Folic Acid Determination in Food Samples Using Green Synthesized Copper Oxide Nanoparticles and Electro-Poly (Methyl Orange) Sensor

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Original Research

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Abstract
Copper (II) oxide nanoparticles (CuONPs) were green synthesized using Ocimum basilicum leaves aqueous extract in which polyphenols act as reducing and stabilizing agents. The synthesized CuONPs were characterized using X-ray diffraction spectroscopy, Fourier transform infrared spectroscopy, transmission electron microscopy, selected area electron diffraction, and Brunauer–Emmett–Teller (BET) surface area analysis. The analyses indicated the formation of crystalline rod-like monoclinic pure CuONPs with a mean grain size of 15 nm, a surface area of 396 m² g⁻¹, and a total pore volume of 0.71 cm³ g⁻¹. A glassy carbon electrode (GCE) was modified using the synthesized CuONPs and electropolymerized poly(methyl orange) (PMO). The modified PMO/CuONPs/GCE electrode was electrochemically characterized and applied for the estimation of folic acid (FA) by cyclic voltammetry, chronoamperometry, linear sweep voltammetry, and differential pulse voltammetry techniques. The influence of pH (7), scan rate (50 mV/s), supporting electrolyte (0.1 M KCl) and FA concentration has been optimized. FA is precisely determined in the range from 0.01 to 1.5 µM with a low detection limit (0.002 µM), a low quantitation limit (0.068 µM), high reproducibility (RSD 0.37, 10 measurements), and high stability (98% activity after 50 days). FA in food samples was determined by the new sensor with high recoveries from 93 to 108.8%.

Keywords  Copper oxide nanoparticles · Cyclic voltammetry · Differential pulse voltammetry · Folic acid · Glassy carbon electrode · Poly(methyl orange)

Introduction
Folic acid (FA) or pteroylmonoglutamic acid (N-[4-[(2-amino-1,4-dihydro-4-oxo-6-pteridinyl) methyl]-amino]benzoyl]-l-glutamic acid), is a water-soluble B9-vitamin that is supplied naturally in a diverse group of foods such as nuts, broccoli, fruit, cabbage, and also exists in the kidney and liver of animals [1–3]. FA plays a role in a variety of metabolic processes that control genetics, fetal growth, and aging [4]. FA has biological activity only after being converted to dihydrofolic acid in the liver and is related to tetrahydrofolate and its other derivatives. In addition, it is also a necessary component of the hemopoietic system and is the coenzyme that regulates the generation of ferroheme [5–7]. Deficiency of FA is a key aim for health goals that can decrease the risk of neural tube defects (NTDs) [8], leukopenia, neurosis, lightheadedness, gigantocytic, anemia, devolution of mentality, and psychosis of heart attack and stroke resulting from a lack of FA. On the other hand, a lack of FA will cause critical complications during pregnancy which can result in malformations of the spine, brain, and skull [9–11].

Due to the importance of FA several techniques have been used for its determination such as high-performance liquid chromatography [12], spectrophotometry [13], fluorescence [14], chemiluminescence [15], flow-injection [16], ion chromatography [17], capillary electrophoresis [18], and quartz crystal microbalance [19]. Despite this, these methods are difficult to master, time-consuming and expensive [20]. By contrast, electrochemical techniques are effective in determining FA and have attracted increasing attention because of their advantages of simplicity, high sensitivity, good selectivity, and low cost [21, 22]. In electroanalytical chemistry, electrodes have been chemically modified and applied to decrease the overpotential. This is due to their capacity to improve unmodified electrodes’ electron transfer rate, sensitivity, selectivity, and stability [23].

Nanoparticles of metal oxides have many advantages over bulk material, including large surface area, small size,
chemical and optical properties, and good electrical conductivity. As a result, they have a wide range of uses, including catalysis, microelectronics, sensors, nanomedicine, and antipathogens [24–26]. Among them, CuONPs (copper oxide nanoparticles) have sparked interest in several industries, including solar cells, biodiesel, photocatalysis, water treatment, supercapacitors, and electroanalysis [27–29]. The applications of CuONPs in electrocatalysis have been limited despite the methods offering improved sensitivity and selectivity and being more economical.

