

Synthesis and characterization of Silver Nanoparticles

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ABSTRACT: Different methods may be used to produce nanoparticles, for instance in 1951 Turkevich and co-workers proposed that gold nanoparticles can be produced from the reaction of trisodium citrate, which acts as a stabilizing and reducing agent, with chloroauric acid, the source of gold nanoparticles. By changing chloroauric acid to silver nitrate, silver nanoparticles can instead be produced. Despite being widely used, there is a debate in the literature on the way the reagents and conditions, used for the Turkevich method, affect the size and shape of the silver nanoparticles produced. In view of this, silver nanoparticles have been synthesised through the Turkevich method using different reaction conditions, namely the reaction temperature and concentration of sodium citrate used. Characterisation techniques were then used to determine the size and shape of the silver nanoparticles produced. It was found that increasing the temperature increased the size of the nanoparticles through SEM, although DLS showed the opposite trend. Furthermore, at higher temperatures the formation of rod-like particles could be observed, as opposed to more spherical particles at lower temperatures.

Keywords: silver nanoparticles, Turkevich method, synthesis, reaction conditions

I. INTRODUCTION

Nanoparticles, which may be of any shape, have at least one dimension of 100 nm or less [1]. These species can be considered as the smallest and most fundamental component in the production of a nanostructure [2]. They can be produced by a number of different techniques, including chemical, photochemical and electrochemical methods. Nanoparticles have a number of different properties when compared to larger particles, for instance, due to their small size nanoparticles have a high surface area-to-volume ratio, a property which comes in extremely helpful in applications such as the antimicrobial and antifungal activity [1,3-5]. Various methodologies may be employed to produce silver nanoparticles, for instance, silver ions may be reduced to silver nanoparticles using sodium borohydride [6-8], NaBH₄, whilst using polyvinylpyrrolidone (PVP) or polyvinylalcohol (PVA), amongst others, as capping agents [7,8]. Another well-established method that may be used to reduce silver ions to silver nanoparticles is the Turkevich method, which uses trisodium citrate as a reducing agent and a capping agent [9-12]. It has been shown that trisodium citrate can act as a reducing agent at temperatures higher than 60 °C [9-12], which means that for the Turkevich method to work, the reaction temperature must at least be equal to this temperature. In view of the fact that the Turkevich method is a well-known technique [10,14-16] and the reagents needed can be readily acquired, this method was used in this study to produce silver nanoparticles.

Synthesis of silver nanoparticles may result in different shapes and sizes, depending on a number of factors. In the case of the Turkevich method, the most important factors are the reaction temperature and the amount of the reducing / capping agent [17-29]. Although there is a general consensus that an increase in temperature results in an increase in the reaction rate, there is a debate on how temperature affects the size of the nanoparticles when synthesised through the Turkevich method. In fact, various authors report that synthesis of silver nanoparticles at high temperatures results in small nanoparticles sizes [13,15] whilst other studies have reported that an increase in temperature will result in larger silver nanoparticles [14,16].

In view of the above, the aim of this study was to investigate the effect of temperature on the size and shape of the silver nanoparticles produced through the Turkevich method. Furthermore, the effect of varying the concentration of trisodium citrate and reaction time was also investigated. In order to characterise the shape and

size of the silver nanoparticles produced, a number of techniques were employed, namely, dynamic light scattering, scanning electron microscopy and ultraviolet-visible spectroscopy.

II. METHOD

2.1 Chemicals Used

- Extra pure silver nitrate (CAS: 7761-88-8), produced by Scharlau, S.L. with a molecular weight of $169.87 \text{ g mol}^{-1}$.
- Extra pure trisodium citrate dihydrate (CAS: 6132-04-3), produced by Scharlau, S.L. with a molecular weight of $294.10 \text{ g mol}^{-1}$.
- Wash grade acetone (CAS: 67-64-1) produced by the ChemiK Co., with a molecular weight of 58.08 g mol^{-1} .

