Electronic Supplementary Information for:

An optimised glucose oxidase bioelectrode exhibiting high performance direct electron transfer

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Figures

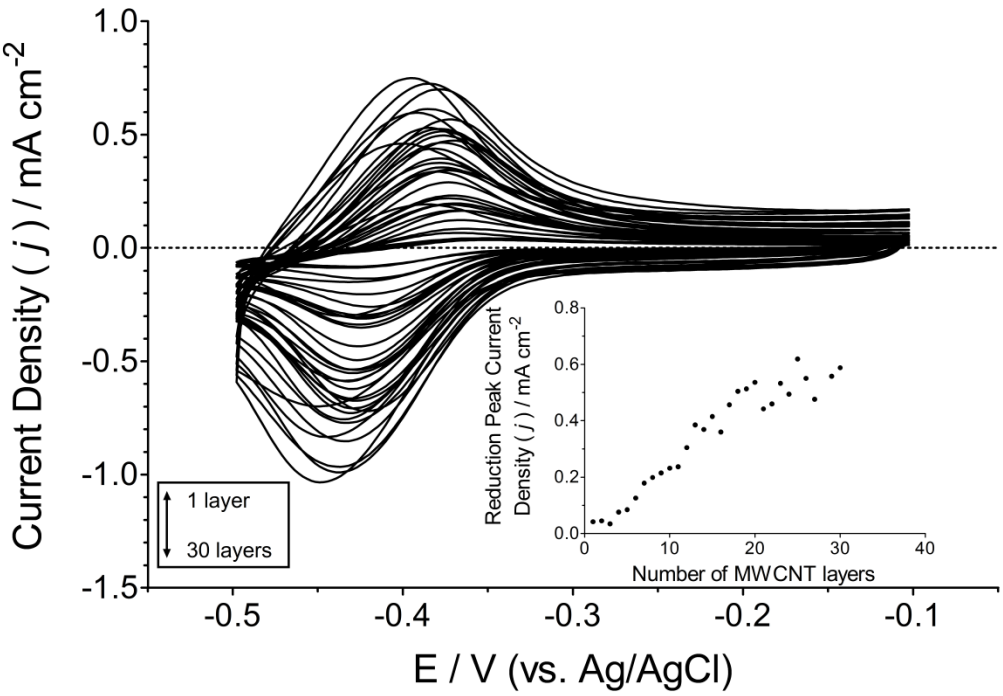

Fig. S.1. – Cyclic voltammograms showing the effect on current intensities by increasing the number of MWCNT "primer" layers), at a scan rate of 100 mV s^{-1} with citrate buffer electrolyte (0.2 mol dm^{-3}, pH = 6) and a GOd loading of 1 mg. The starting potential was -0.1 V.

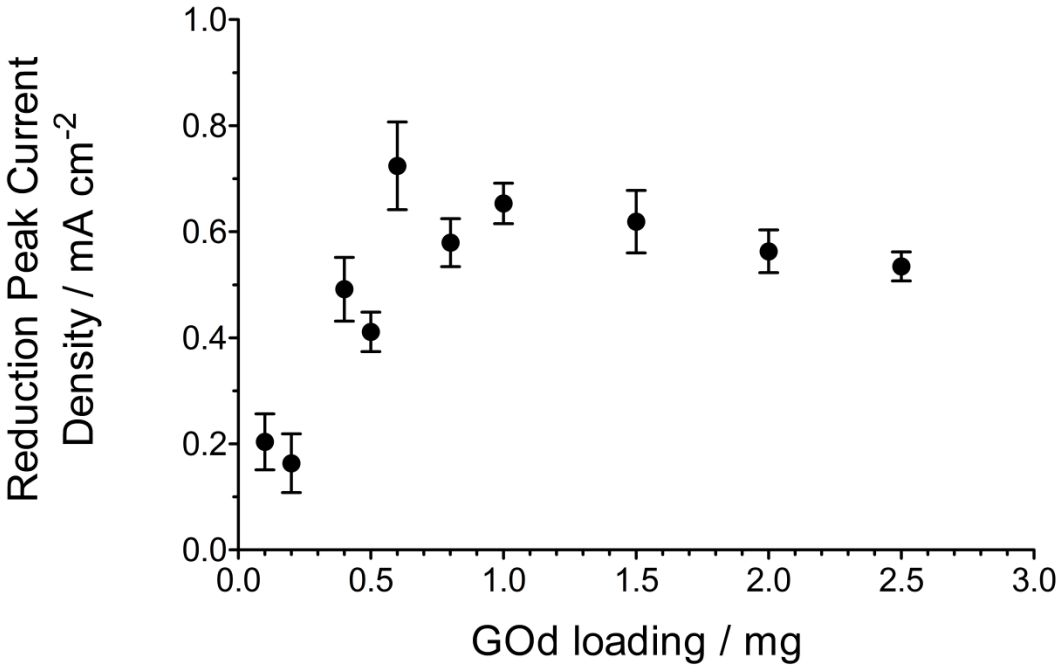

Fig. S.2. – The modulus of reduction peak current *vs.* enzyme loading (GOd) extracted from cyclic voltammograms, at a scan rate of 100 mV s^{-1} with citrate buffer electrolyte (0.2 mol dm^{-3}, pH = 6) and a 20 MWCNT layers, n = 3. The starting potential was -0.1 V.

