“Green Electrodes” Modified with Au Nanoparticles Synthesized in Glycerol, as Electrochemical Nitrite Sensor

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Abstract: A new environmentally friendly Au nanoparticles (Au NPs) synthesis in glycerol by using ultraviolet irradiation and without extra-added stabilizers is described. The synthesis proposed in this work may impact on the non-polluting production of noble nanoparticles with simple chemicals normally found in standard laboratories. These Au NPs were used to modify a carbon paste electrode (CPE) without having to separate them from the reaction medium. This green electrode was used as an electrochemical sensor for the nitrite detection in water. At the optimum conditions the green sensor presented a linear response in the 2.0×10^{-7}–1.5×10^{-5} M concentration range, a good detection sensitivity ($0.268\ \mathrm{A\,L\,mol^{-1}}$), and a low detection limit of 2.0×10^{-7} M of nitrite. The proposed modified green CPE was used to determine nitrite in tap water samples.

Keywords: Green electrodes · Gold nanoparticles · Synthesis in glycerol · Nitrite sensor

1 Introduction

Gold nanoparticles (Au NPs) have attracted considerable interest due to their chemical and physical properties and potential applications in several scientific fields [1]. Different methodologies, such as reflux, microwave and simple heat [2] for NPs preparation in aqueous solution are commonly used. Usually, NPs are formed by promoting a chemical reaction between a metallic precursor (namely gold or silver) and a reducing agent in water. The same procedure is used for the NP synthesis in reverse micelles [3–5], with or without the addition of stabilizers [1,6,7]. Using this idea, but changing all the chemical parameters, namely metallic precursors, reducing agents, stabilizers, etc., a wide variety of synthetic methods for the production of metallic nanoparticles have been reported [1].

The utilization of environmental friendly methods to produce NPs also called “*green synthesis*” have been employed, reactives like alcohols [8], and polyols [9] and have been effectively recognized as reducing agents and/or capping agents of success. Glycerol, an easily biodegradable solvent in aerobic conditions, plays an important role as a moistening agent and a preservative to extend shelf life, moreover the oxidation products of glycerol are also of great interest for the industry. The development of new cost-effective methodologies to be used in the industry applying Au and palladium (Pd) NPs as catalyst has been reported [10–14]. In this sense, it is well known that the glycerol is an attractive reducing agent and presents the ability to be oxidized to aldehydes and carboxylic acids. Thus, it is expected that those carboxylic groups “protect” and act as stabilizer of the NPs surface [2,15,16]. In this way, the effect of the presence of a capping agent (PVP) and the concentration of reactants (glycerol, tetra chloroauric acid, and NaOH) on the size, homogeneity and polydispersion of the Au NPs formed were investigated [16].

Au NPs feature excellent conductivity, high surface area and catalytic properties [17] that make them excellent materials for the electrochemical detection of a wide range of analytes [18–27]. Moreover, Au NPs have the ability to decrease the overpotentials of many electroanalytical reactions and maintain the reversibility of redox reactions [28]. Several approaches such as electrostatic interaction [29,30], electrochemical deposition [31,32], and mixing with components in a composite electrode matrix [33], have been applied to deposit Au NPs on electrode surfaces.

In scientific databases it is possible to find several reports of NPs synthesis in homogenous media [1,6,16], micellar [3–7], liposomes [14] but, surprisingly there are no reports of NPs synthesis in pure glycerol by using a photochemical system such as ultraviolet irradiation. Furthermore, the NPs synthesized in different media need to be subsequently extracted from the reaction medium which results in a laborious and long process step.

On the other hand, nitrite is largely engaged in the chemistry of the environment and health, so the important roles of nitrite in these areas were acknowledged long ago [34]. Nitrite is an adverse human health chemical which would lead to poisoning [34]. Besides, nitrosamines are formed from nitrite. Two important nitrosamines, namely *N*-nitrosodiethylamine (NDEA) and *N*-nitrosodimethylamine (NDMA), are classified as probably carcinogenic to humans (group 2A) by International Agency for Research on Cancer (IARC) [35] . Also, the nitrite may be associated with the formation of methemoglobin in blood, the so-called methemoglobinemia, an adverse health effect especially in infants [36]. Therefore, it is necessary to develop a reliable and sensitive sensor to detect nitrite in food, drinking water and environmental samples.

In this work, it is described a new environmentally friendly synthesis of Au NPs in pure glycerol, without extra-added stabilizers, using only ultraviolet irradiation for a few minutes. In other words, the glycerol acts as the solvent for the reaction medium (dissolve the metallic precursor) as well as the reducing agent and the oxidation products as stabilizers of Au NPs. These NPs were quickly and easily prepared. The results presented here represent a change of paradigm for the formation of NPs. The synthesis proposed in this work may impact on the production of other NPs with simple chemicals normally found in standard laboratories. Their application may be expanded to other fields, such as to cancer therapy and cancer cell imaging. Modified carbon paste electrodes (CPE) were prepared using the Au NPs synthesized in pure glycerol without separating them from the reaction medium, which allowed to designing a practical and non-polluting sensor. The modified CPE has demonstrated to have exceptional catalytic properties in the response of potassium ferrocyanide ($K_4Fe(CN)_6$), a molecule of known electrochemical behavior. Finally, the property of the modified electrode as "*green sensor*" for the detection of nitrite in water was reported.

