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Electrocatalytic Oxidation of Acetaminophen on a PVC/TTF-TCNQ Composite Electrode Modified by Gold Nanoparticles: Application as an Amperometric Sensor

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This work shows the electrocatalytic activity towards the oxidation of acetaminophen of a composite electrode PVC/TTF-TCNQ modified with gold nanoparticles. Owing to a good linear relationship between acetaminophen concentration and current response at lower oxidation potential it is proposed as a new alternative for amperometric determination of acetaminophen. Besides the good catalytic activity (E_{app} = 0.425 V), the proposed sensor shows high reproducibility, long-term stability, low cost and can be used both in batch and in FIA systems. In batch, it was obtained a linear range for acetaminophen over a range of 1 to 800 µM with a detection limit of 0.66 µM and a correlation coefficient of 0.997 with a slope (sensitivity) of 53 mA M^{-1}. In FIA, a sensitivity of 9.9 ± 0.15 mA M^{-1} and a linear regression coefficient (r) of 0.999 were obtained for a linear range for acetaminophen between 1 to 800 µM. The detection limit (s/n = 3) was 8.9 µM.

Keywords: TTF-TCNQ, Composite sensor, Gold nanoparticles, Acetaminophen, Flow injection.

1. INTRODUCTION

Acetaminophen, or paracetamol (N-acetyl-p-aminophenol), is an antipyretic and minor analgesic drug without anti-inflammatory action in practice. It is an effective and safe analgesic agent used worldwide for the relief of mild to moderate pain associated with headaches, backaches, arthritis, and postoperative pains. It is also used for reduction of fevers of bacterial or viral origin [1, 2].

Generally acetaminophen does not exhibit harmful side effects but hypersensitivity or overdoses in few cases leads to the formation of some liver and nephrotoxic metabolites [3, 4]. So it is very important to establish a simple, fast, sensitive and accurate detection method for acetaminophen.

Many methods have been described in the literature for determination of this drug including spectrophotometry [5–7], chemiluminescence [8, 9], liquid chromatography [10], capillary electrophoresis [11, 12], colorimetry [13], titrimetry [14], FTIR-spectrometry [15] and flow injection analysis (FIA) with UV–visible spectrophotometric detection [16–19]. However, these methods require special reagents or are time-consuming, and thus not constitute rapid analytical methods.

Electrochemical methods of determination (voltammetric or amp) represent a rapid and inexpensive analytical alternative [20-23]. These methods can include chemically modified electrodes [24–27] or boron doped diamond electrodes [2, 28] in order to improve the sensitivity. Owing to the some interesting characteristics of gold nanoparticles such as high surface-to-volume ratio and high surface energy, several reports have been published recently for the determination of acetaminophen by using nanoparticles or carbon nanotubes modified electrodes [24, 29-33].

In this work we investigated the electrocatalitic oxidation of acetaminophen on a PVC/TTF-TCNQ composite electrode modified by gold nanoparticles. The presence of these nanoparticles enhanced the oxidation current of acetaminophen and shifted its oxidation potential with respect to the non modified electrode. The affecting variables such as the applied potential, the gold loading into the electrode matrix and the hydrodynamic parameters, were optimized and the resulting electrode is proposed as a suitable electrochemical sensor for the determination of acetaminophen both in batch and in a FIA system.

2. EXPERIMENTAL

2.1. Reagents and solutions

TTF-TCNQ salt (purum grade, Fluka), poly(vinyl chloride) (PVC, low mol. weight, purum grade Fluka) and *N*,*N*´-dimethylformamide (DMF, ≥ 99.7% pure, Merck) were used to prepare the PVC/TTF-TCNQ composite electrode.

Aqueous 1% $HAuCl_4 \cdot 3H_2O$ solution (Sigma, > 49% as gold) and 1% sodium citrate solution were used for the preparation of colloidal gold.

