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Evaluation of Hierarchical Glucose Oxidase/Co₃Mn-CO₃ LDH Modified Electrodes for Glucose Detection

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Abstract

A glucose amperometric biosensor has been prepared by a multilayer deposition on a glassy carbon electrode of Co₃Mn-CO₃ layered double hydroxide, glucose oxidase (GOx) and a biopolymer (carrageenan), respectively. The original transduction step consists in the electrocatalytic oxidation of H₂O₂, enzymatically formed, in the Co₃Mn-CO₃ inner-layer at 0.65 V/Ag-AgCl. The sensitivity and the detection limit, obtained with this optimized bioelectrode (Co₃Mn-CO₃/GOx/carrageenan, 30/20/10 μg), are 12.8 mA M⁻¹ cm⁻² and 0.02 mM, respectively. A 3D-porous bioelectrode was also prepared by impregnation of a carbon felt (CF) with a colloidal suspension of Co₃Mn-CO₃ nanoparticles and then with GOx. The structure and the morphology of the Co₃Mn-CO₃/CF composite were characterized by X-ray diffraction and electron scanning microscopy and the bioelectrode GOx/Co₃Mn-CO₃/CF was finally tested in cyclic voltammetry in the presence of glucose.

Keywords: CoMn Layered Double Hydroxides, Electrocatalyst, Glucose oxidase, Glucose, Hydrogen peroxide.

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1. Introduction

Due to unique high surface-to-volume ratios, 2D materials that display specific electrical or optical properties are very sensitive towards interactions with analytes. Such particular ability to modulate their surface properties make 2D materials suitable for developing sensing platforms [1, 2]. For instance, 2D inorganic compounds, called Layered Double Hydroxides (LDH), have been investigated for their applications in the determination of molecules of biological interests, such as H₂O₂, dopamine or glucose [3, 4]. The structure of LDH is related to that of brucite, M^{II}(OH)₂ in which some of the M^{II} cations are replaced by trivalent cations and intercalated anions between the layers maintain the electroneutrality. Their modularity, concerning i) the nature of metal cations in the sheets, i.e. multi-valent transition metals (TMs) (Mn, Fe, Co or Ni), ii) the nature of interlayer species, i.e. redox active anions, makes LDH, hybrid LDH and, composites based-LDH very good candidate materials as catalysts. These catalytic activities can be coupled advantageously with enzymatic reactions and enable

the development of sensitive analytical devices [4]. Due to these unique properties, LDHs may behave then as multifunctional materials either as supports for redox active anions, host structures for enzyme and/or co-catalysts, as illustrated below with the various glucose oxidase (GOx)/LDH systems.

Due to the physiological importance of glucose, the stability of glucose oxidase and the diversity of sensing methods, this enzyme is often used as a model enzyme to test new biosensors or analytical methods. In this enzymatic reaction, the oxidation of glucose into gluconolactone is carried out by the flavin adenine dinucleotide (FAD) component of GOx which is converted into FADH₂. The latter is re-oxidized to the FAD either by the dioxygen (the GOx cofactor) with the production of hydrogen peroxide (H₂O₂) or by a redox mediator under anaerobic conditions. One of the key points in analytical methods for glucose determination using GOx is therefore an accurate measurement of the H₂O₂ concentration whatever the optical or the electrochemical transduction method selected.

Intrinsic peroxidase-like activity of TM-LDHs, i.e. NiAl [5], CoAl [6, 7], NiCo [8], NiFe [9, 10],

CoFe [11, 12], has been reported in the literature. In the presence of H₂O₂, the oxidation of typical peroxidase substrates, i.e. tetramethylbenzidine (TMB), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS,) *o*-phenylenediamine (OPD) or luminol, is catalyzed by these TM-LDHs, leading to a change in the UV-Vis absorbance spectra that enables an optical quantification of H₂O₂. Exfoliated LDH phases or 3D LDH hierarchically porous structures exhibited higher catalytic activity due to a better accessibility to active sites [6, 7]. This spectrophotometric method for H₂O₂ detection was adapted to monitor glucose. CoAl LDH/TMB [6, 7] and NiCo LDH/ABTS [8] mixtures, incubated with GOx in neutral pH solutions, exhibited sensitive responses to glucose over concentration ranges of 25 - 500 μ M and 0.5 - 100 μ M, respectively. The NiFe LDH/TMB system displayed a catalytic activity in acidic solution (pH 4.0-4.5). Thus the coupling with the GOx reaction must be realized with a two-step procedure where GOx and glucose were first incubated under neutral pH condition and then the UV-Vis measurement was carried out after addition of the NiFe LDH/TMB mixture in an acidic solution [9, 10]. In this case, a linear calibration of glucose was established between 0.5 and 16 μ M [10].

As an alternative to these time-consuming colorimetric methods, the electroanalytical methods offer rapid and more sensitive responses. In the literature, it has been reported that electrodes modified with LDH nanocomposites based on metal oxide nanoparticles (NPs) [13-15], silver NPs [16] or nanodendrites [17], Prussian blue NPs [18] displayed peroxidase-like activity that was applied for H₂O₂ sensing in neutral pH, a pH compatible with GOx activity. However, none of these electrocatalysts was coupled with GOx for the electrochemical detection of glucose.

With the aim of developing glucose amperometric biosensors, GOx was indeed immobilized at the electrode surface by entrapment in inert LDH matrices (i.e. ZnAl-Cl [19], NiAl-NO₃ [20]) and ZnAl-Cl/biopolymers (alginate [21] or chitosan [22]) composites. Most of these GOx/LDH biosensors were prepared by the solvent casting of a defined amount GOx and LDH mixture deposited on an electrode surface, namely Pt or glassy carbon electrode (GCE) [4]. In these studies, GOx was immobilized on the LDH particles by adsorption and a subsequent cross-linking with glutaraldehyde can

be applied to prevent enzyme leaching. Another method of GOx immobilization was proposed by Tonelli's group based on the entrapment of GOx during the electrogeneration of NiAl LDH films on Pt electrode [23, 24].

Different amperometric transduction processes have been tested with these GOx/LDH biosensors. The most popular transduction mode is the direct oxidation of H₂O₂ at the underlying Pt electrode [4]. However, mediated transduction operates generally at lower potential preventing the interference with easily-oxidizable compounds present in real samples. Interestingly, redox active species can be incorporated inside the LDH structure and play the role of an electron relay in electrocatalytic reactions. These electroactive hybrid LDHs can serve as host matrices of enzymes. For instance, Colombari *et al.* report that intercalated anions in LDH, i.e. ferrocene (Fc) sulfonate or carboxylate, allow the mediated oxidation of H₂O₂ formed during the GOx enzymatic reaction with glucose [25].

