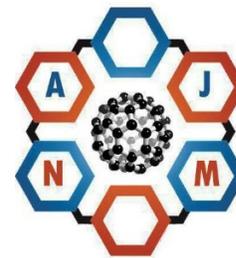




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### Original Research Article

## Optimized synthesis of AgNPs using aqueous extract of celosia argentea and its practical implications on textile dye decolorization

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#### KEYWORDS

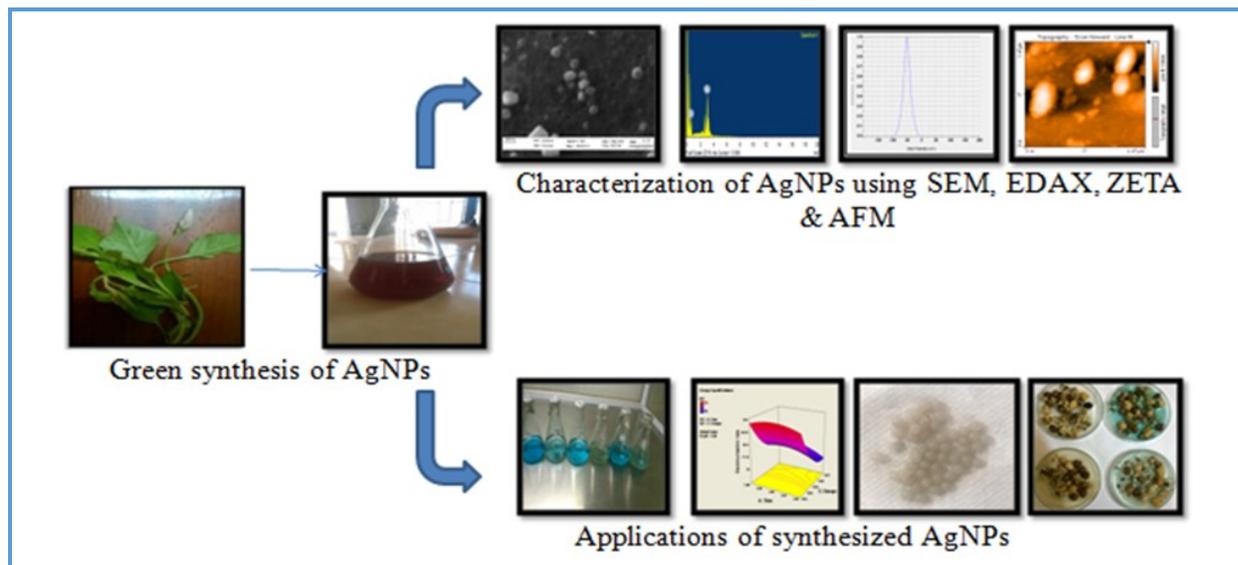
AgNp  
*Celosia argentea*  
Response surface methodology  
SEM-EDX  
Decolourization

#### ABSTRACT

Quickly developing industrialization is one of the significant benefactors of natural contamination as it presents awful difficulties for the earth and the ordinary citizens. Biological synthesis of the silver nanoparticles (AgNPs) at the presence of the aqueous leaf extract of *Celosia argentea*, functioned as the reducing and capping agent. In this research study, we are report the synthesis of the AgNPs with the optimized experimental parameters at the pH. Formation of the AgNPs was confirmed by the UV-Visible spectroscopy and FT-IR analysis. The structural characterization was carried using the SEM-EDX, providing the size of the nanoparticles to be 50-80 nm. The AFM showed the spherical surface topology, the zeta potential whereas, particle size analysis showed stability and the average size was determined to be 50 nm.

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## Graphical Abstract



## Introduction

Rapidly growing industrialization is one of the significant contributors to environmental pollution as it poses dreadful challenges for the environment and the common people [1–4] for example increase in the pollution level of all types of water sources. According to the environmentalists in the coming year's shortage of potable water will have a massive impact on the civilizations worldwide along with India, which is being considered as a water deficiency nation. It is highly important and necessary to create awareness among people about the appropriate use of crisp water and reuse of wastewater, which was generated through various household activities, agricultural practices and, industries. Among various industries, food, paper and pulp, leather industries are primary industries, which cause water pollution [5].

In India, a vast number of leather industries are spread over the South Indian states ranging from Tamil Nadu and Andhra Pradesh. In these industries, dyes used are different for example, reactive dyes, oxidative dyes, organic dyes, azo

dyes. Malachite green, a constituent of numerous chemical dyes, is widely used for the color to paper, silk, and leather. It is highly toxic to the mammalian cell lines as it can induce adverse carcinogenic reactions [6]. The dyeing process of any material includes spinning, bleaching, washing and so forth, during these processes colossal measure of synthetic compounds like sulphur, nitrates, cleansers and substantial metals, for example, arsenic, lead, mercury, cobalt alongside non-biodegradable biting the dust substance are used [7]. The majority of the wastewater discharged from these industries does not undergo proper wastewater treatment and causes water pollution and the recalcitrant compounds present in the wastewater lead to various toxic effects to the plants and animals residing in the vicinity. Reports show that the workers working in these industries suffer from common occupational diseases like chronic infections and irritation of the skin and respiratory system e.g. contact dermatitis upon repeated exposure to the reactive dyes even though it is considered to be negligible amount

[8, 9]. Some of them are also reported to potential mutagenic and genotoxic effects [10].

In this work, the biological reduction of the AgNPs was carried out using the aqueous leaf extract of *Celosia argentea*, which acts as a reducing and capping agent. The AgNPs were then subjected to the physicochemical characterization using the UV-visible spectroscopy, XRD, FT-IR, SEM-EDAX, AFM, Zeta potential and particle size analysis. These AgNPs were used for decolorization of the organic dye Malachite green. The process was optimized by varying pH of the dye, and time is taken for completion of the experiment. Response surface methodology was used to identify the specific dose outcome by statistical and accurate outcome, which was later immobilized in calcium-alginate beads.

## Experimental

### *Sample collection*

The leaf samples were collected from senur village, katpadi and Vellore district, Tamil Nadu, India. Leaf samples were washed with running tap water and transported to the laboratory in sterile polyethylene bags. Under sterile conditions, the leaf samples were rinsed thrice with sterile distilled water and were kept in the shade for drying. The dried leaf samples were then crushed using mortar and pestle and stored for further use [6].

