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Gold Nanoparticles/Cysteic Acid Modified Electrode for Simultaneous Electrochemical Determination of Tramadol and Paracetamol

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Electrochemical deposition of gold nanoparticles (AuNPs) and l-cysteine on glassy carbon electrode was carried out to prepare a modified electrode. The fabricated sensor showed good sensitivity and selectivity for simultaneous determination of paracetamol and tramadol by square wave voltammetry. Linear ranges of 0.10-10.7 μM (paracetamol) and 0.50-63.6 μM (tramadol) were obtained with detection limits of 0.03 and 0.17 μM, respectively. The proposed electrode was used successfully in the simultaneous determination of the drugs in spiked human plasma samples.

Keywords: Paracetamol, Tramadol, Modified electrode, Cysteic acid, Gold nanoparticles, Square wave voltammetry

INTRODUCTION

Paracetamol (PAR, Scheme 1) or acetaminophen is an analgesic and anti-inflammatory drug. PAR lacks many of the side effects of aspirin, so, as an alternative, it is commonly used for the relief of fever, headaches, and other minor aches. Tramadol (TRA, Scheme 1), used for treating moderate to severe pain, contains actions at μ-opioid receptor as well as the noradrenergic and serotonergic systems. Considering complementary mechanisms of action of TRA and PAR, in order to enhance the analgesic effectiveness, a mixture of the drugs is used in a tablet (TRA 37.5 mg and PAR 325 mg) for patients with moderate to severe acute pain and those with chronic painful conditions characterized by intermittent exacerbations of pain [1,2].

However, their overdose is toxic and may cause dizziness, nausea and vomiting. Therefore, the development of a sensitive and selective method for their simultaneous determination is highly desirable for analytical applications and diagnostic research.

Various techniques have been used for PAR and TRA analysis in biological and pharmaceutical samples. These include high-performance liquid chromatography (HPLC) [3-5], liquid chromatography with mass spectrometry detection (LC-MS) [6] and spectrophotometry [7,8]. However, some of them requiring expensive instrumentation, usually are time-consuming and need sample pretreatment,making them unsuitable for routine analyses. Therefore, electrochemical methods, as simple, fast, cost effective, sensitive, and accurate methods, have been developed for determination of these pain killers either simultaneously or individually [9-18].

Chemically modified electrodes (CMEs) present enormous opportunities for sensitive and selective determination of various pharmaceuticals in complex samples [19]. Among different materials, noble metal nanoparticles and amino acids play important roles in constructing CMEs in order to improve selectivity, sensitivity, electrode durability, *etc.*

For instance, the amino acid l-cysteine has been electrochemically converted to cysteic acid, a stable, biocompatible, and conductive polymer that is strongly adsorbed on the electrode surface [20,21]. Among noble metal nanoparticles, gold nanoparticles (AuNPs) have been extensively used in CMEs for their high electrical

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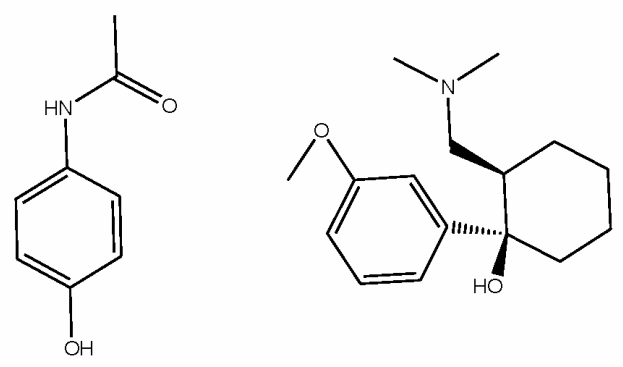

(A) (B)

Scheme 1. Chemical structures of paracetamol (A) and tramadol (B)

conductivity, biocomatibility, stability, *etc.* [22-24].

In this study, l-cysteine was deposited on glassy carbon electrode (GCE) by electrochemical oxidation, followed by modification with AuNPs. The fabricated electrode was used for simultaneous determination of PAR and TRA, successfully. It showed wide linear range, 0.1-10 µM and 0.5-63.5 µM, and low detection limits of 0.03 and 0.17 µM for PAR and TRA, respectively.

EXPERIMENTAl

Apparatus

In this work, cyclic voltammetry (CV) and square wave voltammetry (SWV) were performed using µ-Autolab type III, potentiostat/galvanostat instrument driven by NOVA 1.11 software. The modified GCE was utilized as the working electrode, a platinum wire as the counter electrode, and silver/silver chloride (Ag/AgCl, KCl 3 M) as reference electrode. All electrochemical measurements were carried out at room temperature. Surface morphology of the modified electrode was characterized by field emission scanning electron microscopy (FESEM Vega-Tescan). A pH meter (Jenway, Model 140) with a combined glass electrode was used for pH adjustments.

Reagents and Solutions

All the chemicals were of analytical grade and were used directly without further purification. Both PAR and TRA were purchased from Aldrich. L-cysteine and hydrogen tetrachloroaurate ($HAuCl_4$) were from Merck (Darmstadt, Germany). Phosphate buffer solution (PBS) was prepared from a mixture of phosphoric acid solution and different amounts of sodium hydroxide (0.2 M).

