SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 23(3) (2025) 210-218, DOI: 10.24323/akademik-gida.1793607

Research Paper / Araştırma Makalesi

Electrochemical Determination of Copper in Fish Samples

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Received (Geliş Tarihi): 17.05.2025, Accepted (Kabul Tarihi): 29.09.2025
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ABSTRACT

Toxic metal contamination, particularly by copper Cu (II), poses serious risks to environmental and human health due to its persistence, bioaccumulation, and redox-driven toxicity. This study presents a simple, cost-effective, and reliable electrochemical approach for Cu (II) detection using an unmodified screen-printed electrode (SPE). Electrochemical parameters, including supporting electrolyte type and concentration, as well as scan rate, were systematically optimized using cyclic voltammetry. Among various electrolytes tested, 0.1 M potassium chloride (KCl) provided the most stable and sensitive response. The developed method demonstrated a wide linear range (0.25-10.0 mM), excellent precision (RSD≤5.8%), and a limit of detection (LOD) of 0.080 mM. When applied to complex biological matrices, such as fish liver, the sensor achieved high recovery rates (95.10-105.85%), indicating its applicability in real samples. This study also aimed to determine the effectiveness of solid-phase extraction combined with electrochemical detection for identifying Cu (II) in fish liver samples, and to compare its sensitivity and accuracy with that of inductively coupled plasma optical emission spectrometry (ICP-OES). Results demonstrated that unmodified screen-printed electrodes (SPEs) could reliably detect Cu (II) in complex biological matrices, offering a cost-effective and practical alternative for use in environmental and biological monitoring. Compared to other advanced sensors, this method stood out for its minimalistic design, low cost, and operational simplicity, offering a promising alternative for on-site heavy metal detection in resource-limited settings.

Keywords: Copper detection, Screen-printed electrode (SPE), Cyclic voltammetry (CV), Electrochemical sensor, Fish liver

Balık Numunelerinde Bakırın Elektrokimyasal Tayini

ÖZ

Özellikle bakır Cu (II) kaynaklı toksik metal kontaminasyonu, kalıcılığı, biyolojik birikimi ve redoks kaynaklı toksisitesi nedeniyle çevre ve insan sağlığı için ciddi riskler oluşturmaktadır. Bu çalışma, serigrafi baskılı elektrot (SPE) kullanılarak Cu (II) tayini için basit, uygun maliyetli ve güvenilir bir elektrokimyasal yaklaşım sunmaktadır. Destek elektrolit çözeltisi türü ve konsantrasyonu ile tarama hızı da dahil olmak üzere elektrokimyasal parametreler, döngüsel voltametri kullanılarak sistematik olarak optimize edilmiştir. Deneysel çalışmalar sonucunda çeşitli destek elektrolitleri arasında 0.1 M potasyum klorür (KCl) en kararlı ve hassas yanıtı sağlamıştır. Geliştirilen yöntem geniş bir doğrusal aralık (0.25-10.0 mM), mükemmel hassasiyet (RSD≤%5.8) ve 0.080 mM'lik bir tespit sınırı (LOD) göstermiştir. Balık karaciğeri gibi karmaşık biyolojik matrislere uygulandığında ise, sensör gerçek numunelerde uygulanabilirliğini vurgulayan yüksek geri kazanım oranlarına (%95.10-105.85) ulaşmıştır. Bu çalışma ayrıca balık karaciğeri örneklerinde Cu (II)'yi tanımlamak için elektrokimyasal tespit ile birleştirilmiş katı faz ekstraksiyonunun etkinliğini değerlendirmeyi ve duyarlılığını ve doğruluğunu endüktif olarak eşleştirilmiş plazma optik emisyon spektrometrisi (ICP-OES) ile karşılaştırmayı amaçlamaktadır. Elde edilen sonuçlar, yüzeyi herhangi bir malzeme ile modifiye edilmemiş serigrafi baskılı elektrotların (SPE'ler) karmaşık biyolojik matrislerde Cu (II)'yi güvenilir bir şekilde tespit edebileceğini ve çevresel

ve biyolojik izlemede kullanım için uygun maliyetli ve pratik bir alternatif sunduğunu göstermektedir. Diğer gelişmiş sensörlerle karşılaştırıldığında, bu yöntem minimalist tasarımı, düşük maliyeti ve operasyonel basitliği ile öne çıkmakta ve kaynak sınırlı ortamlarda yerinde ağır metal tespiti için umut verici bir alternatif sunmaktadır.

