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Simultaneous Electrochemical Determination of Serotonin, Melatonin and Tryptophan using A Glassy Carbon Electrode Modified with CuNi-CeO_2-rGO Nanocomposite

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Abstract- In the presented study, for the first time, simultaneous electrochemical measurement of serotonin (Ser), melatonin (Mel) and, tryptophan (Trp) was discussed. The CuNi-CeO_2-rGO 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 CuNi-CeO_2-rGO/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 Transmission electron microscopy (TEM) and, X-ray Diffraction (XRD). At the CuNi-CeO_2-rGO/GCE, three oxidation peaks appeared with I_{pa}= 27, 20.1 and 24 µA for Ser, Mel, and Trp and the peaks separation of ΔE_p (Ser and Mel) =201 mV, and ΔE_p (Mel and Trp) =136 mV in the electrochemical potential window of 0.1-1.0 V. In optimum DPV condition and pH=7, a dynamic range of Ser (0.008-23 µM), Mel (0.008-21 µM) and Trp (0.008-23 µM) with the detection limit of 0.0058, 0.0061 and 0.0063 µM for Ser, Mel, and Trp, respectively, were acquired. The provided modified electrode was successfully used to monitor of the analytes in human biological fluids. The data using the suggested sensor and HPLC method were in good agreement without significant difference.

Keywords- Serotonin; 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 pretreatment, 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 for the determine of 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.

Serotonin (Ser), or 5-hydroxytryptamine is a monoamine and one of the principal neurotransmitters, takes part in controls some of the behavioral and cognitive activities [4]. The abnormally level of Ser in the human body can lead to some diseases, including Alzheimer's disease, inflammatory syndromes, infantile autism, mental retardation, attention-deficit hyperactivity disorder, mood, inflammatory bowel diseases, sleep disorders, carcinoid tumors, appetite and depression, and Parkinson’s disease [5]. Ser was produced by the human brain and is the factor that passing information to the human organs, which does this action via the central nervous system. On the other hand, Ser can control and adjust appetite and, sexuality [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, diarrhoea, 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 prepare 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 of analytes with trace concentration and better signal-to-noise ratio [11].

Graphene has outstanding properties for using in electrical and electrochemical instruments include 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 increase 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 as 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 the recent years, some others metal NPs such as Cu, Ni and Co have 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 (CeO_2) 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]. Also, it can be noted that during synthesis the Metal-Metal-CeO_2 nanocomposite has a propensity for aggregation. So, in order to solve this problem, improve the catalytic efficiency and stability, the nanocomposite could be anchored on the surface of rGO.

In the presented work, a complex nanocomposite containing Cu and Ni metal NPs, CeO_2 metal oxide NPs and, rGO 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 CuNi-CeO_2-rGO nanocomposite was applied for the preparation of a new modified glassy carbon electrode (GCE) for the purpose of attain the best electrochemical analysis figure of merit. For the first time, in this research Ser, Mel, and Trp were determined simultaneously by electrochemical method.

2. EXPERIMENTAL

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 the electrode modifiers such as CuNi-CeO_2-rGO, CuNi-CeO_2, CuNi and rGO nanomaterials include $NaBH_4$, $CuCl_2.2H_2O$, $NiCl_2.6H_2O$, $Ce(NO_3)_3.6H_2O$, graphite powder, H_2SO_4, H_2O_2, HCl, $KMnO_4$, and other using materials were bought from Merck Company. The target analytes (Ser, 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, CuNi-CeO_2-rGO/GCE, CuNi-CeO_2/GCE, CuNi/GCE, rGO/GCE and 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. Synthesis of modifiers

Graphene oxide sheets (GO) were synthesized from graphite powder by an 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 ^oC for 12 h [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 ^oC for 24 h. The obtained Gr was centrifuged, washed deionized water several times, and dried in a vacuum at 80 ^oC for 12 h [21].

