Full Paper

Disposable Gold Electrode Array for Simultaneous Electrochemical Studies

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Abstract

An efficient and inexpensive eight gold electrode array has been manufactured by a combination of screen printing and gold electrodeposition techniques. Gold electrodeposition was performed in potentiostatic and galvanostatic conditions. Different treatments, involving temperature and polishing control, led to electrodes with different roughness. The electrochemical behavior of the generated gold surface was studied by cyclic voltammetry showing the characteristic response of polycrystalline gold, in contrast with disposable gold electrodes fabricated by screen printing from gold inks. The electrodes were chemically modified through the adsorption of alkanethiols self-assembled monolayers and the coupling of a model protein. Both reactions were followed by cyclic voltammetry and Electrochemical Impedance Spectroscopy (EIS). The electrodes have shown high reproducibility in their electrochemical behavior as well as in their modifications.

Keywords: Gold disposable electrodes, Screen printing, Gold electrodeposition, Electrode array/sensors

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1. Introduction

Electrochemically deposited gold has satisfied several demands of the electronic industry. Gold has the third best electrical conductivity of all metals at room temperature and its inertness prevents the formation of insulating surface oxides. On the other hand, gold surface modification with thiolic compounds opens a myriad of opportunities for the construction of modified electrodes by using a few well known and efficient reactions. These allow the introduction of practically any functional group to the electrode surface providing for the molecular design of specific tasks on a surface. This technology has been used as a departing point for a new generation of electrochemical [1], surface plasmon resonance [2, 3] and electrochemical quartz crystal microbalance-based transducers [4].

The development of electrochemical sensors is one of the areas of greater and faster growth in analytical chemistry that led to an increasing search for devices with improved reproducibility, sensitivity and stability [5]. Despite the great versatility and perspectives shown by electrochemical sensors, the utility of an electrode is usually limited by the gradual fouling of its surface. Such problem can be minimized with the use of disposable electrodes. Some basic characteristics for an electrode to be considered a disposable device are: low cost of construction, reproducibility and possibility of large-scale production [6]. Although the requirements for different applications vary, gold deposits are always required to have high purity, controlled roughness and good adhesion to the substrate.

Screen printing technology is perhaps the most suitable technology for the construction of disposable electrodes. For example, graphite screen printed electrodes are part of our daily life, they can be found in glucose meters. The device consists in a graphite area exposed to the sample (working electrode) and an insulated graphite track that connects the electrode to the meter. In the scientific literature several examples of graphite screen printed electrodes can be found [7–9]; in most of these works, its application is limited to amperometric studies at a constant potential as in glucose sensors. It would be very convenient to have access to disposable electrodes able to be used in different electrochemical techniques, e.g.: cyclic voltammetry, differential pulse voltammetry, electrochemical impedance spectroscopy, in which the classical theoretical framework could be applied. One of the limitations when tracks and electrodes are made with graphite ink is its relative low conductivity, which in the case of cyclic voltammetry produces the equivalent effect of an ohmic drop, limiting their use. On the other hand, gold screen printed electrodes, even though they present a good conductivity are expensive and present a behavior that corresponds to an electrode surface partially covered [10].

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In this work, to overcome these problems and with the purpose of designing adequate surfaces for biosensor development, a new strategy to manufacture gold electrode arrays combining silver and graphite screen-printing with gold electrodeposition is presented. The combination of silver and graphite inks improves the conductivity in the tracks of the device. Thus clean gold electrode surfaces can be generated by the electrodeposition on graphite avoiding the formation of silver-gold alloys. Cyclic voltammetry and electrochemical impedance spectroscopy (EIS) experiments performed on these gold electrodes show good reproducibility in roughness, electrochemical response and chemical modification.