### *Synthesis of silver nanoparticles*

The *Celosia argentea* leaf powder was heated with 100 mL of Milli-Q water at 60 °C for 5 min to obtain aqueous leaf extract. The resulting extract was filtered from the filtrate, 10 mL was added to 10 mL of 0.01 M silver nitrate solution and it was maintained at 60 °C for 10 min. An individual control was setup by taking 10 mL *C.*

*argentea* leaf extract without silver nitrate solution [6].

### *Optimization of AgNPs production*

#### *Effect of leaf extract and silver nitrate solution on bioreduction of AgNO<sub>3</sub>*

The ratio of the aqueous leaf extract to silver nitrate was optimized by increasing the volume of the leaf extract (1, 2, and 3 mL) in 1 mL of 0.01 M silver nitrate (ratio 1:1, 2:1, 3:1). After 10 min of heating at 60 °C the absorbance of the solution was measured using the UV-Visible spectrophotometer. Similarly, the ratio of the silver nitrate to leaf extract was optimized with increasing the volume of the silver nitrate solution (1, 2, and 3 mL) in a constant volume of leaf extract (1 mL). The mixture was heated for 10 min at 60 °C and a UV-visible spectrophotometer measured the absorbance of the resultant.

#### *Effect of pH on Bioreduction of AgNO<sub>3</sub>*

The pH of the reaction was optimized by varying pH as 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 by addition of 0.1 N HCl or 0.1 N NaOH according to the requirement. The mixture was heated for 10 min, and the absorbance of the resulting solution was measured using the UV spectrophotometer.

### *Experimental design*

#### *Characterization of silver nanoparticles*

Synthesis of the AgNPs was confirmed visually by a color change from yellow to brown, which was later confirmed by the help of UV-visible spectroscopy from 200-800 nm [11]. The solution was then subjected to 10,000 rpm for 15 min and the pellet was washed and stored for further usage. The AgNPs were characterized by the KBr method and the spectral scanning was

carried out using the FT-IR spectroscopy using Nicolet FT-IT (AVATAR-330) with a narrow band of mercury/cadmium/telluride [12, 13]. The synthesized AgNPs were further subjected to phase identification using X-ray diffraction. Samples were placed in the BRUKER D8 ADVANCE, Germany operated at a voltage of 40 kV, current of 30 mA with Cu K $\alpha$  radiation. The FWHM was used with Scherrer's formula as given below in Equation 1.

$$d = \frac{0.9\lambda}{\beta \cos\theta} \quad (1)$$

Where,  $d$  is the mean diameter of nanoparticles,  $\lambda$  is wavelength of the X-ray source,  $\beta$  is the angular of full width at half maximum at angle  $\theta$  [11]. SEM-EDAX was used to evaluate the AgNPs morphology and the elemental composition of the AgNPs using the ZEISS (EV018) was used for the SEM studies. The average size, stability, and shape morphology were confirmed by zeta potential, particle size analysis and atomic force microscopy [12, 13].

#### *Optimization by response surface methodology (RSM)*

In the present study, the D-optimal form of RSM was used for optimizing the parameters. To evaluate the influence of the reaction parameters on the decolorization, three main factors were selected: (a) reaction time (b) dosage (c) pH, a total of 20 experiments were introduced with Design Expert 7.0.0 [14].

#### *Preparation of Ca-Ag beads and immobilization of AgNPs*

For the preparation of the calcium alginate beads, 4% of sodium alginate was stirred continuously in distilled water [15]. The resultant sodium alginate solution was slowly dropped into a 0.2 M calcium chloride solution and was kept for 2 h in polymerizing solution [16]. Similarly, 4% of AgNPs were added into

the Ca-Ag mixture with continuous stirring for immobilization. The beads were kept for 2 h in a polymerizing solution. Finally, the beads were washed with distilled water and stored at 4 °C. SEM characterization was also carried out for three setups, namely a) distilled water, b) dye solution, c) phosphate buffer was used to check to swell of the beads. Approximately 2 g of beads were added in those three setups for 2 h then the beads were separated; dried with tissue paper and weighed [17] in Equation 2.

$$\text{Swelling percentage} = \frac{W_f - W_i \times 100}{W_i} \quad (2)$$

Where  $W_f$  is the final weight of swollen beads, and  $W_i$  is the initial weight of swollen beads.

#### *Batch decolorization studies*

Decolourisation of the synthetic dye Malachite Green by using AgNPs as a decolorizing agent was studied with setting up the experiment in various batches. The batches were set up in a manner to allow studying the effect of pH of dye solution, the concentration of AgNPs in the solution, and the effect of the time of contact on the decolorization process. To observe the effect of pH of solution on decolorization 0.01 g of AgNPs was added to the 100 mL of 100 ppm of Malachite green solution and the reaction mixture was agitated for 2 h. The pH of the dye solution was varied from 3 to 8. A control experiment was setup where no AgNPs were added to 100 mL of 100 ppm of Malachite Green solution and further was used as a control for all the batch experiments [6]. The effect of particle dosage i.e. effect of concentration of AgNPs, was studied by varying the concentration of particles from 0.01 to 0.05 g/100 mL of the 100 ppm solution of Malachite Green. To study the effect of contact time samples were collected at various time intervals from the reaction mixture and the final

concentration of dye was determined using UV-Visible spectrophotometer at  $\lambda_{\max}$  617 nm. Dye removal percentage was calculated as Equation 3.

$$\text{Percentage of dye decolourization} = \frac{C_i - C_f \times 100}{C_i} \quad (3)$$

Where  $C_i$  is the initial concentration of dye before the study and  $C_f$  is the final concentration of dye after study.

#### *Effect of synthesized AgNPs upon leaf extract to decolorization dye*

To assess the activity of synthesized AgNPs, two separate reactions were carried out [18]. Briefly, 1 mL of the Malachite Green of  $1 \times 10^{-4}$  M was mixed with 0.2 mL of aqueous leaf extract of *Celosia argentea* and 1.8 mL of distilled water and the reaction was monitored after 30 min. In the second reaction, the 1 mL of Malachite Green of  $1 \times 10^{-4}$  M was added to 0.2 mL of aqueous leaf extract of *Celosia argentea* and 1.8 mL of synthesized AgNPs (100 mg/mL) and the reaction was monitored after 30 min. The reactions were carried out in triplicates and were compared to the control dye solution.

