Chapter 12

Amperometric Biosensor for Diagnosis of Disease

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http://dx.doi.org/10.5772/53656

1. Introduction

Our main interest is discussing amperometric biosensors with application in certain disease diagnosis. These biosensors are based on the affinity reaction between antigen/antibody (immunosensor) or DNA/DNA (genosensor) or enzymatic catalytic reaction. The selective interactions will be also discussed in this chapter. In this first part, the central goal is to present and discuss some aspects of working electrode (WE) surface preparation and characterization, electrochemical cell arrangements and (chrono)amperometry as a simple electrochemical technique to evaluate some types of biosensors.

It is well known that in chemical sensors the chemical information is transformed into useful analytical signal. The chemical information can be associated with the concentration of a specific component present in the sample. In a simple way, a molecular receptor in series with a physico-chemical transducer characterizes what is called chemical sensor [1]. When the molecular receptor involves a biochemical component, a biosensor is obtained [2]. In a biosensor, the biological component is responsible for the selectivity while the characteristics of the electrochemical detector determine the sensitivity. It means that the electrochemical detector (transducer) must be carefully selected and prepared. In its selection, the mechanism nature of the biosensor must be known. This mechanism depends basically on the type of active components involved and on the mode of signal transduction. For in-

stance, in enzymatic biosensors, the active site of the enzyme must be preserved after immobilization and satisfactory electrochemical communicability between the redox site and the electrode should be guaranteed.

Different electrochemical techniques can be used to characterize and evaluate biosensors: chrono(amperometry), chronopotentiometry, linear potential sweep (LPS), cyclic voltammetry (CV) (DC techniques), and electrochemical impedance spectroscopy (EIS) and AC voltammetries (AC techniques). For electrochemical characterization of electrode processes, CV and EIS are probably the most used electrochemical techniques. In general, when pre-treated surfaces and modified electrodes are characterized using these techniques, the reversibility, the electron charge transfer (*e.c.t.*) rate of a redox couple such as $Fe(CN)_6^{3-/4-}$, $Ru(NH_3)_6^{3+}$, etc., and the diffusion coefficient of the electroactive species are determined and compared with their standard behavior. If the electrochemical response is given by species in a stagnant solution containing an excess of supporting electrolyte, the response corresponds to a reversible *e.c.t.* controlled by diffusion of the electroactive species to or from the electrode surface. If the electrode is partially blocked with self-assembled monolayers (SAMs) or non-electroactive surface modifiers in the examined potential range, the electrochemical response may vary depending on the shape and size of the access to the active sites by the electroactive species from the solution. Generally, an electrochemical response resembling a less reversible system is observed. The reversibility of the system may decrease as the blocked fraction of surface area increases. It is easily detected by the increase of the difference between anodic and cathodic peak potentials and by the decrease of peak currents. For EIS studies, the decrease in the reversibility is observed by the increase in the modulus of electrochemical impedance and the decrease in the *e.c.t.* reaction rate [3]. However, if the electroactive species is attached or adsorbed on the electrode surface, the electrochemical response will depend on the distance between the redox center and the surface and accessibility of electrons to this redox center, on the position of the redox center into the molecule attached to the electrode surface, and the nature and state of the electrode surface. Generally, if a monolayer of the redox species is attached to the electrode surface and a reversible *e.c.t.* process takes place, the CV shows peak potential separation near zero. Similar response shows a film with several monolayers of the redox species and the electron exchange between the layers reversibly occurs [4].

Details on cyclic voltammetry and its applications are displayed in some textbooks [4-6]. Fundamentals and mathematical analysis of electrochemical impedance spectroscopy can be found in [7,8]. For some applications of CV and EIS to immunosensors characterization, the readers are referred to [9].

The main reasons for the large use of (chrono)amperometry are its simplicity in data collection due to the apparent facility in measuring the current related to the *e.c.t.* associated with the biosensor response. For example, if one compares the amperometric technique with the EIS [9], also used in biosensors characterization and monitoring, there is no doubt that the

later is much more laborious; it is also true that EIS is a better technique to deeply investigate the global behavior of the system [3,10].

The electrochemical techniques are not able to identify the chemical nature of the products or reactants involved in certain electrochemical process, and then some non-electrochemical techniques complement and help us to understand the electrochemical processes. They can be associated to surface or bulk (solid, liquid or gas) analysis.

Amperometry is a voltammetric method in which two- or three-electrode cell configurations are used, and the potential applied between the WE and the auxiliary electrode (AE) results in a constant potential at the working *versus* a reference electrode (RE). If the current is measured as function of time we have the chronoamperometry technique. In this technique, a drastic and immediate change in the WE potential from an initial potential value E_i (where no faradaic reactions take place) to a final potential E_f (where the faradaic reaction of interest occurs) and the current is continuously measured. The analysis of the current-time (I-t) transients can be used to study many electrochemical processes as *e.c.t.* involving species in solution, new phase formation, adsorption, diffusion coefficient determination and so on. For chemical analysis, time-independent current is interesting to be obtained, which can be attained if the diffusion layer thickness is constant. It is possible to obtain by convection transport or using micro or mainly ultramicroelectrodes [11,12].

In order to develop an amperometric biosensor, special attention should be devoted to choose the WE, to conveniently prepare and modify its surface, and identify the electrochemical response related to the specific reaction involving any electroactive species present in the biosensor system, which may unequivocally indicate the presence of certain disease. The performance of the biosensor is strongly dependent on the *e.c.t.* reaction rate. The current generated at the WE measured using a two- or three-electrode cell configuration depends on the reaction rate. The steady-state current is proportional to the analyte concentration in the bulk, c_{bulk}. The area of RE (two-electrode configuration) or the AE (three-electrode configuration) needs to be at least 10 times wider than the WE, and then the reaction occurring at the AE is fast compared to that one occurring at the WE. To fulfill its role, the AE must be a good conductor of electricity and it must be placed in the cell in order to guarantee good distribution of electric field [13].

In order for facilitating the analyses of I-t curves and for getting the best sensitivity for the appropriate electrochemical reaction, the applied potential value can be chosen in such way that the surface concentration (c_{surf}) of the investigated species is zero. If c_{surf} is not zero the current will be lower and dependent on the potential and time. The corresponding equations and mathematical details can be found in [14-17].

Based on the comments presented before, some aspects about the transducer in amperometric biosensors should be considered:

- chemical nature of the working electrode, surface preparation and characterization;
- choosing the potential value of the working electrode;
- repeatability and sensitivity in (chrono)amperometry measurements.

1.1. Chemical nature of the working electrode, surface preparation and characterization

In this section, it will be presented different materials that have been used as transducers, mainly for amperometric immunosensors construction, electrode surface preparation and pre-treatments (when used), and electrochemical cell configurations.

Among these different materials, some can be mentioned, such as: gold, CD-trode, screen-printed electrodes, silver, mercury, graphite, glassy carbon, carbon nanotubes, gold nanowires, gold nanoparticles, metallic oxide nanoparticles, carbon paste, boron-doped diamond and composites. These surfaces can be transformed with different modifiers to form SAMs, and composites which carry or incorporate the active components desired to construct the biosensor.

In aqueous medium, gold presents some advantages compared to platinum since it does not adsorb hydrogen and it has high overpotential for hydrogen-evolution reaction, which is appropriate to study cathodic processes. The real surface area can be determined measuring the charge involved in the reduction of the gold oxide layer formed at high overpotentials. This area can be very different from the geometrical one. In the case of carbon paste electrode, the main advantages are ease of preparation, versatility in the chemical modification and its rapid renewal. Glassy carbon electrode has low cost, high resistivity to chemical attack, very low permeability to gas, large potential window, it is easily polished and treated via potential scanning and it may improve the kinetic of some charge transfer reactions [4].

It is well known that the response of a solid electrode is strongly dependent on the surface preparation, i.e., the mechanical, chemical and electrochemical pre-treatment applied. Different from the liquid electrodes (Hg, Tl), the rate of *e.c.t.* at solid electrodes is extremely dependent on the surface condition. To a general procedure for surface preparation of solid electrodes, the readers may consult the literature [13]. Probably, the more critical consequence of this behavior of solid electrodes is the difficulty in renewing the surface in order to obtain reproducible electrochemical response. Also, preparation, characterization and control of the transducer surface play an important role in the following steps of the sensor construction, stability, quality of response and amount of SAM or other modifier component, and the success or fail of the developed device. These steps and properties are crucial for the immobilization of the biological molecules or other material on the transducer surface and subsequent interaction between the modified surface and the analyte, i.e., the final electrochemical response of the biosensor.

Massive or modified gold was also used to produce immunosensors. A gold electrode was repeatedly polished with 1.0 and 0.3 μm alumina slurry, successively sonicated in bi-distilled water and ethanol for 5 min, and dried in air [18]. Kheiri et al. [19] used similar procedure to pre-treat the gold electrode before modifying it with carbon nanotubes (CNTs) and other modifiers. The gold electrode was polished with 0.3 and 0.05 mm alumina powders in succession, thoroughly rinsed with double distilled water between each polishing step, successively sonicated with acetone and double distilled water, and dried at room temperature. Another strategy was adopted to clean and pre-treat the gold electrode surface to construct immunosensors [20]. Gold electrodes were first polished with aqueous alumina slurries of

25 and 1 μm, rinsed with MilliQ water, sonicated for 1 min, dried with argon, treated with cold piranha solution for 30 s, washed with Milli-Q water and argon dried. Afterwards, a preliminary electrochemical cleaning was performed by LPS between −0.2 and −1.8 V in 0.1 mol L^{-1} KOH, followed by CV in 2 mol L^{-1} H_2SO_4 at 0.2 V s^{-1} for 30 cycles or until stable CVs were recorded.

Gold electrodes array, consisting of 16 gold working electrodes where each WE was placed between an Ag pseudo-reference and a gold AE, were used to prepare amperometric immunosensors for tumor detecting [21]. A thin film of gold or platinum was modified with CNTs to construct an amperometric immunosensor for rheumatoid arthritis [22]. To construct amperometric immunosensors for detection of Chagas disease, transducers were prepared by sputtering gold on Si and Si_3N_4 in argon atmosphere [23]. The silicon-gold slices were annealed at 1000 °C for 5 s, cooled at air atmosphere and room temperature, vigorously washed with distilled water and dried with purified compressed air.

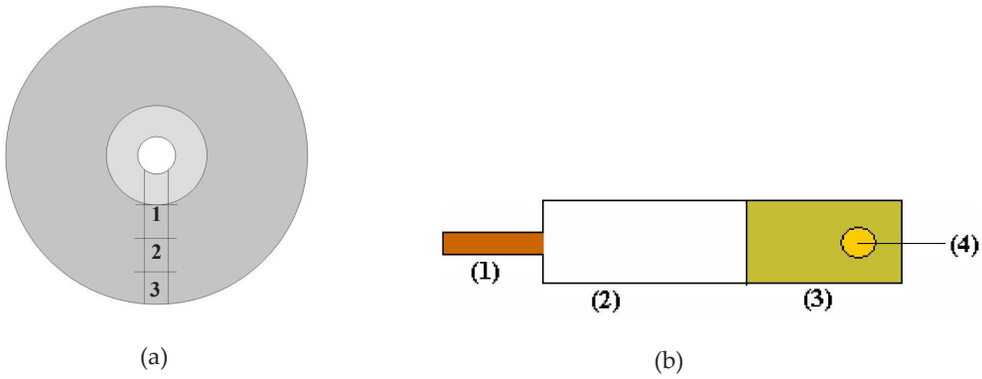

Figure 1. Regions of CD-Rs: (1) inner, (2) central and (3) out border. (B) CDtrode: (1) electric contact of copper, (2) PTFE tape to fix the electric contact, (3) 1KFA25 Kapton tape® applied on the surface to delimit the area of the electrode, and (4) area of the working electrode [29].

