RV: Decision on submission to Journal of Pharmaceutical and Biomedical Analysis

Gustavo Rivas <grivas@fcq.unc.edu.ar> 2 de agosto de 2020, 18:01
Para: pablo gallay <pablogallay@hotmail.com>, Marcela Rodríguez <marcela.rodriguez7@gmail.com>, gustavo rivas <rivasgus@yahoo.com.ar>, grivas@fcq.unc.edu.ar

Hola chicos!
Quiero compartir very good news!!!! Hubo una segunda revisión para disminuir el nro de referencias, así que pasé las tablas a la información suplementaria y reordené las referencias. Les mando la última versión del paper.
Que terminen bien el finde!
UN abrazo!!
Gus

-----Mensaje original-----
De: em.jpba.0.6d0890.6ddaea91@editorialmanager.com [mailto:em.jpba.0.6d0890.6ddaea91@editorialmanager.com] En nombre de Journal of Pharmaceutical and Biomedical Analysis
Enviado el: domingo, 2 de agosto de 2020 16:06
Para: Gustavo Rivas <grivas@fcq.unc.edu.ar>
Asunto: Decision on submission to Journal of Pharmaceutical and Biomedical Analysis

Manuscript Number: JPBA-D-20-00510R2

DOBLE ROLE OF BATHOCUPROINE DISULFONIC ACID AS MULTI-WALLED CARBON NANOTUBES DISPERSING AGENT AND COPPER PRECONCENTRATION LIGAND: ANALYTICAL APPLICATIONS FOR THE DEVELOPMENT OF HYDROGEN PEROXIDE AND GLUCOSE ELECTROCHEMICAL SENSORS

Dear Professor Rivas,

Thank you for submitting your manuscript to Journal of Pharmaceutical and Biomedical Analysis.

I am pleased to inform you that your manuscript has been accepted for publication.

My comments, and any reviewer comments, are below. Your accepted manuscript will now be transferred to our production department. We will create a proof which you will be asked to check, and you will also be asked to complete a number of online forms required for publication. If we need additional information from you during the production process, we will contact you directly.

We appreciate you submitting your manuscript to Journal of Pharmaceutical and Biomedical Analysis and hope you will consider us again for future submissions.

Kind regards,
Sibel Ozkan
Editor

Journal of Pharmaceutical and Biomedical Analysis

Editor and Reviewer comments:

Reviewer #1: Authors have addressed perfectly all my previous minor concerns and I strongly recommend publication of this interesting manuscript in this Journal without further changes.

Reviewer #3: The authors welcomed all my suggestions therefore I recommend approval.

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Journal of Pharmaceutical and Biomedical Analysis

DOBLE ROLE OF BATHOCUPROINE DISULFONIC ACID AS MULTI-WALLED CARBON NANOTUBES DISPERSING AGENT AND COPPER PRECONCENTRATION LIGAND: ANALYTICAL APPLICATIONS FOR THE DEVELOPMENT OF HYDROGEN PEROXIDE AND GLUCOSE ELECTROCHEMICAL SENSORS

--Manuscript Draft--

Manuscript Number:	JPBA-D-20-00510R2
Article Type:	Full length article
Section/Category:	Bioanalytical Applications
Keywords:	Carbon nanotubes; Bathocuproine disulfonic acid; Copper; Hydrogen peroxide electrochemical sensor; Glucose electrochemical biosensor.
Corresponding Author:	Gustavo Rivas Full Professor Córdoba, Argentina
First Author:	Pablo Gallay
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	Marcela Rodríguez
	Marcos Eguílaz
	Gustavo Rivas
Abstract:	We are reporting a new strategy for preparing carbon nanotubes (CNTs)-based hydrogen peroxide and glucose amperometric sensors by taking advantage of the dual role of bathocuproine disulfonic acid (BCS) as dispersing agent of multi-walled carbon nanotubes (MWCNTs) and as ligand for the preconcentration of Cu(II). The platform was obtained by casting glassy carbon electrodes (GCE) with the dispersion of MWCNTs in BCS (MWCNTs-BCS) followed by the preconcentration of Cu(II) by surface complex formation at open circuit potential (GCE/MWCNTs-BCS/Cu). The resulting electrode was used for the sensitive amperometric quantification of hydrogen peroxide at 0.400 V catalyzed by the preconcentrated copper, with a linear range between 5.0×10^{-7} and 7.4×10^{-6} M, a sensitivity of 24.3 mA.M^{-1}, and a detection limit of 0.2 mM. The adsorption of GOx at GCE/MWCNTs-BCS/Cu followed by the immobilization of Nafion (Naf), allowed the construction of a sensitive and selective amperometric glucose biosensor with a linear range between 5.0×10^{-6} M and 4.9×10^{-4} M, a sensitivity of (477 ± 3) mA.M^{-1} and a detection limit of 2 mM. The proposed (bio)sensors were successfully used for the quantification of hydrogen peroxide in enriched milk samples and glucose in milk and commercial beverages without any pretreatment.
Suggested Reviewers:	ARTURO SQUELLA Universidad de Chile Facultad de Ciencias Quimicas y Farmaceuticas asquella@ciq.uchile.cl Expert in the field
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	Concepción PARRADO Universidad Complutense de Madrid cparrado@ucm.es Expert in the field
Response to Reviewers:	

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Cordoba, July 31, 2020

Editor of Journal of Pharmaceutical and
Biomedical Analysis
Prof. Sibel Özkan

Dear Editor:

As corresponding author, I am submitting the corrected version of the manuscript entitled **"Doble role of bathocuproinedisulfonic acid as multi-walled carbon nanotubes dispersing agent and copper preconcentration ligand: analytical applications for the development of hydrogen peroxide and glucose electrochemical sensors"** by Gallay, Rodríguez, Eguílaz and Rivas to be considered for publication in **Journal of Pharmaceutical and Biomedical Analysis** as a full paper. We are also submitting a highlighted version of the manuscript in order to facilitate the evaluation of the changes performed. The paper is unpublished and it has not been submitted for publication elsewhere.

We have found very useful the comments of the editor and reviewers and we have corrected the manuscript accordingly.

The response to the comments is the following:

Editor:

1. Please reply all the comments in detail.

We have replied all the comments.

2. Reference style is as follows: Please keep in your mind the reference number should not exceed 30 for full-length (research) manuscript and 20 for short communication.

We have reorganized the references in order to follow the rules of the journal. Therefore, Tables and the corresponding references have been moved to the Supplementary Information that we have now included to the submission.

Reviewer #1:

Authors report here a new strategy for preparing carbon nanotubes (CNTs) by taking advantage of the dual role of bathocuproine disulfonic acid (BCS) as dispersing agent of multiwalled carbon nanotubes (MWCNTs) and as ligand for the preconcentration of Cu(II) and their potential to be used as GCE modifiers to develop (bio)sensors for sensitive amperometric determination of H2O2 and GOx-assisted glucose by exploiting the catalytic activity of the accumulated copper on hydrogen peroxide oxidation. Both (bio)sensores demonstrated competitive analytical characteristics with other electrochemical (bio)sensors reported in the literatura (mainly in terms of simplicity and cost) and potential to perform the analysis in enriched milk samples and commercial beverages.

Apart from the interesting results, the manuscript is well structured and the experiments, well planned, executed and discussed (including the required controls, in the absence of Cu(II)), support perfectly the conclusions. Therefore, I consider it deserves publication in this Journal after addressing the following minor concerns:

- Have the authors evaluated how many measurements can be made with the same (bio)sensor and their storage stability?.

We appreciate very much the comments of the reviewer about our work.

Regarding the repeatability, we have evaluated the sensitivity towards hydrogen peroxide for the same GCE/MWCNTs-BCS/Cu after successive amperometric determinations at 0.400 V. The sensitivity largely decreases even after the second use of the sensor, indicating that the repeatability/short-term stability of the sensor is poor. Similar behavior was observed for GCE/MWCNTs-BCS/Cu/GOx/Naf after successive amperometric determinations of glucose at 0.400 V. Therefore, considering this poor repeatability/short-term stability, the platforms were thought as single-use (bio)sensors.

- As far as I understand, both samples are analyzed without dilution or matrix effect? Please clarify these advantages from the practical applicability point of view in section 3.5.

The samples were used without any pretreatment or dilution. In the case of hydrogen peroxide, we evaluated the recovery percentage in an enriched milk sample while in the case of glucose determination in milk and beverages, the quantification was performed by standard addition method. We don´t have the Section 3.5 in the manuscript as indicated by the reviewer; therefore, we have included the corresponding information in the Experimental Section (2.4 Procedure) and at the end of 3.2. and 3.3. in Results and Discussions. We have also clarified in the Abstract and Conclusions that the samples evaluated in the manuscript were untreated.

Experimental Section:

"Hydrogen peroxide was quantified in a milk sample (La Serenísima®) enriched with 1.7 $x10^{-3}$ M hydrogen peroxide by transferring a given aliquot of the milk enriched sample to the electrochemical cell containing 5.0 mL of 0.050 M phosphate buffer solution pH 7.40, performing the quanitification by amperometry at 0.400 V using GCE/MWCNTs-BCS/Cu.

Glucose was quantified in milk (La Serenísima®) and two commercial drinks, Gatorade® and Red-Bull®. The beverages and milk were obtained from a local supermarket. An aliquot of the given sample was directly transferred to the electrochemical cell containing 5.0 mL of 0.050 M phosphate buffer pH 7.40 and the determination of glucose was carried out by amperometry at 0.400 V at GCE/MWCNTs-BCS/Cu/GOx/Naf biosensor using the standard addition method in the three cases."

Results and Discussion:

At the end of 3.2…" The recovery percentage was (94 ± 9) % demonstrating the analytical usefulness of the proposed sensor for the highly sensitive and selective quantification of hydrogen peroxide in untreated milk samples."

At the end of 3.3…" These results confirmed the analytical usefulness of GCE/MWCNTs-BCS/Cu/GOx/Naf nanohybrid platform for the development of an efficient electrochemical glucose biosensor that demonstrate practical applicability for the quantification in several untreated samples."

Reviewer #2:

The manuscript (JPBA-D-20-00510) with entitled 'DOBLE ROLE OF BATHOCUPROINE DISULFONIC ACID AS MULTI-WALLED CARBON NANOTUBES DISPERSING AGENT AND COPPER PRECONCENTRATION LIGAND: ANALYTICAL APPLICATIONS FOR THE DEVELOPMENT OF HYDROGEN PEROXIDE AND GLUCOSE ELECTROCHEMICAL SENSORS' have been performed by Pablo Gallay, Marcela Rodríguez, Marcos Eguílaz, Gustavo Rivas.

