

NOVEL VOLTAMMETRIC METHODS IN HEAVILY PASSIVATING MEDIA

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Abstract

The work contained in this thesis demonstrates that the introduction of power ultrasound into electrochemical systems can broaden the scope of techniques such as anodic stripping voltammetry. Analysis is facilitated by the well-documented phenomena associated with ultrasound, cavitation and acoustic streaming. Cavitation is caused by the collapse of voids created by ultrasonic compression and rarefaction of the solution and results in both localised high temperatures and pressures in the bulk solution and microjetting on the surface. Precise mechanistic details are still open to debate but there is evidence to suggest that shear forces at the interface when the electrode is flushed are responsible for removing organic material (particularly large species such as proteins) facilitating continuous cleaning and activation of the surface. Acoustic streaming has been shown to give diffusion layer thicknesses of the order of 1-10 μm depending on solvent and ultrasound power. The increased mass transport at such low diffusion layer thicknesses results in shorter preconcentration periods in electrochemical stripping voltammetry, greater sensitivity and a correspondingly lowered detection limit. Often ultrasound has been applied to highly passivating systems where conventional silent electroanalysis fails with the effect of transforming invisible or tiny voltammetric signals into large and quantitative responses suitable for sensitive and accurate analytical purposes.

In Section II the relative contributions of acoustic streaming and cavitationaly induced microjetting to the sonovoltammetric response is assessed. Chronoamperometry at insonated electrodes of both micro and macro dimensions, and differential pulse voltammetry (DPV) were used to explore the frequency and violence of cavitational events and the nature of the diffusion layer prevailing under steady-state electrolysis. The results lead to a physical model of an insonated electrode which may be described as a steady diffusion layer a few microns thick brought about by acoustic streaming which is occasionally and randomly punctuated by a cavitational event. The frequency and violence of the event is dependent on the solvent and ultrasound power, except at very short electrode-to-horn separation where the cavitational contribution becomes substantial.

Section III concentrates on the implementation of the technique with applications to the detection of heavy metals in biofluids. In Chapter 4 sonoelectroanalysis is applied to the detection of copper bound within human blood protein and whole blood. It is shown that the enhancement of square wave anodic stripping peaks observed in ceruloplasmin and whole blood is not simply due to mass transport enhancement and cavitational cleaning effects alone but also the liberation of copper from the active sites in which it is bound prior to preconcentration. The results of a quantitative determination of total copper in two samples of whole blood were 1300 $\mu\text{g L}^{-1}$ and 620 $\mu\text{g L}^{-1}$, verified by independent blind analysis using atomic absorption spectroscopy (AAS).

The need for rapid analysis of environmental samples can also be fulfilled using sonoelectroanalysis. In Chapter 5 fish gill mucus is used as a non-destructive biomarker for the detection of heavy metals by sono-square wave anodic stripping voltammetry (sono-SWASV). A quantitative assessment of copper content yielded values of $16 \mu\text{g L}^{-1}$ and $21 \mu\text{g L}^{-1}$ which compared favourably with independent blind analysis by AAS. The potential of the technique for detection of other heavy metals for example lead, was also demonstrated.

Lead poisoning is recognized as a major environmental health risk and in Chapter 6 quantitative analysis of lead in artificial saliva from a realistic sputum volume, $220 \mu\text{L}$, introduced to acetate buffer is investigated. An insonated preconcentration obviates the need for lengthy or degradative sample pretreatment by liberating the lead from the glycoproteins and other materials to which it binds in solution. Quantitative depassivation of the electrode surface by cavitation shearing maintained the analytical signal throughout the experiment where under silent conditions the signal diminished to zero with time. The detection limit in the analyte is $0.25 \mu\text{g L}^{-1}$. Following this proof of concept, Chapter 7 goes on to apply the technique to the quantitative determination of lead and cadmium in real human saliva. Close agreement between lead concentration determined by sono-SWASV and independent and blind ICP-MS is reported for human saliva samples with detection limits of $0.5 \mu\text{g L}^{-1}$ lead and $1 \mu\text{g L}^{-1}$ cadmium in saliva.

Section IV harnesses the benefits of acoustic emulsification. Power ultrasound is capable of forming droplets of micron dimensions with lower energy consumption than conventional emulsifiers. Chapter 8 acoustic emulsification is employed in the detection of vanillin (4-hydroxy-3-methoxybenzaldehyde) in vanilla essence using ethyl acetoacetate as a novel electrochemical and sonoelectrochemical solvent. Contrasting with silent voltammetry, ultrasound facilitates emulsification and extraction of vanillin in the extract permitting an analytical square wave voltammetric signal to be obtained. Close agreement with a blind analysis of the samples using HPLC-UV is observed with a limit of detection in the biphasic medium of 0.020 mM .

In Chapter 9 acoustic emulsification is utilized in the solvent extraction of copper. Ultrasonic emulsification of an aqueous phase containing copper ions with the N-benzoyl-N-phenyl-hydroxylamine ligand in an organic ethyl acetate phase was shown to facilitate the extraction of copper into the organic phase at 25°C . Subsequent emulsification with 1 M acid “back-extracts” or “re-strips” the copper into the aqueous phase prior to analysis via sono-SWASV. The technique necessarily removes contaminants present in the test solution since these will prefer to remain in the initial aqueous phase, or will transfer to the organic phase but are unlikely to be doubly transferred into the “clean” final aqueous phase. In Chapter 10 the technique is applied to the analysis of copper in the soft drink “Ribena® Light” with a

detection limit of $2 \mu\text{g L}^{-1}$ and copper in blood with a detection limit of $0.16 \mu\text{g L}^{-1}$. In the latter case the analysis required a sample volume of $100 \mu\text{L}$ suggesting that a pinprick test may be feasible.

Finally in Section V, the analysis of copper in the presence of surfactants is investigated. Surfactant adsorption has been shown to have a passivating effect on the electrode surface during anodic stripping voltammetric measurements. Effluent, both industrial and domestic, is commonly contaminated with surfactant and electroanalysis of heavy metals is frequently precluded. It is therefore desirable to formulate a new analytical strategy in waste water. This is facilitated by controlled experiments with known amounts of anionic, cationic or neutral surfactant which also provides an ideal model system for comparing the techniques introduced in earlier Chapters. "Direct" sonoelectroanalysis in the medium of interest is compared with biphasic sono-solvent double extraction and both are appraised in the light of novel sonotrode technology.

This thesis concludes that sonoelectroanalysis is a powerful and versatile analytical tool that can be employed in an expanding range of applications. The examples given herein are pertinent to the major fields of medical diagnosis and the assessment of environmental pollution. They demonstrate that sonoelectroanalysis is a viable alternative to conventional analytical techniques which have the disadvantages of more lengthy sample pretreatment and cost. Sonoelectroanalysis has the potential to become a portable technique especially in the light of the encouraging results presented using the single probe sonotrode, an instrument which is now commercially available in collaboration with Windsor Scientific Ltd.

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Space restrictions dictate that having reached the end of the page I should probably stop, so to everyone I have been unable to mention...

Thank you

Novel Voltammetric Methods in Heavily Passivating Media

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Section I

Chapter 1 – Introduction

Part 1

General Electrochemistry

1.1 General Electrochemistry

The aim of this section is to introduce concepts underpinning reactions which take place at an electrode in solution. It includes a discussion of the structure of the interfacial region of the electrode and the solution. Subsequently it introduces the concept of an electrochemical cell and equilibrium redox potentials and addresses the thermodynamics of such a cell. Finally it considers the standard hydrogen electrode and formal potentials.

1.1.1. Brief History

Electrochemistry is most succinctly described as “the branch of chemistry concerned with the interrelation of electrical and chemical effects”¹. The field was well established by the 20th century both scientifically and industrially following the advances of Luigi Galvani (1737-1798), Alessandro Volta (1745-1827), Sir Humphrey Davy (1778-1829) and Michael Faraday (1791-1867). The principal advances in electrochemistry came some years later with *inter alia* the extensive work of Wilhelm Ostwald (1853-1932) who founded the German Electrochemical Society in 1894, Walther Nernst (1864-1941) who is noted, *inter alia*, for his theory of the electrode potential, Svante Arrhenius (1859-1927) who is associated with the concept of activation energy as well as first proposing the theory of electrolytic dissociation, and Jacobus van’t Hoff (1852-1911) who made the discovery that solutions that readily conduct an electric current, termed electrolytes, possess distinctive colligative properties. Since the foundation of the Electrochemical Society² in 1902 the field has continued to grow and now encompasses fifteen dedicated international journals reporting on technical innovations that play an important part in the everyday lives of millions for example in recovery of metals from ores, electroplating of metals, corrosion, electrochromic displays, electroanalytical sensors, batteries and fuel cells.

1.1.2 Interfacial Phenomena

The processes which occur at the electrode can be categorised as Faradaic and non-Faradaic. A Faradaic process involves a reaction where charge is transferred across the metal-solution interface. This charge transfer is responsible for oxidising or reducing a species at the interface. The process is termed Faradaic by virtue of its adherence to Faraday's law, that is, the “amount” of chemical reaction caused by the flow of current is proportional to the “amount” of electricity passed. Non-Faradaic processes include adsorption and desorption. The structure of the electrode-solution interface changes with a change in potential or solution composition.

Non-Faradaic Processes – The electrode-solution interface

The electrode-solution interface has been shown experimentally to behave like a capacitor. At a potential, ϕ^M a charge q^M , which represents an excess or deficiency of electrons, will exist on the metal surface and a charge q^S , which is made up of an excess of either cations or anions in the vicinity of the electrode surface, will exist in the solution. At all times $q^M = -q^S$.

q^M and q^S can be expressed as charge densities with

$$\sigma^M = \frac{q^M}{A} \quad (1)$$

where σ^M is the charge density, usually expressed in $\mu\text{C cm}^{-2}$, and A is the electrode area.

The array of charged species and oriented dipoles at the electrode-solution interface is termed the electrical double layer³⁻⁷ and is illustrated schematically in **Figure 1**. Its structure is important as it can affect the rates of electrode processes. The thickness of the diffuse layer depends on the ionic strength of the solution, for concentrations greater than $10^{-2} \text{ mol dm}^{-3}$ the thickness is less than $100 \text{ \AA}$. At a given potential, the electrode-solution interface is characterised by a double layer capacitance, C_d , typically in the range $10\text{-}40 \mu\text{F cm}^{-2}$ for aqueous solutions. One cannot normally neglect the existence of this double-layer capacitance C_d or the presence of a charging current in electrochemical experiments.

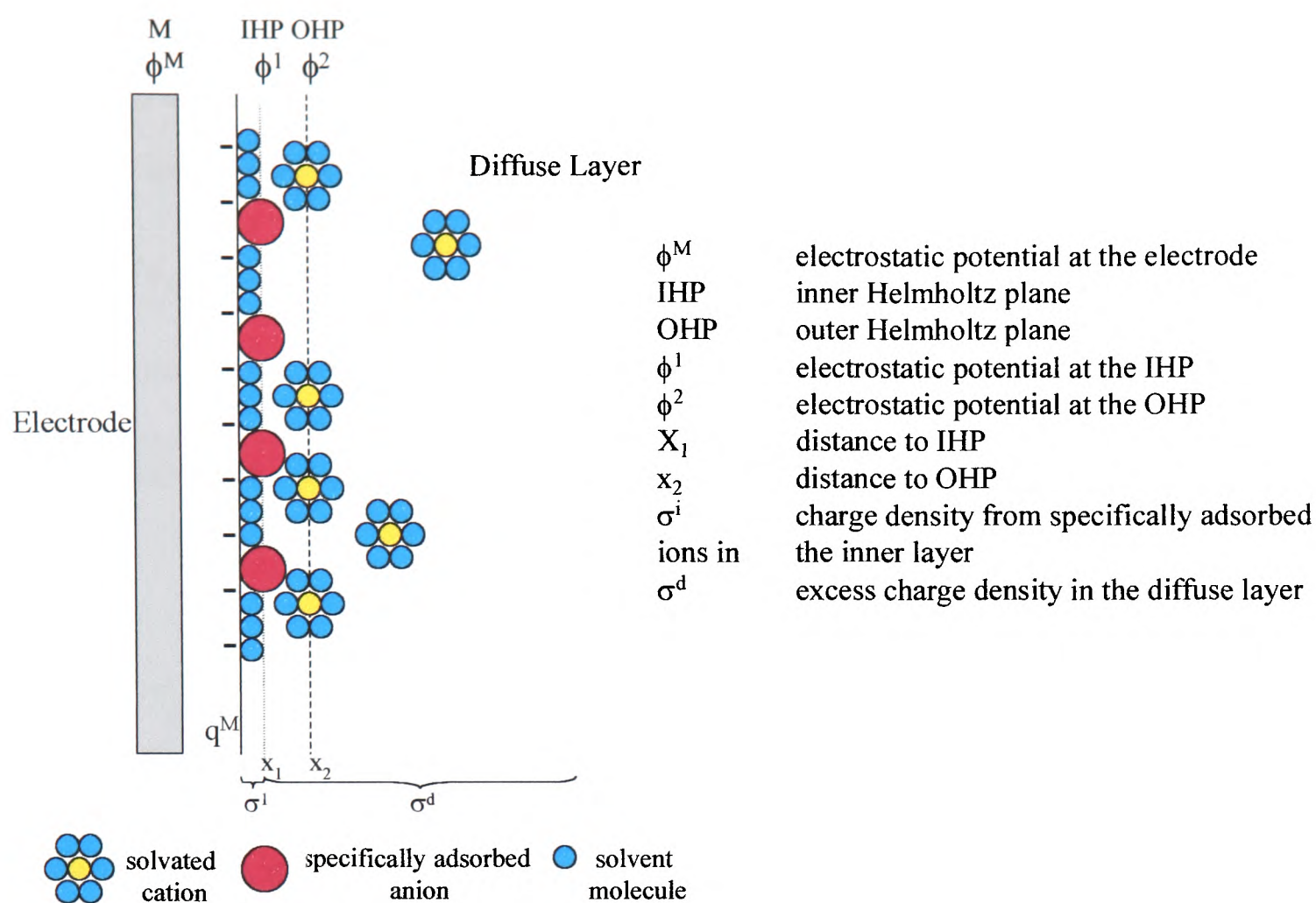


Figure 1 – Schematic model¹ of the double layer region under conditions where anions are specifically adsorbed

1.1.3 Thermodynamics of Electrochemical Equilibrium

A Faradaic electrode process involves the transfer of charge across the interface between a metallic electrode (m) and a solution phase (aq) species as illustrated by the following example:



It is apparent that in order to achieve equilibrium a net charge separation must develop between the source of the electrons, the electrode, and the solution. The resulting potential drop across the electrode|solution interface $\Delta\phi_{\text{m|s}}$ is given by:

$$\Delta\phi_{\text{m|s}} = \phi_{\text{m}} - \phi_{\text{s}} \quad (3)$$

When the system given in equation 2 is at equilibrium the total Gibbs free energy of the system will be at a minimum: the total electrochemical potential of the reactants will equal the total electrochemical potential of the products.

The electrochemical potential^{8,9}, μ_A of species A can be defined as:

$$\bar{\mu}_A = \mu_A + z_A F \phi \quad (4)$$

where μ_A is the chemical potential of species A and z_A is the charge on a molecule of A, ϕ is the potential of the phase, electrode or solution, in which species A can be found and F is the Faraday constant. Hence for equation 2:

$$(\mu_{\text{Fe}^{3+}} + 3F\phi_s) + (\mu_{e^-} - F\phi_m) = (\mu_{\text{Fe}^{2+}} + 2F\phi_s) \quad (5)$$

which rearranges to give

$$F\Delta\phi_{m|s} = \mu_{\text{Fe}^{3+}} + \mu_{e^-} - \mu_{\text{Fe}^{2+}} \quad (6)$$

Since

$$\mu_{\text{Fe}^{3+}} = \mu_{\text{Fe}^{3+}}^\circ + RT \ln a_{\text{Fe}^{3+}} \quad (7)$$

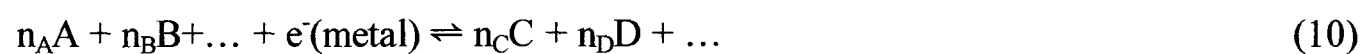
$$\mu_{\text{Fe}^{2+}} = \mu_{\text{Fe}^{2+}}^\circ + RT \ln a_{\text{Fe}^{2+}} \quad (8)$$

where μ_i° denotes the standard chemical potential of species i and a_i denotes the activity of species i, then we can write:

$$\Delta\phi_{m|s} = \Delta\phi^\circ - \frac{RT}{F} \ln \left\{ \frac{a_{\text{Fe}^{2+}}}{a_{\text{Fe}^{3+}}} \right\} \quad (9)$$

$$\text{where } \Delta\phi^\circ = \frac{1}{F} (\mu_{\text{Fe}^{3+}}^\circ + \mu_{e^-} - \mu_{\text{Fe}^{2+}}^\circ) = \text{constant}$$

This is the Nernst equation appropriate to the single electrode|solution interface. More generally for the equilibrium denoted by equation 10 it takes the form given in equation 11:



where n_i is the stoichiometric coefficient of species i

$$\Delta\phi_{m|s} = \Delta\phi^\circ - \frac{RT}{F} \ln \left\{ \frac{a_C^{n_C} a_D^{n_D} \dots}{a_A^{n_A} a_B^{n_B} \dots} \right\} \quad (11)$$

1.1.4 The Electrochemical Cell

Direct measurement of $\Delta\phi_{ms}$ at a single interface is not experimentally feasible. Measurements can only be made if at least one further electrode is introduced. This additional electrode will also possess a potential drop. Hence if the electrodes are connected to a digital voltmeter (DVM) or other voltage measuring device, then the difference between the potential drops at the two electrode|solution interfaces will be measured. The problem is addressed by the introduction of a *reference electrode*. A reference electrode maintains a constant potential drop $\Delta\phi_{ms}$ across its interface with the solution. The measurement of potential difference, E , given by the DVM in this situation is clearly:

$$E = (\phi_m - \phi_s) + \text{constant} \quad (12)$$

Hence any changes in $(\phi_m - \phi_s)$ are manifested by an equivalent change in the measured potential, E . Since $(\phi_{\text{reference}} - \phi_{\text{solution}})$ must be a fixed value for a reference electrode then the following must be satisfied:

- i) the chemical composition of the electrode and the solution to which the electrode is directly exposed must be fixed. One of the implications of this is that current should not be passed through the reference electrode since this would perturb the concentrations of the species involved in the potential determining equilibrium;
- ii) thermodynamic equilibrium should be attained rapidly, i.e. fast electrode kinetics.

Standard Hydrogen Electrode¹ (SHE) and Standard Electrode Potential¹ (SEP)

The SHE has been selected as the reference electrode for reporting electrode potentials. The electrode consists of a platinum electrode coated with finely divided platinum (platinum black) over which H_2 gas is bubbled. The half-cell reaction for this electrode is:

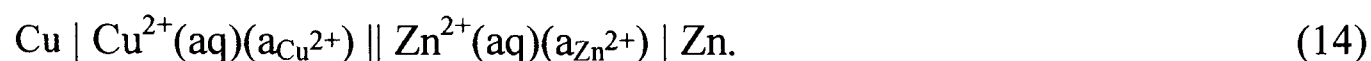


By convention the SHE is defined as possessing an absolute potential of zero.

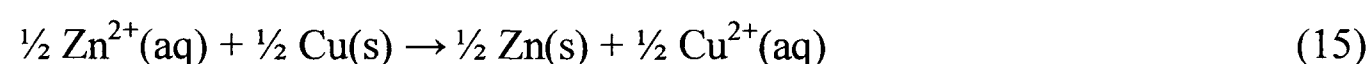
The standard electrode potential, E° , of any system is defined as the measured potential difference between the two electrodes of a cell in which the potential of the electrode of interest is measured against the SHE and in which all the chemical species are present at a concentration corresponding to unit activity in the case of a solution phase species, or to unit pressure in the case of a gas. By

measuring the potentials of cells containing the SHE it is possible to compile extensive tables of E° thus allowing the predictions of the potentials of electrochemical cells.

It is convenient to represent an electrochemical cell as in the example shown below:



In this representation a single line $|$ is indicative of a phase boundary and a double line $||$ denotes a salt bridge. It is also convention that the cell potential is that of the right hand electrode relative to that of the left hand electrode. Hence in this case the formal cell reaction is



and

$$E_{\text{cell}}^\circ = E_{\text{Zn}^{2+}|\text{Zn}}^\circ - E_{\text{Cu}^{2+}|\text{Cu}}^\circ \quad (16)$$

Furthermore since E° is related to the standard Gibbs free energy change by

$$\Delta G^\circ = -nFE^\circ = -RT \ln K \quad (17)$$

then it can be used to calculate ΔG° predict the direction of spontaneity or calculate the equilibrium constant.

The Nernst equation can be given for a general electrochemical cell, which will predict the cell potential for any values of the activities of the reactants and products. For the ‘formal cell reaction’



where R_i represent the reactant species and P_j represent the product species and n_i and n_j are their stoichiometric coefficients, the Nernst equation can be written:

$$E_{\text{cell}} = E_{\text{cell}}^\circ + \frac{RT}{F} \ln \frac{\prod a_{R_i}^{n_i}}{\prod a_{P_j}^{n_j}} \quad (19)$$

where $E_{cell} = E_{right}^{\circ} - E_{left}^{\circ}$, and E_{right}° and E_{left}° are the E° of the half cell reactions for the right and left hand electrodes as drawn in equation 14 above.

Since activity coefficients may be unknown, it becomes inconvenient to deal with activities when evaluating half-cell potentials. The formal potential $E^{0'}$ is defined so as to avoid them. It is the measured potential of the half-cell vs. SHE when the species R and P are present at concentrations such that $\frac{[R]^{n_i}}{[P]^{n_j}} = 1$, and other miscellaneous components of the medium are present at designated

concentrations. The Nernst relation given in equation 19 can be written:

$$E_{cell} = E_{cell}^{\circ} + \frac{RT}{F} \ln \frac{\prod \gamma_{R_i}^{n_i} [R_i]^{n_i}}{\prod \gamma_{P_j}^{n_j} [P_j]^{n_j}} \quad (20)$$

which can be formulated in terms of $E^{0'}$ as follows:

$$E_{cell} = E^{0'} + \frac{RT}{F} \ln \frac{\prod [R_i]^{n_i}}{\prod [P_j]^{n_j}} \quad (21)$$

where

$$E^{0'} = E_{cell}^{\circ} + \frac{RT}{F} \ln \frac{\prod \gamma_{R_i}^{n_i}}{\prod \gamma_{P_j}^{n_j}} \quad (22)$$

$E^{0'}$ is therefore dependent on the activity coefficients and consequently varies with different media and different ionic strengths. When working with formal electrode potentials it is therefore important to maintain a constant ionic strength, this is achieved by the addition of an electrolyte of concentration much greater than that of the reacting species, which remains nominally constant throughout the reaction.

1.1.5 Electrochemical Cells and Cell Resistance

In the experiments described above it is important that no current flows (so as not to disturb the concentration of species in the potential determining equilibrium). Since no current flows, no net Faradaic reaction occurs and the potential is frequently governed by the thermodynamic properties of the system. If a voltage exceeding the open circuit potential of the cell is supplied by an external power supply (E_a) then the cell behaves as an electrolytic cell and a current flows. When the potential

of an electrode is measured against a non-polarisable reference electrode during the passage of current, a voltage drop equal to iR_s forms part of the measured value, where R_s is the solution resistance between the two electrodes. If E_a is greater than the open circuit potential the difference is distributed in two parts:

- i) to deliver the current, the potential of the working electrode, E_w , must shift to a new value;
- ii) the remainder of E_a represents the ohmic drop caused by current flow in the solution;

hence

$$E_a = E_w - iR_s \quad (23)$$

The ohmic potential drop in the solution is characteristic of the bulk solution and not the electrode reaction it can therefore be minimised by careful selection of the bulk solution, termed the background electrolyte. When iR_s is small (less than 1-2 mV) a two electrode cell comprising working and reference electrode can be used to record a current/voltage profile. Should the solution be more resistive and iR_s correspondingly larger (examples would include non-aqueous solvents), a very small electrode (an ultramicroelectrode) must be used if a two electrode cell is to be employed successfully. For such an electrode, currents of the order of 1 nA are typical, hence R_s values even of the order of M Ω can be acceptable. Microelectrodes are delicate and not suitable for all experimental arrangements. Hence in experiments where iR_s may be high, a three electrode cell is preferable. In this arrangement, the current is passed between the working electrode and a counter (or auxiliary electrode). A typical material for an auxiliary electrode is a platinum wire since it will not produce substances by electrolysis that could reach the working electrode. The potential of the working electrode is measured relative to a separate reference electrode by a device with a high input impedance so that a negligible current is drawn through the reference electrode. Consequently its potential will remain constant and equal to its open circuit value. This three electrode arrangement is used in most electrochemical experiments undertaken in subsequent chapters.

1.1.6 Other Reference Electrodes

The SHE introduced in s.1.1.4 is not the only reference electrode which can be used experimentally. It is more convenient to use a saturated calomel electrode or silver/silver chloride reference electrode.

1.1.6.1 Saturated Calomel Electrode¹

The potential determining equilibrium of this electrode is:



so that the potential developed between the mercury (M) and the solution (S) is:

$$\phi_M - \phi_S = \text{constant} - RT/F \ln a_{\text{Cl}^-}$$

The potential is clearly dependent on the activity of the chloride ions; a constant activity is achieved by maintaining the KCl solution inside the electrode at a saturated level by the addition of KCl crystals. The electrode contacts the solution by means of a porous frit which acts as a salt bridge mostly eliminating problems due to a liquid junction. The electrode potential of the $\text{Hg}_2\text{Cl}_2/\text{Hg}$ couple in contact with saturated chloride ions is 0.242 V vs. SHE at 25 °C.

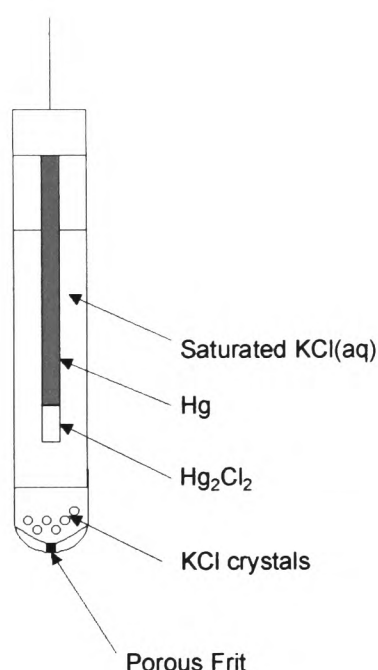


Figure 2 – Saturated Calomel Electrode

1.1.6.2 Silver/Silver Chloride Electrode¹

Similar in design to the saturated calomel electrode, the potential determining equilibrium of the silver/silver chloride electrode is:



The equilibrium is established at the silver/silver chloride boundary. It is therefore important that the silver chloride coat is porous so that the saturated aqueous solution containing the chloride ions penetrates to the boundary. The potential of the Ag/AgCl reference electrode in contact with saturated KCl is 0.197 V vs. SHE at 25 °C.

1.2 Dynamic Electrochemistry

1.2.1 Electron Transfer Kinetics

For reaction 26 in which the oxidised and reduced species are interconverted by a single electron transfer at the electrode



k_{red} and k_{ox} , describe the first order heterogeneous rate constants for the reductive and oxidative electron transfer reactions. The current for the oxidative and reductive components of reaction 25 can be written:

$$i_{\text{red}} = F A k_{\text{red}} [\text{O}]_0 \quad (27)$$

$$i_{\text{ox}} = F A k_{\text{ox}} [\text{R}]_0 \quad (28)$$

where A is the electrode area and $k_{\text{red}}[\text{O}]_0$ and $k_{\text{ox}}[\text{R}]_0$ are the fluxes of material to the electrode surface. The net current flowing is the sum of these two currents hence

$$i = F A (k_{\text{red}} [\text{O}]_0 - k_{\text{ox}} [\text{R}]_0) \quad (29)$$

Transition state theory predicts the rates of the oxidation and reduction will take the Arrhenius form expressed as

$$k_{\text{ox}} = A_{\text{ox}} \exp \left\{ \frac{-\Delta G_{\text{ox}}^*}{RT} \right\} \quad (30)$$

$$k_{\text{red}} = A_{\text{red}} \exp \left\{ \frac{-\Delta G_{\text{red}}^*}{RT} \right\} \quad (31)$$

where ΔG^* is the free energy of activation and A is a frequency factor which accounts for the rate of collision of the electroactive molecule with the electrode surface. This is illustrated in **Figure 3**.

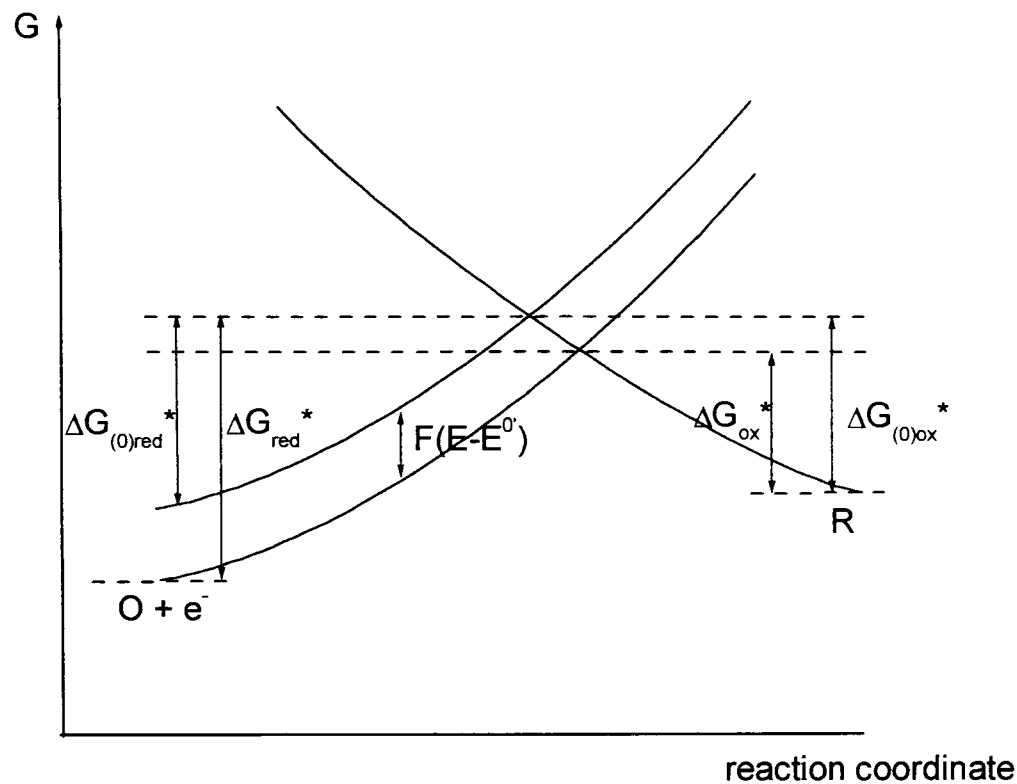


Figure 3 – Effects of a potential change on the standard free energies of activation for oxidation and reduction

If the potential is changed by ΔE the relative energy of the electron “resident” on the electrode changes by $-F\Delta E = -F(E - E^{0'})$, hence the $(O + e)$ curve in **Figure 3** moves up or down by that amount (illustrated for a positive change by **Figure 3**). It is apparent that the barrier to oxidation ΔG_{ox}^* has become less than $\Delta G_{0(ox)}^*$ by a fraction of the total energy change, this is termed $(1 - \alpha)$ where $0 < \alpha < 1$ depending on the shape of the intersection region.

Hence

$$\Delta G_{ox}^* = \Delta G_{0(ox)}^* - (1 - \alpha)F(E - E^{0'}) \quad (32)$$

Furthermore, the barrier to reduction ΔG_{red}^* is higher than $\Delta G_{0(red)}^*$ by $\alpha F(E - E^{0'})$ therefore:

$$\Delta G_{red}^* = \Delta G_{0(red)}^* + \alpha F(E - E^{0'}) \quad (33)$$

Inserting equations (30) and (31) into (32) and (33) gives:

$$k_{ox} = A_{ox} \exp\left(\frac{\Delta G_{0(ox)}^*}{RT}\right) \exp\left(\frac{(1 - \alpha)F}{RT}(E - E^{0'})\right) \quad (34)$$

and

$$k_{red} = A_{red} \exp\left(\frac{\Delta G_{0(red)}^*}{RT}\right) \exp\left(\frac{-\alpha F}{RT}(E - E^{0'})\right) \quad (35)$$

When the interface is at equilibrium with a solution in which $[O]_0 = [R]_0$ then $E = E^{0'}$, $k_{red}[O]_0 = k_{ox}[R]_0$ and $k_{red} = k_{ox} = k^0$. Thus $E^{0'}$ is the potential where the forward and reverse rate constants have the same value, k^0 , known as the *standard rate constant*. This leads to:

$$k_{ox} = k^0 \exp\left(\frac{(1-\alpha)F}{RT}(E - E^{0'})\right) \quad (36)$$

$$k_{red} = k^0 \exp\left(\frac{-\alpha F}{RT}(E - E^{0'})\right) \quad (37)$$

Substitution of these relations into equation 29 yields an equation that can be used to describe the current-potential characteristics of a reaction at an electrode:

$$i = F A k^0 \left\{ \left[[O]_0 \exp\left[\frac{-\alpha F}{RT}(E - E^{0'})\right] \right] - \left[[R]_0 \exp\left[(1-\alpha)\frac{F}{RT}(E - E^{0'})\right] \right] \right\} \quad (38)$$

This essential relation is termed the Butler-Volmer^{10,11} formulation of electrode kinetics.

The overpotential η can be defined as:

$$\eta = E - E^{0'} \quad (39)$$

When η is small, the kinetics are described as “reversible” since little or no applied potential is required to drive the reaction; from equation 38 it can be seen that current will flow readily both anodically and cathodically. Conversely at large η the kinetics can be described as irreversible. When the back reaction contributes less than 1 % of the current this is described as Tafel behaviour.

1.2.2 Tafel Analysis

The Tafel relationship¹², given in equation (40), was originally derived empirically from experiment. It is frequently able to describe the electrode kinetics:

$$\eta = a + b \log i \quad (40)$$

where a and b are the empirical Tafel constants (see **Figure 4**).

A plot of $\log i$ versus η is known as a Tafel plot and is a useful device for evaluating kinetic parameters. Such a plot is illustrated in **Figure 4**. The deviation from linear behaviour at low η is indicative that the back reaction can no longer be regarded as negligible. The gradient of the slope, b for $\alpha = 0.5$ is 0.118 V, a value often quoted as a “typical” Tafel slope for an electrochemically irreversible process.

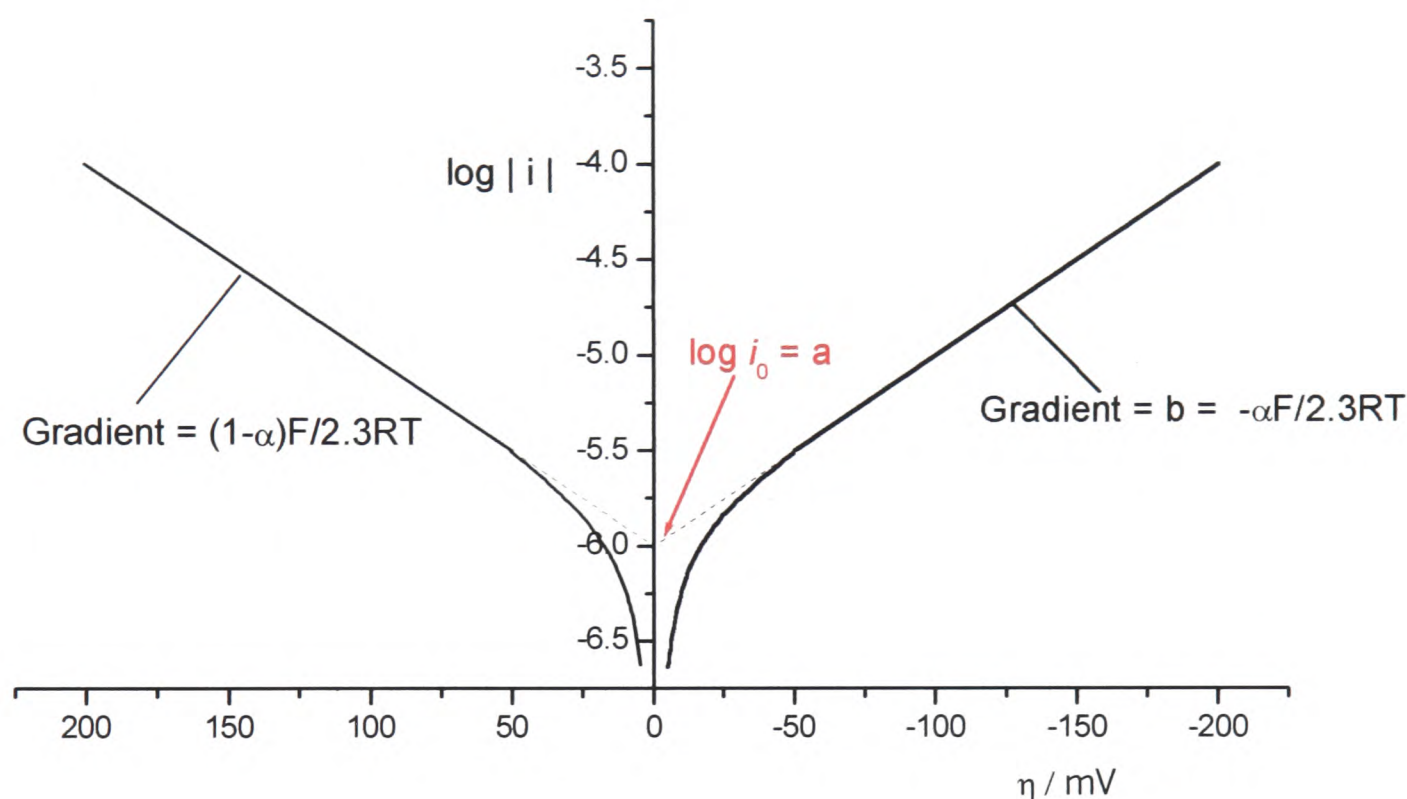


Figure 4 – A typical Tafel plot for anodic and cathodic branches of the current-overpotential curve for a single electron oxidation or reduction as in equation 26, with $\alpha = 0.5$. Both linear segments extrapolate to an intercept of $\log i_0$

1.2.3 Mass Transport

In order for a Faradaic reaction to proceed, the reactant molecules must be transported from the bulk solution to the electrode interface and the reaction products, once formed, must be transported away from the electrode. The rate of the reaction is determined by the slowest step which may be the electron transfer kinetics discussed in s.1.2.1 above, or the rate at which material is brought to or from the electrode through mass transport. The significant transport properties are diffusion, convection and migration.

1.2.3.1 Diffusion

The rate of diffusion at a given point in solution is dependent on the concentration gradient at that particular location. Fick first described the relationship now known as Fick's 1st law of diffusion (equation 41) by considering the simple case of linear diffusion of species B to a planar surface. See appendix 1 for a derivation of this law.

$$j = -D_B \frac{\partial[B]}{\partial x} \quad (41)$$

j is the diffusional flux ($\text{mol m}^{-2} \text{s}^{-1}$), D_B is a constant termed the diffusion coefficient which is characteristic of the diffusing species, B.

In practice we are more concerned with the change in concentration at a point adjacent to the electrode surface with time. This is established by considering the difference in the flux of B entering through the plane at x and the flux leaving through the plane at $(x + dx)$ during a time (dt) which leads to Fick's 2nd law of diffusion (equation 42), allowing the prediction of concentration changes close to the electrode surface (appendix 1).

$$\frac{\partial[B]}{\partial t} = D_B \left(\frac{\partial^2[B]}{\partial x^2} \right) \quad (42)$$

1.2.3.2 Convection

Movement due to convection may arise from natural thermal gradients or density differences and also external forces such as bubbling of gas or stirring. The introduction of forced convection has the effect of swamping any natural convection and makes experiments more reproducible. The effect of ultrasound in convective mass transport is discussed in s.1.4.3 Any such forced convection is usually spatially arranged so as to possess well-defined hydrodynamic behaviour. This enables a quantitative description of the flow in solution to be established and the pattern of mass transport to the electrode to be rigorously predicted. Mathematically, the convection analogue of equation 42 is

$$\frac{\partial[B]}{\partial t} = -v_x \left(\frac{\partial[B]}{\partial x} \right) \quad (43)$$

where v_x is the velocity of the movement of solution in cm s^{-1} in the x direction.

1.2.3.3 Nernst Model of the Diffusion Layer

When an electrode is connected to a potential source sufficient to oxidise or reduce species in solution, a concentration gradient is induced between the electrode and the bulk solution. The concentration gradient is perpendicular to the electrode surface and acts to force a flux of species from the bulk solution to flow towards the electrode. Gradually a ‘diffusion layer’ is established close to the electrode (**Figure 5**). As electrolysis proceeds the thickness of this layer becomes progressively larger. In principle it could grow without limit until it exhausts all the reactive species in the container. In practice, the bulk solution experiences some mixing and natural convection, which limits the expansion of the diffusion layer. It therefore evolves into a steady state thickness controlled by the extent of mixing in the bulk solution. Under these steady state conditions transport within the layer occurs by diffusion alone.

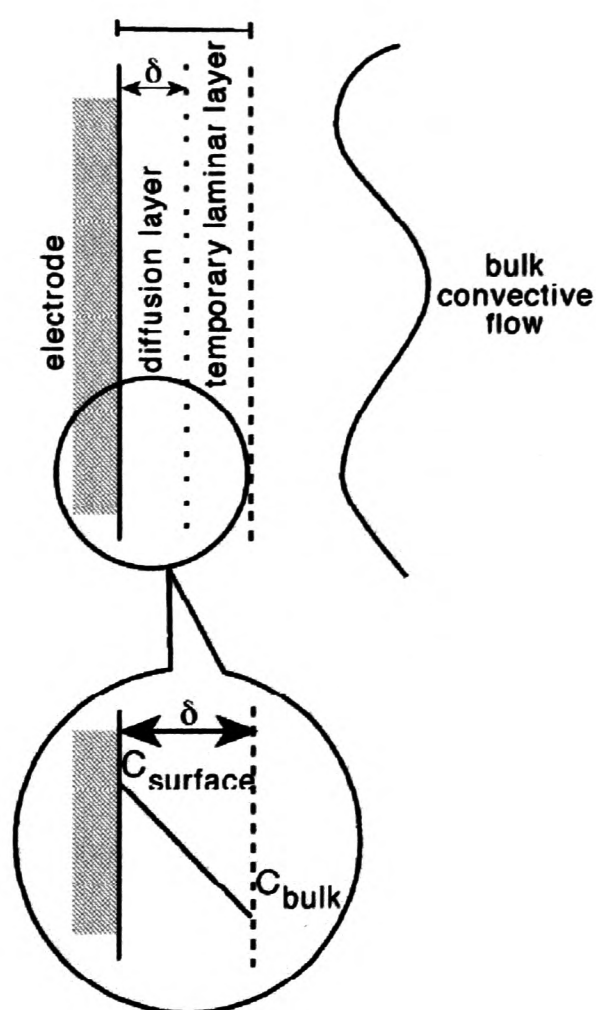


Figure 5 - Schematic representation of the diffusion and boundary layers at the electrode-solution interface as applied in the diffusion layer model

Nernst estimated the size of the diffusion layer thickness, δ , by extrapolating the linear region of **Figure 6** until it reaches a concentration corresponding to that of the bulk solution.

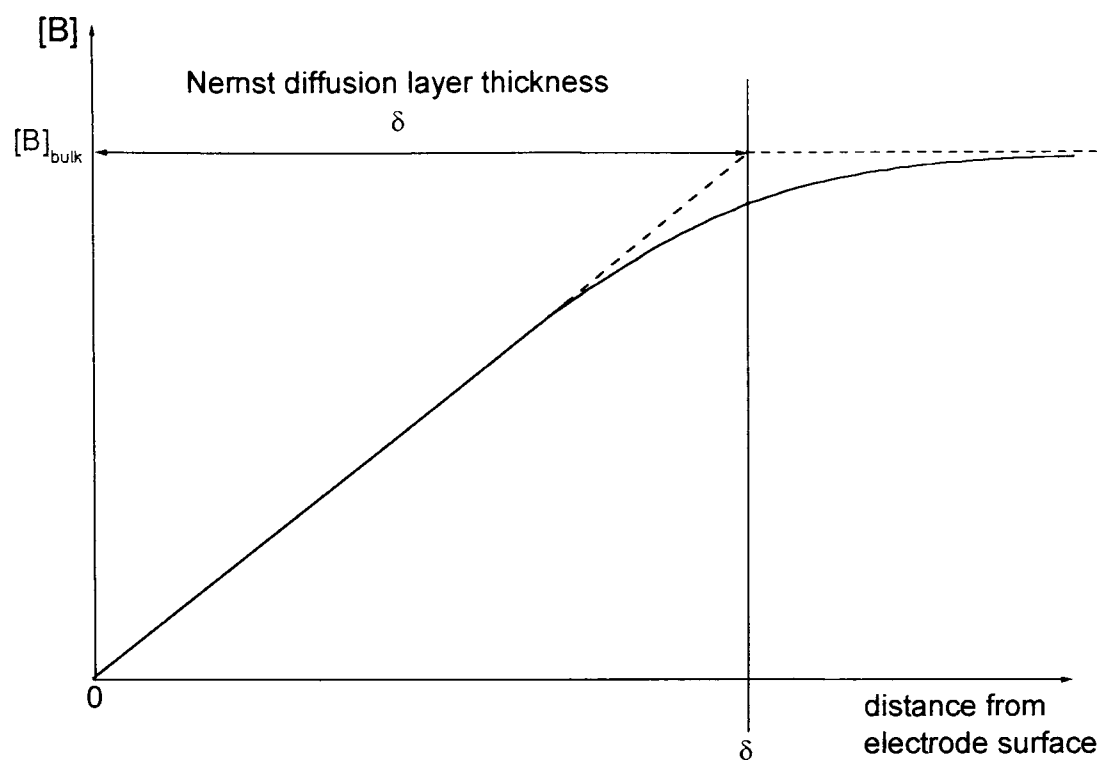


Figure 6 – Nernst diffusion layer thickness

The Nernst approach leads to an estimate of the flux of material to the electrode surface enabling the current due to linear diffusion perpendicular to the electrode to be found.

$$\frac{i}{FA} = j_d = D_B \frac{[B]_{bulk} - [B]_0}{\delta} \quad (44)$$

where i is the current, A the area of the electrode and $[B]_0$ is the concentration of B at the electrode surface.

1.2.3.4 Migration

The drop in electrostatic potential between the electrode and the solution results in an electric field ($d\phi/dx$) at the interface between the two phases. This induces movement of ions to and from the electrode by exerting an electrostatic force (equation 45) on charged species within the interfacial region. These effects are known as ‘migration’ effects and can be quantified in terms of the migratory flux, j_m (equation 46).

$$Force = \frac{zF}{N_A} \frac{\partial \phi}{\partial x} \quad (45)$$

$$j_m \propto -u[B] \frac{\partial \phi}{\partial x} \quad (46)$$

where the ionic mobility, u , is dependent on the ionic charge and size and solution viscosity. In order to simplify the interpretation of such complex mass transport properties a background electrolyte is added (the range $0.01\text{--}1\text{ mol dm}^{-3}$ is adequate depending on the solvent and the desired potential window)^{13,14}. This acts to ensure that at the interfacial region there are sufficient ions to maintain near electroneutrality by a slight redistribution of the anions and cations making up the background electrolyte. Electric fields ($d\phi/dx$) do not build up in the solution as electrolysis proceeds and the system is not perturbed by the small charge injection that arises from the electrolytic reaction, meaning that migratory mass transport effects can be neglected.

Further benefits of adding a background electrolyte include:

- lowering of the electrical resistivity of the solution, otherwise the passage of current through the cell is limited by the bulk conductivity rather than the interfacial region of interest (see s.1.1.5);
- the ‘electric double layer’ (see s. 1.1.2) across which the interfacial potential drop occurs is compressed to $10\text{--}20\text{ \AA}$ permitting electron transfer between the electrode and the electroactive species via quantum mechanical tunnelling;

the ionic strength of the solution is effectively constant hence the activity coefficients of the reactants and products are also constant. As a result the electrode potentials are constant and are quoted as formal electrode potentials (see s.1.1.4).

1.2.4 Voltammetric Methods

1.2.4.1 Chronoamperometry

When a process is occurring at the electrode, a concentration gradient is created by the consumption of an electroactive species at the electrode surface. Chronoamperometry is often concerned with the variation of current with time for $t > 0$ when the potential is stepped at $t = 0$ from a value where no electroactive species react, to a value where all the species that reach the electrode react. For a uniformly accessible planar electrode the resulting variation of current with time, the solution of the diffusion equation, Fick’s Second Law, is the Cottrell equation^{1,15} the validity of which was verified by Kolthoff and Laitinen^{16,17}:

$$i = nFAJ = \frac{nFAD_B^{1/2}[B]_{bulk}}{(\pi t)^{1/2}} \quad (47)$$

where i is the current measured at time t at an electrode of area A , and $[B]_{\text{bulk}}$ is the bulk concentration of electroactive species B . The Cottrell equation is particularly important owing to the widespread use of the pulse techniques detailed in s.1.2.4.4.

For a hydrodynamic electrode or microelectrode, discussed in s.1.2.5, $(\partial c / \partial t) = 0$ which means that currents do not vary with time under steady state conditions. However, this is only the case for steady state conditions, $(\partial c / \partial t) \neq 0$, for example, in fast cyclic voltammetry at a microelectrode.

1.2.4.2 Potential Sweep Techniques

Potential sweep voltammetry is a rapid and useful technique, which can potentially provide a large amount of information on the electrochemical system under scrutiny. The principle behind cyclic voltammetry (CV) and linear sweep voltammetry (LSV) is scanning a region of potential and measuring the current response arising from the electron transfer reactions that occur. Since the measured current is proportional to the concentration of electroactive species they are also able to give a quantitative response. The potential waveform for cyclic voltammetry is shown in **Figure 7**.

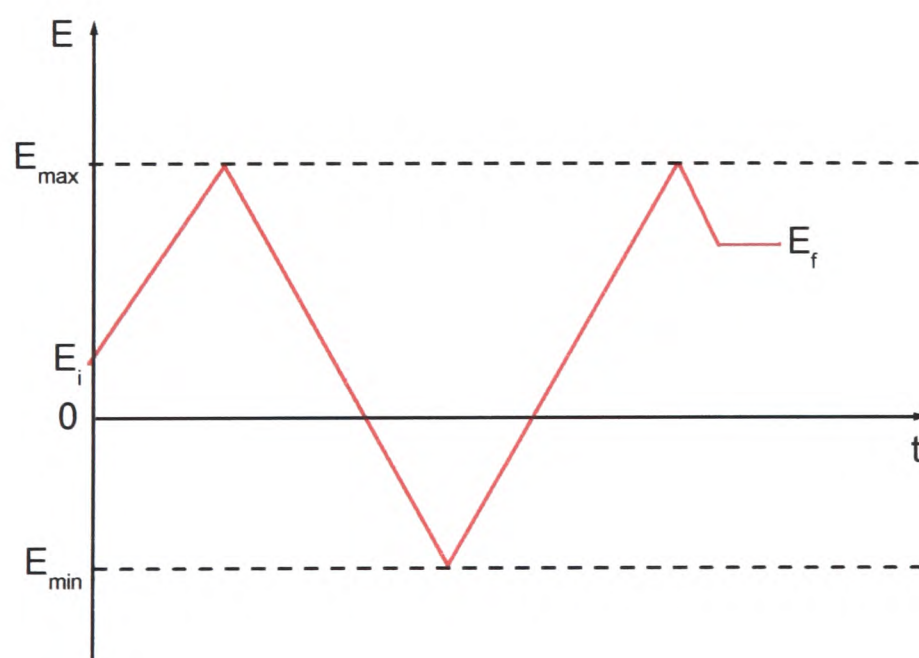


Figure 7 – Potential waveform for cyclic voltammetry: E_i is the initial potential, E_f the final potential, E_{max} and E_{min} the maximum and minimum potentials respectively.

A linear sweep corresponds to the first half of the triangular waveform. The adjustable parameters of the sweep include scan range (the initial and final potentials), sweep rate, v , (equation 48) and potential step.

$$v = \left| \frac{dE}{dt} \right| \quad (48)$$

The sweep rate, v , is particularly important with typical values varying between 1 mV s^{-1} and 1 V s^{-1} , although it is possible to implement higher sweep rates at microelectrodes. The waveform depicted in **Figure 7** is the analogue “ideal”, however since LSV and CV are routinely implemented using digital instruments it follows that the closest approximation to the voltage ramp shown in **Figure 7** is a succession of small voltage steps. Consequently it is important to ensure that the voltage steps are small enough so that the digital approach is completely equivalent.

The measured current is equivalent to the sum of a contribution from the Faradaic reaction (i_f) and a small capacitive contribution (i_c) and can be described as follows¹⁴:

$$i = i_c + i_f = C_d \frac{dE}{dt} + i_f = vC_d + i_f \quad (49)$$

Since i_c is proportional to the scan rate, v , and i_f is proportional to $v^{1/2}$ the capacitive contribution to the measured current, i , must be subtracted when the scan rate is high. This can be achieved by subtraction of the blank response with no electroactive species present.

The current/potential trace obtained using a linear sweep can be described as follows: the current rises as potentials are reached when electrode reactions can occur, a concentration gradient is formed which draws in more electroactive species from the bulk until depletion effects set in and the current falls again. This is illustrated by **Figure 8** which shows a cyclic voltammetric waveform for a reversible reaction. The technique affords detection limits of the order of 10^{-5} M .

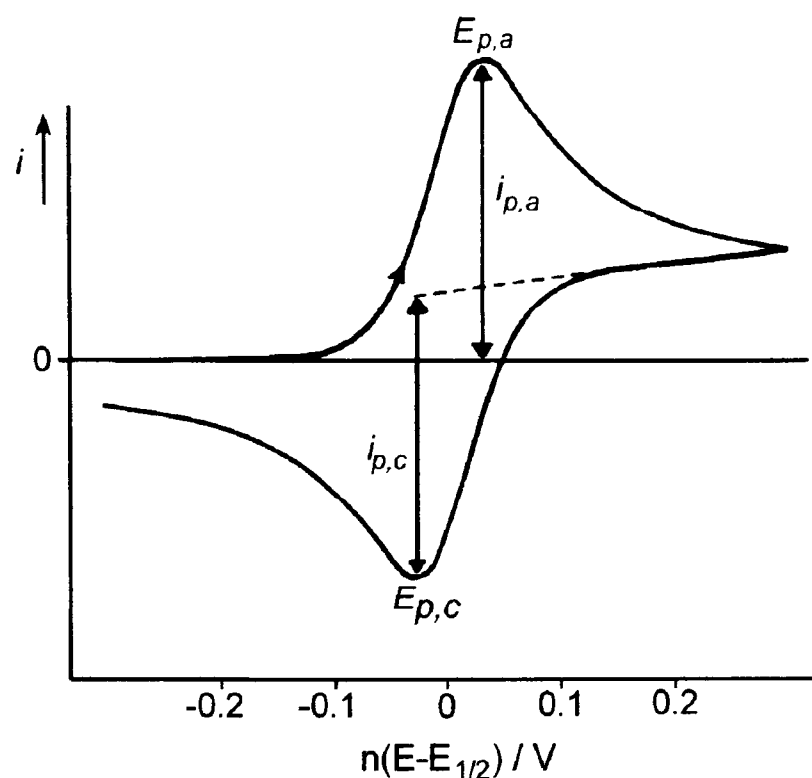


Figure 8 – Cyclic voltammogram for a reversible reaction (adapted from ref 14)

Reversible Reactions

In cyclic voltammetry of ‘reversible’ reactions, that is those with kinetics comparable with or faster than the timescale of the mass transport, the product of the initial oxidation or reduction is correspondingly reduced or oxidised when the scan direction is reversed. The waveform shown in **Figure 8** can be analysed in order to give the following result for the peak current for the oxidation of species B¹⁴ (T=298 K):

$$i_{p,a} = 2.69 \times 10^5 n^{3/2} A D_B^{1/2} [B]_{\infty} \nu^{1/2} \quad (50)$$

where A is the electrode area in cm², D_B is the diffusion coefficient for species B in cm² s⁻¹, [B] is the concentration of species B in mmol cm⁻³, n is the number of electrons transferred in the reaction and ν is the scan rate in V s⁻¹. It is clear from equation 50 that the peak current is proportional to the square root of the scan rate.

For a reversible wave the *peak potential*, E_p , is independent of scan rate. It is sometimes convenient to report the potential at $i_{p/2}$ which is termed the *half peak potential* $E_{p/2}$ which is:

$$E_{p/2} = E_{1/2} + 1.09 \frac{RT}{F} = E_{1/2} + \frac{28.0}{n} \text{ mV at 298 K} \quad (51)$$

A convenient diagnostic for a Nernstian (reversible) wave is:

$$|E_p - E_{p/2}| = 2.20 \frac{RT}{nF} = \frac{56.5}{n} \text{ mV at 298 K} \quad (52)$$

Irreversible Reactions

For completely irreversible reactions only the oxidation or reduction corresponding to the initial sweep direction appears and no reverse peak is observed (see **Figure 9**). This follows since re-reduction or re-oxidation cannot occur.

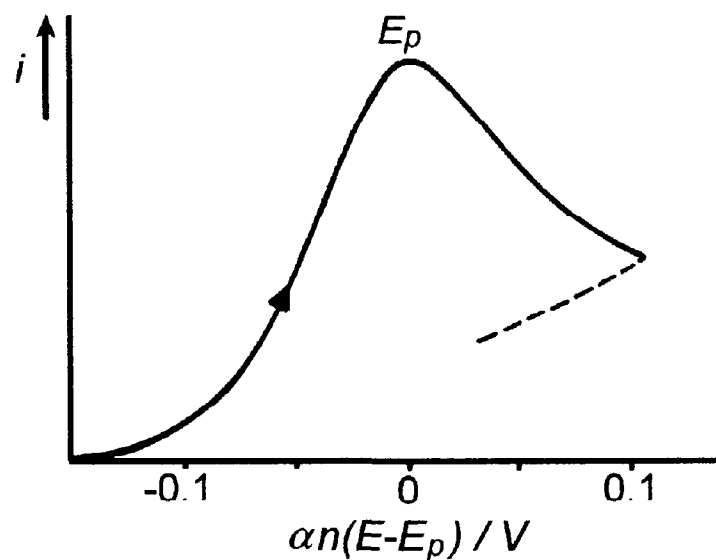


Figure 9 – Linear sweep voltammogram for an irreversible reaction. The dotted line corresponds to the continuation of current decay observed after the potential sweep is reversed (adapted from ref 14)

The peak current $i_{p,a}$ for linear sweep of an irreversible process, again given for an oxidation ($T = 298$ K) is¹⁴:

$$i_{p,a} = 2.99 \times 10^5 n(\alpha_a n')^{1/2} A[B]_{\infty} D_B^{1/2} \nu^{1/2} \quad (53)$$

Where n' is the number of electrons involved in the rate determining step with anodic transfer coefficient, α , and the units are as before. For a totally irreversible wave, E_p varies with the scan rate, ν , exemplified by equation 54¹.

$$E_p = E^{\circ'} - \frac{RT}{\alpha_a n' F} \left[0.780 + \ln \frac{D_B^{1/2}}{k^0} + 0.5 \ln \left(\frac{\alpha_a n' F \nu}{RT} \right) \right] \quad (54)$$

where k^0 is the standard heterogeneous rate constant. The dependence of E_p on ν is the effect of the kinetic limitations shifting an oxidation to more positive potentials and a reduction to more negative potentials compared to the thermodynamic values.

A redox couple may display *quasi-reversible* behaviour, manifested by a reverse peak that appears but is smaller than the forward peak. By measuring the peak potentials for forward and reverse scans standard rate constants can be deduced for simple electrode processes from the peak-to-peak separation.

1.2.4.3 Potential Step Techniques

Potential steps techniques find wide application for use with digitally based potentiostats. Consequently numerous techniques have been devised including square wave voltammetry and differential pulse voltammetry. The Faradaic response to a potential step is a pulse of current which decays with time as the electroactive species in the vicinity of the electrode is consumed. Superimposed on this response is a capacitive contribution due to double layer charging (see s.1.1.2) this dies away more quickly, often within 50 μ s.

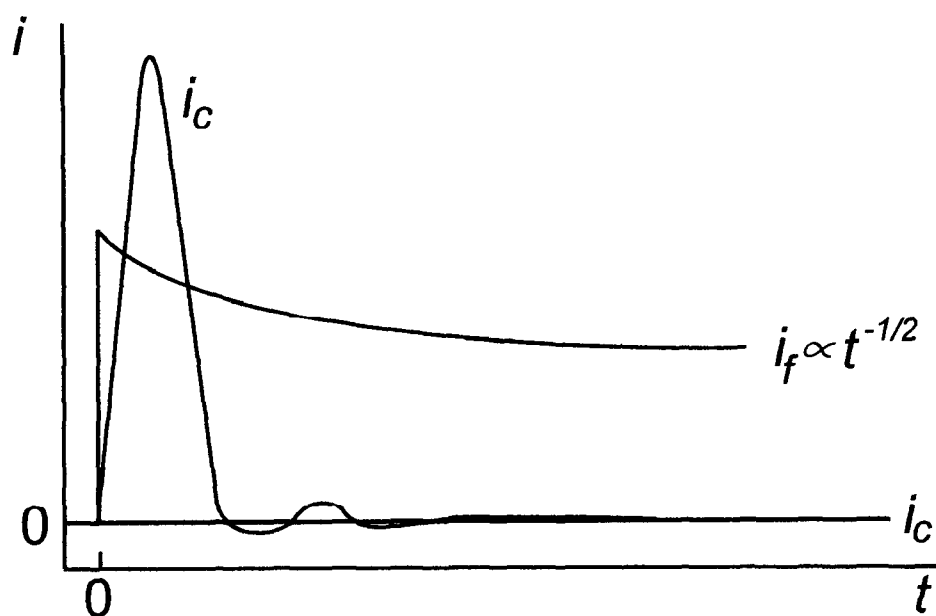


Figure 10 – Evolution of capacitive, i_c and Faradaic, i_f currents following the application of a potential step

After i_c has diminished the current for a reversible system is described by a modified form of the Cottrell equation (see s.1.2.4.1) in which $i \propto t^{-1/2}$ and the charge, $Q \propto t^{1/2}$. Pulse and step techniques can be parameterised so as to sample the current after the capacitive current has died away and also to ensure that time is not allowed for natural convection effects to influence the response.

Differential pulse and square wave techniques find wide use for trace analysis and are exploited extensively in this thesis. They rank among the most sensitive means for the direct evaluation of concentration and when they can be applied they are, as will be demonstrated herein, far more sensitive than molecular or atomic absorption spectroscopy or most chromatographic approaches.

They can also provide more detailed information about the system for example in the identification of oxidation states and detecting complexation effects.

1.2.4.3.1 Differential Pulse Voltammetry

Differential pulse voltammetry¹⁸⁻²¹ (DPV) is a technique typically capable of achieving detection limits of the order of 10^{-7} M. The potential waveform applied during the course of a DPV experiment is shown schematically as a waveform of pulses superimposed on a staircase in **Figure 11**.

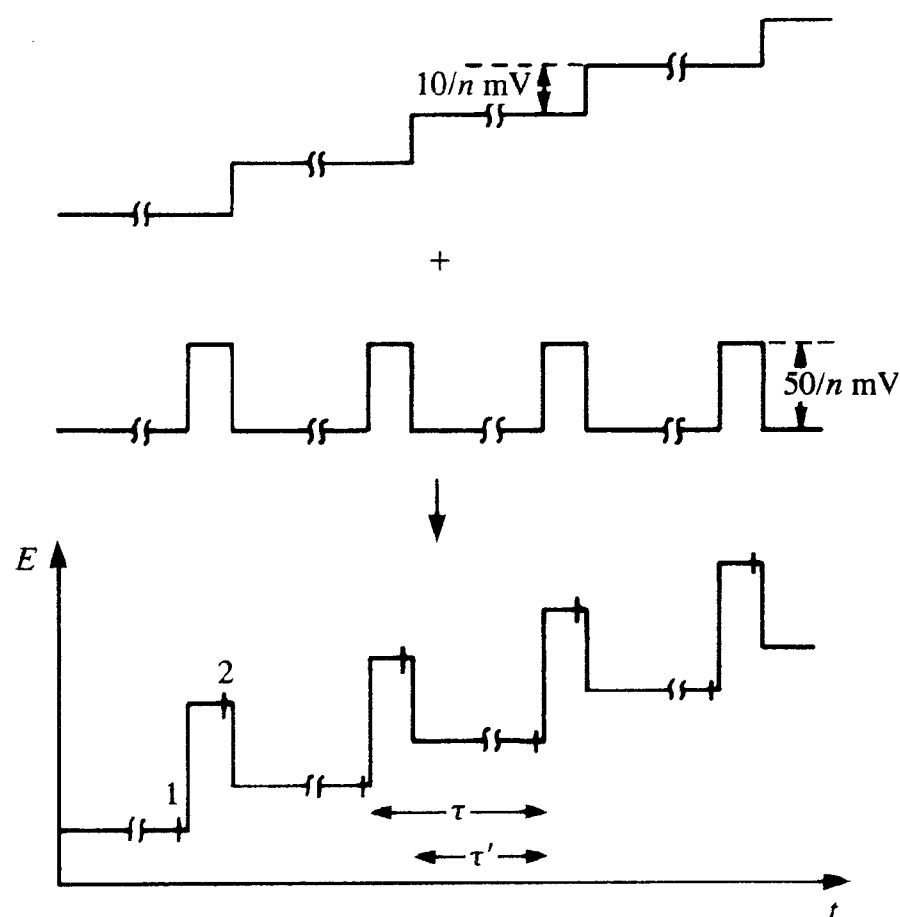


Figure 11 – Schematic waveform of pulses superimposed on a staircase where $\tau \sim 0.5 \rightarrow 5 \text{ s}$ and $(\tau - \tau') \sim 5 - 100 \text{ ms}$ (adapted from ref. 21)

In a DPV experiment the difference the current just before the end of the pulse (**Figure 11** point 2) and the current just before pulse application (**Figure 11** point 1). The pulse duration ($\tau - \tau'$) is at least ten times shorter than the period of the staircase waveform, τ' . The difference between current 1 and current 2 is plotted against the staircase potential and leads to a Gaussian like peak shaped profile as schematically illustrated in **Figure 12**.

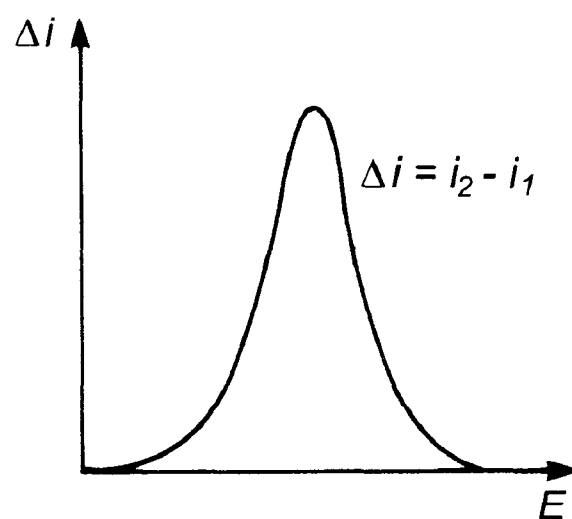


Figure 12 – Schematic illustration of the voltammetric profile resulting from a DPV scan (adapted from ref 14)

The peak potential, E_p , for a reversible system is given by¹:

$$E_p = E_{1/2} - \frac{\Delta E}{2} \quad (55)$$

where $E_{1/2}$ is the “reversible” half wave potential, ΔE is the pulse amplitude. The corresponding peak current, i_p can be found using the expression¹:

$$i = \frac{nFAD_B^{1/2}[B]_{bulk}}{(\pi(\tau - \tau'))^{1/2}} \left(\frac{1 - \sigma}{1 + \sigma} \right) \quad (56)$$

where

$$\sigma = \exp\left(\frac{nF}{RT} \frac{\Delta E}{2}\right) \quad (57)$$

It is evident from equation 57 that the quotient $(1 - \sigma)/(1 + \sigma)$ increases with increasing $|\Delta E|$ and has a value of ± 1 for large ΔE (reduction or oxidation respectively). DPV eliminates the contribution of non-Faradaic (principally capacitive) processes to the recorded signal, cancelling them out by subtracting i_1 from i_2 . It is effective because the background current from interfacial capacitance or interfering Faradaic processes differs little from the first current sample to the second.

The response of DPV can be useful in resolving signals due to two species with close half-wave potentials since the peak potential can be adjusted by changing ΔE , the amplitude of the waveform (see equation 55). However, the width of the peak at half height, $W_{1/2}$ increases with increasing ΔE . Hence amplitudes greater than 100 mV are rarely used because the resolution is degraded to an unacceptable level. The expression for $W_{1/2}$ as ΔE approaches is given by the limiting relationship:

$$W_{1/2} = \frac{3.52RT}{nF} \quad (58)$$

At 298 K the limiting width for $n = 1$ is 90.4 mV however real peaks are often wider especially if the pulse height is comparable to or larger than the limiting width.

1.2.4.3.2 Square Wave Voltammetry

Square wave voltammetry (SWV) was invented by Ramaley and Krause²² and has been developed extensively since²³⁻²⁵. An extremely versatile technique, it has been extensively reviewed by Osteryoung and O'Dea²⁵.

The square wave voltammetric waveform is, as its name suggests, a square-wave which is superimposed on a potential staircase as illustrated in the schematic diagram shown below (**Figure 13**).

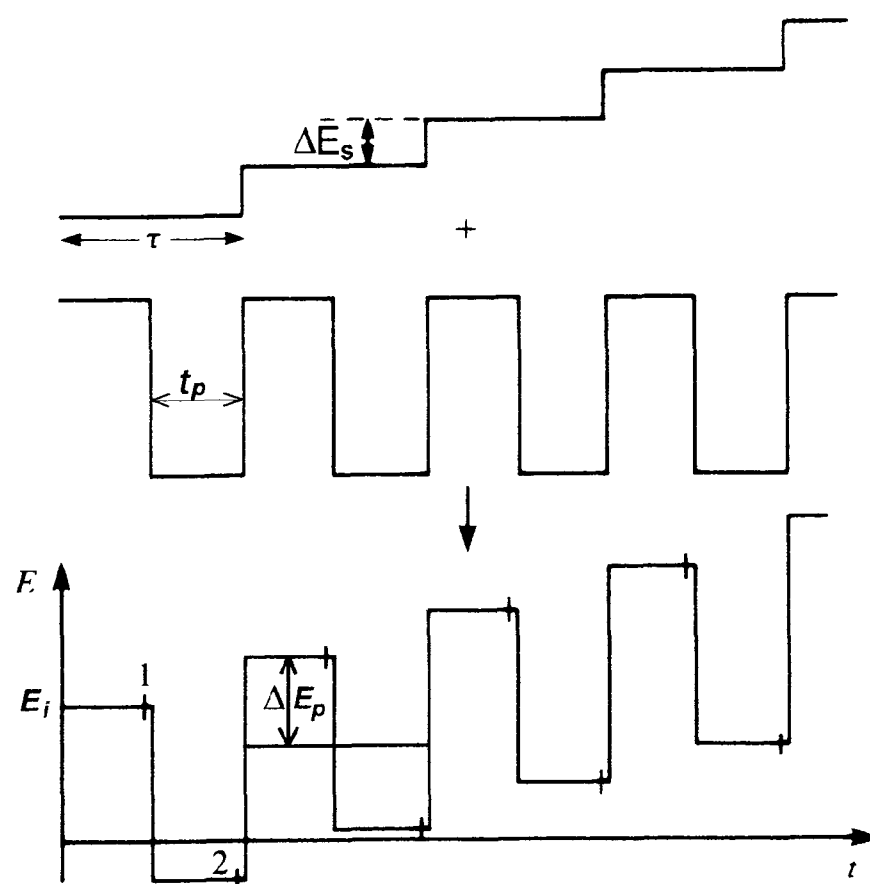


Figure 13 – Waveform and measurement scheme for SWV.
Shows schematically the sum of a staircase and a square wave (adapted from ref. 14)

The square wave is characterised by a pulse height or square wave amplitude, ΔE_p , the staircase height, ΔE_s , and pulse width, t_p , for each cycle τ . Alternatively the pulse width can be expressed in terms of the square wave frequency, $f = 1/2t_p$. The staircase shifts by ΔE_s at the beginning of each cycle;

hence the scan rate $\nu = \frac{\Delta E_s}{2t_p} = f\Delta E_s$. In normal practice ΔE_s is significantly less than ΔE_p , which

defines the span of interrogation in each cycle and therefore determines the resolution of voltammetric features along the potential axis. The procedure measures the currents at the end of the forward, i_f , and reverse, i_r , pulses as a function of the potential (**Figure 13** currents 1 and 2). The difference between these two currents, $i_f - i_r$, which is effectively the net current, is calculated, and the variation of Δi is plotted against the staircase potential as the principal result of the experiment. However, the result of a single SWV run is actually three voltammograms showing forward, reverse, and difference currents against the potential on the corresponding staircase (**Figure 14**).

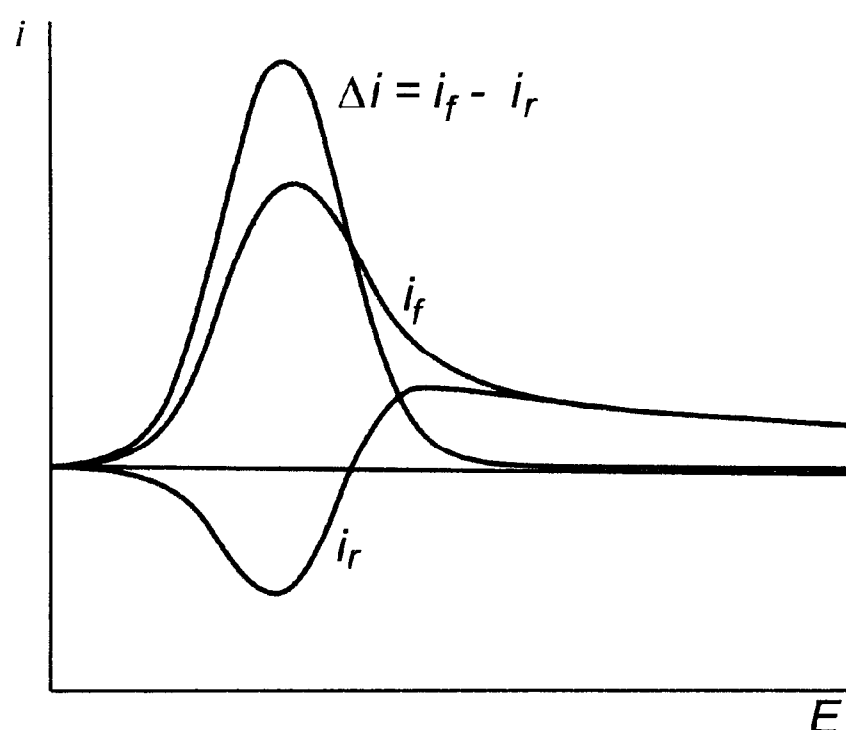


Figure 14 – Schematic voltammetric profiles of the current measured during the forward and reverse pulses and resultant difference, Δi , plotted against the staircase potential E

One of the main benefits of square wave voltammetry is the substantial annulation of capacitive contributions to the current. This is because over the small potential range between forward and reverse pulses the capacity is constant and is cancelled by subtraction. This also allows shorter pulses and faster scan rates than for other pulse techniques such as DPV. Detection limits of $10^{-8} \text{ mol dm}^{-3}$ can be attained under optimum conditions. Other advantages include a lower consumption of electroactive species and less electrode passivation/fouling due to the high scan rate. Furthermore in many electroanalytical applications in regions of negative potential, oxygen does not have to be purged from the solution when using SWV unless there is direct interference with the reaction taking place at the electrode. At negative potentials, in the limiting current region for oxygen reduction, the forward and reverse currents are equal hence Δi is zero. When scanning in the positive direction from negative

potentials, the fast effective scan rate means that no electroactive species have time to diffuse to the electrode surface from the bulk solution.

