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Development of an electrochemical biosensor for the determination of triglycerides in serum samples based on a lipase/magnetite-chitosan/copper oxide nanoparticles/multiwalled carbon nanotubes/pectin composite

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ABSTRACT

A very sensitive electrochemical biosensor to determine totals triglycerides (TGs) in serum samples has been developed. It is based on the electrochemical oxidation of glycerol at glassy carbon electrodes modified with magnetic nanoparticles bonded to lipase enzyme and copper oxide nanoparticles, both supported on a multiwalled carbon nanotubes/pectin dispersion. Glycerol is produced by enzymatic reaction between the TGs present in samples and the lipase immobilized. The quantification of triglycerides was performed by amperometric measurements. The proposed electrochemical biosensor improves the performance of others methods developed for the TGs quantification. The determination of TGs does not need a pretreatment of serum samples. The PLS-1 algorithm was used for the quantification of TGs. According to this algorithm, the of detection and quantification limits were from 3.2×10^{-3} g L^{-1} to 3.6×10^{-3} g L^{-1}, and from 9.6×10^{-3} to 1.1×10^{-2} g L^{-1}, respectively. The sensitivity was 1.64×10^{-6} A L g^{-1}. The proposed electrochemical biosensor exhibited a very good performance, a stability of 20 days, very good reproducibility and repeatability, and it is presented as a very good alternative for the determination of TGs in human serum clinical samples.

1. Introduction

Triglycerides (TGs) are known as natural fats. TGs are esters obtained by a molecule of glycerol bonded to three molecules of fatty acids (saturated/unsaturated or both). The TGs play a vital role in metabolism as energy sources and transporters of dietary fat [1]. Thus, an important indicator of diseases associated with the lipid metabolism is the TGs level in serum. High levels of TGs along with cholesterol are well-known to cause atherosclerosis, hypertension and coronary artery diseases [2]. Normal ranges of total TGs are 40–160 mg dL^{-1} and 35–135 mg dL^{-1} for men and women, respectively [3]. Several standard clinical methods for the TGs determination were developed, such as colorimetric [4], spectrophotometric [5], chromatographic [6], fluorometric [7], titrimetric [8], nuclear magnetic resonance [9] and the enzymatic colorimetric method [10]. However, the most of them are expensive and complicated, and require a complex treatment of sample before the analysis. Therefore, simple, selective and reliable methodologies that allow a rapid diagnosis of TGs are required. A promising alternative for the quantification of TGs is the use of electrochemical biosensors. They are devices of easy design, economical and, in some cases disposable, miniaturizable and very sensitive, specific and fast for routine analysis [11]. They are generated by a modification of the working electrode with a biological component (enzymes, antibodies, DNAs, etc.), which reacts with the analyte. The transductor transforms the interaction between the analyte with biological compound in an electronic signal [12].

The development of biosensors based on lipases is an important alternative in the clinical area [13]. Lipases (EC 3.1.1.3) are part of the family of hydrolases, which catalyze the hydrolysis of esters, producing fatty acids and glycerol [14].

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There are several paper in the literature related to the TGs determination. Most of them use a mixture of enzymes, such as glycerol kinase, glycerol-3-phosphate oxidase and lipase, where the H_2O_2 produced in a cascade reaction is oxidized at electrode surface [15,16]. In addition, there are some potentiometric biosensors, which are based om the pH changes due to the TGs hydrolysis [17]. A review about the TGs determination has recently been published [1].

An interesting alternative for the quantitation of TGs is to determine the glycerol produced by the reaction between lipase and TGs. Therefore, glycerol can be quantified using an electrochemical biosensor based on glycerol oxidase [18] through amperometric measurements, or by colorimetric measurements using a multienzymatic system formed by glycerol kinase, pyruvate kinase and lactate dehydrogenase [19]. Thus, the electrochemical determination of glycerol produced by the lipase is presented as a promising alternative. However, the direct oxidation of glycerol at bare electrodes is not suited for analytical applications. Thus, the development of chemically modified electrodes is of practical significance [20,21]. Since glycerol has a high oxidation potential, different electrodes, such as glassy carbon, gold, platinum, etc., have been modified with metallic or bimetallic nanostructures of gold, ruthenium and platinum, mainly, as electroactive surfaces to alcohols oxidation [22–25]. In addition, there are platforms made of a composite of copper and its oxides as a suitable catalyst for the oxidation of alcohols [26,27]. We recently developed a very sensitive electrochemical sensor to determine glycerol in biodiesel samples by direct oxidation of glycerol on the modified electrode [28]. It was based on the electrochemical oxidation of glycerol, in pH 8 phosphate buffer solutions, on glassy carbon electrodes modified with copper oxide nanoparticles supported on a multiwalled carbon nanotubes/pectin composite.

On the other hand, it is well known that in some cases interferences on proper signal affect the quantitation of a given analite. An alternative to resolve this problem is the use of chemometric tools. Regression in partial least squares (PLS) is a well- known first-order multivariate calibration methodology. It has been widely applied for different types of instrumental data (i.e. spectrophotometric, chromatographic, electrochemical, etc.) [29,30]. This method involves a two-step procedure. First, a calibration is performed, where the comparison between the instrumental signal and reference concentrations is established from a set of standard samples or a reference method and, second, a prediction, in which the calibration results are used to estimate the component concentrations in unknown samples from its instrumental profile [31]. For electrochemical biosensors, there are few works where amperometric measurements are modeled using PLS-1 [32].

