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## Original Article

# Ultra-sensitive electrochemical sensing of acetaminophen and codeine in biological fluids using CuO/CuFe<sub>2</sub>O<sub>4</sub> nanoparticles as a novel electrocatalyst



Foroozan Hasanpour\*, Masoumeh Taei, Somayeh Tahmasebi

Chemistry Department, Payame Noor University, Tehran 19395-3697, Iran

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## ABSTRACT

Copper ferrite–copper oxide (CuO–CuFe<sub>2</sub>O<sub>4</sub>) nanoparticles as a semiconductor composite with p–n junction were synthesized by co-precipitation reaction. Then, a novel CuO–CuFe<sub>2</sub>O<sub>4</sub> carbon paste modified electrode was fabricated which displays an effectual electrocatalytic response to the oxidation of acetaminophen (AC) and codeine (CO). A linear range of 0.01–1.5 μmol L<sup>-1</sup> and 0.06–10.0 μmol L<sup>-1</sup> with the detection limits of 0.007 μmol L<sup>-1</sup> and 0.01 μmol L<sup>-1</sup> were achieved for AC and CO, respectively. The practical usage of the proposed sensor revealed reasonable results for quantification of AC and CO in biological fluids.

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## 1. Introduction

Nanotechnology is one of the most innovative fields in the current century [1]. Nanomaterials based sensing systems offer a novel class of fast and low cost detection. In recent years, spinel ferrites nanoparticles with general formula MFe<sub>2</sub>O<sub>4</sub> (M is divalent transition metal) have revealed noticeable potentials in electrochemical sensor and biosensor [2]. Among the ferrites, CuFe<sub>2</sub>O<sub>4</sub>, the n-type semiconductor, has been focused owing to its high electronic conductivity, high

thermal stability, and effective catalytic activity [3,4]. Cupric oxide (CuO) is p-type semiconductor with a fine band gap energy which widely applied in designing of superconductors, catalysts, sensors materials. Incorporation p–n junction can improve the magnetic, electrochemical and electrical properties [5]. It is reported that the addition of Fe<sup>3+</sup> ion in copper oxide leads to CuO/CuFe<sub>2</sub>O<sub>4</sub> nanocomposites. Acetaminophen (N-acetyl-p-aminophenol, AC) is an analgesic drug and a suitable alternative for aspirin as a pain reliever and fever reducer [6]. It does not exhibit any harmful side effects, but overdoses are known to cause severe liver and kidney

\* Corresponding author. Fax: +98 383 4226230.

E-mail address: [f.hasanpour@pnu.ac.ir](mailto:f.hasanpour@pnu.ac.ir) (F. Hasanpour).

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damages, and its adverse effects include rashes and blood dyscrasia [7]. AC is often used in the presence of other drugs like aspirin, cetirizine, tramadol, and codeine. Combinations of AC with codeine, produce a significant increase in analgesia compared to AC alone, and it is listed as an antipyretic drug in the European and United States Pharmacopoeia [8,9]. These pharmaceutical formulations have accounted for 20% of total non-opiate analgesics during the last decade [10]. Codeine (methyl morphine), a natural opiate alkaloid prepared from poppy or from morphine by methylation, has long been used as an effective analgesic and antitussive agent [11]. Its phosphate form is usually used for the treatment of gent or moderate pains in clinical medication [12]. Ascorbic acid (AA), Vitamin C, is one of electroactive species and has important role for hydroxylation reactions for metabolic pathway in human body [13]. As some fruit and vegetables have high ascorbic acid content, it could be found with high concentration in biological fluid. To date, various methods such as high-performance liquid chromatography [14,15], spectrophotometry [16], and electrophoresis [17–19] have been developed for the simultaneous determination AC and CO. However, these methods usually have many drawbacks such as complicated sample pretreatment that is laborious and time-consuming. Electrochemical methods have also received much interest due to their higher selectivity, lower cost, and faster operation than other methods [20–23]. In this research, CuO/CuFe<sub>2</sub>O<sub>4</sub> nanocomposite was prepared using co-precipitation reaction. The synthesized nanocomposite then used as modifier in carbon paste electrode (CPE) for the sensitive quantification of AC and CO in the biological fluids.

## 2. Experimental

### 2.1. Apparatus

Electrochemical system was the Autolab PGSTAT101 with NOVA software (Ecochemie, Utrecht, The Netherlands), and applied a conventional three-electrode cell assemblage containing an Ag/AgCl electrode (reference electrode), a platinum wire (counter electrode) and the CuO/CuFe<sub>2</sub>O<sub>4</sub>/CPE as the working electrode. The pH of the solutions was controlled with a Corning pH meter (model 146). The nature and morphology of the synthesized nanocomposite were characterized by using XRD (Holland Philips Xpert, X-ray diffractometer with Cu-K<sub>α</sub> radiation) and FE-SEM (Hitachi S-4160). FT-IR was recorded using a JASCO FT-IR (680 plus). The analysis of chemical composition of the modified electrode was performed using an energy dispersive spectrometer (EDX).

