



*Citation for published version:*

Du Toit, H & Di Lorenzo, M 2014, 'Glucose oxidase directly immobilized onto highly porous gold electrodes for sensing and fuel cell applications', *Electrochimica Acta*, vol. 138, pp. 86-92.  
<https://doi.org/10.1016/j.electacta.2014.06.074>

*DOI:*

[10.1016/j.electacta.2014.06.074](https://doi.org/10.1016/j.electacta.2014.06.074)

*Publication date:*

2014

*Document Version*

Publisher's PDF, also known as Version of record

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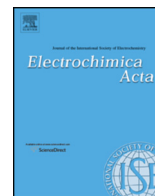
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# Glucose Oxidase Directly Immobilized onto Highly Porous Gold Electrodes for Sensing and Fuel Cell applications



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## ARTICLE INFO

### Article history:

Received 12 April 2014

Received in revised form 11 June 2014

Accepted 12 June 2014

Available online 19 June 2014

### Keywords:

Biofuel cell  
glucose oxidase  
laccase  
porous gold  
glucose sensor

## ABSTRACT

The successful implementation of redox-enzyme electrodes in biosensors and enzymatic biofuel cells has been the subject of extensive research.

For high sensitivity and high energy-conversion efficiency, the effective electron transfer at the protein-electrode interface has a key role. This is difficult to achieve in the case of glucose oxidase, due to the fact that for this enzyme the redox centre is buried inside the structure, far from any feasible electrode binding sites.

This study reports, a simple and rapid methodology for the direct immobilisation of glucose oxidase into highly porous gold electrodes. When the resulting electrode was tested as glucose sensor, a Michaelis-Menten kinetic trend was observed, with a detection limit of 25  $\mu\text{M}$ . The bioelectrode sensitivity, calculated against the superficial surface area of the bioelectrode, was of  $22.7 \pm 0.1 \mu\text{A mM}^{-1} \text{cm}^{-2}$ .

This glucose oxidase electrode was also tested as an anode in a glucose/ $\text{O}_2$  enzymatic biofuel cell, leading to a peak power density of  $6 \mu\text{W cm}^{-2}$  at a potential of 0.2 V.

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## 1. Introduction

The functional immobilisation of redox enzymes, such as laccase and glucose oxidase, onto electrode material surfaces is of keen interest for sensors and biofuel cells development.

Enzymes are the most common bioreceptor molecules used in biosensors due to their extremely high specificity that leads to minimal risk of false positive responses. The implementation of enzymes in biofuel cells allows for the development of membraneless and compartment-less devices, which not only can be easily miniaturised, but can also be used in situations where it is not feasible to separate the fuel and oxidant [1].

For these applications, the achievement of efficient electron transfer between the enzyme active centre and the electrode is critical. Usually a mediated electron transfer (MET) mechanism is required. This might involve the use of small redox active particles and polymers as electron carriers (mediators), such as organic dyes, ferrocene and its derivatives, modified vitamin complexes, and conducting salts [2]. If the mediator is in solution their diffusion to the electrode surface allows for a more rapid electron transfer compared to the direct transfer from the enzyme itself [3]. Alternatively, the mediators can be polymerised directly onto the

electrode surface or co-immobilised with the reacting enzymes to further enhance the rate of electron transfer [4,5].

However, the use of redox active electron carriers can have several drawbacks, such as short lifespans, poor biocompatibility, risk of leaching away from the electrode surface, potential toxicity. Consequently, the achievement of a direct electron transfer (DET) process is preferred.

Major advancements have come as far back as the 1980s from examining transition metal rich enzymes, such as laccase (LAC), capable of catalysing the reduction of oxygen to water through DET [6]. This is because LAC contains several copper centres that allow the electrons transport through the enzyme redox centre to the electrode surface. Consequently, the achievement of DET with LAC-immobilised electrodes is now well established [7–9].

In the case of glucose oxidase (GOx), DET is more difficult to achieve, due to the fact that the GOx redox centre is buried inside the enzyme structure, and is far from any feasible electrode binding sites. To achieve efficient electron transfer, the use of GOx has been often combined with mediator compounds, of which ferrocene is the most common [10]. Most of the GOx immobilisation protocols reported, while effective, are usually very expensive, due to the reagents required. These protocols are often very laborious, involving multi-steps in the immobilization procedure that can be sources of experimental errors [11–14]. Moreover the resulting bioelectrodes can be unstable and inefficient with limited opportunity for practical implementations, due to the leaching of

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the mediator implemented and the dependence of the electron transfer process on the capability of the mediator to be rapidly oxidised and reduced [15].

