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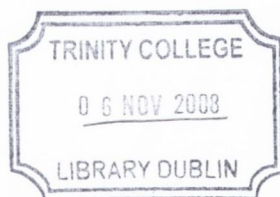
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The Electroanalytical Applications of Carbon Nanotube-Modified Electrodes

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A Thesis submitted to
the University of Dublin
for the degree of
Doctor of Philosophy

School of Chemistry
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May 2008

Thesis
8621

Declaration

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A handwritten signature in cursive script that reads "Gareth Keeley". The letters are fluid and connected, with a prominent 'G' and 'K'.

Gareth Keeley

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Tiomnú

Tá an tráchtas seo tiomnaithe ina iomláine ar son m'athair, Charles Keeley (1923-2005), duine a thug spreagadh dom i ngach uile rud a rinne mé, go háirithe mo chuid staidéir. Fad a mhair sé, chreid m'athair in obair dian agus an rud ceart a dhéanamh. Is iad na smaointí seo a bhfuil mar ionspráid dom i ngach gné den shaol agus mairfidh siad liom go brách.

Abbreviations and Acronyms

AA	ascorbic acid
AFM	atomic force microscopy
AC	alternating current
bpHOPG	basal-plane highly-ordered pyrolytic graphite
BCNT	'bamboo' carbon nanotube
CNT	carbon nanotube
CNTPME	carbon nanotube powder microelectrode
CV	cyclic voltammetry
CVD	deoxyribonucleic acid
DNA	epinephrine
EP	edge-plane highly-ordered pyrolytic graphite
epHOPG	electron transfer
ET	multi-walled carbon nanotube
FAD	ferrocene carboxylic acid
FCA	Fourier transform infrared spectroscopy
FTIR	glassy carbon electrode
MWCNT	glucose oxidase
GOx	high performance liquid chromatography
HPLC	horseradish peroxidase
HRP	linear sweep voltammetry
LSV	flavin adenine dinucleotide
GCE	glassy carbon electrode
GOx	glucose oxidase
HPLC	high performance liquid chromatography
HRP	horseradish peroxidase
LSV	linear sweep voltammetry
MWCNT	multi-walled carbon nanotube
NADH	nicotinamide adenine dinucleotide
NE	norepinephrine
NLLS	non-linear least squares
NPs	nanoparticles
ORR	oxygen reduction reaction
PPy	polypyrrole
RDS	rate-determining step
R-S	<i>Randles-Sevcik</i>
SAM	self-assembled monolayer
SCE	transmission electron microscopy
SEM	scanning electron microscopy
STM	scanning tunneling microscopy
SWCNT	single-walled carbon nanotube
TEM	saturated calomel electrode

Summary

In this study, cyclic voltammetry, potential step chronoamperometry, batch amperometry, hydrodynamic voltammetry and electrochemical impedance spectroscopy were used to investigate the physical and chemical properties of glassy carbon macro-electrodes modified using HiPCo[®] single-walled carbon nanotubes. The latter were themselves characterised using atomic force microscopy, infrared and Raman spectroscopy and thermo-gravimetric analysis. The electrochemistry of redox-active functionalities present on the nanotube surface was probed. The modified electrodes were used to determine ferrocyanide, dopamine, epinephrine, norepinephrine, ascorbic acid, NADH, oxygen and hydrogen peroxide. Comparisons between the electro-catalytic properties of nanotubes and conventional graphite were also performed. The suitability of a number of different solvents for the dispersion and casting of nanotubes was evaluated.

The SWCNT-modified electrodes were further modified using the redox enzyme glucose oxidase and the nanotube / enzyme composites were used to study the direct electron transfer of the enzyme. Several kinetic models were evaluated and developed with a view to the calculation of rate constants for the redox process. Particular significance was attributed to the ability of potential step techniques to identify dispersions in kinetic activity. The performance of the GC/SWCNT/GOx ensembles as glucose biosensors was evaluated, using both ferrocene carboxylic acid and oxygen to mediate the electron transfer.

Publications and Presentations

Peer-Reviewed Articles

Carbon nanotube based modified electrode biosensors. Part 1. Electrochemical studies of the flavin group redox kinetics at SWCNT/Glucose oxidase composite modified electrodes.

M.E.G. Lyons, G.P. Keeley, *Int. J. Electrochem. Sci.* **3** (2008) 819.

Immobilised Enzyme / Single-Walled Carbon Nanotube Composites for Amperometric Glucose Detection at Very Low Applied Potential

M.E.G. Lyons, G.P. Keeley, *Chem. Commun.* (2008) 2529.

The Redox Behaviour of Randomly Dispersed Single-Walled Carbon Nanotubes both in the Absence and in the Presence of Adsorbed Glucose Oxidase

M.E.G. Lyons, G.P. Keeley, *Sensors* **6** (2006) 1791.

Oral Presentations

A Peroxide Biosensor based on Carbon Nanotube-Modified Electrodes

Trinity College Departmental Seminar (2007)

Direct Electrochemistry of Glucose Oxidase at Electrodes modified with Carbon Nanotubes

58th Irish Universities Chemistry Colloquium, NUI Galway (2006)

Poster Presentations

Direct Electrochemistry of Glucose Oxidase at Carbon Nanotube-Modified Electrodes

Electrochem 2005, Northumbria University

Electrochemistry at Carbon Nanotube-Modified Electrodes

57th Irish Universities Chemistry Colloquium, NUI Maynooth (2005)

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Chapter One: Introduction to Carbon Nanotube Electrochemistry

This opening chapter will begin with a description of glassy carbon, the electrode material on which carbon nanotubes were deposited in this work. It will then move on to a basic introduction to nanotubes and a brief mention of their applications in areas beyond the realm of electrochemistry, before focussing on the importance of nanotubes in electroanalysis. The main aim of the chapter is to review the considerable body of literature (chiefly from the last ten years) which exists on the subject of nanotube electrochemistry, and in doing so place the work in a context which will justify the motives behind it. Broadly speaking, work in this area can either involve the use of nanotube electrodes to analyse redox species in solution, or the use of nanotube electrodes further modified by adsorbed species (typically enzymes) in order to investigate the direct electron transfer of the latter or to use the composite systems as amperometric biosensors. In the writing of this introduction, reviews by Wang [1] and Gooding [2] have proved to be very useful sources.

1.1 Glassy Carbon Electrodes

Carbon electrodes are used widely in electroanalysis due to their low background currents, wide potential windows, chemical inertness and low cost [3]. Glassy carbon is a mechanically stable, compact solid. It is comprised of interwoven ribbons of graphite (each a few nm wide) and a representation of its structure is shown in Figure 1.1.

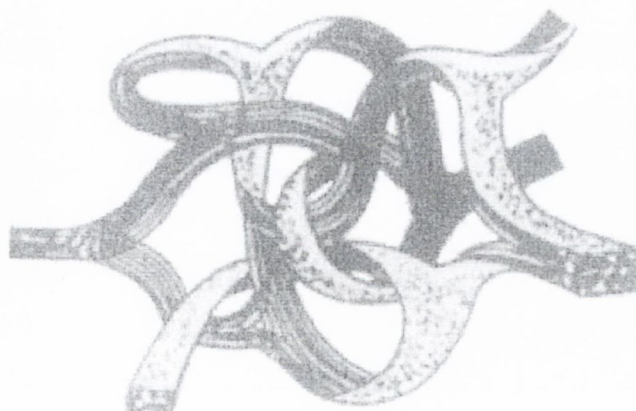


Fig. 1.1. The structure of GC, consisting of interwoven graphite ribbons. Reproduced from [37].

Glassy carbon is considerably harder than conventional graphite and is impermeable to gases and fluids. In electrochemical studies involving carbon nanotube-modified electrodes, glassy carbon is the most commonly selected electrode because it interacts with nanotubes *via* van der Waals forces [4], yielding arrays which are sufficiently robust for electrochemical measurements and yet can be easily removed by simple abrasion to restore the electrode to its original condition. Like any electrodes composed of graphitic carbon, glassy carbon electrodes have been shown to offer the possibility of decoration with oxygen-containing surface groups, notably carboxylic acids, alcohols and quinones [5].

1.2 Carbon Nanotubes

One of the many unusual and interesting features of carbon nanotubes is the confusion which prevails concerning who discovered them! Virtually all papers cite the work in 1991 of Iijima [6], and credit him with their discovery. However, carbon fibres as small as 4 nm in diameter (*viz.* nanotubes) were found on a graphite electrode by Wiles and Abrahamson [7] in 1978. It is fair to say though that the explosion of interest in these structures did not occur until after their *identification* by Iijima. This frenzy of research has indeed been dramatic, and at the time of writing there are well over fifteen thousand papers in the literature involving these remarkable structures. This interest has been due chiefly to their unique structural, mechanical and electronic properties. These properties include high chemical and thermal stability, high elasticity, high tensile strength and, in certain cases, metallic conductivity. This conductivity makes nanotubes suitable for use as molecular wires [8]. With diameters as low as less than one nanometre [9], they are the smallest available electrodes. Metallic CNTs can carry phenomenal current densities of up to 100 MA cm^{-2} , making them over one hundred times more conductive than copper.

Carbon nanotubes can be thought of as graphite sheets of sp^2 -hybridised carbon atoms rolled up into seamless, hollow tubes (see Figure 1.2.1). These may be capped at the ends by fullerene-like hemispheres and have lengths ranging from tens of nanometres to several microns [10]. Broadly speaking, nanotubes may be classified as either single-walled carbon nanotubes (SWCNTs) or multi-walled carbon nanotubes (MWCNTs). The former are single, hollow tubes with diameters between 0.4 and 2 nm, while the latter are

concentric tubes, 0.34 nm apart [10,11]. The average diameter of a multi-walled nanotube is around fifteen nanometres.

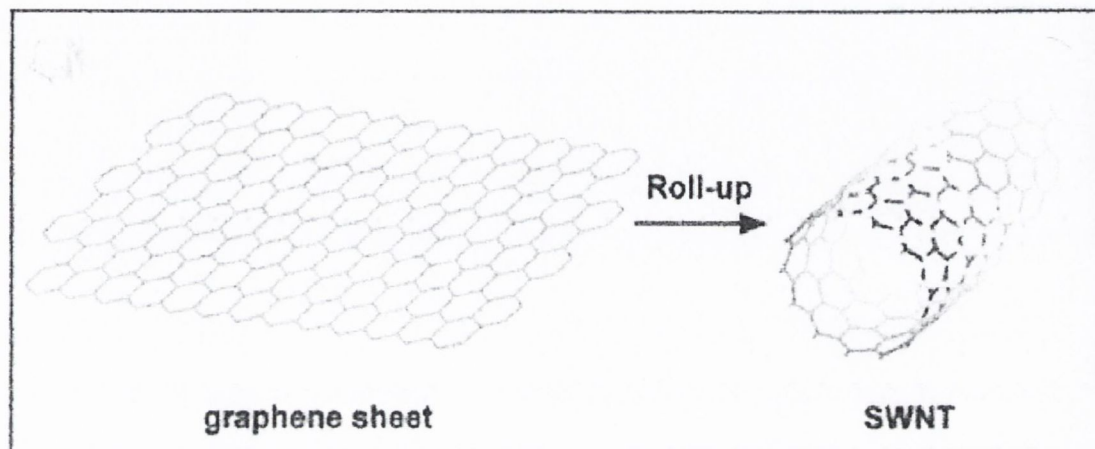


Fig. 1.2.1. The graphite structure of carbon nanotubes. Reproduced from [12].

Nanotubes are usually produced either by the carbon arc discharge process or by chemical vapour deposition. The former involves the striking of an arc between graphite electrodes in a hydrogen or helium atmosphere. The result is that the carbon anode is deposited at the carbon cathode in the form of nanotubes [13]. Chemical vapour deposition consists of the decomposition of various hydrocarbons (commonly ethene) under an inert atmosphere over either an iron/graphite, cobalt/graphite or iron/silica catalyst. It has been shown [14] that the size of the metal catalyst particles determines the diameter of the tubes. With both arc and CVD, defects in the nanotube structure are commonly observed [15]. These defects are believed to play a crucial role in both physical and chemical interactions with other species and will be referred to many times throughout this thesis.

Another method of nanotube synthesis which has become popular in recent years is the HiPCo[®] (high pressure carbon monoxide) process. This involves the rapid mixing of a gaseous catalyst precursor (such as iron carbonyl) with a flow of carbon monoxide in a chamber at high temperature and pressure. The precursor decomposes and metal nanoparticles form from the decomposition products. These NPs serve as a catalyst for nanotube growth. On their surfaces carbon monoxide reacts to form CO₂ and carbon atoms, which combine to form nanotubes. This process produces single-walled nanotubes

only. Purification of the nanotubes is done by treatment with strong acid, which introduces surface oxides to the nanotube surfaces at defect sites. The SWCNTs used in this work were produced using this approach (see *Section 2.8*). It should be pointed out that as yet there is no consensus on the mechanism by which nanotubes grow. The experimental conditions under which synthesis is performed are too chaotic to permit meaningful fitting to existing growth models [11].

Carbon nanotubes have been used as tips for AFM and STM [16]. For this purpose they offer a number of advantages over ‘traditional’ metal and silicon tips. Firstly, their small diameters and high aspect ratios allow them to penetrate deep crevices and trench structures. Secondly, their ability to buckle elastically means that the probe has a reduced impact on the sample, which lowers the risk of deformation or damage to biological or organic subjects. Finally, nanotube ends may be modified to create functional probes [17]. The latter can be used for molecular recognition and chemically sensitive imaging in chemistry and biology.

The conductivity of nanotubes, of course, is of particular interest to electrochemists. Multi-walled nanotubes are regarded as entirely metallic conductors and as such are highly attractive electrode materials. Single-walled nanotubes can be either metallic or semiconducting, depending on their diameter and chirality [10-12]. In the context of nanotubes, the term ‘chirality’ refers to the angle at which the graphite sheets are ‘rolled up’ and hence the alignment of the carbon atoms’ π -orbitals. In a typical sample of SWCNTs, around two thirds are found to be semiconducting, with the remainder exhibiting metallic conductivity. This variation in conductivity might create the impression that single-walled nanotubes are less suitable electrode materials than their multi-walled counterparts. However, SWCNTs are more well-defined systems and their electrochemical behaviour is easier to understand. The various components of a MWCNT have different chiralities and this complicates the interpretation of its electron transfer characteristics. Over the years, a number of methods have been reported by which metallic and semiconducting SWCNTs may be separated by utilisation of their dielectric properties [18], reactivity [19,20] and more recently, by centrifugation [21]. These attempts have met with various degrees of success and have yet to be adopted by other workers.

Kong *et al.* [22] demonstrated that the conductivity of semiconducting single-walled nanotubes could be substantially increased or decreased by exposure to gaseous NO₂ or NH₃, respectively. Another paper by this group [23] showed that molecular hydrogen sensors could be fabricated based on SWCNTs decorated with palladium nanoparticles. These sensors were found to have a fast response time and high sensitivity. Collins and co-workers [24] have reported that semiconducting SWCNTs could be converted to metallic nanotubes through the adsorption of oxygen at room temperature. These workers found that the conductivity of the nanotubes could be decreased by up to fifty per cent if adsorbed oxygen was completely removed. This is indeed an interesting observation, and these authors warn against attributing unique properties to nanotubes without thorough investigation of the effects of other species.

The methods by which carbon nanotubes are synthesised and purified greatly affect their conductivity and hence their suitability for electrochemical applications. Nanotubes produced by chemical vapour deposition are usually purified by treatment with strong acid, which causes the reactive, highly-strained caps to be removed, resulting in open-ended tubes. These open ends are paramount to the electrochemical performance of the nanotubes. Such nanotubes contain either dangling bonds in organic solvents [25] or oxygenated functional groups such as quinones or carboxylic acids in polar solvents [26]. It has been shown [27,28] that the electrochemistry of nanotube-modified electrodes and the rate of electron transfer are greatly influenced by these surface oxides. It should also be pointed out that, while the latter are paramount to the electroanalytical applications of CNTs, their presence might also cause a decrease in nanotube conductivity because when they form, the hybridisation of the carbon atoms involved changes from sp^2 to sp^3 . It is likely, however, that in most samples, the amount of tetrahedral carbon atoms would not be large enough to significantly reduce conductivity values.

The ends of carbon nanotubes, being terminated in oxygenated species, are hydrophilic but the walls, which comprise the vast majority of their mass, are very much hydrophobic. This means that it is difficult to disperse CNTs uniformly in most solvents and represents a major challenge when it comes to the controlled modification of electrode surfaces. Nanotubes may be regarded as large, rigid molecules with a low entropy of mixing. In polar solvents, they coagulate rapidly although Sano *et al.* [29]

showed that their dispersion time may be increased by acid-shortening. The explanation for this is simply that shorter nanotubes have a greater ratio of end-to-wall regions and therefore are more hydrophilic.

Nanotubes have traditionally been dispersed in non-polar organic solvents such as DMF [30-34]. In 2000, Ausman *et al.* [35] showed that CNTs may be dispersed in *N*-methyl-2-pyrrolidone (NMP), whose structure is presented in Figure 1.2.2. This solvent is now widely regarded as the most suitable for nanotube dispersion, thanks largely to a very thorough study carried out by Giordani and co-workers [36]. However, in electrochemical studies it has never been used to our knowledge. The reasons for it being overlooked have never been given. In *Section 3.3* solvent issues will be discussed on the basis of experiments carried out in the course of the present work, and an attempt will be made to provide an explanation for this.

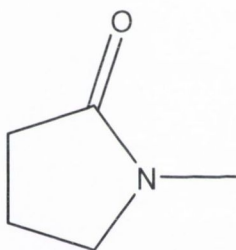


Fig. 1.2.2. Structure of *N*-methyl-2-pyrrolidone (NMP).

Electrochemists have consistently used the hydrophobicity of nanotubes to their advantage by drop-coating electrodes with dispersions of CNTs in organic solvents and evaporating the solvent [34,37-40]. The resulting nanotube-modified electrodes show excellent stability in aqueous systems, indicating that the NTs are strongly adsorbed onto the electrode surface. This method of drop-coating is the most commonly employed in the field but there are several others, which are described in the next section.

1.3 Modifying Electrode Surfaces using Carbon Nanotubes

The first application of nanotubes in electrochemistry was reported in 1996 by Britto *et al.* [41], who, using bromoform as a binder, packed a paste of NTs into a glass tube in order to study the oxidation of dopamine. They found that, at the paste electrode, dopamine underwent a virtually ideally reversible redox process. The reaction occurred at

a much faster rate than those observed using other catalytic surfaces. Subsequently, many other groups have attributed electro-catalytic properties to carbon nanotubes [30,37,42-49]. The number of publications concerning nanotube electrochemistry increased slowly after 1996 before really taking off in 2002, when Musameh and co-workers [38] observed the low-potential stable detection of NADH at a carbon nanotube-modified glassy carbon electrode.

Britto's pioneering method resulted in a randomly-oriented CNT array. Since then, the vast majority of publications in this area have involved such disordered arrangements of nanotubes. These systems have been prepared using a number of approaches, including drop-coating [34,37-40], the use of a nanotube paper as the electrode [42,50] and abrasion onto the basal planes of pyrolytic graphite [44].

In the construction of carbon nanotube-modified electrodes, an elusive goal has always been the fabrication of perfectly aligned, reproducible and robust arrays. Several attempts have been made. Nakashima *et al.* [51] dispersed shortened SWCNTs using an electro-active surfactant. These workers held a gold working electrode at 500 mV (*vs.* SCE) in an aqueous solution containing the dispersed nanotubes, resulting in the deposition of the latter. The amount of deposited nanotubes was controlled by the time spent at 500 mV and TEM images indicated that these arrays were more ordered than those observed using drop-coating. Wohlstadter *et al.* [52] produced a polymer-nanotube composite, which they extruded to yield aligned arrays. These composites were used to immobilise proteins for a sandwich assay in which the protein contained a chemiluminescent label.

The nanotube alignment was improved on by Nugent and co-workers [53] using the boule that forms on the electrode during arc discharge synthesis of multi-walled nanotubes. In this work conductive silver paint was used to attach aligned NTs to the ends of copper electrodes, in such a way that the ends of the nanotubes were exposed to the electrolyte. As mentioned above, the ends are believed to be paramount to the electrochemical performance of the nanotubes. These authors showed that the redox probe ferri/ferrocyanide displayed virtually ideal electrochemistry at the aligned MWCNT electrode.

In 2000, Liu *et al.* [54] published a paper reporting the self-assembly of an aligned array of single-walled nanotubes. The nanotubes were first shortened using strong acid, resulting in additional carboxylic acid functionalities forming at the ends of the tubes. These groups were then reacted with amines on cysteamine ($\text{NH}_2\text{CH}_2\text{CH}_2\text{SH}$). The resulting thiolated nanotubes then adsorbed onto a gold surface just like alkane-thiol self-assembled monolayers. The rate of adsorption of the nanotubes was found to be inversely proportional to the tube length. The AFM images of these arrays are impressive (see Figure 1.3.1), showing vertically aligned nanotube ‘brushes’. However, real applications of such arrangements in electrochemistry have not yet become widespread, possibly because the aligned nanotubes have little support and as such are not sufficiently robust. Despite this, Yu and co-workers [55] successfully adsorbed the enzyme horseradish peroxidase onto a vertically aligned single-walled nanotube array for use as a hydrogen peroxide sensor. They reported that these electrodes were stable for several weeks.

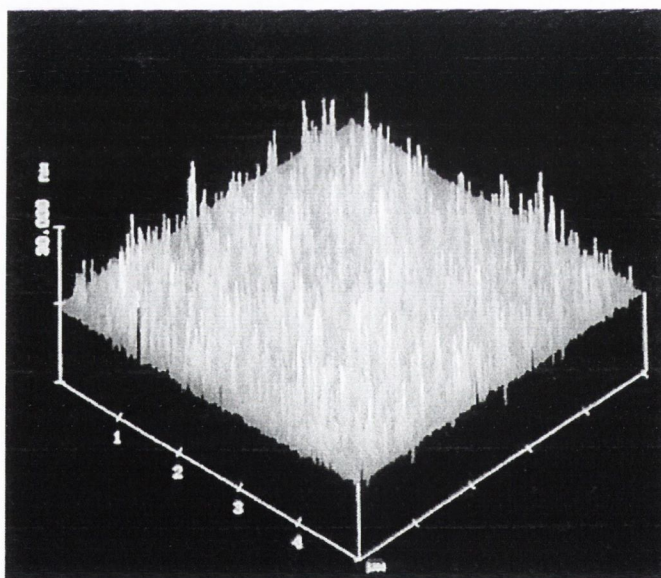


Fig. 1.3.1 Atomic force microscopy image of single-walled carbon nanotubes self-assembled on a gold surface *via* a wet chemistry approach. Reproduced from [54].

As well as self-assembly, electrodes modified with aligned nanotubes may also be created by direct growth on the substrate. The group of Dai has achieved this by growing MWCNTs on a quartz plate and transferring the tubes to a gold surface [56,57]. The growth of aligned nanotubes directly onto an electrode surface was accomplished by

Sotiropoulou and Chaniotakis [58], using chemical vapour deposition (CVD) at a platinum electrode. This arrangement was used to immobilise glucose oxidase, resulting in an amperometric glucose biosensor.

Li *et al.* [59] used CVD to grow aligned MWCNT arrays on silicon with a nickel catalyst. By controlling the size of the catalyst spots, they found they could control the amount of nanotubes grown at a given location. These electrodes were then used in the detection of DNA. All of these papers suggest that aligned nanotubes possess favourable electrochemical properties but, to the best of our knowledge, it was not until 2005 that the catalytic properties of a random dispersion of nanotubes were directly compared with an aligned array of the same tubes. Chou and co-workers [27,60] further modified a cysteamine-modified gold electrode using both random and aligned single-walled nanotubes. Using the ferri/ferrocyanide redox probe, they showed that the aligned arrays catalyse the redox process more efficiently, giving rise to smaller voltammetric peak separations. In this work the aligned tubes were acid-shortened and this process further functionalised the NTs with oxygenated species. It is noted however that this additional functionalisation perhaps undermines the legitimacy of the comparison!

The papers definitively attribute the observed superior electro-catalytic properties to the presence of these groups. The interpretation offered is that alignment results in more surface functionalities being presented to species in solution and these increase the rate of electron transfer, although these functionalities occur not just at the ends but the walls also [61,62]. Despite the arguments put forth in these publications, in most of the papers published between then and the time of writing, no attempts have been made to align nanotube arrays [37,39,42,63-65]. The methods described for fabrication of such ensembles are complicated and unavailable to many researchers [66]. Of course, it is also possible that when a current is passed through a disordered array of nanotubes, the array becomes more ordered, due to the forces which exist between current-carrying molecular wires. This possibility has never been investigated, since it would be difficult to obtain a useful image of an assembly on an electrode during the course of an electrochemical experiment.

The most common approach used when modifying electrodes with carbon nanotubes is to disperse the tubes in an appropriate solvent, drop a small volume of the

dispersion on the electrode surface and evaporate the solvent to give what is usually referred to as a nanotube ‘film’. This was the method employed in the present work. In 2004, Moore, Banks and Compton [44] modified bpHOPG electrodes by rubbing the latter on fine quality paper supporting carbon nanotubes. The catalytic properties of these ‘abrasively’ modified electrodes were compared with those of film electrodes using the redox probes NADH, epinephrine and norepinephrine. Decreases in peak separations relative to the bare electrode were found to be similar but interestingly, the different modification procedures gave rise to very different peak shapes (see Figure 1.3.2).

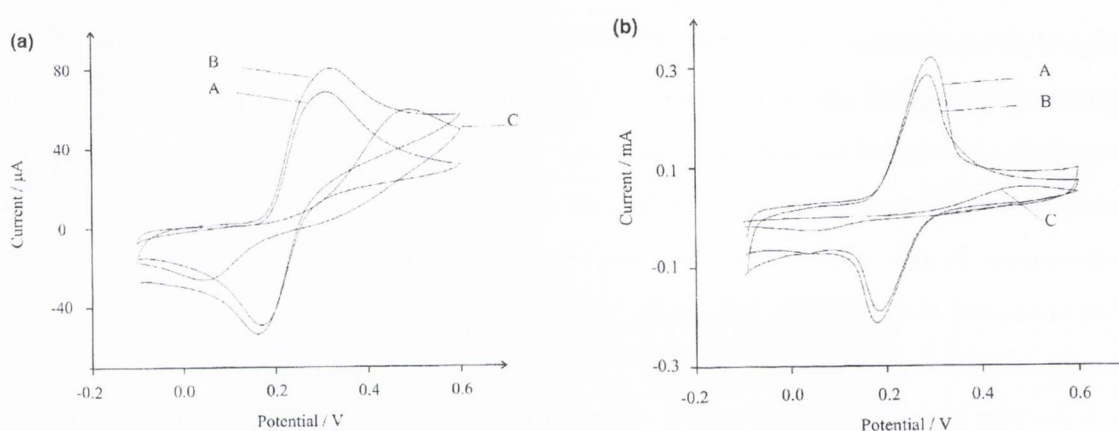


Fig. 1.3.2(a) Voltammograms for norepinephrine at abrasively-modified (A) graphite-, (B) NT-modified and (C) bare bpHOPG electrodes. **(b)** Same using film-modified electrodes. Reproduced from [44].

While the abrasively-modified electrode showed ‘diffusional’ peaks similar to the bare electrode, the film-modified electrode exhibited results more consistent with ‘thin layer’ behaviour. These workers attributed this difference to the porous nature of the nanotube film. The latter allows the leaking of small amounts of the electrolyte into it. This means that redox species can diffuse through a thin layer of solution to react at the electrode, rather than from bulk, as they must do when confronted with a ‘water-tight’ electrode. It may also be that these peaks are not the result of diffusive species at all, and are the result of redox processes associated with adsorbed analyte, as such reactions are also characterised by more symmetrical peaks and the absence of a diffusional tail. This possibility is commonly overlooked in the literature and will be addressed on the basis of our experiments in *Chapter Four*.

In 2005, Heng and co-workers [66] reported on the electroanalytical properties of ‘bamboo-like’ carbon nanotubes (BCNTs). These nanotubes look like bamboo due to the large number of transverse walls which occur at regular intervals along their length (see TEM image in Figure 1.3.3). They are produced either by ball milling and annealing of graphite powder, or by pyrolysis of iron phthalocyanine. By directly comparing glassy carbon electrodes modified with drop-coated, randomly oriented arrays of both BCNTs and HiPCo[®] SWCNTs, these workers showed that the former are endowed with superior electroanalytical properties. These were attributed to the greater density of edge plane sites along the walls of BCNTs, which occur at intersections between side-walls and transverse walls. They suggested that ‘bamboo-like’ nanotubes should be considered as an alternative to short or aligned nanotubes, as they provide a high number of edge plane defects per unit area, without the need for elaborate electrode modification procedures. This is indeed a significant finding, and further investigations into the properties of BCNTs will surely come in the future.

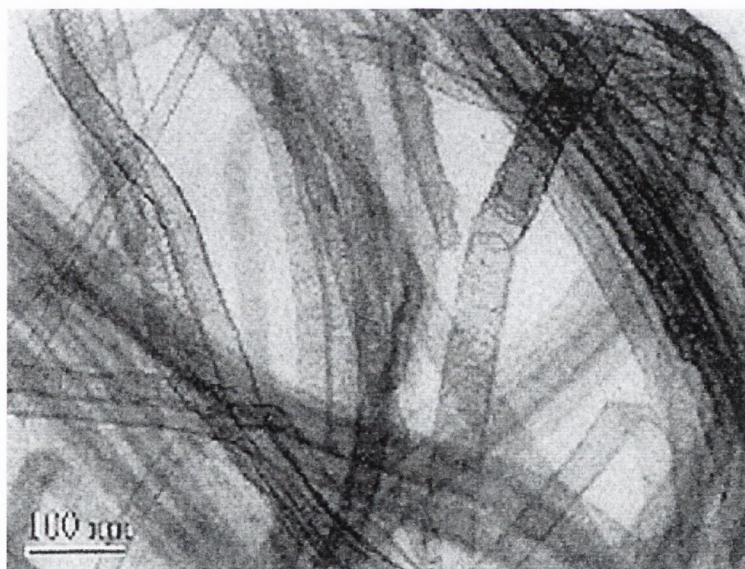


Fig. 1.3.3 TEM image of ‘bamboo-like’ carbon nanotubes, showing the division of nanotubes into segments by transverse internal walls. Reproduced from [66].

An interesting consideration when using carbon nanotube-modified electrodes is that of what is referred to in the literature as electrode ‘activation’. This is the pre-treatment of an electrode in a particular way before using it to catalyse a redox process. For example, Li

et al. [62] reported that nanotube electrodes were found to contain thin air films at the surface when submerged in aqueous electrolytes, due to the hydrophobicity of the tubes. To remove these films, they submerged the electrodes in 1 M nitric acid for five minutes. This was followed by several cycles in the aqueous electrolyte before measurements were performed. Wang *et al.* [32] cycled their electrodes for 1.5 minutes before experiments. These workers claimed that this resulted in the removal of contaminants or layers which inhibit electron transfer and also that it increased surface roughness and surface oxides. Some workers [67] have even gone so far as to activate glassy carbon electrodes prior to nanotube deposition. However, it should be pointed out that the majority of publications in the field make no mention of any such activation pre-treatment.

In most of the literature regarding the applications of CNTs in electrochemistry, it is standard practice to include some non-electrochemical techniques for analysis of the nanotubes being used, the results of which will hopefully be useful in the interpretation of electroanalytical results. These are typically imaging techniques [32,37,42,45,60,68], infrared spectroscopy [4,37,68-70], Raman spectroscopy [4,68,70,71] and X-ray photoelectron spectroscopy [37].

Imaging techniques (AFM, SEM and TEM) are useful for showing the orientation of the nanotubes (whether they are aligned or randomly arranged), the presence of amorphous carbon impurities, and providing an estimate of the nanotube dimensions. Infrared spectra are used primarily to show the presence of carboxylic acid functionalities (COOH), which are found around 1720 cm^{-1} . They are also used to identify carboxylate (COO⁻) but reported wave numbers for this species in these papers range from 1575 to 1620 cm^{-1} . While Raman may be used to estimate the diameter and electronic properties of carbon nanotubes [72], publications in electrochemistry are content to merely identify the Raman-allowed graphitic E_{2g} peak (1585 cm^{-1}) and the D band at 1340 cm^{-1} , indicating the presence of amorphous carbon. It should also be pointed out that some of these papers do not show the IR and / or Raman spectra despite inclusion in results. X-ray photoelectron spectroscopy is used, like FTIR, to indicate the presence of surface oxides, which, as mentioned previously, are believed to be so important to nanotube electrochemistry.

1.4 Carbon Nanotubes in Electrochemistry

With carbon electrodes, the presence of surface functionalities can greatly affect the rate at which electrons are transferred to or from species reacting at the surface. Carbon nanotubes have two distinct types of surface: the walls and the ends. As mentioned above, nanotubes may be likened to rolled-up sheets of graphite. This means that the structure and hence electrochemical properties of nanotube walls can be thought of as analagous to the basal planes of highly ordered pyrolytic graphite (see Fig. 1.4.1). By a similar argument, the ends of the tubes, which bear a variety of oxygenated species, should behave like the *edge* planes of highly ordered pyrolytic graphite [59]. It has been established that edge plane graphite exhibits considerably faster electron transfer kinetics than basal plane [61]. Having said this, NT walls may commonly also be decorated with surface oxides, especially if the tubes are long and curved, as they are in this work (see the AFM images reported in *Section 3.2*). Another factor which contributes to the presence of oxides on nanotube walls is diameter. Thin tubes are more strained and reactive and so have a larger number of defect sites per unit area.

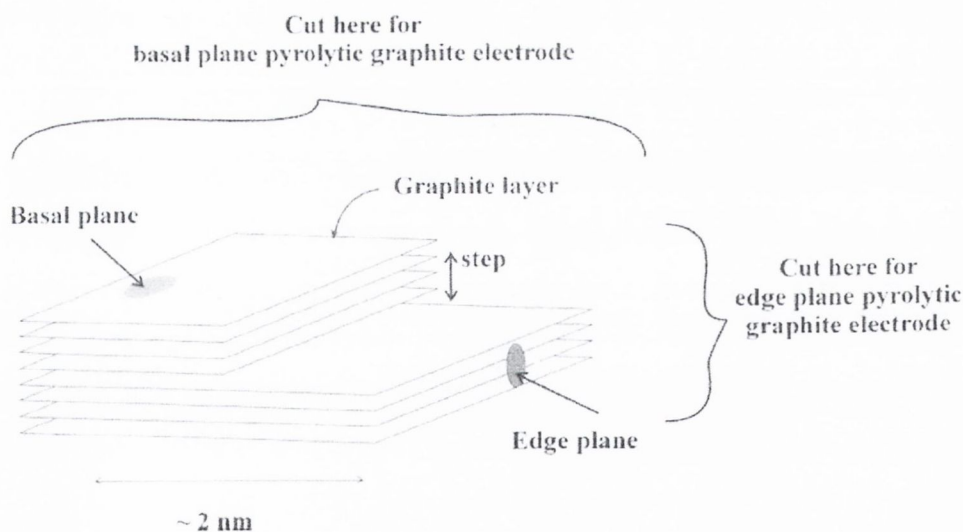


Fig. 1.4.1 Illustration of how bpHOPG and epHOPG are produced from graphite. Reproduced from [61].

Using the ferri/ferrocyanide standard redox probe, Nugent *et al.* [53] reported ideal voltammetric peak separations of 59 mV using copper electrodes modified with aligned

multi-walled carbon nanotubes. They found slightly larger peak separations using epHOPG and very much larger ΔE_p values using basal plane highly ordered pyrolytic graphite. These findings suggested that, for this particular redox couple, the aligned MWCNT-modified electrode had superior electro-catalytic properties to both epHOPG and bpHOPG electrodes.

By contrast, Li and co-workers [62] showed larger than ideal peak separations using SWCNT papers (96 mV) and far larger than ideal ΔE_p values using aligned MWCNT arrays (230 mV). The results for the aligned MWCNTs showed that the peak currents depended on the lengths of the tubes, indicating that the electro-catalytic performance of the nanotubes was dominated by the walls rather than the ends. The SWCNT data also indicated that the walls dictated the electrochemistry. These authors attributed the better performance of the single-walled nanotubes to the acid treatment step in the purification process introducing defects in the walls comprising of oxygenated species, which increased the rate of electron transfer.

The discrepancy between the findings in these two publications gives rise to a number of questions. The first is whether it is the walls or the ends which dominate nanotube electrochemistry. One set of results suggests that nanotube arrays show superior electro-catalytic properties when their ends are presented to redox species in solution, while the other indicates that it is desirable to have mostly walls as the catalytic surface. The second issue is the suitability of ferricyanide as a redox probe. In 1995, Chen *et al.* [73] showed that this species can be significantly affected by interactions with carbon electrodes. The third and most simple point is that the nanotubes used in these publications were neither synthesised nor purified in the same way and as such are not strictly suitable for direct comparison. This represents something of a problem in nanotube electrochemistry in general and it means that synthesis and purification of nanotubes ought to be described as explicitly as possible in any published work.

In 2005, Chou and co-workers [27] presented evidence that electron transfer between carbon nanotubes and redox species occurs *via* the oxygenated functionalities created during acid-shortening. They prepared bed electrodes using SWCNTs acid-shortened for various time periods. They found that the ferri/ferrocyanide couple reacted more readily as the acid-treatment time increased, because it meant shorter nanotubes and

higher ratios of oxygenated functionalities to defect-free regions on the walls. These workers also made the point that, while nanotubes may be no more electro-catalytic than epHOPG electrodes, they have the advantage that their small size allows them to penetrate places a pyrolytic graphite electrode could never reach, such as within the protein shell of an enzyme. Density functional theory calculations and molecular dynamics simulations carried out by Britto *et al.* [74] support the view that electron transfer occurs *via* oxygenated functionalities on the nanotube surface.

Also in 2005, Lawrence and co-workers [75] investigated the variation in electrochemical performance using five different commercially-available nanotubes, produced by both CVD and ARC methodologies. Using glassy carbon as the underlying electrode, it was shown that there were significant differences in background currents and catalysis with respect to the redox probes NADH and hydrogen peroxide, depending not only on the method of nanotube synthesis, but on the choice of solvent for NT dispersion prior to deposition on the electrode. These workers concluded that nanotubes produced using CVD were more suitable for electro-catalytic applications because of their larger proportion of edge plane-like defects (and hence oxygenated functionalities) due to greater curvature straining the carbon-carbon bonds. These nanotubes are also open-ended, while the caps of ARC-produced NTs remain intact [76]. They point out, however, that the precise identity of the catalytic surface oxides responsible and the mechanistic explanation of their catalysis of these reactions are not yet known.

As mentioned in *Section 1.2*, only around one third of a typical sample of SWCNTs shows metallic conductivity, while the rest may be thought of as semiconducting. This represents something of an ‘elephant in the corner’ which is largely ignored in publications. It might not be a serious issue however if the semiconducting tubes are reasonably conducting or if the metallic nanotubes dominate the electrochemistry. Another possibility is that, during the purification stages, the semiconducting tubes are made more conducting. Koehne and co-workers [77] showed that nanotube conductivity can be increased by doping. In most studies nanotubes are ruptured with strong acid. This might lead to ions entering the tubes and altering their electronic properties by injecting electrons or holes into their valence or conduction bands [78].

So it is clear that the electrochemical applications of carbon nanotubes depend very much on how they are made and/or purified. In this field the most common and important step in purification is undoubtedly acid treatment. As mentioned above, this leads to enhanced rates of electron transfer but there is strong evidence to suggest that the functionalities responsible for this advantage themselves give rise to redox peaks in voltammetry which might interfere with those of an analyte [37,62,75,79-81]. If these species play a crucial role in electro-catalysis, it is important to try to identify them, calculate their surface coverages and attempt to explain the mechanisms *via* which they interact with analyte species. It has been suggested [77] that the open ends of carbon nanotubes consist of sp^2 carbon atoms, which are converted to oxygenated functionalities such as carboxylic acids, alcohols and quinones. In several papers, X-ray photoelectron spectroscopy and FTIR studies have provided evidence of the existence of carboxylate and carboxylic acid groups on nanotubes [4,37,68-70].

Luo and co-workers [37] reported broad peaks between -0.2 and +0.2 V (vs. SCE) which they attributed to the four electron reduction of carboxylic acid groups to alcohols and the reverse oxidation process. These were observed when a randomly-dispersed SWCNT film on a glassy carbon electrode was scanned in phosphate buffer solution. Most studies however do not report redox processes observed for surface functionalities. This is another indication that the results of experiments depend on the history of the nanotubes used and any claims made about their electrochemical properties may not necessarily be extended to NTs from other sources.

A striking feature of carbon nanotube-modified electrodes is the large capacitance shown in voltammetry when compared with the bare electrode. This is particularly true of SWCNT films, which Azamian and co-workers [82] reported have capacitances roughly twice those shown by aligned arrays of multi-walled nanotubes. As mentioned above, surface oxides may give rise to their own faradaic processes so the observed capacitance depends on the amount of these functionalities present. Charging of the double layer was given considerable attention in the present work and results concerning this important issue will be presented in *Section 3.5*.

It is interesting to note that the shapes of typical voltammograms for solution species diffusing to CNT-modified electrodes are usually similar to those expected for

planar macro-electrodes, despite the potential for each nanotube bundle to act as an individual micro/nano-electrode. This is due to the high surface coverage of nanotubes in typical arrays. In most cases the tubes are so close together that their diffusion layers overlap and the effect of this is that redox species in solution diffuse linearly as if approaching a planar electrode. Li *et al.* [59,62,77] demonstrated the effect of nanotube surface coverage very nicely (see Figure 1.4.2). These workers used the ferricyanide redox probe to investigate high- and low-density aligned arrays of MWCNTs embedded within a silica matrix. The shape of the voltammogram was similar to that expected for a planar electrode at high surface coverages but became sigmoidal at low surface coverages, indicating that the nanotubes behaved like an array of micro-electrodes, without significant overlap between their diffusion layers.

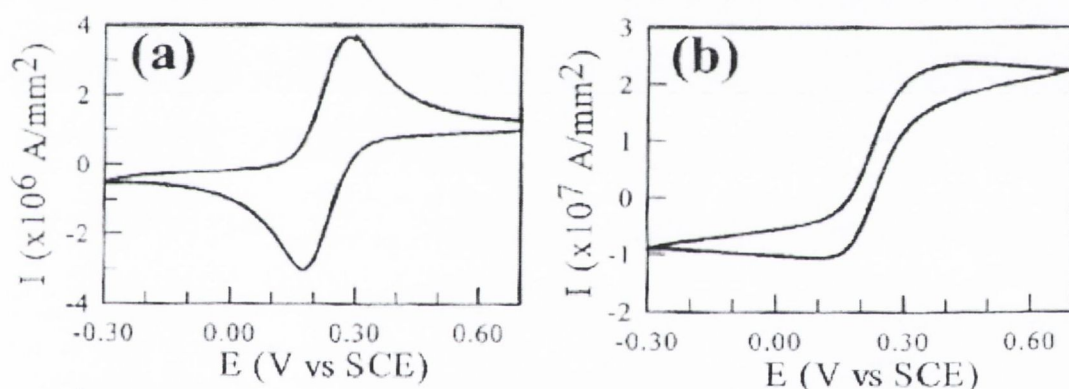


Fig. 1.4.2 Voltammograms in 1 mM potassium ferricyanide in 1 M KCl at a **(a)** high-density MWNT array ($\sim 2 \times 10^9$ NTcm⁻²) and **(b)** low-density MWNT array ($\sim 7 \times 10^7$ NTcm⁻²). Reprinted in part from [59].

There can be no doubt that modification of electrodes using carbon nanotubes can result in the enhancement of electro-catalytic properties with respect to certain redox processes. However, one should not be too quick to assign this ability exclusively to nanotubes. In recent years, several excellent papers have been published by Banks *et al.* [61,78-80] which have questioned the tendency in the literature to claim that nanotubes are endowed with ‘unique’ catalytic properties. These workers used bpHOPG electrodes to investigate the oxidations of the biomolecules NADH, epinephrine and norepinephrine. Their results showed that the catalytic benefits of MWCNT modification of bpHOPG were no better than those seen using ordinary graphite! It should also be pointed out, however, that

Wang and Musameh [83] have found the properties of NT/Teflon composites to compare very favourably with those of graphite/Teflon in the sensing of NADH and hydrogen peroxide.

Also, Banks *et al.* reported that edge plane-like defects on the nanotubes (oxygenated functionalities occur at these sites) were responsible for their electrocatalytic properties. They concluded that epHOPG electrodes might be preferable to carbon nanotube-modified electrodes for use in electroanalysis because they are re-usable, whereas the latter must be freshly prepared for each study. In 2006 Wang *et al.* [84] immobilised glucose oxidase on an unmodified epHOPG electrode for use as a glucose biosensor. Using this very direct approach which requires no 'messenger' between enzyme and underlying electrode, they reported a detection limit equal to that found by Sotiropoulou and Chaniotakis [58] for glucose oxidase immobilised at an aligned array of MWCNTs on a platinum electrode. Despite this, a large volume of literature exists on the investigation of the electroanalytical applications of carbon nanotubes and this is the subject of the next section.

1.5 Electroanalytical Applications of NT-Modified Electrodes

In the field of carbon nanotube electrochemistry, the most basic voltammetric experiments involve a CNT-modified electrode (typically glassy carbon) being used in a solution (typically phosphate buffer) containing small concentrations of various bio-molecules. The most popular of these bio-molecules are dopamine [37,41,45,48,49], ascorbic acid [37,45-49], uric acid [46,47,85], NADH [34,38,44,78,83,86], norepinephrine [44,47-49] and epinephrine [37,44,48,49]. Commonly it is shown that nanotube modification decreases peak separations (or oxidation potentials in the case of irreversible processes) and increases peak currents for the various redox couples. In these papers the variation of peak currents with the square root of the scan rate is often used to show that the redox species undergoes a diffusion-controlled process. Sometimes diffusion coefficients are also calculated for the analyte. Modification of common electrodes with nanotubes gives rise to greater signal-to-noise ratios when used in electroanalysis. It has been proposed that this is because noise depends only on the active

surface area of the sensor (defect sites and / or metallic impurities), while the signal is determined by the total area of the electrode [87].

Wang and co-workers [31] have reported that SWCNT-modified electrodes display considerable electro-catalytic activity towards the oxidation of 3,4-dihydroxyphenyl acetic acid (DOPAC). Oxidation peak currents were found to vary in a linear manner with DOPAC concentration in the range 1 to 120 μM . These systems were also shown to be capable of separating the electrochemical signals of DOPAC and 5-hydroxytryptamine, which coexist in real systems. These authors suggested that nanotube-modified electrodes showed great promise as DOPAC sensors. The group of Rubianes and Rivas has also reported a dramatic increase in the reversibility of the DOPAC reaction at carbon nanotube paste electrodes compared with classical carbon (graphite) paste electrodes [46,49]. This improvement was evident on the basis of decreased peak separations and peak current ratios closer to unity.

1.6 NTs in the Determination of Dopamine, EP and NE

Dopamine, epinephrine and norepinephrine are very important catecholamine neurotransmitters in the mammalian central nervous system and are often monitored electrochemically *in vivo* with microfibre electrodes. The oxidation of these compounds occurs in the human body. Catecholamine drugs are used to treat many ailments including heart disease and asthma.

An understanding of the electrochemical reactions of these three structurally similar compounds is necessary in the development of methods for studying their physiological function, and to aid in the diagnosis of some diseases, because in clinical medicine it is often desirable to develop an electroanalytical method to investigate electron transfer processes. The latter are proposed for these biomolecules to occur *via* the following half-reactions [88]:

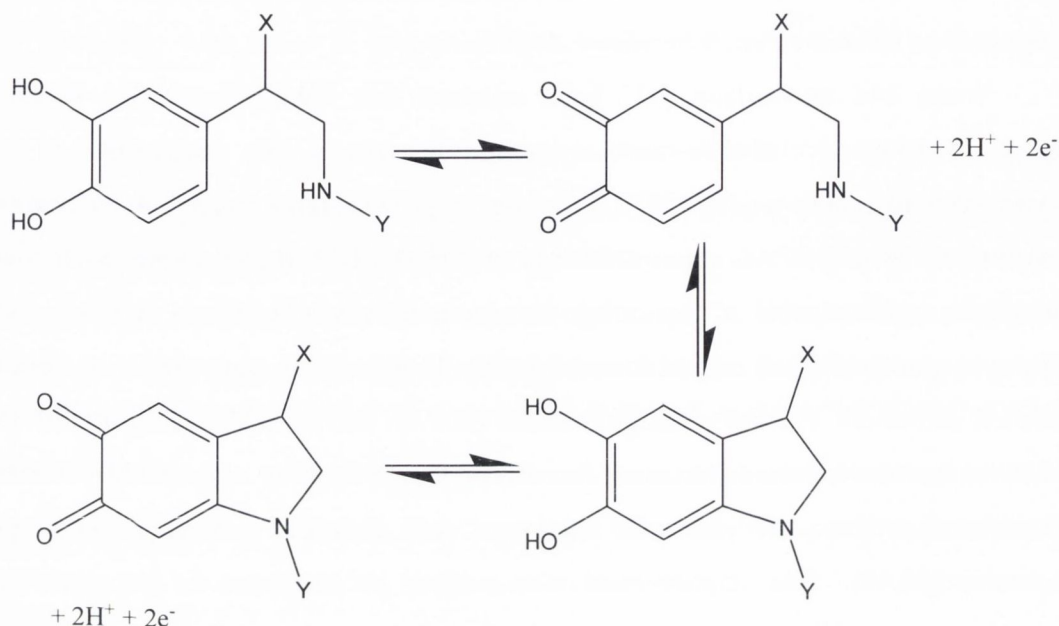


Fig. 1.6 Proposed scheme for the oxidation of epinephrine ($X = \text{OH}$, $Y = \text{CH}_3$), norepinephrine ($X = \text{OH}$, $Y = \text{H}$) and dopamine ($X = \text{H}$, $Y = \text{H}$).

In neutral aqueous solution these oxidations include two sequential electron transfer processes. In the first of these the compound is oxidised to an open-chain quinone (cyclic diketone) which then transfers to a leucochrome. Finally, the latter is oxidised to form the cyclisation product.

In the new millennium there have been several publications concerning the use of carbon nanotube-modified electrodes in the determination of these three compounds. The underlying electrodes used have included glassy carbon [37,48], basal-plane HOPG [44] and gold [47]. Typically these papers have simply reported lower voltammetric peak separations for dopamine and norepinephrine at the modified electrode when compared with the bare electrode, and substantial negative shifts in the (irreversible) oxidation peak of epinephrine, which they attribute to the favourable biocatalytic properties of nanotubes. Again, it should be said that little has been offered by way of an explanation of the observed electro-catalysis, other than the identification of the importance of surface oxides present at edge plane-like defects.

1.7 Sensors for Ascorbic Acid

Ascorbic acid (also known as vitamin C) is used extensively in the food and drink industry. The accurate determination of its concentration is of considerable importance. It is also used in health care where it can increase high-density lipoprotein production, down-regulate cholesterol and lower blood sugar and insulin requirements, thus reducing the risk of cardiovascular diseases [89]. The reduced and oxidised structures of ascorbic acid are shown in Figure 1.7.

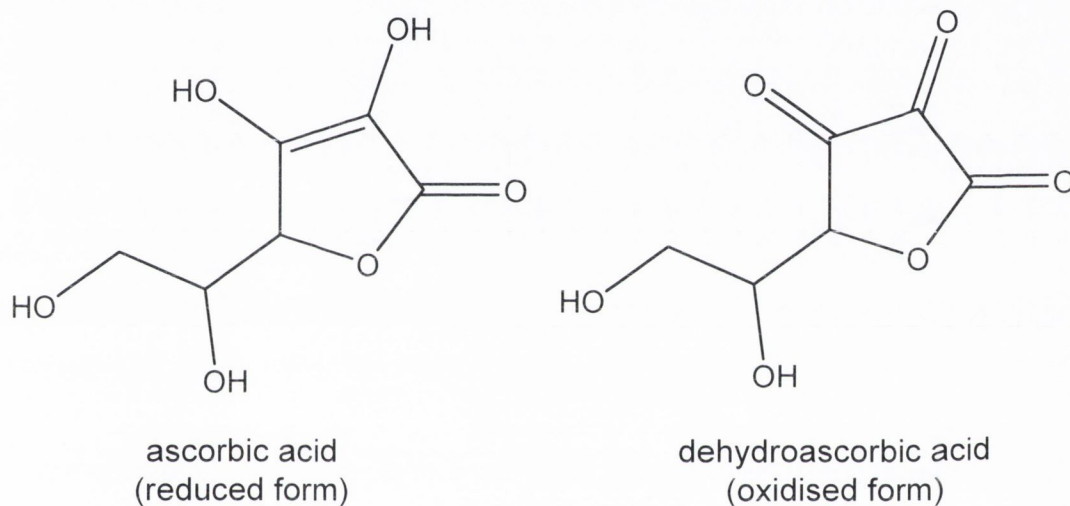


Fig. 1.7 Structures of the reduced and oxidised forms of ascorbic acid.

In the past, ascorbic acid has been determined using high performance liquid chromatography [90], capillary zone electrophoresis [91] and spectrophotometry [92]. Esteve and co-workers [93] compared HPLC with electrochemical techniques in the determination of ascorbic acid. They concluded that the latter were preferable for reasons of cheapness and simplicity.

The reliable sensing of ascorbic acid using conventional carbon electrodes has been hindered by the large overpotentials required and electrode fouling by oxidation products [94]. A large body of literature exists in which conventional electrodes are modified with a view to lowering the oxidation potential (E_{pa}) of ascorbic acid. Wring *et al.* [95] modified a graphite-epoxy composite using cobalt phthalocyanide, resulting in an E_{pa} decrease of 150 mV for ascorbic acid. Ojani and Pournaghi-Azar [96] modified glassy carbon electrodes using ferrocene derivatives and achieved a decrease of 300 millivolts.