This study proposed a rapid, sensitive, selective and user friendly nanohybrid sensor for hydrogen peroxide and glucose. Multiwalled carbon nanotubes were used due to its high surface area and efficient immobilization of BCS and Glucose oxidase. Copper catalyzed the redox oxidation of H2O2. This study can be helpful for designing a portabile sensor. This study has most sensitive results in all glucose hydrogen peroxide sensor at literature.

The figures were designed with high resolution. The all manuscript was well organized. The real application was performed with milk and beverages. This study is proper for aim and scope of JPBA.

We appreciate very much the comments about our work.

Reviewer #3:

This manuscript reveals a new electrodic platform obtained by casting glassy carbon electrodes (GCE) with a dispersion of MWCNTs in BCS (MWCNTs-BCS) followed by the preconcentration of Cu(II) by surface complex formation at OCP (GCE/MWCNTs-BCS/Cu). The proposed platforms were successfully used for the quantification of hydrogen peroxide in enriched milk samples and glucose in milk and commercial beverages. The manuscript is well planned and well executed. The research team has a lot of experience in these topics.

The only aspect that deserves to be clarified has to do with recovery. In page 11 the authors inform a recovery percentage of 94.1% but in the experimental section there is no information on how this study was conducted. What is the standard deviation of the recovery?. Please include this information in the revised version. Furthermore, Include information about the reproducibility and repeatability of the platforms. Also discuss about reusing platforms.

We appreciate very much the comments about our work.

We have added in the Experimental Section (2.4. Procedure) a paragraph about the procedure for real samples.

Experimental Section:

"Hydrogen peroxide was quantified in a milk sample (La Serenísima®) enriched with 1.7 $x10^{-3}$ M hydrogen peroxide by transferring a given aliquot of the milk enriched sample to the electrochemical cell containing 5.0 mL of 0.050 M phosphate buffer solution pH 7.40, performing the quanitification by amperometry at 0.400 V using GCE/MWCNTs-BCS/Cu.

Glucose was quantified in milk (La Serenísima®) and two commercial drinks, Gatorade® and Red-Bull®. The beverages and milk were obtained from a local supermarket. An aliquot of the given sample was directly transferred to the electrochemical cell containing 5.0 mL of 0.050 M phosphate buffer pH 7.40 and the determination of glucose was carried out by amperometry at 0.400 V at GCE/MWCNTs-BCS/Cu/GOx/Naf biosensor using the standard addition method in the three cases."

Recovery percentage:

"The recovery percentage for hydrogen peroxide in milk samples was (94 ± 9) %."

Reproducibility:

Hydrogen peroxide: "The reproducibility obtained for 5 electrodes modified with the same MWCNTs-BCS dispersion was 7.1 %. "

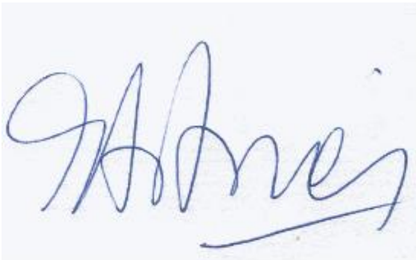
Glucose: "The reproducibility, obtained from the sensitivity of 5 biosensors, was 9.3% using the same MWCNTs-BCS dispersion. "

Repeatability/Reusability:

Regarding the repeatability, we have evaluated the sensitivity towards hydrogen peroxide for the same GCE/MWCNTs-BCS/Cu after successive amperometric determinations at 0.400 V. The sensitivity largely decreases even after the second use of the sensor, indicating that the repeatability/short-term stability and, consequently the reusability of the sensor are poor. Similar behavior was observed for the GCE/MWCNTs-BCS/Cu/GOx/Naf after successive amperometric determinations of glucose at 0.400 V. Therefore, considering this poor repeatability/short-term stability, the platform was thought as a single-use (bio)sensor.

Thanking for your consideration of our paper, we shall be looking forward to receiving further news.

Sincerely yours,



Prof. Dr. Gustavo A. Rivas
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DOBLE ROLE OF BATHOCUPROINE DISULFONIC ACID AS MULTI-WALLED CARBON NANOTUBES DISPERSING AGENT AND COPPER PRECONCENTRATION LIGAND: ANALYTICAL APPLICATIONS FOR THE DEVELOPMENT OF HYDROGEN PEROXIDE AND GLUCOSE ELECTROCHEMICAL SENSORS

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ABSTRACT

We are reporting a new strategy for preparing carbon nanotubes (CNTs)-based hydrogen peroxide and glucose amperometric sensors by taking advantage of the dual role of bathocuproine disulfonic acid (BCS) as dispersing agent of multi-walled carbon nanotubes (MWCNTs) and as ligand for the preconcentration of Cu(II). The platform was obtained by casting glassy carbon electrodes (GCE) with the dispersion of MWCNTs in BCS (MWCNTs-BCS) followed by the preconcentration of Cu(II) by surface complex formation at open circuit potential (GCE/MWCNTs-BCS/Cu). The resulting electrode was used for the sensitive amperometric quantification of hydrogen peroxide at 0.400 V catalyzed by the preconcentrated copper, with a linear range between 5.0 x 10^{-7} and 7.4 x 10^{-6} M, a sensitivity of 24.3 mA.M^{-1}, and a detection limit of 0.2 μM. The adsorption ~~incorporation~~ of GOx ~~by adsorption~~ at GCE/MWCNTs-BCS/Cu followed by the immobilization of Nafion (Naf), allowed the construction of a sensitive and selective amperometric glucose biosensor with a linear range between 5.0 x 10^{-6} M and 4.9 x 10^{-4} M, a sensitivity of (477 ± 3) μA.M^{-1} and a detection limit of 2 μM. The proposed (bio)sensors were successfully used for the quantification of hydrogen peroxide in enriched milk samples and glucose in milk and commercial beverages without any pretreatment.

Keywords: Carbon nanotubes; Bathocuproine disulfonic acid; Copper; Hydrogen peroxide electrochemical sensor; Glucose electrochemical biosensor.

1. INTRODUCTION

Nanomaterials have played a key role in the development of electrochemical (bio)sensors due to their multiple advantages to build (bio)analytical platforms and improve the transduction of (bio)recognition events [1-3]. Particularly, the use of carbon nanotubes (CNTs) for the development of electrochemical sensors, have demonstrated to be a highly successful strategy due to their well-known properties mainly connected with the large surface area, good conductivity, catalytic activity towards the oxidation/reduction of different analytes, and multiple possibilities of functionalization [4, 5] [~~4-6~~]. However, despite these unique properties, CNTs require a functionalization step to disaggregate the bundles before the incorporation in the electrochemical sensors [6] ~~[7,8~~]. This functionalization, either covalent or non-covalent, has two main goals, the obvious one, to exfoliate the nanostructures and allow their dispersion in aqueous media, and the other one, more challenging, to give particular properties to the dissaggregated nanostructures [7] ~~[9].~~ In fact, depending on the nature of the dispersing agents, these properties can be connected to special groups and/or to the biorecognition ability that will allow the anchoring/preconcentration of diverse species and the direct biosensing/bioaffinity interaction, respectively [7] ~~[9].~~

Different strategies have been proposed for de-bundling and functionalizing CNTs, using ionic liquids, polymers, biomolecules, organic molecules and euthectyc mixtures, among others [8, 9] ~~[10,11,12~~]. Polymers like polyhistidine, polylysine [7] [~~13~~], polyarginine [10] ~~[14]~~, ~~polylysine [15~~], polytyrosine [11] ~~[16]~~, small biomolecules like cysteine [12] ~~[17]~~, and biomacromolecules such as glucose oxidase [13] ~~[18]~~, cytochrome c [14] ~~[19]~~, calf-thymus double stranded DNA (dsDNA) [15] [20], avidin [16] [21] and concanavalin A [17] [22] have been successfully used for dissagregating the MWCNTs and building different (bio)sensors.

Hydrogen peroxide is a very important analyte that is receiving increasing attention due to the connection with important metabolic routes, the importance in different industries, the significance as biomarker of diverse pathologies mainly associated with cancer and degenerative processes [18] [23], and its widespread use as indicator for transducing different biorecognition events [19] [24]. In this sense, the most typical example is the use of hydrogen peroxide as indicator for glucose oxidase(GOx)-based first generation electrochemical glucose biosensors. C Considering However, is important to remark that the oxidation and reduction of hydrogen peroxide at carbon electrodes require elevated overvoltages [20] [25], different strategies have been used to overcome this problem. Among them, the incorporation of transition metal nano/micro-particles, like Cu [21] [26], Au [22] [27], Ir [23] [28], Pd [24] [29], Rh [25] [30], Ru [26] [31], metals mixtures [32] and alloys like Cu@PtPd/C [27] [33] core-shell nanoparticles, has demonstrated to be highly successful due to the catalytic activity of these metals for the oxidation and reduction of hydrogen peroxide.

Recently [28] [34], we have reported an electrochemical sensor for the highly sensitive and selective quantification of Cu(II) through the use of MWCNTs non-covalently functionalized with bathocuproine disulfonic acid (BCS), a compound analogue to the ligand bathocuproine (BC), that is an excellent ligand for complexing Cu(I) (Cu(I)-BCS, log β = 19.8) and Cu(II) (Cu(II)-BCS log β_2 = 11.9) [29, 30] [35, 36].

Here, we propose the use of GCE modified with MWCNTs non-covalently functionalized with BCS as Cu(II)-preconcentration layer (GCE/MWCNTs-BCS/Cu) for the development of hydrogen peroxide sensors and glucose biosensors previous incorporation of glucose oxidase (GOx), based on the catalytic activity of the accumulated copper on hydrogen peroxide oxidation. In the following sections we discuss the optimization of the preparation conditions for GCE/MWCNTs-BCS/Cu, the construction of the glucose biosensor enzymatic layer for the quantification of glucose and the analytical performance of the resulting bioanalytical platforms sensors for the quantification of hydrogen peroxide and glucose.

2. MATERIALS AND METHODS

2.1. Chemicals and solutions

Carbon nanotubes (MWCNTs, (30 ± 15) nm diameter, (1-5) µm length and 95.5 % purity, bathocuproine disulfonic acid disodium salt (BCS), glucose, lactose, fructose, galactose, maltose, glucose oxidase (from Aspergillus niger, (EC 1.1.3.4 163,400 units/g of solid)), copper atomic absorption standard solution (1010 μg.mL^{-1} in 5 % HCl) and Nafion (Naf) were supplied from Sigma-Aldrich. Hydrogen peroxide was acquired from Carlo Erba. Other chemicals were of analytical grade and used without further purification.