In recent years, some progress has been reported when using nanostructured electrodes, such as carbon nanotubes (CNTs), as electron acceptors. Due to their size and shape, these electrodes are able to intertwine with the enzyme and come in very close proximity to the enzyme FAD centre. Successful DET of GOx immobilised onto CNTs-based electrodes has been reported [16–18]. High surface area microelectrodes are also preferred in the context of an increasing trend towards the miniaturization of bioelectrochemical devices for applications such as implantable healthcare devices. However, the not yet fully addressed potential long-term toxicity of CNTs leads to controversial opinions on the feasibility of using CNTs for *in vivo* or cutaneous applications [19–23].

Nano-porous gold (nPG) electrodes (porous gold electrodes with a pore size distribution limited to the nanometre range) are considered a very promising alternative for the development of new generation bioelectrochemical devices with implantable capability. These non-toxic electrodes have remarkable properties, such as high conductivity, large surface area, three-dimensional open porosity, and biocompatibility [24]. In this context, an even more promising material for the production of high sensitivity biosensors is highly porous gold (hPG). While retaining the morphology observed with nPG, hPG electrodes present large micro-pores that are lined with nano-pores themselves [25]. As a consequence, hPG electrodes have a very wide pore size distribution, leading to extremely large surface areas and hence larger current densities in comparison to conventional nPG.

A new and rapid method of producing hPG electrodes by direct electrodeposition of porous gold films onto gold electrodes was recently reported [26]. These electrodes were characterised by a 3D foam-like structure, with a wide pore size distribution (ranging from 10 nm to 30  $\mu\text{m}$ ), and a roughness factor (calculated in terms of electrochemically effective surface area) approximately  $10^3$  times higher than polished gold. The hPG electrodes showed excellent glucose electrooxidation activity with a detection limit as low as 5  $\mu\text{M}$  [26]. However, the high specificity required for some applications, such as implantable biofuel cell devices where the fuel (e.g. glucose) and the oxidant (e.g. oxygen) are fed to the system as a mixture, demands the implementation of enzymatic electrodes [27].

The large surface area of hPG electrodes and their complex morphology make them an ideal support for enzyme immobilisation at high loadings. This hypothesis is encouraged by the successful production of GOx-immobilised nPG electrodes recently reported. In these cases the GOx immobilisation protocols involved the nPG functionalization with thiol-linker molecules or with conductive polymers, such as poly(3,4-ethylenedioxythiophene), to enhance the electron transfer process [24,28,29].

This study reports for the first time, an efficient, simple, cost-effective, and rapid method for the functional immobilisation of GOx onto hPG surfaces. The immobilisation protocol does not require any electrode pre-treatments with linker molecules or polymers and it is simple to reproduce. In particular, GOx is immobilised onto the hPG surface *via* a one-step electrochemical adsorption process in a phosphate buffer with no additional chemicals. The use of the resulting GOx-immobilised hPG electrode is tested for glucose sensing and for energy harvesting in a glucose/ $\text{O}_2$  enzymatic biofuel cell.

## 2. Experimental

### 2.1. Materials

Glucose Oxidase (GOx) from *Aspergillus niger*, Laccase (LAC) from *Trametes versicolor*, and all other reagents used were of

analytical grade and purchased from Sigma-Aldrich. All aqueous solutions were prepared with reverse osmosis purified water. Gold disk electrodes (2 mm diameter), saturated calomel electrodes (SCE) and platinum counter electrodes were purchased from IJCambria Ltd. Gold disk electrodes were polished between uses with a 0.3 micron aluminium oxide polish (Buehler).

All analytical experiments were performed in phosphate buffered saline (PBS). This was prepared with the following constituents: 137 mM NaCl, 2.7 mM KCl, 10 mM  $\text{Na}_2\text{HPO}_4$ , 2 mM  $\text{KH}_2\text{PO}_4$ . The pH of this solution was then adjusted to 7 with the drop wise addition of 1 M solutions of HCl and NaOH.

### 2.2. Deposition of highly Porous Gold (hPG) onto gold electrodes

The hPG was fabricated with a two-step potential process as previously described [26]. Briefly, the gold disk electrodes were immersed in an aqueous electrolyte consisting of 0.1 M  $\text{HAuCl}_4$  and 1 M  $\text{NH}_4\text{Cl}$ . Gold was then potentiostatically deposited in two steps. Firstly, the system was set at a working potential of -0.7 V (vs. SCE) for 5 s. Afterwards, the potential was stepped down to a value of -4.0 V (vs. SCE) for 15 s.

### 2.3. Immobilisation of GOx onto hPG electrodes

GOx was electrochemically adsorbed onto the prepared hPG disk electrodes by conducting a total of 6 CV scans between 0.42 V and 0.60 V (vs. SCE) at a scan rate of 1  $\text{mV s}^{-1}$ , in a PBS solution containing 0.45  $\text{mg ml}^{-1}$  GOx (approximately 8  $\text{U ml}^{-1}$  as per activity rating of manufacturer).

