C H A R G E T R A N S F E R R E A C T I O N S
A T T H E I N T E R F A C E
B E T W E E N T W O I M M I S C I B L E
E L E C T R O L Y T E S O L U T I O N S

by

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ABSTRACT

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CHARGE TRANSFER REACTIONS AT THE INTERFACE BETWEEN TWO IMMISCIBLE ELECTROLYTE SOLUTIONS

by Carl Antony Beltrán

Electron transfer between aqueous and immiscible non-aqueous solutions of redox mediating species has been demonstrated. 1,1'-Hexadecyl-4,4'-bipyridinium, di-tetra-kis(4-chlorophenyl)borate, $[C_{16}V^{2+}(TPhBCl^{-})_2]$, was used in the organic phase, viz. 1,2-dichloroethane in contact with an aqueous ferro-ferric citrate reducing redox couple.

From simple four electrode cyclic voltammetric experiments at the 1,2-dichloroethane/water interface a marked difference in the behaviour of the system was observed for the reduction of $C_{16}V^{2+}$ to the radical cation, $C_{16}V^{+\cdot}$, depending on whether either anaerobic or aerobic conditions were employed. In the absence of the aqueous couple, that is, in the presence of aqueous base electrolyte only, $C_{16}V^{2+}$ exhibited an interesting adsorption phenomenon at the interface. The latter is akin to that of viologen film formation/reduction or metal deposition at a metal electrode.

Many important biological compounds exhibit irreversible redox behaviour at electrode surfaces as a result of slow heterogeneous electron transfer. Redox mediator compounds, by facilitating the electron transfer process, can provide a technique by which the electrochemical behaviour of biological redox species can be studied. The successful oxidation of the coenzyme, β-dihydronicotinamide adenine dinucleotide, [NADH], chosen by virtue of its biological importance as a soluble cofactor for many redox enzymes in electron transport chains, has been achieved at the 1,2-dichloroethane/water interface using a cationic hydrophobic redox mediator, Meldola's Blue (a benzophenoxazine derivative), and similarly, though less efficiently with the thionine derivative, Methylene Blue. A more stongly hydrophobic dihexylamino-substituted thionine derivative was also studied. The experiments were carried out using partitioning ions in both phases to fix the interfacial Galvani potential, and also, preliminary voltammetric studies with these dyes have been conducted.

CONTENTS

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DEDICATION

To my mother and father.

CHAPTER ONE

INTRODUCTION

1.1.1 Bioelectrochemistry

In recent years there has been a renewed interest in the application of electrochemical techniques to attempt to furnish solutions to problems of biological interest, viz. bioelectrochemistry. The main topics of bioelectrochemistry have been outlined by Koryta [1,2] and can be briefly summarised as follows: (i) Thermodynamics and kinetics of redox and proton-transfer processes of components of biological systems, particularly biological membranes, and their analogues. These include charge transport in cell respiration and photosynthesis as well as ion transport in the channels of excitable cells. (ii) Electrokinetic phenomena, mechanoelectrical phenomema and dielectric properties of biological membranes. (iii) Applied bioelectrochemistry: (a) electroanalytical methods including biosensors and ionophore based ion-selective electrodes, (b) biocatalysis for electrosynthesis, and (c) biological fuel and other galvanic cells. (iv) Applications of electrochemistry in biological systems: (a) electrochemical assay in vivo (neurological applications) using ion-selective or amperometric microelectrodes, (b) power sources and stimulation electrodes (heart pacemakers), and (c) electrochemical detoxification and cofactor regeneration in biosynthesis.

Numerous papers devoted to these last mentioned subdivisions of bioelectrochemistry have been published. The subject has also been dealt with in several recent specialist publications, mainly monographs [3-12] and conference proceedings [13-18].

1.1.2 Aims of the Project and Research Strategy

The major objectives of the present project were to develop a simple

model membrane system in order to investigate heterogeneous electron transfer reactions occurring between redox centres present in different media. This process has been demonstrated recently [19-25]. The work used primarily voltammetric techniques for the study of the physical chemistry of charge transfer processes occurring at the interface between two immiscible electrolyte solutions, known by the acronym ITIES. More than 200 publications have been dedicated to the study of the polarisation phenomena and charge transfer at the ITIES since Guastalla and Gavach [26] pioneered the use of electrochemical techniques to polarise the water/nitrobenzene interface. The area has been reviewed extensively in over fifteen review articles and specialist publications to date. The latest and most comprehensive reviews are those of Girault and Schiffrin [27], Koryta [28], Samec [29] and Volkov [30]. The physical properties and current ideas advocated for the structure of the ITIES are summarised in the next section (1.2). The studies of the ITIES throughout the project have been based largely upon the recent successful advances in the study of heterogeneous electron transfer at the ITIES (water/1,2-dichloroethane) by Geblewicz et al. [24,25] who showed that from microelectrode voltammetric experiments, the replacement of a metal electrode by a concentrated solution of a fast redox couple leads to the same half-wave potential ($E_{\frac{1}{2}}$) for electron transfer in the corresponding ITIES system. Also, a simple formalism for a two phase redox equilibrium system in the presence of a partitioning ion (viz. a redox phase transfer catalyst, PTC) is presented. The role of the PTC is shown to fix the interfacial Galvani potential and hence determine the position of equilibrium. More importantly, these results were shown to correlate well with voltammetric data, i.e. where the interface is polarised externally by the use of electrodes placed in each phase.

As initially proposed by Koryta et al. [31], by presenting a barrier to charge transfer between electrolyte solutions, the ITIES could serve within certain limits as a model for half of a biological membrane. How one regards the membrane as an ITIES system depends on whether one considers the microscopic or macroscopic properties of the membrane, viz. for an ITIES system one really has to consider the membrane microscopically as a very thin barrier between aqueous bulk phases.

Ultimately, it is hoped that an ITIES model can provide an easily accessible biomimetic model for studying the elementary steps of biological charge transfer reactions. Of particular importance is the oxidation of the coenzyme β-dihydronicotinamide adenine dinucleotide (NADH),(Fig. 1.1). In the electron transfer chain (Fig.1.2) in mitochondria, NADH is oxidised by dioxygen through a series of intermediate heterogeneous electron transfers between membrane constituents and redox coenzymes. The largest group of redox enzymes known to date, the dehydrogenases, is where the enzymatic process depends on a soluble nicotinamide coenzyme; approximately 250 depend on NAD^+/NADH and 150 on $NADP^+$/NADPH. It is the heterogeneous oxidation of NADH in the context of a two phase redox ITIES system, by driving the reaction electrochemically (and chemically) which constituted the ultimate objective of the project.

Biological oxidation-reduction couples, such as the above mentioned NAD^+/NADH couple, are not expected to react significantly with a solid electrode, typically metal. Indeed, the nicotinamide coenzymes are excellent examples of biological redox species which exhibit irreversible redox behaviour at electrode surfaces, as a result of slow heterogeneous electron transfer, and also show a strict mode of homogeneous electron transfer with other redox species. The latter is

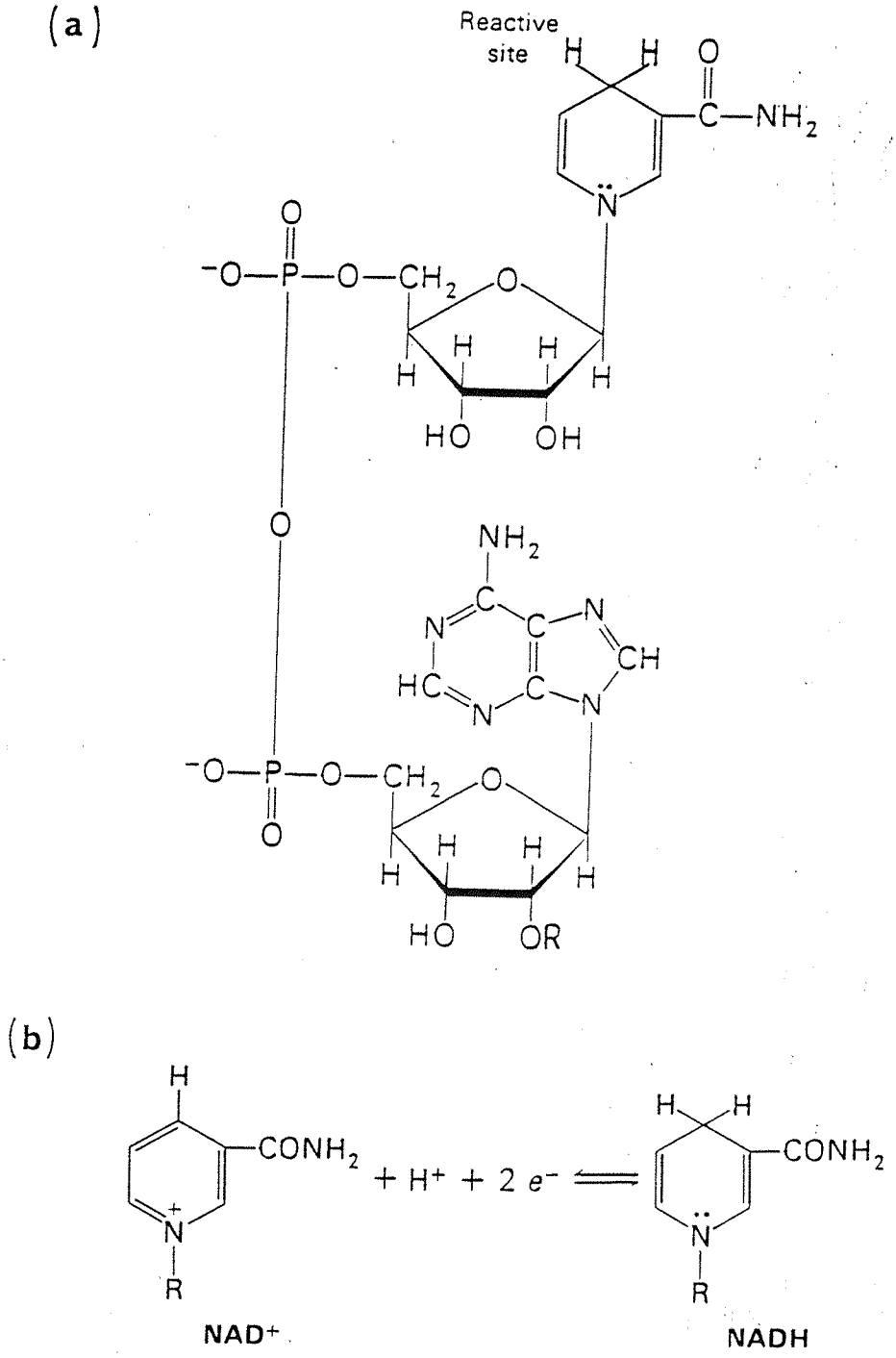


Figure 1.1:
(a) Structure of the reduced forms of nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH). In NADH, R = H; in NADPH, R = PO_3^{2-}.

(b) Simple representation of overall redox process occurring at the redox active site of NAD/NADH (Standard Potential at pH7.0 is -320mV vs. SHE)

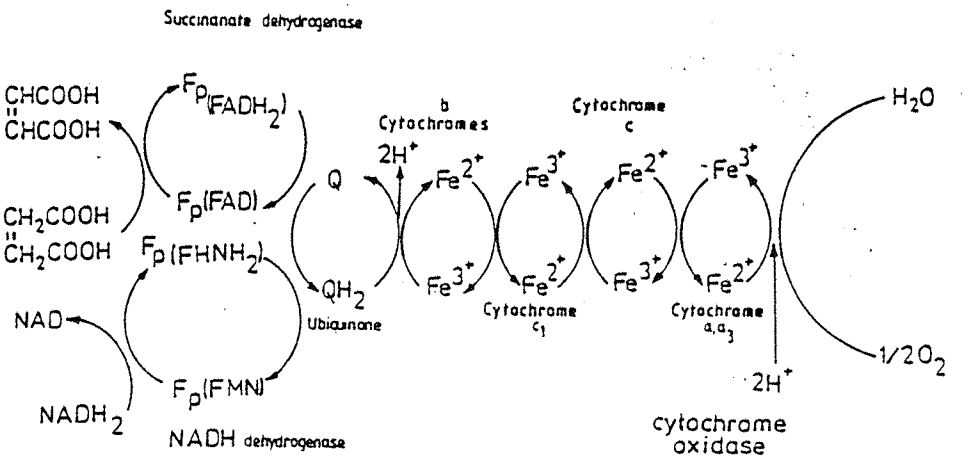
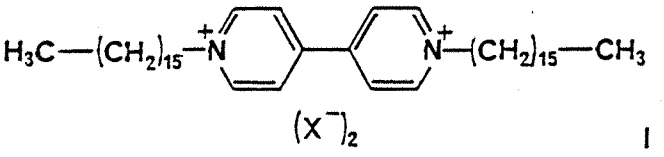


Figure 1.2:
A simple scheme for the respiratory chain in which NADH is one of the major substrates.

not surprising since in nicotinamide-dependent dehydrogenases, the coenzyme appears to be well embedded into the enzyme. There is a cleft for very specific binding of the soluble coenzyme. Hence, the probability of a direct reaction at an electrode material is low due to non-specific binding at the electrode surface.

The large reaction barriers or steric hindrance prevent electron transfer between the cofactor and electrode. This inaccessibility of charge is most likely to be attributable to a natural selection process which ensures selectivity within the electron transfer chain of the cells [32].

In order to alleviate the latter-mentioned problem of charge inaccessibility, redox mediators may be employed to react effectively with both the electrode and the component under assay and to promote electrochemical equilibrium [33,34]. Methods of electrochemical oxidation of (free) NADH, the latest mechanistic theories and the general criteria for a successful redox mediator, firstly, in a homogeneous system and secondly, in the less well studied heterogeneous two phase system are set out in the following sections (1.3). Several cationic hydrophobic mediating redox dyes were synthesised as their tetrakis(4-chlorophenyl) borate salts ($TPhBCl^-$) with a view to their utility as organic based mediators in the ITIES system (Fig.1.1). These were chosen to act as oxidising agents in contact with aqueous based reducing couples, for example, a fast hydrophilic ferric-ferrous complex couple or prefer ably, NADH. The major reason for using $TPhBCl^-$ salts of the dyes was primarily to safeguard the hydrophobicity of the mediator, i.e. to ensure that the mediator remained in the organic phase, and thus to permit true interfacial heterogeneous electron transfer to occur. The first and in fact major part of the investigation was concerned with illustrating



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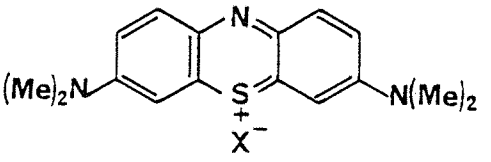

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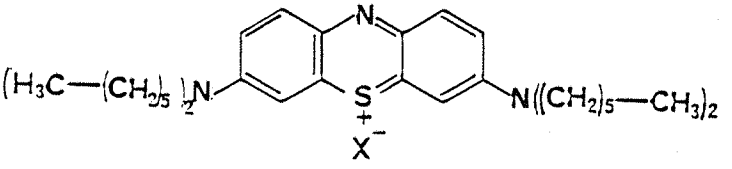
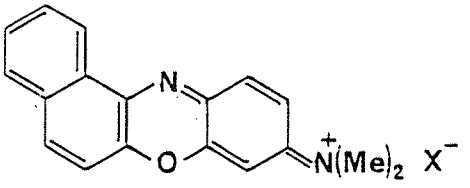


III



IV

$X^- = TPhBCl^-$

Figure 1.3:
The structures of the four redox dyes used throughout the study.

that heterogeneous electron transfers can be observed at the ITIES (water/1,2-dichloroethane) in the case of the reduction of a mediator in the organic phase. For this purpose, a simple hydrophobic viologen dication was used (simple in the fact that the reduction only involves two well-separated 1 electron reduction steps, the first easily accessible reduction to the radical cation was the process of interest), viz. 1,1'-dihexadecyl-4,4'-bipyridinium, di-tetrakis(4-chlorophenyl)-borate; (hexadecylviologen/$C_{16}V^{2+}$); (I), (Fig. 1.3). Secondly, attention was focussed on attempting to oxidise NADH at an ITIES. For this, three monocationic dye species were chosen, (II-IV) (Fig. 1.3), all of which involved a 2 electron/1 proton reduction process (more complex than the viologen mediator). It was hoped that these dyes might be well suited to the mediation of oxidation of NADH, whose own overall oxidation process also involves 2 electrons and 1 proton. In a similar fashion to the viologen, these dyes were examined voltammetrically. However, more use was made of partitioning ions to fix the interfacial potentials between the water/1,2-DCE phases. This allowed the spectrophotometric monitoring of NADH, from the decrease in its characteristic absorption maximum at 340nm. Throughout this study, microelectrode measurements have provided a useful technique for obtaining the redox potentials of the dyes in the organic medium.

1.2.1 A Review of the ITIES

The electrochemical phenomena at the ITIES have attracted substantial interest owing to the extensive range of applications of these systems in chemistry and biology. In particular, the major driving force for their study is the concept that these relatively simple systems might help to elucidate the more complex behaviour of biological cell

membranes. At the ITIES, there exists a boundary potential difference, which in the case of a liquid/liquid interface is closely related to the distribution of ionic and dipolar constituents across the interface. By consideration of the electroneutrality condition, an excess of electrical charge on one side of the interface has to be compensated by the equivalent excess opposite charge on the other side of the interface. These effects give rise to the electrical double layer at the ITIES. The advances in the comprehension of this aforementioned concept are discussed further on in this section. The first direct electrochemical study of the ITIES is attributed to Nernst and Riesenfeld [35] in 1902 who attempted to model a biomembrane, which consisted of a phenol solution between two aqueous phases with the same inorganic electrolyte dissolved in both phases (viz. KI_3^-, K_2CrO_4, $Fe(SCN)_3$, etc). In this pioneering study, they theoretically and experimentally proved the phenomena of accumulation or depletion of the transported electrolyte at the ITIES as being fundamentally dependent on the direction of the current and on transport numbers. During the ensuing decades the research on the ITIES was essentially limited to the investigation of equilibrium potential differences between water and non-aqueous phases in the presence of different electrolytes [36,37-41]. It was not until the mid-1950's that liquid-liquid interfaces under non-equilibrium conditions were again studied and this is most probably attributable to the slow development of the exact thermodynamic descriptions of the ITIES [42-45] and a practical answer to the problem of eliminating liquid junction potentials [45,46].

Guastalla [47-48] studied the water/nitrobenzene interface in the presence of a cationic surfactant, viz. hexadecyltrimethylammonium bromide ($CTMA^+Br^-$), under current flow conditions in which it was found

that the interfacial tension changes. The effect was referred to as electroadsorption. He proposed that the latter phenomenon arises from the interfacial electric field created during current flow. However, these results and those of similar systems [49-53] were only explained satisfactorily by Blank [54] who showed that electroadsorption is entirely a mass transport effect produced by accumulation or depletion of the surfactant at the interface, (as in the Nernst approach [35]) which depended on the concentration of the ions in both phases, the transport numbers and the current density. These studies influenced the discovery of mechanical interfacial movement at the ITIES. Subsequent studies using long-chained cationic surfactants by Dupeyrat [55], Gavach [56,57] and Joos [58,59], provided strong evidence for the explanations offered by Blank. In 1968, Gavach et al. [26] showed for the first time that an ITIES can be polarised. It was shown that for the interface between two immiscible solutions containing a common organic cation, the interface was not polarisable. Steady state polarisation curves for a cell consisting of aqueous potassium chloride (hydrophilic salt) in contact with alkyl(C_{12},C_{14},C_{16}) trimethylammonium picrate (R^+Pi^-) (hydrophobic salt) in nitrobenzene were obtained. Guastalla [60] pursued the phenomenon by measuring the current flowing across the liquid/liquid interface as the response to a triangular voltage scan. However, the lack of potentiostatic control in these experiments, gave seriously distorted voltammograms. Gavach et al. [61-66] obtained chronopotentiograms for polarised and non-polarised systems which were found to be akin in many aspects to those of the metal-electrolyte interface. The kinetic analysis of these data was conducted as in the case of the metal-electrolyte interface, and was also used at high current densities where complications arise due to interfacial salt precipitation [66].

Melroy and Buck resumed these studies [67-70] on the nitrobenzene/water system both in the presence and absence of the base electrolyte. Equilibrium electrochemical principles were applied and an extensive mathematical treatement to interpret ion transfer kinetics by considering interferring ions, partitioning ions, free energies of ion transfer (obtained from partition experiments) and limited potential windows was derived. These previous elementary studies served to illustrate some of the important differences between the ITIES and the charge transfer at metal-electrolyte interfaces [71], viz. (i) Diffusion and migration of ions (Nernst-Planck equation) must be taken into account. (ii) Interfacial transport of ions of opposite signs must be considered because of salt partition equilibria. (iii) Partition equilibrium is a non-linear ionic process which can lead to insoluble equations.

Besides the original chronopotentiometric techniques, modern electrochemical methods have been applied to the study of the ITIES, such as a.c. impedance [61], polarography [31] and cyclic voltammetry [72]. Cyclic voltammetry is used extensively in modern ITIES experiments, particularly in the estimation of Gibbs energies of ion transfer. As in conventional electrochemistry, the interfacial potential must be measured accurately. The voltage clamp technique used in electrophysiology [73], led to the development by Samec and co-workers, in 1979, of the four electrode potentiostat [74]. The problem of the resistance of the electrolyte solutions ('iR' drop) associated mainly with the organic phase, was overcome by the introduction of positive feedback into the potentiostat design, thus permitting iR compensation [77]. Four electrode potentiostats have paved the way since 1979 for successful potential-controlled experiments, besides cyclic voltammetry at the

ITIES, viz. polarography [72, 76], differential pulse-stripping voltammetry [77], chronopotentiometry [78] and a.c.impedance [79]. A six electrode set-up has also been used [80], basically to eliminate side electrolytic phenomena at the ITIES, but the four electrode system has been found to be adequate for most ITIES studies. It is important to note that the measurements of kinetic parameters using these systems are made difficult by the problem of iR drop, particularly as stressed before, in the organic phase, which cannot be fully compensated. This is also coupled with the problem associated with the organic supporting electrolyte which is required to reduce the iR drop; in high concentrations (10^{-3}M to 10^{-1}M), as used typically in most four electrode ITIES, the strong ion-pairing phenomenon of the counterion of the electrolyte and the ion studied perturbs the experiment. This is due to the low dielectric medium.

Prior to the consideration of categories of charge transfer reactions at the ITIES, it is necessary to consider the structure of the double layer at the ITIES, which hitherto, has not been fully appreciated. This is essential since mechanisms of charge transfer cannot be accurately described without an adequate, well-defined model of the double layer which has really only been available over the last six years. The criteria established for ITIES systems and the development of new solvent and supporting electrolyte systems are also briefly reviewed.

The two major limitations to the study of the ITIES are the limitations in the potential window and solvent suitability. The former problem has been addressed very recently where the use of highly hydrophobic ions in the organic phase has been successful in extending the polarisation window. Typically, these salts are crystal violet [81], ethyl violet [82], μ-nitrido-bis(triphenylphosphorus) chloride; (PNP^+Cl^-) [83]

cations, with corresponding counter anions such as $TPhBCl^-$ [24], (the latter is used owing to the increased solubility and extended potential window with respect to tetraphenyl borate; ($TPhB^-$), 1,2-dicarbollylcobaltate(III); (DCC^-) [84] and dipicrylaminate; (DA^-) [84]. Conversely, the principle of salting out of the organic electrolyte has been applied [85] by using concentrated solutions of $MgSO_4$ and Li_2SO_4 to extend the available potential window. For the latter problem mentioned, the requirements imposed for the suitability of the non-aqueous solvent have slowed down the progress of the development of new solvents, viz. (i) the solvent must be immiscible with water and should differ in its density significantly from that of water to permit the formation of a stable interface, (ii) it should be sufficiently polar, with minimum dielectric constant about 10, to allow sufficient conductivity of the non-aqueous phase. Nitrobenzene (nb) and 1,2-dichloroethane (1,2-DCE) meet these criteria well and consequently have been the most commonly used solvents for ITIES experiments. However, these solvents are toxic, and new solvent systems for the investigation of bio-components at the ITIES would be welcome [86].

The validity of the extrathermodynamic assumption advocated by Parker [87] must be examined with respect to new solvents. On the basis of this assumption, which states that the anion and cation of tetraphenylarsonium tetraphenylborate (TPhAsTPhB) have equal standard Gibbs energies of transfer between any arbitrary pair of solvents, a scale for standard Gibbs energies of transfer of ions from one solvent to another can be calculated. The latter is analogous to the standard electrode potential. Girault and Schiffrin [88] have suggested an alternative approach for calculating the energy scale, which has been based on the current theory developed for the double layer at the ITIES.

It was suggested that a Gibbs energy scale could be established from the potential of zero charge at the liquid/liquid interface, assuming that in the absence of specific adsorption, the interfacial Galvani potential difference at this point is zero, and thus, the Gibbs energies of transfer can be referred to this potential. The dependence of the Gibbs energies of ion transfer on the solvent has meant that each new system has had to be reinvestigated as far as the transfer energies are concerned. Most investigators have tended to continue their studies with the established solvent systems. Geblewicz et al. [89,90] have outlined many of the differences between the nb and 1,2-DCE systems. A systematic approach to the choice of solvent has only really been adopted recently, i.e. taking into consideration ion solubilities (from size parameters), immiscibility and conductivity. Other ITIES solvents which have been used and have met with varying degrees of success, are propriophenone [91], 4-isopropyl-1-methyl-2-nitrobenzene [92,93], dichloro-methane [94], methylbutylketone [95], isobutylmethylketone [96], o-nitrophenyloctylether [97], adiponitrile, dibutylcarbonate and dioctylcarbonate [92], 1,2-dichlorobenzene [98], benzonitrile [99] and acetophenone [100].

