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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING THE EXTRACT OF LEAFS OF *BETA VULGARIS* CROP

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ABSTRACT
The silver nanoparticles AgNPs was prepared using a simple, safe and cheap method, as called the green synthesis. The bio compounds of *Beta vulgaris* crop are acting as stabilising and reducing agents of silver ion ($Ag^{+} \rightarrow Ag^{0}$). The prepared nanoparticles were characterised by infrared spectroscopy IR, ultra violet spectroscopy UV and atomic force microscopy AFM. The investigations revealed that the silver nanoparticles with a brownish colour were prepared successfully. The functional groups that exist in the AgNPs were illustrated using the IR spectroscopy. The AFM analysis indicated that the average diameter of aggregated spherical particles was (81-99) nm. A kinetic study of AgNPs showed that the optimal conditions to achieve maximum yield and small size of AgNPs are (0.001 M of silver ion, (6:10) volume ratio of extract to silver nitrate, with 8 of PH and 40 C°). And the surface plasmon resonance band is placed at 450 nm.

KEYWORDS: *Beta Vulgaris,* AgNPs, green synthesis, nanoparticles, biosynthesis.

INTRODUCTION

Universally, researchers acquired the nanotechnology a massive attention. Nano materials have identifiable properties such as shape, size and distribution. Their properties are owing to their significance physicochemical properties and their surface to volume ratio with (1-100 nm) diameter which caused a significant difference in their physical, structural[1] electrical, optical, magnetic and chemical properties[2-4]. These properties enabled the nanotechnology to be used in a different area of applications[3] and research prospects[1] that can advance living standard of human beings[5]. The solutions of technological and environmental problems in the fields of solar energy conversion, catalysis, biology, biomedical science and water treatment were provided by the nanomaterials[5,6]. Three main methods are presumed to produce nanoparticles. These methods are physical, chemical and biological[3,7]. The physical and chemical methods of synthesis include electrochemical reduction, chemical reduction, heat evaporation and microwave irradiation. The biological methods used bacteria, fungi, alage and plants to achieve the synthesis of nanoparticles[8,9]. Green nanotechnology affords more applicable synthesis with anticipated produces[2].The green synthesis uses extracellular of plants and their derivatives. It has more advantages than the chemical and microbe methods. Environmentally, the green synthesis is more favourable. Hence, it is safe, non-toxic and economical[2]. The reducing, capping and stabilising agents of synthesis are existent in flavonoids, alkaloids and polyphonic compounds, the components of plants[2,5]. A great attention is given to the green synthesis of metal nanoparticles because their unique properties. Many studies are achieved on the nanoparticles of noble metals such as Ru, Pd, Ag, Pt and Au[1] because it's strong optical property of absorption in the visible region[10]. One of the most important nobel metals is silver[8]. The silver nanoparticles AgNPs showed essential qualifications such as physical, chemical and biological properties. The inhibitory and antibacterial effects of AgNPs are well known and studied significantly by researchers of nano-biotechnology. In addition, AgNPs have important electrical and optical applications[5]. A demonstration of the optimal conditions of green synthesis of silver nanoparticles with maximum product and small size was carried out by G. Annadurai *et al* [2].The study showed that the 00.1 M concentration of metal ion, high temperatures and the alkaline P^{H} are favourable. In the present work, a green synthesis method was used to produce AgNPs using an extract of leaf of *Beta vulgaris sub sp. cicla.* Furthermore, the kinetic study of synthesis reaction was take place. The Beta vulgaris has medicinal benefits as phytochemicals health protection in addition to its availability and its low cost. Four flavonoid compounds are found in this plant which discovered by a group of Egyptian researchers. Recently, new two flavonoids which have a protective effect of liver were discovered by a new group of Egyptian researchers. These flavonoids are flavonoid carbon glycoside and flavonoid herbicten-3A, β-zylozyl glycoside[11].

