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INVESTIGATION OF PEROXIDASE-MIMICKING PROPERTIES OF FeNiCo METAL-ORGANIC FRAMEWORK DECORATED MWCNT COMPOSITE NANOMATERIAL

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Abstract

Enzyme-mimicking materials replicate natural enzyme functions, offering more durable, cost-effective, and eco-friendly alternatives, making them important for biotechnology and industrial applications. In this study, the peroxidase like properties of a FeNiCo metal-organic framework-decorated multi-walled carbon nanotube (FeNiCo MOF-MWCNT) composite nanomaterial were investigated for the first time. The nanocomposite was synthesized and characterized using Scanning Electron Microscopy (SEM), SEM-Mapping, SEM-EDS, XRD, and FT-IR techniques. Its enzyme-mimicking activity was evaluated using a colorimetric, non-enzymatic H_2O_2 biosensor with 3,3',5,5'-Tetramethylbenzidine (TMB) as the substrate. The biosensor comprises optimized amounts of FeNiCo MOF-MWCNT and TMB. Upon H_2O_2 addition, the nanocomposite mimics peroxidase activity by reducing H_2O_2 to H_2O, while oxidizing TMB to its blue-colored form, TMB(ox), showing absorbance at 654 nm. After optimizing conditions, the biosensor exhibited a linear response to H_2O_2 concentrations ranging from 0.1 mM to 12 mM. It also demonstrated high selectivity, with negligible interference from substances like KCl, ascorbic acid, urea, and NaCl. Accuracy tests using commercial H_2O_2 disinfectant showed results between 100% and 103%, confirming the biosensor's reliability for practical applications.

Keywords: Metal organic frameworks, enzyme mimicking, non-enzymatic H_2O_2 biosensor, colorimetric biosensor

FeNiCo METAL-ORGANİK ÇERÇEVE İLE MODİFİYE EDİLMİŞ MWCNT KOMPOZİT NANOMALZEMENİN PEROKSİDAZ ENZİM TAKLİT ÖZELLİKLERİNİN İNCELENMESİ

Özet

Enzim taklit eden malzemeler, doğal enzimlerin işlevlerini taklit ederek daha dayanıklı, ekonomik ve çevre dostu alternatifler sunar; bu da biyoteknoloji ve endüstriyel uygulamalar için oldukça önemlidir. Bu çalışmada, FeNiCo metal-organik çerçeve modifiye çok duvarlı karbon nanotüp (FeNiCo MOF-MWCNT) kompozit nanomalzemenin, peroksidaz enzim benzeri özellikleri ilk kez incelenmiştir. Nanokompozit, Taramalı Elektron Mikroskobu (SEM), SEM Haritalama, SEM-EDS, X-Işını Difraksiyonu (XRD) ve Fourier Dönüşümlü Kızılötesi Spektroskopisi (FT-IR) teknikleriyle karakterize edilmiştir. FeNiCo MOF-MWCNT'nin enzim taklit özelliği, substrat olarak 3,3',5,5'-Tetrametilbenzidin (TMB) kullanılan enzim içermeyen kolorimetrik bir H_2O_2 biyosensörü ile incelenmiştir. Biyosensör çözeltisi, optimize edilmiş miktarda FeNiCo MOF-MWCNT ve TMB içermektedir. Çözeltiye H_2O_2 eklendiğinde, nanokompozit H_2O_2'yi suya indirgerken peroksidaz enzim aktivitesini taklit eder ve aynı anda TMB'yi oksitler, mavi renkli TMB(ox) formuna dönüşür; bu form 654 nm'de absorbsiyon gösterir. Deneysel koşullar optimize edildikten sonra biyosensör, 0.1 mM ile 12 mM arasındaki H_2O_2 derişimlerinde doğrusal yanıt vermiştir. Geliştirilen enzimsiz biyosensör üzerinde, KCl, askorbik asit, üre ve NaCl gibi girişimci maddelerden kaynaklı anlamlı bir etki gözlemlenmemiştir. Ticari H_2O_2 dezenfektanlarıyla yapılan doğruluk testlerinde sonuçlar %100–103 arasında bulunmuştur.

Anahtar Kelimeler: Metal organik çerçeveler, enzim taklit, enzimsiz H_2O_2 biyosensörü, kolorimetrik biyosensör

