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HEAVY METALS AND HUMAN HEALTH: ELECTROCHEMICAL METHOD FOR MEASURING TOXIC IRON

AĞIR METAL VE İNSAN SAĞLIĞI: TOKSİK DEMİRİ ÖLÇMEK İÇİN ELEKTROKİMYASAL YÖNTEM

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ABSTRACT

Objective: Water resources on Earth are essential for life. Today, heavy metals are released into the ecosystem as a result of various factors, such as increasing industrialization. Iron ions have toxic properties in cells in various ways. Iron ions cause the formation of hydroxyl radicals through Fenton reactions. By participating in reactions within cells, they can lead to the formation of reactive oxygen species and lead to the development of diseases related to oxidative stress. Therefore, the assessment of iron concentrations is of great importance in biomedical and environmental analyses.

Methods: Hydrogen peroxidase enzyme (HRP), bovine serum albümin (BSA), glutaraldehyde, and gelatin were added to the Au electrode in 10 mL increments using an adjustable pipette. This mixture, which will form the bioactive layer, was captured on the electrode using an excimer laser. Electrochemical measurements were taken by sequentially adding Fe^{+2} prepared using stock iron nitrate and hydrogen peroxide (H_2O_2 was used in the study; 10 ml of a 100 g/dL stock solution was used for each measurement).

Results: Optimization studies determined the optimum pH as 6.4; optimum glutaraldehyde percentage as 2.5%; optimum gelatin concentration as 50 mg/dL; and optimum BSA as 45 mg/dL. SEM images showed that the crosslinker bound to the polymers in the bioactive layer, exhibiting a compact structure on the electrode surface. The layers formed were interlocked. Applications at different iron concentrations (0.2 mg/dL, 0.6 mg/dL, 1 mg/dL, 2 mg/dL, 3 mg/dL, and 4 mg/dL) demonstrated the sensitivity and specificity of the electrode.

Conclusion: In this study, a new and sensitive sensor was developed for the rapid and precise detection of Fe ions (Fe^{+2}) in water. The sensor developed in this study was determined to have high sensitivity, low detection limit, and sensitivity for Fe^{+2} detection. The developed biosensor can be used as a low-cost, practical, and reliable analytical method for the detection of iron ions.

Keywords: Bioactive Layer, Biosensor, Heavy Metals, Iron Determination.

ÖZET

Amaç: Dünya üzerinde yer alan su kaynakları canlı yaşamı için vazgeçilmezdir. Günümüzde artan sanayileşme gibi çeşitli faktörler sonucunda ağır metaller ekosisteme salınmaktadır. Demir iyonları çeşitli şekillerde hücrelerde toksik özelliklere sahiptir. Demir iyonları Fenton reaksiyonları ile hidroksil radikallerinin oluşmasına neden olmaktadır. Hücrelerde gerçekleşen reaksiyonlara katılarak reaktif oksijen türlerinin oluşmasına neden olabilir ve oksidatif strese bağlı hastalıkların gelişmesine sebebiyet verebilir. Bu nedenle, demir konsantrasyonlarının değerlendirilmesi biyomedikal ve çevresel analizlerde büyük öneme sahiptir.

Gereç ve Yöntem: Hidrojen peroksidaz enzimi (HRP), sığır serum albümini (BSA), glutaraldehit ve jelatin, ayarlanabilir bir pipet kullanılarak 10 mL'lik artışlarla Au elektroda eklendi. Biyoaktif tabakayı oluşturacak olan bu karışım, eksimer lazer kullanılarak elektrot üzerinde yakalandı. Elektrokimyasal ölçümler, stok demir nitrat ve hidrojen peroksit (çalışmada H_2O_2 kullanıldı; her ölçüm için 100 g/dL'lik stok çözeltinin 10 ml'si kullanıldı) kullanılarak hazırlanan Fe^{+2}'nin sırayla eklenmesiyle alındı.

