GREEN SYNTHESIS OF SILVER NANOPARTICLES USING SPINACIA OLERACEA LEAVE EXTRACT AND THEIR PHYSICAL VERIFICATION

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ABSTRACT

Silver (Ag) nanoparticles synthesized from the eco-friendly green method plays pivotal role for its use in many versatile applications. The green chemistry approach has been successfully adopted to fabricate silver nanoparticles with an average diameter of 10 nm in size from the spinach (Spinacia oleracea) leave extract. The effect of heating in the synthesis process was checked for any change in the size of the silver nanoparticles, which is not pronounced much. Characterization of four samples of silver nanoparticles with different concentrations of the salt and the extract was achieved through UV-VIS, EDX and SEM measurements.

Keywords: *Nanotechnology, Nanoparticles, Spinacia Oleracea, Green Synthesis, SEM*

INTRODUCTION

Nanotechnology has revolutionized the modern research with the approach to design, synthesize and control the particle arrangement ranging from 1-100 nm. Fast growing methods in this prominent technology has opened new frontiers for the researchers to overcome the ever growing issues of the nature. Due to its diversity and command, nanotechnology is gaining a wide range of importance in all fields of life doing through health care to industrial applications. The great interest in field of nanoparticles is largely due to the exhibition of size and shape related properties required in miniaturization of the technology in bulk of the applications (Zhang, Sun, Banis, Cai & Sun, 2011). Noble metal nanostructures especially silver have considerable interest due to their unique electrical, optical and thermal properties with the promise of potential claims in optoelectronic devices, catalysis, biosensors, composite materials, cosmetic products and antimicrobial applications (Korbekandi & Iravani, 2012).

In the context of healthy ecological world, there is a growing need of the environment friendly, green synthesis for the nanoparticles and their systems of the metal nanoparticles and etc. Silver nanoparticles have been used broadly in the form of as antimicrobial agents in food industry, textile composition and in many environmental applications. Due to these properties, silver nanoparticles are becoming prime materials for medicines, various industries, animal husbandry, accessories, health and military. Silver nanoparticles demonstrate prospective antimicrobial effects against the infectious organisms such as

Escherichia coli, Bacillus subtilis, Vibria cholera and many more (Duran, Marcato, Souza, Alves & Esposito, 2007).

LITERATURE REVIEW

Fabrication of such using a plant extract as a reducing agent is far more advantageous than photochemical reaction, heat evaporation, electrochemical reduction and hazardous chemical procedures. The nanoparticles fabricated by the green method are non-volatile to human health, since the human body is exposed to a fast rate of small systems entering through lungs, intestinal tract and the skin (Albrecht, Evans & Raston, 2006). With such an exposure one can think of the harms they can provide to human organs and the ultimate crumbling the nature. Several bio-logical systems including bacteria, fungi, and yeast have been used in the synthesis of these nanoparticles. Because of wide range of applications, numerous methods relating to the formation of silver nanoparticles, and silver-related compounds containing ionic silver (Ag^{+})/metallic silver (Ag^{0}) have been developed using the plant leaves extracts.

Green synthesis of silver nanoparticles have been reported by many plant leaves extracts (whose botanical names) (Awwad & Salem, 2012) like mangosteen, Rosa rugosa, Stevia rebaudiana, Chenopodium, Macrotylomauniforum, Acalypha indica, Ficusbenghalensis, Trianthemadecandra, Cycas, Catharanthusroseus, Piper longum, Nico-tiana tobaccum and different other leaf plants. The other important parameter in the green synthesis of metal nanoparticles which is very little found in literature is variation of the size and morphology of the later with the amount of salt and leave extract. Maria, Devadiga, Kodialbail and Saidutta (2015) has related the amount of salt with the pH of the solution containing the solvent & soluble and in terns to the size of the silver nanoparticles synthesized. From the chemistry point of view, the percentage of the leave extract and ionic salt should result in the variation of the size of the crystalline material obtained, with the approach, that increase in salt amount could be possible reason for the increase in the clustering process of atoms and of the size of nanoparticles, if the amount of extract is kept fixed and vice versa.

