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Immobilized enzyme/single –wall carbon nanotube composites for amperometric glucose detection at very low applied potential.

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In this document issues relating to the stability and catalytic integrity of the adsorbed enzyme and the mechanism of catalysis at the SWCNT surface are addressed in more detail. These topics were brought to the attention of the authors during manuscript review by two of the referees. We address the concerns and queries posed by the referees here and a complete discussion of these topics will be presented in a forthcoming publication.

What is the form of flavin present at the SWCNT surface?

One referee queried whether the flavin dissociated when glucose oxidase is adsorbed on the surface of the nanotube. The flavin (either present in the solution or adsorbed on the nanotube surface) could then function as an inefficient redox mediator. In a recent paper Baughman and co-workers[1] examined the redox behaviour of both the free flavin molecule in the adsorbed state and when it is bound within glucose oxidase at single walled carbon nanotube modified electrodes using potential sweep voltammetry. The standard potential of the $FAD/FADH_2$ transition for both forms of flavin environment were similar, typically – 0.45 V vs Ag/AgCl (in good agreement with our data (E^0 = - 0.396 V see figure 2)) and so it is difficult to differentiate between adsorbed flavin and adsorbed protein bound flavin. Both forms give rise to characteristic bell shaped responses when subjected to a linear potential sweep. However if the flavin is free in solution then the voltammetric response observed should be quite different. A characteristic diffusive response is expected in the voltammogram. This is not observed in our work (again refer to fig.2). Furthermore if the voltammetric response observed in figure 2 arose from ET activity associated with adsorbed flavin molecules then one would

expect that the voltammogram would be considerably distorted because of the fact that the electron would have to tunnel through a blocking layer of adsorbed holo-enzyme molecules which would also be present on the nanotube surface[2]. This distortion in the voltammetric profile is not observed.

The denaturation of glucose oxidase on a metal electrode surface has been experimentally examined using ellipsometry by Bockris and co-workers[2]. In this work two orientations of the enzyme molecule (shaped as a prolate ellipsoid with a major axis of 140 Å and minor axis of 50 Å) on the surface were observed: the major axis could be perpendicular to the surface (labelled the standing position) or parallel to the surface (termed the lying position). It was determined that above a certain coverage enzymes in the standing position were not stable and undergo a transition to the lying position due to increasing intermolecular interaction. In the lying position the enzyme/substrate contact area is large and a gradual unfolding of the glycoprotein sheath occurs brought about largely by significant electrostrictive forces operating in the double layer region. The catalytic function of the enzyme is adversely effected because of this and eventually catalytic activity is lost. Hence a key determining factor in determining enzyme structural stability is the manner in which the enzyme is orientated with respect to the support electrode surface.

Finally we have noted in our experiments that catalytic activity with respect to glucose oxidation is observed only when a soluble mediator such as oxygen or ferrocene monocarboxylic acid is present in the solution. Catalytic glucose oxidation does not occur if adsorbed glucose oxidase is only present. The homogeneous mediator is required to ensure efficient charge shuttling between the flavin active site buried deep within the protein sheath and the underlying carbon nanotube sidewall. Oxygen and ferrocene molecules are of the correct size to enter the glycoprotein sheath effectively and interact with the flavin group.

It is important to note that the carbon nanotube and the enzyme molecule share a similar length scale and so the enzyme is able to adsorb on the nanotube sidewall without losing its biologically active shape, form and function. Indeed Baughman and co-workers have suggested the striking analogy of piercing a balloon with a long sharp needle such that the balloon does not burst. Instead by a gentle twisting action the needle

can be made to enter the balloon without catastrophe. Similarly it was proposed that some number of nanotubes are able to pierce the glycoprotein shell of glucose oxidase and gain access to the flavin prosthetic group such that the electron tunnelling distance is minimized and consequently electron transfer probability optimized. Such access is not generally afforded with traditional smooth electrodes.

Mechanism of catalysis at SWCNT surface.

A second referee enquired whether the SWCNT's were acting as molecular wires and sought information regarding the mechanism of operation of the SWCNT modified electrode. These questions are extremely pertinent at the present time since the mechanisms by which carbon nanotubes achieve catalysis are the subject of considerable attention by a number of groups most notably that of Compton [3] and Dekker and co-workers [4,5].

McCreery and co-workers[6] have noted that electrode kinetics at carbon electrodes depend greatly on surface history and noted that the kinetics of solution phase redox species at basal plane highly ordered pyrolytic graphite electrodes depends largely on the density of defects present on the latter and that slow electron transfer kinetics can be attributed by the lack of specific chemical sites on the basal plane or the low density of electronic states exhibited by low-defect HOPG. An important conclusion from this work was that catalysis of reactions via a mediation mechanism involving reactive surface groups or via a pathway involving the electronic properties of the material in terms of a low density of states are conceptually distinct. Highly ordered surfaces would have a low defect density and hence few reactive surface groups and so one would expect that the catalysis of solution phase redox reactions could be attributed largely to electronic density of states effects similar to that seen in semiconductor electrochemistry as noted by Gerisher[7]. It can be very difficult to separate the two mechanisms responsible for rate enhancement at carbon electrode surfaces.

Dekker and co-workers[4,5] have addressed the effect that the electronic density of states has on the kinetics of interfacial electron transfer at SWCNT using ideas developed in the Marcus-Gerisher model for electron transfer reactions at semiconductor electrode/electrolyte solution interfaces[8]. SWCNT may be p-doped when placed in

contact with an electrolyte solution and so the Fermi level may be located below the band gap region. The density of electron stares for a solution phase reactant exhibits a Gaussian profile which can be broad. Application of an oxidizing potential sweep during voltammetry causes the Fermi level to be lowered more in energy which causes an increase in the number of unoccupied electronic states available to accept electrons from the reductant in solution. Hence the degree of energy overlap between solution phase reductant and acceptor states in the nanotube will be significant thereby promoting electron transfer. It should also be notes that a significant energy overlap will also exist for states located away from the Fermi level due to the inherent width of the reductant Gaussian energy profile (typically 1 eV).

Compton and co-workers[3] have recently concluded from a plethora of careful and insightful studies that much of the catalytic activity, electron transfer and chemical reactivity of graphitic carbon electrodes is at surface defect sites and in particular edge-plane like defect sites. They particularly noted that the observed catalytic activity of MWCNT materials could be ascribed to the existence of edge plane defect sites, probably oxygen containing functional groups such as quinones[9]. It is quite probable that the sidewalls of SWCNT's would also contain considerable quantities of oxygen containing functional groups with a similar reactivity, especially since the tubes are subject to acid oxidation and sonication. If such a scenario is accepted then molecular oxygen will be reduced readily at the sidewall of the nanotube most probably via a surface redox catalysis mechanism of the type described by Schiffrin and co-workers[10] for oxygen reduction at glassy carbon electrodes as outlined below.

$$Q(ads) + 2e^- + 2H^+ \rightleftarrows H_2Q(ads)$$
$$H_2Q(ads) + O_2(aq) \rightarrow Q(ads) + H_2O_2(aq)$$

The issue of determining which of the routes, redox catalysis via reactive surface groups, or nanotube electronic state density, determine the observed rate enhancement at SWCNT modified electrode is yet to be resolved.

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