### 2.2. Chemicals

Acetaminophen and codeine phosphate were purchased from Temad Co, Iran. Stock solutions of AC and CO (0.001 mol L<sup>-1</sup>) were prepared daily by dissolving suitable amounts of them in doubly distilled water in a 10-mL volumetric flask.

Phosphate buffer solutions (0.10 mol L<sup>-1</sup>) with different pH (3–8) values were used. Pure graphite powder (particle size <50 μm) was purchased from Sigma–Aldrich. High-viscosity

paraffin (d = 0.88 kg L<sup>-1</sup>) was used for the preparation of paste electrodes.

### 2.3. Synthesis procedure for CuO/CuFe<sub>2</sub>O<sub>4</sub> preparation

About 0.76 g Cu(NO<sub>3</sub>)<sub>2</sub>·3H<sub>2</sub>O and 0.85 g FeCl<sub>3</sub>·6H<sub>2</sub>O were dissolved together in 90 mL distilled deionized water to get a well-mixed solution. While this mixture stirring, the ammonium 25% was added dropwise until pH adjusted to 9.0. The generated precipitate was allowed to stirring about 1 h. The precipitate was washed with ethanol/distilled water several time and dried at 70 °C. Finally the powder was calcinated at 750 °C for 4 h.

### 2.4. Preparation of CuO/CuFe<sub>2</sub>O<sub>4</sub> modified electrode

The modified electrode was prepared by mixing 50 mg of CuO/CuFe<sub>2</sub>O<sub>4</sub> and 800 mg of graphite powder. Then, diethyl ether was added to achieve uniform mixture. After vaporization of diethyl ether, 200 mg paraffin oil was added and mixed with mortar and pestle to get a uniformly wetted paste. The resulting paste was pressed into the hole at the end of the electrode.

### 2.5. Real sample preparation

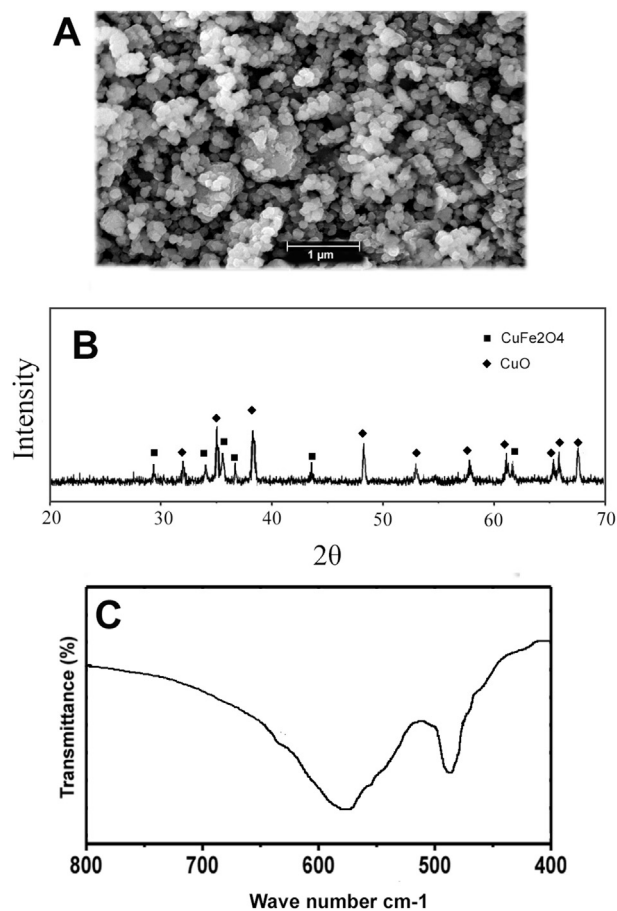
Human plasma and urine samples were originally obtained from volunteers who had taken AC/CO tablets (325/30 mg). The samples were collected 2 h after intake of tablets. Plasma samples were deproteinated using acetonitrile [24]. In order to precipitate proteins in the plasma samples, 5.0 mL of the samples was treated with 10 mL acetonitrile. Then, the mixture was vortexed for a further 30 s and after that it was centrifuged at 3000 rpm for 10 min. The supernatant was transferred to a small flask and evaporated with a stream of nitrogen. The dry residue was diluted to final volume of 20 mL with phosphate buffer solution of pH 5.0 and transferred into the voltammetric cell to be analyzed without any further pretreatment. Standard addition method was used for the determination of AC and CO in the samples.

