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Investigation of Voltammetric Behavior of 3-Nitro-Tyrosine and Tyrosine at Glassy Carbon and Carbon Paste Electrodes

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Abstract- Nitro-tyrosine is produced via tyrosine nitration by reactive nitrogen species such as peroxynitrite and NO_2. Nitrotyrosine presence in the body is an indicator for cell damage and inflammation issue. Hence, the monitoring of this biomarker is an important task. Since nitro-tyrosine is produced from tyrosine, these two molecules are present concurrently in the real samples. In this work electrooxidation of nitro-tyrosine and tyrosine was checked on glassy carbon and carbon paste electrodes (CPE) utilizing cyclic voltammetry and differential pulse voltammetry methods. It was found that nitro-tyrosine creates an oxidation signal at the GC electrode, whereas, no significant oxidation peak was found for tyrosine in the GC electrode. In the case of CPE, However, both nitro-tyrosine and tyrosine showed oxidation peaks which were separated by about 0.1 V/Ag/AgCl. Applying DPV led to better resolution of oxidation peaks of tyrosine and nitro-tyrosine. Moreover, the separation of the peaks was improved when DPV was replaced with CV. Solution pH and the binder content of the carbon paste electrode were optimized in order to achieve maximum signal for both compounds. The calibration curves were established utilizing CPE and DPV techniques.

Keywords- Nitro-tyrosine; Tyrosine; Carbon paste electrode; Simultaneous Determination; Electroxidation

1. INTRODUCTION

Highly reactive oxygen and nitrogen species (ROS and RNS) are naturally created in the body as a result of cellular metabolism. However, some other external sources such as energetic radiation and reactive chemicals interred in the body can lead to producing such active species in the body. Highly reactive oxygen and nitrogen species are beneficial at trace concentrations in the organism; since, they play some important roles such as energy-producing, controlling of cell growth, intercellular signaling, and production of influential biological substances. However, the superfluous of these species results in oxidative harm in several biologically important molecules such as DNA [1-4]. The free radicals nitric oxide ($NO^{\bullet}$) and superoxide ($O_2^{\bullet -}$) can react producing RNS, such as peroxynitrite ($ONOO^-$), a highly reactive anion that can interact with tyrosine residues in proteins, generating 3-nitro-tyrosine (3-NO_2-Tyr) [5-7]. The generation of 3-NO_2-Tyr has been associated with different types of diseases, such as acute lung injury, neurodegeneration, atherosclerosis, and some cancers [8].

Current evidence suggests the incorporation of this tyrosine into proteins, producing changes in cellular activities, increased protein degradation, stimulation of apoptosis, and inhibition of mitogenic pathways, and diseases [9]. It was also determined, that the presence of the nitro group (electron-withdrawing group) at the 3-nitro-tyrosine molecule makes its phenolic group more acidic (pK_a ~ 7.0), when compared with p-Tyr (pKa ~ 10). Hence, 3-nitro-tyrosine neutral form is predominant at pH < pKa, while at alkaline medium the ionized form is major and it is much more polar [8]. It is therefore extremely important to study the electrochemical properties of tyrosine and 3-nitro-tyrosine [7]. 3-nitro-tyrosine is also an important biomarker of oxidative stress it is stable and easily quantified in blood, tissue, and urine [9].

Voltammetric techniques are currently used in redox characterization studies of different species and in the development of electroanalytical methodologies [10-27].

The anodic behavior of tyrosine was investigated using voltammetric techniques and associated with the oxidation of its phenolic group [28-31]. However, few electroanalytical methods for 3-nitro-tyrosine quantification have been described [32,33]. Therefore, additional work should be done to fully characterize the redox reactions of 3-nitro-tyrosine.

Carbon paste electrode (CPE) is one of the most important electrochemical transducers used in electrochemistry research due to its unique characteristics such as a large potential window on both anode and the cathode region, ease of its preparation, low cost, its capability of modification [34,35].

Taking in to account the importance of tyrosine and nitro-tyrosine determination, in this study we tried to investigate the electroanalytical characteristic of these compounds at two traditionally used working electrodes, namely, glassy carbon electrode and carbon paste electrode.

2. EXPERIMENTAL

2.1. Instruments and reagents

Electrochemical data were obtained with a three-electrode system using a potentiostat/galvanostat model PGSTAT302, Metrohm. The CPE was used as a working electrode. A platinum wire and an Ag/AgCl electrode were used as the counter and reference electrodes, respectively. Tyrosine, 3-nitro-tyrosine, and n-eicosane were supplied by Sigma–Aldrich (Munich, Germany), and used as received. Graphite was purchased from Fluka (Buchs, Switzerland). Other chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany).

