RESEARCH PAPER

Development of a Biosensor for Real-time Detection of Spermine as a Cancer Biomarker using Copper oxide Nanoparticles and Aptamer-Modified Paper Electrodes

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ABSTRACT

Polyamines such as spermine are critically involved in cellular proliferation and are frequently overexpressed in various malignancies, making them promising biomarkers for early cancer diagnosis and prognosis. In this study, we report the development of a sensitive, selective, and low-cost electrochemical biosensor for real-time detection of spermine using copper oxide (CuO) nanoparticle–decorated, aptamer-modified paper-based electrodes. CuO nanoparticles were employed to enhance the effective surface area and electrocatalytic activity of the electrode, facilitating rapid electron transfer, while a spermine-specific DNA aptamer provided high molecular recognition and binding affinity toward the target analyte. The synergistic integration of CuO nanoparticles with aptamer functionalisation enabled efficient immobilisation, improved signal amplification, and excellent analytical performance. The fabricated biosensor exhibited a wide linear detection range with a low limit of detection, high sensitivity, and good reproducibility. Importantly, the sensor demonstrated rapid response time and strong selectivity against structurally related polyamines and common interfering species. Real-sample applicability was validated by successful detection of spermine in spiked biological samples, indicating its potential for non- invasive cancer biomarker analysis. Owing to its simplicity, portability, and real-time sensing capability, the proposed CuO nanoparticle–aptamer paper electrode biosensor offers a promising platform for point-of-care cancer diagnostics and early disease monitoring.

Keywords: Cancer, Spermine, Nanoparticles, Biosensor.

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INTRODUCTION

In mammals, the primary polyamines-putrescine, spermidine, and spermine play vital role in cellular function or survival, playing key roles in DNA replication, transcription, translation, and post - translational modifications. [1] Overexpression of spermine has been extensively studied as a significant biomarker for cancer diagnosis and therapy. However, the real-time bioimaging of spermine in living cells continues to present considerable challenges. [2] Detecting biogenic amines like spermine through traditional methods like GC-MS, HPLC, and capillary electrophoresis, while sensitive, require complex sample preparation, costly equipment, and lengthy procedures, making them less practical for rapid or point-of-care use. To address these limitations, molecular aptamer-based sensors and nanoparticles have emerged as promising alternatives, offering simple, cost-effective, and portable detection with improved sensitivity and real-time monitoring capabilities. [3]

Nanotechnological advances have enabled the development of nanoscale biosensors that directly interact with biomolecules or analytes, offering superior properties such as enhanced conductivity, high sensitivity, rapid response, and customizable magnetic, electrical, and optical characteristics compared to traditional biosensors. [4]

Biosensors are sophisticated analytical devices that has incorporated a range of recognition elements, including enzymes, aptamers, antibodies, and specific functional proteins. [5] The conjugation of aptamers on nanomaterials has enabled the development of highly sensitive and selective aptasensors. [6]

In the aptamer-based biosensor, the aptamer serves as the biosensor recognition element, detecting and binding the target with high specificity and affinity, while avoiding interference from other biological matrices. [7]

Aptamers are increasingly recognized as versatile molecular recognition agents with broad applications in diagnostics, therapeutics, and biosensing. Their unique properties-such as high target specificity, minimal immunogenicity along with ease of chemical modification- which make an attractive alternatives to conventional antibodies.

Copper (II) oxide nanomaterial have obtained broad prominence in biosensor research due to their

exceptional physicochemical and electrochemical characteristics, making them ideal candidates for electrode modification and biomarker detection. [8] CuO is a p-type semiconductor having low band gap, high charge mobility additionally superior catalytic properties, which promote efficient electron transfer during electrochemical reactions. [9]

The monoclinic crystalline structure of CuO facilitates rapid electron–hole pair separation, enhancing its electrochemical responsiveness when used in nanoscale biosensing interfaces.[10] various synthesis pathways, for example sol-gel, hydrothermal, and co-precipitation, and green synthesis have been widely employed to achieve CuO Nanosized materials with controlled particle size, morphology as well as crystallinity. [11] Among these, biologically mediated or Green preparation approaches are now extensively adopted due to their minimal environmental impact and affordability, utilizing plant or microbial extracts to stabilize nanoparticle growth and reduce toxicity. [12] The synthesized CuO nanoparticles typically exhibit spherical or rod-like structures ranging between 10–50 nm, that exhibit a high surface area compared to their volume and numerous active sites for biomolecule immobilization.[13]

In biosensor applications, CuO nanoparticles serve as an efficient electron mediator at the electrode interface, enhancing electrochemical signal transduction and sensitivity.[14] When integrated with biorecognition elements such as aptamers, the CuO surface facilitates strong immobilization through functional groups like –COOH and –OH, maintaining aptamer stability and bioactivity. [15] Aptamer-functionalized CuO nanocomposites have demonstrated superior selectivity and sensitivity toward small biomolecules including spermine, which is a polyamine biomarker associated with several cancers such as prostate and colorectal cancer.[16] So the aim of study was to develop a biosensor for real time detection of spermine as a cancer biomarker using copper oxide nanoparticles and aptamer modified paper electrodes, and objectives were : i) To synthesize and characterization of copper oxide nanoparticles, ii) Immobilization of aptamer and copper oxide nanoparticles on paper electrode to construct as a working electrode, detection of spermine in spike sample by using working electrode.

