Green-Synthesized Rice-Shaped Copper Oxide Nanoparticles Using Caesalpinia bonducella Seed Extract and Their Applications

Saranya Sukumar, Agneeswaran Rudrasenan, and Deepa Padmanabhan Nambiar*

Department of Analytical Chemistry, University of Madras, Guindy Campus, Chennai 600025, Tamilnadu, India

ABSTRACT: Copper oxide nanoparticles (CuO Nps) were synthesized using Caesalpinia bonducella seed extract via a green synthetic pathway and were evaluated for electrocatalytic properties by carrying out electrochemical detection of riboflavin [vitamin B₂ (VB₂)]. The seeds of C. bonducella are known to have strong antioxidant properties arising due to the presence of various components, including citrulline, phytosterinin, β-carotene, and flavonoids, which serve as reducing, stabilizing, and capping agents. The synthesized CuO Nps were characterized using UV–visible spectroscopy, Fourier transform infrared spectroscopy, thermogravimetric analysis–differential thermal analysis, X-ray diffraction spectroscopy, X-ray photoelectron spectroscopy, and scanning electron microscopy and further used as a modifier for a graphite electrode surface. The modified electrode was electrochemically characterized by cyclic voltammetry, square-wave voltammetry, and chronamperometry techniques and then assessed for electrocatalysis by carrying out the detection of VB₂. The electrochemical sensor could be used for nanomolar detection of VB₂ with an observed linear range of 3.13–56.3 nM with a limit of detection of 1.04 nM. The electrode showed good stability and reproducibility over a period of 120 days. The CuO Nps were further analyzed for antibacterial effect with Gram-positive and Gram-negative bacteria, and in both cases, high antibacterial activity was clearly observed. The newly synthesized nanoparticles, thus, proved to be an interesting material for electrochemical and biological studies.

INTRODUCTION

Metal nanoparticles (Nps) find applications in microelectronics, sensors, catalysis, and various areas of nanotechnology. These particles are found to have advantages arising due to their small size, large surface area, chemical and optical properties, and good electrical conductivity. Among them, copper oxide nanoparticles (CuO Nps) have acquired great interest in research fields, such as solar cells, biodiesel, photocatalysis, water pollutant removal, supercapacitors, electrocatalysis, etc., due to their desirable properties, such as low cost, nontoxicity, and easy preparation. The number of electrocatalytic applications of CuO Nps has been limited in spite of the methods offering improved sensitivity and selectivity and being more economical.

CuO Nps have been synthesized by various methods, including microwave irradiation, precipitation pyrolysis, and thermal decomposition. However, the green synthesis of CuO Nps offers distinct advantages. Green chemistry is sustainable chemistry, used to minimize the generation of hazardous substances. Green synthesis of MO Nps is one of the simplest and ecofriendly methods, among which plant-mediated synthesis has attracted attention owing to its nontoxicity and also being an economical and facile process. Some of the recent reports on synthesis of CuO Nps using plant sources include the use of Saraca indica with fluorescence properties, Aglaia elaeagnoidea with catalytic and recyclability properties, Fortunella japonica for electrocatalytic detection of 4-nitrophenol, Rheum palmatum. L. root extract for catalytic reduction of 4-nitrophenol, Madhuca longifolia for waste water treatment, Tridax procumbens leaf extract for mosquito larvicidal activity, and synthesis of ultrasmall copper nanoparticles using lemongrass tea extract.

We have synthesized CuO Nps using Caesalpinia bonducella seed extract. The other prevalent names for C. bonducella include bonduc nut, fevernut, or molucca bean and is a medicinal plant widely found in Sri Lanka and India. The various parts of the plant have been found to have therapeutic uses including antioxidant, anti-hyperglycaemic, anti-inflammatory, anti-inflamatory, anti-convulsive, antifilarial, antibacterial, antifungal, anti-diabetic, and diuretic functions. The seeds of C. bonducella are reported to have strong antioxidant properties and contain various chemical compounds, including citrulline, phytosterinin, β-carotene, flavonoids, bonducellin, aspartic acid, β-sitosterol, arginine, and furanoditerpenes. These components could serve as reducing, stabilizing, and capping agents. Due to the vast application possibilities, nanoparticle synthesis using the seed extract was carried out, and since copper has interesting biological and electrical conduction properties, we have chosen it for this work.

Riboflavin [vitamin B₂ (VB₂)] is a central module for coenzymes, such as flavin adenine dinucleotide (FAD) and...
flavin mononucleotide (FMN), and plays an essential role in the sequence of protein, carbohydrate and fat metabolism. It is a water-soluble vitamin necessary for human health, and it is very much required for the proper functioning of human organs, but an excess of riboflavin is hazardous to human health, leading to oxidative damage to DNA and tissue when exposed to UV radiation. Meanwhile, lack of VB2 leads to skin disorders and eye lesions. Most importantly, it cannot be produced by the human body; instead it is provided through dietary supplements and medicinal products. Therefore, it is essential to monitor and determine VB2 in situ in real samples.

In the past few years, various methods have been used to determine VB2, such as high-performance liquid chromatography, microbiological methods, fluorescence spectroscopy, and so on. Even though these methods are highly sensitive and selective, they have certain demerits, such as the high cost, portability issues, and tedious sample preparation. Hence, it is essential to explore sensitive, selective, and low-cost instrumentation. Electrochemical techniques have many attractive properties, such as simplicity, low cost, speed, a wide linear range, low detection limit, high stability, and minimal space and power requirements. They have found a broad range of applications in various fields, including clinical, industrial, environmental and agricultural analyses. Metal and metal oxide nanoparticles have been applied for catalytic applications, e.g., polychitosan gold nanoparticles were employed for the reduction of 4-nitrophenol, CuO Nps were used for electrocatalytic glucose sensing application, copper cobaltite nanoplates were applied for electrochemical determination of acetylcholine, copper oxide nanosheets have been applied for the electrochemical simultaneous detection of environmentally hazardous anions, heterostructural CuO–ZnO nanocomposites were used for the electrochemical determination of NO3−, a CuO nanoparticle-inkjet-printed electrode was employed for glucose sensing, etc. CuO Nps were also shown to exhibit good catalytic efficiency when compared to cobalt or nickel nanoparticles. CuO Nps are a well-known p-type semiconductor with a direct band gap, which has excellent electrical properties, and these semiconducting metal oxides have gained immense attention in electrochemical sensing because of their chemical stability, valence variation, and excellent electrocatalytic activity.

