Catharanthus roseus leaves as a green source for facile synthesis of silver nanoparticles and their efficacy towards catalytic and adsorption kinetics studies of methylene blue dye

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Abstract

A simple, low-cost, eco-friendly route for synthesis of metal nanoparticles had gained attention and emerged as offshoot approach of nanoscience using herbal plant extracts. In the current investigation, we report the reducing and capping potential of aqueous extract from leaf infusion of Catharanthus roseus for the synthesis of silver nanoparticle (AgNPs). The inclined properties of prepared AgNPs were typified by optical examination, UV-Vis spectroscopy, FT-IR, SEM-EDAX and X-ray Diffraction (XRD) studies. The color change of the reaction mixture from light yellow to dark brown was observed within 60 minutes, which indicated primary formation of silver nanoparticles.

The average range of particle size calculated using Dynamic Light Scattering measurements (DLS) was found to be 18.17 nm. The rapid electro kinetic behavior of the silver was evaluated using zeta potential (appox.-27.7mV). The catalytic efficiency of the prepared leaf broth, AgNPs was also investigated on Methylene Blue (MB) dye degradation. Adsorption kinetic studies at 10 ppm concentration suggest good correlation with pseudo second order model (R^2=0.991) and the result demonstrates excellent catalytic properties.

Keywords: *Catharanthus roseus,* Methylene Blue dye, DLS, XRD.

Introduction

Synthesis of silver nanoparticles via green approach is one of the emerging area of interest using herbal extracts with wide number of applications[1]. Even though, numerous metals exist, silver gained an importance due to their unique properties and multiple applications in areas of bio-imaging, catalyst properties and anti-microbial nature and also waste-water treatment[2].

Plenty of literature is available regarding the synthesis of silver nanoparticles from varied plant sources such as stem, leaves, roots fruits buds and latex due to their incredible biological constituents present in them which are responsible for the reducing nature of nanoparticles. Some act as capping agents also for their synthesis that can reduce metal ion and stabilize the nanoparticles to desired shapes and sizes[3]. Silver nanoparticles act as good antimicrobial agent against human clinical pathogens, viruses and other microbes[4].

Catharanthus roseus L. (G.) Don, is an imperative evergreen herbaceous medicinal plant native to its Apocynaceae family (dicotyledonous angiosperm); commonly known as Periwinkle", "Nayantara" or "Sadabahar", derives its name from Greek language *Catharanthus* means "pure flower" while *roseus* means red, rose or rosy. Based on its specified properties, they exhibit their efficacy towards anti-cancer, anti-diabetic, anti-microbial, anti-oxidant, anti-helminthic, anti-ulcer, hypotensive, anti-diarrheal, wound healing property, hypolipidimic effect and memory enhancement activity[5].

In the present study, rapid and eco-friendly synthesis of AgNPs from the aqueous leaf extract of *Catharanthus roseus* was studied. The synthesized AgNPs were characterized and evaluated for their catalytic activity using methylene blue dye.

Material and Methods

Collection of leaves: Healthy leaves of *Catharanthus roseus* were collected in Yogi Vemana University Campus taxonomically identified and authenticated by Dept. of Botany, Yogi Vemana University, Kadapa. The plant material was washed repeatedly with running tap water twice, rinsed with distilled water, allowed to dry under shade for 7-14 days. After drying, the leaves were pulverized and grinded to a fine powder and kept in a well closed container in a dry place.

Preparation of aqueous leaf extracts of *Catharanthus roseus*: 10 g of fine dried leaf powder was weighed and boiled in 100 mL of distilled water at 90ºC for 15 minutes and then the extract was cooled to room temperature followed by filtering with Whatmann No.1 filter paper and directly used for experiments.

Preparation of 1 mM silver nitrate solution: Accurately weigh 0.017 g (1mM $AgNO_3$ Sigma-Aldrich) dissolved in 100 mL of distilled water was stored in amber-colored bottle until further use.

Bio-synthesis of silver nanoparticles: Biogenic silver nanoparticles can be synthesized by accumulating 10 mL of leaf extract drop wise to the 90 mL of $AgNO_3$ solution under constant stirring with magnetic stirrer. Incubate the solution in dark for 24 h at room temperature. The visual color change of the solution from pale yellow to dark brown indicates the formation of silver nanoparticles. The reduction of silver ions can be monitored by using UV-Visible spectrophotometer (Thermo scientific Evolution 201) with a wavelength range 200 nm to 700 nm. The synthesized AgNPs powder was stored and employed for further characterization process.

