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# Catalytic degradation of Methyl Orange and anti-bacterial activity of biosynthesized gold nanoparticles

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**ABSTRACT**

The study reports an investigation of the microwave-assisted synthesis of gold nanoparticles (GNPs) using neem gum. The neem gum acts as both reducing and anti-agglomeration agents. The formation of GNPs was identified by instrumental analysis consists of UV-Vis spectrophotometry, Fourier-transform infrared (FTIR) spectrophotometry, X-ray diffraction (XRD), and transmission electron microscopy (TEM). The synthesized GNPs were found to be crystalline and spherical with an average size of 12±2 nm. These GNPs were assessed for the catalytic reduction of Methyl Orange (MO) and anti-bacterial activity. It was established that the reduction reaction follows the pseudo-first order kinetics with a reaction rate constant of 0.212 $min^{-1}$.

## INTRODUCTION

Nowadays nanoscience is a burning field for the researchers. Nanoscience deals with the nanoparticles having a size of 1-100 nm in one dimension are being significantly used in chemistry, medicine, physics, and all other known fields [1]. Nanoparticles are used immensely due to its physical properties, small size and orientation. Among the several metal nanoparticles, gold nanoparticles show distinguished surface plasmon resonance (SPR) absorption properties which are strongly related to their size, shape and interparticle distance [2]. Gold nanoparticles are of great interest owing to their application in imaging, optoelectronics, electrochemistry, chemical and biological sensing, catalysis, and as non-toxic carriers for drug and gene delivery applications [3-6].

Gold nanoparticles can be easily prepared by different physical, chemical, and biological approaches. Among the several synthesis methods, biosynthesis of GNPs has received increased attention to develop environmentally benign technologies as it has several advantages such as simplicity, cost-effectiveness, and biocompatibility [7]. These biomolecules act as reducing and capping agents in the large-scale commercial production of GNPs. Several biomolecules such as leaf extract, natural gums and fruit extract can actively reduce and stabilize GNPs in an eco-friendly manner [8, 9]. Neem gum is obtained from the trees of Azadirachta indica (family: Meliaceae). It is composed of arabinose, xylose, glucosamine, mannose, fucose, galactose, and glucose. Neem gum is used as a stabilizer, thickener and emulsifier. Due to its non-toxic and biocompatible properties, Neem gum is widely used in food and pharmaceutical industries [10, 11].

In the present investigation, we report a facile, simple and fast method for the synthesis of GNPs using a natural polymer Neem gum (without any additional reducing and stabilizing agents) and the synthesized nanoparticles were characterized by using UV-Vis, FTIR, XRD and TEM and the green synthesized GNPs were showed good catalytic activity for the reduction of MO.

## MATERIALS AND METHODS

### Materials

The aqueous solution was prepared with double-distilled water. Chloroauric acid ($HAuCl_4 3H_2O$) was purchased from Merck, India. All other chemicals used in the present experiment were of analytical grade.

### Synthesis of GNPs

In order to synthesize neem gum capped GNPs, 2ml of 1mM $HAuCl_4.3H_2O$ and 6 ml of gum solution were taken in a boiling

tube. The mixture was then heated in a microwave oven for complete bio reduction at a power of 300W for 4 mins. A commercial MW oven with a 2.45 GHz frequency was used. The resulting red colour of the solution indicated the formation of the gold nanoparticles.

### Characterization of GNPs

Synthesized GNPs were characterized by using various techniques such as UV-Vis spectroscopy (UV-3600, Shimadzu), FTIR spectra (IR Affinity-1), X-ray diffraction ((Rigaku, Miniflex) and Transmission electron microscopy (JEOL 2000 FX-II TEM).

### Catalytic reduction of MO dye

In typical reduction reactions, the reduction of MO was carried out in the presence of $NaBH_4$ and GNPs used as a catalyst. In this procedure, 3 mL of 1 mM MO solution was mixed with 1 ml of 9 mM $NaBH_4$ and the reaction mixture was made up to 10 mL using DD water and stirred for 5 min. 4 ml of these mixtures were taken in a cuvette, sufficient quantities of GNPs were added and UV-Vis spectra were recorded at different time intervals[11] and the results were analyzed.

