

DEXTRAN SULFATE STABILIZED SILVER NANOPARTICLE: NEXT GENERATION EFFICIENT THERAPY FOR CANCER

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ABSTRACT

Objective: Synthesize silver nanoparticles using a green synthesis approach and encapsulate silver nanoparticles with a naturally occurring polymer, particularly of an-hydroglucose type, like dextran sulfate sodium salt and to study its anticancer activity.

Methods: Green synthesis approach is been employed in the synthesis of silver nanoparticles using *Psidium guajava* leaf extract. The nanoparticles were then encapsulated with dextran sulfate biopolymer and the nanoparticles were subjected to different characterization techniques. The structure of the synthesized nanoparticles was analyzed using X-ray diffraction analysis, the presence of different functional groups was analyzed by FTIR studies. Size and morphology of the prepared nanoparticles were investigated using FESEM analysis. Anticancer activity of the synthesized nanoparticles was tested against the MCF-cell line.

Results: The XRD analysis shows the crystalline nature of the synthesized nanoparticles. The stretching and vibrating modes of different functional groups were confirmed by FTIR result. The SEM image confirmed the presence of spherical shaped nanoparticles and the TEM image confirmed the average size of the particles to be around 24 nm. The Ag-DS NPs showed 91% cell inhibition for the concentration of 100 µg/ml, indicating the cytotoxicity of the nanoparticles against MCF-7 cell line.

Conclusion: Dextran sulfate stabilized silver nanoparticles show potent anticancer activity against MCF-7 cell line.

Keywords: Silver nanoparticles, Biopolymer, Dextran sulfate, Anticancer activity

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INTRODUCTION

Nanoparticles are of great scientific interest among the research community because they form an effective bridge between bulk materials and atomic or molecular structures. Metal nanoparticles possess unique properties, for which they are used in many applications like catalysis, sensing and imaging etc., [1-3]. Silver nanoparticle is one of the most vital and fascinating nanomaterials among various metal nanoparticles. The use of silver nanoparticles for different applications depends on various factors including surface chemistry, size, size distribution, shape, particles morphology, particles composition, coating/capping, agglomeration, dissolution rate, particles reactivity in solution, efficiency of ion release and, type of reducing agent [4]. Encapsulating the synthesized nanoparticles in the polymeric matrix which is made from biodegradable and biocompatible polymer can be used for a controlled drug delivery system [5].

Silver nanoparticles tend to adhere to each other to form agglomeration because of Vander Waals forces that act over a short distance. Agglomeration due to Brownian motion is classified as coagulation [6]. To avoid agglomeration, a most suitable dispersing and binder agent dextran sulfate is used. Dextran sulfate is a polymer of an-hydroglucose which are soluble and stable in water. Dextran sulfate contains 17% sulfur, which is equivalent to 2.3 sulfate groups per glucose residue. Dextran sulfate is a biopolymer having a well-defined structure that differs from a synthetic polymer having more random structure [7]. Silver nanoparticles or silver ions coordinate with carboxyl group and have a physical-steric interaction. Dextran derivative such as carboxymethyl dextran or dextran sulfate contains one or more carboxyl or sulpho groups, therefore allowing conditions for forming complex compounds of different compositions and coordination with silver ions [8].

The aim of the present study is to synthesize silver nanoparticles by green synthesis approach using *Psidium guajava* leaf extract as a

reducing agent and to encapsulate these nanoparticles with the dextran sulfate matrix. The synthesized Ag-DS NPs were subjected to various characterization techniques. Finally, the anticancer activity of the green synthesized Ag-DS NPs were analyzed. *In vitro* cell line analysis on MCF7 cell line was carried out using MTT assay.

MATERIALS AND METHODS

Chemicals and reagents

Silver nitrate (99% purity) and all other chemicals used in the synthesis of Ag-DS NPs were purchased from Himedia India Private limited, Dextran sulfate was purchased from Sigma Aldrich chemical, India. All the chemicals used were of analytical grade purity. *Psidium guajava* leaves used were collected from local residents around Coimbatore.

