USO DE LA BENZOQUINONA PARA LA DETERMINACIÓN DE MOLÉCULAS DE TIOL EN UNA SUPERFICIE DE ORO MODIFICADA

USE OF BENZOQUINONE FOR THE DETERMINATION OF THIOL MOLECULES ON A MODIFIED GOLD SURFACE

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RESUMEN

La 4-benzoquinona (BQ) se utiliza en la determinación electroquímica de moléculas de tiol, debido a la interacción del par redox hidroquinona/benzoquinona con los tioles. Este compuesto puede alterar las vías de señalización y afectar a la integridad cromosómica. Se han realizado numerosos intentos para comprender los mecanismos citotóxicos influenciados por la estructura química de la BQ que generan roturas del ADN y apoptosis. También es un fuerte veneno para la topoisomerasa II. Su toxicidad se debe principalmente al estrés oxidativo y/o a la formación de aductos tipo Michael con las proteínas y el glutatión (GSH). Debido a las importantes funciones fisiológicas de las quinonas, como la hidroquinona y la 1,4 benzoquinona, y de los tioles reducidos como el GSH, es necesario desarrollar métodos sensibles y selectivos para la medición de estas sustancias con el fin de estudiar los mecanismos celulares de la toxicidad inducida por sustancias químicas. Para ello, se estudió la modificación de la superficie del oro con 1-propanotiol (1-PT), 2-propeno-1-tiol (2-P-1-T) y 1,3-propanoditiol (1,3-PDT), dejando el grupo tiol (-SH) en la superficie. Después, se hace reaccionar el BQ con el tiol (-SH) de la superficie de oro modificada. La adición de nucleófilos de azufre a quinonas ha sido estudiada y caracterizada como adición 1,4-reductiva de tipo Michael. Las quinonas, que contienen un doble enlace polarizado, se consideran aceptores de Michael, donde los donantes de Michael son nucleófilos de tiol. El producto final de esta reacción es la hidroquinona con un átomo de azufre en posición orto. Si una superficie metálica se modifica con un ditiol (para formar un tiolato con la superficie metálica, dejando un tiol libre, -HS) molécula, la reacción con BQ será fácil para producir la hidroquinona (H2Q) moiety. Este último producto puede utilizarse para determinar electroquímicamente los compuestos que contienen tiol en su estructura.

Palabras clave: 2-propeno-1-tiol, 1,3-propaneditiol, 1,4-benzoquinona, hidroquinona, XPS.

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ABSTRACT

1,4-benzoquinone (BQ) is used in the electrochemical determination of thiol molecules because of the interaction of the hydroquinone/benzoquinone redox couple with thiols. This compound can alter signaling pathways and affect chromosomal integrity. Numerous attempts have been made to understand cytotoxic mechanisms influenced by the chemical structure of BQ generate DNA breaks and apoptosis. It is also a strong topoisomerase II poison. Its toxicity is mainly due to oxidative stress and/or forming Michael-type adducts with proteins and glutathione (GSH). Owing to the important physiological roles of quinones, such as hydroquinone, 1,4 benzoquinone, and reduced thiols such as GSH, it is necessary to develop sensitive and selective methods for the measurement of those substances to study cellular mechanisms of chemical-induced toxicity. For this purpose, was studied the modification of the gold surface with 1-propanethiol (1-PT), 2-propene-1-thiol (2-P-1-T) and 1,3-propanedithiol (1,3-PDT), leaving the thiol (-SH) group on the surface. After, it is putted to react the BQ with the thiol (-SH) from modified gold surface. The Addition of sulfur nucleophiles to quinones has been studied and characterized as 1,4-reductive addition of the Michael type. Quinones, containing a polarized double bond, are considered Michael acceptors, where the Michael donors are thiol nucleophiles. The final product of this reaction is hydroquinone with a sulfur atom in the ortho position. If a metal surface is modified with a dithiol (to form a thiolate with the metal surface, leaving a free thiol,-HS) molecule, the reaction with BQ will be easy to produce hydroquinone (H2Q) moiety. The latter product can be used to determine electrochemically thiol-containing compounds in its structure.

Keywords: 2-propene-1-thiol, 1,3-propanedithiol, 1,4-benzoquinone, hydroquinone, XPS.

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INTRODUCTION

Thiols play an important role in metabolic processes of all living cells and, take part in a wide variety of intracellular oxidation–reduction reactions[1,2]. These compounds are commonly known as mercaptans, a class of organic compounds containing sulphydryl groups (–SH) attached to a carbon atom[3]. Among mercaptans, glutathione (GSH), cysteine (Cys), homocysteine (Hcys), cysteinylglycine and γ-glutamylcysteine are the most important endogenous aminothiols in human biofluids[4]. The presence of N-acetylcysteine and thioglycolic acid in urine has been confirmed and these compounds are assumed to be endogenous constituents of human urine[5,6].

