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► To cite this version:

R Sathiya Priya, D Geetha, P S Ramesh, V Aroulmoji. Synthesis and Characterization of Nano Silver for Different Temperatures and their Antimicrobial Activity. International journal of advanced Science and Engineering, 2017. ⟨hal-03093658⟩

HAL Id: hal-03093658

<https://hal.science/hal-03093658v1>

Submitted on 20 Jan 2021

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Synthesis and Characterization of Nano Silver for Different Temperatures and their Antimicrobial Activity

R. Sathiyapriya^{1*}, D. Geetha², P.S. Ramesh³ and V. Aroulmoji¹

¹ Department of Physics & Center for Research & Development, Mahendra Engineering College, Namakkal-637503.

² Department of Physics, Annamalai University, Annamalai Nagar- 608 002, Tamil Nadu, India.

³ Physics Wing DDE, Annamalai University, Annamalai Nagar 608002, Tamil Nadu, India.

ABSTRACT: Silver nanoparticles were synthesized at four different calcinations temperature viz., 150°C, 200°C, 250°C and 300°C by chemical reduction method. Silver nitrate was taken as precursor and sodium citrate as a reducing agent. The prepared silver nanoparticles were identified by UV-Vis absorption spectroscopy. Structural, morphological and size of the synthesized nanoparticles were characterized by XRD, SEM/EDS and particle analysis showed that the nano silver (Ag) samples are spherical with particle sizes of 0-95 nm by using particles size analyzer. Also we confirmed that the size of the silver nanoparticles was affected by the calcinations temperature. Antimicrobial activity of silver nanoparticles is important to control conventional antimicrobial agents. Bacterial and fungal contamination is a serious problem. This work evaluates the potential of nano silver to remove bacterial and fungal contaminants. The Experiment involved with three different bacteria viz., *Escherichia coli* sp., *Pseudomonas* sp., and *Streptococcus* sp., in MHA medium and three different fungus viz., *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., in SDA medium. The results show that nano silver has a good potential for removing of the bacterial and fungal contaminants in human.

KEYWORDS: Nanostructures, Chemical synthesis and Anti- microbial activity

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1. INTRODUCTION

Inorganic nanoparticles, whose structures exhibit significantly novel and improved physical, chemical, and biological properties, phenomena and functionality due to their nanoscale size, have elicited much interest. Nano structural materials are attracting a great deal of attention because of their potential for achieving specific processes and selectivity especially in biological and pharmaceutical applications [1]. Among inorganic anti-microbial agents, silver has been employed most extensively to fight infections and control spoilage. The antibacterial and antifungal action of silver, silver ions, and silver compounds have been thoroughly investigated by many researchers. The antimicrobial property of silver has been investigated and employed more extensively than any other inorganic antibacterial agent [2]. Toxicity of silver ion and its compounds towards microbes is well established and this property was used in wound dressings, silver loaded zeolites etc., because of its higher stability, stronger antibacterial activity [3]. In particular, because of the recent advances in research on metal nanoparticles, Ag-Nps have received special attention as a possible antimicrobial agent [4-9]. It has been known that silver and its compounds have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities for bacteria, fungi, and virus since ancient times. Silver is a nontoxic, safe inorganic antibacterial agent used for centuries and is capable of killing about 650 types of diseases causing microorganisms [10]. An opportunistic fungal strain has increased dramatically [11-13]. Fungi also cause a number of plant and animal diseases: in humans, ringworm, athlete's foot, and several more serious diseases

are caused by fungi, because fungi are more chemically and genetically similar to animals than other organisms, this makes fungal diseases very difficult to treat. Different types of nanomaterials like copper, zinc, titanium, magnesium, gold, alginate and silver have been developed but silver nanoparticles (Nano-Ag) have proved to be most effective as they exhibit potent antimicrobial efficacy against bacteria, viruses and eukaryotic micro-organisms [14-17]. The use of nano-sized silver particles are, as they are antimicrobial agents become more common as technological advances, make their production more economical. Silver may be less toxic to humans and animals than synthetic fungicides. Antimicrobial activity of silver nanoparticles is gaining importance due its broad spectrum of targets in cell compared to conventional antimicrobial agents. Hence, Ag-Nps have been applied to a wide range of healthcare products, such as burn dressings, scaffold, water purification systems, and medical devices [18, 19].