Raoof and co-workers [94] lowered the oxidation peak potential by 250 mV by modifying a carbon paste electrode using ferrocene carboxylic acid.

. By modifying glassy carbon electrodes with single-walled carbon nanotubes, decreases of 80 mV [37] and 110 mV [48] in the oxidation potential of ascorbic acid have been reported. Rubianes and Rivas [46] achieved a decrease of 230 mV by modifying carbon paste electrodes with multi-walled nanotubes. Wantz, Banks and Compton [89] have also shown that unmodified edge-plane pyrolytic graphite electrodes exhibit impressive sensing properties with respect to ascorbic acid. The response compared favourably with several other allotropes of carbon (although not nanotubes).

1.8 Sensors for NADH

The electrochemical oxidation of nicotinamide adenine dinucleotide (see Figure 1.8 for its structure) is of considerable interest because it is required as a cofactor in a large number of dehydrogenase-based biosensors [97]. For example, NADH-dependent dehydrogenases catalyse the oxidation of compounds such as ethanol and lactic acid. For most solid electrodes, the oxidation of this compound requires a rather large overpotential (*ca.* $> +0.5$ V), meaning that these systems often show poor selectivity in the presence of interfering species. Another serious problem is the irreversible adsorption of oxidation products on the electrode surface. The oxidised form of NADH, NAD^+ , adsorbs onto carbon surfaces. This can lead to a parasitic signal due to the adsorption-controlled faradaic process. Moiroux and Elving [150] have described a simple procedure for the elimination of this problem. This involved poisoning the carbon electrode at a strongly anodic potential for one hour in an agitated NADH solution. The resulting layer of adsorbed NAD^+ did not hinder the sensing of solution NADH in subsequent experiments.

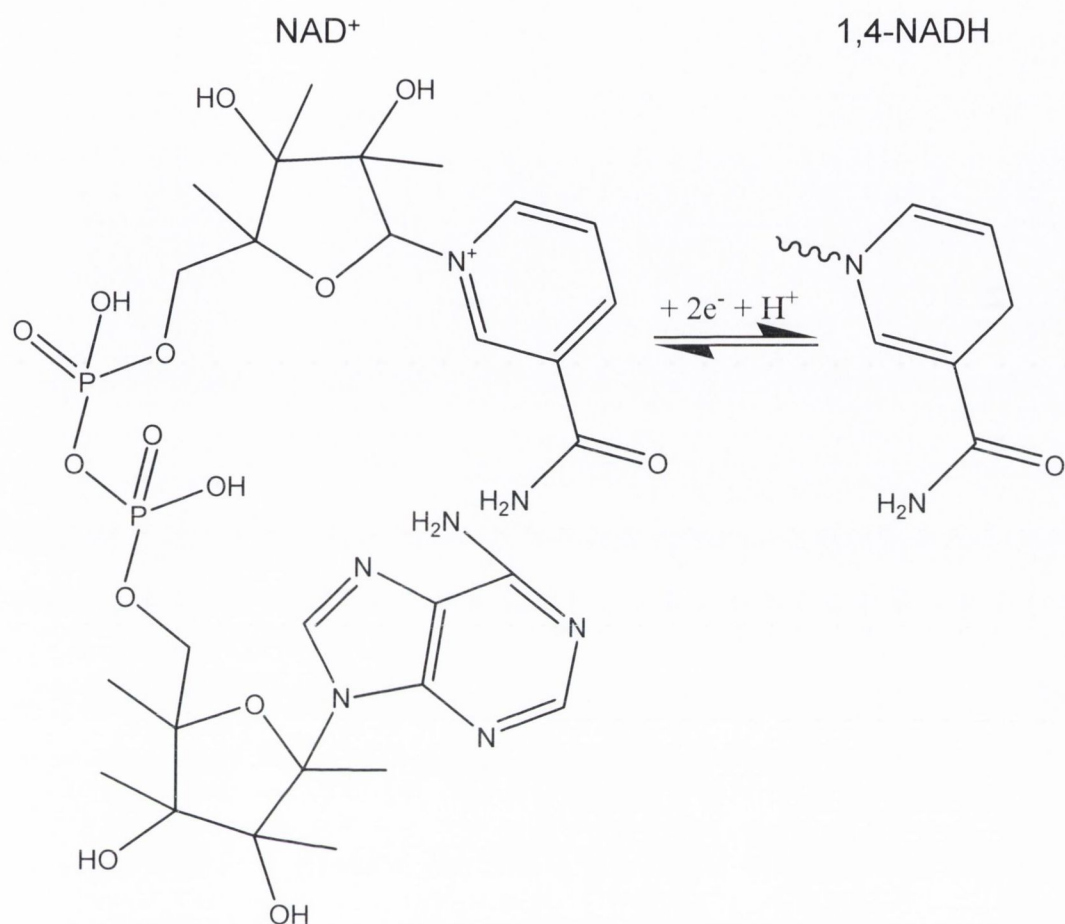


Fig. 1.8 Structural formulae and redox reaction of the biomolecule NAD⁺ and its reduced, enzymatically active form, 1,4-NADH.

As a result, electrode materials which can oxidise NADH at low overpotentials and also resist fouling are increasingly sought. A popular approach is to modify the electrode surface with an appropriate mediator. Kumar and Chen [98] have recently published an extensive review on the use of carbon nanotubes and conducting polymers as electrode modifying agents for NADH detection. In 1997, Bartlett *et al.* [99] modified a glassy carbon rotating disc electrode using a poly(aniline)-poly(vinylsulphonate) composite in order to probe NADH oxidation. These workers developed a kinetic model for the process which incorporated diffusion within the polymer film and competition between oxidised and reduced forms for catalytic sites. Prieto-Simon and Fabregas [100] have systematically studied a range of commonly-used mediators for NADH oxidation as well

as strategies for incorporating each of the electro-catalysts into a dehydrogenase-based biosensor based on epoxy-graphite composites. They concluded that the optimum immobilisation strategy was to electro-polymerise *o*-phenylenediamine on the composite electrode. This was found to resist fouling and exhibit a high sensitivity towards NADH oxidation. However, the overpotential required remained large.

There has also been a number of publications on the use of carbon nanotube-modified electrodes in the electroanalysis of NADH oxidation. To our knowledge this was first done by Musameh and co-workers [38] in 2002, who used both SWCNTs and MWCNTs to modify glassy carbon electrodes. These authors reported a significant decrease in the overpotential required for oxidation, along with impressive resistance to fouling. This work was extended by Chen *et al.* [71], who achieved a detection limit of 0.5 μM using GC electrodes modified with aligned arrays of nanotubes. These authors reported decreases as large as 645 mV in oxidation overpotential. A recent paper by Arvinte *et al.* [34] has reported a decrease in oxidation potential of 400 mV for a GC/SWCNT electrode relative to bare glassy carbon. It is interesting to note the large differences between the various peak shifts observed in the literature. Of course, not every glassy carbon electrode is the same, but it is likely that the disparity is primarily due to the variations which exist among different batches of nanotubes, depending on how they are made and purified. This will be a recurring theme throughout this thesis.

Carbon nanotubes for NADH sensing have also been utilised in paste electrodes [101] and solubilised in biopolymers [102]. Many other approaches to NADH sensing are likely to be conceived in the future. Banks and Compton [80] have used an unmodified epHOPG electrode to determine NADH, achieving detection limits of 5 and 0.3 μM using cyclic voltammetry and batch amperometry, respectively. Interestingly, by comparing these electrodes with basal-plane HOPG, these workers have shown that edge-plane sites are responsible for catalysing the oxidation of NADH, from which it may be inferred that these are the sites on carbon nanotubes which govern the electro-catalysis of this process.

1.9 The Oxygen Reduction Reaction

The oxygen reduction reaction is central to processes such as metal corrosion, and has applications in areas like fuel cells and batteries. It is one of the most studied reactions in

electrochemistry and as such, numerous electrode materials have been proposed for its catalysis [103]. Bockris and Khan [104] have remarked that ‘were one to choose a single electrode reaction having the greatest breadth of practical relevance, it would certainly have to be the oxygen reduction reaction’. Applications of carbon-based and modified carbon electrodes have attracted considerable interest recently [105]. Voltammetric studies of the ORR often reveal two distinct cathodic processes. As proposed by Yeager [106], the reduction of oxygen in aqueous solution generally proceeds by either of these two broad pathways. One involves the direct reduction of molecular oxygen to water by the acceptance of four electrons. The other involves two separate two-electron reductions, the first of which yields hydrogen peroxide:



In the second step, peroxide is reduced to water:



To our knowledge the first report of the application of carbon nanotubes in the study of oxygen reduction was by Che *et al.* [107] in 1998. These workers modified glassy carbon electrodes using nanotubes decorated with platinum nanoparticles and reported favourable catalytic activity towards the reduction of oxygen. In 1999, Britto and co-workers [74] used a MWCNT microelectrode to study the ORR in acidic and neutral electrolytes. Using molecular dynamics simulations, these authors proposed that pentagons at the nanotube tips, pentagon-heptagon defect pairs in the lattice and, to a lesser extent, curvature were responsible for the observed electro-catalysis.

Since then there have been several publications regarding the use of CNT-modified glassy carbon electrodes in the study of the irreversible reduction of oxygen in both acidic and basic media [108-110]. While all of these display evidence of impressive catalytic activity, it is still not known by what mechanism this complicated reaction is catalysed by the nanotubes.

1.10 Sensors for DNA

The need for rapid, simple and inexpensive testing of genetic and infectious diseases has led to considerable research into the development of DNA-modified carbon nanotube biosensors. These are based on nucleic acid recognition events. They rely on the

immobilisation of single-stranded DNA onto the nanotube surface, which upon hybridisation with the complementary strand, produces a useful chemical signal [111]. The importance of nanotubes in these systems has been attributed to enhanced detection of the target guanine or of the product of an enzyme label.

Surface-bound MWCNTs have been shown to be useful in facilitating the adsorptive accumulation of the guanine nucleobase and enhancing its oxidation signal [112]. These nanotube-modified glassy carbon electrodes have been shown to be superior to bare glassy carbon, carbon paste and graphite pencil electrodes. The dramatic amplification of the guanine signal has been combined with a label-free electrical detection of DNA hybridisation. It is worth noting that, while the signal is enhanced, the oxidation potential is not lowered, unlike the biomolecules discussed in previous sections. The enhancement is therefore attributed to an accumulation of guanine at the high-surface area nanotubes rather than an increased rate of electron transfer. A similar enhancement was reported for guanine-rich DNA using MWCNT paste [113] and SWCNT-modified glassy carbon [114] electrodes. Trace ($\mu\text{g L}^{-1}$) amounts of nucleic acids have thus been detected following short accumulation times. Using multi-walled nanotubes as nano-wires to connect target DNA strands to a carbon paste electrode, Kerman and co-workers [115] achieved enhanced voltammetric DNA hybridisation detection down to $0.01 \mu\text{g L}^{-1}$, which is compatible with the required detection limit for genetic testing.

Li and co-workers [59] have shown that an array of vertically aligned MWCNTs embedded in SiO_2 can be useful in the detection of DNA hybridisation. These authors detected $[\text{Ru}(\text{bpy})_3]^{2+}$ mediated guanine oxidation utilising a nanotube array. The sensitivity was improved by using daunomycin, a well-known redox intercalator [116]. A MWCNT-modified glassy carbon electrode was used for this task along with a 5'-amino group functionalised oligonucleotide probe and pulse-voltammetric transduction.

Cai *et al.* [117] have reported indicator-free AC impedance measurements of DNA hybridisation based on a doped polypyrrole film over a multi-walled carbon nanotube layer. The hybridisation event led to a decrease in impedance values, reflecting the lowering of electrode resistance. A five-fold sensitivity enhancement was observed when compared with measurements made in the absence of nanotubes.

The point has now been reached at which the applications of nanotube electrodes in enzyme studies and biosensors will be reviewed. Before doing so, it is prudent to digress briefly from discussions of the literature in order to provide an introduction to the redox enzyme glucose oxidase, as this will feature prominently in the results described in *Chapter Four*.

1.11 Glucose Oxidase

Glucose oxidase (GOx) is a large, dimeric protein with a formula weight of around 160 kDa. It has been described [118] as an ideal model enzyme for bioelectrochemistry and catalyses the oxidation of glucose according to:

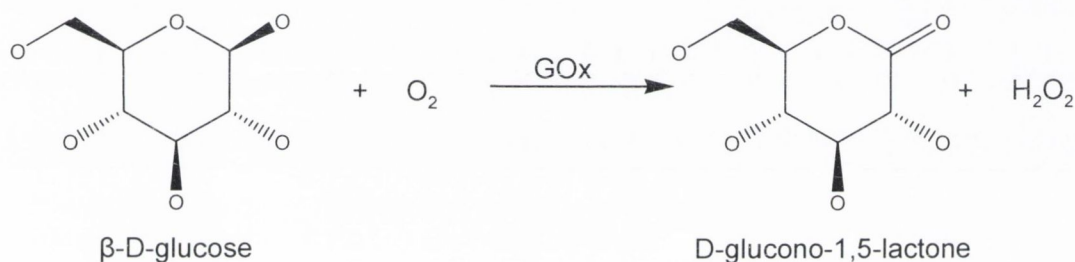


Fig. 1.11.1 Oxidation of glucose in the presence of the enzyme glucose oxidase.

The dimensions of the dimer are roughly $8 \times 6 \times 5$ nm [119], comparable to those of a typical carbon nanotube bundle. Glucose oxidase contains one tightly-bound flavin adenine dinucleotide (FAD) unit per monomer. These redox-active prosthetic groups are not covalently bound and may be released from the protein during denaturation. In this study, the redox chemistry and kinetics of the FAD/FADH₂ redox couple are probed using electrochemical techniques. FAD is an electron carrier in energy metabolism and it exists as a disodium salt. Figure 1.11.2 shows its reduced and oxidised forms.

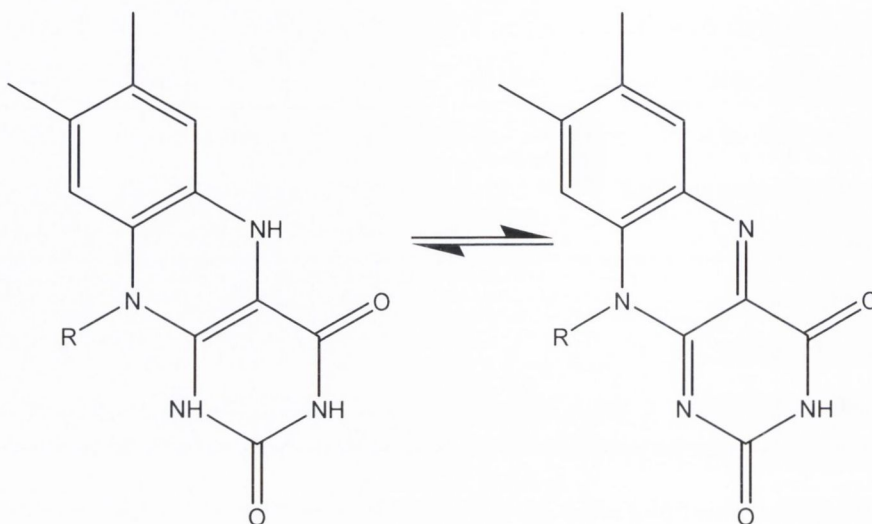


Fig. 1.11.2 The reduced (left) and oxidised (right) forms of the FAD prosthetic group.

The alkyl (R) group in the molecule is a large chain which plays no part in the redox transition but for clarity the structure of the entire compound is shown in Figure 1.11.3.

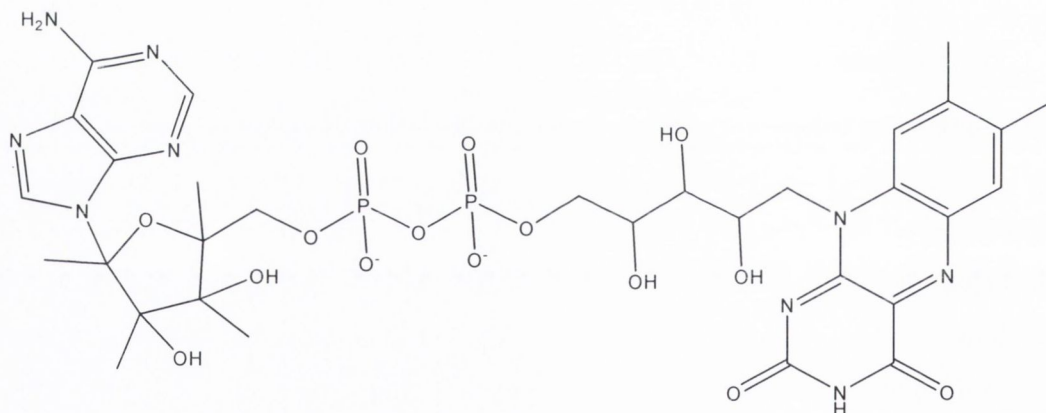


Fig 1.11.3 Unabridged structure of FAD (oxidised form).

Having provided a description of glucose oxidase, the literature review is now revisited, with particular emphasis on studies of the direct electron transfer behaviour of glucose oxidase and other enzymes, followed by electrochemical biosensors for both glucose and hydrogen peroxide.

1.12 CNTs in the Direct ET of GOx and Other Redox Proteins

Immobilised enzymes can serve as model systems by which some of the principles underlying the kinetic behaviour of membrane-bound enzymes can be deduced [120]. To our knowledge, the first report of direct electron transfer between carbon nanotubes and redox proteins was in 1997 when Davis *et al.* [121] used a carbon nanotube paste electrode to study adsorbed cytochrome *c* and azurin. Since then nanotubes have been used to study a number of such systems including peroxidases [30,55,122], cytochrome *c* [32,121,123,124], myoglobin [55,125] and catalase [126]. The Fe(III) / Fe(II) redox centres of these proteins are located close to the outer surface of the molecule, as distinct from glucose oxidase, in which the prosthetic group is embedded deep within the surrounding protein.

Redox proteins have been deposited on nanotube electrodes using several different methodologies. These include depositing a combined solution of nanotubes and enzyme on an electrode and leaving to dry [4], incubating a NT-modified electrode in an enzyme solution overnight [42], and electrochemical deposition [123]. The approach used in this work was to simply drop-coat an enzyme solution (in phosphate buffer) on a nanotube-modified electrode and leave it to dry at room temperature [33,40].

Gooding *et al.* [30] have studied the direct electrochemistry of the enzyme microperoxidase 11 by immobilising it on an aligned SWCNT array (see Figure 1.12), while Yu and co-workers [127] have immobilised both myoglobin and horseradish peroxidase on similar arrays for use as hydrogen peroxide biosensors. The nanotubes facilitate electron transfer between the redox enzyme and the underlying electrode without the assistance of a mediator. The large surface area of CNT-modified electrodes means that more enzyme can be adsorbed than with bare electrodes.

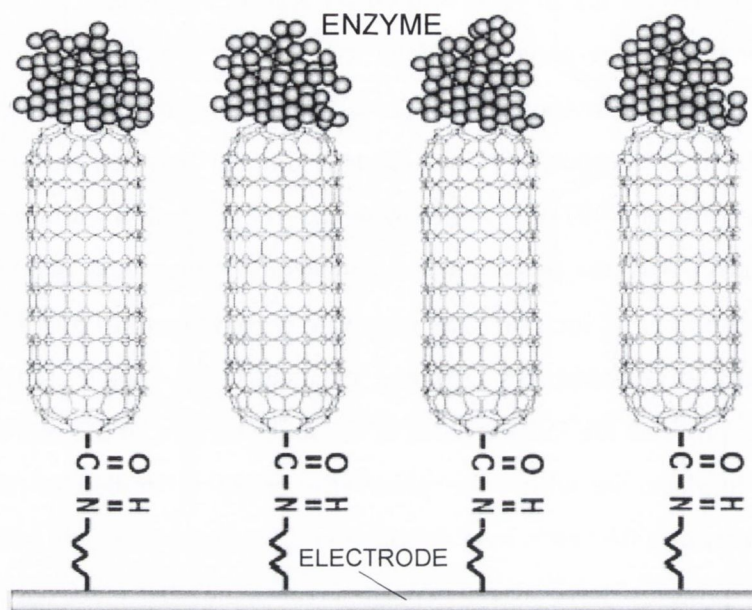


Fig. 1.12 Aligned SWCNTs providing enzyme immobilisation platform. Reproduced from [30].

As mentioned above, the redox centre of glucose oxidase is less accessible than for the heme proteins, being electrically insulated by the protein shell. This makes it difficult for the enzyme to communicate with conventional electrodes in the absence of mediating small molecules [33]. In 2002, Guiseppi-Elie *et al.* [42] reported on the direct electron transfer between single-walled carbon nanotubes and adsorbed glucose oxidase. Both the enzyme and the FAD prosthetic group were found separately to spontaneously adsorb onto a disordered array of NTs on a glassy carbon electrode, showing what was reported as a quasi-reversible *one-electron* transfer. It was assumed that the nanotubes came within tunneling distance of the prosthetic group, without the occurrence of significant enzyme denaturation.

In 2004, Patolsky and co-workers [128] showed that reconstituted GOx on an aligned array of SWCNTs communicates with an electrode and that the rate constant for electron transfer, estimated using the method of Laviron [129], was determined by the length of the nanotubes, contradicting a conclusion drawn by Gooding *et al.* [30] in 2003 using microperoxidase MP-11 as the enzyme. Using glucose oxidase adsorbed onto the surface of CNTs on a glassy carbon electrode, with Nafion as a binder, Yin and co-workers [4] have shown the direct electron transfer of the enzyme. These workers claimed

that the redox process involved the transfer of *two electrons* coupled with two protons. Gooding [30] and Yu [55] have reported on the direct electron transfer of GOx covalently attached to aligned SWCNT arrays. These workers concluded that the latter behaved like metals, transporting electrons from the external circuit to the redox centres of the enzyme.

Once it has been established that an adsorbed enzyme can communicate with an underlying electrode, an obvious possibility to investigate is whether it retains its enzymatic activity for its natural analyte in a biosensor. Before the reported findings on amperometric biosensors involving carbon nanotubes are reviewed, a brief digression will first be made in order to discuss the fundamental concepts of biosensors.

1.13 Electrochemical Biosensors

A biosensor consists of a biological receptor microstructure (typically an immobilised enzyme which has the capacity to interact specifically with an analyte species) coupled to an electronic transducer, which converts biochemical activity into electrical signals which can be amplified, stored, displayed and manipulated. Research into these devices began in 1962 with the report of a biosensor for glucose by Clark and Lyons [130]. Most commercially available biosensors are capable of detecting analyte concentrations of less than 1 mM. The most important analyte in enzyme sensing is glucose [1]. The glucose biosensor is used by diabetics to monitor blood sugar levels and, despite the many biosensors which are used in research laboratories, the glucose biosensor is really the only commercially available biosensor sufficiently reliable and simple to be used by patients and doctors. In nature, FAD oxidises glucose (see Figure 1.11.1), and in the process is reduced to FADH₂. In the presence of oxygen, the active oxidised form is regenerated and hydrogen peroxide is produced. This naturally-occurring ping-pong mechanism is utilised by the glucose biosensor and illustrated in Figure 1.13.

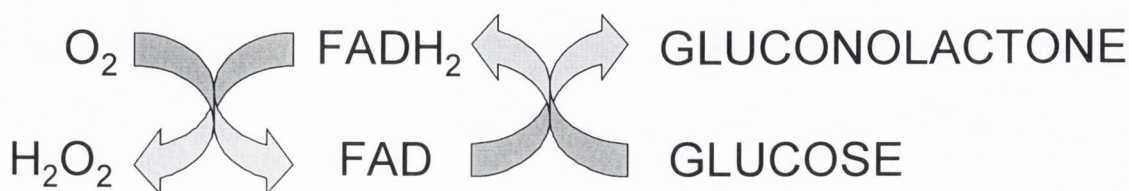


Fig. 1.13 Ping-pong mechanism for the oxidation of glucose, catalysed by FAD.

In typical glucose biosensors, the progress of the biochemical reaction is followed by oxidising the hydrogen peroxide produced. A potential problem here is the high operating voltage (around +0.8 V *vs.* Ag / AgCl) required, which can result in interference from species such as ascorbate, urate and paracetamol. The use of mediators (species which can shuttle electrons between the electrode and the redox centre of the enzyme) can alleviate this problem because, if the right mediator is used, it will be regenerated at lower operating voltages. Ideally for practical purposes a mediator should be of low molecular mass, reversible, fast-reacting, regenerated at a low potential, pH independent, stable in both oxidised and reduced forms, non-toxic and unreactive with oxygen [94]. At present, the most important mediators are arguably compounds based on ferrocene and its derivatives.

In many biosensors, the enzyme is held on the electrode by a polymer film. This film should prevent loss of the protein to the electrolyte solution and ideally have the ability to reject interferent species while allowing a well-defined response to the analyte. In the present work, the polymer Nafion was used for this purpose, so what follows is a brief description of this important material.

1.14 Nafion

Nafion is an ionically conducting perfluorinated polymer of relatively recent origin. Since its invention in 1962, it has featured in many electrochemical studies. Because of their unique ion-exchange, discriminative and biocompatibility properties, Nafion films have been used extensively for the modification of electrode surfaces and the construction of amperometric biosensors [43]. They are stable up to 190 °C and in extreme oxidising conditions. They are highly conductive and combine the ability to act as a separation matrix between two phases with the chemical and electrochemical properties of ion exchangers. In the synthesis of Nafion, a vinyl ether co-monomer is co-polymerised with tetrafluoroethylene to form the precursor copolymer. Hydrolysis with sodium hydroxide is then performed to yield the structure shown in Figure 1.14.

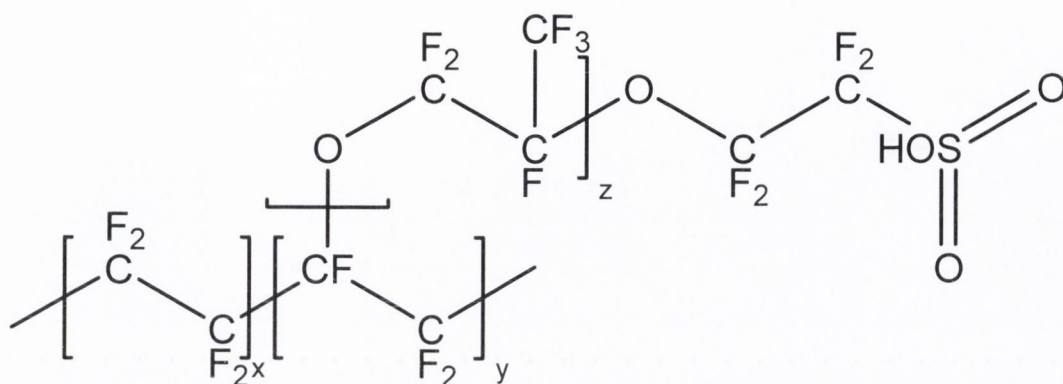


Fig. 1.14 Structure of Nafion.

Nafion is easily cast on electrode surfaces by drop-coating commercially available solutions of it in low molecular mass alcohols and allowing the solvent to evaporate. It is also easily removed by simple abrasion. The films are stable when used in aqueous electrolytes (as they were in the present work) but may decompose in less polar solutions. They are highly conductive to protons and simple cations such as sodium and potassium. The ionomer has been shown to have the ability to exchange and transfer counter-ions while rejecting co-ions (ions of the same charge as the fixed site). Thus, Nafion is considered to be perm-selective, making it an attractive component for electrochemical biosensors. Nafion has been shown [43] to wrap around carbon nanotubes without impairing their electro-catalytic properties, so the polymer is also suitable for nanotube-modified electrodes.

The progress of biosensor research has been impeded by the lack of surface architectures showing sufficiently sensitive responses to the desired biochemical event. In real samples, there can be considerable variations in pH and ionic strength, which seriously affect the responses of certain biosensors [131]. This has given rise to considerable research into the incorporation of nano-materials into sensors, the idea being that shrinking the dimensions of sensor elements produces higher signal-to-noise ratios. If nanotube biosensors are to become a reality, the chemical and physical properties of these structures must be controlled. The oxygenated functionalities mentioned previously are believed to interact with protein shells in the immobilisation of enzymes, with the formation of amide linkages between these functionalities and the lysine residue of the

protein [55,132]. The most commonly used enzyme in the field is glucose oxidase and the next section describes some of the significant findings reported for this system.

1.15 Carbon Nanotubes in Glucose Sensing

The diagnosis and treatment of diabetes require accurate monitoring of blood glucose concentration, which is normally between 3 and 8 mM [133]. Electrochemical biosensors play an important role in this area. Nanotubes have been shown to facilitate the direct electron transfer of glucose oxidase and the detection of hydrogen peroxide at low operating potentials, so they are regarded as attractive components for glucose biosensors. Because of their small diameters, NTs can penetrate the enzyme protein shell and get close to the FAD prosthetic group [42].

Typically, glucose sensors involve the immobilisation of glucose oxidase on an electrode surface. The electron transfer mediator may be present either in solution with the substrate, or it might (preferably) be immobilised on the electrode, providing a platform on which the enzyme adsorbs. Willner's group has published work of some importance on the latter approach. These workers fabricated efficient biosensors based on glucose oxidase reconstituted on monolayers of the mediators boronic acid [134] and rotaxane [135] on gold electrodes. The term *reconstituted* means FAD was initially adsorbed and then the protein (*apo*-GOx) was introduced around it to give an adsorbed layer of glucose oxidase. Using rotaxane as a mediator, it was shown that glucose could be determined at a potential similar to the enzyme's standard redox potential. This offered significant practical advantages, and was certainly an important consideration within the context of the experiments which will be discussed in *Section 5.3*.

Interestingly, nanotube/Nafion-coated electrodes, coupling the electro-catalytic detection of hydrogen peroxide with the perm-selectivity of Nafion, have been found to act as highly selective low-potential (-50 mV vs. Ag /AgCl) *non-enzymatic* glucose sensors [43]. These were shown to discriminate against a number of interferent species when tested. Non-enzymatic glucose sensing has also been reported in a recent paper by Tan and co-workers [136], using multiple-branching MWCNT forests attached to gold electrodes. Wang and Musameh [137] designed a needle microsensor for the amperometric monitoring of glucose based on packing a binderless CNT/GOx composite

within a 21-gauge needle. The resulting biosensor showed low-potential, selective and sensitive glucose determination. This was achieved over a large range of glucose concentrations (up to 35 mM).

Lin and co-workers [132] reported on glucose biosensors based on carbon nanotube ensembles. The low potential (-0.2 V vs. SCE or -0.15 V vs. Ag / AgCl) reductive detection of the hydrogen peroxide produced led to the highly selective monitoring of the substrate, along with linearity up to 30 mM glucose and a detection limit of 0.08 mM. Ye *et al.* [138] reported on the non-enzymatic detection of glucose at an aligned MWCNT-modified electrode. Strong electro-catalytic activity towards glucose in alkaline media was observed with a low operating potential of $+0.2$ V and a useful range of 0-20 mM glucose. Using platinum nanoparticles and single-walled nanotubes dispersed in Nafion and cast on a glassy carbon electrode, Hrapovic and co-workers [67] reported a glucose sensor linear in the range 0.0005-5 mM, with an operating potential of $+0.55$ V and a sensitivity of $2.11 \mu\text{A} / \text{mM}$.

The above are but some of many publications concerning the use of carbon nanotubes in glucose sensing. The following table summarises the findings of these and several others not mentioned.

Electrode	Operating Potential/V	Linear Range/mM	Sensitivity / nAmM^{-1}	Detection Limit/ μM	Reference
GC/CNT	0.0	1-20	not given	not given	[43]
Pt/CNT	+0.8	0.25-2.5	*	190	[58]
CNT/GOx paste	-0.1	up to 30	11.3	600	[46]
CNT/GOx	-0.2	up to 30	not given	80	[132]
GC/CNT/Pt NPs	+0.55	0.0005-5	2110	0.5	[67]
CNT/GOx needle	-0.1	up to 40	2.3	not given	[137]
GC/CNT	+0.2	0.002-11	210	1	[138]
Au/CNT/GOx/PPy	+0.45	up to 20	not given	not given	[139]

(*) Sensitivity given in terms of electrode surface area but a value for the latter was not quoted.

Table 1.15 Summary of some publications regarding the use of carbon nanotubes in glucose sensors. Operating potentials are given relative to the Ag / AgCl couple.

1.16 Sensors for Hydrogen Peroxide

Hydrogen peroxide is an important analyte in food, pharmaceutical, clinical, industrial and environmental analysis. In the past it has been determined using a number of techniques, including titrimetry [140], spectrometry [141] and chemiluminescence [142]. These techniques are hampered by interferences, long analysis time and expensive reagents.

Liu *et al.* [143] reported for the first time the preparation of enzyme nanoparticles, which they successfully incorporated into a reproducible peroxide biosensor. After desolvation with ethanol, the enzyme horseradish peroxidase (HRP) was cross-linked with glutaraldehyde. The enzyme particles were then functionalised with thiols, enabling immobilisation on a gold electrode. The detection limit using this configuration was found to be 0.1 μM .

A peroxide biosensor based on HRP-labelled gold colloids immobilised on gold electrodes by cysteamine monolayers has been reported by Xiao and co-workers [144]. Through the use of a catechol mediator in solution, this sensor was found to operate at the very low potential of -100 mV *vs.* SCE (-55 mV *vs.* Ag / AgCl). The response was linear in the range 0.39 to 330 μM and the response time was five seconds. A detection limit of 0.15 μM was achieved.

In 1996, Li and co-workers [145] reported on a peroxide biosensor based on horseradish peroxidase immobilised in a silica sol-gel matrix on a carbon paste electrode. This work involved the use of hexacyanoferrate (II) as an electron transfer mediator. The sensor was useful for H_2O_2 concentrations ranging from 0.02 to 2.6 mM at an operating potential of -100 mV (*vs.* Ag/AgCl). It also showed impressive selectivity in the presence of various interferent species.

Wang and Dong [146] constructed a peroxide biosensor by coating a sol-gel-peroxidase layer onto a Nafion / methylene green-modified glassy carbon electrode. These workers reported a sensitivity and detection limit of 13.5 $\mu\text{A mM}^{-1}$ and 10^{-7} M, respectively. The sensor was useful for H_2O_2 concentrations between 0.0005 and 1.6 mM at an operating potential of -150 mV (*vs.* Ag / AgCl). More recently, Li *et al.* [147] used screen-printed carbon paste electrodes modified by the electropolymerisation of pyrrole with horseradish peroxidase entrapped. The sensor was useful for peroxide concentrations

between 0.1 and 2.0 mM at a significantly more negative operating potential of -300 mV (vs. Ag / AgCl).

To our knowledge the first application of carbon nanotubes in peroxide sensing came in 2003 when Wang and Musameh [83] constructed a sensor based on a dispersion of multi-walled nanotubes within a Teflon binder. The operating potential was +400 mV (vs. Ag / AgCl). In the same year, Yu and co-workers [55] reported on two different peroxide biosensors based on myoglobin and horseradish peroxidase adsorbed onto vertically aligned single-walled carbon nanotubes. These authors attributed the enzyme adsorption to the formation of amide linkages between the protein and surface oxides present on the nanotubes. Subsequently Zhao *et al.* [123] used a MWCNT-modified glassy carbon electrode to study the direct electrochemistry of cytochrome *c* and to cathodically determine hydrogen peroxide following the adsorption of the enzyme on the nanotube surface. At an operating potential of -200 mV (vs. SCE) the useful range of this sensor was found to be between 2 and 420 μ M peroxide. Impressive selectivity was observed when the system was exposed to a number of interferent species.

Hydrogen peroxide is a particularly interesting analyte in the field of nanotube electrochemistry because its detection by NT-modified electrodes has recently been shown to be dominated by metallic impurities present in the nanotube film, and *not* by oxygenated functionalities present at defect sites [139,148]. This was done by direct comparison between an edge-plane HOPG electrode and a HiPCo[®] SWCNT-modified bpHOPG electrode. While the nanotube electrode gave rise to a peroxide reduction peak, no response was obtained using epHOPG, therefore it was concluded that edge plane sites did not figure in the electro-catalysis. The iron impurities were shown by XPS to be most likely in the form of Fe₃O₄. These impurities cannot be easily removed [149] so their role is difficult to verify absolutely but it is very likely that they are responsible for the catalysis of the peroxide reaction, meaning that nanotubes are attractive candidates for non-enzymatic peroxide sensors. This possibility will be investigated in *Chapter Three*.

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Chapter Two: Experimental Theory and Methodology

This chapter will begin with theoretical descriptions of some fundamental electrochemical concepts and the various electrochemical techniques used in this work, with emphasis on the differences between results predicted for species in solution and those for species adsorbed on electrode surfaces. It will then move on to a detailed description of the electrodes, instruments and reagents used, before finishing with accounts of the electrode modification procedures employed in the present work. It is hoped that this treatise will be sufficiently transparent to provide a complete understanding of how systems were prepared, as important details are often omitted from publications. This chapter is indebted to the excellent book by the Southampton electrochemistry group [1] for its concise descriptions of electrochemical techniques. It has been attempted, where feasible, to draw original figures but in some cases straight copies from said text have been included, for which, of course, these authors are credited.

2.1 Fundamental Electrochemistry

Broadly speaking, electrochemical reactions may be viewed as sequences of several events. These are the diffusion of a reactant to an electrode surface, rearrangement of ions at the interface, adsorption of the reactant onto the electrode, electron transfer between reactant and electrode, and finally diffusion of product away from the electrode. When one of these steps is rate-determining, the dependence of the current on the applied potential is greatly simplified. For an electrochemical process involving the transfer of n electrons, the steady-state current is given by

$$i = nFAk_D \left(\frac{k_c C_O}{k_c + k_D} - \frac{k_a C_R}{k_a + k_D} \right) \quad (2.1.1),$$

where A is the surface area of the electrode, k_D is the diffusional rate constant (cm s^{-1}), and C_O and C_R are the surface concentrations (mol cm^{-3}) of the oxidised and reduced forms of the redox couple, as distinct from their bulk concentrations. The electron transfer rate constants (cm s^{-1}) for the cathodic and anodic processes are given the symbols k_c and k_a , respectively.

The first term in the brackets in this equation gives the cathodic current when multiplied by $nFAk_D$, while the second term describes the anodic current. When the reaction is close to equilibrium these currents are comparable in magnitude and flow in opposite directions. The equilibrium potential (sometimes referred to as the *rest potential*) of a cell is the potential at which the net current is zero. The *over-potential*, η , is defined as the difference between the applied potential, E , and this equilibrium potential:

$$\eta = E - E_{eq} \quad (2.1.2).$$

The kinetics of electron transfer, characterised by k_c and k_a , are highly sensitive to η , whereas k_D is independent of the over-potential. Hence, the magnitude of the applied potential determines whether the reaction is under surface (electrode kinetics) control or mass transport (diffusion) control. The issue of whether a reaction is controlled by one or the other is of course an important one, and the two types of system are analysed in different ways. This point will now be illustrated within the context of potential sweep techniques.

2.2 Potential Sweep Techniques

In electrochemistry, potential sweep techniques are the most commonly employed in preliminary mechanistic investigations. The most popular of these is undoubtedly cyclic voltammetry, but linear sweep voltammetry (LSV) is sometimes used. Voltammetry may be thought of as electrochemical spectroscopy, the results of which indicate clearly the potentials at which oxidation and reduction processes occur. While other techniques may be more useful in the determination of precise kinetic data, the simplicity of voltammetry and the fundamental importance of its results mean that it is virtually always the first technique used when studying a new system. The variation of applied potential with time for a sweep experiment is shown in Figure 2.2. It is easy to see why the technique is sometimes called *cyclic triangular wave voltammetry*.

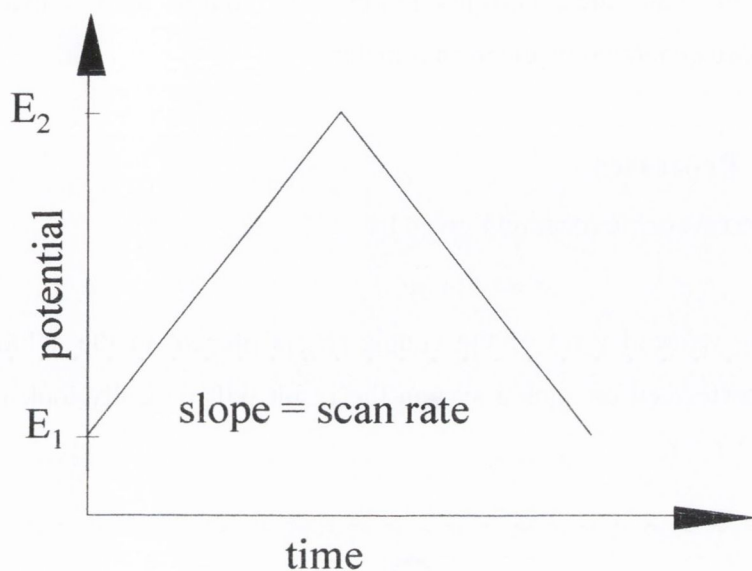


Fig. 2.2 The variation of applied potential with time for a cyclic voltammetry experiment.

In LSV the potential is swept from one value to another at a known rate called the *sweep rate* (symbol v). The value of the latter is given simply by the magnitude of the slope of the E - t profile. In cyclic voltammetry, on reaching E_2 , the potential is swept back again. The final potential need not necessarily be the same as the initial but this will be the case in the experiments described in this work. In sweep voltammetry, the cell current is recorded as a function of the applied potential. Typically, scan rates range from a few mV s^{-1} to a few V s^{-1} . Higher values invite serious practical problems in relation to the effects of double layer charging and solution resistance. The use of microelectrodes can help with these issues.

The electrical double layer is the interface between electrode and electrolyte. Most of the potential drop occurs across this region and its width is typically around one nanometre. It is formed by the segregation of positive and negative charge. These charges may be associated in the form of dipolar molecules or polarised atoms, or they may be free as holes, electrons or ions. Segregation may occur through the preferential adsorption of either positive or negative ions at the interface, the transfer of charge across the interface or the deformation of polarisable molecules by the asymmetrical force field at the interface. Ideally, an electrostatic equilibrium is established like that in a parallel plate

capacitor. At high scan rates, currents required to charge these capacitors can be comparable to those generated by electron transfer.

2.2.1 Solution Processes

Consider a simple reversible oxidation given by



Initially, only the reduced form of the couple (R) is present in the solution. If cyclic voltammetry is performed on such a system the result will typically look like the curve shown in Figure 2.2.1.1.

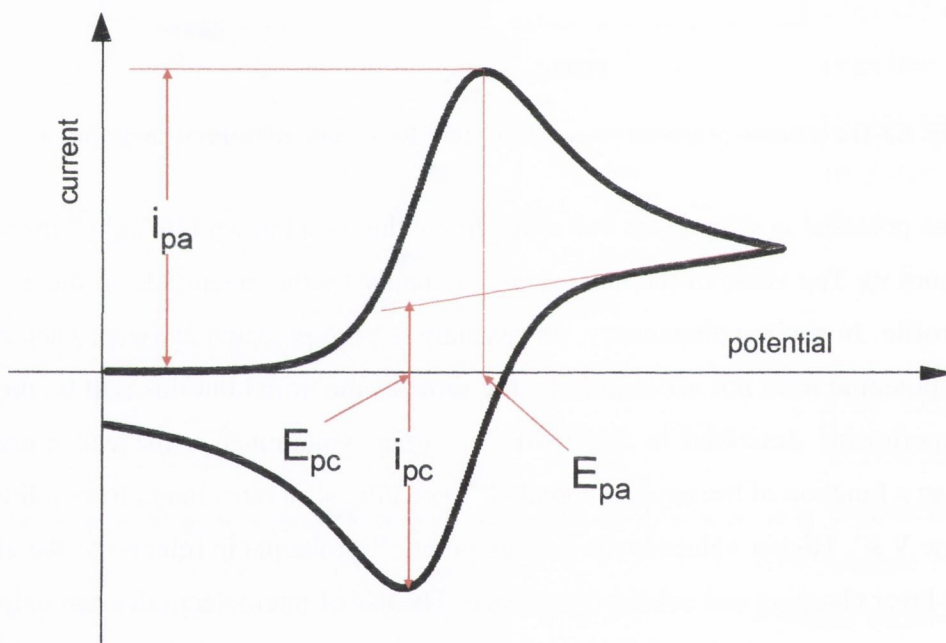


Fig. 2.2.1.1 Typical cyclic voltammetry result for a reversible system. Peak currents and potentials are measured as indicated.

The potential is initially swept anodically, resulting in an oxidation peak representing the process as written in Equation 2.2.1.1. The potential is then swept cathodically back to its initial value and the resulting reduction peak corresponds to the reverse process. Note that the cathodic peak current (i_{pc}) is (crudely) measured relative to a tangent drawn to the curve before the peak begins. Technically the same procedure should be used when calculating the anodic peak current (i_{pa}).

For a reversible redox process, the ratio of the concentrations of the oxidised and reduced forms of the couple (C_O / C_R), within the region next to the electrode surface, varies with the applied potential in accordance with the Nernst equation:

$$E = E^0 + \frac{RT}{nF} \ln \frac{C_O}{C_R} \quad (2.2.1.2)$$

where E^0 is the formal potential of the redox couple. So when the applied potential is moved to a value anodic of the standard potential, this ratio increases. In practical terms, systems which behave reversibly may be defined as those which show rates of electron transfer which are fast compared to the scan rate being used. The current response in Figure 2.2.1.1 represents the current passing between the working electrode and the counter electrode. This current, i , is proportional to the concentration gradient at the electrode surface according to Fick's First Law:

$$i = nFAD \frac{d}{dx} C_R \quad (2.2.1.3)$$

where A is the geometric area of the working electrode, D is the diffusion coefficient of the electroactive species and x is the distance from the electrode surface. In order to rationalise the shape of the curve in Figure 2.2.1.1, consider the concentration profiles shown in Figure 2.2.1.2.

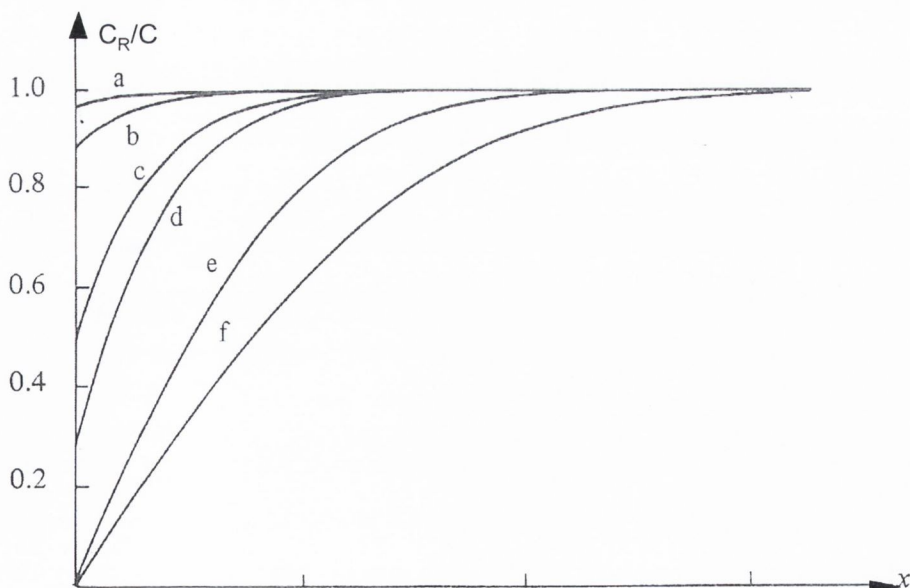


Fig. 2.2.1.2 Concentration-distance profiles for the electroactive species, R , in the oxidation process during a linear potential sweep. The curves correspond to the potentials (a) $E^0 - 90$ mV, (b) $E^0 - 50$ mV, (c) E^0 , (d) $E^0 + 30$ mV, (e) $E^0 + 130$ mV, (f) $E^0 + 280$ mV. Reproduced from [1].

Initially, at cathodic potentials, the concentration of R at the electrode surface is the same as C , its concentration in bulk solution. No oxidation process is occurring here so no significant current flows. As the potential is moved anodically towards the formal redox potential, C_R decreases at the electrode surface. A concentration gradient is thus established between this region and bulk, resulting in current flow. As the potential approaches the oxidation peak potential, R is consumed most rapidly and the current reaches a maximum value. Beyond this point diffusion of the reduced form to the electrode brings down the concentration gradient and the current decreases. This explains the occurrence of a peak corresponding to the anodic process. The form of the cathodic peak may be accounted for by a similar argument, although its analysis is complicated somewhat by the fact that some of the oxidised form is lost to bulk solution before it can be reduced. This also accounts for the fact that the charge transferred during the reduction process (given by the area under the peak) is generally less than that recorded for oxidation. Of course, if only the oxidised form is present in solution and the initial sweep is cathodic, the opposite should be observed.

When the scan rate is increased, the diffusion layer is not given sufficient time to relax, resulting in a more rapid consumption of species closer to the electrode surface. As a result, peak currents are observed to increase with sweep rate. In such experiments the response is controlled by the diffusion of electroactive species from bulk solution to the electrode surface. Migration effects are precluded by the use of relatively large concentrations of background electrolyte, and convection is avoided by carrying out experiments in unstirred solutions. The form of the current response is thus controlled by the scan rate, applied potential, electron transfer rate parameters, diffusion coefficient, bulk concentration, and finally the nature and geometry of the working electrode.

Under the conditions of semi-infinite linear diffusion, the peak current is given by

$$i_p = 0.4463nFA\sqrt{\frac{nF}{RT}}C^\infty\sqrt{D\nu} \quad (2.2.1.4)$$

where C^∞ is the bulk concentration of the reacting species. This is the *Randles-Sevcik equation*. The factor 0.4463 occurs when Fick's Second Law is solved for an ideal system [2]. It should be noted, however, that the diffusion characteristics of a system may only hold for a short time, as thermal and concentration gradients may induce random

convection processes, resulting in current fluctuations. For this reason, only data obtained from the first sweep are normally used in the evaluation of kinetic parameters.

The interface between the working electrode and the electrolyte solution is commonly referred to as the electrical *double layer* and the electrolyte acts like a capacitor. A current is therefore required to change the potential applied to the working electrode, and this is known as the *charging current*. Since the potential in a CV experiment is changing at a constant rate (the scan rate), there is a reasonably constant charging current throughout the experiment that makes a major contribution to the background current. This charging current is proportional to v , whereas peak currents are proportional to $v^{1/2}$ (see Equation 2.2.1.4). This means that the charging current limits the maximum scan rate which can be used to obtain well-defined peaks.

Another problem which can occur in cyclic voltammetry and lead to confusion in the interpretation of data is iR_u drop. This causes the applied voltage to change from its desired value, E , to a value given by $(E - iR_u)$. Because the current varies during a scan, this means that the sweep is no longer linear. This can result in decreases in peak currents and increases in peak separations. Since these are also characteristics of slow electron transfer kinetics, it is essential that these manifestations are not mistakenly attributed to the effects of solution resistance. The best way to do this is to scan a system which is known to behave reversibly (ferrocene, for example) under identical experimental conditions and check that it gives the expected response. During experiments, it is advisable to minimise the distance between the working electrode and the tip of the reference electrode as a defence against this hazard.

In order for a system to be accepted as being electrochemically reversible, the following conditions must be satisfied (at 25 °C):

1. Peak separation should be $59 / n$ mV.
2. $|E_p - E_{p/2}|$ should also be $59 / n$ mV.
3. $|i_{pa} / i_{pc}|$ should be unity.
4. Peak currents should be proportional to $v^{1/2}$.
5. Peak potentials should be independent of scan rate.

Note: $E_{p/2}$ is the value of potential at which the faradaic current has climbed to half of its maximum value.

In reality, such systems are unusual. Failure to satisfy one or more of these conditions suggests that the electron transfer is not reversible on the experimental timescale. When the scan rate is low, the rate of ET may be sufficient to ensure that the surface concentrations of each form of the redox couple have values consistent with Equation 2.2.1.2 and a 'reversible' scan is obtained, even if the process is irreversible. For this reason, it is important to investigate a large range of scan rates, preferably covering two orders of magnitude or as large a range as the charging current permits.

With totally irreversible systems, rates of electron transfer are always slower than those of mass transfer, regardless of the scan rate employed. This means that Nernstian equilibrium cannot be maintained at the electrode surface. The most apparent indication that a process is irreversible is the complete absence of a reverse peak, although this should not be mistaken for definitive proof. An irreversible process will also show a linear variation of peak current with sweep rate. Frequently it is observed that systems which appear reversible at low scan rates show themselves to become irreversible as the latter is increased. So for these systems, known as *quasi-reversible* systems, i_p - $v^{1/2}$ plots show two separate regions of linearity but as a whole are not linear, as shown in Figure 2.2.1.3. For irreversible systems, peak currents are less because the peaks themselves are more 'drawn out'. This is because the surface concentration of the electroactive species changes more slowly, meaning that the concentration gradients established are less severe.

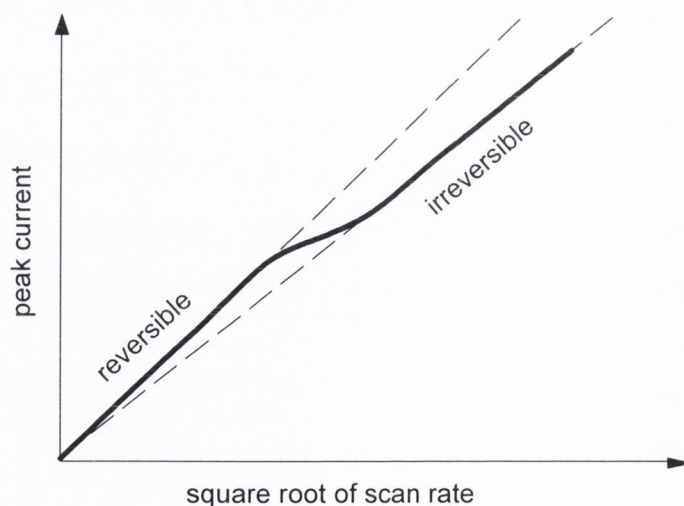


Fig. 2.2.1.3 The variation of peak current with the square root of scan rate, showing the transition from reversible to irreversible behaviour with increasing sweep rate.

