

Fabrication of Polyvinyl Alcohol Doped CuO Thin Films for Improved Amperometric-Non Enzymatic Hydrogen Peroxide Sensing

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Abstract. Nanotechnology is an emerging field in science and technology that primarily focus on nanoparticles ranging from 1–100 nm in diameter, which exhibit distinctive properties owing to their small size and large surface area. Among them, copper oxide nanostructures, are major metal oxide nanoparticles, widely used in various fields especially in the development of biosensors due to their unique structural characteristics and biological effects. In this work, copper oxide nanoparticles were synthesized using a simple chemical reduction method and characterized using XRD to study the morphological and structural properties. Those nanoparticles were doped with PVA by sol-gel process and the electrode was fabricated using the spin coating technique on the precleaned glass slide. CV studies showed that the CuO NPs electrode was effective in detecting hydrogen peroxide with high selectivity even in the presence of other substances. A high-level sensitivity of $0.002 \text{ mA} \cdot \text{mM}^{-1} \text{cm}^{-2}$ and a 0.5 mM to 1.5 mM quick linear response was accomplished due to the large specific surface areas and efficient electron transport in the corresponding reactions, making this electrode a very promising candidate for efficient and accurate non-enzymatic detection of hydrogen peroxide (H_2O_2). © 2023 Journal of Biomedical Photonics & Engineering.

Keywords: copper oxide nanoparticles; hydrogen peroxide; polyvinyl alcohol; electrochemical sensor; amperometric detection.

Paper #7782 received 24 Feb 2023; revised manuscript received 28 Jun 2023; accepted for publication 8 Jul 2023; published online 23 Aug 2023. [doi: 10.18287/JBPE23.09.030309](https://doi.org/10.18287/JBPE23.09.030309).

1 Introduction

Hydrogen peroxide (H_2O_2) plays a crucial role as a mediator in biological systems, where it contributes to the regulation of cell growth. It is involved in various cellular processes, including cell signaling, immune activation, and apoptosis. This is primarily attributed to the reactivity of H_2O_2 with other oxygen species present in the system [1]. It has numerous applications in diverse fields, such as controlling microbes, bleaching textiles and paper, cosmetics, food processing, pharmaceuticals, and environmental practices. In addition, H_2O_2 has many applications, including treating wastewater, oxidizing cellulose, enhancing rocket fuels, recycling waste paper, detoxifying organic pollutants, and manufacturing

various chemicals and plastics that could potentially contaminate the environment. It is also crucial for various enzymatic reactions in the body, and the H_2O_2 layer serves as the initial defense mechanism against different pathogens [2]. However, excess H_2O_2 leakage in biological organisms can be harmful, causing gastrointestinal tract damage, skin and stomach irritation, and protein degradation. Specifically, excessive amounts of H_2O_2 in the body have been linked to several diseases, including fragmentation of DNA, diabetes, membrane damage, cardiovascular issues, cancer, Parkinson's, Alzheimer's, neurodegeneration, tissue damage, and aging problems [3]. It is also utilized as an oxidant in various fields, including environmental monitoring, food safety, and medical applications. These conditions make

