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Review Article

Peroxidase Like Activity of Green Mediated Synthesised Copper Oxide Nano Particles

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Abstract

Readily water soluble CuO nano particles (NPs) were synthesised via combustion method using *Synadenium grantii* latex as reducing agent and used as peroxidase enzyme mimetic. The synthesised NPs were characterized by XRD, XPS, UV-Vis and SEM with EDX. It was found that the obtained CuO NPs possess an intrinsic enzyme like catalytic activity similar to that of natural peroxidase. Thus synthesised CuO NPs can be used in the treatment of water and as a detection tool.

Introduction

In recent years, nanotechnology is getting lot of importance, interdisciplinary with, chemistry, physics, biology, material science and drugs. Green Chemistry method highlights the development of an eco-friendly approach for the synthesis of metal oxide nanoparticles [1,2]. It emphasizes the usage of natural plant products like plant leaf extract, stem extract, flower extract, other organisms like bacteria, fungi and enzymes for the synthesis of metal oxide nanoparticles [3,4]. *Euphorbia* is the family which contains lot of flowering plants. Generally these plants are with white milky latex. In this method, we have selected the latex of *Synadenium grantii* which acts as reducing and capping agent [5].

Recently, copper oxide nanoparticles have achieved significant importance due to their distinctive properties [6]. Copper oxide nanoparticles are used as gas sensors [7-10], batteries [11], catalysis [12], solar energy exchange tools [13], etc. Copper oxide nanoparticles are synthesized through diverse methods such as sol-gel [14], alkoxide-based method [15], thermal decomposition of precursor [16], one step solid-state reaction method [17], precipitation-pyrolysis [18] etc. Chemical synthesis methods lead to absorption of unwanted Chemicals on the surface that may cause undesirable effects in the medical applications [19] and in the manufacturer of materials. To reduce the toxicity and unwanted properties, to enhance the required and desired properties, we have focussed our attention on green synthesis of nanoparticles. Currently, gold and silver nano particles, zinc oxide and copper oxide nanoparticles are synthesized by green Chemistry method using plants materials [20,21].

Enzymes are the efficient biocatalysts which play vital role in almost all biochemical reactions. These enzymes efficiently catalyses the reactions with very mild reaction conditions. Because of these characteristics they find wide applications in the field of medicine, chemical technology, environmental chemistry and bio chemistry. On the other hand, nature will restrict the applications of the compound by its availability, its instability at different conditions and high cost of production and purification. Hence there is a requirement of new stable, ecofriendly, low cost material which can replace the natural enzymes in their properties and activity [22]. However many researchers have synthesized different enzymes like dioxigenase [23], protease [24], aldolase [25], ligases [26], hydrolyses [27] etc.

Now a day's peroxidase mimetic has drawn much attention in this regard, because it plays vital role in the treatment of sewage water and oxidation process. Some peroxidase mimetic like hemin [28], Schiff base [29], carboxyl groups containing mesoporous polymers [30] have been used for enzyme analysis. As we stated earlier, still there is need for better peroxidase enzyme mimetic.

Experimental Procedure

One gram of pure copper nitrate was dissolved in 2ml, 4ml and 6ml of *Synadenium grantii* plant latex and 10ml of water. This mixture is kept in pre heated muffle furnace at 350°C for 30min. Then, obtained product was kept for calcination at 600°C for an hour. The end product was fine black powder which was characterized using XRD, XPS, UV-Vis and SEM.

Results and Discussion

Powder X-Ray Diffraction (PXRD) of CuO NPs

Fig. 1 demonstrates the typical PXRD pattern of CuO nano particles (NPs) using 2ml, 4ml and 6ml *Synadenium grantii* plant latex as fuel and the observed pattern was in well agreement with the standard JCPDS file 41-0254. No diffraction peaks corresponding to other impurities were observed. The average particle size calculated from the broadening of diffraction peaks and were found to be 26nm, 24nm and 23nm as shown in the Table1.The diffraction peaks were observed at scattering angle (2θ) of 27.3°, 30.48°, 33.5°, 36.86°, 46.89°, 51.67°, 56.45° and 59.8° corresponding to reflection (110), (002), (111), (200), (202), (020), (202) and (113) crystal planes as shown in the Fig 1.