2.2.2 Surface Processes

If voltammetric peaks exhibit a shape or sweep rate dependence unlike those discussed so far, it is likely that surface processes such as adsorption might be complicating the situation. Of course, in some experiments, such as the investigation of the direct electron transfer of redox enzymes (see *Chapter Five*), electroactive species are deliberately adsorbed onto the electrode surface and are not present in the electrolyte at all. For such systems the response should be similar to that shown in Figure 2.2.2, which depicts an ideal scenario in which charging currents are not significant, electron transfer is totally reversible, and both forms of the redox couple are strongly adsorbed. Peaks are sharper and more symmetrical and peak separations are low when compared to solution processes. Also, after the initial sweep, there should be no significant loss of product to the solution, meaning that the charges transferred ought to be similar for both sweeps. Quasi-reversible adsorbed systems show less symmetrical peaks and significant peak separations, particularly at high sweep rates.

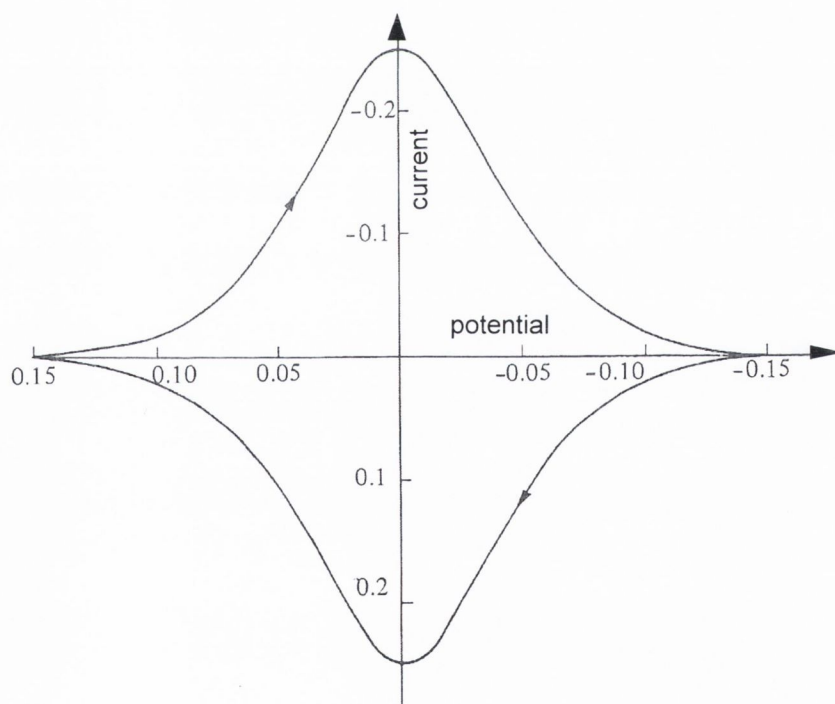


Fig. 2.2.2 Theoretical voltammogram for the redox process of adsorbed species. Reproduced from [1].

2.3 Potential Step Chronoamperometry

Using this technique, the potential of the working electrode is changed instantaneously, rather than swept, to a new value. The response of the current to the perturbation is then recorded as a function of time. Consider again an oxidation reaction in which only the reduced form is present in solution, as in *Equation 2.2.1.1*. The potential-time profile for a step experiment is shown in Figure 2.3.

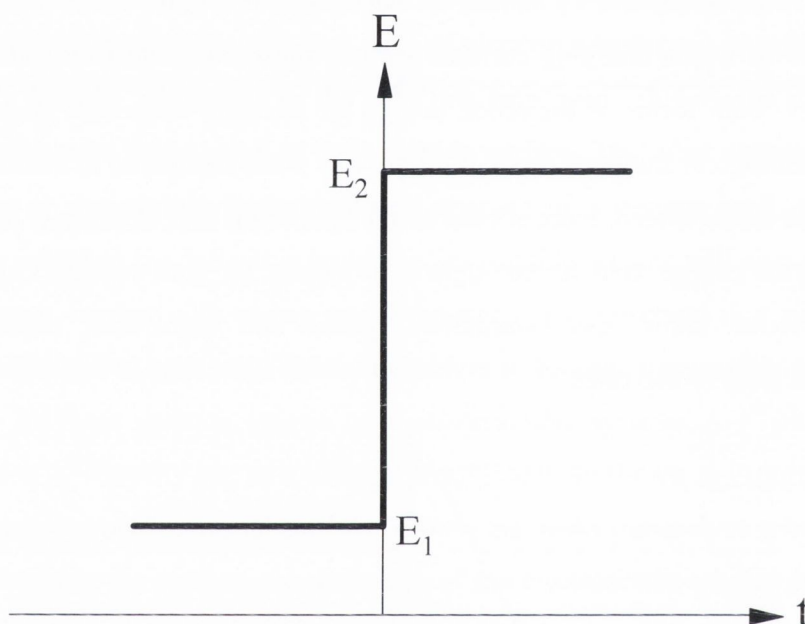


Fig. 2.3 The potential-time profile for a single potential step chronoamperometric experiment.

The initial potential is chosen such that no oxidation is occurring. The final potential is chosen such that the anodic process occurs at a diffusion-controlled rate. Immediately after the potential is stepped, species next to the electrode surface are oxidised. Subsequently, the current response will be governed by the diffusion of species from bulk solution. It can be shown that, for a species diffusing from bulk solution to an electrode, the current magnitude will decay with time according to the Cottrell equation [3]:

$$|I| = \frac{nFAD^{1/2}C_R}{\pi^{1/2}t^{1/2}} \quad (2.3.1)$$

Plots of current against $t^{-1/2}$ should therefore be linear and pass through the origin. This is frequently used as a test for diffusion control. Slopes of these plots also permit the calculation of diffusion coefficients.

In the potential step experiments described in this work, however, we will be concerned primarily with electroactive species adsorbed on the electrode surface rather than those present in the electrolyte. The variation of current with time for these systems is not the same, and what follows is a brief derivation of the expected i - t response, based on some of our published work [4,5]. Again an oxidation process in which only the reduced form of the couple is present is considered (this time as a monolayer on the electrode surface). At any time after the potential is stepped to a value sufficiently anodic for oxidation to occur, we may define the total surface coverage as the sum of contributions made by the two components of the redox couple:

$$\Gamma_{\Sigma} = \Gamma_R + \Gamma_O \quad (2.3.2).$$

Initially, of course, Γ_O is zero and the total coverage is simply Γ_R . After the step, assuming no loss of species to solution, the total coverage is maintained at a constant value by decreases in Γ_R and corresponding increases in Γ_O . The rate of the oxidation process is proportional to the surface coverage of R . This rate decreases as time elapses after the sweep so for any such time it may be stated that

$$\text{Rate} = -\frac{d\Gamma_R}{dt} = +\frac{d\Gamma_O}{dt} = k\Gamma_R \quad (2.3.3),$$

where k is the reaction rate constant. The current flowing is proportional to the rate at which R is losing electrons:

$$\begin{aligned} i &\propto k\Gamma_R \\ \Rightarrow i &= nFAk\Gamma_R \end{aligned} \quad (2.3.4).$$

Integration of Equation 2.3.3 subject to the initial condition that $\Gamma_{\Sigma} = \Gamma_R$ when $t = 0$ gives

$$\Gamma_R = \Gamma_{\Sigma} e^{-kt} \quad (2.3.5).$$

Combination of this with Equation 2.3.4 yields a simple expression for first-order kinetics:

$$\begin{aligned} i &= nFAk\Gamma_{\Sigma} e^{-kt} \\ \Rightarrow i &= Qke^{-kt} \end{aligned} \quad (2.3.6).$$

Here, Q is the total charge transferred when R has been completely oxidised. So it is clear that, for this idealised scenario in which all R molecules are adsorbed at energetically equivalent sites, plots of $\ln i$ against t should be linear. The slopes of these give values for the rate constant and the charge transferred may then be obtained from the y -intercept. Real systems often deviate from this model behaviour and in *Chapter Five*, such departures will be discussed in terms of results obtained during the course of the present work.

2.4 Electrochemical Impedance Spectroscopy

This technique involves the use of very small excitation amplitudes (five to ten millivolts) to generate alternating currents. These cause minimal perturbation to the system so data obtained are relatively reliable. Impedance is simply the AC equivalent of resistance. The response is monitored as a function of perturbation frequency. The interface between an electrode and an electrolyte may be represented by an equivalent circuit comprised of resistors and capacitors (see Figure 2.4.1). This circuit is known as the *Randles equivalent circuit* [6]. It incorporates the double layer capacitance as described in *Section 2.2.1*. The resistance of the electrochemical reaction is in parallel with C_{dl} and represented by a charge transfer resistance and a Warburg impedance due to diffusional mass transport. The relative size of these two contributions is a measure of the balance between kinetic and diffusion control. Kinetically sluggish systems have large R_{ct} values. If there is any uncompensated solution resistance between the point at which the potential is measured (the tip of the reference electrode) and the working electrode, this too appears as part of the measured total impedance.

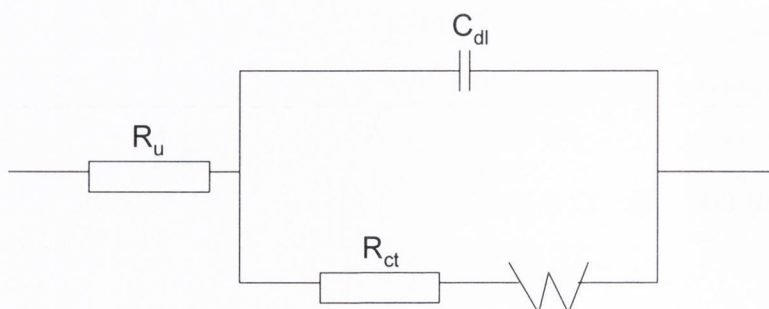


Fig. 2.4.1 Equivalent circuit for an electrode reaction with double layer capacitance C_{dl} and uncompensated solution resistance R_u . The reaction resistance has been resolved into the charge transfer resistance R_{ct} and a Warburg diffusional impedance.

With alternating current, potential and current can show a phase difference, which can be represented by an impedance Z , consisting of a combination of resistances and capacitances. This resistance is conveniently represented on a complex plane and has a real part, Z' , and an imaginary part, Z'' . In an Argand diagram the *Randles* equivalent circuit would appear as in Figure 2.4.2. This representation of impedance data is known as a *Nyquist plot*.

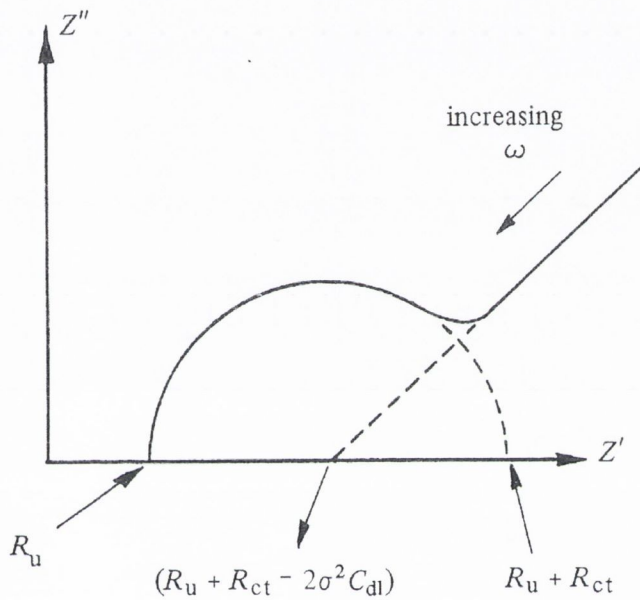


Fig. 2.4.2 Complex impedance plot for the Randles equivalent circuit. Reproduced from [1].

In the figure σ is the *Warburg coefficient*. When the frequency, ω , is high, the plot shows a semicircular region with a centre given by $Z' = R_u + \frac{1}{2}R_{ct}$ and a radius of $\frac{1}{2}R_{ct}$. In this region, the system is under kinetic control. With the current alternating rapidly, impedance is dominated by the double layer charging and charge transfer processes. Diffusion is a much slower process, so the Warburg impedance in this region is negligible.

At low frequencies, diffusion dominates and a linear relationship between the real and imaginary components is observed according to

$$Z'' = Z' - R_u - R_{ct} + 2\sigma^2 C_{dl} \quad (2.4.1).$$

This line is of unit slope for reversible systems, in which R_{ct} may be neglected. For kinetically sluggish systems, this region should be relatively small, and the slope of the

line will deviate from unity due to the contribution of charge transfer resistance. Impedance spectroscopy is a steady-state technique conducted at equilibrium, as opposed to sweep techniques, in which the potential of the working electrode is driven far from its equilibrium value. At equilibrium, the charge transfer resistance and Warburg coefficient are given by

$$R_{ct} = \frac{RT}{nFi_0} \quad (2.4.2)$$

$$\text{and } \sigma = \frac{RT}{2^{1/2} n^2 F^2 AD^{1/2}} \left(\frac{1}{C_O} + \frac{1}{C_R} \right) \quad (2.4.3).$$

It can be seen from these that R_{ct} yields a value for the exchange current i_0 , which is an indication of the electron transfer kinetics and its activation barrier. The exchange current is defined as the magnitude of the oxidation and reduction currents at equilibrium, which, of course, are equal and opposite. On the other hand, as long as the bulk concentrations of electroactive species are known (C_O and C_R), the Warburg coefficient permits calculation of the diffusion coefficient.

2.5 Batch Amperometry

This is a simple technique used to determine the suitability of an electrode for the sensing of a particular analyte. It involves holding the applied potential at a constant value and measuring the current response to increases in the concentration of electroactive species. The potential, of course, is chosen such that the desired process takes place, and its value is most easily indicated by voltammetry. It is desirable that the operating potential should be as low as possible because strong potentials invite interfering signals from other species which may be present in the solution. When using stationary electrodes, as in the present work, convective transport is usually supplied by magnetic stirring while known aliquots of the substrate are literally dropped into the electrolyte. The current is allowed to stabilise before each addition, and an ideal experimental output is shown in Figure 2.5.

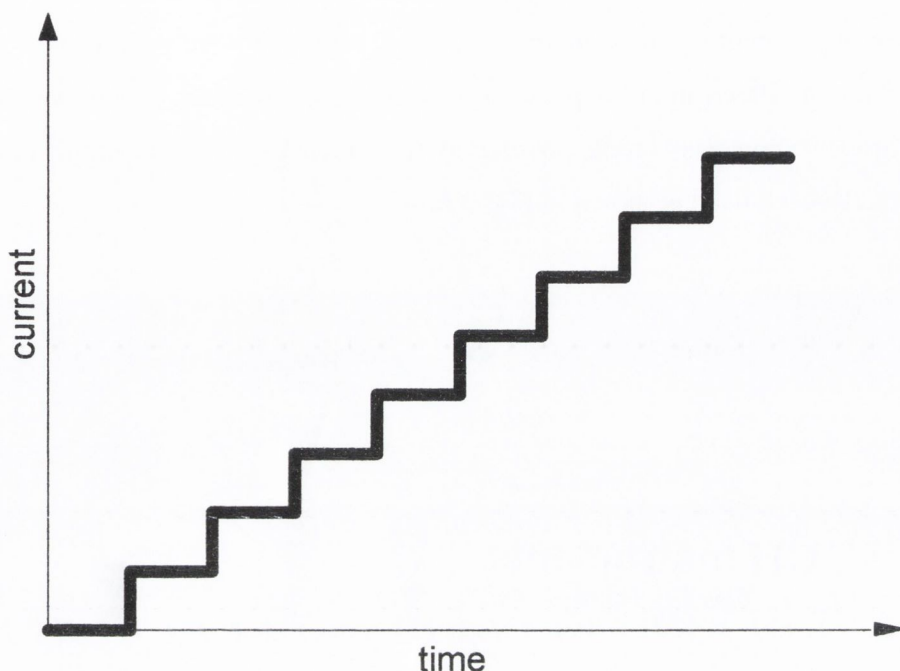


Fig. 2.5 Ideal experimental output of a constant-potential batch amperometry experiment. Steep increases in current indicate a fast response time.

The figure shows how each addition leads to an increase in the measured current. In reality, the initial current is of course not zero, due to the background response. Data are analysed using a *calibration plot*, which is a graph of current against the concentration of the electroactive species. If the background current is subtracted, this should be a straight line through the origin, the slope of which gives the sensitivity of the sensor. Another important characteristic of a sensor is, of course, its limit of detection. This is the smallest increase in concentration to which the current responds significantly. Stirring of the solution can lead to significant noise, so detection limits are commonly reported on the basis of signal-to-noise ratios of *three*.

2.6 The Electrochemical Cell

All electrochemical experiments reported in the present work were conducted in a cell consisting of a glass working vessel (maximum capacity $\sim 15 \text{ cm}^3$), three electrodes and an electrolyte solution. The cell was sealed with a cover which contained holes for the three electrodes and a fourth hole which was used for maintaining an atmosphere of

nitrogen over the solution (when required) and additions of analytes in the batch amperometry experiments. Unless otherwise stated, solutions were purged with nitrogen for a minimum of fifteen minutes prior to measurements in order to remove dissolved oxygen. Nitrogen shows no redox process in the potential windows employed in the present work. The cell used is shown in Fig. 2.6.



Fig. 2.6 The electrochemical cell used in the present work, along with three electrodes and a nitrogen inlet. Behind it is the CH Instruments potentiostat.

In electrochemistry, data obtained occur due to processes taking place at the surface of the working electrode. This electrode ideally is smooth and chemically inert with respect to the solvent and any species dissolved in the electrolyte. The useful potential range of a working electrode is difficult to state accurately as complications due to the evolution of hydrogen or oxygen from water or the decomposition of whatever solvent is being used can occur. Glassy carbon macroelectrodes (model CHI104) were supplied by IJ Cambria. These consisted of glassy carbon discs (radius 1 mm) supported on the polymer Kel-F. The latter is chemically similar to Teflon. In accordance with the manufacturer's instructions, these electrodes were neither exposed to temperatures exceeding 60 °C (this can deform Kel-F) nor cleaned using an ultrasonic bath (this can damage the contact between the GC disc and the external circuit).

The purpose of the reference electrode is to provide a stable potential which does not change during an experiment. The potential of the working electrode may then be measured with respect to this fixed value. In the present work, a Ag / AgCl reference (IJ Cambria, model CHI111) was used. This consisted of a piece of silver wire coated with a layer of silver chloride, immersed in 3 M potassium chloride. It was stored in 3 M KCl when not in use. Another reference electrode which will be referred to from time to time when discussing work carried out by others is the saturated calomel electrode (SCE). A description of how to convert between these two systems can be found in *Section 3.4*.

The purpose of the counter (sometimes called *auxiliary*) electrode is to supply the current required by the working electrode. Basically, its function in the cell is to protect the reference electrode because if this task were the responsibility of the latter, the stability of its potential could be jeopardised. It is important to note that the surface area of the counter electrode should be considerably larger than that of the working electrode. This ensures that no significant polarisation of the former occurs and the characteristics of the auxiliary electrode do not impose themselves on the response of the cell. In the present work a platinum wire counter supplied by IJ Cambria (model CHI115) was used.

An electrolyte solution should contain a large concentration of background ions in order to reduce the resistance between the working and counter electrodes. Also, if the electroactive species is present in solution, the supporting electrolyte will minimise the effect of migration on it by taking on the burden of charge transfer through the solution. In this work electrolyte solutions were either potassium chloride (0.1 or 0.2 M) or 50 mM phosphate buffer.

2.7 Instrumentation

A CH Instruments Model 440 potentiostat was used to perform all electrochemical experiments. The system contains a fast digital function generator, high speed acquisition circuitry and a galvanostat. The potential control range of the instrument is ± 10 mV and the current range is ± 250 mA. A pH meter (model 632, Metrohm) was used to read the pH of buffered solutions.

Infrared spectra were recorded using a Mattson Genesis II FTIR spectrometer. Measurements were performed in a Harrick demountable liquid cell in which the liquid

sample was placed between two calcium fluoride plates separated by a Teflon spacer. Large numbers of accumulations were acquired (typically 64) and spectra were suitably baseline-adjusted.

Raman spectra were recorded in ambient conditions using a Reinshaw 1000 micro-Raman system equipped with a Leica microscope. The 50× magnifying objective of the latter focussed a laser beam onto a spot roughly one micron in diameter. The excitation wavelength was 514.5 nm from an Ar⁺ laser (Laser Physics Reliant 150 Select Multi-Line) with a typical laser power of around 0.5 mW in order to avoid excessive heating. An 1800 line / mm diffraction grating was used in all measurements, which permitted a resolution of approximately 1 cm⁻¹.

Atomic force microscopy images of SWCNT films on gold surfaces were recorded in ambient conditions using an Asylum MFP-3D microscope. Imaging conditions were such that an attractive force was maintained between the tip and the film in order to minimise disruption of the sample. The probe used was a Nanosensors PPP-EFM lever with spring constant 0.1 Nm⁻¹, resonance frequency 69 kHz and maximum radius ten nanometres.

2.8 Materials and Reagents

Purified single-walled carbon nanotubes (HiPCo[®]) were purchased from Carbon Nanotechnologies, Inc., and used as supplied (lot no. PO289). It should be pointed out that these came with a disclaimer of warranties and were sold 'as is' and 'that there may be variations in the characteristics of products, and CNI expressly disclaims any warranties related to any samples that CNI may from time to time provide.' [7]. Using thermo-gravimetric analysis, it was shown that the iron content of these nanotubes was around 12 wt %.

Glucose oxidase (type VII, 221 kUg⁻¹, from *Aspergillus niger*), ascorbic acid (99.0 %), potassium ferricyanide (99.0 %), potassium ferrocyanide (99.0 %), potassium chloride (99.0 %), norepinephrine (98.0 %), NADH (99.0 %), ferrocene carboxylic acid (>97.0 %), graphite (particles < 20 microns), Nafion (~5 % in volatile alcohols), dopamine hydrochloride and epinephrine were purchased from Sigma-Aldrich. Disodium

hydrogen phosphate dodecahydrate (>98.5 %) and monosodium dihydrogen phosphate dihydrate (>98.0 %) were purchased from Merck.

β -D-glucose was purchased from ICN Biomedicals, Inc. Glucose stock solutions were stored overnight at room temperature before use to allow equilibration of anomers. Hydrogen peroxide (30 wt %) was purchased from *Sigma-Aldrich*. This was diluted down to the appropriate concentration freshly each day and used immediately. Oxygen-saturated solutions were made by purging with high-purity O_2 for at least ten minutes. The concentration of saturated aqueous oxygen solutions at room temperature is roughly 1.2 mM [8]. All solutions were prepared with water (18.2 M Ω cm) from an Elix[®] Millipore system.

A 50 mM pH 7.0 phosphate buffer was purchased from *Sigma-Aldrich* and used as background electrolyte for neutral pH experiments. For studies in which pH had to be varied, buffers of various pH values were prepared by dissolving different ratios of monosodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$) and disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) in millipore water. The amounts of each substance used to obtain a particular pH value are listed in *Table 2.8*.

pH	mass of $NaH_2PO_4 \cdot 2H_2O$ / g	mass of $Na_2HPO_4 \cdot 12H_2O$ / g
5.2	0.7636	0.0379
5.8	0.7183	0.1419
6.4	0.5809	0.4571
7.6	0.1213	1.5123
8.2	0.0345	1.7116
8.8	8.954×10^{-3}	1.7701

Table 2.8 The amounts of each component used to prepare 50 mM phosphate buffers of various pH values. The two substances were dissolved in 100 cm³ of solution.

2.9 Electrode Modification Techniques

In this work the surfaces of the glassy carbon electrodes were renewed by carrying out a standard polishing procedure prior to experiments in order to yield a reproducible surface

morphology and freedom from adsorbed impurities, as verified by cyclic voltammetry. The polishing kit used was purchased from IJ Cambria and contained a range of alumina polishing powders (1.0, 0.3 and 0.05 microns) and a range of polishing pads made from different materials (Nylon and Microcloth). Pads were 73 mm in diameter.

Initially, electrodes were polished by a 'figure-of-eight' motion on Nylon pads covered with a paste of 1.0 micron alumina in millipore water (see Figure 2.9.1). After thorough rinsing with the latter, they were polished in the same way using 0.3 micron alumina on Nylon pads. Finally, after more rinsing, they were polished once more, using 0.05 micron alumina on Microcloth pads. When carrying out measurements using bare glassy carbon, the electrodes were placed immediately in the electrochemical cell. When further modification was required, they were blown dry using a stream of nitrogen and promptly modified.

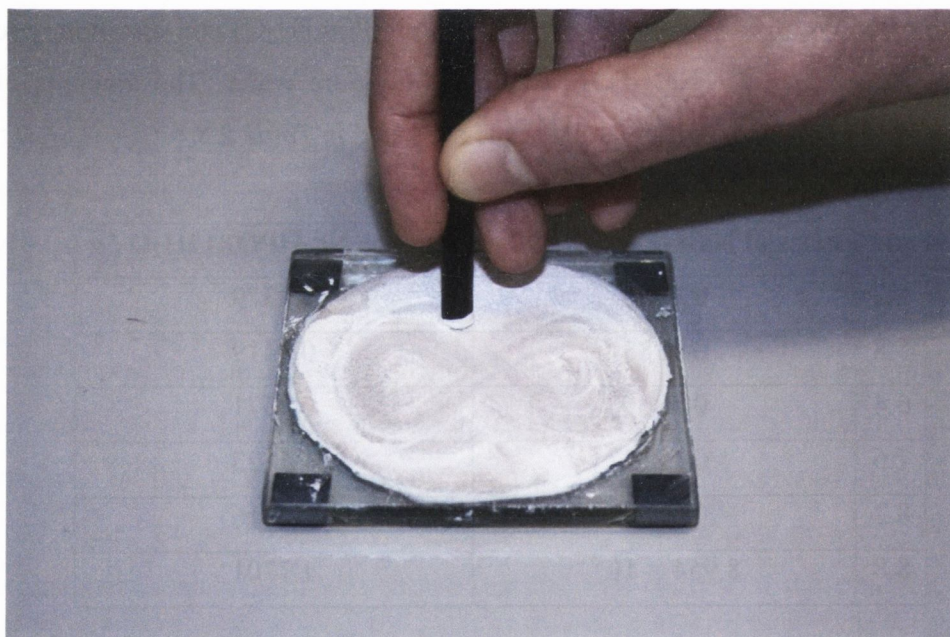


Fig. 2.9.1 The 'figure-of-eight' polishing procedure used for cleaning the surface of the working electrode.

Suspensions of carbon nanotubes were prepared by adding SWCNTs (1 mg) to DMF or NMP (10 cm^3) as required and sonicating for five minutes to yield uniform dispersions which were found to be stable for many months (see Figure 2.9.2).

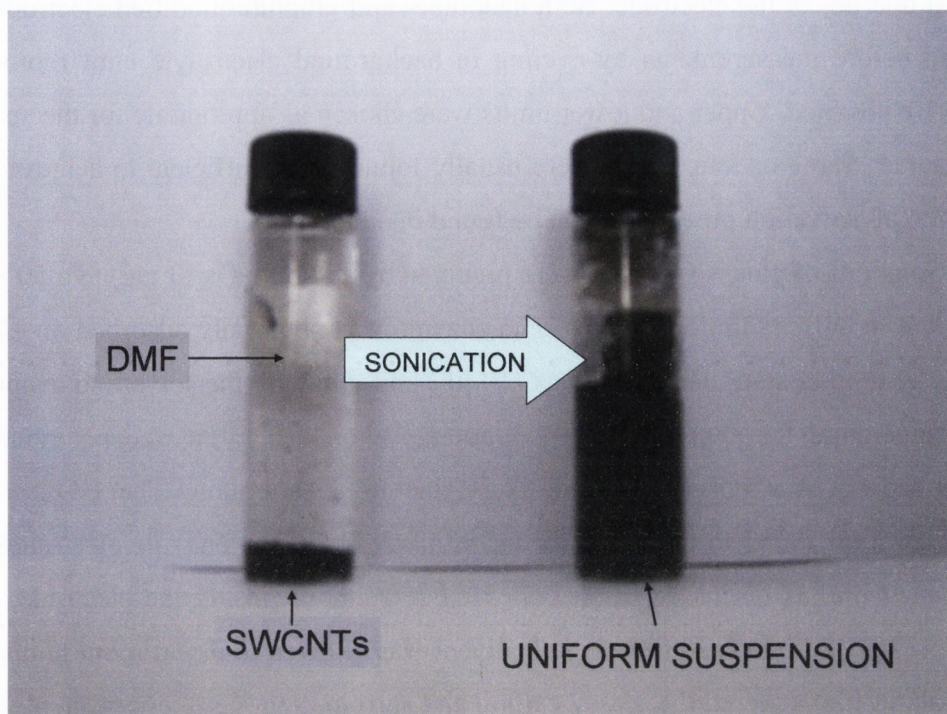


Fig. 2.9.2 Photograph of single-walled carbon nanotubes in DMF before and after sonication.

In order to fabricate GC/SWCNT electrodes, these suspensions were dropped ($4\ \mu\text{L}$) on inverted glassy carbon electrodes. The latter were then covered using clamped sample tubes and the solvents were evaporated by exposure to a fan heater at $40\ ^\circ\text{C}$. This resulted in the deposition of roughly $0.4\ \mu\text{g}$ of nanotubes on the electrode surface. Tubes were often seen to ‘spill over’ onto the Kel-F casing so this value of $0.4\ \mu\text{g}$ may be interpreted as a maximum. The films were clearly visible to the naked eye. It was found that $13\ \mu\text{g cm}^{-2}$ ($0.4\ \mu\text{g}$ on an electrode with geometric area $0.03\ \text{cm}^2$) gave optimal electrochemical performance, with greater coverages sometimes resulting in evidence of passivation of the electrode surface, possibly due to the poor conductivity across the walls of the tubes compared to along the walls. The GC/SWCNT electrodes were rinsed sequentially with millipore water and working electrolyte before experiments.

Glassy carbon /graphite electrodes were prepared in the same way except that the concentration of the graphite suspensions was $3\ \text{mg}$ in $10\ \text{cm}^3$. When lower concentrations were used, the dispersions were very pale compared to those obtained using nanotubes and they were found to have little or no effect on the electrochemical

properties of the electrode. Both nanotube- and graphite-modified electrodes were activated before measurements by cycling in background electrolyte until reproducible scans were obtained. Upper and lower limits were chosen as appropriate for the species to be measured. Three or four cycles were usually found to be sufficient to achieve this. A discussion of activation procedures can be found in *Section 1.3*.

Solutions of glucose oxidase were prepared by adding GOx (3 mg) to a 50 mM pH 7.0 phosphate buffer solution (1 cm³). The enzyme was physically adsorbed on electrode surfaces by drop-coating these solutions (10 μ L) and allowing the solvent to evaporate at room temperature for approximately 2.5 hours. When heat was used no evidence of enzyme activity was observed, most likely due to denaturation. The GOx solutions themselves were found to be stable for up to a week, but the enzyme electrodes had a shorter shelf-life, as discussed in *Section 5.2.1.1*. Without rinsing the electrode, Nafion solution (1 μ L) was cast and the alcohol solvent was allowed to dry at room temperature. The resulting film covered the glassy carbon and surrounding Kel-F encasement. Finally, the modified electrode was rinsed with buffer solution and, with residual buffer still covering the electrode surface, transferred immediately to the cell.

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Chapter Three: Characterisation of Carbon Nanotubes

As already mentioned, the electrochemical properties of carbon nanotubes depend very much on how they are made and purified. It is therefore considered pertinent to begin the discussion of the results obtained in the present work with a thorough characterisation of the HiPCo[®] single-walled nanotubes used in the experiments, before reporting on their electroanalytical applications in later chapters. The aim of this chapter is to provide a clear picture of what the architecture is like on a NT-modified glassy carbon electrode, which will aid the understanding of the electro-catalytic properties found for the latter. It begins with physical characterisation before discussing the electrochemical experiments performed with a view to the elucidation of such important features as surface area and redox-active functionalities present on the nanotube surface.

3.1 Infrared and Raman Spectroscopy

In the large body of literature existing on the subject of nanotube electrochemistry, it is common for authors to mention findings obtained using FTIR, and sometimes Raman, spectroscopy. An infrared spectrum of the single-walled carbon nanotubes used in this work was obtained and is shown in Figure 3.1.1. The dominant peak found at 1625 cm^{-1} is attributed to carbonyl groups in carboxylate and the smaller peak around 1690 cm^{-1} to carbonyl groups in carboxylic acid functionalities, in accordance with previous reports [1,2,3]. These species were introduced to the nanotubes during acid purification. Since the purification process involved no base, it is suggested that the existence of the carboxylate groups may be due to the electro-ionisation of the carboxylic acid functionalities [4]. It should be pointed out that the spectrum shown is the result of considerable baseline adjustment and that the raw data had a very small signal-to-noise ratio. Also, while the assignments agree with those given by some workers, there are discrepancies in the literature as to where these functionalities appear in infrared spectra. For example, Chou and co-workers [5] assign a wave number of 1730 cm^{-1} to the carbonyl in carboxylic acids. It is noted that the carbon nanotube does not lend itself to infrared spectroscopy, since the number of carbonyl bonds is very small compared to the number of C-C bonds.

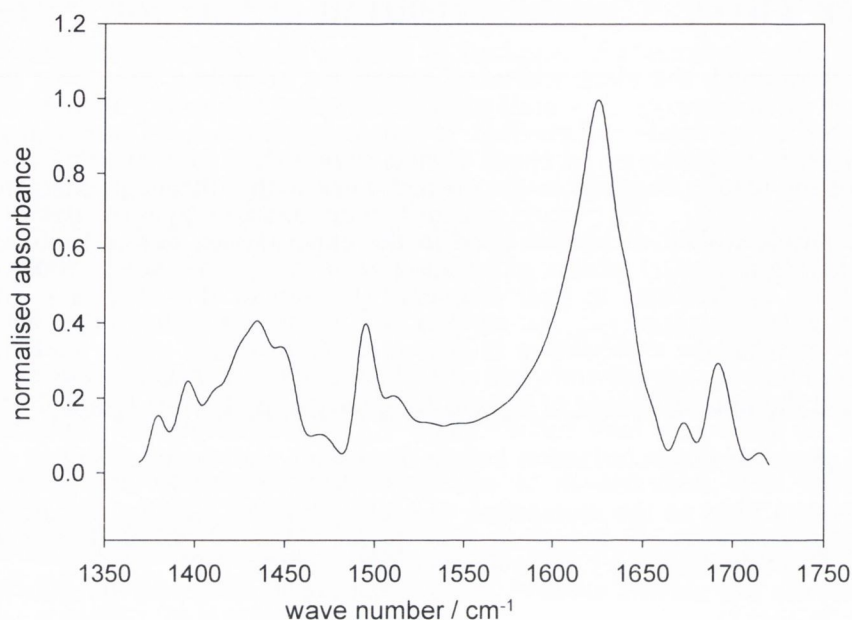


Fig. 3.1.1 FTIR spectrum of single-walled carbon nanotubes.

The single-walled nanotubes were also characterised using Raman spectroscopy, which has been identified as a sensitive probe for the structure of carbon materials [6]. Spectra were recorded over the range 1300-1700 cm⁻¹, covering the region where both the tangential modes derived from the in-plane Raman vibrations in graphite (*G* band, 1500-1600 cm⁻¹) and the disorder modes (*D* band, 1300-1400 cm⁻¹) are seen [47]. A typical spectrum is presented in Figure 3.1.2. Two distinct peaks in the range 1000 to 2000 cm⁻¹ were observed. The Raman-allowed E_{2g} graphitic peak appeared around 1580 cm⁻¹ (*G* band), representing the vibration of sp^2 -hybridised carbon atoms in a two-dimensional hexagonal lattice. The *D* band at 1380 cm⁻¹ was attributed to the presence of amorphous carbon in the sample, as has been done in previous reports [2,7-10]. It should be pointed out that only SWCNTs of a certain diameter, in resonance with the 2.41 eV laser excitation energy, appear in the Raman spectrum [10].

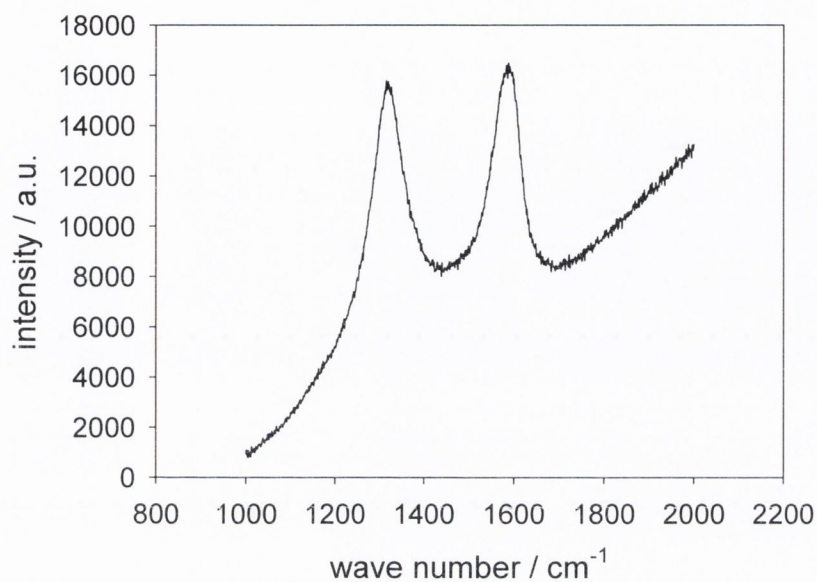


Fig. 3.1.2 Raman spectrum of single-walled carbon nanotubes.

3.2 Atomic Force Microscopy

An AFM image recorded for the carbon nanotube dispersion on a gold substrate is presented in Figure 3.2, illustrating the random orientation of the nanotube assembly.

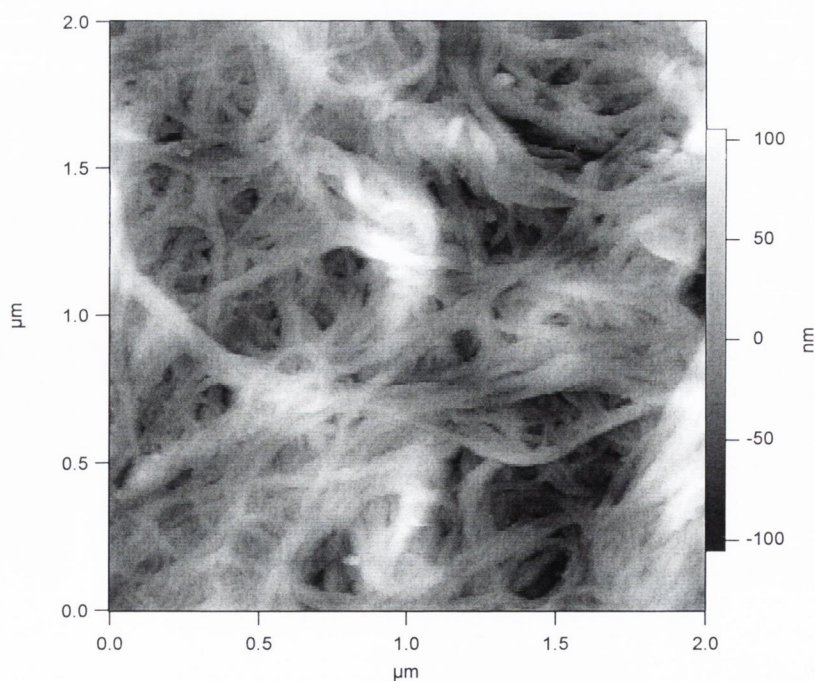


Fig. 3.2 AFM image recorded for SWCNT ensemble dispersed on gold surface.

Many SWCNT bundles with diameters roughly between twenty and thirty nanometers can be observed. The lengths of these bundles could not be determined because a single image does not show both ends of any one bundle. The image shows that some bundles are twisted together and indiscernible from each other. The existence of these bundles is due to the large surface energies of nanotubes. The bundles are held together by strong π - π stacking interactions [11]. The image also shows the porosity of the films, and that there can be gaps between bundles sufficiently large to permit the entry of electrolyte during an electrochemical experiment. In the next chapter, the effect of this leakage on the voltammetric response will be discussed.

This concludes the physical characterisation of the nanotubes used in this work. The chapter will now move on to the electrochemical behaviour of glassy carbon electrodes modified with drop-cast dispersions of these SWCNTs but before doing so, it is worth discussing the factors which led to the decision to choose DMF as the solvent for these nanotube suspensions.

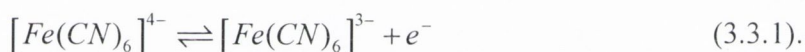
3.3 Solvent Issues

As mentioned in *Section 1.2*, the hydrophobicity of carbon nanotubes means that the choice of solvent requires more careful consideration than with other electrode modifying agents. A number of solvents were tried for the nanotubes used in the present work. Suspensions were prepared using a high-frequency sonicating tip. Use of the latter was found to heat the solvent so acetone was ruled out due its volatility. Acetonitrile was also eliminated as these nanotubes were found to take a long time (twenty minutes) to disperse in it and the resulting suspensions were stable for no more than one day. DMF showed promising results, forming very stable suspensions within five minutes of sonication. NMP proved even more promising, with sonication producing stable dispersions within one minute. This meant that *N*-methyl-2-pyrrolidone was originally favoured, especially since its low boiling point (79 °C compared with 153 °C for DMF) suggested that it would be easily evaporated from the electrode surface once cast.

However, when it came to attempts at electrode modification, NMP showed itself to be quite obtuse when compared with DMF. It was surprising to see that, despite its lower boiling point, NMP was found to take far longer to come off the electrode surface

(one day compared with fifteen minutes), using a fan heater at 40 °C. Not only is this inconvenient, but potentially damaging to the electrode, as heat can break the contact at the glassy carbon disc (see *Section 2.6*). It is suggested that the affinity of NMP for nanotubes is so much stronger that it overcomes the difference in boiling point, and a large amount of energy is required to remove it from the nanotubes. This point will be revisited at the end of this section.

As well as these obvious practical concerns, the final decision to go with DMF was reached on the basis of electrochemical measurements. Cyclic voltammetry was performed at various scan rates using a solution containing 1 mM potassium ferrocyanide, with 0.1 M KCl as supporting electrolyte. The ferro/ferricyanide redox couple has served as a benchmark system in fundamental electrochemistry, since the reaction involves the transfer of a single electron and exhibits near-ideal quasi-reversible outer sphere kinetic behaviour, especially at carbon electrodes, at which it experiences little or no bonding/adsorption interaction. Its redox reaction may be written as



It will be used to probe several important features of the modified electrodes in this and the next chapter. Here, the aim was to evaluate the kinetic facility of this reaction at electrodes modified using both NMP and DMF nanotube suspensions, thereby gaining an insight into their suitability (or lack thereof) for electrochemical applications. Before doing so, it will be necessary to explain the kinetic model employed.

The cyclic voltammetric response of a solution-phase quasi-reversible electron transfer reaction has been the subject of quantitative mathematical analysis, especially in a classic 1965 paper by Nicholson [12]. The approach described therein provides a rapid and simple way to evaluate electrode kinetics on the basis of the variation of peak separation with scan rate. This is done *via* the dimensionless kinetic parameter Ψ , which may be obtained for any voltammogram simply by using the peak separation in conjunction with the working curve presented in Figure 3.3.1. The points used to plot this curve arise from the numerical calculation of current-voltage profiles derived by Nicholson for a diffusion-controlled electrochemical reaction.

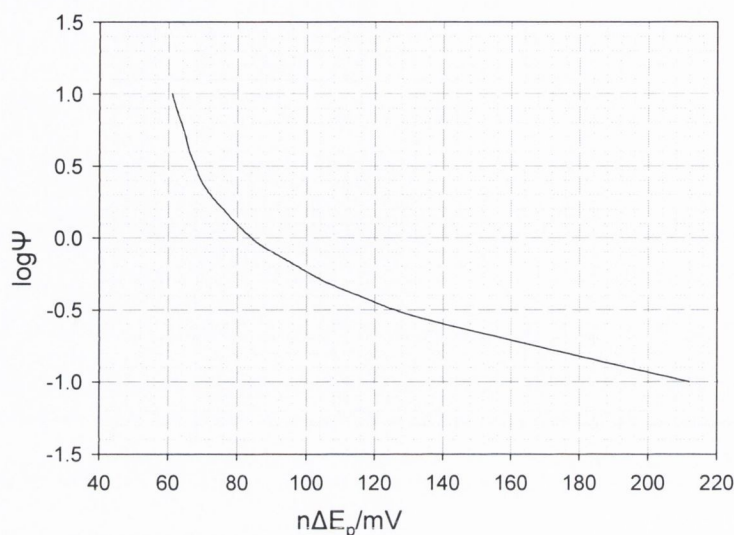


Fig. 3.3.1 The variation of the kinetic parameter Ψ with voltammetric peak separation.

For $n\Delta E_p > 140$ mV, this plot is essentially linear, and Ψ may be calculated using the following fitted linear equation:

$$\log \Psi = -5.36 \times 10^{-3} \times n\Delta E_p + 0.15 \quad (3.3.2),$$

in which ΔE_p has units of millivolts. The analysis of Nicholson [12] showed that Ψ is related to the standard electrochemical rate constant according to

$$\Psi = \frac{\left(\frac{D_O}{D_R}\right)^{\alpha/2}}{\sqrt{\frac{\pi D_O F \nu}{RT}}} k^0 \approx \frac{k^0}{\sqrt{\frac{\pi D_O F \nu}{RT}}} \quad (3.3.3),$$

where it has been assumed for simplicity that the diffusion coefficients of the oxidised (O) and reduced (R) forms of the redox couple are equal (certainly a valid assumption for ferri/ferrocyanide) and that the transfer coefficient (α) has a value of 0.5. The points used to draw the working curve were calculated using a value of 0.5 for α , but it is worth noting that the variation of Ψ with peak separation is virtually independent of the transfer coefficient for scan rates at which the system displays quasi-reversible behaviour [12]. Since it is defined solely for the convenience of the derivation in Nicholson's paper, not much can be said for the physical interpretation of Ψ , apart from the obvious point that it provides an indication of the reaction kinetics.

Before continuing it is important to clarify the physical interpretation of the *standard electrochemical rate constant*, as this is commonly overlooked in the literature. This quantity is a measure of the kinetic facility of the reaction which occurs at the electrode surface. Its magnitude may vary over a very large range, from around $10^{-2} \text{ cm s}^{-1}$ for simple reactions involving rapid electron transfer to $\sim 10^{-13} \text{ cm s}^{-1}$ for complex, multi-step mechanisms involving significant molecular rearrangement. It should be noted that k^0 says nothing about the rate of electron transfer itself, as, according to the Franck-Condon principle, this occurs with extraordinary rapidity (*ca.* 10^{-16} seconds). Rather, it is a measure of the time taken for the reactant species to arrange themselves and their ionic surroundings such that ET may occur.

Returning to Nicholson's analysis, it can be seen from *Equation 3.3.3* that it is straightforward to determine the standard heterogeneous rate constant for an interfacial electron transfer reaction by noting the voltammetric peak separation at a given scan rate, and using the working curve to determine the corresponding value of the Ψ parameter. The rate constant may then be calculated provided the diffusion coefficient of the redox species is known. Of course, the smaller the observed ΔE_p , the larger the rate constant, and the more kinetically facile the redox process occurring at the electrode surface. Figure 3.3.2(a) shows a comparison between voltammograms obtained for the ferrocyanide reaction at electrodes modified using suspensions which were identical in every way except the solvent in which the nanotubes were dispersed.

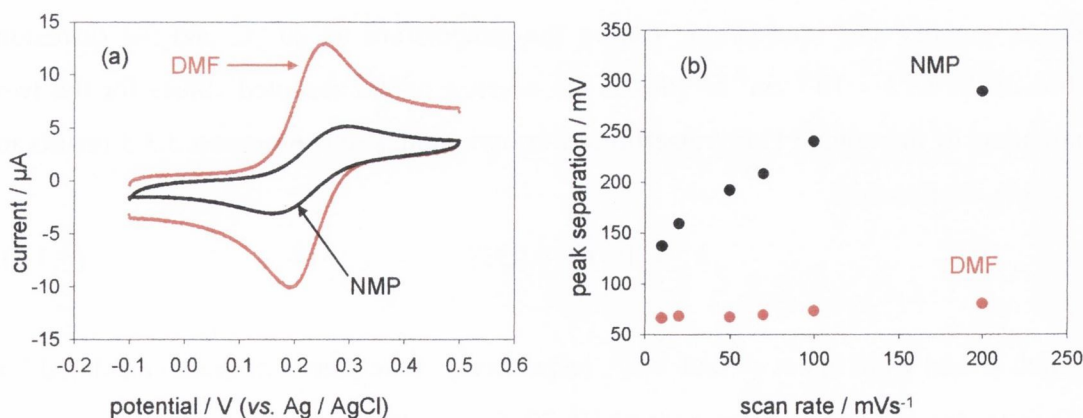


Fig. 3.3.2(a) Cyclic voltammograms for 1mM potassium ferrocyanide in 0.1 M KCl at glassy carbon electrodes modified using suspensions of SWCNTs in both DMF and NMP. The scan rate employed was 10 mV s^{-1} . **(b)** The variation of peak separation with scan rate for the two electrodes.

To digress briefly from solvent issues, it is interesting to note the overall shape of the response obtained for these nanotube-modified electrodes. The latter may be regarded as ensembles of nano-electrodes. The response shapes at such arrays are governed not only by the scan rate but by the distance between the NT bundles on the electrode surface [13]. These electrodes are clearly producing profiles characteristic of what is referred to as 'total overlap'. The diffusion layers of the nanotube bundles overlap in such a way as to produce an overall diffusion layer for the ensemble, so the response has the shape one would expect from a conventional, planar electrode, despite the potential for each bundle to act as an individual nano-electrode. In the latter case, the shape would be sigmoidal, as discussed in *Section 1.4*. It is concluded, then, that these arrays are quite dense. This view is supported by the AFM images in *Section 3.2*.

Returning to the main theme of this section, it is clear from Figure 3.3.2(a) that the response at what will hereupon be referred to as the *NMP electrode* is more sluggish, with smaller, broader peaks and larger peak separations. Figure 3.3.2(b) shows how peak separations varied with scan rate. For the NMP electrode, they are larger and increase more steeply. In fact, above 200 mV s^{-1} , peaks were found to be so broad as to preclude the accurate measurement of ΔE_p values. It is concluded then that at this electrode, the behaviour of the reaction moves from quasi-reversible towards irreversible using these larger scan rates.

So, qualitatively at least, the results indicate that the use of NMP creates a kinetic sluggishness compared with DMF. Using the Nicholson method, an attempt will now be made to quantify this retardation. Taking the temperature as 20°C and the diffusion coefficient as $8.12 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ (this is the average of the accepted values for the two components of the couple [14]), insertion of known values into *Equation 3.3.3* results in the simple relationship

$$k^0 = \Psi \sqrt{\nu} \times 0.0319 \quad (3.3.4),$$

in which k^0 and ν are in cm s^{-1} and V s^{-1} , respectively. Rate constants were calculated for both electrodes at all of the scan rates of 10, 20, 50, 70, 100 and 200 mV s^{-1} . The average values obtained were 8.1×10^{-4} and $1.9 \times 10^{-2} \text{ cm s}^{-1}$ for NMP and DMF, respectively. Taking into account the assumptions used in the derivation of the model by Nicholson,

and those used here in its application, it is prudent to not attach massive significance to the numbers themselves but it is felt that a difference of two orders of magnitude in the standard rate constant is sufficient to show that this quasi-reversible one-electron transfer was severely hindered at the NMP electrode.

It is difficult to say with any great certainty why NMP has such an effect on the electrochemical properties of the nanotubes. It is suggested that there may be some NMP present within the nanotube film, despite the fact that heat was applied until the electrode was visibly dry. The remarkable obstinance of the solvent observed when applying this heat would support this claim. If present within the film, it is likely that the solvent is held there by strong interactions with surface oxides present on the nanotubes. These are, of course, the sites at which electron transfer occurs so it is conceivable that the redox probe would have greater difficulty reacting at these defects. The smaller background currents found for the NMP electrodes would certainly support the view that the redox probe has access to a smaller active surface area. Whatever the explanation, it is felt that the choice of DMF has now been justified, and the chapter may move on to look in more detail at the electrochemical properties of the SWCNT-modified electrodes.