it crucial for the determination of hydrogen peroxide [4]. Various techniques have been developed to detect H_2O_2 , including colorimetry, photoelectrochemical, light detection, titration, chromatography, and electrochemical methods. However, chromatography and titration methods are not suitable for *in vitro* and *in vivo* detection of H_2O_2 . Similarly, light detection and chemiluminescent techniques are inconvenient for H_2O_2 detection because of the differences in excitation methods and the interference of H_2O_2 with chemical compounds during fluorescence measurements. As a result, the electrochemical method has gained popularity in recent years due to its simplicity, accuracy, speed, affordability, and user-friendliness [5]. Extensive research has been conducted on enzyme-based electrochemical biosensors, primarily due to their high sensitivity and excellent selectivity [6]. However, these biosensors suffer from a significant drawback due to a lack of stability and poor reproducibility caused by the inherent fragility of enzymes, which can be easily damaged during biosensor fabrication and testing. Consequently, recent studies have focused on enzyme-free electrochemical biosensors that offer advantages such as high sensitivity, rapid response, affordability, and good stability [7]. Numerous materials have been investigated for enzyme-free electrochemical biosensors, including transition metal nanoparticles, carbon nanotubes, semiconducting metal oxides, graphene, ordered mesoporous carbon, and their composites. These materials possess large specific surface areas and enhanced catalytic activities, enabling sensitive and rapid detection of hydrogen peroxide. Among them, copper oxide stands out as an excellent material due to its remarkable redox properties, non-toxicity, high stability, and conductivity [8]. Conducting polymers have also been employed as modifiers in the fabrication of electrochemical sensors due to their exceptional stability, good conductivity, and ability to maintain stability on the electrode surface [9, 10]. In the present study, we developed enzyme-free H_2O_2 electrochemical biosensing materials using CuO nanoparticles synthesized through a simple reduction method, which were then doped into polyvinyl alcohol polymer to create a thin film. Our findings demonstrate that the CuO nanostructure enhances the electrochemical performance for H_2O_2 detection, primarily due to its large specific surface area and efficient electron charge transfer properties.

2 Experimental Section

2.1 Materials

All the chemicals such as Copper (II) sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$), Sodium hydroxide (NaOH), Ascorbic acid, Polyvinyl Alcohol, (PVA, average molecular weight of 85000), and Distilled water were purchased of analytical grade from Kesari Scientific chemicals (Chennai). Nonionic detergent and deionized water were used to clean all glassware and oven-dried before use.

2.2 Copper Oxide Nanoparticle Synthesis

To synthesize the copper oxide nanoparticles, a simple chemical reduction method was employed. The procedure involved the gradual addition of 25 ml of a 10 mM $CuSO_4 \cdot 5H_2O$ solution, drop by drop, into 50 ml of a 20 mM NaOH solution with vigorous stirring. Subsequently, 25 ml of a 10 mM ascorbic acid solution was added dropwise while continuously stirring the mixture for 1 hour at room temperature. As a result of the reduction facilitated by sodium hydroxide and ascorbic acid, the solution underwent a color change and formed a yellow precipitate containing copper oxide nanoparticles. The particles were then separated by filtration and subjected to thorough washing with distilled water and acetone. Finally, the washed nanoparticles were left to dry naturally at room temperature for 24 h. Distilled water served as the solvent for the preparation of the solution used in the synthesis process [11].

2.3 PVA Doping with Sol-Gel Technique

To incorporate the copper oxide nanoparticles into the polymer matrix, a sol-gel process was employed. The first step involved preparing a 5% solution of polyvinyl alcohol (PVA) by stirring it for 3 h at 60 °C and subsequently allowing it to cool down to room temperature. Once the PVA solution was prepared, the previously synthesized CuO nanoparticles were added to it. During this addition, a magnetic stirrer was used to ensure rapid and continuous stirring, preventing the nanoparticles from aggregating or clumping together. This process resulted in the formation of a CuO nanoparticle sol that was effectively incorporated within the PVA matrix. Fig. 1 represents the complete flow for the preparation of copper oxide nanoparticle sol.

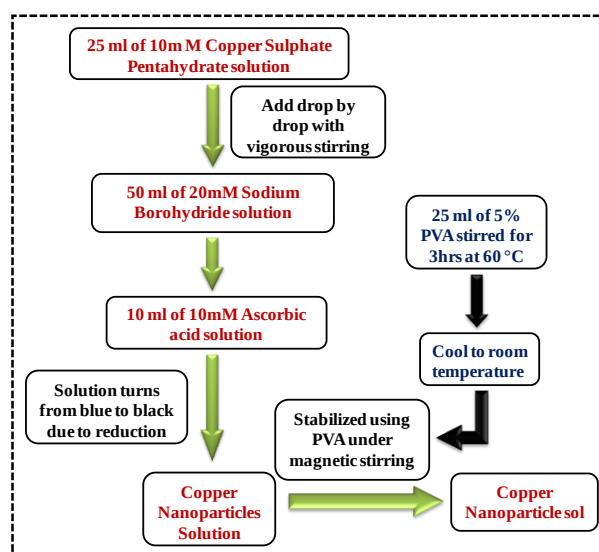


Fig. 1 Flowchart for Copper oxide nanoparticle doped PVA Sol preparation.