For synthesis of CuNi-CeO_2-rGO nanocomposite, 13.6, 4.8 and 7 mg of $CuCl_2.2H_2O$, $NiCl_2.6H_2O$ and $Ce(NO_3)_3.6H_2O$, respectively, were added to 20 ml suspension of GO in water (0.5 mg/ml). The suspension was mixed for 2 h. In the next step, an excess amount of $NaBH_4$ was added to the suspension while it was mixed quickly under N_2 atmosphere for 4h. The black formed precipitate was collected and washed three times by deionized water, then dried in vacuum at 60 ^oC [22]. The CuNi-CeO_2 NPs were prepared by the same protocol but GO was not charged to the suspension, also for CuNi NPs synthesis, the $CuCl_2.2H_2O$ and $NiCl_2.6H_2O$ were used only.

2.3. Fabrication of the electrode

The bare GCE was firstly cleared and polished with different size of Al_2O_3 and cleaned by ultrasonic in ethanol/water (50:50 V:V) to obtain mirror-like surface. Then, the provided GCE was immersed in HNO_3/HCl (50:50 V:V) for 30 min and finally washed with deionized water 3 times [23]. The modified electrodes (CuNi-CeO_2-rGO/GCE, CuNi-CeO_2/GCE, CuNi/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 was 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 using. For adjusting the pH, 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=7 [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 1 a, the XRD patterns of GO and rGO nanosheet, due to the amorphous nature, showed a broad peak at about $2\theta=10°$ 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.

The XRD pattern of CuNi NPs represents two peaks in 44.6° and 50.9° which are well agree with JCPDS No. 04–0836 (for Cu) [27] and JCPDS No. 04–0850 (for Ni) [28] and confirm the CuNi nanocomposite generation. In addition, when the CeO_2 NPs were doped in

CuNi nanocomposite, in XRD pattern a broad peak at 27.7° was appeared which is relating to amorphous CeO_2 NPs and matching perfectly with JCPDS No. 49–1415 [29]. It can be noted that the intensity of peaks related to Cu and Ni reduced in comparison to pure CuNi nanocomposite, because of decrease in crystallinity of CuNi alloy with doping the amorphous CeO_2 NPs. The XRD pattern of CuNi-CeO_2-rGO nanocomposite displays three characteristic peaks, a broad peak at $2\theta = 22$ to 32°, two peaks approximately at 43° and 51°, which can be related to rGO, CeO_2 nanoparticles and, CuNi nanoparticles.