In this study, novel CuO Nps using C. bonducella seed extract were synthesized and used as an electrocatalyst toward sensing VB2. The synthesized nanoparticles were evaluated using UV–visible (UV–vis) spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, thermogravimetric analysis–differential thermal analysis (TGA–DTA), X-ray diffraction (XRD) spectroscopy, X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM) and electrochemical studies, and the antibacterial properties of CuO Nps were established evidently.

RESULTS AND DISCUSSION

The major approach of this work was to make use of C. bonducella seed extract to convert Cu2+ ions to CuO Nps. Flavonoids present in the seed extract can precisely search for the active oxygen species (ROS). The flavonoids have the ability to donate the hydrogen or electron, and the phenolics exhibit a chelating effect on the metal ions, which is responsible for the reduction of Cu2+ from Cu(NO3)2·3H2O to copper oxide nanoparticles. The formation of the latter was confirmed by SEM, UV–vis, FT-IR, TGA and DTA, XRD, and XPS techniques.

Scanning Electron Microscopy. The structural features of the synthesized particles were investigated using field emission scanning electron microscopy (FESEM). The observed images at different magnifications are shown in Figure 1a,b. The nanostructured particles apparently exhibit a rice-grain-shaped morphology. The images show that the copper nanoparticles are evenly distributed and homogeneous in nature. This result is similar to those in previous reports on the morphology of Cu Nps and CuO Nps. The elemental composition of the prepared nanoparticles shows prominently larger peaks for copper (Cu) and oxygen (O) and much smaller peaks corresponding to impurities, aluminium (Al), silicon (Si), iron (Fe), and nickel (Ni). The weight percentage of copper and oxygen was calculated to be 73.15 and 22.17%, respectively. The strong and narrow diffraction peaks of CuO

Figure 1. (a, b) SEM image of CuO Nps at different magnifications.
Nps suggest that the resultant particles are highly crystalline in nature.

**UV–Vis Absorption Spectroscopy for Copper Oxide Nanoparticles.** Formation of CuO Nps was initially confirmed using UV–vis absorption spectroscopy. Figure 2 clearly shows a sharp absorption peak, corresponding to the surface plasmon resonance (SPR) for CuO Nps at 250 nm.53 This is attributed to the oscillation of surface conduction electrons, which are excited by the incident electromagnetic radiation. Another weak and broad spectrum in the range of 320–600 nm was also observed, attributed to the SPR band of some Cu colloid formations of the nonoxidized Cu Nps.54

**Fourier Transform Infrared (FT-IR) Spectroscopy.** FT-IR analysis was carried out to detect the probable biomolecules that are responsible for the formation of CuO Nps. Figure 3 represents the FT-IR spectrum of CuO Nps recorded in the range of 400–4000 cm⁻¹. As observed, a strong peak around 3427 cm⁻¹ corresponds to the −OH stretching of phenolic compounds present in the seed extract, and the band at 1635 cm⁻¹ corresponds to the C=O stretching of ketones or acids.55 The fine peak at 2923 cm⁻¹ was attributed to the alkane C–H stretching mode.55 The sharp peak around 1405 cm⁻¹ shows the presence of −COO carboxylic acid and the absorption peak at 1103 cm⁻¹ corresponds to the C−NH₂ primary aliphatic amines present in the seed extract.55 The narrow bands at 457, 526, 600, and 784 cm⁻¹ confirm the formation of pure CuO Nps.56 This indicates that the biological molecule in the seed extract has a dual function of stabilization and formation of CuO Nps.57

**Thermogravimetric Analysis.** TGA was carried out to further characterize the nanoparticles. Figure 4a represents the TGA curve of CuO Nps on heating from 0 to 800 °C under oxygen. The TGA curve exhibits the two-step decomposition of CuO Nps, with weight loss occurring in the temperature ranges of 80–130 and 260–400 °C. The former weight loss is attributed to the removal of moisture content, and the latter is due to the combustion of the organic solvent compound, and COₓ (x = 1, 2) or organic gases will be left over. Figure 4b shows a minor endothermic DTA peak around the region of 130 °C, which corresponds to the first mass loss in the TGA curve. The second major mass loss occurring around 260–400 °C in TGA is associated with a prominent DTA exothermic peak around the region of 265 °C.56

**X-ray Diffraction (XRD) Crystallography.** To investigate the crystallographic nature and phase purity of the synthesized CuO Nps, XRD analysis was performed, as shown in Figure 5. Major diffraction peaks occurring at the 2θ values of 34.3, 38.7, 48.7, 51.3, 58.2, 61.5, 65.8 were assigned to (0,0,2), (1,1,1), (2,0,−2), (1,1,2), (2,0,2), (1,−1,3), and (0,2,2) miller indices plane. A sharp peak at 2θ = 38.7° with the diffraction of the (111) plane corresponds to CuO Nps, which indicates that they are monoclinic crystals with the space group of C2/c (15). XRD analysis illustrates that the synthesized CuO Nps are monoclinic and crystalline in nature. The assigned lattice parameters are a = 4.6883 Å, b = 3.4229 Å, and c = 5.1319 Å, and these values agreed well with the standard JCPDS No. 00-048-1548. The size of the crystalline nanoparticles was enumerated using the Debye–Scherrer formula for the maximum intensity peak59

\[
D = \frac{0.9}{\lambda} \beta \cos \theta
\]

where λ is the X-ray wavelength (0.1546 nm), β is the full width at half-maximum (FWHM) (line broadening at half-maximum) in radians, and θ is Bragg’s angle. On the basis of the highest 2θ peak, we calculated the crystalline size to be ca. 13.07 nm for CuO Nps. Also, due to the smaller crystalline size, the XRD pattern appears noisy.