3.4 Electrochemical Behaviour of the SWCNT Film

A pair of peaks was observed when the nanotube-modified glassy carbon electrode was scanned using a pH 7.0, 50 mM phosphate buffer solution as the electrolyte in the range 0.2 to -0.7 V (vs. Ag / AgCl). The initial sweep in each case was cathodic. Peak potentials remained reasonably stable in subsequent cycles but peak currents decreased a little (see Figure 3.4.1). This deterioration of peak currents with multiple scans is commonly observed, and it is (tentatively) attributed to incomplete redox of the film at short experimental timescales. At a scan rate of 100 mV s⁻¹, the cathodic and anodic peak potentials were found to be -0.390 (± 0.011) and -0.160 (± 0.006) V (vs. Ag / AgCl) respectively. The standard deviations given were calculated using data from six independently-prepared electrodes. The figure shows that the peaks were quite broad so it is not surprising that there is a significant error in the identification of peak potentials. Similar results were obtained using nanotube-modified *gold* electrodes, suggesting that the observed electrochemistry was due entirely to the nanotubes and was independent of

the underlying substrate. The E^0 value of around -0.28 V is significantly more negative than the value of -0.07 V vs. SCE (-0.02 V vs. Ag / AgCl) reported by Luo and co-workers [4] for SWCNTs immobilised at a glassy carbon electrode in pH 6.9 buffer, so it cannot be stated with certainty that the observed peaks are due to the same species. *Before continuing, it should be pointed out that the potential of the Ag / AgCl electrode is -0.045 V relative to the saturated calomel electrode [15]. This correction will be used regularly throughout these chapters when comparing results from different sources.*

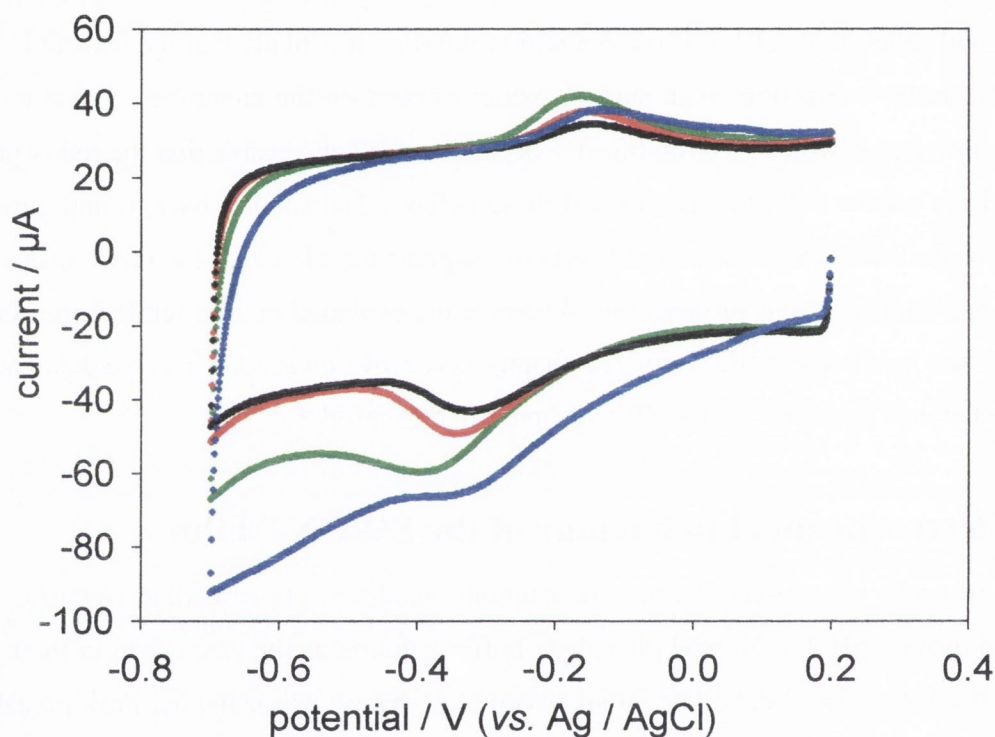


Fig. 3.4.1 Cyclic voltammograms of the single-walled carbon nanotube film on a glassy carbon electrode in pH 7.0 phosphate buffer. The green, red and black scans represent the first, second and third cycles, respectively. The blue scan was obtained after twenty-four hours. The scan rate was 100 mV s^{-1} .

After these scans were performed, the modified electrode was removed from the solution. It was then rinsed with water and ethanol. After 24 hours of exposure to air, the experiment was repeated and the result is also shown in Figure 3.4.1. Peak potentials and currents changed little. These results indicate that the nanotube film is endowed with reasonable stability. The peaks themselves are attributed to oxygen-containing functionalities on the nanotube surface, in accordance with previous reports [4,16-21].

Luo *et al.* [4] have attributed these to carboxylic acid groups, a claim supported by infrared spectroscopy studies, which showed a significant decrease in COOH stretch after the nanotube film had been poised at a strongly cathodic potential for ten minutes and scraped off the electrode. These workers claimed that the electrode process involved four electrons, the rate-determining step being a one-electron reduction. They also proposed a mechanism for the redox process. Li and co-workers [17] have claimed that these peaks are most likely due to quinones but did not offer any evidence. The identity of the species responsible remains debatable and may even vary depending on the history of the nanotubes. Interestingly, the peaks do not appear when the electrolyte is KCl solution and not phosphate buffer. However, this cannot be accounted for at present.

The effect of pH on these peaks was also investigated. Voltammetry was carried out using 50 mM phosphate buffer solutions of various pH values, prepared as described in Section 2.8. The decrease in peak currents with multiple scans mentioned above meant that it was difficult to obtain well-defined peaks at all of the pH values. Broad as they were, it was evident that the peaks shifted negatively with increasing pH, as shown in Figure 3.4.2.

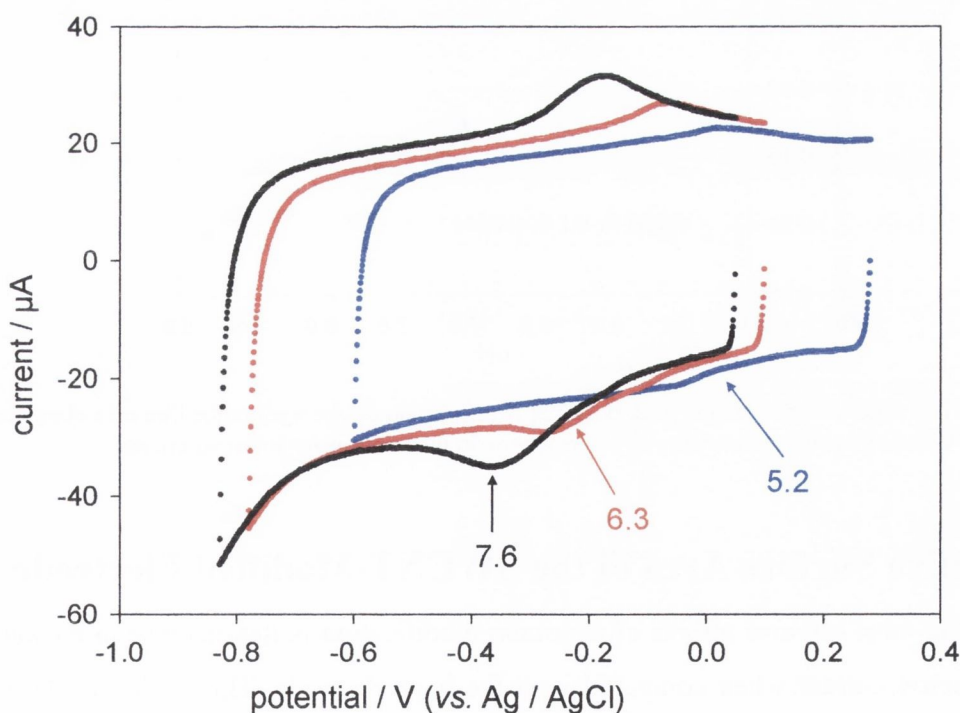


Fig. 3.4.2 Cyclic voltammograms for the SWCNT-modified glassy carbon electrode at 100 mV s^{-1} in 50 mM phosphate buffer solutions of various pH values.

Over the pH range studied, oxidation peaks were found to be sharper than those for reduction, possibly due to interference from the reduction of oxygen trapped within the nanotube film. A plot of the oxidation peak potential against pH is shown in Figure 3.4.3. The slope of this plot was found to be -61.5 mV pH^{-1} , which is close to the theoretical value of -59 mV pH^{-1} for a reversible process at 20°C [2]. The linearity of the variation indicates that the electron transfer is accompanied by an associated protonation / deprotonation equilibrium. The low correlation coefficient is acknowledged, but this is not surprising, given the errors inherent in picking such broad voltammetric peaks. The pH study does not reveal a great deal about the functionalities present on the tubes, but is deemed worthy of inclusion on the basis that it has been reported by others [4]. It is worth noting that these workers provide neither plots nor r^2 values when claiming near-ideal variations of peak potential with pH.

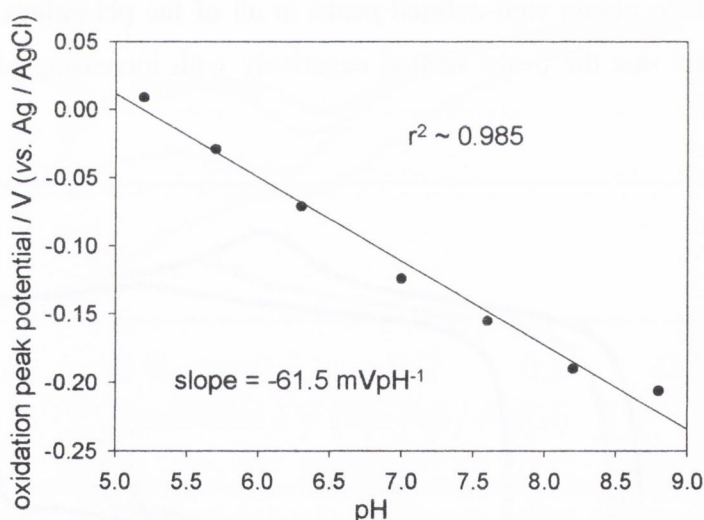


Fig. 3.4.3 The variation of oxidation peak potential with solution pH for a nanotube film on a glassy carbon electrode, based on cyclic voltammetry at 100 mVs^{-1} in 50 mM phosphate buffer solutions.

3.5 Active Surface Area of the SWCNT-Modified Electrode

One of the most obvious effects of nanotube modification is the increase in background voltammetric current when compared with the bare electrode. This is due to the tubes' large, catalytically active surface area (typically between 1200 and $1600 \text{ m}^2\text{g}^{-1}$) and high aspect ratio, which effectively combine to increase the area of the electrode and the

capacitive current required to charge the interface. It has also been suggested [17,20,21] that there is a significant faradaic contribution to this background response due to the presence of surface oxides on the nanotubes, sometimes referred to as a 'pseudocapacitance'. The work presented in this section is concerned with attempts to quantify the increases in active surface area caused by nanotube modification, and to estimate a value for the surface-specific capacitance of the modified electrode. For the former the ferri/ferrocyanide standard redox probe was once again called upon. Cyclic voltammetry was performed using a solution containing 20 mM potassium ferrocyanide and 200 mM potassium chloride. A relatively large concentration of the redox probe was used in order to ensure that its concentration was known very accurately, as this quantity features in the surface area calculation. Figure 3.5.1 shows a comparison between a bare glassy carbon electrode and the same electrode modified using a film of single-walled carbon nanotubes. Initial sweeps in each case were oxidative.

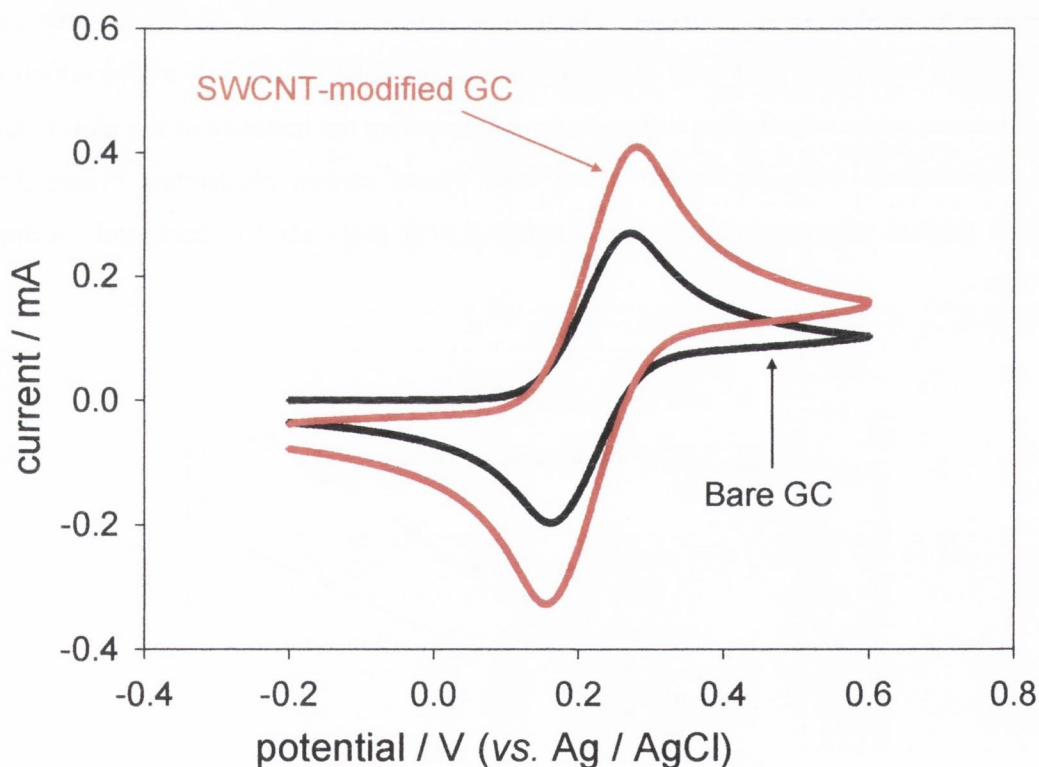


Fig. 3.5.1 Comparison between voltammograms obtained using a bare and SWCNT-modified glassy carbon working electrode. The scan rate employed was 100 mV s^{-1} . The electrolyte used was 20 mM potassium ferrocyanide in 200 mM potassium chloride solution.

Well-defined peaks were evident around +0.27 and +0.16 V (vs. Ag / AgCl) for the ferro/ferricyanide redox process given by Equation 3.3.1. The figure clearly shows the enhancement of peak currents due to the greater active surface area of the modified electrode. The extent of the increase in surface area may be quantified using the *Randles-Sevcik* equation (see Equation 2.2.1.4). By this equation, plots of peak current against square root of scan rate should be linear and pass through the origin. Slopes of these lines are given by

$$m_{R-S} = 0.4463nFA\sqrt{\frac{nF}{RT}}C^\infty\sqrt{D} \quad (3.5.1).$$

Substitution of appropriate values (including an accepted value of $7.35 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ for the diffusion coefficient of ferrocyanide [14]) yields the simple expression

$$m_{R-S} = 0.147 \times A \quad (3.5.2)$$

which gives the surface area in cm^2 when the *Randles-Sevcik* slope is in $\text{A} / (\text{V s}^{-1})^{1/2}$. Four nanotube-modified glassy carbon electrodes were prepared independently and voltammetry was performed over the scan rate range of 10 to 200 mV s^{-1} , in which the redox process was controlled by diffusion (as indicated by the linearity of the plots). Also, these experiments were performed using bare glassy carbon electrodes. Figure 3.5.2 shows a typical comparison between *Randles-Sevcik* plots for the bare and modified electrodes.

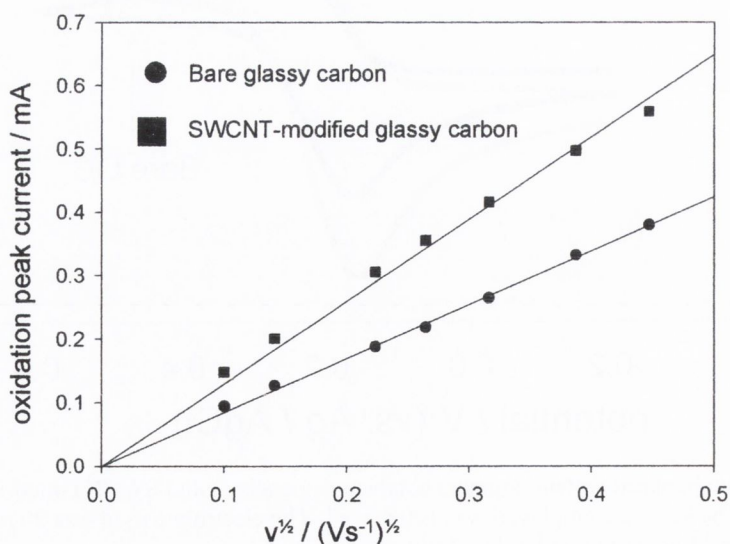


Fig. 3.5.2 Comparison between *Randles-Sevcik* plots obtained using a bare and SWCNT-modified electrode. The electrolyte used was 20 mM potassium ferrocyanide in 200 mM KCl solution.

Inspection of these plots clearly shows a greater slope (and hence area) for the modified electrode. This conclusion that the area increases is reached on the basis that all other terms in *Equation 3.5.1* remain unchanged by modification of the electrode. Before quantifying this increase it is interesting to note the roughness of the *unmodified* glassy carbon electrode. With a radius of one millimetre, this electrode has a geometric surface area of $3.14 \times 10^{-2} \text{ cm}^2$. However, taking into account the four glassy carbon electrodes tested, the *Randles-Sevcik* analysis yields an average active surface area of $(5.66 \pm 0.11) \times 10^{-2} \text{ cm}^2$, meaning a roughness factor of around 1.8. Having said this, surface roughness is of little consequence in most voltammetric work. For electrolysis times greater than one second, the diffusion layer thickness is substantially larger than the characteristic dimensions of roughness. This thickness may be crudely approximated to the square root of the product Dt (typically microns). For example, taking a typical diffusion coefficient value of $10^{-5} \text{ cm}^2 \text{ s}^{-1}$ and a time of one second, the diffusion layer thickness is estimated to be around thirty microns.

The nanotube-modified glassy carbon electrodes were found to have an average surface area of $(9.76 \pm 0.98) \times 10^{-2} \text{ cm}^2$. This relative standard deviation of 10 % is considerably larger than the 2 % found for the unmodified electrode. The lack of reproducibility is hardly surprising though, given the random nature of the nanotube film. However, this analysis is undoubtedly sufficient to show that CNT modification leads to a considerable increase in the active surface area of the electrode, and there are several publications in this field which have reported similar findings [7,22,23]. It is suggested that this value for the area of the modified electrode might incorporate not only its outer surface, but also a significant area of its inner structure, since at these moderate scan rates, there may be sufficient time for some diffusion of the electrolyte into the porous nanotube ensemble, as suggested by Lawrence and co-workers [20]. However, it should be pointed out that the strikingly diffusional shape of the response suggests that, with this particular redox probe, the NT film does not permit this to any great extent. These peaks are characteristic of diffusion from bulk solution, and not from within a confined space ('thin layer' behaviour). In *Section 2.9* it was reported that the modified electrodes typically had a significant amount of nanotubes in contact with the Kel-F casing surrounding the glassy

carbon. The possibility that these could communicate with the GC *via* other nanotubes is acknowledged.

Cyclic voltammetry was also used to estimate the capacitance of the immobilised film by examining the background responses of a SWCNT-modified glassy carbon electrode in pH 7.0 phosphate buffer solution over a range of sweep rates (25–200 mV s^{-1}). Some of the responses obtained are presented in Figure 3.5.3(a). In the potential window of -0.1 to +0.7 V (vs. Ag / AgCl), the voltammograms were found to be featureless, showing no discernible redox peaks. Slight increases in anodic current were observed at high potentials and these were attributed to the partial oxidation of the aqueous solvent. In order to evaluate the capacitance, values for the anodic current measured at a potential of +0.1 V were plotted against scan rate, as shown in Figure 3.5.3(b). This low potential was selected so possible contributions from solvent oxidation could be ruled out.

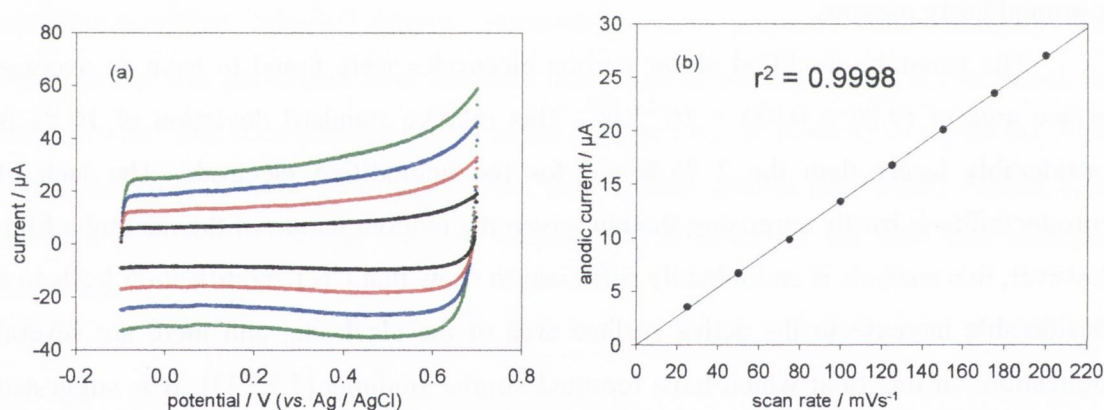


Fig. 3.5.3(a) Cyclic voltammograms of a carbon nanotube-modified glassy carbon electrode in a pH 7.0 phosphate buffer solution. Scan rates were (from inner to outer) 50, 100, 150 and 200 mV s^{-1} . **(b)** The resulting plot of anodic peak current at +0.1 V against scan rate.

The linearity of the plot indicates that the baseline current corresponded to the capacitive charge-discharge current. It can be easily shown that the slope of this graph has units of capacitance and therefore that it provides an estimate for the capacitance of the electrical double layer. In this case the slope was found to be 0.134 millifarads. Combining this with the calculated surface area of $(9.76 \pm 0.98) \times 10^{-2} \text{ cm}^2$, a surface specific capacitance of roughly 1.4 mF cm^{-2} is obtained, which is within the range of values (0.45 to 7.78 mF cm^{-2}) found elsewhere for a variety of nanotubes cast from DMF suspensions [20].

However, the possibility that this value incorporates a ‘pseudocapacitive’ contribution from faradaic processes involving surface functionalities is not ruled out, despite the absence of voltammetric peaks.

In *Chapter Five*, considerable attention will be paid to the analysis of potential step data obtained for the direct electron transfer of the enzyme glucose oxidase adsorbed onto nanotube-modified electrodes. Such data are typically recorded over short timescales (less than one second), and for this reason it is considered prudent at this point to estimate a value for the *cell time constant*. Bard and Faulkner [15] define this as the ‘shortest time domain over which the cell will accept a significant perturbation’. In terms of data obtained using potential steps, it is more useful to think of this as indicating the time period immediately after a step during which the measured current contains a significant (parasitic) contribution from that required to charge the electrical double layer. This is given by the product $R_u C_d$, where R_u is the solution resistance and C_d is the double layer capacitance. This quantity can be easily shown to have units of time. In order to estimate a value for the solution resistance, electrochemical impedance spectroscopy was carried out using a SWCNT-modified glassy carbon electrode. In the enzyme studies described in *Chapter Five*, the electrolyte will be 50 mM phosphate buffer (pH 7.0), so this was used as the electrolyte here. The AC signal applied to the electrode was 5 mV in amplitude. Data were collected at the open circuit potential (+0.17 V vs. Ag / AgCl) in the frequency range 10^{-2} to 10^5 Hz. The resulting Nyquist plot (Figure 3.5.4) was dominated by a large, well-defined, linear Warburg diffusive feature, indicating that the transfer of charge across the buffer / nanotube interface was quite facile.

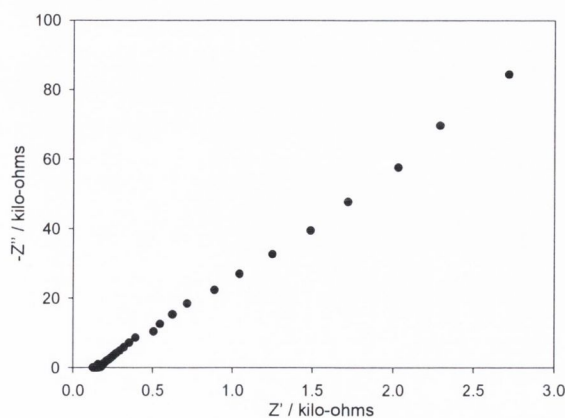


Fig. 3.5.4 Complex impedance Nyquist plot for a nanotube-modified glassy carbon electrode at + 0.17 V (vs. Ag / AgCl) in a pH 7.0, 50 mM phosphate buffer solution.

As discussed in *Section 2.4*, the horizontal intercept of such a plot provides a value for the solution resistance. In this case it was found to be around 125 ohms. Combining this with the calculated double layer capacitance of 0.134 mF, a value of around 17 milliseconds for the cell time constant was obtained.

Potential step chronoamperometry was also used to provide a value for both the solution resistance and the cell time constant. For this, the electrochemical circuit is modelled on the elementary arrangement displayed in Figure 3.5.5, consisting of the series circuit elements given by the solution resistance and the double layer capacitance.

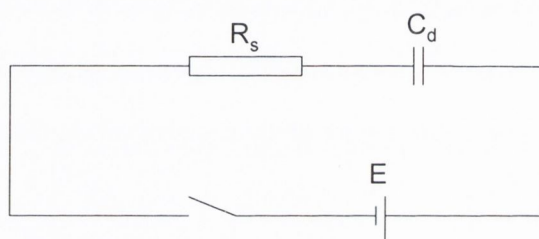


Fig. 3.5.5 Potential step experiment for an RC circuit.

If a potential step of magnitude E is applied to the cell, it is dissipated *via* losses incurred overcoming the barriers created by both the solution resistance and the charging of the double layer. In short, the step magnitude is the sum of the potential drops across these two circuit elements:

$$E = E_C + E_R \quad (3.5.3),$$

where E_C and E_R are the potential drops across the capacitor and resistor, respectively. Using Ohm's Law and the general expression for the voltage across a capacitor,

$$E = \frac{q}{C_d} + iR_u \quad (3.5.4),$$

in which q is the charge transferred and i is the current flowing at any time. Remembering that the latter is the rate at which the charge is being transferred, the following (variables inseparable) differential equation is obtained:

$$\frac{dq}{dt} = \frac{E}{R_u} - \frac{q}{R_u C_d} \quad (3.5.5).$$

The solution of this equation is provided, but not explained, by Bard and Faulkner [15]. It yields an expression which permits the estimation of the solution resistance and cell time constant from a simple current decay resulting from a potential step of magnitude E :

$$i = \frac{E}{R_u} \exp\left(-\frac{t}{R_u C_d}\right) \quad (3.5.6).$$

Plots of $\ln i$ against time should therefore be linear. If the magnitude of the step is known, the intercept gives a value for the solution resistance. The slope provides a value for the cell time constant τ , which is equal to the product $R_u C_d$. With this in mind, potential steps of various magnitudes were performed using a GC/SWCNT electrode in phosphate buffer. The initial potential in each case was 0 V (vs. Ag / AgCl) and the potential was stepped anodically. A typical output is shown in Figure 3.5.6, along with the corresponding plot drawn according to Equation 3.5.6.

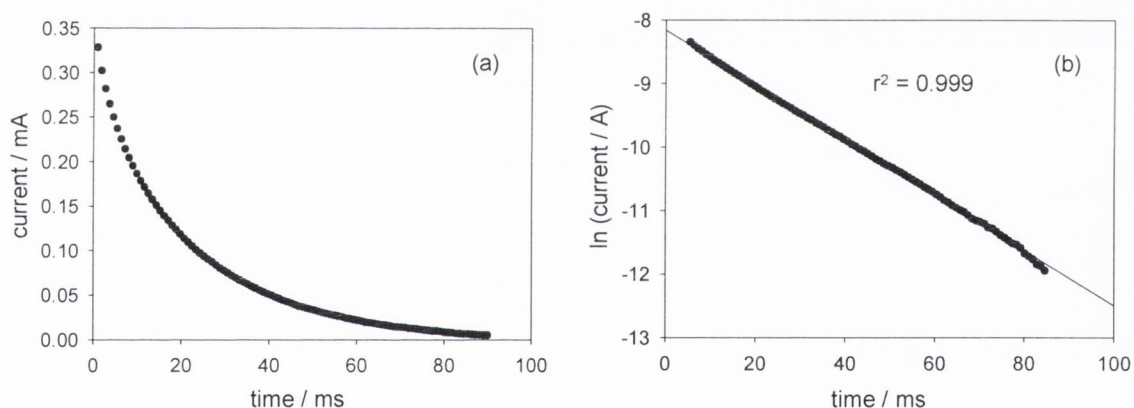


Fig. 3.5.6(a) Raw chronoamperometric data obtained following a potential step from 0 to 50 mV using a GC/SWCNT electrode in 50 mM phosphate buffer. **(b)** The resulting semi-logarithmic plot.

As an example of how the data were processed, consider those obtained from the figure, for which the step magnitude, E , was fifty millivolts. The intercept of the plot was -8.14, so

$$\ln\left(\frac{E}{R_u}\right) = -8.14$$

$$\therefore R_u = 171\Omega$$

The slope of the graph was -43.9 s^{-1} , which yielded a τ value of 23 milliseconds. The results obtained using four different E values are summarised in Table 3.5.

E / mV	R_u / Ω	τ / ms
50	171	23
100	186	25
150	191	27
200	196	28

Table 3.5 Solution resistances and cell time constants calculated from potential step chronoamperometry using a GC/SWCNT electrode in a pH 7.0, 50 mM phosphate buffer solution.

It is not surprising that neither of the calculated values varied a great deal with the size of the perturbation. A similar approach using a *bare* glassy carbon electrode yielded values of 650 Ω and 11 milliseconds for R_u and τ , respectively. It is interesting (and, admittedly, perplexing!) to note that the nature of the electrode surface appeared to influence the measured value for the solution resistance. It is satisfying to see also that the presence of the nanotubes caused a considerable increase in the time required to charge the double layer, as one would expect on the basis of the increase in surface area calculated above. The cell time constant will be revisited in *Chapter Five* in relation to potential step data for adsorbed glucose oxidase.

At this stage a reasonably clear picture of nanotube-modified electrodes as having large surface areas as well as their own (redox-active) functionalities has been provided. The next chapter will investigate the catalytic behaviour of these electrodes with respect to various redox systems which are commonly used in electrochemistry to illustrate the effects of electrode modification.

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Chapter Four: Catalytic Properties of NT-Modified Electrodes

Having characterised the nanotubes used in this work in the previous chapter, provided a physical description of the modified electrode and shown that the GC/SWCNT electrode exhibits a stable electrochemical response, this chapter will focus on the use of various redox probes to show how the electro-catalytic performance of a glassy carbon electrode may be enhanced by the deposition of a nanotube film. Particular attention will be given to the applications of this system in the analysis of bio-molecules. The chapter will then conclude with comparisons between NTs and conventional graphite.

4.1 Ferrocyanide

The first redox probe to be discussed with a view to revealing the effects of nanotube modification on glassy carbon electrodes is ferrocyanide, which has already been seen in *Sections 3.3* and *3.5*. In these sections it was used to probe solvent effects and the surface area of nanotube-modified electrodes. Here it will be used to investigate the electro-catalytic effects of NT modification. Cyclic voltammetry was performed as described in *Section 3.3*, and results for the bare and modified glassy carbon electrodes were compared. Well-defined voltammetric peaks were obtained up to scan rates of around 1.5 V s^{-1} at the bare electrodes. When the scan rate was greater than 0.5 V s^{-1} , charging currents began to swamp the faradaic responses at the NT-modified glassy carbon, as one would expect for an electrode with a large surface area. However, since data for the modified electrode have already been analysed in detail in *Section 3.3*, this discussion for both systems will be restricted to the same range of $10\text{--}200 \text{ mV s}^{-1}$, so data for the two systems may be justifiably compared. In *Figure 4.1.1(a)* a comparison between voltammograms obtained for the bare and modified electrodes is shown. Again, the most immediately striking features are the larger currents due to the increased surface area of the modified electrode, but this has already been discussed fully in *Section 3.5*.

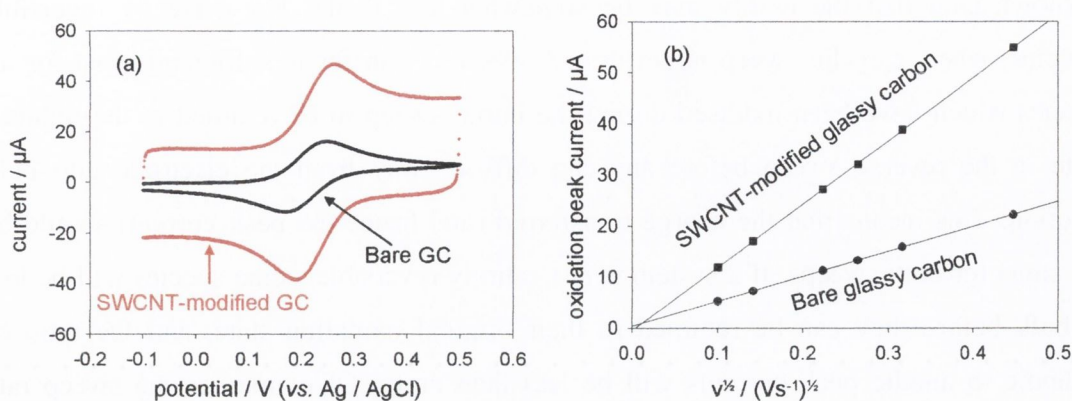


Fig. 4.1.1(a) Cyclic voltammograms for 1 mM potassium ferrocyanide in 0.1 M KCl at bare and SWCNT-modified glassy carbon working electrodes. The scan rate employed was 100 mV s^{-1} . **(b)** Comparison between Randles-Sevcik plots obtained for the two electrodes.

Figure 4.1.1(b) shows a comparison between *Randles-Sevcik* plots obtained for ferrocyanide oxidation at the two electrodes. Again the greater slope obtained for the modified electrode is noted. The plots are linear, each with a correlation coefficient greater than 0.9999, indicating that the reaction is under the control of diffusion at both electrodes. Peak separations were slightly less for the modified electrode, and for both systems increased with scan rate, suggesting a quasi-reversible reaction. When the ΔE_p values for the bare electrode were subjected to the Nicholson [1] analysis (described in detail in *Section 3.3*), the standard electrochemical rate constant for the redox reaction was found to be $1.1 \times 10^{-2} \text{ cm s}^{-1}$, which is slower than the $1.9 \times 10^{-2} \text{ cm s}^{-1}$ reported in *Section 3.3* for the modified electrode. It is proposed therefore that the rate of the redox process is enhanced at the latter, due to the presence of reactive surface oxides. It is noted, however, that these groups have been shown [2], using functionalised glassy carbon, to have little influence on peak potentials for ferrocyanide when compared with more complex redox probes such as ascorbic acid and oxygen. This view will be supported by data presented later in the present chapter. Peak separations were larger than the ideal value of 59 mV s^{-1} even at the lowest scan rate, but this is commonly found with carbon electrodes which have not been substantially pre-treated [3].

As mentioned in *Section 2.2.1*, it was pointed out that, for a reversible electrochemical system, the ratio of voltammetric peak currents should be unity at all scan rates. The following is suggested as an explanation for this phenomenon, although it is

acknowledged that the reality may be somewhat less facile. For perfectly reversible systems, when a cyclic sweep is performed, electron transfer is sufficiently fast for all species which have been oxidised during the initial sweep to be returned to the reduced state in the reverse sweep before any can diffuse away from the electrode into bulk solution. This means that the charge transferred (and hence the peak current) should be the same for both sweeps. If a system is not entirely reversible, some species will be lost to bulk before they can be returned to their original oxidation state, and the ratio of cathodic to anodic peak currents will be less than unity. Of course, as the sweep rate increases, species are given less time to transfer electrons and this number departs further from its ideal value. Hence, the variation of the $i_{pc} : i_{pa}$ ratio with scan rate is in itself a simple indication of the kinetic facility of a redox process. Figure 4.1.2 shows how this fraction varied with ν for the bare and modified electrodes.

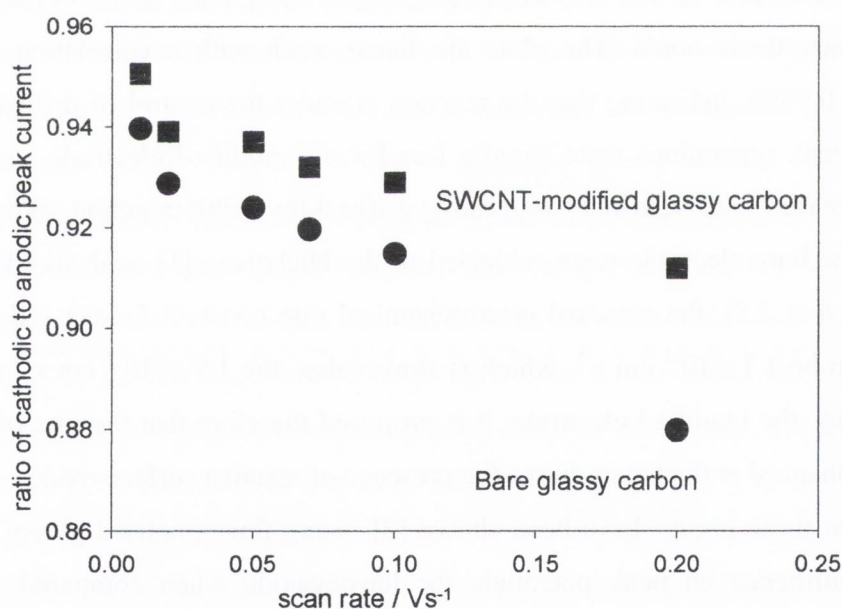


Fig. 4.1.2 The variation of the ratio of the cathodic to anodic peak currents with scan rate for a bare (●) and SWCNT-modified (■) glassy carbon working electrode. The electrolyte used was a 1 mM aqueous solution of potassium ferrocyanide in 0.1 M potassium chloride.

The number decreases with scan rate for both systems, again indicating quasi-reversible electron transfer. However, the departure from unity was more pronounced for the bare electrode. This indicates that ET occurs more readily at the modified electrode,

presumably due to mediation by oxygenated functionalities present at edge-plane-like defects on the nanotube surface. It is suggested that there must be a significant amount of these defects present, in order to overcome what is probably not a very favourable contact between aqueous electrolyte and the hydrophobic basal plane regions of the nanotubes. Zhao *et al.* [4] have proposed, quite rightly, that negative charge associated with surface oxides at these defects must limit the access of the ferrocyanide anion to the electrode. The results shown in the present work suggest, however, that the ability of these reactive sites to exchange electrons with the redox couple is sufficient to overcome this repulsion.

It is reasonable to suppose that the large π -conjugated system within the carbon nanotube 'molecule' confers properties of both electron acceptor and donor on the latter. Under cathodic polarisation, the adsorbed NTs accept electrons from the glassy carbon, thereby increasing their numbers of filled energy levels and allowing electrons to be donated to redox species in solution. By a similar argument, at anodic potentials, NTs donate electrons to the underlying glassy carbon, while simultaneously accepting electrons from the reduced form of the redox couple. The large numbers of energy states, both occupied and unoccupied, within the nanotubes, permit not only facile electron transfer between the adsorbed nanotubes and the solution redox couple, but also efficient electron tunneling between the tubes and the support electrode.

The cyclic voltammetry results indicate that the reaction is more kinetically facile at the modified electrode. This finding was supported by electrochemical impedance spectroscopy experiments. A description of this technique and the *Randles* equivalent circuit on which data interpretation is based can be found in *Section 2.4*. The AC signal applied to the electrodes was 5 mV in amplitude. Data were collected at the standard redox potential (+0.22 V vs. Ag / AgCl, as determined by CV) in the frequency range 10^{-2} to 10^5 Hz. The resulting Nyquist plots for both systems were dominated by large, well-defined, linear Warburg diffusive features, as one would expect for a reaction which has already been shown to be under the control of diffusion. However, closer inspection of the high-frequency region reveals an interesting difference, and such a close-up is presented in Figure 4.1.3. Each data point corresponds to a discrete frequency measurement. The depressed semicircular features corresponding to kinetic control at the bare electrode are

erased when the latter is modified by carbon nanotubes, providing further evidence of enhanced reaction kinetics.

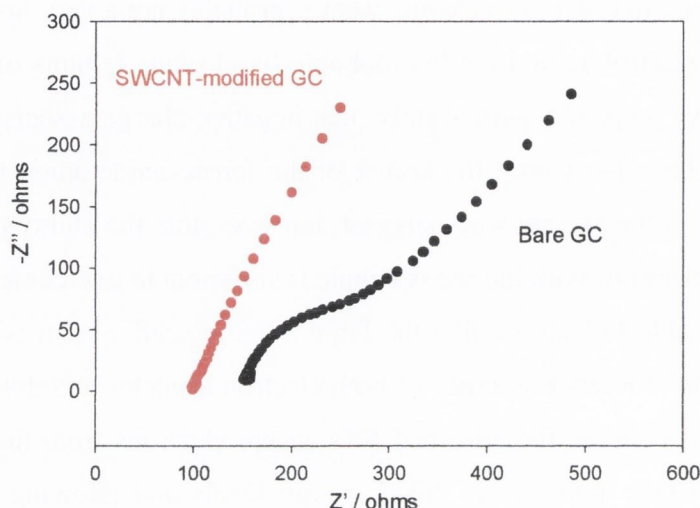


Fig. 4.1.3 Complex impedance Nyquist plots for bare and nanotube-modified glassy carbon electrodes at +0.22 V (vs. Ag / AgCl) in a solution containing 1 mM ferro/ferricyanide and 0.1 M KCl.

4.2 Dopamine

Firstly, voltammetry was performed using a 50 μ M solution of dopamine in phosphate buffer (pH 7.0). Figure 4.2.1 shows a comparison between voltammograms obtained using a bare and nanotube-modified glassy carbon electrode. Pairs of redox peaks corresponding to the reaction of dopamine were observed, with a standard redox potential of +0.17 V (vs. Ag / AgCl). Note the larger peak currents for the modified electrode, which are again attributed to the large surface area of the nanotubes.

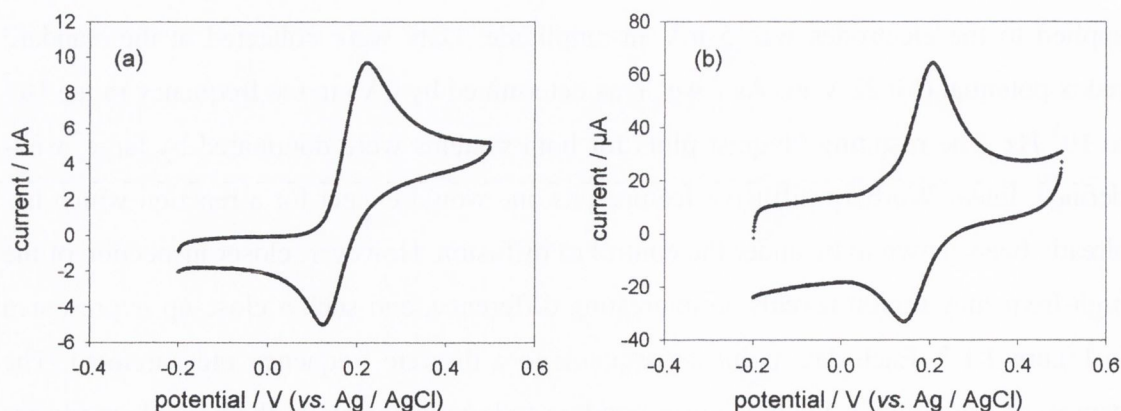


Fig. 4.2.1(a) Voltammogram for 50 μ M dopamine in 50 mM phosphate buffer solution (pH 7.0) at a bare glassy carbon electrode. **(b)** Same for a SWCNT-modified glassy carbon electrode.

The shape of the dopamine oxidation peak at the modified electrode indicated that comparisons between the two electrodes might not be as straightforward as those discussed for ferrocyanide in the previous section. The peak is considerably more symmetrical than that observed at the bare electrode. While the diffusional tail remains evident at the GC/NT electrode, it is suggested that this peak might be the aggregate of contributions from up to three different processes. These are the expected oxidation of dopamine diffusing from bulk, dopamine diffusing through thin layers of solution within the porous nanotube film, and perhaps even dopamine adsorbed onto the surface of the nanotubes. While the large capacitive background current of the modified electrode contributed to the symmetry of the peaks, it was felt that this possibility merited further investigation. Before any experiments involving dopamine were performed, the modified electrode was scanned in phosphate buffer. After a series of dopamine experiments (which will be described shortly), the electrode was thoroughly rinsed and scanned once again in buffer. The result is presented in Figure 4.2.2(a). Undeniably, the electrode had been modified irreversibly during the scans involving dopamine. A redox couple was observed, which is attributed to a species either adsorbed on the NT surface, or present within thin layers of electrolyte trapped within the film. The E^0 value for this couple was found to be around 20 mV lower than that observed for dopamine in solution, so it cannot be said with certainty that this species is dopamine and not some intermediate in, or product of, its oxidation. Figure 4.2.2(b) also shows that these peaks were significantly smaller than those obtained when 50 μM dopamine was present in solution.

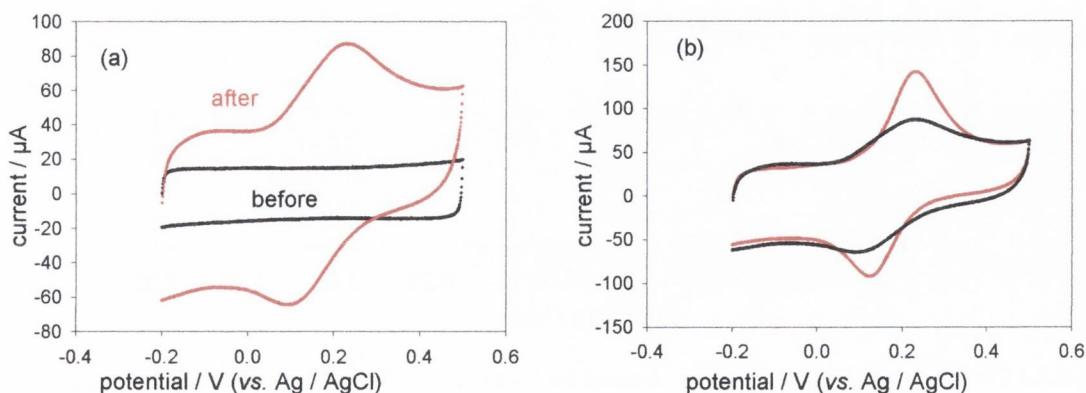


Fig. 4.2.2(a) Cyclic voltammograms obtained for SWCNT-modified GC in pH 7.0 phosphate buffer solution before and after dopamine experiments. **(b)** Comparison showing the relative size of the peaks in (a) and those obtained when dopamine was present in solution. The scan rate in each case was 50 mV s^{-1} .

Clearly, a greater quantity of charge is transferred when dopamine is present in solution, which might explain the survival of the diffusional tail. However, the point is that when the electrode is used to catalyse the redox reaction of dopamine, one must be aware that the obtained response contains some contribution from this parasitic process. Scan rate studies were attempted on the interfering peaks in the hope of establishing whether or not they might be attributed to adsorbed species, but they were found to diminish significantly with multiple cycles. To our knowledge, such complications involving the use of nanotube-modified electrodes have not been discussed widely in the literature, but recently Huang and co-workers [5] have also suggested that precautions are necessary when using NT-modified electrodes. These workers determined haloperidol and hydroxyzine using GC/MWCNT electrodes. They suggested that before every scan, the modified electrode should be renewed by repeated cycles in blank buffer until the unwanted peaks disappeared, and that only the first cycle should be analysed. The confusing effects of these complications can be seen in the comparison between peak separations shown in Figure 4.2.3.

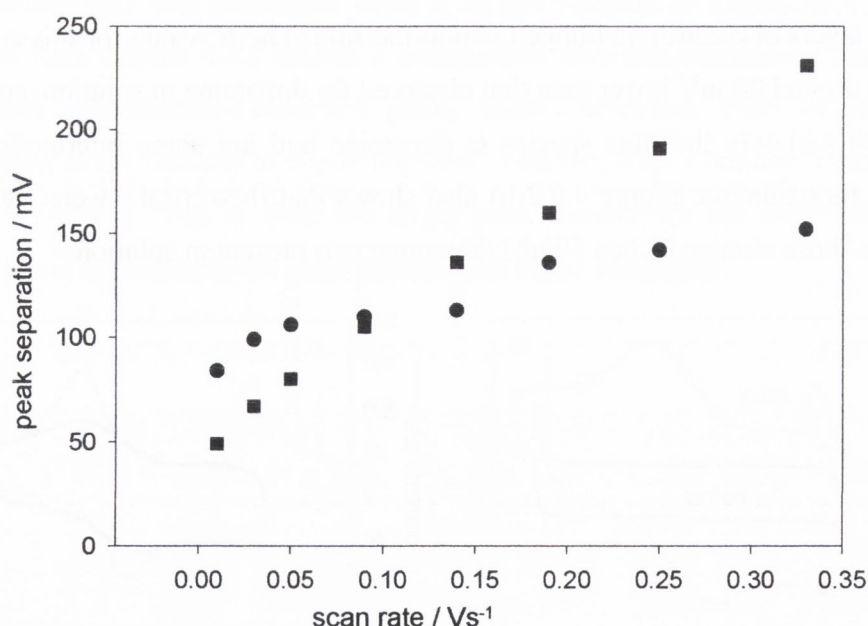


Fig. 4.2.3 Comparison of peak separations obtained for bare (●) and SWCNT-modified (■) glassy carbon electrodes in 50 μ M dopamine in 50 mM pH 7.0 phosphate buffer solution at various sweep rates.

In papers concerning the use of modified electrodes to determine dopamine, it is common to simply show that the modified electrode gives rise to smaller peak separations and therefore conclude that it has superior electro-catalytic properties when compared with the unmodified electrode. In this case however, the data do not adhere to such a facile interpretation. The figure suggests that the modified electrode allows for a faster reaction at low scan rates, but the opposite situation prevails at high v values. Reasons for this are debatable but it is suggested that, at high sweep rates, there is not enough time for diffusion through the narrow pores in the film and therefore no significant contribution from thin layer behaviour. At low scan rates, however, there is sufficient time for the NT film to behave as a three-dimensional electrode, with significant contributions from species diffusing within thin layers. It is envisaged that the reaction occurs not only at the outer surface of the nanotubes but also at reactive sites within the inner structure of the adsorbed assembly. Like adsorbed species, thin layer species give rise to smaller ΔE_p values [6] and it is conceivable that peak separations appear lower than they would if they were due exclusively to species diffusing from bulk. This suggestion is supported by the ‘more diffusional’ appearance of the peaks at higher scan rates. A comparison between scans obtained using a GC/SWCNT electrode in dopamine solution at high and low sweep rates is shown in Figure 4.2.4. Of course, peak currents and separations are higher for larger scan rates, but the difference in the *shape* of the peaks is noted with particular interest.

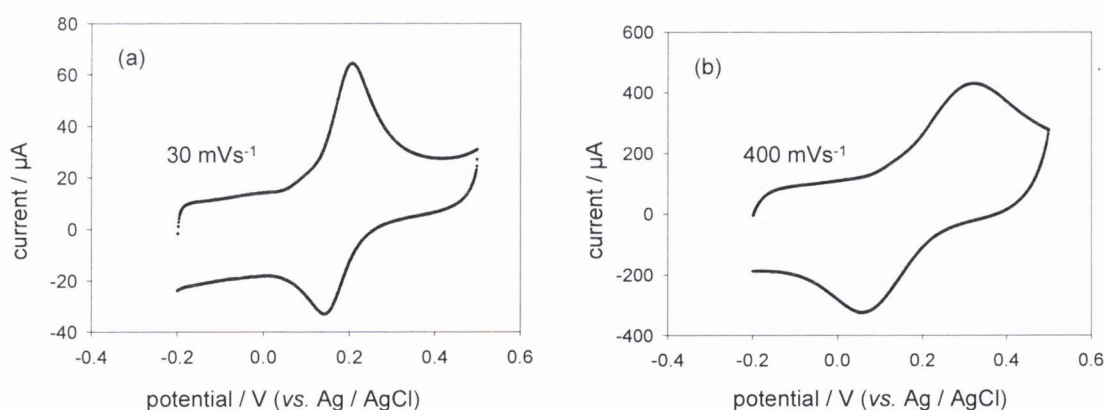


Fig. 4.2.4(a) Cyclic voltammogram obtained at 30 mV s^{-1} using a GC/SWCNT electrode in $50 \text{ }\mu\text{M}$ dopamine in pH 7.0 phosphate buffer solution. **(b)** Same at a scan rate of 400 mV s^{-1} .

Caution is urged, therefore, when assigning ‘catalytic’ properties to nanotubes on the basis of voltammetric peak separation before contributions from thin layer and/or adsorbed species are ruled out. It is suggested that these complications do not occur with ferrocyanide (see previous section), because for this redox probe, peak shapes were found to be diffusional at all scan rates, and a ‘before and after’ test revealed no evidence of thin layer or adsorbed species. The reasons for this may be electrostatic. The oxygenated functionalities on the nanotubes have sufficient negative charge to create an inhospitable environment for the ferro/ferricyanide anions.

The variation of the dopamine oxidation peak current with sweep rate also indicated that the system was influenced somewhat by processes other than the oxidation of dopamine diffusing from bulk solution. Figure 4.2.5 shows that the peak current at the bare electrode varied linearly with $v^{1/2}$ (correlation coefficient ~ 0.998), whereas the behaviour was a little scattered at the modified electrode ($r^2 \sim 0.994$). While this hardly constitutes definitive evidence, it does add some support to the view that the electrochemical response at the modified electrode is somewhat more complicated.