A 0.050 M phosphate buffer solution pH 7.40 was used as supporting electrolyte. Ultrapure water (ρ = 18.2 MΩ cm) from a Millipore-MilliQ system was used for preparing all aqueous solutions.

2.2. Apparatus

Ultra-sonication was carried out with an ultrasonic processor VCX 130W, Sonics and Materials, Inc. of 20 kHz frequency with a microtip of titanium alloy of 3 mm-diameter.

Electrochemical experiments were performed with a TEQ_04 potentiostat. Glassy carbon electrodes (GCE, CH Instruments, 3mm-diameter) modified with MWCNTs dispersed in BCS containing the preconcentrated Cu(II) (GCE/MWCNTs-BCS/Cu) and GCE/MWCNTs-BCS/Cu modified with GOx and Naf (GCE/MWCNTs-BCS/Cu/GOx/Naf) were used as working electrodes. A Pt wire and Ag/AgCl, 3 M NaCl (BAS) were used as auxiliary and reference electrodes, respectively. All potentials are referred to this reference electrode.

Scanning Electron Microscopy (SEM) images were obtained with a Field Emission Gun Scanning Electron Microscope (FE-SEM, Zeiss, ΣIGMA model) equipped with secondary and back-scattered electron detectors. The samples were prepared by drop-coating of the MWCNT-BCS dispersion onto GCE disks followed by the accumulation of Cu(II) at open circuit potential previous evaporation of the solvent at room temperature.

2.3. Preparation of the modified electrodes

2.3.1. Preparation of GCE modified with MWCNTs-BCS and Cu (GCE/MWCNTs-BCS/Cu): this electrode was prepared according to reference [28] ~~[34~~]. Briefly, MWCNTs (0.5 mg.mL^{-1}) were dispersed in 1.0 mg mL^{-1} BCS using a sonicator probe with amplitude of 50% for 10 min while keeping in an ice-bath. GCE/MWCNTs-BCS was obtained by casting 10 µL of MWCNTs-BCS on the top of GCE previously polished with alumina slurries of 1.0, 0.3 and 0.05 µm, rinsed thoroughly with deionized water, sonicated for 30 s in water, and finally dried under a N_2 stream. The preconcentration of Cu was performed at open circuit potential (ocp) by immersion of GCE/MWCNTs-BCS in a 2.5 ppm Cu(II) solution prepared in 0.020 M acetate buffer solution pH 5.00 for 3.0 min under stirring conditions.

2.3.2. Preparation of GCE/MWCNTs-BCS/Cu modified with GOx and Naf (GCE/MWCNTs-BCS/Cu/GOx/Naf): the biosensor was prepared by drop-coating 2.0 mg mL^{-1} GOx onto GCE/MWCNTs-BCS/Cu, followed by the deposition of ~~. Finally, GCE/MWCNTs-BCS/Cu/GOx was coated with~~ 5 μL of 0.5 % w/v Naf. Figure 1 shows the scheme for the preparation of the different platforms.

2.4. Procedure

The quantification of hydrogen peroxide and glucose was performed by amperometry at 0.400 V. All electrochemical experiments were conducted at room temperature in a 0.050 M phosphate buffer solution pH 7.40.

Hydrogen peroxide was quantified in a milk sample (La Serenísima®) enriched with 1.7 x10^{-3} M hydrogen peroxide by transferring a given aliquot of the milk enriched sample to the electrochemical cell containing 5.0 mL of 0.050 M phosphate buffer solution pH 7.40, performing the quanitification by amperometry at 0.400 V using GCE/MWCNTs-BCS/Cu.

Glucose was quantified in milk (La Serenísima®) and two commercial drinks, Gatorade® and Red-Bull®. The beverages and milk were obtained from a local supermarket. An aliquot of the given sample was directly transferred to the electrochemical cell containing 5.0 mL of 0.050 M phosphate buffer pH 7.40 and the determination of glucose was carried out by amperometry at 0.400 V at GCE/MWCNTs-BCS/Cu/GOx/Naf biosensor using the standard addition method in the three cases.

3. RESULTS AND DISCUSSION

3.1. Characterization of GCE/MWCNTs-BCS/Cu

Figure 2A shows SEM pictures of GCE/MWCNTs-BCS/Cu prepared by modification of GCE with a dispersion of 0.50 mgmL^{-1} MWCNTs in 1.0 mg mL^{-1} BCS, followed by the preconcentration of Cu (II) by immersion in a 2.5 ppm Cu (II) solution for 3.0 min at ocp. The whole surface of the glassy carbon disk is covered by MWCNTs-BCS although, in agreement with the pattern obtained for other MWCNTs-modified GCEs [7] [9] there are areas with different density of MWCNTs. As it was previously demonstrated [28] [34], BCS largely contributes to the exfoliation of MWCNTs due the facilitated interaction with the aqueous solvent through the sulfonate groups of the BCS that supports the MWCNTs. The EDX map of the glassy carbon disk shown in Figure 2A (GCE/MWCNTs-BCS/Cu), demonstrates that copper is distributed in the whole surface, confirming the efficient preconcentration of Cu(II) at GCE/MWCNTs-BCS (Figure 2B).

Figure 2C displays the cyclic voltammetric profiles of GCE/MWCNTs-BCS (black line) and GCE/MWCNTs-BCS/Cu (red line) in a 0.050 M phosphate buffer solution pH 7.40. No peaks are observed at GCE/MWCNTs-BCS, while in the presence of Cu at the electrode surface, there are two anodic peaks due to the oxidation of Cu to Cu(I) (0.194 V) and Cu(I) to Cu(II) (0.416 V). The corresponding reduction of Cu(II) to Cu(I) is observed at 0.407 V, while the reduction of Cu(I) to Cu mostly occurs at potentials close to 0 V. Successive voltammograms performed with GCE/MWCNTs-BCS/Cu in buffer solution did not show significant differences in the peak currents for the oxidation and reduction of Cu, clearly evidencing that BCS retains copper in a very robust way (not shown). Therefore, BCS successfully works in the double role of MWCNTs disaggregation agent and surface copper preconcentration element.

3.2. Analytical application of GCE/MWCNTs-BCS/Cu for the quantification of hydrogen peroxide

Figure 3A displays the potentiodynamic i-E profiles obtained at GCE/MWCNTs-BCS/Cu in a ~~0.020~~ 0.050 M phosphate buffer solution pH 7.40 without (black line) and with (red line) 2.0 x 10^{-2} M hydrogen peroxide. The cyclic voltammogram obtained in the absence of hydrogen peroxide presents the expected profile according to Figure 2C. The voltammetric response for 2.0x10^{-2} M hydrogen peroxide shows a huge increment of the oxidation and reduction currents due to the catalytic activity of copper [21] ~~[26]~~. Figure 3B displays the hydrodynamic voltammograms for 2.0 x 10^{-4} M hydrogen peroxide at GCE/MWCNTs-BCS (black) and GCE/MWCNTs-BCS/Cu (red). In agreement with Figure 3A, the presence of copper at the electrode surface produces a drastic decrease in the overvoltages for the oxidation and reduction of hydrogen peroxide and a noticeable increment in the associated currents~~,~~ confirming, once more, the excellent catalytic activity of the copper preconcentrated at the BCS that supports the MWCNTs.

The effect of the accumulation time of 2.5 ppm Cu(II) at GCE/MWCNTs-BCS on the sensitivity for the oxidation of hydrogen peroxide at 0.400 V is shown in Figure 4A. The sensitivity increases with the interaction time and reaches a maximum after 3.0 min, suggesting a saturation of the available sites of the BCS that supports the MWCNTs for complex formation. We also evaluated the influence of Cu(II) concentration used for the preconcentration ~~during the accumulation~~ ~~for 3.0 min~~ at GCE/MWCNTs-BCS for 3.0 min on the sensitivity for hydrogen peroxide oxidation (Figure 4B). It increases with the concentration of Cu(II), reaching a maximum after 2.5 ppm Cu(II). Therefore, the selected conditions for the preconcentration of Cu at the surface of GCE/MWCNTs-BCS were an interaction time of 3.0 min at ocp using a 2.5 ppm Cu(II) solution.

The amperometric response of H_2O_2 at GCE/MWCNTs-BCS/Cu at a working potential of 0.400 V is displayed in Figure 5A. After the addition of H_2O_2, the current rapidly increases and reaches the steady-state after 3 seconds. The inset shows the amperometric response for the lower concentrations range. The corresponding calibration plot is depicted in Figure 5B. The linear range goes from 5.0×10^{-7} M to 7.4×10^{-6} M, with a sensitivity of 24.3 $mA.M^{-1}$ ($r^2 = 0.990$), and a detection limit of 0.2 µM (taken as 3.3 σ/S, where σ is the blank signal-standard deviation and S the sensitivity). The reproducibility obtained for 5 electrodes modified with the same MWCNTs-BCS dispersion was 7.1 %.

Table 1-SI (Supplementary Information) compares the analytical performance of our H_2O_2 sensor with the most relevant non-enzymatic hydrogen peroxide amperometric sensors reported since 2017. The proposed H_2O_2 sensor possesses a competitive detection limit which is lower than those ~~the detection limits~~ obtained in [1-6, 10, 12] ~~[37-42, 46, 48]~~, comparable to those reported in

the references [24, 7, 8, 11] ~~[29, 43, 44, 47]~~ and higher than those presented in [26, 9, 14] ~~[31, 45, 50]~~. However, even when the detection limit of the proposed sensor is higher than those reported in ref. [9, 14] ~~[45 and 50]~~, is important to remark that, these sensors require a more complex and expensive preparation, either using GCE modified with MWCNTs-SnO_2 nanofibers, hemoglobin and chitosan [45] or CF@N-CNTAs-AuNPs [50]. In addition, the working potentials for these sensors ~~in the case of ref.~~ [9, 14] ~~[45 and 50] and [50], the working potentials~~ are very negative (-0.40 and -0.30 V, respectively), making necessary the desoxygenation of the solution and longer times to stabilize the base line currents. GCE/MWCNTs-Av/Ru [26] ~~[31]~~ was proposed recently by our group and allowed to reach detection limits three times smaller than GCE/MWCNTs-BCS/Cu, with a considerably wider linear range, although the sensitivity is comparable to that of our sensor. One advantage of our sensor, is that the element responsible for the catalytic activity (Cu) is considerably cheaper than the one used in the case of GCE/MWCNTs-Av/Ru. In summary, the sensor proposed in this work is a very competitive alternative compared to the already existing ones.

