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THE ELECTROCHEMISTRY OF POLY-5-CARBOXYINDOLE

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A thesis submitted for the degree of Doctor of Philosophy of the
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TO MY MUM AND DAD

**TO CLIMB STEEP HILLS,
REQUIRES SLOW PACE AT FIRST.**

Henry VIII, Act I Scene 1

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DECLARATION

The work presented in this thesis was conducted solely by the author. Where the work of other authors has been discussed this is clearly indicated.

This work was conducted in the Department of Chemistry, University of Warwick, under the supervision of Dr P. N. Bartlett.

Signed.....

SUMMARY

The properties of the conducting polymer poly-5-carboxyindole in aqueous solution have been investigated.

The pH dependence of the electrochemistry of the polymer is explored and an application as a pH sensor proposed.

The use of the polymer as an electrode material to investigate the bioelectrochemistry of the redox protein cytochrome c is shown.

Finally the results of investigations using inorganic redox couples in order to probe the mechanism of electron transfer between the polymer and solution species are presented.

CHAPTER 1

INTRODUCTION

1.0 CONDUCTING POLYMERS

The essential property of all electrode materials is the ability to transfer charge. The methods by which charge transfer is achieved, however, can be quite diverse:

- a) in metals the mobile species is the electron within the conduction band,
- b) in semiconductors it may be electrons in the conduction band or positive holes in the valence band,
- c) in some oxides, sulphides etc., the charge may be carried by the movement of ions through the lattice,
- d) many modified electrodes are redox polymers wherein the electron moves by transfer between discrete centres,
- e) in conducting polymers charge transfer is achieved by the coupled motion of electrons and ions within the matrix of the polymer.

The development of modified electrodes, started in 1975 by Murray's group,¹ conferred a new freedom on the electrochemist. A chemically modified electrode is one which has been deliberately coated with a film of foreign molecules so that direct contact between solution species and the electrode is prevented, i.e. electron transfer is mediated by the molecules constituting the coat. The ability to vary the properties of the surface coat

enabled the construction of electrodes with desirable kinetic characteristics, and the capacity to catalyse or block specific reactions of interest. Many forms of modification can be found in the literature and the interest here is in a group of materials known as conducting polymers. These materials are characterised by electronic conductivities that may be as high as $10^4 \text{ ohm}^{-1} \text{ cm}^{-1}$, but are of a level such that they can act as their own current collector. The concept of the modified electrode is perhaps one of the more exciting developments of the last 25 years.

In conducting polymers the source of the charge carriers is believed to be related to the charge-transfer induced geometric modifications of the polymer chain, which lead to the formation of radical cations (polarons), and dications (bipolarons).²⁻⁴ Most conducting polymers have extensive conjugated π -systems and to become electroactive it is generally necessary to oxidise the polymer chain (although in some cases reduction is required) by removing an electron. The result is a localised lattice distortion resulting in positive charge formation on the chain, a polaron. The distortion involves the switching of the positions of the carbon-carbon double and single bonds. If a second electron is now removed, calculations have been made to show that it is energetically more favourable to remove this further electron from the polaron to form a bipolaron, rather than form a second polaron.⁵ Electron spin resonance spectroscopy (E.S.R.) and optical experiments⁶ have detected the presence of both polarons and bipolarons in the conducting polymer polypyrrole. At low conductivities E.S.R. signals are found for the polaron acting as

the charge carrier (signal increasing with conductivity). At high conductivities no E.S.R. signal is seen and this is consistent with the bipolaron acting as the charge carrier, this will not be detected by E.S.R. as they have no spin and consequently are E.S.R. silent. The majority of applications require the polymer to be capable of cycling between the oxidised and reduced states, for the polymer to hold charge during periods at open circuit, and for the change of oxidation state to be accompanied by strong changes in both conductivity and spectral properties. Possible applications include their use as battery electrodes,⁷ in display devices,⁸ in electronic devices, and as components of solar cells.⁹

The work in this thesis is concerned with the conducting polymer, poly-5-carboxyindole. Investigation of the properties of the polymer in aqueous solution and use as a promoter with a biological redox system will be presented.

1.1 HISTORICAL BACKGROUND

Conducting polymers are usually produced by the electrochemical polymerisation of aromatic compounds of the type first reported with the oxidation of pyrrole to polypyrrole.¹⁰ Dall'Olio and coworkers obtained 'pyrrole black' on electrochemical oxidation of pyrrole in aqueous sulphuric acid. The material produced was a powdery insoluble precipitate on the platinum electrode. Elemental analysis of the material showed that it consisted of 76% 'polypyrrole' with the remainder being sulphate ions, thus indicating a cationic polymer. It was found to have a conductivity

of $8 \text{ ohm}^{-1} \text{ cm}^{-1}$. In 1979 the importance of the electrochemical approach to the synthesis of conducting polymers became apparent.¹¹ The main difference in these experiments compared to those done previously was that they yielded continuous films which could be peeled off the electrode to give a free standing easily manageable air stable film. Also the electrical conductivities were found to be much higher, in the order in $100 \text{ ohm}^{-1} \text{ cm}^{-1}$.

1.2 GENERAL REQUIREMENTS

1.2.1 AROMATIC MONOMERS

Most conducting polymers are formed by the electrochemical oxidative polymerisation of a monomeric molecule. These molecules have several common features. Firstly, they are aromatic and are oxidised at relatively low anodic potentials. This is advantageous as it avoids complications that may arise from oxidative degradation of solvent and electrolyte. Potentials for the electropolymerisation of some aromatic monomers are given in table 1.1.

Secondly the monomer undergoes electrophilic substitution reactions where the aromatic structure is maintained. The polymerisation reaction proceeds via radical coupling, thus the susceptibility to electrophilic attack gives a reasonable indication as to whether a given compound will electropolymerise.

Compound	E_{pa} (V)	n	Reference
pyrrole	1.2	2.2-2.4	12
thiophene	2.07	2.06	13, 14
azulene	0.91	2.2	13
indole	1.26, 1.58	2.1	15
5-carboxyindole	1.4	1.92	15

Table 1.1: Oxidation potentials for some aromatic monomers vs saturated calomel electrode (SCE).

1.2.2 ELECTROLYTE CONDITIONS

On the basis that the polymerisation reaction occurs via radical cation intermediates, the nucleophilicity of the environment surrounding the electrode becomes a concern. The intermediates formed by the process of polymerisation will be sensitive to nucleophilic attack. This has the effect of restricting the use of solvents. For such reasons most studies are performed in aprotic solvents, as these are poor nucleophiles, the most common of these being acetonitrile. Similarly the choice of electrolyte salt is influenced by its degree of dissociation and nucleophilicity. This leads to the use of tetraalkylammonium salts, perchlorates and toluenesulphonic acids since they are soluble in aprotic solvents and are highly dissociated.

1.2.3 ELECTRODES

For high quality films it is best to have the working and reference electrodes separated from the counter electrode, usually by a glass frit. It is the nature of the working electrode that is of most importance. Since the films are generally formed by an oxidation, it is necessary for the working electrode not to undergo oxidation concurrently with the monomer. It is for this reason that most working electrodes are gold or platinum. However other materials have been used successfully such as tin oxide¹⁶ and graphite.¹⁷

1.2.4 GENERAL MECHANISM

The polymer chains consist of linked aromatic units, which in the case of pyrrole and thiophene are primarily 2,5-coupled.^{18,19} In the case of indole and 5-carboxyindole the coupling sites are thought to be the 1 and 3 positions,¹⁵ although the actual structures of these polymers are still unknown. Let us consider the electropolymerisation of pyrrole as a general model for the type of mechanism involved in the formation of conducting polymers. Figure 1.1 shows the proposed mechanism for the electropolymerisation of polypyrrole. After the initial oxidation step, there is a coupling reaction followed by a de-protonation and a one electron oxidation to regenerate the aromatic system.¹⁸ The initial reaction will involve monomer coupling to monomer to form dimeric intermediates, there is also the steady state reaction where dimers react to form

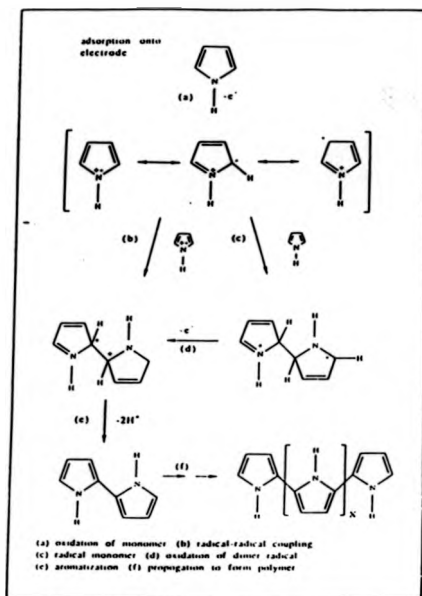


Figure 1.1: Scheme for the electropolymerisation of polypyrrole.²⁰

oligomers and polymeric intermediates. On oxidation, the originally formed radical cation can undergo reaction with another radical to form a dimer or it may act as an electrophile and add to a neutral monomer. Of the two possible routes for polymer formation the radical-radical one is the least likely. Although polymer formation is only observed when the potential is sufficient to oxidise the monomer insufficient to oxidise all the monomer present at the electrode. It is more likely that the radical acts as an electrophile and adds to a neutral monomer. The resultant dimer is more readily oxidised than the monomer giving rise to dimer coupling to radical and to dimer to form oligomers and ultimately polymer. Let us consider the growth of a polymer on an electrode during electropolymerisation. A typical current-time transient for such an experiment is shown in figure 1.2. The transient can be divided up into three distinct sections (as marked I, II and III). Each section represents a different stage in polymer formation. A qualitative scheme for polymer formation of polythiophene has been proposed by Hillman and Mallen.²¹ The first region I, is thought to be associated with monolayer formation on the electrode surface. The second region II, is thought to be due to instantaneous nucleation of the 'organic monolayer' followed by three dimensional growth of the polymer at the site of nucleation. Three dimensional growth is thought to continue at these sites until overlap occurs between nucleation sites. Once overlap has occurred growth continues in a manner perpendicular to the electrode surface, corresponding to region III on the current-time transient.

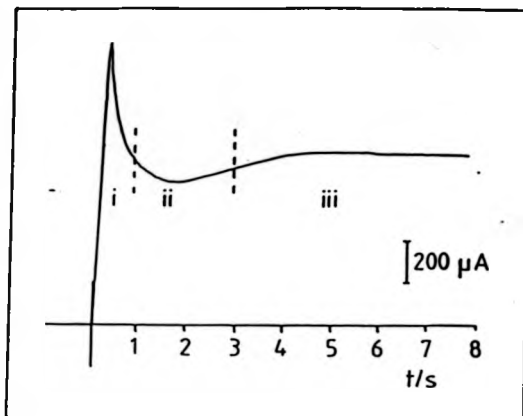


Figure 1.2: Typical current-time transient for the electropolymerisation of a conducting polymer, (growth of polythiophene by potential step from 0 - +1.74 V vs SCE from a 10 mmol dm^{-3} solution on a gold disc electrode area 0.4 cm^2).²¹

Studies of the growth of polythiophene have been made using spectroelectrochemical²² and ellipsometric²³ techniques and have provided evidence consistent with the proposed reaction scheme. The same conclusions as to the general mechanism of polymer growth have been reached by Asavapiriyant *et al.* for pyrrole²⁴ and N-methylpyrrole.²⁵

1.2.5 ELECTROACTIVITY

Many of these electrochemically synthesised polymers are electroactive and can be switched from their neutral, non-conducting, (usually reduced) state to their active, conducting, (usually oxidised) state. This property was first demonstrated with thin films of polypyrrole (0.2-0.4 nm thick).²⁶ The choice of solvent and electrolyte is of importance for the switching reaction. The process of switching in these polymers is a complicated one, and the nature of the polymer will be affected by the nature of the counter ion.²⁷ However the nature of the solvent and co-ions must also be taken into account. Consideration of the counter ions alone corresponds to the assumption that electroneutrality alone determines ion populations within the polymer.²⁸ In this situation the polymer would act in a permselective manner. As most electrochemical experiments are done in conditions of high ionic strength this would be unlikely behaviour for the polymer. It is necessary to consider thermodynamic aspects of the process as well. Studies of charge transport in conducting polymers have been done using the electrochemical quartz crystal microbalance

(E.Q.C.M.)^{28,29} and ellipsometry.²⁹ Based on these studies a general model describing the process has been proposed by Bruckenstein and Hillman.²⁹ They find, qualitatively, that redox switching changes the concentration of fixed charge usually by several molar. The concentration of all charged mobile species must therefore adjust to balance this. This balance is governed by thermodynamics. This approach does not exclude the possibility of the formation of neutral species (ion pairs and/or undissociated molecules). The significant consequences of this model are:

- i) solvent movement is generally expected,
- ii) integral relationships between the number of electrons involved in the redox process and any single species entering or leaving the polymer are not required,
- iii) if there is a configurational change accompanying the charge transfer process, there must be a linked movement of protons, counter ions and solvent into or out of the polymer phase.

Similarly Diaz and Bargon³⁰ propose that the switching reaction involves the oxidation of an organic structure to produce a delocalised cationic intermediate, but do not provide any further explanation as to the other processes involved.

1.2.6 CONDUCTIVITIES

The electrical conductivities of these materials vary considerably. Considering polymers of indole and 5-carboxyindole; these have conductivities of 0.020 and 0.024 $\text{ohm}^{-1} \text{cm}^{-1}$

respectively,¹⁵ which are considerably lower than those for polythiophene 29-100 $\text{ohm}^{-1} \text{cm}^{-1}$ ²⁷ and polypyrrole 10-100 $\text{ohm}^{-1} \text{cm}^{-1}$.³¹ Figure 1.3 shows a comparison of conductivities of conducting polymers with other materials.

1.3 INDOLE AND SOME DERIVATIVES

The electropolymerisation of indole and its derivatives is a little studied area. Only a few papers on the subject have been published. A summary of data from an investigation performed by Waltman and coworkers¹⁵ on the electropolymerisation of indole and a range of substituted indole monomers is given in table 2.1 overleaf.

The n values for the oxidation reaction were estimated from the Nicholson and Shain treatment^{32,33} for a totally irreversible electron transfer process. In general it was found that the monomers that produced polymeric films have an n value of approximately 2. These values were consistent with values obtained from elemental analysis¹⁵ of the polymer films. Cyclic voltammetry of a number of monomers (see table 1.2), shows irreversible oxidation peaks for all, with many giving rise to coloured solutions on electrooxidation. Electropolymerisation of indole can be achieved by constant potential oxidation. This results in a light green solution and a deposit at the electrode surface as reported by Neilson *et al.*³⁴ Of the other monomers used Waltman and coworkers found that the best polymer films were obtained from 5-cyanoindole and indole-5-carboxylic acid.

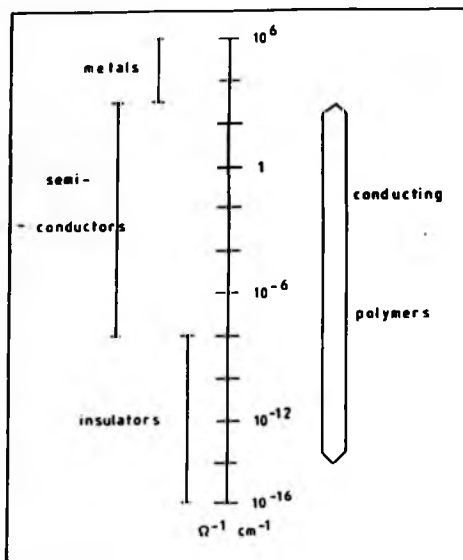


Figure 1.3: Comparison of the conductivity of conducting polymers with other materials.

Compound	Epa (V)	n	Polymer
Indole	1.26 1.58	2.10	+
1-methylindole	1.37 1.74		-
2-methylindole	1.25 1.51	1.40	-
3-methylindole	1.24 1.43	1.25	-
4-methylindole	1.45 1.84		+
5-methylindole	1.14 1.45	1.97	-
6-methylindole	1.70		+
7-methylindole	1.15 1.35	2.07	+
Indole-2-carboxylic acid	1.50	2.24	-
3-cyanoindole	1.71	1.90	-
5-aminoindole	0.64 1.38		-
5-hydroxyindole	0.90 1.35		-
5-methoxyindole	1.12 1.38	1.85	-
5-bromoindole	1.30 1.60	2.07	+
5-fluoroindole	1.52	1.79	+
Indole-5-carboxylic acid	1.40	1.92	+
5-cyanoindole	1.59 1.75	2.06	+
5-nitroindole	1.70	2.39	-
5-chloroindole	1.31 1.55	1.99	+

Table 1.2: Cyclic voltammetric data of indole monomers
in 0.1 mol dm⁻³ TEA.TFB in acetonitrile vs SCE. (after Waltman *et al.*, 15).

From elemental analysis of the polymers formed they obtained values for n of 1.7, 2.0 and 2.5 for 5-cyanoindole, indole-5-carboxylic acid and indole respectively. These were in good agreement with values obtained from the Nicholson and Shain treatment (see table 2.1). These values indicate that the reaction involves approximately 2 electrons per monomer unit. Any excess charge is assumed to come from partial oxidation of the polymer. The lower n values obtained for 5-cyanoindole and 5-carboxyindole are due to the fact that these polymers contain counter anions, as shown by elemental analysis, and thus a fractional charge of approximately 0.25.

1.3.1 SUBSTITUENT EFFECTS

The linkage sites for the polymerisation of indole have not been established yet, although they are thought to be the 1 and 3 positions.¹⁵ Evidence to support the possible linkage sites for indole comes from the fact that monomers of indole with substituent groups, such as methyl, cyano and carboxylic acid, on the 5-membered ring have not yielded polymers under conditions of electropolymerisation tried to date. However polymers have been obtained from monomers with substituents on the benzene ring. Waltman *et al.* suggested that the possible linkage sites were the 1 and 3 positions, and were further confirmed by their spin density calculations.¹⁵ These found high spin at the 1 and 3 positions identifying them as the reactive sites. To investigate substituent effects further, Waltman and coworkers adopted the approach of

comparing the peak oxidation potential E_{pa} of some 5-substituted indole monomers with the corresponding Hammett substituent constants,³⁵ as shown in figure 1.4. As was found from a similar study with substituted thiophenes, a linear relationship was obtained. The positive slope indicates that as the substituents take on a more electrophilic nature, the harder it becomes to electrooxidise the monomer. The slope obtained for the indole series, 0.56, is smaller than that for the thiophene series, 0.80, (from figure 1.4), and also for an aniline series 0.70.¹⁵ This suggests a less pronounced substituent effect for the indoles which can be attributed to its larger π -structure, as well as the fact that the substituent groups are removed from the active sites on the adjacent benzene ring. It was observed for the 5-substituted series that polymers were formed from monomers with electron withdrawing groups but not with electron donating groups, under the experimental conditions investigated. Waltman *et al.* explain this by suggesting that with the more strongly electron donating substituents, such as methyl or methoxy groups, the indole radical cation intermediate becomes sufficiently stabilised to diffuse away from the electrode, thus reducing the probability of radical/radical reactions or radical/monomer reactions occurring in favour of nucleophilic attack in solution away from the electrode region. Conversely the electron withdrawing substituents, such as carboxylic acid and cyano groups, allow electropolymerisation to occur as more reactive radicals are produced.

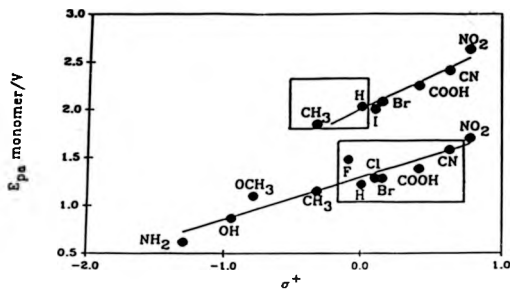


Figure 1.4: Electrochemical peak oxidation of substituted indoles (lower line) and thiophenes (upper line) versus their respective Hammett substitution constants. Monomers which electropolymerise are enclosed within a box.¹⁵

1.4 SELF-DOPED POLYMERS

Recently modifications to the properties of conducting polymers have been achieved by the immobilisation of doping anions within the polymer. These materials are able to act as a 'charge controllable membrane',³⁶ in contrast to the anion exchange properties of the classical polymer. There are three main ways to immobilise the anion dopant:

- a) the use of bulky anionically charged molecules as dopant during the electropolymerisation process,
- b) the formation of a composite between a conducting polymer and an anionically derived polymer that acts as a macro-dopant,
- c) the covalent linkage of the doping anion to the backbone of the conducting polymer.