The interpretation of square wave data is not always straightforward. If the electrode kinetics are slow then the forward current is shifted to a higher overpotential than the reverse current and the reverse current may disappear entirely. For more complex voltammograms, two peaks may appear in the net current voltammogram which could lead to the erroneous conclusion that two species are reacting unless the individual profiles are monitored.

1.2.5 Microelectrodes

The utility of microelectrodes has already been alluded to in s.1.1.5 and s.1.2.4.1. Microelectrodes are smaller than a conventional electrode, which might have dimensions of metres, centimetres or millimetres. Diffusion to such conventional electrodes is assumed to be planar since the electrode dimensions are large compared with the diffusion layer thickness. For very small electrodes the radial component becomes important and, with time, eventually dominates as illustrated schematically in **Figure 15**.

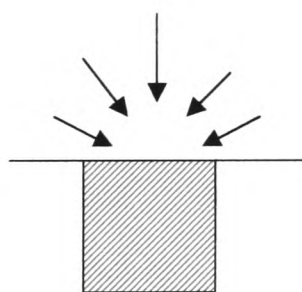


Figure 15 - cross-section of a planar disk microelectrode with a representation of the way in which diffusion of electroactive species to the electrode occurs

Applications may also take advantage of the low ohmic drops that are characteristic of such small electrodes (s.1.1.5) for example in order to perform experiments in non-aqueous solvents. The most common type of microelectrode is the planar disk microelectrode, and it is this type of electrode which is occasionally used in the work reported in this thesis. This type of microelectrodes can be manufactured by sealing a fine wire in an insulator such as glass or plastic resin and then exposing and polishing a cross section of that wire.

The smaller size of the microelectrode leads to lower capacitive contributions to the total current and the possibility of attaining a steady state current in relatively short times. At very short times, where δ

is small compared to the radius of the electrode, the current follows the Cottrell equation (equation 47) and semi-infinite linear diffusion applies. At longer times, where δ is large compared to the critical dimension, the current approaches a steady state. For a planar disk microelectrode this current is given by¹:

$$i_{ss} = 4nFD_B [B]_{bulk} r_0 \quad (59)$$

where i_{ss} is the steady state current and r_0 is the radius of the disk.

Miniaturisation carries with it the advantages of the possibility of introducing electrodes into small volumes of solution and with submicrometre electrodes recording currents of the order of picoamperes the need for any background electrolyte is significantly diminished. However unsurprisingly such electrodes lack mechanical robustness and are rapidly fouled when experiments are conducted in “real” media, for example biological samples or industrial waste.

1.3 Electrode Materials

The choice of working electrode material is determined by the potential range of the material in the relevant solvent and also its environmental impact. It is also necessary for material to be inert over the potential range of study. Common electrode materials include inert metals such as platinum²⁶⁻²⁸, gold and iridium, mercury^{18,29}, boron-doped diamond³⁰⁻³² and carbon³³. The properties of some electrode materials of particular interest are exemplified below.

1.3.1 Glassy Carbon

Carbon electrodes are commonly used in electroanalytical experiments due to their low background current, wide potential window, chemical inertness and low cost³⁴⁻³⁶. Various types of carbon can be used as electrode materials including glassy (vitreous) carbon³⁷, carbon fibres³⁸, carbon black^{39,40}, carbon paste⁴¹ and even glassy-carbon paste electrodes⁴² (**Figure 16**).

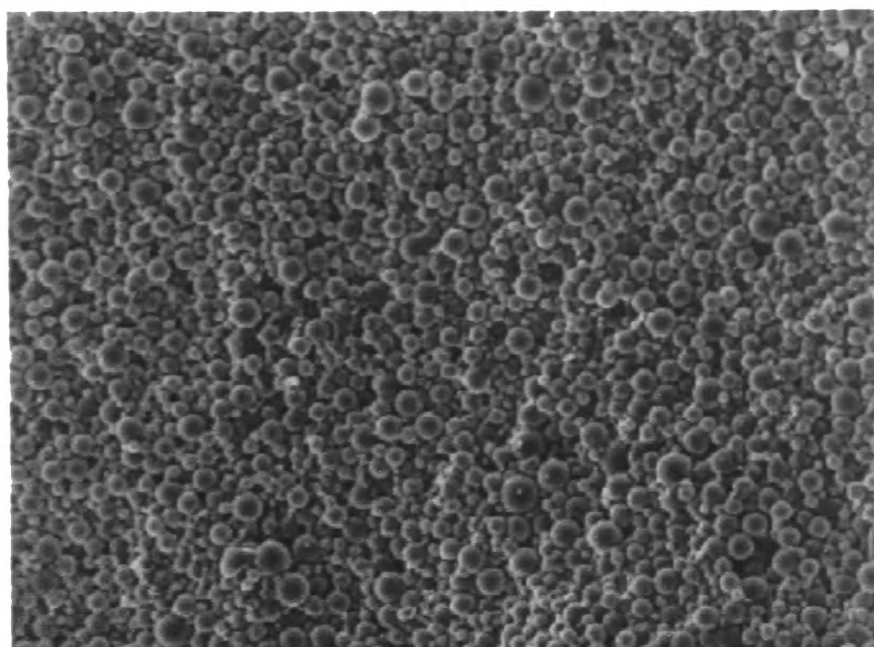


Figure 16 – Scanning Electron Microscopy image⁴² of a glassy carbon paste electrode based on 0.4 – 12 μm particles

The most widely used form of carbon for electroanalysis is glassy carbon, which is isotropic but has the disadvantage of being hard and fragile. Glassy carbon is usually manufactured by heat treatment of polymeric resins such as polyacrylonitrile or phenol/formaldehyde polymers^{43,44}. The bulk has a complex structure of interwoven graphite ribbons, shown schematically in **Figure 17**, and the basic structural units are planar aromatic structures with basal and edge plane⁴³.

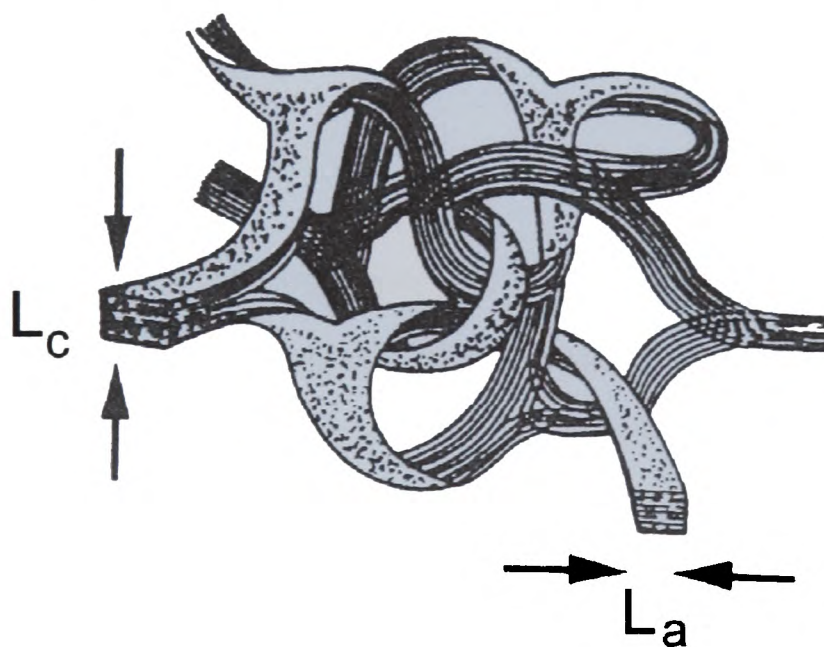


Figure 17 – Schematic representation⁴⁵ of the interwoven graphite ribbon structure of glassy carbon. L_c and L_a refer to the interplanar microcrystallite size and for glassy carbon both lie between 500-1500 Å

The density of glassy carbon is approximately 1.5 cm^{-3} and its porosity is about 35 %^{43,44} however it is impermeable for gases and liquids which suggests that the void space is small and unconnected⁴³.

Figure 18 shows a scanning tunnelling microscopic image of the surface of polished glassy carbon, it shows that the surface is rough at the nm level with ellipsoidal particles of average size 25 nm corresponding to graphite microcrystallinities⁴⁶.

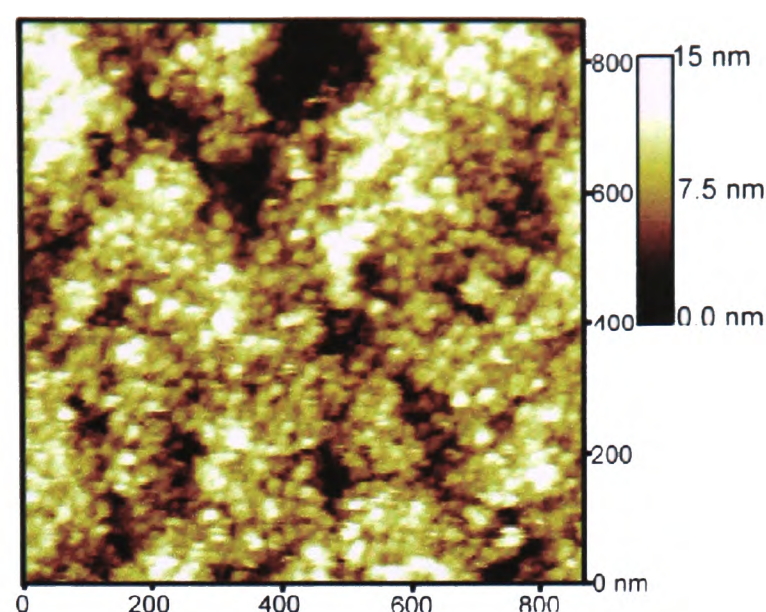


Figure 18 – Scanning tunnelling microscopic image⁴⁵ of polished glassy carbon

Glassy carbon electrodes usually undergo a pretreatment process in order to obtain a reproducible or heightened electrochemical response⁴⁶, this may be mechanical polishing⁴⁷, the method employed in this thesis, or electrochemical activation⁴⁶. The effect of electrochemical activation on the surface structure of glassy carbon has recently been explored using scanning tunnelling microscopy STM⁴⁵ and it is proposed that the electrochemical behaviour of an activated glassy carbon electrode is related to the void volume and the size of the electroactive species.

1.3.2 Boron-Doped Diamond

Boron-doped diamond (BDD) is a relatively new electrode material, which has recently gained in popularity as an effective substrate for a number of electrochemical applications⁴⁸⁻⁵³ by virtue of the robustness of diamond, both structurally and chemically³². Diamond grown by gas phase chemical vapour deposition (CVD) becomes semi-metallic when highly boron doped, typically 10^{20} - 10^{21} cm⁻³. It has low levels of background interference which allows it to be useful over a wide potential window of approximately 3.5 V in aqueous solution. The unique properties of BDD make it ideally suited to stripping voltammetric techniques, however, BDD is only just beginning to be exploited for electroanalytical applications⁵⁴⁻⁶⁰.

1.3.3 Mercury Plated Electrodes

Mercury thin film electrodes (MFE) are well established as an electrode material for electroanalytical applications⁶¹⁻⁶⁴. They consist of a thin layer of mercury covering an inert support. Their increased sensitivity over the hanging mercury drop electrode (HMDE) or the static mercury drop electrode

(SMDE) is largely a result of their high surface area to volume ratio⁶¹. Furthermore, faster rates of mass transport during the deposition step of anodic stripping voltammetry (s.2.2.3.1) can be achieved due to their greater mechanical stability when compared with HMDE's or SMDE's.

1.3.3.1 Mercury on Glassy Carbon

Glassy carbon is a common support for MFE's, its principal advantages being the ease of preparation, high sensitivity, wide potential window and low interaction with the mercury⁶¹⁻⁶⁴. However, poor mechanical properties are a result of weak adhesion to mercury. Mercury on glassy carbon does not resemble a film; it is deposited in the form of randomly distributed tiny droplets⁶³⁻⁶⁶. Alternative substrates, such as platinum, copper and silver, provide mercury thin films with improved mechanical properties. However the formation of intermetallic compounds due to the dissolution of the substrate in mercury can place limitations on their utility^{67,68}.

1.3.3.2 Polymer Coated Mercury Electrodes

Electrodes coated with semi-permeable polymer films that allow passage of the electroactive species to the electrode, while restricting the approach of surface-active compounds, have been reported to yield improved voltammetric signals in the electroanalytical determination of trace heavy metals⁶⁹⁻⁷⁴. This ability is attributed to the size or charge discrimination properties of polymer films, which allow the comparatively small heavy metal ions to be transported across the polymer film while excluding large surfactant molecules, proteins or suspended organic material. Dialysis membranes, cellulose acetate and Nafion® are examples of polymer films which have been used for this purpose⁷⁵⁻⁸¹. In particular, Nafion® coated electrodes have been successfully employed for the detection of heavy metals in foodstuffs and biological fluids^{75,78,79}. As well as an increase in voltammetric signal the polymer coated mercury electrode, usually with a glassy carbon substrate exhibits greater mechanical stability than in the absence of the film allowing devices such as an ultrasonic horn to be introduced⁶⁶.

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Chapter 1 - Introduction

Part 2

Sonoelectrochemistry

1.4 Physical Aspects of Sonoelectrochemistry

1.4.1.1 Introduction to Power Ultrasound

Normal human hearing detects sound waves of frequencies between 16 Hz and 16 kHz. Ultrasound is defined as sound waves of frequencies higher than the human ear can detect (i.e. greater than 16 kHz). The frequencies of ultrasonic sources cover a huge range, from 16 kHz to 500 MHz (**Figure 1**). The lower frequency, high power ultrasound region lies between 20 kHz and 500 kHz. It is this region of the sonic spectrum which can be synergistically coupled with electrochemistry.

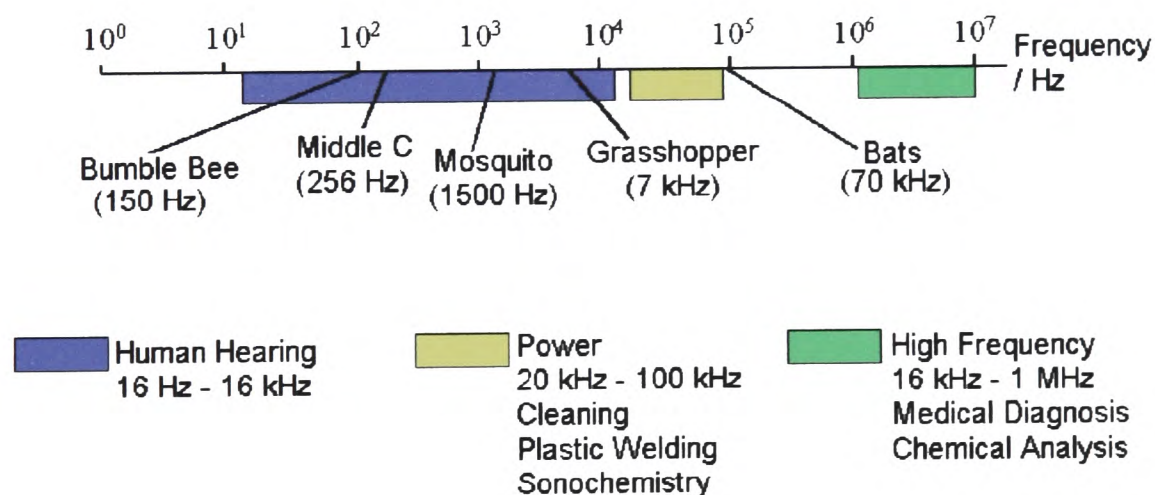


Figure 1 – Ultrasound Spectrum¹

Ultrasound is generated by utilising the inverse piezoelectric effect discovered in 1880^{2,3}. The ultrasonic transducer functions by producing a change in the dimension of a material by the application of an electrical potential across opposite faces. By alternating the potential across the faces at high frequency the crystal converts the electrical energy to mechanical energy. At sufficiently high frequency of alternating potential this will result in the generation of ultrasound⁴. The natural resonance frequency of response to piezoelectric stimulation provides the optimum ultrasonic performance. Target ultrasonic frequencies can be generated by the use of different crystal size, material and cut. Examples of commonly used materials are barium titanate, BaTiO_3 , and lead zirconate titanate, $\text{Pb}(\text{Zr},\text{Ti})\text{O}_3$ ⁵.

Ultrasound has long been used for a wide range of applications⁶. These include *inter alia* the welding of plastics⁷, ultrasonic imaging in medicine⁸, the dispersion of pigments and solids⁹, preparation of tomato ketchup and mayonnaise, cleaning both on an industrial scale¹⁰ and in precision applications such as dentistry¹¹ and in catalyst manufacture¹². Sonoelectrochemistry^{4,13,14} is concerned with the coupling of power ultrasound to electrochemical systems in order to both achieve and develop new processes and applications.

1.4.2 Approaches to the Coupling of Ultrasound with Electrochemical Methods

There are numerous methods of introducing ultrasound into an electrochemical cell. These include whistle reactors, ultrasonic baths and ultrasonic horns (also known as probes)^{4,15}. Of these techniques, the ultrasonic bath is of great commercial use in cleaning applications and in sonochemical reactors. An ultrasonic bath normally consists of a number of fixed frequency (typically 20-100 kHz) transducers beneath the physical exterior of the bath unit. The bath unit is filled with distilled water and an electrochemical cell can be immersed in the bath in a fixed position¹⁶. The transducer is electrically isolated from the cell which is advantageous. However the results have been found to depend strongly on positioning of the cell within the bath and bath design which is clearly undesirable¹⁷. The ultrasonic horn transducer (**Figure 2**) provides an alternative to the bath, it delivers ultrasound directly to the solution within the cell using a metal horn tip.



Figure 2 – 20 KHz ultrasonic horn equipped with a titanium alloy microtip of 3 mm diameter

The horn acts as an amplifier with the shape of the tip determining the mechanical amplification of the piezoelectric vibration. For this reason the tip has a length (typically 12.5 cm for a 20 kHz horn) which corresponds to a multiple of half wavelengths of the ultrasonic wave. The most common material for horn construction is a titanium alloy ($\text{Ti}_6\text{Al}_4\text{V}$). This is chosen because of its strength, durability and relative chemical inertness. The horn is then placed in the electroactive solution of

interest to a depth of not less than 1-2 cm. This arrangement results in the delivery of high intensity ultrasound at, and in the vicinity of, the tip of the horn.

The advantages of the use of the horn over the bath are the higher ultrasonic intensities available (up to 1000 W cm^{-2}), the ease with which the intensity delivered to the system can be varied and the small effect on observed electrode processes resulting from the variation of cell geometry and dimensions. Problems arising from the heating of the cell, resulting from high intensity ultrasound, can be regulated by the introduction of a cooling coil connected to a thermostatted water bath. The need for bipotentiostatic¹⁸ control is removed by the insulation of the horn from the electrochemical cell by the use of a screw thread machined from Delrin®. The ultrasonic frequency of a piezo driven horn is fixed and the most commonly used frequency is 20 kHz. Such equipment is readily available commercially and is easily introduced into the electrochemical cell. A simple design for a three electrode cell is shown in **Figure 3**.

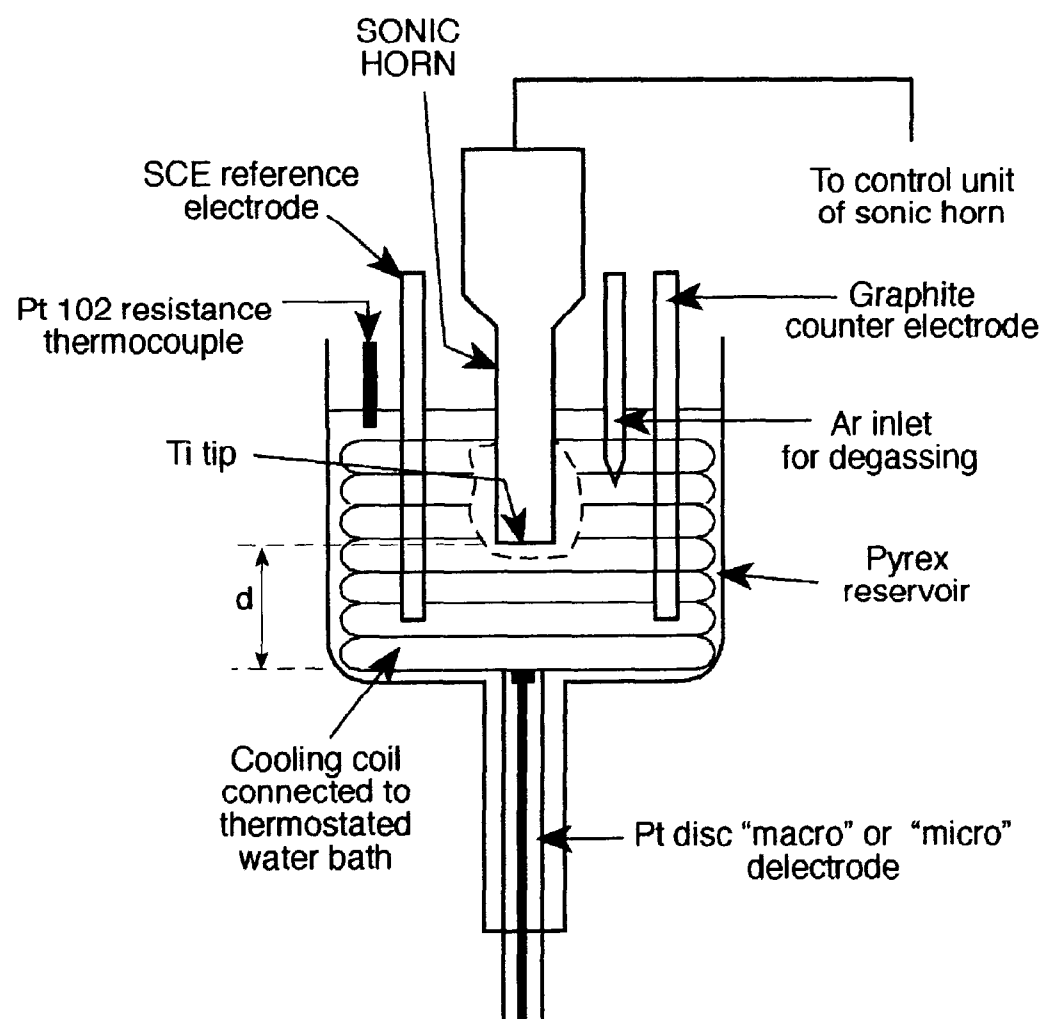


Figure 3 – Simple design for a three electrode cell incorporating an ultrasonic horn. The dotted line shows a “cut-away” of the cooling coil to reveal the titanium tip

Experiments may be conducted using alternative geometries of the ultrasonic horn relative to the working electrode, which permit the study of the processes governing sonovoltammetry. The “face-on” and “sonotrode” geometries are illustrated in **Figure 4**. The “face-on” geometry is the most

conventional and analytically useful. It is primarily this geometry which is exploited and described in subsequent sections.

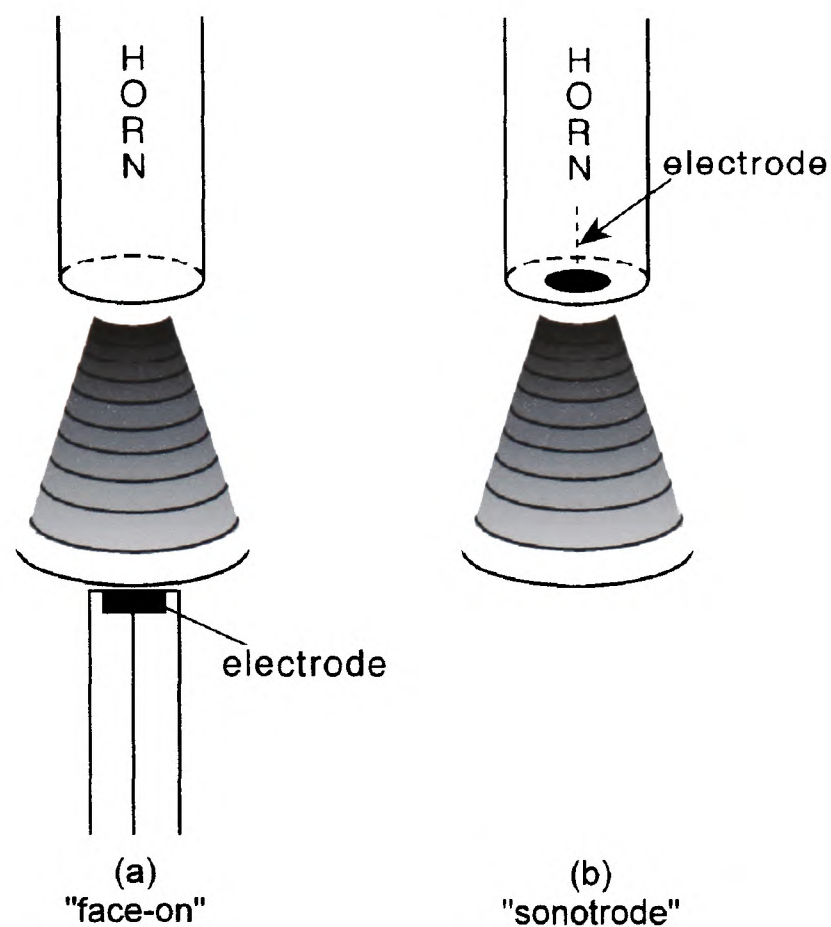


Figure 4 – Typical horn – electrode geometries

In the sonotrode arrangement (**Figure 5**) the working electrode is incorporated in the tip of the ultrasonic horn¹⁹.

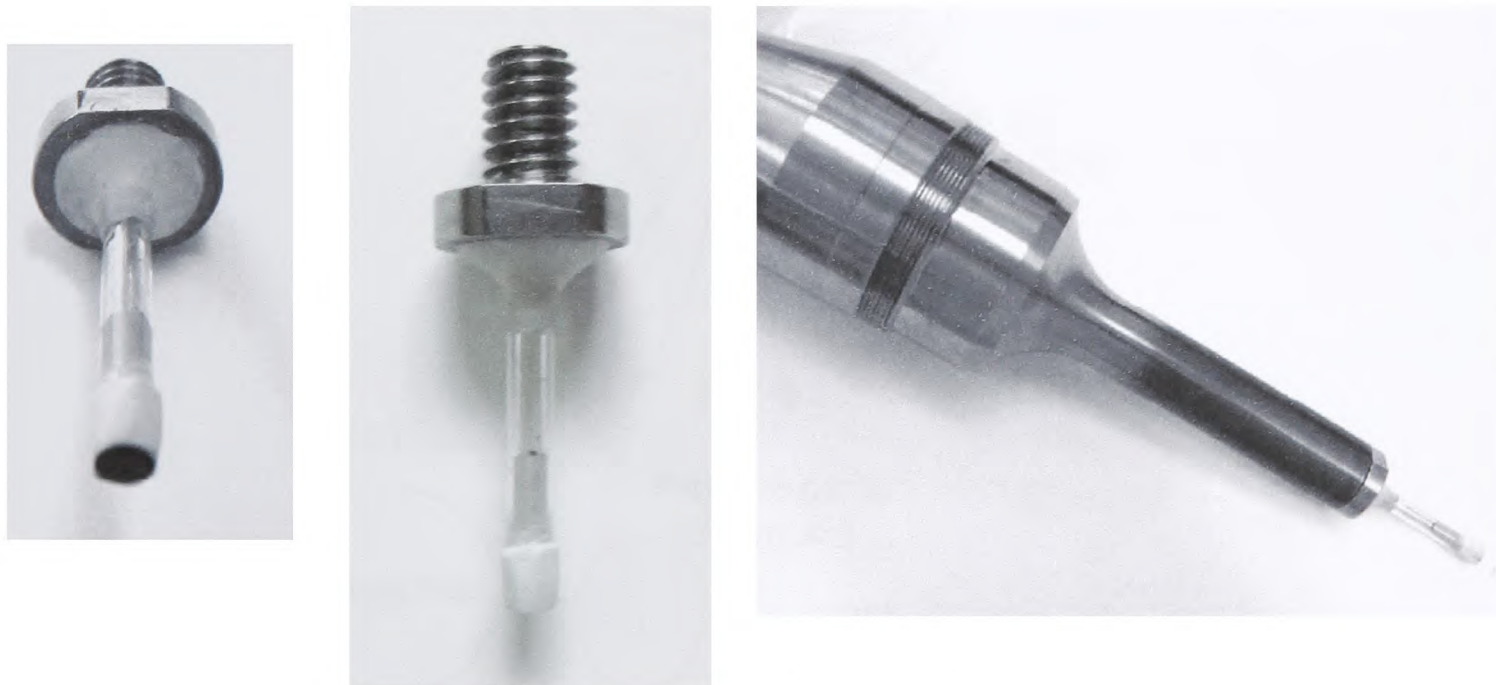


Figure 5a – 3mm diameter glassy carbon sonotrode²⁰

It is proposed that in this arrangement, mass transport to the electrode surface can be maximised and extreme limiting currents corresponding to diffusion layer thicknesses of less than 1 μm have been inferred^{19,21,22}.

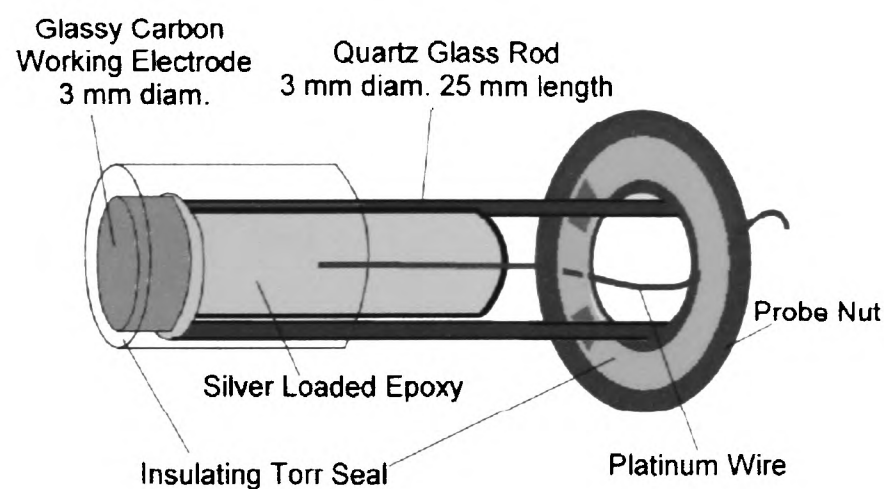


Figure 5 b – Schematic diagram of a 3mm diameter glassy carbon sonotrode²⁰

1.4.3 The Influence of Ultrasound on Mass Transport at the Electrode

Power ultrasound, of frequencies between 20 kHz and 500 kHz is known to cause a multitude of complex phenomena in solution; these include acoustic streaming²³, bubble oscillation and cavitation²⁴ with a resulting generation of microjets²⁵ and shockwaves occurring preferentially at the solid-liquid interface. **Figure 6** shows a schematic diagram of the most significant effects observed in sonoelectrochemical systems, acoustic streaming and cavitation.

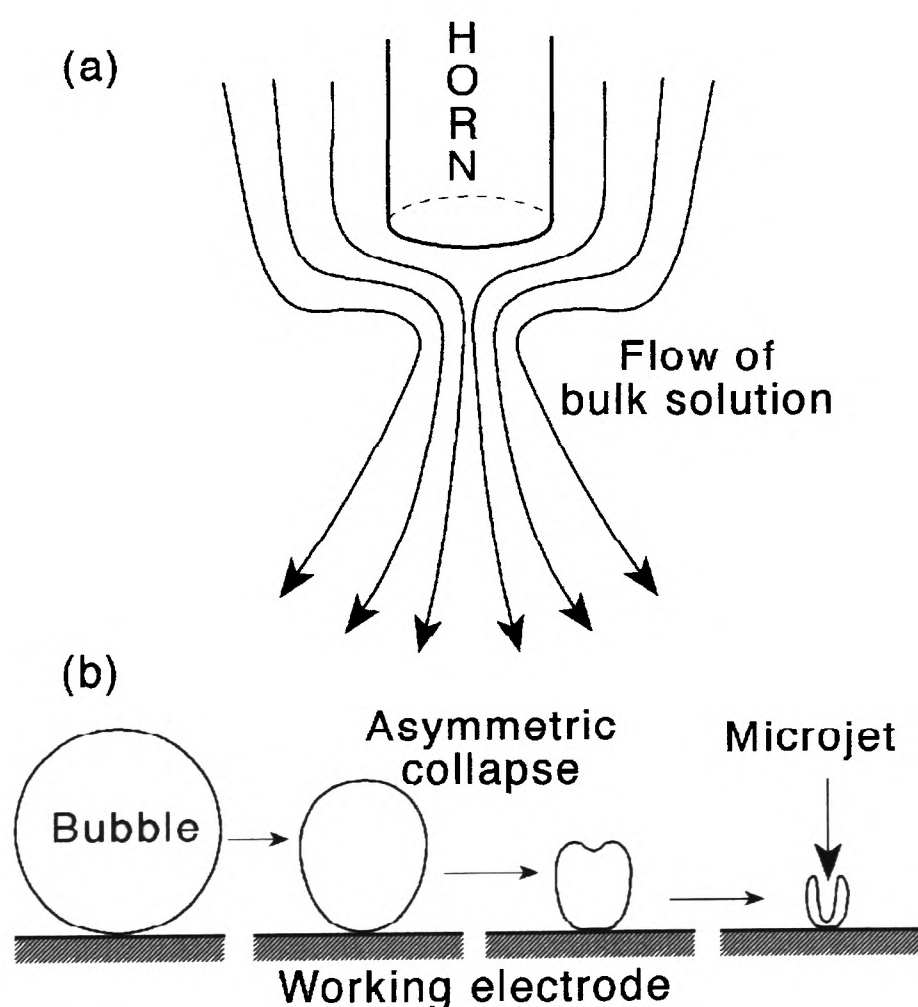


Figure 6 – Schematic diagram illustrating the following phenomena:
a) acoustic streaming, b) surface cavitation

The application of power ultrasound to a solution drastically increases the rate of mass transport of material to the solid surface. **Figure 7** shows two voltammograms obtained at a 3 mm glassy carbon

electrode for the reduction of 1.0 mM $\text{Ru}(\text{NH}_3)_6\text{Cl}_3$ in aqueous solution with 0.1 M KCl as supporting electrolyte which is a well defined, model voltammetric system:

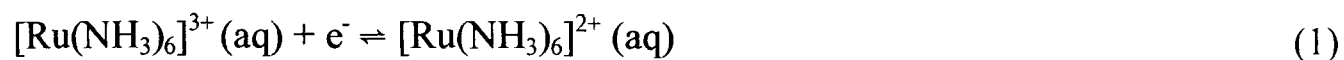


Figure 7(a) shows a cyclic voltammogram recorded under silent conditions which has the characteristic peak shape appearance expected for a reversible one electron reduction with a half wave potential $E_{1/2} = -0.2$ V vs. SCE and a magnitude consistent with a value of $D_{\text{Ru}} = 0.91 \times 10^{-9} \text{ m}^2\text{s}^{-1}$ ²⁶.

Figure 7(b) shows an analogous measurement in the presence of 20 kHz ultrasound of intensity $15 \pm 5 \text{ W cm}^{-2}$ directed in a face on arrangement with the electrode surface at 10 ± 2 mm horn to electrode separation.

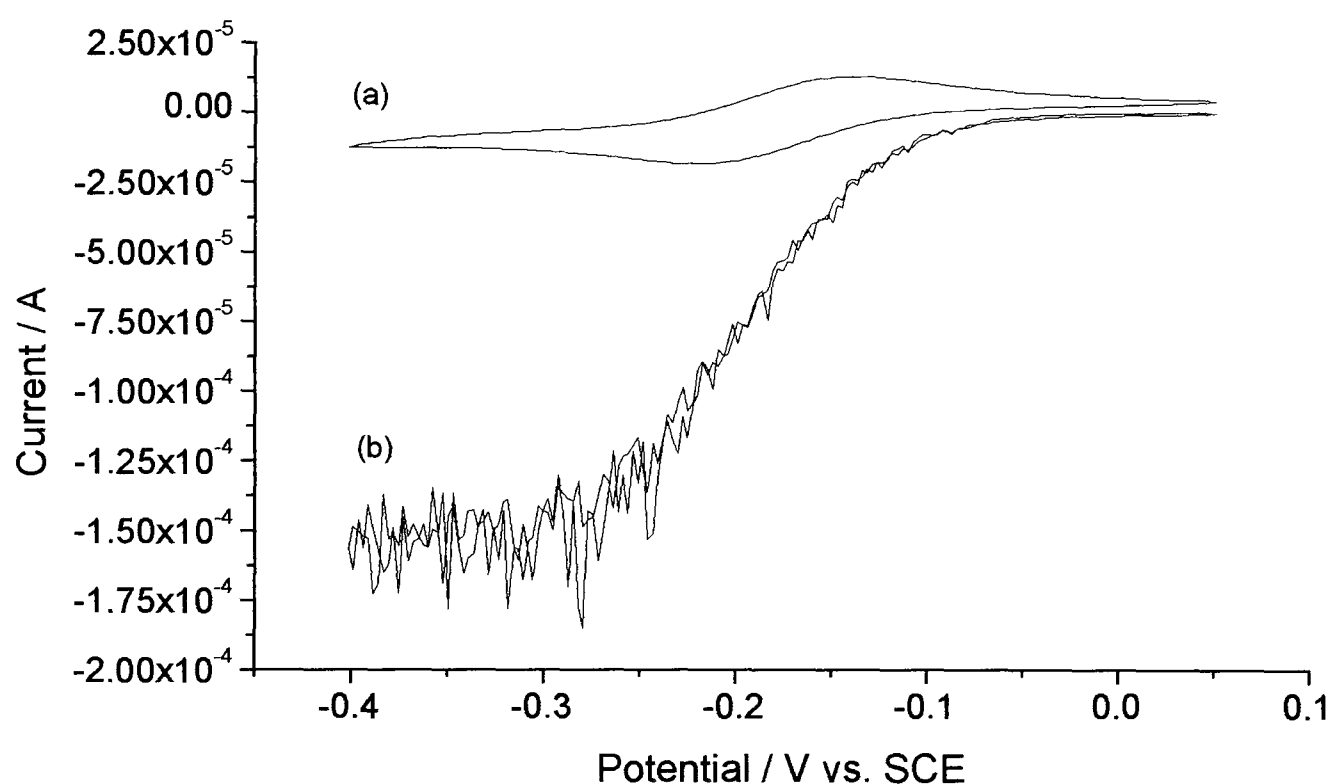


Figure 7 - voltammograms obtained at a 3 mm glassy carbon electrode for the reduction of 1.0 mM $\text{Ru}(\text{NH}_3)_6\text{Cl}_3$ in aqueous solution

a) cyclic voltammogram recorded under silent conditions

b) analogous measurement in the presence of 20 kHz ultrasound of intensity $15 \pm 5 \text{ W cm}^{-2}$ directed in a face on arrangement with the electrode surface at 10 ± 2 mm horn to electrode separation.

It can be seen that the average transport limited current is significantly enhanced when compared to the silent case. Furthermore the form of the voltammogram has qualitatively changed. A familiar cyclic voltammogram is no longer observed but a sigmoidal voltammogram indicative of a constant rate of transport of electroactive species to the electrode surface thus sustaining an average steady current. The magnitude of the limiting current observed in the presence of ultrasound is found to increase on the reduction of the horn to electrode separation or an increase in the magnitude of the ultrasonic intensity applied to the system²⁷. A quantitative model used to parameterise the mass

transport to both insonated microdisc and macrodisc electrodes assumes that the application of power ultrasound results in a truncated or thinned Nernst diffusion layer (see s.1.2.3.3).

The diffusion layer model allows a crude description of the mass transport at the electrode-liquid interface by assuming a laminar sublayer close to the surface and an approximately linear concentration gradient across a thin layer adjacent to the electrode. In systems where there is convection as well as diffusion, in this case due to the acoustic streaming effect of ultrasound, extra material is brought to the electrode surface than when transport is controlled by diffusion alone. The diffusion layer thickness, δ , is now smaller than before and depends on the rate of convection. Maximum observable current is limited by the rate at which reactant reaches the electrode-solution interface. If the electrode potential is gradually increased such that the concentration of reacting species at the electrode surface becomes zero, a limiting current i_{lim} is reached (equation 2). This equation consequently describes transport to the insonated electrode.

$$i_{lim} = \frac{nFD_B A[B]_{bulk}}{\delta} \quad (2)$$

where the limiting current, i_{lim} , is related to the number of transferred electrons, n , the Faraday constant, F , the diffusion coefficient, D_B , the electrode area, A , the concentration, $[B]_{bulk}$, and the diffusion layer thickness, δ . Under these conditions all the electroactive material transported to the electrode is converted into products. It is found experimentally for the “face on” geometry²¹ that:

$$i_{lim} \propto D_B^{2/3} A[B]_{bulk} \quad (3)$$

hence:

$$\delta \propto D^{1/3} \quad (4)$$

This result implies that the diffusion layer thickness is controlled by both diffusion and convection. By comparison with experimental results for hydrodynamic electrodes such as the rotating disc electrode²⁸, it can be shown that stationary electrodes with convective mass transport to the electrode surface exhibit $D^{2/3}$ dependence for i_{lim} , likewise $\delta \propto D^{1/3}$ is expected for channel electrodes and wall-jet electrodes. In the case of the insonated electrode acoustic streaming contributes significantly to this convective flux²⁶.

1.4.4 Cavitation at the Solid / Liquid Interface

A major factor in ultrasound-induced processes is the presence of cavitation both in the bulk solution and at interfaces. The phenomenon caused by voids or gas bubbles in the solution phase being coupled to the oscillating pressure field, is responsible for ‘hot spot’ processes and microjetting. There are different types of cavitation notably stable cavitation (violently oscillating bubbles) or transient cavitation (collapsing bubbles)²⁹. The ultrasound frequency and intensity determine the type and violence of the process. Cavitation at the solid-liquid interface in the vicinity of the electrode surface occurs more readily compared to that in the bulk solution due to the typically weaker molecular interactions. At the interface, crevices and active sites also exist where vapour bubbles readily form. In the presence of hydrodynamic flow, the interfacial tension is increased and the cavities collapse asymmetrically resulting in a microjet of electrolyte being cast against the surface. The nature of the microjets generated by cavitation events in the vicinity of the solid-liquid interface has been the focus of much attention with both experimental and theoretical approaches; increased mass transport and depassivation of electrodes resulting from jet speeds of up to 100 m s^{-1} have been reported³⁰⁻³².

Chronoamperometry at microelectrodes and macroelectrodes permits a direct comparison between the effects of cavitation in different solvents and at different ultrasound intensities and forms the basis for the results presented in Chapter 3. The following discussion is intended only to provide a brief introduction to the use of chronoamperometry to discern the properties of interfacial acoustic cavitation.

Figure 8 shows current-time measurements recorded at an insonated $50 \text{ }\mu\text{m}$ diameter platinum electrode in ammonia, water and DMF (at 298 and 218 K). In each case a well-characterised simple one-electron redox couple was employed as given in **Table 1**.

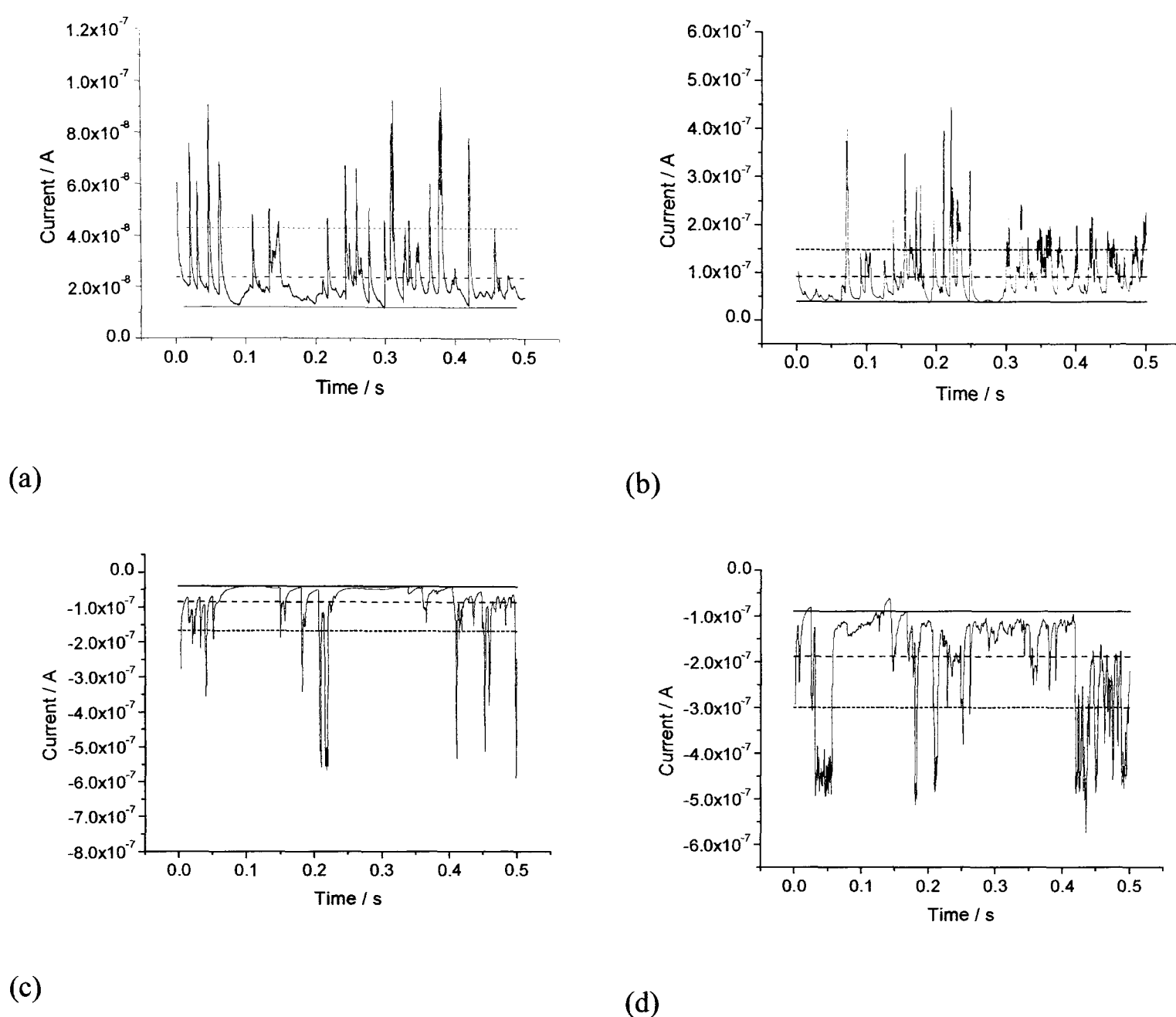


Figure 8 - Chronoamperometric experiments at a 50 μm diameter Pt disc in the presence of *ca.* 250 W cm^{-2} ultrasound in different media and temperatures.

(a) 1mM ferrocene in DMF at 218 K

(b) 1 mM ferrocene in DMF at 298 K

(c) 1 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ in water at 298 K

(d) 1 mM nitrotoluene in liquid ammonia at 218 K.

The additional lines superimposed on the traces correspond to the calculated baseline current (unbroken line) calculated average current (dashed line) and standard deviation (dotted line).

Solvent	Redox System	Supporting electrolyte	Silent Current / nA	$D / \text{cm}^2 \text{s}^{-1}$	$E_{1/2} / \text{V}$
DMF (218 K)	Ferrocene	TBAHFP	4	2.6×10^{-6}	0.774 vs. Ag
DMF (298 K)	Ferrocene	TBAHFP	12	1.2×10^{-5}	0.470 vs. Ag
H_2O (298 K)	$\text{Ru}(\text{NH}_3)_6^{3+}$	KCl	13	9.1×10^{-6}	-0.171 vs. SCE
NH_3 (218 K)	Nitrotoluene	KI	18	1.4×10^{-5}	-0.390 vs. Ag

Table 1 - Redox systems studied at a 50 μm platinum disc electrode under silent conditions.

The electrode was held at a potential corresponding to the transport limited electrolysis of the substrate of interest. Sharp spikes are observed, superimposed on a background current much enhanced in comparison with the steady state current seen under silent conditions. The background or baseline current is attributed to enhanced mass transport brought about by acoustic streaming. The spikes, caused by cavitation activity in the vicinity of the electrode, have been shown to comprise of many thinner spikes, whose rise time can be less than one microsecond³³. The conventional interpretation of this phenomenon is that on the collapse of a cavitation bubble the jet of electrolyte which is cast against the electrode surface brings new electroactive material to the electrode surface giving an enhanced current. In this way one can record direct hits or near misses of these microjets of solution as individual current transients of differing height and shape. For example, for a microjet impinging directly on the electrode surface, the current observed in the corresponding spike may be higher than if the jet had hit the edge of the electrode.

The frequency and violence of cavitation events is different from one medium to the next reflecting the differences in physical properties between solvents, primarily vapour pressure and viscosity. Cavitation bubbles are allowed to grow larger in media of high vapour pressure³⁴ and therefore when the bubble collapses the implosion is less violent due to the cushioning effect arising from its larger size. Large bubbles may also have more chance to move away from the surface of the electrode reducing the frequency of events. Conversely, much more violent events are brought about in solvents of low vapour pressure³⁴. More energy is required to disrupt the medium in order to create a void resulting in small bubbles and a reduction in the “cushioning effect”. The chance of the smaller bubbles escaping from the surface is lessened, the resulting cavitation collapses are not only more violent but also more frequent. Greater stability of bubbles in low vapour pressure can result in prolonged stability of oscillating bubbles on the surface as in the case of liquid ammonia. The viscosity is also a contributing factor with a higher viscosity resulting in much less efficient replacement of solution at the electrode surface on cavitation collapse and therefore a smaller current enhancement.

Macroelectrodes have been used to investigate the variation of cavitation processes with ultrasound intensity with the advantage that the most violent conditions can be attained with only small effects of the local erosion damage on the overall current response³⁵. Chronoamperometry at a macroelectrode permits quantitative analysis of cavitation events occurring at the surface of the electrode at differing ultrasound powers by recording the deviation of individual current data points from the mean

background current. **Figure 9** shows a plot of the percentage of current data points deviating more than 10 % from the mean for the simple one electron oxidation of ferrocene in dimethyl formamide with a background electrolyte of 0.1 M NBu₄PF₆.

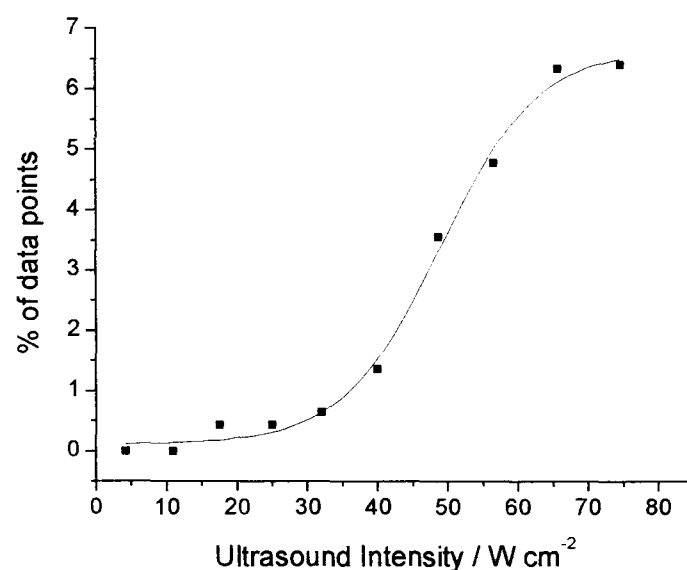


Figure 9 - Plot of the fraction of current readings for the sonoelectrochemical oxidation of 1 mM ferrocene in dimethyl formamide at a Pt disc macroelectrode with a deviation from the average current response of more than 10 % versus the ultrasound intensity employed.

It can be seen that the effect of increasing ultrasound intensity is to promote a non-linear increase in the deviation from the mean with an appearance consistent with the existence of a cavitation threshold³⁴.

1.4.4.1 Surface Acoustic Cavitation Understood via Nanosecond Electrochemistry

Recently, the use of ultra-fast electrochemical equipment has facilitated monitoring bubble activity in real time, enabling the determination of bubble sizes and giving an insight into bubble dynamics^{33,36,37}. Chronoamperometry at a 29 μm diameter platinum electrode revealed spikes in the signal with durations from 50 μs (1 acoustic cycle, transient cavitation) up to a few milliseconds (stable cavitation). Decreasing the horn-electrode separation and thereby increasing the local acoustic pressure resulted in more numerous transient cavitation events. This transition has already been well described in homogeneous solution^{29,38} but has only recently been observed at the electrode surface³³. Experiments with a single microelectrode show that the cavitation activity is complex. Oscillations at harmonics of the driving frequency are observed, and cyclic voltammetry confirms that the bubble is in contact with at least a part of the electrode. However, due to this complexity the special dependence of a single cavitation event is difficult to assess.

Microelectrode arrays have permitted direct visualisation of the spatial extension of the bubbles. Synchronous events on different electrodes have been reported. Since it is very unlikely that two different bubbles would appear at the same time on different electrodes, and given the same peak shape and lifetime, such synchronous signals on adjacent electrodes have been attributed to the same bubble. Similarly, cavitation events have been observed that are localised in the vicinity of one of the electrodes. This leads to the conclusion that bubble sizes can vary at least between 15 μm and 0.8 mm, results that were confirmed by photographic detection. The same kind of chronoamperometric signal (spikes) with almost the same current amplification was observed on all adjacent electrodes affected by the bubble. This casts doubt on the previously accepted microjet model since different signals would be expected for electrode positions below the jet, below the toroidal bubble, and outside the bubble. The authors³³, while not ruling out the presence of microjetting, concluded that it was unlikely to play a predominant role in the action of 20 kHz ultrasound. Microelectrode array experiments reveal that the localised increase in current due to cavitation is most likely due to the bubble wall freeing the electrode from the bubble and then flushing the electrode.

1.4.4.2 Ultrasound Induced Surface Effects

The cleaning effect of ultrasound harnessed in the use of ultrasonic baths is dependent upon cavitation. The same is true at the surface of an electrode. The combination of ultrasonic frequency, intensity and the electrochemical solvent as described above can give rise to considerable release of energy occurring during cavitation events and damage to a metallic electrode surface is possible. There is also evidence for the surface activation of insonated glassy carbon electrodes^{39,40}. A wide range of ex situ and in situ methods are available for the investigation of surface properties. Atomic force microscopy (AFM) may be used for the investigation of various types of electrode surfaces^{41,42}. Images of a platinum electrode polished before (**Figure 10(a)**), and after insonation for 2 min (**Figure 10(b)**) demonstrate the effect which causes a slow but significant roughening due to pitting depending on the applied intensity.

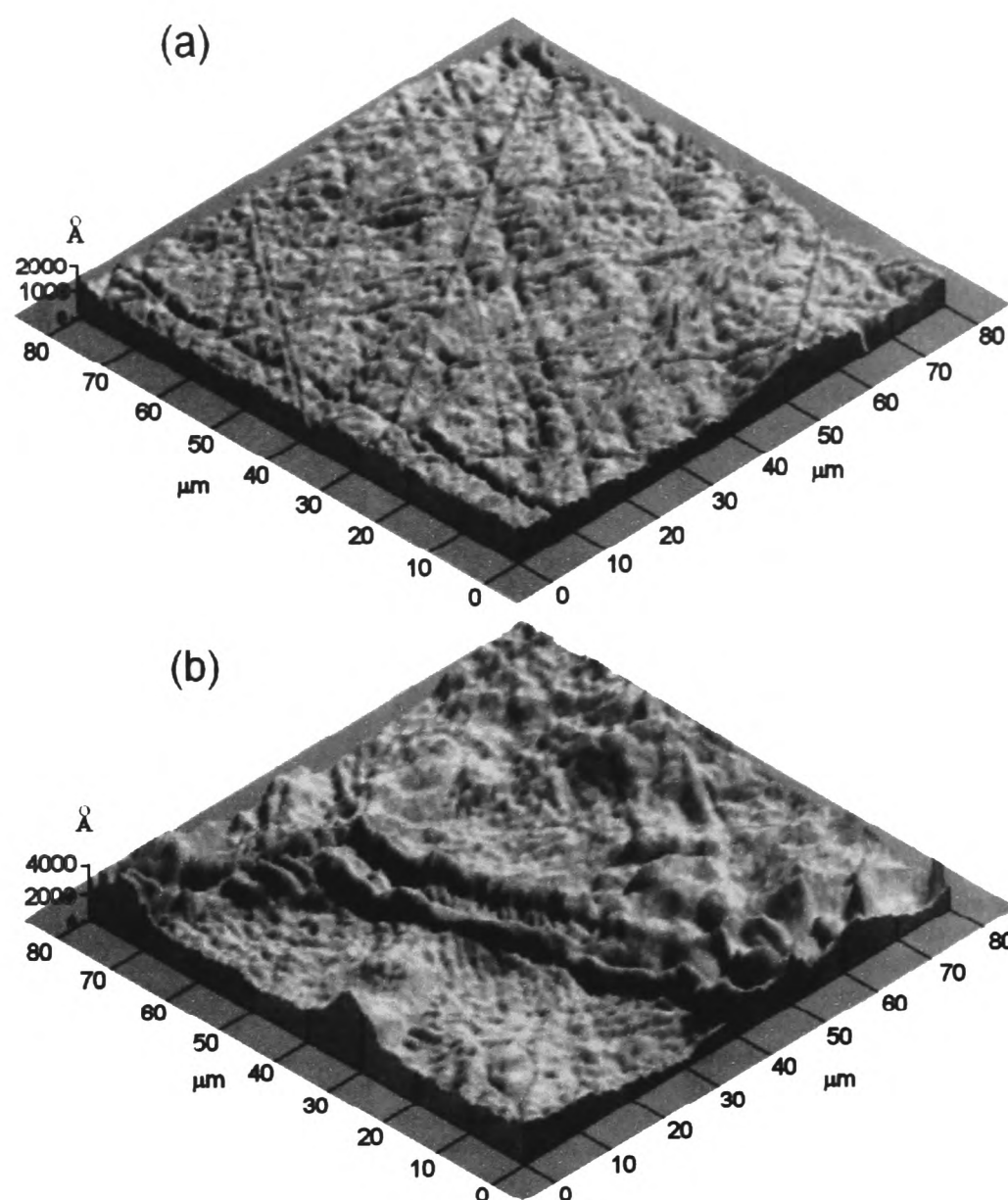


Figure 10 - Atomic force microscopy images of

a) Pt electrode surface freshly polished with 0.1 μM diamond lapping compound (maximum height of the surface topology - 2000 Å);

b) the same Pt electrode after application of power ultrasound in a solution of acetonitrile (maximum height of the surface topology - 4000 Å)

As well as the sound intensity and the liquid media used in the experiments, the erosion process depends on the hardness of the electrode material⁴³. For glassy carbon and gold electrodes considerable damage at high intensities applied in aqueous electrolyte media occurs. In voltammetric experiments however, the timescale is much shorter and the ultrasound intensity much lower meaning that the effect of erosion effect remains acceptable⁴¹ and subtler changes in the surface properties are observed.

Associated with electrode damage the “cleaning” or “depasivation of electrode surfaces is an important aspect of sonoelectrochemistry. This effect has been documented for the depasivation of

iron electrodes³⁰, the removal of deposits⁴³, and observed when polymer films are present at the electrode surface⁴⁴, it also a vital aspect of sonoelectroanalytical methods (see s.1.5). The oxidation of the carbonyl complex $\text{Cr}(\text{CO})_6$ is an example of an electrochemical system which is prone to electrode passivation⁴⁰. In the presence of ultrasound sustained voltammetric currents can be obtained which suggested both depassivation and/or a suppression of precipitation; this is in contrast to the corresponding silent voltammetry where these phenomena preclude “clean” voltammetry.

Electrochemical AC impedance methods are well suited for in situ monitoring of changes in surface roughness and capacitance. Cole-Cole⁴¹ plots obtained at 0.0 V (vs. SCE) for an insonated glassy carbon surface immersed in argon saturated 0.1 M NaOH solution show a marked deviation from the ideal behaviour. This occurs as a direct result of cavitationaly induced surface roughening. Further electrochemical manifestation of the effect is seen for the oxidation of a nickel electrode surface in alkaline aqueous media⁴⁰. In the presence of ultrasound the magnitude of the observed current and overall shape of the signal remains unchanged which is consistent with surface confined processes independent of mass transport in the solution phase. The pH dependant, lowest potential for the formation of a surface oxidised layer is termed the Fladé potential, E_F . Upon insonation E_F is shifted approximately 60 mV towards more anodic potentials. This shift has been attributed to ultrasound causing surface erosion which competes with the growth of the oxide film.

1.4.5 The Influence of Ultrasound on Chemical Processes

The use of ultrasound to enhance the rate or change the pathway of both homogeneous and heterogeneous chemical reactions has been exploited for a number of years⁴⁵. There are a wide variety of processes involved in the alteration of reaction rate or pathway. These vary from surface activation of solid reactants to the generation of active radical species. It is possible to bring about gross changes in the transport leading to new dominant reaction pathways and in many cases the processes behind such changes are not fully understood. Ultrasound might affect chemical reactions which occur in a simple liquid phase mainly via four possible routes:

- enhanced mass transport;
- thermal fluctuations caused by cavitation or “hot spots”;
- the formation of highly reactive intermediates, for example radical species in the solution near a “hot spot”;
- strong shear forces which can affect large molecules, particles or surfaces.

1.4.5.1 Generation of Active Radical Species

The hydroxyl radical has been shown to be responsible for many of the chemical effects of ultrasound in homogeneous systems. This has recently been harnessed for the treatment of waste water⁴⁶. In particular the sonochemical degradation of chlorophenols⁴⁷, chlorohydrocarbons^{48,49} and cyanide⁵⁰ have been the focus of studies aimed at the development of new methods for the elimination of traces of toxic materials. The mechanism proposed for these sonochemical processes is based on the formation of short-lived radical species (equation 6) which are generated in violent cavitation events.



Elegant spin trapping work by Riesz and co-workers⁵¹ comparing pulse radiolysis and sonolysis has provided firm evidence for the hydroxyl radical. Recently Birkin et al presented indirect electrochemical evidence of the hydrogen radical produced by cavitation as the result of ultrasonic irradiation of an aqueous solution⁵². An electrochemical method affording quantitative assessment of sonochemically produced radicals has also been reported⁵³ with detection limits of $\text{nmol dm}^{-3} \text{ s}^{-1}$. In the absence of reactive organic compounds, the sonochemical formation of $\text{OH}^\bullet$ radicals is followed by recombination processes which lead to the formation of hydrogen peroxide⁵⁴. The production of hydrogen peroxide can therefore provide an indirect indication of the presence of high energy intermediates allowing the measurement of a relative sonochemical activity index⁵⁰. Other reaction pathways are likely to be complementary such as the direct pyrolysis of unstable compounds for example nitrophenol⁵⁵ and direct oxidation induced by supercritical water⁵⁶.

The effect of 20 kHz ultrasound on the concentration of cyanide in aqueous 0.1 M NaOH is first order decay in the cyanide concentration over a period of 2.5 hours with an apparent rate constant $1.3 (\pm 0.2) \times 10^{-4} \text{ s}^{-1}$ for a solution volume of 400 mL and ultrasound intensity of 60 W cm^{-2} . However, changing the volume of solution insonated in the reactor dramatically changes the apparent rate constant with a decrease in volume causing an approximately inversely proportional increase in the rate constant for cyanide destruction. This can be understood if the sonochemical reaction does not proceed homogeneously throughout the reactor volume but is localised in a volume close to the ultrasound emitting horn tip. This volume is approximately $3 \times 10^{-6} \text{ m}^3$, with a rate constant for the destruction of cyanide within this reaction volume of $\approx 2 \times 10^{-2} \text{ s}^{-1}$.

1.4.5.2 Rates of Heterogeneous Electron Transfer

The kinetics of heterogeneous electron transfer reaction in the presence of insonation at the electrode surface has been quantified by analysing the wave shape of the sonovoltammetric signal. Huck¹⁷ investigated one electron redox systems such as $\text{Fe}^{3+/2+}$ and $\text{Fe}(\text{CN})_6^{3-/4-}$ and reported consistent results between silent rotating disc voltammetry and sonovoltammetry performed in an ultrasonic bath. Measurements have also been performed in the cell described in **Figure 1** with the working electrode placed opposite the immersed ultrasonic horn⁵⁷. The limiting currents which are observed for the simple one electron reduction of $\text{Ru}(\text{NH}_3)_6^{3+}$ and of Eu^{3+} in aqueous media at various electrode materials can be used to determine the diffusion layer thickness, δ , by applying equation 2. Calculation of the rate of electron transfer was found to give good agreement with analogous measurements under silent conditions²⁶. In all cases the sole effect of ultrasound within experimental error was to change the apparent reversibility of the electron transfer process by promoting mass transport. When the effect of the latter is corrected for, no change in the heterogeneous rate constants for electron transfer were seen in the presence of ultrasound. Consistent with these observations, Fontana *et al*⁵⁸ reported for two reversible systems, $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ and $\text{Fe}^{2+}/\text{Fe}^{3+}$, that ultrasound improves only the mass transport. However for a less reversible quinone-hydroquinone system, ultrasound improved the reversibility by a factor of 2. This improvement, if real, may be due to either an increase in the exchange current density, or to an increase in the speed of the intermediate chemical steps.

1.4.5.3 Sonovoltammetric Study of Homogeneous Chemical Reactions

The use of well-known model systems has been undertaken to confirm the validity and define the limits of the treatment of sonovoltammetry in line with the model of a uniformly accessible electrode and also to assess the influence of ultrasound on homogeneous chemical reactions coupled to the electron transfer. The uniformly accessible electrode model allows the introduction of a reaction layer which has also been successfully employed for rotating disc voltammetry, in studies using channel electrodes⁵⁹ and in a slightly more complex form for studies in turbulent voltammetry⁶⁰.

A physical model has been proposed³⁰⁻³² (see s. 1.4.4) which may be used to describe the processes which occur within the electrochemically important layer of *ca.* 100 μm adjacent to the electrode surface in the presence of power ultrasound. The current may be separated into a steady component and a transient component suggesting a significant local fluctuation of the current density. The

physical process responsible for the fluctuations is the collapse of cavitation bubbles and the formation of microjets. The number and magnitude of these jets impinging onto the electrode surface determines whether the simple stagnant diffusion layer model, or a more sophisticated model is necessary.

Many model systems, which have been studied in detail by electroanalytical methods, are available for comparison with sonovoltammetric measurements. The reduction of halogenated aromatic compounds is known to cause in many cases the cleavage of the carbon halide bond with a first order rate constant determined by the properties of the molecule. From the known range of accessible diffusion layer thicknesses in sonovoltammetry, *ca* 1 – 15 μm , unimolecular rate constants ranging from 10 to 10^4 s^{-1} are accessible. The reduction of 3-bromobenzophenone and ortho-bromonitrobenzene in dimethylformamide⁶¹ may be described by the ECE type mechanism given in equations 7a-d.



Of the two chemical reaction steps, equations 7b and 7c, usually 7b is rate limiting^{62,63}. In this mechanism an electron transfer step at the electrode surface is followed by a chemical step in homogeneous solution, in this case the cleavage of a carbon bromide bond. The sigma radical $\text{Ar}^{\cdot}$ is very reactive and may undergo H-atom transfer with the solvent or electrolyte present⁶⁴ to form the parent aromatic compound. This molecule can undergo further reduction at the electrode surface permitting a second electron to be transferred where the kinetics of the chemical step is fast enough to compete with the mass transport at the electrode surface. Two voltammetric signals are observed for the reduction of 3-bromobenzophenone in dimethylformamide (0.1 M NBu_4PF_6) at a 3 mm glassy carbon electrode in silent conditions. The first process with $E_p^{\text{red}} = -1.56 \text{ V}$ (vs. SCE) corresponds to the one electron reduction process described in equation 7a. The second chemically reversible reduction process occurs at $E_p^{\text{red}} = -1.73 \text{ V}$ (vs. SCE) a potential where benzophenone itself is reduced and therefore corresponds to the process described in 7d. At the insonated electrode the ratio between the measured limiting current for the first and second processes decreases with decreasing horn to

electrode separation hence thinner diffusion layer thickness. A quantitative description for the effective number of electrons, n_{eff} , transferred at a uniformly accessible electrode for variable diffusion layer thickness is given in Equation 8⁶⁵.

$$n_{eff} = \frac{i_{lim}}{i_{lim}^0} = 2 - \frac{\sqrt{\tanh(\delta^2 k / D)}}{\sqrt{\delta^2 k / D}} \quad (8)$$

In this equation i_{lim}^0 is the purely transport controlled limiting current, D is the diffusion coefficient assumed to be identical for all species, and k is the first order rate constant for the chemical reaction step. A plot of the observed limiting currents versus δ^{-1} determined from the first one-electrode process is shown in **Figure 11**.

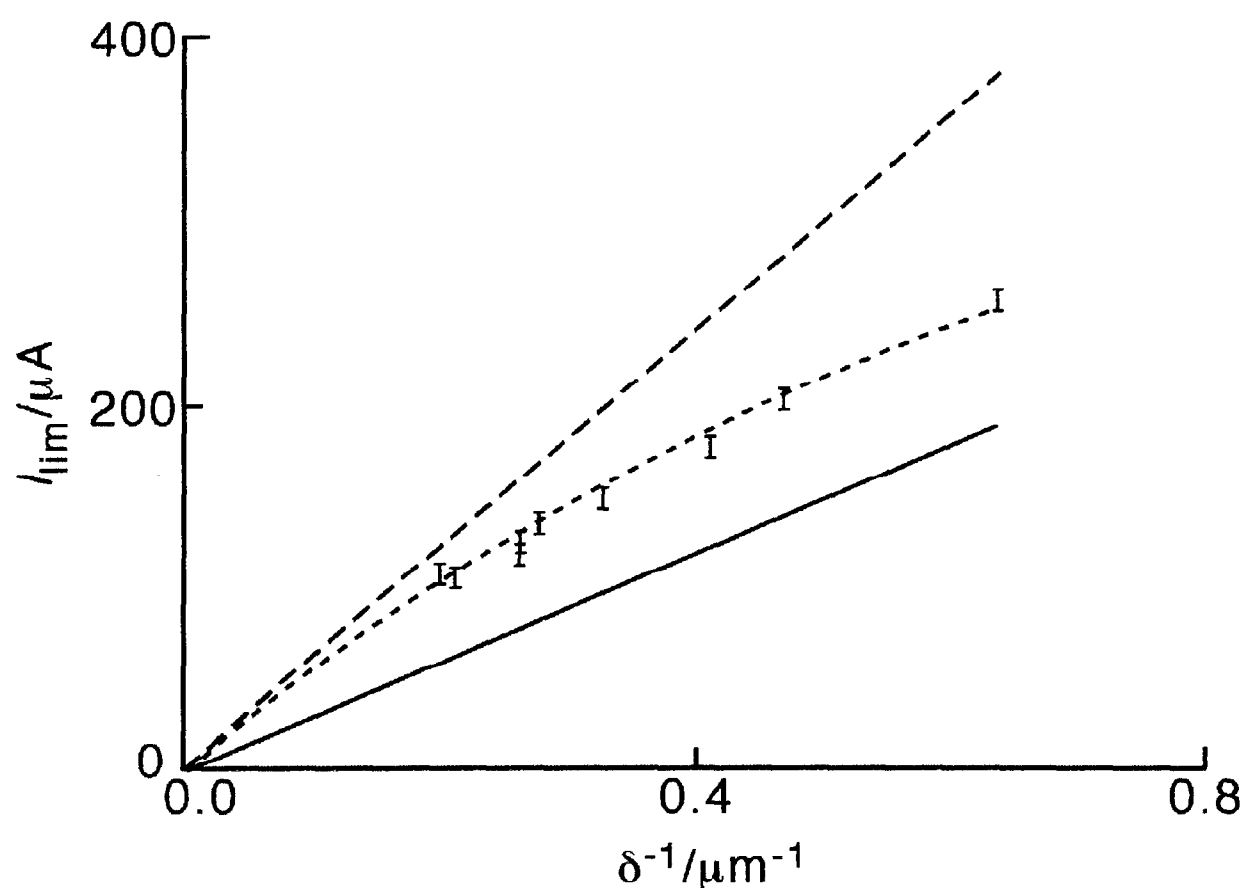


Figure 11 - Plot of sonovoltammetrically obtained limiting currents versus the reciprocal diffusion layer thickness for reduction of 3-bromobenzophenone in dimethylformamide at a glassy carbon electrode. The theoretically predicted curves for $k \rightarrow \infty$ (dashed line) $k \rightarrow 0$ (solid line) and for $k = 6 \times 10^2 \text{ s}^{-1}$ (dotted line) are also shown.

A theoretical line can be fitted to the experimental points and a rate constant of $k = 600 \pm 100 \text{ s}^{-1}$ is in good agreement with the value reported in the literature, $k = 800 \pm 200 \text{ s}^{-1}$ ⁶². For the reduction of *ortho*-bromonitrobenzene a rate constant of $k = 200 \pm 50 \text{ s}^{-1}$ was determined which is also in close agreement with the literature value of $k = 250 \text{ s}^{-1}$ ⁶⁶. The implication is that ultrasound facilitates the measurement of fast rate constants under steady state conditions at macroelectrodes. These would otherwise only be measurable using steady state microelectrode studies or *via* transient techniques

such as fast scan cyclic voltammetry. Furthermore the results suggest first a physical reality to the diffusion layer concept, and second no sonochemical enhancement of the reaction, at least in the two cases reported, as compared to silent conditions. However, in extreme conditions this may not always be the case.

1.4.5.4 Extreme Conditions and Deviation from Hydrodynamic Behaviour

It has been shown in section 1.4.4 that when the source of ultrasound is brought very close to the working electrode, 5 mm horn to electrode separation or less, then the cavitation events induced can be very violent generating sharp spikes in the current transient. On systematic variation of horn to electrode separation and ultrasound power it becomes apparent that there is a threshold for the detection of violent cavitation activity³⁵ see **Figure 9**. This leads to the hypothesis that the cavitation threshold may coincide with the breakdown of the simple planar diffusion layer model, even when calibration of the diffusion layer thickness is employed³⁵. The transition to a more complex mass transport model would obviously be expected for very extreme conditions with strong cavitation interference. In **Figure 12** data from a series of sonovoltammetric experiments are shown, analogous to the data on this reaction given in s.1.4.5.3 above but extending the range of diffusion layer thicknesses into the range of extreme ultrasonic conditions.

