NOVEL CNT SUPPORTED RU-CO CATALYST FOR DETECTION OF THREONINE IN ITS NATURAL ENVIRONMENT

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ABSTRACT

In this study, catalysts consisting of ruthenium (Ru95%) and cobalt (Co5%) supported on carbon nanotubes (CNTs) were prepared using the $NaBH_4$ reduction method. After the Ru-Co/CNT catalysts were prepared, an efficient and sensitive electrochemical sensor was developed using glassy carbon electrode (GCE) modified with Ru-Co/CNT catalysts. The electrochemical behavior of Ru-Co/CNT-modified GCE electrodes was investigated using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Electrochemical results show that the GCE electrode modified with Ru-Co/CNT has a sensitivity of 0.009 mA/cm^2.mM, limit of detection (LOD) 0.06 µM a limit of quantification of (LOQ) 0.18 µM for threonine. In conclusion, the results show that the Ru-Co/CNT-modified GCE electrode has been synthesized for the first time in the literature and is a promising catalyst for the sensitive detection of threonine.

Keywords: threonine, ruthenium, cobalt, carbon nanotube, amino acid.

INTRODUCTION

Amino acids are essential for important processes in the body, such as the synthesis of proteins, hormones, and neurotransmitters (Nelson DL, 2005). Amino acids are organic molecules that contain an amino, carboxyl, and side chain in their structure. Amino acids exist in two forms: D and L forms. Generally, amino acids are found in the L form, but the appearance of the D form can be a sign of aging or disease, which is considered a negative condition (Minami et al, 2013). There are an average of 20 amino acids; they are divided into two different groups: essential and non-essential amino acids. Essential amino acids can not be synthesized metabolically by the body and are obtained externally through the diet. Essential amino acids consist of isoleucine, leucine, valine, lysine, methionine, phenylalanine, threonine, and tryptophan. Non-essential amino acids can be produced naturally by the body. Non-

essential amino acids include cysteine, asparagine, alanine, arginine, aspartic acid, glutamic acid, glutamine, glycine, proline, serine and tyrosine (Silva et al. 2012).

Threonine is an essential amino acid and its molecular formula is $C_4H_9NO_3$. Threonine, ensures the normal functioning of the human organism, especially the functioning of the immune, central nervous and cardiovascular systems, and also participates in the production of muscle tissue, collagen and elastin 8 Vukstich et al. 2022). It also plays a role in the synthesis of Glycine and Serine amino acids. This amino acid ensures the formation of strong bone and tooth enamel structure, and also supports the immune system and ensures rapid recovery after trauma (Tsuzuki, T., Harper, D.O. and Hunt, H., 1958; Wu et al, 1993). Today, as a result of the increasing interest in the diagnosis and treatment of diseases, new methods for amino acid analysis have been developed. Today, the most used methods to detect and characterize amino acids are based on spectroscopic (Lee et al. 2015), chromatographic (Wang, J., Chatrathi, M.P. and Tian, B., 2000), or electrochemical approaches (Vardanega, D. and Girardet, C., 2009).A variety of electrode materials have been used to increase the electrochemical selectivity of carbon-based electrodes for aminoacids in a wide range of electrochemical sensor studies in the literature due to its lower background current, low cost, chemical inertness, and wide potential range (Kazici, H., Salman, F. and Kivrak, H. 2017). Electrochemical techniques such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) are highly sensitive and selective with low detection limits. In general, each method has advantages and disadvantages, and the choice of method depends on the specific application and requirements for amino acid detection.

Many electrochemical sensor studies have been conducted in the literature on glucose (Tian, K., Alex, S., Siegel, G. and Tiwari, A., 2015), amino acid (Gumerov et al.2022), hydrogen peroxide (Kazıcı et al. 2018), and cancer biomarkers (Ferhan et al, 2018). For amino acid detection, electrochemical sensor studies involve modification of electrodes with amino acid-specific catalysts such as carbon nanotubes, metal nanoparticles, nanopolymers, and nanocomposites that enhance the electrochemical response to the amino acid (Imanzadeh et al. 2023). Table 1 summarizes the nanomaterials used in electrochemical sensor studies in the literature, their aminoacid, linear range, limit of detection (LOD), and limit of quantification (LOQ).

Table 1. Electrochemical sensors based on nanomaterials available in the literature for the determination of amino acids.