Urine samples were stored in a refrigerator immediately after their collection. A 5.0 mL of the sample was centrifuged for 10 min at 2000 rpm. The supernatant was filtered using a 0.45 μm filter and then diluted 4-times with PBS pH 5.0 and reaching a final volume of 20 mL. The solution was transferred into the voltammetric cell to be analyzed without any further pretreatment. Standard addition method was used to determine the CO and AC content of the sample.

## 3. Results and discussion

### 3.1. Characterization of CuO/CuFe<sub>2</sub>O<sub>4</sub>

Fig. 1A shows the morphology of CuO/CuFe<sub>2</sub>O<sub>4</sub> nanoparticles which has spherical like structure with mean diameter of 90 nm. The crystallite phases of CuO/CuFe<sub>2</sub>O<sub>4</sub> was identified by X-ray diffraction (Fig. 1B) in 2θ range of 20–70° are in a good agreement with the standard XRD pattern of copper ferrite (tetragonal-type)/copper oxide nanoparticles [5]. Fig. 1C shows



**Fig. 1** – SEM image of synthesized CuO/CuFe<sub>2</sub>O<sub>4</sub> nanoparticles (A); the corresponding XRD patterns (B) and FT-IR spectra of CuO/CuFe<sub>2</sub>O<sub>4</sub> nanoparticles (C).

the FT-IR absorption spectra of CuFe<sub>2</sub>O<sub>4</sub> samples. Two main adsorption bands between 800 and 400 cm<sup>-1</sup> can be assigned to the metal-oxide (M-O) stretching vibration, characteristic of CuO and CuFe<sub>2</sub>O<sub>4</sub> metal oxides. The absorption broad band at ~482 cm<sup>-1</sup> represents the stretching vibrations of Cu–O bond. The main adsorption bands at ~580 cm<sup>-1</sup> can be assigned to stretching vibration of coordinated Fe<sup>3+</sup> to O<sup>2-</sup> ions in CuFe<sub>2</sub>O<sub>4</sub> crystal lattices [25].

### 3.2. Electrochemical characterization of CuO/CuFe<sub>2</sub>O<sub>4</sub>/CPE

The enhancement of real surface area caused by CuO/CuFe<sub>2</sub>O<sub>4</sub>/CPE was determined by the Randles-Sevcik formula:

$$I_{pa} = 2.69 \times 10^5 n^{3/2} A C_0 D^{1/2} \nu^{1/2} \quad \text{Eq. (1)}$$

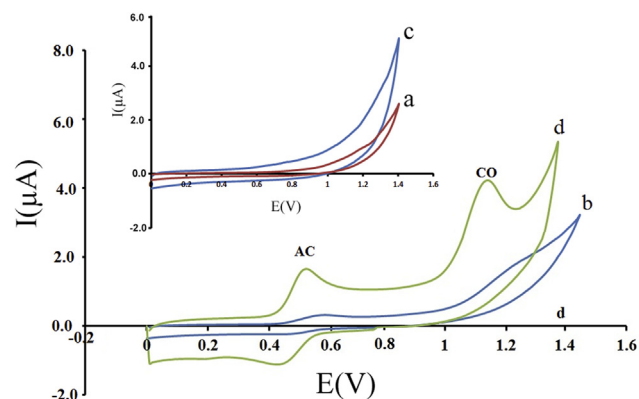
Where  $I_{pa}$  (A) is anodic peak current,  $n$  is the electron transfer number,  $A$  is the surface area of the electrode,  $D$  is the diffusion coefficient,  $C_0$  (mol cm<sup>-3</sup>) is the concentration of K<sub>3</sub>Fe(CN)<sub>6</sub> and  $\nu$  is the scan rate. For 1 mmol L<sup>-1</sup> K<sub>3</sub>Fe(CN)<sub>6</sub> in the 0.1 mol L<sup>-1</sup> KCl electrolyte:  $n = 1$  and  $D_R = 7.6 \times 10^{-6}$  cm<sup>2</sup> s<sup>-1</sup>. Then, from the slope of the  $I_{pa} - \nu^{1/2}$  relation, the real surface area was calculated. The results

showed that the electrode surface area was 0.163 cm<sup>2</sup> for the unmodified carbon paste electrode, and 0.85 cm<sup>2</sup> for CuO/CuFe<sub>2</sub>O<sub>4</sub>/CPE, which is 5.21 times greater than that for the CPE.