2. Experimental

2.1. Materials

Conducting inks were from Ercon Inc. and the insulating ink from Electroquímica Delta (Buenos Aires, Argentina), details are given below. Gold atomic spectroscopy standard concentrate solution, $HAuCl_4$, was provided by Fluka, the cyanide bath used in galvanostatic electrodeposition conditions was supplied by Vilmet (Buenos Aires, Argentina). ENP was supplied by Associates of Cape Code (Seikagaku America), this protein is a recombinant version of the Limulus anti-LPS factor (LALF); pI: 8.5; MW: 12.2 kDa (105 AA residues). Ferrocene carboxylic acid (FCA), cystamine dihydrochloride (Cys), 3-mercaptopropionic acid (MPA), 11-mercaptoundecanoic acid (MUA), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were provided by Sigma. All other reagents were analytical grade. All solutions were prepared with pure water purified through a Milli-Q system (Millipore, Bedford, M.A.).

2.2. Construction of Multielectrode Array

Scheme 1 outlines the construction of the electrode array. Mylar plates (350 μm thickness) were screen printed with conductive silver ink (Ercon Inc., cat # E1660–136) to improve the conductivity of the tracks. Then, they were covered with graphite ink (Ercon Inc., cat # E3456) which extents to the electrode zone where gold will be deposited. Then, insulating ink (acrylic insulating varnish for high voltage electrical motors provided by Electroquímica Delta S.R.L, Argentina) was used in order to obtain 0.6 cm^2 working electrodes (8 electrodes in each plate). In some cases, this graphite electrode array was consecutively polished with 0.3 and 0.05 μm alumina. The electrodes were immersed in 1 M NaOH and cleaned by applying −4.0 V during 90 s, and were alternatively rinsed with MilliQ water and 5% v/v H_2SO_4.

The gold electrodeposition was performed in potentiostatic or galvanostatic conditions. After deposition some plates were heated at 140 °C during 8 hours (TT = thermal treatment).

The behavior for each electrode in the multielectrode array was compared to 1 cm^2 clean polycrystalline gold flag electrodes (massive gold). For all-purpose, clean electrodes were gently rinsed with MilliQ water and the surface cleanness was checked by cyclic voltammetry in 1.8 M H_2SO_4 between 0 to 1.5 V vs. Ag/AgCl at 0.05 V s^{-1}. When the cleaning step was satisfactorily verified, the electrodes were used in further studies.

2.2.1. *Potentiostatic Gold Electrodeposition*

The thick film graphite electrodes were coated with a gold film, prepared by applying a 2 Hz square wave potential, between −0.2 V and 0.8 V vs. Ag/AgCl, during 60 minutes with orbital shake to 50 rpm, in a solution containing 1.2 mg mL^{-1} $HAuCl_4$, 1.5% (w/v) HCl and 0.1 M NaCl [11].

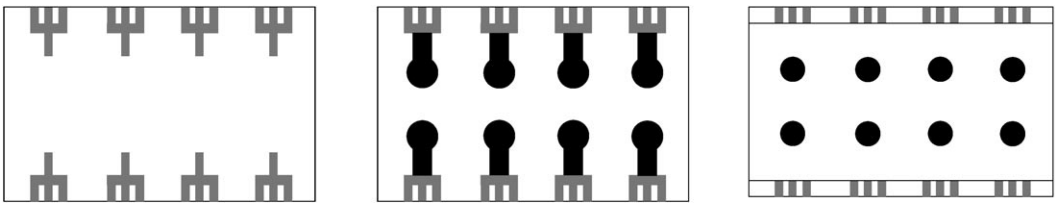
2.2.2. *Galvanostatic Gold Electrodeposition*

In this case, the experiments were carried out in a gold bath composed by 15 mg mL^{-1} $KAu(CN)_2$ and 115 mg mL^{-1} citric acid, adjusted with KOH to pH 4. A current of 5 mA cm^{-2} was applied through the cell during 15 minutes with orbital shaking of 80 rpm.

2.3. Modification of the Multielectrode Array

Modification of gold electrodes was carried out in a specially designed acrylic cell where only the gold surface of the plate is in contact with the reactants.

Thiol adsorption (Au/Cys, Au/MPA and Au/MUA): Gold clean electrodes were immersed separately overnight in 20 mM Cys, MPA or MUA solutions in absolute ethanol/water (9:1). Then, they were rinsed with ethanol and MilliQ water. Fresh solutions were used in each preparation.