Various biohybrid materials based on hemoglobin (Hb) or horseradish peroxidase (HRP) entrapped in LDH have been used for the amperometric detection of H₂O₂ at neutral pH [26]. However, only one bienzymatic electrode, coupling the activities of GOx and HRP immobilized in the ZnCr-ABTS LDH, was reported so far in the literature [27]. In this example, the intercalated ABTS plays the role of an electron shuttle between the redox center of HRP and the electrode. Finally, glucose determination was also performed under anaerobic condition where FADH₂ was regenerated to FAD by redox mediators. Ferrocene derivatives, immobilized in the LDH structure [28] or in solution [29], have been also used in this second generation of GOx/LDH biosensors. We have reported the performance of all these GOx/LDH biosensors in a review published elsewhere [4].

It is reported in the literature that CoAl, NiAl and, NiFe LDH exhibit electrocatalytic properties for H₂O₂ oxidation in strong basic medium (pH 13) [30, 31]. Unfortunately, this pH condition is not compatible with the GOx enzymatic activity. However very recently, we have demonstrated the electrocatalytic activity of a novel Co₃Mn-CO₃ LDH for the oxidation of H₂O₂ at neutral pH [32]. Based on the literature and at this point of our knowledge on the use of LDHs for electrochemical application, a critical stage can be passed to design fully

integrated LDH-based biosensors. Simplicity and innovation of our approach lie on the simple combination of $\text{Co}_3\text{Mn-CO}_3$ LDH and GOx, chosen as a model enzyme, and an original transduction pathway based on the electrocatalytic properties of this LDH. This study is also focused on the control of the LDH/GOx assemblies in order to improve the biohybrid interface and enhance the electrochemical response. An optimization of the biosensor response for glucose determination was achieved by tuning various GOx/ Co_3Mn multilayer configurations, associating or not biopolymers (alginate or carrageenan). For the first time, a 3D-porous electrode was finally prepared by impregnation of a carbon felt (CF) with a colloidal suspension of $\text{Co}_3\text{Mn-CO}_3$ nanoparticles. The morphology and the structure of $\text{Co}_3\text{Mn/CF}$ composite were characterized by SEM and XRD. The $\text{Co}_3\text{Mn-CO}_3/\text{CF}$ electrode was then used as a support to adsorb GOx. The resulting GOx/ $\text{Co}_3\text{Mn-CO}_3/\text{CF}$ bioelectrode was tested for the electrochemical glucose oxidation.

2. Materials and methods

2.1. Chemicals

The reagents used for the preparation of the LDH phases, cobalt nitrate hexahydrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 98%), manganese nitrate tetrahydrate ($\text{Mn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 97%) and sodium carbonate (Na_2CO_3) were purchased from Honeywell and sodium hydroxide (NaOH , 98%) from Acros. The buffer electrolyte solutions (pH 7.0) was prepared with Tris(hydroxymethyl)aminomethane (trizma base 99.9%) from Sigma-Aldrich. α -D(+)-Glucose (99+% anhydrous) was from Acros. Alginate and *ι*-carrageenan, glucose oxidase from *Aspergillus niger* (252 U mg^{-1}) were purchased from Sigma-Aldrich.

2.2. $\text{Co}_3\text{Mn-CO}_3$ LDH synthesis

The $\text{Co}_3\text{Mn-CO}_3$ LDH was synthesized using the coprecipitation method at a fixed pH of 10.0, as previously described [32]. Typically, 100 mL of a mixed $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and $\text{Mn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ solution, with a total metal concentration equal to 0.16 M and $\text{Co(II)/Mn(II)} = 3$, was added to 100 mL of 0.2 M NaOH / 0.1 M NaCO_3 solution, previously purged for 1 h with N_2 . Addition was conducted over

a period of 20 min in an N_2 atmosphere and under a vigorous stirring. Crystallization was favored thanks to the use of a reflux at 50 °C. The resulting precipitate was washed with deionized water and centrifuged three times. A part of this solid was kept in suspension in water for further uses, and another was dried at room temperature in the darkness for 12 h for XRD characterization. $\text{Zn}_2\text{Al-NO}_3$ and $\text{Zn}_2\text{Al-DDS}$ (DDS = dodecylsulfate) were prepared as reported elsewhere [33].

2.3. GOx/ $\text{Co}_3\text{Mn-CO}_3$ modified bioelectrodes

These bioelectrodes were prepared using a multilayer procedure. A $\text{Co}_3\text{Mn-CO}_3$ (2 $\text{mg}\cdot\text{ml}^{-1}$) colloid suspension was dispersed overnight under stirring conditions in pure water. GOx, alginate, carrageenan, were dissolved in pure water with a concentration of 2 $\text{mg}\cdot\text{ml}^{-1}$. The working electrode was a glassy carbon electrode (GCE) ($\Phi = 0.5$ cm) polished with an alumina slurry paste (0.05 μm) and rinsed with ethanol and water. The latter was modified by the deposition of 3 successive layers of LDH/GOx/LDH or LDH/GOx/biopolymer. A fixed volume (5-20 μl) of the LDH, GOx or biopolymer aqueous solutions was deposited successively on the surface of the GCE electrode. Between each deposition step, the coatings were dried under vacuum at room temperature. The bioelectrodes were stored at 6°C overnight before use. For all GOx/LDH biomembranes, the deposited amount of GOx was fixed at 20 μg ; the enzyme leaching after rehydration of the bioelectrodes in 1 mL Tris buffer for 10 min was measured by UV-Vis at 278 nm. Then, the amperometric measurements were performed with a potentiostat EA161 (EDAQ). A saturated Ag-AgCl electrode was used as reference electrode and a Pt wire was used as counter electrode. The steady-state chronoamperograms (i_{lim} vs t) were recorded under a rotating speed of 500 rpm in 20 mL of air saturated 0.1 M Tris buffer solution (pH 7.0, 30°C). After a base line stabilization under applied potential ($E_{\text{app}} = 0.65$ V/Ag-AgCl), the glucose concentration was increased stepwise between 20 and 800 μM by adding with a micropipette fixed volumes of a concentrated stock solution (10 mM). The current response was measured after its stabilisation. The linear range of the calibration plots was fixed according to the best correlation coefficient (R^2); the sensitivity was expressed as the

slope of these plots. The limit of detection was calculated from the linear fit ($LoD = (a+3\sigma a)/b$). I_{max} , corresponded to the pseudo-plateau observed in the calibration curves, is related to the total amount of active enzyme in the film. For the best biosensor configurations, the reported values corresponded to the mean values of independent measurements recorded with three different electrodes.

