Title	Effect of individual plasma components on the performance of a glucose enzyme electrode based on redox polymer mediation of a flavin adenine dinucleotide-dependent glucose dehydrogenase
Author(s)	Bennett, Richard; Blochouse, Estelle; Leech, Dónal
Publication Date	2019-02-11
Publication Information	Bennett, Richard, Blochouse, Estelle, & Leech, Dónal. (2019). Effect of individual plasma components on the performance of a glucose enzyme electrode based on redox polymer mediation of a flavin adenine dinucleotide-dependent glucose dehydrogenase. Electrochimica Acta, 302, 270-276. doi: https://doi.org/10.1016/j.electacta.2019.02.039
Publisher	Elsevier
Link to publisher's version	https://doi.org/10.1016/j.electacta.2019.02.039
Item record	http://hdl.handle.net/10379/15247
DOI	http://dx.doi.org/10.1016/j.electacta.2019.02.039

Effect of individual plasma components on the performance of a glucose enzyme electrode based on redox polymer mediation of a flavin adenine dinucleotide-dependent glucose dehydrogenase

*Richard Bennett, Estelle Blochouse, Dónal Leech**

School of Chemistry & Ryan Institute, National University of Ireland Galway, University Road, Galway, Ireland

Abstract

The performance of glucose enzyme electrodes, consisting of crosslinked flavin adenine dinucleotide glucose dehydrogenase (FADGDH), an osmium redox polymer and multi-walled carbon nanotubes on graphite electrodes, was tested in phosphate buffered saline, artificial plasma and the individual components of artificial plasma to assess the effect of each component on current response and operational stability of the response to better understand the decrease in electrode performance observed in blood. Electrodes tested in artificial plasma show a significant decrease in current response in 5 mM glucose, and operational stability of the response in 100 mM glucose, compared to electrodes tested in buffer. The lowest current response for the enzyme electrodes was observed in the presence of physiological level of uric acid although the largest alteration to enzyme affinity, as estimated from the apparent Michaelis-Menten constant, occurred upon addition of physiological level of sodium bicarbonate. The operational stability observed in the presence of uric acid was the lowest of all components tested, with only 46 % of initial current response after 12 hours, and was comparable to the 27% of current remaining after 12 hours for electrodes operating in artificial plasma. The effect of uric acid on glucose oxidation by enzyme electrodes prepared using both glucose oxidase (GOx) and a recombinant cellobiose dehydrogenase (CDH) was assessed. The maximum current decreased for both FADGDH and GOx enzyme electrodes in the presence of uric acid, with no significant change to the enzyme affinity, suggesting non-competitive inhibition. The CDH based electrodes provided highest stability of current signal in buffer, with 86 % of the initial signal present after 12 hours, but display significant change enzyme affinity, maximum current and operational stability, dropping to only 33%, in the presence of uric acid. In contrast the operational stability of the GOx-based enzyme electrodes was unaffected by the presence of physiological level of uric acid. As uric acid and sodium bicarbonate are present in blood, these results highlight the importance of enzyme selection for *in vivo* biosensing and biofuel cell applications. Further work is required to understand the mechanism of uric acid inhibition on each of the enzymes.

Keywords

Biosensor; Osmium polymer; Glucose oxidising enzyme; Plasma; Uric acid

1 Introduction

A fuel cell couples the electrocatalytic oxidation of a fuel at an electrode with the reduction of an oxidant at a separate electrode to generate power. These reactions occur readily at electrodes using traditional metal based catalysts. However, numerous disadvantages are associated with implementation of non-specific transition metals as catalysts: the electrode can be easily poisoned, lacks specificity, operates most efficiently under harsh conditions (temperature and pH) and requires a membrane to separate the electrode compartments.[1–4] The use of enzymes as catalysts immobilised at electrode surfaces offers solutions to these drawbacks. Enzyme electrodes are specific, operate under physiological conditions and do not require a membrane to operate, allowing for miniaturisation of enzymatic fuel cells (EFCs). EFCs operate by using enzymes to oxidise a fuel at the anode such as glucose while reducing an oxidant, such as molecular oxygen, at the cathode.[5–10] As the fuel and oxidant are readily available in the human body, powering of implantable or semi-implantable devices by miniaturised EFCs using immobilised enzyme biofilms is one potential application.[1,11–15]

Efficient electron transfer from the enzyme active site to the electrode surface is required in order for sufficient current generation by the electrode. Direct electron transfer (DET) and mediated electron transfer (MET) are the two routes of electron transfer between enzyme and electrode. If the active site of the enzyme is greater than 2 nm from the electrode surface DET becomes difficult to achieve. [16–18] For this reason, redox centres are immobilised in the biofilm as mediators to shuttle electrons from the active site to the surface. Osmium based redox polymers co-immobilised at the electrode surface are widely used mediators as they are relatively stable in the Os(II)/Os(III) states, have tunable redox potentials and the polymer films allow fast charge and mass transport resulting in high current generation.[19–22]

Inclusion of a multifunctional crosslinker such as poly(ethylene glycol) diglycidyl ether (PEGDGE) and nano-supports such as multi-walled carbon nanotubes (MWCNTs) in the biofilm improves the stability and magnitude of current signal generated from the glucose oxidising electrodes.[21,23] The MWCNTs provide a larger surface area for the electrode, leading to higher production of current density.[24,25] Electrodes consisting of co-

immobilised osmium-based redox centres with nano-supports and enzyme to form enzymatic fuel cells have been widely reported.[26–30]

We recently reported on a design of experiments-based optimisation process for maximising current generation from glucose oxidising electrodes prepared using a flavin adenine dinucleotide dependent glucose dehydrogenase (FADGDH), osmium based redox polymer, MWCNTs and PEGDGE. Current densities of 1.2 ± 0.1 $mAcm^{-2}$ in 5 mM glucose in the absence of oxygen and 0.8 ± 0.2 $mAcm^{-2}$ in the presence of oxygen were achieved. For fuel cell testing, oxygen reducing cathodes consisting of *Myrothecium verrucaria* bilirubin oxidase, MWCNTs and osmium redox polymer were coupled to the glucose oxidising electrodes yielding power densities of 285 μWcm^{-2} in phosphate buffered saline (PBS), and 146 μWcm^{-2} in artificial plasma.[31] The poor long term stability, coupled to the low power output, from EFC remain major roadblocks towards continuous use implantable and semi-implantable EFC devices.[32–35] One of the major reasons for low current output and lack of stability is the impact that interfering molecules have on performance of the electrodes.[36–42]

Here we report on testing of glucose oxidising enzyme electrodes in PBS, artificial plasma and stability of the glucose oxidation current over a 12-hour window in the presence of each interfering molecule present in artificial plasma is measured to assess the impact on enzyme electrode performance. In addition FADGDH was replaced as glucose oxidising enzyme by glucose oxidase (GOx) or cellobiose dehydrogenase (CDH) in the presence of an interfering molecule to gain greater understanding of the importance of enzyme catalyst on the deterioration of current response and stability of the biofilms.