Anahtar Kelimeler: Bakır tespiti, Serigrafi baskılı elektrot (SPE), Döngüsel voltametri (CV), Elektrokimyasal sensör, Balık karaciğeri

INTRODUCTION

Toxic metals (often called “heavy metals”) such as arsenic, cadmium, chromium, lead, mercury, and copper occur naturally in the earth’s crust, but human activities have massively increased their environmental release [1]. Mining, smelting, and metallurgical operations release large quantities of metals into the air, water, and soil. Combustion of coal and oil, metal fabrication, battery recycling, and waste incineration are also major emission sources [1, 2]. Once released, metals travel via multiple pathways: they can deposit from the air as dust, leach through soils into groundwater and rivers, and enter food chains through plants and aquatic organisms [3]. Importantly, toxic metals are persistent in the environment; they do not break down over time, so contamination can last for decades or longer [4]. Copper is a globally prevalent metal, with over 15 million tons used annually, largely from mining [4]. It is classified as a priority pollutant, with strict environmental quality criteria. For humans, copper is essential in small amounts (1–2.5 mg/day) but becomes toxic at higher doses [4]. Acute overexposure can cause gastrointestinal distress, liver and kidney damage, and in extreme cases, death [5-7]. Copper’s harmful effects are largely due to its redox activity, which, in excess, overwhelms the body’s regulatory systems and induces oxidative stress [3].

Released into aquatic environments, copper can bioaccumulate in organisms and affect entire ecosystems. Copper enters freshwater and marine habitats through industrial discharges and runoff, where it is absorbed by fish via gills and ingestion of contaminated food or sediments [4]. Fish tend to concentrate copper in their gills, liver, and kidneys, with lesser amounts in muscle, while filter-feeding shellfish like mussels and oysters can accumulate copper to levels far exceeding the surrounding water. Sublethal copper levels impair fish growth, reproduction, immune function, and behavior [8]. Copper-rich sediments also reduce invertebrate biodiversity and abundance. As fish are a major food source for humans, copper accumulation poses food safety risks, especially in polluted areas where levels may exceed health guidelines. The liver, being central to metabolism and detoxification, is particularly prone to copper accumulation, making it a critical tissue for monitoring contamination [9]. Measuring copper in fish liver not only reflects ecosystem health, given fish serve as bioindicators, but also informs food safety assessments [10, 11]. Consequently, regular monitoring of copper levels in fish, especially liver tissues, is vital for environmental surveillance, regulatory compliance, and public health protection. According to the Turkish Food Codex (TFC), the maximum Cu concentration allowed in the meat of fish was determined as 20 µg/g from the sea and lakes. The maximum level of Cu is listed as 30 µg/g and 20 µg/g at the Food and Agriculture Organization (FAO) and WHO, respectively. In the study by Tuzen (2003), copper (Cu) concentrations in fish liver tissues from the middle Black Sea were reported to range between 5.05 and 36.22 µg/g, likely on a dry weight basis, indicating a considerable level of metal accumulation in hepatic tissue [12]. Supporting this, Turkmen et al. (2007-2008) consistently observed that liver samples had the highest copper concentrations compared to other tissues, such as muscle, across fish species collected from various Turkish seas. In a related analysis, the liver of *Mugil auratus*, a species native to the Black Sea, was found to contain Cu levels between 0.49 and 1.30 µg/g dry weight, reflecting species- and region-specific differences in bioaccumulation [13, 14]. These findings collectively underscore the liver’s role as a key organ for metal storage and detoxification and highlight the potential use of liver tissue as a reliable biomonitoring for environmental metal contamination.

Traditional techniques for detecting heavy metals, such as Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), and ICP-OES, are known for their high sensitivity, accuracy, and reliability, and are commonly used in environmental monitoring, food safety, and clinical diagnostics [15, 16]. However, these methods involve expensive instrumentation, complex sample preparation, and require skilled personnel, which collectively increase the cost and time of analysis and hinder their use in real-time or field-based applications. Samples often need to be transported to centralized labs, potentially compromising their integrity and delaying results. Consequently, there is a growing demand for alternative detection approaches that are low-cost, portable, and capable of providing rapid results at the point of need. Electrochemical sensors have emerged as a promising solution in this context. They offer high sensitivity, fast response times, and the ability to detect trace levels of heavy metals on-site without the need for elaborate sample preparation. Fabricated from inexpensive materials like carbon-based or screen-printed electrodes, these sensors are cost-effective, easy to operate, and particularly well-suited for deployment in resource-limited settings and large-scale environmental or food safety monitoring programs [17-19].

