Anal. Bioanal. Electrochem., Vol. 14, No. 2, 2022, 201-215

Analytical &
Bioanalytical
Electrochemistry

2022 by CEE
www.abechem.com

Full Paper

Simultaneous Determination of Trace Amounts Ascorbic Acid, Melatonin and Tryptophan using Modified Glassy Carbon Electrode Based on Cuo-CeO_2-rGO-MWCNTS Nanocomposites

Ashkan Faridan, Manochehr Bahmaei*, and Amirabdolah Mehrdad Sharif

Department of Analytical Chemistry, Faculty of Chemistry, Islamic Azad University, North Tehran Branch, Tehran, Iran

*Corresponding Author, Tel.: +98 9121488481

E-Mail: M_bahmaei@iau-tnb.ac.ir

Received: 24 October 2021 / Accepted with minor revision: 25 January 2022 /

Abstract- In the presented study, for the first time, simultaneous electrochemical measurements of ascorbic acid (AA), melatonin (Mel), and tryptophan (Trp) were discussed. The CuO-CeO_2-rGO-MWCNTs nanocomposite was prepared, then applied for amendment of glassy carbon electrode (GCE) surface to the measurement of target analytes using differential pulse voltammetry (DPV) technique. Electrical impedance spectroscopy (EIS) techniques displayed that CuO-CeO_2-rGO-MWCNTs/GCE has the lowest electron transfer resistance (R_{ct}) in comparison to GCE and was suitable for electrochemical applications. The synthesized compounds were analyzed by powerful methods including Scanning Electron Microscopy (SEM) and, X-ray Diffraction (XRD). At the CuO-CeO_2-rGO-MWCNTs/GCE, three oxidation peaks appeared at 0.309, 0.631, and 0.855 V for AA, Mel, and Trp and the peaks separation of ΔE_p (AA and Mel)=322 mV, and ΔE_p (Mel and Trp)=224 mV in the electrochemical potential window of 0.0-1.1 V. In optimum DPV condition and pH=5.0, a dynamic range of AA (0.01-28 µM), Mel (0.01-12.6 µM) and Trp (0.01-13.5 µM) with the detection limit of 9, 8 and 7.3 nM for AA, Mel, and Trp, respectively, were acquired. The provided modified electrode was successfully used to monitor the analytes in human biological fluids.

Keywords- Ascorbic acid; Melatonin; Tryptophan; Glassy carbon electrode; Real sample; Voltammetry

---

1. INTRODUCTION

For accepting a new analytical method as a routine method in the laboratory, it must have acceptable accuracy, sensitivity, selectivity, and, long-time stability. Capillary electrophoresis, high-performance liquid chromatography, enzyme-based immunoassay, chemiluminescence, and, mass spectrometry are the popular methods for the determination of some biomolecules [1, 2]. Although these methods are powerful and sensitive, they have disadvantages such as tedious processes, expensive instruments, and, complex sample pre-treatment, which limits their usage. Among analytical methods, electrochemical methods have some advantages such as relatively inexpensive instrumental, technical simplicity, portability, high sensitivity, and selectivity [3]. Nowadays, constructing the electrochemical sensor which has been used the determine the drugs, ions, and biomolecules is one of the most remarkable techniques. So, it can be one of the best-suggested methods for determining specific compounds in complex mixtures.

AA is very well-known for both its anti-oxidant features and widespread use for preventing and treating the common cold, mental disease, AIDs, infertilities, and cancer [4]. Due to its large overpotential and electrode fouling, AA cannot be directly determined by the electrochemical method on conventional bare electrodes [5,6].

Melatonin (Mel) or N-acetyl-5-methoxytryptamine, as a derivative of serotonin, is a hormone that controls the circadian rhythm of organisms and has an effect on the immune system. It uses for the treatment of sleep disorders and is known as an anti-inflammatory or antioxidant agent and free radical scavenger. Also, the effect of Mel on the treatment of mental disorders and breast cancer has recently been proven. Also, a high level of Mel in the human body can lead to headache and anxiety, diarrhea, dizziness, shaking, joint pain, and, nausea [7].

Tryptophan (Trp) or (S)-2-Amino-3-(3-indolyl) propionic acid, as one of the eight most crucial amino acids, controls the serotonin levels in the human brain [8]. In the human body, Trp participates in the synthesis of melatonin and niacin. The amount of this amino acid is necessary for equilibrating the nitrogen level and, the maintenance of muscle mass and body weight in humans [9]. Since the human body is unable to supply tryptophan through its synthesis, it must be added to the foods used by humans [9]. The abnormal level of this biomolecule resulting depression, delusions, schizophrenia, and hepatic disease. So, the determination of Ser, Mel, and Trp in a biological fluid such as serum and urine have become one of the confirmatory tests for some diseases in clinics.

The nanoparticles (NPs) have distinct chemical, physical, and electronic features compared to bulk materials [10]. Because of these features, NPs are one of the best candidates for preparing a sensing layer in order to construct the electrochemical sensors [11]. Owning to the extensive surface area, conductivity and, specific surface free energy of NPs, the NPs-modified electrochemical sensors could be used to determine analytes with trace concentration and better signal-to-noise ratio.[11]

Graphene has outstanding properties for use in electrical and electrochemical instruments including excellent electric conductivity and, high surface area [12]. Because of their strong mechanical strength, the graphene-modified electrochemical sensors have high-performance, long-time stability, stable response, and, acceptable reproducibility [12]. Reduced graphene oxide (rGO) was synthesized by chemical or electrochemical reduction of graphene oxide, has some lattice defects which lead to increased electrical conductivity. In recent years, scientists for increasing the efficiency of rGO, multi-metallic or multi-metal oxides NPs were doped on the rGO because of the synergistic effect of interactions between metal/metal oxide NPs and rGO [13]. These nanomaterials have different properties and, catalytic activities compare to their component counterparts which are the result of the intrinsic changes of the surface geometric and electronic properties.[14]

Among metal NPs, Au and Ag have represented high electrocatalytic activity but their applications were limited because of low earth abundance and high cost [15]. In recent years, some other metal NPs such as Cu, Ni, and Ce has been proposed as economic and, cost-effective catalysts with high activities [16]. In many cases, bimetallic nanocomposites have better electrocatalytic activity in comparison to monometallic NPs which could be owing to the powerful synergistic effect of metal NPs-metal NPs [17,18]. Some research reported that doping the cerium oxide (CeO2) as metal oxide NPs in monometallic or bimetallic NPs can appreciably enhance the activity of these metal oxide NPs for use in the electrochemical application [19,20].