Physical, chemical, and biological techniques have all been used to produce CuONPs. Physicochemical methods are laborious and use and yield hazardous materials. However, the biological synthesis using plants; fungi, algae, and bacteria; is simple, cost-effective, and environment-friendly [27]. Plant-mediated synthesis is the simplest and cheapest biological method [30]. Recently, CuONPs have been synthesized using some plant sources such as Eupatorium adenophorum [31], Rubia cordifolia [32], Hyptis suaveolens [33], and Trichosanthes kirilowii [34].

Nanoparticles of metal oxides such as Fe₂O₃, CuO, MnO₂, TiO₂, etc., are widely used as supporting materials in sensor applications [35, 36]. Among other metal oxides, CuONPs are a type of transition and p-type semiconductor that has specific properties like stability, environmental friendliness, nontoxicity, cheapness, and fascinating electrochemical and catalytic properties [37, 38].

Methyl orange (MO) is an anionic terminated component that serves as a mediator for electron reactions and proton transfer related to the oxidation of the amino terminated substrates [39]. As a result, MO illustrates a high activity for the electrocatalytic oxidation of FA. Thus, it is expected that a glassy carbon electrode (GCE) has been modified using CuONPs and a poly (methyl orange) (PMO) will result in higher activity.

In this study, CuONPs were green synthesized using basil leaves extract (BLE) and applied; in combination with electropolymerized PMO; to modify the GCE. The behavior of the resulting PMO/CuONPs/GCE sensor was electrochemically studied and optimized for analysis of FA in real samples using different voltammetry techniques. The sensor achieved low detection limits, good sensitivity and better anti-interference ability.

Materials and Methods

Chemicals and Reagents

All chemicals are analytical grade, used as received and purchased from Merck. Stock solution 1 × 10⁻⁴ M of FA was daily prepared by dissolving the required weight of FA in sodium hydroxide (pH 7.0), then prepare 0.1 M potassium chloride, Britton–Robinson buffer (BRB), acetate buffer (ABS), and PBS, which made from NaH₂PO₄.H₂O (ADWIC, 98% purity) and were used as supporting electrolyte. Basil leaves aqueous extract was applied to produce CuONPs from copper (II) acetate monohydrate; Cu(CH₃CO₂)₂.H₂O (ADWIC, 98% purity). The solutions in this experiment were made from double-distilled water. 1.0 M NaOH and 1.0 M HCl solutions were employed to control the pH values. All experiments were accomplished at room temperature (25 °C).

Plant Collection, CuONPs Synthesis, and Characterization

Basil (Ocimum basilicum) leaves were collected from the University botanical garden in summer, cleaned with tap water, rinsed with bidistilled water, and dried in shade. The aqueous BLE was prepared by heating 10 g of dried leaves in 200 mL distilled water for 30 min, soaking overnight, and filtration. The CuONPs were synthesized by stirring and heating 5 g Cu(CH₃CO₂)₂.H₂O in 60 mL plant extract (1:12 m/V) for 5 min, then 60 mL 5% NaOH was added in portions and the reaction continued further for 30 min when the black CuO was completely precipitated (Scheme 1). The CuONPs were isolated through centrifugation at 6000 rpm for 12 min, followed by washing by bidistilled H₂O and ethanol three times each and drying at 80 °C for 3 h. The resulting product was crushed using a pestle and mortar and stored in a sealed container.

The synthesized CuONPs were characterized for phase structure and size analysis using a powder X-ray diffractometer (X’Pert3 Powder, PANalytical, the Netherlands) which was operated at 40 kV voltage and 30 mA current, using monochromatic radiation (Cu–Kα, 1.5406 Å) with diffraction angle in the 20–80° range. The crystallite size was determined using the Scherrer equation (D = Kλ/βcosθ). To evaluate the possible role of phytochemicals in the synthesis of CuONPs, FTIR spectra were taken from 400 to 4000 cm⁻¹ (Shimadzu FTIR, Kyoto, Japan). The size and shape of CuONPs were checked by TEM analysis.