Cite

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1. Introduction

Enzymes are natural protein-based catalysts that are frequently used in the field of biotechnology. However, due to major reasons such as difficult storage conditions and high production costs, the use of alternative synthetic enzymes has become quite popular recently [1,2]. At this point, nanomaterial-based enzyme mimicking materials which are called as "Nanoenzymes" have become particularly promising [2,3]. Noble metal nanoparticles, metal oxides, and especially carbon-based nanomaterials are structures that have proven enzyme mimicking capabilities [2,4]. Although nanoenzymes are highly advantageous compared to natural enzymes due to their simple preparation procedures, low cost, good thermal stability, and adjustable catalytic activities, they generally have lower catalytic activity than natural enzymes [2,5]. Additionally, Metal-Organic Frameworks (MOFs), formed by the combination of metal atoms and organic ligands with high porosity, surface area, tunable pore sizes, ease in functionalization, better biodegradability, and biocompatibility, have been attracting attention in recent years due to their enzyme mimicking properties [6–8]. Thanks to these unique properties, MOF structures are widely used in areas such as energy storage, drug delivery, separation, catalytic applications, purification procedures, sensing systems, gas storage applications, and imaging processes [8–12]. MOF structures containing metal atoms such as Cu, Ce, Zr, Fe, Ni, and Co are widely used types of MOFs with enzyme mimicking properties [8,13–17]. MOF structures, due to the metal atoms in their frameworks, can catalyze chemical reactions like an enzyme, especially oxidation reactions. For example, in a study by Kıyıkçı et al., it was stated that the FeCo bimetallic enzyme mimics both oxidase and peroxidase enzymes depending on the changes in the pH of the environment [8]. Compared to commonly studied nanozymes such as Fe_3O_4, CeO_2 nanoparticles, noble metal nanoparticles, and carbon-based materials like graphene oxide, multimetallic MOF composite structures like as the FeNiCo MOF-MWCNT composite offers several distinct advantages [18–20]. The multi-metallic MOF structure provides synergistic catalytic effects that enhance peroxidase-like activity beyond that of single-metal nanozymes. Additionally, the integration of MWCNTs improves electrical conductivity and structural stability, facilitating more efficient electron transfer and preventing catalyst aggregation. This combination results in higher catalytic efficiency, better stability under various reaction conditions, and improved recyclability. However, the synthesis of FeNiCo MOF-MWCNT can be more complex and costly compared to simpler nanozymes, and optimization of synthesis parameters is required to avoid particle aggregation and ensure uniform dispersion. Despite these challenges, the unique structural and functional advantages of FeNiCo MOF-MWCNT nanozymes make them a superior choice across a wide range of applications, offering enhanced catalytic performance and durability that other nanozymes may not consistently provide [18–20] .

In this study, a composite material has been synthesized by combining a nanomaterial with a trimetallic MOF structure, which exhibits high peroxidase-like activity. The synthesized FeNiCo MOF-MWCNT composite structure was structurally characterized using scanning electron microscopy (SEM), SEM-mapping, energy dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD), and fourier transform infrared spectroscopy (FTIR) techniques. After characterization, the peroxidase-like activity of the material was investigated using a H_2O_2 biosensor. Moreover, the analysis of H_2O_2 plays an important role in various fields, including biological, industrial, environmental, and clinical areas. The developed H_2O_2 non-enzymatic biosensor is a colorimetric biosensor, which can be used for qualitative analysis with the naked eye and for quantitative analysis using a UV-Visible spectrophotometer. The responses of the colorimetric biosensor were monitored through the specific absorbance value of the oxidized form of 3,3',5,5'-Tetramethylbenzidine (TMB) color reagent at 654 nm. In this system, the FeNiCo MOF-MWCNT composite material reduces H_2O_2 to water, while TMB in the environment is oxidized to its TMB(oX) form. During this transformation, the color of the colorimetric biosensor solution changes from colorless to blue, and absorbance occurs at 654 nm.

2. Experimental

2.1. Materials and Method

$CoCl_2·6H_2O$, $Ni(NO_3)_2·6H_2O$, $Fe(NO_3)_3.9H_2O$, H_3BTC, TMB were obtained from Sigma-Aldrich, while DMF, hydrofluoric acid (HF), nitric acid and sulfuric acid were purchased from Merck. Moreover MWCNT powder was obtained from Nanografi. All solutions used were prepared with distilled water, unless otherwise stated. All chemicals used are of analytical grade.

All spectrophotometric measurements with the developed colorimetric non-enzymatic H_2O_2 biosensor were performed using the DLAB SP-UV1100 device in the visible range between 200-900 nm. All spectrophotometric measurements were performed using a glass square cuvette with a 10 mm light path.

2.2. Synthesis of FeNiCo MOF- MWCNT

The first step in the synthesis of the FeNiCo MWCNT composite material is the synthesis of the FeNiCo MOF structure [6,21]. For this purpose, 2.5 mM $CoCl_2·6H_2O$, 2.5 mM $Ni(NO_3)_2·6H_2O$, and 2.5 mM $Fe(NO_3)_3.9H_2O$, along with 4.95 mM H_3BTC and 35 mL of double distilled water, were added and mixed. Then, HF was added to the mixture to achieve a concentration of 3 mM, and the mixture was stirred for 60 minutes. A hydrothermal synthesis procedure was applied using a Teflon-lined stainless steel reactor at 160 °C for 72 hours. After natural cooling, the supernatant was separated via centrifuge and then heated at 80 °C for 10 hours with the addition of double distilled water. Subsequently, heating was carried out with anhydrous ethanol at 60 °C for 8 hours. Finally, the precipitate was dried overnight in a vacuum oven at 70 °C. After the drying process, the FeNiCo MOF structure is ready for composite fabrication experiments with MWCNT.