GSH, discovered in 1888, is a ubiquitous thiol-containing tripeptide, and the major component of cellular antioxidant defenses[7,8], protects cells from oxidative stress and nitrosative damage[9]. As a key modulator of cell functions has important roles in redox regulation of proteins and maintaining redox homeostasis that is critical for proper function of cellular processes, including apoptosis[10]. Its levels in human tissues normally range from 0.1 to 10 mM with high concentrations in liver (up to 10 mM) and in the spleen, kidney, lens, erythrocytes, and leukocytes[11]. The GSH system, present in both cytoplasm and mitochondria, provides most of the H_2O_2 removing capacity in majority of cells[12].

Under oxidative conditions GSH is oxidized to its disulfide (GSSG) and exported from cells when its concentration increases. The decrease in GSH and the concomitant decrease in the GSH/GSSG ratio have been suggested to be important contributing factors to some human diseases such as lung inflammation, amyotrophiclateralsclerosis, chronic renal failure, malignant disorders, diabetes, Parkinson's disease, Alzheimer's disease, catar act formation as well as to the ageing process[13,14,15].

In children with severe malnutrition, it has consistently been shown that the concentration of GSH in plasma and whole blood is lower than normal in those with edema but not in those without edema[16,17]. These results point out that a lower concentration of GSH is indicative of impaired antioxidant capacity[7].

Compared with GSH, Cys is a very abundant extracellular aminothiol[7] that is found in large amounts (0.5–10 mM) inside the cell[18]. This amino acid participates in several biochemical processes, and is involved in several important cellular functions, including protein synthesis, detoxification, and metabolism[19]. It is crucial to determine the concentrations of Cys in plasma and urine because elevated level of Cys in plasma is considered as a vascular disease risk factor[20-22] and is associated with neurotoxicity[23]. On the contrary, deficiency of Cys may result in some serious diseases such as hematopoiesis decrease, leucocyte loss, and psoriasis[24]. Moreover, altered level of Cys has been implicated in hyperhomocysteinemia[25], which has been linked to the increased risks of Alzheimer's disease[26], neural tube defect[27], and osteoporosis[28]. Hence, the detection of Cys continues to be of interest[29].

During the biosynthesis of the essential amino acid methionine to cysteine, homocysteine (Hcy) is formed as an intermediate, which is at the intersection of two main metabolic pathways: remethylation and trans-sulfuration, regulated by methylenetetrahydrofolate reductase and cystathionine beta-synthase[30]. Several studies have revealed that elevated levels of total homocysteine in plasma in fasting is associated with an increased risk for atherosclerotic and tromboembolic vascular disease[31,32]. One of the major mechanisms of Hcy toxicity seemed to be auto-oxidation of Hcy, which reduces disulfide to free thiol, followed by the metalindependent oxidation of the free thiol to generate oxidants such as superoxide and hydrogen peroxide[33].

Other thiols commonly used as drugs in the treatment of many diseases are Cysteamine, N-acetylcysteine, captopril, mesna, 2-mercaptopropionylglycine, D-penicillamine, thyreostats and thiopurines. However, severe adverse reactions to oral thiol-drugs use have been described in subjects in which

abrupt incremental dosing of the drugs was started[4].

The information mentioned above suggests that there is a need for the development of simple yet selective protocols for thiol determination to implement preventative actions before the manifestation of physical symptoms. The classical method currently used to quantify thiols is the Ellman's test, which is a spectrophotometric method based on an exchange reaction between the thiol and the disulfide DTNB (5,50-dithiobis-(2-nitrobenzoic acid)). The sulfide produced shows a characteristic yellow color. However, this test and more generally spectrophotometric methods require a pre-treatment when thiols must be assayed in colored samples and their results may be influenced by variable levels of specific enzyme activities such as glutathione S-transferase or g-glutamyltransferase[34].

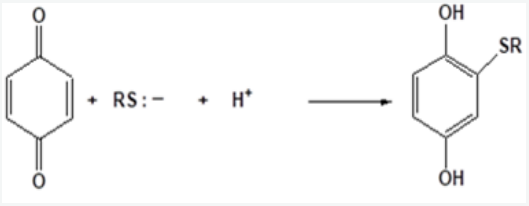
Other intensive studies have been evaluated to the detection of biological thiols, more particularly glutathione. Among them, several quinone systems have been investigated as potential indicators for reduced thiol species[35,36]. The addition of thiols to quinone moieties represents the basis of numerous spectroscopic[37,38] and pre-column chromatographic derivatization protocols[39,36].

A simple protocol has also been described to determine biological thiols by a colorimetric analysis based on the interaction of the magenta color with 5,5'-dithiobis(2-nitrobenzoic acid)[37]. A nanosensor was developed to discriminate cysteine (Cys) from homocysteine (Hcy) and glutathione (GSH) with multiple signals: colorimetric, photoluminescence (PL), and up-conversional photoluminescence (UCP)[38].

Was establish a electrochemical platform based on the Au–Se interface (Au–Se electrochemical platform) for high-fidelity biological detection. Compared with that of the electrochemical platform based on the Au–S interface (Au–S electrochemical platform), the Au–Se electrochemical platform shows a higher charge transfer rate and excellent stability in millimolar levels of GSH[39].

The analytical community has focused its attention on the redox properties of the aminothiols Hcy, Cys, and GSH3[5,42,43]. Electrochemical techniques hold much potential for the in vitro analysis of these aminothiols because such methods offer the advantage of minimal sample pretreatment, rapid analysis time, and simple experimental approach. Numerous electroanalytical strategies have been employed to aid the quantification of thiols and various merits and limitations of the approaches have been reviewed[44,42,34].

