Enzyme electrodes for glucose oxidation are of increasing interest due to their potential applications as biosensors and as anodes in membrane-less cells operating on sugar as a fuel. Such enzymatic fuel cells (EFC) can use enzymes as specific catalysts to oxidize glucose at the anode and reduce oxygen at the cathode, that when combined as a fuel cell convert chemical energy into electrical power.1–3 The advantages to the use of enzyme-based catalysts are substrate specificity, which can eliminate the need for casings and ion exchange membranes in assembled fuel cells, and of being capable of operating under moderate ambient conditions, compared to metal catalysts.1,2

A substantial body of research exists on approaches for maximising catalytic current capture as a result of enzyme redox reactions in enzyme electrodes through the co-immobilization of enzymes and electron-shuttling mediators within redox-conducting hydrogels on solid electrodes. For example, over the past two decades, Heller and co-workers have pioneered use of epoxy cross-linking of electrostatic adducts of redox enzyme and osmium redox polymers, using polyvinylimidazole (PVI) as the polymer backbone, at electrode surfaces to provide “wired” enzyme electrodes capable of producing glucose oxidation current. Mediated electron transfer by electron-hopping self-exchange within these hydrogels allows connection between redox active sites of enzymes and electrode surfaces thus generating bioelectrocatalytic current.4–6 As mediators, osmium based polypyridyl complexes to carboxymethyl dextran (CMD) or polyacrylic acid polymers previously used.4,5 Although immobilization of osmium complexes and enzymes to a polyallylamine support using a di-epoxide reagent.6 Here, we report on the preparation and comparison of enzyme electrodes based on coupling of osmium complexes that contain an aldehyde functional group distal to the metal co-ordinating site, and glucose oxidase (GOx), to carboxymethyl dextran (CMD) polymers previously anchored to amine-functionalised electrode surfaces. More recently,19 Conghaile et al. reported on immobilization of amine-functionalised osmium complexes and enzymes to a polyallylamine support using a di-epoxide reagent.

Recent approaches to glucose enzyme electrode preparation have focused on improving the current signal and stability by co-immobilization and crosslinking of a range mediators within polymer matrices at electrode surfaces.12–14 Addition of multi-walled carbon nanotubes (MWCNT) to the enzyme electrode preparation step results in improved operational output and stability under pseudo-physiological conditions.15–17 These nanostructures provide a support that acts as a scaffold for improved retention of enzymes and redox complexes.10,13,14 A difficulty with use of PVI as the polymer backbone for preparation of redox polymers is the lack of commercial availability of PVI, and laboratory-scale synthesis of PVI by bulk free radical polymerisation15 results in wide molecular weight distribution which affects the physical properties of polymers such as solubility, density etc. In addition, osmium complex loading on the PVI backbone by ligand substitution is also difficult to control and replicate, leading to batch-to-batch variation in enzyme electrode performance using these redox polymers.2,6,17 We, and others, have sought to use commercially available and well characterized polymer supports for preparation of enzyme electrodes. Danilowicz et al.1 reported on coupling of osmium complexes that contain an aldehyde functional group distal to the metal co-ordinating site to amine-based polymers and enzymes in films on electrode to provide enzyme electrodes. We have reported20 on coupling of osmium complexes that contain an amine functional group distal to the metal co-ordinating site, and glucose oxidase (GOx), to carboxymethyl dextran (CMD) polymers previously anchored to amine-functionalised electrode surfaces. More recently, O’Conghaile et al. reported on immobilization of amine-functionalised osmium complexes and enzymes to a polyallylamine support using a di-epoxide reagent.

Here, we report on the preparation and comparison of enzyme electrodes based on coupling of GOx and amine-functionalised osmium complexes to carboxymethyl dextran (CMD) and polyacrylic acid polymer supports. Acid treated MWCNTs are added to the drop-coating solutions to attempt to provide increased surface area and higher current response.15–17 Furthermore, variation in osmium complex redox potential is investigated by synthesis of complexes with more electron donating ligands19,20 and the response of enzyme electrodes prepared using such osmium complexes immobilized with GOx, MWCNT and CMD as a support evaluated.

