

## DEVELOPMENT OF CARBON NANOTUBE-BASED ELECTROCHEMICAL BIOCATALYST BY ONE-STEP AS PLATFORM FOR BIOSENSING

A. Quintero-Jaime<sup>1,\*</sup>, F. Conzuelo<sup>3</sup>, D. Cazorla-Amorós<sup>2</sup>, W. Schuhmann<sup>3</sup>, E. Morallón<sup>1</sup>

<sup>1</sup> *Departamento de Química Física e Instituto Universitario de Materiales, Universidad de Alicante, Apartado 99, 03080, Alicante, Spain*

<sup>2</sup> *Departamento de Química Inorgánica e Instituto Universitario de Materiales, Universidad de Alicante, Apartado 99, 03080, Alicante, Spain*

<sup>3</sup> *Analytical Chemistry – Center for Electrochemical Sciences (CES), Faculty of Chemistry and Biochemistry, Ruhr-Universität Bochum, Universitätsstr. 150, D-44780 Bochum, Germany  
e-mail: andres.quintero@ua.es*

\*Presenting author's e-mail: andres.quintero@ua.es

### Introduction

The development of biofunctional electrodes for energy generation under green conditions (neutral pH, room temperature and source of energy) based on Enzymatic biofuel cell (EBFC) systems, and enzyme-based biosensor for detection of human being analytes for medical interest, have been intensively investigated. One important aspect for a proper performance is the effective interaction between the electrode surface and the enzymatic element, providing high sensitivity, high current density and low on-set potential. In this sense, electron-transfer from the reaction between the enzymatic element and its substrate towards the electrode is a triggering factor for the operation of the device. Even though, carbon nanotubes (CNTs) have been considered a promising material for this kind of application, due to the remarkable chemical stability, biocompatibility, and catalytic and electronic properties, functionalization with surface species are required to guarantee a proper immobilization of the element, to reduce the distance or to connect the active-center with the electrode surface. In this work, electrochemical modification of SWCNTs surface with N and P functionalities and encapsulation of a model enzyme (soluble glucose dehydrogenase (GDH) pyrroloquinoline quinone (PQQ)), is carried out in one step to synthesize a bioelectrode with electrocatalytic activity towards glucose oxidation.

### Materials and Methods

Glassy-carbon electrodes were modified by drop-casting with 10  $\mu\text{L}$  of a SWCNTs dispersion ( $1 \text{ mg}\cdot\text{mL}^{-1}$ ) in DMF. For electrosynthesis by one-step, anodic polarization in a standard three-electrode cell configuration was performed in a 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) Buffer solution (pH=7.0) with 150 mM  $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$  + 0.1M KCl + 1 mM 4-APPA + 5  $\text{mg}\cdot\text{mL}^{-1}$  GDH-s-PQQ, applying different oxidation potentials (0.97, 1.17 and 1.37 V vs. Ag/AgCl).

Once electrodes are synthesized, electrocatalytic activity is evaluated by chronoamperometry, applying a steady potential of 0.35 V vs. Ag/AgCl in 0.1M PBS (pH=7.2) solution, under stirring condition. Aliquots of 0.1M glucose solution were added to the electrochemical cell, obtaining different concentrations values between 0.005 to 10 mM of glucose.

## Results and Discussion

Electrosynthesis of the bioelectrode is based on previous electrochemical procedures developed in which, electrooxidation of the 4-aminophenyl phosphonic acid (4-APPA) promotes the formation of different electroactive species anchored and adsorbed in the CNTs surface. In this sense, **Fig. 1-a** shows the CVs of the electromodification of

SWCNTs where formation of different surface redox processes are observed after the first cycle of electrooxidation. During the cycling, the increase of both the charge of the redox processes and the double-layer capacitance, suggest the existence of surface process associated with the oxidation of 4-APPA entrapping the GDH-s-PQQ.

After the electrochemical synthesis, bioelectrodes show the presence of a high reversible redox process, characteristic of the 2 electron-transfer of the PQQ-redox centre in the holo-enzyme at  $-0.11$  V vs. Ag/AgCl, confirming the entrapment of the enzyme in the species produced onto the SWCNT surfaces<sup>1</sup>. Once, glucose is added in solution, an over oxidation current is observed at higher potential, as result of the reaction from glucose to gluconolactone catalysed by the GDH-s-PQQ (**See Fig. 1-b**). Interestingly, no damage in the structure of the enzyme is observed, since it maintains the catalytic activity towards glucose oxidation with low values of apparent Michaelis-Menten constant value.

## Conclusions

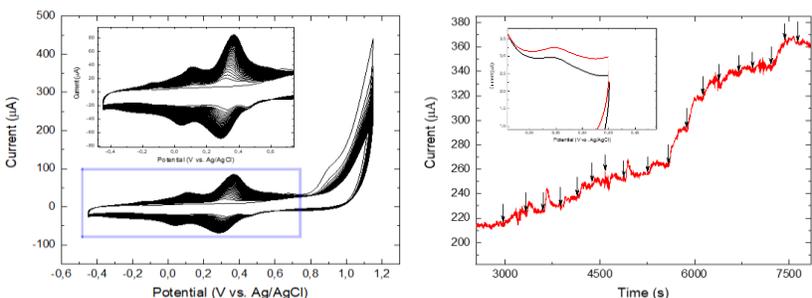
Bioelectrodes based on the entrapment of GDH-s-PQQ in SWCNT modified with N and P species have been successfully synthesized by a one-step method. The obtained biosensor shows electrocatalytic activity towards glucose oxidation with good substrate-enzyme interaction.

## Acknowledgment

The authors would like to thank MINECO and FEDER (MAT2016-76595-R and ENE2017-90932-REDT) for the financial support. A.F.Q.J. gratefully acknowledges Generalitat Valenciana for the Santiago Grisolia grant (GRISOLIA/2016/084).

## References

1. Sarauli, D. Xu, C. Dietzel, B. Schulz, B. Lisdat, F. (2013). Differently substituted sulfonated polyanilines: The role of polymer compositions in electron transfer with pyrroloquinoline quinone-dependent glucose dehydrogenase. *Acta Biomaterialia* 9, 8290-8298.



**Figure 1. a) CV of Electrooxidation of bioelectrodes at 1.17 V vs. Ag/AgCl based on SWCNT-4-APPA-GDH-s-PQQ during 50 cycles at  $50 \text{ mV}\cdot\text{s}^{-1}$  and b) Chronoamperometric response of bioelectrode at 0.35 V vs. Ag/AgCl under stirring conditions after continuous additions of glucose, inset: CV of the electrode in absence (black line) and presence (red line) of 10 mM of glucose at  $5 \text{ mV}\cdot\text{s}^{-1}$ .**