2.4. GOx/Co₃Mn-CO₃/CF modified electrodes

A piece of carbon felt (CF) RVC 2000 from Mersen (3 cm x 3 cm, thickness 6 mm) was pretreated with concentrated HNO₃ for 180 min and then an ultrasonic cleaning was performed in acetone, ethanol and deionized water for 15 min and left to dry at room temperature overnight. The pretreated CF was impregnated with 100 mL of fresh aqueous suspension of 0.08 M Co₃Mn-CO₃ by a percolation process using a filtration apparatus. The mass-loading of LDH deposited on the CF was measured from weight difference between dried clean CF before and after impregnation; the average loading was 0.1 mg/mg CF. The immobilization of GOx on the as-prepared Co₃Mn-CO₃/CF was realized by soaking the electrode cylinders with a diameter (ϕ) of 5 mm in 4 mL GOx solution (2 mg.mL⁻¹) overnight at 6°C. The amount of active immobilized enzyme ($\pm 6 \mu\text{g}$ or $\pm 0.26 \mu\text{g/mg}$ LDH/CF) was estimated by measuring the enzymatic activity in comparison to defined amounts of GOx in solution. The GOx activity was determined with a spectrophotometric method following the kinetics of formation of H₂O₂ by the HRP/ABTS system monitored at 420 nm ($\epsilon = 36000 \text{ M}^{-1} \text{ cm}^{-1}$) [34]. These electrodes have been characterized by scanning electron microscopy (SEM) using a JSM-7500F Field Emission Scanning Electron Microscope operating at an acceleration voltage of 3 KV. X-ray diffraction pattern was recorded using theta-theta PANalytical X'Pert Pro diffractometer equipped with a Cu anticathode ($\lambda K\alpha_1 = 1.540598 \text{ \AA}$, $\lambda K\alpha_2 = 1.544426 \text{ \AA}$) and a X'Celerator detector.

CF, Co₃Mn-CO₃/CF and GOx/Co₃Mn-CO₃/CF electrodes ($\Phi = 5 \text{ mm}$) were characterized in cyclic voltammetry (CV) with a potentiostat Autolab PGSTAT204 controlled by the NOVA software

(Metrohm) using a home-made three electrode cell (Fig.S1). The working electrodes were fixed on the bottom of the cell in contact with a glassy carbon plate connected to a copper wire to assure the electrical contact. The cell was filled with 2 mL of Tris buffer solution and the reference electrode and the counter electrode were fixed on the top of the working electrode. The electroactive surface area (ESCA) of a bare CF cylinder ($\Phi = 5 \text{ mm}$, external surface area 1.33 cm^2 , $21 \pm 0.3 \text{ mg}$) was determined from the Randles-Sevcik relation: $i_p = 2.69 \cdot 10^5 \cdot n^{3/2} \cdot (ESCA) \cdot C \cdot \nu^{1/2} \cdot D^{1/2}$, where $n = 1e^-$ transfer, $C =$ the concentration of the electroactive probe (mol cm^{-3}) and $D =$ the diffusion coefficients ($D_{K_4Fe(CN)_6} = 0.703$ [35]). The CVs were recorded in a 1 mM K₄Fe(CN)₆ + 0.1 M KCl solution at different scan rates ($10 < \nu < 50 \text{ mVs}^{-1}$) (Fig.S2). The ESCA was estimated at 11.8 cm^2 ($560 \text{ cm}^2 \text{ g}^{-1}$). The electrochemical responses of CF, Co₃Mn-CO₃/CF and GOx/Co₃Mn-CO₃/CF upon additions of H₂O₂ or glucose were recorded in 0.1 M Tris buffer solution (pH 7.0).

3. Results and discussion

3.1. Optimization of GOx/Co₃Mn-CO₃ bioelectrodes

Co₃Mn-CO₃ LDH displays the typical rhomboedral structure (R-3m) with a and c cell parameters equal respectively to $3.11450(2) \text{ \AA}$ and $22.7748(1) \text{ \AA}$ (Fig. 1A). The short interlayer distance (7.600 \AA) confirms the intercalation of CO₃²⁻ anions. Both EDX and XPS analyses give a Co/Mn molar ratio equal to 3.1. Valence states of Co and Mn, determined by XPS, correspond to 90 % Co(II), 10 % Co(III) and 100 % Mn(III) [32]. Once coated on a carbon electrode, 2D platelets undergo a preferential orientation with faces parallel to the support leading to an increase of the (003) and (006) X ray diffraction lines (Fig. 1A). As shown on SEM images (Fig. 1B), deposit of Co₃Mn-CO₃ on GCE forms a homogeneous and dense film made of well identified, individual hexagonal monodispersed platelets with an average width and thickness of about 400 nm and 50 nm, respectively.

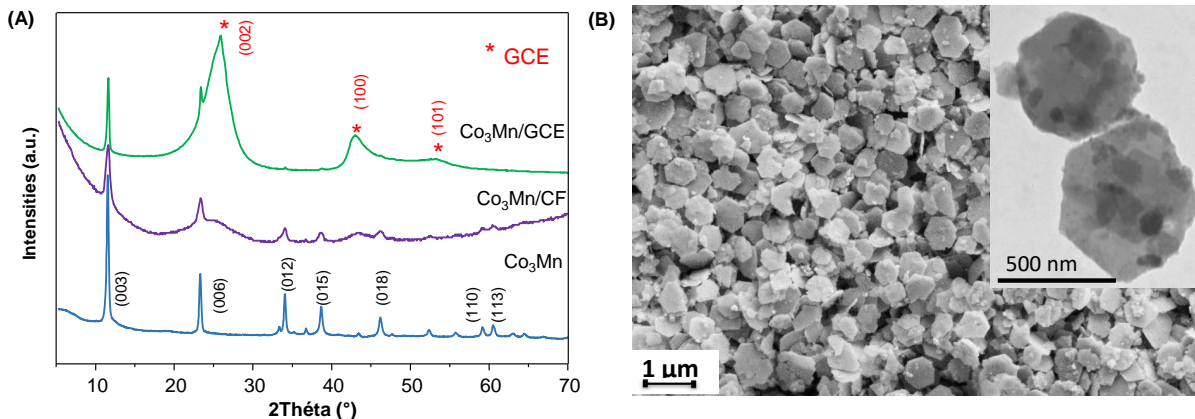


Figure 1: A) XRD patterns of $\text{Co}_3\text{Mn-CO}_3$ LDH, $\text{Co}_3\text{Mn-CO}_3/\text{GCE}$ and $\text{Co}_3\text{Mn-CO}_3/\text{CF}$. B) SEM and TEM images of $\text{Co}_3\text{Mn-CO}_3/\text{GCE}$.

