LOW-DENATURAZING GLUCOSE OXIDASE IMMOBILIZATION ONTO GRAPHITE ELECTRODES

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GOx electrochemical characterization

The bioelectrodes obtained from the incubation in chitosan solutions of GOx of 10 mg/ml were named LDG-CH10. The bioelectrodes prepared by incubating in PBS solutions will be referred to as LDG-PBS10. For all the electrodes assayed the concentration of the enzyme and the electron transfer rate constant have been calculated (see details in Table S1).

Table S1. Enzyme coverage and electron transfer rate constant obtained for PBS- and Chitosan-incubated bioelectrodes.

BIOELECTRODE	INCUBATION SOLUTION	GOx (mg/ml)	Γ (nmol/cm^2)	k (s^{-1})
LDG -CH10	CHITOSAN	10	3.99	2.2
LDG -PBS10	PBS	10	3.01	1.1

Regarding electron transfer kinetics, the redox peaks appear in a region that might be consistent with oxidation/reduction of the GOx active site (FAD) since the redox potential of GOx in solution determined by Vogt and co-workers by UV/vis spectroelectrochemistry was -0.385 V vs Ag/AgCl at pH = 7.4 [48]. These kinetics rates obtained are also consistent with previously reported electron transfer kinetics rates (k) of adsorption of FAD in GOx in graphite electrodes [40]. Thus, it has been established that the k values obtained are probably due to a positive effect of chitosan acting as "wire" for the electrons from the enzyme to the electrode [45]. Additionally, it is worth to mention that these values are inconsistent with direct electron transfer effect since long range electron transfer over 1.7 nm would be required to transfer electrons directly from FAD to the electrode surface which dismiss direct electron transfer effect. Additionally, the electron transfer kinetics obtained by Laviron model indicate again that there is no evidence of DET, as previously reported in literature since k values in this case would be higher than 6 s^{-1} [9].

KCl influence in LDG electrode surface

Firstly, FAD irreversible absorption using a concentration of FAD of 10 µg/ml has been tested using KCl (Figure S1). As can be seen in Figure S1A, FAD peaks do not disappear after the treatment which proves the irreversible adsorption of the cofactor. It is worth to mention that, although the electrodic surface of LDG are always homogenized before each modification by coarse and fine emery paper followed by sonication (see experimental section for more details), KCl influence electrodic surfaces. Thus, it leads to an increasement in capacitive current as can be seen in Figure S1B.

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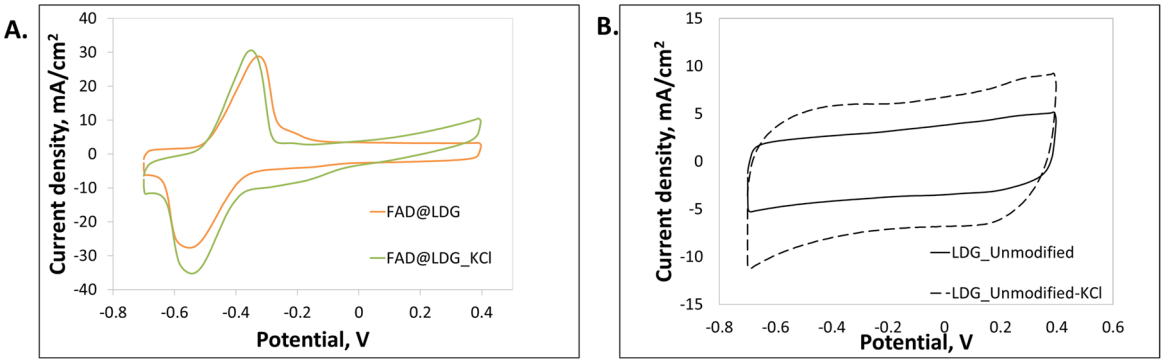

Figure S1. KCl treatment effects in different electrodes: (A) FAD modified electrodes (orange solid line) and FAD modified electrodes treated with KCl (green solid line) and, (B) bare LDG electrodes before (solid line) and after KCl treatment (dashed line).