FIGURE 1: The graph of *Beta vulgaris*

METHODOLOGY

The biosynthesis of silver nanoparticles (Ag NPs)[1,2,5,7,8]

The fresh leafs of *Beta vulgaris* crop were collected locally. The leafs were washed using tap water several times to remove the contaminant. After the wash, 10g of leafs was cut into small pieces and then immersed into 100 mL distilled water. These leaves were boiled at 80°C for 30 min and filtered to get the extract. The extract was stored in refrigerator at 4°C for 24 hrs. Then, 10 mL of leaf extract was added drop wise with 0.001 M aqueous solution of silver nitrate (Ag NO_3). The mixtures were shacked for 2 min to get colloids and leaf for 24 hours. After 10 min, a dark brown colour was visually observed. This change of colour of mixture indicated the formation of Ag NPs.

The kinetic study of the bio reduction reaction[2,10]

Five of parameters; the concentration of silver ion, the ratio of leafs extract to $AgNO_3$, the PH, The temperature and the time of synthesis of AgNPs were varied to examine the best conditions of AgNPs biosynthesis.

The effect of concentration of silver ion

The solutions of silver nitrate $AgNO_3$ with the concentrations of (0.001, 0.002, 0.003, 0.004 M) were prepared and mixed with 10 ml of leafs extract to study the effect of concentration of $AgNO_3$ on the preparation of AgNPs.

The effect of volume ratio of leaf extract

(2, 4, 6, and 8) ml of extract was added separately to 10 ml of the aqueous solution of (0.001) M $AgNO_3$ as the ratio (2:10, 4:10, 6:10 and 8:10) ml to examine the influence of volume of extract on the nanoparticles formation reaction.

The effect of PH

The AgNPs was prepared at different environment of PH (2, 4, 6, 7 and 8). The PH was adjusted using (0.1 N) NaOH and (0.1 N) HCl.

The effect of temperature

The reduction process was carried out at different temperatures (25, 30, 35, and 40) C°.

The effect of time

The preparation of AgNPs was observed at 5, 10, 20, 30, 40, 50, and 60 min. The UV spectroscopy was used to investigate the effect of time on reduction reaction.

The characterization

In addition to optical detection, the preparation reactions and kinetic study were diagnosed using FT-IR spectroscopy, UV-VIS spectroscopy and atomic force microscopy AFM and scanning probe microscope.

RESULTS & DISCUSSION

The investigations of prepared AgNPs

The optical diagnosis

The formation of AgNPs using the extract of *Beta vulgaris* leafs was indicated by a change of colour of colloidal mixture to brown. The change as explained in literature [13 cited in 6] is result from the excitation of conduction electrons in nanoparticles induced by an electromagnetic field.

IR spectroscopy

The IR spectroscopy was carried out before and after preparation to investigate the functional groups of compounds. The spectrum of extract, Fig. 2 showed that there are narrow bands at 833 cm^{-1} assign to C-H vibration of phenyl group (alkene), 717 cm^{-1} assign to C-H vibration of aromatic group (benzene), 1172 cm^{-1} assign to C-O vibration of tertiary alcohol group, 1246 cm^{-1} assign to C-O vibration of ether, 1319 cm^{-1} assign to NO_2 functional group, 1450 cm^{-1} assign to C=C stretching vibration of aromatic group, 1523 cm^{-1} assign to N-O stretching vibration, 1639 cm^{-1} assign to C=N stretching vibration, 3275 cm^{-1} assign to O-H stretching vibration of alcohols and phenols and 3414 cm^{-1} assign to N-H stretching vibration of primary amine group.

The IR spectroscopy showed the functional groups of AgNPs. The spectrum reveals that there are narrow bands at 1054 cm^{-1} assign to N-H vibration of aliphatic amine group, 1130 cm^{-1} assign to C-O vibration of ester, 1381 cm^{-1} assign to NO_2 functional group and 1562 cm^{-1} assign to C=O stretching vibration of carboxyl group, Fig. 3.