This study presents a straightforward and efficient electrochemical method for the detection of Cu (II) using an unmodified screen-printed electrode (SPE), with performance compared to ICP-OES. By systematically optimizing key parameters such as the type and concentration of the supporting electrolyte and the scan rate, the method achieves high sensitivity, well-defined peak resolution, and reliable quantification without requiring complex surface modifications or nanomaterial

enhancements. The SPE-based technique offers a simpler, faster, and more cost-effective alternative to the time-consuming sample preparation involved in ICP-OES, making it a promising tool for the routine monitoring of Cu (II) in fish (*Mugil cephalus*) liver samples and enhancing accessibility in food and environmental safety programs.

MATERIALS and METHODS

Materials

Chemicals used in this study are sodium acetate (CH_3COONa, purity>99%, Sigma-Aldrich), acetic acid (CH_3COOH, purity>99%, Sigma-Aldrich), potassium nitrate (KNO_3, purity>99%, Sigma-Aldrich), phosphate-buffered saline (PBS) tablets, pH 7.2–7.6 (Sigma–P4417), ethanol (C_2H_6O, purity>99.99%, IsoLab), potassium ferrocyanide ($K_4Fe(CN)_6 \cdot 3H_2O$, purity>98%, Sigma-Aldrich), potassium ferricyanide ($K_3Fe(CN)_6$, purity>99%, Sigma-Aldrich), boric acid (H_3BO_3, purity>99.55%, Sigma-Aldrich), phosphoric acid (H_3PO_4, purity>99%, Sigma-Aldrich), potassium hydrogen phosphate (K_2HPO_4, purity>99%, Sigma-Aldrich), potassium dihydrogen phosphate (KH_2PO_4, purity>99%, Sigma-Aldrich), and copper sulfate ($CuSO_4$, purity>99%, Sigma-Aldrich). All reagents were of analytical grade and used as received without further purification. Solutions were prepared and diluted using ultra-pure deionized water (resistivity: 18.2 MΩ·cm) obtained from a reverse osmosis system (Human Corp., Seoul, South Korea). All experiments were performed at room temperature.

Instrumentations

Electrochemical measurement, comprising cyclic voltammetry (CV) was carried out using a portable potentiostat (DropSens uSTAT I400, Metrohm, Switzerland) connected to screen-printed electrodes (Methrom, DRP-110-U75, Number of WEs: 1 WE Working electrode material: Carbon Working electrode diameter: 0.40 cm Working electrode position: In the middle of the strip Auxiliary electrode material: Carbon Reference electrode material: Silver Contacts material: Silver Substrate material: Ceramic Substrate size: Length: 3.38 cm; Width: 1.02 cm Substrate thickness: 0.05 cm). All voltametric experiments were performed under standard laboratory conditions, following the manufacturer's protocols. CEM MARS-6, a microwave digestion system, was used for sample preparation of fish (Matthews, NC, USA). Perkin Elmer Optima 2100 DV, Inductively Coupled Argon Plasma-Optical Emission Spectrometer was used for spectroscopic determination.

Additional equipment included a digital pH meter with a combined glass-calomel electrode (WTW 720, Weilheim, Germany) for accurate pH adjustments. A centrifuge (NF400, Nüve, Türkiye), was used for sample preparation, while an analytical balance (XB 220A, Sweden) ensured precise mass measurements. Thermal treatments were conducted in a laboratory oven (FN 055, Nüve, İstanbul, Türkiye), and sample homogenization was achieved using an orbital shaker (VWR, USA). The concentration of calibration solutions was prepared with ICP-OES standard solutions that were provided by Perkin Elmer.

Optimization Procedure

Key analytical parameters were systematically evaluated and optimized in this study using a one-variable-at-a-time (OVAT) approach. Parameters investigated included the type and concentration of the supporting electrolyte and the scan rate. All experiments were performed at room temperature. In a typical batch analysis, 15 µL of a 0.1 M potassium chloride (KCl) solution containing Cu (II) ions was deposited onto the surface of the unmodified screen-printed electrode, followed by electrochemical analysis using CV. CV measurements were conducted over a potential range of +1.0 V to –1.0 V, with a step size of 2 mV and a scan rate of 50 mV/s. Each measurement was performed in triplicate to ensure reproducibility.