As a term of comparison of performance, GOx was also immobilised by absorption. In this case, the hPG electrodes were incubated with the GOx solution in PBS (0.45  $\text{mg ml}^{-1}$ ) for 1 hour at room temperature, without conducting any CV scans. In both cases, the GOx-hPG electrodes were then thoroughly rinsed three times with PBS to remove any weakly bonded enzyme, and stored in PBS at 4 °C until used.

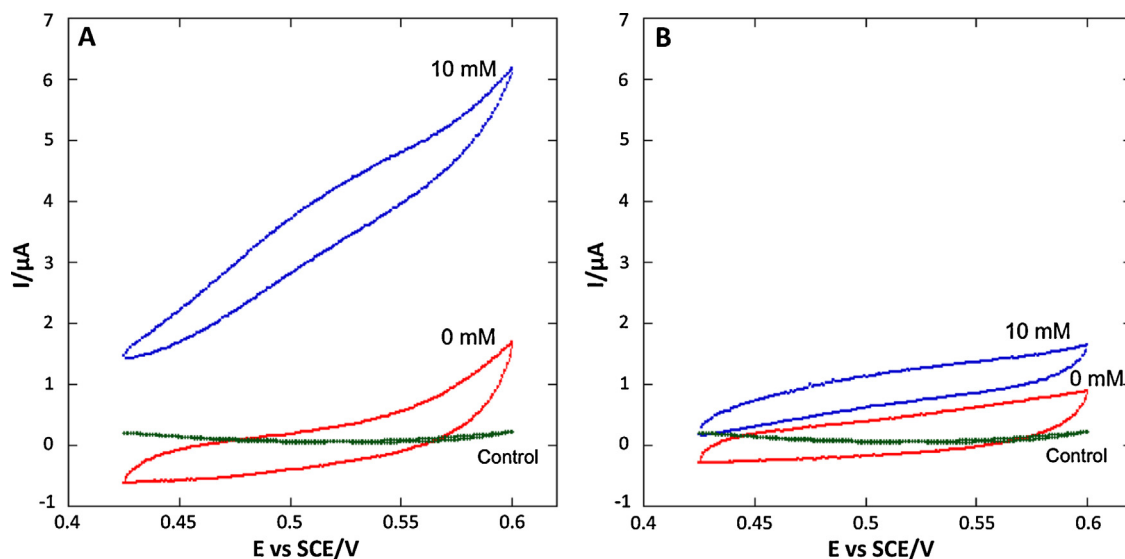
The amount of GOx immobilised onto the hPG electrodes, was estimated by performing a kinetic assay (provided by Megazyme Ltd.) of the enzyme solution before and after the immobilisation procedure and assuming no enzyme losses during the process.

### 2.4. Immobilisation of LAC onto hPG electrodes

The surface of the hPG electrodes was modified with a layer of amino-phenyl groups through a simple two-stage process. Firstly, a layer of nitro-phenyl groups was attached by performing two reductive CV scans at 100  $\text{mV s}^{-1}$  from 0.6 V to -0.6 V (vs. SCE), in the presence of an acetone electrolyte containing 2 mM *p*-nitrophenyldiazonium tetrafluoroborate and 100 mM tetrabutylammonium tetrafluoroborate. In the second stage, the nitro groups were exchanged for amino groups by conducting two reductive CV scans at 50  $\text{mV s}^{-1}$  from 0.0 V to -1.4 V (vs. SCE), in an aqueous electrolyte containing 10% v/v EtOH and 0.1 M KCl. Afterwards, the electrodes were transferred to a PBS solution containing 80  $\text{U ml}^{-1}$  of LAC. The electrodes were kept in this solution overnight at 4 °C in order to facilitate the covalent linkage between the amine functional groups on the gold, and the carboxylate groups on LAC. Finally, the electrodes were rinsed three times with PBS and stored in PBS at 4 °C until required.

### 2.5. Electrochemical Characterisation

All electrochemical processes were conducted using the Autolab PGSTAT128 N (Metrohm, UK) potentiostat. Cyclic voltammetry (CV) and amperometry tests were performed in a three-electrode electrochemical set-up with a SCE reference electrode and a platinum



**Fig. 1.** Electrochemical response to glucose in PBS of the hPG electrodes. A: CV scans of the  $\text{GOx}_{\text{ads}}$ -hPG electrode in the absence and presence of 10 mM of glucose. B: CV scans of the  $\text{GOx}_{\text{abs}}$ -hPG electrode in the absence and presence of 10 mM of glucose. The control was performed with the hPG electrode (no GOx) in the presence of 10 mM of glucose. The scan rate for each test was  $1 \text{ mV s}^{-1}$ .

rod counter electrode. CV scans were performed at a scan rate of  $1 \text{ mV s}^{-1}$  in the potential range of 0.42 – 0.60 V (vs. SCE) in order to determine the optimum potential for the amperometric analysis (this range incorporates the normal oxidation potential of  $\text{H}_2\text{O}_2$  on polished gold). CV analyses were repeated over a period of three days to determine the stability of the GOx-hPG electrodes.

