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Copper Nanoparticle-Reduced Graphene Oxide Film for Electrochemical Detection of Ascorbic Acid

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Abstract

We develop a non-enzymatic sensor for the detection of ascorbic acid (AA) based on cupric nanoparticles-reduced graphene oxide (CuNPs-rGO) nanocomposite. As a vital antioxidant, ascorbic acid contributes to the prevention of oxidative stress, and its abnormal concentrations have been associated with different health problems. Thus, the high-sensitivity detection of AA is important for clinical diagnosis and health monitoring. In this design, CuNPs act as catalytic sites for oxidation and allow the supply of electrons to the rGO material, resulting in excellent electrical conductivity and fast transfer of electrons. The 2D structure of rGO provides it very high surface area, which supports AA adsorption. The CuNPs–rGO nanocomposite was prepared by simple and inexpensive electrochemical synthesis, which provided good uniformity and reproducibility of the material. The fabricated sensor shows a good linear response to the concentration of ascorbic from 0.1–1 mM ($R^2 = 0.97$). A high sensitivity (0.98 mA mM^{-1} cm^{-2}) and low detection limit (0.16 mM) was achieved, manifesting superior sensing performance. Moreover, the design of flexible film-type electrodes improves mechanical flexibility and practical use. In general, the findings further demonstrate that the CuNPs–rGO nanocomposite is a very promising material for non-enzymatic electrochemical ascorbic sensors to achieve high-performance.

Keywords: *Non-enzymatic, Nanocomposite, Ascorbic, Cu Nanoparticle, rGO.*

1. Introduction

Ascorbic acid (AA), the most well-known form of vitamin C, is an essential nutrient that exhibits potent antioxidant activity and has a protective effect against oxidative stress in human cells. It is essential for overall health, as it supports immune function, collagen synthesis, and iron absorption (Huang et al., 2019). Therefore, AA is widely used in biomedical studies, clinical diagnosis, and the food industry. Due to its crucial function, optimal levels of AA are important for human health, and disturbances from this optimal level can be

correlated with a range of pathologies, including scurvy, cardiovascular diseases, and neurodegenerative disorders **(Dhara et al., 2025)**.

Precise determination of AA content is important for clinical diagnosis and food quality monitoring. Nonetheless, due to the abundance of other electroactive substances in biological surroundings (e.g., dopamine (DA), uric acid (UA), and glucose), this process is challenged since these chemicals have similar electrochemical characteristics compared to AA **(Pardakhty et al., 2016)**. The interferants make specific AA detection (i.e., no cross-reactivity) difficult. Although advances in analytical chemistry are significant, there is still a challenge to separate the electrochemical signals of AA from interfering substances **(Razmi & Bahadori, 2021)**.

The detection of small biomolecules, such as AA, via electrochemical sensing techniques is gaining momentum due to the high sensitivity, rapid response, and low cost. These techniques are useful for real-time detection of biomolecules in biological fluids **(Rossato et al., 2022)**. For example, electrochemical sensors rely on the oxidation or reduction of target molecules and produce a current proportional to the concentration of the analyte. Electrochemical methods are promising for AA detection, since by modifying the electrode with functional materials, the electrochemical response of AA is distinguished from other coexisting substances **(Motsaathebe & Fayemi, 2022)**.

The performance of electrochemical sensors can be significantly enhanced by using materials with high conductivity and catalytic properties. Graphene oxide (GO) and reduced graphene oxide (rGO) have gained attention due to their large surface area, good conductivity, and ease of functionalization. rGO, in particular, provides an excellent platform for enhancing electron transfer, which is crucial for the electrochemical detection of AA. Additionally, copper nanoparticles (CuNPs) have been widely explored for their catalytic activity and their ability to facilitate electron transfer during AA oxidation. A combination of CuNPs and rGO into a composite material offers a synergistic effect that improves both the sensitivity and selectivity of the sensor. CuNPs enhance the oxidation of AA by acting as catalytic sites, while rGO provides a conductive platform for efficient electron transfer, resulting in a high-performance sensor for AA detection.