In the presented work, a complex nanocomposite containing CuO and CeO_2 metal oxide NPs and, rGO and MWCNTs was synthesized. At the surface of bare carbon-based electrodes, simultaneous measurement of analytes is difficult and the peaks may overlap when they appear at the close potentials. Owing to exceptional mechanical and, chemical stability, electrical conductivity, surface area, one effective way to improve the signal-to-noise ratio in the electrochemical methods is using nanoparticles or nanocomposites for modification of electrodes. The CuO-CeO_2-rGO-MWCNTs nanocomposites were applied for the preparation of a new modified glassy carbon electrode (GCE) for the purpose of attaining the best electrochemical analysis figure of merit. For the first time, in this research AA, Mel, and Trp were determined simultaneously by the electrochemical method.

2. EXPERIMENTAL SECTION

2.1. Reagents and apparatus

All used reagents were of the analytical grade and, were applied without subsequent purification. The raw materials for the synthesis of the electrode modifiers and other using materials were bought from Merck Company. The target analytes (AA, Mel, and Trp) were purchased from Aldrich Company. Deionized water was used to prepare all aqueous solutions. Britton-Robinson universal buffer solution (B-R buffer solution) was used as a supporting electrolyte and, pH controlling agent**.**

The electrochemical experiments were done at the ambient temperature by Autolab electrochemical system (Model PGSTAT 302 N potentiostat/galvanostat, EcoChemie, The Netherlands). A three-electrode system includes Ag/AgCl/KCl (3 M) electrode as a reference electrode, modified GCE and bare GCE as the working electrode, and Pt wire as the counter electrode were used for voltammetric investigations. The electrochemical impedance spectra and voltammograms were analyzed using the Nova 1.7 software. The Metrohm pH meter (model 713-Switzerland) was applied for Checking the pH. TEM image was taken using Zeiss EM902A (Germany). FTIR spectra were obtained by a Perkin-Elmer spectrophotometer (Spectrum GX), and XRD patterns were determined by an XRD (38066 Riva, d/G.Via M. Misone, 11/D (TN) Italy) at ambient temperature.

2.2. Instrumentation

Graphene oxide sheets (GO) were synthesized from graphite powder by a modified Hummer-Offeman's method. Briefly, 160 ml sulfuric acid was mixed with 40 ml orthophosphoric acid, and 1.6 g graphite powder was added to it and mixed for 1 h. Then, 9 g potassium permanganate was added to the mixture and kept under stirring for 72 h at ambient temperature. In the next step, hydrogen peroxide 30% was added dropwise to the mixture until turns into yellow color. The obtained solution was centrifuged and washed with 1 M HCl solution, deionized water/ethanol three times. The collected GO was dried in a vacuum at 80 ^{o}C for 12h [21].

The rGO was synthesized by the addition of a chemical reduction reagent to GO suspension. In a typical method, 150 mg prepared GO was dispersed into 150 ml deionized water, then 2 ml hydrazine hydrate was added to the suspension and kept at 100 ^{o}C for 24 h. The obtained Gr was centrifuged, washed deionized water several times, and dried in a vacuum at 80 ^{o}C for 12 h [21].

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. Afterward, the prepared mixture was filtered by Whatman No. 42 filter paper and washed with 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 .

For the synthesis of CuO-CeO_2-rGO-MWCNTs nanocomposite, 10, and 10 mg of $Cu(NO_3)_2{\cdot}3H_2O$ and $Ce(NO_3)_3{\cdot}6H_2O$, respectively, were added to 20 ml suspension of GO and MWCNTs in water (0.5 mg/ml). The suspension was mixed for 2 h. In the next step, an excess amount of NaBH4 was added to the suspension while it was mixed quickly under N_2

atmosphere for 4 h. The black formed precipitate was collected and washed three times by deionized water, then dried in a vacuum at 60 °C [22]. The CuO-CeO_2 NPs were prepared by the same protocol but GO and MWCNTs were not charged to the suspension.

2.3. Fabrication of the electrode

The bare GCE was firstly cleared and polished with different sizes of Al_2O_3 and cleaned by ultrasonic in ethanol/water (50:50 V:V) to obtain a mirror-like surface. Then, the provided GCE was immersed in HNO3/HCl (50:50 V:V) for 30 min and finally washed with deionized water 3 times [23]. The modified electrodes (CuO-CeO_2-rGO-MWCNTs/GCE, CuO-CeO_2/GCE, rGO-MWCNTs/GCE, and rGO/GCE) were constructed by dropping 10 µl suspension of the modifiers (1 mg nanocomposite or nanoparticles in 1 ml DMF) on the surface of GCE and dried at laboratory temperature [24].

2.4. Preparation of human plasma and urine samples

Human blood and urine samples were collected from our laboratory staff. Owning to remove proteins from the collected blood sample, centrifuged at 5000 rpm for 120 min at ambient temperature. The separated plasma was stored in a refrigerator before use. For adjusting the pH, a B-R buffer solution was added to the obtained sample. The urine sample was centrifuged at 5000 rpm (30 min), then it was filtered. Before analysis, the urine sample was diluted five times with B-R buffer solution pH=5 [25].

3. RESULTS AND DISCUSSION

3.1. Characterization of the synthesized nanomaterials

Characterization of the synthesized nanomaterials was carried out by XRD analysis and TEM, the results are shown in Figure 1.

As shown in Figure 1a, the XRD patterns of GO and rGO nanosheet, due to their amorphous nature, showed a broad peak at about 2θ=10° and 25°, respectively. These results are well matched with previous reports [24,26] and confirm the reduction of GO to rGO with the used method. In the case of MWCNTs, the observed peak at 2θ=25.86° and 43.98° are related to the graphite plate of MWCNTs. The XRD pattern of CuO-CeO_2 NPs represents two peaks in 29, 33, 43, 48, 56, 70, and 76° [27,28]. In addition, the XRD pattern of CuO-CeO_2-rGO-MWCNTs showed a new peak at about 22° [29].

