Int J Pharm Bio Sci 2014 Oct; 5(4): (B) 759 - 770

Research Article Nanotechnology

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

# GREEN SYNTHESIS OPTIMIZATION AND CHARACTERIZATION OF SILVER NANOPARTICLE SUSING AQUEOUS EXTRACT OF *CROCUS SATIVUS L.*

**NERAN ALI THAMER AND LAMIA A. ALMASHHEDY***

*Iraqi Center for Cancer and Medical Genetic Research/ University of Almustansiria*
**College of Science/University of Babylon*

## ABSTRACT

Green chemistry means utilization of various plant resources for the synthesis of metallic nanoparticles. In the present study silver nanoparticles was synthesized in a single step by a green biosynthetic method using an extract of *Crocus Sativus L* act as a reducing and capping agents. The nanoparticles were characterized usingUV-Vis, X-ray diffraction (XRD) and Dynamic light scattering (DLS) .The prepared silver nanoparticles(AgNPs) showed surface Plasmon resonance centered at 440 nm. The XRD pattern showed the face centered cubic structure of silver nanoparticles. The size rangefrom19.9nm to 28.1nm was determined by using laser particle size analyzer. The current research work investigates the findings of optimization of different experimental variable conditions like time,temperature,reaction pH, silver nitrate concentration and the mixing ratio of the reactants on silver nanoparticles synthesized. The optimized condition for the synthesis of silver nanoparticles revealed that silver nitrate concentration was 1 mM, temperature was70°C, PH was 9, incubation time 1 h and aqueous extract and silver nitrate ratio was 5:95.

**KEYWORDS**: ***Crocus Sativus L,*** **silver nanoparticles, XRD,Dynamic light scattering**

**LAMIA A. ALMASHHEDY**
**College of Science/University of Babylon**

# 1. INTRODUCTION

Nanotechnology is the field of science which involves the synthesis and development of various nanomaterials with size less than100 nm[1].In the field of nanotechnology different concepts of engineering,electronics, and material science are applied in molecular or submicron level[2]. Colloidal particles are starting point for the generation of micro and nanostructures[3]. Metal nanoparticles have attracted much attention in the fields of physics, chemistry, electronics and biology [4] because of their unique electrical [5] chemical [6], optical [7]and photo electrochemical[8] properties. Properties of metal nanoparticles are different from those ofbulk materials made from the same atoms which could be attributed to their size, distribution and morphology[9]. Silver has the highest electrical and thermal conductivity among all metals[10].Different physical , chemical and biological methods have been developed for synthesis of nanoparticles[11].Biological methods using fungi, bacteria, algae[12] and plant extracts have advantages over other methods because of their systems are single step in nature, environment friendly (green chemistry), no need for, hazardous chemicals, high pressure, energy, power and simply scaled up for large –scale synthesis[13].In biological synthesis the reducing agent can be any bio source[14].Many plants are becoming probable sources for reducing and stabilizing agents for green synthesis of nanoparticles[15-17]. Silver nanoparticlres have shown great attention because of their unusual physical , chemical, electronic, catalytic, magnetic antibacterial and biological activities[18].Its were used in broad band of applications like biomedical[19] ,drug delivery[20] food industries[21],agriculture[22] ,water treatment[23] ,as an antioxidant[24], antimicrobial[25] and anti-cancer[26]. Plant or plant extract, it is always more suitable than organisms as to avoid its pathogens, laborious ,time consuming and hygiene maintenance procedures of cell culture[27].Synthesis of silver nanoparticles has been reported from different plant extracts [28-34] Saffron , the dried stigma of *Crocus Sativus L.*is aplant widely used as herbal medicine or food coloring,and as aflavoring agent[35]. The main substances of saffron are crocins,picrocrocin , safranal, ketoisophorone, isophorone and glycosidic terpenoids[36]. Beside its traditional medicine[37-40] saffron have demonstrated its antitumor effects[41],free radical scavenging properties[42], hypolipaemic effects[43]and in neurodegenerative disorder accompanying memory impairment[44]In the present investigation, the synthesisof sliver nanoparticles emphasizes the potentiality of *Crocus sativus*(saffron) in reducing and stabilizing silver nanoparticles.

# 2. MATERIALS AND METHODS

### *2.1 Materials*

Silver nitrate, AgNO3 , was obtained from SigmaAldrich and used without further purification.All other reagents were of analytical grade with maximum purity.All glassware's were properly washed with distilled water and oven dried before use.Saffron has been collected from the market.

### *2.2 Preparation of aqueous extract of C. Sativus*

Fifty g of *C.sativus* stigmas (saffron stigmas) were macerated for 3 days in one liter of deionized water at room temperature .The aqueous extract was filtered using filter paper. The filtered extract was stored at room temperature for further use.

### *2.3 Preparation of silver nanoparticles*

Silver nitrate aqueous solution (1 mM ) was prepared and used for synthesis of silver nanoparticles. 5 ml of aqueous extract of *C .sativus* was added to 95 ml of aqueous solution of 1 mM Ag $NO_3$ and heated with stirrer at 70 °C for 60 min .The formation of brown color was indicated synthesis of silver nanoparticles. To study the optimum factors for silver nanoparticles synthesis , the experiments were carried out in different conditions are silver ion concentration ( 0.25, 0.5 ,1 ,2and3 m M ) pH(1 ,3 , 5 , 7 , 9 ,and 11) ,temperature (40 °C ,50°C ,60°C ,70°C and 80°C),time( 10 min ,20 min ,40 min , 60 min and 80 min )and the *C .sativus* aqueous extract to silver nitrate ratio ( 10:90 , 5 :95 , 2.5 :97.5 ,1 : 99 and 0.5 :99.5 ). The pH of the reaction was adjusted by using 0, 1 N sodium hydroxide and 0.1 N Hydrochloric acid.The effect of these parameters on the synthesis of silver nanoparticles was monitored by UV-Vis spectrophotometer.

