Van Yuzuncu Yil University Journal of Engineering Faculty Volume 2, Number 2, Page 22-30, 2024

Van Yüzüncü Yıl Üniversitesi
Mühendislik Fakültesi Dergisi
https://dergipark.org.tr/tr/pub/vyyumfd

CNT Supported Bimetallic Ru-Mo Catalyst for the Detection of L-Histidine

Omruye OZOK ARICI[a]* , Hilal KIVRAK[b], Aykut CAGLAR[c], Arif KIVRAK[d]

[a] *Department of Biomedical Engineering, Faculty of Engineering and Architectural Sciences, Eskisehir Osmangazi University, Eskisehir, Turkey Orcid no: 0000-0002-4164-8650*
[b] *Department of Chemical Engineering, Faculty of Engineering and Architectural Sciences, Eskisehir Osmangazi University, Eskisehir, Turkey, Orcid no: 0000-0001-8001-7854*
[c] *Department of Biotechnology, Faculty of Science, Bartın University, Bartın, Turkey, Orcid no: 0000-0002-0681-1096*
[d] *Department of Chemistry, Faculty of Sciences and Arts, Eskisehir Osmangazi University, Eskişehir, Turkey, Orcid no: 0000-0003-4770-2686*

ABSTRACT

In this study, a voltammetric L-Histidine (His) sensor is being developed with glassy carbon electrode (GCE) modified with carbon nanotube (CNT) supported %3 Ru-Mo(80-20) bimetallic catalyst. The Ru-Mo/CNT catalyst was prepared by sodium borohydride reduction method. It was identified by surface analytical methods like scanning electron microscopy (SEM) and X-ray diffraction (XRD). Characterization results reveal that this catalyst was successfully synthesized. The modified GCE's electrochemical activity was investigated using cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS). The Ru-Mo/CNT-modified GCE electrode has a sensitivity of 0.0002 mA/cm^2, a limit of detection (LOD) of 0.02 µM for histidine, and a lower limit of detection (LOQ) of 0.06 µM, according to electrochemical data. The findings demonstrate that the Ru-Mo/CNT-modified GCE electrode is a promising catalyst for the sensitive detection of L-histidine and has been manufactured for the first time in the literature.

Keywords: *Amino acid, , Carbon nanotube, Electrochemical sensor, Histidine*

L-Histidinin tespiti için CNT Destekli Bimetalik Ru-Mo Katalizörü

Ömrüye ÖZOK ARICI[a]* , Hilal KIVRAK[b], Aykut ÇAĞLAR[c], Arif KIVRAK[d]

[a] *Biyomedikal Mühendisliği Bölümü, Mühendislik- Mimarlık Fakültesi, Osmangazi Üniversitesi, Eskişehir, Türkiye, ORCID: 0000-0002-4164-8650*
[b] *Kimya Mühendisliği Bölümü, Mühendislik- Mimarlık Fakültesi, Osmangazi Üniversitesi, Eskişehir, Türkiye ORCID: 0000-0000-0001-8001-7854*
[c] *Biyoteknoloji Bölümü, Fen Fakültesi, Bartın Üniversitesi, Bartın, Türkiye, ORCID: 0000-0002-0681-1096*
[d] *Kimya Bölümü, Fen Fakültesi, Osmangazi Üniversitesi, Eskişehir, Türkiye, ORCID: 0000-0003-4770-2686*

