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Ethnopharmacological Damdei plant extract assisted synthesis of copper nanoparticles and evaluation in non-enzymatic kinetics of o-dianisidine oxidation

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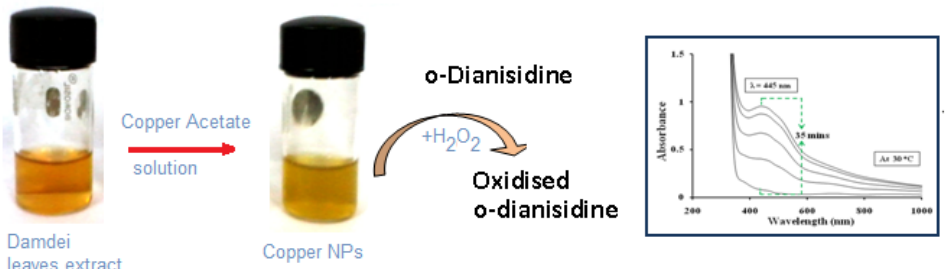
ABSTRACT



Nanosize copper particles were prepared using Damdei green leaves extract without involving any harsh chemicals and special capping agent in aqueous phase. The particles are spherical with an average diameter of 5 nm as determined by HRTEM. These ensuing copper nanoparticles (NPs) possess significant catalytic potential for oxidation of o-dianisidine in presence of hydrogen peroxide. This oxidation reaction itself is extremely slow but the inclusion of copper NPs enhance the reaction rate efficiently even at room temperature. This report may be useful in analytical industries as well as clinical chemistry for successful estimation of hydrogen peroxide present in the surrounding environment.

Keywords: Green approach, Damdei leaves, copper nanoparticles, oxidation, o-dianisidine, hydrogen peroxide

INTRODUCTION

Considerable efforts have been devoted to the creation of nanoscale particles with variety of morphologies and controlled size under soft conditions and witnessed tremendous growth due to unusual chemical and physical properties associated with them.[1-4] These nanoscopic particles possess many potential applications in the field of optoelectronics, semiconductors, sensor, catalyst, photocatalyst, fabrication, drug delivery and so on.[5-10] Although extensive research work has been carried out on metal nanoparticles (NPs), however, major efforts have been on synthesis of heavy metal nanoparticles such as gold, silver, platinum, and palladium. Further, there is an increased interest on nanosized materials of first row transition metals because of their efficient and wide range of potential applications in catalysis, fabrication, magneto-optic and in various other biological field.[11-14] Cu is an essential micronutrient and required in small quantity by all life forms. Cu being a transition metal and involved in many biological processes like mitochondrial respiration, embryonic development, regulation of hemoglobin levels as well as neuronal and hepatocyte functions. The efficacy of nanoparticles as catalyst generally relies on various factors [15-17] such as morphology, average size distribution, porosity and phase composition. Modifying and

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controlling the size and shape of nanocrystalline materials are key issues in catalysis. The catalytic activity of nanoparticulate materials is mainly because of their high surface area to volume ratio contributing to enormous number of active sites for the reactant molecules to interact.[18-20] Therefore, techniques of fabrication for nanomaterials with controlled size and morphology play a crucial role in controlling their properties. There are diverse routes for the synthesis of metal NPs[21-25] in the size range of 10–100 nm but the subsequent use of these particles for various applications are hampered either because of the drastic method of synthesis or due to difficulty in elimination of hazardous chemicals used during synthesis. Compared to other conventional methods, recently research efforts has oriented towards soft route for synthesis of metal NPs because of safety and environmental concerns.[26-29] *Croton caudatum* commonly known as Damdei, displayed in figure 1, grow mainly in the hilly region of Lamka, Manipur, India. It has curative medicinal application in treatment of cancer, diabetes, malaria, and indigestion.[30] It belongs to Euphorbiaceae family and its leaves are claimed to have anticancer properties. Presence of dotriacontamol, bamyrin and b-sitosterol in the roots and barks of this plant have been detected. Some of the phytochemicals such as flavonoids, cyanogenetic glycosides, alkaloids and phenolic compounds present in Damdei green leaves extract have the ability of biogenic growth of metal NPs.