The morphology of synthesized CuO-CeO_2-rGO-MWCNTs nanocomposite was investigated using SEM (Figure 1b). The CuO-CeO_2 NPs were deposited uniformly at the surface of rGO-MWCNTs without significant agglomeration. The average diameter of the deposited NPs on the surface of rGO was 20 nm.

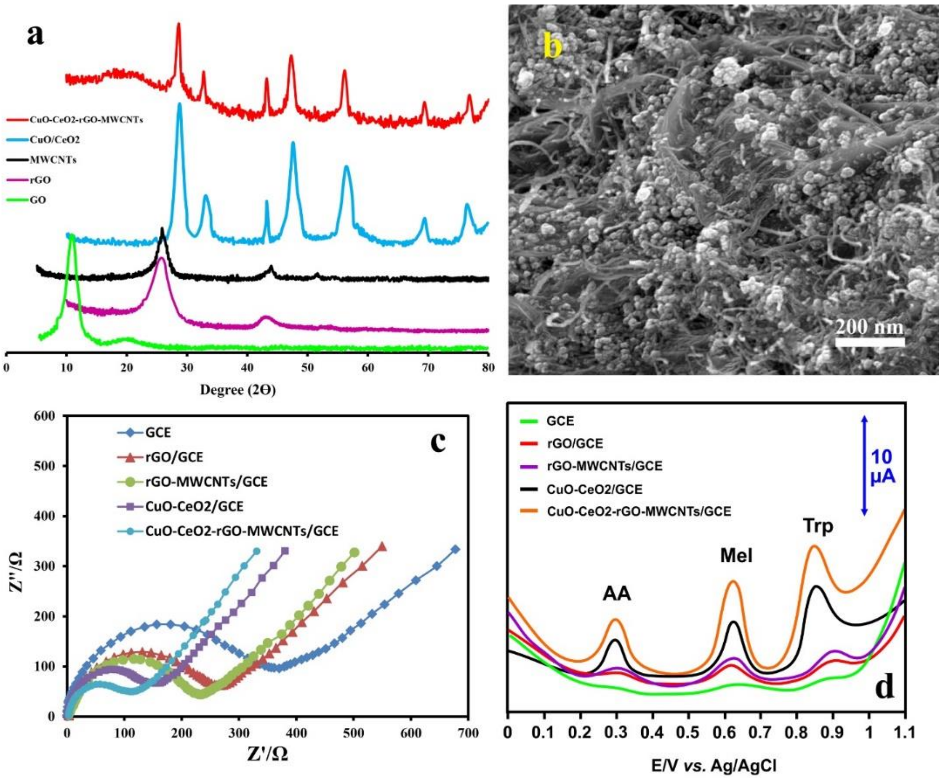


Figure 1. (a) The XRD patterns of GO, rGO, MWCNTs, CuO−CeO_2 and CuO−CeO_2−rGO−MWCNTs nanocomposite, (b) the SEM image of CuO−CeO_2−rGO−MWCNTs nanocomposite, (c) Nyquist plots of the bare GCE, rGO/GCE, rGO−MWCNTs/GCE, CuO−CeO_2/GCE, and CuO−CeO_2−rGO−MWCNTs/GCE in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ and 1.0 M KCl, (d) The DPV of 5 µM AA, Mel and Trp, in B-R buffer solution pH=5, at GCE, rGO/GCE, rGO−MWCNTs/GCE, CuO−CeO_2/GCE, and CuO−CeO_2−rGO−MWCNTs/GCE

3.2. Electrochemical investigate of fabricated electrodes

In the other study, for investigation of the surface property of the different modified and unmodified GCEs the Electrochemical impedance spectroscopy technique was used. Fig. 1d illustrates the recorded EIS plots for CuO-CeO_2-rGO-MWCNTs/GCE, rGO-MWCNTs/GCE, rGO/GCE, CuO-CeO_2/GCE and, GCE electrodes in the present of 1.0 mol L^{-1} KCl and 5.0 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ solution. The diameter of the semicircle of the Nyquist plot represents electron transfer resistance (R_{ct}) of the working electrodes that were changed by the different electrodes. The bare GCE represents a large semicircle diameter and the estimated R_{ct} value was about 330 Ω indicating that it has large charge transfer resistance and low the electron transfer ability. In comparison to GCE, the R_{ct} for rGO/GCE has decreased significantly and was about 250 Ω, whereas the modified GCE with rGO-MWCNTs produced lower semicircle diameter with the R_{ct} value of 230 Ω because the electron transport occurred rapidly. The CuO-CeO_2/GCE has shown the lower R_{ct} (about 135 Ω) which can be due to the high diffusion rate at the surface of this modified electrode. The lowest R_{ct} obtained when the GCE was modified by CuO-CeO_2-rGO-MWCNTs (100 Ω), which ascribed to the growth electrochemical active surface area, enhancement the electrode conductivity and boost catalytic ability of the CuO-CeO_2-rGO-MWCNTs/GCE. Accordingly, the CuO-CeO_2-rGO-MWCNTs/GCE is certified satisfactory efficient for using in electrochemical application.

3.3. Investigation the effect of modifier on electrochemical oxidation of AA, Mel and Trp

In order to improve the conductivity of sensing layer and removing the mass transport limitations onto the surface of the electrodes, the modification of working electrodes was done. To investigation of the effect of kind of modifier, the electrochemical behavior of 1 μM AA, Mel and Trp, in B-R buffer solution pH=5, at the surface of different prepared electrodes include CuO-CeO_2-rGO-MWCNTs/GCE, CuO-CeO_2/GCE, rGO-MWCNTs/GCE, rGO/GCE, and, GCE were checked by DPV technique. The DPV voltammograms are depicted in Figure 2a.