Stock solutions of 0.05 M Acetaminophen, (Sigma ultra, minimum 99%) were prepared in 0.05 M phosphate buffer (pH 7.4). More diluted standards were prepared by suitable dilutions with the same phosphate solution. All solutions were prepared freshly. All other chemicals were from Merck or Fluka (analytical reagent-grade) and used as received.

All solutions were made in water from a Millipore Milli-Q system (18 mΩ).

2.2. Instrumentation and electrodes

Electrochemical measurements were made by using an Autolab PSTAT 12 potentiostat using the software package GPES 4.9. A three electrodes cell (10 ml glass electrochemical cell) equipped

with a Pt wire counter electrode, a BAS MF-2079 Ag/AgCl 3 M KCl reference electrode, and a PVC/TTF-TCNQ/Au_{coll} as the working electrode, was used. All tests were performed at ambient temperature (*ca.* 25 ºC).

The UV-Visible spectra of nanoparticles of Au colloidal were recorded by using a Cary 100 Bio spectrophotometer equipped with a 1 cm path length cell.

The flow system (Figure 1)consists of a Dynamax peristaltic pump model RP-1 (Rainin), a six-way injection valve (Omnifit), PTFE tubes of 0.5 mm inner diameter (Omnifit) and an amperometric detector integrating both the working and counter electrodes. The flow cell was a Metrohm model 6.5303.020 wall-jet cell. The injection volume (sampling loop) was 60 µl.

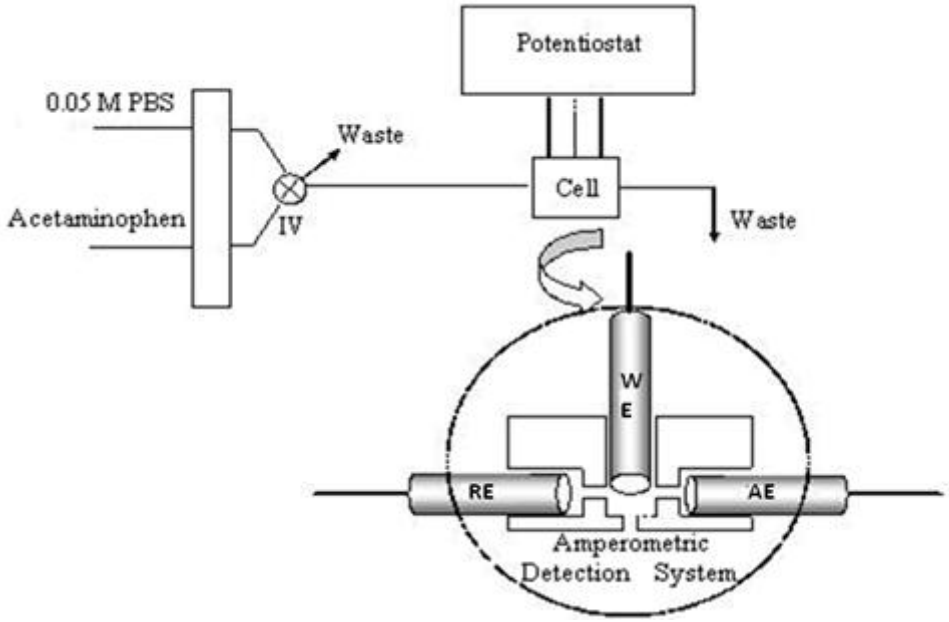


Figure 1. Manifold employed for acetaminophen detection. P: peristaltic pump, IV: six-way injection valve, RE, AE, and WE: reference, auxiliary and working electrodes, respectively.