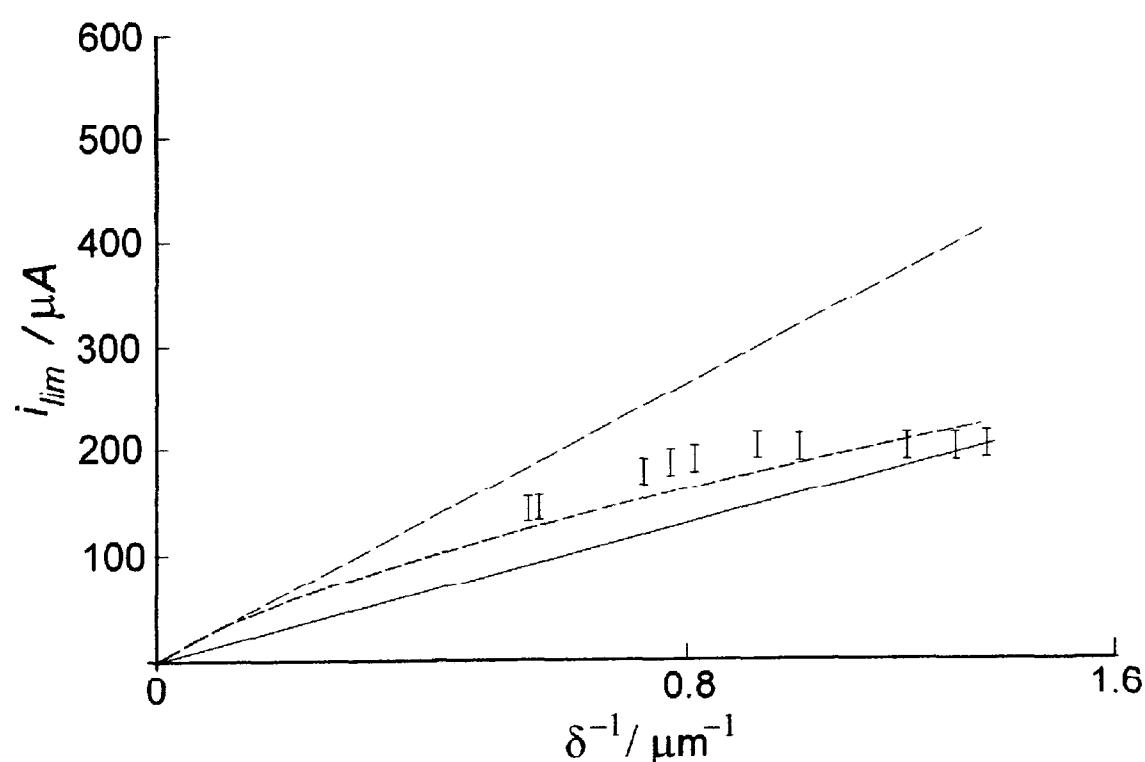


Figure 12 - Plot of sonovoltammetrically obtained limiting currents versus the reciprocal diffusion layer thickness for reduction of 3-bromobenzophenone in dimethylformamide at a glassy carbon electrode. The theoretically predicted curves for $k \rightarrow \infty$ (dashed line), $k \rightarrow 0$ (solid line) and for $k = 8 \times 10^2 \text{ s}^{-1}$ (dotted line) are also shown.

The solid line indicates the average sonovoltammetric limiting current for a one-electron transfer process, and the upper dashed line corresponds to the limiting current associated with a two-electron

process. The intermediate dashed line corresponds to the limiting current expected for the ECE process with a rate constant $k = 800 \text{ s}^{-1}$ (literature value⁶²). Analysis of the limiting current data obtained under conditions, which are more extreme compared to those described in s.1.4.5.3 above, suggesting a marked deviation from the model. As expected, the data points followed a trace consistent with a transfer of between one and two electrons. In a range of intermediate diffusion layer thickness, $\delta = 0.8 - 1.6 \text{ }\mu\text{m}$, the data points for the limiting current are higher than expected, probably due to a thermal effect. This kind of thermal effect has been noted by Coury *et al*⁶⁷ in aqueous systems, and occurs locally at the electrode/solution interface. However, under even more extreme conditions, the data points deviate markedly from the model and, apparently, a smaller number of electrons are transferred. The purely thermal effect would result in the opposite, and therefore, the negative deviation has been attributed to a breakdown of the simple mass transport model³⁵.

In practice the diffusion layer prevailing under insonated conditions arises from interplay between the effects of acoustic streaming and cavitationally induced microjetting, which control the overall sonovoltammetric response. The results presented in Chapter 3 describe the use of DPV as a sensitive means of exploring the diffusion layer under steady-state electrolysis at insonated electrodes. In order to provide a complete introduction to this subject the results of those experiments are briefly summarised here.

The effect of convection on the DPV response can be predicted by a theoretical comparison between the limit of a Nernstian diffusion layer in which the solution is fully stagnant and a rotating disc electrode^{68,69}. The general trend is as the speed of rotation increases (which corresponds to a reduction in diffusion layer thickness in the static simulation), the peak height increases, due to convection increasing mass transport to the electrode. Turning to sonovoltammetry the simulations predict, by analogy, that at a given diffusion layer thickness, if the main effect of insonation is an enhancement of mass-transport to the electrode via convection from acoustic streaming, a larger peak current will flow than that which would be measured under pure, entirely stagnant “Nernstian” diffusion layer conditions.

Figure 13(a-d) shows a comparison of experimental peak currents and theoretical peak currents for the solvents DMF, water and ammonia at a range of diffusion later thicknesses.

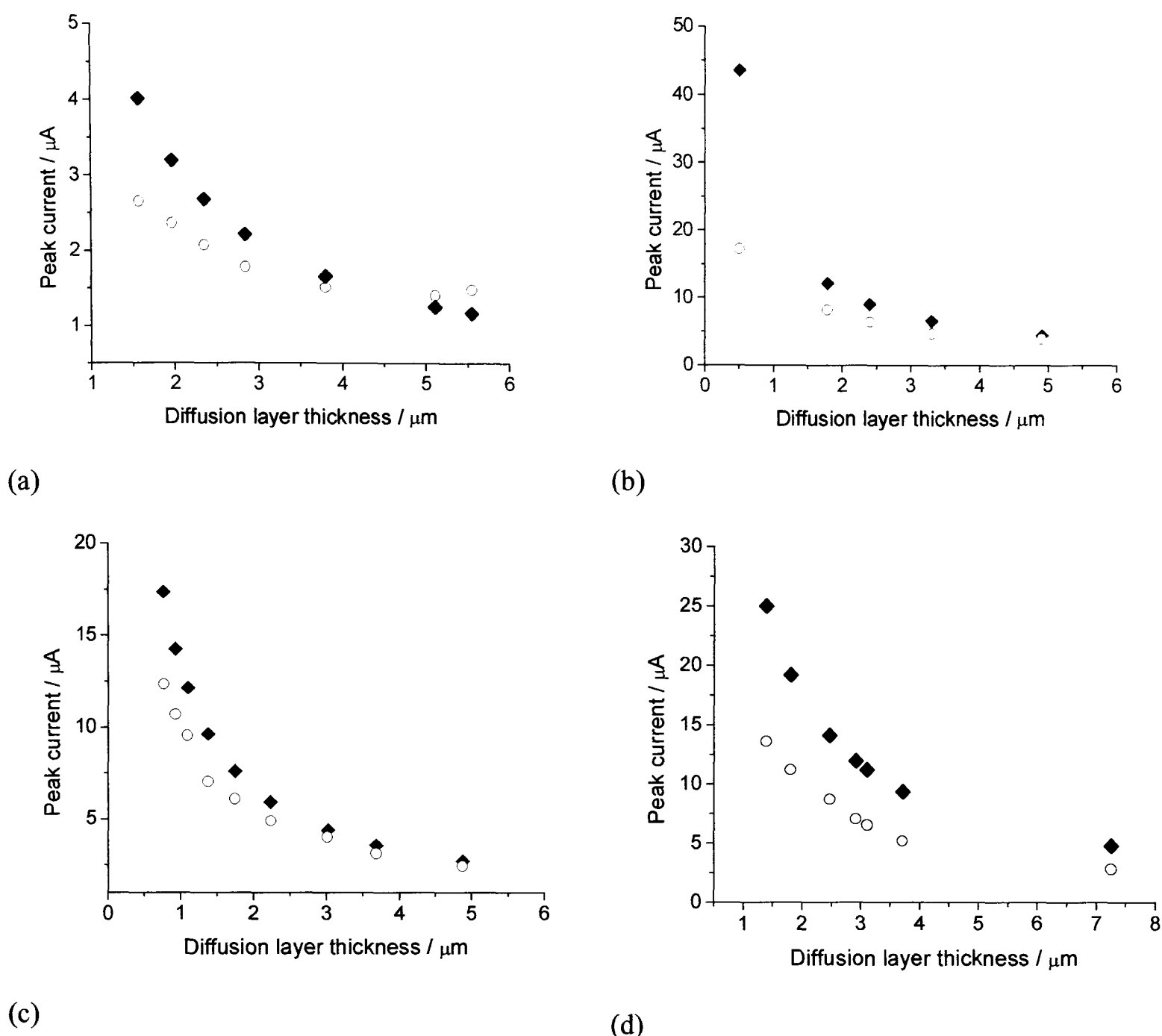


Figure 13 – Theoretical stagnant Nernst diffusion model($\blacklozenge$) and experimental ($\circ$) DPV peak currents for
 (a) 1 mM ferrocene in DMF at 218 K
 (b) 1 mM ferrocene in DMF at 298 K
 (c) 1 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ in water at 298 K
 (d) 1 mM nitrotoluene in liquid ammonia at 218 K.

It is evident from **Figure 13** that experimentally obtained peak currents are smaller than those theoretically predicted at a given diffusion layer thickness, deviating most markedly at small diffusion layer thicknesses where the ultrasonic perturbation is greatest. This is the opposite of the predictions of the rotating disc where increasing mass transport via pure convection (higher rotation speeds) would lead to a larger peak current than that which would be measured under “Nernstian” diffusion layer conditions. It is therefore likely that cavitation not acoustic streaming is responsible for this deviation due to the turbulent collapse of bubbles perturbing or even replacing the diffusion layer. The conditions where it is believed that cavitation is strongest (see **Figure 8**) result in the greatest deviation between theory and experiment. For all the solvents the largest deviation is observed at the smallest diffusion layer thickness, corresponding to the smallest horn to electrode separation and highest power of ultrasound. The correlation between cavitation and the deviation observed in the

DPV confirms that the contribution from cavitation perturbation of the diffusion layer, from both bubble formation and microjetting, is significant in comparison with acoustic streaming under more extreme conditions. Nevertheless it is also clear that for mild ultrasonic stimulation, where the diffusion layer thickness is relatively large, then the Nernst diffusion model is physically realistic as testified by the good agreement between theory and experiment in the high diffusion layer thickness limit.

The resulting physical model of an insonated electrode can be described as a steady diffusion layer a few microns thick brought about by acoustic streaming, which is occasionally and randomly punctuated by a cavitation event the frequency and violence of which is dependent on the solvent and ultrasound intensity. At high intensity and short horn to electrode separation the cavitation contribution becomes substantial with violent cavitation activity giving a pronounced deviation from the steady diffusion layer model.

1.5 Sonoelectroanalysis

1.5.1 Analysis by Classical Electrochemical Techniques

Electrochemical methods of analysis are extremely sensitive and have been exploited to permit the detection of a wide range of analytical targets down to concentrations of the order 10^{-10} M in favourable conditions. The relative inexpense of these electroanalytical techniques when compared with conventional techniques such as ICP-MS (Inductively Coupled Plasma-Mass Spectrometry) and AAS (Atomic Absorption Spectroscopy) has led to the use of electrochemical stripping voltammetry and linear sweep voltammetry for the detection of both inorganic and organic species⁷⁰⁻⁷⁵. Target analytes which have been documented include heavy metals (Bi, Cu, Cd, Ga, Mn, Pb, Sb, Sn, V, Zn), cardiac and anticancer drugs, vitamins and pesticides. However, the limits of applicability for these ‘silent’ classical electrochemical techniques have been compromised by four main drawbacks:

- Lack of rapidity with long accumulation steps⁷⁶⁻⁷⁸;
- Deactivation and complete fouling of the electrode surfaces due to passivating adsorbates⁷⁹⁻⁸⁷;
- Low sensitivity in “real media” in the absence of time consuming substrate pretreatments⁸⁴⁻⁸⁸;
- Techniques are often dependent on toxic mercury electrodes.

In attempts to overcome these problems a range of techniques have been employed. The use of rotating disk electrodes reduces the accumulation time required for sensitive stripping analysis, however, the benefits are restricted to rotation speeds up to the limit where non-laminar flow hinders reliable quantification⁸⁹. Microelectrodes, with diameters of 1-100 μm have further enhanced the applicability of electroanalysis but not without drawbacks. The main difficulties are primarily their fragility, and its consequent complications for cell design and robustness, and also dimensional instability resulting from erosion or the dissolution of electrode substrate into deposited metals⁹⁰. The use either of these techniques is limited in environmental, biological or food samples due to the surface passivating effects of proteins, sugars, soluble polymers and solid particular matter. Such electrode fouling can be overcome in part by the use of a hanging-mercury drop electrode (HMDE) which permits continuous regeneration of the surface by slowly allowing mercury to drop from the electrode. The technique has been used for a wide range of applications, but is still plagued by the drawbacks associated with long accumulation times, furthermore, the use of mercury in high quantities is undesirable due to its toxicity and future restrictions on its use^{91,92}.

1.5.2 Coupling Ultrasound with Electroanalysis

The synergistic coupling of ultrasound with electroanalytical measurements has been the subject of detailed study for many years^{15,16} and the technique is now emerging as a powerful electroanalytical tool^{16,40,76,77,93-97}. The cavitation effects of ultrasound are well documented^{15,40,50,91,97} (see **Figure 3**) and have been shown to facilitate the determination of trace metals in highly passivating media such as alcoholic beverages^{15,91,93,94,96,98}. Cavities, created by ultrasonic compression and rarefaction of the solution, collapse causing both localised high pressures and temperatures in the bulk solution and microjetting and flushing⁹⁷ on the electrode surface with jet speeds of up to 100 m s⁻¹. This results in continuous cleaning of the electrode surface and removal of adsorbed passivating organic complexes. In addition acoustic streaming increases the mass transport of the system resulting in shorter deposition times, greater sensitivity and a correspondingly lower detection limit^{15,27,76,77}. Ultrasonically enhanced stripping and linear sweep voltammetry has therefore been successfully applied to *inter alia* the determination of lead in wine⁹⁸, copper in beer⁹¹, lead in river sediment⁹⁹, manganese in tea¹⁰⁰, nitrite in egg¹⁰¹ and copper in blood¹⁰². **Table 2** provides an overview of the scope of sonoelectroanalysis demonstrating the diversity of targets and the accuracy of results.

Analysis	Type of voltammetry	Ultrasound Power (Wcm ⁻²)	Tip Area (cm)	Sono method	Detection Limit	Independent method	Independent method Results
Lead in wine	sono-ASV	26	1.32	22 ± 6µg/L	2 µg/L	AAS	24 ± 4 µg/L 27 ± 4 µg/L
Copper in beer	sono-ASV	200	0.07	222 ± 31 µg/L 139 ± 4 µg/L	32 µg/L	AAS	230 µg/L 140 µg/L
Lead in river sediment	sono-CSV	14	1.32	187.1 mg/Kg	10 mg/Kg	ICP-MS	206.1 mg/Kg
Nitrite in egg	sono-LSV	50	0.07	1.2 ± 0.05 mg/Kg 1.4 ± 0.05 mg/Kg	0.045mg/Kg	MAFF Data	1.7 ± 0.4mg/Kg
Nitrate in Sewage	Sono-LSV	70	0.07	469 µM		Griess Protocol	479 µM
Lead in petrol	sono-ASV	52	0.07	380 ± 40 mg/L		AAS	400 ± 20 mg/L
Manganese in instant tea	sono-CSV	14	1.32	1859 µg/g 914 µg/g	3 µg/g	AAS	1800 µg/g 1000 µg/g
Cadmium in presence of 10ppm Triton-X	sono-ASV	40	0.07		0.05 µM	Additions of known concentration	
Lead in Lead-Copper Alloy	sono-CSV	14	1.32		1.6 nM	Additions of known concentration	
‡Copper in blood	sono-ASV	300	0.07	1300 ± 300 µg/L 620 ± 60 µg/L	90 µg/L	AAS	1300 µg/L 690 µg/L
‡Copper in fish gill mucous	sono-BASV	200	0.07	16 µg/L 21 µg/L	3 µg/L	AAS	17µg/L 25 µg/L
‡Vanillin in food flavouring	sono-biphasic	200	0.07	9.09 ± 0.21 mM 9.24 ± 0.23 mM	0.020 mM	HPLC	9.17 mM 9.30 mM
‡Copper in ribena®	sono-SEASV	70	0.07	45 ± 5 µg/L	2 µg/L	Std. Addn.	45 ± 5 µg/L
‡Copper in blood	sono-SEASV	70	0.07	1637 ± 26 µg/L 1616 ± 25 µg/L	7 µg/L	Sono-ASV	1620 ± 160 µg/L
Aqueous silver ions	sono-ASV	14	1.32		1 nM	Additions of known concentration	
‡Copper in presence of 1000ppm SDS	sono-SEASV	70	0.07		0.3 µM	% recovery from spiked sample	94.1%
‡Copper in presence of 1000ppm TX-100	sono-SEASV	70	0.07		0.3 µM	% recovery from spiked sample	94.0 %
5-aminosalicylic acid in tissue culture	sono-LSV	140	0.07		0.3µM	% recovery from spiked sample	102 %
Copper in Industrial Effluent	sono-ASV	100	0.07	2.3 ppm 8.6 ppm		ICP-MS	2.5 ppm 8.1 ppm
Aqueous 4-chlorophenol	sono-CV	14	1.32	uncontaminated	1 µM	Additions of known concentration	
Dopamine in egg homogenate	Sono-LSV	140	0.07	uncontaminated	2 µM	Additions of known concentration	
‡Lead in Artificial Saliva	Sono-ASV	30	0.07	uncontaminated	0.25 µg/L	Additions to blank synthetic	
‡Lead in Human Saliva	Sono-ASV	40	0.07	0.92 ± 0.2 µg/L 5.10 ± 1.0 µg/L	0.50 µg/L	ICP-MS	0.92 ± 0.1 µg/L 4.80 ± 0.5 µg/L
‡Cadmium in Human Saliva	Sono-ASV	40	0.07	2.5 ± 0.5 µg/L 4.9 ± 0.05 µg/L	1 µg/L	ICP-MS	2.5 ± 0.05 µg/L 5.0 ± 0.05 µg/L

Table 2 – Summary of sonoelectroanalytical targets and detection limits. Results subsequently reported in this thesis are indicated by the symbol ‡

Key:
ASV – Anodic Stripping voltammetry
SEASV – Solvent Extraction ASV
CSV – Cathodic Stripping Voltammetry
LSV – Linear Sweep Voltammetry

1.5.3 Sonoelectroanalytical Stripping Voltammetry

Anodic stripping voltammetry (ASV), adsorptive stripping voltammetry (AdSV) and cathodic stripping voltammetry (CSV) have been coupled with ultrasound for the detection of target analytes in “real” media. The three techniques involve two distinct steps. Initially, in a preconcentration step, where mass transport is increased by the presence of ultrasound, the analytical target is accumulated from solution onto a suitable working electrode substrate. This can be achieved either by electrochemical deposition (reduction or oxidation) or physical adsorption. In the second step the potential of the working electrode is reversed in order to liberate the analyte from the electrode with the resulting Faradaic current permitting direct quantification of the amount of each analyte present.

1.5.3.1 Anodic Stripping Voltammetry

Anodic stripping voltammetry permits quantitative analysis of cations in solution. It is applied principally to the detection of heavy metal cations, which are accumulated at the electrode by reduction during the preconcentration step:



The analytical stripping step is the reverse of the preconcentration step:



and is accomplished by employing a linear sweep (s.1.2.4.2), square wave (s.1.2.4.3.2) or differential pulse (s.1.2.4.3.1) scan. Most common is the square wave voltage scan, the technique consequently referred to as SWASV. The advantages of employing a square-wave stripping step are its rapidity and high sensitivity (s.1.2.4.3.2). The resulting Faradaic current permits direct quantification of the analyte preconcentrated at the surface.

The ASV response resulting from standard microadditions⁹⁸

Heavy metals in media such as alcoholic beverages, environmental samples and blood are present as M^{n+} in either a state “bound” to the matrices of the substrate or “free” in solution. The quantity of metal ions detected by ASV may not be the total amount of metal in the sample but merely reflect the unbound M^{n+} . The following equilibrium can be considered:



where $M^{n+}_{(bound)}$ is the metal - substrate complex

The equilibrium constant, K_{eq} for the above reaction is given by

$$K_{eq} = \frac{[M_{bound}^{n+}]}{[M_{free}^{n+}][S]} \quad (12)$$

and the total concentration of metal, $[M_{tot}^{n+}]$ is given by

$$[M_{tot}^{n+}] = [M_{free}^{n+}] + [M_{bound}^{n+}] \quad (13)$$

If a small volume of aqueous standard solution (experimentally of the order of 10-100 μL) is added to the sample (a “standard microaddition”) then the total composition can be described by

$$[M_{tot}^{n+}] = [M_{substrate}^{n+}] + [M_{\mu a}^{n+}] \quad (14)$$

where $[M_{substrate}^{n+}]$ is the initial total concentration in the sample to be analysed and $[M_{\mu a}^{n+}]$ is the additional total concentration due to the microaddition. Assuming only M_{free}^{n+} is detected by ASV then to proceed, equation (13) is substituted into equation (12) to give

$$K_{eq}[S] = \frac{[M_{tot}^{n+}] - [M_{free}^{n+}]}{[M_{free}^{n+}]} \quad (15)$$

Substituting equation (14) into equation (15) gives

$$K_{eq}[S] = \left\{ [M_{substrate}^{n+}] + [M_{\mu a}^{n+}] \right\} \left(\frac{1}{[M_{free}^{n+}]} \right) - 1 \quad (16)$$

Since $[S]$ remains approximately constant throughout an experiment because the overall volume of solution is changed only minimally by the microaddition, it can therefore be incorporated into the equilibrium constant to give a new constant, $K = K_{eq}[S]$, permitting equation (16) to be rearranged into the following form:

$$[M_{free}^{n+}] = \frac{1}{K+1} [M_{\mu a}^{n+}] + \frac{1}{K+1} [M_{substrate}^{n+}] \quad (17)$$

it suggests that by plotting $[M_{free}^{n+}]$ versus $[M_{\mu a}^{n+}]$ the concentration of the metal ions in the substrate is given by

$$[M_{\text{substrate}}^{n+}] = (\text{intercept})/(\text{gradient}) \quad (18)$$

The value of $[M_{\text{free}}^{n+}]$ is directly proportional to a stripping peak area, A_{peak} (C), for a linear sweep stripping scan, and the ASV results can be analysed by plotting a graph of A_{peak} vs. $[M_{\mu\text{a}}^{n+}]$

1.5.3.2 Applications of Sono-Anodic Stripping Voltammetry (Sono-ASV)

1.5.3.2.1 Detection of Lead in Wine⁹⁸

Monitoring trace amounts of lead in wine was shown to be possible using sono-ASV. Samples of a white Italian Chardonnay were introduced to the cell described in **Figure 1** with a separation of 10 mm between the ultrasonic horn and the surface of a 4 mm diameter Hg/Pt electrode. Following acidification and argon degassing of the sample, lead ions can be reduced at the electrode at -1.0 V (vs. SCE) to form a Hg-Pb amalgam. The enhanced mass transport associated with power ultrasound at 26 W cm^{-2} yields efficient preconcentration of lead over a 240 s deposition period. The ultrasound was then switched off in order to perform a linear anodic potential sweep at a scan rate of 50 mV s^{-1} . Integration of the peak yielded the amount of charge deposited onto the electrode and the microaddition technique described above was implemented with a 10 min insonation between each deposition step in order to allow equilibration and complete mixing to occur. The value of $[\text{Pb}^{2+}_{(\text{wine})}]$ yielded by sono-ASV was $22 \pm 6 \mu\text{g L}^{-1}$. For comparison, the total lead content in the wine was obtained by AAS, the currently accepted standard method¹⁰³ for total lead detection in wine samples, and the sono-ASV results were found to be in excellent agreement (see **Table 2**). Previous attempts to employ ASV for lead detection have been unsuccessful, in that typically only $\sim 50\%$ of the lead levels detected by AAS were recorded⁸³. The benefits of insonation are first, the maintenance of a clean, active electrode surface to ensure the uptake of metal from the range of complex lead cation species labelled $\text{Pb}^{2+}_{(\text{free})}$, and second, maintenance of the equilibrium between “free” and “bound” ions as described by the equilibrium constant K_{eq} .

1.5.3.2.2 Determination of Copper in Beer⁹¹

Leaching of copper ions from the fermentation vessel into beer is a side effect of the brewing process, its rapid evaluation is therefore of importance to the industry. Mercury-free electrodes were used to determine the amount of copper in Marstons “Pedigree Bitter” (Marston’s Brewery, Burton-on-Trent, Staffs., UK) using a 3 mm diameter glassy carbon electrode. Deposition of copper on the glassy carbon substrate was performed at -1.5 V (vs. SCE) under power ultrasound of intensity 200 W cm^{-2}

from a microtipped horn probe at 5 mm from the surface of the working electrode. The anodic stripping step was conducted using a square wave scan (s.1.2.4.3.2) with a pulse amplitude of 25 mV, potential step of 5 mV and a frequency of 60 Hz. Typical square wave voltammograms are shown in **Figure 14** for microadditions to the beer sample.

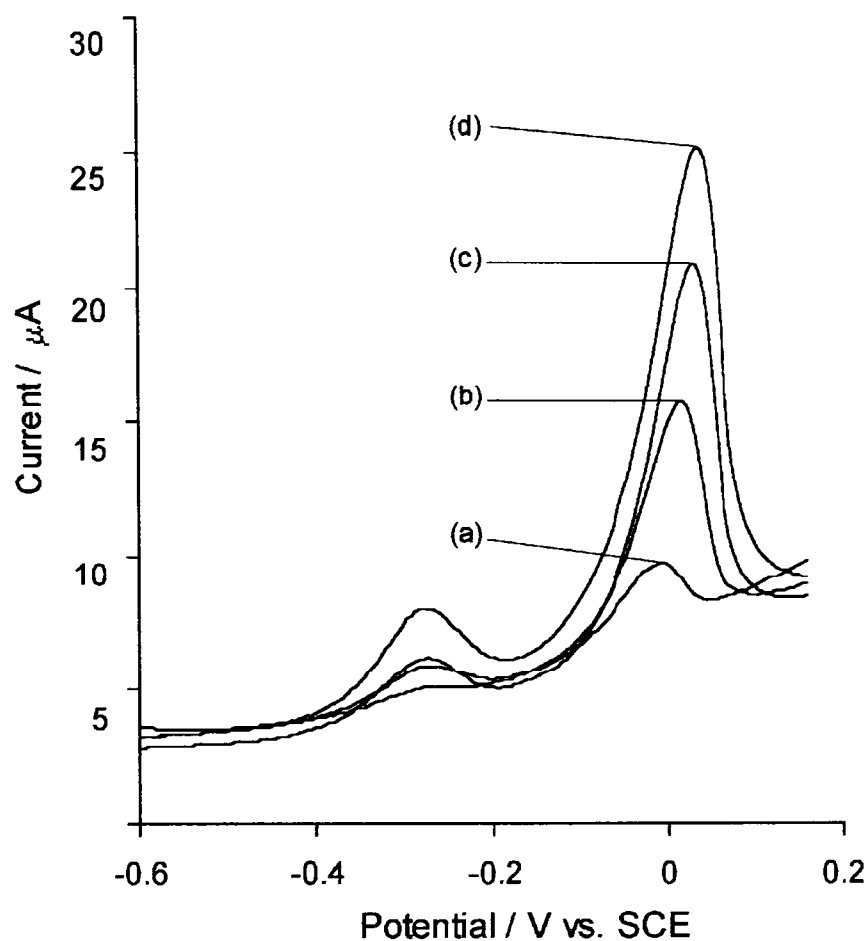


Figure 14 Square wave anodic stripping voltammograms of aqueous solutions of “Pedigree” beer following deposition:

- a) blank beer 240 s insonated at 200 W cm^{-2}
- b) $48 \mu\text{g L}^{-1}$ standard addition of copper insonated at 200 W cm^{-2} ;
- c) $96 \mu\text{g L}^{-1}$ standard addition of copper insonated at 200 W cm^{-2} ;
- d) $144 \mu\text{g L}^{-1}$ standard addition of copper insonated at 200 W cm^{-2} ;

The wave at *ca.* (0 V vs. SCE) increases with added copper concentration and corresponds to the anodic stripping peak of solid copper deposited on the electrode surface. Total copper content levels in the range of $100\text{--}300 \mu\text{g L}^{-1}$ were determined using this technique with the calculated results lying within one standard deviation the independent analysis conducted via atomic absorption spectroscopy (see **Table 2**).

1.5.3.3 Adsorptive Stripping Voltammetry (AdSV)

In cases where the target cannot be usefully preconcentrated by electrolysis at electrode surfaces, such as transition metals with numerous high stable oxidation states or metals which show complicated amalgam formation, adsorptive stripping voltammetry (AdSV) can be a valuable alternative. AdSV is based on the adsorption accumulation of a complex between the target analyte and a complexing agent at the electrode surface, which may be facilitated by an applied potential.



The determination step typically involves reduction of the metal ion from the adsorbed complex.

Since adsorption follows a Langmuir-type isotherm the adsorption time is significant and an adsorption time can be reached which corresponds to the equilibrium saturation of the complex⁷⁰. Equilibrium saturation is generally avoided in favour of the initial part of the isotherm where the adsorbed amount varies linearly with accumulation time. The extent of preconcentration depends on the length of time over which the adsorption is allowed to proceed and consequently, detection limits depend on accumulation times⁷⁸.

AdSV has been applied to the detection of metals such as Co, Ni, Mo, U and V (following their preconcentration as metal complexes) and a wide variety of organic compounds including drugs such as codeine, herbicides such as atrazine and vitamins such as folic acid and riboflavin⁷⁵.

1.5.3.4 Characterisation of sono-AdSV for a Vanadium (V) Catechol Complex⁷⁶

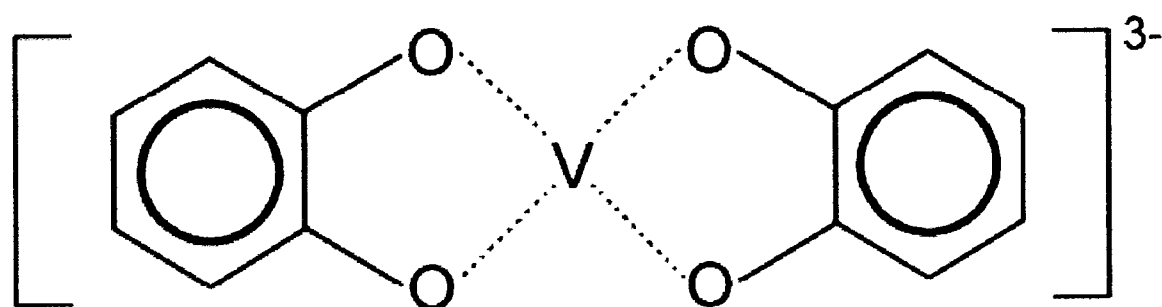


Figure 15 – Possible structure of the vanadium catechol complex

Ultrasound was found to significantly improve the sensitivity of the AdSV of Vanadium by increasing the rate at which the complex was deposited onto a mercury surface in comparison with silent conditions. Maximum responses can be achieved for an insonated system in less than one tenth the time required for conventional AdSV under silent conditions. Calibration data was obtained from a mercury thin film copper electrode for concentrations of vanadium (V) ranging from 0 to 7×10^{-7} M in a solution containing 0.1 M KCl, 8mM PIPES [piperazine-N,N'-bis(2-ethanesulfonic acid)] and 3×10^{-4} M catechol at pH 6.8. The considerable enhancement of the VL₂ differential pulse stripping signal in the sono-AdSV experiments with respect to silent conditions permitted a short preconcentration time of 10 s at -0.3 V (vs. SCE) under 20 W cm^{-2} ultrasound intensity for a horn to

electrode separation of 35 mm. Good linearity is obtained up to vanadium concentrations of ca. 3×10^{-7} M, with a correlation coefficient of 0.99, a sensitivity of 0.4 nA nM^{-1} and detection limit of 2×10^{-8} M. These observations are an improvement to data obtained under silent conditions¹⁰⁴ for a HMDE where a linear range up to 2×10^{-7} M and sensitivity of 0.45 nA nM^{-1} were reported for a longer 60 s preconcentration.

1.5.3.5 Cathodic Stripping Voltammetry (CSV)

Cathodic stripping voltammetry of metal ions is a complementary technique to anodic stripping voltammetry; the analyte is accumulated as an oxidised species on the electrode at a positive potential and then quantified during a cathodic potential stripping step. Equation 20 describes the chemical reaction taking place during the electrochemical stripping of the oxide species.



The benefits of CSV over ASV are avoidance of interactions between metals, such as the formation of alloys on the surface of solid electrodes⁹¹, and an alternative to the use of mercury electrodes. However, the applications of CSV reported in literature are few, considering the potential benefits of the technique. Early studies of CSV utilised platinum electrodes although these methods invariably involved lengthy deposition times and a limited range of applications¹⁰⁵⁻¹⁰⁷. Alternative electrode materials such as conducting glass¹⁰⁸ and tin oxide¹⁰⁹ proved to be more successful although the applications were limited to relatively clean samples such as rain water.

1.5.3.6 Applications of Sono-Cathodic Stripping Voltammetry (Sono-CSV)

The following applications describe the use of ultrasound to broaden the range of analytical targets accessible by CSV when coupled with the mechanically and chemically robust BDD electrode¹¹⁰ (see s.1.3.2).

1.5.3.6.1 Determination of Lead in River Bed Sediment⁹⁹

In the determination of lead by ASV, intermetallic species and overlapping signals can often lead to complications. The effect of possible interferences when using sono-CSV, from other ionic metallic species often found in industrial waste, such as chromium, copper, nickel, iron, cadmium and zinc, was investigated on a BDD electrode by the addition of these heavy metals to a cell containing $1.0 \text{ }\mu\text{M}$ Pb^{2+} in 1.0 M nitric acid. The procedure involved insonated electrode cleaning and mixing for 120 s at 0.0V (vs. SCE), and a 60 s insonated accumulation (10 W cm^{-2}) at $+5.0 \text{ V}$. Following accumulation,

square wave stripping (amplitude 100 mV, step potential 10 mV and frequency 50 Hz) provided the analytical signal. Cr, Ni, Cd and Zn were not detected by this technique. Cu and Fe had no effect on the Pb response, however, both were detected at potentials more cathodic than for Pb reduction. **Figure 16** shows the responses observed for Cu, Pb and Fe, demonstrating a very high elemental specificity for lead (1 μ M) over Cu (150 μ M) and Fe (300 μ M).

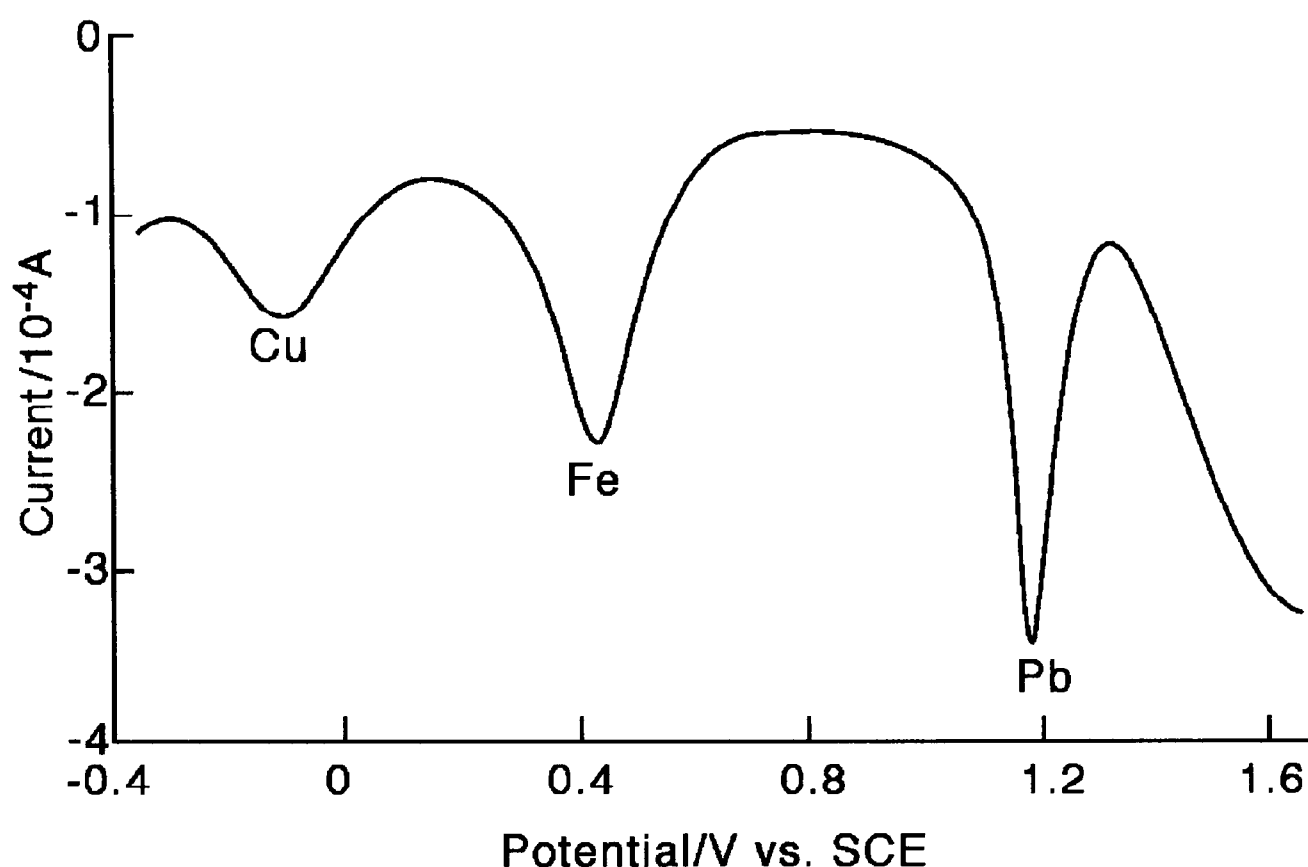


Figure 16 - Square-wave sono-cathodic stripping voltammetry demonstrating the simultaneous detection of copper (150 μ M), iron (300 μ M) and lead (1 μ M) at a boron doped diamond electrode.

The river sediment sample was taken from a region downstream of a location extensively exploited for metalliferous mining, which consequently contained significant heavy metal contamination. The coarse sample was initially air dried, then passed through stainless steel sieves of successively finer mesh until a powder of less than 63 μ m diameter particles was obtained. Following ultrasonic digestion in a mixture of concentrated nitric and perchloric acid, an aliquot of the digest was diluted with dilute nitric acid and introduced to the electrochemical cell. The microaddition technique facilitated quantification of the lead in the acid digest via the sono-CSV procedure described above. This was worked up to give a lead content of 187.1 mg/Kg in the sediment sample which is in agreement with an independent analysis by ICP-MS (see **Table 2**).

1.5.3.6.2 Determination of Manganese in Instant Tea¹⁰⁰

Tea is a popular beverage, in its various forms, on a global scale and is a rich source of dietary manganese¹¹¹, with tea drinkers ingesting between 21 and 45 % more manganese than in the average diet¹¹². Alarming, recent literature has hypothesised that the new prion diseases that have emerged in

the last 15 years, bovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob Disease (variant CJD) could be linked to environmental factors including manganese¹¹³.

The detection limits of sono-CSV analysis of manganese were obtained by trace additions of Mn^{2+} standard solution to 0.5 M ammonium nitrate at pH 7. The analytical stripping response of Mn^{2+} was measured using differential-pulse sono-CSV (sono-DPCSV) in two steps. First accumulation of MnO_2 via a 120 s insonated deposition (14 W cm^{-2}) and potential of +0.85 V (vs. SCE), secondly, the deposited MnO_2 was quantified using an insonated-differential pulse stripping scan (14 W cm^{-2}) over the range +0.85 V to -0.3 V with a step potential of 10 mV, interval time 0.1 s, modulation amplitude 150 mV, and modulation time 0.02s. The electrode was cleaned ultrasonically for 30 s at 0.0V between each scan to ensure complete mixing and removal of any surface material prior to the next scan. The use of ultrasound in both stages of the sono-CSV of manganese is essential to the efficiency of the technique, with corresponding silent deposition of MnO_2 resulting in near total loss of analytical signal, highlighting the dramatic effect of ultrasound on mass transport. An insonated stripping step promotes rapid removal of reduced Mn^{2+} ions from the surface of the electrode ensuring a sharp analytical stripping peak is obtained. This technique was found to give a linear increase in peak height for increasing concentration of aqueous Mn^{2+} over the range 1×10^{-11} to 3×10^{-7} M.

The manganese content of two samples of instant tea was determined by addition of an aliquot containing 250 μg of aqueous instant tea to 250 mL supporting electrolyte. Microadditions of Mn^{2+} permitted the quantification of Mn in instant tea using the sono-DPCSV method outlined above. The samples were also analysed independently using AAS, with good correlation being observed between the sono-DPCSV technique, AAS and the reported Ministry of Agriculture Fisheries and Food (MAFF, UK) data¹¹⁴ (see **Table 2**).

1.5.4 Sonoelectroanalytical Linear Sweep Voltammetry

1.5.4.1 Detection of Nitrite in Egg¹⁰¹

The determination of nitrite in egg highlights the beneficial aspects of sonoelectrochemical analysis in difficult matrices. The presence of high concentrations of protein and fat serve to foul the electrode and have traditionally prevented direct electrochemical probing of such foodstuffs without recourse to substantial sample pre-treatment. 20 kHz power ultrasound in combination with linear sweep technologies is presented as an alternative to sample manipulation beyond simple homogenisation.

Detection is based upon the reaction of acidified nitrite with an activated phenolic compound (phloroglucinol) where the reduction of the resulting nitroso species at a 3 mm glassy carbon electrode provides the analytical signal^{115,116}.

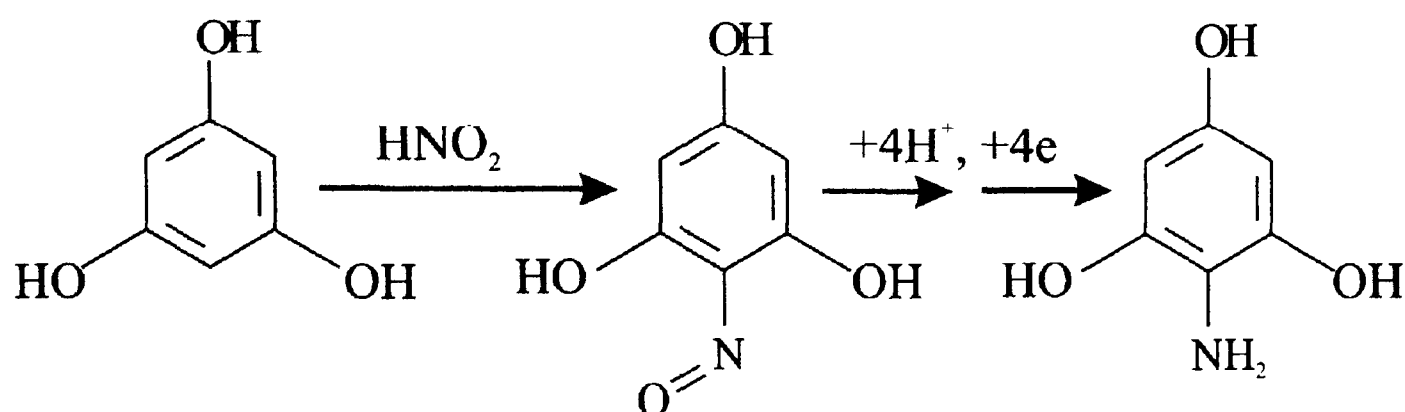


Figure 17 Reaction of phloroglucinol with nitrite and its subsequent reduction to the corresponding aminophloroglucinol derivative.

The analytical procedure consisted of a standard addition method in which a 40 cm³ of homogenised egg (white and yolk) mixture was added to 50 cm³ 0.2 M KCl containing 30 mM phloroglucinol. The pH and volume were then adjusted to pH 3 and 100 cm³ respectively. 20 cm³ of the egg sample was introduced to a cell and standard additions of nitrite (typically 25 µL of either 0.004 M or 0.01 M stock solution) were introduced. In the absence of ultrasound the electrode response to these microadditions is non-linear with no consistent increase in the height of the nitroso reduction peak. This is attributed to electrode fouling which is further complicated by the reduction of other electroactive constituents in the egg adsorbed to the surface of the electrode being reduced at a similar potential to the nitroso reduction.

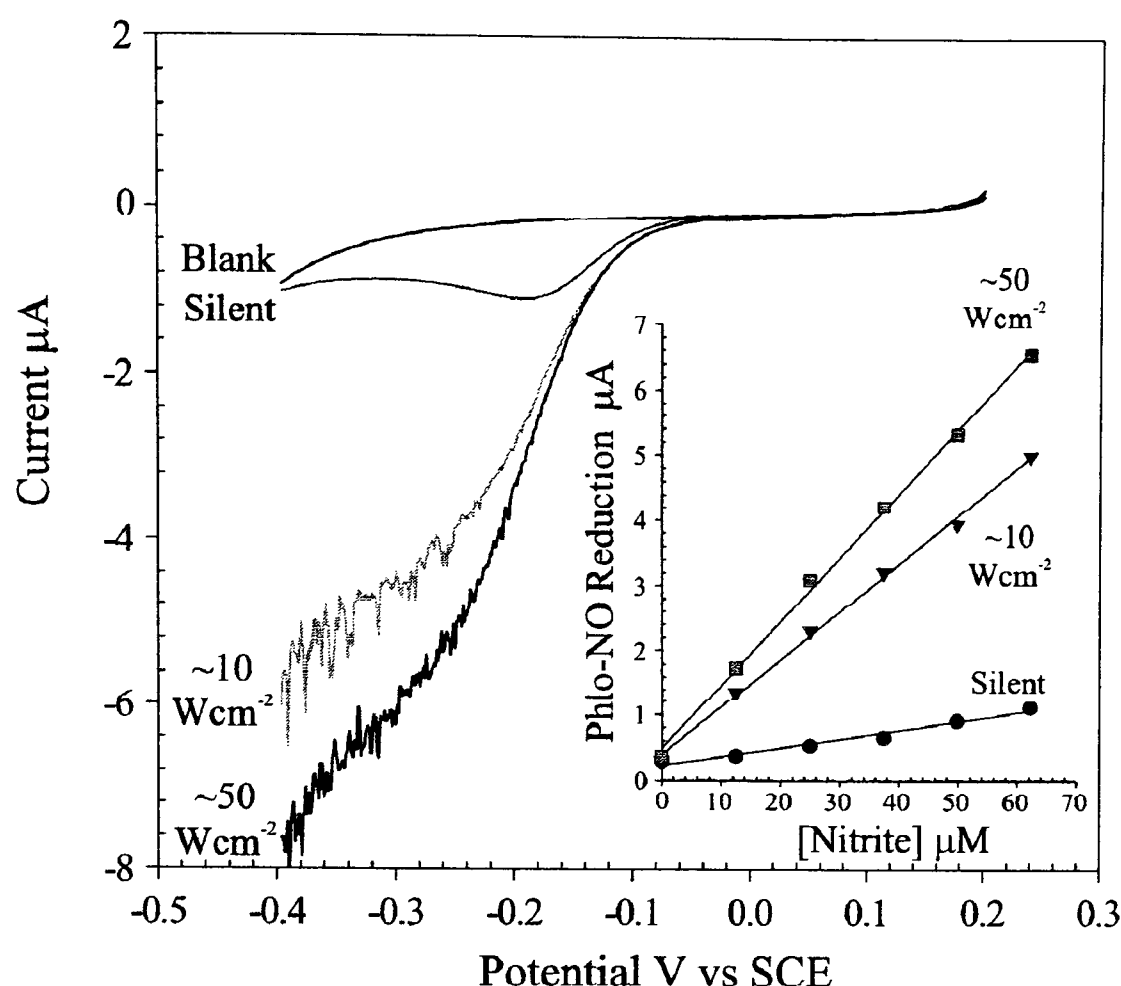


Figure 18 - Linear sweep diagrams comparing the silent and sonochemically enhanced electrode response to nitroso reduction (62mM). Inset : quantitative comparison of the relative procedural sensitivities towards nitroso reduction.

Ablation of the electrode surface by cavitation events, when insonated at 50 W cm^{-2} and 5 mm horn to electrode separation, both during and prior to the linear sweep exerts an in situ cleaning action and is responsible for an improvement in the resolution of the nitroso reduction signal which permitted quantitative measurements to be obtained. The results from two different batches of egg are shown in **Table 2** and were found to be in accordance with MAFF estimates of nitrite levels in egg¹¹⁷. Moreover the advantages of the technique include significant simplification over the liquid chromatographic procedure currently employed for nitrite detection¹¹² which involves hot water extraction, protein precipitation and solid-phase extraction/purification processes.

1.5.4.2 Detection of Nitrate at a Copper Modified Electrode¹¹⁸

The necessity for nitrate monitoring is recognised by most health authorities due to legislation levied on permissible levels in drinking water and food produce¹¹⁹. Existing silent electrochemical methods are based on an electrodeposited copper layer that can provide a linear response over 10-200 μM nitrate¹²⁰. While this range is acceptable for a number of applications it is inadequate when considering the analysis of physiological nitrate concentrations¹²¹. Surface fouling, whether in physiological or environmental samples can also severely restrict the application of the system¹²².

The stability of electrodeposited copper under the influence of ultrasound was examined using a low power setting of 10 W cm^{-2} and 30 mm horn to electrode separation. Mild conditions were selected to minimise cavitation erosion of the surface. It was evident that the introduction of ultrasound removed the fern like growth of electrodeposited copper, but the response to nitrate remained greater than that for a bare electrode suggesting that a catalytic compact layer persists. The layer was found to be stable to repeated pulses of ultrasound facilitating a comparison between silent linear sweep voltammetry and sonovoltammograms recorded at the copper modified electrode. The introduction of ultrasound provides a dramatic improvement in the sensitivity of the electrode towards nitrate above the silent response obtained with the compact copper layer which is illustrated by **Figure 19**.

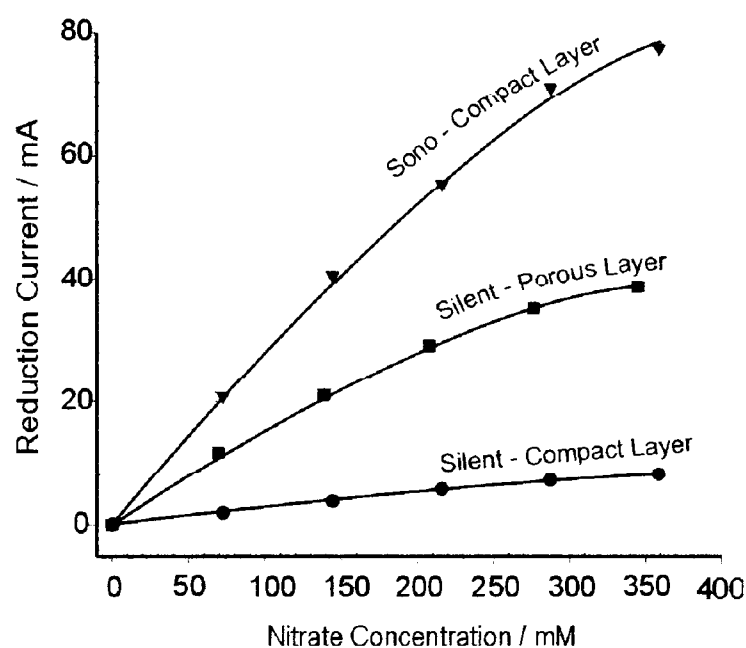


Figure 19 - A quantitative comparison of the compact deposit modified electrode response to increasing nitrate concentration under silent and sonicated conditions. The response obtained at an intact porous, copper modified electrode under silent conditions is also included.

The sonochemical response is also significantly greater than that obtained with the intact, macroporous surface suggesting that lower detection limits may be possible. The fragility of the macroporous electrode is a drawback to its utility and therefore the ability to use the more robust compact layer as a sensing system is a major advantage.

The analytical utility of the sonochemical approach was assessed by evaluation of nitrate in treated sewage effluent by the standard addition protocol under both silent and insonated conditions. The calculated results were $481 \mu\text{M}$ and $469 \mu\text{M}$ for the silent and sonochemical analysis respectively. This compared favourably with an independent spectroscopic analysis based on Cu-Cd reduction/Griess assay¹¹⁸ (see **Table 2**). 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 needed to release the analyte and provide the enhanced detection.

1.5.5 Biphasic Sonoelectroanalysis

Ultrasound is known to facilitate emulsification in biphasic systems¹²³ with benefits including smaller drop sizes down to $0.3\ \mu\text{m}$ ¹²⁴, lower energy consumption and more stable emulsions that are less polydispersed¹²⁴. Cavitation processes occur preferentially at the phase boundary^{125,126}, the imploding cavitation bubbles cause intensive shock waves in the surrounding liquid and the formation of liquid jets of high velocity¹²⁷. These mechanical forces act to divide droplets again and again forming microdroplets and eventually ‘homogenising’ even in the absence of surfactant.

Electrochemistry in biphasic systems is an area which has only been minimally exploited due to the difficulties in creating and maintaining emulsions in the absence of surfactant which can detrimentally affect the analysis^{128,129}. The use of ultrasound to form emulsions ensures that regardless of the relative densities of the two liquids, droplets of both are in constant contact with the electrode surface during voltammetric analysis.

This thesis will exploit the emulsification properties of ultrasound in order to perform the quantitative analysis of vanillin in an aqueous ethanolic solution of vanilla essence (Chapter 8). Chapters 9 and 10 report the development of a novel technique which utilises emulsification by ultrasound to facilitate the solvent extraction of target electroactive species for quantitative interrogation by sonoelectroanalysis.

1.5.5.1 Determination of Lead in Petrol

Owing to the continuing focus on total lead concentrations in petrol in recent years there is increasing demand for simple and rapid methods of determination. Existing methods include AAS¹³⁰⁻¹³², ICP-MS¹³³ and electrochemical stripping voltammetry although these techniques involve elaborate sample preparation, for example, decomposition of the organolead compounds with concentrated nitric acid and then ashing at $300\ ^\circ\text{C}$ ¹³⁴ or dilution in ethanol, nitric acid, mercury ions and detergent¹³⁵. To provide a rapid and facile alternative the determination of lead using biphasic sono-ASV has been established using a 12 mm Hg/Pt electrode. The protocol for obtaining an ASV response from additions of petrol to 0.1 M nitric acid involved holding the potential 0.0 V (vs. SCE) to clean any residual target metal from the electrode surface, followed by an accumulation step at $-1.0\ \text{V}$ for 240 s under $52\ \text{W cm}^{-2}$ ultrasound power for 7 mm horn to electrode separation. Each accumulation step was followed by a single ‘silent’ linear sweep stripping scan at $100\ \text{mVs}^{-1}$. The addition of aliquots of petrol to the cell prior to each accumulation step resulted in the formation of an emulsion on

insonation from which Pb could be plated onto the surface of the electrode. The peak area increased with increasing petrol additions and also behaved linearly with accumulation time over the range 7 s to 240 s.

The mechanism proposed for the detection of lead in the petrol aliquots involves the following steps: first, the extraction of the tetraalkyl lead from the petrol phase;



followed by reduction of lead at the surface of the electrode at a potential of -1.0 V;



and analytically sensitive stripping of Pb to form Pb^{2+} in solution



Insonation in the absence of electrolysis did not lead to the release of detectable Pb^{2+} which supports the assumption that the lead tetraalkyl is transferred from the organic phase into the aqueous phase (equation 21) prior to electrolysis, rather than the alternative homogeneous liberation of Pb^{2+} into solution.

Using the method given above, the microaddition technique was employed to give a value of 380 ± 40 mg L^{-1} for the concentration of lead in petrol detected by sono-ASV which can be compared to independent analysis using atomic absorption spectroscopy according to the Institute of Petroleum method no. 362/93¹³⁶. Excellent quantitative agreement was observed (see **Table 2**).

1.5.6 Aims

In this section a comprehensive introduction to the incorporation of ultrasound in electrochemical systems has been given. Subsequent sections will develop both the fundamental principles behind the technique and will provide novel examples of electroanalytical applications in “real” biological and environmental media.

Section 2 comprising chapter 3 will provide a detailed electrochemical investigation into the nature of the diffusion layer prevailing under insonated conditions for a range of solvents with different

colligative properties. The aim of this section is to achieve a thorough understanding of the interplay between cavitation and acoustic streaming at the electrode-solution interface.

Section 3 comprises chapters 4-7 which develop novel sonoelectroanalytical protocols for the quantitative determination of trace heavy metals in whole blood, fish gill mucous, artificial saliva (bovine) and human saliva. This aim of this section is to demonstrate the viability of sonoelectroanalysis for medical and environmental applications by emphasising the benefits of a relatively low cost, facile, rapid yet reliable technique with the potential for developing portable technology.

Section 4 comprising chapters 8-10 turns to biphasic sonoelectroanalysis and begins with the quantitative determination of vanillin in food flavouring. This application highlights the versatility of ultrasound with analysis of an electroactive organic species in non-aqueous and biphasic media. Chapters 9 and 10 of section 4 develop the concept of biphasic sono-double extraction into a viable technique capable of isolating trace heavy metal from low volumes of highly passivating media such as soft drinks and blood. Complexation of the metal ions in the “dirty” aqueous phase followed by extraction into a “clean” organic phase removes the passivating organic species. Subsequently the target metal ions are ultrasonically stripping into “clean” dilute acid prior to analysis by sonoelectroanalysis. The advantage of the technique is that passivating media will prefer to reside in the organic or aqueous phases but is unlikely to be doubly transferred into the final aqueous phase.

Section 5 comprising chapter 11 is a concluding section which employs the analysis of copper in anionic, cationic and non-ionic surfactant as a model for the analysis of industrial and domestic waste, which commonly contains high concentrations of surface active compounds. It is also a comparative section which provides a critical and quantitative evaluation of the techniques introduced in sections 3 and 4. The sonoelectroanalytical approaches are compared both with one another and electroanalysis in silent (no ultrasound) conditions where a voltammetric signal was often entirely precluded by the surfactant. The potential for sonoelectroanalysis and biphasic sonoelectroanalysis to be marketed as commercially viable techniques is emphasised.

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Chapter 1 - Introduction

Part 3

The Toxicity of Trace Heavy Metals

1.6 Medical Impact of Trace Heavy Metals

1.6.1 Copper Related Conditions

Copper is an essential mineral present in the bloodstream which is vital for infant growth, host defence mechanisms, bone strength, red and white cell maturation, iron transport, cholesterol and glucose metabolism, myocardial contractility and brain development¹⁻⁸.

Copper is distributed widely in foodstuffs and even the most mediocre diet furnishes 2-5 mg a day, which can be compared to the Recommended Dietary Allowance (RDA) subcommittee which proposed that 900 µg per day is adequate⁹. Consequently overt copper deficiency is not believed to be a widespread public health concern for most population groups. However, under certain circumstances, clinical conditions may predispose individuals to the risk of copper deficiency or copper excess¹⁰. Examples include deficiency in bottle fed^{11,12} or malnourished infants and children and conditions predisposing to excessive copper losses¹⁰. More unusual cases have seen zinc induced copper deficiency brought about by deliberate ingestion of metallic objects such as pennies¹³.



Figure 1 - Endoscopic photograph¹³ showing ingested coins in greater curvature gastric pouch of a 58-yr-old man with a 25-yr history of paranoid schizophrenia and metal pica, especially coins. He was diagnosed with zinc induced copper deficiency.

Adults receiving total parenteral nutrition (TPN) are also at risk particularly due to the removal of copper from TPN in cholestatic conditions¹⁴⁻¹⁶. It has been recognised that it is vital to monitor copper levels in such patients^{12,15}. In contrast, increases in copper concentrations have been reported in response to stress, inflammation, and infection¹⁷, in Parkinson disease and diabetes mellitus, and in conditions involving an obstruction to bile flow.

The inability to metabolise copper normally can lead to a number of potentially fatal conditions. The principal examples are is the expression of Menkes syndrome¹⁸⁻²³ and Wilson's Disease⁶. Menkes Syndrome is an X-linked, recessive disturbance of copper metabolism associated with a progressive clinical course and abnormal hair²⁴ (**Figure 2**). The disease is dominated by neurological symptoms combined with connective tissue manifestations, most of which can be explained by the lack of important copper enzymes²⁵.



Figure 2 – Scanning electron micrograph²⁴ of kinky hair exhibited by a sufferer of Menke's Syndrome

Wilson's disease is a progressive and uniformly fatal disorder that causes toxic concentrations of copper to accumulate in the liver and brain. It affects approximately 1 in 30,000 people who inherit a mutant pair of the gene ATP7B located on chromosome 13²⁶. The copper toxicosis of Wilson's disease is characterised by an average concentration of hepatic copper twenty times higher than normal²⁷. Neurologic symptoms may be progressively severe tremors²⁸, or incapacitating rigidity of the head, arms, hands or legs. As copper moves from the liver to brain, some of it deposits in Descemet's membrane of the cornea, producing gold or greenish gold Kayser-Fleischer²⁹ rings (**Figure 3**) or crescents. Misdiagnosis can be fatal and some patients exhibit neither Kayser-Fleischer rings nor low ceruloplasmin concentrations³⁰. Assessment of total copper status is therefore vital.

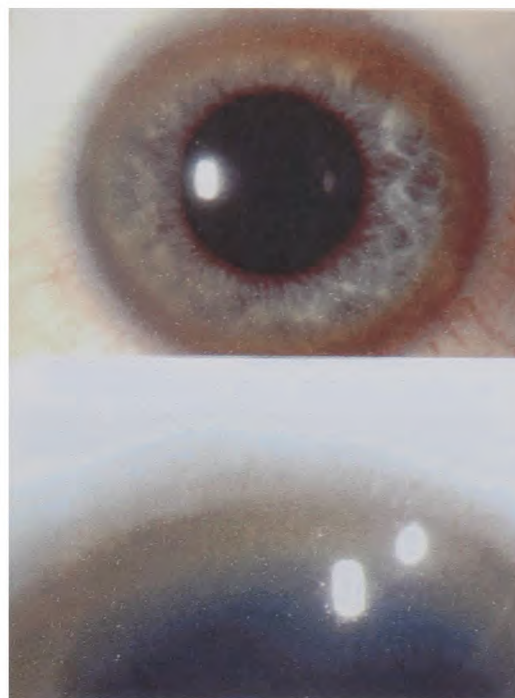


Figure 3 – Slit lamp biomicroscopy²⁹ shows peripheral golden brown Kayser-Fleischer deposits

Further conditions linked with copper deficiency include: occipital horn syndrome (OHS)²¹⁻²³, microcytic anaemia and neutropenia^{31,32}, bone disorders such as osteoporosis in children with radiological abnormalities³³⁻³⁵. Copper deficiency has even been linked to some forms of cardiovascular disease³⁶ and sudden infant death syndrome (SIDS)¹¹. Copper deficiency in children is often misdiagnosed as scurvy^{34,37} and in some cases the radiologic abnormalities and unexplained spontaneous fractures (**Figure 4**) have led to the erroneous conclusion of child abuse³³⁻³⁵. Had the conditions been correctly diagnosed, anaemia and neutropenia are quickly corrected by the administration of 10 %, by mass, aqueous copper sulphate solution.

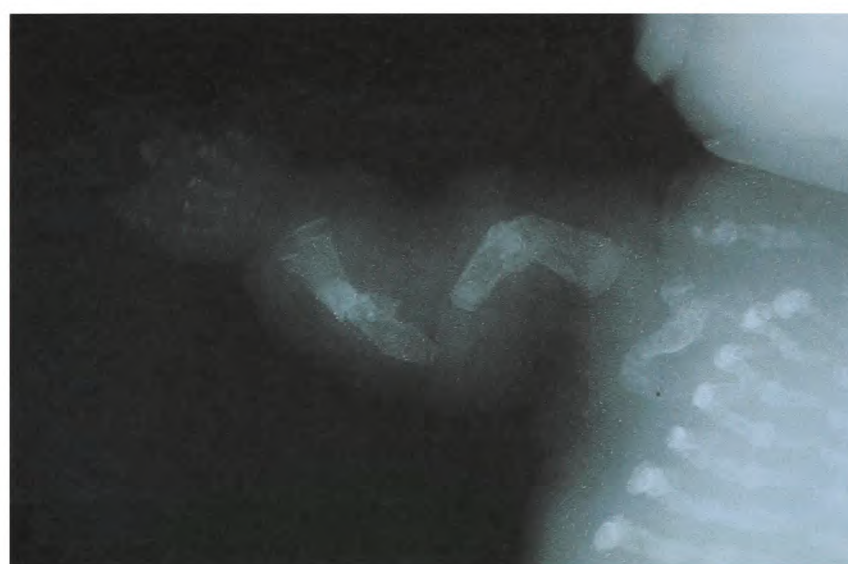


Figure 4 – Radiological abnormalities exhibited in an infant suffering from copper deficiency

Studies have shown that the variation in trace copper content in whole human blood is between 800 and 1400 $\mu\text{g L}^{-1}$ ⁶ depending on age and sex. More than 90% of this copper is bound to ceruloplasmin, an α_2 -globulin with ferroxidase activity (**Figure 5**). The diagnosis of copper deficiency at present is

established by the demonstration of a low serum ceruloplasmin level and less commonly by atomic absorption spectroscopy of serum copper content⁷. A ceruloplasmin level of less than 150 mg/L or 400µg/L copper can normally indicate deficiency in small infants and in later infancy, childhood and adulthood levels should exceed 700 µg/L¹.

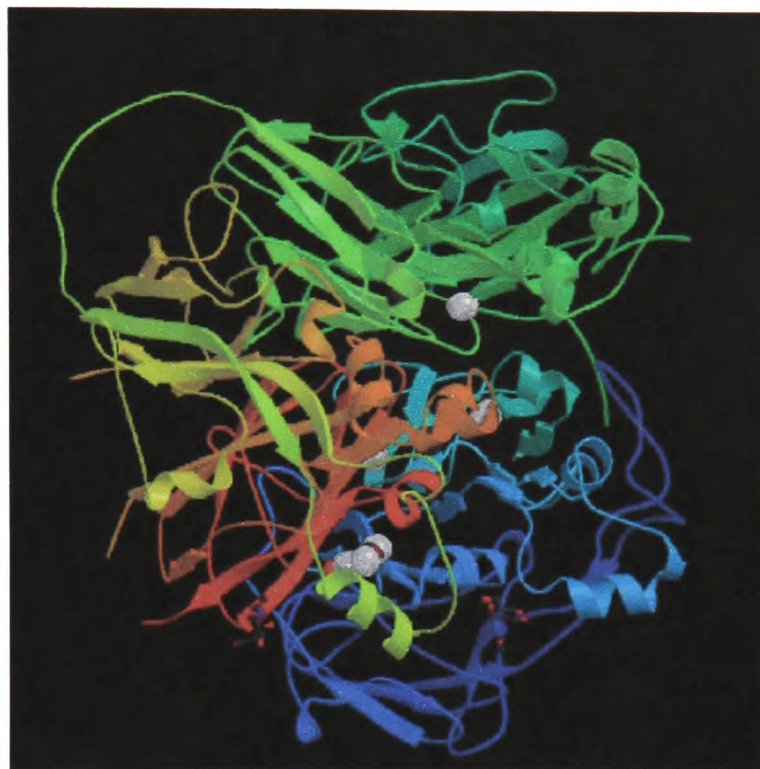


Figure 5 – Ceruloplasmin³⁸

1.6.2 Lead Related Conditions

Lead is a dangerous and important environmental pollutant, existing naturally in elemental, inorganic and organic forms. Its toxicity is well documented and it is recognized as a major environmental health risk³⁹⁻⁴². The major toxic effects of lead are referable to the bone marrow and to the gastrointestinal and nervous systems⁴³. **Figure 6** shows the symptoms expected for increasing blood lead concentration.

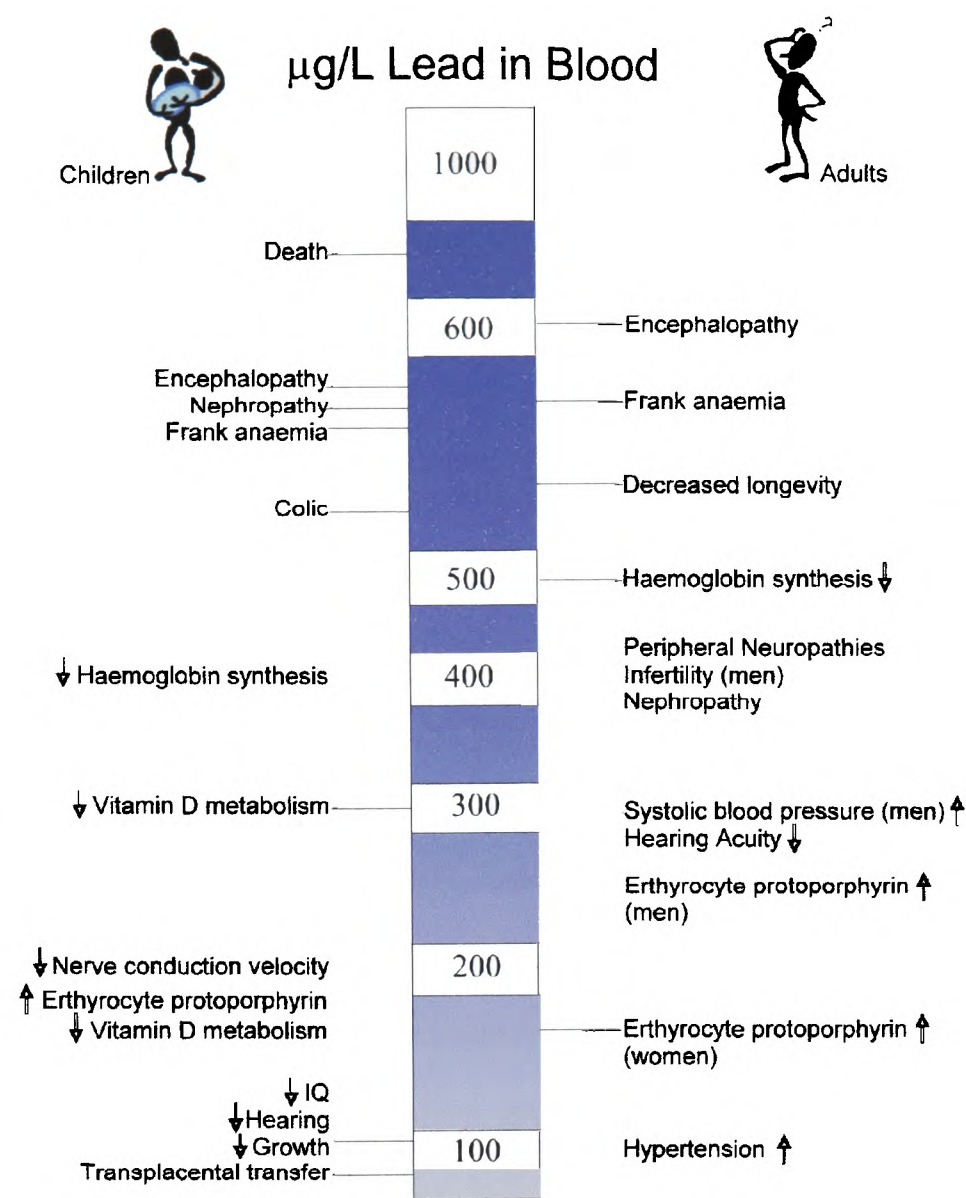


Figure 6 – Schematic diagram illustrating the symptoms associated with lead poisoning for given levels of lead in blood⁴⁴ - arrows indicate acceleration or depression of symptoms

Sources of lead include lead paints and dusts produced when these paints are old and chipped as well as lead based industries for example lead smelting, refining and battery manufacturing^{1,45,46}.



Figure 7 – Lead poisoning in this individual arose from the numerous ingested items including lead paint chips which are evident on the X-ray image

Lead in petrol and petrol emissions also provide cause for concern and are subject to European regulations^{47,48}. Within the UK the Control of Substances Hazardous to Health regulations (COSHH) and Control of Lead at Work regulations (CLAW) have recently been updated in order to implement the stringent requirements of the European Union Chemical Agents Directive^{49,50}. Most States of the USA have enacted detailed legislation, in particular for the protection of children against the hazards of lead poisoning⁵¹.

It is recognized that young children are highly susceptible to lead poisoning and it is one of the most common paediatric health problems in the United States⁵², affecting approximately 890,000 children nationwide at any given time⁵³. The sources of this exposure are primarily leaded paint, which was not banned in the United States until 1978, and contaminated soil⁵⁴. Lead from these sources exists or evolves into a variety of Pb^{2+} compounds which are persistent in the environment and difficult and expensive to remediate. At high concentrations, lead poisoning causes coma convulsions and even death. There is now a scientific consensus that exposure to even low levels of lead reduces IQ and causes profound developmental disorders in children⁵⁵ including reduction in attention span, reading and learning disabilities, hyperactivity, impaired growth and hearing loss⁵⁶. **Figure 8** shows bone abnormalities known as “lead lines” which are indicative of an infant’s exposure to lead in periods during their growth.



Figure 8 – “lead growth lines” evident at the joints of an exposed infant

1.6.3 Cadmium Related Conditions

The toxicity of cadmium resembles that of arsenic, mercury and lead and is well documented particularly because of widespread exposure arising from cigarette smoking and passive inhalation⁵⁷⁻⁵⁹. A known human carcinogen it is also nephrotoxic⁶⁰, causes renal dysfunction⁶¹ and has recently been

connected with osteomalacia and/or osteoporosis⁶². As with lead, health risks are particularly associated with exposure in utero and the early years of life⁶³ since adsorption and retention is significantly greater in infants than adults and the developing child is at greater risk from permanent damage. Diabetes sufferers also exhibit increased susceptibility to cadmium poisoning⁶⁴. Even very low concentrations of cadmium ($0.4 \mu\text{gL}^{-1}$) may have an impact on human fertility with sperm motility, linear and curvilinear velocity significantly correlated with semen cadmium levels⁶⁵. Cadmium remains in the body for a long period after ingestion and is mainly stored in the liver and kidneys. It manifests as a tubular dysfunction with increased proteinuria and calciuria⁶⁶. Long term exposure to cadmium has adverse effects on bone with a severe clinical manifestation being Itai-Itai disease (translated⁶⁷ this means “that hurts-that hurts”)⁶⁸. The main symptoms of this are severe bone pain and pathological fractures because of osteomalacia, waddling gait, aminoaciduria and glycosuria⁶⁹, see **Figures 9,10**.



Figure 9 – A patient suffering from the crippling bone deformation of itai-itai disease⁷⁰



Figure 10 – A pelvic x-ray showing a layer of bone change in both thigh bones (femur) where the arrows are pointing^{67,71}

The World Health Organisation (WHO) has established a provisional tolerable weekly intake (PTWI) for cadmium at $7 \mu\text{g/kg}$ of body weight⁷². In the non-smoking members of the general population, ingestion is the main route of entry for cadmium (approximately $1.4\text{--}25 \mu\text{g}$ per day)⁶⁶. Individuals who smoke typically double their total cadmium intake by the inhalation of cadmium oxide⁷³. In the occupational setting, workers may be exposed to cadmium oxide fumes generated during heating or welding of cadmium containing materials, or by inhalation of particles of metal, cadmium oxide and pigment dust. Such exposure has been connected with the motor industry and spray-painting vehicles, the zinc smelting industry where the pure metal is produced, the manufacture of cadmium pigments, nickel-cadmium batteries and the production of alloys^{74,75}. In such workers typical symptoms have included the alteration of lung function and there is a possible connection with lung and prostate cancers⁶⁶.

1.7 Environmental Pollution by Heavy Metals

Industrial and domestic waste introduces a range of potentially harmful substances into the aquatic environment including pesticides, heavy metals and hydrocarbons. High levels of discharge may have an immediate impact including death of aquatic organisms, for examples fish and plants. Lower levels of discharge can result in accumulation in aquatic organisms with consequences such as immunosuppression, reduced metabolism, and damage to gills and epithelia⁷⁶. Specific alterations to gill ultrastructure have been associated with damage by copper and cadmium⁷⁷. Exposure to as little as 0.1 mg L^{-1} copper is responsible for stimulating hyperactivity of mucous cells resulting in a thick layer of mucus on the gills at 0.5 mg L^{-1} (**Figure 11**).

