



Development of an electrochemical sensor for the determination of glycerol based on glassy carbon electrodes modified with a copper oxide nanoparticles/multiwalled carbon nanotubes/pectin composite

Fernando Javier Arévalo^a, Yolanda Osuna-Sánchez^c, José Sandoval-Cortés^{d,*}, Aylén Di Tocco^a, Adrián Marcelo Granero^a, Sebastián Noel Robledo^b, María Alicia Zon^a, Nelio Roberto Vettorazzi^a, José Luis Martínez^c, Elda Patricia Segura^c, Anna Iliná^c, Héctor Fernández^{a,*}

^a Departamento de Química, Grupo de Electroanalítica (GEANA), Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Agencia Postal N° 3, 5800 Río Cuarto, Argentina

^b Departamento de Tecnología Química, Grupo de Electroanalítica (GEANA), Facultad de Ingeniería, Universidad Nacional de Río Cuarto, Agencia Postal N° 3, 5800 Río Cuarto, Argentina

^c Cuerpo Académico de Nanobiociencias, Facultad de Ciencias Químicas, Universidad Autónoma de Coahuila, Boulevard V. Carranza y José Cárdenas Valdés, 25280, Saltillo, Coahuila, Mexico

^d Cuerpo Académico de Química Analítica, Facultad de Ciencias Químicas, Universidad Autónoma de Coahuila, Boulevard V. Carranza y José Cárdenas Valdés, 25280 Saltillo, COAH, Mexico

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ABSTRACT

A very sensitive electrochemical sensor to determine glycerol (GLY) in biodiesel samples has been developed. It is based on the electrochemical oxidation of GLY at glassy carbon electrodes modified with copper oxide nanoparticles supported on multiwalled carbon nanotubes/pectin composite. The electrochemical oxidation of GLY was studied in pH 8 phosphate buffer solutions. The quantification of GLY was performed by amperometric measurements. The proposed electrochemical sensor improves the performance of others methods developed for the quantitation of GLY. A low limit of detection of 5.8×10^{-6} g dm⁻³ and a percent coefficient of variation smaller than 10% was found, along with a reasonable linear range of 9×10^{-6} – 1×10^{-3} g dm⁻³. The proposed electrochemical sensor exhibited good performance, stability, reproducibility, repetitiveness and a good detection limit and a linear concentration range to quantify GLY in biodiesel samples.

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1. Introduction

Glycerol (GLY) is a very important substance commonly used in many areas of daily life, such as food, textiles, automobiles, bio-combustibles (bio-fuels), cosmetics, pharmaceutical industry, etc. In recent years, biodiesel (novel alternative fuel derived from lipids of vegetable oils or animal fats) has gained importance due to the rapid decline of fossil fuels and the consequent impact on the increase in fuel prices, which has led to the urgent need to massively develop the use of renewable energies such as solar, wind, hydro, biomass, etc. One of the alternatives to address the demand

for fossil fuels, especially for agriculture and transport, is the use of biomass to produce biodiesel, in particular obtained from oil seeds other than for the production of food.

Biodiesel is produced by transesterification the parent oil or fat with an alcohol, usually methanol, in the presence of a catalyst, usually a strong base such as sodium or potassium hydroxide, or, preferably and increasingly more commonly, alkoxides [1,2]. Therefore, the resulting product can contain not only the desired alkyl ester but also unreacted starting material, residual alcohol and catalyst. GLY is formed as a by-product and it is separated from biodiesel in the production process, however, traces thereof can be found in the final biodiesel product [3].

Due to the rising importance of biodiesel as alternative fuel in many countries, it is absolutely necessary to establish standards for the description of the quality of the product. A higher content of free GLY may cause problems during the storage or in the fuel

* Corresponding authors.

E-mail addresses: josesandoval@uadec.edu.mx (J. Sandoval-Cortés), hfernandez@exa.unrc.edu.ar, hfernandezster@gmail.com (H. Fernández).

system, due to separation of GLY, or can lead to injector fouling and lower engine performance, or the formation of higher aldehyde emissions [4]. Thus, it is important to develop sensitive and reliable analytical methods for the quantification of free GLY in biodiesel. Therefore, biodiesel standards related to the presence of GLY have been established in various countries and regions around the world, such as United States of America (ASTM 6584–00), Europe (EN 14105:2003), China (GB/T20828–2007), Germany (DIN V51606), etc. [5]. Argentine uses the same standard that United States of America. All these specifications in biodiesel standards mentioned above impose a maximum concentration of free GLY of 0.02% (200 mg kg⁻¹). The ASTM standard for GLY determination is based on gas chromatography. This standard specifies a method for determining the content of free GLY and mono-, di- and triglycerides residual methyl esters of fatty acids. The technique consists in the transformation of free GLY, mono- and di-glycerides in the silylated derivatives, which are compounds more volatile in the presence of pyridine and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide [6].

Several analytical techniques have been proposed to determine GLY such as electrochemistry [7,8], biosensor [9–15], spectrophotometry, chemiluminescence [16–18] and chromatographic ones [19,20]. Electrochemical methods have been proved to be an effective and inexpensive tool to determine different compounds. However, it has been proved that the direct oxidation of GLY at bare electrodes is not suited for analytical applications. Thus, the development of chemically modified electrodes (CME) is of practical significance.

The alcohol oxidations have widely been studied to design fuel cells through the methanol and ethanol oxidation [21]. Due to the high oxidation potentials of GLY, electrodes of expensive materials (i. e. platinum, ruthenium, gold, etc.) have been modified by metallic or bimetallic nanostructures, [21–24]. Thus, various platforms pointing to different alcohols determination have been proposed [21,25–29]. However, many efforts have been undertaken to reduce the cost of production of electrodes for the oxidation of alcohols with the use of less expensive electrodes, which also have an equally high efficiency. One of the platforms promising is that which considers the composite of copper and its oxides as a catalyst suitable for the oxidation of alcohols [30–32] and other important compounds, such as glucose [33] and H₂O₂ [34]. The sensors developed by nanostructures/structures of metallic electrodes, such as copper and nickel, have the advantage of using an inexpensive metal and environmentally friendly. The electrode processes in alkaline solutions involve the electrochemical formation of metal oxide or hydroxide films of higher oxidative states, i.e., NiO(OH) and CuO(OH), which have been proposed to behave as redox mediators associated with the analyte oxidations [35]. In addition, chemically modified electrodes containing surface-bound inorganic redox species have been described, which demonstrate an unusual stability and reactivity for aliphatic compounds [30].