ÖZET

Bu çalışmada, karbon nanotüp (CNT) destekli Ru-Mo bimetalik katalizörle modifiye edilmiş camsı karbon elektrot (GCE) ile bir voltametrik L-Histidin (His) sensörü geliştirilmektedir. Ru-Mo/CNT, katalizörü, sodyum borohidrit indirgeme yöntemiyle hazırlandı. X-ışını kırınımı (XRD) ve taramalı elektron mikroskobu (SEM) gibi yüzey analitik teknikleri ile karakterize edildi. Karakterizasyon sonuçları bu katalizörün başarıyla sentezlendiğini ortaya koymaktadır. Modifiye edilmiş GCE'nin elektrokimyasal davranışını incelemek için elektrokimyasal empedans spektroskopisi (EIS), diferansiyel darbe voltametrisi (DPV) ve döngüsel voltametri (CV) kullanılmıştır. Elektrokimyasal sonuçlar, Ru-Mo/CNT ile modifiye edilmiş GCE elektrotunun 0.0002 mA/cm^2 duyarlılığına, histidin için tespit limiti (LOD) 0.02 µM ve alt tayin sınırına (LOQ) 0.06 µM'ye sahip olduğunu göstermektedir. Sonuç olarak, sonuçlar Ru-Mo/CNT ile modifiye edilmiş GCE elektrotunun literatürde ilk kez sentezlendiğini ve L-histidinin hassas tespiti için umut verici bir katalizör olduğunu göstermektedir.

Anahtar Kelimeler: *Amino asit, Karbon Nanotüp, Elektrokimyasal sensör, Histidin.*

**Ömrüue Özok Arıcı*
E-mail: o.ozok@hotmail.com

Received 29 August 2024
Accepted 14 October 2024

1. Introduction

Amino acids constitute the basic components that run protein metabolism in the body and are responsible for maintaining acid-base and fluid balances in the body. As amino acids are involved in important physiological processes, identifying minute quantities of amino acids is crucial for food analysis, disease detection, and biomedical research [1]. Among these, histidine (His), a vital amino acid, is necessary for the development of tissue and the transfer of metal elements [2].

L-Histidine (L-His,His) is an essential amino acid contains an imidazole side chain play significant roles in numerous biological systems. It has two different forms: L-Histidine and D-Histidine. The L-Histidine isomer is found in nature. Abnormal amount of histidine in the body can cause lung and chronic kidney diseases, stress and psychological disorders [3]. Detection of L-His is important for the identification and treatment of L-His deficiency.

Nowadays, new methods have been developed in the diagnosis and treatment of diseases. The most used methods to detect and characterize amino acids are based on spectroscopic [4], chromatographic [5] or electrochemical approaches [6]. Of these, electrochemical technologies have several noteworthy advantages, such as the capacity to regulate the rate of reaction, the capability to precisely measure the oxidation or reduction phase of an element, the cost-effectiveness of the employed devices, and their ecological sustainability [7].

Many electrochemical sensor studies have been conducted in the literature, such as amino acid [8], hydrogen peroxide [9], glucose [10], and cancer biomarkers [11-12]. These include the mechanisms by which certain catalysts, including organic compounds, metal nanoparticles, carbon nanotubes, nanocomposites, and nanopolymers, interact with electrodes to enhance the electrochemical response to amino acids, hence facilitating the detection of amino acids in the context of electrochemical sensor research. Nanomaterials and their amino acids, linear ranges, limit of detection (LOD) and limit of quantification (LOQ) used in electrochemical sensor studies in the literature are shown in Table 1. Looking at the studies in the literature, Heidari et al., in a study they conducted in 2019, examined CuFe2O4/rGO-Au nanomaterial against L-cysteine and found LOD/LOQ values for the sensors range from 0.383µM and 1.14 µM [13]. In another study, the sensor effect of Ta2O5/RGO/GCE nano material against L-lysine was examined and LOD/LOQ values of 0.01/0.03 µM were found [14].

For this reason, not many electrochemical sensor studies have been done on histidine before. To obtain a sensitive electrochemical histidine sensor, the LOD of the histidine sensor needs to be improved. Additionally, new electrode materials need to be created to commercialize the histidine sensor. CNT-supported Ru-Mo catalyst may be promising nanocatalyst as histidine sensor due to the unique properties of CNT.