In this paper, the development of an electrochemical biosensor for the determination of TGs in lyophilized serum samples through the electrochemical oxidation of glycerol produced in the enzymatic hydrolysis between TGs and lipase, which is immobilized on the biosensor surface was performed (Scheme 1). The electrochemical biosensor consists of a composite based on lipase immobilized on chitosan coated magnetic nanoparticles (CNP-L) on a dispersion of multiwalled carbon nanotubes/pectin (MWCNT/Pe) modified with copper oxide nanoparticles (CuONP) on a glassy carbon electrode (GC). The electrochemical biosensor performance was checked by the determination of TGs in standard human serum samples. Cyclic voltammetry and amperometry were the electrochemical techniques used. A PLS-1 algorithm for the determination of TGs in the presence of a modeled interferer (uric acid) was used.

2. Material and methods

2.1. Chemicals and reagents

All chemicals were of analytical grade. Cupric chloride, $CuCl_2$ x $2H_2O$ (Carlo Erba) was used to generate copper deposit. The supporting electrolyte to generate copper deposit was 0.1 mol L^{-1} KCl solution (JT Baker). Oxide generation was carried out in 0.1 mol L^{-1} NaOH (Merck p.a.). Triolein, glycerol, pectin (Pe), uric acid (UA) and glucose (Glu) were obtained from Sigma-Aldrich. pH 8 PBS was prepared from their salts (Merck p.a): 1.25×10^{-2} mol L^{-1} Na_2HPO_4 and 1.25×10^{-2} mol L^{-1} NaH_2PO_4. pH was adjusted with HCl (Merck p.a.) solution. A lyophilized serum for precision control in Analytical Chemistry (Standatrol S-E 2 levels, Wiener Lab®) was used as TGs standard.

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

The lipase immobilized on magnetic nanoparticles (CNP-L) was obtained by a methodology previously reported by us [14]. The nanoparticles have a diameter of 9.9 ± 0.2 nm, as determined by high-resolution transmission electron microscopy (HRTEM).

2.2. Instrumentation

Amperometric and cyclic voltammetry (CV) measurements were performed with an EPSILON potentiostat (BASi Bioanalytical System, USA) coupled to a PC with software incorporated. Scanning electron microscopy (SEM) as well as quantitative elemental analysis were performed using a field emission scanning electron microscope JSM −740-1F. Conditions to record micrographs were: accelerating voltage of 6 kV and a working distance between 6 and 8 mm. Operating conditions for quantitative elemental analysis were: accelerating voltage of 15 kV and 8 mm working distance. Model generation for partial least squares (PLS) algorithm was implemented from MATLAB 7.8 software. The PLS-1 algorithm was applied using the multivariate calibration (MVC1) written for MATLAB [33]. Another algorithm, also written in MATLAB, was used for generate ellipses [34].

2.3. Methods

2.3.1. Preparation of MWCNT/Pe/GC electrode

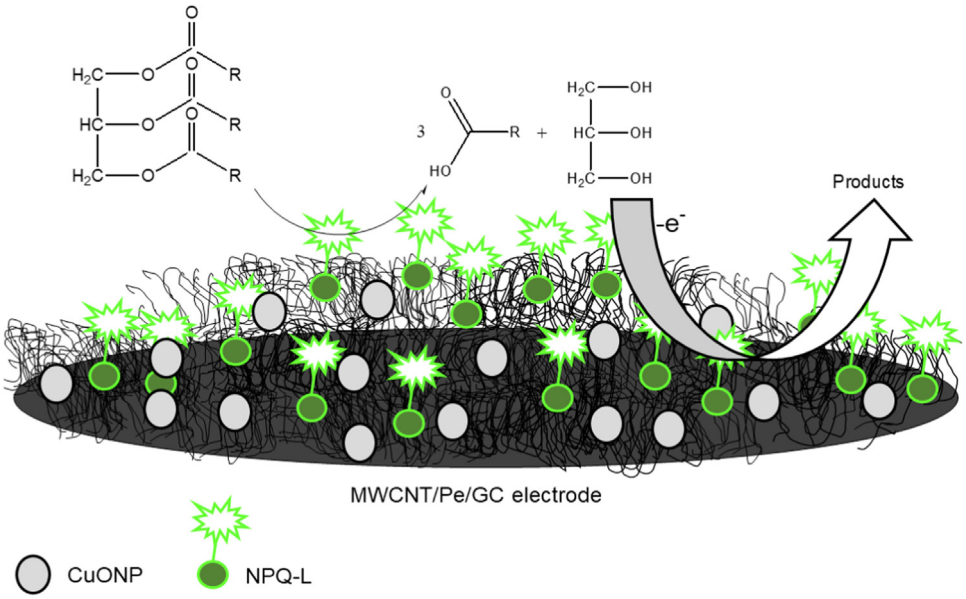
A dispersion of MWCNT/Pe was prepared as it was previously described by us [28] to generate the MWCNT/Pe/GC electrode. Briefly, 2 mg mL^{-1} of MWCNT was added to a solution of 1 mg mL^{-1} of pectin and three ultrasonic cycles of three min each one with manual agitation between each cycle was applied. Then, 20 μL of the MWCNT/Pe dispersion was dropped onto the GC electrode surface. Finally, it was dried in an oven at 40 °C during 20 min.