Bulgular: Yapılan optimizasyon çalışmalarında optimum Ph 6.4; optimum gluteraldehit yüzdesi %2.5; optimum jelatin konsantrasyonu 50mg/dL ve optimum BSA 45mg/dL olarak belirlendi. Sem görüntüleri çapraz bağlayıcının biyoaktif tabakadaki polimerlere bağlanarak elektrot yüzeyinde kompakt bir yapı sergiledi. Katmanlar halinde olşan tabakalar birbirine kenetlenmişti. Farklı demir konsantrasyonlarında (0.2 mg/dL, 0.6 mg/dL, 1 mg/dL, 2 mg/dL, 3 mg/dL, and 4 mg/dL) yapılan ugulamalar elektrotun hassasiyetini ve özgüllüğünü gösterdi.

Sonuç: Bu çalışma kapsamında, sulardaki Fe iyonlarının (Fe^{+2}) hızlı ve hassas bir şekilde tespiti için yeni ve hassas bir sensör geliştirilmiştir. Çalışma kapsamında hazırlanan sensör Fe^{+2} tespiti için yüksek duyarlılık düşük algılama limiti ve hassasiyete sahip olduğu belirlenmiştir. Geliştirilen biyosensör, demir iyonlarının tespiti için düşük maliyetli, pratik ve güvenilir bir analitik yöntem olarak kullanılabilir.

Anahtar kelime: Ağır Metaller, Biyoaktif Tabaka, Biyosensör, Demir Tayini.

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INTRODUCTION

Water constitutes over 71% of the Earth's surface. It is the most vital element for sustaining life. Nevertheless, merely 2.5% of the global water supply is classified as freshwater. Additionally, more than two-thirds of this 2.5% is trapped in glaciers, which is inadequate to satisfy the increasing demands of society(Mishra, 2023; Musie & Gonfa, 2023; Stephens et al., 2020). The right to access clean, unpolluted water is fundamental for every person on the planet. Water contamination impacts drinking supplies, rivers, lakes, and oceans globally, endangering living organisms, human health, and the ecosystem. As the use of various metals in both industry and daily life rises, exposure to heavy metals has also escalated. The issues stemming from heavy metal pollution have reached alarming levels across the globe. While water pollution may not produce immediate health effects, it can lead to toxic and potentially fatal outcomes over time(Gambhir, Vinod Kapoor, Ashutosh Nirola, Raman Sohi, & Vikram Bansal, 2012). Heavy metals released from industrial activities can accumulate in adjacent lakes and rivers, affecting marine life, other animals that ingest this contaminated water, and humans who consume animal products via the food chain. Toxins present in industrial waste can lead to immune system suppression, reproductive issues, or acute poisoning(Chouhan, Meena, & Poonar, 2016).

Heavy metals represent a significant contributor to global water pollution. These metals are characterized as metallic elements with a high density (greater than 4 g/cm^3, or five times that of water) and are considered hazardous or toxic even in minimal concentrations(Jomova, Alomar, Nepovimova, Kuca, & Valko, 2025). Heavy metals can infiltrate the human body through multiple pathways, such as the ingestion of contaminated food or water, inhalation of polluted air, and absorption through the skin, leading to detrimental effects on various biological processes(Tchounwou, Yedjou, Patlolla, & Sutton, 2012).

Essential heavy metals serve as cofactors in a variety of biological functions. For instance, Co, Fe, Cu, and Zn are crucial for oxygen utilization, cellular growth, numerous enzymatic reactions, biomolecular synthesis, and the immune system of the body. Iron is a component of hemoglobin, myoglobin, cytochromes (a, b, c), catalase, aconitase, succinate dehydrogenase, aldehyde oxidase, peroxidases, tryptophan 2,3-dioxygenase, and many other enzymes(Kim, Kim, & Kumar, 2019). Consequently, the metabolism of iron within the human body is typically maintained in a relative equilibrium to ensure both sufficiency for essential functions and safeguard against toxicity. To uphold iron homeostasis, the body absorbs around 1–2 mg of iron daily, mainly from dietary sources. Dietary iron is found in two forms: divalent ferrous iron (Fe^{2+}) and trivalent ferric iron (Fe^{3+}). The metabolism of iron encompasses several tightly controlled processes, including absorption, transport, utilization, circulation, regulation, and storage(Hentze, Muckenthaler, & Andrews, 2004; Pasricha, Tye-Din, Muckenthaler, & Swinkels, 2021).