***2.4 Characterization of green synthesis silver nanoparticles***

***2.4.1 By color change***

The color change in the reaction mixture was recorded through visual observation.The color change from yellow to dark brown indicated that the silver nanoparticles were synthesized.

***2.4.2 UV-Visible spectral analysis***

The reduction of silver ions ($Ag^{+}$) to silver nanoparticles ($Ag^{0}$) was spectrometerically identified by double beam UV-Vis spectrophotometer (PD-303 UV) at different wavelength( 300-800 nm ) .The graph of wavelength on X-axis and absorbance on Y-axis.Using deionized water as a reference .

***2.4.3 X- Ray diffraction studies (XRD)***

The synthesized silver nanoparticles were centrifuged at 15,000 rpm for 15 min. and collect the pellet. The pellet was washed with distilled water to remove any purity and dried to get the powder. The X-ray diffraction assay was performed for the detection of crystalline nature of the metal nanoparticles was done by X-ray diffractometer Shimadzu XRD-6000 AS (3K .NOPC).

***2.4.4Dynamic light scattering,***

Dynamic light scattering (DLS) is an important technique used to investigate the size distribution pattern and average particles size of the biosynthesis AgNPs present in suspension or solution(45). The prepared sample was dispersed in deionised water followed by ultrasonication.Dynamic light scattering (DLS) measurements were carried out on ABT-9000 Nano particle size analyzer

# 3. RESULTS AND DISCUSSION

***Characterization of silver nanoparticles***

***3.1. By color change***

The checking of formation of AgNPs was primary well known by the color change from yellow to brown (Figure 1 ) while adding *Crocus sativus* extract into silver nitrate solution.The color formation occurred within 10 minutes.This change in color due to the excitation of free electrons in nanoperticles(45)which gives the surface plasmon resonance (SPR) absorption band by the combined vibration of electrons of metal NPs in resonance with light wave(46).Metal nanoparticles exhibits different colors in solution due to their optical properties(47).The solution was stored at room temperature for 24 hours for the complete settlement of nanoparticles. After 24 hours the reaction mixture was centrifuged at 15000 rpm for 15 min and pellets were collected followed by washing with deionized water then freeze-dried,powdered and use for XRD.

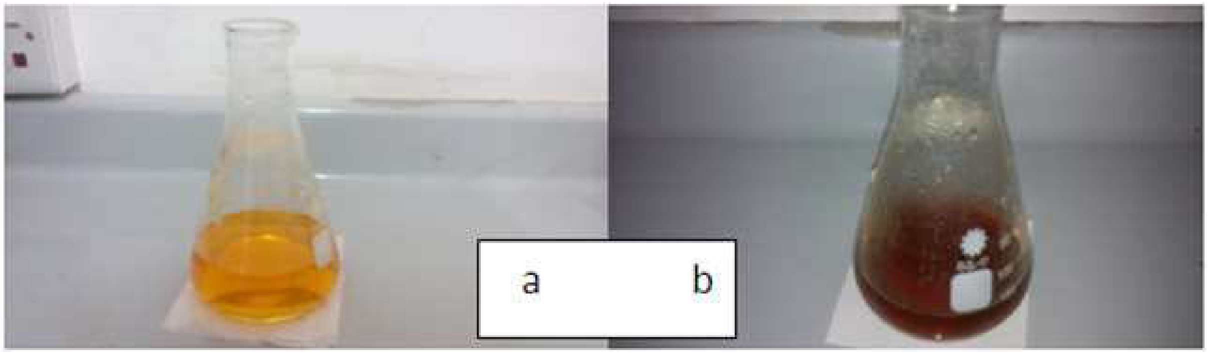



**Figure 1**
***(a) Crocus sativus(saffron) extract ,(b) brown color indicate the formation of silver nanoparticles*.**

***3.2. UV-Visible spectral analysis***

***3.2 .1 Effect of silver ion concentration***

The UV –Vis spectrum (Figure 2) shows the effect of silver nitrate concentration in the silver nanoparticles synthesis by using *Crocus sativus* extract .Characteristic surface Plasmon absorption band was observed at 440 nm for the brown colouredsilver nanoparticles synthesized from the 1 mM silver nitrate. The absorption was increased while increasing the concentration of silver ions from 0.25 mM to 1mM ,but by increase the concentration of silver ion there was a fall in absorbance.The study of Srivastava et al(48)has been found that the maximum peak was observed in 0.5 mM silver nitrate . In another study the optimum concentration of silver nitrate was observed 0.7 mM (49).In the present study 1 mM concentration was the optimum condition for AgNPs as shown in (Figure 2). The study of Ravichandran et al(50) has been found that the maximum peak was observedin1 mM silver nitrate solution support our data. Bashir et al(51) was found that the optimum concentration of silver nitrate is 1mM, these observations were another study in agreement with our finding.