The amperometry tests were performed at a fixed potential (0.52 V) during the step-wise increase of glucose concentration. After each concentration increase, the solution was agitated for 20 s and then left for 270 s to allow the amperometric response under convective mixing to stabilise. The average amperometric response over a period of 10 s was then reported. Three replicates were performed per each experiment. In each case, a glucose concentration range of  $5 \mu\text{M}$  – 50 mM was considered.

## 2.6. Biofuel Cell Setup

The biofuel cell consisted of a glass vial (28 ml in volume) containing a  $\text{GOx}_{\text{abs}}$ -hPG electrode and a LAC-hPG electrode as anode and cathode respectively. The electrode spacing was approximately 0.5 cm. The system was fed with 12 ml of an aerated PBS solution containing 27.8 mM glucose at pH 7. The potential difference ( $\Delta V$ ) between the two electrodes was measured in open circuit mode until steady state conditions were achieved. Afterwards, the cell potential was measured under a range of different external resistances applied to the system.

## 3. Results and Discussion

### 3.1. GOx-hPG electrodes

The purpose of this study was to investigate the possibility to develop a more rapid and reproducible immobilization protocol of GOx onto hPG electrodes with respect to those so far reported that involve the use of thiol groups or conductive polymers [24,28]. This was achieved by running a series of CV scans of a hPG electrode in a GOx buffer solution, with the aim of increasing enzyme loading and affinity through the use of electrostatic forces.

The performance of the resulting bioelectrode was compared with a hPG electrode with GOx immobilised by absorption, as described in Section 2.3. Fig. 1 compares the CV curves produced with the two GOx-hPG electrodes in the presence of glucose. In

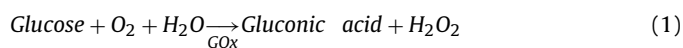
both cases, no mediators were added to the electrolyte solution. As shown, when the  $\text{GOx}_{\text{ads}}$ -hPG electrode was prepared electrochemically ( $\text{GOx}_{\text{ads}}$ -hPG electrode) a more prominent response to glucose was observed compared to the electrode produced by absorption ( $\text{GOx}_{\text{abs}}$ -hPG electrode). The CV scan of the  $\text{GOx}_{\text{ads}}$ -hPG electrode shows in particular a shoulder peak between 0.5 V and 0.525 V vs. SCE.

The difference in the CV curves for the two electrodes suggests that a different type of linkage between the enzyme and hPG electrode surface occurs in the two cases. The immobilisation mechanism achieved during the CV scans in the presence of GOx is not merely *absorption*, but an electrochemically driven physical *adsorption*. Since GOx is anionic at pH 7.0, and since a relatively high positive scan range is used, the CV scans are likely to promote an electrostatic attraction between the gold surface and the free enzyme. In contrast, when the electrodes are simply placed in contact with the enzymatic solution, absorption occurs into the pores of the electrode. Consequently, the resulting electrode is less stable as GOx can easily leach out.

The CV scans might actively draw the enzyme to the surface of the electrode, thus significantly increasing the loading. For the case of electrochemical adsorption in fact an average of 31.7% reduction of activity in solution after the immobilisation process was observed. On the other hand, the amount of enzyme immobilised by absorption was so low that no significant changes were observed with the activity tests in solution prior and after the incubation with the hPG electrode.

The amperometric response of the two GOx-hPG electrodes to glucose (10 mM) was tested and compared at a potential of 0.52 V vs SCE (the potential at which the electrodes showed the highest sensitivity to glucose).

This potential is within the 0.4 – 0.6 V range of values previously reported for the oxidation peak of  $\text{H}_2\text{O}_2$  on polished gold [11,24].  $\text{H}_2\text{O}_2$  is formed through the oxidation of glucose by GOx and, since its formation is related to the amount of glucose in solution (see Equation (1)), many glucose sensors rely on the resultant oxidation of  $\text{H}_2\text{O}_2$  on gold to determine the concentration of glucose in a sample [30].



**Table 1**

Comparison of the amperometric response at 0.52 V vs SCE of the GOx-hPG electrode to 10 mM of glucose, according to the immobilisation methodology. Standard deviation refers to three replicates.