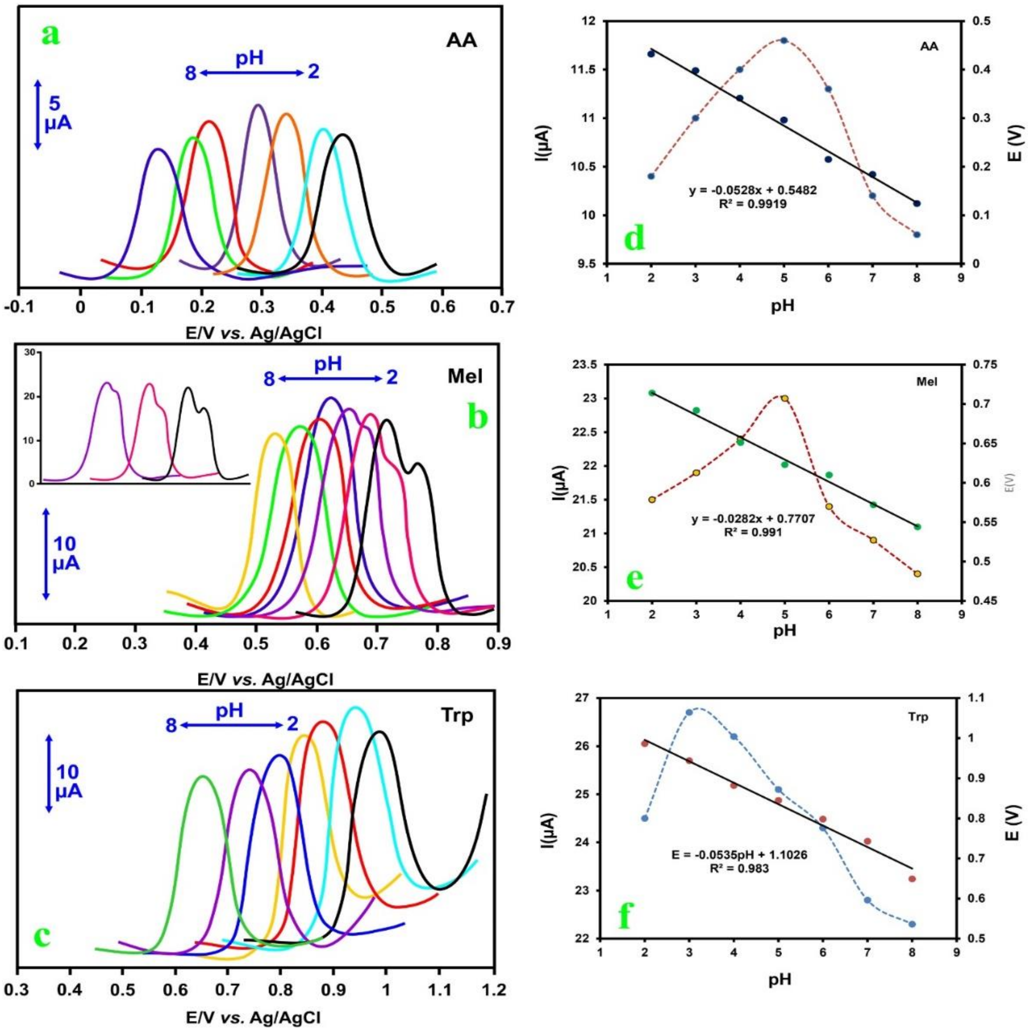


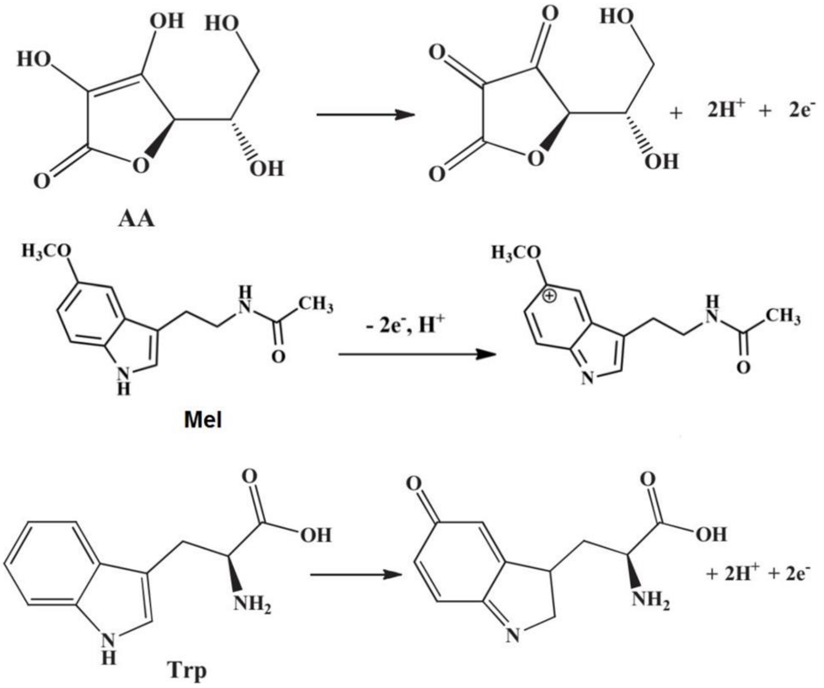
Figure 2. The DPV of 1 μM (a) AA, (b) Mel and (c) Trp at CuO-CeO_2-rGO-MWCNTs/GCE at different pH from 2 to 8, The effect of pH on response of the electrode and potential of the peak for (d) AA, (e) Mel and (f) Trp

It can be seen that by using bare GCE three weak and broad peaks was observed for electro-oxidation of AA, Mel and Trp. By modification of GCE with rGO, the response of electrode for AA, Mel and Trp have increased slightly which could be owing to an increase in the surface area of the electrode. It can be seen that a considerable increase in DPV responses was induced while the GCE was modified by rGO-MWCNTs (E_p: 0.309, 0.631 and 0.855 mV for AA, Mel and Trp, respectively), which the peak potential of oxidation peaks has shifted to the negative values. By using CuO-CeO_2/GCE as sensing electrode, three separated and sharp anodic peaks appeared at the same potential in comparison to rGO-MWCNTs/GCE. At the surface of CeO_2-rGO-MWCNTs/GCE three sharp, and well-defined peaks for AA (I_p=3.5 µA), Mel (I_p=7.1 µA) and Trp (I_p=7.2 µA) was observed. Because the large separation peaks and the maximum oxidation peak currents for the target analytes was obtained at the surface of CuO-CeO_2-rGO-MWCNTs/GCE, it was chosen as the best working electrode for subsequent experiments for achieve the lowest detection limit (DL) and maximum sensitivity.

