such as ECE reactions. The application of the RFCC as a tool for mechanistic studies is discussed with the investigation of the well characterised ECE reaction of m-iodo-nitrobenzene in acetonitrile, giving a value of $80 \pm 5 \text{ kJ mol}^{-1}$ for the activation energy of the rate constant for the decomposition of the m-iodo-nitrobenzene radical anion. This represents, the first example of a quantitative, fully modelled investigation of a mechanistically complex electrochemical process at a locally heated electrode. The FIDAPTM computer simulations presented in this chapter were performed in collaboration with Dr. B. A. Coles, PTCL, Oxford University.

8.1 INTRODUCTION

As illustrated in chapter 7, a novel high temperature channel flow cell has been presented, comprising of a 40 μm thick platinum electrode exposed to 8 MHz radio frequency (RF) radiation. As a result, the induced eddy currents cause the direct heating of the platinum electrode. Since the RF-channel cell (RFCC) operates under “non-

isothermal" conditions, the relation between the limiting current and the volume flow rate (and hence the diffusion coefficient) cannot be described by an analytical equation. The mass and heat flow must be calculated by computer simulation in order to describe the voltammetric experiments, taking into account the changes in viscosity and diffusivity in the electrochemical system. The necessary simulations were described in reference [1].

Previous work involving the use of the RFCC has focussed on electrochemical processes in aqueous conditions [1,2]. In this chapter, a non-aqueous study of one and two electron redox reactions in a wide range of temperatures and flow rates in acetonitrile solvent is presented. The diffusion activation parameters obtained are in excellent agreement with independent experimental data carried out at a microelectrode and are corroborated with published data. Success in using non-aqueous redox systems has allowed us to develop the RFCC experiments for the mechanistic investigation of more complex electrochemical reactions, such as the ECE reaction of m-iodo-nitrobenzene. This has demonstrated that full quantitative modelling of mechanistically complex processes using a locally heated electrode is feasible and practicable, and represents the first example of such an investigation.

8.2 EXPERIMENTAL

All of the following reagents were of the highest grade available and used without further purification; ferrocene (Aldrich), N,N,N',N'tetramethylphenylenediamine (TMPD) (Aldrich, 98 % purity), tris(4-bromophenyl)amine (TBPA)(Aldrich, 98 %), m-iodo-nitrobenzene (m-INB)(Aldrich, 99 %) and tetrabutylammonium hexafluorophosphate (TBAHFP)(Fluka, 99 %). All solutions were prepared using acetonitrile (Fisher Scientific) and were deoxygenated prior to use, achieved by bubbling with argon (Pureshield, BOC) in the microelectrode cell or with argon at a reduced pressure (ca. 10 mm Hg) for 10 minutes when working with the RFCC. In both cases the solutions were maintained under argon during the experiments.

The RFCC experimental set-up was the same as described previously [1,2] with a saturated calomel electrode (SCE) employed as reference electrode (Radiometer, Copenhagen). The working electrode comprised of 40 μm thick platinum foil (99.99 %, Copenhagen).

Goodfellow Cambridge, UK) of 4.0×4.1 mm and platinum mesh was used as a counter electrode. The potentiostat, thermocouple and the RF system were controlled via a PC. The RF generator was a Radyne C 12/B unit (Radyne Ltd., Wokingham, England) modified by replacement of the single-phase power supply with a three-phase unit and with a driver circuit added to allow fast on-off control by the PC. FIDAPTM (version 8.52 Fluent Europe Ltd., Sheffield, UK, <http://www.fluent.com>), a commercial finite element fluid dynamics software package executed on a Silicon Graphics Origin 2000 was used for the numerical simulations. A detailed description of the simulation procedure has been given by Qiu et al [1].

The microelectrode high temperature experiments were carried out using a cell approximately 20 cm^3 in volume, mounted in an oven with forced air circulation, as described previously [2]. A Pt resistance thermometer was used to control the air temperature and a thermocouple in contact with the solution was used to read the temperature during the voltammetric scans. A $25 \text{ }\mu\text{m}$ diameter platinum disc (99.99 %, Goodfellow Cambridge, UK) sealed in Pyrex glass was used as the working microelectrode, with a platinum coil counter electrode and a SCE maintained at room temperature used as the reference electrode. The electrochemical measurements were conducted using a μ Autolab computer-controlled potentiostat (Eco-Chemie, Utrecht, Netherlands). The Pt microelectrode was polished before use with 1 and $0.3 \text{ }\mu\text{m}$ alumina powder (Buehler Micropolish II, USA).

8.3 MASS AND HEAT TRANSPORT

8.3.1 RF CHANNEL CELL

Under constant temperature and laminar hydrodynamic conditions, the Levich equation, previously mentioned in chapter 7, stipulates the relationship between limiting current and volume flow rate for the channel flow cell [3]:

$$I_{\text{lim}} = 0.925 n F C (D_c x_e)^{2/3} w_e (v_f / d h^2)^{1/3} \quad (8.1)$$

where n , F , C and D_c are the number of electrons, the Faraday constant, the bulk concentration and the diffusion coefficient of the electroactive species respectively. The

other parameters represent the length, x_e , and width, w_e , of the electrode, the channel width, d , the channel height, $2h$, and the volume flow rate, v_f .

In the absence of any heating effects, a parabolic velocity profile and limiting currents (I_{lim}) consistent with equation (8.1) were obtained from the simulation [1]. On the application of RF-heating, the flowing solution cools the electrode so that the temperature of the leading (upstream) edge is relatively low. The temperature of the electrode consequently increases along the length of the electrode as the solution, itself, becomes heated. It was shown in previous work [2], that the assumption of a uniform heat density over the electrode was not sufficiently accurate for the electrode thickness and the frequency employed, as measurements on a similar sized platinum foil using two thermocouples indicated transverse temperature gradients with higher temperatures nearer the periphery of the foil. Therefore, in the simulations the thermocouple temperature was replaced by an “effective” temperature, T_{Eff} , calculated from the half-wave potential shift ($E_{1/2}$) of ferrocene observed in the experiments with the RFCC, using the factor obtained for the same system under isothermal conditions at the heated microelectrode, 0.53 mV K^{-1} (figure 8.1). For a thermocouple reading of $50 \text{ }^\circ\text{C}$, T_{Eff} is approximately $55 \text{ }^\circ\text{C}$ as shown in table 8.1. This is substantially less than observed in the aqueous experiments where T_{Eff} was approximately $75 \text{ }^\circ\text{C}$ indicating that the electrode achieves a more even heating effect in the acetonitrile solvent than with water alone, due to the greater relative contribution of the platinum thermal conductivity.

Flow Rate / $\text{cm}^3 \text{ s}^{-1}$	RFCC		$T_{Eff} / \text{ }^\circ\text{C}$
	$[T_{Therm} = 19 \text{ }^\circ\text{C}]$ $E_{1/2} / \text{V}$	$[T_{Therm} = 50 \text{ }^\circ\text{C}]$ $E_{1/2} / \text{V}$	
0.447	0.4083	0.4271	54.5
0.321	0.4076	0.4269	55.4
0.221	0.4063	0.4263	56.7
0.121	0.4028	0.4217	54.7
0.036	0.4002	0.4188	54.1

Table 8.1 - Half-wave potential ($E_{1/2}$) shift calibration data for the RFCC electrode temperature using 1.002 mM ferrocene and 0.1 M TBAHFP in MeCN.

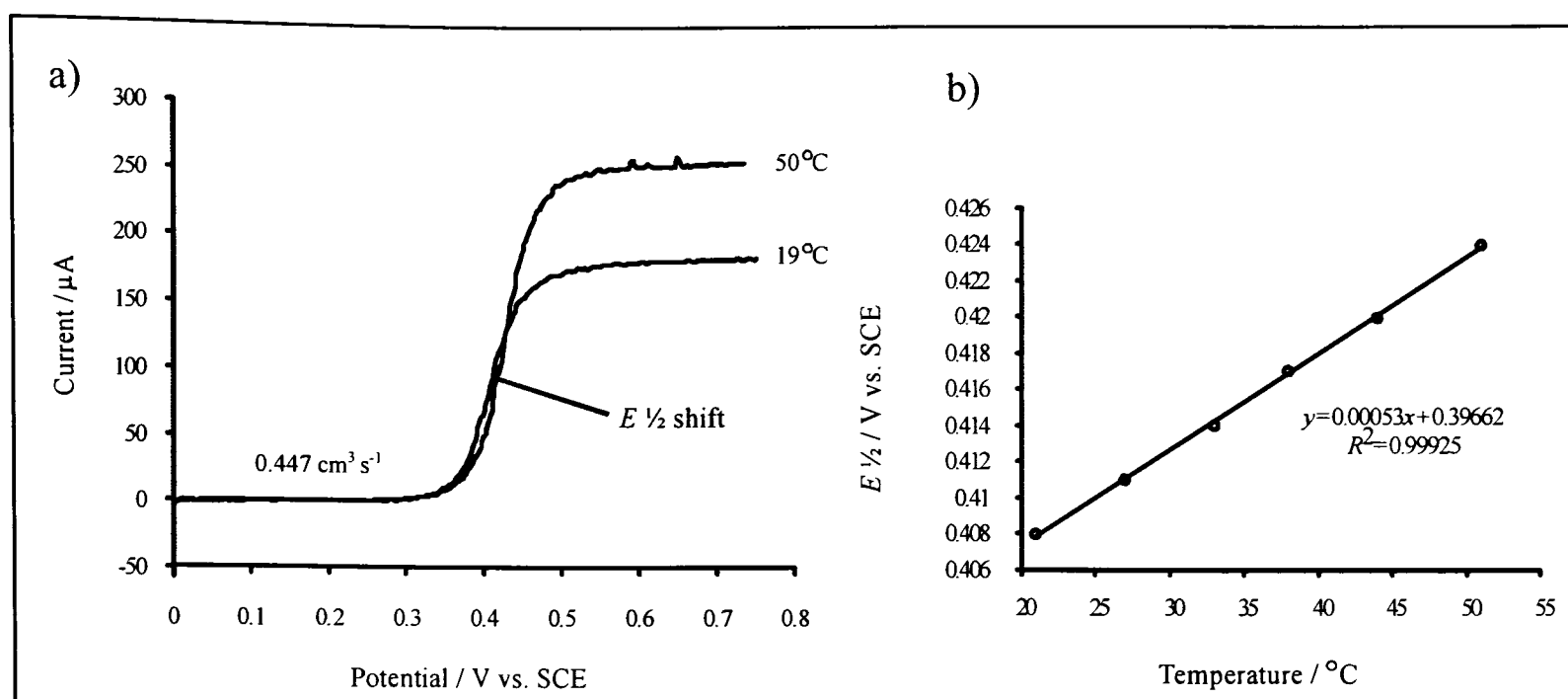


Fig. 8.1 - Calibration of RFCC using a) RFCC LSVs of 1.002 mM ferrocene and b) Microelectrode half-wave potential shift ($E_{1/2}$) of 1.005 mM ferrocene both in 0.1 M TBAHFP in MeCN.

For the same flow rates and thermocouple temperatures, the ferrocene-calibrated effective temperature values were used to estimate the actual temperatures for the other redox systems. Published values for the temperature-dependent viscosity, density, thermal conductivity and specific heat values of acetonitrile [4] were used for the FIDAP simulation. When a steady state thermal gradient is established and the “effective” temperature calculated, the two-dimensional model simulation indicates that the relationship between I_{lim} and $v_f^{1/3}$ is linear to a good approximation.

8.3.2 MICROELECTRODE CELL

By using the following equation for a microdisk electrode of radius r_0 [3], the diffusion coefficients were calculated from the steady-state limiting currents:

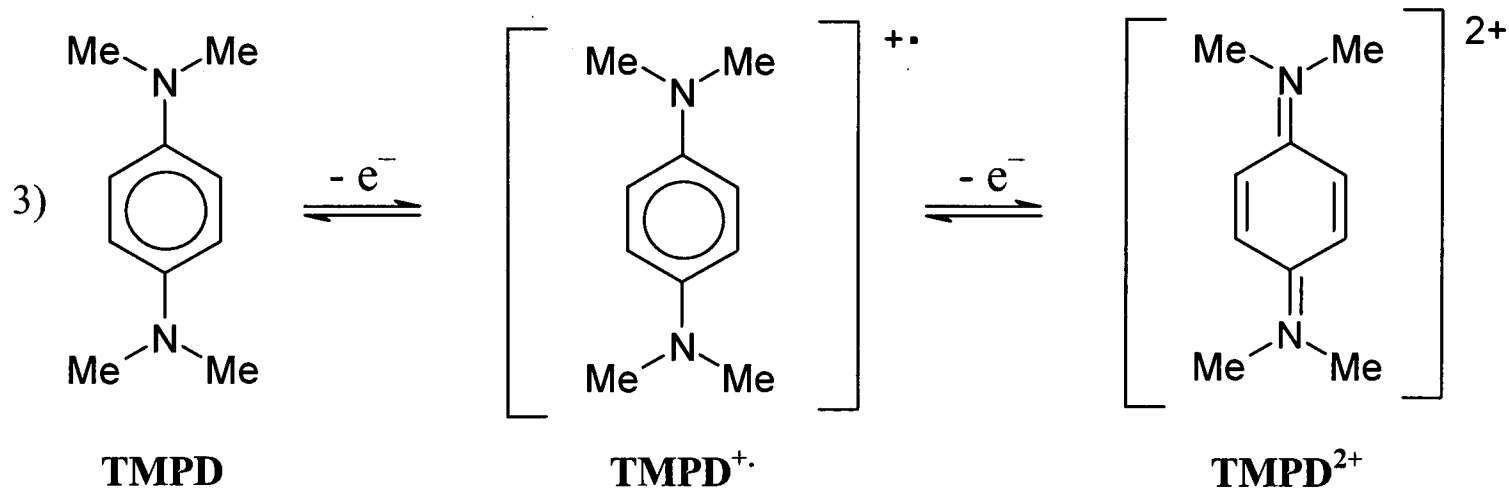
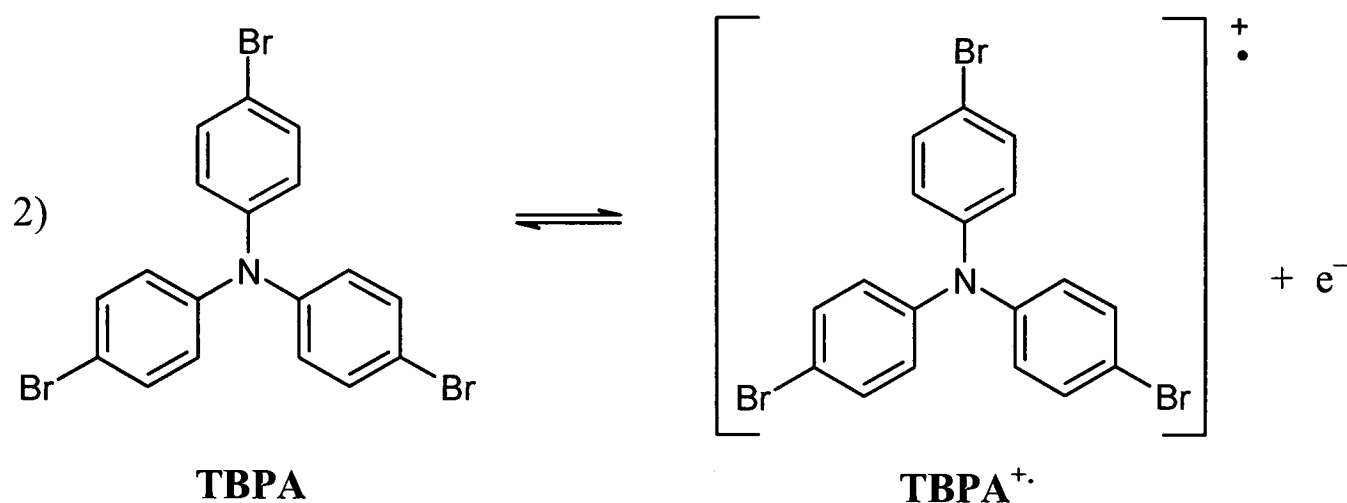
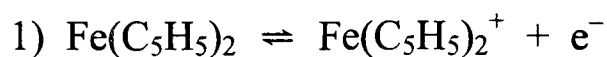
$$I_{lim} = 4 n F D C r_0 \quad (8.2)$$

The microelectrode experiments were limited to temperatures below 70 °C. Above this temperature, interpretation of the voltammograms proved problematic due to the formation of bubbles on the surface of the electrode giving poorly defined limiting plateaux. The electrode surface was polished prior to each voltammetric scan and was systematically checked using a microscope that the surface was not only clean and free

from passivated material, but also flush with the glass ensuring the Pt wire had not recessed.

8.4 RESULTS AND DISCUSSION

The redox equations for the three electrochemical systems of investigation are shown below.



8.4.1 MICROELECTRODE RESULTS

The above systems were investigated at the microelectrode set-up using linear sweep voltammetry (LSV) and a scan rate of 1 mV s^{-1} . A typical example of the resulting voltammetry is shown in figure 8.2, where the limiting current for TMPD increases systematically with temperature. Data for TMPD were obtained from the first oxidation step (formation of the cation radical) as the plateau for the 2nd electron transfer lies at ca. 1.0 V, which can be problematic due to the formation of platinum oxide and solvent oxidation.

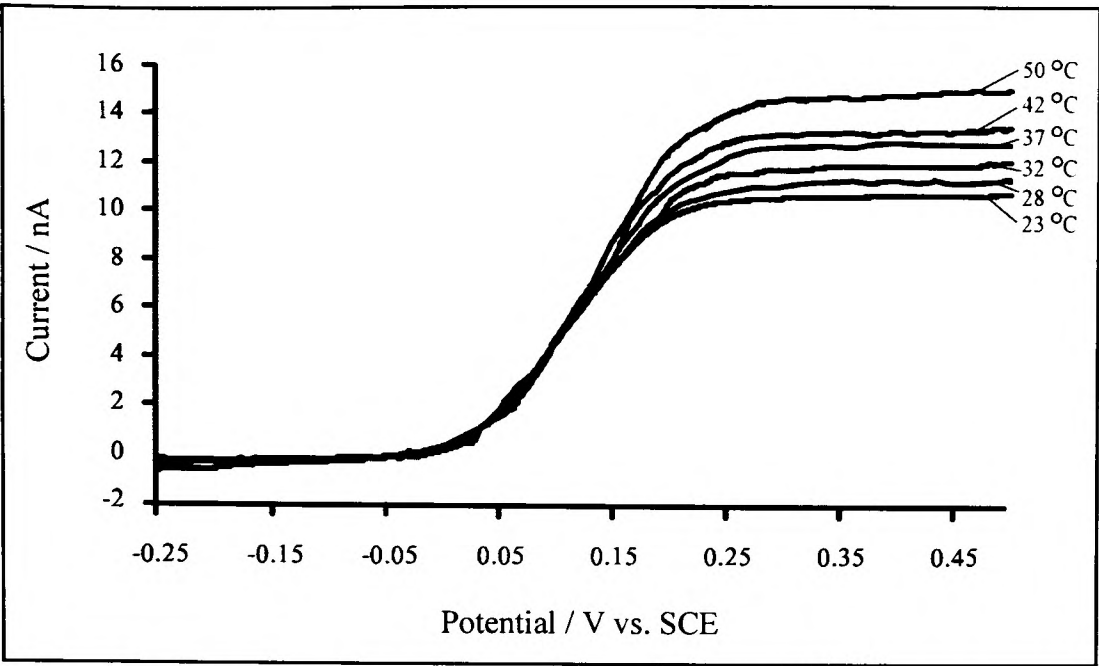


Fig. 8.2 - Microelectrode variable temperature LSVs of 1.011 mM TMPD in 0.1 M TBAHFP in MeCN.

Diffusion coefficients were calculated using equation (8.2) and the resulting data was then analysed in terms of an Arrhenius-type relation:

$$D_c = D_o \exp (- E_A / RT) \tag{8.3}$$

plotting $\ln D_c$ against $1/T$ (figure 8.3), the slopes of which are related to the activation energies shown in table 8.2.

System	RF-Channel Cell			Microelectrode Cell			Published Data		
	D_o $10^4 /$ $cm^2 s^{-1}$	E_A $/ kJ$ mol^{-1}	$D_{(RT)}$ $10^5 /$ $cm^2 s^{-1}$	D_o $10^4 /$ $cm^2 s^{-1}$	E_A $/ kJ$ mol^{-1}	$D_{(RT)}$ $10^5 /$ $cm^2 s^{-1}$	E_A $/ kJ$ mol^{-1}	$D_{(RT)}$ $10^5 /$ $cm^2 s^{-1}$	Ref
Ferrocene	4.77	7.0	2.33 15°C	5.78	7.4	2.52 21°C	6.9	2.37 24°C	[5]
TMPD	9.02	9.0	2.39 25°C	10.7	9.6	2.19 23°C	10.0	2.39 24°C	[5, 11, 12]
TBPA	5.01	8.4	1.69 25°C	5.95	8.4	1.85 20°C	8.7	1.50 22°C	[5]

Table 8.2 - Diffusion activation parameters of ferrocene, TMPD and TBPA in 0.1 M TBAHFP in acetonitrile from RFCC and microelectrode high temperature experiments.

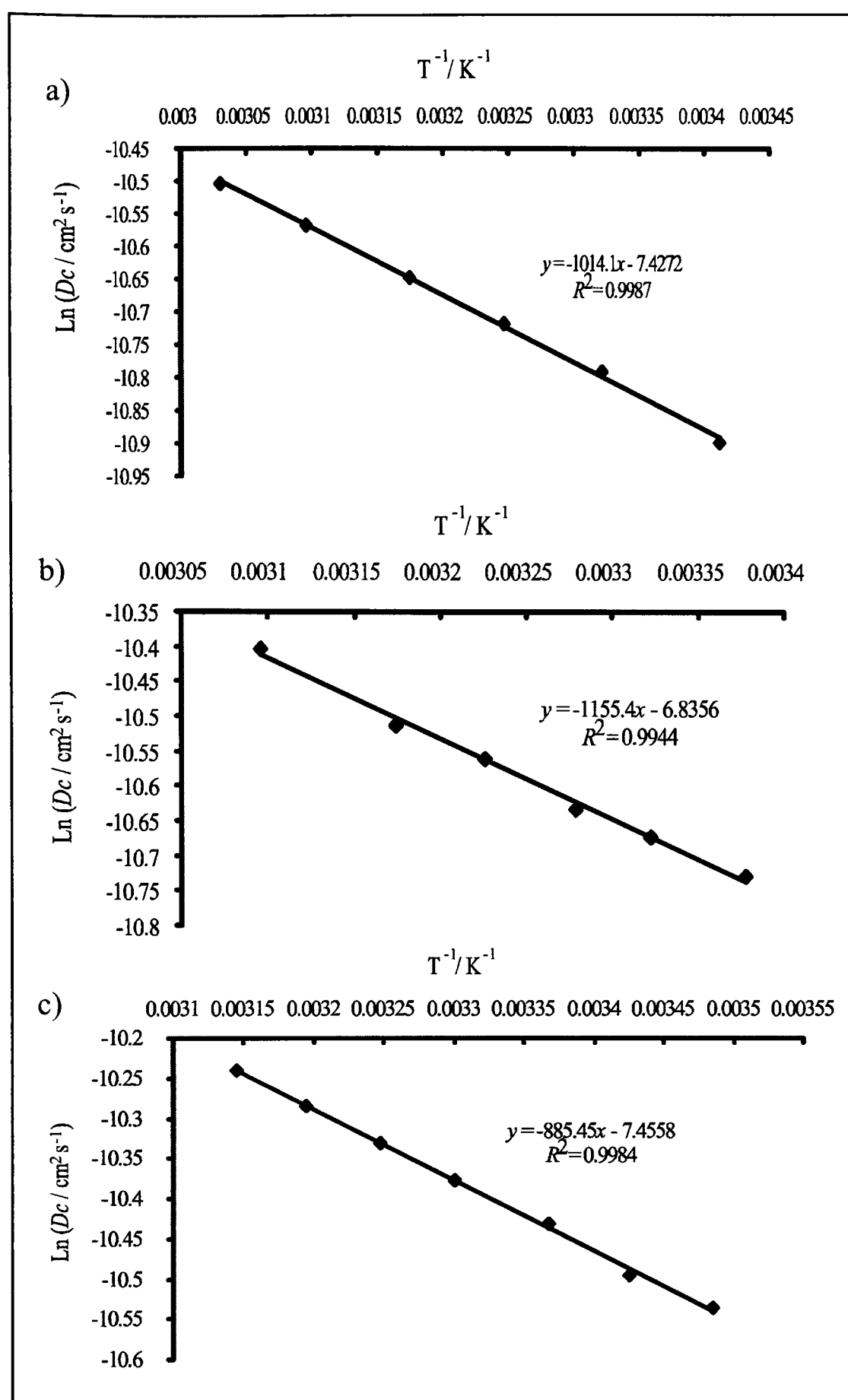


Fig. 8.3 - Arrhenius plots for microelectrode data of a) 1.010 mM TBPA b) 1.011 mM TMPD and c) 1.011 mM ferrocene in 0.1 M TBAHFP in MeCN. The likely error in the current is ± 0.1 nA.

8.4.2 RFCC RESULTS

The RFCC was thermally-calibrated using the half-wave potential shift of the oxidation wave of ferrocene as a function of temperature, as measured with the microelectrode apparatus in conjunction with a SCE reference electrode. Data was obtained for the three

redox systems using LSV with a sweep rate of 5 mV s^{-1} at flow rates varying from 0.036 to $0.447 \text{ cm}^3 \text{ s}^{-1}$ at a variety of temperatures. The voltammograms for the oxidation of TMPD as a function of flow rate and temperature are shown in figure 8.4. The oxidation of ferrocene was adopted as the reference system and used to evaluate the simulation model based on the assumption of an effective temperature for a given volume flow rate [2]. The experimental steady-state limiting currents under defined flow rate conditions were accurately reproduced by adopting the parameters of $E_A = 7.0$ and $D_{(15^\circ\text{C})} = 2.33 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ in the simulation (figure 8.5 c). This data was in excellent agreement with an E_A of 7.4 kJ mol^{-1} and a $D_{(21^\circ\text{C})}$ of $2.52 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ obtained under isothermal conditions at the microelectrode cell, and also with E_A of 6.9 kJ mol^{-1} and a $D_{(24^\circ\text{C})}$ of $2.37 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ from published data [5].