2 Experimental

2.1 Materials

The redox polymer $[Os(2,2'\text{-bipyridine})_2(\text{poly-vinylimidazole})_{10}Cl]^+$ (Os(bpy)PVI), was synthesised by modification of procedures found in the literature. [43,44] All chemicals were purchased from Sigma-Aldrich, unless otherwise stated. The flavin dependent glucose dehydrogenase is from *Aspergillus sp.* (FADGDH 1.1.99.10, Sekisui, Cambridge, USA; product GLDE-70-1192). The glucose oxidase is from *Aspergillus niger (*GOx, EC 1.1.3.4., Sigma-Aldrich). The flavodehydrogenase domain of *Corynascus thermophilus* CDH

(r*Ct*CDH) was heterologously expressed in the methylotrophic yeast *Pichia pastoris* and purified as previously described. [45] The MWCNTs (Sigma-Aldrich) were pretreated in concentrated nitric acid under reflux for 6 hours followed by washing and filtration. The PEGDGE was purchased from Sigma-Aldrich (average Mn~526). Milli-Q water (18 MΩcm) was used to prepare all aqueous solutions unless otherwise stated.

2.2 Methods

Graphite electrodes were prepared to give a geometric working surface area of 0.0707 cm^2 by insulating graphite rods (Graphite store, USA, 3.0 mm diameter, NC001295) with heat shrink tubing and polishing the exposed surface on fine grit paper. Enzyme electrodes were prepared through deposition of 16 µL of a 5 mg mL^{-1} redox polymer aqueous solution, 2 µL of a 15 mg mL^{-1} PEGDGE aqueous solution, 8.88 µL of a 46.25 mg mL^{-1} MWCNT aqueous dispersion and sufficient quantity of enzyme (aqueous solution) to achieve a deposition of 0.1 mg of enzyme per electrode. All electrodes were allowed to stand for 24 hours to ensure the biofilm had cured.

Electrochemical testing was performed using a CH Instrument 1030a multichannel potentiostat with a three electrode cell containing 50 mM PBS (150 mM NaCl, pH 7.4, 37ºC). Graphite rods were used as working electrodes with a platinum mesh as counter electrode (Goodfellow) and a custom built Ag/AgCl (3M KCl) as reference electrode. All electrochemical responses are an average of the response obtained for 4 separate electrodes.

The artificial plasma contained uric acid (68.5 mg L^{-1}), ascorbic acid (9.5 mg L^{-1}), fructose (36 mg L^{-1}), lactose (5.5 mg L^{-1}), urea (267 mg L^{-1}), cysteine (18 mg L^{-1}), sodium chloride (6.75 g L^{-1}), sodium bicarbonate (2.138 g L^{-1}), calcium sulfate (23.8 mg L^{-1}), magnesium sulfate (104.5 mg L^{-1}) and bovine serum albumin (7 g L^{-1}). [46]

3 Results and Discussion

Previous studies wiring FADGDH with osmium redox polymers at electrode surfaces have focused on optimising current density for application as anodes in EFCs. [28,31,47–49] Substantial changes to current density, or EFC power, responses are observed when anodes or EFCs are operated in physiological fluids, compared to operation in buffer solutions. [31,38,50–52] We report the effect of selected blood plasma components on the performance of wired glucose oxidising electrodes, in an attempt to understand and hence mitigate against

any effect. The performance of the electrodes is assessed by measuring current response in the presence of increasing concentration of glucose as well as the stability of the signal over a 12-hour timeframe. Control testing is carried out in PBS with no other plasma component present. Enzyme electrodes are produced by coating graphite electrodes with an amount of enzyme, redox polymer, MWCNTs and crosslinker, with amounts selected based on results from previous work.[31] Slow-scan cyclic voltammetry (CV) in the presence and absence of glucose substrate is used to monitor the response of the enzyme electrodes (Figure 1). In the absence of glucose, oxidation and reduction peaks for Os(bpy)PVI centre at ~ 0.22 V vs Ag/AgCl, in agreement with previously reported values for the Os(II/III) redox transition for this redox polymer at an electrode surface.[53,54] Peak currents vary linearly with scan rate at slow scan rates (<20 mVs^{-1}) in the absence of substrate which indicates a surface confined response. At higher scan rates (>20 mVs^{-1}) peak currents vary linearly with the square root of scan rate indicative of semi-infinite diffusion which is expected from multi-layer biofilms at an electrode surface. Slow scan CV recorded in the presence of glucose shows a sigmoidal response for the enzyme electrodes (Figure 1), characteristic of an electrocatalytic process (EC'). The half-wave potential ($E_{1/2}$) of the catalytic response is negatively shifted by approx. 100 mV when compared to the response in the absence of substrate. At low substrate concentration a separation of the response between the plateau-shaped catalytic wave and a reversible mediator wave is also evident. This shift and separation may be due to transport limitation of the glucose and occurs for a mixed case between substrate-limited and kinetic-limited conditions, particularly for high local mediator concentrations relative to the Michaelis-Menten kinetics for the enzyme-mediator interaction, as is the case for these redox polymer films. [55–57].

Insert Figure 1 here

Plateau currents observed in 100 mM glucose concentrations in Figure 1 are used to select an electrode potential of 0.45 V to be applied for steady state amperometry measurements. The amperometric response of enzyme electrodes is then measured as a function of increasing glucose concentration (Figure 2). Current densities observed in steady state amperometry are slightly higher than those for the same potential in slow scan CVs, due to the stirring of the solution during amperometric measurements implemented to avoid depletion of the substrate at the electrode surface, as reported previously.[49]

Parallel amperometric responses of enzyme electrodes are measured in PBS, in artificial plasma as well as in PBS containing only one of the components of artificial plasma. Oxidation current responses increase with increasing glucose concentrations for all enzyme electrodes, with some variation in magnitude of current response in the presence of plasma components. Representative current vs concentration plots are shown in Figure 2 and the response in the presence of 5 mM and 100 mM glucose, representing response under typical human physiological and under typical substrate saturated conditions, shown in Table 1.