2.2 Synthesis of silver nanoparticles at different temperatures and citrate ion concentration

A $0.005 \text{ mol dm}^{-3}$ silver nitrate stock solution was first prepared. This was done by first drying the silver nitrate crystals to constant weight in an oven set at $100 \text{ }^\circ\text{C}$. 0.425 g of the dried silver nitrate crystals was weighed using a duly calibrated balance (Kern, S/N: WF114939). This mass was then transferred to a 500 mL amber volumetric flask and filled with distilled water up to the mark. All transfers were followed by three washings. This stock solution was then diluted by transferring 100 mL of the stock silver nitrate solution to a second 500 mL amber volumetric flask and filling the volumetric flask to the mark with distilled water. This resulted in a diluted solution having a concentration of $0.001 \text{ mol dm}^{-3}$.

A $0.034 \text{ mol dm}^{-3}$ solution of trisodium citrate was then prepared by transferring 1.00 g of trisodium citrate dihydrate into a 100 mL volumetric flask and filling the volumetric flask up to the mark with distilled water. As in the above case, all transfers were followed by three washings.

A volume of 300 mL of diluted silver nitrate solution were measured and transferred to a 500 mL three-necked round-bottomed flask. This was placed in a heating mantle (Witeg, S/N: 1000974140D001) with a condenser placed in a reflux mode. 8 mL (2.72×10^{-4} moles) of the trisodium citrate solution was placed in a dropping funnel, which was attached to the three necked round bottom flask.

The sodium nitrate solution was heated to $100 \text{ }^\circ\text{C}$ ($\pm 1 \text{ }^\circ\text{C}$) by using a temperature controller (Witeg, S/N: 040677712AP002) attached to the heating mantle. The solution was stirred throughout the heating process. Upon reaching this temperature, the trisodium citrate present in the dropping funnel was added to the silver nitrate solution whilst still stirring. The time taken for the solution to change colour (t_1 , verified through UV spectroscopy as explained below) was noted, and the reaction was continued to be heated for double of this time, i.e. the reaction was heated for $3 \times t_1$. After this time, the reaction mixture was allowed to cool to room temperature.

The above procedure was then repeated using different temperatures and amounts of trisodium citrate. More specifically, the temperatures used were $60, 70, 80, 90$ and $100 \text{ }^\circ\text{C}$. For each of these temperatures, three different amounts of trisodium citrate were used, namely, 2.72×10^{-4} , 4.08×10^{-4} and 5.44×10^{-4} moles of trisodium citrate. This resulted in a total of 15 experiments. For each of these experiments, three samples were collected, where, the first sample was taken when the solution changed colour (time t_1), the second was taken at time $2 \times t_1$ whilst the third reading was taken at time $3 \times t_1$. In all cases, the nanoparticles were characterised using U.V., DLS and SEM as explained below. Due to the number of trials done, the DLS experiments were only performed for the second sample (i.e. the sample taken at time $2 \times t_1$) for each case, while the SEM experiments were only performed for the samples obtained when using 4.08×10^{-4} mol of trisodium citrate and taken at a time $2 \times t_1$.

2.3 U.V. Visible Spectroscopy

UV – Vis experiments were carried out using a V-650 UV-Visible spectrophotometer (Jasco Inc), which was set to scan over a range of 700 to 300 nm . A quartz cuvette was used as a sample holder, making sure that the sides of the cuvette were completely clean and clear. A background scan was first carried out using distilled water after which the three samples, for each experiment, were characterised.

2.4 Dynamic Light Scattering Spectroscopy

Dynamic light scattering (DLS) experiments were carried out using a Malvern Zetasizer (Malvern Instruments Ltd., S/N: MAL1100322). A small volume of the nanoparticles solution (dispersed in water) was added to a cuvette after which the latter was introduced into the sample holder of the Zetasizer. Experiments were carried out at $20 \text{ }^\circ\text{C}$. The refractive index and the absorption value of the silver nanoparticles were obtained through literature as 1.07 and 0.01 respectively [12].