2 Experimental

2.1 Chemicals and Solutions

Au NPs were synthesized using gold (III) chloride hydrate ($HAuCl_4$) from SIGMA, USA. Glycerol was Sintorgan, HPLC grade. Sodium perchlorate ($NaClO_4$), sodium nitrite ($NaNO_2$) and potassium ferrocyanide ($K_4Fe(CN)_6$) from Sigma were used as received. The following buffer solution (pH 7.00, PBS) was prepared from their salts (Merck, p.a.): 1×10^{-2} M phosphate buffer solutions, Na_2HPO_4 and NaH_2PO_4, 0.137 M NaCl and 2.7×10^{-3} M KCl. Real samples (tap water) used in the determination of nitrite were prepared in buffer solution pH 7 and used without pretreatment. The results obtained with the developed green sensor were validated and compared with those obtained by using the Griess protocol [37].

2.2 Spectroscopy Measurements

The absorption spectra were measured using a Shimadzu 2401 equipment at 25.0 ± 0.18 unless otherwise indicated. The path length used in the absorption experiment was 1 cm. 1_{max} was measured by taking the midpoint between the two positions of the spectrum where the absorbance is equal to 0.9 A_{max}. The uncertainties in 1_{max} are about 0.1 nm.

2.3 Transmission Electron Microscopy

The TEM micrographs were recorded by using a PHILIPS CM-12 microscope at 20–120 kV with a Megaview-II Docu camera and SIS NT Docu software. For TEM analysis, a drop of the AuNPs was suspended onto copper coated grid. The grids were dried in a desiccator one day and then they were examined.

2.4 Electrochemical Characterization

The measuring system for the electrochemical detection was composed by an EG&G PARC Model 273 potentiostat/galvanostat equipped with a PAR270 electrochemical analysis software. Cyclic voltammograms (CV) were obtained in the range of 10 to 200 $\mathrm{mV\,s^{-1}}$. Characteristic parameters used in most of the experiments to obtain square wave voltammograms (SWV) were: $\mathrm{D}E_{\mathrm{SW}} =$ 25 mV, the staircase step height ($\mathrm{D}E_{\mathrm{s}}$) = 5 mV and the frequency was 20 Hz. Electrochemical measurements were performed in a two-compartment Pyrex cell. The working electrode was carbon paste (CP) disks.

The CP electrode (Bioanalytical Systems, Inc., 1.6 mm i.d., 3 mm o.d.) was pretreated by placing a new thin layer of CP prior to each experiment and then polishing the CPE in a soft glass paper. The CP was composed of uniform graphite particles mixed with paraffin binder (Bioanalytical Systems, Inc.). The CPE surfaces obtained were electrochemically highly reproducible. The modified CPE were prepared as follows: 170 mg carbon paste was homogeneously mixed with different volumes (mL) of Au NPs prepared in glycerol. For the NO_2^- assessment, it was used 250 mL of Au NPs. The reference electrode was an aqueous saturated calomel electrode (SCE) fitted with a fine glass Luggin capillary containing a bridge solution identical to that of the sample being measured. Solutions were deaerated by bubbling purified nitrogen for at least 10 minutes prior to the measurements.

2.5 Preparation of Gold Nanoparticles (Au NPs) in Glycerol

For the Au NPs synthesis, a solution of 1.15 mM $HAuCl_4$ in glycerol was placed in a 2 mL quartz cuvette and exposed to ultraviolet irradiation (300 nm) during 8 minutes. The Rayo Net chamber lamps (Model RPR-100) are controlled by dual switches located on the front panel. Reactor chamber is 16” deep, 10” in diameter, with 2/8” openings at top.

The Au NPs colloidal solutions showed a wine-red color and were stored in a dark glass bottle at + 6 8C for further use. The Au NPs are stable for two month and their stability was checked through UV-visible spectroscopy following the band corresponding to the surface plasmon resonance (SPR) around 560 nm. Note that Au NPs were not extracted from the reaction medium, that means, the Au NPs were used as synthesized and blended (like this) to the CPE for the electrochemical studies.

3 Results and Discussion

3.1 Au NPs Synthesis in Glycerol

In contrast to many studies where Au NPs are formed by the contact of two or more reactants solubilized in water [6] or micellar systems [3–5,7] and then the NPs are separated from the reaction media in order to use them, in this work, it is reported a simple approach to synthesize Au NPs in glycerol by using ultraviolet irradiation for a few minutes without having the need to separate them from the reaction medium. It was verified that an irradiation time of 8 minutes it was enough to obtain Au NPs, which is an advantage in comparison with other reported reaction media [2]. In order to characterize morphologically the synthesized Au NPs, it was used different approaches such as spectroscopic technique and transmission electron microscopy (TEM).