Iron deficiency represents a prevalent nutritional shortfall that generally arises from inadequate dietary iron intake, poor iron absorption, or iron loss. Severe cases of iron deficiency can result in hypochromic anemia, which is marked by a reduction in hemoglobin production(Dutt, Hamza, & Bartnikas, 2022). Heme, an iron-containing tetrapyrrole, is vital for numerous biological processes. It is crucial for oxygen transport, gas sensing, oxidative metabolism, xenobiotic detoxification, and the processing of microRNA(Beavers et al., 2023). A reduction in hemoglobin levels due to iron deficiency has a direct impact on oxygen transport and the utilization of oxygen in tissues. For instance, diminished performance in aerobic endurance exercises is often noted, particularly among adolescent and female athletes who are more vulnerable to iron deficiency(Roy, Kück, Radziwolek, & Kerling, 2022). Additionally, heme levels can influence the transcription of target genes across various pathways, such as circadian rhythms, cell proliferation, apoptosis, responses to oxidative stress, ion channel function, and mitochondrial respiration(Beavers et al., 2023; Chambers, Willoughby, Hamza, & Reddi, 2021).

While iron is a crucial micronutrient in human nutrition and is vital for various cellular functions and metabolic activities, an excess of iron can be extremely harmful. Iron overload leads to the production of reactive oxygen species (ROS), which in turn causes cellular dysfunction, oxidative stress, and damage to tissues and organs. The toxicity associated with excess iron is mainly due to its capacity to transition between the ferrous (Fe^{2+}) and ferric (Fe^{3+}) forms, enabling redox reactions that can harm DNA, proteins, lipids, and organelles such as mitochondria and lysosomes. When transferrin saturation surpasses approximately 60–70%, the binding capacity is overwhelmed, resulting in the generation of non-transferrin-bound iron (NTBI). NTBI can easily penetrate cells, especially cardiomyocytes, through

calcium channels, where it accumulates and progressively leads to tissue damage(Fleming & Ponka, 2012).

Chronic liver iron overload is a contributing factor to fibrosis, cirrhosis, and hepatocellular carcinoma. Myocardial iron accumulation leads to mitochondrial dysfunction, which can result in heart failure and arrhythmias. Additionally, it is believed that iron overload is linked to cardiovascular disease through the promotion of oxidative stress, inflammation, and atherosclerosis(Ru et al., 2024). The involvement of iron in the oxidation of low-density lipoprotein (LDL) and the formation of plaques remains a topic of debate; however, there is evidence indicating that iron accumulation within macrophages and vascular tissues plays a role in vascular damage. Iron dysregulation has also been associated with neurological disorders such as Huntington's disease, Parkinson's disease, Alzheimer's disease, and autism, as well as secondary brain injury following intracerebral hemorrhage. In these cases, excess iron leads to lipid peroxidation, ferroptosis, and damage to neuronal mitochondria, which aggravates neurodegeneration and cerebral edema. Likewise, iron overload impacts immune function by promoting the growth of pathogens, thereby heightening the risk of infections(Ru et al., 2024).

Considering the extensive systemic implications, accurately assessing body iron levels holds considerable clinical and therapeutic significance. In this context, electrochemical techniques offer a swift and straightforward approach for quantifying toxic Fe^{+2} concentrations in contrast to conventional methods. The assessment of toxic Fe^{+2} levels facilitates the evaluation of drinking water quality. This research focused on creating a biosensor capable of rapid and highly sensitive identification of toxic Fe^{+2} ions in drinking water.

MATERIALS AND METHODS

Preparation of the working electrode and measurement of samples

HRP, BSA, glutaraldehyde, and gelatin were added to the Au electrode in 10 mL increments using an adjustable pipette. This mixture, which will form the bioactive layer, was captured on the electrode using an excimer laser. HRP enzyme was used at a concentration of 0.25 mg/dL in each step of our study.

50 mL of sodium phosphate buffer was added to the measurement cell. The prepared electrode was then placed in the measurement cell. Electrochemical measurements were taken by sequentially adding Fe^{+2} prepared using stock iron nitrate and hydrogen peroxide (H_2O_2 was used in the study; 10 ml of a 100 g/dL stock solution was used for each measurement). Measurements were taken with different concentrations of Fe^{+2} to determine the measurement range of the electrode prepared for the electrochemical method.

