

Copper nanoparticle synthesis using *Picea glauca* 'Conica'

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SUMMARY

Copper nanoparticles (CuNPs) are frequently used as catalysts, antibacterial agents, electromagnetic interference shielding, and more, and are relatively inexpensive. Typically, synthesizing CuNPs requires toxic chemicals. Additionally, millions of *Picea glauca* 'Conica', or spruce, trees and plants are sold at Christmas every year, enjoyed in-season, and then discarded. There is great potential to keep these trees and plants out of landfills by using their leaves in alternate applications. We hypothesized that *P. glauca* leaves could be used as a non-toxic reducing agent to synthesize CuNPs due to their high antioxidant content. We synthesized CuNPs by adding an extract of *P. glauca* to copper sulfate, using starch as a capping agent. We obtained transmission electron microscope (TEM) images of the CuNPs and analyzed their morphologies using ImageJ software. The particles were predominantly spherical in shape, with an average diameter of 18.79 ± 5.99 nm. Energy-dispersive (EDS) showed absorption peaks of copper (Cu). We conclude that *P. glauca* leaf extract acts as an excellent reducing agent. Furthermore, biosynthesis using *P. glauca* is environmentally friendly, does not use toxic chemicals, and takes advantage of copper as an abundant resource. Ultimately, the CuNPs produced will be beneficial, as CuNPs are integral to medicine, electronics, and a variety of industries.

INTRODUCTION

Nanoparticles, defined as particles with a dimension less than 100 nm, have recently gained popularity in scientific research due to their enhanced optical properties, magnetism, catalysis functions, and more (1). Copper has a high natural abundance and a low cost (2, 3). Copper nanoparticles (CuNPs) are a potential alternative to using rare, expensive metals (4). They are used in a variety of industries as they exhibit high antibacterial activity and can be mixed with several polymers (5, 6). For example, CuNPs are often used as catalysts in organic reactions, as antibacterial agents against pathogens, and as shielding against electromagnetic interference in electronics (5, 7, 8).

The main parameters studied for nanoparticles are size, shape, and homogeneity. Nanoparticles' sizes and shapes are closely related to their properties and, thus, their applications. For example, size and shape determine how nanoparticles are taken up by cells, especially in nanomedicine (9). Spherical

nanoparticles, in particular, have many desirable properties that allow for applications in a variety of fields. Spherical particles have less friction (10). Because of this, they can be used in nanofluids, which save energy in heat transfer processes. Spherical nanoparticles are also vital for drug delivery since they can be delivered a variety of ways (11). In addition to this, their large surface area to volume ratio further assists their role in drug delivery. Thus, sphericity is essential to a nanoparticle's function. Homogeneity is also highly necessary to perform clear studies on how a nanoparticle's morphology relates to its function.

A specialized method to measure nanoparticle size, distribution, shape, and morphology is transmission electron microscopy (TEM) (12). TEM uses a beam of electrons to generate magnified images that are as much as 2 million times larger than the particle (13). Energy-dispersive X-ray spectroscopy (EDS) is used to analyze the composition of nanoparticle samples (14). In an EDS system, X-rays eject electrons from an atom. Removing these electrons from the system will leave behind a hole that a higher energy electron can fill in. The energy released by the higher energy electron is unique to each element on the periodic table and identifies elements present as well as what proportion they are present in.

A popular approach to synthesizing nanoparticles involves a chemical reduction of a metal salt with a reducing agent (15). However, this approach results in toxic byproducts, and the resultant nanoparticles cannot be used in a clinical setting (16). Additionally, many chemicals used in synthesis, such as sodium borohydride, 2,2 diphenyl-1-picrylhydrazyl hydrate, methanol, and ethanol, are toxic in their own right. This has led to growing interest in using biological routes to synthesize nanoparticles.

