

## Research Article

## Peroxidase Like Activity of Quinic Acid Stabilized Copper Oxide Nanosheets

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## Abstract

Water-soluble copper oxide nanosheets (CuO nanosheets) were synthesized via a chemical reduction method and used as peroxidase enzyme mimetics. The synthesized CuO nanosheets were characterized by using XRD and TEM. The obtained CuO nanosheets in fact possessed an intrinsic enzyme like catalytic activity identical to that found in natural peroxidases, which have applications as detection tools and in water treatment.

**Keywords:** Water soluble CuO nanosheets; Peroxidase enzyme activity; Quinic acid

## Introduction

Enzymes, a kind of proficient biocatalyst play key role in almost all *in vivo* reactions. Reactions catalyzed by enzymes are of extensive significance due to their high specificity, efficiency and need of mild reaction conditions. Additionally, enzyme based analysis has a broadscope of applications in various fields such as biochemistry, chemical technologies, clinical diagnosis and in environmental science. On the other hand, the availability of limited natural sources, instability at different temperatures, P<sup>H</sup> and high cost of purification has made enzymes restricted to limited applications. Hence there is a need for the construction of new stable, temperature and P<sup>H</sup> resistant, low cost materials which can replace the natural enzymes in their function and properties [1]. However, various enzyme mimetics have been synthesized for different enzymes such as serine protease [2], hydrolase [3], superoxide dismutase [4], dioxygenase [5,6], lipase [7], nitrile hydratases [8], phosphodiesterase [9-12], aldolase [13], cytochrome P450 [14], ligase [15] and acylase [16].

Now-a-days, much attention has been focused on the development of peroxidase mimetics due to its high importance in enzymatic analysis and in waste water treatment. Several peroxidase mimetics such as metal porphyrin [17], hemin [18], metal hexacyanoferrate [19,20], Schiff base complex [21], metal phthalocyanine [22], hemeatin [23] and carboxyl groups containing mesoporous polymers [24] have been used for enzymatic analysis.

Recently, scientists have proved that the iron oxide magnetic nanoparticles are found to possess intrinsic peroxidase mimetic activity [25]. Later, several nanoscale peroxidase mimetics have been developed to their potential applications [26-39]. The present study describes the intrinsic peroxidase activity of quinic acid stabilized copper oxide nanosheets sheets. Here, we have synthesized the water soluble copper oxide nanosheets and used as peroxidase enzyme mimics.

## Chemicals and Materials

## Experimental procedure

Copper (II) sulfate, Sodium hydroxide, Sodium borohydride,

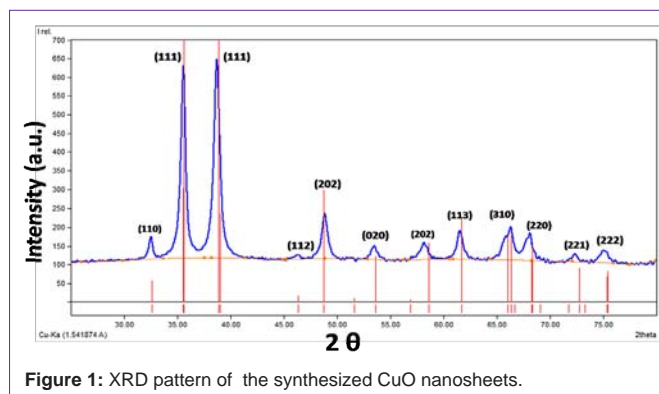
Quinic acid, Hydrogen peroxide, O-Dianisidine and all other solvents were obtained from Sigma-Aldrich Chemicals, Bangalore.

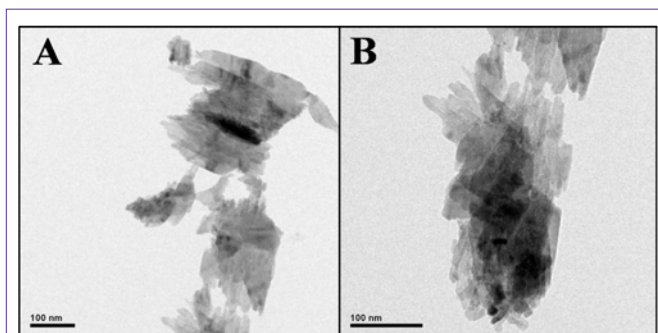
## Preparation of CuO nanosheets

The copper oxide nanosheets were synthesized as per our reported procedure elsewhere [40]. Briefly, equal volume of aqueous NaOH (0.01 M) and NaBH<sub>4</sub> (0.1 M) were added into a beaker containing the same amount of CuSO<sub>4</sub> (0.01 M) and Quinic acid (0.02 M) mixture and stirred vigorously at room temperature (30°C). Reaction was continued for about a half an hour with stirring to obtain copper oxide nanosheets. The product was then purified by repeatedly washing with double distilled water and ethanol which was finally dried at 60°C in hot air oven.

