Anti Fungal Studies of Tea Leaf mediated Silver Nano Colloid

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Abstract. Silver nano colloids are prepared using Tea leaf as reducing and stabilizing agent. Samples with different concentrations of the reducing agents are irradiated with gamma rays of various doses. Transmission Electron Microscope images of the samples show agglomerated clusters of nano particles before irradiation while the images of after irradiation show chain free individual nano particles with reduced size and spherical morphology. The Selected Area Electron Diffraction pattern confirms the crystal nature and face centred cubic structure of nano silver. UV-Visible spectroscopy gives absorption peaks in the blue region. Gamma irradiation results in the reduction in peak intensity without any change in wavelength. A statistical approach is done on treating the gamma irradiated colloidal sample with the as prepared silver nano colloid for measuring the affected area of fungal infection on a potato-agar medium. It is found that the fungal growth is declined rapidly with the introduction of gamma irradiated silver nano colloid than the as prepared colloid.

Keywords: silver nano colloid, Tea powder, gamma irradiation, spherical morphology, antifungal studies.

1. Introduction

Silver nanoparticles are noble metal nano particle most often used in the fields of green chemistry and nano technology branches due to their high surface to volume ratio [1]. Silver nanoparticle is an essential component in electronic industries due to semiconducting properties and fast switching mechanism [2]. Its unique properties make it nearly impossible to substitute and are used in a wide range of applications in consumer products like antifungal creams, edible packages and in medicines [3]. Solubility and stability of silver nano particle with polymers and carbon compounds are excellent. Silver can be tuned in research for improving the physico-chemical properties [4]. In Biomedical research, silver is highly used as fluorescent nano particle for drug delivery applications [5, 6]. Silver nano particle usually prepared from plant extract shows fluorescent quenching effect [7, 8]. Antimicrobial properties of silver nanoparticles and silver ions have been known for last few centuries [9]. Silver nanomaterials have also been shown to have antifungal properties. Most important findings were related to toxic effects towards Candida albicans species [10]. It was shown that colloidal silver nanoparticles in very low concentrations may have substantial antifungal impact in vitro. Even now the mechanisms of antibacterial nano silver action are not completely understood. It is supposed that silver inhibits the function of proteins and DNA replication [11]. Another theory is that silver causes damage to the bacterial membrane by incorporating itself into-membrane structure and interacting with the major building elements [12]. This structural damage causes change in membrane permeability to water and various ions, which leads to bacterial death.

Aim of this work is to enhance the antifungal property of silver nano particle by irradiation treatment. The attempt is also made to reduce the agglomeration and to maintain the average particle size in nano regime by suitable irradiation of highly energetic rays like gamma ray.
2. Experimental Details

Silver nitrate powder (Sigma Aldrich Co. USA: purity-99.9999999%) is used as the source material for our study. In order to prepare Silver nitrate solution, weigh out 10 mg silver nitrate powder using electronic weighing balance and is dissolved with 150ml de-ionized water using a magnetic stirrer. 10 grams of tea leaves from the Kannandevan hills, Kerala are cleaned well using tap water several times and then with double distilled water. Particular leaf extract is taken by boiling it with 100ml deionized water. Extract is double filtered using Whatman NA 125mm Transfer Paper. Different concentration like 2ml, 4ml, 6ml, 8ml and 10ml of filtered tea-leaf extract is added to each beaker containing 30ml AgNO3 solution. Magnetic stirring is provided for uniform concentration for 10 minutes while adding the extract. Keep the solution for 2-3 days under constant room temperature to get colloidal silver nano particle. The colour of the solution changes from colourless to yellowish brown.

The as prepared silver nano particles are exposed to Co-60 irradiator- model no. LDI 2000-BRIT. It is having an irradiation rate at a maximum order of 2 gray/minute with an attenuation of 50%. The samples are irradiated at a dose rate of 1 Gray/min. Varying dosages like 4Gray; 6Gray and 8Gray are given to silver nano colloids by controlling the exposure time. A visible colour change from dark red to pale yellow is observed for the colloidal samples.