Figure 5C compares the sensitivity for hydrogen peroxide obtained from amperometric recordings at 0.400 V at GCE/MWCNTs-BCS and GCE/MWCNTs-BCS/Cu. As expected, the sensitivity for hydrogen peroxide obtained at GCE/MWCNTs-BTC is negligible compared to the one obtained at GCE/MWCNTs-BCS/Cu.

We evaluate the analytical application of the sensor, determining the recovery of hydrogen peroxide in a milk sample enriched with 1.7 x 10^{-3} M hydrogen peroxide~~,~~ without any pre-treatment. ~~The samples were enriched with~~

~~1.7 x 10^{-3} M hydrogen peroxide.~~ The recovery percentage was ~~94.1%~~ (94 ± 9) % demonstrating the analytical usefulness of the proposed sensor for the highly sensitive and selective quantification of hydrogen peroxide in untreated milk samples.

3.3. Analytical applications of GCE/MWCNTs-BCS/Cu/GOx/Naf for the quantification of glucose

Figure 6A displays the amperometric response of glucose at GCE/MWCNTs-BCS/Cu/GOx/Naf at 0.400 V. A well-defined and fast response is observed after each addition of glucose. The corresponding calibration plot, displayed in Figure 6B, shows a linear range between 1.0 x 10^{-5} M and 4.9 x 10^{-4} M, with a sensitivity of (477 ± 3) μAM^{-1} (r^2 = 0.9996), and a limit of detection of 2 µM (calculated as it was previously indicated).

Different concentrations of GOx and dilutions of Naf were evaluated and the best compromise between sensitivity, stability and reproducibility was obtained using 2.0 mg/mL GOx and 0.5 % w/v Naf (results not shown).

The reproducibility, obtained from the sensitivity of 5 biosensors, was 9.3% using the same MWCNTs-BCS dispersion. The selectivity of GCE/MWCNTs-BCS/Cu/GOx/Naf was evaluated in the presence of 1.0 x 10^{-4} M lactose, galactose, fructose and maltose. No interference was obtained for lactose and maltose, while for galatose and fructose it was just (7.7 ± 0.9) % and (7.2 ± 0.1) %, respectively, demonstrating the selectivity of the biosensor in the presence of other sugars.

Table 2-SI (Supplementary Information) summarizes the analytical performance of the most representative ~~electrochemical~~ enzymatic glucose

biosensors with electrochemical trasnduction reported since 2018. The detection limit of our biosensor is better than those ~~the detection limits~~ reported in references [23-28, 31-33] ~~[59-64, 67-69]~~, comparable to the ones obtained in [17-22, 30] ~~[53-58, 66]~~ and higher than those reported in references [15, 16, 29, 34] ~~[51, 52, 65, 70]~~. GCE/AuNF/GS-IL-AuNRs/GOx/GA/Naf [16] ~~[52]~~, Pt/GOx/gelatin [29] ~~[65]~~ and Pt/IrNPs/Ludox/GOx [34] ~~[70]~~ involve expensive noble metals like Au~~-NR~~, Pt, and Pt-Ir, respectively. Therefore, our biosensor represents a competitive bioanalytical platform for glucose quantification with a relatively simple procedure for the preparation of the bioanalytical platform ~~electrode~~.

The practical application of the biosensor was evaluated using milk (La Serenísima®) and two beverages (Gatorade® and Red Bull®). The average concentration of glucose in milk, obtained from 5 determinations, was (2.0 ± 0.1) g/100 mL, value that is in excellent agreement with the one reported by the company (1.9 g.mL^{-1}). The glucose contents in Gatorade and Red Bull obtained with our biosensor were (2.5 ± 0.3) g/100 mL and (3.4 ± 0.3) g/100 mL, respectively, values that also show an excellent correlation with the reported values (2.3 g/100mL and 3.6 g/100 mL, respectively). These results confirmed the analytical usefulness of GCE/MWCNTs-BCS/Cu/GOx/Naf nanohybrid platform for the development of an efficient electrochemical glucose biosensor that demonstrate practical applicability for the quantification in several untreated samples.

4. CONCLUSIONS

The platforms proposed in this work represent a fast, easy-to-prepare, reproducible, sensitive and selective alternative to develop hydrogen peroxide and glucose sensors with very competitive analytical performance and interesting practical applications in untreated samples, without the requirement of sophisticated instruments or complicated protocols. These platforms are the result of an efficient integration of MWCNTs, that offer a large surface and the robustness for the efficient immobilization of BCS and GOx; BCS, that allows the disaggregation of the carbon nanostructures and the preconcentration of the catalyst; and Cu, that efficiently catalyzes the oxidation of hydrogen peroxide.

This strategy to build a biosensing platform can be considered a prototype for further developments of other (bio)sensors, either based on the catalytic activity of copper for building non-enzymatic sensors, or biosensors based on hydrogen peroxide-producing oxidases.

DECLARATION OF COMPETING INTEREST

On behalf of the authors of the manuscript “doble role of bathocuproine disulfonic acid as multi-walled carbon nanotubes dispersing agent and copper preconcentration ligand: analytical applications for the development of hydrogen peroxide and glucose electrochemical sensors” by Gallay, Rodríguez, Eguílaz and Rivas, I declare that there are no conflicts of interest.

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LEGENDS OF THE FIGURES

Figure 1: Schematic representation of the steps involved in the functionalization of MWCNTs with BCS and copper.

Figure 2: (A) SEM micrograph of a glassy carbon disk modified with a dispersion of 0.50 $mgmL^{-1}$ MWCNTs in 1.0 mg mL^{-1} BCS, followed by the preconcentration of Cu (II) by immersion in a 2.5 ppm Cu (II) solution for 3.0 min at ocp. Magnification: 10,000 X (B) EDX of the GCE/MWCNTs-BCS/Cu. C) Cyclic voltammograms obtained at GCE/MWCNTs-BCS (black) and at GCE/MWCNTs-BCS/Cu (red) in phosphate buffer solution 0.050 M pH 7.40. Scan rate: 50 $mV.s^{-1}$. Modification of GCE: same conditions as in Figure 2A.

Figure 3: (A) Cyclic voltammograms obtained at GCE/MWCNTs-BCS/Cu in phosphate buffer solution 0.050 M pH 7.40 (black) and 2.0 x 10^{-2} M H_2O_2(–). Scan rate: 0.050 V s^{-1}. (B) Hydrodynamic voltagram for 2.0 x 10^{-4} M H_2O_2 at GCE/MWCNTs-BCS/Cu (black) and GCE/MWCNTs-BCS/Cu (red).

Figure 4: Sensitivities for hydrogen peroxide obtained from amperometric recordings at GCE/MWCNTs-BCS/Cu as a function of (A) the accumulation time and (B) Cu(II) concentration. Working potential: +0.400 V. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40. Cu(II) concentration (A): 2.5 ppm. Accumulation time (B): 3.0 min.

Figure 5: (A) Amperometric recording obtained at GCE/MWCNTs-BCS/Cu for successive additions of 5.0 x 10^{-7} M (a), 1.0 x 10^{-6} M (b), 5.0x10^{-6} M (c), and 1.0 x10^{-5} (d) M H_2O_2. Inset: amperometric recording for the lower concentrations range. (B) Calibration plot obtained from the amperometric recording shown in Figure 5 A. Inset: calibration plot in a more restricted concentrations range. (C) Sensitivities for H_2O_2 obtained from amperometric experiments using GCE/MWCNTs-BCS and CGE/MWCNTs-BCS/Cu. Inset: amperometric recordings for successive additions of hydrogen peroxide at GCE/MWCNTs-BCS (black) and GCE/MWCNTs-BCS-Cu (red). Working potential: +0.400 V. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

Figure 6: (A) Amperometric recording obtained at GCE/MWCNTs-BCS/Cu/GOx/Nf for successive additions of 1.0 x 10^{-5} M (a), 5.0 x 10^{-5} M (b), and 1.0x10^{-4} M (c) glucose. Inset: shows the amperometric recording for the lower concentrations range. (B) Calibration plot obtained from the amperometric recording shown in Figure 6 A. Inset: calibration plot in a more restricted concentrations range.

Table 1: Comparison of the analytical performance of GCE/MWCNTs-BCS/Cu with those of the the most relevant non-enzymatic electrochemical hydrogen peroxide sensors reported since 2017.

Table 2: Comparison of the analytical performance of GCE/MWCNTs-BCS/Cu/GOx/Naf with those of the the most relevant amperometric enzymatic glucose biosensors reported in the period 2018-2020.

Highlights-RIVAS ET AL

- BCS presents the double role of exfoliating MWCNTs and accumulating Cu.
- The nanohybrid MWCNTs-BCS was successfully used to accumulate Cu in a robust way.
- GCE/MWCNTs-BCS/Cu makes possible the sensitive quantification of H_2O_2.
- GCE/MWCNT-BCS/Cu/GOx/Naf is successfully used to quantify glucose in milk and beverages.

Abstract

We are reporting a new strategy for preparing carbon nanotubes (CNTs)-based hydrogen peroxide and glucose amperometric sensors by taking advantage of the dual role of bathocuproine disulfonic acid (BCS) as dispersing agent of multi-walled carbon nanotubes (MWCNTs) and as ligand for the preconcentration of Cu(II). The platform was obtained by casting glassy carbon electrodes (GCE) with the dispersion of MWCNTs in BCS (MWCNTs-BCS) followed by the preconcentration of Cu(II) by surface complex formation at open circuit potential (GCE/MWCNTs-BCS/Cu). The resulting electrode was used for the sensitive amperometric quantification of hydrogen peroxide at 0.400 V catalyzed by the preconcentrated copper, with a linear range between 5.0×10^{-7} and 7.4×10^{-6} M, a sensitivity of 24.3 $mA.M^{-1}$, and a detection limit of 0.2 μM. The adsorption of GOx at GCE/MWCNTs-BCS/Cu followed by the immobilization of Nafion (Naf), allowed the construction of a sensitive and selective amperometric glucose biosensor with a linear range between 5.0×10^{-6} M and 4.9×10^{-4} M, a sensitivity of (477 ± 3) $\mu A.M^{-1}$ and a detection limit of 2 μM. The proposed (bio)sensors were successfully used for the quantification of hydrogen peroxide in enriched milk samples and glucose in milk and commercial beverages without any pretreatment.