2. Objectives of the Study

In this regard, we report the synthesis of Cu NPs through green methods and their incorporation into rGO to develop CuNP- rGO nanocomposites. The key contributions of this work are:

- Green synthesis of CuNPs using eco-friendly methods for sustainable nanomaterial production.
- CuNPs-rGO nanocomposite integrating green- synthesized copper nanoparticles with conducting reduced graphene oxide.
- Fabrication of a flexible film-type biosensor by coating the CuNPs/rGO nanocomposite onto copper tape substrates, offering potential for wearable and practical applications in health monitoring.
- Innovative manufacturing strategy combining green-synthesized CuNPs with carbon hybrid for AA sensors. This approach provides a sustainable pathway for high-performance, flexible AA sensors.

3. Literature Review

The present trends of electrochemical sensors for ascorbic acid (AA) emphasize nanomaterials theoretically with increased sensitivity, selectivity, and detection limits. For example, screen-printed electrodes modified with graphene/CuPc/PANI nanocomposites exhibited potent electrocatalytic properties for AA oxidation at low potentials **(Pakapongpan et al., 2012)**. The nanocomposites, such as the PdNPs/rGO on glassy carbon electrodes (GCE), facilitate co-detection of AA, dopamine, and uric acid owing to the enhanced conductivity and surface area from metal nanoparticles atop rGO **(Wei et al., 2020)**. The resultant heterostructure-based materials (3D CuO–ZnO NPs/PPy/RGO: ZnOCuO p–n junction with polypyrrole nanofibers on the rGOs) demonstrated low limits of detection for AA (0.024 μM), as well as wide linear ranges owing to high surface area and synergistic effects **(Ghanbari & Bonyadi, 2018)**. Cu-rGO composites, one of the most effective materials for electrochemical sensors and fabricated through a one-pot electrodeposition technique with minute amounts of CuNP (0.01% wt %) can provide an effective strategy for replacing noble metal- decorated rGO

(ERGO) as a support material to enhance nucleation rates at the electroactive surface via copper oxidation states such as δ CuO and η-Cu2O **(Sookhakian et al., 2021)**, leading in delicate electron transfer compared to Cu oxides on ERGO, also enable concurrent detection of AA and dopamine through methodologies presented from manuscript reference. For glucose, they have optimized rGO-wrapped Cu nanoparticles (ERGO/Cu NPs), but emphasize copper-graphene synergies for electrocatalysis. Nonetheless, tensions remain with CuNP-rGO systems in achieving the most linear ranges together with reproducibility and flexible formats at the same time solely for AA.

There are rare reports that elaborate on the method in a direct and facile manner with electrochemical synthesis of CuNP-rGO films that can be used for AC sensing on a flexible substrate **(Xie et al., 2014)**. Here, we report a CuNP-rGO film sensor by first optimizing electrodeposition conditions for reproducibility followed with unique properties in flexibility and performance.

4. Materials and Methodology

4.1 Leaf and Chemicals used

Fresh leaves of neem and Tulsi were collected from vicinity, used as the naturally occurring bio-reductants and stabilizers for copper oxide nanoparticles formation. Copper nitrate dihydrate ($Cu(NO_3)_2 \cdot 2H_2O$, 98% purity, Merck) was used as metal precursor. Millipore-Q deionized water (DI, 18.2 MΩ·cm), N-methyl-2-pyrrolidone (NMP, Macklin) as solvent when needed. Residual contaminants were washed away with iso-propyl alcohol (IPA, Merk). ***(View of Synthesis Of Copper Oxide Nanoparticles From Tulsi and Neem Leaf Extract And Study Their Antibecterial Effect., 2000.)***.

4.2 Green synthesis of Copper Nanoparticles (CuNPs)

Fresh leaves of neem (Azadirachta indica) and Tulsi (Ocimum sanctum) were collected, washed for several times with water and deionized water to remove dirt. Copper (Cu) was synthesized, 40 mL of both the neem extract and Tulsi leaf extract were mixed with an aqueous solution composed of copper nitrate trihydrate ($Cu(NO_3)2 \cdot 3H2O$, 100 mL), in a ratio by volume of 9:4:6 **(Chokhawala & K.C.Poria, 2023)**. An in is reaction room temperature for 5 h indicated that a color change of the indicates development of nanoparticles. The resulting suspension was centrifuged for 60 min at a speed of 8000 rpm to collect the Cu nanoparticles. The obtained nanoparticles were washed with deionized water and Isopropyl Alcohol in order to wash away remains of impurities, followed by drying as for characterization. The schematic representation of the synthesis process of CuNPs is illustrated in Fig - 1.