In this study, CNT-supported Ru-Mo catalysts were first synthesized by the (NaBH4) reduction method. To characterize the prepared bimetallic catalysts, they were analyzed by advanced surface analytical technique such as (SEM). Ru-Mo/CNT catalyst were used to modify GCE in order to conduct electrochemical tests. CV and EIS were used to assess the changed GCE's electrochemical behavior. In the last step, sensitivity and selectivity measurements were made on GCE electrodes modified with Ru-Mo/CNT, and LOD and LOQ values were calculated as a result.

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

Aminoacid	**Electrode**	**Linear range**	**LOD/LOQ**	**Ref**
L-Cysteine	$CuFe_2O_4$/rGO-Au	0.05–0.2 µM	0.383/1.14 µM	[13]
Trp	Ta_2O_5/RGO/GCE	1.0–8.0 µM 8.0–80 µM 80–800 µM	0.84/2.52 µM	[14]
L-Lys	LOxNPs/GrONPs/PGE	0.01–1000 µM	0.01/0.03 µM	[15]
L-Serine	Ni−NiO HNT/BDDE	0.2–6.54 µM	0.1/0.3 µM	[16]
L-Proline	P-Cys/GQDs/GNPs/GCE	0.0005–10000 µM	0.0001/0.0003 µM	[17]
D-Aln	Fe3O4 @Au@Ag@CuxO NPs	0.0001–10 µM	0.000052/0.0015 µM	[18]
L-Histidine	Ru-Mo/CNT	0.0002 mA/cm^2	0.02/0.06 µM	**This study**

2. Material and Method

2.1.The synthesis of Ru-Mo/CNT catalyst

For the catalyst synthesis, the sodium borohydride ($NaBH_4$) reduction method was used in accordance with the procedure in the literature [19]. Distilled water, Ru (80%) precursor ($RuCl_3.3H_2O$) and Mo (20%) precursor $Mo_2(CH_3COO)_4$ were first added to a beaker and mixed homogeneously. It was mixed in an ultrasonic bath until it dispersed. CNT was added as a support material on metals dispersed homogeneously in distilled water. This prepared mixture was mixed in an ultrasonic bath and magnetic stirrer for approximately 2 hours. Finally, sodium borohydride (30 eq.) 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.

2.2. Electrochemical studies

Electrochemical studies were performed on Ru-Mo/CNT, modified GCE electrode by CV, EIS, and DPV by employing CHI 660 E potentiostat in a three-electrode system. Glassy carbon (GCE), Pt wire, and Ag/AgCl were used as the working, the counter, and reference electrode, respectively. By dispersing 5 mg nanocatalyst in 1 mL of Nafion, a nanocatalyst ink was obtained and 3 µl of nanocatalyst ink was transferred on GCE electrode and dried. As a result, Ru-Mo/ CNT modified GCE electrode were obtained.

3.Results

3.1. Physical characterization

SEM and XRD analyzes were performed to examine the surface structure and crystal structure of the 3% Ru-Mo(80-20)/CNT catalyst. Figure 1 shows the formation of CNT structures and metal nanoparticles from SEM images. Except for a few points, metals are distributed homogeneously on the CNT surface. In XRD analysis, the peak occurring at approximately $2\theta = 25.5^\circ$ corresponds to the hexagonal structure of carbon [20]. Additionally, the diffraction peaks indicating the formation of face-centered cubic (fcc)

Ru(0) crystals are observed at $2\theta = 43.1^\circ$, 52.7°, 67.0° and 78.5°, respectively, as Ru (1 0 1), Ru (1 0 2), Ru (1). 1 0) and Ru (1 0 3) planes were formed [21]. The reason why the presence of Mo cannot be observed in the XRD analysis may be due to the Ru ratio being approximately 4 times that of Mo and the high intensity of the C peak. However, in the literature study, as a result of the XRD analysis of the Ru/CNT catalyst, the Ru (1 0 1) plane occurred at approximately 44º [22]. For the 3% Ru-Mo(80-20)/CNT catalyst, a negative shift of approximately 0.9º of the Ru (1 0 1) (43.1º) plane indicates that an interaction occurs between Ru and Mo.

