

Ag-CuO Nanoparticles Synthesis Using *Citrus limetta* Peels and Its Applications in Congo Red Removal

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ABSTRACT

In this study, Ag-CuO bimetallic nanoparticles were synthesized from *Citrus limetta* peel extract. Synthesized Ag-CuO nanoparticles were characterized by UV-Visible spectroscopy, FE-SEM, EDS, XRD, and zeta potential analysis. UV-Vis spectrum demonstrated surface plasmon resonance peaks at 340 nm and 282 nm for silver and copper oxide nanoparticles, respectively. FE-SEM micrography indicated a non-spherical, irregular morphology with uniform distribution and apparent surface roughness, and EDS showed the elemental ratio with 97.93% Ag and 2.07% Cu. Synthesized nanoparticles showed potential catalytic performance towards the degradation of Congo red (CR) with NaBH₄ up to 85%. In summary, *Citrus limetta* peel mediated Ag-CuO nanoparticles provide a green, economically feasible, and scalable platform for environmental remediation and antibacterial applications.

Keywords- Ag-CuO, biogenic synthesis, Congo red

I. INTRODUCTION

Nanoparticles are measure between 1 to 100 nanometers and they possess very special characteristics that distinguish them from bulk materials of larger size. High surface-to-volume ratio makes them chemically more reactive and display special optical and quantum effects. These interesting features make them useful in different scientific fields such as materials science, medicine, electronics, renewable energy, and cleaning up the environment [1]. Silver nanoparticles have been commonly reported due to their broad-spectrum antimicrobial activity, their ability to accelerate wound healing, and in anticancer applications. All these activities are mediated by ROS-producing mechanisms, interaction with microbial membranes, and biomolecule-specific interactions with cancer cell molecules [2]. Similarly, copper oxide (CuO) nanoparticles have also gained widespread interest they exhibit good photocatalytic, antibacterial, and environmental remediation applications [3].

In bimetallic nanoparticles, through the combination of two different metallic elements at the nanoscale show synergistic effects that results in higher catalytic capability compared to their single metal counterparts. This improvement is in the form of higher reaction efficiency, lesser catalyst loading, and better cost-effectiveness [4]. Among various approaches used for the synthesis of bimetallic nanoparticles, including thermal fusion, physiochemical reduction, and microemulsions, biogenic synthesis methods are attracting interest due to their eco-friendliness and sustainability. Biological materials like plant extract- or microbial-based routes, facilitate nanoparticle synthesis without the need for hazardous chemicals commonly employed in traditional methodologies [5]. Among these, plant-mediated synthesis is of choice because it is simple, scalable, and has ready access to phytochemically active precursors. Microbial based processes, on the other hand, tend to have demanding growth and maintenance requirements, which makes them less practically applicable. Interestingly, the application of agriculture-based and food processing waste as raw material in

nanoparticle synthesis is a new and eco-friendly practice that solves waste management and material sustainability at the same time [6]. Peels of *Citrus limetta* (also referred to as mosambi) contains phytochemicals which are rich in reducing sugars, polyphenols, flavonoids, saponins, tannins, and cardiac glycosides that may function both as reducing as well as capping agents while synthesizing nanoparticles. In this work *Citrus limetta* peel extract was used for the silver-copper oxide (Ag-CuO) bimetallic nanoparticle synthesis. Further the synthesized nanoparticles were used for dye degradation and antibacterial applications.

II. MATERIALS AND METHODS

Materials

Finely powdered, desiccated peels of *Citrus limetta* (sweet lime or mosambi) functioned as the natural bio-reducing and capping agent for the green synthesis of nanoparticles. Silver nitrate (AgNO_3) and copper sulfate (CuSO_4) were the metal precursors to generate bimetallic nanoparticles. For further studies on the degradation of dyes, analytical-grade Congo red (CR) dye was obtained

from SRL Chemicals. All the metal salt solutions and plant extracts were prepared with deionized distilled water to avoid any contamination risk and maintain experimental accuracy.