2. MATERIALS AND METHODS

The chemicals used for the study are silver nitrate (AgNO_3), citrate of sodium was purchased from Hi-media, Pondicherry. Received chemicals were used as such; De-ionized water was used throughout the study. Human pathogens viz., *E.coli* sp., *Pseudomonas* sp., *Streptococcus* sp., Similarly fungi viz., *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., NA Broth, Muller Hinton Agar, SDA solution, silver nanoparticles are prepared by using chemical reduction method.

2.1. Synthesis of silver nanoparticle

Silver nanoparticles are prepared from the reduction of

*Corresponding Author: sathiyapriya29.r@gmail.com

Received: 15.09.2017

Accepted: 10.11.2017

Published on: 12.12.2017

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International Journal of Advanced Science and Engineering

www.mahendrapublications.com

silver nitrate by sodium citrate. This process has been done in four different temperatures 150°C, 200°C, 250°C and 300°C. 1M of silver nitrate was prepared by dissolving in de-ionized water. Similarly, 0.1ml of sodium citrate solution was prepared. The transparent colorless solution of silver nitrate solution was changed into pale white, when sodium citrate was added into it. The mixed solution was kept in a magnetic stirrer for 24 hours. After that, the precipitation was collected, filtered and washed several times using de-ionized water and ethanol. The precipitate was kept at four different temperatures viz., 150°C, 200°C, 250°C and 300°C for 30, 25, 20 and 15 minutes respectively. The colour of the prepared samples was changed into pale brown (which is the indication of Nano silver).

3. CHARACTERIZATIONS

Ultra-Violet-Visible Spectroscopy (UV-Vis) was performed in a Shimadzu UV-1650PC Spectrophotometer. To determine the crystalline structure X-ray diffraction (XRD) is used and the data were obtained using a XPERT-PRO diffractometer. The studies of morphology of nanoparticles were performed in JEOL-JSM-5610LV with INCA EDS by scanning electron microscopy (SEM). Particle analyzer was done to measure the particle size using U2475ES Nano. Ultra.

4. RESULTS AND DISCUSSION

4.1. Optical Studies

UV-Vis spectroscopy is one of the most widely used techniques for structural characterization of silver nanoparticles. The absorption spectrum (Fig. 1) of the pale brown silver colloids prepared by chemical reduction showed a surface Plasmon absorption band with a maximum of 425 nm, 420 nm, 414 nm and 402 nm for 150°C, 200°C, 250°C and 300°C respectively is the indication the presence of spherical or roughly spherical silver nanoparticles, UV-Vis absorption spectrum reveals that, the position and shape of the Plasmon absorption depends on the particle size, shape and the dielectric constant of the surrounding medium. It may be observed that surface Plasmon absorption maximum is at 425 nm and 420 nm for 150°C and 200°C but at the temperatures 250°C and 300°C the absorption maximum at 414 nm and 402 nm. From the figure 1 it may be observed that the shift is to lower wavelength with two temperatures (250°C and 300°C). The spectrum shows a smooth variation.

4.2. X-ray Diffraction Studies

The formation of silver nanoparticles in the chemical reduction method has been demonstrated by X-ray diffraction studies. XRD data indicate well-defined characteristic patterns of silver nanoparticles present in

the sample [20]. The observed diffraction peaks are found at 38.1, 44.26, 64.50 and 77.42 and assigned to (111), (200), (220), and (311) planes of free silver particles (Fig 2). The crystallinity of the silver nano-clusters was established by performing the powder X-ray diffraction, the data of which was collected for four different temperatures (150°C, 200°C, 250°C and 300°C). The four diffraction peaks obtained at an angle 39.98, 44.28, 64.46 and 77.32 respectively, could be completely attributed to the face-centered cubic crystalline nature of the sample. As shown in the figure, the highly intense diffraction peak was located at $2\theta=39.98$. This peak is from the (111) plane of the Face Centered Cubic silver. Referring to the above pattern, the lattice constant for the Ag was estimated to be $a=4.0875\text{\AA}$ which was consistent with the reported values ($a=4.0875\text{\AA}$); JCPDS File no (04-0783). The (200), (220) and (311) peaks are less intense. So, in the case, it might conclude that the Nano-assemblies were mainly composed of (111) lattice planes.