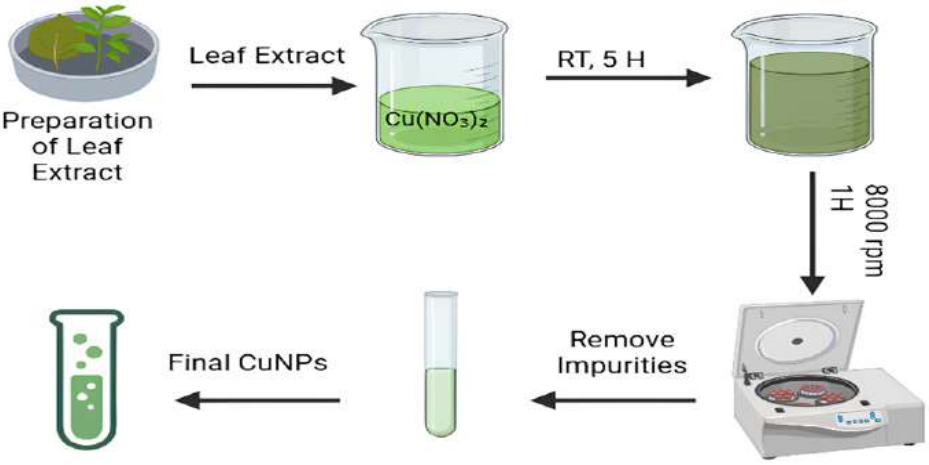

Fig – 1: Schematic representation of the green synthesis process of copper oxide nanoparticles (CuO NPs) using neem and tulsi leaf extracts.

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4.3 Preparation of reduced graphene oxide (rGO)

Graphene oxide (GO) was synthesized according to a Tour method by oxidizing graphite powder with deep H_2SO_4 and H_3PO_4 (9:1 v/v) under stirring in an ice-water bath, then adding $KMnO_4$, which was subsequently diluted—by deionized water—and further treated with H_2O_2 to decompose residual permanganate (Marcano et al., 2010). The product of graphite oxide was filtered and washed with 5% HCl to remove sulfate ions, followed by exfoliation in water at 60°C for 12 h. The resulting GO (0.1mg/mL) was then reduced in the presence of ascorbic acid (10:1 weight ratio) at 60 °C for 60min. H2O2 in excess was introduced to remove the free ascorbic acid and centrifuged, followed by washing with ethanol solution and deionized water and dried at 120 °C for 24 h. rGO. Schematic of the synthesis process of rGO as shown in Fig- 2.

4.4 Fabrication of CuNPs-rGO composite film

The ternary nanocomposite was prepared by mixing reduced graphene oxide and the green-synthesized copper nanoparticles (CuNPs) in a weight ratio of ~1:1. Specifically, 5 mL of rGO dispersion (0.1 mg/mL) and 5 mL of CuNPs suspension were combined with DI. The dispersion was gently stirred and sonicated for 30–60 min to provide a uniform mixed suspension. To produce a thin-film composite was drop-cast or coated onto the Copper tape substrate and dried either at room temperature or low heating (40–60 °C) to remove solvent. The composite film exhibited wonderful electrochemical performance for the sensitive detection of ascorbic acid.
(Yao, 2022).