#### *Preliminary toxicity study of decolorized dye effluent*

The study carried out on the toxicity of decolorized products by the germination assay method earlier [19]. The decolorized dye solution was separated from silver nanoparticles by a simple centrifugation method at the end of the reaction. Then the seeds were surface sterilized with the sterile distilled water and placed in different sterile Petri plates those containing (a) wet filter paper with distilled water (served as a control for seed germination), (b) AgNPs with distilled water,

(c) dye solution and (d) decolorized dye solution. For five days, 5 mL of corresponding solutions were added to the respective petriplates daily. All the Petri plates were observed for the germination of seeds. Toxicity was calculated based on a number of germinated seeds and plumule length [19, 20]. Seed germination was calculated using the Equation 4.

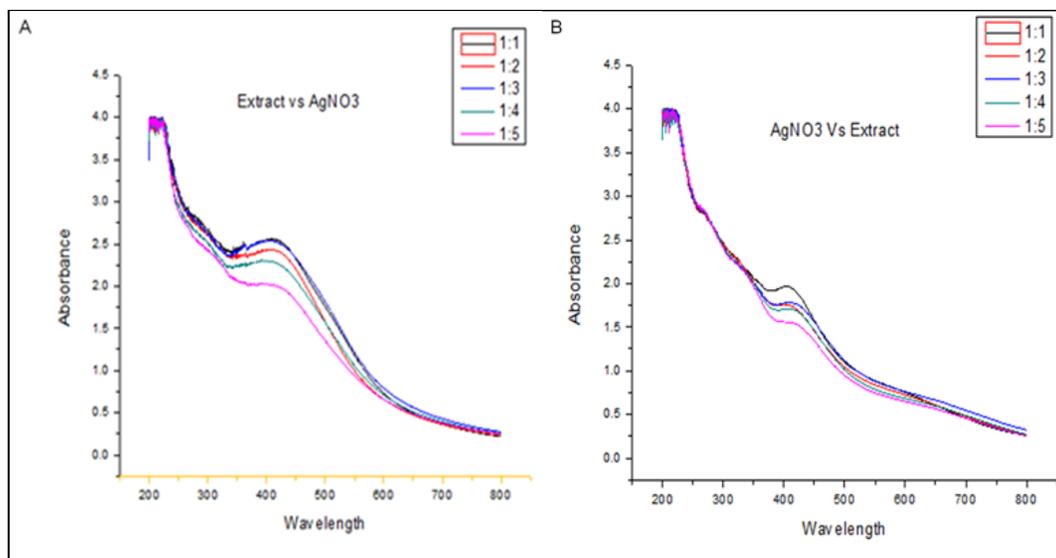
$$\text{Relative germination\%} = \frac{\text{Number of seeds germinated} \times 100}{\text{Number of seed sowed}} \quad (4)$$

## **Results and Discussion**

Many reports have documented color change of the dye solution from yellow to light brown, and after some time, color may further darken to blackish brown [21–24]. The presence of the UV excitation peaks from 400–450 nm was due to the surface plasma resonance interaction of the electromagnetic radiation and the electrons present around the nanoparticles.

#### *Effect of leaf extract ratio and silver nitrate solution ratio on bioreduction of $\text{AgNO}_3$*

Optimization process for the synthesis of the AgNPs was confirmed by UV-Visible spectroscopy. It was observed that, by enhancing the quantity of leaf extract, the absorption increased, shifted the peak from 450 nm to 405 nm. Formation of the spherical and homogenous distribution of the AgNPs was observed with the reduction in the mean diameter of the silver nanoparticles, which was further depicted with the blue shift (Figure 1a, b). These results were consistent with the previous results [25–28]. Aggregation of nanoparticles is dependent on the bioactive compounds present in the leaf extract which act as reducing and capping agents.



**Figure 1.** a) Effect of leaf extract ratio upon silver nitrate (1:1, 1:2, 1:3, 1:4, 1:5) b) effect of silver nitrate ratio upon extract as (1:1, 1:2, 1:3, 1:4, 1:5) at pH 7 and 0.01 mM of  $\text{AgNO}_3$  solution. b). When the ratio of silver nitrate increases aggregation of nanoparticles occurred which is due to lower availability of leaf broth for the reduction of silver ions

#### *Effect of pH on bioreduction of $\text{AgNO}_3$*

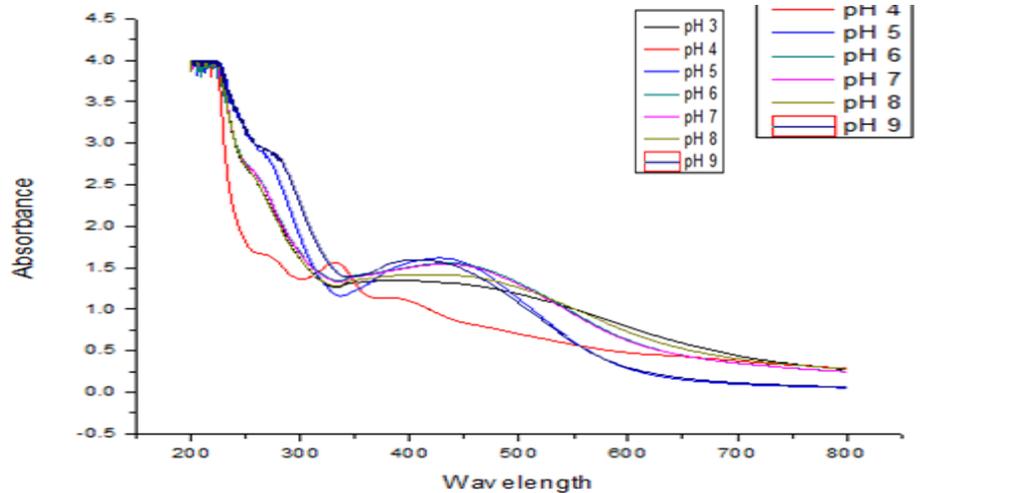
The presence of the specific pH plays a crucial role in the formation of the AgNPs. The pH range was fixed from pH 3 to 9. Figure 2 reveals formation of the AgNPs, which indicated its dependency on the pH of reactions mixture, the absorbance value was found to be increasing as there was a change in pH value from 3 to 9, and the maximum synthesis of AgNPs was observed at basic pH.