Electrochemical Apparatus of PMO/CuONPs/GCE

Before the modification, the bare GCE was refined with a fine polishing cloth and then refined with alumina slurry (0.05 µm), followed by sonication in nitric acid, acetone, and cleaned with distilled water. For the fabrication of the PMO/CuONPs/GCE modified electrode, 1 mg/mL of CuONPs was dispersed in ethanol, then the suspension was subjected to ultrasonication for a few minutes, and then 5.0 µL of the colloidal was drop-cast on the GCE surface and left to dry. Then electropolymerization of MO on the CuONPs/GCE surface was carried out according to our previous publication [40]. Briefly, the fabricated
CuONPs/GCE was immersed in 0.1 M PBS (pH 7.0) containing 5.0 mM of MO monomer. Then, the polymer film of MO was electrodeposited onto the CuONPs/GCE surface through consecutive cyclic voltammetry by scanning the potential from −1.25 to +1.25 V (vs. Ag/AgCl) for 9.0 cycles at a scan rate of 100 mV/s. Finally, the synthesized electrodes were carefully cleaned with deionized water to remove any unreacted particles and were dried at room temperature.

**Preparation of Real Samples**

Fresh citrus juices (orange, tomato, and strawberry; 5 g each) were weighed, and were obtained using a squeezer. The juices obtained were filtered through a normal filter paper and then centrifuged for 15 min. Finally, 1.0 mL of the filtrate was added to 100 mL of 0.1 M KCl (pH 7.0) in a voltammetric cell. The standard addition method was applied to quantify FA in the samples.

**Results and Discussion**

**Characterization of CuONPs**

Figure 1A shows the XRD of the synthesized CuONPs to identify the phase and crystal structure. The sample showed several diffraction peaks characteristic of the crystalline nanoparticles at 2θ values of 32.52, 35.44, 38.68, 48.63, 53.5, 58.18, 61.45, 66.23, 68.14, 72.47, 75.16, and corresponding to respective Miller indices of (−1−10), (00−2), (−1−1−1), (0−20), (−202), (−20−2), (−1−13), (−3−11), (−2−20), (−3−1−1), and (−2−22). These values were well matched with COD file no. 9015924 of tenorite (volume-81.29, system-monoclinic, space group-C 1 2/c 1 (15), cell parameter $a=4.6832$, $b=3.4288$, and $c=5.1297$). The CuONPs have a crystallite size of about 14.86 nm using the Scherrer equation (Table 1). FTIR spectroscopy investigations were used to determine the involvement of biological entities in the extract in the reduction of Copper salt and
Table 1 Physical characteristics of the synthesized CuONPs

| Sample | Crystallite size (XRD) | Crystallite size (TEM) | Surface area | Mean pore diameter | Pore volume, cm³ g⁻¹ |
|--------|------------------------|------------------------|-------------|--------------------|----------------------|
| CuO BLE | 14.86 nm               | 18 × 5 nm              | 396.07 m² g⁻¹ | 7.1689 nm          | 0.7098 0.6121        |

Fig. 2 A TEM image and B SAED pattern of CuONPs

Fig. 3 A Adsorption/desorption isotherm and B NLDFT/GCMC pore size distribution analysis of CuONPs

Fig. 4 CVs of 5.0 mM MO in 0.1 M PBS (pH 7.0) on the CuONPs/GCE surface at a scan rate of 100 mV/s
Capping of nanoparticles. Absorption peaks in the wavenumber region of 400 to 4000 cm\(^{-1}\) are visible in comparison to FTIR spectra (Fig. 1B). The BLE FTIR spectrum contained major peaks in the range of 3424 and 1659 cm\(^{-1}\). These peaks are attributed to O–H stretching and the in-plane bending vibrations of hydroxyl groups or possible stretching of the N–H group of amides. The peaks at 2927, 1722, and 1075 cm\(^{-1}\) point to C–N (unsaturated), C=O, and C–H bending vibration, respectively. The spectrum of CuONPs (Fig. 1B) displayed peaks at 3425, 1633, and 1048 cm\(^{-1}\) indicating the OH stretching, OH bending, and C–H bending, respectively. Besides, the characteristic Cu–O peak was noticed at 542 cm\(^{-1}\) [41]. These results confirm the reducing and capping actions of organic moieties in BLE.