RESULTS

pH Optimization of sodium phosphate buffer

To determine the optimum pH of the sodium phosphate buffer to be used in the study, measurements were made at different pH values (pH 5.6, 6.0, 6.4, 6.8, and 7.2). As a result of the measurements, the highest current was observed at pH 6.4, and the optimum pH value was determined to be 6.4. The following sections of the study were conducted based on the optimum pH value (Figure 1).

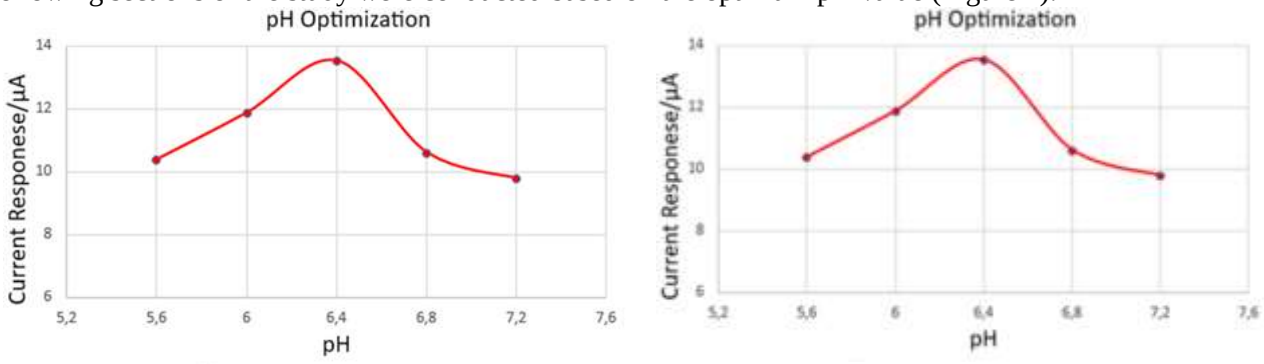

Figure 1. Graph showing the optimum pH value of the buffer.

Optimization of the Bioactive Layer

Determination of the Optimum Amount of Glutaraldehyde

To determine the optimum amount of glutaraldehyde, measurements were made using electrodes prepared using different concentrations of glutaraldehyde (2%, 2.5%, and 3%). Measurements were made by adding 10 µL of iron nitrate solution containing 100 µg/dL iron to sodium phosphate buffer (pH 6.4). The highest current value was obtained in the solution containing 2.5% glutaraldehyde, and therefore, the optimum amount of glutaraldehyde was determined to be 2.5%. The next part of the study continued using 2.5% glutaraldehyde for the bioactive layer (Figure 2).

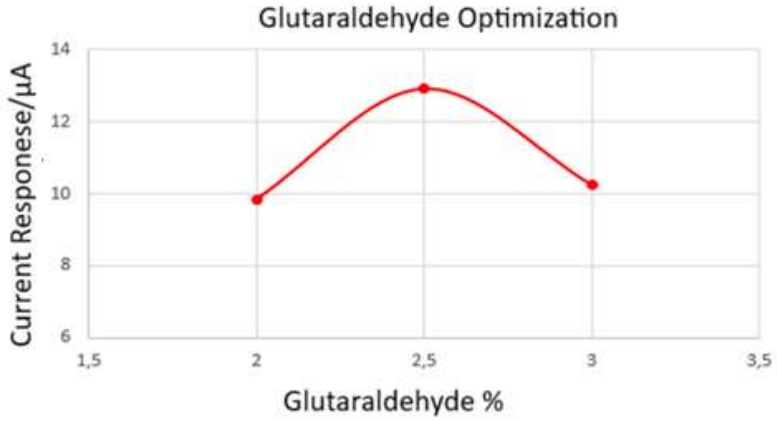

Figure 2. Graph showing optimum glutaraldehyde concentration.

Determining the Optimum Amount of Gelatin

To determine the optimum amount of gelatin, measurements were made using electrodes prepared using gelatin at different concentrations (25 mg/dL, 50 mg/dL, 100 mg/dL, and 150 mg/dL) (other parameters were kept constant during electrode preparation). As a result of the measurements, the highest current was obtained with the electrode prepared using 50 mg/dL gelatin, and the optimum amount of gelatin was determined to be 50 mg/dL (Figure 3). The next part of the study continued using 50 mg/dL gelatin for the bioactive layer.