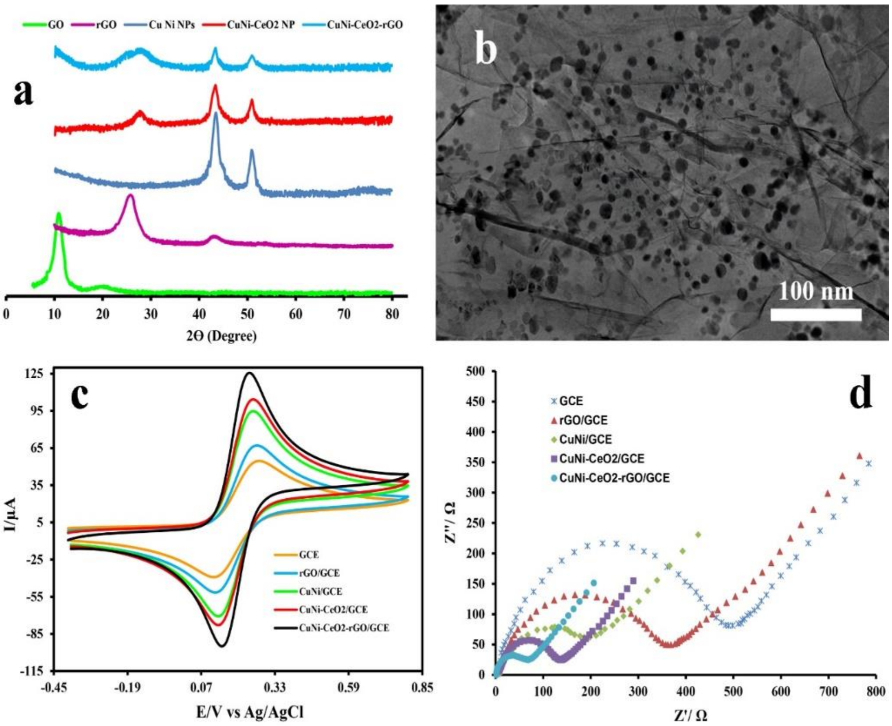


Figure 1. (a) The XRD patterns of GO, rGO, CuNi, CuNi-CeO_2 and CuNi-CeO_2-rGO nanocomposite, (b) the TEM image of CuNi-CeO_2-rGO nanocomposite, (c) CVs (d) (b) Nyquist plots of the bare GCE, rGO/GCE, CuNi/GCE, CuNi-CeO_2/GCE, CuNi-CeO_2-rGO/GCE in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ and 1.0 M KCl

The morphology of synthesized CuNi-CeO_2-rGO nanocomposite was investigated using TEM (Figure 1b). As can be seen, the rGO nanosheets illustrate a cloudy agglomerated and, wrinkled structure, because of elimination of oxygen functional groups. Also, the NPs were deposited uniformly at the surface of rGO without significant agglomeration. The average diameter of the deposited NPs on the surface of rGO was 18 nm.

3.2. Electrochemical investigate of fabricated electrodes

The voltammetric appraisement of the proposed CuNi-CeO_2-rGO/GCE, CuNi-CeO_2/GCE, CuNi/GCE, rGO/GCE and, GCE electrodes were investigated in the presence of 1.0 mol L^{-1} KCl and 5.0 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$. The data were presented in Figure 1c.

The voltammograms obtained with the sweep rate of 100 mV s^{-1} by GCE represent faradaic redox peaks at 275 mV (anodic peak) and 113 mV (cathodic peak), ΔE_p=162 mV. The alteration of oxidation and reduction potential could be dependent on the electron transfer rate between $[Fe(CN)_6]^{3-/4-}$ and the electrochemical reaction sites on the electrode surface. The rGO/GCE depicted the ΔE_p=129 mV with oxidation and reduction peak currents of 38 and 30 µA, respectively. By using CuNi/GCE as working electrode, the ΔE_p has reduced to 118 mV, while the oxidation and reduction currents have increased significantly to 76 and 65 µA, respectively. At the surface of CuNi-CeO_2/GCE, the obtained currents have boosted ($I_{p(ox)}$=85 µA, $I_{p(red)}$=77 µA) and the ΔEp was reduced to 112 mV. The CuNi-CeO_2-rGO/GCE displayed a ΔE_p close to 97 mV, with the oxidation peak to reduction peak current ratio of 1.1, which represents a reversible system.

In the other study, for investigation of the surface property of the different modified and unmodified GCEs the Electrochemical impedance spectroscopy technique was used. Figure 1d illustrates the recorded EIS plots for CuNi-CeO_2-rGO/GCE, CuNi-CeO_2/GCE, CuNi/GCE, rGO/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 500 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 350 Ω, whereas the modified GCE with CuNi produced lower semicircle diameter with the R_{ct} value of 200 Ω because the electron transport occurred rapidly. The CuNi-CeO_2/GCE has shown the lower R_{ct} (about 140 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 CuNi-CeO_2-rGO (65 Ω), which ascribed to the growth electrochemical active surface area, enhancement the electrode conductivity and boost catalytic ability of the CuNi-CeO_2-rGO/GCE. Accordingly, the CuNi-CeO_2-rGO/GCE is certified satisfactory efficient for using in electrochemical application.

3.3. Investigation the effect of modifier on electrochemical oxidation of Ser, 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 5 µM Ser, Mel and Trp, in B-R buffer solution pH=7, at the surface of different prepared electrodes include CuNi-CeO_2-rGO/GCE, CuNi-CeO_2/GCE, CuNi/GCE, rGO/GCE and GCE were checked by DPV technique. The DPV voltammograms are depicted in Figure 2a.

It can be seen that by using bare GCE three weak and broad peaks was observed for electro-oxidation of Ser, Mel and Trp. By modification of GCE with rGO, the response of electrode for Ser, Mel and Trp (E_p: 385, 595 and 716 mV, respectively) have increased slightly (I_p: 5.5, 4.6 and 3.6 µA, respectively) which could be owing to an increase in the surface area of the electrode.