Fish *(Mugil cephalus)* Liver Sample Preparation

Three samples of *Mugil cephalu*s fishes were procured from fisherman in İzmir and brought to the laboratory in polyethylene containers at -4°C. Upon arrival, the samples were washed with distilled water and preserved at -80°C until extraction. 1 g of each fish sample was dried in a laboratory oven. The solubilization process for liver tissues of each fish sample was conducted using a microwave digestion system before analysis by electrochemical measurements and ICP-OES [20]. In the One Touch Food Methodology, 10 mL of HNO_3 and H_2O_2 in a 1:1 (v/v) ratio were introduced to 0.1 g (dried weight) of liver tissue after its measurement. Iprep pots were employed for digestion purposes. The procedure has a ramp time of 30 min at 210°C, during which the samples maintain this temperature for 15 min. The concentrations of the calibration solutions were 10, 50, 100, 250, and 500 µg/kg, respectively.

RESULTS and DISCUSSION

Electrolyte Type

The selection of a suitable supporting electrolyte is crucial in shaping the redox behavior of metal ions during electrochemical analysis. Both the ionic composition and solvent environment of the electrolyte influence key factors such as metal–electrolyte complexation and the stability of the electrode interface. An ideal supporting electrolyte should promote high sensitivity through elevated peak currents, provide well-defined and reproducible redox signals, minimize background current and noise, and exhibit minimal interfering electrochemical activity. To determine the most appropriate electrolyte for Cu (II) detection, four different supporting electrolytes were evaluated: acetate buffer (pH 4.0), potassium chloride (KCl), potassium nitrate (KNO_3), and phosphate-buffered saline (PBS).

As depicted in Figure 1, the acetate buffer yielded only a single, poorly defined reduction peak, suggesting limited electrochemical responsiveness and weak interaction with Cu (II) under the tested conditions. KNO_3 showed a generally low current response, indicating suboptimal

ionic conductivity and inadequate support for efficient electron transfer. In contrast, both KCl and PBS exhibited more distinct and well-defined redox features, with similar peak currents and the same number of redox events. However, the voltammogram recorded in PBS displayed soliton resistance, which can introduce artifacts that compromise measurement accuracy and reproducibility. Given its stable current response, minimal interference, and clearly resolved redox peaks, KCl was identified as the most suitable supporting electrolyte for Cu (II) detection in this study. Over the potential range −1.0 to 1.0 V, two pairs of redox peaks, P1 and P2, were observed for Cu (II). The oxidation peak OP1 appeared at -0.04 V, the oxidation peak OP2 appeared at 0.1 V, and the reduction peaks RP1 and RP2 were observed at -0.31 and -0.08 V, respectively. The findings of this study are consistent with previous literature on the influence of supporting electrolytes on Cu (II) electrochemistry. The voltammetric results obtained in this study align well with the existing literature comparing the performance of different supporting electrolytes for Cu (II) detection. Both KCl and PBS exhibited clearly defined redox peaks; however, the voltammogram recorded in KCl demonstrated sharper peak profiles, a more stable baseline, and less capacitive interference compared to PBS. These differences can be attributed to the simpler ionic composition and higher conductivity of KCl, which minimizes solution resistance and enhances electron transfer kinetics. In contrast, PBS often introduces soliton resistance and baseline drift due to its multicomponent buffer system, which may lead to distorted peak shapes and reduced measurement reproducibility. Similar observations have been reported by Shaikh et al., who found that KCl facilitates well-resolved Cu (II)/Cu (I) and Cu (I)/Cu (0) redox transitions, while PBS tends to suppress or broaden these peaks [21]. Moreover, Liberato et al. emphasized that KCl is preferable in voltammetric studies due to its inertness, high ionic strength, and minimal background interference [22]. Therefore, consistent with both experimental results and literature findings, KCl was identified as the most suitable supporting electrolyte for reliable and accurate Cu (II) detection in this study.