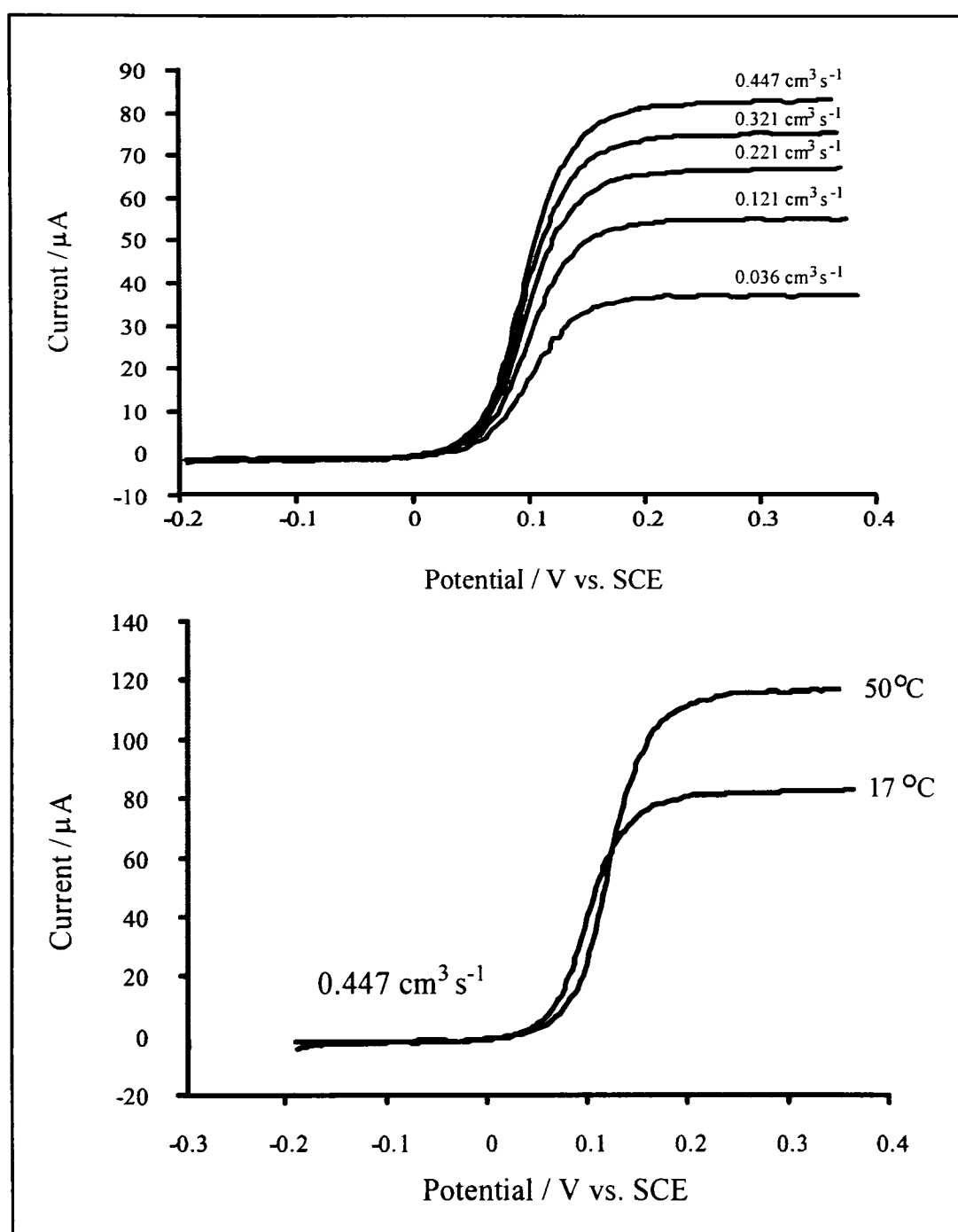


Fig. 8.4 - RFCC LSVs of 0.499 mM TMPD in 0.1 M TBAHFP in MeCN at a wide range of flow rates and temperatures.

The RFCC experimental data for TBPA and TMPD electrochemical systems were analysed using the above model over a wide range of volume flow rates and temperatures. Figure 8.5 presents data of experimental and simulated limiting currents for ferrocene, TBPA and TMPD as functions of flow rate and temperature. Good agreement between the experimental data (points) and simulated limited current (lines) at 55 °C is achieved when the RF power is adjusted to give an identical temperature rise at different flow rates.

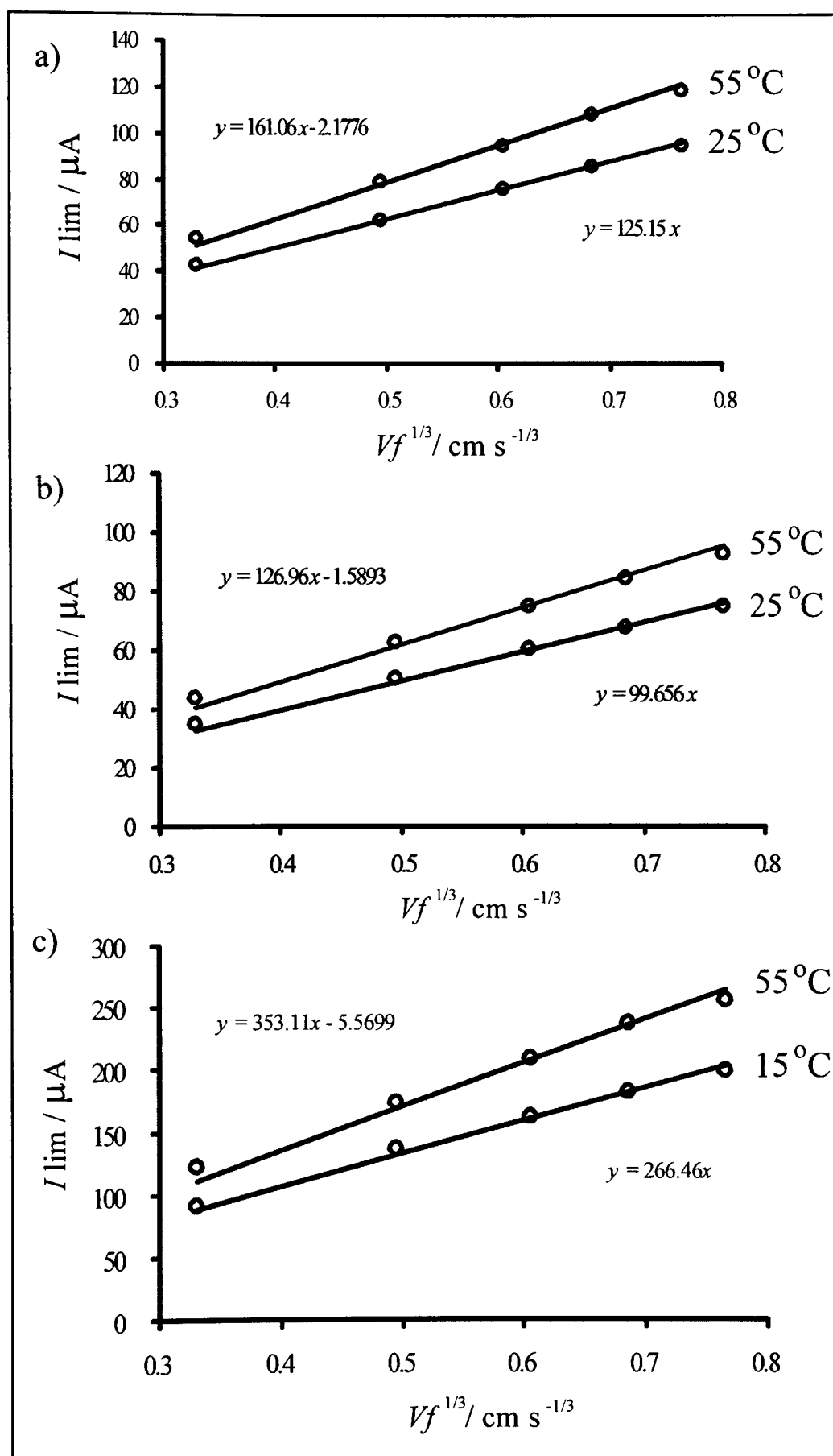


Fig. 8.5 - RFCC experimental (○) and simulated (—) limiting currents for the oxidation of a) TMPD, b) TBPA and c) ferrocene in 0.1 M TBAHFP in MeCN as a function of flow rate and effective temperature.

The results in figure 8.5 were used to determine diffusion coefficients under isothermal conditions (i.e. using the room temperature data) from equation (8.1). Diffusion activation energies (E_A) were calculated by minimising the deviation between simulation and experimentally obtained limiting current data over the wide range of flow rates. The results are summarised in table 8.2.

The Stokes-Einstein relationship can also be employed to analyse the temperature dependence of the diffusion coefficient:

$$D = k_B T / \pi n \eta r \quad (8.4)$$

where k_B is the Boltzman constant, η is the macroscopic dynamic viscosity of the solution, r , defines the molecular “effective” radius and n is a constant that can either take the value of 6, assuming that the fluid “sticks” to the diffusing particle surface (i.e. infinite frictional coefficient) or 4, assuming the “slip” condition of a zero surface friction.

Excellent linear plots of D against T / η were obtained for TMPD, TBPA and ferrocene using data from both the microelectrode and RFCC apparatus, shown in figure 8.6. The effective radii, r , of TMPD, TBPA and ferrocene calculated from the best linear regression lines in figure 8.6 and using both slip and stick boundary conditions are shown in table 8.3. The effective radius, measured using the RFCC and simulation data for TMPD in acetonitrile at both slip and stick conditions, is shown to be 3.95 and 2.63 Å respectively, which is in good agreement with values of 3.7 and 2.5 Å quoted from published data [5,6].

		TMPD	TBPA	Ferrocene
		$r / \text{\AA}$	$r / \text{\AA}$	$r / \text{\AA}$
<i>Slip ($n = 4$)</i>	Microelectrode	4.19	4.81	3.11
	RFCC	3.95	5.63	3.44
<i>Stick ($n = 6$)</i>	Microelectrode	2.79	3.21	2.07
	RFCC	2.63	3.76	2.29

Table 8.3 - Stokes-Einstein data for TMPD, TBPA and ferrocene in MeCN derived from RFCC and microelectrode results using both slip and stick boundary conditions.

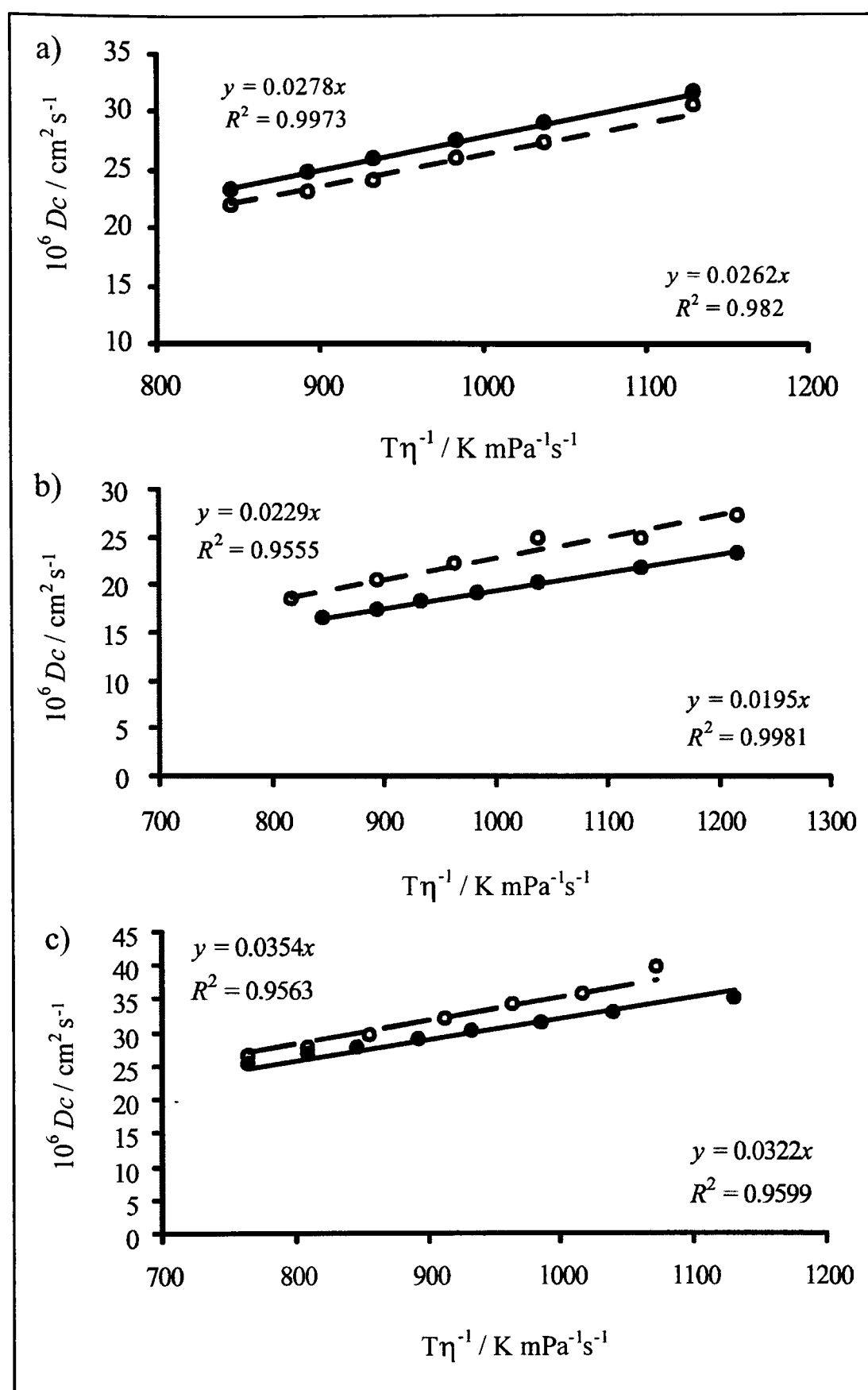
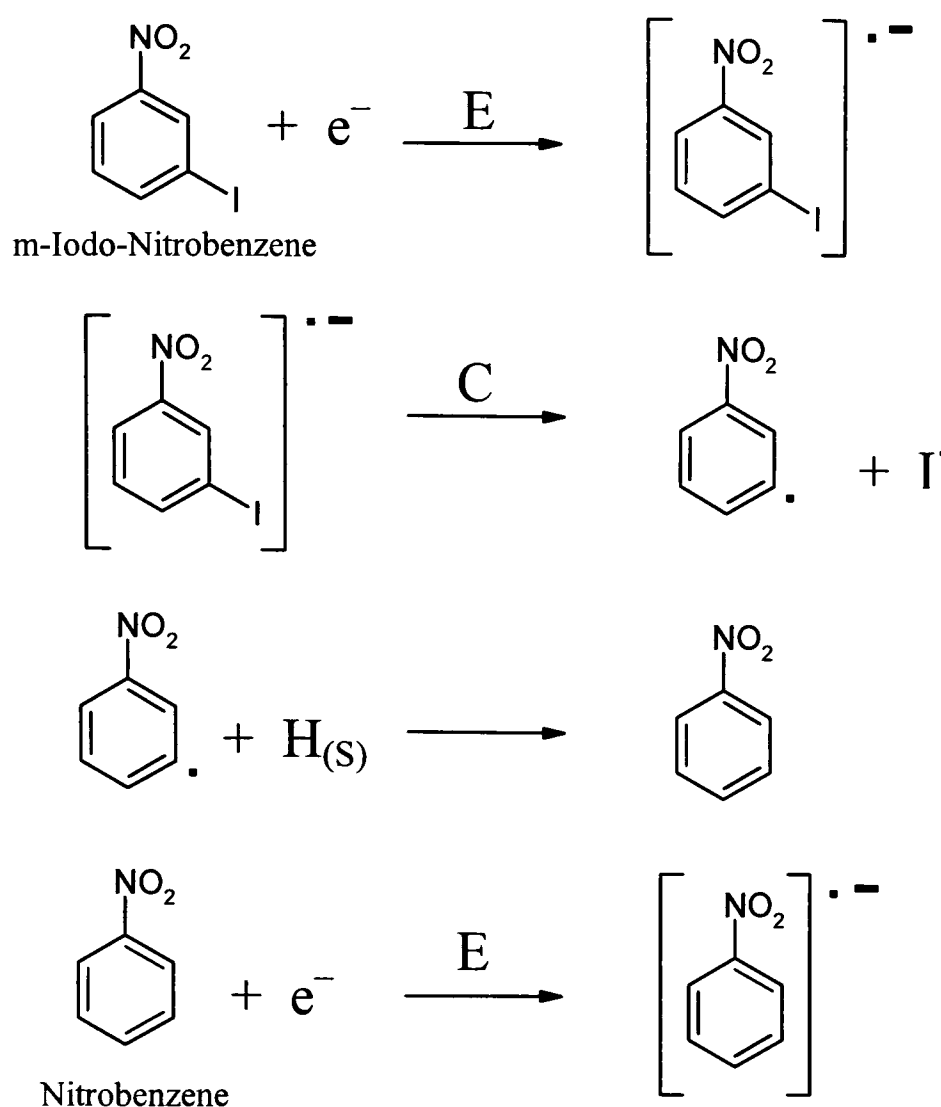


Fig. 8.6 - Stokes-Einstein plots for both microelectrode (○) and RFCC (●) data of a) TMPD, b) TBPA and c) ferrocene in 0.1 M TBAHFP in MeCN.

8.4.3 ECE REACTION OF M-iodo-NITROBENZENE

The reaction of m-INB in acetonitrile solvent has been shown to undergo a mechanistically complex electro-reduction at platinum electrodes [7] and is consistent with an ECE process [8,9]. The reaction scheme is outlined below:



where $\text{H}_{(\text{s})}$ denotes the solvent-supporting electrolyte system and the decomposition of the radical anion $[\text{m-I-C}_6\text{H}_4\text{-NO}_2]^{\cdot -}$ is rate-determining.

Values of $E_{\text{A}} = 7.4 \text{ kJ mol}^{-1}$ and $D_0 = 5.7 \times 10^{-4} \text{ cm}^2 \text{ s}^{-1}$ for the diffusion coefficient of m-INB were derived from microelectrode and RFCC data at temperatures below 30 °C by comparison with ferrocene. Values were taken from literature for the diffusion coefficient of nitrobenzene of $E_{\text{A}} = 8.3 \text{ kJ mol}^{-1}$ and $D_0 = 5.7 \times 10^{-4} \text{ cm}^2 \text{ s}^{-1}$ [10], and for $k = 0.30 \text{ s}^{-1}$ at 298 K for the rate constant of the C-step [8]. Using these values, the reaction scheme was modelled with FIDAPTM to calculate the currents from the reductions of the m-INB and of the nitrobenzene for a range of temperatures and flow rates. The activation energy for the C-step rate constant was treated as a variable parameter while maintaining the 298 K value of 0.30 s^{-1} for k , and good agreement with experiment was obtained for $E_{\text{A}} = 80 \pm 5 \text{ kJ mol}^{-1}$ (figure 8.7). **This represents the first observation of an ECE or mechanistically complex reaction at a locally heated electrode.**

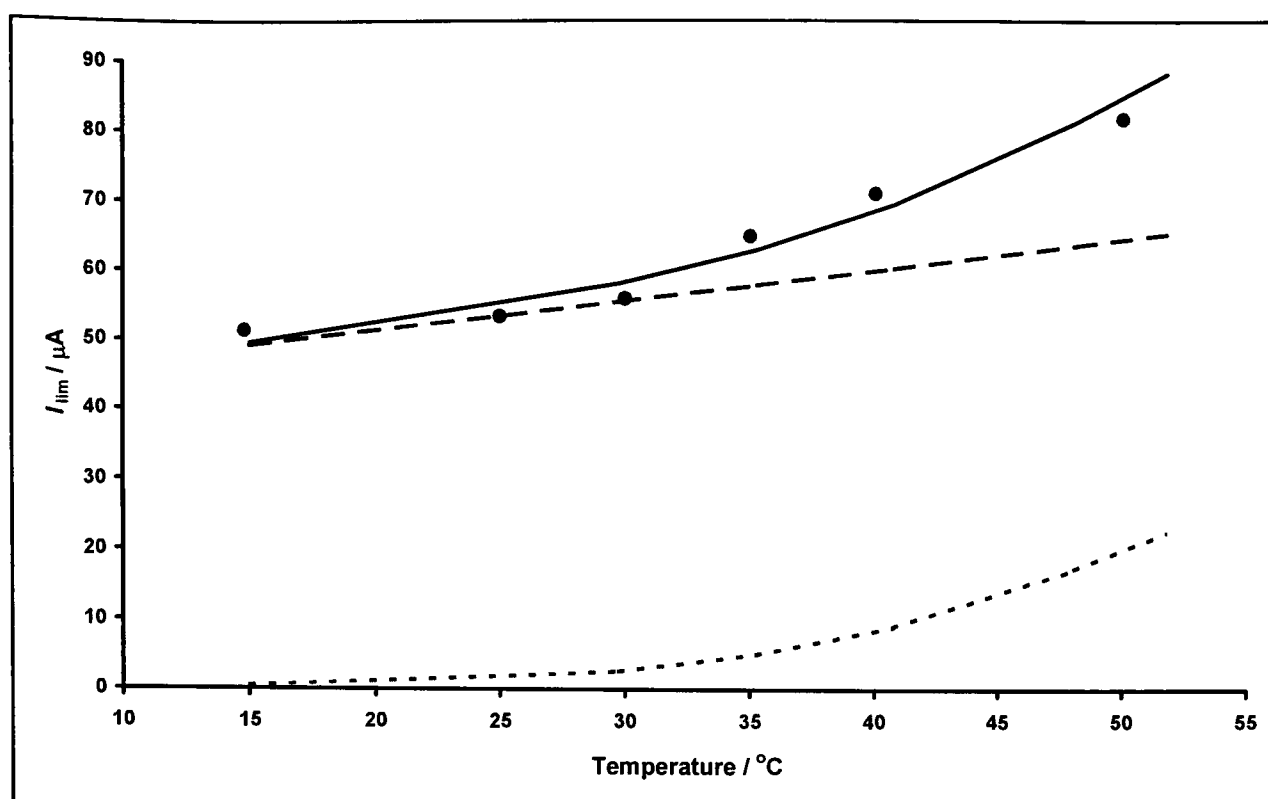


Fig. 8.7 - RFCC simulated currents: m-INB (---), nitrobenzene (·····), and total (—), and experimental values (●) as a function of thermocouple temperature, for a flow rate of $0.22 \text{ cm}^3 \text{ s}^{-1}$.

8.5 CONCLUSIONS

It was shown in this chapter that the comprehensive characterisation of the RF-heated channel cell electrode could be extended to incorporate solvents other than aqueous solutions. This was achieved by use of the two-dimensional finite element model in conjunction with an “effective” temperature determined from the response of a known reference system. Using limiting currents derived from RFCC experimental data as a function of flow rate and temperature, it was possible to obtain diffusion activation energies from simulation which were in excellent agreement with independent microelectrode experiments and published data. Data derived from the Stokes-Einstein model of both RFCC and microelectrode results were also shown to be consistent with the literature. The versatility of the RFCC was shown not only to be useful in the study of simple 1- and 2-electron reductions/oxidations but also to encompass more complicated electrode processes with coupled homogeneous kinetics such as ECE reaction mechanisms. The results obtained from the RFCC study of m-iodo-nitrobenzene are in good agreement with microelectrode and published data where available.

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CHAPTER 9

ELECTROCHEMICAL REDUCTION OF THE ANAESTHETIC GAS ENFLURANE (2-CHLORO-1,1,2-TRIFLUOROETHYL DIFLUOROMETHYL ETHER)

*The work presented in this chapter has been published in
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The work presented in the final two chapters of this thesis examines the enhanced activation achieved from modification of the electrode geometry, and in particular the application of microelectrodes to the development of electroanalytical techniques. The electrochemical reduction of the inhalation anaesthetic agent enflurane is reported at a variety of microelectrode substrates (Au, Ag, Cu, Pt and glassy carbon) with electrode dimensions varying from 10 to 60 μm . The solvents water, dimethyl sulfoxide and acetonitrile were investigated along with the supporting electrolytes potassium chloride, tetrabutylammonium hexafluorophosphate and various tetraalkylammonium perchlorates. The use of a gold microelectrode with dimethyl sulfoxide solvent and tetraethylammonium perchlorate as the supporting electrolyte was found to give well-defined voltammetry. Linear calibration curves were obtained between 0 and 2 % v/v (gaseous additions) or up to 135 mM (gravimetric additions), offering scope for the development of a rapid, inexpensive electrochemical gas sensor. Impedance measurements were carried out in collaboration with Dr. Cesar Prado, PTCL, Oxford University.

9.1 INTRODUCTION

Enflurane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether) is a stable, non-flammable inhalation anaesthetic agent which is a member of a family of fluorinated anaesthetics, first discovered for its anaesthetic properties in 1963 and introduced into clinical use ten years later [1-4]. Other than a brief assertion in the patent literature [5], to the best of our knowledge there are no reports of the direct electrochemical investigation of enflurane, although in 1988, Northing et al reported the indirect reduction of isoflurane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether), an isomer of enflurane, mediated using the

radical anion of fluoranthene or in the form of a polymer-coated modified electrode based on poly-vinylfluoranthene [6-8].

In contrast, a predecessor of enflurane, halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) has been extensively studied electrochemically due to the ease at which the carbon-bromine bond can be cleaved [9-14]. The structures of halothane, enflurane and isoflurane are shown in figure 9.1.

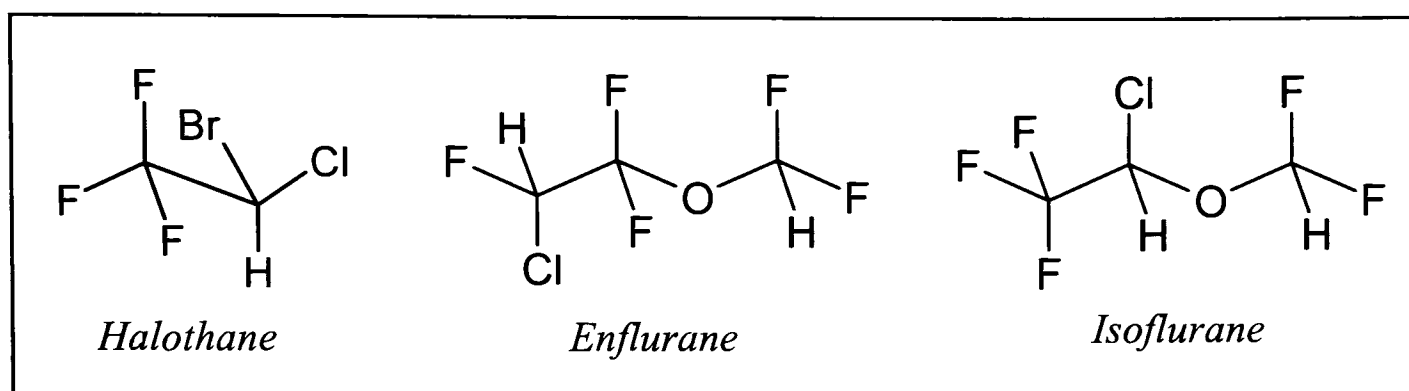


Fig. 9.1 - Structures of some anaesthetic gases.