**Experimental**

Materials.— All chemicals were purchased from Sigma-Aldrich (Dublin, Ireland) and used as received unless otherwise stated. All solutions were prepared from Milli-Q (18.2 MΩ cm) water. The complexes, [Os(N-N)2(4-aminoethylpyridine)Cl]PF6 and [Os(N-N)2(4-aminoethylpyridine)Cl]PF6, where N-N is either 2,2′-bipyridine or 4,4′-dimethoxy-2,2′-bipyridine, were synthesized by ligand substitution.

**Coupling of Amine-Containing Osmium Complexes and Glucose Oxidase with Carboxylic Acid Polymer and Carbon Nanotube Matrix to Provide Enzyme Electrodes for Glucose Oxidation**

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tion of one Cl ligand of [Os(N-N)2Cl2] with 4-aminomethylpyridine by heating of the complex in an ethylene glycol solution containing 1.1 mole equivalents of ligand at reflux, with ligand substitution monitored by cyclic voltammetry and differential pulse voltammetry, as reported on previously.9,20 The [Os(N-N)2Cl2] complexes were prepared from (NH4)2OsCl6 according to literature pulse voltammetry, as reported on previously.9,20

The [Os(N-N)2Cl2] or 4-aminoethylpyridine by heating of the complex in an ethylene glycol solution (average molecular mass of 15,000 Da) were purchased from Sigma-Aldrich. The MWCNTs (Sigma) were acid-treated by heating 20 microl L of 4.5 mM aqueous solution) to the eppendorf. Enzyme electrodes were prepared by drop coating 20 microl L of polymer (5 mg ml−1), CMD (average molecular mass of 15,000 Da) and polyacrylic acid (average molecular mass of 450,000 Da) were purchased from Sigma-Aldrich. The MWCNTs (Sigma) were acid-treated by heating 20 mg mL−1 in HNO3 at reflux for 6 hours at ∼150 °C.17

Methods.— CH Instruments 600 series or 1032 multichannel potentiostat (JJ Cambria) coupled to a thermostatted electrochemical cell was used to perform all electrochemical measurements. Custom built Ag/AgCl reference electrodes (3 M KCl) and platinum foil counter electrodes (Goodfellow) were used in the cell. Graphite disc electrodes (3 mm diameter) were prepared by shrouding graphite rods (Graphite store, part # NC01295) in heat-shrinkable tubing and polishing the exposed disk on 1200 grit silicon carbide paper (Buehler) followed by thorough rinsing with Milli-Q water. Working electrodes were sonicated in Milli-Q water for 10 min and dried under nitrogen gas prior to use. All electrochemical measurements were performed in phosphate buffer saline (PBS, 0.05 M phosphate, pH 7.4, 0.15 M NaCl) at 37 °C. GOx activity was determined using the o-dianisidine and horseradish peroxidase-coupled spectrophotometric assay. The reaction was monitored on an Agilent 6453 UV/Vis spectrophotometer at 460 nm.22

The enzyme electrode preparation protocol is illustrated in Scheme 1. Prior to enzyme electrode preparation, carboxylic acid functional groups of the polymer and the acid treated MWCNT were activated for coupling to amine functional groups by incubation of 5 μL of polymer (5 mg ml−1) and 5 μL aqueous suspension (0.23 mg) of acid treated MWCNT with 4 μL of an aqueous solution of 40 mM N-[3-dimethylaminopropyl]-N′-ethylcarbodiimide (EDC) and 10 mM N-hydroxsuccinimide (NHS) in an eppendorf for 12 minutes. This was followed by addition of GOx (5 μL of 10 mg ml−1) and redox complex (5 μL of 4.5 mM aqueous solution) to the eppendorf. Enzyme electrodes were prepared by drop coating 20 μL of the resulting solution onto the graphite disk, with the dropcoat dried for 18 hours at room temperature to allow crosslinking, followed by rinsing of the enzyme electrode in PBS for ∼5 seconds before testing commenced.