The most important developments have come from the latter two methods. For the conducting polymer/anionic polymer composite the enhanced mechanical properties and the cathodic shift (250 mV) along with the cationic exchange properties exhibited³⁷ have made these materials promising for applications as batteries³⁸ and water desalination.³⁹ Conducting polymers with the doping anion linked to the backbone have been found to exhibit interesting new properties such as solubility in water⁴⁰ and sensitivity to pH.⁴¹ These materials promise to have many interesting applications and will warrant further investigations.

An area of great interest to us is the possible application of conducting polymers as electrode modifiers to enable the direct electrochemistry of biological molecules, especially redox proteins. The following section discusses the requirements for this.

1.5 CYTOCHROME C

Studies of electron transfer reactions of redox proteins have, in recent years, attracted widespread attention, the main features of interest being the nature of protein-protein interactions and the properties of these proteins as electron carriers in biological electron transport systems. It is extremely difficult to achieve the direct electrochemistry of redox proteins, ie: without promotion or mediation by small electron carriers. Reasons for this include irreversible adsorption and denaturation of the proteins at the electrode/electrolyte interface, slow rates of diffusion and the need to achieve correct protein/electrode orientation for electron transfer. To achieve stable reproducible diffusion dominated electrochemistry the following criteria need to be met:

- i) diffusion of reactant protein to electrode surface,
- ii) association of protein with the electrode surface in an orientation suitable for electron transfer (usually by promoters or mediators),
- iii) electron transfer,
- iv) dissociation of the protein from the electrode surface,
- v) diffusion of product protein away from the electrode surface.

Many redox proteins have been looked at, such as plastocyanine, flavodoxin and azurin, but the most widely studied has been cytochrome c.

1.5.1 STRUCTURE OF CYTOCHROME C MOLECULE

The cytochrome c molecule is a ubiquitous electron transport protein and has a molecular mass of around 12,000 Daltons dependant on origin. It is a roughly spherical molecule with an approximate diameter of 3.4 nm. The haem group is surrounded by many tightly packed hydrophobic side chains. The iron atom is bound to the sulphur atom of a methionine residue and to the nitrogen atom of a histidine residue, see figure 1.5. The overall structure of the molecule can be characterised as a shell, one amino acid residue thick, surrounding the haem group. The cytochromes play a vital role in the process of respiration. They are associated with the electron transport chain found in the inner mitochondrial membrane. In the respiratory chain of eukaryotes cytochrome c receives electrons from a cytochrome reductase complex, and passes electrons onto a cytochrome oxidase complex which ultimately uses the electrons along with protons from solution to reduce oxygen to water. Figure 1.6 shows a schematic representation of the overall process. Cytochrome c contains the same haem prosthetic group as haemoglobin and myoglobin. The haem is an iron porphyrin complex. The chemical environment surrounding this complex is very important as it is this environment which is responsible for delocalising the electron, which is active in the reaction, from the iron atom.

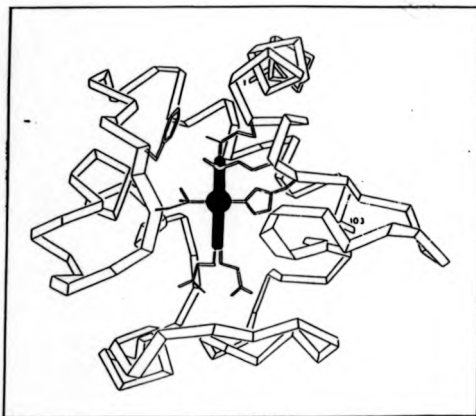


Figure 1.5: A general view of cytochrome *c* molecule from tuna showing the haem group in the centre and the amino acid backbone in ribbon representation.

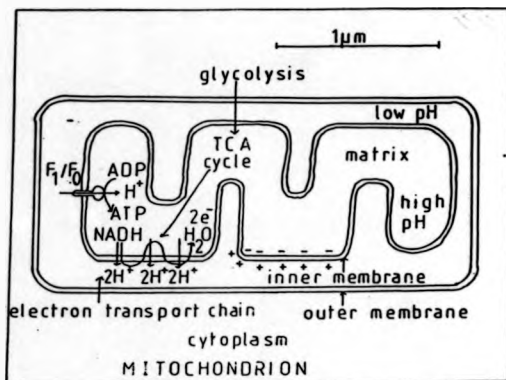


Figure 1.6: Overall scheme of respiration showing location of reactions within the mitochondrion.

Thus it moves to the edge of the cytochrome molecule and is transferred to another redox system with a higher affinity for electrons. The method of interaction is thought to be associated with the distribution of charged residues on the surface of the protein. Cytochrome *c* molecules of all species so far studied have clusters of lysine residues (positively charged at pH 7.0) around the haem cravice on one face of the molecule. This distribution of charges on the surface of cytochrome *c* almost certainly plays a role in the recognition and binding of the reductase and oxidase.⁴²⁻⁴⁴

1.6 NATURE OF ELECTRON TRANSFER

There are two possible means by which electron transfer may be achieved in the natural system. An electron could be transferred between different proteins by a relay of aromatic side chains. Alternatively, direct transfer could occur from one haem to another. It is important to remember that the electron in a haem group is not confined to the iron atom, as previously stated it is in a delocalised state. Hence, an electron can be transferred from one haem to another. This direct mechanism for electron transfer seems more likely as the free energy required to form a free radical anion of an aromatic side chain is very high. This proposed method of interaction is supported by investigations by Smith, Ahmed and Millat.⁴⁵ They looked at the reaction of horse heart cytochrome *c* with succinate cytochrome *c* reductase and cytochrome *c* oxidase. A semi-empirical relationship for the electrostatic energy of

interaction between cytochrome *c* and its reduction and oxidation partners was developed. In this, specific complementary charge pair interactions between lysine amino groups on cytochrome *c* and negatively charged carboxylate groups on the other proteins are assumed to dominate the interaction. It was found that specific modification of lysine groups surrounding the haem crevice on the cytochrome *c* molecule decreased the rate of reaction between cytochrome oxidase and cytochrome *c*₁. The electrostatic energies of interaction between cytochrome *c* and its reductase and oxidase were found to be nearly the same, providing evidence that the two reactions take place at similar sites on cytochrome *c*, and also the location of the reaction site. It is important to remember the negative charge on the reaction partners of cytochrome *c* for it is this that orientates the molecule correctly for electron transfer to occur as it approaches its redox partner.

1.6.1 ELECTRON TRANSFER DISTANCES

The distance of the cytochrome *c* molecule from an electrode surface will effect the probability of an electron transfer reaction occurring. Due to the size and shape of the molecule direct electron transfer is unlikely to occur with a clean electrode. The haem group is situated asymmetrically to one side of the protein close to the surface. Consequently for electron transfer to occur the molecule needs to be orientated with the haem group towards the electrode, see figure 1.7. It is assumed that electron transfer occurs through the exposed, but recessed haem edge.

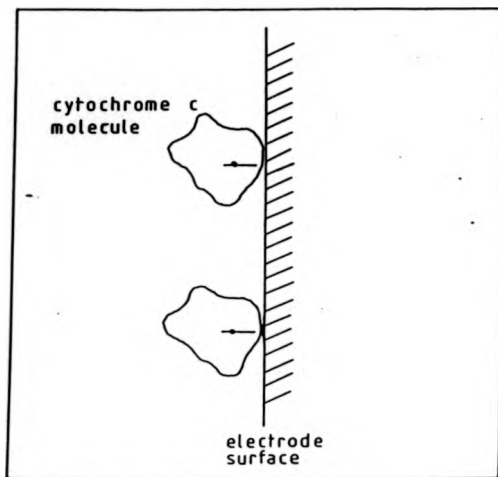


Figure 1.7: Required orientation of the cytochrome *c* molecule for electron transfer to occur.

The effective area of the haem crevice is approximately 0.5×1.8 nm⁴⁶ occupying about 3% of the total surface of the molecule. It is clear that for electrochemical measurements to be made at an electrode it is the interface region that will be most important. If we take a clean gold electrode, it is generally assumed to have an inner monolayer of ordered water molecules and a second layer that is not so ordered.⁴⁷ Hydrated electrolyte ions are found concentrated adjacent to this inner water layer to balance any excess charge. If the highly charged haem edge of the cytochrome *c* molecule is thought to contact this layer a distance for electron transfer of 0.6 nm is then found, this assumes a 0.3 nm thick layer of water and a distance 0.3 nm for the haem edge below the cytochrome *c* molecular surface.⁴⁸ The figure of 0.6 nm will correspond to an electron transfer intersite distance of 0.9-1.0 nm⁴⁹ by including van der Waals radii of haem carbon and surface gold atoms giving a combined value of 0.3-0.4 nm. Also reported is an intersite electron transfer distance of 0.8-1.0 nm for the photooxidation of Chromalium cytochrome *c*₅₅₂.⁵⁰ Overall it is thus concluded that electron transfer between an electrode surface and the exposed haem edge of a properly orientated cytochrome *c* molecule involves maximum distances of about 0.6-0.9 nm. For comparison Marcus and Sutin⁴⁷ obtain a value for the electron transfer distance in cytochrome *c* of 0.64 nm. This value is based on computer models of the cytochrome-cytochrome molecule orientation. In these models a haem edge to haem edge complex is found to offer the most favourable orientation for electron transfer to occur. This orientation has also been found in independent X-ray

studies.⁵¹ Other intersite distances proposed are approximately 0.7 nm for electron transfer between the haem edge and non-penetrating non-binding reactants such as $[\text{Fe}(\text{EDTA})]^{2-}$ and $[\text{Ru}(\text{NH}_3)_6]^{2+}$.⁵² Marcus and Sutin⁴⁷ have applied classical electron transport theory to systems of biological interest, and they found it necessary, in so doing, to include additional features in the theory such as the distances and orientations of the reactants. They find that the correlation between the predicted and the calculated rates for reactions involving redox proteins can be very good, as found with the reaction of cytochrome *c* with various inorganic reactants.⁴⁷ In the case of intramolecular transfer they find that results are consistent with an electron transfer rate that falls off exponentially with distance.

1.7 ELECTROCHEMICAL STUDIES OF CYTOCHROME C

Early work on cytochrome *c* was carried out at mercury electrodes, chosen because of the reproducibility of the electrode surface. At mercury the reaction of cytochrome *c* from horse heart was found to be irreversible.^{53,54} The protein absorbs strongly and irreversibly to the electrode surface and interferes with the redox reaction. As a result of this layer of absorbed protein the observed electrochemistry is complex and interpretation difficult. More recent work carried out at polished and electrochemically roughened silver surfaces⁵⁵ has shown that it is possible to observe, and interpret, the electrochemistry of cytochrome *c* at a bare silver electrodes whereas previous reports have shown problems

with slow kinetics and irreversible adsorption. Reed and Hawkrige⁵⁶ demonstrated that when using silver electrodes it is the purity of the cytochrome *c* which governs the stability and reversibility of the electron transfer process. Previous reports of poisoning, assumed to be due to protein adsorption, were overcome by using chromatographically purified cytochrome *c*. Other workers have examined the electrochemistry of cytochrome *c* at gold and glassy carbon electrodes. Bond *et al.*⁵⁷ concluded that there is a conformational equilibrium in solution between two forms of the protein and that it is a biologically inactive form which adsorbs at the electrode blocking the electrochemistry of the biologically active form. The poor results obtained at bare electrodes are most likely to be the result of a combination of this adsorption effect coupled with electrostatic incompatibility of the electrode surface with the cytochrome *c* molecule. The problem of protein adsorption seems to be overcome by using an electrode material with intrinsic functional groups or modifying the electrode surface with a promoter of some kind. This has the effect of making the active interface presented to the cytochrome *c* molecule more acceptable to it. Promoters should be distinguished from mediators in that a promoter is a species which is electrochemically inactive in the potential range being studied but facilitates the electron transfer of the redox protein, whereas a mediator is a species which itself undergoes redox reactions in the course of catalysis of the redox protein electrochemistry.

1.7.1. USE OF PROMOTERS WITH CYTOCHROME C

From the above discussion the necessity of presenting the redox protein with an electrode surface which mimics the electrostatic interactions required in the natural system is evident. It is therefore necessary to be able to modify the electrode surface to achieve such a situation. The possibility of electrostatic promotion is thus raised. Pyrolytic graphite has been observed to promote the rapid heterogeneous electron transfer of a number of proteins.⁵⁸⁻⁶² For negatively charged proteins or negatively charged electron transfer sites the electrode surface presented should be positive in charge. The edge-plane pyrolytic graphite electrode carries a negative charge at neutral pH and modest potentials so that cation promoters are required to mask this negative charge. Without these cationic promoters in solution the electrode may be used to promote electrostatic interaction with proteins carrying a net positive surface charge. The results obtained from the study of redox proteins in this manner appear to have reduced the sensitivity of the electron transfer process to impurities. However the use of ions as promoters is limited. The use of simple organic molecules as promoters would be a more flexible and advantageous method. These molecules could be tailored by conventional organic chemistry as promoters for the electron transfer process. As with the cationic promoters these simple organic compounds must be chosen so that they orientate the redox protein at the electrode surface to facilitate electron transfer. It is this type of promoter, the modified electrode, in which we are

interested that are described later. The first demonstration of quasi-reversible, non-degradative electrochemistry of cytochrome *c* was by Yeh and Kuwana⁶³ who showed that a good cyclic voltammetric response could be obtained from horse heart cytochrome *c* at a tin-doped indium oxide electrode. At about the same time Eddowes and Hill,⁶⁴ using a modified gold electrode, also demonstrated reversible cyclic voltammetry with a peak separation close to 60 mV, from horse heart cytochrome *c*. The modifying agent Eddowes and Hill used was a monolayer of the reagent 4,4'-bipyridyl. Faradaic currents were found to vary linearly with (scan rate)^{1/2}. These results show that electron transport is effectively controlled by diffusion of the cytochrome *c* to and from the electrode. They also suggest that it is the exact nature of the protein/electrode interface that determines the type of reaction occurring at the electrode. Hill et al. followed this line of reasoning and performed a study of 54 bifunctional organic molecules⁶⁵ to assess their ability to promote the electrochemistry of cytochrome *c*. A general thesis for surface modification resulted from this study. As functional groups are required for the promotion of the electrochemistry of cytochrome *c*, a general class of bifunctional molecules X-Y should be used, where X represents a surface active group that will adsorb/bind to the electrode surface and Y represents the functional group that will, hopefully, promote electron transfer. A pictorial representation is shown in figure 1.8. These bifunctional molecules (X-Y) where the X group is designed to adsorb to a gold electrode surface and the Y group is designed to interact with the protein⁶⁵⁻⁶⁷ showed that certain

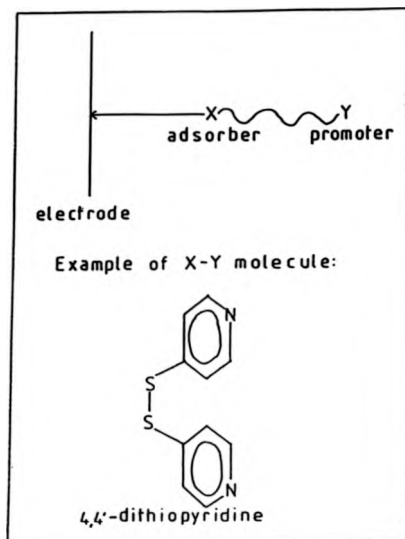


Figure 1.8: Orientation of bifunctional promoter molecule at electrode.

structural factors were required for a molecule to act as a good promoter. These can be summarised as follows:⁶²

- i) X should be able to bind to the electrode surface through N, P or S,
- ii) Y should permit favourable interaction with the electron transfer domain of the redox protein,
- iii) not only must Y be present at the interface but it must also project outwards from the electrode surface,
- iv) X-Y can be aromatic or aliphatic, rigid or flexible and it appears that the length of the molecule has little effect on the rate of electron transfer.

The nature of the interaction between the protein and the promoter is one of interest. For example when the promoter bis-(4-pyridyl)disulphide is used and progressively 'diluted' by substitution with the 'inert' analogue thiophenol, the result gives more than just the expected corresponding loss in cytochrome c activity; the observed current density drops faster than expected on the basis of slower kinetics alone.⁶⁸ The conclusion is that there must be some minimum number of active promoters required, in any given ensemble of molecules on the surface to permit cytochrome c electrochemistry. It is therefore best to consider the interface between the promoter on the surface and the protein to be *dynamic* and most important, for diffusion dominated electrochemistry, the protein must dissociate from the electrode surface after electron transfer.⁶⁹

1.8 RECENT DEVELOPMENTS

In recent investigations the direct electrochemistry of cytochrome c has been reported in which there is no need for chemical modification of the electrode surface.^{70,71} This has been done using graphite electrodes. X-ray photoelectron spectroscopy⁷¹ has established the presence of C-O functional groups on the graphite surface (carbonyl, phenolic, carboxylic and ether like groups are also thought to be present). Bond and coworkers^{72,73} have recently proposed a microscopic model for the electron transfer of cytochrome c at graphite electrodes. The model attempts to explain the different responses obtained at edge- and basal-plane graphite electrodes. The model proposes the concept of very rapid electron transfer (at the electrode interface) at arrays of oxygen-containing functionalised electroactive sites which are of microscopic dimensions. No electron transfer can occur at bare non-functionalised carbon. The sites are generated by surface abrasion. The surface density of such sites at basal-plane electrodes is low and at edge-plane electrodes high. The difference in the voltammograms observed from the two types of electrodes is explained on the basis that reaction at a single microscopic site will have radial mass transport and thus produce a sigmoidal current voltage curve, as is seen for basal-plane electrodes. In contrast when these microscopic sites overlap, as at edge-plane electrodes, the reaction will have linear mass transport and the usual current voltage curve will be observed, which is the case. Thus it is the

number of functionalised sites that determines the response obtained from a graphite electrode.

1.9 CONCLUSION

In general it is necessary for a promoter to bind the protein without deactivation. The binding should orientate the redox protein to favour electron transfer and should be 'reversible', thus forming a transient protein/electrode complex that enhances the rate of electron transfer analogous to the natural system of a protein/protein complex. The need to ensure that the interaction between the redox protein and the electrode surface is closer to that which occurs in nature has led to interest in biologically compatible interfaces. A direct result of this is the use of an amino acid promoter based on L-cysteine⁷⁴ with cytochrome *c*. Quasi-reversible and persistent electrochemistry of the cytochrome *c* was observed. It was assumed that these electrodes function by orientating the acid and amine groups into the bulk solution and as such fit the requirements previously outlined.

The study of redox proteins such as cytochrome *c* has led to the consideration of the nature of the electrode/protein interface. A greater understanding of such interfaces is of considerable importance. The ability to link into biological electron transfer systems should lead to a greater understanding of the *in vivo* behaviour of these proteins and provide a good basis for the development of practical bioelectrocatalytic systems. Further

development of protein electrochemistry could also lead to its application in analytical devices.

1.10 OUTLINE OF THE REST OF THIS THESIS

The following chapter of this thesis describes the experimental techniques used in the course of this work.

In chapter 3 the properties of the polymer in aqueous solution are discussed and a possible application of the polymer is described.

In chapter 4 results are presented for the use of the polymer as a promoter for the bioelectrochemistry of a redox protein.

In chapter 5 the results of investigation of redox couples at a polymer modified electrode are given.

The final chapter is a summary chapter and includes proposals for future work on the polymer.

CHAPTER 2

EXPERIMENTAL

All experimental techniques and apparatus used in the course of this work are described below. This includes all the instrumentation used for electrochemistry.