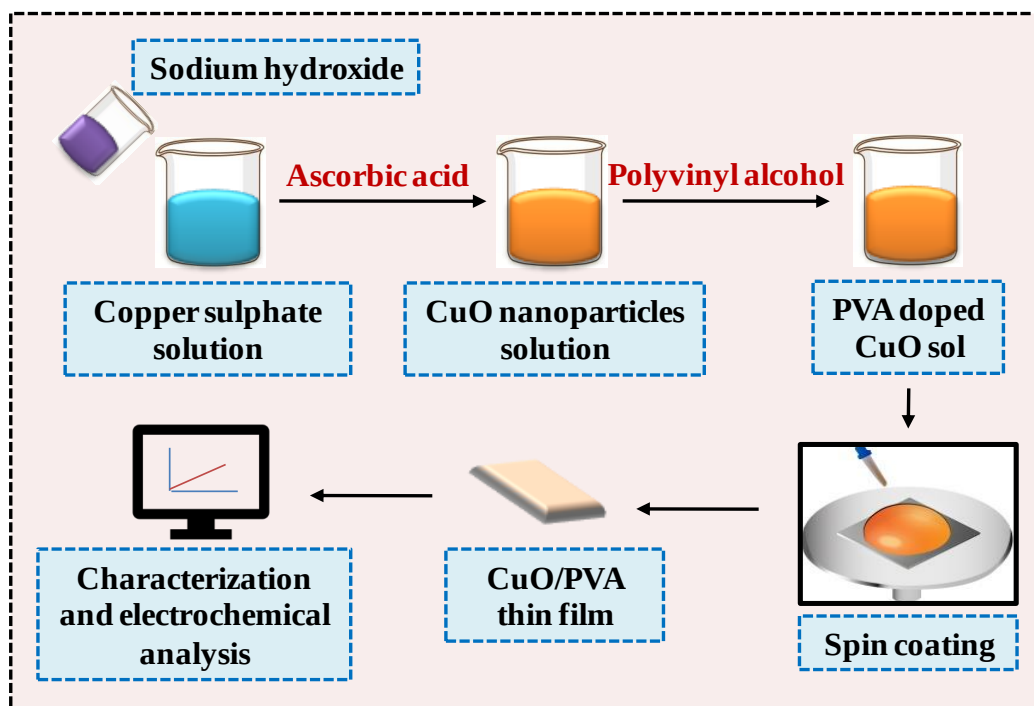


Fig. 2 Schematic representation for preparation of CuO NPs/PVA thin film.

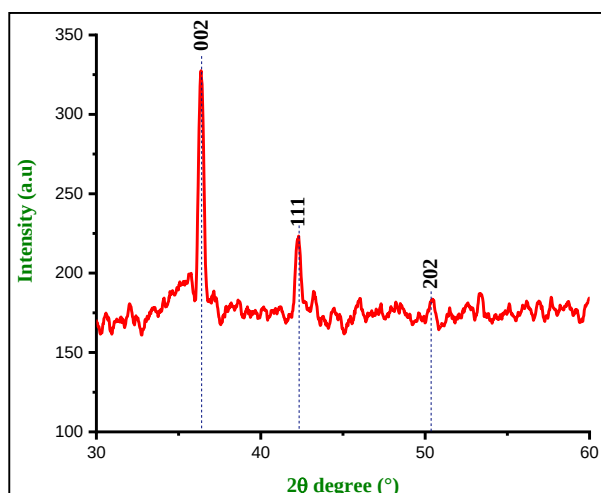


Fig. 3 X-ray diffraction pattern for CuO NPs/PVA thin film.