2.3. Procedure

2.3.1. Preparation of Au_{coll}

Gold colloids were prepared according to the procedure previously described in reference [34]. After cleaning all glassware used in a bath of freshly prepared 3:1 HNO_3-HCl, rinsing thoroughly in twice-distilled water, and drying in air, 2.5 ml of sodium citrate solution were added to 100 ml of a boiling aqueous solution containing 1 ml 1% (w/w) $HAuCl_4 \cdot 3H_2O$. The surface plasmon absorption band of the stable red colloidal solution obtained was observed by UV-vis spectra. The absorption spectra presented a maximum of the SPR band close to 510 nm (Figure 2). So, the diameter of the Au_{coll} particles must be in the nanometers range [35, 36]. Preparations were stored in dark glass bottles at 4 ºC for further use.

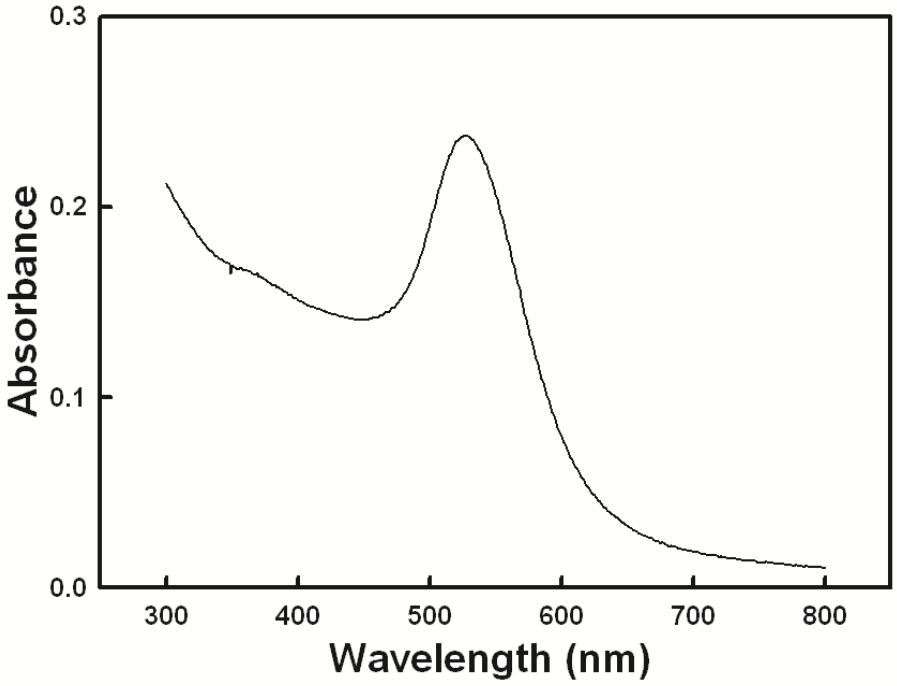


Figure 2. UV-Vis spectra of gold nanoparticles in water measured in the range of wavelength 300 – 800 nm with a 1 cm path length cell.

2.3.2. PVC/TTF-TCNQ/Au$_{coll}$ electrode

The PVC/TTF-TCNQ/Au$_{coll}$ composite electrode was prepared as follows: Polyvinyl chloride was thoroughly dissolved in DMF and then the TTF-TCNQ salt was added to obtain a homogeneous PVC/TTF-TCNQ mixture. Subsequently, the suspension was separated, and different volumes (0.5, 2 y 5 mL) of the colloidal gold suspension were added and dried in order to obtain the composite powder.

The composite powder was pressed into cylindrical pellets 4 mm in diameter and 2.5 mm thick with an active surface area of 12 mm^2. Composite pellets were inserted by press-fitting into a Teflon tube furnished with a copper wire to establish electrical contact.

The electrode surface was regenerated as required by polishing with P-1200 emery paper to obtain a flat surface, followed by polishing with glossy paper to obtain a uniform surface and rinsing with water.

