Green Synthesis and Characterization of Copper Oxide Nanoparticles Using *Psidium guajava* Leaf Extract

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Abstract Green synthesis of nanoparticles has gained enormous attention in today’s world due to ongoing demand to develop safe, sustainable, cost-effective and environmentally friendly process for synthesizing a wide variety of materials including metal/metal oxides nanoparticles, hybrid and bioinspired materials. In the current study, we have carried out green synthesis of copper oxide (CuO) nanoparticles (NPs) using *psidium guajava* leaf extract as capping agent and copper acetate as metal precursor. The biosynthesized nanoparticles were characterized using wide variety of techniques i.e. powder X-ray diffraction (PXRD), FTIR, TGA and HRTEM. The PXRD result confirms the synthesis of copper oxide nanoparticles in pure phase having monoclinic symmetry. The average particle size using PXRD was found out to be ~33 nm. **Key words:** ecofriendly, cost-effective, sustainable, nanoparticles and metal oxides

1. Introduction

Over the last few decades, synthesis of nanomaterials (such as metal NPs, carbon nanotubes (CNTs), quantum dots (QDs), graphene, etc.) having dimension within the range of 10-100
nm have become an extensive area of research due to their potential applications in broad area of science and technology [1-6]. Though bulk synthesis of nanoparticles can be carried out rapidly using conventional methods (i.e. physical and chemical methods), they require use of hazardous chemicals [7-9]. Therefore keeping this in mind, use of green synthesis approach that makes use of mild reaction conditions and non-hazardous precursors have been emphasized for promoting environmental sustainability [10-15]. The biological systems that are actively used in the green synthesis of NPs include plants and their derivatives (phytosynthesis), microorganisms like fungi, bacteria, algae and yeast [16-17]. Among all the available methods, phytosynthesis is the ideal method for the synthesis of metal oxide NPs owing to several benefits associated with it i.e. safe, efficient, cost-effective, simple (does not involve complex & multistage process like microbial isolation, culturing, etc.) and provides better control over shape, size & dispersion of metal nanoparticles in comparison to bacteria and/or fungi mediated synthesis [18].

Among a variety of transition metal oxides, CuO is one amongst the potential p-type semiconductors that have gained considerable attention attributed to its excellent physical, electrical, optical, and magnetic properties. CuO with a narrow band gap of 1.2 eV is widely used in catalysis [19-22], electrochemistry [23-24], solar energy conversion [25], sensors/biosensors [26-28], energy storage [29], biocidal agents [30-31] etc. In literature, green synthesis of CuO NPs is reported using plants such as Calotropsis procera leaf extract [32], Abutilon indicum leaf extract [33], aloe vera leaf extract [34], karaya gum [35], Cassia alata flower extract etc. In the current study, Psidium guajava leaves have been used for carrying out the synthesis of copper oxide NPs due to the presence of high amount of phytochemicals in it [36-37]. Psidium guajava is a medicinal plant commonly known as guava & has a place in Myrtle family as Myrtaceae [38]. Psidium guajava is found in various areas of the tropical and subtropical zones and has been reported to have antidiarrheal [39], antispasmodic [40], antioxidant [41], anti-allergy [42], antibacterial [43], anti-cough [44], anti-inflammatory [45], and anticancer activities [46].

2. Materials and methods

Copper acetate monohydrate (Merck, ≥ 98.0 %), Sodium lauryl sulfate (Sisco Research Laboratories ≥ 98.0 %) and fresh psidium guajava leaves collected from the Delhi Technological University (DTU) campus.
2.1. Extraction of *psidium guajava* Leaf Extract:

Fresh *psidium guajava* leaves collected from the DTU campus were first cut into small pieces. These leaves were then washed thoroughly using double distilled water to eliminate debris. These cleaned leaves were then dried in the presence of sunlight for 5 days. After that the dried leaves were ground into a fine powder using pestle mortar & stored in dry place. *Psidium guajava* leaf extract was prepared by heating the solution of *psidium guajava* leaf powder and distilled water (1:10 wt. %) at 100°C for 30 minutes in Erlenmeyer flask till the colour of solution changes to dark brown (indicating phytochemical extraction). After that the solution was allowed to cool down to room temperature and filtered through Whatman No.1 filter paper. The filtrate was used for the synthesis of copper oxide NPs.