2.3.2. Generation of CuONP on the MWCNT/Pe/GC electrode

The generation of copper oxide nanoparticles (CuONP) on the MWCNT/Pe/GC electrode was previously described by us [28]. Briefly, MWCNT/Pe/GC electrode was immersed in 1×10^{-4} mol L^{-1} $CuCl_2x2H_2O$ + 0.1 mol L^{-1} KCl aqueous solution in the absence of oxygen. A potential step of −0.4 V vs. Ag/AgCl was applied during 180 s in an unstirred solution. Then, the electrode was rinsed with water and dried under a stream of N_2 and it was immersed in a 0.1 mol L^{-1} NaOH solution. The generation of copper oxide nanoparticles was carried out by 160 successive cycles in the potential range from - 0.5 V to 0.3V vs. Ag/AgCl at 0.1 V s^{-1}.

2.3.3. Generation of CNP-L/CuONP/MWCNT/Pe/GC electrode

For the development of the electrochemical biosensor, the immobilization of lipase on CuONP/MWCNT/Pe/GC electrode surface was performed. Thus, magnetic nanoparticles modified with the lipase enzyme (CNP-L) were used. Dispersions of CNP-L in water were prepared in the dilution range of 1:20–1:1000. Then, 20 μL of this dispersion was dropped on the CuONP/MWCNT/Pe/GC electrode surface. Finally, the modified electrode was dried in an oven at 40 °C during 20 min. Thus, the CNP-L/CuONP/MWCNT/Pe/GC electrode was formed.



Scheme 1. Schematic representation of the electrochemical biosensor to determine triglycerides in serum samples using the CNP-L/CuONP/MWCNT/Pe/GC electrode.

2.3.4. Chemometric calculations

2.3.4.1. Preparation of standards. For training and validation of the PLS-1 model, standard solutions were prepared by mixing aliquots of different concentrations of triolein ($c^*_{triolein}$) and UA (c^*_{UA}) in pH 8 PBS (Table 1). Samples 1–10 were designed to obtain the calibration set. Their concentrations were established through an experimental central composite design (CCD) for two factors, of type 2^2 + star, rotatable, and with two central points in the concentration range from 1×10^{-3} to 5×10^{-2} g L^{-1} and 8×10^{-3} to 0.207 g L^{-1}, respectively. Samples 11–15 were designed to obtain the validation set with random amount of each analyte in the same concentration range used for the calibration set. All samples were measured by triplicate in a random order. Finally, four samples of lyophilized human serum standards were measured using the model obtained by PLS-1.

2.3.4.2. Validation of results. The root mean square error (RMSE) between nominal and estimated concentrations for each substrate and the relative errors of predictions (RER %) were calculated in order to evaluate the quality of quatitative predictions of concentrations obtained from the PLS-1 algorithm. In addition, given that the slope and the intercept are not statistically independent, there is always some degree of correlation between them. Thus, we also analyzed if the point (1,0) was included in the elliptical region of the joint confidence of slope and intercept. Thus, it was evaluated whether or not concentrations estimated by PLS-1 differ statistically from nominal concentrations [34].

The limits of detection (LOD) and quantification (LOQ), estimate by PLS-1 approach, has been suggested to be available in the form of a range of values, whose lower and upper limits for LOD are given by [34]:

$$LOD_{min} = 3.3\sqrt{\frac{var(x)(1+h_{0min})}{\|\beta\|^2} + h_{0min}\ var(c_{cal})}$$

$$LOD_{max} = 3.3\sqrt{\frac{var(x)(1+h_{0max})}{\|\beta\|^2} + h_{0max}\ var(c_{cal})}$$

where

$$h_{0min} = \frac{\bar{c}^2_{cal}}{\sum_{i=1}^{I}(c_{cal} - \bar{c}_{cal})^2}$$

and

$$h_{0max} = \max\left(h_i + h_{omin}\left[1 - \left(\frac{c_i - \bar{c}_{cal}}{\bar{c}_{cal}}\right)^2\right]\right)$$

β, the vector of regression coefficients, indicates the norm or vector length, var(x) is the variance in the instrumental signal, var(c_{cal}) is the variance in calibration concentrations, and h_{0min} and h_{0max} are the minimum and maximum values of the leverage at the blank level, hi and ci are the leverage and (uncentered) analyte concentration of a generic calibration sample, respectively (mean-centering is assumed in building the PLS-1 model).

A MATLAB software for first-order calibration is available, which includes the estimation of figures of merit [35].

Table 1
Concentrations of TGs and UA prepared in pH 8 PBS used for calibration and validation sets.