2.5 Scanning Electron Microscopy

A strip of double-sided carbon tape was placed on the SEM sample holder, on top of which a gold leaf was placed. The dispersion of silver nanoparticles was diluted with acetone (1:6 dilution). One drop of the diluted silver nanoparticles dispersion was placed on the gold coated substrate and allowed to dry.

The sample was then introduced in a Carl Zeiss Merlin Field Emission SEM. The magnification used was 50,000, with an InLens signal detector, a scan speed of 8, probe current of 125 pA and an EHT of 5 kV. 10 micrographs were taken from random positions in the area where the nanoparticles were present.

The SEM images were first 'cleaned' using a photo editing software (Gimp v 2.8) by removing any shines produced by the gold substrate and then analysed using MATLAB, in order to determine the sizes of silver nanoparticles produced. This was achieved by assuming spherical nanoparticles by averaging the 2 radii obtained from the elliptical fit in the case of each nanoparticle.

III.RESULTS AND DISCUSSION

The synthesis of silver nanoparticles at the various temperatures and volumes of trisodium citrate used in these experiments proceeded as expected, where in all cases a change in colour (checked through UV-Vis spectroscopy in order to ensure consistency) was observed. The time taken for a change in colour to occur was found to be dependent on the reaction temperature but not on the volume of trisodium citrate used. The solutions at lower temperatures needed higher reaction times for the colour change to occur. Referring to Figure 1, the relation between the reaction temperature and time needed for the reaction mixture to change colour can be described by an Arrhenius-type exponential fit.

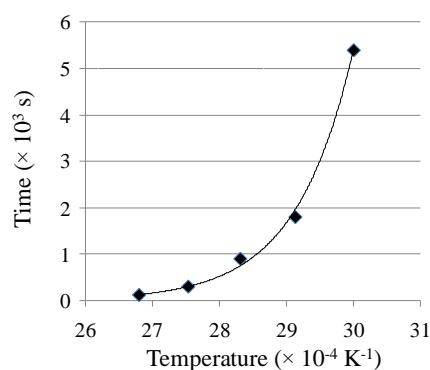


Figure 1: An exponential fit showing the relation between the time needed for the reaction mixture to change colour and the inverse of the absolute temperature. The line equation of the exponential fit is $3 \times 10^{-12} e^{11683x}$, while the R^2 for the fit is 0.995.

The UV spectra which were carried out on the nanoparticles dispersions give an indication of the nanoparticle's size, concentration and distribution. Referring to Figure 2, in all cases considered in this study, the absorbance of the nanoparticle dispersion increased with increasing reaction time, indicating that the number and / or size of nanoparticles produced increased with reaction time. Furthermore, it was noticed that for each temperature considered, a red shift in the UV spectrum occurred as the reaction time increased, indicating that the nanoparticles may be becoming larger [14,16,22,24,27-33].

Furthermore, it can be clearly observed from Figure 2 that in most cases, upon an increase in temperature and / or volume of trisodium citrate, the absorbance increased. The increase in absorbance at higher temperatures may be a result of the increased reaction rate. The increase in absorbance with an increase in trisodium citrate volume may be attributed to a higher presence of citrate ions. These will cap the silver nanoparticles more rapidly, preventing them from aggregating together.