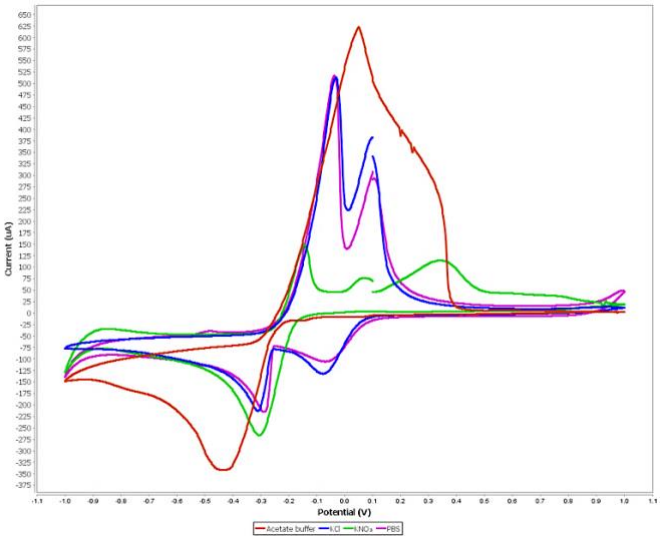

Figure 1. Cyclic voltammograms obtained using different supporting electrolytes at 0.1M. (Cu (II) Concentration: 5 mM; scan range: 1 V to –1 V; step size: 2 mV; scan rate: 50 mV/s).

Electrolyte Concentration

Electrolyte concentration is a key factor in ensuring the accuracy and reliability of electrochemical measurements, particularly in techniques like voltammetry. The cyclic voltammograms presented in Figure 2 illustrate the effect of varying KCl concentrations (ranging from 0.1 M to 3 M) on the electrochemical behavior of Cu (II) ions. As observed, the anodic and cathodic peak positions remain largely unchanged across different electrolyte concentrations, and the overall shape of the voltammograms is preserved. Essentially, a high concentration of supporting electrolyte reduces solution resistance and minimizes the iR drop, thereby preventing distortion of applied potential. This enhanced ionic conduction stabilizes the electrochemical system and improves signal clarity, which is especially important when detecting analytes at low concentrations. As we observed in this study, while there are slight variations in current intensity, these differences are not systematic or significant enough to suggest a meaningful influence from supporting electrolyte concentration. This indicates that the ionic strength provided even by the lowest tested concentration (0.1 M KCl) is sufficient to ensure stable electrochemical conditions, such as adequate ionic conductivity and minimized solution resistance. Based on this finding, 0.1 M KCl was selected as the optimal concentration, as it is sufficient to support the redox process without unnecessary excess of electrolyte.

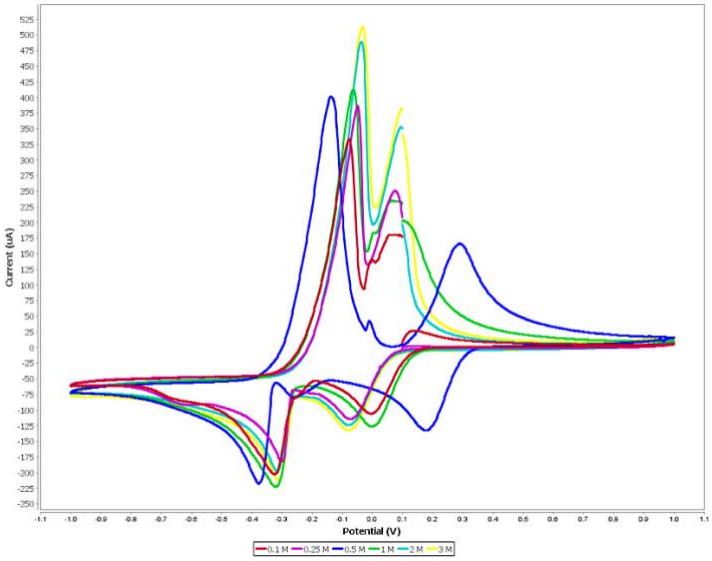

Figure 2. Cyclic voltammograms obtained using different Concentration of KCl. (Cu (II) Concentration: 5 mM; scan range: 1 V to –1 V; step size: 2 mV; scan rate: 50 mV/s).

Scan Rate

The scan rate in CV is a crucial parameter that significantly influences the shape and characteristics of the voltammogram. By varying the scan rate, researchers can gain insights into the kinetics of redox reactions and the electrochemical behavior of the analyte under different dynamic conditions. Higher scan rates can uncover intermediate or transient species that may not be observable at slower rates, providing a more detailed understanding of the reaction mechanism. Therefore, scan rate studies are essential for evaluating and optimizing the performance and efficiency of electrochemical systems.