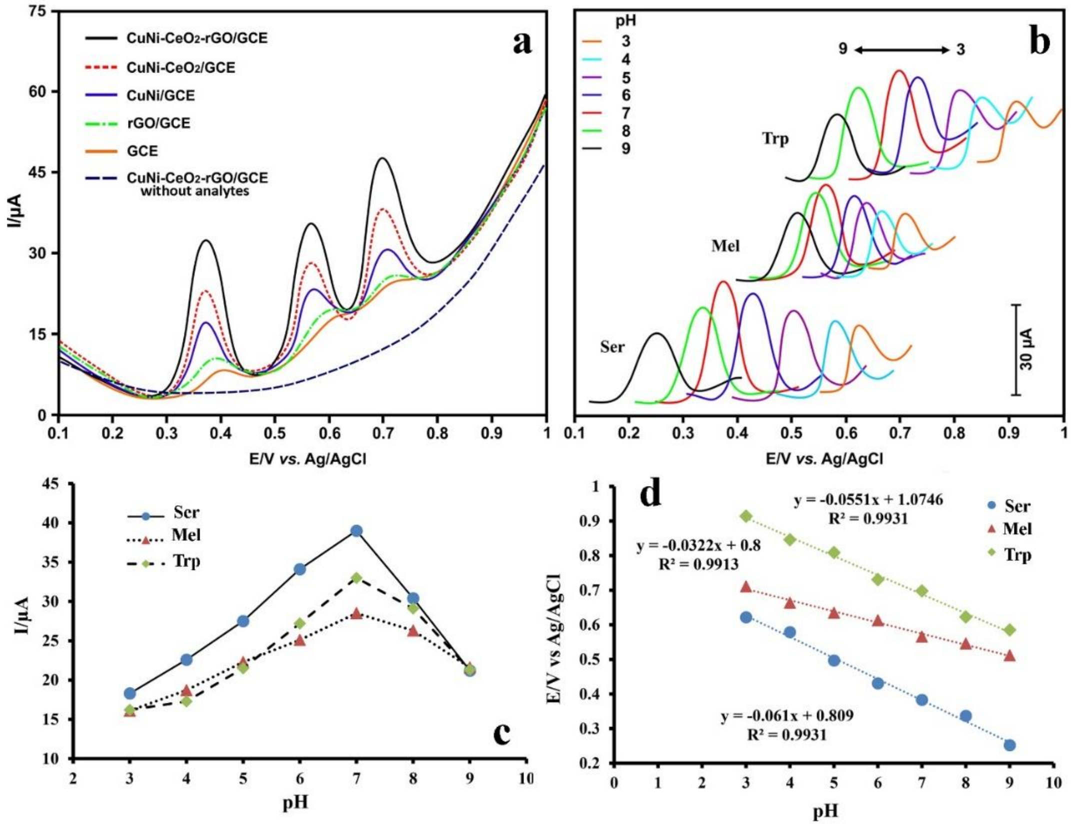


Figure 2. (a) The DPV of 5 µM Ser, Mel and Trp, in B-R buffer solution pH=7, at CuNi-CeO_2-rGO/GCE, CuNi-CeO_2/GCE, CuNi/GCE, rGO/GCE and GCE, (b) The DPV of 8 µM Ser, Mel and Trp at CuNi-CeO_2-rGO/GCE at different pH from 3 to 9, The effect of pH on (c) response of the electrode and (d) potential of the peak