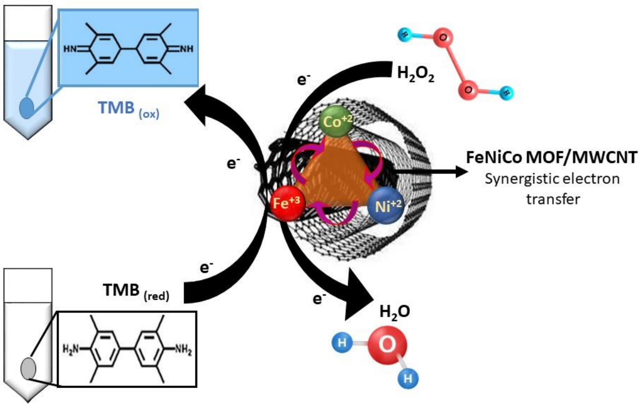
For the MWCNT structures to form a composite with the MOF structure, they need to undergo a pretreatment with acid [14]. For this purpose, the MWCNT was carboxylated by heating with concentrated nitric acid and sulfuric acid at 120 °C for 8 hours. Then, approximately 100 mg of MWCNT-COOH was dispersed in 40 mL of DMF by ultrasonication. Next, 1.2 mg of FeNiCo MOF was added to the MWCNT-COOH dispersion, and ultrasonication was continued for an additional hour. The mixture was transferred to a hydrothermal synthesis reactor and heated at 170 °C for 12 hours. The solid phase of the FeNiCo MOF-MWCNT mixture was separated via centrifugation, and the product was dried at 120 °C for 24 hours. The FeNiCo MOF-MWCNT composite suspension at a concentration of 0.001 g/mL was prepared using pH: 4 acetate buffer for use in non-enzymatic biosensor applications.

2.3. Preparation of colorimetric non-enzymatic H_2O_2 biosensor

The peroxidase enzyme mimetic activity of the synthesized FeNiCo MOF-MWCNT composite structure was elucidated using a colorimetric H_2O_2 biosensor. For this purpose, a biosensor was prepared by adding 100 µL of TMB, 100 µL of FeNiCo MOF-MWCNT composite structure, and 600 µL of acetate buffer into an Eppendorf tube. The responses of the developed biosensor were tested by adding 100 µL of H_2O_2 solution at specific concentrations into the Eppendorf tube.

2.4. Working principle of develeoped non-enzymatic H_2O_2 biosensor

The responses of the developed colorimetric non-enzymatic H_2O_2 biosensor were monitored using UV-Visible spectrophotometry. The TMB in the biosensor solution is colorless in its reduced form. When H_2O_2 is added to the solution, the FeNiCo MOF-MWCNT structure mimics peroxidase activity by reducing H_2O_2 to H_2O, while TMB simultaneously oxidizes and transforms into its oxidized form, TMB (ox) [22]. The oxidized form of TMB is blue in color and exhibits a specific absorbance at 654 nm. The peroxidase enzyme mimetic activity of the FeNiCo MOF-MWCNT structure, along with the responses of the developed non-enzymatic biosensor, were investigated based on the absorbance values obtained at 654 nm.