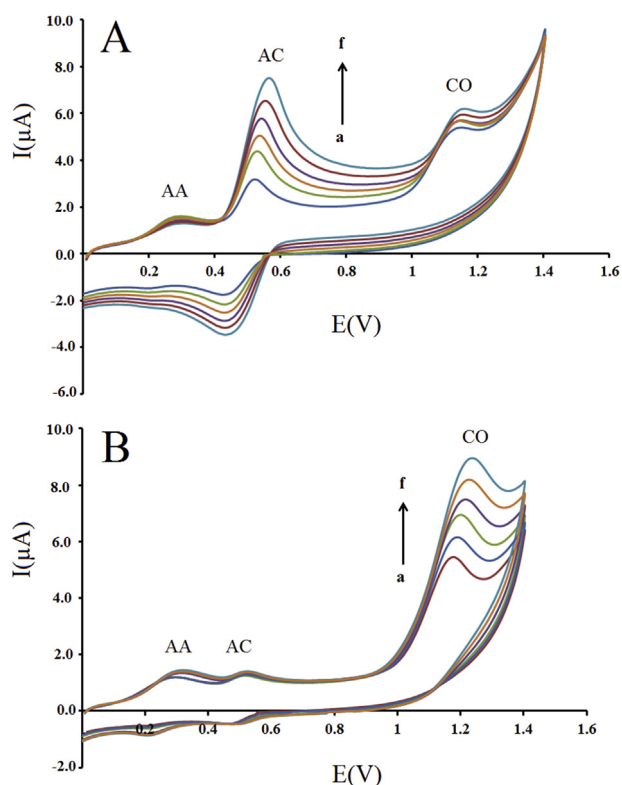
### 3.3. Electrochemical behavior of AC and CO at the surface of modified electrodes

Fig. 2 show cyclic voltammograms (CVs) of the blank solution at the surface of CPE (a); at CuO/CuFe<sub>2</sub>O<sub>4</sub>/CPE (c) and CVs of 0.25 μmol L<sup>-1</sup> AC and 1.5 μmol L<sup>-1</sup> CO in phosphate buffer (pH 4.0) at CPE (b) and CuO/CuFe<sub>2</sub>O<sub>4</sub>/CPE (d). At the CPE (curve b), there was low electrochemical responses for AC and CO, representing that slow electron transfer process. The increases in the peak currents at the surface of the CuO/CuFe<sub>2</sub>O<sub>4</sub>/CPE are about 6.1 and 6.3 times greater than CPE for AC and CO, respectively. A notable amplification in peak currents was obtained at CuO/CuFe<sub>2</sub>O<sub>4</sub>/CPE, which demonstrated the effective facilitation of electron transfer for AC and CO. As can be seen, the background currents for modified electrodes are higher than that of the CPE. This is because nanoparticles increase the surface area of electrode, and because the non-faradic current mainly depends on the surface area of electrodes [26]. For confirmation the role of CuO/CuFe<sub>2</sub>O<sub>4</sub>, the differential pulse voltammograms (DPVs) of AC and CO were recorded and compared to CuO/CPE or CuFe<sub>2</sub>O<sub>4</sub>/CPE alone (Fig. 1S). For 0.25 μmol L<sup>-1</sup> AC and 1.5 μmol L<sup>-1</sup> CO in PBS (pH = 4.0), analysis of DPV results (by NOVA 1.5 software) show at CuO/CPE the currents were 2.2 and 1.5 μA, respectively. At CuFe<sub>2</sub>O<sub>4</sub>/CPE these currents obtained as 2.3 μA and 1.8 μA. While at CuO/CuFe<sub>2</sub>O<sub>4</sub>/CPE the oxidation current for AC and CO obtained as 5.6 and 4.5 μA, respectively. Therefore, it can be concluded that CuO/CuFe<sub>2</sub>O<sub>4</sub> nanoparticles had catalyst effect on AC and CO beyond only increase in the microscopic surface area.

The CVs of various concentrations of AC in the presence of 20.0 μmol L<sup>-1</sup> AA and 1.0 μmol L<sup>-1</sup> CO were recorded (Fig. 3A). As the results showed, the peak current of AC increased by increasing its concentration, while the peak currents of CO and AA remained almost constant. Similar patterns were observed for different concentrations of CO at fixed



**Fig. 2** – CVs of (a) the PBS(pH = 4.0); (b) 0.25 μmol L<sup>-1</sup> AC and 1.5 μmol L<sup>-1</sup> CO in PBS at CPE; and CVs of (c) PBS and (d) 0.25 μmol L<sup>-1</sup> AC and 1.5 μmol L<sup>-1</sup> CO at CuO/CuFe<sub>2</sub>O<sub>4</sub>/CPE.