1,4-Benzoquinone (BQ) is also used in the electrochemical determination of thiol molecules because of the interaction of the hydroquinone/benzoquinone redox couple with thiols, thus benzoquinone behaves as an electron mediator for thiols detection in the electroanalytical approach[35,45-47]. The addition of sulfur nucleophiles to quinones has been studied and characterized as 1, 4-reductive addition of the Michael type (Eq. 1). Quinones, containing a polarized double bond, are considered Michael acceptors, where the Michael donors are thiol nucleophiles. The final product of this reaction is hydroquinone with a sulfur atom in the ortho position. If a metal surface is modified with a dithiol (to form a thiolate with the metal surface, leaving a free thiol,-HS) molecule, the reaction with BQ will be easy to monitor through the production of hydroquinone (H_2Q) moiety[44,45].



(1)

The product orientation in the addition of sulfur nucleophiles to quinones is determined by the substituent and the occurrence of the new sulfur substituent controls in large extent the subsequent chemistry.

The reduction potential of quinoid compounds is, among the intrinsic

properties of quinones, the most important factor affecting the rate of electron transfer between donor and acceptor. Moreover, the reduction potential represents the simplest control over free radical reactivity[48].

Because quinones are one- and two-electron redox species, following reduction potentials at pH 7 can be considered: the one-electron reduction potential [E(BQ/BQ•$^-$)], the two-electron reduction potential [E(BQ/BQ^{2-})], and the reduction potential of the intermediate step [E(BQ•-/BQ^{2-})]. The latter, E(BQ•-/BQ^{2-}), is not easily measured in protic media but it can be calculated according to the equation[49]:

$$E(BQ^{\bullet -}/BQ^{2-}) = 2\,E(BQ/BQ^{2-}) - E(BQ/BQ^{\bullet -})$$

The reduction potential of quinones is influenced by the substituents, dissociation constants, H^+ concentration, and solvent effects[50,51]. The redox potential of quinones is dependent on the H^+ concentration of the medium and the dissociation constants of the species. At pH 7, semiquinones, having pK values between 4 and 5, will be in their anionic form and participate readily in electron transfer reactions; the so-called stability of hydroquinones, however, will be partly because they are protonated at this pH, hence slowing down electron-transfer processes[52].The latter product can be used to determine electrochemically thiol-containing compounds in its structure[53,54]. Moreover, the gold electrodes have demonstrated to provide a suitable support

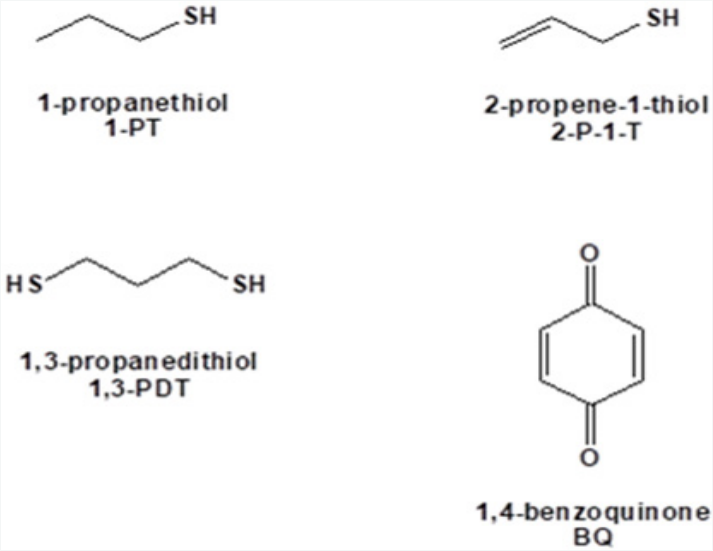


Figure 1-A. Structures of compounds used to modify gold surface.

for the thiol immobilization, the chemical modification of surface can be carried out with a very good reproducibility, suitable for the sensor development[55].

The electrochemical kinetics of the BQ/H_2Q redox couple in aqueous solutions has been the subject of numerous investigations[56-63]. In the electrochemical determination of thiols, by their ability to operate as biomarkers, BQ plays an important role in these studies. White et al.[64] studied different quinoid intermediates and their subsequent reaction with sulphydryl thiols (RSH), using glassy carbon as the working electrode. Others researcher has studied potentiometric response of the reaction of BQ and thiols[65-69].

Self-assembled monolayers (SAMs) are ordered molecular assemblies formed by the adsorption of active surfactants on a solid surface. This is a technique that provides an elegant route to the preparation of well-defined organic assemblies on solid surfaces70. The preparation of high-ordered monolayer by molecular self-assembly has been employed extensively as a surface derivatization procedure. This method has a wide use in the preparation of modified surfaces[70]. The advantages of SAMs include their ease of preparation, stability, and the

possibility of introducing different chemical functionalities. The incorporation of the appropriate chemical functionality with some molecular level control into the highly ordered monolayer allows the preparation of surfaces with tailor-made properties [100].