Electrode	$I/\mu\text{A}$	Sensitivity/ $\mu\text{A mM}^{-1} \text{cm}^{-2}$
hPG	$0.07 \pm 0.03$	
GOx <sub>abs</sub> -hPG	$1.6 \pm 0.2$	$2.42 \pm 0.05$
GOx <sub>ads</sub> -hPG	$6.10 \pm 0.12$	$22.7 \pm 0.1$

The amperometric tests confirmed that the GOx<sub>ads</sub>-hPG electrode performed better than the GOx<sub>abs</sub>-hPG electrode. As reported in Table 1, the response of the GOx<sub>ads</sub>-hPG electrode is in fact approximately four times higher than the GOx<sub>abs</sub>-hPG electrode, with a sensitivity of  $22.7 \pm 0.1 \mu\text{A mM}^{-1} \text{cm}^{-2}$ , calculated against the superficial surface area of the bioelectrode ( $0.03 \text{cm}^2$ ). In comparison, the amperometric response of a blank hPG electrode to glucose at 0.52 V is negligible. This is because the current peak related to glucose electrooxidation by the non-enzymatic hPG electrode occurs at a potential of 0.15 V [26].

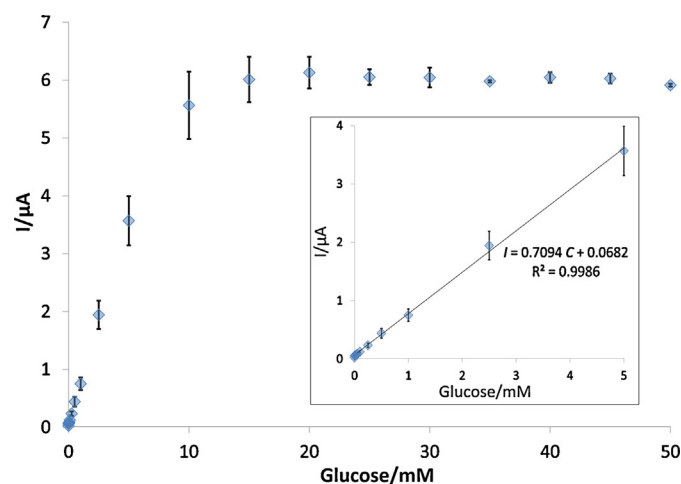
### 3.2. The use of the GOx<sub>ads</sub>-hPG electrode as glucose sensor

The amperometric response of the GOx<sub>ads</sub>-hPG electrode to increasing concentrations of glucose at a constant potential of 0.52 V (vs SCE) was investigated. A typical Michaelis-Menten kinetic trend was observed. This was characterised by a linear increase in the current output up to a concentration of 10 mM as shown in Fig. 2. The detection limit the system was found to be  $25 \mu\text{M}$ , which is lower compared to other GOx electrodes reported previously [28,31]. At least three replicates were performed using different electrodes, showing a good reproducibility of the electrode performance with a maximum standard deviation factor of 0.38 (at  $5 \mu\text{M}$  of glucose).

The enzymatic affinity towards glucose was estimated in terms of the apparent Michaelis-Menten constant ( $K_M^{\text{app}}$ ), and was calculated by using the electrochemical version of the Lineweaver-Burk equation of enzyme kinetics (Equation (2)) [13].

$$\frac{1}{i} = \frac{1}{i_{\text{max}}} + \frac{K_M^{\text{app}}}{i_{\text{max}}c} \quad (2)$$

Where  $i$  is the steady-state current observed after the addition of glucose;  $i_{\text{max}}$  is the maximum current under the saturated concentration of glucose;  $c$  is the glucose concentration. The reciprocal of the current was plotted versus the reciprocal of the glucose



**Fig. 2.** Amperometric tests (at 0.52 V vs SCE) of the GOx<sub>ads</sub>-hPG electrode in the presence of various glucose concentrations in the electrolyte (range of  $5 \mu\text{M}$  - 50 mM). Error bars refer to three replicates.

concentration in the range of 0.005–10 mM. This gave a value of  $K_M^{\text{app}}$  equal to  $6.3 \pm 0.7 \text{mM}$ , which was calculated from the slope ( $K_M^{\text{app}}/i_{\text{max}}$ ) and the intercept ( $1/i_{\text{max}}$ ) of the plot. This value is much lower than the one reported for the native GOx from *Aspergillus niger* in solution (27 mM) [32]. This indicates that the immobilised GOx onto hPG electrodes has high enzymatic activity and higher affinity for glucose than the soluble enzyme with a low diffusion barrier. Although this value is slightly higher than the value of 1.5 mM recently reported for a GOx/chitosan/ferrocene carbon nanotubes electrode [13], it is, however, comparable with the value (6.3 mM) reported for a ferrocene-modified multiwalled carbon nanotube [12], and much lower than other reported GOx electrodes [33,34].