Figures 1a and b show the absorption spectra and pictures of glycerol solutions of the i) metallic precursor, ii) AuNPs synthesized after 8 minutes of irradiation iii) Au NPs aggregated after two month of storage, respectively. As shown, when the Au NPs were formed, the particles suspensions turned pink along the time and appeared a band corresponding to the surface plasmon resonance (SPR) around 560 nm, similar to that observed in Au NPs formed into glycerol-water mixtures [16]. It is known that the optical properties of spherical Au NPs can be analyzed, both theoretically and experimentally in water following the maxima wavelength [38,39]. Moreover, Chen and collaborators [40] suspended Au NPs with different morphologies (nanospheres, nanocubes, nanobranches, nanorods, nanobipyramids) in water-glycerol mixtures and studied the relationship with the refractive index of the mixtures. Furthermore, the wavelength of NPs synthesized by Chen matches the morphology of Au nanospheres that were suspended and could indicate that the Au NP synthesized in this work present a similar morphology. The colloidal solution prepared without the addition of any extra-added stabilizer remains stable in glycerol for a period of two months. After this period of time the suspension turned blue due to the agglomeration of the Au NPs (Figure 1a (iii) and Figure 1b (iii)).

The spectra (Figure 1a (iii)) shows that the absorbance corresponding to the nanoparticles decreases and shifts to the red, indicating that the Au NPs are aggregated. Pandian et al. [2] also synthesized Au NPs in glycerol but using reflux and microwave systems, in both cases small amounts of PVP were required for stabilization of Au NPs for two months. In addition, another research [15] have suggested that the presence of oxygen in the glycerol leads to a short induction period, indicative of the primordial use of some glycerol radicals in the system, to consume the dissolved oxygen. In this work, we thought that the fast exposing to ultraviolet irradiation, the viscosity of glycerol and, the oxidation products of glycerol (aldehydes and carboxylic acids) are responsible for the high stability of the Au NPs.

Figure 2 shows TEM images of the Au NPs synthesized in glycerol after one day of dried.

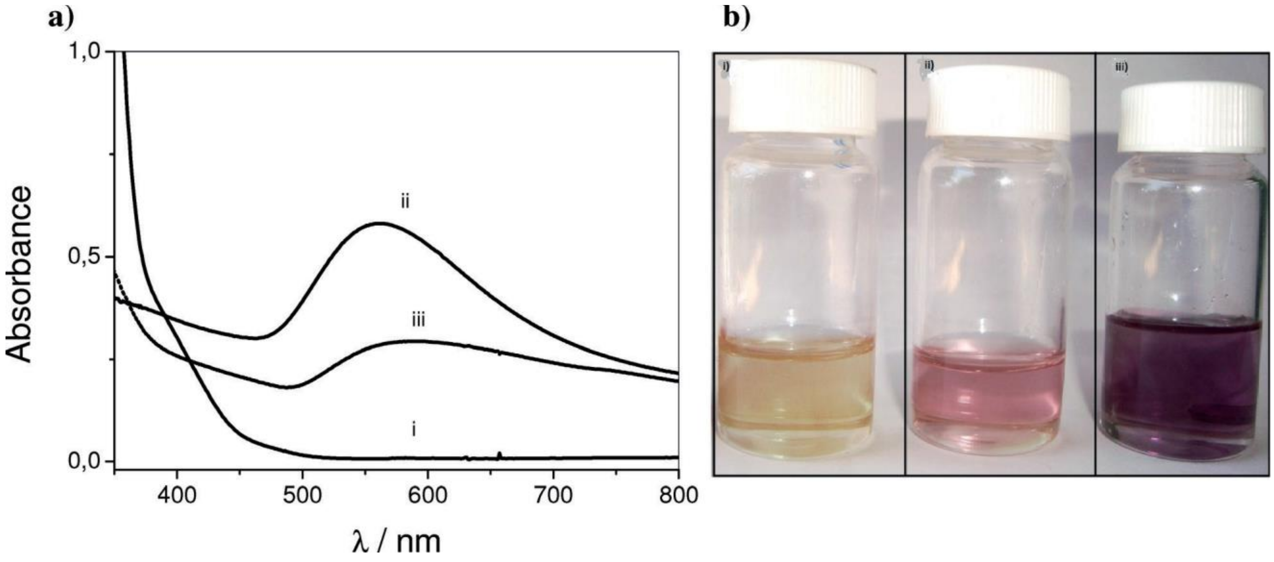

Fig. 1. a) UV-Vis spectra in glycerol. i) the $HClAu_4$ precursor, ii) the Au NPs synthesized and iii) the Au Nps aggregate. b) Images corresponding to UV-Vis spectra i) the $HClAu_4$ precursor, ii) the Au NPs synthesized and iii) the Au NPs aggregate.