The study of the electrochemical double layer at the ITIES has received much attention and has been examined using interfacial tension measurements [36,101-107], a.c. impedance [108-113] and galvanostatic pulse techniques [114-116]. The results of these studies have led to the elaboration of a rather well-defined model of the ITIES. The Verwey-Niessen model [36], represented the double layer as a diffuse double layer and the respective space charge regions were analysed with the help of the Gouy [117] and Chapman [118] theory (GC). By analogy with Stern's modification of the GC theory, Gavach and co-workers [119]

extended the VN model in which they introduced the concept of an ion-free compact layer of oriented solvent molecules separating the two diffuse layers (the MVN model). It was assumed that the applied potential difference existed across this layer. From this model and assumption, the theories developed previously for heterogeneous electrochemical kinetics were applied to the ITIES [120,121]. Based upon the evidence of very recent studies, the latter model, although supported by several investigations [105,122], is now thought to be incorrect. Strong evidence against the compact layer hypothesis originates from the fact that the potential drop across the layer is small (~0-50mV) [104,106,107,109] but on the other hand, the inner layer capacitance is significantly larger ($\sim 1 F.m^{-2}$) [107,110,112,123]. Further investigations by Girault and Schiffrin on the solvent surface excess [105], in order to derive the "inner layer" thickness of the ITIES and of the potential of zero charge [124], endorse the latter measurements. By consideration of these results, these investigators [105] have postulated a model in which the interface can be regarded as consisting of a mixed solvent layer, as opposed to two well-defined regions of pure solvent, with no preferential orientation of the dipolar solvent molecules. Confirmation of this model of interfacial solvent mixing, has been obtained by Samec et al. [112], who analysed capacity measurements using the Gouy-Chapman theory. It was demonstrated that most of the ITIES potential drop is confined to within the two diffuse double layers. This overall approach has been incorporated into several mathematical interpretations of the double layer [125-127], which illustrate that for high surface charge densities, the Gouy-Chapman model fails. A qualitative model of the ITIES mixed solvent model is given in Fig. 1.4. If this model is correct, the classical heterogeneous charge transfer theories are inapplicable to

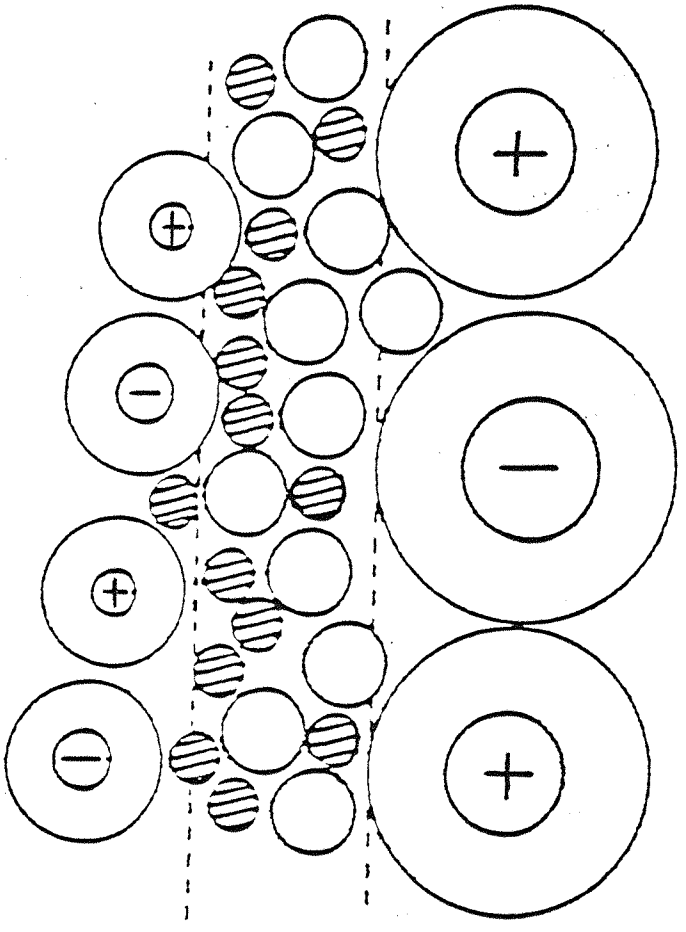


Figure 1.4;
Mixed solvent layer model for the double layer at the polarisable water/ 1,2-DCE interface, where, base electrolytes used are KCl/ Tetrabutylammonium Tetraphenylborate.

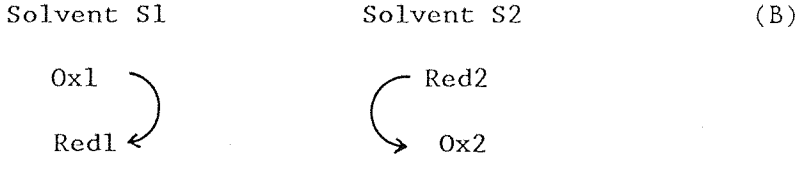
the ITIES. Although the interfacial potential difference across the mixed layer is small in comparison to the Galvani potential difference between the two phases, Girault and Schiffrin [128] have developed kinetic theories for ion transfer, and more recently for electron transfer [129], where the driving force is the total applied potential difference, which excludes the need to refer to a compact inner layer. A different elaborate approach to the kinetics of ion transfer has been taken by Gurevich and Kharkats [130], in which the potential energy barrier of ion transfer at the ITIES was taken into consideration and altered by the applied potential. This model was used to calculate transfer rate constants [131] from which it was concluded that a potential energy barrier was likely to exist. It must be stressed, however, that there is little agreement on the interfacial structure. Recently, Samec [29] has claimed that a mixed solvent model is at variance with recent Monte Carlo (MC) simulations [132]. Obviously, more work is required to elucidate these problems.

1.2.2 Charge Transfer at the ITIES

With the development of electrochemical methodology for the study of the ITIES (as described in 1.2), charge transfer reactions have been studied in detail, viz., (i) ion transfer (Scheme A):

$$\text{Ion in solvent S1} \longrightarrow \text{Ion in Solvent S2} \qquad \text{(A)}$$

and to a less extent, (ii) electron transfer (Scheme B):



It must also be noted that these two processes may be affected by one another.

(i) ION TRANSFER

a) Simple Ion Transfer

Initial ion transfer experiments at ITIES systems were centred on the study of the transfer of the tetramethylammonium ion (TMA^+) from water to nb performed chronopotentiometrically [133]. The process was shown to be diffusionally controlled. Cyclic voltammetry confirmed this reversible behaviour [66]. Similarly, the transfer of the picrate anion was also shown to be reversible. On the other hand, these early experiments conducted with perchlorate, thiocyanate and nitrate, showed only transfer currents from nb to water, which can be accounted for by the limitations imposed by the simultaneous transfer of the base electrolyte used, ie tetrabutylammonium (TBA^+) transfer from water to nb. By using new more hydrophobic supporting electrolyte cations, the latter-mentioned anions were shown to behave reversibly [81,134] and standard Gibbs transfer energies and diffusion coefficients were estimated. Examples of the many cation transfers studied to date at the water/nb ITIES are those of tetraalkylammonium ions [92,107], choline and acetylcholine [135,136], Cs^+ [75,137], tris(2,2'-bipyridine)Ru(II), dialkyl(C_1-C_7)viologens [94,138] and K^+ [139]. Similarly, the anion transfers from water to nb investigated have been: picrate [92,134], perchlorate, laurylsulphate and octoate [140], iodide, nitrate and thiocyanate [81], tetrafluoroborate [141], and very recently for the heteropolyanion, 12 tungstosilicate (charge -4) [142]. At the water/1,2-dichloroethane (1,2-DCE) interface, the transfers of TMA^+, tetraethylammonium (TEA^+), TBA^+ and Cs^+ ions have been studied by Geblewicz et al. [143,144] and also by Wang and co-workers [145]. Tris-

(2,2'-bipyridine)Ru(II) and dialkyl (C_1 to C_7) viologen cations (the latter two also at the water/dichloromethane interface [138]) have also been investigated and simple reversible ion transfers have been observed. The rate constants for ion transfer for tetralkylammonium ions, Cs^+ and acetylcholine/choline [137,146] have been measured, but the values obtained are very much dependent on the double layer model chosen.

Alemu and Solomon have investigated simple ion transfers across the o-nitrotoluene/water [147] and more recently the water/benzonitrile [99] interfaces. The standard Gibbs energies of transfer were analysed using Abraham's solvation model [148], which employs a semi-empirical treatment to calculate the transfer energy. In recent work on ion transfers at the electrolyte dropping electrode, Kihara et al. [149] studied the transfer of halides, their oxoacid anions and other polyatomic anions at the water/nb and water/1,2-DCE interfaces. A pH dependence was noted for the transfer of tetracyclines [150], such as terramycin [151], where the transferred ion is the protonated tetracycline cation. This exists predominantly at pH > pK_1 as the zwitterion in aqueous solution, and hence, the mechanism involves transfer of the species to the interface, with subsequent cation formation, followed by transfer. Thus, in acidic media, simple ion transfer is observed. A similar effect has also been noted for aniline and other amines [179,152], and for 1,10-phenanthroline derivatives [153]. Wang et al [154] has reviewed studies of many analytically important acidic dye transfers at water/nb, water/1,2-DCE and mixed organic solvent/water interfaces, eg Bromocresol Green and Bromophenol Blue. The dyes were identified spectroscopically, and displayed complicated transfers depending on the transfer of the anionic and cationic components. Sabela and co-workers [155] have also observed the

same effect for the transfer of negericin at the water/nb interface. In general, where the ion transferred is the salt of a weak acid, the transfer is pH dependent.

Due to the inherently large errors associated with high ohmic resistances for ion transfer, Taylor and Girault [156] developed a micro-ITIES (micro electrode technique), which has offered a useful solution to the study of fast transfer kinetics at the ITIES. Girault et al.[157] have applied this technique to the determination of facilitated ion transfer at micro-ITIES. Very recent work, also by Girault [158,159] and Marecek et al.[160] has illustrated photochemical ion transfer acoss the ITIES, whereby irradiation of the ITIES gives rise to a photopotential or photocurrent which has been shown to be due to the transfer of excited states of the base electrolyte ions. The influence of specific adsorption of organic compounds on ion transfer rates leading to a decrease in rate constants, has been demonstrated quantitatively by Cunnane et al. [161]. TEA^+ ion was used as a probe ion. The activation energy for ion transfer was found to be that of pore formation in a monolayer of adsorbed lecithin at the water/1,2-DCE interface. The pore radius was shown to be in very good agreement with the unhydrated ionic radius of TEA^+. These investigations were carried out at pH 8.6 and in the potential region where no lipid desorption occurred. Earlier work by Samec et al. [162], had addressed the latter system using an a.c.impedance method. This illustrated a strong potential dependence of adsorption of the lipid and that the lipid desorbs within certain regions of the polarisation window. The behaviour of hydrophobic moieties, eg. $HTMA^+$ [163], derivatised affinity dyes [164] and amphiphilic species (lipids [162]) at the ITIES, has illustrated well-defined potential dependencies of adsorption [105]. Schiffrin et al. [165] have shown the strong connection between the pH,

interfacial potential and interfacial tension of phospholipid monolayers at the ITIES. It has been proposed that the coupling between these functions may provide a simple driving force for cell and organelle motility in living organisms. Similar work has also been conducted by Makinko et al. [166].

(b) Assisted (or facilitated/mediated) Ion Transfer.

The second type of ion transfer at the ITIES currently attracting a great deal of interest, is assisted ion transfer. The transfer of ions can be attained at the ITIES by the presence of ionophores or charge carriers in the non-aqueous phase. Two subdivisions of these studies can be considered in the use of: (i) synthetic or (ii) natural ionophores. A detailed review of these systems can be found in publication by Girault and Schiffrin [27]. The former approach is associated with the use of crown ether ionophores to transport ions, such as Li^+, Cd^{2+}, Na^+, K^+, Cs^+ and H^+, for which complex association mechanisms and stability constants have been formulated. Ionophore diffusion from the bulk to the interface has been observed as the rate determining step. The site of ionophore complexation remains controversial, with proposals for both interfacial [167] and aqueous-sited complexation [168] having been put forward. Very recently, Girault et al. [157] have studied the kinetics of transfer of K^+ by dibenzo-18-crown-6 from water to 1,2-DCE using micro-ITIES and have demonstrated an interfacial (E) type complexation. Other synthetic ionophores which have been studied are 1,10-phenanthroline and 2,2'-bypyridine [169] for Fe^{2+}, Fe^{3+}, Ni^{2+} and Zn^{2+}; 2,2'-bypridine for Cd^{2+} [170], (viz. solvent extraction of metals), and proton facilitated transfer by organic bases as mentioned previously,

eg aniline [152] and acridine [154]. The study of natural ionophores has been concerned with the transfer of antibiotics and their analytical detection, as reviewed by Wang and Sun [154], since the levels of accuracy of detection using an ITIES have been found to be comparable with microbiological assay techniques. Some of the systems studied have been those of negericin [155], valinomycin [168], erythromycin [171], monesin [172] and mydecamycin [173]. As in the case of synthetic ionophores, the site of complexation remains unclear in many respects for the latter two antibiotics. However, monesin [172] is now thought to be complexed in the organic phase and it has been shown to behave as a dual transfer agent for H^+ and M^+ (Li^+, Na^+ or K^+).

(ii) ELECTRON TRANSFER.

As mentioned briefly in the first section, the driving force behind the study of heterogeneous electron transfer at the ITIES, has been the idea that the ITIES represents a simple approach to modelling membranes, and it is known that electron transfer reactions occur in living organisms between redox centres present in media of different polarity. Heterogeneous electron transfer was first reported by Guainazzi et al. [93] in 1975, in a somewhat isolated study. The process studied was the reduction of aqueous Cu(II) to Cu using TBA^+(hexacarbonylvanadate$^-$) in 1,2-DCE, by passing a current through the interface. Subsequent work by Samec et al. [21,22] up until 1982 was focussed on the electron transfer reaction between ferrocene (FC) in nb and aqueous ferricyanide. However, these experiments failed to appreciate the fact that the electron transfer was complicated by simultaneous ion transfer. This was borne out in later studies of the transfer of the ferricenium ion across the ITIES by Hanzlik et al. [23]. More recently, electron transfer has been

studied by Geblewicz et al. [24,25]. In order to exclude the problems associated with partitioning of the organic based redox species in the FC system, very hydrophobic organic redox agents with readily accessible redox states were studied. Lutetium diphthalocyanine [24] and tin(IV)diphthalocyanine [25] in 1,2-DCE were used. Makrlik [174] has also investigated the copper/FC system and also developed a theory for electron transfer using a electrolyte dropping electrode (EDE) [175] and chronopontentiometry [174]. Samec et al. [177] have also developed theoretical treatments for the voltammetry at a stationary ITIES and for polarography at the EDE. Very recent work by Kihara et al. [176] in which current scan polarography at the aqueous EDE has been used, has aimed to develop a systematic elucidation of the general features of electron transfer. The oxidising/reducing agents employed in this latter study were 7,7,8,8-tetracyanoquinodimethane (TCNQ), tetra-thiafulvalene (TTF) and FC. Again, it is important to notice that these redox centres are not very hydrophobic, unlike those employed by Geblewicz [24,25], and the polarograms obtained, to some extent, may not express a true heterogenous electron transfer. The kinetics of electron transfer have been predicted theoretically by Samec [178], and as discussed previously, more recently by Girault and Schiffrin [129] ,on the basis of a pre-encounter equilibrium model.

In summary, criteria which have been highlighted by these few studies for true interfacial (heterogeneous) electron transfer are: (i) Highly hydrophobic and correspondingly highly hydrophilic redox components are required in the respective phases. (ii) Components of both phases should be sufficiently redox active. (iii) For successful electron transfers, (a) the formal potentials of both components should be matched closely and, (b) the separation between the two redox components should be small

to allow interaction of the two centres.

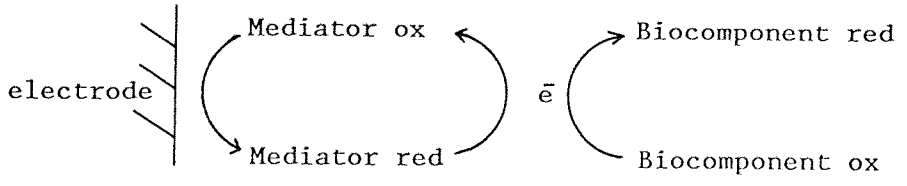
1.3 Redox Mediating Compounds

The importance and role of redox mediators has already been emphasised in 1.1.2. A great deal of attention has recently been focussed on the use of redox mediators in coupled oxidation reactions, amperometric biosensors, microbial fuel cells and bioelectrochemical syntheses using redox enzymes [179]. It is important to distinguish between the different classes of mediator which are possible.

(i) Homogeneous mediation: by far the most widely studied, where the biocomponent and redox mediator are present in soluble form in the same phase.

(ii) Heterogeneous mediation: (a) where the mediator is immobilised at an electrode or membrane surface in contact with an aqueous solution of the biocomponent; (b) where the mediator is soluble, confined in the organic phase, in contact with an aqueous solution of the biocomponent (ITIES system), ie one of the aims of this project.

Many homogeneous redox mediator compounds have been investigated electrochemically to date, mainly in conjunction with biological redox compounds, as shown in the following scheme (C):



(C)

Many comprehensive reviews on the desirable homogeneous mediator characteristics have been published; in particular, those of Dutton and Wilson [33], Clarke [34] and Szentrimay [12], and more recently, by Fultz and Durst [180]. The latter lists many mediators and their

corresponding applications to particular biosystems. The criteria for an 'ideal' homogeneous mediator are listed below:

(i) Well-defined electron stoichiometry.

(ii) Known formal potential, E', which should be close to that of the biocomponent under investigation.

(iii) Fast heterogeneous and homogeneous electron transfer.

(iv) Ready solubility in both oxidised and reduced forms.

(v) Stability in both oxidised and reduced forms.

(vi) No optical interference where optical monitoring of the biocomponent is used and no interaction with the biocomponent in a manner which alters the redox potential.

In order to assess the criteria for a heterogeneous redox mediator for use in a two phase system, the characteristics of the latter case of homogeneous mediators should be compared to those general criteria outlined for the ITIES. This is achieved simply by considering heterogeneous mediation as an extension of the homogeneous case with unidimensional inhomogeneity. Points (i)-(iii) and (v) above remain pertinent to both systems. For the ITIES mediated electron transfers, the redox mediator is required to be highly hydrophobic, ie as far as possible totally insoluble in the aqueous environment. Consequently, since the redox mediator and the biocomponent are present in different phases, organic and aqueous respectively, then condition (vi) presents no problem to the ITIES case. Some specific examples of redox mediators are discussed below, and form the basis for the experimental ITIES investigations throughout the present project.

(i) 1,1'-Disubstituted-4,4'-bipyridinium Dications. (THE VIOLOGENS)

The viologens have received a great deal of interest as a result of their redox properties. Originally, these compounds were investigated

as redox indicators in biology [181], by virtue of their having the most cathodic redox potential relative to any other organic compounds, and their significant electrochemical reversibility. The herbicidal effect of viologens was discovered in 1965 [182], and since attributed to their redox properties [183]. Many recent investigations have been carried out to examine the electrochromic properties of the viologens [184]. The development of radical film formation at metal electrodes from aqueous viologen solutions has been conducted and examined spectroelectrochemically, in particular by Bewick et al. [185]. The simple reduction of the viologens presented in the scheme (D) below:

$$C_nV^{2+}(X^-)_2 \overset{e^-}{=} C_nV^{+}\cdot X^- + X^- \overset{e^-}{=} C_nV + X^- \quad (D)$$

In the case where the alkyl substituent chain length n=C_1 to C_8 was used, then violet-coloured cation films were formed, whereas for n=C_5 to C_6, an aqueous insoluble film resulted. The electrochemical properties of the viologens have been discussed by Bird and Kuhn [186] and Summers [187]. The viologen redox system is also currently extensively utilised as one of the links in redox chains of both homogeneous [188] and heterogeneous [189,190] model systems for solar to electrical energy conversion and photosynthesis mimetic models. The role of the viologen, again, as a consequence of its reversibility, is that of a relay or trapping compound, which maintains the charge separation through the electron transfer from a photosensitive redox couple to an electron acceptor. Photoinduced reaction of $NADP^+$ using methyl viologen as an electron carrier has been shown [191]. Also, on a biological theme, viologens have been used in conjunction with enzymically-modified electrodes to mediate the bioreduction of NAD^+ to NADH [192]. The electrochemical transport across liquid membranes by 1,1'-dihexadecyl-

viologen located at one interface of the membrane has been achieved [193], and later studies have demonstrated this effect in vesicles [194]. Similarly, methyl viologen mediated redox across dihexadecylphosphate vesicles has been observed [195]. The amphiphile, 1,1'-dihexadecylviologen, was chosen for electron transfer studies for its strongly hydrophobic nature and its general electrochemical reversibility (simple 1 electron reduction to the more hydrophobic radical cation). The problem with smaller alkyl-chained moieties, eg. heptylviologen, is that they are transferable at the ITIES (as illustrated by Samec et al.[138]). By investigating the reduction of this apparently simple mediator at the ITIES, with a fast aqueous redox couple it was hoped to establish a simple working model of interfacial reduction of mediators.

(ii) The Oxidation of NADH

As stressed previously, due to its biological importance, the oxidation of NADH has been studied extensively. Methods of electrochemical oxidation of this coenzyme have been discussed by Gorton [196] in 1986. These can be described as follows:

(a) Direct electrochemical oxidation at solid electrode surfaces.

This has been described in very many recent papers, and was first studied quantitatively by Burnett [197]. Many mechanistic studies have been conducted with NADH or on model compounds. Discussions have centred on whether the oxidation involves a two electron transfer or in fact two one-electron steps. Preliminary investigations at Pt electrodes employing high NADH concentations, indicated a second order pH-dependent outer sphere electrochemical reaction [198], as represented in the following

scheme (E):

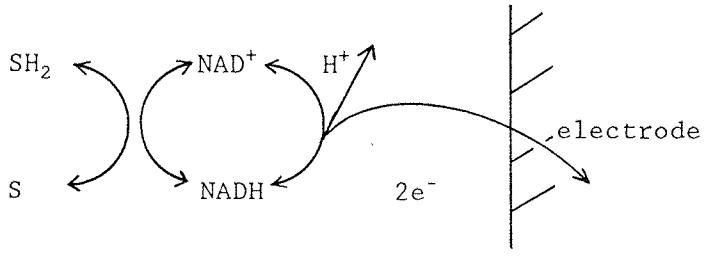
(a)

$$NADH \xrightarrow{slow} NADH^{+}\cdot + e^{-}$$

$$NADH^{+} \rightleftharpoons NAD\cdot + H^{+}$$

$$NAD\cdot \overset{fast}{\rightleftharpoons} NAD^{+} + e^{-}$$

(b) A simple overall representation: (E)



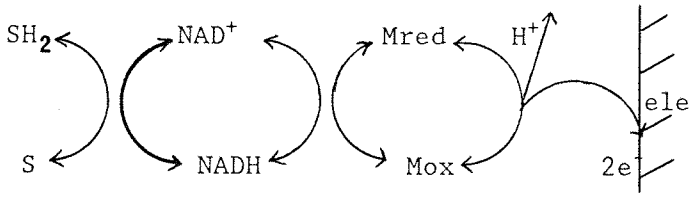
where, SH_2 and S are the reduced and oxidised forms of the particular substrate under consideration. However, this mechanism did not fully explain all observations. It has been concluded that the details of the mechanism are uncertain. In further mechanistic investigations by Studnickova et al. [199] by means of controlled potential electrolysis, a reductive cleavage of NADH was proposed as an alternative mechanism to form NAD^{+} and $NAD\cdot$.

(b) Mediated NADH Oxidation

(i) Soluble Homogeneous Mediator:

In order to increase the rate of electron transfer, the homogeneous mediator must provide a very fast reaction with NADH, followed by electrochemical reoxidation at a potential much lower than that at which NADH is oxidised. As a consequence of this, only a few compounds have sufficed to provide redox coupling (Scheme F) between the biocomponent and electrodes [12,200]. For NADH, the most important mediators have been o- and p-quinones, quinone imines and phenylene dimines [180] whose

structures are incorporated into the larger mediators, in indophenols, phenazines and phenoxazines. Homogeneous mediators have been used analytically for the successful detection of NADH, as described in [196].



(F)

where, Mox and Mred are the oxidised and reduced forms of the mediator respectively. Bennetto et al. [179] have investigated the kinetics of reduction by β-NADH of thionine (TH) and several of its alkyl-substituted derivatives in an aqueous homogeneous system, in order to access their use for biofuel cells. The electrochemical reduction by 'free' NADH was shown to be considerably faster for thionine than its analogues, implying some favourable mechanism. The rate of TH reduction was found to be unaffected by the addition of diaphorase enzyme, whereas the rates of the analogues, with values up to 2 to 3 orders of magnitude smaller than TH in the absence of the enzyme, were enhanced up to six fold with enzyme addition. The large difference in rates was attributed to steric inhibition of the alkyl side chains for NADH reductions carried out with microorganisms [201] which were shown to exhibit no large differences in the reaction rates for TH and its analogues. This tended to suggest that the electron shuttle from organism to mediator is limited by penetration of the cell membranes and not the intracellular NADH-mediator interaction.

(ii) Mediators Immobilized on the Electrode Surface:

This is a rapidly expanding branch of electrochemistry whereby the soluble mediator functionalities, mentioned in part (b.i), can be surface-immobilised in a chemically modified electrode, (CME) [200]

leading to a 'reusable' heterogeneous mediator for NADH. A useful list of mediators used for NADH oxidation and the criteria for successful mediator CME's can be found in Gorton's review [196]. Gorton et al. [202] have investigated adsorbed phenoxazines as mediators on graphite, of which the graphite electrode modified with Meldola's Blue (MB), proved to be the most successful mediator for NADH. The reaction is thought to proceed by a charge-transfer complex (Scheme G) of the immobilised dye with NADH, which decomposes to NAD^+ and the reduced form of the MB, viz. MBH.

$$NADH + Mox = [NADH\ M] \longrightarrow NAD^+ + Mred \quad (G)$$

By analogy with the CME heterogeneous mediation results for NADH and the soluble homogeneous mediators available for NADH oxidation, it was decided to utilise some of these dyes and analogues in the present project. These were used as cationic oil-based mediators in the water/1,2-DCE ITIES system in an attempt to achieve heterogeneous oxidation of NADH. The closest example akin to this type of process is the study carried out by Boguslavsky et al. [203], where the Volta potential difference for the octane/water interface was measured for Vitamin K_3 (electron acceptor) and a etioporphyrin complex (catalyst) in octane in contact with a buffered aqueous NADH solution. For the preliminary investigations, Methylene Blue and Meldola's Blue were examined as organic based mediators for NADH by preparation of their $TPhBCl^-$ salts, (II) and (IV) respectively. The use of more desirable hydrophobic mediators of these and other redox dyes, of which the di-hexyl derivative of thionine (III) was prepared for this preliminary work, is subject to further investigation.

CHAPTER TWO

THEORY OF THE ITIES

2.1. Thermodynamics of the ITIES

The interface between two immiscible electrolyte solutions is normally classified according to different categories, depending on to what extent it is permeable to charged species of the system.

(a) A non-polarised interface: where the interfacial potential is determined by the activities of ions present in both phases in contact.

(b) An ideally polarised interface: where there appears to be an extra degree of freedom in the system, viz. the interfacial charge or potential.