A general mechanism proposed for the oxidation of aliphatic compounds, as GLY, at the CuO, CuO(OH) and Cu(III) surfaces involves several steps, where there is an adsorption of alcohol and a strong dependence on the OH⁻ concentration [30]. The adsorption of alcohol conforms to a Langmuir isotherm model, being the rate-determining step the abstraction of a hydrogen atom from the α -carbon according to Fleischmann [36]. The mechanism includes the formation of a CuO layer from Cu₂O oxidation, where the chemisorption of hydroxide ions on the CuO surface lattice is followed by the oxidation of the hydroxide to a hydroxyl radical. Meanwhile, at adjacent lattice site, the analyte also adsorbs onto the electrode surface forming a bridged cyclic intermediate with a subsequent abstraction of a hydrogen atom from the carbon in the α -position to the functional group. After the α -hydrogen abstraction the analyte radical is further rapidly oxidized to carboxylate or other product [30].

The nanostructured CuO and Cu₂O are promising in the development of sensors for alcohol oxidation because of their highly specific surface area, good electrochemical activity, and the possibility of promoting electron transfer reactions at a lower overpotential [37–40]. The methods to synthesize nanostructured CuO and Cu₂O mainly include hydrothermal synthesis in solution [38,41], sonochemical route [39], heating copper substrates at elevated temperature [42,43] and electrodeposition with electrochemical oxidation *in situ* [33,44]. Thus, it is possible to generate an electrochemical sensor based on glassy carbon (GC) electrodes modified with CuO, CuO(OH) nanostructures to oxidize GLY to lower overpotentials. As far as we know, it has not yet been discussed in literature any composite sensor based on copper nanostructures to determine GLY.

In the development of electrochemical sensors, multi-walled carbon nanotubes (MWCNT) are an important option. It has been demonstrated that electrodes modified with MWCNT show an increment in the linear analytical response and lower the detection limit [45,46]. However, it is necessary to work with homogeneous dispersions of MWCNT for obtaining reproducible results. Therefore, homogeneous dispersions can be obtained using different solvents such as DMF, DMSO or dioxolane [47,48]. In addition, if water dispersions are used it is necessary to use soluble or hydrophilic polymers to obtain a homogenous dispersions [49,50]. Another alternative to obtain homogeneous dispersions is the use of natural polymers such as pectin or chitosan in aqueous solutions. On the other hand, the use of pectin allows attracting copper ions (Cu²⁺) in solution towards the pectin/MWCNT composite at the electrode surface. It is well known the ability of pectin to interact with divalent ions as Cu²⁺ [51,52], and the ability of some carbohydrates to disperse MWCNT [53–55].

In this paper, we discuss the development of an electrochemical sensor to determine free GLY in biodiesel samples in pH 8 phosphate buffer solutions (PBS). The sensor is based on GC electrodes modified with CuO nanoparticles (CuONP) supported on a multi-walled carbon nanotubes (MWCNT) + pectin (Pe) composite (CuONP/Pe(MWCNT)/GC). The GLY oxidation was promoted by CuONP supported on MWCNT. The sensor developed properly uses the benefits of nanomaterials in order to obtain an advantageous method to quantify free GLY in biodiesel samples.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals used were of analytical grade. CuCl₂·2H₂O (Carlo Erba Reagents) was used to generate copper oxides. The supporting electrolyte to generate copper oxide nanoparticles was 0.1 mol dm⁻³ KCl solutions (JT Baker). Oxide generation was carried out in 0.1 mol dm⁻³ NaOH (Merck p.a.) solution. GLY, pectin and anhydrous heptane were obtained from Sigma-Aldrich. A 1 × 10⁻³ mol dm⁻³ stock solution of GLY was prepared in distilled water, and it was fractionated and stored frozen until use.

pH 8 2.5 × 10⁻² mol dm⁻³ PBS was prepared from their salts (Merck p.a): 1.25 × 10⁻² mol dm⁻³ of dibasic sodium phosphate dihydrate (Na₂HPO₄) and 1.25 × 10⁻² mol dm⁻³ of monobasic sodium phosphate monohydrate (NaH₂PO₄). The pH value was adjusted with HCl (Merck p.a.) solution.

MWCNT 85% purity, l. 5–15 μ m, dia. 10–30 nm, were industrial grade (Alfa Changdu Nanotechnology Co. Ltd.).

The standards to determine GLY by gas chromatography were Sigma-Aldrich, under ASTM D-6584. They were 1,3-diolein, monoolein, tricaprin and 1,2,4-butanetriol, all of them as pyridine solutions. The derivatizing agent used was *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide, from Sigma-Aldrich.

Biodiesel samples were obtained from the Departamento de Ingeniería Química, Facultad de Ingeniería, Universidad Nacional de Río Cuarto, and were spiked with different amounts of GLY. For free GLY determination in biodiesel samples using the electrochemical sensor it was necessary to perform the GLY extraction with aqueous solution, considering that GLY solubility in water is high (about 1 g dm^{-3}). Thus 5 g of biodiesel were added to 45 g of pH 8 PBS. The mixture was stirred during 5 min, then left to stand for 5 min. Once achieved the separation of the aqueous and organic phases, the aqueous phase was extracted and diluted by a factor of 1:2000. Then, GLY standard aliquots were added for electrochemical measurements.

2.2. Instrumentation

All electrochemical measurements were performed in a three electrode C3 stand cell (BASi Bioanalytical System, USA). A GC disk (3 mm dia., CH Instruments), polished with alumina ($0.05 \mu\text{m}$ in diameter) and sonicated in water during two min before modification was used as working electrode. Ag/AgCl (BAS) and a Pt wire were used as the reference and the counter electrodes, respectively. All potentials are referred to this reference electrode. Amperometric and cyclic voltammetry (CV) measurements were performed with an EPSILON potentiostat (BASi Bioanalytical System, USA) coupled to a PC with software incorporated. To perform scanning electron microscopy (SEM) as well as quantitative elemental analysis, a field emission scanning electron microscope JSM-740-1F was used. Conditions to record micrographs were: accelerating voltage of 6 kV and 8 mm working distance. Operating conditions to carry out elemental analysis were: accelerating voltage of 15 kV and 8 mm working distance.