Insert Figure 2 here

Electrodes tested in artificial plasma show a significant decrease in performance compared to electrodes tested in PBS, as reported previously. [31,50,51] Testing of electrodes in PBS containing magnesium sulfate, cysteine and calcium sulfate produced marginally higher glucose oxidation current densities at 5 mM and 100 mM glucose when compared with testing in PBS. The observed increase in current density is in agreement with work published previously on the effect of cations in solution on glucose oxidising enzymes. [58–60] The effect of individual plasma components on biosensor or bioanode performance has been reported previously, although not extensively studied. [61–64] Current densities recorded for enzyme electrodes in the individual presence of either urea, fructose, lactose, ascorbic acid, BSA, sodium bicarbonate or uric acid are lower than responses in PBS, Table 1. Nonlinear least-squares fitting of the data points obtained to the Michaelis-Menten equation is used to estimate K_m^{app} and j_{max} values for the enzyme electrode, to permit comparison of performance, Table 1. The estimated K_m^{app} values obtained in the presence of individual plasma components are similar to that obtained in PBS, with the exception of that obtained in the presence of sodium bicarbonate which is significantly higher than the value obtained in PBS. This suggests that the significant increase in K_m^{app} observed for FADGDH enzyme electrodes in artificial plasma, compared to buffer, is due to the presence of sodium bicarbonate. It is unclear as yet if the observed change in K_m^{app} is due to a specific effect of sodium bicarbonate on the enzyme or on the redox polymer. The estimated j_{max} value in the presence of uric acid is significantly lower than values for enzyme electrodes tested in PBS, and in all other plasma components. Uric acid and ascorbic acid are capable of undergoing direct oxidation at electrodes. [65,66] There is evidence of direct oxidation of uric acid at these physiological concentrations at the enzyme electrode, but not of ascorbic acid. Although oxidation of uric acid should provide for increased enzyme electrode response in its

presence it is possible that the allantoin product of uric acid oxidation is accumulated within the enzyme electrode, and therefore decreases enzyme electrode response.

Further testing of the enzyme electrodes is performed to assess the operational stability in buffer, artificial plasma, and the individual components of artificial plasma, given the importance of stability of signal for application to continuous enzyme electrode operation as a biosensor or EFC. Amperometry was performed at 0.45 V vs. Ag/AgCl in solutions containing 100 mM glucose with stirring at 150 rpm (pH 7.4 @ 37 ^{o}C), immediately following recording of the slow-scan CV (see Figure 1). Comparison of the current obtained 10 min after testing was commenced with that after a 12-hour timeframe is used to assess stability of signal. The percentage of current remaining after 12 hours is used to report on the performance of enzyme electrodes in the presence of plasma components (Table 2).

Table 1: The current densities observed at 5 mM and 100 mM for electrodes consisting of FADGDH (100 µg), Os(bpy)PVI (80 µg), MWCNTs (410 µg) and PEGDGE (30 µg) tested in the presence of an individual plasma component (n=4). Apparent Michaelis-Menten constants (K_m^{app}) and j_{max} values for each experimental run is included below. Amperometry performed at 0.45V vs. Ag/AgCl in PBS with stirring at 150 rpm (pH 7.4 @ 37 ^oC).

Plasma component	Current density at 5 mM glucose (mA cm^{-2})	Current density at 100 mM glucose (mA cm^{-2})	K_m^{app} (mM)	j_{max} (mA cm^{-2})
Magnesium sulfate	1.6 ± 0.2	8.9 ± 1.0	28.9 ± 2.8	12.1 ± 0.4
Cysteine	1.5 ± 0.1	8.8 ± 0.6	28.4 ± 1.9	11.8 ± 0.3
Calcium sulfate	1.5 ± 0.2	9.2 ± 2.0	25.3 ± 4.3	12.3 ± 0.7
None (PBS)	1.5 ± 0.1	7.8 ± 0.5	23.4 ± 1.8	10.3 ± 0.3
Urea	1.3 ± 0.1	7.8 ± 0.6	25.7 ± 2.5	10.5 ± 0.3
Fructose	1.3 ± 0.1	8.0 ± 0.7	26.9 ± 3.3	10.7 ± 0.5
Lactose	1.3 ± 0.1	7.9 ± 0.6	28.5 ± 1.8	10.8 ± 0.3
Ascorbic acid	1.2 ± 0.1	7.4 ± 0.4	29.7± 1.8	9.9 ± 0.2
Artificial plasma	1.2 ± 0.1	7.5 ± 0.5	45.5 ± 2.8	11.1 ± 0.3
BSA	1.2 ± 0.2	7.0 ± 0.8	24.4 ± 2.7	9.3 ± 0.3
Sodium bicarbonate	1.2 ± 0.2	9.5 ± 1.3	48.9 ± 6.1	14.4 ± 0.8
Uric acid	0.9 ± 0.1	5.1 ± 0.3	22.4 ± 1.7	6.5 ± 0.2

Table 2: Current densities recorded for electrodes consisting of FADGDH (100 µg), Os(bpy)PVI (80 µg), MWCNTs (410 µg) and PEGDGE (30 µg) in PBS (pH 7.4 @ 37 ºC) containing 100 mM glucose and in the presence of an individual plasma component after 10 minutes and after 12 hours of continuous operation. Amperometry performed at 0.45V vs. Ag/AgCl with stirring at 150 rpm.

Plasma component	Initial current after 10 minutes (mA cm^{-2})	Current after 12 hours (mA cm^{-2})	% current after 12 hours
Fructose	7.7 ± 0.6	7.1 ± 0.5	92
Urea	8.3 ± 0.8	6. 8 ± 0.6	82
Lactose	7.5 ± 0.6	5.9 ± 0.5	80
BSA	7.3 ± 0.7	5.8 ± 0.4	79
Calcium sulfate	9.2 ± 2.1	7.1 ± 0.7	77
Magnesium sulfate	8.7 ± 0.9	6.5 ± 0.4	75
Sodium bicarbonate	8.5 ± 1.3	6.3 ± 1.0	74
None (PBS)	8.2 ± 0.3	5.9 ± 0.2	72
Cysteine	8.5 ± 0.5	5.3 ± 0.3	63
Ascorbic acid	7.3 ± 0.5	4.6 ± 0.4	62
Uric acid	4.7 ± 0.2	2.2 ± 0.4	46
Artificial plasma	7.0 ± 0.2	1.9 ± 0.1	27

Enzyme electrodes tested in PBS, with no additional plasma component, retain 72 % of initial current after 12 hours. This performance is in agreement with that reported previously for similar enzyme electrodes.[49] However, enzyme electrodes tested in artificial plasma retain only 27% of initial current after 12 hours of continuous operation. Previous work carried out on EFC response in complex solutions, such as artificial tears, report a similar decrease in operational stability over time. [63] Interestingly, highest operational stability for glucose oxidation, 92 % of initial current remaining after 12 hours, is achieved for enzyme electrodes operating in PBS and fructose at physiological levels (36 mg L^{-1}). It is unclear why the presence of fructose in solution stabilises the current response over this time period. For example, the FADGDH enzyme shows no activity towards fructose. [67,68] Of all the individual components present in artificial plasma, the presence of uric acid at physiological

levels (68.5 mg L^{-1}) results in the greatest % decrease in current over 12 hours operation, as well as the lowest initial current response in the presence of 5 mM or 100 mM glucose, and lowest j_{max}. It is known that uric acid is responsible for interfering signals to *in-vivo* electrochemical sensing as a result of direct oxidation of uric acid at electrodes (0.59 V vs. NHE) [41,42,69] If the effect of uric acid on these enzyme electrodes was due to direct oxidation, an increase in current in its presence is anticipated, not a decrease. The results obtained in this study suggest that uric acid inhibits enzyme electrode performance resulting in decreased current production and operational stability (Table 1 and Table 2).