(c) The case where there is charge transfer through the interface. When two immiscible solvents are placed in contact, both containing ions of species i, the work required to transfer an ion from the bulk of one solvent to the bulk of the other will be equal to the difference of the electrochemical potential of the ion in each phase.

$$\Delta\tilde{G}_{t,i}^{\alpha\rightarrow\beta} = \tilde{\mu}_i^{\beta} - \tilde{\mu}_i^{\alpha}$$

$$= (\mu_i^{\beta} - \mu_i^{\alpha}) + [Z_iF(\phi\beta-\phi\alpha)] \qquad \ldots.(2.1.1)$$

In order to analyse an electrochemical system, the electrochemical potential of an ion is considered as consisting of a chemical and electrochemical part (This has no meaningful physical significance): (i) The electrical energy of transfer, where this term is connected with the

transfer of a charge z_iF from the potential $\phi\alpha$ to potential $\phi\beta$, and (ii) The chemical energy of transfer arising from the change in interaction of the ion with its media. $\phi\alpha$ and $\phi\beta$ are the electrochemical potentials of the interior of the α and β phases, known as the inner Galvani potentials, z_i is the ionic charge, and F the Faraday constant. The difference $(\phi\alpha - \phi\beta)$ is called the Galvani potential difference, $\Delta^{\beta}_{\alpha}\phi$ The Gibbs energy of transfer, $\Delta G^{\alpha\rightarrow\beta}_{t,i}(=\Delta^{\beta}_{\alpha}G_{t,i})$, $\phi\alpha$ and $\phi\beta$ are not directly measurable quantities (an inherent restriction arising from the thermodynamics), and thus, they have to be determined with the use of an extrathermodynamic assumption. Many different assumptions have been proposed. The quite widely accepted assumption (described in Chapter 1), due to Parker [87], is considered. This states that:

$$\Delta G_t^{\theta,\alpha\rightarrow\beta}(TPhAs^+) = \Delta G_t^{\theta,\alpha\rightarrow\beta}(TPhB^-) = \tfrac{1}{2}\Delta G_t^{\theta,\alpha\rightarrow\beta}(TPhAsTPhB) \quad \ldots.(2.1.2)$$

The assumption is based on the fact that $TPhAs^+$ and $TPhB^-$ are symmetrical ions, of much the same size and shape, where the charge is buried under the phenyl groups. On the basis of this assumption, a scale for the standard Gibbs energies of transfer can be obtained, viz.

$$\Delta^{\alpha}_{\beta}\phi = -\Delta^{\alpha}_{\beta}G/z_iF \quad \ldots.(2.1.3)$$

In the case of a non-polarisable interface, the equilibrium between the two phases involving single ion i partition is given by:

$$\Delta^{\alpha}_{\beta}\phi_i = \Delta^{\alpha}_{\beta}\phi_i^{\ominus} + (RT/z_iF)\ln(a_i(\beta)/a_i(\alpha)) \quad \ldots.(2.1.4)$$

where $a_i(\alpha)$ and $a_i(\beta)$ are the activities of i in the phases α and β respectively. The transfer of a cation from α to β (typically water (α) and organic (β) phase), occurs at a more positive potential than that of the equilibrium potential. The converse is true for the anion.
A comprehensive list of Gibbs energies of transfer can be found in recent reviews by Volkov [204] and Girault and Schiffrin [27]. For the general case of a non-polarised ITIES (cation B^+ and anion A^-) consisting of the electrolytes (Scheme 2A),

α	β	
BA	BA	SCHEME 2A

assuming electroneutrality in both phases, for dilute solutions:

$$\Delta^{\alpha}_{\beta}\phi = \tfrac{1}{2}(\Delta G^{\theta,\alpha\rightarrow\beta}_{t,A^-} - \Delta G^{\theta,\alpha\rightarrow\beta}_{t,B^+}) \qquad ....(2.1.5)$$

is the distribution potential and is dependent on the ability of ions B^+ and A^- to transfer from α to β. If the transfer of the anion from α to β is easier than cation transfer from α to β, then,

$$\Delta G^{\alpha->\beta}_{t,A^-} < \Delta G^{\alpha->\beta}_{t,B^+} \qquad ....(2.1.6)$$

A more commonly encountered case for single ion transfer is illustrated schematically below:

α	β	
B_3A_1	B_3A_2	SCHEME 2B

Transfers of A_1^- from α to β($\Delta^{\beta}_{\alpha}\phi_{A_2^-}$ large and positive) and that of A_2^- ($\Delta^{\beta}_{\alpha}\phi_{A_1^-}$ large and negative) in the opposite direction, are difficult with

respect to transfer of the common ion B_3^+, as indicated by the following inequalities:

$$\Delta G^{\theta,\alpha\to\beta}_{t,A_1^-} >> 0 \; ; \quad \Delta G^{\theta,\alpha\to\beta}_{t,A_2^-} << 0$$

$$\Delta G^{\theta,\alpha\to\beta}_{t,A_2^-} << \Delta G^{\theta,\alpha\to\beta}_{t,B_3^+} << \Delta G^{\theta,\alpha\to\beta}_{t,A_1^-} \qquad \ldots.(2.1.7)$$

$$\left|\Delta G^{\theta,\alpha\to\beta}_{t,B_3^+}\right| << \Delta G^{\theta,\alpha\to\beta}_{t,A_1^-} \; ; \quad \left|\Delta G^{\theta,\alpha\to\beta}_{t,B_3^+}\right| << -\Delta G^{\theta,\alpha\to\beta}_{t,A_2^-}$$

At equilibrium, the Galvani potential difference is determined only by the activity of B_3^+. That is, if the concentrations of B_3^+ in α and β are comparable and the activities of $A_1^-(\beta)$ and $A_2^-(\alpha)$ are considered to be very small.

An ideally polarised interface is illustrated below (scheme 2C). This consists of two immiscible solvents and containing electrolytes B_1A_1 and B_2A_2 which are essentially confined to their respective phases.

$$\underset{B_1A_1}{\alpha} \;\Big\|\; \underset{B_2A_2}{\beta} \qquad \text{SCHEME 2C}$$

The criteria for an ideally polarised interface are described below (with respect to Scheme 2C); α represents the aqueous phase, and β the non-aqueous phase.

(a) The aqueous electrolyte B_1A_1 (assumed to be completely dissociated into B_1^+ and A_1^-) is strongly hydrophilic, and conversely, B_2A_2 (assumed to be completely dissociated into B_2^+ and A_1^-) is strongly hydrophobic.

(b) The equilibrium exchange processes which are shifted to the left

hand side, are summarised as follows:

$$B_1{+}(w) \; + \; B_2^{+}(o) \rightleftharpoons B_1^{+}(o) \; + \; B_2^{+}(w) \qquad \ldots(2.1.8)$$

$$A_1^{-}(w) \; + \; A_2^{-}(o) \rightleftharpoons A_1^{-}(o) \; + \; A_2^{-}(w) \qquad \ldots(2.1.9)$$

$\Delta^{w}_{o}\phi$ for the cation in (w) and the anion in (o) must be as positive as possible, and conversely, for the cation in (o) and the anion in (w), $\Delta^{w}_{o}\phi$ must be as negative as possible. A sufficiently large difference between the less positive value of $\Delta^{w}_{o}\phi$ for the first pair (2.1.8), and the least negative value of $\Delta^{w}_{o}\phi$ for the second pair (2.1.9). Therefore, it follows from these criteria, that a definite potential range exists in which the interfacial Galvani potential can be fixed externally without passing a charge across the interface. This is referred to as the 'potential window' (or polarisation window).

2.2. Redox System Equilibrium

The general redox process in a two phase system may be expressed as:

$$n_2O_1^{(w)} + n_1R_2^{(o)} \rightleftharpoons n_2R_1^{(w)} + n_1O_2^{(o)} \qquad (2.2.1)$$

where the stoichiometric n_1 and n_2 are related to the separate redox couples:

$$O_1^{(w)} + n_1e \rightleftharpoons R_1^{(w)} \qquad \ldots.(2.2.2)$$

and

$$O_2^{(o)} + n_2e = R_2^{(o)} \qquad \ldots.(2.2.3)$$

The equilibrium conditions for the redox process described in (2.2.1) can be represented in terms of individual ionic activities and the Galvani potential difference of each phase, giving:

$$\Delta^w_o\phi = E^\theta(R_2/O_2) - E^\theta(R_1/O_2) + (RT/n_1n_2F)\ \ln J_a \qquad \ldots.(2.2.4)$$

where J_a is given by:

$$J_a = (a^{n_2}_{R_1} a^{n_1}_{O_2}) \Big/ (a^{n_2}_{O_1} a^{n_1}_{R_2}) \qquad \ldots.(2.2.5)$$

The interfacial Galvani potential difference $\Delta^w_o\phi$ is given by:

$$\Delta^w_o\phi_i = (\phi_i^{(w)} - \phi_i^{(o)}) \qquad \ldots.(2.2.6)$$

where ϕ_i is the inner potential difference of phase (i), E^θ_i is the standard potential referred always to the same standard reference electrode in one phase, R is the universal gas constant and T the absolute temperature. From the relationship (2.2.6), the position of equilibrium in the two phase redox system is given by Ja which is dependent on E^θ and $\Delta^w_o\phi$. This is the key difference from homogeneous redox equilibria and arises from the extra degree of freedom of a two-phase system. This latter-mentioned further degree of freedom in this heterogeneous system is very important, since for a given set of redox couples, the position of equilibrium will be imposed by the externally imposed Galvani potential $\Delta^w_o\phi$ can be fixed using standard electrochemical instrumentation, or by the use of a partitioning ion (which is not part of the redox couple). The Galvani potential difference can be estimated for a particular partitioning ion (2.1.4), where the standard potential of transfer is defined by equation (2.1.3).

From the equilibrium condition (2.2.4) and equation (2.1.4), the

position of equilibrium for a two phase redox system in the presence of a partitioning ion will be given by equation (2.2.7).

$$\log J_a = (n_1 n_2 F/(2.303RT)\ [E^{\theta}(R_1/O_1) - E^{\theta}R_2/O_2) + \Delta^{w}_{o}\phi] + (n_1 n_2/z_i)\ \log\ (a_i(o)/a_i(w)) \quad \ldots.(2.2.7)$$

Equation (2.2.7) relates the thermodynamic properties of the redox compounds and those of the partitioning ions to the position of equilibrium. The applicability of this system has been demonstrated successfully by the author and co-workers [25] for a reversible two phase redox system. This relationship has been used in the present work as a simple predictive tool for establishing the range of standard potentials of the aqueous redox couple to be chosen. This, of course, represents the minimum thermodynamic requirement, but unfortunately, does not tell us anything about the rates at which the two phase reactions will occur. At present, the complete formalisms to analyse the various possible interfacial reaction schemes, when irreversible chemical reaction steps are present, have not been established, and this will be a subject of further study.

Throughout the work, the Galvani potential differences were calculated using equation (2.1.4), for a partitioning ion in both phases.

Activity coefficients were considered in the non-aqueous phase (1,2-DCE) phase only.

These were calculated from the extended Debye-Huckel Theory for associated electrolytes, [205].

$$-\log \gamma_i = A \sqrt{(\alpha_i c_i)}/(1 + Ba \sqrt{(\alpha_i c_i)}) \qquad \ldots.(2.2.8)$$

where A and B are constants, α_i is the degree of dissociation and a is the ion size parameter. The value of α_i was calculated from the association constant given by,

$$K_{assoc} = (1-\alpha_i)/(c_i \alpha_i^2 \gamma_i) \qquad \ldots.(2.2.9)$$

The system of equations (2.2.8) and (2.2.9) was solved by an iterative technique on a BBC$^+$ microcomputer [206].

CHAPTER THREE

EXPERIMENTAL

3.1. Chemicals and Preparation of Reagents

3.1.1 Solvents:

(a) Organic:

1,2-Dichloroethane, 1,2-DCE (Aldrich, 99+% spectrophotometric grade) was used as received and utilised as the non-aqueous solvent in all the ITIES experiments. This was stored in the dark and under nitrogen.

All the solvents used for recrystallisations of the supporting electrolytes were of AnalaR quality. Absolute ethanol (J. Burrough (FAD) Ltd., A.R. grade 99.7%), acetone (Aldrich HPLC grade 99.9%) were used as received. When necessary, the solvents used were purified and dried according to the methods of Perrin, Armarego and Perrin [207].

(b) Aqueous:

Owing to the extreme sensitivity of the techniques used to impurities, particularly organics, it was found necessary to use triply distilled water in the preparation of all aqueous supporting electrolytes and buffer solutions. Millipore water was distilled from dilute $KMnO_4$, then H_3PO_4 followed by a final distillation.

3.1.2 Aqueous supporting electrolytes:

The chemical used were : Lithium sulphate monohydrate (Fluka Biochemika Microselect, >99.0%), Lithium chloride anhydrous (Aldrich Chem. Co., >99.0%), tri-Sodium Citrate (AnalaR, BDH, 99.0%), di-Potassium hydrogen phosphate and Potassium dihydrogen phosphate (Fluka Biochemika Microselect).

3.1.3 Organic supporting electrolytes and organic reference supporting electrolytes:

Tetraphenylarsonium chloride (Fluka p.a. 90-95%), TPhAsCl), Potassium tetrakis(4-chlorophenyl)borate (Fluka selectophore), (KTPhBCl), Sodium tetraphenylborate (Fluka puriss p.a.), (NaTPhB) and Ethylviolet, chloride salt (Aldrich), (EVCl) were used as organic reference electrolytes and for the preparation of the supporting electrolytes in the organic phase. Tetraphenylarsonium tetrakis(4-chlorophenyl)borate, (TPhAsTPhBCl) was prepared by precipitation from equimolar solutions of TPhAsCl and KTPhBCl from an ethanol water mixture (1:2), filtered and washed thoroughly with water and recrystallised twice from acetone. In an analogous method to this latter salt, both Tetraphenylarsonium tetraphenylborate, (TPhAsTPhB), and Ethylviolet tetrakis(4-chlorophenyl)borate, (EVThBCl) were prepared from TPhAsCl and NaTPhB and EVCl and KTPhBCl respectively and purified by recrystallisation from acetone. μ-Nitrido-bis(triphenylphosphorus)tetraphenylborate, (PNPTPhB) was a gift from Dr. H.M. Alemu (Addis Ababa University) and was used as received. All these salts were vacuum dried (50°C, 500mm Hg) and stored over silica gel in a desiccator prior to their use.

3.1.4 Salts used as partitioning ions in the non-electrochemical (Phase Transfer Catalysis) experiments:

Tetra-n-butylammonium tetraphenylborate (TBATPhB), tetra-n-propylammonium tetraphenylborate (TPATPhB), tetra-ethylammonium tetraphenylborate (TEATPhB) and TPhAsTPhBCl were used as electrolytes in the organic phase (1,2-DCE), whereas their corresponding chlorides (supplied by Fluka (puriss)) were used in the aqueous phase. The organic salts were prepared

in an analogous manner to TPhAsTPhBCl. Again, due to the hygroscopic nature of the chlorides, each salt was dried under vacuum prior to use.

3.1.5 Aqueous redox species used:

(a) The Iron(II)/Iron(III) citrate aqueous redox couple was prepared by dissolution of iron(II) sulphate.$7H_2O$ (Fluka puriss) and iron(III) sulphate.$5H_2O$ (Fluka purum) in a concentrated solution of sodium citrate (1M),[208]. The equilibrium redox potential of the couple was adjusted by varying the Fe(II): Fe(III) ratio, and was measured on a mercury electrode versus the saturated calomel electrode (S.C.E.) for the deaerated solutions. Owing to their chemical reactivity, each solution was freshly prepared immediately before use and the solutions were protected against light. Similarly, the pH was checked using a calibrated Philips PW9421 pH meter.

(b) β-Dihydronicotinamide adenine dinucleotide (NADH) disodium salt (supplied by Boehringer-Mannheim, grade II 98%, from yeast) was stored at 4°C and sealed under argon. This was employed in the second part of the work. Owing to the light/ moisture sensitivity of the NADH each solution used was freshly prepared in 10^{-2}M phosphate buffer (pH7.0) and kept in the dark prior to use. The purity of each solution was checked spectrophotometrically from the ratio of the characteristic absorbances of NADH at 260nm and 340nm, [209].

The phosphate buffer (10^{-2}M) employed was freshly prepared by dissolving equimolar quantities of di-potassium hydrogen phosphate (10^{-2}M) and potassium dihydrogen phosphate (10^{-2}M) in deaerated triply distilled water. The pH of the each solution was measured prior to use.

3.1.6 Preparation and characterisation of the organic redox mediating dyes used for the liquid-liquid studies:

All elemental analyses were conducted at the micro-analytical laboratory, University College, London. Ultraviolet/visible spectra were recorded on a Phillips PU8800 Spectrophotometer controlled with an Acorn BBC+ microcomputer.

Infra-red spectra were recorded as nujol mulls on a Perkin-Elmer 1600 F.T. series spectrometer (± $5cm^{-1}$ accuracy). Proton nuclear magnetic resonance spectra were recorded at 360MHz on a Bruker AM360 instrument. All n.m.r spectra are reported as parts per million (ppm) downfield shift from TMS and are reported consecutively as position (δH) relative integral multiplicity and coupling constant (JHz). Mass spectra were recorded at Southampton University on a Kratos MS 30, or a VG 70 250 SE spectrometer. Major fragments are given as percentages of the base peak density (100%). Fast atom bombardment (FAB) spectra were recorded using either glycerol or 3-nitrobenzyl alcohol as matrix.

Analytical thin layer chromatography (tlc) was conducted on precoated 0.25mm alumina or silica plates and compounds were visualised by fluorescence, iodine or aqueous potassium permanganate.

(a) 1,1'-Dihexadecyl-4,4'-bipyridinium, di-tetrakis(4-chloro-phenyl borate, (I): $C_{16}V^{2+}.2(TPhBCl^{-})$.

Starting materials:

4,4'-Bipyridyl, anhydrous (Fluka purum), 1-Bromohexadecane (Fluka practical grade), dimethylformamide (DMF) (Fluka puriss).

The synthesis of (I) was carried out via an adaptation to the method used for shorter alkyl chain viologens [210]. A mixture of 4,4'-bipyridyl (4.9g) and bromohexadecane (49.1g, 5x excess) and DMF (250ml) was refluxed for 12 hrs under nitrogen with continuous stirring. The mixture was cooled to room temperature and the resulting precipitate was filtered off, leaving an amber coloured solution of the monohexadecyl derivative and unreacted reagents. The product was purified by recrystallisation twice from ethanol followed by acetonitrile, which gave the dibromide salt of the required product as fine bright yellow crystals (22g, 90%). The resulting salt (5.0g) was taken up in DMF (250ml), mixed with a two molar equivalent of KTPhBCl (3.3g) (recrystallised from an ethanol/water mixture) in 100 ml of ethanol in order to exchange the bromide counter anion (metathesis). The required di-$TPhBCl^-$ salt crystallised out slowly as fine bright orange crystals which were filtered under vacuum and washed thoroughly with triply distilled water until free of potassium bromide. The product was triply recrystallised from absolute ethanol, and subsequently dried for 6 hrs in a vacuum oven at 50°C, 500mm Hg. m.p. 155-157°C.

IR (nujol) 2360(w), 1630(w), 1080(m), 1010(m), 800(m) cm^{-1}; UV (1,2-DCE) 239(ϵ=55500$dm^3.mol^{-1}$ cm^{-1}) nm; PMR (360MHz; d7-DMF) 9.62 4H,s broad), (2-H,2'-H)), 8.89 (4H,s broad),(3-H,3'-H)), 7.2 (16H, m (counter anion)), 7.08 16H, m (counter anion)), 2.15 (4H,s,(-CH_2-N)), 1.30 (56H,s,(-CH_2-)), 0.95 (6H,s,(-CH_3)). m/z (FAB^+) 607(($M+H)^+$, 100% cation), 592(20), 381(45), 157(18), 55(25). Anal. found: C,71.0; H,7.1; N,1.9; Cl,18.4. $C_{90}H_{106}B_2Cl_8N_2$ requires C,71.1; H,7.0; N,1.8; Cl,18.6.

(b) 3,7-Bis(dimethylamino)-phenothiazin-5-ium, tetrakis(4-chlorophenyl)borate, (II): (Methylene Blue)$^{+}$TPhBCl^{-}. Commercially available Methylene Blue, chloride salt, (1.0g, 3mmol) (supplied by Fluka (puriss)) dissolved in triply distilled water (50ml) was mixed with an equimolar quantity of KTPhBCl (1.6g) in ethanol (50ml). Deep blue crystals of the desired salt precipitated slowly, and were washed thoroughly with triply distilled water, followed by recrystallisation twice from acetone. The crystals were dried under vacuum at 50°C, 500mm Hg for 12 hrs and stored in a desiccator.

IR (nujol) 2360(w), 1600(s), 1335(s), 1140(m), 790(s) cm^{-1}; UV (1,2-DCE) 658(ϵ=128700$dm^3.mol^{-1}$ cm^{-1}), 607 sh.(39600), 294(55000) nm; NMR (360MHz; $CDCl_3$) 7.95 (2H,d,J 10Hz,(9-H,1-H)), 7.25 (8H,dq,J 8,3Hz,(B"-2'-H, B"-6'-H) (counter anion)), 7.18 (2H,dd,J 3,9Hz,(2-H,8-H)), 6.96 (8H,d,J 8Hz,(3'-H,5'-H) (counter anion)), 6.74 (2H,d,J 3Hz, (4-H,6-H)), 3.18 (12H,s,(-$N(CH_3)_2$); m/z (FAB^{+}) 284((M+H)$^{+}$,81% cation), 241(5.0), 154(100), 107(24.7), 89(23.9), 63(12.5). Anal. found: C,64.1; H,4.4; N,5.6. $C_{40}H_{34}N_3SBCl_4$ requires C,64.8; H,4.6; N,5.6.

(c) 3,7-bis(dihexylamino)-phenothiazin-5-ium, tetrakis-(4-chlorophenyl)borate, (III): $C_6TH^{+}TPhBCl^{-}$

Starting materials:

Phenothiazine (Fluka purum), glacial acetic acid (BDH AnalaR) bromine, di-n-hexylamine (97%) and diethyl ether (Aldrich), n-hexane (Fluka puriss). All chemicals were used without any further purification.

The method used is an adaptation of that used for similar dyes [211,212]. However, the preparation has been extended with further purification

stages. Phenothiazine (4g, 0.02mol) was ground up with a pestle and mortar and suspended in glacial acetic acid (150ml) with vigorous stirring. The reaction vessel was maintained at 25°C with a water jacket. A solution of 5% bromine in acetic acid was added to the mixture over a period of 20 mins and the solid phenothiazonium perbromide was filtered off and washed with diethyl ether until free of bromine. The red/brown solid obtained was suspended in 100 mls of absolute ethanol. A solution of di-n-hexylamine (23g, 0.124mole) in ethanol (100ml) was added over a period of 30mins. The mixture rapidly became bright blue in colour, and the solvent was removed in vacuo. The resulting crude dye was extracted with ether, followed by a Soxhlet extraction with n-hexane to remove the bromamide formed in the reaction, which afforded the dye as a blue solid (200mg). Further purification of the dye was necessary using column chromatography (basic alumina, (Prolabo), EtOAc). The resulting solid (100mg) was recrystallised three times from absolute ethanol and dried under vacuum (50°C, 500mm Hg), m.p 128-130°C.

IR (nujol) 2360(w), 1590(s), 1300(s), 1240(m), 1140(m), 865(m), 800(s) cm^{-1}; UV (1,2-DCE) 657(ϵ=8660dm^3 mol^{-1} cm^{-1}), 615 sh.(6540) nm; PMR (360MHz; $CDCl_3$) 7.9 (2H,m (weak), aromatics), 7.45 (8H,m (counter anion)), 7.25 (2H,m, aromatics), 7.10 (8H,m (counter anion)), 6.90-6.60 (2H,m, aromatics), 3.50-2.85 (8H,m,(-CH_2-)), 2.0-1.30 (32H,m broad, (-CH_2-)), 0.88 (12H,s broad,(-CH_3)); m/z (CI^+) 566(M^+, 100% cation), 520(5.0), 482(25.0), 409(7.5), 159(19.0), 114(20.0), 75(11.0), 41(17.5). Anal. Found: C,70.2; H,7.6, N,4.3; S,2.9. $C_{60}H_{74}N_3BCl_4S$ requires C,70.5; H,7.3; N,4.1; S,3.1.

(d) 7-Dimethylamino-1,2-benzophenoxazine, tetrakis(4-chlorophenyl)borate, (IV): (Meldola's Blue)$^{+}$TPhBCl^{-}

Compound (IV) was prepared by metathesis from the commercially available Meldola's Blue, chloride salt and KTPhBCl in an identical method to compound (II). The product was obtained as deep blue/black fine crystals and was purified by recrystallisation from acetone, followed by vacuum drying (50°C, 500mm Hg).

IR (nujol) 2360(w), 1630(m), 1080(m), 1010(m), 800(s) cm^{-1}; UV (1,2-DCE) 620 sh.(ϵ=17880dm^3 mol^{-1} cm^{-1}), 580(24600), 550 sh.(18200) nm; PMR (360MHz; $CDCl_3$) 9.07 (1H,d,J 8Hz), 8.45 (1H,d,J 8Hz), 8.10-7.81 (3H,m), 7.70 (1H,d,J 8Hz), 7.56-7.40 (2H,m), 7.18 (8H,s broad (counter anion)), 6.96 (8H,s broad (counter anion)), 6.87 (1H,m), 3.41 (3H,s,(-CH_3)), 3.35 (3H,s,(-CH_3)). **m/z** (FAB^{+}) 275((M+H)$^{+}$, 100% cation), 259(14), 242(12), 226(11), 202(7), 152(15), 136(61), 107(32), 89(41), 77(47), 63(20). Anal. found: C,68.0; H,4.3; N,3.9. $C_{42}H_{31}N_2OBCl_4$ requires C,68.9; H,4.3; N,3.8.

3.2. Instrumental and Cell Configurations and Cell Arrangements

3.2.1 Preparation of glassware

All the glassware for each experiment was routinely cleaned according to the following procedure: Glassware soaked in a potassium permanganate, concentrated sulphuric acid mixture overnight, then rinsed thoroughly with triply distilled water, followed by placing it in an atmosphere of steam for 3 hrs. This was then baked in a vacuum oven (300°C, 500mm Hg) for 6 hrs and allowed to cool down to room temperature in a desiccator prior to use.