**X-ray Photoelectron Spectroscopy (XPS).** The synthesized CuO Nps were characterized by XPS analysis to understand the chemical composition of the particles and is shown in Figure 6. The survey scan spectrum in Figure 6a shows peaks corresponding to C 1s (59.9%), Cu2p₁ (8.9%), and O 1s (31.2%). Figure 6b shows the corresponding O 1s region. Figure 6c shows the strong C 1s spectra with high resolution. Figure 6d represents the strong Gaussian fitting peak of Cu 2p in the range of 934–954 eV. The two peaks around 943 and 963 eV are the corresponding satellite peaks.58 The peak at 934 eV with a lower binding energy is assigned to CuO, corresponding to the Cu 2p₁/₂ state, and the other peak at 954 eV corresponds to the Cu 2p₃/₂ state, which is similar to the result in earlier reports.59–61 The oxidation state of Cu was thus Cu(0), which can be easily oxidized by atmospheric oxygen to Cu(I) or Cu(II) to form the CuO Nps.62

**Antibacterial Activity of CuO Nps.** The antibacterial properties of CuO Nps were examined against *Staphylococcus aureus* and *Aeromonas*. CuO Nps exhibited good properties for inhibiting the bacterial growth; Figure 7 clearly shows the zone of clearance against both the pathogens. It is revealed that
CuO NPs showed better antibacterial properties against *S. aureus* when compared to *Aeromonas*. The antibacterial properties of CuO nanoparticles might be due to the interaction between the rice-grain-shaped nanoparticles with the bacterial surface, which easily leads to cell damage due to the ripping of the bacterial cell wall. The good activity seen toward Gram-positive and Gram-negative bacteria could be due to the generation of reactive oxygen species (ROS) from CuO. The obtained zone of inhibition was measured and presented in Figure 8. The results presented are the mean of three parallel experiments and are presented as mean ± standard deviation.

**Electrochemical Behaviour of a Copper Oxide Nanoparticle Modified Electrode.** A novel electrochemical sensor
for the modified electrode in comparison to the unmodified bare PIG electrode in the presence of riboflavin. This indicated the modifier CuO Nps have a good catalytic effect and faster electron transfer kinetics. The modified electrode shows a redox peak with an $E_{pa}$ value of 0.1 V and an $E_{pc}$ value of −0.15 V, and a corresponding $ΔE_p$ value of 0.25 V within a potential window of 0.4 to −0.4 V at a scan rate of 50 mV s$^{-1}$. The surface coverage concentration of the modified electrode was found to be $7.4207 \times 10^{-13}$ mol cm$^{-2}$ using the equation

$$\Gamma = \frac{Q}{nFEA}$$

where $Q$ is the charge, $\Gamma$ is the surface coverage (mol cm$^{-2}$), $n$ is the number of transferred electrons, $F$ is the Faraday constant (96 485.3329 C mol$^{-1}$), and $A$ is the electrode surface area (cm$^2$).

**Effect of pH on the Electrochemical Determination of Riboflavin.** The effect of pH on the electrocatalysis of riboflavin by CuO Nps under different pH conditions (3.0–8.0) was studied. The pH was maintained using 0.1 M acetate or phosphate buffers. The voltammogram was recorded with the modified electrode as the working electrode in 30 μM VB$_2$ at a scan rate of 50 mV s$^{-1}$. Figure 10a shows the cyclic voltammograms for the oxidation of VB$_2$ under different pH conditions. The maximum anodic peak current was observed at a pH of 5. Above or below this pH, the peak current was found to be reduced. The highest current response at pH 5 may be due to two factors: the protonation of the acidic buffer (ABS) at higher pH values and the fact that the redox mechanism of riboflavin involves a proton, thus resulting in the reduction in current at higher pH values. Hence, pH 5 was considered as optimum for further determination of VB$_2$ using the CuO-Np-modified electrode.

Figure 10b shows the plot of pH vs anodic potential $E_{pa}$ (V) for riboflavin oxidation, which gives a linear response with correlation coefficient $R^2 = 0.9698$. Figure 10c represents the plot of pH vs current $I$ (μA).

**Effect of Scan Rate.** A scan rate study plays a significant role in the rate and kinetic studies of electrochemical reactions. To analyze the nature of the electrocatalytic action of the CuO Nps/PIG-modified electrode on VB$_2$ reduction, the effect of scan rate on the anodic peak potential and current was investigated in the presence of 50 μM VB$_2$ at scan rates ranging from 50 to 250 mV s$^{-1}$ in a pH 5 acetate buffer solution. As shown in Figure 11a, the anodic and cathodic peak current increases gradually with increasing scan rates and the anodic peak potential shifts toward the positive side, which suggests the redox process to be quasi-reversible. The potential shift is due to the kinetic limitation, wherein the active species and electrolyte ions get enough time to interact with each other at a lower scan rate, but the interaction was not good enough at a higher scan rate. The corresponding plots of the observed current (for both anodic and cathodic peaks) vs scan rate are clearly shown in Figure 11b. The obtained correlation coefficient for the anodic current ($I_{pa}$) $R^2 = 0.9776$ and for the cathodic current ($I_{pc}$) $R^2 = 0.9292$. The plot shows very good linearity, where the slope value is close to 1, and this confirms that the electron transfer reaction is a diffusion-controlled process.

The electrocatalytic redox reaction of VB$_2$ involves two protons coupled with two electrons. Figure 12 exhibits the possible mechanism of VB$_2$ reduction at the CuO-Np-modified electrode

$$\text{VB}_2 + 2\text{H}^+ + 2e^- \rightarrow \text{VB}_4\text{H}_2$$

**Determination of Riboflavin: Cyclic Voltammetry Study.** VB$_2$ was added into the electrolyte solution containing 0.1 M ABS of pH 5, and the voltammogram was recorded with the CuO-modified PIG electrode as the working electrode. The standard addition method was used to ascertain the linearity and detection limit for riboflavin oxidation at the modified electrode. The potential was scanned in the region of −0.4 to 0.4 V at a scan rate of 50 mV s$^{-1}$. Figure 13a shows the

Figure 7. (a, b) Antibacterial activity of CuO Nps against S. aureus and Aeromonas.

Figure 8. Zone of inhibition of CuO Nps against S. aureus and Aeromonas.

Figure 9. Cyclic voltammograms of bare and modified electrodes with 30 μM riboflavin in 0.1 M acetate buffer at pH 5.
Cyclic voltammograms for the VB₂ redox process. The redox peak current is linearly enhanced with an increase in VB₂ concentration. A well-distinguished anodic peak is observed at 0.1 V and a cathodic peak at –0.15 V. The observed redox process is a two-electron process with one electron involved in the conversion of the oxidized flavin molecule to semiquinone and another electron in the conversion of half reduced dihydroflavin to a fully reduced dihydroflavin molecule.49

The obtained linear range for VB₂ is from $6.27 \times 10^{-7}$ to $75 \times 10^{-7}$ M with a detection limit of $2 \times 10^{-7}$ M. Figure 13b shows the calibration plot for VB₂ exhibiting linear dependency. The linearity was found to be from 3.13 to 56.3 nM with a detection limit of 1.04 nM. The corresponding correlation coefficient was $R^2 = 0.9760$.