The cyclic voltammogram in Figure 3 displays the electrochemical behavior of a 5 mM Cu (II) solution recorded at various scan rates (10, 25, 50, 100, and 250 mV/s). A key observation is that as the scan rate increases, the current response, both anodic and cathodic peak currents, is preserved. Importantly, the redox peaks are preserved across all scan rates, indicating the stability and reproducibility of the electrochemical process and chemical behavior of Cu (II) solution recorded at various scan rates. This increase in peak current with scan rate can be explained by the Randles-Sevcik equation, which describes how the peak current (Ip) in a diffusion-controlled electrochemical system is proportional to the square root of the scan rate ($v^{1/2}$). As the scan rate increases, the time available for diffusion decreases, resulting in a thinner diffusion layer and a steeper concentration gradient of electroactive species at the electrode surface. This enhanced gradient leads to a higher flux of species toward the electrode, thereby increasing the current response.

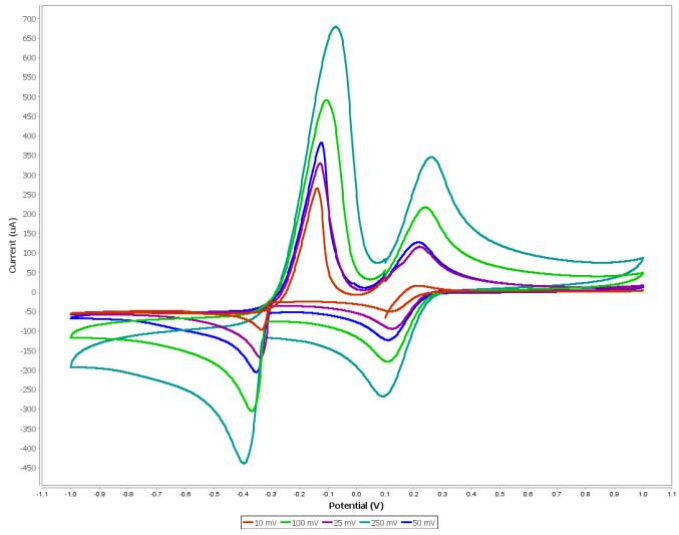

Figure 3. Cyclic voltammograms obtained using different scan rates. (Cu (II) Concentration: 5 mM; scan range: 1 V to –1 V; step size: 2 mV; scan rate: 50 mV/s).

To investigate whether copper is weakly adsorbed on the electrode surface, the slope of the log v - log Ip graph obtained from different scan rates in Figure 4a was found to be 0.2911 and 0.3962 for anodic and cathodic peaks, respectively. according to the Randles-Sevcik equation, this further supports a diffusion-controlled process. If the slope value is in between 0.2 and 0.6, then the process would be diffusion-controlled, and if the slope value lies in the range of 0.75-1.0, then it should be pure adsorption-controlled [23, 24]. The fact that the correlation coefficient (r) of the $v^{1/2}$–i_p plot given in Figure 4b is 0.9989 (close to 1).

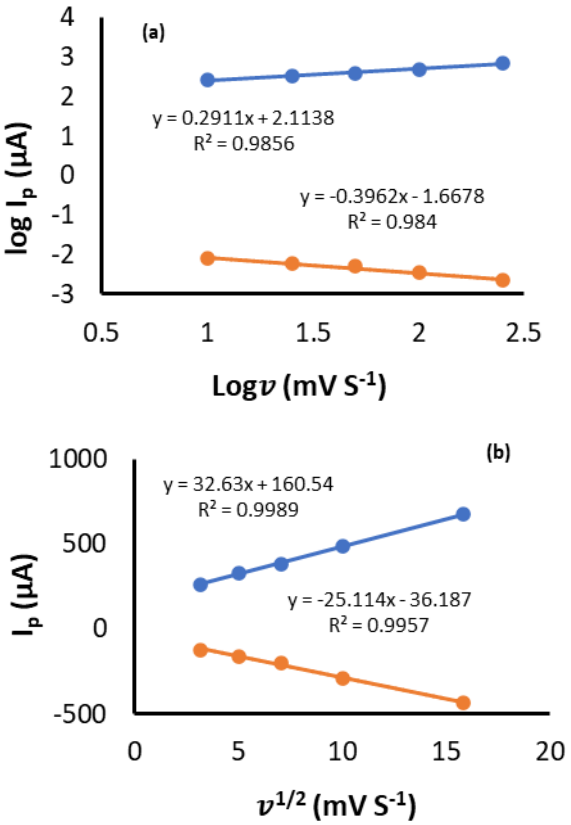

Figure 4. (a) Plot of log anodic and cathodic peak currents versus the log scan rate, (b) plot of anodic and cathodic peak currents versus the square root of the scan rate.