2.4 Electrode Fabrication

The CuO NPs thin films were deposited onto glass substrates via a sol-gel/spin coating process. The glass slides were pre-treated with alumina slurry and washed with deionized water and acetone. The CuO NPs sol was deposited onto the glass slides using a spin coater at 2500 rpm for 30 s, followed by drying and annealing at different temperatures (100–400 °C) for 5 min. Fig. 2 represents the complete flow for the preparation of thin film CuO NPs working electrode.

3 Result and Discussion

The structural properties of the coated films were determined using a Phillips X'Pert Pro X-ray

diffractometer with Cu K α 1 radiation (1.5406). The electrochemical measurements, specifically the cyclic voltammetry (CV) curve and the CV VS H₂O₂ concentration, were performed in a three-electrode cell system at room temperature. The platinum was used as the counter electrode, Ag/AgCl saturated with potassium chloride was used as the reference electrode, and the newly developed thin film CuO NPs/PVA slide acted as the working electrode, with a diameter of 3mm. The Wireless potentiostat was used for the electrochemical measurements.

3.1 Materials Characterization

The crystal structure was identified with the aid of XRD analysis, which allowed for the detection of diffraction peaks in the copper oxide. The diffraction peaks at $2\theta = 36.36^\circ$, 42.18° , and 50.91° (JCPDS 45-0937) were attributed to the (002), (111), and (202) crystallographic planes of the CuO monoclinic crystal structure, as shown in Fig. 3. These diffraction peaks were found to be responsible for the characteristic pattern of the copper oxide, confirming the crystalline nature of the copper nanostructures that were present in PVP.

The mean size of the nanoparticles was determined using the Scherrer formula based on the high-resolution X-ray diffraction peaks. The Scherrer formula is a mathematical equation that relates the size of crystalline domains to the width of X-ray diffraction peaks. It can be expressed as follows in Eq. (1):

$$\tau = \frac{K\lambda}{\beta \cos\theta} \quad (1)$$

In Eq. (1), τ represents the mean size of the ordered (crystalline) domains, K is a dimensionless shape factor (approximately unity), λ is the X-ray wavelength, β is the line broadening at half the maximum intensity (full width at half maximum, FWHM, in radians) represented as 2θ , and θ is the Bragg angle. By performing the calculations using the Scherrer formula, the average size of the nanoparticles was determined to be approximately 19.72 nm.

3.2 Electrochemical Analysis of H_2O_2

3.2.1 Mechanism of Interaction

Despite the distinct morphological properties of copper oxide (CuO), it is capable of forming a compound with the polymer polyvinyl alcohol (PVA). Fig. 4 illustrates that the hydroxyl group present in PVA interacts with the oxygen atoms of CuO nanoparticles, leading to the formation of CuO-doped PVA through hydrogen bonding. This interaction between the polymer and nanoparticles allows for their integration at a molecular level. However, when a reactive species such as hydrogen peroxide (H_2O_2) comes into contact with the surface of CuO, it serves as a redox carrier and facilitates a chemical reduction process. This interaction with H_2O_2 can lead to changes in the properties or behavior of the CuO nanoparticles, potentially impacting their functionality or reactivity in certain applications. This

electroreduction generates free electrons, corresponding to the concentration of H_2O_2 .

When hydrogen peroxide (H_2O_2) molecules come into contact with the electrode surface, they undergo a reaction where they release $2H^+ + 2e^-$ ions. This process is accompanied by the production of an electric current when a potential is applied to the electrode. Fig. 5 depicts a schematic representation of this electrochemical process. By measuring the resulting current value, it is possible to estimate the quantity or concentration of H_2O_2 present in the sample.