2.1 ELECTRONIC COMPONENTS

The controlling electronic components are in the form of individual modular components housed in a rack type system. Power is supplied via a mains run transformer to give a 15 V source. The rack system and other components in a typical experimental set up are shown in plate 2.1. Modules used were:

- Potentiostat
- Triangular Wave Generator
- Voltage Source

The potentiostat was of in-house construction, the major components of which were:

- R.S. Components Strip Board
- Operational Amplifier 747 (b, c)
- Operational Amplifier LM 11 (a, d, e)
- Burr Brown Electronics Operational Amplifier 3627 Am (f, g),

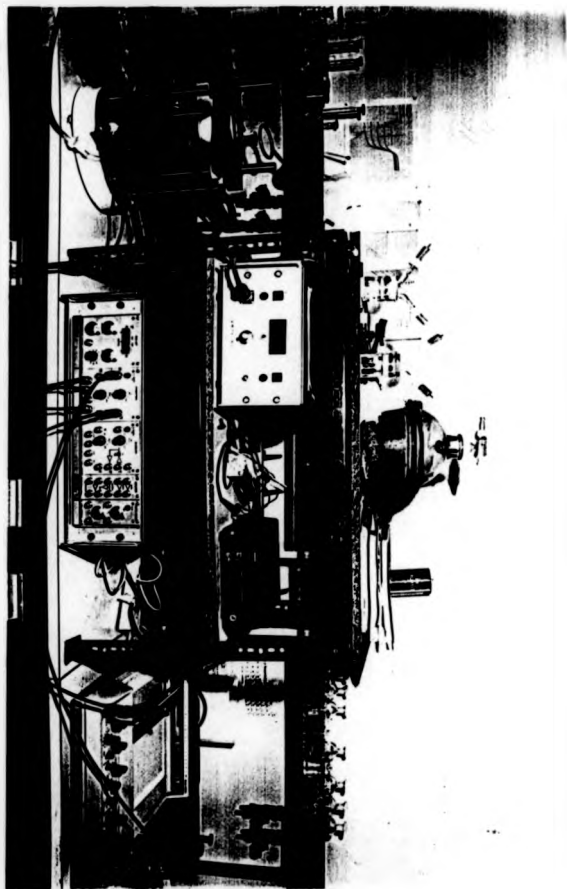


Plate 2.1: Typical experimental set up.

A circuit diagram of the potentiostat is given in figure 2.1. The triangular wave generator and voltage source were supplied by Oxford Electrodes.

Voltage outputs were recorded on a Bryans series 60000 XY-t recorder or alternatively measured using a Keithley 197 digital multimeter.

2.2 ELECTROCHEMICAL TECHNIQUES

All the techniques and methods used during the course of this work were standard and can be found in most text books of electrochemistry. The interested reader is referred to texts by Bard and Faulkner,⁷⁵ Albery and Hitchman,⁷⁶ Albery,⁷⁷ and the Southampton Electrochemistry group.⁷⁸

2.3 ELECTRODES

Platinum rotating disc (area = 0.385 cm^2) and gold rotating disc (area = 0.385 cm^2) electrodes were supplied by Oxford Electrodes. Electrodes supplied by Oxford Electrodes were initially polished with $25 \mu\text{m}$ aluminium oxide powder (Buehler) in glycerol on a polishing pad using a purpose built polishing device. Subsequent polishing, after a thorough wash in distilled water to prevent transfer of abrasive polishing particles, was performed to produce a mirror finish. This employed lapping cloth sprayed with $6 \mu\text{m}$ and $3 \mu\text{m}$ lapping spray (Engis) along with Hyprez lubrication fluid (Engis), followed by a final hand polish with a slurry of $1.0 \mu\text{m}$

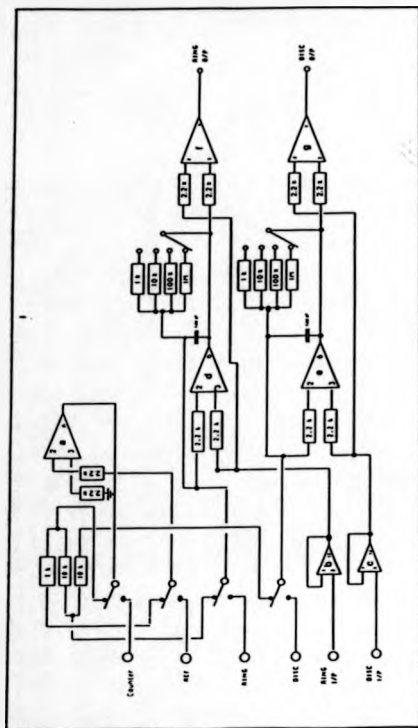


Figure 2.1: Circuit diagram for potentiostat.

and 0.3 μm alumina (Banner Scientific) in water. This was applied by cotton wool. It is important to keep all polishing materials free of dust during polishing and to store them in a manner to prevent contamination with particles that may cause unwanted abrasion. Polishing with 0.3 μm alumina was repeated prior to each experiment.

Electrode areas were calculated using the mean of the measurements of ten randomly selected diameters, performed with a travelling microscope. Platinum wire (thickness 125 μm) microelectrodes were constructed in house to the design shown in figure 2.2. A clean surface was obtained by slicing a small section off with a razor when required. Counter electrodes were made from a piece of platinum gauze (area approximately 2 cm^2) which was spot welded onto a length of platinum wire (external diameter 0.5 mm) to allow external connection. Alternatively a length of platinum wire was used in conjunction with the small volume cells.

Potentials were measured with respect to a saturated calomel electrode (SCE). The calomel electrode was of in-house construction to a specific design incorporating a screw cap to prevent leakage of the internal filling fluid and ultimate device failure through solder contact dissolution. Contact to the external solution is made via a low porosity ceramic frit (a gift from Kent Industrial Measurements Ltd.). The design of this type of electrode is shown in figure 2.3. The potential of the home made calomel electrodes were checked against a commercial model (Radiometer). If deviations of greater than ± 2 mV were found the electrode was repacked with calomel and retested before further use.

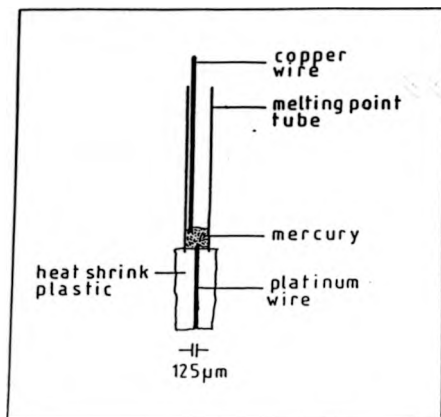


Figure 2.2: Design of platinum micro-electrodes produced by Warwick Electrochemistry Group.

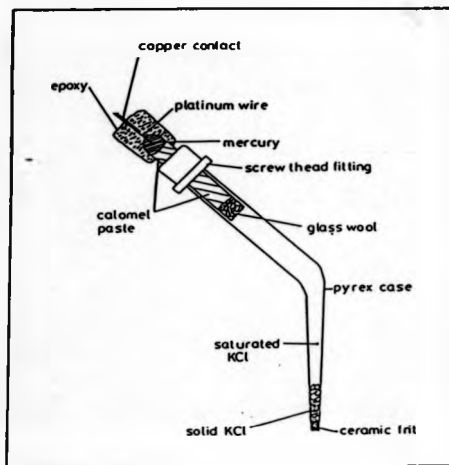


Figure 2.3: Design of reference electrode.

2.4 ELECTRODE ROTATOR

Rotating disc electrodes were mounted on a bearing block (Oxford Electrodes) by means of a internal brass screw thread contact. Rotation is achieved by direct drive from a printed armature d.c. motor and motor controller (Oxford Electrodes). The rotation speed of the electrode is continuously monitored by means of a slotted opto switch connected to the motor drive shaft and displayed in a digital form. This control system allows the accurate determination of electrode rotation speed, giving speeds of between 1 and 50 Hz (± 0.01 Hz).

2.5 ELECTROCHEMICAL CELLS

Two types of specially designed electrochemical cell were used in the course of this work. The majority of work was carried out in a two compartment, water jacketed, Pyrex cell having a volume of 15 cm³, used in conjunction with a rotating disc electrode. The cell is shown in figure 2.4. The design of this cell enables a stream of nitrogen to be passed through the solution in the inner cell compartment prior to the start of an experiment. Oxygen is then prevented from diffusing back into the solution, during the experiment, by maintaining a positive pressure of nitrogen in the cell. The counter electrode is placed in a second compartment behind a high porosity glass frit. This prevents contamination of the bulk solution by products of the counter electrode reaction.

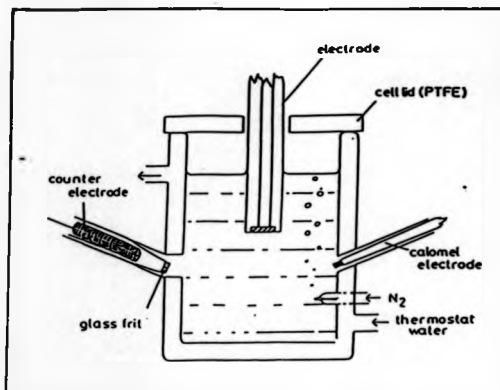


Figure 2.4: Design of electrochemical cell.

The cells are covered with a close fitting teflon lid. For experiments on small solution volumes, 2-5 cm³, a small two compartment Pyrex cell was employed. In this cell the reference electrode is contacted to the bulk solution by means of a pin hole.

2.6 DEOXYGENATION SOLUTION AND TEMPERATURE CONTROL

Deoxygenation of solutions used for electrochemistry was achieved by purging with nitrogen which had previously had traces of oxygen removed by passage through a train of dreschel bottles containing a caustic solution of anthraquinone-2-sulphonate in contact with zinc amalgam, followed by saturation in either acetonitrile or water, dependent on the solution under investigation. The use of anthraquinone reagent also has the advantage of being self indicating turning from deep red to straw yellow when exhausted. Deoxygenation was continued for at least 20 minutes prior to the start of an experiment. All solutions used for electrochemistry were treated in this way unless stated otherwise. Solutions were thermostatted at 25 °C (± 0.2 °C) by use of a water bath circulator (Grant Scientific (Cambridge) Ltd. model SE15), unless specified otherwise.

2.7 SOLUTIONS AND CHEMICALS

Non-aqueous solutions for the growth of the polymer and subsequent voltammetry were prepared using acetonitrile (Rathburn Chemicals,

HPLC grade) distilled over calcium hydride and stored over 3 Å molecular sieves. All other solutions were prepared using water from a Whatman WRSO RO/Deioniser water purification system, which produces water of $1.0 \mu\text{S cm}^{-1}$ conductivity. All glassware was cleaned by immersion in Decon 90 overnight, followed by a thorough rinsing with purified water. Glassware to be used with organic solvents was then dried in an oven prior to use.

All simple inorganic chemicals were of analytical reagent grade and were used without further purification unless stated otherwise.

Indole-5-carboxylic acid (Aldrich) was recrystallised once from water and dried over silica gel. Tetraethylammonium tetrafluoroborate (TEA.BF_4) (Aldrich) was recrystallised three times from methanol and dried over silica gel. Tris (2,2'-bipyridine) iron (II) perchlorate was synthesised in the department (a gift from V.M.Eastwick-Field) and was recrystallised once from ethanol and dried on a Schlenk line.

All buffer reagents were supplied by Fisons or BDH and were of analytical reagent grade. Amino acids in the protected ester form (Aldrich) were used as supplied. Horse heart Cytochrome c (Sigma) type III was used as supplied whereas type VI was purified by column chromatography (see section 2.8).

2.8 PURIFICATION OF CYTOCHROME C TYPE VI

Purification of horse heart Cytochrome c type VI was performed by ion exchange column chromatography following the method of Brautigan *et al.*⁷⁹ Ion exchange chromatography was performed on CM-Sephadex

C-25 (Pharmacia) in a 1.6 x 50 cm glass column (Pharmacia GK16). The packing material was pre-swollen at room temperature in sodium phosphate buffer, $0.085 \text{ mol dm}^{-3}$ (initial elution concentration), pH 7.0 for 24 hours prior to column packing. After decanting fine particles from the surface of the packing material and washing with the initial elution buffer the column was packed by continuous pouring under gravity. The column was then stabilised by running buffer through it for 2 hours to ensure complete settling of the packing material. The column was then checked for air pockets and cracks with a 100 W electric lamp. The sample was applied to the top of the column and elution achieved with $0.085\text{-}0.120 \text{ mol dm}^{-3}$ sodium phosphate buffer pH 7.0 fed from a home made gradient mixer via a peristaltic pump (Pharmacia P-1) at a flow rate of $10 \text{ cm}^3 \text{ h}^{-1}$. Collection of eluted fractions was done with an automatic fraction collector (Pharmacia Frac-200). Samples were stored in sealed tubes in the (fridge at 4°C) prior to determination of concentration and subsequent use for electrochemical experiments.

2.9 SPECTROSCOPIC DETERMINATION OF PURIFIED CYTOCHROME C CONCENTRATION

By measuring the absorbance at 550nm (A_{550}) of a solution of purified cytochrome c and with knowledge of the molar absorption coefficient for the molecule, the concentration can be determined using a equation derived from the Beer-Lambert law.⁸⁰

$$A = \epsilon c l \dots \dots \dots (2.1)$$

Where:-

A is the absorbance,

ϵ is the molar extinction coefficient ($\text{dm}^{-3} \text{mol}^{-1} \text{cm}^{-1}$),

c is the molar concentration,

l is the path length (cm).

The molar concentration for purified cytochrome c is therefore given by:

$$c = A \cdot 550 / \epsilon l \dots \dots (2.2)$$

Where $l = 1 \text{ cm}$ and $\epsilon = 21,100 \text{ dm}^{-3} \text{mol}^{-1} \text{cm}^{-1}$.⁸¹ This fixed wavelength determination was the method used in this work.

2.10 SYNTHESIS OF AMINO ACID SUBSTITUTED MONOMERS

The procedure used in the synthesis of substituted monomers was based on a method by Sheehan et al.⁸² using the coupling reagent: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride EDC (Aldrich).

The general mechanism for amide bond formation using a carbodiimide coupling reagent is given in figure 2.5. Equimolar amounts of the starting materials (monomer, amino acid and coupling reagent) were weighed out and dissolved separately in dichloromethane. The three solutions were then added to the reaction vessel and left stirring overnight. The solution is then washed successively with dilute hydrochloric acid, saturated sodium bicarbonate solution and finally water. The washed solution was dried over magnesium sulphate followed by rotary evaporation to give

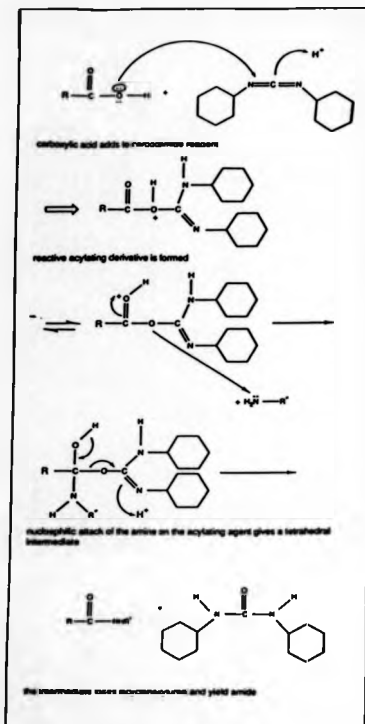


Figure 2.5: General reaction mechanism for formation of amide bond using carbodiimide coupling reagent. 23

the product. The product was then recrystallised from water and dried over silica gel.

Deprotection of the product was achieved by dissolving (300 mg) of the crystalline product in a mixture of 95% ethanol and 1 mol dm⁻³ sodium hydroxide in a ratio of approximately 4:1 (200 ml:50 ml). This solution was reduced to about 1/20 of the initial volume by rotary evaporation. This was then added to water at pH 3.0 (70 ml) and stirred for a few minutes followed by addition of an equal volume (70 ml) of 1 mol dm⁻³ sodium hydroxide. The product of the deprotection reaction was extracted with ethyl acetate (dried over magnesium sulphate) and evaporated under reduced pressure to give crystalline product. Products were characterised using the following techniques:

- i) n.m.r. Perkin Elmer R34 220 MHz (continuous wave),
- ii) mass spectrometry Kraytos MS 50,
- iii) infra red spectroscopy Perkin Elmer 580B Infra-red spectrophotometer.

The following is a list of the amino acid substituted monomers synthesised together with the spectral assignments.

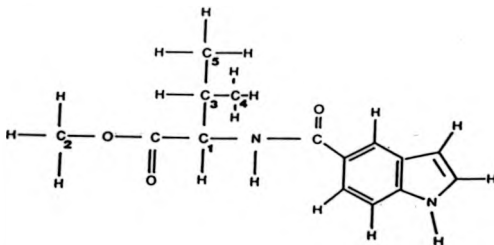
1) methyl ester of N-(indole-5'-carboxamido) valine

n.m.r. solvent CD_3CN .

- 1.1 (m) 6H 2 x CH_3 [4,5].
- 2.5 (m) 1H 1 x CH [3].
- 3.7 (d) 1H 1 x CH [1].
- 3.8 (s) 3H 1 x CH_3 [2].
- 6.6 (s); 7.4 (d); 7.5 (d); 7.7 (d); 7.9 (d); 8.3(s) [Indole ring].

mass spec. m/z (CI) = calc. 274, found 275 56% (MH^+).

i.r. cm^{-1} nujol mull 1740 (C=O), 1640 (N-H).



2) ethyl ester of N-(indole-5'-carboxamido) alanine

n.m.r. solvent CD_3CN .

= 1.1 (t) 3H 1 x CH_3 [4].

= 1.5 (d) 3H 1 x CH_3 [2].

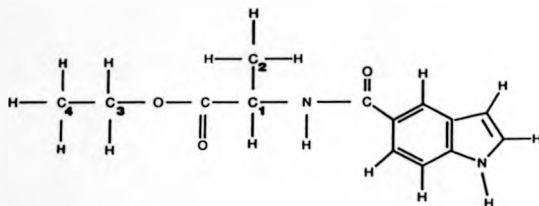
= 3.8 (d) 1H 1 x CH [1].

= 4.1 (q) 2H 1 x CH_2 [3].

= 6.6 (s); 7.45 (d); 7.5 (d); 7.65 (d); 8.3 (s) [indole ring].

mass spec. m/z (CI) = calc. 260, found 261 10% (MH^+).

i.r. cm^{-1} nujol mull 1690 (C=O), 1640 (N-H).



3) methyl ester of N-(indole-5'-carboxyamido) glutamic acid

n.m.r. solvent CD_3CN .

= 2.2 (m) 3H 1 x CH, 1 x CH_2 [1,2].

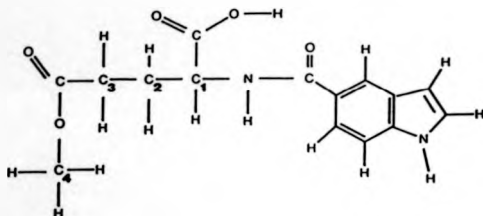
= 3.6 (t) 2H 1 x CH_2 [3].

= 3.75 (s) 1 x CH_3 [4].

= 6.7 (s); 7.35 (d); 7.55 (d); 7.7 (d); 7.95 (d); 8.35 (s) [Indole ring]. -

mass spec. m/z (EI) = calc 304, found 260 55% (M-COOH).

i.r. cm^{-1} nujol mull 1670 (C=O), 1610 (N-H).



4) methyl ester of *N*-(indole-5'-carboxamido) glycine

n.m.r. solvent CD_3CN .

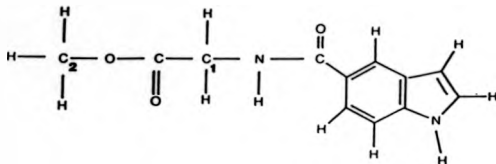
- 2.6 (s) 2H 1 x CH_2 [1].

- 3.8 (s) 3H 1 x CH_3 [2].

- 6.6 (s); 7.45 (d); 7.6 (d); 7.75 (d); 7.95 (d); 8.35(s) [Indole ring].

mass spec. m/z (CI) = calc. 232, found 233 26% (MH^+).

i.r. cm^{-1} nujol mull 1670 (C=O), 1610 (N-H).



5) N-(indole-5'-carboxamido) valine

n.m.r. solvent CD_3CN

= 1.1 (m) 6H 2 x CH_3 [3,4].

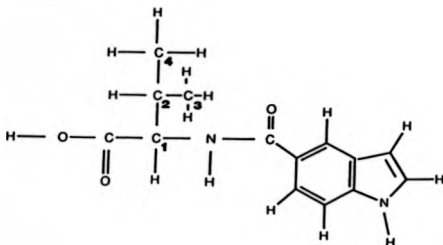
= 2.5 (m) 1H 1 x CH [2].

= 3.7 (d) 1H 1 x CH [1].

= 6.6 (s); 7.4 (d); 7.55 (d); 7.7 (d); 7.85 (d); 8.45 (s) [Indole ring]. -

mass spec. m/z (FAB) = calc. 260, found 261 28% (MH^+).

i.r. cm^{-1} nujol mull 1740 (C=O), 1600 (N-H).



6) ethyl ester of N-(indole-5'-carboxyamido) cysteine

n.m.r. solvent CD_3CN .

- 1.2 (t) 3H 1 x CH_3 [4].

- 3.5 (m) 2H 1 x CH_2 [2].