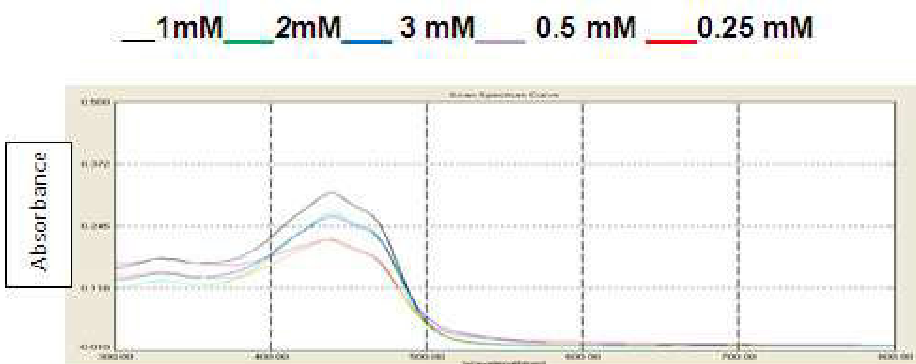
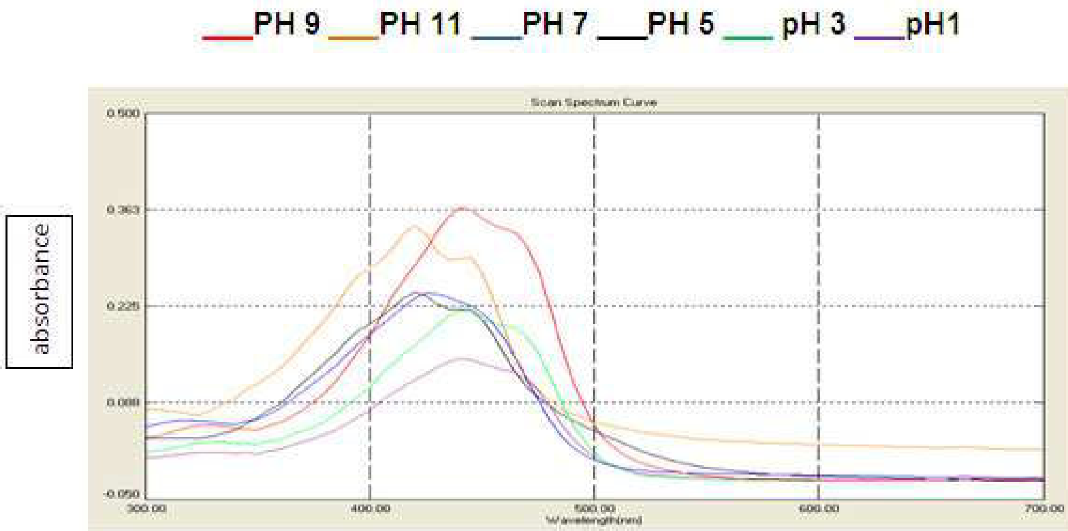



**Figure (2)**
***UV spectrum of synthesis of silver nanoparticles at different concentration of $AgNO_3$***

***3.2.2Effect of PH***

PH play an important role in the nanoparticles synthesis ,the effect of pH on synthesis of silver nanoparticles was tested under a different pH range( PH1-11) as shown in Figure (3).




**Figur (3)**
***UV spectra show the effect of PH in nano particles synthesis***

The UV-Visible absorption spectra shows that the maximum absorption at PH 9. Increase in pH the observed decrease in absorption. So in current study the optimize pH observe was 9 .In low pH ,small with broadening shape of silver nanoparticles(52).Sathishkumer et al (53SPR band was formed indicates formation of large size of nanoparticles(47),while the sharp peak indicates formation of spherical) observations study the effect of pH on the shape and size of AgNPs and found that great number of particle formation in alkaline pH while in acidic pH increase aggregation of nuclei instead of formation .The present investigation indicates alkaline pH is more suitable for synthesis of silver nanoparticle , the blue shift in absorption pattern confirmed formation of relatively smaller NPs .G.Annadural et al(47)reported that silver nanoparticles show maximum stability at the PH 8.2 ( very close to our finding).

### *3.2.3 Effect of temperature*

Temperature another physical factor which play an important role to control the nucleation process of nanoparticle formation. As shown in Figure 4 the absorbance increase by increase the temperature from 40° C to 70° C. and then decrease at higher temperatures.The study of Amit ,Abhishek and Uttam(54)has been found that the absorbance increased with increase of temperature from 25 to 45°C and then decrease at higher temperature .these follow the same pattern as follow in our study.Again, our study finding was close agreement of the previous study (50). The higher rate of reduction was occurred at higher temperature due to the consumption of silver ions in the formation of nuclei, whereas the secondary reduction was stopped on the surface performed nuclei (55). Bashir et al(51) was observed that the maximum formation of AgNPs was observed at75° C( very close to our finding).