**Chronoamperometric and Impedance Study.** To determine the operating potential for amperometric detection of VB₂ at the CuO-modified PIG electrode, a hydrodynamic study was carried out. Based on the study, an operating potential of 0.1 V was chosen as the optimum for carrying out the electrocatalysis of VB₂ at the CuO-modified PIG electrode was studied using SWV, as it is a more sensitive technique for such an analysis. VB₂ was added into the electrolyte solution in the volume range of 5–90 μL, and voltammograms were recorded within a potential window of –0.2 to 0.3 V, as shown in Figure 14a. A sharp distinct peak was observed around 0.03 V, the current values for which were enhanced on increasing the VB₂ concentration. Figure 14b shows the calibration plot for VB₂ exhibiting linear dependency. The linearity was found to be from 3.13 to 56.3 nM with a detection limit of 1.04 nM. The corresponding correlation coefficient was $R^2 = 0.9760$. The electrocatalytic activity of the CuO-modified PIG electrode.
the reduction of VB$_2$ at the modified electrode. Figure 15a shows the chronoamperogram obtained for the reduction of VB$_2$ on the CuO-modified PIG electrode at a fixed potential of 0.1 V under stirring conditions of 300 rpm in 0.1 M ABS, pH 5. With successive additions of VB$_2$, the CuO-modified PIG electrode showed a stepwise increment of the catalytic current with a linear range of 1.2 to 8.58 $\mu$M. Figure 15b shows the corresponding calibration plot exhibiting linearity with a correlation coefficient of $R^2 = 0.9646$. Figure 15c shows the double-step chronoamperometric result of the CuO-modified PIG electrode to investigate the electrocatalysis of VB$_2$ using a chemically modified electrode. The study was carried out with a pH 5 electrolyte by standard addition of the VB$_2$ solution. The working potential was fixed at 0.1 V for the first potential step and $-0.1$ V for the second potential step. On increasing the concentration of VB$_2$, the anodic current increases for the first potential step and the cathodic current increases for the second potential step; both steps consume equal charge for oxidation and reduction of the redox couple in the CuO-modified PIG electrode.66,67

Figure 16a shows the electrochemical impedance spectra (EIS) of the bare and CuO-modified PIG electrodes in the presence of 5 mM of [Fe(CN)$_6$]$_3^{-}/4^{-}$ in 0.1 M KCl as the supporting electrolyte. In the Nyquist diagram, the semicircle diameter shows the electron transfer kinetics of the [Fe(CN)$_6$]$_{3^{-}/4^{-}}$ redox scan on the electrode surface, which is equal to the electron transfer resistance.68 The resistance of the modified electrode is lower when compared to the bare electrode, suggesting the lower resistance to electron transfer due to the electrocatalytic effect of the CuO Nps. The impedance spectra fitted well with the Randles circuit shown in Figure 16b. $R_s$ (electrolyte resistance) is connected with $R_{ct}$ (charge resistance) and $W$ (Warburg resistance), and it is parallel to $C_d$ (double-layer capacitance).69 It is clear that the CuO-modified PIG electrode provides enhancement of the charge transfer efficiency when compared to the bare electrode.70

The electrochemical response of the modified electrode for VB$_2$ detection has been correlated with other electrochemical methods reported in the literature. The comparison is presented in Table 1, which shows that the CuO-modified PIG electrode exhibits an excellent linear range and lower detection limit compared to previous reports.

**Interference.** An important parameter for evaluating sensor performance is its ability to distinguish any interfering compounds in relation to the analyte of interest. To determine the selectivity of the CuO-modified PIG electrode toward VB$_2$, possible interfering species such as glucose, ascorbic acid, dopamine, uric acid, salicylic acid, and tartaric acid were added to the test solution. A 10-fold excess of ascorbic acid and dopamine and a 50-fold excess of uric acid, salicylic acid, tartaric acid, and glucose show minimal interference in detecting VB$_2$ and is shown in Figure 17. The result suggests the excellent selectivity of the CuO-modified PIG electrode toward VB$_2$ determination.

**Stability and Reproducibility.** The stability and reproducibility of the proposed CuO-modified PIG electrode sensor were evaluated for 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, and 120 days using an optimized experimental method. Figure 18 shows the current response of the CuO-modified PIG electrode over a period of 120 days. Therefore, the mentioned results indicate that the CuO-modified PIG electrode is a sensor with good stability and reproducibility for the determination of VB$_2$.

**Real-Sample Analysis.** The CuO-modified PIG electrode potential was evaluated for the detection of VB$_2$ in B-complex tablets, milk powder, and egg yolk. The corresponding analytical parameters are presented in Table 2. The concentration of VB$_2$ was evaluated by the standard addition method, and the recovery values range from 99 to 99.75%. The obtained result shows that the proposed method can be employed for real-sample analysis.

---

**Figure 12.** Possible redox mechanism for VB$_2$ on the CuO-Np-modified electrode.

**Figure 13.** (a, b) Cyclic voltammogram for the determination of VB$_2$ and the corresponding calibration plot of anodic and cathodic peak currents vs concentration of VB$_2$. 

---

**Table 1.** Comparison of different methods for the determination of VB$_2$ in different samples.

**Table 2.** Analytical parameters for the determination of VB$_2$ in real samples.
EXPERIMENTAL METHODS

Chemicals. Cupric nitrate trihydrate [Cu(NO₃)₂·3H₂O], riboflavin (C₁₇H₂₀N₄O₆), and ammonia (NH₃) were purchased from Sigma-Aldrich.