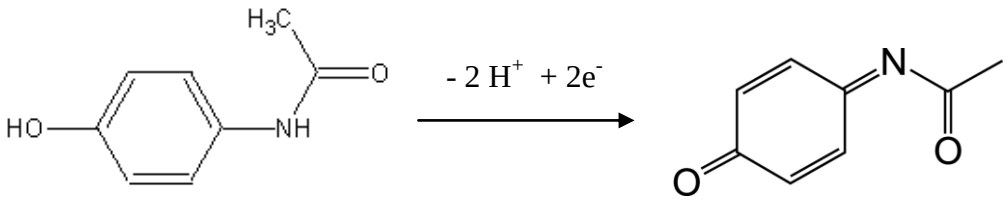
3. RESULTS AND DISCUSSIONS

3.1. Cyclic voltammetry studies of PVC/TTF-TCNQ/Au$_{coll}$ electrodes for detection of acetaminophen

First of all, in order to delimit the potential range in which there is no risk of decomposition of the organic salt, a cyclic voltammogram was registered by using the PVC/TTF-TCNQ/Au$_{coll}$ in the PBS buffer. The TTF-TCNQ salt started becoming oxidized at 0.450 V vs. Ag/AgCl, and the reduction

started at –0.15 V. Therefore, it was possible to polarize the PVC/TTF-TCNQ/Au_{coll} at any potential between –0.1 and 0.450 V without the occurrence of problems associated with the salt decomposition.

Kissinger et al. [37, 38] investigated the electrochemical oxidation of acetaminophen through cyclic voltammetric studies. The first reaction step is an electrochemical oxidation involving two electrons and two protons to generate *N*-acetyl-*p*-quinoneimine:



All subsequent reaction steps are non-electrochemical.

Figure 3 shows cyclic voltammograms obtained at a scan rate of 0.01 Vs^{-1} in 0.05 M PBS at pH 7.4 and different Au_{coll} loading into the electrode matrix (expressed as ml of the colloidal solution added to the composite electrode). The electrocatalytic effect of gold nanoparticles is significant. Without Au_{coll} loading into the electrode matrix (Au 0 ml curve in Fig. 3) the current due to oxidation of acetaminophen is practically negligible within the working range of the electrode. However, in the presence of Au_{coll} loading into the electrode matrix the oxidation of acetaminophen starts about 0.375 V and the intensity increases with the potential, although it does not give rise to a well defined peak within of this potentials range.