Preparation of *Citrus limetta* peels Extract

Citrus limetta (mosambi) peels were obtained from a juice shop and thoroughly washed using deionized distilled water to remove surface impurities. The washed peels underwent oven drying under a heat setting of 70–80 °C until they were completely dehydrated. The desiccated substance was thereafter pulverised with a sterile mortar and pestle and transferred into a sterile, sealed zip-lock plastic bag to avert contamination. In the process of preparing the extract from the plant, an amount of 5 g of the peel powder was added to the sterile glass tube. A total amount of 100 mL of deionized distilled water was then added to this mixture, and it was heated inside a mantle for 15 minutes. The extract was obtained by filtering the mixture using Whatman grade 1 filtration paper once it had cooled to room temperature. After that, the filtrate was stored at 4 °C in cold storage, and the resulting extract was utilised to create nanoparticles [7].

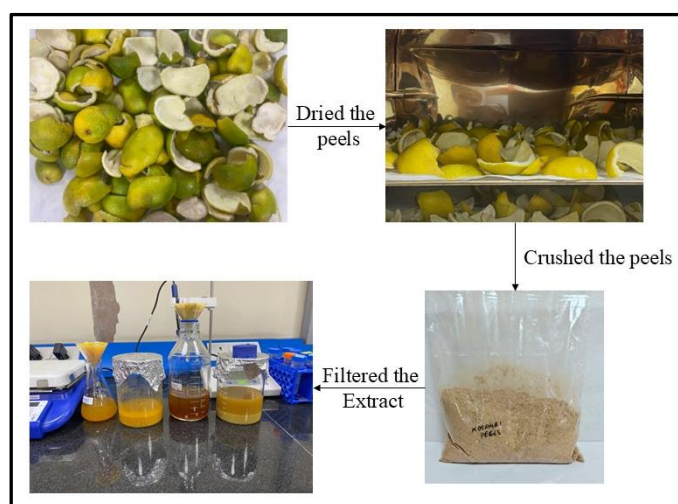


Figure 1: Representation of the synthesis of plant extract

Preparation of Metallic Salts

Double distilled water was used for making aqueous solutions of 5 mm silver nitrate (AgNO_3) and copper sulfate (CuSO_4). Prepared metal salt solutions were stored under sterile conditions at a temperature of 4 °C before being used in the experiment for nanoparticle preparation [8].

Green Synthesis of Ag-CuO Bimetallic Nanoparticles

Bimetallic nanoparticles of Ag-CuO were synthesized by using the technique of co-reduction at the same time. The *Citrus limetta* (mosambi) peel extract was first diluted in a 1:9 ratio with a 5 mm solution of silver nitrate (AgNO_3). This blend was kept at room temperature under constant stirring and then heated at 70–80 °C. Silver nanoparticle formation was observed by the apparent color change from colorless to yellowish-brown. And it

was subsequently mixed with the same volume of 5 mm copper sulfate (CuSO_4) solution along with more of the mosambi extract. The reaction was heated once more for one hour, during which period the solution was changed from bright yellow to dark gray, which indicated the successful synthesis of Ag-CuO bimetallic nanoparticles. Notably, even on prolonged heating, the stability of the black color was maintained, which guaranteed nanoparticle stability. Underwent centrifugation at 10,000 rpm for 5 minutes after which the supernatant was carefully discarded. The pellet was rinsed using deionized-distilled water and then again centrifuged under the same conditions. The desiccated nanoparticles were kept at 70°-80°C. The whole process of synthesis and purification was performed twice to ensure

repeatability. The dried nanoparticles thus obtained were kept in a sealed container for future uses [9].

Ag-CuO Bimetallic Nanoparticle Characterization

The synthesized Ag-CuO bimetallic NPs was validated by several analytical techniques to examine their existence, composition, and stability. “Ultraviolet-Visible spectroscopy (Thermo Fisher Scientific, Genesys 50)” in the wavelength of 200-800 nm was used to observe the characteristic peaks of SP resonances for both AgNPs and CuONPs. The shape and elemental structure of Ag-CuO bimetallic NPs were characterized via FESEM (ZEISS) and EDS, whereas the crystalline phase was observed by means of “X-ray diffraction technique (XRD)” on an EMPYREAN XRD machine in a 2θ range of 20° - 80° . Moreover, colloidal stability of the NPs in different environments was studied by a “particle size analyzer (Litesizer 500)” to estimate the zeta potential of NPs [10].