4.3. SEM Analysis

The scanning electron microscopy (SEM) image of the synthesized silver is presented in Fig.3 which indicated more or less spherical. The average size of these particles is approximately 38 for 150°C, 34 for 200°C, 29 for 250°C and 20 for 300°C. In comparison with particle size obtained in various temperatures (Table 1) the mean diameter increase significantly at two different temperatures namely 200°C and 300°C. At the same time the shifting of surface Plasmon absorption maximum from 425 nm to 420 nm and 414 nm to 402 nm could be due to the difference in particles shape and size, there is a decrease in absorption indicating a little aggregation of silver nanoparticles. The SEM images for the samples, prepared at 200°C and 300°C are most interesting because of the equal proportion of spherical nanoparticles and flat nano disks prevailing at this temperature. It is to be noted that the width of individual nano disk is in the range 40-95 nm. The spherical nano globules have dimensions as large as 95 nm, but are compositions of smaller spherical nanoparticles, which become as small as 29 nm. Fig.3. (a, b, c, d) depicts the SEM images of samples obtained from the powdered silver nanoparticles prepared at 150°C, 200°C, 250°C and 300°C.

Fig.3 (a) is the SEM image of sample prepared from the Ag colloidal solution of 150°C that shows the existence of very few spherical nanoparticles. At 200°C the SEM feature [Fig. 3(b)] clearly reveals the globular nano clusters in the diameter range 20 nm. This is the dominant morphology at this temperature, but a small number of stacked nano-disks could also be seen. Fig.3 (c) is the SEM images of the samples corresponding to 250°C colloidal silver solutions. In this temperature also the predominant morphology is the spherical nano aggregates but the proportion of nano-disks is higher compared to the 200°C.

Table 1 Particle sizes from XRD, Particle size analyzer and surface plasmon absorption maximum silver nanoparticles at different temperature by salt reduction method.

Temperature	UV-VIS wavelength	X-Ray Diffraction Particle size	Particle Analyzer Particle size
150°C	425 nm	38 nm	95 nm
200°C	420 nm	34 nm	90 nm
250°C	414 nm	29 nm	75 nm
300°C	402 nm	20 nm	65 nm

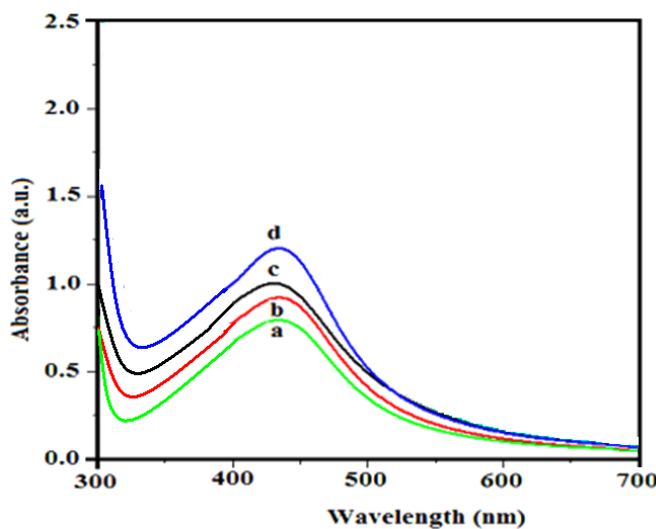


Fig. 1. UV-Visible spectrum of silver nanoparticles a) 425 nm at 150°C, b) 420 nm at 200°C, c) 414 nm at 250°C and d) 402 nm at 300°C