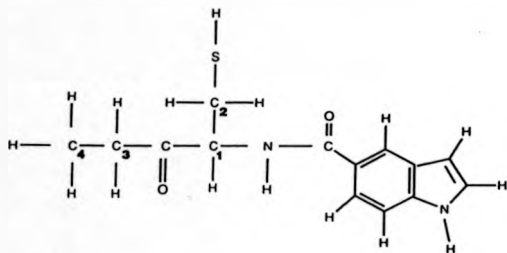
- 3.7 (q) 1H 1 x CH [1].

- 4.2 (q) 2H 1 x CH_2 [3].

- 6.55 (s); 7.45 (d); 7.5 (d); 7.7 (d); 7.9 (d); 8.3 (s) [Indole ring].

mass spec. m/z (CI) = calc. 292, found 248 10% $(\text{M}-\text{OC}_2\text{H}_5)^+$.

i.r. cm^{-1} nujol mull 1670 (C=O), 1610 (N-H).



2.11 OTHER TECHNIQUES

All pH measurements were made using a Corning pH meter 145 in conjunction with a Corning micro combination electrode 306L.

Jandel (Scientific) Sigma Plot version 4.0 was used to generate lines of best fit to data presented in chapter 5. User functions are given in the appropriate sections in chapter 5.

CHAPTER 3

INTRODUCTION

3.1 POLY-5-CARBOXYINDOLE IN AQUEOUS SOLUTION

The conducting polymer poly-5-carboxyindole is of interest in aqueous solution as it has the possibility of being a self compensating polymer at neutral pH. The negative charge of the carboxylate functions should balance the positive charge of the conducting backbone. The presence of the carboxylate group may also mean that the polymer exhibits some form of pH dependence. In addition it may be possible to use poly-5-carboxyindole as a promoter with biological redox proteins, such as cytochrome c, to allow the direct electrochemistry of these molecules. It was therefore necessary to see if the polymer was capable of either being grown from aqueous solution or transferred to aqueous solution after growth in aprotic solution. This chapter describes this process and the properties of the polymer mainly in aqueous solution.

3.2 GROWTH OF POLYMER

The conducting polymer, poly-5-carboxyindole, may be grown electrochemically in two ways, either by holding the electrode at a potential sufficient to oxidise the monomer or by sweeping the potential back and forth between zero and this potential. The

former, potentiostatic, method was preferred for the production of consistently stable films. It was also found necessary to rotate the electrode to obtain even films. The conducting polymer films were grown by electrochemical polymerisation from acetonitrile on platinum or gold rotating disc electrodes (area = 0.38 cm^2) or onto $125 \mu\text{m}$ diameter platinum microelectrodes. The polymer films were grown from a solution of 5 mmol dm^{-3} 5-carboxyindole dissolved in acetonitrile containing 0.1 mol dm^{-3} TEA.TFB. All films were grown potentiostatically at $+1.4 \text{ V}$ vs SCE, with the electrode rotating at 4 Hz (for microelectrodes the solution was stirred with a magnetic stirrer), for between 15 and 60 seconds.

Electropolymerisation was attempted from aqueous solution using a monomer concentration in the range 5 to 35 mmol dm^{-3} in high purity water containing 0.1 mol dm^{-3} TEA.TFB. Potential steps from 0.0 V to potentials in the range 0 to $+0.95$ to $+1.3 \text{ V}$ versus SCE were used at a platinum disc electrode rotating at 4 Hz . All attempts to grow polymer from an aqueous solution of 5-carboxyindole were unsuccessful under these conditions.

3.3 INITIAL FINDINGS IN ACETONITRILE

The postulated structure for poly-5-carboxyindole¹⁵ is shown in figure 3.1. The polymer contains one carboxylate group for each monomer unit. Cyclic voltammetry of the films in acetonitrile containing TEA.TFB shows evidence of two redox processes, as shown in figure 3.2. The amount of charge associated with each peak is found to be equal (ratio 1:1).

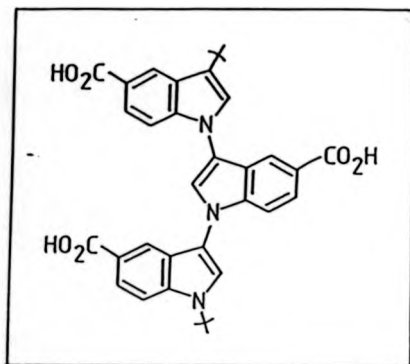


Figure 3.1: Proposed structure of poly-5-carboxyindole.¹⁵

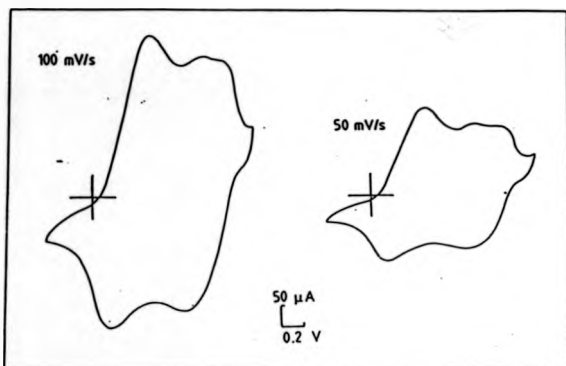


Figure 3.2: Cyclic voltammogram of poly-5-carboxyindole in acetonitrile containing TEA.TFB.

Table 3.1 summarises the data for several different polymer films, grown for different times.

Growth times s	Thickness nm	Q/ mC peak 1	Q/ mC peak 2	Ratio
30	212.8	3.99	3.86	1:1.03
60	410.7	6.97	5.45	1:1.27
90	652.7	7.46	8.55	1:1.15
120	803.6	9.12	9.28	1:1.02
180	1307.6	22.13	21.24	1:1.04

Table 3.1 Ratio of charges associated with the two peaks of poly-5-carboxyindole in 0.1 mol dm⁻³ TEA.TFB in acetonitrile at a sweep rate of 20 mV s⁻¹. Charges were calculated by measuring the area under the oxidation and reduction peaks for each process and subtracting the area obtained from a clean platinum electrode in the same solution. The peaks were separated by drawing a line splitting the cyclic voltammogram at the trough between the two peaks.

The peak height sweep rate dependence was found to be linear for both peaks in acetonitrile as is shown in figure 3.3. Like other conducting polymers, the charge passed in depositing the film is directly related to the film thickness this is shown in figure 3.4. This shows that the time of growth can be used as a good measure for the amount of polymer deposited.

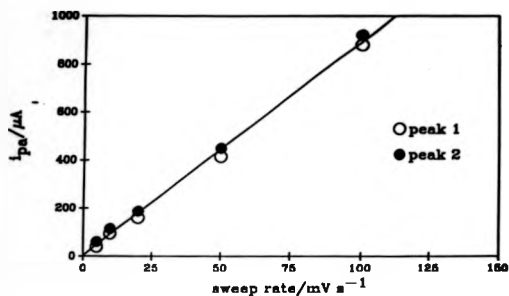


Figure 3.3: Sweep rate dependence of I_{pa} for the two peaks observed for poly-5-carboxyindole in acetonitrile containing 0.1 mol dm^{-3} TEA.TFB.

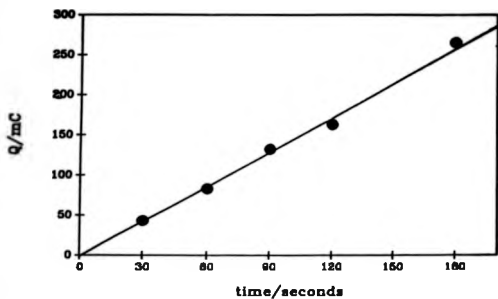


Figure 3.4: Charge passed during growth of poly-5-carboxyindole by potential step (0- +1.4 V vs SCE) from a 5 mmol dm^{-3} solution of monomer in acetonitrile containing 0.1 mol dm^{-3} TEA.TFB at a rotating platinum electrode (4 Hz) versus deposition time.

Film thicknesses may be estimated using the equation:

$$\text{thickness} = \frac{VQL}{nFA}$$

where

V = molecular volume of the molecule = $8.00 \times 10^{-30} \text{ m}^3$

(estimated by using known or approximated bond lengths for indole).⁷⁶

n = number of electrons = 2.67 (growth)*.

L = Avogadro's number = $6.02 \times 10^{23} \text{ mol}^{-1}$.

F = Faraday = $9.64 \times 10^4 \text{ C mol}^{-1}$.

A = geometric area of electrode = 0.38 cm^2 .

Q = charge passed during deposition = x mC.

* This value for n is arrived at by comparing the amount of charge passed in growing the polymer to that passed when the polymer is cycled in buffer solution, this ratio is found to be 4:1, and assuming in growth that the current is 100% Faradaic and the oxidation of each monomer unit involves 2 electrons.

The data for figure 3.4 is given in table 3.2.

Growth time s	Charge for growth/ mC	Thickness nm
30	43.17	212.8
60	83.30	410.7
90	132.40	652.7
120	163.00	803.6
180	265.10	1307.6

Table 3.2: Data for the growth of poly-5-carboxyindole by potential step, (0- +1.4 V vs SCE).

3.4 TRANSFER TO AQUEOUS SOLUTION

Initial attempts of transferring the polymer films to aqueous solution directly were unsuccessful in that the films cracked, flaked and fell off the electrode. The change of environment from acetonitrile to water results in large internal forces in the polymer, causing it to break up. A more gradual transfer procedure was devised. After film deposition the electrodes were removed from the growth solution and washed in mixtures of acetonitrile/water containing progressively greater amounts of water (20%, 50%, 80% and finally 100% water). Washing consisted of rotating the electrodes in each solution, typically at 10 Hz, for 10 seconds. This procedure was successful for films grown for periods up to 90 seconds. Thicker films required a much longer transfer time between solutions, typically 60 seconds in each one.

3.5 CYCLIC VOLTAMMETRY IN AQUEOUS SOLUTION

After washing the films were transferred to a buffered aqueous solution containing $0.1 \text{ mol dm}^{-3} \text{ NaCl}$ and were cycled between -0.2 and $+1.0 \text{ V}$ vs SCE to obtain reproducible voltammetry. This typically takes less than 15 minutes. The cyclic voltammetry differs when the polymer is cycled in aqueous solution to that in acetonitrile. There are however no gradual changes observed between the cyclic voltammograms obtained in each solution. The changes are immediate. This is due to the fact that the polymer has been conditioned to the aqueous environment by the washing procedure.

The peaks of the polymer on transfer to aqueous solution are seen to sharpen up over the initial cycles as the polymer exchanges the counter ions present in solution for those already present in the polymer from the growth procedure. In aqueous buffered solution the cyclic voltammetry of the film is markedly pH dependent as shown in figure 3.5. Initial experiments were carried out using a disodium hydrogen orthophosphate buffer solution adjusted to the correct pH value with either 5 mol dm^{-3} HCl or 5 mol dm^{-3} NaOH. This was found to be unsatisfactory as the buffering capacity is insufficient in the region pH 5.0 to 3.0. The polymer needs to be buffered dynamically as when it becomes conducting there is a large flux of protons into solution. Hence the buffer must be capable of compensating for this large increase in protons at the polymer surface. Subsequently all experiments were performed using a phosphate/citrate buffer (McIlvaine)⁸⁵ solution containing 0.1 mol dm^{-3} NaCl as additional electrolyte, which also keeps the ionic strength of the solution constant. This solution was found to fulfil the requirements. The position of the anodic and cathodic peaks for the second oxidation process shift by approximately 100 mV per pH unit. This is shown in figure 3.6. As a check to ensure the shifts observed were a real effect and not due to the buffer being used, an alternative buffer, sodium acetate/acetic acid, was also used. This established that the shifts were a real effect (see figure 3.6). The movement of the peaks associated with the second oxidation process of the polymer presumably involves the loss or gain of protons and it is this which confers the pH sensitivity. The cyclic voltammetry of the films is stable over the region

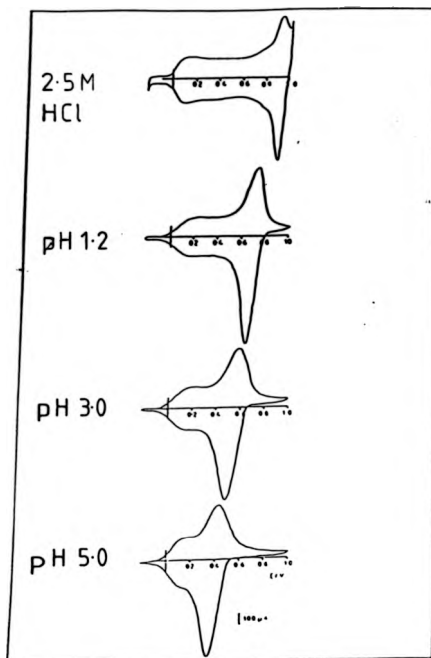


Figure 3.5: pH dependence for a 410 nm thick film of poly-5-carboxyindole in a McIlvaine buffer containing 0.1 mol dm^{-3} NaCl at a sweep rate of 10 mV s^{-1} .

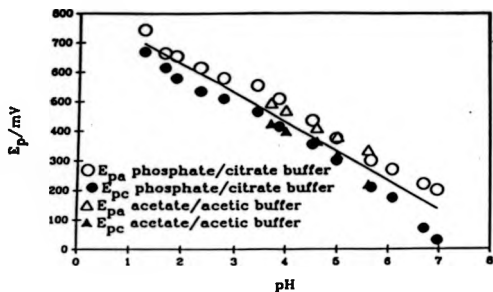


Figure 3.6: Variation of E_{pa} and E_{pc} of second oxidation peak with pH in two buffers of a 410 nm thick film of poly-5-carboxyindole at a sweep rate of 10 mV s^{-1} .

pH 1.0 to 5.0. In the region 5.0 to 7.0 the voltammetry decays away slowly. Above pH 7.0 no voltammetry is observed and polymeric material can be seen streaming off the electrode as the polymer dissolves.

The peak separation for the second oxidation peak was found to be relatively pH independent at 78 ± 14 mV for the pH range 1.0 to 6.0. This shows that electron transfer is rapid and reversible. The first oxidation peak does not exhibit such marked pH dependence and remains relatively static around 200 mV versus SCE.

3.6 DISCUSSION

The electrochemistry of poly-5-carboxyindole has been found to display a marked pH dependence. Other conducting polymers that exhibit similar behaviour, which have been reported, are polyaniline^{86,87} and the ion exchange polymer poly-(3-methylpyrrole-4-carboxylic acid).⁸⁸ In the case of polyaniline it seems to be the protonation of the partially oxidised form of polyaniline that causes the polymer to exhibit conductivity. The protonation enables the part of the polymer in contact with the electrode surface to undergo electrochemical redox reactions and would serve as a conduction medium for electron transport to the remainder of the polyaniline film.⁸⁵ Studies have shown that the resistance of polyaniline (in acidic aqueous solution) is at a minimum when the polymer is approximately half oxidised.⁸⁶ The electrochemical oxidation and reduction of polyaniline in aqueous solution of varying pH shows two classes of redox processes, occurring at

different potentials, which differ from each other by the extent to which the processes are accompanied by deprotonation (on oxidation) and reprotonation (on reduction).⁸⁶ Polyaniline is found to be electroactive over the pH range 1.0 to 4.0 while above this the polymer is found to be inactive, this is thought to be due to the fact that essentially no protonation of the polymer occurs at these slightly acidic conditions and hence very little conductivity can be imparted to the polymer.⁸⁶ The proposed structure of polyaniline is a quinoidal³⁰ one formed upon the oxidation and associated deprotonation of the polymer, this is shown in figure 3.7. In the case of poly-(3-methylpyrrole-4-carboxylic acid) the pH dependence is also explained by the degree of protonation of the carboxylic acid group, and the oxidation state of the polymer.⁸⁸ Pickup assumed, by analogy to polypyrrole,²⁰ that approximately one pyrrole unit in four can be oxidised, thus he proposed that the loss of one electron during oxidation is accompanied by the deprotonation of two carboxylic acid groups. From a plot of E versus pH for such a system a theoretical slope of 118 mV per pH unit would be obtained.⁸⁶ The observed slope of 115 mV per pH unit obtained from his experimental data is in good agreement with this value. The range over which this slope is observed is pH 1.0 to 7.0, above this range electrochemical responses are found but are not pH dependent.

If we consider poly-5-carboxyindole it is seen that the pH dependence is associated with the second redox process (figure 3.5). We can thus draw comparisons with the other materials in that the oxidation state of the polymer is involved in the process, and similarly the protonation state of the carboxylic acid functions

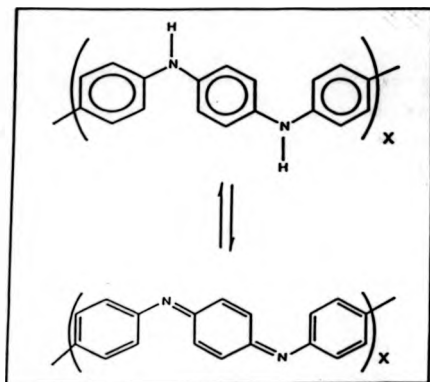


Figure 3.7: Structure of polyaniline.

must also be involved. The assumption that the protonation state of the carboxylate functions plays an important role in the response of the polymer to variations in pH is confirmed by the fact that polyindole is found to exhibit no electrochemical response, in buffered aqueous solution over the pH range used in the study of poly-5-carboxyindole. Figure 3.8 shows a typical cyclic voltammogram of poly-indole in acetonitrile and one in buffered aqueous solution.

In the cyclic voltammetry of poly-5-carboxyindole in aqueous solution the first oxidation process is seen to be unaffected by pH and is probably associated, with the formation of a partially oxidised conducting state. For the second oxidation process the peak height sweep rate dependence was found to be linear for the anodic peak all across the pH range, whereas for the cathodic peak the peak height was found to be linearly dependent on sweep rate only in strong acid solutions (2.5 mol dm^{-3}), and to become non-linearly dependent on sweep rate in weakly acidic solutions (pH 5.0). Figure 3.9 shows a comparison of sweep rate versus peak height for the second oxidation process of poly-5-carboxyindole in 2.5 mol dm^{-3} HCl and a pH 5.0 buffered solution. The deviation of the cathodic reaction from linear behaviour implies that the electron transfer on reduction is slow when the polymer is in solutions $>\text{pH } 1.0$. This is probably associated with the fact that re-protonation of the polymer will not occur as readily under weakly acid conditions.

For the second oxidation process a plot of E_p versus pH gives a

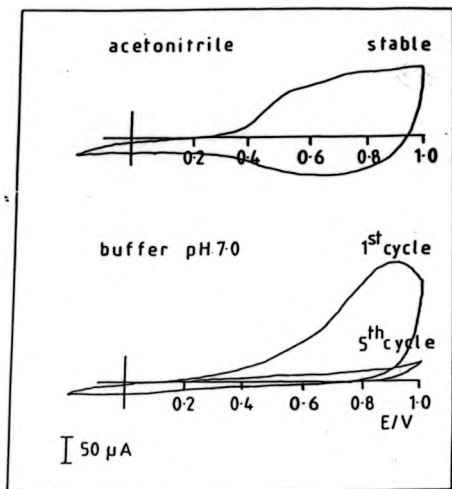


Figure 3.8: Typical cyclic voltammogram of poly-indole in acetonitrile containing 0.1 mol dm^{-3} TEA.TFB and in buffered solution pH 7.0 containing 0.1 mol dm^{-3} NaCl at a sweep rate of 20 mV s^{-1} .

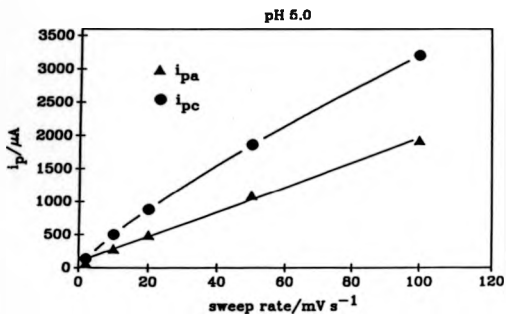
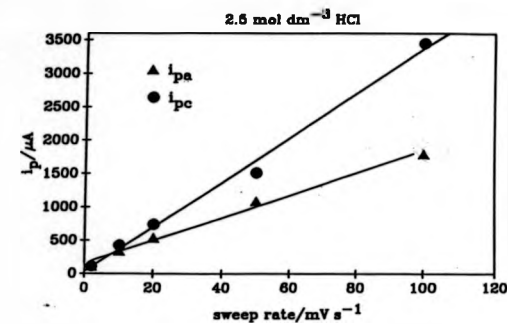


Figure 3.9: Comparison of sweep rate dependence of second oxidation process in strong and weak acid conditions.

straight line in the region pH 1.0 to 5.0 (figure 3.6) with a slope of 99.3 mV per pH unit.