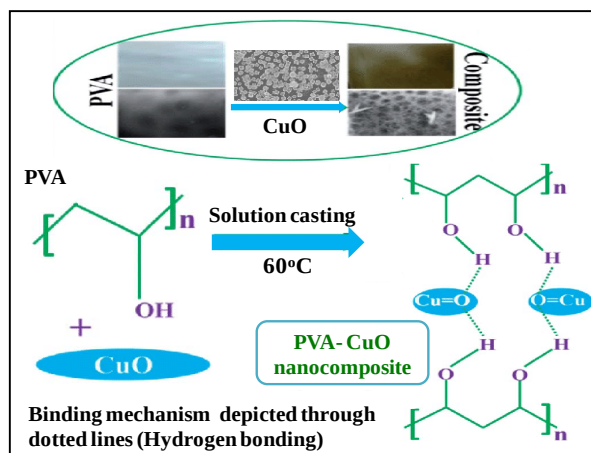


Fig. 4 Reaction mechanism between CuO and PVA [12].

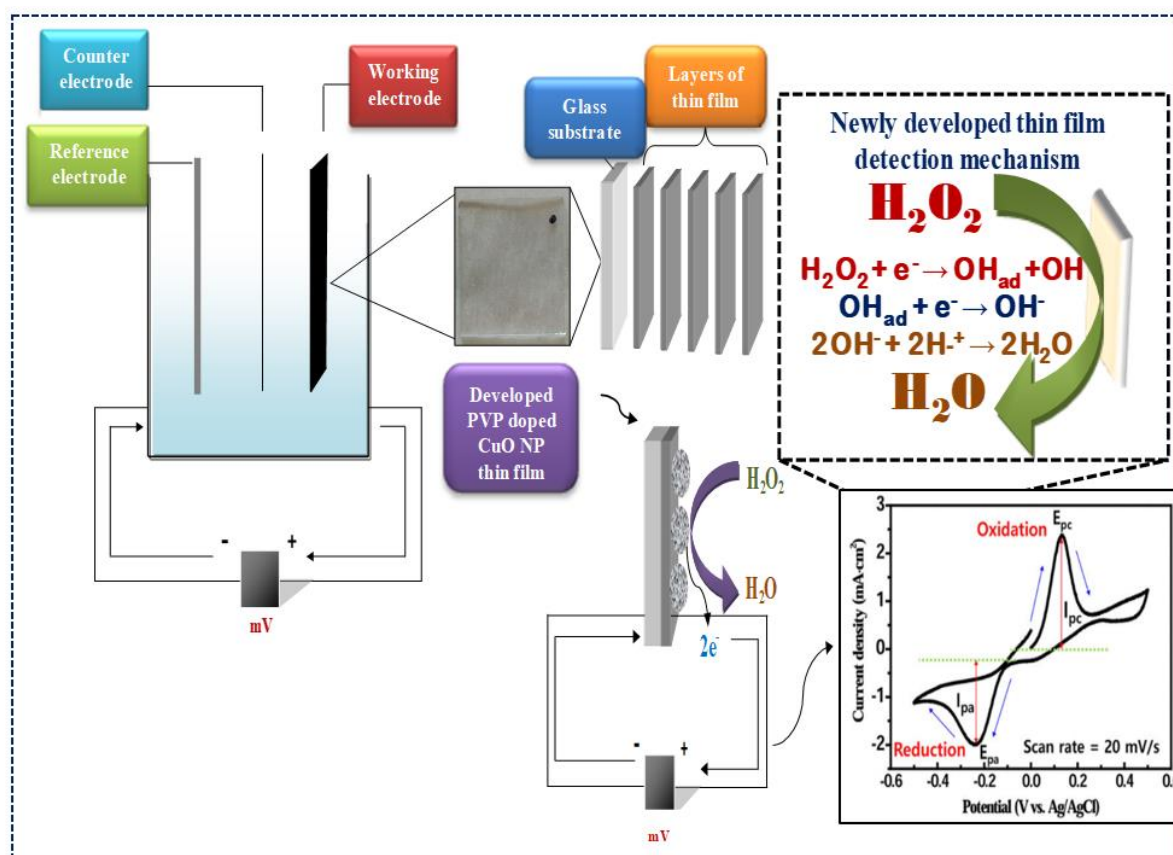


Fig. 5 Schematic representation of the working mechanism of developed CuO NPs/PVA thin film.

