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Electrochemical Determination of Dopamine, Acetaminophen and Tryptophan using AuPdCu-rGO-MWCNTs Nanocomposite as the Sensing Layer

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Abstract-In this study, AuPdCu-rGO-MWCNTs nanocomposite was synthesised by a chemical reduction method which was applied to fabricate an electrochemical sensor for simultaneous determination Dopamine (DA), Acetaminophen (AC) and Tryptophan (Trp) by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. The formation of Au, Pd, and Cu nanoparticles on rGO-MWCNTs nanocomposite are characterized by XRD, FTIR, and SEM techniques. The relationship between the concentration of DA, AC, and Trp with the response of AuPdCu-rGO-MWCNTs/CPE was linear in the range of 10 nM to 8.3 µM, 20 nM to 12.5 µM and 10 nM to 9.4 µM by DPV technique, and 0.007-8.0, 0.011-11.6 and 0.008-8.8 µM by amperometric method, respectively. Also, the detection limits for DA, AC, and Trp were calculated to be 3, 7 and 4 nM, respectively by the voltammetric method and 2, 5 and 3 nM for DA, AC and Trp, by amperometric method. The investigations show that AuPdCu-rGO-MWCNTs/CPE has acceptable selectivity, stability, repeatability, and reproducibility. Finally, AuPdCu-rGO-MWCNTs/CPE was successfully applied to determine the analytes in the urine, blood plasma, and AC Tablet by desirable percentage of relative standard deviation and recovery.

Keywords- Voltammetry; Dopamine; Acetaminophen; Tryptophan; Graphene; Nanocomposite; Determination

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1. INTRODUCTION

Dopamine (DA), which is naturally produced in human brain and kidney, is one of the main neurotransmitters which plays a remarkable role in central and peripheral nervous systems, renal, hormonal and cardiovascular systems. DA act as a neurotransmitter in the brain also controls the amount of sodium excretion in the kidneys. The research has shown that low level of DA may lead to Parkinson's disease, schizophrenia and renal parenchymal disease. In the other hand,a high dosage of DA can increase heart rate and blood pressure [1].

Acetaminophen (AC) or paracetamol has been known as a safe analgesic and antipyretic drug since 1953 to treat fever, cold, cough and pain such as headache, toothache and backache. However, the impressment mechanism of AC is still debated; it has been assumed that AC activates descending serotonergic pathways, which leads to the inhibition of prostaglandin (PG) synthesis. Prostaglandins cause pain and inflammation after cell injury by several mechanisms [2].

Inappropriate dosing, acetaminophen could be considered as a safe painkiller available, with minimal side effect while serious poisoning has been reported due to excessive consumption of prescribed doses, liver failure and kidney damage, for instance. Therefore, this would be a compelling reason to provide a sensitive and accurate method to measure crucial doses of acetaminophen to improve the quality of presenting medicine [2,3].

Tryptophan (Trp) as one of the most important amino acid for the human body and herbivores. Trp controls serotonin levels in the brain. Also, it is a precursor of melatonin and niacin. The amount of Trp is necessary for equilibrating the nitrogen level and the maintenance of muscle mass and body weight in humans. Some people cannot metabolize Trp properly, so the possibility of schizophrenia has increased dramatically in these people.
Also, the abnormal level of Trp in plasma leads to hepatic disease. Forasmuch as the human body cannot able to synthesize Trp directly, so it must add to food products and pharmaceutical formulas. Therefore, detection of the concentrations of these molecules with a simple, rapid, sensitive, selective, and inexpensive method is a great interest for analytical chemists [4].

Hitherto, several analytical techniques have been used for the determination of DA, AC, and Trp such as mass spectrometry and high-performance liquid chromatography [5], and spectrophotometry [6]. These methods are laborious, with intricate preparation steps and likewise the comparatively expensive; however, the mentioned methods are relatively sensitive, selective, and with high accuracy. These disadvantage of the procedure limit their usage to the measurement of analytes [7,8].

In recent years methods that are based on electrochemistry have been used for the measurement of many drugs, biomolecules, heavy metals, and other compounds. The various superiorities such as cost-effective, sensitive, selective, and rapid, with comparatively simple tools are the reason for much consideration to electrochemical methods [9,10].

Since some of the electroactive species have same oxidation potential and also because of pronounced electrode fouling, simultaneous determination of some drugs at the surface of bare electrodes such as carbon paste electrode (CPE), glassy carbon electrode (GCE) and screen-printed electrode (SPE) is difficult, besides, the selectivity and reproducibility at these electrodes is poor.

Diverse kinds of electrodes have been applied for simultaneous measurement of ions and molecules to overcome the mentioned hindrance, which using carbon-based materials, enzymes, nanocomposites or other modifiers such as ligands and their complex with the heavy metal, Molecularly Imprinted Polymer (MIP) and Nafion [11-13].

Amongst these modification ways, the pleasant technique for ameliorating the electrochemical sensor efficiency is using nanomaterials for the procurement of the sensing layer. In electrochemical usages of metal nanoparticles, they supply multiple particular functions, such as electrocatalytic activity and amelioration of conductivity [14,15].

Gold NPs (Au NPs) have unique attributes in comparison with its bulk form, such as supreme electrical conductivity, and accordingly, has been used in sensing systems to measure toxic or non-toxic compounds [16].

Palladium NPs (Pd NPs) play a crucial responsibility in many technologies and are applied in plentiful applications such as gas sensing and electro-oxidation reactions [17].

Copper NPs (Cu NPs) is one of the important choices for applying in electrochemical sensor because of the high conductivity and low cost in comparison wih the noble metal nanoparticles [18].

Other kinds of nanomaterials used in the electrochemical sensing layer are carbon-based nanomaterials like Graphene (Gr) and Multiwall Carbon Nanotubes (MWCNTs). Gr is a two-dimensional monolayer of carbon atoms that are bonded together in a honeycomb crystal lattice. Gr suggestion particular specifications like the flexibility, superbelectrical conductivity [19], 2630 m2/g surface area, 1,100 GPa Young's modulus, and 125 GPa fracture strength, based on these properties Gris a noteworthy candidate for the construction of working electrodes [20].

Based on previous studies, the physical properties of metal nanoparticles (NPs) are distinct from those of bulk metal and depend on the size of particles [16].

MWCNTs show particular features like good electrical conductivity (103–106 S/cm) and fast electron transfer rate. The unique properties of MWCNTs are originated from the activity of the edge-plane-like graphite sites at their ends [21], broad surface area as a consequence of high surface to volume ratio, super mechanical and chemical stability. Therefore, the MWCNTs are a conspicuous candidate for employing in electronic industries as basic material for the construction of a new electronic apparatus.