Calculation of the Turnover Rate (TR)

$$i_P = \frac{n^2F^2}{4RT}\nu A\Gamma^*_O \qquad \text{Equation } 1^1$$

The surface coverage of the Method 4 bioelectrode was determined using Equation 1, where i_P = peak current, n = number of electrons transferred, F = Faraday's constant, R = gas constant, T = temperature, ν = scan rate, A = area of electrode and Γ^*_O = surface coverage. The surface coverage of GOd on the method 4 bioelectrode was calculated to be 1.398 x10^{-12} mol cm^{-2}.

$$TR = \frac{I_{cat}/_A}{nF\Gamma_{GOd}} \qquad \text{Equation } 2^2$$

The turnover rate at a saturated electrode was calculated by using equation 2, where I_{cat} is the current at the glucose saturation. The turnover rate was calculated to be 74.1 s^{-1}.

Chemicals

Nanocyl–3100 MWCNTs were supplied by Nanocyl (Belgium). GOd (Aspergillus niger, 200 U mg^{-1}), glucose, microcrystalline cellulose, 1–ethyl–3–methylimidazolium acetate

(EMIM–acetate), citric acid, and sodium citrate were purchased from Sigma–Aldrich (UK). Partially oxidised MWCNTs were prepared by refluxing in aqueous HNO_3 (2.6 mol dm^{-3}) for 10 h, rinsing with water until a neutral pH was obtained and drying the product in an oven at 80°C for 12 h[3]. Glucose solutions were allowed to mutarotate for 1 d and were kept refrigerated. Ultrapure water was used to prepare all solutions (18.2 MΩ cm^{-1}, Select Fusion system from Purite, U.K.).

Instrumentation and Procedures

Electrochemical analyses were carried out using an Autolab PGSTAT302N (EcoChemie, Netherlands) in a 15 cm^3 three electrode water–jacketed cell at 22 ± 1°C with a Pt wire counter electrode, and an Ag/AgCl (NaCl(aq), 3 mol dm^{-3}, +0.196 V vs. SHE at 298 K) reference electrode. An aqueous citrate buffer (0.2 mol dm^{-3}, pH = 6) purged with either high–purity nitrogen or air was used as electrolyte.
Before use, glassy carbon electrodes (GC, diameter = 3 mm) were polished with 0.5 µm alumina, and ultrasonically washed in deionised water. Stable MWCNT dispersions in water (0.1 mg cm^{-3}) were obtained by sonication for 5 min. The EMIM–acetate/MWCNT/cellulose matrix was prepared as follows: cellulose (3%w/w) was dissolved in EMIM–acetate at 70°C for 1 h under sonication, and this solution was then ground with MWCNT (0.1%w/w) in an agate mortar under nitrogen. The resulting dispersion was transferred to a sealed tube and stored in a desiccator until use.

In method 1, 10 µL of the MWCNT/EMIM–acetate/cellulose matrix was applied to the electrode surface; the MWCNT/EMIM–acetate/cellulose matrix modified electrode was then soaked in ultrapure water to remove the EMIM–acetate from the matrix, yielding a MWCNT–embedded cellulose layer on the electrode surface. A 10 µL GOd aliquot (80 mg cm^{-3}) was then added onto the MWCNT/cellulose layer.

Method 2 involved the direct dissolution of the lyophilized enzyme powder (80 mg cm^{-3}) with the MWCNT/EMIM–acetate/cellulose matrix. The mixture was sonicated for 1 h to maximise dispersion. The matrix was added to the electrode and the EMIM–acetate was removed as before.

Method 3 involved enzyme adsorption in reverse order to method 1. A 10 µL aliquot of the GOd solution (80 mg cm^{-3}) was added to the electrode surface and dried. The MWCNT/EMIM–acetate/cellulose layer was then added to the electrode and the EMIM–acetate removed as before.

Method 4 involved the pre–treatment of the GC electrodes with MWCNT by depositing successive layers (20 layers) of 10 µL aliquots of aqueous MWCNT dispersion (0.1 mg cm^{-3}). The electrodes were then prepared in an identical fashion to method 3.

During bioelectrode preparation, good surface coverage was achieved for method 4 following the addition of the MWCNT/cellulose layer, whilst surface defects were observed by eye for methods 1 – 3. Method 2 exhibited poor repeatability and the low magnitude of the observed currents is most probably due to loss of enzyme activity

during sonication. Desorption of GOd from the MWCNT/cellulose matrix was observed during electrochemical analysis of method 1 bioelectrodes, which again highlights the importance of the MWCNT/cellulose encapsulation.

References

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