3.4. Influence of pH and sweep rate on electrooxidation of AA, Mel and Trp

In order to attain the best sensitivity, stability and maximum selectivity in simultaneous electrochemical determination of AA, Mel and Trp, selection of optimized pH is necessary. The pH value of analytes solution can change the peak potentials and currents, so the solution pH was optimized via DPV technique in 0.2 M B–R buffer solutions and the presence of 5 µM AA, Mel and Trp in the pH range of 3 to 8. The voltammograms are depicted in Figure 2b.
The data shows that the response of CuO-CeO_2-rGO-MWCNTs/GCE for the AA and Mel have growth considerably by changing the pH value from 2 to 5, and after that the oxidation current decreased between pH=5 and 8 (Figure 2c). It can be concluded that, because the highest oxidation current was obtained in pH=5, so the best sensitivity achieves in this optimized pH. In the other hand, the peak current of Trp has increased significantly when the pH increases from 2 to 3 and after that the oxidation current decrease by increasing the pH from 3 to 8.

The impact of the pH value on the anodic peak potential (E_p) of AA, Mel and Trp was checked, the Ep of three analytes have changed linearity by increasing the pH from 3 to 8 (Figure 2d). The linear relationship between Ep and pH can be represented by the following equations: for AA, E=-0.0528pH+0.5482 (R^2 = 0.992), for Mel, E = -0.0282pH + 0.7707 (R^2 = 0.991) and for Trp, E=-0.0535pH+1.1026 (R^2 = 0.983). As can be seen, the acquired slope values for AA, Mel and Trp were -0.0528, -0.0282 and -0.0535 mV/pH, respectively. Based on the Nernst equation, the obtained slope values for AA, and Trp corroborated that the ratio between number of protons and electrons which participated in electrochemical oxidation of these molecules is one. In the other hand, the slope of -0.0282 mV/pH for Mel suggests that number of transferred protons and electrons was not equal and the number of electrons was twice that of protons which result is in a good settlement with previous researches. The suggested electro-oxidation mechanism of Ser, Mel and Trp at the surface of CuO-CeO_2-rGO-MWCNTs/GCE are presented in Scheme1 [30-32].



Scheme 1. Reported electrochemical oxidation mechanism for AA, Mel and Trp

The kinetics of electrochemical reaction on the surface of CuO-CeO_2-rGO-MWCNTs/GCE was studied by using CV technique at different scan rates in the range of 25 to 300 mV/s in 0.2 M B–R buffer solutions pH=5 containing 5 μM AA, Mel and Trp, which the voltammograms are represented in Figure 3a. The electrode responses for oxidation of AA, Mel and Trp have increased with the increasing scan rate values. The oxidation peak currents of analytes changed in a linear relationship with the square root of scan rate with a calibration equation of $Ip = 1.2849\ \upsilon^{1/2} - 6.3553$ ($R^2 = 0.9962$) for AA, $I_p = 1.4034\ \upsilon^{1/2} - 1.9158$ ($R^2 = 0.9951$) for Mel and $I_p = 1.5586\ \upsilon^{1/2} - 1.2670$ ($R^2 = 0.9951$) for Trp, which demonstrate that the surface reaction on CuO-CeO_2-rGO-MWCNTs/GCE for these analytes was a diffusion-controlled process (Figure 3b) [21].

3.5. Effect of concentration and calibration curves

In order to confirm the performance of CuO-CeO_2-rGO-MWCNTs/GCE in the individual and simultaneous determination of the AA, Mel and Trp with the DPV technique, various concentrations of the target molecules were measured in pH 5.

