



Impact of Successive Addition of Precursor in Synthesis of Copper and Silver Nanoparticles using the *Tulsi* leaf extract

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ABSTRACT

Investigating the impact of successive introduction of precursor in making of silver-copper bimetallic green nanoparticles utilizing *Tulsi* leaf extract both as a stabilizing and reducing agent. Green synthesis methods using plants and plant-based extract are more beneficial and biodegradable than any other synthesis methods. Compared to other approaches plants generate nanoparticles which are more stable, and scaling them up is very easy. The impact of electronegativity of Ag and Cu metals on the formation and growth of these bimetallic nanoparticles has been studied. Energy Dispersive X-ray analysis (EDX) and scanning electron microscopy (SEM) were used to investigate the biosynthesized Ag-Cu nanoparticles and Cu-Ag nanoparticles.

Keywords: Bimetallic, Electronegativity, Green synthesis nanoparticles.

INTRODUCTION

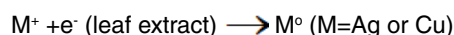
Nanotechnology which deals with materials at the nanoscale. It is used to formulate and engineer devices for many applications. The key components of nanotechnology are nanoparticles. These nanoparticles possess exceptional properties like surface to volume ratio, make nanoparticles chemically more sensitive and at particle size, less than 10nm optical and electronic properties differ due to quantum confinement.¹ Amalgamation of two different metal elements is known as bi-metallic nanoparticle which exhibit better properties and can

be applied for innovative applications. Bimetallic are divided into types depending on how their atoms are organized, such as alloy, core-shell, intermetallic, and subcluster. The characteristics of a bimetallic nanoparticle depend on the metallic nanoparticles it includes.² In recent years, there has been a growing attention in bimetallic nanoparticles due to their physical structures, chemical, mechanical, thermal, optical, catalytic, and magnetic properties^{3,4,5,6,7}. The bimetallic nanoparticles offer more properties over monometallic nanoparticles^{8,9,10}. The bimetallic nanoparticles find applications in a wide array of fields, including nanomedicine, wastewater



treatment, oil industry, agriculture, biosensors, gas industry, biomedical, drug delivery agents, imaging, food processing^{11,12,13,14,15,16,17}. Furthermore, extensive research has been carried to find the antimicrobial and anticancer efficiency of bimetallic nanoparticles. The conventional methods for synthesizing nanomaterials, like physical or chemical methods, tend to be expensive, labor-intensive, slow, environmentally hazardous, pose risks to human health. Hence, there is a need for environmentally friendly, efficient, clean, reliable, simple to scale up and inexpensive synthesis technique that can control these drawbacks. The natural synthesis method also the 'green synthesis method,' emerges as a substitute for synthesizing nanomaterials. In this method the nanoparticles are synthesized using natural sources like plants or microorganisms, resulting in an end product that is environmentally friendly as well as biocompatible. Numerous types of nanoparticles, ranging from mono to quad metallic nanoparticles, have been successfully synthesized utilizing plant extracts. Nanoparticles produced through this approach are commonly referred to as biogenic nanoparticles or nanomaterials³. In the biosynthesis of bimetallic nanoparticles using plant-based extract, is affected by several factors including the process kinetics, adsorption kinetics, the pH of the plant extract, length of incubation, temperature, the concentrations of both the plant extract and metal salts. During the synthesis method these factors play a significant role in determining the magnitude and morphology of the nanoparticles¹⁸. Plant-mediated synthesis presents several advantages compared to microbial synthesis like it eliminates the necessity for a bacterial culture, fast process, occurs at room temperature and the plant extracts has various bioactive components serve as both reducing and stabilizing agents, this phenomenon is referred to as nano-phytotechnology. These bioactive components comprise proteins, carbohydrates, vitamins, amino acids, flavonoids, alkaloids, terpenoids, ketones, tannins, aldehydes, amides, polysaccharides, polyphenols, carboxylic acids, and phenolic acids^{19,20}. Copper-silver (Cu-Ag) nanoparticles were synthesized using an aqueous extract derived from date palm tree, synthesis process involved heating at 95°C with continuous stirring for a duration of 1 h resulting in the production of nanoparticles with an approximate size of 26 nanometers²¹. Amaranthus tricolor, another Argo waste, was employed as both a reducing and stabilizing agent in the synthesis of

Ag-Cu bimetallic nanoparticles²². Cu-Ag nanoparticles were synthesized using *Kigelia Africana* fruit as the precursor material, the synthesis technique was managed at a temperature of 120°C under reflux conditions^{20,23}. Aloe vera leaf extract was employed in the synthesis of Ag-Cu nanoparticles directly on cotton fabric, making it suitable for application as a wound dressing material²⁴. The dimensions and shape of the particles are strongly dependent on the sequence in which the precursors are introduced. Herein, discovered the impact of successive precursor introduction on the size dimensions of Ag-Cu nanoparticles using *Tulsi* leaf extract. *Tulsi* or holy basil is "The Queen of Herbs," rich in secondary metabolites like flavonoids, phenols etc. The *Tulsi* leaf extract is incorporated to the metal precursor solution containing the respective metal compounds to initiate the green synthesis of green nanoparticles. The possible reduction mechanism could be as follows.