Scheme 1. Construction of electrode array on mylar. From left to right: silver, graphite, and insulating ink deposition.

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ENP modification (Au/MUA/ENP): The binding of ENP to the thiol modified electrodes were carried out through the formation of an amide bond. Au/MUA electrodes were immersed in a solution containing 100 mM EDC and 100 mM NHS in 50 mM MES buffer (pH 5.5) for 30 minutes to activate of carboxylate groups. Then, they were rinsed with MilliQ water and immersed in 10 μg mL^{-1} ENP solution in 10 mM HEPES buffer (pH 8.0) and shaken for 3 hours at room temperature. After that, the ENP modified electrodes were immersed in a 1 M ethanolamine solution to quench the activated carboxylate groups and finally rinsed with MilliQ water and measurement buffer.

2.4. Cyclic Voltammetry Measurements

The multielectrode array was used with a purpose-built operational amplifier multipotentiostat (MultiTEQ) and an acrylic cell was designed to ensure fixed position for all the electrodes in the array. Reference electrode was Ag/AgCl/3 M KCl, and as counter electrode a gold sheet was placed in front of the working electrodes. The charge of the gold oxide reduction obtained for each working electrode, in the acidic conditions detailed before, was used to calculate its roughness factor, dividing its value by the charge of the oxide reduction peak for a clean gold mirror, 450 μC cm^{-2} [12].

2.5. Electrochemical Impedance Spectroscopy (EIS) Measurements.

EIS measurements were performed using a μAUTOLAB type III impedance analyzer. The cell, reference and counter electrodes used in these experiments are the same as described in the previous section. Unless mentioned, as redox probe $Fe(CN)_6^{3-/4-}$ in equimolar concentrations, 10 mM, was used in a 10 mM phosphate buffer, pH 6 containing 0.2 M KNO_3 as supporting electrolyte. The impedance spectra were recorded within a frequency range of 1 Hz – 10 kHz, at the formal potential of the redox probe, 225 mV. The amplitude of the alternative voltage was 10 mV. 9 points per frequency decade were taken with logarithmic distribution.

The Randles modified equivalent circuit [1, 13] was used to fit the impedance spectroscopy and to determine electrical parameter values for each experiment. The circuit includes the electrolyte resistance between working and reference electrodes (R_s), Warburg impedance (W), electron-transfer resistance (R_{et}), and the constant phase element Q, as function of Y_0 and n (instead of the double layer capacitance) to take into account the frequency dispersion often related directly to electrode roughness [14].

3. Results and Discussion

3.1. Array Construction

A conductive surface using silver and graphite inks is generated on a mylar plate using a conventional screen-printing technique (Scheme 1, left and center). It can be observed that the silver surface does not overlap with the graphite circle (working electrode area) to avoid silver diffusion to the surface and later alloy formation with gold. The improvement of the conductivity in the graphite working electrode area will be achieved once gold is deposited. Finally, the plate is masked with an insulating ink to leave only the graphite circle and the silver contacts exposed (Scheme 1, right).

For gold electrodeposition, a plate is sandwiched in an acrylic cell (Fig. 1). The lower part works as support for the plate, while the upper part, where the reference and counter electrodes are placed, is filled with the gold bath. The working electrodes are connected in parallel to the potentiostat (or galvanostat) through the silver tracks at the plate borders. For electrochemical studies, the described acrylic cell is also used; and the working electrodes are connected simultaneously to a multipotentiostat where the response for each electrode can be recorded through different channels.

Two electrochemical gold deposition methods, potentiostatic and galvanostatic, were systematically studied with the purpose of reaching the desired electrode properties: good appearance, mechanical resistance, controlled roughness, deposit uniformity and good adhesion to the substrate. Both methods turned out to be simple, fast and inexpensive to obtain reproducible electrode surfaces, however the galvanostatic method reduces four times the experimental time to obtain a suitable gold deposit. Table 1 resumes the surface properties of the multielectrode arrays constructed under different methods.