Table 2 compares the sensitivity to glucose of the GOx<sub>ads</sub>-hPG electrode with other GOx electrodes created by using much more complex immobilisation procedures [12–14,21,35,36]. As reported, the GOx<sub>ads</sub>-hPG electrode sensitivity is greater than the majority of the other methods reviewed, including some that boast direct electron transfer between the FAD centre of GOx and the electrode surface.

### 3.3. Stability

The GOx<sub>ads</sub>-hPG electrodes were stored in PBS at  $4^\circ\text{C}$  and tested again at regular intervals of time to investigate their stability. In particular, Fig. 3A compares the CV scans obtained immediately after the electrode fabrication, and after 24 and 48 hours, respectively.

A reduction in the current signal was observed within the three days, with the largest variation occurring after 24 hours of storage. After the first 24 hours of storage, a 35% reduction in the current peak at 0.52 V was observed. Interestingly, after the first 24 hours of storage, a marked change in the blank (e.g. CV test in the absence of glucose) was also observed, while no further changes occurred in the following days. The reduction in the response observed was lower after 48 hours, with a 25% decrease in the current peak at 0.52 V with respect to data collected at 24 hours.

This suggests that the initial variation in the activity of the bioelectrodes might be caused by a reduction in the hPG sensitivity towards  $\text{H}_2\text{O}_2$ , rather than simply to enzyme deactivation and/or leaching. The reduction in the electrochemical activity of nPG structures with the time has been previously reported and attributed to a coarsening process [37]. This hypothesis was supported by stability tests carried out on the hPG electrode with no enzyme immobilised. When the hPG electrode was tested in PBS after one day, a change in the CV curve was observed, which was minimal after another day of storage (see supplementary data, Figure S1).

The stability of the GOx<sub>ads</sub>-hPG electrodes in terms of amperometric response was also investigated during a period of time of five days. As shown in Fig. 3B, the linearity range and the detection limit did not change. However, the magnitude of the amperometric response diminished by a maximum factor of 3.60 over the 5 days tested. In considering practical applications, the GOx<sub>ads</sub>-hPG electrode response should be normalised and calibrated prior to use to achieve concurrent data sets. After data normalisation, the variance observed between data sets obtained on different days for concentrations of glucose between  $50 \mu\text{M}$  and 5 mM was minimal (see supplementary data, Figure S2).

### 3.4. GOx electrode in enzymatic biofuel cell

The functional immobilisation of LAC was achieved by activating the hPG surface with amino-phenyl groups as previously reported [38]. The steps in the immobilisation protocols are reported in Fig. 4. Firstly, nitrophenyl groups were electrochemically attached to the gold by conducting two reductive CV scans in the presence of p-nitrophenyldiazonium salt. In order to limit the production



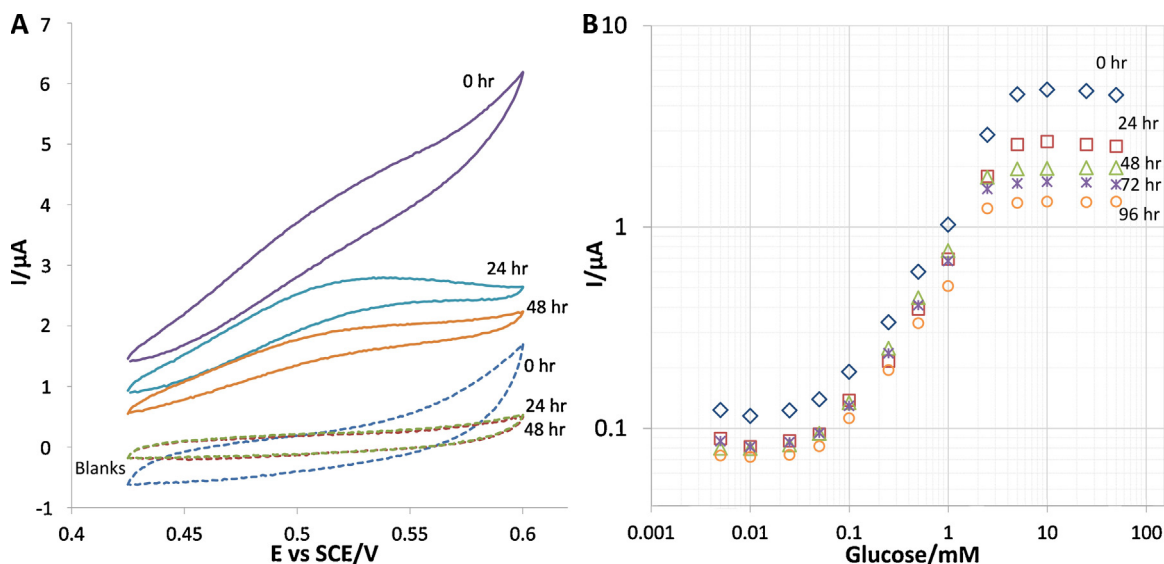
**Table 2**  
Comparison of the sensitivity to glucose of several GOx electrodes. \*Standard deviation refers to three replicates. \*\*Methods which claim to achieve direct electron transfer.