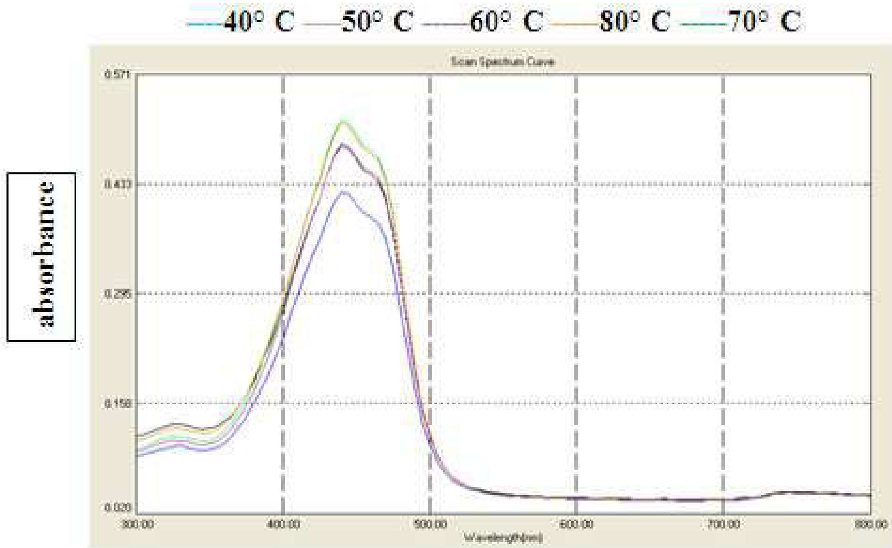



**Figure 4**
***UV-Visible spectra of AgNPs showing effect of different reaction temperature***

***3.2.4 Effect of time***

Time is another factor which effect the formation of silver nanoparticles.Figure 5 shows the increasing of reaction time resulted in gradual increasing of absorbance spectrum with SPR at 440 nm and the color intensity increased with the duration of incubation and the maximum absorbance was investigated at 1 h of reaction time. The intensity of the SPR peak increased as the reaction time increased which indicated the increased concentration of the silver nanoparticles .This result means that the silver nanoparticles prepared by this green synthesis method is very stable without aggregation. The study of Chandran et al [56] found that absorbance of silver colloid solution increased with span of time and maximum absorption was observed after 12 hrs of reaction. In the present study the optimum duration condition 60 min..Preeti,D. et al[57] was found The formation of AgNPs within 30 min incubation.The intensity of SPR increase up to 8 hrs and there was no changein SPR within the reaction time period Another study[50]the optimum duration necessary for the end of reaction was 60 min, which is a close agreement to our finding.

***3.2.5 Concentration ratio of silver nitrate and saffron extract***

The effect of extract concentration in the mixed solution on the biosynthesis of silver nanoparticles was investigated. The difference concentration of extract in the mixed solution was obtained by changing the volume of the added extract solution different volumes(0.5 - 10 ml ) of extract were added to 100ml of 1 mM silver nitrate . Optimization of concentration ratio of silver nitrate and extract was observed at ratio 5: 95 and at high ratio (10:90) ,the absorption was decreased as shown in Figure 6. It was reported in literature[50]that optimal extract and metal salt ratio was necessary for the symmetrical nanoparticles preparation is 1:19 ,these observation was a close agreement to our results. Another study reported [58] that the optimal 6 ml of leaves extract and 50 ml of 1 mM silver nitrarte. It was reported that[50]5:95 ratio of leaves extract in the mixture of reaction was efficient for the synthesis of silver nanoparticles( a close to our finding).