For both methods, electrodes with and without thermal treatment (heated at 140 °C for 8 h.) were tested. In the case of electrodes constructed under galvanostatic conditions, electrodeposition was carried out on graphite electrodes with and without previous polishing with 0.3 and 0.05 μm alumina. Those constructed under galvanostatic conditions

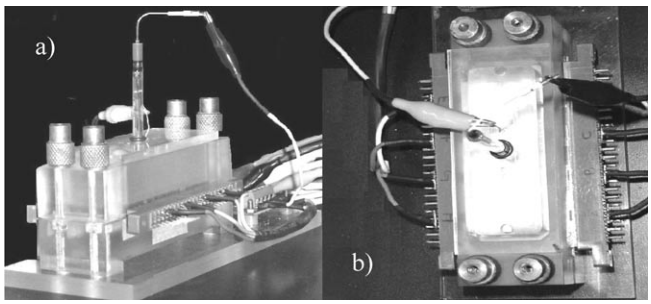

Fig. 1. Side (a) and upper (b) views of the acrylic cell with the connected working, reference and counter electrodes.

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Table 1. Surface properties for the multielectrode arrays. TT: thermal treatment

	Potentiostatic without polishing		Galvanostatic without polishing		Galvanostatic with polishing	
	without TT	with TT	without TT	with TT	without TT	with TT
Roughness factor	7.7 ± 0.6	4.7 ± 0.3	6.0 ± 0.5	3.0 ± 0.2	5.5 ± 0.3	2.0 ± 0.2
Adherence	adequate [a]	adequate	excellent [b]	excellent	excellent	excellent

[a] After some experiment, detachment of gold by mechanical action is observed. [b] There are no detachments of gold.

with polishing and thermal treatment were used in the following studies.

3.2. Electrochemical Characterization and Applications

To check roughness and cleanness of the electrode surfaces and the reproducibility among them, cyclic voltammograms of the 8 gold electrode array were simultaneously performed in 1.8 M H_2SO_4 at 50 mV s^{-1} with a multipotentiostat where the response can be separately recorded. Figure 2 shows a typical voltammogram indicating a clean gold surface. For the eight electrodes, the voltammetric responses are almost identical. For clarity, in these figure only the response of one electrode is shown. The reproducibility of the procedure employed in the electrodes construction was evaluated by comparing the charge of the gold oxide reduction peak, the result was 903 ± 13 μC cm^{-2} ($n = 8$).

Ferrocene carboxylic acid (FCA) was used to investigate the electron transfer behavior of a reversible redox couple in solution. FCA is commonly used as an electrochemical probe since it has excellent electrochemical properties: stable in both oxidation states, electrochemically reversible and presents a fast electron transfer with redox enzymes. Simultaneous cyclic voltammograms for FCA were performed in a buffer solution pH 7 showing a typical Nernstian behavior (Fig. 3). The resultant relevant parameters, $E^{\circ\prime} = (312 \pm 1)$ mV, $\Delta E_p = 71 \pm 6$ mV and $i_p^a/i_p^c = 1.02$ ($n = 8$), clearly reflect that the response of the electrodes is reproducible and similar to the one of massive gold electrode; in this way, dynamic techniques as cyclic voltammetry can be used without any signal distortion. This result differs from those observed in previous works on screen-

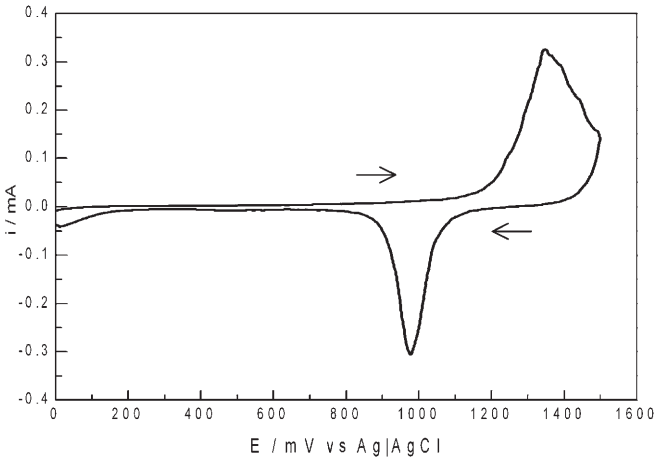

Fig. 2. Cyclic voltammogram for one of eight gold electrode array in H_2SO_4 1.8 M, at 50 mV s^{-1}