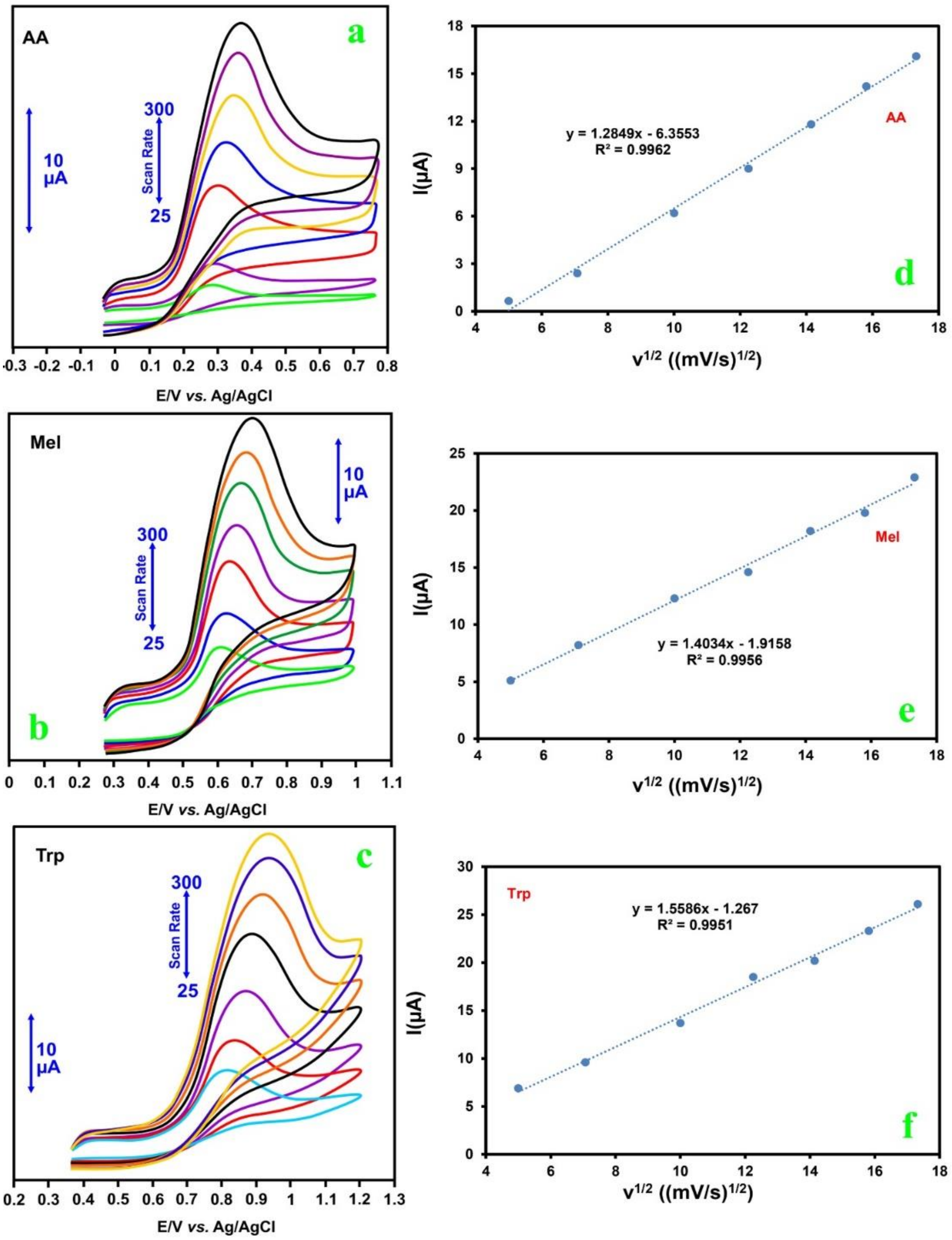


Figure 3. CVs at CuO-CeO$_2$-rGO-MWCNTs/GCE in B-R buffer solution with pH=5 containing 5 μM (a) AA, (b) Mel and (c) Trp with scan rates ranging from 25 to 300 mV/s, The linear relationship between the peak current vs. square root of the scan rate ($\upsilon^{1/2}$) for (d) AA, (e) Mel and (f) Trp

Since, one of the main obstacles in simultaneous electrochemical measurement is that one target analyte can interfere with the determination of the others of analytes. Therefore, interferent of target species on each other at the CuO-CeO$_2$-rGO-MWCNTs/GCE was investigated with electrode response measurement by fixing the concentration of two analytes at a certain amount and change the concentration of the other analyte.

It can be seen that, a remarkable increase of the response of CuO-CeO_2-rGO-MWCNTs/GCE was occurred while the concentration of one of the analytes has increased and concentration of the other analytes were almost unchanged. The voltammograms are represented in Figure 4a-c and the linear dynamic range for AA (0.01-28 µM), Mel (0.01-12.5 µM) and Trp (0.01-13.5 µM) were observed with calibration equations of I_p=1.7757C (AA)+1.5177 (R^2=0.9987), I_p=4.0647C(Mel)+1.7219 and I_p=4.4112C(Trp)+1.469, respectively (Figure 4d-f).

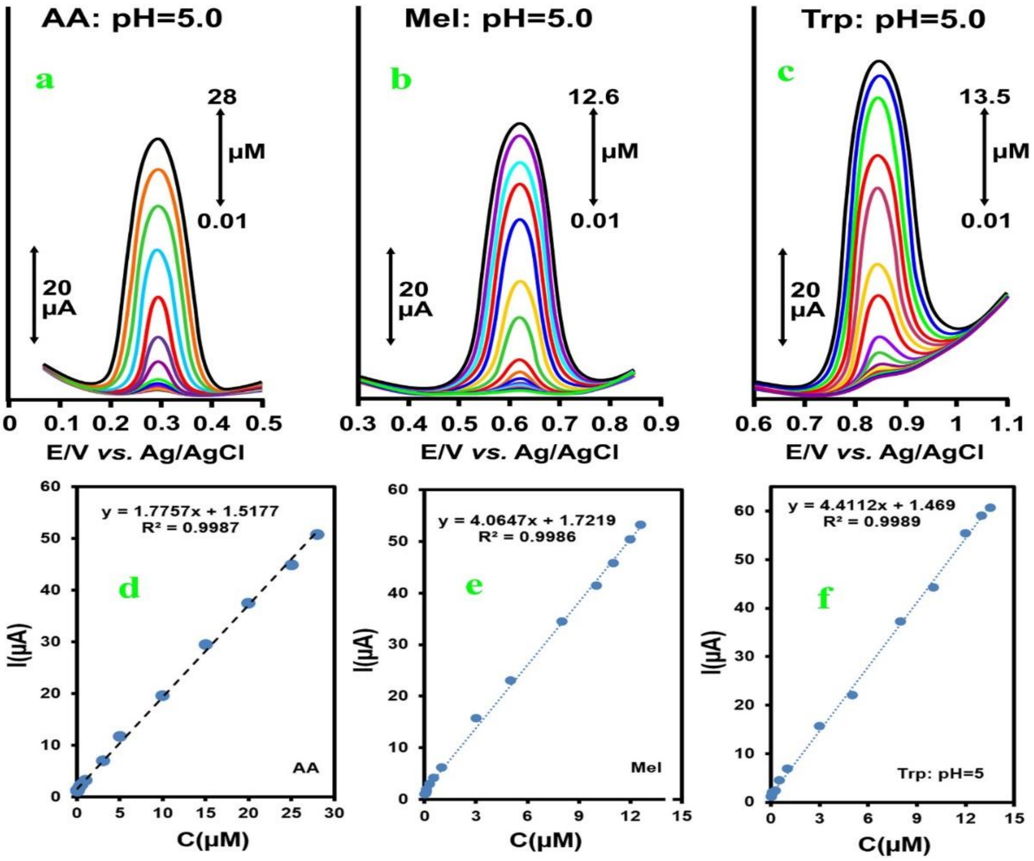


Figure 4. DPVs at CuO-CeO_2-rGO-MWCNTs/GCE in B-R buffer solution with pH=5 for (a) AA, (b) Mel and (c) Trp, Calibration curve for (d) AA, (e) Mel and (f) Trp

In the other study, the concentrations were varied simultaneously from 0.01 to 28 µM for AA, 0.01 to 12.5 µM for Mel and 0.01 to 13.5 µM for Trp. The recorded voltammograms are depicted in Fig. 5a. As observed, by increasing the concentrations of AA, Mel and Trp in mentioned concentrations the oxidation peak currents have enhancement linearly with the regression equation of I_p=1.7312C+1.6448 (R^2=0.9982) for AA, I_p=3.9714C+1.9 (R^2 = 0.9983) for Mel, and I_p=4.3406C+1.6505 (R^2=0.9988) for Trp (Figure 5b-d). The DLs were calculated as the ratio of three times of standard deviation of 10 measurements of a blank sample per slope of calibration curve equations (3Sb/m). The DLs of electrochemical determination of AA, Mel and Trp by CuO-CeO$_2$-rGO-MWCNTs/GCE were calculated to be 9, 8 and 7.3 nM, respectively.