Catalytic Degradation of Congo Red

The catalytic efficiency of the Ag-CuO catalytic nanomaterials with regard to the breakdown of the dye was determined using Congo red (CR) as the target pollutant. The aqueous mixture of Ag-CuO nanoparticles containing 1 mg mL^{-1} concentration, 10 mM NaBH_4 , and 1 mM CR dye was used in the reaction. Specifically, 1 mL of CR stock solution and 1 mL of NaBH_4 were added to a test tube. A 4 mL combination was then created by adding 2 mL of deionised distilled water. Finally, 1 mL of Ag-CuO solution was added to the remaining 5 mL of mixed volume. The effectiveness of the nanoparticles as catalysts in breaking down the dye was assessed using UV-visible spectroscopy within the wavelength range of 200-800 nm. The results showed a significant decrease in the absorbance maximum of Congo red with the addition of NaBH_4 and Ag-CuO nanoparticles, indicating a high catalytic efficiency in the degradation of Congo red [11].

III. RESULTS AND DISCUSSIONS

UV-Visible Spectrophotometer Analysis

The analysis of UV-Vis spectroscopy was performed initially to characterize the synthesis of Ag-CuO nanoparticles which were prepared using *Citrus limetta* peel extract. Optical absorption spectrum for both the peel extract of *Citrus limetta* as well as the nanoparticles prepared from them has been measured in the region of 200 to 800 nm as shown in Fig. 2. Remarkably, there was notable change in color from the precursor solution to a darker solution due to reduction in the metal ions and formation of nanoparticles. This change in color has been substantiated further with the help of UV-Visible spectroscopic data. Two peaks have been observed at around 282 nm and 340 nm for the Ag-CuO nanoparticles synthesized while there is no presence of such absorption peaks in the precursor solution and even in the extract of *Citrus limetta* peel [12,13].

In this regard, the absorption peak found around 282 nm could be linked to the copper oxide-based electron

transition, while the broader absorption peak obtained around 340 nm might be due to the incorporation of silver in the Ag-CuO bimetallic system. From the broad nature of the absorption peak, it is clear that the synthesis of nanoparticles of heterogeneous morphologies with different sizes and different surface environments has been obtained. This characteristic is commonly observed in the environmentally friendly production of nanoparticles, wherein naturally occurring phytoconstituents serve as stabilizing and reducing agents throughout the metal nanoparticle creation process. The extracts from *Citrus limetta* fruit peels are recognized for containing many phytochemicals, including phenols, flavonoids, tannins, and other active compounds. These phytochemicals may alter the local dielectric environment surrounding the nanoparticles and contribute to the observed absorption characteristics.

The optical behavior observed in the present study may also be influenced by the synergistic interaction between silver and copper oxide components within the bimetallic nanostructure. Charge transfer interactions between the component metals in bimetallic systems can alter the electronic structure and, as a result, the material's absorption characteristics. Furthermore, particle aggregation, surface roughness, crystallinity, and capping by plant-derived biomolecules may contribute to broadening and shifting of absorption bands. Therefore, the observed spectral features are likely the combined result of nanoparticle formation, metal-metal oxide interactions, and phytochemical stabilization. Similar observations have been reported for plant-mediated bimetallic nanomaterials, where the optical characteristics differed from those of the corresponding monometallic nanoparticles due to synergistic effects and surface modifications induced by biological reducing agents. The present findings align with previous research conducted on green synthesised Ag-CuO nanoparticles. The production of biogenic Ag-CuO nanoparticles was accomplished via [14], which were characterized by their UV-visible spectral absorbance features confirming the formation of the bimetallic nanomaterials. According to the authors, the optical behavior depended significantly on the interactions of the silver and copper oxide phases along with the biomolecules present on the surface of the nanoparticles. Similarly, [15] synthesized eco-friendly Ag-CuO nanoparticles and reported distinct absorption bands associated with nanoparticle formation, which were subsequently validated through complementary characterization techniques. These studies support the interpretation that the absorption features observed in the present work are consistent with the successful formation of Ag-CuO nanostructures.