Gold-based substrates produced by sputtering can be substituted, with advantages, by metallic substrates obtained from recordable compact discs (CD-Rs) [24]. These devices present comparable electrochemical performance to commercial gold electrodes, they are easily constructed and versatile, of low cost to be used and discarded in cases of fouling, surface oxidation, irreversible adsorption, and so on, and are user-friendly because electrode polishing is not necessary [25,26]. In general, the gold CD-R has a gold film thickness of 50-100 nm and it can be also used as WE (CDtrode). As-received CD-R pieces may be treated with 69-70% HNO_3 for 5-10 min to remove the polymeric layers, cleaned with 95-98% sulfuric acid and abundantly washed with ethanol and/or water. Recently, Foguel et al. (2011) [27,28] developed an amperometric immunosensor for Chagas disease using CDtrode prepared by the procedure described above. It was observed that the voltammetric response of CDtrode depends on the procedure applied to remove the protective polymeric-based layer, the subsequent chemical or electrochemical treatments, trade of CD-R and also sometimes the region of the CD-R. Foguel [29] also investigated in more detail the use of different CD-R trades, nominated as AA, BB and CC, and different regions (out border, center and inner) of

CD-Rs (Fig. 1a). The polymeric layers covering the gold surface were removed by different procedures: (a) careful manual removal with tweezers, vigorous washed with distilled water and dried with purified compressed air; (b) the procedure described by Lowinsohn et al. [26]; (c) the procedure described in (b) and the area of the electrode limited by a mask of toner. Figure 1b illustrates the final setup of the electrode.

Surface roughness, CD track height and thickness, and the distance between CD tracks (cavities thickness) were measured by AFM. For AA CD-R the measured parameters were almost invariable in the inner, center and out border regions of the CD-R; BB CD-R presented almost the same surface roughness and CD track height in all regions, high difference in track thickness, the inner border tracks are thicker and the distance between them is higher; the inner and border parts of the CC CD-R showed similar tracks height, thickness and roughness values, but varied the distance between CD tracks among the different regions of the CD-R, and different values for all parameters in the center compared with the other regions. These results indicated that the AA CD-R is the only one that showed a more homogeneous gold surface and, therefore, it should present the best electrochemical behavior. FE-SEM analysis showed differences in the CDtrodes surface: CD tracks were better defined when the polymeric layers were manually removed and flatter when concentrated HNO_3 was used. Unmodified electrode surfaces were initially characterized by CV of 1 x 10^{-3} mol L^{-1} $Fe(CN)_6^{4-}$ in 0.5 mol L^{-1} H_2SO_4 aqueous solution (higher *e.c.t.* rate) at 50 mV s^{-1} without or with an application of 10 cycles from +0.2 to +1.5 V / Ag|AgCl|$KCl_{sat.}$ in 0.5 mol L^{-1} H_2SO_4 solution at 50 mV s^{-1}. When the polymeric layers were manually removed the I-E profiles were bad-defined and this procedure was abandoned.

Figure 2 shows cyclic voltammograms (CVs) recorded for unmodified CDtrode, constructed from BB CD-R, in 0.1 mol L^{-1} phosphate buffer (PB) solution at pH 7.0 containing 1 x 10^{-3} mol L^{-1} $Fe(CN)_6^{3-/4-}$ at 50 mV s^{-1}: (A) after removal the protective layers from the gold surface using the procedure (b); (B) after applying the procedure (b) followed by 10 cycles from +0.2 to +1.5 V / Ag|AgCl|$KCl_{sat.}$ in 0.5 mol L^{-1} H_2SO_4 solution at 100 mV s^{-1} and 10 cycles from −0.4 to +0.7 V / Ag|AgCl|$KCl_{sat.}$ in 1.0 x 10^{-3} mol L^{-1} $Fe(CN)_6^{3-/4-}$ + 0.1 mol L^{-1} PB solution at pH 7, at 50 mV s^{-1}.

It is clear that the I-E profile described in Fig. 2B resembles the response of a reversible charge transfer process, while the I-E profile in Fig. 2A suggests a non-reversible charge transfer process. Many factors can be involved in this electrochemical response. All of them are related to the surface nature of the solid electrode: the presence of protective material residues and other dirt, contaminants, oxides generated during the acid attack, defects and heterogeneities on the surface present in the original material or caused by the chemical attack. In this case, the mechanical procedure which is applied in many solid electrodes is not applicable. The chemical etching recommended to gold by using "piranha" or strong alkaline solution may also damage the delicate surface mainly at stressed regions of gold deposit. Therefore, the chemical etching is not recommended for CDtrodes. The adsorbed species can be removed and the electrode surface activated by potential cycling between the potentials of H_2 and O_2 evolution reactions. This process makes the surface reproducible and repeatable, and may improve the reversibility of the electrode process, as observed in Figure 2.

http://dx.doi.org/10.5772/53656

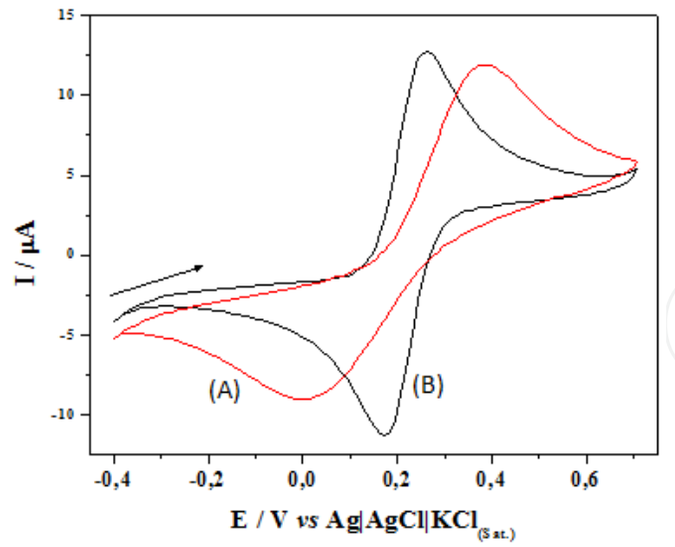

Figure 2. CVs of 1.0 x 10^{-3} mol L^{-1} $Fe(CN)_6^{3-/4-}$ in 0.1 mol L^{-1} PB solution at pH 7.0, 50 mV s^{-1} on gold CDtrode in which the protective layers were removed by procedure *b* (A) and *b* followed by 10 cycles in 0.5 mol L^{-1} H_2SO_4 solution from +0.2 to +1.5 V / Ag|AgCl|KCl_{sat} at 100 mV s^{-1} and 10 cycles in 1.0 x 10^{-3} mol L^{-1} $Fe(CN)_6^{3-/4-}$ in 0.1 mol L^{-1} PB solution at pH 7.0 from –0.4 to +0.7 V / Ag|AgCl|KCl_{sat} at 50 mV s^{-1} (B) [29].

In the case of screen-printed electrodes (SPEs), special care should be taken during handling to avoid irreversible damage. For instance, in recent studies [3,10], screen-printed gold-based electrodes were used as-received. These SPEs are received in aluminum sealed package individually isolated from the atmosphere. The package of each electrode was opened just before using and avoiding surface contamination. Chemical etching is not recommended for SPE gold electrodes. Therefore, the SPEs were thoroughly washed with ethanol and Milli-Q water for further procedures. Similar procedure has been recommended in literature [30,31]. It was observed that some immobilization or electrochemical processes are not significantly influenced by surface pre-treatments [32], and, some cases, they are used as-produced or -received, without pre-treatment [33].

Carbon based materials (graphite, glassy carbon, carbon fibers, carbon-SPE, carbon-epoxy resin composites, nanotubes and boron-doped diamond) have been used in both unmodified and modified forms by incorporation of gold nanoparticles (GNP) or iron oxides nanoparticles (NPs) dispersed in a polystyrene polymer matrix to construct amperometric or other biosensors. Iron oxides NPs exhibit magnetic properties and are constituted by paramagnetic γ-Fe_2O_3 and Fe_3O_4 or modified with some specific groups or can be a core-shell structure, with a core (γ-Fe_2O_3) and shell (styrene-based copolymer). In a recent work, bare graphite electrodes were mechanically treated by wet polished on emery paper, thoroughly washed with distilled water and modified to construct an amperometric biosensor [34]. Glassy carbon was successively wet polished with 1.0, 0.3 and 0.05 mm alumina slurry until a mirror-like surface, and the surface was thoroughly rinsed between each polishing step with doubly distilled water. Afterwards, it was successively sonicated in 1:1 nitric acid, acetone and doubly distilled water, and allowed to dry at room temperature [35]. Carbon fiber electrodes are produced, mainly in connection with the preparation of high-strength compo-

sites by high-temperature pyrolysis of polymer textiles or via catalytic chemical vapor deposition [36]. A chitosan-modified carbon fiber electrode was used to develop a biosensor for dengue virus envelope protein detection [37]. The carbon fibers surfaces were sonicated in ultrasonic bath with 10% HNO_3 solution for 10 min, rinsed with distilled water and conveniently modified. Commercial available carbon SPE was treated by applying an anodic current of 25 μA for 2 min in 50 μL of 0.1 mol L^{-1} H_2SO_4 solution dropped on the SPE carbon electrodes and washed with 0.1 mol L^{-1} Tris buffer pH 7.2 [38].

Graphite powder may be used to prepare composites which can be modified by NPs and/or magneto NPs and used in amperometric sensor. Recently, graphite powder and epoxy resin were used by Pividori, et al. to develop a sandwich magneto immunoassay [39]. The modified magnetic NPs are captured by the magnetic field on the magneto electrode. Arrays of carbon-SPE electrodes were also used to construct immunosensors. The arrangement was washed with water to remove any adsorbed species and characterized by CV in 5.0 mmol L^{-1} $Fe(CN)_6^{3-}$ solutions [40].

The development, properties (good electrical conductivity, nanometer size, high aspect ratio and structure, electrochemical stability, high specific area and surface chemistry) and applications of CNTs, mainly in biosensors construction, were deeply discussed recently [41]. Both its high specific area, which allows the analyte to be accumulated on the surface, and the capability of increasing *e.c.t.* reaction rate increase the response signal and diminish the overpotential for some electrode reactions. However, the fundamental reasons for that are not still well-established. The electrochemical behavior of graphitic materials is in great part defined by edge defects and oxygen functionalities at the surface, and the properties of the CNTs are similar to the high oriented pyrolytic graphite (HOPG). Details of CNT growth, working electrode preparation, surface modification and its application to construct specific enzymatic biosensors and genosensors were described [41]. In general, the CNT electrodes are subjected to electrochemical treatments based on three different electrolytes: 0.1 mol L^{-1} HNO_3, 10 s at 1 V; 0.1 mol L^{-1} KCl, 60 s at 1.75 V and 1 mol L^{-1} NaOH, 60 s at 1 V / Ag|AgCl|KCl; the last one seems to be the best. The CNTs cleaning is based on oxidation of the amorphous carbon, and carboxylic moieties generation for further covalent functionalization. SPEs made of commercial or homemade carbon inks were modified with multiwall carbon nanotubes (MWCNTs) and Au NPs to construct immunosensors [42]. These electrodes are pre-treated by applying +1.5 V / Ag|AgCl|$KCl_{sat.}$ for 5 min in 0.1 mol L^{-1} NaOH solution, and chemical treated by 3:1 concentrated H_2SO_4 and HNO_3 solution. This modified surface is immersed in 0.5 mol L^{-1} H_2SO_4 solution containing 0.1 mmol L^{-1} $HAuCl_4$ and gold NPs which are deposited by applying 15 cycles from +1.0 to 0.0 V / Ag|AgCl|$KCl_{sat.}$ at 50 mV/s. The resulting surface is rinsed with deionized water and stored in 0.1 mol L^{-1} phosphate buffer saline (pH 7.0) before characterization. CNTs can be conveniently functionalized with amino groups, deposited GNP, generated an appropriate composite and applied on massive gold electrodes [19]. Composites of MWCNT-polystyrene were modified and also applied on gold or on platinum thin film, and used to construct an amperometric immunosensor for rheumatoid arthritis diagnosis [22].

1.2. Choosing the potential value of the working electrode

Several factors influence the choice of the best potential value to be applied to the working electrode in order to get the best sensitivity of the biosensor. Some criteria may be adopted: (a) all steps of the biosensor construction should be carefully characterized by electrochemical and non-electrochemical techniques; (b) the current peak or wave responsible for the biosensor response must be unequivocally determined; (c) the stability and repeatability of the system should be investigated by obtaining enough number of I-E curves or CVs for a series of biosensors prepared by the same methodology.

Theoretically, the potential to be applied should reduce to zero the surface concentration of active centers responsible for the amperometric biosensor response. At this potential current, is directly proportional to the analyte concentration and the effective electrode surface area. In practice, this potential value frequently corresponds to the peak potential of CV, which does not mean that the surface concentration is zero; it depends on the electrode process. As the current generated at this potential is the sum of all faradaic processes occurring, supposing that no significant charging current is present, the reaction of interest identification may not be easy. Getting satisfactory reproducibility and repeatability of the biosensor response may be a hard task, mainly if low currents are generated, which may require more sophisticated setup and/or more expensive instrumentation. Different studies have applied the peak potential obtained from the CVs or the peak current of CVs at a constant scan rate to evaluate the biosensor response.