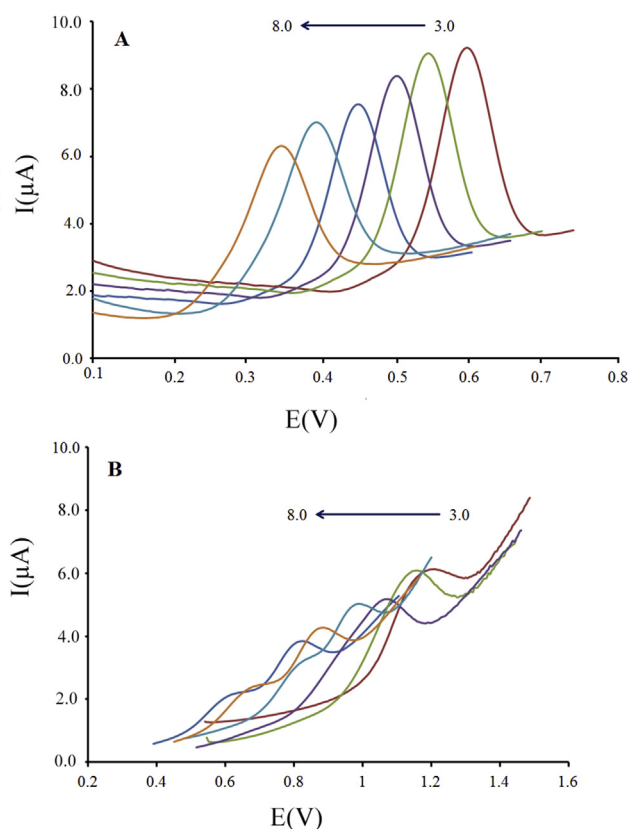


**Fig. 3** – Cyclic voltammograms of (A): (a) 0.08; (b) 0.11; (c) 0.15; (d) 0.2; (e) 0.25; (f) 0.3  $\mu\text{mol L}^{-1}$  of AC in the presence of 20.0  $\mu\text{mol L}^{-1}$  AA and 1.0  $\mu\text{mol L}^{-1}$  CO, (B): (a) 0.8; (b) 2.0 (c) 3.0; (d) 4.0; (e) 5.0; (f) 6.0  $\mu\text{mol L}^{-1}$  of CO in the presence of 20.0  $\mu\text{mol L}^{-1}$  AA and 0.06  $\mu\text{mol L}^{-1}$  AC.

concentration of AC and AA without any mutual interference from AC (Fig. 3B). This phenomenon proved that the electrochemical signals of AC and CO do not interfere with each other or in presence of AA. The Cottrellian behavior for electro-oxidation of AC and CO was demonstrated using the plot of current versus square root of scan rate ( $v^{1/2}$ ) in the ranges of 10–110  $\text{mVs}^{-1}$  at the surface of  $\text{CuO/CuFe}_2\text{O}_4/\text{CPE}$ . The results showed that the anodic peak currents increased linearly with  $v^{1/2}$  for two drugs, which confirm. Diffusion coefficients ( $D$ ) of AC and CO were determined using single potential step chronoamperometry. The mean value of the  $D$  is found to be  $5.2 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$  and  $6.7 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$  for AC and CO, respectively.

### 3.4. Optimization of measurement conditions

With the aim of reach to maximum sensitivity, the effect of pH (PBS from 3 to 8) as a main variable on electrochemical behavior of AC and CO was investigated. The results show that the peak currents of CO increased by increasing the solution pH from 3.0 to 4.0 (Fig. 4). From pH 3.0 to 4.0, one anodic wave was observed that can be the result of the sum of two close peaks. This peak related to the oxidation of the tertiary amine and the 6-hydroxy groups of CO, which are almost overlaid. At above pH 4, the oxidation peaks of the tertiary amine and the 6-hydroxy groups were separated [27,28]. The optimum pH value for AC was gained at pH 4. Therefore, pH 4.0 was



**Fig. 4** – Effect of pH on the oxidation peak potential of 0.3  $\mu\text{mol L}^{-1}$  AC (A) and 0.6  $\mu\text{mol L}^{-1}$  CO (B) at the  $\text{CuO/CuFe}_2\text{O}_4/\text{CPE}$ .

selected to achieve the best sensitivity to both AC and CO in the following experiments. The oxidation potential of two drugs has negative shifts with increasing pH, indicating that electrochemical oxidation involved protons transfer for these two species. The slope of the peaks potential vs. pH was found to be 53 and 52 mV per decade for AC and CO, respectively which are close to the Nernstian theoretical value. This suggests that equal numbers of electrons and protons participate in redox reaction [29].