There are several unusual features about the cyclic voltammogram of poly-5-carboxyindole in aqueous solution. There is no definite peak found for the first oxidation process and only a small shoulder is observed. This is followed by a flat region and then the peaks for the second oxidation process are clearly seen. The length of the flat region and the position of the peaks associated with the second redox process are highly pH dependant.

An analogy may be drawn between the flat region seen in the cyclic voltammogram for poly-5-carboxyindole and that observed for pyrrole. Feldberg has explained this flat region by proposing that it was due to capacitive current associated with the electrochemical switching of the film.⁸⁹ He made three basic assumptions:

- i) the differential capacitance is proportional to the amount of oxidised material,
- ii) the differential capacitance is independent of potential,
- iii) the differential capacitance of the platinum electrode is ignorably small.

If we apply these assumptions to our system we find that they do not explain our observations. The flat region observed in the cyclic voltammogram of poly-5-carboxyindole in aqueous solution (see figure 3.5) according to Feldberg's model would have to be solely due to the differential capacitance of the polymer. This would mean that the charge passed in the first oxidation process would have to be extremely small so as not to be obvious in the cyclic voltammogram. It seems unlikely that the first oxidation process of the polymer

will be associated with such a small amount of charge we therefore propose an alternative possible explanation as follows. The Feldberg model assumes that the faradaic and capacitive currents cannot be distinguished. We agree with this in respect of the present system but the interpretation of what is the faradaic and the capacitive current is the important factor. We assume that the faradaic current is produced by the passage of charge associated with the intercalation of ions into the matrix of the polymer whereas the capacitive charge is due to the collection of charge on the surface of the polymer. Making these assumptions we interpret the cyclic voltammogram as follows. In the neutral state (reduced) the polymer will possess a combination of protonated and deprotonated carboxylate groups. When the first oxidation process occurs a positive charge is produced on the polymer backbone, this is associated with some intercalation of counterions into the polymer causing a faradaic current to pass. However there is also an associated deprotonation of the polymer which results in interaction between the negatively charged carboxylate groups and the positively charged Na^+ ions in solution to form a 'semi polymer electrolyte' like structure. This surface process will cause a capacitive current to pass. The deprotonation of the carboxylate groups will continue as the potential is taken more positive resulting in more negative sites allowing the extension of the 'polymer electrolyte' structure. This process will continue until the surface is totally deprotonated. This behaviour will account for the flat region observed in the cyclic voltammogram. When the second oxidation process occurs this can only be associated with the

intercalation of ions into the matrix of the polymer thus only a faradaic current will pass and the normal shape for a redox process will be observed in the cyclic voltammogram. The possible structures postulated above are shown in figure 3.10.

It is obvious that the processes involved in the switching reaction of poly-5-carboxyindole are highly complex and an absolute description at this time cannot be given.

3.7 POLY-5-CARBOXYINDOLE AS A PH SENSOR

As the response to pH was so marked it was thought that the polymer may be successfully used as a pH sensor. The polymer has several features that make it promising for this type of application:

- ease of electrochemical deposition
- small size of device (125 μm or less)
- rapid response times
- reasonable storage stability

3.7.1 GROWTH AND CALIBRATION

Films were grown as in section 3.2 and transferred to a pH 2.0 buffer solution. Once the voltammetry had stabilised the films were held at +800 mV versus SCE in this solution and the current allowed to decay to zero. This process 'calibrates' the films so that they give a response of +800 mV versus SCE at this pH.

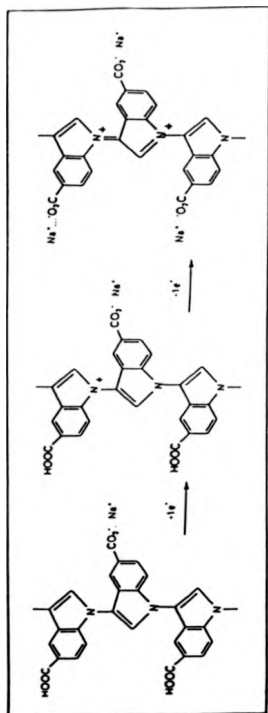


Figure 3.10: Possible structures of poly-5-carboxyindole as the polymer is oxidised.

3.7.2. PH MEASUREMENTS

After 'calibration' the electrodes were transferred to the test solution. The experimental arrangement is shown schematically in figure 3.11. pH measurements were made using a Corning 145 pH meter reading absolute mV, (input impedance 10^{12} ohms), with the polymer coated electrode and a SCE reference electrode. The pH of the test solution was varied by adding small amounts of 5 mol dm^{-3} HCl or 5 mol dm^{-3} NaOH (with mixing by magnetic stirrer). The mV output of the Corning pH meter was fed to a Bryans 60000 series XV/t recorder for response time measurements. The pH of the solution was simultaneously measured using a commercial combination pH electrode and separate pH meter.

3.7.3 RESPONSES

Typical results for the measurement of pH are shown in figure 3.12 for a coated platinum electrode. Figure 3.13 shows a comparison between coated platinum and coated gold electrodes. The responses are essentially linear over the range pH 1.0 to 5.0 with a slope of 83 mV per pH unit. The response of these devices to pH is reasonable rapid, probably better than 10 seconds as shown in figure 3.14.

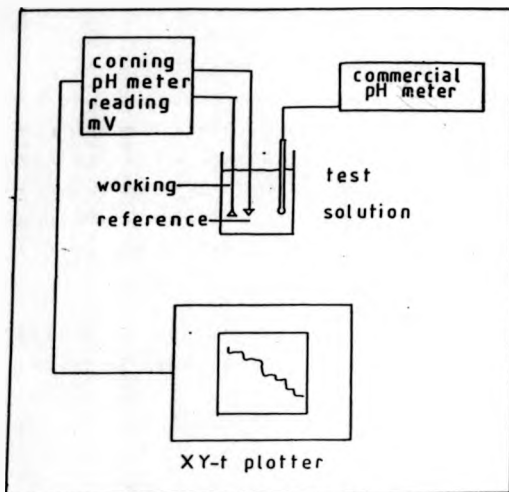


Figure 3.11: Schematic representation of the experimental set up for measuring pH responses.

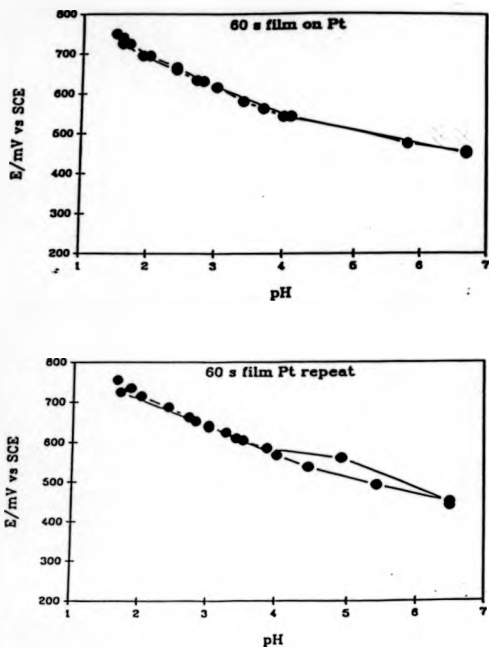


Figure 3.12: Typical pH responses for poly-5-carboxyindole on a rotating disc electrode (area = 0.38 cm^2) in aqueous solution containing $0.1 \text{ mol dm}^{-3} \text{ NaCl}$, pH adjusted by adding strong acid or strong alkali.

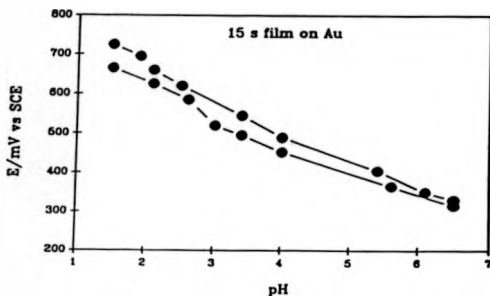
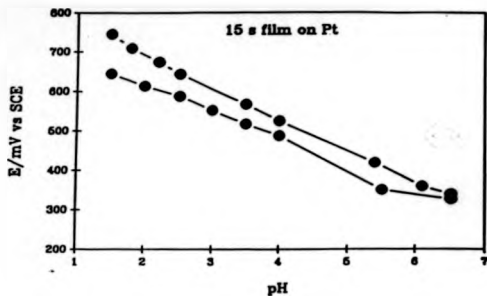


Figure 3.13: Comparison of pH response using platinum and gold as the electrode material.

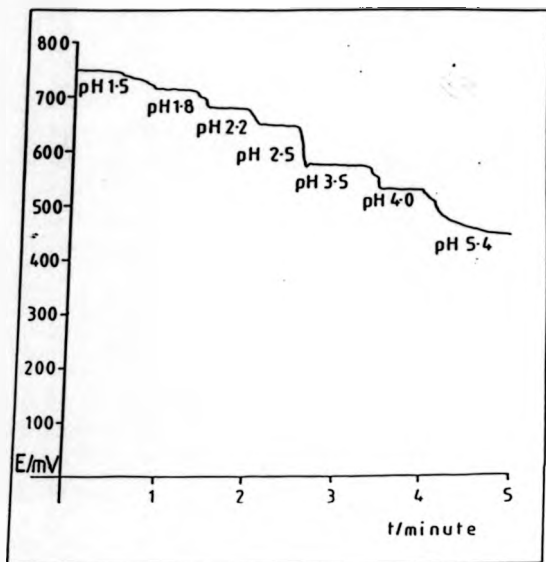


Figure 3.14: Actual pH responses obtained from poly-5-carboxyindole showing short response time.

3.7.4 SENSITIVITY TO OTHER IONS

The devices were found to be essentially insensitive to the concentration of Na^+ in the range 0.01 to 1 mol dm^{-3} NaCl as shown in figure 3.15. The devices were also found to be insensitive to Mg^{2+} and Pb^{2+} over a similar range. These latter two ions were chosen for study as they have large binding constants with benzoic acid and it was therefore felt that they might bind strongly to the polymer.

3.7.5 STABILITY OF RESPONSE

The devices were found to be stable for periods of approximately 2 hours of continuous use provided the pH was kept below about 7.0. Over longer periods the response declines. The response may be restored by 're-calibrating' at +800 mV in pH 2.0 buffer.

3.7.6 MICROELECTRODE DEVICES

The storage stability of a set of microelectrode devices (for design see experimental chapter section 2.2) was investigated by 'calibrating' a set of electrodes and then storing them in a dry state for one week. The devices were then re-tested first without 're-calibration' and then after 're-calibration'. Figure 3.16 shows the effect of storage on the response of a coated platinum microelectrode. Without 're-calibration' the response of the device declines. After 're-calibration' the response returns to the

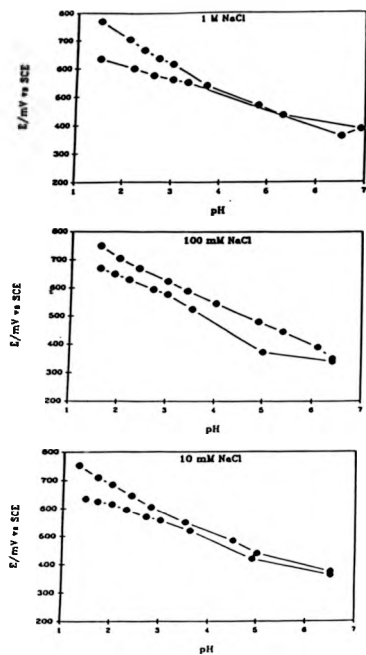


Figure 3.15: Effect of Na^+ concentration on pH response.

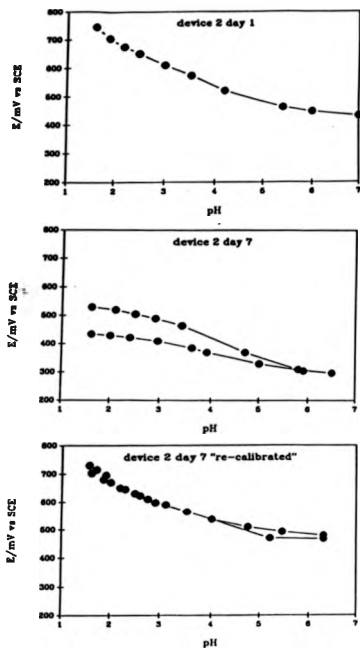


Figure 3.16: Responses obtained from a micro device before and after 1 weeks storage and 're-calibration'.

Initial value.

The disadvantages at present are the working range pH 1.0 to 5.0 due to solubility of the polymer in basic solution and the possibility of redox interference. It should be possible to overcome the solubility by either forming a copolymer or crosslinking the polymer. Redox interference could be solved by coating the polymer with a film of poly-phenol which will act as a impermeable barrier to most molecules but is permeable to protons.

CHAPTER 4

INTRODUCTION

4.1 ELECTROCHEMISTRY OF CYTOCHROME C AT POLY-5-CARBOXYINDOLE

The direct electrochemistry of cytochrome *c* has been investigated by many groups at a wide variety of electrode surfaces.^{53,90,91} Much of this work has highlighted the need for productive binding between the redox protein and the electrode surface in order to allow rapid electron transfer.^{92,93} In an elegant series of studies Hill and co-workers have shown that this can be achieved by the addition of promoters, such as 4,4'-dithiopyridine to the solution.⁶⁵ These promoters function by adsorbing at the electrode and thus providing a surface which possesses some of the characteristics of the protein's natural redox partner. We are interested in the application of conducting polymers, as electrode materials, to bioelectrochemistry. These materials are attractive because they are easily chemically modified and can be deposited at electrode surfaces in a controlled manner. We reasoned that it should be possible to achieve the direct electrochemistry of cytochrome *c* using a polymer chosen, or derivatised, in such a manner as to mimic the redox protein's natural partner. Poly-5-carboxyindole fulfils these requirements as it has carboxylate groups attached to the polymer chain.

This chapter presents the work based on this reasoning and also work which attempts to produce a conducting polymer which presents a 'biological surface' by coupling amino acid residues to the parent monomer 5-carboxyindole.

4.2 PROCEDURE FOR MEASUREMENT OF THE DIRECT ELECTROCHEMISTRY OF CYTOCHROME C AT A POLYMER MODIFIED ELECTRODE

All electrochemical measurements were carried out at $25^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ using a conventional three electrode system. Horse heart cytochrome c was either used as received or purified following the method of Brautigan *et al.*⁷⁹ (see section 2.8). The concentration of the purified material was determined spectrophotometrically as described in section 2.9. All aqueous solutions were prepared using high purity water (conductivity $<1\ \mu\text{S}$). Phosphate buffer solutions were prepared from di-sodium hydrogen orthophosphate ($85\ \text{mmol dm}^{-3}$) adjusted to pH 7.0 with $5\ \text{mol dm}^{-3}$ hydrochloric acid. Poly-5-carboxyindole films were deposited electrochemically on platinum or glassy carbon electrodes from a solution of $5\ \text{mmol dm}^{-3}$ 5-carboxyindole in acetonitrile containing $0.1\ \text{mol dm}^{-3}$ TEA.TFB. The films were grown by stepping the potential from 0 to +1.4 V versus SCE at an electrode rotating at 4 Hz. After deposition for between 15 and 20 seconds the electrode was removed and transferred to water by washing the electrode as previously described in section 3.4. Poly-indole films were grown from a $5\ \text{mmol dm}^{-3}$ solution of indole in acetonitrile containing $0.1\ \text{mol dm}^{-3}$ TEA.TFB at a stationary

electrode by stepping the potential from 0 to +1.3 V versus SCE. The films were washed with water prior to use.

4.3 COMPARISON OF POLY-5-CARBOXYINDOLE AND POLY-INDOLE IN CYTOCHROME C SOLUTION

When the platinum electrode was coated with a poly-indole film no electrochemistry was observed for cytochrome c in solution, similarly in a blank experiment 5-carboxyindole was not found to promote the electrochemistry of cytochrome c when present in solution. Figure 4.1 shows a typical voltammogram for poly-indole in background electrolyte at pH 7.0 with and without the presence of the crude cytochrome c. When the electrode was coated with poly-5-carboxyindole markedly different voltammetry is obtained. Figure 4.1 shows a typical cyclic voltammogram for the coated electrode in background electrolyte at pH 7.0 with and without the presence of crude cytochrome c. In the absence of cytochrome c the voltammogram shows only the background charging current associated with the polymer present on the electrode surface. The magnitude of this current is strongly dependent on the amount of polymer deposited. It was therefore necessary to use thin polymer films (short growth times) to avoid excessively large background currents. When cytochrome c is present in solution, a pair of peaks is clearly visible in the voltammogram close to the potential reported for the redox protein in this buffer.^{65,94} Identical results were obtained from poly-5-carboxyindole when either platinum or glassy carbon were used as the underlying electrode material. The responses were

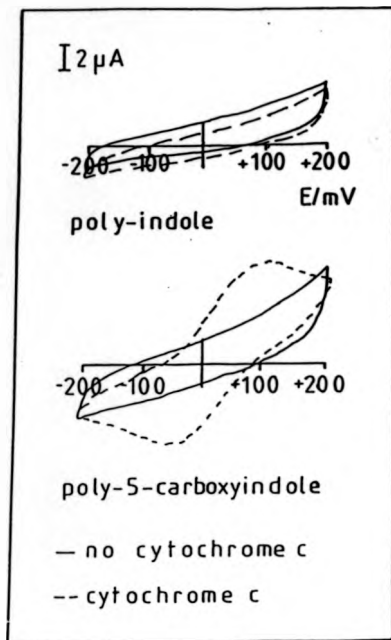


Figure 4.1: Comparison of poly-indole and poly-5-carboxyindole in a solution of $100\ \mu\text{mol dm}^{-3}$ crude cytochrome c pH 7.0, (sweep rate $10\ \text{mV s}^{-1}$, electrode area $0.39\ \text{cm}^2$).

reasonably stable with typically a 10% decrease in peak height after 20 minutes continuous cycling.

Table 4.1 summarises the cyclic voltammetry of crude cytochrome *c* at two different poly-5-carboxyindole films.

Film 1

Sweep rate mV s ⁻¹	<i>i</i> _{pa} μA	<i>i</i> _{pc} μA	E _{1/2} mV	ΔE mV
5	2.4	1.5	5.6	66
10	4.6	3.8	7.5	65
20	8.5	8.2	10.0	80
50	19.5	19.5	20.0	130
100	30.0	29.0	12.5	155

Film 2

2	2.2	1.5	7.5	45
5	4.1	3.4	10.0	100
10	6.8	5.9	10.0	140
20	11.5	11.0	15.0	180
50	20.0	18.7	22.2	225
100	29.5	27.7	20.0	240
200	44.2	39.0	20.0	260

Table 4.1: Cyclic voltammetry data of 50 μmol dm⁻³ crude cytochrome *c* pH 7.0 at two poly-5-carboxyindole films.

The sweep rate peak height dependence of the cytochrome *c* redox process at poly-5-carboxyindole is shown in figure 4.2. Background currents for the polymer alone have not been subtracted this is due to the difficulty in estimating the contribution that the

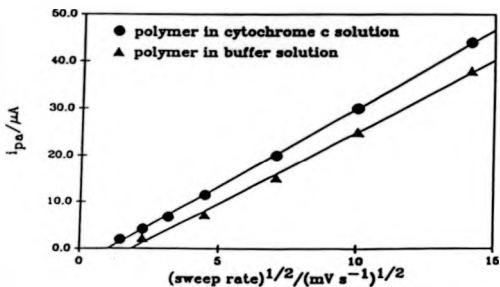
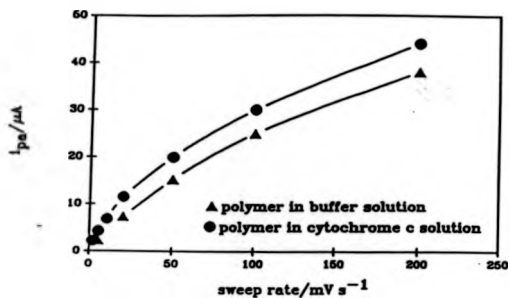


Figure 4.2: Sweep rate dependence of cytochrome c at poly-5-carboxyindole coated platinum electrode (area = 0.39 cm^2).

polymer makes in this region. As can be seen in Figure 4.2 the sweep rate dependence is a combination of the polymer and the cytochrome *c*. The results obtained suggest that there is some limiting parameter involved in the reaction (see section 4.5).