Characterization of AgNPs: The reduction of silver ions into silver particles was monitored by UV-Visible spectrophotometer. An aliquot of 0.1mL of the sample was diluted with 1 mL distilled water and absorption spectrum was recorded between 200-700 nm. X-ray diffractometer (RIGAKU, SMARTLAB) operated at 30 Kv 100 Ma with Cu K_β as a radiation source was used to record XRD patterns of dried AgNPs to confirm the crystalline nature.

Scanning Electron Microscopic (SEM) images were obtained using HITACHI Model SEM-JEOL machine at 120 kv. EDAX Spectra was observed with the help of JEOL analysis station at an acceleration voltage of 20-40 keV. Thin films of samples were prepared on a gold coated grid by just dropping a very small amount of sample on the grid.

Extra solution was removed using blotting paper and then films on SEM grid were allowed to dry by putting it under mercury lamp for 5 min, later examined at various magnifications to focus the surface morphology of AgNPs nanoparticles. The FT-IR spectrum was recorded using Perklin-Elmer (Spectrum Two model), UK instrument at wavelength ranges from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. Dynamic Light Scattering (DLS) analysis was carried out on Zetasizer S-90, Malvern, UK by taking dilute dispersion solution of AgNPs in distilled water.

Separation of silver nanoparticles: The synthesized silver nanoparticles were separated by centrifugation (REMI centrifuge). The process was carried out thrice to get rid of any un-coordinated biomolecules. The supernatant liquid was resuspended in the sterile double distilled water every time and a pellet was collected. The desired pellet solution was dried and stored at 4^oC for further use.

Evaluation of effect of synthesized *Catharanthus roseus* AgNPs on the reduction of methylene blue (MB) dye: Methylene blue purchased from Sigma, India was used to assess the catalytic activity of synthesized AgNPs as described by Edison and Sethuraman[6]. Both reactions were carried out in 3 mL cuvette and absorbance was recorded using UV-Visible spectrophotometer (Thermo-scientific Evolution 201).

At first reaction, 1 mL (1×10^{-4} M) of MB was mixed with 200 µL of aqueous leaf extract and 1800 µL of water and the reaction mixture was monitored. After some time in second reaction, 1 mL (1×10^{-4} M) of MB was mixed with 200 µL of aqueous leaf extract of *Catharanthus roseus* and 1800 µL of synthesized AgNPs (100 mg/mL) and the reaction was monitored at three regular intervals such as 30, 45 and 60 min. In both the reactions, the volume was made up to 3 mL. The values of absorption maxima of the reaction mixture were determined by using UV-Visible spectrophotometer and then compared with MB dye (664 nm).

Adsorption kinetics of methylene blue using silver nanoparticles: To evaluate adsorption kinetics for silver nanoparticles derived from *Catharanthus roseus,* leaf extract by MB dye was investigated by preparing fresh stock solutions of 10 ppm MB dye and AgNPs (1 mg/mL). In a reaction mixture, 500 µL MB dye and 5 mL of CR-AgNPs from stock solutions were mixed properly using ultra-sonicator and final volume was made to 10 mL with distilled water and absorbance was recorded using UV-Visible spectrometer as a function of time and to study kinetics, Langergren first order and Pseudo second order model were chosen to our current investigation and calculated by using following formula:

The Lagergren first-order rate equation:

$$\ln(q_e - q_t) = \ln q_t - k_1 t \quad (1)$$

where qt (mg/g) is the quantity of the metal ions adsorbed at t (min) and k_1 is the rate constant of the adsorption (1/min).

The pseudo-second-order kinetic model equation expressed as:

$$t/qt = 1/k_2 q_t^2 + (1/qe)t \quad (2)$$

where k_2 (mg/g min) represents the rate constant of the pseudo-second order equation.

Results and Discussion

The synthesized silver nanoparticles were initially confirmed through visual color change from pale-yellow to dark-brown in color. Further, the reduction of silver ions into AgNPs is the presence of aqueous leaf infusion of *Catharanthus roseus*. The particles were formed within 60 min. and spectrum was recorded at regular intervals using UV-Visible spectra and the results are depicted in figure 1.

A strong peak at 440-480 nm was observed and intensity of peak increased with increase in reaction time which is the characteristic feature of AgNPs due to surface plasmon resonance[7].