The role of copper oxide as a catalyst for oxidation of hydroxyl group substituted aromatics has been proven [30]. The cyclic voltammograms of  $\text{CuO/CuFe}_2\text{O}_4$  (Fig. 2S) casted on glassy carbon electrode (PBS, pH = 4) exhibit redox peaks at about 0.02 and  $-0.1$  V which can be assigned to redox couple of  $\text{Cu(I)/Cu(II)}$  [31]. As redox currents and corresponding potentials of copper ions in absence and presence of drugs were almost the same, the role of nanoparticles can be only catalysis of oxidation reaction of AC or CO. The possible pathways for oxidation of these drugs can be as [32,33]:

At cathode the most likely reaction is the reduction of hydrogen ions by gaining the produced electrons and protons.

### 3.5. Simultaneous determination of AC and CO

The next aim was made to determine AC and CO simultaneously using  $\text{CuO/CuFe}_2\text{O}_4$  modified electrode. Since differential pulse voltammetry almost has a much higher current



sensitivity than cyclic voltammetry it was used to plot of calibration curves [34]. Fig. 5 shows the DPVs of different concentrations for AC and CO at the CuO/CuFe<sub>2</sub>O<sub>4</sub>. As can be seen, there are two well discrete anodic peaks at potentials of 0.57 V, and 1.15 V corresponding to the oxidation of AC and CO, respectively. The calibration curves for AC and CO were linear for the concentration ranges of 0.01–1.5 μmol L<sup>-1</sup> of AC and 0.06–10.0 μmol L<sup>-1</sup> of CO with detection limits of 0.007 and 0.01 μmol L<sup>-1</sup> (signal to noise ratio of 3) for AC and CO, respectively. The sensitivity (slope of calibration curve) of the modified electrode for AC and CO detection in the presence and absence of the other two analytes is almost the same. Therefore, it is possible to individually or simultaneously determine AC and CO in mixed samples at CuO/CuFe<sub>2</sub>O<sub>4</sub> without any cross interferences. The detection limit and linear dynamic range of AC and CO at CuO/CuFe<sub>2</sub>O<sub>4</sub> are compared with those recently voltammetric methods

developed for simultaneous determination of these drugs [12,28,35–43]. Although some modified electrodes had lower detection limit and wider linear range than our proposed method, these methods measured AC or CO alone (Table 1). The linear dynamic ranges of our proposed method for AC and CO are better than other methods, which determine AC and CO simultaneously. Our work has superiority over some previously reported methods [12,42] because it used nontoxic modifier and is free from interference of cysteine and ascorbic acid.

The stability of CuO/CuFe<sub>2</sub>O<sub>4</sub> was checked out over a four-week period using 1.0 μmol L<sup>-1</sup> of AC. The DPV of AC at the surface of the modified electrode (stored in the laboratory at room temperature) shows that the oxidation peak current of AC was decreased less than 5.3% of the initial value without any alteration in the peak potential. Nanoparticles with p–n junction were reported in the literature as the most