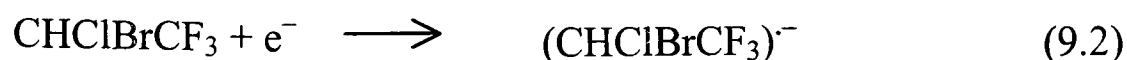
The electrochemical reduction of halothane was first reported in aqueous media at silver electrodes some twenty years ago [9] and has since been studied at other metal surfaces, including copper, gold, zinc, cadmium, mercury, gallium, indium, thallium, tin, antimony, tungsten, nickel, palladium, platinum and glassy carbon in a variety of solvents, including water (both under acidic and alkaline conditions), propylene carbonate, acetonitrile, methanol, acetone and tetrahydrofuran [10-14]. There are varied opinions in the literature as to whether the reaction is surface specific. Albery et al first reported the halothane reduction reaction in aqueous alkaline media (1 M KOH) to be



alluding to the specific affinity of gold and silver electrodes for bromide and suggesting the reduction proceeds via the intermediate ($\text{Ag}\cdots\text{Br}\cdots\text{CHClCF}_3^-$) [9]. These observations are not inconsistent with more recent reports that silver has an electrocatalytic effect on the reduction of organic halides, based on the idea of a halide/silver specific interaction and of an "attenuated radical" intermediate ($\text{R}\cdots\text{X}\cdots\text{Ag}$) [15-19].

In contrast, based on studies performed at silver, platinum, gold, glassy carbon and copper electrodes, Mount et al suggested that the reaction proceeds via an ECEC type

mechanism in aqueous alkali media (0.1 M KOH) comprising only intermediates located in the solution, with no surface-specific interaction [10]:



The use of halothane in clinical anaesthesia [20] has declined due to the development of significant toxicities with this agent, and because the newer fluorinated ethers (enflurane, isoflurane, sevoflurane and desflurane) have more desirable properties. These newer agents have lower blood-gas solubilities compared to halothane, allowing more rapid onset and offset of anaesthetic action; less sensitivity of the heart to the effects of epinephrine (and so causing so fewer cardiac arrhythmias); and reduced hepatic toxicities.

Enflurane is metabolised in the body to the extent of about 2-3 % and is used to maintain anaesthesia in inspired concentrations of between 0.5 and 2.0 % v/v, usually in conjunction with nitrous oxide and oxygen. While lower doses of enflurane may produce inadequate anaesthesia, higher doses can lead to irreversible renal damage due to the degradation of enflurane to produce fluoride ions that are hepatotoxic. The need for accurate detection of enflurane (as well as the other fluorinated ethers) in inspired air is therefore of paramount importance, and this is achieved currently by the use of expensive infrared gas analysers. A simple electrochemical method to measure the inspired gas concentrations of inhalational anaesthetic agents would therefore be highly desirable.

In this paper, the electroreduction of enflurane in DMSO at gold and other microelectrodes is reported. The decision to use microelectrodes was made partly in light of the recent publications that have been shown to resolve the electrochemical reduction signals of oxygen, carbon dioxide and nitrous oxide (an anaesthetic gas) when performed at Au microelectrodes [21-26]. This is an important feature worth considering for future development of a gas-based sensor for anaesthetic agents used in conjunction with the above gases.

9.2 EXPERIMENTAL

Chemical reagents used throughout the experiments were all obtained in the highest commercially available grade and used without further purification. For the electrochemical experiments supporting electrolytes were tetraethylammonium perchlorate (TEAP), tetrabutylammonium perchlorate (TBAP), tetrahexylammonium perchlorate (THAP) and tetrabutylammonium hexafluorophosphate (TBAHFP); all obtained from Fluka. KCl and $\text{K}_4\text{Fe}(\text{CN})_6$ were both obtained from Aldrich. The enflurane anaesthetic agent was supplied by Abbott Laboratories Ltd. under the commercial name of Ethrane[®]. Solvents used for the electrochemical experiments were dimethyl sulfoxide (DMSO, Spectrosol grade, BDH Chemicals Ltd.), acetonitrile (Fisher) or deionised water with a resistivity of not less than $18 \text{ M}\Omega \text{ cm}$ (Elgastat, USF Ltd., Bucks., U.K.)

A computer-controlled potentiostat was used for the electrochemical measurements (EG&G Princeton Applied Research model 273) using a conventional two-electrode arrangement with a silver wire pseudo-reference/counter electrode, giving a measured potential of 220 mV more negative than the saturated calomel electrode (SCE). The electrochemical cell of conventional design was thermostatted at 25°C ($\pm 0.5^\circ\text{C}$) via a pump-driven water bath. The gold working microdisk electrodes were all obtained from Microglass Instruments, Greensborough, Australia and included 60, 10, 5 μm geometries. The remaining platinum (10 μm), silver (25 μm), copper (25 μm) and glassy carbon (GC, 5 μm) microelectrodes were fabricated in house. The microelectrodes were all polished [27] at the start and end of each experiment first using a 1.0 μm alumina-water slurry followed by 0.3 μm alumina (both Buehler, Lake Bluff, IL) on a microelectrode polishing pad (Kemet, Kent, U.K.). The microelectrodes were thoroughly inspected using an optical microscope to check that they had not recessed into the glass surface. Diameters of the working microelectrodes were calibrated electrochemically using 10 mM ferrocyanide in 0.1 M KCl with a value of the diffusion coefficient (at 25°C) taken to be $6.9 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ [28]. All solutions were degassed with pure nitrogen gas (BOC) prior to use. Linear sweep voltammetry (LSV) was employed typically operating at a scan rate of 100 mV s^{-1} or conditions corresponding to near steady-state transport limited voltammetry.

The enflurane anaesthetic agent was introduced into solution either as gravimetric aliquots in liquid form or via the gas, bubbled through the solution prior to each electrochemical sweep. In the latter case the enflurane gas was produced using a commercial clinical vaporiser (PPV enflurane vaporiser, Penlon, Ltd.) and introduced into the cell as a v/v % dilution with pure N₂ gas (BOC). A respiratory gas monitor (Hewlett Packard model M1025A, Bracknell, U.K.) was used to accurately determine the concentration of the anaesthetic agent prior to dissolution into the solvent. The gas was bubbled through the solution for a period of no less than 20 minutes to ensure that full equilibration of the gas and solvent was achieved. Cessation of the gas supply during the voltammetric sweeps was important to ensure that measurements were taken in stationary solution, under diffusion-controlled conditions.

Finally, a.c. impedance measurements were performed with a computer-controlled Frequency Response Analyser (Solartron 1250) coupled to a potentiostat (Solartron 1286, both Hampshire, UK). The experiments were performed by fixing the potential between 0 and -3.0 V and then sweeping the frequency between 20 kHz and 10 Hz.

9.3 RESULTS AND DISCUSSION

9.3.1 REDUCTION OF ENFLURANE IN DMSO AT GOLD MICROELECTRODES

The reduction of gaseous enflurane was investigated at a 60 μm Au microelectrode in a solution of 0.2 M TBAP and DMSO. Gas concentrations between 0.14 to 2.0 % v/v were equilibrated with the solution and linear sweep voltammograms recorded between potentials of 0.0 and -3.0 V (vs. Ag), shown in figure 9.2 (a). The enflurane reduction wave was visible between potentials of -1.7 and -2.8 V (vs. Ag) with a well-defined limiting current. A linear calibration plot, correlating limiting current with enflurane concentration, is shown in figure 9.2 (b). Similarly, gravimetric additions of liquid enflurane were introduced into a solution of 0.2 M TBAP in DMSO and reduced at a 60 μm gold microelectrode. Figure 9.3 (a) shows voltammograms with the enflurane concentration increasing from 13.7 to 135 mM; a linear calibration plot is shown in figure 9.3 (b).

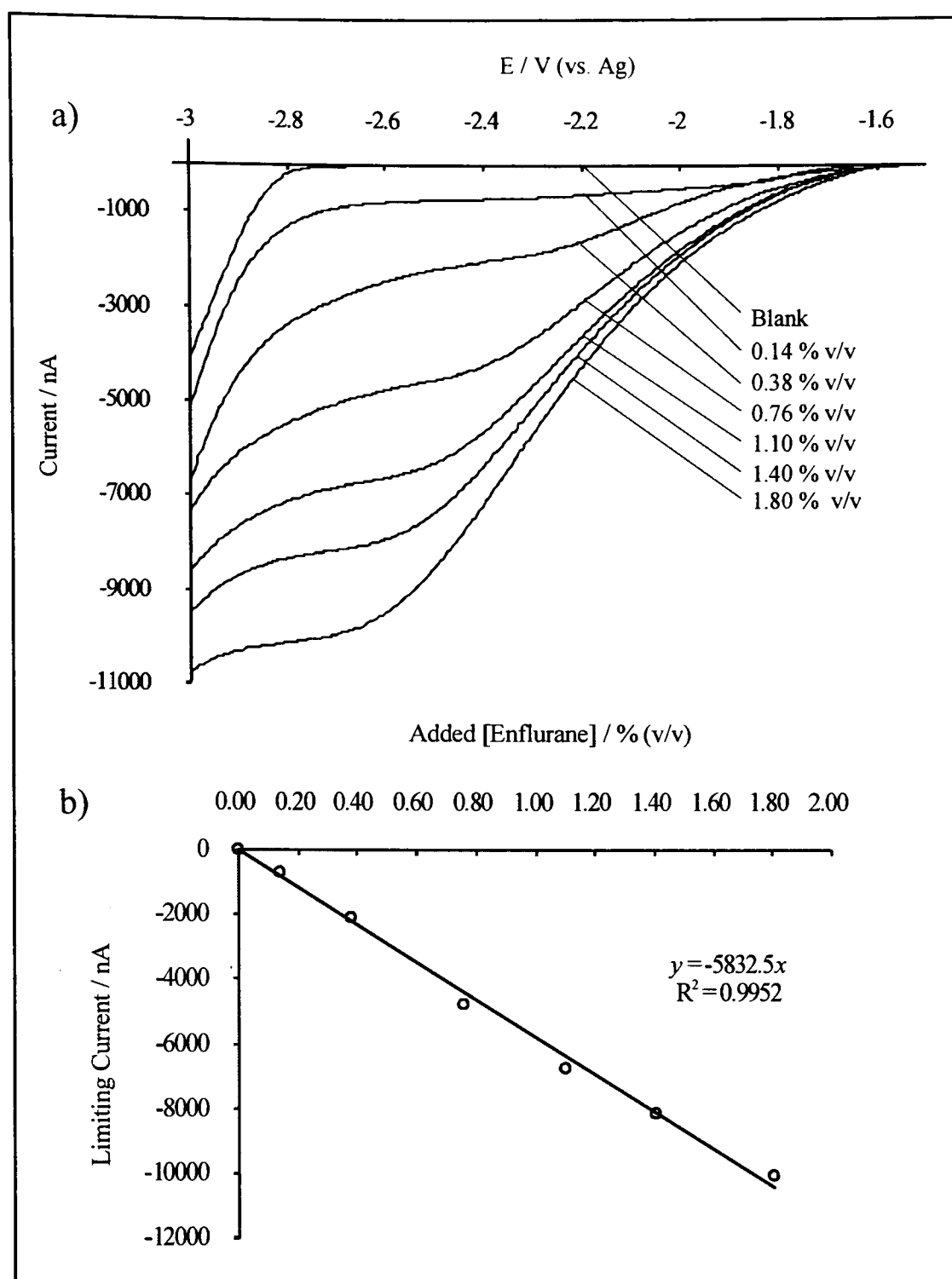


Fig. 9.2 - a) Reduction of 0.14 to 1.80 % v/v gaseous enflurane immediately following equilibration with 0.2 M TBAP and DMSO at a 60 μm Au microelectrode and b) corresponding linear calibration curve plot.

Using the gravimetric data, the diffusion coefficient of enflurane can be calculated using the following equation for the transport limited current at a microelectrode [29]

$$I_{\text{LIM}} = 4 n F D_c [B] r \quad (9.5)$$

where n is the number of electrons transferred, F is the Faraday constant, D_c is the diffusion coefficient, $[B]$ is the bulk concentration of enflurane and r is the radius of the electrode. Using the electrochemically-measured diameter (63.7 μm) and assuming $n = 1$ or 2, the resulting diffusion coefficients were found to be 6.7×10^{-6} and $3.3 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ respectively. These compare with the value of $4.0 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ estimated using the

Wilke-Chang empirical model (see Appendix C) [30] and with $6.0 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ obtained for halothane in aqueous solution [9,10,13]. These values were essentially unchanged when deduced in an analogous fashion at a $5 \text{ }\mu\text{m}$ diameter electrode implying the reaction is truly a 1- or 2-electron process (and not for example an ECE process where the effective number of electrons would be radius sensitive).

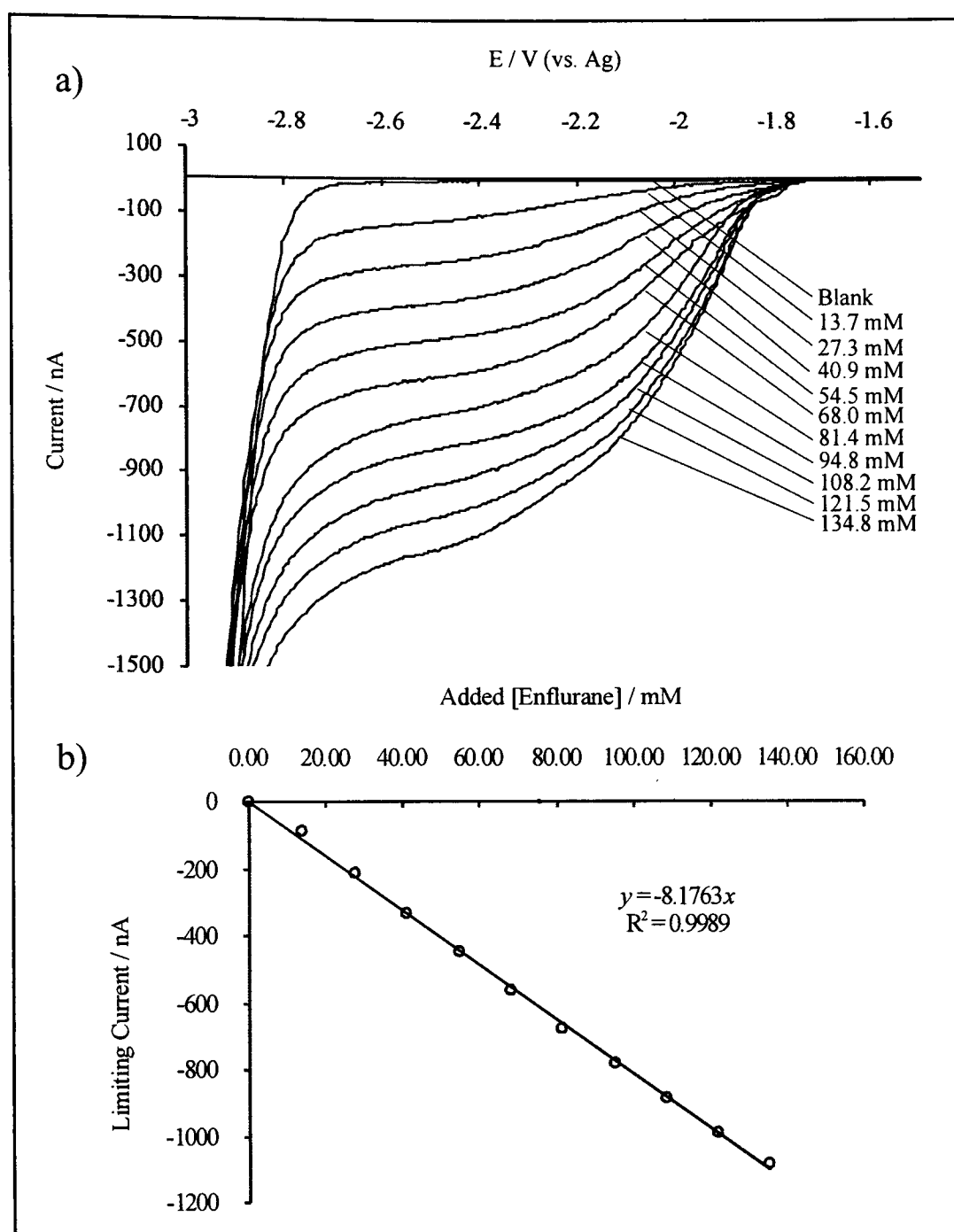


Fig. 9.3 - a) Reduction of 13.7 to 135 mM additions of enflurane in 0.2 M TBAP and DMSO at a $60 \text{ }\mu\text{m}$ diameter Au microelectrode and b) corresponding linear calibration curve plot.

The relationship between the gas concentration (in v/v % or partial pressure) and the dissolved solution concentration (in mol dm^{-3}) is stated by Henry's law, in that the dissolved concentration of a gas, A, in a solution is directly proportional to the partial pressure of the gas above it, forming the relationship

$$P_A = [A] \times K_H \quad (9.6)$$

where P_A is the partial pressure of the gas, $[A]$ is the solution concentration and K_H is the Henry's law constant (the reciprocal of the molar solubility). An empirical correlation between gaseous enflurane (in v/v %) and dissolved solution concentration (in millimolar) was obtained using gravimetric data (e.g. from figure 9.3, 1 mM = 8.2 nA) to calibrate the enflurane gas concentrations (figure 9.4). From the gradient, K_H for enflurane is calculated to be $0.014 \text{ atm mol}^{-1} \text{ dm}^3$.

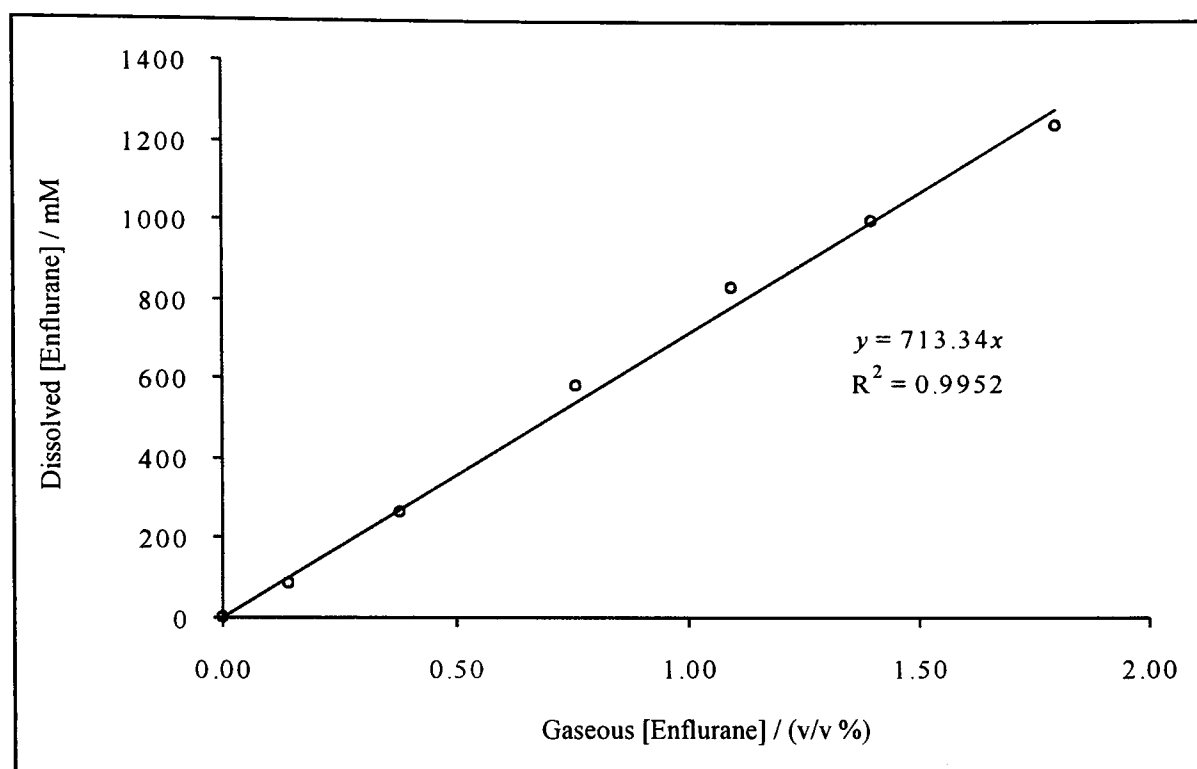


Fig. 9.4 - Calibration of dissolved enflurane concentration as a function of gaseous concentration derived from gravimetric data.

9.3.2 SOLVENT EFFECTS

In addition to DMSO, the reduction of enflurane was investigated at Au microelectrodes in the solvents MeCN, H₂O and a H₂O/DMSO mixture (20 % H₂O 80 % DMSO) in supporting electrolytes of 0.1 M TBAHFP, 0.1 M KCl and 0.2 M TBAP respectively. A range of enflurane concentrations (from 0 to 2 % v/v) were dissolved into the respective solvents followed by subsequent LSV analysis. The results from the reduction of 0.75 % v/v enflurane in the various solvents are directly compared in figure 9.5 and the corresponding $E_{1/2}$ data for MeCN, DMSO and H₂O/DMSO are -2.42, -2.14 and -1.93 V (vs. Ag) respectively.

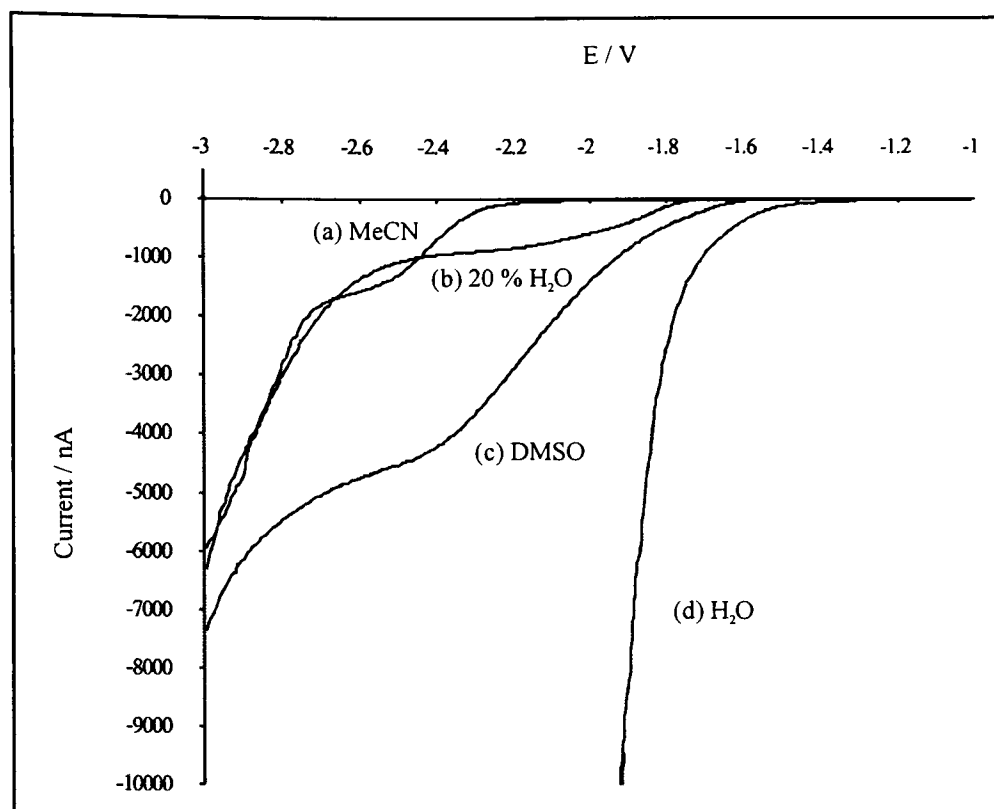


Fig. 9.5 - Reduction of 0.75 % v/v enflurane gas after equilibration in a) MeCN with 0.1 M TBAHFP, b) 20 % H₂O and 80 % DMSO with 0.2 M TBAP, c) DMSO with 0.2 M TBAP and d) H₂O with 0.1 M KCl performed at a 60 μ m diameter Au microelectrode.

Enflurane in the above solvents, with the exception of water, exhibits well-defined voltammetry. The limiting currents obtained from the H₂O/DMSO mixture are linear with increasing enflurane concentration and have been compared against the data obtained from pure DMSO in figure 9.6.