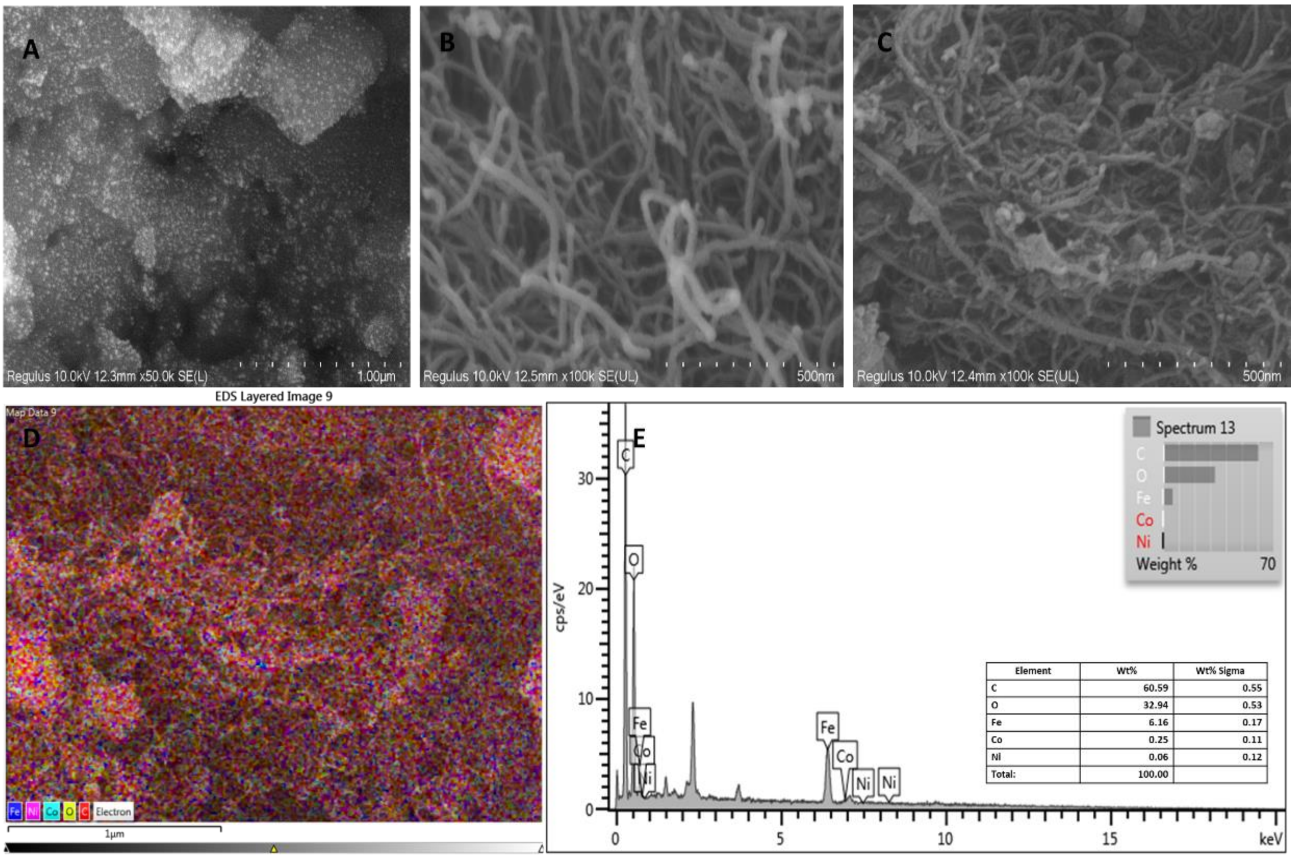


Scheme 1. Schematic Representation of the working principle of the developed non-enzymatic colorimetric H_2O_2 biosensor.

3. Results and Discussion

3.1. Structural characterization of the FeNiCo MOF-MWCNT

The structural characterization of the synthesized FeNiCo MOF-MWCNT composite structure was performed using SEM, EDS, EDS mapping, XRD, and FT-IR methods. Due to the magnetic properties of the sample, which caused issues during measurements at very high energy levels, all samples were gold-coated before the imaging was performed during the SEM measurements. In the SEM image of the FeNiCo MOF structure in Figure 1A, the white spherical/cubic structures are attributed to metal nanostructures. Irregular cubic formations are observed, resulting from the combination of the amorphous structure with irregular aggregation from Fe and the cubic structure from Co [8]. This morphology is highly characteristic of the MOF structure formed by Fe, Co, and Ni metals with the BTC organic ligand [15, 23]. Furthermore, while the SEM images of commercially purchased MWCNT are shown in Figure 1B, the images of the FeNiCo MOF-MWCNT composite structure are presented in Figure 1C. In the SEM imaging analysis of the MWCNT, long tubular structures positioned in a network-like pattern are clearly observed. On the other hand, as a result of the positioning of the FeNiCo MOF structure onto the MWCNT, disruptions in the clarity of the long tubular structures are observed, and white, dot-like structures are seen to aggregate and position themselves on the tubular structures. The white aggregated amorphous structures, which are occasionally spherical and sometimes cubic, on the tubular structures in Figure 1C, are characteristic of the general MOF-MWCNT structures [14]. Looking at the SEM mapping images in Figure 1D, it is possible to observe the morphologically characteristic tubular structure of the MWCNT and identify the C and O atoms within the MWCNT structure as red and yellow, respectively.



Element	Wt%	Wt% Sigma
C	60.59	0.55
O	32.94	0.53
Fe	6.16	0.17
Co	0.25	0.11
Ni	0.06	0.12
Total:	100.00	

Figure 1. SEM images of A. FeNiCo MOF, B. MWCNT and C. FeNiCo MOF-MWCNT composite material. D. SEM-Mapping image and E. EDS analysis result of FeNiCo MOF-MWCNT composite material.