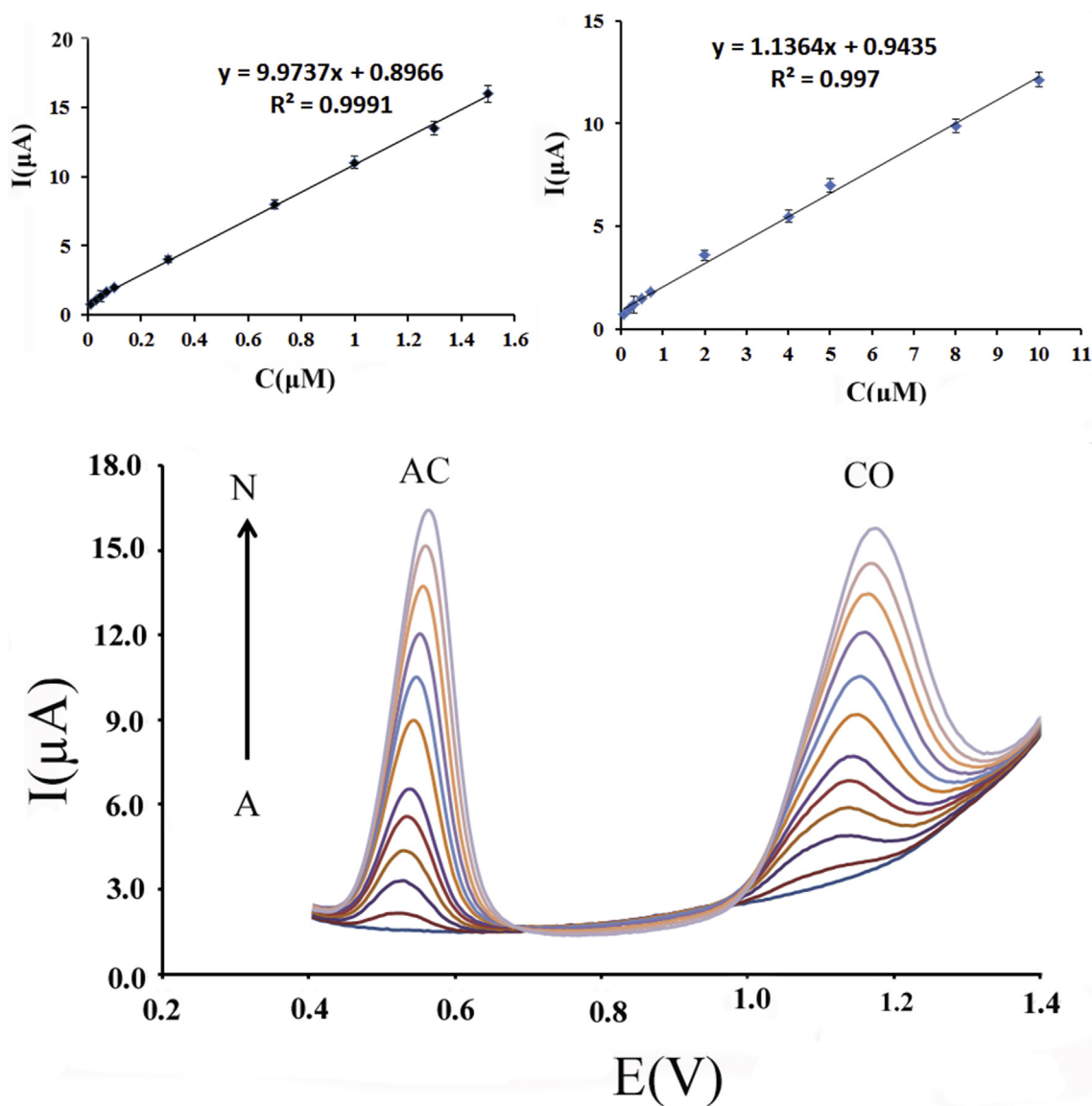


Fig. 5 – DPVs for different concentrations of AC and CO in pH 4.0. PBS on the surface of the CuO/CuFe<sub>2</sub>O<sub>4</sub>/GPE. Concentrations of AC from (A) to (L): 0, 0.01, 0.03, 0.05, 0.07, 0.1, 0.3, 0.7, 1.0, 1.3, 1.5 μmol L<sup>-1</sup>; Concentrations of CO from (A) to (L): 0, 0.06, 0.1, 0.3, 0.5, 0.7, 2.0, 4.0, 5.0, 8.0, and 10 μmol L<sup>-1</sup>.

**Table 1 – Comparison of some characteristics of the different modified electrodes for the determination of AC and CO.**

Working electrode	Limit of detection ( $\mu\text{mol L}^{-1}$ )		Linear dynamic range ( $\mu\text{mol L}^{-1}$ )		References
	AC	CO	AC	CO	
PSi/Pd/CNTPE	0.4	0.3	1–700	1–700	[28]
TiO <sub>2</sub> Nanoparticles	0.05	0.018	0.6–110.6	0.07–100	[12]
AlEMPd	5	5	100–3000	100–3000	[35]
Glassy carbon electrode modified with MWCNTs	0.19	0.2	5–400	5–240	[36]
BDD modified electrode	–	0.08	–	0.1–60	[37]
SWCNT/GNS	0.038	–	0.05–64.5	–	[38]
PEDOT/GO	0.57	–	10–60	–	[39]
GRBME	–	0.015	–	0.05–30	[32]
NiONPs-CB-DHP/GCE	3.0–47.8	0.83–38.3	0.12	0.48	[40]
Cathodically pretreated BDD Electrode	0.001	0.018	0.20–95.3	0.40–9.58	[41]
BDD-poly crystalline silicon wafer	–	1.0	–	7–36	[42]
Anodically pretreated BDD Electrode	–	0.34	–	20–100.7	[43]
HTP-MWCNTCPE	Not reported	0.063	0.95–125.8	0.2–31.4	[44]
CuFe <sub>2</sub> O <sub>4</sub> /MWCNTs paste electrode	0.007	0.01	0.01–1.5	0.06–10	This work