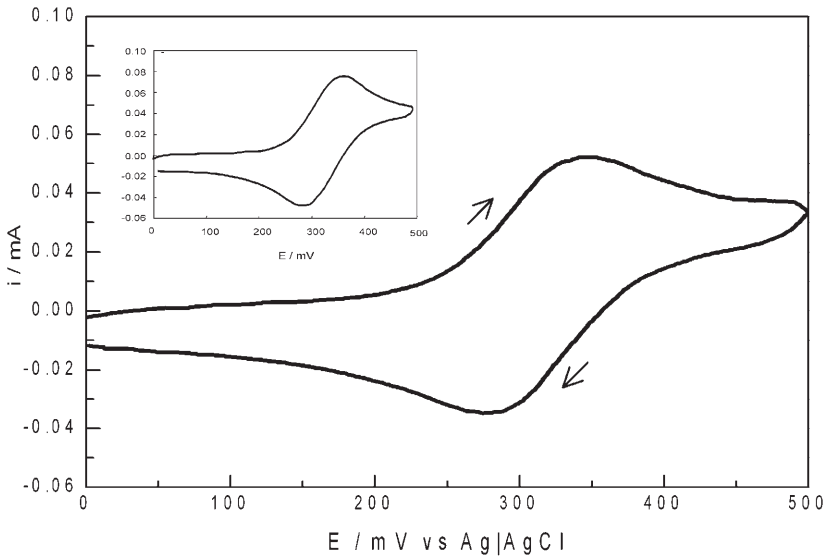

Fig. 3. Cyclic voltammogram for one of eight gold electrode array with 1 mM ferrocene carboxylic acid in 50 mM buffer TRIS at pH 7 with 0.2 M KNO_3 as supporting electrolyte, at 50 mV s^{-1}. The inset shows the same experiment on a massive gold.

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printed Au and Pt electrodes, in which the difference between anodic and cathodic peak potentials is greater than the expected for a reversible couple. The deviations from theoretical values are attributed to the binder in the ink composition covering some active sites of the electrodes [10, 15]. To explain this behavior a model was developed by Amatore et al. based on nonlinear diffusion effects due partial coverage [16].

The combination of graphite with silver for track production, to avoid ohmic drop, and the electrodeposition of gold yields disposable gold electrodes in which the classical electrochemical theoretical framework for cyclic voltammetry can be applied.

In order to explore the performance of the manufactured electrodes and to show the applicability of such a disposable device, its chemical modification was studied.

Spontaneous adsorption of organic molecules from solution onto solid surfaces can give rise to self-assembled monolayers (SAMs). Thiol compounds spontaneously adsorb onto gold surfaces to form close-packed oriented SAMs [17]. In the literature, many aspects related to the adsorption of thiols are described [18–20]. Electrodes were modified using three alkanethiols with different chain length, cystamine (Cys, 2C), 3-mercaptopropionic acid (MPA, 3C) and 11-mercaptoundecanoic acid (MUA, 11C).

To evaluate the Cys adsorption on the electrodes, we used the method proposed by Walczak et al. [21] based in the reductive desorption of thiols at basic pH. The electrodes were modified with Cys, (Au/Cys) (according to the procedure described in the Experimental), were rinsed with absolute ethanol and MilliQ water and finally dried with a nitrogen flow. Cyclic voltammograms were obtained sweeping the applied potential between −0.2 V and −1.3 V vs. Ag/AgCl at 100 mV s^{-1} in 0.5 M KOH solution previously degassed with nitrogen. Figure 4 shows the cyclic voltammogram obtained in the conditions previously detailed. In this figure, three suggested peaks can be observed due to the reductive desorption of the self-assembled cystamine, showing great similarity with the behavior of a massive gold electrode (inset) and to the one reported for cysteine and cystamine by El-Deab et al. [22]. These peaks are assigned to desorption of the thiol from domains (111), (100), (110) in polycrystalline gold.