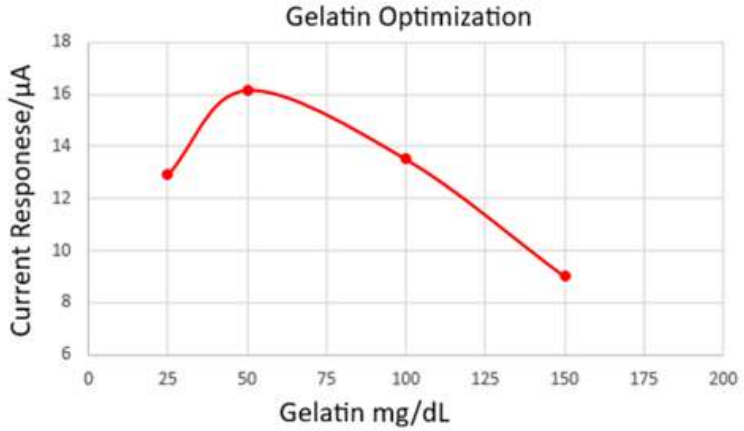

Figure 3. Graph showing optimum glutaraldehyde concentration

Determining the Optimum Amount of BSA

The optimum BSA concentration was determined by measurements using electrodes prepared using different BSA concentrations (30 mg/dL, 45 mg/dL, and 60 mg/dL) (other parameters were kept constant during electrode preparation). Measurements were obtained by adding 10 µL of iron nitrate solution containing 100 µg/dL iron to 50 mL of sodium phosphate buffer with a pH of 6.4. The measurements determined the optimum BSA amount to be 45 mg/dL (Figure 4). The following sections of the study continued using 45 mg/dL BSA for the bioactive layer.

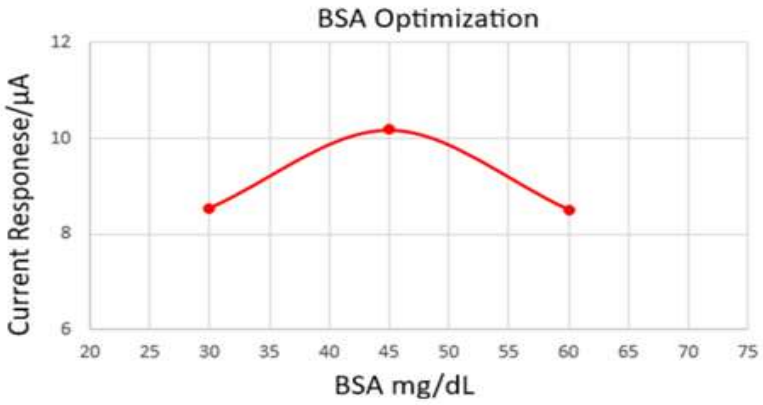

Figure 4. Graph showing optimum BSA concentration.

Scanning Electron Microscopy (SEM) Analysis of the Bioactive Layer

The surface morphologies of bioactive layers composed of HRP enzyme, BSA, glutaraldehyde, and gelatin were determined by SEM analysis. SEM images show that glutaraldehyde, used as a cross-linker, binds to the polymers within the bioactive layer, forming a compact structure, and the polymers forming the bioactive layer are interlocked (Figure 5).

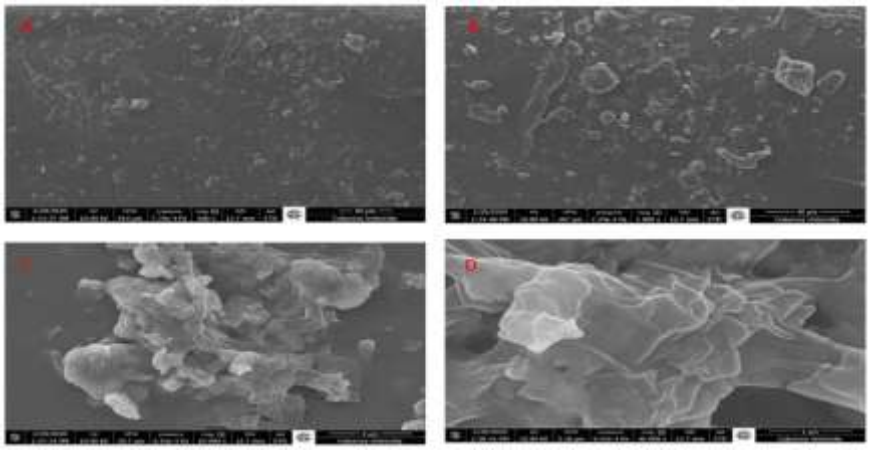

Figure 5. A) SEM image of the bioactive layer (50 µm). B) SEM image of the bioactive layer (40 µm). C) SEM image of the bioactive layer (4 µm). D) SEM image of the bioactive layer (1 µm).