Sample	Calibration (g L^{-1})		Sample	Validation (g L^{-1})	
	Triolein	Uric acid		Triolein	Uric acid
1	0.052	0.108	11	0.25	0.21
2	0.027	0.207	12	0.41	0.05
3	0.003	0.200	13	0.05	0.45
4	0.050	0.200	14	0.53	0.29
5	0.027	0.008	15	0.09	0.17
6	0.050	0.015			
7	0.027	0.108			
8	0.027	0.108			
9	0.003	0.015			
10	0.001	0.108			

2.3.5. Electrochemical measurements for TGs quantification

TGs quantification was performed by amperometric measurements. CNP-L/CuONP/MWCNT/Pe/GC electrode prepared as indicated in Section 2.3.3, was introduced in the electrochemical cell, which contains 3 mL of pH 8 PBS. The solution was stirred to 350 rpm. Then, a potential of 0.8 V vs. Ag/AgCl was applied. A steady state current (I_{ss}) is reached after 400 s. Then, 10 μL of TGs sample was injected in intervals of 50 s. The time necessary to reach the I_{ss} was 40 s.

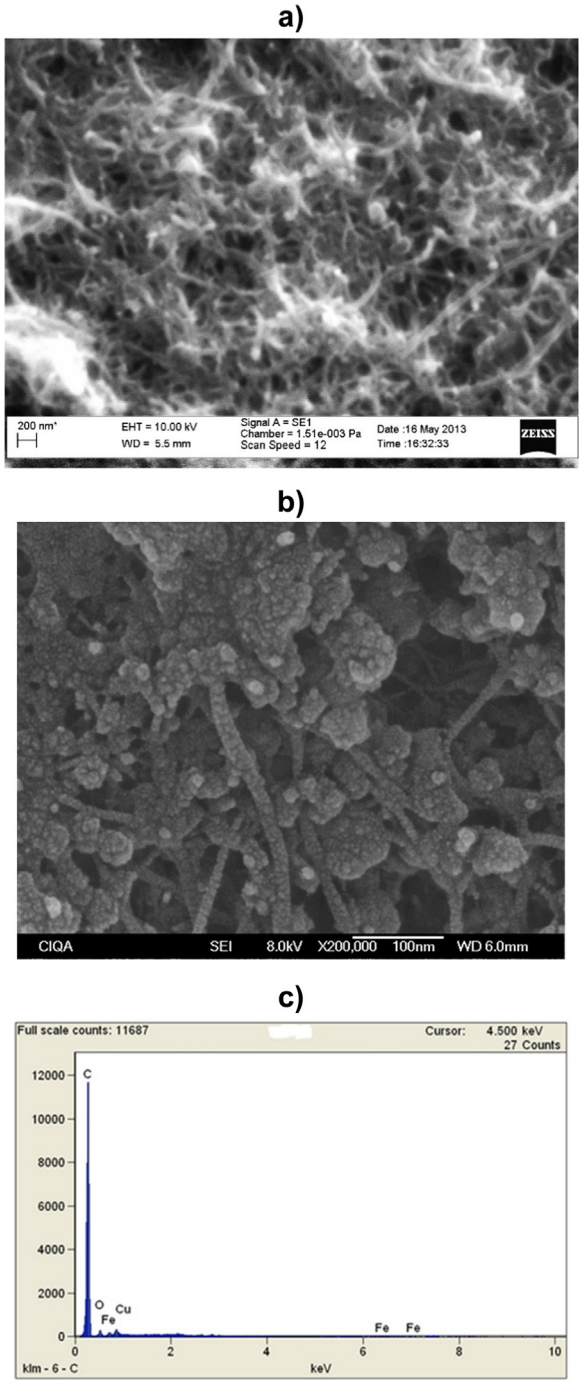


Fig. 1. SEM images of the surface of the GC electrode modified with a) MWCNT/Pe and b) CNP-L/CuONP/MWCNT/Pe, respectively. c) Quantitative elemental analysis of the CNP-L/CuONP/MWCNT/Pe/GC electrode surface.

3. Results and discussions

The TGs quantification is based on the electrochemical oxidation of the glycerol formed in the reaction between the TGs of sample and lipase immobilized on the electrode surface via the magnetic nanoparticles. The quantification of TGs was performed in pH 8 PBS. The maximum activity for the lipase immobilized on magnetic nanoparticles is obtained at this pH [14]. More alkaline pH´s would allow higher oxidation currents for glycerol, but the activity of the enzyme decreases significantly. Triolein was used as model triglyceride. The effect of interferences such as UA and GLU was also analyzed and evaluated by PLS-1 algorithm.