DOBLE ROLE OF BATHOCUPROINE DISULFONIC ACID AS MULTI-WALLED CARBON NANOTUBES DISPERSING AGENT AND COPPER PRECONCENTRATION LIGAND: ANALYTICAL APPLICATIONS FOR THE DEVELOPMENT OF HYDROGEN PEROXIDE AND GLUCOSE ELECTROCHEMICAL SENSORS

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ABSTRACT

We are reporting a new strategy for preparing carbon nanotubes (CNTs)-based hydrogen peroxide and glucose amperometric sensors by taking advantage of the dual role of bathocuproine disulfonic acid (BCS) as dispersing agent of multi-walled carbon nanotubes (MWCNTs) and as ligand for the preconcentration of Cu(II). The platform was obtained by casting glassy carbon electrodes (GCE) with the dispersion of MWCNTs in BCS (MWCNTs-BCS) followed by the preconcentration of Cu(II) by surface complex formation at open circuit potential (GCE/MWCNTs-BCS/Cu). The resulting electrode was used for the sensitive amperometric quantification of hydrogen peroxide at 0.400 V catalyzed by the preconcentrated copper, with a linear range between 5.0×10^{-7} and 7.4×10^{-6} M, a sensitivity of 24.3 $mA.M^{-1}$, and a detection limit of 0.2 μM. The adsorption of GOx at GCE/MWCNTs-BCS/Cu followed by the immobilization of Nafion (Naf), allowed the construction of a sensitive and selective amperometric glucose biosensor with a linear range between 5.0×10^{-6} M and 4.9×10^{-4} M, a sensitivity of (477 ± 3) $\mu A.M^{-1}$ and a detection limit of 2 μM. The proposed (bio)sensors were successfully used for the quantification of hydrogen peroxide in enriched milk samples and glucose in milk and commercial beverages without any pretreatment.

Keywords: Carbon nanotubes; Bathocuproine disulfonic acid; Copper; Hydrogen peroxide electrochemical sensor; Glucose electrochemical biosensor.

1. INTRODUCTION

Nanomaterials have played a key role in the development of electrochemical (bio)sensors due to their multiple advantages to build (bio)analytical platforms and improve the transduction of (bio)recognition events [1-3]. Particularly, the use of carbon nanotubes (CNTs) for the development of electrochemical sensors, have demonstrated to be a highly successful strategy due to their well-known properties mainly connected with the large surface area, good conductivity, catalytic activity towards the oxidation/reduction of different analytes, and multiple possibilities of functionalization [4, 5]. However, despite these unique properties, CNTs require a functionalization step to disaggregate the bundles before the incorporation in the electrochemical sensors [6]. This functionalization, either covalent or non-covalent, has two main goals, the obvious one, to exfoliate the nanostructures and allow their dispersion in aqueous media, and the other one, more challenging, to give particular properties to the dissaggregated nanostructures [7]. In fact, depending on the nature of the dispersing agents, these properties can be connected to special groups and/or to the biorecognition ability that will allow the anchoring/preconcentration of diverse species and the direct biosensing/bioaffinity interaction, respectively [7].

Different strategies have been proposed for de-bundling and functionalizing CNTs, using ionic liquids, polymers, biomolecules, organic molecules and euthectyc mixtures, among others [8, 9]. Polymers like polyhistidine, polylysine [7], polyarginine [10], polytyrosine [11], small biomolecules like cysteine [12], and biomacromolecules such as glucose oxidase [13], cytochrome c [14], calf-thymus double stranded DNA (dsDNA) [15], avidin

[16] and concanavalin A [17] have been successfully used for dissagregating the MWCNTs and building different (bio)sensors.

Hydrogen peroxide is a very important analyte that is receiving increasing attention due to the connection with important metabolic routes, the importance in different industries, the significance as biomarker of diverse pathologies mainly associated with cancer and degenerative processes [18], and its widespread use as indicator for transducing different biorecognition events [19]. In this sense, the most typical example is the use of hydrogen peroxide as indicator for glucose oxidase(GOx)-based first generation electrochemical glucose biosensors. C Considering that the oxidation and reduction of hydrogen peroxide at carbon electrodes require elevated overvoltages [20], different strategies have been used to overcome this problem. Among them, the incorporation of transition metal nano/micro-particles, like Cu [21], Au [22], Ir [23], Pd [24], Rh [25], Ru [26], and alloys like Cu@PtPd/C [27] core-shell nanoparticles, has demonstrated to be highly successful due to the catalytic activity of these metals for the oxidation and reduction of hydrogen peroxide.

Recently [28], we have reported an electrochemical sensor for the highly sensitive and selective quantification of Cu(II) through the use of MWCNTs non-covalently functionalized with bathocuproine disulfonic acid (BCS), a compound analogue to the ligand bathocuproine (BC), that is an excellent ligand for complexing Cu(I) (Cu(I)-BCS, log $\beta = 19.8$) and Cu(II) (Cu(II)-BCS log $\beta_2 = 11.9$) [29, 30].

Here, we propose the use of GCE modified with MWCNTs non-covalently functionalized with BCS as Cu(II)-preconcentration layer (GCE/MWCNTs-BCS/Cu) for the development of hydrogen peroxide sensors and glucose biosensors previous incorporation of glucose oxidase (GOx), based on the catalytic activity of the accumulated copper on hydrogen peroxide oxidation. In the following sections we discuss the optimization of the preparation conditions for GCE/MWCNTs-BCS/Cu, the construction of the glucose biosensor and the analytical performance of the resulting bioanalytical platforms for the quantification of hydrogen peroxide and glucose.

2. MATERIALS AND METHODS

2.1. Chemicals and solutions

Carbon nanotubes (MWCNTs, (30 ± 15) nm diameter, (1-5) µm length and 95.5 % purity, bathocuproine disulfonic acid disodium salt (BCS), glucose, lactose, fructose, galactose, maltose, glucose oxidase from Aspergillus niger EC 1.1.3.4 163,400 units/g of solid)), copper atomic absorption standard solution (1010 $\mu g.mL^{-1}$ in 5 % HCl) and Nafion (Naf) were supplied from Sigma-Aldrich. Hydrogen peroxide was acquired from Carlo Erba. Other chemicals were of analytical grade and used without further purification.

A 0.050 M phosphate buffer solution pH 7.40 was used as supporting electrolyte. Ultrapure water (ρ = 18.2 MΩ cm) from a Millipore-MilliQ system was used for preparing all aqueous solutions.

2.2. Apparatus

Ultra-sonication was carried out with an ultrasonic processor VCX 130W, Sonics and Materials, Inc. of 20 kHz frequency with a microtip of titanium alloy of 3 mm-diameter.

Electrochemical experiments were performed with a TEQ_04 potentiostat. Glassy carbon electrodes (GCE, CH Instruments, 3mm-diameter) modified with MWCNTs dispersed in BCS containing the preconcentrated Cu(II) (GCE/MWCNTs-BCS/Cu) and GCE/MWCNTs-BCS/Cu modified with GOx and Naf (GCE/MWCNTs-BCS/Cu/GOx/Naf) were used as working electrodes. A Pt wire and Ag/AgCl, 3 M NaCl (BAS) were used as auxiliary and reference electrodes, respectively. All potentials are referred to this reference electrode.

Scanning Electron Microscopy (SEM) images were obtained with a Field Emission Gun Scanning Electron Microscope (FE-SEM, Zeiss, ΣIGMA model) equipped with secondary and back-scattered electron detectors. The samples were prepared by drop-coating of the MWCNT-BCS dispersion onto GCE disks followed by the accumulation of Cu(II) at open circuit potential previous evaporation of the solvent at room temperature.

2.3. Preparation of the modified electrodes

2.3.1. Preparation of GCE modified with MWCNTs-BCS and Cu (GCE/MWCNTs-BCS/Cu): this electrode was prepared according to reference [28]. Briefly, MWCNTs (0.5 $mg.mL^{-1}$) were dispersed in 1.0 mg mL^{-1} BCS using a sonicator probe with amplitude of 50% for 10 min while keeping in an ice-bath. GCE/MWCNTs-BCS was obtained by casting 10 µL of MWCNTs-BCS on the top of GCE previously polished with alumina slurries of 1.0, 0.3 and 0.05 µm, rinsed thoroughly with deionized water, sonicated for 30 s in water, and finally dried under a N_2 stream. The preconcentration of Cu was performed at open circuit potential (ocp) by immersion of GCE/MWCNTs-BCS in a 2.5 ppm Cu(II) solution prepared in 0.020 M acetate buffer solution pH 5.00 for 3.0 min under stirring conditions.

2.3.2. Preparation of GCE/MWCNTs-BCS/Cu modified with GOx and Naf (GCE/MWCNTs-BCS/Cu/GOx/Naf): the biosensor was prepared by drop-coating 2.0 mg mL^{-1} GOx onto GCE/MWCNTs-BCS/Cu, followed by the deposition of 5 μL of 0.5 % w/v Naf. Figure 1 shows the scheme for the preparation of the different platforms.

2.4. Procedure

The quantification of hydrogen peroxide and glucose was performed by amperometry at 0.400 V. All electrochemical experiments were conducted at room temperature in a 0.050 M phosphate buffer solution pH 7.40.

Hydrogen peroxide was quantified in a milk sample (La Serenísima®) enriched with 1.7 $x10^{-3}$ M hydrogen peroxide by transferring a given aliquot of the milk enriched sample to the electrochemical cell containing 5.0 mL of 0.050 M phosphate buffer solution pH 7.40, performing the quanitification by amperometry at 0.400 V using GCE/MWCNTs-BCS/Cu.

Glucose was quantified in milk (La Serenísima®) and two commercial drinks, Gatorade® and Red-Bull®. The beverages and milk were obtained from a local supermarket. An aliquot of the given sample was directly transferred to the electrochemical cell containing 5.0 mL of 0.050 M phosphate buffer pH 7.40 and the determination of glucose was carried out by amperometry at 0.400 V at GCE/MWCNTs-BCS/Cu/GOx/Naf biosensor using the standard addition method in the three cases.

3. RESULTS AND DISCUSSION

3.1. Characterization of GCE/MWCNTs-BCS/Cu

Figure 2A shows SEM pictures of GCE/MWCNTs-BCS/Cu prepared by modification of GCE with a dispersion of 0.50 mgmL^{-1} MWCNTs in 1.0 mg mL^{-1} BCS, followed by the preconcentration of Cu (II) by immersion in a 2.5 ppm Cu (II) solution for 3.0 min at ocp. The whole surface of the glassy carbon disk is covered by MWCNTs-BCS although, in agreement with the pattern obtained for other MWCNTs-modified GCEs [7] there are areas with different density of MWCNTs. As it was previously demonstrated [28], BCS largely contributes to the exfoliation of MWCNTs due the facilitated interaction with the aqueous solvent through the sulfonate groups of the BCS that supports the MWCNTs. The EDX map of the glassy carbon disk shown in Figure 2A (GCE/MWCNTs-BCS/Cu), demonstrates that copper is distributed in the whole surface, confirming the efficient preconcentration of Cu(II) at GCE/MWCNTs-BCS (Figure 2B).