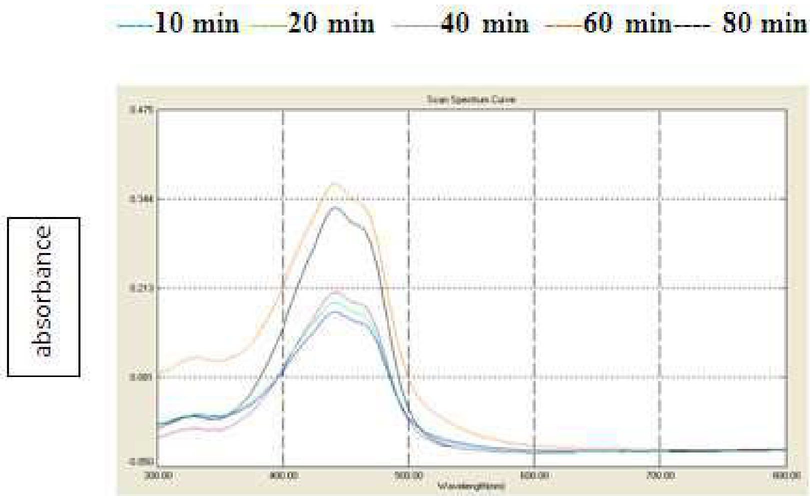



**Figure 5**
***UV-Visible spectra of AgNPs showing effect of reaction time***

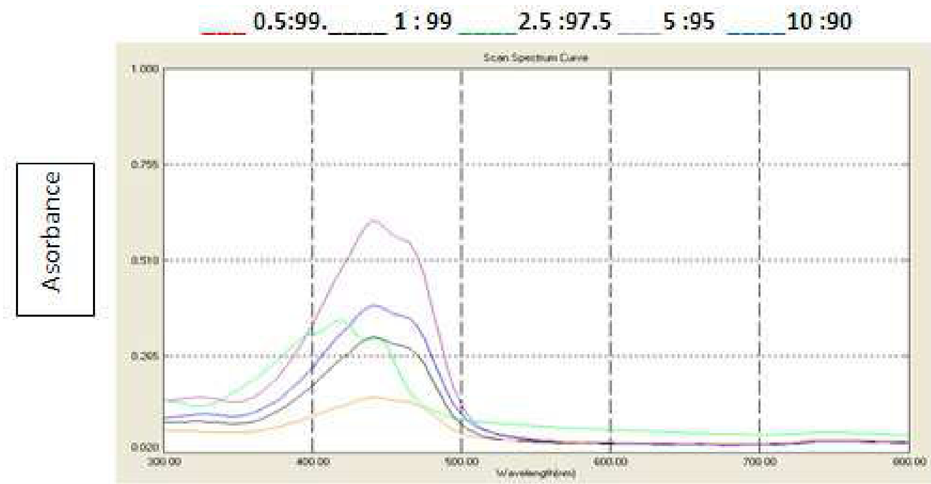



**Figure 6**
***UV-Visible spectra of AgNPs showing the effect of saffron extract and $AgNO_3$***

***3.3X-ray diffraction(XRD)***

Figure 7 shows the X-ray diffraction (XRD) patterns of dried silver nanoparticles synthesized using saffron extract.The diffracted intensities from30° to 80° at 2θ. The XRD patterns of Ag/ extract indicate that the structure of silver nanoparticles is face-centered cubic(fcc)(59). In ddition , the XRD peaks at 2θ of 38.17° ,44.31° , 64.44° ,77.34° and 81.33° could be attributed to the 111 ,200 ,311and 222 crystallographic planes.XRD pattern confirmed that the silver particles formed in our experiment were in the form of nanoparticles , as evidence by the peaks at 2θ values and planes for silver respectively.The present study is inconsistence with Mustafa et al,where silver nanoparticle were synthesized using olive leaf extract(60).The crystalline like particles were synthesized using various plant extracts and also reported by Jagtap and Bapa(61),Das et al (62),Edison and Sethuraman(63) Bindhu and Umadevi(64).

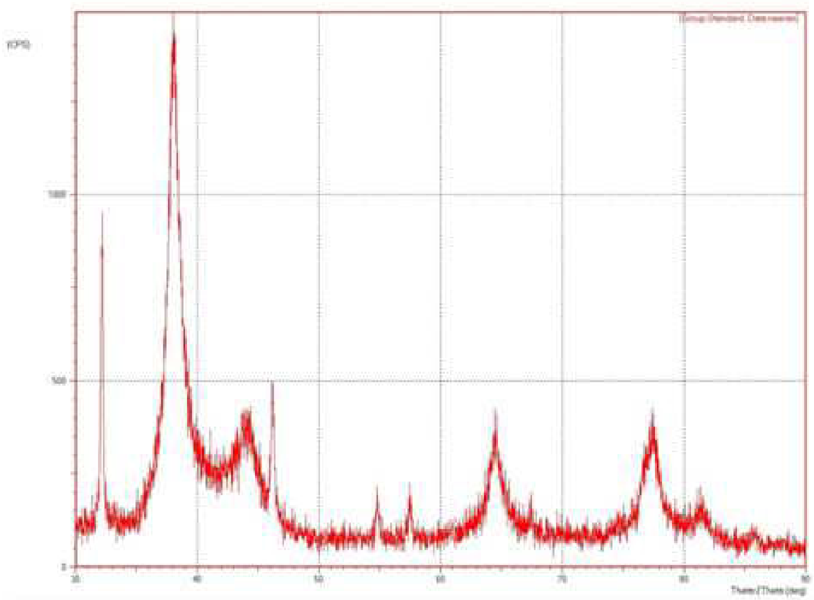


**Figure 7**
***XRD patterns of silver nanoparticles .The XRD pattern showed 4 intense peaks in the whole spectrum of 2θ values ranging from 30-80.***

***3.4 Dynamic light scattering, DLS***

Size analysis by DLS utilizes the Brownian motion that particles, emulsions, and molecules in suspension undergo as a result of bombardment by solvent molecules [65]. If The particles are illuminated by a laser, the intensity of the scattered light fluctuatesat a rate that is dependent upon the size of the particles. This is because smallerThe particles are "hit" more frequently by the solvent molecules and move more rapidly.Analysis of these intensity fluctuations yields the velocity of the Brownian motionand hence the particle size using the Stokes-Einstein relationship[66]. Based on the DLS analysis(table 1) that about 63% of the particles The smaller the particle is the faster the movement is, so the range is bigger.The bigger the particle is ,the slower the movement,so the range is smaller.

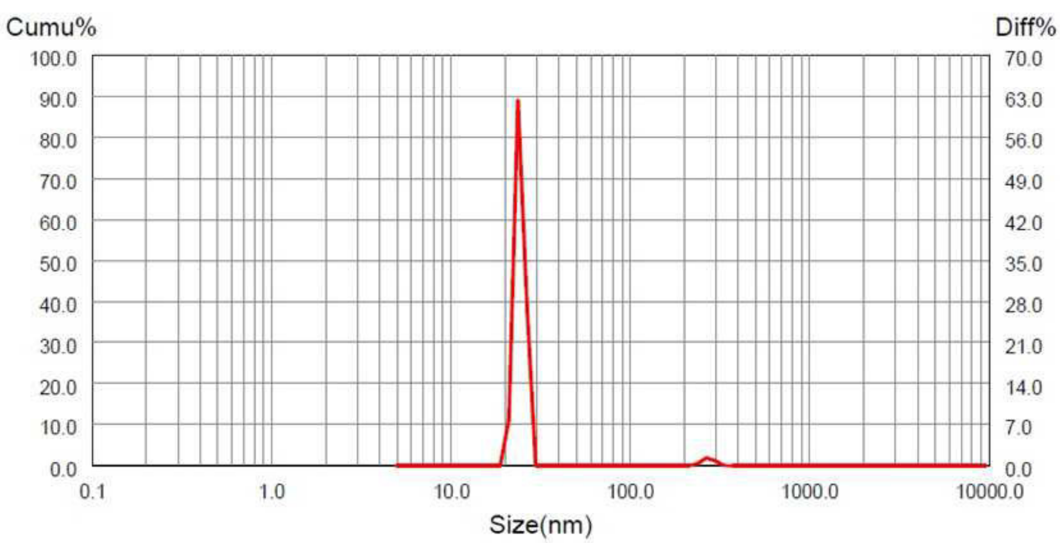



**Figure 8**
***Histogram of particle size distribution of silver nanoparticles***

Figure 8 shows the DLS pattern of the biologically synthesized nanoparticles after separating them from the reaction mixture by ultracentrifugation. It clearly shows that the colloidal solution of silver nanoparticles contains particles with the average size range of nanoparticles varied within19.9 to 28.1