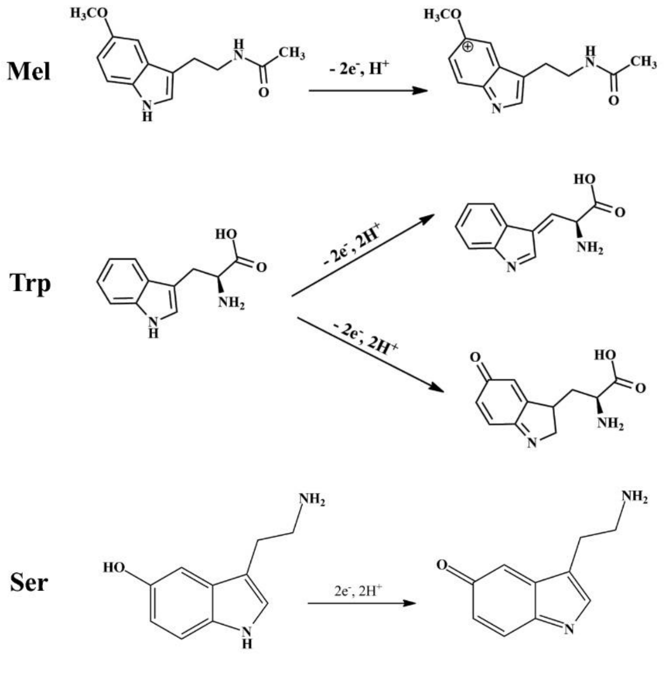
It can be seen that a considerable increase in DPV responses was induced while the GCE was modified by CuNi NPs (I_p: 11.8, 8.4 and 9.2 µA for Ser, Mel and Trp, respectively), also the peak potential of oxidation peaks has shifted to the negative values (E_p: 369, 570 and 706 for Ser, Mel and Trp, respectively). By using CuNi-CeO_2/GCE as sensing electrode, three separated and sharp anodic peaks appeared at the same potential in comparison to CuNi/GCE with 17.9, 14.4 and 16.5 µA oxidation current. At the surface of CuNi-CeO_2-rGO/GCE three sharp, and well-defined peaks for Ser (I_p=27 µA), Mel (I_p=20.1 µA) and Trp (I_p=24 µA) with the peak's separation of ΔE_p (Ser, Mel) =201 mV, and ΔE_p (Mel, Trp) =136 mV was observed. Because the large separation peaks and the maximum oxidation peak currents for the target analytes was obtained at the surface of CuNi-CeO_2-rGO/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 Ser, Mel and Trp

In order to attain the best sensitivity, stability and maximum selectivity in simultaneous electrochemical determination of Ser, 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 8 μM Ser, Mel and Trp in the pH range of 3 to 9. The voltammograms are depicted in Figure 2a.

The data shows that the response of CuNi-CeO_2-rGO/GCE for the three analytes have growth considerably by changing the pH value from 3 to 7, and after that the oxidation current decreased between pH=7 and 9 (Figure 2b). It can be concluded that, because the highest oxidation current was obtained in pH=7, so the best sensitivity achieves in this optimized pH (Figure 2c). The impact of the pH value on the anodic peak potential (E_p) of Ser, Mel and Trp was checked, the E_p of three analytes have changed linearity by increasing the pH from 3 to 9 (Figure 2d).

The linear relationship between E_p and pH can be represented by the following equations: for Ser, E_p = -0.061pH + 0.809 (R^2 = 0.9931), for Mel, E_p = -0.0322 pH + 0.8 (R^2 = 0.9913) and for Trp, E_p = -0.0551 pH + 1.0746 (R^2 = 0.9931). As can be seen, the acquired slope values for Ser, Mel and Trp were -0.061, -0.0322 and -0.0551 mV/pH, respectively.



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

Based on the Nernst equation, the obtained slope values for Ser, 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 -0.0322 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 CuNi-CeO_2-rGO/GCE are presented in Scheme1 [30-32].

The kinetics of electrochemical reaction on the surface of CuNi-CeO_2-rGO/GCE was studied by using CV technique at different scan rates in the range of 10 to 250 mV/s in 0.2 M B–R buffer solutions pH=7 containing 8 μM Ser, Mel and Trp, which the voltammograms are represented in Figure 3a. The electrode responses for oxidation of Ser, 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 $I = 1.3716\ \upsilon^{1/2} + 0.9701$ ($R^2 = 0.9964$) for Ser, $I = 1.076\ \upsilon^{1/2} + 0.2008$ ($R^2 = 0.9982$) for Mel and $I = 1.1744\ \upsilon^{1/2} + 1.0217$ ($R^2 = 0.9979$) for Trp, which demonstrate that the surface reaction on CuNi-CeO_2-rGO/GCE for these analytes was a diffusion-controlled process (Figure 3b) [21].