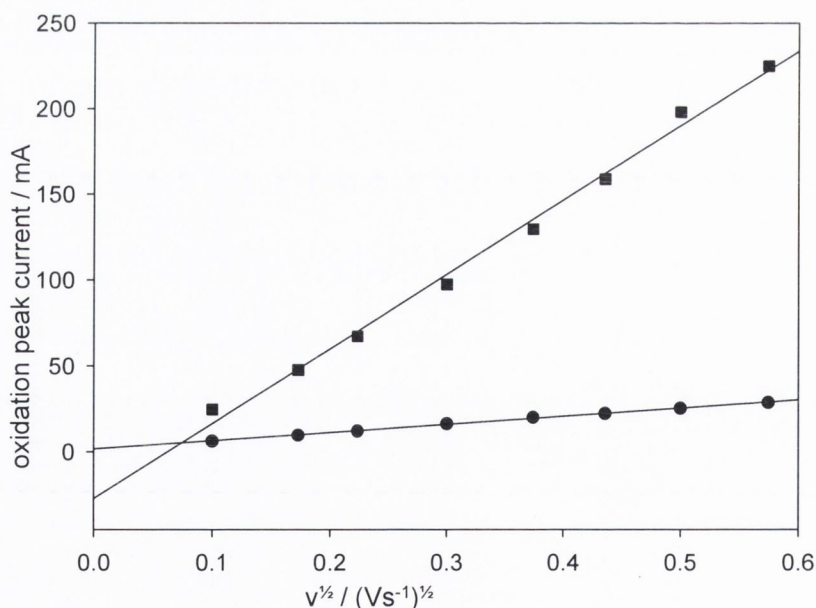


Fig. 4.2.5 The variation of oxidation peak current with square root of scan rate for a bare (●) and SWCNT-modified (■) electrode. The electrolyte used was a 50 μM solution of dopamine in 50 mM PBS (pH 7.0).

Further evidence of the absence of diffusion control at the modified electrode was provided by potential step chronoamperometry. A description of this technique can be found in *Section 2.3*. In these experiments, the potential was held at 0 V for 25 seconds to ensure only the reduced form of dopamine was present near the electrode surface. The potential was then stepped to +0.3 V (vs. Ag / AgCl) and the response was recorded. The pulse width was four seconds, which was found to be sufficient to allow currents to decay to steady-state values, as shown by the raw data in Figure 4.2.6(a). These do not reveal a great deal. Of course, currents are higher for the modified electrode. As mentioned in *Section 2.3*, for a reaction under the control of diffusion, plots of $|I|$ against $t^{-1/2}$ should be linear and pass through the origin. Such plots for the two electrodes are presented in Figure 4.2.6(b). It is immediately obvious that the reaction at the bare electrode is under the control of dopamine diffusion (correlation coefficient 0.995). However, the GC/SWCNT data deviate drastically from the Cottrell model, suggesting that there are other processes controlling the reaction rate.

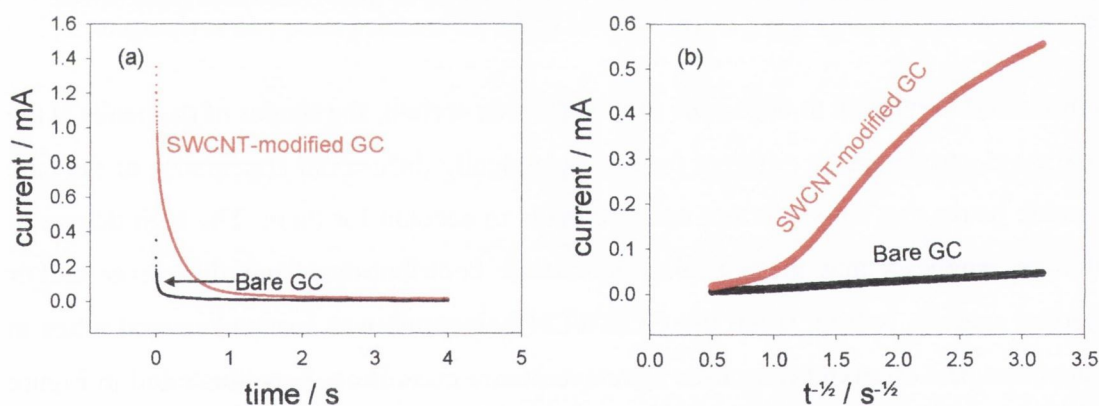


Fig4.2.6(a) Chronoamperometric responses of 50 μ M dopamine at bare and SWCNT-modified glassy carbon electrodes to a potential step from 0 to +0.3 V after poisoning the electrodes at 0 V for 25 seconds. Pulse widths were four seconds. **(b)** The resulting Cottrell plots.

4.3 Epinephrine

The nanotube-modified electrode was also used to catalyse the oxidation of epinephrine (EP). At the bare glassy carbon electrode, this process was found to occur at a potential of +0.31 V (vs. Ag / AgCl), while at the the modified electrode, this peak shifted negatively to +0.20 V, and the oxidation peak current increased dramatically. The superior electro-

catalytic properties of the modified electrode are believed to be due to the nanotube dimensions, their electronic structure and to topological defects present on the NT surface [7]. Figure 4.3.1 shows a comparison between voltammograms (first cycles only) obtained at the bare and modified electrodes. The E_{pa} shift of 110 mV is considerably larger than the 70 mV reported by Luo *et al.* [8] and the 50 mV reported by Wang and co-workers [9] for SWCNTs immobilised on a glassy carbon electrode.

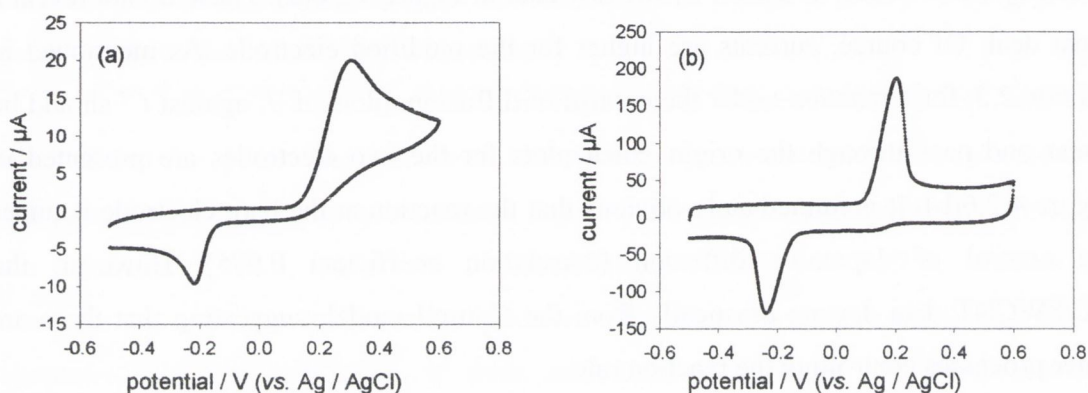


Fig. 4.3.1 (a) Cyclic voltammogram for 0.4 mM EP in phosphate buffer (pH 7.0) at a bare glassy carbon electrode. The scan rate was 100 mV s^{-1} . (b) Same for a SWCNT-modified glassy carbon electrode.

As discussed in relation to dopamine in the previous section, the shapes of the peaks at the modified electrode are so different from the classically diffusional appearance of the bare electrode peaks that some attempt must be made to account for them. The high degree of symmetry suggests that these peaks incorporate contributions from thin layer and/or adsorbed species. Indeed, when the GC/SWCNT electrode was scanned several times in epinephrine, the observed responses were even more complicated, as illustrated in Figure 4.3.2. The decrease in the oxidation peak current for EP and the appearance of a pre-peak suggest the adsorption of an oxidation product (or perhaps intermediate) at the modified electrode. Adsorbed products are stabilised with respect to the electrode reaction and therefore react more readily [10]. A post-peak would indicate the presence of an adsorbed reactant.

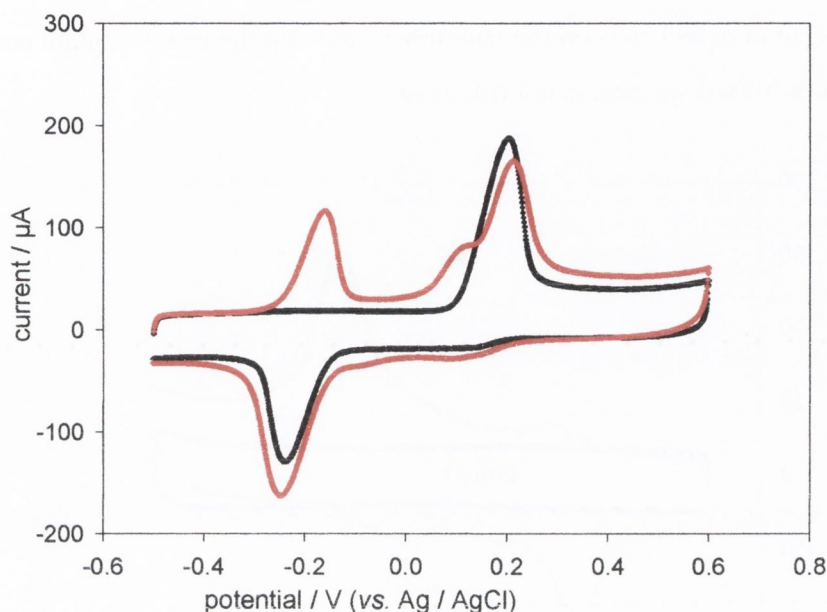


Fig. 4.3.2 Cyclic voltammograms for 0.4 mM EP in PBS (pH 7.0) at a GC/SWCNT electrode. The black and red responses represent the initial and a subsequent cycle, respectively. The sweep rate was 100 mVs^{-1} .

Even more striking than the appearance of this shoulder is the appearance of a ‘new’ oxidation peak at -0.16 V (vs. Ag / AgCl). The considerable increase in the charge transferred in the cathodic sweep suggests that this species reduces at the same potential as epinephrine, but the large separation between the two dominant anodic processes is not something which can be explained satisfactorily at this point. Moore, Banks and Compton [11] have also shown the shoulder and second oxidation peak at basal plane HOPG electrodes modified using MWCNT films, but these workers made no attempt to identify the species responsible. Interestingly, in the same paper, they showed that these complications do not occur at abrasively modified electrodes. The latter are not porous, so the most which can be said is that these parasitic features are probably due to thin layer behaviour within the porous nanotube film. When the modified electrode was removed, thoroughly rinsed and scanned in buffer, peaks corresponding to all of these processes were observed, as shown in Figure 4.3.3. In the absence of solution epinephrine, these were, of course, considerably smaller but nonetheless sufficient to again show that irreversible alteration of the nanotube electrode occurred during the EP experiments. Such behaviour was not observed at bare glassy carbon, and the most likely explanation is

simply the porosity of the film. The possibility, however, that adsorption of certain species can occur at defect sites on the nanotubes, and that the peaks contain contributions from these immobilised species, is not ruled out.

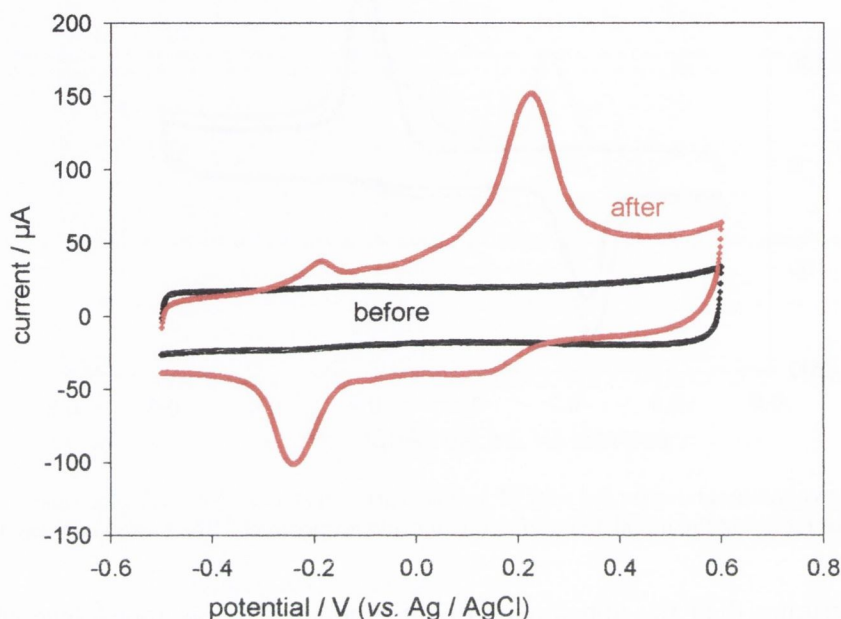


Fig. 4.3.3 Cyclic voltammograms obtained for a SWCNT-modified glassy carbon electrode in pH 7.0 phosphate buffer solution before and after epinephrine experiments. The sweep rate was 100 mV s^{-1} .

4.4 Norepinephrine

The ability of SWCNT film-modified glassy carbon electrodes to catalyse the redox process of the important neurotransmitter norepinephrine (NE) is now reported. Figure 4.4.1 shows a comparison between voltammograms obtained using bare and modified electrodes in the absence and presence of 1 mM norepinephrine. The data shown represent the first scans obtained immediately on placing the electrodes in solution, for reasons which will be explained later in this section. At the bare glassy carbon electrode, norepinephrine showed quasi-reversible behaviour, with a peak separation of 200 millivolts at a scan rate of 100 mV s^{-1} . This ΔE_p value was lowered to 160 mV when the electrode was modified using carbon nanotubes. This decrease of 40 mV suggests an enhancement of reaction kinetics but it should be pointed out that it is not as dramatic as the 100 mV reported by Wang *et al.* [9] for norepinephrine at glassy carbon electrodes modified using arc discharge single-walled nanotubes.

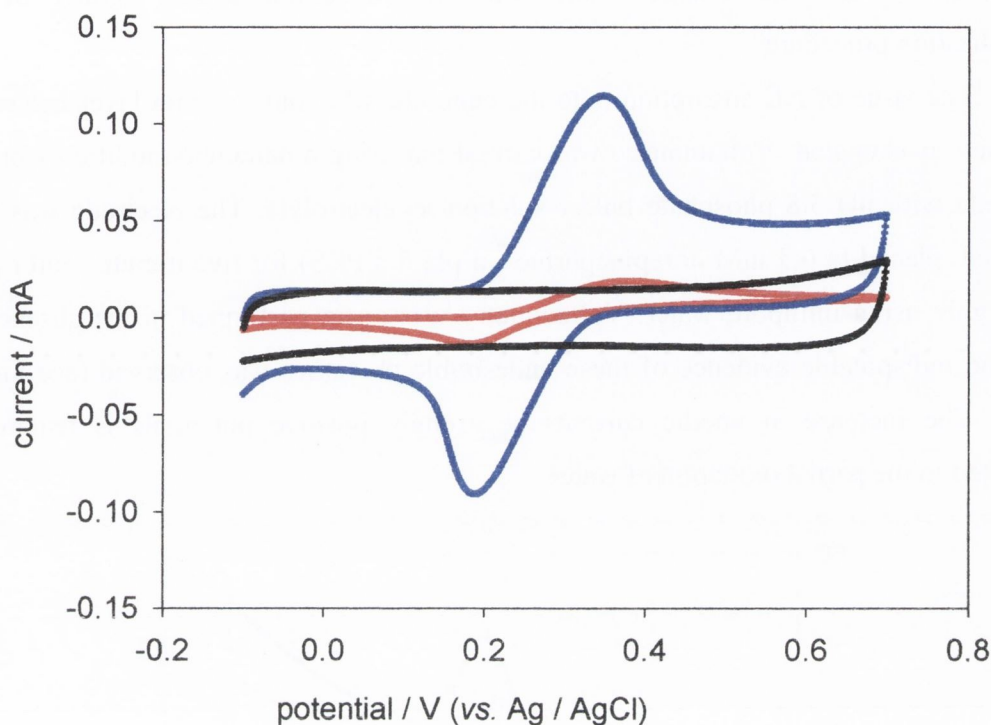


Fig. 4.4.1 Cyclic voltammograms at a bare glassy carbon electrode (red) and a SWCNT-modified electrode (black and blue) in the absence of norepinephrine (black) and in 1 mM norepinephrine (red and blue) in pH 5.8 phosphate buffer solution. The scan rate employed in each case was 100 mV s^{-1} .

The figure also illustrates the huge increase in peak currents caused by nanotube modification, which is again attributed to an enhancement of the electro-catalytic response and the large active surface area of the modified electrode. The film-modified electrode showed no peaks in the absence of NE in this potential window (black). Strictly speaking it is not correct to claim an enhancement of the electro-catalytic response on the basis of larger peaks, unless all currents are corrected for active surface area. However, this precaution is virtually never exercised in published studies of nanotube electrochemistry so it will not be mentioned again. It is noted that, when current *densities* are compared, based on the surface areas calculated in the previous chapter, the steeper and larger peaks remain evident at the modified electrode. An argument against the use of normalisation with respect to surface area would be the porosity of the modified electrode. At high scan rates, species do not have as much time to gain access to the inner surface of the nanotube ensemble. This means, effectively, that the active surface area of

the modified electrode changes with scan rate, undermining the rigidity of the normalisation procedure.

The issue of NE adsorption onto the nanotube film and / or thin layer behaviour was then investigated. Voltammetry was carried out using a nanotube-modified working electrode with pH 5.8 phosphate buffer solution as electrolyte. The electrode was then removed, placed in 0.5 mM norepinephrine (in pH 5.8 PBS) for five minutes and rinsed thoroughly using millipore water. Voltammetry was again performed in the absence of NE, and indisputable evidence of these undesirable processes was observed (see Figure 4.4.2). The increase in anodic currents at strongly positive potentials is tentatively attributed to the partial oxidation of water.

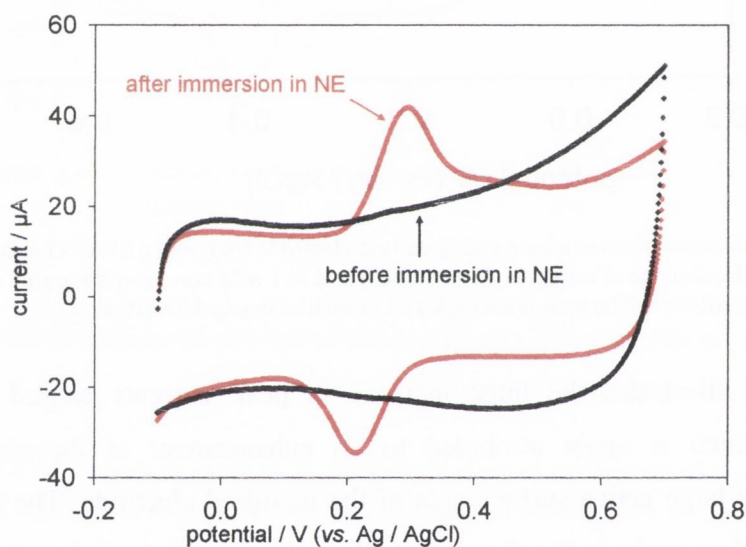


Fig. 4.4.2 Voltammograms obtained using a SWCNT-modified glassy carbon electrode in pH 5.8 PBS. No norepinephrine was present in the electrolyte in either experiment. The red scan was obtained after the electrode had been immersed in 0.5 mM NE for five minutes. The scan rate employed was 100 mV s^{-1} .

These results indicate that norepinephrine either spontaneously adsorbs onto the surface of the carbon nanotubes used in this work, or the porous film retains small volumes of solution. If adsorption occurs, the nature of this interaction would be difficult to elucidate definitively. It is suggested at this point that it is electrostatic. Adsorption was not observed using a bare glassy carbon electrode. Oxygenated defects present on the nanotubes' surface are the most obvious sites at which such interactions might occur but again proof of this is far from trivial.

Regardless of the precise explanation, the fact is that these complications *do* occur and this represents a most serious consideration in the interpretation of data regarding ‘electro-catalytic’ properties of nanotubes. It is interesting to note that the peak separation for adsorbed / thin-layer norepinephrine was a good deal smaller than when the molecule was diffusing through the electrolyte to react at the electrode surface (80 compared to 160 mV), as expected for such processes. This means that one must be absolutely certain that one is looking at a bulk solution process when, for example, comparing peak separations for bare and modified electrodes. This is the reason that the scans presented in Figure 4.4.1 were the first scans obtained immediately on placing the electrode in solution and not subsequent scans, for which it would be likely that significant contributions from parasitic processes might be made, resulting in more symmetrical peaks, and, more seriously, deceptively small peak separations.

Despite concerns regarding these unwanted contributions, the influence of scan rate on the oxidation process was also investigated. Inspection of Figures 4.4.1 and 4.4.2 shows that peak currents were far larger for solution NE than for the adsorbed / thin-layer species so, while there can be no doubt that oxidation of the latter occurs as more scans are performed, the observed signal is dominated by the norepinephrine present in the bulk solution. The oxidation peak current was found to vary in a linear manner with the square root of scan rate (see Figure 4.4.3) within the range of 20 to 200 mV s^{-1} , indicating that the signal is indeed due to a diffusion-controlled process.

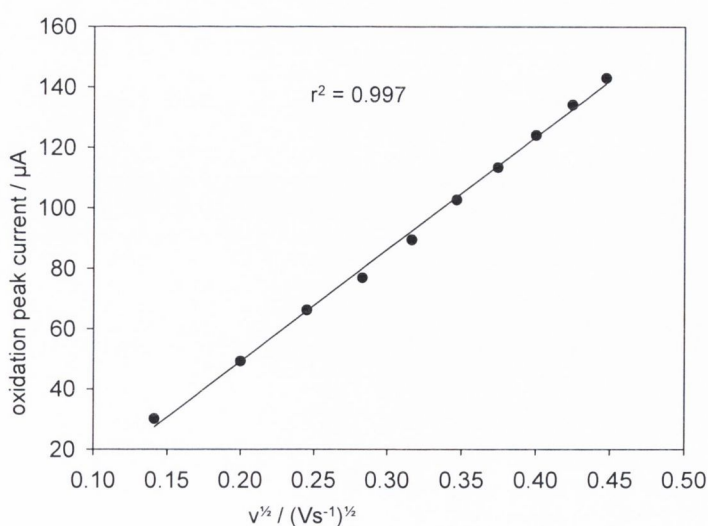


Fig. 4.4.3 The relationship between oxidation peak current and scan rate for 0.5 mM norepinephrine in pH 5.8, 50 mM phosphate buffer solution. The working electrode was SWCNT-modified glassy carbon.

It was also found that oxidation peak currents varied linearly with the concentration of norepinephrine (see Figure 4.4.4). This was investigated by adding known amounts of NE to the electrolyte, stirring to ensure homogeneous mixing and performing cyclic voltammetry. The concentration range over which linearity was observed was between 1.2 and 18 μM , with a correlation coefficient of 0.997 ($n = 12$). The detection limit (the lowest concentration for which a measurable peak current was obtained) was found to be around 1 μM .

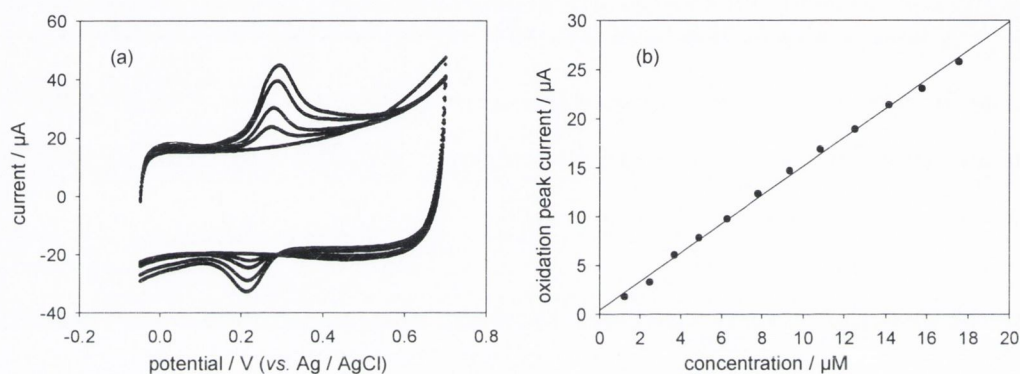


Fig. 4.4.4(a) Cyclic voltammograms of norepinephrine at concentrations of (from inner to outer) 0.0, 2.5, 6.3, 13 and 18 μM . **(b)** The resulting plot of electrocatalytic peak currents against norepinephrine concentration. Background electrolyte was a pH 5.8 PBS and the scan rate in each case was 100 mV s^{-1} .

4.5 Ascorbic Acid

In the case of ascorbic acid (AA), an irreversible oxidation peak was observed at $+0.21 (\pm 0.01)$ V using a bare glassy carbon electrode. Standard deviations were calculated on the basis of data obtained using four independently-prepared electrodes. The structure of ascorbic acid can be found in *Section 1.7*. When the electrode was modified using a film of SWCNTs, this peak shifted negatively to $-0.02 (\pm 0.02)$ V, and a slight increase in oxidation peak current was observed (see Figure 4.5.1).

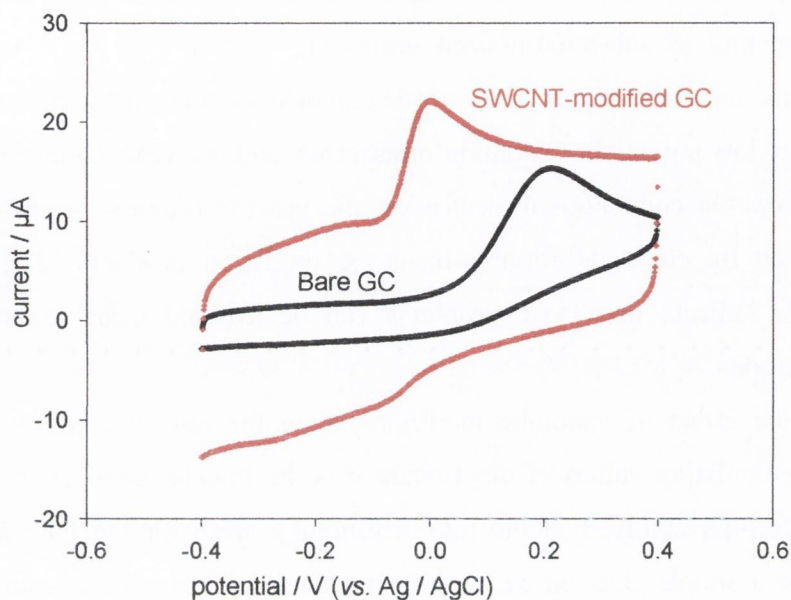


Figure 4.5.1 Cyclic voltammograms recorded at a bare glassy carbon electrode and a SWCNT-modified electrode in 0.5 mM ascorbic acid in pH 7.0 phosphate buffer solution. The scan rate was 100 mV s^{-1} .

As discussed in *Chapter One*, there exists a large body of literature concerning the determination of AA using electrodes modified with carbon nanotubes and various other materials. In the present work a shift of 230 mV in the oxidation potential is reported due to modification which, as *Table 4.5* shows, compares favourably with many of these published findings.

Substrate	Modifier	AA Oxidation Peak Shift / mV	Reference
GC	SWCNTs	80	[8]
CPE	MWCNTs	230	[11]
GC	SWCNTs	112	[9]
GC	Ferrocene derivatives	300	[13]
Carbon	Plasma polymerised vinylpyridine + pentachloroiridite	230	[14]
Pt	Ferrocene	150	[15]
Graphite-epoxy composite	Cobalt phthalocyanide	150	[16]
Microdisk gold	3,4-Dihydroxybenzoic acid	200	[17]
CPE	FCA	248	[18]

Table 4.5 Comparison of the efficiency of some modified electrodes used for the determination of ascorbic acid. All work was carried out in aqueous solution.

Poh *et al.* [19] have reported that the oxidation of AA occurs at a boron-doped diamond electrode consisting of sub-micron-sized grains at +0.45 V (vs. Ag / AgCl), so it is apparent that the nanotube-modified electrode can achieve ascorbic acid determination at an impressively low potential. Oxidation of ascorbic acid is a real challenge in analytical electrochemistry. On conventional electrodes, the process requires large overpotentials and is hindered by electrode fouling from the oxidation products [20]. The results presented here indicate that these problems can be avoided using carbon nanotube-modified electrodes.

Also, the effect of nanotube modification on the rate of electron transfer was estimated by calculating values of αn_a (where α is the transfer coefficient and n_a is the number of electrons involved in the rate-determining step) for the bare and modified electrodes. For a simple electron exchange between a solution species and an electrode the first-order heterogeneous rate constant for the oxidation process may be expressed using the empirical equation [10]

$$k = k^0 \exp\left(\frac{\alpha n_a F E}{RT}\right) \quad (4.5.1).$$

In this expression k^0 is the standard rate constant for the anodic reaction and E is the sum of the overpotential (η) and the equilibrium potential (E_e). From this expression it is clear that a larger value of αn_a implies a larger value for the rate constant. Values for αn_a at 100 mV s⁻¹ were obtained using the expression [21]

$$\alpha n_a = \frac{0.048}{E_p - E_{p/2}} \quad (4.5.2).$$

Here E_p is the peak potential and $E_{p/2}$ is the potential when the current is half-way between the maximum height and the baseline against which it is measured. Values for αn_a were found to be 0.29 and 0.59 for the bare and modified electrodes, respectively. These values confirm that, not only is the oxidation peak potential decreased, but the rate of electron transfer is significantly increased.

Furthermore, it was found that peak currents varied linearly with the concentration of ascorbic acid (see Figure 4.5.2). This was investigated by adding known amounts of AA to the electrolyte, stirring to ensure homogeneous mixing and performing cyclic voltammetry. The concentration range over which linearity was observed was between

0.12 and 1.75 mM with a correlation coefficient of 0.9991 ($N = 12$). The detection limit (the lowest concentration for which a measurable peak current was obtained) was found to be around 12 μM .

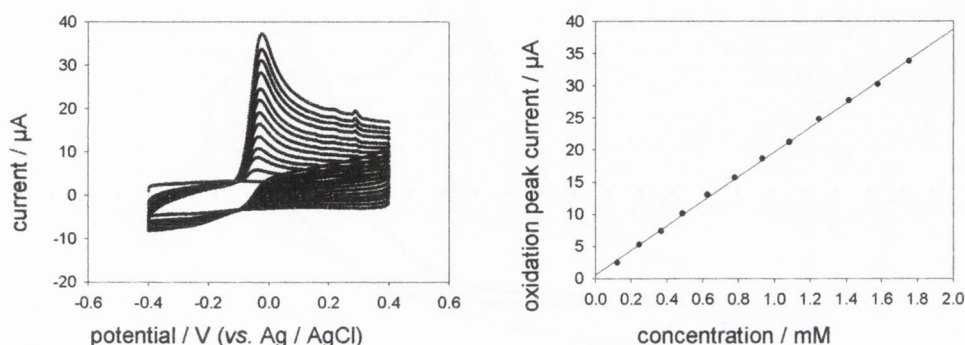


Fig. 4.5.2 Cyclic voltammograms of ascorbic acid at various concentrations ranging (from inner to outer) from 0.12 to 1.75 mM (left) and the resulting plot of electrocatalytic peak currents against AA concentration (right). Background electrolyte was a pH 7 PBS and the scan rate in each case was 20 mV s^{-1} .

4.6 NADH

The next biomolecule investigated was β -nicotinamide adenine dinucleotide (NADH). A brief introduction to this compound and its importance in electroanalysis are provided in Section 1.8. In the past, anodic detection of NADH has been hindered by problems such as the high potential required using conventional electrodes [22] and surface fouling due to the accumulation of the NAD^+ produced by the oxidation process [23-26]. As a result, the search for modified electrodes which can minimise these effects has become an area of interest. Figure 4.6.1 compares voltammograms for NADH, recorded at 50 mV s^{-1} , at bare and SWCNT-modified glassy carbon electrodes. The response of the modified electrode is typical of a NT electrode formed by drop-coating, in which ‘thin layer’ behaviour is observed with the loss of the ‘diffusional tail’ [11]. The symmetry of the peak is undermined somewhat by the large currents at strongly anodic potentials, which are again attributed to the partial oxidation of the aqueous solvent. By comparing CNT-modified electrodes with ePHOPG and bPHOPG electrodes, Banks and Compton [27] have shown that the oxidation of NADH occurs at oxygenated functionalities present at the edge-plane sites of nanotubes (tube ends and defects). It is as yet unclear what the

precise identity of the oxide(s) responsible is, but is worth noting that *o*-quinones have been shown to act as efficient mediators in the electro-oxidation of NADH [28].

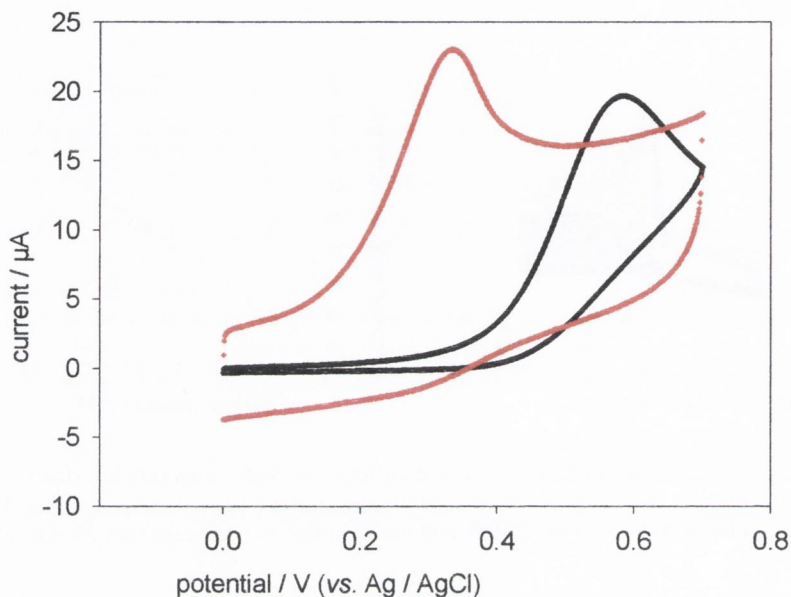


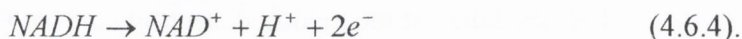
Figure 4.6.1 Cyclic voltammograms at a bare glassy carbon electrode (black) and a SWCNT-modified electrode (red) in 2 mM NADH in pH 7.0 phosphate buffer solution. The scan rate was 50 mV s^{-1} .

On the basis of four independent experiments, the irreversible oxidation of NADH was found to occur at $+0.33 (\pm 0.01) \text{ V}$ (vs. Ag / AgCl) using the modified electrode, compared with $+0.58 (\pm 0.01) \text{ V}$ using the bare glassy carbon electrode. The figure also shows the sharper peak shape at the GC/SWCNT electrode, as well as the slightly enhanced peak current. Again the observed decrease in oxidation potential is attributed to the presence of oxygenated functionalities, which have been known to mediate NADH electron transfer [29]. This value for the oxidation potential of NADH at the nanotube electrode agrees exactly with that reported by Musameh *et al.* [24] using GC/SWCNT and GC/MWCNT electrodes. However, these workers report a significantly higher value for the E_{pa} at the bare electrode ($+0.82 \text{ V}$ vs. Ag / AgCl) and hence a larger shift. Banks and Compton [27] have reported an NADH oxidation peak potential of $+0.40 \text{ V}$ vs. SCE ($+0.45 \text{ V}$ vs. Ag / AgCl) at epHOPG electrodes, whereas Rao *et al.* [25] have shown that this process occurs at $+0.60 \text{ V}$ vs. SCE ($+0.65 \text{ V}$ vs. Ag / AgCl) using boron-doped diamond electrodes.

The mechanism of the electrochemical oxidation of NADH is thought to be as follows [30]:



Firstly, in the rate-determining step, NADH is irreversibly oxidised to give the cation radical $NADH^{\bullet+}$. This then deprotonates to give a neutral radical which, at a sufficiently positive potential, is immediately oxidised to NAD^+ at the electrode surface in the third and final step. $NAD^{\bullet}$ may also lose an electron to a cation radical in bulk solution [30]. The overall oxidation process may be represented as



It was also shown, using steady-state amperometry, that the nanotube-modified electrodes exhibit a highly stable amperometric NADH response. A typical result is shown in Figure 4.6.2. Phosphate buffer (pH 7.0) was placed in the cell and the potential was fixed at +0.35 V (vs. Ag / AgCl). After allowing the current to stabilise for around two minutes, a known amount of NADH solution was added, making the concentration in the cell around 0.2 mM. The current increased as a result and was allowed to decay for another thirty minutes.

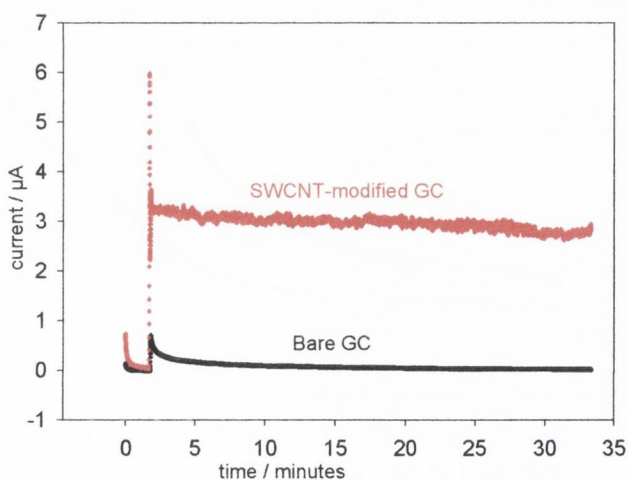


Fig. 4.6.2 Stability of the response to 0.2 mM NADH using bare and SWCNT-modified glassy carbon electrodes. The operating potential was + 0.35 V (vs. Ag / AgCl) and the stirring rate was 100 rpm. The electrolyte used was a pH 7.0 phosphate buffer solution.

As can be seen, the response of the modified electrode to the addition of NADH is more substantial, an effect which is again attributed to the increased surface area of the modified electrode and the fact that, at this low operating potential, there is little oxidation of NADH occurring at the bare electrode (see voltammetry results). It is also interesting to note the superior stability of the signal. The bare glassy carbon response current decays rapidly, losing 82 %, 90 % and 98 % of its initial value after five, fifteen and thirty minutes respectively, suggesting virtually complete inhibition of the oxidation process. On the contrary, the response of the modified electrode is remarkably robust throughout the entire experiment, having lost only 5 %, 8 % and 14 % of its initial magnitude at these times. These results suggest that the nanotube-modified electrode offers superior resistance to surface fouling compared to bare glassy carbon.

The resistance of the modified electrode to NAD^+ fouling was also probed using cyclic voltammetry. The voltammetric response of the *bare* glassy carbon electrode before and after being left in 1 mM NADH for one hour was investigated, and the results obtained are presented in Figure 4.6.3.

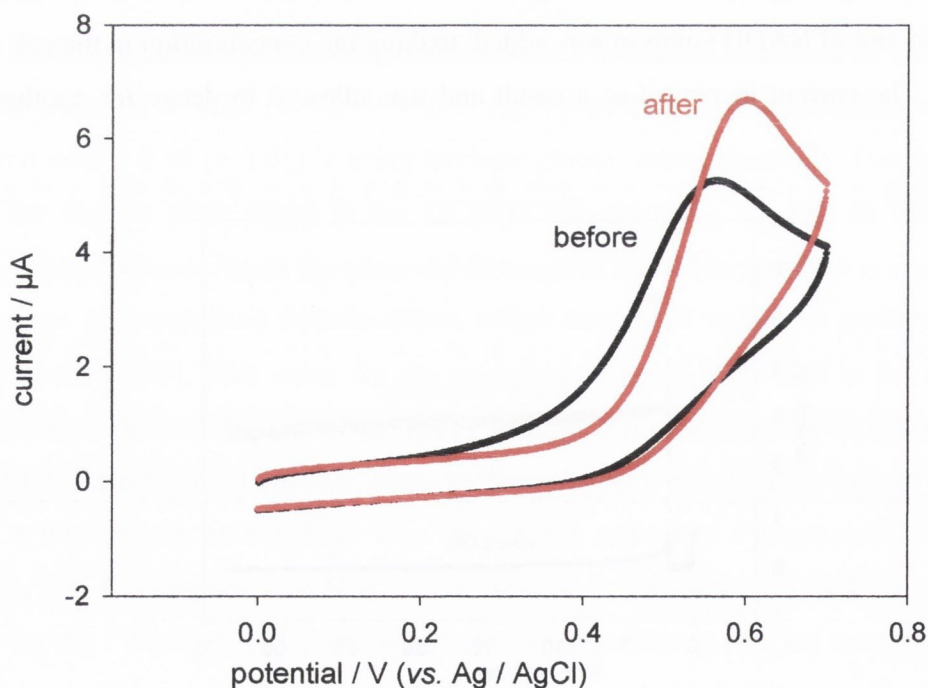


Fig. 4.6.3 Voltammetry before and after leaving a bare glassy carbon electrode in a pH 7.0 PBS containing 1 mM NADH for one hour. After the initial scan, the electrode was left in the same solution for one hour. The solution was then stirred before performing the second scan. The scan rate was 50 mV s^{-1} .

The figure shows that an hour of exposure of the bare electrode to NADH caused the oxidation potential to shift to a value some 40 mV more positive. A similar increase in oxidation potential was observed when the freshly polished glassy carbon electrode was scanned once in NADH, rinsed with water and allowed to dry in air for one hour. Such an increase is attributed to adsorption phenomena. The NAD^+ bound to the glassy carbon surface inhibits the approach of NADH, and a higher potential is necessary for oxidation to occur. It is acknowledged, however, that the increase in peak current appears to contradict this view. Pariente *et al.* [26] have reported shifts of 300 mV due to adsorption processes on glassy carbon. The GC/SWCNT electrode was found to be immune to such problems, as shown in Figure 4.6.4. No appreciable change in oxidation potential was observed. The peak current also increased considerably, which is interpreted as an indication of resistance to fouling. This behaviour was found to be reproducible when carried out on four independently-prepared modified electrodes. It is therefore evident that nanotube-modified glassy carbon electrodes not only permit the oxidation of NADH at significantly lower potentials than bare electrodes, but also display impressive resistance to fouling. These findings suggest possible sensing applications.

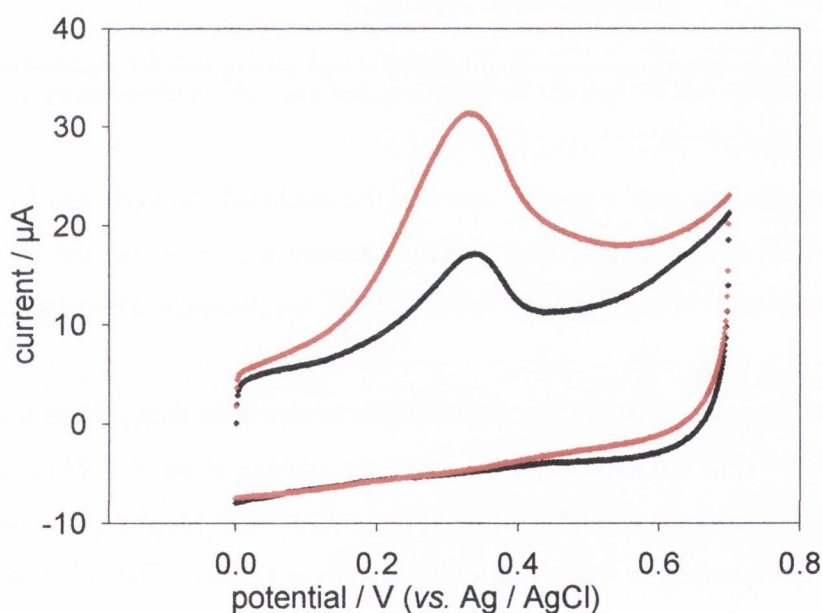


Fig. 4.6.4 Voltammetry before (black) and after (red) leaving a GC/SWCNT electrode in a pH 7 PBS containing 1 mM NADH for one hour. After the initial scan, the electrode was left in the same solution for one hour. The solution was then stirred before performing the second scan. The scan rate was 50 mV s^{-1} .

Biosensing applications commonly rely on amperometric measurements of stirred sample solutions. Hydrodynamic voltammetry was therefore carried out in order to examine the electro-catalytic behaviour of the modified electrode under these conditions. A typical result is shown in Figure 4.6.5.

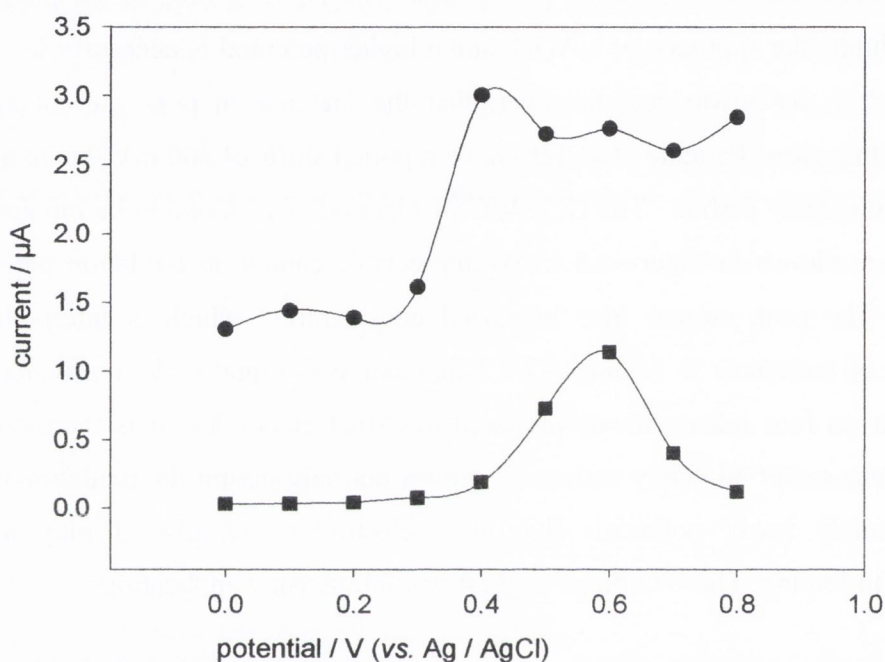


Fig. 4.6.5 Hydrodynamic voltammograms for 2 mM NADH at bare (■) and SWCNT-modified (●) GC electrodes. The stirring rate was 300 rpm and the electrolyte used was a pH 7.0 phosphate buffer.

Like with cyclic voltammetry, it can be seen that the modified electrode can facilitate the oxidation of NADH at a lower potential. The fluctuations in current after the GC/SWCNT peak reflect the complicated oxidation route and the involvement of surface processes [24].

The sensing possibilities of the modified electrodes were then probed using cyclic voltammetry. This work involved carrying out scans starting at an NADH concentration of 3.2 mM. After each scan, a small volume of 8.5 mM NADH (in phosphate buffer) was added in order to increase the concentration by a known amount. The solution was then stirred to ensure uniformity before scanning at the raised concentration. All scans were recorded at a sweep rate of 50 mV s^{-1} and are presented in Figure 4.6.6.

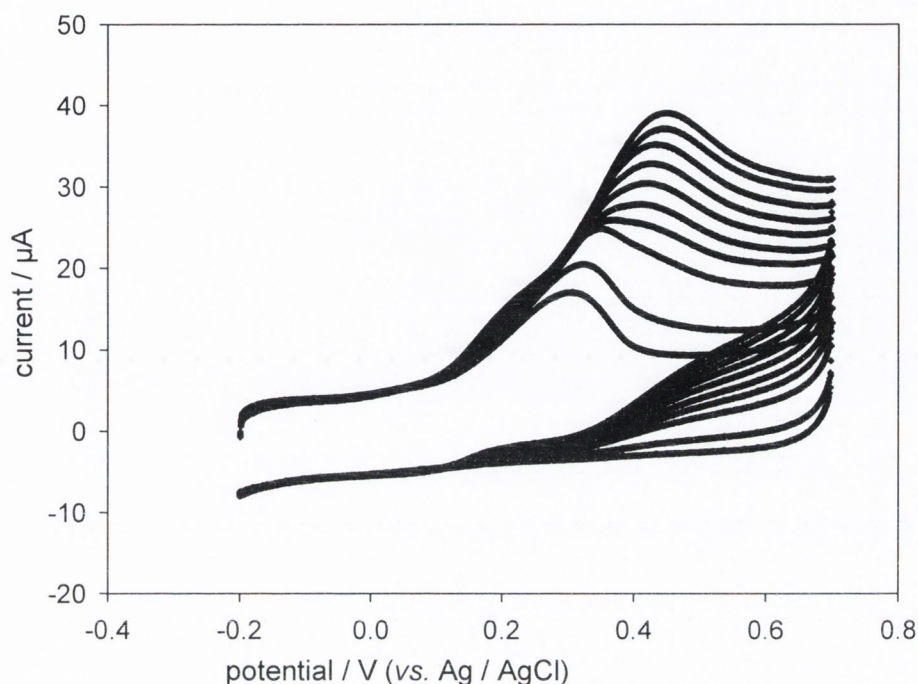


Fig. 4.6.6 Voltammetric responses observed at a SWCNT-modified glassy carbon electrode from additions of NADH to a pH 7.0 PBS from NADH concentrations of 3.21 to 23.5 μM . Scan rate was 50 mV s^{-1} .

The figure shows clearly the increase in oxidative peak current with increasing NADH concentration. A calibration curve was thus drawn (see Figure 4.6.7) which produced the following linear regression: $i_{pa} / \text{A} = 9.38 \times 10^{-2} \times ([\text{NADH}] / \text{M}) + 3.79 \times 10^{-7} \text{ A}$; $r^2 = 0.99$, $N = 11$). Peak currents were measured as defined in *Chapter Two*, with a baseline drawn tangent to the curve just before the start of the peak ($\sim 0.1 \text{ V}$ in this case). The values for i_{pa} were taken as the vertical difference between the current maximum and this baseline. The limit of detection (the smallest value of the NADH concentration for which a well-defined and easily quantifiable peak current was obtained, based on a signal-to-noise ratio of three) was found to be 1.8 μM , which compares favourably with the 4.5 μM reported by Banks and Compton [27] using voltammetry with edge-plane HOPG electrodes. Rao *et al.* [25] have reported well-defined voltammetric peaks for NADH concentrations as low as 1 μM using boron-doped diamond electrodes.

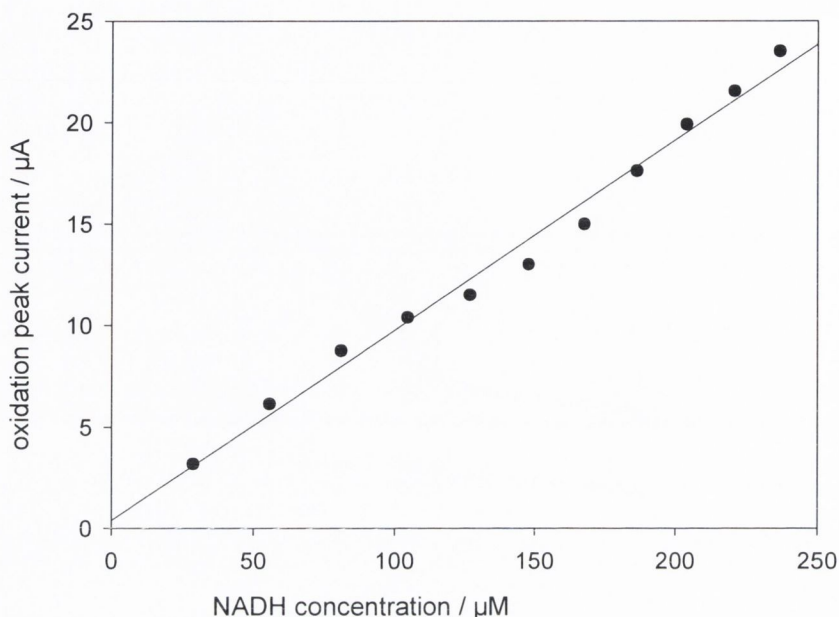


Fig. 4.6.7 Typical calibration plot of peak current versus NADH concentration for cyclic voltammetry experiments carried out at a sweep rate of 50 mV s^{-1} .

An issue which ought to be raised at this point is the increase in oxidation potential as more and more experiments are performed (as shown in Figure 4.6.6), which is attributed to a degree of surface fouling. As mentioned previously, the nanotube edge-plane sites are believed to be paramount to the electro-catalytic response of the modified electrodes with respect to NADH oxidation. However, it is these very regions which are thought to contribute to fouling [27]. These sites are decorated with oxygenated functionalities (as was shown using several techniques in the previous chapter) for which the NAD^+ formed has a considerable affinity [8,11]. This problem was not observed by Banks and Compton [27] using edge-plane HOPG electrodes. A possible explanation for this is that the SWCNTs used in this work may have contained fewer edge-plane sites per unit area than epHOPG electrodes. So, while there must indeed be a degree of fouling with the latter, there are so many active sites that this does not significantly affect the kinetics of electron transfer over this number of experiments. It is conceivable that these nanotubes may have contained relatively few edge-plane sites as they were prepared using the HiPCo[®] method. To our knowledge there are no reports on the edge-plane defect content of nanotubes produced in this way. It is likely, however, that the density of edge-plane-like sites on a nanotube ensemble, while sufficient to enhance the catalytic response, is less

than that of an electrode composed entirely of edge-plane graphite. Taking all this into account, caution is urged when interpreting the voltammetric data, as the evidence suggests that the electrode surface after several experiments differs significantly from its initial condition.

The system was next tested using steady-state amperometry. This involved periodic additions of NADH solution to a stirred phosphate buffer, while holding the potential at + 0.35 V vs. Ag / AgCl (see Figure 4.6.8). This potential was chosen because the voltammetric data indicated that the oxidation process occurred at around this value at the modified electrode.