Preparation of leaf extract

Psidium guajava leaves were collected from the local residents in and around Coimbatore and the plant materials were identified and authenticated by Botanical Survey of India, Coimbatore, TamilNadu (Voucher No: SI/SRC/5/23/2010-11/Tech. 1785). Fresh leaves of *Psidium guajava* were collected and cut into small pieces. These leaves were washed thoroughly for several times with double distilled water to remove dust and other particles attached to the surface of leaves. *Psidium guajava* leaf extract was prepared by weighing 20 g of freshly cut leaves with 200 ml of double-distilled water boiled at 60 °C in Erlenmeyer flask for 20 min [9]. The *Psidium guajava* leaf extract was then filtered through Whatman No.1 filter paper [10]. The filtered was used for the synthesis of nanoparticles in room temperature.

Green synthesis of Ag-DS NPs

Silver nanoparticles were synthesized by mixing 100 ml of 1×10^{-3} M, aqueous silver nitrate with 5 ml of *Psidium guajava* leaf extract and

the mixture were stirred vigorously for 20 min in a magnetic stirrer at room temperature [11]. It was observed that color of the reaction mixture changed from light yellow to reddish-brown that indicating the formation of AgNPs [12].

Initially, 0.005 gms of dextran sulfate was mixed with 5 ml of double-distilled water (1 mmol) and stirred on a magnetic stirrer for 20 min at room temperature. The green synthesized AgNPs using *Psidium guajava* leaf extract as a reducing agent was then added with dextran sulfate biopolymer. For each of 10 ml of colloidal green synthesized Ag nanoparticle solution, 2 drops of dextran sulfate were added and stirred on a magnetic stirrer for 1 h at room temperature. Finally, the synthesized nanoparticles were subjected to various characterization techniques.

Characterization of Ag-DS NPs

The final products obtained by chemical synthesis and green synthesis were characterized using X-ray Diffractometer (XRD, Bruker D8 Advance, CuK α ($k = 1.540593 \text{ \AA}$), Fourier Transform Infrared Spectroscopy (FTIR, IR-Affinity 1, Shimadzu), Field Emission Scanning Electron Microscope (FESEM, JEOL-JSM-6490LA), Energy Dispersive Spectroscopy (EDS, JEOL-JSM-6490LA), and High resolution Transmission Electron Microscope (HRTEM, JEOL-JEM-2100).

In vitro anticancer activity

The human breast cancer cells (MCF-7) were obtained from National Center for Cell Science (NCCS), Pune and the cells were treated with serial concentration (12.5, 25, 50 and 100 $\mu\text{g/ml}$) of the green synthesized Ag-DS NPs. The plates containing the synthesized nanoparticles were added with 5 mg/ml in phosphate buffer saline (PBS) solution. The plates were incubated at 37 $^{\circ}\text{C}$ for 4 h. MTT assay the medium without samples was served as control and triplicate was maintained for all concentrations.

The % cell inhibition was determined using the following formula.

$$\frac{\text{Mean absorbance of test sample}}{100 - \text{Mean absorbance at control}} \times 100 \text{---Eqn. 1.1}$$

A nonlinear regression graph was plotted between % cell inhibition and Log concentration and IC₅₀ was determined using GraphPad Prism software.

RESULTS AND DISCUSSION

X-ray diffraction analysis

The XRD peaks of the green route synthesized Ag-DS NPs are shown in fig. 1. The XRD spectrum shows the main predominant Bragg's diffraction peaks, which are positioned at 2θ value of 66.3° corresponding to (220) plane. The orientation plane (200), (104), and (311) was also observed at $2\theta = 45.3^{\circ}$, 54.9° , and 78.4° . These planes correspond to facets of metallic silver. Due to the crystal symmetry and relative phase velocity, the common crystal habitat of silver nanoparticles showed a face-centered cubic shape [13, 14]. These diffraction peaks are consistent with the standard Ag crystals (JCPDS Card No.4-0783), indicating that the synthesized AgNPs are of crystalline in nature. These results are in good correlation with previously reported results of AgNPs [15, 16].

The crystallite size of the green synthesis AgNPs was calculated by the Debye-Scherrer equation, $D = K\lambda/\beta \cos \theta$. Where D = average crystallite size, K-shape factor (0.9), λ -wavelength of Cu K α radiation (1.541 \AA), β -FWHM of reflection (in radians) located at 2θ and θ -angle of reflection (in degrees) was used to relate the crystallite size to the line broadening. The average crystallite size of prepared AgNPs was found to be about 20 nm.