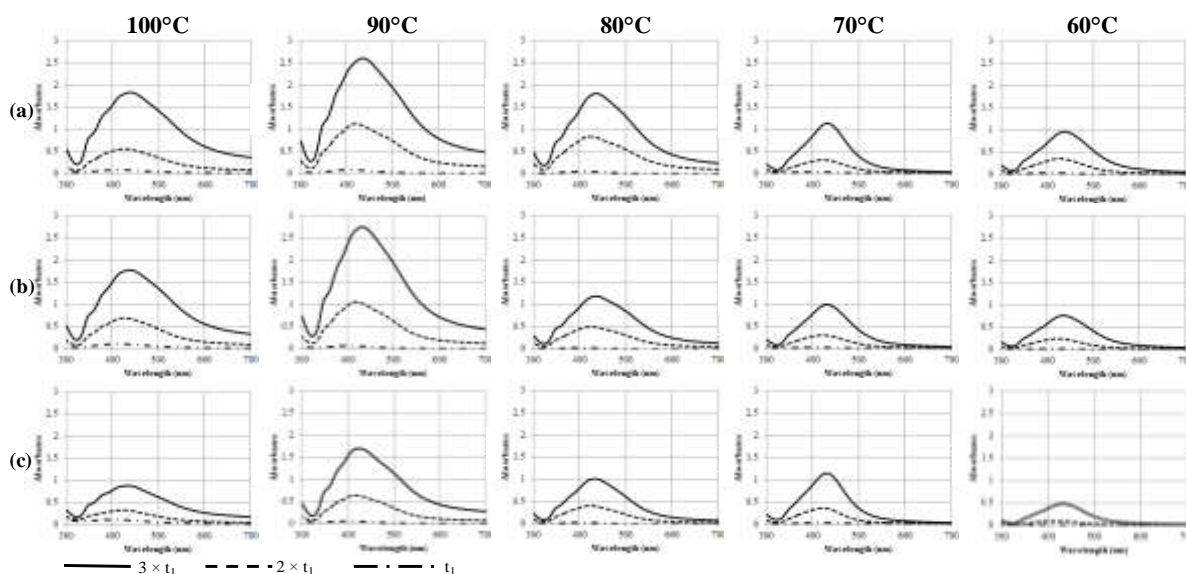


Figure 2: Plots of absorbance on the y-axis against wavelength (nm) on the x-axis for the temperatures used to synthesise silver nanoparticles. Each temperature consists of 3 graphs representing the different amounts of citrate used where (a) 5.44×10^{-4} moles (b) 4.08×10^{-4} moles (c) 2.72×10^{-4} moles.

The second technique used to characterise the nanoparticles was DLS spectroscopy. Various parameters can be obtained from this technique, which include the z-average and the polydispersity index (PDI). The z-average is the main and most stable parameter produced by the DLS spectrometer, however care must be taken when interpreting this value as it is sensitive to even a small quantity of aggregation [34]. On the other hand, the PDI can indicate an increase in broadness of molecular weight or non-spherical shapes of the dispersed particles. PDI values larger than 0.7 indicate a very broad size distribution of the sample and probably would not be suitable for the DLS technique. PDI values of 0.1-0.2 indicate a monodispersed sample, i.e. almost all particles have the same size and shape [34]. As may be observed from Figure 3, the average nanoparticle diameter decreased whilst the polydispersity index (PDI) increased upon an increase in temperature. Due to the high PDIs, the z-averages obtained from the DLS do not necessarily represent the true sizes of the nanoparticles produced. This is due to a number of limitations posed by this technique, including the assumption that all particles dispersed in solution are of spherical shape [34]. This means if different nanoparticle shapes are produced, this technique will still assume that all particles are spherical, which may result in over or under estimation of the average nanoparticle diameter. Furthermore, the z-average measures what is known as the hydrodynamic size of the nanoparticle, which is based on a hard sphere model. In reality, the nanoparticles may be solvated, i.e. surrounded by a layer of solvent molecules, in which case the DLS will record the diameter of the solvated particle (meaning that the z-average recorded would be higher than the actual size of the nanoparticle). Another consideration that has to be taken into account when interpreting results obtained by the DLS is that the scattering of light is more pronounced when large particles are present. Even when large particles are present in small amounts, these exhibit the largest scattering which may result in an overestimation of larger particles present [34].

