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A FACILE, GREEN APPROACH FOR ENHANCED SYNTHESIS OF SILVER NANOPARTICLES BY *WEISSELLA CONFUSA* PRODUCED DEXTRAN

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Abstract

Green, economical, facile and a highly productive approach to synthesize silver nanoparticles (AgNPs) are devised. Aqueous extract of *Aloevera* with dextran produced by *Weissella confusa* as a reducing and stabilising agent is utilized for the synthesis. Parameters like concentration of silver nitrate (1,5,10 and 20mM), plant extract dilutions (4:1, 3:2, 2:3 and 1:4), concentration of both the commercial (CDx) and microbial (MDx) dextrans, incubation time and temperature were studied to understand their influence in the AgNPs synthesis. UV-Vis spectrum shows surface Plasmon resonance at 450nm. The average particle size ranged from 101-58.1nm without dextran and 82.1- 49.6nm with dextran and a mean particle size of about 20nm respectively. All the particles were spherical with variable size. Both CDx and MDx had enhanced AgNPs productivity. Dextran mediated AgNPs suspension was highly stable even after 30 days. Present attempt may be commercially significant for enhanced production of stable smaller sized AgNPs which could have several applications.

Key words: Green synthesis, silver nanoparticles, dextran, *Weissella confusa*.

Introduction

Nanoparticles have unique properties as a consequence of their size, distribution and morphology. Metal nanoparticles such as gold and silver have received more attraction compared with other nanoparticles because of their catalytic (Mallik *et al*., 2005), optical (Liz-Marzan *et al*., 2006) and electronic properties (Kamat *et al.,* 2002) making them to have wide variety of applications in medical, pharmaceutical and agricultural fields respectively. Silver in its nitrate form has a strong antimicrobial effect, but when silver nanoparticles (AgNPs) are used they are more effective as the surface area exposed to microbes increases considerably (T.M. Abdelghany *et al*., 2018) and also they do not induce any surface modification of the living cells nor do they cause any instance of microbial resistance. AgNPs can be synthesized by different methods like laser ablation of silver precursors in a solvent, photochemical reduction, electrochemical method and sonochemical deposition which are not eco-friendly (Jayaprakash *et al.,* 2019). AgNPs can be green synthesized by biological methods using biomaterials (bioenzymes/polymers, carbohydrates, alkaloids, metabolites, proteins, phenols) or microorganisms like bacteria, fungi, algae and yeast or different plant extracts (Ahmed *et al.,* 2016).

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Polysaccharides are natural biopolymers that are gaining importance for the synthesis of metallic nanoparticles (MNPs) because of their outstanding biocompatible and biodegradable properties. Polysaccharides are diverse in size and structure that makes them suitable for the reduction and stabilization of MNPs. For example dextran is a polymer of glucose produced by the microorganisms like *Weissella confusa, Leuconostoc mesenteroids,* and *Lactobacillus sps.* etc. (N.H. Maina *et al*., 2008). It not only has wide range of applications in food, pharmaceuticals and other industries but also a good reducing and capping agent that aids in nanoparticles synthesis (S. Patel and A. Goyal *et al.,* 2011).

The present study focusses on enhanced green synthesis of AgNPs with the use of *Aloe vera* plant extract and polysaccharide like dextran. *Aloe vera* gel contains potentially active component like hydroquinones that act as a reducing agent (Emaga *et al*., 2008). Dextran is a natural exopolysaccharide composed of α-(1-6) linked D- glucose molecules of varying length (Naessens *et al.,* 2005) which behaves as a flexible an extended polymer that can provide template for AgNPs adherence. Dextran enhances production as it acts both as a reducing agent and as a stabilizer, respectively. Generally it is well known phenomenon that nanoparticles without stabilizers are thermodynamically unstable. The quality, type and characteristics of green synthesized AgNPs are greatly influence by the concentration of $AgNO_3$, concentration of plant extract, incubation time and temperature (Kuchibhatla *et al.,* 2012). Therefore the work is highly concentrated on standardizing these parameters to develop a cost effective process for enhanced production using dextran, which also increases the stability of AgNPs.