1.3. Repeatability and sensitivity in (chrono)amperometry measurements

For surface-controlled electrode processes (adsorption, new phase formation, surface modifications and so on) the current-time curves recorded at constant potential are strongly dependent on the nature of the substrate, and the reproducibility is strictly related to the similarity between previous and renewed surfaces. At constant temperature and solution composition, the structure of the monoatomic layers at the renewed surface are not strictly similar to that recorded to the previous surface, which may leave to different I-E profiles. Therefore, the best practice is recording a great number of current transients for each investigated condition and using the average current value. In minor grade, the surface conditions also influence the current values even if electroactive species are in solution, due to changes in the surface roughness or adsorption of active or inactive species on the surface. The response of modified surfaces may also depend on the surface roughness, defects, heterogeneities, coating stability, impurities in the medium, etc.

Some techniques are more sensitive than others for specific properties of the system. For instance, EIS presents high sensitivity to any change on the electrode surface. Amperometric response for diffusion-controlled processes depends on c_{bulk}, diffusion coefficient, number of electrons/particle, applied potential and effective surface area, size and geometry of the working electrode, and it is inversely proportional to the square root of time. Therefore, the higher current is obtained at short measuring time and, in general, it exponentially decays, tending to a stationary value. The capacitive current contribution is higher at very short time, the faradaic current depends on the kinetic of the electrode process, and the total cur-

rent reaches a stationary value for longer times. These two characteristics of the technique may result in lower sensitivity when compared to some other electrochemical techniques. The analytical current density can be increased by convection (flux, stirring or jet the analyte) during the electrolysis or using micro or ultramicroelectrodes. Decreasing the analyte concentration, the faradaic current decreases and approximates to the current background (charging current, surface oxidation or reduction processes, noise). Therefore, in classical polarography the charging current limits the detection from 5×10^{-6} to 1×10^{-5} mol L^{-1} interval. However, techniques with time dependences for capacitive and analytical currents favoring the analytical one (pulse polarography techniques) may offer lower limit of detection. All these pulse techniques are based on a sampled current potential-step (chronoamperometric) experiment [36].

Also, higher faradaic/capacitive currents ratio (lower limit of detection) can be obtained for redox processes which occur near the potential of zero charge of the working electrode. Therefore, if possible, the working electrode that must be chosen is the one with a potential of zero charge closest to the redox potential of the analyte.

In order to optimize the working conditions of the developed biosensor, other parameters and/or properties influencing its response should be investigated such as pH, operating potential, temperature, stability, repeatability, cut off, limit of detection and sensitivity.

Following biosensor for disease diagnosis based on antigen/antibody (immunosensor) or DNA/DNA (genosensor) or enzymatic catalytic reaction will be described.

2. Amperometric immunosensors

The immobilization of antigens or antibodies on the surface of electrochemical transducers led to the development of immunosensors for several substrates of interest in the biological, clinical and industrial areas [43-45]. Immunosensors combine the advantages of the electrode process and the high specificity of immunologic reactions [46]. The methods are very rapid, they have the advantage of requiring small sample volumes affording an increase in the number of analyzed samples, and enabling versatile transducers and different techniques for monitoring, thus lowering costs compared with conventional analytical methods.

The immunosensor is classified as optical, mass-sensitive or electrochemical according to the technique. The electrochemical immunosensor, according to the monitoring, is classified as amperometric, potenciometric, impedimetric and condutometric. As mentioned before, chrono-amperometric technique for the development of amperometric immunosensor compared with other electrochemical techniques, is simple, cheap, sensitive, its potential applied not affected sample and possibly portable measuring amperometric system.

Several amperometric immnunosensors have been developed for disease diagnosis as shown in Table 1.

Cavalcanti et al. [37] developed a chitosan modified fiber electrode for dengue virus envelope (DENV). Antibodies against DENV were covalently immobilized on the chitosan ma-

trix after activation with sodium periodate. Amperometric response of the competitive immunoassays was generated by hydrogen peroxide with peroxidase conjugated to DENV and 2′-azino-bis-(-3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) as mediator. The immunosensor showed a lower limit of detection for DENV (0.94 ng mL^{-1}) than previously described and a linear range from 1.0 to 175 ng mL^{-1}, in concentration levels clinically relevant for dengue virus diagnosis.

A novel amperometric immunosensor for the detection of the p24 antigen (p24Ag) from HIV-1 using gold nanoparticles (GNP), multiwalled carbon nanotubes (MWCNTs), and an acetone extracted propolis (AEP) film was developed by Kheiri et al. The GNP/CNT/AEP film provided a suitable surface for the immobilization of antibodies and prevented direct contact of the biomolecules with the substrate. Moreover, GNPs were synthesized in situ on the amino functionalized MWCNTs (MWCNTNH2) for antibody immobilization, which also improved the electrochemical signal of HRP-anti p24 Ab, thus enhancing the detection sensitivity of the reduction of H_2O_2 [19].

Two methods to diagnose hepatitis B [18,35] are described in Table 1 and both methods determine hepatitis B surface antigen based on gold nanoparticle. The method developed by Zhuo et al. [18] is based on the gold nanoparticles and horseradish peroxidase (HRP)-modified gold electrode for the determination of hepatitis B surface antigen (HBsAg). The system was optimized for a reliable determination of HBsAg in the range of 2.56-563.2 ng mL^{-1} with a limit of detection 0.85 ng mL^{-1}. Qiu et al. [35] also determined hepatitis B surface antigen using a glassy carbon electrode modified with an assembly of positively charged poly(allylamine)-branched ferrocene (PAA-Fc) and negatively charged gold nanoparticle. The concentration of the antigen can be quantified in the range 0.1 and 150 ng mL^{-1}, with a limit of detection 40 pg mL^{-1}.

González et al. [38] used screen-printed carbon electrodes to detect pneumolysin (PLY) in human urine. The voltammetric immunosensor is based on the electrochemical detection of indigo blue, produced by alkaline phosphatase (AP) when 3-indoxyl phosphatase (3-IP) is used as enzymatic substrate. It is prepared and evaluated for measuring this toxin in human urine samples. The single-use immunosensor is fabricated by deposition of biotinylated anti-PLY monoclonal antibodies onto pre-oxidised streptavidin coated screen-printed carbon electrodes (SPCEs). Rabbit polyclonal IgGs anti-PLY are used in combination with an anti-rabbit IgG alkaline phosphatase conjugate as detection antibodies.

The determination of the antigliadin antibodies from human serum samples is of vital importance for the diagnosis of an autoimmune disease such as celiac disease. Therefore, Rivera et al. determined antigliadin antibodies in real human serum using an electrochemical immunosensor with control over the orientation and packing of gliadin antigen molecules on the surface of gold electrodes. The orientation of the antigen on the surface has been achieved using a carboxylic ended bipodal alkanethiol that is covalently linked with amino groups of the antigen protein. Amperometric evaluation of the sensor with polyclonal antigliadin antibodies showed stable and reproducible low limits of detection (46 ng mL^{-1}; % RSD = 8.2, n = 5) [20].

Disease or infectious agent	Electrode / immobilization/Sample	Limit of detection
Dengue	Carbon fiber electrode / chitosan with antibody against dengue virus envelope protein / Hs [37]	0.94 ng mL^{-1}
Malaria falciparum	Graphite epoxy composite electrode / magnetic nanoparticle modified with monoclonal antibody against HRP2 / Hs [39]	0.36 ng mL^{-1}
	Screen-printed electrodes / multiwall carbon nanotubes and Au nanoparticles with rabbit anti-PfHRP-2 antibody / Hs [42]	8 ng mL^{-1}
HIV-1 p24 antigen	Multi-walled carbon nanotubes / Au nanoparticles with p 24 antibody / Hs [19]	0.0064 ng mL^{-1}
Hepatitis B	Glassy carbon electrode / assembly of positively charged poly(allylamine)-branched ferrocene (PAA-F_c) and hepatitis B surface antibody / Hs [35]	40 pg mL^{-1}
	Gold electrode / Au nanoparticles / HRP and hepatitis B surface antibody / Hs [18]	0.85 ng mL^{-1}
Pneumonia	Screen-printed carbon electrodes / biotinylated anti-pneumolysin monoclonal antibodies Hu [38]	0.12 ng mL^{-1}
Celiac disease	Gold electrode / gliadin antigen / Hs [20]	46 ng mL^{-1}
Urinary infection	Gold electrodes array / alkanethiolate SAM / monoclonal antibody anti-lactoferrin / Hu [47]	145 pg mL^{-1}
Tumor markers	Carbon screen-printed / capture antibody / Hs [40]	0.03-0.05 ng mL^{-1}
Colon cancer	Gold electrode arrays / anti-carcinoembryonic antibody / Hs [21]	0.2 ng mL^{-1}
Rheumatoid arthritis	Carbon nanotube composite electrodes / anti-citrullinated peptide antibody / Hs [22]	> 1:200 dilution Hs
Chagas disease	Au-SPE / antigenic protein (epimastigote membranes) / Hs [51]	−0.104 μA (cut-off)
	Au-SPE / antigenic Tc85 protein (trypomastigote membranes) / Hs [23]	−0.158 μA (cut-off)
	Au-CD-R transducer / antigenic Tc85 protein (trypomastigote membranes) / Hs [27]	−0.949 μA (cut-off)
	Gold electrode / anti-*Trypanosoma cruzi* G / Hs [52]	62 ng mL^{-1}

Human serum (Hs); Human urine (Hu)

Human serum (Hs); Human urine (Hu)

Table 1. Amperometric biosensors for diseases or infectious agents based on immunosensors.

Pan et al. [47] reported the development of an electrochemical immunosensor for direct detection of the urinary tract infection (UTI) biomarker lactoferrin from infected clinical samples. The electrode surfaces were coated with either a SAM of 11-mercaptoundecanoic acid (MUDA) or a mixed of MUDA and 6-mercapto-1-hexanol. A sandwich amperometric immunoassay was developed for detection of lactoferrin from urine, with a limit of detection 145 pg mL^{-1}.

Honglan et al. developed an electrochemical immunosensor array for the simultaneous detection of multiple tumor markers by incorporating electrochemically addressing immobilization and one signal antibody strategy. As a proof-of-principle, an eight-electrode array including six carbon screen-printed working electrodes was used as a base array for the

analysis of two important tumor markers, carcinoembryonic antigen (CEA) and a-fetoprotein (AFP) and a horseradish peroxidase-labeled antibody was used as a signal antibody. The result showed that the steady current density was directly proportional to the concentration of target CEA/AFP in the range from 0.10 to 50 ng mL^{-1} with a limit of detection 0.03 and 0.05 ng mL^{-1} for CEA and AFP, respectively [48].

Laboria et al. [21] reported on the development of an amperometric biosensor for detecting CEA in colon cancer detection based on the immobilization of anti-CEA monoclonal antibody on a novel class of bipodal thiolated self-assembled monolayers containing reactive N-hydroxysuccinimide ester end groups. The current variations showed a linear relationship with the concentration of CEA over the range of 0-200 ng mL^{-1} with a sensitivity of 3.8 nA mL ng^{-1} and a limit of detection 0.2 ng mL^{-1}, which is much below the commonly accepted concentration threshold (5 ng mL^{-1}) used in clinical diagnosis.

A simple amperometric immunosensor was constructed to be potentially used for the detection of serum anticitrullinated peptide antibodies, which are specific for rheumatoid arthritis (RA) autoimmune disease. Sera of RA patients contain antibodies to different citrullinated peptides and proteins such as fibrin or filaggrin. Herein, a chimeric fibrin-filaggrin synthetic peptide was used as a recognition element anchored to the surface of a multiwalled carbon nanotube-polystyrene-based electrochemical transducer [22].

2.1. Amperometric immunosensors for malaria

Malaria is a serious tropical disease transmitted to humans via the female *Anopheles* mosquito and is caused by 4 species of protozoal parasites from the *Plasmodium* genus: *P. falciparum, P. vivax, P. ovale* and *P. malariae. P. falciparum* causes the most severe form of the disease and can be fatal if not correctly treated.

The *P. falciparum* parasite synthesizes several proteins containing large amount of amino acid histidine, which are commonly referred to as histidine-rich proteins (HRP). One of these, HRP2, with 34% histidine and 37% alanine shows a markedly high density among proteins [42].

In recent years, devices for the diagnosis of *P. falciparum* malaria based on HRP2 have significantly gained importance. The abundance of the antigen and the resulting high sensitivity of the diagnostic devices combined with the simplicity of their application make them an obvious alternative in settings where microscopy is not available or not of sufficiently high quality standard [48].