Further the dislocation density (δ) of CuO NPs are calculated by William and Small man's equation $\delta = 1/D^2$ where D is the particle size in nm. The average dislocation density for 2ml, 4ml and 6ml *Synadenium grantii* was found to be $1.4792X10^{15}$ to $1.8903X10^{15}$. The small δ for CuO NPs indicates higher crystallization of the sample. Thus 2ml shows high level of surface defects and deteriorates crystal quality. But 4ml and 6ml, CuO NPs shows the low level of surface defects. The average crystallite size of CuO NPs were determined from Scherer equation

$$D = \frac{k\lambda}{\beta \cos\theta}$$

Where k; is constant(0.9),
λ; X ray wavelength ($1.5405x10^{-10}$m),
β; is the full width half maximum and
θ; is half diffraction angle.
Stress is calculated using the equation $\sigma = \varepsilon Y$.

Table 1: *Crystallite size, strain, Dislocation density and stress of CuO nano particles prepared by various concentration of Synadenium grantii plant milky latex.*

Sample CuO ml	Scherrer Equation D nm	Strain ε x 10^{-3}	Dislocation density $\delta = 1/D^2$ x10^{15}	Stress $\sigma = \varepsilon Y$ x 10^6 Nm^{-2}
2	26	1.203	1.4792	180.45
4	24	1.320	1.7361	198
6	23	1.34	1.8903	201

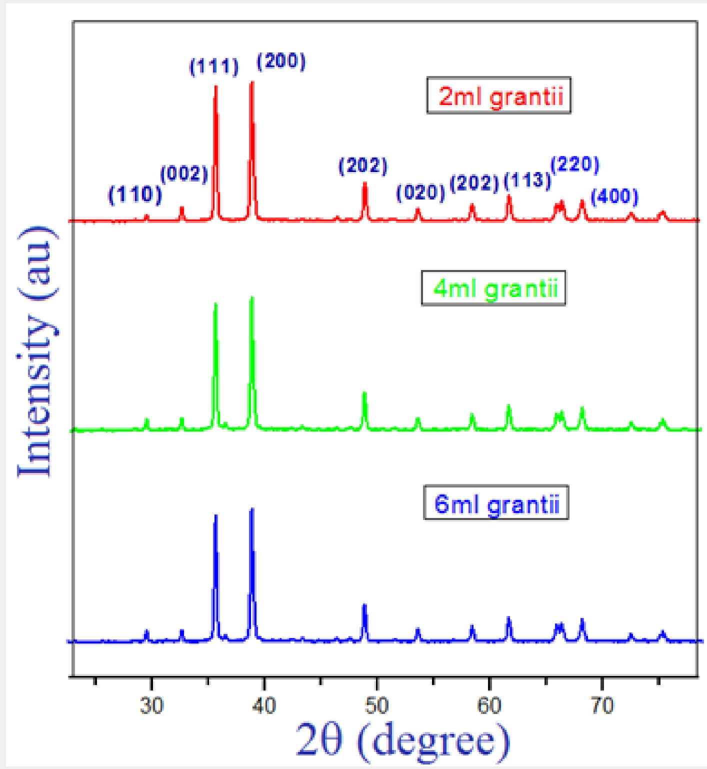


Figure 1: *XRD pattern of CuO.*

X-Ray Photoelectron Spectroscopy (XPS) of CuO NPs

Fig. 2 shows XPS analysis of green mediated synthesized CuO NPs. Fig 2(a), 2(b), 2(c) and 2(d) shows the peaks correspond to wide spectra, Cu, O and C respectively. Comparing the peaks of the spectra with the wide spectra, no impurity peaks were found. The high resolution XPS spectra of Cu 2p shows peaks at 952.5 eV and 954.5 eV. 952.5 eV corresponds to $2P_{3/2}$ state and 954.5 eV corresponds to $2P_{1/2}$ state [31]. In addition to this two more peaks are observed at 943.8 eV and 962.4 eV. These peaks are evidence of an open $3d^9$ shell corresponding to the Cu^+ state. Fig 2(c) corresponds to O 1s state. This core-level spectrum is broad and consists of three peaks that can be assigned to the O^{2-}. The main peak at the lower binding energy of 529.4 eV is attributed to Cu-O, which is consistent with the literature and the other peaks at 531.4 eV and 532.5 eV was due to chemisorbed oxygen caused by surface hydroxyl [32]. Fig 2(d) shows the high resolution XPS spectra of C 1s.