Structural and morphological properties are analysed through Transmission Electron Microscope (TEM) Model JEOL JEM 2100 operated at 200KV and Selected Area Electron Diffractogram (SAED) is recorded. Optical absorption spectra are performed in a wavelength ranging from 300 to 900 nm using a double beam UV-Visible spectrophotometer, JASCO-V550 with deionized water as the reference. HRTEM images are further analysed using Image J software to get the average particle size and lattice spacing.

Antifungal activity of gamma irradiated biosynthesized silver nano particle is performed using Dextrose Agar media according to well diffusion method. Antifungal activity was assessed against Rhizopus Stolonifer. 30ml of gamma irradiated silver nano particle is added in wells of 1.5 mm diameter wounds in Petri plates with potato- dextrose –Agar (PDA). A colony of Stock cultures from Rhizopus Stolonifer already prepared and maintained in PDA slants were transferred to the medium. Zone of inhibition is continuously examined using a meter ruler. A histogram is plotted based on the statistical analysis for a period of one week. During the period the specimen were well maintained at constant temperature of 200C.

3. Result and Discussions

Green synthesis of silver NPs is done with tea-leaf extract as reducing agent. The appearance of yellowish-red colour of the samples suggests the formation of nano silver. As the concentration of the extract increases the nano silver colloidal solution changes from orange to dark red in colour. This may be due to the production of large amount of silver nano particles in colloidal suspensions. Silver nano colloids with varying concentrations of reducing agents undergo gamma irradiation at different doses like 4 gray, 6 gray and 8 gray. The Figure 1 shows the colloidal samples with gamma irradiation.

![Figure 1: Gamma irradiated samples of silver nano particles](image-url)
The morphology, size and lattice spacing of synthesized silver nanoparticle is determined by TEM, HRTEM and SAED pattern. Figure 2 shows TEM, HRTEM and SAED image of silver nanoparticle under 20 nm and 2 nm (inset: HRTEM) resolution after irradiation. It reveals the lattice spacing between adjacent fringes is 0.82 nm. SAED analysis can index with the help of C-Spot software treatment and found to have FCC lattice with orientation of peaks along (111), (220) and (311).

![Figure 2: TEM, HRTEM and SAED pattern of Gamma irradiated samples of silver nano particles](image)

The UV-Visible absorption diagram of gamma irradiated nano silver colloid and Tauc plot is shown in Figure 3. From the figure, it is found that the intensity of absorption of gamma irradiated sample becomes 1.35 and from Tauc plot the band gap energy is found to be 2.11±0.02 eV. It may be due to the colour change from dark brown to pale yellow.

![Figure 3: Optical absorption spectra and Tauc plot for Gamma irradiated silver nano particle](image)

Antifungal studies are done on the Potato Agar solution by making three wounds of 1-1.5 mm. The control is taken as tea-leaf extract media only and is inserted in one wound. The other two is filled with silver nano particles before irradiation and the last with gamma irradiated silver nano particles. The study is repeated for 4 Gray and 8 Gray samples of 2ml, 4ml and 8ml tea-leaf extract added samples. The best inhibition rate is obtained for 8gray-8ml tea leaf extract mediated sample. The affected area of fungal growth is monitored in a continuous duration of 24 hrs and is continued for one week. The diameter of affected area is measured using a meter ruler and histogram is plotted. Figure 4 represents the fungal affected areas on different media and the histogram of timely response.
4. Conclusion

In this work, green synthesis of silver nanoparticles is prepared through tea-leaf extract of different concentrations. Gamma irradiation of various dosages are done on samples and are found to have a reduction in yellowish-brown colour in aqueous solution to pale yellow due to a reduction in average particle size to controlled nano regime. The characteristic colour is also an indication of excitation due to surface plasmon vibrations in silver nanoparticles. From TEM analysis of nano silver samples without irradiation, it is found that morphology is core-shell in nature with agglomerated nano particles. It got disappeared in gamma irradiated samples and individual nano particles are found with a reduction in average particle size. The uniform distribution of nano particle in gamma irradiation is due to the effective photo induced reduction of particle size by irradiation effect. The SAED pattern of silver nanoparticles with and without gamma irradiation show white ring like pattern. The ring-like diffraction pattern indicates that the particles are crystalline. The diffraction rings could be indexed on the basis of image software and are found of having FCC structure for the nano silver. Optical studies of bio synthesized silver peaks show almost similar broadness in variety of samples. Prepared nano silver is found to have a direct allowed transition with a wide band gap. Effect of gamma irradiation further reduces the peak intensity and broadness while the band gap energy is not much affected. From the antifungal studies of silver nano particles, the culture media alone shows a drastic growth in fungi for an observation period of one week while in the samples incorporated with gamma irradiated silver nano particles, the growth is much reduced.