In the present study we designed a simple, cost-effective and an environment protected synthesis method of the silver nanoparticles (Ag NPs) at ambient conditions using the spinach (Spinacia oleracea) leave extract as a reducing and stabilizing agent. The other important parameter in the green synthesis of metal nanoparticles which is very little found in literature is variation of the size and morphology of the later with the amount of salt and leave extract. Moreover with different characterization tools, it was tried to correlate the morphology & size of the small Ag nanoparticles with the quantity of salt and leave extract. The effect of length of time on the reduction of silver nanoparticles was also investigated for couple of samples with same concentration of the solvent and the soluble.

MATERIAL AND METHODS

Silver nanoparticles were synthesized using the spinach leave extract freshly collected from the local fields of Baffa near Hazara University, Mansehra. All other materials including the chemical, silver nitrate ($AgNO_3$), Whatman filter paper, distilled water and de-ionized water etc were purchased from the super chemical and scientific traders Rawalpindi, Pakistan. All glassware were washed with sterile distilled water many times and dried in an oven before use in order to remove any residue contaminants.

Preparation of Spinach Leaves Extract

Figure 1: Picture of the spinach (Spinacia oleracea) leaves obtained from the fields of Baffa near Hazara University, Mansehra. Fresh leaves were obtained for all the samples used in this case.

Fresh spinach leaves as shown in the figure 1, were washed few times through water in order to get rid of the dust particles and then dried in natural source of light to remove the residual moisture. The botanical name of the spinach is Spinacia oleracea and the leaves are rich in iron and contain potassium and some part of vitamins. There are carbohydrates, fats and proteins which make up the larger contents of the leaves. In the next step, spinach leaves were washed with distilled water for any possible impurity. The leaves extract used for the reduction of silver nitrate to silver nanoparticles (Ag) was prepared by mixing the 10 g of fresh spinach leaves dried up, cut into very small pieces with 100 ml de-ionized water in 250 ml beaker and the mixture was stirred at 100^{o} C for 30 minutes continuously. After boiling, the mixture was cooled and then filtered with Whatman filter paper and then centrifuged for 30 mints at 4000 rpm. For further experiments the extract was collected and stored at room temperature. The details are mentioned in the table 1.

Table 1. Preparation scheme of spinach leave extract for synthesis of silver nanoparticles

Spinach leaves	De-ionized water	Temperature	Stirring	Centrifuge (4000 rpm)
10 gm	100 ml	100^{o} C	30 minutes	30 minutes

Synthesis of Silver Nanoparticles

In a typical reaction procedure, the 1 m Mole (mM) of $AgNO_3$ aqueous solution was prepared in100 ml de-ionized water in 250 ml beaker and was used for the production of silver NPs. The 50 ml of 1 mM $AgNO_3$ solution was taken and treated with 5 ml of spinach leaves filtrate and heated the mixture for 1 hour at 90^0C for reduction into Ag ions. For 1 mM silver nitrate solution the extract acts as reducing and stabilizing agent. The resulting solution becomes grey-black in color after 60 minutes, indicating the formation of Ag NPs (Awwad & Salem, 2012). The concentrations of AgNO3 solution and spinach leaves extract were also varied from 50ml to 150ml $AgNo_3$ solution and 5ml to 50ml leaves extract as well as temperature and time duration is also varied detail is shown in table given below. The formation of silver nanoparticles from the Spinacia oleracea is likely to be affected by the amount of carbohydrates present in the leaves and may acting as reducing agent to cause the formation of silver ions and nanoparticles.