Figure 2C displays the cyclic voltammetric profiles of GCE/MWCNTs-BCS (black line) and GCE/MWCNTs-BCS/Cu (red line) in a 0.050 M phosphate buffer solution pH 7.40. No peaks are observed at GCE/MWCNTs-BCS, while in the presence of Cu at the electrode surface, there are two anodic peaks due to the oxidation of Cu to Cu(I) (0.194 V) and Cu(I) to Cu(II) (0.416 V). The corresponding reduction of Cu(II) to Cu(I) is observed at 0.407 V, while the reduction of Cu(I) to Cu mostly occurs at potentials close to 0 V. Successive voltammograms performed with GCE/MWCNTs-BCS/Cu in buffer solution did not show significant differences in the peak currents for the oxidation and reduction of Cu, clearly evidencing that BCS retains copper in a very robust way (not shown). Therefore, BCS successfully works in the double role of MWCNTs disaggregation agent and surface copper preconcentration element.

3.2. Analytical application of GCE/MWCNTs-BCS/Cu for the quantification of hydrogen peroxide

Figure 3A displays the potentiodynamic i-E profiles obtained at GCE/MWCNTs-BCS/Cu in a 0.050 M phosphate buffer solution pH 7.40 without (black line) and with (red line) 2.0 x 10^{-2} M hydrogen peroxide. The cyclic voltammogram obtained in the absence of hydrogen peroxide presents the expected profile according to Figure 2C. The voltammetric response for 2.0x10^{-2} M hydrogen peroxide shows a huge increment of the oxidation and reduction currents due to the catalytic activity of copper [21]. Figure 3B displays the hydrodynamic voltammograms for 2.0 x 10^{-4} M hydrogen peroxide at GCE/MWCNTs-BCS (black) and GCE/MWCNTs-BCS/Cu (red). In agreement with Figure 3A, the presence of copper at the electrode surface produces a drastic decrease in the overvoltages for the oxidation and reduction of hydrogen peroxide and a noticeable increment in the associated currents, confirming, once more, the excellent catalytic activity of the copper preconcentrated at the BCS that supports the MWCNTs.

The effect of the accumulation time of 2.5 ppm Cu(II) at GCE/MWCNTs-BCS on the sensitivity for the oxidation of hydrogen peroxide at 0.400 V is shown in Figure 4A. The sensitivity increases with the interaction time and reaches a maximum after 3.0 min, suggesting a saturation of the available sites of the BCS that supports the MWCNTs for complex formation. We also evaluated the influence of Cu(II) concentration used for the preconcentration at GCE/MWCNTs-

BCS for 3.0 min on the sensitivity for hydrogen peroxide oxidation (Figure 4B). It increases with the concentration of Cu(II), reaching a maximum after 2.5 ppm Cu(II). Therefore, the selected conditions for the preconcentration of Cu at the surface of GCE/MWCNTs-BCS were an interaction time of 3.0 min at ocp using a 2.5 ppm Cu(II) solution.

The amperometric response of H_2O_2 at GCE/MWCNTs-BCS/Cu at a working potential of 0.400 V is displayed in Figure 5A. After the addition of H_2O_2, the current rapidly increases and reaches the steady-state after 3 seconds. The inset shows the amperometric response for the lower concentrations range. The corresponding calibration plot is depicted in Figure 5B. The linear range goes from 5.0 x 10^{-7} M to 7.4 x 10^{-6} M, with a sensitivity of 24.3 mA.M^{-1} (r^2 = 0.990), and a detection limit of 0.2 µM (taken as 3.3 σ/S, where σ is the blank signal-standard deviation and S the sensitivity). The reproducibility obtained for 5 electrodes modified with the same MWCNTs-BCS dispersion was 7.1 %.

Table 1-SI (Supplementary Information) compares the analytical performance of our H_2O_2 sensor with the most relevant non-enzymatic hydrogen peroxide amperometric sensors reported since 2017. The proposed H_2O_2 sensor possesses a competitive detection limit which is lower than those obtained in [1-6, 10, 12], comparable to those reported in the references [24, 7, 8, 11] and higher than those presented in [26, 9, 14]. However, even when the detection limit of the proposed sensor is higher than those reported in ref. [9, 14], is important to remark that, these sensors require a more complex and expensive preparation, either using GCE modified with MWCNTs-SnO_2 nanofibers, hemoglobin and chitosan [45] or CF@N-CNTAs-AuNPs [50]. In addition, the working potentials for these sensors [9, 14] are very negative (-0.40 and -0.30 V, respectively),

making necessary the desoxygenation of the solution and longer times to stabilize the base line currents. GCE/MWCNTs-Av/Ru [26] was proposed recently by our group and allowed to reach detection limits three times smaller than GCE/MWCNTs-BCS/Cu, with a considerably wider linear range, although the sensitivity is comparable to that of our sensor. One advantage of our sensor, is that the element responsible for the catalytic activity (Cu) is considerably cheaper than the one used in the case of GCE/MWCNTs-Av/Ru. In summary, the sensor proposed in this work is very competitive alternative compared to the already existing ones.

Figure 5C compares the sensitivity for hydrogen peroxide obtained from amperometric recordings at 0.400 V at GCE/MWCNTs-BCS and GCE/MWCNTs-BCS/Cu. As expected, the sensitivity for hydrogen peroxide obtained at GCE/MWCNTs-BTC is negligible compared to the one obtained at GCE/MWCNTs-BCS/Cu.

We evaluate the analytical application of the sensor, determining the recovery of hydrogen peroxide in a milk sample enriched with 1.7 x 10^{-3} M hydrogen peroxide, without any pre-treatment. The recovery percentage was (94 ± 9) % demonstrating the analytical usefulness of the proposed sensor for the highly sensitive and selective quantification of hydrogen peroxide in untreated milk samples.

3.3. Analytical applications of GCE/MWCNTs-BCS/Cu/GOx/Naf for the quantification of glucose

Figure 6A displays the amperometric response of glucose at GCE/MWCNTs-BCS/Cu/GOx/Naf at 0.400 V. A well-defined and fast response is observed after each addition of glucose. The corresponding calibration plot, displayed in Figure 6B, shows a linear range between 1.0 x 10^{-5} M and 4.9 x 10^{-4} M, with a sensitivity of (477 ± 3) μAM^{-1} (r^2 = 0.9996), and a limit of detection of 2 µM (calculated as it was previously indicated).

Different concentrations of GOx and dilutions of Naf were evaluated and the best compromise between sensitivity, stability and reproducibility was obtained using 2.0 mg/mL GOx and 0.5 % w/v Naf (results not shown).

The reproducibility, obtained from the sensitivity of 5 biosensors, was 9.3% using the same MWCNTs-BCS dispersion. The selectivity of GCE/MWCNTs-BCS/Cu/GOx/Naf was evaluated in the presence of 1.0 x 10^{-4} M lactose, galactose, fructose and maltose. No interference was obtained for lactose and maltose, while for galatose and fructose it was just (7.7 ± 0.9) % and (7.2 ± 0.1) %, respectively, demonstrating the selectivity of the biosensor in the presence of other sugars.

Table 2-SI (Supplementary Information) summarizes the analytical performance of the most representative enzymatic glucose biosensors with electrochemical trasnduction reported since 2018. The detection limit of our biosensor is better than those reported in references [23-28, 31-33], comparable to the ones obtained in [17-22, 30] and higher than those reported in references [15, 16, 29, 34]. GCE/AuNF/GS-IL-AuNRs/GOx/GA/Naf [16], Pt/GOx/gelatin [29] and Pt/IrNPs/Ludox/GOx [34] involve expensive noble metals like Au, Pt, and Pt-Ir, respectively. Therefore, our biosensor represents a competitive bioanalytical platform for glucose quantification with a relatively simple procedure for the preparation of the bioanalytical platform.

The practical application of the biosensor was evaluated using milk (La Serenísima®) and two beverages (Gatorade® and Red Bull®). The average concentration of glucose in milk, obtained from 5 determinations, was (2.0 ± 0.1) g/100 mL, value that is in excellent agreement with the one reported by the company (1.9 $g.mL^{-1}$). The glucose contents in Gatorade and Red Bull obtained with our biosensor were (2.5 ± 0.3) g/100 mL and (3.4 ± 0.3) g/100 mL, respectively, values that also show an excellent correlation with the reported values (2.3 g/100mL and 3.6 g/100 mL, respectively). These results confirmed the analytical usefulness of GCE/MWCNTs-BCS/Cu/GOx/Naf nanohybrid platform for the development of an efficient electrochemical glucose biosensor that demonstrate practical applicability for the quantification in several untreated samples.

4. CONCLUSIONS

The platforms proposed in this work represent a fast, easy-to-prepare, reproducible, sensitive and selective alternative to develop hydrogen peroxide and glucose sensors with very competitive analytical performance and interesting practical applications in untreated samples, without the requirement of sophisticated instruments or complicated protocols. These platforms are the result of an efficient integration of MWCNTs, that offer a large surface and the robustness for the efficient immobilization of BCS and GOx; BCS, that allows the disaggregation of the carbon nanostructures and the preconcentration of the catalyst; and Cu, that efficiently catalyzes the oxidation of hydrogen peroxide.

This strategy to build a biosensing platform can be considered a prototype for further developments of other (bio)sensors, either based on the catalytic activity of copper for building non-enzymatic sensors, or biosensors based on hydrogen peroxide-producing oxidases.

DECLARATION OF COMPETING INTEREST

On behalf of the authors of the manuscript “doble role of bathocuproine disulfonic acid as multi-walled carbon nanotubes dispersing agent and copper preconcentration ligand: analytical applications for the development of hydrogen peroxide and glucose electrochemical sensors” by Gallay, Rodríguez, Eguílaz and Rivas, I declare that there are no conflicts of interest.

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LEGENDS OF THE FIGURES

Figure 1: Schematic representation of the steps involved in the functionalization of MWCNTs with BCS and copper.

Figure 2: (A) SEM micrograph of a glassy carbon disk modified with a dispersion of 0.50 mgmL^{-1} MWCNTs in 1.0 mg mL^{-1} BCS, followed by the preconcentration of Cu (II) by immersion in a 2.5 ppm Cu (II) solution for 3.0 min at ocp. Magnification: 10,000 X (B) EDX of the GCE/MWCNTs-BCS/Cu. C) Cyclic voltammograms obtained at GCE/MWCNTs-BCS (black) and at GCE/MWCNTs-BCS/Cu (red) in phosphate buffer solution 0.050 M pH 7.40. Scan rate: 50 mV.s^{-1}. Modification of GCE: same conditions as in Figure 2A.