Apparatus Required. All electrochemical experiments were performed on an electrochemical workstation (CHI 6095E Instruments Austin, TX) with a conventional three-electrode system, in which the paraffin-impregnated graphite electrode (PIGE) was used as the working electrode, a platinum electrode was employed as the counter electrode, and a saturated calomel electrode (SCE) was used as the reference electrode. For the impedance study, the applied potential was 0.3 V and was recorded with an amplitude of 5 mV over the frequency range of 0.1 Hz to 100 kHz. All electrochemical studies were carried out at room temperature (25 °C). The synthesized compound was characterized by SEM, UV−vis, FT-IR, TGA and DTA, XRD, and XPS techniques. SEM was recorded with a Quattro S Instrument, ThermoFisher Scientific; FT-IR spectra were recorded with an AGILENT Instrument; UV−vis spectra were recorded with a JAZ, Ocean optics; TGA–DTA curves were recorded with a FTA2500 Instrument, Netzsch; XRD spectra were recorded with a SmartLab SE X-Ray Instruments, Rigaku; and XPS spectra were recorded with a ULVAC-PHI Versaprobe-II. The details of the antibacterial studies are given below.

Preparation of Seed Extract. The C. bonducella seed (bonduc seed) coat was removed, and the kernel was finely ground using a mortar and pestle. About 0.5 mg of the ground seed was taken in 50 mL of deionized water and allowed to sonicate for 30 min and filtered.

Synthesis of Copper Oxide Nanoparticles. Various percentage solutions of the seed extract, ammonia solution (25%), and 0.01 M cupric nitrate trihydrate were used for the synthesis of copper nanoparticles. The pH conditions were examined and optimized on the basis of the obtained product yield. Briefly, 40 mL of the cupric solution was taken in a 100 mL beaker and 40 µL of the freshly prepared seed extract was added dropwise for carrying out the reduction of copper ions, followed by the addition of 200 µL of ammonia solution under constant stirring. The blue-colored solution turned green over a period of 5 h, and finally, a dark brown precipitate formed at about 7 h, as shown in Scheme 1. The formation of the brown

Figure 14. (a, b) Square-wave voltammogram obtained with the modified electrode for VB₂ oxidation with the corresponding calibration plot.

Figure 15. (a−c) Chronoamperometric study of VB₂ reduction with the corresponding calibration plot and double-step chronoamperogram of VB₂ reduction and oxidation using the CuO-modified PIG electrode.
precipitate indicated the complete reduction of copper ions and the formation of CuO Nps. The obtained solution was then centrifuged and washed several times using deionized water, and the sample was heated at 450 °C for an hour.

Antibacterial Activity. The antibacterial properties of CuO Nps were investigated against S. aureus (Gram-positive) and Aeromonas (Gram-negative) bacteria by the agar diffusion method. Each bacterial strain was smeared evenly on the sterilized agar Petri plates and allowed to dry. Two wells each of ∼5 mm in diameter were made on petri dish after inoculum of strain and 30 μL of CuO-NPs were loaded in the wells. To observe the zone of clearance, the plates were incubated at 37 °C for 24 h, and the zone of inhibition was measured and photographed the next day.

Preparation of Real Samples. The egg was allowed to boil in a water bath for 6 min and the yolk was taken separately. Then, 1 g of egg yolk was added to 20 mL of 0.02 M acetic acid and sonicated for 15 min at room temperature. The extract was filtered using Whatman filter paper No. 1 (55 mm diameter). Vitamin B-complex tablets were purchased from a local pharmaceutical store; it was ground, and 10 mg of

Table 1. Comparison of Electrochemical Behavior of the Experimental Sensors for VB₂ Detection

| modified electrode                  | linear range   | LOD (nM) | reference |
|-------------------------------------|----------------|----------|-----------|
| rMoS₂/graphene/Au electrode         | 0.025–2.25 μM  | 20       | 71        |
| (OMC/GCE)                           | 0.4–1.0 μM     | 20       | 65        |
| (HMHP/CPE)                          | 0.03–1.5 μM    | 11       | 32        |
| SnO₂/RGO                            | 0.1–150 μM     | 34       | 50        |
| BiFe/SwAdSV                         | 0.3–0.8 μM     | 100      | 72        |
| nano-Zr-ZSM-5/GCE                  | 30–500 μM      | 5        | 73        |
| PTN/GC                              | 0.01–65 μM     | 3        | 74        |
| Cys-MWNTs-GO-GCE                    | 0.002–1500     | 1.2      | 75        |
| α-Fe₂O₃/MWCNT/Au Nps                | 0.3–0.006 μM   | 6        | 76        |
| N-CQD/SnO₂/SPCE                     | 0.05–306 μM    | 8        | 77        |
| CuO-modified PIGE                   | 3.13–56.3 nM   | 1.04     | this work |

Figure 18. Current response at the CuO-Np-modified electrode toward 30 μM VB₂ over 120 days in ABS (pH 5) at a scan rate of 50 mV s⁻¹.

Table 2. Determination of VB₂ in B-Complex, Milk Powder, and Egg Yolk Samples (μM)

| sample              | real sample | added (μM) | detected (μM) | RSD (%) | recovery (%) |
|---------------------|-------------|------------|---------------|---------|--------------|
| VB₂                 | B-complex   | 4          | 4.03          | 0.66    | 99.25        |
|                     |             | 5          | 5.03          | 1.66    | 99.40        |
|                     |             | 6          | 6.04          | 0.53    | 99.33        |
| milk powder         | 3           | 3.03       | 1.15          | 99.00   |
|                     | 4           | 4.01       | 0.43          | 99.75   |
|                     | 5           | 5.02       | 0.52          | 99.60   |
| egg yolk            | 2           | 2.01       | 0.74          | 99.00   |
|                     | 3           | 3.02       | 0.70          | 99.33   |
|                     | 4           | 4.02       | 0.52          | 99.50   |

Scheme 1. Photographs Taken during the Formation of CuO Nanoparticles

Antibacterial Activity. The antibacterial properties of CuO Nps were investigated against S. aureus (Gram-positive) and Aeromonas (Gram-negative) bacteria by the agar diffusion method. Each bacterial strain was smeared evenly on the sterilized agar Petri plates and allowed to dry. Two wells each of ∼5 mm in diameter were made on petri dish after inoculum of strain and 30 μL of CuO-NPs were loaded in the wells. To observe the zone of clearance, the plates were incubated at 37 °C for 24 h, and the zone of inhibition was measured and photographed the next day.