3.1. Glycerol oxidation on CNP-L/CuONP/MWCNT/Pe/GC electrode

As described in the Section 2.3.1, the GC electrode was modified with MWCNT/Pe dispersion. Fig. 1.a shows a micrograph of the MWCNT/Pe/GC electrode morphology by SEM, were a uniform dispersion was observed. Then, the MWCNT/Pe/GC electrode was modified to facilitate the oxidation of glycerol. We have developed an electrochemical sensor for of glycerol using a GC electrode modified with CuONP/MWCNT/Pe. On this surface, glycerol starts to oxidize from 0.55 V vs. Ag/AgC [28]. It is already been accepted a general mechanism for the oxidation of aliphatic alcohols at the CuO, CuO(OH) and Cu(III) surfaces, where several steps are involved, such as the adsorption of alcohols and a strong dependence on the OH − concentration [36]. On this electroactive surface, the CNP-L was added to form CNP-L/CuONP/MWCNT/Pe/GC electrode. Fig. 1.b shows a SEM micrograph of CNP-L/CuONP/MWCNT/Pe/GC electrode. A remarkably difference is observed respect to the MWCNT/Pe/GC electrode due to the presence of CuONP and CNP-L nanoparticles deposited on the surface. A good uniformity is reached. On the other hand, copper and iron from copper oxide and magnetic nanoparticles, respectively, on CNP-L/CuONP/MWCNT/Pe/GC electrode surface were confirmed by quantitative analysis (Fig. 1c). Fig. 2 shows cyclic voltammograms obtained for CuONP/MWCNT/Pe and CNP-L/CuONP/MWCNT/Pe/GC electrodes in the presence of glycerol. Glycerol oxidation started at about 0.6 V vs Ag/AgCl, with the absence of any peak, showing a characteristic behavior of a catalytic processes. Oxidation currents of glycerol are similar in both voltamperogramms. This result indicates that passivation produced by CNP-L is negligible.

3.2. Triolein quantification using the CNP-L/CuONP/MWCNT/Pe/GC electrode

Triolein is a widely known triglyceride. Its hydrolysis produces glycerol and oleic acid. When the triolein reacts with lipase bound to the magnetic beads (CNP-L), a glycerol molecule is generated which is oxidized on the surface of the electrode when the applied potential is higher than 0.6 V vs. Ag/AgCl. Fig. 3.a shows an amperogram for different concentration of triolein when a potential of 0.8 V vs. Ag/AgCl was applied. A I_{ss} was obtained for a time lesser than 50 s. The oxidation current was proportional to triolein concentration. For a triolein concentration higher than 1×10^{-2} g L^{-1}, the I_{ss} increases with a lower

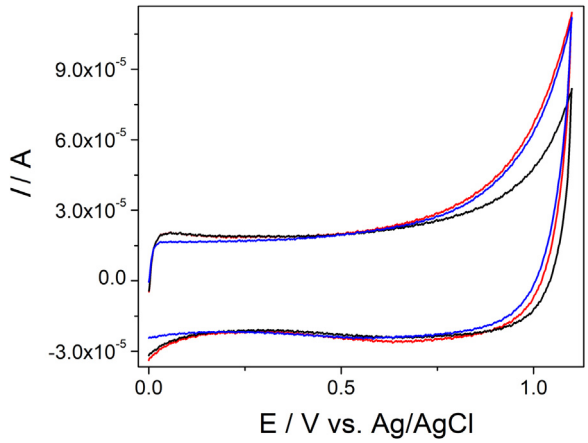


Fig. 2. Cyclic voltammograms obtained for —) CuONP/MWCNT/Pe/GC and —) NPQ-L/CuONP/MWCNT/Pe/GC electrodes recorded in 1×10^{-3} M glycerol + pH 8 PBS solution. —) Cyclic voltammogram obtained using CNP-L/CuONP/MWCNT/Pe/GC electrode recorded in pH 8 PBS solution. v = 0.1 V s^{-1}.

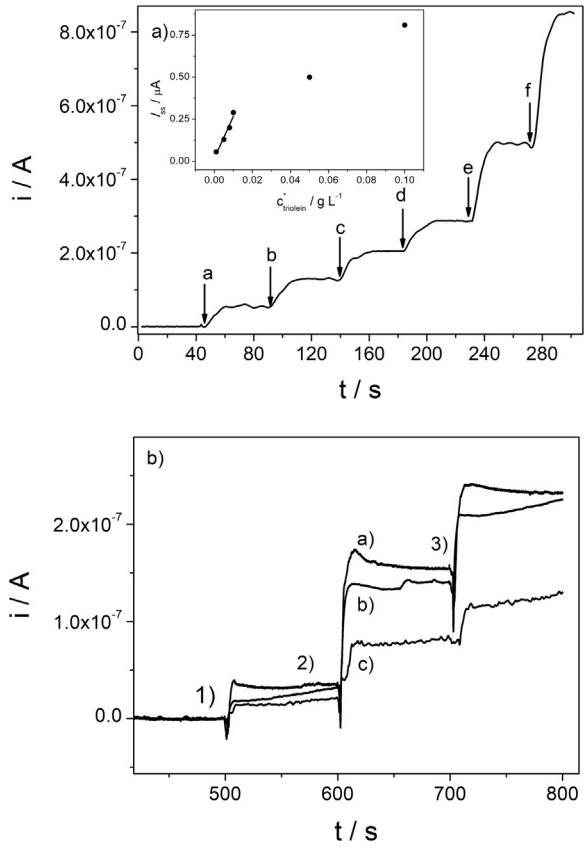


Fig. 3. a) Amperometric measurements performed in pH 8 PBS for different triolein concentration. $c^{*}_{triolein}$: a) 1×10^{-3}, b) 5×10^{-3}, c) 8×10^{-3}, d) 1×10^{-2}, e) 5×10^{-2} and f) 1×10^{-1} g L^{-1}. Inset: calibration curve. The parameters of linear regression are: slope = (25 ± 3) μA g^{-1} L, intercept = (1.9 ± 0.2) x 10^{-2} μA, r = 0.9709. b) Amperometric measurements performed for different dilutions of CNP-L: a) 1:100, b) 1:1000 and c) 1:20 for aggregates of triolein of 1) 1×10^{-3}, 2) 5×10^{-3} and c) 8×10^{-3} g L^{-1}, in pH 8 PBS. E_{app} = 0.8 V. Stirring speed: 350 rpm.

slope. This is due to the saturation of the electrode surface with the enzymatically generated glycerol [36].