Table 2. Detailed information listed for the synthesis of silver nanoparticles production

Sample	$AgNO_3$ concentration	$AgNo_3$ solution	Extract	Time	Temperature	Centrifuge (4000 rpm)
Sample 1&2	1 mM	50 ml	5 ml	1 hour	90^0C	30 minutes
Sample 3	1 mM	150 ml	15 ml	1 hour	90^0C	30 minutes
Sample 4	1 mM	150 ml	50 ml	5 days	At room temperature	30 minutes

Characterization Techniques

UV-VIS spectroscopy is valuable technique to characterize the transmission, absorption and reflectivity of a number of compound and for scientifically important material, such as coating and pigments etc. The Ultraviolet and Visible spectrum have wide features that are of use for sample identification but are extremely helpful for quantitative measurements. The other method used for the determination of silver nanoparticles was scanning electron microscopy (SEM) analysis. The resolution and quality of SEM images are function of three main parameters: (i) specimen nature, (ii) performance of instrument and (iii) imaging parameters selection. All the three aspects run parallel and neither of them must or can be unobserved or over emphasized. One of the most amazing aspects of SEM is the obvious ease by which Scanning Electron Microscopy images of three dimensional substances can be interpreted by some spectator by no previous information of the apparatus. This is rather amazing in view of the strange method in which image is created, which seem to be different greatly from usual human being knowledge by images created by light and view by eye.

RESULTS AND DISCUSSION

The bio-reduction of silver nitrate to silver nanoparticles was analyzed mainly by the UV-VIS spectroscopy and SEM analysis with EDX for the morphological and elemental investigation. The study is interesting since the percentage of the amount of salt and leave extract in samples is the main line and the particle size is related with this.

Visual Observation and UV-VIS Spectroscopy Data

The production of silver (Ag) nanoparticles by the procedure of biosynthesis using the spinach leaves extract was checked and confirmed through UV-Vis spectrometer to determine the formation, beside from the color identification. In figure 2 the absorption spectra is plotted against the range of wavelength, here from 200-800 nm. The stability and production of Ag NPs have been shown by the surface Plasmon resonance appearing around 425 nm, a specific value for the observation of silver nanoparticles (Korbekandi & Iravani, 2012). The UV-Vis spectrum was repeated as a function of the course of reaction time, as the mixture of leaves extract was mixed with the silver nitrate and was used to record the formation of Ag nanoparticles, has been shown in figure 3(a). The spectra is recorded for different reaction times shows that at longer times the Ag NPs settled well and display a higher peak in the absorption spectrum, also there is an increase in the number and size of the Ag NPs as previously recorded (Awwad & Salem, 2012).

The overall broadness of the spectra and shift in the absorbance peak indicates that silver nanoparticles require some time to settle down, which mean the reaction takes time to complete but as far as the size of these particles are concerned, it has been mentioned that their size grows slightly in comparison to initial stage of the reaction when the extract is being mixed with the salt. In figure 3(b), the same procedure is repeated for all the samples reported in this paper and the formation of the Ag Nps was confirmed in the three cases (Roopan, Madhumitha, Rahuman, Kamaraj, Bharathi & Surendra, 2013). UV-Vis spectra for the other three samples obtained by the same synthesis procedure adopted for the first sample. It can be seen that each sample roughly peaks around the absorption resonance calculated for the silver nanoparticles, thus confirms the presence of the Ag NPs.

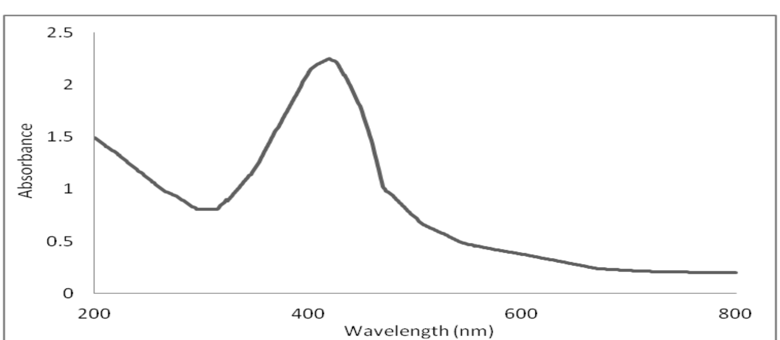
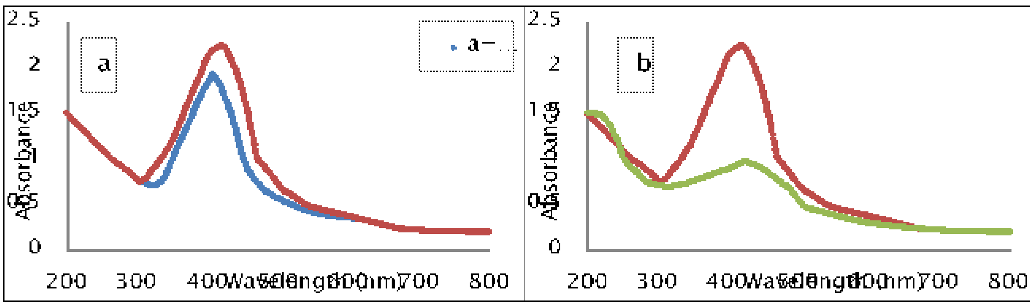