2.2. Preparation of the sensors

The binder material (n-eicosane0 was melted in a dish at 45–50˚C and mixed with graphite powder using a stainless steel spatula. The procured paste was utilized to fill a hole (2.00mm in diameter, 3 mm in depth) at the end of a Teflon body; the excess solidified material was removed with the assistance of a paper sheet. The electrode was reused after each experiment by moving the electrode surface on paper in order to extrude a thin layer of the electrode surface.

3. RESULTS AND DISCUSSIONS

3.1. Cyclic voltammetry of tyrosine and nitro-tyrosine at glassy carbon electrode

Figure 1 represents the cyclic voltammetry responses of both tyrosine and nitro-tyrosine at glassy carbon electrodes. According to the depicted voltammograms tyrosine shows no significant response at GC electrode when scanning the potential of the electrode from 0.0 to 1.1 V versus Ag/AgCl reference electrode.

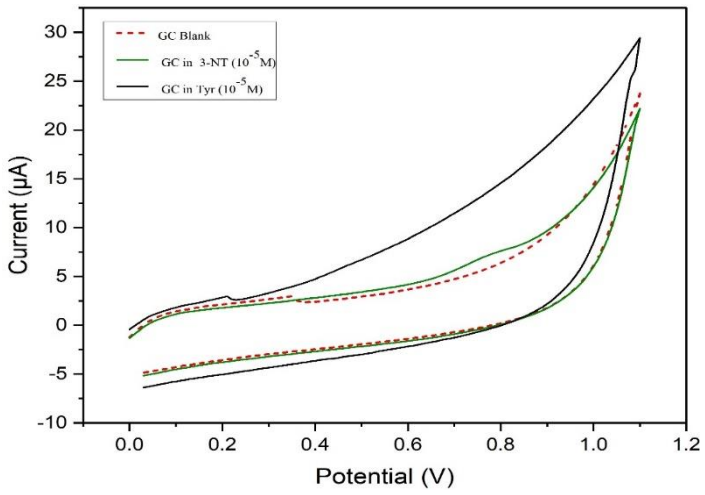

Figure 1. Cyclic Voltammetry responses of tyrosine and 3-nitrotyrosine (10^{-5} M) in PBS (0.05 M) at GC electrode

However, in the case of nitro-tyrosine, an oxidative peak can be observed at a potential of about 0.75V versus Ag/AgCl.

3.2. Cyclic voltammetry of tyrosine and nitro-tyrosine at carbon paste electrode

Figure 2 represents the cyclic voltammetry responses of tyrosine and nitro-tyrosine at the carbon paste electrode. As seen, both tyrosine and nitro-tyrosine exhibit significant voltammetric responses at the carbon paste electrode when scanning the potential of the electrode from 0.0 to 1.1 V versus Ag/AgCl reference electrode. Cyclic voltammetry of tyrosine at CPE shows a distinct oxidative peak at about 0.77 V/(Ag/AgCl). As can be seen, no reductive peak appears when reversing the potential scanning at the CPE for tyrosine, indicating that the oxidation of tyrosine at the electrode is electrochemically irreversible. On the other hand, the voltammogram of nitro-tyrosine recorded using the CPE exhibits a significant oxidative peak which is located at much positive potential (0.86V/(Ag/AgCl) compared to that of tyrosine indicating that the oxidation of nitro-tyrosine occurs fairly harder than the electrooxidation of tyrosine. Moreover, the cyclic voltammetry of nitro-tyrosine does not show a reductive peak when inversing the potential scanning, indicating that electrooxidation of nitro-tyrosine is an irreversible electrochemical reaction like the electrooxidation of tyrosine.