Table 1: Influence of the coating composition of GOx/LDH bioelectrodes on the amperometric performance for glucose detection

Coating composition (μg)	Immobilized GOx (μg (%))	Sensitivity ($\text{mA M}^{-1} \text{cm}^{-2}$)	R^2 (n)	I_{max} ($\mu\text{A cm}^{-2}$)
Inner $\text{Co}_3\text{Mn-CO}_3/\text{GOx}/\text{Outer } \text{Co}_3\text{Mn-CO}_3$				
10/20/10	7.6 (38)	4.8	0.9974 (8)	1.4
20/20/10	9.2 (46)	7.7	0.9971 (5)	1.8
30/20/10	7.6 (38)	8.0	0.9950 (5)	2.1
40/20/10	7.8 (39)	9.9	0.9961 (5)	2.0
30/20/20	11.0 (55)	8.3	0.9976 (5)	2.0
30/20/30	11.0 (55)	9.8	0.9978 (5)	2.1
30/10/10/10/10	12.2 (61)	6.0	0.9980 (6)	1.5
Inner 30/20/Outer 10*				
$\text{Co}_3\text{Mn-CO}_3/\text{GOx}/\text{Co}_3\text{Mn-CO}_3$	9.2 (38 \pm 1)	8.0 \pm 0.3	0.9950 (5)	2.1 \pm 0.1
$\text{Co}_3\text{Mn-CO}_3/\text{GOx}/\text{Zn}_2\text{Al-NO}_3$	9.2 (46 \pm 14)	17.0 \pm 0.7	0.994 (5)	4.0 \pm 1
$\text{Co}_3\text{Mn-CO}_3/\text{GOx}/\text{Zn}_2\text{Al-DDS}$	12.4 (62 \pm 0.5)	15.5 \pm 0.5	0.997 (5)	3.2 \pm 0.2
$\text{Co}_3\text{Mn-CO}_3/\text{GOx}/\text{Alginate}$	12.6 (63 \pm 1)	7.5 \pm 0.3	0.994 (6)	2.2 \pm 0.2
$\text{Co}_3\text{Mn-CO}_3/\text{GOx}/\text{Carrageenan}$	18.0 (90 \pm 2)	12.8 \pm 0.1	0.994 (6)	4.0 \pm 0.5

*Measurements performed in triplicate

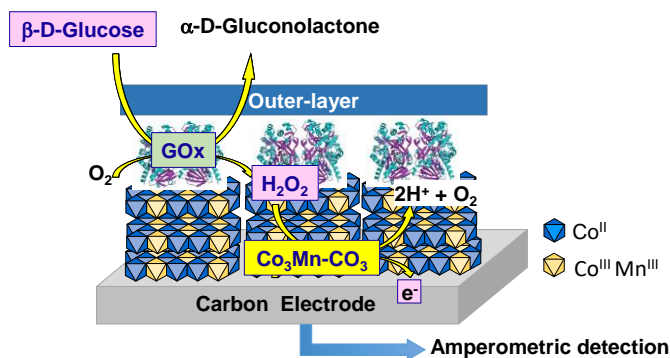


Figure 2: Design of $\text{GOx}/\text{Co}_3\text{Mn-CO}_3$ based bioelectrodes

In a recent study [32], we have shown that hydrogen peroxide can be oxidized in neutral pH at a

carbon electrode modified with 20 μg of $\text{Co}_3\text{Mn-CO}_3$ LDH. The sensitivity was found to be $30 \pm 0.7 \text{ mA M}^{-1} \text{cm}^{-2}$, with a linear concentration range of 0.11 - 1.20 mM. With the aim to benefit of the $\text{Co}_3\text{Mn-CO}_3$ electrocatalytic behavior, we have co-immobilized GOx and $\text{Co}_3\text{Mn-CO}_3$ LDH on GCE using the layer by layer method. The biosensor configuration results from the immobilization of GOx between two layers of LDH. The outer-coating prevents the enzyme release, whereas the enzymatically generated H_2O_2 is oxidized in the underlying $\text{Co}_3\text{Mn-CO}_3$ LDH coating (Fig. 2). Glucose calibration curves were therefore obtained by measuring the oxidation current of H_2O_2 under steady state condition at $E_{\text{app}} = 0.65 \text{ V}/\text{Ag-AgCl}$ by chronoamperometry (Fig. S3). In order to optimize the biosensor composition, a systematic study was conducted by varying the amount of LDH

deposited in each layer (Table 1). In all cases, the deposited amount of GOx on the working electrode was fixed at 20 μg . The amount of enzyme truly immobilized was calculated from the difference between the deposited amount, namely 20 μg , and the amount of protein released in the buffer solution determined by spectrophotometry. Regardless the amount of $\text{Co}_3\text{Mn-CO}_3$ deposited in the outer-layer, only 38-55 % of GOx remain adsorbed on the LDH particles after the rehydration of the bioelectrodes in Tris buffer solution (Table 1). As we have observed previously with the H_2O_2 sensors [32], the biosensor response increases by increasing the amount of $\text{Co}_3\text{Mn-CO}_3$ in the inner-layer (Table 1). A best sensitivity was obtained with 40 μg . However in our previous work, we have noticed that for the thicker film (40 μg) the response to H_2O_2 became less reproducible [32]. Therefore, we decided to use 30 μg for further experiments.

On the other hand, it appears clearly that the outer-coating based on $\text{Co}_3\text{Mn-CO}_3$ does not sufficiently prevent GOx leaching. Indeed, by increasing the amount of LDH in the outer-layer from 10 μg to 30 μg , only 17% more GOx are immobilized. In all cases, the pseudo-plateau observed in the calibration curves (Fig. S3), which can be related to the total amount of active enzyme in the LDH film, corresponds to the same value of I_{max} ($\approx 2.0 \mu\text{A cm}^{-2}$) (Table 1). As seen on SEM image (Fig. 2B), $\text{Co}_3\text{Mn-CO}_3$ films are porous due to the LDH morphology and assembly. Another multilayer configuration was also tested with the same amount of GOx (2x10 μg) but dispersed between 2 LDH layers of 10 μg each, the inner layer being fixed at 30 μg (30 LDH/10 GOx/10 LDH/10 GOx/10 LDH). In this case, the rate of GOx immobilization was increased up to 61 % but the electrochemical response was worse, with a decrease of the sensitivity and I_{max} values (Table 1). In fact, the diffusion of glucose through the film to reach the enzyme dispersed in the LDH coatings seems to be limited. Hence, the manufacturing reproducibility of the biosensors was tested for the configuration $\text{Co}_3\text{Mn/GOx/Co}_3\text{Mn}$ (30/20/10) with three different electrodes prepared by the same procedure (Fig. 3).