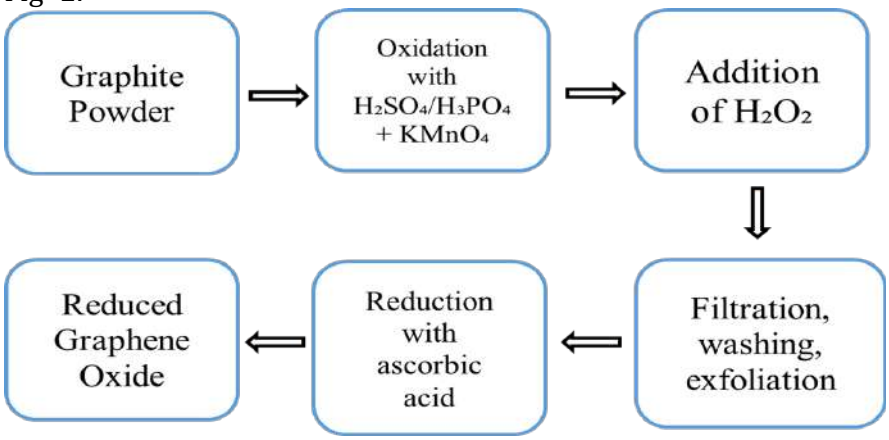

Fig -2: Schematic representation of the synthesis of reduced graphene oxide (rGO) from graphite powder.

5. MECHANISM OF ASCORBIC ACID SENSING

Electrochemical oxidation of ascorbic acid at the surface-modified Cu tape/CuNPs–rGO electrode results in its detection. Molecules of ascorbic acid diffuse from the solution and get adsorbed on the electrode, wherein embedded rGO contributes a high-surface-area conductive network for accelerated electro-transfer. CuNPs serve as catalysts, and the copper tape substrate facilitates conductivity and electron transport.

Ascorbic acid is oxidized to dehydroascorbic acid under an applied potential, producing electrons and protons as shown in Fig - 3. Cu (CuNPs and substrate) reduces the oxidation overpotential and enhances reaction kinetics. These generated electrons are quickly transferred pathway via the CuNPs–rGO network to the electrode generates an enhanced current response **(Wang et al., 2015)**.

As the concentration of ascorbic acid increases, the oxidation peak current also rises linearly, guaranteeing detectable and trustworthy determination.

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Electrochemical Reaction:

$$\text{Ascorbic Acid} \rightarrow \text{Dehydroascorbic Acid} + 2H^{+} + 2e^{-}$$

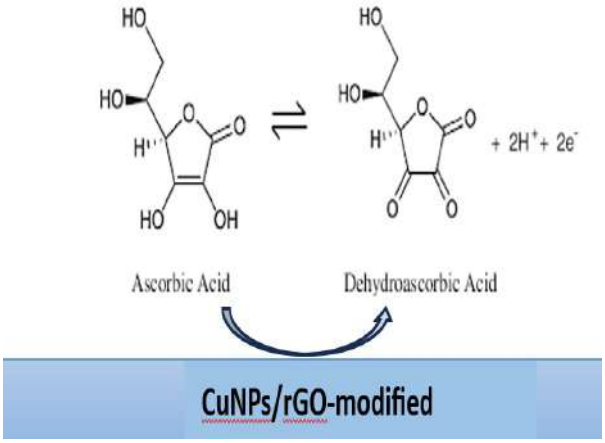

Fig – 3: Schematic representation of the mechanism of ascorbic acid (AA) sensing at the CuNPs/rGO-modified electrode

5.1 Electrochemical Characterization

The oxidation of ascorbic acid electrochemically at the CuNP/rGO/Cu-modified electrode generated on copper tape is prompted by the synergistic catalytic activity enabled through the conductive rGO network and also copper nanoparticles **(Marcano et al., 2010)**. Together, the rGO has a high surface area and an excellent electron transfer rate while CuNPs can also serve as effective electrocatalytic sites for oxidation of ascorbic acid. As illustrated in Fig - 4(a), after the modification, the CuNPs/rGO electrode shows a remarkable increase of current response compared to the bare Cu tape, confirming that there is an enhanced electrochemical activity with its modification.

Finally, the first broad peak in the 0.2–0.4 V region can be assigned to surface-related redox behaviour of copper ($Cu/Cu^{+}/Cu^{2+}$), as well as activation of catalytic sites which increase adsorption and/or charge transfer for ascorbic acid. With the applied potential incremented to 0.4–0.6 V, ascorbic acid starts its main oxidation reaction toward dehydroascorbic acid, yielding a prominent anodic peak (as shown in Fig - 4(b). According to these mechanisms, at higher potentials (0.6–0.8 V), it becomes possible that several oxidation processes involving copper species and surface interactions occur, which would contribute to the final electrochemical response and stability of the sensing platform. CuNPs reduce the oxidation overpotential and enhance electron transfer kinetics with rGO serving as a conductive path to quickly transport electrons to the electrode surface.