3.2.2 Four-electrode ITIES experimental arrangement

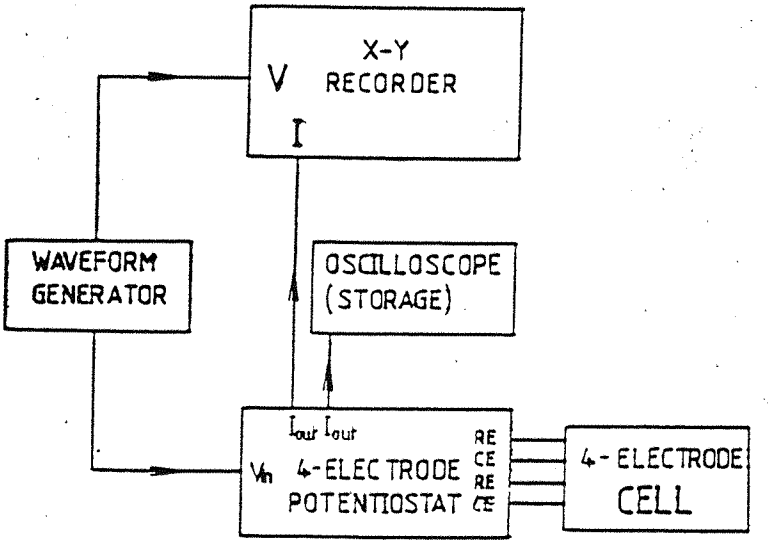
(a) Cell Design and Setup:

The cell employed for the four electrode ITIES work was similar to that used by Samec et al. [112] (see figure 3.1.b.). The surface area of the interface was 0.126 cm^2, and the volumes of the upper (aq.) and lower (org.) compartments were 5.0cm^3. The two current-supplying counter electrodes were Pt gauze discs (10mm diameter) positioned parallel with respect to the interface in both phases. The potential difference in two horizontal planes close to the interface is indicated by means of the two reference electrodes connected via luggin capillaries to each phase: the capillary immersed in the aqueous phase contains aqueous base electrolyte and the organic reference compartment contains aqueous supporting electrolyte solution consisting of the same cation (or in some cases anion) as is present in the non-aqueous phase (shaded in the diagram) in equimolar quantities. This latter arrangement determines the potential of the organic reference arm. The reference electrodes employed were: (a) For the aqueous compartment, (i) Ag/AgCl where the common ion present was chloride; however, (ii) in the majority of cases encountered in the present project, the S.C.E. electrode was employed where the aqueous base electrolytes used were sodium citrate or lithium sulphate; (b) For the organic reference, (i) in the majority of cases, where the chloride salt was used in the aqueous side of the liquid/liquid junction, a Ag/AgCl electrode was employed, otherwise, (ii) in the case of the liquid junction containing a common anion then the saturated sodium chloride electrode (S.S.C.E.) was used.

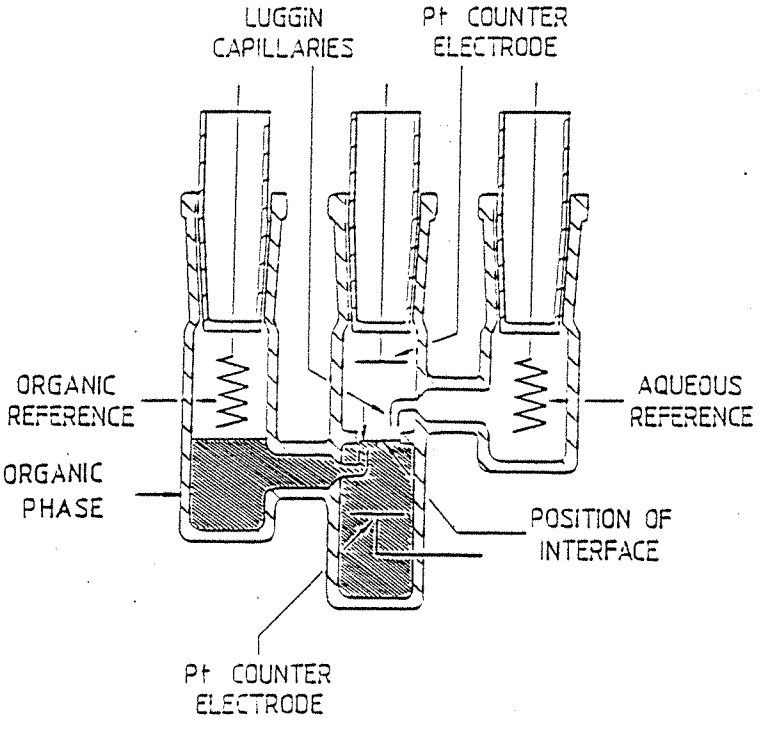
(b) Electronic Instrumentation:

The potential difference was applied using a battery operated four-electrode potentiostat with positive feedback ohmic compensation.

(a)



(b)



4-Electrode Liquid / Liquid Cell

Figure 3.1:
(a) Instrumental configuration for the cyclic voltammetric and chronoamperometric studies of the water / 1,2-DCE interface.
(b) Diagram of the four electrode cell used for the ITIES studies. The shaded area represents the organic (1,2-DCE) phase.

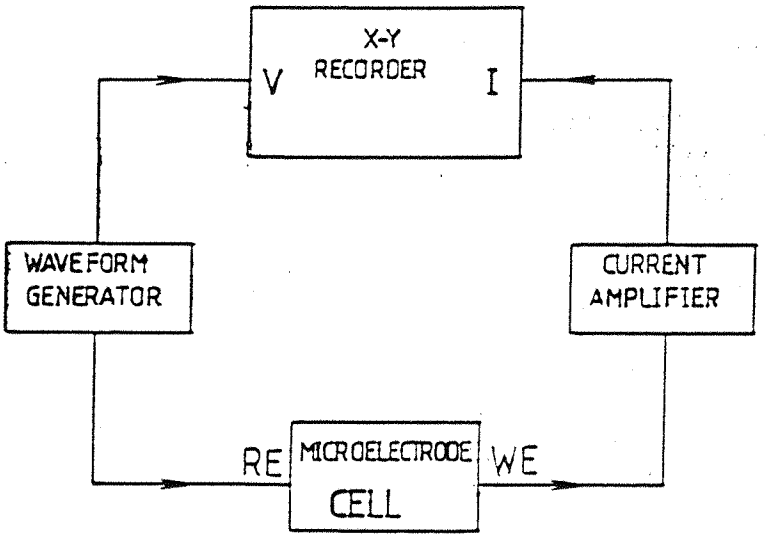
This was constructed by the author from a design similar to that of Samec et al. [75,213]. In order to obtain good ohmic compensation, it was necessary to enclose both the potentiostat and cell in a Faraday cage. A triangular waveform for cyclic voltammetry was applied with a Hi-Tek PPR1 waveform generator and the resulting cyclic voltammograms were recorded on a X-Y recorder (Bryans 26000A3) as illustrated in figure 3.1.a. The potential step measurements were also performed with the latter equipment and were stored on digital storage oscilloscope (Gould OS4100) and then downloaded onto the X-Y recorder. (c) Sign convention for the transfer of charge at ITIES: The standard convention used throughout is that the potentials reported are those of the aqueous phase with respect to the organic phase. A positive current corresponds to the transfer of positive charge from the aqueous to the organic phase.

3.2.3 Microelectrode experimental arrangement

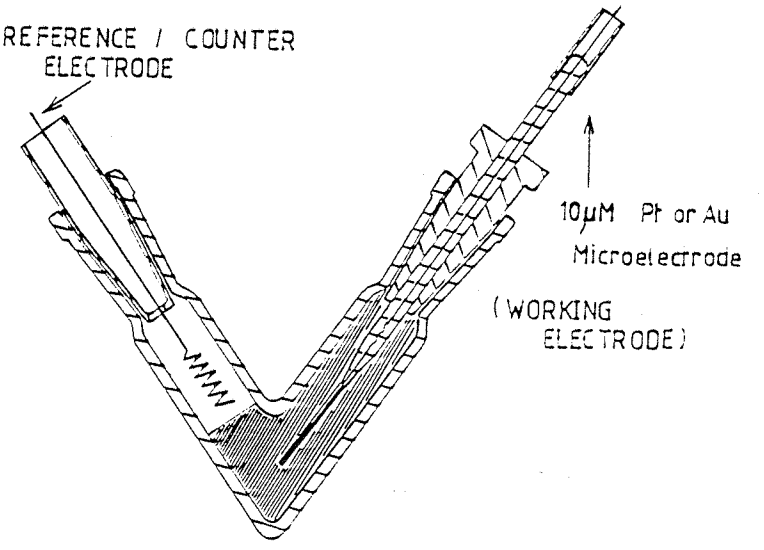
(a) Cell design and Setup:

The simple V-shaped [214] cell presented in figure 3.2.b was used throughout the work in order to study the reduction of the redox mediator dyes at a metal electrode in 1,2-DCE. A 10μm diameter microelectrode either Pt or Au (fabricated as in [215]) was used in the organic phase ($5.0cm^3$) (shaded in the diagram) with supporting electrolytes, typically, (1) TPhAsTPhBCl or (2) TPhAsTPhB, as used in the ITIES case. The microelectrodes were very carefully cleaned prior to use and after any internal adjustments had been made to the cell, by polishing consecutively with three grades of alumina powder: 1.0μm, 0.3μm and 0.05μm (Buehler UK). Triply distilled water was used as the suspension medium and the polishing was carried out on a microcloth (Buehler UK). The electrodes were sonicated in water for approximately 1 minute between each grade of abrasive, and were dried with acetone (A.R. grade)

(a)



(b)



Microelectrode Cell

Figure 3.2:
(a) Instrumental configuration for the study of the reduction of the redox dyes at microelectrodes.
(b) Diagram of the simple cell used for the microelectrode studies on the dyes. The shaded area represents the organic (1,2-DCE) phase.

before positioning in the cell. The current-supplying electrode and reference electrode was either Ag/AgCl in TPhAsCl for (1) or SSCE in NaTPhB in case (2). Equal concentrations of electrolyte the aqueous and the organic phase were used, and the potential of the water/1,2-DCE junction was calculated in the same way as for the ITIES case.

(b) Electronic Instrumentation:

A Hi-Tek PPR1 waveform generator served as the source of potential and a Keithley 427 current amplifier was used to measure the currents as illustrated in figure 3.2.a. The resulting steady state polarisation curves were recorded on a X-Y recorder (Bryans 26000A3). These were analysed using the standard equations for the limiting current, I_l at a microelectrode which is given by [215,216]:

$$I_l = 4nFDCr \qquad (3.2.1)$$

where n is the number of electrons, F is the Faraday constant, D is the diffusion coefficient, C is the concentration of electroactive species and r is the microelectrode radius. Semilogarithmic analysis of the steady state polarisation curves was carried out in order to ascertain the reversibility of the reactions studied. For a reversible reaction,

$$E = E_{\frac{1}{2}} + (RT/nF)\ \ln(I_l/I - 1) \qquad ...(3.2.2)$$

where $E_{\frac{1}{2}}$ is the half wave potential, I_l is the limiting current and I is the current at potential E.

3.3. Liquid-liquid Experiments Conducted with the Redox Dyes (I-IV):

All experiments were carried out at 20± 2°C, and all the solutions were air saturated unless otherwise stated. Prior to conducting each experiment, the two phases were allowed to equilibrate for 15 minutes and the potential of the reference electrodes used was checked carefully. Throughout, unless otherwise stipulated, cyclic voltammetry was used as the principal electrochemical technique to study the redox dyes.

3.3.1 $C_{16}V^{2+}(TPhBCl^-)_2$, (I):

(a) Microelectrode studies of (I):

The simple microelectrode setup, as previously illustrated was used with the following CELL (1):

Ag	AgCl	TPAsCl(w) 1mM	TPhAsTPhBCl(o) 1mM $C_{16}V^{2+}(TPhBCl^-)_2$ xM	Au (10μm Ø)
0				E(1)

The low concentration of supporting electrolytes was chosen because at higher concentrations (> 5mM) the base currents measured became very large. The organic phase (5ml) was injected with a 50μl aliquot of a 5mM stock solution of the dye (I) in 1,2-DCE, dispensed from a sampler micropipette (Oxford Instruments). After having allowed the dye to homogenise by leaving the system to equilibrate for 5 minutes, a desired concentration of 50μM in the dye was obtained. The steady state polarisation curve for the dye was recorded at a low sweep rate, typically $5mVs^{-1}$ to avoid problems of hysteresis associated with higher sweep rates.

(b) The behaviour of (I) at the ITIES in the absence of aqueous redox species.

The four-electrode configuration previously described was used for the following CELL (2).

Ag	AgCl	TPhAsCl 10mM	TPhAsTPhBCl(o) 10mM $C_{16}V^{2+}(TPhBCl^-)_2$ xM	δ	1.0M Li_2SO_4 (w)	SCE
O						E(2)

where, δ is the interface under investigation. The high concentration of

aqueous supporting electrolyte was chosen to increase the available potential window by the salting out of the organic electrolyte [85]. The potential window was located, and dependence of the base electrolyte system was investigated as a function of sweep rate in the range 1 to $100mVs^{-1}$. After having established the limits of a well iR compensated potential window for the base electrolyte, the organic phase (5.0ml) was 'spiked' with a 50μl aliquot of a 5mM stock solution of the redox dye (I) by carefully passing the micropipette through the interface via the aqueous compartment. This gave a concentration of 50μM of dye in the organic phase. Before measurements, the solution in the cell was allowed to stand for five minutes for equilibration. This 'spiking' technique was used throughout every ITIES cell and similarly in the non-electrochemical (partitioning ion/PTC) experiments in order to administer the dye to the organic phase. The behaviour of the dye within the potential window was investigated by cyclic voltammetry. The sweep was started at the positive limit. The sweep rate dependence of the system and the base electrolyte was also measured.

The behaviour of an analogous base electrolyte system was examined as in Cell(2) to establish any general features due to the dye (I), CELL(3):

Ag	AgCl	TPhAsCl 10mM	TPhAsTPhBCl(o) 10mM $C_{16}V^{2+}(TPhBCl^-)_2$ xM	$(Na)_3Citrate(w)$ 1M	SCE
0					E(3)

Similarly, the effects of different organic and aqueous supporting electrolytes were qualitatively investigated to ascertain any general noticable phenomena attributable to the dye. The systems studied were:

(i) Ag/AgCl/EVCl(10mM)/EVTPhBCl(10mM)//Na_3citrate(1M)/SCE

(ii) Ag/AgCl/EVCl(10mM)/EVTPhBCl(10mM)//Li_2SO_4(10mM)/SCE

(iii) SSCE/NaTPhB(2mM)/PNPTPhB(2mM)//Na_3citrate(1M)/SCE

(iv) Ag/AgCl/TPhAsCl(10mM)/TPhAsTPhBCl(10mM)//LiCl(10mM)/AgCl/Ag

To try to minimise the error associated with the problems of uncompensated resistance in the organic phase, the systems shown in CELL(2) and CELL(3) were repeated, but using 50 mM TPhAsTPhBCl in the organic phase with correspondingly, 50 mM TPhAsCl in the organic reference solution. The new systems are referred to as Cell(4) and Cell(5) respectively. Potential step techniques were conducted on cell(4) to confirm data extracted from the cyclic voltammetric experiment. CELLS (4) and (5) were also carried out under conditions where each phase was deaerated with argon gas as a control for later experiments, to demonstrate the effect, if any, on the behaviour of dye(I).

(c) The study of interfacial electron transfer between dye (I) and a fast aqueous redox couple at the ITIES:

(i) Qualitative investigation of the reduction of dye (I): After having established the reduction potential of the dye from the microelectrode studies, suitable hydrophilic reducing agents were shaken with the hydrophobic dye (I) in 1,2-DCE. The effects of both deaeration with argon and saturation with oxygen of the two phases in contact (and separated) was noted visually by observing any colour changes and also, spectrophotometrically. It was hoped that the reagents would meet the requirements for interfacial electron transport at the ITIES (see Chapter 1). The reagents tried were:

(a) Alkaline hydrazine aq. (Hydrazine sulphate (10mM) and sodium

carbonate (2mM) in water)

(b) Sodium dithionite aq.(10mM);

(c) Iron(II)/iron(III) citrate (as described in 3.1.5).

(ii) Electron transfer carried out under anaerobic conditions:

The measured potentials for electron transfer correspond to the following CELL (6):

Ag	AgCl	TPhAsCl 50mM	TPAsTPhBCl 50mM $C_{16}V^{2+}(TPhBCl^-)_2$ 50μM	$(Na)_3$Citrate (1M) $Fe(II)SO_4$ (0.04M) $(Fe(III))_2(SO_4)_3$ (0.01M)	SCE
0					E(6)

Each solution was deae rated for ten minutes with argon, and particular care was taken with the organic phase on account of the appreciable solubility of oxygen in most organic solvents. The pH and the equilibrium potential were recorded for the redox couple as previously described. The cell was filled under a stream of argon and care was taken to avoid air entering the system when adjustment of the interface was necessary and during the spiking procedures.

The sweep rate dependence and effects of the redox couple on the base electrolyte system relative to Cell (3) and any accompanying changes in the nature of the interface were investigated. The effect of air having entered the system was also recorded.

A new cell, CELL (7) was set up under identical conditions to the latter, but the concentration of the dye was increased twofold. Again, both the potential and pH of the couple were re-measured and cyclic voltammetry conducted in the usual manner.

The effect of varying the equilibrium potential of the aqueous redox

couple was investigated by increasing in CELL (8), the ratio of iron(II) to iron(III) 100:1 with respect to Cell (7).

(iii) Electron transfer conducted under aerobic conditions:

The potentials measured correspond to the following general cell, CELL (9), where the ratio of Fe(II) to Fe(III) in the couple was varied as listed below (TABLE I). All the solutions were air saturated.

TABLE I

	Ratio Fe(II) : Fe(III)	Concentration/(mol.dm^{-3}) x : y
(i)	1 : 100	3×10^{-4} : 3×10^{-2}
(ii)	1 : 1	10^{-2} : 10^{-2}
(iii)	2 : 1	2×10^{-2} : 10^{-2}
(iv)	5 : 1	5×10^{-2} : 10^{-2}
(v)	10 : 1	0.1 : 10^{-2}
(vi)	100 : 1	0.1 : 10^{-3}

CELL (9):

Ag	AgCl	TPhAsCl 50mM	TPhAsTPhBCl(o) 50mM $C_{16}V^{2+}(TPhBCl^{-})_2$ 50μM	δ	$(Na^{+})_3$citrate(1M)(w) Fe(II)sulphate(xM) Fe(III)sulphate(yM)	SCE
0						E(9i-vi)

The behaviour at interfaces in CELL(9i-vi) was recorded cyclic voltammetrically in the manner previously described.

3.3.2 The Behaviour of Dyes (II-IV) and Preliminary Investigation on the Oxidation of NADH:

(i) Microelectrode studies of the dyes (II-IV).

The same techniques which were employed for dye (I) were also used for dyes II to IV. In the case of the hydrophobic dye (III), ($C_6TH^+TPhBCl^-$), the potentials refer to the following cell:

CELL (10)

Ag	AgCl	TPhAsCl(w) 1mM	TPhAsTPhBCl(o) 1mM $C_6TH^+(TPhBCl^-)$ 50μM	Au (10μm ϕ)
0				E(10)

However, in the case of the two semi-hydrophobic dyes (II) and (IV), it was necessary to select $TPhB^-$ as the reference ion in the liquid junction of the organic reference electrode($\Delta^w_o\phi$ = +360 mV) to avoid partitioning of these dyes into the reference electrode compartment. This would have happened if TPhAs had been used , since the standard transfer potential of this ion is $\Delta^w_o\phi$-360 mV, ie, making the water negative with respect to the oil phase. The following cell was used:

CELL (11)

SSCE	NaTPhB 1mM	TPhAsTPhB 1mM $Mox^+(TPhBCl^-)$ 50μM	Au (10μm ϕ)
0			E(11)

where Mox^+ represents, (i) $(Methylene\ Blue)^+$ or

(ii) $(Meldola's\ Blue)^+$

The half wave potentials obtained from the microelectrode experiments were correlated with the values obtained for the liquid/liquid partition experiments described by the equations in Chapter 2.

(ii) Non-electrochemical, 'Phase transfer catalysis' experiments at the liquid/liquid interface.

As described in Chapter 1, the use of different potential determining ions in varying concentrations to fix the interfacial Galvani potential and thus affect the rate of electron transfer, has been successfully accomplished by Geblewicz et al. [24]. From equation (2.2.7) it is posssible to match the standard potentials of the redox couples in the two phases to the transfer potential of the partitioning ion and its activity in both phases.

The Galvani potential difference was calculated from [217]:

$$\Delta^{w}_{o}\phi_i = \Delta^{w}_{o}\phi_i^{\theta} + (RT/z_iF)\ \ln(\alpha_i c_i(o)\gamma(o)/c_i(w)) \qquad ....(3.3.1)$$

where c_i is the concentration of the ion i, αi is the degree of dissociation, γi is the activity coefficient and z_i is the ionic charge. Partial dissociation of electrolytes was considered in the non-aqueous solution only [217]. $\Delta^{w}_{o}\phi$ values for the partitioning ions were calculated using values of α_i and γ_i, which were calculated from the extended Debye-Huckel Theory for an associated electrolyte, (as described in Chapter Two). Association constant values of $TPhB^-$ or $TPhBCl^-$ with $TPhAs^+$, TEA^+, $TPrA^+$ and TBA^+ of 600, 2500, 2100 and 1715 respectively were used, as given by Abraham and De Namor [218]. No association constant between $TPhB^-$ and $TPhBCl^-$ was considered. In the case of the

redox dyes II and IV, due to their 'semi'-hydrophobic nature mentioned above, it was necessary to select suitable partitioning ions to avoid partitioning of the dyes into the aqueous phase, but at the same time fix the interfacial potential to values as negative as possible. For dyes (II) and (IV), the negative limit to which $\Delta^{w}_{o}\phi$ could be fixed, was established by investigating the transfer of the dyes with several partitioning cations, and the results are given in Table II (A)-(C). This was performed using a non-electrochemical system (12), in which the aqueous phase was examined both visually and spectrophotometrically in the region 550 to 660nm to check for dye partition. In the case of dye (IV), by virtue of its hydrophobicity, $TPhAs^{+}$ was investigated as a common ion, Table II (D) and System (13). The methodology for these systems is presented in the following sections.

System (12):

1,2-DCE		$(10^{-2}M)$ Phosphate Buffer [pH 7.0]
(0.5mM) $TRA^{+}TPhB^{-}$	‖	(0.5mM) $TRA^{+}Cl^{-}$
(0.1mM) $Mox^{+}(TPhBCl^{-})$	‖	

where R is Et, n-Pr or n-Bu, and Mox is Dye II or IV. System (13):

1,2-DCE		$(10^{-2}M)$ Phosphate Buffer [pH 7.0]
(0.5mM) TPhAsTPhBCl	‖	(0.5mM) TPAsCl
(100μM) $C_6TH^{+}(TPhBCl^{-})$	‖	

TABLE II

	Concentration Partitioning Ion/(mM)		$\Delta^{w}_{o}\phi$ (calculated)/ V
(A)	0.5	(TEATPhB	+0.025

		(TEACl	
(B)	0.5	(TPrATPhB	-0.109
		(TPrACl	
(C)	0.5	(TBuATPhB	-0.243
		(TBuACl	
(D)	0.5	(TPhAsTPhBCl	-0.378
		(TPhAsCl	

From the results of these semi-quantitative partition experiments, it was possible to choose suitable partitioning ions to use in a two phase non-electrochemically controlled redox system consisting of the redox dye (org.) and NADH (aq.). Preliminary work was conducted typically with a 5 fold excess of the partitioning ions relative to the redox species under study. Similarly, a 5:1 excess of the dye (org.) was utilised with respect to the NADH (aq.) for the following general system. System (14):

1,2-DCE	‖	(10^{-2}M) Phosphate Buffer
(0.5mM) PTC^+TPhB^-	‖	(0.5mM) PTC^+Cl^-
(100μM) $Mox^+(TPhBCl^-)$	‖	(20μM) NADH

where Mox represents either dye II, III or IV, and PTC corresponds to the chosen phase transfer catalyst.

Methodology: All the chemicals were weighed directly into their respective flasks using a Sartorious five-decimal place ($\pm 10^{-4}$g) balance and solutions prepared as previously described. The experiments were carried out in a darkroom. All experiments were carried out at room

temperature (20 ± 2°C) The solution were allowed 20 mins. equilibration time prior to making measurements and to assume room temperature. NADH (20μM) in phosphate buffer (10^{-2}M, pH7.0) was prepared and its U.V. spectrum recorded to ascertain the purity of the NADH [209]. The base-line for the experiments consisted of the buffer solution only. The spectrometer was that previously mentioned, and all spectra were stored for later analysis. 10 ml of a 20 μM NADH and 20 μM PTC^+Cl^- solution was placed in contact with 10 ml of a freshly prepared solution of PTC^+TPhB^- in 1,2-DCE (10ml). The container used was a simple glass cylinder with an area of 4.5cm^2 and a glass lid. The mixture was gently stirred for 20 mins. After equilibration, the top phase was carefully extracted, without disturbing the interface, and pipetted into a quartz cuvette. The UV spectrum of the solution in the characteristic region (340-250nm) for NADH absorbance was recorded, after which the solution was returned to the reaction vessel. The organic phase of the system was spiked with the redox dye (200μl of a 5mM stock of II, III or IV = 100μM) and again the system was allowed to equilibrate with stirring for twenty minutes. The UV spectrum was recorded in the same manner as before and the solution returned to the reaction vessel. The system was monitored over a period of time. In addition to the above experiment, several control experiments were conducted simultaneously under identical conditions to the one above:

(1) NADH (20μM) in buffered solution (10ml) in contact with 1,2-DCE, a general control.

(2) NADH (20μM) + PTC^+Cl^-(0.5mM) in aqueous buffer in contact with PTC^+TPhB^- (0.5mM) in 1,2-DCE;

(3) NADH (20μM) in aqueous buffer in contact with redox dye (100μM) in 1,2-DCE (with no potential control; no PTC).