The morphology of the as-synthesized CuONPs was studied by TEM (Fig. 2A). The image shows the rod-shaped CuONPs aggregates with an average length of 18 nm and a width of 5 nm (Table 1). The selected area electron diffraction (SAED) pattern (Fig. 2B) reveals the crystalline nature of the CuONPs.

The electrochemical activity is affected by the material surface area where the reactions occur. The higher surface area normally corresponds to higher activity. The Brunauer–Emmett–Teller (BET) surface area and the nonlocal density functional theory/grand canonical Monte Carlo (NLDFT/GCMC) pore size of the CuONPs have been investigated using \(\text{N}_2\) adsorption/desorption analysis at 77 K. According to the IUPAC classification, the \(\text{N}_2\) sorption

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**Scheme 2** Biological electrocatalytic oxidation of FA at the C(9)–N(10) bond at PMO/CuONPs/GCE
isotherm of CuONPs at P/P₀ = 0.982 (Fig. 3A) is classified as type IV with a single point final plateau and a type H3 hysteresis loop, which refers to non-rigid aggregates of mesoporous particles leading to slit-shaped pores [42]. Moreover, the pore radius is 7.169 nm as calculated by the NLDFT/GCMC pore size distribution curve (Fig. 3B), confirming the mesoporous structure of CuONPs. Adopting a 16.2 Å for the cross-section area of the N₂ molecule, the calculated BET-specific surface area is very high (396.07 m² g⁻¹), indicating high electrochemical activity. Additionally, the total pore volume at saturation pressure is 0.7098 and 0.6121 cm³ g⁻¹ according to BET and NLDFT/GCMC, respectively (Table 1).
Electropolymerization of MO on the CuONPs/GCE Surface

Figure 4 displays the continuous cyclic voltammograms (CVs) of the electropolymerization of 5.0 mM of MO in 0.1 M PBS (pH 7.0) using the CuONPs/GCE at a scan rate 100 mV/s for 9.0 cycles. From the first cycle, MO exhibits its redox behavior, where an anodic peak at a potential +0.77 V is caused by the electro-oxidation of the MO monomer. Also, the redox peaks that appeared at 0.33 and +0.17 V are corresponding to the formation of PMO film [40]. Meanwhile, the cathodic peak at −0.48 V may be related to the redox couple Cu(II)/Cu(0) species. As the number of cycles increases, an increase in peak current is observed, which implies a gradual growth of the polymer film of MO on the CuONPs/GCE surface.
Electrochemical Catalysis of the PMO/CuONPs/GCE for FA

Figure 5 depicts the CVs for the electrochemical oxidation of 1.5 μM FA in 0.1 M KCl (pH 7.0) at a scan rate of 50 mV/s at bare GCE, CuONPs/GCE, PMO/GCE, PMO/CuONPs/GCE, and electrodes without polymer film or FA. A slight oxidation peak was found at bare GCE, CuONPs/GCE, and PMO/GCE. A well-defined irreversible oxidation peak at 1.2 V is shown at PMO/CuONPs/GCE, which is shifted in a negative direction [43]. In addition, a rapid increase in anodic current was observed for FA in comparison with bare GCE, CuONPs/GCE, and PMO/GCE due to the existence of CuONPs of the larger surface area responsible for the FA electrocatalytic oxidation [23]. The results indicate that the application of PMO/CuONPs/GCE improves the FA oxidation signal. The efficient electrocatalytic activity of CuONPs and the polymer film of MO plays a vital role in accelerating electron transfer between the electrode and FA in the solution. Based on the literature [29], a possible mechanism for enhanced catalytic activity of the synthesized CuONPs is described (Scheme 2). The electrocatalytic oxidation of FA to dihydrofolic acid occurs at the C(9)–N(10) bonds of FA, resulting in these peaks [20].