Figure 3: (A) Cyclic voltammograms obtained at GCE/MWCNTs-BCS/Cu in phosphate buffer solution 0.050 M pH 7.40 (black) and 2.0 x 10^{-2} M H_2O_2(–). Scan rate: 0.050 V s^{-1}. (B) Hydrodynamic voltagram for 2.0 x 10^{-4} M H_2O_2 at GCE/MWCNTs-BCS/Cu (black) and GCE/MWCNTs-BCS/Cu (red).

Figure 4: Sensitivities for hydrogen peroxide obtained from amperometric recordings at GCE/MWCNTs-BCS/Cu as a function of (A) the accumulation time and (B) Cu(II) concentration. Working potential: +0.400 V. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40. Cu(II) concentration (A): 2.5 ppm. Accumulation time (B): 3.0 min.

Figure 5: (A) Amperometric recording obtained at GCE/MWCNTs-BCS/Cu for successive additions of 5.0 x 10^{-7} M (a), 1.0 x 10^{-6} M (b), 5.0x10^{-6} M (c), and 1.0

x10^{-5} (d) M H_2O_2. Inset: amperometric recording for the lower concentrations range. (B) Calibration plot obtained from the amperometric recording shown in Figure 5 A. Inset: calibration plot in a more restricted concentrations range. (C) Sensitivities for H_2O_2 obtained from amperometric experiments using GCE/MWCNTs-BCS and CGE/MWCNTs-BCS/Cu. Inset: amperometric recordings for successive additions of hydrogen peroxide at GCE/MWCNTs-BCS (black) and GCE/MWCNTs-BCS-Cu (red). Working potential: +0.400 V. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

Figure 6: (A) Amperometric recording obtained at GCE/MWCNTs-BCS/Cu/GOx/Nf for successive additions of 1.0 x 10^{-5} M (a), 5.0 x 10^{-5} M (b), and 1.0x10^{-4} M (c) glucose. Inset: shows the amperometric recording for the lower concentrations range. (B) Calibration plot obtained from the amperometric recording shown in Figure 6 A. Inset: calibration plot in a more restricted concentrations range.

Table 1: Comparison of the analytical performance of GCE/MWCNTs-BCS/Cu with those of the the most relevant non-enzymatic electrochemical hydrogen peroxide sensors reported since 2017.

Table 2: Comparison of the analytical performance of GCE/MWCNTs-BCS/Cu/GOx/Naf with those of the the most relevant amperometric enzymatic glucose biosensors reported in the period 2018-2020.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Figure

Figure 1- Rivas et al.

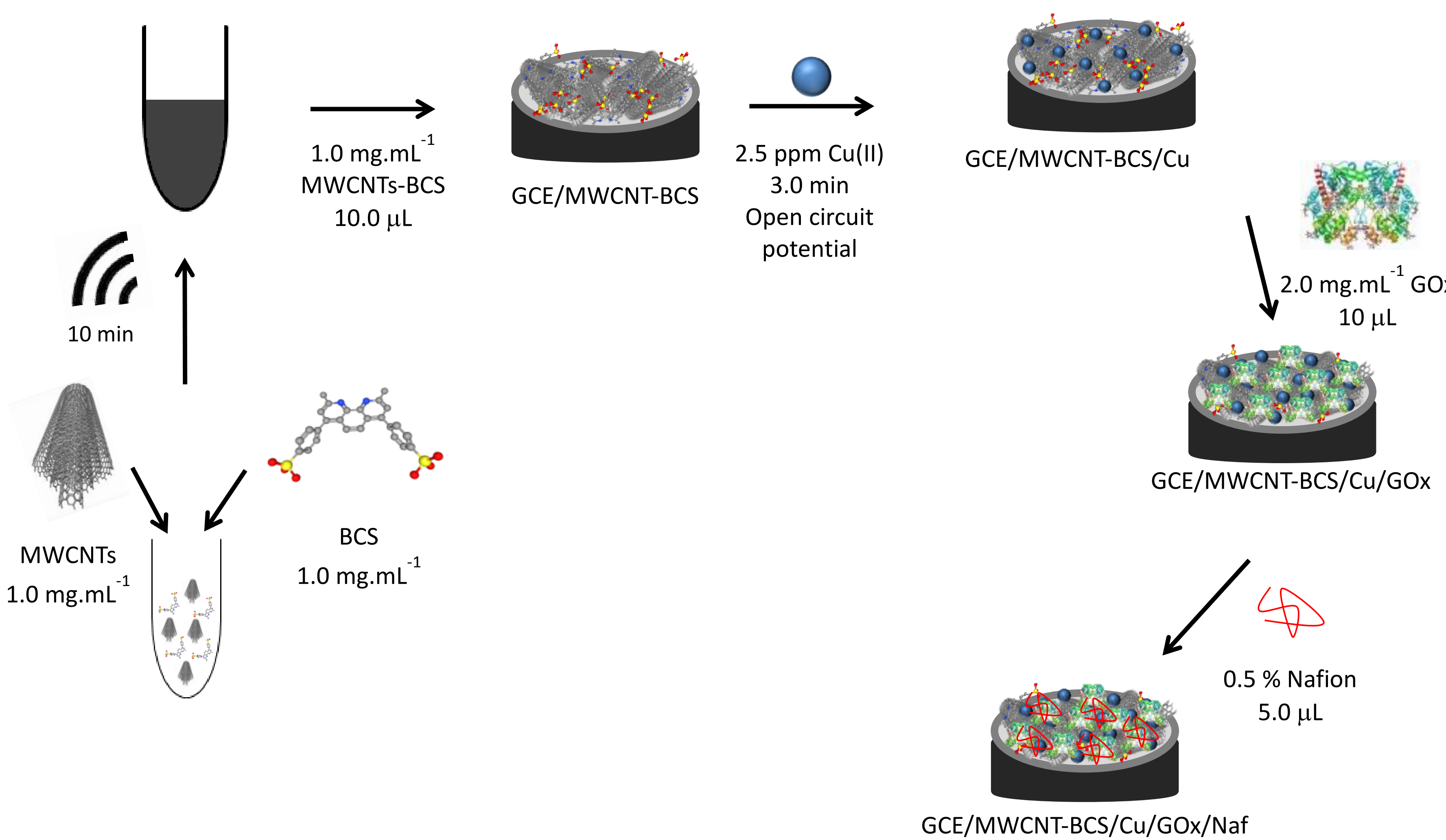
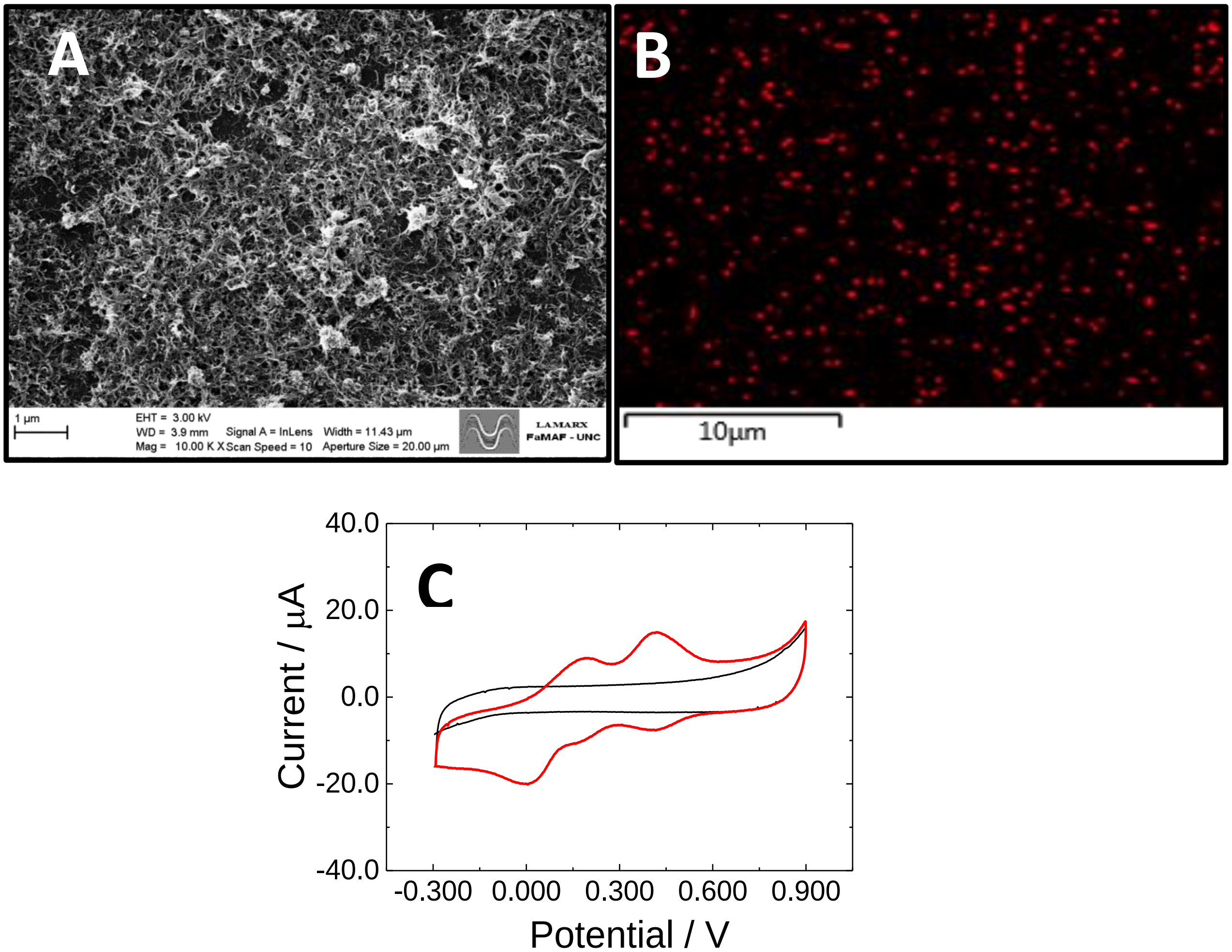


Figure 2- Rivas et al.