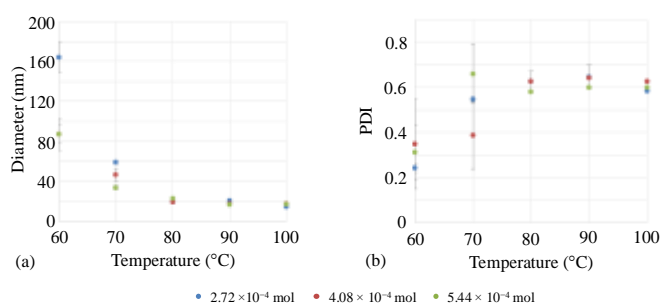


Figure 3: Results obtained using the DLS technique showing (a) the average silver nanoparticle diameter and (b) the P.D.I. obtained at each temperature and for each amount of trisodium citrate used. The error bars represent the standard deviation between 3 repeats.

Furthermore according to the DLS measurements (Figure 3), the volume of trisodium citrate added to the reaction mixture seems to have an effect when the reaction is carried out at low temperatures. In fact, the nanoparticle diameters produced at temperatures of 80 °C and higher seem to be independent of the volume of trisodium citrate used. The highest PDI's (ca. 0.6) were obtained for reaction temperatures higher than 80 °C. This indicates that the distribution of silver nanoparticles, particularly, for the nanoparticles produced at these temperatures, is not homogenous, with a large size distribution being present. This observation is in agreement with the results obtained from the UV spectrometer, where the peaks became broader at higher reaction temperatures.

Analyses of the scans obtained by the SEM (see Figure 4) show that the magnification used (50,000 times) was enough to visualise nanoparticles as small as 10 nm. These scans also show that the dilution used was enough so as not to obtain aggregation of the nanoparticles. In order to quantify the average size of the particles produced, the SEM images were analysed in MATLAB, with the assumption that all particles are spherical, the same assumption that is made by the DLS. This assumption is valid for the spherical and irregular particles, while the rod-shaped particles are not well represented. In the case of the results obtained through the SEM technique the mean sizes of nanoparticles appear to become larger with increasing temperature (see Table 1). This is in accordance with the work of Jiang *et al.* [14] who argue that at high temperatures the nanoparticles 'fuse' together, due to the high kinetic energy these particles possess. Furthermore, it may be noted from Table 1 that as the temperature increases, the standard deviation computed for the diameters measured through the SEM technique also increases. This indicates that as the temperature increases, the size distribution of silver nanoparticles also increases, in agreement with the DLS measurements. As the reaction temperature is reduced, there is a shift towards the smaller sizes of the silver nanoparticles.

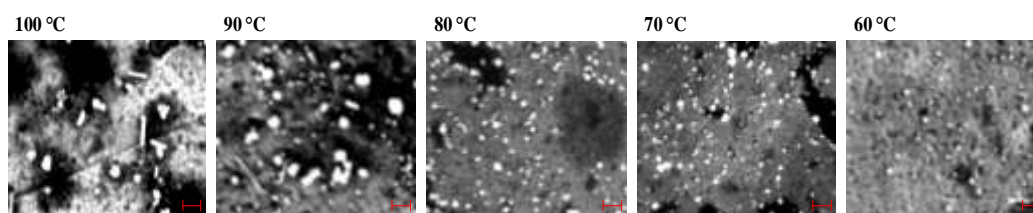


Figure 4: The SEM images of silver nanoparticles obtained at the various reaction temperatures. In each case, 4.08×10^{-4} moles of trisodium citrate were used. Note that the scale bar represents 200 nm.

Table 1: Table showing the average silver nanoparticles sizes obtained at each reaction temperature using the SEM and DLS techniques. In each case, 4.08×10^{-4} moles of trisodium citrate were used. The standard deviation for each measurement is given in the bracket.