Healthy human blood plasma samples were obtained from Imam Reza hospital (Kermanshah, Iran). Pretreatment of serum samples was carried out by adding methanol (2% v/v) for protein precipitation and centrifuged at 3,000 rpm for 30 min. The supernatant was diluted (100 times) by PBS (0.1 M, pH 7) and spiked with PAR and TRA. Standard addition was used for evaluating the proposed method.

Fabrication of the Working Electrode

GCE was polished on alumina slurry (mesh size, 0.05 µm), followed by immersing in ethanol solution (1:1 v/v% ethanol + H_2O) and putting in an ultrasonic bath for 1 min to clean the electrode surface from alumina and other adsorbed residues. The shiny polished GCE was then used for electrode modification by l-cysteine [20]. Briefly, GCE was immersed in l-cysteine solution (10 mM in PBS 0.1 M, pH 7.0) and consecutive cyclic voltammetry (20 cycles) was applied in a potential range of -0.8-2.2 V (against Ag/AgCl, KCl 3 M). Then, the modified electrode (Cysteic acid/GCE) was washed with double distilled water and allowed to dry at room temperature. In order to deposit AuNPs, cysteic acid/GCE was immersed in a solution of $HAuCl_4$ (1 mM) and a constant potential (-0.4 V against Ag/AgCl, KCl 3 M) was applied for 600 s [25]. The modified electrode (AuNPs/Cysteic acid/GCE) was then rinsed with a plenty of water to eliminate unreacted salt from the surface. The modified electrode was stored in a refrigerator (4 °C) when

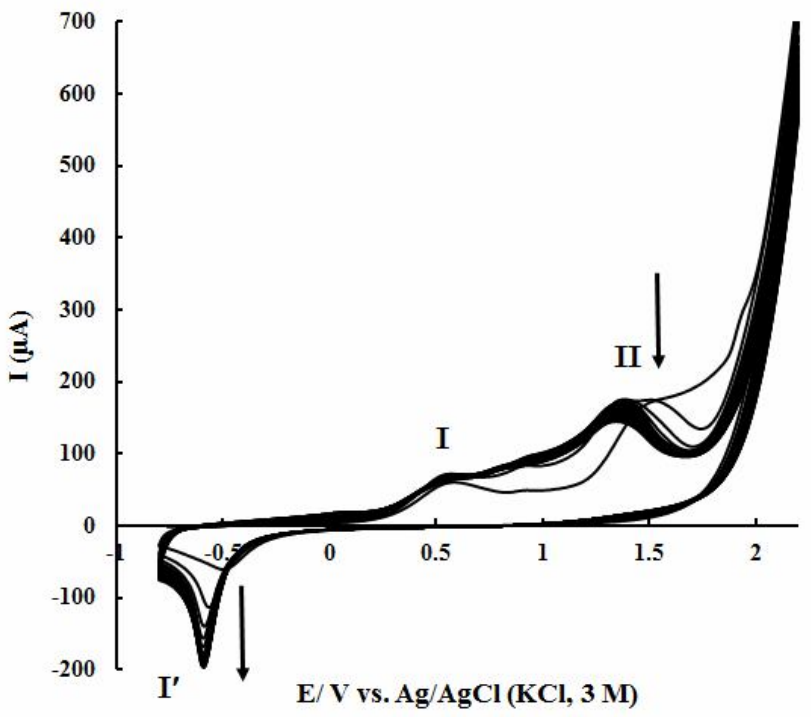

Fig. 1. Electrodeposition of l-cysteine (10 mM in PBS pH 7.0, 0.1 M) on GCE. Scan rate: 100 mV s^{-1}; Number of potential cycles: 20.

not in use.

RESULTS AND DISCUSSION

Electrode Modification

Cyclic voltammetry (20 potential cycles) was used for modification of GCE by applying a potential range of -0.8-2.2 V (against Ag/AgCl, KCl 3 M) in an aqueous solution of l-cysteine (10 mM). A quasi-reversible redox pair (referred to as I and I′ in Fig. 1) was observed. Peak (I) at +0.50 V is assigned to the oxidation of l-cysteine (CySH) to a cation radical ($CyS^{\bullet}$) followed by dimerization to cystine (CySSCy) [26]. The cathodic peak at -0.6 V (I′) was the result of cystine reduction back to the cation radical. The overall mechanism is summarized below [27-29]:

$$CySH \longrightarrow CyS^{-} + H^{+} \quad (1)$$

$$CyS^{-} \longrightarrow CyS^{\bullet}_{ads} + e^{-} \quad (2)$$

$$2CyS^{\bullet}_{ads} \rightleftharpoons CySSCy \quad (3)$$

In this mechanism, $CyS^{\bullet}_{ads}$ refers to adsorbed cation radical. This mechanism is frequently reported for redox behavior of thiol-containing compounds.

A second anodic peak (Fig. 1, labeled as II) was appeared irreversibly at more positive potentials (+1.3 V) which was assigned to further oxidation of CySSCy to cysteic acid ($CySO_3H$) [30]:

$$CySSCy + 3H_2O \longrightarrow CySO_3H + 5H^{+} + 5e^{-} \quad (4)$$

Strong adsorption of $CySO_3H$ on electrode surface was reported [26], indicating the long-term stability of the modified electrode. The prepared electrode was then immersed in a solution of $HAuCl_4$ (1 mM) and a constant potential (-0.4 V against Ag/AgCl, KCl 3 M) was applied for 600 s. AuNPs were deposited on Cysteic acid/GCE.