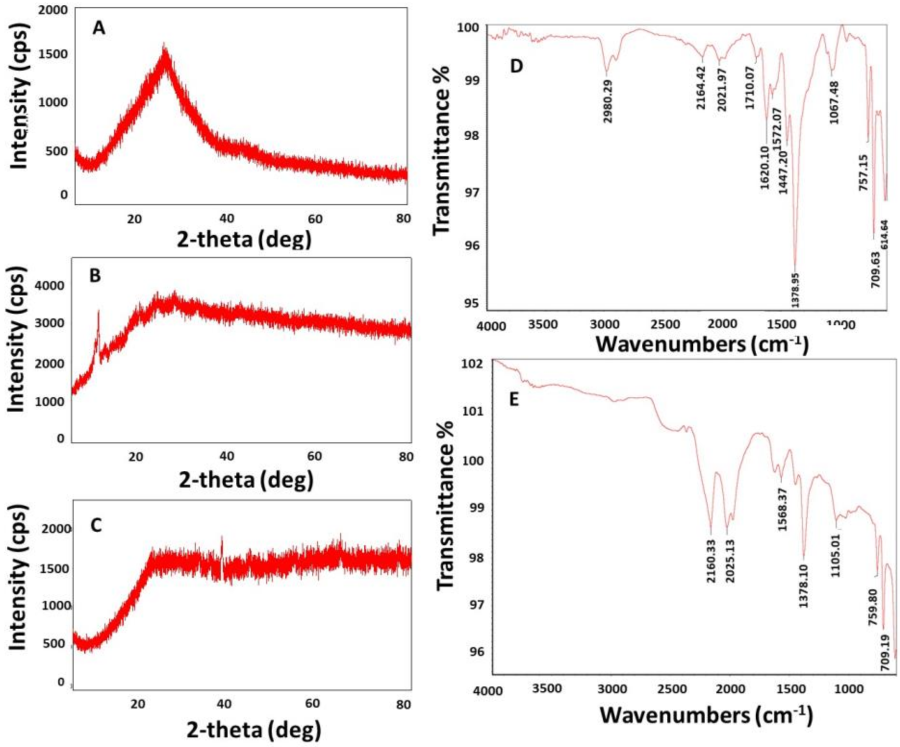


Figure 2. XRD patterns of A. MWCNT, B. FeNiCo MOF and C. FeNiCo MOF-MWCNT composite structure. FTIR spectra of D. FeNiCo MOF and E. FeNiCo MOF-MWCNT composite structure.

On the other hand, the Fe, Co, and Ni atoms from the FeNiCo MOF structure are shown in blue, green, and pink, respectively. From the mapping images, it can be concluded that the Fe atoms are more concentrated in the composite structure compared to Co and Ni. Moreover, the image of the region where the EDS measurement was performed for the FeNiCo MOF-MWCNT structure and the corresponding measurement graph of the EDS analysis are presented in Figure 1E. According to the EDS analysis results, the composition contains 60.59% C, 32.94% O, 6.16% Fe, 0.25% Co, and 0.06% Ni by mass. It is evident that the Fe element is positioned significantly more effectively in the composite structure compared to the other metal atoms.

The crystal structures of the synthesized material was elucidated through XRD analysis. The results of the XRD analysis, conducted in the 2θ range of 0-80°, for all the synthesized structures are presented in Figure 2. As seen in Figure 2A, the strong diffraction pattern observed at $2\theta = 27°$ corresponds to the graphitic-layered structure of MWCNT. On the other hand, in the XRD analysis results of the FeNiCo MOF structure shown in Figure 2B, the diffraction pattern observed at around 13° indicates the crystalline structure of the FeNiCo-BTC MOF, while the broad diffraction signal near at $2\theta = 23°$ suggests the presence of an amorphous carbon phase, indicating that the Fe, Ni, and Co metal centers are successfully coordinated within the MOF framework. Additionally, a broad diffraction signal observed in the 0-10° range of 2θ is evidence of Co atom and Ni atom presence, which is consistent with the data in the literature [14,15,23]. The XRD analysis graph obtained after the formation of the composite structure of FeNiCo MOF and MWCNT is presented in Figure 2C. After the formation of the FeNiCo MOF-MWCNT composite structure, the sharp pattern intensity associated with the Fe atom decreases, although it is still partially observed. This is due to the relatively high percentage of Fe atoms in the structure. The specific patterns of the other metal atoms are not observed, as they are suppressed by the specific peaks of the MWCNT structure [16].

The bond tensions and vibration energies of the synthesized structures were measured using FT-IR analysis, with % Transmittance values recorded in the wavenumber range of 0-4000 cm^{-1}. Figure 2D shows the FTIR analysis results of the FeNiCo MOF structure, while Figure 2E presents the FTIR analysis results of the FeNiCo MOF-MWCNT structure. The bands observed around 3000 cm^{-1} are attributed to the C-H bonds in the aromatic ring. Additionally, the peaks in the 1600-1700 cm^{-1} range and around 700 cm^{-1} are also associated with stretching vibrations of the aromatic structure. These aromatic peaks are attributed to the γ(C−H) and δ(C−H) vibrations in the BTC ligand. The sharp peak around 1770 cm^{-1} is due to the stretching vibrations of the −COOH functional group in the MOF structure. On the other hand, the peak in the 1100-1060 cm^{-1} range is attributed to C-O stretching vibrations. Furthermore, the three narrow bands observed at 614, 709, and 757 cm^{-1} are identified as characteristic bands for the binding of Fe to the BDC group and the Fe-O bond [15, 24]. The distinct characterization data for the Fe atom in all composite structures are due to the high percentage of Fe atoms in the structure. Upon examining the FTIR results in Figure 2E, after the composite formation of MWCNT with the FeNiCo MOF structure, carboxyl and phenolic C-O stretching vibrations from the MWCNT structure are observed around 1500 cm^{-1}, while characteristic C-C stretching bands are detected around 1100 cm^{-1} [16].