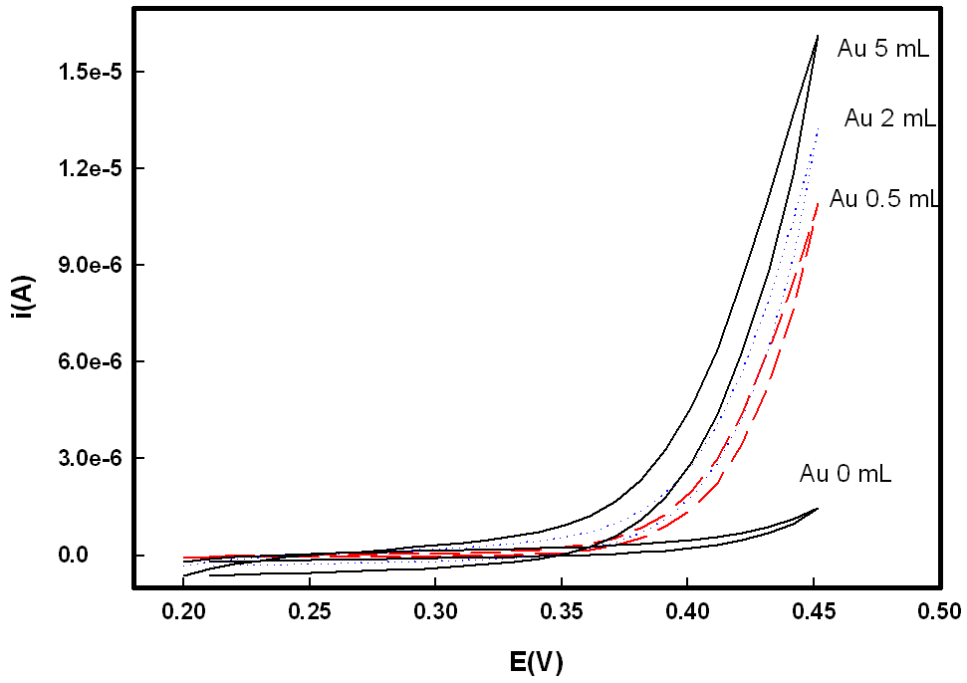


Figure 3. Cyclic voltammograms corresponding to the different Au_{coll} concentrations loading into the composite electrode: 0, 0.5, 2 and 5 ml, for 0.8 mM acetaminophen. PBS solution (0.05 M, pH 7.4).

This electrocatalytic effect provides a potential oxidation of acetaminophen less positive than those obtained from other electrodes modified with gold nanoparticles, such as carbon paste [29], indium tin oxide [27] or 1,6 hexanedithiol modified Au electrode [30], what makes this system interesting as possible amperometric detector to be working at a lower overpotential.

In Figure 3 we can observe that the current response is proportional to the Au_{coll} concentration. Nevertheless, it must be pointed out that there are reproducibility problems when sensors prepared with amounts above 5 ml Au_{coll} are used. This is due to a lack of homogeneity and that decreases the mechanical stability, hardness and compaction of the composite electrode.

According to these results, the best response for electrocatalytic oxidation of acetaminophen under voltammetric conditions is obtained with the PVC/TTF-TCNQ/Au_{coll} 5 mL electrode.

3.2. Chronoamperometric detection of acetaminophen

The acetaminophen calibration curves were obtained from the corresponding chronoamperometric response recorded in the range 0.400 – 0.450 V after successive additions of acetaminophen under stirring conditions, with different Au_{coll} loading into the electrode matrix.

Based on the results, we can derive the following comments:

a) The higher the potential applied to the chronoamperometric calibration the greater intensity obtained. However, for values above 0.425–0.450 V, records began to be somewhat influenced by the rupture of the TCNQ-TTF salt. Therefore, it has been chosen the potential of 0.425 V as the optimal compromise value.

b) The more gold loadings into the electrode matrix the better the response intensity, similarly to the results obtained in voltammetry (Figure 3). Furthermore, the analytical characteristics are also improved by increasing the amount of colloidal gold (Figure 4, Table 1). This confirms the catalytic role that carries the gold in the oxidation of acetaminophen. Therefore, the PVC/TTF-TCNQ/Au_{coll} 5 mL electrode was selected for this study.

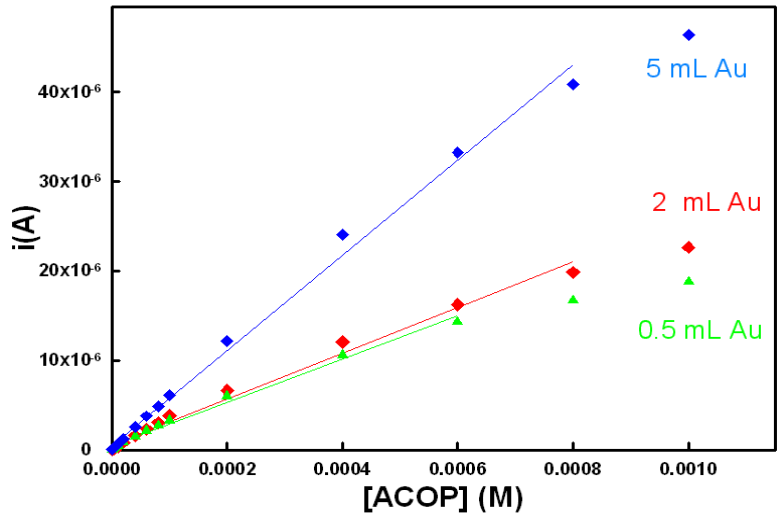


Figure 4. Acetaminophen calibration curves corresponding to the different Au_{coll} concentrations loading into the composite electrode. PBS solution at pH 7.4. The applied potential is 0.425 V.