(iii) Preliminary four electrode investigations of the dyes (II) to (IV)

Preliminary four-electrode investigations were conducted. The behaviour of the Meldola's Blue $TPhBCl^-$ salt at the water/1,2-DCE interface was recorded voltammetrically in the following Cell (15):

				δ		
Ag	AgCl	TPhAsCl 10mM	TPhAsTPhBCl 10mM $MB^+TPhBCl^-$ xM		H_2KO_4P (10mM) HK_2O_4P (10mM) pH 7.0	SCE
0						E(15)

The baseline for the system was recorded and the response to the addition of the dye (50μM) into the organic compartment was observed. The effect of increasing the concentration was examined and the effect of replacing the aqueous phase with a solution of buffered NADH (20μM) was investigated. The drawback of this system is the possibility of the Meldola's Blue diffusing in through the organic luggin capillary and partitioning into the aqueous component of the organic reference. This would give rise to a mixed and poorly defined interfacial potential. The cyclic voltammetric behaviour of both dyes II and IV at the ITIES is the subject of further work.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Studies of $C_{16}V^{2+}(TPhBCl^-)_2$

4.1.1 The reduction of $C_{16}V^{2+}$ at a microelectrode

A typical microelectrode baseline obtained for CELL (1) is shown in Figure 4.1.a. The polarisation window is well-defined. After the addition of 50μM of $C_{16}V^{2+}(TPhBCl^-)_2$ to the organic compartment of the cell, two well separated reduction steady state polarisation waves are obtained. The half-wave potential of the first process occurs at $E_{\frac{1}{2}}(1)$= -0.050V in the centre of the window. The second is less well-defined since it lies at the far negative limit, with a half wave potential of $E_{\frac{1}{2}}(1)$ = -0.650V. The first wave is shown in figure 4.1.b. The semilogarithmic analysis of the polarisation curve based on equation 3.2.2 (Figure 4.2), gives a linear response with a slope of -60mV/decade, which is close to the value expected for a Nernstian one electron reversible process of, -58mV/decade. The first process corresponds to the first reduction of $C_{16}V^{2+}$ to the mono-cation radical, $C_{16}V^{+\cdot}$. This is akin to the behaviour of the smaller alkyl-chain viologen dications, such as the well-studied diheptylviologen [219]. The formal potential of the $C_{16}V^{+\cdot}/C_{16}V^{2+}$ couple in 1,2-DCE with respect to the standard hydrogen electrode (SHE) can be calculated from the analysis of the half-wave potential of Cell (1), from which

$$E^{\theta}(C_{16}V^{+\cdot}/C_{16}V^{2+}) = E_{\frac{1}{2}}(1) + \Delta^{w}_{o}\phi(TPhAs^{+}) + E_{(Ag/AgCl)} - (RT/F)\ln(\gamma C_{16}V^{2+})/\gamma(C_{16}V^{+\cdot}) \quad ...(4.1.1)$$

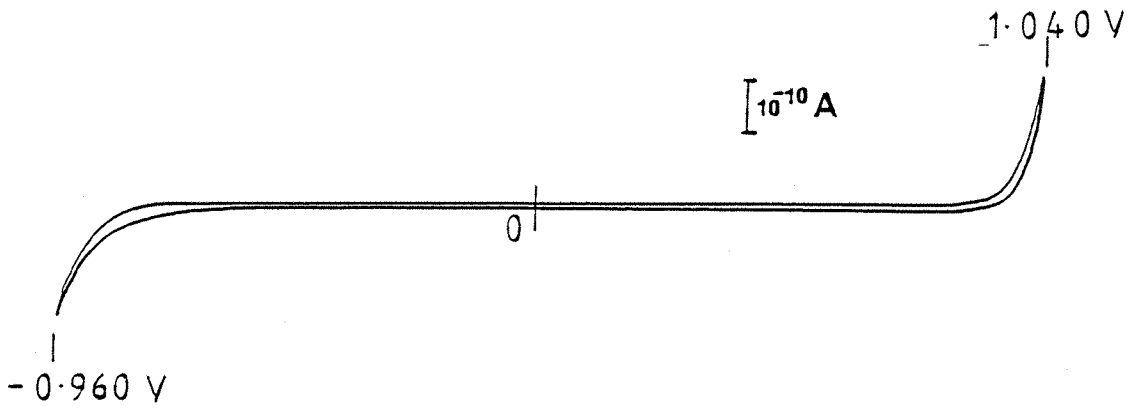


Figure 4.1.a:
The microelectrode baseline polarisation curve in the absence of the electroactive species for cell(I). Potentials refer to Cell (I).

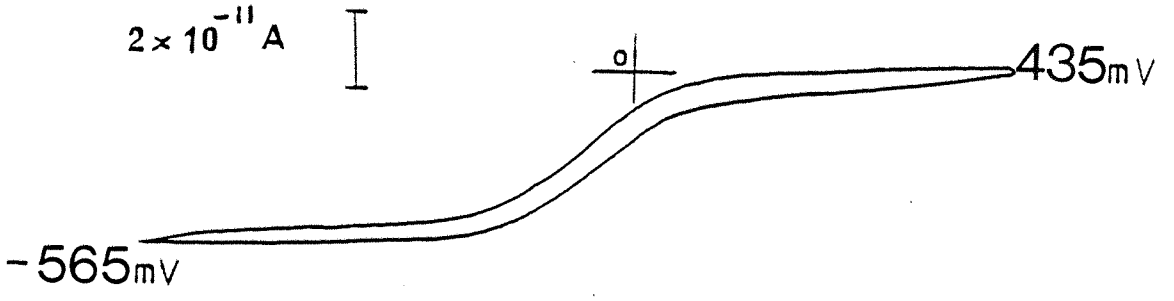


Figure 4.1.b:
The polarisation curve for the reduction of $C_{16}V^{2+}$ (50μM) on a Au microelectrode. Potentials refer to Cell (I)

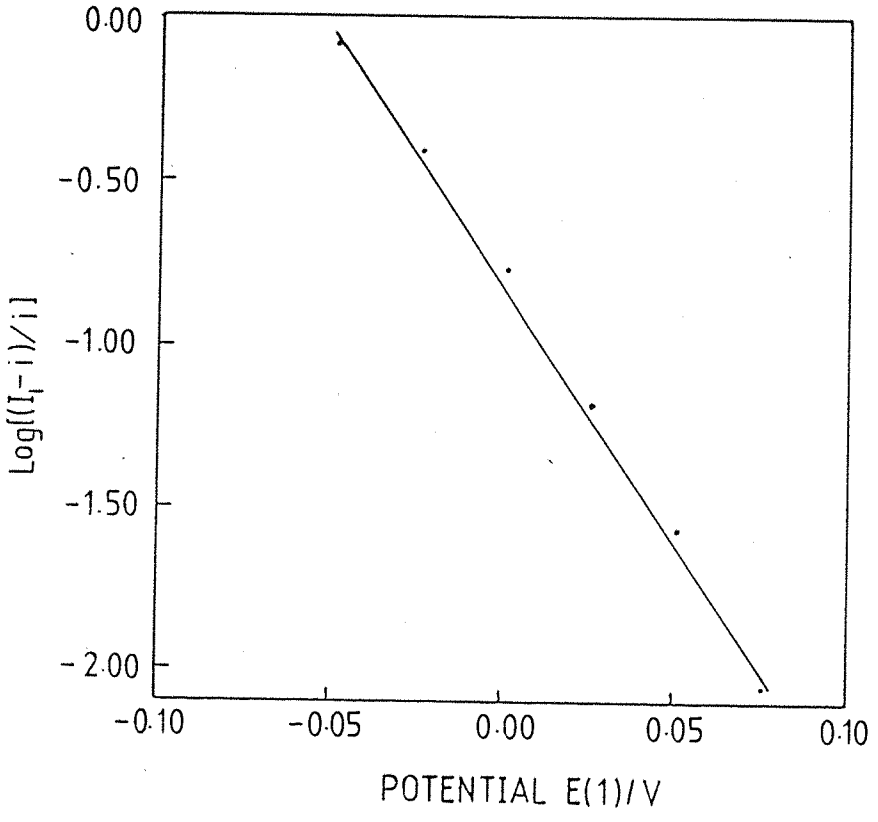


Figure 4.2
A semilogarithmic analysis of the steady state polarization curve obtained for the reduction of $C_{16}V^{2+}(TPhBCl^{-})_2$ in 1,2-DCE at a 10 μm Au microelectrode.

where $E^{\theta}(c_{16}V^{+\cdot}/C_{16}V^{2+})$ is the formal potential of the organic couple with respect to the aqueous SHE, $\Delta^{w}_{o}\phi(TPhAs^{+})$ is the Galvani potential difference across the reference junction, $E_{(Ag/AgCl)}$ is the potential of the silver/silver chloride with respect to the SHE and γ is the activity coefficient. At the half-wave potential, the concentrations of both the oxidised and reduced species are assumed to be equal. The ratio of their activities will be close to unity for the dilute solutions studied and thus the final term in equation (4.1.2) can be taken as approximately zero. The value of $\Delta^{w}_{o}\phi$ $(TPhAs^{+})$ = -0.383V was calculated using equation 3.3.1; the value of $E_{(Ag/AgCl)}$ was 0.397V, using activity coefficients calculated from the extended Debye-Huckel theory. From these values, $E^{\theta}(C_{16}V^{+\cdot}/C_{16}V^{2+})$ = -0.036mV. The diffusion coefficient for the dication in 1,2-DCE, estimated from the limiting current and equation (3.2.1) was found to be 2.5×10^{-6} $cm^{2}.s^{-1}$.

4.1.2 The behaviour of $C_{16}V^{2+}$ at the water/1,2-DCE interface

All the potentials of the liquid-liquid system are referred to the oil phase, i.e., these are given by the difference $(\phi^{(w)}-\phi^{(o)})$. For comparing the results obtained from the different cells, the formal Galvani potential difference was used. This was obtained from the individual contributions to the cells (2) to (5) and is represented by the general equation

$$E(n) = E_{(SCE)} - E_{(Ag/AgCl)} - \Delta^{w}_{o}\phi(TPhAs^{+}) + \Delta^{w}_{o}\phi(n) \quad ....(4.1.2)$$

where E(n) is the measured potential (water vs. oil), $E_{(SCE)}$ is the potential of the saturated calomel electrode taken as 0.244V at 20°C, $\Delta^{w}_{o}\phi$ is the potential on the formal Galvani potential scale. $\Delta^{w}_{o}\phi(TPhAs^{+})$ was calculated as previously described, giving -0.404 for Cells (2) and (3) where the concentration of organic cation was 10mM, and -0.423V for

Cells (4) and (5) where the concentration used was 50mM. $E_{(Ag/AgCl)}$ was taken as +0.341V and +0.302V for Cells 2 and 3 and for Cells 4 and 5 respectively.

Figure 4.3.a shows a typical baseline voltammogram for the ITIES at a sweep rate of $10mV.s^{-1}$ in the absence of electroactive species. Figure 4.3.b illustrates the typical sweep rate dependence of the baseline capacitative currents for Cell (1). The shape and sweep rate dependence are characteristic of all the liquid/liquid baseline voltammograms of the systems which were investigated. The limits of the polarisation window result from the transfer of the supporting electrolytes and are determined by their activities and Gibbs energies of transfer in accordance with the criteria given in Chapter 2 for an ideally polarised ITIES. In the case of the baseline of Cell (2) (figure 4.3.a), the negative limit can be attributed to the transfer of $TPhAs^+$ (o→w) and the positive limit due to that of $TPhBCl^-$ (o→w). The effect of increasing the base electrolyte concentration in going from Cells (2 and 3) to Cells (4 and 5) is a slight decrease in the span of the polarisation window. However, the electronic compensation required in (4) and (5) is negligible compared to (2) and (3). This overriding desirable property of Cells (4) and (5) helped to avoid the problem of the error associated with uncompensated solution resistance (Chapter 1). This is particularly important if the potential is to be measured accurately. $Na_3^+(citrate^{3-})$ gave the same baseline as the Li_2SO_4 solution, (cells 2 and 3, and cells 4 and 5)

Figure 4.4.a shows the linear sweep voltammetric response from the positive to the negative region for a concentration of 50μM of $C_{16}V^{2+}$ in Cell (2). This response was highly reproducible, provided that the potential was held at a positive value with respect to the observed peaks

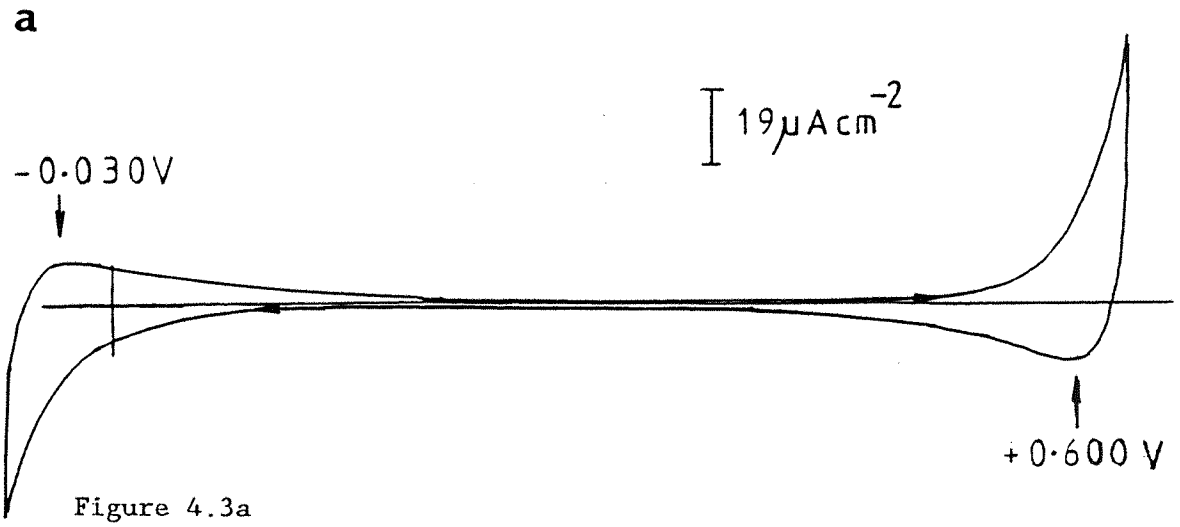


Figure 4.3a
Cyclic voltammogram for the base electrolyte in the absence of the electroactive species in CELL (2) at a sweep rate of 10 mVs^{-1}.

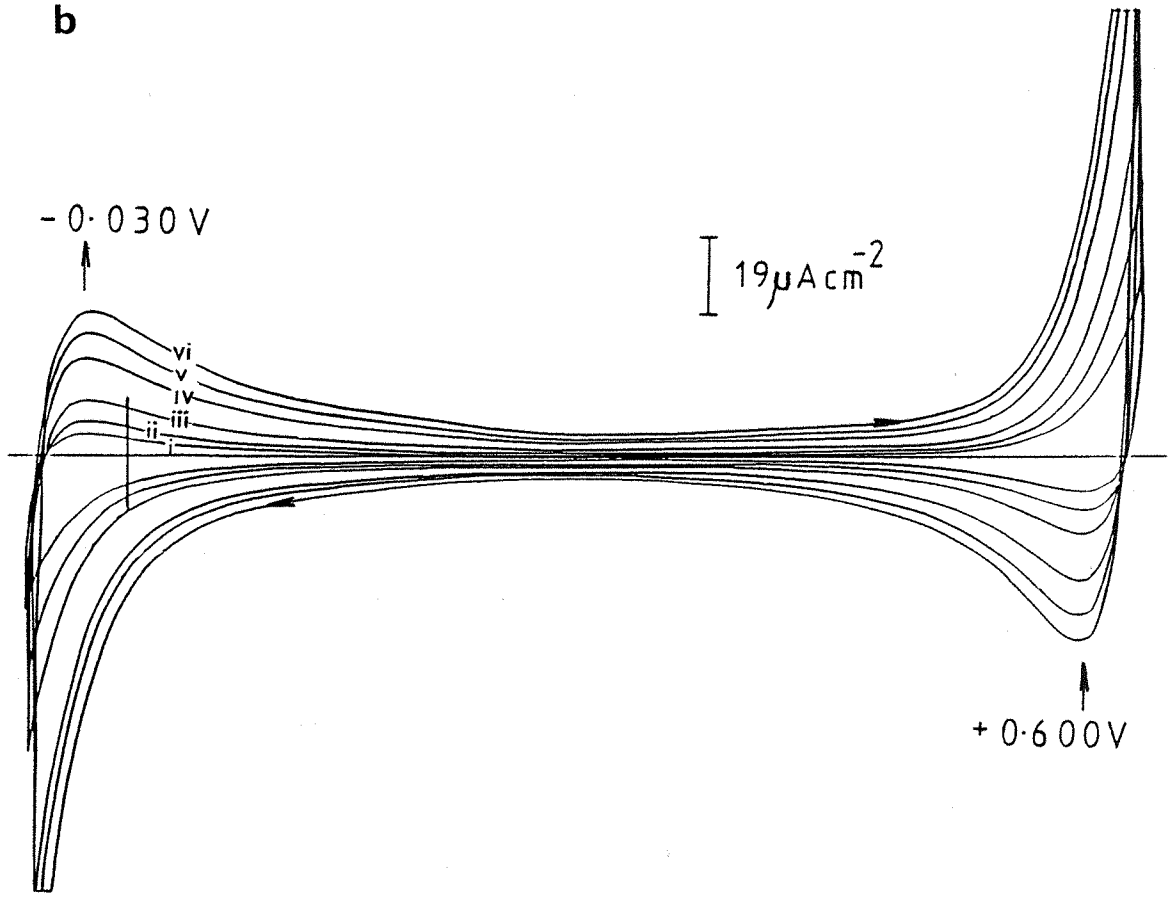


Figure 4.3b
Dependence of the baseline currents in the absence of electroactive species on the sweep rate. Sweep rates: (i) 5, (ii) 10, (iii) 20, (iv) 50, (v) 75 and (vi) 100 mVs^{-1}.

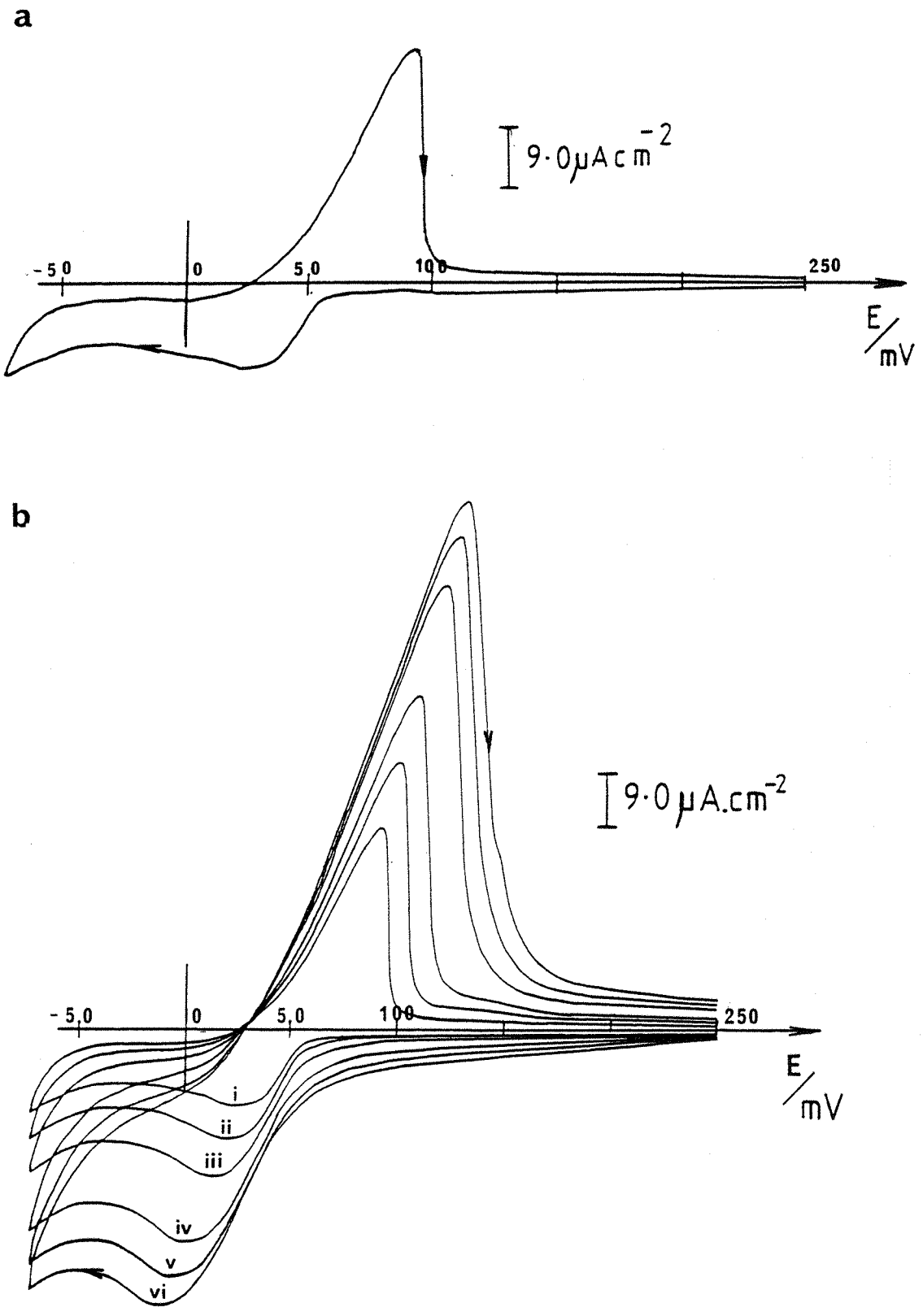


Figure 4.4
(a) Cyclic voltammetry for CELL (2) for a 50 μM solution of $C_{16}V^{2+}$; the aqueous electrolyte was 1M Li_2SO_4.
(b) Sweep rate dependence of the above; (i) 5, (ii) 10, (iii) 20, (iv) 50, (v) 75, and (vi) 100 mVs^{-1}.

for a minimum of five minutes prior to each sweep.

An unusual voltammetric response is observed, having a strong resemblance to the deposition of insoluble films [222]. The charge transfer phenomena occurred in the same potential range in all four cells studied. If the peak potential at $5mVs^{-1}$ is considered, then from equation (4.1.2), the formal peak Galvani potential differences for the 10 mM organic supporting electrolyte systems (2) and (3), $\Delta^{w}_{o}\phi$, were calculated as -0.282V, and in the case of the 50 mM organic supporting electrolyte for cells (4) and (5), as -0.295V. The difference in these values is small, and it is likely to be due to the errors associated with the estimation of the liquid junction potentials. For the forward sweep towards the negative water region, the onset of a rapidly increasing current is observed, which reaches a minimum and is followed by a falling section towards negative potentials. The effect of reversing the sweep, thus making the water more positive relative to the oil, results in much higher peaks with a very rapidly falling portion and sharp cut off which unusually has the characteristics of a stripping peak. This behaviour is very characteristic of that seen for a reversible metal deposition with preceding nucleation [220] at metal electrode surfaces and perhaps more appropriately, resembles the behaviour of radical cation film formation, such as that of the diheptylviologen radical [219] at a metal surface. The mechanisms for these two latter processes have been studied in considerable detail. The first direct analogy between metal deposition and viologen film formation at a metal electrode from aqueous solutions was made by Bruinink and Kregting [221].

It is reasonable to propose a similar type of film formation for the liquid/liquid systems, which might explain the observed.This is the first

time that a phase formation process has been observed electrochemically at a liquid-liquid interface. The relative magnitudes of the forward and reverse peaks currents for a given sweep rate are approximately twice the magnitude for the Li_2SO_4 case as compared with the Na^+_3citrate solutions. Thus, it appears that the film formation is dependent to some extent on the anion in the aqueous phase. The fact that the citrate is a large tri-anionic species relative to the di-anionic sulphate could possibly explain this effect, ie packing of the smaller sulphate at the interface is more favourable than that of the more voluminous and highly charged citrate anion.

For the voltammetric responses of Cells (2) to (5), it can be seen that there is a slight shift in the negative direction of the forward peak potential (as shown in Figure 4.4.b, for Cell (4)) for increasing sweep rates, and this effect is more pronounced at higher sweep rates. At higher sweep rates, however, mechanical oscillations of the interface are likely, and therefore, for the purposes of analysis the lower sweep rates, where film formation is not greatly subjected to these interferences, were analysed. The model for insoluble film deposition was applied to all the systems, viz. the formation peak current dependence on sweep rate was analysed from [222]:

$$I_p = 1.082nFc(\ fvD/\pi)^{\frac{1}{2}} \qquad \ldots(4.1.3)$$

where I_p is the peak current density $f = nF/RT$, n = charge of species (=2 for $C_{16}V^{2+}$), and c is the concentration of the viologen ($molcm^{-3}$). In all the cells the peak heights measured were corrected for the background capacitive currents. The behaviour was typically that shown in Figure 4.5 , for Cell (2), which shows the initial linear dependence of current

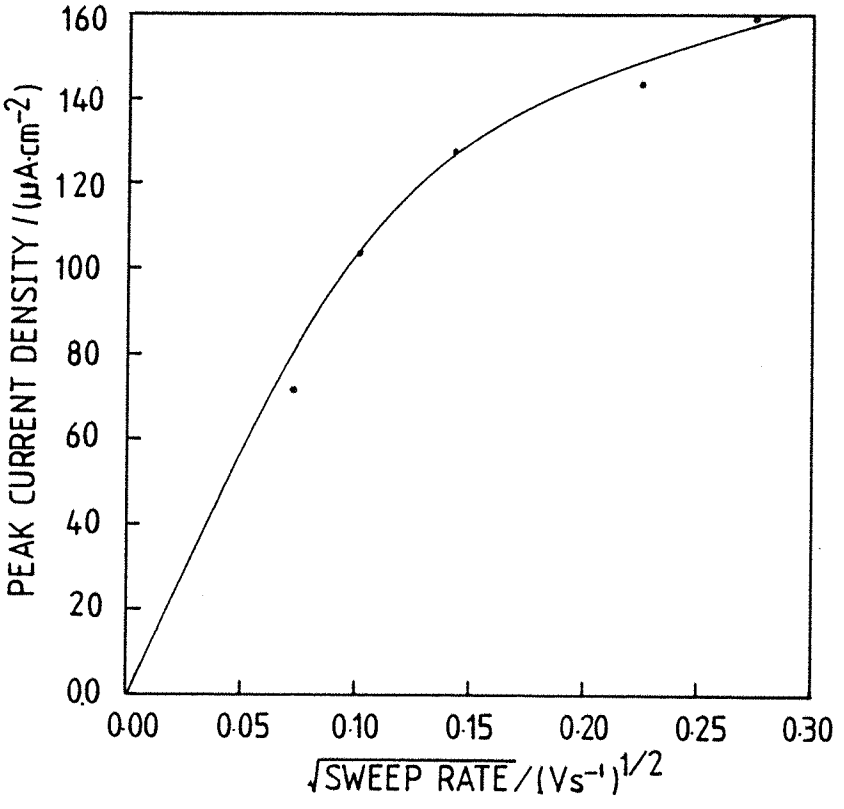


Figure 4.5
Sweep rate dependence of the peak current for the results shown in Fig. 4.4b

density up to 20 mVs^{-1} followed by deviation to smaller currents than predicted by equation (4.1.3) at higher sweep rates. Analysis of the linear portion of the plot gave a value of the diffusion coefficient of $D = 3.1 \times 10^{-6}$ cm^2 s^{-1} which is close to that obtained from the microelectrode experiments. However, the analysis of the citrate systems gave values of D of 7×10^{-5} cm^2 s^{-1} (Cells (3 and 5)). Although great care was taken in the purification of the viologen, the inconsistency of the results obtained could not be accounted for, and further work is required.

Potential step techniques were employed to investigate the layer formation. Potential steps from +0.350V to +0.050V and 0.0V were studied as well as the baseline transients in the abscence of viologen. The I vs t transients, corrected for the background currents were analysed for times greater than 10ms using the Cottrell equation [223]

$$I(t) = nFc(D/\pi t)^{\frac{1}{2}} \qquad \ldots(4.1.4)$$

where n is the charge of the species considered (=2 for $C_{16}V^{2+}$), t is the time and I(t) is the current density. A typical transient and a test of the Cottrell equation is illustrated in figure 4.6. The resulting values of D were similar to those obtained from cyclic voltammetry for the same system.

Qualitative experiments with other base electrolytes are listed in 3.3.1.b. In all cases, the voltammetric response was similar to that described above, showing that the unusual effects observed are independent of the base electrolyte. All attempts to observe the current-time transients characteristic of a two dimensional nucleation process were not successful.