Aminoacid	Electrode	Linear range	LOD/LOQ	Ref
tryptophan	Nano-MIP/MWCNTs-GCE	0.06–0.4 µM	0.0071 / 0.021 µM	(Alizadeh, T. and Amjadi, S., 2017)
methionine	CuO NFs/GCE	1.0–300 µM	0.3/0.9 µM	(Ziyatdinova, G. and Gimadutdinova, L., 2023)
histidin	HRP/GNPs-Thi/Chit-GCE	0.01–103 ng/mL	3.3/9.9 pg/mL	(Ren, R., Lu, D. and Pang, G., 2020)
L-serine	Ni−NiO HNT/BDDE	0.2–6.54 µM	0.1/0.3 µM	(Dai, et al.2015)
L-cysteine	Mo/CNT/GCE	0–150 µM	0.20./0.60 µM	(Selçuk, K., Kivrak, H. and Aktaş, N., 2021)
Threonine	Ru-Co/CNT	100–500 mM	0.06/0.018 µM	This study

In this study, CNT-supported Ru-Co catalysts were first synthesized by sodium borohydride ($NaBH_4$) reduction method. For electrochemical measurements, GCE was modified with Ru-Co/CNT catalysts, and the electrochemical behavior of the modified GCE was determined by CV, and EIS. Finally, sensitivity, interference, and selectivity measurements were performed on GCE electrodes modified with Ru-Co/CNT, and as a result, LOD and LOQ values were obtained.

EXPERIMENTAL

1. The synthesis of RuCo/CNT catalyst

The sodium borohydride reduction method was used for catalyst synthesis. The distilled water, Ru precursor ($RuCl_3.3H_2O$), and Co precursor ($Co(CH_3COO)_2$) were first added into a beaker and kept in an ultrasonic bath until homogeneously dispersed. CNT was added as a support material onto metal precursors dispersed homogeneously in distilled water. This prepared mixture was mixed in the ultrasonic bath and the magnetic stirrer for approximately 2 hours. Finally, sodium borohydride (30 equiv.) was added to this mixture as a reducing agent and stirred for another hour. It was filtered, washed, and dried at 85 °C for approximately 12 hours.

2. Electrochemical studies

Electrochemical studies were carried out on the Ru-Co/CNT bimetallic catalyst by CV and EIS. The effect of amino acid concentration, scan rate, and pH was investigated by CV on Ru-Co/CNT bimetallic catalysts on GCE electrode. Sensitivity of electrodes for threonine detection was defined using EIS. All electrochemical measurements were performed using a three-electrode system using a CHI 660E potentiostat. Glassy carbon (GCE) was used as the working electrode, (Ag/AgCl) and Pt wire ternary system were used as the reference electrode, respectively. By dispersing 5 mg bimetallic catalyst in 0.5 mL of nafion, a nanocatalyst ink was obtained and 3 µl of bimetallic catalyst ink was transferred on GCE and dried. Consequently, Ru-Co/CNT modified GCE electrodes were obtained. CV measurements were performed on the Ru-Co/CNT modified electrode, 0.1 M PBS +5 mM threonine solution at different pHs (pH:5.5, pH:7.2, pH:10.5) were obtained in the (-1V/1V) range at room temperature (scan rate = 100 mV s^{-1}). Following the pH effect study, the effect of concentration on the electro-oxidation of threonine on the GCE electrode modified with bimetallic Ru-Co/CNT was investigated. Cyclic voltammograms 5mM threonine in PBS (pH = 7.2) at 10-100 mV s^{-1} scan rates to define the scan rate effect towards threonine electro-oxidation.

RESULT AND DISCUSSION

The modified Ru-Co/CNT catalyst system was used to modify a GCE for measuring threonine. The performances of the electrooxidation of threonine amino acid were examined on the modified GCE electrode in 0.1 M PBS at three different pH levels. It was observed that the current was better at pH: 7.2 for threonine amino acid. (Figure 1).

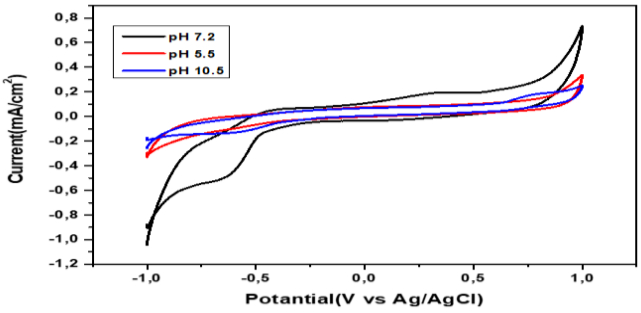


Figure 1. Cyclic Voltammograms obtained at pH=5.5, pH=7.2, pH=10.5 in a) 0.1 M PBS + 5 mM

Threonine, solution at 100 mV/s scan rate on Ru-Co/CNT modified GCE electrode.

