Electrochemical enzyme-free sensing of oxalic acid using amine mediated synthesis of CuS nanosphere

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Abstract

**CuS nanosphere** (CuS NS) were prepared by solvothermal method with the support of p-phenylene diamine as a structure direct agent. The formation of CuS NS was evaluated using XRD, FE-SEM, HR-TEM, XPS, and electrochemical methods. The CuS NS modified electrode demonstrated excellent electro-catalytic behavior for the electro-oxidation of oxalic acid (OA). The modified electrode showed good linear range (50 to 700 µM), high sensitivity (0.0353 µAµM⁻¹ cm⁻²), low detection limit (35.6 µM), long term stability and good anti-interference behavior.

**Key words:** copper sulfide; solvothermal; oxalic acid sensor; electrochemical detection, modified electrode
Introduction

The development of sensor for oxalic acid detection is highly desirable in many areas including medical, food, environmental and so on. It is largely available in spinach, ginger, tomato and animals, microbes and easily amalgamated with Ca, Mg, Fe, Na or K to form less soluble salts such as metal oxalates [1-4]. The consumption of these salts could create several health complications such as digestive problem, kidney stone formation, irritation in kidneys and stomach, hypocalcaemia, nephrotoxicity, neurotoxicity. Further, the excess intake of OA may create neural and heart problems through intake of calcium from blood [4-9]. Industries also largely release OA in the environment [10]. Hence, development OA sensor with simple protocol and continuous monitoring is urgently required. Obviously, the electrochemical detection has several advantages when compared to the conventional detection methods such as spectrophotometry, titration, gas chromatography and HPLC [11-15]. Currently, several researchers are devoting on the development of electrochemical sensors by using different protocols. For example, an enzymatic detection of OA was demonstrated by Hang et al. In this work, oxalate oxidase enzyme has been used for the detection of OA with good sensitivity and selectivity [4]. However, the enzymatic detection has increased the cost of the detection and also several drawbacks with enzyme including stability, complicated protocol for enzyme immobilization, and reproducibility. The non-enzymatic detection has provided several advantages over the enzymatic methods including low-cost, high stability, simple electrode modification and so on [16-18]. However, the surface fouling and poor sensing property are the major hurdles during the electrochemical detection of OA. In order to overcome these problems, different nanomaterials have been used as surface modifier to improve the sensitivity, restrict the surface fouling, stability, and reproducibility due to their special properties. In the past few years,
several nanomaterials have been used for the electrochemical detection of OA. For example, Chen et al. fabricated Pt nanoparticles decorated graphene sheets as an electrode material for oxalic acid detection. The modified electrode was demonstrated with good electro-catalytic properties for the electro-oxidation of OA and reported good analytical performances [18]. Liu et al. prepared Pd loaded carbon fibers and applied for the electrochemical detection of OA. The modified electrode exhibited good sensitivity and selectivity [16]. Raoof et al. developed Pd incorporated mesoporous silica SBA-15 as electrode material in carbon paste electrode configuration and applied for OA detection with good sensing performance [19]. Ivandiniet al. used boron doped diamond electrode for the electrochemical oxidation of oxalic acid [1]. Similarly, the electro-deposited Pd-Au alloys have been used as an electrode material for the electrochemical determination of oxalic acid and applied to the determination of OA in spinach samples [3]. Sharma et al. reported the ternary composite consist of Au nanoparticles embedded polypyrole/rGO composite as a modifier for oxalic acid detection with good performance [6]. Maiyilagan et al. reported the tungsten carbide nanotubes supported Pt nanoparticles and used for electro-oxidation of oxalic acid [2]. It may be noted here that most of the electrode materials used for OA detection are expensive due to the usage of noble metals and followed by a complicated preparation protocol. However, simple fabrication of nanomaterials with inexpensive and attractive properties for OA detection is still lacking. Among the different types of nanomaterials, transition metal based materials are playing key role and applied in many applications due to their good electronic conductivity, low cost, easy preparation [20,21]. In the past, copper sulfide (CuS) of special structural property was used for various electrochemical sensor applications. Because, it has many attractive properties over other transition metal sulfides.
including tunable conductivity, high surface area, good redox properties, electro-catalytic activity, simple preparation with tunable morphologies, good stability and so on [22-31].