Although UV-Visible spectroscopy provides valuable information regarding nanoparticle formation and optical behavior, it should be considered a preliminary characterization technique rather than a definitive tool for phase identification. Hence, in order to verify the findings of UV-Visible, different analytical

methods like XRD, FE-SEM, EDS, and zeta potential measurement were done. The cumulative result of all these techniques showed that Ag-CuO nanoparticles were successfully prepared using the green method through *Citrus limetta* peel extract. The plant extract exhibited significant efficacy as a reducing and stabilising agent. [16]

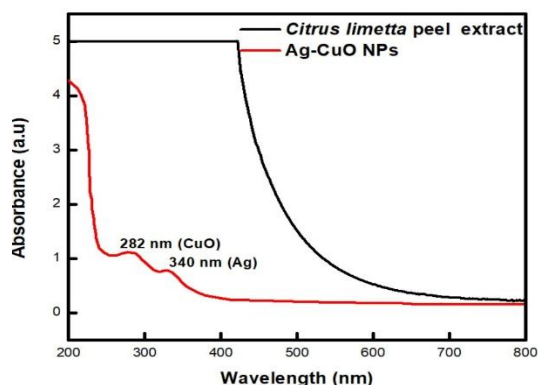


Figure 1: UV Analysis of Ag-CuO Bimetallic NPs

3.2 FE-SEM and EDS Analysis

The technique of FE-SEM was utilized for examining the morphological structure and microstructure of the biogenic Ag-CuO nanoparticles produced using a green process. The FE-SEM images showed the presence of heterogeneously formed nanostructures that were rough in surface morphology and had various structural features randomly distributed in the bulk material. It was noted that the particles were not uniformly spherical but had different structures of various sizes. Morphological heterogeneity in the production of nanomaterials via plant-based synthesis is common due to the presence of various phytochemicals [17]. Furthermore, FE-SEM analysis also revealed that the obtained nanostructures appeared mostly as clustered groups and not entirely as separate and distinct nanoparticles. This might be attributed to the particle attraction because of the Van der Waals forces of attraction as well as adsorption of phytochemicals from *Citrus limetta* fruit peel on nanosurfaces. While aggregation took place in some areas, the overall dispersion of the nanomaterials across the selected surface still occurred efficiently, which indicates that synthesis of Ag-CuO nanomaterials took place successfully. Formation of clustered groups in the form of agglomerates is often observed in green synthesis of metal and metal oxide nanoparticles.

A rough texture and clustering of the formed Ag-CuO nanoparticles are likely to improve the catalytic activity and the antibacterial properties of the particles. Irregular surfaces and clustered structures enable an increase in active sites that facilitate the interaction of the particles with the dye molecules during degradation reactions. In addition, increased interactions with microbial cells through disruption of the cell membrane, ROS production, and resulting cell damage may account

for enhanced antibacterial effects. These findings corroborate previous studies on green-synthesized Ag-CuO nanoparticles, which reported the impact of irregular morphology and particle clustering on catalytic and antibacterial activity. It is essential to perform additional research on the impact of Ag-CuO nanoparticles concerning size measurement based on FE-SEM images [18].

EDS technique was used to determine the composition of the synthesized Ag-CuO nanoparticles. Thus, the presence of Ag and Cu in the sample was established as the main constituent elements. This finding proves that the expected bimetallic Ag-CuO system was synthesized successfully. Quantification of the elements via EDS analysis demonstrated the elemental content of 97.93 wt.% Ag and 2.07 wt.% Cu. Thus, silver was determined to be more prevalent in the synthesized samples than copper. The obtained data is in accordance with the precursor ratio used for the synthesis of the bimetallic nanoparticles. The absence of other peaks in the EDS pattern indicates the purity of the synthesized product. It should be also noted that the EDS results are fully consistent with UV-Visible spectroscopy, XRD analysis, and FE-SEM findings. Namely, the presence of Ag and Cu signals proves the successful fabrication of the nanomaterials. In addition, the proportion between the two metals can affect the properties of the bimetallic systems in various applications.