The formation of high order monolayers is often done by the spontaneous adsorption of n-alkanethiols or their derivatives to gold surfaces[101-115].

In our laboratory was studied the modification of the gold surface with 1-propanethiol (1-PT), 2-propene-1-thiol (2-P-1-T) and 1,3-propanedithiol (1,3-PDT), leaving the thiol (-SH) group on the surface (see fig 1-A and fig 1-B). After, it is putted to react the 1,4-benzoquinone (BQ) with the thiol (-SH) from surface, by Michael addition reaction, forming the hydroquinone (H_2Q) moiety. Here is presented results of the electrochemical behavior, using cyclic voltammetry, and a study of surface analysis, using X-ray photoelectron spectroscopy (XPS).

For this chapter is of great interest to contribute with new modifications of gold surfaces and their possible applications in the field of biomarkers.

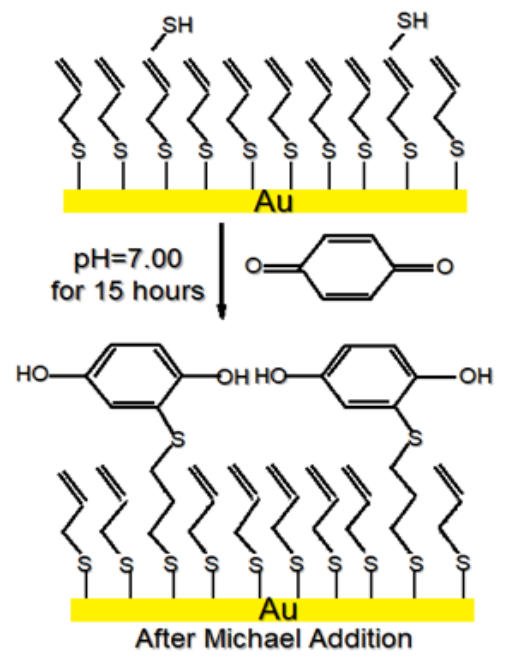


Figure 1-B. Scheme 1

ELECTROCHEMICAL AND XPS STUDIES OF GOLD SURFACE MODIFIED WITH BQ

Pre-treatment and substrates modification for electrochemical study.

The electrochemical cell employed was a conventional three-electrode cell using platinum gauze as a counter electrode. All potentials are reported with respect to Ag/AgCl reference electrode.

All substrates used were new and before their modification they were submitted to a cleaning treatment.

Electrode cleanness was verified by cycling the potential in a 1.0 M H_2SO_4 solution between hydrogen and oxygen regions until characteristic cyclic voltammogram (CV) were observed.

Was verified its cleanness by cyclic voltammetry before being chemically modified. Figure 2 shows a cyclic voltammogram of a clean gold electrode of 1.6 mm of diameter in 1.0 M H_2SO_4, at 100 mV/s scan rate. The current-potential behavior shown in this figure is characteristic of a clean Au surface in a clean test solution[106-109].

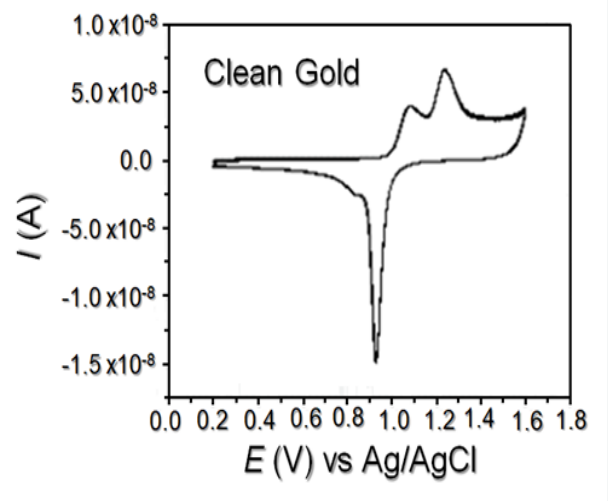


Figure 2. Cyclic voltammogram of clean gold surface in 1.0 M H_2SO_4 solution. Scan rate 100 mV s^{-1}. The solution was degassed with N_2 flow for least 30 min. Measurements were performed at 21 + 1 °C.

Self-assembly of 1-PT (or 2-P-1-T) and 1,3-PDT was done on Au surfaces using commercial

gold disk electrodes of 1.6 mm of diameter to carry out the electrochemical measurements. The previously cleaned substrates were submerged in a 3.0 mM 1-PT (or 2-P-1-T) ethanol solution for 30 min. afterward, the substrates were washed with ethanol, dried under nitrogen gas flow, and submerged in a 3.0 mM 1,3-PDT ethanol solution for 5 h.

The modified electrodes were immersed in a 2.01 mM 1,4-benzoquinone (BQ) solution, in Britton-Robinson buffer (acetic, boric, and phosphoric acids, each at a concentration of 0.04 M and adjusted to pH 7 through the addition of sodium hydroxide)[112], for 15 h. In this last solution, the BQ compound react through a nucleophilic 1,4-Michael addition to form the corresponding reduced thiol conjugate on gold surface (shown in scheme 1), forming the hydroquinone (H_2Q) moiety.