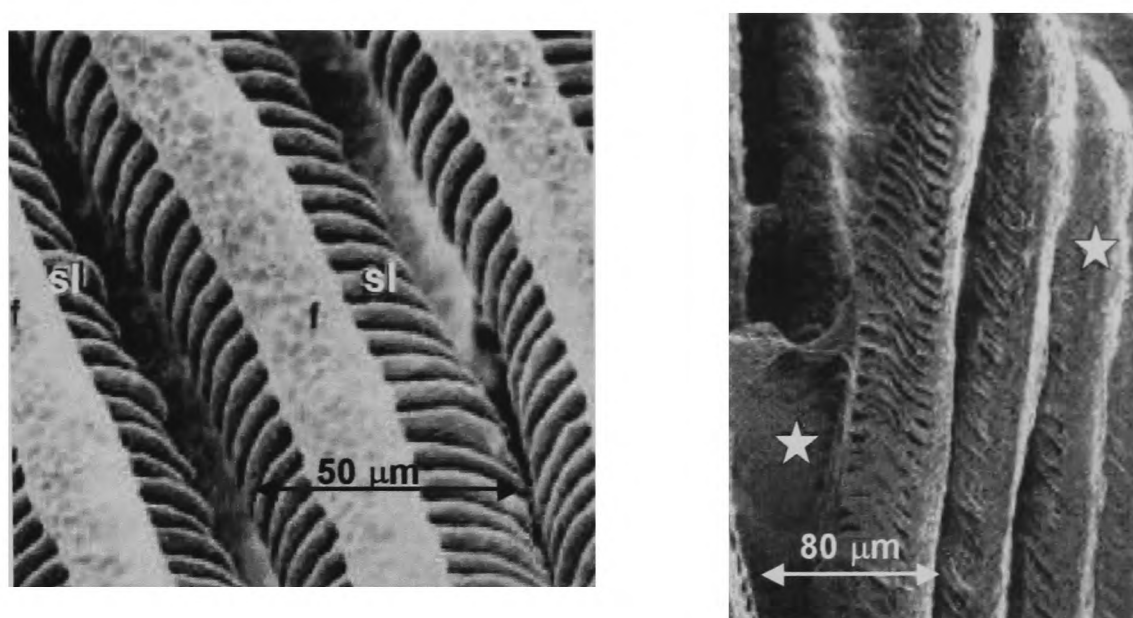


Figure 11 – Normal healthy gills (shown left) and gills affected by 0.1 mg L^{-1} copper contamination⁷⁷ stars show hyperactive mucus production “sl” indicates secondary lamellae

Cadmium damage at 1.0 mg L^{-1} was associated with the formation of large subepithelial spaces within the secondary lamellae (**Figure 12**) and at 10.0 mg L^{-1} with lamellar aneurism.

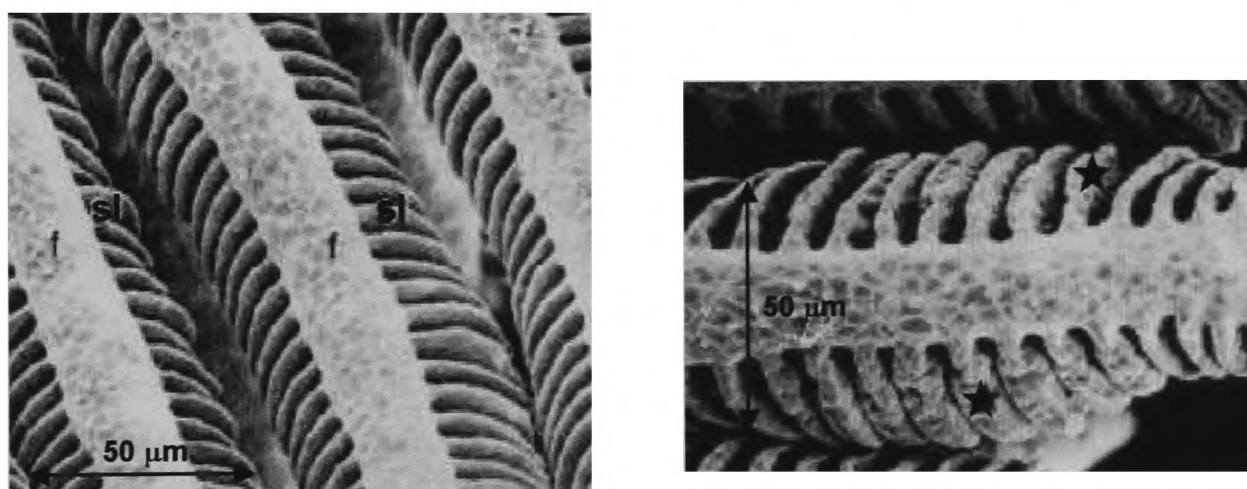


Figure 12 – Normal healthy gills (shown left) and gills affected by 1 mg L^{-1} aqueous cadmium⁷⁷ the stars show the formation of subepithelial spaces within the secondary lamellae (“sl”)

Road “runoff” represents a significant problem to aquatic environments bordering heavily used roads such as motorways⁷⁸. Heavy metals such as lead, zinc, copper and cadmium are persistent pollutants. Their impact on aquatic flora and fauna is dependent on the speciation of metals with the soluble salts of copper and cadmium being readily absorbed by fish as illustrated above. Insoluble lead compounds remain in particulate matter and high levels accumulate in silt and riverbed sediment⁷⁹.

Histopathological studies of fish exposed to pollutants have shown that fish gills are efficient indicators of water quality since they are external organs with a large surface area^{77,80,81}. Impairment of gill function can also seriously damage the health of the fish. Gills may therefore be considered to be the most appropriate indicators of water pollution levels^{77,80,81}. Aqueous metal interactions at fish gills have been modelled to demonstrate the binding and rate of uptake of different heavy metals into the

gills^{82,83}, and it has been proposed that fish gill mucus plays a large part in these interactions via the glycoproteins within the layer of mucus covering these organs⁸⁴.

As well as the aquatic environment, heavy metals are often bioaccumulated in plants. Willow, a source of food for Rocky Mountain ptarmigan, acts to “biomagnify” the concentration of cadmium⁸⁵. Once ingested by the birds the cadmium accumulates in the kidneys and as in the human itai-itai disease (see s.1.6.3), calcium levels in the blood drop and the body “borrows” calcium from the bones.



Figure 13 – This X-ray image of a ptarmigan shows a fracture caused by calcium deficiency triggered by cadmium damaged kidneys

The human health risk from eating ptarmigan is low unless the internal organs are consumed. A greater threat is posed to predators such as eagles, hawks, foxes and coyotes that may consume the whole bird.

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Chapter 2 – Experimental

2.1 Cell Design

The most convenient arrangement for delivering ultrasound to the electrochemical cell, for both ease of manipulation and control, is the ‘face on’ orientation of horn and electrode illustrated in **Figure 4** of section 1.4.2. The custom made Pyrex® cells illustrated in **Figures 1** and **2** are designed to accommodate this arrangement.

There is a need for thermal control dictated by the temperature dependence of reaction rates, diffusion coefficients and formal electrode potentials. However, insonation has the inevitable consequence of heating the solution. For experiments in aqueous solution the cell was thermostatted by introducing a stainless steel cooling coil through which water was circulated from a bath at constant temperature (see **Figure 1**). This enabled voltammetric measurements to be conducted at an approximately constant temperature ($25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$). For experiments below room temperature in non-aqueous solvent, thermostating of the cell was accomplished by means of an ethanol–dry ice mixture in a stainless steel bath (see **Figure 2**) allowing experiments to be conducted over a wide range of low temperatures (from $-60\text{ }^{\circ}\text{C}$ up to room temperature) correct to within $2\text{ }^{\circ}\text{C}$.

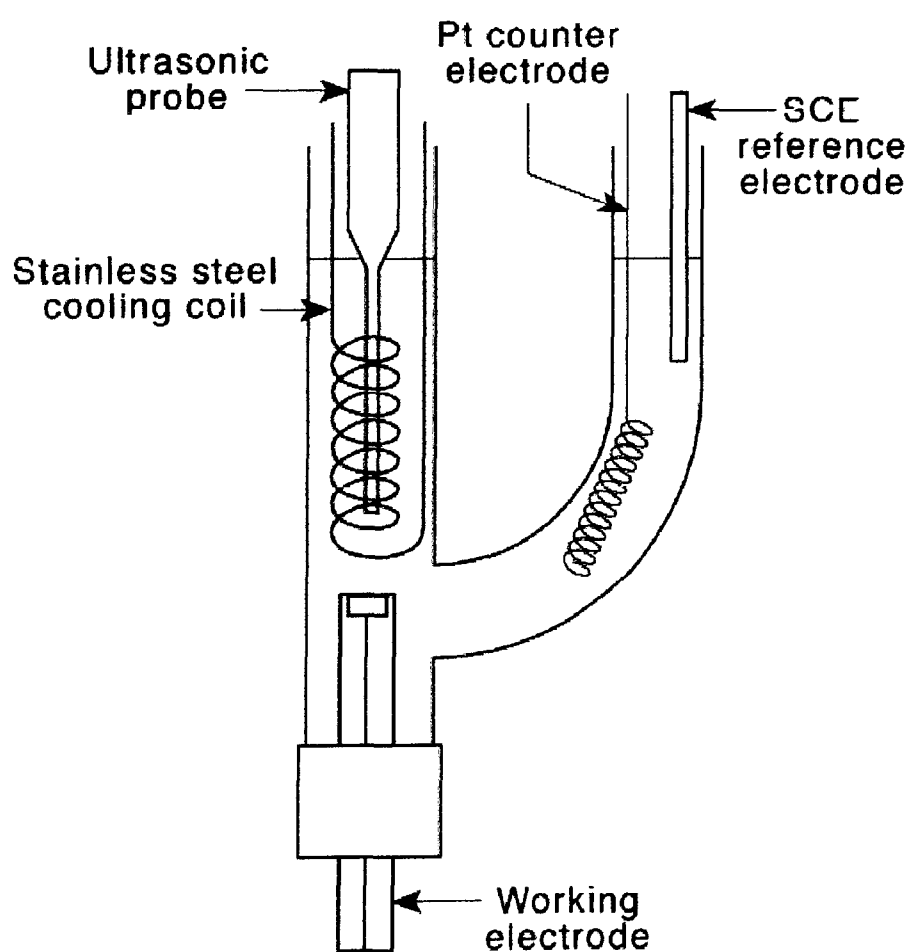


Figure 1 - Schematic diagram of the experimental arrangement used for a cell volume of 25-35 cm^3 and a horn tip area of 0.07 cm^2

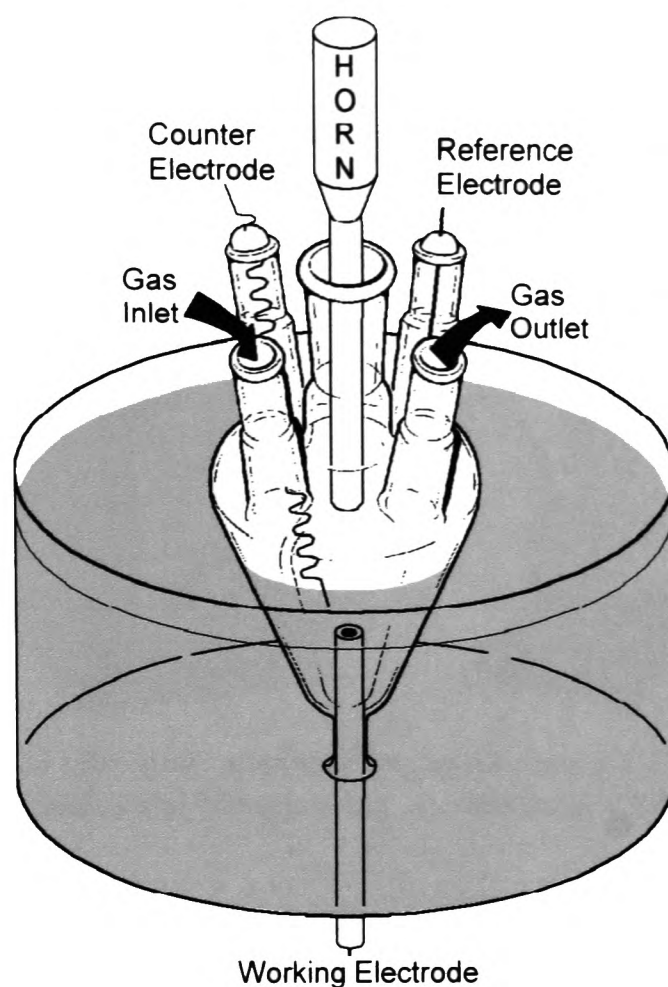


Figure 2 – Schematic diagram of the experimental arrangement used for working with solvents at low temperature such as liquid ammonia and dimethylformamide (DMF) at 218 K. The cell is housed within a cooling bath adjusted to the appropriate temperature. The gas inlet and outlet provides for circulation of argon in order to maintain an inert argon atmosphere when working with low temperature ammonia and DMF. The horn illustrated has a cross sectional tip area of 0.07 cm^2 and the maximum cell volume is 75 cm^3

2.2 The Ultrasonic Horn

The working electrode was oriented directly opposite the ultrasonic horn with an adjustable horn to electrode separation of up to 3 cm. Two models of ultrasonic horn have been used for the work reported in this thesis. The first, shown in **Figure 4**, is a Model CV 26 ultrasonic processor (Sonics and Materials Inc. Connecticut, USA) with an operating frequency of 20 kHz. It was equipped with a titanium alloy microtip (Ti 90%, Al 6% and V 4%) (Aldrich, UK) of cross-sectional area 0.07 cm^2 as illustrated schematically in **Figure 3**. The benefits of using titanium alloy are its high fatigue strength, low acoustic loss and chemical inertness.

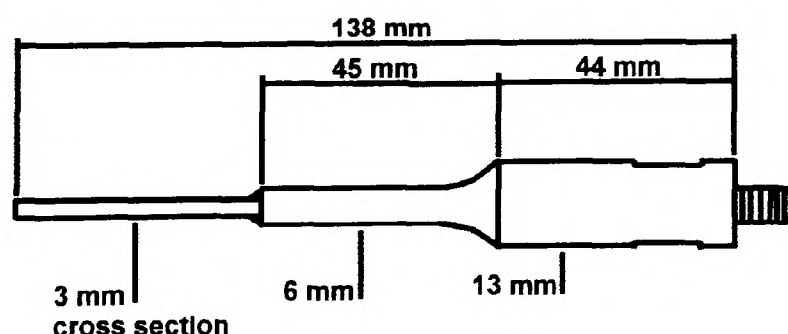


Figure 3 – Schematic diagram of a titanium alloy stepped microtip with a cross sectional diameter of 3 mm



Figure 4 – Model CV 26 ultrasonic processor¹ equipped with a 1.4 cm² cross sectional area titanium alloy microtip (Sonics and Materials Inc., Connecticut, USA)

The second ultrasonic horn employed was a dr. Hielscher GmbH Model UP 50H (Stuttgart, Germany) with an operating frequency of 30 kHz. It was equipped with a titanium microtip of cross sectional area 0.07 cm² (model MS3 dr. Hielscher GmbH, Stuttgart, Germany). Again, the ultrasonic intensity was calibrated calorimetrically according to the procedure of Margulis^{3,4} over the range 10-80 W cm⁻². The unit is portable with the generator and the transducer combined in one unit weighing 0.75 Kg, and can be operated whilst hand held. It has a lower range of accessible ultrasound intensities (10-80 W cm⁻²) than the CV 26 ultrasonic processor described above, which places a limitation on its utility for electroanalytical applications.



Figure 5 – Model UP50 H ultrasonic processor² (dr.hielscher GmbH, Stuttgart, Germany)

	Sonic and Materials	dr. Hielscher
	Model CV 26	Model UP50H
Operating Frequency / kHz	20	30
Net power output ^a / W	500	50
Weight of ultrasonic probe / Kg	1.24	0.75
Solution Volume /cm ³	10-50	10-50
Maximum dislocation amplitude ^a / μm	378	not reported
Intensity ^b / W cm ⁻²		
	Maximum	80
	Typical ^c	30
Generated temperature in water / °C min ⁻¹		
	Maximum	20
	Typical ^c	2.8

a reported in technical specifications - http://www.sonicsandmaterials.com/VCX-500-750/VCX-500750_Specs/vcx-500750_specs.html and http://www.hielscher.com/ultrasonics/50h_p.htm respectively (accessed 9 August 2002)

b determined calorimetrically (see s.2.3) for a maximum setting of 100% (large horn) or 35% (small horn)

c intermediate intensities (50% large horn and 10% small horn) allow a wider range of mass transports to be investigated

Table 1 – Technical data and characteristics of the Sonics and Materials Model CV26 and dr. Hielscher Model UP50H ultrasonic processors

2.3 Calorimetric Calibration of the Ultrasonic Horn

Calibration of the power output of the sonic horn is essential to provide an accurate measure of the level of ultrasound delivered to the solution, since the operationally adjustable parameter of amplitude (%) is uninformative. In aqueous solution, the method used was that of Margulis et al^{3,4} which relies on measuring the change in temperature of water as a function of time.

Initially the horn was tuned in air in order to draw the minimum electrical power for the amplitude to be used. The horn was then lowered to 3 cm depth into 50 cm³ of water contained in a 75 cm³ thermally lagged beaker. Ultrasound was delivered at the amplitude of interest for 5 minutes and the temperature was recorded every 30 s. The amplitude range over which the measurements can be taken is 5-35 % for the 0.07 cm² micro-tip. A calorimetric plot of temperature versus time permits the initial slope dT/dt to be calculated for given amplitude. The power output can therefore be calculated using equation 1

$$P = mC_p \left(\frac{dT}{dt} \right)_{t=0} \quad (1)$$

where P is the ultrasound power (W), m is the mass of water (g) and C_p is the specific heat capacity ($4.184 \text{ J K}^{-1} \text{ g}^{-1}$ for water)⁵. From the power output corresponding to the amplitude setting the intensity (W cm^{-2}) can be calculated:

$$\text{Intensity} = \frac{\text{Power}}{\text{Area}} \quad (2)$$

where the units are power (W) and area (cm^2).

This enables a plot of amplitude versus intensity to be obtained as illustrated in **Figure 6**. The polynomial trend in such a plot is used to convert amplitude to intensity (W cm^{-2}) giving subsequent measurements in a meaningful form.

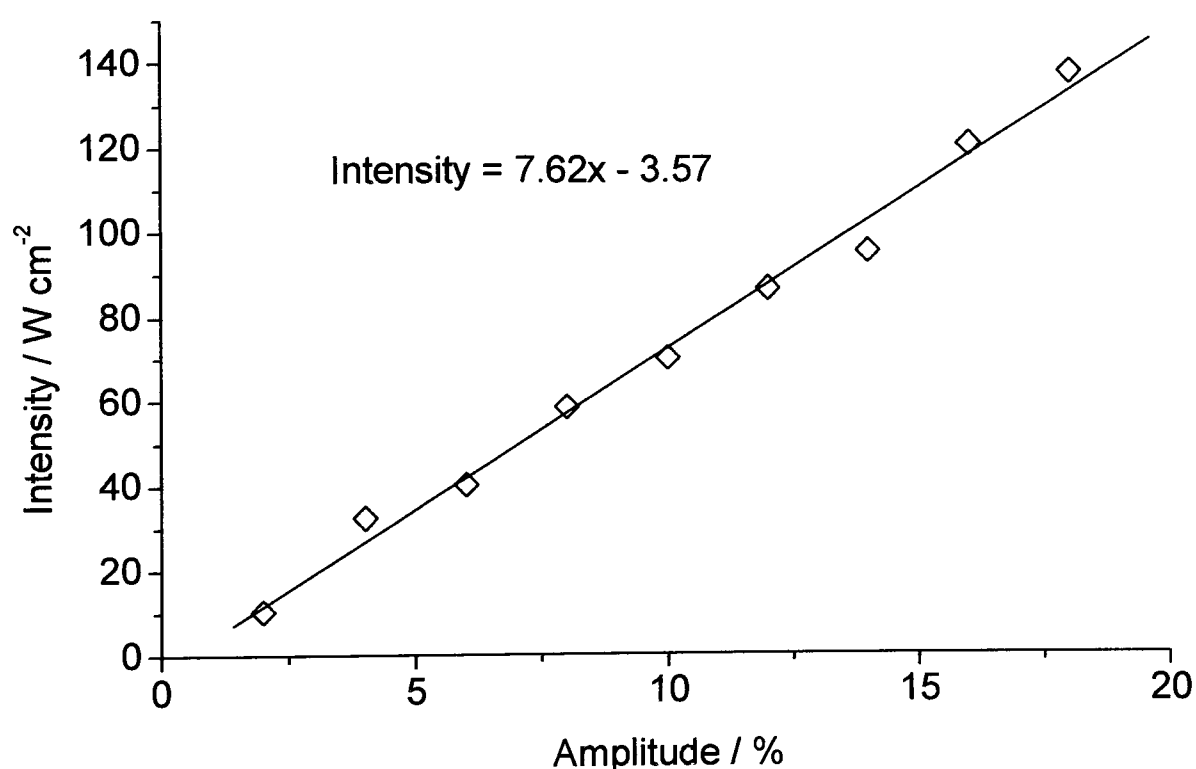


Figure 6 – Calorimetric calibration for the Sonics and Materials Inc. CV 26 horn equipped with a 0.07 cm^2 titanium alloy microtip, for operational amplitudes of 2-18 %

A calibration of this kind is required for each horn and transducer, it is also repeated periodically throughout the lifetime of a titanium alloy tip since in general there is a slow reduction in power output over the lifetime of the tip due to cavitation erosion of the tip.

For the ultrasound intensity calibration in liquid ammonia, conventional calorimetric measurements as described above are not applicable and a method based on the measurement of the electrical power consumed by the transducer has been developed⁶. Given the following assumptions:

- i) the energy dissipated in air is negligible compared to that dissipated in the solvent; and
 - ii) the energy lost due to heat dissipation in the transducer is constant when the tip is tuned correctly;
- then the power dissipated in the solvent is given by the difference between the total consumed power in air and in the solvent under study.

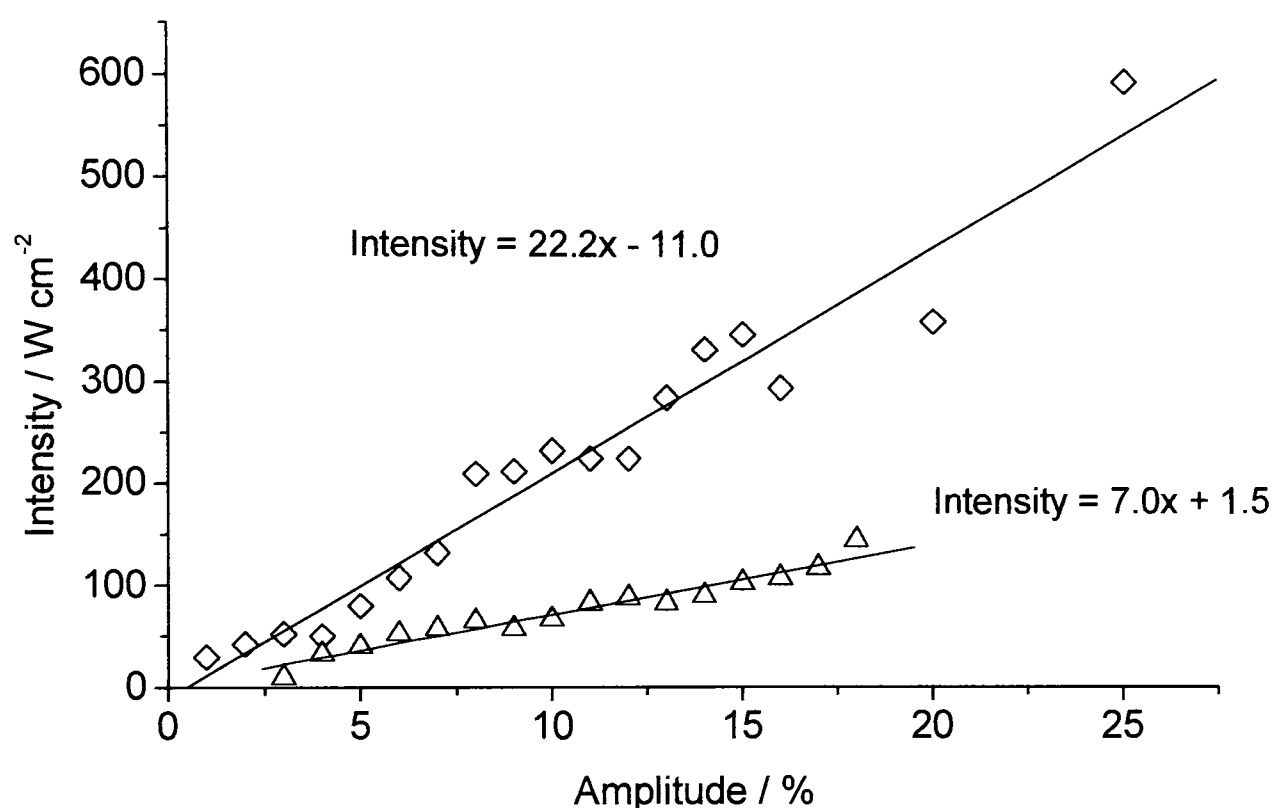


Figure 7 – Plot showing the ultrasound intensity emitted from the horn probe versus the amplitude, for water at room temperature (◇) and for liquid ammonia at –35 °C (Δ) (adapted from ref. 6 – for a Sonics and Materials Inc. VCX-400)

Figure 7 illustrates the linear dependence of intensity and amplitude which, for aqueous solution, is in agreement with the calorimetric method described earlier. It is evident from **Figure 6** that the energy dissipated in liquid ammonia at –35 °C is considerably lower than that dissipated in water at room temperature. This has been attributed to the difference in viscosity and the level of cavitation activity⁶. The intensity dissipated in liquid ammonia increases with decreasing temperature with ammonia at –60 °C absorbing ultrasound to a similar extent to water at room temperature⁶.

2.4 Potential Control

As introduced in s.1.1.5, in electrochemical research it is the process at a single electrode, the working electrode, which is of prime interest. In the work reported in this thesis the potential of this electrode was controlled with respect to the reference electrode (s.1.1.6) in a three electrode cell with a platinum

auxiliary (counter) electrode (s.1.1.5), by means of an Eco-Chemie μ -AUTOLAB II® (Utrecht, Netherlands). This is a compact potentiostat for general electrochemical research and electroanalysis⁷. It has a current limit of 80 mA and can perform all the electrochemical techniques described in s.1.2.4.

2.5 Electrode Preparation

An introduction to working electrode materials was given in s.1.3. Preparation of all the working electrodes used in this thesis began with the bare electrode being polished according to the procedure of Bond et al⁸ using alumina up to a grade of 0.1 μ m (Buehler Ltd, Evanston, US).

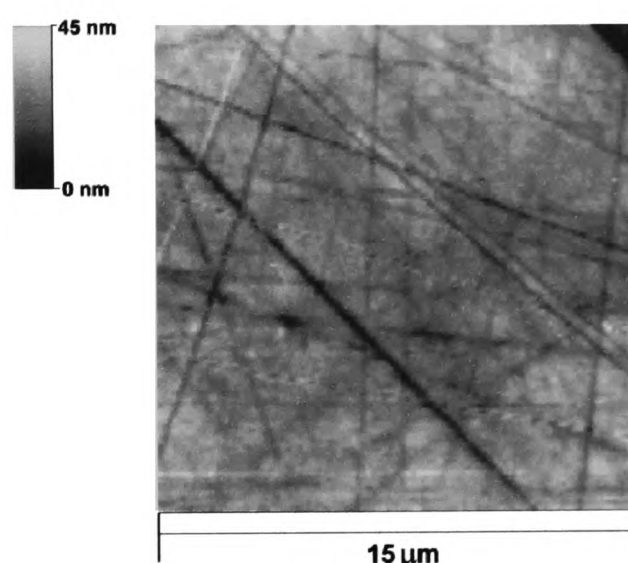


Figure 8 – atomic force microscopy image of a platinum electrode polished to 0.1 μ m grade

In Chapter 6 a polymer coated working electrode is used. Nafion® coating was achieved according to the procedure detailed by Brett et al⁹ by applying 10 μ L of 0.2 % (w/v) freshly prepared Nafion solution to the electrode surface, followed immediately by 5 μ L DMF as a casting solvent, with a micropipette. The electrode was then rotated at 50 rpm while the solvents were evaporated in a warm stream ($\sim 40^{\circ}\text{C}$) from an air gun positioned 20 cm from the electrode surface. When the solvents are fully evaporated a bluish sheen can be seen to coat the electrode surface. The polymer film was then cured for 60 s in a hot airstream ($\sim 70^{\circ}\text{C}$) with the air gun a few centimetres from the electrode surface. Mercury deposition was achieved in situ during anodic stripping voltammetry (see s.1.5.3) by the addition of between 70 – 100 μ M mercury (II) acetate to the cell contents.

2.6 Chemical Inventory

Reagents used were all of analytical grade or the highest commercially available purity (see appendix 2 for a full list of chemicals used). Solutions were prepared using UHQ grade water of resistivity not less than 18 M Ω cm (Elgastat, High Wycombe, Bucks., UK). The detection limits of electrochemical

measurements could be affected by other electrode reactions occurring in the same potential range. The most prevalent of these, and one which interferes with the study of reduction processes, was the reduction of oxygen, since its solubility in aqueous solutions open to the atmosphere is up to 10^{-4} M⁵. Where necessary oxygen was purged from the solution using nitrogen or argon gas (PureShield, BOC, UK) prior to electrolysis.

¹ <http://www.sonicsandmaterials.com/gateway/gateway.html>, Sonics and Materials Inc, Connecticut, USA, accessed 9 August 2002

² http://www.hielscher.com/ultrasonics/50h_p.htm, dr. Hielscher, Stuttgart, Germany, accessed 9 August 2002

³ M.A. Margulis, A.N. Maltsev, *Russ. J. Phys. Chem.*, **1969**, 43, 592

⁴ T.J. Mason, J.P. Lorimer, D.M. Bates, *Ultrason.*, **1992**, 30, 140

⁵ G.W.C. Kaye, T.H. Laby. “*Tables of Physical and Chemical Constants*” 15th Ed., Longman Scientific and Technical, Harlow, UK, **1986**

⁶ F. J. Del Campo, A. Neudeck, R.G. Compton, F. Marken, *J. Electroanal. Chem.*, **1999**, 477, 71

⁷ <http://www.ecochemie.nl>, Eco-Chemie, Netherlands, accessed 9 August 2002

⁸ T.J. Cardwell, J. Mocak, J.H. Santos, A.M. Bond, *Analyst*, **1996**, 121, 357

⁹ F. -M. Matysik, S. Matysik, A.M. Oliveira Brett, C.A. Brett, *Anal. Chem.*, **1997**, 69, 1651

Section II

**Physical Effects of Ultrasound as Probed
Voltammetrically**

Chapter 3 – Differential Pulse and Chronoamperometric Studies of Insonated Systems: Acoustic Streaming and Cavitation Effects

3.1 Introduction

The often dramatic increase in mass transport limited currents seen on insonation with a 20 kHz probe is attributed to both cavitation phenomena and acoustic streaming (see ss. 1.4.3 and 1.4.4). Cavitation is caused by the collapse of voids created by ultrasonic compression and rarefaction of the solution and results in localised high pressures and temperatures throughout the bulk solution and microjetting at the surface of the electrode. Acoustic streaming is a non-linear effect which allows high intensity sound energy to be converted into kinetic energy in the liquid surrounding the sound source. The resulting turbulent solution flow can give rise to significant sustained currents.

The aim of this chapter is to assess the relative contributions of acoustic streaming and cavitationally induced microjetting to the sono-voltammetric response of well-characterised simple redox couples measured in the solvents water, dimethylformamide (DMF) and ammonia. The wide liquid range of DMF ($T_m = -60\text{ }^{\circ}\text{C}$, $T_b = 156\text{ }^{\circ}\text{C}$) permits comparative data to be obtained at temperatures similar to those used both for the water and ammonia based systems. In addition to simple voltammetric data recorded under transport limited currents at both micron and millimetre dimensioned electrodes, differential pulse voltammetry (DPV) is used as a sensitive means of exploring the nature of the diffusion layer prevailing under steady-state electrolysis at insonated electrodes. The potential sequence defining this form of voltammetry is given in s.1.2.4.3.1. Given that in DPV it is a difference in current which is monitored, it follows that the technique will be sensitive to the transport conditions prevailing during the interval between current sampling times. In particular, in a cavitation free sono-voltammetric experiment it would be expected¹⁻³ that a diffusion layer would be established in which convection was reduced as compared to bulk solution. The transport of material within this diffusion layer would therefore control the DPV response. However, in a cavitationally highly active situation, the frequent disruption of the diffusion layer by bubble activity would reduce the voltammetric sensitivity if the frequency is such that bubbles are regularly formed in the time period between sampling times.

Initially, a computer simulation of DPV is used to explore the effect of convection on the voltammetric response. First, the limit of a Nernstian diffusion layer in which the solution is fully stagnant is considered. This is compared with the use of a rotating disc electrode (RDE) where the influence of well-defined convection with the diffusion layer can be quantified. James L Melville carried out the computational simulation⁴ the theory can be found in Appendix 3. The results of the theoretical investigation permit the resolution of convective and cavitation effects in experimental sono-voltammetry reported in s.3.4.

3.2 Simulation Theory Results

The computer simulation of DPV at a RDE was run under physical conditions that applied to the reduction of $\text{Ru}(\text{NH}_3)_6^{3+}$ in water. The parameters used can be found in **table 1**.

Temperature / K	298	$[\text{Ru}(\text{NH}_3)_6^{3+}]_{\text{initial}} / \text{mol cm}^{-3}$	10^{-6}
Kinematic viscosity / $\text{cm}^2 \text{s}^{-1}$	10^{-2}	Diffusion Coefficient [†] $\text{Ru}(\text{NH}_3)_6^{3+/2+} / \text{cm}^2 \text{s}^{-1}$	9.1×10^{-6}
Scan range / V	-0.3 $\rightarrow$ 0.0	Formal potential E^0 / V	-0.171
Amplitude E_a / V	0.01995	Electrode radius [‡] / cm	5.00×10^{-2}
Step potential E_s / V	0.00195	Frequency of disc rotation / Hz	1 $\rightarrow$ 50
Pulse duration ($\tau - \tau'$) / s	0.05	Computational Parameters (see appendix 4)	
Rest period (τ') / s	0.1	NJ	500
		NL	500

[†] see reference 4

[‡] The radius of the electrode used was nominally set as 5.00×10^{-2} cm, in order to directly compare with results obtained with a static electrode. It should be noted that this is not the size of a practical RDE, but the currents simply scale with the area of the electrode.

Table 1- parameters used for the computer simulation (see appendix 3) of DPV at an RDE

The diffusion layer thickness that is created by the motion of the RDE is predicted by the following equation⁶:

$$\delta = 0.643 \nu^{1/6} D_{\text{RED}}^{1/3} W^{-1/2} \quad (1)$$

The variation of peak current with rotation speed for a rotating disc which has convective contribution to the mass transport characterised by equation (1) and the same electrode in the absence of convection with a Nernstian diffusion layer (see s. 1.2.3.3) of the same length, δ , is shown in **Figure 1**.

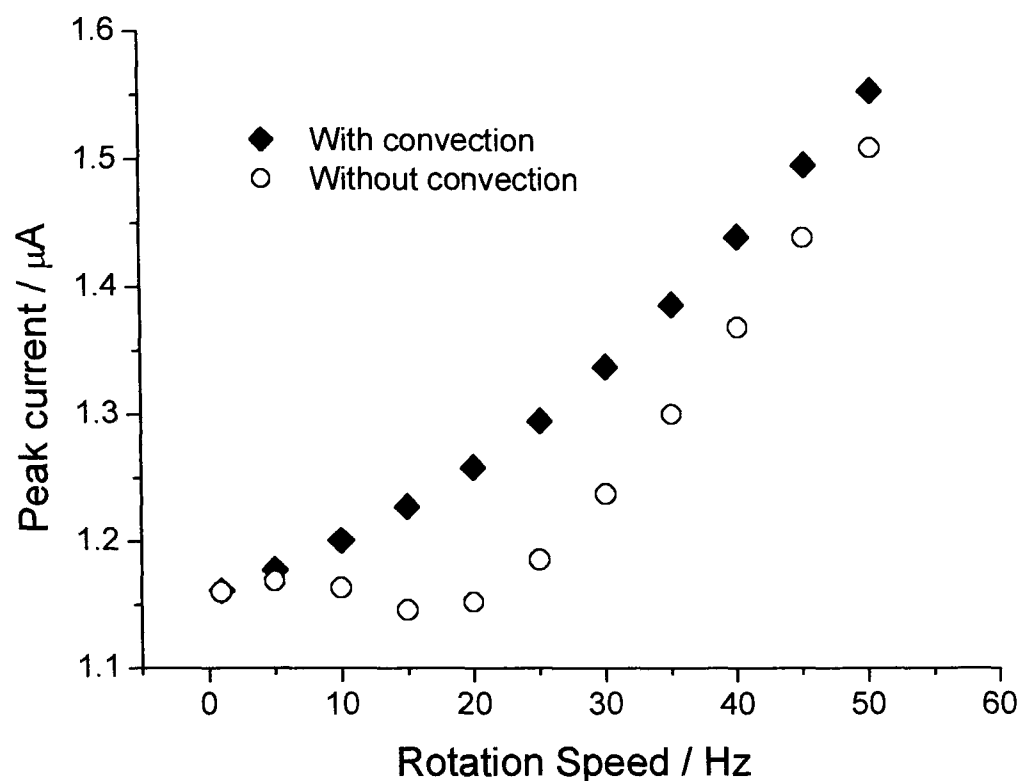


Figure 1 - Results for the simulation of DPV of the reduction of $\text{Ru}(\text{NH}_3)_6^{3+}$ in water at (○) a static electrode and (◆) a Rotating Disc Electrode. The parameters used are tabulated above. In the case of a static electrode, diffusion layer thicknesses were set equal to the values that would be expected at a specific disc rotation speed by equation 1.

The results in **Figure 1** are plotted to show the change in peak current against the rotation speed of the RDE required to achieve the relevant diffusion layer thickness predicted by equation 1. The general trend is as the speed of rotation increases (which corresponds to a reduction in diffusion layer thickness in the static simulation), the peak height increases, due to convection increasing mass transport to the electrode.

Turning to sonovoltammetry the simulations predict, by analogy, that at a given diffusion thickness, if the main effect of sonication is an enhancement of mass-transfer to the electrode via convection from acoustic streaming, a larger peak current will flow than that which would be measured under pure, entirely stagnant, “Nernstian” diffusion layer conditions.

3.3 Experimental

All reagents used were electrochemical grade, or the highest available purity, see appendix 2 for full details. Aqueous solutions were prepared using UHQ grade water of resistivity of not less than 18 MΩ cm (Elga, High Wycombe, Bucks, UK).

The working electrodes used were macro (1 mm) and micro (50 μm) platinum discs mounted in glass. Teflon was avoided as electrode shrouding material due to its large coefficient of thermal expansion,

which caused the Pt electrode to protrude in low temperature experiments, thus exposing an unknown surface area. The working electrode was polished prior to measurements according to the procedure of Bond et al (see s.2.5) with alumina of increasing grade down to 1 μm particle size. A gold foil served as the counter electrode and a silver wire (Aldrich, 99.9% 0.5 mm diameter) was used as a pseudo-reference electrode in ammonia and DMF. In aqueous solution all potentials are quoted with respect to the saturated calomel electrode (SCE, Radiometer, Copenhagen). The electrochemical cell used throughout is illustrated in s.2.1 Figure 2 and was thermostatted as described previously (s.2.1). The ultrasonic horn was a VCX400[‡] (Sonic and Materials Inc. Connecticut, USA) with a stepped 3 mm diameter titanium alloy microtip (see s.2.2 Figure 3).

Electrochemical measurements in liquid ammonia and DMF were carried out under dry conditions in an inert atmosphere of argon. This is especially important in liquid ammonia, where traces of water may preclude meaningful voltammetric measurement. The supporting electrolyte in ammonia, potassium iodide, was pre-treated by grinding into a fine powder and drying in an oven at 130 - 140 °C overnight. The electrodes were also stored in a dessicator prior to use. The ammonia was dried by condensing it into a flask containing pieces of lithium, 50 mL was then distilled under argon into the electrochemical cell containing the supporting electrolyte, KI.

The diffusion coefficient for ferrocene in DMF at low temperature was determined by means of a series of potential step experiments at a microelectrode (50 μm) under silent conditions and at different temperatures. The diffusion coefficient was found to behave according to an Arrhenius-type relation. A plot of $\ln D$ vs. $1/T$ for diffusion in DMF gave an activation energy of 10500 J mol⁻¹. This permits the calculation of diffusion coefficient at any required temperature using equation 2.

$$D_{T_2} = D_{T_1} \left[\frac{E_a}{R} \left(\frac{1}{T_1} - \frac{1}{T_2} \right) \right] \quad (2)$$

D_{T_2} is the diffusion coefficient in m²s⁻¹ at the desired Kelvin temperature, T_2 , D_{T_1} is the known diffusion coefficient for that species at a given Kelvin temperature, T_1 , E_a is the activation energy for diffusion in J mol⁻¹ in a particular solvent (in this case DMF), R is the gas constant.

[‡] VCX 400 is the new product code for the CV26 described in s.2.2, Sonics and Materials Inc.

The ultrasonic intensity was calibrated at room temperature and low temperature conditions over the range 40-550 W cm⁻² as described in s.2.3. In order to achieve different diffusion layer thicknesses for the DPV experiments, both the distance between the ultrasonic horn and the electrode, and the ultrasonic intensity were varied according to need.

The DPV parameters typically employed were amplitude, Ea, 0.025 V, step potential, Es, 0.00495 V, pulse duration ($\tau - \tau'$), 0.05 s, rest time, τ' , 0.1 s (see s.1.2.4.3.1). Time resolved experiments were conducted at both macro and micro electrodes under the presence of power ultrasound. The duration of the experiments ranged from 100 ms up to 500 ms, with a timescale resolution of 50 μ s.

3.4 Results and Discussion

3.4.1 Insonated Chronoamperometry at a 50 μ m Platinum Electrode

Chronoamperometric measurements were recorded at a 50 μ m diameter platinum electrode in ammonia, water and DMF (at 298 and 218 K) for the well-characterised simple one electron redox couples given in table 2.

Solvent	Redox System	Supporting electrolyte	Silent Current / nA	$D_B / \text{cm}^2 \text{s}^{-1}$	$E_{1/2} / \text{V}$
DMF (218 K)	Ferrocene	TBAHFP	4	2.6×10^{-6}	0.774 vs. Ag
DMF (298 K)	Ferrocene	TBAHFP	12	1.2×10^{-5}	0.470 vs. Ag
H ₂ O	$\text{Ru}(\text{NH}_3)_6^{3+}$	KCl	13	9.1×10^{-6}	-0.171 vs.SCE
NH ₃	Nitrotoluene	KI	18	1.4×10^{-5}	-0.390 vs. Ag

Table 2- Redox systems studied at a 50 μ m platinum disc electrode under silent conditions.

For all experiments the electrode was held at a potential corresponding to the transport limited electrolysis of the substrate of interest. Under silent conditions, steady, near constant currents were observed (see s.1.2.5) consistent with the equation:

$$I_{\text{lim}} = 4nFD_B [B]r \tag{3}$$

where r is the radius of the electrode and D_B corresponds to the diffusion coefficients given in table 2. Note that the currents are of the order of several nano-amperes. When insonated, the current-time behaviour for the same systems is shown in **Figure 2**.

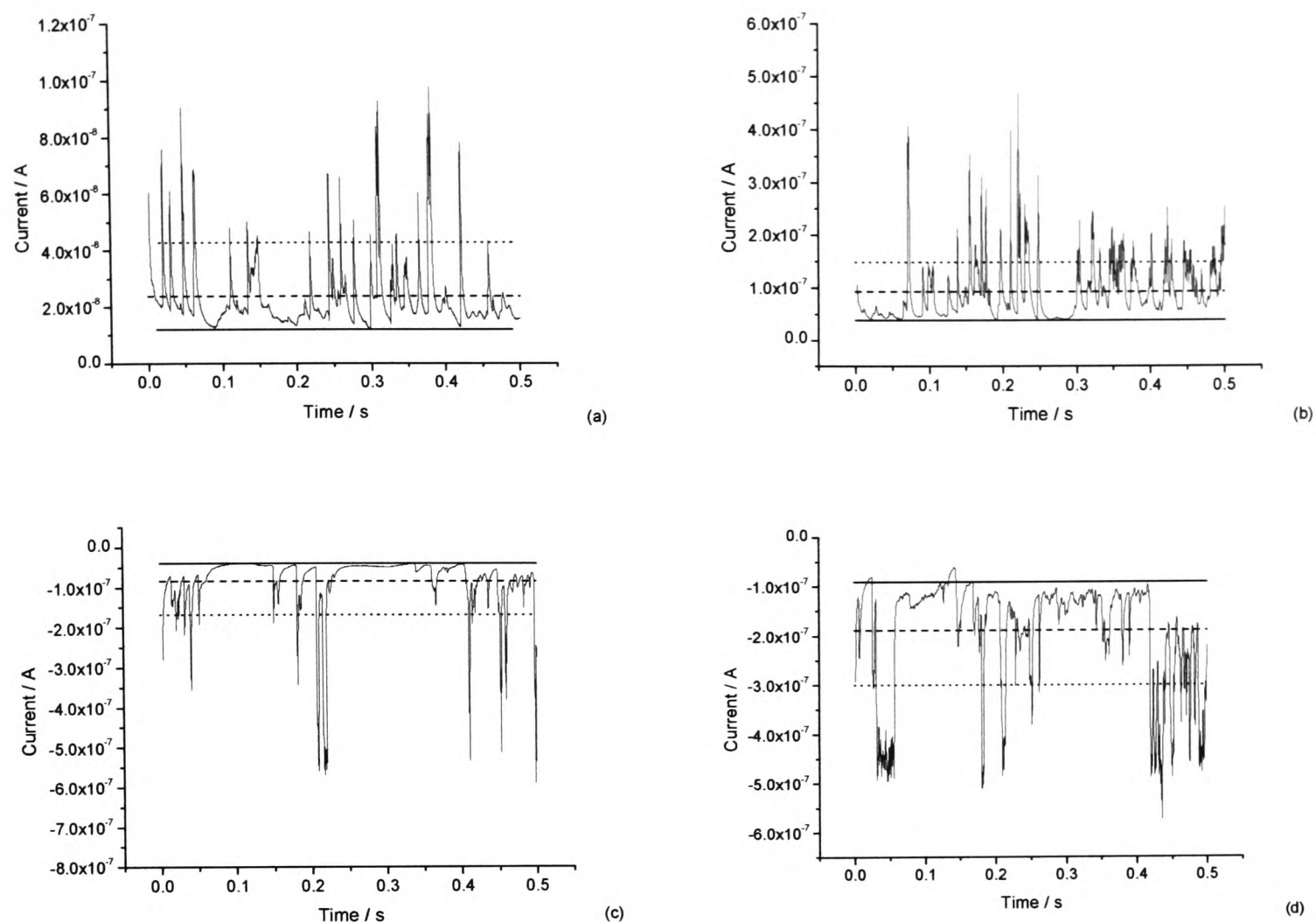


Figure 2 - Chronoamperometric experiments at a 50 μm diameter Pt disc in the presence of 250 W cm^{-2} ultrasound in different media and temperatures.

(a) 1mM ferrocene in DMF at 218 K.

(b) 1mM ferrocene in DMF at 298 K.

(c) 1mM $\text{Ru}(\text{NH}_3)_6^{3+}$ in water at 298 K

(d) 1 mM nitrotoluene in liquid ammonia at 218 K.

The additional lines superimposed on the traces correspond to the calculated baseline current (—) calculated average current (---) and standard deviation (.....)

Figure 2a, which shows the behaviour found in DMF at 218 K, is considered first. Sharp spikes are evident, superimposed on a background current which is much enhanced in comparison with the steady state current seen under silent conditions. The average current and the estimated background current are shown in **Figure 2a** together with an indication of the magnitude of the standard deviation. The spikes are caused by the collapse of single cavitation bubbles in the vicinity of the electrode. When a cavitation bubble collapses near the electrode surface, a jet of electrolyte is cast against it, bringing new electroactive material and thus giving an enhanced current. This way, one can record direct hits or near misses of these microjets of solution as individual current transients of differing height and shape. Therefore, for a microjet impinging directly on the electrode surface, the current observed in the corresponding spike may be higher than if the jet had hit the edge of the electrode. These observations and inferences are consistent with the work of Birkin⁷ Degrand⁸ and Alkire^{9,10}.

Figures 2b, 2c and 2d show analogous experiments to those reported for DMF at 218 K but correspond to DMF and water at 298 K, and ammonia at 218 K. Again the traces are annotated to show the estimated “background” current and the standard deviation. Comparison of the former with the “silent” limiting currents reported in table 2 reveal a substantial increase under all conditions indicating that acoustic streaming contributes measurably to the mass transport resulting in a steady convective flow augmenting that from diffusion to the microelectrode. However comparison of “baseline” and “average” currents show a significant contribution from cavitation in all cases.

Comparing **Figures 2a, b, c and d**, the frequency and violence of cavitational events is different from one medium to the next. This reflects differences in physical properties between solvents, primarily the vapour pressure and viscosity. These parameters are reported in table 2. It is possible to assess the “violence” of imploding of cavitational bubbles by observing surface erosion and the limiting current of insonated redox processes when physical conditions promote an increase in the force of cavitational collapse. The violence of each cavitational event is known to change with both applied pressure^{11,12} and vapour pressure¹². It is thought that cavitational bubbles are allowed to grow larger in media of high vapour pressure¹¹ and therefore when the bubble collapses the implosion is less violent due to the cushioning effect arising from its larger size. This has been termed “soft cavitation”¹². Large bubbles may also have greater opportunity to move away from the surface of the electrode. Conversely sonication within a solvent of low vapour pressure brings about much more violent cavitational events¹¹. In this case more energy is required to disrupt the medium in order to create a void. The bubbles formed are therefore much smaller, reducing the “cushioning effect”. The chance of these small bubbles escaping from the surface is lessened, the resulting cavitational collapses become not only more frequent but also violent.

Solvent	NH ₃ (218 K)	Water (298 K)	DMF (298 K)	DMF (218 K)
Vapour Pressure / mmHg	200	24	4	2
Viscosity / cP	0.4	0.9	0.95	1.0

Table 2 - Vapour Pressure and Viscosity Data¹³⁻¹⁵

In water and liquid ammonia (**Figure 2 c and d**), where vapour pressure is higher fewer spikes are observed than in DMF at 298 K possibly since bubbles are allowed to grow large enough to rise up and

leave the solution without collapsing. Greater stability of bubbles in the solvent with the highest vapour pressure (ammonia) is also evident (**Figure 2d**) where a broad cluster of spikes during the first 50 μs may be interpreted as oscillation of a large bubble. The viscosity of the solvent is also a contributing factor to the nature of the observed cavitation. A comparison of **Figure 2a** and **b** demonstrates that although the vapour pressure of DMF is lower at 218 K than at 298 K the violence and frequency of cavitation does not increase. The peak sizes are in fact much smaller and less frequent in the low temperature case. The viscosity of DMF at low temperature is higher than at room temperature, and it is proposed that there is less efficient replacement of solution at the electrode surface on cavitational collapse and therefore a smaller current enhancement when the microjet impinges on the surface. The frequency of cavitational collapse is reduced in comparison with the higher temperature case due to the increased energy required to create a void in the more viscous solution of low vapour pressure. The observations described above are shown schematically in **Figure 3** and showed good reproducibility across different batches of electrodes.

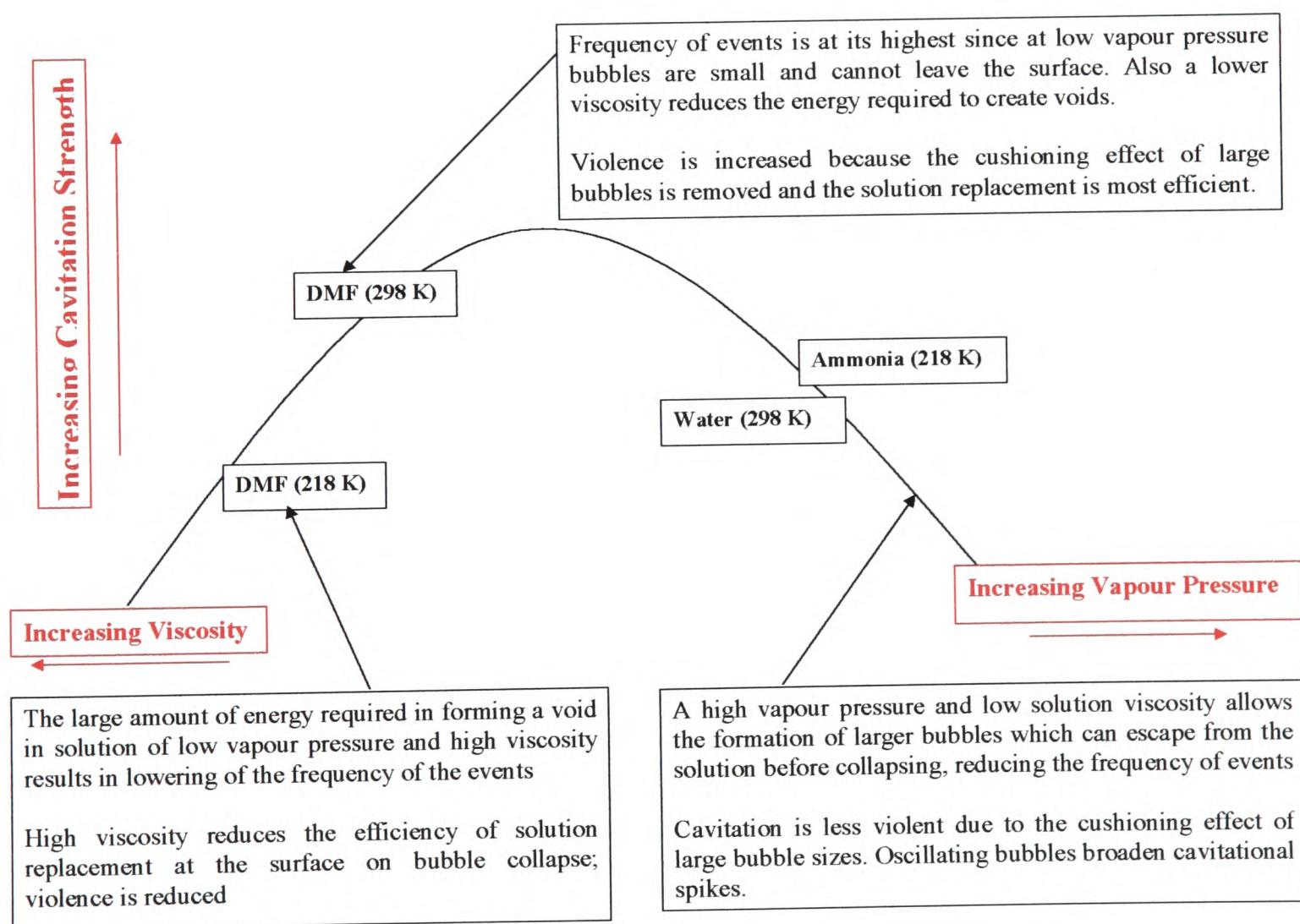


Figure 3 – Schematic diagram illustrating the effect of vapour pressure and viscosity on the observed cavitation frequency and violence.

3.4.2 Insonated Chronoamperometry at a 1 mm Platinum Electrode

Macroelectrode experiments (1 mm diameter Pt disc) were conducted in which chronoamperometric measurements were recorded in water (298 K), DMF (298 and 218 K) and NH_3 (218 K) in the presence of ultrasound. The electrode was held at potentials such that reduction (nitrotoluene in NH_3 and $\text{Ru}(\text{NH}_3)_6^{3+}$ in water) or oxidation (ferrocene in DMF) of electroactive species was controlled by mass transport. The observed current traces are shown in **Figure 4**.

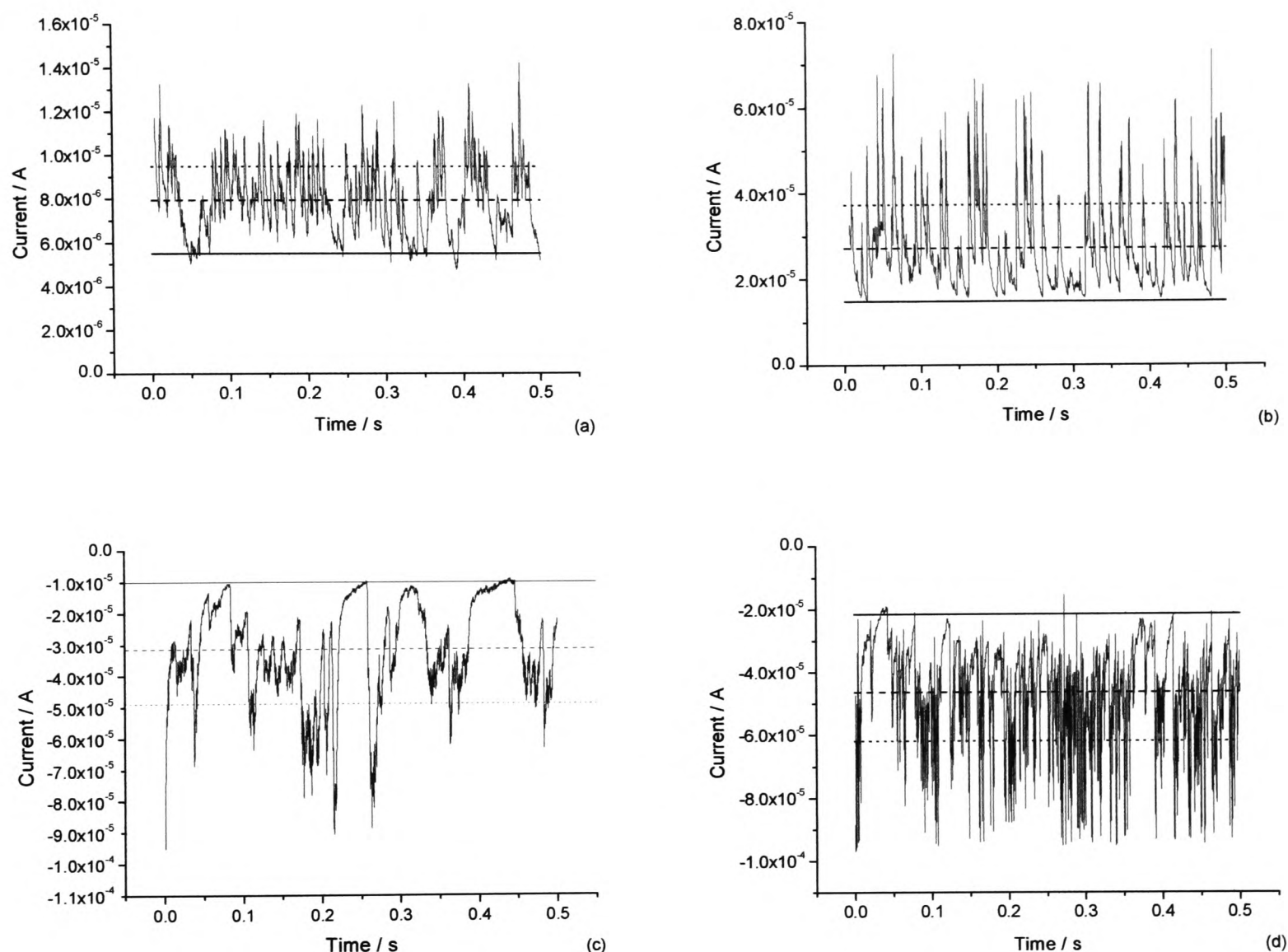


Figure 4 - Chronoamperometric experiments at a 1 mm diameter Pt disc in the presence of 250 W cm^{-2} ultrasound in different media and temperatures.

(a) 1mM ferrocene in DMF at 218 K.

(b) 1mM ferrocene in DMF at 298 K.

(c) 1mM $\text{Ru}(\text{NH}_3)_6^{3+}$ in water at 298 K

(d) 1 mM nitrotoluene in liquid ammonia at 218 K.

The additional lines superimposed on the traces correspond to the calculated baseline current (—) calculated average current (---) and standard deviation (.....)

In this set of experiments, three ultrasonic probe-electrode distances were used (5, 10 and 15 mm; see table 3), but the ultrasonic power employed remained constant at 250 Wcm^{-2} . Although the traces resemble those from the microelectrode experiments, there is a noticeable difference between them. Those obtained at the macroelectrode are much “noisier” than their microelectrode counterparts regardless of the medium. It is still possible to see sharp spikes for the case of DMF, and the presence

of large oscillating bubbles in water and ammonia may also be inferred. The spikes, however, no longer correspond to single events but relate to a multiplicity of collapses occurring at similar times. The current arising from one acoustic bubble can go almost unnoticed at an electrode of millimetre dimensions due to the magnitude of the overall current. The sono-currents at the macroelectrode (**Figure 4**) are the order of microamps, whilst the currents caused by single cavitational collapses (**Figure 2**) are the order of nanoamps. In addition, given that the area of the macroelectrode is *ca.* 400 times larger than the microelectrode, it is reasonable to conclude that at a macroelectrode more than one event will happen at the same time on surface, given the frequency of single bubble events recorded on the microelectrode.

Solvent	Horn-electrode distance / mm	T / K	Baseline / μA	Average current / μA	Standard deviation [†] / μA	Diffusion layer [‡] / μm
DMF	5	218	4	6	2	5
	10		3	4	1	7
	15		2	3	1	10
DMF	5	298	18	27	7	5
	10		10	18	12	10
	15		10	13	7	10
H ₂ O	5	298	22	33	5	3
	10		13	17	4	5
	15		11	16	6	6
NH ₃	5	218	20	38	12	5
	10		18	29	17	6
	15		16	26	9	7

[†]relating to the average current.
[‡] values calculated using the baseline current via equation 4

Table 3- Currents obtained at a 1 mm Pt. Electrode under 250 W cm⁻² ultrasound in different media. Diffusion layer thicknesses have been calculated using equation 4 and *D* values given in table 2.

The baseline currents shown in **Figure 4** were estimated and can be attributed to the effect of acoustic streaming alone. These currents were then used to calculate the diffusion layer thicknesses quoted in table 3 using equation 4, which presumes a simple Nernstian diffusion layer model (see s. 1.2.3.3).

$$I_{lim} = \frac{nFAD_B[B]_{bulk}}{\delta}$$

(4)

In this equation *I_{lim}* is the baseline current, *A* the electrode area, *F* the Faraday constant and [*B*]_{bulk} the bulk concentration. Table 3 also contains the average current and standard deviation values as defined in **Figure 4**. The contribution of cavitation to the traces such as in **Figure 4** may be quantified by two factors: the standard deviation of the current from the average current, and the difference between the

baseline current and the average current. Both factors follow the same trend as they do for the microelectrode case discussed above. In particular the relative contribution of cavitation decreases as the horn-electrode separation increases, consistent with the known localisation of cavitation events near to the tip of the sonic horn. However, on examining **Figure 4** it is important to observe the “multiplet” nature of the spikes recorded at a macroelectrode contrasting with the discrete spikes observed in the case of a microelectrode. One can infer from this that a multitude of stable oscillating bubbles¹⁶ on the surface contribute to the observed transient. This highlights the difficulties in quantifying cavitation at a macroelectrode.

3.4.3 Insonated Differential Pulse Voltammetry at a 1 mm Platinum Electrode

In this section DPV is used as a sensitive means of probing the physical nature of the diffusion layer prevailing at an insonated macro-electrode to facilitate assessment of the relative contributions of acoustic streaming and cavitation in different media.

Linear sweep voltammograms at a scan rate of 25 mV s^{-1} were recorded using a 1 mm platinum macroelectrode electrode in a face on arrangement with an ultrasonic horn at a range of ultrasound powers from 50 to 500 W cm^{-2} and horn to electrode separations of 2 to 15 mm for the simple redox couples described in table 2. Determination of the baseline currents allowed equation 4 to be employed to calculate the diffusion layer thickness associated with a stagnant diffusion layer for each combination of horn electrode separation and power. The results of this calibration gave a range of diffusion layer thicknesses from *ca.* 7 μm down to *ca.* 0.5 μm which could be effected by a known combination of horn to electrode separation and ultrasound power in each solvent.

Differential pulse voltammograms were then recorded (see s.1.2.4.3.1 Figure 11) ($\tau - \tau' = 0.05 \text{ s}$, $\tau' = 0.1 \text{ s}$ $E_a = 0.025 \text{ V}$, $E_s = 0.00495 \text{ V}$) for an insonated 1 mm platinum electrode over the range of diffusion layer thicknesses calibrated. **Figure 5** shows a typical sono- DPV voltammogram for ferrocene in DMF at 298 K.

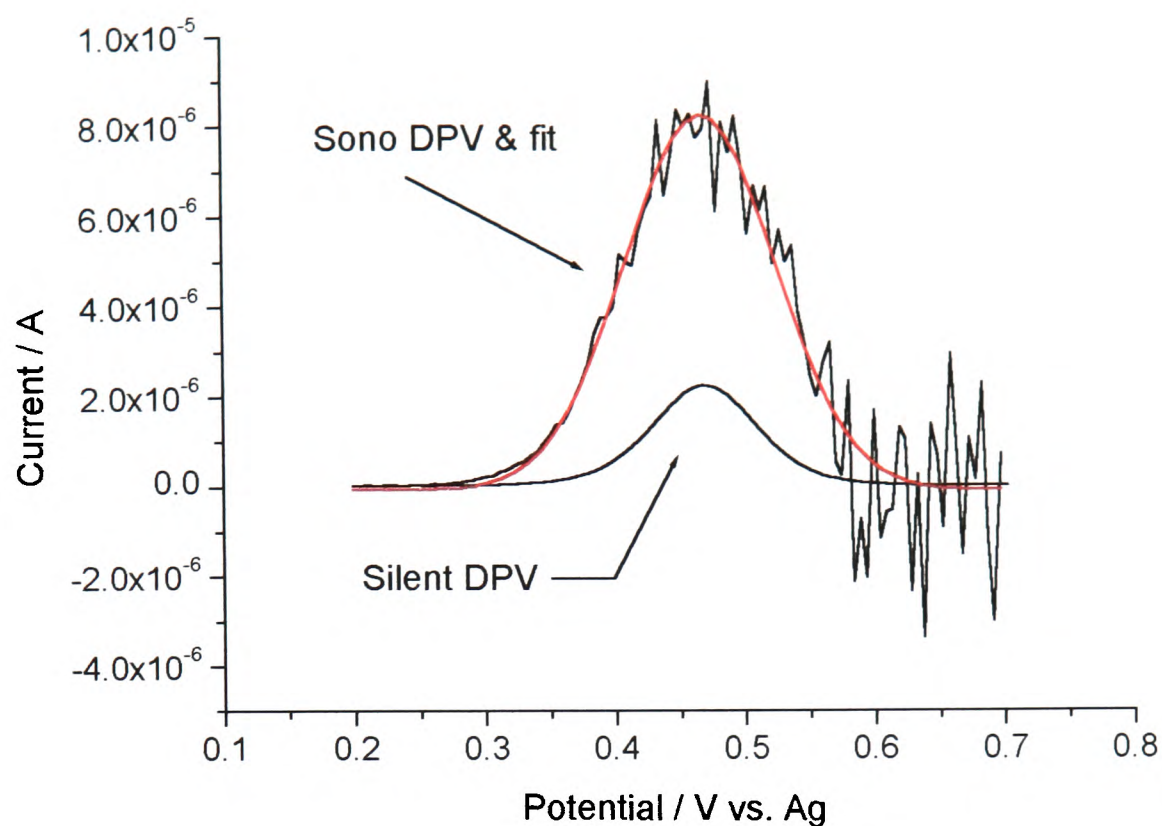


Figure 5 - Comparison between sono and silent DPV of 1 mM ferrocene in DMF at 298 K

The "noisy" data was fitted to the gaussian form typical of a silent voltammogram using Origin 6.0 (Microcal, MA, USA) which allowed the determination of the peak current in each of the insonated cases. Note the enhancement of the insonated signal in **Figure 5** when compared with silent conditions.

Simulation of DPV as outlined in s.3.2 and appendix 3 was used to calculate the theoretical peak current for each of the diffusion layer thicknesses (estimated as described in s.3.4.2 above) in the insonated experiment. Given the assumption made in the theory we estimate that the theoretical predictions are likely to be accurate to within 5 to 10%¹². The parameters of the DPV simulation corresponded to those employed experimentally. **Figure 6 (a-d)** shows a comparison of experimental peak currents and theoretical peak currents for the solvents DMF, water and ammonia at a range of diffusion layer thicknesses.

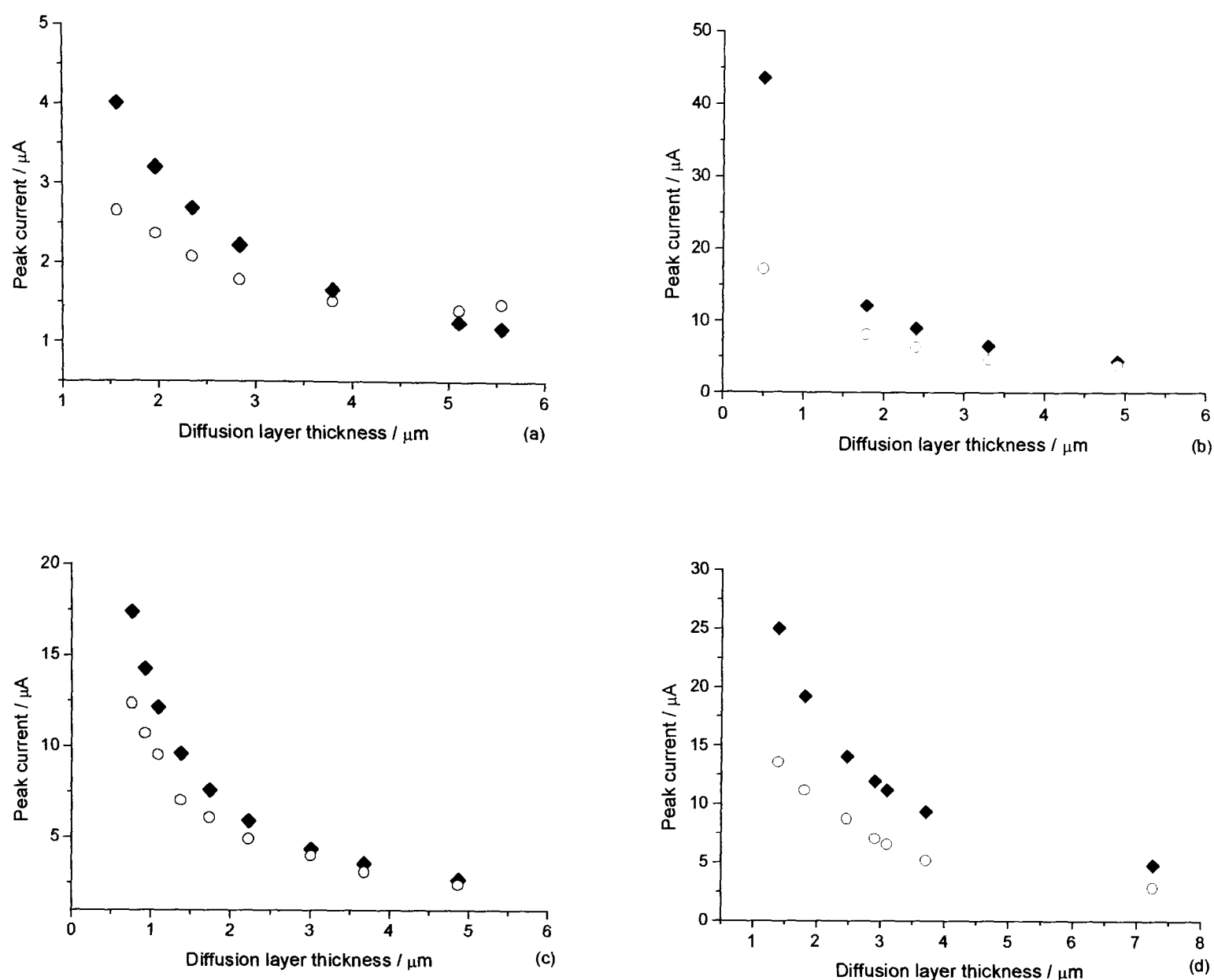


Figure 6 - Theoretical stagnant Nernst diffusion model($\blacklozenge$) and experimental ($\circ$) DPV peak currents for (a) 1mM ferrocene in DMF at 218 K
 (b) 1mM ferrocene in DMF at 298 K
 (c) 1mM $\text{Ru}(\text{NH}_3)_6^{3+}$ in water at 298 K
 (d) 1 mM nitrotoluene in liquid ammonia at 218 K

It is evident from **Figure 6** that experimentally obtained peak currents are smaller than those theoretically predicted at a given diffusion layer thickness, deviating most markedly at small diffusion layer thicknesses where the ultrasonic perturbation is greatest. It was predicted from the simulations for the rotating disk reported above that the increasing mass transport via pure convection (higher rotation speeds) would lead to a larger peak current than that which would be measured under "Nernstian" diffusion layer conditions. It is therefore likely that cavitation, not acoustic streaming, is responsible for this deviation due to the turbulent collapse of bubbles perturbing or even replacing the diffusion layer. For all the solvents the largest deviation is observed at the smallest diffusion layer thickness, corresponding to the smallest horn to electrode separation and highest ultrasound intensity. A comparison of the extent of the deviation across the different solvents presents the largest deviation for DMF at 298 K and the smallest deviation for DMF at 218 K. These observations follow a trend

consistent with the frequency and violence of cavitation shown in the chronoamperometry of **Figure 2**, the most extreme cavitation taking place in the solvent with the lowest vapour pressure and viscosity combination (see **Figure 3**). The correlation between cavitation and the deviation observed in the DPV confirms that the contribution from cavitation perturbation of the diffusion layer, from both bubble formation and microjetting, is significant in comparison with acoustic streaming under these conditions. Nevertheless it is also clear that for mild ultrasonic stimulation, where the diffusion layer thickness is relatively large, then the Nernst diffusion model is physically realistic as testified by the good agreement between theory and experiment in the high diffusion layer thickness limit.

3.5 Conclusions

Chronoamperometry at insonated electrodes results in characteristic transients comprised of sharp spikes superimposed on a background current which is much enhanced in comparison with the steady state current observed under silent conditions. At a microelectrode these spikes are caused by the collapse of an individual cavitation bubble in the vicinity of the electrode surface which casts a jet of electrolyte against it, bringing new electroactive material and giving the enhanced current observed on the transient. The size and multiplet nature of the spikes on the transient, show that they correspond to multiple collapses which occur at nearly similar times. The enhanced background current is attributable to acoustic streaming.

The size and frequency of the spikes on the microelectrode traces represent the amount and violence of the cavitation taking place in the solution. Calculation of the baseline current, the average current and the standard deviation of the current allows a quantitative assessment of the amount of cavitation taking place relative to the mass transport contribution from acoustic streaming.

Differential pulse voltammetry was employed as a sensitive means, in parallel with chronoamperometry, of exploring the nature of the diffusion layer prevailing under steady state electrolysis at insonated macro-electrodes, resulting in a physical model of an insonated electrode which may be described as a steady diffusion layer a few microns thick brought about by acoustic streaming which is occasionally and randomly punctuated by a cavitation event the frequency and violence of which is dependent on the solvent and ultrasound delivered, except at very short electrode-to-horn separation where the cavitation contribution becomes substantial.

The benefits of introducing ultrasound into electrochemical systems are wide ranging both in electroanalytical and electrosynthetic fields. Advancement in the field has opened up the need for a greater understanding of the relationship between cavitation and acoustic streaming which is vital if greater selectivity in analysis and synthesis is to be pursued. As a result, this has recently provoked greater discussion about the origin and nature of these phenomena and factors which influence their operation in a range of physical conditions. The results described, provide a greater understanding of acoustic streaming, which may prove to be as important and widespread as the well-established phenomenon of cavitation.