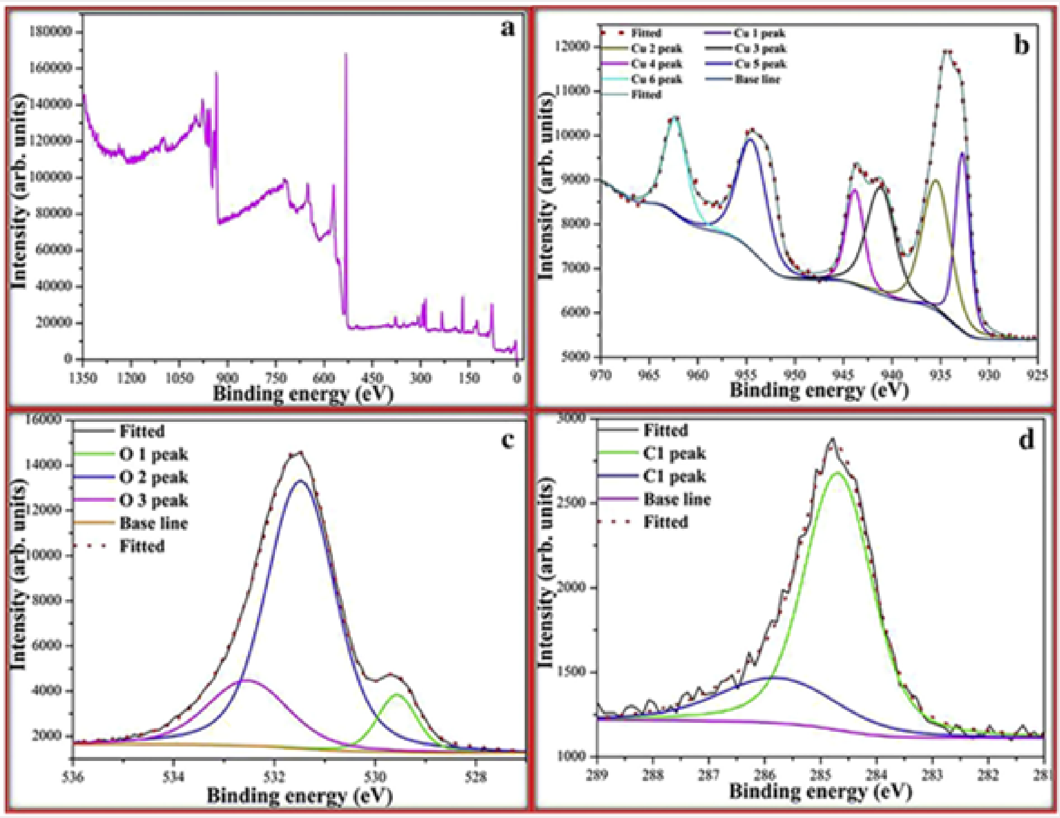


Figure 2: *XPS analysis of green mediated synthesized CuO NPs (a) wide spectra (b) Cu 2p (c) O 1s and (d) C 1s*

Peroxidase Like Activity of CuO NPs

For studying steady state experiment, fixed concentration of O-dianisidine and 10ml to 50ml H_2O_2 prepared in phosphate buffer (pH 7). The change in absorbance was measured using UV-Visible spectrometer. Then using Michaelis-Menten equation the catalytic parameters were calculated. Fig. 3 shows UV-Visible absorption spectrum of the system containing CuO NPs, H_2O_2 and O-dianisidine. The graph shows maximum absorbance at 430nm characteristic to the oxidized product of O-dianisidine, which represents the typical peroxidase like activity of CuO NPs. However, the experiment was repeated taking different concentration of H_2O_2 without CuO NPs, but no significant colour change was observed. This indicates H_2O_2 efficiently oxidises O-dianisidine in the presence of CuO NPs. In the presence of CuO NPs, the compound diazo-bis-o-dianisidine (shown below) is formed, which is responsible for orange brown colour (430nm).