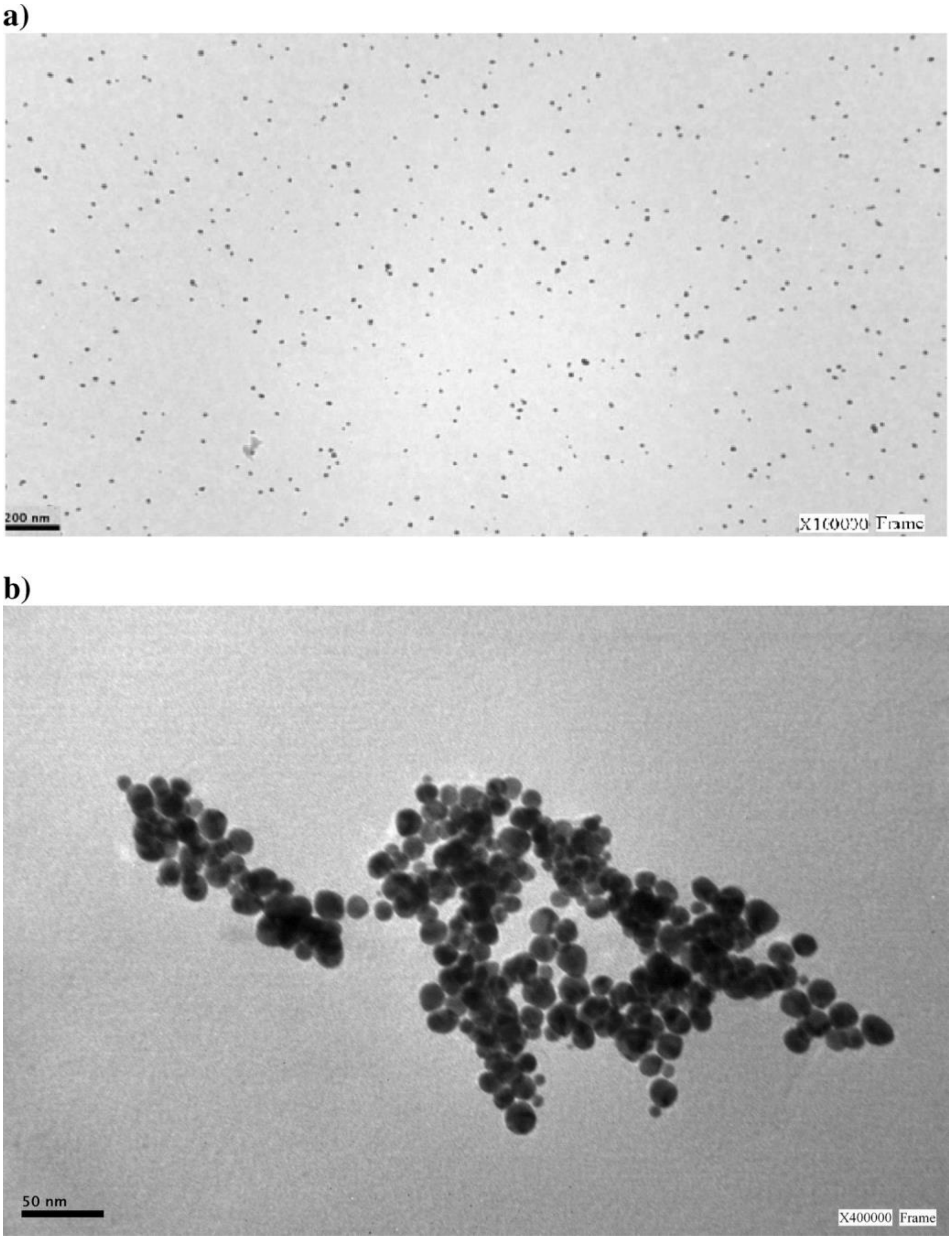

Fig. 2. TEM images at different scales of Au NPs synthesized in glycerol at 8 minutes by ultraviolet irradiation. a) 200 nm, b) 50 nm.

As it is possible to observe, spherical shape Au NPs with size less than 15 nm were obtained. It is supposed that due to the homogeneous and quick irradiation, the reduction and nucleation of the metal was accelerated. Thus, many of Au(0) nuclei was formed and higher crystallization and reproducibility was obtained in glycerol.

This characterization allows us to conclude that the method used for the formation of Au NPs is fast and easy. In addition, a high degree of reproducibility and homogeneous distribution of small Au NPs was obtained.

3.2 Electrocatalytic Properties of Au NPs Synthesized in Glycerol

Due to the simple and well-defined responses of redox models {e.g., the $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$} couples, they have been widely used to characterize the surface properties of different nanoparticles modified electrodes [41]. For this reason, an electrochemical studies using $K_4Fe(CN)_6$ as molecular probe and the modified CPE with the Au NPs synthesized in glycerol, were carried out. Also, the electrochemical response using unmodified carbon paste electrodes were investigated for comparison.