Materials and Methods

Production of microbial dextran

The microbial culture of *Weissella confusa* was inoculated into Cortezi medium containing sucrose as a main carbon source (Cortezi *et al*., 2005). Production of microbial dextran (MDx) was carried out in 250mL flask using 5% inoculum in 50mL medium with an incubation temperature of 37°C for 24hrs (B.Srinivas *et al.,* 2016). After incubation dextran was recovered from the fermented broth by alcohol precipitation (Farwa *et al*., 2008) and was further used for AgNPs synthesis protocol.

Preparation of plant extract

Fresh leaves of *Aloe vera* were collected from local garden and washed with tap water followed by double distilled water to remove any surface contaminants. Approximately 20gms of air-dried and crushed leaves along with gel were mixed with 100mL of double distilled water in a 250mL beaker and boiled for about 10-15min and then cooled and filtered using Whatmann No.1 filter paper (Tanveer *et al.,* 2018). The prepared aqueous leaf extract sample was stored at 4°C for further use.

Preparation of silver nanoparticles

$AgNO_3$ solutions (1mM, 5mM, 10mM and 20mM) were prepared separately using double distilled water along with aqueous dilutions of plant extract in the ratios of 4:1, 3:2, 2:3 and 1:4 (plant extract: water). The prepared $AgNO_3$ solutions were mixed with different percentages of CDx and MDx (2.5%, 5% and 7.5%) followed by addition of various dilutions of plant extract at room temperature and then incubated for 24hrs. The clear solution was turned to brown color indicating the formation of AgNPs. The synthesized AgNPs were separated by centrifugation at 10,000 rpm for 10min and the produced pellet was washed with hexane to remove the moisture and was later kept for further drying and same used for analysis.

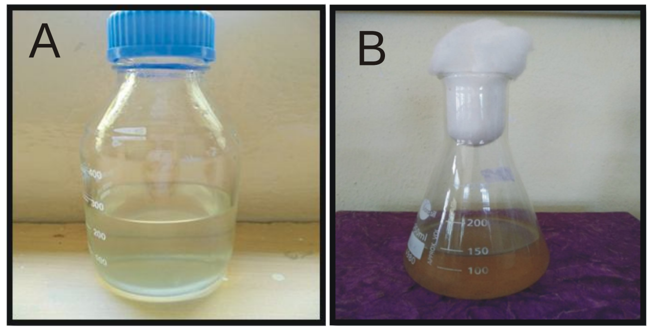

Fig. 1: Formation of silver nanoparticles by using *Aloe vera* plant extract (A) Mixture of silver nitrate, plant extract and dextran (B) Formation of brown color indicating AgNPs synthesis.

Characterization of AgNPs

The color change from transparent to brown color in dextran-*Aloe vera* -$AgNO_3$ mixed solution indicates the formation of AgNPs which were monitored by UV-Vis spectrophotometer in the range of 300-500nm to determine the absorption maxima(surface Plasmon resonance=SPR) of the samples which was observed to be 450nm. The size and shape of synthesized AgNPs were determined by Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) using the dried AgNPs pellets.

Results and Discussion

The results indicates that $AgNO_3$ upon reaction with *Aloe vera* plant extract solution in presence of dextran resulted in a gradual color change that could be read spectrophotometrically using a UV-Vis spectrophoto-meter (Fig. 1). The green synthesized AgNPs exhibit brown color in aqueous solution due to excitation of free electrons in AgNPs known as surface plasmon resonance (Bankura *et al*., 2012). The AgNPs prepared from both commercial dextran and microbial dextran (5% dextran, 3:2 plant extract,10mM $AgNO_3$) exhibit a sharp emission peak at 450nm (Fig. 2). Similar spectrographic results were obtained on the effect of different concentrations

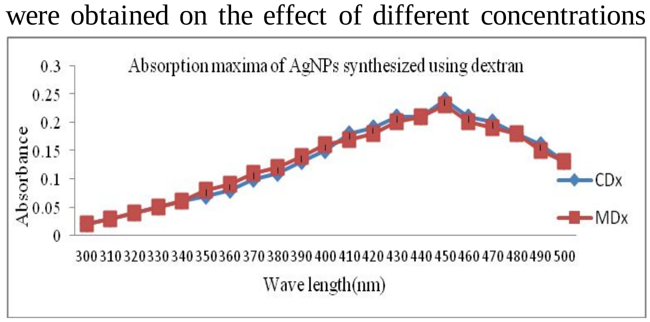

Fig. 2: Absorption maxima of silver nanoparticles synthesized from commercial dextran (CDx) and microbial dextran (MDx).