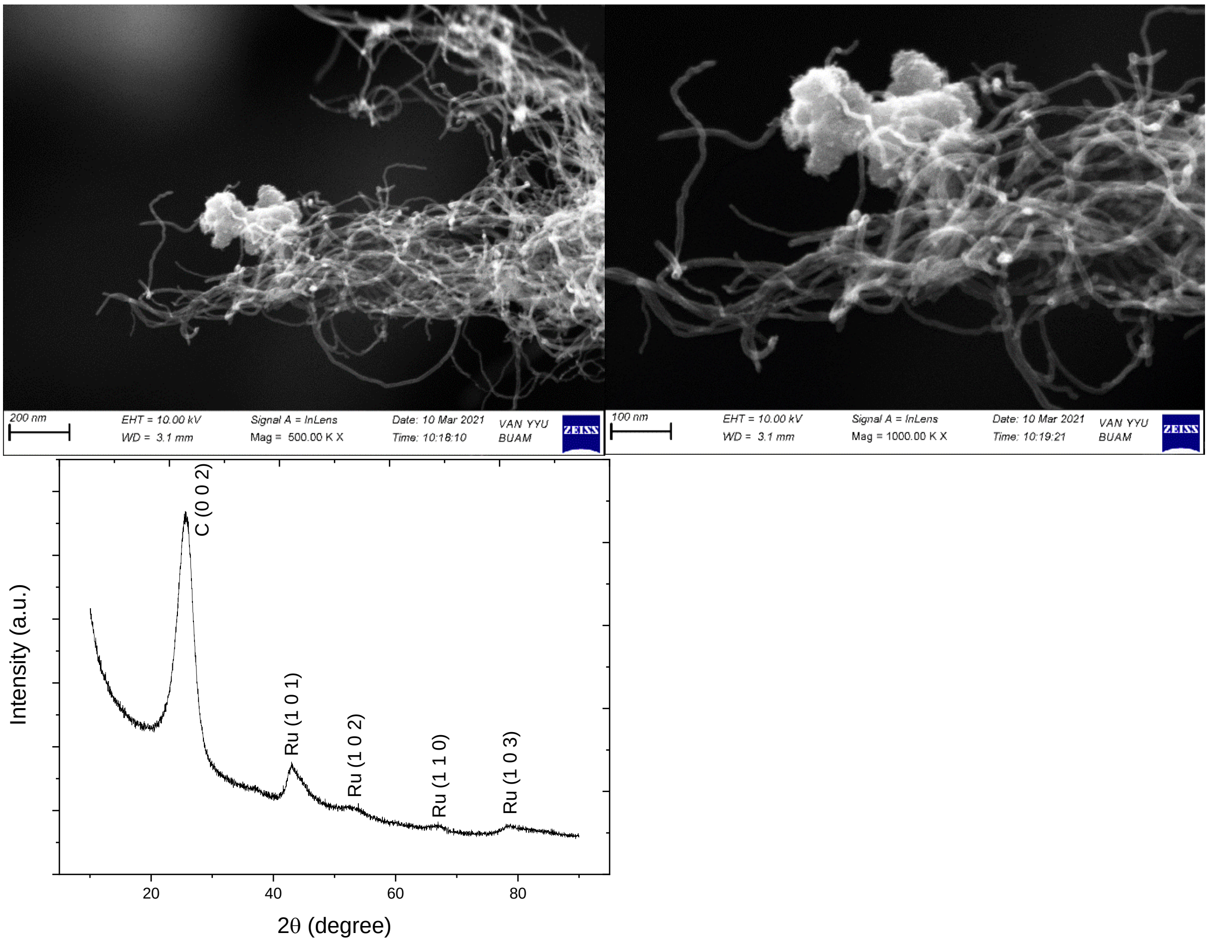

Figure 1. SEM images and XRD analysis of Ru-Mo/CNT catalyst.