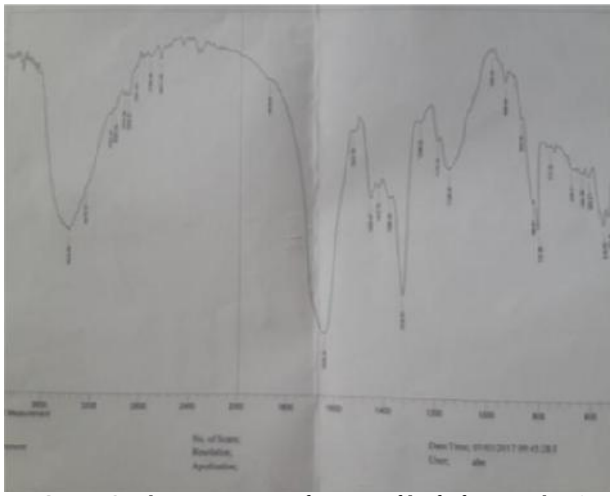

FIGURE 2: The IR spectrum of extract of leaf of *Beta vulgaris*

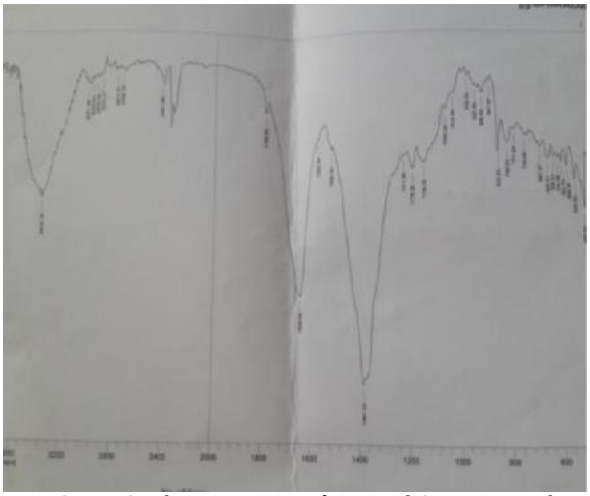

FIGURE 3: The IR spectrum of prepared AgNPs using the extract of leaf of *Beta vulgaris*

AFM analysis and scanning probe microscope

The shape, size and distribution of prepared nanoparticles were diagnosed using the atomic force microscopy AFM for two samples of AgNPs. The first sample was with the volume ratio of extract to $AgNO_3$ (6:10) ml. The result showed that the prepared particles were aggregated and spherical with average diameter equal to 81 nm, Fig. 4. The other one was with the volume ratio of extract to $AgNO_3$ (2:10) ml. The prepared particles were also spherical with average diameter equal 99 nm, Fig. 5. The aggregation of particles was interpreted as a result of existence of cell components on the surface of nanoparticles that act as capping agent.[2, 10]