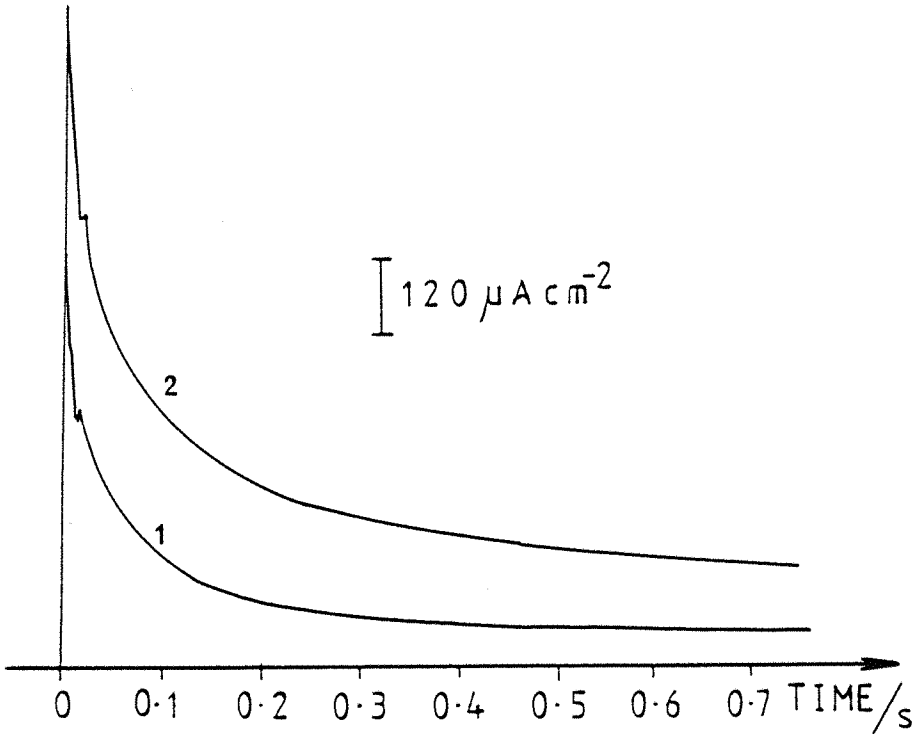


Figure 4.6a
Current-time transients in the absence (1) and presence (2) of 50 μM $C_{16}V^{2+}(TPhBCl^-)_2$ in CELL 4. Potential step from 0.350 to 0V.

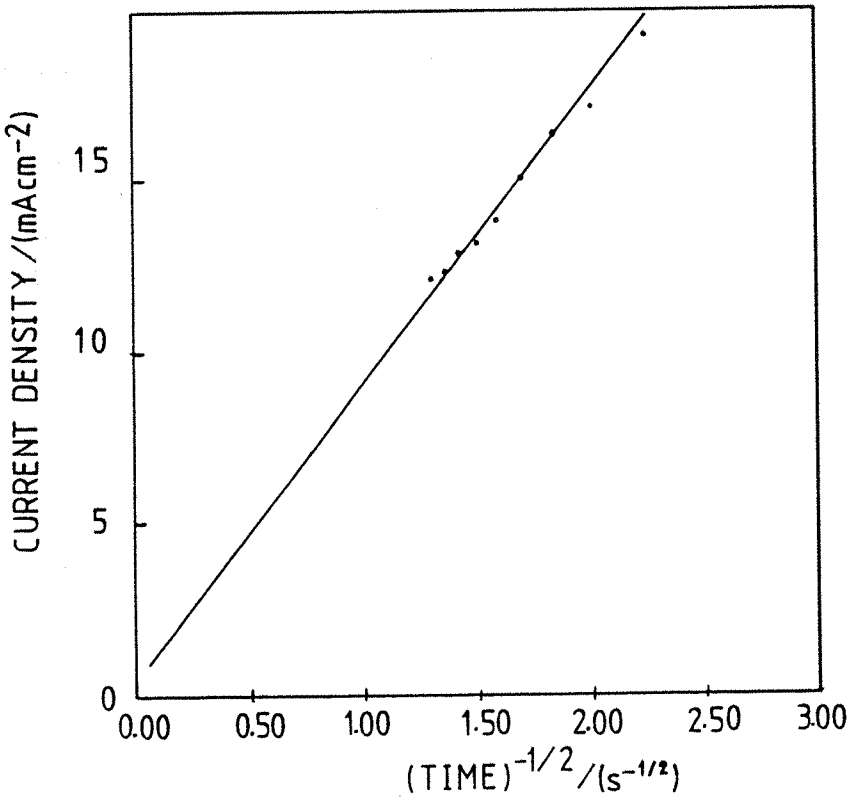


Figure 4.6b
Test of the Cottrell equation for the results shown in Fig 4.6a.

In summary, the phase formation observed for the viologen dication at the 1,2-DCE/water interface, is a purely non-redox reaction, which is akin to well-investigated nucleative metal deposition and insoluble film formation, as for instance, viologen radical salts formed at metal electrode surfaces. Further work is required to elucidate more clearly the mechanistic features of this phase formation and the unexpected irreproducibility in the values of the diffusion coefficient. Work is currently being undertaken to investigate the behaviour of other amphiphilic species at the 1,2-DCE.

4.1.3 Interfacial electron transfer between $C_{16}V^{2+}(TPhBCl^-)_2$ and a fast aqueous redox couple at the ITIES.

(a) Qualitative investigations of the reduction of $C_{16}V^{2+}$

The reduction of $C_{16}V^{2+}$ was initially examined qualitatively using simple aqueous reducing agents. The study yielded valuable information as to the nature of the radical cation formed by the one electron reduction of the dication. Alkaline hydrazine and dithionite in water were used as reducing agents. Shaking a 100 μM solution of $C_{16}V^{2+}$ in 1,2-DCE resulted in a colour change of the organic phase from colourless/light orange to deep blue/purple. The deep blue colour observed is characteristic of viologen cation radicals. When the organic reduced phase was extracted, an immediate colour change from blue back to the original colour was noted, which is attributable to the rapid aerial oxidation of the cation radical back to the dication. No significant change to these latter observations was noted if both phases were carefully deareated with argon, prior and during the experiment.

The same simple procedure carried out above was utilised for an 100:1 Fe(II) : Fe(III) citrate redox couple, whose preparation was described

in section 3.1.5. On placing air-saturated solutions of the viologen dication in 1,2-DCE in contact with the aqueous couple formed the blue radical cation colour very readily. However, after the extraction of this blue phase, no instantaneous decolourisation was noted. The blue solution remained stable for a sufficiently long period of time, such that the UV-vis spectrum was easily obtained (Figure 4.7.a. (the dication) and 4.7.b. (the radical cation)). The spectrum obtained for the radical cation resembles closely that of that recorded for the aqueous methyl viologen radical cation [187]. The addition of hydrogen peroxide to the blue solution results in its immediate decolouration. Further work is required to analyse the mechanism of these reactions. These simple experiments served to indicate that the two phase reduction of the hydrophobic bipyridyl compound was possible.

(b) Interfacial anaerobic electron transfer at the ITIES

The Fe(II)/Fe(III) citrate system was chosen as the hydrophilic redox couple for the two phase redox system in Cell (6). The equilibrium concentration of this couple could be easily changed so as to match the half wave potential of dihexadecyl viologen, obtained from the microelectrode experiments (See equation 2.2.4). Each solution was carefully deaerated prior to the experiment. The concentration of the aqueous redox couple was chosen much higher than that of the organic in order to simplify the diffusional problem, i.e., only the diffusion of the viologen dication need be considered. The redox couple in Cell (6) had no effect on the baseline.

From the voltammetry of Cell (6), after the addition of $C_{16}V^{2+}$ under an argon atmosphere, electron transfer between the two phases is evident as shown in Figure 4.8. No interfacial phase formation as noted previously in the absence of the aqueous redox couple was observed. A

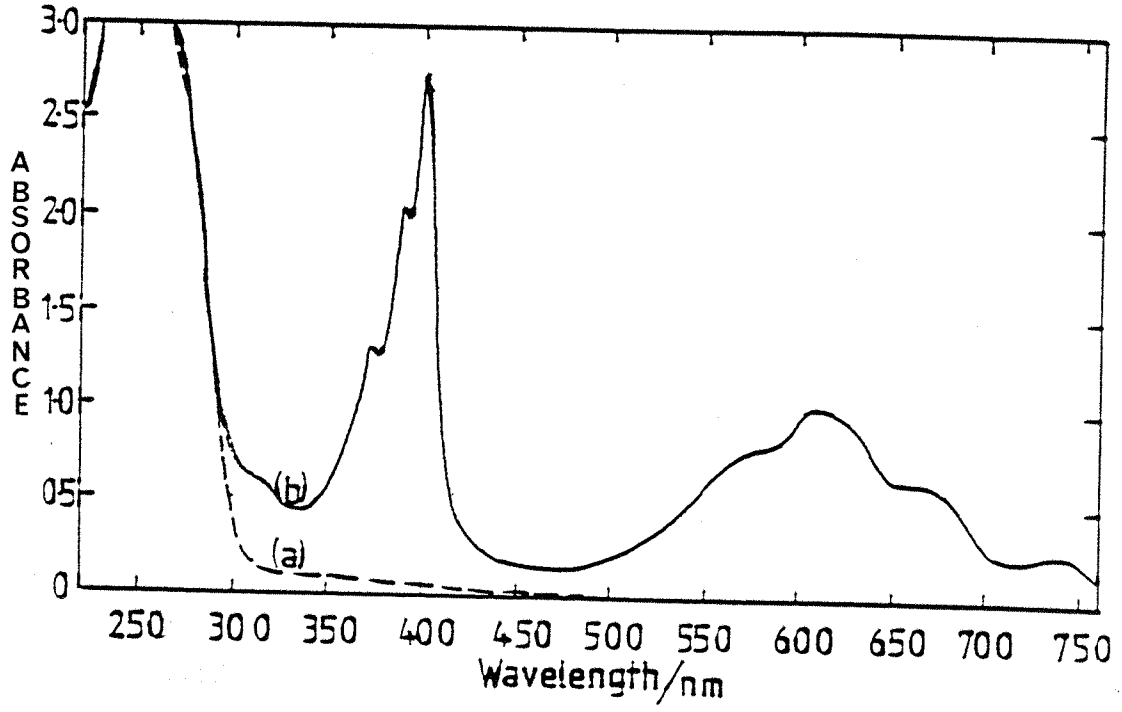


Figure 4.7
(a) UV/vis spectrum of 100 μM $C_{16}V^{2+}(TPhBCl^{-})_2$ in 1,2-DCE.

(b) UV/vis spectrum of 100 μM $C_{16}V^{2+}(TPhBCl^{-})_2$ in 1,2-DCE after reduction with an aqueous Fe(II)/Fe(III) (ratio 100:1) citrate couple.

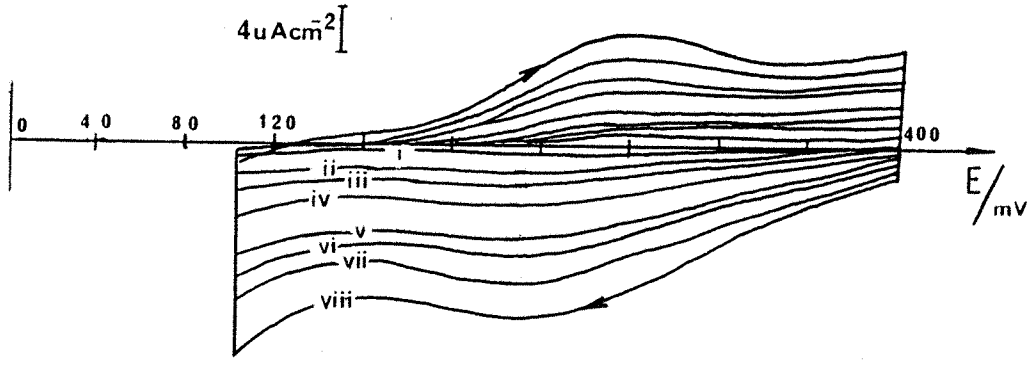


Figure 4.8
Cyclic voltammetry for reversible electron transfer across the 1,2-DCE/aqueous solution interface, between 100 μM $C_{16}V^{2+}(TPhBCl^{-})_2$ and the iron citrate redox system. Potentials and conditions refer to CELL 6. Sweep rates:(i) 1, (ii) 5, (iii) 10, (iv) 20, (v) 40, (vi) 50, (vii) 75 and (viii) 100. The experiment was carried out in the absence of dissolved oxygen.

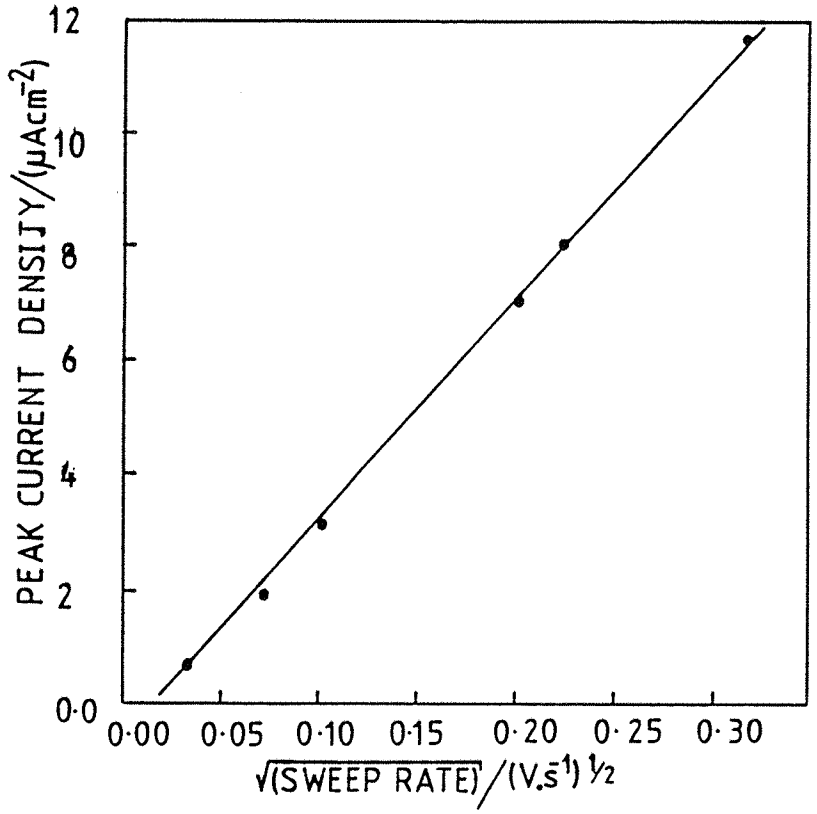


Figure 4.9
Sweep rate dependence of the peak currents of the results shown in Figure 4.8.

peak to peak separation of 58mV, close to the Nernstian response for an expected one electron reversible transfer, was obtained. The currents correspond to the reaction:

$$C_{16}V^{2+} + Fe(II) \rightleftharpoons C_{16}V^{+\cdot} + Fe(III) \quad ...(4.1.5)$$

The measured half wave potential from the electron transfer experiments in Cell (6) was $E_{\frac{1}{2}}(6) = +0.210V$. The Galvani potential difference at $E_{\frac{1}{2}}$ can be calculated from the individual contributions to Cell (6):

$$E_{\frac{1}{2}}(6) = E_{SCE} - E_{(Ag/AgCl)} - \Delta^{w}_{o}\phi(TPhAs^{+}) + \Delta^{w}_{o}\phi_{\frac{1}{2}} \quad (6)$$

$$....(4.1.6)$$

$\Delta^{w}_{o}\phi(TPhAs^{+})$ was taken as -0.423V (from equation (3.1.1)) and $E_{(Ag/AgCl)}$, +0.302V vs. SHE. Thus the half wave potential on the Galvani potential difference scale for electron transfer in Cell (6), was $\Delta^{w}_{o}\phi_{\frac{1}{2}} = -0.155V$. From equation 2.2.7 and the measured potential of the Fe(II)/Fe(III) couple, of -0.264Vvs SCE, $E^{\theta}(C_{16}V^{+\cdot}/C_{16}V^{2+}) = -0.175V$, which must be compared with a value of -0.036V measured with in the microelectrode experiment. Previous work with the diphthalocyanines showed that the formal potential from these two experiments should be the same [24,25]. This difference cannot be accounted for by experimental error. A possible explanation is the difference in the state of the viologen molecule at the interface. As discussed above, strong interactions between $C_{16}V^{2+}$ and the double or triply charged anion of the aqueous electrolyte are known to occur. These, of course, are absent in the reduction experiments at the Au microelectrode.

Assuming a reversible one electron reduction, the peak current corrected

for the charging currents showed a linear dependence with the square root of the sweep rate (v) as shown in Figure 4.9. These results were analysed using the Randles-Sevcik equation [223]:

$$I_p = 2.69 \times 10^5 D^{\frac{1}{2}} c v^{\frac{1}{2}} \qquad \ldots(4.1.7)$$

A value of $D = 2.0 \times 10^{-6}$ cm^2 s^{-1} was obtained from the results in Figure 4.9, which is in good agreement with that previously obtained from the microelectrode experiment for the diffusion of the dication.

In Cell (7) the concentration of the dye was increased two fold with respect to Cell (6). As expected, this led directly to a two-fold increase in the peak current densities obtained and overall, the same behaviour as for Cell (6) was observed. Attempts to obtain a full range of concentrations of the dye for a single experiment were thwarted by the problem of oxygen entering the system.

In order to ascertain whether or not the process was unequivocally due to electron transfer, the redox couple was made more reducing by increasing the ratio of Fe(II) to Fe (III) to 100 : 1 in Cell (8) using a concentration of 50 μM of the dye as in Cell (6). The shape of the voltammogram obtained and the sweep rate dependence were equivalent to that of Cell (6) but with half-wave potential shifted. The pH of the aqueous solution was kept constant for each different ratio of Fe(II) to Fe(III) used. The measured half-wave potential, $E_{\frac{1}{2}}(8)$ was +0.270V and from equation 4.1.6, the value of $\Delta^w_o\phi_{\frac{1}{2}}$ was calculated as +0.095V. As expected, from equations (2.2.4 and 2.2.5) the effect of increasing the concentration of Fe(II) with respect to Fe(III) should make the logarithmic term more positive such that for electron transfer should become more positive by approximately 58mV per decade assuming a one

electron stoichiometry. To analyse this point, the potentials of these redox couples were measured and $E_{(Fe(II)/Fe(III)}$ was -0.264 and -0.351V vs SCE for the 1:4 and 1:100 Fe(II).Fe(II) ratios respectively. The difference in $\Delta^{w}_{o}\phi_{\frac{1}{2}}$ was of 60 mV, whereas that of $E_{Fe(II)/Fe(III)}$ was 87 mV. The electron transfer results are in reasonable agreement with theoretical expectations.

(b) Electron transfer under aerobic conditions.

It is quite interesting to note the effect on the reversible voltammogram when oxygen was allowed into the system, as shown in Figure 4.10. Firstly the system appears to be completely irreversible. The peak potential is enhanced greatly as seen in Figure 4.10 and occurs at a more negative potential with respect the reversible wave for the same cell.

In order to study the effect of oxygen in the system the procedure was relatively simple, as the majority of reactions at the ITIES employ conditions where the solutions are air-saturated. The reduction wave observed occurs at potentials where the radical cation is formed. It is proposed that the reduction wave observed is due to a redox catalytic process involving the $C_{16}V^{+\cdot}$ radical. From the initial experiments, oxygen appears to react quite readily with the viologen radical, and a possible reaction sequence would be:

$$C_{16}V^{2+} + Fe(II) \rightleftharpoons C_{16}V^{+\cdot} + Fe(III) \quad ...(4.1.5)$$

$$2\ C_{16}V^{+\cdot}(o) + O_2 + 2\ H_2O \rightarrow 2\ C_{16}V^{2+}(o) + H_2O_2 + 2\ OH^- \quad ...(4.1.8)$$

followed by further reduction of H_2O_2 by the radical. From the preliminary experiments, the latter reaction is known to be very fast. A second mechanism could involve the reduction of oxygen to peroxide by

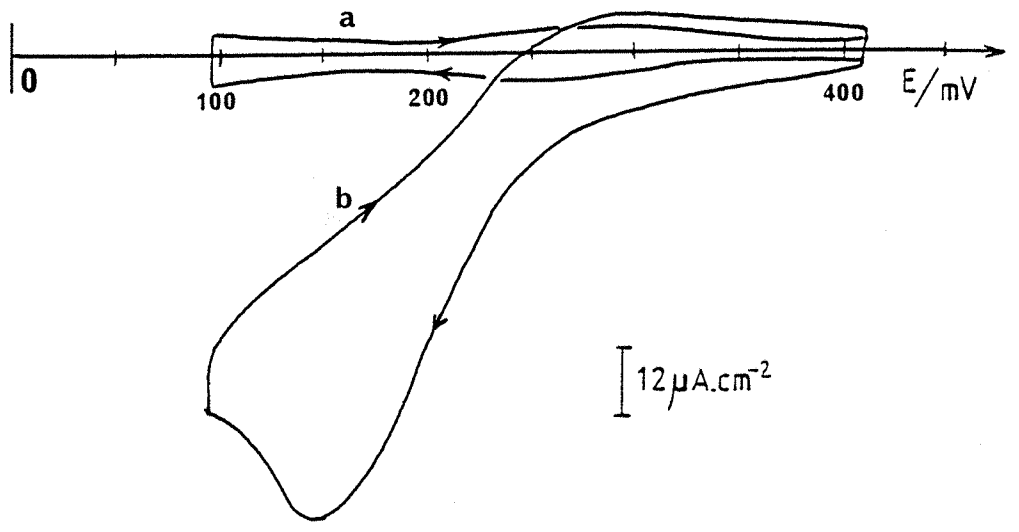


Figure 4.10
Effect of oxygen on the cyclic voltammetry between 100 μM $C_{16}V^{2+}(TPhBCl^-)_2$ and the iron citrate redox system. Potentials and conditions refer to CELL 6; sweep rate = 20 mVs^{-1}. (a) in the absence of oxygen, (b) Air saturated solutions.

Fe(II), followed by its further reduction at the interface by $C_{16}V^{+\cdot}$. These mechanisms need further investigation, but it is very important to notice the similarity of these two phase transfer reactions with redox catalysis at metallic electrodes.

The cyclic voltammetric responses when the ratio of Fe(II) to Fe(III) was varied as given in Table I, (Chapter 3) for Cells (9i) to (9vi), was recorded. The same general behaviour is noted as in the system above. The measured redox potentials of the couples employed and peak potentials for the wave recorded at 10 mV s^{-1} in each of the cells, were measured and the results are given in TABLE III. Also, the potential limits are given in this Table. Using these values, the formal Galvani peak potential difference was calculated from the cell analysis given in (4.1.6).

TABLE III

CELL	$E_{eq(Fe(II)/Fe(III))}$ /V(vs SCE)	pH	POTENTIAL LIMITS / V	PEAK POTENTIAL @ 10mVs^{-1} / V
9(i)	-0.177	6.3	+0.035 — +0.600	
9(ii)	-0.226	6.6	+0.050 — +0.628	+0.112
9(iii)	-0.260	6.6	+0.035 — +0.630	+0.142
9(iv)	-0.272	6.6	+0.030 — +0.632	+0.150
9(v)	-0.298	6.6	+0.030 — +0.632	+0.162
9(vi)	-0.351	6.6	+0.038 — +0.632	+0.172

As in the case of the reversible electron transfer, it can be clearly seen that the peak potential shifts to more positive values with an increase in the concentration of Fe(II) relative to that of Fe(III), making the redox potential of the aqueous couple more negative. The variation of the potential limits of the system measured is small and the pH is essentially constant throughout. Figure 4.11 shows the

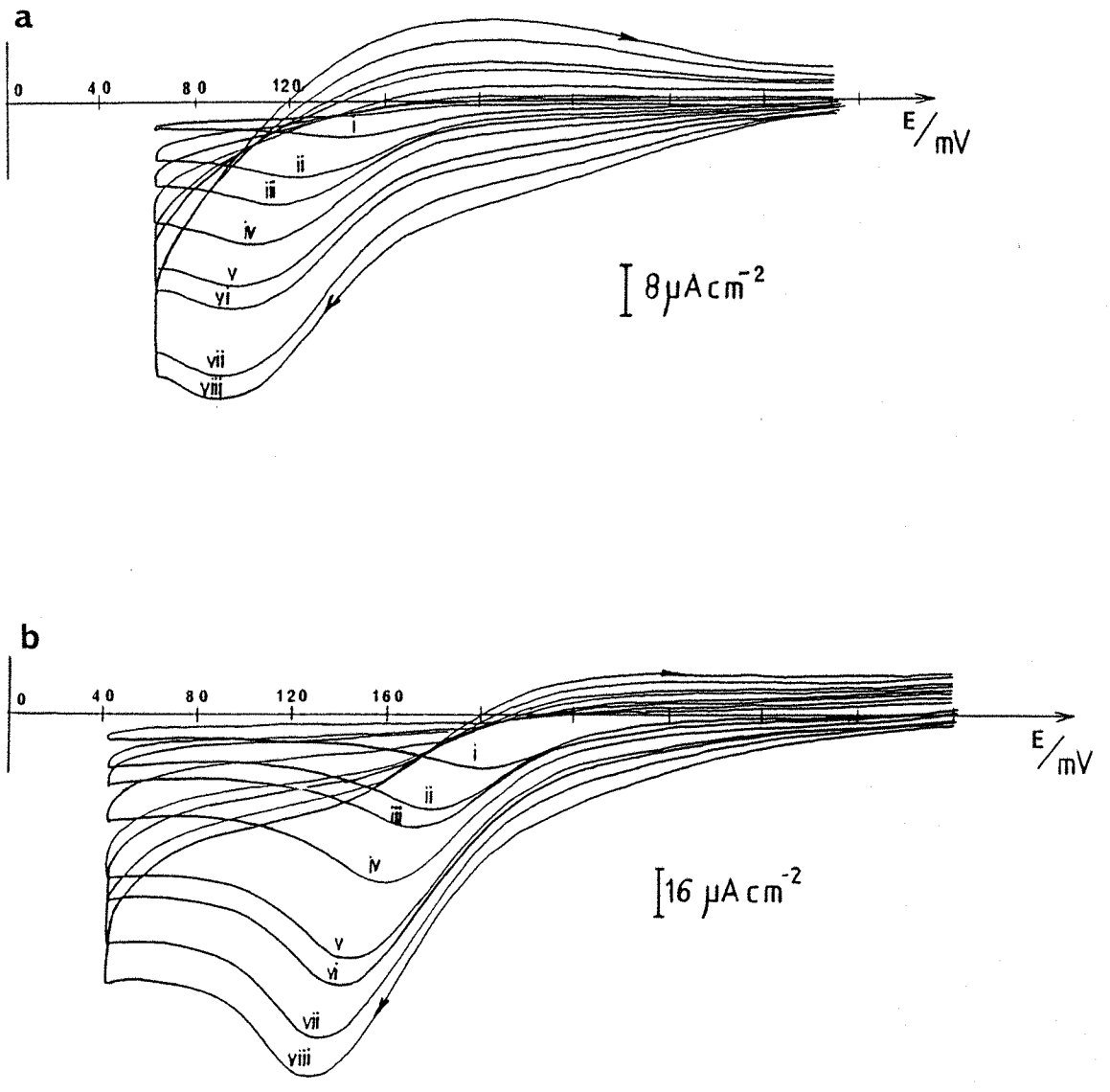


Figure 4.11
Dependence of the catalytic oxygen reduction wave on the potential of the aqueous redox couple for 50 μM $C_{16}V^{2+}(TPhBCl^{-})_2$ and different ratios of Fe(II)/Fe(III). Sweep rates = (i) 1, (ii) 5, (iii) 10, (iv) 20, (v) 40, (vi) 50, (vii) 75 and (viii) 100 mVs^{-1}. (a) CELL 9(ii) with E = -0.226V; (b) E = -0.351V, CELL 9(iv).

voltammograms obtained for Cells (9ii) and (9vi). Cell (9i) was an exceptional case in that the concentration of Fe(II) used was negligible in comparison to Fe(III) and no electron transfer was noted, but instead, the phase formation (equivalent to Cell(5)) phenomenon was obtained. The variation of peak current density with sweep rate, illustrated for Cell (9v) in Figure 4.12, gives an apparently linear response, which tends to suggest that the electrocatalytic process occurring is diffusionally controlled.