In order to study the surface morphology of the modified electrodes, field emission scanning electron microscopy (FESEM) was used (Fig. 2). Comparison of images A and B clearly shows the evenly distributed AuNPs

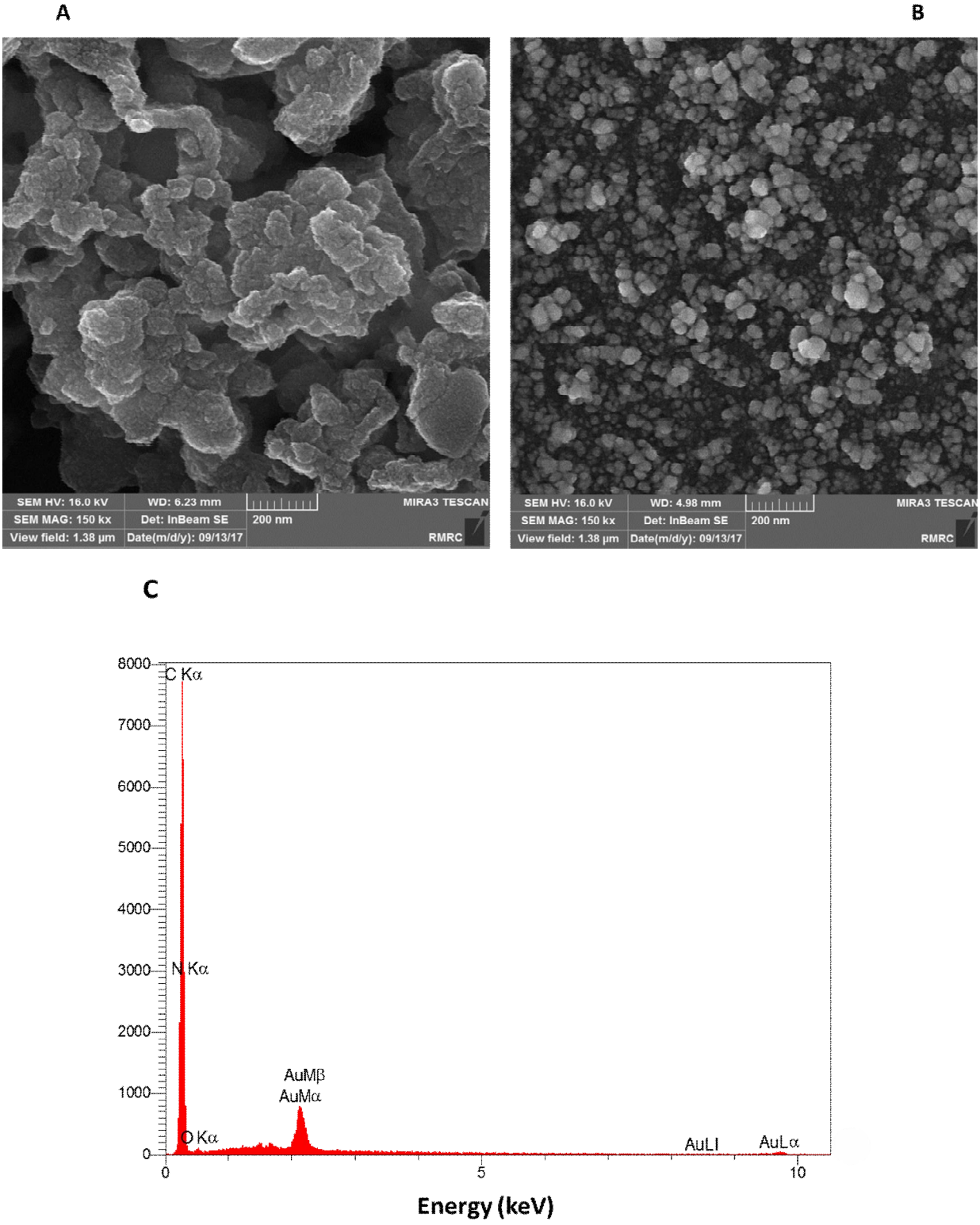

Fig. 2. FESEM images of (A) cysteic acid/GCE, (B) AuNPs/cysteic acid/GCE, (C) EDS spectrum of AuNPs/cysteic acid/GCE.