Plants are rich in phytoconstituents. These phytochemicals, especially phenols and flavonoids, can act as antioxidants in the reduction of copper ions to CuNPs (17). Thus, they can be used to synthesize metal nanoparticles. Plant-based synthesis is a cost-effective, environmentally friendly, and less toxic alternative to most chemical and physical methods as it does not require hazardous chemicals during synthesis (18). Other plant-based extracts which have been used for CuNP synthesis are lemon, gooseberry, eucalyptus, and hibiscus due to their high antioxidant content (19). Dried spruce leaves also have a high antioxidant content of 29.31 mmol/100g per the antioxidant food table and are hypothesized to be an

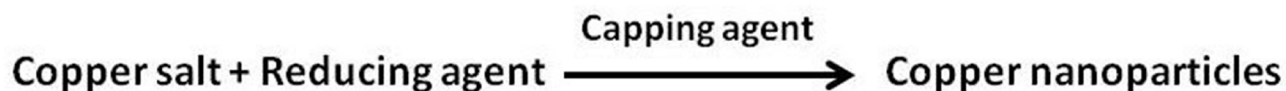


Figure 1: Chemical reaction of reduction experiment. Copper salt (copper sulfate) upon addition of reducing agent (plant extract of *P. glauca*) in the presence of a capping agent (starch) will form copper nanoparticles.

excellent reducing agent (20).

A major source of spruce leaves are Christmas trees and plants, millions of which are sold every year (21). These trees are enjoyed during the holidays just to be discarded after. Many waste management providers as well as non-profits pick up discarded trees for a few weeks following Christmas. *Picea glauca* 'Conica' is a coniferous spruce shrub sold as a mini-Christmas tree (22). The leaves of *P. glauca* contain a high level of phytochemicals such as flavonoids (23).

Given the ready availability of *P. glauca* and its role as a recyclable waste product, we studied the synthesis of CuNPs using an extract of *P. glauca* leaves. As *P. glauca* leaves have a high antioxidant value, we hypothesized that they would be an excellent, environmentally friendly reducing agent needed in the synthesis of CuNPs. The results of our experiment showed that CuNPs with consistent morphologies formed when an extract of *P. glauca* was added to a solution of copper sulfate, supporting our hypothesis. TEM and EDS analysis of the resulting nanoparticles showed that the synthesized CuNPs exhibited consistent morphologies (size and shape). The homogeneity of the CuNPs demonstrates the predictability of the process studied.

RESULTS

We first sought to prepare an extract of *P. glauca* for use in CuNP synthesis. We accomplished this by preparing and filtering the extract overnight. We used starch as a capping agent to stabilize the synthesis, prevent overgrowth of nanoparticles, and prevent oxidation (24). We added the extract and starch to a copper sulfate solution to synthesize the CuNPs (Figure 1). To investigate the formation of the CuNPs, we performed visual and chemical analyses, TEM imaging, and EDS. We set up a negative control group with a copper sulfate solution, deionized water, and starch.

Visual Analysis

When we added *P. glauca* extract to the copper sulfate solution, the mixture changed from a bright blue to a light brown color. While the solution heated, reddish-brown precipitates were created, indicating the reduction of copper sulfate and the formation of CuNPs (Figure 2A, B). Additional precipitates continued to form while the solution sat at room temperature for 36 hours. No precipitates formed in the control set, and there was no color change (Figure 2C, D).