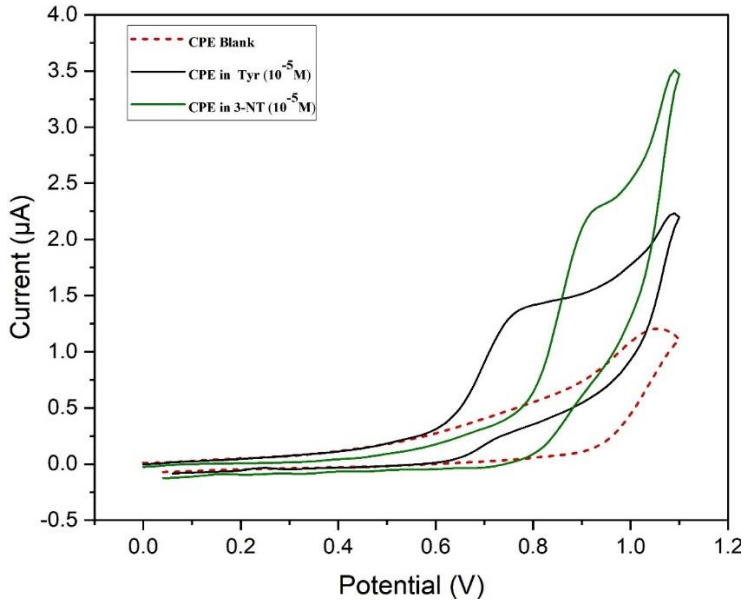

Figure 2. Cyclic Voltammetry responses of tyrosine and 3-nitrotyrosine (10^{-5} M) in PBS (0.05 M) at the CPE electrode

According to the previous reports [36], the electrooxidation of tyrosine and nitro-tyrosine includes oxidation of phenolic group of the molecules and its conversion to a radical anion species which subsequently is dimerized or polymerized (Figure 3).

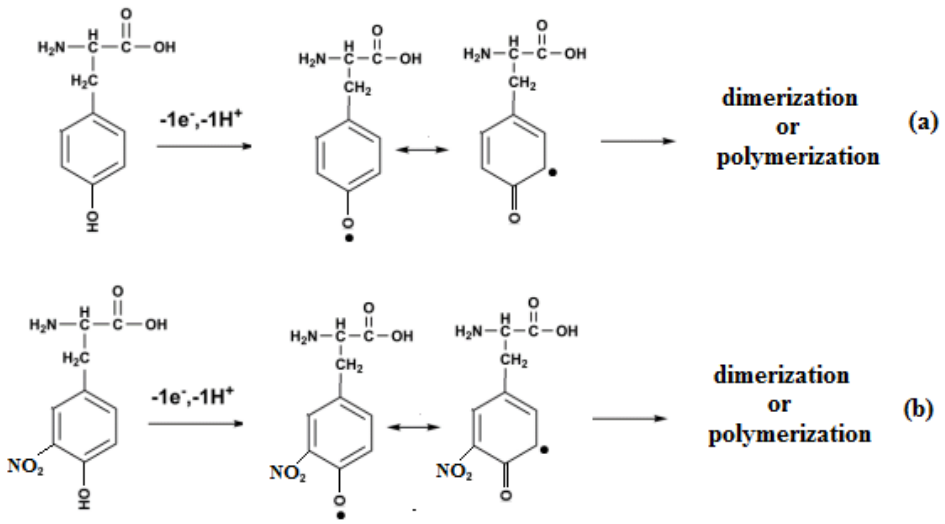

Figure 3. Electrochemical oxidation mechanism of tyrosine and nitro-tyrosine

Based on the as described results, CPE is a better electrode for voltammetric determination of nitro-tyrosine and tyrosine, compared to the glassy carbon electrode. The reasons for this assertion can be cited as below.

The CPE shows distinct oxidative peaks for both tyrosine and nitro-tyrosine; whereas GC exhibits no oxidation signal for nitro-tyrosine Furthermore, the peak potentials of tyrosine and nitro-tyrosine are separated considerably (about 1.0 V) at CPE which make it suitable for simultaneous determination of both compounds in a real sample. However, in spite of the above-mentioned advantages of CPE, the cyclic voltammetry peaks of both tyrosine and nitro-tyrosine are broadened significantly making difficult peaks resolution of these compounds when they exist simultaneously in a sample. Fig. 4 represents the cyclic voltammetry recorded in a solution containing both tyrosine and nitro-tyrosine. As seen, although both molecules show oxidative peaks but the related peaks are overlapped considerably.

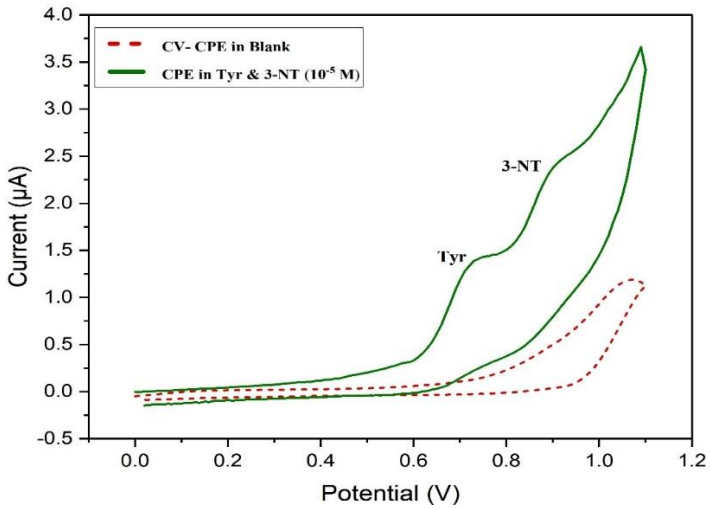

Figure 4. Cyclic voltammetry responses of tyrosine and 3-nitrotyrosine (10^{-5} M) at the CPE electrode when existing simultaneously in a solution of PBS (0.05 M)