2.2. Synthesis of CuO Nanoparticles:

To synthesise CuO NPs 1 M copper acetate monohydrate was dissolved in 10 ml of distilled water at room temperature using magnetic stirrer (250 rpm). After that, 30 ml of *psidium guajava* leaf extract was added into the copper acetate solution drop wise, with continuous stirring. Later the solution was kept on magnetic stirrer (250 rpm) for 24 hrs at 60 °C leading to the formation of gel that was dried in hot air oven at temperature of 60 °C. The green precipitate thus obtained was calcined at temperatures of 400 °C for 6 hrs in the muffle furnace, forming black CuO nanoparticles.

2.3. Synthesis of CuO Nanoparticles in presence of sodium lauryl sulfate (SLS)

For the synthesis of CuO nanoparticles in presence of SLS, SLS (4 g) was added into *psidium guajava* leaf extract (30 ml) and the resulting solution was stirred for five minutes. Rest of the procedure followed was same as mentioned above.

PXRD pattern of synthesized sample was recorded using high-resolution Bruker D8 Advanced X Ray diffractometer employing Cu Kα radiation (λ = 1.5418 Å) over the range of 2θ = 10-80°. Thermo gravimetric analysis (TGA) was conducted using the Pyris 1® TGA in the range 50-800°C under flowing nitrogen (50 mL min⁻¹) at a heating rate of 10 °C min⁻¹. FTIR spectra of the sample was recorded using a Perkin-Elmer 2000 FTIR spectrometer.
employing KBr disks. TEM of the sample was recorded using Jeol/JEM 2100 HRTEM operated at an acceleration voltage of 200KV.

3. Results and discussion

3.1. Qualitative Phytochemical Screening:

Phytochemical screening of freshly prepared *psidium guajava* leaf extract was carried out using simple chemical tests to identify the presence of active phytoconstituent i.e. polyphenol, alkanoids, flavonoids, saponins, tannins, etc. in the sample (Table 1).

| Component          | Procedure                                                                 | Result                                                                 |
|--------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------|
| Flavonoids         | A portion of aqueous extract was added to 5 ml of aq ammonia solution, followed by the addition of Conc. H$_2$SO$_4$  | Absence of yellow colour indicating the absence of flavonoids.         |
| Gum & Mucilages    | 10 ml of extract was added to 25 ml of ethanol with constant stirring      | No white or cloudy precipitate indicating the absence of Gum & Mucilages|
| Phenolic Compounds | To 5 ml of leaf extract few drops of 5% neutral FeCl$_3$ was added         | formation of dark green colour indicating the presence of Phenolic Compounds |
| Terpenoids         | To 5 ml of leaf extract 2 ml of chloroform was added followed by the addition of 2 ml of Conc. H$_2$SO$_4$ carefully along the sides of the test tube to form a layer | No formation of a reddish brown colour at the interface indicates the absence of terpenoids. |
| Saponins           | A 1 ml aliquot of the leaf extract was diluted using 20 ml of distilled water and was then shaken vigorously for 15 minutes in a vortex | Persistent foaming indicates the presence of saponins. |
| Glycosides         | Few drops of aqueous NaOH was added into a 1ml of leaf extract            | No formation of yellow color indicates the absence of Glycosides. |
Steroids

| 5 ml of extract was added to a 2 ml of chloroform & Conc. H$_2$SO$_4$ solution and was shaken thoroughly $^{[51]}$. | No formation of a reddish brown colour at the lower chloroform layer indicates the absence of steroids. |

Phytoconstituent are involved in the green synthesis of metal oxide nanoparticles and can acts as both effective reducing as well as stabilizing agents. Therefore in order to understand the mechanism of nanoparticles synthesis screening of phytochemical is inevitable. Phytochemical analysis exhibit positive result for phenolic & saponins content in the leaf extract inferring its significant properties and shows negative results for flavonoids, gum & mucilages, terpenoids, glycosides and steroids.