3.3. Optimization of amount of CNP-L on CuONP/MWCNT/Pe/GC electrode

The amount of CNP-L added on CuONP/MWCNT/Pe/GC electrode was studied. Dispersions of CNP-L in water were prepared in the dilution range of 1:20–1:1000, as was previously described in Section 2.3.3. The greatest I_{ss} obtained was for a 1:100 dilution of CNP-L (see Fig. 3.b). For a 1:20 dilution of CNP-L, an unstable surface was obtained because the formation of CNP-L agglomerations. On the other hand, when 1:1000 dilution of CNP-L was used, a decrease of current respected to the 1:100 dilution was observed. Therefore, a 1:100 dilution of CNP-L was chosen for the construction of the CNP-L/CuONP/MWCNT/Pe/GC electrode.

3.4. Effects of stirring rate of the solution in the electrochemical cell

The stirring rate was evaluated in the range from 50 to 500 rpm. We found that for stirring rates less than 250 rpm, the I_{ss} is reached for times between 70 and 125 s. When the stirring rates increase from 250 rpm, I_{ss} is reached more rapidly and its values also increase. I_{ss} reaches the maximum value in the shortest time for a stirring speed of 350 rpm. For stirring rate > 350 rpm, no change was observed in I_{ss} (data not shown). Thus, a stirring rate of 350 rpm was chosen for all experiments.

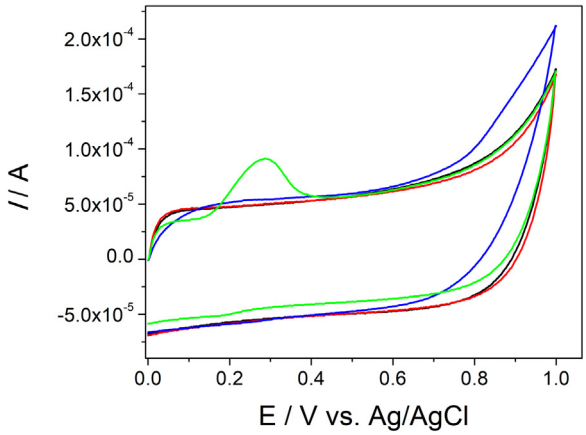


Fig. 4. Cyclic voltammograms obtained using NPQ-L/CuONP/MWCNT/Pe/GC electrode recorded at: —) pH 8 PBS, —) 0.85 mg L^{-1} glucose, —) 1.7×10^{-2} mg L^{-1} UA, both in pH 8 PBS, and —) 1:500 dilution of standard human serum in pH 8 PBS. v = 0.1 V s^{-1}.

3.5. Interferer study

Several substances present in the serum samples can interfere in the quantification of TGs. The most common compounds in these samples are albumin, bilirubin, cholesterol, creatinine, proteins, urea, inorganic phosphorus, metals such as iron, calcium, magnesium, and GLU and UA. The last two are presented as possible interferences. It is known that glucose needs a high over-potential (> 1 V) for its oxidation on GC electrodes. However, when copper oxide particles are present, the over-potential decreases significantly when a solution of pH > 12 is used [37,38]. On the other hand, when pH decreases, the over-potential increases significantly. At pH = 8, no GLU oxidation current is observed in the range of potential from 0 to 1 V vs. Ag/AgCl (Fig. 4). On the other hand, a peak for the oxidation of UA is observed at about 0.28 V vs Ag/AgCl under the same conditions. Thus, when an aliquot of serum standard was analyzed, a shoulder centered at about 0.3 V and a remarkable increase in current from 0.6 V were observed, corresponding to UA and TGs, respectively. Therefore, the detection of TGs by amperometry cannot be performed from a univariate methodology, since the I_{ss} obtained shows contributions of the oxidation of TGs and UA.

3.6. PLS-1 Calibration and prediction of TGs in the presence of acid uric

Two data sets were used in PLS-1 calibration. Thus, a calibration set was used to build the regression model and a validation set check the prediction ability of the PLS-1 model after all calibration parameters have been optimized. Fig. 5.a and b show the chronoamperograms used in the calibration and validation sets, respectively, and Fig. 5.c shows the chronoamperograms of standard human serum.

In the PLS-1, all model parameters are optimized for the determination of one analyte at a given time. During the model-training step, the calibration data are decomposed by an iterative algorithm, which correlates the data with the calibration concentrations using a so-called 'inverse' model [31]. Thus, current vs. concentration relationship for TGs was analyzed by PLS-1 through the multivariate calibration 1 (MVC1) [33].