#### *Characterization*

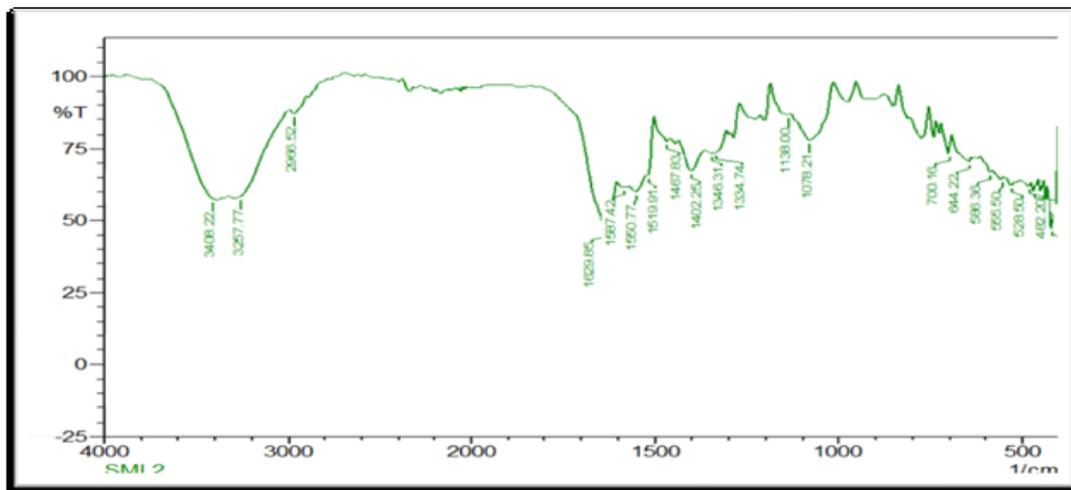
The UV-Visible spectroscopy confirmed the formation of the AgNPs at the maxima 406 nm. Further characterization of the AgNPs was carried out and the FT-IR confirmed the presence of the organic compounds which act as a capping agent for the synthesized AgNPs. These organic compounds were found to be present in the leaf extract (Figure 3). The functional groups may act as a capping agent with strong bands at 3408 and 3257  $\text{cm}^{-1}$  stretching showed the presence of phenols (O-

H group), the band 3257.77  $\text{cm}^{-1}$  showed ester (C=O), the band at 1550.77  $\text{cm}^{-1}$  displayed aromatic compounds (C=C) which were compared with standard FT-IR table. XRD depicted the peak at 38.192° (111) suggesting a strong indication of the AgNPs which highly coincides with the previous reports [29] (Figure 4). The XRD confirmed the crystalline nature of NPs and its lattice constant estimated to be  $\alpha=4.082$ , which was consistent with  $\alpha=4.0862$  Å reported by JCPDS file no 04-0783. The SEM analysis of the AgNPs was found to be spherical (Figure 5) with the absence of the aggregates of the NPs which confirmed the presence capped AgNPs and was similar to earlier reported results [28].

The average particle size of the synthesized AgNPs was found to be 50.3 nm (Figure 6), and the stability of the NPs was found to be at -49.6 mV, which might be due to the presence of the polyphenolic compounds that act as capping agent [30]. The nanoparticles were found to be spherical and monodispersed as evident from the SEM and AFM images (Figure 7).