**Table 1**
***shows the percent of particles distribution according to their size***

| Diam nm ofAgNPS | Diff % | Cumu % |
|---|---|---|
| 19.9-22.3 | 7.86 | 7.86 |
| 22.3-25.0 | 62.38 | 70.24 |
| 25.0-28.1 | 27.15 | 97.39 |
| 223-250 | 0.34 | 97.73 |
| 250-281 | 1.35 | 99.08 |
| 281-315 | 0.86 | 99.94 |
| 315-353 | 0.06 | 100 |

Based on the DLS analysis ( Table 1 )that about 63% of the particles with a range of 22.3-15 NM then 27% of the particles have the range 25-28 nm .The smaller the particles the faster the movement is, so the range is bigger. The bigger the particle is ,the slower the movement,so the range is smaller. Sizes and shape of metal nanoparticles are influenced by a number of factors, including PH, Precursor concentration, reductant concentration, time of incubation , temperature as well as the method of preparation [(65)].

## 4. CONCLUSION

The biosynthesis of silver nanoparticles using *crocus sativus* extract were shown to be rapid and produce particles of fairly uniform size and shape. Following the addition of extracts to the silver nitrate solution, silver nanoparticles began to form within 10 min and the reaction neared completion at 1 h, as shown by the UV-Vis spectroscopy. The reduction os silver ions to silver nanoparticles was to be optimized at a ratio of 5:95 extract to l mM silver nitrate and at 70°C with alkaline PH .The synthesized particles ranged in size from 19.9-28.1 nm as shown in DLS. This green synthesis method is rapid, less time consuming and environmentally safe can be used in various biotechnological applications.

## 5. REFERENCES

1. Basavarj U ,Praveenkumar N , Sabiha T S , Rupali Sh , Samprita B .Synthesis and characterization of silver nanoparticles .International Journal of Pharmacy and Biological Sciences 2:10-14 (2012).
2. Supra S,Mohmmama AS,Chakraavarthy N, GayaprakashPA,Sagadavan E,Kainathan MK ,Sindhu S, Arumugam P.Green synthesis of silver nanoparticles from*Cynodon dactylon* leaf extract.International Journal of Chem Tech Research .Vol .5,No.1,271-277(2013).
3. Asta S ,Igoris P , Judita P,Algimantas J ,Asta G. Analysis of silver nanoparticles produced by chemical reduction of silver salt solution .1392-1320.Material Science.Vol. 12, No. 4,(2006) .
4. AmrutnS L,Satish J S,Ramchandra BP .Synthesis of silver nanoparticles ; a safer alternative to conventional antimicrobial and antibacterial agent.JChem Pharm Res,2 (3);478-483(2010).
5. Udapudi B,Naik P,Savadatti St,Sharma R,Balgi S.Synthesis and characterization of silver nanoparticles.Int J Pharm Bio Sci Vol.2 ,10-14(2012) .
6. Karunakar R K,R Manisha RD,Preshanthi Y,Ramchander M, Pratap M P..Synthesis of silver nanoparticles using plant*Allmanaia nadiflora* and evaluation of its antimicrobial activites.Int J Res Pharm Sci,4(4)504-511(2013).