of metal precursor $AgNO_3$ (1mM, 5mM, 10mM and 20mM), various dilutions of *Aloe vera* plant extract (4:1, 3:2, 2:3 and 1:4) and different concentrations of dextran (2.5%, 5%, 7.5% of both CDx and MDx) at 450nm. The results indicated that as concentration of $AgNO_3$, plant extract, dextran increases the optical density (absorbance) also increases which infers enhanced AgNPs synthesis. Higher the molarity of any compound more will be the concentration of atoms in it, which could in turn provide more raw materials for any synthesis. The same aspect is reflected in our experiments where the synthesis of AgNPs increased with increasing molarity of $AgNO_3$ (Ahmed A. Moosa *et al.*, 2015) until it reaches a higher threshold limit (which was found to be 10mM) indicated in fig. 3. The active component like hydroquinones in *Aloe vera* plant extract could promote more AgNPs synthesis. Henceforth higher the concentration of plant extract higher would be the AgNPs obtained (Emaga *et al.*, 2008). This was also clearly indicated in results with 3:2 ratios as optimum (plant extract: water) (Fig. 4). Any reaction needs optimum temperature and time of

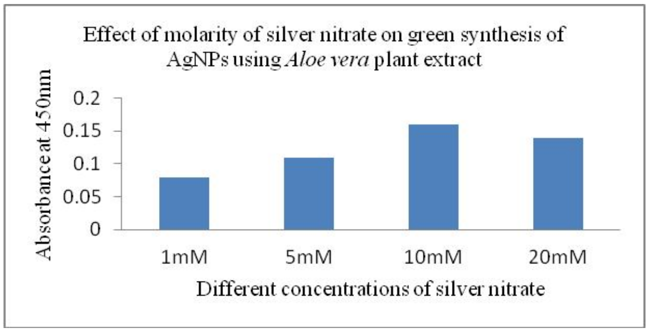

Fig. 3: Effect of molarity of silver nitrate on green synthesis of AgNPs using *Aloe vera* plant extract.

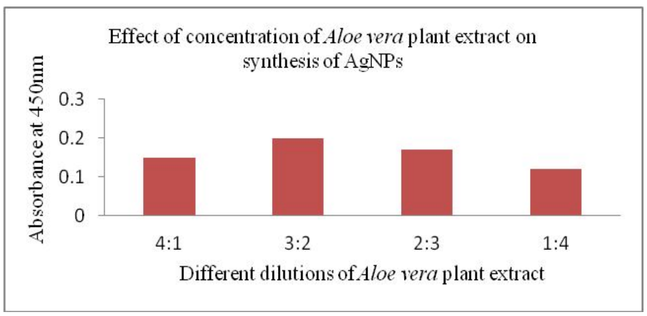

Fig. 4: Effect of concentration of *Aloe vera* plant extract on synthesis of AgNPs.

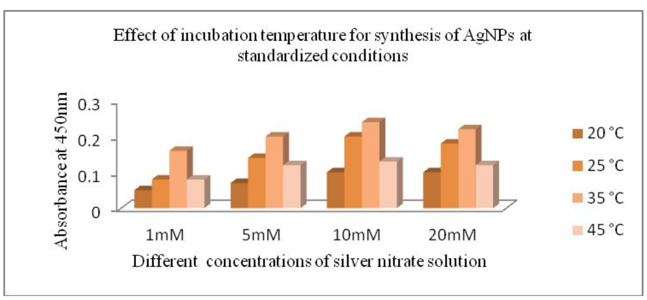

Fig. 5: Effect of incubation temperature period during synthesis of AgNPs.

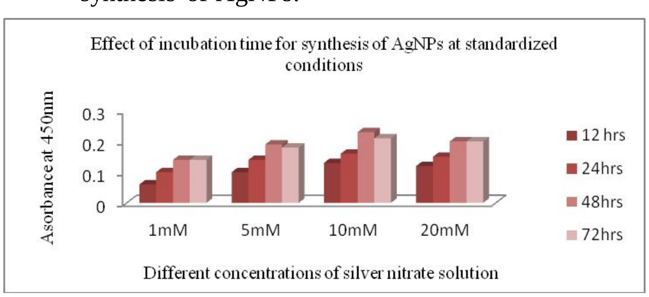

Fig. 6: Effect of incubation time period during synthesis of AgNPs.