Determining the Measurement Range

To determine the measurement range, measurements were made using Fe^{+2} amounts at different concentrations (0.2 mg/dL, 0.6 mg/dL, 1 mg/dL, 2 mg/dL, 3 mg/dL, and 4 mg/dL). As a result of the measurements, it was determined that the prepared sensor had high sensitivity and spesivicity (Figure 6).

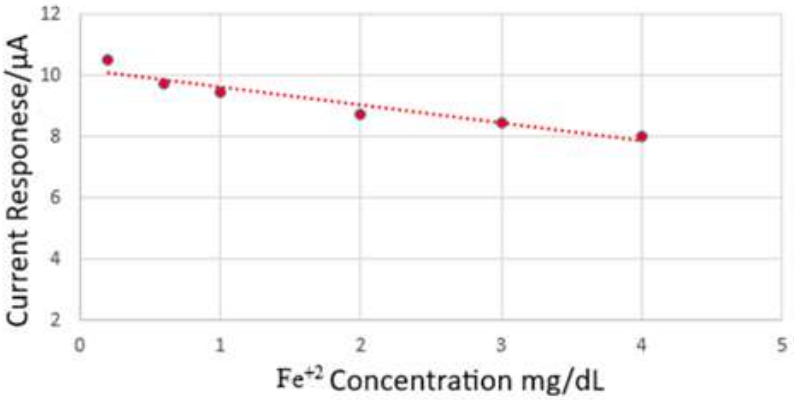

Figure 6. Measuring range of electrochemical method

DISCUSSION

Due to the rising trends of urbanization and industrialization, there is a notable increase in the concentration of heavy metals within water resources. Toxic heavy metals are frequently detected in wastewater originating from industrial and urban sources. These heavy metals pose significant risks to both human health and ecological systems. The discharge of heavy metals into the environment represents one of the most pressing environmental challenges faced by developing and emerging economies today. When numerous hazardous chemical elements or heavy metals are released into the environment, they tend to accumulate in soil and sediment within water bodies. These accumulated heavy metals or chemicals are subsequently absorbed by living organisms, thereby entering the food chain through various species inhabiting the ecosystem. Consequently, humans also face exposure to heavy metals via the food chain(Farh, Seghier, & Zayed, 2023; Salehi, 2022).

The levels of toxicity associated with heavy metals differ based on the specific type of metal and the organisms that come into contact with it. The heavy metals most frequently found in drinking water include lead, iron, cadmium, copper, zinc, and chromium. Among these heavy metals, copper is recognized as an essential trace element. Nevertheless, elevated concentrations of copper in drinking water can result in toxic effects. Cadmium, another heavy metal, is extremely toxic even at minimal concentrations and tends to bioaccumulate within organisms and ecosystems. Research has shown that cadmium possesses a prolonged biological half-life in the human body, which can range from 10 to 30 years(Briffa, Sinagra, & Blundell, 2020; Peana et al., 2022).

The known fatal consequences of heavy metal toxicity in drinking water encompass impairment or reduction of mental and central nervous system functions, as well as diminished energy levels(Kardar, Shemirani, & Zadmard, 2020). Additionally, heavy metals induce disorders in blood composition. They adversely affect essential organs, including the kidneys and liver. Prolonged exposure to heavy metals results in physical, muscular, and neurological degenerative processes that can culminate in conditions such as Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis (MS)(Mohod & Dhote, 2013).