Comparing of the slopes in individual and simultaneous determination for each species shows that there is no significant difference in the DPV current responses and the slopes are approximately equal to each other in these two studies, concluding that the three analytes do not have any interference in the determination of each other.

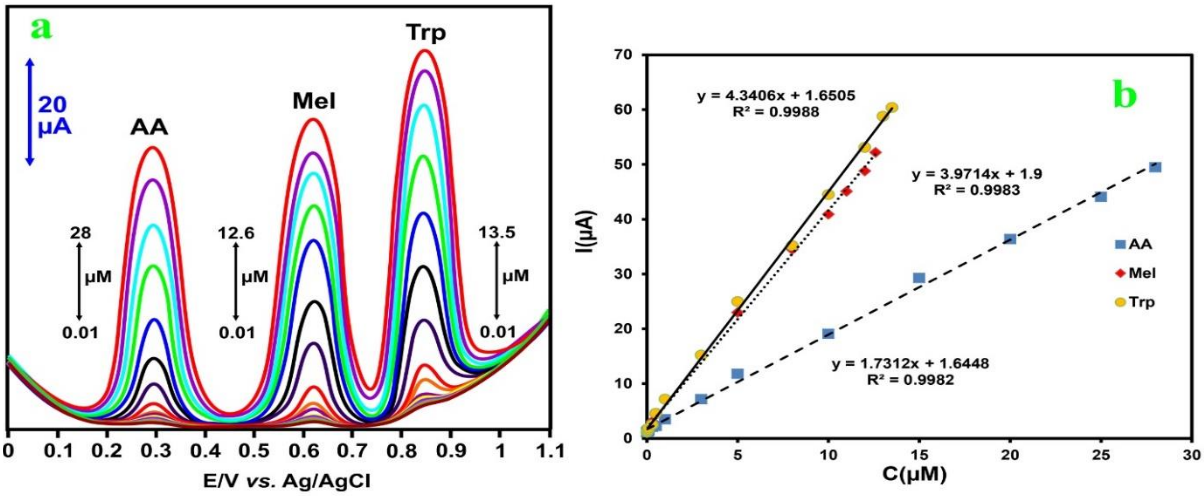


Figure 5. (a) DPVs at CuO-CeO$_2$-rGO-MWCNTs/GCE in B-R buffer solution with pH=5 for simultaneous measurement of AA, Mel and Trp; (b) Calibration plots for AA, Mel and Trp

3.6. Interferences and selectivity

The selectivity of the presented electrochemical sensor towards 5 µM AA, Mel and Trp in the presence of possible interfering species is a critical parameter in analytical techniques. To appraise the selectivity of the method some interfering compounds were investigated. The selectivity of the CuO-CeO$_2$-rGO-MWCNTs/GCE in AA, Mel and Trp detection was appraised in the presence of Tyrosine ($C_{Interferent}/C_{Analyte}$=135), and uric acid ($C_{Interferent}/C_{Analyte}$=180), Dopamine ($C_{Interferent}/C_{Analyte}$=150), Glucose, Lactose and Sucrose ($C_{Interferent}/C_{Analyte}$=400), Na^+, K^+, Cl^-, Ca^{2+}, Zn^{2+}, and Al^{3+} ($C_{Interferent}/C_{Analyte}$=1000). The obtained data show that no changes of more than 5% in the voltammetry oxidation currents of the analytes in the presence of the mentioned interferences were observed. These results indicate the sufficient selectivity of CuO-CeO$_2$-rGO-MWCNTs/GCE in the voltammetric measurement of AA, Mel and Trp.

3.7. Repeatability, and reproducibility of the CuNi-CeO$_2$-rGO/GCE

Since the construction of the modified electrode will not affect the efficiency of the method, this assay is expected to have repeatability, and reproducibility. The response repeatability of the CuNi-CeO$_2$-rGO/GCE for determination of 5 µM of AA, Mel and Trp were studied by ten successive replicates determination. The relative standard deviations (RSDs) of the performed experiments for AA, Mel and Trp were obtained to be 2.56, 2.27 and 2.91%, respectively. These data demonstrate the provided sensor has satisfactory repeatability results. In order to check the reproducibility of CuO-CeO_2-rGO-MWCNTs/GCE, five electrochemical sensors were constructed by the same procedure and the response of the electrodes for determination of 5 µM of AA, Mel and Trp were recorded. The RSD values were calculated to be 3.44, 3.18 and 3.65% which demonstrate the reproducibility of the fabrication method of the presented electrode.

3.8. Testing the presented electrode on real samples

To confirm the applicability of CuO-CeO_2-rGO-MWCNTs/GCE, detection of AA, Mel and Trp in human urine and blood serum samples was performed. In order to decrease the sample matrix effect, the urine and blood serum samples were diluted with B-R buffer solution pH=5. The monitoring of AA, Mel and Trp in the samples were calculated by standard addition method. The data of analysis are presented in Table 1. The recovery percentages indicate that the presented modified electrode is trustworthy and efficient for real sample analysis.