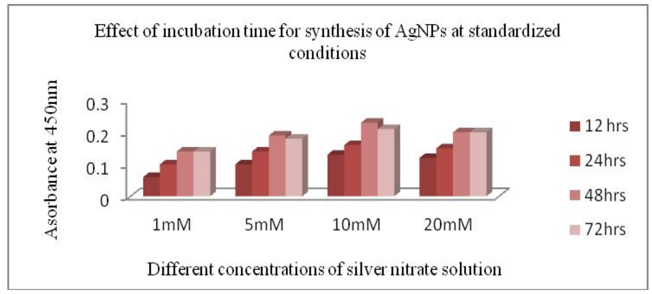

Fig. 7: Effect of concentration of both commercial and microbial dextran on green synthesis of AgNPs.

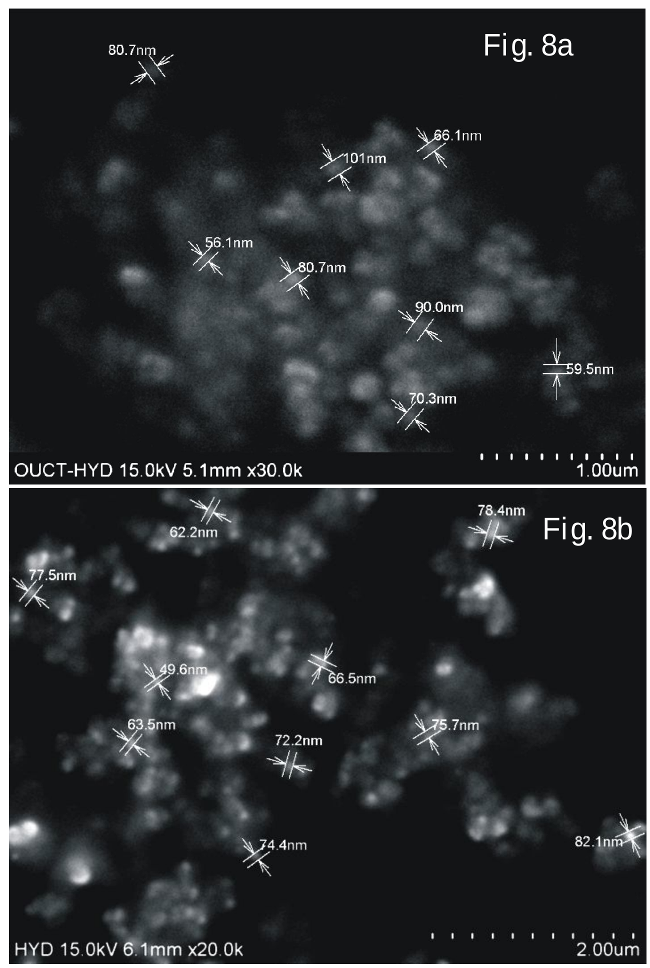

Fig. 8: SEM images of AgNPs synthesized without dextran (8a) and with dextran sample (8b).

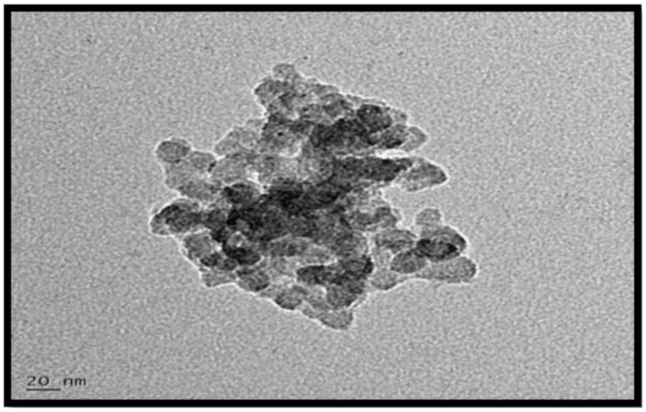

Fig. 9: TEM image of dextran mediated AgNPs.

incubation for maximum production of compound. The same was reflected in the present study, where the temperature effected the reaction between $AgNO_3$ and plant extract where the optimum reaction temperature for high productivity of AgNPs being 35ºC for all samples and similarly the optimum incubation period was found to be 48hrs (Fig. 5 & 6) respectively.