### Test for anti-bacterial activity

The synthesized nanoparticles were tested for their anti-bacterial properties against gram-positive and gram-negative bacteria's i.e. respectively using disc diffusion method [12]. The zone of inhibition (ZOI) that appeared around the disc was measured and recorded for the evaluation of antibacterial activity of neem gum capped AuNPs. Luria-Bertani agar medium was sterilized by autoclaving at a pressure of 15 psi and 120$^0$C temperature for 30 mins. Then the medium was transferred to sterilized Petri dishes and allowed for solidification. After solidification of media, overnight cultures of *E. coli* and *S. aureus* were evenly spread over the surface of the agar media. The sterilized discs were placed on the inoculated plates. The sample solutions were added on the discs and incubated at 37$^0$C for 24 hours in the incubator. After 24 hours, the inoculated plates were observed for the evolution of the antibacterial activity of the compounds.

## RESULTS

### UV-Vis spectroscopy

UV-Vis spectroscopy is a simple and sensitive technique for the characterization of GNPs due to its extension to SPR. The formation of GNPs was optimized by using varying concentrations of the gum and $HAuCl_4$. Figure.1 shows the UVVis spectra of the synthesized GNPs with different concentrations of neem gum (0.11 %) with 2 mM $HAuCl_4$. The production of GNPs with different concentrations of $HAuCl_4$ at the fixed concentration of gum was shown in Figure.2.

### FTIR

The active sites on gums involved in the reduction of gold ions and GNPs formation were investigated by using Fourier transform infrared spectroscopy. Figure. 3 demonstrated that the FTIR spectra of neem gum shows the major peaks located at 3401, 2948, 1794, 1685, 1492, and 1107 $cm^{-1}$. The absorption bands of neem gum capped GNPs were detected at 3210, 2947, 1781, 1627, 1447, 1259 and 1076 $cm^{-1}$.

### XRD

The XRD technique was used to determine and confirm the crystalline structure of the prepared GNPs. XRD pattern of gum capped GNPs were shown in Figure 4. GNPs exhibited four well-defined peaks at 2θ = 38.8, 44.1, 64.8, and 77.2.

### TEM

The shape, size and surface morphology of prepared GNPs were analyzed by TEM (Figure.5). To obtain size distributions of GNPs, approximately 58 particles were counted and then converted into histograms. Figure 6 presents a histogram of the particle size distribution of GNPs. Most of the particles were in the size around 12 nm.

### Catalytic reduction of MO dye

The catalytic reduction of MO dye by $NaBH_4$ was chosen to evaluate the performance of the synthesized GNPs as a catalyst.

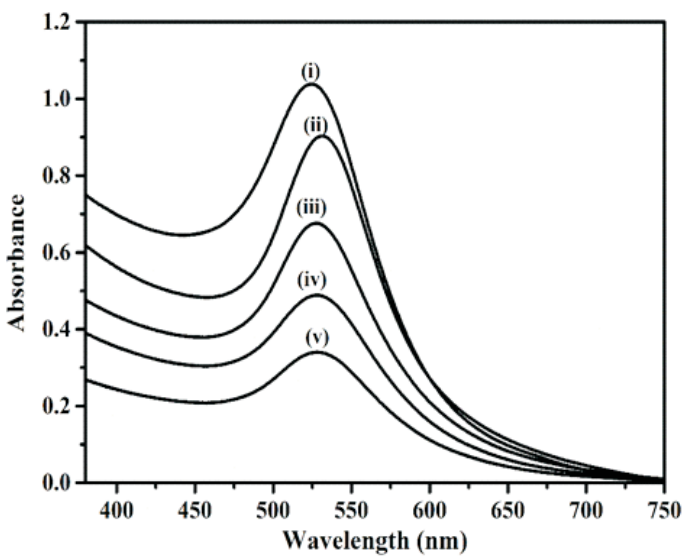


**Fig. 1 :** Absorption spectra of GNPs synthesized at different neem gum concentrations (i-1%, ii-0.75%, iii: 0.5%, iv-0.25% and v-0.1%)