Sharma et al. developed amperometric immunosensor for the detection of HRP2 in the sera of humans with *P. falciparum* malaria. For this purpose, disposable screen-printed electrodes were modified with multiwall carbon nanotubes and Au nanoparticles. Nano-Au/MWCNT/SPEs yielded the highest-level immunosensing performance among the electrodes, with a limit of detection 8 ng mL^{-1} [42].

Castilho et al. [39] used, for the first time, magneto immunoassay-based strategies for the detection of *P. falciparum* histidine-rich protein 2 related to malaria using magnetic micro-

nanoparticles. The immunological reaction for the protein PfHRP-2 was successfully performed in a sandwich assay on magnetic micro- and nanoparticles by using a second monoclonal antibody labeled with the enzyme horseradish peroxidase (HRP). Then the modified magnetic particles were easily captured by a magneto sensor made of graphite-epoxy composite (m-GEC) which was also used as the transducer for the electrochemical detection. The schematic representation for the detection of the *P. falciparum* antigen related to malaria disease in human serum based on a sandwich assay performed on magnetic beads or nanoparticles modified with a IgM monoclonal antibody (anti-HRP2-MB and anti-HRP2-MNP, respectively) and using a second IgG monoclonal antibody labeled with the enzyme horseradish peroxidase (anti-HRP2-HRP) electrochemical signal is showed in the Figure 3.

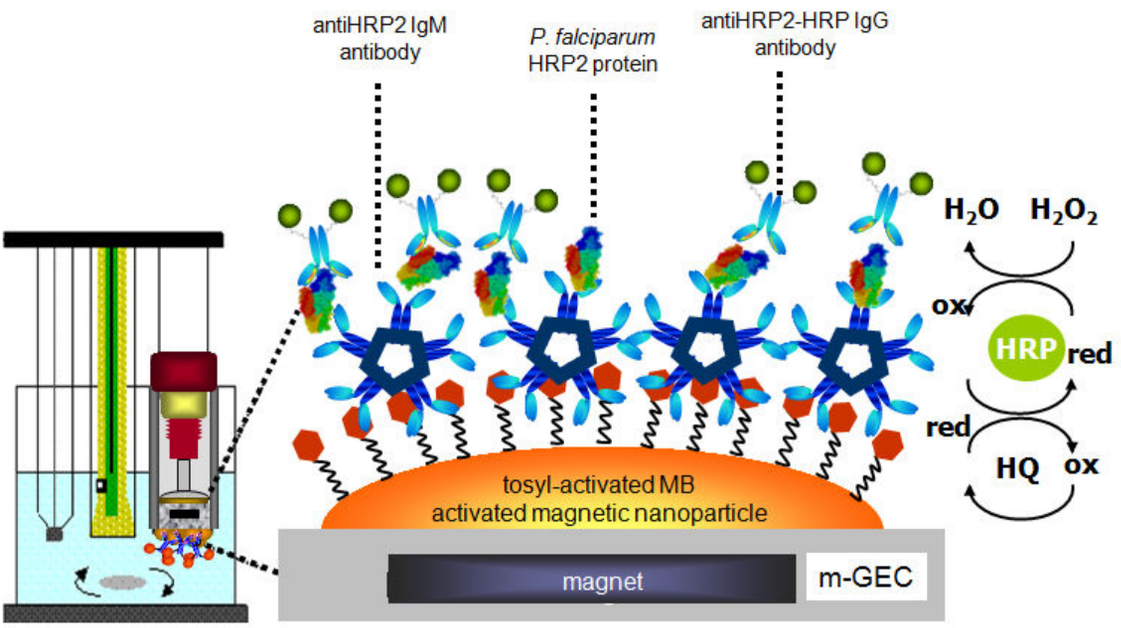

Figure 3. Schematic representation of the experimental details for the electrochemical magneto immunosensor [39].

The electrochemical signal was determined by polarizing the m-GEC electrode at a working potential of −0.100 V / Ag|AgCl. The electrochemical signal was based on the enzymatic activity of the HRP after the addition of hydrogen peroxide as the substrate and hydroquinone as a mediator. The electrochemical magneto immunosensor coupled with magnetic nanoparticles have shown a limit of detection 0.36 ng mL^{-1} [39].

2.2. Amperometric immunosensors for Chagas disease

Chagas disease, also known as American trypanosomiasis, is a neglected tropical disease caused by the hemoflagellate *Trypanosoma cruzi* (*T. cruzi*). An estimated 10-15 million people are infected worldwide, mostly in Latin America where Chagas disease is endemic. More than 25 million people are at risk of the disease. There is no vaccine for Chagas disease; therefore, vector control and diagnostic tests are effective methods of preventing Chagas

disease. Blood screening is necessary to prevent infection through transfusion and organ transplantation [49].

The detection of antigen in the blood sera could be useful just for the acute phase of Chagas disease. Detection of anti-*T. cruzi* antibodies in the serologic investigation is the method of choice for the etiological diagnosis of Chagas disease in the chronic phase, considering the specificity and sensitivity of the tests used in the clinical analysis routine. Traditional in clinical practice are the following serological tests using *T. cruzi* antigens: indirect hemaglutination, indirect immunofluorescence and enzyme-linked immunosorbent assay (ELISA) [50].

The methodology for clinical diagnosis must be sensitive and with high reproducibility and repeatability. Different analytical methodologies were developed and amperometric immunosensors were constructed and applied for diagnosis of various diseases stages.

Antigenic proteins (Ag) of *T. cruzi* epimastigote membranes were used for construction of an amperometric immunosensor for serological diagnosis. Proteins with molecular mass ranging from 30 to 100 kDa were immobilized on gold surface of screen-printed electrode treated with self- assembled monoyers (SAMs) of cysteamine (CYS) and glutaraldehyde (GA). Antibodies (Ab) present in the serum of patients with Chagas disease were captured by the immobilized antigens and the affinity interaction was monitored by chronoamperometry at a potential of −400 mV / Ag|AgCl|KCl_{sat}. using peroxidase-labeled IgG (Ac*) conjugate and hydrogen peroxide, iodide substrate. Figure 4 shows a scheme of the reactions involved in the steps of SAMs formation, antigen *T. cruzi* immobilization on GA-CYS SAMs and immunoassays. The incubation time to allow maximum antigen-antibody and antibody-peroxidase-labeled IgG interactions was 20 min with a reactivity threshold at −0.104 μA [51]. Another amperometric immunosensor was developed using a specific glycoprotein of the trypomastigote surface (Tc85). The purified recombinant antigen also was immobilized on cysteamine and glutaraldehyde self-assembled monolayers. The affinity reaction was monitored directly using amperometry through a secondary antibody tagged to peroxidase at −400 mV / Ag|AgCl|$KCl_{sat.}$ [23]. In both amperometric immunosensors, peroxidase enzyme catalyses the I_2 formation in the presence of hydrogen peroxide and potassium iodide, and the reduction current intensity was measured at a given potential with screen-printed electrodes. The immunosensor was applied to sera of chagasic patients and patients having different systemic diseases with a reactivity threshold at −0.158 μA. Amperometric immunosensor also was developed for determination of Chagas disease through a gold based electrode obtained from a recordable compact disc (CD-R transducer) modified with 4-(methylmercapto)benzaldehyde for the immobilization of Tc85 protein of the *T. cruzi*. The immunoassays were carried out using positive and negative sera from Chagas disease patients and immunoglobulin conjugated with peroxidase enzyme. The immunosensor presented −0.949 μA as cut-off value and was applied in sera samples [27]. It is important to note that the cut-off value obtained for each immunosensor is different because the transducer modifications are not the same.

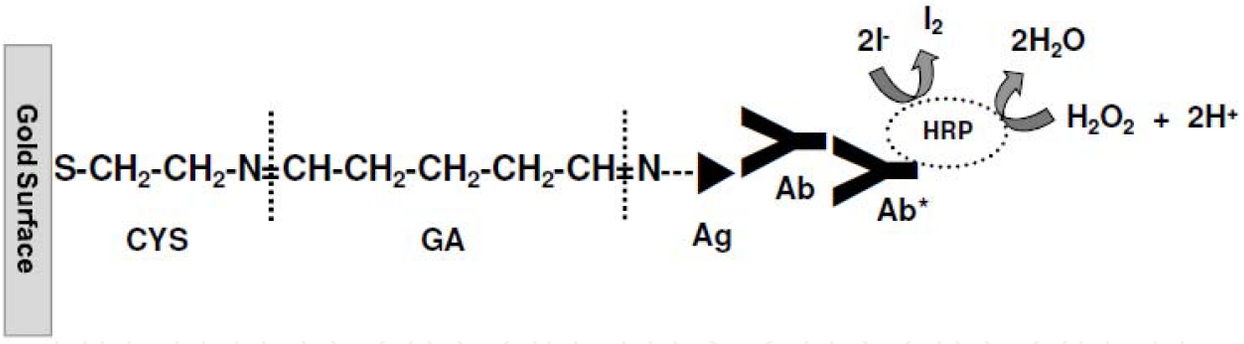

Figure 4. Scheme of the immobilization of antigenic protein on gold modified with SAMs and immunoassays.

Recently, Belluzo et al. applied strategy orientation recombinant proteins to develop amperometric biosensors to diagnose Chagas disease. The gold electrode was modified with thiol and activated the thiolated surface with carbodiimide which allow the subsequent reaction with the amine moieties of the protein Lys residues. The immunoassay involved serum sample anti-*T. cruzi* (analyte), peroxidase-conjugated anti-human immunoglobulin G and with 62 ng mL^{-1} limit of detection [52].

3. DNA based biosensors

Electrochemical biosensors that use DNA, also called genosensors, can be used for analysis and determination of base sequences of DNA to diseases diagnose. DNA molecule has structural features that allow its immobilization on electrode surfaces as single or double helix [53]. Several electrode materials can be modified with DNA, and DNA biosensors can be used for hybridization studies in order for disease diagnosis, mutation detection [54] and also for DNA damage [55] analysis and for detection of antioxidant capacity of many compounds [56]. In this part of the chapter, the focus is on amperometric biosensors for hybridization studies.

DNA hybridization technology has been applied in biosensor systems for diagnosis and it can be considered rapid, with simplicity of execution and lower cost. Hybridization process involves the formation of the DNA duplex by annealing two complementary single strands. The single-stranded DNA (ss-DNA) modified electrode identifies the complementary sequence of nucleic acid in the sample solution leading to the formation of a hybrid double-stranded (ds-DNA). This identification is effective and specific even in the presence of non-complementary sequences [57]. The stability of the hybridization depends on the nucleotide sequences of both strands. A perfect match in the sequence of nucleotides produces very stable ds-DNA, whereas one or more base mismatches impart increasing instability that can lead to weak hybridization of strands [58].

The ability to immobilize the probe DNA in a predictable manner while maintaining their affinity for complementary DNA is an important aspect of genosensors development. The appropriate immobilization is strictly dependent on the characteristics of the transducer,

since each of the different immobilization strategies can lead to the proper orientation of biomolecules, allowing to control the probes conformational freedom, making them accessible for interaction with target DNA and providing minimal steric hindrance. Random DNA attachment to the electrode surface can result in chemical modifications of genetic material basic components, which consequently may cause the decrease in the specificity of layer recognition.

The hybridization event can be direct or indirectly monitored [57,59,60]. Direct detection or label-free detection involves the measurement of changes in electrochemical signals related to the electroactivity of DNA bases, most commonly guanine oxidation. After the hybridization, the steric conformation of the DNA molecule protects the guanine oxidation, causing an electrochemical signal decrease, since the oxidation sites of the base are in the internal parts ds-DNA molecule [61]. Although this method is simple and sensitive, the direct oxidation of DNA requires relatively high potential. Other disadvantage is that such measurement of the decreased anodic signal of the immobilized probe cannot be used for detecting targets containing guanine bases. An alternative is the use of inosine-substituted probes. Guanines in the probe sequence are substituted by inosine residues (pairing with cytosines) and the appearance of a guanine signal upon hybridization with the target enables a new detection method for DNA hybridization [62].