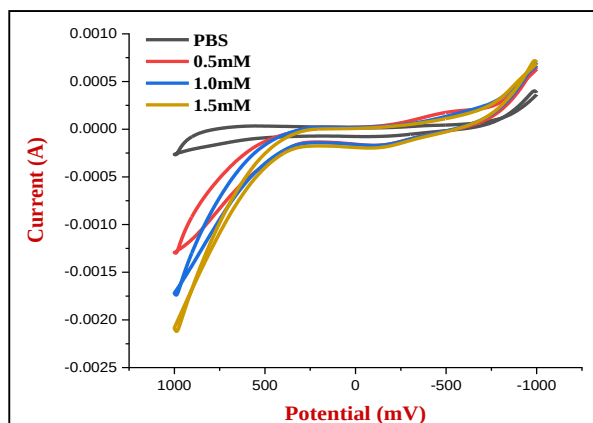


Fig. 6 Cyclic Voltammogram of H_2O_2 in CuO NPs/PVA thin film electrode at different concentrations with 500 mV/sec scan rate.

This mechanism forms the basis for the development of electrochemical sensors specifically designed for the detection and quantification of H_2O_2 . These sensors utilize the electrochemical response generated by the interaction between H_2O_2 and the electrode surface to provide a means of detecting and measuring H_2O_2 levels in various applications.

3.2.2 Different Concentration

Cyclic Voltammetric measurements were performed on a CuO-doped PVA thin film working electrode in 10 mM PBS (pH 7.4) with 0.5 mM to 1.5 mM H_2O_2 . The newly generated CuO NPs doped PVA thin film electrode displayed a significant response in reduction current. Prior to this, the CV results were obtained for the PBS solution without H_2O_2 for comparison. The cathodic peak observed between -0.4 to 0.2 V confirmed the electrocatalytic behavior of the thin film in H_2O_2 electroreduction. Fig. 6 displays the CVs of a CuO thin film electrode for varying concentrations of H_2O_2 in 10 mM PBS (pH 7.4) with a 500 mV/sec scan rate. The results reveal that the catalytic reduction current increased with increasing H_2O_2 concentrations (0.5–1.5 mM), indicating that the sensor is suitable for quantitative analysis.

3.2.3 Different Scan Rate

Fig. 7 illustrates the cyclic voltammograms (CVs) obtained from a CuO-doped PVA thin film electrode in 10 mM phosphate-buffered saline (PBS) with a pH of 7.4 and 1.0 mM hydrogen peroxide (H_2O_2) at 500 mV/sec scan rate. The cathodic reduction current of H_2O_2 exhibits a linear correlation with the square root of the scan rate. This suggests that the electron transfer reaction at the thin film electrode surface is diffusion-controlled. In other words, the rate of the electron transfer reaction is limited by the diffusion of H_2O_2 to the electrode surface. As the scan rate increases, the diffusion layer thickness decreases, allowing more H_2O_2 molecules to access the electrode surface, leading to a higher reduction current. This linear relationship between the reduction current

and the square root of the scan rate agrees with the Randles-Sevcik equation, which describes the behavior of electrochemical systems under diffusion-controlled conditions. The Randles-Sevcik equation is an empirical relationship used in electrochemistry to describe the behavior of a reversible redox reaction at an electrode surface. It relates the peak current of a cyclic voltammogram to the concentration of the electroactive species, the scan rate, and the electrochemical properties of the system.

The Eq. (2) is expressed as:

$$i_p = (2.69 \times 10^5) n^3 A C (D\nu)^{1/2}, \quad (2)$$

where i_p is the peak current (in A), n is the number of electrons involved in the redox reaction, A is the electrode surface area (in cm^2), D is the diffusion coefficient of the electroactive species (in cm^2/s), C is the concentration of the electroactive species (in mol/cm^3), and ν is the scan rate (in V/s).