Ru-Co/CNT modified GCE electrode was used to investigate threonine concentration effect on electrooxidation activity. Two different concentrations (0 and 5 mM) were tested to determine the current density of threonine electrooxidation, presented in Figure 2. The findings showed that the current density values for electrooxidation of threonine amino acid gave the best current at 5 mM concentration.

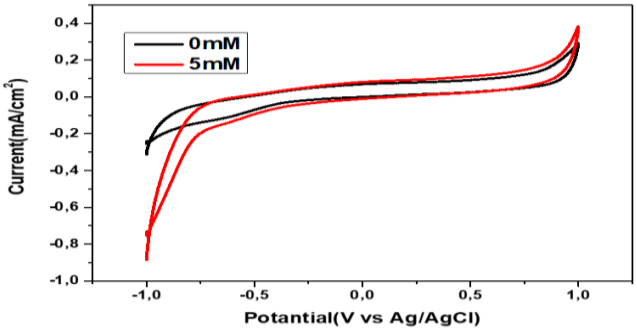


Figure 2. Cyclic Voltammograms obtained at various threonine concentrations (0-5 mM) in 0.1 M PBS (pH=7.2) solution at 100 mV/s scan rate on Ru-Co/CNT modified GCE electrode.

Threonine on a GCE electrode modified with Ru-Co/CNT using CV. Figure 3 presents the results showing that the electrochemical oxidation current of threonine increases in direct proportion to the scan rate. This can be explained by the emergence of diffusion-controlled formation. To evaluate the effectiveness of the electrode's electroactive surface area, the researchers used CV to measure it at various scan rates ranging from 10 to 500 mV/s in a phosphate buffer solution (Figure 3b). They used the Randles-Sevcik equation to calculate the effective surface area, solution concentration, square root of scan rate, phosphate diffusion coefficient, and number of electrons. The results showed that the electrode had an effective surface area of (R^2 = 0.90).

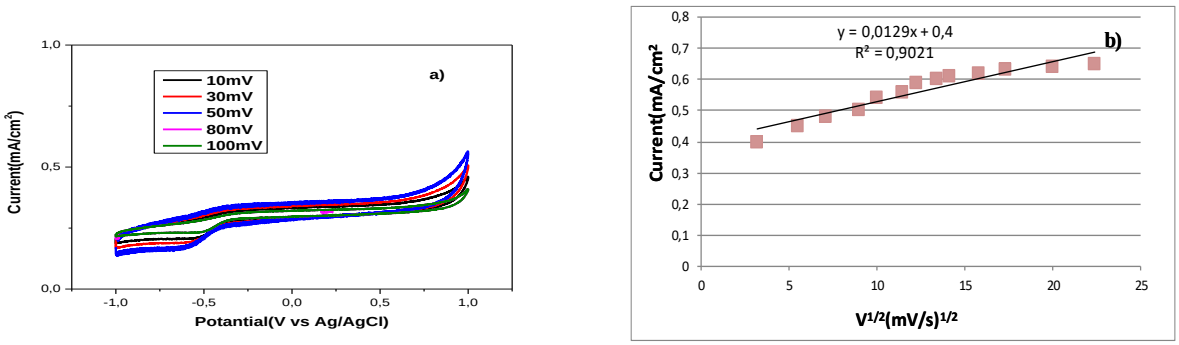
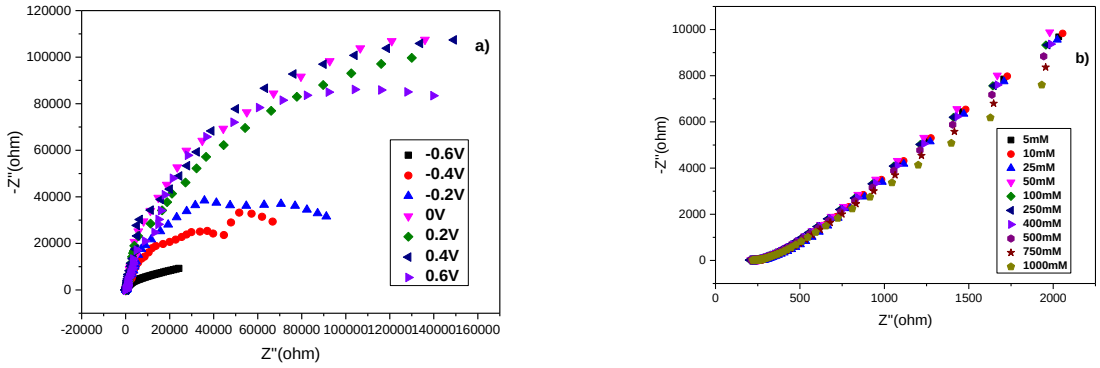