The relative standard deviation (RSD), calculated over the whole calibration curve, was 4 %. The amperometric current is proportional to glucose concentration over a range of 2×10^{-5} - 1.2×10^{-4} M. The sensitivity and the detection limit (LoD) were $8.0 \text{ mA.M}^{-1}\text{cm}^{-2}$ and of 0.01 mM, respectively.

In order to improve the immobilization efficiency of GOx, we have tested different outer-layers. Quantities for each component ($\text{Co}_3\text{Mn-CO}_3/\text{GOx}/\text{outer-layer}$) deposited on the electrode surface were fixed at 30/20/10 μg , respectively. $\text{Co}_3\text{Mn-CO}_3$ was replaced by other LDHs ($\text{Zn}_2\text{Al-NO}_3$, $\text{Zn}_2\text{Al-DDS}$) (Fig. 3A). These LDHs have been chosen for their good biocompatibility toward enzymes [4, 36], in particular with GOx [19, 37], and for their morphology with smaller and polydispersed particle sizes. Moreover, $\text{Zn}_2\text{Al-DDS}$ was also tested as an organo-modified LDH where dodecylsulfate (DDS) may act as a hydrophobic barrier against GOx leaching. Even though the immobilization percentage of GOx was increased with the latter LDH (62 %), the most sensitive response was obtained with the $\text{Zn}_2\text{Al-NO}_3$ outer-layer (Table 1). However this biosensor configuration suffered of a lack of reproducibility as shown in figure 3A (RSD = 27 %). As reported in the literature, biopolymers, such as chitosan, alginate and carrageenan, have been also used in the preparation of enzyme/LDH biosensors to increase the biocompatibility and the adhesive properties of LDH coatings [21, 22, 38, 39]. Hence, we have also tested biopolymers, i.e. alginate and carrageenan, as an alternative to the LDH outer-layer. The efficiency of GOx immobilization was indeed improved, reaching 90 % with the outer-layer based on carrageenan, resulting in an enhancement of the I_{max} value ($4.0 \mu\text{A cm}^{-2}$) (Table 1). With the carrageenan outer-layer, the reproducibility of the calibration curve was 7 % (Fig. 3B). This biosensor configuration provides a linear amperometric response between 2×10^{-5} and 1.6×10^{-4} M with an improved sensitivity of $12.8 \text{ mA M}^{-1} \text{ cm}^{-2}$ and a LoD of 0.02 mM.

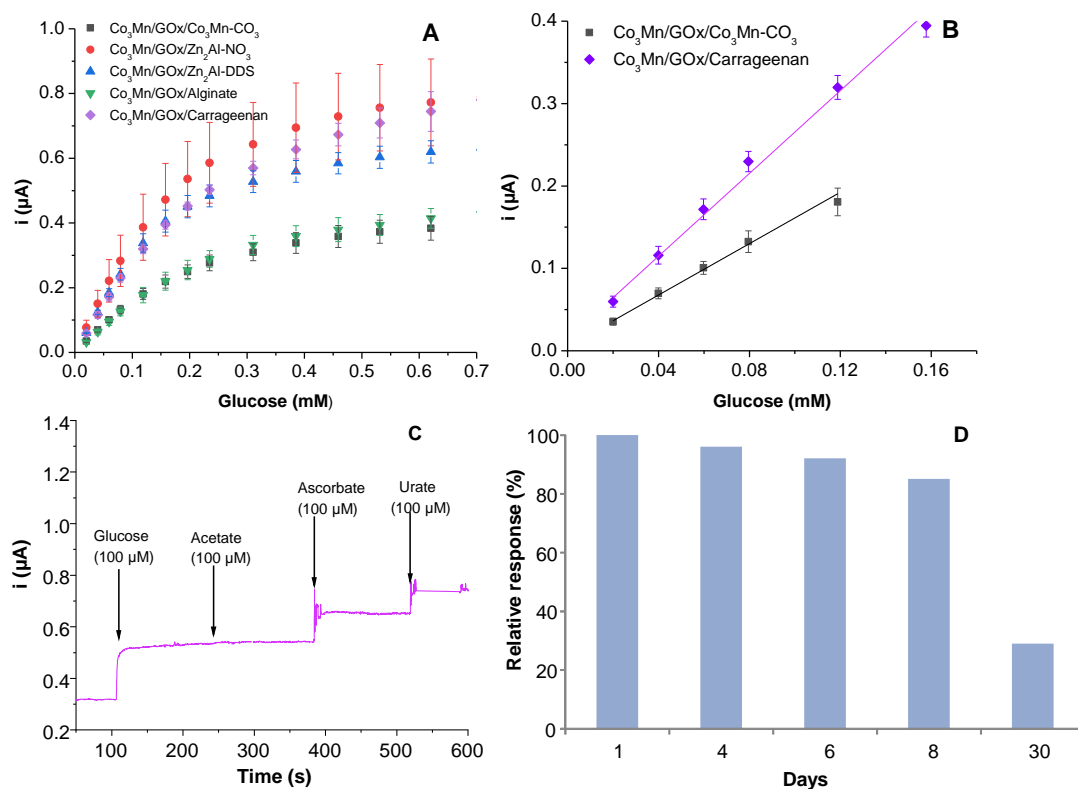


Figure 3 A) Glucose calibrations curves of (30/20/10 μg) $\text{Co}_3\text{Mn}-\text{CO}_3/\text{GOx}$ biosensors as a function of the outer-layer a) $\text{Co}_3\text{Mn}-\text{CO}_3$ and b) $\text{Zn}_2\text{Al}-\text{NO}_3$, c) $\text{Zn}_2\text{Al}-\text{DDS}$, d) Alginate, e) Carrageenan. **B)** Linear range obtained with a) $\text{Co}_3\text{Mn}-\text{CO}_3/\text{GOx}/\text{Co}_3\text{Mn}-\text{CO}_3$ and b) $\text{Co}_3\text{Mn}-\text{CO}_3/\text{GOx}/\text{carrageenan}$ biosensors (Error bars are determined by the standard deviation for 3 different electrodes), **C)** Steady state amperometric response as function of time of the $\text{Co}_3\text{Mn}-\text{CO}_3/\text{GOx}/\text{carrageenan}$ biosensor after successive addition of 100 μM of glucose, acetate, ascorbate and urate. **D)** Relative response of $\text{Co}_3\text{Mn}-\text{CO}_3/\text{GOx}/\text{carrageenan}$ biosensors under storage at 6°C (each point corresponds to a new electrode). (Experimental conditions: Tris buffer solution (pH 7.0), $E_{\text{app}} = 0.65\text{V}/\text{Ag}-\text{AgCl}$).