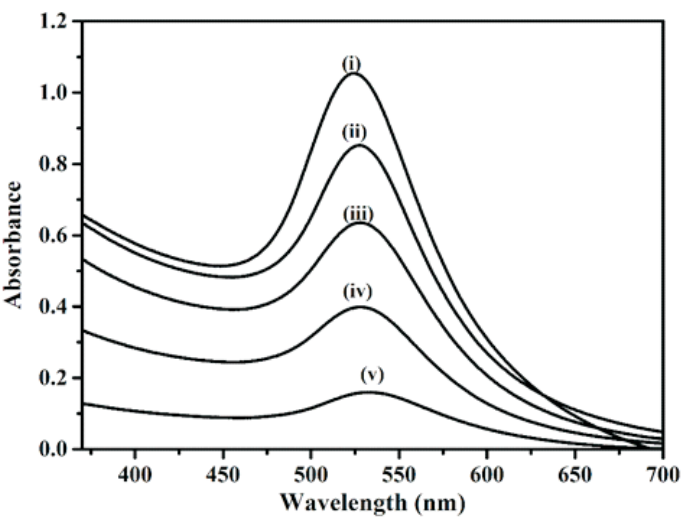


**Fig. 2 :** Different concentrations of $HAuCl_4$ (i-1mM, ii-0.75 mM, iii: 0.5 mM, iv-0.25 mM, v-0.1 mM).

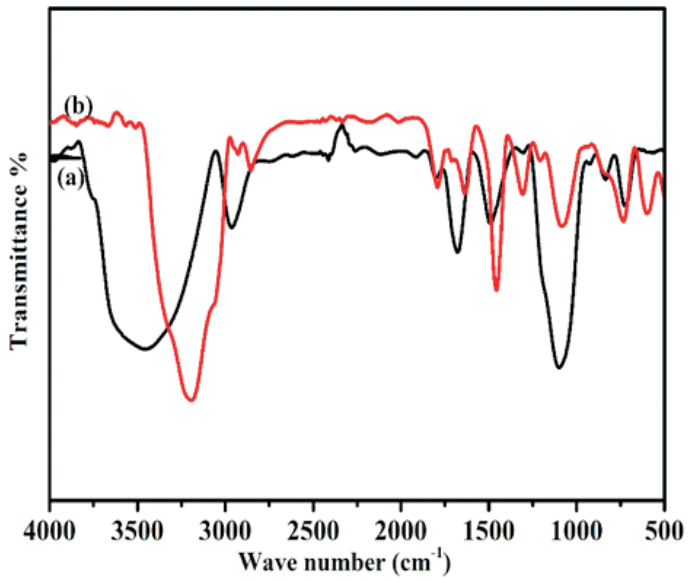


**Fig. 3 :** FTIR spectra of the Neem gum (a) and Neem gum capped GNPs (b)

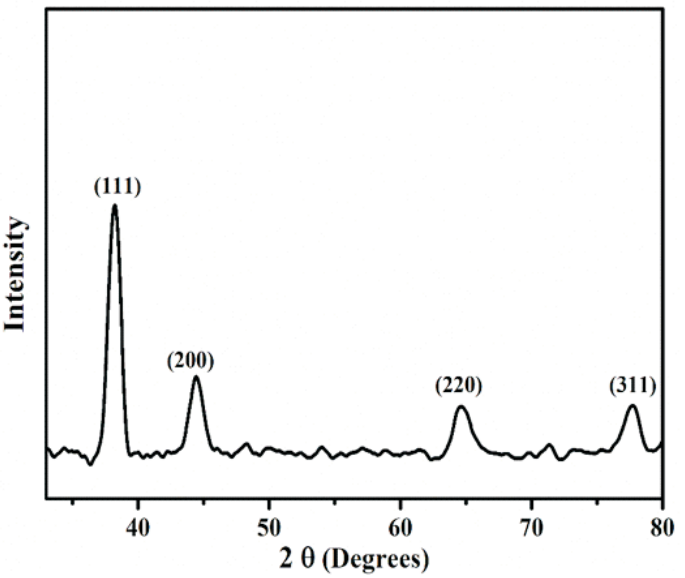


**Fig. 4 :** XRD pattern of synthesized GNPs.

The reaction progress was monitored by UVVis spectroscopy. In aqueous medium, MO shows the absorption peak at 462 nm [15]. Figure. 7 shows the UV-Vis spectra of MO with $NaBH_4$ recorded in the absence of GNPs for a period of 120 min at room temperature. When the GNPs were added to the mixture of MO and $NaBH_4$, the absorption intensity of MO rapidly decreased at absorption peak at 452 nm in the presence of GNPs. The reduction reaction was completed within 12 min. Figure. 8 shows a continuous decrease of MO absorption peak at 462 nm by increasing time. The rate constant (k) was determined from the linear plot of ln ($A_0/A_t$) versus reduction time (Figure.9). The rate constant was found to be 0.212 $min^{-1}$.

### Anti-bacterial activity

In the present investigation, the anti-bacterial activity of neem gum capped AuNPs and neem gum alone were analysed by disc diffusion method using Gram-positive and negative strains of