MATERIALS

For experiments, Silver Nitrate (AgNO_3), Copper (II) Sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), were purchased from, Abids, India. Deionized water was provided the laboratory. 25 g of fresh *Tulsi* leaves were collected from botanical garden at Kondapur, Hyderabad, India.

Experimental procedure

Tulsi leaf extract preparation

25 g of *Tulsi* leaves were washed thrice with distilled water to get rid of undesirable dust particles present on the *Tulsi* leaves. After washing *Tulsi* leaves were air dried and chopped, and were placed in a 250 mL flask containing 100 mL of deionized water. The temperature of the solution was raised to 40°C using magnetic stirrer.

Addition of $\text{CuCO}_3 \cdot 5\text{H}_2\text{O}$ followed by AgNO_3 in *tulsi* leaf extract

In this experiment, a colour change from light green to dark green was observed when Copper nanoparticles were primarily synthesized by introducing 0.01M $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution into a 30 mL of *Tulsi* leaf extract. Using a magnetic stirrer the solution was agitated for one minute. Subsequently, 0.01M silver nitrate solution was

incorporated into the solution containing copper nanoparticles, and the mixture was further heated and stirred. The progression of Cu-Ag nanoparticle growth was then examined using SEM and EDX analysis. The resulting particles were denoted as Cu-Ag nanoparticles.

Addition of AgNO_3 followed by $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in *Tulsi* leaf extract

In this method, we introduced a modification in the sequence of precursor addition. Initially, a solution of 30 mL of *Tulsi* leaf extract was combined with 0.01 M AgNO_3 solution, a variation in the color of the solution from transparent to brown colour was observed indicating generation of silver (Ag^0) nanoparticles from silver ions (Ag^+). Following this, 0.01M copper sulfate solution was incorporated into the solution containing silver nanoparticles and heated for 1 h using a magnetic stirrer. The resultant particles were named as Ag-Cu nanoparticles. Subsequently, SEM and EDX were employed to investigate the characteristics of Ag-Cu particles.

Characterization

SEM

Scanning Electron Microscopy plays a crucial role, while analyzing the green nanoparticles. Scanning Electron Microscopy is an advanced microscopy technique that employs a beam of electrons to scan the surface of a specimen. It provides highly magnified, three-dimensional pictures, allowing researchers to study the morphology, size, and dispersal of nanoparticles.

EDX

EDX is a multipurpose tool in nanoparticle synthesis and characterization, providing essential information about the elemental composition and distribution within materials. It is instrumental in verifying the success of synthesis processes and ensuring the desired properties of nanoparticles for various applications.

RESULTS AND DISCUSSION

Possible Bio-reduction mechanism for synthesis of the green nanoparticles

There are three basic stages in the bio-reduction mechanism of metal nanoparticles in plants and plant extracts²⁵. The first stage is activation, during which cations in the metal salt

are reduced into metal atoms and are nucleated. The two stages are the growth stage, where metals atoms move arbitrarily and colloid with cations, results in the creation of clusters. At this stage, starting concentration of precursors is controlled because nuclei generation must grow quicker and slower progress of nuclei. The nucleus is formed through continuous collision of clusters, atoms and cations, and the termination stage, nucleus grows to a critical size and lastly formation of nanoparticle.

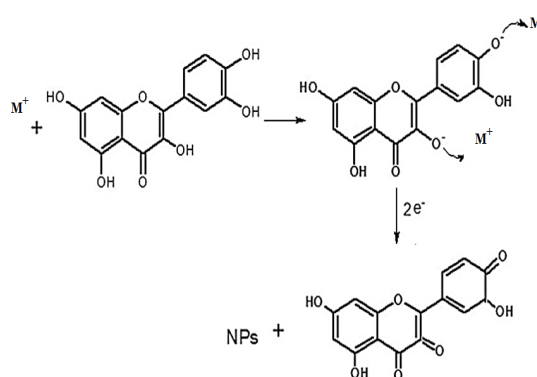


Fig. 1. Formation of Green Nanoparticles

From Fig. 1²⁶ when metal salts are dissolved in distilled water, splits into metal ions. These metal ions interact with the *Tulsi* leaf extract which contains high number of flavonoids and a charge transfer occurs between metal ion and flavonoids results in the formation of capped nanoparticles.