The new investigations represent that mixture of some nanoscale metals in order to form an alloy lead to betterment the final properties such as thermodynamic stability,

electrochemical, and electrocatalytic characteristics.

The acidic pretreatment of MWCNTs and Graphene oxide (GO) to modifythem with some types of an oxygen-containing functional group can act as a conductive protecting agent [22]. Coating the MWCNTs and Grnanomaterials by metal nanoparticles is the specific way for the improvement of the electrochemical properties and the construction of the working electrodes [18].

In the present research, we described the synthesis of a nanocomposite by using three metals and two carbon base materials to the construction of an efficacious electrochemical sensor (AuPdCu-MWCTs/Gr/CPE) for determination of DA, AC, and Trp. The response of the modified electrode compared to a bare electrode has remarkably increased. The analytical performance AuPdCu-MWCTs/Gr/CPE was successfully evaluated by differential pulse voltammetry (DPV) method in different real samples.

2. EXPERIMENTAL

2.1. Reagents and equipment

The using materials in the presented research have the analytical grade and were used without any further purification. MWCNTs, Graphite powder (<20 μm), H_2SO_4, HCl, H_2O_2, $KMnO_4$, $(CH_2OH)_2$ (ethylene glycol), $HAuCl_4.4H_2O$,HNO_3,$PdCl_2$, $NaBH_4$,and $CuSO_4.5H_2O$ were procured from Aldrich Company. DA, AC, and Trp and the chemical interferences compounds were purchased from Merck Company. Deionized water was used for the preparation of the solutions. Britton-Robinson universal buffer solution (B-R buffer solution) was used as supporting electrolytes and pH adjustment agent. Glassware laboratory equipment was kept in the 10% v/v HNO_3 solution, then washed by deionized water before using.

All electrochemical measurement were done by Autolab electrochemical system (302 N, Utrecht, Netherlands)in the ambient temperature with a conventional three-electrode system containing an Ag/AgCl/KCl (3 M) electrode (reference electrode), modified or bare GCE (working electrode) and Pt wire (counter electrode). The pH of solutions was measured by a Metrohm pH meter (model 713-Switzerland). In order to prepare SEM images, FTIR spectra and XRD patterns an SEM (SEM-EDX, XL30, Philips Netherland), a Perkin-Elmer spectrophotometer (Spectrum GX) and XRD (38066 Riva, d/G. Via M. Misone, 11/D (TN) Italy) were used, respectively.

2.2. Synthesize of modifiers and fabrication of modified electrodes

GO sheet was synthesized a modified Hummer-Offeman's method using graphite powder [23,24]. In order to increase the oxygen-containing functional group and remove the impurities (graphite or metal) of MWCNTs, the acidic pretreatment process was done.

Firstly, 0.4 g MWCNTs were heated at 400 °C under an airflow of 12 ml/min for 90 min. Then, 0.2 g cooled MWCNTs were dispersed in 100 mL HCl (6.0 M) and sonicated for 4 h at ambient temperature. Afterwards, the prepared mixture was filtered by Whattman No. 42 filter paper and washed by deionized water to reach the pH=7, and for drying the wet MWCNTs, the IR lamp was used.

The rGO/MWCNTs mixture was made by sonicating the suspension of prepared GO and MWCNTs and the addition of $NaBH_4$ as a chemical reduction agent. In addition, the AuPdCu-rGO-MWCNTs nanocomposite was synthesized by the same procedure. Firstly, 8.98 mg $PdCl_2$, 20.61 mg $HAuCl_4.4H_2O$, and 25.25 mg $CuSO_4.5H_2O$ was added to ethylene glycol and kept under stirring until the metal salts completely dissolved. In the next step, 32.33 mg rGO-MWCNTs was dispersed in the prepared solution by sonication for 3 h. To the reduction of Au, Pd, and Cu ions on the surface of rGO-MWCNTs, the additional amount of $NaBH_4$ was added to the mixture one drop at a time and strenuously stirred for 4 h to place the Au, Pd and Cu metal nanoparticles uniformly onto the surface of rGO-MWCNTs. After finishing the stirring, the suspension was centrifuged and washed by deionized water/ethanol for three times [27].

CPEs were constructed by the common method; a mixture of graphite powder and paraffin oil were prepared in 3:1 w/w ratio into a mortar using a pestle. To make the homogeneous modified paste, 12% w/w of modifiers (include rGO, rGO-MWCNTs, or AuPdCu-rGO-MWCNTs nanocomposite) were mixed truly with the graphite powder and paraffin oil in 12:63:25 w/w%, respectively. Eventually, unmodified or modified carbon pastes were attentively packed into a piston-driven carbon paste electrode holder so that air bubbles come out of the layers. The sensing layer of the electrodes was renewed mechanically by scraping out the used surface with a filter paper.

2.3. Spiked and Real samples preparation

The applicability of the AuPdCu-MWCTs/Gr/CPE for measuring DA, AC, and Trp in different real samples was examined using the standard addition technique. The commercial pharmaceutical tablets were bought from a local pharmacy in Tehran. Iran. Ten tablets were weighed accurately, powdered, and homogenized in a mortar, and then a sufficient amount of the prepared powders was weighed and dissolved in deionized water using stirring of magnetic stirrer for 30 min to ensure that powdered tablets were dissolved totally. The mixture was filtered and transferred to a 100 mL volumetric flask and stored at 4 °C. Supporting electrolytes was used for diluting samples to electrochemical analysis [25].

For the preparation of a urine sample, 20 ml of the urine sample was centrifuged for 40 min at 2000 rpm; then, the supernatant was filtered using a 0.45 mm filter. The obtained sample was diluted 5-times with the supporting electrolyte of pH 7.5. The prepared urine sample was stored in a refrigerator before using [25].

For removing redundant proteins in the blood plasma samples, 1.0 mL of the blood samples were mixed with 0.15 ml perchloric acid and stirred for 1 min. In the next step, the sample was centrifuged at 2000 rpm for 40 min. The obtained supernatant was added to a 10 ml B-R buffer solution of pH=7.5 and transferred into the electrochemical cell [26].

3. RESULTS AND DISCUSSION

3.1. Surface characterization of the differently prepared nanocomposites

In order to investigate the phase composition and phase structure of the synthesized nanoparticles and nanocomposites, they were examined by XRD analysis. Fig.1a illustrates the XRD patterns of the synthesized GO, rGO, MWCNTs, rGO-MWCNTs, and AuPdCu/rGO-MWCNTs.