Results and Discussion

Cyclic voltammetry (CV) is used to characterize the redox complexes within polymer supported and cross-linked films on graphite electrodes. For example, the redox complex [Os(2,2′-bipyridine)(4-aminomethylpyridine)Cl]PF6 (Os(bpy)24AMP) co-immobilized with GOx, MWCNT and CMD or polyacrylic acid as a polymer support on an electrode surface exhibits oxidation and reduction peaks corresponding to the osmium(II/III) transition at 0.30 V (versus Ag/AgCl), which is similar to the redox potential observed for Os(bpy)24AMP in phosphate buffer.23 This redox potential is also close to that reported on for the complex immobilized by coupling either directly to a carbon electrode using electrochemical oxidation of the alkylamine functional group,24 or by reaction of the alkylamine with carboxylic acid groups introduced to carbon25 or boron-doped diamond26 electrodes, and for the complex immobilized within CMD films coupled to a carbon electrode.18 The similarity of redox potential between solution-phase and immobilized complex indicates that the Os(II/III) redox transition is generally not affected by the immobilization procedure.

All enzyme electrodes display CV response in PBS, in the absence of glucose substrate, where the Os(II/III) peak currents vary linearly with scan rate, at slow scan rates (<20 mVs−1), indicative of a surface-controlled response.27 At higher scan rates the peak currents scale linearly with the square root of scan rate, indicative of semi-infinite diffusion control of the response.8,28,29 An estimation of redox complex surface coverage (θsurf) on the electrode can be obtained by integration of the charge passed during electrolysis of the complexes within films on the electrode using slow scan rate voltammetry in PBS solution.20 For example, enzyme electrodes prepared using CMD, MWCNT and GOx result in redox complex surface coverage of 90 ± 14 nmoles cm−2, comparable to the value obtained previously for enzyme electrodes prepared by co-immobilization of osmium redox polymers and enzymes,21,30 and approximately one thousand-fold that expected for monolayer coverage of osmium pyridyl complexes31 on electrode surfaces. Upon addition of glucose, sigmoidal-shaped cyclic voltammograms, characteristic of catalytic oxidation of glucose by the enzyme, with electron transfer mediated by the redox complex from the enzyme active site to the electrode are obtained for all enzyme electrodes,29 as shown for example in Figure 1 for the enzyme electrode prepared using Os(bpy)24AMP, MWCNT, GOx and either CMD or polyacrylic acid as support.

Enzyme electrodes are prepared using Os(bpy)24AMP, GOx and either CMD or polyacrylic acid as polymer support and the area of the enzyme active site to the electrode are obtained for all enzyme electrodes,29 as shown for example in Figure 1. The enzyme electrode prepared using Os(bpy)24AMP, MWCNT and either CMD or polyacrylic acid as support compares well with the amperometric 1.0 ± 0.2 mA cm−2 current density obtained at 0.45 V applied potential, Procedure 1A and Figure 2. In the initial comparison of the effect of polymer support on enzyme electrode response, increased glucose oxidation current density is obtained from CVs in 5 mM glucose in PBS for enzyme electrodes prepared using CMD as support, 0.72 ± 0.09 mA cm−2, over that obtained using polyacrylic acid as support, 0.23 ± 0.04 mA cm−2.

A comparison of glucose oxidation current density, extracted from amperometric measurements at 0.45 V vs. Ag/AgCl, as function of glucose concentration for CMD-based and polyacrylic acid-based enzyme electrodes is shown in Figure 2. Substrate saturation is observed,
Figure 1. CVs recorded at 1 mV s\(^{-1}\) in the presence (red, solid) and absence (black, dashed) of 5 mM glucose in PBS (pH 7.4, 37 °C) for enzyme electrodes prepared from Os(bpy)_24AMP, MWCNT, GOx and either (A) CMD or (B) polyacrylic acid. CVs recorded under the same conditions, in the absence of glucose, for electrodes prepared from MWCNT only (green, dotted) are shown for comparison.