Figure 2: UV-Vis spectra for the pure Ag NPs resulted after the mixing of the leaves extract in salt solution. The maximum appears about 425 nm, which is roughly in the range for the absorption spectra of the synthesized (reported) silver nanoparticles.

Figure 3: UV-Vis spectra for the pure Ag NPs resulted after mixing of the leaves extract in salt solution. (a) This is for the sample 1 with respect to time as mentioned; while (b) is for the other samples.



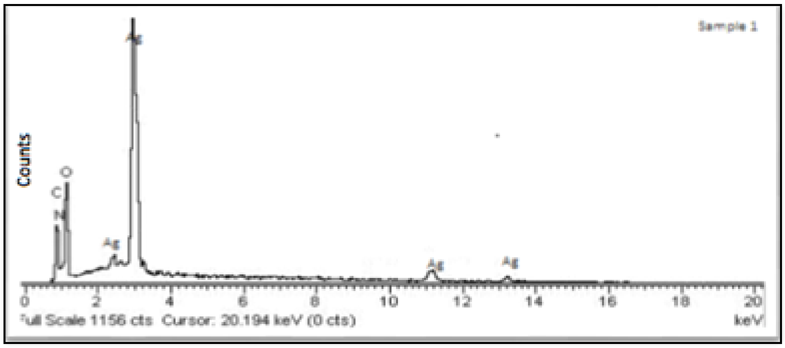
Structural & Morphological Analysis using SEM

Systems composed of nanoparticles have huge physical significance that comes from the fact that we can manipulate and treat these compounds at very minute level and with their intrinsic/built-in properties in contact. This is largely achieved with the help of microscopy measurements of such systems. Scanning electron microscopy is a tool that allows seeing the invisible world of micro & nano-space. The structure and morphology of small scaled systems is inevitable to access through light microscope, so we have an alternate (SEM) with which it is possible to carry out all those measurements enabling to tell the inside story. Scanning electron microscopy is used extensively in this thesis for the analysis of all samples containing silver (Ag) nanoparticles made from 1mM $AgNo_3$ salt concentration.

EDX Analysis

The compositional study of the prepared nanopowder of Ag nanoparticles was done through the energy dispersive x-ray (EDX) study of the samples under consideration, as shown by the figure 4. The EDX spectrum showed the presence of Ag elemental peak along with small clinks of carbon and oxygen, related to the surface and may be moisture in the sample. The emission energies of the Ag are in the range of 2-4 KeV, which are consistent with already found from the same process (Torresday, Gomez, Videa, Parsons & Troiani, 2003).

Figure 4: The EDX analysis diagram indicating the Ag element in the sample 1 and the same has been obtained for all the samples.



SEM Analysis

In this portion the morphology and the size of the Ag nanoparticles from the spinach (Spinacia oleracea) leave extract is discussed using the scanning electron microscopy data obtained for different samples. The SEM analysis of the samples was made using the HITACHI SU-1500, from the CIIT, Islamabad, Pakistan.