3.6 Surface Acoustic Cavitation Further Understood via Nanosecond Electrochemistry

Ultrafast and high-resolution electrochemical equipment ($\sim 2800 \text{ V s}^{-1} / 1 \text{ ns}$) allowed the authors of subsequent investigations^{17,18} into acoustic cavitation to monitor the bubble activity in real time to determine the bubble size and to gain an insight into bubble dynamics. Analogous measurements to those described in s. 3.4.1 were reported at a $29 \text{ }\mu\text{m}$ diameter platinum electrode. However the equipment was capable of sub-microsecond resolution, when compared with the $50 \text{ }\mu\text{s}$ that could be achieved with the Eco-Chemie μ -Autolab used in the work described above. However, similar transients were observed with the overall cavitation peak varying from around $50 \text{ }\mu\text{s}$ (1 acoustic cycle, transient cavitation) up to a few milliseconds (stable cavitation), suggesting $50 \text{ }\mu\text{s}$ was sufficient resolution for the purposes of the discussion given in s.3.4.1 above. At times outside the occurrence of the peaks, the current is approximately constant, but much greater than the spherical diffusion-limited current expected for the microelectrode under silent conditions. This quasi-steady-state current, termed the baseline current, is proportional to the electrode area for electrode diameters ranging from $25 \text{ }\mu\text{m}$ to 0.4 mm and is attributed to macroscopic acoustic streaming². The resulting diffusion layer had a thickness of $8 \pm 2 \text{ }\mu\text{m}$. Since any peak inducing a variation of more than 10 % in the steady-state current would be detected, this result reveals that in mild conditions such as this (8.9 W cm^{-2}) there is no cavitation contribution to the measured steady-state current. This upholds the results of the differential pulse investigations described above. It is clear from **Figure 6 c** that for diffusion layer thicknesses over $5 \text{ }\mu\text{m}$, no deviation from the Nernstian predictions would be expected.

In s.3.4.2, insonated chronoamperometry at a macroelectrode was reported. The transients, shown in **Figure 4**, could all be described as multiplets, contrasting with the appearance of individual spikes in the case of a microelectrode. It was inferred that a multitude of stable oscillating bubbles¹⁹ on the surface contribute to the observed transient. Later work employing multielectrode arrays¹⁷ permitted direct visualisation of the spatial extent of the bubbles. The arrays were comprised of 29 μm electrodes with an interelectrode distance of 103 μm . Chronoamperometry at all three electrodes revealed the presence of cavitation peaks on all three simultaneously. The authors inferred that since it is very unlikely that two different bubbles appear at the same time on different electrodes with the same peak shape and lifetime, the signals could be attributed to the same bubble. Synchronous events were recorded for distances as large as 0.8 mm by varying the electrode-electrode separation. Conversely, signals isolated on a single electrode with an interelectrode separation of 5 μm were also observed. This suggests a wider range of bubble sizes than previously thought²⁰ and upholds the description given in s.3.4.2 above of complex multi-event activity on a 1 mm macroelectrode with the possibility of a single large oscillating bubble with a diameter as large as 0.8 mm and small events with bubble diameters less than 40 μm .

It is concluded that the model inferred from the experimental work in s.3.5 which describes the nature of cavitation and its effect on the diffusion layer brought about by acoustic streaming, is supported by this nanosecond interrogation.

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Section III

Sonoelectroanalysis

Chapter 4 – Liberation and Sonoelectroanalytical Determination of Copper in Whole Blood

4.1 Introduction

Copper is an essential mineral present in the bloodstream. Its impact on human health has been discussed in s.1.6.1. The trace copper content in whole human blood varies between 800 and 1400 $\mu\text{g L}^{-1}$ depending on age and sex¹. More than 90% of this copper is bound to ceruloplasmin, an α 2-globulin with ferroxidase activity^{2,3} (see s.1.6.1). The diagnosis of copper deficiency at present is established by the demonstration of a low serum ceruloplasmin level and less commonly by atomic absorption spectroscopy (AAS) of serum copper content^{2,4}. This is an expensive technique involving a sample digestion pretreatment. However the diagnosis of marginal copper deficiency by these methods has not been perfected and can be highly unreliable. Potential alternatives involving the determination the activities of copper containing enzymes such as erythrocyte superoxide dismutase and platelet cytochrome-c oxidase^{5,6} in blood cells are generally unavailable except within very specialised laboratories. A need has therefore been identified for a technique to accurately and rapidly determine copper stores in whole blood that is cheaper than spectroscopic or enzymatic techniques and requires little or no sample pretreatment.

Detection of copper within whole blood using voltammetric methods is advantageous due to the compact size of the equipment allowing the potential for portable determination and relative inexpense when compared to spectroscopy. However this has until now required complicated and lengthy pretreatment of the blood sample prior to analysis, examples of such treatments include: electrochemical⁷, wet oxidative digestion⁸ filtering of acidified blood on nitrocellulose⁹, and dilution¹⁰ with a metexchange reagent¹¹. In addition all these determinations utilise mercury based electrodes which are environmentally undesirable and result in a toxic and biohazardous mixture of blood and mercury for disposal.

In this chapter the versatility of anodic stripping voltammetry (see s. 1.5.3.1) when enhanced with power ultrasound is exploited for the determination of copper stores in whole blood.

4.2 Experimental

The electrochemical cell used throughout is described in s.2.1 Figure 1. The ultrasonic horn was a Model CV 26 (Sonics and Materials Inc, Connecticut, USA) located at a horn to electrode separation of 3 mm. The ultrasonic intensity was calibrated calorimetrically according to the procedure given in s.2.3 and the intensity used was $300 \pm 5 \text{ W cm}^{-2}$ unless otherwise stated. The cell was thermostatted as described in s.2.1 and limiting the sonication time to no more than 5 min enabled voltammetric measurements to be conducted at approximately constant temperature ($25 \pm 3 \text{ }^{\circ}\text{C}$). Experiments were conducted using a bare 3 mm diameter glassy carbon disc electrode (BAS, US). This electrode was prepared according to the procedure given in s.2.5. All potentials are quoted with respect to the saturated calomel electrode, SCE (Radiometer, Copenhagen). The counter electrode employed in the three-electrode system was a coiled platinum wire which was flame annealed prior to each experiment. The analytical square wave stripping step required the following parameters: frequency (f) 25 Hz, step potential (ΔE_s) of 2.0 mV and an amplitude (ΔE_p) of 50 mV. Reagents were all of electrochemical grade or the highest available purity, see Appendix 2 for full details.

4.3 Results and Discussion

4.3.1 Cyclic Voltammetry of Human Ceruloplasmin

A comparison of square wave anodic stripping voltammetry (SWASV) in silent and insonated conditions was undertaken for solutions of human ceruloplasmin and whole horse blood. Ceruloplasmin solutions were prepared by dissolving 1.0 mg pure protein in 25 cm^3 0.1 M HNO_3 which acted as background electrolyte. This corresponded to a solution of approximately 10 % of the typical concentration of ceruloplasmin in human blood (40 mg dL^{-1})². Cyclic voltammetry of the protein under silent conditions revealed a copper stripping peak visible at -0.025 V in **Figure 1**.

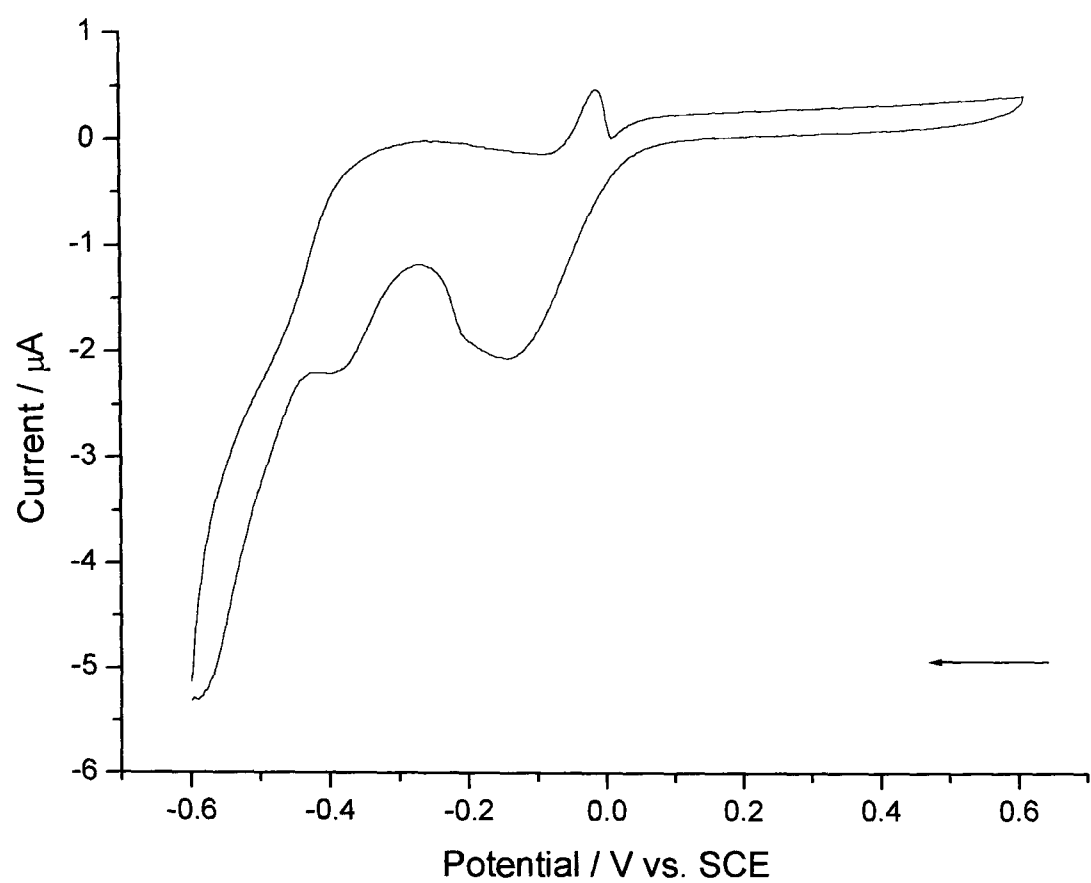


Figure 1 – Steady state cyclic voltammogram of 3.3 $\mu\text{g dL}^{-1}$ human ceruloplasmin in 0.1 M HNO_3 on a glassy carbon working electrode at a scan rate of 5 mV s^{-1} . The arrow shows the direction of the scan which began at 0.6 V vs. SCE.

4.3.2 Square Wave Anodic Stripping Voltammetry of Human Ceruloplasmin

The copper stripping peak evident in the cyclic voltammetry of human ceruloplasmin was used to investigate the effect of ultrasound on the protein via square wave anodic stripping voltammetry (SWASV). **Figure 2** shows square wave stripping scans (frequency, $f = 25 \text{ Hz}$, step potential $\Delta E_s = 0.002 \text{ V}$ and amplitude, $\Delta E_p = 0.05 \text{ V}$) which followed preconcentration periods where the working electrode was held at a reducing potential of -1.5 V the duration of which was 15, 30 and 60 s in silent conditions and 15 s insonated at 200 W cm^{-2} ultrasound intensity.

Preconcentration Period / s	Preconcentration Conditions	Square Wave Peak Height / μA
15	silent	7.84
30	silent	12.6
60	silent	18.1
15	200 W cm^{-2} insonation	65.2

Table 1 – Comparison of square wave stripping peak heights following different preconcentration periods and conditions as shown in Figure 2

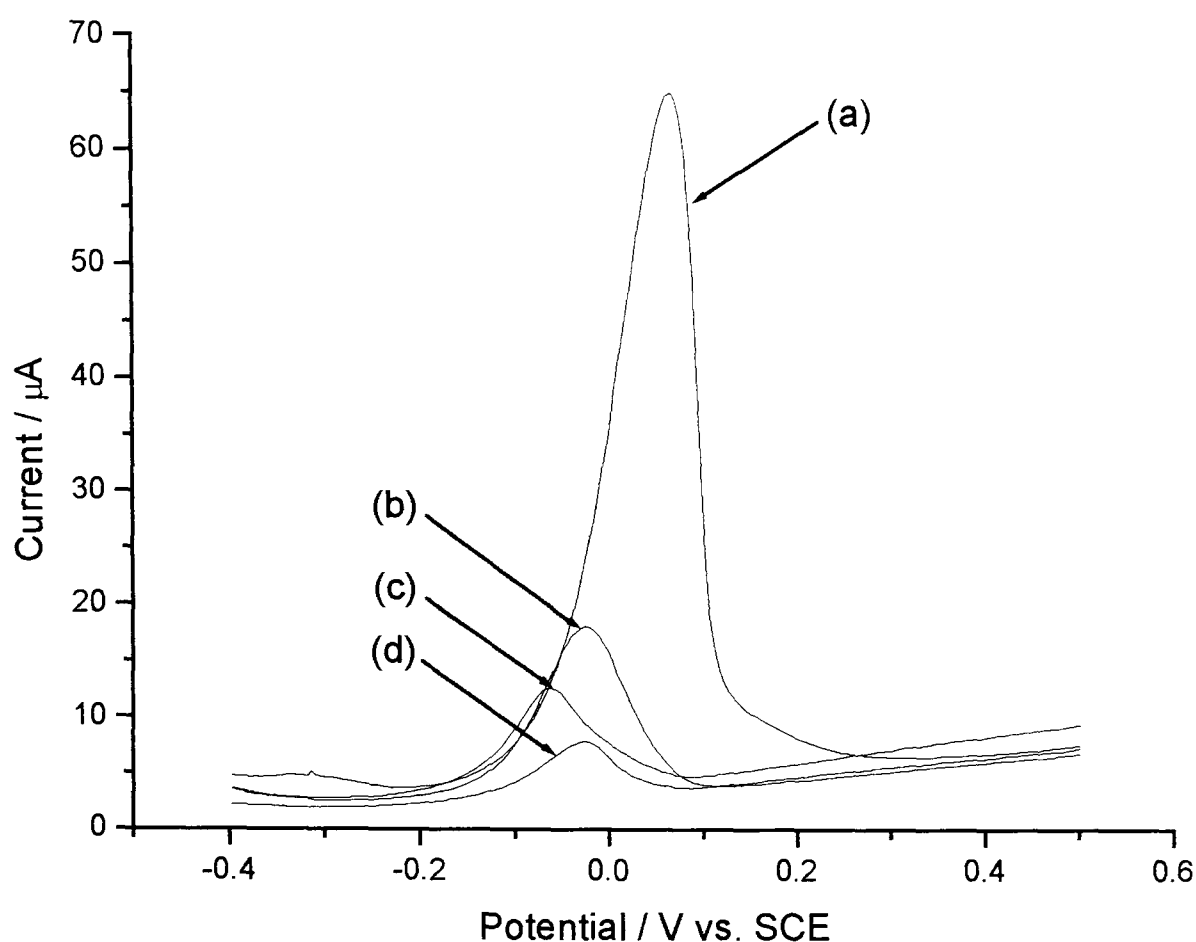


Figure 2 - Square wave scan ($f = 25$ Hz, $\Delta E_s = 2$ mV V, $\Delta E_p = 50$ mV) of $3.3 \mu\text{g dL}^{-1}$ human ceruloplasmin in 0.1 M HNO_3 on a glassy carbon working electrode following a preconcentration period at -1.5 V for
 (a) 15 s insonated at 200 W cm^{-2} ;
 (b) 60 s silent;
 (c) 30 s silent;
 (d) 15 s silent

It is apparent from the results shown in **Figure 2** that the detection of copper via SWASV in human ceruloplasmin is significantly enhanced when an insonated preconcentration is employed. The peak heights given in Table 1 show an approximately linear increase with increasing preconcentration time for silent deposition, however, an insonated preconcentration of 15 s yields a peak height of $65.2 \mu\text{A}$, eight times larger than the silent case with a peak height of $7.84 \mu\text{A}$.

4.3.3 Square Wave Anodic Stripping Voltammetry of Whole Blood

A solution of 10 % by volume whole horse blood was prepared using 0.1 M HNO_3 as background electrolyte and similar analyses as in the case of ceruloplasmin were executed. Since whole blood was more passivating than pure protein on the surface of the electrode it was necessary to lengthen the preconcentration time and the results are shown for an optimised preconcentration time of 240 s in both silent and insonated conditions. The preconcentration voltage and parameters characterising the square wave stripping step remained the same as in s.4.3.2 for the pure protein. The results can be seen in

Figure 3 and again confirm the strong enhancement of the stripping peaks that can be observed following an insonated preconcentration step. In this case the peak height for an insonated preconcentration is five times greater (58.1 μA) than in the silent case (11.6 μA).

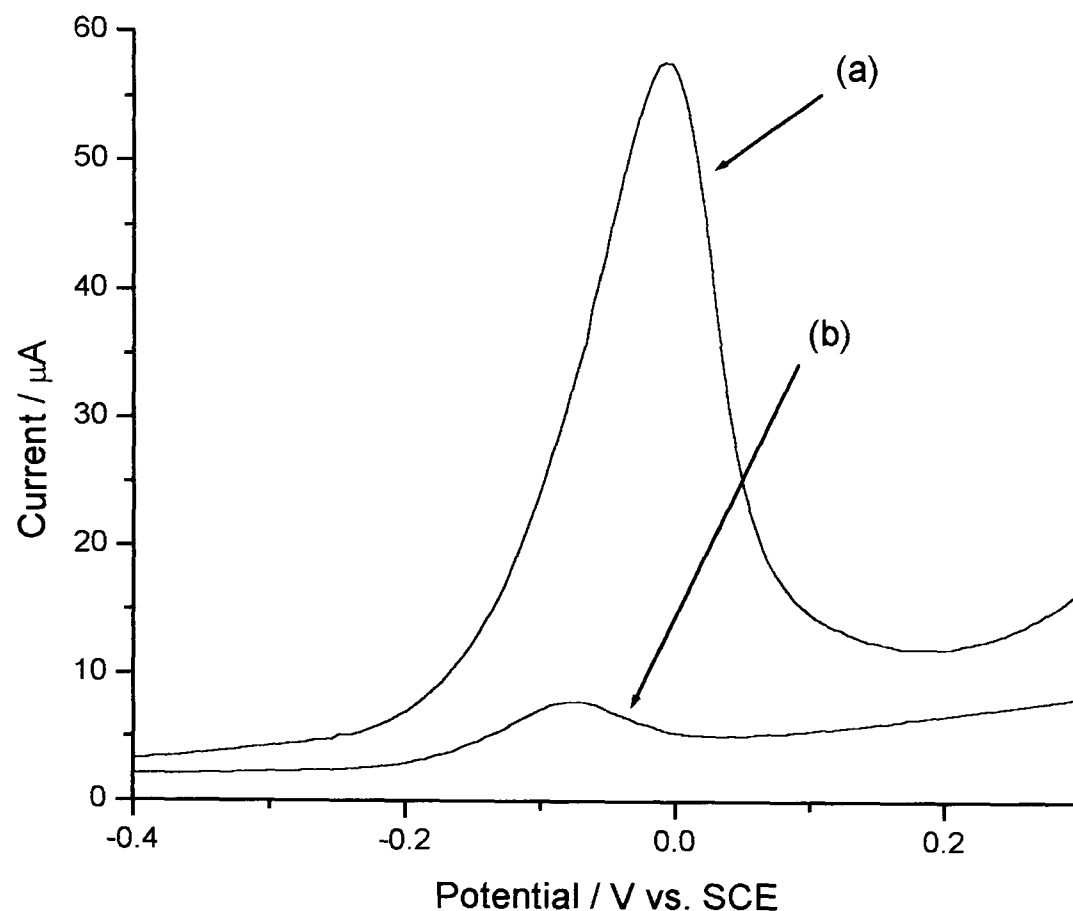


Figure 3 - Square wave scan ($f = 25 \text{ Hz}$, $\Delta E_s = 0.002 \text{ V}$, $\Delta E_p = 0.05 \text{ V}$) of 10 % blood in 0.1 M HNO_3 on a glassy carbon working electrode following deposition at -1.5 V for a) 240 s insonated at 200 W cm^{-2} ; b) 240 s silent

4.3.4 Ultrasonic Pretreatment of Whole Horse Blood

It is clear from the results presented in ss. 4.3.2 and 4.3.3 that ultrasound is beneficial in the electroanalysis of copper in solutions of ceruloplasmin and whole blood. However, these results do not provide an insight into the role of ultrasound during SWASV: is the enhancement due to the liberation of copper from the active sites in which it is bound? Or is it purely the effect of increased mass transport to the electrode surface during the preconcentration step? The following experiment was conducted with the aim of resolving this question.

Initially the solution of whole horse blood (10 % by volume in 0.1 M HNO_3) was insonated for a known period of time from 240 s to 960 s whilst the electrode was held at $+0.1 \text{ V}$ to ensure that no copper was deposited on the electrode during this period. This “pretreatment” was followed by a silent preconcentration period of 240 s -1.5 V during which the mass transfer of electroactive species to the

electrode was controlled by diffusion alone. Finally a square wave stripping step yielded the analytical signals which can be seen in **Figure 4**.

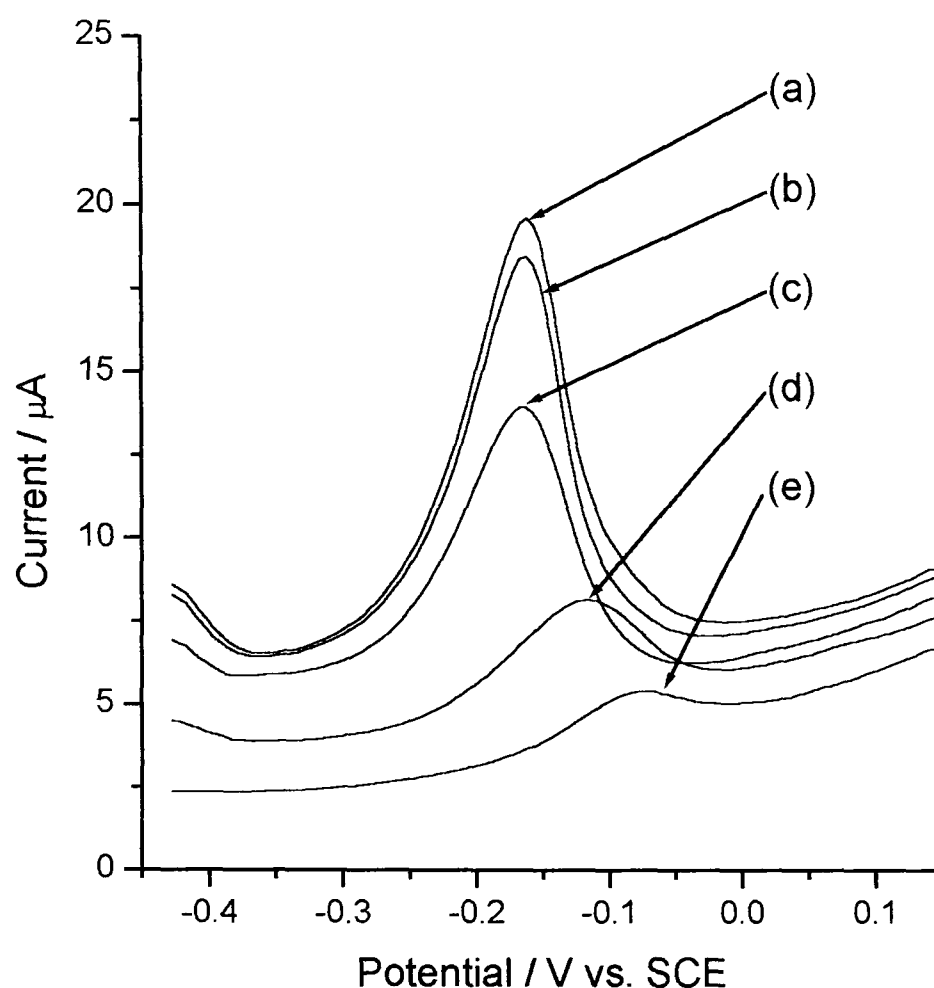


Figure 4 - Square wave scan ($f = 25 \text{ Hz}$, $\Delta E_s = 0.002 \text{ V}$, $\Delta E_p = 0.05 \text{ V}$) of 10 % blood in 0.1 M HNO_3 on a glassy carbon working electrode following deposition at -1.5 V for 240 s in silent conditions for an initial pretreatment at +0.1 V of

- (a) 960 s insonated at 200 W cm^{-2}
- (b) 720 s insonated at 200 W cm^{-2}
- (c) 480 s insonated at 200 W cm^{-2}
- (d) 240 s insonated at 200 W cm^{-2}
- (e) 240 s silent

Figure 4 shows that increasing the insonation period, prior to a silent preconcentration, increases the measured square wave stripping peak. This provides evidence that the function of ultrasound in the enhancement of stripping peaks in whole blood is not due to mass transport and cavitation cleaning alone. Indeed for copper deposited under silent conditions where diffusion alone controls the mass transport to the electrode surface, the size of the stripping peak is related to the duration of insonation prior to the deposition. This increase in peak size reaches a plateau and maximum after 960 s of insonation prior to the deposition step. It should be noted that the total insonation time was attained in periods of 240 s to ensure that temperature could be maintained at $25 \pm 3 \text{ }^\circ\text{C}$. We conclude that ultrasound facilitates the liberation of copper from the active sites in which it is bound within the

ceruloplasmin, and other enzymes, in blood. This type of liberation resulting from insonation precludes the necessity for complex and degradative sample pretreatments aimed at performing this function.

4.3.5 Quantitative Determination of Total Copper in Whole Horse Blood

A determination of concentration of copper in two samples of horse blood was then undertaken by means of the standard addition technique described in s.1.5.3.1. A 15 mL solution of 10 % by volume whole blood in 0.1 M HNO₃ was prepared and the solution was insonated for 960 seconds at +0.1 V to ensure all the copper was liberated from the ceruloplasmin prior to the determination. This removed the possibility of a slow release of copper from the protein compromising the results. SWASV followed with a 300 W cm⁻² insonated deposition time of 240 s, 3 mm horn to electrode separation, and a fixed potential of -1.5V. The scan was repeated for each standard addition of 100 µg L⁻¹ copper solution. The standard addition volume was constant throughout each experiment and was based on the magnitude of the copper signal prior to any external additions, for the results reported below the addition volume lay within the range of 5-50 µL. There was no observed interference in the stripping voltammetry with other metals known to be present in blood, for example cobalt, nickel, zinc and iron.

Copper Content (µg L ⁻¹)			
Blood sample no.	SWASV‡ method	Independent laboratory AAS method	% RSD SWASV method
1	1300 ± 300	1300	23.1
2	620 ± 60	690	9.7

‡SWASV stripping step (f = 25 Hz, ΔE_s = 0.002 V, ΔE_p = 0.05 V) following ultrasonic liberation of copper for 960 s at 300 W cm⁻² and 240 s insonated deposition step at -1.5 V and 300 W cm⁻² ultrasound intensity

Table 2 - A comparison of copper content by sono-SWASV and independent analysis by electrothermal AAS. The values quoted are the mean (2 s.f.) and standard deviation for a set of 4 separate copper determinations on each sample.

The results obtained using sono-SWASV were compared with independent laboratory analyses of copper content by electrothermal atomic absorption spectroscopy and the results can be seen in table 2. Sonoelectroanalysis shows close agreement with the independent analyses of both samples confirming the applicability of sono-SWASV to the rapid and facile determination of blood copper content.

4.4 Conclusions

Insonation of whole blood facilitates the determination of copper content via square wave anodic stripping voltammetry. It enhances the mass transport to the electrode by acoustic streaming and cavitationaly depassivates the electrode surface during preconcentration. Ultrasound has also been shown to liberate copper from the active sites in which it is bound ensuring results are not compromised by slow release of copper over the duration of the experiment.

We have demonstrated that in an ultrasound enhanced electroanalytical system it is possible to determine the concentration of copper both accurately and rapidly, obviating the need for mercury electrodes or a prior sample degradation step, both of which complicate and lengthen the analytical protocol. The method is optimised for the determination of copper in a 15 cm³ solution of 1-10 % by volume whole blood, a quantity suitable for analysis from standard blood test samples. This contrasts with higher concentration samples used in the determination of lead in whole blood by DPV conducted by Jagner et al¹⁰. SWASV test results compare favourably to those obtained by independent analysis using electrothermal atomic absorption spectroscopy, a recognised technique often employed in clinical analysis. We conclude that due to the facile experimental procedure, portability of the apparatus and relative inexpense, sonoelectroanalysis has the capacity to become a powerful clinical laboratory tool.

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Chapter 5 – Sonoelectroanalytical Determination of Heavy Metals in Fish Gill Mucus

5.1 Introduction

The environmental impact of heavy metals was discussed in s.1.7. Fish are commonly used as environmental biomarkers to assess the levels of pollution in waterways. The extent of heavy metal contamination using the analysis of fish tissue is well documented and the following methods exist: metallothionein determination¹⁻³, intracellular zinc determination⁴, heavy metal analysis of fish parasites such as intestinal nematode worms⁵ and analysis of fish tissue primarily, liver and gill⁶⁻⁸. The first method is the most widely recognized biomarker and is based on an increase in metallothionein, a stress protein particularly responsive to heavy metals. The disadvantages of this method are that it is not specific to the heavy element causing the stress, it is a destructive technique which requires a hepatic sample of fish tissue and it is also subject to variation across species², which means that an interspecies comparison cannot easily be made. Intracellular zinc determination relies on similar principles⁴, an increase in the concentration of zinc on exposure to heavy metals, but it has been minimally researched and is subject to the same disadvantages as metallothioneins. The analysis of fish parasites for heavy metal content involves a complicated procedure and is also species specific as parasitic types can vary significantly, although it does have the advantage of giving individual element concentrations. Of the existing methods available, direct determination of metal concentrations in fish tissue samples is the most universal indicator of pollution levels as it is the least species specific and provides precise elemental values⁶⁻⁸, it is however a destructive technique that involves complicated methodology and dissection.

The damage to fish gills caused by pollution was discussed in s.1.7 along with elegant structural studies by Alazemi et al⁹. As external organs with a large surface area, gills are first to come into contact with pollutant heavy metals and it has been suggested that binding to mucus glycoproteins is one of the principal modes of uptake¹⁰⁻¹².

This study is based on the application of 20 kHz power ultrasound to an extract of mucus solution from *Salmo gairdneri* (rainbow trout) followed by square wave anodic stripping voltammetry (SWASV)

using an in situ plated mercury thin film glassy carbon electrode. This directly facilitates a rapid quantitative determination of total copper content and qualitative heavy metal contamination within the extract. The result is the elimination of complex sample pretreatment and most importantly it provides a non destructive biomarker for heavy metal pollution which might easily be applied in the field.

5.2 Experimental

The electrochemical cell used throughout is described in s.2.1 Figure 1. The ultrasonic horn was a Model CV26, (Sonics and Materials Inc., Connecticut, USA) located at a horn to electrode separation of 5 mm. The ultrasonic intensity was calibrated calorimetrically according to the procedure given in s.2.3 and the intensity used was 200 W cm^{-2} unless otherwise stated. The cell was thermostatted as described in s.2.1 and limiting the sonication time to no more than 5 minutes enabled voltammetric measurements to be conducted at approximately constant temperature ($25 \pm 3 \text{ }^{\circ}\text{C}$). Experiments were conducted using a 3 mm diameter glassy carbon disc electrode (BAS, US) plated in situ with mercury from a 1mM solution (see s.1.3.3 which addresses the properties of mercury plated electrodes). This electrode was prepared according to the procedure given in s.2.5. All potentials are quoted with respect to the saturated calomel electrode, SCE (Radiometer, Copenhagen). The counter electrode employed in the three-electrode system was a coiled platinum wire which was flame annealed prior to each experiment. The analytical square wave stripping step required the following parameters: frequency (f) 25 Hz, step potential (ΔE_s) of 2.0 mV and an amplitude (ΔE_p) of 50 mV. Reagents were all of electrochemical grade or the highest available purity, see appendix 2 for full details.

Mucus solution was extracted using the method outlined by Pärt et al¹¹. In our case deceased rainbow trout (*Salmo Gairdneri*) (Haydens, Oxford) were weighed and the gill mucus extracted by gently rinsing behind the gill flaps with 250 cm^3 borate buffer from a wash bottle (50 mM pH 7). The resulting solution was collected in a tray. The stabilizing effect of borate buffer on the glycoproteins¹³ allowed the mucus extract to be kept for up to three weeks in a refrigerator at a temperature of between 3-5 $^{\circ}\text{C}$ without degradation.

5.3 Results and Discussion

5.3.1 Borate Buffer in Biological and Electrochemical Systems

Borate buffer is a common biological buffer due to its convenient pH range and its stabilizing effect on glycoproteins¹³. Voltammetric analyses of heavy metals in this medium however have not been reported and square wave anodic stripping voltammetry (SWASV) of copper in borate buffer was initially investigated. **Figure 1** shows copper standard additions to a solution of 0.5 mM Cu²⁺ in 150 mM borate buffer and demonstrates that sensitive quantitative measurements of copper content could be obtained.

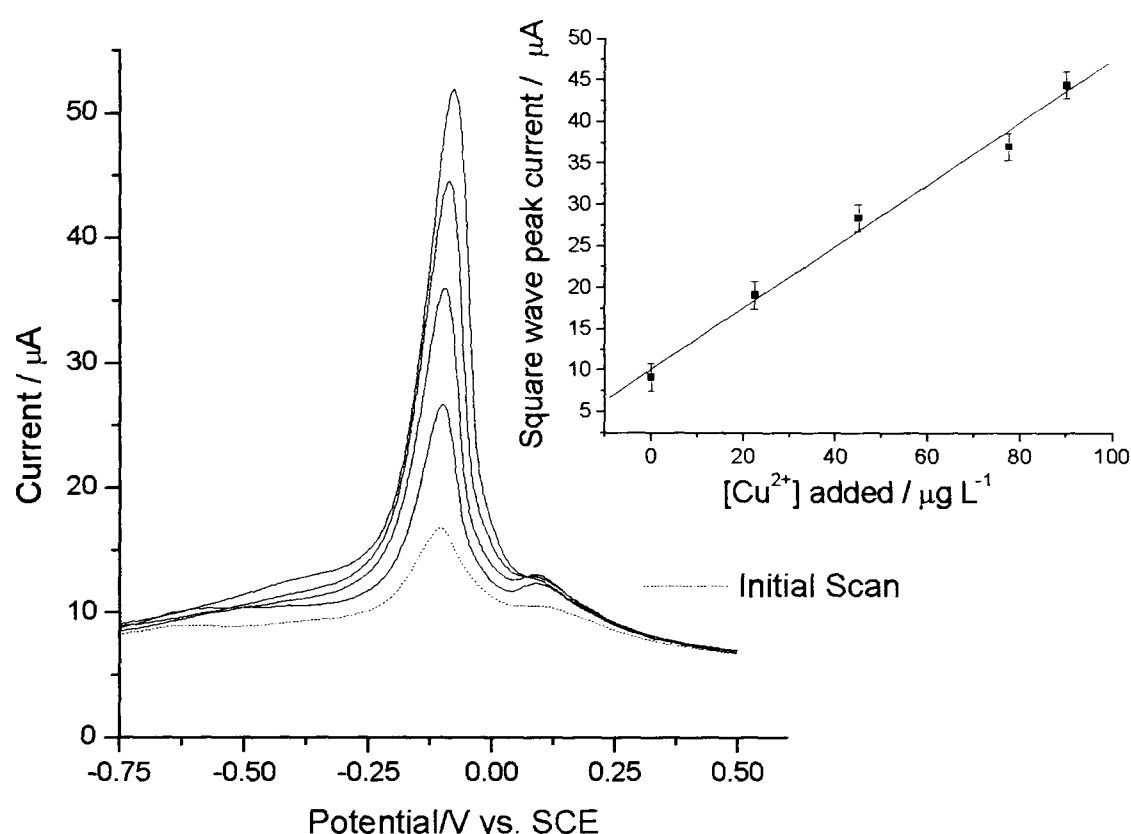


Figure 1 - SWASV of a solution of 0.5 mM Cu²⁺ in 150 mM borate buffer following a 120 s deposition step at -1.0 V and 200 W cm⁻² ultrasound power. The initial scan is shown with four successive scans which represent standard additions of 22.5 μg L⁻¹ Cu²⁺. Inset: standard addition calibration plot with R² = 0.9897

5.3.2 Optimum Deposition Potential and Ultrasound Power for Sono-SWASV of Copper

Depression of current signal due to passivation is evident for electrochemical analyses in full strength mucus extract. It was found that ultrasound effected quantitative depassivation of the electrode in a solution of one part mucus to nine parts 0.1 M HNO₃ acting to prolong the lifetime of the electrode and improve sensitivity. In order to determine the optimum ultrasound power and deposition potential for copper analysis the variation in square wave anodic stripping peak heights for deposition potentials from -0.5 to -2.0 V in silent conditions and ultrasound up to 200 W cm⁻² were obtained (**Figure 2**).

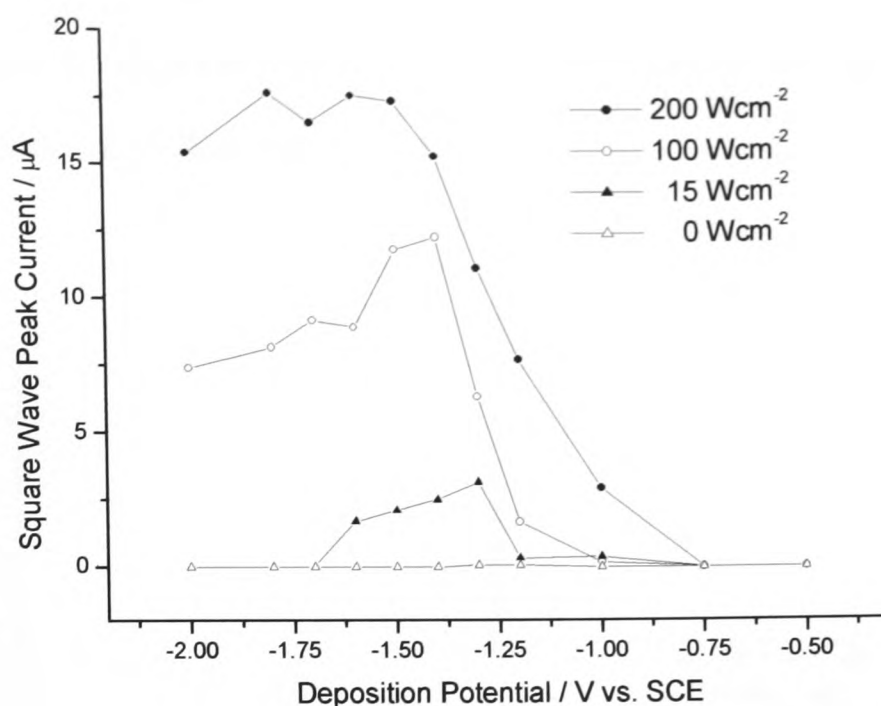


Figure 2 - Variation in square wave peak currents for copper deposition from a solution of one part rainbow trout mucus to nine parts 0.1 M HNO₃. Shown for a 120 s deposition at potentials from -0.5 to -2.0 V and ultrasound powers from 0 to 200 W cm⁻²

It is evident that at higher ultrasound powers the optimum deposition potential becomes more negative. We conclude that stability of the codeposited copper mercury film on the surface of the electrode is dependent on the cavitation activity at the electrode surface, which also acts to depassivate the electrode in the presence of glycoproteins from the mucus. Acoustic streaming, the effects of which increases with increasing ultrasound power, is responsible for the increase in the average peak height on going from silent to 200 W cm⁻² ultrasound intensity. Subsequent anodic stripping measurements were made using the optimized ultrasound power of 200 Wcm⁻² with a deposition potential of -1.6 V.

5.3.3 Quantitative Determination of Copper in Fish Gill Mucus

A determination of copper in the rainbow trout mucus sample and in the blank borate buffer solution was then undertaken by means of the standard addition technique described in s.1.5.3.1. 20 mL of 10 % by volume gill mucus solution was prepared using one part mucus to nine parts 0.1 M HNO₃ with a concentration of 1 mM Hg²⁺. This solution was insonated for 120 s at 0.1 V to initiate the decomposition of the glycoproteins bound to the metal ions in the mucus solution. After the initial pretreatment an insonated preconcentration period of 120 s at -1.6 V and 200 W cm⁻² ultrasound intensity was followed by a silent square wave stripping step ($f = 25$ Hz, $\Delta E_s = 0.002$ V and $\Delta E_p = 0.05$ V). Standard additions of standard 10 µg L⁻¹ Cu²⁺ were added and corresponding square wave stripping peak heights recorded (**Figure 3**) and used to determine the initial concentration of copper in the sample.

A shift in peak potential is observed in **Figure 3** for increasing standard additions. This arises because the reference electrode is immersed directly in the experimental test solution and the chemical composition of the solution may fluctuate (see s.1.1.4 i).

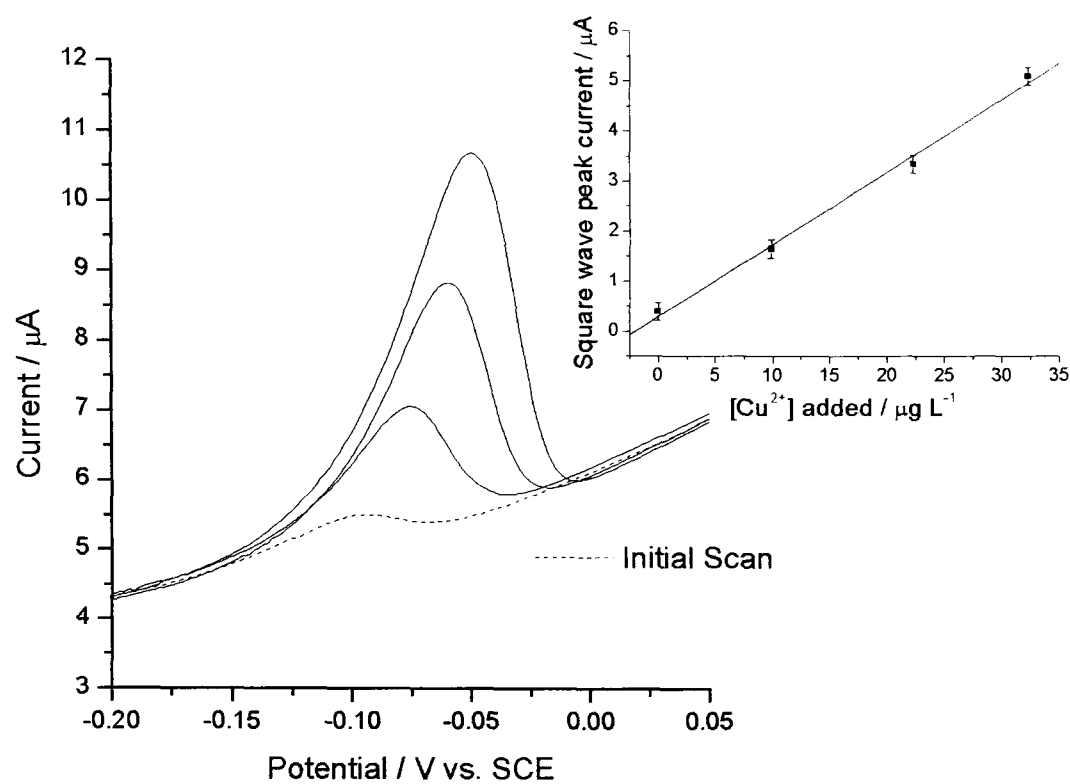


Figure 3 - SWASV of a solution of one part rainbow trout mucus to nine parts 0.1 M HNO₃ after pretreatment at 200 W cm⁻² ultrasound intensity for 120 s. The initial scan followed by three standard additions of 10 μg L⁻¹ Cu²⁺ are shown for a deposition at -1.6 V and 200 W cm⁻² ultrasound intensity for 120 s. Inset: Standard addition calibration plot with R² = 0.9974

The results obtained using SWASV show good intrasample reproducibility and compare favourably with independent analysis of the sample by electrothermal atomic absorption spectroscopy (table 1).

Mucous Sample No.	Copper Content (μg L ⁻¹) Independent Laboratory AAS method	Copper Content (μg L ⁻¹) SWASV [‡] method	% RSD [†] (n=3) for SWASV technique
1	17	16 ± 0.8	5.00
2	25	21 ± 0.4	1.90

[†] Relative standard deviation (RSD), expressed as a percentage is obtained by multiplying the standard deviation by 100 and dividing this product by the average

[‡] SWASV stripping step (f = 25 Hz, ΔE_s = 0.002 V and ΔE_p = 0.05 V) following ultrasonic liberation of copper for 120 s at 200 W cm⁻² and 120 s insonated deposition step at -1.6 V and 200 W cm⁻² ultrasound intensity.

Table 1 - A comparison of copper content by sono-SWASV and independent analysis by electrothermal AAS for mucus samples from two different rainbow trout. The values quoted are the mean and RSD for each full strength mucus sample, corrected for the blank borate/mercury solution result of 10 μg L⁻¹ (obtained by independent AAS). The detection limit of this method is 4 μg L⁻¹.

We propose that quantitative comparisons of the copper content of different fish specimens could be made by measuring the protein concentration in the mucus sample using the Bio-Rad assay procedure¹⁴ (Bio Rad Laboratories, Bromley, UK). This procedure can be used to assay samples in most common buffers. The colorimetric procedure is based on an observed shift in the absorbance maximum when Coomassie® Brilliant Blue G-250 dye reacts with protein. Bradford¹⁵ first demonstrated the usefulness of this principle in protein assay. Spector¹⁶ found that the extinction coefficient of a dye-albumin complex was constant over a 10-fold concentration range. Thus, Beer's law may be applied for accurate quantification of protein by selecting an appropriate ratio of dye volume to sample concentration. As with any colorimetric protein assay, there is variation in colour response to different proteins. Both the colour change due to dye binding and the variation in colour response can be explained by the dye having three absorbing species: a red cationic species, a green neutral species and a blue anionic species.

5.3.4 Optimum Deposition Potential and Ultrasound Power for Sono-SWASV of Lead

The potential for further development of the technique for the determination of other heavy metals, for example lead, which are present in the gill mucus of fish was then investigated. The characteristic stripping potential of lead in this media was determined by standard additions of lead ions to the sample which resulted in the assignment of peaks at $-0.4 \rightarrow -0.5$ V. **Figure 4** shows the variation of square wave peak heights with deposition potential for preconcentration at 200 W cm^{-2} and 100 W cm^{-2} from a dilute solution of mucus.

It is evident by a comparison of **Figures 2** and **4** that the parameters effecting the optimum of copper with mercury *in situ* discussed in s.7.2.3, differ from those which give the optimum of lead with mercury *in situ*. In particular, lead appears to be more readily ablated from the glassy carbon surface by ultrasound than copper since the greatest peak height for copper arises after an insonated preconcentration at 200 W cm^{-2} whereas the greatest peak height for lead arises under 100 W cm^{-2} ultrasound. In order to optimise the sonoelectroanalytical methodology for other heavy metals it is clear that the need to maximise acoustic streaming and cavitational cleaning is finely balanced against ablating the deposit of the target material from the surface.

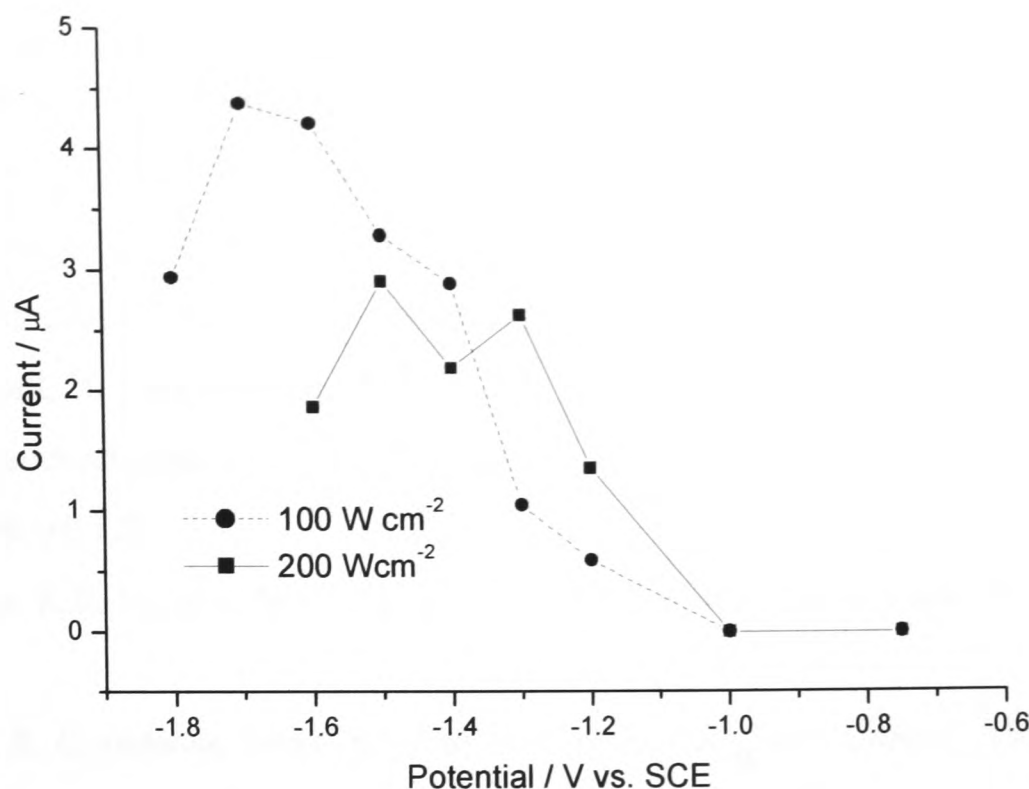


Figure 4 - Variation in square wave peak currents for lead deposition from a dilute solution of rainbow trout mucus in 0.1 M HNO₃. Shown for a 120 s deposition at potentials from -1.0 to -1.8 V and ultrasound powers of 100 and 200 W cm⁻².

5.4 Conclusions

By using the extraction of fish gill mucus into buffer solution followed by sonoelectroanalysis, a rapid assessment of the heavy metal status of fish has been demonstrated. Insonation of the mucus solution facilitates voltammetric analysis by SWASV with acoustic streaming providing enhanced mass transport to the electrode surface and cavitation depassivation maintaining surface activation during preconcentration. By combining an optimum deposition potential and ultrasound power it was possible to determine the concentration of copper both accurately and rapidly using sono-SWASV. The analytical results show good intrasample reproducibility and compare favourably with independent analysis of the mucus extract by electrothermal atomic absorption spectroscopy. A detection limit of 4 μg L⁻¹ was established. The potential for extending the technique to the determination of lead has also been demonstrated. To conclude, sonoelectroanalysis facilitates the use of gill mucus as a non-destructive biomarker with the advantages of minimal experimental procedure, elimination of sample pretreatment, possibility for portable apparatus and relative inexpense compared with either ICP-MS or AAS.

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Chapter 6 – Sonoelectroanalytical Determination of Lead in Artificial Saliva

6.1 Introduction

The toxicity of lead is well documented and it is recognized as a major environmental health risk with lead poisoning being one of the most common health problems in the United States both paediatric and through occupational exposure see s.1.6.2. Urinary lead excretion is not a true reflection of bodily lead levels. Therefore the usual means of interrogation is a direct blood test¹. However it has been found that workers occupationally exposed to lead can have extremely high levels of lead in sweat and saliva, even though their lead level in blood is moderately elevated². Luconi and Silva found an average of $50 \mu\text{g L}^{-1}$ in the human saliva samples they tested³. Salivary lead concentration is closely related to recent lead exposure^{4,5} and may provide an efficient and non invasive method of analyzing workers who are occupationally exposed³ and children who have come into contact with lead paints.

Conventional techniques for the analysis of trace heavy metals include electrothermal atomic absorption spectroscopy ETAAS⁶. However, this is expensive and generally involves a sample digestion pre-treatment. Inductively coupled plasma mass spectroscopy (ICP-MS) is widely regarded as the optimum technique with a detection limit of the order of $1\text{-}50 \text{ ng L}^{-1}$ ⁷. However it is extremely costly and only available in some specialist laboratories.

The introduction of power ultrasound into electrochemical systems has broadened the scope of techniques such as anodic stripping voltammetry and has been shown to facilitate the determination of trace metals in highly passivating media see s. 1.5.2. In the previous two chapters, the quantitative determination of copper in both blood and fish gill mucus were reported, illustrating the applicability of sonoelectroanalysis to biological media. Ultrasound introduces the well-documented phenomena of cavitation and acoustic streaming. Hemispherical cavitation voids are formed on the electrode surface by ultrasonic rarefaction of the solution and oscillate at the driving frequency⁸. Localised increases in current are observed at the surface when the bubble wall frees from the surface and ‘flushes’ the electrode. Both processes can shear passivating organic material (especially large molecules) from the surface resulting in continuous cleaning or activation of the electrode. Acoustic streaming increases the mass transport of the system, resulting in shorter deposition times, greater sensitivity and a corresponding lowered detection limit⁹⁻¹². In Chapter 4 cavitation was shown to liberate copper ions

from the carrier protein ceruloplasmin and a similar effect was observed when performing the quantitative analysis of copper in the presence of glycoproteins in Chapter 5. In s.5.3.4 the potential for quantitative analysis of lead in biological media by sonoelectroanalysis was examined. It was clear that the stability of codeposited lead and mercury would play a large role in the formulation of any sonoelectroanalytical technique.

Mercury thin film electrodes (MFE) on a solid glassy carbon support, discussed in s.1.3.3, are a well-established electrode material for anodic stripping voltammetry. However, weak adhesion results in poor mechanical properties (s.1.3.3.1). This problem can be overcome by coating the glassy carbon electrode with polymer films such as Nafion® (s.1.3.3.2). Such electrodes often exhibit higher sensitivity and resistance to electrode fouling by excluding negatively charged organic species¹³⁻¹⁵.

In the present Chapter the feasibility of sono-SWASV for lead in saliva is assessed using an artificial saliva solution containing the minerals and mucin found in saliva. The performance of a bare glassy carbon electrode under “silent” conditions is contrasted with voltammetric measurements made with an insonated preconcentration at an ultrasound intensity of 30 W cm^{-2} . Measurements at this ultrasound intensity were made directly in the saliva solution at an insonated Nafion® coated mercury thin film deposited on a glassy carbon electrode.

6.1 Experimental

The electrochemical cell used throughout is described in s.2.1 Figure 1. The ultrasonic horn was a Model UP 50H (dr.hielscher GmbH, Stuttgart, Germany) located at a horn to electrode separation of 3 mm. Insulating the transducer from the probe with a thin Teflon™ disc and connecting the two with a screw thread insulated with a thin sheet of Mylar™ eliminated the need for bipotentiostatic control of the titanium horn. The ultrasonic intensity was calibrated calorimetrically according to the procedure given in s.2.3 over the range $10\text{-}80 \text{ W cm}^{-2}$ and the intensity used was 30 W cm^{-2} unless otherwise stated. The cell was thermostatted as described in s.2.1 and limiting the sonication time to no more than 5 mins enabled voltammetric measurements to be conducted at approximately constant temperature ($25 \pm 3 \text{ }^{\circ}\text{C}$). Experiments were conducted using a 3 mm diameter glassy carbon disc electrode (BAS, Indiana, US) or a Nafion® coated mercury thin film glassy carbon electrode. The procedure for the preparation of both electrode materials began with the bare electrode being polished as detailed in s.2.5. Nafion® coating was achieved according to the procedure of Brett given in s.2.5.

Mercury deposition took place *in situ* by the addition of 0.6 mL 5mM mercury (II) acetate to the cell contents to give a total volume of 35 mL.

All potentials are quoted with respect to the saturated calomel electrode, SCE (Radiometer, Copenhagen). The counter electrode employed in the three-electrode system was a coiled platinum wire which was flame annealed prior to each experiment. Sono-square wave anodic stripping voltammetry (sono-SWASV) involved a silent oxidative pretreatment at 0.6 V for 60 s in order to clean and activate the electrode surface followed by an insonated preconcentration for 120 s at -1.5 V vs. SCE unless otherwise stated. The analytical square wave stripping step required the following parameters: frequency (f) 50 Hz, initial potential -0.725 V, final potential -0.3 V, step potential (ΔE_s) of 1.0 mV and an amplitude (ΔE_p) of 50 mV.

Reagents were all of electrochemical grade or the highest available purity, see Appendix 2 for full details. The artificial saliva was prepared according to the recipe of Leung and Darvell^{16,17}, as shown in Table 1. This recipe was advocated in subsequent trials of synthetic saliva¹⁸. As is common in some clinical studies, Leung and Darvell did not consider the organic fraction of the mixture and made no attempt to simulate the viscosity of natural saliva; they included only the mineral components of natural saliva. The necessity to account for the organic components which consists mainly of glycoproteins¹⁹ was discussed extensively by Gal et al¹⁸. Shellis²⁰ used the extract of salivary glycoproteins (mucin) from bovine submandibular glands. The similarity of saliva substitutes containing porcine gastric mucin (PGM) and / or bovine submandibular mucin (BSM) to authentic human saliva has been reported²¹ for a concentration of 30 g L^{-1} mucin. This concentration of mucin is therefore reflected in our artificial saliva recipe. The pH of authentic saliva is known to be 6.8 after emission from the canals¹⁸. Our artificial saliva was therefore adjusted with tiny amounts of dilute nitric acid or sodium hydroxide to correspond to this pH.

Name	Conc. / g L ⁻¹	Chemical Formula	CAS [‡] Number	Supplier	Grade
Sodium chloride	1.594	NaCl	[7647-14-5]	BDH	≥99.9% pure ≤0.0002% Pb
Ammonium nitrate	0.328	NH ₄ NO ₃	[6484-52-2]	BDH	≥98% pure ≤0.001% Pb
Potassium phosphate	0.636	KH ₂ PO ₄	[7778-77-0]	Sigma	≥99.9% pure ≤0.001% Pb
Potassium chloride	0.202	KCl	[7447-40-7]	BDH	≥99.5% pure ≤0.01ppm Pb
Potassium citrate	0.308	K ₃ C ₆ H ₅ O ₇ ·H ₂ O	[6100-05-6]	BDH	≥99.05 pure ≤0.0005% Pb
Uric acid sodium salt	0.021	C ₅ H ₃ N ₄ O ₃ Na	[1198-77-2]	Sigma	
Urea	0.198	H ₂ NCONH ₂	[57-13-6]	Aldrich	≥99%
Lactic acid sodium salt	0.146	C ₃ H ₅ O ₃ Na	[312-85-6]	Sigma	60% w/w syrup
Bovine submaxillary gland mucin Type I-S	30.000		[84195-52-8]	Sigma	
Water		H ₂ O		Elgastat, High Wycombe, Bucks.	UHQ Resistivity ≥18MΩcm.

[‡] CAS- Chemical Abstracts Service Registry Number

Table 1 – Recipe for Artificial Saliva

6.3 Results and Discussion

6.3.1 “Silent” Voltammetry of Lead at a Bare Glassy Carbon Electrode

First linear sweep anodic stripping voltammetry²² for standard additions of lead to a solution of 0.1 M HNO₃ with a “silent” (no ultrasound) preconcentration step was carried out at a bare glassy carbon electrode. For linear sweep voltammetry the area of the stripping peak is proportional to the amount of lead preconcentrated at the surface and to be analytically useful would be directly proportional to the bulk concentration of lead. A plot of peak area versus total lead concentration is expected to demonstrate a linear relationship passing through the origin where the concentration is zero. LS-ASV for lead standard additions over the ranges 500-2500 µg L⁻¹ and 250-1500 µg L⁻¹ are shown in **Figure 1**. Corresponding linear standard addition calibration plots however do not intersect the origin but at -0.52 µC and -0.38 µC respectively.

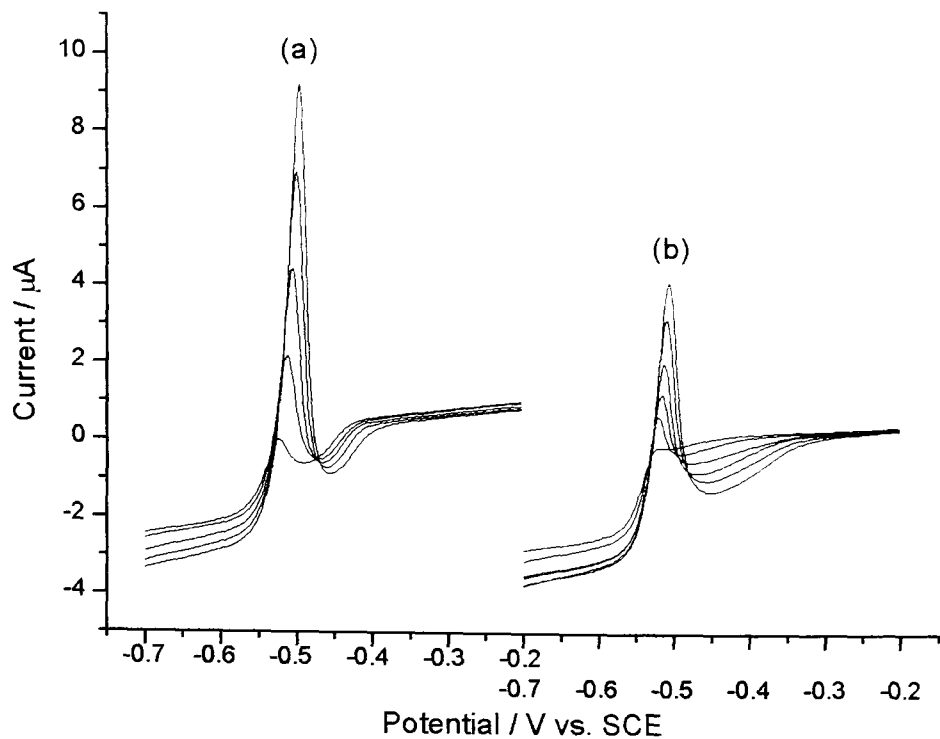


Figure 1 - Silent preconcentration at -1.5 V for 60 s followed by a linear potential scan (0.1 V s^{-1}) at a bare glassy carbon electrode. Standard additions of $\text{Pb}^{2+}(\text{aq})$ to 0.1 M HNO_3 over the range
a) $500\text{-}2500 \mu\text{g L}^{-1}$
b) $250\text{-}1500 \mu\text{g L}^{-1}$

It is evident from **Figure 1** that the baseline is not flat and is characteristic of the anodic stripping peak superimposed on the reduction wave for lead deposited on the surface. That is, the formation and stripping of lead proceeds with very little overpotential in either direction on this substrate, illustrated schematically in **Figure 2**.

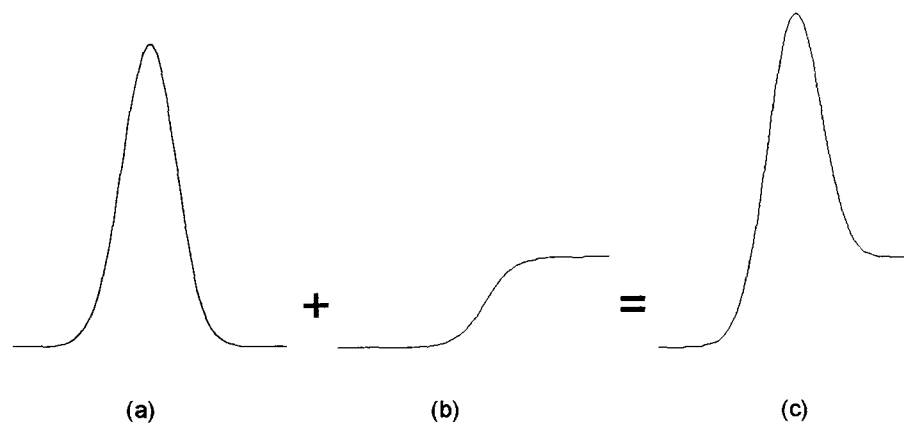


Figure 2 – Schematic diagram illustrating the superimposition of a reduction wave corresponding to the deposition of lead (b), upon the anodic stripping peak (a), with the resulting voltammogram having the appearance illustrated in (c), evident in the voltammetry of Figure 1

6.3.2 Insonated voltammetry of lead at a bare glassy carbon electrode

Ultrasound was introduced during the preconcentration step of LS-ASV. The promotion of mass transport, via acoustic streaming and cavitation, is evident in the increase of peak size (area and

height) which is clearly shown in **Figure 3** for standard additions over the range $250\text{--}1250\ \mu\text{g L}^{-1}$. For a concentration of $1250\ \mu\text{g L}^{-1}$ the peak area corresponding to a silent preconcentration ($1.78\ \mu\text{C}$) is *ca.* thirty-six times smaller than the peak area ($63.6\ \mu\text{C}$) observed following an insonated deposition at $30\ \text{W cm}^{-2}$. The simultaneous oxidation and reduction of lead at the electrode surface is still evident in the insonated case although it is small in comparison to the total peak height.

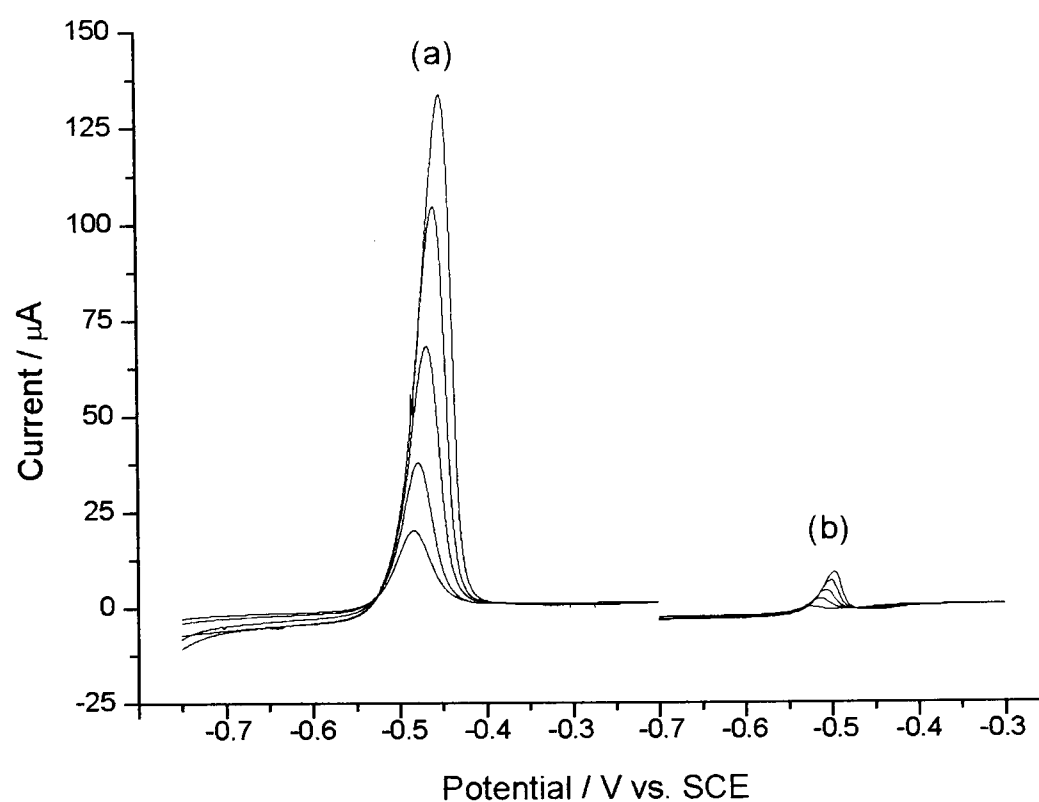


Figure 3 - Linear sweep anodic stripping voltammetry at a scan rate of $0.1\ \text{V s}^{-1}$ for standard additions of $250\ \mu\text{g L}^{-1}\ \text{Pb}^{2+}(\text{aq})$ to $0.1\ \text{M HNO}_3$

- a) insonated preconcentration at $-1.5\ \text{V}$ for $60\ \text{s}$ with a horn to electrode separation of $5\ \text{mm}$
- b) silent preconcentration at $-1.5\ \text{V}$ for $60\ \text{s}$.

The concentration range of lead in saliva is of the order of $0\text{--}300\ \mu\text{g L}^{-1}$ and is higher than blood lead levels where the patient has only recently been exposed. The onset of lead poisoning is known to occur at a blood lead concentration of $100\ \mu\text{g L}^{-1}$. For a realistic sputum size of $0.2\text{--}0.5\ \text{mL}$ of saliva to a total cell volume of $35\ \text{mL}$ the concentration range in the analyte is therefore of the order of $0\text{--}10\ \mu\text{g L}^{-1}$. The sensitivity of LS-ASV to standard additions over the range $1.25\text{--}11.25\ \mu\text{g L}^{-1}$ at a bare glassy carbon electrode was evaluated for an insonated preconcentration at $30\ \text{W cm}^{-2}$ and was found to be insufficient to yield a satisfactory calibration with $R^2 = 0.941$ and an intercept of $-0.5\ \text{nC}$. Although square wave voltammetry would afford greater sensitivity its interpretation is not straightforward due to the simultaneous oxidation and reduction occurring at the bare glassy carbon surface. A Nafion® coated mercury thin film glassy carbon electrode was therefore employed as a possible substrate.

6.3.2 Detection of Lead at a Nafion® coated mercury thin film glassy carbon electrode

The Nafion® coated mercury thin film glassy carbon electrodes (NCMTFGCE) were prepared according to the procedure detailed in the experimental section. In accordance with the recommendations of Brett et al²³ and Compton et al²⁴ the supporting electrolyte was 0.1 M acetate buffer at pH 4.6. The mercury thin film was plated in situ by the addition of 85.7 μM mercury (II) acetate. The Nafion® coating enabled a mechanically stable mercury film to be formed on the surface that remained intact when insonated^{23,24}.

The flat baseline of linear sweep anodic stripping voltammetry at this substrate confirmed that the oxidation of lead on the surface proceeded with a much higher overpotential than at the bare glassy carbon surface. Sensitive square wave anodic stripping voltammetry (SW-ASV) could therefore be employed with the following parameters: silent pretreatment at 0.6 V for 60 s followed by an insonated preconcentration at -1.5 V for 120 s with a horn to electrode separation of 5 mm. The square wave analytical stripping step had a frequency of 50 Hz, a scan range of -0.725 to -0.3 V a step potential of 1 mV and an amplitude of 50 mV. **Figure 4** shows SW-ASV for standard additions of $1.25 - 6.25 \mu\text{g L}^{-1}$ with a linear calibration $R^2 = 0.993$ shown inset. This demonstrates the high sensitivity of the square wave technique at this substrate in the concentration range of interest.

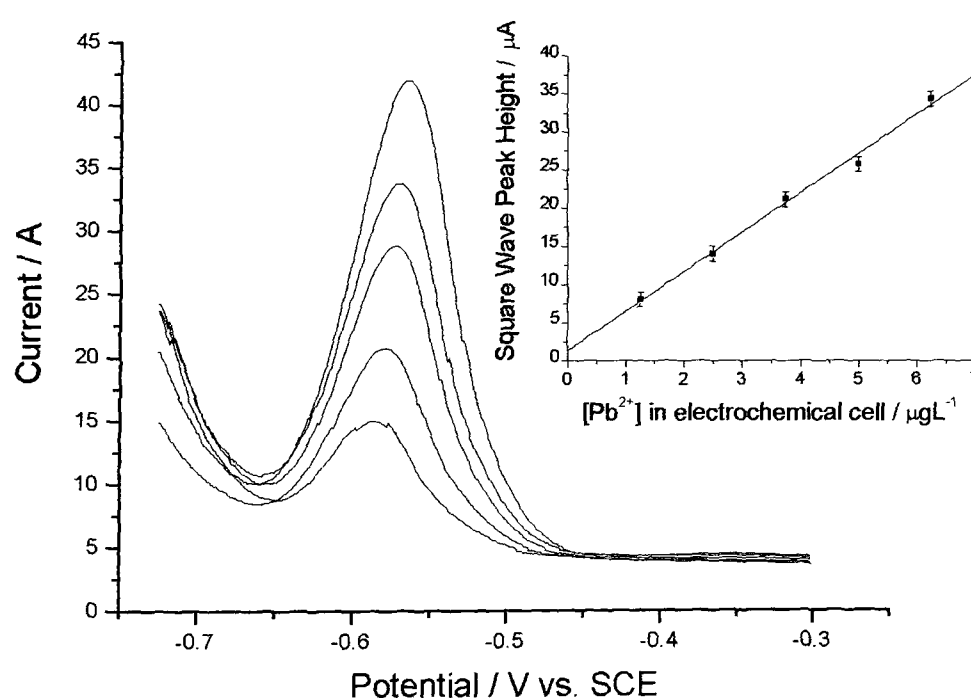


Figure 4 - Standard additions of $1.25 \mu\text{g L}^{-1} \text{Pb}^{2+}(\text{aq})$ to 0.1 M acetate buffer (pH 4.6) containing 85.7 mM $\text{Hg}^{2+}(\text{aq})$. Silent pretreatment for 60 s at 0.6 V and an insonated preconcentration of lead at -1.5 V for 120 s, followed by square wave anodic stripping voltammetry ($f = 50$ Hz, $\Delta E_s = 1.0$ mV, $\Delta E_p = 50$ mV) at a

Nafion® coated mercury thin film glassy carbon electrode. Inset: standard addition calibration plot with $R^2 = 0.9930$

6.3.4 Voltammetry in the presence of artificial saliva at the NCMTFGCE

A comparison between the square wave signal obtained for an insonated preconcentration for 120 s and silent preconcentration of the same duration, in the presence of 875 μL artificial saliva, is seen in **Figure 5** for a lead concentration of $6.25 \mu\text{g L}^{-1}$ in a cell volume of 35 mL. The increased passivation leading to a very low signal for a silent preconcentration is contrasted with the larger signal in the insonated case. This is attributed to the increase in mass transport due to acoustic streaming and cavitation shearing of glycoproteins and other active materials from the electrode surface.

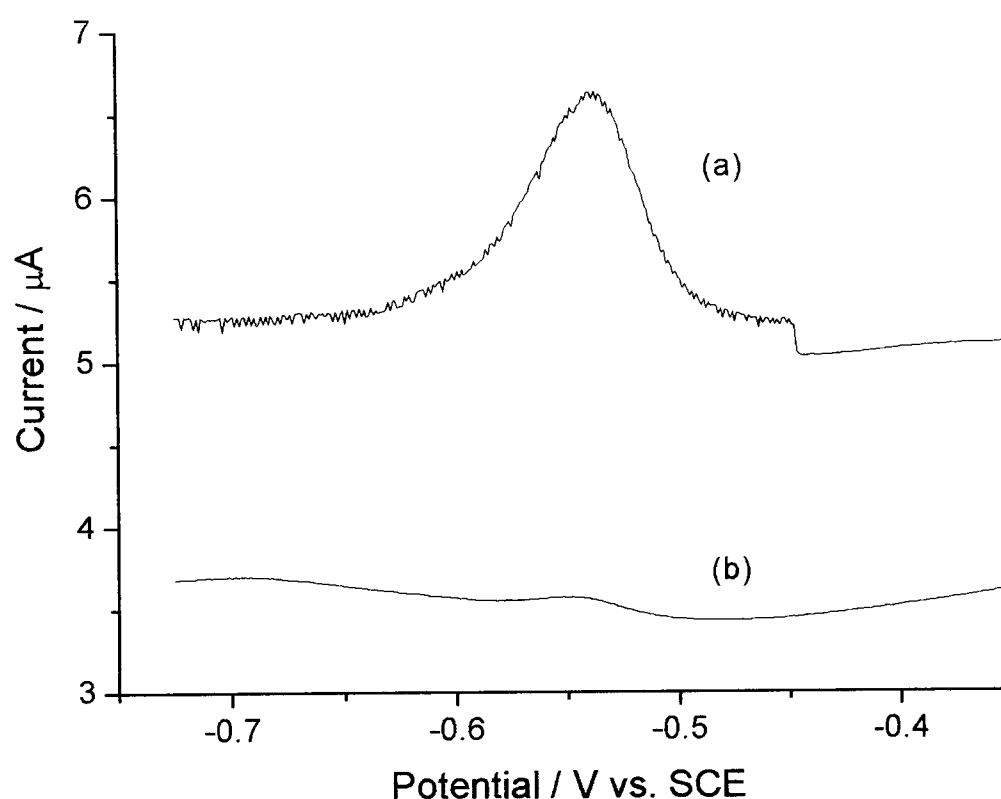


Figure 5 - SWASV at a Nafion® coated mercury thin film glassy carbon electrode for a concentration of $6.25 \mu\text{g L}^{-1}$ $\text{Pb}^{2+}(\text{aq})$ in 0.1 M acetate buffer (pH 4.6) containing 85.7 mM $\text{Hg}^{2+}(\text{aq})$ and 875 μL artificial saliva. Silent pretreatment for 60 s at 0.6 V followed by
a) insonated preconcentration (30 Wcm^{-2}) at -1.5 V for 120 s
b) silent preconcentration at -1.5 V for 120 s.