MATERIALS AND METHOD

The present study was carried out at the Maharishi Markandeshwar Institute of Medical Sciences and Research, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala, Haryana. The institution provided all the requisite infrastructure and research facilities necessary for successful execution of the study. Prior to initiation, ethical approval was obtained from the Institutional Ethics Committee.

1. Chemicals and Reagents

Copper sulfate, sodium hydroxide, ethanol, and acetone were purchased from LOBA Chemie and utilized for preparing phosphate-buffered saline (PBS, pH 7.2) containing 28 mM KCl, 136 mM NaCl, 17 mM KH_2PO_4, and 0.9 mM Na_2HPO_4. The spermine-specific aptamer, potassium ferricyanide, and Tris buffer were obtained from Sigma-Aldrich. Milli-Q grade water was used for the preparation of all reagents and solutions. The nucleotide sequence of the spermine aptamer was 5′-TCATCTATCTATGGTACATTACTATCTGTAATG TGATATG-3′.[14] Screen-printed paper electrodes (SPPEs) were procured from Class One Systems, New Delhi, India. Subsequent surface modification and fabrication steps were performed at the Biosensor Technology Laboratory, Central Research Laboratory, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala, Haryana.

2. Methods

2.1 Synthesis of Copper Oxide nanoparticles

CuO-NPs were synthesized via the hydrothermal technique. Initially, copper sulfate (1 g) was dissolve in 40 mL of deionized water to obtain a uniform blue-colored solution. The solution was stirred using a magnetic stirrer for several minutes to ensure complete dissolution. Next, 1 g of potassium hydroxide (KOH) was dissolved in 90 mL of deionized water. The KOH solution was then added dropwise to the copper sulfate solution until the pH reached 10. The addition of potassium hydroxide induced a noticeable color change in the solution. The resulting mixture was stirred at 90 °C for 2 hours, leading to the transformation of the blue solution into a dark precipitate. The precipitate was then centrifuged three times at 3500 rpm for 10 minutes and washed with double-distilled water (DDW). The obtained black product was dried in an oven at 80 °C for 18 hours. Finally, the dried powder was calcined at 400 °C for 4 hours to obtain CuO-NPs.[17]

2.2 Instruments

Sample separation was performed using a remi centrifuge, and dispersion of materials was achieved with an ultrasonic probe sonicator (Athena, Athena Technology). Accurate weighing of chemicals was done using a Wesnar digital weighing machine, while solution preparation was carried out on a remi magnetic stirrer. Optical analysis was performed using a UV–Visible spectrophotometer (UV-2600). Structural characterization was carried out by X-ray diffraction (XRD) at Panjab University, Chandigarh. The pH of solutions was measured using a Labtronics pH meter, and sterilization was done using an Apex autoclave. Electrochemical studies was conducted using a Palsenspotentiostat/galvanostat, and functional group analysis was performed using FTIR spectroscopy with a QATR-S accessory.

2.3 Characterization of Copper Oxide nanoparticles

Characterization of copper oxide (CuO) nanoparticles was carried out using UV–Visible spectroscopy, X-ray diffraction (XRD), and Fourier transform infrared (FTIR) spectroscopy. UV–Visible analysis was performed to study the optical properties and confirm

the formation of CuO nanoparticles by observing the characteristic absorption band in the UV region. The crystalline nature, phase purity, and average crystallite size of the synthesized nanoparticles were determined using XRD analysis, where diffraction peaks corresponding to the monoclinic structure of CuO confirmed successful synthesis. The presence of surface functional groups and Cu–O stretching bands in CuO nanoparticles was confirmed using FTIR spectroscopy. Together, these techniques provided broad knowledge about optical, structural and chemical characteristics of the CuO nanoparticles.

2.4 Electrochemical Activation of Screen-Printed Paper Electrode (SPPE)

Screen-printed carbon electrodes was purchased from Class One systems. Activation of SPCEs consisted of 25 repetitive cyclic voltammetric at the 10mVs−1 scan rate between +1.0 and −0.7V (vs Ag) in 10mM H2O2 (in 0.1MPBS, pH7). After activation, electrodes were rinsed with deionized water and left to dry naturally.

2.5 Modification of Screen-Printed Paper Electrode using CuO-NPs

For fabrication of the electrochemical biosensor, a carbon-based screen-printed paper electrode comprising a working electrode, counter electrode, and an Ag/AgCl reference electrode was utilized. Before modification, the working electrode was carefully cleaned using 70% ethanol followed by distilled water to remove surface-bound contaminants. A homogeneous suspension of CuO nanoparticles (CuO-NPs) was prepared in ethanol, and 6 µL of this suspension was drop-cast onto the surface of the working electrode and allowed to dry at ambient temperature. After drying, the electrode was gently rinsed with distilled water to remove any weakly attached CuO-NPs.