The enzyme electrodes prepared using the Os(bpy)_24AMP redox complex require relatively high potentials to oxidize glucose and therefore may not the most suitable electrodes for application to glucose biosensors or enzymatic biofuel cell anodes.\(^1\) Synthesis of osmium redox complexes having lower redox potential can be achieved by alteration in the co-ordinating ligands of the complex.\(^{19,33}\) For example, replacement of the 4,4′-H on both bipyridines, by a more electron-donating methoxy-functional group can shift the redox potential of the osmium oxidation by ∼−0.25 V.\(^{10,21,36}\) Thus synthesis of [Os(4′,4′-dimethoxy-2,2′-bipyridine)\(_2\)(4-aminoethylpyridine)\(_2\)]PF\(_6\) (Os(dmobpy)_24AEP) and [Os(4,4′-dimethoxy-2,2′-bipyridine)\(_2\)(4-aminoethylpyridine)\(_2\)]PF\(_6\) (Os(dmobpy)_24AEP) redox complexes is targeted to provide enzyme electrodes for mediated oxidation of glucose at lower potentials. For enzyme electrodes prepared by co-immobilization of these redox complexes with GOx, MWCNT and CMD polymer as support on electrode surfaces, oxidation and reduction peaks for the Os(II/III) transition at 0.05 V vs. Ag/AgCl are obtained, Figure 3A, similar to the redox potential previously observed for an Os(dmobpy)_24AEP complex in solution.\(^{19,37}\) As before, slow scan CVs (<20 mV s\(^{-1}\)) in the absence of glucose display peak currents that vary linearly with scan rate, permitting estimation of \(\Gamma_{\text{Ox}}\) of 67 ± 6 nmoles cm\(^{-2}\) and 57 ± 18 nmoles cm\(^{-2}\) for the enzyme electrodes containing GOx, MWCNT, CMD and Os(dmobpy)_24AEP or Os(dmobpy)_24AEP complexes, respectively. These values are comparable to those seen for the Os(bpy)_24AMP complex, and for those reported previously for osmium redox polymers and enzyme based electrodes.\(^{17,38}\)

Upon addition of 5 mM glucose, sigmoidal shaped cyclic voltammograms are obtained, Figure 3B, characteristic of electrocatalytic

1. The average \(K_M\) and \(I_{\text{max}}\), Table I, for all enzyme electrodes is 9.3 ± 1.7 mM which compares well with the reported \(K_M\) value of 10 ± 5 mM for other GOx-based enzyme electrodes.\(^{18,33}\) The CMD and polyacrylic acid-based enzyme electrodes display maximum current densities, \(I_{\text{max}}\), of 4.5 ± 0.9 mA cm\(^{-2}\) and 0.34 ± 0.1 mA cm\(^{-2}\), respectively, Table I, confirming the improved performance of enzyme electrodes using CMD over those prepared with polyacrylic acid. The inclusion of MWCNTs was undertaken based on results from previous studies using polyvinylimidazole-based osmium redox polymers that demonstrated an increase in glucose oxidation current density upon MWCNT addition to enzyme electrodes.\(^{17,34}\) The current densities of all enzyme electrodes increase after inclusion of MWCNT in the preparation steps, Table I, even when no polymer support matrix is included. Enzyme electrodes without CMD, but with added MWCNT produce a \(I_{\text{max}}\) of 0.85 ± 0.08 mA cm\(^{-2}\) at 0.45 V vs Ag/AgCl, higher than the current density for enzyme electrodes with CMD only as support (no MWCNT), and for any of the polyacrylic acid-based enzyme electrodes, with or without MWCNT. The main contributing factor for higher current at enzyme electrodes based on MWCNT and CMD is the higher retained enzymatic activity, as presented in the data in Table I. For example, enzyme electrodes based on GOx co-immobilized with CMD with 12 U of enzyme activity deposited in the electrode preparation step show 9.0 ± 0.1 U retained activity, using the peroxidase-coupled activity assay, compared to 2 ± 1 units for GOx co-immobilized with polyacrylic acid, perhaps because of lower reactivity of the polyacrylic acid carboxylate to EDC/NHS activation or decreased access of the GOx for immobilization to the activated carboxylate within the polyacrylic acid film. This results in the ten-fold increase in glucose oxidation current density for the CMD-based enzyme electrodes over the polyacrylic acid-based electrodes.

2. Comparison of this performance with other glucose-oxidizing enzyme electrodes is rendered difficult due to different methodologies used for film preparation, and different test conditions. As examples, Daniłowicz et al.\(^7\) report glucose oxidation current density of 60 μA cm\(^{-2}\) extracted from 5 mV s\(^{-1}\) CV for enzyme electrodes using an osmium complex attached to a poly(allylamine) support, co-immobilized with GOx in the presence of 50 mM glucose compared to a current density of 4.3 ± 0.9 mA cm\(^{-2}\) from amperometry in 50 mM glucose for the CMD-based enzyme electrodes co-immobilized with Os(bpy)_24AMP, MWCNT and GOx here. The response of these electrodes of 4.5 ± 1.0 mA cm\(^{-2}\) in 100 mM glucose and 1.0 ± 0.2 mA cm\(^{-2}\) in 5 mM glucose also compares well with reported glucose oxidation current density of 0.29 mA cm\(^{-2}\) in 100 mM glucose or 0.12 mA cm\(^{-2}\) in 5 mM glucose for enzyme electrodes of Os(bpy)_24AMP co-immobilized by crosslinking with GOx and a poly(allylamine) support.\(^{39}\) From the results obtained, co-immobilization of redox complex and GOx with the CMD polymer and MWCNT is selected for subsequent studies on the basis of higher glucose oxidation current density compared to other polymer supported enzyme electrodes.