3.2. Electrochemical measurements

The modified Ru-Mo/CNT catalyst system was used to modify a GCE for L-His measurement. The electrooxidation performances of histidine amino acid were examined at three different pH levels on a modified GCE electrode in 0.1 M PBS (Phosphate Buffer Saline). It was observed that the best current for L-His amino acid was better at pH: 7.2 (Figure 2).

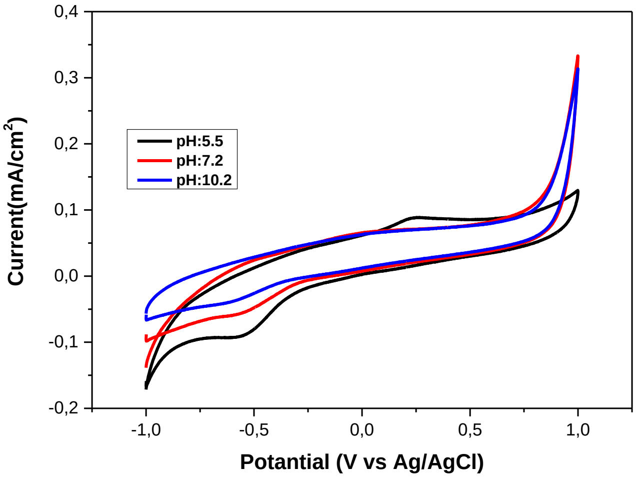

Figure 2. Cyclic voltammograms recorded at 100 mV/s on the Ru-Mo/CNT modified GCE electrode in 0.1 M PBS + 5 mM L-His solution at three distinct pHs (pH = 5.5, pH = 7.2, and pH = 10.5).

The impact of L-His concentration on electrooxidation activity was examined using Ru-Mo/CNT modified GCE electrode. The current density of histidine electrooxidation was determined by testing five different concentrations, as shown in Figure 3. The results demonstrated that the optimal current was obtained at a concentration of 5 mM for the electrooxidation of L-His amino acid.

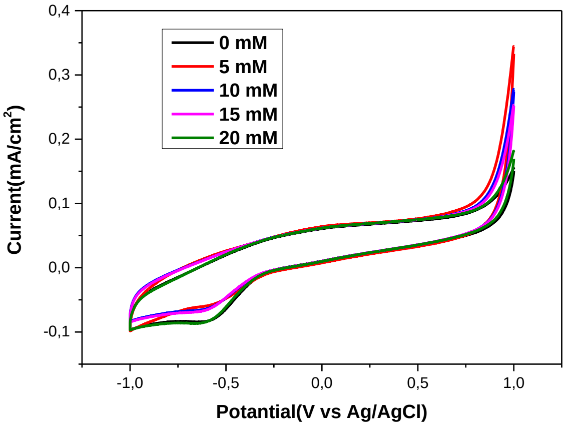

Figure 3. Cyclic Voltammograms obtained at various L-His concentration (0-5-10-15-20 mM) in 0.1 M PBS (pH=7.2) solution at 100 mV/s scan rate on Ru-Mo/CNT modified GCE electrode

L-His employing CV to modify a GCE electrode with Ru-Mo/CNT. The data in Figure 4 demonstrate that there is a direct correlation between the scan rate and the increase in the L-His electrochemical oxidation current. According to these findings, diffusion-controlled formation is to blame. The researchers measured the electroactive surface area of the modified electrode using CV at different scan rates ranging from (10-500 mV/s) in 0.1 M PBS in order to assess its efficacy (Figure 4a). The concentration of the solution, effective surface area, square root of the scan rate, phosphate diffusion coefficient, and electron count were all determined using the Randles-Sevcik equation. Assuming this, the data demonstrated the electrode's effective surface area (R2 = 0.97) (Fig. 4b).