This method enabled effective immobilization of CuO-NPs on the working electrode surface, facilitating the assembly of the electrochemical biosensor. The stability and reproducibility of the CuO-NPs-modified electrodes were systematically assessed. The integration of CuO-NPs markedly increases the effective surface area, sensitivity, and selectivity of the biosensor, thereby enhancing its analytical performance for target biomolecule detection. Furthermore, non-covalent functionalization of CuO-NPs offers a significant advantage by introducing surface functional groups without altering their structural or electronic integrity. Consequently, this strategy maintains the inherent properties of CuO-NPs while allowing flexible surface modification for a wide range of biosensing applications.

2.6 Immobilization of aptamers onto copper oxide/Paper electrode

Spermine-specific aptamers were immobilized onto the copper oxide nanoparticle (CuO-NPs) modified paper electrode using a drop-casting method. Briefly, 6 µL of the aptamer solution was drop-cast onto the surface of the CuO-NPs/paper electrode and allowed to incubate for 12 h in the dark at 4 °C to ensure effective immobilization. After incubation, the electrode was thoroughly rinsed with Tris-EDTA buffer, followed by washing with double-distilled water, to remove any physically adsorbed or unbound aptamer molecules. The modified electrode was then dried at room temperature. Electrochemical characterization of the fabricated aptamer/CuO-NPs/paper electrode was carried out using cyclic voltammetry (CV) in the presence of a redox indicator (methylene blue) to confirm successful aptamer immobilization and assess the electrochemical activity of the electrode.

2.7 Spiked Samples for Spermine Detection

For method validation and performance evaluation of the developed aptasensor, spiked samples of spermine were commercially purchased from Sigma-Aldrich. These samples was used to represent known concentrations of spermine under controlled conditions. The samples were subsequently employed for cyclic voltammetric measurements to evaluate the sensitivity, selectivity, and practical applicability of the CuO nanoparticle–aptamer-modified paper electrode for spermine detection.

2.8 Cyclic voltametric measurement and testing of aptasensor

Cyclic voltammetry measurements were recorded using a potentiostat–galvanostat with an aptamer/CuO-NPs/paper electrode as the working electrode, Ag/AgCl as the reference electrode, and platinum as the counter electrode. The measurements were carried out in the potential range of −0.2 to 0.8 V in 15 mL of 0.05 M sodium phosphate buffer (pH 7.0) containing 0.15 mM copper (0.5 mL). The addition of the spermine solution initiated the reaction, which was monitored at the optimal voltage.

RESULTS AND DISCUSSION

Morphological and Structural Characterization of Copper Oxide Nanoparticles by Transmission Electron Microscopy

Transmission Electron Microscopy (TEM) was employed to investigate the morphology and size distribution of the synthesized copper oxide (CuO) nanoparticles is 50 nm. The particles appear well dispersed with relatively uniform contrast, indicating good crystallinity. The average particle size is in the nanometer range, as confirmed by the scale bar of 50 nm, demonstrating successful synthesis of nanosized CuO (Fig. 1) The nanoscale dimensions and high surface area of the CuO nanoparticles are advantageous for biosensor applications, as they facilitate efficient electron transfer and provide an enhanced surface for aptamer immobilization, thereby improving the sensitivity of spermine detection as a cancer biomarker

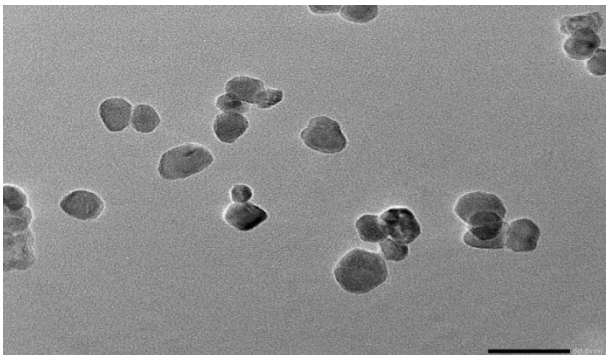

Figure 1. TEM images prepared CuO nanoparticles

Morphological Characterization of Copper Oxide (CuO) Nanoparticles by Scanning Electron Microscopy (SEM)

SEM analysis was performed to evaluate the surface morphology and microstructural features of the synthesized CuO nanoparticles. The SEM study confirms that CuO nanoparticles were successfully formed and have suitable size and structure for use in biosensor and electrochemical applications. The particles are distributed unevenly, forming clusters of varying sizes, which can be attributed to strong interparticle interactions and high surface energy inherent to metal oxide nanoparticles. The rough and porous surface texture observed in the SEM image is advantageous for electrochemical biosensor applications, as it provides an increased effective surface area for aptamer immobilization and facilitates enhanced electron transfer (Fig .2). Such morphological characteristics are expected to improve the sensitivity and real-time detection capability of the CuO nanoparticle-based paper electrode for spermine sensing as a cancer biomarker.