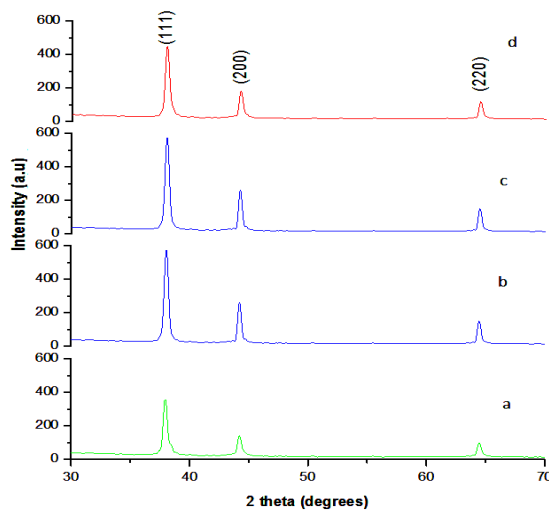


Fig.2. X-ray diffraction spectrum of silver nanoparticles at 150°C, b) 200°C, c) 250°C and d) 300°C

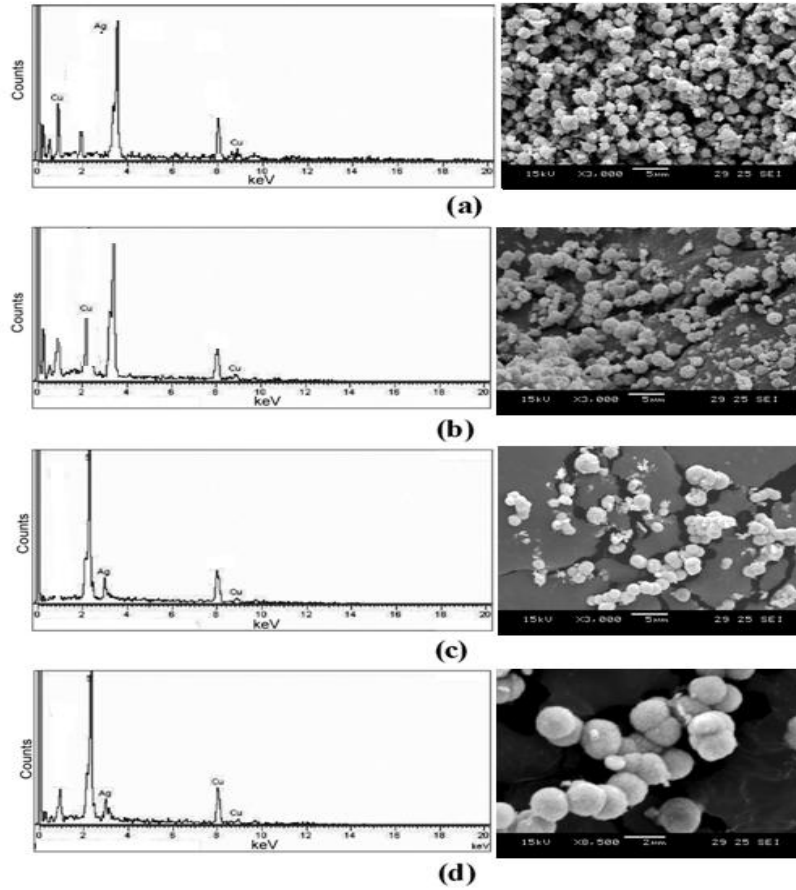
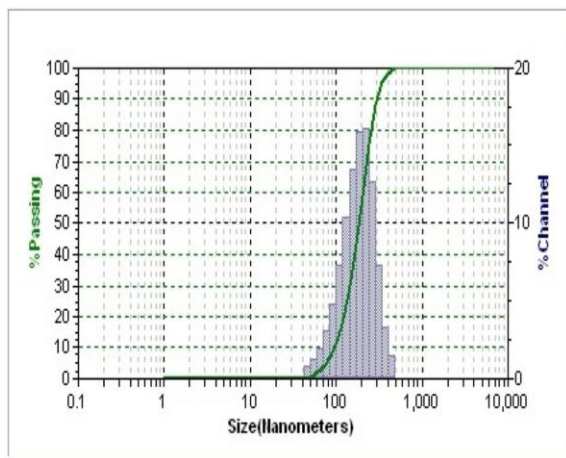
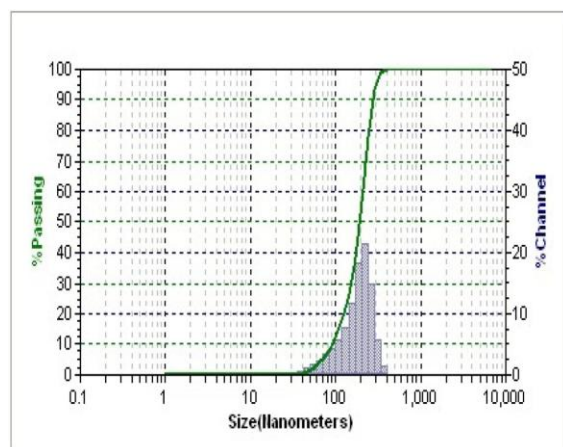