Figure 1. Damdei plant

Oxidation of many biological substances in body fluids produces a certain amount of hydrogen peroxide (H_2O_2). H_2O_2 at high concentration results in cell damages and its accumulation causes oxidation of cellular moieties such as DNA, proteins and lipids leading to mutagenesis and/or cell death. Further, the content of hydrogen peroxide in the environment is important because it is a key species in the reaction of the troposphere [31]. So a sensitive assay for H_2O_2 has aroused great interest in analytical chemistry.

In the view of the above information, we directed a simple strategy for the fabrication of copper NPs by the reduction of copper salt with Damdei (*Croton caudatum*) green leaves extract at room temperature in aqueous medium following the basic principle of green chemistry. The particles formation occurs soon after the addition of copper salt solution into the leaves extract. Transfer of electrons play a pivotal role during oxidation of o-dianisidine (ODA) with H_2O_2. These copper NPs have the ability to catalyze oxidation of ODA with subsequent reduction of H_2O_2. To the best of our knowledge, this is the first report about significant synthesis of copper NPs utilizing Damdei green leave extract and the use of these metal particles as effective catalyst for the oxidation of ODA with H_2O_2 in aqueous medium without employing enzymes or other metal complexes that have the potential to catalyze the above mentioned oxidation reaction.

EXPERIMENTAL DETAILS

MATERIALS

All the chemical reagents used were of AR grade. Hydrogen peroxide (H_2O_2) was obtained from Rankem, India and o-dianisidine (ODA) along with horse radish peroxidise, type VI, from Sigma Aldrich. Damdei green leaves were purchased from local market of Lamka, Manipur, India. Additionally copper acetate [$(CH_3COO)_2Cu.H_2O$] was purchased from SRL, India and syringe filters were a product of Sartorius. All the solutions were prepared in double distilled water and the chemicals were used as such without any further purification.

Synthesis of copper nanoparticles (NPs): a green approach

Preparation of stable copper NPs was accomplished by reacting copper acetate solution with Damdei green leaves extract. The leave extract was prepared by stirring 100 mg of properly cleaned and dried Damdei green leaves in 6 ml of double distilled water for 30 minutes at room temperature. This extract to be used as reaction medium was then separated from Damdei leaves solid material using a syringe filter. The extract obtained was a clear transparent light yellowish solution with a pleasant odour. Now, the copper acetate solution (200µl of 1.5 % w/v) was added to the reaction medium (4.6 ml extract) with continuous stirring. The reaction mixture was further stirred for another 10 minutes. The color of the solution changes from yellowish to greenish indicating the formation of copper particles. The synthesized copper NPs were characterized for their size, morphology, thermal stability, optical behaviour and then finally utilized as such as nanocatalyst for the oxidation of ODA with H_2O_2.

Kinetic study for the oxidation of ODA with H_2O_2

The reaction was studied by measuring the increase in absorbance at 445 nm, a characteristic peak of the brown colour oxidised product. It was carried out in a 3.5ml capacity quartz cuvette of optiglass. To 2.65 ml of double distilled water taken in quartz cuvette, ODA (100 µl, 1.64×10^{-2} M), H_2O_2 (50 µl of 30 %) and freshly prepared copper NPs (200 µl of 1.67×10^{-4} % (w/v)) were added, and the change in absorbance at 445 nm with respect to time was noted. The same procedure was followed for the uncatalyzed reaction where 200 µl of copper NPs was replaced with 200 µl of double distilled water to maintain the total volume of the reaction mixture at 3 ml. The reaction was carried out at different temperatures in the range of

20-50°C for determining rate constants and activation energy of the reaction.

CHARACTERIZATION OF THE COPPER NPS

Ultraviolet-Vis (UV-Vis) spectrum

All UV-Vis spectra were recorded on Shimadzu-1601 UV-Vis spectrophotometer. The temperature of the cell holders of spectrophotometer were maintained constant by circulating water around them using a water circulator from Haake instruments. Absorption spectrum of the prepared copper NPs was recorded by taking the aqueous dispersion of NPs scanned in the range of 190 to 1100 nm.

High resolution transmission electron microscopy (HRTEM)

TEM pictures were obtained with TECNAIG^2-30 U TWIN instrument. After preparation, the metal nanoparticles were centrifuged at 10,000 rpm for 9 minutes and re-dispersed in double distilled water by sonication for 3-4 minutes. A drop of dilute solution was put on the copper grid and the grid was dried under ambient conditions. After complete drying of the grid, HRTEM picture of the copper NPs was taken.