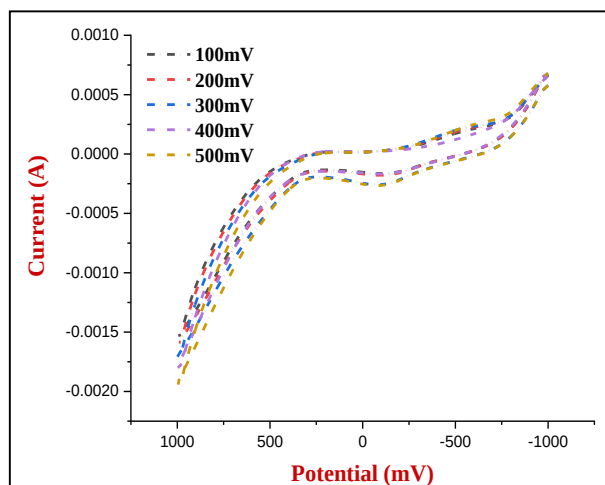


Fig. 7 Cyclic Voltammogram of H_2O_2 at different scan rates with 1.0 mM concentration.

3.2.4 Effect of Annealing Temperature

To investigate the impact of annealing temperature on the performance of the CuO-doped PVA thin film electrode, a series of thin films were prepared and subjected to annealing at different temperatures ranging from 100°C to 400°C . The cyclic voltammograms of these films were recorded and subjected to analysis. The outcomes, as shown in Fig. 8, indicate that an increase in annealing temperature resulted in a corresponding increase in current within the current-voltage response curve. This observation can be attributed to the heightened reactivity between CuO nanoparticles and H_2O_2 molecules, facilitated by the greater surface area of the CuO agglomerates formed at higher annealing temperatures. The enhanced reactivity leads to increased current generation during the electrochemical reaction. These findings are consistent with prior research [13–15], which has demonstrated the influence of annealing on the structural and morphological properties of thin films,

subsequently affecting their electrochemical behavior. By optimizing the annealing temperature, it becomes possible to customize the performance of the thin film electrode to meet specific application requirements. These results have significant implications for the advancement of electrochemical sensors that aim to achieve sensitive and selective detection of H₂O₂.

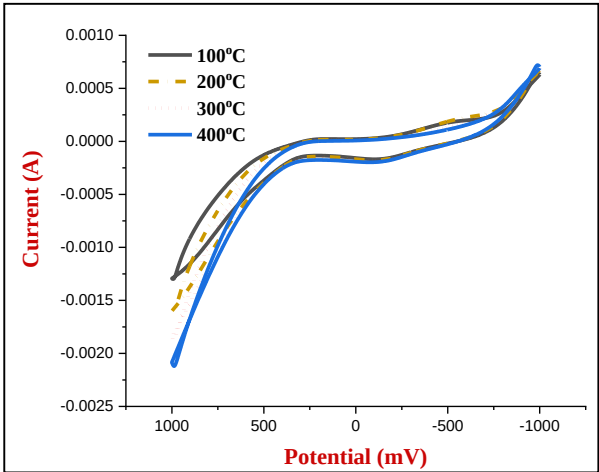


Fig. 8 Cyclic Voltammogram of H₂O₂ with CuO NPs/PVA thin films annealed at various temperatures.

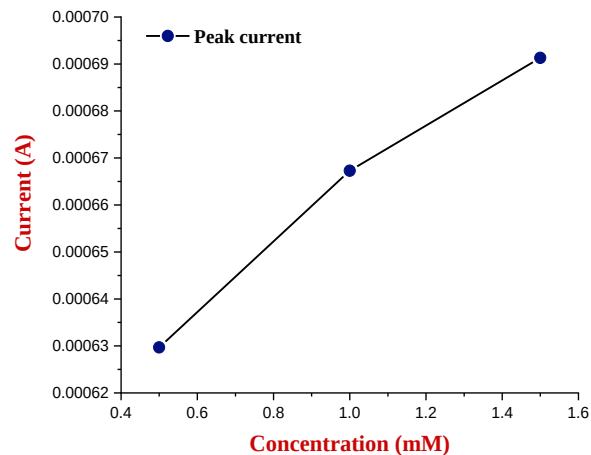


Fig. 9 Peak current density VS H₂O₂ concentration.