A basic assumption for application of multivariate calibration model is the data bi-linearity, which may be compromised by possible interactions between analytes or analytes and the electrochemical surface. However, the use of flexible latent variables in PLS-1 allows to take into account slight deviations of the bi-linearity, when sufficient information is provided in the calibration phase of the algorithm.

Results of the PLS-1 model application to validation set obtained to predict TGs concentrations are given in Table 2. Note that the optimum

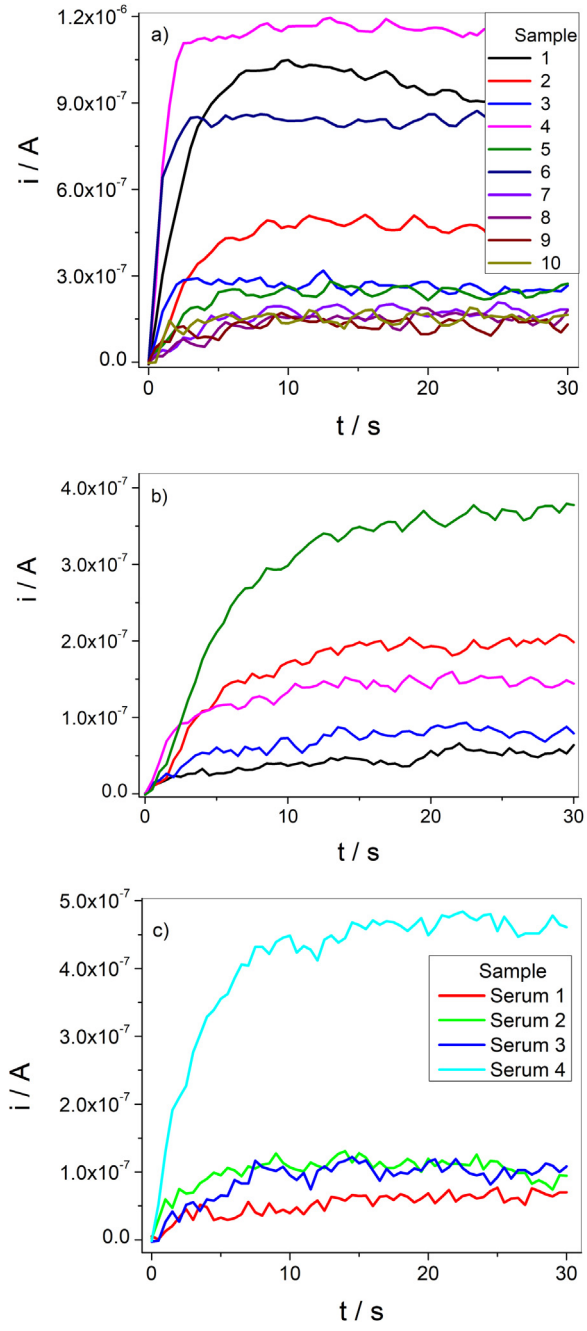


Fig. 5. Corrected chronoamperometric currents related to a) calibration sets (triolein and UA concentrations are indicated in Table 1). b) Validation sets using the following concentration pairs of triolein and UA: —) 1×10^{-3}, 5×10^{-2}, —) 5×10^{-3}, 0.2, —) 8×10^{-3}, 5×10^{-3}, —) 1×10^{-2}, 1.5×10^{-2}, —) 5×10^{-2}, 0.1 g L^{-1}, respectively. c) Standard human serum samples.

number of latent variables PLS-1 in the modeling (Nc = 4) was greater than the expected theoretical value of two, i.e. TGs and UA, because to the need to model additional phenomena, as mentioned above.

In addition, Fig. 6.a shows the regression of predicted concentrations vs. nominal values based on weighted least squares (WLS) method corresponding to the calibration and validation sets, respectively, and Fig. 6.b shows the corresponding ellipse of the Elliptical joint confidence regions (EJCR) analyses for TGs in the validation set. Results obtained for TGs by the PLS-1 method include the ideal point, whereby the first-order calibration model exhibits an adequate predictive

Table 2
Result of PLS-1 model applied to the validation set.

Analite	Nc[a]	RMSEC[b] / g L^{-1}	RMSEP[c] / g L^{-1}	REP[d] (%)	R^{2}[e]
TGs	4	1.3×10^{-4}	1.6×10^{-3}	5.9	0.9967

[a] Nc is the number of components or latent variables used in the PLS model.
[b] RMSEC is the root mean square error of calibration.
[c] RMSEP is the root mean square error of prediction.
[d] REP (%) is the relative prediction of the percentage error in concentration.
[e] R^2 is the coefficient of correlation between predicted and nominal concentration values of validation samples.