Dynamic light scattering (DLS)

The measurement was done with a NicompTM 380 ZLS instrument (NICOMP Co, Santa Barbara, CA) with an argon-ion air-cooled laser (488 nm) as a light source and recorded at a scattering angle of 90° and at room temperature (25°C). The hydrodynamic diameter (d) of the NPs was calculated from the diffusion of the particles using Stoke–Einstein equation.

X-ray diffraction (XRD) analysis

XRD patterns of the prepared particles were estimated after complete drying of the particles at room temperature. X-ray diffraction analysis of the particles was carried out using Bruker D8 discover X-ray diffractometer employing Cu Kα radiation and scanned in the 2θ range of 20-80°.

Thermo Gravimetric Analysis (TGA)

The sample was monitored with Perkin Elmer DTA/TGA/DSC instrument by taking an adequate amount of copper nanoparticles, which were dried at room temperature, on the sample holder of the instrument in nitrogen atmosphere. The change in weight of the subjected materials with respect to temperature in the range 0-1000°C was carried out.

RESULTS AND DISCUSSIONS

There is an increasing demand of environmentally benign synthetic approach which uses less hazardous chemicals. Various naturally grown abundant plant species can be used as environment friendly reservoirs for the production of metal NPs. We have adopted a greener route for the synthesis of copper NPs purely in aqueous phase using Damdei green leaves extract without involving any strong and harmful reducing agents. The methodology is simple and depicted schematically in figure 2.

Figure 3 shows UV-Vis absorption spectrum of the colloidal dispersion of copper NPs in water. However, no prominent characteristic absorption peak of the particles was observed in the UV-Visible region. There are reports available in the literature on large copper particles exhibiting plasmon

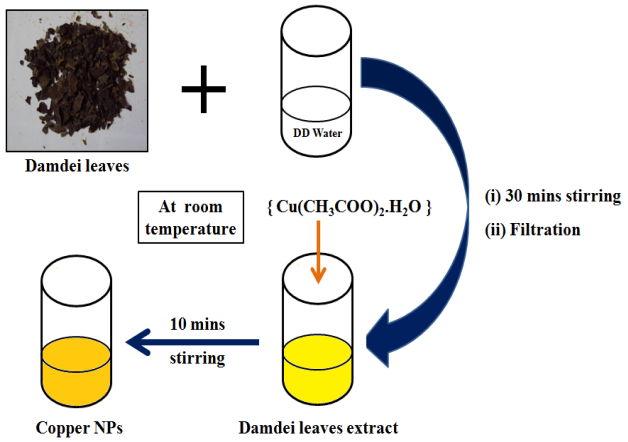

Figure 2. Synthesis scheme of copper NPs using cleaned and dried Damdei leaves extract

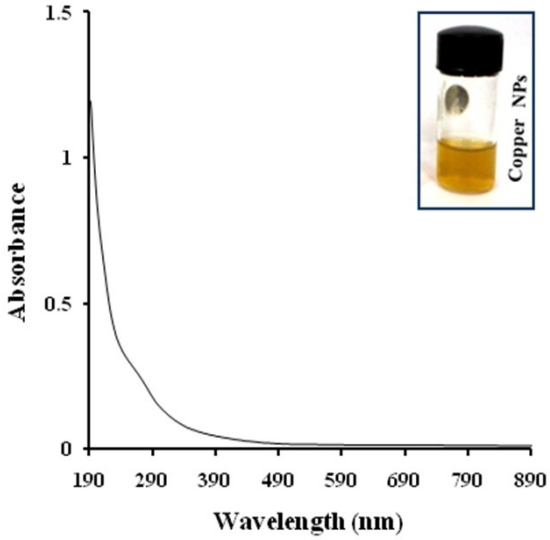

Figure 3. UV-Vis spectrum of copper NPs; inset: digital photograph of the synthesized copper NPs dispersed in water.

resonances at 560-580 nm range and their disappearance on size reduction has been clearly demonstrated.[32] The copper NPs are reasonably monodispersed and their average diameter estimated by high resolution transmission electron microscopy (HRTEM) comes out to be around 5 nm with spherical morphology as shown in figure 4(a). Selected area electron diffraction pattern (SAED) exhibits poor ring patterns which clearly depicts the particles are weakly crystalline in nature, figure 4(b). However, average size of the copper NPs determined from DLS measurement is 9 nm as displayed in figure 5. This difference in the average diameter as measured by DLS and TEM can be attributed to the fact that in TEM we use dry form of the synthesized particles but in DLS the size of the particle is estimated in aqueous dispersion which gives the hydrodynamic diameter of the particles.