Figure 3. Cyclic Voltammograms on Ru-Co/CNT modified GCE electrode in 0.1 M PBS (pH=7.2) + 5 mM threonine solution at different scan rates (10, 30, 50, 80, 100, mV/s),b) linear regression of peak currents vs the square root of scan rates.

The EIS method is used to determine the electrode capacitance on the electrode, determine the charge transfer resistance, and reveal the diffusion properties simultaneously (Sahin, O., Duzenli, D. and Kivrak, H., 2016). The Nyquist plot for EIS consists of semicircular and linear parts. The semicircular part shows the charge transfer, while the linear part shows the diffusion controlled process.

As seen in Figure 4a, the uptake of Ru-Co/CNT-modified GCE and threonine electrooxidation in the solution prepared in 0.1 M PBS and 5 mM threonine shows that it has the best charge transfer resistance at 0.2V. In Figure 4b, the charge transfer resistance of 0.1M PBS and 5mM threonine at different concentrations at 0.2V was examined and the best charge transfer resistance was shown at 1000mM.

In Fig. 4c, the Ru-Co/CNT-based threonine sensor showed a current sensitivity of 0.0009 mA/cm2,mM, and (LOD) 0,06 µM and (LOQ) value 0,18µM. In Figure 4d, the charge transfer resistance (Rct) values of the Ru-Co/CNT modified GCE electrode in 1000 mM (371.8 Ω) > 750 mM (361.6 Ω) > 500 mM (331.1 Ω) > 400 mM (314.3 Ω), > 250 mM (244.6 Ω), > 100 mM (228.9 Ω), > 50 mM (228.3 Ω), 25 mM (218.5 Ω), > 10 mM (213.9 Ω), > 5 mM (206 Ω) was found from the different concentration equivalent circuit model. Since the 5mM concentration has the lowest semicircular shape and Rct, it has the highest carrier transfer performance compared to other potentials.



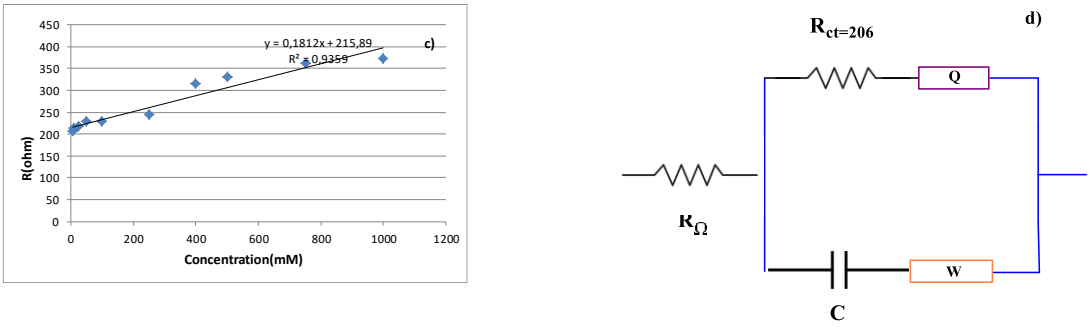


Figure 4.a) Electrochemical impedance spectrum and equivalent circuit model at different potentials of Ru-Co/CNT modified GCE electrode at 0.1 M PBS (pH 7.2) + 5 mM threonin, b) Concentration comparison of 0.1M PBS (pH 7.2) + 5 mM threonine at 0.2V, c) Linear regression of maximum current versus threonine concentration, d) Rct value in 0.1M PBS (pH 7.2) + 5 mM threonine at 0.2V.

CONCLUSIONS

- The Ru-Co/CNT catalyst was produced by applying the $NaBH_4$ reduction method and modified to contain threonine amino acid sensor property. To evaluate the performance of the modified Ru-Co/CNT GCE electrode, including evaluation of detection limits.
- The $NaBH_4$ reduction method played a critical role in the creation of the sensor of threoine and gave positive results. The Ru-Co/CNT modified GCE electrode showed high sensitivity, stability, and selectivity towards threonine. These new study, the detection of threonine for the first time, contribute significantly to the literature

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