Sample 1 & 2

As mentioned in portion 2.2, in the first of such samples contain 1mM of the salt in treatment with 5ml of the leave extract. The SEM image of both the samples is displayed in figure 5, with sample 1 (heated) and sample 2 (non-heated) and of the same concentration as mentioned.

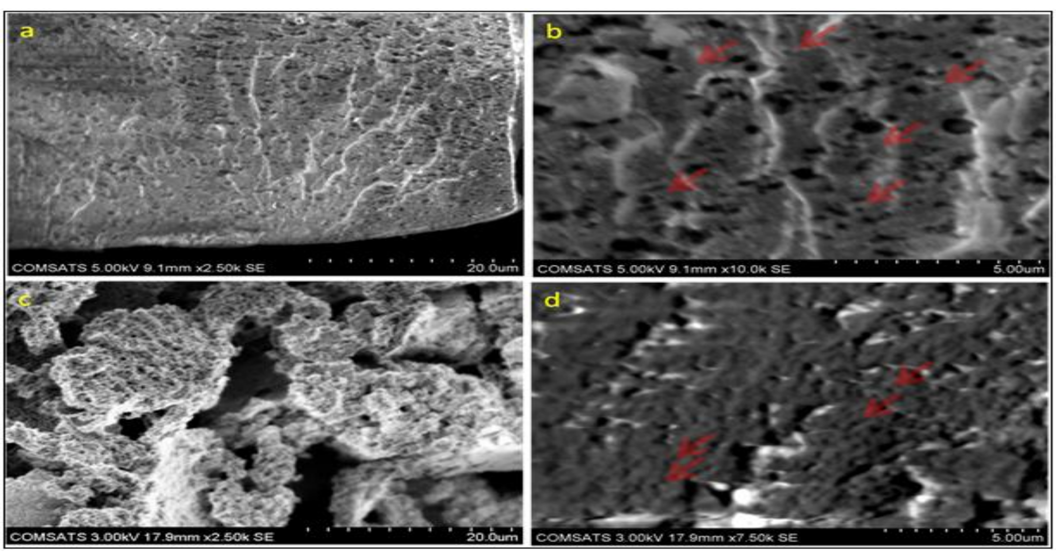


Figure 5: SEM images of sample 1&2 with 1mM $AgNO_3$ salt concentration and 5ml of Spinacia oleracea leave extract. Figure 5(a & b) are for sample 1, with lower and higher magnification respectively; whereas figure 5(c & d) are for sample 2. SEM images show

the circular shape Ag nanoparticles with almost uniform size distribution, except for agglomeration.

The SEM images of the Ag nanoparticles show the formation of spherical shape particles with very small size distribution. The formation effect related to heat is affected a bit, with the heated sample producing large number of particles as compared with the non-heated sample. The size distribution in both the cases is much less pronounced with heat as can be seen in figure 6, displaying the histograms of the samples. The SEM images were analyzed using the MATLAB and also the ImageJ software. For the first sample the analysis reveal 18 bit depth with the image resolution of 739×552 and the file size of 1.6 MB in the image format of Tiff. The same for sample 2 resulted in 20 bit depth with the image resolution of 802×639 and the file size obtained was 2 MB in the Tiff image format. The data from both the software was consistent and also with the previous work (Nazeruddin, Prasad, Prasad, Garadkar & Nayak, 2014; and Forough & Farhadi, 2010).

The average size of the Ag nanoparticles remain in the low nanometer range as shown in the below figure. In the figure the histograms of the samples were estimated from the ImageJ software, where one can select a portion of the SEM image which is evenly illuminated and can get the particle size. During this method the large formation of nanoparticles due to agglomeration as can be spotted in the SEM images of the samples is neglected and a portion with small distribution of the size of nanoparticles were taken and estimated. This data really helps us to highlight the size of Ag Np's and was found to be really interesting since the average particle size is around 10 nm.