Preparation of Real Samples. The egg was allowed to boil in a water bath for 6 min and the yolk was taken separately. Then, 1 g of egg yolk was added to 20 mL of 0.02 M acetic acid and sonicated for 15 min at room temperature. The extract was filtered using Whatman filter paper No. 1 (55 mm diameter). Vitamin B-complex tablets were purchased from a local pharmaceutical store; it was ground, and 10 mg of...
the powdered sample was dissolved in 25 mL of 0.05 M phosphate-buffered saline (PBS) pH 5 solution.\textsuperscript{50} The milk powder was weighed and dissolved in boiling water; by the addition of acetic acid, the proteins present in the milk powder were precipitated and digested on the water bath for 15 min and was filtered through Whatman filter paper No. 1 (55 mm diameter).\textsuperscript{76,78}

**CONCLUSIONS**

In summary, this study provides a convenient and inexpensive method to synthesize CuO NPs using C. bonducella as a novel reducing and stabilizing agent. The CuO NPs were characterized by SEM, UV-vis, FT-IR, TGA–DTA, XRD, and XPS spectroscopy. The CuO-Np-modified PIG electrode was used as a suitable sensor for the determination of nanomolar concentration of VB\textsubscript{2}. The stability of the CuO-Np-modified PI electrode seemed to be satisfactory for over a period of 120 days. The practical applicability of the modified electrode was checked with real samples, such as egg yolk, milk powder, and B-complex tablets. The results obtained showed very good recovery, and the method could be extended for further practical applications.

**AUTHOR INFORMATION**

**Corresponding Author**
*E-mail: pndeepa@hotmail.com.*

**ORCID**
Deepa Padmanabhan Nambiar: 0000-0002-7467-5612

**Funding**
This work was not supported by any funding agencies.

**Notes**
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**
The authors would like to thank Dr. Saju Pillai and Peer Mohamed, CSIR—National Institute for Interdisciplinary Science and Technology (NIIST), Kerala, for the XPS study, K. Krishna Kumar, Central Instrumentation Laboratory, VELS Institute of Science, Technology & Advanced Studies, Chennai, for the SEM analysis, Preethi Ramadoss, Crystal Growth Centre, Anna University, Chennai, for the antibacterial studies, and GNR Instrumentation Centre, University of Madras, Guindy Campus, Chennai, for UV-vis, FT-IR, TGA–DTA, and XRD spectroscopic analysis.

**REFERENCES**

1. Mott, D.; Galkowski, J.; Wang, L.; Luo, J.; Zhong, C.-J. Synthesis of Size-Controlled and Shaped Copper Nanoparticles. *Am. Chem. Soc.* 2007, 23, 5740–5745.

2. Sharma, J. K.; Akhtar, M. S.; Ameen, S.; Pratihba, S.; Guridip, S. Green synthesis of CuO nanoparticles with leaf extract of Calotropis gigantea and its dye-sensitized solar cells applications. *J. Alloys Compd.* 2015, 632, 321–325.

3. Rintu, V.; Joy Prabu, H.; Johnson, I. Green Synthesis and Characterizations of Flower Shaped CuO Nanoparticles for Biodiesel Application. *Sens. Transducers J.* 2017, 210, 38–41.

4. Manjari, G.; Saran, S.; Arun, T.; Vijaya Bhaskara Rao, A.; Sulaj DeviPriya, P. Catalytic and Recyclability Properties of Phytogenic Copper Oxide Nanoparticles Derived from Aglaia elaeagnoidea Flower Extract. *J. Saudi Chem. Soc.* 2017, 21, 610–618.

5. Peternela, J.; Silva, M. F.; Vieira, M. F.; Bergamasco, R.; Vieira, A. M. S. Synthesis and Impregnation of Copper Oxide Nanoparticles on Activated Carbon through Green Synthesis for Water Pollutant Removal. *Mater. Res.* 2018, 21, No. e20160460.

6. Afšin, P.; Mir Fazlollah, M.; Mohammad Sani, R. Fabrication of anchored copper oxide nanoparticles on graphene oxide nanosheets via an electrostatic coprecipitation and its application as supercapacitor. *Electrochim. Acta* 2013, 88, 347–357.

7. Ramachandran, A.; Arya Nair, J. S.; Yesodha, S. K. PolyaniLine-Derived Nitrogen-Doped Graphene Quantum Dots for the Ultratrace Level Electrochemical Detection of Trinitrophenol and the Effective Differentiation of Nitroaromatics: Structure Matters. *ACS Sustainable Chem. Eng.* 2019, 7, 6732–6743.

8. Shaalan, N. M.; Rashad, M.; Abdel-Rahim, M. A. CuO nanoparticles synthesized by microwave-assisted method for methane sensing. *Opt. Quantum Electron.* 2016, 48, No. 531.

9. Mohammad Shafee, M. R.; Kargar, M.; Ghashang, M. Simple Synthesis of Copper Oxide Nanoparticles in the Presence of Extractive Rosmarinus Officinalis leaves. *J. Nanostruct.* 2016, 6, 28–31.

10. Tamaekong, N.; Liewhiran, C.; Phantomphant, S. Synthesis of Thermally Spherical CuO Nanoparticles. *J. Nanomater.* 2014, No. 407978.

11. Wacławek, S.; Padil, V. V. T.; Cernik, M. Major advances and challenges in heterogeneous catalysis for environmental applications: a review. *Ecol. Chem. Eng. S* 2018, 5, 9–34.

12. Verma, N.; Kumar, N. Synthesis and Biomedical Applications of Copper Oxide Nanoparticles: An Expanding Horizon. *ACS Biomater. Sci. Eng.* 2019, 5, 1170–1188.

13. Prasad, K. S.; Patra, A.; Shruthi, G.; Chandan, S. Aqueous Extract of Saraca indica Leaves in the Synthesis of Copper Oxide Nanoparticles: Finding a Way towards Going Green. *J. Nanotechnol.* 2017, No. 7502610.

14. Singh, S.; Kumar, N.; Kumar, M.; Jyoti; Agarwal, A.; Mizaikoff, B. Electrochemical sensing and remediation of 4-nitrophenol using bio-synthesized copper oxide nanoparticles. *Chem. Eng. J.* 2016, 31, 283–292.

15. Bordbar, M.; Sharifiz-Zarchi, Z.; Khodadadi, B. Green synthesis of copper oxide nanoparticles/dinophtolitë using Rheum palmatum L. root extract: high catalytic activity for reduction of 4-nitro phenol, rhodamine B, and methylene blue. *J. Sol–Gel Sci. Technol.* 2017, 72, 66–73.