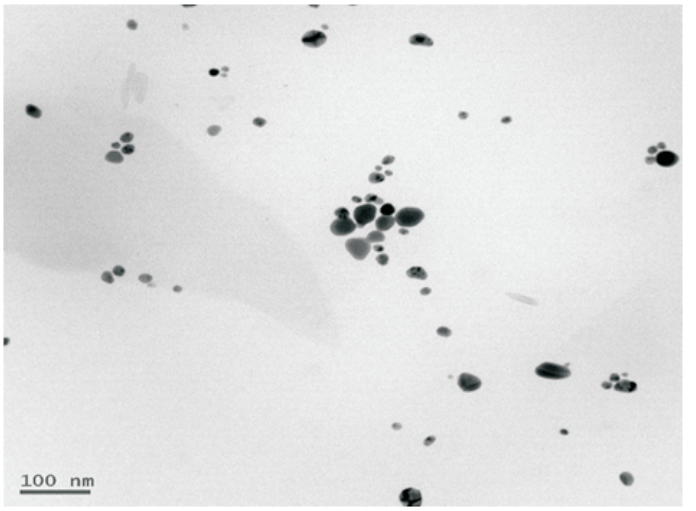


**Fig. 5 :** TEM images of synthesized GNPs

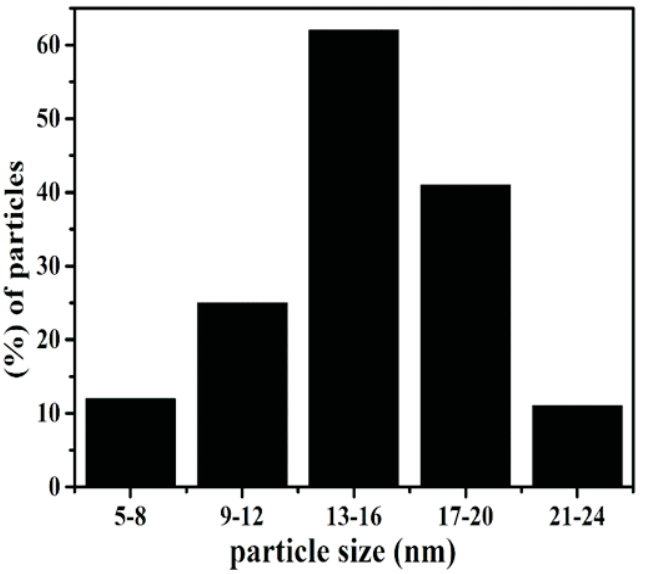


**Fig. 6 :** Particle size distribution histogram of GNPs

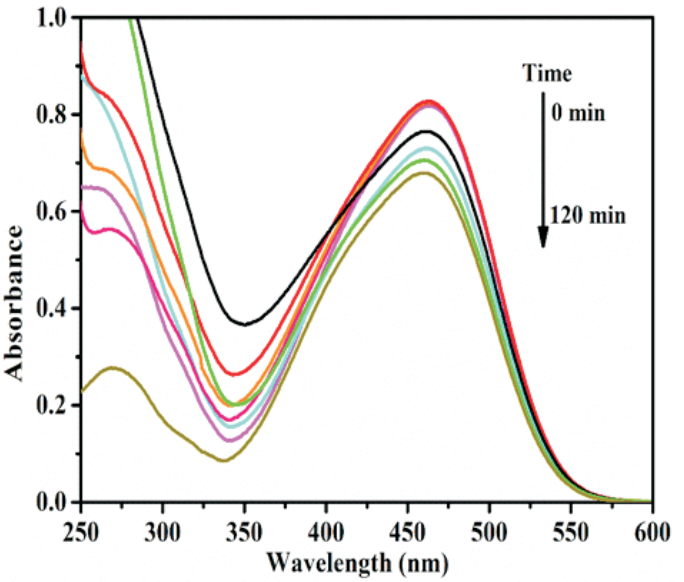


**Fig. 7 :** Reduction of MO dye in the presence of $NaBH_4$ and absence of GNPs

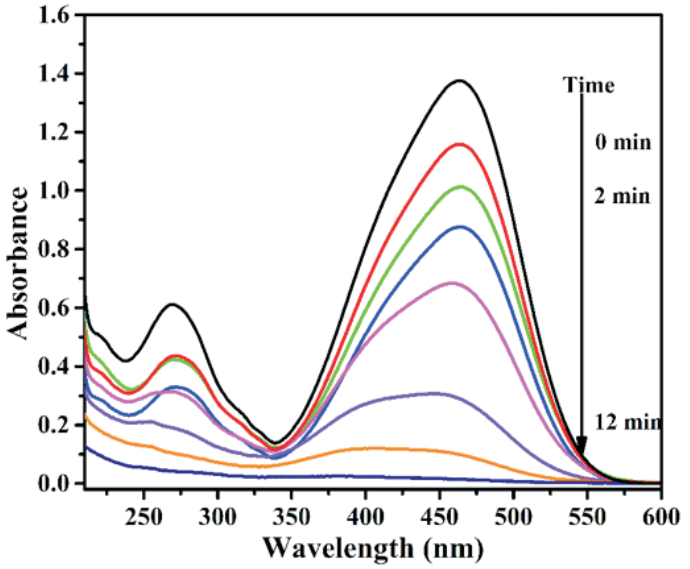


**Fig. 8 :** Time-dependent UVVis spectra for the catalytic reduction of MO by $NaBH_4$ in the presence of GNPs

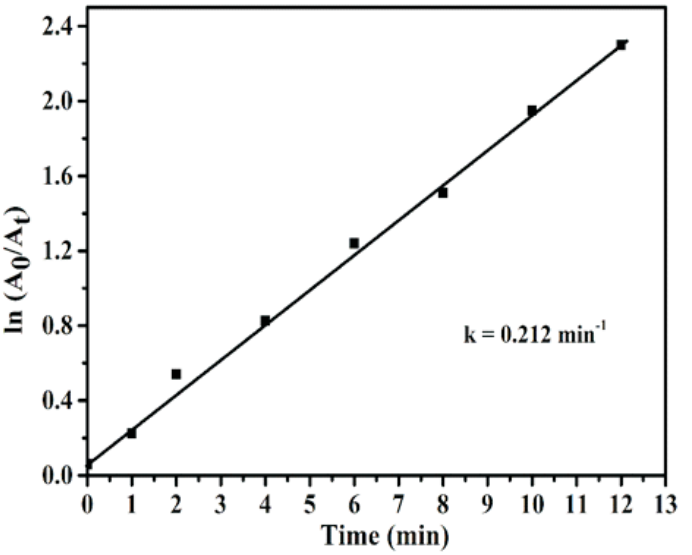


**Fig. 9 :** The plot of $\ln(A_0/A_t)$ versus time for the reduction of MO

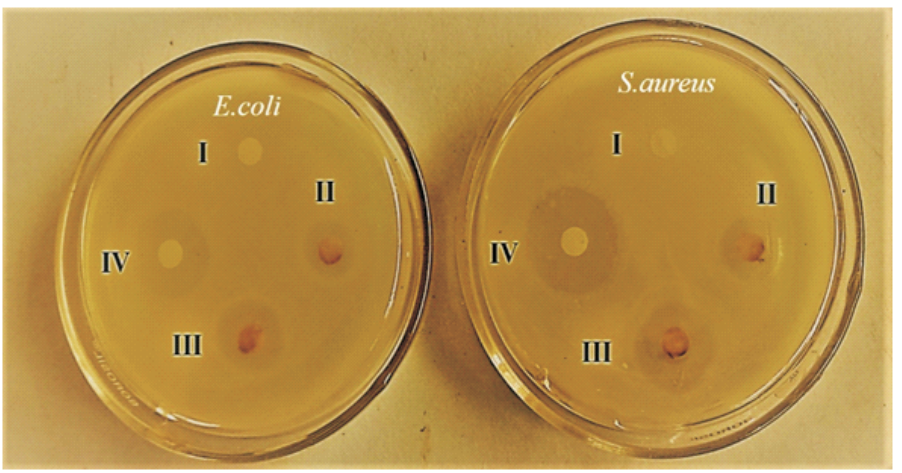


**Fig. 10 :** Anti-bacterial activity of AuNPs against E.coli and S.aureus after 24 hours of incubation. I-IV represents 10 mg of neem gum, 5 mg of ampicillin, 5 mg of neem GNPs and 10 mg of neem GNPs solutions.

bacteria's such as *E. coli* and *S. aureus* respectively (Figure.10) and the diameter of zonal inhibition values was recorded. The Zone of inhibition appeared around the disc was construed as a measure of the effectiveness of neem gum capped AuNPs. The inhibition zone (about 14 mm diameter) for 10 mg/ml of neem gum capped AuNPs, and the inhibition zone (about10 mm diameter) for 5 mg/ml neem gum capped AuNPs was observed for both Gram + ve and Gram -ve bacterias.