The enzyme electrodes prepared using the Os(bpy)_24AMP redox complex require relatively high potentials to oxidize glucose and therefore may not the most suitable electrodes for application to glucose biosensors or enzymatic biofuel cell anodes.\(^1\) Synthesis of osmium redox complexes having lower redox potential can be achieved by alteration in the co-ordinating ligands of the complex.\(^{19,33}\) For example, replacement of the 4,4′-H on both bipyridines, by a more electron-donating methoxy-functional group can shift the redox potential of the osmium oxidation by ∼−0.25 V.\(^{10,21,36}\) Thus synthesis of [Os(4,4′-dimethoxy-2,2′-bipyridine)\(_2\)(4-aminoethylpyridine)\(_2\)]PF\(_6\) (Os(dmobpy)_24AEP) and [Os(4,4′-dimethoxy-2,2′-bipyridine)\(_2\)(4-aminoethylpyridine)\(_2\)]PF\(_6\) (Os(dmobpy)_24AEP) redox complexes is targeted to provide enzyme electrodes for mediated oxidation of glucose at lower potentials. For enzyme electrodes prepared by co-immobilization of these redox complexes with GOx, MWCNT and CMD polymer as support on electrode surfaces, oxidation and reduction peaks for the Os(II/III) transition at 0.05 V vs. Ag/AgCl are obtained, Figure 3A, similar to the redox potential previously observed for an Os(dmobpy)_24AEP complex in solution.\(^{19,37}\) As before, slow scan CVs (<20 mV s\(^{-1}\)) in the absence of glucose display peak currents that vary linearly with scan rate, permitting estimation of \(\Gamma_{\text{Ox}}\) of 67 ± 6 nmoles cm\(^{-2}\) and 57 ± 18 nmoles cm\(^{-2}\) for the enzyme electrodes containing GOx, MWCNT, CMD and Os(dmobpy)_24AEP or Os(dmobpy)_24AEP complexes, respectively. These values are comparable to those seen for the Os(bpy)_24AMP complex, and for those reported previously for osmium redox polymers and enzyme based electrodes.\(^{17,38}\)