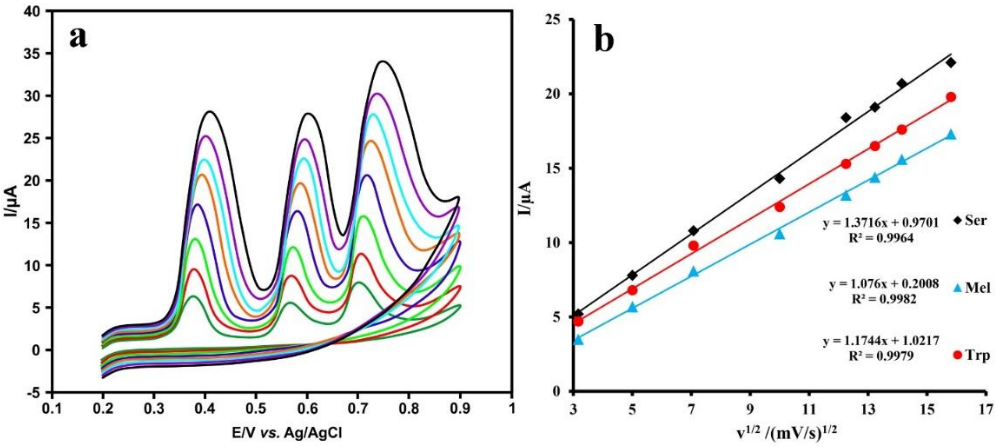


Figure 3. (a) CVs at CuNi-CeO2-rGO/GCE in B-R buffer solution with pH=7 containing 8 μM Ser, Mel and Trp with scan rates ranging from 10 to 250 mV/s, (b) The linear relationship between the peak current vs. square root of the scan rate ($\upsilon^{1/2}$) for Ser, Mel and Trp

3.5. Effect of concentration and calibration curves

In order to confirm the performance of CuNi-CeO_2-rGO/GCE in the individual and simultaneous determination of the Ser, Mel and Trp with the DPV technique, various concentrations of the target molecules were measured in pH 7.

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 CuNi-CeO_2-rGO/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 CuNi-CeO$_2$-rGO/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 Figures 4a-c and the linear dynamic range for Ser (0.008-23 µM), Mel (0.008-21 µM) and Trp (0.008-23 µM) were observed with calibration equations of $I_{pa} = 4.7349C_{(Ser)} + 2.09$ ($R^2 = 0.9982$), $I_{pa} = 3.7389C_{(Mel)} + 1.7606$ and $I_{pa} = 4.1944C_{(Trp)} + 1.6263$ ($R^2 = 0.9983$), respectively (Figures 4d-f).