As observed in Fig - 4b, the peak current of oxidation amplitudes monotonically increases with increasing concentrations of ascorbic acid indicating a significant concentration-dependent response. These synergistic roles lead to better-defined and higher oxidation peaks in cyclic voltammetry, thus confirming that the prepared CuNPs/rGO-modified electrode is a highly sensitive and reliable platform for ascorbic acid detection herein.

5.2 Amperometric characteristics

As shown in Fig – 5 (a), there is a linear relationship between the electrode response and ascorbic acid concentration, demonstrating that the fabricated CuNPs/rGO-modified Cu tape electrode has good electrochemical performance. The calibration curve obtained is linear ($R^2 = 0.97$) within the tested concentration range, with regression equation y = 0.98x + 0.39. From this calibration, the sensor sensitivity was determined to be around 0.98 mA mM^{-1} cm^{-2} using an electrode surface area of 1 cm^2. The limit of detection (LOD) for dopamine, calculated using the formula:

$$LOD = 3.3 \times (\sigma/S)$$

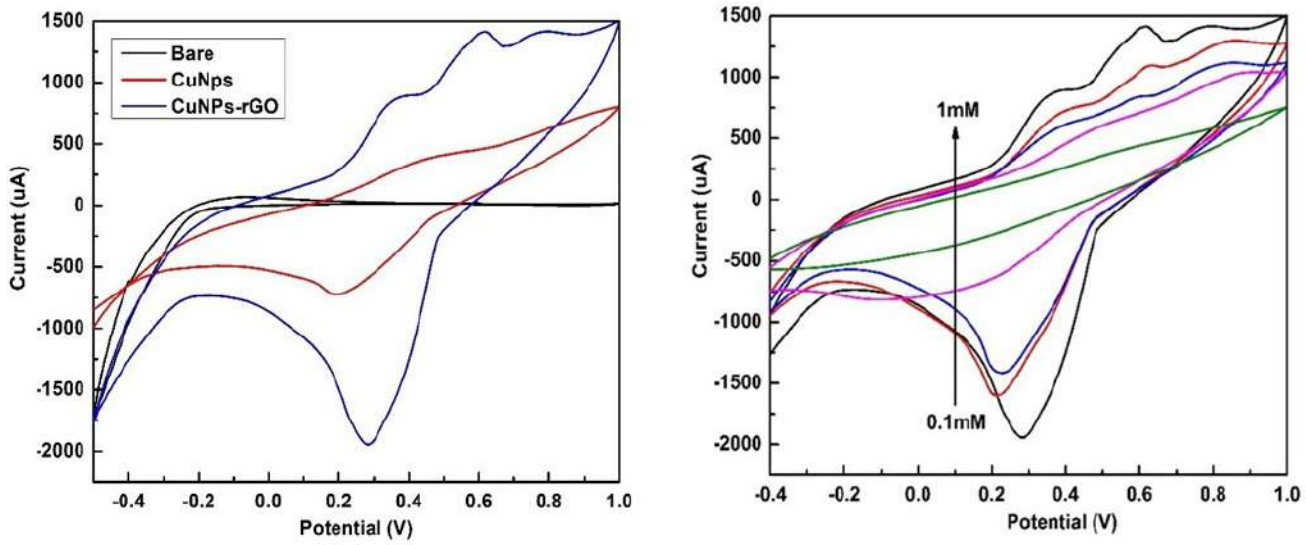

Fig – 4: (a) Cyclic voltammograms (CVs) of bare Cu tape and CuNPs/rGO-modified electrodes demonstrating enhanced electrochemical activity after surface modification. (b) CV responses of the CuNPs/rGO-modified electrode toward ascorbic acid at different concentrations recorded at a scan rate of 10 mV/s, showing a concentration-dependent increase in oxidation peak current.