2.3. Methods

2.3.1. Preparation of multi-walled carbon nanotubes/pectin dispersions

The Pe(MWCNT) dispersion was studied and optimized by varying the concentration of MWCNT and pectin from 0.5 to 3 mg dm^{-3} and from 1 to 3 mg dm^{-3} , respectively. The sonication time was also studied from 3 to 20 min. Different combinations of concentrations and sonication times were checked. Thus, the resulting dispersion was the one that showed the highest homogeneity. This was obtained by adding 2 mg mL^{-1} of MWCNT to a solution of 1 mg mL^{-1} of pectin. Three cycles of three min each with manual agitation between each cycle were optimal conditions for sonication. The resulting dispersion was stable by six month. Then, it started to show the formation of aggregates. The resulting dispersion was stored in darkness at room temperature. Then, $2 \times 10^{-5} \text{ dm}^3$ of the dispersion was deposited onto the surface of the GC electrode and dried in an oven at 40°C during 20 min. Thus, the Pe(MWCNT)/GC electrode was obtained. Fig. 1 shows a SEM micrograph about the morphology of Pe(MWCNT) on GC electrode. As shown in Fig. 1, a uniform dispersion on the electrode surface was obtained, where the absence of aggregates is observed. It is also possible to observe the presence of bigger MWCNT, which can be due to the use of industrial grade carbon nanotubes, of pureness of 95%, which are significantly cheaper than those of higher purity.

2.3.2. Generation of copper oxide nanoparticles (CuONP)

Fig. 2 shows cyclic voltammograms recorded at the bare GC electrode (a) and the Pe(MWCNT)/GC electrode (b) in a $1 \times 10^{-4} \text{ mol dm}^{-3} \text{ CuCl}_2 \cdot 2\text{H}_2\text{O} + 0.1 \text{ mol dm}^{-3} \text{ KCl}$ aqueous solution, which was previously bubbled with Ar during 15 min. In both cases, there are two pairs of peaks attributed to two quasi-reversible redox reactions, i.e., Cu(I)/Cu(0) and Cu(II)/Cu(I), respectively. The difference between capacitive currents of both

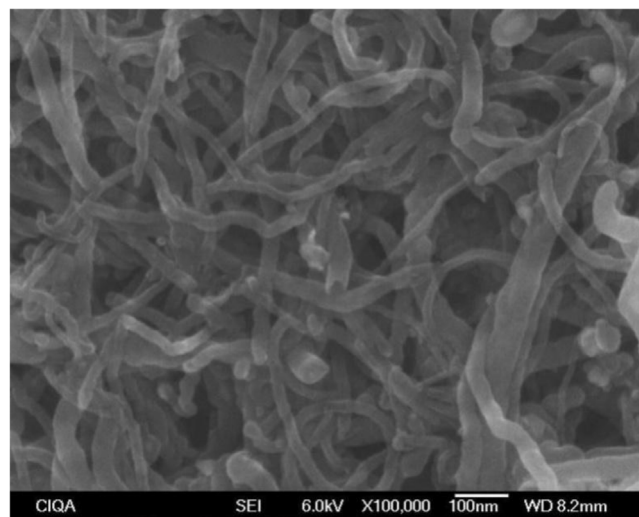


Fig. 1. SEM image of the GC/Pe(MWCNT) electrode surface. 100,000 \times .

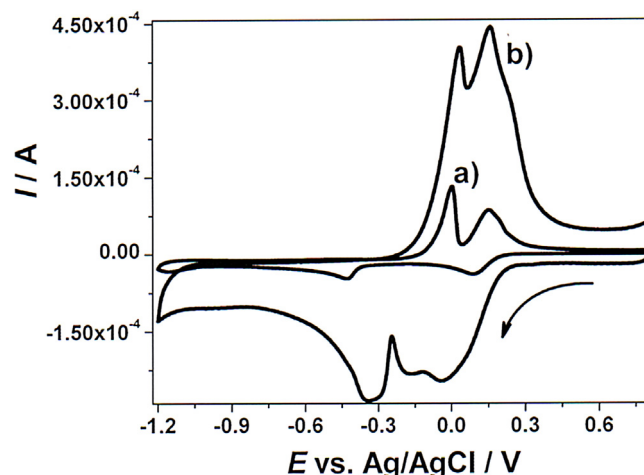


Fig. 2. Cyclic voltammograms recorded at GC (a) and Pe(MWCNT)/GC (b) in $100 \mu\text{mol dm}^{-3} \text{ CuCl}_2 \cdot 2\text{H}_2\text{O} + 0.1 \text{ mol dm}^{-3} \text{ KCl}$ at $v = 0.1 \text{ V s}^{-1}$. The arrow indicates the direction of the potential sweep.

GC and Pe(MWCNT)/GC electrodes is due to the presence of Pe(MWCNT), which produces an increment in the electroactive area. To generate the copper deposition on Pe(MWCNT)/GC electrode, a potential step of -0.4 V was imposed during 180 s on the electrode surface in the absence of oxygen in not stirred solution. The applied potential allowed a complete reduction of copper generating deposits on the Pe(MWCNT)/GC electrode surface.

Subsequently, the electrode was rinsed with water and dried under a stream of N_2 . Then, the electrode was immersed in a $0.1 \text{ mol dm}^{-3} \text{ NaOH}$ solution and a cyclic voltammogram were recorded in the potential range from -1.1 V to 0.4 V (Fig. 3a). Two well defined oxidation peaks (a_1 and b_1) were found in the anodic sweep. The a_1 peak is attributed to the oxidation of Cu to form Cu_2O and (CuOH) , in two independent reactions. The b_1 and c peaks are due the generation of CuO from products formed in the a_1 peak. In the cathodic sweep, the reduction of CuO to Cu_2O and CuOH (b_2 peak) and the reduction of Cu_2O and (CuOH) to Cu (a_2 peak) are observed, in close agreement with results found for the deposition of copper on GC [31,44].

Oxidized copper provides the active surface for the oxidation of aliphatic compounds [30]. Thus, the generation of copper oxide nanoparticles was carried out by successive cycles in $0.1 \text{ mol dm}^{-3} \text{ NaOH}$ solution, in the potential range from -0.5 V to 0.3 V at