In order to determine whether uric acid affects the FADGDH enzyme, or any of the other components in the enzyme electrode, enzyme electrodes prepared using alternate glucose oxidising enzymes (glucose oxidase or recombinant cellobiose dehydrogenase at a loading of 0.1 mg per electrode) within otherwise identical biofilms of osmium polymer, MWCNTs and PEGDGE are tested. Steady state amperometry is used to record current responses as a function of increasing glucose concentration, with testing in the presence and absence of uric acid at physiological level (Figure 3), with K_m^{app} and j_{max} values estimated as before, Table 3.

Insert Figure 3 here

Table 3: Apparent Michaelis-Menten constants (K_m^{app}),j_{max} values, initial current, remaining current and percentage remaining current for enzyme electrodes (n=4) prepared using a glucose oxidising enzyme (100 µg), Os(bpy)PVI (80 µg), MWCNTs (410 µg) and PEGDGE (30 µg) from currents in the presence of glucose recorded under steady state amperometry at 0.45V vs. Ag/AgCl in PBS with stirring at 150 rpm (pH 7.4 @ 37 ^{o}C). Uric acid concentration was 68.5 mg L^{-1}.

Enzyme	K_m^{app} (mM)	j_{max} (mA cm^{-2})	Initial current after 10 minutes (mA cm^{-2})	Remaining current after 12 hours (mA cm^{-2})	Remaining current (%)
FADGDH	23.4 ± 1.8	10.3 ± 0.3	8.2 ± 0.3	5.9 ± 0.2	72
in uric acid	22.4 ± 1.7	6.5 ± 0.2	4.7 ± 0.2	2.2 ± 0.4	46
GOx	18.0 ± 4.2	8.8 ± 0.6	6.0 ± 1.1	4.3 ± 1.1	70
in uric acid	17.7 ± 4.9	7.1 ± 0.6	4.5 ± 1.3	3.1 ± 1.0	70
CDH	80.0 ± 5.8	8.8 ± 0.4	4.3 ± 0.2	3.7 ± 0.1	86
in uric acid	40.1 ± 5.2	5.5 ± 0.3	3.5 ± 0.4	1.2 ± 0.1	33

K_m^{app} values are similar for enzyme electrodes produced using FADGDH or GOx when operated in the presence or absence of uric acid. The j_{max} value for enzyme electrodes using GOx is 20% lower in the presence of uric acid than that in PBS buffer alone. This difference is not as significant as the 37% decrease observed for enzyme electrodes based on FADGDH in the presence of uric acid. A decrease in j_{max} while maintaining a similar K_m^{app} is indicative of non-competitive enzyme inhibition [70–72] thus uric acid inhibits non-competitively both FADGDH and GOx activity although GOx is not as badly affected as the FADGDH enzyme. For comparison, both K_m^{app} and j_{max} values decrease significantly for enzyme electrodes produced using a CDH enzyme when operated in the presence of uric acid instead of PBS buffer alone. The decrease in both K_m^{app} and j_{max} suggests uncompetitive inhibition [70] occurs for the CDH based enzyme electrode in the presence of uric acid. The effect of interfering compounds on the CDH enzyme has been reported on previously, although uric acid was not investigated. [73]

The proportion of initial current remaining after 12 hr operation dropped from 72 % to 46 % in the presence of uric acid, compared to operation in PBS buffer alone, for the FADGDH based electrodes. The stability of the current response is unaltered in the presence of uric acid for the GOx based enzyme electrodes, although the initial current density, in the absence of uric acid, is lower than that observed for FADGDH based enzyme electrodes. As reported on previously CDH electrodes, in the absence of uric acid, produce the most stable current, retaining 86% of initial current after 12 hr operation in PBS. [49,74] However, in the presence of uric acid, CDH based enzyme electrodes retain only 33 % of initial current after 12 hr operation. Again, the results, in combination with the observed changes to K_m^{app} and j_{max}, suggest that the uric acid acts as an uncompetitive inhibitor of the CDH enzyme for the oxidation of glucose. From the three glucose oxidising enzymes tested, the operational stability of GOx based electrodes in the presence of uric acid was least affected by the presence of uric acid, with however significant changes to estimated maximum current density of all three enzyme electrodes in the presence of uric acid.

4 Conclusion

Enzyme electrodes consisting of co-immobilised FADGDH, Os(bpy)PVI, MWCNTs and PEGDGE were tested in PBS, artificial plasma and the individual components of the artificial plasma to gain a better understanding of the reason for a decrease in electrode performance

observed in artificial serum and human serum.[31,38,50–52] The lowest current response for these electrodes was observed in the presence of physiological level of uric acid although the largest change to K_m^{app} occurred upon addition of physiological level of sodium bicarbonate. The lowest operational stability observed in the presence of a single plasma component was recorded in the presence of uric acid, with only 46 % of initial current response after 12 hours: this compares to only 27% of current remaining after 12 hours for electrodes operating in artificial plasma. Electrodes were prepared using alternate glucose oxidising enzymes, GOx and CDH, and tested in the presence of uric acid. For both FADGDH and GOx enzyme electrodes, j_{max} decreased significantly with no significant change to the K_m^{app} suggesting non-competitive inhibition. The CDH based electrodes provide highest stability of current signal in PBS, with 86 % of the initial signal present after 12 hours, but demonstrate significant change to the K_m^{app} and j_{max} values as well as operational stability dropping to only 33% in the presence of uric acid. In contrast the operational stability of the GOx based enzyme electrodes was unaffected by the presence of physiological level of uric acid. As uric acid and sodium bicarbonate are present in blood, careful consideration is required in choosing a glucose oxidising enzyme for both glucose sensing and EFC device assembly. Further work is required to understand the mechanism of uric acid inhibition on each of the FADGDH, GOx and CDH enzymes.

Acknowledgments

R. Bennett acknowledges support through an NUI Galway College of Science fellowship and an Irish Research Council Postgraduate Scholarship (GOIPG/2016/505). Donation of recombinant CDH produced with financial support from the European Commission (“Bioenergy” PEOPLE-2013-ITN-607793) by Roland Ludwig (University of Boku) is gratefully acknowledged.

References

[1] S. Calabrese Barton, J. Gallaway, P. Atanassov, Enzymatic Biofuel Cells for Implantable and Microscale Devices, Chem. Rev. 104 (2004) 4867–4886. doi:10.1021/cr020719k.

[2] D. Leech, P. Kavanagh, W. Schuhmann, Enzymatic fuel cells: Recent progress,

Electrochim. Acta. 84 (2012) 223–234. doi:10.1016/j.electacta.2012.02.087.