3.2. Structural Analysis of CuO Nanoparticles

The PXRD pattern of CuO nanoparticles synthesized using psidium guajava leaf extract in absence and in presence of SLS is presented in Figure 1. The presence of PXRD peaks at 2θ value of 32.53°, 35.63°, 38.74°, 48.83°, 53.56°, 58.36°, 61.51°, 65.85°, 66.42°, 68.11°, 72.34° and 75.33° confirms the formation of CuO having monoclinic symmetry with lattice constant $a = 4.683$, $b = 3.428$, $c = 5.129$ Å (JCPDS 80-1268) $^{[52]}$. Since no additional peak due to the presence of any other phase was observed in absence of SLS this suggests the formation of copper oxide nanoparticles in pure phase.

The average particle size of synthesized copper oxide nanoparticles was calculated using Debye- Scherrer's formula, i.e.,

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

Where, D, λ, β, θ represents average particle size (nm), wavelength of x-ray (0.15406 nm), full width half maximum of the intense peak, Bragg’s angle and k is taken as 0.89 (constant), respectively. Using the above equation, the average particle size of copper oxide nanoparticles was found out to be ~33 nm.
Further confirmation of internal structure, accurate measurement of particle size and morphology was done using TEM analysis (Figure: 2). Both the average particle size & interplanar spacing values were in close agreement to that obtained from XRD data. However SAED pattern confirms the crystalline nature of synthesized copper oxide nanoparticles.

The FTIR spectrum of synthesized copper oxide nanoparticles is presented in Figure 3. The peaks around 784, 624 and 529 cm\(^{-1}\) corresponds to the Cu–O stretching vibration of copper oxide nanoparticles in the monoclinic structure. The absorption peaks at 3439 cm\(^{-1}\) and 1628 cm\(^{-1}\) corresponds to the OH stretching vibration and HOH bending mode of adsorbed water molecules, since the nano crystalline materials possess high surface area to volume ratio.
leading to the absorption of moisture in the lattice. The absorption band at 1107 cm\(^{-1}\) corresponds to the C–O stretching of phenol and alcoholic compounds.

The TGA/DTG profile of copper oxide nanoparticles is presented in Figure 4. The TGA curve showed a three-step decomposition process of the precursor to form CuO NPs. The first weight loss below 166°C (~9%) corresponds to the removal of moisture and organic solvent molecules. The second and third weight loss in the region from 166 – 403°C may be due to pyrolysis and combustion of organic compounds form precursor and solvent that leaves the system in the form of CO, CO\(_2\) and other organic gases. At further higher temperatures, no significant weight loss was observed, thereby supporting the formation of copper oxide NPs with high purity.
Hence, based on TGA and phytochemical analysis, the mechanism of biochemical reduction & stabilization of CuO nanoparticles might be as follows:

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\text{Ellagic Acid} \quad \underset{60^\circ C}{\overset{400^\circ C, 6hrs}{\longrightarrow}} \quad \text{CuO NPs}
\]

(Figure 5: Mechanism of the synthesis of CuO nanoparticles)

4. Conclusion

The present study reports the biosynthesis of copper oxide nanoparticles by making use of using *psidium guajava* leaf extract as both capping & reducing agent. The presence of phytoconstituent (phenolic compounds) in the leaf extract plays major role in the biochemical reduction & stabilization of copper oxide at nano scale level. The PXRD spectrum confirms the formation of CuO in monoclinic phase having average crystallite size of ~33nm. The HRTEM revealed spherical morphology of CuO nanoparticles. The methodology used for the
synthesis is green and viable because of its ease, efficiency, cost-efficient and eco-friendly nature in comparison to other methods.

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