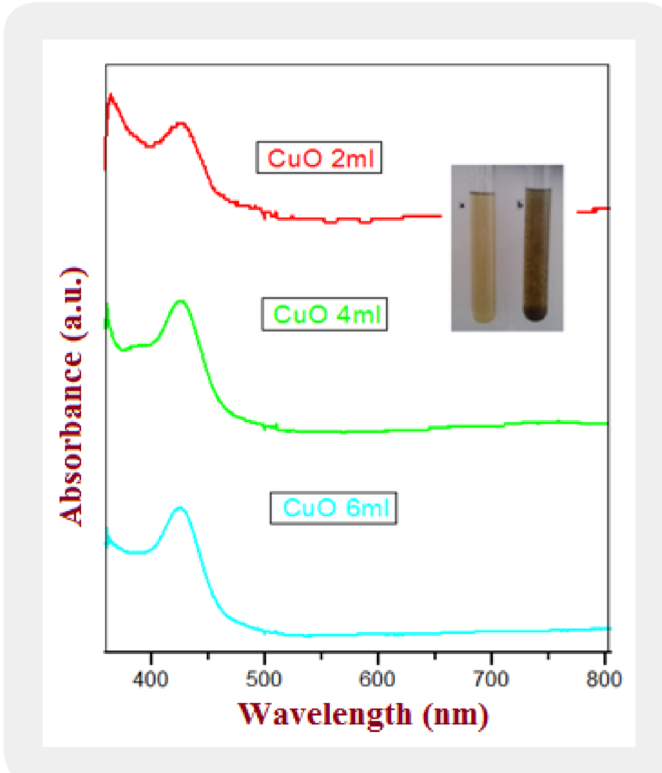
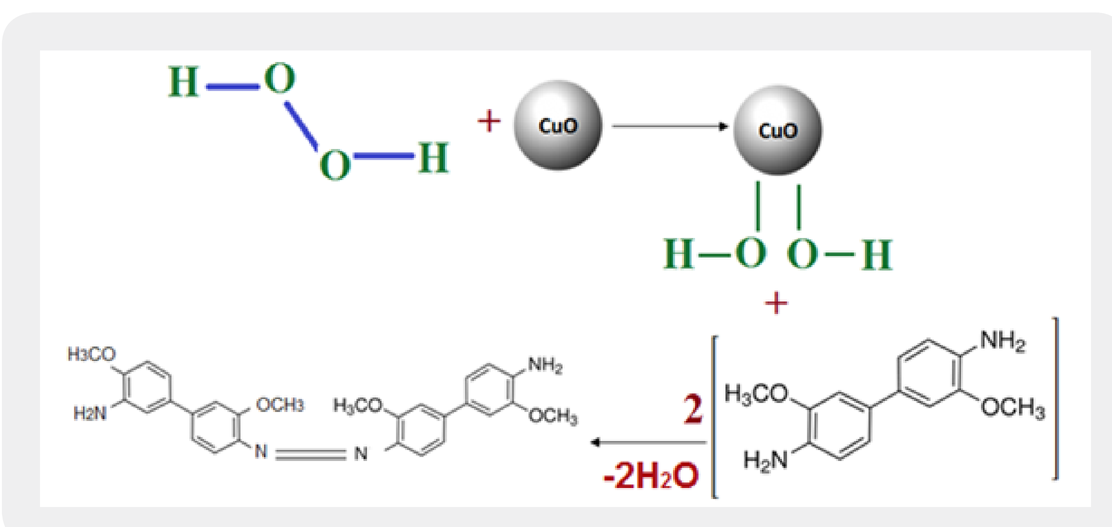


Figure 3: *UV – Visible spectra of CuO, H_2O_2 and O – dianisidine nano reaction system*



In addition to this, the enzyme activity of CuO NPs was further examined by taking steady state kinetics. The experiment was conducted keeping O-dianisidine concentration constant by varying H_2O_2 concentration. Fig. 4 shows Michaelis-Menten curve. From the graph V_{max} and K_m were recorded and values were compared with the literature (Table 2). We found that our work shows better enzymatic activity than the others.