The crystalline nature of synthesized AgNPs was presented in figure 2 which shows Braggs reflections with 2θ range of 20-80^0 values as 38.10^0, 44.40^0, 64.48^0 and 77.55^0 sets correspond to indexed planes of crystals of AgNPs to (111), (200), (220) and (311) which represent the face centered cubic (fcc) lattice of silver respectively, which confirmed the crystalline nature of synthesized AgNPs (JCPDS No-65-

2871). Siva Sankar[7] also reported the crystalline nature of silver using *Grewia flaviscences* leaf extract.

Representive SEM micrograph depicted in figure 3(a) showed that AgNPs are spherical in shape and well dispersed with average size of 60-80 nm and few particles are also agglomerated. EDX spectrum displayed in figure 3(b) exhibited strong signal at the energy of 3 keV indicating the presence of AgNPs. and also few signals of Si, C, O and Cl were also obtained.

FTIR spectrum was used to identify the potential functional groups present in the moiety of biosynthesized AgNPs and *Catharanthus roseus* leaf extract. Figure 4 shows the absorption bands at 3498, 2925, 1735, 1049 and 584 cm^{-1}. The bands at 3498 cm^{-1} and 2925 cm^{-1} correspond to OH stretching vibration and aldehydic CH stretching vibration respectively. The band at 1735 cm^{-1} could be attributed to C=C aromatic vibrations[8].The band at 584 cm^{-1} was assigned for the presence of alkyl halides. From the above spectral analysis, OH-group present in the leaf extract of *Catharanthus roseus* was involved in the reduction of silver ion to silver between $AgNO_3$ and the alcoholic groups present in the leaf can be represented as -:

$$2\,Ag^{0} + RO + HNO_3 \longrightarrow 2\,Ag + NO_3^{-} + ROH$$

It could be confirmed that the proteins and carbonyl group of amino acids had the stronger ability to bind with silver nanoparticles and may form a layer on the surface of AgNPs. Hence, the surface capped biomolecules suggest that the biological molecules might act as both reducing and capping for silver nanoparticles[9,10].

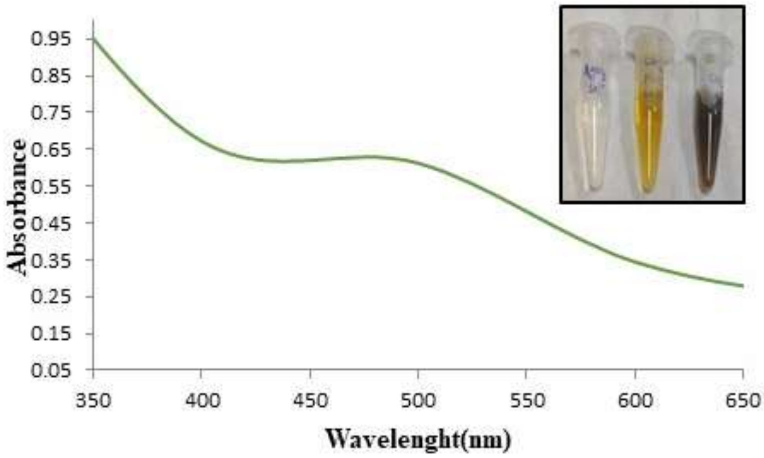

Figure 1: Visual observation and UV-Visible absorption spectra of instantly synthesized AgNPs (*Inset* color change from pale-yellow to dark-brown)

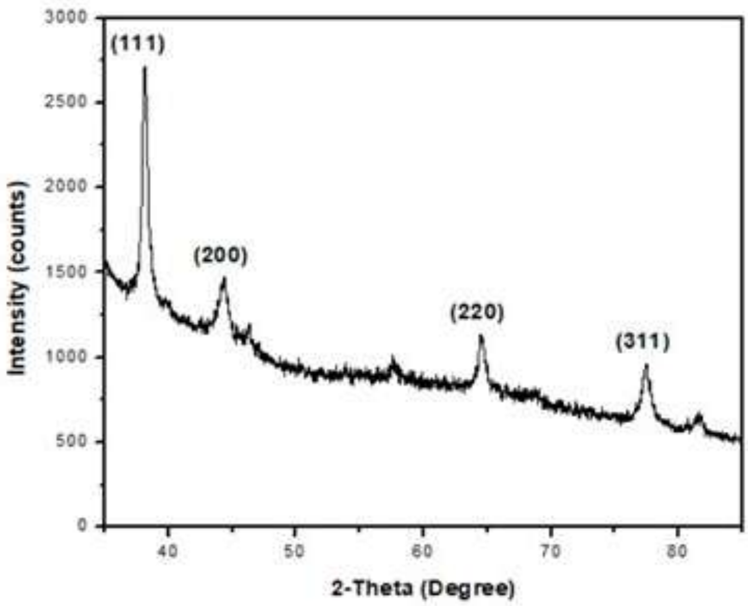

Figure 2: XRD pattern of the AgNPs synthesized from the leaf extract of *Catharanthus roseus.*