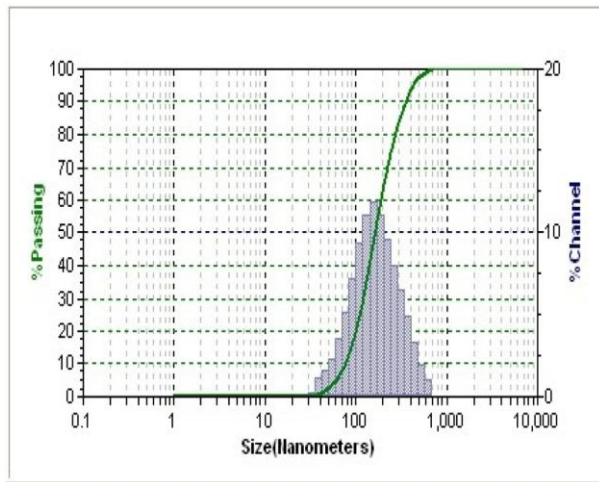
Fig. 3. SEM photographs of silver nanoparticles with EDS at different temperature (a) 150°C (b) 200°C (c) 250°C (d) 300°C



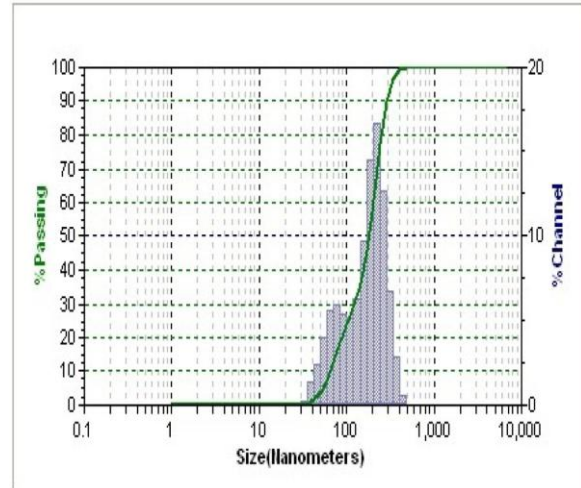
(a)



(b)



(c)



(d)

Fig .4.Shows the particle size analyzer spectrum a) 150°C, b) 200°C, c) 250° C, d) 300°C