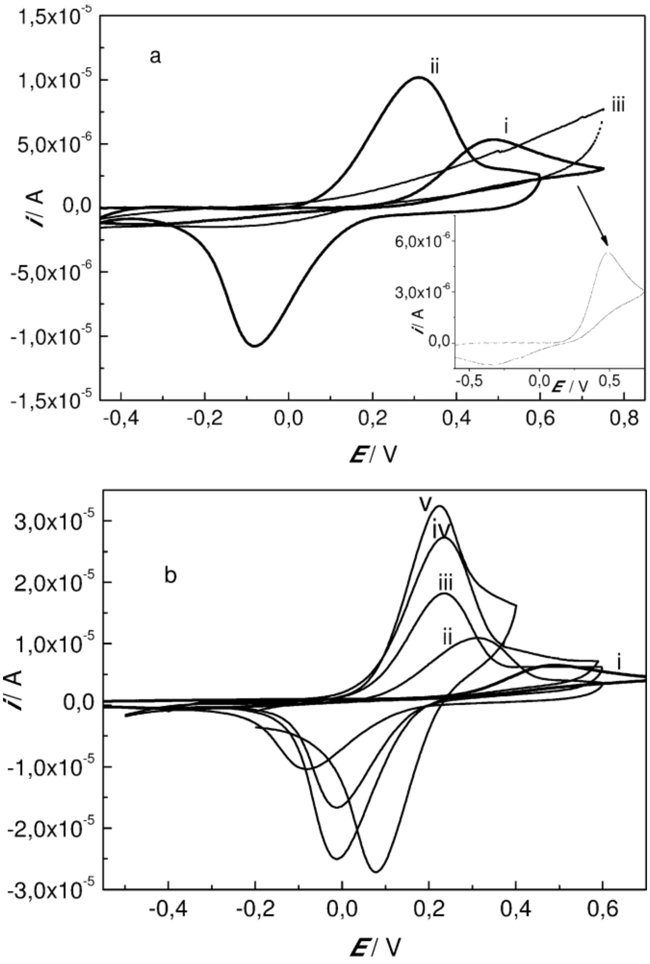

Fig. 3. Cyclic voltammograms of $K_4Fe(CN)_6$ generated in water using 0.1 M $NaClO_4$ as supported electrolyte for a) (i) unmodified CPE, (ii) modified CPE with 170 mg carbon paste + 100 mL Au NPs in glycerol and (iii) modified CPE with 170 mgr carbon paste+ 100 mL glycerol without Au NPs. b) modified electrodes with different carbon paste: (i) unmodified CPE, (ii) modified CPE with 100 mL, (iii) 150 mL, (iv) 200 mL and (v) 250 mL Au NPs in glycerol. $c^{*}_{K4Fe(CN)6}$ 1×10^{-3} M; $v = 0.05$ V s^{-1}.

The voltammetry of the standard redox process was examined in aqueous solutions using 0.1 M $NaClO_4$ as supported electrolyte in order to investigate how the Au NPs in the modified electrodes affect the electron-transfer kinetics in comparison with the one obtained using the unmodified electrodes. The electrochemical properties of Au NPs modified electrodes significantly depend on their surface properties which also depend on the NPs size [17].

The Figure 3a shows a cyclic voltammogram of $K_4Fe(CN)_6$ generated in water using 0.1 M $NaClO_4$ as supported electrolyte for different CPE. In Figure 3a (i) it can be possible to see, that when an unmodified CPE is used, an oxidation peak is observed whose anodic potential peak (E_{pa}) value is 450 mV, and an reduction peak at −344 mV. Figure 3a (ii) shows the same experiment but using the modified CPE with 100 ml of the Au NPs synthesized in glycerol. As it is observed, the E_{pa} appears around 300 mV and shows the corresponding reduction potential peak (E_{pr}) at −80 mV which means that the electron transfer becomes more reversible because there is a decreases of the E_{pa} (around 150 mV) and the difference in the peak potential (D$E_p = E_{pa} - E_{pr}$) also decreases [42].

In Figure 3a (iii) a cyclic voltammogram recorded using a CPE with 100 mL of glycerol without Au NPs is also represented. In this case, an increase in the anodic current was observed, but no oxidation or reduction peaks were defined. It seems that the presence of glycerol hinders the $K_4Fe(CN)_6$ signal definition. This result confirms that the liable for the discharge observed in the cyclic voltammogram of Figure 3a (ii) is due to the presence of the Au NPs in the modified CPE.

Cyclic voltammograms were carried out for CPE modified with different volume of Au NPs synthesized in glycerol. The data plotted in Figure 3b (i–v) shows that the redox couple becomes more reversible and the peak current (i_p) increases markedly with the increasing of the Au NPs volume in the mixture. Furthermore, the anodic peak (i_{pa}) and the reduction peak (i_{pr}) current separation and the electron-transfer kinetics of Au NPs modified electrodes changes with the amount of Au NPs used in the CPE. An excess amount of NPs usually gives no further improvement or increases the resistance of the double-layer capacitance of the modified electrode, lead-ing to a decrease in the electrochemical sensitivity and in- crease in peak-to-peak separation [43].