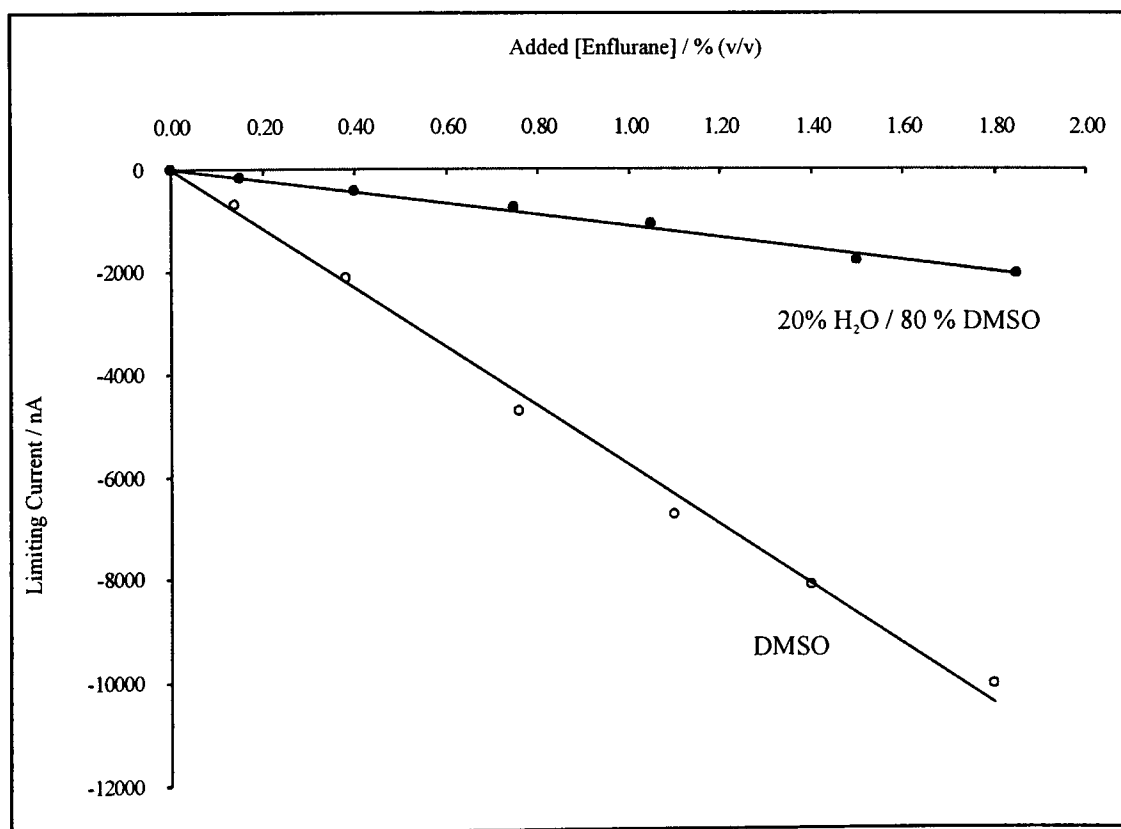


Fig. 9.6 - Comparison of gaseous enflurane reduction at 60 μ m diameter Au and 0.2 M TBAP in pure DMSO and H₂O/DMSO mixture.

Significantly higher currents are seen when using pure DMSO than with using the aqueous dilution, reflecting the greater solubility of gaseous enflurane in non-aqueous solvents compared to water. The relative solubility of enflurane in MeCN was experimentally derived using gravimetric additions in an analogous procedure as outlined with DMSO. Calibration of the 0.75 % v/v gaseous data (from gravimetric, 1 mM = 25 nA) gave a solution concentration of 35 mM in MeCN; approximately 17 times lower in comparison with pure DMSO at the same gas concentration (at 25 °C). When using pure water as the solvent the working range of the electrode was significantly narrowed (limited to potentials lower than -2.0 V) due to the onset of gas evolution from solvent breakdown: no enflurane reduction was observed.

9.3.3 ROLE OF THE ELECTRODE SURFACE

Further experiments were carried out to explore the role of the electrode surface in the reduction mechanism in light of speculation that Au and Ag electrodes possess catalytic properties towards the reductive dehalogenation of organic halides [9,15-19]. The electrode surface properties were investigated by using a variety of electrode substrates (GC, Cu, Pt, Ag and Au), each one scrutinised by comparison of their voltammetric response to gravimetric enflurane additions (13.7 - 135 mM) in 0.2 M TBAP and DMSO. The activity of Ag and Cu microelectrodes towards enflurane reduction is highlighted in figure 9.7, where linear sweep voltammograms are shown at increasing concentrations of enflurane. Figure 9.8 (a) shows the comparative reduction of 135 mM enflurane at Au, Ag, Cu and GC substrates whilst figure 9.8 (b) shows the calibration plot data. The diffusion coefficients calculated from the calibration plots for Ag and Cu are 6.5×10^{-6} and $6.8 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ and corresponding $E_{1/2}$ data for Au, Ag and Cu substrates are -2.00, -2.19 and -2.51 V (vs. Ag) respectively. Pt microelectrodes, however, showed irreproducible baselines due to gas evolution formed via solvent breakdown beginning at -2.2 V, and were not considered further. GC microelectrodes were found to show no activity towards enflurane within the working potential range from 0 to -3.0 V (vs. Ag).

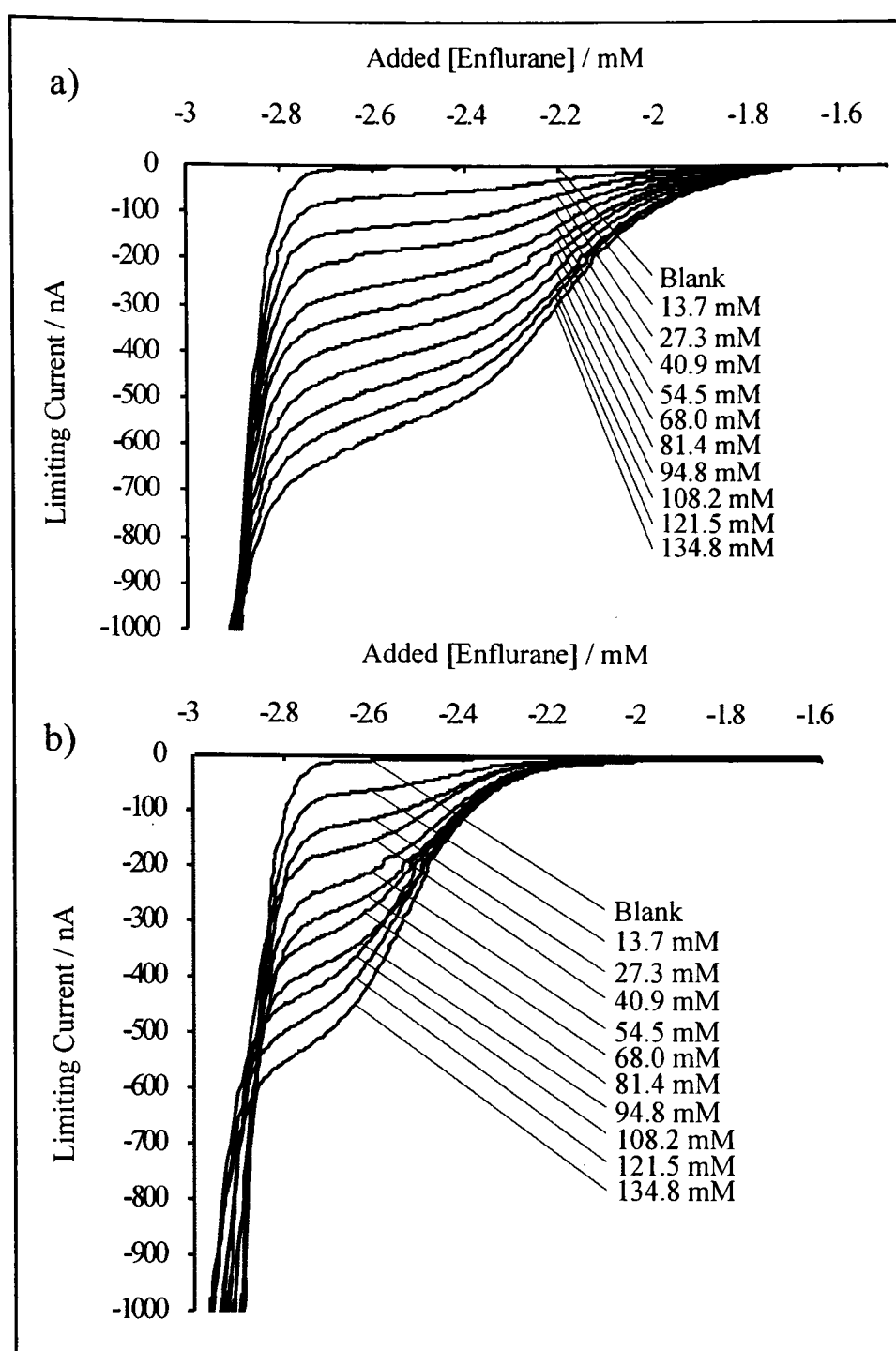


Fig. 9.7 - Reduction of increasing concentrations of enflurane at a) 33.0 μm Ag and b) 29.0 μm Cu substrates in 0.2 M TBAP and DMSO.

From inspection of both the voltammetric and linear calibration curve data it is clear that currents obtained from the Au, Ag and Cu substrates show significant activity towards enflurane reduction whereas GC microelectrodes are inert. In addition, the variation in $E_{1/2}$ potential confirms that less reducing potentials are required with Au and Ag substrates compared to those obtained with Cu. Taking these observations into account, and making reference to that fact that this has also been reported in the literature when looking at the electrochemical reduction of other organic halides, it is not inconsistent with the suggestion that Au and Ag surfaces possess specific catalytic activity when compared to the glassy carbon electrode surface [15-19].

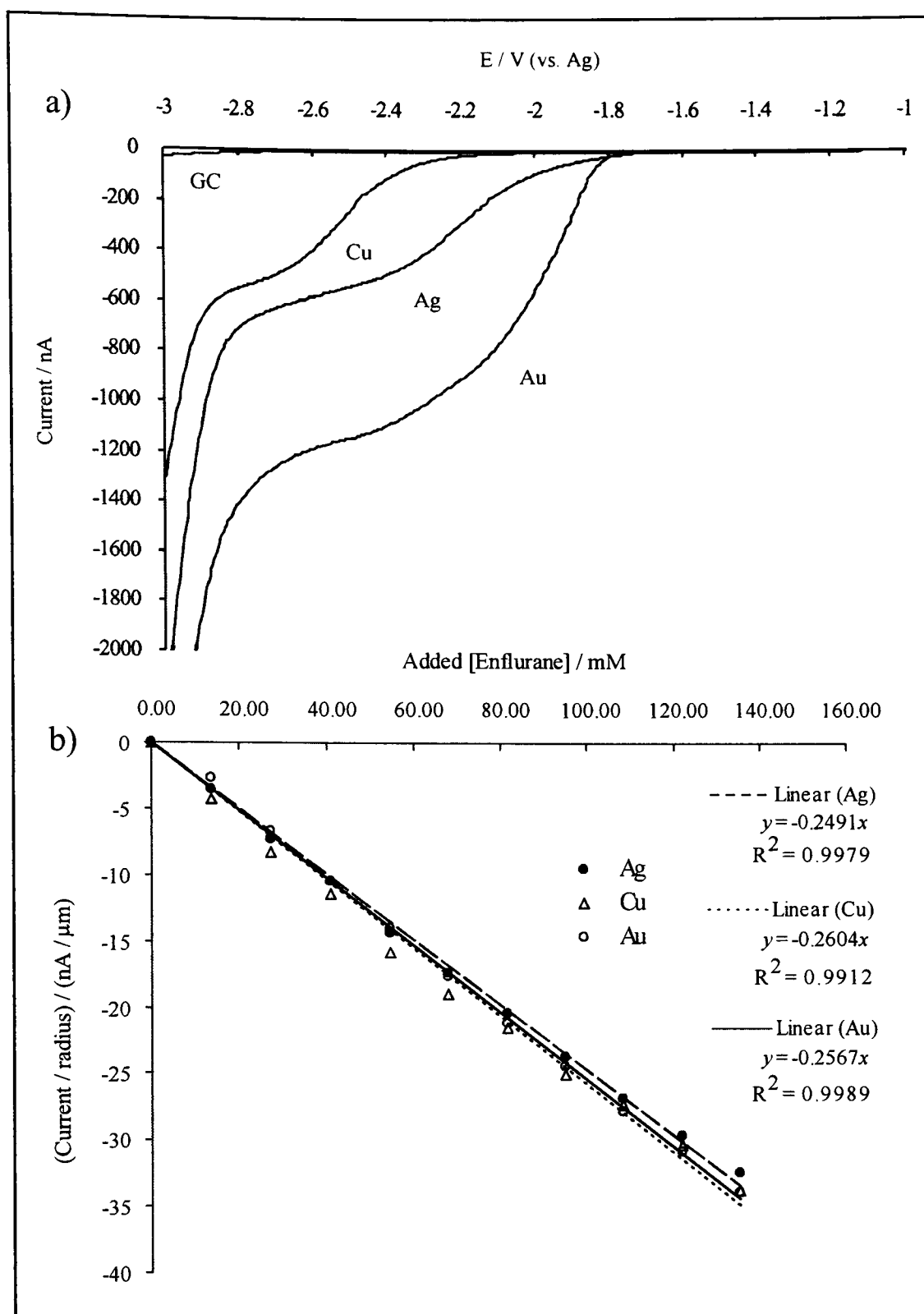


Fig. 9.8 - Comparison of electrode surface activity through a) reduction of 135 mM enflurane in 0.2 M TBAP and DMSO and b) linear calibration curve plots recorded at Au, Ag and Cu microelectrodes of 63.7, 33.0 and 29.0 μm diameters respectively.

9.3.4 ROLE OF THE SUPPORTING ELECTROLYTE

Last we compared the electrochemistry of enflurane in the presence of TEAP, TBAP and THAP supporting electrolytes. In the literature there are reports that the choice of the supporting electrolyte not only influences the position of the reduction wave for organic halides, but also plays a key role in any charge-transfer mechanisms by changing the charge and size of the Inner Helmholtz Plate (IHP) [15,16]. It is suggested that using an electrolyte with anions that are entirely non-absorbing on the Au or Ag substrate (e.g.

perchlorate or tetrafluoroborate anions), the TEA^+ cations are specifically adsorbed onto the surface so that the IHP is the locus of positive charges [15,16]. This supposedly sets up an effective screening of the cathode surface facilitating the approach of the halide atom on the organic molecule affecting the activation energy profile at the electrode|solution interface and changing the kinetics for electron transfer [31,32]. The effect of changing the length of the alkyl chain (from TEAP to TBAP) results in more bulky TBA^+ cations adsorbed on the electrode, resulting in a drop in the observed peak reduction current due to hindered approach of the organic halide. The result is the formation of a lipophilic film with an inhibiting effect related to its thickness (alkyl chain length) [15].

In contrast, the presence of a specifically absorbing anion (e.g. $\text{X}^- = \text{Cl}^-$, Br^- , etc) supplied either from the supporting electrolyte or from the reduction of the organic halide may lead to a reversal of the IHP charge from positive (TEA^+ absorbed) to negative (X^- absorbed) and a subsequent shift of the reduction wave is observed to more reducing potentials [15]. Figure 9.9 shows the effects of increasing TEAP and TBAP electrolyte concentration on the reduction of 13.7 mM enflurane. It can be seen that the effect of changing the alkyl chain length from TEA^+ to TBA^+ has the effect of both shifting the position of the reduction wave to more reducing potentials, consistent with the idea that more negative potentials are required with increasing chain length [33] and also lowering the peak reduction current [15].

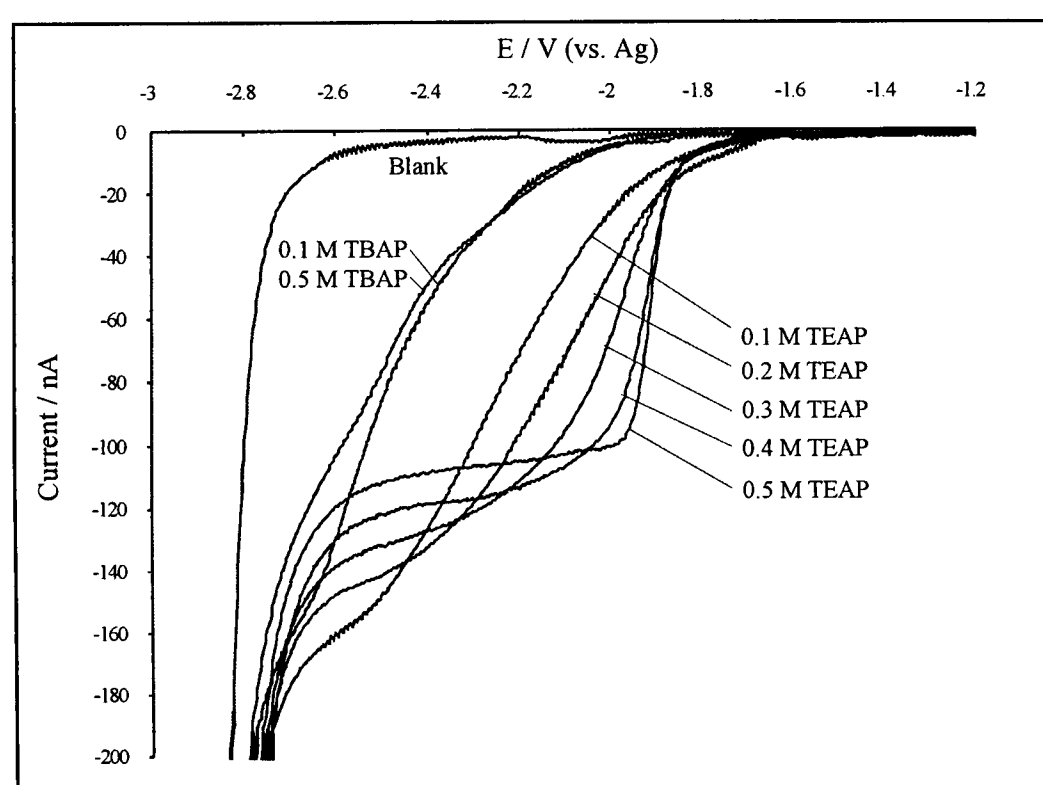


Fig. 9.9 - 13.7 mM enflurane reduction in DMSO solvent and a variety of supporting electrolyte concentrations.

Without detailed knowledge of the stoichiometry of the electrode reaction, analysis of the current-voltage curves in figure 9.9 is difficult. Mass transfer-corrected Tafel plots of E against $\ln (I_{\text{lim}} - I) / I$ were used to give an approximate measure of the degree of electrochemical reversibility. In the case of simple electron transfer processes, the charge transfer coefficient can be obtained from the gradient and is equal to $RT/\alpha nF$ [34].

Such analyses showed an increase in the apparent electrochemical reversibility with increasing TEAP supporting electrolyte concentration as is implicit in figure 9.9 and is not inconsistent with the idea that the electrode can be more effectively screened by the increased number of adsorbed TEA^+ cations, creating a more positive IHP and facilitating closer enflurane approach.

A decrease of reversibility when changing from 0.5 M TEAP to 0.5 M TBAP was seen and is not inconsistent with the supposition that the approach of the enflurane molecule is sterically hindered from the bulky TBA^+ anions that reside on the electrode surface and IHP. Consistent with this the literature reports no observed reduction of organic halides on Ag when using TBAP/MeCN as the supporting electrolyte [15].

Figure 9.10 shows the reduction of enflurane in a mixture of 0.2 M TEAP with additions of 3.4 mM TBAP. From these results it suggests a higher degree of reversibility is obtained using either a large concentration of TEAP electrolyte (ca. 0.5 M), or conversely 0.2 M TEAP with a small addition of TBAP (25.0 mM). In TEAP alone, halide ions are reported to perturb the TEA^+ film, reversing the charge on the IHP and changing the electron-transfer kinetics [15]. We speculate that the likely Cl^- reduction product from enflurane may adsorb onto the electrode surface and modify the charge on the IHP. In TBAP supporting electrolyte, the TBA^+ cations are more difficult to displace.

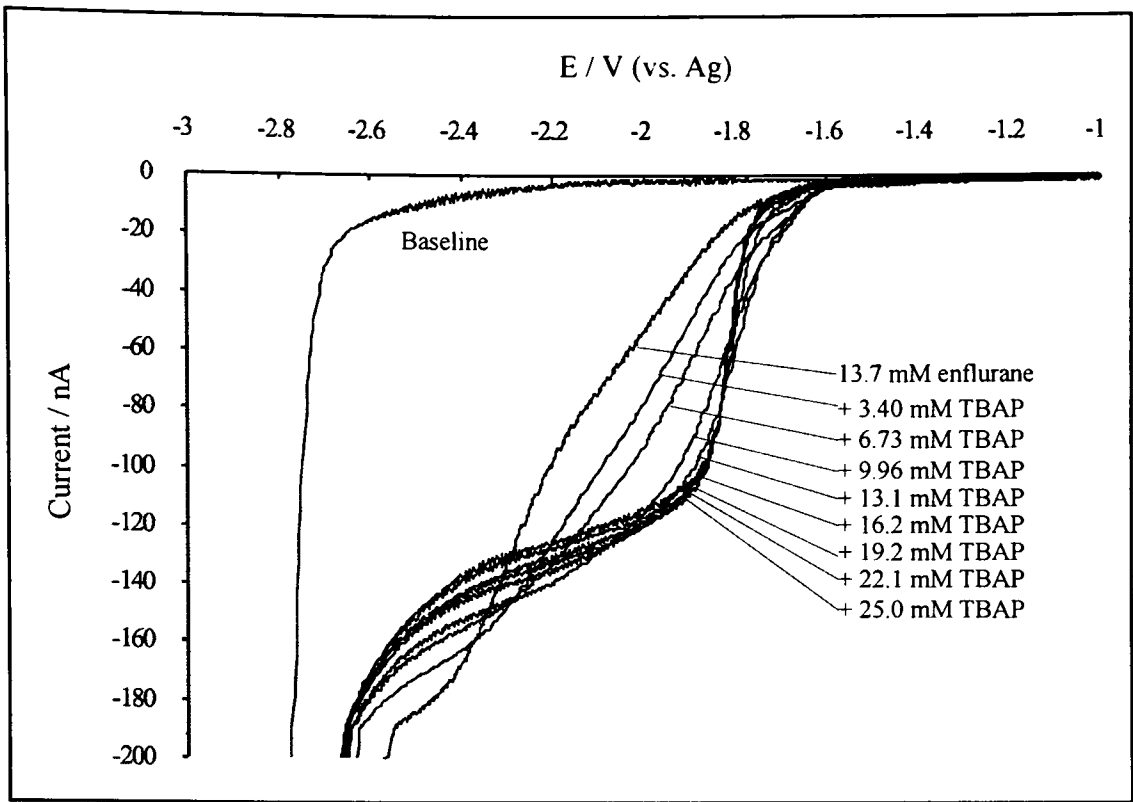


Fig. 9.10 - 13.7 mM enflurane in 0.2 M TEAP and DMSO with aliquots of TBAP at a 60 μm diameter Au electrode.

Similar observations were found changing from TBAP to THAP supporting electrolyte. Introduction of millimolar THAP additions to a 0.2 M TEAP solution containing 13.7 mM enflurane gave a change in the voltammetry, similar to TBAP, shown in figure 9.11.

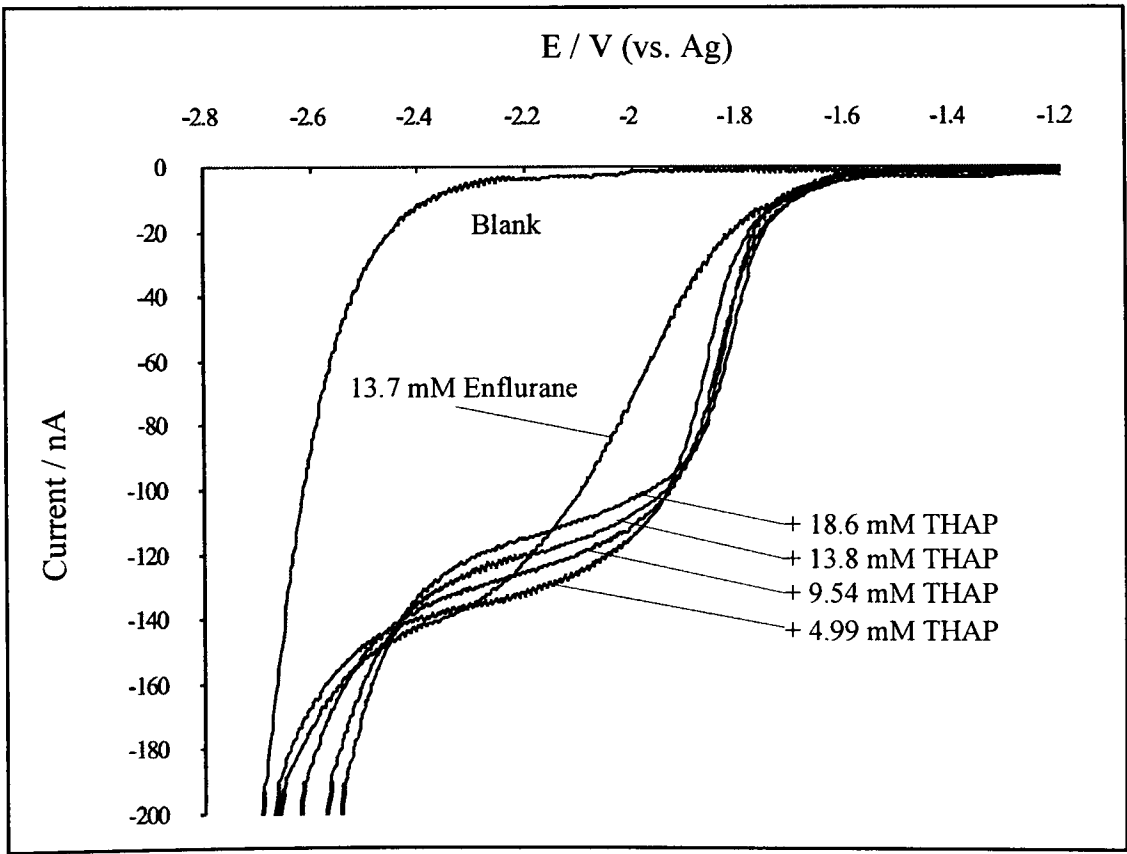


Fig. 9.11 - Aliquots of THAP to 13.7 mM enflurane in 0.2 M TEAP and DMSO at a 60 μm diameter Au electrode.

To further investigate $\text{TEA}^+/\text{TBA}^+/\text{THA}^+$ adsorption onto the electrode surface, a.c. impedance techniques were used. The studied potentials are more negative than the reported potential of zero charge (pzc) of 165 mV [35] and therefore the electrode surface is negatively charged, facilitating the cation adsorption. Capacity curves for 0.5 M TEAP, TBAP and THAP supporting electrolyte obtained with a 60 μm Au microelectrode in DMSO solvent are shown in figure 9.12. It was observed that between 0 and -2.70 V (vs. Ag) the impedance behaviour was purely capacitive, however, at more negative potentials faradaic processes were observed due to solvent breakdown.