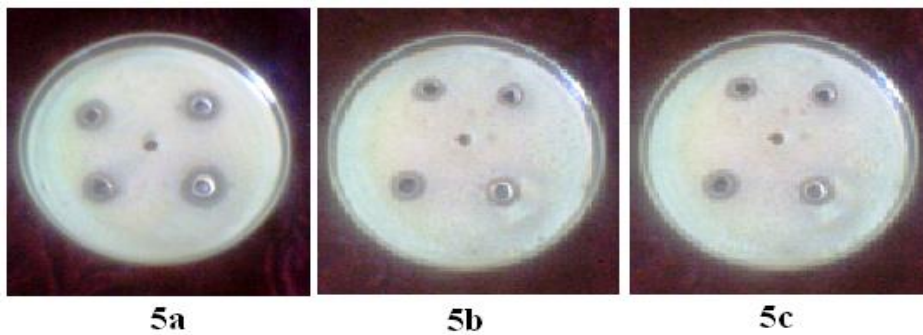


Fig .5.shows the antimicrobial activity of silver nanoparticles prepared at 150°C,200°C, 250°C, and 300°C on bacteria such as a) E.coli sp. b) Pseudomonas sp. and c) Streptococcus sp.

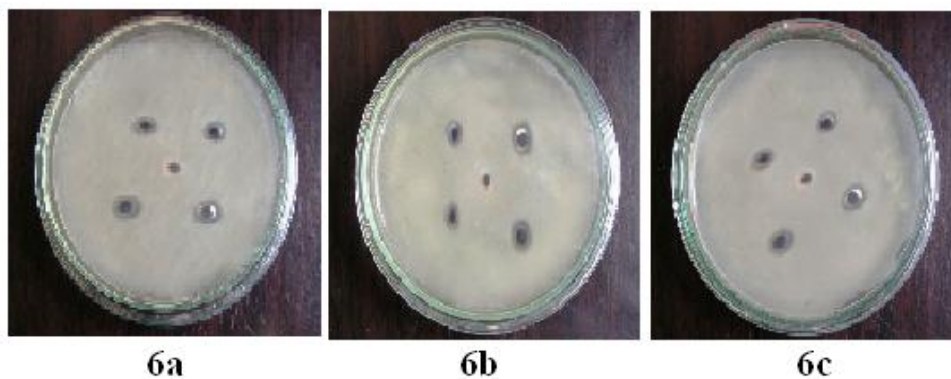


Fig. 6.Shows the antimicrobial activity of silver nanoparticles prepared at 150°C, 200°C, 250°C, and 300°C on fungus such as a) Aspergillus sp. b) Pencillium sp. c) Rhizopus sp.

Table 2 Zone of inhibition (mm) of silver nanoparticles sols prepared at different conditions against test strain (Bacteria)

Bacteria	150°C/95	200°C/90	250°C/75	300°C/65
E.coli sp.	16 mm	17 mm	21 mm	22 mm
Pseudomonas sp.	10 mm	11 mm	12 mm	15 mm
Streptococcus sp.	9 mm	10 mm	11 mm	13 mm

Table 3 Zone of inhibition (mm) of silver nanoparticles sols prepared at different conditions against test strain (Fungi)

Fungus	150°C/95	200°C/90	250°C/75	300°C/65
Aspergillus sp.	9 mm	10 mm	11 mm	12 mm
Pencillium sp.	10 mm	11 mm	12 mm	13 mm
Rhizopus sp.	12 mm	13 mm	14 mm	15 mm

4.4. Particle size analyzer

Laser diffraction particle size analyzer provides the detail about the particle nature, such as monodispersed, di-dispersed and poly-dispersed [21]. This investigation revealed that nanoparticles are in poly-dispersed mixture, with the various sized ranges from 60-100 nm. The obtained nano silver analysis with particle size analyzer, with various temperatures of 150°C, 200°C, 250°C and 300°C is showed in Fig. 4 Particle-size analysis reveals that the prepared nano silver has a diameter of 95. It is noteworthy that the diameter of the particles measured by particle-size analysis higher than the size estimated from XRD predominantly because of high swelling capacity of silver nanoparticles. Fig.4 showed the correlation between temperature and nano silver particle diameter. In varied temperatures of 150°C, 200°C, 250°C and 300°C they obtained nanosilver diameter were 95, 90, 75, and 65 nm respectively. These four points of temperature variations was chosen considering the boiling point of water as the solvent and the melting point (444°C) of silver.