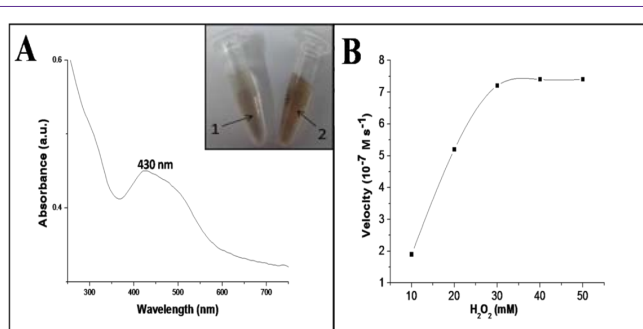
## Investigation of peroxidase like catalytic activity of CuO nanosheets

To examine the peroxidase like catalytic activity of the synthesized CuO nanosheets, the catalytic oxidation of o-dianisidine (as colourless peroxidase substrate, reduced form) was tested in the presence of H<sub>2</sub>O<sub>2</sub> as oxidizing agent. The oxidation of o-dianisidine was carried out using phosphate buffer (p<sup>H</sup> 7.0) in the presence of CuO nanosheets (100 μl) for 200 seconds at 25°C. Later, hydrogen peroxide (10mM) was added to start the reaction. The catalytic activity of CuO nanosheets was carefully measured by the development of colour due to oxidation of o-dianisidine using UV-Visible spectrophotometer at 430 nm.

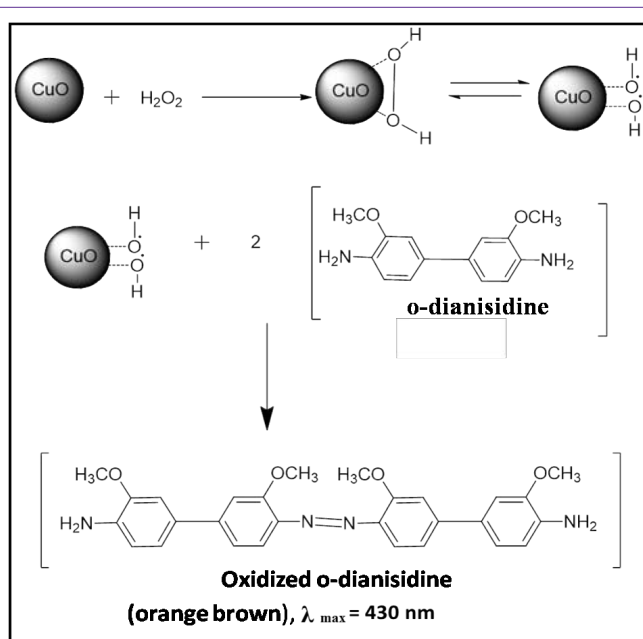




**Figure 2:** HR-TEM images of the synthesized CuO nanosheets at 100 nm magnifications (A, B).



**Figure 3:** Typical UV-Visible spectra of o-dianisidine-H<sub>2</sub>O<sub>2</sub>-CuO nano reaction system (A) kinetic analysis of CuO nanodispersion with H<sub>2</sub>O<sub>2</sub> as substrate (B); Inset: 1=without CuO nanosheets and 2= with CuO nanosheets.



**Figure 4:** Chemical reaction mechanism of CuO nanosheets relative to o-dianisidine-H<sub>2</sub>O<sub>2</sub> system.

### Kinetic parameters analysis

Steady-state kinetic experiments were done by changing the H<sub>2</sub>O<sub>2</sub> concentration from 10 to 50mM at a fixed concentration of o-dianisidine (0.7mM) prepared in phosphate buffer (pH 7.0). The change in the absorbance was measured using a UV-Visible

spectrophotometer in the time scan mode at 430 nm. The catalytic parameters were then obtained by fitting the absorbance data to the Michaelis–Menten equation as,

$$V = V_{\max} [C] / K_m + [C]$$

Where  $V_{\max}$  is the maximal velocity of reaction,  $V$  is the initial velocity and  $C$  represents the substrate concentration, and  $K_m$  is the Michaelis-Menten constant

## Result and Discussion

### Characterization of the copper oxide nanosheets

The copper oxide nanosheets were synthesized via a chemical reduction method. Figure 1 shows the XRD pattern of the produced CuO nanosheets which represents the formation of CuO nanosheets with a monoclinic structure (JCPDS No. 96-410-5686). Peaks in standard X-ray diffractogram of CuO obtained from JCPDS database had been matched with that of the obtained CuO nanosheets to find out any impurity present or not. Figure 1 shows the absence of characteristic impurity peaks in the XRD diffractogram of CuO nanosheets after comparison with standard diffractogram of CuO nanosheets which confirm the formation of pure CuO nanophase. The average crystalline size of the CuO nanosheets was calculated by using Debye-Scherrer formula as about 192 nm.