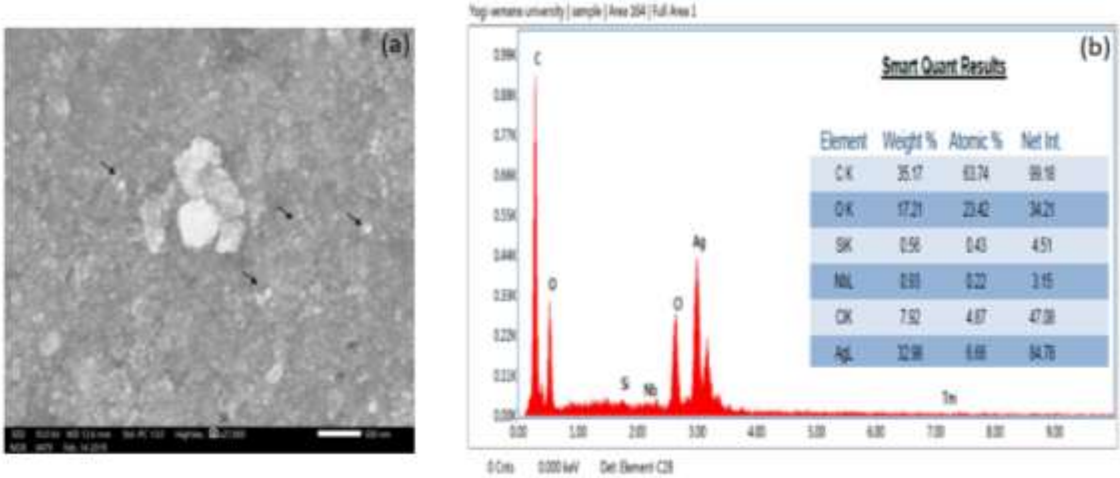

Figure 3: (a) SEM micrograph (b) EDX spectrum of synthesized AgNPs of *Catharanthus roseus.*

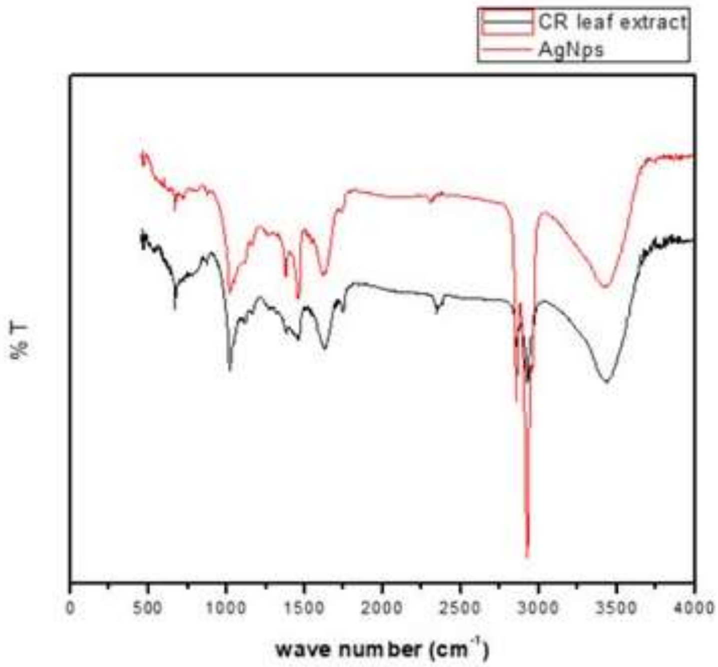

Figure 4: FTIR spectrum recorded by making a KBr pellet with leaf extract and synthesized AgNPs of *Catharanthus roseus.*

The DLS size distribution image of AgNPs (pH 7) using *Catharanthus roseus* leaf extract was shown in figure 5. The result of DLS analysis reveals that the average range of particle size distribution of AgNPs was 18.17-43.82 nm. The rapid electro kinetic behavior of the silver was evaluated using zeta potential (appox.-27.7mV). The larger negative potential value could be due to the capping of phytoconstituents exist in the leaf extract. Although the biosynthesis of AgNPs using *Terminalia chebulla* was found to be the average particle size as 25 nm[6].