Finally, the electrochemical study of this latter system formed on the gold surface, is done in Britton-Robinson buffer solution without the presence of BQ in the solution.

Results of cyclic voltammetry studies.

The electrochemical characterization of the modified gold surfaces can reveal the presence of a full coverage of the electrode. Figure 3 present a cyclic voltammogram (CV) in 2.5 mM $K_3Fe(CN)_6$ in 0.1 M KCl solution: (a) for clean gold, (b) gold surface modified with 3.0 mM 2-P-1-T for 30 min and (c) gold surface modified with 3.0 mM 2-P-1-T for 30 min and 3.0 mM 1,3-PDT for 5 h (d), in alcoholic solution. Is observed that increasing the number of organic molecules on the gold surface the reduction and oxidation current of ferricyanide species become smaller, indicating a blockade of the surface.

Using small molecules containing a thiol (-SH) group facilitates the transfer of electrons from the metal surface to the active group that is on this surface. Furthermore, if these molecules are unsaturated with one double bond (C=C), promotes electron transfer, which was demonstrated in this study.
Is placed the 1-PT (or 2-P-1-T) molecule on the gold surface to create pinholes between these molecules and then arranged inside these pinholes the 1,3-PDT molecule, leaving a thiol group (-SH) on the surface, which reacts with the BQ to form hydroquinone moiety, staying anchored to the surface (see scheme 1). This system would be used to recognize molecules containing a thiol (-SH) group in its structure, with an activity electrochemistry.

Figure 4 shows voltammograms for a 2.01 mM 1,4-benzoquinone (BQ) solution, in Britton-Robinson buffer, at pH 7, on clean gold surface. As is obvious, in this potential window, in figure 3(b), a peak of reduction (E_{pc}) of +0.008 V and peak of oxidation (E_{pa}) of +0.121 V, with a peak-to-peak separation (ΔE_p) of 0.112 V, were observed. This behavior may be related to the one-electron redox potential [E(BQ/BQ•$^-$)], with kinetic limitation. An anodic peak current (i_{pa}) of 16.4 µA and a cathodic peak current (i_{pc}) of -21.4 µA, for an $|i_{pa}/i_{pc}|$ of 0.76, describing this system as quasi-reversible electrochemically, also were observed. The BQ is reduced on clean gold surface, but in their oxidation chemical reactions coupled to the electrode process perturb the electron transfer.

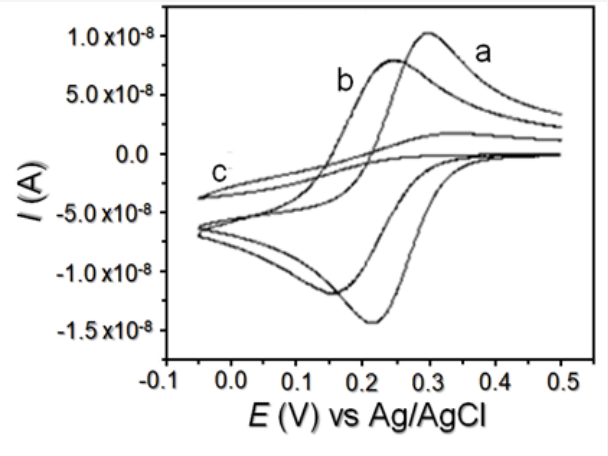


Figure 3. Cyclic voltammograms of 2.5 mM $K_3Fe(CN)_6$ in 0.1 M KCl solution: (a) for clean gold, (b) gold surface modified with 3.0 mM 2-P-1-T for 30 min and (c) gold surface modified with 3.0 mM 2-P-1-T for 30 min and 3.0 mM 1,3-PDT for 5 h (d), in alcoholic solution. Scan rate 100 mV s^{-1}. Measurements were performed at 21 + 1 °C.

Gold surfaces were modified with 1-PT, 2-P-1-T, and 1,3-PDT. The gold modified surfaces were immersed in 2.01 mM 1,4-benzoquinone (BQ) solution, in Britton-

Robinson buffer, at pH 7, for 15 h, without applying any potential. Afterward, the gold modified surfaces dropped out of nucleophile solution, washed with water, and immersed in Britton-Robinson buffer without BQ molecules. In this process, the BQ reacts with the thiol (-SH) group of the 1,3-PDT molecules, to form hydroquinone (H_2Q) moiety (see scheme 1), after Michael addition.

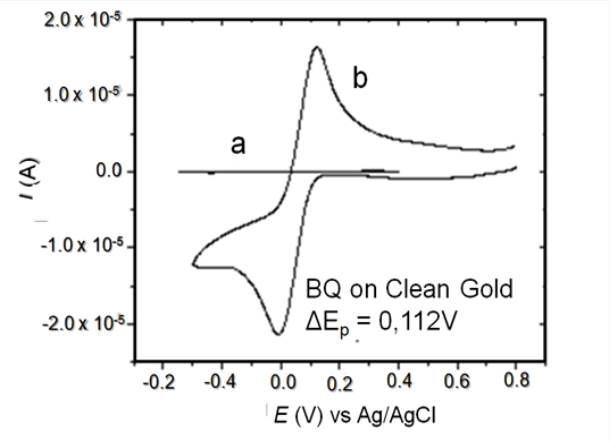


Figure 4. Cyclic voltammograms for a 2.01 mM 1, 4-benzoquinone (BQ) solution, in Britton-Robinson buffer, at pH 7, on clean gold surface. The solution was degassed with N2 flow for least 30 min. Scan rate 100 mV s^{-1}. Measurements were performed at 21+1 °C.