Indirect hybridization detection protocol can be based on the incorporation of electroactive indicators. These compounds, usually cationic metal complexes or organic compounds, have different affinities for the double-stranded DNA (formed after the hybridization process) when compared with single-stranded DNA, preferentially binding with ds-DNA in the groove, by intercalation or electrostatic interaction. Due to variation of redox indicator concentrations near the electrode surface, the resulting current signal indicates the hybridization event. An example of this kind of biosensor is described by Gao & Tansil [63]. After hybridization, a threading intercalator called PIND-Ru was introduced into the biosensor. PIND-Ru selectively intercalated with double-stranded DNA (ds-DNA) and became immobilized on the biosensor surface. The redox moieties of the interacted PIND-Ru showed excellent catalytic activity towards oxidation of amines observed by amperometry at 0.65 V / Ag|AgCl. The current was proportional to the target DNA concentration and a limit of detection 1.5 pmol L^{-1} was determined.

The use of enzymes has shown a good sensitivity for indirect electrochemical hybridization detection. The target DNA sequence is previously labeled with a redox active enzyme which catalyses a redox reaction and further generates an electrochemical change [64]. An electrochemical genosensor array for the individual and simultaneous detection of two high-risk human papillomavirus (HPV) DNA sequences using horseradish peroxidase enzyme (HRP) labeled DNA probes was developed by Civit et al. [65,66]. Using polymerase chain reaction (PCR) products of three specific high-risk HPV sequences, HPV 16, 18 and 45, it was possible to detect DNA in picomolar range. A high specificity of the sensor array was observed with negligible hybridization signal with the non-specific target.

A DNA sensor for West Nile Virus (WNV) was developed by Ionescu et al. [67]. In this work, aminated DNA probe was immobilized on the electrode, followed by hybridization of

the WNV complementary DNA target and an additional hybridization process with a complementary biotinylated WNV DNA, resulting in an extremely sensitive detection limit (1 fg mL^{-1}) of WNV DNA target.

Genosensors based on enzyme label have also been applied for diagnosis of some kind of cancer, for example, acute promyelocytic leukemia. Lin et al. [68] employed oligonucleotide derivative that hybridizes with very high affinity to perfectly complementary targets. Hybridization event was monitored by the HRP. The biosensor was applied in PCR amplicon from the fusion gene, which plays an important role in leukemogenesis. Another DNA biosensor for detection of promyelocytic leukemia/retinoic acid receptor alpha fusion gene is described by Wang et al. [69]. This biosensor, based on a 'sandwich' sensing mode, involves a pair of capture probe immobilized at electrode surface and biotinyl reporter probe as an affinity tag for streptavidin-horseradish peroxidase. It allowed detecting the complementary DNA standard concentration range from 0.05 to 5.0 nmol L^{-1}. A large number of studies describe the use of enzymes to monitor amperometrically DNA or RNA hybridization in order to analyze other diseases or infectious agents and some of them are included in Table 2.

As described above, there are many works about DNA biosensor for disease detection or diagnosis purposes. In our research group, we have been working in the development of genosensors for hepatitis C virus (HCV) detection. According to World Health Organization (WHO), hepatitis C affects about 170 million people worldwide and more than 350,000 people die from hepatitis C-related liver diseases each year. Since it rarely causes specific symptoms, hepatitis C is one of the most serious public health problems [70]. In general, the goal of a detection strategy is the simplification of the analytical methodology to a practical level, with a minimum demand of operator skills. In this way, HCV biosensors have become an alternative for diagnosis.

In the first work, we studied a piezoelectric biosensor [71]. Gold electrodes from quartz crystal microbalance were modified with oligonucleotides for detection of hepatitis C virus in serum. Avidin or streptavidin were immobilized and used for attachment of biotinylated DNA probes from four different sequences. The piezoelectric biosensors were used to monitor the DNA resulting from samples from HCV contaminated patients and the results compared with the standard RT-PCR procedure (test kit Roche Amplicor®). The samples characterized as positive in the Amplicor test were able to hybridize with at least one of the four probes immobilized on the piezosensor. However, some of the samples appearing as negative in the Amplicor assay also provided hybridization with some of the immobilized probes. This inconsistency might be explained by different sequences of probes used in the piezosensor assay and in the Amplicor assay (sequence unknown). These results are considered preliminary as not all parameters affecting the hybridization reaction were optimized and the effect of temperature on the double strand formation and stability of hybridized complex on the surface of piezosensor is critical. In our case, all measurements were carried out at room temperature (25 °C), thus allowing for hybridization and duplex formation probably even in the case of only a partial matching between the probe and the amplicon.

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Disease or infectious agent	Electrode / immobilization	Sample	Limit of detection	Reference
Colorectal Cancer	Gold / SAM	Synthetic oligonucleotides	5.85 pmol L^{-1}	[72]
Celiac Disease	Gold electrode / SAM	Synthetic oligonucleotides	0.01 nmol L^{-1}	[73]
Pseudomonas aeruginosa	Gold / SAM	Total RNA isolated from *P. aeruginosa*	0.012 pg μL^{-1}	[74]
Uropathogenic bacteria	Gold array / SAM	16S rRNA from bacterial lysis	0.3 fmol L^{-1}	[75]
	Gold array / SAM	16S rRNA from bacterial lysis	0.5 ng μL^{-1} for *E. coli* total RNA	[76]
	Biosensor array	16S rRNA from bacterial lysis	10^4 cfu mL^{-1}	[77]
	Gold SPE / SAM	16S rRNA from bacterial lysis	---	[78]
Escherichia coli	Fe_2O_3@Au core/shell nanoparticle / SAM	*E. coli* genomic DNA	0.01 pmol L^{-1}	[79]
	Screen-printed electrodes- magnetic beads / STA-biotin	PCR products	0.01 cfu mL^{-1}	[80]
	Gold electrode array / STA-biotin	rRNA from *E. coli*	1000 cells without PCR	[81]
Staphylococcus aureus	Graphite-epoxy electrodes / adsorption onto a nylon membrane	Synthetic oligonucleotides	---	[82]
Enterobacteriaceae family	Gold screen-printed electrodes - magnetic beads / Tetrathiafulvalene	PCR products	5.7 fmol	[83]
Streptococcus pneumoniae	Gold electrode and magnetic beads / STA-biotin	PCR products	1.1 nmol L^{-1}	[84]

SAM: self-assembled monolayer; STA: streptavidin; PCR: polymerase chain reaction; rRNA: Ribosomal ribonucleic acid; cfu: colony-forming unit.

Table 2. Amperometric biosensors for diseases or infectious agents based on DNA or RNA hybridization.

A selective and sensitive label free electrochemical detection method of DNA hybridization for HCV was proposed in cooperation with Dr. M. Josowicz's research group [85]. DNA probes of specific sequence HCV type-1 were immobilized on polypyrrole films deposited on Pt microelectrodes. The monitoring of the hybridization with the complementary DNA was based on electrostatic modulation of the ion-exchange kinetics of the polypyrrole film and it allowed the detection of HCV-1 with a limit of detection 1.82×10^{-21} mol L^{-1}. With this

biosensor, HCV-1 DNA detection did not show unspecific interactions in the presence of mismatched sequences from different HCV genotypes as 2a/c, 2b, and 3.

An advantage of the construction of DNA biosensors is the use of disposable electrodes. These electrodes have a low construction cost, good reproducibility of the area, the possibility of large scale production, and the absence of surface inactivation. Different disposable electrodes as recordable gold CD-R and pencil graphite electrodes (PGE) have being used.

Using PGE, we developed a disposable HCV genossensor with thin films siloxane-poly(propylene oxide) hybrids prepared by sol-gel method and deposited on the electrode surface by dip-coating process [86]. The streptavidin (STA) was encapsulated in the films and biotinylated 18-mer DNA probes for hepatitis C virus (genotypes 1, 2a/c, 2b and 3) were immobilized through STA, since strong interaction occurs between the avidin (or streptavidin) and biotin. The complementary DNA was hybridized to the target-specific oligonucleotide probe immobilized and followed by avidin-peroxidase labeling. Hybridization event was detected by amperometrically monitoring the enzymatic response at −0.45V / Ag|AgCl using H_2O_2 as enzyme substrate and KI as electron mediator. Negative and positive controls and positive samples of sera patients were analyzed and the HCV 1, 2a/c, 2b and 3 oligonucleotide probes immobilized on PGE were able to distinguish positive and negative sera samples.

Chemometric studies were applied to the development of another biosensor for hepatitis C virus using PGE [87]. Fractional factorial and factorial with center point design were applied in order to simultaneously evaluate the variables of interest that have significant influence on the biosensor response. MINITAB software generated level combinations for all factors used in the assays. Then the sensor current was measured by controlled potential amperometric technique for each of these level combinations. This strategy had several advantages, such as a reduced number of experimental runs, more information obtained and biosensor delineation, in which the biosensor response permitted the optimal experimental conditions to be determined. It was possible to optimized concentration and incubation time for all biomolecules studied with this biosensor using the developed methodology. We also demonstrated the applicability of full factorial and fractional factorial designs to the immobilization of DNA molecules at a gold electrode built using a recordable compact disc (CDtrode) [88].

For DNA immobilization on electrode surfaces, the optimization of many parameters is necessary, such as: biomolecules concentration and incubation time. In this way, the biosensor for HCV, illustrated in Figure 5, was developed using chemometric experiments applied to steps 4-6 (Figure 5). The evaluated variables were the degree of dilution and incubation time of DNA probes for HCV-1, dilution and incubation time of complementary DNA, and concentration and incubation time of conjugate avidin-HRP, which was the label for hybridization accompanied by amperometry measurements. After establishment of all optimized parameters for biomolecule immobilization, the amperometric genosensor was applied to HCV-1 DNA detection in different HCV-infected patients, which had been previously analyzed by the standard qualitative Amplicor hepatitis C Virus Test. The results showed that the current intensities for the positive samples were higher than those for the negative samples. The factorial design procedure enables identification of critical parameters, while knowledge of the chemistry involved enables further refinement of the technique, where

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necessary. Full and fractional factorial design methods were employed for the optimization of a biosensor for hepatitis C diagnosis, and could be extended to other types of DNA-based biosensors.

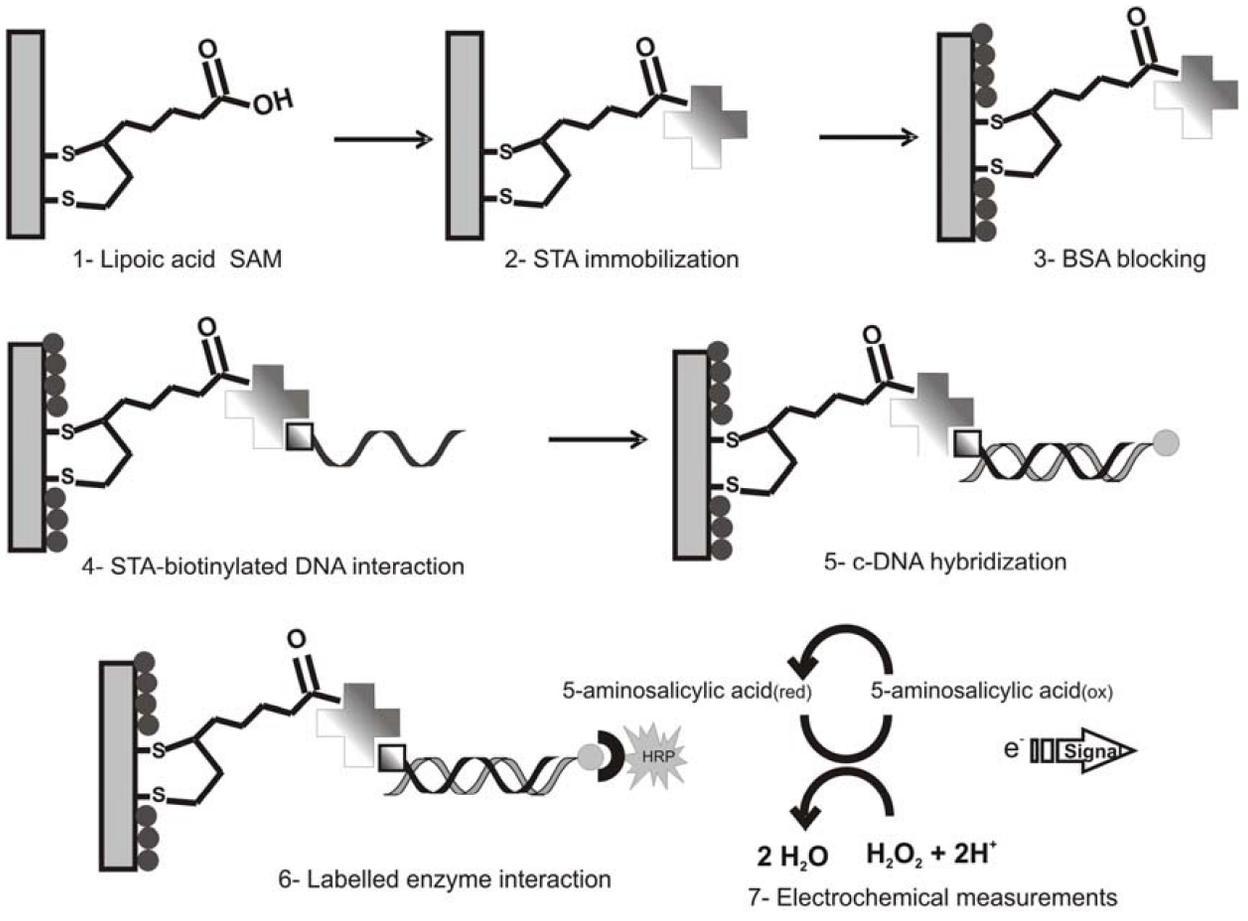

Figure 5. Scheme of DNA biosensor construction with gold CDtrodes [88].