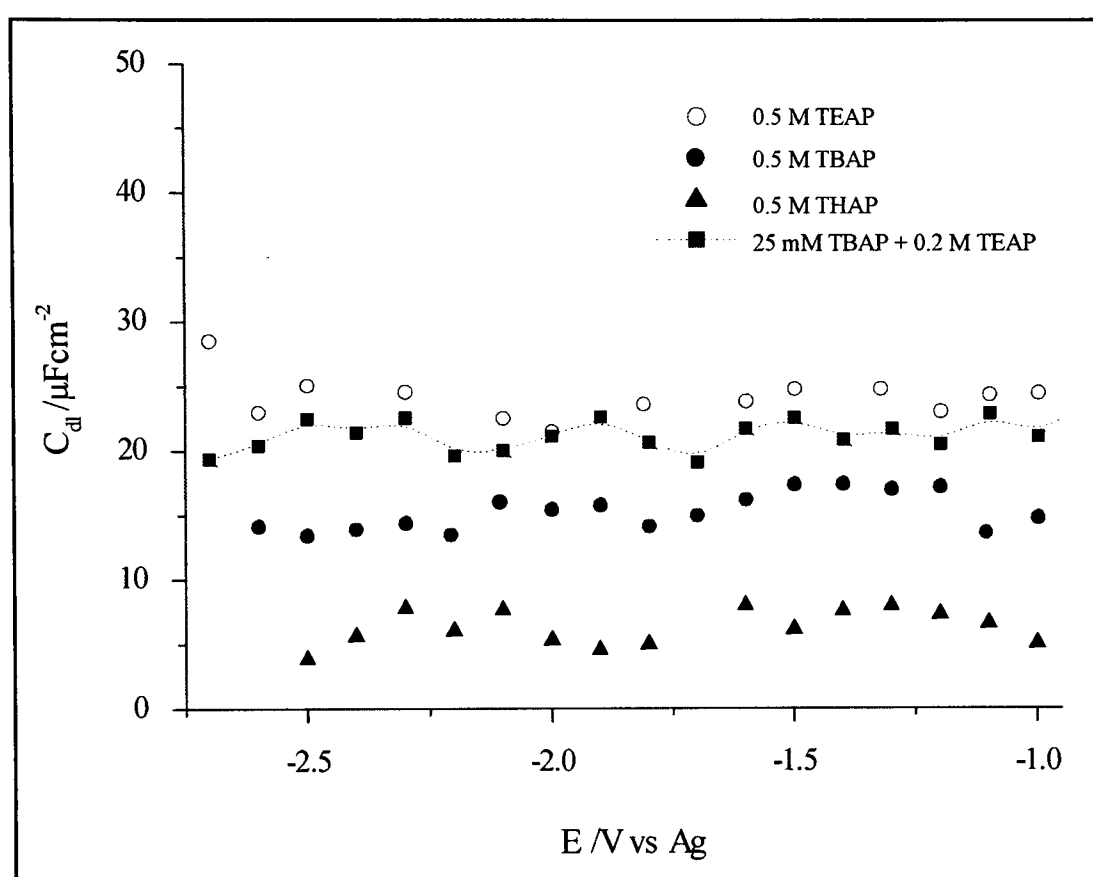


Fig. 9.12 - A.C. impedance measurements ($\nu = 100$ Hz) performed in 0.5 M TEAP, TBAP and THAP supporting electrolytes in DMSO at a 60 μm Au microelectrode. The double layer capacity for the 25.0 mM TBAP / 0.2 M TEAP mixture is also shown for comparison.

As the interface in the capacitive region can be expressed in terms of a simple two-plate-capacitor, the different values for the various supporting electrolytes can therefore be explained by the differences in IHP thickness. From the data, it is observed that when using THAP as the supporting electrolyte, a capacitance value is generated that is approximately 6 times lower than TEAP and 4 times lower than TBAP supporting electrolytes respectively.

9.4 CONCLUSIONS

The data reported in this chapter offers scope for the development of an electrochemical sensor that can accurately monitor enflurane concentrations in the gaseous phase within the clinically relevant range between 0 and 2 % v/v. Well-defined voltammetry was achieved using 0.5 M TEAP supporting electrolyte with DMSO solvent in combination with a 60 μm gold microelectrode. Reduction occurs on Au, Ag and Cu substrates whilst there is mechanistic information that supports the view that the reduction of enflurane is catalytic on both gold and silver electrodes in DMSO solvent. The choice of the supporting electrolyte medium affects both the reduction potential and reversibility of the electrode reaction. The work presented next follows on from the studies presented in this chapter and investigates the versatility of the electrochemical approach to the design and fabrication of an anaesthetic gas sensor through study of the electrochemistry of enflurane in the presence of O_2 and N_2O gases.

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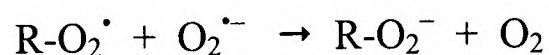
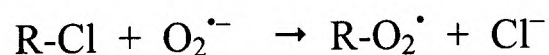
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CHAPTER 10

ELECTROCHEMICAL STUDIES OF THE ANAESTHETIC AGENT (2-CHLORO-1,1,2-TRIFLUOROETHYL DIFLUOROMETHYL ETHER) IN THE PRESENCE OF OXYGEN: REACTION WITH ELECTROGENERATED SUPEROXIDE

*The work presented in this chapter has been accepted for publication in
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The work presented in this chapter extends the work previously covered in the last chapter where the electrochemical reduction of the anaesthetic agent enflurane was investigated at Au, Ag and Cu microelectrodes in DMSO solvent and tetraalkylammonium perchlorate supporting electrolytes, with the long-term aim of the production of a rapid, inexpensive electrochemical sensor for the anaesthetic gas. During clinical anaesthesia, it is typical to receive doses of enflurane (between 1 to 5 % v/v) in gaseous mixtures of oxygen ranging from 30 to 100 % v/v and therefore in order to realistically assess the design of any electrochemical sensor, it is necessary to study the electrode behaviour of enflurane in the presence of oxygen. Moreover, it is desirable to be able to monitor both gases using the same amperometric sensor. The work reported in this paper provides the mechanistic basis for such a protocol. The superoxide anion radical, formed from the electro-reduction of dissolved oxygen, is shown to react with enflurane complicating their simultaneous detection. The kinetics of the enflurane / superoxide reaction are found to be first order with respect to both superoxide and enflurane with a rate constant of $0.25 \text{ M}^{-1} \text{ s}^{-1}$ determined by three independent methods: steady-state voltammetry, digital simulation of cyclic voltammetric data and UV/Vis spectroscopic analysis. The likely reaction scheme is outlined below (where R = $\text{CHF}_2\text{CF}_2\text{OCHF}_2$).



or



The electro-reduction of oxygen in the presence of enflurane is found to be dependent on electrode size and enflurane concentration. Using small electrode sizes, the reduction is shown to be a single one-electron process, since the homogeneous reaction kinetics are effectively "out-run". At larger electrode sizes, or with higher concentrations of enflurane, the reduction current is enhanced via the catalytic formation of oxygen, formed from the reaction between superoxide and enflurane.

10.1 INTRODUCTION

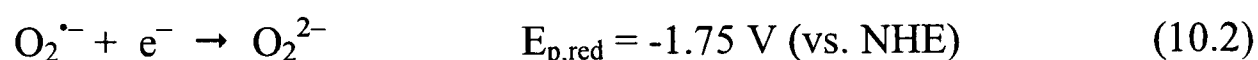
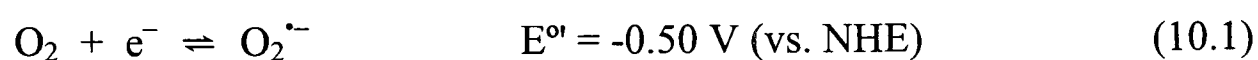
The electrochemical reduction of the inhalation anaesthetic agent enflurane has been reported at Au, Ag and Cu microelectrodes in DMSO solvent and tetraalkylammonium perchlorate supporting electrolytes, with the long-term aim of the production of a rapid, inexpensive electrochemical sensor for the anaesthetic gas [1]. However, during clinical anaesthesia, it is typical to receive doses of enflurane (between 1 to 5 % v/v) in gaseous mixtures of oxygen ranging from 30 to 100 % v/v [2]. Therefore, in order to realistically assess the design of any electrochemical sensor, it is necessary to study the electrode behaviour of enflurane in the presence of oxygen. Moreover, it is desirable to be able to monitor both gases using the same amperometric sensor. The work reported in this paper provides the mechanistic basis for such a protocol.

The separate reduction of enflurane and oxygen gases has been investigated in various solvents, with well-defined voltammetry achieved through use of non-aqueous media, in particular DMSO [1,3]. The advantages in the use of non-aqueous solvents over aqueous-based systems manifest themselves in wider cathodic potential windows, therefore avoiding solvent breakdown as a competing reaction, and the greater solubility of both gases.

The reduction of enflurane was reported in chapter 9 to show catalytic activity at Au and Ag electrodes with half-wave potentials ($E_{1/2}$) of -2.0 and -2.2 V (vs. Ag) respectively [1]. Linear calibration curves were obtained at a 60 μm Au microelectrode between 0 and 2 % v/v (gaseous additions) or up to 135 mM (gravimetric additions) using DMSO solvent and TEAP electrolyte. An empirical correlation between gaseous enflurane (in v/v %) and dissolved solution concentration (in molar) was obtained using gravimetric data.

From these measurements, the Henry's law constant, K_H , for enflurane was calculated to be $0.014 \text{ atm mol}^{-1} \text{ dm}^3$ indicating a high solubility in DMSO.

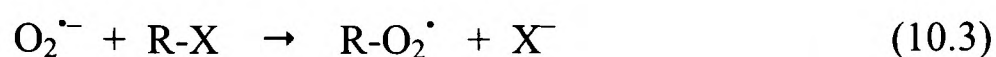
The electrochemical reduction of oxygen to superoxide in aprotic solvents has been studied since 1965 and is currently a method-of-choice for in-situ superoxide generation [4-7]. At Au and Pt electrodes in DMSO solvent, oxygen is reduced in an electrochemically reversible one-electron process, generating the superoxide anion radical [8]. This, in turn, can be reduced by a second successive one-electron process forming the peroxide anion, which may react with the solvent [7,9].



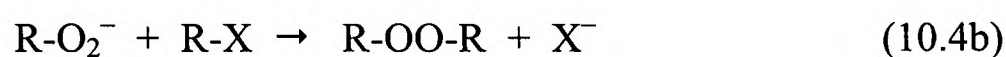
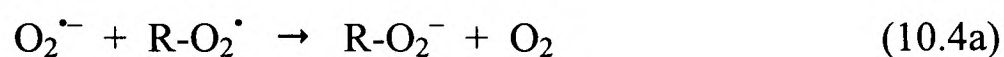
The superoxide radical has been shown to be unstable in aqueous environments where it readily undergoes disproportionation with fast second order rate constants between 10^7 - $10^{10} \text{ M}^{-1} \text{ s}^{-1}$ [10,11]. Superoxide in the form of its potassium and sodium salts is reported to have very low solubility in DMSO and is practically insoluble in all other organic solvents [12,13]. Solubility can be increased through the use of tetraalkyl ammonium salts and these are routinely used as supporting electrolytes in electrochemical protocols. The use of quaternary ammonium salts is reported to not only increase the stability of the electrogenerated superoxide anion, but also its nucleophilicity [12,14]. Wadhawan et al recently reported the electrochemical reduction of dissolved oxygen in DMSO solvent with TEAP supporting electrolyte at Au microelectrodes [3]. Two well-defined signals corresponding to the reduction of oxygen and superoxide were observed with half-wave potentials of -0.4 and -1.6 V (vs. Ag) respectively. After equilibrium with saturated O_2 gas passing over the solvent, a maximum solution concentration of 2.1 mM can be reached [5,15]. In comparison, enflurane has a much greater solubility and solution concentrations of 1.5 M can be reached after equilibration with only 2.0 % v/v gas mixture (in N_2) [1].

The reduction of enflurane in the presence of oxygen in non-aqueous solvents is likely to be complicated by the fact that oxygen is reduced more easily than the enflurane species [1,3]. The chemistry of the superoxide anion radical has been extensively studied for the past 30 years. In addition to the excellent reviews published on the chemistry of the

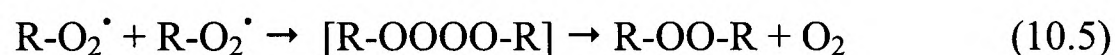
superoxide anion [16,17] there have been a number of publications based on the reactivity of superoxide with a variety of organic halides in aprotic solvents [12-14,18-23]. In particular, superoxide is reported to be a strong nucleophile and to react vigorously with alkyl halides in an S_N2 -type mechanism as shown below



resulting in the loss of the halide ion [12-14,18-23]. The $R-O_2^{\bullet}$ radical has been shown to react further with either additional superoxide radicals, or itself, leading to the formation of the ether, ROOR, and regenerating oxygen [13,14,16,18,20]



or



The electron transfer reaction shown in (10.4a) is reported for 1-bromobutane in DMF to have a rate constant an order of magnitude greater than for the self-reaction radical pathway shown in (10.5) [13,14,19]. Table 10.1 lists the rate constants for the reaction between superoxide in aprotic solvents with a variety of organic halides.

Organic Halide	Solvent	Rate Constant / $M^{-1} s^{-1}$	Reference
1-chlorobutane	DMSO	3.2	[12]
2-chlorobutane	DMSO	0.6	[12]
bromomethane	DMSO	670	[24]
bromoethane	DMSO	350	[24]
1-bromobutane	DMSO	150	[24]
2-bromopropane	DMSO	65	[24]
1-chlorobutane	Pyridine	2.3	[16]
2-chlorobutane	Pyridine	0.5	[16]
2-chloro-2-methylpropane	Pyridine	<0.003	[16]
1-bromobutane	Pyridine	90	[16]
2-bromobutane	Pyridine	54	[16]
1-bromo-2-methylpropane	Pyridine	35	[16]
2-bromo-2-methylpropane	Pyridine	<0.01	[16]
1-chlorobutane	DMF	6.6	[18]
2-chlorobutane	DMF	1.0	[18]
bromoethane	DMF	1350	[18]
1-bromobutane	DMF	950	[18]
2-bromobutane	DMF	174	[18]
1-bromo-2,2-dimethylpropane	DMF	0.045	[18]
benzyl chloride	DMF	440	[18]

benzyl bromide	DMF	14 000	[18]
(1-bromo-2,2-dimethyl propyl) benzene	DMF	0.11	[18]
1-iodoadamantane	DMF	$<10^{-5}$	[18]

Table 10.1 - Kinetic parameters for the reaction of $O_2^{\cdot -}$ with various organic halides in aprotic media

In DMSO solvent, there has also been speculation that the $R-O_2^{\cdot -}$ anion can further react with the solvent to yield the alcohol and dimethyl sulfone [13,16,21,23,25]



This chapter aims to investigate and characterise the reduction of enflurane and oxygen in a clinically-relevant binary gas mixture, after dissolution into DMSO solvent. The reaction between superoxide and enflurane is quantitatively characterised through steady-state voltammetry and digital simulation of cyclic voltammetric data in the light of UV/Vis spectroscopic analysis.

10.2 EXPERIMENTAL

Chemical reagents used throughout the experiments were all obtained in the highest commercially available grades. Tetraethylammonium perchlorate (TEAP) was obtained from Fluka; KCl, $K_4Fe(CN)_6$ and KO_2 were obtained from Aldrich. The enflurane anaesthetic agent was supplied by Abbott Laboratories Ltd. under the commercial name of Ethrane[®]. Solvents used for the electrochemical experiments were dimethyl sulfoxide (DMSO, Spectrosol grade, BDH Chemicals Ltd.) and deionised water with a resistivity of not less than 18 M Ω cm (Elgastat, USF Ltd., Bucks., UK). The DMSO was dried using the method outlined in reference 26.

Solutions of superoxide exhibit a pale yellow colour with λ_{max} reported between 250-300 nm depending on the solvent [16,27-29] and this absorbance has been ascribed to the $2\pi_g \rightarrow 2\pi_u$ electron transition of $O_2^{\cdot -}$ [30-32]. On addition of an alkyl halide, the solution rapidly discolours due to the loss of the superoxide species [27]. The reaction can be monitored by the decay in the absorbance between 250-300 nm offering direct determination of reaction kinetics. The reaction of superoxide with enflurane was therefore followed using UV/Vis spectroscopy. Concentrations of up to 10 mM KO_2

were dissolved into dried solutions of DMSO containing 0.1 M TEAP and reacted with varying amounts (between 20-80 mM) of enflurane added into the same solution. The reaction was monitored at 300 nm absorbance using a Unicam UV2 spectrophotometer (ATI Unicam) and a quartz cell of 1 cm optical path length in agreement with procedures previously outlined elsewhere [27-29].

A computer-controlled potentiostat was used for the electrochemical measurements (PGSTAT 30, Eco Chemie, Netherlands) using a conventional two-electrode arrangement with a silver wire pseudo-reference/counter electrode. The electrochemical cell of conventional design was thermostatted at 25 °C (± 0.1 °C) via a pump-driven water bath. The gold working microdisk electrodes was obtained from Microglass Instruments (Greensborough, Australia) and were nominally of 5, 10, 60, 125 and 500 μm diameters. The microelectrodes were all polished [33] at the start and end of each experiment first using a 1.0 μm alumina-water slurry followed by 0.3 μm alumina (both Buehler, Lake Bluff, IL) on a microelectrode polishing pad (Kemet, Kent, U.K.). The microelectrodes were thoroughly inspected using an optical microscope to check that they had not recessed into the glass surface. Diameters of the working microelectrodes were calibrated electrochemically using 2 mM ferrocyanide in aqueous 0.1 M KCl with a value of the diffusion coefficient (at 25 °C) taken to be $6.9 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ [34]. All solutions were degassed with pure nitrogen gas (BOC) prior to use.

The oxygen and enflurane anaesthetic agent were introduced into solution as gas, bubbled through the solution prior to each electrochemical sweep, using a commercial clinical vaporiser (PPV enflurane vaporiser, Penlon, Ltd.) and introduced into the cell as a v/v % dilution with the N_2/O_2 carrier gas (both BOC). A respiratory gas monitor (Capnomac Ultima™, Datex-Ohmeda, Finland) was used to accurately determine the concentration of both the anaesthetic agent (± 0.1 %) and O_2 (± 1 %) prior to dissolution into the solvent. The gases were bubbled through the solution for a period of no less than 20 minutes to ensure that full equilibration of the gas and solvent was achieved. Cessation of the gas supply during the voltammetric sweeps was important to ensure that measurements were taken in stationary solution, under diffusion-controlled conditions. 'Standard addition' plots were made by incrementing the oxygen levels and monitoring the current response. The potential at which the latter is measured is specified in the relevant section in the text or in the relevant figure legend.

10.3 RESULTS AND DISCUSSION

10.3.1 SIMULTANEOUS ELECTROCHEMICAL REDUCTION OF OXYGEN AND ENFLURANE AT AU MICRODISC ELECTRODES IN DMSO

2.0 % enflurane vapour in N_2 and N_2/O_2 mixtures was equilibrated with a 0.5 M TEAP DMSO solution and its electrochemistry interrogated using linear sweep voltammetry (LSV) at a 60 μm Au microelectrode at potentials between 0 and -3.0 V (vs. Ag). O_2 gas additions, ranging from 0 to 100 % v/v (equivalent to 0 to 2.1 mM solution concentrations) were systematically equilibrated with the DMSO solution with subsequent LSV analysis. Figure 10.1 shows the linear sweep voltammograms (LSVs) of enflurane both in the presence and absence of oxygen together with the LSVs of pure oxygen for comparison.

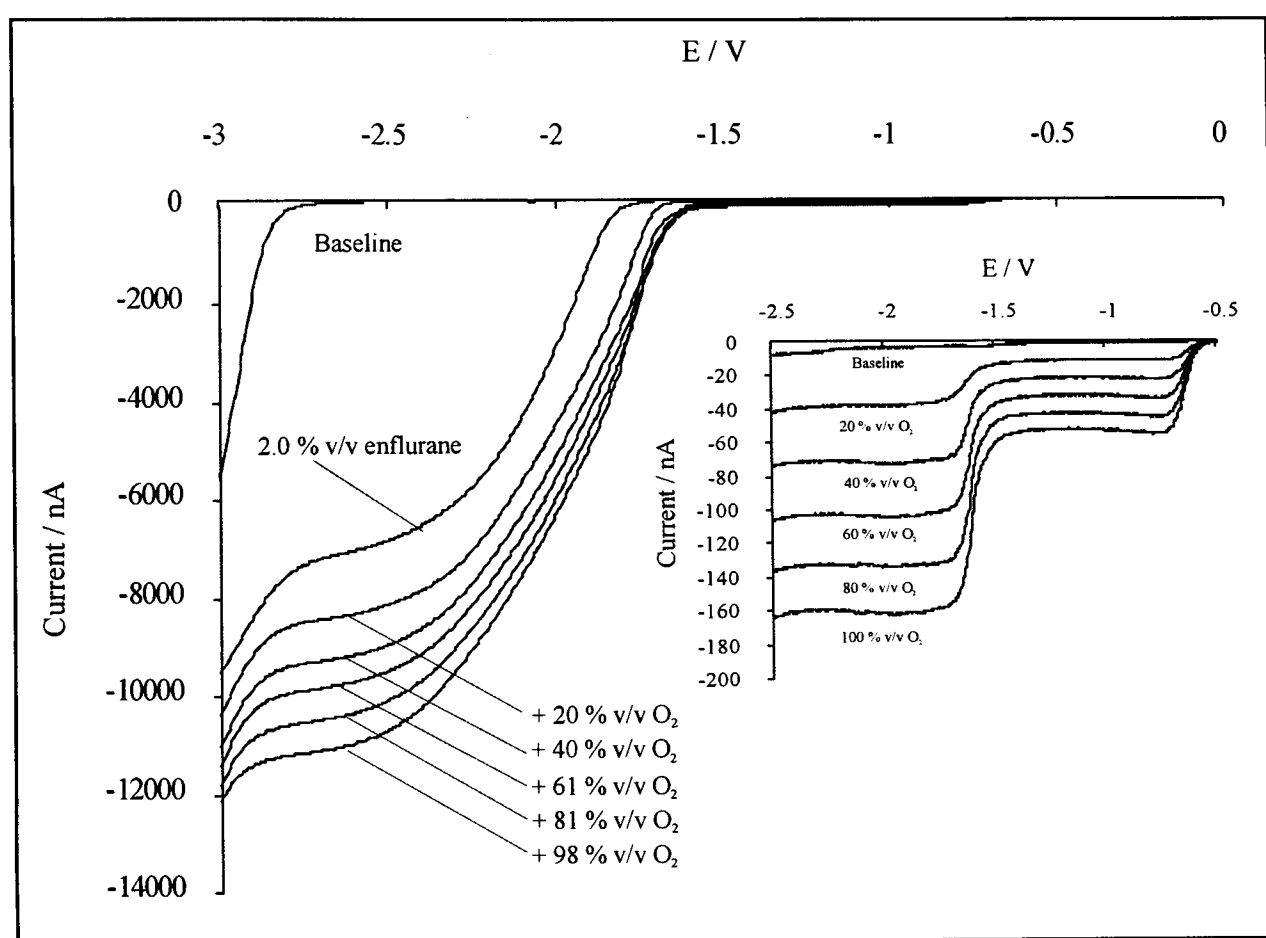


Fig. 10.1 - 2.0 % v/v enflurane dissolved into 0.5 M TEAP in DMSO at a 60 μm diameter Au microelectrode in the presence of 0, 20, 40, 61, 81 and 98 % v/v O_2 . Inset: Comparison of pure O_2 reduction between -0.5 and -2.5 V (vs. Ag).

From figure 10.1, it can be seen that the first reduction wave for oxygen occurs with a half-wave potential of -0.60 V (vs. Ag) in the potential range where enflurane is not

electroactive, whereas in the range between -1.7 and -2.8 V (vs. Ag), there is an overlap between the reductive currents of enflurane and the second reduction of O_2 . Figure 10.2 shows the enhancement of the limiting reduction current of enflurane (ΔI_{LIM}) through standard additions of O_2 and is compared to the standard addition plot for pure O_2 (in the absence of enflurane), both dissolved into 0.5 M TEAP DMSO at a 60 μm Au microelectrode. It can be seen that the current enhancement of the enflurane wave on increasing O_2 concentrations cannot simply be explained by the addition of the two new reductive waves for the O_2 and $O_2^{\cdot-}$ species.

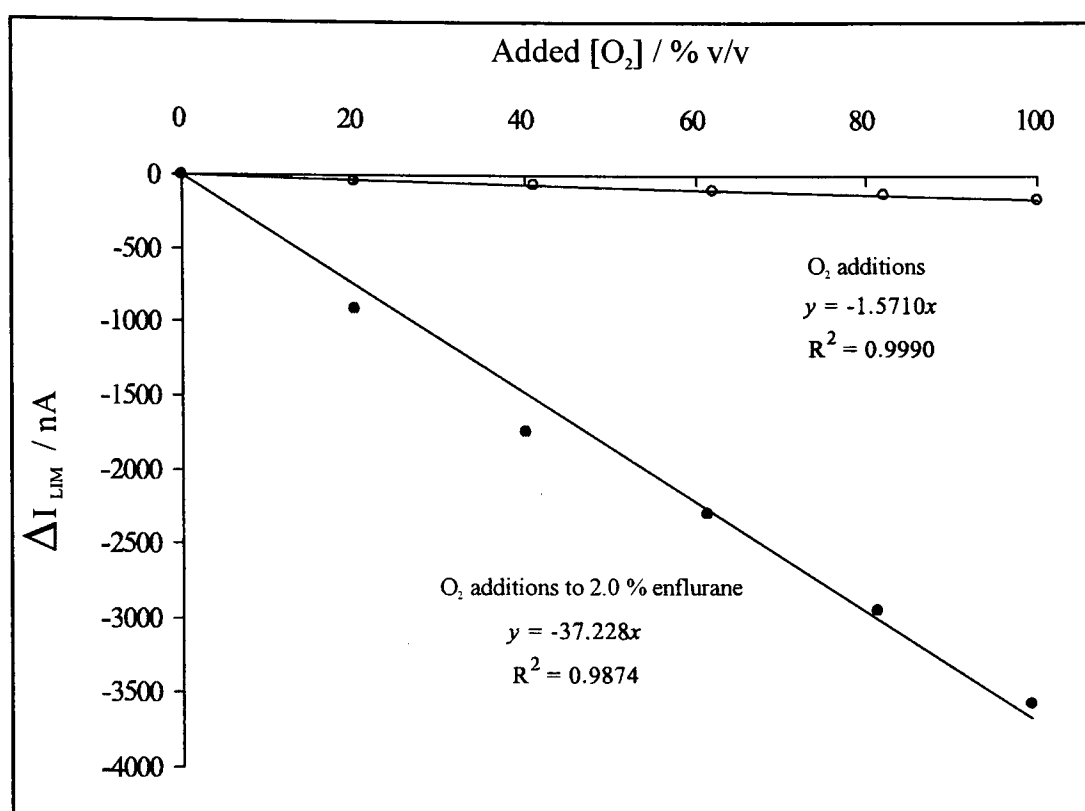


Fig. 10.2 - Enhancement in the reduction current between -1.7 and -2.7 V (vs. Ag) as a function of $[O_2]$ standard additions in the presence (2.0 % v/v) and absence of enflurane.