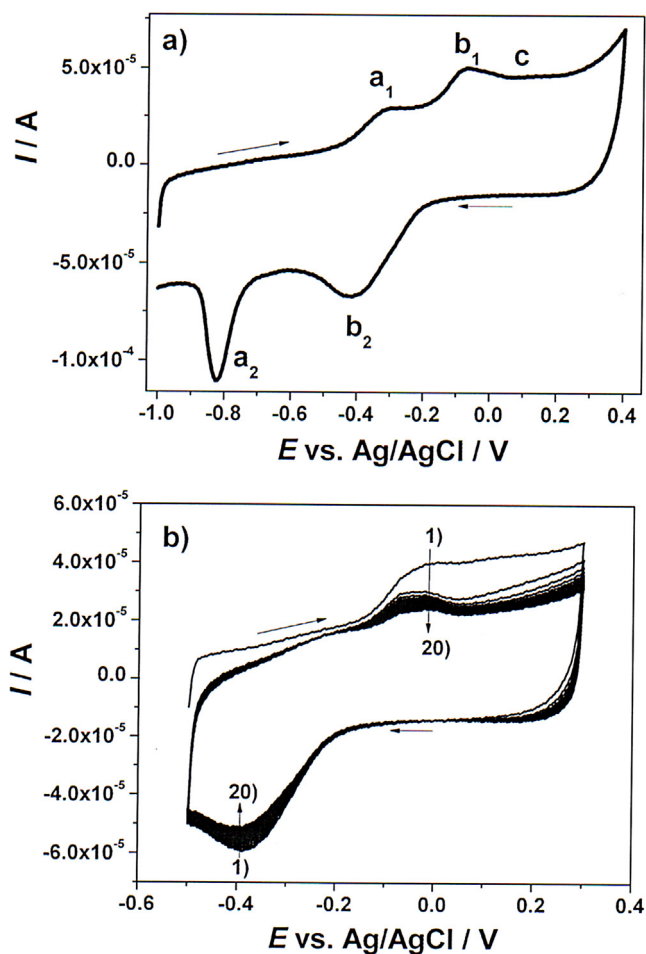


Fig. 3. a) Cyclic voltammograms recorded at the CuPe(MWCNT)/GC electrode and b) at the CuONP/Pe(MWCNT)/GC electrode in 0.1 mol dm^{-3} NaOH at $v=0.1 \text{ V s}^{-1}$. Number of cycles = 20. The arrows indicate the direction of the potential sweep.

0.1 V s^{-1} . Cathodic potential limit involves the no generation of Cu(0), which prevents the reduction of copper oxides to Cu, so the copper is always in the form of oxides and/or hydroxides. As it is shown in Fig. 3b, when the number of cycles increases, progressively the generation of oxides and the corresponding reduction peak decreases, indicating that copper oxide structures were successfully formed. Thus, the CuONP/Pe(MWCNT)/GC electrode was electro-generated.

The presence of Cu at the CuONP/Pe(MWCNT)/GC electrode surface was analyzed by quantitative chemical analysis and SEM. Fig. 4a and b shows the quantitative chemical analysis and the SEM image, respectively. The presence of copper on the surface of the Pe(MWCNT)/GC is confirmed by quantitative analysis (Fig. 4a). The CuONP are non-uniform and they are not uniformly distributed on the surface of Pe(MWCNT)/GC (Fig. 4b).

3. Results and discussion

3.1. Glycerol oxidation. Preliminary results

The oxidations of aliphatic alcohols on GC electrodes are practically not possible due to high over-potentials required for oxidation to occur (higher than 1V). Thus, it is necessary to work on an active modified GC surface for this purpose. It is known that the oxidation of aliphatic alcohols occurs at carbon electrodes modified with Pt and/or Au nanoparticles [28,30,56]. Oxidation of GLY

in 0.1 mol dm^{-3} NaOH at the CuNP/Pe(MWCNT)/GC electrode shows an oxidation current at about 0.70V. When the CuONP/Pe(MWCNT)/GC electrode is used, an oxidation current starts to appear at approximately 0.5V (Fig. A in Supplementary Material). The current difference between these two electrodes is approximately 87% higher at a potential about 0.8V when copper oxide nanoparticles are present.

3.2. Glycerol oxidation on CuONP/Pe(MWCNT)/GC electrode

The oxidation of GLY was studied using both the bare and the modified GC electrodes in each stage of modification. No oxidation of glycerol was observed neither on bare GC nor on Pe(MWCNT)/GC electrodes in $1 \times 10^{-3} \text{ mol dm}^{-3}$ GLY + pH 8 PBS (Fig. 5A, line a). When the Pe(MWCNT)/GC electrode was further modified with copper oxide nanoparticles to form the CuONP/Pe(MWCNT)/GC electrode a significant increase in current was found in the same solution (Fig. 5A, line b). Despite an oxidation peak was not observed, an increase in current was observed at potentials above 0.6V, due to the presence of copper oxide nanoparticles, which undoubtedly promote the oxidation of GLY. This increase in oxidation currents is more evident at higher GLY concentrations. Thus, studies of the current as a function of GLY concentration, C_{GLY}^* , were performed in the GLY concentration range from $1 \times 10^{-6} \text{ mol dm}^{-3}$ to $1 \times 10^{-3} \text{ mol dm}^{-3}$ in pH 8 PBS (Fig. 5B). The dependence between the GLY oxidation charge (Q) obtained by the integration of cyclic voltammograms and C_{GLY}^* was linear up to $5 \times 10^{-4} \text{ mol dm}^{-3}$ (data not shown). At C_{GLY}^* higher than $5 \times 10^{-4} \text{ mol dm}^{-3}$ Q grows more slowly and reaches a saturation value. This behavior is characteristic of processes where the reactant is adsorbed on the electrode surface [57].

3.3. Optimization of copper oxide nanoparticles generation

The magnitude of the Q obtained for the oxidation of GLY depends on the amount of deposited electro-generated copper oxide nanoparticles. Therefore, CuCl₂ concentration, copper electro-deposition time and the number of cycles required to obtain their oxides were optimized. As GLY does not show any oxidation peak, the GLY oxidation charge was used to optimize the generation of the copper oxide nanoparticles. The integration of anodic sweeps was performed after subtraction of the corresponding blank currents.

3.3.1. Dependence of Cu electrodeposit with the electrodeposition time

CuCl₂ concentration was varied in the concentration range from $1 \times 10^{-6} \text{ mol dm}^{-3}$ to $1 \times 10^{-3} \text{ mol dm}^{-3}$. The GLY oxidation current decreased as the CuCl₂ concentration increased (results not shown). Thus, a concentration of $1 \times 10^{-6} \text{ mol dm}^{-3}$ was chosen for the CuONP formation. Moreover, different copper electro-deposition times (t_{dep}) were tested in a $1 \times 10^{-6} \text{ mol dm}^{-3}$ CuCl₂ + 0.1 mol dm^{-3} KCl non-stirred solution. The t_{dep} was varied from 90 to 360 s. The GLY oxidation was carried out pH 8 PBS at a GLY given concentration after the generation of CuONP in alkaline solution. A maximum charge was reached at $t_{\text{dep}} = 180 \text{ s}$ (Fig. 6). The electrode surface activity decreased for t_{dep} higher than 180 s.

