Gold nanoparticle-modified ultramicroelectrode arrays for environmental applications

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Abstract
Ultramicroelectrode arrays (UMEAs) exhibit a greatly enhanced mass transport and very high current densities compared with conventional microelectrodes. The combination of these sensors with gold nanoparticles provides excellent prospects for the fabrication of chemical transducers thanks to their unique physical and chemical properties and their potential application in interdisciplinary fields. Taking advantage of these features, these devices have been developed to be applied in environmental monitoring and clinical diagnostic. On one hand, the gold nanoparticle-modified microelectrode array has been applied for detection of copper (II) in samples of environmental interest. On the other hand, a couple of strategies for immobilization of biorecognition elements over the surface of the UMEAs have been proposed. The latter includes either a thiol-modified oligonucleotide or an enzyme attached to the arrays. The benefits of the resultant devices are highlighted for the detection of phenolic compounds in aqueous solutions.

Keywords: Ultramicroelectrode arrays, gold nanoparticles, copper, phenolic compounds, environmental monitoring.

Introduction
Ultramicroelectrode arrays (UMEAs) have been explored for the development of electrochemical sensors in the field of environmental and diagnostic applications [1, 2]. However, any strategy aimed to enlarge the current density measured with these devices is highly appreciate when either very high sensitivity or further improvement of the detection limits are required. In this context, gold nanoparticles provide excellent prospects for electronic signal transduction thanks to their unique physical properties [3].

Recently, modification of UMEAS by electrochemical deposition of gold nanoparticles has been performed by our group. The main achievement expected by combining gold nanoparticles and UMEAs is the potential increase of sensitivity and the enhancement of detection levels. On the other hand, anchorage of biorecognition elements over the UMEAs surface by means of gold nanoparticles can increase their selectivity towards certain target analyte.

Different methodologies have been used for the anchorage of gold nanoparticles on electrode surfaces. The resulting modified UMEAs showed improved analytical performance by increasing their active area while keeping the microelectrode properties [4].

Experimental
In the present communication, the development and characterisation of gold nanoparticle-modified UMEAs for the sensitive detection of copper (II) is described. The copper content of contaminated soil extract samples was effectively determined with the modified UMEAs and results are in good agreement with those obtained using a standard method.

On the other hand, anchorage of an oligonucleotide on the UMEA surface through gold nanoparticles was successfully performed and characterized by optical methods. An enzyme was also immobilized over the gold nanoparticle-modified UMEAs surface and the resultant device used for the detection of phenolic compounds in aqueous solutions.

Equipment and devices
Au microelectrode arrays were fabricated at the IMB-CNM, (CSIC) according to standard photolithographic techniques using Si/SiO2/metal structures as described elsewhere [4]. Table 1 shows the area for each array. The electrochemical cell consisted of an UMEA working electrode and an on-chip counter electrode. A Ag/AgCl/10% (w/v) KNO3 reference electrode (Metrohm 0726 100) completed the setup.
Volratmic experiments were performed at room temperature using a type III μ-Autolab potentiostat (Ecochemie), controlled with GPES 4.7 (General Purpose Electrochemical System) software package. Voltammograms in 0.1 M KNO₃ containing 1 mM K₄Fe(CN)₆.3H₂O, at 0.1 V.s⁻¹ were recorded to evaluate UMEA geometric features and their performance.

Reagents and methodology

Electrodeposition of gold nanoparticles was performed by dropping 10 µl of a commercial suspension of gold nanoparticles over the surface of the array and applying a constant potential of +1.6 V vs. a miniaturized Ag/AgCl pseudo-reference electrode for 20 min. Response of UMEAs to changes in copper concentration was evaluated by UPD-ASV, at a scan rate of 0.1 Vs⁻¹, after addition of different volumes of 10 mM Cu stock solution.

Deposition of Cu was performed by underpotential deposition-anodic stripping voltammetry (UPD-ASV) by applying a potential of -0.1 V (vs. a Ag/AgCl reference electrode) in a 1 µM Cu aqueous sulphuric acid solution and varying the pre-concentration time between 0 s and 400 s, at a scan rate of 0.1 Vs⁻¹.

Conjugation of the oligonucleotide to gold nanoparticles was performed using a 172.5 µM stock solution of a thiolated oligonucleotide labelled with (5'thiol-AAGAAC ACCAGCACAGACGC-3' Rhodamine red), purchased from MWG-BiotechAG (United Kingdom).

Anchorage of an enzyme, Horseradish Peroxidase (HRP), was performed over a UMEA previously modified with a self assembled monolayer (SAM). Two thiol molecules, 3-3' Dithiodipropionic acid di (N-hidroxy succinimide ester) (DTSP) and mercaptoundecanoic acid, were studied for this purpose. Immobilization of the enzyme was carried out in a 10 mg.ml⁻¹ HRP solution.

Results and discussion

Modification of UMEAs with GNP

We previously reported that electrodeposition of gold nanoparticles on the UMEAs surface not only enabled the estimation of the yielding of the fabrication process, but also increased their surface microscopic area while keeping the microelectrode features [4]. Gold nanoparticles exhibit a net negative charge induced in their preparation process, which was used to deposit them over the UMEAs surface by applying a positive potential high enough to induce the discharge of the nanoparticles by anodic oxidation. During the electrodeposition process at +1.6 V, a temporary decrease in the current was recorded, which can be explained as an oscillating electrode surface during the growth of the nanostructured gold layer. The electrodeposition was stopped when a minimum and constant current was reached, which means that deposition of as many gold nanoparticles as possible (lower than a monolayer) is guaranteed. The time and charge necessary to reach such stage was evaluated with 10 UMEAs and values were 20 min and 2.41 mC (s.d = 0.16) respectively.