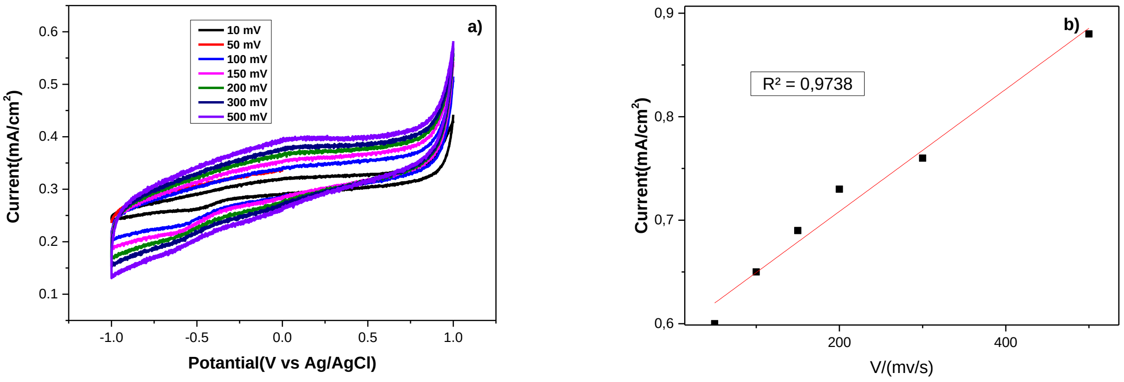

Figure 4. a) Ru-Mo/CNT modified GCE electrode cyclic voltage clamps in 0.1 M PBS (pH=7.2) + 5 mM L-His solution at varying scan rates (10-500, mV/s), b) linear regression of peak currents vs the square root of scan rates.

A study was carried out to assess the sensitivity of an L-His detection approach employing DPV. The results presented in Figure 5 showed that when a GCE electrode was replaced with Ru-Mo/CNT, 0.1 M PBS, and L-His concentrations ranging from 0 to 750 µM, the response increased as the L-His concentration increased, even at low concentrations. The maximum current density values obtained from the concentration curve were plotted and presented in Figure 5b. A linear response was observed in the range of 0.0002 mA/cm^2 current sensitivity, 0.02 µM LOD, and 0.06 µM LOQ signal-50-400 µM for the Ru-Mo/CNT-based L-His sensor. These findings demonstrate that the approach is sensitive to L-His and can pick it up at low concentrations.

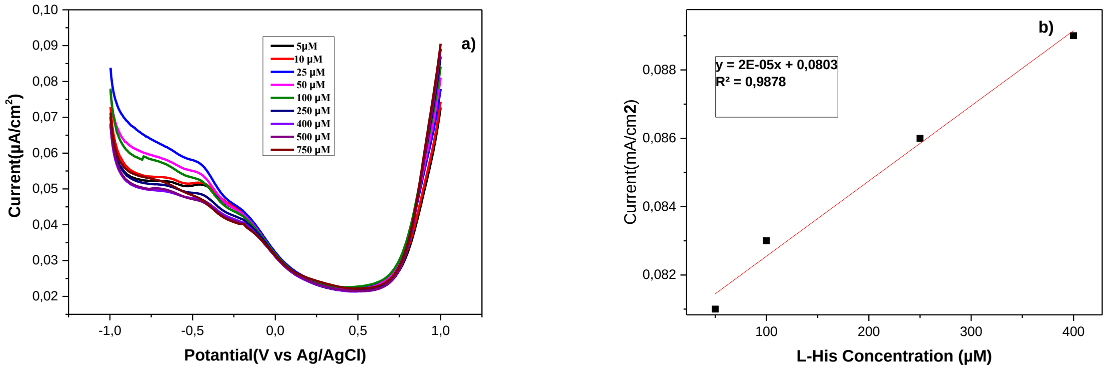

Figure 5. a) DPV measurements on the modified Ru-Mo/CNT GCE electrode in the presence of (5 – 750 µM) L-His solution in 0.1 M PBS (pH=7.2), b) Linear regression of maximum current versus L-His concentration.