The optimum saliva sample size was evaluated from additions of artificial saliva to a solution of background electrolyte spiked with $1.25 \mu\text{g L}^{-1}$ lead. The results can be seen in Figure 6 for consecutive 100 μL additions of saliva.

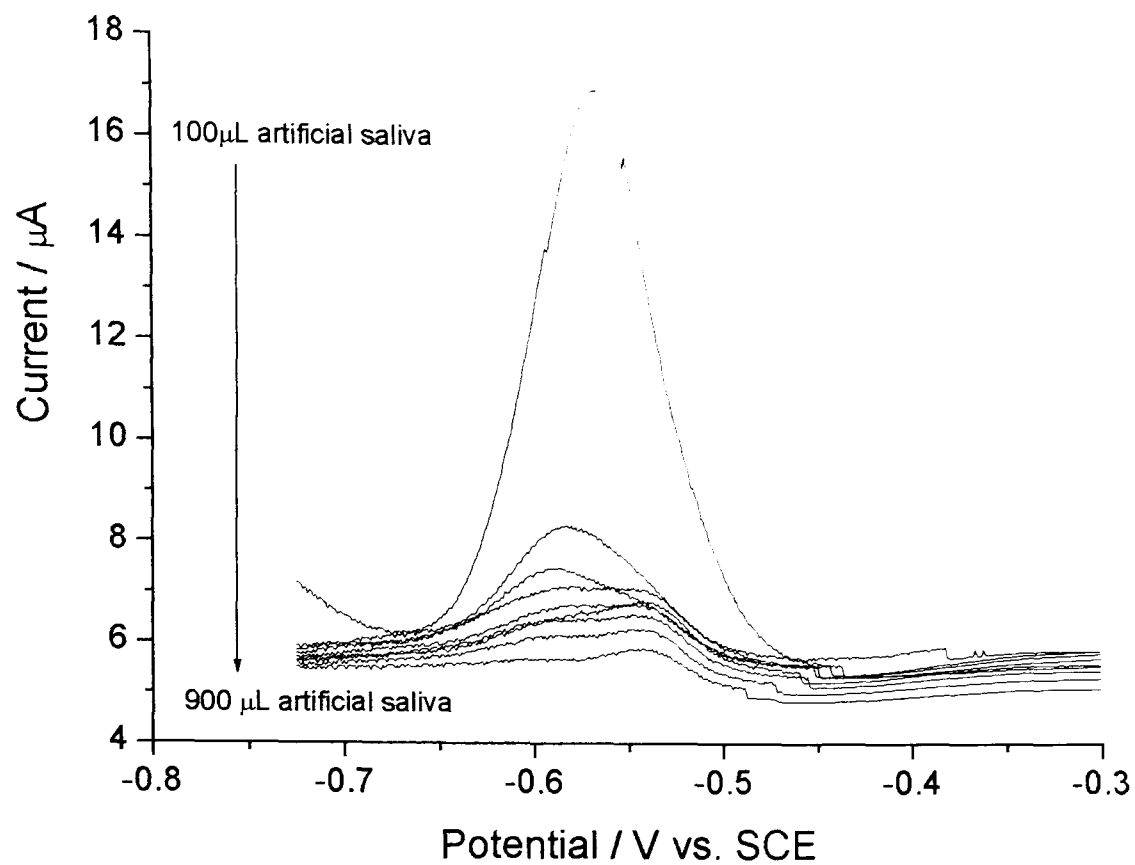


Figure 6 - Consecutive 100 μL additions of artificial saliva to a fixed concentration of lead, $1.25 \mu\text{g L}^{-1}$, in 0.1 M acetate buffer (pH 4.6) containing $85.7 \text{ mM Hg}^{2+}(\text{aq})$. Square wave anodic stripping voltammetry ($f = 50 \text{ Hz}$, $\Delta E_s = 1.0 \text{ mV}$, $\Delta E_p = 50 \text{ mV}$) at a Nafion® coated mercury thin film glassy carbon electrode followed silent pretreatment for 60 s at 0.6 V and insonated preconcentration of lead at -1.5 V for 120 s at 30 Wcm^{-2}

It is apparent that the effect of saliva is to rapidly passivate the electrode, even for an insonated preconcentration, with almost complete passivation evident after 9 additions of saliva. The optimum sputum size employed in all further experiments was $220 \mu\text{L}$ a compromise between maximizing the concentration of lead introduced to the cell and minimizing the electrode passivation in order to maintain quantitative ultrasonic depassivation.

Figure 7 shows standard additions of $0.6 \mu\text{g L}^{-1} \text{ Pb}^{2+}$ to $220 \mu\text{L}$ artificial saliva in acetate buffer at pH 4.6. The linear calibration shown inset, intersected the origin as anticipated for the uncontaminated artificial saliva and had an R^2 of 0.9981. The results showed good intrasample reproducibility with repeat measurements for the same addition of lead overlaying precisely.

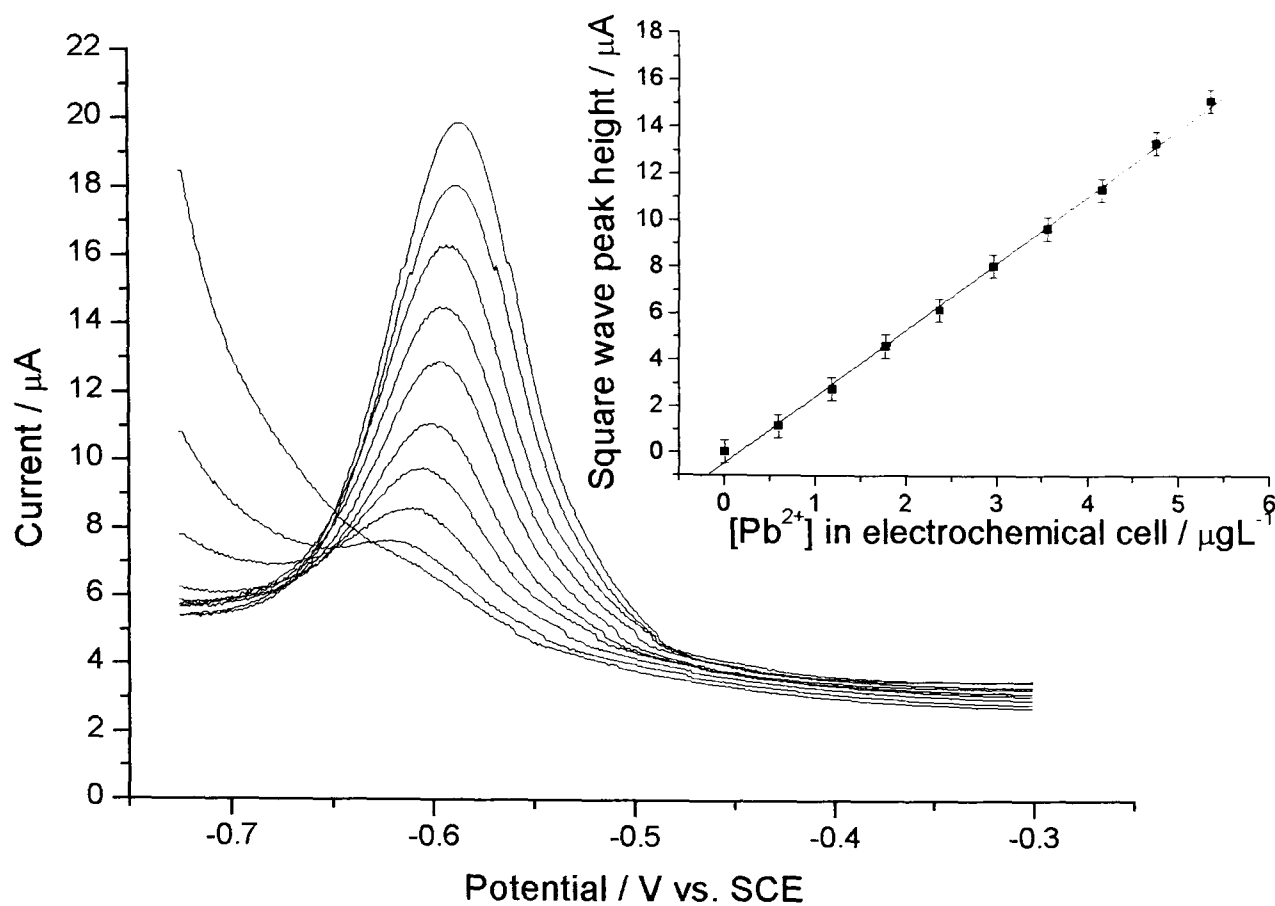


Figure 7 - Standard additions of $0.6 \mu\text{g L}^{-1}$ to $220 \mu\text{L}$ artificial saliva in 0.1 M acetate buffer at $\text{pH } 4.6$ containing $85.7 \text{ mM Hg}^{2+}(\text{aq})$. Square wave anodic stripping voltammetry ($f = 50 \text{ Hz}$, $\delta V = 1.0 \text{ mV}$, $A = 50 \text{ mV}$) at a Nafion® coated mercury thin film glassy carbon electrode followed a silent pretreatment for 60 s at 0.6 V and an insonated preconcentration period of 120 s at -1.5 V and 30 W cm^{-2} ultrasound intensity. Inset: standard addition calibration plot with $R^2 = 0.9980$

Careful and consistent preparation of the Nafion® coated glassy carbon electrode played an important role in the intersample reproducibility with subsequent standard addition experiments giving R^2 values of 0.9888 and 0.9933 . The theoretical detection limit (1) defined as²⁵ :

$$\text{LOD} = 3S_b/b \quad (1)$$

where S_b is the standard error of the y-residuals of the line of best fit and b is the gradient for the detection of lead in our artificial saliva sample, is $0.25 \mu\text{g L}^{-1}$. Previous electrochemical analyses for lead in biological media such as blood using a vibrating glassy carbon electrode²⁶ have yielded accurate results over the range $22\text{--}616 \mu\text{g L}^{-1}$. Ultrasonically enhanced square wave anodic stripping voltammetry therefore presents a significant increase in sensitivity for analysis of lead in the presence of biological complexants.

6.4 Conclusions

Quantitative determination of lead via sono-anodic stripping voltammetry at a bare glassy carbon electrode has been contrasted with an *in situ* plated Nafion® coated mercury thin film. A difficulty involved in the determination of lead at bare glassy carbon was the simultaneous oxidation and reduction of lead, which proceeds with very little overpotential in either direction at this substrate. The benefits afforded by the Nafion® coated mercury thin film on glassy carbon are high surface area leading to increased sensitivity and mechanical robustness of the film when insonated. The overpotential problems observed at the bare glassy carbon electrode are not evident at this substrate, hence an insonated preconcentration can be synergistically coupled with sensitive square wave voltammetry, enabling the technique to access the concentration range of interest 1-10 $\mu\text{g L}^{-1}$.

The determination of lead in artificial saliva without the need for sample pretreatment has been shown to be possible at an NCMTFGCE. Employing an insonated preconcentration period is vital at the low concentrations, 0-10 $\mu\text{g L}^{-1}$ in order to liberate the lead from the glycoproteins to which it binds in solution and to maximize the material preconcentrated at the surface over a short time (120 s). Cavitation shearing of large organic proteins effected depassivation of the surface. A linear standard addition plot intersects the origin as predicted for samples uncontaminated by lead and exhibits R^2 values between 0.988 and 0.994 depending on the quality of the Nafion® film. The theoretical detection limit in the analyte is 0.25 $\mu\text{g L}^{-1}$ which for a 220 μL sputum size corresponds to 38 $\mu\text{g L}^{-1}$. With the onset of lead poisoning at a blood lead concentration of 100 $\mu\text{g L}^{-1}$ this technique therefore accesses biologically relevant lead levels. The rapidity of the technique, potential for portability, relative low cost and sensitivity are favourable for the introduction of routine lead screening in occupational health and paediatric care.

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Chapter 7 - The Membrane Free Sonoelectroanalytical Determination of Trace Levels of Lead and Cadmium in Human Saliva

7.1 Introduction

Anodic stripping voltammetry (ASV), synergistically combined with power ultrasound has enabled the quantitative analysis of a wide range of analytical targets in media considered to be highly passivating a summary of which can be found in s.1.5.2. Specific biofluid examples have been documented in Chapters 4-6, the determination of copper in blood¹ and fish gill mucous² and lead in artificial saliva³.

Chapter 6 reported quantitative analysis of lead in artificial saliva from a sputum volume of 220 μL using an *in situ* plated mercury thin film glassy carbon electrode. Cavitation depassivation of the electrode surface maintained the analytical signal throughout the experiment where under silent conditions the signal diminished to zero with time. The precise mechanical details of cavitation are still open to debate⁴ and there is evidence to suggest that shear forces at the interface when the electrode is flushed are responsible for removing organic material (particularly large species such as proteins) facilitating continuous cleaning and activation of the surface. Acoustic streaming increased the mass transport of the system resulting in shorter deposition times, greater sensitivity and a detection limit in the analyte of $0.25 \mu\text{g L}^{-1}$ which corresponded to a salivary lead concentration of $38 \mu\text{g L}^{-1}$. With the onset of adverse effects at a blood lead concentration of $50\text{-}100 \mu\text{g L}^{-1}$ this technique was deemed to access biologically relevant levels.

It was noted in the Chapter 6 that the reproducibility of the Nafion® polymer film at the electrode played a large part in the intersample reproducibility. One of the aims of the work presented in this chapter is to improve the technique by obviating the necessity for such a film. It also presents an extension to Chapter 6 by incorporating a protocol for the analysis of cadmium and lead in human saliva validated using independent analysis by ICP-MS.

7.1 Experimental

The electrochemical cell used throughout is described in s.2.1 Figure 1. The ultrasonic horn was a Model CV-26 (Sonics and Materials Inc, Connecticut, USA) located at a horn to electrode separation of 3 mm. The ultrasonic intensity was calibrated calorimetrically according to the procedure given in s.2.3 over the range 10-138 W cm⁻².

Electrochemical measurements were carried out using a 3 mm diameter glassy carbon working electrode plated *in situ* by the addition of 0.6 mL 5 mM mercury (II) acetate to the cell contents to give a total cell volume of 35 mL. The background electrolyte was 0.1 M acetate buffer at pH 4.6. All potentials are quoted with respect to a saturated calomel electrode, SCE (Radiometer, Denmark). The counter electrode was a coiled platinum wire which was flame annealed prior to each experiment. Sono-square wave anodic stripping voltammetry (sono-SWASV) involved a silent oxidative pre-treatment at 0.1 V for 2 s, followed by an insonated preconcentration for 60 s at -1.5 V vs. SCE. The analytical square wave stripping step was parameterised as follows: frequency (f) 25 Hz, step potential (ΔE_s) 5 mV, amplitude (ΔE_p) 50 mV.

Reagents used were all of analytical grade or the highest available purity see Appendix 2 for full details. The artificial saliva was prepared according to the recipe of Leung and Darvell^{5,6} and, as described previously in Chapter 6³, a bovine submandibular gland mucin concentration of 30 g/L reflected the necessity to allow for the organic component of saliva consisting mainly of glycoproteins⁷. The pH was adjusted to 6.8 by additions of small volumes of dilute HCl or NaOH, in order to correspond to the pH of authentic saliva after emission from the canals. Samples of human saliva were collected in clean glass vials following two oral rinses with deionised water. It is preferable to request that the subjects do not eat for up to two hours prior to obtaining the sample to reduce the likelihood of the analysis being contaminated with particulate food.

7.3 Results and Discussion

7.3.1 Obviating the necessity of the polymer film

Mercury thin films on glassy carbon consist of sub-micrometer⁸ sized droplets spread across the surface⁹⁻¹¹ and weak adhesion results in poor mechanical properties. A Nafion® film was used in the preceding chapter to improve the mechanical stability and act as a barrier to fouling by large

negatively charged organic species¹²⁻¹⁴. It was recognised that the reproducibility of the spin coated Nafion® film affected the intersample reproducibility of the quantitative determination of lead in artificial saliva. The thickness of the polymer film was critical, and it was apparent that a thicker film resulted in the depression of measured peak heights. It is also vital to simplify the process of electrode preparation in order to optimise commercial viability.

Ex situ plated mercury films exhibited rapid removal of deposited mercury when insonated, even at low powers. However *in situ* plating of the mercury film on a glassy carbon electrode proved to be more promising with a surface that was continuously renewed during preconcentration of lead and cadmium. Careful manipulation of the duration of the preconcentration and the ultrasound power to which the electrode was exposed, illustrated in **Figure 1**, allowed parameters to be identified where the necessity of the polymer film was obviated.

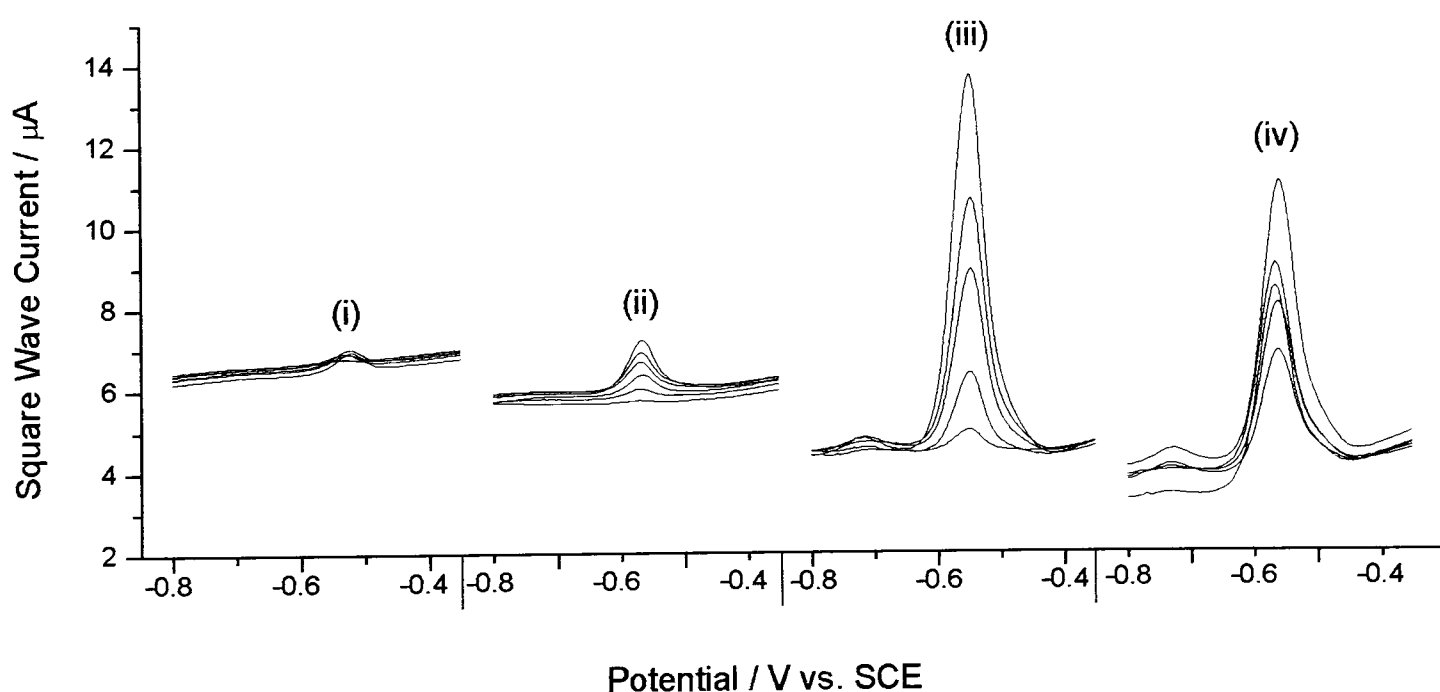


Figure 1a - Square wave anodic stripping voltammetry (SWASV) for microadditions of $0.625 \mu\text{g L}^{-1}$ lead to $200 \mu\text{L}$ artificial saliva in 35 mL 0.1 M acetate buffer at pH 4.6. A silent oxidative pre-treatment at 0.1 V for 2 s was followed by preconcentration step for 60 s at -1.5 V vs. SCE and an analytical square wave stripping step ($f = 25 \text{ Hz}$, $\Delta E_s = 5 \text{ mV}$, $\Delta E_p = 50 \text{ mV}$). The preconcentration step was carried out under the following conditions:

- i) Silent
- ii) Insonated at 30 W cm^{-2}
- iii) Insonated at 40 W cm^{-2}
- iv) Insonated at 70 W cm^{-2}

Figure 1a shows square wave stripping voltammograms following a 60 s preconcentration step at -1.5 V for microadditions of $0.625 \mu\text{g L}^{-1}$ to $200 \mu\text{L}$ artificial saliva in 35 mL background electrolyte for

silent (i) and insonated (ii-iv) preconcentration at applied ultrasound powers of 30-70 W cm⁻². Ultrasound is advantageous in producing both a stable and lower baseline current by cavitationally reducing electrode fouling and enhanced square wave peak current due to the increased mass transport afforded by acoustic streaming.

Since clean artificial saliva was used the initial concentration of lead in the sample was zero, hence the ratio of intercept : gradient for the calibration curves corresponding to the voltammetry shown in Figure 1, should be close to zero. Improvement is seen on going from a silent preconcentration, where an elevated value is indicative of electrode fouling, to 40 W cm⁻², as shown in Table 1.

	R ²	Intercept / A	Gradient / A/μgL ⁻¹	Ratio I/G / μgL ⁻¹
Silent	1	3.37 x 10 ⁻⁸	1.23 x 10 ⁻⁷	0.27
30 W cm ⁻²	0.9966	2.459 x 10 ⁻⁸	3.38 x 10 ⁻⁷	0.07
40 W cm ⁻²	0.9881	1.86 x 10 ⁻⁷	3.39 x 10 ⁻⁶	0.05

Table 1 – Comparison of the calibration data corresponding to the voltammetry shown in Figure 2

However, for an applied ultrasound power of 70 W cm⁻² there is initially a large enhancement in the peak current which rapidly falls off through the duration of the experiment as illustrated in **Figure 1b**.

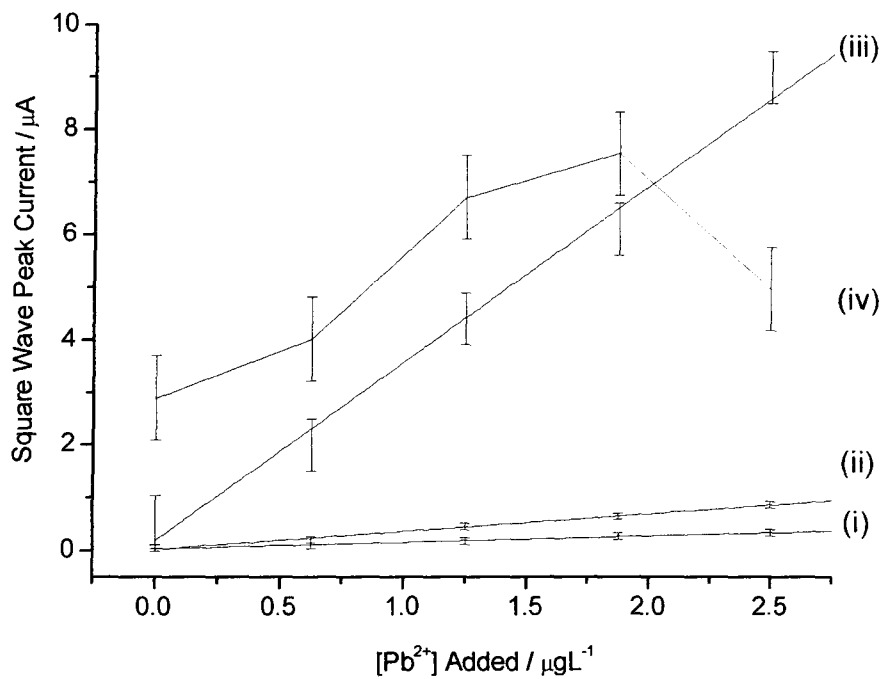


Figure 1b – Plot of Square wave peak current versus total [Pb²⁺] introduced for each microaddition with the parameters described in Figure 2a. The preconcentration conditions (i)-(iv) correspond with the voltammetry in Figure 2a.

This has been attributed to the ablation of mercury from the surface, resulting in correspondingly lowered accumulation of lead at higher power.

The optimum parameters were identified as a preconcentration time of 60 s and an ultrasound power of 10-40 W cm⁻². A comparison between microadditions of 0.625 µg L⁻¹ lead to an *in situ* plated mercury thin film GCE and a Nafion® coated mercury thin film glassy carbon electrode in a solution of artificial saliva can be seen in **Figure 2** for an ultrasound power of 30 W cm⁻². It is evident that if the mercury film can be maintained on the surface without the need for a polymer film then the peak heights and peak widths are improved.

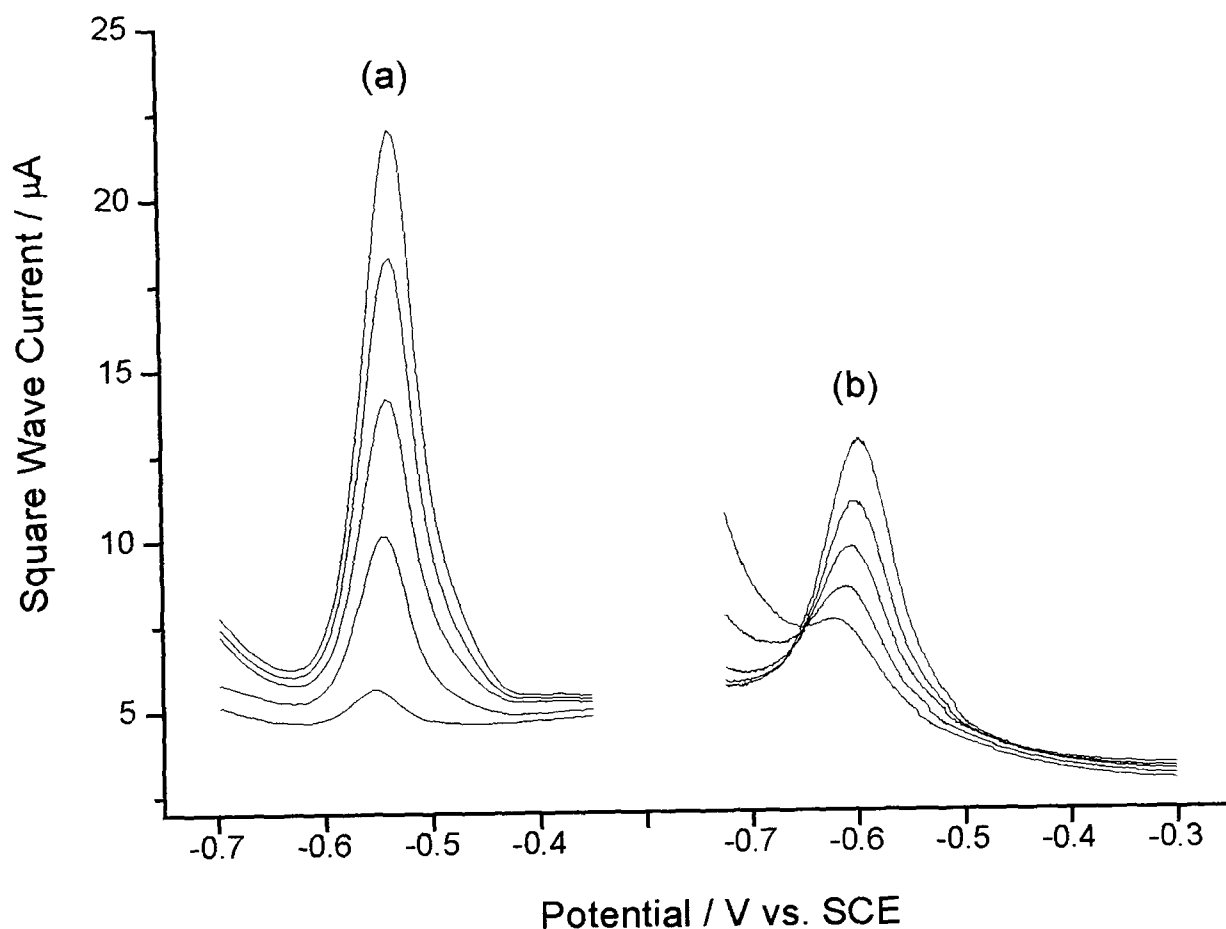


Figure 2 - SWASV for microadditions of 0.625 µg L⁻¹ lead to 200 µL artificial saliva in 35 mL 0.1 M acetate buffer at pH 4.6. A silent oxidative pre-treatment at 0.1 V for 2 s was followed by an insonated preconcentration with a power of 30 W cm⁻² for 60 s at -1.5 V vs. SCE and an analytical square wave stripping step ($f = 25$ Hz, $\Delta E_s = 5$ mV, $\Delta E_p = 50$ mV). The working electrodes employed were:

- a) *in situ* plated mercury thin film glassy carbon
- b) Nafion® coated mercury thin film glassy carbon

The quantitative accuracy afforded by the *in situ* plated mercury thin film glassy carbon electrode was verified using microadditions of 0.625 µg L⁻¹ to a clean (saliva free) solution of background electrolyte with an insonated preconcentration at 30 W cm⁻² and is shown in **Figure 3**. The corresponding linear calibration can be seen to intersect the origin (**Figure 3 inset**), and presents an R^2 value of 0.9897. The protocol is applied below to the detection of lead in authentic saliva.

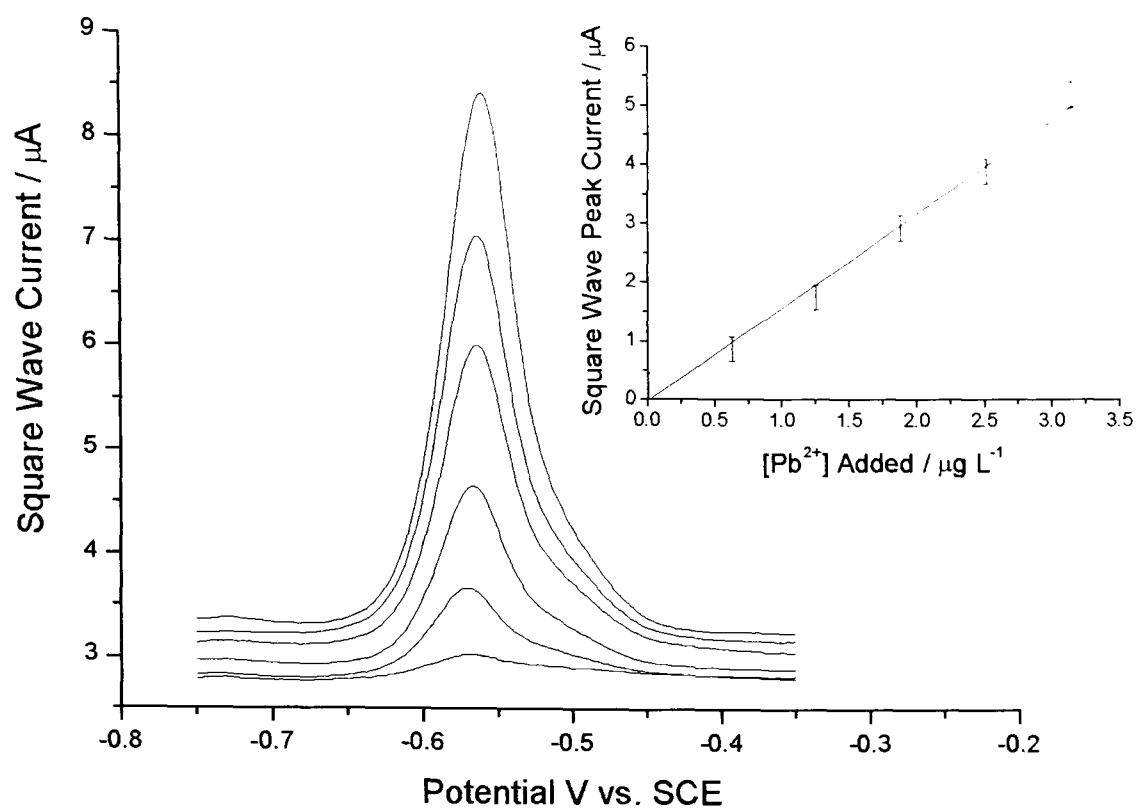


Figure 3 - SWASV for microadditions of $0.625 \mu\text{g L}^{-1}$ lead to 35 mL 0.1 M acetate buffer at pH 4.6. A silent oxidative pre-treatment at 0.1 V for 2 s was followed by an insonated preconcentration step with a power of 30 W cm^{-2} for 60 s at -1.5 V vs. SCE and an analytical square wave stripping step ($f = 25 \text{ Hz}$, $\Delta E_s = 5 \text{ mV}$, $\Delta E_p = 50 \text{ mV}$). A linear calibration plot is shown inset which intersects the origin with $R^2 = 0.9897$.

7.3.2 The Detection and Determination of Lead in Human Saliva

Saliva samples referred to as saliva A and saliva B were collected according to the procedure detailed above in s.7.2. Half of each sample was retained, the other half to be tested independently and blind by ICP-MS. Preliminary tests on both samples suggested concentrations of lead of less than $10 \mu\text{g L}^{-1}$. For these low concentrations the analytical protocol required modification with an increased saliva sample size of $400 \mu\text{L}$ to maximise the concentration of lead in the cell. In order to mitigate the effects of increased electrode fouling, evidenced by peak broadening and elevated background current, the ultrasound power used in the preconcentration step was 40 W cm^{-2} (illustrated in **Figure 1a(iii)**). Furthermore, the microaddition size was reduced tenfold to $0.0625 \mu\text{g L}^{-1}$ which was lower than the anticipated levels of lead in the electrochemical cell. A typical plot showing square wave voltammetry for microadditions to saliva A can be seen in **Figure 4** with a linear calibration graph shown inset with $R^2 = 0.9983$.

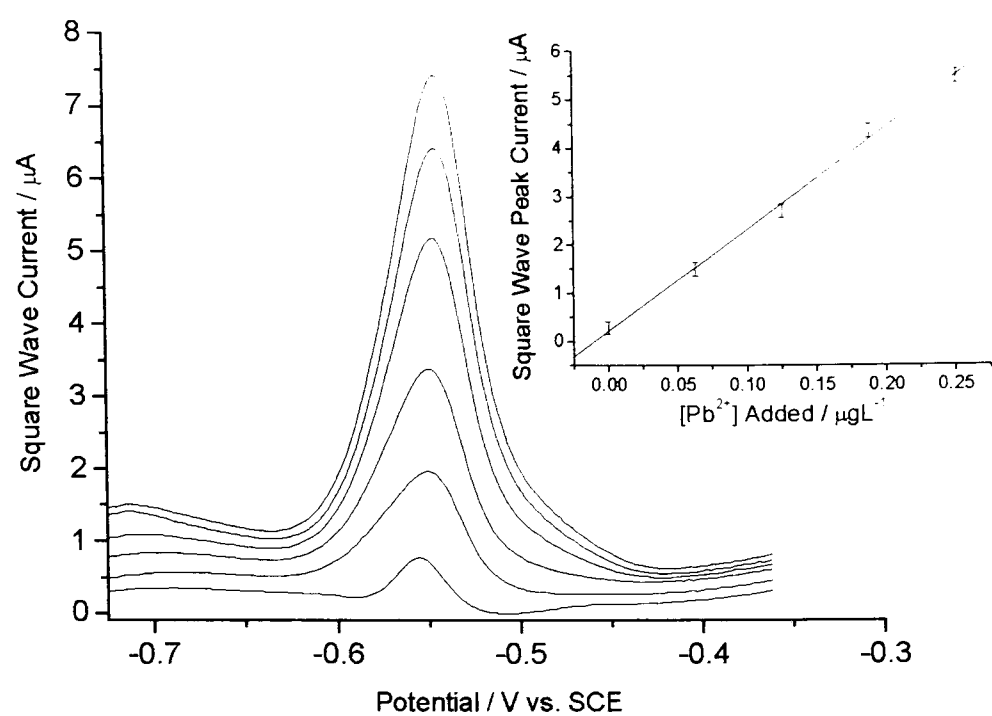


Figure 4 - SWASV for microadditions of 0.0625 $\mu\text{g L}^{-1}$ lead to 400 μL human saliva sample A in 35 mL 0.1 M acetate buffer at pH 4.6. A silent oxidative pre-treatment at 0.1 V for 2 s was followed by an insonated preconcentration step with a power of 40 W cm^{-2} for 60 s at -1.5 V vs. SCE and an analytical square wave stripping step ($f = 25$ Hz, $\Delta E_s = 5$ mV, $\Delta E_p = 50$ mV). The linear calibration plot is shown inset with $R^2 = 0.9983$.

The concentration was found to be $0.92 \pm 0.4 \mu\text{g L}^{-1}$ which can be compared with $0.92 \pm 0.1 \mu\text{g L}^{-1}$ determined independently by ICP-MS (see Table 2). Saliva B yielded a value of $5.1 \pm 0.4 \mu\text{g L}^{-1}$ compared with $4.8 \pm 0.5 \mu\text{g L}^{-1}$ determined independently by ICP-MS (see Table 2). These results were typical of lead levels associated with unexposed individuals^{15,16}. In order to test the accuracy of the technique at higher lead levels saliva A and B were spiked to give a total concentration of $20 \mu\text{g L}^{-1}$ and $10 \mu\text{g L}^{-1}$ respectively. The results are reported in Table 2 and encouragingly provide an accurate reflection of the elevated levels of lead introduced to the cell.

	Saliva A	Saliva B	Spiked Saliva 10 $\mu\text{g L}^{-1}$	Spiked Saliva 20 $\mu\text{g L}^{-1}$
Sono-SWASV Mean $[\text{Pb}^{2+}] / \mu\text{g L}^{-1}$ (n=4)	0.92 ± 0.2	5.1 ± 1.0	11.2 ± 2.5	19.4 ± 2.5
ICP-MS Mean $[\text{Pb}^{2+}] / \mu\text{g L}^{-1}$ (n=4)	0.92 ± 0.1	4.8 ± 0.5	-	-

Table 2 – Comparison of Sono-Square Wave Anodic Stripping Voltammetry and ICP-MS Results for the Determination of Lead in Human Saliva

The analytical protocol reported herein has a detection limit of $0.5 \mu\text{g L}^{-1}$ in saliva based on the minimum concentration of lead which produces a measurable stripping peak, this corresponds to

detecting $0.006 \mu\text{g L}^{-1}$ in the analyte. This is significantly lower than both the detection limit in artificial saliva at a Nafion® coated electrode, reported to be $38 \mu\text{g L}^{-1}$ ³ ($0.25 \mu\text{g L}^{-1}$ in the analyte) and the detection limit in artificial saliva at a membrane free electrode, calculated to be $8 \mu\text{g L}^{-1}$ ($0.05 \mu\text{g L}^{-1}$ in the analyte). The greater sensitivity in human saliva is attributed to the improved performance of the membrane free electrode and is also evidence that the artificial saliva prepared for the previous work fouls the electrode more readily than authentic saliva.

7.3.3 The Detection and Determination of Cadmium in Human Saliva

The potential for quantitatively determining cadmium using the *in situ* plated mercury thin film working electrode and the sonoelectroanalytical protocol detailed above for lead was investigated. Microadditions of $0.625 \mu\text{g L}^{-1}$ cadmium were added to clean background electrolyte and the square wave voltammetric stripping step, which followed preconcentration for 60 s at -1.5 V and 40 W cm^{-2} , is shown in Figure 5 along with a linear calibration plot (inset) of $R^2 = 0.9993$, intersecting the origin as anticipated (intercept = 1.54×10^{-8} , gradient = 4.58×10^{-7}).

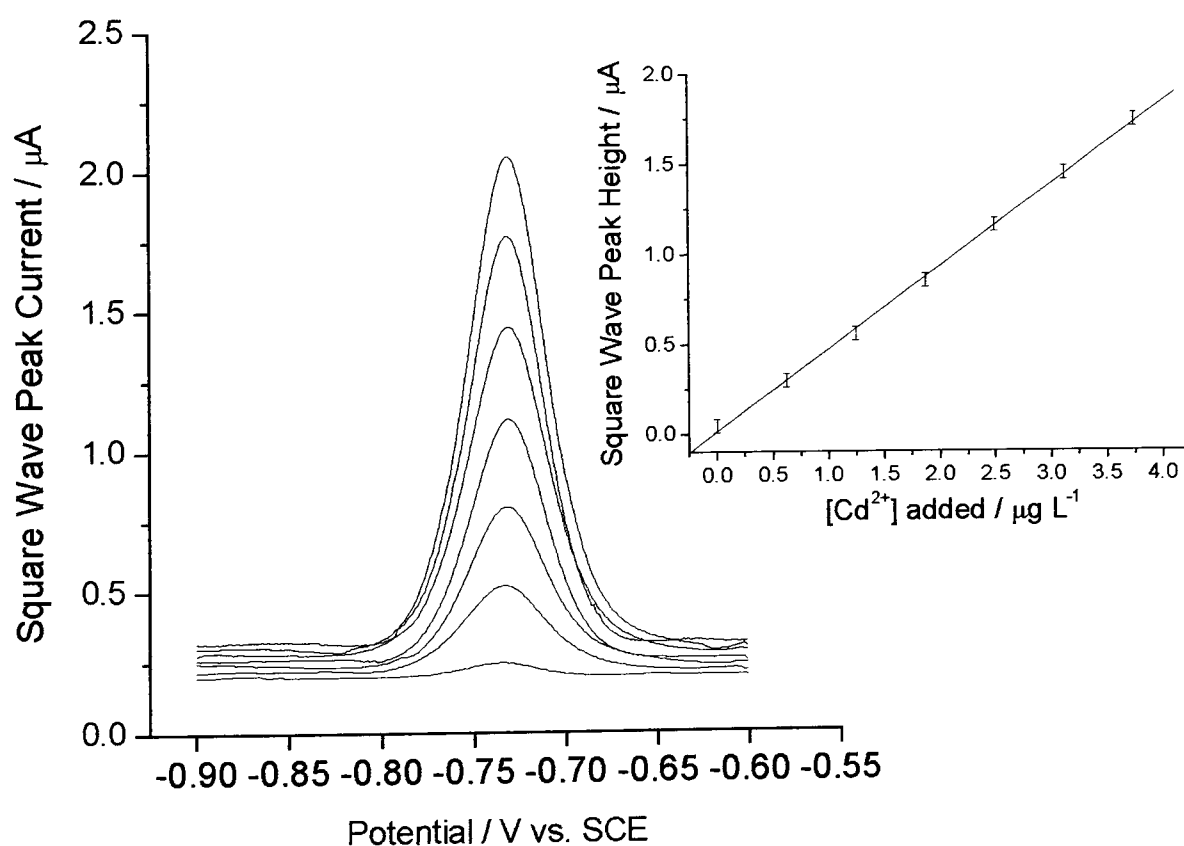


Figure 5 - SWASV for microadditions of $0.625 \mu\text{g L}^{-1}$ cadmium 35 mL 0.1 M acetate buffer at pH 4.6. A silent oxidative pre-treatment at 0.1 V for 2 s was followed by an insonated preconcentration step with a power of 40 W cm^{-2} for 60 s at -1.5 V vs. SCE and an analytical square wave stripping step ($f = 25 \text{ Hz}$, $\Delta E_s = 5 \text{ mV}$, $\Delta E_p = 50 \text{ mV}$) The linear calibration plot is shown inset and intersects the origin with $R^2 = 0.9993$.

The quantitative determination of cadmium in the saliva samples B and C was attempted using the above parameters (preconcentration at -1.5 V for 60 s insonated at 40 W cm^{-2}) however no measurable square wave stripping peaks were observed for either sample. The samples were then spiked with $2.5 \mu\text{g L}^{-1}$ and $5 \mu\text{g L}^{-1}$ cadmium such that their concentrations lay within the range anticipated for recently exposed workers but well below the levels where there is evidence for the onset of cadmium poisoning. Microadditions of $0.0125 \mu\text{g L}^{-1}$ to saliva C spiked with $5 \mu\text{g L}^{-1}$ can be seen in Figure 6 with a linear calibration shown inset of $R^2 = 0.9990$.

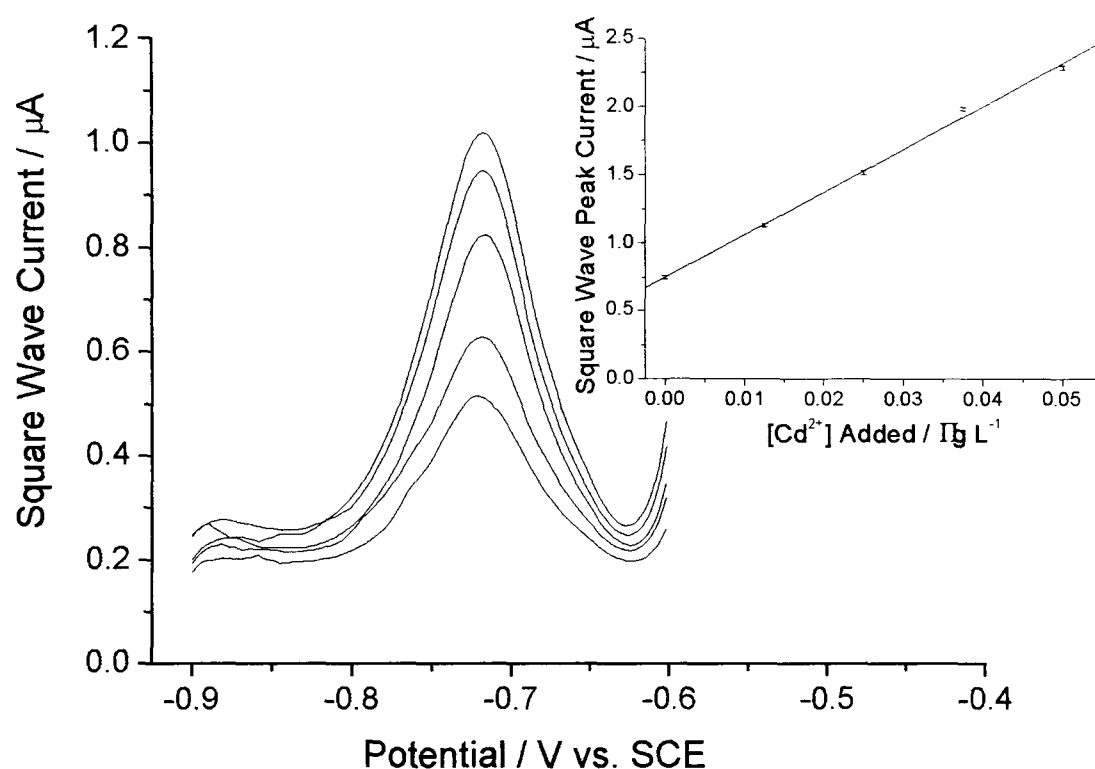


Figure 6 - SWASV for microadditions of $0.0125 \mu\text{g L}^{-1}$ cadmium to $200 \mu\text{L}$ human saliva sample C in 35 mL 0.1 M acetate buffer at pH 4.6. A silent oxidative pre-treatment at 0.1 V for 2 s was followed by an insonated preconcentration step with a power of 40 W cm^{-2} for 60 s at -1.5 V vs. SCE and an analytical square wave stripping step ($f = 25 \text{ Hz}$, 5 mV , $\Delta E_p = 50 \text{ mV}$). The linear calibration plot is shown inset with $R^2 = 0.9990$.

The concentration of cadmium determined using this method was $4.9 \pm 0.14 \mu\text{g L}^{-1}$ (see Table 3) which can be compared with the “unspiked” results obtained using ICP-MS which suggested a concentration of $0.15 \mu\text{g L}^{-1}$. Similar microaddition data was recorded for saliva B spiked with $2.5 \mu\text{g L}^{-1}$ cadmium. The linear calibration plot ($R^2 = 0.9930$) was indicative of a concentration of $2.5 \pm 0.5 \mu\text{g L}^{-1}$ (see Table 3) which can be compared with the “unspiked” results using ICP-MS which suggested a concentration of $0.03 \mu\text{g L}^{-1}$. The results provide evidence that additions of $0.0125 \mu\text{g L}^{-1}$, corresponding to $1 \mu\text{g L}^{-1}$ in saliva can easily be followed using this sonoelectroanalytical protocol.

	Saliva B	Saliva C	Spiked Saliva 2.5 µg L ⁻¹	Spiked Saliva 5 µg L ⁻¹
Sono-SWASV Mean [Cd ²⁺] / µg L ⁻¹ (n=4)	No peak	No Peak	2.5 ± 0.5	4.9 ± 0.05
ICP-MS Mean [Cd ²⁺] / µg L ⁻¹ (n=4)	0.03 ± 0.05	0.15 ± 0.05	-	-

Table 3 - Comparison of Sono-Square Wave Anodic Stripping Voltammetry and ICP-MS Results for the Determination of Cadmium in Human Saliva

7.4 Conclusions

Sono-square wave voltammetry at a membrane free electrode has been applied to the detection of lead and cadmium in authentic saliva with cavitation essential in maintaining a clean and activated electrode surface. In contrast, for a silent preconcentration, electrode fouling led to fluctuations in the baseline current and signal depression, which precluded accurate quantitative analyses. Acoustic streaming permitted low sampling times by increasing the mass transport of electroactive material to the electrode surface. Accurate responses, verified independently by ICP-MS have been reported over the ranges 0.9 – 20 µg L⁻¹ for lead and 2.5 – 5 µg L⁻¹ for cadmium. The technique is therefore presented as a potential alternative to conventional and costly analysis such as ICP-MS or AAS.

7.5 Acknowledgements

I would like to thank Dr David A. Worsley of the University of Swansea for facilitating the analysis of human saliva samples using a Perkin Elmer Elan DRC^{Plus} Inductively Coupled Plasma Spectrophotometer (ICP-MS). I would also like to thank Dr. Gareth R. Davies of Corus RD&T, for conducting the aforementioned analysis at the Welsh Technology Centre, Port Talbot, UK. I am also indebted to the volunteers, who provided saliva samples for analysis.

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Section IV

Biphasic Sonoelectroanalysis

Section IV

Chapter 8 – Simultaneous Extraction from, and Determination of Vanillin in Food Flavouring

8.1 Introduction

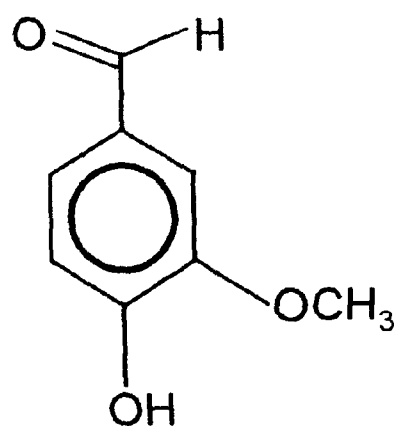
Ultrasound has long been known to facilitate emulsification in biphasic systems, being reported first in 1927 by Wood and Loomis¹. The benefits of emulsification by ultrasound include smaller drop sizes² down to 0.3 μm and lower energy consumption than the traditional mechanical approach. It also produces more stable emulsions that are less polydispersed². Several mechanisms have been proposed for the disruption and creation of droplets under the influence of ultrasound³⁻⁹. Cavitation, however, is the most widely recognised⁹⁻¹². Cavitational processes occur preferentially at the phase boundary^{11,13}, the imploding cavitation bubbles cause intensive shock waves in the surrounding liquid and the formation of liquid jets of high velocity¹⁴. These mechanical forces act to divide droplets again and again forming microdroplets and rapidly ‘homogenizing’ that heterogeneous system, even in the absence of surfactant.

Electroanalysis in biphasic systems is an area which has only been minimally exploited due to the difficulties in creating and maintaining emulsions in the absence of surfactant which, if present, can detrimentally affect the analysis. The use of ultrasound to form emulsions ensures that regardless of the relative densities of the two liquids, droplets of both are in constant contact with the electrode surface during voltammetric analysis. The potential of this technique was demonstrated by the analysis of lead in petrol by Blythe *et al*¹¹ using sono-anodic stripping voltammetry. The method reduced electrode fouling by cavitational depassivation and ultrasonic emulsification of petrol and water permitted the analysis of total lead without the need for elaborate sample pretreatments.

Sono-emulsion electrosynthesis¹⁵, for example the Kolbe electrosynthetic process^{16,17}, is a further significant area where acoustic emulsification presents advantages over traditional techniques such as emulsification with surfactants^{18,19} or turbulent flow²⁰ in particular for ease of separation of products and the minimal energy required in order to generate the emulsion.

Most recently Banks et al²¹ reported “nanosecond” chronoamperometry under variable potentiostatic conditions in a two-phase system, water and heptane, with an electro-inactive electrolyte under acoustic emulsification. The spikes observed in the current transient were attributed to heptane droplets, with a mean droplet size of 3 μm , impacting on the electrode surface. A change in polarity of the spikes was observed when the electrode potential travelled through the accepted potential of zero charge (pzc) of the electrode material in the pertinent aqueous medium. Banks et al address the possibility of using this technique to determine the pzc’s of electrode-electrolyte interfaces.

This chapter demonstrates the extraction, via acoustic emulsification, and sonoelectroanalysis of vanillin from food flavouring. Vanillin (4-hydroxy-3-methoxybenzaldehyde), see **Structure 1**, is used extensively in confectionery, beverages, pharmaceuticals, foods and perfumery. It is reported to have beneficial health effects such as inhibition of the oxidation of human low density lipoproteins leading to lower rates of cardiac disease mortality²² and an antisickling effect in sickle cell anaemia sufferers²³.



Structure 1 - Vanillin (4-hydroxy-3-methoxybenzaldehyde)

The classical methods of analysis for quality control purposes can be costly and lengthy involving complex sample pretreatments. They include high-performance liquid chromatography (HPLC)¹⁶, micellar electrokinetic chromatography (MEKC)²⁴, supported liquid membranes with amperometric detection²⁵ and square wave voltammetry using cylindrical carbon fibre electrodes²⁶.

8.2 Experimental

The electrochemical cell used throughout is described in s.2.1 Figure 1. The ultrasonic horn was a Model CV-26 (Sonics and Materials Inc, Connecticut, USA) located at a horn to electrode separation of 5 mm. The ultrasonic intensity was calibrated calorimetrically according to the procedure given in

s.2.3 over the range 35-200 W cm⁻². The intensity used was 200 ± 5 W cm⁻² unless otherwise stated. Electrochemical measurements were carried out using a 3 mm diameter glassy carbon working electrode and glassy carbon microelectrodes fabricated in house to a range of diameters. Microelectrode size was calibrated using a system of known diffusion coefficient, ferrocene in dimethyl formamide (DMF) via the equation for steady state currents at a microelectrode (see ss. 1.2.4.1 and 1.2.5 equation 65). All potentials are quoted with respect to a silver wire pseudo reference electrode. The counter electrode was a coiled platinum wire which was flame annealed prior to each experiment.

Reagents used were all of electrochemical grade or the highest available purity see Appendix 2 for full details. Vanilla extract was supplied by Culpepper Ltd. (Cambridge, UK).

8.3 Results and Discussion

8.3.1 Characterisation of Ethyl Acetoacetate as an Electrochemical and Sonoelectrochemical Solvent

Ethyl acetoacetate is a versatile solvent which is useful for extraction techniques due to the high solubility of a range of non ionic organic species such as vanillin and its ease of handling with a boiling point of 68-70 °C. However, to our knowledge the use of ethyl acetoacetate as an electrochemical solvent has not previously been exploited. It was therefore necessary to assess whether meaningful electrochemical measurements could be made in this solvent of low dielectric constant ($\epsilon = 15.9$)²⁷. Cyclic voltammetry of ferrocene with a background electrolyte of tetrabutylammonium nitrate (TBAN) was recorded at both micro and macro electrodes. Ferrocene (FeCp₂) is oxidised to the ferrocenium ion (FeCp₂⁺) in a single electron redox process. TBAN was chosen due to its high solubility in ethyl acetoacetate, permitting electrolyte concentrations of 0.1 M. **Figure 1** shows linear sweep voltammetry at 10 mV s⁻¹, of 1 mM ferrocene in ethyl acetoacetate containing 0.1 M TBAN at two different glassy carbon microelectrode diameters. The voltammograms exhibit a close to ideal steady state sigmoidal shape.

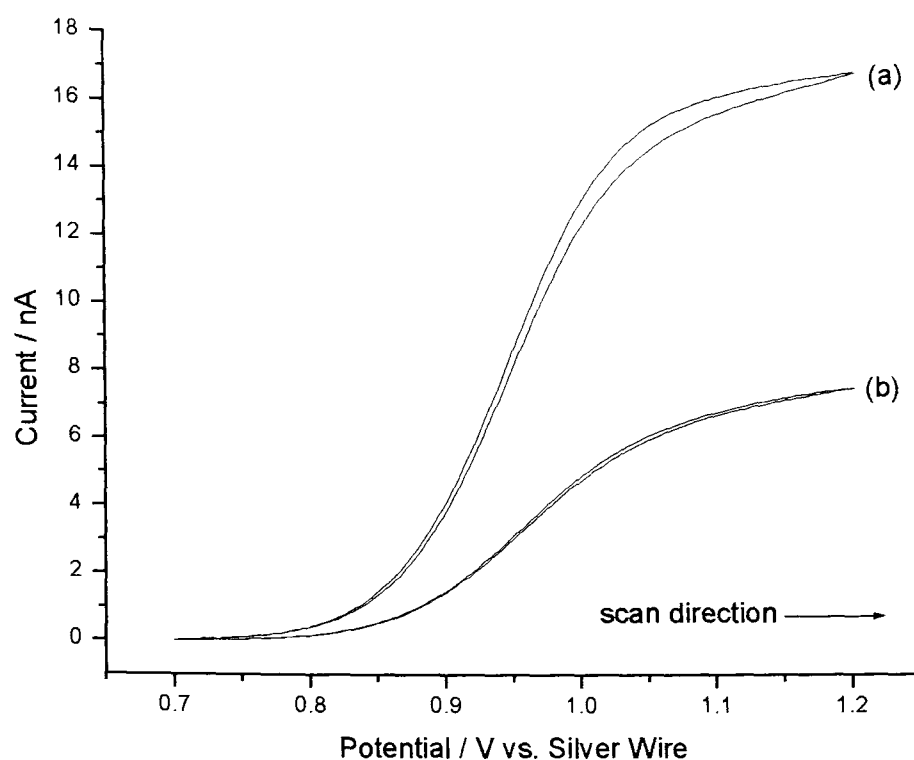


Figure 1 – Steady state cyclic voltammetry of 1 mM ferrocene in ethyl acetate with 0.1 M TBAN at a scan rate of 10 mV s^{-1} :
 (a) at a $60 \text{ }\mu\text{m}$ glassy carbon disc microelectrode
 (b) at a $30 \text{ }\mu\text{m}$ glassy carbon disc microelectrode

For an electrochemically reversible voltammetric wave a plot of measured potential, E , versus $\log[(i_{\text{lim}}/i)-1]$ will be a straight line with a gradient of $\approx 0.06 \text{ V}$ for $n = 1$ at 298 K and an intercept of $E_{1/2}$, the half wave potential, see s.1.2.2.

$$E = E_{1/2} + \frac{RT}{nF} \ln \frac{i_{\text{lim } c} - i}{i - i_{\text{lim } a}} \quad (1)$$

where E is the potential, $E_{1/2}$ is the half wave potential, $i_{\text{lim } c}$ is the cathodic limiting current and $i_{\text{lim } a}$ is the anodic limiting current. Tafel analysis of the voltammograms shown in **Figure 1** is shown in **Figure 2** and is indicative of good agreement with equation 1.

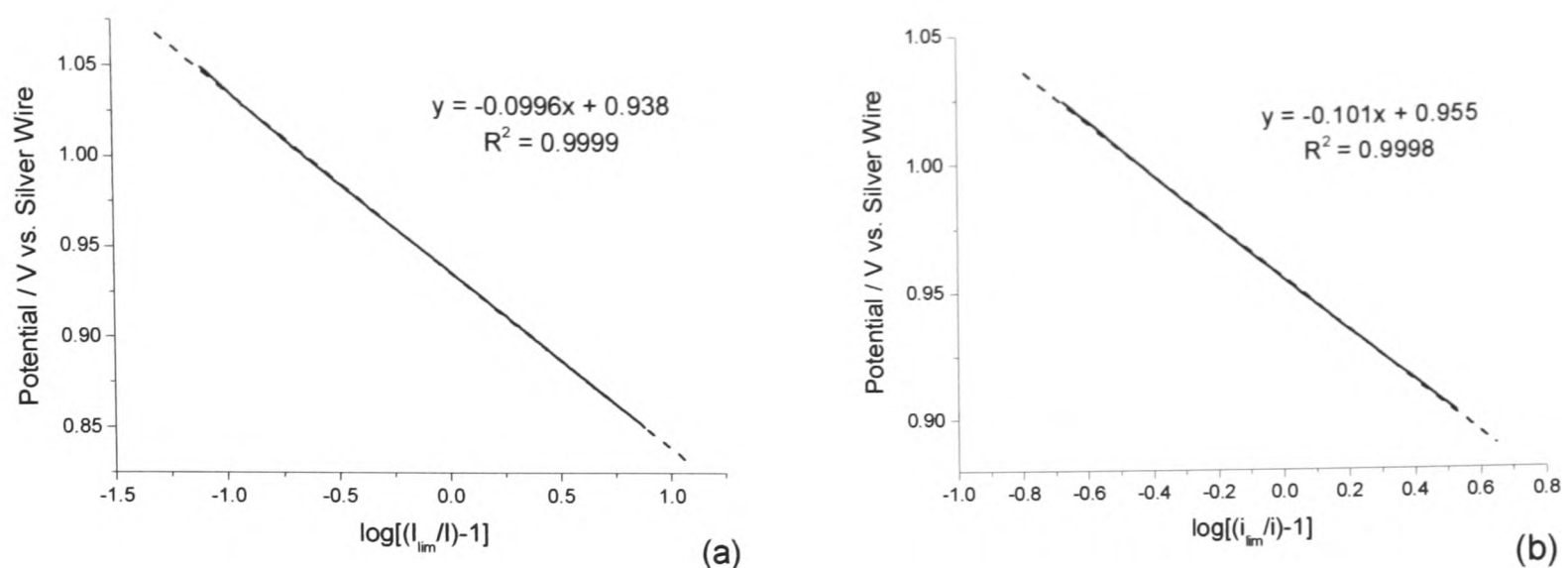


Figure 2 – Tafel analysis of the voltammograms shown in Figure 1 for
 (a) a 60 μm glassy carbon disc microelectrode
 (b) a 30 μm glassy carbon disc microelectrode

The average gradient over the two electrode diameters is 0.100 V. The difference between this value and the predicted value from equation 1 can be attributed to ohmic distortion resulting from the low conductivity of ethyl acetoacetate. The average half wave potential measured on oxidation scans against a silver wire pseudo reference electrode was 0.947 V.

The diffusion coefficient of ferrocene was calculated using equation 2 for steady state currents at a microelectrode (s. 1.2.5):

$$i_{\text{lim}} = 4nFD_{\text{FeCp}_2} [\text{FeCp}_2]_{\text{bulk}} r_0 \quad (2)$$

where D_{FeCp_2} is the diffusion coefficient of ferrocene, $[\text{FeCp}_2]_{\text{bulk}}$ is the bulk concentration of ferrocene and r_0 is the electrode radius. A range of microelectrode diameters were used from 5 to 70 μm , the mean value of D calculated was $1.41 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ with a standard deviation, $\sigma = 0.05 \text{ cm}^2 \text{ s}^{-1}$ for $n = 6$.

The cyclic voltammetry of ferrocene in ethyl acetoacetate at a 3 mm glassy carbon macroelectrode is shown in **Figure 3**. The voltammogram over the range -2 V to $+2 \text{ V}$ demonstrates the wide potential window of ethyl acetoacetate prior to the onset of solvent breakdown or oxygen reduction. Scanning over a narrower potential range (**Figure 3 - inset**) demonstrates the well defined near reversible voltammetry that can be obtained at a 3 mm glassy carbon in ethyl acetoacetate, facilitating the use of cheaper and less fragile macroelectrodes for electroanalysis in this medium.

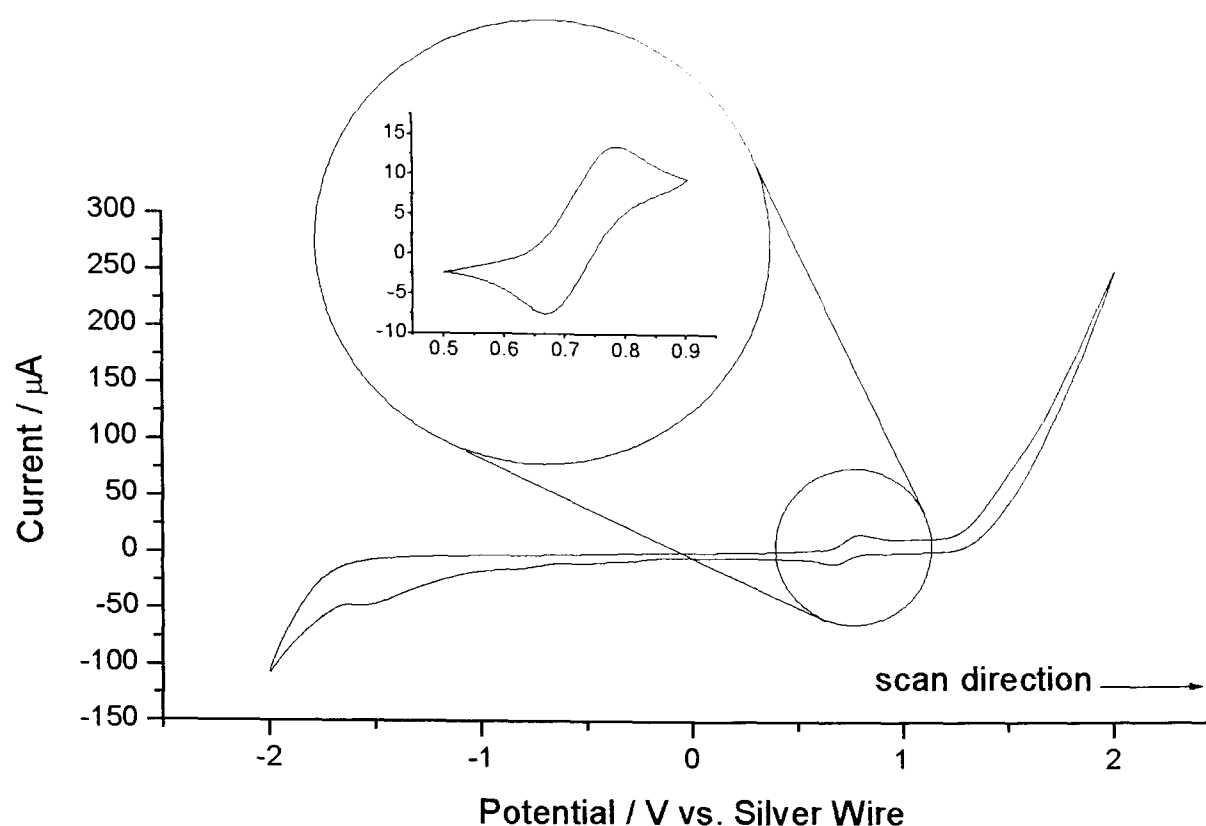


Figure 3 – Steady state cyclic voltammetry of 1 mM ferrocene in ethyl acetoacetate with 0.1 M TBAN with a scan rate of 100 mV s^{-1} at a 3 mm glassy carbon electrode, over a wide potential range of -2 to $+2$ V and narrow potential range of 0.5 to 0.9 V (inset).

The effect of ultrasound in ethyl acetoacetate was investigated by calibration of the diffusion layer thickness attainable at a range of ultrasound power and horn to electrode separation. The mean limiting current of the sono-voltammograms recorded for 1 mM ferrocene can be attributed to both acoustic streaming and cavitation collapse²⁸, see s.3.4.2. The contribution of acoustic streaming alone was calculated using the baseline of the limiting current. These limiting currents were then used to calculate the diffusion layer thickness quoted in table 1 using equation 3, which presumes a simple Nernstian diffusion layer model (see s.1.4.3).

$$i_{\text{lim}} = \frac{nFAD_B[B]_{\text{bulk}}}{\delta} \quad (3)$$

Horn to electrode separation / mm	Ultrasound Power %	Mean i_{lim} / A n = 3	Diffusion layer thickness δ / μm
10	35	1.30×10^{-4}	7.4
	73	1.66×10^{-4}	5.8
	111	2.03×10^{-4}	4.7
	150	2.52×10^{-4}	3.8
20	35	8.09×10^{-5}	11.9
	73	1.31×10^{-4}	7.3
	111	1.52×10^{-4}	6.3
	150	2.10×10^{-4}	4.6
30	35	6.70×10^{-5}	14.3
	73	9.83×10^{-5}	9.8
	111	1.23×10^{-4}	7.8
	150	1.67×10^{-4}	5.7

Table 1 – Calculated δ (μm) for a range of ultrasonic intensities

The range of diffusion layer thicknesses attainable using ultrasound power up to 20 % and horn to electrode separations of 10 mm, 20 mm and 30 mm is shown in Figure 4. The observed trend follows that predicted from other solvents^{28,29} showing that diffusion layer thicknesses under 10 μm are readily attainable in this solvent without the necessity for harsh conditions (very small horn to electrode separation or high power).

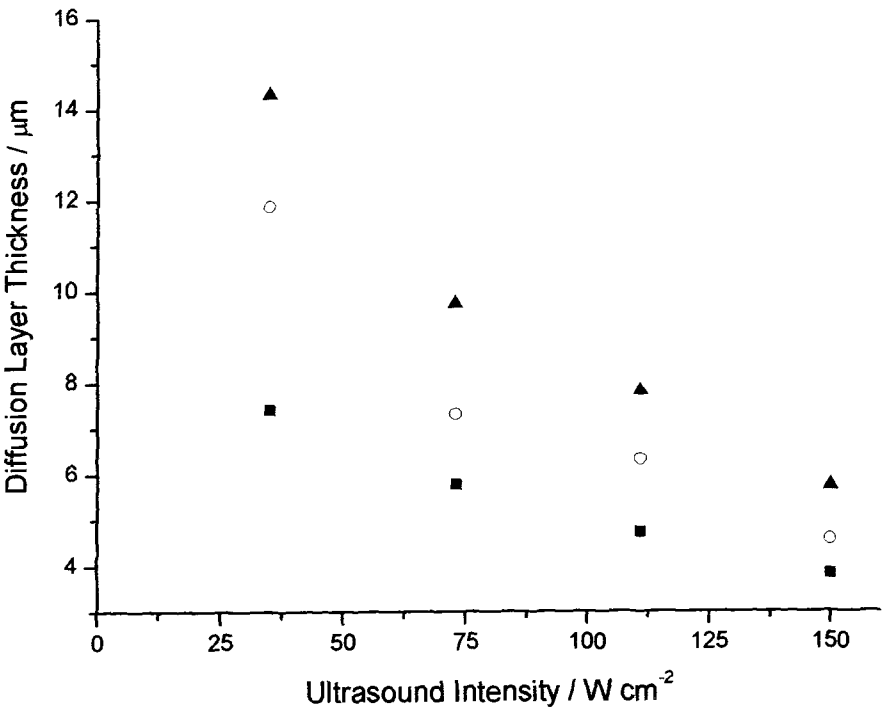


Figure 4 -Variation of diffusion layer thickness with ultrasound intensity for a 1mM solution of ferrocene in ethyl acetoacetate and 0.1 M TBAN for horn to electrode separations of:

- 10 mm
- 20 mm
- ▲ 30 mm

8.3.2 Voltammetry of Vanillin in Ethyl Acetoacetate

Natural vanilla extract purchased as food flavouring is a solution in aqueous ethanol mixture (approximately 10 % EtOH). The electrochemical oxidation of vanillin in aqueous ethanol (10 % by volume EtOH) was studied and linear sweep voltammetry yielded irreproducible peak heights and was insensitive to microadditions. Square wave voltammetry yielded no better results since the redox chemistry of vanillin in aqueous ethanol was essentially irreversible. In contrast linear sweep voltammetry of vanillin in ethyl acetoacetate gave oxidative peak heights sensitive to microadditions. In addition the near-reversible kinetics in ethyl acetoacetate facilitated sensitive square wave voltammetry. However, electrode passivation led to a diminishing of peak height for consecutive scans at the same concentration of vanillin. Pretreatment of the electrode by holding it at a positive potential of +2 V for 4 seconds prior to the square wave scan resulted in some improvement although this still lacked reproducibility. **Figure 5a** shows three consecutive square wave scans for a 1 mM solution of vanillin in ethyl acetoacetate with electrode passivation apparent in the decreasing peak height. By employing ultrasound at a power of 200 W cm^{-2} for 30 s in between each scan, consistent peak heights were maintained. **Figure 5b** shows three consecutive square wave scans of the same solution of 1 mM vanillin in ethyl acetoacetate where it can be seen that ultrasound between scans effected quantitative depassivation of the electrode surface.

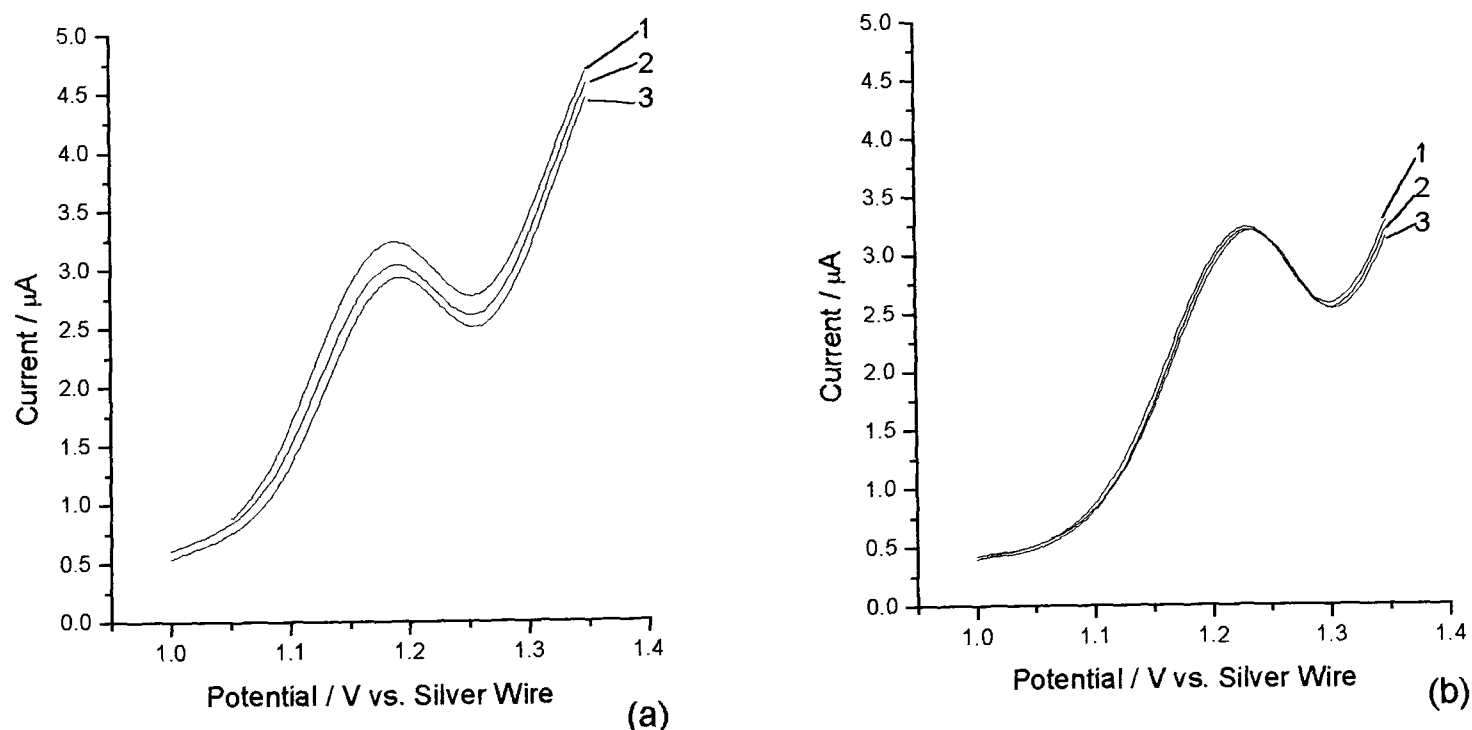


Figure 5 - Square wave voltammetry ($\Delta E_p = 15 \text{ mV}$, $\Delta E_s = 1.5 \text{ mV}$ and $f = 10 \text{ Hz}$) in a 1 mM solution of vanillin in ethyl acetoacetate and 0.1 M TBAN at a 3 mm glassy carbon electrode with electrode conditioning at +2 V for 4 sec prior to each scan.