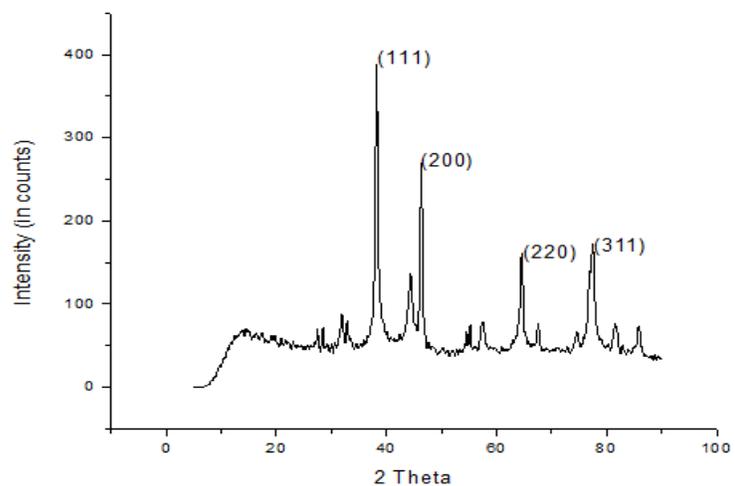


**Figure 2.** Effects of pH on reduction of silver nitrate to silver nanoparticles in the presence of leaf extract



**Figure 3.** Shows the reducing and capping agent compounds present in AgNPs by leaf extract

**Figure 4.** XRD micrograph of synthesized silver nanoparticles



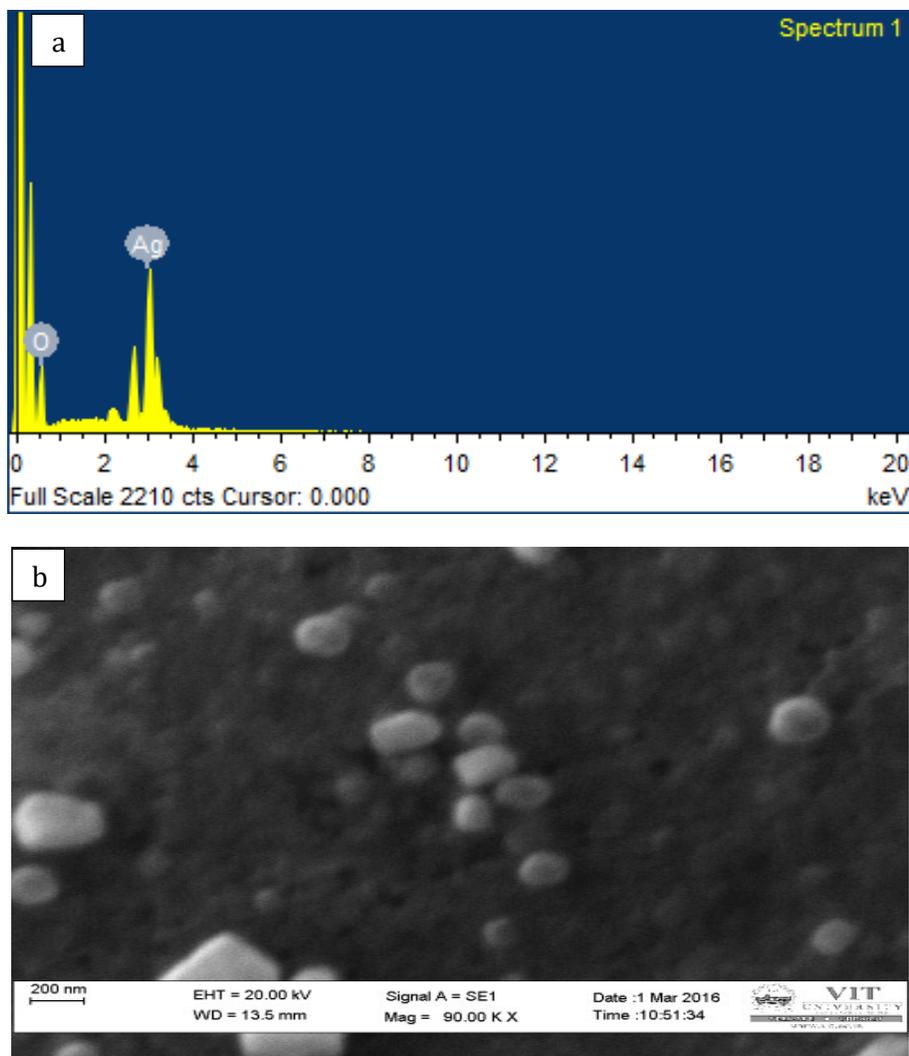


Figure 5. a) SEM and b) EDAX image of the synthesized silver nanoparticles

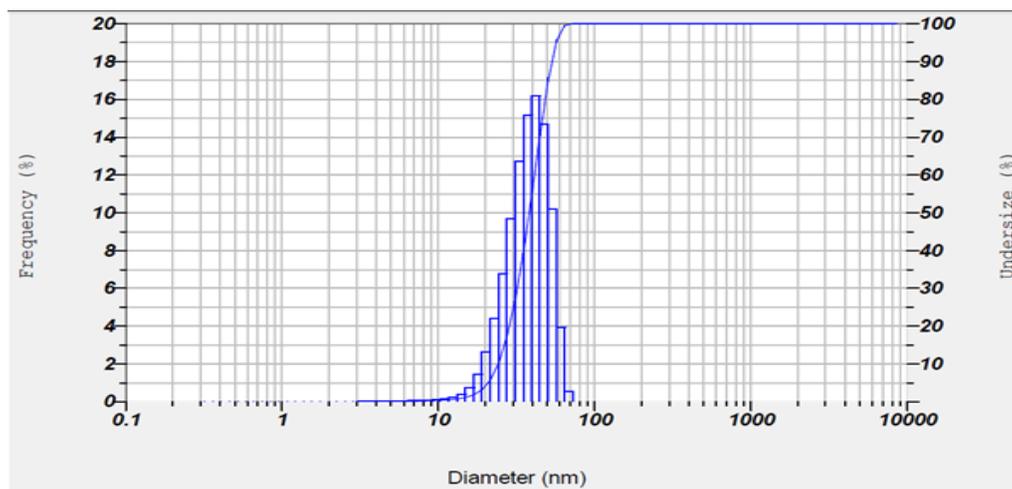
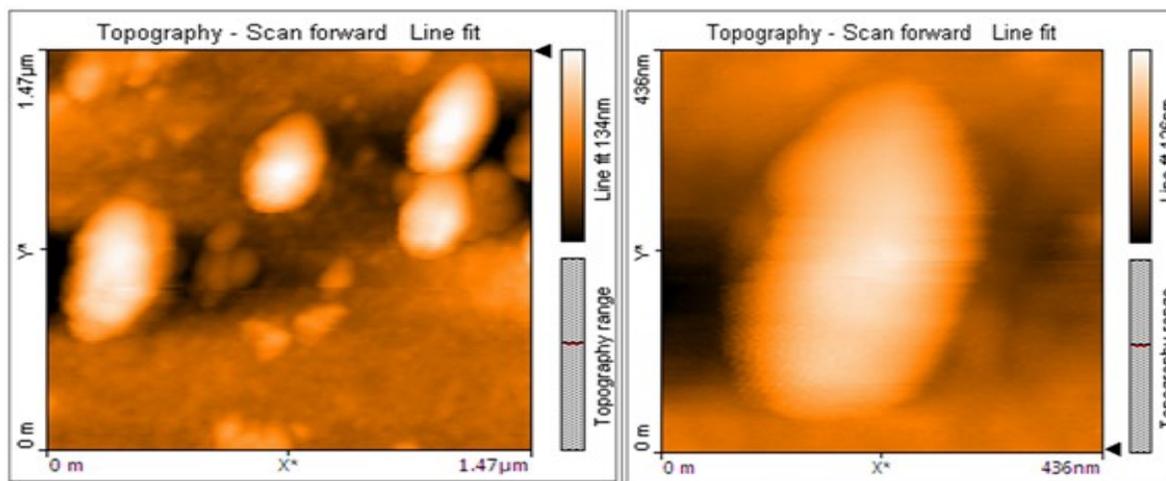


Figure 6. Particle size analysis of the synthesized silver nanoparticles



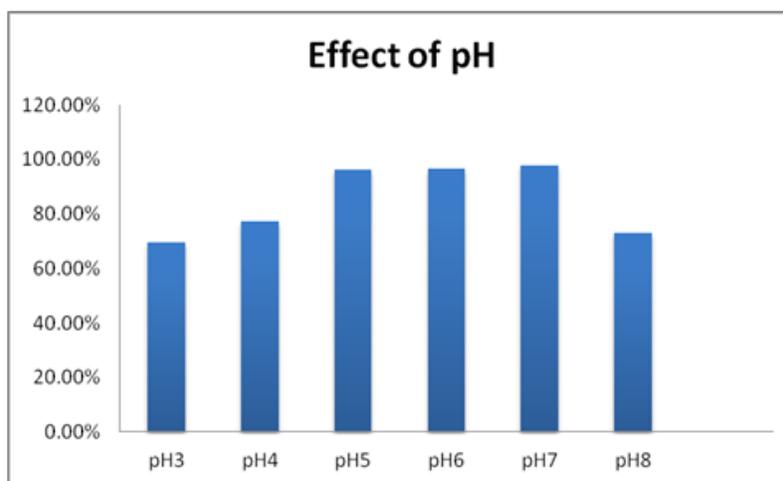
**Figure 7.** AFM analysis of the synthesized silver nanoparticles