The EIS method was used to determine the electrode capacitance and charge transfer resistance on the electrode. As seen in Figure 6, the current of electrooxidation of Ru-Mo/CNT-modified GCE and L-His in the solution prepared in 0.1 M PBS and 5 mM L-His shows that it has the best charge transfer resistance at -0.4V.

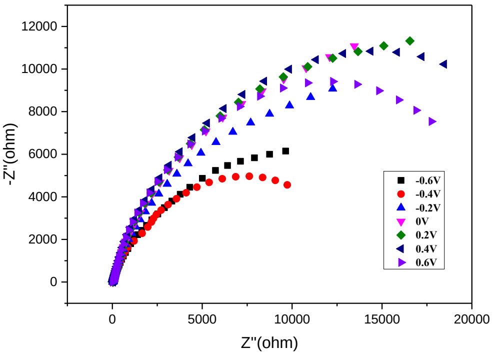

Figure 6. Ru-Mo/CNT modified GCE electrode EIS and equivalent circuit model at various potentials at 0.1 M PBS (pH 7.2) + 5 mM L-His

4. Conclusion

At present, Ru-Mo/CNT catalyst has been prepared by NaBH4 reduction method. The characterization of this bimetallic Ru-Mo/CNT catalyst and its application as L-His sensor have been carried out. The characterization results revealed that Ru-Mo/CNT modified GCE electrode was prepared with desired Ru and Mo loading. The results obtained from XRD and SEM and NaBH4 reduction method are shown to be an effective method for the preparation of L-His sensor material. Ru-Mo/CNT modified GCE electrode showed good electrochemical responses towards L-His with high sensitivity, stability and selectivity. This work is novel and will contribute to the literature for constructing an L-His sensor.

Acknowledgment

Omrüye Ozok Arici writing—original draft preparation, review and editing, Aykut Caglar, formal analysis, Hilal Kivrak, visualization, Arif Kivrak supervision. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] S. Shahrokhian, (2001). Lead phthalocyanine as a selective carrier for preparation of a cysteine-selective electrode. Analytical Chemistry, 73(24), 5972-5978.
[2] C.G. Nan, W.X. Ping, D.J. Ping, C.H. Qing, (1999). A study on electrochemistry of histidine and its metabolites based on the diazo coupling reaction. Talanta, 49(2), 319-330.
[3] A. Torrado, G.K. Walkup, B. Imperiali, (1998). Exploiting polypeptide motifs for the design of selective Cu (II) ion chemosensors. Journal of the American Chemical Society, 120(3), 609-610.

[4] C.S. Lee, P.F. Teng, W.L. Wong, H.L. Kwong, A.S. Chan, (2005). New C2-symmetric 2, 2′-bipyridine crown macrocycles for enantioselective recognition of amino acid derivatives. Tetrahedron, 61(33), 7924-7930.

[5] J. Wang, M.P. Chatrathi, B. Tian, (2000). Micromachined separation chips with a precolumn reactor and end-column electrochemical detector. Analytical chemistry, 72(23), 5774-5778.

[6] D. Vardanega, C. Girardet, (2009). Nonlinear polarization effects in superchiral nanotube sensors of amino acids. Chemical Physics Letters, 469(1-3), 172-176.

[7] S. Ouchemoukh, N. Amessis-Ouchemoukh, M. Gómez-Romero, F. Aboud, A. Giuseppe, A. Fernández-Gutiérrez, A. Segura-Carretero, (2017). Characterisation of phenolic compounds in Algerian honeys by RP-HPLC coupled to electrospray time-of-flight mass spectrometry. LWT-Food Science and Technology, 85, 460-469.

[8] V. M. Gumerov, E. P. Andrianova, M. A. Matilla, K. M. Page, E. Monteagudo-Cascales, A. C. Dolphin, I. B. Zhulin, (2022). Amino acid sensor conserved from bacteria to humans. Proceedings of the National Academy of Sciences, 119(10), e2110415119.