Electrode	Sensitivity/ $\mu\text{A mM}^{-1} \text{cm}^{-2}$	E vs SCE/V	Reference
GOx <sub>ads</sub> -hPG *	22.7 ± 0.1	0.52	This Study
Naf/GOx-NPG/GCE	0.697	0.40	[14]
Fc/GOx-MWCNT/Chi-BSA- AuE	7.77 ± 0.08	0.17	[13]
GOx-Fc/MWCNT/Chi-GCE	25	0.31	[12]
Gel/GOx-WMCNT/GCE **	2.47	-0.48	[35]
AuNPs/GOx/Biofilm-MWCNT/GCE **	16.6	-0.40	[36]
PDDA/AuNPs-MWCNT/GE **	29.72	-0.49	[21]

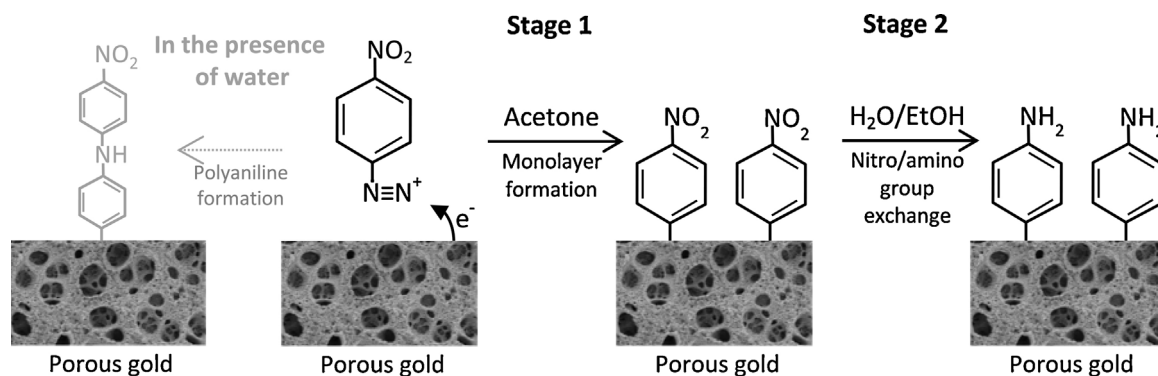
Naf: Nafion; GCE: glassy carbon electrode; Fc: Ferrocene; MWCNT: multi-walled carbon nano-tubes; Chi: Chitosan; BSA: bovine serum albumin; AuE: gold electrode; Gel: gelatine; AuNPs: gold nano-particles; PDDA: poly(diallyldimethylammonium chloride); GE: graphene electrode

of polyaniline (by the polymerisation of the phenyl groups through hydrolysis), and to promote the formation of a monolayer, this stage was conducted in an acetone-based electrolyte. The CV scans conducted show two peaks (at 400 mV and -200 mV vs. SCE) on the first scan that greatly decrease on the second scan (Figure S3). These peaks are attributed to the reduction of the diazonium salt on the different planar surfaces of gold and crystalline gold as previously suggested for the case of an acetonitrile electrolyte [39]. Since the phenyl-gold bond formed is not oxidised back in this scan range, the large decrease in the magnitude of the peaks on the second scan suggests that the surface is quickly becoming saturated with the nitro-phenyl groups [8,39,40].