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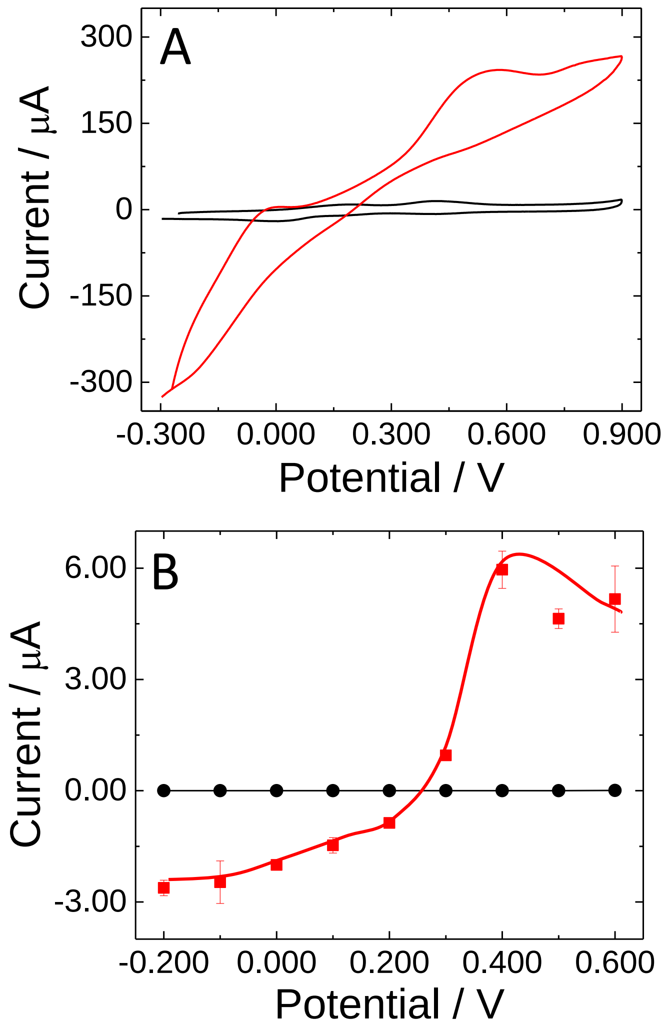


Figure 4 – Rivas et al.

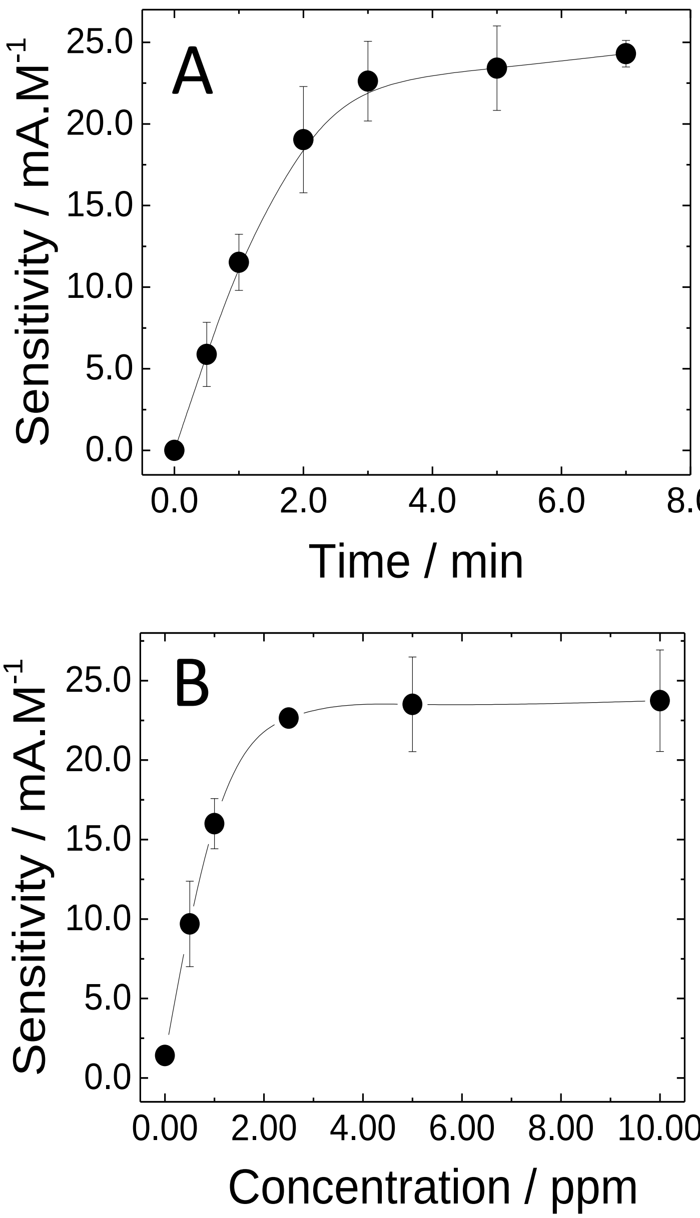
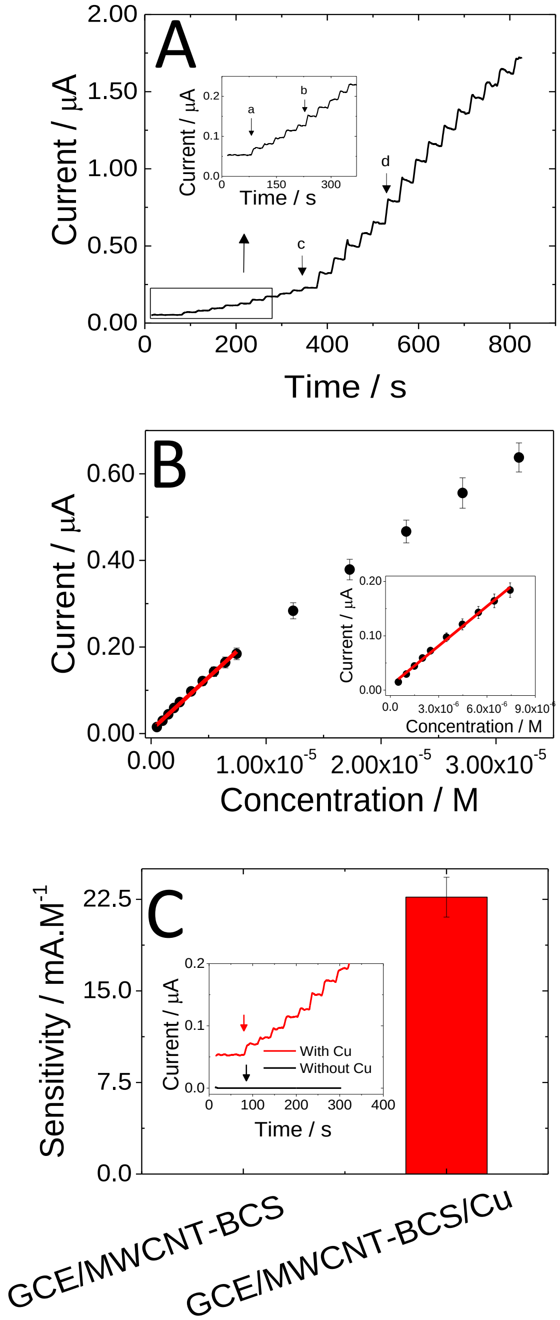


Figure 5 – Rivas et al.



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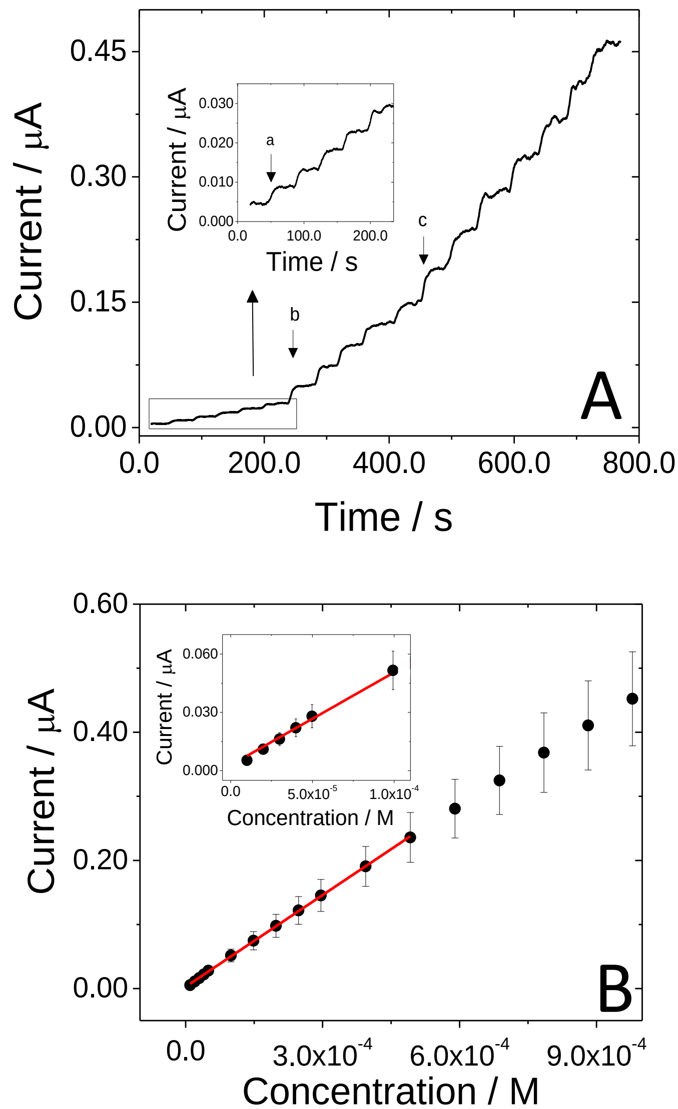

AUTHORSHIP STATEMENT

Manuscript title: __**"Doble role of bathocuproinedisulfonic acid as multi-walled carbon nanotubes dispersing agent and copper preconcentration ligand: analytical applications for the development of hydrogen peroxide and glucose electrochemical sensors"**

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the Hong Kong Journal of Occupational Therapy.

Authorship contributions

Please indicate the specific contributions made by each author (list the authors' initials followed by their surnames, e.g., Y.L. Cheung). The name of each author must appear at least once in each of the three categories below.

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Category 3

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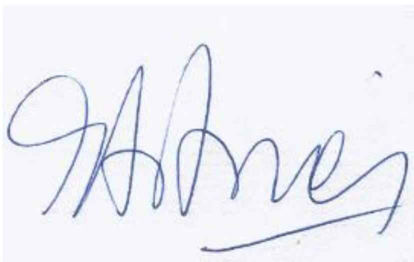

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