In order to characterize the electronic transfer process, it was analysed the effect of the potential scan rate (v) on the i_{pa} using freshly prepared CPE. In this way, cyclic voltammograms were obtained for 3×10^{-3} M $K_4Fe(CN)_6$ aqueous solution at different v. The relationship between the i_{pa} and the v was studied and it was verified that there is no a linear behavior between i_{pa} with $v^{1/2}$ or v, which indicates that the electron transfer process is neither diffusional nor adsorption pure (data not shown). i_{pa} $v^{-1/2}$ versus $v^{1/2}$ plot was linear, (slope: $(9.4 \pm 0.3) \times 10^{-4}$ A s V^{-1}, intercept: $(1.97 \pm 0.1) \times 10^{-4}$ $AV^{-1/2}s^{1/2}$, $r = 0.99$), showing a mixed control for the electrode process [44]. This result confirms that, under these conditions, there are two processes involved for the electroactive species: adsorptive and diffusional.

Figures 4a and 4b shows that with the increasing of the amount of the Au NPs in the modified CPE a decreases of the E_{pa} and DE_p is verified This means that Au NPs confers electrocatalytic properties for the $K_4Fe(CN)_6$ discharge and turn the system into a more reversible process.

Figure 4c shows the effect of the amount of Au NPs used in the CPE on the i_{pa}. As it is possible to verify, the current increases with the amount of Au NPs present in the CPE, indicating that the presence of the Au NPs are responsible for the increase in the electrochemical current response. There is a current increase of five times more when compared with those obtained by using unmodified electrodes.

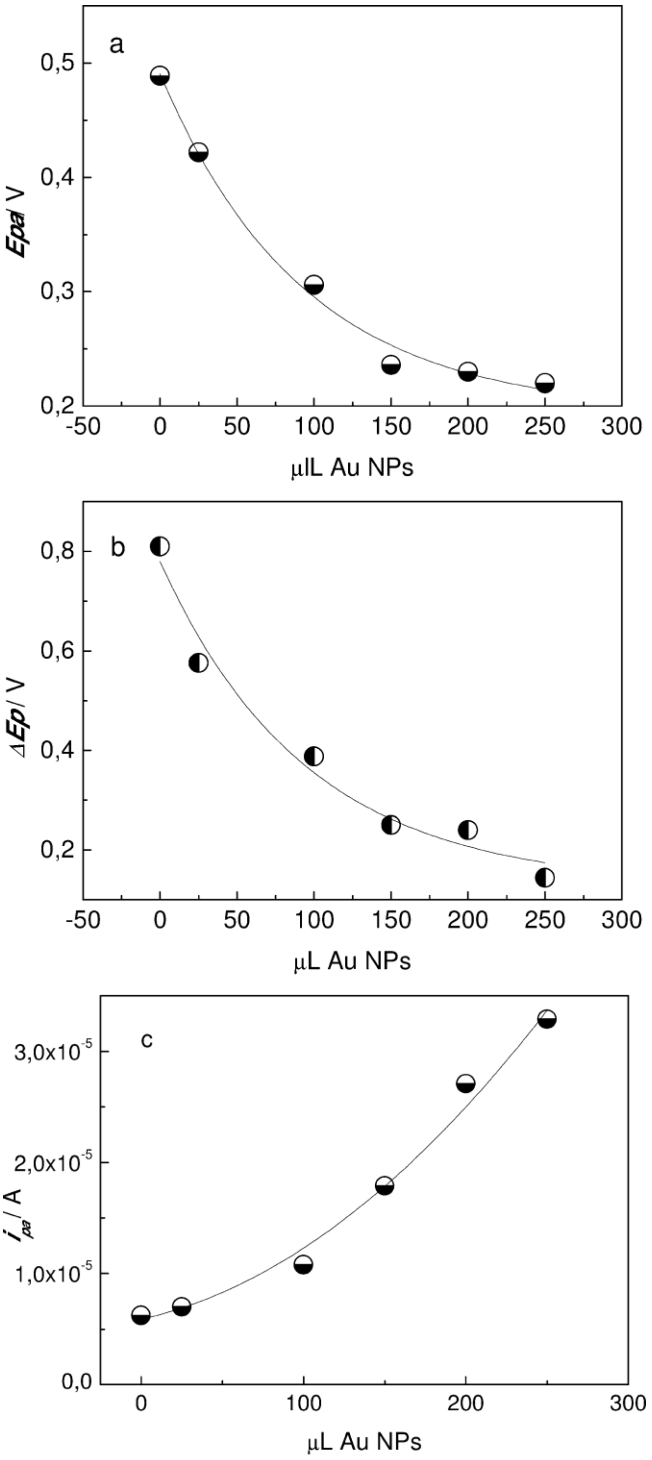

Fig. 4. Variation of the E_{pa} (a), DE_p (b) and I_{pa} (c) increasing the mL of Au NPs in 170 mgr carbon paste. $c^*_{K4Fe(CN)6}$ 1×10^{-3} M; $v = 0.05$ V s^{-1}

This drastic gain in the electrochemical current has never reported for Au NPs modified CPE which infer that this increase is due to the presence of Au NPs in glycerol contained in the modified CPE. It is acceptable that the smaller particles modified electrode with higher active surface area is expected to show a better electron-transfer process.