In addition, the changes in the interfacial properties of electrodes by thiols and biomolecules incorporation were evaluated using the ferro/ferricyanide redox couple as a probe in EIS experiments. Impedance spectroscopy is a sensitive and non-destructive technique widely used for the characterization of functionalized electrodes. The immobilization of biomaterials on electrodes alters the capacitance and interfacial electron transfer resistance of the conductive surfaces, thus, EIS is a very powerful tool for the analysis of interfacial property changes on modified electrodes [23–25].

In particular, the electron transfer behavior of a redox couple on SAMs modified electrodes is essentially related to the surface morphology and defects [26]. The EIS data indicated the formation of a SAMs on gold. Figure 5 illustrates the results obtained by immobilization of short chain thiols (MPA) and a long chain thiol (MUA) onto electrode surface. The impedance plot for the bare gold electrode shows a negligible semicircle domain implying very fast electron-transfer process with a diffusional limiting step. The semicircular diameter enlarged due the alkanethiol incorporation at the surface, is more significant for a thiol with longer carbon chain. MUA presents the greatest impedance change, entailing a high electron transfer resistance due to the increased thickness and compactness, as well as the fewer pinhole defects on the electrode surface. On the other hand, the short chain thiol, MPA, forms a self-assembled monolayer containing many pinholes and the redox probe is sufficiently small to freely penetrate through them [27, 28]. Our results agree with previous works carried out on massive gold [1] where the R_{et} dependence with the chain length on modified Au electrodes is reported.

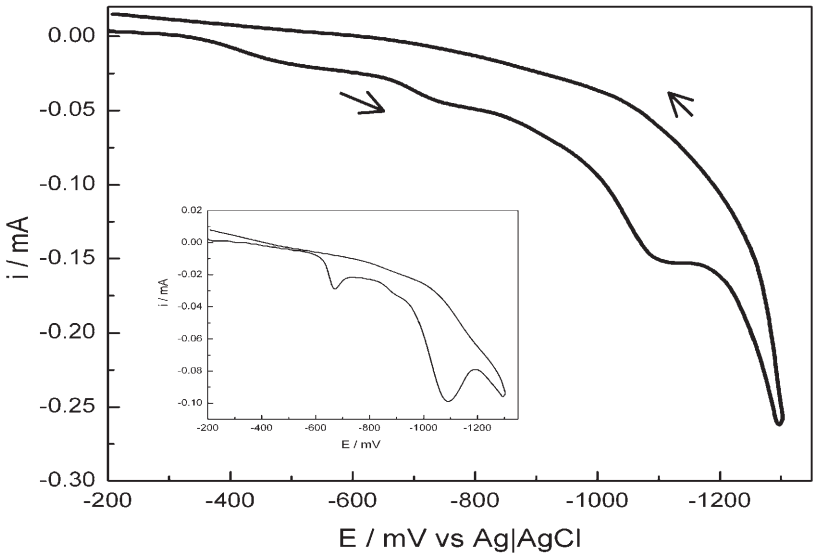

Fig. 4. Cyclic voltammetric of immobilized cystamine in 0.5 M KOH at 100 mV s^{-1}. The inset shows the same experiment on the massive gold.

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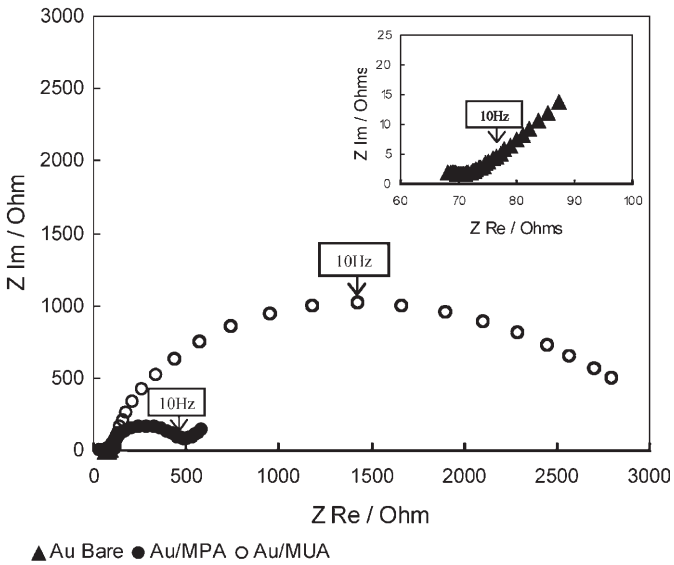