The XRD patterns of GO and rGO represent typical characteristic peaks at 2θ=10.81° and 25.78°, respectively, which they are fit well with previous researches for the GO and rGO nanomaterials. So, as a result, rGO was formed from GO by the addition of NaBH4. In the case of MWCNTs, the observed peak at 2θ=25.86° and 43.98° are related to the graphite plate of MWCNTs. The appeared to peak about 2θ=25 and 43° in the rGO-MWCNT nanocomposite, which is most likely from the MWCNTs and has a little shifted to a lower angle, also, no related peak of GO is detected in rGO-MWCNT.

The XRD pattern of AuPdCu/rGO-MWCNT demonstrates five peaks at about 24.79, 36.82, 43.34, 64.09 and 77.12owhich correspond to the (002) the plane of rGO-MWCNTs, and (111), (200), (220) and (311) the plane of face-centred cubic (fcc) crystalline structure [27].

The obtain XRD data confirm the synthesis of AuPdCu/rGO-MWCNT nanocomposite.
For more investigation, the FTIR spectra of the synthesized nanomaterials were evaluated, and the spectra are presented in Fig. 1b. The FTIR spectrum of GO illustrates some peaks at 3431.08, 2970.25, 1646.85, 1646.11, 1401.19, and 1248.38 cm^{-1}, which are related to O−H stretching, C−H stretching, C=O stretching, C-O-H vibration, C−O stretching related to the phenolic group, and C=C stretching related to aromatic rings, respectively.

The FTIR spectrum of acidic-MWCNTs represents four main peaks at 3732.75, 3449.15, 2356.63, and 1559.25 cm^{-1} which is related to the free hydroxyl groups, the O–H stretch of O=C−OH and C−OH (corresponds to carboxyl groups), O−H stretch of the hydrogen-bond carboxylic acid group and carboxylate anion stretch mode, respectively [28]. The presence of oxygen-containing functional group on the surface of GO and acidic-MWCNTs can improve wetting characteristics and trapping of metal particles, and plays the role as an anchoring site of Au, Pd, and Cu metal ions. The absorption band of oxygen-containing functional groupsin the spectrum of rGO has decreased remarkably after the addition of $NaBH_4$. The FTIR spectrum of the GO-MWCNTs mixture shows the main peaks of both GO and MWCNTs.

In addition, the FTIR spectrum of AuPdCu/rGO-MWCNT shows a peak at 2876.59, which is related to C-H symmetric stretching, and the combination band becomes visible at 2665.38 cm^{-1}. The broad peak of O-H, which appeared at about 3500 cm^{-1}, is completely removed, indicates the reduction of ions and oxygenated groups with $NaBH_4$. Moreover, other peaks of GO have appeared with lower intensity in comparison to the original spectrum.

SEM image investigated the morphology of the synthesized AuPdCu/rGO-MWCNT nanocomposite. Fig. 1c illustrates the formation of the AuPdCu nanoalloy with an average size of 26 nm on the surface area of the rGO-MWCNTs nanocomposite. Moreover, the EDS pattern of AuPdCu/rGO-MWCNT was recorded to investigate the composition of the nanocomposite and is depicted in Fig. 2b inset. The EDS analysis exhibits the presence of Au, Pd, Cu, and C elements, which authenticates the presence of the three kind metals on the synthesized composite.

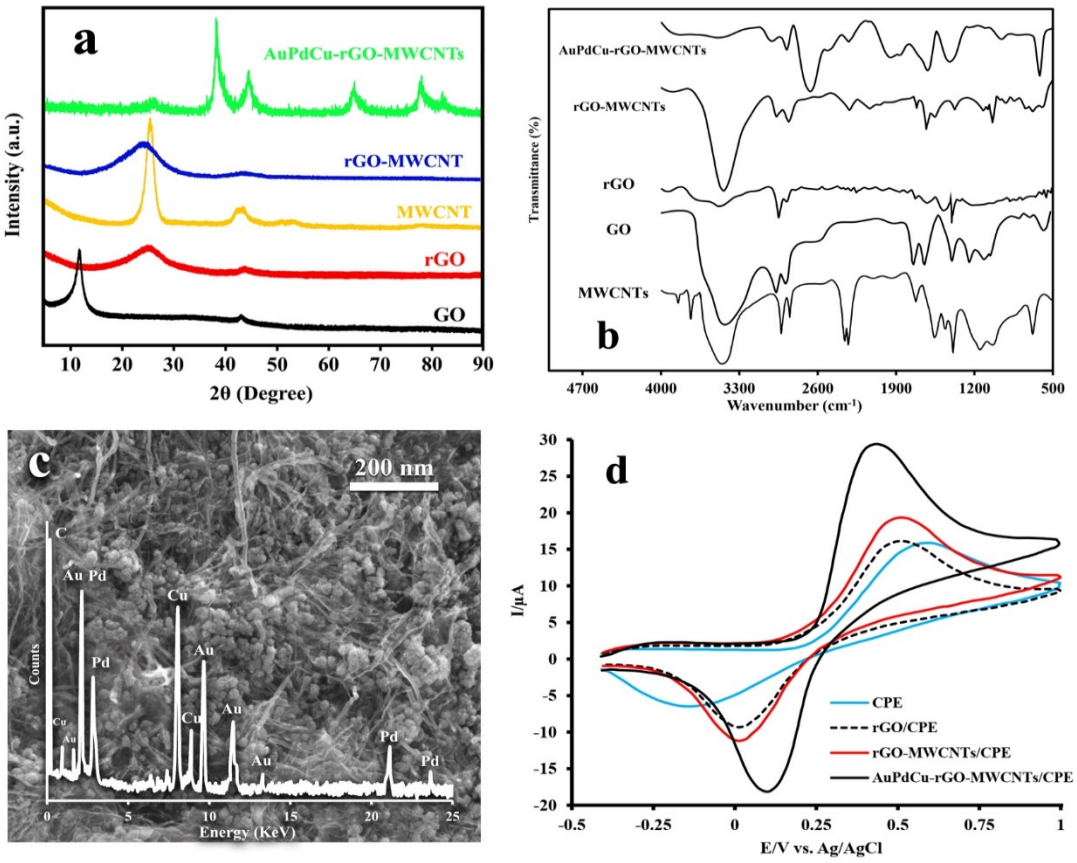


Fig. 1 (a) The XRD patterns **(b)** the FTIR spectra of the synthesised GO, rGO, MWCNTs, rGO-MWCNTs, AuPdCu-rGO-MWCNTs; **(c)** SEM image of AuPdCu-rGO-MWCNTs/CPE (Inset: the EDS pattern); **(d)** CVs and **(e)** Nyquist plots of the bare CPE, rGO/CPE, rGO-MWCNTs/CPE, and AuPdCu-rGO-MWCNTs/CPE in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ and 1.0 M KCl (Scan Rate=100 mV/s); **(f)** CVs of AuPdCu-rGO-MWCNTs/CPE at different scan rates from 20 to 400 mVs-1 in 5.0 mM [Fe(CN)6]3-/4- and 1.0 M KCl.