Based on a series of characterization experiments and a comprehensive literature review, a potential reaction mechanism occurring between the MWCNT and MOF structures during the formation of the FeNiCo-MWCNT composite can be proposed as follows: The formation of the FeNiCo MOF-MWCNT composite is primarily driven by a combination of electrostatic interactions, π–π stacking between aromatic ligands of the MOF and the graphitic surface of the MWCNTs, and coordination bonding through functional groups (–COOH) present on acid-treated MWCNTs. Since the MOF was pre-synthesized prior to combination, physical mixing and surface adsorption govern the composite formation, enabling efficient interfacial contact and uniform dispersion, which in turn enhance the catalytic performance of the material [16].

3.2. The characterization of the peroxidase mimetic behavior of FeNiCo MOF MWCNT composite

For this purpose, two colorimetric biosensor solutions were prepared by applying the procedure outlined in the "Preparation of Colorimetric H_2O_2 Biosensor" section: one in the presence of FeNiCo MOF MWCNT and one in the absence of FeNiCo MOF MWCNT.

Both colorimetric biosensor solutions were each added with 2 mM of 100 µl H_2O_2 solution, and after 30 minutes, spectrophotometric measurements were performed in the visible region. As can be seen from the spectrum in Figure 3, after the addition of H_2O_2, a high absorbance peak is obtained with the colorimetric biosensor solution containing FeNiCo MOF, while no absorbance peak is observed with the colorimetric biosensor solution without FeNiCo MOF. When H_2O_2 is added to the biosensor solution containing the FeNiCo MOF MWCNT composite structure, the FeNiCo MOF MWCNT structure mimics peroxidase by reducing H_2O_2 to H_2O, while the TMB(red) structure is oxidized to TMB(ox). As TMB transitions from its colorless reduced form to its blue-colored oxidized form, it exhibits a specific absorbance at 654 nm.

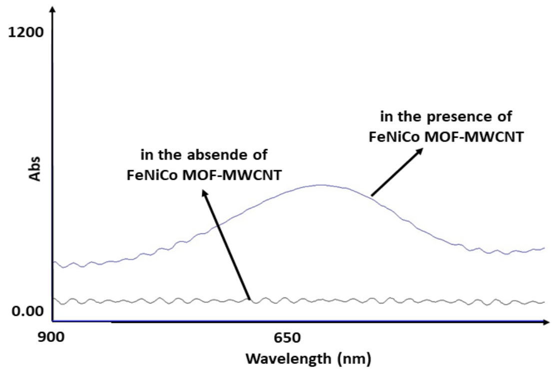


Figure 3. The UV-Vis spectrum for characterizing the mimicry property of the FeNiCo MWCNT composite material.

On the other hand, in the other colorimetric biosensor solution, when the FeNiCo MOF MWCNT composite structure, which mimics peroxidase, is not present, the mentioned sequential reactions could not occur, and no absorbance was detected at 654 nm. As a result, the peroxidase mimetic behavior of the FeNiCo MOF MWCNT composite structure has been characterized by observing the specific absorbance at 654 nm.

3.3. Proposed mechanism of peroxidase-mimicking activity via Fenton-like reaction

The FeNiCo MOF-MWCNT composite exhibits significant peroxidase-like catalytic activity, primarily attributed to the multivalent transition metal centers (Fe, Ni, Co) within the structure. This activity is mainly governed by a Fenton-like mechanism, in which the composite catalyzes the decomposition of H_2O_2 to generate reactive oxygen species (ROS), particularly hydroxyl radicals (•OH), which can oxidize various substrates.

The general Fenton-like reactions for each metal ion involved are as follows:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\cdot}OH + OH^{-} \quad (2)$$

$$Co^{2+} + H_2O_2 \rightarrow Co^{3+} + {}^{\cdot}OH + OH^{-} \quad (3)$$

$$Ni^{2+} + H_2O_2 \rightarrow Ni^{3+} + {}^{\cdot}OH + OH^{-} \quad (4)$$

$${}^{\cdot}OH + H_2O_2 \rightarrow HO_2{}^{\cdot} + H_2O \quad (5)$$

$$TMB_{(colorless)} + {}^{\cdot}OH \rightarrow (ox)TMB_{(\text{blue-colored})} \quad (6)$$

While these metal-mediated reactions lead to the formation of •OH or other ROS, which in turn oxidize chromogenic substrates such as TMB, H_2O_2 is decomposed into H_2O and O_2 in side reactions catalyzed by the metal centers.

This oxidation produces a blue-colored product (oxTMB), which can be monitored spectrophotometrically and serves as a measurable indicator of peroxidase-like activity. In this catalytic system, trimetalic MOF ensures high surface area and well-dispersed active sites, while the MWCNTs enhance electron transport and structural stability. The synergy between these components facilitates efficient ROS generation and substrate oxidation [25, 26].