Temperature/°C	SEM	DLS	
	Mean (nm)	Z-Average (nm)	P.D.I
60	27.38 (18.20)	87.15 (15.77)	0.348 (0.199)
70	30.90 (24.66)	46.63 (6.01)	0.387 (0.148)
80	34.82 (20.05)	25.96 (0.34)	0.559 (0.003)
100	63.68 (43.58)	17.83 (0.14)	0.626 (0.004)

Referring to Figure 4, rod-like silver nanoparticles start to form, apart from spherical and irregularly shaped silver nanoparticles, at high temperatures. This is in agreement with the literature [14,16,22,24,27-33] where at high temperatures, different shapes of nanoparticles are reported to exist. More specifically, at a reaction temperature of 90 °C the shape of some nanoparticles changes from being spherical (or irregular) to rod-like. This observation becomes even more evident when the reaction temperature is increased to 100 °C, as the number of rod like nanoparticles increases.

IV. CONCLUSIONS

In this work, silver nanoparticles have been synthesised through the Turkevich method, a well-known technique for producing such species, which employs the use of citrate ions and silver nitrate. Reaction conditions, namely temperature and concentration of trisodium citrate, have been varied in order to study their

effect on the morphological features of silver nanoparticles, i.e. their size and shape distribution. Such features were studied through the use of a number of characterisation techniques, more specifically scanning electron microscopy, ultra-violet visible spectroscopy and dynamic light scattering spectroscopy. The reaction rate was found to have an Arrhenius-like exponential relation to the temperature, and was independent of the trisodium citrate concentration. It was also shown that at higher temperatures the size of the silver nanoparticles increased, as characterised by the SEM images. This result was not in agreement with the DLS results which showed a decrease in particle diameter with an increase in temperature. The results obtained through the SEM were deemed to be more reliable than the DLS results since the latter had high PDI values. At high temperatures, the occurrence of more than one shape was shown by the SEM images whereby spherical and rod shaped nanoparticles were recorded. The results obtained here are very important as they highlight ways how to obtain different shapes of nanoparticles, particularly, spherical and rod-shaped particles through a simple chemical method. Furthermore, they also shed light on how one may obtain low distributions of nanoparticles. This is not trivial as the properties of the silver nanoparticles may be affected by their size and shape. For example several studies have shown that silver nanoparticles are very good antimicrobial agents [1,3-5]. In this respect, a detailed investigation can be carried out on the antifungal and antibacterial properties of silver nanoparticles, particularly the effect of different sizes and shapes on their activity.