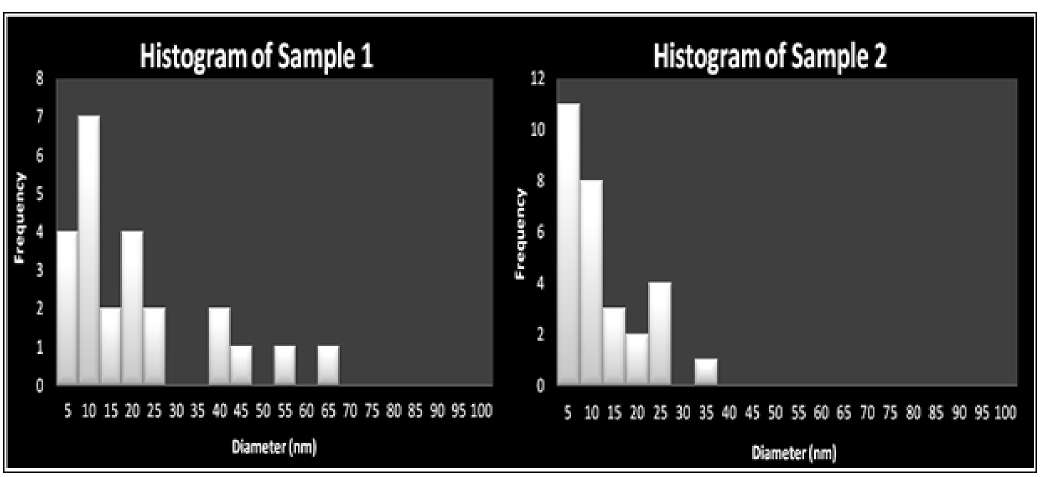


Figure 6: Histogram of both samples. The data is obtained from the SEM images at higher resolution of respective sample and using the ImageJ software. The average size of the Ag nanoparticles in samples is almost 10 nm.

Sample 3 & 4

Next we increase both the solvent amount and also the solvable, with exact 150ml of 1mM $AgNO_3$ plus 15ml of Spinacia oleracea leave extract. In this case sample 3 is heated while sample 4 was non-heated to observe any change. The SEM images of these samples and the histogram are shown in figure 7 and 8 respectively.

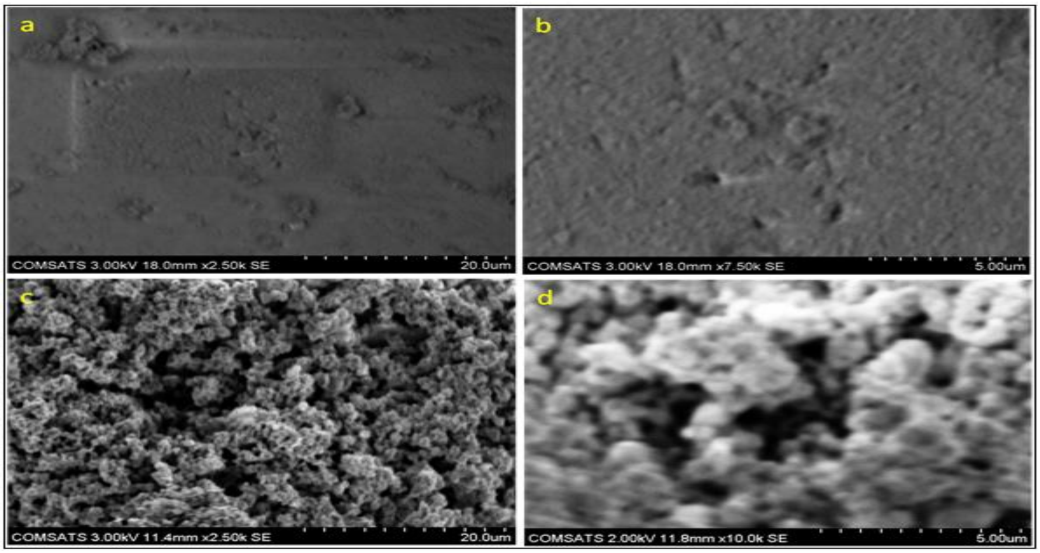


Figure 7: SEM images of sample 3 & 4 with 1mM $AgNO_3$ salt concentration and 15ml of Spinacia oleracea leave extract. Figure 7(a & b) are for sample 3, with lower and higher magnification respectively; whereas figure 7(c & d) are for sample 4. SEM images show the circular shape Ag nanoparticles with almost uniform size distribution, except for agglomeration.