Table 2: Analytical performance of GOx/LDH amperometric biosensors for glucose detection.

LDHs	E_{app} (V)	Sensitivity ($\text{mA M}^{-1} \text{cm}^{-2}$)	LR (M)	Ref
Direct oxidation of H_2O_2 on Pt				
$\text{Zn}_2\text{Cr}-\text{Cl}$	0.6	19	-	[27]
$\text{Zn}_3\text{Al}-\text{Cl}$	0.7	55	1×10^{-6} - 3×10^{-3}	[19]
$\text{Zn}_3\text{Al}-\text{Cl}+\text{chitosan}$	0.6	35	1×10^{-6} - 1×10^{-2}	[37]
$\text{Zn}_3\text{Al}-\text{Cl}+\text{alginate}$	0.6	63	1×10^{-6} - 3×10^{-3}	[22]
$\text{Ni}_2\text{Al}-\text{NO}_3$	0.45	6.2	5×10^{-4} - 1×10^{-2}	[53]
Mediated detection of H_2O_2				
$\text{Mg}_2\text{Al}-\text{FcSO}_3$	0.5	2.1	-	[25]
$\text{Mg}_2\text{Al}-\text{FcCO}_2$	0.4	4.8	-	
$\text{Co}_3\text{Mn}-\text{CO}_3/\text{carrageenan}$	0.65	12.8	2×10^{-5} - 1.6×10^{-4}	This work
$\text{HRP}@Zn_2\text{Cr}-\text{ABTS}$	0.0	23	3×10^{-8} - 8×10^{-6}	[27]
Anaerobic regeneration of FAD				
$\text{Zn}_3\text{Al}-\text{Cl}+\text{FcMeOH}$	0.25	60	7×10^{-6} - 4×10^{-4}	[29]
$\text{Zn}_2\text{Cr}-\text{FcPSO}_3$	0.5	68	1×10^{-7} - 3×10^{-5}	[28]

Since the amperometric detection of H_2O_2 requires high over potentials and faces interferences of easily oxidizable compounds, the glucose selectivity was evaluated towards three interfering anions, namely acetate, ascorbate and urate, added to the electrolyte solution at the same concentration (100 μM) than glucose. Using the $\text{Co}_3\text{Mn-CO}_3/\text{GOx}/\text{carrageenan}$ biosensor, the percentages of interfering currents were 0, 50 and 43 %, respectively (Fig. 3C). We have observed the same interference phenomenon for the detection H_2O_2 at Co_3Mn modified electrode [32]. Unfortunately, the presence of the carrageenan outer-layer does not hinder the diffusion of those anionic species through the film to reach the electrode surface. This problem may probably be solved by increasing the thickness of the carrageenan film or, as reported in [40], by using other negatively charged and more hydrophobic polymers, such as Nafion or Kodac AQ ionomers. Finally, five different bioelectrodes were stored at 6 $^\circ\text{C}$ to study their storage stability. The biosensor responses were stable for one week and after one month the response was around 30 % of the initial value (Fig. 3D).

These biosensor characteristics can be compared to the values reported for other GOx/LDH biosensors (Table 2), showing that this original amperometric transduction pathway gives a good sensitivity within a reliable concentration range of glucose. Indeed, the use of TM-LDH as an electrocatalyst for H_2O_2 oxidation can be considered as an alternative to the use of expensive Pt electrodes. Moreover, it does not require the immobilization of redox mediators or the presence of peroxidase substrates in solution, as reported for the mediated amperometric detection (Table 2) or the spectroscopic methods described in the introduction. For all these examples, the upper limit of glucose concentration is slightly lower than the recommended values (20-30 mM). Of course, as mentioned by Wang et al [41] for non-enzymatic glucose electrochemical sensors, especially those based on LDH , these sensor performance cannot be directly transposed to clinical conditions but there is room for further improvements and adaptability to other oxidase enzymes.

3.2. 3D- $\text{GOx}/\text{Co}_3\text{Mn-CO}_3/\text{CF}$ electrodes

The development of biointerfaces can be also applied to energy production. It concerns biofuel cells that convert chemical energy into electrical one by means of an enzymatic reaction. Since glucose and oxygen are both present in blood and extracellular fluids, glucose can be oxidized by an enzyme, i.e. GOx , and releases electrons at high voltage enabling oxygen to be reduced to provide bioelectricity [42, 43]. 3D-nanostructured bioelectrodes having a great surface area are investigated as these configurations are essential to increase the produced power energy. In previous works, we have demonstrated the possibility to use redox active LDH in energy conversion. That was the first examples of glucose/ O_2 biofuel cells using GOx and laccase as enzymes and LDH materials to build both anode and cathode [44, 45]. Electrocatalytic activity was improved by depositing the enzyme/ LDH films on carbon porous tubes or on single-walled carbon nanotubes (SWCNTs) modified electrodes, with enhanced electroactive area. Few papers report the coating of LDH on carbon felts (CFs) and all concern LDHs containing mixed valence transition metals ($\text{Ni}^{\text{II}}\text{Al}$ [46], $\text{Ni}^{\text{II}}\text{Co}^{\text{III}}$ [47], $\text{Fe}^{\text{II}}\text{Fe}^{\text{III}}$ [48-50] and $\text{Co}^{\text{II}}\text{Fe}^{\text{III}}$ [51]). Applications target mainly energy production or advanced heterogeneous electro-Fenton processes for wastewater cleaning. CFs were also used in order to increase the electroactive surface area of TM-LDH materials. *In-situ* precipitation at the CF surface under solvothermal conditions was the main strategy employed to prepare those LDH/CF nanocomposites.