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References

- [1]. S. Gurunathan, K. Kalishwaralal, R. Vaidyanathan, V. Deepak, S.R.K. Pandian, J. Muniyandi, N. Hariharan, S.H. Eom, Biosynthesis, purification and characterisation of silver nanoparticles using *Escherichia coli*, *International Journal of Colloids and Surfaces B*, 74(1), 2009, 328-335.
- [2]. RSC Nanoscience & Nanotechnology, Introductory Aspects of Soft Nanoparticles, in J. Estelrich, M. Quesada-Perez, J. Forcada, J. Callejas-Fernandez (Ed.), *Soft Nanoparticles for Biomedical Applications*, (Cambridge, The Royal Society of Chemistry, 2014) 1–18.
- [3]. A. Gupta, S. Silver, Silver as a biocide: Will resistance become a problem?, *Nature Biotechnology*, 16(10), 1998, 888.
- [4]. K. Kurihara, C. Rockstuhl, S. Petit, Y. Yamakawa, J. Tominaga, Plasmonic Devices with Controllable Resonances - An Avenue towards High-Speed and Mass Fabrication of Optical Meta-Materials, *Journal of Microscopy*, 229(3), 2008, 396–401.
- [5]. S. Pal, Y.K. Tak, J.M. Song, Does the Antibacterial Activity of Silver Nanoparticles Depend on the Shape of the Nanoparticle? A Study of the Gram-Negative Bacterium *Escherichia Coli*, *Journal of Applied and Environmental Microbiology*, 73(6), 2007, 1712–1720.
- [6]. S.D. Solomon, L. Mulfinger, M. Bahadory, A.V. Jeyarajasingam, S.A. Rutkowsky, C. Boritz, Synthesis and Study of Silver Nanoparticles, *Journal of Chemical Education*, 84(2), 2007, 322.
- [7]. K. Mavani, M. Shah, Synthesis of Silver Nanoparticles by Using Sodium Borohydride as a Reducing Agent, *International Journal of Engineering Research and Technology*, 2(3), 2013, 1–5.
- [8]. A. Zielińska, E. Skwarek, A. Zaleska, M. Gazda, J. Hupka, Preparation of Silver Nanoparticles with Controlled Particle Size, *Procedia Chemistry*, 1(2), 2009, 1560–1566.
- [9]. Ratyakshi, R.P. Chauhan, Colloidal Synthesis of Silver Nano Particles, *Asian Journal of Chemistry*, 21(10), 2009, 113–116.
- [10]. J. Turkevich, P.C. Stevenson, J.A. Hillier, Study of the Nucleation and Growth Processes in the Synthesis of Colloidal Gold, *Discussions of the Faraday Society*, 11(1), 1951, 55–75.
- [11]. H., Yin, T. Yamamoto, Y. Wada, S. Yanagida, Large-Scale and Size-Controlled Synthesis of Silver Nanoparticles under Microwave Irradiation, *Journal of Materials Chemistry and Physics*, 83(1), 2004, 66–70.
- [12]. W. Zhang, Y. Yao, K. Li, Y. Huang, Y. Chen, Influence of Dissolved Oxygen on Aggregation Kinetics of Citrate-Coated Silver Nanoparticles, *Journal of Environmental Pollution*, 159(12), 2011, 3757–3762.
- [13]. S. Agnihotri, S. Mukherji, Size-controlled silver nanoparticles synthesised over the range 5-100 nm using the same protocol and their antibacterial efficacy, *RSC Advances*, 4(8), 2014, 3974.
- [14]. X.C. Jiang, W.M. Chen, C.Y. Chen, S.X. Xiong, A.B. Yu, Role of Temperature in the Growth of Silver Nanoparticles Through a Synergetic Reduction Approach, *Journal of Nanoscale Research Letters*, 6(1), 2011, 1–9.
- [15]. Lee, S.I. Shin, Y.C. Kim, S.G. Oh, Preparation of Silver Nanorods through the Control of Temperature and pH of Reaction Medium, *Journal of Materials Chemistry and Physics*, 84(2), 2004, 197–204.
- [16]. Y. Lee, S. Chen, Finding a Facile Method to Synthesise Decahedral Silver Nanoparticles through a Systematic Study of Temperature Effect on Photomediated Silver Nanostructure Growth, *Journal of the Chinese Chemical Society*, 57(3), 2010, 325–331.