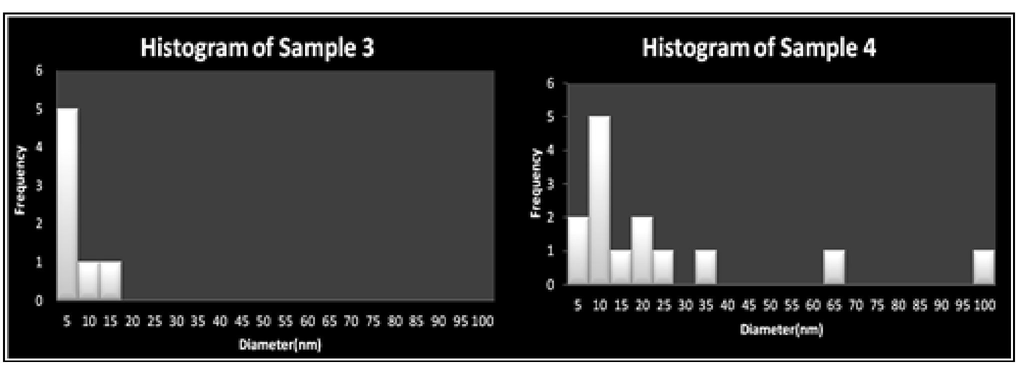


Figure 8: Histogram of samples 3 & 4. The data is obtained from the SEM images at higher resolution of respective sample and using the ImageJ software. The average size of the Ag nanoparticles in samples is around 10 nm (consistent with the first two samples).

It is observed that the increase in the amounts of salt and leave extract does not affect much on the size of the silver nanoparticles and as was thought the increase in the number of nanoparticles produces is seen from figure 7. The heating adopts the same pattern as for the first two samples, that in this case sample with heating has lot more increased number of particles as compared to the non-heated one (sample 4). Moreover sample 4 was left for couple of days, but the time effect cannot be pronounced from the given results. The histogram of these two samples is shown in figure 8 and it can be said that the particle diameter is unaffected with the amount of solvent and soluble and remains closed to 10 nm on average.

The analysis of SEM image for sample 3 reveals 21 bit depth with the image resolution of 727×565 and the file size of 1.6 MB in the image format of Tiff. The same for sample 4 results in 15 bit depth with the image resolution of 642×481 and the file size of 1.2 MB in the image format of Tiff. The effect of heat, concentration and molar ratio on the synthesis of nanoparticles has been investigated by many authors Maria el at., 2015; Torresday et al., 2003; Ghorbani & Soltani, 2015 and Veerasamy, Xin, Gunasagaran, Yang, Jeyakumar & Dhanaraj, 2011) but not from the Spinacia oleracea leave extract synthesis of Ag Np's. Moreover it was further revealed that the average size of the nanoparticles from the present study is much smaller as compared with the previous results.

CONCLUSION

In this study we have synthesized the small scale silver nanoparticles from the fresh spinach (Spinacia oleracea) leave extract from the silver nitrate salt by the non-toxic, non-hazardous green synthesis technique. The confirmation of the Ag nanoparticles was done by the help of UV-VIS and EDX analysis, which confirms that the sample under study contain silver metal with no other contaminated material. We have investigated four samples, two with same molar ratio and the other two with increased amount of solvent and the soluble but with same molar ratio. The SEM analysis of the samples results extra small size Ag nanoparticles with a median size of 10 nm, which makes this green method a useful technique to develop small particles for multiple use with no toxic or damaging effects to the environment. The results also confirms that the heating does not affect the size of the nanoparticles for the same amount of salt and extract but only increases the number of particles as compared with the non-heated sample. Silver nanoparticles synthesized by this method have very useful applications in many versatile spaces.

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