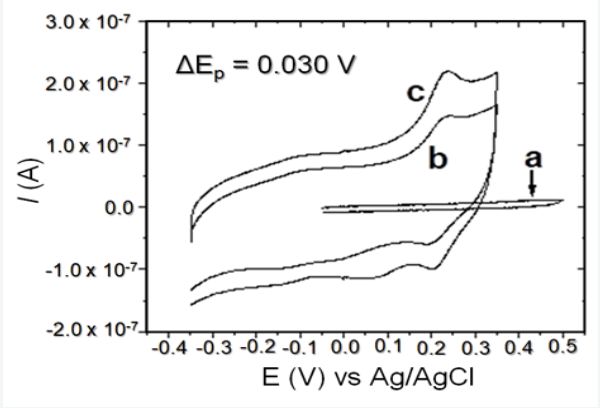


Figure 5. Cyclic voltammograms for the gold modified with: (a) 3.0 mM 2-P-1-T for 30 min and 3.0 mM 1,3-PDT for 5 h; (b) 3.0 mM 1-PT for 30 min, 3.0 mM 1,3-PDT for 5 h, and hydroquinone (H_2Q) moiety; (c) 2-P-1-T for 30 min, 3.0 mM 1,3-PDT for 5 h and hydroquinone (H_2Q) moiety. The solution was degassed with N_2 flow for least 30 min. Scan rate 100 mV s^{-1}. Measurements were performed at 21 + 1 °C

Figure 5 shows the CVs for the gold modified with (a) 3.0 mM 2-P-1-T for 30 min and 3.0 mM 1,3-PDT for 5 h; (b) 3.0 mM 1-PT for 30 min, 3.0 mM 1,3-PDT for 5 h, and hydroquinone (H2Q) moiety; (c) 2-P-1-T for 30 min, 3.0 mM 1,3-PDT for 5 h and hydroquinone (H2Q) moiety. The scan potential was from -0.35 V to +0.35 V, at 100 mV/s.

In figure 5(a) redox couple was not observed, indicating that the thiol (-SH) group on gold surface is not active at this potential window. In Figures 5(b) and 5(c), both have the same behavior en la oxidation and reduction of H_2Q on gold surface modified:

$$H_2Q \rightleftarrows Q^{2-} + H^+$$

However, in the Figure 5c was increased the anodic and cathodic peak currents, possibly due to double bond (C=C) in 2-P-1-T. The only difference between these two systems is changing 2-P-1-T by 1-PT, maintaining the same 1.3-PDT and H_2Q on the surface. A cathodic peak potential (E_{pc}) of +0.210 V, an anodic peak potential (E_{pa}) of +0.240 V, and a peak separation (ΔE_p) of 0.030 V, were observed. This Peak-to-peak separation indicates that the overall process is kinetically fast for two-electron redox potential; however, an anodic peak current (i_{pa}) of 0.22 µA and a cathodic peak current (i_{pc}) of -0.10 µA, for a $| i_{pa}/i_{pc} |$ of 2.2, shows a quasi-reversible system. There are substantial differences between the electrochemistry of BQ on clean gold surface and H_2Q moiety on gold surface modified. This system has lower reversibility than the BQ on clean gold surfaces.

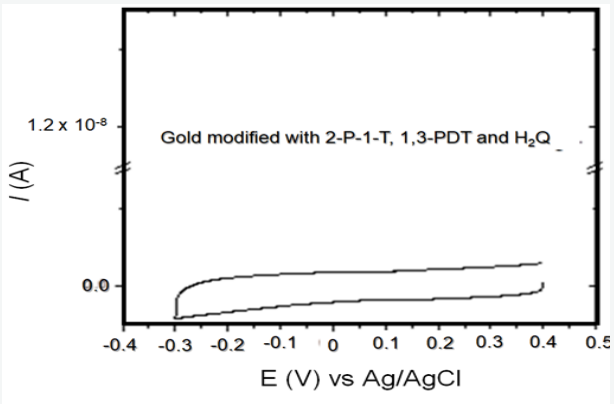


Figure 6. Cyclic voltammograms for the gold modified with 3.0 mM 2-P-1-T for 30 min and 3.0 mM 1,3-PDT for 5 h. The solution was degassed with N_2 flow for least 30 min. Scan rate 100 mV s^{-1}. Measurements were performed at 21 + 1 °C.

To confirm that the Michael addition reaction between the BQ and the thiol (-SH) (from 1,3-PDT) was reached, was voltammetric sweeps from +0.40 V to -0.30 V. Thus, is excluded the presence of BQ, indicating the formation of H_2Q moiety on the gold surface modified. Figure 6 shows the absence of redox behavior for the BQ molecule (as is observed in Figure 5) because this molecule is reduced to H_2Q on the modified gold surface.