## DISCUSSION

The characterization of GNPs by UV-Vis spectroscopy clearly indicates that the formation of nanoparticles was increased with an increase in gum concentration and it is also observed that the amount of nanoparticles formation increases with an increase in the concentration of $HAuCl_4$. These findings are in accordance with the previous results [13]. Fourtier transform infrared spectroscopy showed that the peaks were shifted from 3401 to 3210, 1794 to 1781, 1685 to 1627 and 1492 to 1447 $cm^{-1}$ when compared to that of in neem gum and other peaks were found to be unchanged (Figure.3). The obtained results indicate that hydroxyl groups and the carboxyl groups were involved in the synthesis and stabilization of GNPs [14]. In the XRD, all the four peaks correspond to standard Bragg reflections for (111), (200), (220), and (311) planes of the face centred cubic crystal structure of metallic gold. The existence of diffraction peaks was matched to the standard data files (JCPDS card No. 04-0784) for all reflections.TEM results indicates that the prepared GNPs were found to be spherical in shape and well dispersed in gum matrix and an average particle size was 12 nm.

In the present results, a continuous decrease of MO absorption peak at 462 nm by increasing time indicates that the dye has been degraded slowly. Hence, the catalytic degradation of methyl orange followed the pseudo-first-order kinetics. The obtained results explicated that the zone of inhibition increases with an increase in the concentration of GNPs. But, the zone of inhibition in gram-positive bacteria is observed more than the gram-negative bacteria because gram-negative bacteria consist thick cell wall when compare with gram-positive bacteria. The pure neem gum (negative control) was shown no inhibition zone. The results corroborates with the earlier reports [17, 18]. Hence, the results clearly indicate that the neem gum capped AuNPs possess

potent antibacterial activity.

## CONCLUSION

The study reports the green aqueous synthesis of spherical gold nanoparticles having an average diameter of 12±2 nm using neem gum. The neem gum acts as both reducing and anti-agglomeration agent. The XRD pattern showed that the synthesized GNPs were essentially crystalline. It is found that both hydroxyl and carbonyl groups of neem gum are involved in the synthesis and stabilization of GNPs. The gold nanoparticles have been used for the catalytic degradation of methyl orange in the presence of $NaBH_4$.Thus by utilizing a naturally available material in the ecosystem, a strong and stable catalyst like gold nanoparticles can be synthesized and used for the treatment of wastewater containing toxic dyes and other organic pollutants.

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