Both at a 60 μm diameter Au microelectrode in 0.5 M TEAP DMSO.

Figure 10.3a shows the comparison of the first reduction wave of oxygen, in both the presence and absence of enflurane with the corresponding standard addition plots presented in figure 10.3b. It can again be seen that there is an enhancement of these waves in the presence of enflurane.

Gravimetric aliquots of enflurane ranging from 14 to 214 mM were added to a 0.5 M TEAP DMSO solution that had been equilibrated with 100 % O_2 v/v, and the resulting LSVs recorded at a 60 μm Au microelectrode are shown in figure 10.4a. The inset shows the change in the first O_2 reduction current as a function of increasing enflurane concentration. It is observed that at successively higher enflurane concentrations, there is a gradual increase in the reduction current of oxygen. Turning to the reduction of

enflurane, a standard addition plot (after subtraction of the oxygen/superoxide reduction component) is shown in figure 10.4b. In comparison with the plots obtained with enflurane in the absence of oxygen [1], the observed reduction currents for the enflurane wave (after correction for the oxygen/superoxide component) are higher in the presence of oxygen (a gradient of -9.5 nA mM^{-1} vs. -8.2 nA mM^{-1}).

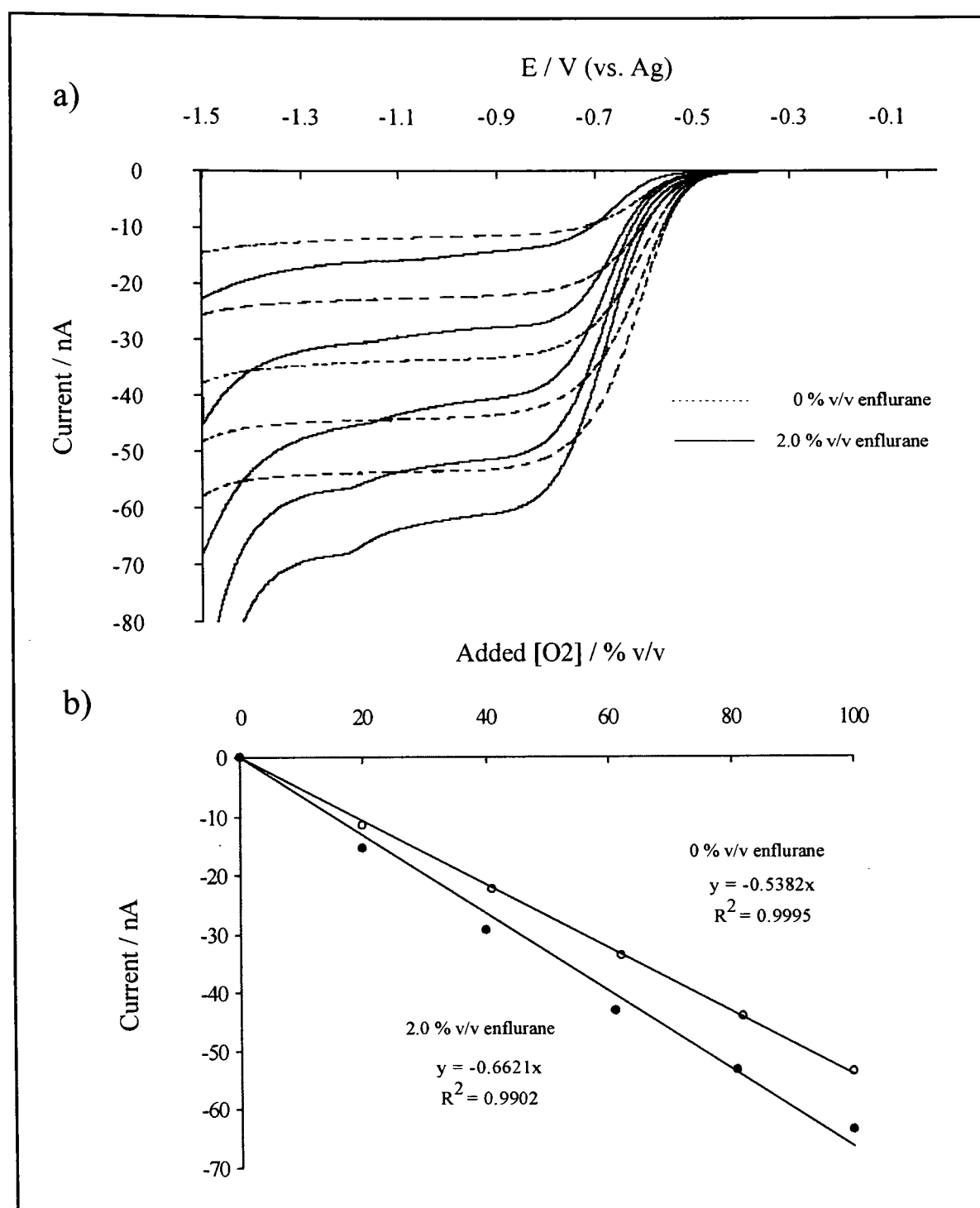


Fig. 10.3 - a) Oxygen reduction wave for [O₂] of 20, 40, 61, 81 and 98 % v/v in 0 % and 2.0 % v/v enflurane at a 60 μm diameter Au microelectrode in TEAP/DMSO and b) corresponding standard addition plots.

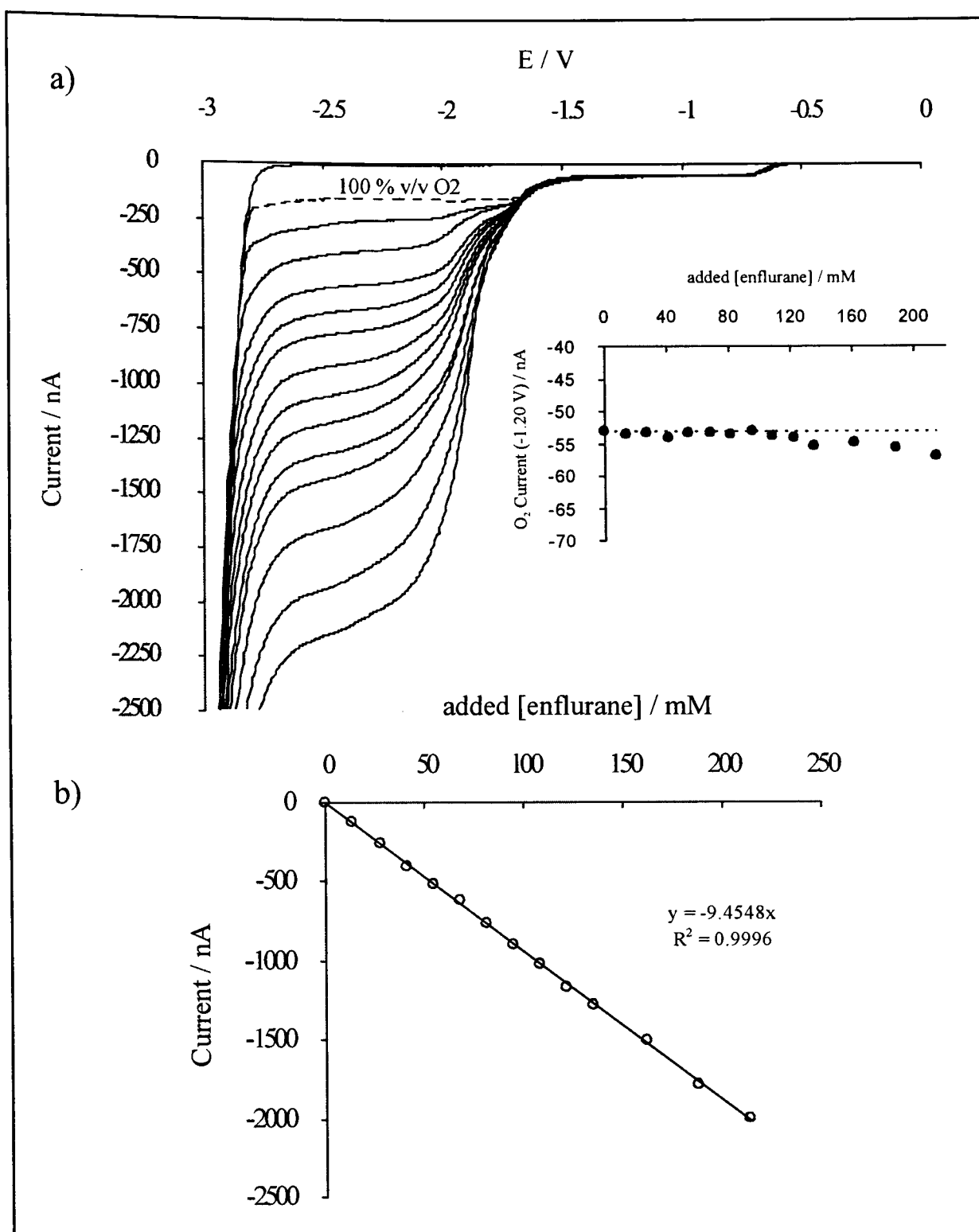


Fig. 10.4 - a) Gravimetric standard additions of enflurane between 13.7 and 214 mM (—) to 100 % v/v O₂ (-----) dissolved in 0.5 M TEAP/DMSO at a 60 μ m Au microelectrode. Inset: Change in first O₂ reduction wave at -1.20 V with increasing [enflurane]. b) Corresponding standard addition plot for enflurane reduction wave between -1.5 and -2.7 V after subtraction of O₂ current. The likely error is ca. ± 1 nA.

Voltammetry of 214 mM enflurane in 0.5 M TEAP DMSO at a 60 μ m Au electrode was investigated in both the presence and absence of 100 % O₂. The results, shown in figure 10.5, offer further evidence that the enhancement of the enflurane wave in the presence of 100% O₂ cannot simply be attributed to the addition of the O₂ / O₂^{•-} reduction waves. Analysis of the limiting current obtained from the reduction of 214 mM enflurane in the

absence of oxygen at -2.50 V (on the limiting current, before solvent breakdown), using the electrochemically-measured diameter (62.12 μm) and the equation below [35]

$$I = 4 n D F [B] r \quad (10.7)$$

where n is the number of electrons transferred, D is the diffusion coefficient, F is the Faraday constant, $[B]$ is the concentration of enflurane and r is the electrode radius, yields a diffusion coefficient equal to $6.5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ (assuming $n = 1$) which is in good agreement with the value of $6.7 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ previously reported [1].

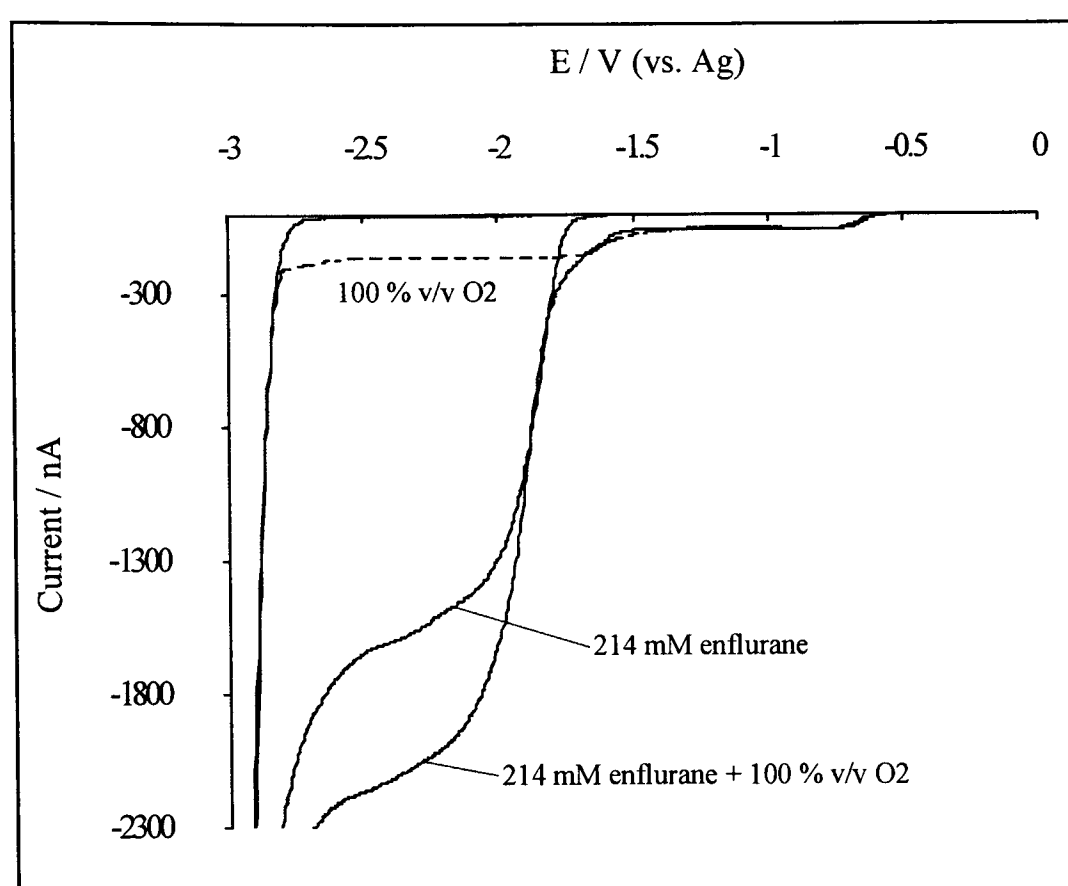
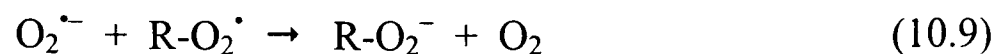


Fig. 10.5 - Reduction of 214 mM enflurane in the presence of 0 and 100 % v/v O_2 (—) vs. reduction of 100 % v/v O_2 only (-----) in 0.5 M TEAP / DMSO at a 60 μm Au microelectrode.

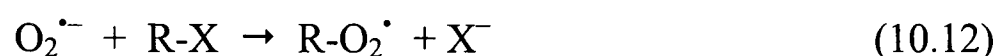
All the observations presented above suggest a reaction between both the superoxide and peroxide anions, electro-generated in-situ from oxygen, and enflurane. The enhancement of the first O_2 reduction wave (between -0.5 and -1.5 V), seen in the voltammetry (fig. 10.3 and 10.4), is not inconsistent with equations (10.3) and (10.4a) whereby oxygen is formed catalytically as an end result of the reaction between $\text{O}_2^{\bullet-}$ and R-X. We therefore speculate that in our case, we observe the following reaction (where $\text{R} = \text{CHF CF}_2\text{OCHF}_2$)



with the loss of a chloride ion from the enflurane. Alternatively, the increased currents observed at the first oxygen reduction wave could be explained via the further reduction of the $\text{R-O}_2^{\bullet}$ moiety at the electrode surface, generated through equations (10.3) and (10.8).



This suggestion reflects reports suggesting the existence of a possible ECE-type pathway, shown below, when oxygen is reduced in the presence of alkyl halides [13,14].



In order to verify or otherwise assess the proposed mechanisms, a number of experiments are next presented in an attempt to further support the reaction between enflurane and superoxide. These experiments aim not only to offer further evidence of the above mechanisms but to also present supplementary kinetic information.

10.3.2 DETERMINATION OF THE RATE CONSTANT FOR THE REACTION OF SUPEROXIDE WITH ENFLURANE IN DMSO: UV/VIS SPECTROSCOPIC STUDY

Absorbance spectra were obtained between 200-400 nm for concentrations between 2 and 13 mM of KO_2 in 0.1 M TEAP/DMSO. Consistent with the Beer-Lambert expression,

$$\text{Absorbance} = \epsilon [\text{O}_2^{\bullet -}] l \quad (10.14)$$

where ϵ is the molar absorption coefficient (or extinction coefficient) and l is the optical path length, linear plots of absorbance ($\lambda = 300$ nm) vs. $[\text{O}_2^{\bullet -}]$ were obtained (fig. 10.6).

An extinction coefficient of $306 \text{ M}^{-1} \text{ cm}^{-1}$ was obtained and this compares to values of 1483 and $2686 \text{ M}^{-1} \text{ cm}^{-1}$ obtained in acetonitrile and DMSO-18-crown-6 solvents respectively [27,29]: the differences reflecting contrasting solvation. A deviation from the above relationship occurs when using concentrations greater than ca. 10 mM and on inspection a precipitate is observed indicating that the maximum solubility of KO_2 in TEAP/DMSO has been attained.

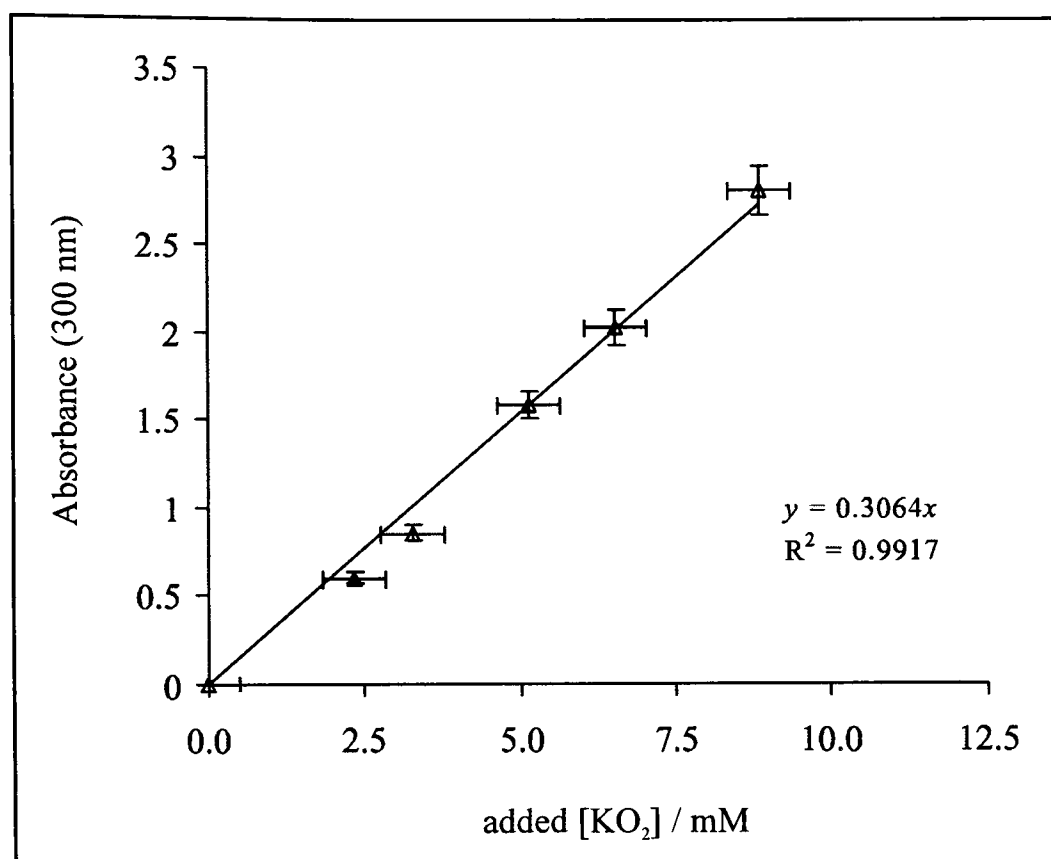


Fig. 10.6 - Beer-Lambert plot for KO_2 dissolved in 0.1 M TEAP in DMSO recorded at 300 nm wavelength.

Solutions of ca. 8 mM KO_2 were prepared with varying amounts of enflurane added in 2:1, 5:1 and 10:1 stoichiometric ratios (enflurane : KO_2). Upon mixing with enflurane, the solution rapidly discolours with effervescence, consistent with the release of oxygen shown in equations (10.4a and 10.9). This is in agreement with observations reported using similar alkyl halides, such as 1-bromobutane [19,27]. Figure 10.7 shows a plot of the decay of $\text{O}_2^{\bullet-}$ as a function of time for a solution containing 19.1 mM enflurane dissolved in 8.42 mM KO_2 (both in 0.1 M TEAP DMSO). The data was analysed assuming 2nd order kinetics, using equation (10.15) [36]

$$\ln \frac{[B]/[B]_0}{[A]/[A]_0} = kt([B]_0 - [A]_0) \quad (10.15)$$

where A and B represent $\text{O}_2^{\bullet-}$ and enflurane respectively and k is the 2nd order rate constant and has the units $\text{M}^{-1} \text{ s}^{-1}$. The rate constant for the reaction of $\text{O}_2^{\bullet-}$ with

enflurane as shown in equation (10.8), can be derived based on the assumption that 2 molecules of superoxide react with 1 molecule of enflurane as shown in equations (10.8 and 10.9) with equation (10.8) as the rate-determining step. Figure 10.8 shows a typical plot of $\ln ([B]/[B]_0)/([A]/[A]_0)$ against $t([B]_0 - [A]_0)$ for times up to 330 seconds, such that the linear dependence seen is consistent with equation (10.15). Analysis of figure 10.8 yields a rate constant equal to $0.25 \pm 0.03 \text{ M}^{-1} \text{ s}^{-1}$ (at 25°C). Similar results were obtained for 5:1 and 10:1 stoichiometric ratios (enflurane: KO_2).

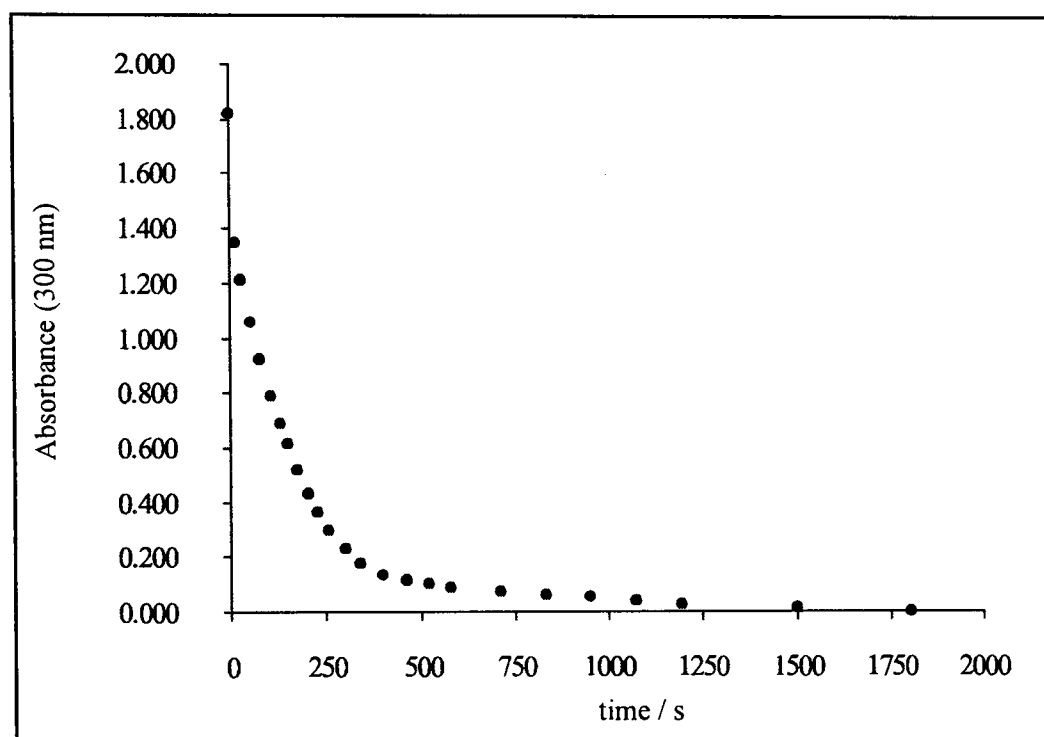


Fig. 10.7 - Decay in the absorbance of 8.42 mM O_2^- at 300 nm as a function of time after the addition of 19.1 mM enflurane in 0.1 M TEAP DMSO (data corrected for background DMSO absorbance).

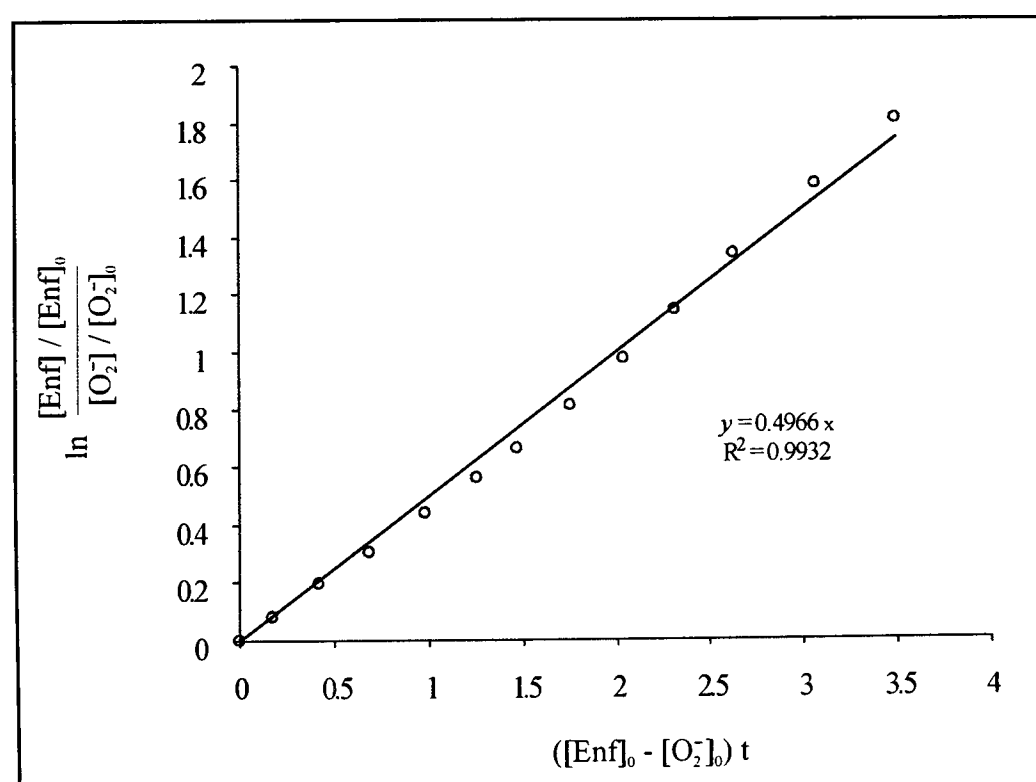
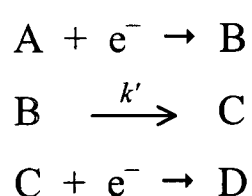


Fig. 10.8 - 2nd order plot for the determination of the rate constant for the reaction between 19.1 mM enflurane and 8.42 mM O_2^- dissolved in 0.1 M TEAP DMSO.