The current study aims at the reduction of methylene blue dye by the natural green aqueous extract of *Catharanthus roseus* consisting of AgNPs. Pure methylene blue has a maximum absorbance value at 664 nm. It is a well-known fact that AgNPs and their composites have greater surface area thereby show greater catalytic activity in areas of dye removal and degradation process. Reduction of MB by arsine using silver nano was studied by Pal et al[10].

Witcomb et al[11] evaluate catalytic degradation using phenosaffarin dye was studied. After some time, the combination of leaf extract to the dye gradually decreases and then shifted towards higher wavelength. The decrease in absorbance is an indication to the capacity of phytoextract to degrade methylene blue. The reaction mixture containing leaf extract, AgNPs at the end of 30, 45 min and upto 300 min. time interval which display a remarkable decrease in the absorbance of methylene blue and increase of SPR peak of AgNPs at 664 in figure 6 which often shows the electron relay effect[12] in which electron transfer occurs in between leaf extract, AgNPs and methylene blue dye.

Figure 7a and 7b illustrates the linear expression of Lagergren first and pseudo-second order for adsorption of methylene blue by *Catharanthus roseus* AgNPs. Our results interpret the absorption of *Catharanthus roseus* AgNPs with rate constant K_1, K_2 as 0.046, 0.007. The result of the pseudo second kinetic model displayed good correlation (R^2= 0.991) with experimental data (q_e(exp.)= 19.5 mg/g) and the equilibrium data (q_e(cal.)= 27.7 mg/g) was satisfied and kinetics parameters remained concise and shown in table 1 and 2. Similar work was also reported by using Almond Gum for Cd (II) biosorption kinetics[13].

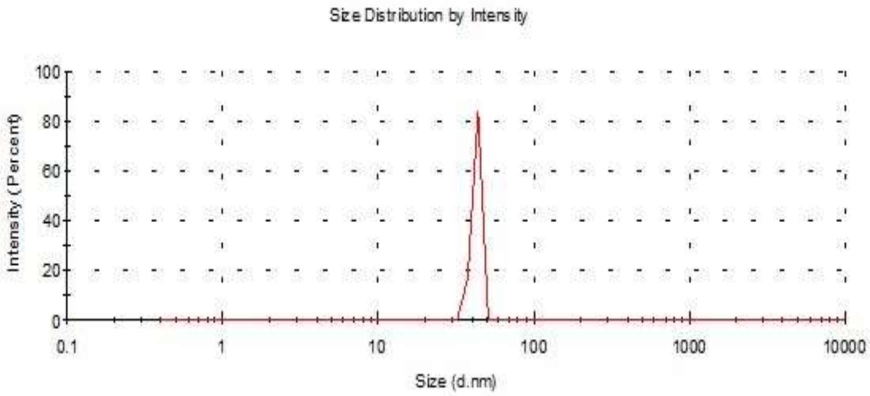

Figure 5: DLS of AgNPs synthesized from *Catharanthus roseus* leaf extract at pH-7.