Table 1. Analytical characteristics obtained from calibration plots for acetaminophen which different colloidal gold loading into in the composite electrode. Applied potential: 0.425 V.

SENSOR (Au_{coll} loading)	Limit of detection (μM)	Slope (mA/M)	Linear range (μM)	r^2
0.5 mL	1.1	24 ± 0.6	1 - 600	0.9913
2 mL	0.83	28 ± 0.7	1 - 800	0.991
5 mL	0.66	53 ± 1	1 - 800	0.997

3.3. Amperometric detection of acetaminophen in a FIA system

Prior to the application in a FIA system, it was examined the influence of hydrodynamic variables such as flow rate and injected volume on the response of the proposed sensor.

The best results in terms of sensitivity and reproducibility were obtained at a flow rate of 1 ml min^{-1} and an injected volume (sampling loop) of 60 μL. Moreover, the most suitable potential was found to be 0.425 V for these conditions.

The reproducibility (n=5) was checked on several consecutive injections of a 0.1 mM acetaminophen solution. A relative standard deviation of 1.9 % was obtained, which indicates a good reproducibility and therefore a good mechanical resistance of the electrode under flowing conditions.

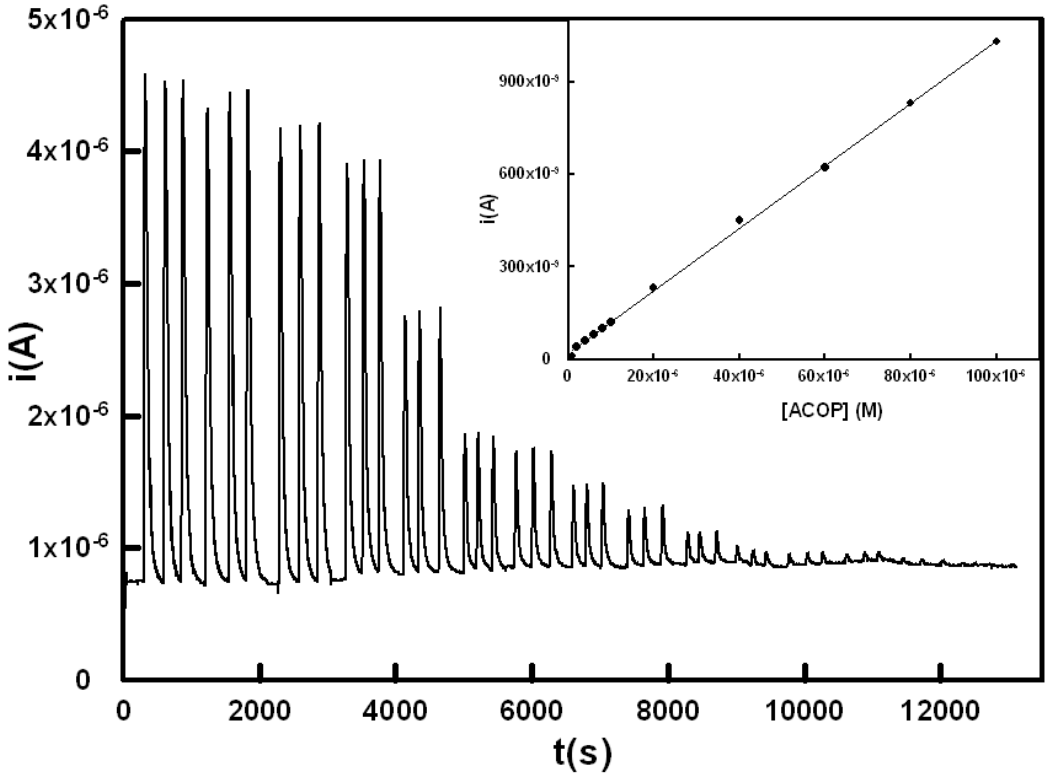


Figure 5. FIA peaks obtained at the sensor for triple injections of acetaminophen standards (ranging from 2 μM to 1 mM acetaminophen) and the corresponding calibration curve (inset). Carrier solution: PBS buffer (0.05 M pH 7.4). Flow rate: 1 ml min^{-1}. Injected volume (sampling loop): 60 μl. Applied potential: 0.425 V.