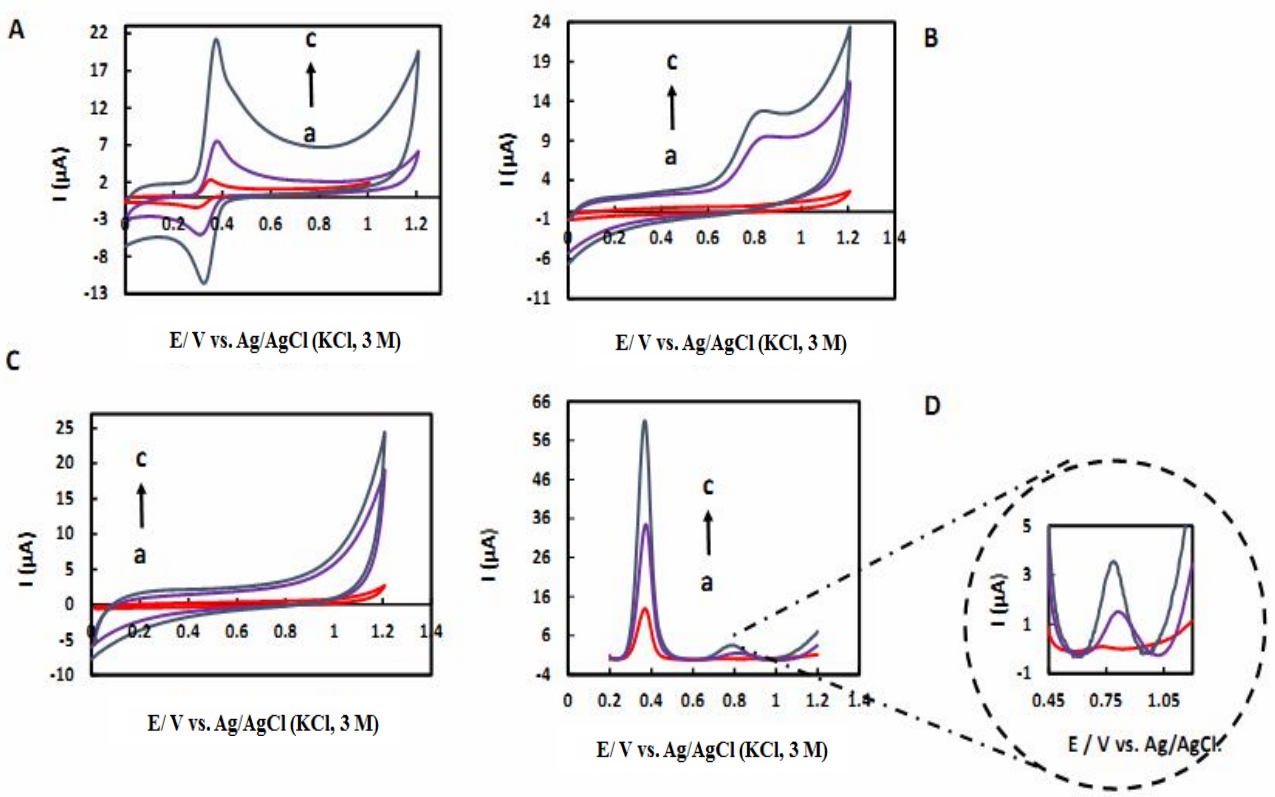

Fig. 3. Cyclic voltammetry of (A) PAR (10 μM), (B) TRA (30 μM), and (C) supporting electrolyte on different electrodes. In each figure: (a) bare GCE; (b) Cysteic acid/GCE; and (c) AuNPs/cysteic acid/GCE and (D) SWV in a mixture of PAR and TRA.. Supporting electrolyte: PBS (0.1 M, pH 7.0). Scan rate: 100 mV s^{-1}.

on the cysteic acid layer. Deposition of AuNPs was further confirmed by EDS spectrum (Fig. 2C). Strong interaction between Au and cysteine contributes to the stabilization of the nanocomposite formed.

Cyclic Voltammetric Behavior of PAR and TRA at AuNPs/Cysteic Acid/GCE

The prepared electrode was used in simultaneous determination of PAR and TRA. Figure 3 shows cyclic voltammetry curves (CVs) in the presence of each of the two drugs at the surface of different electrodes (Figs. 3A and 3B). The superiority of peak currents on AuNPs/Cysteic acid/GCE (curve a) is observed compared to Cysteic acid/GCE (curve b) and GCE (curve c). Slightly negative shift in oxidation potential is also observed in the case of TRA. For comparison, the corresponding CVs in blank solution are shown in Fig. 3C. Enlarged surface area of the electrode by cysteic acid and AuNPs formation, and electrocatalytic properties of the nanocomposite were responsible for current and potential improvements. In Fig. 3D, square wave voltammetry (SWV) was applied to a mixture of TRA and PAR using the same electrodes. AuNPs/Cysteic acid/GCE shows the most sensitive results.

Optimization of Experimental Conditions

Effect of pH. The effect of pH (2-11) was studied on the redox behavior of PAR and TRA by cyclic voltammetry (Fig. 4A). The variation of peak current and potential against pH was plotted in Fig. 4B (for TRA) and Fig. 4C (for PAR). At pH 7, the oxidation peak current reached a maximum for TRA. In the case of PAR, a steady decrease of peak current was observed by increasing pH (Fig. C). So, pH 7 was selected as the proper buffer (PBS 0.1 M) for further experiments.