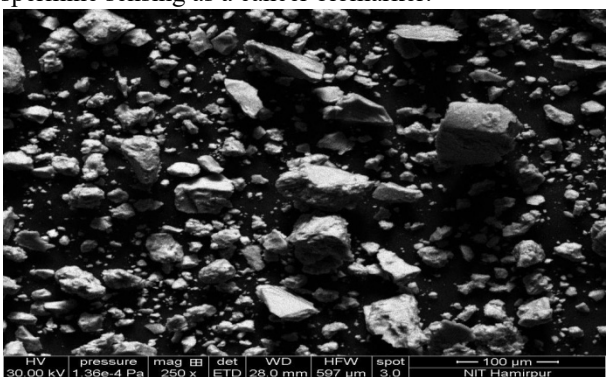

Figure 2. SEM images of characterized CuOnanoparticlesFunctional Group

Analysis of CuO Nanoparticles by Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was used to identify the functional groups and confirm the chemical composition of the synthesized copper oxide (CuO) nanoparticles. The FTIR spectrum exhibits a broad absorption band around 3449 cm^{-1}, which is attributed to the stretching vibrations of hydroxyl (–OH) groups arising from adsorbed moisture or surface hydroxylation. The peak observed at 1702 cm^{-1} may be associated with residual carbonyl (C=O) stretching from precursor materials, while bands at 1647, 1550, and 1423 cm^{-1} correspond to bending vibrations of adsorbed water molecules and possible surface-bound organic species (Fig. 3). Additional peaks in the region of 1363–1092 cm^{-1} indicate C–O and C–N stretching vibrations, suggesting the presence of trace organic residues that may assist in nanoparticle stabilization. Most importantly, the characteristic absorption bands observed in the low wavenumber region between 487 and 407 cm^{-1} confirm the Cu–O stretching vibrations, validating the successful formation of CuO nanoparticles. The presence of surface functional groups is beneficial for aptamer immobilization, enhancing the interaction between CuO-modified paper electrodes and spermine, thereby improving the biosensor's sensitivity and real-time detection performance.

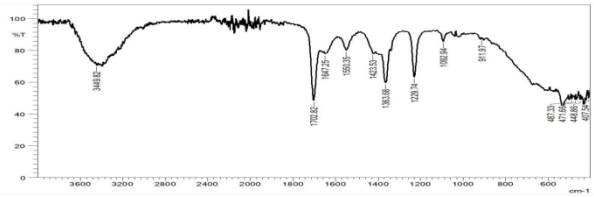

Figure 3. Fourier Transform Infrared (FTIR) spectra of synthesized copper oxide (CuO)Crystalline

Structure Analysis of Copper Oxide (CuO) Nanoparticles by X-Ray Diffraction (XRD)

X-ray Diffraction (XRD) analysis was carried out to investigate the crystalline structure and phase purity of the synthesized copper oxide (CuO) nanoparticles. In this study the standard XRD pattern exhibits well-defined diffraction peaks at 2θ values corresponding to the crystallographic planes (110), (−111), (002), (111), (200), (−202), (020), (−113), (022), (−311), (311), (220), (004), and (222). These peaks are in good agreement with the monoclinic crystal structure of CuO and match the standard JCPDS card no. 45-0937, confirming the successful formation of phase-pure CuO nanoparticles without detectable impurity phases. The sharp and intense diffraction peaks indicate high crystallinity of the synthesized nanoparticles, which is desirable for electrochemical applications. The crystalline nature of CuO facilitates efficient charge transport and electron transfer at the electrode interface, thereby enhancing the electrochemical performance of the aptamer- modified paper electrode for real-time detection of spermine as a cancer biomarker (Fig. 4).

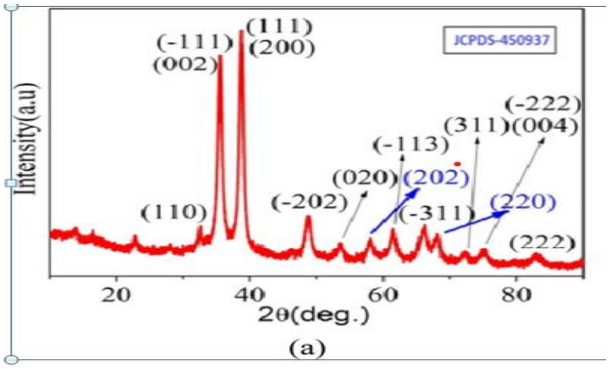

Figure 4. Standard XRD spectra of CuO nanoparticles

Intensity

Cyclic voltametric response of the bare screen-printed paper electrode (Baseline measurement)

Cyclic voltammetric (CV) response of the bare paper electrode recorded in buffer solution under control conditions, without modification by copper oxide nanoparticles or aptamer immobilization. The voltammogram exhibits a relatively low and broad redox current response with poorly defined anodic and cathodic peaks, indicating limited electrochemical activity of the unmodified paper electrode. This behavior is attributed to the absence of catalytic nanomaterials and recognition elements on the electrode surface, resulting in slow electron transfer kinetics. The observed baseline current serves as a reference for evaluating the enhancement in electrochemical performance after CuO nanoparticle deposition and aptamer functionalization (Fig.5). Establishing this control response is essential to demonstrate the role of CuO nanoparticles in improving conductivity and the specificity of the aptamer-modified paper electrode toward the real-time detection of spermine as a cancer biomarker.