In the present work, we have synthesized copper sulfide nanosphere (CuS NS) using simple solvothermal method with the support of p-phenylenediamine as structure directing agent for the first time. The prepared CuS nanosphere showed good electro-catalytic properties towards electro-oxidation of oxalic acid.

Experimental

Materials and Instrumentation

Copper acetate, sodium sulfate and Nafion solution were obtained from Sigma Aldrich. Sulfur powder, oxalic acid and ethanol were procured from Merck. p-phenylenediamine received from Alfa Aesar. All the reagents used in this work were of analytical grade without any modification. The solution was prepared using deionized (DI) water (Elix water purifier) of 18.2 MΩ resistivity.

Instrumentation

The prepared material was characterized using various techniques including X-ray diffraction (XRD) using Bruker D8 Advance Instrument, field emission scanning electron microscope (FE-SEM) using Carl Zeiss AG Ins), Tecnai G2F20S high resolution transmission electron microscopy (HR-TEM) Instrument, and ESCA 2000 X-ray photo electron spectroscopy. The electrochemical measurements were recorded using Autolab PGSTAT 30 electrochemical workstation. A conventional three-electrode configuration was applied for the electrochemical measurements consisting copper sulfide coated glassy carbon (3mm; GC) electrode as working electrode, Ag/AgCl and Pt foil electrode as reference and counter electrode respectively.
**Preparation of CuS nanosphere**

The CuS nanosphere was synthesized through well-known solvothermal reaction explained as follows: First, copper acetate (0.7 mmol) and sulfur powder (2.1 mmol), were mixed in 40 mL ethanol. Then, p-phenylenediammine (0.35 mmol) dissolved separately in 30 mL of ethanol. Finally, the two solutions were transferred into a Teflon-lined stateless-steel autoclave and kept in muffle furnace at 160°C for 12 h (Scheme-1). The black precipitate was collected by centrifugation at 8000 rpm for 15 min after cooling to room temperature and dried at 50°C in an oven.

**Glassy carbon working electrode preparation using CuS nanosphere**

Initially, CuS (2 mg) was dispersed in 900 µL water and 100 µL Nafion solution and sonicated for 15 min. Followed by, the solution was coated over the pre-cleaned GC electrode and air-dried at room temperature.

**Results and Discussion**

**Materials characterization**

The CuS product with nanosphere morphology was simply achieved by solvothermal method in the presence of copper acetate, sulfur powder, and p-phenylenediammine as Cu, S and structure-directing agent (Scheme 1). The obtained product was investigated by FE-SEM. Fig. 1A&B shows the FE-SEM images of CuS product and observed sphere-like structure (80 ± 20 nm) consisting of several primary nanoparticles.

< Scheme 1 >

The deep morphologies of CuS product further examined by HR-TEM (Fig. 1C&D) where the observed spherical morphology is in good agreement with FE-SEM investigations. The average diameter of the CuS nanosphere and nanoparticles over the CuS sphere is estimated
to be 70 ± 5 nm and 6 ± 3 nm, respectively. The HR-TEM image of CuS nanosphere (Fig. 1D) suggests the good crystalline nature of the product.

< Fig. 1 >

These morphologies studies clearly show that the nanoparticles are initially formed under solvothermal method and the reaction prolonged till the aggregation of nanoparticles into a uniform sphere-like structure with the support of structure directing reagent. Further, the small pore created between the nanoparticles aggregate in the CuS nanosphere is highly useful to obtain better electrochemical response via easy diffusion of the electrolyte.

The phase purity and crystalline characteristics of the CuS nanosphere was verified by XRD. Fig. 2 shows the XRD spectrum of CuS product and the peaks are perfectly matches with the hexagonal crystal phase of CuS (JCPDS: 00-001-1281;P63/mmc space group) [32].