Effect of Supporting Electrolyte and pH on PMO/CuONPs/GCE Performance

We studied the effects of several supporting electrolytes with identical concentrations and pH, such as phosphate buffer, acetate buffer, BRB, and KCl solution, on the peak current of FA oxidation. The 0.1 M solution of KCl was chosen as the support because it resulted in a higher FA peak current with a well-defined shape, as illustrated in Fig. 6. The influence of pH on the FA oxidation peak was studied at PMO/CuONPs/GCE at pH values of 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, and 8.0. Figure 7 illustrates linear sweep voltammograms (LSV) of 1.4 μM FA in 0.1 M KCl at different pH values. The peak current increased as pH increased from 4 to 7, but it decreased above pH 7. Hence, pH 7.0 was chosen as the optimum pH value of the working solution. Furthermore, the FA peak potential shifted gradually in the negative direction with increasing pH (Fig. 7), showing the

Table 2 Comparison the performance of different modified electrodes for determination of FA

| Method | Modified electrode | Linear range, μM | DL, μM | Ref |
|--------|--------------------|-----------------|-------|-----|
| DPV    | ZnO@GO nanocomposites/GCE | 1–400 | 0.04 | [43] |
| SWV<sup>a</sup> | CuNPs-GO-CB-PEDOT: PSS/GCE<sup>b</sup> | 5.0–31 | 1.0 | [45] |
| DPV<sup>c</sup> | DVB/CNT/CPE<sup>d</sup> | 10–50 | 0.640 | [46] |
| APM<sup>e</sup> | Cu<sub>2</sub>O/CuO/GO/CPE | 0.01–24 | 0.004 | [37] |
| DPV | Cu<sup>2+</sup>/PANI/Nano-ZSM-5/GC<sup>f</sup> | 0.013–700 | 0.005 | [47] |
| CV    | PtNPs/GNPs/MWCNTs-β/CD/GCE<sup>g</sup> | 20–500 | 0.48 | [48] |
| CV    | NiFe<sub>2</sub>O<sub>4</sub>/rGO/ILCPE<sup>h</sup> | 0.02–285 | 0.006 | [49] |
| DPV   | ZrO<sub>2</sub>/ZnO nanocomposite | 2–480 | 0.29 | [50] |
| DPV   | PMO/CuONPs/GCE | 0.01–1.5 | 0.002 | This work |

<sup>a</sup> Square wave voltammetry

<sup>b</sup> copper nanoparticles-graphene oxide–carbon black-poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate)/glassy carbon electrode

<sup>c</sup> differential pulse voltammetry

<sup>d</sup> 2,2'-[1,7-heptanediylbis(nitrilomethylidene)]-bis(4-hydroxyphenol) and carbon nanotubes modified carbon paste electrode

<sup>e</sup> amperometry

<sup>f</sup> transition metal ion-exchanged polyaniline–zeolite organic–inorganic hybrid materials

<sup>g</sup> platinum nanoparticles/graphene nanoplatelets/multiwalled carbon nanotubes/β-cyclodextrin composite modified carbon glass electrode

<sup>h</sup> NiFe<sub>2</sub>O<sub>4</sub>/reduced graphene oxide/ionic liquid CPE
involvement of a proton in the electrochemical process. The relationship between peak potential and pH is linear, as indicated in the inset of Fig. 7 with the equation: \( E_p(V) = -0.053 \text{ pH} + 1.562 \) \( (R^2=0.9908) \), indicating this slope is quite near to the Nernstian value (0.059 V/pH at 25°C), indicating that the protons/electrons ratio is 1.

**Effect of Scan Rate on PMO/CuONPs/GCE Performance**

Figure 8A shows CVs obtained for PMO/CuONPs/GCE in 0.1 M KCl (pH 7.0), including 1.3 µM FA at different scan rates. The peak current increased as the scan rate was raised from 10 to 500 mV/s, suggesting that the FA oxidation follows a diffusion-controlled mechanism [44], and the regression equation could be written as \( I_p = 0.026 n^{1/2} \text{ (mV/s)}^{1/2} + 0.148 \) \( (R^2=0.9844) \). Increasing the scan rate shifted the peak potential to positive values (inset Fig. 8A).