According to the literature, biosensors rank fourth among the techniques used for the detection and classification of pathogens, behind the polymerase chain reaction (PCR), culture and colony counting and ELISA methods [89]. The reason for that is DNA biosensors offer several advantages, such as the ability to analyze complex fluids, high sensitivity, compatibility with compact instrumentation technology and portability, becoming a good alternative for application in clinical chemical analysis.

4. Enzyme based biosensor

Enzymes play a critical role in the metabolic activities of all living organisms and are widely applied in biotechnology. Abnormality of the enzyme metabolism systems leads to a number of metabolic diseases [90]. Diseases associated with components of the enzyme metabo-

lism or with the enzyme activities are broadly applied in clinical examinations as special markers as some examples displayed on Table 3.

Disease	Enzyme	Electrode / immobilization	Limit of detection	Reference
Diabetes mellitus	glucose oxidase	Gold nanocomposite/poly(pyrrole propylic acid) Graphene/nafion Film	50 mmol L^{-1} 30 mmol L^{-1}	[91] [92]
Uremia	urease	Rhodium nanoparticles/acrylonitrile copolymer membrane Platinum and graphite composite/ urease covered with dialysis membrane	500 mmol L^{-1} ---	[93] [94]
Heart failure, Respiratory insufficiency, Metabolic Disorders	lactate oxidase	Carbon screen-printed/mesoporous silica Carbon screen-printed/polysulfone-carbon nanotubes	18.3 µmol L^{-1} 1.5 mmol L^{-1} 3.46 µmol L^{-1}	[95] [96]
Idiopathic urolithiasis, intestinal diseases	oxalate oxidase	Gold electrode/multi-walled carbon nanotube-gold nanoparticle composite Platinum/multi-walled carbon nanotubes-polyaniline composite film	1 µmol L^{-1} 3 µmol L^{-1}	[97] [98]
Muscle damage	creatinine amidohydrolase	Platinum/multi-walled carbon nanotube-polyaniline composite film Platinum/PbO_2 layer-polyurethane membrane	0.1 µmol L^{-1} 0.8 µmol L^{-1}	[99] [100]

Table 3. Amperometric biosensor for disease based on enzyme.

4.1. Biosensor for substrate determination

Cholesterol and its fatty acid ester are extremely important compounds for human beings since they are components of neural and brain cells and are precursors of other biological materials, such as bile acid and steroid hormones. However, high cholesterol accumulation in blood due to excessive ingestion results in fatal diseases, such as arteriosclerosis, cerebral thrombosis, myocardial infarction, coronary diseases and lipid metabolism dysfunction [101]. Brahim et al. [102] developed a rapid, two-step method for constructing cholesterol biosensors by entrapment of cholesterol oxidase within a composite poly(2-hydroxyethyl methacrylate) (p(HEMA))/polypyrrole (p(pyrrole)) membrane. The optimized cholesterol biosensor exhibited a linear response range from 500 µmol L^{-1} to 15 mmol L^{-1} and limit of detection 120 µmol L^{-1} toward cholesterol and was applied in the analysis of serum samples from hospitalized patients. A review on cholesterol biosensor is published by Arya [103].

Choline is used as a marker of cholinergic activity in brain tissue, especially in the field of clinic detection of neurodegenerative disorder diseases, such as Parkinson's and Alzheimer's diseases. Zhang et al. [104] presented an electrochemical approach for the detection of choline

based on prussian blue (PB) modified iron phosphate nanostructures (PB-$FePO_4$), being the amperometric choline biosensor developed by immobilizing the enzyme choline oxidase on the PB-$FePO_4$ nanostructures and monitoring the formation of H_2O_2. The biosensor exhibited a low limit of detection (0.4 ± 0.05 μmol L^{-1}) and a wide linear range (2 μmol L^{-1} to 3.2 mmol L^{-1}). López et al. [105] designed a choline amperometric biosensor using as biological component choline oxidase entrapped in polyacrylamide microgels. The working electrode was prepared by holding the enzyme loaded microgels on a platinum electrode by a dialysis membrane. Under optimal conditions the biosensor presented high sensitivity for choline with limit of detection 8 μmol L^{-1}, and the response linear range from 20 μmol L^{-1} to 0.2 mmol L^{-1}. On the other hand, Lenigk et al. proposed methodology for the clinical purpose of evaluating anti-Alzheimer medicine based on the inhibition of acetylcholinesterase [106].

Phenylketonuria is a disease characterized by not metabolizing phenylalanine resulting in brain damage and mental retardation in children. A carbon paste electrode composed by paraffin oil, NAD^+, phenyalanine dehydrogenase, uricase and electron mediator was proposed [107] for aminoacid determination in urine sample. The reagentless biosensor presented a limit of detection 0.5 mmol L^{-1}.

Among biosensors for substrate determination, the most investigated and more successful on the commercial point of view is for glucose determination; probably because the diabetes mellitus is a world health problem, but also due to the stability of glucose oxidase (GOX).

The stability of enzymatic biosensors is important for the success of these devices as analytical instruments, and it is mainly dependent on the lifetime, or the rate of denaturation or inactivation of the immobilized enzyme [95]. Depending on the conditions of storage, temperature and method of immobilization, the enzyme can retain the activity from days to months [91-100], and is often one of the most important factors to take into account for the commercial viability of such device.

4.2. Biosensor for enzyme activities determination

Abnormal enzymes concentration can be related to diseases as shown.

Trypsin and trypsinogen levels are increased with pancreatitis disease like acute pancreatitis, cystic fibroses. Radioimmunoassay tests estimated 248 ± 94,9; 1100 ± 548 and 1399 ± 618 μg L^{-1} for healthy, chronic renal failure and acute pancreatitis, respectively. Ionescu et al. proposed a biosensor based on the suppression of GOX by steric hindrance due to a gelatin membrane and its reactivation by trypsin digestion of blocking membrane: the GOX was previously mixed with pyrrole and adsorved onto platinum electrode after that the enzyme was entrapped into the polypyrrole film by electropolimerization at +0.8 V / Ag|AgCl| KCl_{sat}. LOD was 42 pmol L^{-1} and response time 10 min [108].

Aspartate aminotranferase is an enzyme to diagnose acute myocardial infarction [109]. A biosensor based on Os-HRP layer and a layer composed by hydroxiethylcellulose, microcrystalline cellulose, aspartic acid, cetoglutaric acid and pyridoxil onto the gold electrode was proposed by Guo, et al. [110]. The LOD was 10 U L^{-1}, shelf stability 2 months, response time 120 s.

Adenosine deaminase (ADA) level is a biomarker for liver disease. A printed Ir/C was modified by xanthine oxidase and purine nucleoside phosphorylase; through the H_2O_2 measurement at potential of +0.27 V / Ag|AgCl the ADA activities in blood sample were determined. Linear calibration curve from 0 to 36 U L^{-1} was obtained, which is suitable for discriminating a healthy individual from a person suffering of liver disease, 18 and 31.6 U L^{-1}, respectively [111].

Reviews on age-related disease [112], clinical chemistry [113], cancer clinical testing [114], technology of commercial glucose monitoring [115] and glucose biosensor based on carbon nanomaterials [116] have been recently published.

5. Concluding remarks

Two aspects are very important to consider in biosensor development: the biological component determines the selectivity while the transducer determines the sensitivity. To guarantee the maximum selectivity, the active center of a biological molecule must be chemically and/or physically accessible and as freer as possible of steric effects. The surface preparation and modification of the transducer need to be thought mainly to reach this goal. In this case, the affinity reaction between different molecules such as antigen/antibody or DNA/DNA or enzymatic catalytic reaction can be used for quantification of biological substances which are important for the medicine and clinical analysis. The tendency is to produce more and more sophisticated and specific surface transducers using surface engineering and nanotechnological tools to get the best biosensor device. If this happens, health workers will believe more in this bioanalytical methodology and they may get benefits from it in the instant of giving to the patient an unequivocal diagnostic of disease.

Acknowledgment

The authors thank to FAPESP (Proc. 2008/08990-1, 2011/10707-9, 2008/07729-8, 2010/04663-6), CNPq (Proc. 305890/2010-7, 313307/2009-1), CAPES and PROPe-UNESP for financial support.

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References

[1] Thevenot D.R.; Toth K.; Durst R.A.; Wilson G.S. Electrochemical biosensors: recommended definitions and classifications. Pure and Applied Chemistry 1999; 12, 2333-2348.

[2] Cammann K. Bio-sensors based on ion-selective electrodes, Fresenius Zeitschrift Analytical Chemistry 1977; 287, 1-9.

[3] Ferreira A.A.P.; Fugivara C.S.; Barrozo S.; Suegama P.H.; Yamanaka H.; Benedetti A.V.; Electrochemical and spectroscopic characterization of screen-printed gold based electrodes modified with self-assembled monolayers and Tc85 protein. Journal of Electroanalytical Chemistry 2009; 634, 111-122.

[4] Noel M.; Vasu K.I. Cyclic Votammetry and the Frontiers of Electrochemistry, Aspect Publications Ltd., 1990.

[5] Gosser Jr. D.K. Cylic Voltammetry - Simulation and Analysis of Reaction Mechanisms, VCH Publishers, 1993.

[6] Compton R.G., Banks C.E. Undestanding Voltammetry, Word Scientific ed., London, 2009.

[7] Barsoukov E., Macdonald J.R. Impedance spectroscopy theory, experiment, and applications, John Wiley & Sons, USA, 2005.

[8] Orazem M. E.; Tribollet B. Electrochemical impedance spectroscopy. John Wiley & Sons, Inc., Hoboken, N. J., 2008.

[9] Ferreira A.A.P.; Fugivara C.S.; Yamanaka H.; Benedetti A.V. Preparation and characterization of immunosensors for disease diagnosis. In: Serra A.P. (ed). Biosensors for Health, Environment and Biosecurity. Rijeka: In Tech; 2011. p.183-214.

[10] Ferreira A.A.P.; Alves M.J.M.; Barrozo S.; Yamanaka H.; Benedetti A.V. Optimization of incubation time of protein Tc85 in the construction of biosensor: Is the EIS a good tool? Journal of Electroanalytical Chemistry 2010; 643, 1-8.

[11] Bagotsky V.S., Fundamentals of Electrochemistry, 2nd edition, John Wiley & Sons. Inc., NJ, 2006.

[12] Wightman R.M.; Wipf D.O. In: Bard A.J. (ed.). Electroanalytical Chemistry, vol. 15, Marcel Dekker, New York, 1989.

[13] Westbroek P.; Priniotakis G.; Kiekens P. Analytical electrochemistry in textiles, CRC Press, NY, 2005.

[14] Bard A.J.; Faulkner L.R. Electrochemical Methods – Fundamentals and Applications, 2nd edition, John Wiley & Sons. Inc., NY, 2001.

[15] Macdonald D.D. Transient Techniques in Electrochemistry, 1st edition, NY, 1977.

[16] Fleishmann M.; Pons S.; Robson D.; Schmidt P.P. Ultramicroelectrodes. Datatech Science Morganton, N. C., 1987.

[17] Wightman R.M.; Wipf D.O. Voltammetry at Ultramicroelectrodes In: Bard A.J. (ed.). Electroanalytical Chemistry,15., New York: Marcel, 1989.

[18] Zhuo, Y.; Yuan, R.; Chai, Y.; Zhang, Y.; Li, X. I.; Zhu, Q.; Wang, N. An amperometric immunosensor based on immobilization of hepatitis B surface antibody on gold electrode modified gold nanoparticles and horseradish peroxidase. Analytica Chimica Acta 2005; 548, 205-210.