Figure 4.13 shows a plot of the variation of the Galvani peak potential difference for Cells (9ii to 9vi) versus the potential of the redox couple, which clearly demonstrates that an electron transfer process occurs for these systems. The relationship is not linear; since the reaction is not reversible.

4.2. Preliminary Investigations of Dyes II to IV and the oxidation of NADH

4.2.1 Reduction of Dyes (II) to (IV).

In a analogous manner to the experiments with Cell (1), a sigmoidal shaped reduction curve was obtained for the microelectrode reduction of $C_6TH^+TPhBCl^-$ (Dye (IV)), with $E_{\frac{1}{2}}(10) = -0.170V$. A semilogarithmic analysis gave a slope of 0.130V, far different from the expected response for the simple one electron reversible electron transfer seen for the viologen. This suggests that the dye reduction is kinetically slow. The other two semi-hydrophobic dyes gave reduction and their semilogarithmic plots gave slopes corresponding to reversible one electron transfers and an example of this is shown in Figure 4.14. In the latter two cases the common ion used in the reference junction was $TPhB^-$, because of the problem of dye transfer at the reference junction, as noted previously.

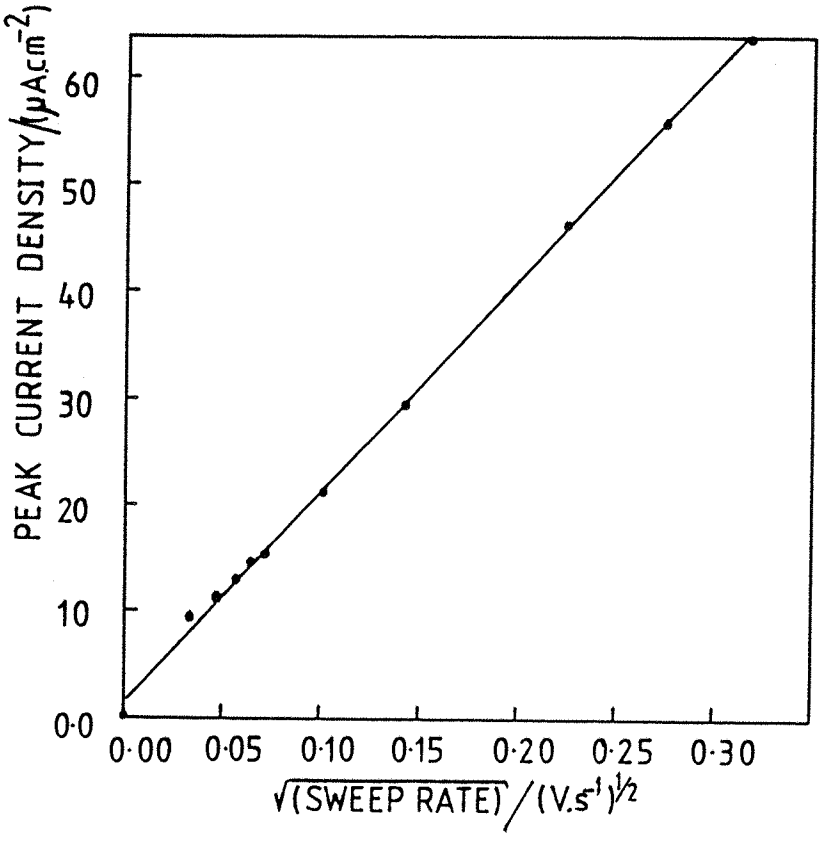


Figure 4.12
Sweep rate dependence of the peak current for the results from CELL 9v for 50 μM $C_{16}V^{2+}(TPhBCl^{-})_2$ and an equilibrium potential of the aqueous redox couple of -0.298 vs SCE.

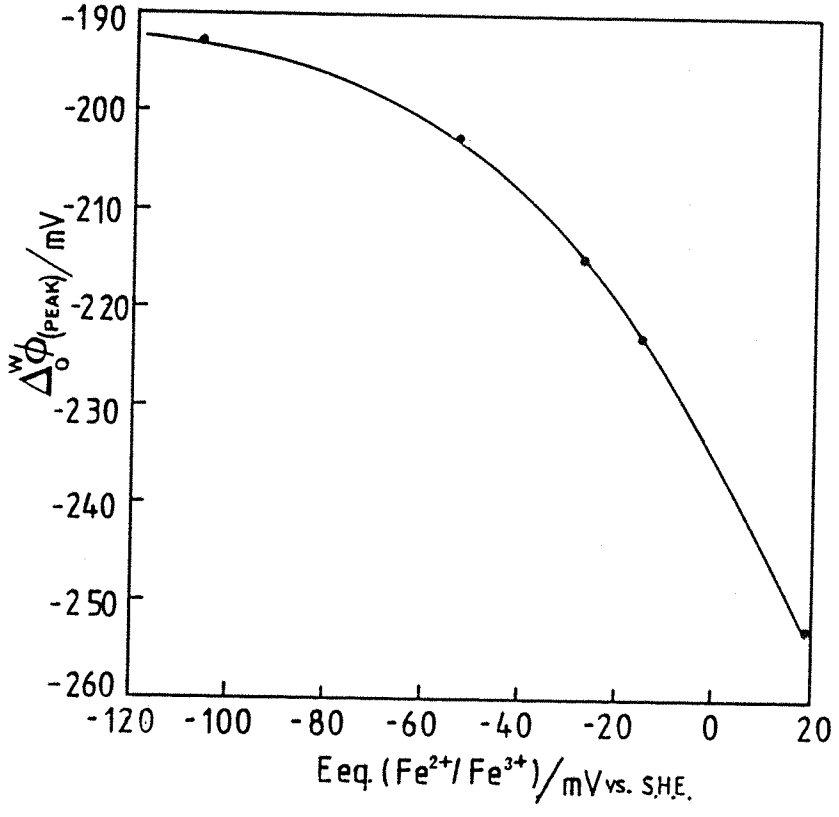


Figure 4.13
Dependence of the Galvani peak potential difference $\Delta^{w}_{o}\phi$ measured at 10 mVs^{-1} for CELLS 9(ii)→9(vi) on the measured equilibrium potential of the aqueous redox couple (vs SHE). Details are given in Table I, Chapter 3.

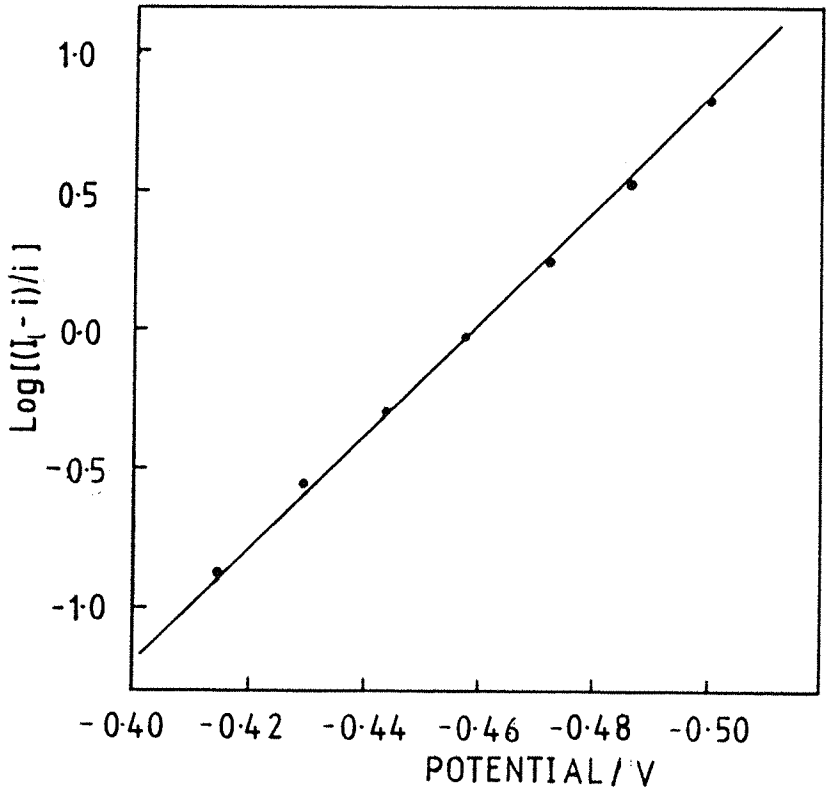


Figure 4.14
Semilogarithmic analysis for the steady state polarization curve for the reduction of (Meldola Blue)$^{+}$TPhBCl^{-} in 1,2-DCE at a 10 μm Au microelectrode (CELL 1), to test for a one electron reversible process.

The diffusion coefficient for the two dyes in 1,2-DCE was estimated using equation (3.2.1) and the results are shown in Table IV. An analysis of the measured half-wave potentials was carried out by considering the individual components of Cell (11):

$$E^{o}Mox = E_{\frac{1}{2}}(11) + \Delta^{w}_{o}\phi(TPhB^{-}) + E_{(SSCE)} - (RT/F)\ln(\gamma(Mox)/\gamma(Mred)) \quad \ldots(4.2.1)$$

where Mox and Mred correspond to the oxidised forms of (Meldola Blue)$^{+}$TPhBCl^{-} or (Methylene Blue)$^{+}$TPhBCl^{-}. The measured halfwave potentials were -0.620V in the case of Methylene Blue and -0.460V for Meldola Blue. The ratio of the activity coefficients was taken as unity as in the other cases where both the oxidised and reduced forms of the dye were charged. The value of $\Delta^{w}_{o}\phi(TPhB^{-})$ was calculated from equation 3.3.1 as +0.383V.

The data obtained from the analysis above is summarised in TABLE IV:

TABLE IV

Dye	E° vs SHE/V	D/(cm^2.s^{-1})	
(Methylene Blue)$^{+}$ DYE (II)	-0.001	7.3 x 10^{-6}	reversible
C_6TH^{+} DYE (III)	-0.156	------	irreversible
(Meldola Blue)$^{+}$ DYE (IV)	+0.159	6.6 x 10^{-6}	reversible

From the point of view of matching the potentials to that of the biocomponent of interest, viz NADH (E = -0.320V vs SHE), the standard potentials of these three dyes are not ideally matched. From equation (2.2.7), it is clear that the best approach is to have similar values for the standard potentials of the aqueous and organic couples.

The transfer potential of the dyes (II) and (IV) was examined in system 12, described in section 3.3.2, using the conditions in Table II of

Chapter 3 to fix the interfacial Galvani potential. The results are presented in TABLE V. A transfer denotes the observed partitioning of the dye from 1,2-DCE to water.

TABLE V

	TEA+	TPrA+	TBuA+	TPhAs+
DYE (II)	-	-	transfer	transfer
DYE (III)	-	-	-	-
DYE (IV)	-	-	transfer	transfer

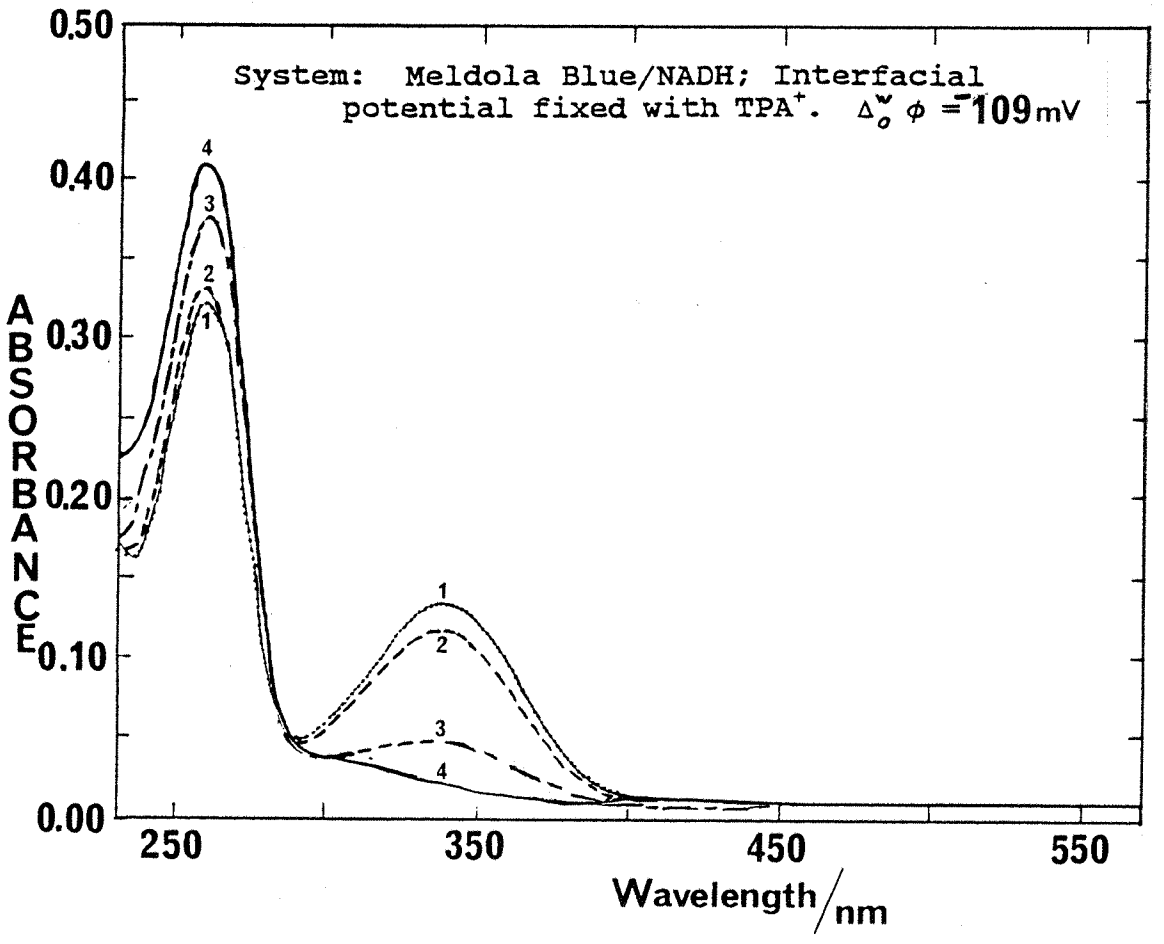
From Table V it can be seen that the semihydrophobic dyes (II) and (IV) have transfer potentials close to that of TBA^+ ($\Delta^w_o\phi(TBA^+)$ = -0.243V), and hence, the number of partitioning tetraalkylammonium salts which can be used is limited essentially to $TPrA^+$ and TEA^+. The hydrophobic dye (III), C_6TH^+ presents no transfer problem under the given conditions. From equation (3.3.1), the ratio of the activities can be made smaller and the Galvani potential thus more negative, or vice versa, and therefore if necessary a whole range of potentials can be imposed up to the dye transfer limit using TEA^+ and $TPrA^+$. However, for simplification the values and concentrations given for the partition systems in Table (II) were used for the non-electrochemical system (14) used with NADH. For the control experiments, NADH in a buffered solution at pH 7 was placed in contact with 1,2-DCE. Only a small decay was noted after a very long time (2 days). No observed effects were apparent on the NADH in the presence of the partitioning ions which were used to confer the required potential control to the interface. The purpose of these measurements was to establish the conditions required for the two phase oxidation of NADH, using equation 2.2.7.

The successful two phase oxidation of NADH was observed in the case of

dye (IV), Meldola Blue. In the absence of interfacial potential control, a very slow negligible decay was noted, when no phase transfer catalyst (PTC) was used for fixing the interfacial potential. When $TPrA^+$ was used, a relatively fast oxidation of NADH was noted as illustrated in Figure 4.15. A significant oxidation was observed within forty minutes of equilibrating the immiscible organic phase containing the dye with the NADH solution. The use of TEA^+ resulted in a much slower decay, as expected from equation 2.2.7, since this ion makes the aqueous phase less negative than in the case of the $TPrA^+$, hence decreasing the driving force for the reaction. $(Meldola\ Blue)^+$ appears to be an effective mediator for NADH oxidation in the context of a two phase system.

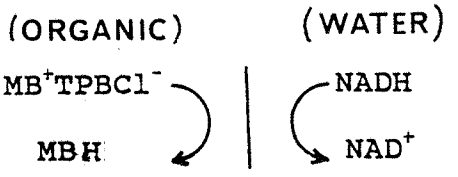
When $(Methylene\ Blue)^+$ was used as the mediator, no significant decay was noted in the NADH peak at 340nm in the absence of PTC, in experiments carried out under the same conditions as above . After a very long time (24hrs) a very slight partitioning of the dye was observec spectrophotometrically. Under potential control conferred by the use of $TPrA^+$, a slow oxidation of the NADH is seen which is still greater than that observed in the corresponding control experiments. Again, at very long times, greater than 24 hours, a slight partitioning of the dye in the aqueous phase was noted. The reaction in the presence of TEA^+ as the PTC was very slow relative to the last system, but again greater than that of the corresponding control experiment, and similarly as before, a very slight partitioning of the dye was noted at times greater than 24 hrs. The partitioning phenomenon in the case of Methylene Blue suggests that this dye is slightly less hydrophobic than Meldola Blue where no partitioning of the dye was seen. The effect is only observed at very long times (>24hrs) and may result from decomposition of the organic electrolyte.

Figure 4.15



Change of absorbance of NADH with time.

1 No redox dye
2 after 40 mins
3 after 90 mins
4 after 3 hours



For the very hydrophobic dye, C_6TH^+ (III), no significant oxidation of NADH could be found both in the absence and presence of $TPhAs^+$, used as the phase transfer catalyst, with respect to the control experiments.

If the above results are compared, although $E_{\frac{1}{2}}$ of C_6TH^+ is not ideally matched to that of NADH, it is closer than for the two semihydrophobic dyes. However, as compared to these other dyes, the rate of NADH oxidation by C_6TH^+ was very much slower, and thus it is likely for this reason that the reaction of C_6TH^+ with NADH was not observed.

The slow kinetics can be attributed to the much greater degree of steric hindrance, and hence shielding, of the C_6TH^+ redox centre which is conferred by the four long alkyl chains of the amino substituents, rather than the extra delocalisation of the cationic charge by the introduction of the positively inducing alkyl chains, as compared to the analogue, Methylene Blue with methyl substituents. The positive inductive stabilising effect of the substituents only acts over a short range, approximately for the first three methylene groups in the substituent chain. In the case of Meldola's Blue, if its redox potential alone is considered, it would appear to be less favourable than Methylene blue as a mediator for NADH oxidation. Clearly, the reaction of Meldola Blue with NADH is enhanced significantly with respect to that of Methylene Blue with a potential more negative than that of Meldola Blue. An explanation for this probably lies with the fact the Meldola Blue forms a more favourable interfacial charge transfer intermediate with NADH at the interface as compared to that of Methylene Blue.

From the few studies which have been conducted, it is difficult therefore to outline any general trends regarding mediation of the oxidation of NADH in the two phase system as yet. Although the ideas underlying the effect of the Galvani potential difference on NADH oxidation rates appear

to be correct, clearly more dyes need to be investigated systematically to illustrate such trends. It appears that the orientation of the mediating species plays a very important role regarding the interfacial interaction of the two redox centres.

Preliminary voltammetric studies of Meldola Blue at liquid-liquid interfaces were carried out using Cell (9). The initial experiment using Cell (9) was complicated by the transfer of the dye into the organic reference junction leading to mixed ill-defined junction potentials. The baseline obtained for the supporting electrolyte system was in the range +0.35 to +0.610V. The effect of the addition of the dye to the organic phases did not afford, as what might have been expected, a clear transfer of the dye. Instead the dye was seen to transfer over the whole potential range, leading to broad capacitive currents. The effect of increasing the concentration of the dye was an increase in the latter effect of large capacitive currents. The system as it stands was unsuitable for the study of NADH oxidation, i.e., to study the very small electron transfer currents expected in the presence of large background currents. From the effect of the addition of NADH (20 μM) in the buffer solution (pH7.0) to the aqueous section, it was not possible to distinguish properly any electron transfer from the large background currents obtained.

The voltammetric study of the interfacial oxidation of NADH with Meldola Blue in the organic phase requires further investigation using alternative techniques to that of four electrode cyclic voltammetry.

4.3. Conclusions

The hydrophobic dye dihexadecyl viologen, $C_{16}V^{2+}(TPhBCl^{-})_2$, has been shown to exhibit a very reproducible film formation phenomena with the anion of the aqueous electrolyte at the water/1,2-DCE interface. The results

suggest a diffusionally controlled process and the analogy between this behaviour at the liquid/liquid interface and that of viologen radical cation film or metal deposition at metal electrodes has been drawn. The solution to the mathematical description for metal deposition was applied to the viologen system at a liquid/liquid interface, but does not appear to be entirely applicable to the film formation observed, which is not totally unexpected since in the liquid/liquid case the diffusion of species at two sides needs to be considered. Interfacial reversible electron transfer between the above organic based dye, $C_{16}V^{2+}(TPhBCl^{-})_2$, and a fast aqueous Fe(II)/Fe(III) citrate redox couple has been demonstrated, under conditions where both phases in the two phase system were fully deaerated. Conversely, the effect of using air-saturated solutions afforded an irreversible cyclic voltammogram with a large enhanced single wave at increasing negative potentials. This suggests that oxygen electrocatalyses the reduction of the viologen and this idea is supported by simple qualitative two phase tests carried out with the viologen. After having demonstrated the reduction of an organic based redox dye, viz. the viologen, at the ITIES, it was hoped to transpose the ideas to the oxidation of NADH.

It must be noted, however that the above studies are fundamentally different from the studies with NADH since that the previous voltammetric studies employed a concentrated aqueous redox couple, but in the case of the biocomponent system, NADH, the aqueous redox component was utilised in small concentrations.

By the use of partitioning ions to fix the interfacial potential difference at the water/1,2-DCE interface it has been demonstrated that interfacial electron transfer between NADH and redox active dyes can be observed. In particular the use of the hydrophobic dye, C_6TH^{+}, (as

synthesised in this project to a high purity), appeared to be relatively ineffective in reducing NADH, which is probably accountable by the observed slow kinetics of reduction of the dye from microelectrode experiments.

By comparison with the semi-hydrophobic analogue of the di-hexylthionine derivative used ((Methylene Blue)$^+$TPhBCl$^-$), which reacted interfacially with NADH, albeit slowly relative to (Meldola Blue)$^+$TPhBCl$^-$, it appears that at the expense of making the species more desirably hydrophobic in the case of C_6TH^+ using long alkyl chain, there is a significant increase in the steric hindrance on the redox site of the molecule due to the substituents . Also, the charge is less localised on the dye owing to the positive inductive stabilising effect of the alkyl chains on the 3- and 7-position amino substituents on the phenothiazine backbone in C_6TH^+. This increases the availability of the lone pair of electrons on the nitrogens, relative to the case where the substituent is methyl (in (II)), and consequently, the cationic charge residing on the ring system can be mesomerically stabilised. The less hydrophobic analogue, (Methylene)$^+$TPhBCl$^-$, was not affected to as large an extent by the steric problem of alkyl substituents and showed characteristic reversible electron transfer at the microelectrode. However, as a mediator, it proved to be slow, probably due to steric, orientational factors or incorrect value of the redox potential for electron transfer. The final mediator tried was the semi hydrophobic, (Meldola Blue)$^+$TPhBCl$^-$, (IV), which was by far the most successful of the three redox dyes chosen for the two phase study of NADH oxidation. This compound, as in the case (Methylene Blue)$^+$, displayed a reversible one electron reduction at the metal surface, and from a steric point of view, it would appear that it can interact more easily with NADH than the other dyes. In future work,

it is hoped to obtain slightly more hydrophobic redox analogues of Meldola Blue for use in this system .

In order to elucidate the mechanism for the above processes, more quantitative measurement are required and indeed the behaviour of more analogues of these species and other hydrophobic dyes will have to be investigated systematically in the two phase system. The important outcome of the study is that for the first time it has been shown that by the use of partitioning ions in both phases to impose an interfacial Galvani potential, it is possible to drive the reduction of a biologically important redox active compound, such as NADH, with the use of a soluble organic-based redox placed in the organic phase of a two phase system. This demonstrates one of the aims of the project, viz.the use of a two phase system, the ITIES as a simple half-biological membrane model. The last stage of these preliminary investigations , the behaviour of (Meldola Blue)$^{+}$ at the ITIES was studied voltammetrically but this investigation was curtailed somewhat by the strong adsorption of the dye over the whole potential range. From the large capacitive current it was difficult to ascertain whether any electron transport process was occurring after placing NADH in the aqueous phase. The other dyes have yet to be investigated with respect to their behaviour at the ITIES. As noted above, the major difference between the ITIES four electrode study of the viologen, where the concentration of the aqueous couple was much greater than that of the violcgen, and the NADH study, is the smaller concentration of aqueous redox component with respect to organic component. Therefore, diffusional behaviour of both the aqueous and the organic redox species has to be considered.

Suggestions for further work

(i) It is hoped to re-investigate the mechanism of viologen film formation at the ITIES using alternative methodology, such as fast techniques, and to examine other similar amphiphilic species at the ITIES.

(ii) Clearly, many more quantitative measurements are required to investigate the heterogenous oxidation of NADH driven by the use of phase transfer catalysts with redox dyes. The investigation of more hydrophobic redox and more highly reversible redox species with potentials ideally as close to that of NADH need to be examined systematically so that a clearer basis for the oxidation of NADH and perhaps other important biocomponents can be ascertained. If this is achieved, then it is desirable to re-examine these species voltammetrically using the four electrode arrangement and thus attempt to characterise the nature of the interfacial charge transfer interaction of the redox species electrochemically.

(iii) A slightly different approach could be adopted to the study of the redox chemistry of elementary biocomponents at the ITIES. This could involve the study of the synthesised mediators incorporated into simple monolayers of lipids at the ITIES surface and thus try to attain a model closer to that of the natural biomembrane environment than is presented by the simple ITIES system, in which to examine electrochemically charge transfer reactions .

REFERENCES

[1] J. Koryta, Electrochim. Acta, 29:9 (1984) p.1291.