< Fig. 2>

There is no additional peak was observed in the XRD profile, confirmed the phase purity and good crystalline nature of the CuS under simple solvothermal method. Further, X-ray photoelectron spectroscopy XPS was used to investigate the oxidation state and chemical composition of the CuS product (Fig. 3). Fig. S1 shows the survey spectrum of the as-prepared CuS product. As expected, all the peaks appeared in the spectrum confirm the successful formation of CuS product under simple solvothermal condition. Fig. 3A shows the high resolution spectrum of the Cu 2p region, where it shows four distinguished peaks for the Cu 2p region. The main peak of CuS observed at 933.8 eV and 954.2 eV corresponds to the Cu 2p3/2 and Cu 2p1/2 respectively. The observed additional peaks at 942.4 and 961.8 eV attributed to the Cu satellite peaks, which represent the presence of copper as Cu²⁺ oxidation state in the CuS
product. Similarly, the high resolution spectrum of S 2p region shown in Fig. 3B, the observed two peaks at 161.7 and 163.7 eV related to the binding energies of S 2p3/2 and S 2p1/2, respectively. It further confirms the successful formation CuS product and the obtained peaks are perfectly matches with the literature reports [33-36].

< Fig. 3>

Electrochemical characterization of CuS nanosphere modified electrode

The prepared CuS NS initially characterized by cyclic voltammogram (CV) as shown in Fig. 4. The CVs recorded in the potential range between 0 to 1.5 V at a scan rate of 50 mVs−1 in 0.1 M Na2SO4 as supporting electrolyte. The CuS modified electrode does not show any characteristic peak (curve a) in 0.1 M Na2SO4 solution, whereas after the injection of 5mM oxalic acid (OA), a clear anodic oxidation peak (17.2 µA) was observed at the oxidation potential of 1.1 V. In the identical condition, the bare GC electrode shows the oxidation peak potential and peak current value of 1.3 V, and 16.1 µA, respectively. The obtained OA oxidation peak potentials at CuS NS modified surface is lower than the other nanomaterials modified electrodes reported in the literatures [1, 6, 17, 19]. The increased oxidation peak current and decreased oxidation potential of CuS NS is due to the porous structure and excellent catalytic behaviour of CuS nanosphere.

< Fig. 4>

The scan rate effect of CuS NS modified electrode was investigated by CVs in the scan rate between 10 to 100 mVs−1. The obtained CVs profiles were shown in Fig. 5, indicated the electro-oxidation peak current of OA increased gradually with increasing scan rate. Further, good linear behavior was observed while plotting between peak current vs. square root of scan rate with a correlation coefficient of 0.9856 (Inset of Fig. 5). It suggests that the electro-oxidation
reaction of OA at CuS NS electrode is diffusion controlled process. Further, the electro-oxidation of OA behaviour studied using linear sweep voltammetry (LSV) at different concentration of OA from 0 to 2.5 mM. The obtained LSV profile displayed in Fig. 6 where the oxidation peak current of OA at CuS electrode was found to increase with the OA concentration (each addition 100 µM). The obtained peak current vs. concentration of OA provide a linear behaviour with a correlation coefficient 0.9920 (Inset of Fig. 6).

< Fig. 5 & Fig. 6>

In order to verify the sensitivity of the present CuS electrode, amperometry measurements were recorded at an applied overpotential of 1.1 V in 0.1 M Na₂SO₄ solution. The obtained amperometric profile was displayed in Fig. 7A, where the current signal increases gradually with the injection of OA concentration from 50 µM to 700 µM by each addition of 50 µM of OA. The amperometric steady state current reaches within 4s at a low concentration, suggesting the good electro-catalytic behavior of CuS nanosphere towards electro-oxidation of OA. The amperometric current linearly increased from 50 to 700 µM and obtained good linear behavior with a correlation coefficient of 0.9933 (Fig. 7B). The limit of detection and sensitivity of the CuS electrode was estimated to be 35.6 µM and 0.0353 µA µM⁻¹ cm⁻², respectively from the amperometric calibration plot. The obtained linear range, detection limit and sensitivity are very much comparable and also superior with several reports related to OA detection using various modified electrodes (Table S1).