7. StevenL Ch Rong –T W.Preparation of silver nanoparticles with different particles size for low-temperature sintering.I P C B E E.Vol 2 22-58 (2011).
8. Mehrdad F, Khalil F.Biological and green synthesis of silver nanoparticles.Turkish J Eng Env Sci.34,281-287(2010).
9. Smith AM,Duna H,Rhyner MN, Runa G ,Nie S.Asystem examination of surface coating on the optical and chen properties of semiconductor quantum dots .Physical Chem Chemical Physics ,33:3895-3903,doi :10,1039/B606572B(2006).
10. Kasi G,Shanmugam G ,Ayyakannu A.Phytosynthesis of silver nanoparticles using *Pterocarpus* santalinus leaf extract and their antibacterial properties.J of Nanostructure in Chemistry 3:68 1-7(2013)
11. Anandini R ,Padan K J, Umesh K P ,Birendra K B. Green synthesis of silver nanoparticles using leaves extract of *Centella asiatica L*. for studies against human pathogens.Int J Pharm Bio Sci Oct:4(4) 661-674 (2013).
12. Konishi N, Deutzmann R,Sumper M .Polycationic peptides from diatom biosilica that direct silica nanosphere formation .Science 289:1129-1132 (1999).
13. Frattini A,Pellegri N,Nicastro D,Sanctics DO.Preparationo amine coated silver nanoparticles using triethylenetrtramine.Mater Chem Phys,94:148-152(2005).
14. Santosh K,Raisa M D,Mwkthang S,Anjali B,sudhesh K,Mukesh S.Biosynthesis of silver nanoparticles using *Premna herbacea* leaf extract and evaluation of its antimicrobial activity against bacteria causing dysentery.Int J Bio Sci Oct 378-384(2013).
15. Singh A , Upadhyay DJ,Khandelwal MK, Verma HN. Green synthesis of silver nanoparticles using *Argemone Mexicana* leaf extracts and evaluation of their antimicrobial activities .Digest Jof Nanomaterials and Biostruct, 5: 483-489,(2010).
16. Xu H ,Yao L,Sung H , Wu L . Chemical compositionand antitumor activity of different polysaccharides from the roots of *Actinidia eriantha*.Carbohydr.Pol 78: 316-322 (2009).
17. Huang J ,Li Q, Sun D ,Lu Y ,Su Y ,Yang X. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf .Nanotechnol.18:104-105 (2007).
18. Sharma VK, Yugard RA,Lin Y. Silver nanoparticles green synthesis and their antimicrobial activities.Adv Colloid Interface Sci 145 ,83-96 (2006).
19. Chaloupla K , Malam Y , Selfalan AN. Nanosilver as a new generation of nanoproduct in biomedical application.Trends Biochnol 28,580-588 (2010).
20. Prow TW,Grice JE ,Lin LL, Faya R , Bulter M ,Becker W , Wum EMT , YoogC , Robestson TA,Soyer HP, Roberts MS . Nanoparticles and microparticles for skin drug delivery,Adv Drug DelvRev 63,470-491(2011).
21. Chaudhry Q,Castle L. Food application of nanoparticles an overview of opportunities and challenges for developing countries.Trends Food Sci Tech (22),595-603 (2011).
22. Najr R, Varghese SH,Nair BG,MaekawaT,YoshidaY,Sakchi KD. Nanoparticles material delivery to plants.Plant Sci 169,154-163(2010).
23. Dankovich TA ,Gray DG .Bactericidal paper impregnated with silver nanoparticles for point of use water treatment Environ Sci,Tech 45 ,1992-1998(2011).
24. Nilaimathhi KL,SudhaV,LavanyaR,BrindhaP. Biosynthesis of silver nanoparticles using *Aiternanthea sesslis L*. extract and their antimicrobial antioxidant activities .Colloid Surface B102,288-291(2013).
25. Sankar R,arthik A, Prabu A,Karthik S,Shivashangari KS ,Ravkumar V. *Onganumvulgare* mediated biosynthesis of silver nanoparticles for its antibacterial and anticancer activity .Colloid Surface B108,80-84(2013).
26. Boca SC,Potara M,Gabudean AM,JuhemA,Baldeck PL,AstilenS.Chitosan-coated triangular silver nanoparticles as a noval class of biocompatible highly effective photochemical transducers for in vitro cancer cell therapy .Cancer Lett31,131-140(2011).

27. Lekshmi NCJ,Sumi S B,Jeeva S,Brindha R. Antibacterial activity of nanoparticles from Allium sp. J Microbiol. Biotech Res.,2(1) :115-119(2012) .
28. Sulochana , Palanlyandi K,Sivaranjani K.Synthesis of silver nanopoarticles using leaf extract of *Andrographis paniculata*. J of Pharmacology and Toxicology 7(6): 251-258,(2012).
29. Mukunthan KS, Elumalala EK,Patel TN,Murthy VR Catharanthus roseus ;A nature source for the synthesis of silver nanoparticles.Asian Journal of Tropical Biomedicine 270-274,(2011).
30. Mahendran V,Guruswamy A *Coleus aromaticus* leaf extract mediated synthesis of silver nanoparticles and its bactericidal activity.Appl. Nanosci Vol3(3)217-223(2013).
31. Mano Priya .Green synthesis of silver nanoparticles from the lesf extracts of Euphorbia hirta*,Nerium indicum*.Digest journal of nanomaterials and biostructuresvol.6, No2. p.869-877,(2011).
32. Ramteke Ch, Chakrabarti T,Ketan B,Pandey R A. Synthesis of silver nanoparticles from the aqueous extract of leaves of *Ocimum sanctum* for enhanced antibacterial activity .Journal of chemistry .vol 2013 (2013).
33. Pour B M,Sasidharan S. In vivo toxicity study of Lantana camara . sian pacfic journal of tropical biomedicine..doi:10.1016/s2221-1691(11)60033-6 (2011).
34. Dinesh ,Karthikeyan S Arumugam P.Biosynthesis of silver nanoparticles from *Glycyrrhiza glabra* root extracts.Archives of applied Science Research,4910:178-187.(2012).
35. Abdullaev ,F.I Frenkel GD.Saffron is biological and medical research In:Negbi ,M.(ed).saffron ,*Crocus sativus L*.Harwood academic publisher ,Amsterdam p103-113 (1999).
36. Tarantilis PA,Tsoupras G ,Polissiou M.Determination of saffron (*crocus sativusL*) component in crude plant extract using high-performance liquid chromatography UV-Visible photodiode-array detection –mass spectroscopy .J Chromatogr A,699 : 107 -118 (1995) .
37. Rios J L, Recio M C,Ginger R M ,Manz S. An update review of saffron and its active constituents .PhytotherapyResearch vol 10 no.3 ,189- 193 (1996).
38. Abdullaev F I,Espinosa-Aguirre J. Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials .Cancer Detection and Prevention vol 28 ,No.6 426-432 (2004) .
39. Schmidt M, Betti G ,Hensel A. Saffron in phytotherapy pharmacology and clinical uses . Wiener Medizinische Wochenschrift vol 157 no.13,315-319 (2007).
40. Kianbakht S .Asystematic review on pharmacology of saffron and its active constituents .Journal of Medicinal Plants vol 7 No. 28 ,1-23 (2008) .
41. Salomi M J,Nair SC, Panikkar AR. Inhibitor effect of Niegilla sativa and saffon ( Crocus sativus ) on chemical carcenogenesis in mice .Nutr Cancer 16 67-72 (1991).
42. Nair SC,Kurumboor SK ,Hasegawa J H .Saffron chemoprevention in biology and medicine review .Cancer Biother 10,257-264(1995)
43. Marderosian D A,Review of natural products .facts and comparison ;Missouri 520-528(2001).
44. Abe K,Saito H .Effects of a saffron extract and its constituent crocin on learing behavior and long tempotentiation .Phytother Res.14 ,149-152(2000).
45. Roy K, Biswas S,Banaejee P C.Green synthesis of silver nanoparticles by using grape*Vistis vinifera* fruit extract :Characterization of the particles and study of antibacterial activity. Res J Phar Bio Chem Sci .Vol 4 (1) march 1271-1278(2013)
46. Mulvancy P.Surface Plasmon spectroscopy of nanosized metal particles.Langmiur 12: 788-800(1996).
47. Samir A A,Ishnava K I.Plant mediated synthesis of silver nanoparticles by using dried stem power of *Tinospora Cordifolia*, its antibacterial activity and comparison with antibiotics.Int J Phar Bio Sci Oct ;4 (4):849-863 (2013).
48. Vanaja M, Rajeshkumar S, Paulkumar K,Gnanajobitha G, Malarkodi C, Annadurai G. Kinetic study on green synthesis of silver nanoparticles using *Coleus aromaticus* leaf extract. Advances