4.3.1 EFFECT OF PURIFICATION OF CYTOCHROME *c*

Most workers using cytochrome *c* find it necessary to purify the material to obtain good, reproducible electrochemistry. All the results so far reported have been with crude material which with poly-5-carboxyindole has given very good responses. Purification of cytochrome *c* should thus have the effect of improving the responses obtained. This proved the case with the only difference between crude and pure material being greater magnitude for the redox peaks from the pure material. Table 4.2 summarise the voltammetric data for pure and crude cytochrome *c*.

Sweep rate mV s ⁻¹	crude		pure	
	<i>i</i> _{pa} μA	<i>i</i> _{pc} μA	<i>i</i> _{pa} μA	<i>i</i> _{pc} μA
20	11.20	11.00	25.00	24.00
50	21.00	21.75	49.00	49.00
100	37.50	34.50	88.00	86.00
200	50.00	52.00	147.00	148.00

Table 4.2: Cyclic voltammetric data for poly-5-carboxyindole coated electrode in 22 μmol dm⁻³ cytochrome *c* solution at pH 7.0.

4.4 COMPARISON WITH CHEMICAL MODIFIER

Having established that it was possible to observe the electrochemistry of cytochrome *c* with a poly-5-carboxyindole coated electrode it was decided to use a well characterised chemical modifier as a comparison. Modification of a gold electrode was achieved by dipping the electrode in a 1 mmol dm^{-3} solution of the modifier 4,4'-dithiopyridine⁶⁵ for approximately 2 minutes followed by rinsing with pH 7.0 buffer solution. A comparison of the cyclic voltammograms obtained from the two modified electrodes is shown in figure 4.3 for a $100 \text{ } \mu\text{mol dm}^{-3}$ solution of crude cytochrome *c* (type VI). The electrochemistry of cytochrome *c* is clearly seen with the poly-5-carboxyindole coated electrode. In fact it is found to be better than the chemical modifier giving anodic and cathodic peaks of greater magnitude.

4.4.1 ADDITIONAL INFORMATION

The magnitude of the peak current was found to be essentially independent of the thickness of the polymer film and insensitive to the cytochrome *c* concentration over the range 20 to $200 \text{ } \mu\text{mol dm}^{-3}$. Figure 4.4 shows a plot of cytochrome *c* concentration against i_{pa} obtained from cyclic voltammetry at a sweep rate of 20 mV s^{-1} . The intercept on the y axis represents the background current for the polymer. The film thickness dependence is shown in figure 4.5.

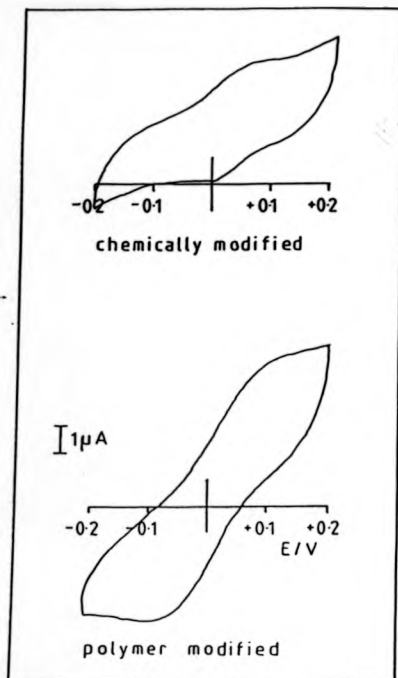


Figure 4.3: Comparison of chemically modified electrode to a poly-5-carboxyindole coated electrode in a $50 \mu\text{mol dm}^{-3}$ solution of crude cytochrome *c* pH 7.0.

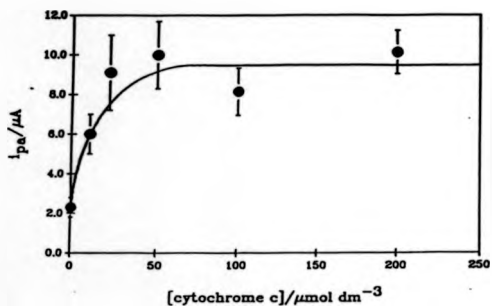


Figure 4.4: Concentration of cytochrome c against i_{pa} (no correction for background) for a sweep rate of 20 mV s^{-1} (film thickness = 240 nm).

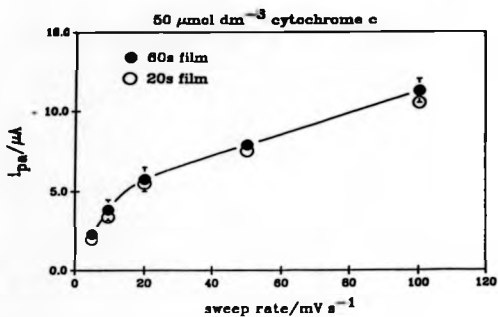


Figure 4.5: Film thickness dependence of cytochrome c at a poly-5-carboxyindole modified electrode.

On transferring the polymer coated electrode from cytochrome *c* solution to a fresh buffer solution at the same pH the cyclic voltammetric peaks associated reaction of cytochrome *c* were observed to persist for a few cycles and then to decay away, as shown in figure 4.6. This suggest that the desorption of the protein from the electrode surface is slow.

4.5 DISCUSSION

The results indicate that the presence of the carboxylate groups on the conducting polymer poly-5-carboxyindole facilitates the electrochemistry of cytochrome *c*.

The proposed structure of poly-5-carboxyindole (see figure 3.1) leaves the carboxylate groups relatively unhindered and presumably accessible to the redox protein. We believe that the binding occurs between these carboxylate groups and the lysine residues clustered around the haem crevice of the cytochrome *c* molecule. At pH 7.0, the working pH for these experiments, we would expect the bulk of the polymer to be in a deprotonated state. However the surface of the polymer maybe different from the bulk. As mediation of the electrochemistry of cytochrome *c* is observed the carboxylate groups on the surface of the polymer must be in a deprotonated state to fulfil the requirements needed for mediation to occur. Thus the charge on these residues will be negative. This negative charge will be electrostatically attractive to the positively charged lysine residues of the cytochrome *c* molecule. This electrostatic

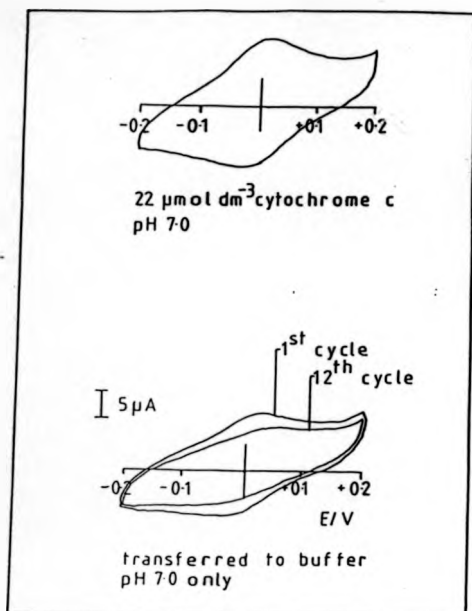


Figure 4.6: Desorption of cytochrome c from polymer surface when placed in a pH 7.0 buffer solution.

attraction orientates and binds the molecule to facilitate the formation of a polymer/cytochrome c transient to enable electron transfer to occur in a manner analogous to that of the normal interaction of cytochrome c/cytochrome c oxidase in nature.⁹⁵

As shown earlier (figure 4.2) the sweep rate dependence of cytochrome c at the polymer does not exhibit the behaviour expected from a bound species. The involvement of a limiting parameter is seen. The cytochrome c couple comes in the region which corresponds to the first oxidation process of the polymer, see figure 4.7. This region is thought to be associated with the polymer in a partially oxidised state and thus not ideally conducting. It seems reasonable to assume that it is the rate of electron transfer to the film that is the limiting factor. This is supported by figure 4.2. The sweep rate dependence for the polymer goes from linear to square root. This is consistent with the rate of electron transfer in the film being the limiting process. At slow sweep rates the transfer will be rapid but at higher sweep rates ($>20 \text{ mV s}^{-1}$) diffusion of electrons within the film will become an important factor.

The magnitude of the peaks obtained for the oxidation/reduction of cytochrome c when using a poly-5-carboxyindole modified electrode are found to be greater than those found for a chemically modified electrodes. For a poly-5-carboxyindole modified electrode, typical peak currents of 12-15 μA are obtained, compared to 2-3 μA for a chemically modified electrode (for the same sweep rate). Table 4.3 gives a comparison of peak currents obtained from a poly-5-carboxyindole modified electrode and a chemically modified electrode, modifier 4,4'-(dithiopyridine), in a cytochrome c

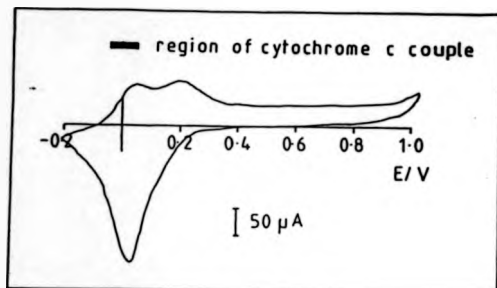


Figure 4.7: Region of cytochrome *c* couple in comparison to the cyclic voltammogram of poly-5-carboxyindole in pH 7.0 buffer.

Sweep rate mV s^{-1}	chemical		polymer	
	i_{pa} μA	i_{pc} μA	i_{pa} μA	i_{pc} μA
10	1.30	1.25	6.90	4.80
20	2.00	1.85	11.70	4.00
50	3.80	3.40	21.0	9.50

Table 4.3: Peak currents obtained from a $100 \mu\text{mol dm}^{-3}$ solution of cytochrome c with a chemically and a polymer modified electrode.

solution.

An explanation for this is found by considering the coverage of cytochrome c. Albery *et al.*⁹⁶ found a limiting coverage of $\Gamma_L = 1.2 \times 10^{-6} \text{ mol m}^{-2}$, assuming the cytochrome c molecule to be a sphere with a radius of 1.5 nm and a surface roughness factor for their electrode of 4. An estimate of our coverage of electroactive cytochrome c is obtained by integrating the charge in the cyclic voltammogram. Using our electrode area of 0.39 cm^2 and $\Gamma_{\text{monolayer}} = 3.0 \times 10^{-7} \text{ mol m}^{-2}$ we calculate a coverage in the region of 40 monolayers. The surface roughness of the polymer must make a large contribution to this. This larger surface coverage would explain the larger currents which we observe in cyclic voltammetry as compared to those observed with a chemically modified electrode.

An estimate of the concentration at which saturation occurs (from figure 4.4) was found to be in the range $20 - 30 \mu\text{mol dm}^{-3}$. Comparing this with the data of Albery *et al.*, who find a value of

approximately $200 \mu\text{mol dm}^{-3}$, this shows that cytochrome *c* binds almost 10 times more strongly to the polymer as to a chemical modified (4,4'-bipyridyl) electrode surface.

Attempts to use rotating disc voltammetry were unsuccessful as, upon rotation of the electrode, the polymer was observed to detach from the electrode surface.

The strong binding and slow desorption of cytochrome *c* from the polymer surface (see figure 4.6) can be a disadvantage in that electron transfer between the bound redox protein and the polymer is reasonably facile and persistent. It is suggested that to overcome this problem of strong adsorption that the surface carboxylate groups of the polymer be 'diluted' in some way, thus reducing the number of sites available for binding with the redox protein. Preliminary studies have shown that the formation of co-polymers of poly-indole and poly-5-carboxyindole may offer a solution to this problem.

The ability to control the properties of the polymer is a significant advantage which can be exploited to produce electrode surfaces which mimic the natural macromolecular redox partner of a redox protein of interest. A logical extension is to couple an amino acid to a conducting polymer to produce a 'biologically acceptable' surface, thus allowing the investigation of bioelectrochemical systems.

4.6 AMINO ACIDS

An amino acid consists of an amine group, a carboxyl group, a hydrogen atom and a distinctive R-group bound to a carbon atom which is called the α -carbon. The R-group is referred to as the side chain. Amino acids form a very important group of compounds as they are the basic structural units of proteins. There are twenty kinds of side chains varying in size, shape, charge, hydrogen bonding capacity and chemical reactivity, commonly found in proteins. In fact, all proteins in all species, from bacteria to humans are composed of the same twenty amino acids. This fundamental alphabet from which proteins are made has been around for at least 2 billion years. The remarkable range of functions mediated by proteins results from the diversity and versatility of these structural units.

4.7 AMINO ACIDS COUPLED WITH CONDUCTING POLYMERS

The rationale behind coupling an amino acid with a conducting polymer is to produce a material that can easily be deposited by electropolymerisation to give a biologically compatible modified electrode. The range of properties of amino acids would make these materials of interest for the following reasons and in the following possible applications:

i) Coupling of amino acids to form peptides is a well documented procedure. Sheehan et al.⁸² developed a simple quick procedure using (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride. This method should be adaptable to enable coupling of a protected amino acid (methyl or ethyl ester) to the monomer of a conducting polymer prior to electropolymerisation, (i) There is a need to develop new materials for use in small cheap chemical sensors (gas and solution phase), suitable for use with microprocessors for monitoring and control, (ii) Amino acid derivatised conducting polymers are of interest in the development of bioelectronic devices and biosensors in which biological redox species are used in combination with electronic devices as sensors. An example of such an approach is the immobilisation of enzyme within the conducting polymer.⁹⁷

4.8 ELECTROCHEMICAL PROCEDURE

The synthesis of amino acid modified monomers of indole has been described in section 2.10 along with the spectral assignments for these molecules. In this section we describe the attempts to obtain conducting polymer films by electropolymerisation using a potential sweep method. All electrochemical measurements were carried out using a standard three electrode system with a saturated calomel reference electrode (SCE), platinum gauze counter electrode and platinum rotating disc working electrode. Electropolymerisation was

attempted from 5 mmol dm^{-3} solutions monomer (except for N-(indole-5'-carboxyamido)valine where a saturated solution of approximately 0.5 mmol dm^{-3} was used) in acetonitrile containing 0.1 mol dm^{-3} TEA.TFB by a potential sweep from 0 to +2.0 V versus SCE at a electrode rotating at 4 Hz (except for ethyl ester of N-(indole-5'-carboxyamido)cysteine where a stationary electrode was used). A typical example of a cyclic voltammogram obtained is shown in figure 4.8.

All compounds gave similar results; a large initial oxidation peak with no associated reduction peak on the first sweep. Successive sweeps showed little or no signs of any electrochemistry. Table 4.4 overleaf summaries the electrochemical data.

4.9 DISCUSSION

Attempts to obtain an amino acid modified conducting polymer by firstly modifying the parent monomer proved unsuccessful. Using the approach of Waltman *et al.*¹⁵ a plot of electrochemical peak oxidation potential of the modified indole monomer versus the corresponding Hammett substituent constant was constructed. A value for the substituent group $-\text{CONH}_2$ was used. This is chosen as it is the closest group found in the literature that has a Hammett constant quoted to the actual group CONH-R . Figure 4.9 shows the plot, using the Hammett substituent constant of 0.28 for the group $-\text{CONH}_2$.⁹⁸ Waltman *et al.* found that the substituted monomers enclosed in the box electropolymerised. Some of the amino acid modified indoles are found to lie inside this box and some outside.

Compound	[] mmol dm ⁻³	W Hz	E _{pa} /V sweep rate/mV s ⁻¹		
			20	50	100
methyl ester of N-(indole-5'-carboxy amido)valine	5	4	1.64	1.69	1.85
ethyl ester of N-(indole-5'-carboxy amido)alanine	5	4	1.59	1.67	1.75
methyl ester of N-(indole-5'-carboxy amido)glutamic acid	5	4	1.64	1.72	1.79
methyl ester of N-(indole-5'-carboxy amido)glycine	5	4	1.61	1.67	1.86
N-(indole-5'-carboxy amido)valine	0.5	4	1.40	1.42	1.44
ethyl ester of N-(indole-5'-carboxy amido)cysteine	5	0	1.45	1.51	1.64

Table 4.4: Electrochemical data for amino acid modified indole monomers.

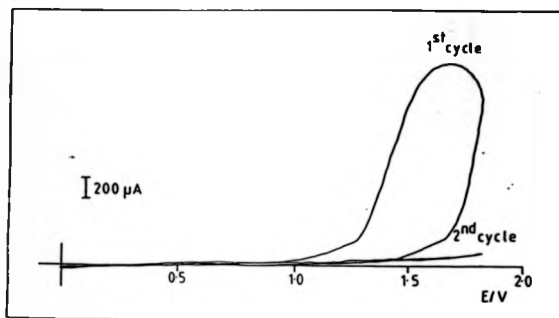
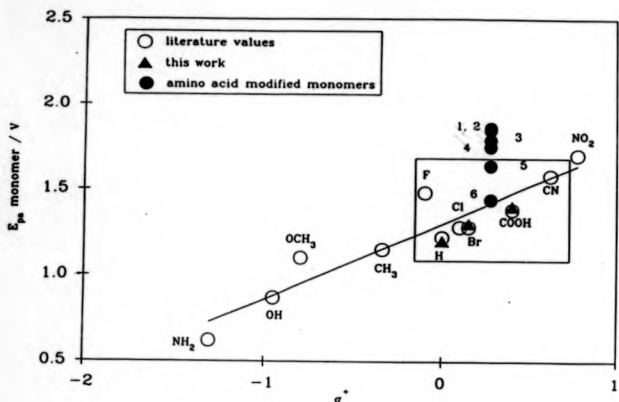


Figure 4.8: Typical cyclic voltammogram of an amino acid modified indole (sweep rate 50 mV s^{-1}).



1. Methyl ester of N-(indole-5'-carboxyamido)glycine.
2. Methyl ester of N-(indole-5'-carboxyamido)valine.
3. Methyl ester of N-(indole-5'-carboxyamido)glutamic acid.
4. Ethyl ester of N-(indole-5'-carboxyamido)alanine.
5. Ethyl ester of N-(indole-5'-carboxyamido)cysteine.
6. N-(indole-5'-carboxyamido)valine.

Figure 4.9: Electrochemical peak oxidation potential (vs SCE) of substituted indole monomers versus Hammett substitution constants.

Monomers which electropolymerise are enclosed within box, (after Waltman et al.¹⁵).

However all fail to yield polymers. This is possibly due to steric hindrance, and this explanation is favoured as we obtain the same electrochemical peak oxidation potentials as Waltman *et al.* for indole and indoles substituted with carboxylic acid and bromine (see figure 4.10). Thus ruling out experimental factors for the failure of the amino acid monomers to electropolymerise. An alternative approach that may prove successful is to electropolymerise poly-5-carboxyindole first and attach the amino acid groups to the polymer afterwards. This approach is suggested for future investigations. Also there is the possibility of forming copolymers of poly-5-carboxyindole and the amino acid modified monomers.

CHAPTER 5

INTRODUCTION

5.1 INVESTIGATION OF REDOX COUPLES AT POLY-5-CARBOXYINDOLE

This chapter presents the work done on the investigation of redox couples at the polymer film. Redox couples were used to probe the mechanism of electron transfer between the film and solution species.

The results are analysed using the model of Alberly and Hillman for the kinetics and mechanism of reactions at modified electrodes.⁹

5.2 THE ALBERLY AND HILLMAN MODEL

The model was devised to characterise the mechanism and kinetics of reactions at modified electrodes coated with thin films containing redox groups. The process of electron transfer between the solution species and the metal electrode is 'mediated' by these redox groups.

Our system differs, but is analogous to these films, in that the conjugated system of double and single bonds within the polymer structure act as the pathway for electron transfer to occur between solution species and the electrode.

Figure 5.1 shows the general model adopted by Alberly and Hillman for the processes taking place in the film. The redox couple

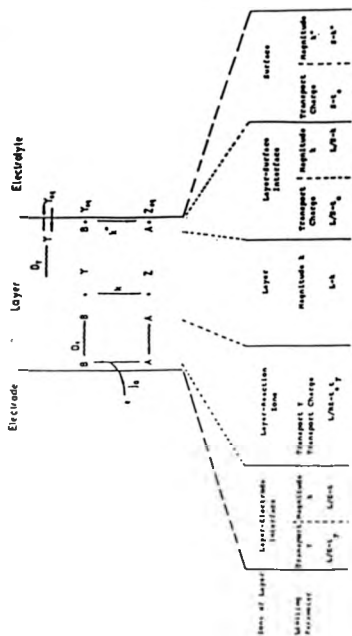


Figure 5.1: General model for the reaction of a redox couple at a modified electrode, (after Albery and Hillman⁹).