Electrochemical characterization of graphite modified electrodes.

Cyclic voltammetry of the modified graphite electrodes in oxygen-free electrolyte and plot of the anodic and cathodic currents obtained versus the scan rate for the chitosan-mediated GOX electrodes is shown in Figure S2. Additionally, the shift in the anodic and cathodic peaks with the scan rate and the linear relationship between the peak currents and scan rate show for the four different bioelectrodes prepared, using 4 and 10 mg/mL GOx in PBS and chitosan-mediated immobilization, showing the typical behaviour of a surface confined redox process.

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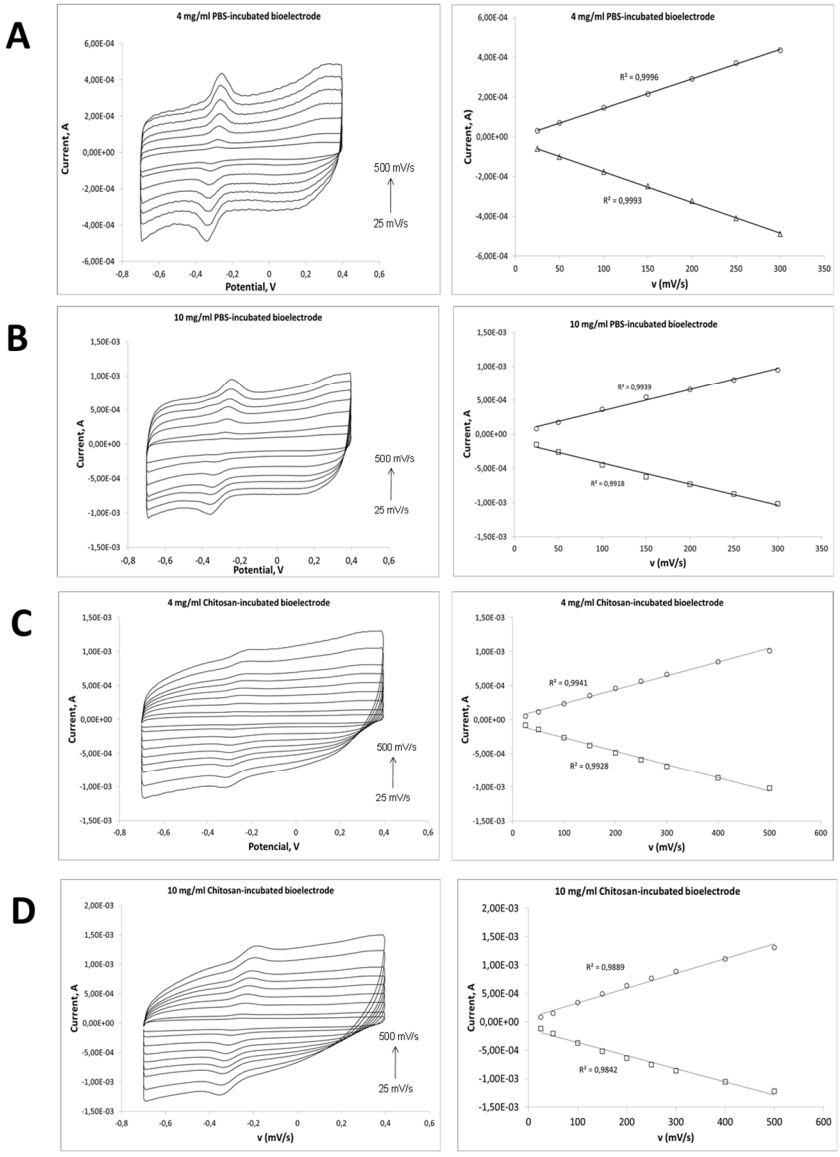

Figure S2. GOx immobilization for in PBS for (A) 4mg/ml GOx (LDG-PBS4) and (B) 10mg/mL GOx, (LDG-PBS10) and in chitosan for (C) 4mg/ml GOx (LDG-CH4) and (B) 10mg/mL GOx, (LDG-CH10). (Left) cyclic voltammetry in an O_2 free atmosphere at 25, 50, 100, 150, 200, 250, 300, 400 and 500 mV/s. and (rigt) Evolution of peak current with scan rate.