Pre-treatment and substrates modification for surface analysis.

For X-ray photoelectron spectroscopy (XPS) studies, the sample analyses were performed using the Al Kα (15.0 kV at 350 watts) source of a PHI 5600 ci spectrometer. This instrument has a hemispherical analyzer, a toroidal monochromator, and a multichannel detector. The base pressure in the chamber during analysis was less than $1x10^{-9}$ Torr.

The binding energy values reported in this study were corrected using the C 1s signal of the atmospheric contaminants (285 eV).

High-resolution spectra were recorded at a take-off angle of 450 and 11.75 eV pass energy, with at least 5 minutes for each element.

Different experiment setups were used for the XPS studies. The conditions for each setup are summarized in results of surface analyses.

All substrates used were new and before their modification they were submitted to a cleaning treatment. MAXTEK® Au substrates were used. The polycrystalline gold electrodes used for the XPS measurements were prepared by polishing a 0.32 cm^2 geometric area electrode with 1.0, 0.3, and 0.05 mm Al_2O_3 paste, and then rinsed copiously with nano-pure water. Electrode cleanness was verified by cycling the potential in a 1.0 M H_2SO_4 solution, at 100 mV/s scan rate, between hydrogen and oxygen regions until characteristic CV were observed (See Figure 2). The electrode was polished with Al_2O_3 paste, again and washed copiously with nano-pure water. Finally, they were dried with an argon gas flow. We verified its cleanness by cyclic voltammetry before being chemically modified.

The previously cleaned substrates were submerged in a 3.0 mM 2-P-1-T ethanol solution for 30 min. afterward, the substrates were washed with ethanol, dried under nitrogen gas flow, and submerged in a 3.0 mM 1, 3-PDT ethanol solution for 5 h.

Finally, the modified electrodes were immersed in a 2.01 mM 1,4-benzoquinone (BQ) solution, in Britton-Robinson buffer for 15 h. In this last solution, the BQ compound react through a nucleophilic 1,4-Michael addition to form the corresponding reduced thiol conjugate on gold surface.

Results of spectroscopic studies.

XPS has been widely used to study the composition of self-assembled monolayers on metallic surfaces. This technique was used to confirm the 2-P-1-T, 1,3-PDT, and H_2Q organization on the gold surface. The XPS spectrum of the clean gold substrates is shown in Figure 7. This spectrum showed photoemission peaks that were attributed to the Au and C atoms that are present on the clean gold surface.

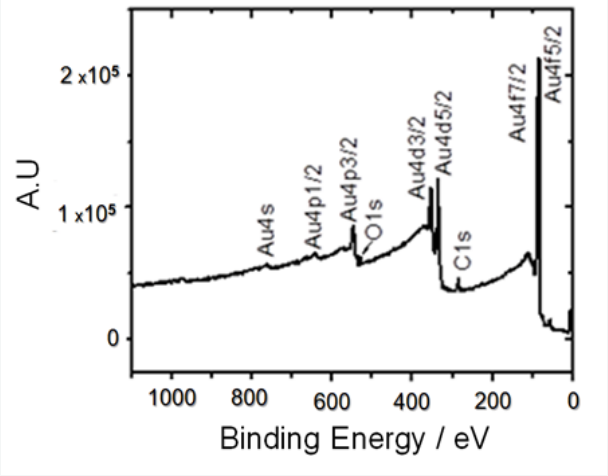


Figure 7. Survey of X-ray photoelectron spectroscopy (XPS) for clean Au surface.

High-resolution XPS spectra for the core binding energy region of gold are shown in Figure 8. The spectra, taken under the same instrumental conditions, reveal how

the signal of gold is reduced when the Au surface was modified with 2-P-1-T, 1,3-PDT, and H_2Q. This indicates that in some way these molecules are adsorbed on the surface and are blocking the XPS detection of Au 4f peaks. In the figure 9 the presence of carbon on the gold surface is observed. The clean gold surface does not present this atom, the signal of carbon from atmospheric contamination only.

Figure 10 shows XPS spectra for the O (1s) region at take-off angles of 45o. Reveal how the Oxygen signal is increased when the Au surface was modified with 2-P-1-T, 1,3-PDT, and H_2Q, respectively. The O (1s) peak (Figure 10a) appear at a binding energy in accordance with oxides and to a H_2O signal.[111-115] Changes in the area of each O (1s) signal were observed (Figure 10b and 10c), indicating that the concentration of the different types of compounds without oxygen is remarkable (2-P-1-T and 1,3-PDT compounds). After the reaction of the 1,3-PDT and BQ compounds, increases the presence of oxygen, indicating that the H_2Q has been formed on the modified surface.