3.3.2. Formation of nanoparticles of copper oxides

The generation of CuONP was conducted by successive voltamperometric cycles in 0.1 mol dm^{-3} NaOH in the potential range from -0.5 to 0.3 V . Oxidation currents to generate CuONP decreases up to a constant value as the number of cycles was increased (Fig. 3b) indicating the transformation of electro-deposited copper to Cu₂O, Cu(OH) and CuO. The charge obtained for the oxidation increased

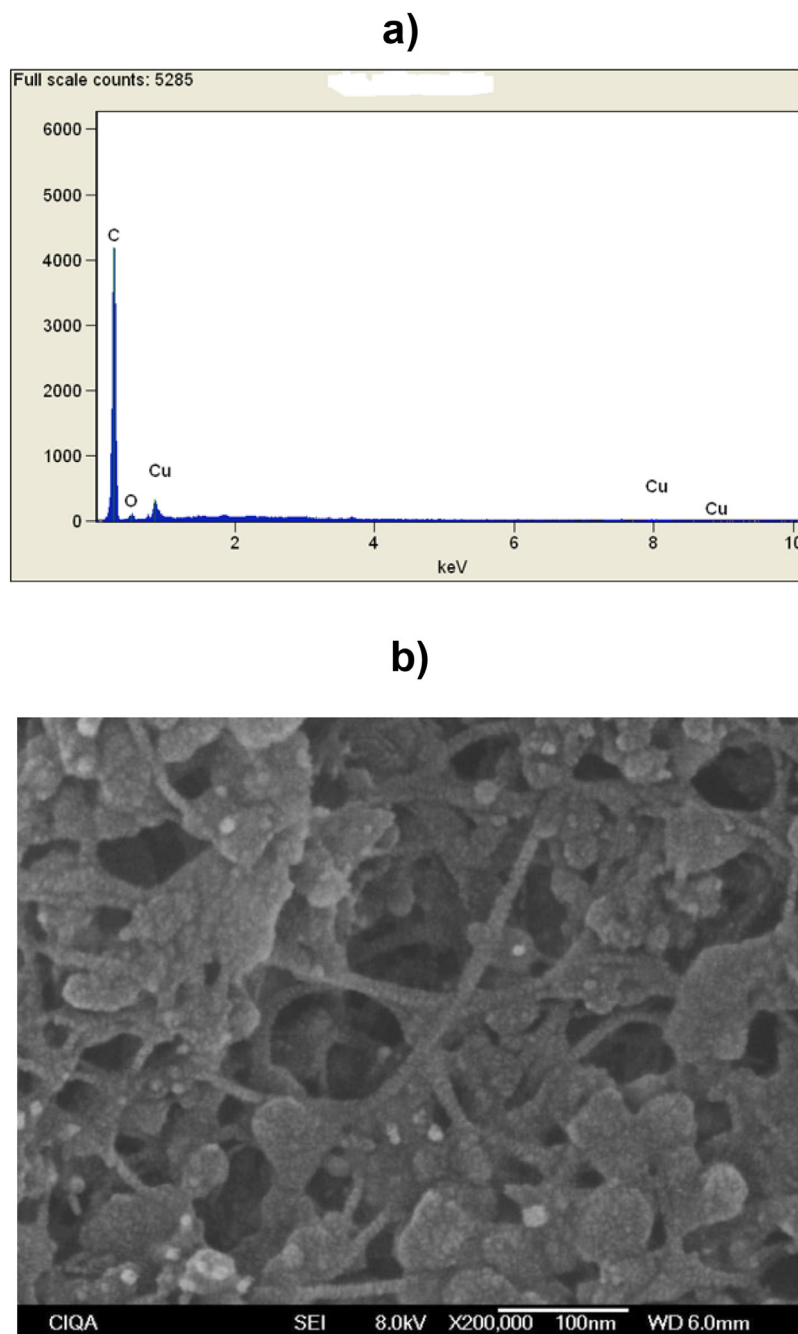


Fig. 4. a) Quantitative elemental analysis of the CuONP/Pe(MWCNT)/GC electrode surface. b) SEM image of the CuONP/Pe(MWCNT)/GC electrode surface. 200,000 \times .

as the amounts of CuONP on the electrode surface was increased. Such behavior is reflected in the variation of the charge obtained from cyclic voltammograms recorded for the GLY oxidation with the number of cycles performed to obtain oxides (Fig. 7). Therefore, a constant GLY oxidation charge was obtained after 160 cycles, and this value was chosen for all subsequent experiments.

3.4. Analytical characteristics of the electrochemical sensor

The response of the CuONP/Pe(MWCNT)/GC electrode towards GLY was evaluated by amperometric experiments under constant stirring. Thus, $1 \times 10^{-5} \text{ dm}^3$ of a GLY standard solution were added to the electrochemical cell containing $3 \times 10^{-3} \text{ dm}^3$ of pH 8 PBS under stirring at 350 rpm. Amperometric experiments were conducted at different values of applied potentials. The GLY oxidation

started at about 0.6 V. Thus, calibration curves were performed by amperometric measurements by applying a potential in the range from 0.6 to 1.1 V. The sensitivity was higher when the potential was close to 1 V (data not shown). Thus, 1 V was used for all subsequent experiments. Fig. 8 shows the current variation after the addition of different aliquots of GLY in pH 8 PBS. Steady state currents (I_{ss}) were reached at 50 s after the addition of GLY. The insert of Fig. 8 shows the calibration curve obtained using the same electrochemical sensor.

The linear range of the calibration curve was from 9×10^{-6} to $1 \times 10^{-3} \text{ g dm}^{-3}$. It can be represented by the following equation:

$$I_{ss}(\text{nA}) = (6.6 \pm 1.6)(\text{nA}) + (5.5 \pm 0.3) \times 10^4 (\text{nAg}^{-1} \text{ dm}^3) c^* \text{GLY}$$