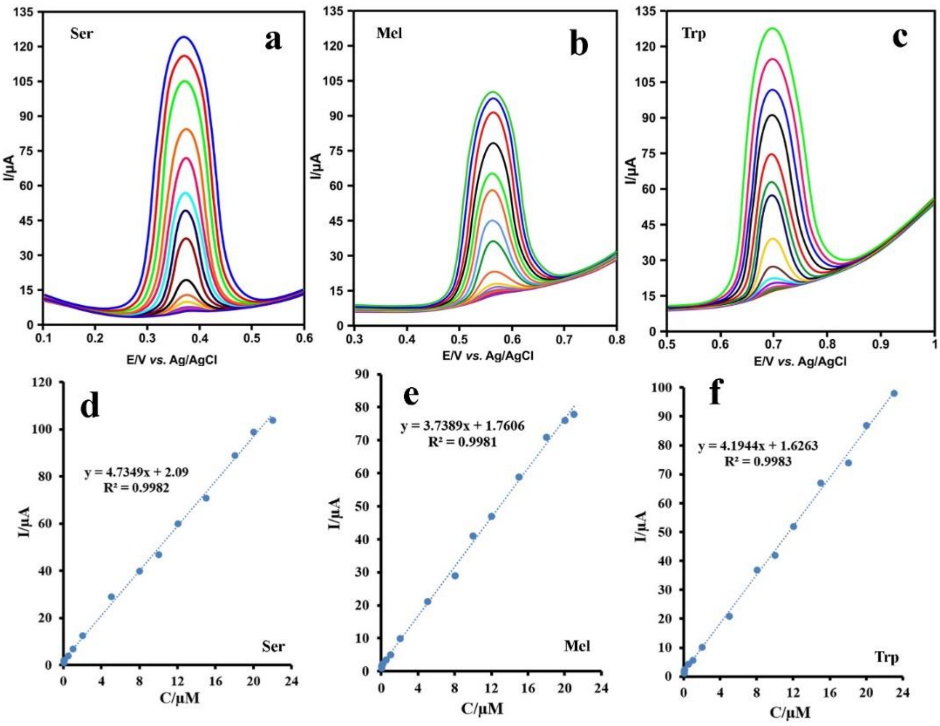


Figure 4. DPVs at CuNi-CeO$_2$-rGO/GCE in B-R buffer solution with pH=7 for (a) Ser (0.008-23 µM), (b) Mel (0.008-21 µM) and (c) Trp (0.008-23 µM), Calibration curve for (d) Ser, (e) Mel and (f) Trp

In the other study, the concentrations were varied simultaneously from 0.008 to 23 µM for Ser, 0.008 to 21 µM for Mel and 0.008 to 23 µM for Trp. The recorded voltammograms are depicted in Figure 5a. As observed, by increasing the concentrations of Ser, Mel and Trp in mentioned concentrations the oxidation peak currents have enhancement linearly with the regression equation of $I_{pa} = 4.6403C + 2.153$ ($R^2 = 0.998$) for Ser, $I_{pa} = 3.7237C + 1.5878$ ($R^2 = 0.9983$) for Mel, and $I_{pa} = 4.1084C + 1.5902$ ($R^2 = 0.9983$) for Trp (Figures 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 Ser, Mel and Trp by CuNi-CeO$_2$-rGO/GCE were calculated to be 0.0058, 0.0061 and 0.0063 µM, 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.

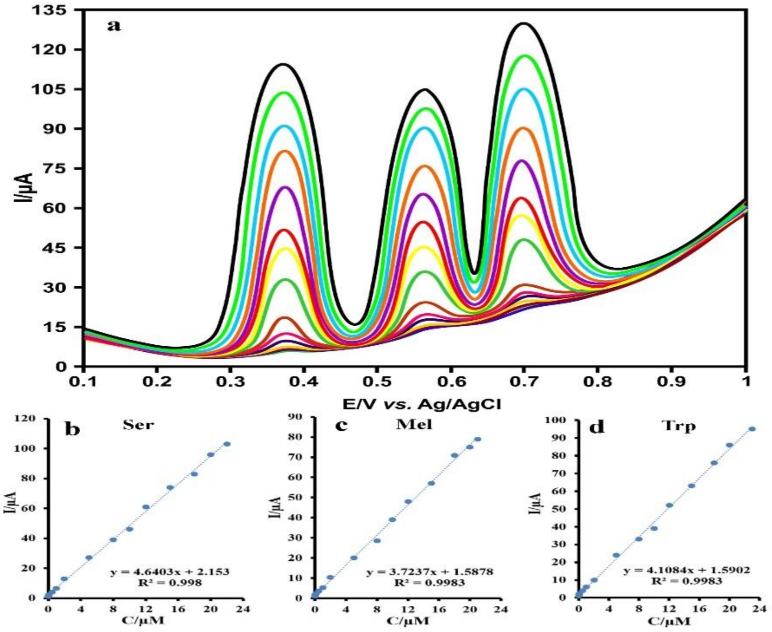


Figure 5. (a) DPVs at CuNi-CeO2-rGO/GCE in B-R buffer solution with pH=7 for simultaneous measurement of Ser, Mel and Trp; Calibration plots for (b) Ser, (c) Mel and (d) Trp