FTIR analysis

To identify the different functional groups present in the synthesized Ag-DS NPs FTIR analysis was carried out. Fig. 2 shows the FTIR spectrum of the green synthesized Ag-DS NPs. The broad absorption peak at 3265 cm^{-1} corresponds to the stretching vibration of the hydrogen bond, which shows the presence of O-H stretching of phenol and hydroxyl group. A weak absorption band at 2923 cm^{-1} might be due to C-C stretching vibration of alkynes. A strong and sharp absorption peak at 1636 cm^{-1} may be due to primary amide NH₂ bending [17]. Medium peak observed at 1451 cm^{-1} is due to symmetric and asymmetric C-H deformation vibration of alkanes group. Medium peak observed at 1518 cm^{-1} , 1327 cm^{-1} and 1242 cm^{-1} corresponds to O-H deformation vibration belonging to alcohol group. Strong peak corresponding to 1022 cm^{-1} shows C-O stretching vibration of alcohol group. A strong absorption peak found at 667 cm^{-1} may be due to C-H plane deformation vibration of alkyl halides and aromatics group. The observed peaks are consistent with previous reports [18, 19].

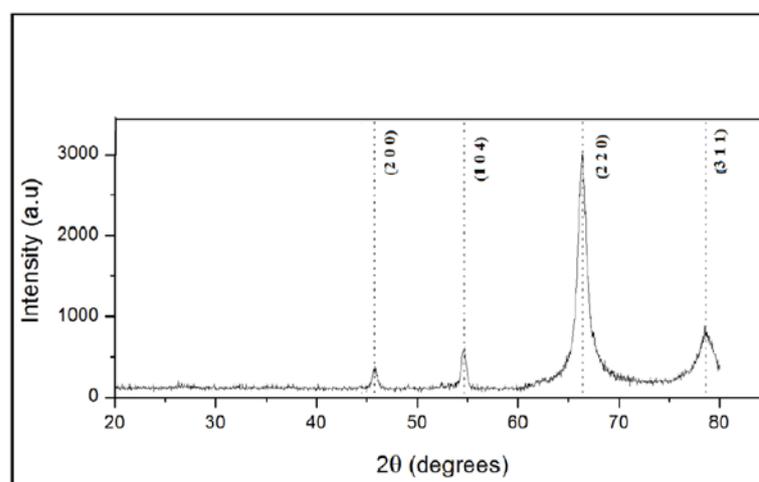


Fig. 1: X-ray diffraction spectrum of Ag-DS NPs

FESEM and EDAX analysis

Fig. 3 shows the field emission scanning electron microscopy image of Ag-DS NPs. The image indicates that the nanoparticles are composed of large scale mono-dispersed, spherical-shaped structure.

The dispersive energy spectrum (EDS) was done along with the FESEM analysis. The EDS spectra of the green route prepared Ag-DS NPs is shown in fig. 4. The compositional analysis confirms the presence of silver along with carbon, silicon, sodium, calcium, magnesium, iron, and oxygen elements. The elemental composition

of the green synthesized silver nanoparticles shows only 39% of elemental silver, along with all other compounds.

Oxygen was observed with a weight percentage with 16%, carbon with 22%, silicon with 15%, sodium with 1%, calcium with 2%, magnesium with 0.65%, and iron with 1%. All the other elements

present in the spectrum i.e., carbon, sodium, calcium, magnesium, iron and oxygen, might be contributed due to the plant's profile and dextran sulfate biopolymer. They might have also arisen due to the synthesis procedure adopted to prepare nanoparticles. The element silicon might have contributed due to the grid used for the holding sample in the electron microscope apparatus.