$$r = 0.9853$$

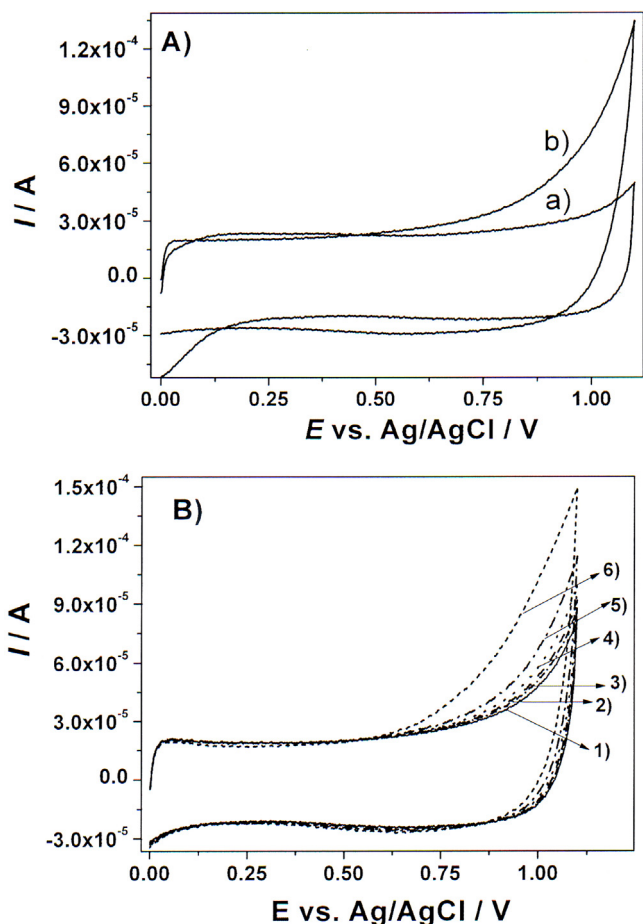


Fig. 5. A) Cyclic voltammograms of $1 \times 10^{-3} \text{ mol dm}^{-3}$ GLY+ pH 8 PBS recorded at: a) Pe(MWCNT)/GC electrode; b) CuONP/Pe(MWCNT)/GC electrode. B) Cyclic voltammograms recorded at different GLY concentrations in pH 8 PBS at the CuONP/Pe(MWCNT)/GC electrode. c_{GLY}^+ = 1) 0, 2) 1×10^{-6} , 3) 1×10^{-5} , 4) 1×10^{-4} , 5) 5×10^{-4} and 6) $1 \times 10^{-3} \text{ mol dm}^{-3}$. $v = 0.1 \text{ V s}^{-1}$.

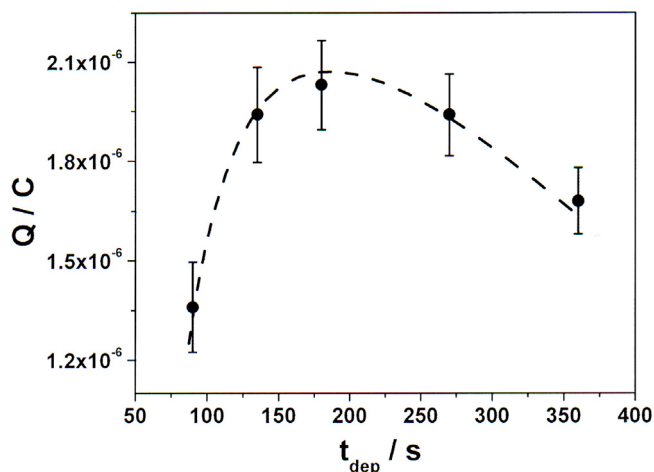


Fig. 6. Charge variation for the GLY oxidation on CuONP/Pe(MWCNT)/GC electrode in 0.1 mol dm^{-3} NaOH with copper electro-deposition time. $c_{\text{CuCl}_2}^+ = 1 \times 10^{-6} \text{ mol dm}^{-3}$. Applied potential for copper deposition = -0.6 V .

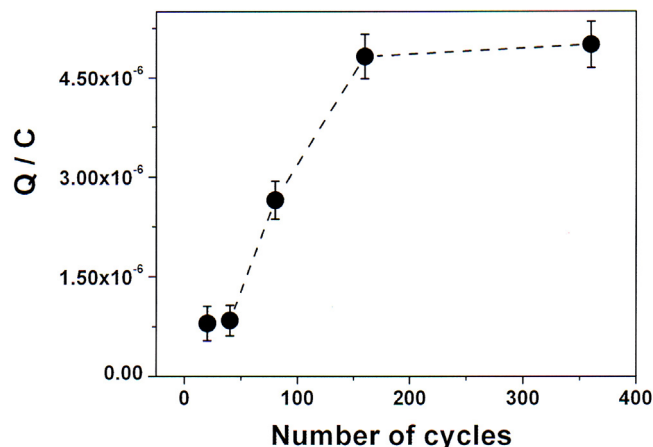


Fig. 7. Variation of GLY oxidation charge obtained from cyclic voltammograms with the number of cycles carried out at the CuONP/Pe(MWCNT)/GC electrode in 0.1 mol dm^{-3} NaOH. $c_{\text{CuCl}_2}^+ = 1 \times 10^{-6} \text{ mol dm}^{-3}$. Applied potential for copper deposition = -0.6 V .

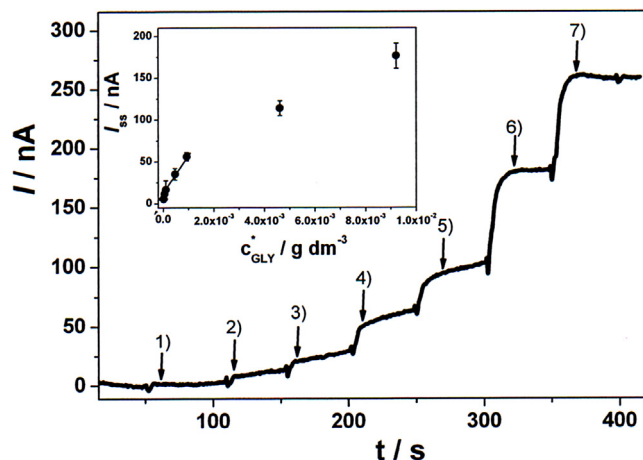


Fig. 8. Amperometric measurements performed in pH 8 PBS for different GLY concentrations. c_{GLY}^+ : 1) 9.21×10^{-6} , 2) 4.60×10^{-5} , 3) 9.21×10^{-5} , 4) 4.60×10^{-4} , 5) 9.21×10^{-4} , 6) 4.60×10^{-3} and 7) $9.21 \times 10^{-3} \text{ g dm}^{-3}$. Insert: calibration curve obtained for GLY determination. Each point represents the average of four replicated measurements. The bars represent corresponding errors. $E_{\text{app}} = 1 \text{ V}$. Stirring speed: 350 rpm.

where r is the linear correlation coefficient.

A limit of detection (LOD) of $5.8 \times 10^{-6} \text{ g dm}^{-3}$ and a limit of quantification (LOQ) of $9.2 \times 10^{-6} \text{ g dm}^{-3}$ were obtained for a signal-to-noise ratio of 3:1 and 10:1, respectively. The LOD was about 3×10^4 fold lower than the permitted limit of glycerol in biodiesel [5] and about 5 fold lower than the lowest LOD reported (Table 1). Statistical coefficients were analyzed for the same sensor and for different sensors in the same day. As summarized in Table 2, the sensor for GLY determination showed a good accuracy and reproducibility. Besides, the linear range of determination is two orders of magnitude lower than those reported previously, which allows to perform dilution of regular samples of GLY for analysis with the consequent diminution from possible interference signals.