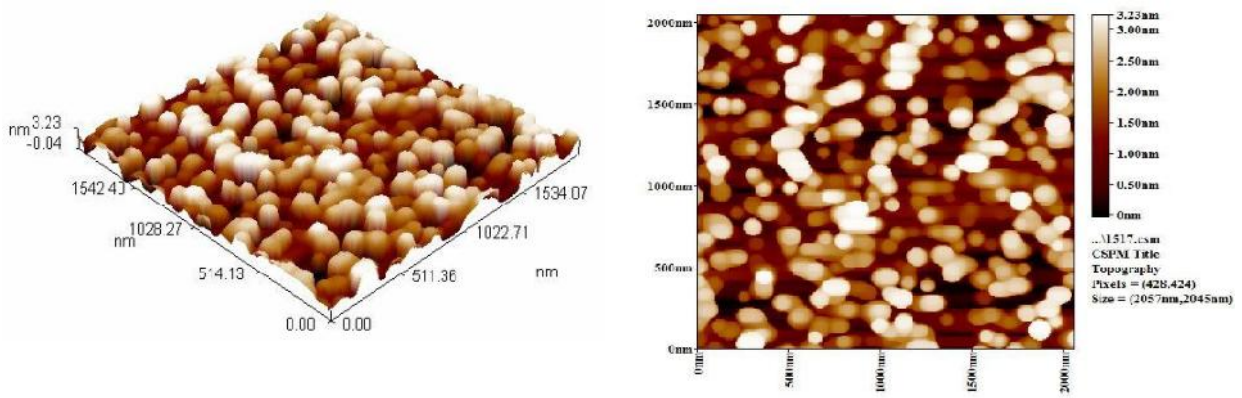

FIGURE 4: The AFM images of AgNPs prepared the volume ratio of extrat to $AgNO_3$ (6:10) ml

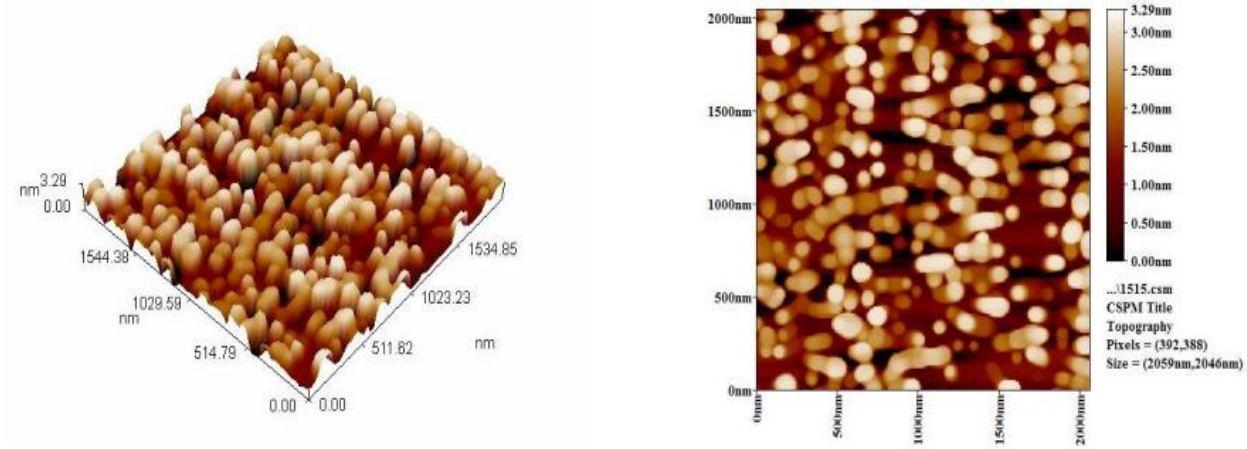

FIGURE 5: The AFM images of AgNPs prepared with the volume ratio of extract to $AgNO_3$ (2:10) ml

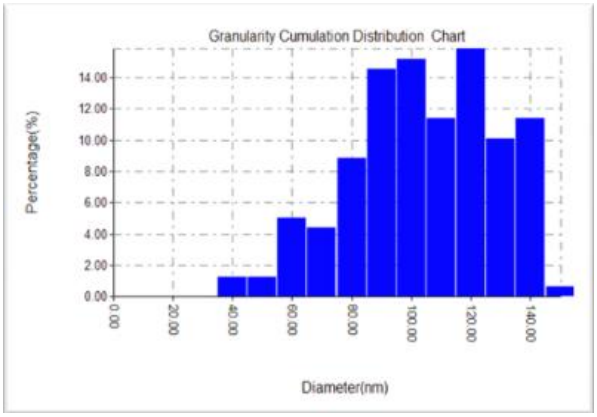

FIGURE 6: The scanning probe microscopy shows the cumutation distribution of granules prepared using 2:10 volume ratio of extract to $AgNO_3$.