3.2. Electrochemical characterization of the constructed sensors

The electrochemical characteristics of the constructed modified and unmodified sensor were investigated in the presence of 5.0 mM $[Fe(CN)6]^{3-/4-}$ and 1.0 M KCl by the cyclic voltammetry technique (CV). The CVs of CPE, rGO/CPE, rGO-MWCNT/CPE, and AuPdCu-rGO-MWCNTs/CPE are shown in Fig. 1d. The probe illustrates a weak redox peak with high electrochemical peak separation at the surface of the CPE; on the other hand, with the addition of rGO in the matrix of the sensing layer, the oxidation and reduction currents have increased while the electrochemical peak separation has reduced to 498 mV. On the rGO-MWCNT/CPE, the using probe shows a good reversible CV with the oxidation and reduction currents of 12 and 11.6 µA accompanied by a 494 mV separation peak. By using AuPdCu-rGO-MWCNTs/CPE as a working electrode, the best result was obtained. The redox currents have growth about 1.6 fold in comparison to rGO-MWCNT/CPE, and the obtained electrochemical peak separation was 330 mV.

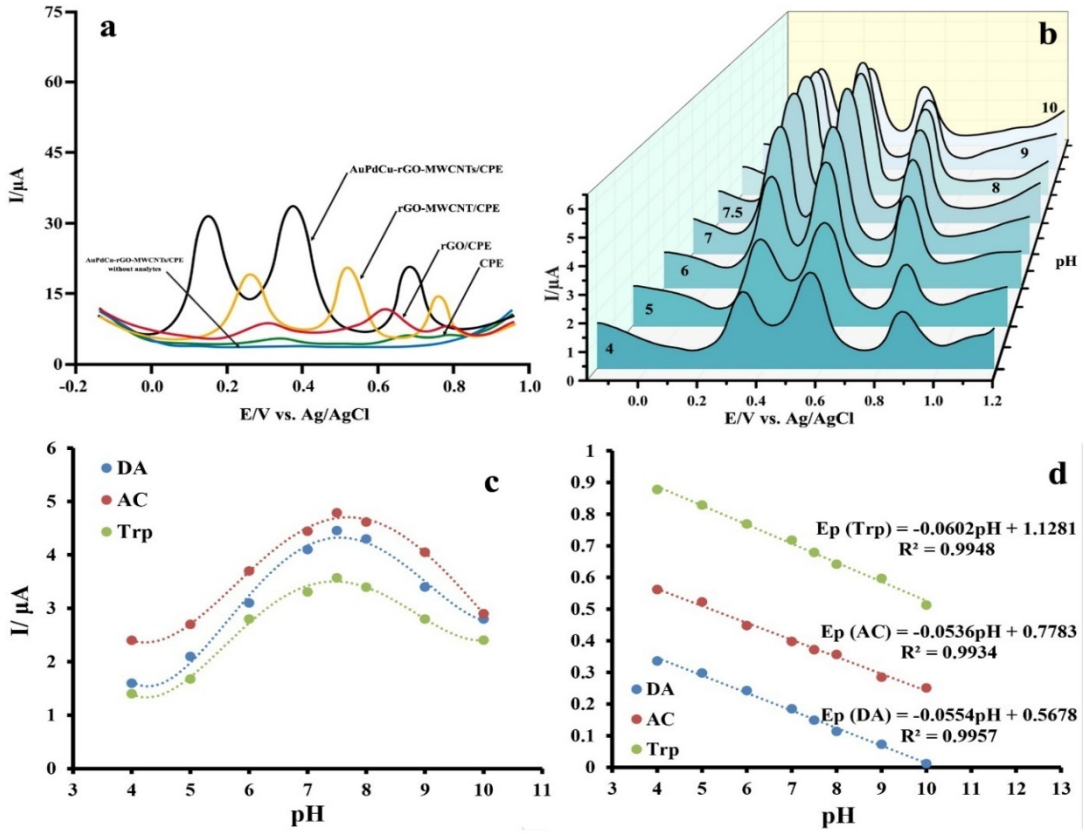


Fig. 2. (a) SWV of CPE, rGO/CPE, rGO-MWCNTs/CPE, and AuPdCu-rGO-MWCNTs/CPE in the presence of 5.0 µM DA, AC and Trp and AuPdCu-rGO-MWCNTs/CPE in the absence of the analytes, **(b)** The SWV of 1µM DA, AC and Trp at AuPdCu-rGO-MWCNTs/CPE at different pH between 4 and 10, **(c)** The influence of pH on (c) current and **(d)** potential of the peak of 1µM DA, AC and Trp.

3.3. Electrochemical behaviour of DA, AC, and Trp at the different electrodes

The electrochemical behaviour 5 μM of the target analytes at bare CPE, rGO/CPE, rGO-MWCNT/CPE, and AuPdCu-rGO-MWCNTs/CPE was studied at the pH= 7.5 by Square wave voltammetry technique (SWV), and the results are depicted in Fig. 2a.

It can be seen; the bare CPE shows three broad oxidation peaks with low oxidation peak currents (I_{pa}) centred at 343, 681, and 779 mV for DA, AC, and Trp, respectively, which are owing to slow electron transfer at the bare CPE. Also, due to oxidation peak potential (E_{pa}) separation between AC and Trp (ΔE_{pa}=98 mV) the simultaneous determination of AC and Trp at CPE was not possible, on the other hand, the simultaneous determination of DA with AC and DA with Trp can be done because ΔE_p (DA-AC)=338 mV and ΔE_p (DA-Trp)=429 mV.

In comparison to bare CPE, it was observed that the E_{pa} of DA, AC, and Trp were a negative shift by using rGO/CPE at 309, 618, and 772 mV, while the I_{pa} for the analytes has increased to 4.5, 7, 4 μA, respectively.

Three sharp oxidation peaks appeared at 261, 520, and 760 mV for DA, AC, and Trp at the surface of rGO-MWCNT/CPE; it can be seen that the ΔEp (DA-AC)=259 mV and ΔE_p (AC-Trp)=240 mV so simultaneous measurement of all analytes can be done. Also, the I_{pa} for DA, AC, and Trp have increased to 3, 2.59, and 2.28 fold compared to rGO/CPE, respectively.