Figure 8B shows the relationship between \( \log(I_p) \) and \( \log(v) \) with the linear equation: \( \log(I_p) = 0.369 \log(v) + 1.9 \); \( R^2=0.9893 \) and a slope of 0.37, which is quite near to theoretical electron transfer coefficient (0.5). From the linear calibration plot \( E_p \) versus \( \log(v) \) with the following equation: \( E_p = 0.072 \log(v) + 1.09 \); \( R^2=0.9813 \) as illustrated in Fig. 8C. The number of participating electros were determined from the Laviron equation \( \Delta E/\Delta \log(v) = 0.059/n \alpha \) (where \( \alpha \) is the electron transfer coefficient) and determined to be 2.
Effect of FA Concentration on PMO/CuONPs/GCE Performance

Differential pulse voltammetry was applied to evaluate the influence of the concentration of FA (at pulse height $25 \times 10^{-3}$ V, pulse width $50 \times 10^{-3}$ s, step time of 0.1 s, and a scan rate of 20 mV/s). Figure 9 illustrates differential pulse voltammograms (DPVs) of FA in 0.1 M KCl solution (pH 7.0) at different concentrations (from 0.01 to 1.5 μM) using PMO/CuONPs/GCE. The figure clearly shows that PMO/CuONPs/GCE has a well-defined oxidation current that increases linearly with FA concentration. Peak current increases due to the availability of more FA molecules at higher concentrations, resulting in a greater number of electrons involved in electrode reactions. It was discovered that the anodic peak current and various FA concentrations have a linear relationship (inset Fig. 9). The calculated detection limit (DL) was 0.002 M and the quantification limit (QL) was 0.068 M.

For comparison purposes, the analytical methods, linear range, and DL were compared with those of other developed sensors for FA analysis (Table 2). Results show that PMO/CuONPs/GCE sensor has comparable or better results than many developed sensors with the simplicity of the preparation method.

Chronoamperometric Measurements

The behavior of FA at different concentrations toward oxidation at PMO/CuONPs/GCE was also studied by chronoamperometry using 0.1 M KCl support (Fig. 10A). This was done by setting the working electrode potential at 0 (first step) and 1.7 V (second step) vs. Ag/AgCl. The Cottrell equation [7, 51] describes the current observed for the electrochemical reaction at the mass transport restricted condition for FA: $I = nFAD^{1/2}C_b/\pi^{-1/2}t^{-1/2}$ where $n$ is the transferred electrons (2), $F$ is the Faraday constant (96,485 C/mol), $A$ is the working electrode surface area (cm$^2$), $D$ is the diffusion coefficient (cm$^2$/s), $t$ is time (s), and $C_b$ is the concentration (M). The plots of $I$ vs. $t^{-1/2}$ for various FA concentrations showed good linearity, as shown in Fig. 10B.
The obtained slopes from the straight lines were then plotted vs. FA concentrations, as shown in Fig. 10C. $D$ was calculated using the Cottrell equation and the resulting slope was $1.79 \times 10^{-5}$ cm$^2$/s.

**Selective Quantification of FA in Presence of Ascorbic Acid and Other Interferences**

As shown in Fig. 11A, DPV was used to detect different concentrations (0.01–1 μM) of FA in 0.1 M KCl solution (pH 7.0) at PMO/CuONPs/GCE while keeping the ascorbic acid (AA) concentration constant at 10 μM. The oxidative peak current of FA increased linearly with increasing concentration, whereas the voltammetric peak of AA remained constant. A linear relationship with the corresponding regression equation $I_p (\mu A) = 102.134 C (\mu M) + 70.203; R^2 = 0.9906$ was obtained (inset in Fig. 11A) and a 0.0018 μM DL was obtained. These results indicate that the oxidation processes of FA and AA at PMO/CuONPs/GCE are independent. Figure 11B demonstrates the DPVs of AA at different concentrations (10–24 μM) and constant concentration of FA (1 μM). Plotting $I_p$ of AA versus concentration (inset Fig. 11B) resulted in a straight line $I_p (\mu A) = 0.373 C (\mu M) + 0.516, R^2 = 0.9985$ with a DL 0.01 μM. Thus, an accurate FA evaluation in the presence of AA at the fabricated electrode is possible. In addition, Fig. 11C depicts the DPVs obtained from different FA and AA concentrations at PMO/CuONPs/GCE. The simultaneous concentration increment of both FA and AA resulted in increasing the peak currents of the two acids linearly, indicating the ability of PMO/CuONPs/GCE to determine both acids.