3.6. Interferences and selectivity

The selectivity of the presented electrochemical sensor towards 0.1 µM Ser, 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 CuNi-CeO_2-rGO/GCE in Ser, Mel and Trp detection was appraised in the presence of tyrosine, dopamine, and adrenaline ($C_{Interferent}/C_{Analyte}$=150), ascorbic acid and caffeine ($C_{Interferent}/C_{Analyte}$=300), glucose, lactose and sucrose ($C_{Interferent}/C_{Analyte}$=480), Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}, ClO_4^-, SCN^-, and NO_3^- ($C_{Interferent}/C_{Analyte}$=500). 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 CuNi-CeO_2-rGO/GCE in the voltammetric measurement of Ser, 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 0.5 µM of Ser, Mel and Trp were studied by ten successive replicates determination. The relative standard deviations (RSDs) of the performed experiments for Ser, Mel and Trp were obtained to be 1.9, 2.1 and 2.6%, respectively.

3.8. Testing the presented electrode on real samples

To confirm the applicability of CuNi-CeO_2-rGO/GCE, detection of Ser, Mel and Trp in human urine and blood plasma samples was performed. In order to decrease the sample matrix effect, the urine and blood plasma samples were diluted with B-R buffer solution pH=7.

Table 1. Determination of the analytes in real samples

Sample	Analyte	Added (µM)	Detected (µM)	Recovery (%)	HPLC
Urine	Ser	2	2.05	102.5	2.09
		10	9.81	98.1	9.93
		20	20.14	100.7	20.23
	Mel	2	1.93	96.5	2.01
		10	10.17	101.7	10.09
		20	20.16	100.8	20.23
	Trp	2	2.08	104	2.06
		10	9.86	98.6	10.04
		20	19.72	98.6	19.83
Blood plasma	Ser	2	2.06	103	2.09
		10	10.34	103.4	10.18
		20	19.75	98.8	20.10
	Mel	2	2.07	103.5	9.98
		10	10.11	101.1	10.00
		20	20.43	102.2	20.25
	Trp	2	2.04	102	2.02
		10	10.23	102.3	10.11
		20	19.69	98.4	19.57

These data demonstrate the provided sensor has satisfactory repeatability results. In order to check the reproducibility of CuNi-CeO_2-rGO/GCE, five electrochemical sensors were constructed by the same procedure and the response of the electrodes for determination of 0.5 µM of Ser, Mel and Trp were recorded. The RSD values were calculated to be 3.0, 3.2 and

3.5% which demonstrate the reproducibility of the fabrication method of the presented electrode.

The monitoring of Ser, Mel and Trp in the samples were calculated by standard addition method. The data of analysis are presented in Table 1, and the obtained results for determination of analytes by electrochemical method was compared with the HPLC method. The obtained data with the electrochemical method and the chromatographic method were in good agreement without significant difference. The recovery percentages indicate that the presented modified electrode is trustworthy and efficient for real sample analysis.

4. CONCLUSIONS

In the presented study, the CuNi-CeO_2 nanocomposite were decorated on rGO and applied to modification the GCE surface. Based on CV and EIS analysis, the CuNi-CeO_2-rGO/GCE has the best electrochemical features, due to the synergistic effect of simultaneous using CuNi, CeO_2 and rGO NPs, compared to the other investigated electrodes. Also, the maximum currents, excellent selectivity and sensitivity for oxidation of Ser, Mel and Trp were obtained when the CuNi-CeO_2-rGO/GCE was applied as working electrode in pH=7. The prepared CuNi-CeO_2-rGO/GCE exhibited satisfied repeatability and reproducibility and high selectivity, which could be effective for the simultaneous monitoring of Ser, Mel and Trp in their mixture. The presented modified electrode was applied for the determination of Ser, Mel and Trp in human biological fluid samples. The recovery percentages for three analytes were achieved in the range of 96.5% to 103.5%. The data of the electrochemical method for the monitoring of analytes in the real samples were close to those of the HPLC method. So, it can be concluded that the developed method with the mentioned properties has the potential for clinical applications.

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