Table 1. Determination of the analytes in the real samples

Sample	**Analyte**	**Added µM**	**Determine µM**	**Recovery %**
Blood Serum	AA	0.5	0.51	102
		5	5.08	101.6
		15	14.76	98.4
	Mel	0.5	0.25	104
		1	0.98	98
		5	4.84	97.2
	Trp	0.5	0.49	98
		5	1.05	105
		15	4.91	98.2
Urine	AA	0.5	0.49	98
		5	5.11	102.2
		15	15.23	101.53
	Mel	0.5	0.49	98
		5	1.03	103
		15	5.06	101.2
	Trp	0.5	0.52	104
		5	1.01	101
		15	4.93	98.6

4. CONCLUSIONS

In the presented study, the CuO-CeO_2 nanocomposite were decorated on rGO-MWCNTs and applied to modification the GCE surface. Based on EIS analysis, the CuO-CeO_2-rGO-MWCNTs/GCE has the best electrochemical features, due to the synergistic effect of simultaneous using CuO, CeO_2, MWCNTs and rGO NPs, compared to the other investigated electrodes. Also, the maximum currents, excellent selectivity and sensitivity for oxidation of AA, Mel and Trp were obtained when the CuO-CeO_2-rGO-MWCNTs/GCE was applied as working electrode in pH=5. The prepared CuO-CeO_2-rGO-MWCNTs/GCE exhibited satisfied repeatability and reproducibility and high selectivity, which could be effective for the simultaneous monitoring of AA, Mel and Trp in their mixture. The presented modified electrode was applied for the determination of AA, Mel and Trp in human biological fluid samples. The recovery percentages for three analytes were achieved in the range of 97.2% to 102.2%. Thus, it can be concluded that the developed method with the mentioned properties has the potential for clinical applications.

REFERENCES

[1] P. Ravisankar, C.N. Navya, D. Pravallika, and D. N. Sri, IOSR J Pharm. 5 (2015) 7.
[2] M. R. Monteiro, A. R. P. Ambrozin, L. M. Lião, and A. G. Ferreira, Talanta 77 (2008) 593.
[3] T. Grygar, F. Marken, U. Schröder, and F. Scholz, Collection of Czechoslovak Chemical Communications 67 (2002) 163.
[4] G. Di Giovanni, and P. De Deurwaerdère, Serotonin research: Crossing scales and boundaries, Elsevier (2020).
[5] A. Kumar, R. M. Russell, R. Pifer, Z. Menezes-Garcia, S. Cuesta, and S. Narayanan, J. B. MacMillan, V. Sperandio, Cell Host & Microbe 28 (2020) 41.
[6] S. Xuev, and A. Ickowicz, J. Canadian Academy of Child and Adolescent Psychiatry 30 (2021) 156.
[7] J. Smajdor, R. Piech, M. Pięk, and B. Paczosa-Bator, J. Electroanal. Chem. 799 (2017) 278.
[8] A. Fiore, and P. J. Murray, Current Opinion in Immunology 70 (2021) 7.
[9] M. Platten, M. Friedrich, D. A. Wainwright, V. Panitz, and C. A. Opitz, Current Opinion in Immunology 70 (2021) 57.
[10] S. D. Steichen, M. Caldorera-Moore, and N. A. Peppas, European J. Pharm. Sci. 48 (2013) 416.
[11] H. Kumar, N. Venkatesh, H. Bhowmik, A. Kuila, Biomed. J. Sci. Tech. Res. 4 (2018) 3765.
[12] W. Choi, I. Lahiri, R. Seelaboyina, Y. S. Kang, Solid State Mater. Sci. 35 (2010) 52.

[13] C. Si, Z. Sun, and F. Liu, Nanoscale, 8 (2016) 3207.

[14] V. Chabot, D. Higgins, A. Yu, X. Xiao, Z. Chen, and J. Zhang, Energy Environ. Sci. 7 (2014) 1564.

[15] M. Sabela, S. Balme, M. Bechelany, J. M. Janot, and K. Bisetty, Advanced Engineering Materials 19 (2017) 1700270.

[16] B. Habibi, and N. Delnavaz, RSC Advances 6 (2016) 31797.

[17] R. Bogue, Assembly Automation 31 (2011) 106.

[18] E. Omanović-Mikličanin, A. Badnjević, A. Kazlagić, and M. Hajlovac, Health and Technology 10 (2020) 51.

[19] Y. Tonbul, S. Akbayrak, and S. Özkar, Int. J. Hydrogen Energy 41 (2016) 11154.

[20] F. Zhang, S. W. Chan, J. E. Spanier, E. Apak, Q. Jin, R. D. Robinson, and I. P. Herman, Applied Phys. Lett. 80 (2002) 127.

[21] H. R. Movahed, M. Rezaei, and Z. Mohagheghzadeh, Anal. Bioanal. Chem. Res. 8 (2021) 453.

[22] Y. H. Zhou, S. Wang, Y. Wan, J. Liang, Y. Chen, S. Luo, and C. Yong, J. Alloys and Compounds 728 (2017) 902.

[23] E. Mahmoudi, A. Hajian, M. Rezaei, A. Afkhami, A. Amine, and H. Bagheri, Microchem. J. 145 (2019) 242.

[24] H. Bagheri, A. Hajian, M. Rezaei, and A. Shirzadmehr, J. Hazard. Mater. 324 (2017) 762.

[25] E. Nikpanje, M. Bahmaei, and A. M. Sharif, J. Electrochem. Sci. Technol. 12 (2021) 173.

[26] A. Shirzadmehr, M. Rezaei, H. Bagheri, and H. Khoshsafar, Int. J. Environ. Anal. Chem. 96 (2016) 929.

[27] M. Gholinejad, F. Saadati, S. Shaybanizadeh, and B. Pullithadathil, RSC Advances 6 (2016) 4983.

[28] X. Li, P.F. Liu, L. Zhang, M.Y. Zu, Y. X. Yang, and H. G. Yang, Chem. Commun. 52 (2016) 10566.

[29] L. Wang, S. Chen, J. Hei, R. Gao, L. Liu, L. Su, G. Li, and Z. Chen, Nano Energy 71 (2020) 104570.

[30] A. Babaei, M. Afrasiabi, and A. Yousefi, Anal. Bioanal. Electrochem. 11 (2019) 1.

[31] H. Bagheri, A. Afkhami, P. Hashemi, and M. Ghanei, RSC Advances 5 (2015) 21659.

[32] O. A. Yokuş, F. Kardaş, O. Akyıldırım, T. Eren, N. Atar, and M. L. Yola, Sens. Actuators B 233 (2016) 47.