Upon addition of 5 mM glucose, sigmoidal shaped cyclic voltammograms are obtained, Figure 3B, characteristic of electrocatalytic
oxidation of glucose.\textsuperscript{29} The comparison of glucose oxidation currents for CMD based enzyme electrodes as a function of glucose concentration was extracted from amperometric measurements at 0.2 V vs. Ag/AgCl, again 150 mV more positive of the Os(II/III) oxidation to ensure mediated glucose oxidation. The characteristic apparent Michaelis-Menten constant, \( K_{M, app} \), and the maximum current, \( I_{max} \), can be estimated from non-linear least-squares curve fitting of these plots to the Michaelis-Menten equation.\textsuperscript{32} The average \( K_{M, app} \), for enzyme electrodes is 14 ± 2 mM which compares well with the value determined for enzyme electrodes based on the Os(bpy)\textsubscript{2}4AMP complex above, and with the reported \( K_{G} \) value of 10 ± 5 mM for other GOx-based enzyme electrodes.\textsuperscript{19,33} From calibration curve which follow the trend expected for steady-state approximation, the maximum current density assuming Michaelis-Menten equation are on the order of 3.4 mA cm\textsuperscript{-2} for Os(dmobpy)\textsubscript{2}4AMP enzyme electrodes can be estimated compared to higher \( I_{max} \) of 4.5 mA cm\textsuperscript{-2} for Os(bpy)\textsubscript{2}4AMP enzyme electrodes. The enzyme electrodes prepared with Os(dmobpy)\textsubscript{2}4AMP produce lower maximum glucose oxidation current density, \( I_{max} \), of 3.4 mA cm\textsuperscript{-2} compared to 4.5 mA cm\textsuperscript{-2} for Os(bpy)\textsubscript{2}4AMP enzyme electrodes. This lower glucose current density may be as reported for a tethered redox polymer, (poly-vinylimidazole\textsubscript{2}4[Os(4,4′-dimethyl-2,2′-bipyridine)\textsubscript{2}(poly-vinylimidazole)\textsubscript{2}Cl])\textsuperscript{+} redox polymer crosslinked with GOx and MWCNT operating in 5 mM glucose.\textsuperscript{10} A current density of 0.21 mA cm\textsuperscript{-2} is reported for enzyme electrodes based on a redox polymer [P20-Os(4,4′-dimethyl-2,2′-bipyridine)\textsubscript{2}(4-aminomethyl pyridine)Cl]PF\textsubscript{6} co-immobilized with an FAD-dependent GDH operating in 5 mM glucose at applied potential of 0.2 V vs. Ag/AgCl.\textsuperscript{38} Carbon fiber enzyme electrodes prepared by co-immobilization of an [Os(4,4′-dimethyl-2,2′-bipyridine)\textsubscript{2}(poly-vinylimidazole)Cl]\textsuperscript{+} redox polymer with GOx achieve a current density of only 0.6 mA cm\textsuperscript{-2} at 0 V vs. Ag/AgCl in 15 mM glucose.\textsuperscript{40} A higher current density of 1.15 mA cm\textsuperscript{-2} is reported for a tethered redox polymer, (poly-vinylpyridine[Os(N,N′-diallylated-2,2′-bi-imidazole)\textsubscript{12/13}] modified carbon fiber electrode at a potential of ~0.1 V vs. Ag/AgCl in 15 mM glucose.\textsuperscript{41} More recently, tethered redox polymer-based enzyme electrodes co-immobilized with pyrroloquinoline quinone-dependent glucose dehydrogenase (PQQGDH) produced a current density of 1.3 mA cm\textsuperscript{-2} at a scan rate of 5 mV s\textsuperscript{-1} in 20 mM glucose solution stirred at 1000 rpm.\textsuperscript{42} There remains however some difficulties with the use of polyvinylimidazole (PVI) based redox polymers, as these systems rely upon synthesis of PVI by bulk free radical polymerisation\textsuperscript{12} that results in a wide molecular weight distribution that then affects the physical properties of polymers such as solubility, density etc. In addition, control of osmium complex loading, by ligand substitution, on PVI-based polymers is difficult and leads to batch-to-batch variations in redox polymer and hence enzyme electrode performance.\textsuperscript{2} We, here and previously,\textsuperscript{14,27} adopt the approach of using commercially available and water soluble polymer supports with suitable functional groups to permit coupling of osmium redox complexes instead of by ligand substitution reactions. For example, enzyme electrodes prepared by coupling of Os(dmobpy)\textsubscript{2}4AMP and GOx to CMD brushes, anchored to diazonium salt-derivated surfaces, yielded a current

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Current density in the presence of increasing concentrations of glucose recorded at an applied potential of 0.45 V, for films prepared using Os(bpy)\textsubscript{2}4AMP co-immobilized with GOx, MWCNT and either CMD or polyacrylic acid polymer support in pH 7.4 PBS at 37 °C, solution stirred at 150 rpm. Error bars represent standard deviation (n = 5).

| Film Component with GOx | \( j_{max, app} \) (mA cm\textsuperscript{-2}) | \( K_{M, app} \) (mM) | Surface Coverage (\( \Gamma_{O} \), nmole cm\textsuperscript{-2}) | Enzymatic Activity (U)\textsuperscript{*} |
|------------------------|---------------------------------|-----------------|-------------------------|-----------------------------|
| CMD, Os(bpy)\textsubscript{2}4AMP | 0.53 ± 0.03 | 10 ± 1 | 47 ± 8 | 2.0 ± 1.0 |
| CMD, Os(bpy)\textsubscript{2}4AMP and MWCNT | 4.5 ± 0.9 | 11 ± 1 | 90 ± 14 | 9.0 ± 0.1 |
| Polyacrylic acid, Os(bpy)\textsubscript{2}4AMP | 0.11 ± 0.02 | 7 ± 1 | 63 ± 2 | 1.0 ± 0.1 |
| Polyacrylic acid, Os(bpy)\textsubscript{2}4AMP and MWCNT | 0.34 ± 0.10 | 9 ± 1 | 60 ± 1 | 2.0 ± 1.0 |
| MWCNT, Os(bpy)\textsubscript{2}4AMP | 0.85 ± 0.08 | 38 ± 17 | | |
| CMD, Os(dmobpy)\textsubscript{2}4AMP and MWCNT | 3.4 ± 0.5 | 14 ± 2 | 67 ± 6 | 5.2 ± 2.1 |