#### *Batch decolorization studies*

#### *Effect of pH on decolorization of Malachite green*

The effect of the initial solution of Malachite Green using silver nanoparticles was represented in (Figure 8). It was observed that there was an increase in the percentage of dye removal from 61% to 83% with the increase in pH of reactive dye from 3 to 5. The pH 5 was kept as constant pH value for further studies. The maxima were compared with the control due to the influence of pH adjusting solution in dye absorbance. It has proven that at lower pH

value, the number of positively charged sites on AgNPs was increased. It was found, that the electrostatic interaction of negatively charged dye constituents and binding sites present on AgNPs loaded with activated carbon increase the uptake rate of anions. When there is an increase in the pH of AgNPs loaded with activated carbon, it becomes more negatively charged and thereby resulting in increased electrostatic repulsion between the dye molecules and the surface of particles which further leads to a decrease in uptake of dye anion molecules [31].



**Figure 8.** Effect of pH on decolourization of Malachite Green using AgNPs. pH: 3, 4, 5, 6, 7, 8. Malachite Green Concentration: 10 ppm, Dosage 0.01 g/100 mL

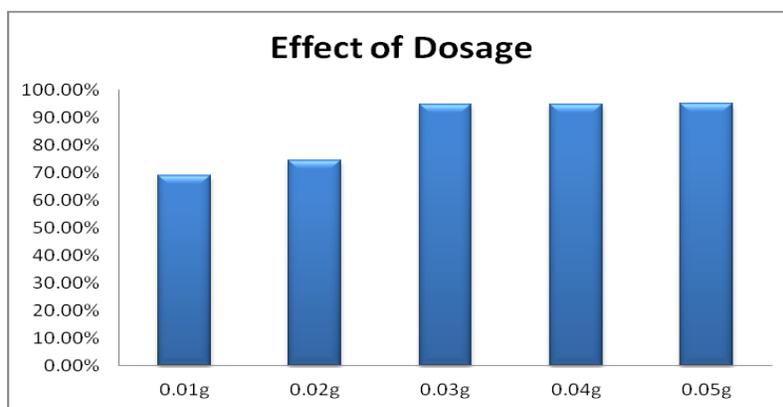
### Effect of nanoparticles dosage on decolorization of Malachite Green dye

The effect of nanoparticle dosage was studied by varying the concentration of nanoparticles in the range from 0.01 g to 0.05 g/100 mL. The amount of dosage is important for the determination of percentage removal of the dye, and also it helps to determine the concentration to be employed for further studies. From the observations of the experiments, it is clear that an increase in decolorization of Malachite green occurred at the increasing amount of nanoparticles and after 0.03 g/100 mL which is comparatively effective to the results obtained by with dosage

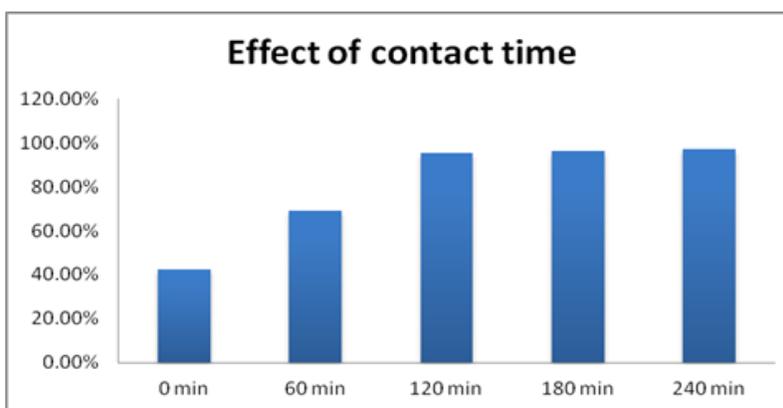
of 0.5 mg/100 mL [10]. The increased rate of decolorization, i.e.95% is due to the increase in the number of available active sites (Figure 9).

### Effect of AgNPs contact time on Malachite Green dye decolorization studies

The effect of the contact time between the AgNPs and Malachite Green dye is the most important strategy that was evaluated from 0 min to 2 min (Figure 10). With the increase in contact time of the AgNPs with Malachite green, the rate of decolorization increases. And, the minimum time, it takes for the reaction of decolorization to start, indicates initiation of desorption process.



**Figure 9.** Effect of nanoparticles dosage on Malachite Green Dye. Ph 5, particle Dosage 0.01 g, 0.02 g, 0.03, 0.04 g and 0.05 g/100 mL