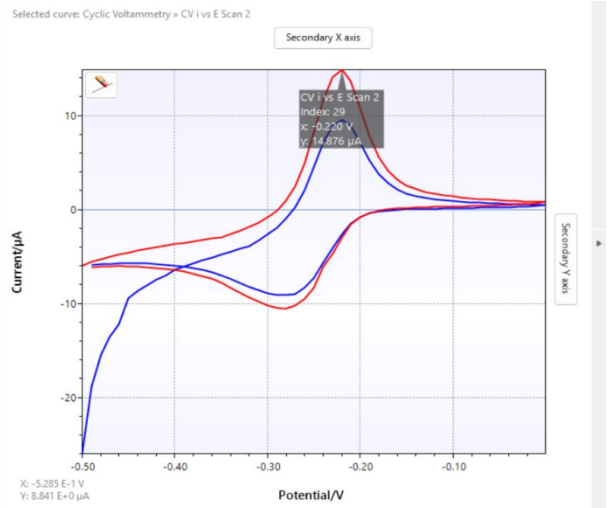

Figure 5. Cyclic voltammetric response of the bare screen-printed paper electrode (baseline measurement)

Cyclic Voltammetric Response of immobilized CuO Nano particles on paper electrode

The cyclic voltammetric (CV) response of the CuO nanoparticle paper electrode recorded in the presence of spermine. Compared to the control electrode, a significant enhancement in both anodic and cathodic peak currents is observed, indicating improved electrochemical activity upon target binding. The well-defined redox peaks and increased current response suggest effective electron transfer facilitated by the CuO nanoparticles and specific interaction between spermine and the immobilized aptamer. The shift in peak potentials and increase in peak current further confirm the successful recognition of spermine at the electrode interface (Fig. 6). These results demonstrate the high sensitivity of the developed biosensor and validate its capability for real-time electrochemical detection of spermine as a potential cancer biomarker.

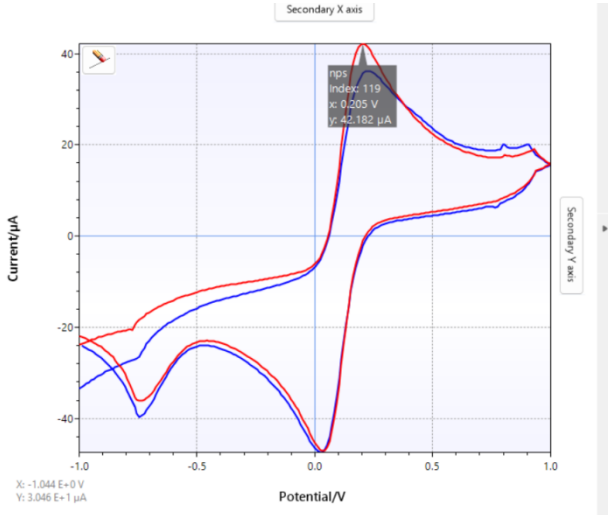

Figure 6. Cyclic voltammetric response of CuO nanoparticle-immobilized paper electrodes

CV response of immobilized spermine specific aptamer

The cyclic voltammetric (CV) response of the aptamer-modified paper electrode recorded in the presence of spermine. The voltammogram exhibits a substantial enhancement in both oxidation and reduction peak currents relative to the unmodified electrode, indicating effective attachment of the aptamer onto the surface of the paper electrode.The enhanced current response and well-defined redox peaks indicate strong and specific interaction between spermine molecules and the surface-bound aptamer. The presence of CuO nanoparticles beneath the aptamer layer facilitates efficient electron transfer, while the aptamer provides molecular recognition, resulting in improved sensitivity and selectivity of the biosensor (Fig. 7). These results demonstrate the effective role of aptamer functionalization in enabling selective and real-time electrochemical detection of spermine as a potential cancer biomarker.

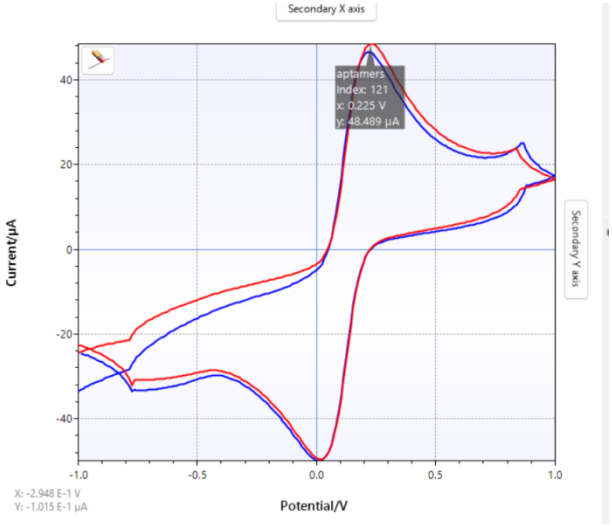

Figure 7. Cyclic voltammetric response of spermine-specific aptamer-immobilized paper electrodes