10.3.3 APPLICATION OF THE RATE CONSTANT FOR THE REACTION OF SUPEROXIDE WITH ENFLURANE IN DMSO: ANALYSIS OF STEADY-STATE VOLTAMMETRY AT GOLD MICROELECTRODES

Alden [37] has previously described the solutions of the mass-transport equations at microdisk electrodes under steady-state, diffusion-only conditions under the assumption of equal diffusion coefficients. He used ILU preconditioned Krylov subspace methods in conjunction with the Amatore and Fosset method for the conformal mapping of the microdisk electrode geometry [38]. The numerical simulations were used to generate working curves for various mechanisms at microdisk electrodes permitting quantitative mechanistic analysis. These working curves represent how the effective number of electrons transferred (N_{Eff}) during the reaction varies as a function of a dimensionless, mechanism-dependent rate constant, K . In the case for the ECE-type mechanism shown below, with k' rate determining



the following relationship applies

$$K_{ECE} = \frac{k' r_e^2}{D} \quad (10.16)$$

where k' is the first-order rate constant, r_e is the radius of the electrode and D is the diffusion coefficient for O_2 .

Linear sweep voltammograms were performed at gold microelectrodes of sizes 5 and 60 μm for the reduction of varying amounts of both oxygen and enflurane in 0.1 M TEAP/DMSO solutions at scans rates of 5 mV s^{-1} to ensure steady-state conditions. Figure 10.9a and 10.9b shows the first reduction wave of oxygen in the presence and absence of 0.5 % enflurane at 5 and 60 μm diameter Au microelectrodes. The effect of the enflurane concentration on the reductive current of first oxygen wave was investigated and the experimental data was quantitatively analysed through determining

an effective number of electrons transferred. This was deduced by the ratio of the oxygen current, recorded at -1.20 V corresponding to transport limited conditions, in the presence ($i_{Lim}^{O_2+enf}$) and absence ($i_{Lim}^{O_2}$) of enflurane, as indicated below.

$$N_{Eff} = \frac{i_{Lim}^{O_2+enf}}{i_{Lim}^{O_2}} \quad (10.17)$$

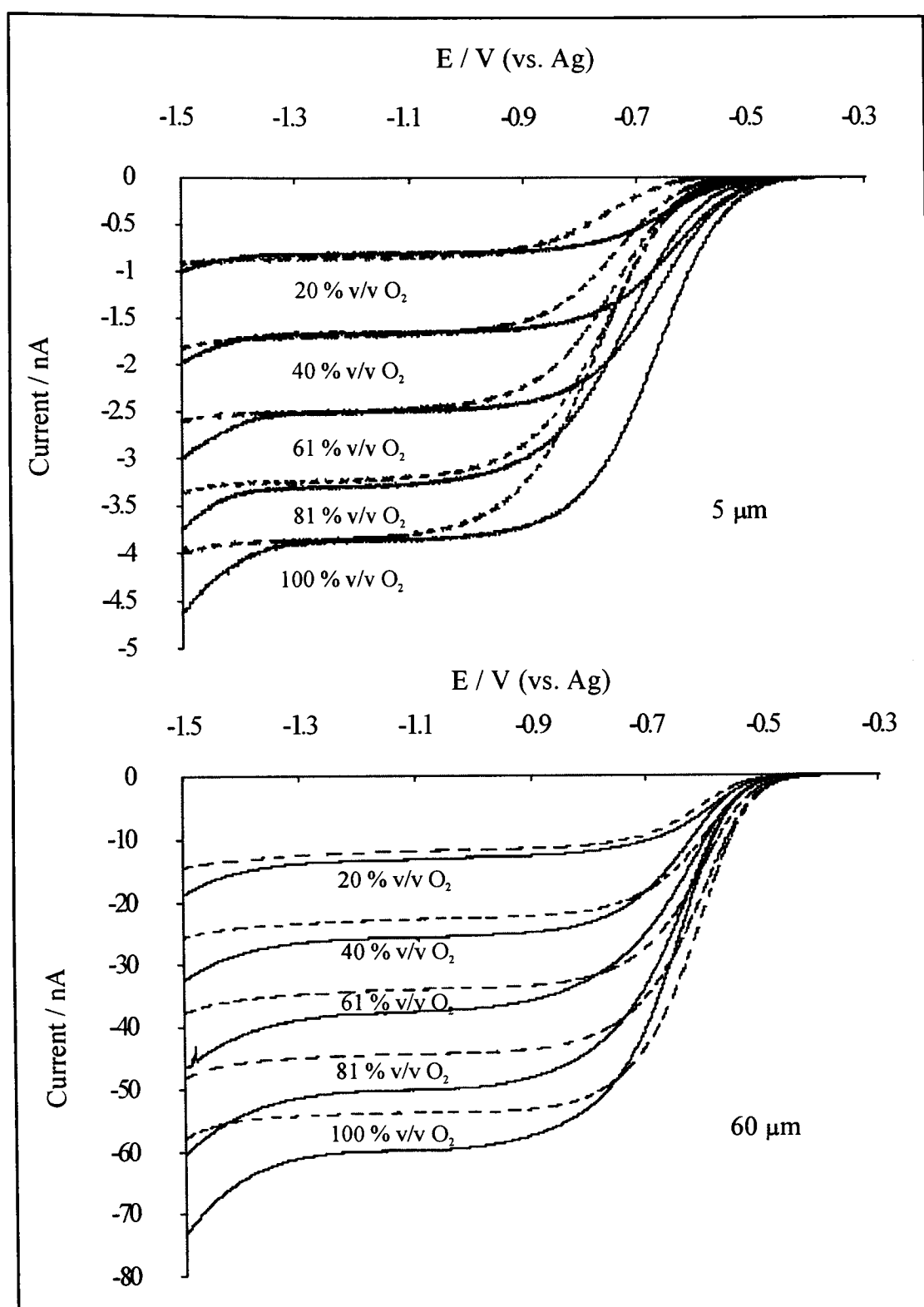


Fig. 10.9 - LSVs of first O_2 wave for 20, 40, 61, 81 and 100 % v/v oxygen recorded in the presence of 0 % (-----) and 0.5 % v/v enflurane (—) at a) 5 μm and b) 60 μm diameter Au microelectrodes. Both experiments conducted in 0.1 M TEAP DMSO and at scan rates of 5 mV s⁻¹.

Figure 10.10 shows a plot of the experimentally obtained N_{Eff} as a function of $[O_2]$ and [enflurane] together with N_{Eff} values obtained through use of the working curves via

numerical simulation at both 5 and 60 μm . The latter were obtained using the following parameters; D_{O_2} was set equal to $2.3 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (derived from experiments conducted in 0.1 M TEAP DMSO and in close agreement with reference 3), calibrated electrode sizes of 62.1 or 4.2 μm diameter and the pseudo first-order rate constant derived using the following expression

$$k' = k [\text{enflurane}] \quad (10.18)$$

where k is the second-order rate constant measured previously as $0.25 \text{ M}^{-1} \text{ s}^{-1}$.

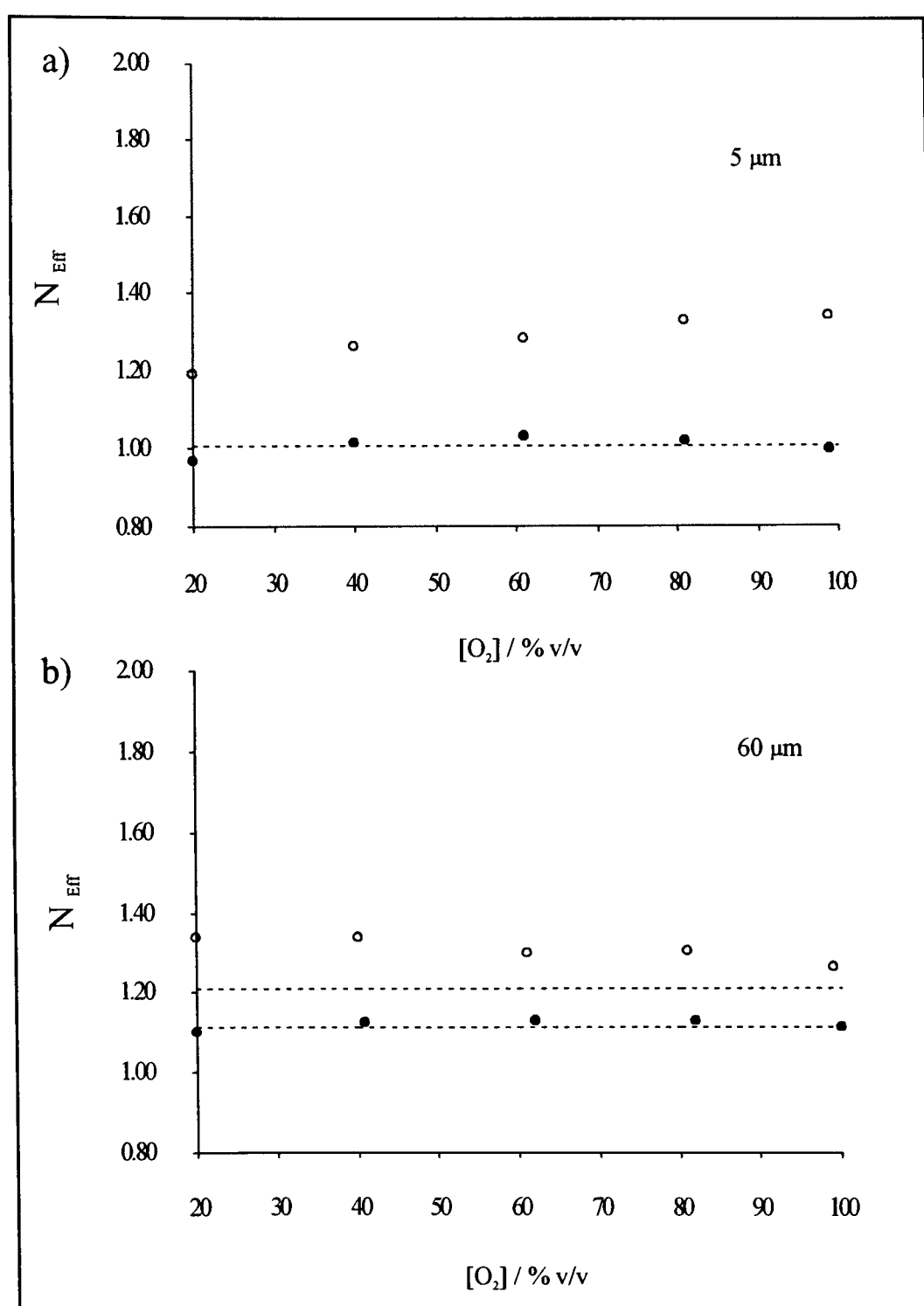


Fig. 10.10 - Plots of experimentally obtained N_{Eff} vs. $[\text{O}_2]$ at 0.5 % (●) and 2.0 % v/v (○) enflurane vs. numerical simulation (-----) at a) 5 μm and b) 60 μm diameter Au microelectrode in 0.1 M TEAP DMSO. The simulated lines for the two enflurane concentrations at 5 μm superimpose.

At low concentrations of enflurane (0.5 % v/v), there is a good agreement between the numerical simulation and the experimentally derived data at both 5 and 60 μm microelectrodes. It can be shown by using smaller microelectrode sizes (5 μm) an $N_{\text{Eff}} \approx 1$ can be achieved, indicating that the coupled homogeneous kinetic step of the ECE-process has been essentially "out-run" by the fast mass transport and thus there is transportational removal of the $\text{O}_2^{\bullet-}$ species into bulk before it can react with the enflurane. This effectively collapses the chemistry to a single one-electron process as shown in equation (10.11). Using larger microelectrodes (60 μm), the rate at which the $\text{O}_2^{\bullet-}$ species is lost into bulk solution is proportionally less, and therefore extra current is observed through the pathways highlighted by equations (10.12) and (10.13).

This use of a 5 μm electrode to 'out-run' the reaction kinetics is specific towards the superoxide / enflurane reaction. Note that in the potential region between -1.7 and -2.8 V where peroxide is formed, current enhancement is still observed, suggesting that there is a reaction between the enflurane and the electrogenerated peroxide anion (O_2^{2-}), which is faster in comparison to the superoxide.

At higher concentrations of enflurane (2.0 % v/v), however, the agreement with the ECE microdisk simulation is noticeably less consistent. In this situation, the excess of enflurane (ca. 700 fold) could have the effect of driving the formation of products in equations (10.8) and (10.9), resulting in the regeneration of some oxygen. This, in effect, generates a complementary pathway. This reaction is addressed in the next section.

10.3.4 DETERMINATION OF THE RATE CONSTANT FOR THE REACTION OF SUPEROXIDE WITH ENFLURANE IN DMSO: DIGITAL SIMULATION OF CYCLIC VOLTAMMETRY

Cyclic voltammograms at varying scan rates were recorded at a 125 μm Au electrode with 100 % v/v oxygen both in the presence and absence of 2.0 % v/v enflurane, dissolved into 0.5 M TEAP DMSO. Figure 10.11 shows the corresponding voltammograms for scan rates of 10 and 50 mV s^{-1} . In the absence of enflurane, two peaks are observed with the forward peak corresponding to the one-electron reduction of oxygen and the reverse peak corresponding to the reoxidation of superoxide. The

difference in peak heights is attributable to the differences in the diffusion coefficients of oxygen and superoxide in DMSO (2.16 and $1.08 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ respectively [3,5]) as inferred from DIGISIM[®] modelling of the voltammetry using the approximation of a hemispherical diffusion regime in which $r_e^{\text{hemisphere}} = (2 / \pi) r_e^{\text{disc}}$ [37]. In the presence of enflurane, an enhancement of the oxygen reduction wave is observed with a concomitant decrease in the reverse peak for the reoxidation of superoxide. Furthermore at scan rates of 10 mV s^{-1} the reverse peak has completely disappeared.

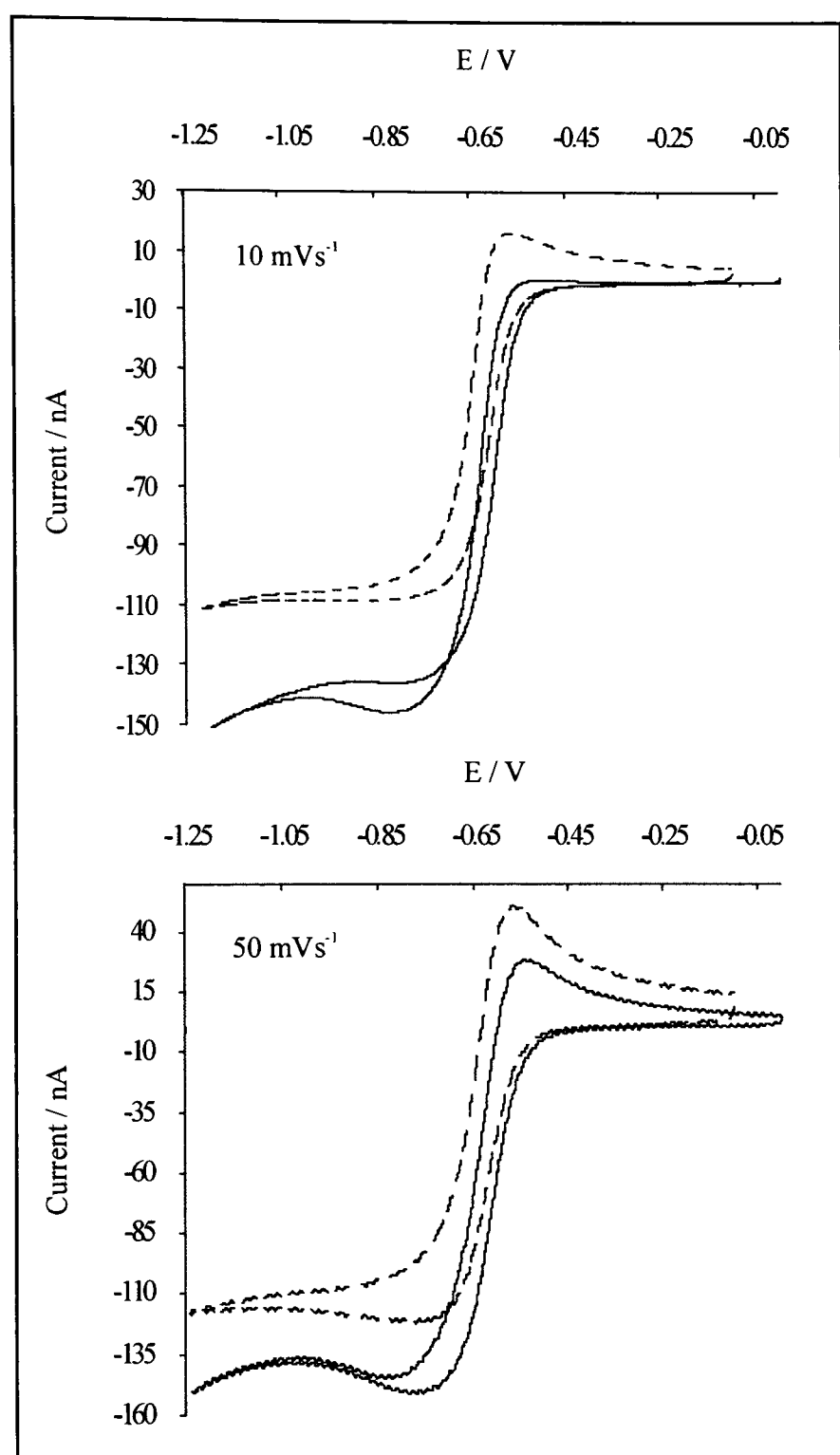
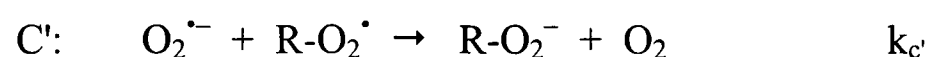
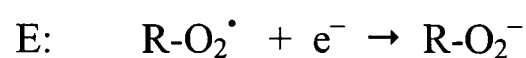


Fig. 10.11 - Cyclic voltammograms at 10 and 50 mV s^{-1} performed in $100\% \text{ v/v O}_2$ with 0% (-----) and $2.0\% \text{ v/v}$ (—) enflurane at a $125 \mu\text{m}$ diameter Au microelectrode in 0.5 M TEAP DMSO .

These observations can be interpreted by the reaction of superoxide with enflurane, involving additional electron transfer processes as discussed below. The disappearance

of the peak due to the reoxidation of superoxide is evident at low scan rates due to "titration" of the electrogenerated superoxide with enflurane. At faster scan rates ($> 50 \text{ mV s}^{-1}$) as the time scale of the experiment is shorter, there is a reduced reaction between the superoxide and enflurane and a reverse peak is observed. At these scan rates a reaction is still observed indicated by the enhancement of the oxygen peak in the presence of enflurane.

DIGISIM[®] 2.0, under the approximation of hemispherical geometry, was used to simulate the cyclic voltammetric data at a $125 \text{ }\mu\text{m}$ microelectrode using diffusion coefficients for oxygen and superoxide of 2.16 and $1.08 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ respectively [3,5], a solubility of 2.1 mM for saturated oxygen in DMSO [5,15] and employing a mixed ECE/DISP reaction scheme (consistent with equations 10.1, 10.8-10.10) as follows



The parameters describing this scheme are k_o , the heterogeneous rate constant for the $\text{O}_2/\text{O}_2^{\bullet -}$ couple and k_c and $k_{c'}$ the second order rate constants for the respective homogeneous kinetic steps. k_c is known from the results reported above and equal to $0.25 \text{ M}^{-1} \text{ s}^{-1}$. The experimental and simulation data for the one-electron reduction of oxygen in the absence of enflurane (k_c and $k_{c'} = 0$) is presented in figure 10.12. A value of k_o equal to 0.015 cm s^{-1} was adopted, which compares with values of 0.032 and 0.028 cm s^{-1} reported at glassy carbon and graphite electrodes, also in DMSO [27].

Figure 10.12 also shows the good agreement between experimental and simulated data for oxygen in the presence of enflurane when using values for k_c and $k_{c'}$ of 0.25 ± 0.03 and $100 \pm 20 \text{ M}^{-1} \text{ s}^{-1}$ respectively. The latter is consistent with rate constants obtained in similar radical reactions [13,14,19].

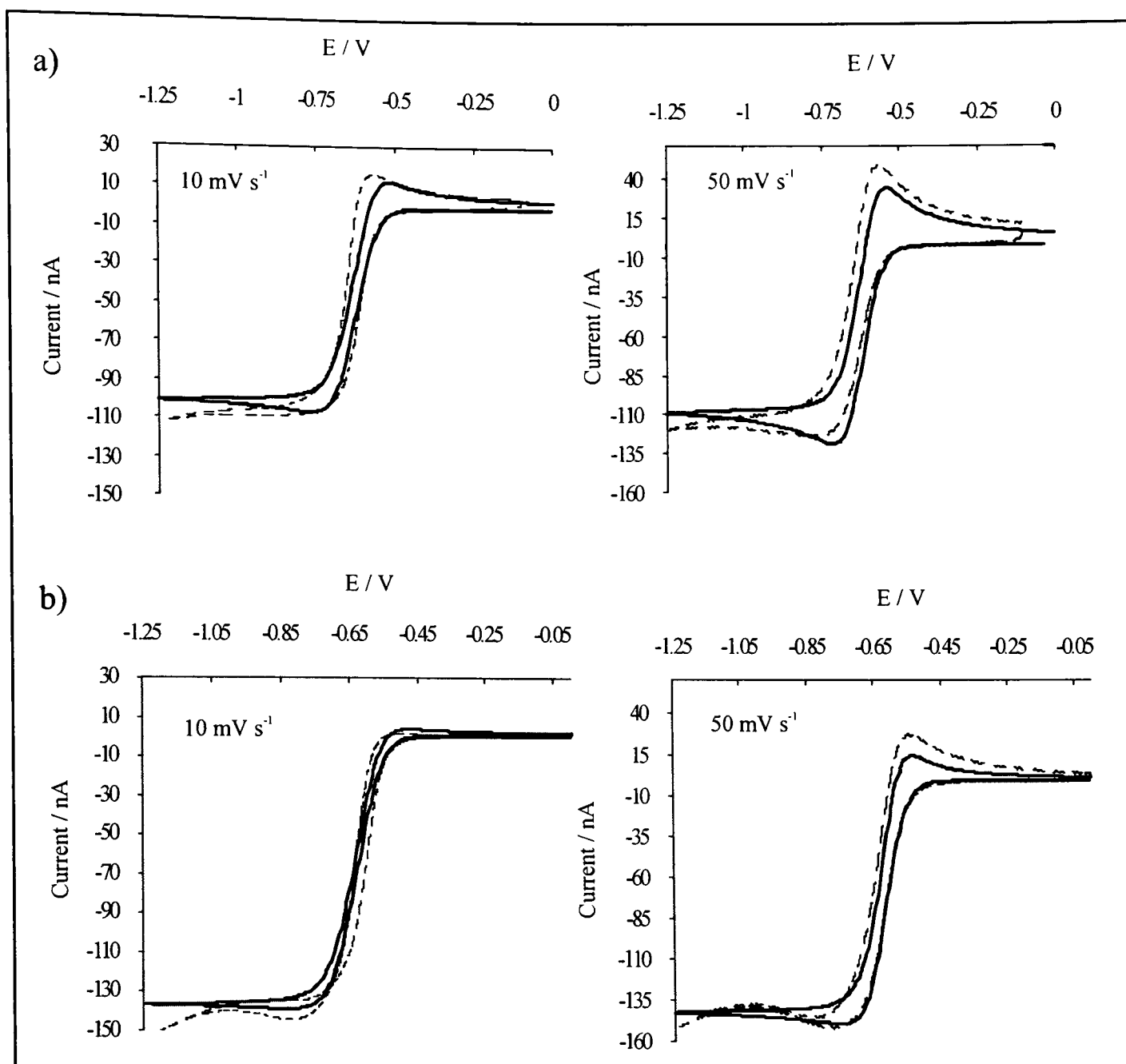


Fig. 10.12 - Comparison of DIGISIM[®] simulation (—) vs. experimental (-----) of 100 % v/v O₂ using parameters of $k_c = 0.25 \text{ M}^{-1} \text{ s}^{-1}$, $k_c' = 100 \text{ M}^{-1} \text{ s}^{-1}$ and $k_o = 0.015 \text{ cm s}^{-1}$ in a) the absence of enflurane (hence k_c and $k_c' = 0$) and b) the presence of 2.0 % v/v enflurane.

10.3.5 QUANTITATIVE SENSING STRATEGY FOR BINARY MIXTURES OF ENFLURANE AND OXYGEN

In light of the work included in this chapter, a sensing methodology can be proposed that offers a procedure for the individual analysis of enflurane and oxygen gases in the presence of each other. The method is based on the simultaneous use of "small" and "large" gold microelectrode diameters in the same electrochemical cell, whereby the potential of both electrodes are held at a fixed value (between -1 and -1.5 V) corresponding to the first reduction of oxygen to superoxide. At the smaller electrode diameter ($< 5 \text{ } \mu\text{m}$), the reaction kinetics between the electrogenerated superoxide and

enflurane are shown to be 'out-run', therefore removing any interference with the oxygen wave. The resulting currents are therefore a direct measurement of the oxygen concentration in the solution and can be quantitatively analysed using the relationship shown in equation (10.7).

At the larger electrode, the observed oxygen reduction currents are shown to be complicated through the reaction of superoxide with the enflurane. A ratio of the currents obtained at the two microelectrodes (when factored for electrode size) gives rise to N_{Eff} values for different concentrations of enflurane. At low concentrations of enflurane, the N_{Eff} values obtained are shown to be in good agreement with numerical simulation (fig. 10.10) and through the use of published ECE working curves [37] and knowledge of the homogeneous rate constant (presented herein), enflurane concentrations can be readily obtained. In practice a range of "larger" sized electrodes might be used within an array to ensure the 'ECE' limit was achieved.