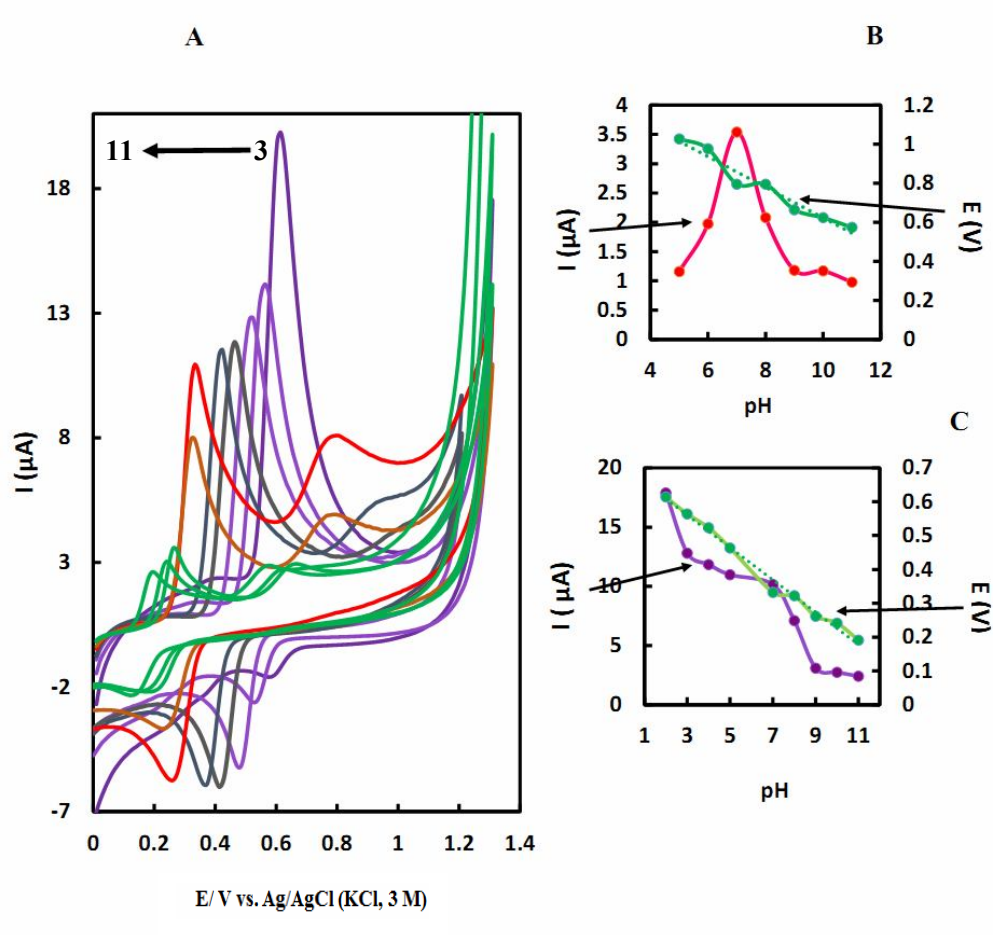

Fig. 4. (A) The effect of pH value on the oxidation current of PAR (10 μM) and TRA (30 μM) in PBS (pH 7, 0.1 M) on AuNPs/cysteic acid/GCE. Plots of peak current and potential against pH for (B) PAR, and (C) TRA. Scan rate: 100 mV s^{-1}.

The anodic peak potential (Ep_a) of PAR and TRA shifted to less positive potentials with increasing pH from 2 to 11, suggesting the involvement of protons in the oxidation reactions. The regression equation of Ep_a against pH was: Ep_a (V) = -0.05 pH + 0.70 and Ep_a (V) = -0.08 pH + 1.41 for PAR and TRA, respectively. The slope of these equations is equal to 0.059 p/n where p is the number of protons and n is the number of electrons involved in the electrode reaction [31,32]. The slopes of -0.05 mV pH^{-1} and -78.4 mV pH^{-1} showed that an equal number of protons and electrons ($2e^-$ and $2H^+$) are involved in the oxidation reactions of PAR, in agreement the results reported in the literature [13,16,33,34], while unequal number of protons and electrons ($2e^-$ and $1H^+$) were concluded from the slope (-0.08 mV pH^{-1}) in the case of TRA oxidation [35].

Effect of scan rate. The effect of scan rate (ν) was studied on redox peaks of PAR and TRA in the range of 0.01-0.3 V s^{-1} on AuNPs/Cysteic acid/GCE (Fig. 5). As expected, the peaks heights increased with scan rate in the case of both drugs; linear relationship between peak currents and $v^{1/2}$ (Figs. 5A and 5B) was obtained which showed the diffusion of the drugs to the surface of the modified electrode as the main route for mass transfer [36].

Determination of PAR and TRA

Square wave voltammetry (SWV) was used for simultaneous analysis of PAR and TRA at the surface of