Chemical Analysis

We filtered part of the precipitate and tested it with

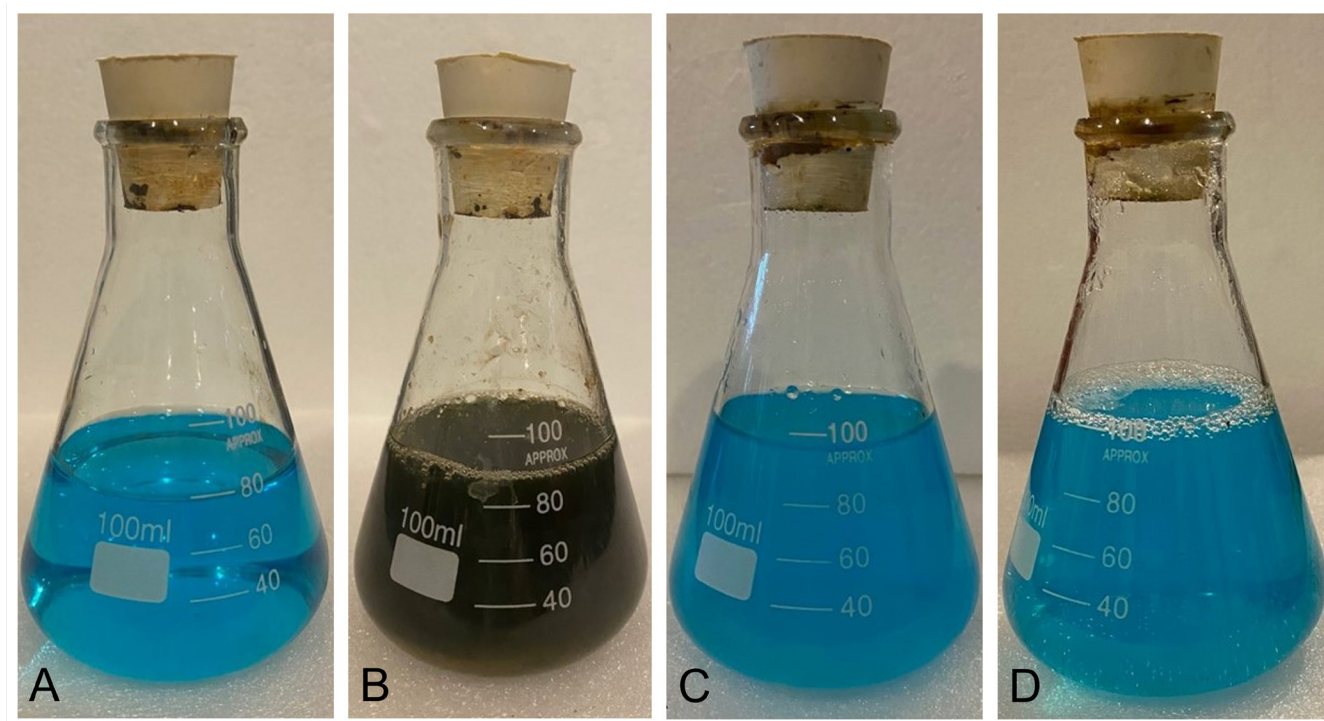


Figure 2: Color change observed due to the formation of CuNPs. (A) Copper sulfate solution before the addition of *P. glauca* extract as a reducing agent. (B) Solution changed to brown upon the addition of *P. glauca* extract and heating for one hour. (C) Copper sulfate solution before the addition of water and starch. (D) Solution remained the same and no precipitates formed upon heating.

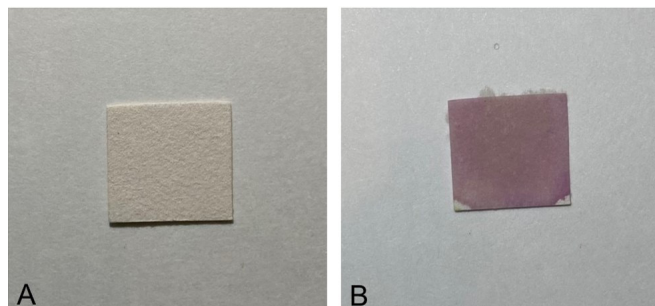


Figure 3: Cuprotesmo test paper color change validating synthesis of CuNPs. (A) Cuprotesmo test paper before precipitates were tested. (B) Change in color to pink when precipitates came into contact with the test paper.

Cuprotesmo test papers, which only reacts with copper, to verify the presence of copper. The precipitate turned the test papers pink, indicating that the precipitate contained metallic copper (Figure 3). The negative control did not form any precipitates and, thus, was not tested with the Cuprotesmo papers.

TEM and EDS Spectra analysis

EDS analysis validated the presence of synthesized CuNPs (Figure 4). EDS spectra shows three distinctive energy peaks, corresponding to the presence of copper in the solution. The samples were placed on a carbon film during imaging, explaining the carbon peak. The sulfur and oxygen peaks result from the usage of copper (II) sulfate in the original solution. The phosphorus peak is caused by the presence of phosphorus in *P. glauca* leaves (25). The presence of

an oxygen peak could imply the formation of copper oxide, which would affect the conductivity of the CuNPs. However, the proportion of copper to oxygen in the EDS spectra implies that the majority of the CuNPs synthesized were not oxidized.