in Applied Science Research,4 (3); 50-55(2013).
49. Srivastava A A, Kulkarni A P, Harpale P M , Zunjarrao R S .Plant mediated synthesis of silver nanoparticles using a bryophyte :Fissidens minutes and its anti-microbial activity .International Journal of Engineering Science and Technology ,3(12) :8342-8327 (2011).
50. Bergers G,Javaherian K, Lo K M,Folkman J HanahanD . Effects of angiogenesis inhibitors on multistage carcenogenesis in mice. Science 284:808-812(1999).
51. Ravichandran V, Tiah Z X , Subashini G, Terence F W X ,Eddy F C Y,Sokkalingam A D .Biosynthesis of silver nanoparticles using *mangosteen* leaf extract and evaluation of their antimicrobial activities. Journal of Saudi Chemical Society ,15 ;113-120 (2011).
52. Ahmad B, Ali J , Bashir Sh. Optimization and effects of different reaction conditions for the bioinspired synthesis of silver nanoparticles using *Hippophaerhamnodes Linn* .leaves aqueous extract. World Applied Science Journal 22(6);836-843 ,(2013)
53. Sathishkumar M,Sneha K,Yun T S.Immobilization of silver nanoparticles synthesized using *Curcuma loga tuber* powder and extract on cotton cloth for bactericidal activity.Bioresource Technology,101;7985-65(2010).
54. Amit K M , Abhishek K ,Uttam C B . Free radical scavenging and antioxidant activity of silver nanoparticles synthesized from flower extract of *Rhododoendron dauricum* .Nano Biomedicine Engineering ,4 (3) ; 118-124 (2012).
55. Chandran S P,Chaudary M,Pasricha R,Ahmad A,Sastry M.Synthesis of gold nanoparticles and silver nanoparticles using *Aloe Vera* plant extract .Bio Technol Prog 232,577-588(2006).
56. Preeti D , Mukhopadhyay M; In –vitro free radical scavenging activity of biosynthesized gold and silver nanoparticles using *Prunus armeniaca* (apricot) fruit extract .Journal of Nanoparticles Research,15; 1366-1376 (2013).
57. Krishnaraj C R,Ramachandran K M , Kalaichelvan PT; Optimization for rapid synthesis of silver nanoparticles and its effect on phytopathogenic fungi.Spectrochimica Acta Part A ; Molecular and Biomolecular Spectroscopy 93: 95-99 (2012).
58. Shame K , Ahmed M B , YunusW M Z W, Ibrahim N A. Synthesis and characterization of silver \talc nanoparticles using the wet chemical reduction methods .Int J. Nanomed,5 743-751(2010).
59. Mostafa M H K, Ismail E H ,El-baghdady K Z,Mohamed D. Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity.Arabian Journal of Chemistry 1-9( 2013).
60. Jagtap U B, Bapq V A.Green synthesis of silver nanoparticles using *Artocarpus heterophyllus Lam*. Seed extract and its antibacterial activity. Ind. Crop. Prod, 46, 132-137 (2013).
61. Das J ,Paul Das M Velusamy P.*Sesbania grandiflora* leaf extract mediated green synthesis of antibacterial silver nanoparticles against selected human pathogens ..Spectrochim Acta A 104 ,265-270 (2013).
62. Edison T J , Sethuraman M G.Biogenic robust synthesis of silver nanoparticles usingPunica granatum peel and its application as a green catalyst for the reduction of an anthropogenic pollutant 4-nitrophenol. Spectrochim Acta A 104, 262-264(2013).
63. Bindhu M R, Umadevi M. Snthesis of monodispersed silver nanoparticles using *Hibiscus cannabinus* leaf extract and its antimicrobial activity .Spectrochim Acta A 101 ,184-190 (2013).
64. Kaszuba M,Mcknigth D,Connah M T Mc-Neil-Waston F K,Nobbmann U.Nonaggregated colloids silver nanoparticlew for surface enhanced resonance Raman spectroscopy .J Nanopart .Res 10 (2008).
65. Umoren S A , Obot I B , Gasem Z M .Green synthesis and characterization of silver nanoparticles using red apple (*Malus domestica)*fruit extract at room temperature. J Mater Envirom Sci5 (3) 907-914(2014).