3.5. Determination of free GLY in biodiesel samples

From the extracts obtained as described in Section 2.1, cyclic voltammetry was performed in pH 8 PBS, in the potential range from 0 to 1.1 V. No peak current appeared in this potential range, but it was observed an increase in current, which corresponds to

Table 1
Analytical performance of several methods for the determination of glycerol in biodiesel samples.

Detection Meth5od	Steps of Gly extraction	LOD/ mg L ⁻¹	Linear range/ mg L ⁻¹	Reference
Spectrophotometric assay	Yes	0.92	5.0–50.0	[17]
Spectrofluorimetric assay	Yes	0.037	0.10–5.0	[18]
DPV, Electrochemical assay	Yes	3.04	46.04–1.10 × 10 ³	[7]
CV, Electrochemical assay	Yes	0.28	3.04–1.56 × 10 ²	[58]
CV, Electrochemical assay	Yes	2.30	3.04–1.56 × 10 ²	[59]
Amperometry, Electrochemical assay	Yes	N.I.	14.73–1.47 × 10 ²	[60]
Amperometry, Electrochemical gas assay	No	0.51	0.92–92	[24]
Amperometry, Electrochemical assay	Yes	5.80 × 10 ⁻³	9.0 × 10 ⁻³ –1.0	This work

DPV: differential pulse voltammetry; CV: cyclic voltammetry.
N.I.: not informed.

Table 2
Reproducibility data obtained for the determination of GLY using the electrochemical sensor.

c _{GLY} [*] /g dm ⁻³	Intra-assay		Inter-assay	
	I _{ss} ^a /nA	%CV ^b	I _{ss} ^a /nA	%CV ^b
4.6 × 10 ⁻⁵	17.8	8.7	18.3	8.8
9.0 × 10 ⁻⁴	60.1	7.9	56.0	10.6

^a Average stationary current.

^b Percent coefficient of variation.

the oxidation of GLY. Methyl esters of linoleic acid and unsaturated acids, methanol, mono-, di- tri acyl glycerols, total glycerol, Na⁺, K⁺, Ca²⁺ and Mg²⁺ cations and phosphates are present in the biodiesel [6]. After extraction of GLY (Section 2.1), it is expected that in the aqueous phase are found alcohols, all cations and phosphate ions. Methanol is a potential interference for the determination of GLY by using our proposed electrochemical sensor. Cyclic voltammograms were obtained in pH 8 PBS in the presence of 3.2 × 10⁻² g dm⁻³ of methanol. No oxidation current was observed. Anyway, to minimize the effects of matrix of the biodiesel extract, the standard addition method was used to determine GLY in biodiesel samples. Thus, the extract obtained from the partition of biodiesel with pH 8 PBS was spiked with different amounts of GLY standard solution in order to achieve desired concentrations. The I_{ss} vs. c_{GLY}^{*} plot obtained for spiked sample was linear in the range from 5 × 10⁻⁶ to 1 × 10⁻³ g dm⁻³. The standard addition curve can be represented by a least-square procedure as:

$$I_{ss}(\text{nA}) = (105 \pm 2)(\text{nA}) + (1.93 \pm 0.03) \times 10^4 [\text{nAg}^{-1}\text{dm}^3] c^*_{\text{GLY}}$$

$$r = 0.9989$$

Thus, the GLY concentration obtained for the biodiesel sample was 1.17 g dm⁻³.

These results were compared with those obtained by gas chromatography/FIT methodology proposed by ASTM [6]. GLY concentration determined by gas chromatography was considered as the reference value. A calibration curve (peak area vs c_{GLY}^{*}) was constructed using standard solutions of GLY and mono and di-glycerols. A concentration of 1.2 g dm⁻³ of free GLY in the biodiesel sample was obtained. Thus, the GLY concentration in the biodiesel sample obtained from both methodologies agrees satisfactorily, indicating a very good performance for the CuONP/Pe(MWCNT)/GC electrochemical sensor.

4. Conclusions

This paper describes the development of a sensitive electrochemical sensor to determine glycerol in biodiesel samples based on the electrochemical oxidation of glycerol at copper oxide nanoparticles supported on a multiwalled carbon nanotubes + pectin composite on glassy carbon electrodes. A very good

limit of detection of 5.8 × 10⁻⁶ g dm⁻³ and a percent coefficient of variation smaller than 10% was found in all concentration range. The limit of detection obtained was about 3 × 10⁴ fold lower than the permitted limit of glycerol in biodiesel and about 5 fold lower than the lowest limit of detection reported. This electrochemical sensor is more sensitive compared with others reported in literature. On the other hand, the construction of the sensor was carried out with a cheap metal, as it is copper, which promotes the electrochemical oxidation of glycerol in pH 8 phosphate buffer solution, opening the possibility of using this platform for the construction of an electrochemical biosensor using the lipase enzyme as the biological recognition element. Therefore, the proposed electrochemical sensor exhibits good performance, stability, reproducibility, repetitiveness, detection limit and a good linear concentration range for quantification of glycerol in biodiesel samples.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.snb.2017.01.093>.

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Biographies

Fernando J. Arévalo obtained his Ph. D. in Chemistry (2009) from Río Cuarto National University (Río Cuarto, Argentina). He is Researcher at Argentine Research Council (CONICET). At present, he also is Assistant Professor at the Chemistry Department, Faculty of Exact, Physico-Chemical and Natural Sciences (Río Cuarto National University). Dr. Arévalo is an active member of the Electroanalytical Group at the Chemistry Department, and his research interest focuses on the development and characterization of electrochemical (bio) sensors based on the use of nano-structured materials.

Yolanda Osuna obtained her Master Degree in Biotechnology specializing in Enzymology (2013) from Chemistry School, Autonomous University of Coahuila (Saltillo, Mexico). She is actually working as responsible of Research and Development Department in the private company Environmental Analysis and Inputs (Coahuila, Mexico). At present she also provides services at Quality Control Department of the Private Company Bioamin (Coahuila, Mexico). She also collaborates in the Development of New Products Department of the same company. Her research interests focus in the innovation of biological products used in agriculture.

José Sandoval received his M.Sc. in Analytical Chemistry in 2003 by the analysis of pharmaceutical bioproducts, later in 2006 he obtained his PhD in Electrochemistry by his work with modified electrodes, both from Universidad de Guanajuato (Guanajuato, México). At present, he is professor of analytical chemistry at the Faculty of Chemical Sciences at Universidad Autónoma de Coahuila (Saltillo, México). His current interests focus on the development of new nanostructured materials, based in carbon nanotubes with synthetic and natural polymers, to be used as electrode materials to detect and quantify species of biological, pharmaceutical or industrial interest.