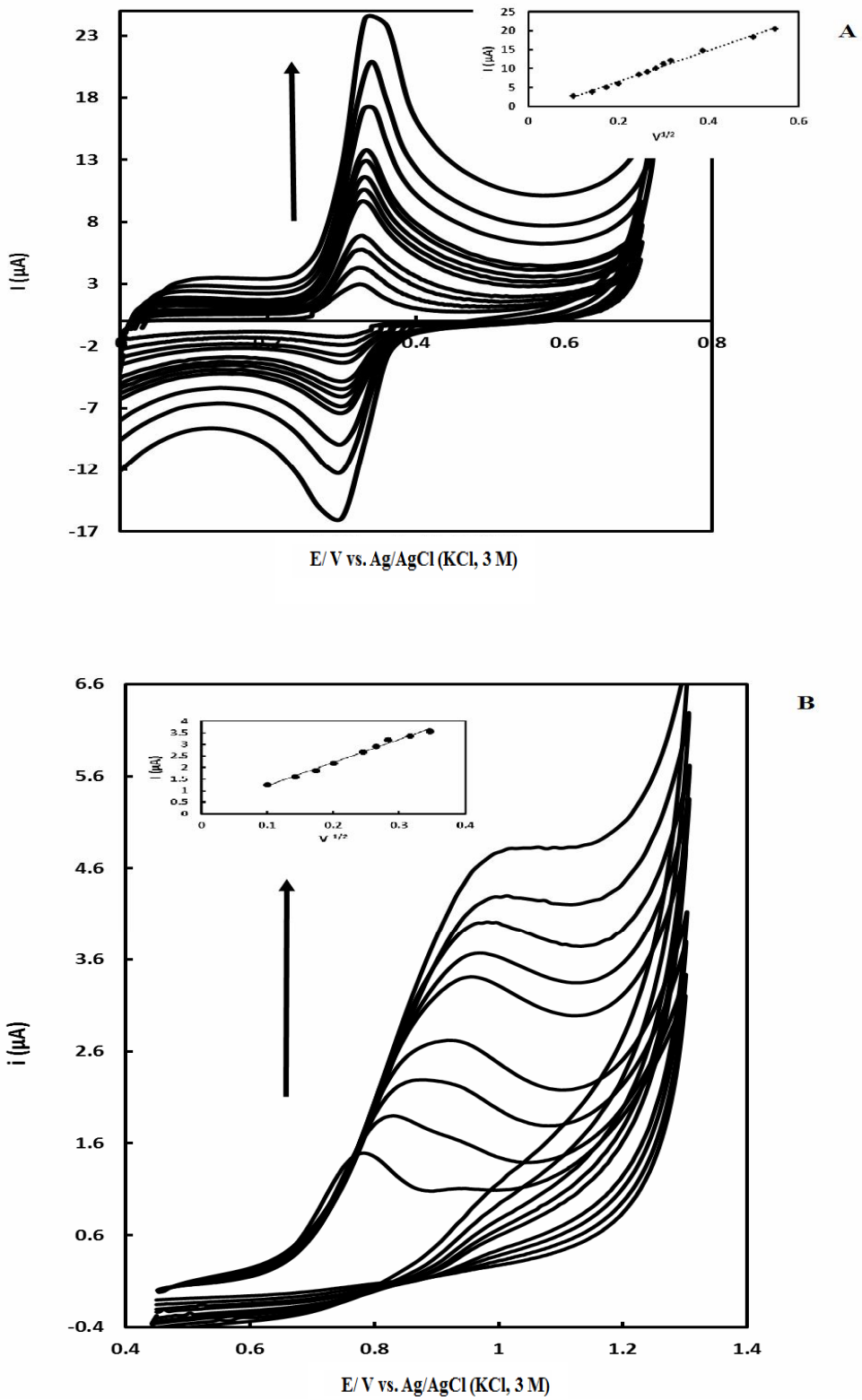

Fig. 5. Cyclic voltammetry at different scan rates on AuNPs/cysteic acid/GCE in the presence of (A) PAR, and (B) TRA in PBS (pH 7.0, 0.1 M). Insets: Linear plots of oxidation peak currents against square root of the scan rate.