Figure 5 shows the calibration curve obtained for standards. Three measurements were performed for each standard solution. A sensitivity of 9.9 ± 0.15 mA M^{-1} and a linear regression coefficient (r) of 0.999 were obtained for a linear range between 1 to 800 μM. The detection limit (s/n = 3) was 8.9 μM.

These results are quite close to those recently reports for a gold nanoparticle modified carbon paste electrode [29]. However, the sensor proposed in this work offers two clear advantages: first, the applied potential was 0.425 V compared with 0.75 V applied in ref. 29, this resulting in the desirability of using the TTF-TCNQ salt instead carbon to prepare the electrode composite and, second, the pH value of 7.4 (close to the physiological pH value) is more appropriate for clinical applications than the pH value of 4.7 (ref. 29).

The electrode stability of PVC/TTF-TCNQ/Au_{coll} is n ot of prime importance in these studies because, after several times of use, a new electrode surface can be easily obtained as indicated in the experimental section.

3.4. Interferences studies

Figure 6 shows the chronoamperogram obtained for 0.1 mM acetaminophen (ACOP) in the presence of the most usual electroactive interferences, such as ascorbic acid (AA) and uric acid (UA).

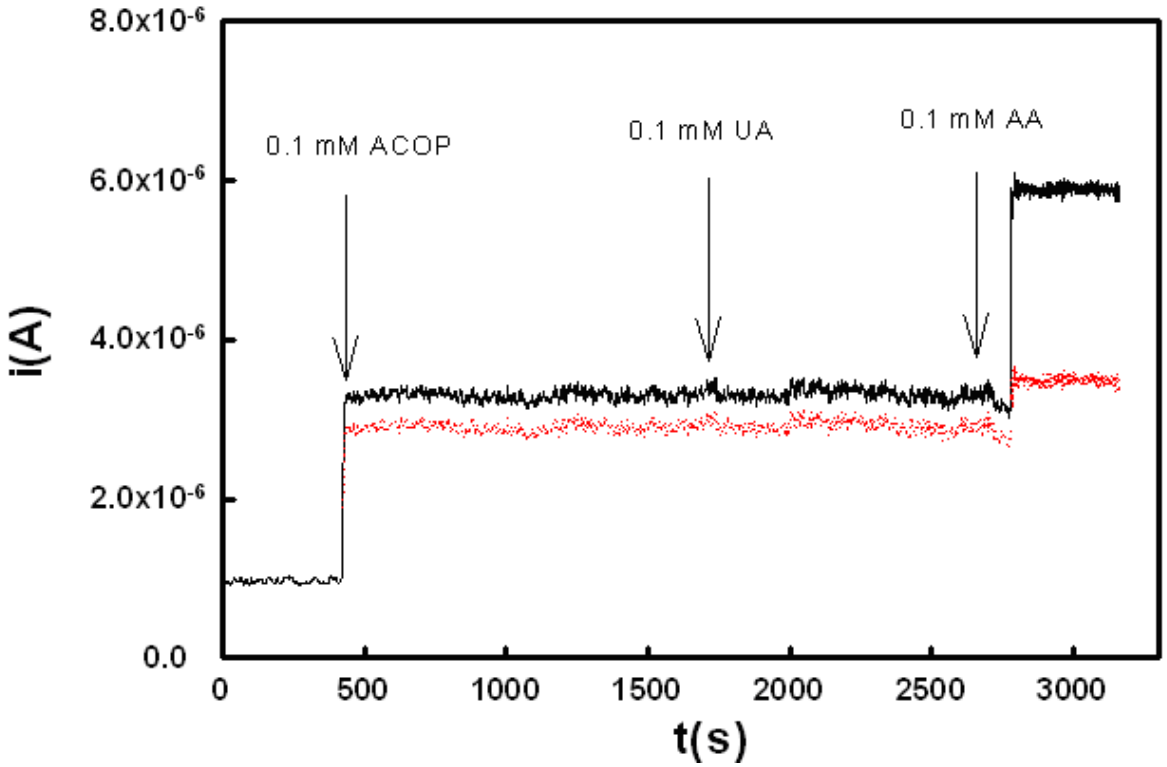


Figure 6. Influence of electroactive interfering compounds at 0.425 V in PBS 0.05 M buffer of pH 7.4. Responses of not Nafion electrode (black line) and Nafion electrode (red line). Arrows indicate the addition of the different compounds.

AA is the only signal obtained at the work potential, so that the UA does not interfere in the determination of acetaminophen. However, the possible interference of AA can be easily eliminated by the usual procedure of coating the sensor with an extra 15 µl layer of 0.5 % Nafion solution, although it entails a decrease in reproducibility an sensitivity of sensor (c.a. 5%) [39]

3.5. Acetaminophen determination in commercial tablets

The developed method was applied to the analysis of two different commercial tablets (Effervescent tablets EFFERALGAN 1 g and Paracetamol Dr Esteve Laboratories S.A. 650 mg). The tablets were weighed, ground into powder, and then dissolved in H_2O. The sample solutions were transferred to a 25 ml flask and diluted with PBS 0.05 M (pH 7.4) until the concentration