With the aim to enhance the electrocatalytic current of the $\text{GOx}/\text{Co}_3\text{Mn-CO}_3$ modified electrodes, we have prepared 3D-porous electrodes by impregnation of a carbon felt with a suspension of $\text{Co}_3\text{Mn-CO}_3$ nanoparticles. Before use, the CF was pretreated with concentrated HNO_3 to increase its wettability and enable the anchoring of positive LDH layers. SEM images of pretreated CF show an interconnected network of carbon fibers of approximately 20 μm diameter (Fig. 4A and B). A porosity of 8.5 $\text{cm}^3 \text{g}^{-1}$, determined by mercury intrusion, was reported by Mazurenko *et al* [52]. The electroactive surface area (ESCA) of a bare cylinder of CF ($\Phi = 5 \text{ mm}$), fixed on the bottom of the electrochemical cell, corresponds to 11.8 cm^2 (560 $\text{cm}^2 \text{g}^{-1}$), a value 8.8 higher than the geometrical

external area (1.33 cm^2). $\text{CO}_3\text{Mn-CO}_3$ nanoparticles were immobilized on the pretreated CF by percolation of a LDH suspension. The average mass-loading of LDH deposited on the CF was 0.1 mg/mg CF that corresponds to $180 \mu\text{g cm}^{-2}$ of LDH anchored in the 5 mm cylinder. As shown on the SEM images, carbon fibers are fully covered by a mosaic of LDH platelets of around 400 nm of diameter (Fig. 4C and D). The LDH/CF morphology resulting from this mild coating process appears far different from that obtained when LDH particles are grown directly onto CF fibers. Indeed, solvothermal process leads to the formation of a heterogeneous coating with pack of LDH nanocrystals [48-51]. The crystallinity of the as-prepared LDH modified CF electrode was characterized by XRD confirming the presence of the LDH particles anchored on the carbon fibers (Fig. 2A).

The electrochemical response of the as-prepared $\text{CO}_3\text{Mn-CO}_3/\text{CF}$ electrode was recorded by cyclic voltammetry (CV) in Tris buffer solution (pH 7.0) without and in the presence of H_2O_2 . In the presence of $50 \mu\text{M}$ H_2O_2 , the $\text{CO}_3\text{Mn-CO}_3/\text{CF}$ exhibited an enhancement of the oxidation current measured at 0.9 V/Ag-AgCl from 0.47 to 1.23 mA ($\Delta i = 0.755 \text{ mA}$) (Fig. 5A). This current increase is the double compare to the current recorded at the bare pretreated CF electrode in the presence of the same amount of H_2O_2 . This confirms the electrocatalytic behaviour of $\text{CO}_3\text{Mn-CO}_3$ as regards to H_2O_2 oxidation. On the other hand, we have verified that glucose was not oxidized at a $\text{CO}_3\text{Mn-CO}_3$ modified electrode [32]. Then, $\text{GOx}/\text{CO}_3\text{Mn-CO}_3/\text{CF}$ electrodes were prepared by the simple adsorption of the enzyme on the composite electrode by soaking $\text{CO}_3\text{Mn-CO}_3/\text{CF}$

cylinders in a GOx aqueous solution (2 mg. mL^{-1}). The amount of immobilized active enzyme was estimated at $\pm 0.26 \mu\text{g/mg}$ LDH/CF that corresponds to a mass ratio in the biocomposite electrode $\text{GOx}/\text{CO}_3\text{Mn-CO}_3/\text{CF}$ of $\pm 0.006/2/21 \text{ mg}$. The electrochemical response was investigated by CV, showing that the adsorption GOx on $\text{CO}_3\text{Mn-CO}_3$ platelets induced a resistive component that slowed down the electron transfer within the LDH coating. The same observation has been already reported by Han *et al* [39]. They have shown by electrochemical impedance measurements that immobilizing polyphenol oxidase in $\text{Zn}_3\text{Al-Cl}/\text{chitosan}$ composite increased the electron transfer resistance in the bioelectrode. Despite this limitation, the as-prepared bioanode exhibited an increase of current (Δi) of 0.34 mA upon addition of $50 \mu\text{M}$ glucose (Fig. 5B). The inset shows that no current response was observed at the bare CF after addition of glucose, confirming that H_2O_2 formed during the GOx reaction with glucose was oxidized by $\text{CO}_3\text{Mn-CO}_3$ electrocatalysts. These preliminary results open new possibilities of applications for this 3D- $\text{GOx}/\text{CO}_3\text{Mn-CO}_3/\text{CF}$ bioelectrode for energy production, for instance. Of course, improvements of current intensity produced by our bioelectrode may be expected i) if we succeed to increase the amount of immobilized enzyme by soaking it in carrageenan solution, for instance, ii) the electrochemical measurements should be carried out under dynamic conditions to increase the flow of glucose through the 3D-bioelectrode and also to remove oxygen bubbles formed at the electrode surface during the electroenzymatic reaction. For this latter perspective, a different flow cell device should be considered.

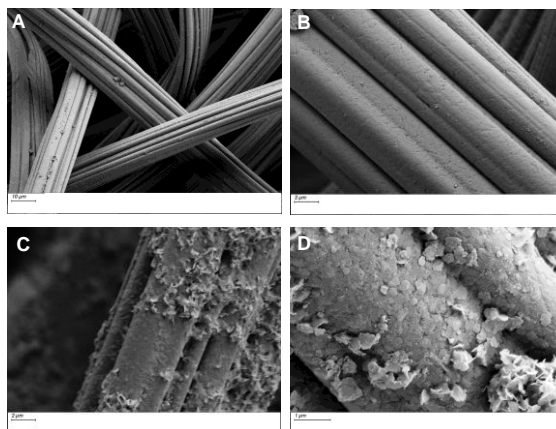


Figure 4: SEM images of pretreated CF (A and B), $\text{CO}_3\text{Mn-CO}_3/\text{CF}$ (C and D).

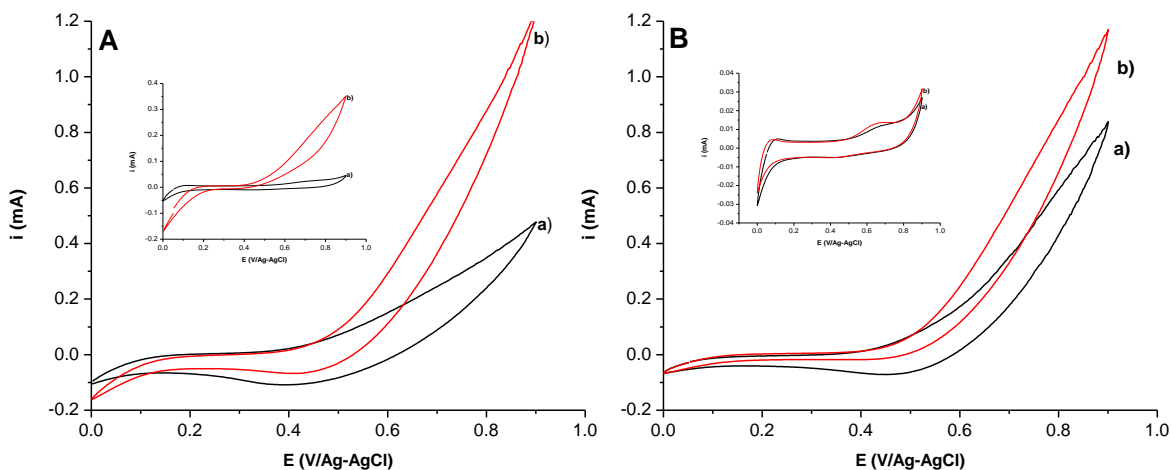


Figure 5: A) Cyclic voltammograms of $\text{Co}_3\text{Mn-CO}_3/\text{CF}$ a) before and b) after additions of $50 \mu\text{M H}_2\text{O}_2$, B) $\text{GOx}/\text{Co}_3\text{Mn-CO}_3/\text{CF}$ a) before b) after additions of $50 \mu\text{M}$ glucose. Insets show the CV recorded at the bare pretreated CF under the same conditions (Experimental conditions: Tris buffer solution (pH 7.0) $\nu = 10 \text{ mV s}^{-1}$).