[2] J. Koryta and J. Dvorak, Principles of Electrochemistry, Chapter 6, J. Wiley and Sons, New York (1987) p.378-428.

[3] R.P. Buck, CRC Crit. Rev. Anal. Chem., 5 (1975) p.323.

[4] J. O'M. Bockris and A.K.N. Reddy, Modern Electrochemistry, Plenum Press, New York (1970).

[5] J. Koryta, J. Dvorak and V. Bohackova, Lehrbuch der Elektochemie, Springer, Vienna (1975); Electrokhimiya Mir, Moscow (1977);
J. Koryta and J. Dvorak, Electrochemie, 3rd Czech edn., Academia, Prague (1983).

[6] J. Koryta, Ions, Electrodes and Membranes, J. Wiley and Sons, Chichester (1982); Mir, Moscow (1983).

[7] J. Kuta and E. Palacek, in 'Topics in Bioelectrochemistry and Bioenergetics', Vol.5, ed, G. Milazzo, Interscience, New York (1983).

[8] F.A. Armstrong, H.A. Hill and N.J. Walton, Quart. Rev.
Biophys., 18 (1986) p.261.

[9] 'Biological Electrochemistry', Vol.1, ed. G. Dryhurst,
K.M. Kadish, F. Scheller and R. Renneberg, Academic Press, New York (1982).

[10] 'Comprehensive Treatise of Electrochemistry', Vol.10,

ed. S. Srinivanasan, Yu. A. Chizmadzhev, J. O'M. Bockris, B.E. Conway, E. Yeager and R.E. White, Plenum Press, New York (1981).

[11] 'Modern Bioelectrochemistry', ed. F. Gutmann and H. Keyser, Plenum Press, New York (1986).

[12] R. Szentrimay, P. Yeh and T. Kuwana, in 'Electrochemical Studies of Biological Systems', ed. D. Sawyer, A.C.S., Washington, D.C. (1977) p.143.

[13] 'Biological Aspects of Electrochemistry', ed. G. Milazzo, Experientia Suppl., Vol.18 (1971).

[14] 'Bioelectrochemistry: Ions, Surfaces, Membranes', ed. M. Blank, A.C.S., Washington, U.S.A. (1980).

[15] 'Bioelectrochemistry', ed. H. Keyser and F Gutmann, Plenum Press, New York (1983).

[16] 'Bioelectrochemistry-I. Biological Redox Reactions', ed. G. Milazzo and M. Blank, Plenum Press, New York (1983).

[17] 'Premier Coloque National de Bioelectrochimie', Groupe Francais de Bioelectrochimie (1983).

[18] L.I. Boguslavsky, Bioelectrochemical Phenomena and Phase Boundary (in Russian), Nauka, Moscow (1978).

[19] L.Y.C. Lee and J.K. Hurst, J. Am. Chem. Soc., 106 (1984) p.7411.

[20] H.T. Li and M.J. Weaver, J. Am. Chem. Soc., 106 (1984) p.6107.

[21] Z. Samec, V. Marecek and J. Weber, J. Electroanal. Chem., 103 (1979) p.11.

[22] Z. Samec, V. Marecek, J. Weber and D. Homolka,

J. Electroanal. Chem., 126 (1981) p.105.

[23] J. Hanzlik, Z. Samec and J. Homolka, J. Electroanal. Chem., 216 (1987) p.303.

[24] G. Geblewicz and D.J. Schiffrin, J. Electroanal. Chem., 244 (1988) p.27.

[25] C.A. Beltran, V.J. Cunnane, G. Geblewicz, D.J. Schiffrin and T. Solomon, J. Electroanal. Chem., 247 (1988) p.203.

[26] C. Gavach, T. Mlodnicka and J. Guastalla, C. R. Acad. Sci., Ser. C., 266 (1968) p.1196.

[27] H.H. Girault and D.J. Schiffrin, in 'Electro-analytical Chemistry', Vol.15, ed. A.J. Bard, Marcel Dekker, Inc., (1989) p.1-141.

[28] J. Koryta, Electrochim. Acta, 34:2 (1989) p.93.

[29] Z. Samec, Chem. Rev., 88 (1988) p.617.

[30] A.G. Volkov and V.S. Markin, J. Colloid and Interface Science, 131:2 (1989) p.382; Electrochem. Acta, 34:2 (1989) p.93.

[31] J. Koryta, P. Vanysek and M. Brezina, J. Electroanal. Chem., 67 (1976) p.263.

[32] C.M. Visser, Origins Life, 12 (1982) p.165.

[33] P.L. Dutton and D.F. Wilson, Biochimica et Biophysica Acta, 346 (1974) p.165.

[34] W.M. Clarke, Oxidation-Reduction Potentials of Organic Systems, Bailliere, Trindall and Cox, Ltd., London (1960).

[35] W. Nernst and E.H. Riesenfeld, Ann. Phys., 8 (1902) p.600.

[36] E.J.W. Verwey and K.F. Niessen, Philos. Mag., 28 (1939) p.435.

[37] M. Cremer, Z. Biologie, 46 (1906) p.562.

[38] F. Haber and Z. Klemenciewicz, Z. Phys. Chem., 67 (1909) p.385.

[39] E.H. Riesenfeld and B. Reinhold, Z. Phys. Chem., 68 (1909) p.59.

[40] R. Beutner, Z. Elektrochem., 19 (1913) p.319,p.467; 24 (1918) p.94.

[41] E. Baur and S. Kronman, Z. Phys. Chem., 92 (1917) p.819.

[42] K.F. Bonhoeffer, M. Kalweit and H. Strehlow, Z. Electrochem., 57 (1953) p.614.

[43] K.F. Bonhoeffer, M. Kalweit and H. Strehlow, Z. Phys. Chem. (Frankfurt), 1 (1954) p.21.

[44] F.M. Karpfen and J.E.B. Randles, Trans. Faraday Soc., 49 (1953) p.823.

[45] M.I. Gugeshashivili, B.T. Lozhkhin, L.I. Boguslavsky, Electrokhimiya, 10 (1974) p.1272.

[46] M.I. Gugeshashivili and L.I. Boguslavsky, Electrokhimiya, 8 (1972) p.1471.

[47] J. Guastalla, J. Chim. Phys., 53 (1956) p.470.

[48] J. Guastalla, Proc. 2nd Int. Congress Surf. Activity, (London), 3 (1957) p.112.

[49] M. Blank and S. Feig, Science, 141 (1963) p.1173.

[50] M. Dupeyrat, J. Chim. Phys., 61 (1964) p.316.

[51] M. Dupeyrat and J. Michel, C. R. Acad. Sci. Paris, 264c (1967) p.1240.

[52] M. Dupeyrat and J. Michel, J. Colloid and Interface Sci., 29 (1969) p.605.

[53] A. Watanabe, M. Matsumoto, H. Tamai and R. Gotch, Kolloid Z. Z. Polym., 220 (1967) p.152.

[54] M. Blank, J. Colloid Interface Sci., 22 (1966) p.51.

[55] M. Dupeyrat and J. Michel, Experientia Suppl., 18 (1971) p.321.

[56] C. Gavach and B. D'Epenoux, C. R. Acad. Sci., 272c (1971) p.321.

[57] C. Gavach, Experientia Suppl., 18 (1971) p.321.

[58] P. Joos and M. Van Bockstable, J. Phys. Chem., 80 (1976) p.1573.

[59] Y. Verburgh and P. Joos, J. Colloid Interface Sci., 74 (1980) p.384.

[60] J. Guastalla, Nature, 227 (1970) p.485.

[61] C. Gavach, P. Seta and F. Henry, Bioelectrochem. Bioenerg., 1 (1974) p.329.

[62] C. Gavach, B. d'Epenoux, J. Electroanal. Chem., 54 (1974) p.361; 55 (1974) p.59.

[63] C. Gavach, C. R. Acad. Sci., Ser. C., 269 (1969) p.1356.

[64] C. Gavach and F. Henry, C. R. Acad. Sci., Ser. C., 274 (1972) p.1545.

[65] C. Gavach, F. Henry and R. Sandreaux, C. R. Acad. Sci., Ser. C., 278 (1974) p.491.

[66] C. Gavach and B. d'Epenoux, J. Electroanal. Chem., 64 (1975) p.107.

[67] O.R. Melroy, R.P. Buck, F.S. Stover and H.C. Hughes,

J. Electroanal. Chem., 121 (1981) p.93.

[68] O.R. Melroy and R.P. Buck, J. Electroanal. Chem., 136 (1982) p.19; 143 (1983) p.23.

[69] W.E. Bronner, O.R. Melroy and R.P. Buck, J. Electroanal. Chem., 162 (1984) p.263.

[70] O.R. Melroy, W.E. Bronner and R.P. Buck, J. Electrochem. Soc., 130 (1983) p.373.

[71] O.R. Melroy, Ph.D. Thesis, The University of North Carolina (1982).

[72] Z. Samec, V. Marecek, J. Weber and D. Homolka, J. Electroanal. Chem., 99 (1979) p.385.

[73] A.L. Hodgkin, A.F. Huxley and B. Katz, Arch. Sci. Physiol., 3 (1949) p.129.

[74] Z. Samec, V. Marecek, J. Koryta and W. Khalil, J. Electroanal. Chem., 83 (1977) p.393.

[75] Z. Samec, V. Marecek and J. Weber, J. Electroanal. Chem., 100 (1979) p.841.

[76] S. Kihara, Z. Yoshida and T. Fujinaga, Bunseki Kagaku, 31 (1982) p.81; 31 (1982) p.297.

[77] D. Homolka, V. Marecek, Z. Samec, K. Base and H. Wendt, J. Electroanal. Chem., 163 (1984) p.159.

[78] T. Kakutani, T. Osakai and M. Senda, Bull. Chem. Soc. Jpn., 56 (1983) p.991.

[79] L.E.A. Berlouis, J. Chatman, H.H. Girault and D.J. Schiffrin, Extended Abstracts, 163rd Meet. Am. Electrochem. Soc., San Francisco (May 1983).

[80] Le. Q. Hung, Chem. Listy, 74 (1980) p.1089.

[81] B. Hundhammer, T. Solomon and B. Alemayehu,

J. Electroanal. Chem., 135 (1982) p.301.

[82] D.J. Schiffrin, private communication.

[83] B. Hundhammer and S. Wilke, J. Electroanal. Chem., 266 (1989) p.133.

[84] J. Koryta, P. Vanysek and M. Brezina, J. Electroanal. Chem., 75 (1977) p.211.

[85] G. Geblewicz, A.K. Kontturi, K. Kontturi and D.J. Schiffrin, J. Electroanal. Chem., 217 (1987) p.261.

[86] P. Vanysek, J.D. Reid, M.A. Craven and R.P. Buck, J. Electrochem. Soc., 131 (1984) p.1788.

[87] A.J. Parker, Electrochim. Acta, 21 (1976) p.671.

[88] H.H. Girault and D.J. Schiffrin, Electrochim. Acta, 31 (1986) p.1341.

[89] G. Geblewicz, Ph.D. Thesis, Warsaw University, Poland (1982).

[90] Z. Koczorowski, G. Geblewicz, J. Electroanal. Chem., 139 (1982) p.177; 152 (1983) p.55.

[91] J. Guastalla, Mem. Ser. Chim. Etat., 41 (1956) p.317.

[92] D. Homolka, Le Quoc Hung, A. Hofmanova, M.W. Khalil, J. Koryta, V. Marecek, Z. Samec, S.K. Sen, P. Vanysek, J. Weber and M. Brezina, Anal. Chem., 52 (1980) p.1606.

[93] M. Guainazzi, G. Silvestri and Serravalle, J. Chem. Soc., Chem. Comm., 6 (1975) p.200.

[94] Z. Samec, D. Homolka, V. Marecek and L. Kavan, J. Electroanal. Chem., 145 (1983) p.213.

[95] A.N. Popov and B. Punns, Latv. P. S. R. Zinat. Akad.

Vestis. Kim. Ser., 2 (1981) p.169.

[96] Z. Koczorowski, G. Geblewicz and M. Panoch, J. Electroanal. Chem., 172 (1984) p.327.

[97] O. Valent, J. Koryta and M. Panoch, J. Electroanal. Chem., 226 (1987) p.21.

[98] H.M. Alemu, private communication.

[99] H.M. Alemu and T. Solomon, J. Electroanal. Chem., 261 (1989) p.297.

[100] H.M. Alemu, M.Sc. Thesis, Addis Ababa University, Ethiopia (1983).

[101] P. Joos and R. Vanden Bogaert, J. Colloid Interface Sci., 56 (1976) p.206.

[102] L.I. Boguslavsky, A.N Frumkin and M.I. Gugeshashvili, Electrokhimiya, 12 (1976) p.858.

[103] P. Seta, B. d'Epenoux and C. Gavach, J. Electroanal. Chem., 95 (1979) p.191.

[104] J.D. Reid, O.R. Melroy and R.P. Buck, J. Electroanal. Chem., 147 (1983) p.71.

[105] H.H. Girault and D.J. Schiffrin, J.Electroanal.Chem., 150 (1983) p.43; 179 (1984) p.277.

[106] M. Gros, S. Cromb and C. Gavach, J.Electroanal.Chem., 89 (1978) p.29.

[107] T. Kakiuchi and M. Senda, Bull. Chem. Soc. Jpn., 56 (1983) p.277, p.991, p.1322.

[108] T. Osakai, T. Kakiuchi and M. Senda, Bull. Chem. Soc. Jpn., 57 (1984) p.370.

[109] Z. Samec, V. Marecek and D. Homolka, J. Electroanal. Chem., 126 (1981) p.121.

[110] P. Hajkova, D. Homolka, V. Marecek and Z. Samec, J. Electroanal. Chem., 151 (1983) p.277; 159 (1983) p.233.

[111] V. Marecek and Z. Samec, J. Electroanal. Chem., 149 (1983) p.185.

[112] Z. Samec, V. Marecek and D. Homolka, Farad. Discuss. Chem. Soc., 77 (1984) p.197.

[113] J.D. Reid, P. Vanysek and R.P. Buck, J. Electroanal. Chem., 147 (1983) p.71; 161 (1984) p.1; 170 (1984) p.109.

[114] Z. Samec, V. Marecek and D. Homolka, J. Electroanal. Chem., 187 (1985) p.31.

[115] V. Marecek and Z. Samec, J. Electroanal. Chem., 185 (1985) p.263.

[116] H.H. Girault and D.J. Schiffrin, J.Electroanal. Chem., 170 (1984) p.127.

[117] G. Gouy, C. R. Acad. Sci., 149 (1910) p.654.

[118] D.L. Chapman, Philos. Mag., 25 (1913) p.475.

[119] C. Gavach, P. Seta and B. d'Epenoux, J. Electroanal. Chem., 83 (1977) p.225.

[120] R.A. Marcus,J. Chem. Phys., 43 (1965) p.679.

[121] V.G. Levich, in 'Advances in Electrochemistry and Electrochemical Engineering', Vol.4, ed. P. Delahay, Interscience, New York (1966) p.299.

[122] M.I. Gugashashvili, M.A. Manvelyan and L.I. Boguslavskii, Electrokhim., 10 (1974) p.819.

[123] Z. Samec, V. Marecek and D. Homolka, J. Electroanal. Chem., 170 (1984) p.383.

[124] H.H. Girault and D.J. Schiffrin, J. Electroanal. Chem., 161 (1984) p.415.

[125] C.W. Outhwaite, L.B. Bhuiyan and S. Levine, J. Chem. Soc. Farad. Trans. II, 76 (1980) p.1388.

[126] S.L. Carnie, D.Y.C. Chan, D.J. Mitchell and B.W. Ninham, J. Chem. Phys., 74 (1981) p.1472.

[127] G.M. Torrie and J.P Valleau, J. Electroanal. Chem., 206 (1986) p.69.

[128] H.H. Girault and D.J. Schiffrin, J. Electroanal. Chem., 195 (1985) p.213.

[129] H.H. Girault and D.J. Schiffrin, J. Electroanal. Chem., 244 (1988) p.15.

[130] Y.Y. Gurevich and Y.I. Kharkats, J. Electroanal. Chem., 200 (1986) p.3.

[131] Z. Samec, Y.I. Kharkats and Y.Y. Gurevich, J. Electroanal. Chem., 204 (1986) p.257.

[132] E. Yurtsever and H. Karaasian, Ber. Bunsen-Ges. Phys. Chem., 91 (1987) p.600.

[133] P. Delahay, New Instrumental Methods in Electrochemistry, Interscience, New York (1954).

[134] D.Homolka and V. Marecek, J. Electroanal. Chem., 112 (1980) p.91.

[135] P. Vanysek and M. Behrendt, J. Electroanal. Chem., 130 (1982) p.287.

[136] V. Marecek and Z. Samec, Anal. Lett., B15 (1981) p.141.

[137] Z. Samec, V. Marecek, J. Weber and D. Homolka, J. Electroanal. Chem., 126 (1981) p.105.

[138] J. Hanzlik and Z. Samec, Collection Czech. Chem. Commun., 52 (1987) p.830.

[139] A.K. Kontturi, K. Kontturi and D.J. Schiffrin, J. Electroanal. Chem., in press.

[140] P. Vanysek, J. Electroanal. Chem., 121 (1988) p.149.

[141] B. Hundhammer and T. Solomon, J. Electroanal. Chem., 157 (1983) p.19.

[142] E. Wang and Y. Liu, J. Chem. Soc., Farad. Trans. I, 84:7 (1988) p.2289.

[143] G. Geblewicz, Z. Koczorowski and Z. Figaszewski, Chemia Analityczna, 31 (1986) p.567.

[144] Z. Koczorowski, G. Geblewicz and Z. Figaszewski, Colloids Surfaces, 6 (1983) p.43.

[145] Z. Pang, C.A. Chang and E. Wang, J. Electroanal. Chem., 234 (1987) p.71.

[146] Z. Samec, V. Marecek and D. Homolka, J. Electroanal. Chem., 158 (1983) p.25.

[147] H. Alemu and T. Solomon, J. Electroanal. Chem., 237 (1987) p.113.

[148] M.H. Abraham and J. Liszi, J. Inorg. Nucl. Chem., 43 (1981) p.143.

[149] S. Kihara, M. Suzuki, K. Maeda, K. Ogura and M. Masui, J. Electroanal. Chem., 210 (1986) p.147.

[150] Yu. N. Kozlov and J. Koryta, Anal. Lett., B16 (1983) p.255.

[151] E. Wang and Y. Liu, J. Electroanal. Chem., 214 (1986) p.459.

[152] E. Makrlik, W. Ruth and P. Vanysek, J. Colloid Interface Sci., 96 (1983) p.548.

[153] Z. Yoshida and H. Freiser, Inorg. Chem., 23 (1984) p.3931.

[154] Z. Sun and E. Wang, Electrochimica Acta, 33:5 (1988) p.603; Trends in Analytical Chemistry, 7:3 (1988) p.99.

[155] A. Sabela, J. Koryta and O. Valent, J. Electroanal. Chem., 204 (1986) p.267.

[156] G. Taylor and H.H.J. Girault, J. Electroanal Chem., 208 (1986) p.179.

[157] J.A. Campbell, A.A. Stewart and H.H. Girault, J. Chem. Soc., Farad. Trans. I, 85:4 (1989) p.843.

[158] F.L. Thomson, L.J. Yellowlees and H.H. Girault, J. Chem. Soc., Chem. Comm., (1988) p.1547.

[159] Z. Samec, A.R. Brown, L.J. Yellowlees, H.H. Girault and K. Base, J. Electroanal. Chem., 259 (1989) p.309.

[160] V. Marecek, A.H. De Armond and M.K. De Armond, J. Electroanal. Chem., 261 (1989) p.287.

[161] V.J. Cunnane, D.J. Schiffrin, M. Fleischmann, G. Geblewicz and D. Williams, J. Electroanal. Chem., 243 (1988) p.455.

[162] T. Wandlowski, S. Racinsky, V. Marecek and Z. Samec, J. Electroanal. Chem., 227 (1987) p.281.

[163] T. Kakiuchi, M. Kobayashi and M. Senda, Bull. Chem. Soc. Jpn., 60 (1987) p.3109.

[164] D.J. Schiffrin, M. Wiles and M.R. Calder, Electrochemical Society Proceedings, Vol. 86:14

p.166.

[165] H.H. Girault and D.J. Schiffrin, Biochim. Biophys. Acta, 857 (1986) p.251.

[166] T. Makino and R. Aogaki, J. Electroanal. Chem., 198 (1986) p.209.

[167] T. Kakutani, Y. Nishiwaki, T. Osakai and M. Senda, Bull. Chem. Soc. Jpn., 59 (1986) p.781.

[168] L. Sinru, Z. Zaofan and H. Freiser, J. Electroanal. Chem., 210 (1986) p.137.

[169] D. Homolka and H. Wendt, Ber. Bunsen- Ges. Phys. Chem., 89 (1985) p.1075.

[170] E. Wang and Y. Liu, J. Electroanal. Chem., 214 (1986) p.465.

[171] E. Wang, Y. Liu and Y. Jiang, Proc. Electrochem. Soc., 86 (1986) p.35.

[172] J. Koryta, D. Guo, D. Ruth and P. Vanysek, Farad. Discuss. Chem. Soc., 77 (1984) p.209.

[173] F. Ruixi and C. Yufei, J. Electroanal. Chem., 256 (1988) p.207.

[174] E. Makrlik, Z. Phys. Chem., 268 (1987) p.200, p.212.

[175] E. Makrlik, J. Electroanal. Chem., 158 (1983) p.295.

[176] S. Kihara, M. Suzuki, K. Maeda, K. Ogura, M. Matsui and Z. Yoshida, J. Electroanal. Chem., 271 (1989) p.107.

[177] Z. Samec, J. Electroanal. Chem., 103 (1979) p.1.

[178] Z. Samec, J. Electroanal. Chem., 99 (1979) p.197.

[179] H.P. Bennetto, J.L. Stirling and K. Tanaka, Chem. Ind., London (1985) p.695.

[180] M.L. Fultz and R.A. Durst, Anal. Chim. Acta, 140 (1982) p.1.

[181] L. Michaelis and E.S. Hill, J. Gen. Physiol., 16 (1933) p.859.

[182] W.R. Boon, Chem. Ind., London (1965) p.782.

[183] L. Popsil, J. Kuta and J. Volke, J. Electroanal. Chem., 58 (1975) p.217.

[184] H.L Landrum, R.T. Salmon and F.M. Hawkridge, J. Am. Chem. Soc., 99 (1977) p.3154.

[185] A. Bewick, A.C. Lowe and C.W. Wederell, Electrochimica Acta, 28:12 (1983) p.1899.

[186] C.L. Bird and A.T. Kuhn, Chem. Soc. Rev., 10-11 (1981) p.49.

[187] L.A. Summers, The Bipyridinium Herbicides, Academic Press, London (1980).

[188] T.J. Meyer, Acc. Chem. Res., 11 (1978) p.94.

[189] P.A. Brugger and M. Gratzel, J. Am. Chem. Soc., 102 (1980) p.2461.

[190] T. Sugimoto, J. Miyazaki, T. Kokubo, S. Tanimoto, M. Okano and M. Matsumoto, J. Chem. Soc., Chem. Comm. (1981) p.210.

[191] S. Aono and I. Okura, Inorganica Chimica Acta, 137 (1987) p.135.

[192] H.C. Chang, T. Matsue, I. Urchida and T. Osa, Chemistry Letters, (1989) p.1119.

[193] T. Sugimoto, J. Miyazaki, T. Kokubo and S. Tanimoto, Tetrahedron Letters, 22 (1981) p.1119.

[194] M.I. Khramov, S.V. Lymar, V.N. Parmon and K.I.

Zamaraev, Dolkady Akad. Naur SSSR, 289:1 (1986) p.146.

[195] B.C. Patterson, D.H. Thompson and J.K. Hurst, J. Am. Chem. Soc., 110 (1988) p.365.

[196] L. Gorton, J. Chem. Soc., Farad. Trans. I, 82 (1986) p.1245.

[197] A.L. Underwood and R.W. Burnett, Biochemistry, 4 (1965) p.2060.

[198] H. Jaegfeldt, J. Electroanal. Chem., 110 (1980) p.295.

[199] M. Studnickova, H.P. Klukanova, J. Twanek and J. Kovar, J. Electroanal. Chem., 252 (1988) p.383.

[200] J. Zak and T. Kuwana, J. Electroanal. Chem., 150 (1983) p.645.

[201] H.P. Bennetto, M.E. Dew, J.L. Stirling and K. Tanaka, Chem. Ind., London (1981) p.776.

[202] B. Persson, L. Gorton and G. Johanssen, Bioelectrochem. and Bioenergetics, 16 (1986) p.479.

[203] A.G. Volkov, M.I. Gugeshashvilli, A.F. Miranov and L.I. Boguslavsky, Bioelectrochem. and Bioenergetics, 10 (1983) p. 485.

[204] V.S. Markin and A.G. Volkov, Electrochim. Acta, 34:2 (1989) p.93.

[205] R.A. Robinson and R.H. Stokes, Electrolyte Solutions, Butterworths, London (1959).

[206] D.J. Schiffrin, Southampton University, "in-house" computer program.

[207] D.D. Perrin, W.L.F. Armaregoe and D.R. Perrin, Purification of Laboratory Chemicals, Pergamon Press, Oxford (1080).

[208] "Encyclopaedia of Electrochemistry of the Elements", ed. A.J. Bard, Vol, 9.

[209] Boehringer-Mannheim Corporation, Biochemical Data Book, (1989) p.209.

[210] J. Bruinink, C.G.A. Kregting and J.J. Ponjee, J. Electrochem. Soc., 124:12 (1977) p.1854.

[211] Du Pont de Nemurs, E.I. & Co., U.K. Patent (Cl.C08f) (1970).

[212] P.J. Parsons, Southampton University, private communication.

[213] T. Vandernoot, The Queen Mary College, London, private communication.

[214] V.J. Cunnane, Southampton University, private communication.

[215] M. Fleischmann, S. Pons, D.R. Rolison and P.P. Schmidt, Ultramicroelectrodes, Datatech Systems

[216] K.B. Oldham, J. Electroanal. Chem., 122 (1981) p.1.

[217] J. Czapkiewicz and B. Czapkiewicz-Tutaj, J. Chem. Soc., Faraday Trans. I, 76 (1980) p.1663.

[218] M.H. Abraham and A.F. Danil de Namor, J. Chem. Soc., Faraday Trans. I, 72 (1976) p.955.

[219] C.W. Wederell, M.Sc. Thesis, University of Southampton (1981).

[220] G.J. Hills, D.J. Schiffrin and J. Thompson, J. Electrochem. Soc., 120:2 (1973) p.157.

[221] D.J. Bruinink and C.G.A. Kregting, J. Electrochem. Soc., 125 (1978) p.1397.

[222] D.J. Schiffrin, J. Electroanal. Chem., 201 (1986) p.199.

[223] A.J. Bard and L.R. Faulkner, Electrochemical Methods, Fundamentals and Applications, J. Wiley and Sons, New York (1980).