16. Das, P.; Ghosh, S.; et al. Madhuca longifolia plant mediated green synthesis of cupric oxide nanoparticles: promising environmentally sustainable material for waste water treatment and efficient. *J. Photochem. Photobiol., B* 2018, 189, 66–73.

17. Anamkwar, B.; Chaudhary, M.; Sastry, M. Gold nano triangles biologically synthesized using tamarind leaf extract and potential application in vapour sensing. *Synth. React. Inorg. Met.-Org. Chem.* 2005, 35, 19–26.

18. Brumbaugh, A. D.; Cohen, K. A.; Angelo, S. K. S. Ultrasmall Copper Nanoparticles Synthesized with a Plant Tea Reducing Agent. *ACS Sustainable Chem. Eng.* 2014, 1933.

19. Shukla, S.; Mehta, A. Antioxidant, Total Phenolics and total flavonoid content of the aqueous extract of Casalpinia bonducella Seeds. *Chang Mai J. Sci.* 2017, 44, 929–938.

20. Sharma, S. R.; Dwivedi, S. K.; Swarup, D. Hypoglycaemic, antihyperglycaemic and hypolipidemic activities of Casalpinia bonducella seeds in rats. *J. Ethnopharmacol.* 1997, 58, 39.

21. Kannur, D. M. Antidiabetic and Antihyperlipidemic Activity of Bonduccela (Casalpinia bonducella) Seeds. *Nuts and Seeds in Health and Disease Prevention* Elsevier, 2011; pp 237–244.

22. Kannur, D. M.; Paranipe, M. P.; Sonavane, L. V.; Dongre, P. P.; Khandelwal, K. R. Evaluation of *Caesalpinia bonducella* (Roxb.) Seeds. *J. Ethnopharmacol.* 2012, 3, 171–175.

23. Ali, A.; Venkat Rao, N.; Shalam, M.; Shivraj Gouda, T.; Shantakumar, S. M. Anticonvulsive Effect of Seed Extract of *Caesalpinia bonducella* (Roxb.). *Iran. J. Pharmocol. Ther.* 2009, 8, 51.

24. Gaur, R. L.; Sahoo, M. K.; Dixit, S.; Fatma, N.; Rastogi, S.; Kulshreshtha, D. K.; Chatterjee, R. K.; Murthy, P. K. Antifilarial
activity of Caesalpinia bonduc on experimental filarial infections. Indian J. Med. Res. 2008, 128, 65–70.
(25) Billah, M. M.; Islam, R.; Khatoon, H.; Parvin, S.; Islam, E.; Islam, A. S. M.; Mia, A. A. Antibacterial, antidiarrhoeal, and cytotoxic activities of methanol extract and its fractions of Caesalpinia bonduc (L.) Roxb leaves. BMC Complementary Altern. Med. 2015, No. 101.
(26) Khan, H.-U.; Ali, I.; Khan, A.-U.; Naz, R.; Gilani, A. H. Antibacterial, antifungal, antispasmodic and Ca++ antagonist effects of Caesalpinia bonduc. J. Nat. Prod. Res. 2011, 25, 444–449.
(27) Kannur, D. M.; Hukkeri, V. I.; Akki, K. S. Antidiabetic effect of Caesalpinia bonduc seed extracts in rats. Fitoterapia 2006, 77, 546–549.
(28) Khedkar, A.; Mandavkar, Y. D.; Shinde, G.; Khalure, P.; Dere, P. Diuretic effect of Caesalpinia bonduc in rats. Bangladesh J. Pharmacol. 2011, 6, 61–63.
(29) Manikandasevi, S.; Vadivel, V.; Brindha, P. Studies on Nutraceutical Properties of Caesalpinia bonduc L.; An Indian Traditional Medicinal Plant. Res. J. Med. Plants 2016, 10, 127–139.
(30) Lilaram; Ahmed, R. N. Effect of Caesalpinia bonduc seed extract on histoarchitecture of some vital organs and clinical chemistry in female albino rats. J. King Saud Univ., Sci. 2013, 25, 1–6.
(31) Yu, Y.-Y.; Wang, J.-X.; Si, R.-W.; Yang, Y.; Zhang, C.-L.; Yong, Y.-C. Sensitive Amperometric Detection of Riboflavin with a Whole-Cell Electrochemical Sensor. Anal. Chim. Acta 2017, 985, 148–154.
(32) Zarei, E.; Jamal, M. R.; Bagheri, J. Electrochemical Determination of Riboflavin by an Ionic Liquid Modified Carbon Paste Electrode as a Sensitive Sensor. Anal. Bioanal. Electrochem. 2018, 10, 642–657.
(33) Diniz, M.; Dias, N.; Andrade, F.; Paulo, B.; Ferreira, A. Isotope dilution method for determination of vitamin B2 in human plasma using liquid chromatography–tandem mass spectrometry. J. Chromatogr. B 2019, 15, 14–19.
(34) Strong, F. M.; Carpenter, L. E. Preparation of Samples for Microbiological Determination of Riboflavin. Ind. Eng. Chem., Anal. Ed. 1942, 14, 909–913.
(35) Monte-Filho, S. S.; Andrade, S. I. E.; Lima, M. B.; Araujo, M. C. U. Synthesis of highly fluorescent carbon dots from lemon and onion juices for determination of riboflavin in multivitamin/mineral supplements. J. Pharm. Anal. 2019, 9, 209–216.
(36) Makkoumi, K.; Bäckman, A. Photodynamic UVA-riboflavin bacterial elimination inantiobiotic-resistant bacteria. Clin. Exp. Ophthalmol. 2016, 44, 582–586.
(37) Aguiar, T. Q.; Silva, R.; Domingues, D. Beyond industrial riboflavin production: A historical perspective and emerging biotechnological applications. Biotechnol. Adv. 2015, 33, 1774–1786.
(38) Pérez-Alvarez, E. P.; Ruiz-González, R.; Nonell, S.; Garde-Cerdán, T. Riboflavin applications to grapevine leaves and berries blue-light post-harvest treatments modifies grape anthocyanins and amino acids contents. Food Res. Int. 2019, 122, 479–486.
(39) González-Santamaría, R.; Ruiz-González, R.; Nonell, S.; Garde-Cerdán, T.; Pérez-Alvarez, E. P. Influence of foliar riboflavin applications to vineyard on grape amino acid content. Food Chem. 2018, 240, 601–606.
(40) Silvestri, D.; Wacławek, S.; et al. A poly(3-hydroxybutyrate)–chitosan polymer conjugate for the synthesis of safer gold nanoparticles and their applications. RSC Green Chem. 2018, 4975.
(41) Jain, S.; Mishra, S.; Sarma, T. K. ZnO-Induced Self-Assembled Growth of Octapodal CuO-ZnO Microcrystals: Multifunctional Applications in Reductive Degradation of Organic Pollutants and Nonenzymatic Electrochemical Sensing of Glucose. ACS Sustainable Chem. Eng. 2018, 6, 9771–9783.
(42) Balaubramanian, P.; Balamurugan, T. S. T.; Chen, S.-M.; Chen, T.-W. Facile Synthesis of Spinel-Type Copper Cobaltite Nanoplates for Enhanced Electrochemical Detection of Acetylyholine. ACS Sustainable Chem. Eng. 2019, 7, 7642–7765.
(43) Sudha, V.; Krishnamoorthy, K.; et al. Copper oxide nanosheet modified electrodes for simultaneous determination of environmentally hazardous anions. J. Alloys Compd. 2018, 959.
(61) Gao, D.; Zhang, J.; Zhu, J.; Qi, J.; Zhang, Z.; Sui, W.; Shi, H.; Xue, D. Vacancy-Mediated Magnetism in Pure Copper Oxide Nanoparticles. *Nanoscale Res. Lett.* 2010, 5, 769–772.