[3] M. Rasmussen, S. Abdellaoui, S.D. Minteer, Enzymatic biofuel cells: 30 years of critical advancements, Biosens. Bioelectron. 76 (2016) 91–102. doi:10.1016/J.BIOS.2015.06.029.

[4] A. Heller, Miniature biofuel cells, Phys. Chem. Chem. Phys. 6 (2004) 209–216. doi:10.1039/b313149a.

[5] S.D. Minteer, B.Y. Liaw, M.J. Cooney, Enzyme-based biofuel cells, Curr. Opin. Biotechnol. 18 (2007) 228–234. doi:10.1016/J.COPBIO.2007.03.007.

[6] A.T. Yahiro, S.M. Lee, D.O. Kimble, Bioelectrochemistry: I. Enzyme utilizing bio-fuel cell studies, Biochim. Biophys. Acta - Spec. Sect. Biophys. Subj. 88 (1964) 375–383. doi:10.1016/0926-6577(64)90192-5.

[7] T. Chen, S.C. Barton, G. Binyamin, Z. Gao, Y. Zhang, H.-H. Kim, A. Heller, A Miniature Biofuel Cell, J. Am. Chem. Soc. 123 (2001) 8630–8631. doi:10.1021/ja0163164.

[8] J.A. Cracknell, K.A. Vincent, F.A. Armstrong, Enzymes as Working or Inspirational Electrocatalysts for Fuel Cells and Electrolysis, Chem. Rev. 108 (2008) 2439–2461. doi:10.1021/cr0680639.

[9] S. Tsujimura, From fundamentals to applications of bioelectrocatalysis: bioelectrocatalytic reactions of FAD-dependent glucose dehydrogenase and bilirubin oxidase, Biosci. Biotechnol. Biochem. (2018) 1–10. doi:10.1080/09168451.2018.1527209.

[10] I. Mazurenko, A. de Poulpiquet, E. Lojou, Recent developments in high surface area bioelectrodes for enzymatic fuel cells, Curr. Opin. Electrochem. 5 (2017) 74–84. doi:10.1016/j.coelec.2017.07.001.

[11] R.F.B. Turner, D.J. Harrison, R.V. Rajotte, H.P. Baltes, A biocompatible enzyme electrode for continuous in vivo glucose monitoring in whole blood, Sensors Actuators B Chem. 1 (1990) 561–564. doi:10.1016/0925-4005(90)80273-3.

[12] C.A. Quinn, R.E. Connor, A. Heller, Biocompatible, glucose-permeable hydrogel for in situ coating of implantable biosensors., Biomaterials. 18 (1997) 1665–1670. http://www.ncbi.nlm.nih.gov/pubmed/9613815 (accessed April 9, 2018).

[13] C.N. Kotanen, F.G. Moussy, S. Carrara, A. Guiseppi-Elie, Implantable enzyme amperometric biosensors, Biosens. Bioelectron. 35 (2012) 14–26. doi:10.1016/j.bios.2012.03.016.

[14] P. Cinquin, C. Gondran, F. Giroud, S. Mazabrard, A. Pellissier, F. Boucher, J.-P. Alcaraz, K. Gorgy, F. Lenouvel, S. Mathé, P. Porcu, S. Cosnier, A Glucose BioFuel Cell Implanted in Rats, PLoS One. 5 (2010) e10476. doi:10.1371/journal.pone.0010476.

[15] S. Cosnier, A.J. Gross, F. Giroud, M. Holzinger, Beyond the hype surrounding biofuel cells: What's the future of enzymatic fuel cells?, Curr. Opin. Electrochem. (2018). doi:10.1016/J.COELEC.2018.06.006.

[16] A.L. Ghindilis, P. Atanasov, E. Wilkins, Enzyme-catalyzed direct electron transfer: Fundamentals and analytical applications, Electroanalysis. 9 (1997) 661–674. doi:10.1002/elan.1140090902.

[17] P. Kavanagh, D. Leech, Mediated electron transfer in glucose oxidising enzyme electrodes for application to biofuel cells: recent progress and perspectives, Phys. Chem. Chem. Phys. 15 (2013) 4859–4869. doi:10.1039/c3cp44617d.

[18] J.C. Pickup, G.W. Shaw, D.J. Claremont, Potentially-implantable, amperometric glucose sensors with mediated electron transfer: improving the operating stability., Biosensors. 4 (1989) 109–119. http://www.ncbi.nlm.nih.gov/pubmed/2719726 (accessed October 5, 2018).

[19] N. Mano, F. Mao, A. Heller, Characteristics of a Miniature Compartment-less Glucose−O_2 Biofuel Cell and Its Operation in a Living Plant, J. Am. Chem. Soc. 125 (2003) 6588–6594. doi:10.1021/ja0346328.

[20] F. Mao, N. Mano, A. Heller, Long Tethers Binding Redox Centers to Polymer Backbones Enhance Electron Transport in Enzyme "Wiring" Hydrogels, J. Am. Chem. Soc. 125 (2003) 4951–4957. doi:10.1021/ja029510e.

[21] T. de Lumley-Woodyear, P. Rocca, J. Lindsay, Y. Dror, A. Freeman, A. Heller, Polyacrylamide-Based Redox Polymer for Connecting Redox Centers of Enzymes to Electrodes, Anal. Chem. 67 (1995) 1332–1338. doi:10.1021/ac00104a006.

[22] P. O Conghaile, D. MacAodha, B. Egan, P. Kavanagh, D. Leech, Tethering Osmium

Complexes within Enzyme Films on Electrodes to Provide a Fully Enzymatic Membrane-Less Glucose/Oxygen Fuel Cell, J. Electrochem. Soc. 160 (2013) G3165–G3170. doi:10.1149/2.026307jes.

[23] B.A. Gregg, A. Heller, Cross-linked redox gels containing glucose oxidase for amperometric biosensor applications, Anal. Chem. 62 (1990) 258–263. doi:10.1021/ac00202a007.

[24] I. Osadebe, D. Leech, Effect of Multi-Walled Carbon Nanotubes on Glucose Oxidation by Glucose Oxidase or a Flavin-Dependent Glucose Dehydrogenase in Redox-Polymer-Mediated Enzymatic Fuel Cell Anodes, ChemElectroChem. 1 (2014) 1988–1993. doi:10.1002/celc.201402136.

[25] D. MacAodha, M.L. Ferrer, P.Ó. Conghaile, P. Kavanagh, D. Leech, Crosslinked redox polymer enzyme electrodes containing carbon nanotubes for high and stable glucose oxidation current, Phys. Chem. Chem. Phys. 14 (2012) 14667–14672. doi:10.1039/c2cp42089a.

[26] S. Boland, P. Kavanagh, D. Leech, Mediated Enzyme Electrodes for Biological Fuel Cell and Biosensor Applications, in: ECS Trans., ECS, 2008: pp. 77–87. doi:10.1149/1.3036213.