[19] Kheiri, F.; Sabzi, R.E.; Jannatdoust, E.; Shojaeefar, E.; Sedghi, H. A novel amperometric immunosensor based on acetone-extracted propolis for the detection of the HIV-1 p24 antigen. Biosensors and Bioelectronics 2011; 26, 4457-4463.

[20] Rosales-Rivera, L.C.; Acero-Sánchez, J.L.; Lozano-Sánchez, P.; Katakis, I.; O´Sullivan, C.K. Electrochemical immunosensor detection of antigliadin antibodies from real human serum. Biosensors and Bioelectronics 2011; 26, 4471-4476.

[21] Laboria, N.; Fragoso, A.; Kemmner, W.; Latta, D.; Nilsson, O.; Botero, M. L.; Drese, K.; O'Sullivan, C. K. Carcinoembryonic antigen in colon cancer samples based on monolayers of dentritic bipodal scaffolds. Analytical Chemistry 2012; 82, 1712-1719.

[22] Villa, M. G.; Jiménez-Jorquera, C.; Haro, I.; Gomara, M. J.; Sanmartí, R.; Fernández-Sánchez, C.; Mendoza, E. Carbon nanotube composite peptide-based biosensors as putative diagnostic tools for rheumatoid arthritis. Biosensors and Bioelectronics 2011; 27, 113-118.

[23] Ferreira, A.A.P.; Colli, W.; Alves, M.J.M.; Oliveira, D.R.; Costa, P.I.; Güell, A.G.; Sanz, F.; Benedetti, A.V.; Yamanaka, H. Investigation of the interaction between Tc85-11 protein and antibody anti-T. cruzi by AFM and amperometric measurements. Electrochimica Acta 2006; 51, 5046-5052.

[24] Angnes L.; Richter E.M.; Augelli M.A.; Kume G.H. Gold electrodes from recordable CDs. Analytical Chemistry 2000; 72, 5503-5506.

[25] Richter E.M.; Augelli M.A.; Kume G.H.; Mioshi R.N.; Angnes L. Gold electrodes from recordable CDs for mercury quantification by flow injection analysis. Fresenius Journal Analytical Chemistry 2000; 366, 444-448.

[26] Lowinsohn D.; Richter M.A.; Angnes L.; Bertotti M. Disposable gold electrodes with reproducible area using recordable CDs and toner masks. Electroanalysis 2006; 18, 89-94.

[27] Foguel, M.V.; Santos, G.P.; Ferreira, A.A.P.; Yamanaka, H.; Benedetti, A.V. Amperometric immunosensor for Chagas'disease using gold CD-R transducer. Electroanalysis 2011; 23, 2555-2561.

[28] Foguel M.V.; Uliana C.V.; Tomaz P.R.U.; Marques P.R.B.O.; Yamanaka H.; Ferreira A. A. P. Avaliação da limpeza de CDtrodo construídos a partir de CD de ouro gravável/fita adesiva de galvanoplastia. Eclética Química 2009; 34, 59-66.

[29] Foguel M.V. Master Dissertation, Instituto de Química-UNESP, 2010.

[30] Kaláb T.; Skládal P. Disposable amperometric immunosensor for 2,4-dichlorophenoxyacetic acid. Analytical Chimica Acta 1995; 304, 361-368.

[31] Navrátilová I.; Skaládal P. The immunosensors for measurement of 2,4-dichlorophenoxyacetic acid based on electrochemical impedance spectroscopy. Bioelectrochemistry 2004; 62, 11-18.

[32] Carpini G.; Lucarelli F.; Marrazza G.; Mascini M. Oligonucleotide-modified screen-printed gold electrodes for enzyme-amplified sensing of nucleic acids. Biosensors and Bioelectronics 2004; 20, 167-175.

[33] García-González R.; Fernández-Abedul M.T.; Pernía A.; Costa-García A. Electrochemical characterization of different screen-printed gold electrodes. Electrochimica Acta 2008; 53, 3242-3249.

[34] Bóka B.; Adányi N.; Virág D.; Sebela M.; Kiss A. Spoilage detection with biogenic amine biosensors, comparison of different enzyme electrodes. Electroanalysis 2012; 24, 181-186.

[35] Qiu, J.D.; Huang, H.; Liang, R.P. Biocompatible and label-free amperometric immunosensor for hepatitis B surface antigen using a sensing film composed of poly (allylamine)-branched ferrocene and gold nanoparticles. Microchimica Acta 2011; 174, 97-105.

[36] Wang J. Analytical Electrochemistry, 2nd ed., Wiley VCH, NY, 2000.

[37] Cavalcanti, I.T.; Silva, B.V.M.; Peres, N.G.; Moura, P.; Sotomayor, M.I.F. G.; Dutra, R. F. (2012). A disposable chitosan-modified carbon fiber electrode for dengue virus envelope protein detection. Talanta 2012; 91, 41-46.

[38] González, M.D., García, M.B.G., García, A.C. Detection of pneumolysin in human urine using an immunosensor on screen-printed carbon electrodes. Sensors and Actuators B 2006; 113, 1005-1011.

[39] Castilho, M.S.; Laube, T.; Yamanaka, H.; Alegret, S.; Pividori, M.I. Magneto immunoassays for plasmodium falciparum histidine-rich protein 2 related to malaria based on magnetic nanoparticles. Analytical Chemistry 2011; 83, 5570-5577.

[40] Honglan, Q., Chen, L., Qingyun, M., Qiang, G., Chengxiao, Z. Sensitive electrochemical immunosensor array for the simultaneous detection of multiple tumor markers. Analyst 2012; 137, 393-399.

[41] Pacios M.; Martín-Fernández I.; Villa R.; Godignon P.; Del Valle M.; Bartrolí J.; Esplandiu M.J. Carbon nanotubes as suitable electrochemical platforms for metallopro-

tein sensors and genosensors. In: Naraghi M. (ed). Carbon Nanotubes – Growth and Applications, Rijeka: InTech; 2011. p. 299-324.

[42] Sharma, M.K.; Rao, V.K.; Agarwal, G.S.; Rai, G.P.; Gopalan, N.; Prakash, S.; Sharma, S.K.; Vijayaraghavan, R. Highly sensitive amperometric immunosensor for detection of Plasmodium falciparum histidine-rich protein 2 in serum of humans with malaria: comparison with a commercial kit. Journal of Clinical Microbiology 2008; 46, 3759-3765.

[43] Luppa, P.B.; Sokoll, L.J.; Chan, D.W. Immunosensors – principles and applications to clinical chemistry. Clinica Chimica Acta 2001; 314, 1-26.

[44] Mello, L.D., Kubota, L.T. Review of the use of biosensors as analytical tools in the food and drink industries. Food Chemistry 2002; 77, 237-256.

[45] Kim, J.H.; Yeo, W.H.; Shu. Z.; Soelberg, S.D.; Inoue, S.; Kalyanasundaram, D.; Ludwig. J.; Furlong. C.E.; Riley, J.J.; Weigel, K.M.; Cangelosi, G.A.; Oh, K.; Lee, K.H.; Gao, D.; Chung, J.H. (2012). Immunosensor towards low-cost, rapid diagnosis of tuberculosis. Lab on a Chip. 2012; 12, 1437-40.

[46] Ramírez, N.B.; Salgado, A.M.; Valdman, B. The evolution and developments of immunosensors for health and environmental monitoring: problems and perspectives. Brazilian Journal of Chemical Engineering 2009; 26, 227-249.

[47] Pan, A.N., Y.; Sonn, G.A; Sin, M.L.Y.; Mach, K.E.; Shih, M-C.; Gau, V.; Wong, P.K.; Liao, J.C. Electrochemical immunosensor detection of urinary lactoferrin in clinical samples for urinary tract infection diagnosis. Biosensors and Bioelectronics 2010; 26, 649-654.

[48] Noedl, H.; Yingyuen, K.; Laoboonchai, A.; Fukuda, M.; Sirichaisinthop, J.; Miller, R.S. (2006). Sensitivity and specificity of an antigen detection ELISA for malaria diagnosis. American Journal of Tropical Medicine and Hygiene 2006; 75, 1205-1208.

[49] World Health Organization (WHO). Chagas disease (American trypanosomiasis). WHO Fact Sheet 2010; 340.

[50] Brener, Z.; Andrade, Z. A.; Barral-Netto, M. Trypanosoma cruzi e doença de Chagas. Guanabara Koogan, Rio de Janeiro, 2000.

[51] Ferreira, A.A.P.; Colli, W.; Costa, P.I.; Yamanaka, H. Immunosensor for the diagnosis of Chagas' disease. Biosensors and Bioelectronics 2005; 21, 175-181.

[52] Belluzo, M.S; Ribone, M.E.; Camussone, C.; Marcipar, I.S.; Lagier, C. M. Favorably orienting recombinant proteins to develop amperometric biosensors to diagnose Chagas' disease. Analytical Chemistry 2011; 408, 86-94.

[53] Yang, M.; Msgovern, M.V.; Thompson, M. Genosensor technology and the detention of interfacial nucleic acid chemistry. Analytica Chimica Acta 1997; 346, 259-275.

[54] Garcia, T.; Fernandez-Barrena, M.G.; Revenga-Parra, M.; Nunez, A.; Casero, E.; Pariente, F.; Prieto, J.; Lorenzo, E. Disposable sensors for rapid screening of mutated genes. Analytical and Bioanalytical Chemistry 2010; 39, 1385-139.

[55] Fojta, M. Detecting DNA damage with electrodes. Perspectives in Bioanalysis 2005; 1, 385-431.

[56] Prieto-Simón, B.; Cortina, M.; Campás, M.; Calas-Blanchard, C. Electrochemical biosensors as a tool for antioxidant capacity assessment. Sensors and Actuators B 2008; 129, 459-466.

[57] Teles, F.R.R.; Fonseca, L.P. Trends in DNA biosensors. Talanta 2008; 77, 606-623.

[58] Fojta, M. Electrochemical sensors for DNA interactions and damage. Electroanalysis 2002; 14, 1494-1500.

[59] Labuda, J.; Brett, A.M.O.; Evtugyn, G.; Fojta, M.; Mascini, M.; Ozsoz, M.; Palchetti, I.; Paleček, E.; Wang, J. Electrochemical nucleic acid-based biosensors: concepts, terms, and methodology (IUPAC Technical Report). Pure and Applied Chemistry 2010; 82, 1161-1187.

[60] Belluzo, M.S.; Ribone, M.E.; Lagier, C.M. Assembling amperometric biosensors for clinical diagnostics. Sensors 2008; 8, 1366-1399.

[61] Kerman, K.; Kobayashi, M.; Tamiya, E. Recent trends in electrochemical DNA biosensor technology. Measurement Science and Technology 2004; 15, R1.

[62] Wang, J.; Rivas, G.; Fernandes, J.R.; Paz, J.L.L.; Jiang, M.; Waymire, R. Indicator-free electrochemical DNA hybridization biosensor. Analytica Chimica Acta 1998; 375, 197-203.

[63] Gao, Z.; Tansil, N. A DNA biosensor based on the electrocatalytic oxidation of amine by a threading intercalator. Analytica Chimica Acta 2009; 636, 77-82.

[64] Sassolas, A.; Leca-Bouvier, B.D.; Blum, L.J. DNA Biosensors and Microarrays. Chemical Reviews 2008; 108, 109-139.

[65] Civit, L.; Fragoso, A.; O'Sullivan, C.K. Electrochemical biosensor for the multiplexed detection of human papillomavirus genes. Biosensors and Bioelectronics 2012; 26, 1684-1687.

[66] Civit, L.; Fragoso, F.; Hölters, S.; Dürst, M.; O'Sullivan, C.K. Electrochemical genosensor array for the simultaneous detection of multiple high-risk human papilloma virus sequences in clinical samples. Analytica Chimica Acta 2012; 715, 93-98.

[67] Ionescu, R. E.; Herrmann, S.; Cosnier, S.; Marks, R. S. A polypyrrole cDNA electrode for the amperometric detection of the West Nile Virus. Electrochemistry Communications 2006; 8, 1741-1748.

[68] Lin, L.; Liu, Q.; Wang, L.; Liu, A.; Weng, S.; Lei, Y.; Chen, W.; Lin, X.; Chen, Y. Enzyme-amplified electrochemical biosensor for detection of PML–RARαfusion gene based on hairpin LNA probe. Biosensors and Bioelectronics 2011; 28, 277-283.