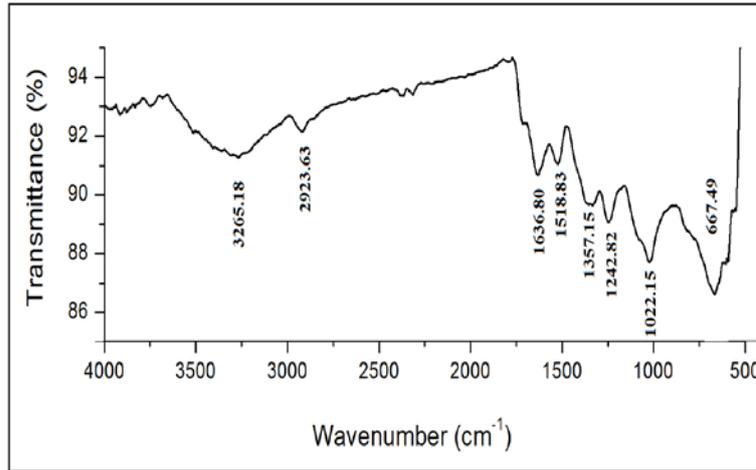


Fig. 2: FTIR spectrum of Ag-DS NPs

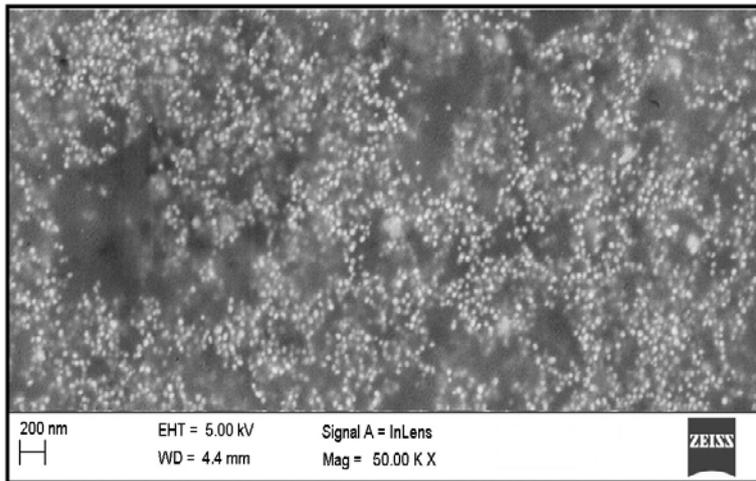


Fig. 3: FESEM micrographic image of Ag-DS NPs

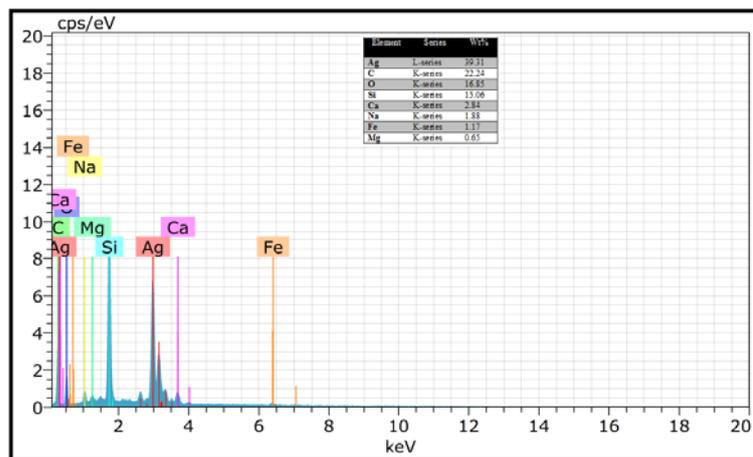


Fig. 4: EDAX analysis of Ag-DS NPs

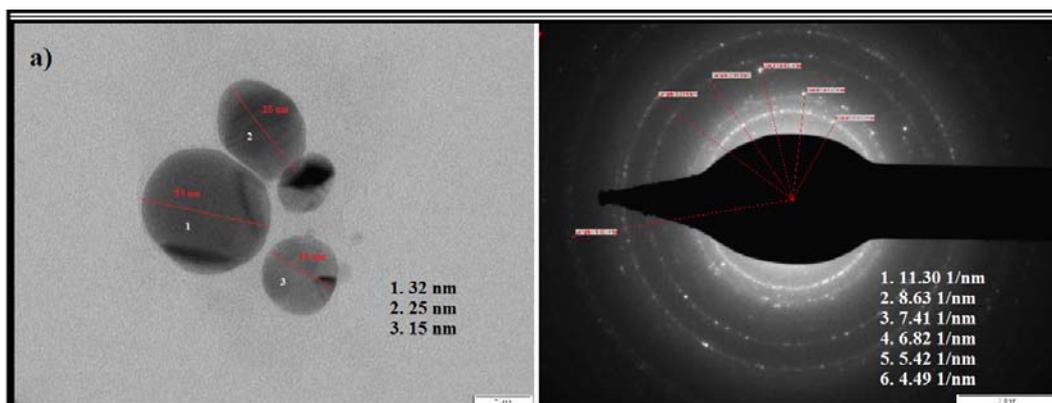


Fig. 5: TEM micrographic image of Ag-DS NPs

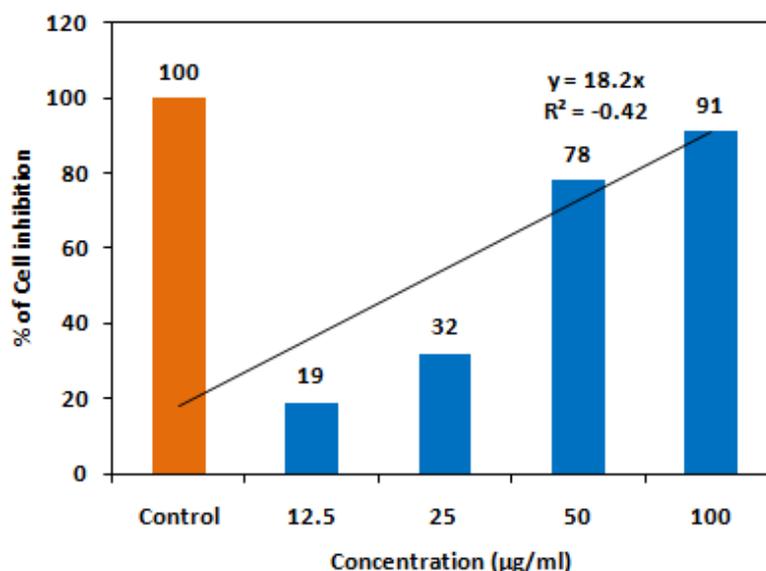


Fig. 6: Anticancer activity of green synthesized Ag-DS NPs on cancer cell line MCF-7

TEM analysis

Fig. 5 shows the TEM image and SAED pattern of green synthesized Ag-DS NPs. The image shows that the green synthesized Ag-DS NPs are spherical in shape with an average particle size of 24 nm.

Anticancer activity

Anticancer activity of the synthesized Ag-DS NPs demonstrated significant cytotoxicity against MCF-7 human breast cancer cell lines at different concentrations (12.5, 25, 50 and 100 µg/ml). Fig. 6 represents 91% of cell death, which was observed in the highest concentration of 100 µg/ml. 78% of cell death was observed for 50 µg/ml concentration. The IC_{50} value of Ag-DS NPs against human breast cancer MCF-7 cells holds around 37.81 µg/ml.

CONCLUSION

Ag-DS NPs were synthesized at room temperature using *Psidium guajava* leaf extract as a reducing agent. The XRD analysis shows high intense diffraction peaks indicating the crystalline nature of synthesized Ag-DS NPs with face-centered cubic structure. The FTIR spectrum shows weaker to medium bands corresponding to different functional groups, which might have arisen due to the characteristics of *Psidium guajava* plant. A spherical shaped structure with uniform distribution of the grains was observed from the micrograph images. The EDAX spectrum shows different elemental composition peaks related to carbon, silicon, sodium, calcium, magnesium, iron and oxygen which would support the

biocompatibility of the green synthesized nanoparticles. Green synthesized Ag-DS NPs have a particles size of about 24 nm. The SAED pattern confirms the crystalline nature of the nanoparticles. The Ag-DS NPs showed 91% cell inhibition for the concentration of 100 µg/ml, indicating the cytotoxicity of the nanoparticles against MCF-7 cell line. *Psidium guajava* leaf extract might also be responsible for the anticancer activity of green synthesized Ag-DS NPs.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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