In contrast, at the AuPdCu-rGO-MWCNTs/CPE, three well-defined peaks were observed, and E_{pa} of DA, AC, and Trp shift negatively to 151, 374 and 683 mV with ΔE_p (DA-AC)=223 mV and ΔE_p (AC-Trp)=309 mV. In addition, I_{pa} for DA, AC and Trp have significant growth to 19.83, 22.45, 13.77 μA, respectively, which are 4.41, 3.21 and 3.44 times higher than at rGO/CPE which makes this electrode the best candidate for simultaneous measurement of the analytes with a low detection limit (DL). The decrement in oxidation overpotential with the enhancement of I_{pa} indicates that AuPdCu-rGO-MWCNTs nanocomposite has boosted electrocatalytic activity towards DA, AC and Trp oxidation. Accordingly, the electrochemical features of the AuPdCu-rGO-MWCNTs nanocomposite are much desirable for the voltammetric measurement of the target analytes, so in the other experiments, AuPdCu-rGO-MWCNTs/CPE is used as a working electrode.

3.4. pH optimisation

The impacts of pH on I_{pa} and E_{pa} of 1 μM DA, AC and Trp at AuPdCu-rGO-MWCNTs/CPE in the range of 4–10 using B-R buffer solutionwere investigated by SWV technique, and the results are shown in Fig. 2b.We can see from Fig. 2b that the peak currents and potential of oxidation peak have changed by variation of solution pH. As shown in Fig. 2c, for DA, AC and Trp with increasing pH value from 4.0 to 7.5, the peak currentshave

growth and reaches the maximum value at pH =7.5,after that have a downtrend with the increasing the pH values between 7.5 and 10.

On the other hand, the peak potentials for the three analytes shifted to negative direction with increasing pH value owning to participate of hydrogen ions in the electrochemical oxidation of the analytes at the surface of AuPdCu-rGO-MWCNTs/CPE (Fig. 2d).

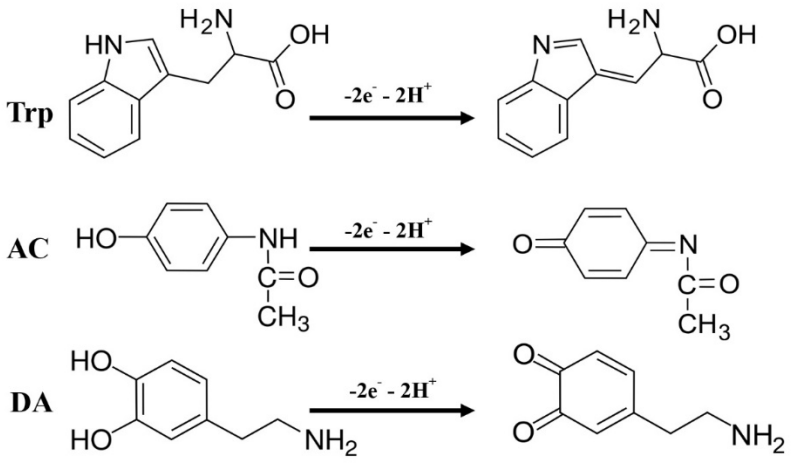
The potentials of DA, AC and Trp followed the linear regression equations with changing the pH value:

DA: E_{pa} = -0.0554pH + 0.5678 R^2 = 0.9957 Eq. 1

AC: E_{pa} = -0.0536pH + 0.7783 R^2 = 0.9934 Eq. 2

Trp: E_{pa} = -0.0602pH + 1.1281 R^2 = 0.9948 Eq. 3

For all target analytes, the slop of E_{pa}-pH equation is very close to the theoretical value of 59 mV/pH at 25 °C expected from the Nernst equation. These results indicate that the ratio between the number of electron and proton which participated in electro-oxidation of DA, AC and Trp is 1 and based on previous reports the reactions are two electron–two proton process [26]. The proposed mechanism for electro-oxidation of DA, AC and Trp are as given in scheme 1 [26].



Scheme 1. Proposed electrochemical oxidation mechanism for DA, AC and Trp

3.5. The effect of scan rate on the electro-oxidation of DA, AC and Trp

To study of reaction kinetics, the effect of scan rate on the electro-oxidation on currents and potentials peak of 5 µM DA, AC and Trp at the AuPdCu-rGO-MWCNTs/CPE was investigated by CVs (Fig 3a(I), b(I) and c(I)).