Analyzing the CuNPs' shape, size, and consistency determines its potential in various applications. TEM images taken at a point resolution of 0.34 nm show that the nanoparticles are uniformly distributed in the solution and are predominantly spherical in shape (Figure 5). Analysis of the images with ImageJ software yielded an average size of 18.79 ± 5.99 nm (mean \pm SD). Ratios for roundness and circularity were assessed as well; analysis showed an average roundness of 0.86 ± 0.13 and average circularity of 0.29 ± 0.12 . Additionally, there was a narrow size distribution, indicating that the synthesized CuNPs were uniform in size (Figure 6).

DISCUSSION

We successfully synthesized morphologically consistent CuNPs using non-toxic chemicals. While sulfur, phosphorus, and carbon were detected in the EDS spectra, we believe they will not affect the performance of the CuNPs, as they are likely only present in the supernatant. As the CuNPs were small, the irregularities in the borders strongly influenced the change in circularity. Therefore, we conclude that the CuNPs synthesized are spherical but not morphologically smooth. This lack of morphological smoothness results in an increased surface area which, in turn, can potentially change the binding properties of the CuNPs. A rough surface could increase the adsorption of catalysts and enzymes in biomedical applications. Further studies could be structured to better understand this (26).

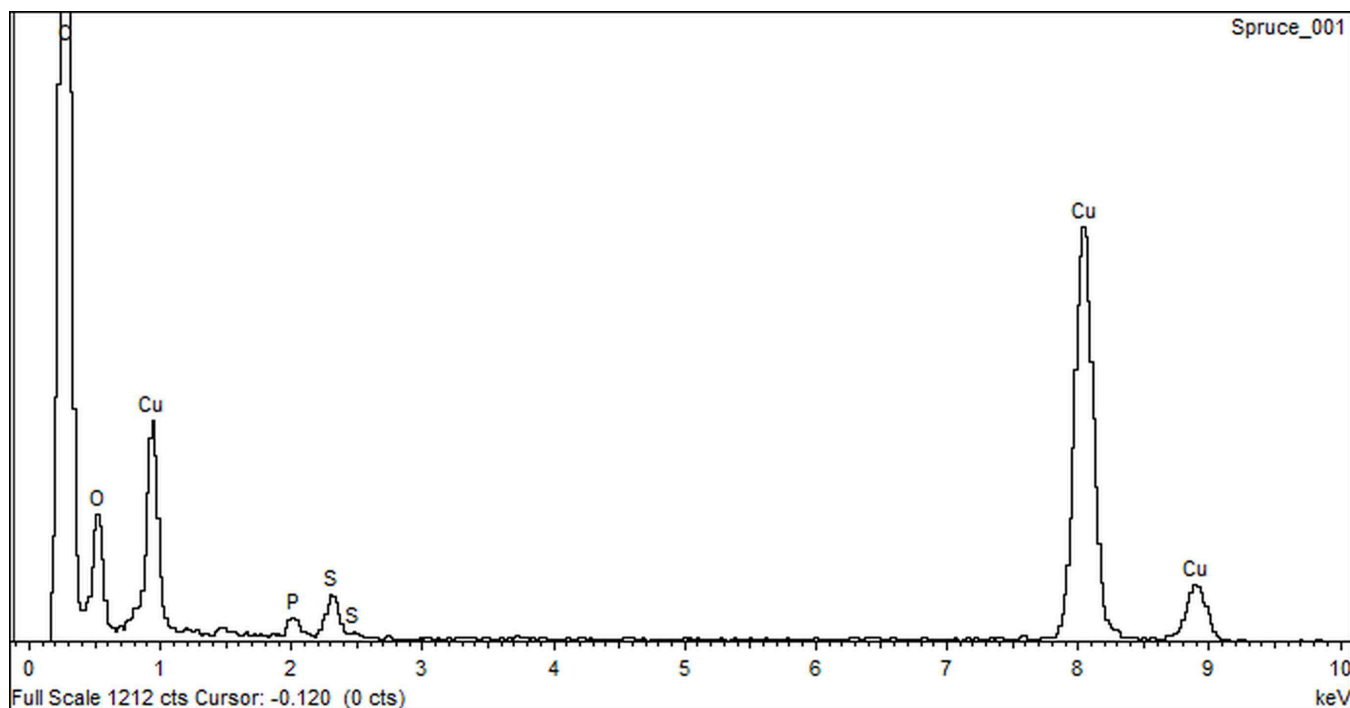


Figure 4: EDS spectra of CuNPs synthesized with *P. glauca*. Energy spectrum as plot of X-ray counts versus energy in keV. Energy peaks show different elements present in final solution. Cu peaks validate presence of CuNPs. C peak results from the use of a carbon grid. O peak results from presence of sulfate ions in the solution and/or oxidation. S and P peaks are from *P. glauca* extract. EDS analysis completed using Oxford INCA EDS system featuring a 10 mm² detector with 140eV resolution.

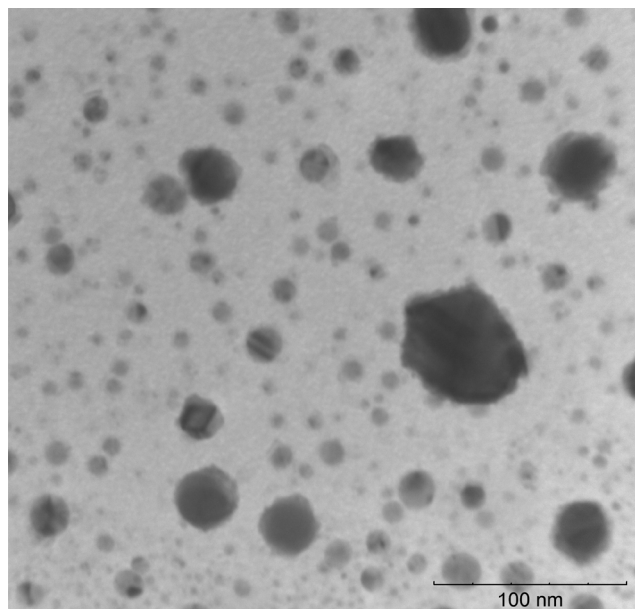


Figure 5: TEM image of CuNPs synthesized with *P. glauca*. Nanoparticles are uniformly distributed and are predominantly spherical in shape. Some clusters are present.