Analytical Figures of Merit

The proposed method was assessed using several key analytical parameters. The linear range of the calibration curves for Cu (II) was determined using Mandel's test at a 95% confidence level. Limits of detection (LOD) and quantification (LOQ) were calculated under the calibration conditions using 15 blank water samples (N=15). To further validate these values, LOD and LOQ were independently confirmed based on signal-to-noise (S/N) ratios, employing the criteria of 3σ for LOD and 10σ for LOQ.

Figure 5 illustrates the calibration voltammogram and corresponding calibration curve of Cu (II), obtained using five standard solutions within the linear concentration range of 0.25-10 mM. As shown in Figure 5, the calibration curve for Cu (II) demonstrated good linearity in the concentration range of 0.25-10.0 mM, with the regression equation A=77.564 [Cu (II)] + 23.270 and a correlation coefficient (R^2=0.997). The percentage recovery was found to be between 95.10 and 105.85%, indicating good accuracy. The method showed acceptable repeatability with an RSD of 4.8%, evaluated at a concentration of 5 mM. LOD and LOQ were determined to be 0.080 mM and 0.244 mM, respectively. Precision calculated using one-way ANOVA revealed a within-day precision of 5.4% and intermediate precision of 6.1%, confirming the reliability of the method.

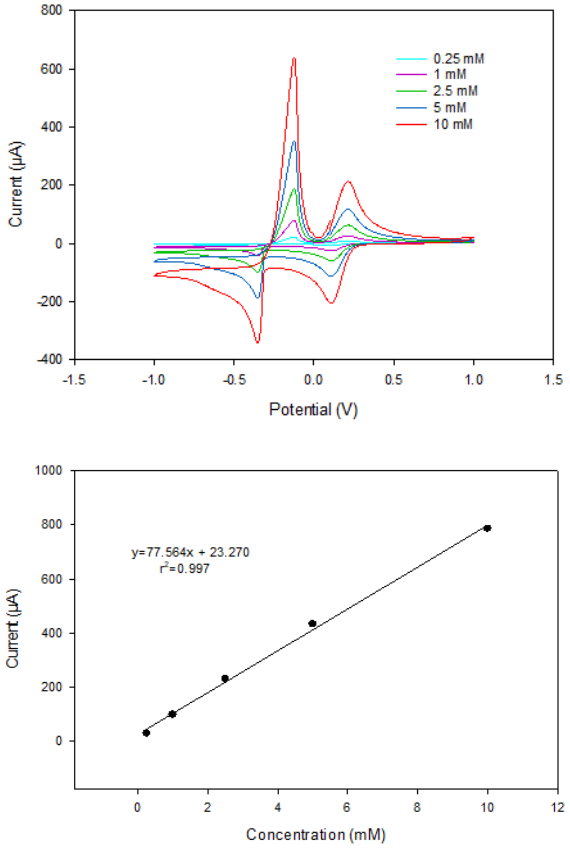

Figure 5. Calibration voltammogram obtained by CV and calibration curve of Cu (II) (Concentration: 0.25 – 10 mM; scan range: 1 V to –1 V; step size: 2 mV; scan rate: 50 mV/s)

Matrix effects (ME) were assessed using Equation 1 by comparing the slopes of two calibration curves: one prepared in pure solvent and the other in a matrix-matched extract.

$$ME\ (\%) = 100 \times \left(\frac{k_a}{k_b} - 1\right) \quad (1)$$

Here, k_a is the slope of the matrix-matched calibration curve, and k_b is the slope of the solvent-based calibration curve. The solvent-based calibration yielded the equation y=77.564x + 23.270 (r^2=0.997) while the matrix-matched calibration produced y=82.256x+22.058 (r^2=0.996). Based on these slopes, the calculated ME (%) (9.43 %) falls within the acceptable range of –20% to +20%, which suggests that no significant signal enhancement or suppression occurred due to matrix effects.

Table 1 presents a comparison between the materials used in this study and various magnetic materials previously reported for Cu (II) removal and preconcentration. In contrast to earlier works, the present study offers a notably simpler and more accessible approach by utilizing SPE without any surface modifications or nanomaterial enhancements. Despite the absence of functional coatings or composite materials, the sensor effectively detects Cu (II) in a complex biological matrix, fish liver, with a LOD of 0.080 mM. Although this LOD is not the lowest among the surveyed studies, it is particularly remarkable given the minimalistic and cost-effective design of the sensor.
Conversely, many previously reported sensors rely extensively on material-based modifications to improve sensitivity and selectivity. Examples include carbon nanotube-modified carbon paste electrodes (1MCPE-CNT) for detecting Cu (II) in water and hair samples [25], hexaazatriphenylene-functionalized SPEs (2HNQP/SPE) for aqueous samples [26], and aurintricarboxylic acid/magnetic bead-modified glassy carbon electrodes (3ATA/MBs/GCE) for marsh and seawater analysis [27]. Additionally, ionophore-modified carbon paste electrodes (Ionophore/MCPE) have been employed for detecting Cu (II) in soil and food simulants [28]. While these advanced sensors achieve impressive LODs, as low as 0.000056 mM, they typically require elaborate preparation procedures, specialized reagents, and higher overall costs.