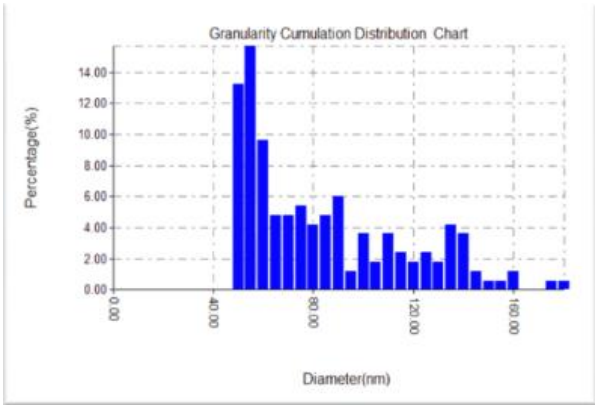

FIGURE 7: The scanning probe microscopy shows the cumulation distribution of granules prepared using 6:10 volume ratio of extract to $AgNO_3$.

The percentage of diameter of particles was determined using the scanning probe microscope of the two samples, Figures 6 and 7. The results showed that distribution of the growth granules of the sample with the volume ratio (6:10) ml was as the average diameter of particles was 81 nm, 130 nm for 90% of particles, and 70 nm for 50% of

particles. And the distribution of growth granules of second sample with (2:10) volume ratio was as the average diameter of particles was 99 nm, 130 nm for 90% of particles, 90 nm for 50% of particles and 60 nm for 10% of particles. Thus the (6:10) ml considers the optimal volume ratio to get small size of particles.

The Kinetic study of bio reduction reaction

The effect of conc. of silve

The results showed that 0.001 M is the optimal concentration to obtain maximum amount of precipitate of AgNPs. This result was monitored by UV spectrum. As shown in Fig. 8, the band of surface plasmon resonance SPR is centred at 450 nm. The maximum peak is related to the minimum conc. 0.001 M. The absorbance was increased at this conc. The narrow peak indicated the reduction of size of AgNPs which is a result of existence of functional groups in the leaf extract[2, 10]. For these reasons, the 0.001 M of silver nitrate considers the optimal concentration for synthesis of AgNPs. Thus, it is used in all later kinetic studies.

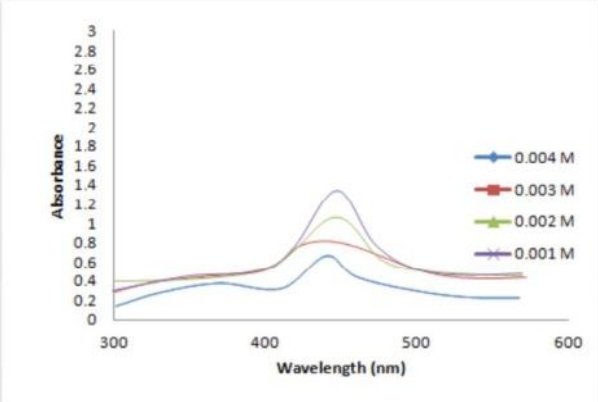

FIGURE 8: The UV spectram of AgNPs prepared with different conc. of silver ion

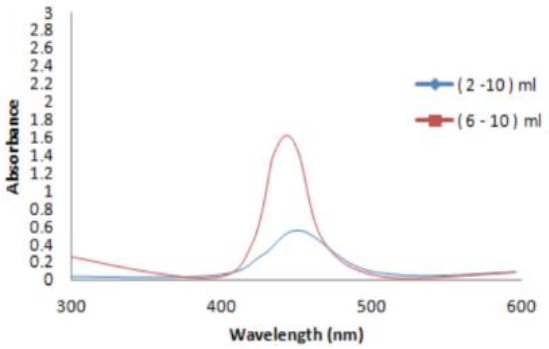

FIGURE 9: The UV spectrum of AgNPs prepared with different volume ratio of extract to $AgNO_3$

The effect of volume ratio of extract to $AgNO_3$

The UV spectrum shows that the maximum peak was related to the AgNPs prepared using the volume ratio (6:10) ml of extract to $AgNO_3$, Fig. 9. The peak is centred at 450 nm. It is nearly narrow indicating that the size of nanoparticles is small. The lower peak related to the AgNPs prepared using the volume ratio (6:10) ml was broad showing that the size of particles is large[2, 10].