Electrochemical studies cyclic voltametric (CV) of prepared biosensor

The electrochemical behavior of the developed biosensor was investigated using cyclic voltammetry (CV) to evaluate each step of electrode modification. The bare paper electrode exhibited a relatively low redox current, indicating limited electroactive surface area and poor electron transfer capability. Upon modification with copper oxide nanoparticles (CuO-NPs), a significant increase in peak current was observed, which can be attributed to the high surface area, excellent conductivity, and enhanced electrocatalytic activity of CuO- NPs, facilitating faster electron transfer at the electrode–electrolyte interface (Fig. 8).

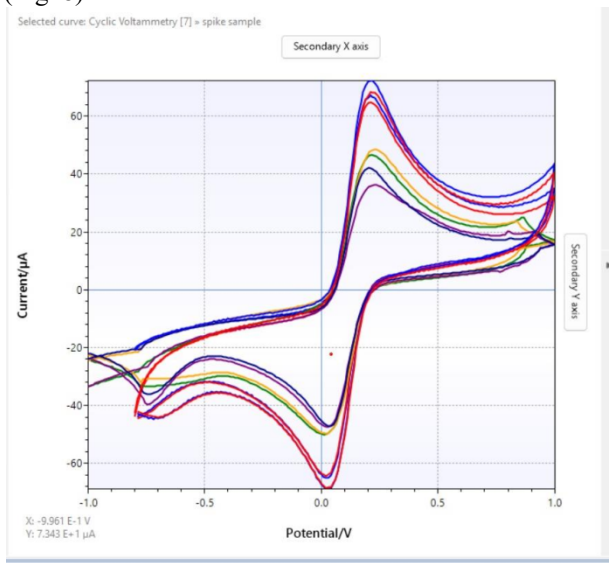

Figure 8. Cyclic voltammetry (CV) response of bare paper electrode, CuO- nanoparticle-modified electrode, aptamer/CuO-NPs-modified electrode, and spermine-spiked samples recorded in phosphate buffer solution.

After immobilization of spermine-specific aptamers onto the CuO-NP-modified electrode, a noticeable decrease in current response was recorded, which is ascribed to the insulating nature of the aptamer layer that partially hinders electron transfer. Furthermore, upon the addition of spermine (spiked samples), distinct changes in the anodic and cathodic peak currents were observed, confirming the specific interaction between spermine and the immobilized aptamers. This binding event alters the interfacial charge transfer behavior, demonstrating the successful fabrication and sensing capability of the aptamer/CuO-NPs- based electrochemical biosensor for spermine detection. Polyamines are small aliphatic molecules that are indispensable for normal cellular physiology, playing critical roles in DNA stabilization, RNA transcription, protein synthesis, and regulation of cell proliferation. Among these, spermine is of particular biological significance due to its higher intracellular concentration and stronger polycationic nature compared to other polyamines. In cancerous conditions, dysregulation of polyamine metabolism leads to elevated spermine levels as a consequence of enhanced biosynthesis and reduced degradation. Such alterations support rapid tumor growth and survival, making spermine a valuable biomarker for cancer detection and progression monitoring. The present study focused on developing a paper-based electrochemical biosensor for real-time detection of spermine using copper oxide nanoparticles and an aptamer-modified working electrode

DISCUSSION

The discussion presented here critically evaluates the experimental findings in relation to the study objectives, with special emphasis on electrochemical behavior observed through potentiostat measurements, the role of nanomaterials and aptamers in signal generation, and the diagnostic relevance of the observed responses.

Cancer-associated dysregulation of polyamine metabolism is now well established, with multiple studies demonstrating that rapidly proliferating tumor cells exhibit enhanced biosynthesis and accumulation of polyamines to sustain growth and survival. Gerner and Meyskens (2004) described polyamines as central regulators of cellular proliferation and transformation, highlighting the overexpression of key enzymes such as ornithine decarboxylase and S-adenosylmethionine decarboxylase in a wide range of malignancies. [18]

Similarly, Jr Cassero et al. (2019) reported that elevated intracellular polyamine pools correlate strongly with tumor aggressiveness and poor clinical outcomes, reinforcing the concept that altered polyamine homeostasis is a hallmark of cancer. Clinical metabolomic investigations further support this association;[19] Hiramatsu et al. (2005) identified urinary N^1,N^{12}-diacetylspermine as a significantly increased metabolite in patients with diverse cancers, [20] while Tse et al. (2022) demonstrated that both spermine and its acetylated derivatives are linked to

cancer staging and diagnosis in colorectal, lung, breast, liver, and urogenital malignancies. [21] These findings are in strong agreement with the present study, which exploits spermine as a target biomarker for cancer detection. The use of aptamers as recognition elements is justified by their high affinity, specificity, and synthetic reproducibility, as first established through the SELEX methodology, and subsequently validated in numerous biosensing applications. Moreover, the incorporation of nanomaterials into electrochemical sensors has been shown to markedly enhance analytical performance.[22]