Based on cyclic voltammetric curves obtained in 0.1 mM H₂SO₄ solution, an estimation of the surface microscopic area was achieved. This was conducted based on the amount of charge consumed during the reduction of the gold surface oxide monolayer. The reported value of 400 µC.cm⁻² was used for the calculations [5]. The values of the surface microscopic area of the UMEA-10, 5A and 5b with electrodeposited GNPs are 3.38 x 10⁻³ (sd=0.1, n=5), 3.50 x 10⁻³ (sd=0.2, n=10) and 37.4 x 10⁻³ (s.d=1.8, n=5), respectively. It is shown that a high area increase was obtained after GNPs electrodeposition, which was similar (more than 43 times) with those UMEA units sharing the same geometric area (UMEA-10 and 5A units). However an increase higher than 100 times was obtained with UMEA-5B units, which have a geometric area 4 times higher than the other two UMEA geometries.

Electrodeposition of GNPs on the electrode surface was confirmed by transmission light microscopy. Figure 1 shows a picture of an UMEA-10 unit after electrodeposition of GNPs. Evident differences between GNP-modified UMEs (vertical arrow) and non-modified ones (horizontal arrow) can be observed. Non-modified UMEs remained passivated (closed) after the fabrication process.

Figure 1. UMEA microscopy image taken after electrodeposition of GNPs (x20).
Electrodeposition of gold nanoparticles was also confirmed by AFM. Figure 2a shows a UMEA-5A device coated with GNPs. The increase of roughness means an increase of the active area and thus, a higher amount of the target analyte can be potentially electrodeposited. The quite uniform distribution of the particles guarantees appropriate electrochemical properties. However, gold nanoparticle electrodeposition does not always take place in this fashion. Figure 2b shows an electrode unit where the gold deposit generates a non uniform surface, which hampers the electronic transfer. This took place in nearly 10% of the experiments and the resulting modified electrodes were rejected for copper quantification.

Next set of experiments were conducted in order to test the feasibility of using GNPs-modified UMEAs for copper detection. The peak current of the stripping voltammetric process of Cu in a 1 μM Cu in 10 mM H₂SO₄ solution was studied as a function of the preconcentration time between 0 and 420 s. Results in Figure 3 reveal an almost linear dependence between peak current and time in the entire time interval. This Figure also shows the results obtained with a bare UMEA unit of the same geometry. The plot is linear up to 60 s deposition time. For longer times, the voltammetric peak current keeps increasing and levels off at times longer than 240 s due to saturation of the electrode surface. This fact demonstrates the improved performance of the modified UMEAs for the electrodeposition of a higher amount of copper, which, a priori, will result in higher sensitivity values and longer linear range intervals. This is in concordance with the increase in surface roughness achieved by gold nanoparticle electrodeposition, as explained above. A linear concentration range of copper five times higher compared with bare UMEAs was achieved. Also, the copper content of contaminated soil extract samples was effectively determined with the modified UMEAs and results are in good agreement with those obtained using a standard method.

Evaluation of GNP-modified UMEAs as platform for the detection of heavy metals

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Evaluation of GNP-modified UMEAs as platform for the immobilization of biomolecules

Oligonucleotide biomolecules

Anchorage of Rodamine red-marked oligonucleotide conjugated with gold nanoparticles over the UMEAs surface was successfully performed using the protocol described in Scheme 1.

The successful anchorage of Rodamine was evidenced by fluorescence microscopy imaging. Figure 4 depicts a picture of an Au UMEA-10 device after modification with the oligonucleotide-modified nanoparticles. Bright red spots are clearly visible on the modified-microelectrode units (vertical arrow). By contrast, no colour is observed on the surface of the passivated ones. This fact demonstrates the effectiveness of the gold UMEA surface modification process.

The same results were observed with the other microelectrode geometries.
Figure 4. UMEA microscopy image taken after electrodeposition of GNPs conjugated with an oligonucleotide attached to the UMEA surface (x20).

Enzyme biomolecules

The GNP-modified UMEAs were also applied to catechol detection. For that, a HRP enzyme were immobilized onto the modified UMEA trough a DTSP SAM as explained in experimental section and showed in Scheme 2.

Scheme 2. Scheme protocol for the anchorage of HRP over the gold nanoparticle-modified UMEA surface.

Cyclic voltammograms, at 0.005 Vs⁻¹, for the prepared electrodes indicate that in all cases they do not show direct electron transfer between a heme group of HRP and the electrode, even in the presence of H₂O₂, resulting in no voltammetric wave. When 0.5 mM catechol as a diffusional mediator was added to the initial PBS background, a dramatic increase of both faradaic and capacitive current density in the oxidative scan sense and a soft increase of them in the reductive one were observed. This is due to the easy accessibility of the diffusional mediator (and oxidation and reduction of it) to the surface of the electrode.

When 0.1 mM H₂O₂ was present in the solution the increase of the cathodic current density evidences the catalytic activity of the HRP-immobilized electrode, which is accompanied by a slight decrease in the anodic current density.

The performance of the prepared electrodes was also evaluated by amperometry in a pH 7.4, 0.05 M PBS background. It was noteworthy a dramatic increase in response of the HRP/DTSP/GNP-modified UMEA respect to the bare UMEAs. Values of the corresponding calibration parameters in the concentration range of linear response show the higher sensitivity of the HRP/DTSP/GNP-modified UMEA (228.6 μAcm⁻²mM⁻¹), which is 3 times higher respect to that attained from the HRP/DTSP/UMEA.

Repeatability and reproducibility of the biosensors preparation procedure were also evaluated using catechol as target analyte. Results have demonstrated the potential usefulness of the obtained platform for the immobilization of biomolecules and the development biosensors.

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