The effects of various interfering species were studied in 0.1 M KCl containing 1.5 μM of FA to determine the selectivity of the PMO/CuONPs/GCE (see Table 3). The presence of 1000-fold concentration ratios of the inorganic ions Na$^+$, K$^+$, Zn$^{2+}$, Cl$^-$, SO$_4^{2-}$, and NO$_3^-$ do not interfere with FA. Also, 100-fold of some physiological substances such as alanine and cysteine, tartaric acid, fumaric acid, and tenfold of glucose, vitamin B6 (VB$_6$) and vitamin B12 (VB$_{12}$), and fivefold ascorbic acid, uric acid, and dopamine leads to a less than ±5% change in the FA peak current. Thus, PMO/CuONPs/GCE is highly selective for the detection of FA even at a high concentration of these interferences.

**Reproducibility, Repeatability, and Stability**

The reproducibility of PMO/CuONPs/GCE was examined using cyclic voltammetry as can be seen in Fig. 12A. The oxidation peak current remained constant after twelve repetitive measurements with a relative standard deviation of 0.37%. This indicates that the PMO/CuONPs/GCE electrode has an excellent reproducibility. The stability of the PMO/CuONPs/GCE modified electrode was tested by measuring CVs of 1.5 μM FA for 50 days (Fig. 12B). The electrode retained 98.4% of its initial response, demonstrating its high stability.

**Real Sample Analysis**

The concentration of FA in strawberry, orange, and tomato samples was determined by the PMO/CuONPs/GCE sensor using the standard addition method. The cyclic voltammetry anodic peak current for each sample was recorded and then converted to concentration using the linear equation of the corresponding calibration curve. The results are given in Table 4 and indicate the successful estimation of FA in all samples with recoveries in the 93–108.8 range.

![Fig. 12 CVs of PMO/CuONPs/GCE in 0.1 M KCl containing 1.5 μM FA at a scan rate of 50 mV/s A reveals the reproducibility and B shows the stability](image-url)
Table 4  Detection of FA in food samples

| Sample     | Added (µm) | Found (µm) | Recovery (%) |
|------------|------------|------------|--------------|
| Orange     | 0          | 0.002      | -            |
|            | 1          | 1.09       | 108.78       |
|            | 1.5        | 1.49       | 99.2         |
| Tomato     | 0          | 0.09       | -            |
|            | 1          | 1.09       | 100          |
|            | 1.3        | 1.29       | 93.0         |
| Strawberry | 0          | 0.01       | -            |
|            | 1          | 1.09       | 107.92       |
|            | 1.2        | 1.2        | 99.17        |

Conclusions

Herein, CuONPs were green synthesized using basil leaves, characterized and applied for the construction of a new PMO/CuONPs/GCE sensor. The synthesized CuONPs are crystalline and mesoporous that have a 15 nm particle size and 396 m² g⁻¹ surface area, resulting in a clear enhancement in the PMO/CuONPs/GCE electrode electrocatalytic activity. Various parameters of the electrocatalytic process were optimized, including supporting electrolyte, scan rate, pH, and FA concentration. The new sensor exhibited low DL (0.002 µM) and QL (0.068µM), wide linear range (0.01 to 1.5 µΜ) and very good stability, sensitivity, and selectivity that allowed the determination of FA in the presence of different interferences. The fabricated sensor has been precisely employed to determine FA in various real samples at 93 to 108.8% recovery.

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Declarations

Conflict of Interest  The authors declare no competing interests.

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