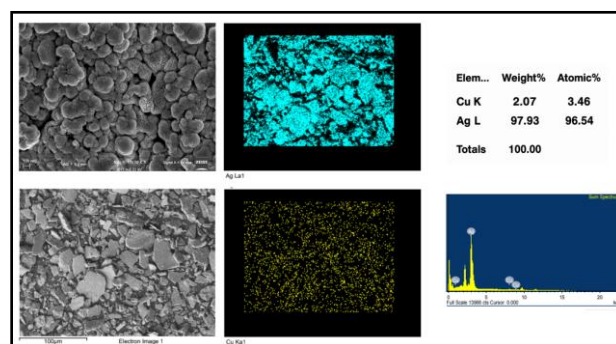


Figure 2: FE-SEM and EDS Analysis of Ag-CuO Bimetallic NPs

XRD Analysis

The crystal structure and phases present in the making of Ag-CuO NPs by green synthesis have been investigated by "X-Ray Diffraction (XRD)", and the XRD spectrum that was obtained has been displayed in Figure 3. It can be seen that diffraction peaks have been observed at $2\theta = 32.36^\circ, 38.26^\circ, 44.43^\circ, 46.35^\circ, 64.61^\circ, 77.55^\circ$, and 81.70° , indicating the crystallinity of the synthesized material. From the sharp and intense diffraction peaks, it could be concluded that the sample had good crystallinity, and nanocrystalline phases had been successfully synthesized. The mean crystallite size estimated from the XRD results by using the Debye-

Scherrer formula was determined to be 23.37 nm, indicating the formation of nanocrystals. The diffracted spots identified at angles 32.36°, 38.26°, and 44.43° were correlated to be associated with Ag crystals (122), (111), and (220), respectively. Out of these diffractions, the one obtained at an angle of 38.26° for Ag (111) showed the greatest intensity value and therefore the most preferred orientation for silver crystals. The high intensity of the Ag (111) plane is evidence that the conversion of Ag⁺ to solid metallic silver has taken place during the course of the phytochemical synthesis reaction. The reflection at an angle of 46.35° was identified as belonging to the Ag₂O (211) plane, showing the potential existence of silver oxide in a small amount. The formation of Ag₂O might have been caused by surface oxidation that may have taken place during the synthesis process, drying, and storage. Surface oxidation often occurs in green-synthesized Ag based nano materials because of their highly reactive surfaces exposed to the air. The average size of AgCuO crystals obtained from Scherrer's equation $D = k\lambda / (\beta \cos\theta)$ is 23.37nm, as indicated in Table 1. K represents the constant (0.94), β denotes the full width at half maximum (FWHM) of the diffracted peaks in radians, and λ signifies the x-ray wavelength (0.154 nm). The particle sizes (D) are in nm [19].

The diffraction peaks located at 64.61°, 77.55°, and 81.70° were indexed to CuO crystalline planes corresponding to (113), (311), and (222), respectively, confirming the successful incorporation of copper oxide into the nanocomposite structure. The presence of these CuO-associated reflections demonstrates that copper existed predominantly in its oxide form rather than as metallic copper. It appears that the occurrence of reflections associated with Ag-, Ag₂O-, and CuO could imply the preparation of an Ag-CuO nanocomposite with multiple phases. It should be mentioned that there could be certain overlaps in some peaks due to the fact that Ag, Ag₂O, and CuO all show peaks in neighboring diffraction regions. Besides, nanocrystalline size, lattice strain, structural defects, and the effect of phytochemicals extracted from *Citrus limetta* peel may result in peak broadening and shifting with respect to those obtained using bulk standards. Likewise, in a similar study on Ag-CuO nanocomposites synthesized by means of plants, synergistic effects observed due to the combination of metallic silver, silver oxide, and copper oxide improved their performance in catalysis and antimicrobial activity. The XRD results were confirmed using EDS analysis, showing that Ag and Cu elements were successfully prepared to form Ag-CuO nanocomposite nanoparticles [20].

Table 1: Crystallite Dimensions for Every Peak in XRD Spectrum and Mean Crystallite Dimensions

Crystal Plane	2θ (degree)	θ	cosθ	FWHM (Degrees)	Radians	D (Crystalline Size)
Ag (122)	32.36	16.17	0.960	0.318	0.0055	27.093
Ag (111)	38.26	19.13	0.944	0.306	0.0053	28.623
Ag (220)	44.43	22.21	0.925	0.433	0.0075	20.676
Ag ₂ O (211)	46.35	23.17	0.919	0.345	0.0060	26.131
CuO (113)	64.61	32.30	0.845	0.464	0.0081	21.134
CuO (311)	77.55	38.77	0.779	0.562	0.0098	18.939
CuO (222)	81.70	40.85	0.756	0.521	0.0091	21.022
Average = 23.37 nm						