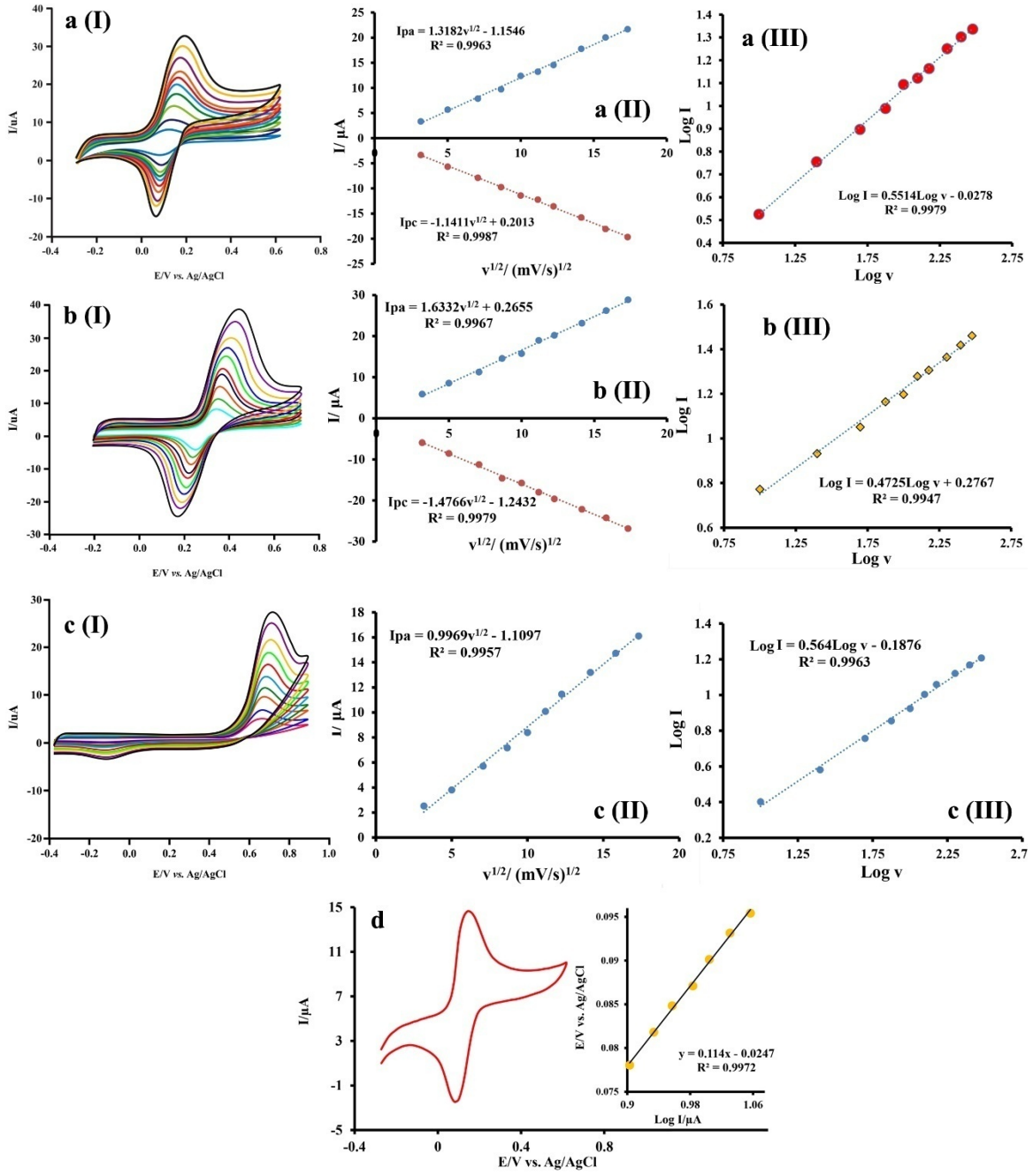


Fig. 3 CVs for AuPdCu-rGO-MWCNTs/CPE in B-R buffer solution with pH 7.5 containing 5 µM **a (I):** DA, **b (I):** AC and **c (I):** Trp with scan rates ranging from 10 to 300 mV s^{-1}; The linear relationship between the peak current vs. square root of the scan rate (υ1/2) for **a (II):** DA, **b (II):** AC and **c (II):** Trp; The linear relationship between the Log peak current vs. Log scan rate for **a (III):** DA, **b (III):** AC and **c (III):** Trp. **(d)**Tafel plot derived from the rising part of CV recorded at a scan rate 50 mV s^{-1} related to DA at AuPdCu-rGO-MWCNTs/CPE in pH=7.5.

It can be seen that for the analytes, the peak currents hadraised when the scan rateincreased in the range of 10-300 mV/s(Fig. 3a(II), b(II) and c(II)) and the oxidation or reduction peak currents changed in a linear relationship with the square root of scan rates by the following equations:

DA:	I_{pa} = 1.3182v1/2 - 1.1546	R^2 = 0.9963	Eq. 4
	I_{pc} = -1.1411 v1/2 + 0.2013	R^2 = 0.9987	Eq. 5
AC:	I_{pa} = 1.6332 v1/2 + 0.2655	R^2 = 0.9967	Eq. 6
	I_{pc} = -1.4766 v1/2 - 1.2432	R^2 = 0.9979	Eq. 7
Trp:	I_{pa} = 0.9969 v1/2 - 1.1097	R^2 = 0.9957	Eq. 8

Also by increasing scan rates, the oxidation peak potentials of the analytes shifted to positive direction while the reduction peak potentials of DA and AC shifted to lower potential values, arising from the kinetic limitations of the electrochemical reaction. The obtained results demonstrate that the electrochemical reactions of analytes at the surface of AuPdCu-rGO-MWCNTs/CPE are diffusion-controlled rather than surface controlled [25].

In addition, the changes in logarithm peak currents with the variation of logarithm scan rate were investigated, and the obtained data indicate that the slop of Log peak currents versus Log scan rate plots were 0.5514, 0.4725 and 0.5640 μA.s/mV for DA, AC and Trp, respectively (Fig. 3a(III), b(III) and c(III)).According to previous reports, when the slope is 0.5, the electrochemical reaction is a diffusion-controlled process, while the slop of 1, the electrochemical reaction occurs via an adsorption-controlled process [29]. From the obtained data, it can be concluded that the electrochemical reactions of DA, AC and Trp are governed by diffusion control process at the using electrode, and the analytes did not foul it.

For more study, in order to obtain information about the rate-determining step of oxidation of DA, the Tafel plot was drawn, as derived from points in the Tafel region of the CV. The Tafel region is affected by electron transfer kinetics between the analyte (DA) and using electrode (AuPdCu-rGO-MWCNTs/CPE). The slope of the Tafel plot was equal to 2.3 RT/n(1−α) F, which came up to 0.1140 V decade−1 for scan rate50mV s−1(Fig. 3d).Therefore, we obtained the mean value of α (charge transfer coefficient) equal to 0.4816.

3.6. Individual and simultaneous determination of DA, AC and Trp

The electrochemical responses of the AuPdCu-rGO-MWCNTs/CPE in order to individual and simultaneous determination of DA, AC and Trp in a 0.1 M B-R buffer solution at pH of 7.5were investigated, and the voltammograms are depicted in Figs. 4.

For the individual determination of DA, AC and Trp, SWV technique was applied for their mixture, keeping the concentration of two species constant while changing the other analyte. The separate measurements of DA, AC and Trp in the concentration range 0.01-8.3,

0.02-12.5 and 0.01-9.4 µM respectively, was done in the separate solutions containing AC/Trp, DA/Trp and AC/Trp at the certain concentration of 5.0 µMrespectively (Fig. 4a-c). The results show that the oxidation peak current of DA increasedlinearly with adding concentrations of it between 10 nM to 8.3 µM, which the linear regression equation is expressed as Ipa = 3.7804CDO + 0.8089 (R^2 = 0.9993); also it can be said that the currents related to AC (20 nM to 12.5 µM) and Trp (10 nM to 9.4 µM)were almost constant with less than 3% change.The voltammetric behaviours of AC and Trp were same as DA, and the regression equationsfor the calibration curves of individual determination of AC and DA can beexpressed byIpa = 3.9886CAC + 1.0886 (R^2 = 0.9991) and Ipa = 2.5724CTrp + 1.1304 (R^2 = 0.9989) respectively.