The effect of P^H

The effect of PH was studied at different values (2, 4, 6 and 8). The highest peak of UV spectrum was related to bio synthesis at PH 8. It is cantered at nearly 450 nm, Fig. 10. And it is narrow. This result revealed that the alkaline environment is optimal to obtain spherical and small size of nanoparticles[2,10].

The effect of temperature

The UV spectrum of AgNPs prepared at different temperatures shows that the maximum peak is related to the nanoparticles prepared at 40 C°. It is centered at nearly 450 nm (violet peak), Fig. 11. This narrow peak indicating that the high temperatures are favoured to achieve a greatest product with a small size. Hence, the consumption of silver ion to form the nuclei is occurred at the high temperatures[2, 10].

The effect of time

A change of colour of mixture at 5, 10, 20, 30, 40, 50, and 60 min was monitored by the UV spectroscopy. It is noticed that the intensity and absorption are increased as the time of preparation increased.

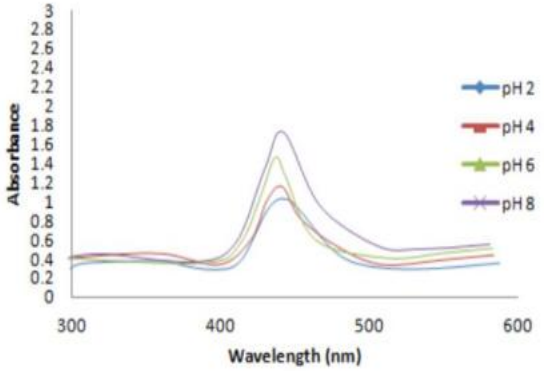

FIGURE 10: The UV spectrum of AgNPs prepared at different PH

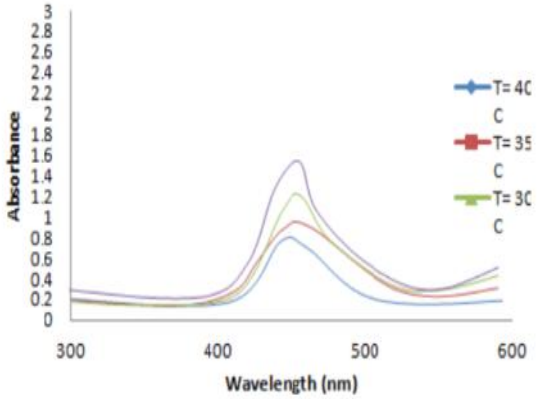

FIGURE 11: The UV spectrum of AgNPs prepared at different T

CONCLUSION

The green synthesis of nanoparticles that used leaf extract as stabilizing and reducing agent of metal ions is safe, easy and low cost method. The brown colour of mixture was observed after 10 min indicating the formation of nanoparticles. The IR spectroscopy and AFM and scanning probe microscope analysis showed that spherical nanoparticles were achieved successfully using the extract of *Beta Vulgaris* crop with silver nitrate. The average diameter of particles was (81-99) nm. The UV spectrum revealed that the conditions of bio synthesis of AgNPs as (0.001 M of $AgNO_3$, the volume ratio of extract to $AgNO_3$ was 6:10, PH=8 and T=40 C°) are optimal to obtain a yield of nanoparticles with small size. And the SPR band is centred at 450 which confirmed the formation of AgNPs.

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