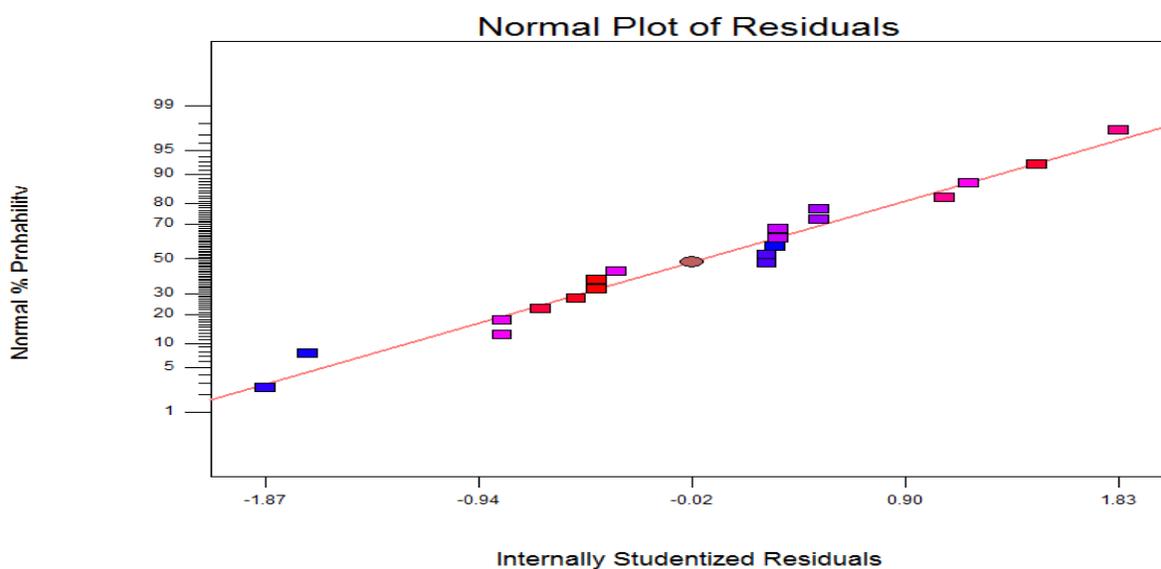
**Figure 10.** Effects of Contact time between AgNPs and Malachite Green dye. pH 5, Dosage: 0.03 g/100 mL, Contact time: 0 min to 2 hr

### Statistical analysis

In statistics, a D-optimal design is an experimental design for developing the quadratic model for varying responses of individual variables. It includes selecting a design, analyzing data using ANOVA, checking for coefficients and confirmation process. Further the data was analyzed for the actual and predicted value to design the model with 20 setups standard order.

### Response Optimization and verification

The optimization and verification showed the combined effectiveness of the given parameters. The curve represented the best outcomes of the reaction keeping two variables as constant in Figure 11 and it varies from one to other [32, 33].



**Figure 11.** Represents the normal response of experimental values by the predicted output

### Checking for the Model

To confirm the integrity of the given experimental design, the regression coefficient analysis was carried out which showed optimal dyedecolorization [34]. It helped to analyze the output of experimental design with 98% precision. The model exposed the relationship between two or more variables are successfully optimized with effective dye decolorization in perturbation curve (Figure 12).

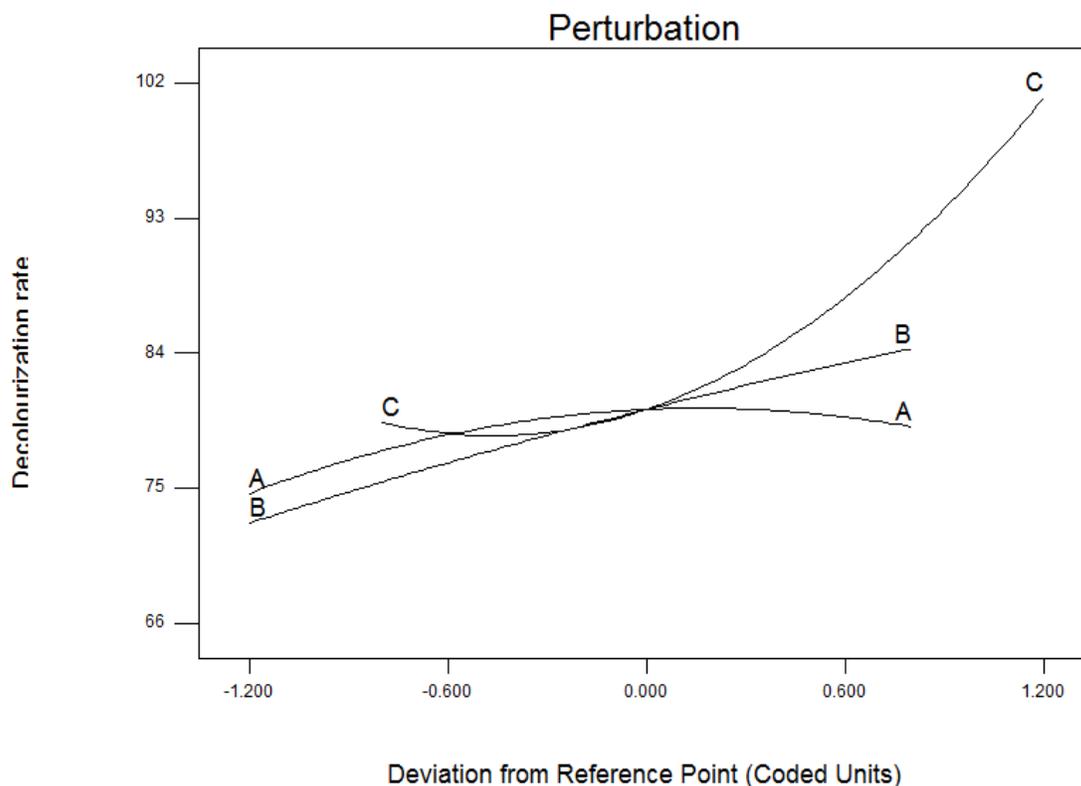
### Preparation of Ca-Ag Beads and Immobilization of AgNPs

The immobilized Ca-Ag beads were of the size of approximately 2 mm, 3 mm, and 4 mm and the immobilized AgNPs beads also showed similar size. Both control and immobilized beads were studied for SEM analysis (Figure 13) along with the EDAX. The swelling rate of Ca-Ag and immobilized AgNPs was calculated after 2 h of incubation. The beads showed a maximum in 16% phosphate buffer, 4% dye solution and 11% water.