of acetaminophen lies in the range calibration plot. Appropriate amounts of the diluted samples were transferred to the electrochemical cell for the amperometric determination. The results are shown in Table 2 and the values obtained are an average of five replicate measurements. The reference values correspond to those given by the tablets specifications.

The statistical data indicate that the above proposed method is precise and reliable with excellent recoveries. Moreover, this method was simple and rapid and it can thus be recommended as amperometric sensor for the analysis of acetaminophen in pharmaceutical preparations.

Table 2. Determination results of acetaminophen in tablets for chronoamperometric response at 0.425 V.

Sample	Reference (mmol l^{-1})	Found (mmol l^{-1})	Recovery (%)	RSD (%)
	0.1	0.098	98	2.1
EFFERALGAN	0.01	0.0105	105	2.6
	0.001	0.00097	97	2
	0.2	0.198	99	2.5
PARACETAMOL	0.02	0.018	90	2.3
	0.002	0.0021	105	2.1

4. CONCLUSIONS

The obtained results confirm that the colloidal gold catalyzes the oxidation of acetaminophen, reducing its redox potential considerably. This catalytic phenomenon has been exploited in this work to propose a composite electrode PVC/TTF-TCNQ modified with gold nanoparticles serving as a good amperometric sensor for the determination of acetaminophen. It can be used both in batch and in FIA systems. Besides the good catalytic activity, the electrode shows high reproducibility, long-term stability and low cost. Also, due to the low working potential, the only possible interference could be

due to AA, an interference that can be easily eliminated by coating the surface with a thin layer of Nafion.

In batch, it was obtained a linear range for acetaminophen over a range of 1 to 800 μM with a detection limit of 0.66 μM and a correlation coefficient of 0.997 with a slope (sensitivity) of 53 mA M^{-1}. In FIA, a sensitivity of 9.9 ± 0.15 mA M^{-1} and a linear regression coefficient (r) of 0.999 were obtained for a linear range for acetaminophen between 1 to 800 μM. The detection limit (s/n = 3) was 8.9 μM.

The proposed method can be applied for the routine analysis of acetaminophen in pharmaceutical industry (commercial drugs) without any sample pre-treatment, such as separation, filtration, extraction or derivatization.

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