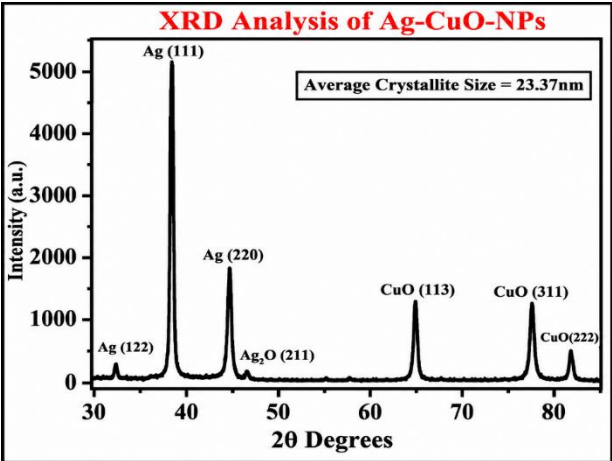


Figure 3: XRD analysis of Ag-CuO NPs

Zeta Analysis

The zeta potential is defined as the electrical potential in the slipping plane of a particle in a colloidal suspension and is one of the major indicators of electrostatic stability in nanoparticle suspensions. Zeta potential is the force of repulsion or attraction of similar charges of neighboring particles. It is directly correlated with colloids' long-term and short-term stability. Large zeta potentials mean high repulsion between particles hence the prevention of agglomeration while lower zeta potentials are the result of less strong forces of repulsion leading to greater probability of agglomeration [21,22]. Litesizer 500 instruments were utilized for the determination of zeta potential in this current study for the Ag-CuO bimetallic nanoparticles. In figure 5, we observed a stable and well dispersed population of

nanoparticles having a negative zeta potential with an average of -11.2 mV peaking at -10.9 mV and a standard deviation of 0 mV. This means that the negative surface charge created would ensure that under normal conditions, there is enough repulsion in electrostatic charges between nanoparticles to prevent agglomeration. Mono metallic silver nanoparticles normally have a zeta potential of -10 mV [23]. On the other hand, copper nanoparticles exhibit properties of around -6.45 [24]. Stability of colloids of Ag-CuO NPs proves particularly useful in applications requiring high colloidal stability, which include catalysis, biosensing, environmental protection, and biomedical applications. The stability of nanoparticles in avoiding flocculation or agglomeration is a definitive sign of their prospective application capabilities [25].

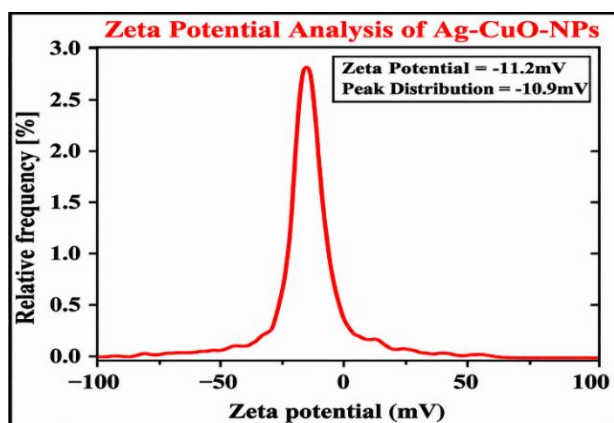


Figure 5: Zeta Potential of Ag-CuO Bimetallic Nanoparticles

Catalytic Degradation of Congo Red

Catalytic degradation of Congo red (CR) was performed through biogenic formation of Ag-CuO NPs, and the entire process of degradation was measured via UV-Vis spectroscopy at varying reaction times (Fig. 6). Congo red displayed an absorption band between 400 –

500 nm, which is characteristic of the azo group that makes up the chromophore giving Congo red its deep color. The addition of Ag-CuO nanoparticles led to a steady reduction in the absorption band intensity over time, indicating effective degradation of Congo red. The degradation percentage after 90 min was determined to be approximately 83 – 85% [26].