Due to the excellent electrocatalytic properties observed with $K_4Fe(CN)_6$ used as a molecular probe, studies was performed in order to apply this modified CPE as sensor for the detection and quantification of nitrite in water.

3.3 Application of Sensor for the Detection of Nitrite in Water

3.3.1 Determination of Nitrite in Phosphate Buffer Solutions

The analyte selected in order to understand the analytical potential of the Au NPs modified CPE was the sodium nitrite ($NaNO_2$). Since in the drinking water pH should be within 6.5 and 8.5 [45], cyclic voltammetric studies by using nitrite solution in phosphate buffer solution at pH 7 and a scan rate of 0.025 V s^{-1} was carried out. This study demonstrated that the oxidation of nitrite was viable using the Au NPs modified CPE as shown in Figure 5 (i). The electrochemical oxidation NO_2^- signal appears at 200 mV less than when an unmodified CPE was used (Figure 5 (ii)). Moreover, the current increases more than four times compared with the unmodified CPE (Figure 5 (ii)). This change shows the practical application of the modified CPE with Au NPs which confers electrocatalytic properties in the detection of nitrites.

The Au NPs provide the necessary conduction pathway besides it act as nanoscale electrode by promoting the electron transfer between the analyte and the CPE surface [46,47].

The net current-potential curve in Square Wave Voltammetry is the most useful analytical signal in electrochemical detection [48], The determination of NO_2^- was carried out on Au NPs modified CPE in unstirred solutions composed by buffer aqueous solution in pH 7 (Figure 6).

A linear relationship between net peak current ($I_{p,n}$) vs c^*_{NaNO2} was obtained at $f = 20$ Hz in the range of 0.20 mM

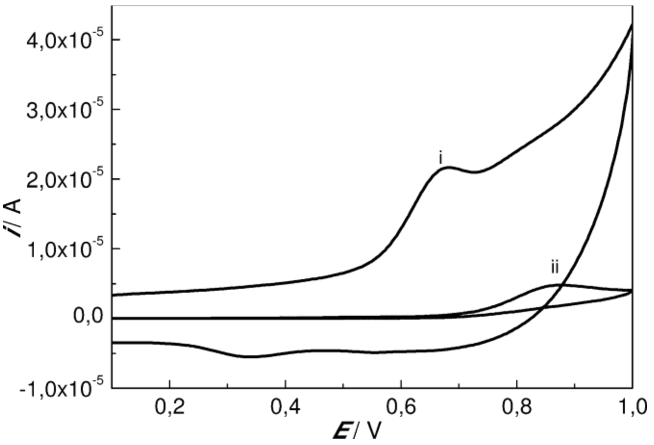

Fig. 5. Cyclic voltammograms of $NaNO_2$ 1.00×10^{-5} M generated in buffer solution pH 7 for (i) modified CPE with 250 mL Au NPs in glycerol (ii) unmodified CPE. $V = 0.025$ V s^{-1}

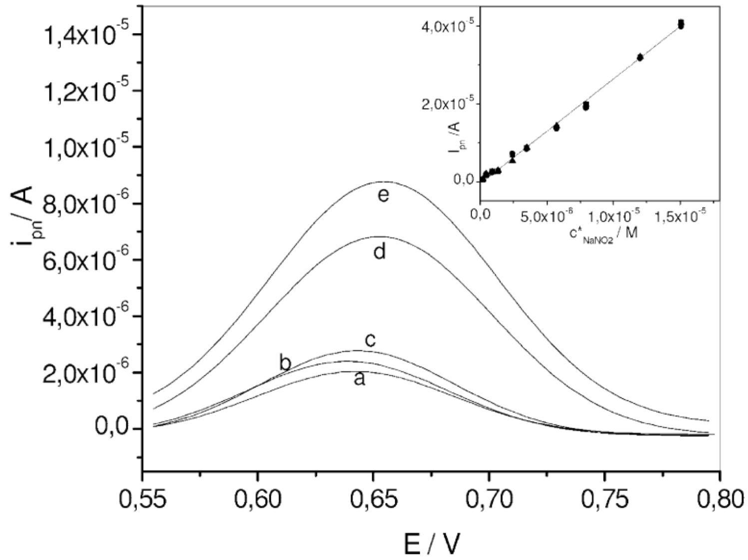

Fig. 6. Square wave voltammograms for $NaNO_2$ in buffer solution pH 7 at different concentrations: i) 4.00×10^{-7} M, ii) 9.02×10^{-7} M, iii) 1.35×10^{-6} M, iv) 2.42×10^{-6} M and v) 3.5×10^{-6} M. Inset: Calibration curve for $NaNO_2$ in buffer solution pH 7. D$E_{SW} = 0.025$ V, D$E_s = 0.005$ V, $f = 20$ Hz.