This dual or multiple microelectrode arrangement has the possibility of eliminating current interference arising from the reactivity of the superoxide radical anion with the enflurane molecule. It also avoids the need to monitor the enflurane wave directly at more negative potentials, where the system is complicated by the 2nd reduction wave of oxygen and formation of the peroxide anion species.

10.3.6 ELECTROCHEMICAL REDUCTION OF NITROUS OXIDE IN THE PRESENCE OF OXYGEN

Further to the studies of enflurane/oxygen binary mixtures, the electrochemistry of the anaesthetic gas, N_2O , was also investigated in the presence of oxygen at Au microelectrodes. Contemporary clinical anaesthesia typically employs the use of tertiary mixtures of the gases enflurane, N_2O and O_2 [2] and therefore the investigation of the electrochemical behaviour of both N_2O and O_2 and the reactivity of the superoxide anion in a binary mixture is of paramount clinical interest.

The reduction of N_2O at various electrode substrates has been investigated in aqueous solutions for the past three decades [39-45] and has been performed in the presence of O_2 without interference [46]. In aprotic media, however, the reduction of N_2O has received

little attention and there have only been a few qualitative reports indicating that N_2O and O_2 can be reduced in the presence of one another at Au microelectrodes in DMSO solvent [47-49]. These reports presented standard addition plots that were linear for both N_2O and O_2 additions in binary mixtures, stating half-wave potentials between -1.7 and -2.0 V (vs. Ag) for N_2O . In aqueous solution, the diffusion coefficient for N_2O is reported to be $7.7 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ [50].

To study the possible reactivity of electrogenerated superoxide with N_2O , 31 % v/v O_2 was dissolved into a 0.5 M TEAP/DMSO solution and interrogated using LSV following subsequent additions of between 10 and 66 % v/v N_2O at a 60 μm Au microelectrode, as shown in figure 10.13(a). The current response of the first O_2 reduction wave was also monitored as a function of added $[\text{N}_2\text{O}]$ (Inset: figure 10.13a) and the corresponding standard addition plot for the reduction of N_2O in the absence and presence of O_2 is shown in figure 10.13(b). The reduction of 10 % v/v N_2O in the presence and absence of 31 % v/v O_2 is further illustrated in figure 10.14 and is shown in comparison with 31 % v/v O_2 only. It can be seen that the reduction of N_2O ($E_{1/2} = -2.0 \text{ V}$) occurs more negative than the first O_2 reduction potential and overlaps with the second reduction wave. The current from the 1st reduction wave of oxygen shows no interference on additions of N_2O and furthermore, the standard addition plots of N_2O in the absence and presence of O_2 are identical. The LSVs presented in figure 10.14 suggest that the current enhancement observed in the N_2O wave in the presence of O_2 , is simply due to the sum of the oxygen and N_2O reduction waves.

The results presented above suggest no reaction occurs between electrogenerated superoxide and nitrous oxide, when both are present in solution at clinically relevant concentrations. These results offer further support to the design of an amperometric sensor that is compatible in the presence of tertiary gas mixtures of enflurane, nitrous oxide and oxygen.

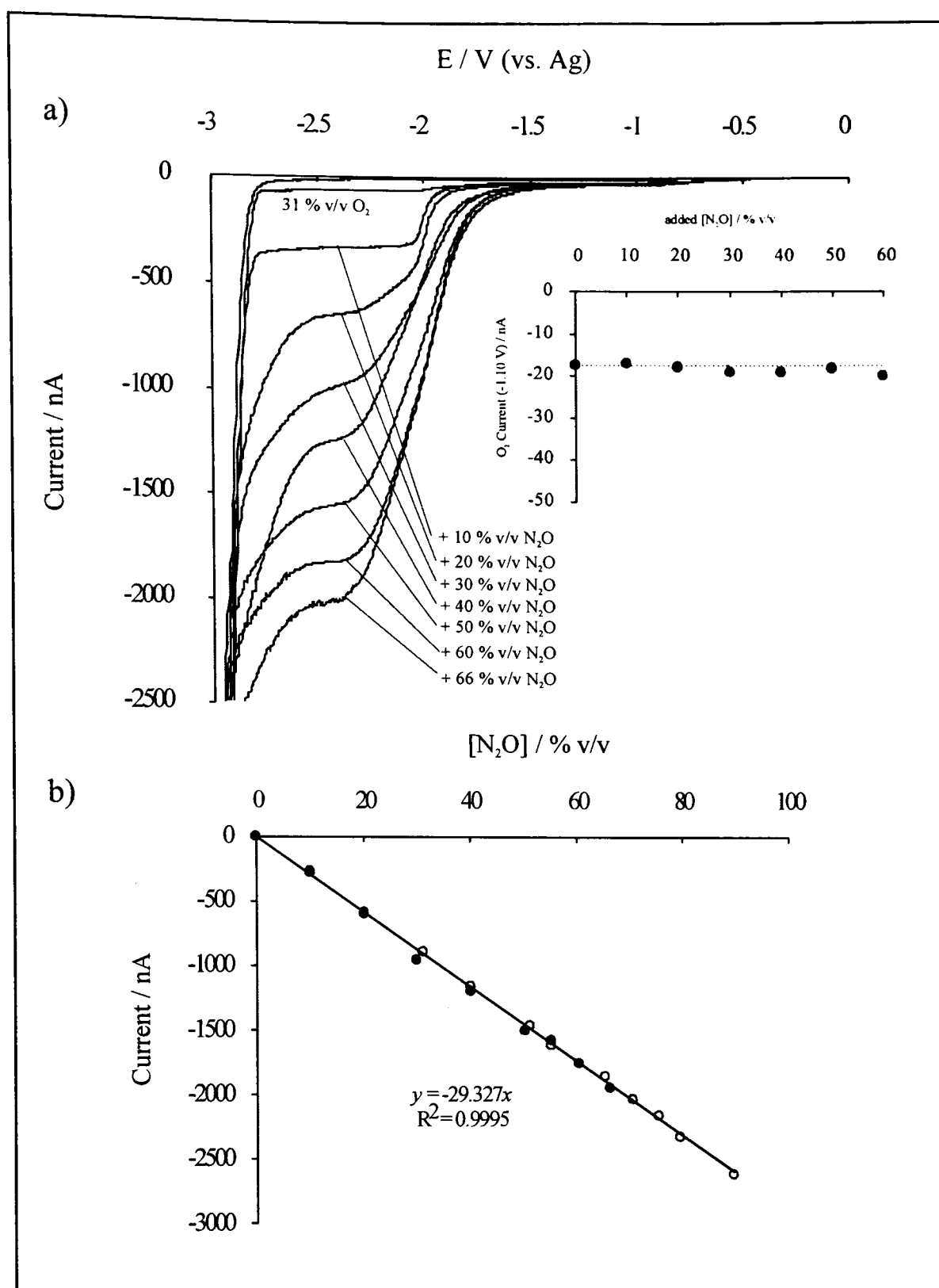


Fig. 10.13 - a) Reduction of 31 % v/v oxygen in the presence of increasing $[\text{N}_2\text{O}]$ between 10 and 66 % v/v performed at a 60 μm diameter Au microelectrode in 0.5 M TEAP/DMSO. Inset: Change in the first reduction wave of oxygen as a function of $[\text{N}_2\text{O}]$. b) Corresponding standard addition plots for N_2O in the absence ($\circ$) and presence ($\bullet$) of 31 % v/v oxygen (after subtraction for O_2 current).

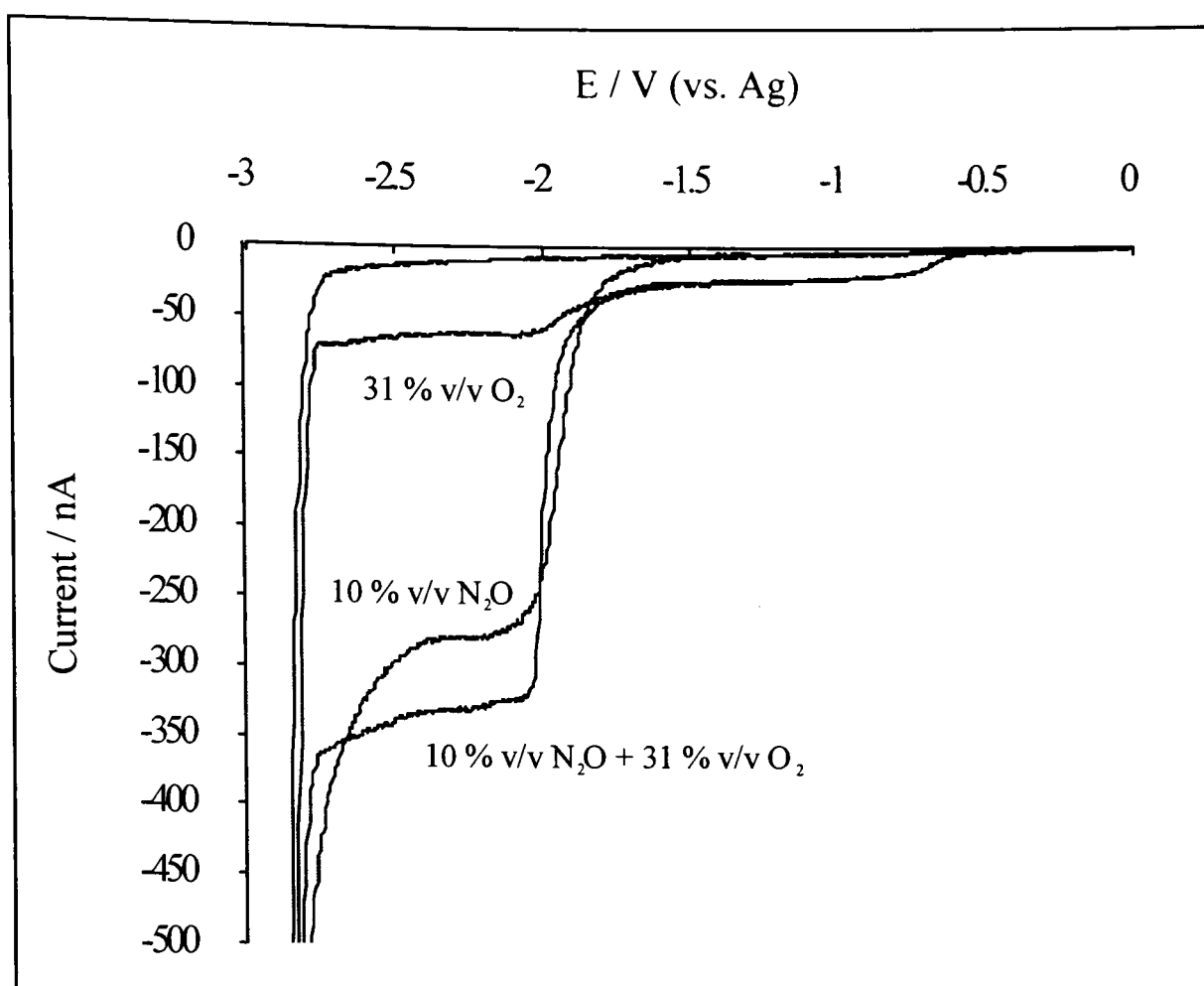


Fig. 10.14 - Reduction of 10 % v/v N_2O in the presence and absence of oxygen vs. the reduction of 31 % v/v O_2 only at a 60 μm diameter Au microelectrode in 0.5 M TEAP/ DMSO. The slight changes in voltammetric shape may reflect adsorption phenomena.

10.4 CONCLUSIONS

In this chapter the electrochemical reduction of enflurane in the presence of oxygen at Au microelectrodes in DMSO solvent, in a clinically-relevant binary gas mixture is reported. Electrogenated superoxide, formed from the reduction of oxygen, is shown to react with the enflurane consistent with an $\text{S}_{\text{N}}2$ -type mechanism resulting in the loss of Cl^- . A second order rate constant of $0.25 \text{ M}^{-1} \text{ s}^{-1}$ has been established using steady-state voltammetry, digital simulation of cyclic voltammetric data and UV/Vis spectroscopic analysis. At low concentrations of enflurane, the reaction is shown to be in good agreement with an ECE-type mechanism, the kinetics of which can be effectively 'out-run' and collapsed to a single one-electron process using microelectrodes of 5 μm diameter and below. At higher concentrations of enflurane, the reaction is shown to be complicated by further reaction processes consistent with the catalytic formation of oxygen. This supplements previous work done in this area and offers further scope for the production of an electrochemical sensor for the anaesthetic gas enflurane that is compatible with oxygen and nitrous oxide in binary and tertiary mixtures.

10.5 CHAPTER 10 REFERENCES

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APPENDIX A

FICK'S LAWS OF DIFFUSION

A.1 FICK'S FIRST LAW OF DIFFUSION

Fick's first law of diffusion can be derived by consideration of the model illustrated below in figure A.1.

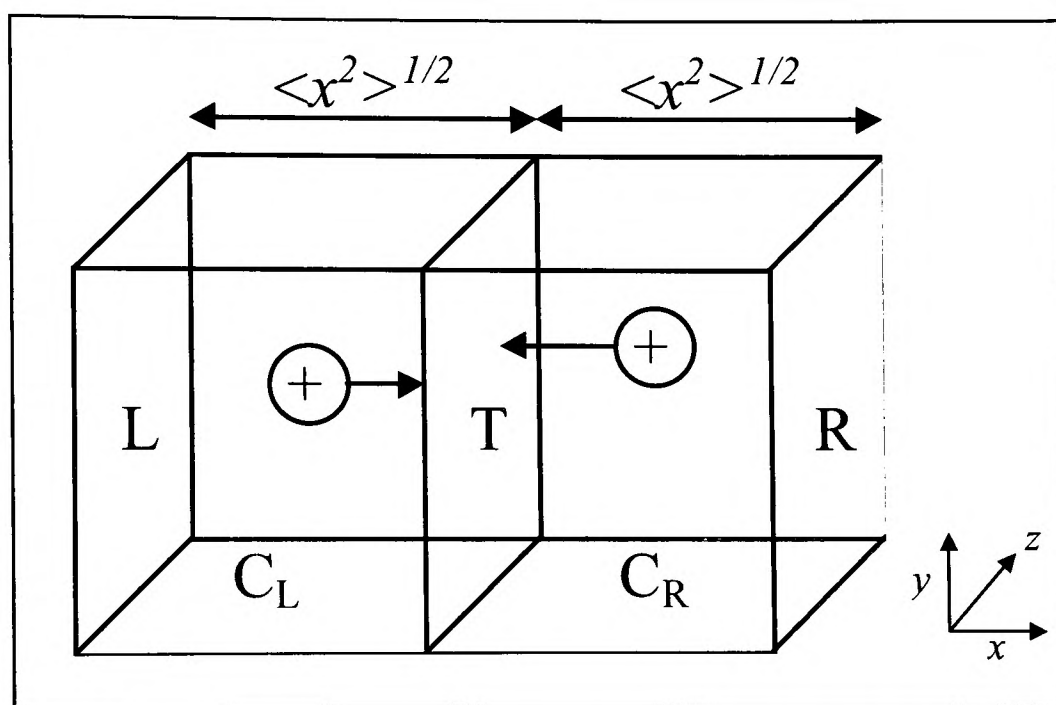


Fig. A.1 - Parallelepiped of solution used in the random walk model.

The parallelepiped of solution shown above can be considered as a volume of electrolyte where the diffusing ionic species varies in the x -direction but remains constant in the yz -plane. The solution can then be divided into two compartments by an imaginary 'transit' plane, T, of area A , such that diffusion occurs normal to this plane. The outer planes L and R are located at a distance of $\sqrt{\langle x^2 \rangle}$ (the mean distance travelled by an ion in a time interval 't') from the transit plane ensuring that all ions in each compartment cross the transit plane, provided the ions are moving in the specified direction.

Assuming the ions are moving in a random walk process, statistically only half of the ions will be moving towards the plane T at any given time. Thus, in a time interval, t ,

Number of ions moving from L $\rightarrow$ T

$$\frac{1}{2} \left(\sqrt{\langle x^2 \rangle} A C_L \right) \quad \text{A.1}$$

Number of ions moving from R $\rightarrow$ T

$$\frac{1}{2} \left(\sqrt{\langle x^2 \rangle} A C_R \right) \quad \text{A.2}$$

where C_L and C_R are the ionic concentrations in each compartment. The total diffusive flux ($\text{mol m}^{-2} \text{s}^{-1}$) of ions across the transit plane in the L $\rightarrow$ R direction is

$$J = \frac{1}{2} \frac{\sqrt{\langle x^2 \rangle}}{t} [C_L - C_R] \quad \text{A.3}$$

The concentration gradient (dc/dx) operating in the L $\rightarrow$ R direction can then be written

$$\frac{dc}{dx} = \frac{[C_R - C_L]}{\sqrt{\langle x^2 \rangle}} = - \frac{[C_L - C_R]}{\sqrt{\langle x^2 \rangle}} \quad \text{A.4}$$

and combined equations (A.1) and (A.2) it can be shown that

$$J = - \frac{1}{2} \frac{\langle x^2 \rangle}{t} \frac{dc}{dx} \quad \text{A.5}$$

This is Fick's first law where

$$D = \frac{\langle x^2 \rangle}{2t} \quad \text{thus} \quad \langle x^2 \rangle = 2Dt \quad \text{A.6}$$

hence the parameters x and D are related.

A.2 FICK'S SECOND LAW OF DIFFUSION

As with the derivation shown above, only planar diffusion is considered. With reference to the model proposed in figure A.1 and considering a small region of volume $A dx$ and the concentration changes which occur in the time interval t and $t + dt$,

$$\boxed{\begin{array}{l} \text{Number of moles} \\ \text{in the region at} \\ \text{time } t + dt \end{array}} - \boxed{\begin{array}{l} \text{Number of moles} \\ \text{in the region at} \\ \text{time } t \end{array}} = \boxed{\begin{array}{l} \text{Number of moles} \\ \text{entering across} \\ \text{plane at } x \end{array}} - \boxed{\begin{array}{l} \text{Number of moles} \\ \text{leaving across} \\ \text{plane at } x + dx \end{array}}$$

which can be expressed as

$$c(x, t + dt)A dx - c(x, t)A dx = J(x, t)A dt - J(x + dx, t)A dt \quad \text{A.7}$$

where $J(x, t)$ is the flux in the x -direction ($\text{mol cm}^{-2} \text{ s}^{-1}$) such that

$$[c(x, t + dt) - c(x, t)]dx = [J(x, t) - J(x + dx, t)]dt \quad \text{A.8}$$

which rearranges to give

$$\frac{\partial}{\partial t} c(x, t) + \frac{\partial}{\partial x} J(x, t) = 0 \quad \text{A.9}$$

Substituting $J(x, t)$ from Fick's first law (A.5) we obtain Fick's second law of diffusion

$$\frac{\partial}{\partial t} c(x, t) = D \frac{\partial^2}{\partial x^2} c(x, t) \quad \text{A.10}$$

Thus, through consideration of a simple statistical model, Fick's first and second laws of diffusion can be derived to mathematically describe the diffusional processes of molecules [1].

A.3 APPENDIX A REFERENCE

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- 1 A. Fick, *Philosophical Magazine and Journal of Science*, 10 (1855) 30.

APPENDIX B

DETECTION LIMITS

B.1 DETECTION LIMITS

Calibration plots can be obtained by constructing graphs of response vs. added concentration of active species. The unknown concentration of a sample can then be inferred either directly from interpolation or through the standard addition method. The latter involves the analysis of a sample with unknown concentration through spiking with aliquots of a standard, building up a standard addition plot as shown in figure B.1.

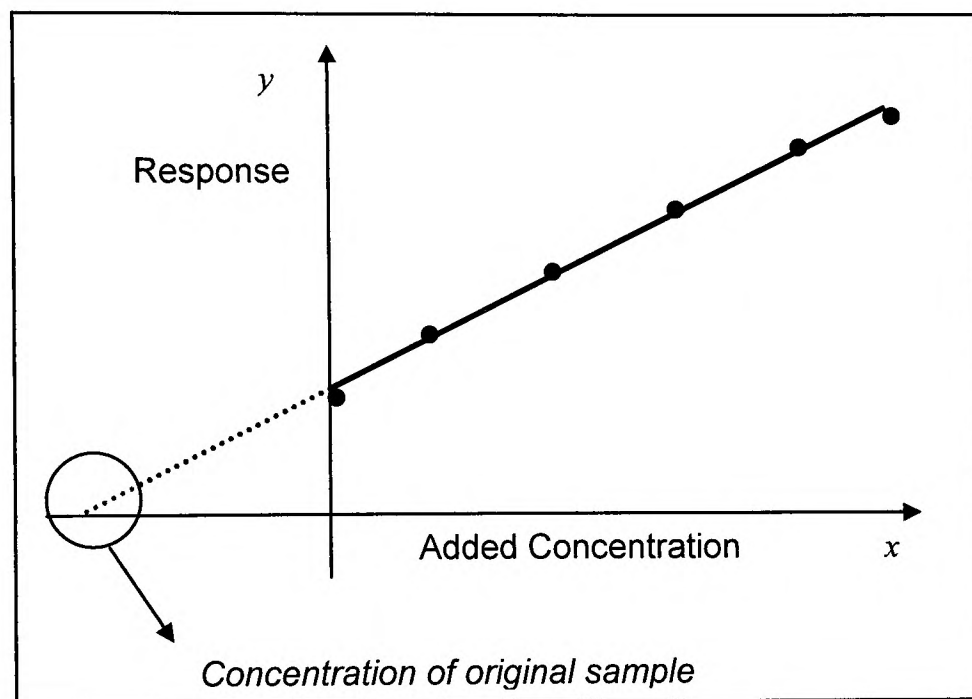


Fig. B.1 - Standard addition method.

The concentration of the sample is then determined through extrapolation to the intercept on the concentration axis. The accuracy of this technique relies on a good linear response from the standard additions and in practice is achieved from best-fit lines with a correlation coefficient, r , (or more commonly quoted as r^2) approaching unity.

The limit of detection (LOD) is defined as the minimum value of the signal from the species being measured that is significantly different from the blank signal. This is normally open to discussion but modern analytical techniques often quote detection limits based on three times the standard deviation of the y -residuals [1]. From the plot in figure B.2, the intercept of the linear plot, $y = a + bx$, is the blank value of the response,

y_B ($= a$), and a LOD for x corresponds to the y -value for $y = y_B + 3s_B$, where s_B is the standard deviation of the y -residuals from the line of best fit.

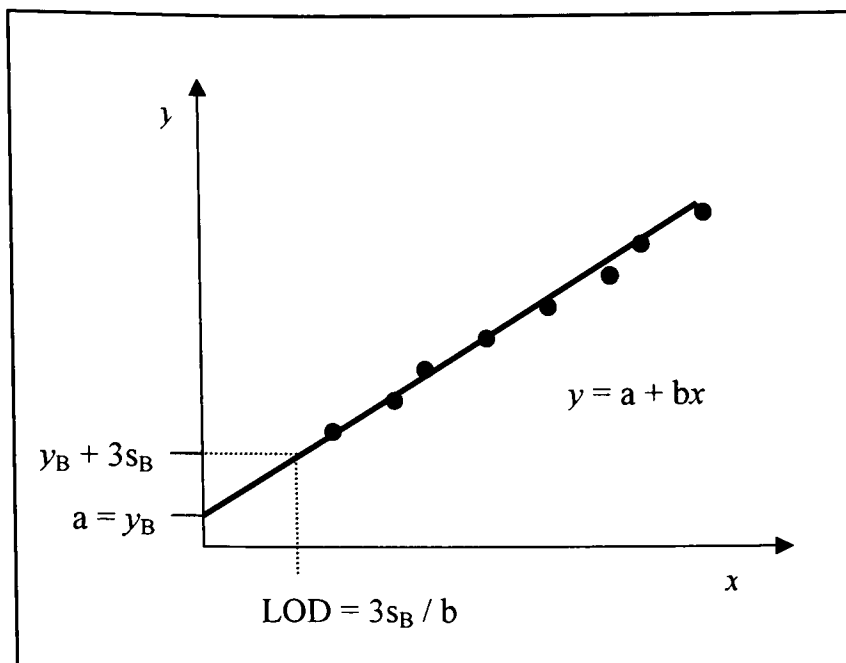


Fig. B.2 - Determination of LOD from a linear calibration plot.

B.2 APPENDIX B REFERENCES

- 1 C. M. A. Brett and A. M. O. Brett, *Electroanalysis*, Oxford University Press, Oxford, 1998.

APPENDIX C

WILKE-CHANG METHOD FOR THE ESTIMATION OF DIFFUSION COEFFICIENTS

C.1 THE WILKE-CHANG METHOD

A semi-empirical equation can be used to correlate the diffusion coefficient of an organic species in dilute aqueous or organic media to the molecular size, proposed by Wilke and Chang in 1955 [1].

$$D = 7.4 \times 10^{-8} \times \frac{T\sqrt{xM}}{\eta V^{0.6}} \quad (\text{C.1})$$

where D is the diffusion coefficient, T is the temperature, x is the solvent association coefficient, M is the molar mass of the solvent, η is the solvent viscosity and V is the atomic volume of the solute. Of the systems studied, the Wilke-Chang correlation (C.1) was found to be consistent to an error of ca. 10 %.

The association parameter, x , is used to define the effective molecular mass of the solvent with respect to the diffusion process, as illustrated in table C.1.

Solvent	Association parameter, x
Water	2.6
Methanol	1.9
Ethanol	1.5
Benzene	1.0
Ether	1.0
Heptane	1.0

Table C.1 - Wilke-Chang association parameter.

The atomic volume of the molecule, V , can be estimated by summation of the individual atoms using values shown in table C.2 [1].

Atom / Functional Moiety	Atomic Volume, $V / \text{cm}^3 \text{mol}^{-1}$
H	3.7
C	14.8
O	7.4
Cl	24.6
Br	27.0
I	37.0
N, primary amine	10.5
N, secondary amine	12.0
N, double bonded	15.6

Table C.2 - Wilke-Chang atomic volumes.

These values must be further modified if the molecule contains a ring configuration, and the corresponding values that must be subtracted are shown in table C.3.

Ring size / Type	Amount to Deduct / $\text{cm}^3 \text{mol}^{-1}$
3	0.6
4	8.5
5	11.5
Pyridine	15
Benzene	15
Naphthalene	30
Anthracene	47.5

Table C.3 - Atomic volume ring deductions.

C.2 APPENDIX C REFERENCE

1 C. R. Wilke and P. Chang, *A. I. Ch. E. Journal*, 1 (1955) 264.