The morphology and size of the produced CuO nanosheets were analyzed by transmission electron microscopy (TEM). The TEM images (Figure 2) clearly show the presence of sheet like CuO nanostructures. However, the average size of the CuO nanosheets was found to be in good agreement with the XRD result.

### Peroxidase like activity of CuO nanosheets

Figure 3A showed the typical UV-visible absorption spectrum of the reaction system containing CuO nanosheets, H<sub>2</sub>O<sub>2</sub> and o-dianisidine shows an increase in the absorbance at 430 nm characteristic to the oxidized product of o-dianisidine, representing the typical peroxidase like activity of the CuO nanosheets. However, the reaction was studied in the absence of CuO nanosheets, with the addition of various concentrations of H<sub>2</sub>O<sub>2</sub>, but no significant color change was observed even at higher concentrations (> 50 mM) indicating inefficiency of H<sub>2</sub>O<sub>2</sub> in oxidizing o-dianisidine. But in the presence of CuO nanosheets the orange brown color appeared which indicates the catalytic activity of CuO nanosheets when added to the o-dianisidine-H<sub>2</sub>O<sub>2</sub> system (Figure 3A inset). The basic catalytic mechanism involves the formation of an intermediate complex by the two electron reduction of H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O in the presence of CuO nanosheets, and the o-dianisidine is bound to the formed intermediate complex by a nucleophilic attack, thus resulting the oxidation reaction to take place with a color change to orange brown (Figure 4).

Additionally, the enzymatic activity of the CuO nanosheets was further examined by taking steady state kinetics. The kinetic experimental information was obtained by changing the H<sub>2</sub>O<sub>2</sub> concentration while keeping the constant o-dianisidine concentration. Figure 3B shows the typical Michaelis–Menten curves obtained for the CuO nanodispersion with H<sub>2</sub>O<sub>2</sub> as substrates.  $V_{\max}$  and the Michaelis constant ( $K_m$ ) are recorded from the graph. The noticeable  $K_m$  value of CuO nanosheets was compared with horseradish peroxidase

**Table 1:** Comparison of the kinetic parameters of CuO nanosheets, HRP and other peroxidase mimics, where.

Catalyst	$K_m$ (mM)	$V_{max} / 10^{-8} \text{ M s}^{-1}$	Reference
HRP	3.7	8.71	41
$\text{Co}_3\text{O}_4$	140.07	12.1	42
$\text{Fe}_3\text{O}_4$	154.0	9.78	41
Prussian blue $\text{Fe}_2\text{O}_3$	323.6	117	43
Palladium nanostructures	1064.0	443	44
$\text{ZnFe}_2\text{O}_4$	1.66	7.74	45
Platinum nanostructures	769	185	46
CuO nanosheets	15.8	0.77	Present work

$V_{max}$  is the maximal reaction velocity and  $K_m$  is the Michaelis-Menten constant (HRP) and other nano peroxidase mimetics, which confirmed that CuO nanosheets exhibits a considerable catalytic behavior to use as peroxidase mimetic (Table 1). The apparent  $K_m$  and  $V_{max}$  value of CuO nanosheets with  $\text{H}_2\text{O}_2$  as substrate was found to be 15.8 mM and  $7.6 \times 10^{-7} \text{ M s}^{-1}$ , whereas that for HRP is 3.7 mM, 87 ( $10^{-7} \text{ M s}^{-1}$ ), respectively under the same experimental conditions.

## Conclusion

In conclusion, water-soluble copper oxide nanosheets were prepared and examined as peroxidase enzyme mimetics. The kinetic catalytic activity of the water-soluble CuO nanosheets is due to the high affinity of copper oxide sheets to hydrogen peroxide. Moreover, the excellent dispersibility of CuO nanosheets in the aqueous systems makes its applications easy in water systems. This work not only proves the intrinsic peroxidase activity of copper oxide nanosheets, but also leaves its scope of applications in medicine, environmental chemistry and biochemistry.

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