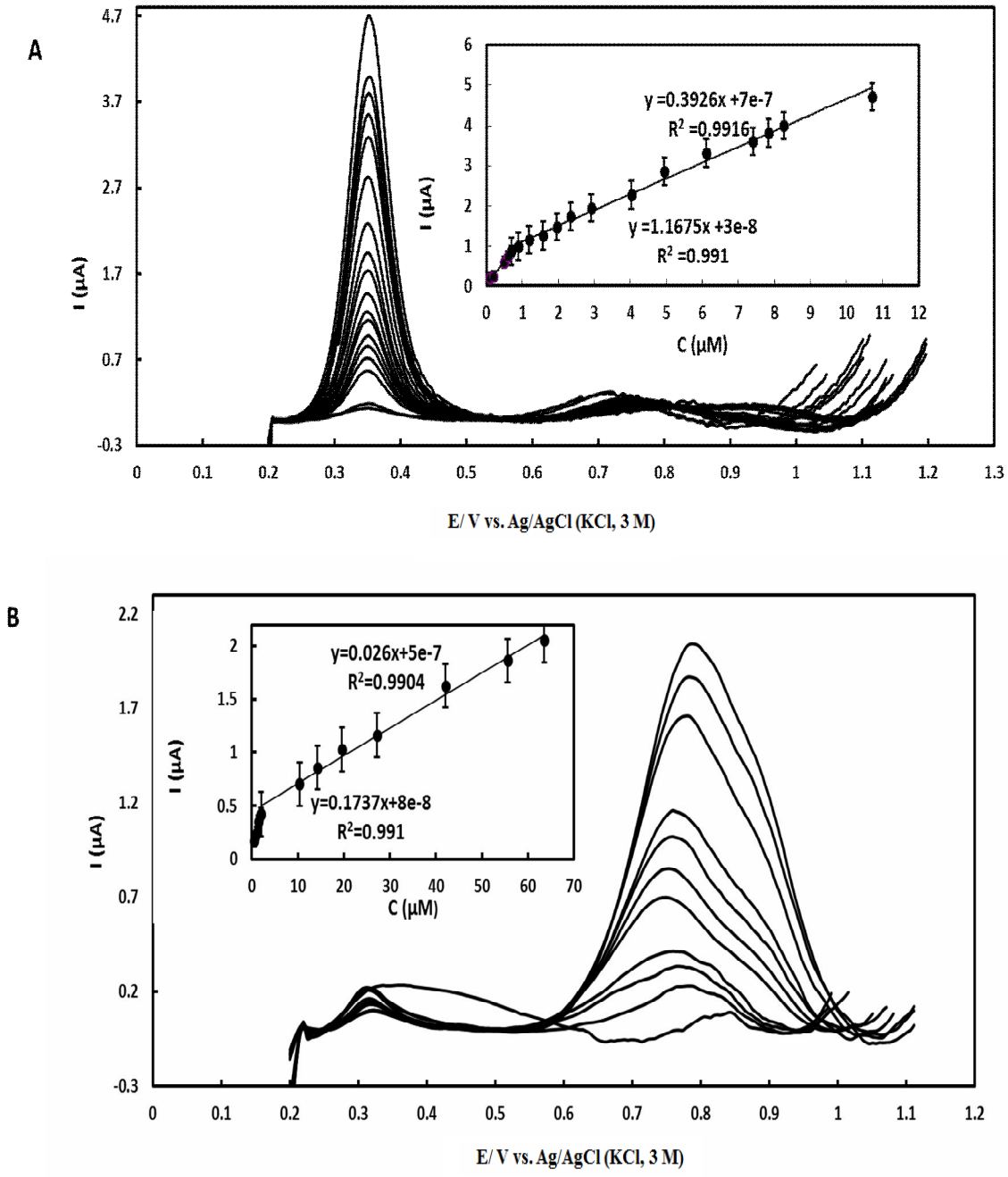

Fig. 6. SWVs on AuNPs/cysteic acid/GCE for (A) different concentrations of PAR in the presence of TRA (1 µM), and (B) different concentrations of TRA in the presence of PAR (0.2 µM). Insets: Calibration plots.

AuNPs/Cysteic acid/GCE (Fig. 6). The experiments were carried out in a constant concentration of the second drug. As observed in this figure, the presence of TRA did not interfere in the determination of PAR, and vice versa. There was a two-segmented linear relationship between the peak current and concentration of PAR in a range from 0.1-10.7

Table 1. Comparison of some Electrochemical Sensors for Determination of PAR and TRA

Modifier (s)	Analyte	Linear range (μM)	Detection limit (μM)	Ref.
SWCNT modified carbon-ceramic electrode	PAR	0.2-150	0.12	[12]
N-(3,4-Dihydroxyphenethyl)-3,5-dinitrobenzamide modified MWCNT	PAR	15-270	10.0	[13]
Carbon nanoparticle/GCE	PAR	0.1-100	0.05	[17]
	TRA	10-1000	5	
Poly (3,4-ethylenedioxythio-phene)-modified screen-printed electrodes	PAR	4.0-400	1.39	[15]
Poly(AY)/Nano-Tio_2/GCE	PAR	12-120	2.0	[14]
Aluminum electrode modified by Thin layer of palladium	PAR	100-5000	5.0	[16]
Cobalt Microparticles Film Modified Platinum Electrode	PAR	0.5-100	0.42	[18]
Graphene/$NiFe_2O_4$	PAR	0.010- 9.0	0.0036	[35]
Nanocomposite	TRA	0.010- 9.0	0.003	
Poly (nile blue) modified	PAR	0.2-16.2	0.08	
Glassy carbon electrode	TRA	1.0-310	0.5	[37]
Au/cysteic acid/GCE	PAR	0.1-10	0.03	
	TRA	0.5-63.5	0.17	Present work

μM (Fig. 6A, inset). In the case of TRA (Fig. 6B, inset), the linear concentration range was 0.5-63.6 μM. Limit of detection (LOD, 3S/N) was calculated as 0.03 μM and 0.17 μM for PAR and TRA, respectively. Some previously reported modified electrodes for PAR and/or TRA were studied and the results were compared with the present work (Table 1). In addition to the comparable linear range and LOD, the simple procedure for electrode modification and its robustness are among the advantages of AuNPs/Cysteic acid/GCE against other modified electrodes.

Interference Studies

To evaluate the selectivity of the method for the simultaneous determination of PAR and TRA in real

Table 2. Selectivity of the Proposed Method in the Simultaneous Determination of PAR (10 µM) and TRA (30 µM)

Interferent	Tolerance limit ($c_{Interferent}/c_{PAR}$)	Tolerance limit ($c_{Interferent}/c_{TRA}$)
Glycine	80	30
Asparagine	150	30
Urea	150	50
Glucose	150	50
Mg^{2+}	200	66
NH_4^+	200	66
Cl^-	200	66