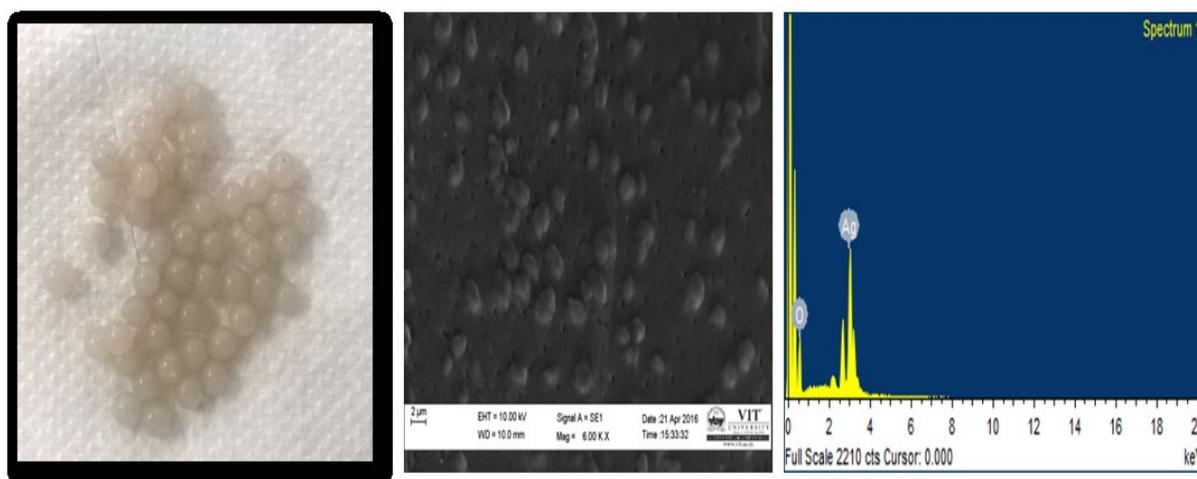
### Decolourisation studies of immobilized beads with and without AgNPs

The decolorization studied without immobilized AgNPs was assessed with beads of 2 mm, 3 mm, and 4 mm. The percentage of the decolourization was calculated. It exposed the

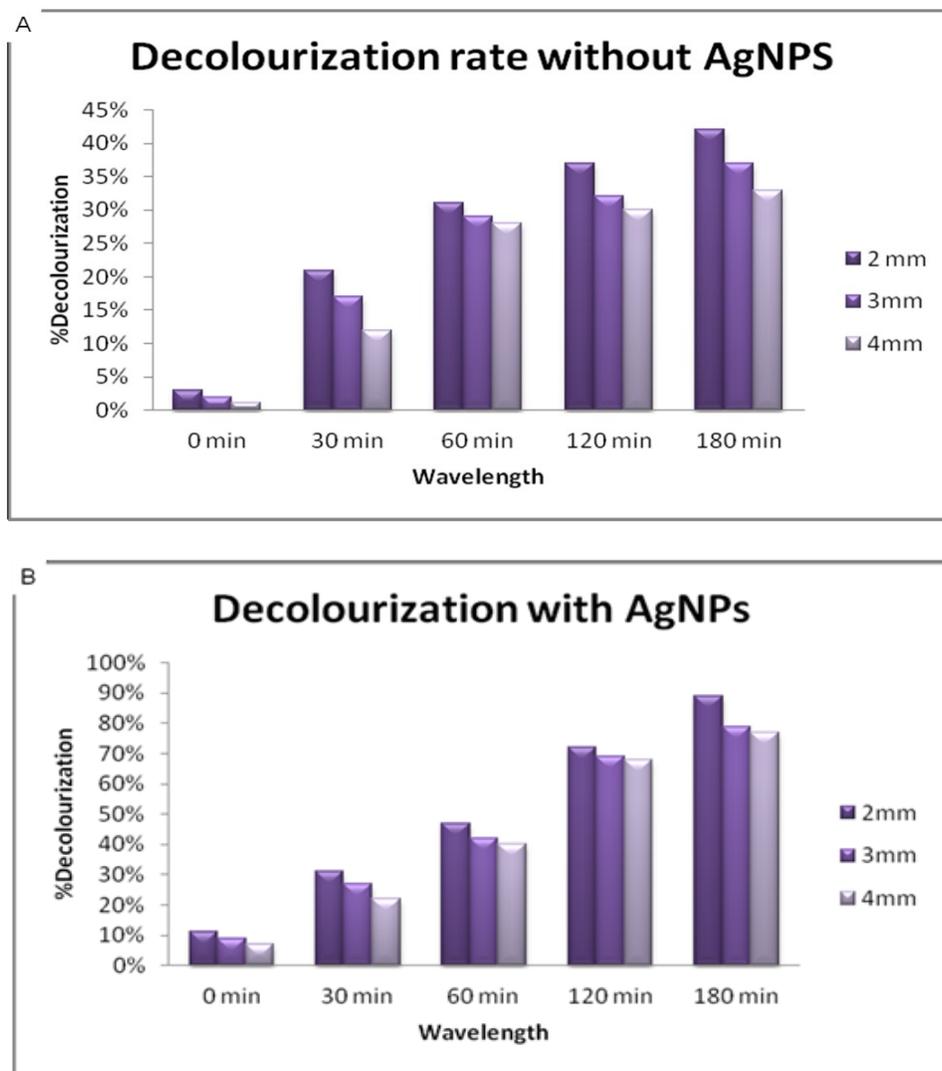
maximum decolourization with 88% in immobilized particles with 2 mm for 2 h (Figure 14 A, B).



**Figure 12.** Represents the perturbation curve of given variables for the effective dye decolourization analysis



**Figure 13.** Comparison of the immobilized Ca-Ag beads with AgNPs and SEM image of Ca-Ag bead with AgNPs



**Figure 14.** a) Percentage of decolourization without AgNPs. b) Percentage of decolourization with immobilized AgNPs

## Conclusions

The goal of this study was to synthesize the AgNPs using plant (green synthesis) and immobilization of Ca-Ag beads with AgNPs. The synthesis of AgNPs was done using aqueous leaf extract of *Celosia argentea*. The observation revealed it is of spherical shape, 50 nm of size and particle analysis result revealed its excellent stability. Effect of leaf extract and silver nitrate solution on bioreduction of  $\text{AgNO}_3$  was checked as a preliminary assay. Its characterization further showed good properties which took us

for application studies. The effect of nanoparticle dose, pH and contact time with Malachite Green dye for decolorization were carried out. Further a model was optimized and the Ca-Ag beads were immobilized with AgNPs. From the above study it has been concluded, Ca-Ag beads when immobilized with AgNPs, showed a higher percentage of (88%) dye decolorization of 2 mm in 2 h. In comparison with beads without immobilized AgNPs showed a lower percentage (42%) of decolorization. Therefore, the result demonstrated a positive

and promising role of AgNPs in the field of decolorization studies. Further research is required to study the mechanism behind the decolorization activity of AgNPs.

### Acknowledgments

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### Disclosure Statement

No potential conflict of interest was reported by the authors.

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