This study does have limitations. Since we conducted the experiment in a home environment, the copper precipitates could not be centrifuged and washed, as done in professional laboratory settings. This made prepping the samples for imaging more difficult. There was also potential for more oxidation to occur, as an inert atmosphere could not be maintained. Additionally, there was limited access to a laboratory, so there was a reliance on a lower number of samples. Within these stated limitations, however, our study provides preliminary evidence that CuNPs can be synthesized using *P. glauca* leaves.

The findings in this study could be explored further by changing the copper precursor (for example, to copper nitrate or copper chloride) and using the same synthetic protocol.

This could potentially alter the shape and morphology of the CuNPs synthesized (27). Another area of exploration would be to remove the capping agent and see if the phytochemicals in spruce leaves can act as a capping agent instead. Alternately, a different capping agent could be used to see if the size of the CuNPs change. Further investigation could reveal ways to alter the morphology of CuNPs and to evaluate yield rates for commercial applications.

Thus, we demonstrated that *P. glauca* leaf extracts provide a novel, environmentally sustainable pathway to synthesize CuNPs. The novelty of the experiment is that we synthesized CuNPs using a recyclable agent that has never been used for this purpose before. Furthermore, *P. glauca* leaves are a waste product from Christmas trees, so this method could help with recycling. Our technique also does not use any toxic compounds and is inexpensive.

MATERIALS AND METHODS

Preparation of plant extract

Dwarf Alberta Spruce (*P. glauca*) leaves were cut from the plant (purchased from Home Depot) and washed in deionized water (Ecoxall LLC) to remove dust. Leaves were then pulverized in a coffee grinder. The leaf extract was prepared by mixing 5 g of the pulverized leaves with 100 mL of water and boiling at 100 °C for 10 min (28). The extract was allowed to sit for 12 hours, after which it was filtered through Whatman No. 1 filter paper. The filtrate was stored in a refrigerator at 4 °C for further use.