[9] H. Çelik Kazıcı, A. Caglar, T. Aydogmus, N. Aktas, H. Kivrak, (2018). Microstructured prealloyed Titanium-Nickel powder as a novel nonenzymatic hydrogen peroxide sensor, Journal of Colloid and Interface Science, 530 353-360.

[10] K. Tian, S. Alex, G. Siegel, A. Tiwari, (2015). Enzymatic glucose sensor based on Au nanoparticle and plant-like ZnO film modified electrode. Materials Science and Engineering: C, 46, 548-552.

[11] H. Kivrak, O. F. Er, O. Ozok, S. Celik, A. Kivrak, (2022). Synthesis and characterization of 4-(2-(4-methoxyphenyl) benzo [b] thiophen-3-yl) benzaldehyde for carbohydrate antigen 125 electrochemical detection and molecular docking modeling. Materials Chemistry and Physics, 281, 125951.

[12] A.R. Ferhan, J.A. Jackman, J.H. Park, N.J. Cho, D.H. Kim, (2018). Nanoplasmonic sensors for detecting circulating cancer biomarkers. Advanced drug delivery reviews, 125, 48-77.

[13] M. Heidari, A. Ghaffarinejad, (2019). Electrochemical sensor for L-cysteine by using a cobalt (II)/aluminum (III) layered double hydroxide as a nanocatalyst. Microchimica Acta, 186, 1-7.

[14] S. Zhou, Z. Deng, Z. Wu, M. Xie, Y. Tian, Y. Wu, J. Liu, G. Li, Q. He, (2019). Ta2O5/rGO nanocomposite modified electrodes for detection of tryptophan through electrochemical route. Nanomaterials, 9(6), 811.

[15] B. Nohwal, R. Chaudhary, C.S. Pundir, (2020). Amperometric l-lysine determination biosensor amplified with l-lysine oxidase nanoparticles and graphene oxide nanoparticles. Process Biochemistry, 97, 57-63.

[16] W. Dai, H. Li, M. Li, C. Li, X. Wu, B. Yang, (2015). Electrochemical imprinted polycrystalline nickel–nickel oxide half-nanotube-modified boron-doped diamond electrode for the detection of l-serine. ACS applied materials & interfaces, 7(41), 22858-22867.

[17] M. Hasanzadeh, H.N. Baghban, N. Shadjou, (2018). Non-enzymatic determination of L-Proline amino acid in unprocessed human plasma sample using hybrid of graphene quantum dots decorated with gold nanoparticles and poly cysteine: a novel signal amplification strategy. Analytical Sciences, 34(3), 355-362.

[18] H. Liu, J. Shao, L. Shi, W. Ke, F. Zheng, Y. Zhao, (2020). Electroactive NPs and D-amino acids oxidase engineered electrochemical chiral sensor for D-alanine detection. Sensors and Actuators B: Chemical, 304, 127333.

[19] B. Ulas, A. Caglar, S. Yılmaz, U. Ecer, Y. Yilmaz, T. Sahan, H. Kivrak, (2019). Towards more active and stable PdAgCr electrocatalysts for formic acid electrooxidation: the role of optimization via response surface methodology. International Journal of Energy Research, 43(15), 8985-9000.

[20] M.J.A. Abbas, A. Caglar, H. Kivrak, (2024). The synthesis and characterization of carbon nanotube-supported CuBi catalyst for H2 production from NaBH4 methanolysis. Process Safety and Environmental Protection, 185, 96-104.

[21] A. Biswas, S. Paul, A. Banerjee, (2015). Carbon nanodots, Ru nanodots and hybrid nanodots: preparation and catalytic properties. Journal of Materials Chemistry A, 3(29), 15074-15081.
[22] G. Zhang, T. Chen, Y. Zhang, T. Liu, G. Wang, (2020). Effective conversion of cellulose to sorbitol catalyzed by mesoporous carbon supported ruthenium combined with zirconium phosphate. Catalysis Letters, 150, 2294-2303.