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References

[1] Alsharach E, Alazzam S, Ahmed F, Arshi N, Al-Hindawi M and Sing G K 2017 Green synthesis of silver nano particle and their reduced graphene oxide nano composites as anti bacterial agents: a bio inspired approach Act. mett. Sini. (English letters) vol. 30 no. 1 pp. 45-52
[2] Zhasnakunov Z A, Satyvaldiev A C, Doolotkeldieva T, Omurzak E, Bobusheva S T, Kelgenbaeva Z and Abdullaeva Z 2018 Synthesis of biologically active silver and copper nano composites Mat. Res. Exp. vol. 5 no. 8 pp. 085404.
[3] Lin S K and Cheng W T 2020 Fabrication and characterization of colloidal silver nano particle via photo chemical synthesis Mat. Lett. vol. 261 no. 15 pp. 127077.
[4] Chang K, Jian X, jeong H M, Kwon Y, Lu Q and Cheng M J 2020 Improving CO₂ electrochemical
reduction to CO using space confinement between gold and silver nano particle J Phys. Chem. Lett. vol. 11, no. 5, pp. 1896-1902.

[5] Valsalam S, Agastian P, Arasu M V, Al-Dhabi N A, Kareem A, Ghilan M, Kaviyarasu K, Ravindran B, Chang S W and Arokiyaraj S 2019 Rapid biosynthesis and characterization of silver nanoparticles from the leaf extract of tropaeolummajus and its enhanced in-vitro antibacterial, antifungal, antioxidant and anticancer properties J. of Photochem. and Photobio B: Bio. vol. 191 pp. 65-74.

[6] Maghima M and AAlharbib S 2020 Green synthesis of silver nanoparticles from curcuma longa and coating on the cotton fabrics for antimicrobial applications and wound healing activity J. of Photochem. and Photobio B: Bio. vol. 204 pp. 111806.

[7] Hosny A M S, Kashef M T, Rasmy S A, Aboul-Magd D S and El-Bazza Z E 2017 Antimicrobial activity of silver nanoparticles synthesized using honey and gamma radiation against silver-resistant bacteria from wounds and burns Adv. in Natural Sciences: Nanoscience and Nanotechnology vol.8 No.4 pp.1-7.

[8] Bozkurt P A 2017 Sonochemical green synthesis of Ag/graphene nano composite Ultrasonics Sonochemistry, Vol. 35, Part A, pp. 397-404.

[9] Nasrollahzadeh M, Shafiei N, Eslamipanah M, Fakhri P, Jaleh B, Orooji Y and Varma R S 2020 Preparation of Au nanoparticles by Q switched laser ablation and their application in 4-nitrophenol reduction Clean Tech. and Env. Policy vol. 22 pp.1715–1724.

[10] Huang W, Yan M, Duan H, Bi Y, Cheng X, and Yu H 2020 Synergistic antifungal activity of green synthesized silver nanoparticles and epoxiconazole against stesosphaeria turicca Journal of Nanomaterial Article ID 9535432.

[11] Li J, Zhu Z, Liu F, Zhu B, Ma Y, Yan J, Lin B, Ke G, Liu R, Zhou L, Tu S and Yang C 216 DNA-mediated morphological control of silver nanoparticles, Small. Vol. 12 no. 39 pp.5449-5487.

[12] Qais F A , Shafiq A, Khan H M, Husain F M , Khan R A, Alenazi B, Alsalme A , and Ahmad I 2019 Antibacterial effect of silver nanoparticles synthesized using murrayakoenigii (L.) against multidrug-resistant pathogens Bioinorg. Chem. and Applied Physics B pp 1565-3633.