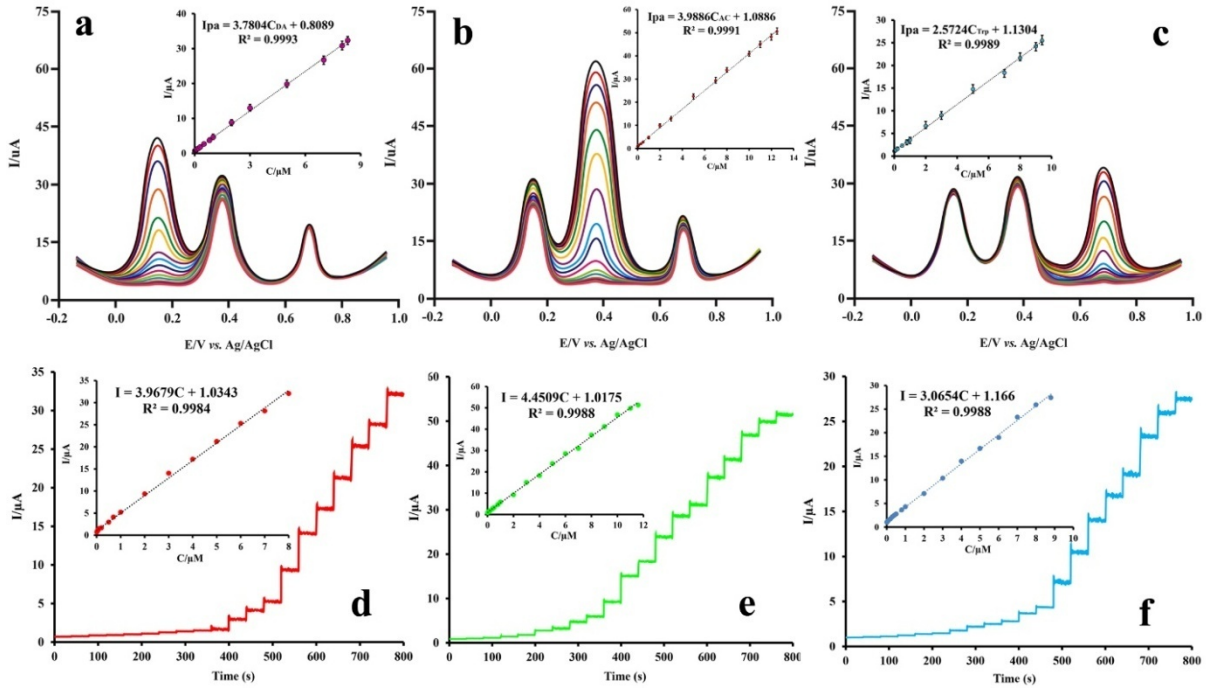


Fig. 4 (a) SWVs in B-R buffer solution pH = 7.5 for DA (0.01-8.3 µM) at the AuPdCu-rGO-MWCNTs/CPE in the presence of 5.0 µM AC and Trp, **(b)** SWVs for AC (0.02-12.5 µM) at the AuPdCu-rGO-MWCNTs/CPE in the presence of 5.0 µM DA and Trp, **(c)** SWVs for Trp (0.01-9.4 µM) at the AuPdCu-rGO-MWCNTs/CPE in the presence of 5.0 µM DA and AC; Amperometric responses of the AuPdCu-rGO-MWCNTs/CPE for determination of **(d)** DA in the range of 0.007-8.0 µM, the applied potential is 151 mV **(e)** AC in the range of 0.011-11.6 µM,the applied potential is 374 mV and **(f)** Trp in the range of 0.008-8.8 µM, the applied potential is 683 mV; in B-R buffer solution pH=7.5 and the constant stirring rate of 150 rpm.

Because Amperometry under stirred conditions has a much higher current sensitivity than some voltammetric techniques, it was used to achieve better DLs. So, the Amperometric determination of DA, AC and Trp were investigated by stirring the B-R buffer solution with pH=7.5 at a constant rate of 150 rpm andthe obtained currents were collected at a fixed

potential of 151, 374 and 683 mV, respectively. Fig. 4d, e and f illustrate the amperometric current vs. time response of the AuPdCu-rGO-MWCNTs/CPE for the determination of DA, AC and Trp, respectively. The AuPdCu-rGO-MWCNTs/CPE demonstrated a linear response to DA,AC and Trp in the concentration range of 7 nM to 8.0 µM, 11 nM-11.6 µM and 8 nM to 8.8 µM with equations of I=3.9679CDA+1.0343 (R2=.9984), I=4.4509CAC+1.0175 (R2=0.9988) and I=3.0654CTrp+1.166 (R2=0.9988) (Inset of Fig. 4d, e and f). Based on S/N = 3, the DLs were determined to be 2, 5 and 3nM for DA, AC and Trp, respectively.

In order to the simultaneous determination of the three analytes, the concentration of DA, AC and Trp have been changed in the range of 10 nM to 8.3 µM, 20 nM to 12.5 µM and 10 nM to 9.4 µM respectively and the recorded voltammograms are depicted in Fig. 5a.The oxidation currents of the analytes at AuPdCu-rGO-MWCNTs/CPE have increased linearly while the concentration of they have grown (Fig. 5b) by the following equations:

DA:	$I_{pa} = 3.7151CDO + 0.8386$	$R^2 = 0.9987$	Eq. 9
AC:	$I_{pa} = 4.089CAC + 0.9021$	$R^2 = 0.9986$	Eq. 10
Trp:	$I_{pa} = 2.6278CTrp + 1.0893$	$R^2 = 0.999$	Eq. 11

The DLs for DA, AC and Trp were calculated to be 3, 7 and 4nM, respectively, which were computed based on S/N = 3.

Also, comparison between the slope of the equations in individual and simultaneous determination for each species shows that the slopes are approximately equal to each other, concluding that the three analytes do not have interfered in the determination of each other.