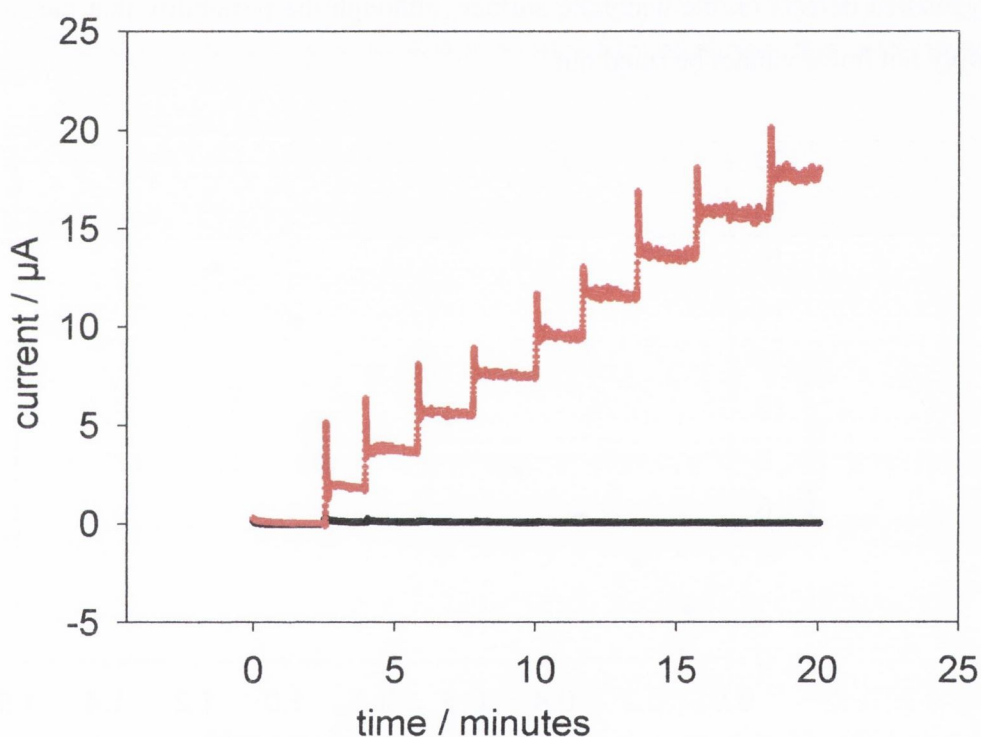


Fig. 4.6.8 Current-time recordings obtained on increasing the NADH concentration in known steps at a bare (black) and SWCNT-modified (red) glassy carbon electrode. The operating potential was + 0.35 V (vs. Ag / AgCl), the stirring rate was 100 rpm and the electrolyte was a pH 7.0 phosphate buffer.

The figure shows clearly the dramatic effect of nanotube modification. At this low oxidation potential, the bare glassy carbon electrode exhibits virtually no response to the addition of NADH, as one would expect on the basis of the cyclic voltammetry results already discussed. On the other hand, the GC/SWCNT electrode shows a very well-defined signal on each successive increase in analyte concentration. The response is also

quite rapid, with signals becoming steady within ten seconds of each addition. A plot of current against NADH concentration was found to be reasonably linear (correlation coefficient 0.996) up to around 1.4 mM, as shown in Figure 4.6.9. Using this technique, NADH concentrations as low as 10 μM were detected. Rao and co-workers [25] have reported detection limits of 10 $n\text{M}$ using boron-doped diamond electrodes, albeit at the significantly greater applied potential of +0.58 V vs. SCE (+0.63 V vs. Ag / AgCl).

The sensitivity to NADH additions decreased slightly as the experiment progressed (this manifested itself in the data in Figure 4.6.9 ‘leaning over’ a little), an effect which is again attributed to a degree of fouling due to NAD^+ adsorption at oxygenated defects on the nanotube surface, although the possibility that the response is simply not linear cannot be ruled out.

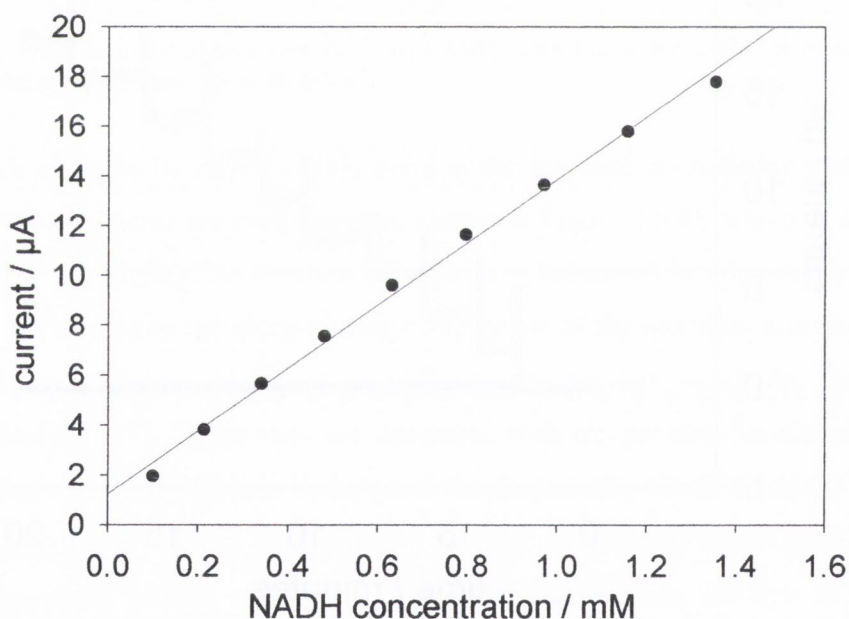


Fig. 4.6.9 The current response to increases in NADH concentration for the nanotube-modified glassy carbon system in the previous figure.

4.7 Oxygen

The effect of nanotube modification on the response to the irreversible reduction of oxygen was investigated both in base and in pH 7.0 phosphate buffer. Firstly, cyclic voltammetry was performed using both bare and modified glassy carbon electrodes in

electrolytes saturated with either nitrogen or oxygen. The results obtained at neutral pH are presented in Figure 4.7.1.

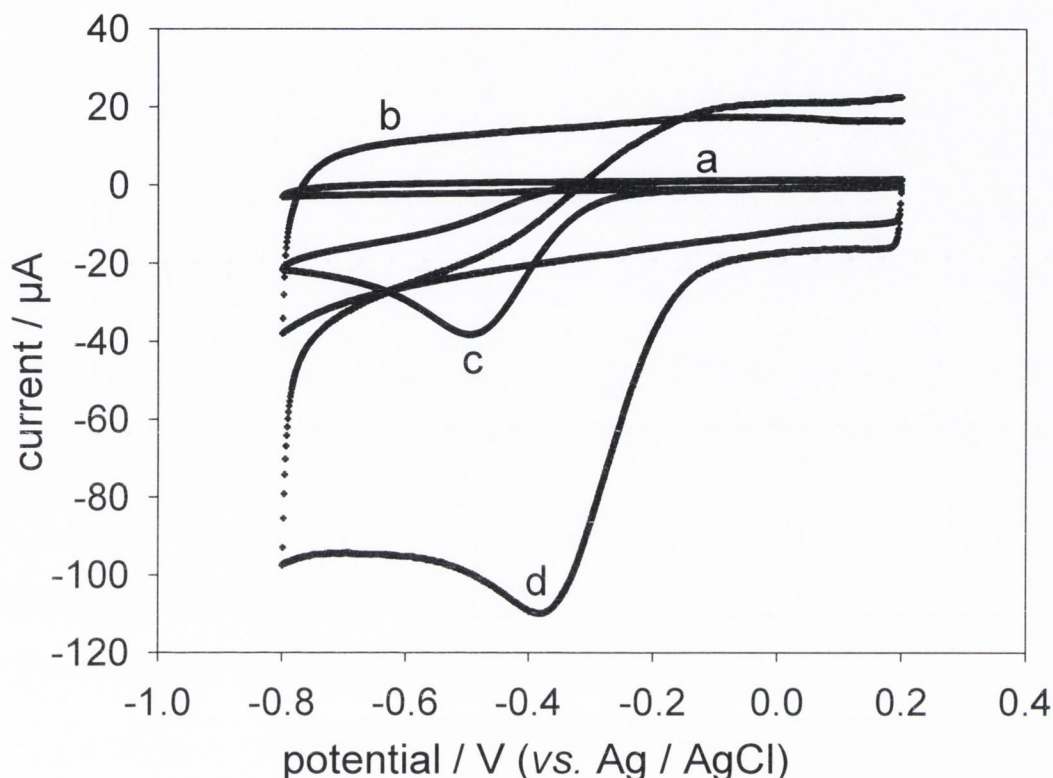


Fig. 4.7.1 Voltammograms obtained at 100 mV s^{-1} using (a and c) bare and (b and d) SWCNT-modified glassy carbon electrodes. The solution was saturated with (a and b) nitrogen and (c and d) oxygen. The electrolyte was a pH 7.0 phosphate buffer solution.

Neither the bare nor modified electrodes showed any evidence of faradaic processes when the solution was saturated with nitrogen. In the presence of oxygen, a reduction peak was observed at -500 mV (vs. Ag / AgCl) using the bare electrode. When the latter was modified using a film of single-walled carbon nanotubes, this peak was found to shift anodically to -380 mV and the reduction peak current increased dramatically. These findings suggest an improved electro-catalytic response, which is again attributed to the mediation of electron transfer by surface oxides present on the nanotubes. The absence of a second cathodic peak suggests that oxygen reduces directly to water without the formation of the peroxide intermediate, as discussed in *Section 1.9*. It is noted however that intermediate formation is generally observed at graphite electrodes [31]. Similar results were obtained in base, although the shift in peak potential was only 70 mV (see

Figure 4.7.2). The peaks for the two electrodes are shifted anodically with respect to those obtained in neutral buffer. Such pH dependence is to be expected for any process involving protonation:

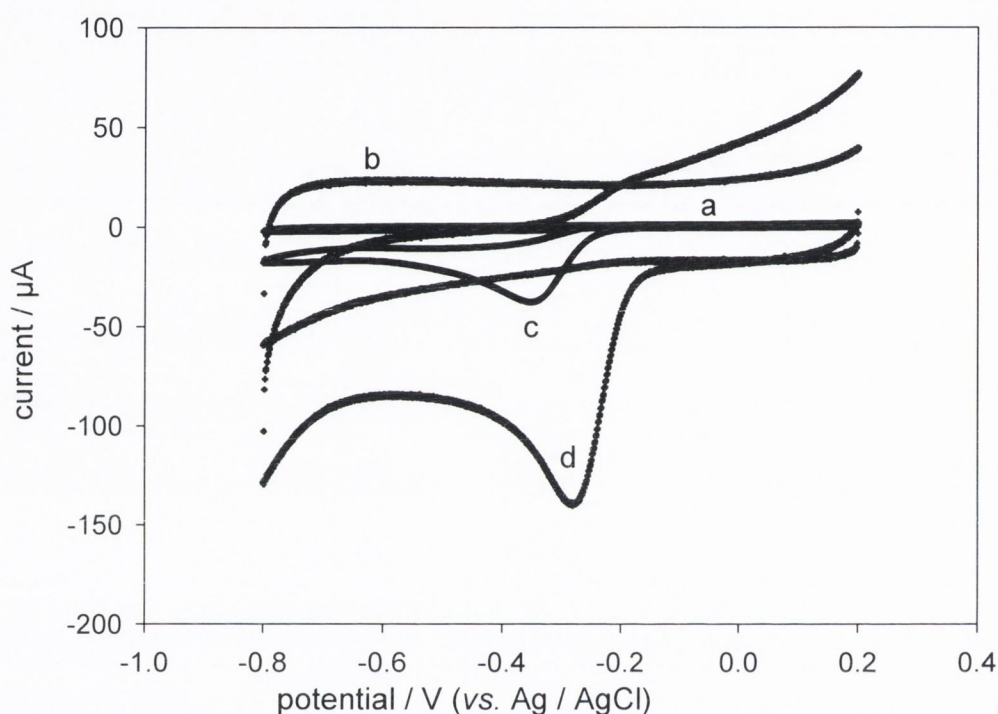


Fig. 4.7.2 Voltammograms obtained at 100 mV s^{-1} using (a and c) bare and (b and d) SWCNT-modified glassy carbon electrodes. The solution was saturated with (a and b) nitrogen and (c and d) oxygen. The electrolyte was a 1 M sodium hydroxide solution.

Tafel plots were drawn using data obtained from linear potential sweeps at a rate of 2 mV s^{-1} . The results found in pH 7.0 buffer are presented in Figure 4.7.3. Using both bare and modified electrodes, Tafel slopes were found to be around the ideal value of $-120 \text{ mV decade}^{-1}$, indicating a value of 0.5 for the transfer coefficient and that the rate-determining step was the first electron transfer for both. Deviations from linearity occurred at strongly cathodic potentials due to mass transport limitations.

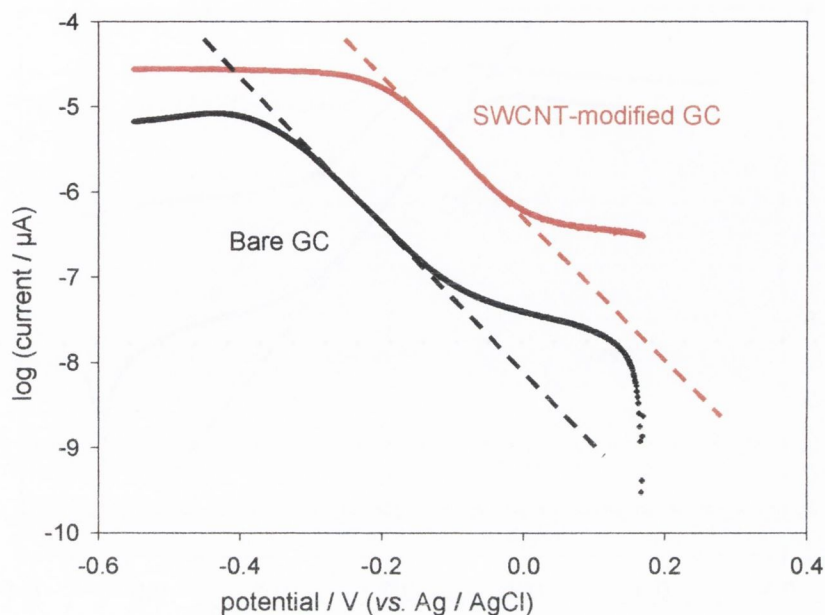
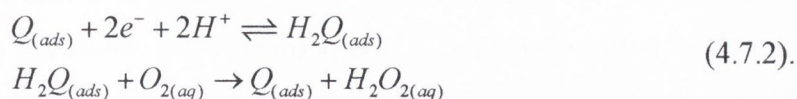


Fig. 4.7.3 Tafel plots obtained in pH 7.0 phosphate buffer solution at a scan rate of 2 mV s^{-1} . The dashed lines show how slopes were estimated.

The same experiments were carried out in base, and these Tafel plots are shown in Figure 4.7.4. It is interesting to note that these slopes are roughly $-60 \text{ mV decade}^{-1}$, suggesting that the RDS in base is a *non-electrochemical* step, assuming a Langmuir adsorption isotherm. The rate of such a step is independent of the applied potential. However, it is acknowledged that Tafel parameters alone cannot fully elucidate reaction mechanisms [32], particularly for the oxygen reduction reaction, for which so many different pathways have been proposed. This would require careful reaction order studies and might also benefit from the use of a rotating disk electrode. It is noted that Tammeveski and co-workers [33] proposed a simple mechanism for the reduction of oxygen at glassy carbon electrodes in the presence of adsorbed quinones:



If the presence of quinones at defect sites on the nanotubes is accepted, the results reported here might be rationalised in terms of this scheme. Experimental verification of this view, however, remains elusive at this point.

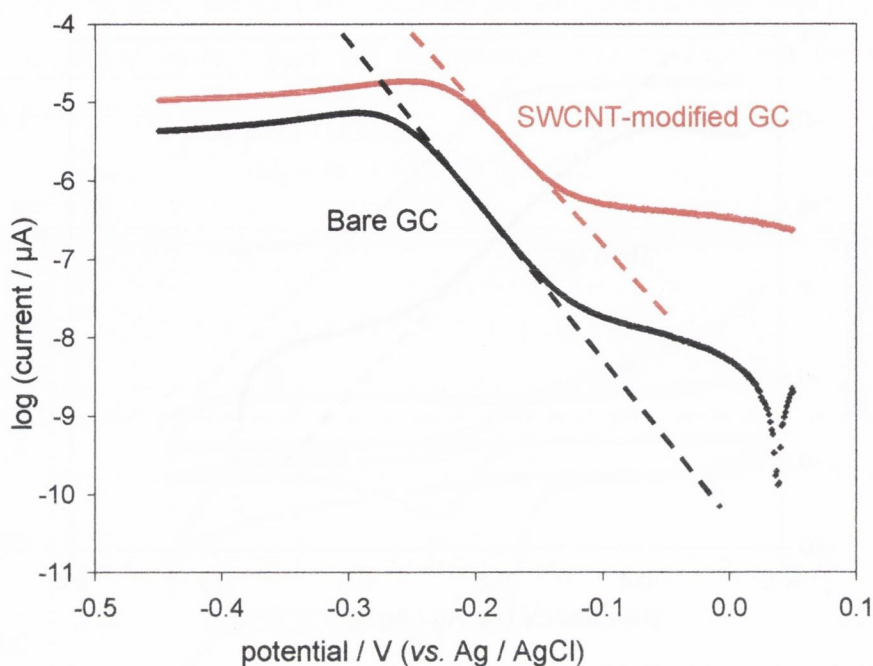


Fig. 4.7.4 Tafel plots obtained in 1 M sodium hydroxide solution at a scan rate of 2 mV s^{-1} . The dashed lines show how slopes were estimated.

4.8 Hydrogen Peroxide

As mentioned in *Section 1.16*, hydrogen peroxide is an important analyte in its own right. However, in the next chapter, the use of nanotube-modified electrodes in glucose biosensors will be reported. Commonly these systems work by oxidising the H_2O_2 produced in the enzymatic reaction. This was the primary motivation behind these investigations into the ability of the GC/SWCNT system to catalyse this anodic process, given by



Firstly, linear sweep voltammetry was performed at a rate of 1 mV s^{-1} in a 50 mM phosphate buffer containing 3 mM peroxide. The results are presented in Figure 4.8.1. The onset of peroxide oxidation at the modified electrode is evident at the low potential of +0.4 V (vs. Ag / AgCl).

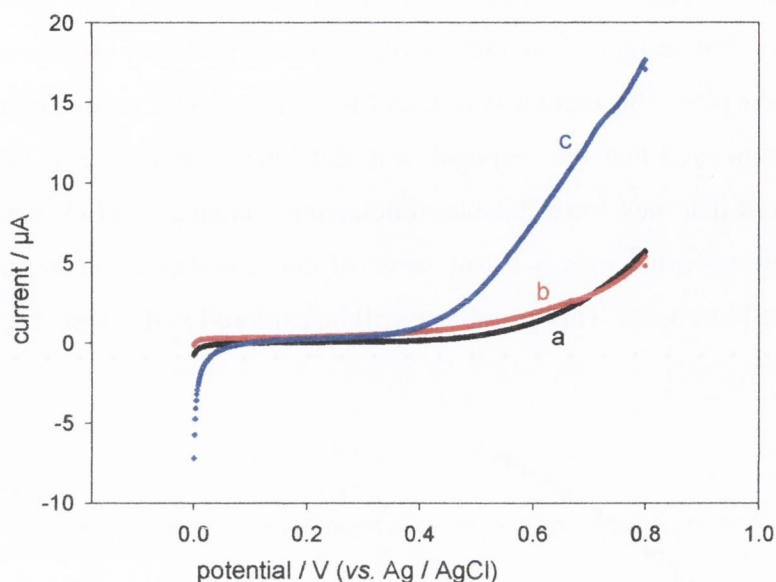


Fig. 4.8.1 Linear sweep voltammograms obtained using a bare (a) and SWCNT-modified (b and c) GC electrode in PBS with (a and c) and without (b) 3 mM peroxide. The sweep rate was 1 mV s^{-1} .

Included in the figure is the response obtained using the modified electrode in blank buffer, confirming that the current increase was indeed due to the oxidation of peroxide and not the aqueous solvent. The figure also shows a scan obtained using a bare glassy carbon electrode in peroxide solution. This response was not nearly as pronounced, indicating that the modified electrode is endowed with superior electro-catalytic properties with respect to this reaction. It is known that the sensitivity of carbon towards peroxide oxidation is low [34], so the enhanced catalytic activity is likely to be due to some additional feature of the nanotubes. Gao and co-workers [35] have reported that the low-potential onset of peroxide oxidation at CNT-modified electrodes is due to the presence of the iron catalyst used in the nanotube synthesis. As mentioned in *Section 2.8*, the SWCNTs used in this work were shown using thermo-gravimetric analysis to have an iron content of around 12 wt%. It is suggested that this residual catalyst gave rise to the enhanced response to peroxide.

The sensitivity of GC/SWCNT electrodes towards peroxide oxidation was further probed using batch amperometry at +0.4 V (vs. Ag / AgCl) in pH 7.0 phosphate buffer. A magnetic stirring bar provided the convective transport during these measurements. The stirring rate was 150 rpm. After initiating the scan, the current was allowed to stabilise for

400 seconds. At this point an aliquot of 0.4 M peroxide solution was added. Additions were made every 100 seconds thereafter. A typical result is shown in Figure 4.8.2, along with a calibration plot. The current was found to increase with peroxide concentration, although it is conceded that the response was not linear. Nonetheless, these modified electrodes showed that they were capable of detecting increases in H_2O_2 concentration at a reasonably low potential over a broad range of concentrations and as such might be useful in glucose biosensors. This possibility will be explored in the next chapter.

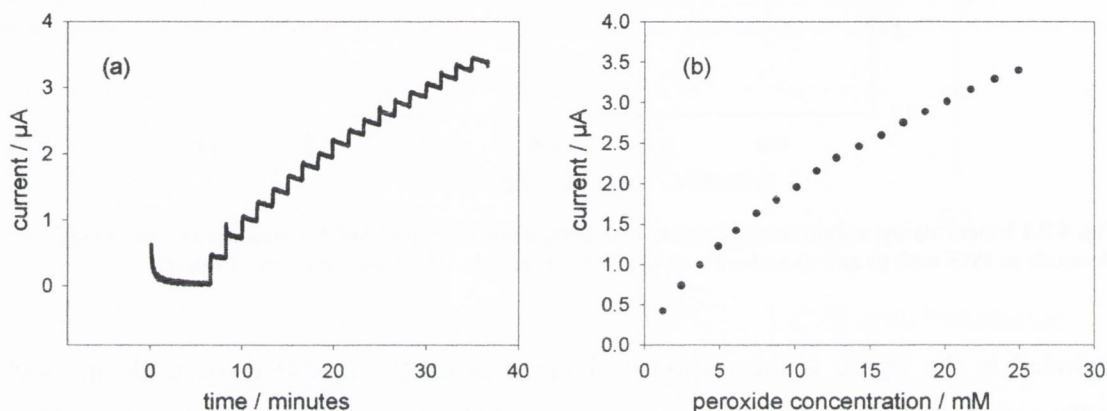


Fig. 4.8.2(a). Batch amperometric response of a SWCNT-modified glassy carbon electrode to additions of hydrogen peroxide. The working electrode was poised at +0.4 V (vs. Ag / AgCl) and the solution was stirred using a magnetic stirrer at 150 rpm. **(b)** The resulting calibration plot.

4.9 Comparisons between CNTs and ‘Ordinary’ Graphite

As discussed in the opening chapter, since carbon nanotubes were first used in electrochemistry by Britto *et al.* [7] in 1996 to study the oxidation of dopamine, a large body of literature has emerged investigating the electro-catalytic properties of nanotube electrodes towards a plethora of redox couples present in solution. Most of these publications have attributed ‘superior’ electro-catalytic properties to these modified electrodes. However, it was not until 2004 that Moore *et al.* [11] thought to run control experiments substituting conventional graphite for carbon nanotubes. These workers reported striking similarities between the observed catalytic properties for the two allotropes towards the oxidation of NADH, epinephrine and norepinephrine. With these findings in mind, we felt it was incumbent upon us to carry out similar comparisons before assigning unique qualities to the nanotubes used in this work.

Firstly the redox behaviour of the ferri/ferrocyanide couple at glassy carbon electrodes modified using both graphite and single-walled nanotubes was investigated. A solution of 1 mM potassium ferricyanide in 0.1 M KCl was employed as the electrolyte and cyclic voltammetry was carried out at scan rates ranging from 10 to 200 mV s^{-1} . The results are presented in Figure 4.9.1.

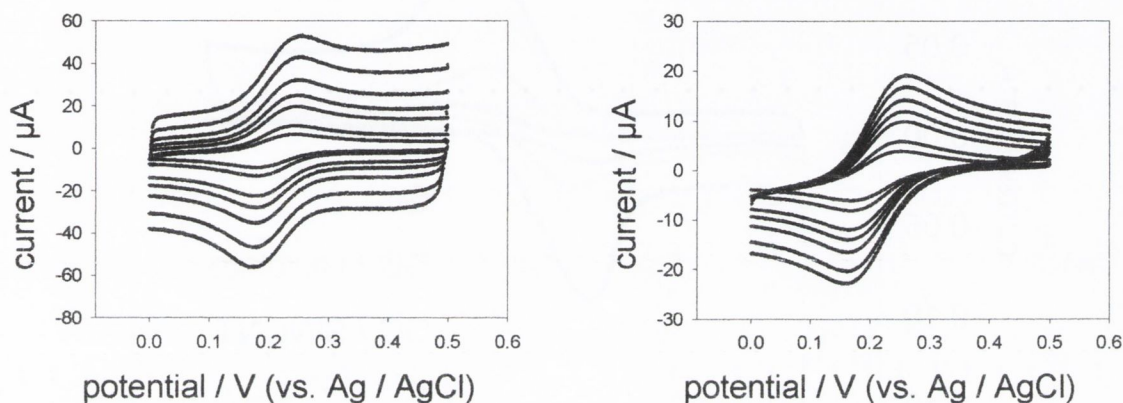


Fig. 4.9.1 Voltammetric responses of (left) a GC/SWCNT and (right) a GC/Graphite electrode to 1 mM ferricyanide in 0.1 M KCl at scan rates of (from inner to outer) 10, 20, 50, 70, 100, 150 and 200 mV s^{-1} .

With both systems, peak separations were observed to increase with sweep rate, indicating quasi-reversible electron transfer. At a scan rate of 100 mV s^{-1} , the ΔE_p value was 91 mV for the graphite electrode and 71 mV for the nanotube electrode, suggesting that the latter permits a more kinetically facile reaction.

It is also interesting to note the difference in the overall forms of the voltammograms obtained using the two electrodes. This reflects the different amounts of material present on the electrode surface. Graphite has a lower surface energy than carbon nanotubes, therefore graphite does not ‘bundle’ like nanotubes and as a result graphite arrays are less robust. During some experiments graphite was observed to partially ‘fall off’ the electrode into the solution. The relatively small surface area of graphite also meant that peak and charging currents were lower.

The next probe used was the neurotransmitter norepinephrine (NE). For these experiments the electrolyte employed was a 1 mM solution of NE in pH 5.8 phosphate buffer. This pH value was selected on the basis of work carried out by Wang *et al.* [9], who reported that the electrochemical response of norepinephrine is optimal at this value.

Comparisons between voltammetric responses obtained using bare and modified glassy carbon electrodes are presented in Figure 4.9.2.

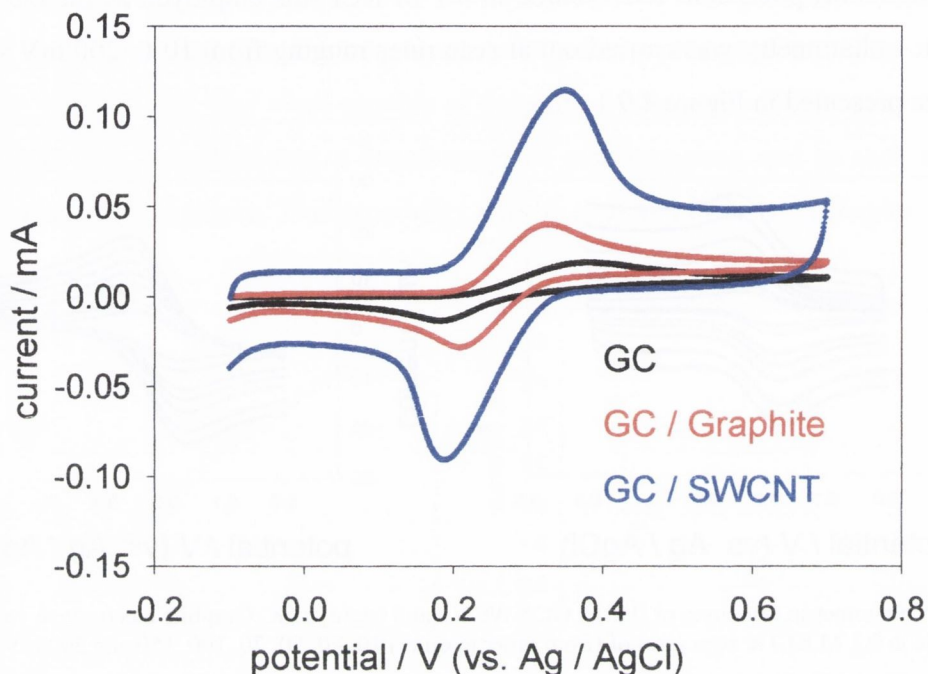


Fig. 4.9.2 Cyclic voltammograms of 1 mM norepinephrine in a pH 5.8 phosphate buffer for GC / SWCNT and GC / Graphite electrodes. Also shown is the response obtained using an unmodified glassy carbon electrode. The sweep rate in each case was 100 mV s^{-1} .

For the bare glassy carbon electrode the process appeared slowest, with a peak separation of 197 mV and a formal potential of around +0.28 V (vs. Ag / AgCl). The nanotube-modified electrode did indeed show superior catalytic properties, lowering the separation to 160 mV and greatly enhancing peak currents. However, this performance was surpassed by the graphite-modified electrode, for which the ΔE_p value was a mere 118 millivolts. These results indicated that nanotubes offer no particular advantage over graphite as electrode modifying agents for the electroanalytical determination of norepinephrine.

As reported in *Section 4.6*, the single-walled nanotubes used in this work show considerable catalytic properties towards the oxidation of β -nicotinamide adenine dinucleotide (NADH). Nanotube modification resulted in a decrease of around 250 mV in the oxidation potential. In order to compare this catalytic activity with graphite,

voltammetry was performed using a 2 mM solution of NADH in pH 7.0 phosphate buffer as electrolyte. The findings are presented in Figure 4.9.3.

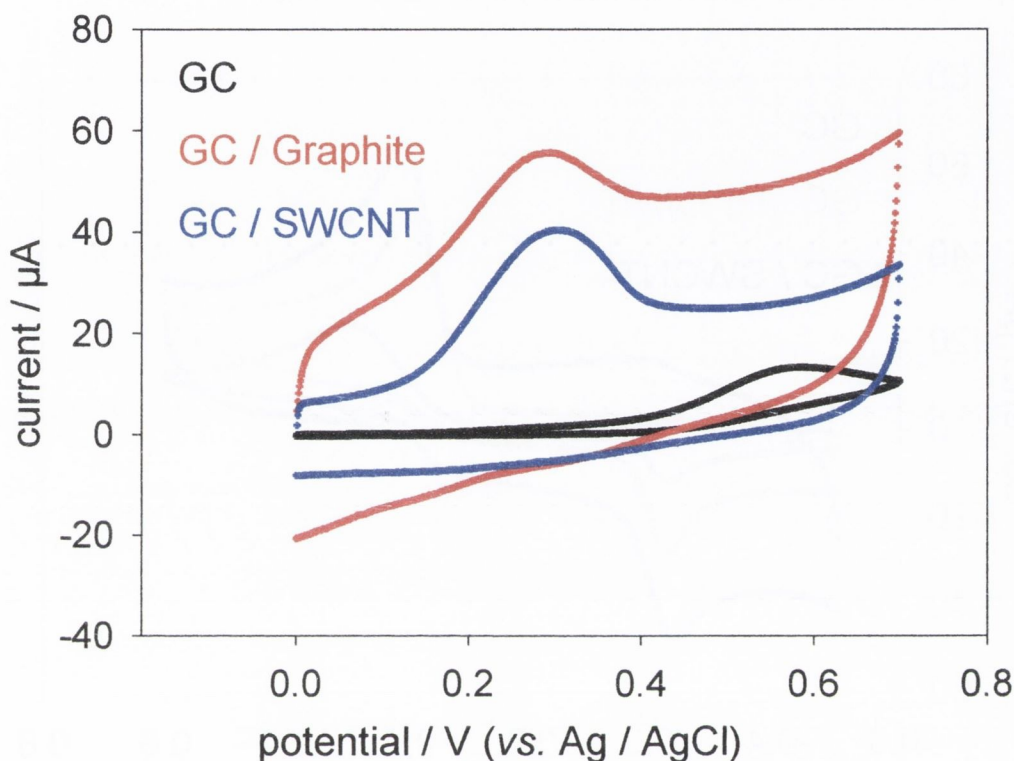


Fig. 4.9.3 Cyclic voltammograms of 2 mM NADH in pH 7.0 phosphate buffer solution for GC / SWCNT and GC / Graphite electrodes. Also shown is the response obtained using an unmodified glassy carbon electrode. The sweep rate employed in each case was 50 mV s^{-1} .

The figure shows that the decrease in oxidation potential due to graphite modification was virtually the same as that brought about by the carbon nanotubes. This result again shows that more caution should be exercised when claiming uniquely ‘catalytic’ behaviour for nanotubes.

Finally, epinephrine was used as a probe to compare graphite- and nanotube-modified electrodes. Cyclic voltammetry was performed using a 0.4 mM solution of epinephrine in pH 7.0 phosphate buffer (see Figure 4.9.4). An irreversible oxidation peak was observed at +298 mV (vs. Ag / AgCl) using a bare glassy carbon electrode. As the figure shows, the two types of modified electrode precipitate virtually identical shifts in peak potential (around 100 mV). This finding again suggests that the electro-catalytic properties of nanotubes may not be as unique as commonly claimed in the literature. The

small oxidation wave around -0.16 V on the GC/SWCNT scan is attributed to oxygenated functionalities present on the nanotube surface, as discussed in Section 3.4. The corresponding reduction wave is obscured by that of epinephrine.

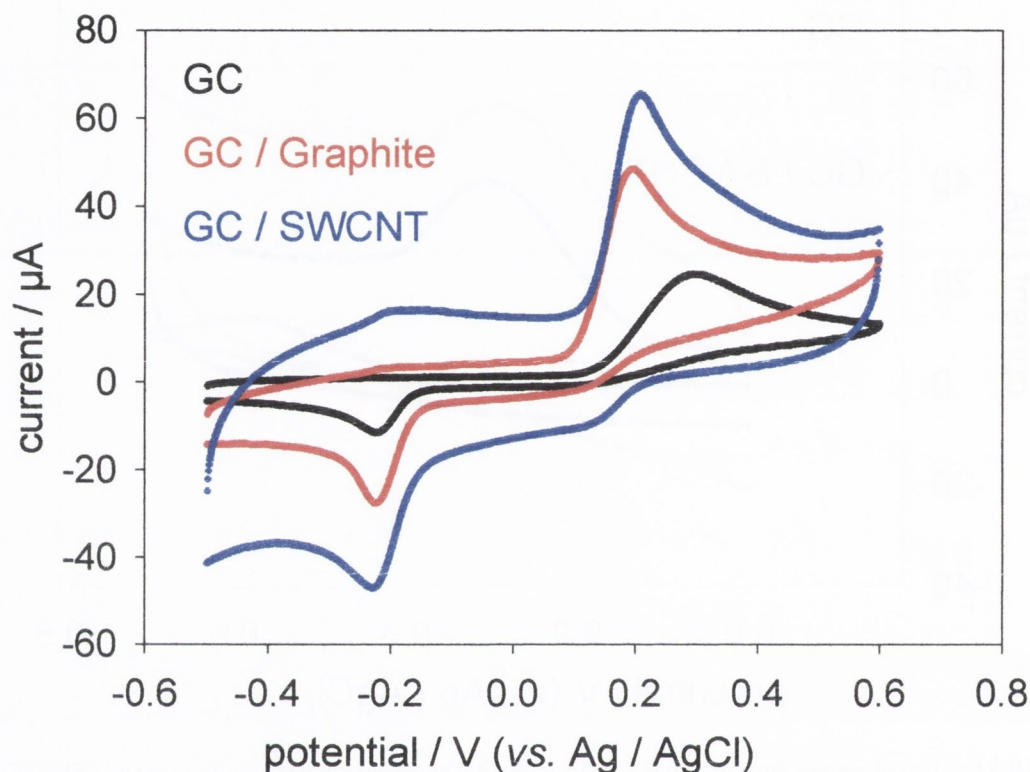


Fig. 4.9.4 Cyclic voltammograms recorded for 0.4 mM epinephrine in pH 7.0 phosphate buffer solution using GC / SWCNT and GC / Graphite electrodes. Also shown is the response obtained using an unmodified glassy carbon electrode. The sweep rate employed in each case was 100 mV s⁻¹.

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Chapter Five: GOx Immobilised on SWCNT-Modified Electrodes

In the previous chapter, nanotube-modified electrodes were used in the analysis of various redox species present in solution. The results discussed in this chapter were obtained when these electrodes were further modified with an adsorbed layer of the enzyme glucose oxidase. Broadly speaking, the chapter will consist of two parts. The first will report on the direct electron transfer of GOx at the carbon nanotube film. The second will investigate the applications of these nanotube / enzyme composite systems in glucose biosensors. Before discussing electrochemical data, however, it is considered pertinent to describe briefly some images obtained using AFM, as these will be of some use when interpreting electrochemical characteristics.

5.1 Atomic Force Microscopy

AFM images recorded for a film of single-walled carbon nanotubes adsorbed on a gold substrate and for glucose oxidase adsorbed onto the nanotubes are presented in Figure 5.1, showing a layer of enzyme on the nanotube array, like a blanket of snow on mountains.

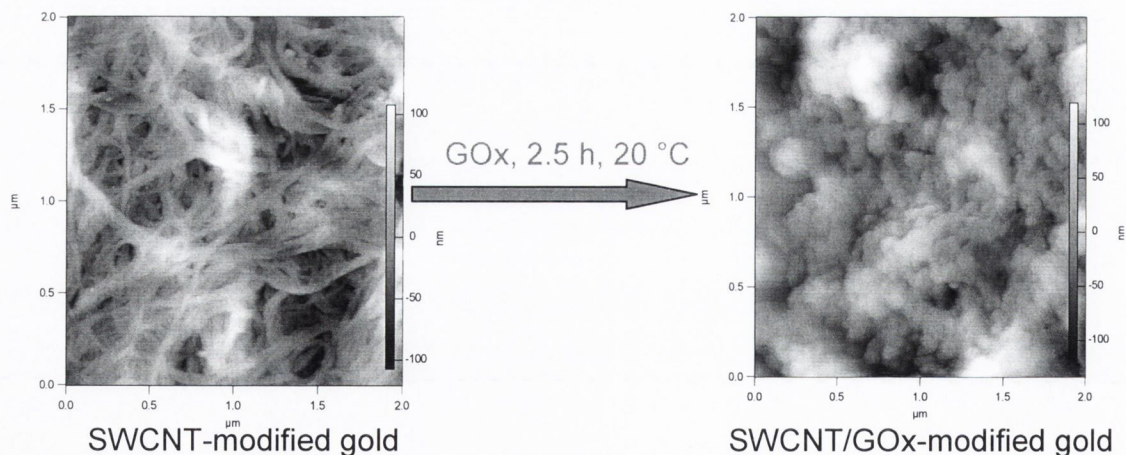


Fig. 5.1 Atomic force microscopy images recorded for (left) a SWCNT film randomly dispersed on a gold surface and (right) glucose oxidase adsorbed onto the nanotube film.

These images suggest that the enzyme fills many of the spaces which exist between the nanotube bundles. The results discussed in this chapter show that this loading is sufficient

to permit electron transfer between enzyme and underlying electrode. It is prudent to note, however, that this arrangement may be different from what prevails during an electrochemical measurement. When Nafion is binding the system to the underlying glassy carbon electrode, a current is passing through the assembly and a double layer has been set up, it is conceivable that there is a change in the orientation of the assembly. Despite this, the images were deemed worthy of inclusion, as it is fairly common for publications in this field to feature such techniques [1-6]. While pictures may be pleasing on an aesthetic level there can be no doubt that electrochemical techniques uncover far more interesting information about such systems. It is these methods which will be focussed on in subsequent sections.

5.2 Direct Electrochemistry of GOx at SWCNT Electrodes

An introduction to glucose oxidase is provided in *Section 1.11*. The present section reports on the use of electrochemical techniques to probe its redox process when adsorbed onto nanotube-modified glassy carbon electrodes. It begins with results obtained using cyclic voltammetry.

The voltammograms in Figure 5.2.1.1 were obtained in a phosphate buffer (pH 7.0) electrolyte using bare, SWCNT/Nafion-modified and SWCNT/GOx/Nafion-modified glassy carbon working electrodes. As can be seen in the figure, the bare GC electrode exhibited virtually no voltammetric response, while a pair of well-defined peaks was observed at the SWCNT/GOx/Nafion electrode. The absence of any peaks in the GC/SWCNT/Nafion voltammogram indicates that these peaks were not given rise to by either Nafion or the nanotubes. The electrochemical response of glucose oxidase immobilised on a heterogeneous surface is due to the redox reaction of the flavin adenine dinucleotide (FAD) prosthetic group [18]. The symmetry of the peaks, small peak separations and similarity between the charge transferred in oxidative and reductive sweeps are all characteristic of processes associated with adsorbed species [8]. Ideally, for a Nernstian process under Langmuir isotherm conditions, the peak width at half the maximum height should be $89/n$ mV at low scan rates for an array of identical sites immobilised on an electrode at 20 °C [9]. The glucose oxidase redox system involves the transfer of two electrons, so a theoretical peak width of around 45 mV is predicted.

Typically, values for this system were found to be in the range 60 – 70 mV, suggesting a spread in kinetic activity among the many enzyme molecules which are active on the nanotube surface. This inequivalence of active enzyme sites is an important consideration, which shall be revisited later when analysing data from potential step experiments.

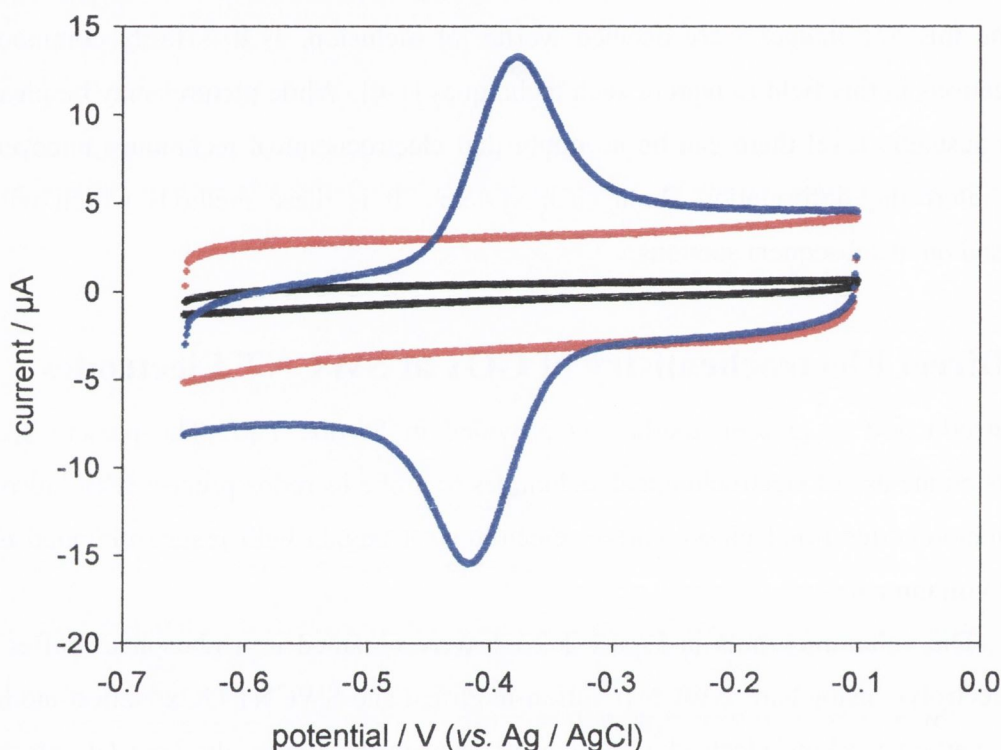


Fig. 5.2.1.1 Cyclic voltammograms of a bare (black), SWCNT/Nafion-modified (red) and SWCNT/GOx/Nafion-modified (blue) glassy carbon working electrode. The electrolyte was a 50 mM phosphate buffer solution (pH 7.0) and the scan rate was 100 mV s⁻¹.

Before going on to discuss this voltammetric data, it is interesting to note the evidence observed regarding the contribution of Nafion to the performance of the system. Figure 5.2.1.2 shows the results of the same experiment carried out using a GC/SWCNT/GOx electrode in the *absence* of Nafion. There were clearly peaks indicating the presence of active glucose oxidase but these were not well-defined and quickly became indiscernable when multiple sweeps were performed [10]. The results suggest that significant amounts of glucose oxidase were lost to the electrolyte when the binder was absent. It was decided therefore that Nafion should always be used when working with adsorbed glucose oxidase. It is also conceivable that Nafion caused some ‘de-bundling’ of the nanotubes,

thereby increasing their active surface area and perhaps improving their ability to interact with the enzyme. Nafion has been shown [11] to wrap around CNTs without impairing their electro-catalytic properties. The wrapping of nanotubes by water-soluble polymers such as Nafion is a general phenomenon driven by a thermodynamic impetus to eliminate the hydrophobic interface between the tubes and the aqueous medium [12], thereby reducing the density of the tangled tube assembly on the electrode surface. This may well increase the permeability of the void space between the nanotubes, allowing increased transport of electrolyte ions and resulting in a change in the interfacial potential distribution and hence on the voltammetric response [13].

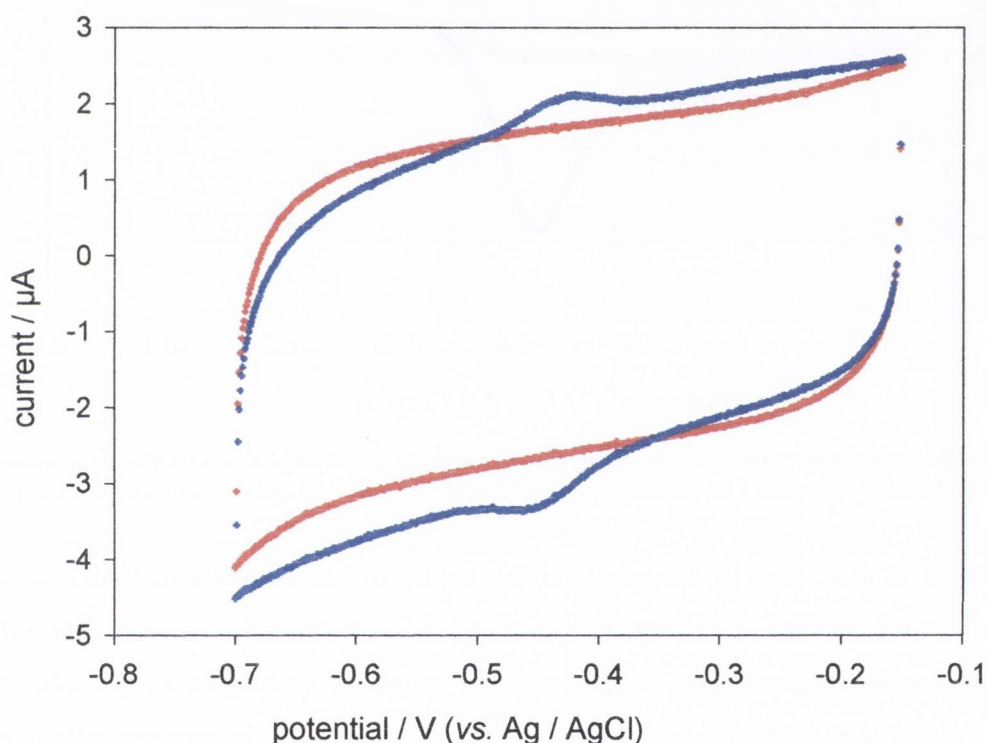


Fig. 5.2.1.2 Cyclic voltammograms of a SWCNT-modified (red) and SWCNT/GOx-modified (blue) glassy carbon working electrode. The electrolyte was a 50 mM PBS (pH 7.0) and the scan rate was 50 mV s^{-1} .

Also, it is worth noting the crucial role played by the nanotubes in the promotion of glucose oxidase electron transfer. Figure 5.2.1.3 shows a comparison between voltammograms obtained with and without carbon nanotubes. Again evidence can be seen of active enzyme on glassy carbon in the absence of NTs but the peaks are not sufficiently defined to be useful for analytical purposes. With the nanotube-modified electrode, the

area beneath the enzyme peaks is relatively huge, indicating a far larger quantity of adsorbed, active enzyme. Britto *et al.* [14] have also reported that nanotubes have superior electrochemical properties when compared with conventional carbon materials.

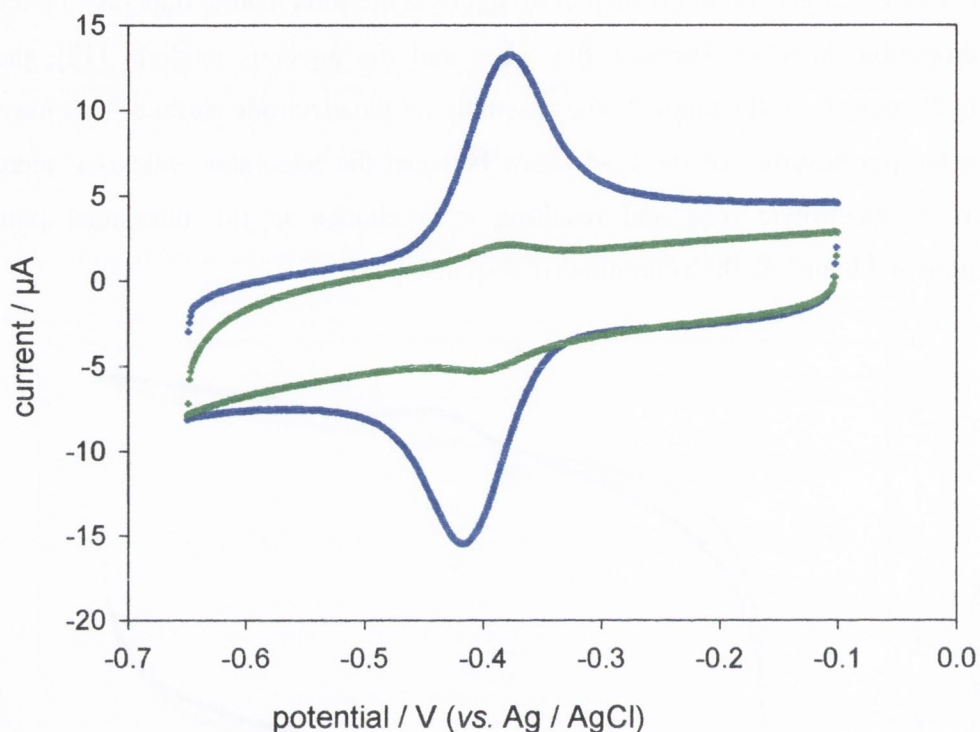


Fig. 5.2.1.3 Cyclic voltammograms of a GOx/Nafion-modified (green) and SWCNT/GOx/Nafion-modified (blue) GC working electrode. The electrolyte was a 50 mM PBS (pH 7.0) and the scan rate was 100 mV s^{-1} .

It is useful at this stage to comment on the nature of the interactions which result in enzyme immobilisation on the nanotube film, as the experiments described here indicate that the modified electrode is clearly superior to glassy carbon as an immobilisation matrix. Wang *et al.* [15] have reported that glucose oxidase adsorbs preferentially to edge-plane sites on nanotubes. It has been established [16,17] that such sites contain a significant amount of oxygenated functionalites. These groups are formed *via* the rupturing of carbon-carbon bonds at the nanotube ends and at defect sites which may occur on the side-walls. They render hydrophilicity and ionic character to the NTs and it is believed that they are responsible for the ‘nesting’ of the protein on the nanotube film. The nanotube bundles and enzyme molecules are of similar dimensions, which facilitates the adsorption of glucose oxidase, without significant loss of its biochemically active shape, form or function. It is suggested that nanotubes can pierce the glycoprotein shell

and gain access to the prosthetic group such that the electron tunneling distance is minimised. Such access is not generally possible with conventional ‘smooth’ electrodes, and significant unfolding of the protein shell can occur, resulting in the loss of biochemical activity. Indeed, Guiseppi-Elie *et al.* [1] have compared the present system to the gentle insertion of a needle into a balloon such that the balloon does not burst. It cannot be said with certainty that the protein shell does not suffer some unfolding on the nanotubes, and that these well-defined peaks are due to adsorbed *FAD* dissociated from the enzyme, as opposed to adsorbed, structurally untainted glucose oxidase. Indeed, it is acknowledged that free *FAD* can adsorb onto carbon electrodes and display a stable voltammetric response [1]. However, if the prosthetic group were ‘pulled out’ of the protein shell and adsorbed onto the nanotubes, it is possible that the response might be somewhat more sluggish than observed, due to the necessity of electron tunneling through a degraded protein film barrier on the electrode surface.