[27] R. Kumar, D. Leech, A glucose anode for enzymatic fuel cells optimized for current production under physiological conditions using a design of experiment approach., Bioelectrochemistry. 106 (2015) 41–46. doi:10.1016/j.bioelechem.2015.06.005.

[28] I. Osadebe, P.Ó. Conghaile, P. Kavanagh, D. Leech, Glucose oxidation by osmium redox polymer mediated enzyme electrodes operating at low potential and in oxygen, for application to enzymatic fuel cells, Electrochim. Acta. 182 (2015) 320–326. doi:10.1016/j.electacta.2015.09.088.

[29] P. Ó Conghaile, M. Falk, D. MacAodha, M.E. Yakovleva, C. Gonaus, C.K. Peterbauer, L. Gorton, S. Shleev, D. Leech, Fully Enzymatic Membraneless Glucose|Oxygen Fuel Cell That Provides 0.275 mA cm $^{-2}$ in 5 mM Glucose, Operates in Human Physiological Solutions, and Powers Transmission of Sensing Data, Anal. Chem. 88 (2016) 2156–2163. doi:10.1021/acs.analchem.5b03745.

[30] M. Shao, M.N. Zafar, M. Falk, R. Ludwig, C. Sygmund, C.K. Peterbauer, D.A.

Guschin, D. MacAodha, P. Ó Conghaile, D. Leech, M.D. Toscano, S. Shleev, W. Schuhmann, L. Gorton, Optimization of a Membraneless Glucose/Oxygen Enzymatic Fuel Cell Based on a Bioanode with High Coulombic Efficiency and Current Density, ChemPhysChem. 14 (2013) 2260–2269. doi:10.1002/cphc.201300046.

[31] R. Bennett, I. Osadebe, R. Kumar, P.Ó. Conghaile, D. Leech, Design of Experiments Approach to Provide Enhanced Glucose-oxidising Enzyme Electrode for Membrane-less Enzymatic Fuel Cells Operating in Human Physiological Fluids, Electroanalysis. 30 (2018) 1438–1445. doi:10.1002/elan.201600402.

[32] S. Cosnier, A. Le Goff, M. Holzinger, Towards glucose biofuel cells implanted in human body for powering artificial organs: Review, Electrochem. Commun. 38 (2014) 19–23. doi:10.1016/J.ELECOM.2013.09.021.

[33] S. Vaddiraju, D.J. Burgess, I. Tomazos, F.C. Jain, F. Papadimitrakopoulos, Technologies for Continuous Glucose Monitoring: Current Problems and Future Promises, J. Diabetes Sci. Technol. 4 (2010) 1540–1562. doi:10.1177/193229681000400632.

[34] S. Rubenwolf, S. Sané, L. Hussein, J. Kestel, F. von Stetten, G. Urban, M. Krueger, R. Zengerle, S. Kerzenmacher, Prolongation of electrode lifetime in biofuel cells by periodic enzyme renewal, Appl. Microbiol. Biotechnol. 96 (2012) 841–849. doi:10.1007/s00253-012-4374-8.

[35] S. Cosnier, A. J. Gross, A. Le Goff, M. Holzinger, Recent advances on enzymatic glucose/oxygen and hydrogen/oxygen biofuel cells: Achievements and limitations, J. Power Sources. 325 (2016) 252–263. doi:10.1016/J.JPOWSOUR.2016.05.133.

[36] X. Li, L. Zhang, L. Su, T. Ohsaka, L. Mao, A Miniature Glucose/O $_2$ Biofuel Cell With a High Tolerance Against Ascorbic Acid, Fuel Cells. 9 (2009) 85–91. doi:10.1002/fuce.200800054.

[37] E. Katz, A.F. Bückmann, I. Willner, Self-Powered Enzyme-Based Biosensors, J. Am. Chem. Soc. 123 (2001) 10752–10753. doi:10.1021/ja0167102.

[38] V. Coman, R. Ludwig, W. Harreither, D. Haltrich, L. Gorton, T. Ruzgas, and S. Shleev, A Direct Electron Transfer-Based Glucose/Oxygen Biofuel Cell Operating in Human Serum, Fuel Cells. 10 (2009) 9-16. doi:10.1002/fuce.200900121.

[39] J.P. Lowry, K. McAteer, S.S. El Atrash, A. Duff, R.D. O'Neill, Characterization of Glucose Oxidase-Modified Poly(phenylenediamine)-Coated Electrodes in vitro and in vivo: Homogeneous Interference by Ascorbic Acid in Hydrogen Peroxide Detection, Anal. Chem. 66 (1994) 1754–1761. doi:10.1021/ac00082a025.

[40] J.P. Lowry, R.D. O'Neill, Homogeneous mechanism of ascorbic acid interference in hydrogen peroxide detection at enzyme-modified electrodes, Anal. Chem. 64 (1992) 453–456. doi:10.1021/ac00028a022.

[41] J. Wang, J. Liu, L. Chen, F. Lu, Highly Selective Membrane-Free, Mediator-Free Glucose Biosensor, Anal. Chem. 66 (1994) 3600–3603. doi:10.1021/ac00093a011.

[42] S. V. Sasso, R.J. Pierce, R. Walla, A.M. Yacynych, Electropolymerized 1,2-diaminobenzene as a means to prevent interferences and fouling and to stabilize immobilized enzyme in electrochemical biosensors, Anal. Chem. 62 (1990) 1111–1117. doi:10.1021/ac00210a004.

[43] R.J. Forster, J.G. Vos, Synthesis, characterization, and properties of a series of osmium- and ruthenium-containing metallopolymers, Macromolecules. 23 (1990) 4372–4377. doi:10.1021/ma00222a008.

[44] E.M. Kober, J. V. Caspar, B.P. Sullivan, T.J. Meyer, Synthetic routes to new polypyridyl complexes of osmium(II), Inorg. Chem. 27 (1988) 4587–4598. doi:10.1021/ic00298a017.

[45] C. Sygmund, P. Staudigl, M. Klausberger, N. Pinotsis, K. Djinović-Carugo, L. Gorton, D. Haltrich, R. Ludwig, Heterologous overexpression of Glomerella cingulata FAD-dependent glucose dehydrogenase in Escherichia coli and Pichia pastoris., Microb. Cell Fact. 10 (2011) 106. doi:10.1186/1475-2859-10-106.

[46] C.A. Burtis, E.R. Ashwood, D.E. Bruns, Tietz Textbook of Clinical Chemistry and Molecular Diagnostics., Elsevier Health Sciences, 2012.

[47] X. Xiao, P.Ó. Conghaile, D. Leech, R. Ludwig, E. Magner, An oxygen-independent and membrane-less glucose biobattery/supercapacitor hybrid device, Biosens. Bioelectron. 98 (2017) 421–427. doi:10.1016/j.bios.2017.07.023.

[48] M.N. Zafar, N. Beden, D. Leech, C. Sygmund, R. Ludwig, L. Gorton, Characterization of different FAD-dependent glucose dehydrogenases for possible use in glucose-based

biosensors and biofuel cells, Anal. Bioanal. Chem. 402 (2012) 2069–2077. doi:10.1007/s00216-011-5650-7.