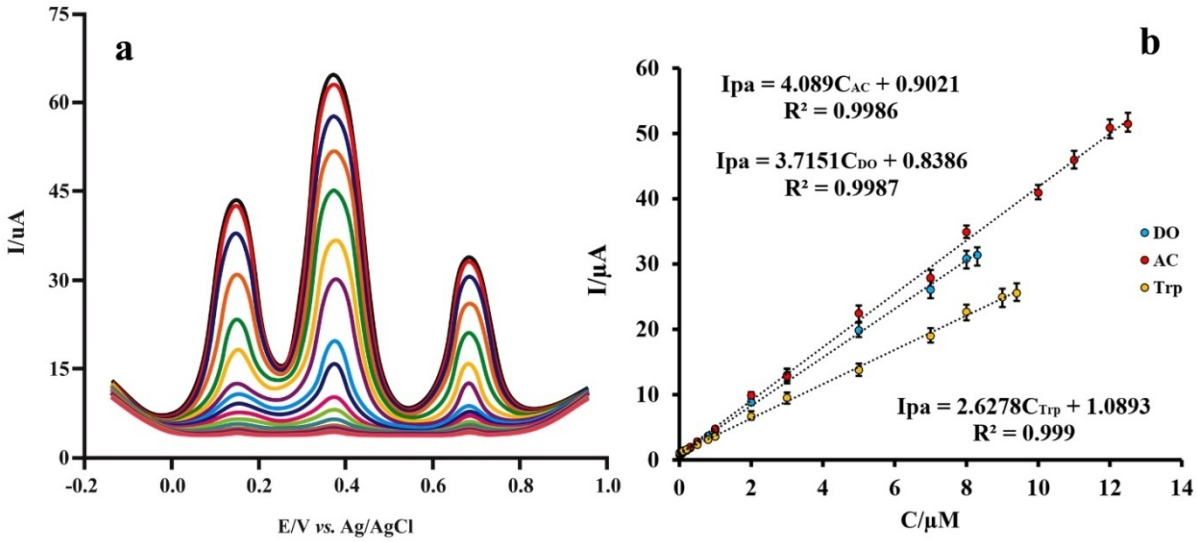


Fig. 5 (a) SWVs for different concentrations of DA, AC and Trpin pH 7.5 at AuPdCu-rGO-MWCNTs/CPE and **(b)** Calibration curves of simultaneous measurement of the analytes.

3.7. Investigation of chemical interferences, stability, repeatability, and reproducibility

The tolerance limits were taken as the maximum amount of the foreign ions and molecules, which lead to an about ±5% relative error in the current of measurements. The selectivity of the proposed method toward the simultaneous determination of 5 μM DA, AC and Trp were investigated by the addition of some species which can change the response of the electrode. It is found that common ions such as Li^+, Na^+, K^+, Cl^-, Ca^{2+}, SO_4^{2-}, CO_3^{2-}, ClO_4^- and HCO_3^{2-} have no interference in the determination of target analytes. The effect of some molecules includes ascorbic acid, uric acid, codeine, melatonin, glucose, cysteine, serotonin, alanine, tyrosine, and phenylalanine were studied, and the results show that except for serotonin and tyrosine, the other molecules have no interfere up to 30 folds. The serotonin shows a change in oxidation currents of DA and AC while the concentration of it was more than 17 folds of DA and AC. Also, 9 folds concentration of tyrosine changed the Trp signal more than 5 %. The obtained data represented the acceptable selectivity of AuPdCu-rGO-MWCNTs/CPE even in the present high concentrations of potential interference species.

The oxidation peak current of 5 μM DA, AC and Trp were determined in different days, and the measurements show that the response of electrode decreased less than 5% after 19 days while it was stored at ambient temperature. This data confirms the high stability of the proposed electrode.

The repeatability of the suggested method was checked out with intra-day and inter-day measurement for 5 μM of DA, AC and Trp, for which the respective relative standard deviations (RSD %) were computed. The calculated RSD% for the intra-day and 5-time determinations of DA, AC and Trp were 2.4, 2.9 and 2.7%, respectively and for the inter-day repeatability were 3.5, 3.8 and 4.0%, respectively.

The reproducibility of AuPdCu-rGO-MWCNTs/CPE was evaluated by analyzing a solution containing 5 μM DA, AC and Trp with ten independently prepared electrodes based on the similar construction procedure. The presented electrode illustrates an appropriate precision with RSD% of 4.1, 4.5 and 4.3% for DA, AC and Trp, respectively.

These obtained values of RSD% indicate good repeatability and reproducibility of AuPdCu-rGO-MWCNTs/CPE for the simultaneous determination of the target drugs.

3.8. Determination of DA, AC and Trp in real samples

To investigate the applicability of the proposed electrochemical sensor, various real samples, include blood plasma, urine and pharmaceutical formulations were tested. The results of this test are summarized comprehensibly in Table 1, with the recoveries of the spiked samples ranged from 96.0% to 104.0%. It was evident that the AuPdCu-rGO-MWCNTs/CPE could be successfully applied for the simultaneous determination of DA, AC and Trp in the chosen real samples. The AuPdCu-rGO-MWCNTs/CPE shows a good potency for the analysis of pharmaceutical formulations and biological samples.

Table 1. Determination of DA, AC and Trp in real samples (n = 5)

Samples	Analyte	Added (μM)	Found (μM)	RSD%	Recovery (%)
Urine	DA	1.00	0.96	2.2	96.0
		2.00	2.05	2.5	102.5
		5.00	5.09	3.1	101.8
	AC	1.00	1.02	2.5	102.0
		2.00	2.07	2.7	103.5
		5.00	5.11	2.4	102.2
	Trp	1.00	0.98	1.9	98.0
		2.00	2.08	2.1	104.0
		5.00	5.19	3.0	103.8
Blood plasma	DA	1.00	1.03	2.8	103.0
		2.00	2.06	1.9	103.0
		5.00	4.92	2.7	98.4
	AC	1.00	1.02	2.4	102.0
		2.00	2.07	1.6	103.5
		5.00	4.89	2.5	97.8
	Trp	1.00	1.03	2.3	103.0
		2.00	2.07	2.4	103.5
		5.00	5.08	2.7	101.6
AC Tablets	DA	1.00	0.97	2.9	97.0
		2.00	1.96	2.5	98.0
		5.00	5.13	3.3	102.6
	AC	0.00	0.61	3.0	-
		1.00	1.59	2.6	96.7
		2.00	2.63	2.4	101.6
	Trp	1.00	0.97	2.5	97.0
		2.00	1.95	2.7	97.5
		5.00	5.12	2.4	102.4

4. CONCLUSION

In the presented work, an unprecedented electrochemical sensor based on AuPdCu-rGO-MWCNTs nanocomposite as a modifier in CPE has constructed that provides a rapid, sensitive, accurate and selective method for the individual and simultaneous determination of DA, AC and Trp. The proposed electrode has a reasonable charge transfer rate with a linear range from range 0.01-8.3, 0.02-12.5 and 0.01-9.4 μM for DA, AC and Trp respectively. The calculated DLs were found 0.003, 0.007 and 0.004 μM for DA, AC and Trp, respectively. The conducted studies show that the potential and currents of oxidation peaks of the target analytes were depended to solution pH so that the best peak shapes and currents were obtained at pH=7.5. The change in the scan rate leading to increase the responses of the electrode in the CV and the currents changed linearly by variation of the square root of the scan rate which it means that the electrochemical reactions of DA, AC and Trp at the surface of AuPdCu-rGO-MWCNTs/CPE are diffusion controlled. Application of the proposed

method for the measurement of DA, AC and Trp in different real samples gave acceptable results without time-consuming extractions. The expressed features suggest that the proposed electrochemical sensor is a potential candidate for practical applications.

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