Four GC/SWCNT/GOx/Nafion electrodes were prepared independently and voltammetry was carried out at a number of different sweep rates using phosphate buffer (pH 7.0) as electrolyte. The average reduction peak current was $5.94 (\pm 1.19) \mu\text{A}$ at a scan rate of 50 mV s^{-1} , much larger than the $0.75 \mu\text{A}$ reported by Liang and Zhoubin [18] using a SWCNT/GOx-modified gold working electrode at 100 mV s^{-1} . However, at around 20%, the standard deviation of these currents was significant. This is hardly surprising though, given the inherently random nature of the system. As shown in *Chapter Three*, the nanotube-modified electrodes are not endowed with reproducible active surface areas. Even if they were, finding a way to control the orientation of the enzyme on the nanotube array would represent a major challenge. It should be pointed out though that Liang and Zhoubin [7] report a deviation of only 6.5 % using a similar electrode preparation procedure. The average value for the ratio of anodic to cathodic peak current was found to be very close to unity (~ 0.98), suggesting a quasi-reversible redox process [18]. Standard redox potentials and peak separations were found to exhibit impressive reproducibility, as shown in *Table 5.2*.

Electrode	E^0 / mV (vs. Ag / AgCl)	ΔE_p @ 50 mV s ⁻¹
1	-442 ± 2	17 mV
2	-439 ± 1	24 mV
3	-442 ± 1	19 mV
4	-443 ± 2	14 mV

Table 5.2 Summary of redox potential and peak separation data obtained for four independently-prepared GC/SWCNT/GOx/Nafion electrodes.

The E^0 values listed are average values over the range of scan rates studied, with appropriate standard deviations, showing that the standard redox potential of the system is independent of sweep rate over this range. Ideally, for a fully reversible system involving no solution reactants, peak separations should be zero [19]. The discrepancy may be due to a potential difference between the electrode and the site of activation (the flavin group). Such a difference would be attributed to the protein acting as a dielectric layer, and if true, indicate that enzyme does not unfold significantly on the nanotubes. These E^0 values are significantly less negative than the -659 mV (vs. Ag / AgCl) reported by Zhao *et al.* [20] for GOx immobilised at a carbon nanotube powder microelectrode (CNTPME). They are, however, similar to those reported by other workers studying the direct electrochemistry of glucose oxidase using various immobilisation methods [1,15,18,21,22]. The prominent background currents observed are a consequence of the large, catalytically active surface area of the modified electrode. The formal potential of the FAD / FADH₂ redox couple is -430 mV vs. Ag / AgCl [23], so it is clear that the E^0 values obtained agree strongly with that of the native molecule. This suggests that this system may have applications as a glucose biosensor. This question will be addressed in *Section 5.3*. For now the attention focuses on the direct electron transfer between GOx and the modified electrode.

5.2.1 Stability of the Glucose Oxidase Electrochemical Response

The stability of the electrochemical response shown by the GC/SWCNT/GOx/Nafion system was probed by carrying out one hundred successive scans at 50 mV s⁻¹ in

phosphate buffer (pH 7.0). Reduction peak currents were normalised with respect to the initial value and plotted against scan number, as shown in Figure 5.2.1.1.

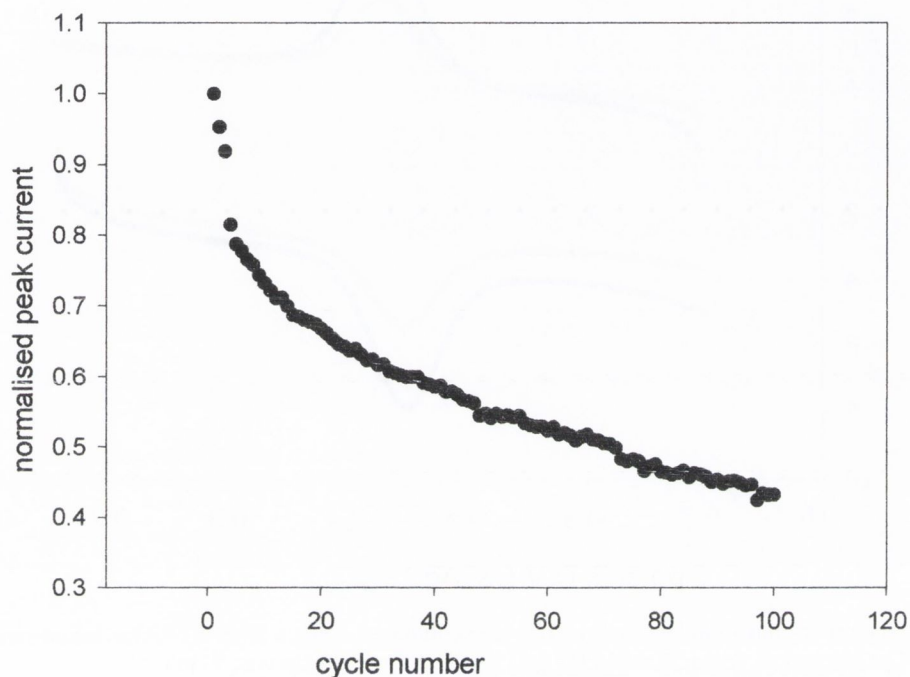


Fig. 5.2.1.1 The stability of the direct electron transfer of glucose oxidase immobilised on a GC/SWCNT electrode for one hundred successive scans in 50 mM phosphate buffer (pH 7.0).

These peak currents were found to drop sharply at first and then more steadily as the number of cycles increased. After one hundred cycles, the signal had decayed to around 45 % of its initial value but remained well-defined, as shown in Figure 5.2.1.2, indicating that the system was suitable for experiments involving large numbers of scans.

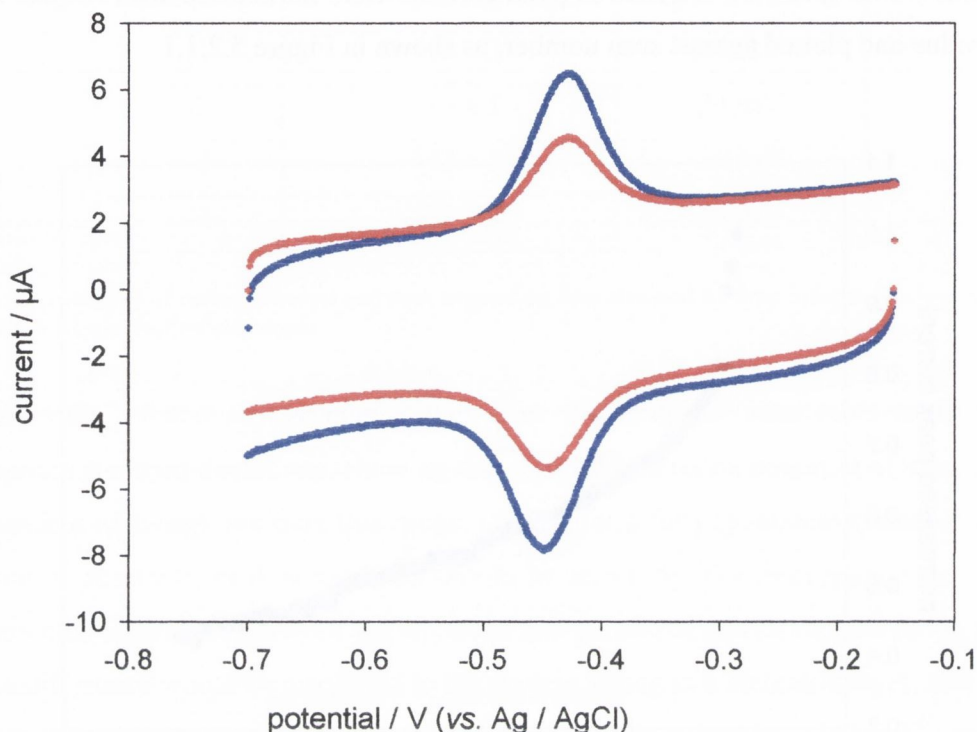


Fig. 5.2.1.2 The first (blue) and last (red) of 100 scans obtained using a SWCNT/GOx/Nafion-modified GC electrode. The electrolyte was a 50 mM PBS (pH 7.0) and the scan rate was 50 mV s^{-1} .

The storage stability of the GC/SWCNT/GOx/Nafion system was investigated by storing an electrode in buffer and scanning in buffer every day for four days. By the third day, peak currents were found to have decreased to less than 10 % of their initial values. As a result, it was decided that fresh electrodes should be prepared daily.

5.2.2 The Effect of pH

The electrochemical response of glucose oxidase immobilised on a heterogeneous surface is due to the redox activity of flavin adenine dinucleotide [7], which is bound to the protein shell. The FAD system involves the transfer of two electrons and an associated protonation / deprotonation equilibrium:



This means that its standard and peak potentials depend on the pH of its environment. Figure 5.2.2.1 shows a number of voltammograms obtained using 50 mM phosphate buffers of various pH values as electrolyte.

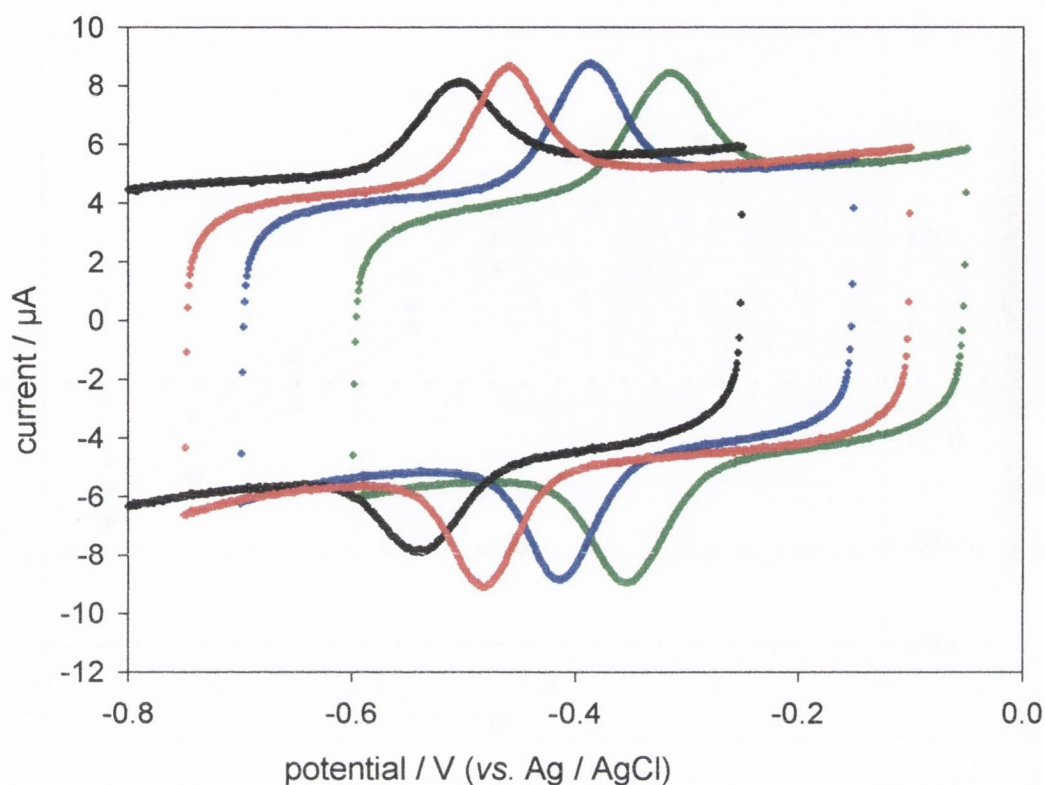


Fig. 5.2.2.1 Voltammograms obtained using a GC/SWCNT/GOx/Nafion electrode in 50 mM phosphate buffers of pH (from left to right) 8.6, 7.5, 6.3 and 5.1. The scan rate employed in each case was 50 mV s^{-1} .

The figure clearly shows that peak potentials are influenced by the pH of the electrolyte solution. All changes in voltammetric peak potentials with pH were found to be reversible, that is, the same values were obtained when electrodes were transferred from a solution with different pH values back to the original solutions in which they were scanned. Four independently-prepared electrodes were investigated at seven pH values. The standard potential and two peak potentials were all found to shift cathodically with increasing pH, as shown in Figure 5.2.2.2. Three such plots were drawn for each electrode. The average slope of the twelve lines was found to be $-53 (\pm 2) \text{ mV/pH}$, which agrees reasonably well with the theoretical value of -59 mV/pH for a reversible, two-proton / two-electron process at 20°C [21].

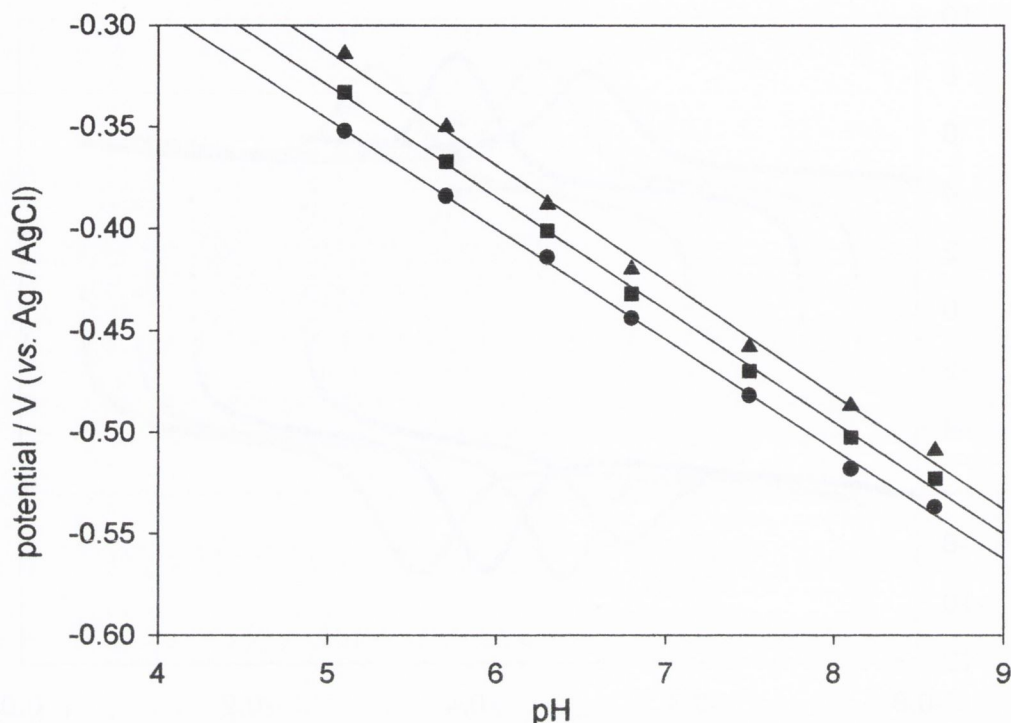


Fig. 5.2.2.2 The variation of E_{pc} (●), E° (■) and E_{pa} (▲) with pH for a GC/SWCNT/GOx/Nafion electrode in 50 mM phosphate buffer solutions of various pH values.

5.2.3 Surface Coverage of Glucose Oxidase on SWCNT Ensembles

In *Section 3.5* it was reported that GC/SWCNT electrodes were found to have an active surface area of $(9.76 \pm 0.98) \times 10^{-2} \text{ cm}^2$. These electrodes were then further modified using glucose oxidase and Nafion. Voltammetry was carried out using phosphate buffer (pH 7.0) as electrolyte and these results were combined with the surface areas already calculated to determine values for the surface coverage (Γ) of glucose oxidase on the nanotube-modified electrodes. An example of one such calculation is now outlined.

The active surface area of one of the GC/SWCNT electrodes was found to be $8.8 \times 10^{-2} \text{ cm}^2$ using the ferro/ferricyanide probe. When this electrode was modified using GOx and Nafion, the area under the cathodic peaks at scan rates for which the process was surface-controlled was $(5.51 \pm 0.33) \mu\text{C}$. This corresponds to the transfer of 5.71×10^{-11} moles of electrons, meaning that 1.43×10^{-11} moles of glucose oxidase were reduced (each glucose oxidase dimer contains two FAD groups, each of which gains two electrons). So the surface coverage is given by

$$\Gamma = \frac{1.43 \times 10^{-11}}{8.8 \times 10^{-2}} = 1.62 \times 10^{-10} \text{ mol cm}^{-2}$$

Table 5.2.3.1 summarises the surface coverage data for GOx on the four electrodes. As described in Section 2.9, these electrodes were prepared by dropping ten microlitres of a 3 mg cm⁻³ GOx solution on the electrodes. If the formula weight of the enzyme is taken to be 1.6 × 10⁵ g mol⁻¹, this corresponds to the addition of around 19 × 10⁻¹¹ moles of glucose oxidase. The table shows that only ~10 % of this amount becomes active on the electrode surfaces. There are probably several reasons for this inefficiency. Most obviously, the solution was seen to ‘spill over’ onto the electrodes’ Kel-F casing, so any enzyme therein could not possibly become active. As regards the amount on the electrode itself, some of this must surely interact very weakly with the nanotubes if in contact with basal plane regions. Also, some adsorbed enzyme may not be orientated in such a way as to permit electron transfer fast enough to occur on the timescale of the experiment. Variations in ET rates within the adsorbed enzyme layer will be discussed in detail in Section 5.2.7.

Electrode	Area of GC/NT (cm ²)	Moles of active GOx	Γ (pmol cm ⁻²)
1	0.088	1.43 × 10 ⁻¹¹	162
2	0.091	0.82 × 10 ⁻¹¹	90
3	0.105	2.71 × 10 ⁻¹¹	258
4	0.107	1.49 × 10 ⁻¹¹	139

Table 5.2.3.1 Summary of surface coverage data obtained for four independently-prepared GC/SWCNT/GOx/Nafion electrodes.

The table reveals a number of interesting points concerning the system. Firstly, it is clear that the method of electrode preparation used offers no control over the surface coverage of glucose oxidase. Secondly, there is no correlation between nanotube area and GOx coverage, meaning that a large active nanotube surface area does not necessarily lead to a large coverage of active enzyme. Table 5.2.3.2 shows that these values for glucose oxidase surface coverage compare favourably with those reported for other

immobilisation matrices. It would appear that the SWCNT electrodes permit the adsorption of a reasonable amount of active enzyme.

System	GOx surface coverage / pmol cm ⁻²	Reference
Au/SWCNT/GOx	52	[18]
epHOPG/GOx	3	[15]
Au/SAM/GOx	1	[24]
CNTPME/GOx	199	[20]

Table 5.2.3.2 GOx surface coverages reported in the literature for various immobilisation matrices.

A closer look at the reported surface coverage values adds to the emerging picture of the system. If a planar electrode is assumed of area $9.76 \times 10^{-2} \text{ cm}^2$ ($9.76 \times 10^{14} \text{ \AA}^2$) and a perfect monolayer of glucose oxidase dimers, each with a contact area $6000 \text{ \AA}^2$ [25], it is found that 0.271 pmol of GOx are present, giving a monolayer surface coverage of around 5.8 pmol cm^{-2} . Values calculated here are significantly larger, which suggests some unfolding of the protein shell to expose FAD or maybe the incorporation of some FAD within the porous nanotube array. This is, of course, mere speculation at this point but the employed method of surface coverage calculation is a common one. Let it suffice to say that the nanotubes provide a matrix for the immobilisation of a large loading of glucose oxidase and the Nafion succeeds in preventing the loss of significant amounts of this enzyme to the solution during electrochemical experiments. Attention will now focus on how the variation of voltammetric data with sweep rate was used to extract further information about this system.

5.2.4 The Effect of Scan Rate

As mentioned in the description of cyclic voltammetry provided in *Section 2.2*, peak currents and potentials change depending on the sweep rate used. The manner in which they respond to the latter forms the basis of the most popular ways of elucidating both qualitative and quantitative information about systems in electrochemistry. Figure 5.2.4.1 shows voltammograms obtained at a number of different scan rates for a

GC/SWCNT/GOx/Nafion ensemble, using phosphate buffer as the electrolyte. The effect of scan rate on peak currents is obvious.

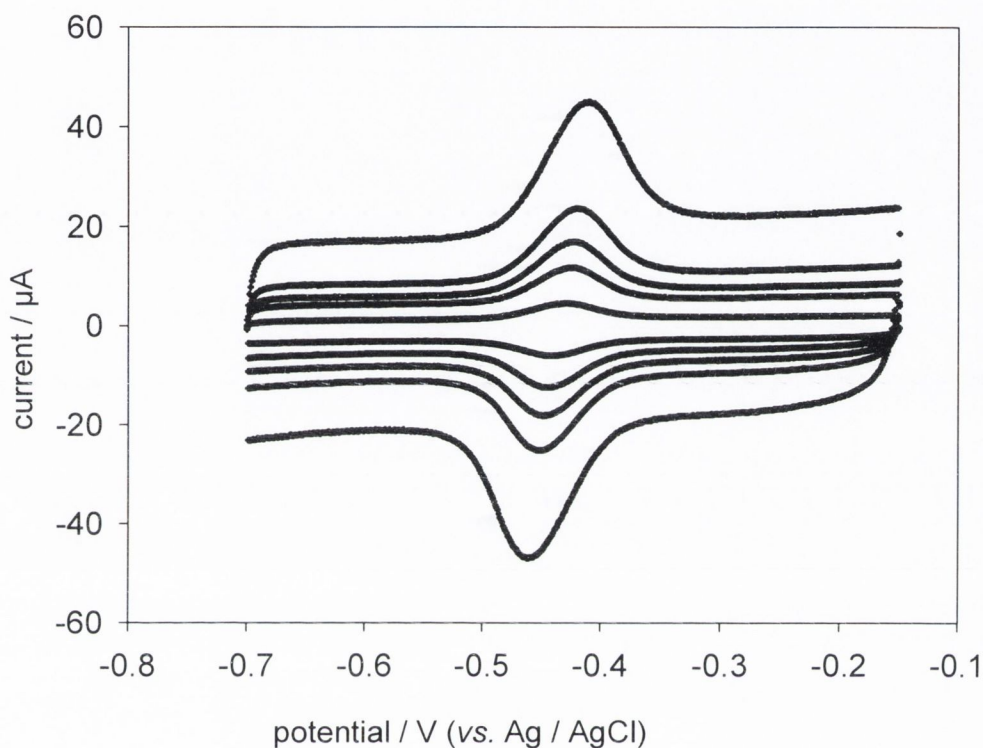


Fig. 5.2.4.1 Cyclic voltammograms obtained using a GC/SWCNT/GOx/Nafion electrode. The electrolyte used was a 50 mM PBS (pH 7). The scan rates (from inner to outer) were 20, 50, 70, 100 and 200 mV s^{-1} .

Firstly, it has already been mentioned that a specific range of scan rates was investigated for the GC/SWCNT/GOx/Nafion system. The variation of peak currents with sweep rate forms a basic test showing whether the current response generated by an electrochemical reaction is under the control of the kinetics at the interface, or diffusion of species from bulk solution. For the former, peak current values are expected to vary in a linear manner with scan rate. Such a plot for the present system is presented in Figure 5.2.4.2.

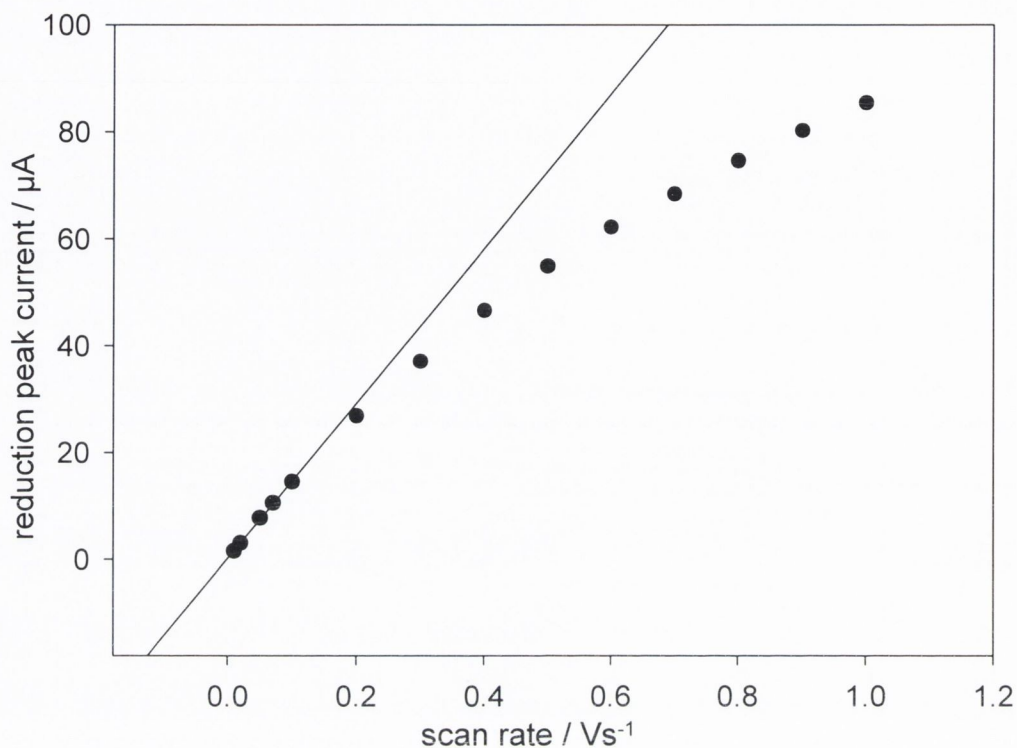


Fig. 5.2.4.2 The variation of cathodic peak current with scan rate over a wide range of scan rates using a GC/SWCNT/GOx/Nafion electrode. The electrolyte was a 50 mM phosphate buffer (pH 7.0).

The figure shows that, up to a scan rate of around 150 mV s^{-1} , the reduction peak current of glucose oxidase varies in a linear manner with sweep rate, indicating a surface-controlled process. At higher scan rates, however, this linearity is clearly lost. In this higher region, the effects of ohmic drop become significant, and the measured peak currents become misleadingly small. Similar conclusions were drawn using data from oxidative sweeps. Of course, this does not necessarily mean that the reaction falls under the control of mass transport at short experimental timescales, but it is clear that voltammetric data obtained using large scan rates ought to be treated with caution. Figure 5.2.4.3 shows a typical variation of cathodic peak current with scan rate in the *low* range, the linearity of which is characteristic of a diffusion-less system.

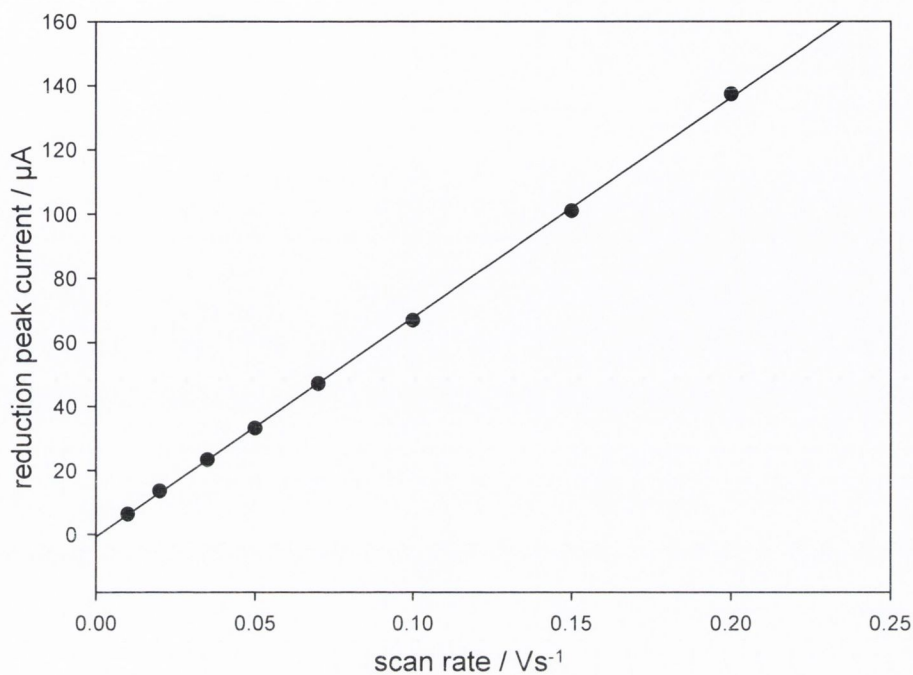


Fig. 5.2.4.3 The variation of cathodic peak current with scan rate for low scan rates using a GC/SWCNT/GOx/Nafion electrode. The electrolyte was a 50 mM PBS (pH 7.0).

For the four independently-prepared electrodes, plots of peak currents against scan rate showed impressive linearity at low scan rates. Their slopes, however, were not at all reproducible, as the peak currents varied each time, as mentioned earlier. The important point is that the system showed itself to be clearly under surface control in this range of scan rates. The possibility of diffusion control at higher sweep rates cannot be ruled out, particularly since voltammetry indicates that the surface reaction is quite facile, but this test for surface control is certainly worthy of inclusion, based on the fact that it is employed in virtually all studies of the direct electron transfer of immobilised enzymes.

Electrode	Slope of i_{pc} vs. scan rate / $\mu A s V^{-1}$	Correlation coefficient
1	153	0.9985
2	57	0.9991
3	94	0.9989
4	685	0.9998

Table 5.2.4 Summary of cathodic peak current data obtained for four independently-prepared GC/SWCNT/GOx/Nafion electrodes.

5.2.5 Calculation of Rate Constants using Cyclic Voltammetry

In 1979, Laviron [26] derived a model which could be used to calculate rate constants for surface-bound electrochemical systems based on the variation of peak potential with scan rate. The range of scan rates which may be used in this analysis is limited at the low end by the condition that peak separations must be greater than $200/n$ mV (for the FAD couple n , the number of electrons in the overall redox process, is two), and at the high end by peak-broadening due to ohmic drop. For this system, the sweep rates analysed were between 1.5 V s^{-1} (below which $\Delta E_p < 100 \text{ mV}$) and 5.0 V s^{-1} (above which the observed peaks were too broad to permit accurate identification of peak potentials). This model has become widely applied in surface electrochemistry, and to the best of our knowledge, is the only analysis used to calculate rate constants for glucose oxidase immobilised on nanotube-modified electrodes [1,15,20-22]. The model defines the relationship between peak potential and scan rate using the following equations:

$$E_{pc} = E^0 + \frac{RT}{\alpha nF} \ln \frac{RTk^0}{\alpha nF} - \frac{RT}{\alpha nF} \ln \nu, \quad (5.2.5.1)$$

$$E_{pa} = E^0 + \frac{RT}{(1-\alpha)nF} \ln \frac{RTk^0}{(1-\alpha)nF} + \frac{RT}{(1-\alpha)nF} \ln \nu.$$

Here, E_{pc} and E_{pa} are the cathodic and anodic peak potentials, T is the temperature of the electrolyte (293 K), α is the electron transfer coefficient, k^0 is the standard rate constant for the surface reaction, n is as defined above and E^0 is the standard redox potential (reported earlier as $-0.44 \text{ V vs. Ag / AgCl}$). These two equations may be combined to yield

$$\Delta E_p = \frac{RT}{(1-\alpha)\alpha nF} [\alpha \ln(1-\alpha) + (1-\alpha) \ln \alpha - \ln \frac{RT}{nF} - \ln k^0] + \frac{RT}{(1-\alpha)\alpha nF} \ln \nu. \quad (5.2.5.2)$$

This means that if peak separations are plotted against natural logarithm of scan rate a straight line should be obtained. The slope of this line allows a value to be calculated for the transfer coefficient, which may then be used along with the y -intercept to calculate a value for the standard ET rate constant. For the four independently-prepared GC/SWCNT/GOx/Nafion electrodes, it was found that when the scan rate was higher than

around 0.05 V s^{-1} ($\ln v = -3$), the cathodic peak potential shifted negatively and the anodic peak potential shifted positively, as shown in Figure 5.2.5.1.

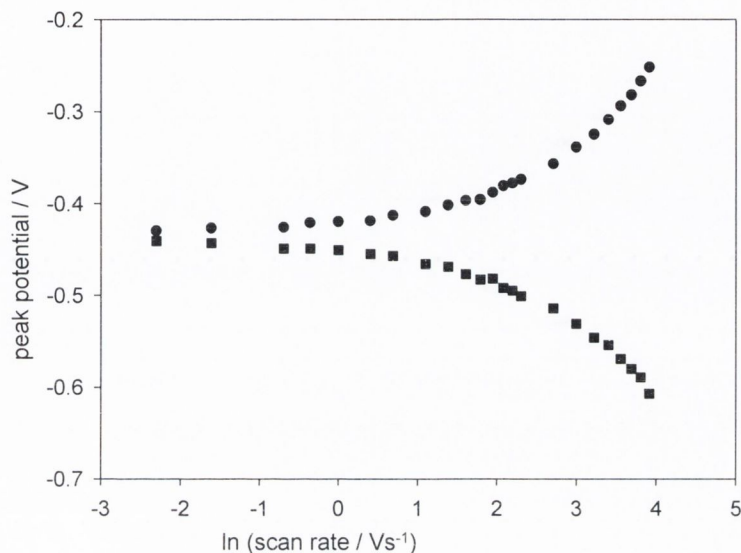


Fig. 5.2.5.1 Plots of anodic (●) and cathodic (■) peak potentials against natural logarithm of scan rate for a GC/SWCNT/GOx/Nafion electrode. The electrolyte was a 50 mM phosphate buffer (pH 7.0).

The figure clearly shows that peak potentials diverge as sweep rate increases. Over the appropriate range of sweep rates, as outlined at the start of this section, peak separations were plotted against natural logarithm of sweep rate and a typical result is presented in Figure 5.2.5.2.

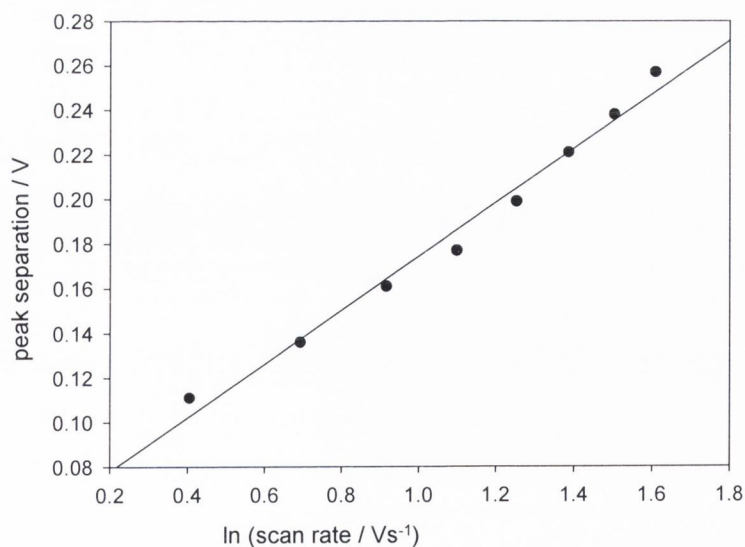


Fig. 5.2.5.2 Plot of peak separation against natural logarithm of scan rate for a GC/SWCNT/GOx/Nafion electrode. The electrolyte was a 50 mM phosphate buffer solution (pH 7.0).

It is now presented in detail how the Laviron analysis was used to calculate rate constants for the system, as this is commonly overlooked in the literature. The slope of one of these plots was found to be 0.121 volts. By *Equation 5.2.5.2*,

$$\frac{RT}{(1-\alpha)\alpha nF} = 0.121.$$

The result was a quadratic equation in α , which was solved to give values of 0.12 and 0.88 for the transfer coefficient. The line was found to intersect the ordinate at 0.054 V, and this intercept was used along with an α value of 0.12 to give, again by *Equation 5.2.5.2*,

$$\frac{RT}{0.88 \times 0.12 \times nF} \left[0.12 \times \ln 0.88 + 0.88 \times \ln 0.12 - \ln \frac{RT}{nF} - \ln k^0 \right] = 0.054,$$

which yielded a value of 7.61 s^{-1} for the rate constant. It should be pointed out that, because their sum is unity, the same value is found using the ‘other’ value obtained for the transfer coefficient. This process was carried out using data obtained for four independently-prepared electrodes and the results are presented in *Table 5.2.5.1*.

Electrode	Intercept / V	Slope / V	Correlation coefficient	k^0 / s^{-1}
1	0.081	0.160	0.98	5.18
2	0.054	0.121	0.98	7.61
3	0.072	0.146	0.97	5.91
4	0.068	0.150	0.98	5.64

Table 5.2.5.1 Summary of rate constants obtained from plots of peak separation against natural logarithm of scan rate for four independently-prepared GC/SWCNT/GOx/Nafion electrodes.

Based on these four values, a rate constant of $6.1 (\pm 1.1) \text{ s}^{-1}$ is reported. This value is greater than those reported in several other studies of the direct electron transfer of glucose oxidase. These literature values are presented in *Table 5.2.5.2*.

System	Rate constant / s ⁻¹	Reference
GC/SWCNT/GOx	1.7	[1]
epHOPG/GOx	2	[15]
GC/NT/GOx/Nafion	1.53 ± 0.45	[22]
GC/NT/GOx/Nafion	1.5 ± 0.5	[21]
Au/SAM/SWCNT/GOx	0.3	[5]
CNTPME/GOx	1.61 ± 0.03	[20]

Table 5.2.5.2 GOx rate constants reported in the literature for various immobilisation matrices.

On the basis of the Laviron model, it is clear that the nanotubes used in this work allow for efficient electron transfer. The large rate constant might be interpreted as an indication that there is a degree of unfolding of the protein shell, resulting in the adsorption of FAD directly onto the NTs, or perhaps even the diffusion of free FAD within the film. The relative standard deviation of the calculated rate constants is large (although not as large as certain literature values presented in *Table 5.2.5.2*) and the plots themselves deviate considerably from linearity (average correlation coefficient around 0.98). It is interesting to note that several of the authors referenced in *Table 5.2.5.2* make no mention of reproducibility (or lack thereof) and most do not show plots of peak separation against natural logarithm of scan rate. In fact, some of these papers offer virtually no indication as to how the model was applied and appear content to merely report values for the rate constant. The most helpful description is provided by Zhao *et al.* [20] but even this fails to report values of correlation coefficients for plots used.

There is further ambiguity on the subject of the transfer coefficient. Most papers make no mention of the value used. While Wang *et al.* [15] assumed a value of 0.5, Zhao and co-workers [20] used plots of peak potentials against *lnv* to obtain a value of 0.47. The lack of clarity which prevails in the literature with respect to the Laviron model is alarming, given that it is the only approach used to calculate rate constants in this field. There appears to be no consensus as to how exactly the analysis should be applied, and this makes it difficult to compare calculated rate constants to reported values with real confidence.

It is suggested that the data simply do not fit the model because it is based on the Butler-Volmer picture of electrochemical kinetics [9], which may be too simplistic to adequately describe the physical reality of surface-immobilised systems. This description assumes that the electron transfer rate constant for each immobilised molecule is the same at any given potential. This is indeed optimistic for glucose oxidase on carbon nanotube electrodes. The AFM image in *Section 5.1* clearly shows that the nanotube film is completely disordered. Also, variations in nanotube conductivity (as described in *Section 1.2*) and the fact that the enzyme is almost certainly not oriented in a uniform manner must surely serve to broaden the spread in kinetic activity. The applicability of the Laviron analysis has been questioned previously by Weber and Creager [27], who reported that data obtained for ferrocene oxidation in mixed monolayers on gold electrodes did not fit the model. The idea that there is not a single common value for the rate constant for all of the active GOx molecules will be pursued now, and the intrinsically dispersive nature of the adsorbed enzyme system will be emphasised. The results will show that *potential step chronoamperometry* provides a convenient way to both identify and quantify kinetic dispersion in surface-adsorbed redox systems.

Cyclic voltammetry does not always provide the optimum route to kinetic parameters. It can be difficult to distinguish between ohmic drop and kinetic effects when investigating the variation of the voltammetric response with sweep rate, as shown by the data in the previous section. Potential step techniques may be used to calculate electron transfer rate constants for redox systems immobilised on electrodes. A simple potential step experiment is now described, the aim of which was to elucidate the kinetics of the oxidation of the adsorbed enzyme. The potential was poised at -0.6 V (vs. Ag / AgCl) for five minutes in order to ensure that only the reduced form of the prosthetic group was present. The potential was then stepped to -0.2 V and the current response was monitored for a pulse width of 0.4 seconds, which was found to be sufficient to allow the signal to decay to a steady-state value. The raw data are presented in Figure 5.2.5.3(a). In *Section 2.3*, a simple expression for first-order kinetics based on a uniform array of adsorbed species was derived. The model permits the calculation of rate constants directly from the slopes of plots of $\ln i$ against time. Such a plot is shown in Figure 5.2.5.3(b).

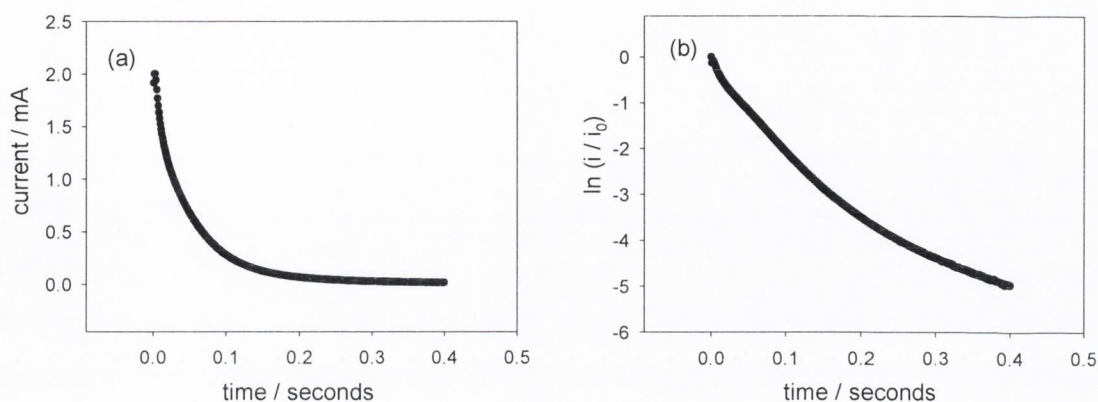


Fig. 5.2.5.3(a) Chronoamperometric response to a potential step experiment across the FAD oxidation potential for a GC/SWCNT/GOx/Nafion electrode. The electrolyte used was a 50 mM PBS (pH 7.0). **(b)** The resulting plot for the determination of the rate constant, based on the assumption of first-order kinetics.

Before continuing, it is considered prudent to make some comments regarding the *cell time constant*, τ , for the modified electrode. In *Section 3.5*, this was found to be around 25 milliseconds. It is noted from *Equation 3.5.6* that when $t = \tau$, the charging current current has decayed to 37 % of its initial value. It is suggested that the measured current may contain a significant charging contribution up to $t = 3\tau$, at which the background current has fallen to 5 % of its initial value. This should be considered when analysing data obtained at short times and will be referred to later.

In Figure 5.2.5.3(b), the current has been normalised with respect to the current at zero time (i_0). The latter is simply estimated using Figure 5.2.5.3(a). Clearly the first-order plot is not linear. Such ideal behaviour does not prevail, and it is concluded that the analysis ought to be adjusted accordingly. The system is clearly too complicated for this simple model. It is proposed therefore that the deviation from linearity is caused by variations in the kinetic facility of the redox process at the active enzyme sites. The spread in kinetic activity is not obvious from voltammetric results, so it is prudent to use potential step techniques in conjunction with the Laviron model when rate constants are sought for adsorbed species.

This complex behaviour is attributed to the kinetic inequivalence of the glucose oxidase molecules on the nanotubes. It is safe to assume that the orientation of the protein structure with respect to the nanotube platform is not uniform. This means that the

FAD sites in each molecule are various distances from the nanotubes, so a dispersion in the magnitude of the rate constant quantifying the redox kinetics between the active flavin site and the nanotube surface is observed. There exists an unknown spatial relationship between the protein and the nanotubes. Furthermore, certain sites on the latter are more active than others, depending on the distribution of oxygenated defects. In particular, ends are likely to be more reactive than side-walls. Also, it has been established that SWCNTs display a range of electrical conductivity [29]. These factors all serve to increase the spread in rate constant values. In situations such as these, in which there is a serious departure from first-order kinetics, current transients and kinetic plots are sometimes analysed on the basis of the *bi-exponential model*, which will now be discussed.

5.2.6 The Bi-exponential Model

The physical interpretation of this model is that every active site in the system is in one of two distinct environments, characterised by the ‘fast’ and ‘slow’ rate constants k_f and k_s . Examples of the use of this approach can be found in the literature [30,31]. It involves replacing the simple single exponential decay with a two-term expression:

$$\frac{i}{i_0} = A \exp(-k_f t) + B \exp(-k_s t) \quad (5.2.6).$$

Here, A and B are coefficients representing the relative weights associated with the processes having the rate constants k_f and k_s , respectively. If the experimental current transients (normalised current against time) are fitted to this equation, a non-linear least squares (NLLS) analysis may be used to extract values for the four parameters k_f , k_s , A and B . The results of the NLLS fitting (using SigmaPlot version 9, www.systat.com) of the equation to the data in Figure 5.2.2(a) yielded ‘fast’ and ‘slow’ rate constants of (90.7 ± 3.7) and $(16.2 \pm 0.1) \text{ s}^{-1}$. The ratio of A to B was found to be around 0.5. The data obtained were found to fit the equation reasonably well (correlation coefficient ~ 0.998). The ratio A/B is an indication of the extent to which each environment contributes to the observed response. The calculated value of 0.5 suggests that there is a larger number of sites towards the ‘slow end’ of the kinetic spread.

The bi-exponential model is certainly preferable to that of Laviron on the basis that it provides some indication of the extremes of the rate constant values and the

relative contributions made by the ‘fast’ and ‘slow’ active sites. However, the assumption on which this model is based is again rather crude. It seems reasonable to expect that the number of distinct environments experienced by the adsorbed enzyme must be considerably larger than two. Of course, the bi-exponential expression may be extended to incorporate any number of environments, resulting in a *multi*-exponential expression, but when this is done, calculated rate constants quickly become unreliable, with very large errors. This is due to the large number of parameters required for fitting. In 1985, Alberly *et al.* [28] proposed an alternative model for the analysis of kinetically dispersed systems which is based on what may be a more reasonable assumption. This will be the subject of the next section.

5.2.7 The Gaussian Model

In this approach, it is assumed that the free energy of activation for the surface redox process is given by

$$\Delta G^\ddagger = \overline{\Delta G^\ddagger} - \gamma x RT \quad (5.2.7.1),$$

where the first term on the right-hand side denotes the *mean* free energy of activation. The quantity x is the coordinate characterising the Gaussian distribution $\exp(-x^2)$, while γ is the dispersion parameter, which indicates how wide the distribution is. It is important to note that, because a Gaussian distribution in the *free energy of activation* is assumed, the model is based on a Gaussian distribution in the *natural logarithm* of the rate constant, distinguishing it from the theory developed by Scott [31], in which there are Gaussian terms in k itself. The dispersion in first-order heterogeneous electron transfer rate constants about a mean value is given by

$$k = \bar{k} \exp(\gamma x) \quad (5.2.7.2).$$

In simple terms, this means that most active sites are characterised by a rate constant similar to this average, a small number have values much smaller and a small number have values much larger. An advantage of this model lies in the fact that it introduces only *two* adjustable parameters: the dispersion (γ) and the mean rate constant, compared with the four variables inherent in the bi-exponential model. The dimensionless time τ is related to the mean rate constant (units s^{-1}) according to

$$\tau = \bar{k}t \quad (5.2.7.3).$$

The first-order rate equation for the adsorbed enzyme may therefore be written as

$$-\frac{d\Gamma}{dt} = k\Gamma = \bar{k}\Gamma \exp(\gamma x) \quad (5.2.7.4),$$

where Γ is the surface coverage of the reactant species. Rearrangement for integration gives

$$\int_{\Gamma_0}^{\Gamma} \Gamma^{-1} d\Gamma = -k \int_0^t dt \quad (5.2.7.5),$$

in which Γ_0 and Γ are the reactant surface coverages at times zero and t , respectively. Integration followed by substitutions involving *Equations 5.2.7.3 and 5.2.7.4* gives

$$\frac{\Gamma}{\Gamma_0} = \exp[-\tau \exp(\gamma x)] \quad (5.2.7.6).$$

Integration across the Gaussian normal distribution $\exp(-x^2)$ yields the following expression for the normalised surface coverage:

$$\frac{\Gamma}{\Gamma_0} = \frac{\int_{-\infty}^{+\infty} \exp(-x^2) \exp[-\tau \exp(\gamma x)] dx}{\int_{-\infty}^{+\infty} \exp(-x^2) dx} = \frac{1}{\sqrt{\pi}} \int_{-\infty}^{+\infty} \exp(-x^2) \exp[-\tau \exp(\gamma x)] dx \quad (5.2.7.7),$$

$$\text{where} \quad \int_{-\infty}^{+\infty} \exp(-x^2) dx = \sqrt{\pi} \quad (5.2.7.8).$$

Albery and co-workers [28] have derived the same expression to describe the normalised *concentration* of a reactant but within the context of species immobilised on electrodes it is more helpful to discuss surface coverages. It is noted that this ratio decays as the reaction proceeds (τ increases) and that the decrease is more pronounced when the dispersion, γ , is large. It should be pointed out that this model may be broken down to the simple first-order kinetics relationship derived in *Section 2.3* when there is no dispersion ($\gamma = 0$):

$$\frac{\Gamma}{\Gamma_0} = \exp(-\tau) = \exp(-\bar{k}t) \quad (5.2.7.9).$$

Equation 5.2.7.7 contains an integral $I(x, \tau, \gamma)$ requiring a numerical procedure which will now be described in detail. It is useful to divide this integral into two separate areas representing $x < 0$ and $x > 0$:

$$I(x, \tau, \gamma) = I_1(x, \tau, \gamma) + I_2(x, \tau, \gamma) \quad (5.2.7.10),$$

$$\text{where} \quad I_1(x, \tau, \gamma) = \int_{-\infty}^0 \exp(-x^2) \exp[-\tau \exp(\gamma x)] dx \quad (5.2.7.11)$$

$$\text{and} \quad I_2(x, \tau, \gamma) = \int_0^{\infty} \exp(-x^2) \exp[-\tau \exp(\gamma x)] dx \quad (5.2.7.12).$$

It is convenient at this point to change the variable from x to λ , where the relationship between these is defined by the following transformation:

$$\begin{aligned} x < 0 \quad x &= \ln \lambda \quad \lambda = \exp(x) \quad dx = \lambda^{-1} d\lambda \\ x > 0 \quad x &= -\ln \lambda \quad \lambda = \exp(-x) \quad dx = -\lambda^{-1} d\lambda. \end{aligned}$$

This transformation means that when $x = \pm\infty$, $\lambda = 0$ and that when $x = 0$, $\lambda = 1$. The advantage in the variable change is that the limits become 0 and 1, rather than $\pm\infty$, which will eventually permit a straightforward application of Simpson's rule to solve the integral. We may now write

$$\begin{aligned} I_1(x, \tau, \gamma) &= \int_0^1 \lambda^{-1} \exp[-(\ln \lambda)^2] \exp[-\tau \exp(\gamma \ln \lambda)] d\lambda \\ &= \int_0^1 \lambda^{-1} \exp[-(\ln \lambda)^2] \exp[-\tau \lambda^\gamma] d\lambda \\ \text{and} \quad I_2(x, \tau, \gamma) &= \int_0^1 \lambda^{-1} \exp[-(\ln \lambda)^2] \exp[-\tau \exp(-\gamma \ln \lambda)] d\lambda \\ &= \int_0^1 \lambda^{-1} \exp[-(\ln \lambda)^2] \exp[-\tau \lambda^{-\gamma}] d\lambda \end{aligned} \quad (5.2.7.13),$$

where the following relations have been used:

$$\begin{aligned} \int_a^b f(x) dx &= - \int_b^a f(x) dx \\ \exp(\gamma \ln \lambda) &= \lambda^\gamma \\ \exp(-\gamma \ln \lambda) &= \lambda^{-\gamma} \end{aligned}$$

This means that
$$I(x, \tau, \gamma) = \int_0^1 g(\lambda) d\lambda \quad (5.2.7.14),$$

where
$$g(\lambda) = \lambda^{-1} \exp\{-[\ln \lambda]^2\} \{\exp[-\tau \lambda^\gamma] + \exp[-\tau \lambda^{-\gamma}]\} \quad (5.2.7.15).$$

The population of a Gaussian distribution of redox states given by *Equation 5.2.7.7* computed numerically *via* the extended Simpson's rule with $\gamma = 2$ is illustrated in the three-dimensional plot in Figure 5.2.7.1.

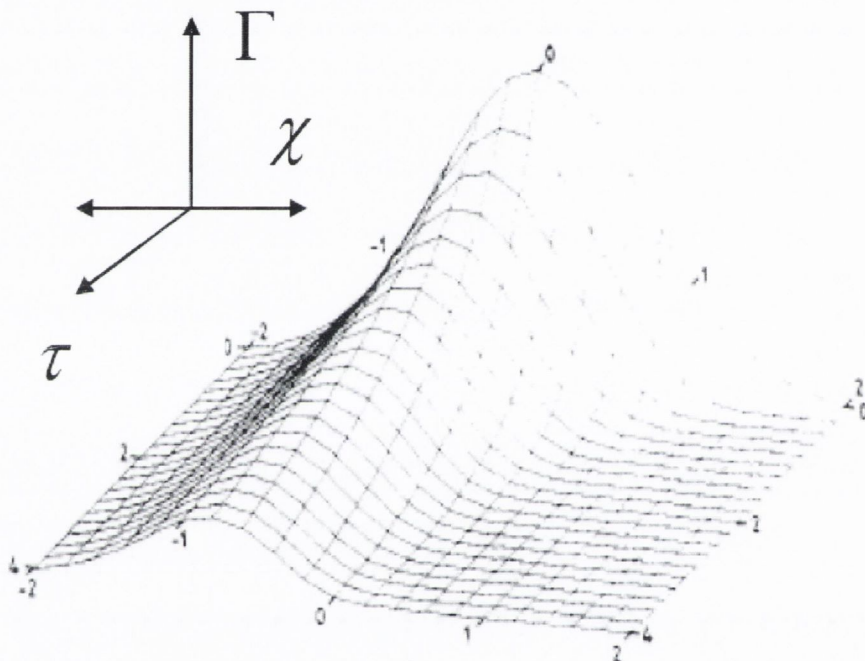


Fig. 5.2.7.1 The decay of the Gaussian population calculated using *Equation 5.2.7.7* with spread parameter $\gamma = 2$. Reproduced from [28].