Furthermore, figure 6 demonstrates the XRD pattern of the as-synthesized particles. The diffraction pattern indicates the

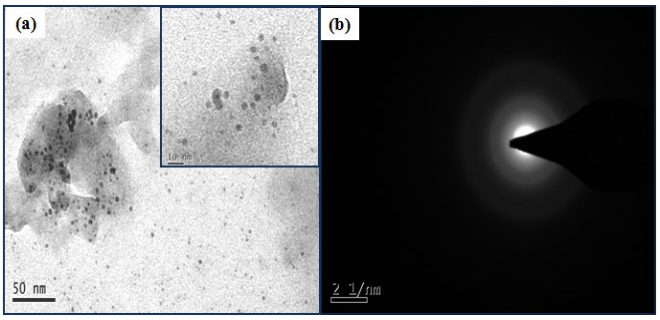

Figure 4. (a) Transmission electron microscopic (TEM) picture of copper NPs showing an average diameter of about 5 nm; inset: shows HRTEM image (b) The selected area electron diffraction (SAED) pattern of copper NPs.

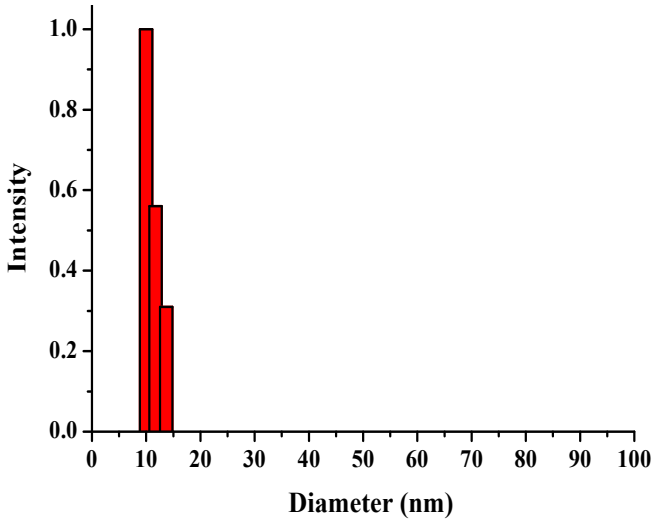

Figure 5. DLS of the generated copper NPs showing an average size of 9 nm.

presence of cupric oxide instead of pure nanosphere copper particles. This observation stems from the very fact that copper particles undergo oxidation to its oxide form during drying process at room temperature since first row transition metals are very prone to oxidation upon aerial contact.

TGA result of the phytochemically stabilized copper NPs obtained under nitrogen atmosphere is presented in figure 7. There is a progressive loss in the weight of NPs that may be related to the decomposition of surface coated phytochemicals, adsorbed water molecules and oxidation of copper NPs upon increasing the temperature. The thermogram shows 50 % weight loss at a temperature of 633°C. The colloidal dispersion of the NPs in water was clear and stable for more than a week as we noticed. These observations vividly indicate that the phytochemicals present in Damdei green leaves extract serve a dual purpose of (a) reducing the copper salt to nanodimensional copper particles and (b) act as capping agent. The chemical frameworks [33] of the phytochemicals present in the leave extract must have sufficient potential to effectively wrap around the copper particles to restrict their growth after a certain stage and provide excellent robustness against agglomeration.

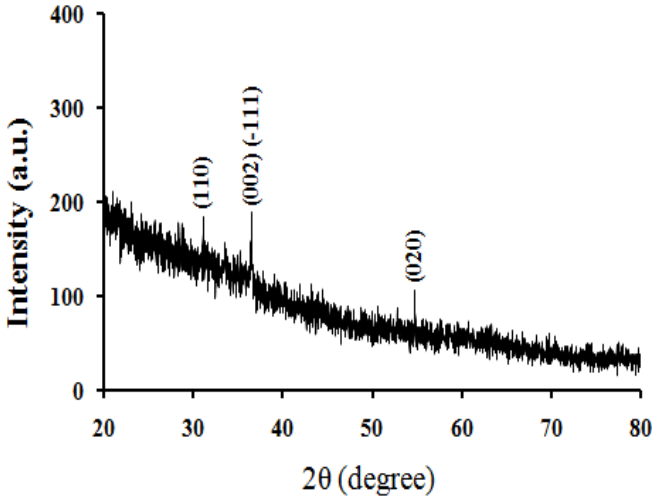

Figure 6. X-Ray Diffraction (XRD) analysis of nanosphere copper particles.