Wang (2006) demonstrated that nanomaterial-modified electrodes significantly improve electron-transfer kinetics and sensitivity,[22] while it as been reported that metal- oxide nanoparticles are particularly effective for detecting small-molecule analytes. Consistent with these reports, the integration of CuO nanoparticles within the present aptamer-based platform contributes to improved signal amplification and lower detection limits. In addition, the selection of screen-printed electrodes is supported by previous work emphasizing their low cost, portability, and suitability for point-of-care [23] diagnostics. Collectively, these literature findings corroborate the design rationale of the present biosensor and highlight its potential as a sensitive, non-invasive, and clinically relevant tool for spermine-based cancer screening.[24]

Definition and Importance of Control in the Present Study

In electrochemical biosensing, the control is essential for establishing baseline behavior and validating sensor specificity. In the present study, the control consisted of a blank electrolyte solution that did not contain spermine. This control system retained all components of the sensing platform, namely the paper electrode, copper oxide nanoparticles, and immobilized aptamer, except for the target analyte.

The purpose of using such a control was to confirm that any electrochemical response observed during potentiostat analysis arises solely from spermine–aptamer interaction and not from nonspecific adsorption, background redox activity, or intrinsic electrochemical behavior of the electrode materials. The absence of any electrochemical peak in control measurements demonstrates that the modified electrode remains electrochemically silent under target -free conditions, thereby establishing a stable and noise-free baseline. [25]

From an analytical standpoint, this behavior is critical, as false-positive signals can severely compromise diagnostic reliability. Clinically, a sensor that produces a signal in the absence of a biomarker would be unsuitable for cancer screening. Therefore, the clear lack of response in the control strongly supports the validity and reliability of the developed biosensor.

Electrochemical Response and Interpretation of Potentiostat Peaks

Electrochemical sensing is based on monitoring changes in current or potential that occur due to redox reactions or interfacial charge transfer at the electrode surface. In potentiostat-based analysis, the appearance of an electrochemical peak represents a distinct electron transfer event, which may arise from analyte oxidation or reduction or from changes in the electrode–electrolyte interface following molecular binding.

In the present study, no electrochemical peak was detected in control samples, whereas a distinct and reproducible peak appeared in spermine-spiked samples. This clear contrast is of central importance. The lack of peaks in the control confirms that copper oxide nanoparticles and aptamer-functionalized paper electrodes do not independently undergo redox reactions within the applied potential range. This observation eliminates background interference and confirms that the sensing platform is inherently stable.The emergence of a peak in spermine-spiked samples indicates that binding of spermine to the aptamer induces a measurable electrochemical change. This peak reflects modifications in electron transfer kinetics at the electrode surface resulting from specific molecular recognition. Thus, the peak serves as a direct and reliable indicator of spermine presence Mechanism of Signal Generation through Spermine–Aptamer Interaction. [26]

Spermine is a highly polycationic molecule containing multiple amine groups, which allows strong electrostatic interactions with the negatively charged phosphate backbone of nucleic acid aptamers. Upon binding to the immobilized aptamer, spermine induces conformational rearrangements in the aptamer structure. These changes modify surface charge distribution and affect accessibility of the electrode surface to the electrolyte. Such interfacial modifications influence the rate and efficiency of electron transfer between the electrode and the surrounding medium, resulting in the appearance of a distinct electrochemical peak. The reproducibility of this peak across repeated measurements indicates that the spermine–aptamer interaction is stable and consistent, reinforcing confidence in the sensing mechanism. [27]

Role of Copper Oxide Nanoparticles in Enhancing Sensor Performance

Copper oxide nanoparticles played a crucial role in enhancing the electrochemical response of the biosensor. Their nanoscale dimensions provide a large effective surface area, allowing higher aptamer loading and increased probability of spermine binding. Additionally, CuO nanoparticles exhibit favorable semiconducting properties that facilitate rapid electron transfer.

Importantly, CuO nanoparticles do not contribute to electrochemical activity in the absence of spermine, as demonstrated by the flat baseline observed in control samples. Upon spermine binding, however, they efficiently transduce molecular interactions into electrical signals, resulting in a well-defined

electrochemical peak. This confirms their role as signal amplifiers rather than independent electroactive entities. [28]

Selectivity of the Biosensor

The clear differentiation between control and spermine-spiked sample responses highlights the high selectivity of the biosensor. Selectivity is especially critical in cancer biomarker detection, as biological samples contain numerous potentially interfering substances. The absence of electrochemical peaks in the control demonstrates that the biosensor does not respond to unrelated molecules present in the sample matrix.