Reduction of copper sulfate

5 g of copper sulfate (Innovating Science, Cat# IS12179) was dissolved in 80 mL of deionized water in an Erlenmeyer flask. 20 mL of the leaf extract and 1 g of starch (Innovating Science, Cat# IS28205) were added to the flask and mixed well. The flask was placed in a boiling water bath for 1 hour, after which it was capped and placed in a dark cabinet for 36 hours to minimize oxidation. A portion of the precipitate formed was transferred into amber glass bottles and delivered to a scientific analysis lab for TEM imaging and EDS. Visual

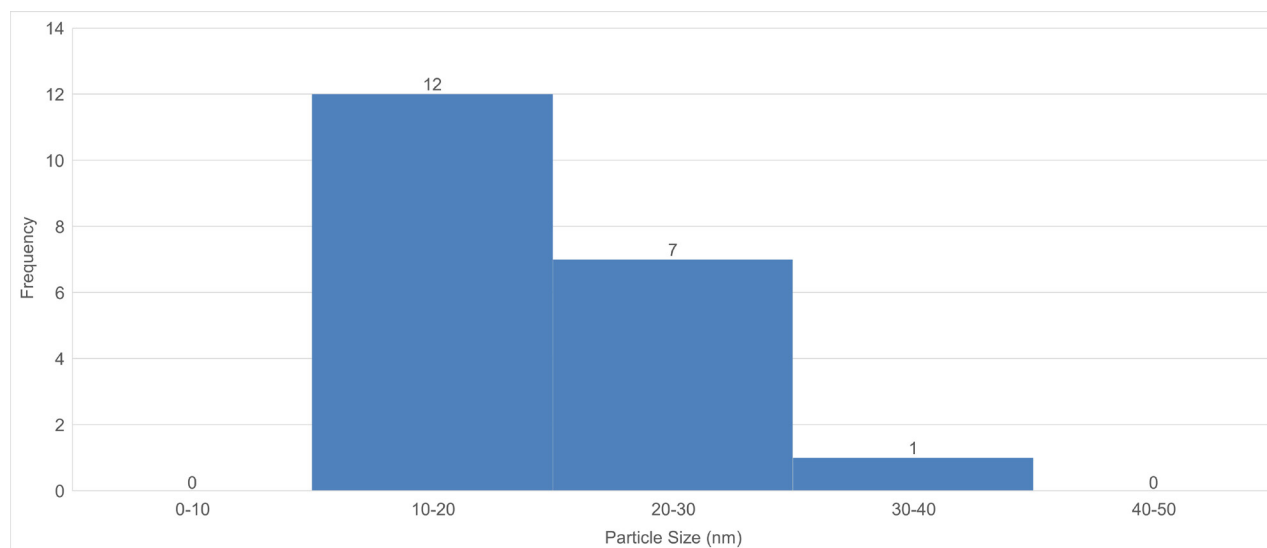


Figure 6: Size distribution of CuNPs. Number of nanoparticles for each particle size within 0–50 nm range (n=20). Sizes determined using TEM image processing and analysis with ImageJ software. The histogram shows a narrow distribution with ± 5.99 nm standard deviation.

and chemical tests were performed on the remainder of the precipitates.

A negative control group was set up with 5 g of copper sulfate dissolved in 80 mL of deionized water. 20 mL of deionized water (instead of plant extracts) and 1 g of starch were added to the control set, placed in a boiling water bath for 1 hour, then capped and placed in a dark cabinet for 36 hours. No plant extracts were added to the control set.

Characterization of CuNPs

Cuprotesmo (CTL Scientific Supply Corp) test papers were utilized to confirm that precipitates contained copper. TEM images were obtained using a Philips CM 120 transmission electron microscope at an accelerating voltage of 100 kV and a point resolution of 0.34 nm. EDS analysis was done using an Oxford INCA EDS system featuring a 10 mm² detector with 140 eV resolution. Once TEM images were returned, images were sized and characterized using ImageJ software. We used analysis functions in ImageJ to measure sphericity of the synthesized CuNPs. Sphericity can be measured both in terms of roundness and circularity. Roundness is a term used for the ratio between major and minor axes of the nanoparticle (29). The value ranges from 0 (straight line) to 1 (perfect circle). Circularity is a similar metric, except that circularity is sensitive to edge irregularities (30).

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