(a) Three consecutive scans in the absence of ultrasound

(b) Three consecutive scans with insonation at for 30 s at 200 W cm^{-2} and 10 mm horn to electrode separation between each scan.

Square wave voltammetry obtained for microadditions of 5.0×10^{-5} M to ethyl acetoacetate is shown in **Figure 6**, at optimised square wave parameters of $\Delta E_p = 15$ mV, $\Delta E_s = 1.5$ mV and $f = 10$ Hz. The true background current in the absence of vanillin was fitted to a 7th order polynomial using Origin 6.0 software (Microcal, MA, USA) which was then used to facilitate the calculation of the background current arising from solvent breakdown (cf. **Figure 3**) for each addition of vanillin. The background fit was performed for each addition due to the slight potential drift observed with vanillin additions due to the instability of the silver wire as a reference electrode. **Figure 6 (inset)** shows the square wave analytical signal and modelled background signal. Subtraction of the background signal gave an approximately Gaussian shaped square wave profile as theoretically predicted^{30,31}.

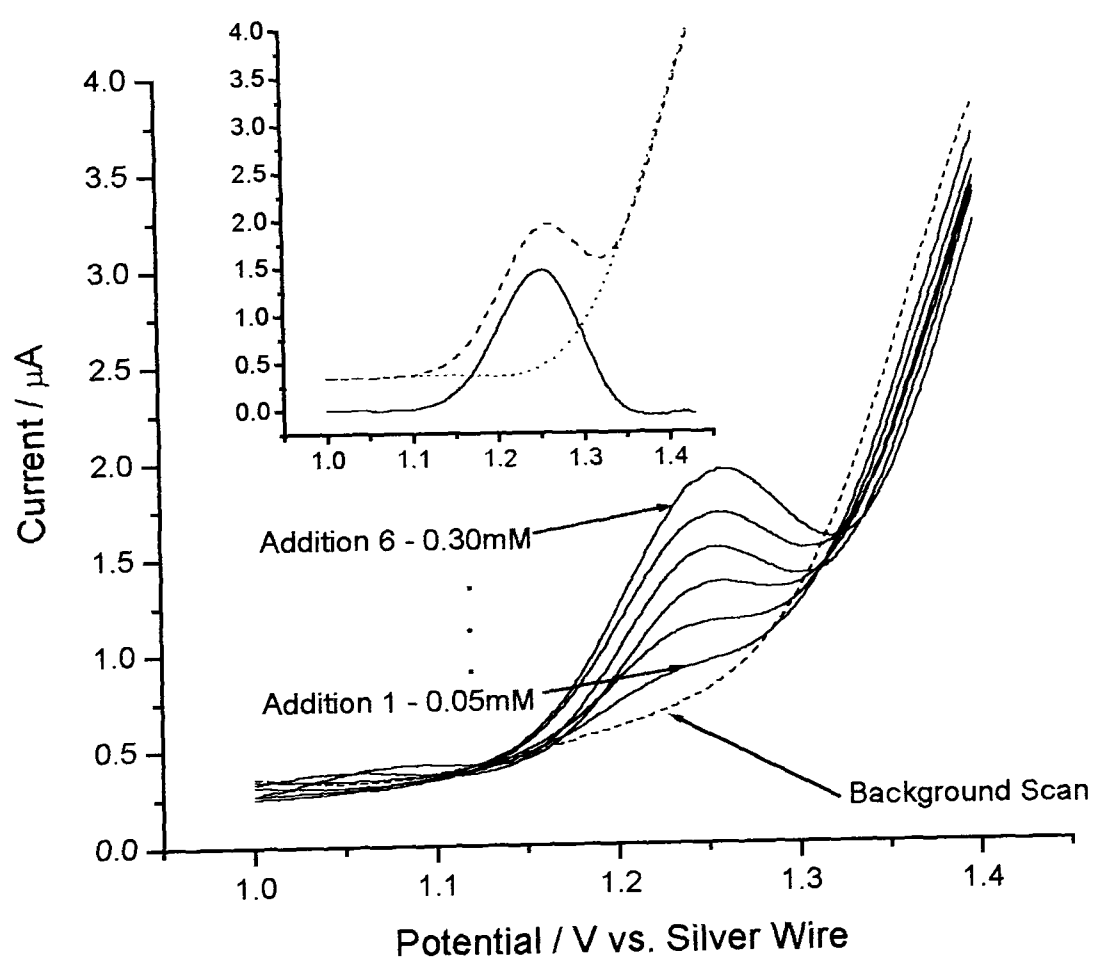


Figure 6 - Square wave voltammetry ($\Delta E_p = 15$ mV, $\Delta E_s = 1.5$ mV and $f = 10$ Hz) for microadditions of 0.05 mM vanillin to ethyl acetoacetate, 0.1 M TBAN at a 3 mm glassy carbon electrode with electrode conditioning at +2 V for 4 s prior to each scan. Ultrasound was employed in between each scan for 30 s at 200 W cm^{-2} and 10 mm horn to electrode separation.

Inset - Background correction of square wave voltammetry for the 6th addition.

- Square wave analytical signal
- Calculated background signal
- Background corrected signal

Following background correction the microadditions of 0.05 mM vanillin can be plotted as shown in figure 7. A linear calibration graph (Figure 7 inset) of $r = 0.9987$ was obtained. The limit of detection of 0.016 mM ($n = 4$) was calculated³² using equation 4.

$$\text{LOD} = 3S_b/b \quad (4)$$

where S_b is the standard error of the y-residuals of the line of best fit and b is the gradient.

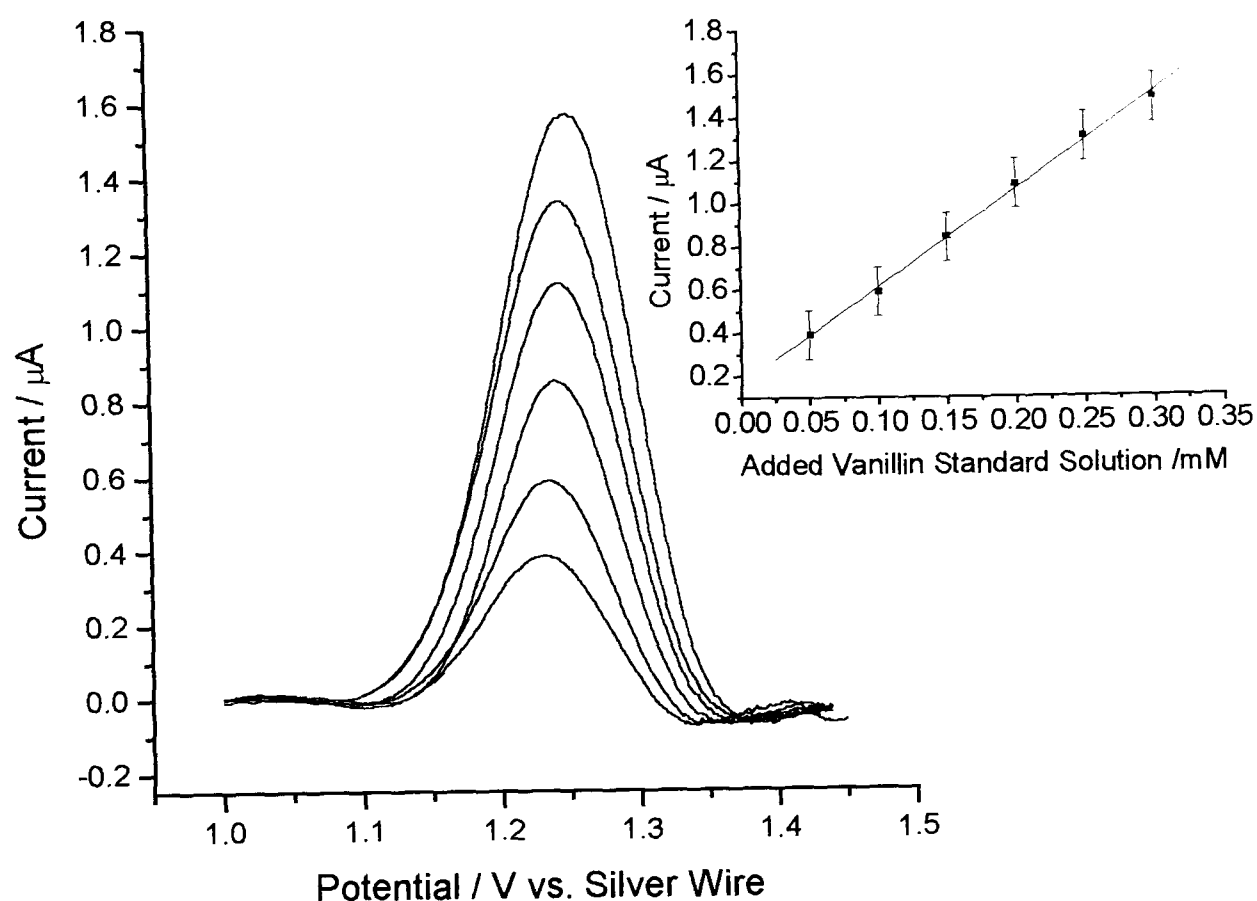


Figure 7 - Background corrected square wave voltammetry of additions of 0.05 mM vanillin to ethyl acetoacetate (0.1 M TBAN) with electrode conditioning at +2 V for 4 s and ultrasound at 200 W cm^{-2} for 30 s prior to each scan. Inset – Standard addition calibration ($R^2 = 0.9987$)

8.3.3 Detection of Vanillin in Natural Vanilla Extract

The determination of vanillin in natural vanilla essence was achieved by the exploitation of biphasic sonoelectrochemistry in ethyl acetoacetate and aqueous ethanolic vanilla pod extract. The droplet size and distribution was investigated using a Leica DMLM microscope (Leica Microsystems AG, Weztlar, Germany) with an Olympus objective lens (ULWD MSPlan 50, OLYMPUS Optical Co. GmbH, Hamburg, Germany) with 0.55 numerical aperture. A biphasic mixture of 400 μL water in 25 mL ethyl acetoacetate was insonated at 200 W cm^{-2} for 60 s. Immediately following insonation 25 μL of the resulting emulsion was placed on a clean glass microscope slide beneath a glass cover slip. Digital photographic images (see **Figure 8**) of the resulting droplets were recorded using a YC-05 video camera (Leica Microsystems AG, Weztlar, Germany) connected to a PC. The images were calibrated against a stage micrometer of 1-100 μm with 20 μm divisions (L4202, Agar Scientific Ltd,

Stanstead, UK). Statistics formulated using a set of four images analogous to that shown in **Figure 8** yielded a mean diameter of water droplets dispersed in ethyl acetoacetate of $1.6 \pm 0.5 \mu\text{m}$.

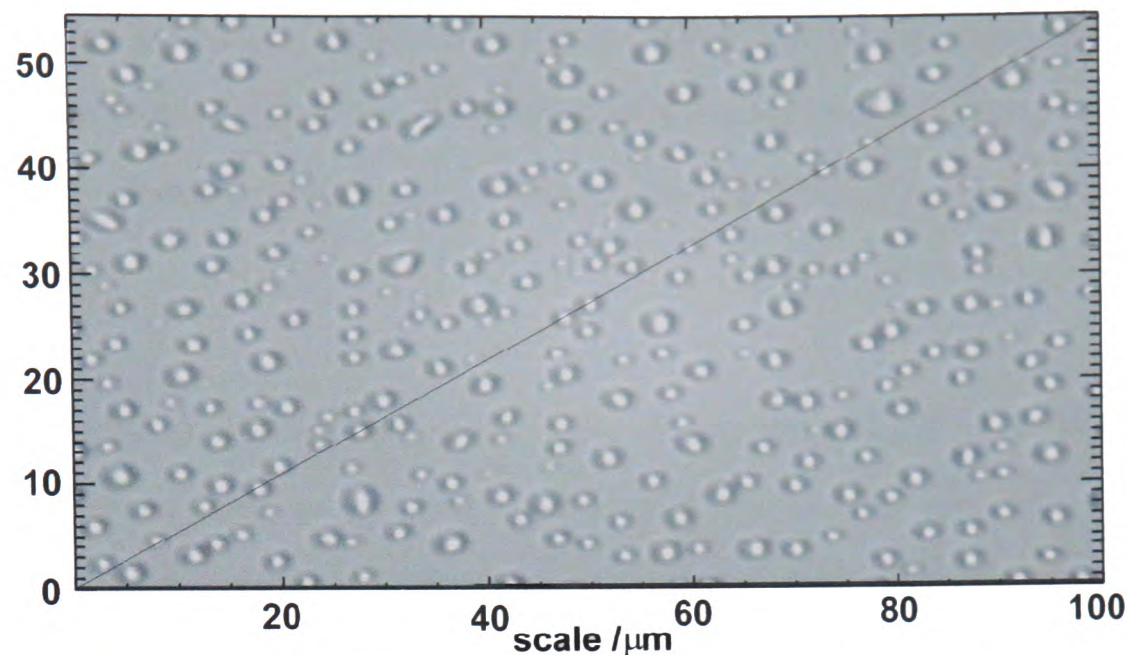


Figure 8 – Microscope photograph of an acoustic emulsion resulting from insonation for 60 s at 200 W cm^{-2} in a biphasic mixture of 400 μL water in 25 mL ethyl acetoacetate.

Square wave voltammetry ($\Delta E_p = 15 \text{ mV}$, $\Delta E_s = 1.5 \text{ mV}$, $f = 10 \text{ Hz}$) followed a 4 s electrode pretreatment at +2 V for a 400 μL addition of vanilla extract to a solution of ethyl acetoacetate (0.1 M TBAN). Under silent conditions no signal was observed for vanillin.

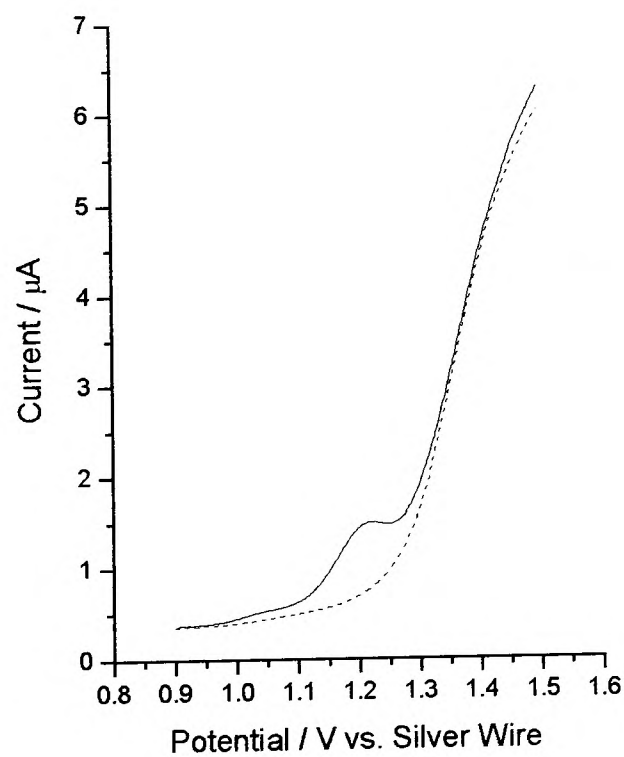


Figure 9 - Square wave voltammetry ($a = 15 \text{ mV}$, $\delta V = 1.5 \text{ mV}$ and $f = 10 \text{ Hz}$) at a 3 mm glassy carbon electrode with electrode conditioning at +2 V for 4 s prior to each scan, for an addition of 400 μL vanilla essence to ethyl acetoacetate (0.1 M TBAN).

- Voltammetry in silent conditions
- Voltammetry following insonation for 1 minute at 200 W cm^{-2} and 10 mm horn to electrode separation

Figure 9 contrasts the result for silent voltammetry with square wave voltammetry following insonation at 200 W cm^{-2} for 60 s. In the former case no analytical signal was observed for vanillin, however in the latter, a strong analytical signal was evident. Furthermore the voltammetric signal in the biphasic mixture was enhanced above that obtained for a monophasic aqueous ethanolic system. It can therefore be concluded that ultrasound facilitates both emulsification and extraction in biphasic sonoelectroanalysis.

Passivation between scans was manifest, as in s.8.3.2 above, suggesting a methodology which initially employed ultrasound to form an emulsion and extract the vanillin and subsequently introduced ultrasound between each scan to maintain the emulsion and depassivate the electrode surface. The optimum volume of vanilla essence to add to the cell was based on the effectiveness of ultrasonic conditioning of the electrode in maintaining the analytical response. If the quantity of vanilla extract became too high, passivation occurred even in the presence of ultrasound. In the present study a volume of 250 μL in 25 mL ethyl acetoacetate and 0.1 M TBAN was found to be optimal.

Microadditions of 0.05 mM vanillin to two separate samples of vanilla essence in ethyl acetoacetate gave square wave voltammetric responses which were background corrected using the same method as described above for pure vanillin in order to achieve an accurate comparison of peak heights. Microaddition calibration plots for sample A and sample B showed good linearity with $R^2_A = 0.9960$ and $R^2_B = 0.9948$ and gave a quantity of vanillin in sample A of $9.09 \pm 0.21\text{ mM}$ and $9.24 \pm 0.23\text{ mM}$ in sample B. The limit of detection in this media was found to be 0.020 mM ($n = 4$). This was compared with a blind analysis of the samples using HPLC-UV (performed by Clare Paterson, Glaxo Wellcome Analytical Technologies Group) which provided a value for sample A of 9.17 mM and 9.30 mM in sample B. The results are compared in table 2. Both methods are seen to be in close agreement supporting the validity of sonoelectroanalysis to this biphasic system.

	Vanillin Content / mM	
	sono-SWV	HPLC – UV method
Vanilla Extract A	9.09 ± 0.21	9.17
Vanilla Extract B	9.24 ± 0.23	9.30

Table 2 – Results of a quantitative determination of vanillin content in two samples of vanilla essence, sono-SWASV is compared against HPLC - UV

8.4 Conclusions

Sono-square wave voltammetry is effectively employed in the analysis of vanillin in the novel electrochemical solvent ethyl acetoacetate. In a monophasic system it is well known that ultrasound facilitates such analyses by cavitation depassivation and activation of the surface of the electrode. In addition, however, ultrasonic emulsification also permits voltammetric measurement in the biphasic system of aqueous ethanol and ethyl acetoacetate, precluded in a silent system. This is complemented by the ultrasonic extraction of vanillin into ethyl acetoacetate enhancing the analytical signal obtained to useful and quantitative levels.

It has been demonstrated that in an ultrasound enhanced electroanalytical system it is possible to determine the concentration of vanillin in a sample of vanilla food flavouring both accurately and rapidly with close agreement with an alternative technique, HPLC-UV. The need for sample degradation or a separation step, which would lengthen and complicate the analytical protocol, is removed leading to the conclusion that biphasic sonoelectroanalysis demonstrates an attractive alternative to currently accepted techniques.

8.5 Acknowledgements

Blind analysis of the vanilla extract samples was provided by Glaxo Wellcome Analytical Technologies Group, using an HP1090 Liquid Chromatograph (Agilent Technologies, Waldbrom, Germany) to perform HPLC analysis with UV detection at 254 nm. The HPLC column was a 100 x 2.1 mm Hypersil (Thermoquest, UK) HyPurity Elite C18 3 μ m.

I would also like to thank members of the Colin Bain group (Physical and Theoretical Chemistry Laboratory, University of Oxford) for facilitating the use of their Leica microscope equipment and their assistance in obtaining the image shown in Figure 8.

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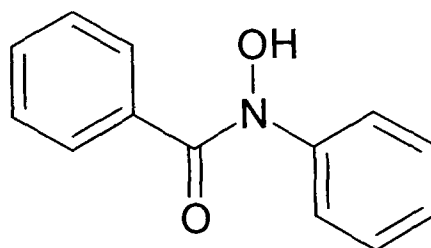
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Chapter 9 -The Electroanalytical Detection and Determination of Copper in Heavily Passivating Media

Ultrasonically Enhanced Solvent Extraction by N-Benzoyl-N-Phenyl-Hydroxylamine in Ethyl Acetate
Coupled with Electrochemical Detection by Sono-Square Wave Stripping Analysis

9.1 Introduction

Solvent extraction has been used extensively on an analytical scale to prepare the substrate for techniques such as atomic absorption spectroscopy¹, spectrophotometry^{2,3} and voltammetry⁴. However the main disadvantage is the time consuming stirring and subsequent separation of the aqueous and organic phases. Potential for the resurgence of the technique when coupled with sensitive voltammetric detection was recognized by Bond et al in a recent paper⁵, which employs the solvent extraction technique in the analysis of biological and river water samples. The method utilizes the extraction of metal-N-benzoyl-N-phenyl-hydroxylamine (metal-ligand) complexes (see Structure 1) into ethyl acetate followed by direct determination using differential pulse polarography in the organic medium.



Structure 1 - N-benzoyl-N-phenyl-hydroxylamine

The work presented in this Chapter demonstrates a significant extension of the solvent extraction technique, which is enhanced by the synergistic use of ultrasound in both the extraction and analytical steps. The benefits of the technique over that presented by Bond et al are rapid room temperature solvent extraction (Bond et al require 40 °C) by sono-emulsification directly from the aqueous phase into the organic phase followed by re-stripping into a clean aqueous phase which is more favourable for quantitative analysis using voltammetric methods. The technique necessarily removes contaminants present in the test solution since these will prefer to remain in the initial aqueous phase, or will transfer to the organic phase but are unlikely to be doubly transferred into the “clean” final aqueous phase.

9.2 Experimental

Ultrasonic Horn

The ultrasonic horn was a Model CV 26 (Sonics and Materials Inc., Connecticut, USA) located at a horn to electrode separation of 5 mm. The ultrasonic intensity was calibrated calorimetrically according to the procedure given in s.2.3 over the range 40-200 Wcm⁻². Unless otherwise stated the intensity used was 140 ± 5 Wcm⁻².

Solvent Extraction

The solvent extraction procedure involved two sonication steps followed by electrochemical detection of extracted copper ions. A schematic diagram of the process is shown in **Figure 1**.

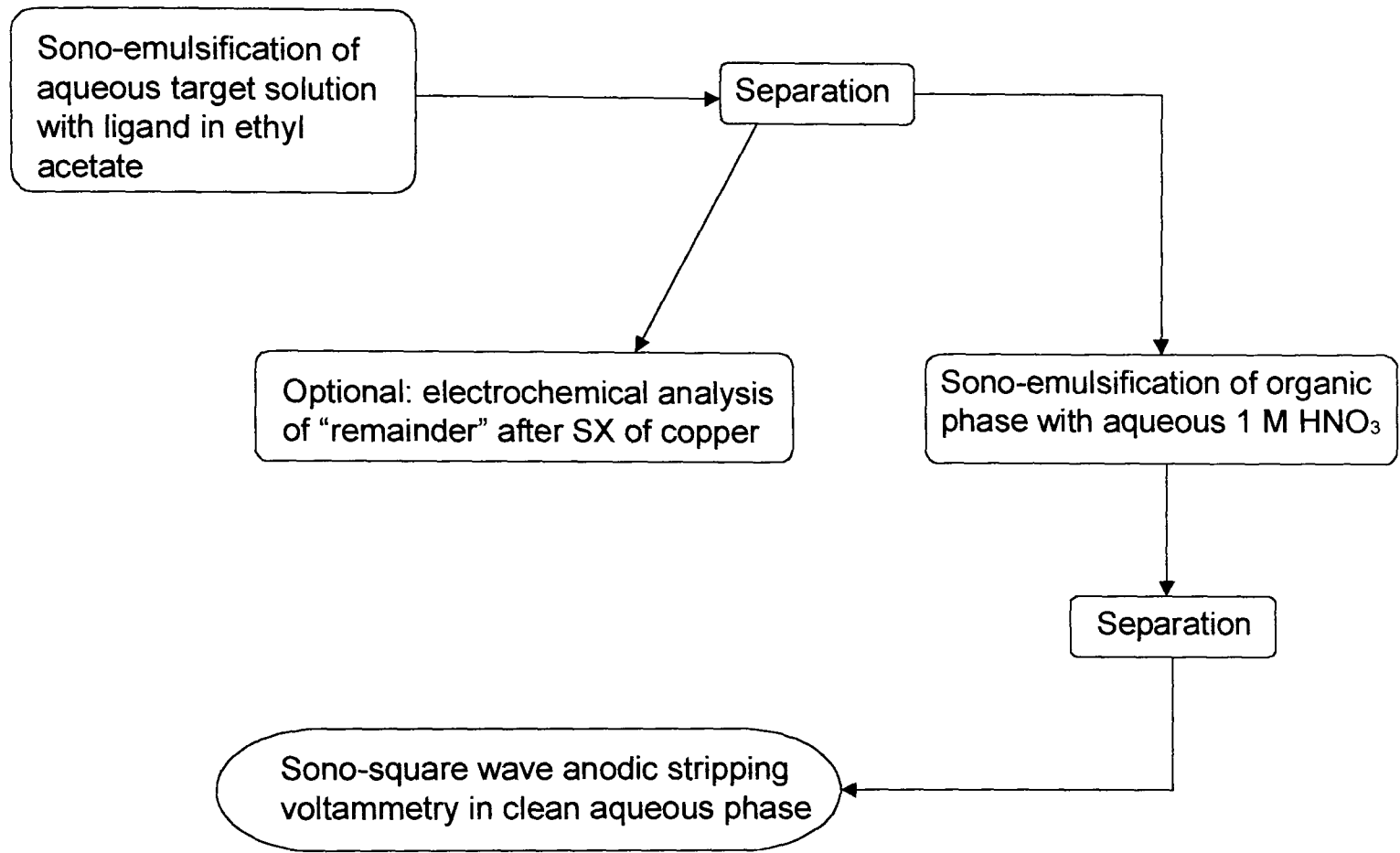


Figure 1 - The process of solvent extraction and electrochemical detection

The first step involves a 4-minute sono-emulsification of 10 mL of the target solution with 10 mL N-benzoyl-N-phenyl-hydroxylamine solution dissolved in ethyl acetate. This stage extracts the copper contained within the target aqueous phase into the organic phase which can then be separated from the aqueous phase. The organic phase containing the copper ligand complex is then sono-emulsified for a further 4 minutes with 10 mL 1 M HNO₃ which "back extracts" or "re-strips" the copper into the aqueous phase.

The emulsification cell, shown in **Figure 2a**, was thermostatted using a glass cooling coil inserted into the cell through which water circulated from a bath at 25 °C. By dividing the 4 minute sonication period into four 1 minute periods with 30 sec between each sonication step, the cell temperature could be maintained at 25 ± 2 °C. This reduced loss of solvent through evaporation. The optimum size of the cell was determined by the overall volume of solution to be insonated, 20 mL and a preference for the step of the microtip to be immersed in the solution as shown in **Figure 2b**. This assists the flow of the two phases in opposing directions enhancing the speed of emulsification.

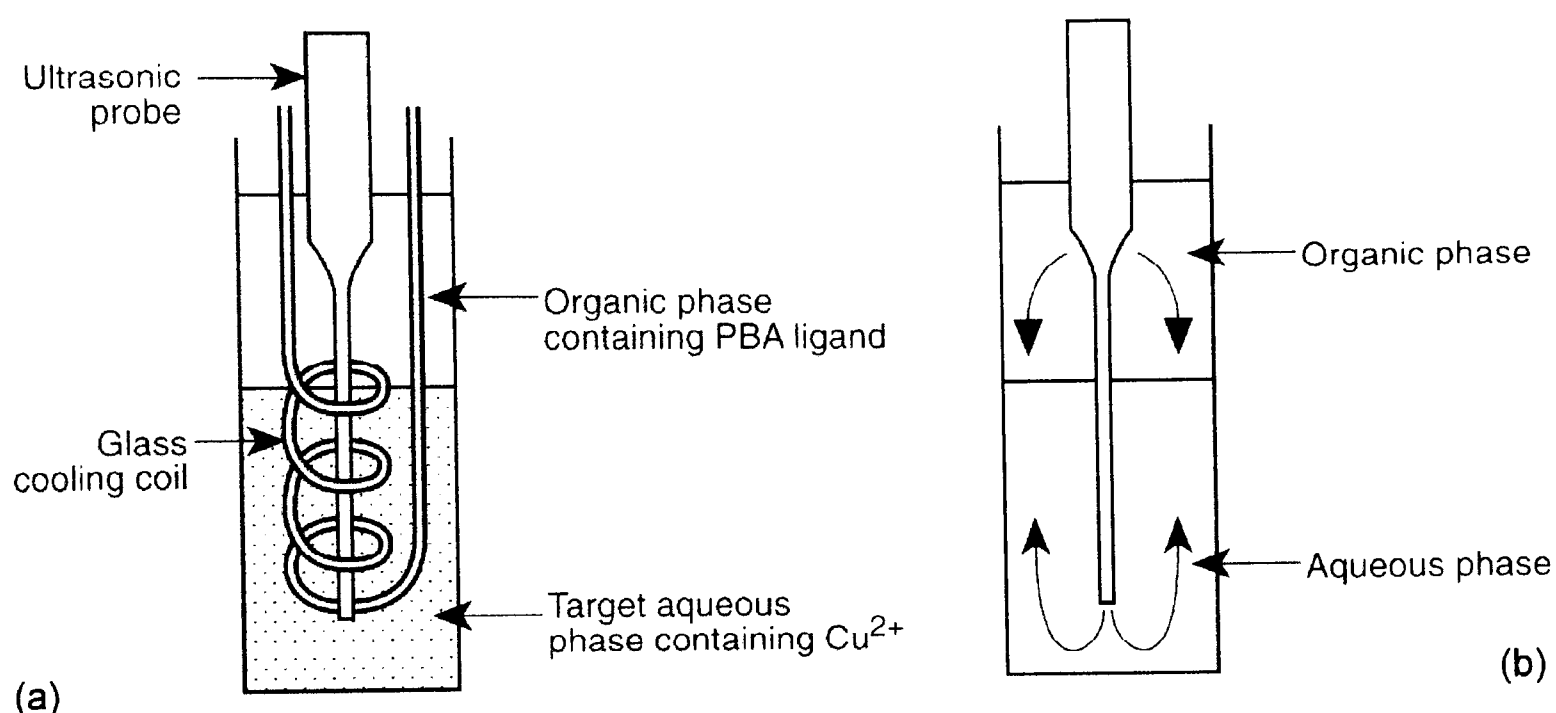


Figure 2 - a) The sono-emulsification cell; b) Schematic diagram illustrating the horn immersed beyond the shoulder of the stepped tip

Electrochemical Detection

The electrochemical cell used throughout is described in s.2.1 Figure 1. Experiments were conducted using a bare 3 mm diameter glassy carbon disc electrode (BAS, Indiana, US). The electrode was polished according to the procedure given in s.2.5. All potentials are quoted with respect to a saturated calomel electrode, SCE (Radiometer, Copenhagen). The counter electrode employed in the three-electrode system was a 20 cm coiled platinum wire which was flame annealed prior to each experiment. Sono-SWASV involved an insonated deposition step of 2 minutes at -1.5 V vs. SCE followed by an analytical square wave stripping step, at the optimized parameters of $f = 10$ Hz, $\Delta E_s = 1.5$ mV and $\Delta E_p = 15$ mV, under silent conditions over a potential range of -0.3 V to 0.3 V vs. SCE.

UV/visible Spectroscopy

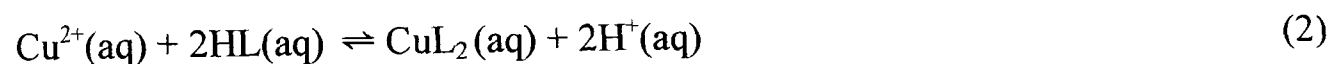
All spectroscopic measurements were made using a Unicam UV2-100 UV/visible spectrometer (Unicam, Cambridge, UK) over the range 190 nm to 900 nm. The sample was contained within a standard quartz cell of 3 mL volume and 1 cm path length.

Reagents

Reagents used were all of electrochemical grade or the highest available purity see appendix 2 for full details. The water used throughout had been treated using a UHQ Elgastat (Elga, UK) and was of resistivity not less than 18 MΩcm.

9.3 Theory

The following solvent extraction scheme is proposed in which the equilibria are facilitated by acoustic emulsification:



where L is the ligand, N-benzoyl-N-phenyl-hydroxylamine. The protonation state of the ligand at each of the stages above is assumed from its reported⁶ pK_a of 8.15.

A numerical model of the system can be constructed based on three parameters where L corresponds to the ligand and the subscripts A and O correspond to the aqueous and organic phases respectively.

First

$$Q = \frac{[\text{HL}]_A}{[\text{HL}]_O} \quad (4)$$

where Q is the partition coefficient corresponding to the equilibrium of ligand in the aqueous and organic phases given in equation 1, second

$$K = \frac{[\text{CuL}_2]_A}{[\text{Cu}]_A [\text{L}]_A^2} \quad (5)$$

where K is the equilibrium constant corresponding to the formation of the complex in the aqueous phase as shown in equation 2, and third

$$P = \frac{[CuL_2]_A}{[CuL_2]_O} \quad (6)$$

where P is the equilibrium constant corresponding to equilibrium of the complex in the aqueous and organic phases given in equation 3. Equations 7 and 8 relate the known total amounts of copper and ligand to the distribution of species between the phases of the system:

$$[Cu]_{tot} = [CuL_2]_A + [CuL_2]_O + [Cu]_A \quad (7)$$

$$[HL]_{tot} = 2[CuL_2]_A + [HL]_A + 2[CuL_2]_O + [HL]_O \quad (8)$$

Substitution of these relations into the equation for the equilibrium constant K (5) by incorporating the partition coefficients P and Q permits the following relation to be derived:

$$K = \frac{P[CuL_2]_O}{([Cu]_{tot} - (P+1)[CuL_2]_O) \left(\left(\frac{Q}{Q+1} \right) ([HL]_{tot} - 2(P+1)[CuL_2]_O) \right)^2} \quad (9)$$

which, on rearrangement, provides an equation from which the theoretical value of $[CuL_2]_O$, the concentration of copper obtained by back extraction from the complex in the organic phase, can be determined:

$$0 = P[CuL_2]_O - K \left[([Cu]_{tot} - (P+1)[CuL_2]_O) \left(\left(\frac{Q}{Q+1} \right) ([HL]_{tot} - 2(P+1)[CuL_2]_O) \right)^2 \right] \quad (10)$$

In the above relation the values of $[Cu]_{tot}$ and $[HL]_{tot}$ are known experimental parameters. Initially unknown are the partition coefficients P and Q and the equilibrium constant K . Realistic values of P , Q and K were chosen in **Figure 3** to show the variation in total copper extracted, $[CuL_2]_O$, for a fixed $[Cu]_{tot}$ of 5 μ M and varying total ligand, $[L]_{tot}$. It is clear that there is a maximum value of $[CuL_2]_O$ and hence a maximum percentage recovery which tends to a limit for all ligand concentrations above a certain excess of $[Cu]_{tot}$.

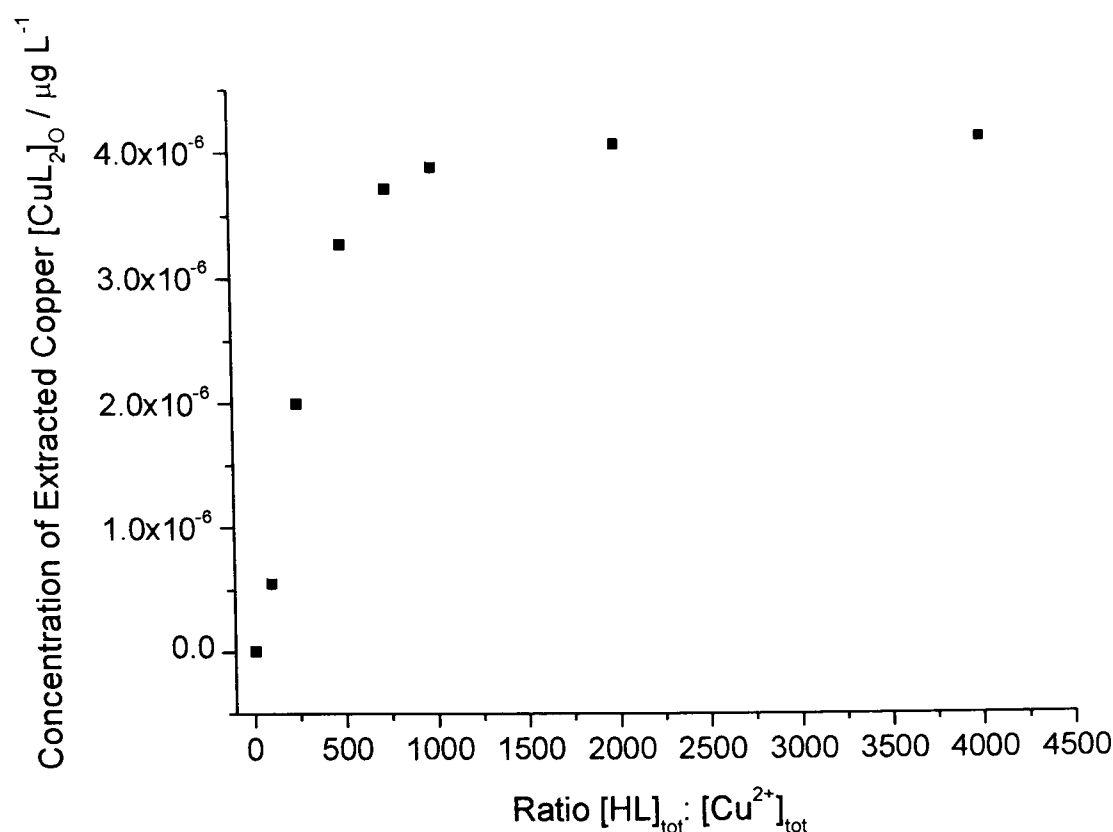


Figure 3 - Theoretical variation in total copper extracted $[\text{CuL}_2]_O$ for a fixed concentration $[\text{Cu}]_{\text{tot}}$ of 5 μM and varying total ligand $[\text{HL}]_{\text{tot}}$ with $P = 0.2$, $Q = 0.01$, $K = 1 \times 10^9 \text{ mol}^{-2}\text{L}^2$

We next turn to the evaluation of P , Q and K for the system of interest.

9.4 Results and Discussion

9.4.1 Measurement of P , Q , and K

In order to determine the partition coefficient Q for the ligand between the aqueous and organic phase by UV/visible spectroscopy it was initially determined that N-benzoyl-N-phenyl-hydroxylamine exhibited an absorbance maximum at 271 nm. This is consistent with literature value for the similar species N-phenyl-benzamide which is reported to have an absorption maximum at 266 nm⁷. Absorbance maxima were obtained over a concentration range of 50-200 μM . **Figure 4** shows the linear increase in absorbance maxima with concentration leading to a calculation of ϵ , the molar extinction coefficient from Beer's Law and Lambert's Law, equation 11.

$$\log_{10} \frac{I_0}{I} = \epsilon \cdot l \cdot c \quad (11)$$

where I_0 and I are the intensities of the incident and transmitted light respectively, l is the path length of the absorbing solution in centimeters and c is the concentration in mol L^{-1} . $\log_{10}(I_0/I)$ is called the absorbance or optical density. This permits the calculation of $\epsilon = 9.0 \times 10^3 \text{ cm}^{-1} \text{ M}^{-1}$, from the gradient of the graph shown in **Figure 4**.

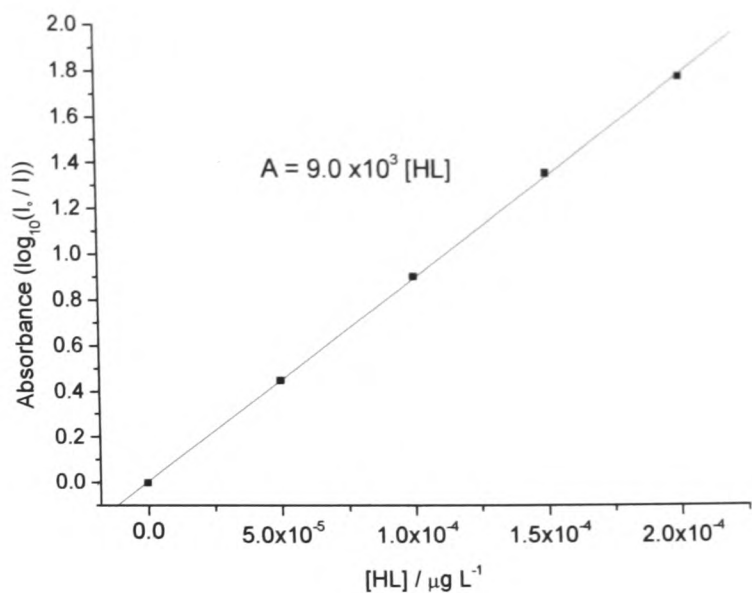


Figure 4 - Beer-Lambert absorbance versus concentration plot for the calculation of the molar extinction coefficient of N-benzoyl-N-phenyl-hydroxylamine

In order to determine Q it was necessary to obtain a value for the concentrations of the ligand both the organic phase and aqueous phase following a four minute insonation period to emulsify the phases as for the extraction procedure. A solution of 0.05 M N-benzoyl-N-phenyl-hydroxylamine in ethyl acetate was emulsified with an equal volume of water and the ligand present in the water on separation was “back-extracted” by sono- emulsification with pure ethyl acetate. An aliquot of this solution of unknown concentration was added to a known amount of ethyl acetate and its UV/visible absorbance at 271 nm was recorded for a range of aliquot volumes. Table 1 shows the values of the absorbance maxima for a given aliquot size to 10 mL ethyl acetate. These values increase linearly with aliquot size with $R^2 = 0.9997$. Extrapolation to a theoretical aliquot size of 10 mL gives a value for the absorbance of the ligand “back extracted” from the water which permits calculation of the corresponding concentration using equation 11. The concentration, c, was found to be $7.0 \times 10^{-4}M$. The partition coefficient $Q = 1.40 \times 10^{-2}$ (25 °C) was calculated via equation 4.

Aliquot Size /μL	UV/visible absorbance at 271 nm
100	0.12
500	0.378
1000	0.675
1500	0.996
10000 (extrapolated)	6.281

Table 1 – Values of the absorbance maxima for a solution comprising an aliquot of N-benzoyl-N-phenyl-hydroxylamine back-extracted from water and made up to 10 mL with pure ethyl acetate. Extrapolation to a theoretical aliquot size of 10 mL gives a value for the absorbance of the ligand back extracted from water from which the concentration can be calculated.

For the determination of P using the method outlined above for Q a sample of CuL_2 was prepared by precipitation of the green solid CuL_2 in water by addition of a solution of 0.75 mM Cu^{2+} to a 1.5 mM solution of N-benzoyl-N-phenyl-hydroxylamine. The quantity of the complex which can be precipitated in a sensible volume of solution, for example 1 L, is limited by the low solubility of the ligand in water (0.04 g per 100 mL at 25 °C⁸). Once precipitation had begun the solution was cooled and stirred until precipitation had ceased. The solution was then filtered and the solid washed with a small amount of ethyl acetate and water to ensure it was free from contamination by excess ligand and Cu^{2+} before being dried thoroughly in an oven at 200 °C overnight. UV/visible spectroscopy of a 100 μM solution of CuL_2 in ethyl acetate yielded an absorbance maximum at 304 nm. Absorbance maxima were obtained over a concentration range of 20-110 μM as in the determination of Q above.

Figure 5 shows the linear increase in absorbance maxima with concentration leading to a calculation of ϵ , the extinction coefficient of $2.0 \times 10^4 \text{ cm}^{-1} \text{ M}^{-1}$ from the gradient of the graph by equation 11. This gave a value of $P = 0.351$ using equation 6.

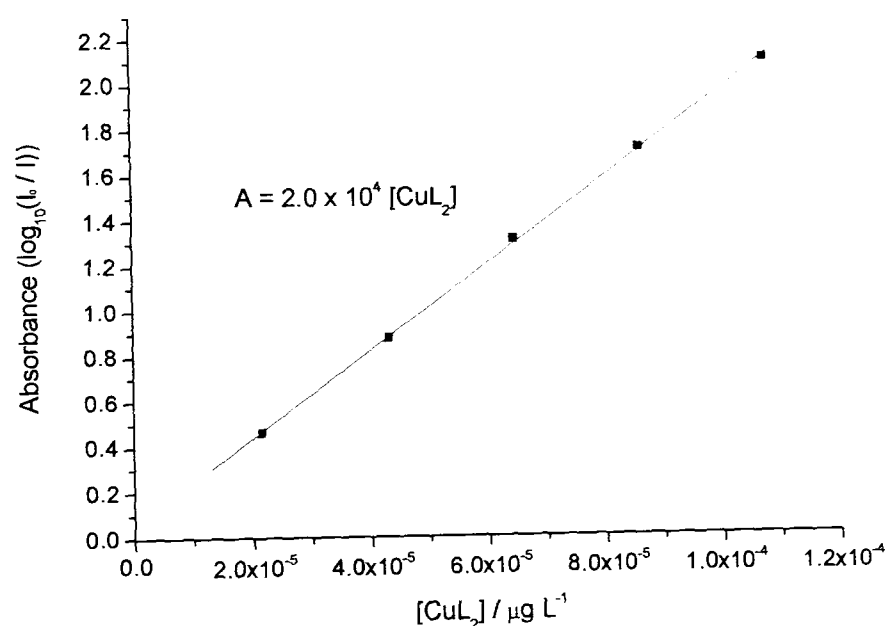


Figure 5 - Beer-Lambert absorbance versus concentration plot for the calculation of the molar extinction coefficient of copper-N-benzoyl-N-phenyl-hydroxylamine complex

An attempt to calculate K was made using Job's Method of continuous variation^{9,10}. This method can, under favourable circumstances, be used to determine the stability constant for a complex from the measured concentrations and absorbance, provided that the extinction coefficient and the formula of the complex are known. The spectra of the two species are recorded over a range of concentrations each solution containing the same total reagent concentration. As the concentration of one of the reactants for example Cu^{2+} increases from zero so does the amount of complex, hence the absorbance of the peak corresponding to the complex increases. Absorbance reaches a maximum in solution in

which Cu^{2+} and ligand are in the same proportions as in the complex, since this solution will contain the highest concentration of complex. Further addition of Cu^{2+} give solutions containing insufficient ligand to complex with all the metal, so absorbance due to the complex then falls. A plot of absorbance as a function of the amount of added ligand should give two straight lines which intersect where Cu^{2+} and ligand are in the correct proportion to give maximum complex formation. K can then be calculated using the following relation:

$$K = \frac{M / \epsilon l}{[H - M / \epsilon l]^3} \quad (12)$$

where ϵ is the molar extinction coefficient of CuL_2 , M is the height of the curve at the absorbance maximum corresponding to the actual amount of equilibrated complex and H is the height of the apex at this composition which corresponds to a solution with 100 % complex formation. The success of Job's method depends on the extent to which Beer's law is followed. In the present case the method was complicated experimentally by the precipitation of CuL_2 in the aqueous phase and also the low solubility of the ligand in this medium. Calculations based on data for high copper concentration where interference from the precipitation of complex was minimized led to a value of K of approximately $2 \times 10^9 \text{ mol}^{-2} \text{ L}^2$.

9.4.2 Extraction, Restripping and Detection of Copper

A series of solvent-extraction and re-strip experiments were carried out using 10 mL aqueous phase of fixed $[\text{Cu}]_{\text{tot}}$, 5.4 μM , and 10 mL ethyl acetate containing a known but varying $[\text{HL}]_{\text{tot}}$, between 0.07 mM and 3.4 mM. The effects of changing the pH of the aqueous phase on the extraction was investigated by Bond *et al*⁵ and it was found that the concentration of solvent extracted copper measured by differential pulse polarography remained constant over the pH ranges 4.5-10.5. Consequently pH 5 was chosen for the aqueous phase and all solutions were prepared accordingly with dilute nitric acid. The solvent extraction technique described above was employed over the range of $[\text{HL}]_{\text{tot}}$. For each value of $[\text{HL}]_{\text{tot}}$, copper concentration was determined by sono-SWASV in the “back-extracted” final aqueous phase and in the “remainder” of the original aqueous solution as illustrated by the schematic diagram, **Figure 1**. This ensured that all copper was accounted for and none remained in the organic phase undetermined.

Sono-SWASV required 1 mL of test solution to be added to the electrochemical cell followed by 19 mL 0.1 M HNO_3 . The dilution reflects the very high sensitivity of sono-SWASV in clean solution. It was also necessary to perform the electroanalytical step at pH 1 to ensure that the only redox process occurring at the electrode is:



since at higher pH reduction to, or oxidation of Cu (I) complicates analysis¹¹.

Sono-SWASV involved an insonated preconcentration step of 2 minutes at -1.5 V vs. SCE followed by an analytical square wave stripping step, at the optimized parameters of $f = 10$ Hz, $\Delta E_s = 1.5$ mV and $\Delta E_p = 15$ mV, under silent conditions over a potential range of -0.3 V to 0.3 V vs. SCE. Total copper content was determined using the standard addition technique with additions of $10 \mu\text{L}$ from a stock solution of 0.3 mM Cu^{2+} . Examples of standard additions to the “back extracted” copper solution and the “remainder” from the original test solution can be seen in **Figure 6** for extraction of $5.38 \mu\text{M}$ copper using $68.5 \mu\text{M}$ N-benzoyl-N-phenyl-hydroxylamine, a ratio of approximately 1:13.

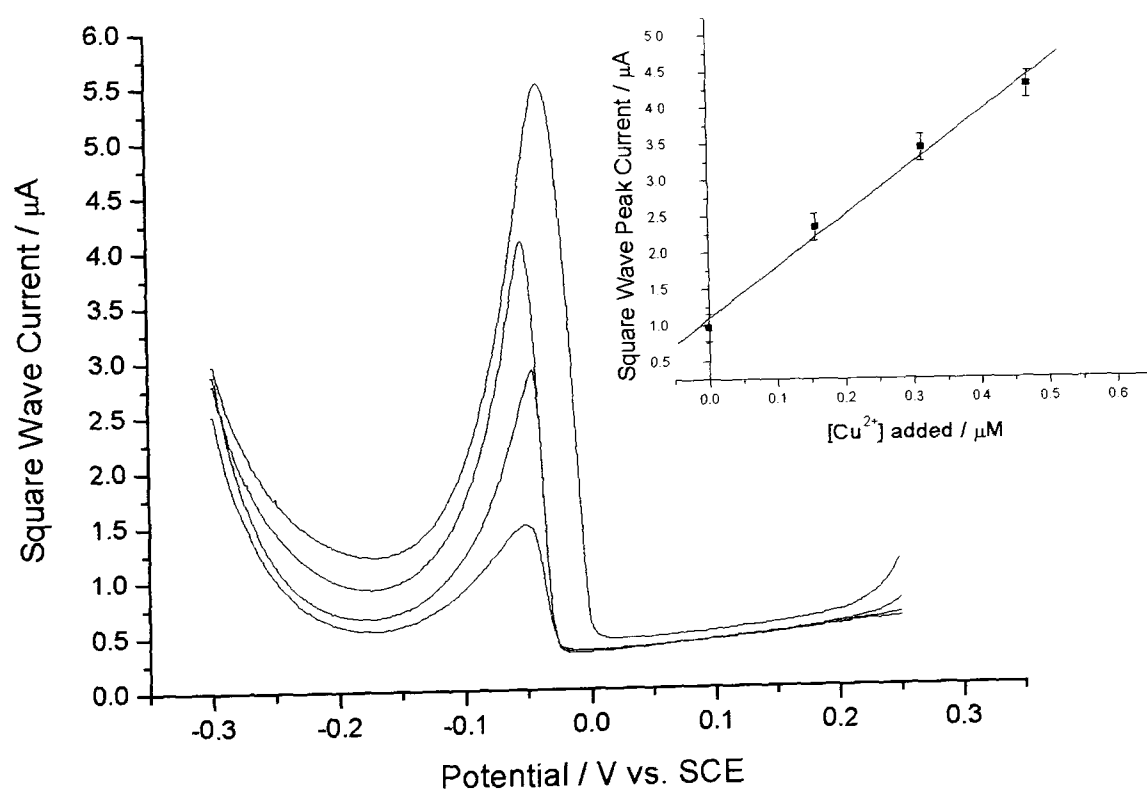


Figure 6a - Standard additions to the “remainder” following the sono-solvent extraction procedure of $5.38 \mu\text{M}$ copper using $68.5 \mu\text{M}$ N-benzoyl-N-phenyl-hydroxylamine, a ratio of approximately 1:13. Calibration data ($R^2=0.9889$) yielded a value of $5.15 \mu\text{M Cu}^{2+}$ remaining in the starting solution

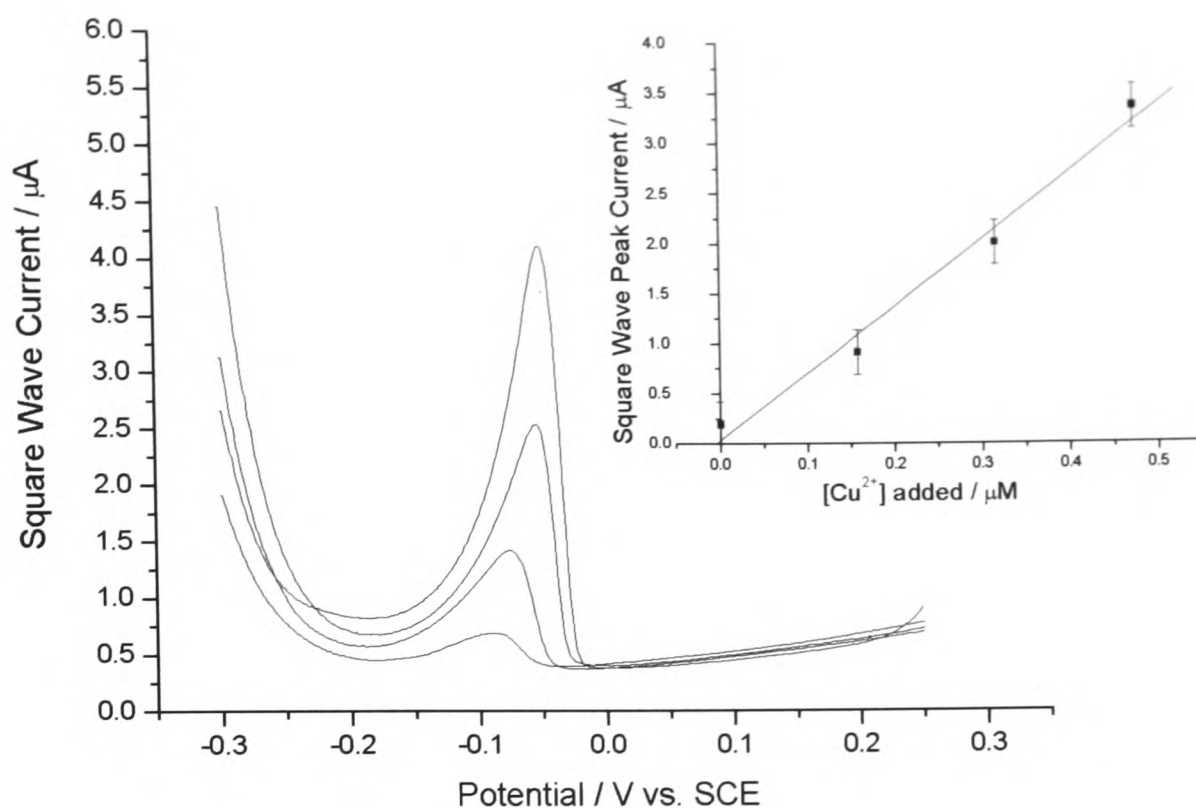


Figure 6b - Micro-additions to the final re-stripped solution following sono-solvent extraction of $5.38 \mu\text{M}$ Cu^{2+} with $68.5 \mu\text{M}$ N-benzoyl-N-phenyl-hydroxylamine. Calibration data ($R^2 = 0.9824$) yielded a value of $0.17 \mu\text{M}$ Cu^{2+} transferred by the extraction procedure into the final test solution.

It is evident that $5.15 \mu\text{M}$ Cu^{2+} remained in the starting solution (**Figure 6a**) and only $0.17 \mu\text{M}$ Cu^{2+} was transferred by the extraction procedure into the final test solution (**Figure 6b**). This can be contrasted with a Cu:ligand ratio of 1:637, where $4.07 \mu\text{M}$ Cu^{2+} was determined in the extracted solution (standard addition calibration data $R^2=0.9886$).

Optimisation of the value of K permitted the theoretical data to be fitted to the experimental data. **Figure 7** shows the variation in percentage of copper extracted with increasing concentration of ligand for the experimental data and theoretical predictions. Close agreement gave a final value for K of $2.6 \times 10^9 \text{ mol}^{-2} \text{L}^2$ which is consistent with the approximated value obtained using Job's method.

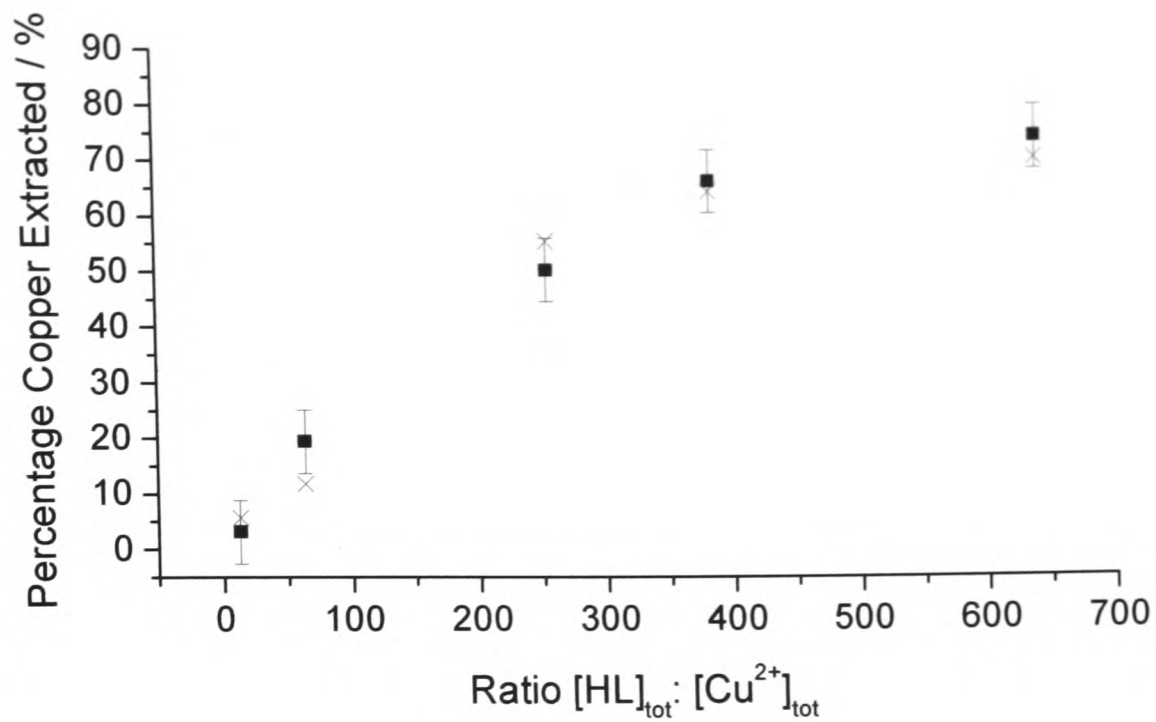


Figure 7 - Variation in percentage of copper extracted with increasing concentration of N-benzoyl-N-phenyl-hydroxylamine for the experimental data and theoretical predictions.

- Experimental Data
- × Theoretical Prediction

It is clear that the maximum percentage of copper which can be extracted using this method is 73 % for ligand concentration in 1000-fold excess of the copper concentration. This changes only very slowly with ligand concentration as illustrated by a value of 74 % copper extracted for a 10,000-fold excess of ligand. In the following Chapter the applicability of this method to the analysis of real samples is investigated.

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Chapter 10 – Application of Sono-Solvent Double Extraction to the Analysis of Real Samples

10.1 Determination of Copper in Ribena

10.1.1 Introduction

In this section the technique described in Chapter 9 is used to determine the concentration of copper in a sample of Ribena® Light. The soft drink contains organic species such as sugars and natural flavourings and colourants and hence is difficult to analyse using classical electrochemical analysis. The use of ultrasound in the analysis of such passivating media has improved the limits of detection with active depassivation of the electrode surface by the phenomenon of acoustic cavitation. However ultrasound is unable to effect quantitative depassivation of the electrode in a sample of untreated acidified Ribena®. The present methodology presents an alternative to direct analysis by producing a “clean”, doubly transferred, sample with few contaminant organic molecules.

10.1.2 Results and Discussion

Analysis of the Ribena® samples was achieved by sono-extraction of copper from a neat solution of Ribena® Light direct from a drink carton and adjusted to pH 4.75. A 2.5 mM solution of ligand in ethyl acetate was used for the extraction which is assumed to be in a greater than 1000-fold excess than the copper content of Ribena® given regulations imposed by the UK government¹. It is interesting to note that following the extraction procedure the purple colouration of the drink remained in the initial aqueous phase and was not transferred via the organic phase into the final “stripped” aqueous phase. This suggests the anticipated advantages of the “double extraction” procedure where contaminants necessarily remain in the initial aqueous phase or the organic phase but are not transferred to the final clean phase.

The resulting solution was analysed using the sono-SWASV procedure detailed in s.9.4.2. The glassy carbon working electrode was insonated during the preconcentration step of sono-SWASV, with cavitation facilitating the removal of any residual ethyl acetate or N-benzoyl-N-phenylhydroxylamine from the surface of the electrode whilst acoustic streaming enhanced the mass transport maximizing peak height and resulting in the correspondingly increased sensitivity and low detection limit. The true background current in the absence of “back-extracted” solution was

fitted to a 6th order polynomial using Origin 6.0 Software (Microcal, MA, USA) which was then used to facilitate the calculation of the background current for each addition of copper. Subtraction of the background signal gave approximately Gaussian shaped square wave profiles as theoretically predicted^{2,3}. 10 μL additions corresponding to an increase in concentration of 0.06 μM ($3.8 \mu\text{g L}^{-1}$) were plotted as shown in **Figure 1**. A linear standard addition calibration graph of $R^2=0.9951$ **Figure 1 (inset)** was obtained.

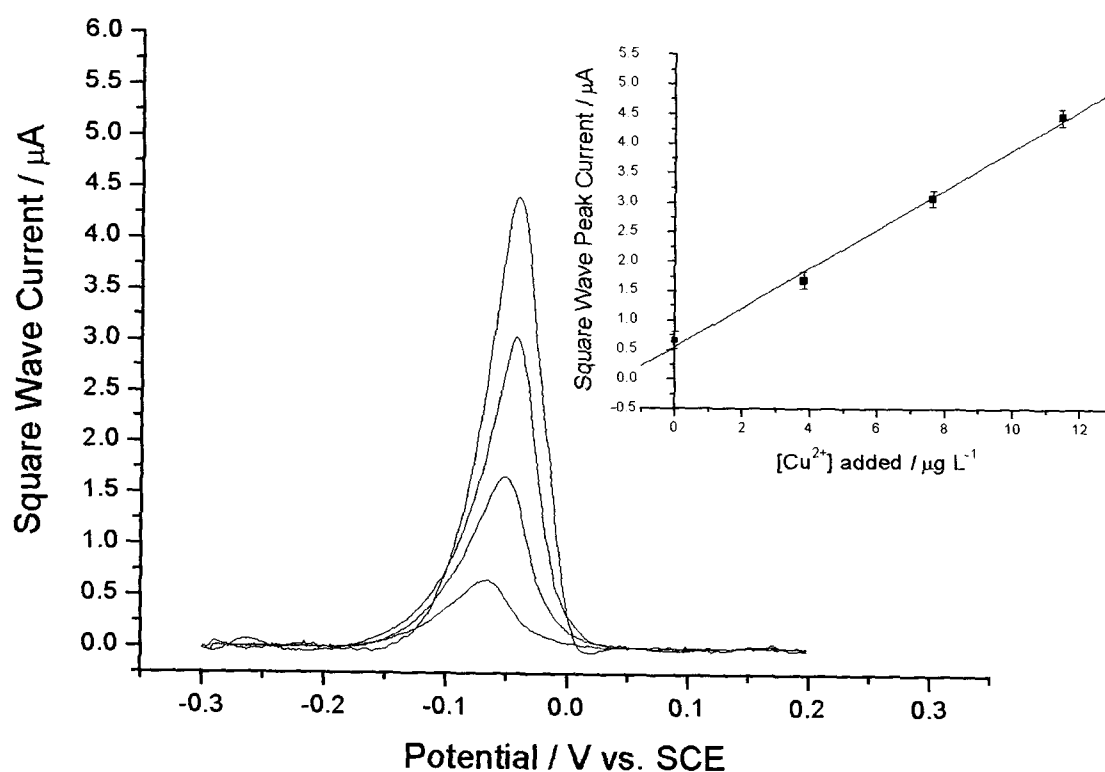


Figure 1 – Background corrected square wave voltammetric scans ($f = 10 \text{ Hz}$, $\Delta E_s = 1.5 \text{ mV}$ and $\Delta E_p = 15 \text{ mV}$) following an insonated preconcentration for 2 minutes at -1.5 V vs. SCE for standard additions to sono-solvent extracted Ribena[®] solution (1 mL extract in 20 mL HNO_3). Each 10 μL addition corresponding to an increase in concentration of 0.06 μM ($3.8 \mu\text{g L}^{-1}$). Calibration graph shown inset ($R^2=0.9950$)

The limit of detection of $1.3 \mu\text{g L}^{-1}$ was calculated using equation 1⁴:

$$\text{LOD} = 3S_b/b \quad (1)$$

where S_b is the standard error of the y-residuals of the line of best fit and b is the gradient. This figure was corrected using the known maximum percentage of 74 % which can be extracted in the light of the numerical analysis presented in Figure 7 of Chapter 9, to give a value of $1.7 \mu\text{g L}^{-1}$. The standard addition data gave a total mean copper concentration in Ribena[®] of $45 \mu\text{g L}^{-1}$ ($n=3$, $\sigma = 1$). This value has again been corrected to take into account the maximum percentage of copper that can be extracted from the numerical modelling of the reaction.

In order to verify the quantitative analysis a known amount of copper was added to the Ribena[®] solution in order to obtain a concentration of $(\alpha + 100\mu\text{g L}^{-1})$ where α is the initial concentration. This solution was then treated via the solvent extraction procedure to produce a clean “back-extracted” solution that was analysed using sono-SWASV. **Figure 2** shows background corrected additions of $3.8\mu\text{g L}^{-1}$. A linear calibration graph of $R^2=0.9960$ **Figure 2 (inset)** was obtained giving a corrected value[‡] of $148\mu\text{g L}^{-1}$ ($n=3$ $\sigma = 3$) which shows close agreement with the known amount of copper added (see Table 2).

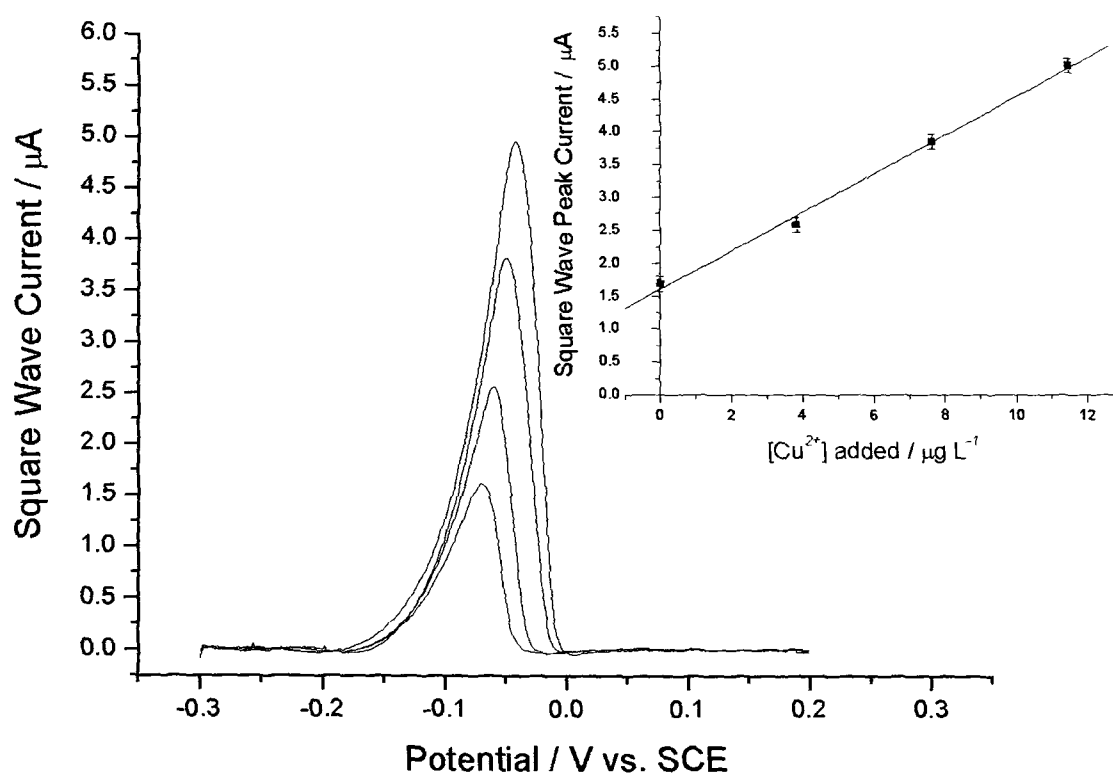


Figure 2 – Background corrected square wave voltammetric scans ($f=10\text{ Hz}$, $\Delta E_s = 1.5\text{ mV}$ and $\Delta E_p = 15\text{ mV}$) following an insonated preconcentration for 2 minutes at -1.5 V vs. SCE for standard additions to sono-solvent extracted Ribena[®] solution spiked with $100\mu\text{g L}^{-1}$ copper (1 mL extract in 20 mL HNO_3). Each $10\mu\text{L}$ addition corresponding to an increase in concentration of $0.06\mu\text{M}$ ($3.8\mu\text{g L}^{-1}$). Calibration graph shown inset ($R^2=0.9960$) giving concentration of $148\mu\text{g L}^{-1}$ ($n=3$ $\sigma = 3$)

	Neat Ribena [®] Light from carton ($\alpha\mu\text{g L}^{-1}$)	Spiked Ribena [®] Light ($\alpha + 100\mu\text{g L}^{-1}$)
Calculated concentration via solvent extraction ($\mu\text{g L}^{-1}$)	45	148
R^2	0.9951	0.9960
Standard deviation ($\mu\text{g L}^{-1}$)	1	3
Limit of Detection ($\mu\text{g L}^{-1}$)	1.7	1.6

Table 2 – Comparison of experimentally measured concentration of copper in neat Ribena[®] and Ribena[®] spiked with $100\mu\text{g L}^{-1}$ copper shown with their respective statistical parameters.

[‡] Since the solvent extraction technique is capable of transferring an approximate maximum of 74 % to the final aqueous phase all values calculated must be corrected accordingly, see s.9.4.2.

The validity of the technique is supported by the results from the spiked sample, since the value of α calculated in both cases agrees to within $3 \mu\text{g L}^{-1}$.

The necessity for ultrasound during the preconcentration step of the analytical SWASV may be questioned given that clean solution is produced for analysis. Similarly the necessity for a standard addition calibration instead of a single point determination should be justified. The principal factor which prevents this is likely to be the less than 100 % efficient separation of the aqueous and organic phases: a small amount of organic solvent remains in the clean stripped aqueous phase and this can hinder analysis in the absence of ultrasound which acts to depassivate the electrode surface. Furthermore, the concentration of target analyte is low. Acoustic streaming improves the mass transport of material to the electrode surface, enabling shorter preconcentration periods and increasing sensitivity.

10.2 Determination of Copper in Blood

10.2.1 Introduction

Following on from the proof of concept described above for Ribena®, this section turns to the determination of copper in blood. In chapter 4 sonoelectroanalysis was applied to the detection of copper bound within the human protein ceruloplasmin and in a sample of whole horse blood⁵. It was demonstrated that the enhancement of stripping peaks observed in ceruloplasmin and whole blood is not simply due to the well documented ultrasonic phenomena of increased mass transport and cavitation cleaning but also the liberation of copper from the sites in which it is bound, prior to preconcentration. Quantitative determination of copper in whole laked horse blood was achieved using the standard addition technique. The volume of blood required for the test in Chapter 4 is 1.5 mL which compares favourably with other electrochemical methods for analysis in blood⁶. However reduction of this volume is clearly desirable if the test is to become routine.

10.2.2 Results and Discussion

Analysis of the blood sample was achieved by sono-extraction of copper from a solution of 100 μL blood in 10 mL UHQ water. The measured pH of this solution was 7.36. This was deemed to be acceptable since the concentration of solvent extracted copper remains constant over the pH range⁷ 4.5-10.5 (see s.9.4.2).

A 0.05 M solution of ligand in ethyl acetate was used for the extraction, a concentration of approximately greater than 1000-fold excess than the copper content of the dilute blood solution given the likely range of copper in blood⁸. It was observed that the red coloration of the solution due to the red blood cells present was not transferred via the organic phase into the final “stripped” aqueous solution. As reported in s.10.1.2 for Ribena®, this provides visual evidence for the advantages of the “double extraction” procedure where contaminants necessarily remain in the initial aqueous phase or the organic phase but are not transferred into the final “clean” aqueous phase.

Sono-SWASV required 1 mL of the stripped solution to be added to the electrochemical cell followed by 19 mL 0.1 M HNO_3 . The dilution reflects the very high sensitivity of sono-SWASV in clean solution. It was also necessary to perform the electroanalytical step at pH 1 for reasons given elsewhere, see s.9.4.2, equation. 13. Sono-SWASV involved an insonated deposition step of 1 minute at -1.5 V vs. SCE followed by an analytical square wave stripping step, at the optimized parameters of $f = 25$ Hz, $\Delta E_s = 1.5$ mV and $\Delta E_p = 15$ mV, under silent conditions over a potential range of -0.3 V to 0.3 V vs. SCE. Total copper content was determined using the standard addition technique with additions of $10\mu\text{L}$ giving an increase in concentration of the test solution corresponding to $0.2\text{ }\mu\text{g L}^{-1}$.

The true background current in the absence of “back-extracted” solution was fitted to a 7th order polynomial using Origin 6.0 Software (Microcal, MA, USA) which was then used to facilitate the calculation of the background current for each addition of copper. Standard additions were plotted as shown in **Figure 3**. For this dataset a linear calibration graph of $R^2=0.9972$ **Figure 3 (inset)** was obtained. The theoretical limit of detection in the test medium of $0.12\text{ }\mu\text{g L}^{-1}$ was calculated using equation 1. This value was corrected using the known maximum percentage of 74 % which can be extracted in accordance with theoretical prediction, to give a value of $0.16\text{ }\mu\text{g L}^{-1}$.