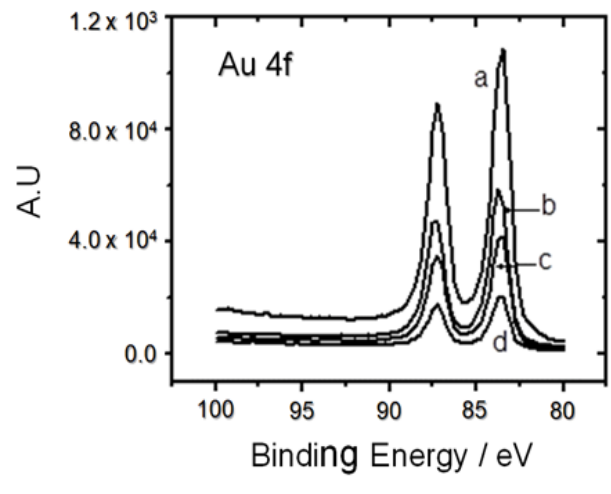


Figure 8. (a) Clean Gold; (b) 3.0 mM 2-P-1-T for 30 min; (c) 3.0 mM 2-P-1-T for 30 min and 3.0 mM 1,3-PDT for 5 h (d) 2-P-1-T for 30 min, 3.0 mM 1,3-PDT for 5 h and hydroquinone (H_2Q) moiety

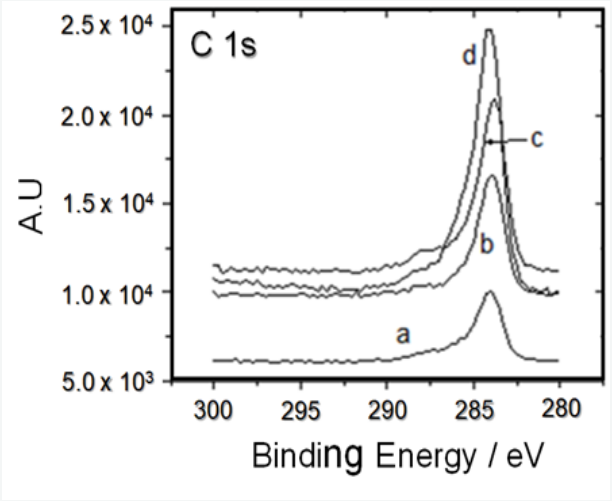


Figure 9. XPS spectra for the C (1s) region (a) Clean Gold; (b) 3.0 mM 2-P-1-T for 30 min; (c) 3.0 mM 2-P-1-T for 30 min and 3.0 mM 1,3-PDT for 5 h (d) 2-P-1-T for 30 min, 3.0 mM 1,3-PDT for 5 h and hydroquinone (H_2Q) moiety. The study made at a 45o take-off angle.

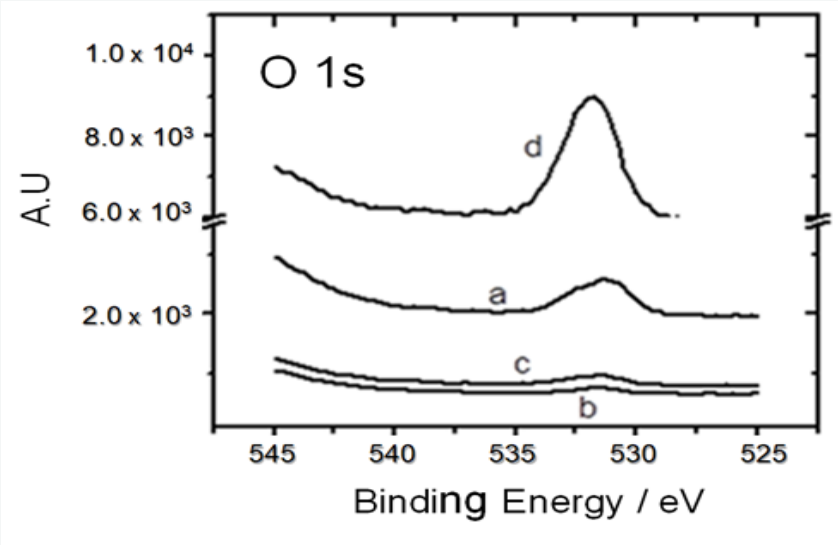


Figure 10. XPS spectra for the O (1s) region (a) Clean Gold; (b) 3.0 mM 2-P-1-T for 30 min; (c) 3.0 mM 2-P-1-T for 30 min and 3.0 mM 1,3-PDT for 5 h (d) 2-P-1-T for 30 min, 3.0 mM 1,3-PDT for 5 h and hydroquinone (H_2Q) moiety. The study made at a 45o take-off angle.

CONCLUSION

The use of modified metal surfaces is very useful in the study of biomarkers due to their ability and stability in performance.

Owing to the important physiological roles of quinones, such as hydroquinone, 1,4-benzoquinone (BQ), and reduced thiols such as GSH, it is necessary to develop sensitive and selective methods for the measurement of those substances to study cellular mechanisms of chemical-induced toxicity. For this purpose, the BQ is used in the electrochemical determination of thiol molecules. The Addition of sulfur nucleophiles to quinones has been studied and characterized as 1,4-reductive addition of the Michael type. Quinones, containing a polarized double bond, are considered Michael acceptors, where the Michael donors are thiol nucleophiles. The final product of this reaction is hydroquinone with a sulfur atom in the ortho position. If a metal surface is modified with a dithiol (to form a thiolate with the metal surface, leaving a free thiol,-HS) molecule, the reaction with BQ will be easy to produce hydroquinone (H_2Q) moiety. The latter product can be used to determine electrochemically thiol-containing compounds in its structure

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