3.3. Simultaneous determinations of tyrosine and nitro-tyrosine at CPE using differential pulse voltammetry

In order to separate the oxidative peaks of tyrosine and nitro-tyrosine, as possible, differential pulse voltammetry (DPV) method was applied. Figure 5 represents the DPV response of tyrosine and nitro-tyrosine when they are simultaneously in a solution. The voltammograms depicted in the figure indicate that both oxidation peaks are sharpened and thus they are speared considerably. The first oxidation peak appeared at a potential of 0.65 V is for tyrosine and that at the potential of 0.85 V belongs to nitro-tyrosine. This means that the potential distance between the peaks is about 0.2V.

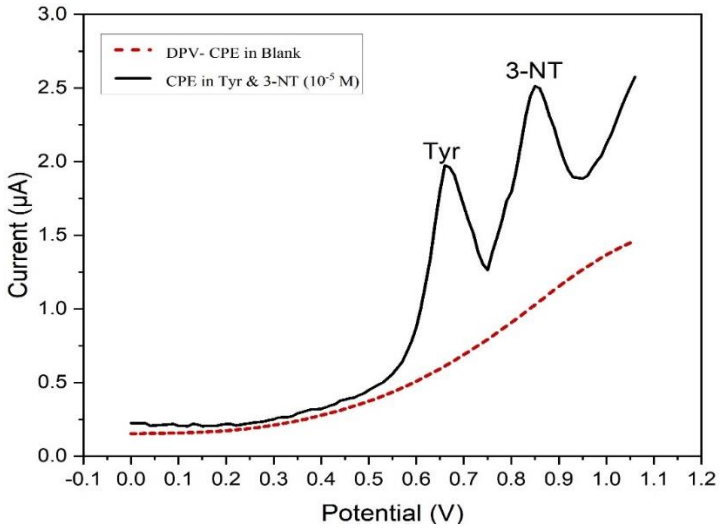

Figure 5. Differential pulse voltammetry response of tyrosine and 3-nitrotyrosine (10^{-5} M) coexisting in a solution at CPE in the presence of PBS (0.05 M)

3.4. Optimization of the important variables effective on the DPV signals of tyrosine and nitro-tyrosine

The effect of solution pH on the DPV response of the CPE electrode for both tyrosine and nitro-tyrosine was checked in order to find the optimal pH condition for the measurement of these compounds. The results obtained are represented in Fig. 6(a). As seen in both cases of tyrosine and nitro-tyrosine the electrode current signal is maximum at neutral pH, meaning that lower and higher than neutral pH the electrode signal to the examined molecules decreases considerably.

The amount of binder (n-eicosane) applied for making the CPE is an important factor in the electrochemical characterization of the electrode fabricated. Fig.6(b) shows the effect of binder content of CPE in the oxidation signal of both tyrosine and nitro-tyrosine. As seen, the DPV signals of both investigated molecules decrease as a result of increasing of binder content of the electrode. Therefore, one can conclude that a minimal amount of binder should be used for electrode making; however, when lower than 15% of the electrode composition was

allocated for n-eicosane the electrode created was mechanically unstable. Hence, it was decided to use a minimal amount of binder (15%) for the electrode fabrication.