The observed gradual reduction of absorbance with increasing reaction time indicates that decomposition of the azo dye takes place continuously. Relatively low reduction in absorbance has been seen at the early stages of the reaction, i.e., from 15 min to 45 min, possibly because of adsorption of dyes on the catalyst and interactions between the catalyst and dyes. But a drastic fall has been noticed from 60 min onwards, indicating that at the later stages of the reaction, faster kinetics of degradation occurs. Such results can be interpreted as fast cleavage of azo bonds by Congo red due to increased availability of surface active sites and electron transfer processes occurring on the surface of Ag-CuO. Such a behavior has also been observed for biogenic metal and metal oxide catalysts, wherein initially a lag phase is followed by fast degradation of dyes due to fast catalytic process on the surface [27–28].

According to the absorbance readings at the specific wavelength, Ag-CuO nanomaterials have successfully exhibited around 83% – 85% degradation in 90 minutes, signifying the strong catalytic ability of Ag-CuO nanomaterials towards dye degradation. The strong degradation performance is due to the interaction of Ag and CuO that allows fast electron exchange reactions. Interestingly, any increase in the duration of the reaction past 90 minutes was found not to yield additional dye degradation. This means that the reaction probably had reached saturation or quasi-equilibrium point, meaning that all the dye molecules that could be broken down were already destroyed, while most of the active catalytic sites were fully occupied. Thus, 90 minutes was taken to be the optimum reaction duration for this particular experiment (Table 2).

Table 2. Previous published studies on dye degradation using various nanoparticles

S no.	Catalyst/system	Synthesis type	Dye degradation/removal	Time	Key comparison
1	AgCuO NPs, present work	Biogenic/green	83 – 85%	90 min	Bimetallic Ag–CuO synergy, green route, good kinetics, $k = 0.0197$
2	Gum-stabilized Au NPs	Green	Catalytic CR reduction; ($k = 0.236$)	Short time	High rate, but uses Au noble metal only [29]
3	MgAl ₂ O ₄ NPs	Sol–gel	Up to 99.27% CR degradation	Optimized conditions	High %, but non-biogenic synthesis route [30]
4	ZnO/CuO nanocomposite	Chemical co-precipitation	95% CR degradation	Noted under optimized conditions	High activity, but chemically synthesized [31]
5	rGO–AgxO	Composite/chemical-	81.9% degradation	—	Present AgCuO gives

	composite	biological			slightly higher degradation, 83–85% [32]
6	Green CuO NPs for MG/MO dyes	Green	~65% degradation for tested dyes	—	Present AgCuO shows higher dye-removal efficiency [33]

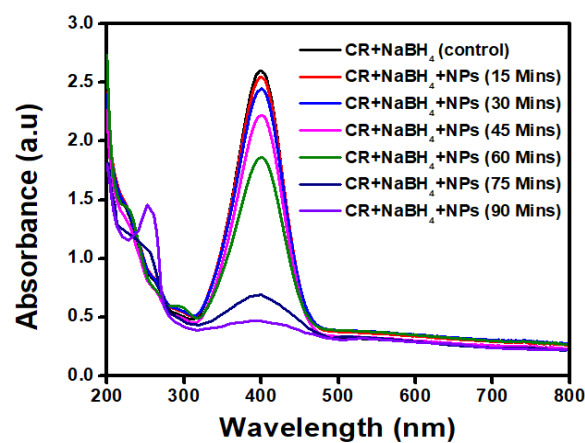


Figure 4: Degradation Percentage of Ag-CuO Bimetallic Nanoparticles

IV. CONCLUSIONS

Citrus limetta peel extract was employed in the present work where it acts as a green stabilizer and reducer for Ag-CuO bimetallic NPs synthesis. Use of peel extract possess non-toxic, cost-effective, and eco-friendly method for nanoparticles synthesis. Phytochemicals present in peels such as flavonoids, tannins, and phenolics played important roles in the reduction of metal ions and capping the formed NPs. Synthesized Ag-CuO nanoparticles with NaBH₄, showed potential catalytic activity in degradation of Congo red, with 85%. The results show a promise of these nanoparticles in wastewater treatment processes, especially for the degradation of toxic organic pollutants. Further research can be done on the development of these bimetallic nanoparticles in other areas like biosensors, anti-cancer therapies, antifungal drugs, and packaging for food preservation. From an environmental standpoint, the present study highlights the importance of integrating agro-waste valorization with sustainable nanomaterial development.

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