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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.