(62) Patil, S. A.; Ryu, C.-H.; Kim, H.-S. Synthesis and Characterization of Copper Nanoparticles (Cu-Nps) using Rongalite as Reducing Agent and Photonic Sintering of Cu-Nps Ink for Printed Electronics. *Int. J. Precis. Eng. Manuf.-Green Technol.* 2018, 8, 239–245.

(63) Ananth, A.; Dharaneeedharan, S.; Heo, M.-S.; Mok, Y. S. Copper oxide nanomaterial: synthesis, characterization and structure-specific antibacterial performance. *Chem. Eng. J.* 2015, 262, 179–188.

(64) Saranya, S.; Jency Feminus, J.; Geetha, B.; Deepa, P. N. Simultaneous detection of glutathione, threonine, and glycine at electrodeposited RuHCF/rGO-modified electrode. *Ionics* 2019, 25, 5537–5550.

(65) Bai, J.; Ndamanisha, J. C.; Liu, L.; Yang, L.; Guo, L. Voltammetric detection of riboflavin based on ordered mesoporous carbonmodified electrode. *J. Solid State Electrochem.* 2010, 14, 2251–2256.

(66) Ojani, R.; Raoof, J. B.; Fathi, S.; et al. Electrochemical behavior of Ni(II) incorporated in zeolite Y-modified carbon electrode: application for electrocatalytic oxidation of methanol in alkaline solution. *J Solid State Electrochem.* 2011, 15, 1935.

(67) Raoof, J. B.; Ojani, R.; Jamali, F.; Hosseini, S. R. Electrochemical Detection of Hydrazine Using a Copper oxide electrode Modified Glassy Carbon Electrode. *Caspian J. Chem.* 2012, 1, 73–85.

(68) Feng, J. J.; Xu, J. J.; Chen, H. Y. Direct electron transfer and electrocatalysis of hemoglobin adsorbed onto electrodeposited mesoporous tungsten oxide. *Electrochem. Commun.* 2006, 8, 77–82.

(69) Ajdari, F. B.; Kowsari, E.; Ehsani, A.; Schorowski, M.; Ameri, T. New synthesized ionic liquid functionalized graphene oxide: Synthesis, characterization and its nanocomposite with conjugated polymer as effective electrode materials in an energy storage device. *Electrochim. Acta* 2018, 292, 789–804.

(70) Muñoz, J.; Céspedes, F.; Baeza, M. Modified multiwalled carbon nanotube/epoxy amperometric nanocomposite sensors with CuO nanoparticles for electrocatalytic detection of free chlorine. *Microchem. J.* 2015, 122, 189–196.

(71) Wang, Y.; Zhuang, Q.; Ni, Y. Fabrication of riboflavin electrochemical sensor based on homoadenine single-stranded DNA/molybdenum disulfide–graphene nanocomposite modified gold electrode. *J. Electroanal. Chem.* 2015, 736, 47–54.

(72) Sá, E. S.; da Silva, P. S.; Jost, C. L.; Spinelli, A. Electrochemical sensor based on bismuth-film electrode for voltammetric studies on vitamin B2 (riboflavin). *SENS. ACTUATORS, B* 2015, 209, 423–430.

(73) Kaur, B.; Srivastava, R. Nanocrystalline Metallosilicate Modified Electrodes for the Simultaneous, Sensitive, and Selective Determination of Riboflavin, Rutin, and Pyridoxine. *Electroanalysis* 2014, 26, 1078–1089.

(74) Hajian, A.; Rafati, A. A.; Afraz, A.; Najaﬁ, M. Electrolysynthesis of high-density polythiophene nanotube arrays and their application for sensing of riboflavin. *J. Mol. Liq.* 2014, 199, 150–155.

(75) Zhang, J.-l.; Zhang, N. Preparation of Novel Cystine-multiwalled Carbon Nanotubes-graphene Oxide-glassy Carbon Electrode and Electrochemical Detection of Riboflavin and Dopamine. *Sens. Mater.* 2018, 30, 2799–2810.

(76) Sumathi, C.; Muthukumarum, P.; Radhakrishnan, S.; Ravi, G.; Wilson, J. Riboflavin detection by α-Fe2O3/MWCNT/AuNPs-based composite and study of the interaction of riboflavin with DNA. *RSC Adv.* 2015, 17888.

(77) Muthusankar, G.; Rajkumar, C.; Chen, S.-M.; Karkuzhali, R.; Gopu, G.; Sangili, A.; Sengottuvelan, N.; Sankar, R. Sonocatalytic driven simple preparation of nitrogen-doped carbon quantum dots/SnO2 nanocomposite: A novel electrocatalyst for sensitive voltammetric determination of riboflavin. *SENS. ACTUATORS, B* 2019, 281, 602–612.

(78) Kowalczyk, A.; Sadowska, M.; Krasnodebska-Ostrega, B.; Nowicka, A. M. Selective and sensitive electrochemical device for direct VB2 determination in real products. *Talanta* 2017, 163, 72–77.