< Fig. 7>

The interference, long-term stability, and reproducibility measurements are the important analytical parameter to apply the fabricated sensors in real applications. The anti-interference characteristic of the present fabricated sensor was evaluated by amperometric measurement at an
applied potential of 1.1 V as shown in Fig. 8. The amperometric profile clearly shows that the present CuS electrode strongly responds for OA (200 µM), whereas negligible current response observed for 4-fold higher concentration (800 µM) of other major interfering compounds including glucose, citric acid, acetic acid, uric acid, formic acid. It confirms that the present CuS modified electrode has good anti-interference behaviour for the major interfering compounds and strongly responds only to OA. The higher selectivity is due to the fact that the tendency of different oxidation potential of the interfering compounds when compared to the oxidation potential of oxalic acid. Further, interaction/repulsion between the interfering compounds and modified electrode surface (Nafion/CuS) is also playing a major role to get higher selectivity.

< Fig. 8>

Further, the electrode reproducibility was verified through fabrication of four CuS NS electrode under identical conditions. The amperometric measurements were performed for the four individual electrodes in the presence of 200 µM OA. The obtained amperometric current responses were given as bar diagram in Fig. S2A. The relative standard deviation of the four modified electrodes was estimated to be 2.80%. Similarly, the stability of the CuS NS modified electrode was evaluated by amperometric measurement in the presence of 200 µM OA for 20 days. The obtained current response for each 5 days was given as bar diagram in Fig.S2B. The current response for 20th days was decreased to ~ 7.0 % from the value of the 1st day response. It confirms the present modified CuS NS has good reproducibility and stability. The attractive features of our newly fabricated oxalic acid sensor is that the CuS nanospheres preparation using organic amine as structure directing agent for first time and simple drop cast method for electrode modification. The modified electrode demonstrated good sensitivity, selectivity,
reproducibility, and stability. The analytical performance of the newly fabricated non-enzymatic oxalic acid sensor is highly comparable with the earlier reported system.

Conclusions

We have reported the newly prepared CuS nanosphere through simple solvothermal method in the presence p-phenylene diamine as a structure direct agent. The prepared CuS nanosphere used as surface modifier for the effective non-enzymatic detection of oxalic acid. The fabricated CuS electrode showed good analytical performance for oxalic acid sensors. The current amine-mediated synthesis of CuS nanostructure may increase the scope of the preparation of other transition metal nanostructures in future.

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Figure captions

**Scheme-1** Schematic illustration of electrochemical oxidation of oxalic acid by CuS nanospheres modified electrode.

**Fig. 1** FE-SEM & HR-TEM images of CuS product

**Fig. 2** XRD spectrum of CuS product

**Fig. 3** High-resolution XPS spectra of (A) Cu 2p and (B) sulfur 2p region

**Fig. 4** Cyclic voltammogram of bare GC (curve c and d) and CuS NS modified electrode (curve a and b) in presence (curve b and d) and absence (curve a and c) of oxalic acid.

**Fig. 5** Scanrate effect of CuS NS modified electrode from 10 mVs⁻¹ to 100 mVs⁻¹ and inset plot shows calibration plot.

**Fig. 6** Linear sweep voltammogram of CuS NS modified electrode at various concentrations from 0.1 mM to 2.5 mM and inset graph shows the related calibration plot.

**Fig. 7** (A) Amperometric response of OA oxidation at CuS modified electrode; each addition 50 µM of OA(B)Corresponding calibration plot.

**Fig. 8** Amperometric interference study of CuS modified electrode at applied potential of 1.1 V.
Scheme-1
Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5
Fig. 6
Fig. 7

(A) Current vs. time plot showing steps at 200 µM and 500 µM concentrations.

(B) Current vs. concentration plot with a linear fit, giving $R^2 = 0.9968$. 

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Fig. 8
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