The effectiveness of Fe(II) as an electron donor and Fe(III) as an electron acceptor renders iron a vital mineral and nutrient. Nevertheless, this characteristic also positions iron as a potential biological threat, as it can easily catalyze the production of harmful radicals under aerobic conditions(Mahurpawar, 2015). The toxicity of iron is primarily attributed to Fenton and Haber-Weiss reactions. Even minimal amounts of iron can facilitate the generation of hydroxyl radicals (OH-) from superoxide (O_2-) and hydrogen peroxide (H_2O_2). Organ damage resulting from chronic iron accumulation poses significant risks due to the diverse range of tissues impacted and the often gradual and insidious emergence of organ dysfunction(Niederau et al., 1985; Papanikolaou & Pantopoulos, 2005). The liver, heart, and pancreatic beta cells are among the most frequently affected organs and cell types in cases of iron overload. Iron toxicity can be categorized as either corrosive or cellular in nature. An excess of iron in the body inflicts direct corrosive harm to the gastrointestinal mucosa, leading to symptoms such as nausea, vomiting, abdominal pain, and diarrhea. Hemorrhagic necrosis of the gastrointestinal mucosa may result in hematemesis, perforation, and peritonitis. On a cellular level, iron interferes with metabolic processes in the heart, liver, and central nervous system. Additionally, free iron accumulates in the mitochondria of cells, disrupts oxidative phosphorylation, catalyzes lipid peroxidation, and produces free radicals through Fenton reactions. The free radicals generated contribute to oxidative stress and are significant in the development of various diseases(Eaton & Qian, 2002).

Atomic absorption spectroscopy (AAS), capillary electrophoresis (CE), inductively coupled plasma/atomic emission spectrometry (ICP-AES), ion chromatography ultraviolet-visible spectroscopy (IC-UV-vis), inductively coupled plasma-mass spectroscopy (ICP-MS), micro probes (MP), and X-ray fluorescence spectroscopy (XFS) are employed for the detection of heavy metals, even at minimal concentrations(Yuen & Becker, 2023; Zaynab et al., 2022). While these conventional techniques exhibit high sensitivity and selectivity, they also present several drawbacks, including elevated instrument costs, complex sample preparation processes, the necessity for skilled personnel, and restrictions on single-component detection and preconcentration methods. These limitations create significant challenges for real-time and continuous analysis.

In contrast, electrochemical methods serve as a potent sensing mechanism for identifying heavy metal ions. This approach presents numerous benefits, such as precision, ease of use, affordability, exceptional sensitivity, efficient multiplex detection, and the ability for in situ detection(Waheed,

Mansha, & Ullah, 2018). The sensitivity and selectivity of an electrochemical sensing system can be further improved by chemically altering the bare electrode with effective electron mediators. The electrical conductivity, extensive surface area, and potential for modification of nanomaterials or other structures render them effective electron mediators and promising candidates for electrode enhancement. A diverse range of nanomaterials, including carbon-based substances, metallic nanoparticles (NPs), and silicon-based compounds, have been employed as electrode modifiers. Additionally, the modified sensors demonstrate size-dependent characteristics and a high level of functionality(Kempahanumakkagari, Deep, Kim, Kailasa, & Yoon, 2017; Zhang et al., 2016).

Biosensors developed using electrochemical methods are generally defined as sensitive instruments that detect, process, and convert a sample into a signal. The measurement principle of the electrochemical method we developed in this study is based on the Fenton reaction. All values obtained in our optimization studies were found to be consistent with the literature(Guan, Huang, & Li, 2022; Guo, Chan, Chen, & Zeng, 2017; Kang et al., 2020; Manoj, Auddy, Nimbkar, Chittibabu, & Shanmugasundaram, 2020). The prepared biosensor structure was found to be capable of rapidly and accurately detecting Fe^{+2} ions in drinking water. According to the findings of this research, we are confident that the developed biosensor possesses the capability to detect heavy metal ions, including Cu^{+2}, Sn^{+2}, and Pb^{+2}.

CONCLUSION

In this study, an electrochemical surface sensor designed for the determination of Fe^{2+} ions in aqueous environments was developed and successfully optimized. The sensor performance was improved by rigorously optimizing the basic experimental parameters of the systematically designed biosensor, such as pH, crosslinker, and biopolymer. Thus, the sensor exhibited good performance and showed sensitivity to Fe^{2+} ions. The developed biosensor can be used as a low-cost, practical, and reliable analytical method for the detection of iron ions.

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