Fig. 5. Impedance spectroscopy of bare gold, immobilized MPA and MUA, in the presence of 10 mM $Fe(CN)_6^{3-/4-}$ as redox probe in 10 mM phosphate buffer at pH 6. Frequency range: 1 Hz – 10 kHz, at the formal potential of the redox probe, 225 mV. The amplitude of the alternative voltage was 10 mV.

Table 2 shows the experimental data obtained at different frequencies for a multielectrode array (8 electrodes) modified with MUA. The low standard deviation observed at each frequency shows once again the reproducibility of this reaction and the construction method for these electrodes.

One of the promising applications of this type of surface is the construction of a platform for recognition assays. To achieve this goal a recognition agent was bound to the electrode. In previous reports, we have used endotoxin neutralizing protein (ENP) as recognition agent to detect pyrogens [29, 30]. In this work, it was used to check the reproducibility of the coupling reaction that is carried out using EDC/NHS.

Gold electrodes were modified with alkanethiolate derivatives bearing carboxylate groups by a simple adsorption procedure; thereafter the recognition protein ENP was bound through amide bond formation by activation of the carboxylate groups present in the surface with EDC/NHS (Scheme 2). Electron transfer studies for $Fe(CN)_6^{4-/3-}$ on these modified electrodes demonstrate that the fabrication of these electrodes, combined with efficient reactions, produce very reproducible surfaces suitable for sensor development.

Table 2. Experimental data for real and imaginary Z for Au/MUA modified multielectrode array ($n = 8$, confidence level = 95%).

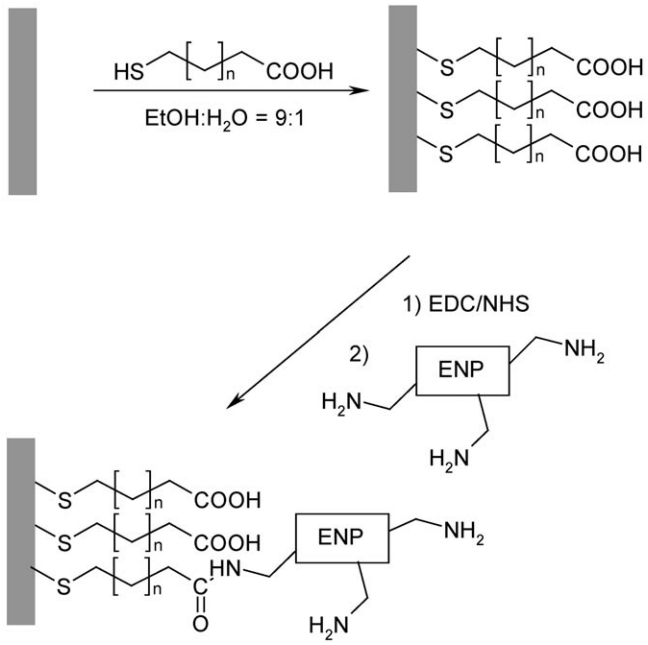
Frequency	Z_{Re} (Ω)	Z_{Im} (Ω)
10 kHz	122 ± 9	7.4 ± 0.6
1 kHz	128 ± 9	39 ± 2
100 Hz	211 ± 13	303 ± 20
10 Hz	1266 ± 98	972 ± 82
1 Hz	2758 ± 192	560 ± 43

Figure 6 shows the results obtained by EIS, performed at the mentioned conditions, for a MUA modified electrode array (Au/MUA) and thereafter with ENP, (Au/MUA/ENP). Table 3 resumes the values of the relevant parameters determined using the Randles modified equivalent circuit for 8 electrodes modified in the same way, showing that the coupling reaction is a reproducible method for sensor construction.