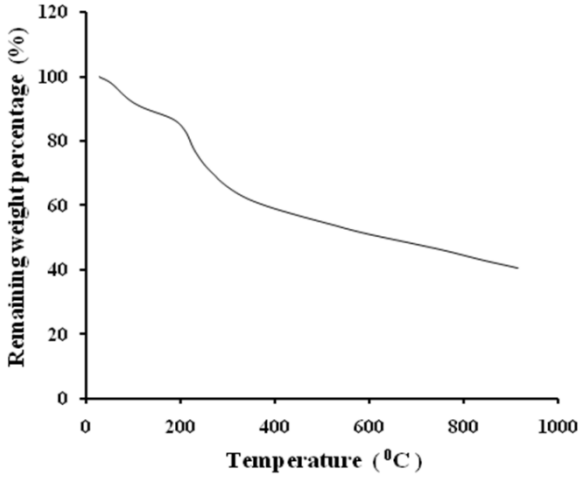

Figure 7. Thermogravimetric Analysis (TGA) of the synthesized copper NPs.

We hypotheses the formation of nanosize copper particles from Cu^{2+} might have occurred through the oxidation of hydroxyl group(s) of the phytochemicals specially polyphenolic compounds to carbonyl groups as shown below in figure 8.

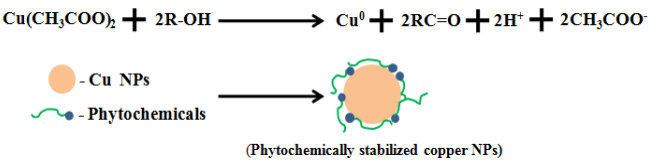

Figure 8. Probable reaction mechanism for the formation of copper NPs.

The so-synthesised copper NPs are employed as such as catalyst for the oxidation of ODA with H_2O_2 in aqueous medium at room temperature. The oxidation reaction results in the formation of a brown coloured product solution as shown in figure 9.

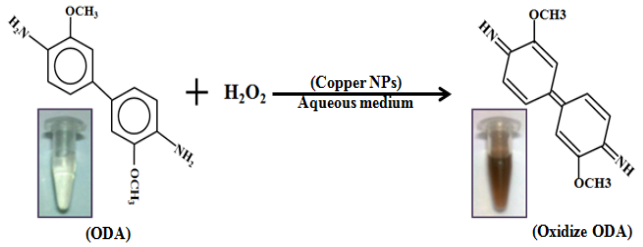

Figure 9. Reaction scheme for the oxidation of o-dianisidine (ODA) in the presence of hydrogen peroxide (H_2O_2).

Oxidation of ODA in presences of H_2O_2 gives quinonediimine, which exhibits a strong absorption maximum at 445 nm[34-36] as displayed in figure 10. The absorption spectrum of ODA is presented in figure. 10(a) while figure. 10(b) and figure 10(c) show absorption spectra of the oxidised product of ODA in presence of horseradish peroxidise (HRP) and in presence of copper NPs as catalyst respectively.

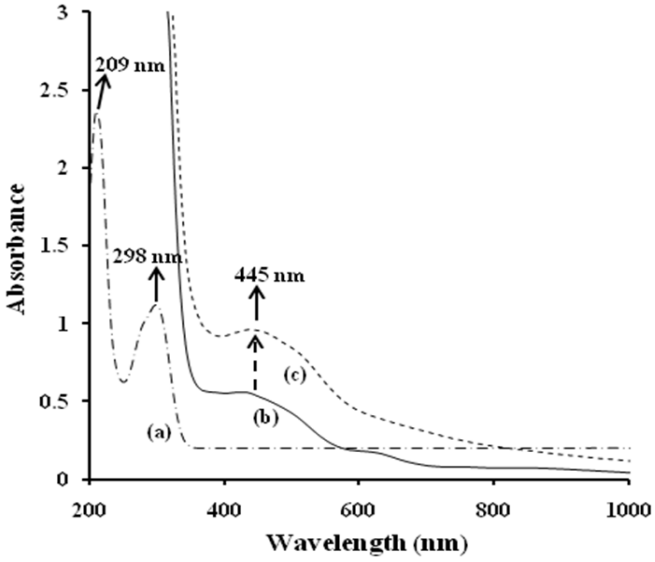

Figure 10. Absorbance spectra of (a) o-dianisidine (ODA) (b) ODA oxidize product in the presence of HRP Enzyme and (c) ODA oxidize product in the presence of copper NPs.