Aylen Di Tocco obtained her degree in Chemistry Engineering (2016) from Río Cuarto National University (Río Cuarto, Argentina). She is a research fellow in electrochemical sensors/biosensors in the Department of Chemistry, Faculty of Exact, Physico-Chemical and Natural Sciences (Río Cuarto National University).

Adrian M. Granero obtained his Ph.D. in Chemistry (2009) from Río Cuarto National University (UNRC, Río Cuarto, Argentina). He carried out a Postdoctoral training (2009–2012) at Río Cuarto National University, and at Córdoba National University (UNC, Córdoba, Argentina). Currently, he is Researcher at Argentine Research Council (CONICET). His research interest focus on several subjects, such as electrochemistry of mycotoxins, natural antioxidants, and the design of sensors/biosensors for the determination of these substances in real matrixes. Actually, he has over fifteen peer-reviewed papers, five book chapters and one book.

Sebastián N. Robledo obtained his Ph. D. in Chemistry (2012) from Río Cuarto National University (Río Cuarto, Argentina). He is a Researcher at Argentine Research Council (CONICET). At present, he is also an Assistant Professor at the Faculty of

Engineering (Río Cuarto National University). Dr. Robledo is an active member of the Electroanalytical Group at the Chemistry Department, and his research interest focuses on several subjects, such as electrochemistry of synthetic and natural antioxidants, as well as chemometrics studies for electroanalytical applications.

María A. Zon obtained her Ph. D. in Chemistry (1985) from Universidad Nacional de Río Cuarto (Río Cuarto, Argentina). She did the postdoctoral training at the Universidad de Córdoba (Córdoba, España) between 1990 and 1992. She is Full Professor at the Universidad Nacional de Río Cuarto and Principal Researcher at the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). She has been the secretary of the Asociación Argentina de Químicos Analíticos (2007–2009). Her research interest focuses on the development of electrochemical (bio) sensors by using nano-materials for the determination of different substrates such as mycotoxins, natural antioxidants and hormones. She has over 70 peer-reviewed papers, seven book chapters and she is co-author of a book. She has been co-editor of an electroanalytical book. Prof. Zon is an AAQA, AAIFQ and SIBAE fellow.

Nelio R. Vettorazzi earned his PhD in Chemistry (1983) at the National University of Río Cuarto (UNRC, Río Cuarto, Argentina). He did his postdoctoral training in the Department of Chemistry, UNRC in the area of electrochemistry (mechanistic electrooxidation studies of organic compounds, liquid interfaces with micelles, vitreous carbon electrodes, biosensors for mycotoxins, antioxidants and bacteria). He is an Associate Professor in the Analytical Chemistry area in the Department of Chemistry, UNRC. He has participated in the publication of scientific papers at conferences, meetings and international journals. Prof. Vettorazzi has co-authored three book chapters and a patent. He is a current member of the Argentinean Association of Analytical Chemists.

José Luis Martínez-Hernández obtained his Ph.D. in Chemical Engineering from Antonio Echeverría Polytechnic Institute, Habana, Cuba (2004). He is a Professor and active member of Nanobioscience Research Group in Chemistry School, Autonomous Coahuila University (Saltillo, Mexico). He is a member of Mexican National Research System and received Miguel Ramos Arizpe medal. Currently, he is chair of PhD Program in Food Science and Technology of Autonomous Coahuila University. His research interests focus on development of nanobiotechnological processes, enzyme and microorganism immobilization, fermentation process engineering, characterization of interactions between biological systems and nanocarriers.

Elda Patricia Segura-Ceniceros obtained her Ph.D. in Materials and Polymers (2008) from Research Center for Advanced Materials (Chihuahua, Mexico). She is a Professor and active member of Nanobioscience Research Group in Chemistry School, Autonomous Coahuila University (Saltillo, Mexico). She is a member of Mexican National Research System. She received Miguel Ramos Arizpe medal and Tecnos – 2006 Award. Currently, she is active member of Postgraduate Program in Food Science and Technology of Autonomous Coahuila University. Her research interests focus on development of materials for biosystems immobilization, enzyme inhibitors testing, nanobiotechnological application of functionalized materials.

Anna Ilyina obtained her Ph.D. in Enzymology, Kinetics and Catalysis from M.V. Lomonosov Moscow State University (1990) and postdoctoral training (2014) in Ufa State University of Economics and Service (2Russia). Currently, she is a Professor and Nanobioscience Research Group Leader in Chemistry School, Autonomous Coahuila University (Saltillo, Mexico). She is a member of Mexican National Research System. She received Dr. Mariano Narvaez medal, Miguel Ramos Arizpe medal, and Tecnos – 2006 Award. She is active member of Postgraduate Program in Food Science and Technology of Autonomous Coahuila University. Her research interests focus on enzymes characterization and applications, their immobilization on nanocarriers, and development of biotechnological processes using bionanostructured systems.

Héctor Fernández obtained his Ph. D. in Chemistry (1978) from Río Cuarto National University (UNRC) (Río Cuarto, Argentina). He did the postdoctoral training (1980–1982) at the University of New York at Buffalo, Buffalo (USA). Currently, he is Full Professor at UNRC and Principal Researcher at Argentine Research Council (CONICET). He was Dean of the Faculty of Exact, Physico-Chemical and Natural Sciences (UNRC, 1992–1999) and Head of the Department of Chemistry at the Faculty of Exact, Physico-Chemical and Natural Sciences (2001–2004). He was President of the Argentinean Society of Analytical Chemists (2007–2009). His research interest focuses on several subjects, such as electrochemistry of mycotoxins, hormones and synthetic and natural antioxidants, studies on ultramicroelectrodes and electrodes modified by self-assembled monolayers of thiols, carbon nanotubes, antibodies, etc and their use for electroanalytical applications. Development of electroanalytical techniques for the determination of antioxidants, mycotoxins and hormones in real matrixes (plants, cereal, foods, sera of animal origin, etc, respectively). Design and characterization of chemical sensors, electrochemical (bio)sensors and immunoelectrodes based on nanostructured materials. He has over eighty peer-reviewed papers, eight book chapters, co-author of a book and has been the editor of a book. Prof. Fernández belongs to the Editorial Board of *J Biosensors and Bioelectronics* and *Polish Journal of Environmental Studies*. He is an AAQA, AAIFQ and SIBAE fellow.