Dextran a branched aqueous soluble polysaccharide of glucose units can be used as a reducing and stabilizing agent (S.I. Easo *et al.*, 2016) and so in present study it was used in two variations like commercial dextran (CDx) and microbial dextran (MDx). Effect of dextrans (CDx and MDx) based samples in different concentrations for AgNPs synthesis when studied indicated that all the dextran samples have shown similar AgNPs synthesis pattern with optimum concentration for high productivity being 5% (Fig. 7). Till date there are several reports indicating the chemical synthesis of AgNPs using dextran and its amino derivatives as capping agent along with other compounds like ascorbic acid (Yang *et al.,* 2009) N, N methylenebisacrylamide (Yang *et al*., 2005) and sodium borohydride (Signori *et al.*, 2010). Until now there are no reports which describe the use of microbial dextran as a reducing and stabilizing agent for the green synthesis of AgNPs under various conditions and parameters as mentioned in this research. Therefore our research has become novel and offers a simple approach for highly productive and enhanced green synthesis of AgNPs.

The size and morphology of green synthesized AgNPs using 10mM $AgNO_3$, 3:2 dilution of plant extract and 5% of dextran was determined by Scanning electron microscopy (SEM) (Fig. 8a & 8b). SEM images indicate that the AgNPs synthesized using dextran as a reducing agent were spherical and comparatively smaller in size (49.6nm) than those AgNPs synthesized without dextran (58.1nm). This may be due to agglomeration or instabiltity. TEM image (Fig. 9) of synthesized AgNPs exhibit a typical spherical morphology with the particle diameter was found to be approximately 20nm. Therefore further stability studies were carried out for about 30 days with and without dextran and the results showed that AgNPs synthesized using dextran were more stable than AgNPs synthesized without dextran respectively.

The resulting stability of AgNPs for a period of 30 days with both the dextran samples as indicated in fig. 10 could be due to its combining with more Ag^+ ions that promoted the reduction reaction and also prevented agglomerization. The hydrophilic nature of dextran could enhance the water dispersion and stability of silver nanoparticles in colloid (Guili Yang *et al.,* 2012).

Conclusion

A novel, facile, economical, non-toxic and green synthesis approach is devised for mass production of AgNPs. The present procedure uses economically viable source of microbial dextran as a reducing, capping agent for easy scale up of the process. The AgNPs synthesized using this protocol has a good stability till 30 days without imparting any physico-chemical changes in their properties. The as-prepared AgNPs can be employed in myriad of applications ranging from medicine to biomedical devices, food storage to packaging, cosmetic to aesthetic industry and for purification applications. By employing this cost effective synthesis approach, a significant amount of small metal oxide nanoparticles like AgNPs, AuNPs, Cu/CuO, SiO2, Fe/Fe_2O_3 etc. can be commercially exploited for other applications respectively.

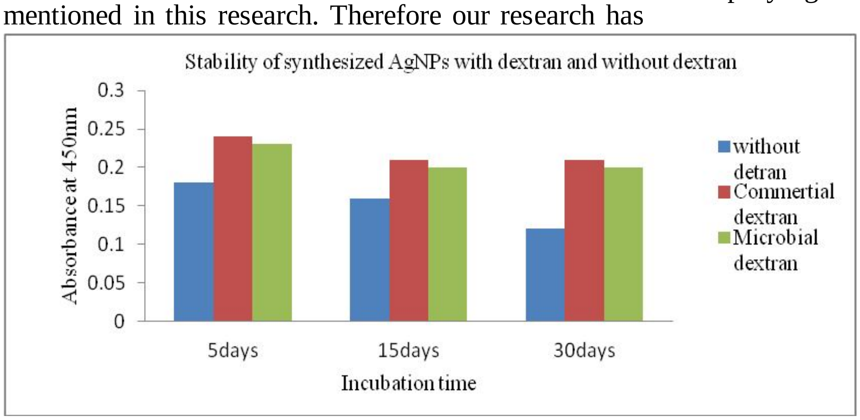

Fig. 10: Stability of green synthesized AgNPs with dextran (CDX & MDx) and without dextran.

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