Absorption spectra of the oxidized ODA in the presence of HRP and copper NPs are identical to each other indicating the formation of same product upon oxidation with H_2O_2. The reaction was monitored spectrophotometrically both in the absence and presence of copper NPs at 30°C and are highlighted in figure 11(A) and 9(B) respectively.

The increase in absorbance of the colour product solution with time occurs at an extremely slow rate for the uncatalyzed reaction which indicates very slow reaction kinetics. However, upon addition of copper NPs oxidation of ODA is greatly enhanced. The oxidation of ODA using H_2O_2 in presence of Damdei green leave extract only (i.e. in absence of nanoscale copper particles) resembled to the curve of uncatalyzed reaction as presented in figure 11(C),

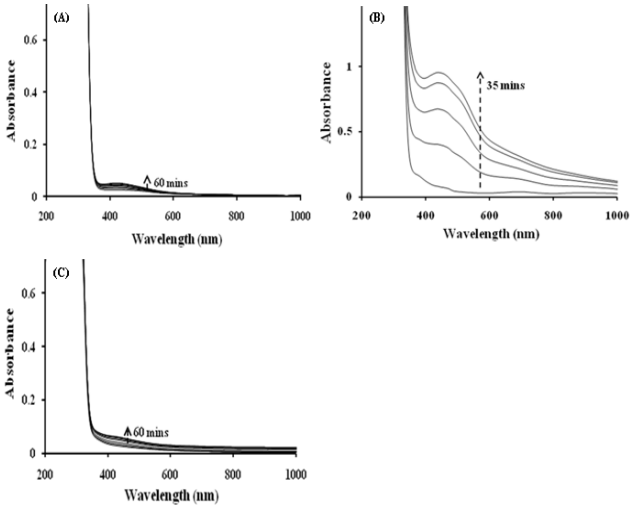

Figure 11. Oxidation of o-dianisidine (ODA) using H_2O_2 (A) in the absence of copper NPs, (B) in the presence of copper NPs as catalyst and (C) in presence of Damdei green leaves extract instead of copper NPs at 30°C in aqueous medium.

From this observation, we can gingerly interpret that the reaction is catalyzed by copper NPs and not by the phytochemicals present in the leaves extract. Copper NPs, thus, act as a mediator facilitating the transfer of electron during the reaction between ODA and H_2O_2. Figure 12 shows the change in absorbance of the reaction at 445 nm, a characteristic absorption peak of the oxidised product of ODA, with time in the temperature range of 20-50°C. The reaction follows first order kinetics and the rate constants were determined from the initial slope of - ln A Vs time graph.

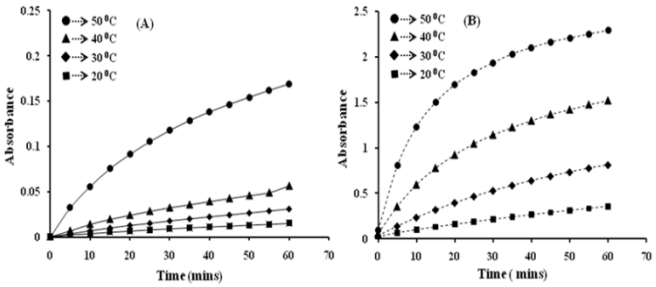

Figure 12. Change in absorbance of the oxidized color product of ODA with time measured at a wavelength of 445 nm at different temperatures for (A) uncatalyzed reaction and (B) reaction catalyzed by copper NPs.

Figure 13 gives the variation of rate constant with temperature and from the Arrhenius plot of -ln k Vs (1/T), shown in figure 14, the values of the corresponding activation energy for the oxidation reaction both in the absence and presence of catalyst were determined.