[69] Wang, K.; Sun, Z.; Feng, M.; Liu, A.; Yang, S.; Chen, Y.; Lin, X. Design of a sandwich mode amperometric biosensor for detection of PML/RARαfusion gene using locked nucleic acids on gold electrode. Biosensors and Bioelectronics 2011; 26, 2870-2876.

[70] World Health Organization. WHO: Hepatitis C. <http://www.who.int/mediacentre/factsheets/fs164/en/>. (accessed 4 June 2012).

[71] Skládal, P.; Riccardi, C.S.; Yamanaka, H.; Costa, P.I. Piezoelectric biosensors for real-time monitoring of hybridization and detection of Hepatitis C virus. Journal of Virological Methods 2004; 117, 145-151.

[72] Wang, Z.; Yang, Y.; Leng, K.; Li, J.; Zheng, F.; Shen, G.; Yu, R. A sequence-selective electrochemical DNA biosensor based on HRP-labeled probe for colorectal cancer DNA detection. Analytical Letters 2008; 41 24-35.

[73] Ortiz, M.; Torréns, M.; Canela, N.; Fragoso, A.; O'Sullivan, C.K. Supramolecular confinement of polymeric electron transfer mediator on gold surface for picomolar detection of DNA. Soft Matter 2011; 7, 10925-10930.

[74] Liu, C.; Zeng, G. M.; Tang, L.; Zhang, Y.; Li, Y. P.; Liu, Y. Y.; Li, Z.; Wu, M.S.; Luo, J. Electrochemical detection of Pseudomonas aeruginosa 16S rRNA using a biosensor based on immobilized stem-loop structured probe. Enzyme and Microbial Technology 2011; 49 266- 71.

[75] Liao, J.C.; Mastali, M.; Gau, V.; Suchard, M.A.; Møller, A.K.; Bruckner, D.A.; Babbitt, J. T.; LI, Y.; Gornbein, J.; Landaw, E. M.; Mccabe, E. R. B.; Churchill, B. M.; Haake, D. A. Use of electrochemical DNA biosensors for rapid molecular identification of uropathogens in clinical urine specimens. Journal of Clinical Microbiology 2006; 44, 561-570.

[76] Elsholz, B.; Worl, R.; Blohm, L.; Albers, J.; Feucht, H.; Grunwald, T.; Jurgen, B.; Schweder, T.; Hintsche, R. Automated detection and quantitation of bacterial RNA by using electrical microarrays. Analytical Chemistry 2006; 78, 4794-4802.

[77] Mach, K. E.; Du, C. B.; Phull, H.; Haake, D. A.; Shih, M. C.; Baron, E. J.; Liao, J. C. Multiplex pathogen identification for polymicrobial urinary tract infections using biosensor technology: A Prospective Clinical Study. The Journal of Urology 2009; 182, 2735-2741.

[78] Liao, J.C.; Mastali, M.; Li, Y.; Gau, V.; Suchard, M. A.; Babbitt, J.; Gornbein, J.; Landaw, E.M.; Mccabe, E.R.B.; Churchill, B.M.; Haake, D.A. Development of an advanced electrochemical DNA biosensor for bacterial pathogen detection. Journal of Molecular Diagnostics 2007; 9, 158-168.

[79] Li, K.; Lai, Y.; Zhang, W.; Jin, L. Fe2O3@Au core/shell nanoparticle-based electrochemical DNA biosensor for Escherichia coli detection. Talanta 2011; 84 607-613.

[80] Loaiza, O.A.; Campuzano, S.; Pedrero, M.; García P.; Pingarrón, J.M. Ultrasensitive detection of coliforms by means of direct asymmetric PCR combined with disposable magnetic amperometric genosensors. Analyst 2009; 134, 34-37.

[81] Gau, J.J.; Lan, E.H.; Dunn, B.; Ho, C.M.; Woo, J.C.S. A MEMS based amperometric detector for E. Coli bacteria using self-assembled monolayers. Biosensors and Bioelectronics 2001; 16, 745-755.

[82] Pividori, M.I.; Merkoçi, A.; Alegret, S. Dot-blot amperometric genosensor for detecting a novel determinant of b-lactamase resistance in Staphylococcus aureus. Analyst 2001; 126, 1551-1557.

[83] Loaiza, O.A.; Campuzano, S.; Pedrero, M.; Pividori, M.I.; García, P.; Pingarrón, J.M. Disposable magnetic DNA sensors for the determination at the attomolar level of a specific Enterobacteriaceae family gene. Analytical Chemistry 2008; 80, 8239-8245.

[84] Campuzano, S.; Pedrero, M.; García, J.L.; García, E.; García, P.; Pingarrón, J.M. Development of amperometric magnetogenosensors coupled to asymmetric PCR for the specific detection of Streptococcus pneumonia. Analytical and Bioanalytical Chemistry 2011; 399, 2413-2420.

[85] Riccardi, C.S.; Kranz, C.; Kowalik, J.; Yamanaka, H.; Mizaikoff, B.; Josowicz, M. Label-free DNA detection of Hepatitis C virus based on modified conducting polypyrrole films at microelectrodes and atomic force microscopy tip-integrated electrodes. Analytical Chemistry 2008; 80, 237-245.

[86] Riccardi, C.S.; Dahmouche, K.; Santilli, C.V.; Costa, P.I.; Yamanaka, H. Immobilization of streptavidin in sol-gel films: application on the diagnosis of Hepatitis C virus. Talanta 2006; 70, 637-643.

[87] Uliana, C.V.; Riccardi, C.S.; Tognolli, J.O.; Yamanaka, H. Optimization of an amperometric biosensor for detection of Hepatitis C virus using fractional factorial designs. Journal of the Brazilian Chemical Society 2008; 19, 782-787.

[88] Uliana, C.V.; Tognolli, J.O.; Yamanaka, H. Application of factorial design experiments to the development of a disposable amperometric DNA biosensor. Electroanalysis 2011; 23, 2607-2615.

[89] Lazcka, O.; Campo, F.J.; Muñoz, F.X. Pathogen detection: A perspective of traditional methods and biosensors. Biosensors and Bioelectronics 2007; 2, 1205-1217.

[90] Raja, M.M.M.; Raja, A.; Imran, M.M.; Santha, A.M.I.; Devasena, K. Enzymes application in diagnostic prospects. Biotechnology 2011; 10, 51-59.

[91] Senel, M.; Nergiz, C. Novel amperometric glucose biosensor based on covalent immobilization of glucose oxidase on poly(pyrrole propylic acid)/Au nanocomposite. Current Applied Physics 2012; 12, 1118-1124.

[92] Zhang, Y.; Fan, Y.; Wang, S.; Tan, Y.; Shen, X.; Shi, Z. Facile Fabrication of a graphene-based electrochemical biosensor for glucose detection. Chinese Journal of Chemistry 2012, 30, 1163-1167.

[93] Gabrovska, K.; Ivanov, J.; Vasileva, I.; Dimova, N.; Godjevargova, T. Immobilization of urease on nanostructured polymer membrane and preparation of urea amperometric biosensor. International Journal of Biological Macromolecules 2011; 48, 620-626.

[94] Pizzariello, A.; Stredansky, M.; Stradanska, S.; Miertus, S. Urea biosensor based on amperometric pH-sensing with hematein as a pH-sensitive redox mediator. Talanta 2001; 54, 763-772.

[95] Shimomura, T.; Sumiya, T.; Ono, M.; Ito, T.; Hanaoka, T. Amperometric L-lactate biosensor based on screen-printed carbon electrode containing cobalt phthalocyanine, coated with lactate oxidase-mesoporous silica conjugate layer. Analytica Chimica Acta 2012; 714, 114-120.

[96] Perez, S.; Sanchez, S.; Fábregas, E. Enzymatic strategies to construct L-lactate biosensors based on polysulfone/carbon nanotubes membranes. Electroanalysis 2012; 24, 967-974.

[97] Pundir, C.S.; Chauhan, N.; Rajneesh; Verma, M.; Ravi. A novel amperometric biosensor for oxalate determination using multi-walled carbon nanotube-gold nanoparticle composite. Sensors and Actuators, B 2011; 155, 796-803.

[98] Yadav, S.; Devi, R.; Kumari, S.; Yadav, S.; Pundir, C.S. An amperometric oxalate biosensor based on sorghum oxalate oxidase bound carboxylated multiwalled carbon nanotubes-polyaniline composite film. Journal of Biotechnology 2011; 151, 212-217.

[99] Yadav, S.; Kumar, A.; Pundir, C.S. Amperometric creatinine biosensor based on covalently coimmobilized enzymes onto carboxylated multiwalled carbon nanotubes/polyaniline composite film. Analytical Biochemistry 2011; 419, 277-283.

[100] Shin, J.H.; Choi, Y.S.; Lee, H.J.; Choi, S.H.; Ha,J.; Yoon, I.J.; Nam, H.; Cha, G.S.A planar amperometric creatinine biosensor employing an insoluble oxidizing agent for removing redox-active interferences. Analytical Chemistry 2011; 73, 5965-5971.

[101] Li, X-R.; Xu, J-J.; Chen, H-Y. Potassium-doped carbon nanotubes toward the direct electrochemistry of cholesterol oxidase and its application in highly sensitive cholesterol biosensor. Electrochimica Acta 2011; 56, 9378-9385.

[102] Brahim, S.; Narinesingh, D.; Guiseppi-Elie, A. Amperometric determination of cholesterol in serum using a biosensor of cholesterol oxidase contained within a polypyrrole–hydrogel membrane. Analytica Chimica Acta 2001; 448, 27-36.

[103] Arya, S.A., Datta, M. Malhotra, B.D. Recent advances in cholesterol biosensor. Biosensors and Bioelectronics 2008; 23, 1083-1100.

[104] Zhang, H.; Yin, Y.; Wu, P.; Cai, C. Indirect electrocatalytic determination of choline by monitoring hydrogen peroxide at the choline oxidase-prussian blue modified iron phosphate nanostructures. Biosensors and Bioelectronics 2012; 31, 244-250.

[105] López, M. S-P.; Pérez, J.P.H.; Lopez-Cabarcos, E.; Lopez-Ruiz, B. Amperometric biosensors based on choline oxidase entrapped in polyacrylamide microgels. Electroanalysis 2007; 19, 370-378.

[106] Lenigk, R.;Lam, E.; Lai, A.; Wang, H.; Han, Y.; Carlier, P.; Rennenberg, R. Enzyme biosensor for studying therapeutics of Alzheimer's disease. Biosensors and Bioelectronics 2000; 15, 541-547.

[107] Weiss, D.J.; Dorris, M.; Loh, A.; Peterson, L. Dehydrogenase based reagentless biosensor for monitoring phenylketonuria. Biosensors and Bioelectronics 2007; 22, 2436-41.

[108] Ionescu, R.E.; Cosnier, S.; Marks, R.S. Protease amperometric sensor. Analytical Chemistry 2006; 78, 6327-31.

[109] Yamanaka, H., Laluce, C., Oliveira Neto, G. Assay of serum glutamic-oxaloacetic transaminase using malate dehydrogenase preparation obtained from S. aureofaciens. Analytical Letters 1995; 28, 2305-2316.

[110] Guo, Z.; Yang, Q.; Liu, H.; Liu, C.; Cai, X. The study of a disposable reagentless biosensor for fast test of aspartate aminotransferase. Electroanalysis 2008; 20, 1135-41.

[111] Bartling, B.; Li, L.; Liu, C. Detection of adenosine deaminase activity with a thick-film screen-printed Ir/C sensor to detect liver disease. Journal of the Electrochemical Society 2010; 157, 130-J134.

[112] He, Y.; Wu, Y.; Mishra, A.; Acha, V.; Andrews, T.; Hornsby, P.J. Biosensor Technology in aging research and age-related diseases. Ageing Research Reviews 2012; 11, 1-9.

[113] D'Orazio, P. Biosensors in clinical chemistry - 2011 update. Clinica Chimica Acta 2011; 412, 1749-1761.

[114] Rasooly, A.; Jacobson, J. Development of biosensors for cancer clinical testing. Biosensors and Bioelectronics 2006; 21, 1851-1858.

[115] Vashist, S.K.; Zheng, D.; Al-Rubeaan, K.; Luong, J.H.T.; Sheu, F. Technology behind commercial devices for blood glucose monitoring in diabetes management: A review. Analytica Chimica Acta 2011; 703, 124-136.

[116] Zhu, Z.; Garcia-Gancedo, L.; Flewitt, A.J.; Xie, H.; Francis Moussy, F.; Milne, W.I. A critical review of glucose biosensors based on carbon nanomaterials: carbon nanotubes and graphene. Sensors 2012; 12, 5996-6022.

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