4.5. Microbial Activity of Nanosilver

Three micro-organisms were chosen as test strains; such as *Escherichia coli sp.*, *Pseudomonas sp.*, *streptococcus sp.* (bacteria) and *Aspergillus sp.*, *Pencillium sp.*, *Rhizopus sp.*, (fungus). In view of the results, it appeared that all tested Ag nanoparticles are the most effective products against the tested bacterial and fungal strains. Finally, the antimicrobial susceptibility of Ag nanoparticles synthesized was investigated. The Kirby-Bauer diffusion method was used as antimicrobial susceptibility testing method. Disposable plates inoculated with the tested bacteria, and fungus at a concentration of 1 ml solution was used for the tests. Zone of inhibition were obtained and measured after 24 hr. (bacteria) and 168 hr. (fungus) of incubation at 35°C.

Fig. 5 shows plates to which a bacterial suspension was applied and Fig.6 shows the plates to which a fungus

suspension was applied (1ml solution). The presence of nanoparticles at a certain level inhibited bacterial growth by more than 90%. The diameter of inhibition zone (in millimeter) around the different silver nanoparticles sols against test strains are shown in Table .2 and 3.

Decrease in particle size of silver nanoparticles show a lower susceptibility. However, it is necessary to determine the minimum inhibitory concentration (MIC) of each sample. The silver sols were diluted with distilled water and applied on the bacteria incubated on the Miller Hinton Agar with the concentration of 1 ml. The minimum inhibitory concentration (MIC) was read after 24 hr. of incubation at 35°C. Similarly the silver sols were applied on the fungus incubated on the Sabourauds Dextrose Agar with the concentration of 1 ml. The MIC was read after 168 hr. of incubation at 35°C. Smaller silver nanoparticles synthesized using citrate of sodium has considerably shows the antimicrobial activity. This phenomenon is related to size of colloidal silver particles. Thus the 20 nm (XRD) silver nanoparticles showed the highest activity against bacteria and fungi.

Silver nanoparticles may attach to the surface of the cell membrane and disturb its power function such as permeability and respiration. It is reasonable to state that the binding of the particle to the bacteria depends on the surface area available for interaction. Smaller particles having the larger surface area available for interaction will give more bactericidal and fungicidal effect than the larger Ag nanoparticles.

5. CONCLUSION

Silver nanoparticles were synthesized using chemical reduction method at four different temperatures. UV-Vis spectra show the characteristic Plasmon absorption peak for silver nanoparticles ranging from 402 to 425 nm. The XRD spectrum reveals the clear peaks at higher temperature (300°C) with particles size of 20 nm. The SEM/EDS of the samples shows the spherical morphology

and confirmed the presence of elemental silver, no peaks of other impurity were detected, and SEM study revealed the temperature dependent morphological variation of the nanoparticles, at 200°C and 300°C temperatures only spherical morphology was observed, while at 150°C and 250°C disk morphology was appeared. Particles size analyzer (PSA) measurements showed that the silver nanoparticles form aggregates. Additionally, the antibacterial and antifungal activity of the nanoparticles dispersion was measured by Kirby-Baur method. The results of this study clearly demonstrated that the colloidal silver nanoparticles inhibited the growth and multiplication of the tested bacteria and fungi, including highly multi-resistant bacteria such as *Escherichia coli* sp., *Pseudomonas* sp., and *streptococcus* sp. and fungus viz., *Aspergillus* sp, *Pencillium* sp., and *Rhizopus* sp. Such high antimicrobial activity was observed at very small sized nano silver.

From the observed results, it may be concluded that, the nano silver fight against the microbes in the order of
(i) Bacteria: Ag Nps: *E.Coli* sp., > *Pseudomonas* sp., > *Streptococcus* sp.

(ii) Fungi: Ag Nps: *Rhizopus* sp., > *Pencillium* sp., > *Aspergillus* sp.

Hence, the *E. coli* (Bacteria) and *Rhizopus* sp., (Fungal) have more inhibition (or) highest zone of clearance.

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