- [17]. D. Chen, X. Qiao, X. Qiu, J. Chen, Synthesis and electrical properties of uniform silver nanoparticles for electronic applications, *Journal of Material Science*, 44(4), 2009, 1076–1081.
- [18]. P. Dhandapani, N. Supraja, Extracellular Synthesis of Silver Nanoparticles by Marine Thermophilic Bacteria, *International Journal of Pharmaceutical & Biological Archives*, 3(6), 2012, 1418–1423.
- [19]. M.B. Kasture, P. Patel, A.A. Prabhune, C.V. Ramana, A.A. Kulkarni, B.L.V. Prasad, Synthesis of Silver Nanoparticles by Sphorolipids: Effect of Temperature and Sphorolipid Structure on the Size of Particles, *Journal of Chemical Sciences*, 120(6), 2008, 515–520.
- [20]. K. Kathiresan, S. Manivannan, M.A. Nabeel, B. Dhivya, Studies on Silver Nanoparticles Synthesised by a Marine Fungus, *Penicillium Fellutanum* Isolated from Coastal Mangrove Sediment, *Colloids and Surfaces B: Biointerfaces*, 71(1), 2009, 133–137.
- [21]. S. Kaviya, J. Santhanalakshmi, B. Viswanathan, Green Synthesis of Silver Nanoparticles Using *Polyalthia Longifolia* Leaf Extract along with D-Sorbitol: Study of Antibacterial Activity, *Journal of Nanotechnology*, 2011(1), 2011, 1-5.
- [22]. S.C.G. Kiruba Daniel, N. Mahalakshmi, J. Sandhiya, K. Nehru, M. Sivakumar, Rapid Synthesis of Ag Nanoparticles Using Henna Extract for the Fabrication of Photoabsorption Enhanced Dye Sensitized Solar Cell (PE-DSSC), *Journal of Advanced Materials Research*, 678, 2013, 349–360.
- [23]. A.K.M.M. Islam, M. Mukherjee, Effect of Temperature in Synthesis of Silver Nanoparticles in Triblock Copolymer Micellar Solution, *Journal of Experimental Nanoscience*, 6(6), 2011, 596–611.
- [24]. A.M. Fayaz, K. Balaji, P.T. Kalaichelvan, R. Venkatesan, Fungal Based Synthesis of Silver Nanoparticles-An Effect of Temperature on the Size of Particles, *Colloids and Surfaces B: Biointerfaces*, 74(1), 2009, 123–126.
- [25]. M. Vanaja, S. Rajeshkumar, K. Paulkumar, G. Gnanajobitha, C. Malarkodi, G. Annadurai, Kinetic Study on Green Synthesis of Silver Nanoparticles Using *Coleus Aromaticus* Leaf Extract. *Journal of Advances in Applied Science Research*, 4(3), 2013, 50–55.
- [26]. M. Vanaja, G. Gnanajobitha, K. Paulkumar, S. Rajeshkumar, C. Malarkodi, G. Annadurai, Phytosynthesis of Silver Nanoparticles by *Cissus Quadrangularis*: Influence of Physicochemical Factors, *Journal of Nanostructure in Chemistry*, 3(1), 2013b, 17.
- [27]. R. Yang, D. Tang, F. Ma, T.X. Tao, Y. Ren, Z. Chen, C. Zhang, F. Li, One-Pot Low Temperature Synthesis of Monodisperse Silver Nanoparticles, *Journal of Nanoscience and Nanotechnology*, 12(9), 2012, 7280–7283.
- [28]. O.A. Yeshchenko, I.M. Dmitruk, A.A. Alexeenko, A.V. Kotko, J. Verdal, A.O. Pinchuk, Size and Temperature Dependence of the Surface Plasmon Resonance in Gold Nanoparticles, *Surface Science*, 608, 2012, 275–281.
- [29]. N. Yıldız, Ç. Ateş, M. Yılmaz, D. Demir, A. Yıldız, A. Çalmlı, Investigation of Lichen Based Green Synthesis of Silver Nanoparticles with Response Surface Methodology, *Green Processing and Synthesis*, 3(4), 2014, 259–270.
- [30]. H.R. Ghorbani, H. Attar, Optimization of Silver Nanoparticles Production by *E. Coli* and the Study of Reaction Kinetics, *Asian Journal of Chemistry*, 23(11), 2011, 5111.
- [31]. N.A.C. Lah, M.R. Johan, Optical and thermodynamic studies of silver nanoparticles stabilized by Daxad 19 surfactant, *International Journal of Materials Research*, 102(3), 2011, 1-8.
- [32]. S. Prakash, N. Soni, Factors Affecting the Geometry of Silver Nanoparticles Synthesis in *Chrysosporium Tropicum* and *Fusarium Oxysporum*, *American Journal Nanotechnology*, 2, 2011, 112-121.
- [33]. M. Sathishkumar, K. Sneha, Y.S. Yun, Immobilization of Silver Nanoparticles Synthesised Using *Curcuma Longa* Tuber Powder and Extract on Cotton Cloth for Bactericidal Activity. *Bioresource Technology*, 101(20), 2010, 7958–7965.
- [34]. Dynamic Light Scattering Common Terms Defined, Malvern Instruments Inform White Paper; 2011. Available at: <http://www.malvern.com/en/support/resource-center/Whitepapers/WP111214DLSTermsDefined.aspx>