Table 1. Comparison with other studies

Sensor	Sample	Recovery (%)	LOD (mM)	Linearity Range	Reference
MCPE-CNT	Water samples (tap, well, river) and human hair	95	0.0011	4-200 ng/mL	[25]
HNQP/SPE	Aqueous solutions	-	0.142	20-100 µM	[26]
ATA/MBs/GCE	Marsh and sea water	104 - 116	0.00017	10^{-6}-10^{-4} M	[27]
Lonophore/MCPE	Different soil samples and food simulants from unlined copperware	-	0.000056	$1.0x10^{-7}$-$5.0x10^{-3}$M	[28]
-	Fish liver	95.10-105.85	0.080	0.25 – 10 mM	Present work

MCPE-CNT: Carbon Nanotube-Modified Carbon Paste Electrode, HNQP: complex hexaazatriphenylene derivative, ATA/MBs: Aurintricarboxylic Acid Ammonium Salt/Magnetic Beads, IIP-CPE: Ionophore-Modified Carbon Paste Electrode.

Application of the Proposed Method with Real Sample Analysis

The proposed general procedure was utilized for the recovery and determination of Cu (II) in real samples (Table 2). The proposed electrochemical approach was applied for the extraction and quantification of Cu (II) in real fish liver samples (*Mugil cephalus*) using an unmodified SPE. To validate the accuracy of the method, a comparative analysis was performed using inductively coupled plasma optical emission spectrometry (ICP-OES). The ICP-OES analysis revealed copper concentrations in the liver tissue samples to be 72.18, 31.02, and 55.35 µg/g. Parallel measurements were conducted using CV with the SPE, and the results were compared with those obtained from ICP-OES to evaluate the reliability and practical applicability of the electrochemical sensor. The SPE method yielded copper concentrations within the range of 0.25 mM to 10.00 mM, and each measurement was performed in triplicate under identical experimental conditions to ensure reproducibility. The method demonstrated low limits of detection (LOD) and quantification (LOQ), indicating high sensitivity and precision. Furthermore, the strong correlation observed between the results of the SPE and ICP-OES analyses confirms the high accuracy and robustness of the SPE-based method for copper detection in biological liver matrices.

Table 2. Analysis of Cu (II) in fish (*Mugil cephalus*) liver tissue samples with ICP-OES and a proposed electrochemical method using SPE.

	SPE			ICP-OES (µg/g)
Samples	Spike (µg/g)	Found (µg/g)	Recovery %	
1	317.5	393.9	105.85	72.18
2	317.5	347.0	95.10	31.02
3	317.5	375.1	104.06	55.35

CONCLUSION

This study demonstrates a simple, cost-effective, and reliable electrochemical method for the detection of Cu (II) using an unmodified SPE. By systematically optimizing key parameters, such as supporting electrolyte type and concentration, and scan rate, the method achieved high sensitivity, distinct peak resolution, and consistent quantification without the need for surface modification or nanomaterial enhancement. These findings highlight the potential of unmodified SPEs for a rapid and electrochemical method for the detection of Cu (II) using an unmodified SPE. The approach exhibited robust analytical performance, characterized by an extensive linear range (0.25-10.0 mM), a minimal detection limit (0.080 mM), and excellent reproducibility, as validated by statistical analysis. The sensor demonstrated significant application in liver samples of *Mugil cephalus*, attaining a range of 95.10-105.85% without requiring surface modification or nanomaterial enhancement.

The accuracy of the SPE method for quantifying copper concentrations was confirmed through comparison with ICP-OES results. A strong correlation between the two sets of data demonstrated the reliability and precision of the SPE for detecting copper in liver samples. Compared to other reported sensors, this method offers a simpler, more reliable alternative, making it well-suited for on-site applications in environmental and food safety monitoring.

CONFLICT of INTEREST

The authors report there are no competing interests to declare.

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