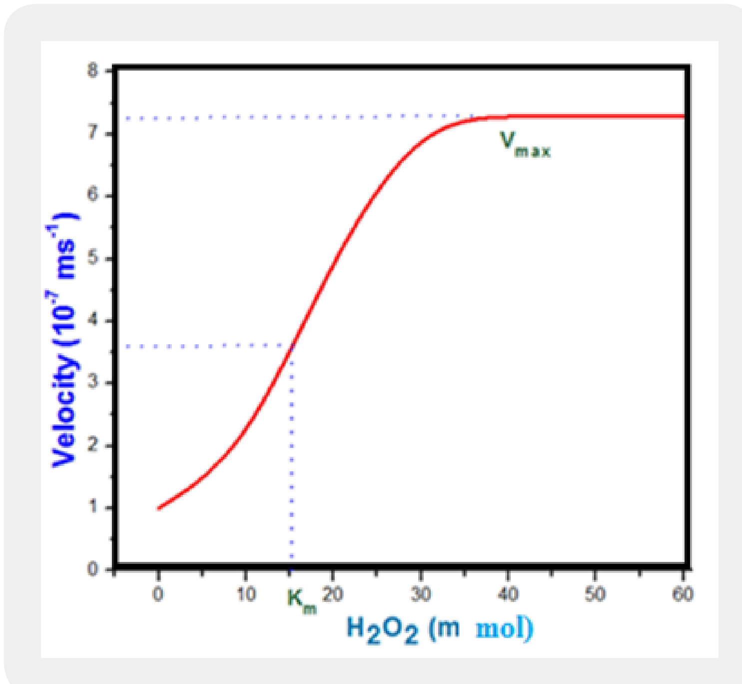


Figure 4: *Kinetic analysis of CuO nano dispersion with H_2O_2 as substrate*

Table 2: *Comparison of the Kinetic parameters of CuO with other mimetic*

Catalyst	V_{max} X10^{-7} ms^{-1}	K_m in m Mol	Reference
HRP	87.1	3.7	[33]
Co_3O_4	12.1	14.07	[34]
Prussian blue Fe_2O_3	11.7	32.3	[35]
CuO NPs	7.3	15.8	[36]

Scanning Electron Microscopy (SEM) of CuO NPs

The morphology of CuO NPs were analysed by SEM. Many researchers reported spherical shape for CuO NPs [37,38]. Mainly the morphology of the particles were affected by synthesis method and the fuel used. By using *Synadenium grantii* latex as the fuel, by combustion synthesis, the particles were found to have flower like morphologys (Fig. 5). The morphology depends on fuel used for the decomposition of $Cu(NO_3)_2$. EDX confirms the purity of the compound.

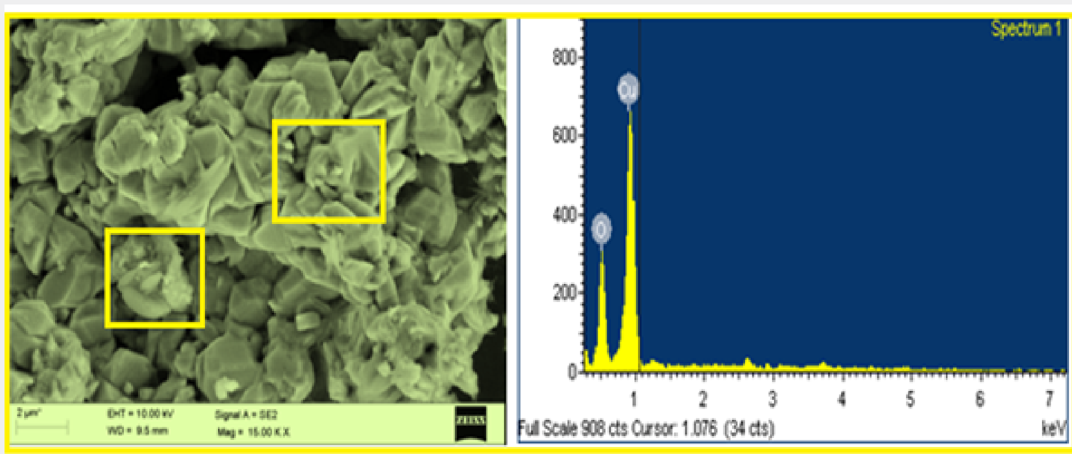


Figure 5: *SEM image of CuO and EDX of CuO NPs.*

Conclusion

Water soluble CuO NPs were synthesised by a new eco-friendly method. The obtained CuO NPs have flower like morphology. EDX confirms the purity of the sample. The kinetic catalytic activity of CuO with H_2O_2 makes its application in water purification. This work not only supports peroxidase like activity of CuO NPs but also leaves lot of scope for its application in the field of Environmental science.

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