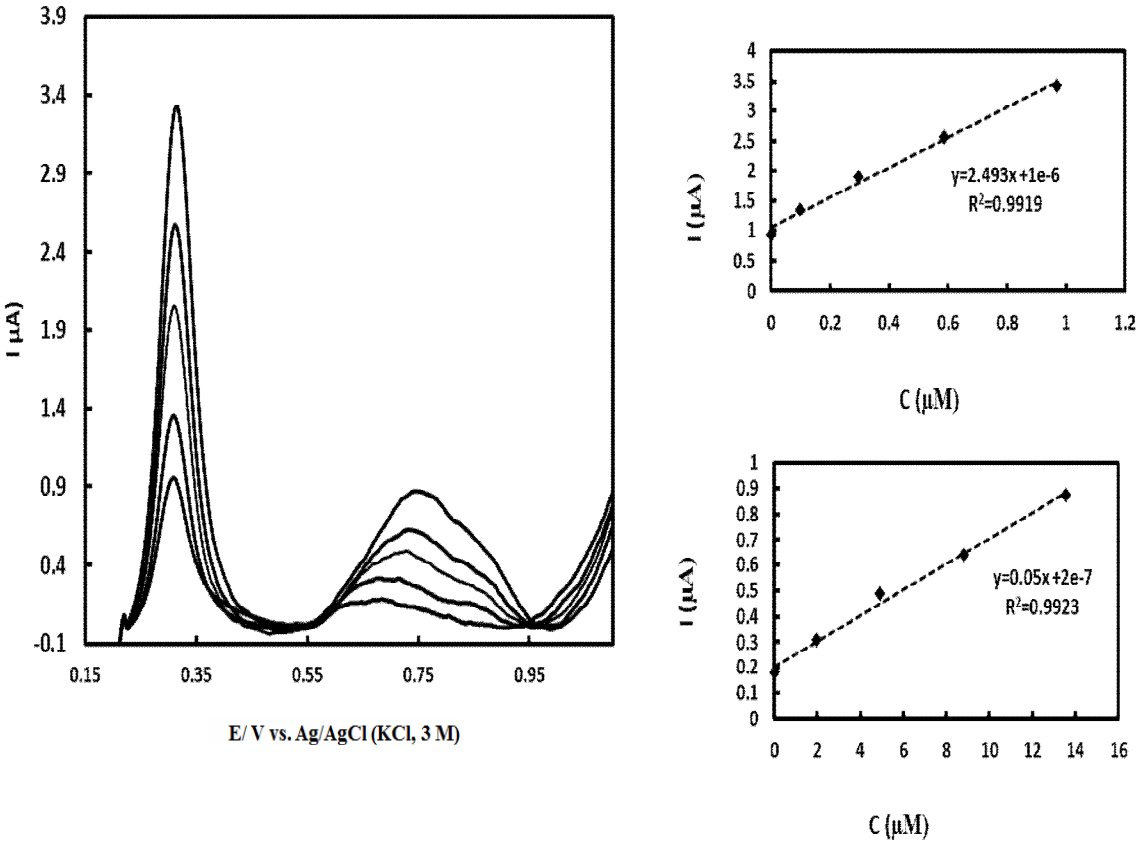

Fig. 7. (A) SWV of spiked human blood plasma with PAR (0.4 µM) and TRA (4.0 µM) at AuNPs/cysteic acid/GCE in PBS (0.1 M, pH 7.0). Inset: Calibration plots for PAR (above) and TRA (below).

samples, the influence of potentially interfering substances was investigated. The tolerance limit for interfering species was considered as the concentration associated with a relative error less than ±5.0% in the concentration of PAR or TRA. From Table 2, it was concluded that the method has good selectivity for the determination of PAR and TRA in the presence of studied compounds.

Repeatability and Reproducibility

The relative standard deviation (RSD%) for simultaneous determinations of PAR (10 µM) and TRA (30 µM) was calculated to be 3.11 and 3.30%, respectively (5 replicate determinations). Moreover, when 3 different electrodes were used, RSD% was 3.92 and 4.02%, respectively. Based on the results, it was concluded that the method has an acceptable precision in the simultaneous determination of PAR and TRA.

Analytical Application

Diluted human blood plasma samples were spiked with known amounts of PAR (0.4 µM) and TRA (4.0 µM) and their recovery was obtained by the proposed method (Fig. 7). The calculated recoveries for PAR and TRA were found to be in the rage 99.5-102%, with RSD% 0.71-1.9%. The results show the selectivity and sensitivity of the proposed method in simultaneous analysis of PAR and TRA in complex biological fluids.

CONCLUSIONS

A nanocomposite of Au nanoparticles and cysteic acid was electrodeposited on a glassy carbon electrode. The favorite adsorption of cysteic acid on the electrode surface, on one hand, and strong interaction between Au and cysteine, on the other hand, made the modified electrode uniform and stable. The electrode was used in the simultaneous determination of analgesic drugs, paracetamol and tramadol, successfully. The enlarged surface area of the electrode and high electrocatalytic activity brought about by the nanocomposite were useful in the sensitive and selective determination of paracetamol and tramadol. The linear dynamic ranges of concentration, obtained by square wave voltammetry, were 0.1-10.7 µM and 0.5-63.5 µM, for paracetamol and tramadol, respectively. Limit of detection (LOD) was calculated as 0.03 µM for paracetamol and 0.17 µM for tramadol. Application of the proposed method in recovery experiments was carried out in spiked human serum samples. The obtained recoveries for both analgesics were 99.5-102 % (RSD% < 5%, n = 3).

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