[49] D. MacAodha, P. Ó Conghaile, B. Egan, P. Kavanagh, C. Sygmund, R. Ludwig, D. Leech, Comparison of Glucose Oxidation by Crosslinked Redox Polymer Enzyme Electrodes Containing Carbon Nanotubes and a Range of Glucose Oxidising Enzymes, Electroanalysis. 25 (2013) 94–100. doi:10.1002/elan.201200536.

[50] T. Siepenkoetter, U. Salaj-Kosla, X. Xiao, P.Ó. Conghaile, M. Pita, R. Ludwig, E. Magner, Immobilization of Redox Enzymes on Nanoporous Gold Electrodes: Applications in Biofuel Cells, Chempluschem. 82 (2017) 553–560. doi:10.1002/cplu.201600455.

[51] D. MacAodha, P.Ó. Conghaile, B. Egan, P. Kavanagh, D. Leech, Membraneless Glucose/Oxygen Enzymatic Fuel Cells Using Redox Hydrogel Films Containing Carbon Nanotubes, ChemPhysChem. 14 (2013) 2302–2307. doi:10.1002/cphc.201300239.

[52] R.D. Milton, K. Lim, D.P. Hickey, S.D. Minteer, Employing FAD-dependent glucose dehydrogenase within a glucose/oxygen enzymatic fuel cell operating in human serum, Bioelectrochemistry. 106 (2015) 56–63. doi:10.1016/J.BIOELECHEM.2015.04.005.

[53] H. Ju, D. Leech, [Os(bpy)2(PVI)10Cl]Cl polymer-modified carbon fiber electrodes for the electrocatalytic oxidation of NADH, Anal. Chim. Acta. 345 (1997) 51–58. doi:10.1016/S0003-2670(97)00093-7.

[54] T.J. Ohara, R. Rajagopalan, A. Heller, Glucose electrodes based on cross-linked bis(2,2’-bipyridine)chloroosmium(+/2+) complexed poly(1-vinylimidazole) films, Anal. Chem. 65 (1993) 3512–3517. doi:10.1021/ac00071a031.

[55] B. Limoges, J. Moiroux, J.-M. Savéant, Kinetic control by the substrate and the cosubstrate in electrochemically monitored redox enzymatic immobilized systems. Catalytic responses in cyclic voltammetry and steady state techniques, J. Electroanal. Chem. 521 (2002) 8–15. doi:10.1016/S0022-0728(02)00658-7.

[56] P.N. Bartlett, K.F.E. Pratt, Theoretical treatment of diffusion and kinetics in amperometric immobilized enzyme electrodes Part I: Redox mediator entrapped within the film, J. Electroanal. Chem. 397 (1995) 61–78. doi:10.1016/0022-0728(95)04236-7.

[57] B. Limoges, J.-M. Savéant, Cyclic voltammetry of immobilized redox enzymes. Interference of steady-state and non-steady-state Michaelis–Menten kinetics of the enzyme–redox cosubstrate system, J. Electroanal. Chem. 549 (2003) 61–70. doi:10.1016/S0022-0728(03)00285-7.

[58] P. Bollella, Y. Hibino, K. Kano, L. Gorton, R. Antiochia, The influence of pH and divalent/monovalent cations on the internal electron transfer (IET), enzymatic activity, and structure of fructose dehydrogenase, Anal. Bioanal. Chem. 410 (2018) 3253–3264. doi:10.1007/s00216-018-0991-0.

[59] D. Kracher, K. Zahma, C. Schulz, C. Sygmund, L. Gorton, R. Ludwig, Inter-domain electron transfer in cellobiose dehydrogenase: modulation by pH and divalent cations, FEBS J. 282 (2015) 3136–3148. doi:10.1111/febs.13310.

[60] C. Schulz, R. Ludwig, P.O. Micheelsen, M. Silow, M.D. Toscano, L. Gorton, Enhancement of enzymatic activity and catalytic current of cellobiose dehydrogenase by calcium ions, Electrochem. Commun. 17 (2012) 71–74. doi:10.1016/j.elecom.2012.01.031.

[61] F. Moussy, D.J. Harrison, D.W. O'Brien, R. V. Rajotte, Performance of subcutaneously implanted needle-type glucose sensors employing a novel trilayer coating, Anal. Chem. 65 (1993) 2072–2077. doi:10.1021/ac00063a023.

[62] D.S. Bindra, Y. Zhang, G.S. Wilson, R. Sternberg, D.R. Thevenot, D. Moatti, G. Reach, Design and in vitro studies of a needle-type glucose sensor for subcutaneous monitoring, Anal. Chem. 63 (1991) 1692–1696. doi:10.1021/ac00017a008.

[63] X. Xiao, T. Siepenkoetter, P.Ó. Conghaile, D. Leech, E. Magner, Nanoporous Gold-Based Biofuel Cells on Contact Lenses, ACS Appl. Mater. Interfaces. 10 (2018) 7107–7116. doi:10.1021/acsami.7b18708.

[64] M. Cadet, S. Gounel, C. Stines-Chaumeil, X. Brilland, J. Rouhana, F. Louerat, N. Mano, Cadet, M., Gounel, S., Stines-Chaumeil, C., Brilland, X., Rouhana, J., Louerat, F., Mano, N., 2016. An enzymatic glucose/O2 biofuel cell operating in human blood. Biosens. Bioelectron. 83, 60–67. doi:10.1016/j.bios.2016.04.016.

[65] R.T. Kachoosangi, C.E. Banks, R.G. Compton, Simultaneous Determination of Uric Acid and Ascorbic Acid Using Edge Plane Pyrolytic Graphite Electrodes,

Electroanalysis. 18 (2006) 741–747. doi:10.1002/elan.200603470.

[66] P. Pinyou, A. Ruff, S. Pöller, S. Ma, R. Ludwig, W. Schuhmann, Design of an Os Complex-Modified Hydrogel with Optimized Redox Potential for Biosensors and Biofuel Cells, Chemistry - A Eur. J. 22 (2016) 5319–5326. doi:10.1002/chem.201504591.

[67] H. Yoshida, G. Sakai, K. Mori, K. Kojima, S. Kamitori, K. Sode, Structural analysis of fungus-derived FAD glucose dehydrogenase, Sci. Rep. 5 (2015) 13498. doi:10.1038/srep13498.

[68] K. Sode, N. Loew, Y. Ohnishi, H. Tsuruta, K. Mori, K. Kojima, W. Tsugawa, J.T. LaBelle, D.C. Klonoff, Novel fungal FAD glucose dehydrogenase derived from Aspergillus niger for glucose enzyme sensor strips, Biosens. Bioelectron. 87 (2017) 305–311. doi:10.1016/j.bios.2016.08.053.