AlEMPd: aluminum electrode modified by thin layer of palladium; BDDF: boron-doped diamond; SWCNT/GNS: single-walled carbon nanotube–graphene nano sheet; PEDOT/GO: poly(3,4-ethylenedioxythiophene)/graphene oxide; GRBME: graphene based modified electrode; HTP-MWCNTCPE: 4-hydroxy-2-(triphenylphosphonio)phenolate-Multiwall Carbon Nanotubes Carbon Paste Electrode; NiONPs-CB-DHP/GCE: nickel oxide nanoparticles-carbonblack- dihexadecylphosphate/Glassy carbon electrode.

performing materials for CO<sub>2</sub> gas sensing [45]. CO<sub>2</sub> gas readily adsorbs to these nanoparticle surfaces and cause some changes in their properties [46]. Decreasing in oxidation peak current of AC after 4-week period can be attributed to this phenomenon. Furthermore, cyclic voltammetry results proved that current of 2.0 mmol L<sup>-1</sup> of [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> (as a redox probe) at CPE modified with fresh prepared CuO/CuFe<sub>2</sub>O<sub>4</sub> is significantly more than old one. The reproducibility of the modified electrode was investigated by comparing the current of DPV response with 1.0  $\mu\text{mol L}^{-1}$  AC and 2.0  $\mu\text{mol L}^{-1}$  CO at six modified electrodes, prepared independently. The relative standard deviations (RSD) of 3.1% and 4.5% were obtained for AC and CO, respectively. This confirmed that the electrode is not poisoned by the oxidation products of either AC or CO.

### 3.6. Interference study

The effects of potentially interfering species, found in biological fluids or pharmaceuticals, were investigated for simultaneous determination of 1.0  $\mu\text{mol L}^{-1}$  AC and CO. The maximum concentration of the potential interfering species,

which causes an error less than 5%, was considered as the tolerance limit. The results show that 1000-fold concentration of glucose, ascorbic acid, cysteine, alanine, phenylalanine, methionine, sucrose; 500-fold concentration of glycine, naproxen and 200-fold phenacetin did not affect the oxidation current of AC or CO. However, the oxidation current of 1.0  $\mu\text{mol L}^{-1}$  AC was decreased to 89% of its initial currents in the presence of 50.0  $\mu\text{mol L}^{-1}$  uric acid. Despite its interference, it isn't present at significant levels in real samples. Furthermore epinephrine and para-amino phenol at one fold level interfered with the AC signal.

### 3.7. Determination of AC and CO in biological fluids

Pharmacokinetic data show concentration of codeine in healthy subjects who took 20 mg–40 mg codeine phosphate after 2 h were in the ranges of 7.4–24.8  $\mu\text{g mL}^{-1}$  [47]. Furthermore, excreted concentration of acetaminophen following oral administration of a single conventional 500-mg tablet to 5 normal healthy volunteers was found about 28  $\mu\text{g mL}^{-1}$  [48]. The practical utility of CuO/CuFe<sub>2</sub>O<sub>4</sub> modified electrode for determination of AC and CO in blood plasma and urine samples was assessed. The recovery percentages were in the range of 95.6–104.6%, indicating outstanding analytical performance of modified electrode (Table 2).

**Table 2 – Simultaneous determination of AC and CO in urine and plasma samples.**

Sample	Analyte	Added ( $\mu\text{mol L}^{-1}$ )	Found ( $\mu\text{mol L}^{-1}$ )	Recovery%
Urine	AC	–	15.1 ± 0.3	–
		10.0	24.3 ± 0.5	96.8
	CO	–	3.3 ± 0.05	–
		1.0	4.5 ± 0.07	104.6
Plasma	AC	–	1.3 ± 0.02	–
		1.0	2.2 ± 0.03	95.6
	CO	–	<Detection limit	98.0
		10.0	9.8 ± 0.08	

±Shows the standard deviation with four replicates determination.

## 4. Conclusions

CuO/CuFe<sub>2</sub>O<sub>4</sub> nanocomposite was synthesized by coprecipitation approach. The nanocomposite modified electrode enhanced oxidation currents of AC and CO noticeably. The electrode had high reproducibility and sensitivity which could be used for routine analysis of AC and CO in urine and plasma samples. The noticeable characteristics of CuO/CuFe<sub>2</sub>O<sub>4</sub> modified electrode were high sensitivity and

reproducibility accompanied by facile preparation. Furthermore, no interference was seen from typical species existing in biological samples which suggest selectivity of the modified electrode for accurate analysis of AC and CO in biological samples.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jfda.2017.10.001>.

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