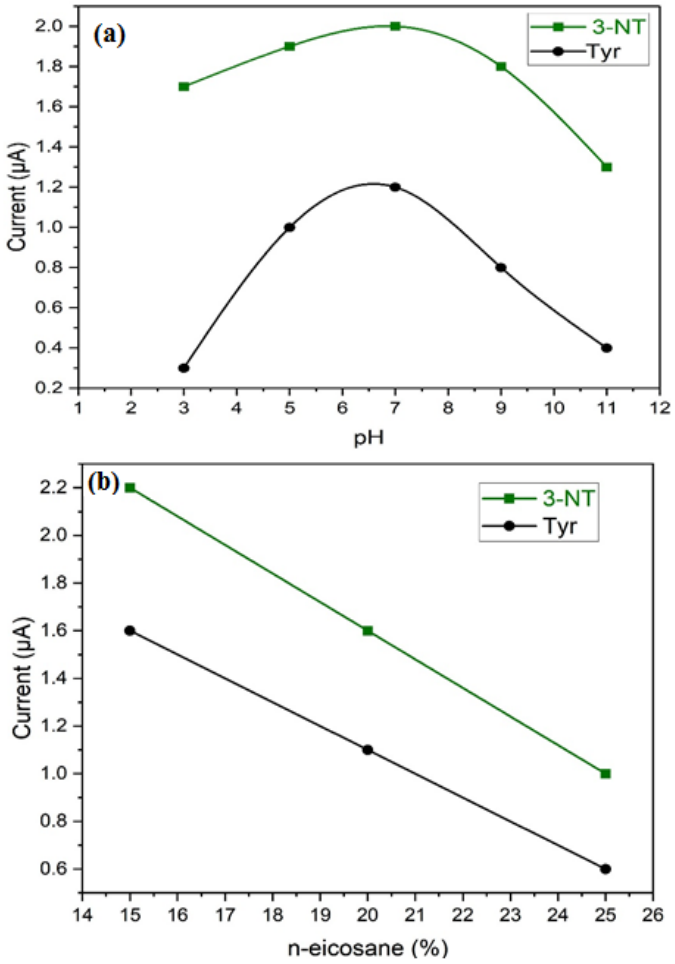

Figure 6. The effect of pH (a) and binder content of the CPE (b) on differential pulse voltammetry response of tyrosine and 3-nitrotyrosine (10^{-5} mol L^{-1})

3.5. correlations between the tyrosine and nitro-tyrosine concentrations and their DPV signals

The relationship between the DPV signal of 3-nitrotyrosine and tyrosine and their various concentrations, when they coexist in a solution, is illustrated in Figure 7. As seen, there is a good correlation between the nitro-tyrosine concentration and DPV signal recorded using CPE at the optimized conditions. The same correlation is also found between the tyrosine DOV signal and its various concentrations. The correlation between both tyrosine and nitro-tyrosine concentrations and their related DPV signal is linear between concentration ranges of 0.1-10 µM.

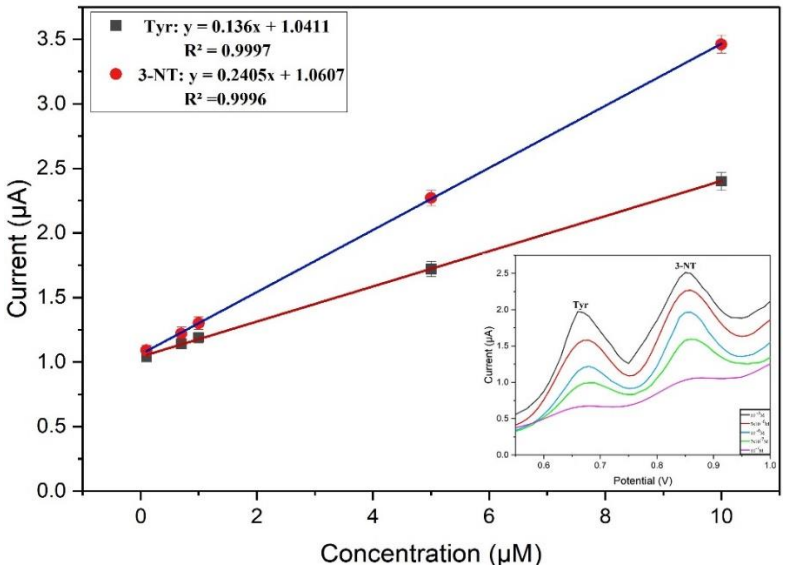

Figure 7. Calibration curve plotted for tyrosine and nitro-tyrosine based on differential pulse voltammetry responses (inset) obtained using CPE at the optimized conditions

4. CONCLUSION

3-nitro-tyrosine and tyrosine voltammetric characteristic was checked on glassy carbon and carbon paste electrodes. It was shown that GC electrodes exhibit an oxidation signal for nitro-tyrosine, whereas, no significant oxidation peak was seen for tyrosine. In the case of CPE, both nitro-tyrosine and tyrosine showed oxidation peaks which were separated by about 0.1 V/Ag/AgCl. Applying DPV led to better resolution of oxidation peaks of tyrosine and nitro-tyrosine. Some effective factors on the CPE response to both tested compounds were examined and optimized in order to achieve maximum signal for both compounds. The calibration curves were established utilizing CPE and DPV techniques.

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