\*Maximum enzyme activity, if all added enzyme activity is retained, is 12 U.
density of 0.22 mA cm\(^{-2}\) in 10 mM glucose, lower than reported here, as the polymer films were of the dimension of the polymer support only.\(^{18}\) Enzyme electrodes based on co-immobilization of PQQGDH and MWCNT with Os(dmobpy)\(_2\)4AMP, but without incorporation of a polymer support, yielded glucose oxidation current density of 0.30 mA cm\(^{-2}\) in 5 mM glucose solutions.\(^{23}\) The substantial glucose oxidation current density of 0.83 ± 0.21 mA cm\(^{-2}\) at 0.2 V applied potential achieved with Os(dmobpy)\(_2\)4AMP coupled within films of the water soluble CMD polymer matrix via EDC/NHS, is perhaps due to improved enzyme loading, and retention of enzyme activity within these CMD hydrogels. In any event, the performance of these enzyme electrodes shows promise for their application as biosensors or as anodes in enzymatic biofuel cells for power generation.

Operational stability of glucose oxidation current generation for selected enzyme electrodes was evaluated from constant potential amperometry at 0.2 V vs. Ag/AgCl for 24 hr in saturated glucose (100 mM) solution while gently stirring to avoid localised substrate depletion. Approximately 68% of initial current density response remains after the 24 hr period for enzyme electrodes prepared from Os(dmobpy)\(_2\)4AMP, GOx, MWCNT and CMD compared to ~45% remaining for the Os(bpy)\(_2\)4AMP based enzyme electrodes. Interestingly, redox site surface coverage is retained to the same extent for Os(bpy)\(_2\)4AMP and Os(dmobpy)\(_2\)4AMP based enzyme electrodes, with 43% and 36% coverage retained, respectively, after 24 hrs. The improved operational stability of Os(dmobpy)\(_2\)4AMP based enzyme electrodes may be as a result of improved retention of glucose oxidase activity over time, as these electrodes retained 60% of initial enzyme activity compared to only 44% for the Os(bpy)\(_2\)4AMP based enzyme electrodes after 24 hrs. Interestingly, higher currents (rate constants) were previously reported for glucose oxidation at enzyme electrodes based on co-adsorption of redox complexes and GOX on graphite when redox complexes containing dmobpy ligands were used, supporting the results obtained here.\(^{33}\)

**Conclusions**

The use of CMD and polyacrylic acid has been investigated as support for co-immobilization of components to provide...
glucose-oxidizing enzyme electrodes. Overall, CMD based enzyme electrodes exhibit higher glucose oxidation current densities, of 1.0 ± 0.2 mA cm⁻² in 5 mM glucose and of 4.5 ± 1.0 mA cm⁻² in saturated glucose solutions in PBS (pH 7.4, 37°C), over electrodes prepared using polyacrylic acid. This substantial current density is, however, achieved at the relatively high applied potential of +0.45 V vs Ag/AgCl. Variation in osmium complex structure to provide a re-doxx complex of lower formal potential results in CMD-based enzyme electrodes producing current densities of 0.83 ± 0.21 mA cm⁻² in 5 mM glucose and of 3.4 ± 0.7 mA cm⁻² in saturated glucose solutions in PBS at an applied potential of +0.2 V vs Ag/AgCl showing promise for application as glucose oxidizing biosensors, and as anodes for in-vivo enzymatic fuel cells. Future work is focused on variation in enzyme selection in the proposed matrix of CMD, Os(dmnbpy)₂AAMP, and MWCNT to further improve the current output signal of such enzyme electrodes.

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