4. Conclusion

Looking for potential electroactive hosts for building-up sensors, we have shown that $\text{Co}_3\text{Mn-CO}_3$ LDH phase can be used to replace Pt electrode in the development of GOx based bioelectrodes. Different compositions of the multilayer GOx/LDH modified electrodes have been tested, showing that H_2O_2 oxidation occurs in the $\text{Co}_3\text{Mn-CO}_3$ inner-layer which depends therefore on the amount of the TM-LDH deposited on the surface of carbon electrode. The use of carrageenan in the outer-layer increases the enzyme immobilization rate to 90% and it improves the electrochemical response upon addition of glucose. This new concept of amperometric transduction consists in an alternative to the GOx/TM-LDH based colorimetric sensors described in the literature. Preliminary results obtained with 3D-TM-LDH/carbon felt electrodes open a new field of applications of these electrodes, for instance in energy production or in electrocatalysis, even though further improvements are still necessary.

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Note: The authors declare that they have no known competing financial interests or personal

relationships that could have appeared to influence the work reported in this paper.

Appendix: Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.electacta.2021.138050. Scheme of the electrochemical cell, CV of $\text{K}_4\text{Fe}(\text{CN})_6$ recorded at a bare CF electrode, glucose calibration curves obtained with $\text{Co}_3\text{Mn-CO}_3/\text{GOx}/\text{Co}_3\text{Mn-CO}_3$ bioelectrodes.

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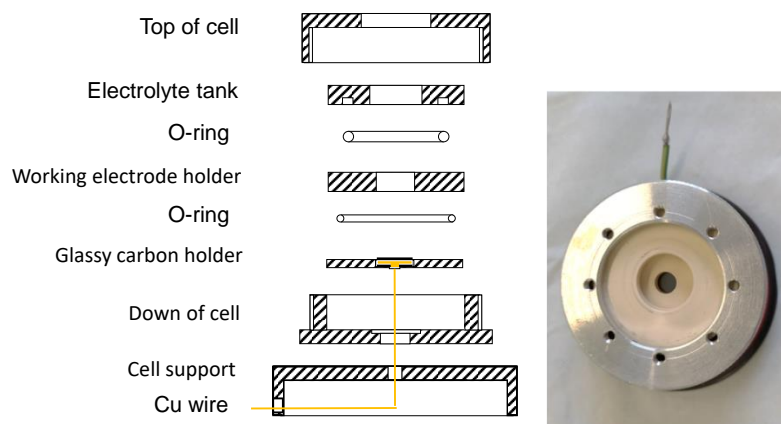


Figure S1: Scheme and photo of the electrochemical cell used with CF modified electrodes.

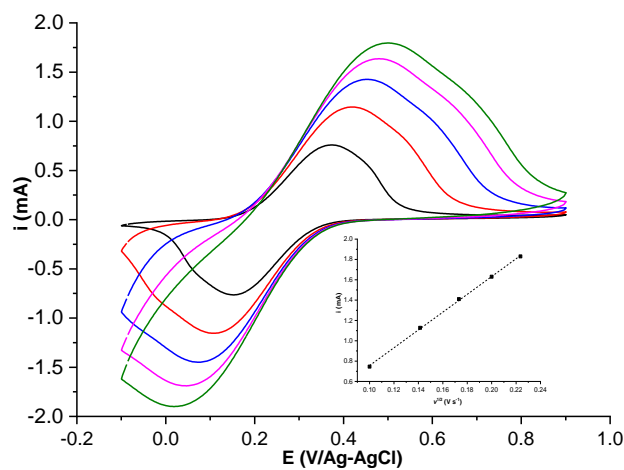


Figure S2: Cyclic voltammograms of 1 mM $\text{K}_4\text{Fe}(\text{CN})_6$ in 0.1 M KCl solution recorded at a bare CF ($\Phi = 5$ mm) electrode ($10 \leq \nu \leq 50 \text{ mVs}^{-1}$). Insert shows the I_{p_a} as a function $\nu^{1/2}$.

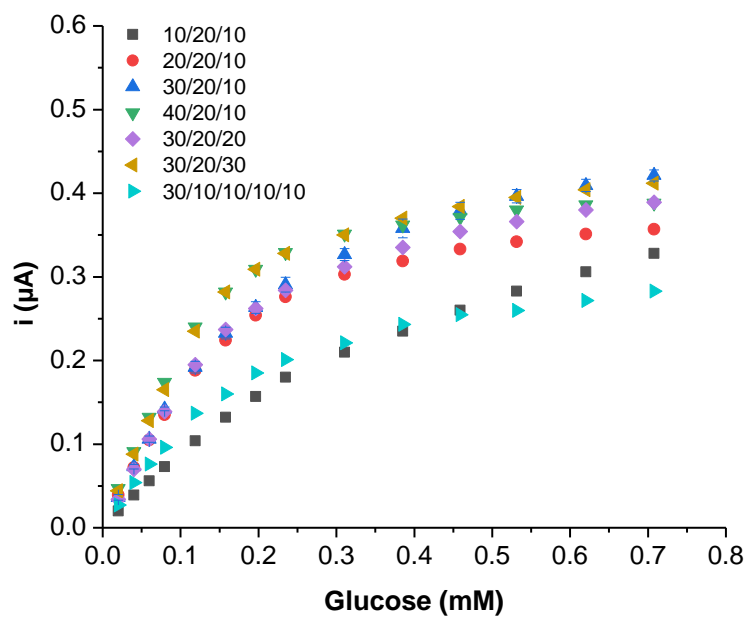


Figure S3: Calibration curves of glucose as a function of the composition $\text{Co}_3\text{Mn-CO}_3/\text{GO}_x/\text{Co}_3\text{Mn-CO}_3$ biosensors ($E_{\text{app}} = 0.65 \text{ V/Ag-AgCl}$ in Tris buffer pH 7.0).