Table 1. Comparison of the characteristic of some carbon paste modified electrodes in the detection of nitrite. CV: cyclic voltammetry; LSV: linear sweep voltammetry, VPD: voltammetry pulse differential; SWV: square wave voltammetry; *LOD*: detection limit.

Detection technique	Range linear (M)	*LOD* (M)	Potential detection (V)	Reference
Amperometry	5.0×10^{-6}–1.5×10^{-2}	8.0×10^{-7}	0.95	[49]
SWV	7.2×10^{-7}–1.0×10^{-4}	6.25×10^{-7}	0.97	[50]
Amperometry	5.0×10^{-6}–1.2×10^{-3}	2.8×10^{-6}	−0.25	[51]
CV	0–1.38×10^{-2}	1.45×10^{-6}	0.9	[52]
DPV	5.0×10^{-7}–1.0×10^{-4}	3.0×10^{-7}	0.83	[53]
LSV	5.0×10^{-7}–1.5×10^{-3}	2.0×10^{-7}	0.78	[54]
SWV	5.0×10^{-8}–2.5×10^{-4}	1.0×10^{-8}	0.95	[55]
SWV	2.0×10^{-7}–1.5×10^{-5}	2.0×10^{-7}	0.65	This work

to 15 mM (see Inset Figure 6). The linear regression can be expressed by a least square procedure as:

$$I_{p,n} = (0.268 \pm 0.002)\ c^{*}_{NaNO2} - (3.9 \pm 0.1) \times 10^{-7} \quad (r = 0.997) \tag{1}$$

where $I_{p,n}$ is expressed in Amperes and c^{*}_{NaNO2} in M.

Data used in the regression analysis of the calibration curve are the average of three replicated measurements (*RSD*: 4 %). The limit of detection defined as *LOD* = 3Sb/m, where *LOD*, Sb and m are the limit of detection, standard deviation of the blank and the slope of the calibration graph, respectively. The *LOD* was found to be 2.0×10^{-7} M (0.014 mg mL^{-1}), this value is lower than the maximum allowed value for drinking water (0.5 mg mL^{-1}) [45]. Sb was estimated by ten replicate determinations of blank signals. The sensor showed a very good stability insomuch as for two months still retaining about 95 % of the sensitivity for the determination of the $NaNO_2$.

The selectivity of the sensor was studied by investigating of the effects of some common cations and anions present in drinking water. The results showed that adding 40-folds of K^{+}, Ca^{2+}, Mg^{2+}, F^{-}, Cl^{-}, NO_3^{-} and SO_4^{2-} not influences in the determination of the nitrite, indicating that the sensor had a good selectivity to nitrite.

There are many works in literature related to the detection of nitrite using modified CPE. Table 1 summarizes some of the more recent [49–55]. Note that the detection limit and the linear range are comparable with other values reported by other studies for nitrite detection with carbon paste modified electrodes but this sensor present the advantage that the detection was performed at lower detection potential than that most of the recently reported (Table 1). Besides, these methods usually include inherent disadvantages such as a strong commitment of time, labor and expense [50–53].

3.3.2 Determination of Nitrite in Tap Water Samples

In order to examine the possible use of the proposed modified CPE, experiments were performed in three tap water samples for determination of nitrite, using the standard addition method. The results were listed in the Table 2. In all cases, the RSD for each sample was less than 5 %. The recoveries for the method were investigat-

Table 2. Determination of nitrite in three tap water samples.

Sample	Content (mg mL^{-1})	Added (mg mL^{-1})	Found (mg mL^{-1})	Recovery (%)
1	0.250	0.069	0.325	101.9
2	0.200	0.069	0.268	99.6
3	0.180	0.069	0.250	100.4

ed and the values obtained were 101.9 %, 99.6 % and 100.4 %, respectively. The experimental data indicated that this proposed electrode could be successfully applied for the detection of nitrite in drinking water samples.

4 Conclusion

For the first time Au NPs were synthesized in pure glycerol by using ultraviolet irradiation without extra-added stabilizers. This synthetic method is quick, easy, inexpensive and non-polluting. Au NPs of small sizes (< 15 nm) were obtained and were stable for two months. The results presented here represent a change of paradigm for the formation of NPs and using a simple chemicals normally found in standard laboratories may impact on the production of NPs.

The modified CPE presented larger surface area, with the advantage that it was not necessary to separate the Au NPs from the reaction medium in order to make the modified electrodes, allowing the practical design of a sensor as well as easy preparation. Thus, it is possible to develop "*green*" electrochemical sensors with lower detection limits for nitrite in water (2.0×10^{-7} M), good linear range, reproducibility and very good stability.

Au NPs synthesized in pure glycerol has shown remarkable electrocatalytic properties for the $K_4Fe(CN)_6$ and $NaNO_2$ discharge, the increases in current are markedly higher than the one found using unmodified electrodes, result that open the possibility of using them in the construction of nanostructured electrodes for electroanalytical purposes.

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