A/B is bound in the film. It is assumed that the electrode potential is such that the net reaction is the reduction of the solution species Y to Z. This reaction can take place at the film/solution interface - a surface reaction, with a rate constant k'' . Alternatively Y can diffuse into the film and reaction can take place in the film. The film reaction will be governed by two different transport processes. Firstly the diffusion of electrons, in the form of B, from the electrode to meet Y, characterised by D_e . (In our case this is analogous to the movement of electrons through the conducting polymer). Secondly the diffusion of Y into the film itself, D_y . It is assumed that there are no kinetic barriers to the partitioning of Y at the film/solution surface.

The model proposes two 'reaction layer thicknesses'. The first layer thickness, x_L , is the distance Y, as it enters the film from electrolyte, will diffuse before undergoing reaction with B. The second layer thickness, x_0 , is the distance an electron, in the form of B, entering the film from the electrode will diffuse before undergoing reaction with Y.

Five possible reaction locations are found. Firstly when electrons (B) penetrate the film more easily than Y, the reaction will take place at the film/surface (L/S case). Secondly if Y penetrates the film more easily than electrons, the reaction will take place at the film/electrode (L/E case). The relative thickness of the film will also determine the reaction site. If the total film thickness is much smaller than the distance Y will diffuse into it, then the whole of the film is used in the reaction, this being the third possible reaction location (L case). The fourth location

is found when the total film thickness is much smaller than the distance electrons (B) will diffuse into it, then the reaction will occur at the surface (S case). A fifth location is found when the total film thickness is greater than either the distance Y or electrons (B) will diffuse into it. In this case the reaction will take place somewhere in a reaction zone in the middle of the film (L/RZ case).

A diagnostic table has been produced to aid the interpretation of results obtained in this type of study. This is reproduced in figure 5.2 and is the method employed in the analysis of data presented in this chapter.

5.3 IRON (II) TRIS 2,2'-DIPYRIDYL PERCHLORATE AT A CLEAN PLATINUM ELECTRODE

Solutions of $\text{Fe}(\text{bipy})_3(\text{ClO}_4)_2$ were made up using buffered (McIlvaine⁸⁵) aqueous solution containing 0.1 mol dm^{-3} sodium chloride as background electrolyte. Solutions were degassed thoroughly before use.

Figure 5.3 shows the cyclic voltammogram obtained from a 2.0 mmol dm^{-3} solution of $\text{Fe}(\text{bipy})_3(\text{ClO}_4)_2$ in aqueous solution at a range of pHs, at a sweep rate of 10 mV s^{-1} . The couple is found to have a constant half wave potential, at each pH, of $+832 (\pm 5) \text{ mV}$. Note the shift in the solvent limit and the increase in the formation of platinum oxide as the pH of the solution shifts towards neutral.

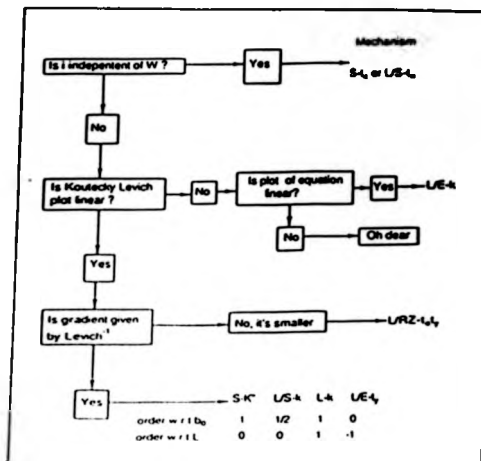


Figure 5.2: Diagnosis scheme for reaction mechanisms at a modified electrode, (after Albery and Hillman⁹).

$$\text{(equation: } (y_m / j)^2 = y_m / k_D \cdot (1 / k''_{HE})^2 \text{)}.$$

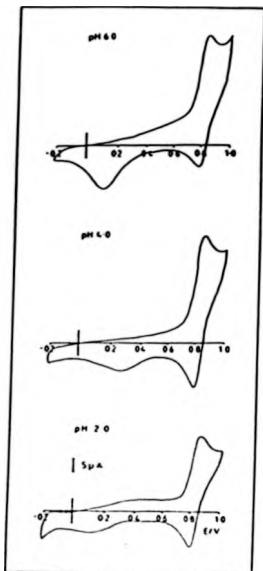


Figure 5.3: Cyclic voltammograms of 2.0 mmol dm⁻³ Fe(bipy)₃(ClO₄)₂ in buffered aqueous solution at a clean platinum electrode at a range of pH, (sweep rate = 10 mV s⁻¹).

The peak current of the couple is found to vary with the square root of sweep rate and a Levich plot for the couple is linear passing through the origin. These are shown in figure 5.4. This gives a calculated diffusion coefficient of $D = 1.76 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ and an estimate of D from cyclic voltammetry of $1.1 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$. Analysis of a polarogram by construction of a corrected Tafel plot gives a slope of 64 mV which indicates a reversible 1 electron process. In conclusion the redox couple $\text{Fe}(\text{bipy})_3(\text{ClO}_4)_2$ in buffered aqueous solution undergoes a outer sphere 1 e^- reversible oxidation at a clean platinum electrode.

5.3.1 POLY-5-CARBOXYINDOLE IN IRON (II) TRIS 2,2'-DIPYRIDYL PERCHLORATE SOLUTION

The electrochemistry of the couple $\text{Fe}(\text{bipy})_3(\text{ClO}_4)_2$ was studied using a polymer modified electrode.

Poly-5-carboxyindole films were grown on a platinum rotating disc electrode (area = 0.38 cm^2) by a potentiostatic method as previously described in section 3.2. Following growth the polymer film was transferred to buffered aqueous solution (see section 3.4).

5.3.2 CYCLIC VOLTAMMETRY OF IRON (II) TRIS 2,2'-DIPYRIDYL PERCHLORATE AT A POLY-5-CARBOXYINDOLE COATED ELECTRODE

Figure 5.5 shows typical cyclic voltammograms obtained from a 2.0 mmol dm^{-3} solution of $\text{Fe}(\text{bipy})_3(\text{ClO}_4)_2$ at pH 2.0 and pH 4.0. In

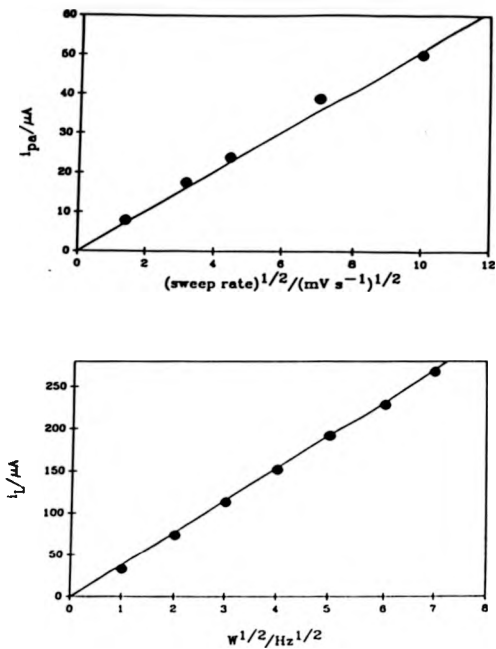


Figure 5.4: Sweep rate dependence of the couple $\text{Fe}(\text{bipy})_3(\text{ClO}_4)_2$ at a clean platinum electrode and Levich behaviour.

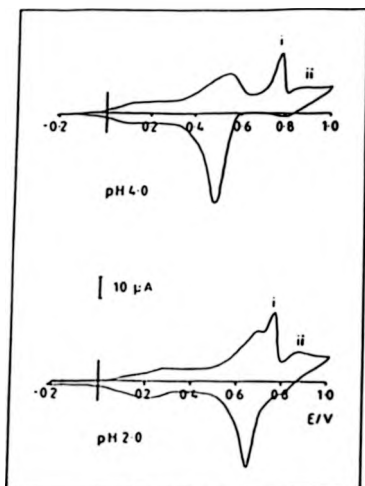


Figure 5.5: Cyclic voltammetry of $2.0 \text{ mmol dm}^{-3} \text{Fe}(\text{bipy})_3(\text{ClO}_4)_2$ in buffered aqueous solution at a poly-5-carboxyindole modified electrode (sweep rate = 2 mV s^{-1}).

addition to the peaks associated with the polymer two sets of redox peaks are seen (labelled {i} and {ii} in figure 5.5). We will consider the origin of these two processes separately starting with the redox peaks labelled {ii}. The couple labelled {ii} is found to have a half wave potential of +832 (± 5) mV. If a plot of i_{pa} versus sweep rate is made the peak current is found to vary linearly with the square root of sweep rate. Figure 5.6 shows the sweep rate dependence of the peak current at a range of film thicknesses. Some deviation from clean electrode behaviour is seen for thicker films. (Fairly good agreement is found between the clean electrode and the coated electrode). If the concentration of the couple is varied the currents obtained scale with concentration. The anodic peak current i_{pa} is not used as there is a merging of the two peaks {i} and {ii} at sweep rate $> 10 \text{ mV s}^{-1}$.

For the second couple labelled {i} the anodic peak current is found to vary linearly with sweep rate, indicating it is associated with a species which is not diffusion limited, possibly being bound, see figure 5.7. The cathodic wave is seen to move with the reduction peak of the polymer, which is pH dependent.

The anodic peak potential of this couple is found to be constant at +770 (± 5) mV over the range of pHs used (pH 2.0-6.0). The charge under the peak remains constant, as the pH of the solution is varied, at $1.52 (\pm 0.08) \text{ mC cm}^{-2}$. The value is also found to remain the same (1.52 mC cm^{-2}) when the concentration of $\text{Fe}(\text{bipy})_3(\text{ClO}_4)_2$ in solution is varied ($0.2\text{-}2.0 \text{ mmol dm}^{-3}$). These data are summarised in table 5.1 overleaf. If the thickness of the polymer

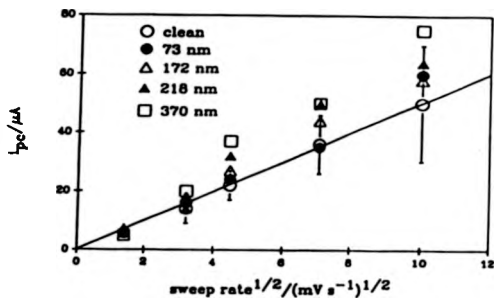


Figure 5.6: Sweep rate dependence of I_{pc} for couple (11) at poly-5-carboxyindole coated electrode in a $2.0 \text{ mmol dm}^{-3} \text{ Fe(bipy)}_3(\text{ClO}_4)_2$ solution at pH 4.0 over a range of different polymer thicknesses.

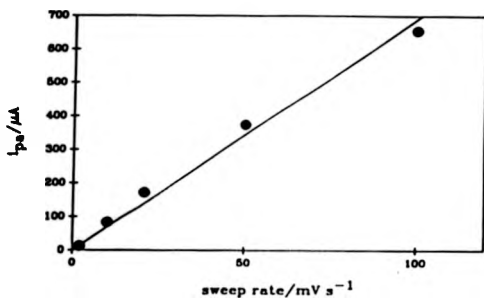


Figure S.7: Sweep rate dependence of i_p for couple (1) at a poly-5-carboxyindole modified electrode in a 2.0 mmol dm^{-3} $\text{Fe}(\text{bipy})_3(\text{ClO}_4)_2$ solution at pH 4.0.

Charge under anodic peak (bound) mC

pH	2 mmol dm ⁻³	0.9 mmol dm ⁻³	0.2 mmol dm ⁻³
2.0	0.55	-	-
4.0	0.59	0.54	0.61
6.0	0.60	-	-

Table 5.1 Charges under anodic peak for couple (1) when pH and couple concentration in solution are varied.

is varied the amount of charge under the anodic wave is found to increase linearly with film thickness as shown in figure 5.8.

5.3.3 DISCUSSION

From the results obtained from cyclic voltammetry it appears as if the couple $\text{Fe}(\text{bipy})_3(\text{ClO}_4)_2$ behaves in a dual manner with the polymer. If we consider the couple labelled (1), this couple seems to react at the surface of the polymer in a manner similar to a clean electrode. This is indicated by the fact that the half wave potential is found to be the same for the polymer coated electrode as that for the clean electrode, +832 (± 5) mV. Also the sweep rate dependence for the couple is found to be square root for both clean and polymer coated electrodes, with the slopes agreeing, indicating a solution species.

If we now consider the couple labelled (2), the voltammetry seems to show that the couple is located within the matrix of the polymer

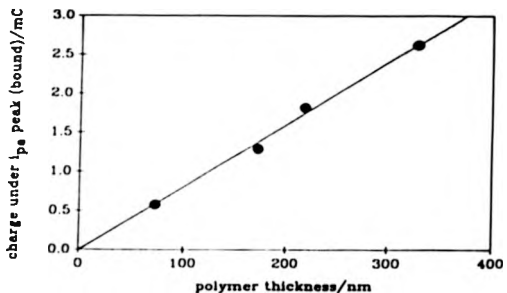


Figure 5.8: Charge under anodic wave for couple (1) versus polymer thickness at a platinum electrode coated with poly-5-carboxyindole films of various thickness at a sweep rate of 10 mV s^{-1} .

and reacts within this matrix. This is indicated by difference in anodic peak potential from the previous couple +770 (± 5) mV (cf. 865 ± 5 mV) and the fact that the sweep rate dependence is found to be linear, a characteristic of bound species. The peak exhibits no pH dependence over the range pH 2.0-6.0. If the coverage of the couple is calculated for a range of polymer thicknesses it is found to be constant at 1 molecule of $\text{Fe}(\text{bipy})_3(\text{ClO}_4)_2$ for every 12 monomer units of the polymer. As the charge under the peak remains constant over the concentration range 0.2-2 mmol dm⁻³ (for a given thickness) this suggests that this level of coverage is the saturation point for the polymer.

The shape of the anodic peak for couple (1) is unusual, an explanation for this may be found by considering the proposals of Feldberg and Rubenstein.⁹⁹ They describe similar shaped voltammograms obtained from a unusual quasi-reversible system, such as Prussian blue. Their explanation of the shape of the voltammogram is based on an n-shaped free energy relationship.

5.3.4 ROTATING DISC ELECTRODE STUDIES

Data obtained from clean rotating disc electrode studies of $\text{Fe}(\text{bipy})_3(\text{ClO}_4)_2$ holding the electrode at a potential of +960 mV (vs SCE) was initially analysed in terms of the Levich equation¹⁰⁰

$$i_L = 1.554nFAD^{2/3}\omega^{1/6}\nu^{1/2}c_{\infty} \dots \dots \dots 5.1$$

where

n = number of electrons involved

A = area of the electrode (cm²)

D = diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$)

ν = kinematic viscosity of aqueous medium ($\text{cm}^2 \text{s}^{-1}$)

ω = rotation speed (Hz)

C_{∞} = bulk concentration (mol cm^{-3})

If we construct a Levich plot (i_L vs $\omega^{1/2}$) for the couple $\text{Fe}(\text{bipy})_3(\text{ClO}_4)_2$ at the polymer coated electrode we obtain a curve (see figure 5.9), indicating that there is some kinetic step involved in the electron transfer reaction.

Further analysis of data was done using the Koutecky-Levich equation⁹

$$1/i_L = 1/k'DC_{\infty} + 1/nFAk'_{ME}C_{\infty} \dots \dots \dots 5.2$$

where

$k'D$ is mass transport rate constant⁹ given by equation ($k'D = 1.554nFAD^{2/3}\nu^{-1/6}\omega^{1/2}$)

k'_{ME} is the rate constant for the rate limiting process occurring at the modified electrode.

If we assume that this is through a film at the electrode surface then

$$k_{ME} = \frac{KD'}{1}$$

D' diffusion in the film

K coefficient for partition of the couple into the matrix of the film

If we now construct Koutecky-Levich (K-L) plots (i_L^{-1} vs $\omega^{-1/2}$) from the data we obtain a series of parallel lines having a slope given by Levich^{-1} , see figure 5.10. From the reciprocal of the

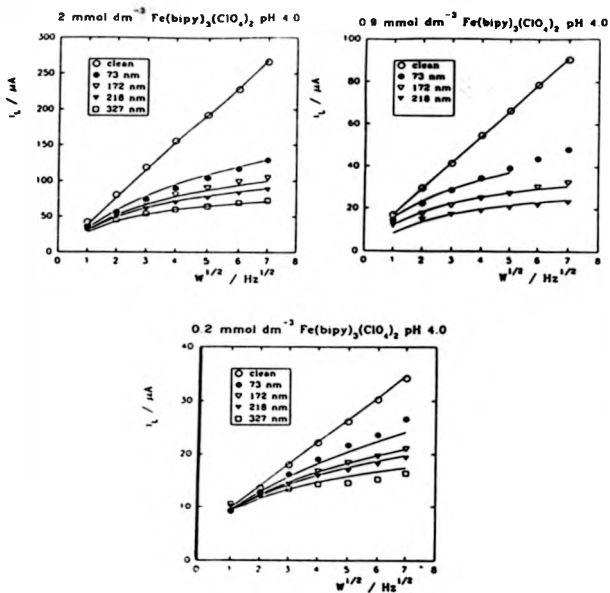


Figure 5.9: Levich plots for a range of film thicknesses at a range of concentrations for the couple $\text{Fe}(\text{bipy})_3(\text{ClO}_4)_2$, electrode potential = +960 mV (vs SCE).

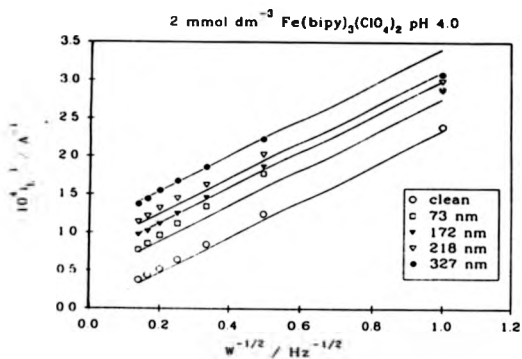


Figure 5.10: Koutecky-Levich plot for a range of polymer thicknesses
in a 2.0 mmol dm^{-3} solution of $\text{Fe(bipy)}_3(\text{ClO}_4)_2$ at pH 4.0.

intercepts of this plot we find that the electrochemical rate constant for the polymer modified electrode, k'_{ME} , decreases with increasing film thickness.

Overall we find that the current is dependent on W , $K-L$ plots being linear with the gradient being given by Levich^{-1} . Using the diagnostic table in figure 5.2 this leads us to 4 possible reaction sites these being $S-K''$, $L/S-k$, $L-k$, $L/E\text{-ty}$. As k'_{ME} is found to vary inversely with film thickness, this gives the reaction site as the film /electrode interface, $L/E\text{-ty}$.

Figure 5.10 shows the $K-L$ behaviour; the symbols represent experimental data and the lines are computer generated using a curve-fitting routine (Jandel Sigma plot 4.0). As can be seen the computer generated fit tends to diverge from the experimental data at low rotation speeds. This is due to the fact that the computer routine puts too much weight on the fit for low rotation speeds (which will be the least accurate). Consequently a least squares analysis approach was then applied. A two-parameter fit for i_L was performed (film thickness and rotation speed allowing for some background current) at each concentration to the following equation

$$i_L - i_{bg} = \frac{1}{\frac{1}{k'D W^{-1} W^{1/2} C_{\infty}} + \frac{1}{D'K C_{\infty}}} \quad \dots 5.3$$

$$(i_L = \{(A/W^{1/2}) + (BxL)\}^{-1} + C)$$

where

$k'D W^{-1}$ is the mass transport rate constant at a electrode rotation speed of 1 Hz

and

$$A = \frac{1}{k'D \omega - 1 C_{\infty}} \quad B = \frac{1}{D'K C_{\infty}} \quad C = i b g$$

Table 5.2 summarises the computer generated results for parameters A and B.

parameter A

[] mmol dm ⁻³	Value $\mu A^{-1} Hz^{-1/2}$	S.D.	C.V. %
2.0	2.61×10^{-2}	1.71×10^{-2}	0.65
0.9	8.10×10^{-2}	1.59×10^{-3}	1.96
0.2	2.42×10^{-1}	5.77×10^{-3}	2.38

parameter B

[] mmol dm ⁻³	Value $\mu A^{-1} nm^{-1}$	S.D.	C.V. %
2.0	5.72×10^{-4}	8.80×10^{-6}	1.54
0.9	2.20×10^{-3}	1.20×10^{-7}	5.60
0.2	2.86×10^{-3}	1.60×10^{-4}	5.61

Table 5.2: Computer generated data for parameters A and B.

S.D. is the standard deviation and C.V. is defined as the standard deviation divided by the parameter value and expressed as a percentage.