It is noted from this surface plot that as the reaction proceeds (τ increases) the original symmetrical Gaussian profile becomes skewed to those species with lower rate constants. These slower species predominate in the latter stages of a potential step experiment, giving rise to the type of curvature seen in Figure 5.2.5.3(b). The 3-D surface also indicates why the Gaussian model may be preferable to the bi-exponential approach in the analysis of chronoamperometric transients. Values for 'fast' and 'slow' rate constants extracted from the latter will vary according to the pulse width (time for which the response is monitored) of the step experiment. The use of the Gaussian model is advocated to calculate an average k value along with the spread parameter. It should be

noted that when analysing current transients the faster redox sites make a greater contribution to the observed current.

In their 1985 paper, Albery and co-workers [28] reported that if the decay of some property λ (where λ is proportional to concentration, c) could be experimentally measured with time, plots of $\ln(\lambda / \lambda_0)$ against $\ln t$ could be matched against working curves of $\ln(c / c_0)$ against $\ln t$ calculated using Equation 5.2.7.7. By adjusting the displacements of the experimental curve on both axes, matching data to a particular theoretical curve produced a value for the dispersion parameter.

In potential step chronoamperometry experiments, the decay of current with time is recorded. It is essential to note that the current flowing in an electrochemical experiment is *not* proportional to the concentration (or, for adsorbed species, surface coverage) of reactant species, but rather to the *rate* at which the surface coverage is changing. This means that it is not valid to simply fit data such as those shown in Figure 5.2.5.3(b) to working curves calculated using Equation 5.2.7.7.

The current flowing across an interface is the rate at which charge is being transferred, so if a current-time transient for a potential step such as the one shown in Figure 5.2.5.3(a) is integrated, the result is a *charge*-time transient showing how the quantity of charge transferred, $Q(t)$, *increases* with time. The result of such an operation is presented in Figure 5.2.7.2(a). If Q_{tot} is the total amount of charge transferred after a potential step, the quantity W may be defined as the fraction of the total charge *remaining* to be transferred at a given time:

$$W = \frac{Q_{tot} - Q(t)}{Q_{tot}} \quad (5.2.7.16).$$

Like the normalised surface coverage Γ / Γ_0 , this number decays from unity to zero during the course of a potential step experiment, as shown in Figure 5.2.7.2(b). The definition of W is perhaps a little confusing, but it is necessary to do this in order to provide a quantity which *decays* with time. The point is that W , unlike the normalised current, for all intents and purposes may be considered as representing the normalised surface coverage. They have the same value at all times during a potential step experiment. This means, for example, that if 80 % of the total charge has been transferred at a given time, W has the value 0.2 and it is correct to infer that 80 % of the active sites have reacted at that time. It

would not be reasonable to claim this on the basis of the *current* losing 80 % of its initial value. So values for the dispersion parameter may be obtained by fitting plots of $\ln W$ against $\ln t$ to theoretical plots of $\ln (\Gamma / \Gamma_0)$ against $\ln t$ drawn using Equation 5.2.7.7.

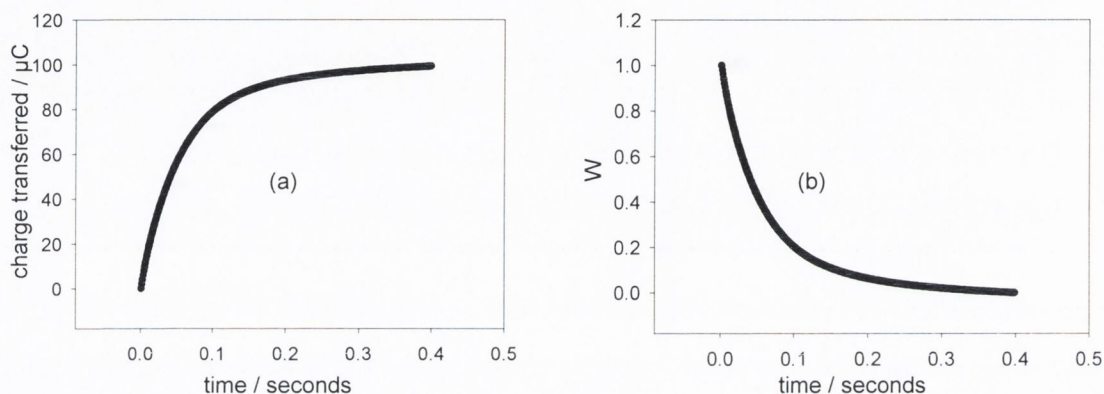


Fig. 5.2.7.2(a) Chronocoulometric response to a potential step experiment across the FAD oxidation potential for a GC/SWCNT/GOx/Nafion electrode, obtained by the integration of the curve shown in Figure 5.2.5.3(a). The electrolyte used was a 50 mM phosphate buffer solution (pH 7.0). **(b)** The resulting plot showing the decay of W , as defined in Equation 5.2.7.16.

Rather than fitting data to working curves, values for the spread parameter and also, importantly, the *mean rate constant* may be found using the procedure originally suggested by Alberly and co-workers [28]. These two values may be obtained using the simple analysis of a semi-logarithmic plot such as that shown in Figure 5.2.7.3(a). The mean rate constant is given by

$$\bar{k} = \frac{1}{t_{1/e}} \quad (5.2.7.17),$$

where $t_{1/e}$ is the time at which W has lost $1/e$ ($\sim 37\%$) of its initial value. In practical terms, this is simply the time at which W is 0.63, or $\ln W$ is around -0.46. Figure 5.2.7.3(b) shows how the plot was used to obtain a $t_{1/e}$ value of 0.028 seconds, which gave a value of 36 s^{-1} for the mean rate constant characterising the enzymatic oxidation process. This is well within the ‘fast’ and ‘slow’ values found using the bi-exponential model. The spread is calculated according to

$$\gamma = 0.92 \times \left(\frac{t_{7/8}}{t_{1/2}} - 3 \right)^{1/2} \quad (5.2.7.18),$$

where $t_{7/8}$ is the time at which W has lost $7/8$ (0.875) of its initial value. This is the time at which W is 0.125, corresponding to $\ln W = -2.08$ in Figure 5.2.7.3(b). The time at which W is half its initial value is $t_{1/2}$, which corresponds to $\ln W = -0.69$ in the same figure. Values of 0.14 and 0.04 seconds for $t_{7/8}$ and $t_{1/2}$, respectively, yielded a value of 0.65 for the spread parameter. Great statistical significance should not be attributed to the latter but it is noted that this γ value is similar to those reported for dyes bound to biological membranes [28]. When the same experiment and analysis were carried out for a cathodic step, poisoning the potential at -0.2 V and stepping to -0.6 V, values of 33 s^{-1} and 0.58 were obtained for the reduction rate constant and spread parameter, respectively. The rate constants were calculated using times similar to the cell time constant (see *Chapter Three*), so they should be regarded as little more than an indication of fast electron transfer, as one would expect for a system showing such well-defined voltammetric peaks. It is this speed which might be viewed as rendering the system unsuitable for this approach. A system with a longer lifetime would have a larger $t_{1/e}$ value, at which distortions due to double layer charging could be safely ruled out.

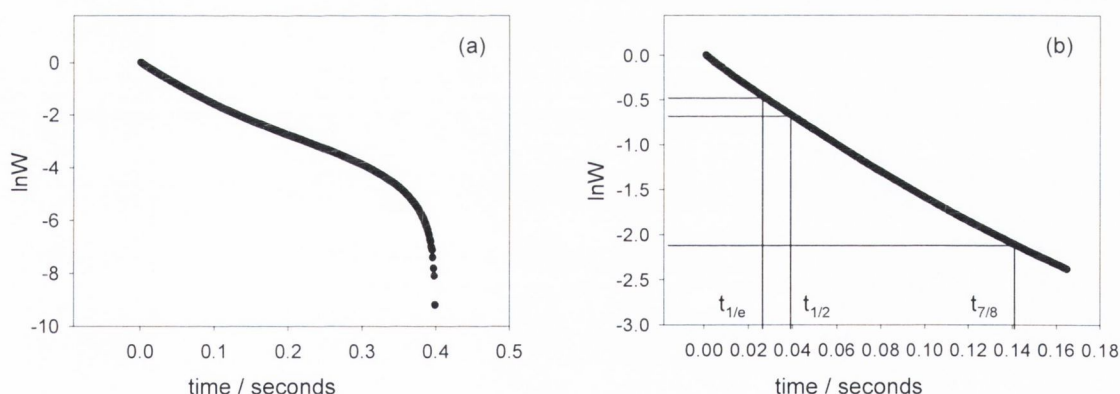


Fig. 5.2.7.3(a) Semi-logarithmic plot showing the decay of W , based on data from the previous figure. **(b)** Close-up of the short-time region, showing how the relevant times were estimated for the calculation of the mean rate constant and the spread parameter.

5.3 Mediated Bio-electro-catalytic Oxidation of Glucose

Having thoroughly investigated the direct electron transfer of glucose oxidase on nanotube-modified glassy carbon electrodes, attention then turned to the issue of whether the immobilised enzyme retained its electro-catalytic activity for the oxidation of glucose.

The quality of the data obtained for the direct ET using deoxygenated buffer as the electrolyte suggested that the system might facilitate the mediator-free sensing of glucose but it was found that mediating small molecules were still necessary. Separate studies were performed using ferrocene carboxylic acid and oxygen to mediate the biosensor signal.

5.3.1 Ferrocene Carboxylic Acid

The ability of the GC/SWCNT/GOx/Nafion ensembles to sense glucose was initially explored by cyclic voltammetry using deoxygenated phosphate buffers containing both glucose and the redox mediator, ferrocene carboxylic acid (FCA). Typical results obtained at the low sweep rate of 1 mV s^{-1} are shown in Figure 5.3.1.1. The sigmoidal shape of the voltammetric responses to glucose is characteristic of radial diffusion [32].

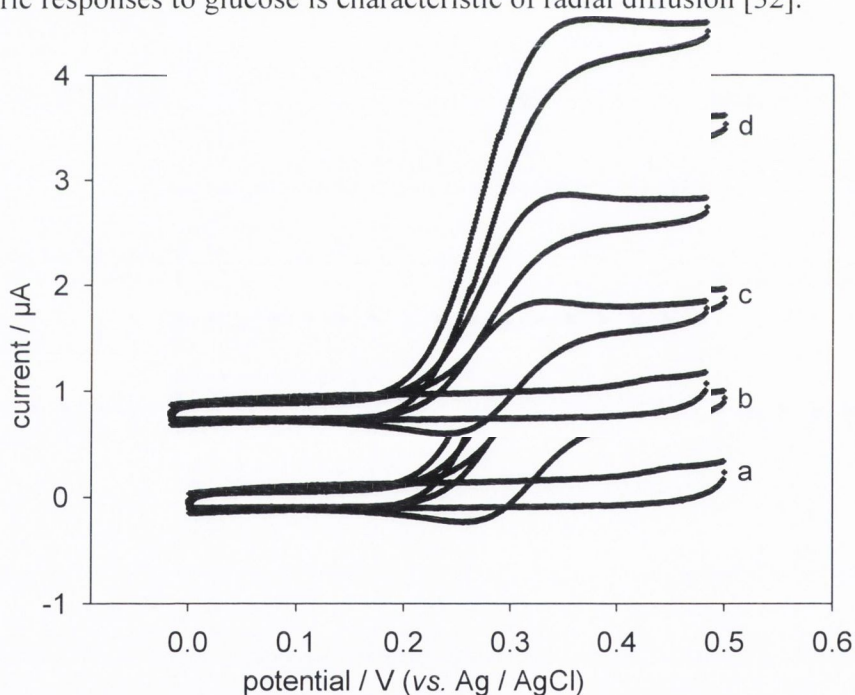


Fig. 5.3.1.1 Voltammetric responses of the GC/SWCNT/GOx/Nafion electrode in (a) 50 mM PBS (pH 7.0), (b) PBS containing 0.5 mM FCA, (c) PBS containing 0.5 mM FCA and 5 mM glucose, and (d) PBS containing 0.5 mM FCA and 10 mM glucose. The scan rate employed was 1 mV s^{-1} .

The figure shows a pair of redox peaks (*b*) corresponding to the redox reaction of FCA, with a standard redox potential of around $+0.3 \text{ V (vs. Ag / AgCl)}$. When ferrocene carboxylic acid was absent, there was no evidence of any such process (*a*). When glucose was introduced to the solution, the anodic current increased dramatically and a quasi-

steady-state electro-catalytic plateau was observed (c). The term ‘quasi-steady-state’ is used because the scan rate used was extremely low. The catalytic current was found to increase with the concentration of glucose (d). The catalytic activity was shown to be due to the immobilised enzyme interacting with glucose by repeating the experiment in the absence of glucose oxidase, as illustrated in Figure 5.3.1.2.

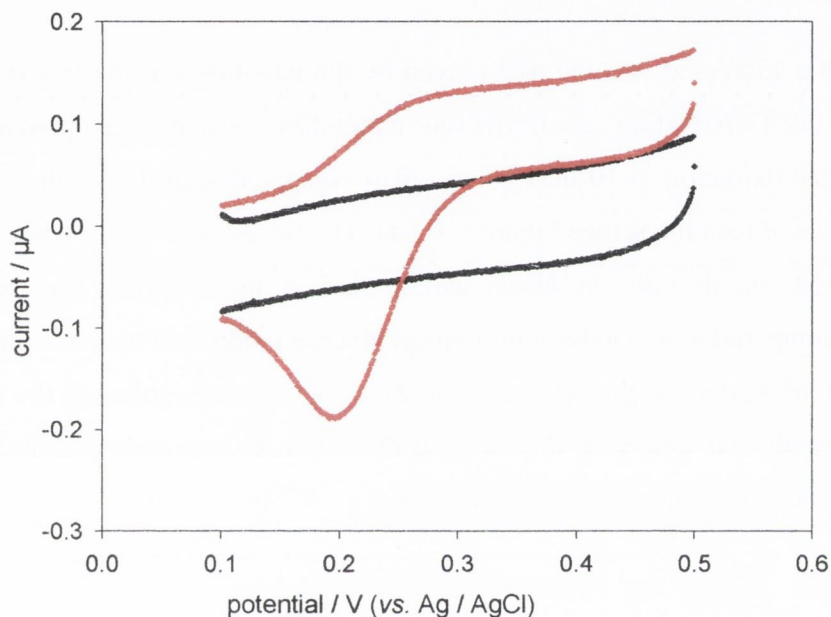


Fig. 5.3.1.2 Voltammetric responses of a GC/SWCNT/Nafion electrode (no GOx) in 50 mM PBS (pH 7.0) containing 10 mM glucose (black), and 50 mM PBS (pH 7.0) containing 10 mM glucose and 0.5 mM ferrocene carboxylic acid (red). The scan rate employed was 1 mV s^{-1} .

From the figure it is clear that, in the absence of the enzyme, the oxidation of glucose does not occur, regardless of the presence of a mediator. So it may be concluded that carbon nanotubes provide an immobilisation matrix which not only promotes the direct electron transfer of glucose oxidase, but permits the retention of the enzyme’s electro-catalytic activity towards the oxidation of glucose.

With this in mind it is suggested that the ‘ping-pong’ mechanism illustrated in Figure 5.3.1.3 provides an appropriate overview of the mediated interaction between electrode and substrate. Glucose is oxidised to gluconolactone *via* an FAD-catalysed reaction with the mediator. The observed signal is attributed to the oxidation of FCA at the electrode surface and increases with the concentration of glucose present in bulk solution.

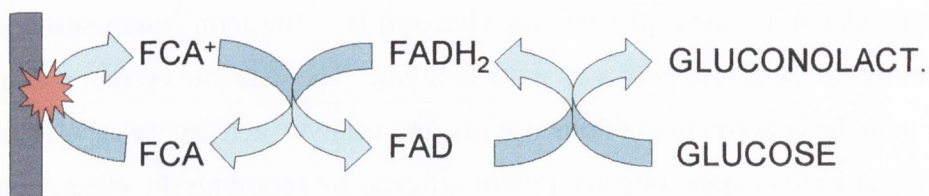


Fig. 5.3.1.3 Proposed 'ping-pong' mechanism for the FCA-mediated oxidation of glucose to gluconolactone via a glucose oxidase-catalysed reaction.

The next issue addressed was the role played by the nanotubes in the observed catalytic activity. A GC/GOx/Nafion electrode (no nanotubes present) was prepared and its response to the oxidation of 10 mM glucose was compared with that of the same system in the presence of nanotubes (see Figure 5.3.1.4). The figure shows that, when the enzyme is immobilised directly onto the glassy carbon surface, its activity is low (although not zero) when compared to nanotube-immobilised glucose oxidase. It may be concluded then that the SWCNTs promote the catalytic activity of the enzyme, although the effect could possibly be explained simply by the fact that the nanotube ensemble permits a far larger enzyme coverage than unmodified glassy carbon.

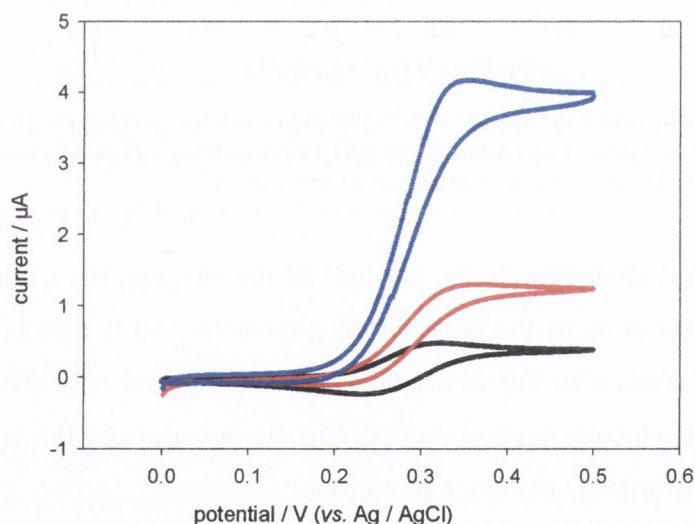


Fig. 5.3.1.4 Cyclic voltammograms of a GC/GOx/Nafion (black, red) and GC/SWCNT/GOx/Nafion (blue) electrode in 50 mM phosphate buffer solution (pH 7.0) containing 0.5 mM ferrocene carboxylic acid in the absence (black) and presence (red, blue) of 10 mM glucose.

The storage stability of the GC/SWCNT/GOx/Nafion response to the oxidation of glucose was also investigated. The results are shown in Figure 5.3.1.5. It was found that the

response decreased by 18 % after only one day's storage in buffer. This finding supports the conclusion drawn in *Section 5.2.1* that fresh electrodes should be prepared daily.

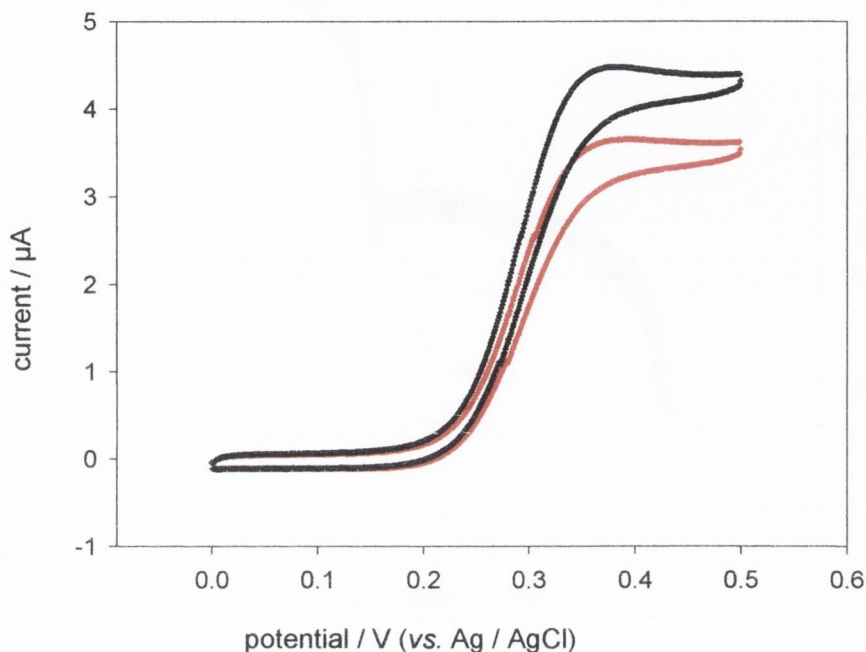


Fig. 5.3.1.5 Bio-electro-catalytic responses of the GC/SWCNT/GOx/Nafion electrode to the oxidation of 10 mM glucose in 50 mM PBS (pH 7.0) in the presence of 0.5 mM ferrocene carboxylic acid. The black curve is the response obtained immediately after the electrode was prepared and the red curve represents the response after the electrode was stored in buffer for one day.

5.3.2 Oxygen

The next avenue explored was the issue of whether the system could show a useful response to glucose in the presence of molecular oxygen, the natural co-substrate of glucose oxidase. Figure 5.3.2.1 confirms that it could. The figure shows the result of a chronoamperometric experiment at an applied constant potential of -475 mV, carried out under constant stirring in oxygen-saturated buffer. The current which initially flows is due to the reduction of oxygen at the electrode surface. When glucose is introduced to the solution, this current drops dramatically. This is attributed to the consumption of oxygen *via* its GOx-catalysed reaction with glucose, as described in *Section 1.11*. The 'ping-pong' mechanism for this system is analogous to the scheme proposed in Figure 5.3.1.3, with oxygen replacing ferrocene carboxylic acid as the electron transfer mediator. In this case the electrical signal is attributed to the reduction of oxygen to hydrogen peroxide.

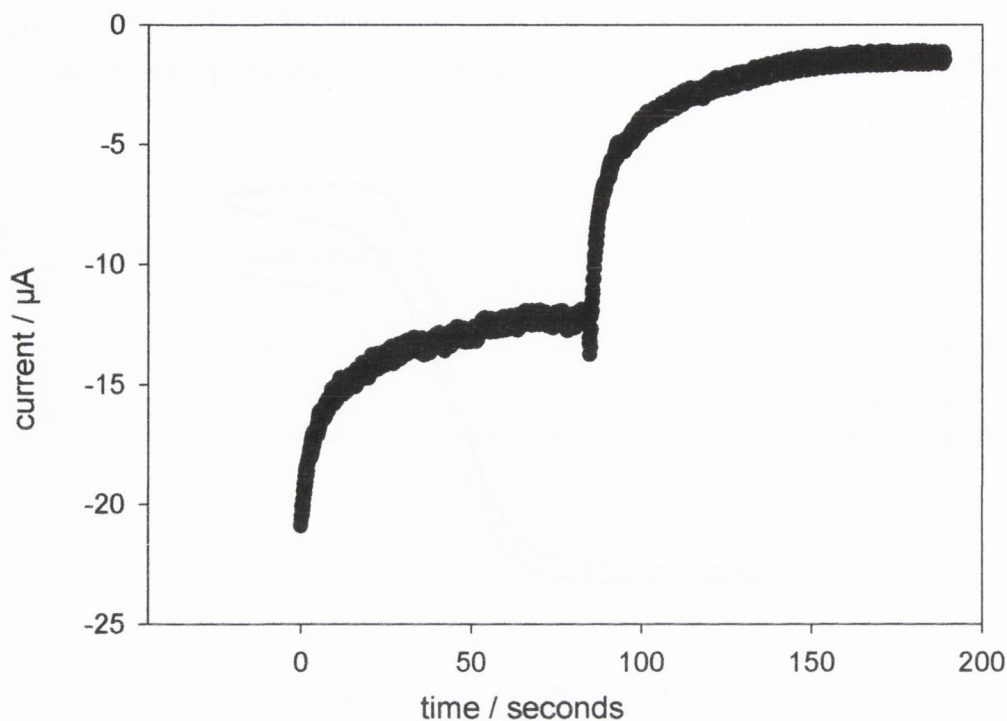


Fig. 5.3.2.1 Chronoamperometric response of the GC/SWCNT/GOx/Nafion electrode in O_2 -saturated PBS (pH 7.0) at a constant potential of -475 mV, with glucose added at 85 s to a final concentration of 25 mM.

Further evidence was found using cyclic voltammetry (see Figure 5.3.2.2). The red scan shows the response of the GC/SWCNT/GOx/Nafion electrode in deoxygenated buffer. When the solution is saturated with oxygen the effect on the signal is dramatic (blue curve). The enhancement of the cathodic peak is attributed to the reduction of oxygen at the electrode surface. Finally, the black voltammogram was obtained after the chronoamperometric experiment described above involving the introduction of glucose to the solution. As can be seen, the oxygen has been consumed entirely and the response is virtually identical to the original scan. So much so, in fact, that it can barely be seen behind the first (red) scan.

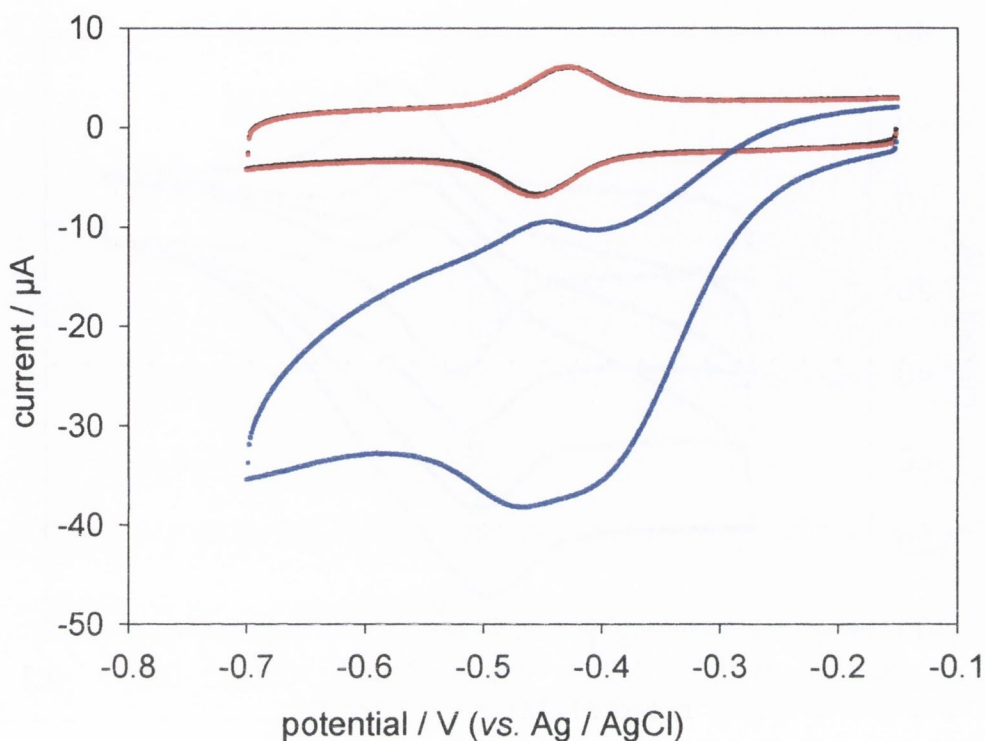


Fig. 5.3.2.2 Cyclic voltammograms of a GC/SWCNT/GOx/Nafion electrode in deoxygenated PBS (red), in O₂-saturated PBS (blue) and after the addition of 25 mM glucose (black). The scan rate was 50 mV s⁻¹.

These results indicate that voltammetry may be used to monitor increases in glucose concentration by observing the *decrease* in oxygen reduction current as it is consumed by glucose. The mediated electro-catalysis of glucose was thus investigated using GC/SWCNT/GOx/Nafion electrodes in pH 7.0 phosphate buffer solution, as shown in Figure 5.3.2.3. When the electrolyte was saturated with oxygen (scan *b*) a dramatic effect was observed, with a large reduction current evident for dissolved oxygen. Upon addition of glucose, this current was seen to decrease (*c* and *d*). This decrease is attributed to the consumption of oxygen by glucose *via* a GOx-catalysed reaction at the electrode surface. The *oxidation* peaks in the figure are attributed to the direct electrochemistry of dissociated flavin. It is important to state that the existence of these oxidation peaks suggests that the electro-active FAD discussed in *Section 5.2* does *not* participate in the enzymatic reaction. The latter is attributed to GOx itself, whereas the direct electrochemistry is due to dissociated flavin, which is commonly found in commercially available supplies of glucose oxidase.

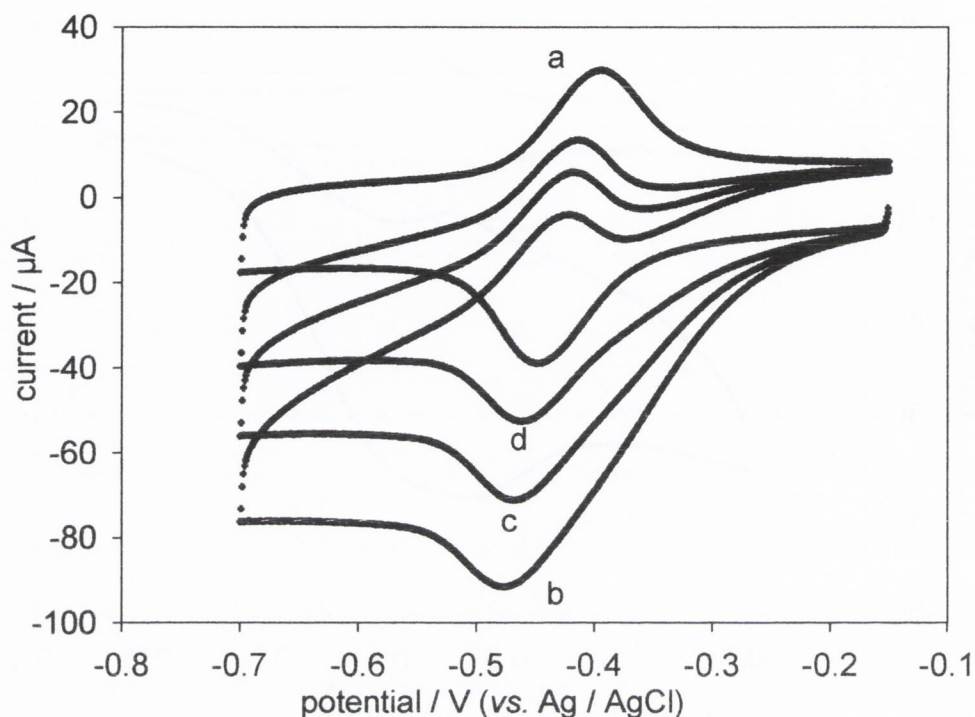


Fig. 5.3.2.3 Voltammograms recorded for a GC/SWCNT/GOx/Nafion electrode in (a) deoxygenated 50 mM pH 7.0 PBS, (b) oxygen-saturated PBS, (c) oxygen-saturated PBS after addition of 1.2 mM glucose and (d) same after addition of 2.4 mM glucose. The scan rate was 50 mV s^{-1} .

This represents an alternative approach to glucose sensing. Glucose biosensors commonly work by monitoring increases in oxidation currents with glucose concentration. In this case glucose was detected by observing *decreases* in reduction currents caused by the consumption of oxygen by glucose. If the difference between the reduction peak current when glucose is present and when it is absent is measured, the results show that this quantity increases with substrate concentration, forming the basis of the calibration plot in Figure 5.3.2.4. The response was linear between 0.61 and 3.0 mM with a correlation coefficient of 0.9992 ($N = 5$). Based on a signal-to-noise ratio of three, the detection limit using this approach was found to be 0.1 mM. A sensitivity value of $16.5 \mu\text{A} / \text{mM}$ was calculated from the slope of the graph. This approach of monitoring voltammetric oxygen reduction peak currents for the purposes of glucose sensing is uncommon but has been reported previously by Wang *et al.* [15]. The system used in the latter study was formed by the immobilisation of glucose oxidase on an *unmodified* edge-plane highly-ordered pyrolytic graphite electrode. It is interesting to note that they reported a detection limit of

0.05 mM but the response was linear up to a glucose concentration of only 1.5 mM. As an explanation it is suggested that this system can detect lower concentrations than what is described here because the enzyme is in direct contact with the underlying electrode. The present system responds linearly over a greater concentration range because the nanotube array permits the adsorption of a larger loading of enzyme than a ‘flat’ electrode. Indeed, these workers report GOx surface coverages which are at best an order of magnitude less than reported here, as discussed in *Section 5.2.1.3*.

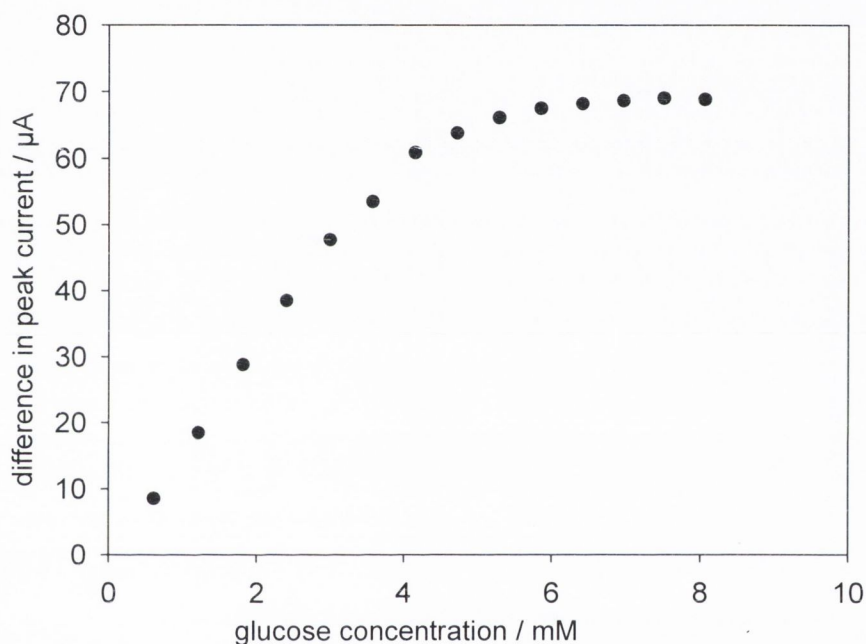


Fig. 5.3.2.4 Calibration plot showing how the oxygen reduction current decreases as the concentration of glucose increases based on cyclic voltammetry at a sweep rate of 50 mV s^{-1} .

Having obtained reasonably encouraging results using cyclic voltammetry, the performance of the system as a glucose biosensor was further probed using batch amperometry with magnetic stirring at a fixed potential of -0.475 V (vs. Ag / AgCl) to monitor the decrease in oxygen reduction current due to additions of glucose. It was thought that this would be an attractive avenue to explore because interfering species such as ascorbic acid are not oxidised at such a negative potential. Furthermore, it is desirable that a biosensor should operate at a potential similar to that at which the enzyme reacts [33]. However, while the current indeed decreased on additions of glucose, it continued to

do so without reaching a steady value due to the depletion of oxygen at the electrode surface, making it impossible to draw a reliable calibration plot.

Instead, glucose sensing was investigated along more traditional lines by holding the potential of the enzyme electrode at +0.6 V in a magnetically stirred (150 rpm), oxygen-saturated pH 7.0 phosphate buffer and measuring the increase in oxidation current with glucose concentration. In this case the electrical signal was due to the oxidation of hydrogen peroxide:



Here the ‘ping-pong’ mechanism is exactly like the one depicted in Figure 5.3.1.3 except that the FCA^+ / FCA redox couple is replaced by O_2 / H_2O_2 . It is common practice to employ a *Michaelis-Menten analysis* when investigating the response of an immobilised enzyme to increases in substrate concentration. Before doing so for the present system, it is considered pertinent to provide a brief derivation of this simple model.

Consider an enzyme, E , which catalyses the reaction of a substrate, S , by initially forming an intermediate species, ES :



The equation shows the rate constants for the forward and reverse processes. It is worth noting that the units of k_1 and k_{-1} are $\text{dm}^3 \text{mol}^{-1} \text{s}^{-1}$ and s^{-1} , respectively. The product, P , then forms and the enzyme is regenerated:



The rate constant for product formation is called the catalytic rate constant (k_c , units s^{-1}) and so the rate equation for this step may be written as

$$R = k_c[ES] \quad (5.3.2.4).$$

Combination of the two steps yields the overall scheme



The rate of formation of the intermediate is $k_1[E][S]$ and that of its decomposition is given by the sum of $k_{-1}[ES]$ and $k_c[ES]$. Analysis of the results reported in the present work involves currents measured under *steady-state conditions*. Under the latter it is reasonable to state that these two rates are equal:

$$k_1[E][S] = k_{-1}[ES] + k_c[ES] \quad (5.3.2.6).$$

We now define $[E]_{\Sigma}$ as the total enzyme concentration (units mol dm⁻³):

$$[E]_{\Sigma} = [E] + [ES] \quad (5.3.2.7).$$

Combining *Equations 5.3.2.6 and 5.3.2.7* to eliminate $[E]$ gives

$$\begin{aligned} [ES] &= \frac{k_1[S][E]_{\Sigma}}{k_1[S] + k_{-1} + k_c} \\ &= \frac{[S][E]_{\Sigma}}{[S] + \left(\frac{k_{-1} + k_c}{k_1} \right)} \\ &= \frac{[S][E]_{\Sigma}}{[S] + K_M} \end{aligned} \quad (5.3.2.8),$$

in which K_M is the *Michaelis-Menten constant* (units mol dm⁻³), defined as:

$$K_M = \frac{k_{-1} + k_c}{k_1} \quad (5.3.2.9).$$

This quantity may be interpreted as an indication of the enzyme-substrate kinetics. Small values suggest that k_1 is large, and the formation of the enzyme-substrate complex is favoured. However, when dealing with enzymes immobilised on electrodes, its value can be affected by micro-environmental and diffusional contributions [34]. In such situations in the literature [35-39] it is common to report instead what is referred to as an *apparent* Michaelis-Menten constant, the physical interpretation of which might not be as straightforward.

Returning to the derivation, *Equations 5.3.2.4 and 5.3.2.8* may be combined to yield the following equation, which predicts how the rate of an enzymatic process varies with substrate concentration:

$$Rate = k_c[E]_{\Sigma} \left(\frac{[S]}{[S] + K_M} \right) \quad (5.3.2.10).$$

For large substrate concentrations (saturated enzyme kinetics), $[S] \gg K_M$, and the term in the brackets may be approximated as unity, giving the following expression for the maximum rate:

$$R_{\max} = k_c[E]_{\Sigma} \quad (5.3.2.11).$$

This is independent of $[S]$, meaning that zeroth-order kinetic behaviour prevails at large substrate concentrations. A combination of *Equations 5.3.2.10* and *5.3.2.11* gives the *Michaelis-Menten equation*:

$$R = R_{\max} \left(\frac{[S]}{[S] + K_M} \right) \quad (5.3.2.12).$$

For low substrate concentrations ($[S] \ll K_M$), the term in brackets may be approximated as $[S] / K_M$ and the rate is given by

$$R = \frac{R_{\max} [S]}{K_M} \quad (5.3.2.13).$$

In this case the rate is proportional to the substrate concentration. The electrochemical equivalent of the *Michaelis-Menten* equation is based on the idea that the steady-state current flowing is proportional to the rate of the enzymatic process occurring in the immobilised film:

$$I_{ss} = I_{\max} \left(\frac{[S]}{[S] + K_M} \right) \quad (5.3.2.14).$$

This equation predicts that the current is initially directly proportional to the substrate concentration, but tends towards an upper limit at larger values. Figure 5.3.2.5(a) shows the current response to additions of aliquots of 0.1 M stock glucose to a stirred, oxygen-saturated solution, with the potential of the enzyme electrode poised at a value sufficiently anodic to oxidise the peroxide produced during the GOx-catalysed reaction. After the scan had begun the current was allowed to stabilise for eight hundred seconds. At this time the first addition was made and the current was allowed to stabilise for two hundred seconds before the second addition. After this, additions of glucose were made every two hundred seconds. Current increases at high concentrations were slight, so the times at which additions were made are indicated by arrows. In the calibration plot the currents are background-corrected. This plot indicates that the system displays *Michaelis-Menten* behaviour, despite the use of oxygen as a mediator, a complication which is not considered by the model.

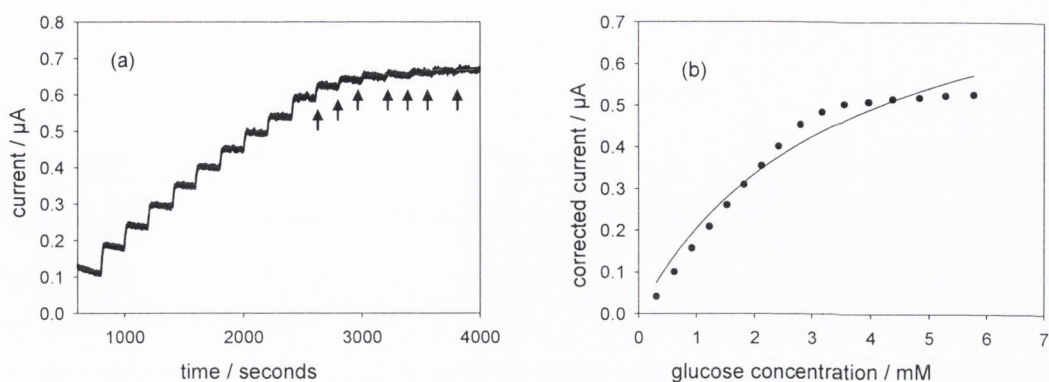


Fig. 5.3.2.5(a) Amperometric response for a GC/SWCNT/GOx/Nafion electrode upon successive additions of glucose solution at a constant potential of +0.6 V (vs. Ag / AgCl). **(b)** The resulting calibration plot, including a curve fitted using the *SigmaPlot* programme. The electrolyte was a magnetically stirred oxygen-saturated pH 7.0, 50 mM phosphate buffer.

The response time and detection limit ($S/N = 3$) were found to be 15 s and 0.05 mM, respectively. The biosensor exhibited linearity from 0.3 to 2.8 mM ($N = 9$, $R^2 = 0.998$) with a sensitivity of $0.17 \mu\text{A mM}^{-1}$. A value for the apparent *Michaelis-Menten* constant was obtained by fitting the data in Figure 5.3.2.5(b) to Equation 5.3.2.14 using a NLLS fitting (*SigmaPlot* version 9, www.systat.com). It is pointed out that the *Michaelis-Menten* equation is of the form

$$y = \frac{ax}{b + x},$$

which describes a rectangular hyperbola. A reasonable fit (correlation coefficient 0.97) was obtained to such a curve, yielding values of $0.9 (\pm 0.1) \mu\text{A}$ and $3.5 (\pm 0.7) \text{mM}$ for I_{max} and the apparent K_M , respectively. The errors in parentheses were produced by the fitting procedure. It should be emphasised again that the latter characterises the enzyme *electrode* and does not represent an intrinsic property of glucose oxidase itself. Its value may be affected by the diffusional constraints to which the substrate is subjected in the immobilised film. The calculated value is smaller than those reported for the soluble enzyme (21–33 mM) [40,41] and GOx entrapped in polypyrrole films (19 mM) [35]. This indicates that glucose oxidase immobilised on carbon nanotubes binds relatively easily with its natural substrate and that these electrodes are suitable for biosensing applications. The low value obtained for the apparent K_M suggests that the bio-catalytic process is under the control of catalysis. Stirring is used to eliminate the possibility of the rate being

limited by the diffusion of substrate from bulk solution. However, the possibility of slow mass transport within the film itself cannot be ruled out. It should be noted that Wang *et al.* [15] have reported a value of 2.5 mM using glucose oxidase immobilised on an edge-plane HOPG electrode, also using oxygen as an electron transfer mediator. These workers attributed the impressive enzyme-substrate affinity to the fact that the glucose oxidase was in direct contact with the unmodified electrode. It is possible that the value reported for the present system may be a little higher due to the interference of Nafion in the interaction between enzyme and substrate.

Wang and co-workers [15] also used a simple calculation to provide a value for the maximum turnover rate (TR_{max}) for glucose oxidase immobilised on an epHOPG electrode. This number is interpreted as the average number of electrons generated by a GOx molecule per second and is given by

$$TR_{max} = \frac{I_{sat}}{Fn\Gamma} \quad (5.3.2.16),$$

in which I_{sat} is the maximum current density reached under saturated enzyme conditions, n is the number of electrons involved in the enzyme process (two for glucose oxidase) and Γ is the enzyme surface coverage. Using Figure 5.3.2.5(b), a value of 0.6 μA was estimated for the maximum current. In Section 3.5 it was shown that the average surface area of a nanotube-modified electrode was 0.0976 cm^2 , so a value of 6.1 $\mu\text{A cm}^{-2}$ was assigned to the maximum current density. In Section 5.2.1.3, an average enzyme surface coverage of 160 pmol cm^{-2} was reported. Insertion of these values into the equation gave a maximum turnover rate of 0.2 s^{-1} , which is less than the 1 s^{-1} found by Wang and co-workers [15], again suggesting that direct immobilisation on an unmodified electrode may be preferable.

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Chapter Six: Conclusions and Future Work

This thesis has reported on the physical characterisation of SWCNT-modified glassy carbon electrodes and their use as sensors for various bio-molecules. These ensembles were also used to study the direct electron transfer of the enzyme glucose oxidase and as components in amperometric glucose biosensors. These areas have attracted considerable interest in the last decade and are likely to continue doing so in the near future. It is hoped that the literature reviewed in *Chapter One* succeeded in its aim of placing the work in a broader context, thereby justifying the motives behind it.

In *Chapter Three*, a number of very different techniques were used with the aim of providing a description of what the surface architecture is like on a nanotube-modified electrode. Atomic force microscopy images showed a disordered array of SWCNTs and this picture proved useful when interpreting certain electrochemical properties in later chapters. Collaborations between electrochemists and surface scientists have become more prevalent in the literature recently and we believe this trend will, and indeed should, continue. It is likely that surface characterisation will become almost a standard feature in electrochemistry publications.

Infrared spectroscopy was used to indicate the presence of surface oxides on the nanotubes, which of course are believed to play such a key role in the electro-catalytic performance of the latter. It is perhaps ironic that the usefulness of nanotubes in electroanalysis depends to a large extent on the existence of these defects. A point which has been emphasised throughout this work is that careful physical characterisation of NTs is essential, as the amount of these functionalities (and also residual iron catalyst) present can vary over a large range, depending on how the tubes are made and /or purified. In the future it is recommended that comparisons between the electrochemical properties of nanotubes before and after vacuum annealing should be investigated. This technique can heal defect sites and remove functional groups.

Cyclic voltammetry was used to elucidate the redox processes attributed to these oxygenated species and to quantify the considerable increase in active surface area precipitated by nanotube deposition, another vital consideration in the electroanalytical applications of nanotubes. We also showed for the first time that, despite its excellence as

a solvent, *N*-methyl-2-pyrrolidone is not suitable as an agent for the modification of electrode surfaces with carbon nanotubes.

In *Chapter Four*, various redox probes were used to highlight the improved electro-catalytic performance of the modified electrodes, most simply by decreases in peak separations and detection potentials. The findings for the different systems are summarised in *Table 6.1*.

	Bare GC Electrode			SWCNT-Modified Electrode		
	E_{pa} / V	E_{pc} / V	$\Delta E_p / V$	E_{pa} / V	E_{pc} / V	$\Delta E_p / V$
ferrocyanide	0.25	0.17	0.08	0.25	0.18	0.07
dopamine	0.23	0.12	0.11	0.24	0.14	0.10
epinephrine	0.31	—	—	0.20	—	—
norepinephrine	0.36	0.16	0.20	0.34	0.18	0.16
ascorbic acid	0.21	—	—	-0.02	—	—
NADH	0.58	—	—	0.33	—	—
oxygen	—	-0.5	—	—	-0.38	—

Table 6.1 Summary of voltammetric data obtained at 100 mVs^{-1} using bare and SWCNT-modified glassy carbon electrodes to detect various redox species.

With several of these probes, evidence of thin-layer behaviour at the GC/SWCNT electrodes was observed. This does not take away from the fact that the bio-molecules were detected at lower potentials, but it was concluded that it may be foolhardy to attribute the observed peak shifts exclusively to enhanced reaction kinetics. These three-dimensional electrode characteristics mean that comparisons between bare and modified electrodes are not as straightforward as portrayed in certain publications. It is recommended that more attempts are made to fabricate aligned, robust arrays of nanotubes on electrode surfaces, as these ought to be considerably less porous, making these comparisons somewhat more justifiable. Also, of course, the increased amount of tube ends presented to redox species in solution might well lead to faster reaction kinetics and the further lowering of peak separations and oxidation potentials. It is also suggested that some experiments should be tried using CNT electrodes in non-aqueous electrolytes, as it is conceivable that electro-catalysis is impeded by the poor contact between water and the notoriously hydrophobic nanotubes. It was also shown in this chapter that the perceived electro-catalytic properties of nanotubes might not be as unique as suggested

elsewhere, and that similar results could be achieved using conventional graphite as the modifying agent. The latter gives rise to less robust ensembles, but it is certainly recommended that future studies in this field should involve control experiments using graphite-modified electrodes. We also advocate the use of edge-plane HOPG electrodes in future work, as these might indicate whether shifts in peak potentials are due to oxygenated defects or the presence of iron catalyst. The latter is of particular relevance to the sensing of hydrogen peroxide.

In *Chapter Five*, glucose oxidase was immobilised on GC/SWCNT electrodes using a simple procedure and its direct electron transfer was extensively probed. It was tentatively suggested that the nanotubes functioned as molecular wires and that the enzyme retained its natural shape, form and function, but it must be conceded that the direct electrochemistry was due to dissociated flavin and not the enzyme itself. Particular attention was given to the application and discussion of three different kinetic models in the calculation of rate constants for the FAD/FADH_2 redox process at the nanotube-modified electrodes. Using the Laviron [1] analysis, a value of around 6 s^{-1} was obtained for the rate constant, but the data did not fit this model well, most likely due to its assumption of kinetic equivalence for all active sites on the electrode. The bi-exponential analysis yielded ‘fast’ and ‘slow’ rate constants of 91 and 16 s^{-1} , but it was again concluded that the assumptions on which this model was based were rather crude.

A sophisticated analysis proposed by Albery and co-workers [2] was then considered. This model allowed curved semi-logarithmic kinetic plots to be fitted based on the assumption of a Gaussian distribution in the free energy of activation for the redox process. We used this model, for the first time, to analyse data obtained from potential step chronocoulometry. A mean rate constant of around 35 s^{-1} , along with a spread parameter in the region of 0.6, were obtained *via* this method. In the future, it would be interesting to use this analysis with GOx immobilised at an *aligned* array of nanotubes, in order to investigate the effect of alignment on the spread parameter. It is also envisaged that aligned arrays will offer improved control over the reproducibility of data. Furthermore, the incorporation of spreads in kinetic activity into the Laviron model would indeed be a goal worth pursuing [3]. A key point made in relation to the direct electron transfer of redox enzymes at modified electrodes was that potential step techniques

readily identified kinetic dispersion. This represents a compelling reason for their use in conjunction with voltammetry in the study of such systems.

Also in this chapter, the use of GC/SWCNT/GOx/Nafion electrodes as glucose biosensors was reported. The retainment of the enzyme's bio-catalytic activity supported the view that the nanotubes acted as molecular wires, without causing significant denaturation of the protein. The ability of the system to function as a biosensor was attributed to glucose oxidase, and it was emphasised that these enzyme molecules were not involved in the direct electron transfer studies. Both oxygen and ferrocene carboxylic acid were used to mediate the transfer of electrons. With oxygen present in solution and operating at a potential of +0.6 V (vs. Ag / AgCl), a detection limit of 50 μ M was achieved. This compares favourably with several of the literature values listed in *Section 1.15*. However, the biosensor was useful for glucose concentrations only up to around 3 mM, which is too low to be of any practical use. Glucose biosensors should ideally be able to detect concentrations up to 20 mM. While other workers have reported the incorporation of nanotubes into biosensors fulfilling this requirement, a recent paper by Hong and Pan [4] has pointed out that few reports exist on the use of NTs in *real* biological systems. Future work must investigate the *in vivo* bio-compatibilities, toxicities and stabilities of carbon nanotubes. Also, the sensitivity of the nanotube-modified electrode to species such as ascorbic acid, as shown in *Chapter Four*, implies that the ensemble might suffer from poor selectivity in real samples.

While research into the applications of nanotubes in a myriad of sophisticated electrochemical systems continues, some fundamental issues regarding the mechanism by which they function as electro-catalysts remain unresolved. A striking reluctance prevails in the literature (and admittedly, in the present work) to commit to saying much more than that the observed electro-catalysis is attributed to 'oxygenated functionalities'. Cline and co-workers [5] have noted that slow electron transfer kinetics at basal-plane HOPG electrodes may be attributed to either the lack of specific chemical sites on the surface or the low density of electronic states exhibited by defect-free graphite. An important consequence of this work was that catalysis *via* a mediation mechanism involving reactive surface groups or by a pathway determined by the density of states are conceptually

distinct. In real terms, however, distinguishing between these two mechanisms at carbon electrodes is far from straightforward.

The most significant contributions towards the search for answers to this challenging question have undoubtedly been made by the group of Compton [6]. Numerous studies carried out by these workers have attributed the catalytic properties of graphitic carbon electrodes to reactive carbonyl groups present at surface defect sites, particularly ‘edge-plane-like defects’. This view is now widely accepted and has been referred to many times in this thesis. Definitive evidence supporting proposed mechanisms is a problem in electro-catalysis in general, and the debate as to precisely how carbon nanotubes function in this capacity seems set to continue.

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