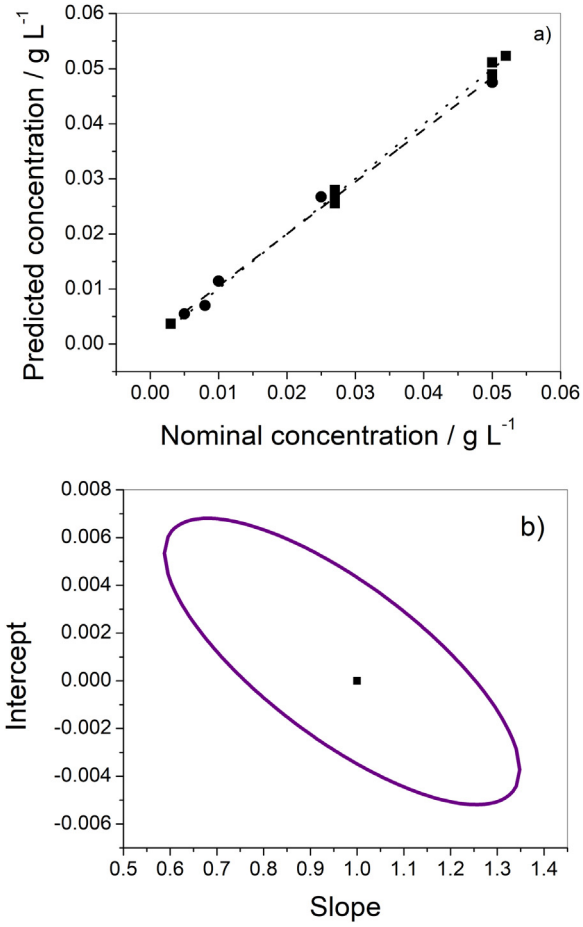


Fig. 6. a) Regression of predicted concentrations vs. nominal values based on weighted least squares (WLS) method corresponding to calibration and validation sets, where: ■) calibration values, •) validation values, ··) fitting of points of the calibration curve and —) fitting of points of the validation valuesb) EJCR (at a 95% confidence level) of the results obtained for TGs by PLS-1 for the validation set.

Table 3
Figures of merit for the determination of TGs in the presence of uric acid (interfering) PLS-1 Algorithm.

Sensitivity/A L g^{-1}	Analytical sensitivity/g L^{-1}	1.64×10^{-6}
		1.68×10^{3}
LOD_{min}	g L^{-1}	3.2×10^{-3}
LOD_{max}		3.6×10^{-3}
LOQ_{min}		9.6×10^{-3}
LOQ_{max}		1.1×10^{-2}

capability for the quantification of TGs in the presence of an interfering (UA).

The statistical results shown in Table 2, with adequate values for

Table 4
Determination de TGs in lyophilized standard human serum samples by PLS − 1.

Sample	Analyte	Nominal concentration/g L^{-1}	Found concentration[a]/g L^{-1}	Recovery (%)
Serum 1	TGs	5.0×10^{-3}	$(4.3 \pm 0.8) \times 10^{-3}$	86
Serum 2	TGs	8.0×10^{-3}	$(8.6 \pm 0.9) \times 10^{-3}$	92.5
Serum 3	TGs	1.0×10^{-2}	$(0.9 \pm 0.1) \times 10^{-2}$	90
Serum 4	TGs	5.0×10^{-2}	$(4.6 \pm 0.3) \times 10^{-2}$	92

[a] Mean value ± standard deviation.

RMSEP and REP (%) for TGs, also support this conclusion. Thus, Table 3 shows figures of merit for the determination of TGs in the presence of UA by PLS-1 algorithm.

3.7. Analysis of real samples

The developed analytical method was applied to determine TGs in lyophilized standard human serum with different concentration levels. The corrected currents, Fig. 5.c, were used to generate the first-order data and were implemented in the PLS-1 model. The REP (%) obtained was 7.2%, somewhat higher than that obtained for the validation set (Table 2). Possibly, this is due to a matrix effect not taken into account in calibration and validation sets, but acceptable due the use of calibration standards in the absence of the matrix.

Finally, recovery studies were performed in order to validate the proposed method. Recovery percentages, between 86% and 92.5%, with an average value of 90% were obtained (Table 4).

On the other hand, the stability of the electrochemical biosensor was studied. The response was evaluated along 30 days. For every day, a triolein determination was performed. Then, the electrochemical biosensor was washed with pH 8 PBS, dried with N_2 and stored at 4 °C. From day 20, the I_{ss} began to decreased. By day 30, I_{ss} decreases by 33%. Thus, it is concluded that the electrochemical biosensor is stable for 20 days (data not shown). Pundir and Narwal [1], show a very complete table comparing the analytical characteristics of different methodologies (optical, potentiometric and amperometric methods) to determine TGs, which contains some mistakes in the reported LOD values. The electrochemical biosensor proposed by us shows a higher analytical performance compared to papers presented in the literature [1]. On the other hand, this electrochemical biosensor does not require pretreatment of the sample, the response is reached quickly (40 s) and has no interfering effects.

4. Conclusions

The biosensor proposed for the determination of triglycerides exhibits good performance, stability, reproducibility, repetitiveness, limit of detection and a good linear concentration range for quantification of triglycerides. On the other hand, the modeling of the chronoamperometric data through the implementation of PLS-1 is suitable for the quantification of triglycerides in samples of lyophilized human serum. Therefore, this electrochemical biosensor is presented as promissory tool for triglycerides quantification in clinical field.

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