Table 1 Parameters for intercept and R-Square calculation.

Equation	$y = a + b \times x$
Plot	B
Weight	No Weighting
Intercept	$6.01167\text{E-}4 \pm 8.48109\text{E-}6$
Slope	$6.16\text{E-}5 \pm 7.85196\text{E-}6$
Residual Sum of Squares	$3.08267\text{E-}11$
Pearson's r	0.99197
R-Square (COD)	0.98401

3.2.5 Sensitivity and Detection Limit

To assess the analytical effectiveness of a CuO-doped PVA thin film, a series of amperometric tests were carried out using known concentrations of H₂O₂ in a 10 mM PBS (pH 7.4) solution. The scan rate of 500 mV/sec was utilized, and standard solutions of H₂O₂ were added in successive amounts to generate a calibration curve. The linear range of the calibration curve was found to be 0.5 mM to 1.5 mM, and the corresponding peak current was determined. The resulting peak current for each concentration of H₂O₂ was plotted in Fig. 9. The relative sensitivity of the proposed sensor was found to be approximately 0.002 mA·mM⁻¹cm⁻². The limit of detection (LOD) was calculated using the 3S/k approach [16] and was determined to be 0.78 mM. Overall, the results demonstrate that the CuO NPs/PVA thin film provides great performance in detecting H₂O₂ and acts as a prominent sensor for this application.

3.2.6 Stability and Reproducibility

Another requirement that a sensor must meet is long-term storage stability, which has been examined for the CuO-doped PVA thin film produced and tested in mM H₂O₂. The modified electrode was stored at room temperature when not in use. Results showed that after two weeks of storage, the electrode retained 96% of its initial reaction, as previously reported, and after a month, it could still sustain 98% of its original current. No reaction was detected over a longer period, indicating the high durability and stability of the newly developed electrode.

The reproducibility of the electrode was evaluated by measuring responses to 1.0 mM H₂O₂ solution for 20 successive measurements, which demonstrated peak currents at ±0.067 mA, as shown in Fig. 10. These results confirm the electrode's long-term stability, durability, and reproducibility.

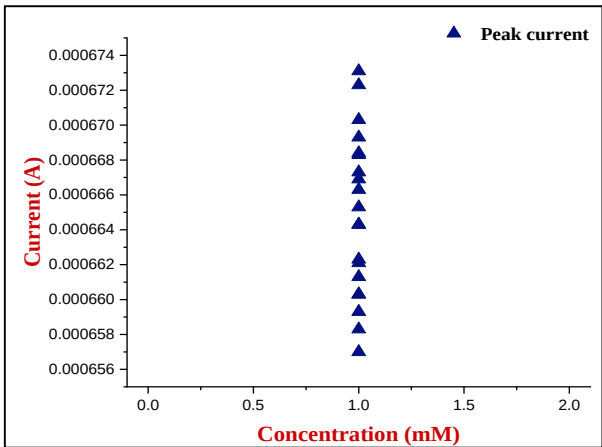


Fig. 10 Peak current VS 1.0 mM H₂O₂ concentration for 20 successive measurements.

4 Conclusion

In summary, a CuO nanostructure-based thin film was successfully synthesized using a reduction method without any additives, and it was incorporated into PVA for the detection of H₂O₂. The electrochemical properties of this material were thoroughly investigated. The analysis revealed that the morphology of the CuO nanostructures primarily depended on the initial amount of CuSO₄. The resulting CuO nanoparticle-doped PVA thin film demonstrated effective non-enzymatic detection of H₂O₂. The modified glass electrode with cubic CuO nanoparticles displayed outstanding catalytic performance towards H₂O₂, exhibiting high sensitivity

(0.002 mA·mM⁻¹cm⁻²), rapid response, relatively low detection limit, good stability, and favorable selectivity. Consequently, this electrode holds great promise for sensing applications due to its advantageous features and ease of production.

Acknowledgment

This work is supported by DST-SERB, New Delhi, Project File number: EEQ/2021/000467EEQ.

Disclosures

The authors declare no conflict of interest.

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