[69] M.G. Simic, S. V. Jovanovic, Antioxidation mechanisms of uric acid, J. Am. Chem. Soc. 111 (1989) 5778–5782. doi:10.1021/ja00197a042.

[70] A. Cornish-Bowden, Fundamentals of enzyme kinetics, Butterworths, 1979.

[71] M. Grattieri, P. Scodeller, C. Adam, E.J. Calvo, Non-Competitive Reversible Inhibition of Laccase by H_2O_2 in Osmium Mediated Layer-By-Layer Multilayer O_2 Biocathodes, J. Electrochem. Soc. 162 (2015) G82–G86. doi:10.1149/2.0831509jes.

[72] R.D. Milton, S.D. Minteer, Investigating the Reversible Inhibition Model of Laccase by Hydrogen Peroxide for Bioelectrocatalytic Applications, J. Electrochem. Soc. 161 (2014) H3011–H3014. doi:10.1149/2.0031413jes.

[73] P. Bollella, L. Gorton, R. Ludwig, R. Antiochia, A Third Generation Glucose Biosensor Based on Cellobiose Dehydrogenase Immobilized on a Glassy Carbon Electrode Decorated with Electrodeposited Gold Nanoparticles: Characterization and Application in Human Saliva, Sensors. 17 (2017) 1912. doi:10.3390/s17081912.

[74] M.N. Zafar, I. Aslam, R. Ludwig, G. Xu, L. Gorton, An efficient and versatile membraneless bioanode for biofuel cells based on Corynascus thermophilus cellobiose dehydrogenase, Electrochim. Acta. 295 (2019) 316–324. doi:10.1016/J.ELECTACTA.2018.10.047.

Figure legends

Figure 1: Slow scan (1 mVs^{-1}) cyclic voltammograms of electrodes in quiescent 50 mM PBS solution (150 mM NaCl, pH 7.4, 37ºC). Electrode biofilms consisted of FADGDH (100 µg), Os(bpy)PVI (80 µg), MWCNTs (410 µg) and PEGDGE (30 µg). CV scans were recorded in the absence of glucose (blue), 5 mM glucose (red) and 100 mM glucose (green).

Figure 2: Current response at 0.45 V (37 ºC) with stirring at 150 rpm for enzyme electrodes (n=4) consisting of FADGDH (100 µg), Os(bpy)PVI (80 µg), MWCNTs (410 µg) and PEGDGE (30 µg) in PBS, pH 7.4 (blue circle), artificial plasma (red diamond) and in the presence of magnesium sulfate (purple star), or uric acid (green triangle)) at the level expected for plasma (see experimental section).

Figure 3: Average current response (n=4) generated for each electrode consisting of glucose oxidising enzyme (100 µg), Os(bpy)PVI (80 µg), MWCNTs (410 µg) and PEGDGE (30 µg). Amperometry performed at 0.45V vs. Ag/AgCl in PBS with stirring at 150 rpm (pH 7.4 @ 37 ºC). Uric acid concentration was 68.5 mg L^{-1}. FADGDH electrodes tested in the presence (red) and absence (blue) of uric acid. GOx electrodes tested in the presence (green) and absence (pink) of uric acid. CDH electrodes tested in the presence (dark green) and absence (light blue) of uric acid.

FIGURE 1

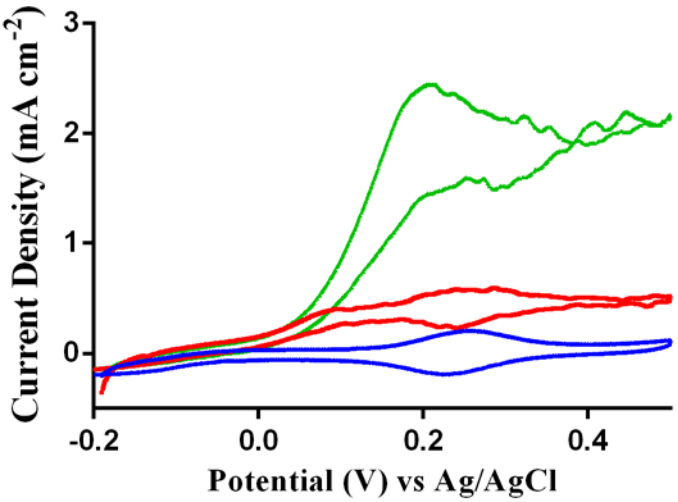


FIGURE 2

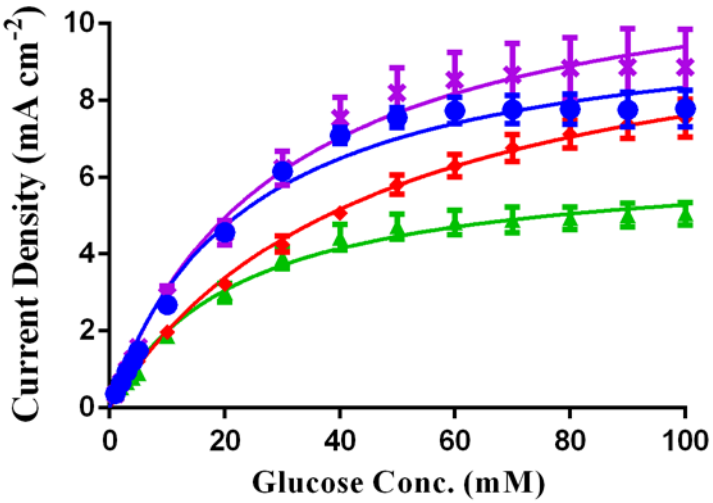
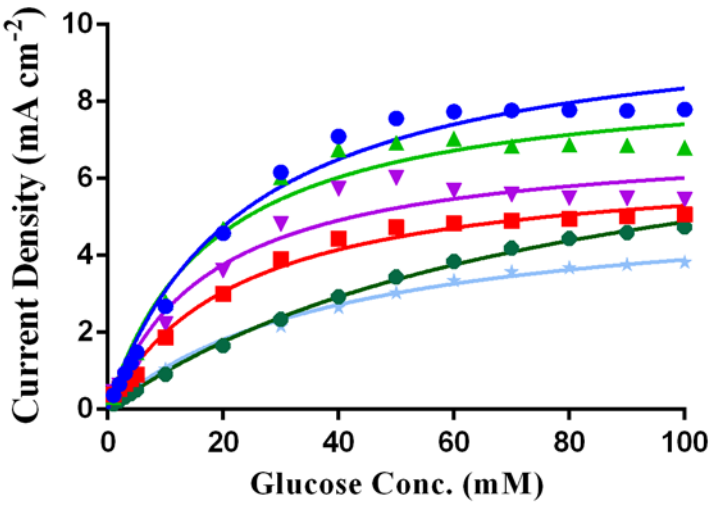


FIGURE 3


Current Density (mA cm-2)
10
8
6
4
2
0
0
20
40
60
80
100
Glucose Conc. (mM)