3.4. Optimization of experimental conditions

The amount of enzyme-mimicking material, reaction temperature, and reaction time are critical factors that significantly impact the catalytic performance of nanozymes. Increasing the FeNiCo MOF/MWCNT loading enhances the availability of active sites, thereby improving peroxidase-like activity up to an optimal threshold; however, excessive amounts may lead to aggregation or hinder substrate diffusion, resulting in decreased efficiency. Reaction temperature influences molecular kinetic energy and catalytic reaction rates. Generally, an increase in temperature accelerates the reaction, but temperatures beyond the optimum can compromise catalyst stability and cause deactivation. Similarly, reaction time governs the extent of substrate conversion; insufficient reaction time leads to incomplete conversion, whereas excessively long durations may cause catalyst degradation or unwanted side reactions. Therefore, careful optimization of these parameters is essential to maximize enzyme-mimicking activity by balancing substrate interaction, catalyst stability, and overall reaction kinetics [13, 27,28] .

3.4.1. Optimization of FeNiCo MOF MWCNT composite amount

Since the FeNiCo MOF MWCNT composite structure in the developed system exhibits peroxidase mimetic properties, the amount in the colorimetric biosensor should be optimized. For this purpose, 25 µL, 50 µL, 100 µL, 200 µL, and 300 µL of the FeNiCo MOF MWCNT solution prepared at a concentration of 0.001 g/mL (in pH:4 acetate buffer) were taken to prepare five different colorimetric non-enzymatic H_2O_2 biosensors, and their responses to 2 mM of 100 µL H_2O_2 solution were investigated. The spectra obtained from the spectrophotometric measurements are presented in Figure 4A. The highest absorbance value at 654 nm was obtained with the colorimetric biosensor containing 200 mL of FeNiCo MOF MWCNT composite structure, and therefore, the optimum FeNiCo MOF MWCNT amount was selected as 200 µL (Figure 4B). As the amount of the FeNiCo MOF MWCNT composite structure increases, the amount of enzyme-mimicking material also increases, resulting in a higher absorbance value at 654 nm due to the formation of TMB(ox). However, since the absorbance value obtained with the biosensor mixture containing more than 200 µL of composite material did not significantly increase, using more material has lost its significance.

3.4.2. Optimization of biosensor response temperature

Since an enzyme-mimicking material is used in the system and temperature is known to have a significant effect on the electron exchange of TMB, another optimized parameter was temperature. For this purpose, three biosensor solutions prepared with the optimum amount of FeNiCo MOF MWCNT composite material were incubated for 30 minutes at 4°C, 25°C, and 50°C after adding 2 mM of 100 µL H_2O_2 solution. The spectra of the spectrophotometric measurements performed as a result of incubations at different temperatures are presented in Figure 4C. Since the highest absorbance value at 654 nm was obtained as a result of incubation at 25°C, the optimum response temperature of the biosensor was determined to be 25°C (Figure 4D).

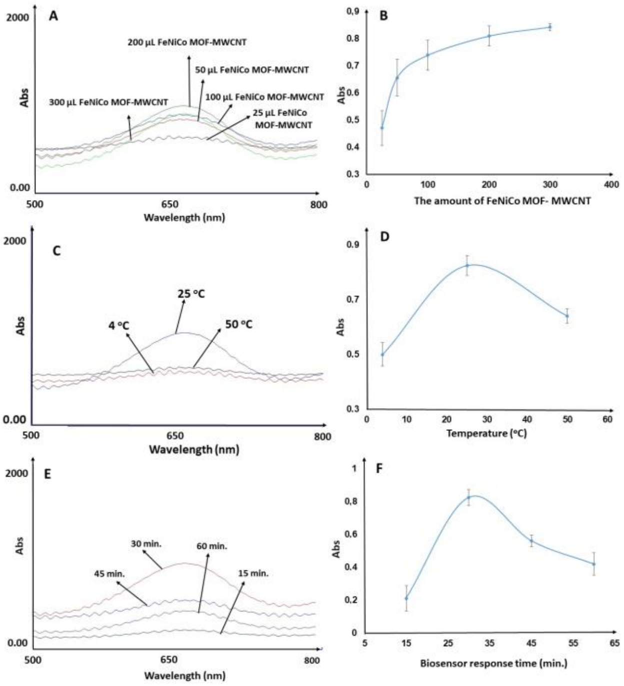


Figure 4. UV-Vis spectrum of A. FeNiCo MOF MWCNT composite amount, C. biosensor response temperature, E. biosensor response time optimization. Optimization graph of B. FeNiCo MOF MWCNT composite amount, D. biosensor response temperature, F. biosensor response time.

The very low absorbance values obtained at 4°C and 50°C suggest that 4°C is too low a temperature for the enzyme-mimicking material to function, while 50°C is too high, potentially causing orientation differences in the structure of the mimetic material, which could lead to the blockage of the active regions responsible for mimicking the enzyme [29].

3.4.3. Optimization of biosensor response time

The response time of a biosensor is of great importance in terms of practicality. For this reason, the response time of the developed non-enzymatic H_2O_2 biosensor has been optimized. For this purpose, the responses of the non-enzymatic H_2O_2 biosensor, prepared under optimal conditions, to the 2 mM H_2O_2 analyte were spectroscopically examined after incubation periods of 15 minutes, 30 minutes, 45 minutes, and 60 minutes (Figure 4E). As observed in the optimization graph in Figure 4F, the highest absorbance value was obtained after 30 minutes of incubation, so 30 minutes was chosen as the optimum response time for the biosensor. Shorter incubation times were insufficient for the FeNiCo MOF MWCNT composite material to mimic peroxidase. At incubation times longer than 30 minutes, due to the extended duration, quenching occurred in the TMB(ox) species, leading to a decrease in the absorbance value 654nm.