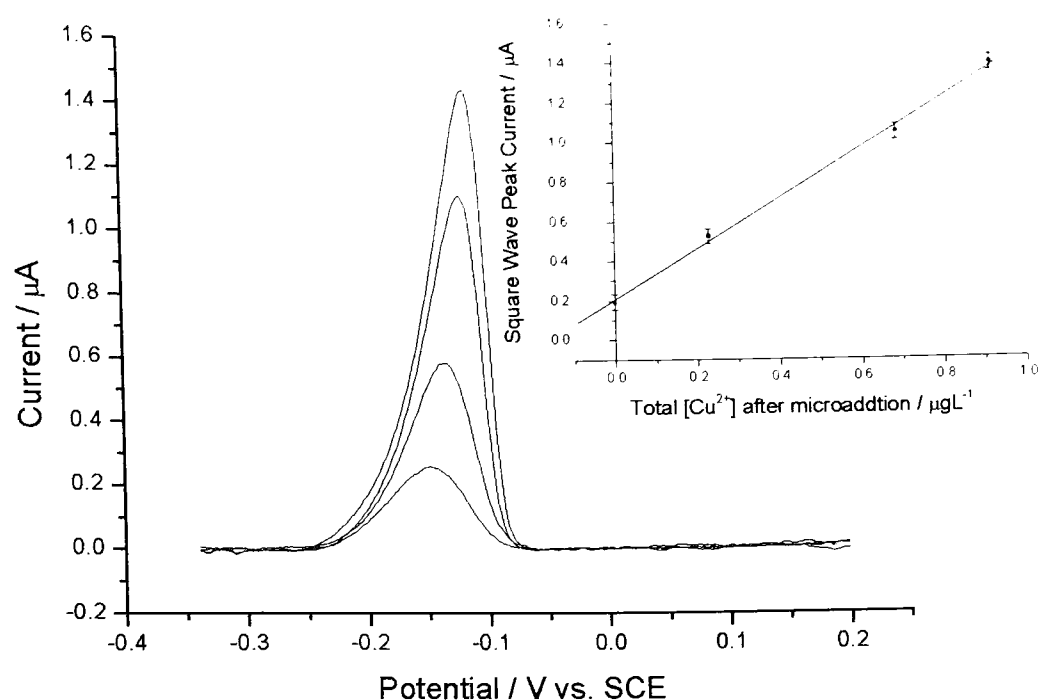


Figure 3 – Background corrected square wave voltammetric scans ($f = 25 \text{ Hz}$, $\Delta E_s = 1.5 \text{ mV}$ and $\Delta E_p = 15 \text{ mV}$) following an insonated preconcentration for 60 s at -1.5 V vs. SCE for standard additions to sono-solvent extracted laked horse blood solution (test solution 0.05 % -by volume- blood). Each $10 \mu\text{L}$ addition corresponding to an increase in concentration of $0.22 \mu\text{g L}^{-1}$. Calibration graph shown inset ($R^2=0.9972$).

The standard addition data gave a total mean copper concentration in blood of $1613 \mu\text{g L}^{-1}$ ($n=3$, $\sigma=26$) see **Table 2**. This value has again been corrected to take into account the maximum percentage of copper that can be extracted from the thermodynamic modelling of the reaction.

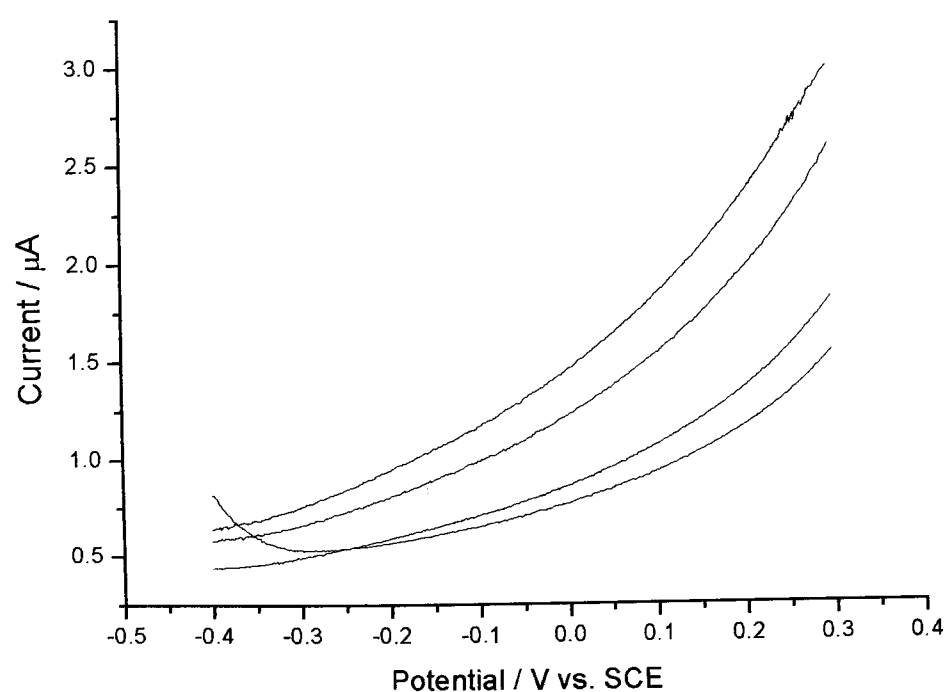


Figure 4 - Square wave anodic stripping voltammetry for a silent deposition step of 60 s at -1.5 V . Traces show standard additions to sono-solvent extracted laked horse blood solution 0.05 % -by volume- blood. Each $10 \mu\text{L}$ addition corresponds to an increase in copper concentration of $0.22 \mu\text{g L}^{-1}$.

In contrast **Figure 4** shows square wave anodic stripping voltammetry following silent deposition for the test solution and three standard additions. Clearly in the absence of ultrasound obtaining a quantitative analytical signal is unfeasible at this low concentration.

The effect of micro volumes of dissolved ethyl acetate remaining in the test solution was also investigated. Under silent conditions it was evident that standard additions of ethyl acetate to aqueous 5 μM copper solution had a passivating effect on the observed voltammetric signal. However by insonating the electrode during the preconcentration period an active electrode surface could be maintained for increasing standard additions of ethyl acetate with reproducible voltammetric signals for copper. This is in agreement with the arguments advanced in s.10.1.2 above.

In order to independently verify quantitative analysis by sono-solvent extraction the sample was also analysed using the previously proven sonoelectroanalytical method published by Compton *et al*⁵ and reported in Chapter 4 of this thesis. Results calculated using this method have been shown to lie within 1 standard deviation of an independent laboratory atomic absorption spectroscopy method⁵ see s.4.3.5. Close agreement is reported between the results obtained using this “direct” sono-SWASV in blood diluted with nitric acid and the results obtained using the solvent extraction prepared sample. A comparison is given in Table 1 which shows the high intrasample reproducibility, low standard deviation and low limit of detection afforded by the solvent extraction technique.

Sono-solvent extraction method						Direct sono-electroanalysis					
n	total copper / $\mu\text{g L}^{-1}$	R^2	$\bar{x}$ / $\mu\text{g L}^{-1}$	σ	LOD / $\mu\text{g L}^{-1}$	n	total copper / $\mu\text{g L}^{-1}$	R^2	$\bar{x}$ / $\mu\text{g L}^{-1}$	σ	LOD / $\mu\text{g L}^{-1}$
1	1637	0.9972				1	1745	0.9673			
2	1616	0.9788	1613	26	0.16	2	1677	0.9978	1619	163	91
3	1585	0.9960				3	1435	0.9988			

n = experiment number, R^2 = 5 point linear regression parameter for microaddition data, $\bar{x}$ = mean total copper (n=3), σ = standard deviation, LOD = limit of detection in square wave electroanalytical test medium

Table 1 – Comparison of quantitative analysis of copper in a sample of horse blood by i) the sono-solvent extraction methodology presented in Chapter 9 and ii) direct sono-electroanalysis⁵ presented in Chapter 4.

10.3 Conclusions

Solvent extraction followed by square wave anodic stripping voltammetry presents an accurate and reproducible analytical tool for the detection of copper both in the soft drink in Ribena® and most importantly, in whole blood from a sample volume of 100 μL .

Since the double extraction methodology necessarily removes contaminants, which are likely to prefer the aqueous phase or the organic phase but are unlikely to be “doubly transferred” into the “clean” stripped aqueous phase, the limit of detection is pushed lower than previously possible (compared with Chapter 4). Ultrasonic treatment in the emulsification stages acts both to place the N-benzoyl-N-phenyl-hydroxylamine ligand in contact with the copper present in the target material but in the case of blood it also ensures that copper is liberated from the active protein sites in which it is bound. The limit of detection for in the clean test medium, $0.16 \mu\text{g L}^{-1}$, suggests analysis of lower sample volumes is possible with a pinprick test feasible.

Close statistical agreement is seen between total copper measurements taken using the solvent-extraction methodology of sample preparation and the previously verified technique employing sono-electroanalytical measurements directly into diluted blood. The advantages of the former are greater intrasample reproducibility, lower limit of detection and lower sample volume, while the latter presents a rapid protocol requiring little pretreatment.

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- ¹ The Water Supply (Water Quality) (England and Wales) Regulations 2000, SI No. 3184
- ² B.A. Brookes, R.G. Compton, *J. Phys. Chem. B*, **1999**, *103*, 9020
- ³ B.A. Brookes, J.C. Ball, R.G. Compton, *J. Phys. Chem. B*, **1999**, *103*, 5289
- ⁴ C.M.A. Brett, A.M.O. Brett, “*Electroanalysis*” OUP, Oxford, UK, **1998**
- ⁵ J.L. Hardcastle, G. Murcott, R.G. Compton, *Electroanal.* **2000**, *12*, 559
- ⁶ D. Jagner, L. Renman, Y. Wang, *Electroanal.*, **1994**, *6*, 285.
- ⁷ A.M. Bond, Y. Nagaosa, T. Menjyo, *Analyst*, **1991**, *116*, 257
- ⁸ M.A. Moreno, C. Marin, F. Vinagre, P. Ostapczuk, *Sci.Tot.Env.*, **1999**, *229*, 209.

Section V

Overview of Sonoelectroanalytical Techniques

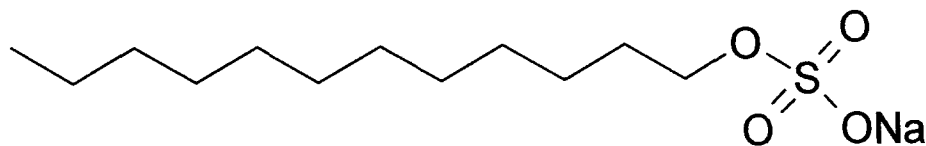
Section V

Chapter 11 – Enhanced Detection and Determination of Copper in the Presence of Surfactants

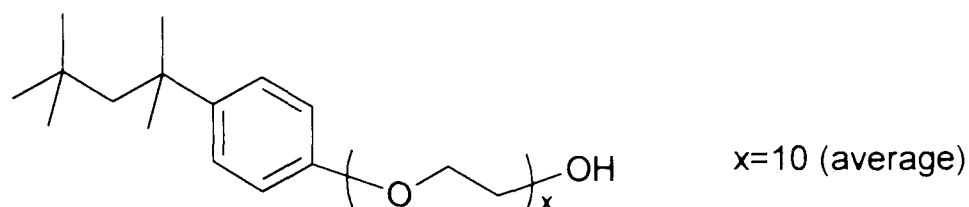
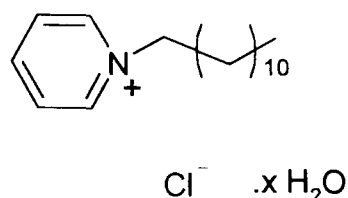
11.1 Introduction

In Chapters 4-10 the introduction of power ultrasound in synergy with anodic stripping voltammetry has been shown to broaden the scope of electroanalysis to cover a wide range of target species without the need for often laborious sample pre-treatments. Analysis in highly passivating media such as blood^{1,2} (Chapters 4 and 10), mucus³ (Chapter 5), saliva^{4,5} (Chapters 6 and 7) food additives⁶ (Chapter 8) and soft drinks⁷ (Chapter 10), has been made possible by the well documented effects of *in situ* cleaning by ultrasonic cavitation and increased mass transport due to acoustic streaming⁸⁻¹⁰ (see Chapter 3) in addition, ultrasound is effective in liberating bound species from proteins and matrices.

This chapter provides a direct comparison of the techniques described in earlier chapters. This is achieved through assessing the feasibility of sono-anodic stripping voltammetry of copper in aqueous solutions containing anionic (sodium dodecyl sulphate – see structure 1), non-ionic (Triton® X-100 – see structure 2) or cationic (dodecyl pyridinium chloride – see structure 3) surfactants at concentrations between 10-1000 ppm. The performance of a bare glassy carbon electrode under “silent” conditions is contrasted with both insonated voltammetric measurements made with the horn in a “face-on” arrangement (see s.1.4.2 Figure 4) and measurements made using a sonotrode (see s.1.4.2 Figures 4 and 5). These measurements made directly in the surfactant solution at ultrasound intensities from 30-260 W cm⁻² are also compared with measurements made following pre-treatment of the samples by sono-solvent double extraction (see Chapters 9 and 10) prior to an insonated analytical stripping step.



Structure 1 – Sodium Dodecyl Sulphate

**Structure 2** – Triton X-100 (average MW = 646)**Structure 3** – Dodecyl Pyridinium Chloride

Surfactant adsorption has a passivating effect on the electrode surface resulting in decreased electron transfer rates and impeded diffusion of analytes to the electrode surface. This in turn is manifested as a depression in the height of stripping responses, peak broadening and shifts in peak potential^{11,12}. In order to permit electrochemical analysis of samples containing surfactants several pretreatment methods have been reported. These include UV digestion¹³, addition of ethanolic suspensions of fumed silica¹⁴, pre-electrolysis¹⁵ and wet ashing¹⁶. All are time consuming and involve extensive interference with the sample. The deleterious effect of surfactant has also been partially surmounted by covering the electrode with a permselective/protective film such as Nafion®¹⁷, agar¹¹, cellulose acetate¹⁸ and polypyrrole¹³. Electrodes coated with semipermeable polymer films allow the passage of the analytes to the electrode while restricting the access of surface-active compounds. The use of such films have been reported to yield improved signal quality in electrochemical stripping analysis^{12,19-24}, with analysis in the presence of up to 10 ppm of the non-ionic surfactant Triton®-X 100 shown to be possible¹². A recent non-invasive technique which has extended the available strategies for electrochemical analysis in the presence of surfactant is stripping voltammetry enhanced by microwave activation during the preconcentration step²⁵. This has also been shown to facilitate the detection of cadmium in the presence of up to 10 ppm Triton® X-100 and 10 ppm sodium dodecyl sulphate.

11.2 Experimental

The ultrasonic horn used was a Model CV 26 (Sonics and Materials Inc., Connecticut, USA). The ultrasonic intensity was calibrated calorimetrically according to the procedure given in s.2.3 over the range 30-260 W cm⁻². The electrochemical cell is described in s.2.1 Figure 1. For experiments

in a “face on” horn to electrode arrangement the separation was 3 mm. For experiments employing the sonotrode a flat-bottomed cell of the same specifications as that shown in s.2.1 was used and the horn tip/electrode was located 3 cm from the base of the vessel.

In order to investigate the efficiency of ultrasonic depassivation in the presence of surfactants the intensity of cavitation behaviour at the surface of the electrode was investigated electrochemically. The system employed for this was the reduction of $\text{Ru}(\text{NH}_3)_6^{3+}$ in aqueous 0.1 M KCl see s.1.4.3 equation 1. Chronoamperometry, over a time interval of 0.25 s with a sample time of 5 μs at a potential of -0.4 V vs. SCE, permits a useful correlation between the frequency of the observed spikes in the current transient and the intensity of cavitation events occurring at the surface of the electrode. In order to quantify the “amount” of cavitation a C++ subroutine²⁶ was written to count the number of spikes in the current transient over 0.5 s at a resolution of 5 μs . The computer program achieved this by defining a spike as a maximum or minimum in the current transient. **Figure 1** shows the number of spikes counted for increasing ultrasound power with a tolerance of $2 \times 10^{-6}\text{A}$, which is the minimum size of a current spike recorded as a cavitation event (this partially eliminates the contribution from small background fluctuations in the current transient).

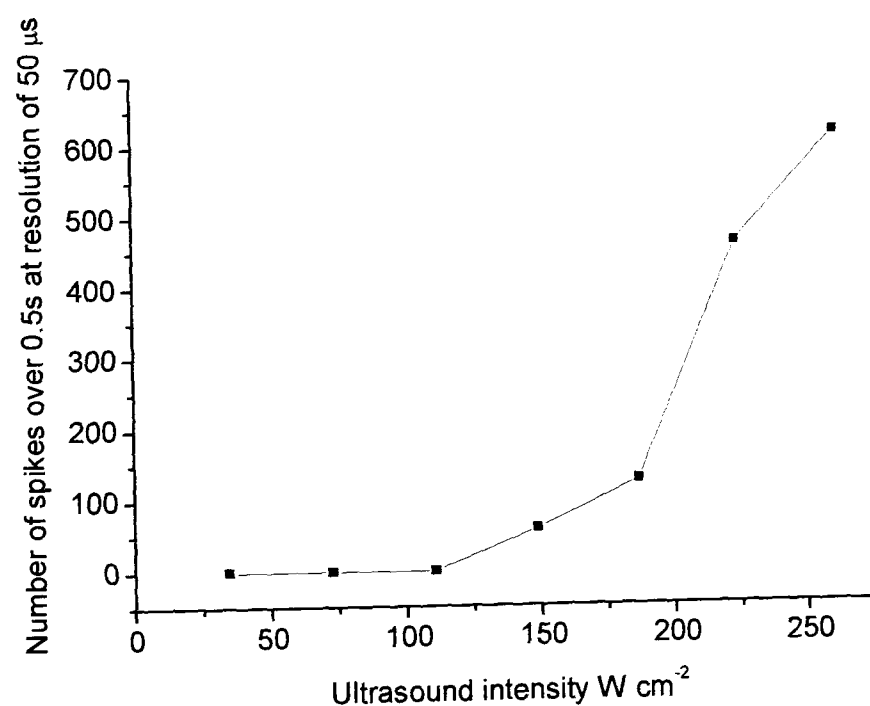


Figure 1 - Cavitation threshold plot describing the relationship between the number of current spikes recorded during chronoamperometry, over a 0.5 s timescale, with 50 μs resolution, and the applied ultrasound intensity at a fixed horn to electrode separation of 3 mm.

It is clear from **Figure 1** that there is an ultrasound intensity beyond which there is a noticeable increase in the intensity of cavitation events with increasing power. The non-linear appearance of

Figure 1 is consistent with the presence of a cavitation threshold^{8,9} as discussed in s.1.4.4 for DMF (Figure 9 s.1.4.4.). Sono-anodic stripping voltammetry of copper in the presence of surfactant was performed with ultrasound intensity above (150 W cm^{-2}) and below (70 W cm^{-2}) this threshold value. Intensities above 150 W cm^{-2} , although maximising the cavitational activity at the electrode surface, can result in substantial erosion of the electrode over the 60 s preconcentration period.

The design for the sonotrode is shown in s.1.4.2 Figure 5. The sonotrode is comprised of a disc shaped glassy carbon electrode (3 mm diameter, 2 mm thick, BAS, Indiana, USA) attached to an insulated wire using conductive epoxy resin (Chemtronics, US) and secured to a 30 mm quartz rod using Torr Seal® (Varian Vacuum Technologies, US). The glassy carbon tipped rod is inserted into a drilled probe tip see s.1.4.2 Figure 5 and is again secured using Torr Seal®. The glassy carbon probe tip assembly is connected to the CV26 ultrasonic horn and the attached insulated wire passes through the probe to the potentiostat. The solvent extraction procedure^{2,7} involved two sonication steps followed by electrochemical detection of extracted copper ions as described in s.9.2 Figure 1.

Electrochemical measurements were carried out using the sonotrode or a 3 mm diameter glassy carbon disc electrode (BAS, Indiana, US). Both were polished according to the procedure given in s.2.5. All potentials are quoted with respect to a saturated calomel electrode, SCE (Radiometer, Copenhagen). The counter electrode employed in the three-electrode system was a 20 cm coiled platinum wire which was flame annealed prior to each experiment. Sono-ASV involved an insonated deposition step of 60 s at -1.5 V vs. SCE followed by a linear sweep stripping step, with a scan rate of 0.1 V s^{-1} over a potential range of -0.3 V to 0.3 V vs. SCE. Reagents used were all of electrochemical grade or the highest available purity see appendix 2 for full details.

11.3 Results and Discussion

11.3.1 Anodic Stripping Voltammetry in the Absence of Ultrasound (Silent)

The performance of a bare glassy carbon electrode under silent conditions in the presence of surfactant was assessed. The aqueous solution was prepared with $6 \text{ }\mu\text{M}$ Cu(II) and surfactant concentrations from 0-1000 ppm in 0.1 M HNO_3 .

ASV involved a “silent” preconcentration for 60 s at -1.5 V vs. SCE followed by linear sweep voltammetry at a scan rate of 0.1 V s^{-1} . The true background current in 0.1 M nitric acid was fitted

to a 7th order polynomial using Origin 6.0 software (Microcal, MA, USA) which was then used to facilitate the calculation of the background current for each addition of copper. Subtraction of the background signal gave approximately Gaussian shaped linear sweep profiles as theoretically predicted. The copper concentration was determined using the standard addition technique with 25 μL additions giving an increase in concentration of the test solution corresponding to 3 μM . For ease of comparison calculated ASV values for copper concentration are expressed as a percentage of total known copper concentration and termed the percentage recovery.

Standard addition traces for increasing concentration of SDS are shown in **Figure 2**. Linear standard addition plots of peak area against added copper at 0 ppm SDS ($R^2 = 0.9969$), 10 ppm SDS ($R^2 = 0.9763$) and 100 ppm SDS ($R^2 = 0.9892$) were indicative of passivation with increasing surfactant concentration with depreciating peak heights and lower overall percentage recovery of copper.

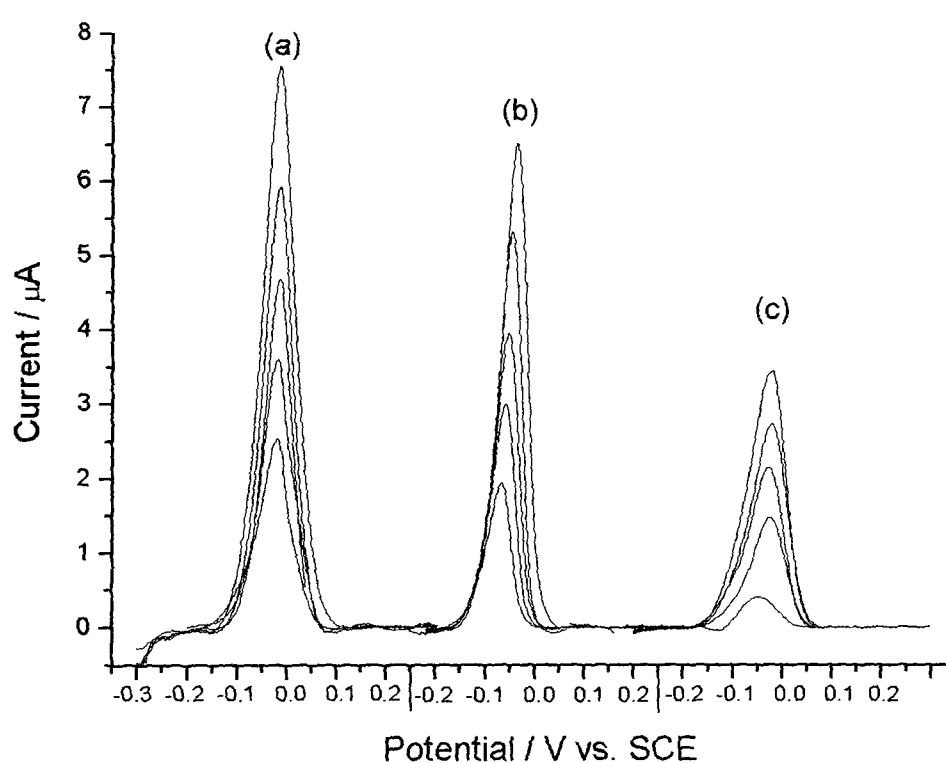


Figure 2 - Standard addition traces for ASV with a “silent” preconcentration for 60 s at -1.5 V vs. SCE followed by linear sweep voltammetry at a scan rate of 0.1 V s^{-1} and step potential of 2.5 mV , shown for increasing concentration of SDS in a solution of 0.1 M HNO_3 and $6 \mu\text{M}$ copper (II). Each addition corresponds to an increase in copper (II) concentration of $3 \mu\text{M}$.

- a) 0 ppm SDS, 97 % recovery
- b) 10 ppm SDS, 73.8 % recovery
- c) 100 ppm SDS 33.7 % recovery

The behaviour in the presence of 1000 ppm SDS was non-linear precluding the determination of apparent copper concentration. It is clear that the presence of as little as 10 ppm SDS has a detrimental effect on the accuracy of the analysis. Similar analyses with 10 ppm TX-100 was

indicative of complete passivation of the electrode as shown in **Figure 3**. In the presence of 10 ppm DPC a signal was obtained for increasing addition of copper **Figure 4**, however the change in peak area was non-linear and of minimal analytical utility. At a concentration of 100 ppm no analytical signal was obtained due to complete passivation of the electrode. It is therefore apparent that surfactants quickly preclude meaningful analysis at unmodified electrode surfaces via traditional electroanalytical techniques.

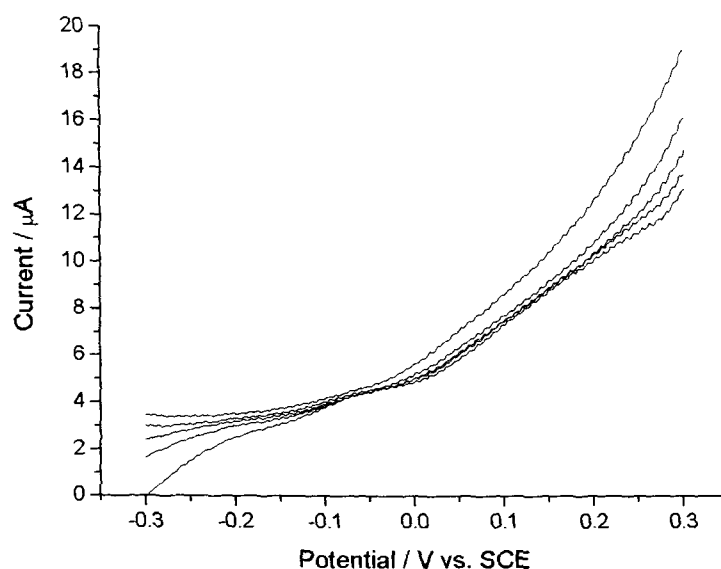


Figure 3 - Standard addition traces for ASV (“silent” preconcentration for 60 s at -1.5 V vs. SCE followed by linear sweep voltammetry at a scan rate of 0.1 V s $^{-1}$) for a solution of 0.1 M HNO $_3$ and 6 μ M copper (II) containing 10 ppm TX-100. Each addition corresponds to an increase in copper (II) concentration of 3 μ M.

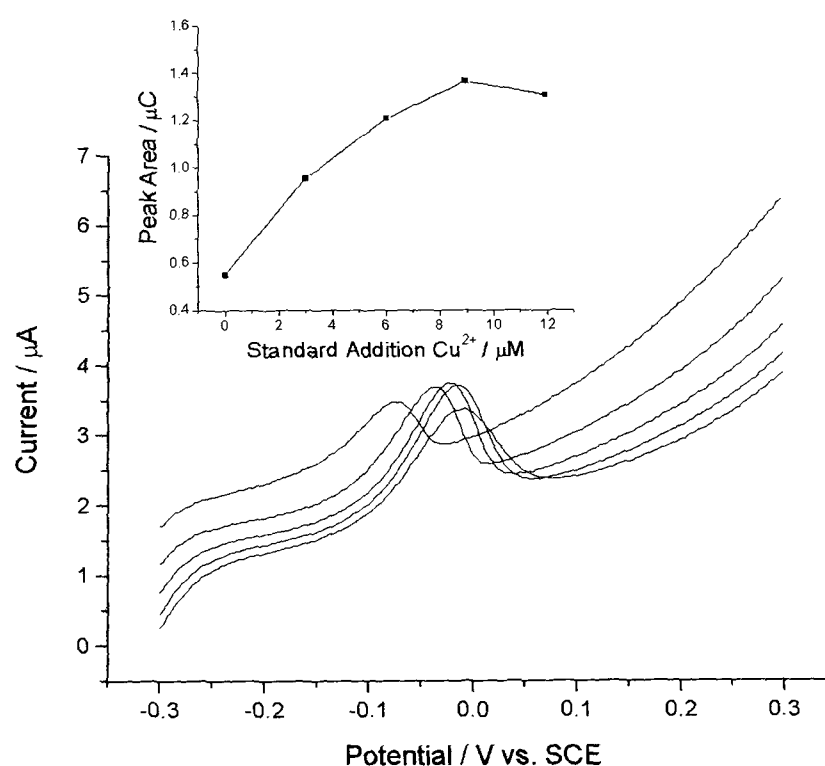


Figure 4 - Standard addition traces for ASV (“silent” preconcentration for 60 s at -1.5 V vs. SCE followed by linear sweep voltammetry at a scan rate of 0.1 V s $^{-1}$) for a solution of 0.1 M HNO $_3$ and 6 μ M copper (II) containing 10 ppm DPC. Each addition corresponds to an increase in copper (II) concentration of 3 μ M. Shown inset is a plot of peak area (μ C) against concentration (μ M) demonstrating the non-linearity of standard additions to this media.

11.3.2 Anodic Stripping Voltammetry with Insonated Preconcentration

11.3.2.1 SDS – anionic surfactant

Introduction of ultrasound into the system during the preconcentration step has the benefits of increased mass transport of bulk material to the electrode and cavitation depassivation of adsorbed species. The enhancement in sensitivity attainable with an insonated preconcentration is clearly illustrated in **Figure 5** for an ultrasound intensity of 70 W cm^{-2} , below the cavitation threshold. Standard addition data gave a value of 99.8 % the total concentration of copper which compares favourably with the 93.7 % obtained under silent conditions.

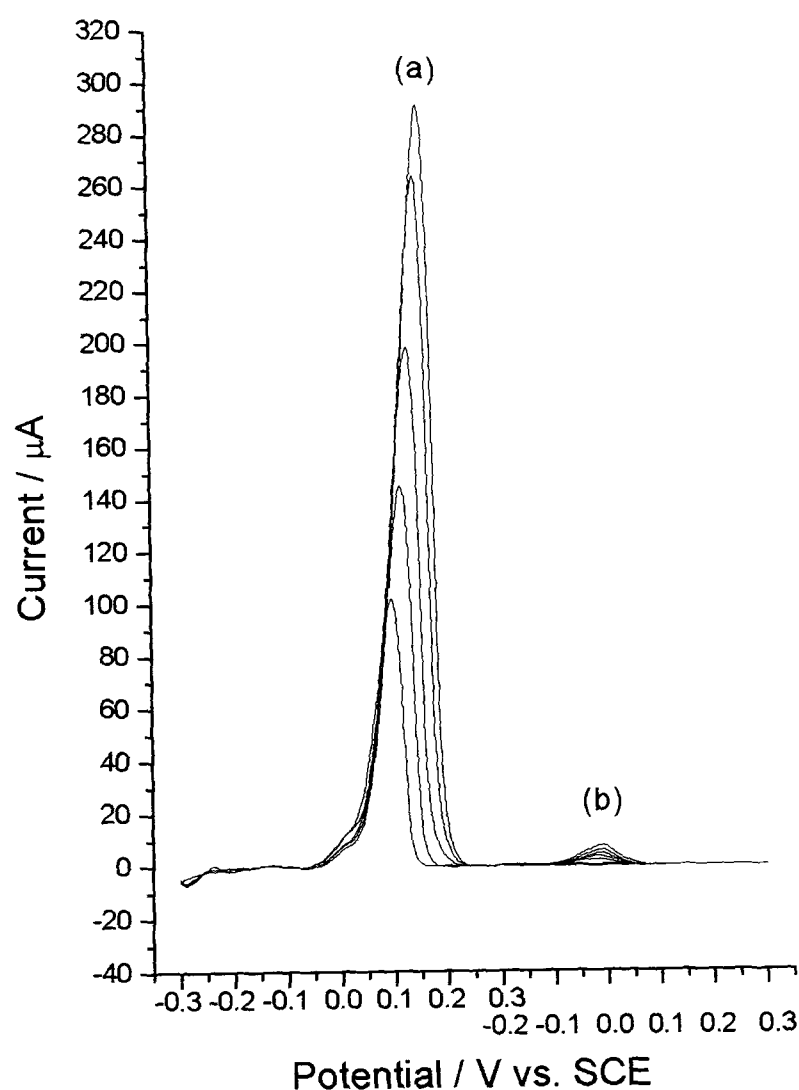


Figure 5 -Standard addition traces for ASV (60 s preconcentration at -1.5 V vs. SCE followed by linear sweep voltammetry at a scan rate of 0.1 V s^{-1}) for a solution of 0.1 M HNO_3 and $6 \mu\text{M}$ copper (II) with $3 \mu\text{M}$ copper (II) additions.

(a) Employing an insonated preconcentration at 70 W cm^{-2}

(b) Employing a silent preconcentration

The ability of ultrasound to depassivate the electrode surface was investigated for each surfactant at ultrasound intensities both above (150 W cm^{-2}) and below (70 W cm^{-2}) the cavitation threshold in a “face-on” geometry, and a sonotrode operating at an intensity of 42.6 W cm^{-2} . For SDS the variation in the calculated total copper concentration for a given surfactant concentration and ultrasound power is given in Table 1.

Surfactant Concentration / ppm	Ultrasound Power / W cm ⁻²	% Recovery‡	R ² of microaddition plot
0	0	93.7	0.9925
0	70	99.8	0.9831
0	150	100	0.9920
10	0	73.8	0.9969
10	43†	93.6	0.9940
10	70	95.7	0.9931
10	150	98.9	0.9887
100	0	33.7	0.9763
100	70	88.5	0.9982
100	150	70.1	0.9620
1000	70	57.2	0.9998
1000	150	75.5	0.9690

† data for sonotrode

‡calculated ASV results for copper concentration expressed as a percentage of total known copper concentration

Table 1 – Anodic stripping voltammetry for the determination of copper concentration in the presence of SDS for a prepared solution of known [Cu²⁺] = 6 µM

Under silent conditions the addition of SDS rapidly precludes the determination of copper concentration. In contrast the presence of ultrasound facilitates analysis in media of SDS concentration up to 100 ppm. For higher surfactant concentration, 1000 ppm, although the percentage recovery was only 57 % an analytical signal with extremely good linearity ($R^2 = 0.9998$) was obtained at an intensity of 70 W cm⁻². The data obtained at 150 W cm⁻² is indicative of a higher percentage recovery of copper than below the cavitation threshold with a 75 % recovery from 1000 ppm SDS. This verifies our expectation that increased cavitational activity both assists the mass transport of material to the surface and promotes efficient removal of passivating surface-active species.

The sonotrode geometry²⁷ can be seen to perform comparably with the face on geometry in this medium with a lower sonotrode intensity of 42.6 W cm⁻² achieving a percentage recovery of 93.6 % compared with 70 W cm⁻² intensity “face-on” which exhibits a percentage recovery of 95.7 %. These values are comparable despite the difference in ultrasound intensity since the actual intensity experienced at the electrode surface in the case of the sonotrode will be higher than in the “face-on” geometry for any given value. This can be explained as follows: in the case of the stepped microtip (illustrated in s.2.2 Figure 3) some of the ultrasonic intensity is introduced into the

solution from the shoulders, located away from the electrode as well as the end of the tip (shown schematically in s.9 Figure 2). Furthermore, in this geometry, the electrode is necessarily located at some distance from the source of the ultrasound, usually only a matter of millimetres. However, it is clear from s.3.4.2 Table 3 that the size of the diffusion layer increases rapidly as horn to electrode separation increases, s.1.4.5.4 also provides evidence of how the cavitation activity increases as horn to electrode separation decreases. In the case of the sonotrode all the ultrasonic intensity is dissipated at the electrode surface leading to a maximum of cavitation activity and acoustic streaming at the electrode surface for any given intensity. Other advantages of a sonotrode also lie in its ease of use. As a single probe analytical instrument it has greater commercial potential²⁸ than ultrasound in the face on geometry.

11.3.2.2 Triton X 100 – non-ionic surfactant

In the absence of ultrasound it was not possible to obtain any analytical signal in the presence of 10 ppm TX-100. However, by employing an insonated preconcentration the percentage recovery was dramatically improved (Table 2) for 10, 100 and 1000 ppm.

Surfactant Concentration / ppm	Ultrasound Power / W cm ⁻²	% Recovery‡	R ² of microaddition plot
0	0	93.7	0.9925
0	70	99.8	0.9831
0	150	100	0.9920
10	0	0	No signal
10	43†	100	0.9990
10	70	62.4	0.9931
10	150	88.5	0.9960
100	0	0	No signal
100	70	34.3	0.9982
100	150	63.4	0.9950
1000	70	18.0	0.9506
1000	150	34.4	0.9920

† data for sonotrode

‡calculated ASV results for copper concentration expressed as a percentage of total known copper concentration

Table 2 - Anodic stripping voltammetry for the determination of copper concentration in the presence of TX-100 for a prepared solution of known [Cu²⁺] = 6 µM

In this case it is clear that the sonotrode performs better than the “face on” geometry for a surfactant concentration of 10 ppm. The sonotrode affords a 100 % recovery for an intensity of 42.6 W cm⁻² which is compared with 88.5 % when insonated in the “face-on” geometry.

For the “face on” geometry, there is a distinct improvement in depassivation beyond the cavitation threshold as observed for SDS with enhancement of percentage recovery at 150 W cm^{-2} much greater than at 70 W cm^{-2} **Figure 6**).

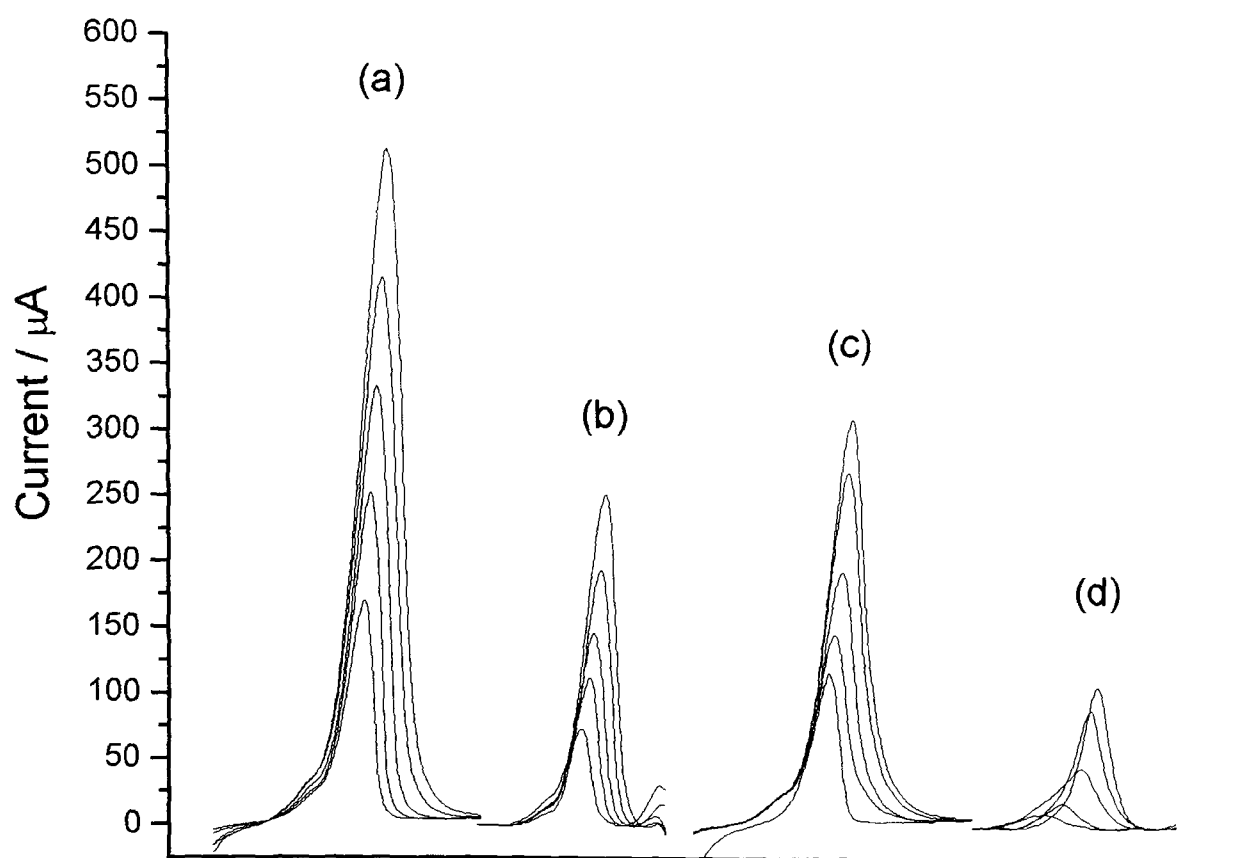


Figure 6 - Standard addition traces for ASV (insonated 60 s preconcentration at -1.5 V vs. SCE followed by linear sweep voltammetry at a scan rate of 0.1 V s^{-1}) for a solution of 0.1 M HNO_3 and $6 \mu\text{M}$ copper (II), containing TX-100. Each addition corresponds to an increase in copper (II) concentration of $3 \mu\text{M}$.

- a) 10 ppm TX-100 , 150 W cm^{-2} , 88% recovery
- b) 10 ppm TX-100 , 70 W cm^{-2} , 62% recovery
- c) 100 ppm TX-100 , 150 W cm^{-2} , 34% recovery
- d) 100 ppm TX-100 , 70 W cm^{-2} , 18% recovery

11.3.2.3 DPC – Cationic Surfactant

There is a contrast between the enhancement of the analytical signal obtained following an insonated deposition step in the presence of SDS and TX-100 and the behaviour in DPC. In 10 ppm DPC with a silent preconcentration a copper stripping peak of area $5.5 \times 10^{-7} \text{ C}$ was obtained. Following an insonated preconcentration period at 70 W cm^{-2} there was no visible analytical signal. Increasing the ultrasound power to 150 W cm^{-2} did not yield any improvement as illustrated in **Figure 7**. Similarly the use of a sonotrode (**Figure 7 (a)**) was unhelpful.

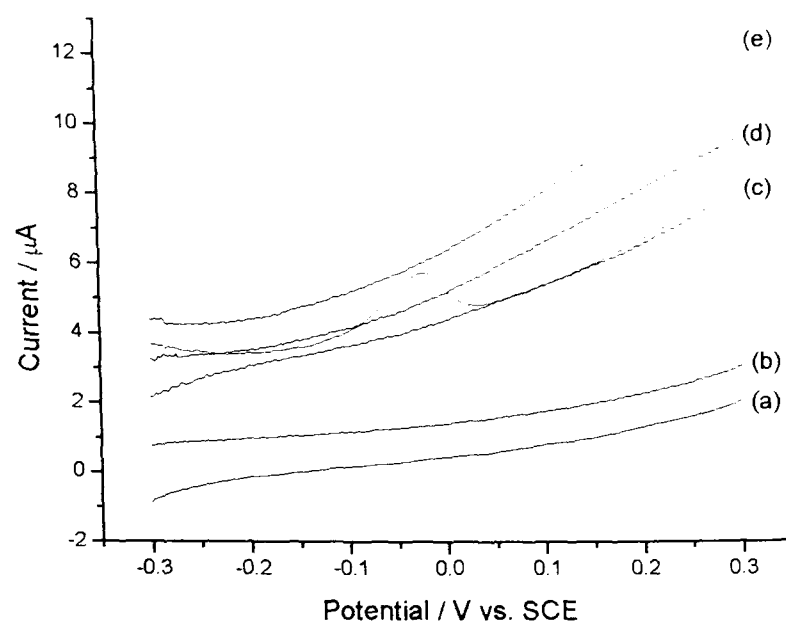


Figure 7 - ASV (60 s preconcentration at -1.5 V vs. SCE, followed by linear sweep voltammetry at a scan rate of 0.1 Vs^{-1} for a solution of 0.1 M HNO_3 containing $6 \text{ } \mu\text{M Cu (II)}$ and 10 ppm DPC
 ----- silent preconcentration, a) sonotrode 42.6 W cm^{-2} b) 70 W cm^{-2} , c) 110 W cm^{-2} , d) 150 W cm^{-2} , e) 185 W cm^{-2}

It is therefore concluded that for a preconcentration at -1.5 V the rate of adsorption of cationic DPC is increased due to the increased mass transport. Increasing the ultrasound power beyond the cavitation threshold did not give any improvement even at ultrasound powers up to 180 W cm^{-2} suggesting that ultrasound cannot be used in synergy with electrochemical interrogation in this medium.

11.3.3 Sono-Biphasic Double Extraction with Electrochemical Detection via Anodic Stripping Voltammetry

Finally the applicability of sono-biphasic double extraction to the determination of trace copper in surfactant-contaminated solutions was assessed. Two copper concentrations were used in the following experiments, $30 \text{ } \mu\text{M}$ with a final test concentration of $6 \text{ } \mu\text{M}$ to facilitate a comparison with the peak heights of the data given in s.11.3.2 above, and $6 \text{ } \mu\text{M}$ with a final test concentration of $0.3 \text{ } \mu\text{M}$. The former was extracted using 0.05 M ligand in ethyl acetate; the latter was extracted using 0.01 M ligand in ethyl acetate in order to ensure that the concentration of ligand was greater than the 1000-fold excess required from theoretical predictions and experimental observations (see s.9.4.2 Figure 7). The concentration of solvent extracted copper remains constant over the pH range 4.5 - 10.5 (see s.9.4.2) the natural pH of the copper nitrate solutions of ~ 6.9 was therefore deemed to be acceptable.

The rapid separation of aqueous and organic layers previously observed for solvent extraction (a matter of seconds in Chapters 9 and 10)^{2,7} was lengthened in the presence of surfactant with separation times of approximately 10 minutes in the presence of 1000 ppm SDS and 12 minutes in the presence of 1000 ppm TX-100. In the presence of 1000 ppm DPC there was no separation of the aqueous and organic phases and a stable emulsion was formed precluding further analysis in this media by this methodology.

For extraction from 1000 ppm TX-100 and 1000 ppm SDS, the isolated organic phase containing the copper ligand complex could then be stripped using 1.0 M HNO₃ to yield a “clean” aqueous phase prior to electroanalytical copper determination. Sono-ASV was carried out as above with an insonated preconcentration at 70 W cm⁻² for 1 min at -1.5 V vs. SCE followed by a linear sweep with a scan rate of 0.1 V s⁻¹ under silent conditions over a potential range of -0.3 V to 0.3 V vs. SCE. The concentration of copper in the final test solutions was obtained using the standard addition technique. It was not possible to use a calibration followed by single measurement since, due to the nature of a surfactant, a small amount is transferred into the final test solution.

From an analyte containing 1000 ppm SDS, voltammetric traces for standard additions to a final test solution of 6 µM copper are shown in **Figure 8** with a linear calibration curve ($R^2 = 0.9980$) inset. The calculated copper concentration was corrected using the known maximum percentage of 74 % which can be extracted in accordance with theoretical prediction^{2,7} (s.9.4.2.), to give a mean ($n = 3$) value of 93.6 % recovery. The calibration data can be compared with **Figure 5** which shows standard additions of 3 µM to a clean solution of 6 µM copper. Their similarity, with only a small depletion in peak size, is indicative of efficient removal of 1000 ppm SDS.

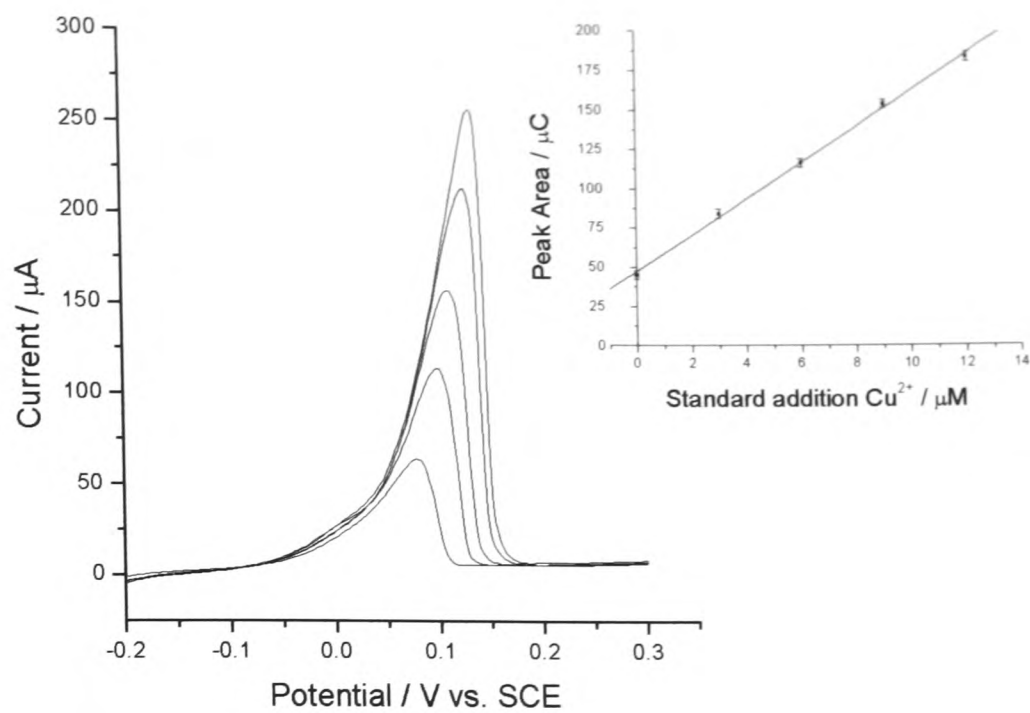


Figure 8 - ASV voltammetric traces for an insonated preconcentration of 60 s at -1.5 V followed by linear sweep voltammetry at a scan rate of 0.1 V s^{-1} and step potential of 2.5 mV . Traces show standard additions to copper solution prepared via sono-solvent double extraction from an aqueous solution containing $30 \text{ }\mu\text{M}$ Cu(II) and 1000 ppm SDS. Each addition corresponds to an increase in copper concentration of $3 \text{ }\mu\text{M}$. Calibration graph shown inset ($R^2 = 0.9980$) gives a recovery of 94%

A second experiment involved analysis of a sample prepared from an aqueous starting solution of $6 \text{ }\mu\text{M}$ copper containing 1000 ppm SDS. Standard additions to a final test solution of $0.3 \text{ }\mu\text{M}$ copper yielded a linear calibration curve ($R^2 = 0.9900$) and a corrected mean ($n = 3$) percentage recovery of 94.1% . This is a dramatic increase in sensitivity over the results attained without a sono-solvent extraction sample pre-treatment where the best result for $[\text{SDS}] = 1000 \text{ ppm}$ was 75.5% recovery for preconcentration at 150 W cm^{-2} , see Table 3.

Surfactant	Concentration / ppm	Technique	Ultrasound power in sono-ASV / W cm^{-2}	% Recovery†	R^2 of microaddition plot
SDS	1000	Sono-ASV	70	57.2	0.9998
SDS	1000	Sono-ASV	150	75.5	0.9690
SDS	1000	Sono-biphasic‡	70	94.1	0.9900
TX-100	1000	Sono-ASV	70	18.0	0.9506
TX-100	1000	Sono-ASV	150	34.4	0.9920
TX-100	1000	Sono-biphasic‡	70	94.0	0.9970

†calculated results for copper concentration expressed as a percentage of total known copper concentration

‡Sono- biphasic double extraction sample preparation followed by sono-anodic stripping voltammetry

Table 3 – Comparison between sono-anodic stripping voltammetry and sono-biphasic double extraction for determination of copper from $6 \text{ }\mu\text{M}$ starting solution in the presence of 1000 ppm surfactant.

Similar analyses were carried out for solutions containing 1000 ppm TX-100. Voltammetric traces for standard additions to a final test solution of 6 μM copper are shown in **Figure 9** with a linear calibration curve ($R^2 = 0.9890$) inset. The calculated copper concentration was corrected using the known maximum percentage of 74 % which can be extracted in accordance with theoretical prediction, to give a value of mean ($n = 3$) value of 88.2 % recovery.

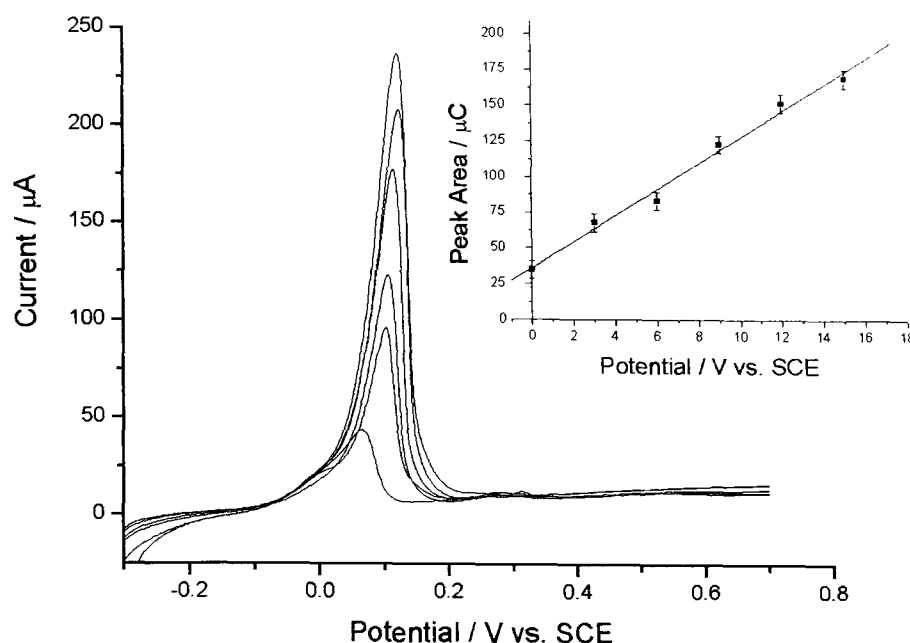


Figure 9 - ASV voltammetric traces for an insonated preconcentration of 60 s at -1.5 V followed by linear sweep voltammetry at a scan rate of 0.1 V s^{-1} and step potential of 2.5 mV . Traces show standard additions to copper solution prepared via sono-solvent double extraction from an aqueous solution containing $30 \mu\text{M}$ Cu(II) and 1000 ppm TX-100. Each addition corresponds to an increase in copper concentration of $3 \mu\text{M}$. Calibration graph shown inset ($R^2=0.9890$) gives a recovery of 88 %

Comparison with the voltammetry of a clean $6 \mu\text{M}$ copper solution, shown in **Figure 5**, is favourable and the small depreciation in peak size is indicative of efficient removal of 1000 ppm from the starting solution. A second experiment involved analysis of a sample prepared from an aqueous starting solution of $6 \mu\text{M}$ copper containing 1000 ppm TX-100. Standard additions to a final test solution of $0.3 \mu\text{M}$ copper yielded a linear calibration curve ($R^2 = 0.9970$) and a corrected mean ($n = 3$) percentage recovery of 94.0 %. Again we observe a distinct increase in sensitivity over analysis without sono-solvent extraction sample pre-treatment where the best result for $[\text{TX-100}] = 1000 \text{ ppm}$ was 34 % recovery at 150 W cm^{-2} , see Table 3.

11.4 Conclusions

The feasibility of sono-anodic stripping analysis for the determination of copper in aqueous media contaminated with surfactant has been studied in order to provide a qualitative and quantitative

comparison between the techniques described earlier in this thesis. In s.11.3.1 effect of surfactant contamination on the analytical sensitivity of anodic stripping voltammetry in the absence of ultrasound (silent) is illustrated. Linear standard addition plots of peak area against added copper at 0-100 ppm sodium dodecyl sulphate were indicative of significant electrode passivation with increasing surfactant concentration. Measurements where the SDS concentration exceeded than 100 ppm were completely precluded. Similarly In Triton X-100 the presence of 10 ppm was sufficient to give complete passivation of the electrode. 10 ppm 1-dodecylpyridinium chloride, while permitting an analytical signal for increasing additions of copper the change in peak area was non-linear and of minimal analytical utility. At a concentration of 100 ppm DPC no analytical signal was obtained due to complete passivation of the electrode.

The analogous sono-electroanalytical response was investigated for each surfactant at ultrasound intensities both above and below the cavitation threshold and using a sonotrode. An insonated preconcentration stage in anodic stripping voltammetry facilitates analysis by increasing the mass transport of material to the electrode and cavitationaly depassivating the surface. Below the cavitation threshold the observed enhancement in the size of the stripping signal is largely due to the increased mass transport due to acoustic streaming. Above the cavitation threshold the intensity of cavitational events is significantly increased and adsorbed surfactant molecules are sheared from the surface. As a result analyses with SDS concentrations up to 100 ppm are possible. This compares favourably with literature at modified electrodes where 1-10 ppm SDS is shown to be strongly passivating^{11,22}. The data obtained for insonation above the cavitation threshold is indicative of a higher percentage recovery of copper consistent with our expectation that increase cavitational activity promotes efficient removal of passivating surface active species. The results obtained using a sonotrode compare favourably with those obtained using the “face on” geometry with the added advantage that this is a single probe technique.

In the absence of ultrasound it was not possible to obtain an analytical signal in the presence of 10 ppm TX-100. However an insonated preconcentration step gave a dramatically improved percentage recovery with an analytical signal visible in solutions of up to 1000 ppm when ultrasound above the cavitational threshold is applied. This can be contrasted with other literature demonstrating analysis in the presence of a maximum of 2 - 10 ppm¹² and 1-10 ppm^{11,22}. Again the

sonotrode provided impressive results with a 100 % recovery from TX-100 at 10 ppm compared with 88.5 % when insonated at 150 W cm^{-2} in the face-on geometry.

In contrast, an insonated preconcentration gave poorer results than a silent preconcentration in the presence of 10 ppm DPC. This cationic surfactant accumulates at the electrode during the -1.5 V preconcentration and its adsorption is enhanced when the mass transport due to insonation is increased. Ultrasound above the cavitation threshold was also unable to depassivate the electrode with the result that meaningful electrochemical measurements are precluded in the presence of this surfactant.

The power of the sono-solvent extraction technique is clearly illustrated by the ability to obtain accurate measurements of copper concentration from starting solutions containing 1000 ppm SDS or TX-100. Solutions of initial concentration $30 \text{ }\mu\text{M}$ and $6 \text{ }\mu\text{M}$ copper were analysed with the final test solution from the former providing a direct analogue in peak size to the clean sono-electroanalytical measurements. There was close comparison between the two indicative of efficient removal of the surfactant from the initial test solution resulting in a much cleaner final test solution. This was exemplified by analysis of the low concentration ($0.3 \text{ }\mu\text{M}$) final test solution from the $6 \text{ }\mu\text{M}$ initial solution which gave a percentage recovery of 94 % in the presence of 1000 ppm TX-100 or 1000 ppm SDS. Measurements in the presence of DPC were again precluded, in this case due to the formation of a stable emulsion when insonated with the ethyl acetate containing the ligand.

It is therefore possible to derive an analytical strategy for the sonoelectroanalytical determination of copper in the presence of anionic and neutral surfactants such as TX-100 and SDS which is transferable into a range of different “real” media. At low surfactant concentration direct sono-anodic stripping voltammetry in the aqueous solution of interest can yield significantly improved results over those obtained in the absence of ultrasound and using modified electrodes. The advantages are ease and rapidity of analysis with no necessity for time-consuming sample pretreatments. Where surfactant concentration is much higher, for example in effluent samples an alternative approach is suggested. Sono-solvent extraction permits analysis of trace copper in the presence of at least 1000 ppm TX-100 and SDS. This methodology for the production of a clean sample for analysis is less destructive than wet ashing and UV digestion and minimises the risk of

introducing impurities into the sample which is crucial when very low concentrations are being analysed.

11.5 Thesis Conclusions

This thesis has demonstrated that sonoelectroanalysis is a powerful and versatile analytical tool that can be employed in an expanding range of real world applications. Better understanding of the phenomena associated with insonation, cavitation and acoustic streaming, has facilitated the successful development of sonoelectroanalytical protocols for application in media generally considered to be highly passivating where analysis using conventional electroanalytical techniques is often precluded.

The sonoelectroanalytical examples in real media given in Chapters 4-11 are pertinent to the major fields of medical diagnosis and the assessment of environmental pollution. They demonstrate that sonoelectroanalysis is a viable alternative to conventional analytical techniques such as AAS or ICP-MS, which have the disadvantages of more lengthy sample pretreatment and cost.

Sonoelectroanalysis is a rapid, facile and cost effective technique, which with continued commercial interest and investment may potentially be rendered portable. This is envisaged especially in the light of the encouraging results presented in Chapter 11 using the single probe sonotrode. Following, *inter alia*, the work presented in Chapter 11, this instrument has become commercially available in collaboration with Windsor Scientific Ltd.

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Fick's Laws of Diffusion

Fick's 1st Law of Diffusion

For this derivation a simplistic model is employed. Consider a volume of electrolyte solution where the concentration of the diffusing ionic species is constant in the yz-plane, but varies in the x-plane (**Figure 1**). The parallelepiped of solution considered is then evenly divided into two compartments by an imaginary 'transit' plane of area A. The diffusion occurs normal to this plane. The outer planes L and R are situated 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 (T) ensuring that, provided the ions are moving in the specified direction, all the ions in each compartment cross the plane T.

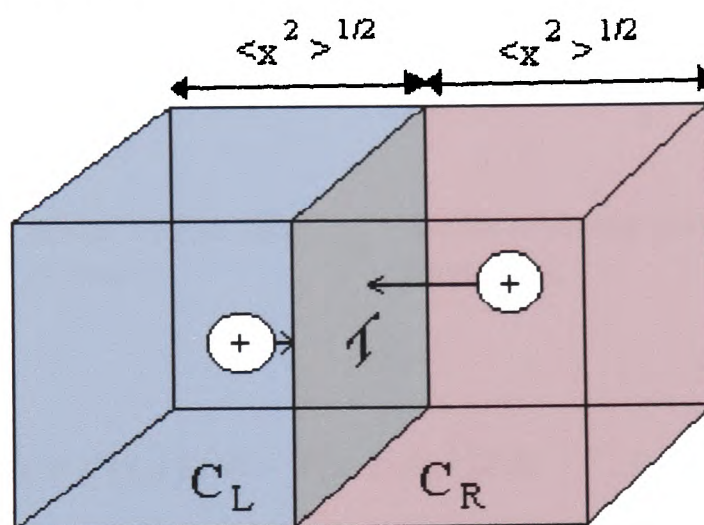


Figure 1 – Parallelepiped of solution used to model the random walk process

As the ions are “randomly walking”, statistically only half the ions will be moving towards the plane T at any given time. Thus in a time interval t,

$$\text{Number of ions making the } L \rightarrow T \text{ crossing is } \frac{1}{2} \sqrt{\langle x^2 \rangle} A C_L \quad (1)$$

$$\text{Number of ions making the } R \rightarrow T \text{ crossing is } \frac{1}{2} \sqrt{\langle x^2 \rangle} A C_R \quad (2)$$

where C_L and C_R are the concentrations of ions in the respective compartments. The total diffusive flux, J , ($\text{mol m}^{-2} \text{s}^{-1}$) of ions across the 'transit' plane in the $L \rightarrow R$ direction is:

$$J = \frac{\sqrt{\langle x^2 \rangle}}{2t} [C_L - C_R] \quad (3)$$

the concentration gradient (dc/dx) in the $L \rightarrow R$ direction can then be written:

$$\frac{dc}{dx} = \frac{[C_R - C_L]}{\sqrt{\langle x^2 \rangle}} \equiv -\frac{[C_L - C_R]}{\sqrt{\langle x^2 \rangle}} \quad (4)$$

Combination of the two expressions results in the following equation:

$$J = -\frac{\langle x^2 \rangle}{2t} \frac{dc}{dx} \quad (5)$$

This is analogous to Fick's 1st law where the diffusion coefficient $D = \frac{\langle x^2 \rangle}{2t}$

Fick's 2nd Law of Diffusion

As for the first law only planar diffusion is considered (**Figure 1**).

For a small volume $A dx$ the concentration changes which occur over the time interval between t and $(t + dt)$ are:

$$\text{Moles within } A dx \text{ at time } (t + dt) - \text{Moles within } A dx \text{ at time } t = \text{Moles entering across plane at } x - \text{Moles leaving across plane at } (x + dx)$$

or

$$c(x, t + dt) A dx - c(x, t) A dx = J(x, t) A dt - J(x + dx, t) A dt \quad (6)$$

where $J(x, t)$ is the flux in the x direction ($\text{mol cm}^{-2} \text{ s}^{-1}$) such that

$$[c(x, t + dt) - c(x, t)] dx = [J(x, t) - J(x + dx, t)] dt \quad (7)$$

rearrangement gives:

$$\frac{\partial}{\partial t} c(x, t) + \frac{\partial}{\partial x} J(x, t) = 0 \quad (8)$$

Finally, substitution of Fick's 1st law for $J(x, t)$ leads to Fick's 2nd law:

$$\frac{\partial}{\partial t} c(x, t) = D \frac{\partial^2}{\partial x^2} c(x, t) \quad (9)$$

Appendix 2

Chemical Inventory

Chemical	Supplier	Purity	CAS‡ Number
1-dodecylpyridinium chloride	Sigma-Aldrich	98 %,	[139549-68-1]
acetic acid	Sigma-Aldrich	≥99.99 %	[64-19-7]
ammonia	BOC	>99.9 %	[7664-41-7]
ammonium nitrate	BDH	≥98 % pure ≤0.001 % Pb	[6484-52-2]
argon	BOC	Pureshield	[7440-37-1]
boric acid	Sigma-Aldrich	Ultra grade	
bovine submaxillary gland mucin	Sigma-Aldrich	Type I-S, Lyophilized powder. Bound sialic acids approx. 12 %	[84195-52-8]
cadmium standard solution	BDH	1000 ppm	[10325-94-7]
ceruloplasmin (human)	CN Biosciences Inc (Nottingham, UK)	> 90 % pure lyophilised powderd	[9031-37-2]
copper (II) nitrate	Sigma-Aldrich	99.99+ % total metallic impurities <100 ppm	[19004-19-4]
DMF	Sigma-Aldrich	99.9 + % HPLC grade	[68-12-2]
ethyl acetate	Sigma-Aldrich	HPLC Grade 99.8 %	[141-78-6]
ethyl acetoacetate	Sigma-Aldrich	≥ 99 %	[141-97-9]
ferrocene	Sigma-Aldrich	98 %	102-54-5
horse blood	Oxoid Ltd (Basingstoke, UK)	Laked	
lactic acid sodium salt	Sigma-Aldrich	60 % w/w syrup	[312-85-6]
lead standard solution	BDH	1000 ppm in nitric acid	[10099-74-8]
lithium wire	Sigma-Aldrich	99.9 %	[7439-93-2]
mercuric acetate	Sigma-Aldrich	≥ 99.999 %foreign heavy metals <0.002 %	[1600-27-7]
Nafion®,	Sigma Aldrich	Fluka brand, at 5 % concentration in a mixture of lower aliphatic alcohols and water	[31175-20-9]
N-benzoyl-N-phenyl-hydroxylamine	Sigma-Aldrich	98 %	[304-88-1]
nitric acid	Sigma Aldrich	70 % PPB/Teflon grade with trace metal impurities in ppt determined by ICP-MS	[1310-73-2]
nitrotoluene	Sigma-Aldrich	99 + %	[88-72-2]
potassium chloride	BDH	≥99.5 % pure ≤0.01ppm Pb	[7447-40-7]
potassium citrate	BDH	≥99.05 pure ≤0.0005 % Pb	[6100-05-6]

potassium iodide	Sigma-Aldrich	99.8 %	[7681-11-0]
potassium phosphate	Sigma-Aldrich	≥ 99.9 % pure ≤ 0.001 % Pb	[7778-77-0]
Ribena® Light™	Smithkline Beechams, UK		
sodium acetate	Sigma-Aldrich	≥ 99.995 %	[127-09-3]
sodium chloride	BDH	≥ 99.9 % pure ≤ 0.0002 % Pb	[7647-14-5]
sodium dodecyl sulphate	Sigma-Aldrich	≥ 99 %	[151-21-3]
sodium hydroxide	Sigma-Aldrich	volumetric standard 1.0 M solution in ASTM reagent grade water	[1310-73-2]
tetrabutylammonium hexafluorophosphate	Fluka	Electrochemical grade	[3109-63-5]
tetrabutylammonium nitrate	Sigma-Aldrich	≥ 99 %	[1941-27-1]
Triton® X-100	Sigma-Aldrich	Average $M_w=646$	[9002-93-1]
urea	Sigma-Aldrich	≥ 99 %	[57-13-6]
uric acid sodium salt	Sigma-Aldrich		[1198-77-2]
vanilla extract	Culpepper Ltd (Cambridge, UK)		
vanillin	Sigma-Aldrich	≥ 98 %	[121-33-5]

‡Chemical Abstracts Registry Number

Appendix 3

Differential Pulse Voltammetry Simulation Theory

The theory of DPV is considered for the following electrochemically reversible redox reaction at a uniformly accessible electrode:



The current generated during a DPV experiment is given by:

$$I = FAJ \quad (2)$$

where A is the area of the electrode, J is the flux to the electrode, defined as:

$$J = D_{\text{R}} \left(\frac{\partial [\text{RED}]}{\partial y} \right)_{y=0} \quad (3)$$

and y is the Cartesian coordinate normal to the electrode surface.

Theory will first be considered for the case of a Nernst diffusion layer, of thickness δ , in which transport is by diffusion alone and outside of which concentrations are maintained at “bulk” values. In this case, the concentration of RED and OX is calculated by applying Fick’s second law of diffusion:

$$\frac{\partial [\text{RED}]}{\partial t} = D_{\text{RED}} \frac{\partial^2 [\text{RED}]}{\partial y^2} \quad (4)$$

$$\frac{\partial [\text{OX}]}{\partial t} = D_{\text{OX}} \frac{\partial^2 [\text{OX}]}{\partial y^2} \quad (5)$$

These equations are solved and concentrations of RED and OX obtained throughout the diffusion layer. In order to solve the equations, boundary conditions are required. The first of these is that initially, the potential is set so that the RED/OX equilibrium lies far to the RED side. In practical terms this means that the concentration of RED is at its bulk value and the concentration of OX is zero everywhere. Algebraically, at $t = 0$, $0 < y < \delta$,

$$[\text{RED}] = [\text{RED}]_{\text{bulk}}, [\text{OX}] = 0 \quad (6)$$

The second boundary condition specifies that the concentration of RED will tend towards its bulk value beyond the edge of the diffusion layer, while that of OX will be zero. At $t > 0$, $y = \delta$,

$$[\text{RED}] = [\text{RED}]_{\text{bulk}}, [\text{OX}] = 0. \quad (7)$$

At the electrode surface, the redox reaction is assumed to be reversible and therefore the concentrations of RED and OX are given by the Nernst equation, at $t > 0$, $y = 0$

$$\frac{[\text{OX}]_0}{[\text{RED}]_0} = e^{\theta} \quad (8)$$

where

$$\theta = \frac{F}{RT}(E - E^{0'}) \quad (9)$$

and $E^{0'}$ is the formal potential of the redox couple. E is the potential at time t , given by the following equation:

$$E = \begin{cases} E_0 + (t-1)E_s/2 & t = 1,3,5... \\ E_0 + (t-2)E_s/2 + E_a & t = 2,4,6... \end{cases} \quad (10)$$

where E_0 is the initial potential and E_s and E_a are the step potential and amplitude potential of the DPV waveform, defined in s.1.2.4.3.1, Figure 11.

The final boundary condition specifies conservation of flux at the electrode surface, when $t > 0$, $y = 0$

$$D_{\text{OX}} \frac{\partial [\text{OX}]}{\partial y} = -D_{\text{RED}} \frac{\partial [\text{RED}]}{\partial y} \quad (11)$$

Computer solutions to these equations were obtained by use of the finite difference backwards implicit (BI)¹⁻³ method with a back-to-back grid. A full explanation of the procedure is given elsewhere⁴, and involves dividing the diffusion layer into $2\text{NJ}+1$ points, where NJ is an integer. These points are then labelled by the coordinate j . The values of RED are stored at values of j from 0 to NJ , the values of OX are stored at the equivalent grid points in negative space, i.e. from -1 to $-\text{NJ}$. A spatial coordinate transform⁵ is employed to increase the efficiency of the simulation.

In the simulations the diffusion layer thickness can be set to a value large enough that a semi-infinite diffusion layer effectively pertains (e.g. 300×10^{-4} cm) or to finite values, for example those which might be created due to the action of ultrasound or rotation of an electrode. With these parameters specified and the boundary conditions in place, time-dependent^{6,7} solutions of the mass transport equations can be obtained by the BI method.

In the case of an experiment using a Rotating Disc Electrode, a convective term is added to the equations describing the mass transport behaviour of RED and OX⁸.

$$\frac{\partial[\text{RED}]}{\partial t} = D_{\text{RED}} \frac{\partial^2[\text{RED}]}{\partial y^2} + Cy^2 \frac{\partial[\text{RED}]}{\partial y} \quad (12)$$

$$\frac{\partial[\text{OX}]}{\partial t} = D_{\text{OX}} \frac{\partial^2[\text{OX}]}{\partial y^2} + Cy^2 \frac{\partial[\text{OX}]}{\partial y} \quad (13)$$

where Cy^2 is the convection term normal to and close to the electrode. The constant C is given by⁹:

$$C = 8.032 W^{3/2} \nu^{-1/2} \quad (14)$$

where W is the speed of rotation of the disc in Hz, and ν is the kinematic viscosity in units of $\text{cm}^2 \text{s}^{-1}$.

The diffusion layer thickness that is created by the motion of the RDE is predicted by the following equation⁹:

$$\delta = 0.643 \nu^{1/6} D_{\text{RED}}^{1/3} W^{-1/2} \quad (15)$$

A coordinate transform, based on the Hale transformation^{8,10,11}, was also used to improve the efficiency of the simulation. The same boundary conditions apply as for the non-convective simulation and the mass transport equations are solved in a similar fashion.

The accuracy of the computer simulation depends on the two parameters: NJ , the number of spatial points over which the equations are solved (the larger NJ , the finer the grid, and hence the more accurate the results) and NL , the number of times at each DPV pulse or rest period, the calculations are carried out. The simulation was first run at extremely high NJ and NL values (= 10000 points) to ensure that the result obtained was free from any errors due to the coarseness of either temporal or spatial measurements. The number of points was then reduced until the simulation ran within 99% accuracy of these results, at NJ and $NL = 500$. This resulted in the simulations running within an

acceptable time (approximately 3 minutes for each voltammogram).

The simulations were compiled using FORTRAN 77 and executed on a Silicon Graphics Origin 2000 server, except the equation involved in the Hale transformation, which was solved using Matlab v.5.1.0.421.

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Appendix 4

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- 1) Anodic stripping voltammetry of copper at insonated glassy carbon based electrodes: application to the determination of copper in beer. *The Analyst* **1999**, *124*, 1053
- 2) The 20 kHz sonochemical degradation of trace cyanide and dyestuffs in aqueous media. *New Journal of Chemistry*, **1999**, *23*, 845
- 3) Sonoelectrochemical and sonochemical effects of cavitation: correlation with interfacial cavitation induced by 20 kHz ultrasound. *Ultrasonics Sonochemistry* **2000**, *7*, 7
- 4) Sonoelectroanalysis: ultrasonically facilitated liberation and determination of copper in whole blood, *Electroanalysis*, **2000**, *12*, 559
- 5) Sonoelectroanalytical determination of heavy metals in fish gill mucous, **2001**, *13*, 89
- 6) Differential pulse and chronoamperometric studies of insonated systems: acoustic streaming and cavitation effects, *J. Phys. Chem. A*, **2001**, *105*, 666.
- 7) Biphasic sonoelectroanalysis: simultaneous extraction from, and determination of vanillin in food flavoring. *Electroanalysis*, **2001**, *13*, 899
- 8) The electroanalytical detection and determination of copper in heavily passivating media: ultrasonically enhanced solvent extraction by N-benzoyl-N-phenyl-hydroxylamine in ethyl acetate coupled with electrochemical detection by sono-square wave stripping voltammetry analysis. *Analyst*, **2001**, *126*, 11
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