This selectivity is primarily attributed to the use of aptamers, which offer high affinity and specificity toward their target molecules. Compared to antibodies, aptamers are more chemically stable, less prone to denaturation, and exhibit lower batch-to-batch variability. These characteristics make aptamers particularly suitable for electrochemical biosensing applications and contribute to the reliable detection of spermine observed in this study. [29]

Detection of Spermine in Spiked Samples

The successful detection of spermine in spiked samples confirms the functional capability of the working electrode. Spiking experiments simulate physiological conditions and provide an initial assessment of sensor performance in complex matrices, where various endogenous components may otherwise interfere with analyte recognition. The appearance of a clear and well-defined electrochemical peak only in spiked samples demonstrates that the biosensor is capable of selectively detecting spermine under conditions relevant to biological analysis, while maintaining a stable baseline in the absence of the target analyte. The reproducibility of the electrochemical response in spiked samples further indicates the reliability and robustness of the sensing platform. Consistent peak generation suggests that the spermine–aptamer interaction is stable and that the electrode modification strategy supports effective signal transduction. [30] These attributes are essential for practical biosensing applications, where repeatability and analytical confidence are critical. This ability to detect spermine rapidly and reliably supports the application of the biosensor for real-time analysis, which is particularly important for clinical monitoring and diagnostic decision-making. Real- time detection enables prompt assessment of biomarker levels, facilitating early intervention and timely evaluation of disease progression or therapeutic response. The rapid electrochemical readout also reduces analysis time compared to conventional laboratory- based methods, highlighting the potential of the developed biosensor for point -of-care and decentralized diagnostic applications. [31]

Clinical Relevance and Point-of-Care Potential

Elevated spermine levels have been reported in multiple cancer types due to increased polyamine biosynthesis and impaired catabolism. Therefore, reliable spermine detection has important diagnostic and prognostic implications. The biosensor developed in this study demonstrates a clear electrochemical response exclusively in the presence of spermine, indicating its potential utility in cancer screening and disease monitoring. The ability of the sensor to generate a distinct electrochemical signal only upon specific spermine recognition enables accurate discrimination between normal and pathological conditions, which is essential for early-stage cancer detection.

Furthermore, the sensitivity of the biosensor to spermine-induced electrochemical changes suggests its applicability in monitoring disease progression and therapeutic response. Variations in spermine concentration over time may reflect tumor growth dynamics or response to treatment, and a rapid detection platform such as the one developed in this study could facilitate timely clinical decision-making. The real-time electrochemical readout also allows repeated measurements without significant sample consumption, which is advantageous for longitudinal patient monitoring. [32] The paper-based nature of the electrode further enhances its suitability for point-of-care applications. Paper electrodes are inexpensive, lightweight, disposable, and easy to fabricate, making them particularly attractive for decentralized testing environments. Their disposable nature minimizes the risk of cross-contamination and eliminates the need for extensive cleaning or regeneration steps between measurements.

Unlike conventional analytical techniques that require complex instrumentation, highly trained personnel, and extensive sample preparation, this platform offers rapid detection with minimal resource requirements. The simplicity of operation and portability of paper-based electrochemical sensors enable their integration with compact, handheld potentiostats, allowing on-site analysis and immediate interpretation of results. This feature is especially valuable in resource-limited settings, outpatient clinics, and large-scale screening programs. Overall, the combination of selective spermine detection, rapid electrochemical response, and a low-cost paper-based platform positions the developed biosensor as a promising tool for practical cancer diagnostics. [33] Its ease of use, affordability, and adaptability highlight its potential to improve access to early cancer detection and to support personalized disease monitoring strategies in real-world clinical settings.

SUMMARY

The present study aimed to develop a simple, selective, and low-cost paper-based electrochemical biosensor for real-time spermine detection using copper oxide (CuO) nanoparticles and an aptamer-modified working electrode.

The biosensor was fabricated on a paper electrode platform to ensure affordability, disposability, and suitability for point-of-care applications. CuO nanoparticles were employed to enhance surface area and electron transfer efficiency, while a spermine-

specific aptamer provided selective molecular recognition. Electrochemical performance was evaluated using potentiostat-based measurements, with emphasis on signal specificity, stability, and diagnostic relevance.

From a clinical perspective, the developed biosensor shows strong potential for cancer diagnostics and disease monitoring. Rapid electrochemical readout enables near real-time analysis, while the paper-based format offers advantages such as low cost, portability, disposability, and ease of use. These features make the platform well suited for point-of-care testing and deployment in resource-limited settings.

Despite its promising performance, further validation using clinical samples is required to establish diagnostic accuracy. Interference studies with structurally related polyamines such as spermidine and putrescine, along with optimization of sensor architecture and assessment of long-term stability, will be essential for translational development.

In conclusion, this study demonstrates the successful development of a CuO nanoparticle- and aptamer-modified paper-based electrochemical biosensor for selective spermine detection. The absence of background signals in control samples and the presence of distinct electrochemical peaks in spermine-containing samples confirm specific molecular recognition and efficient signal transduction. Overall, the findings highlight the potential of this biosensor as a reliable, cost-effective, and practical platform for real-time spermine detection in cancer diagnostics.

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