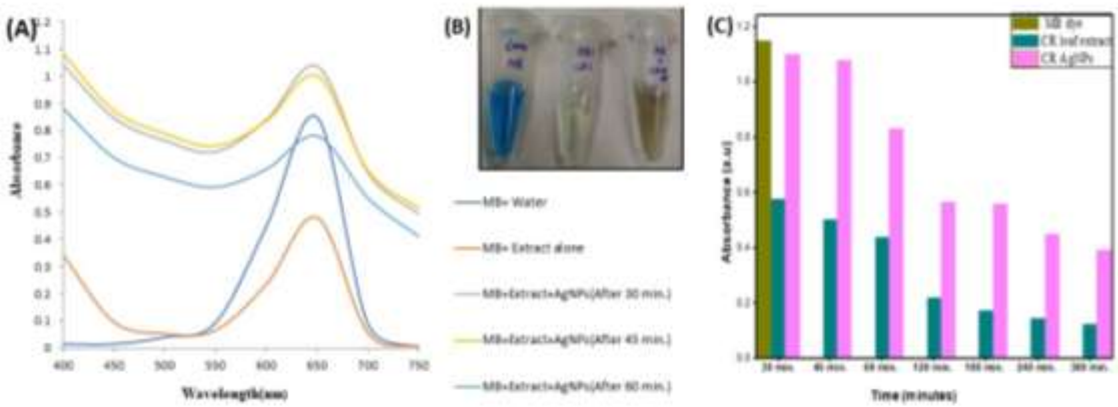

Figure 6: (A) UV-Visible spectra of methylene blue reduction by *Catharanthus roseus* in the presence of AgNPs (B) Inset figure Visual examination of synthesized AgNPs on the reduction of MB dye by *Catharanthus roseus* leaf extract 6 (C) Effect of contact time on the adsorption of MB dye at 664 nm by *Catharanthus roseus* in the presence of AgNPs.

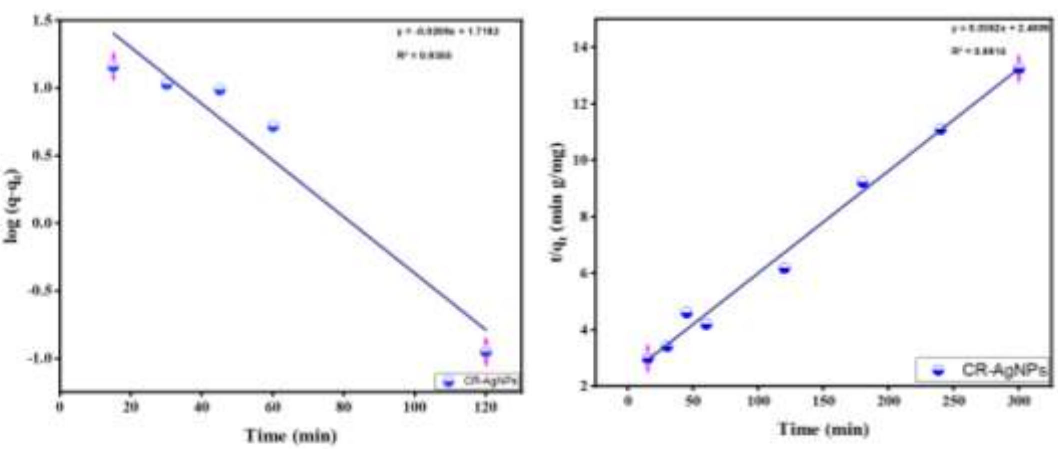

Figure 7: Kinetic studies of AgNPs nanoparticles using leaf extract of *Catharanthus roseus* by methylene blue (A pseudo-first order B) pseudo-second order.

Table 1
Kinetic parameters obtained from Langergren first and pseudo second order for CR-AgNPs by methylene blue at 10 ppm concentration.

S.N.	Time (min.)	First order kinetics	Second order kinetics
		log (qe-q)	t/q(min.mg/g)
1	15	1.1611	2.9962
2	30	1.0311	3.4261
3	45	0.989	4.6153
4	60	0.7186	4.2049
5	120	-0.9488	6.1895
6	180		9.2307
7	240		11.111
8	300		13.256

Table 2
Equilibrium time and q_e values of *Catharanthus roseus* AgNPs of Langergren first and pseudo second order at 10 ppm concentration by methylene blue

Initial conc.(mg/L)	Equilibrium time (min.)	qe (exp.) (mg/L)	First order kinetics			Second order kinetics		
			K_1 (1/min.)	q_e (Cal) mg/g	R^2	K_1 (1/min.)	q_e (Cal) mg/g	R^2
10	120	19.5	0.046	52.24	0.935	0.007	27.77	0.991

Conclusion

The study concludes that AgNPs could be prepared instantly by making use of aqueous extract of *Catharanthus roseus.* The phytoconstituents such as alkaloids and phenolic compounds present in the leaf infusion may be responsible and act as both capping and reducing agent for synthesis of biogenic AgNPs as evident from FT-IR and EDX studies. The biosynthesized AgNPs were found to have crystalline structure with fcc geometry as obtained from XRD analysis.

The SEM and DLS studies confirmed the spherical and average size of nanoparticles around 18.17- 43.82 nm. The biogenic AgNPs acts through electron relay effect have influence on degradation of methylene blue by *Catharanthus roseus* AgNPs, acting as beneficial catalytic agent.

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