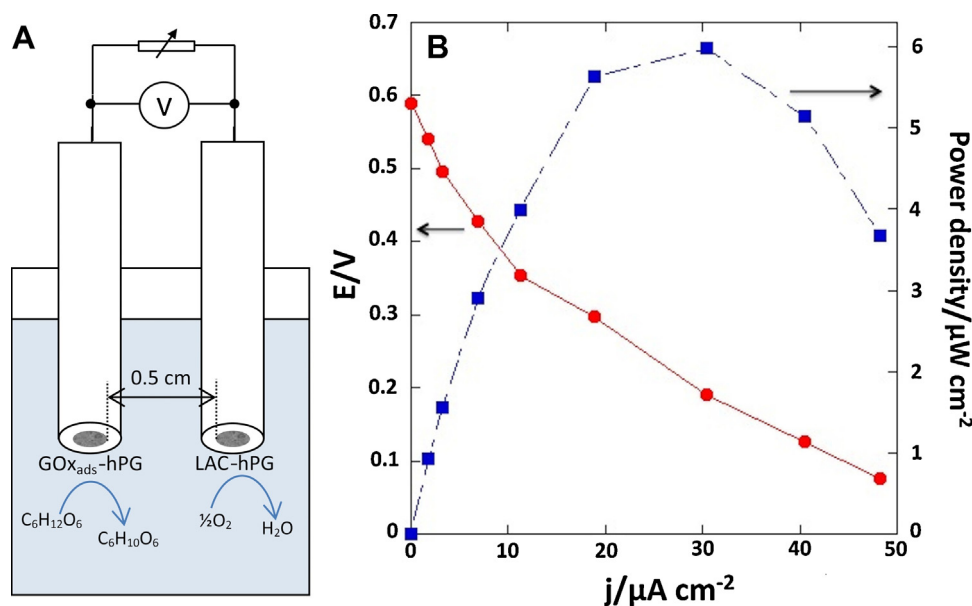
During the second stage, the treated electrodes were immersed in a 10% ethanol and water based electrolyte and two more reductive CV scans were conducted in a much more negative scan range (0.0 V and -1.4 V vs. SCE). This facilitated the exchange of oxygen for protons on the nitro groups resulting in the production of amino and hydroxylamino groups. The voltammogram of the first scan shows a very clear peak at -820 mV which is established as the potential at which nitrophenyl groups are irreversibly reduced to aminophenyl groups [8]. This peak is not evident on the second scan though a small redox peak is observed at -700 mV (Figure S4). This results suggests the presence of hydroxylamine groups amongst the amine groups [41].



**Fig. 3.** Stability tests. A: CV scans of the GOx<sub>ads</sub>-hPG electrode after 0, 24, and 48 hours for a glucose concentration of 10 mM. The blanks were performed in the absence of glucose in the electrolyte. B: Amperometric response of the GOx<sub>ads</sub>-hPG electrode to increasing concentration of glucose over a period of five days of storage.



**Fig. 4.** Schematic of gold surface modification prior to LAC immobilisation.



**Fig. 5.** A: Scheme of the enzymatic biofuel cell set up. B: Polarisation and power density curve of the GOx<sub>ads</sub>-hPG and LAC-hPG biofuel cell. The power and the current density refer to the total superficial surface area of the electrodes used (0.03 cm<sup>2</sup>).

The GOx<sub>ads</sub>-hPG and the LAC-hPG electrodes were subsequently tested as anode and cathode respectively of a glucose/oxygen enzymatic biofuel cell. The electrodes were immersed in an aerated PBS solution containing 27.8 mM of glucose, and the potential difference of the cell was monitored by means of a potentiostat (Fig. 5). An open circuit voltage of 0.58 V was observed, comparable with the value previously reported [27]. Subsequently, the cell was polarised by connecting the electrodes to a range of external resistors. Fig. 5B shows the cell voltage and the power density as a function of the current density. As shown, the peak power density was of 6  $\mu\text{W cm}^{-2}$  at 0.2 V vs SCE. This result, although obtained with a very simple design, is comparable with recently reported miniature enzymatic biofuel cells [27,42].

#### 4. Conclusions

Glucose oxidase was immobilised onto highly porous gold electrodes by electrochemical adsorption, with a rapid one-step protocol that did not involve the use of expensive and/or harsh reagents. The GOx<sub>ads</sub>-hPG electrode exhibits Michaelis-Menten kinetics with a wide linearity range between 50  $\mu\text{M}$  and 10 mM of glucose, and a detection limit of 25  $\mu\text{M}$ .

The sensitivity, calculated against the total superficial surface area of the bioelectrode was of  $22.7 \pm 0.1 \mu\text{A mM}^{-1} \text{cm}^{-2}$ . After five days of storage the bioelectrode amperometric response to glucose was 3.6 times lower compared to the initial value. However, the linearity range and the detection limit were maintained.

Preliminary experiments encourage the implementation of the GOx<sub>ads</sub>-hPG electrode as the anode of an enzymatic biofuel cell. When the electrode was coupled with a LAC-hPG electrode as a cathode, a peak power density of 6  $\mu\text{W cm}^{-2}$  at 0.2 V vs SCE was achieved. Future work will be regarding the implementation of the enzyme hPG electrodes in a micro biofuel cell for healthcare applications.

#### Acknowledgments

The authors acknowledge: Prof Frank Marken, University of Bath, for valuable discussions; the Royal Society Research Grant (RG110344) and EPSRC (EP/K031597/1) for funding.

#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.electacta.2014.06.074>.

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