The low standard deviation in the determined parameters shows that the fabrication process (screen printing, gold electrodeposition, thiol adsorption and protein coupling) can be carried out with great reproducibility, allowing the simultaneous determination of different samples without the need of an internal standard, since results obtained with different electrodes can be compared straightforward. This fact also reduces the experimental time since the calibration process and the measurement of samples can be carried out at the same time; allowing in this way to construct arrays similar to those used in ELISA assays. Also, this system has potential applications in works on combinatorial electrochemistry since the simultaneous study of the response of differently modified electrodes can be carried out [31].

4. Conclusion

We have described a simple and inexpensive method to manufacture disposable gold electrodes. We are able to build electrode arrays in a controlled way, with working surfaces similar to routinely polished gold, which have



Scheme 2. Gold surface modification. First step: adsorption of mercaptocarboxy derivatives. Second step: incorporation of the recognition agent using EDC/NHS. ENP stands for endotoxin neutralizing protein.

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Table 3. Values of the equivalent circuit elements of the fitting curves for Au/MUA and Au/MUA/ENP modified multielectrode array ($n = 8$, confidence level = 95%).

	R_s (Ω)	R_{et} (Ω)	Y_0 (10^{-6})	n	W (10^{-3})
Au/MUA	117 ± 9	2655 ± 47	12.1 ± 1.8	0.86 ± 0.02	2.1 ± 0.2
Au/MUA/ENP	78 ± 6	2294 ± 91	18.6 ± 1.5	0.81 ± 0.01	3.1 ± 0.3

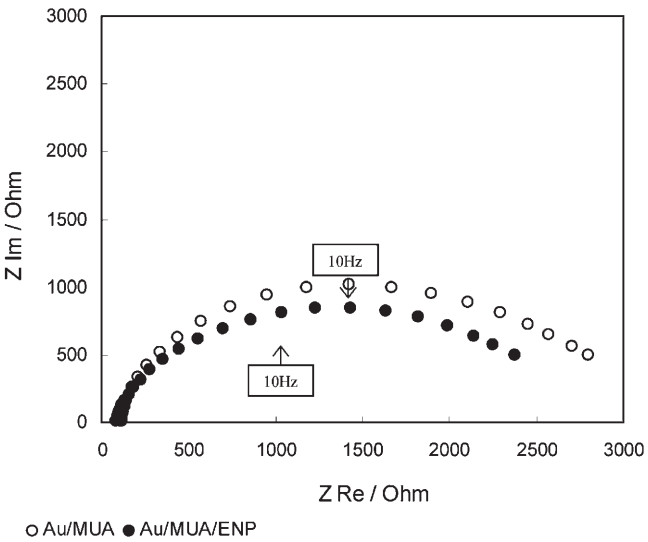

Fig. 6. Nyquist plot for Au/MUA/electrolyte interface (○) and Au/MUA/ENP/electrolyte interface (●) in 20 mM $Fe(CN)_6^{3-/4-}$ (1:1), pH regulated at pH 6, within a frequency range of 1 Hz – 10 kHz, at the formal potential of the redox probe, 225 mV. The amplitude of the alternative voltage was 10 mV.

roughness factors of 2 – 3 [13]. We have observed by optical microscopy that the deposited gold on graphite follows the roughness of the graphite surface (data not shown); therefore, as our results show, polished graphite surfaces and gold annealing help to get roughness factor in that order. The electrodeposition of gold produces clean surfaces with an electrochemical performance comparable to polycrystalline gold; therefore, results can be straightforward analyzed with the well established theoretical framework for the electron transfer process on electrodes.

Also, the array has demonstrated to provide a convenient platform for thiol adsorption and subsequent biomolecule immobilization; in both cases the chemical modification of the surface can be carried out with a very good reproducibility.

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