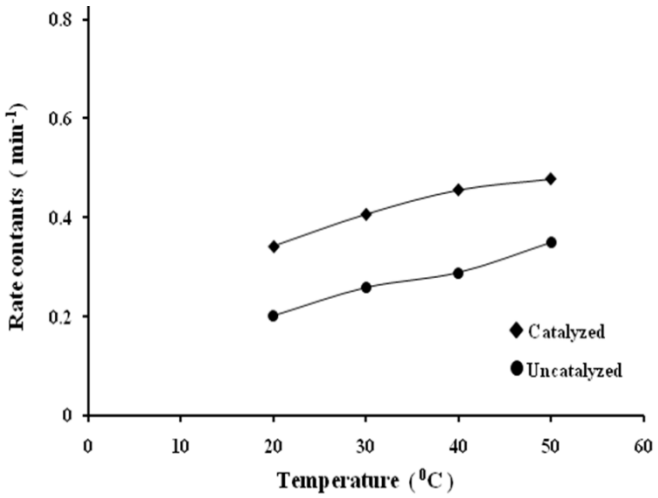

Figure 13: Variation of rate constants with temperature for the catalyzed and uncatalyzed oxidation of ODA with H_2O_2.

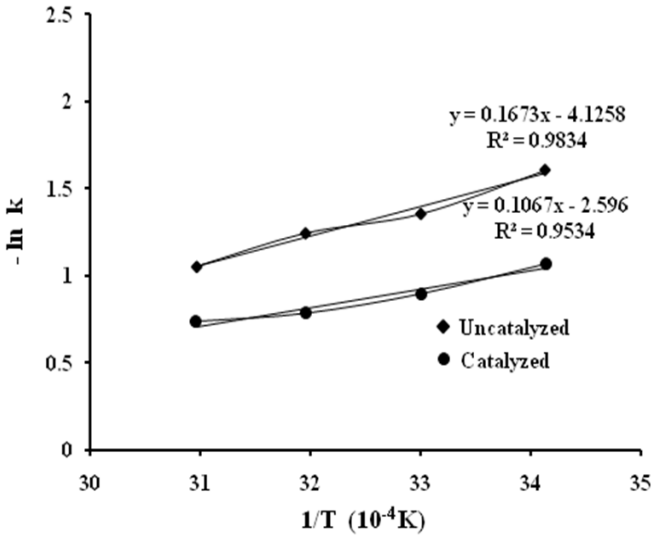

Figure 14. Arrhenius plot of - ln k versus 1/T for copper NPs catalyzed and uncatalyzed oxidation of ODA with H_2O_2 in aqueous medium.

The activation energy comes out to be 8.865 kJmol^{-1} for catalyze reaction and 13.904 kJmol^{-1} for uncatalyzed reaction. From these values of activation energies we can conclude that unlike the uncatalyzed reaction, copper NPs catalyzed reaction follows an alternative pathway. The particles significantly bring down the kinetic energy barrier and accelerate oxidation of ODA reaction thereby acting as an effective catalyst. The difference in the reaction rates between the catalyzed and uncatalyzed reactions can be attributed to the enhanced dispersion and large surface to volume ratio of the nanosized copper particles which in turn increases the number of active sites and provide an alternate pathway with lower energy requirement for the reaction to occur. The tiny copper NPs dominated by surface atoms allow them to interact with more reactant molecules. This single step greener approach of synthesis is general and can be extended to noble metals and other transition metals. This study, therefore, may be promising in analytical chemistry and the self assembled copper NPs may provide new opportunities in myriad of applications such as in sensors, catalysis, fuel cell membrane, food and drug industries, and so forth.

CONCLUSION

We have successfully designed a single step aqueous phase – green synthetic approach for copper NPs with an average diameter of 5 nm by reducing copper acetate solution with Damdei green leaves extract at room temperature. The various phytochemicals present in the green leave extract are effective in both the generation and stabilization of copper NPs for direct application as catalyst in non enzymatic oxidation of ODA with H_2O_2 in aqueous medium. In the absence of copper NPs the reaction occurs extremely slowly. A comparison has been done between the catalyzed and uncatalyzed reaction to highlight the catalytic efficacy of nanodimensional copper particles by measuring the rate constants at different temperatures and determining their corresponding activation energy from the Arrhenius plot. The significantly high catalytic activity of copper NPs has been attributed to their enhanced dispersion in aqueous medium and their small size leading to large surface area to volume ratio.

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