Additionally, pH influence has been also analysed. Here is worth to mention that less acid pH was not possible to be assayed since they will affect GOx stability. Moreover, formal potentials exhibit a linear dependence of pH ranging from 5 to 9 (Inset of Figure S3B) with a slope around to -50 mV/pH (R^2 = 0.9964), a value close to the theoretical -59.2 mV/pH for the two-electron and two-proton coupled reaction [11]. In addition, the linear relationship between peak current and scan rate implies that the bioelectrode presents the typical behavior of a surface-confined redox process with anodic and cathodic peak separation characteristic of quasi-reversible redox systems with a mean value lower than 100 mV [47].

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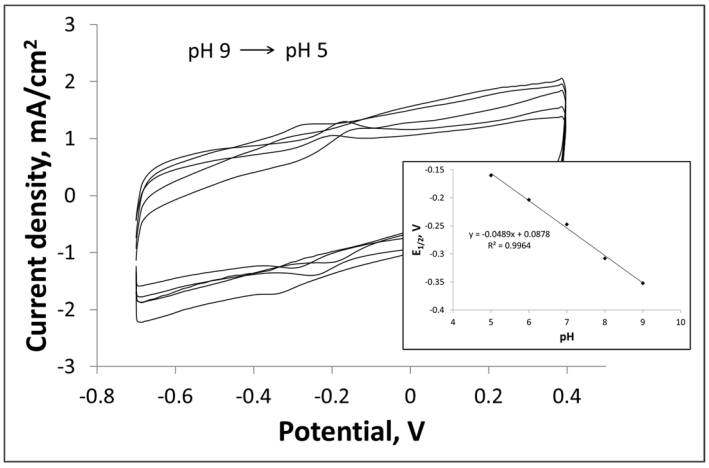

Figure S3. pH dependence of redox peaks in for the LDG-CH10 bioelectrode. Voltammograms acquired at 25 mV/s.

GOx activity and inhibition assays

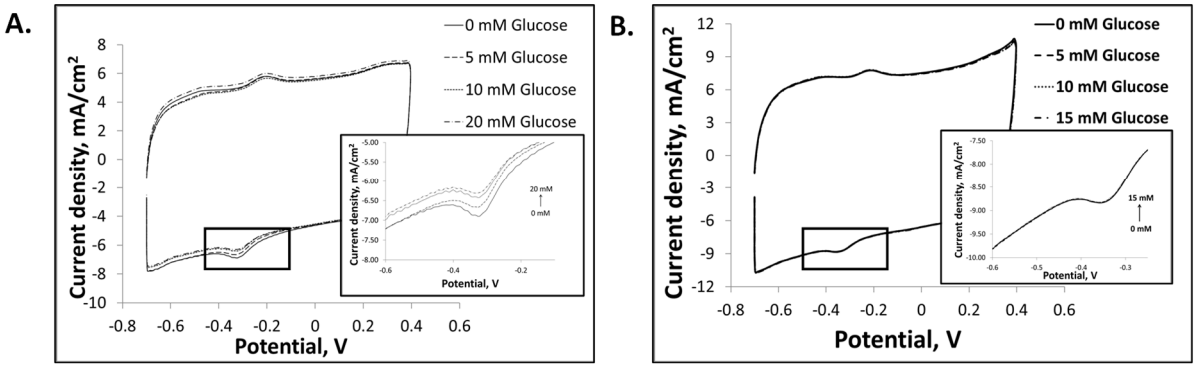

Figure S4. Cyclic voltammetry in oxygen-free PBS solution of LDG-CH4 bioelectrode A) with increasing glucose concentrations and B) with increasing glucose concentration in the presence of the inhibitor Ag+. Voltammograms acquired at 0.1 V/s

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