The intercepts $C / \mu A$ were as follows:-

for 2 mmol dm^{-3} - none

0.9 mmol dm^{-3} - 4.5 (clean)

5.3 (73 nm)

2.6 (172 nm)

0.9 (218 nm)

0.2 mmol dm^{-3} - 5.79

(data for the thickest film (327 nm) has been omitted for the concentration 0.9 mmol dm^{-3}).

From parameter A we can calculate a value for the diffusion coefficient of the couple in solution. In this way we obtain a value of $D = 4 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$. From parameter B a value for k_D' may be calculated, this is found to be $k_D' = 2.8 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$, which compares to values of $1.3 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$ for Nafion.¹⁰¹

The values obtained are reasonable although the diffusion coefficient for the couple in solution is a little high compared to the value obtained from cyclic voltammetry ($D = 1.1 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$).

The results obtained from cyclic voltammetry and rotating disc studies of the redox couple seem to be contradictory with evidence of two sites for reaction. The evidence suggests that peak (i) corresponds to a tightly bound species 'within' the film, hence the shift in anodic peak potential. Peak (ii) corresponds to a species which is able to diffuse through the porous film and react at the electrode, hence no shift in anodic peak potential is observed. This suggests that the polymer is probably insulating at this potential (+960 mV) which would be consistent with the structure proposed for the polymer in a fully oxidised state in chapter 3.

5.4 POTASSIUM FERRICYANIDE AT A CLEAN PLATINUM ELECTRODE

Solutions of potassium ferricyanide were made up using buffered aqueous solution (pH 6.0) containing 0.1 mol dm^{-3} sodium chloride as background electrolyte. Solutions were degassed thoroughly before use.

The couple is found to have a half wave potential of $+192 (\pm 5) \text{ mV}$. The peak current of the couple varies with the square root of sweep rate and a Levich plot gives a straight line passing through the origin, as expected for an outer sphere reaction. A value for the diffusion coefficient at a clean platinum electrode is found to be $D = 6.4 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$.

5.4.1 POTASSIUM FERRICYANIDE AT A POLY-5-CARBOXYINDOLE COATED ELECTRODE

The cyclic voltammetry of the couple at a polymer coated electrode is similar to that at a clean electrode. However there are some minor differences; at slow sweep rates $< 50 \text{ mV s}^{-1}$ a broadening of the anodic wave is seen and the cathodic wave splits. Figure 5.11 shows a comparison of the cyclic voltammograms obtained from a clean and a polymer coated electrode. At a polymer coated electrode the half wave potential of the couple is found to be $+197 (\pm 5) \text{ mV}$. The sweep rate dependence is found to be linear changing to square root with increasing sweep rate. The peak currents obtained are also

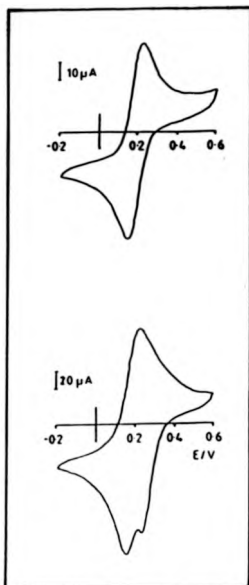


Figure 5.11: Comparison of the cyclic voltammograms obtained for a clean and a polymer coated electrode in a 2.0 mmol dm^{-3} solution of $\text{K}_3\text{Fe}(\text{CN})_6$ at pH 6.0, for a sweep rate of 10 mV s^{-1} , film thickness = 360 nm.

found to be substantially larger than those at the clean electrode. Rotating disc electrode studies were done at a range of polymer thicknesses and over a concentration range of 2.0-11.0 mmol dm⁻³ K₃Fe(CN)₆. A typical Levich plot at a concentration of 9.0 mmol dm⁻³ is shown in figure 5.12. Koutecky-Levich plots are linear with the slope being given by Levich⁻¹.

5.4.2 DISCUSSION

Cyclic voltammetry of the couple at a polymer coated electrode suggests that the reaction is taking place at the surface of the polymer. This is indicated by the half wave potential being the same as at a clean electrode +197 (±5) mV (cf. +192 (±5)).

Although the cyclic voltammograms differ this is thought to be due to an adsorbed layer of ferricyanide on the surface of the polymer (see section 5.5.6). The higher currents obtained for the coated electrode are probably due to the surface roughness of the polymer offering a larger surface area for the couple to react at in comparison to a clean electrode.

Levich plots from rotating disc work (electrode potential = -100 mV vs SCE) are found to be curves indicating some kinetic parameter is involved in the electron transfer process. Figure 5.12 shows a typical set of data for a couple concentration of 9.0 mmol dm⁻³. Symbols represent experimental data and the lines are a computer generated fit, which were obtained by fitting the experimental data to the following equation.

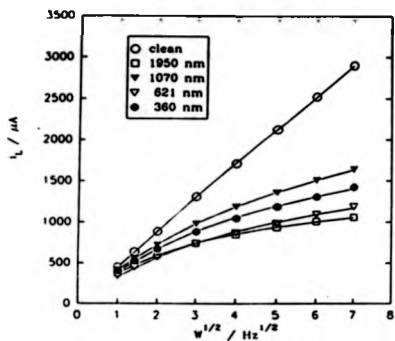


Figure 5.12: Levich plots for a range of polymer thicknesses in a 9.0 mmol dm^{-3} solution of $\text{K}_3\text{Fe}(\text{CN})_6$ at pH 6.0.

From equation 5.3 when $i_{bg} = 0$ then

$$i_L = \frac{k'D W^{-1} W^{1/2} c_{\infty}^2 D'K}{DKc_{\infty} + k'D W^{-1} W^{1/2} c_{\infty}} \quad \text{..... 5.4}$$

A series of slopes is obtained from the computer fit the value for the slope is found to be constant over the range of film thicknesses investigated. The results are summarised in table 5.3.

Polymer thickness nm	Slope $\mu A^{-1} Hz^{-1/2}$	S.D.	C.V. %
CLEAN	455	2.1	0.47
360	460	9.4	2.04
621	388	10.7	2.75
1070	468	3.9	0.83
1950	484	5.3	3.12

Table 5.3: Summary of data obtained from computer fit for poly-5-carboxyindole in a 9.0 mmol dm^{-3} solution of $K_3Fe(CN)_6$ at pH 6.0.

A mean value for the slope of $451 \pm 6.3 \mu A^{-1} Hz^{-1/2}$ is found, giving a value for the diffusion coefficient of $K_3Fe(CN)_6$ in solution of $D = 1.59 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$. Koutecky-Levich plots give straight lines (slope = Levich⁻¹) and the intercepts k'_{ME} are found to be independent of film thickness, see figure 5.13. Using the

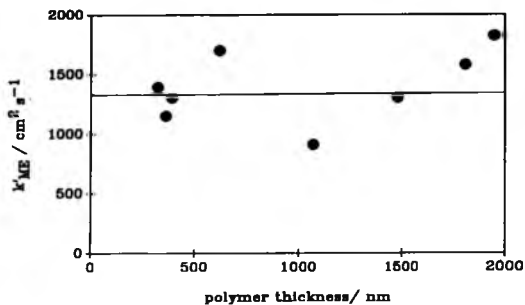


Figure 5.13: k'_{ME} versus film thickness for a 9.0 mmol dm^{-3}
solution of $\text{K}_3\text{Fe}(\text{CN})_6$ at pH 6.0

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5.5 EFFECT OF $\text{NH}_2-(\text{CH}_2)_N-\text{NH}_2$ ON POLY-5-CARBOXYINDOLE

As we believe from experiments with cytochrome *c* that the polymer is able to coordinate to $-\text{NH}_2$ groups it was decided to investigate the effects of diamine groups on the electrochemistry of the polymer. Diamines were chosen as the distance between the NH_2 groups of propane 1,3-diamine was calculated to be similar to that for the $\text{CO}_2^- - \text{CO}_2^-$ distance on the polymer assuming that the polymer has the structure proposed by Waltman *et al.*¹⁵

5.5.1 COMPLEXATION OF POLY-5-CARBOXYINDOLE WITH PROPANE 1,3-DIAMINE

Solutions of propane 1,3-diamine were made up with aqueous buffer at pH 7.0. It is important to ensure that the pH of the solution is kept at 7.0 since the polymer is pH sensitive and at this pH the polymer will possess deprotonated carboxylate groups, which is consistent with the results shown in chapter 4.

Poly-5-carboxyindole was grown in the usual manner and stabilised in aqueous buffer at pH 7.0. The polymer was then transferred to a solution containing propane 1,3-diamine.

Figure 5.14 shows the cyclic voltammograms obtained from the polymer in buffer and in propane 1,3-diamine solution. The outstanding feature is the loss of the peaks associated with the redox activity of the polymer. The cyclic voltammogram eventually looks like that of a clean platinum electrode.

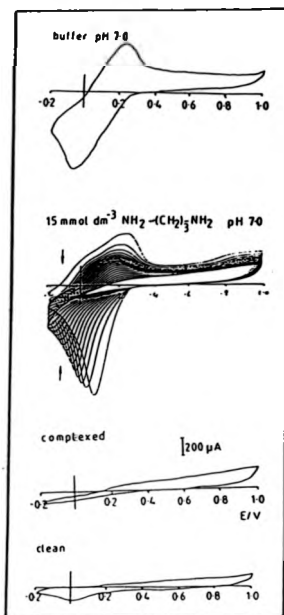


Figure S.14: Complexation of poly-5-carboxyindole with 15 mmol dm⁻³ NH₂-(CH₂)₃-NH₂ solution at pH 7.0.

However the redox peaks of the polymer may be restored if the polymer is transferred to buffer solution at pH 5.0. Figure 5.15 shows the cyclic voltammograms for the polymer in pH 7.0 buffer before complexation, after complexation and on transfer to pH 5.0 buffer. If the pH of the solution containing the propane 1,3-diamine is changed from 7.0 to 5.0 then no effect on the electrochemistry of the polymer is seen, complexation does not occur.

5.5.2 EFFECT OF CONCENTRATION OF PROPANE 1,3-DIAMINE

If the concentration of the propane 1,3-diamine solution is varied the only difference observed is in the time taken for the polymer to become complexed (so that the resultant cyclic voltammetry resembles that for a clean electrode). For a 15 mmol dm^{-3} solution complexation takes approximately 8 minutes compared to 30 minutes and 80 minutes for 1.5 mmol dm^{-3} and $0.15 \text{ mmol dm}^{-3}$ solutions respectively.

5.5.3 VARIATION OF $-(\text{CH}_2)_n-$ CHAIN LENGTH

If the chain length of the $\text{NH}_2-(\text{CH}_2)_n-\text{NH}_2$ molecule is varied ($n = 2, 3$ and 6) identical results are obtained for each n value.

However if the polymer is placed in ammonium chloride (NH_4Cl) solution at pH 7.0 no effect is found on the redox activity of the polymer.

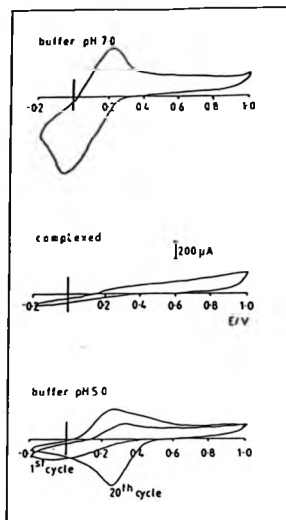


Figure S.15: Complexation of poly-5-carboxyindole with 15 mmol dm^{-3} $\text{NH}_2\text{-(CH}_2\text{)}_3\text{-NH}_2$ solution at pH 7.0 and regeneration of the redox peaks of the polymer in pH 5.0 buffer.

5.5.4 POLY-5-CARBOXYINDOLE COMPLEXED WITH PROPANE 1,3-DIAMINE IN POTASSIUM FERRICYANIDE SOLUTION

Results obtained with a complexed polymer in a 2.0 mmol dm^{-3} $\text{K}_3\text{Fe}(\text{CN})_6$ solution at pH 6.0 were found to be the same as those for a polymer that had not been complexed. The half wave potential was found to be $+195 (\pm 5) \text{ mV}$ (cf. $+197 (\pm 5) \text{ mV}$). The sweep rate dependence of the anodic peak current was also found to go from linear to square root with increasing sweep rate. Figure 5.16 shows a comparison of i_{pa} versus sweep rate for at a normal polymer film and a complexed polymer film of equivalent thickness.

5.5.5 DISCUSSION

If we consider the cyclic voltammetry of the complexed polymer we find the redox peaks associated with the oxidation/reduction of the polymer disappear. The polymer is still conducting; studies of potassium ferricyanide are found to be identical for those of a uncomplexed polymer.

An explanation for this may be found by considering the pK_a^{102} of the complexing molecules. These are given in table 5.5 overleaf.

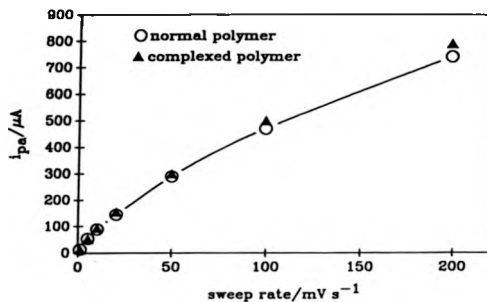
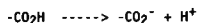


Figure S.16: Sweep rate dependence for a normal polymer and a complexed polymer in a 2.0 mmol dm^{-3} solution of $\text{K}_3\text{Fe}(\text{CN})_6$ at pH 7.0.

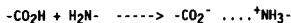
Complexing molecule	pKa
Ethylene diamine	10.7
Propane 1,3-diamine	10.9
Hexamethylene diamine	11.8

Table 5.5: pKa values for diamine molecules used to complex the polymer.

An explanation for the behaviour observed when poly-5-carboxyindole is complexed with a diamine molecule is to assume that the diamine molecules will be able to diffuse into the matrix of the polymer and H-bond to the carboxylate groups of the polymer. This can be thought of as analogous to the protonation of the carboxylic acid group.



and



Due to the basic nature, shown by the pKa, of the molecules they will have the effect of altering the pH of the internal environment of the polymer to a more basic pH. The fact that the diamine molecules are capable of being bound to the polymer, crosslinks the polymer and so prevents the physical dissolution of the polymer at this more basic pH. Due to the pH sensitivity of the polymer the redox peaks will consequently be shifted more negative, and out of

the region shown in the cyclic voltammograms. Thus the cyclic voltammetry of the polymer now resembles that for a clean electrode.

If we now consider the complexed polymer in potassium ferricyanide solution more closely, we find no differences between complexed and uncomplexed polymer. However it becomes apparent with a complexed polymer that the splitting of the cathodic wave, seen in the cyclic voltammetry (see figure 5.11), cannot be due to the redox activity of the polymer as there are now no polymer peaks. As suggested in section 5.4.2 the additional peak seems to be due to pre-adsorption of the redox couple. Evidence for this is found by varying the thickness of the polymer. We then find that the charge under this additional peak remains fairly constant, see figure 5.17. Values for a range of polymer thicknesses are given in table 5.6 overleaf. An average value of 0.72 mC cm^{-2} is found. By making an estimate for the size of the ferricyanide molecule a coverage can be estimated. Using values of $3.99 \times 10^{-10} \text{ m}$ for the C-N distance¹⁰³ and taking the diameter of Fe^{3+} to be $1.308 \times 10^{-10} \text{ m}$ ¹⁰⁴ and assuming the molecule to be roughly spherical, a coverage of approximately 78 monolayers is found. It seems reasonable to assume that this arises from the surface roughness. This estimate for the surface roughness of the polymer is found to be greater here than with cytochrome c. This is only to be expected as ferricyanide is a much smaller molecule and will be able to access the convoluted surface of the polymer more easily.

Complexation of the polymer with a diamine molecule is found to have no effect on the conductive nature of the polymer.

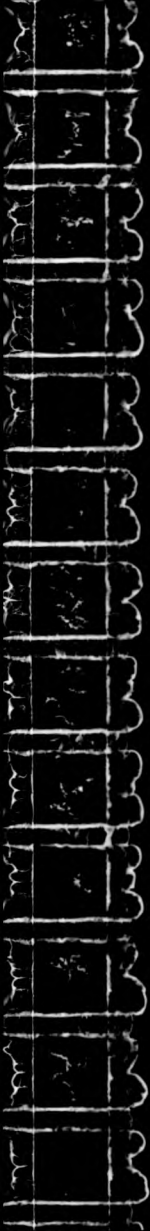
$Q / \mu\text{C cm}^{-2}$	L / nm
0.72	360
0.76	350
0.80	330
0.84	280
0.64	200
0.55	90

Table 5.5: Charge under pre-adsorption peak for potassium ferricyanide at different polymer thicknesses.

5.6 OVERALL CONCLUSIONS

Overall the polymer is found to exhibit two types of behaviour with the redox couples studied.

Firstly, with $\text{Fe}(\text{bipy})_3(\text{ClO}_4)_2$, the evidence suggests that there are two species. Peak (1) is associated with a tightly bound species within the polymer. This is supported by the fact that the anodic peak potential is shifted to a value where the species is easier to oxidise and the sweep rate dependence of the current for this peak is found to be linear and also by the fact that the amount of charge associated with this bound species remains constant on variation of the couple concentration in solution for a given



polymer thickness, suggesting saturation of available sites within the polymer.

Peak (11) is associated with a species that can diffuse through the porous polymer and react at the electrode surface this species will be positively charged. This is possible as the polymer will be negatively charged. Also the polymer may possibly be in an insulating form after the second redox process of the film allowing the species to diffuse through easily. The value found for KD' is in the order $10^{-8} \text{ cm}^2 \text{ s}^{-1}$ which is higher than that reported for Nafion $10^{-9} \text{ cm}^2 \text{ s}^{-1}$ a commonly used electroinactive coating. This is not surprising as the polymer will be far more porous in nature.

Secondly, for $K_3\text{Fe}(\text{CN})_6$, electron transfer is found to occur at the layer/surface; indicating that electrons pass through the film more easily than the couple may penetrate the film. This seems reasonable for at the working pH (6.0) of these experiments the polymer will be predominantly deprotonated, thus possessing a negative charge. It seems unlikely that the redox couple, which is also negatively charged, will be able to easily penetrate the film. However as a pre-adsorption wave is found in the cyclic voltammetry at a polymer modified electrode, this seems to indicate that the polymer is generally porous in nature.

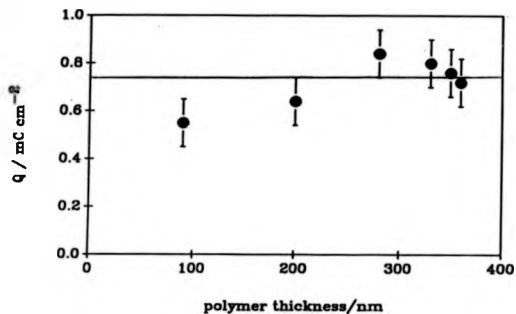


Figure 5.17: Charge found under pre-adsorption peak of $K_3Fe(CN)_6$ for a range of polymer thicknesses.

CHAPTER 6

6.1 GENERAL CONCLUSIONS

Some of the properties of the conducting polymer poly-5-carboxyindole have been investigated.

We have found that the polymer exhibits a remarkable pH dependence in aqueous solutions. This could be exploited to produce a pH sensor although a drawback at the present time is the solubility of the polymer in solutions of $> \text{pH } 7.0$. Further work to combat this problem is suggested either using the approach of 'coating' the polymer with a poly-phenol to prevent dissolution; or by exploring the possibility of using co-polymers of poly-indole and poly-5-carboxyindole.

The processes involved in the switching reaction of the polymer have been shown to be highly complex and somewhat different to other conducting polymers. Although a possible explanation has been proposed further work is needed in this area. The effect of varying the ionic strength of the solution is an obvious starting point for future investigations.

The polymer has been demonstrated to mediate the electron transfer of a biological redox protein, cytochrome c. Investigations of other such biological molecules should be undertaken. The range of molecules that may be studied can be increased with further work on the coupling of modifier groups to the parent monomer/polymer. The approach of coupling a modifying group to the polymer may prove more

successful than the approach used in this study of coupling to the monomer prior to polymerisation.

Finally the probing of the mechanism of electron transfer between the film and solution species has shown that the polymer behaves like a metal electrode in one respect (with $K_3Fe(CN)_6$) but also that the structure of the polymer has a influence on the site of reaction, indicating a porous nature to the polymer. Future investigation should include closer examination of the surface morphology of the polymer using electron microscopy and the use of a greater range of redox couples.

In general this class of materials exhibit many properties that have yet to be fully elucidated and should keep investigators busy for many years to come.

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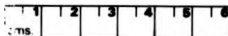
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