3.5. Analytical characteristics

As shown in Figure 5A, the responses of the developed and fully optimized non-enzymatic FeNiCo MOF-MWCNT composite material-based biosensor to increasing concentrations (from 100 µM to 12000 µM) of H_2O_2 were spectroscopically investigated. The obtained absorbance values were plotted against the increasing concentrations of H_2O_2, and the linear response range of the developed biosensor with the equation y: $5x10^{-5}x + 0.3436$ ($R^2 = 0.9898$) for H_2O_2 concentrations between 100 µM and 12000 µM H_2O_2 was determined (Figure 5B). The limit of detection (LOD:3s/m) and the limit of quantification (LOQ:10s/m) values were calculated as 9 µM and 30 µM, respectively. Lastly, in the experiments conducted at a 5000 µM concentration of H_2O_2, the relative standard deviation (RSD) was found to be 2.75%.

3.6. Interference studies

Considering the potential use of the developed non-enzymatic FeNiCo MOF-MWCNT-based colorimetric H_2O_2 biosensor in biological samples, the effect of reagents with interferent potential in serum on the biosensor responses was investigated. For this purpose, a cocktail solution containing interferent agents (KCl, ascorbic acid, urea and NaCl) in serum was prepared, and its effect on the responses during H_2O_2 analysis at equal concentrations was investigated. The analysis results for H_2O_2 at the same concentration with and without the presence of interferents were compared, and the % recovery value was calculated as between 98% and 102%. Looking at the % recovery value, it is observed that the potential interferents in serum do not significantly affect the responses of the developed biosensor.

3.7. Sample application

For real sample experiments, a 3% commercial H_2O_2 solution, which is commonly found in first aid kits and used for wound sterilization, was used. For this purpose, analysis of the commercial H_2O_2 solution was performed using the developed biosensor, and an accuracy between 100% and 103% was determined.

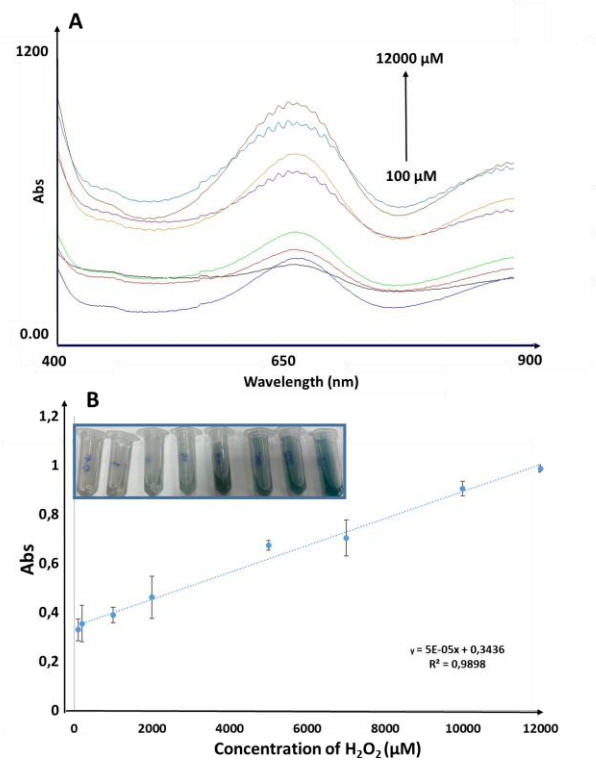


Figure 5. A. The UV-Vis spectrum showing the responses of the developed non-enzymatic H_2O_2 biosensor to increasing concentrations of H_2O_2. B. Calibration graph of the developed H_2O_2 biosensor. Inset: Real image of the responses detectable by the naked eye to increasing concentrations of H_2O_2. Analytical characterization studies were conducted under optimum conditions (using 200 µL FeNiCo MOF-MWCNT, at 25 °C, and for 30 minutes incubation time).

4. Conclusion

The FeNiCo MOF MWCNT composite structure has been synthesized and, for the first time, spectroscopically investigated in terms of peroxidase enzyme mimic properties in this study. After the synthesized FeNiCo MOF MWCNT composite material was comprehensively characterized structurally, an H_2O_2 non-enzymatic biosensor was developed, and the material's peroxidase mimic property was confirmed through the conducted experiments. In the sample tests conducted with a commercial H_2O_2 solution used for wound disinfection, as well as in tests performed in the presence of several potential interfering agents that might be encountered in serum, high % recovery values were obtained, thereby clarifying the accuracy and specificity of the developed non-enzymatic H_2O_2 biosensor. As aresult, structures containing multi-metal ions, such as FeNiCo MOF MWCNT, can be used as enzyme mimic materials by forming composite structures with materials like MWCNT, which enhances their durability due to the redox reaction capability of the metallic structures.

5. References

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