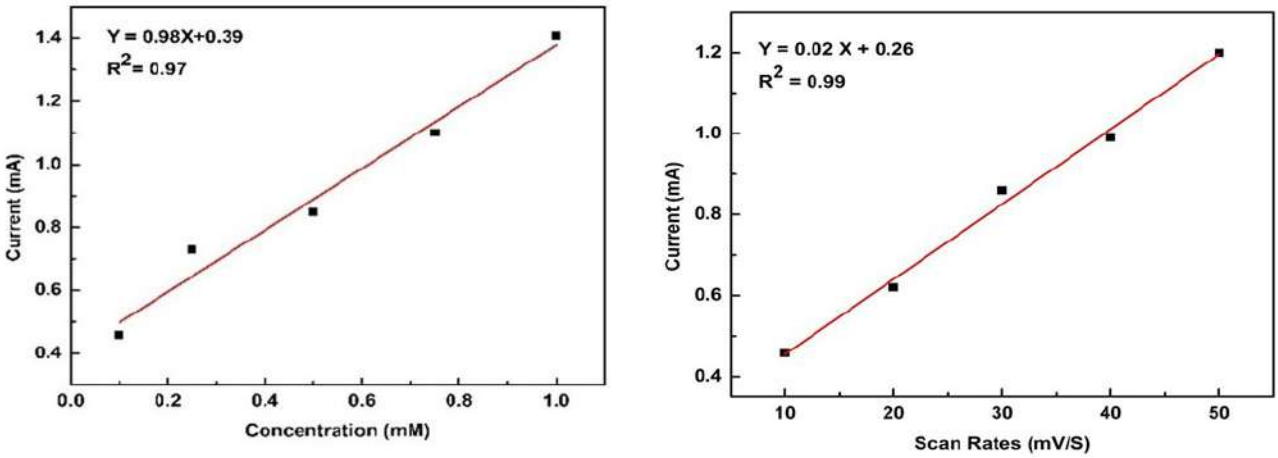

Fig - 5: (a) linear relationship between the peak current and the Concentration of the CuNP - rGO (b) linear relationship between the peak current and the scan rate of the CuNP-rGO.

where σ is the standard deviation of the regression line and S is the slope. The LOD was determined to be 0.16 mM, demonstrating good sensitivity of the sensor. Furthermore, as illustrated in Fig – 5 (b), the current response increases proportionally with scan rate, confirming that the electrochemical process is diffusion-controlled. The cyclic voltametric response of the CuNPs/rGO electrode in electrolyte solution shows a linear relationship between peak current and scan rate, indicating efficient electron transfer kinetics and stable sensor performance.

6. Comparative Analysis With Existing Methods

In addition to the internal evaluation, the proposed model was compared with previously reported methods in the literature. The comparative results, presented in Table 1, show that the proposed approach achieves improved performance over existing methods.

Table 1. Comparison of previous sensors results with the present study

Material	Sensitivity (mA mM^{-1} cm^{-2})	LOD	Linear Range	Reference
ZnO-decorated rGO/GCE	0.42	0.7 µM	5–1100 µM	**(Nithya M, 2015)**
PdNPs/rGO/GCE	0.56	20 µM	50 µM–1.35 mM	**(Wei et al., 2020)**
ZnO-CuO PPy/rGO	1.23	0.024 µM	0.1–1.2 mM	**(Ghanbari & Bonyadi, 2018)**
Graphene-CuPc/PANI	0.35	50 µM	Up to 1 mM	**(Pakapongpan et al., 2012)**
CuNPs-rGO Film	**0.98**	**0.16 mM**	Wide	**This Work**

7. Conclusion

A Cu tape electrode modified with CuNPs/rGO was successfully constructed and used as an electrochemical sensing platform for detection of ascorbic acid. The Cuinanoparticles/rGO synergy not only provided high electrocatalytic activity, but also excellent electron transfer, leading to a one-step oxidation signal of ascorbic acid with an intense and well-defined peak. The sensor showed a sensitivity of ~0.98 mA mM^{-1} cm^{-2} with a limit of detection of 0.16 mM. The linear dependence of peak current on ascorbic acid concentration and scan rate indicates a diffusion-controlled electrochemical process, confirming efficient electron transfer at the electrode/electrolyte interface. Due to its good sensitivity, linearity, and stability, the CuNPs/rGO-modified Cu tape electrode provides a simple low-cost and reliable platform for effective detection of ascorbic acid in practical applications.

Statements and Declarations

Competing Interests: The authors declare no competing interests.

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