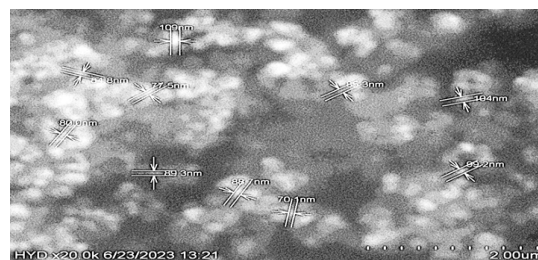


Fig. 2. SEM image of green synthesized Copper-Silver nanoparticles with average size of 70nm-100nm

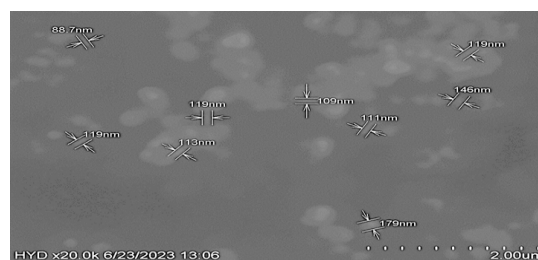


Fig. 3. SEM image of green synthesized Silver-Copper nanoparticles with average size of 130nm

SEM image Fig.2, the observed nanoparticles demonstrate a size range of approximately 70-100nm. Furthermore, SEM image Fig.3 provides additional confirmation of the presence of larger Ag-Cu particles, measuring around 90-150nm. This designates the importance of precursor addition sequence on the formation of green nanoparticles. The distinction between Ag-Cu nanoparticles and Cu-Ag nanoparticles can be attributed to the process kinetics employed in the synthesis procedures. In the synthesis procedure of Cu-Ag nanoparticles, silver nitrate reduces to silver nanoparticles not only due to bio components of Tulsi leaf extract but also experience reduction due to galvanic displacement reaction of copper ions. This implies that, Cu actively helps not only in the reduction of Ag, and but also in the nucleation. Conversely, in the synthesis of Ag-Cu nanoparticles, the Ag nanoparticles formed do not

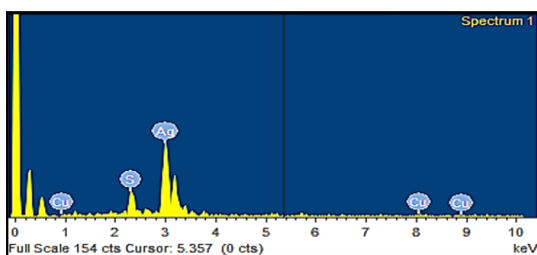


Fig. 4. EDX image of CuAg Nanoparticles

CONCLUSION

Using *Tulsi* leaf extract as reducing agent, we investigated the impact of successive introduction of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and AgNO_3 as precursors. From the reports, it was found that due to electronegativity of the two metallic elements the size of final particles formed depends on the order of introduction of precursor in the synthesis of green nanoparticles. This green synthesis method can be applied to synthesize other bimetallic nanoparticles. Further studies are required to test the anti-bacterial effect

participate in the reduction of Cu ions and do not contribute in nucleation process. Subsequently, the particles formed in this scenario are larger in size compared to those in the Cu-Ag synthesis. These conclusions emphasize the critical role of precursor addition sequence in nanoparticle synthesis procedure. Also, this agrees with the previously published study results²⁷.

EDX

The EDX examination substantiates that the Cu-Ag nanoparticles from Fig. 4 consist of 43.94 wt% Ag and 43.95 wt% Cu. Signals Sulphur presences possibly due to the presence of phytochemicals on the surface of Ag-Cu NPs', were observed. From Fig. 5 Ag-Cu nanoparticles consists of 88.38 wt% Ag and 9.51 wt% Cu. The presences of oxygen is attributed to phytochemicals present in the *Tulsi* leaf extract.

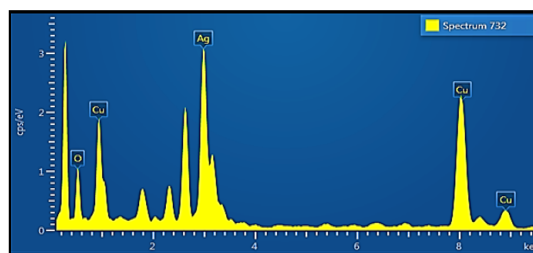


Fig. 5. EDX image of AgCu Nanoparticles

and efficiency of synthesized Silver-Copper and Copper-Silver nanoparticles.

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Conflict of interest

The author declare that we have no conflict of interest.

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