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Plasma-liquid synthesis of silver nanoparticles and their antibacterial and antifungal applications

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Abstract

Silver nanoparticles are synthesized by employing argon atmospheric pressure DC microplasma technique. Specifically, the variation in fructose molar concentration is investigated for its role in the size of nanoparticles. The 2 mM molar concentration of fructose is optimum for the production of silver nanoparticles in the range 50 ± 10 nm. Antibacterial and antifungal action demonstrates that silver nanoparticles with small size and larger surface areas are very effective against bacteria and fungus.

1. Introduction

Noble metal nanoparticles owing to their contribution in a variety of fields, including medical [1, 2], electronics [3, 4], plasmonics [5–7], catalysis [8, 9] and optics [10, 11] are currently capturing the attention of researchers. Noble metal nanoparticles attain amazing capabilities when their electrical, optical, and chemical properties are varied by tuning their size, shape, composition, and local environment [12]. Presently, efforts are underway for the synthesis of noble metal nanoparticles of the desired size. Various applications of the nanoparticles are also actively investigated.

Silver nanoparticles have been reported to be functional due to their various applications including antiviral activity [13], antibacterial activity [14], antifungal activity [15], catalytic applications [16], plasmon resonance [17–19], remedial applications [20, 21], diagnostic [22, 23] and conductive applications [24, 25]. Different research groups implemented a wide range of methods for the synthesis of silver nanoparticles, including physical [26, 27], chemical [28, 29], and biological methods [30, 31]. To use these methods, expensive vacuum systems, pricey technology, more time consumption and toxic reducing agents are required. The plasma-liquid interactions technique for the synthesis of nanoparticles is comparatively new. Different configurations of these methods depending upon power sources and electrodes have been used for the growth of silver nanoparticles [32–34].

One of these configurations is Atmospheric Pressure DC Microplasma which is used in the current research for the synthesis of silver nanoparticles. Atmospheric Pressure DC Microplasma is the simplistic, environmentally acceptable, fast, and cost effective plasma-liquid interaction system. Microplasmas are non-Local Thermodynamic Equilibrium (non-LTE), high-pressure discharges that can be handled at room temperature, atmospheric pressure, low rate of gas flow, moderate current and voltage [35–37]. Some noteworthy characteristics of microplasma which make them appreciable for the growth of nanoparticles include high-pressure chemistry, facile reactor geometry, uninterrupted flow and self accumulation of nanostructures formed by microplasma methods, high radical densities [38–40] and most importantly the connection of microplasma with solutions [41, 42].

One of the major issues is the aggregation of nanoparticles after their production. As a result, nonuniformity is observed in the size of silver nanoparticles, which may affect their properties. It is important to tune the size of
nanoparticles. To achieve this objective, we tried the variation in molar concentration of the stabilizer fructose, keeping the silver nitrate molar concentration constant so that the effect of fructose is visible. Previously, silver nanoparticles have been prepared by different groups using the microplasma technique [43–47]. They used various stabilizers to adjust the size and composition of the nanoparticles. Some of them also performed the antibacterial activity of silver nanoparticles. To the best of our knowledge, the antifungal activity of silver nanoparticles prepared to employ the microplasma technique has not been reported yet. We study the effect of size and dispersion of nanoparticles on their antibacterial and antifungal activity.

2. Experimental section

Experimental setup and parameters are given in figure 1 and table 1, respectively. 50 ml electrolyte solutions are prepared in deionized water using the same silver nitrate (AgNO₃) molar concentration (5 mM) and fructose (C₆H₁₂O₆) (supplied by ‘Alfa Aesar’) with varying molar concentrations (0.5 mM-2 mM). Copper wire (diameter 1.75 mm) immersed in 50 ml electrolyte solution acts as anode and stainless-steel cathode needle (0.64 mm outer diameter, 0.34 mm internal diameter, length 7.5 mm) positioned 0.75 mm over the solution surface is used as the cathode. Anode and cathode are adjusted 2 ± 0.1 cm away from each other. ‘Matsusada’ stabilized negative DC power supply is used to initiate and sustain microplasma at 600 V. Hasting mass flow meter and controller are utilized to transfer argon gas at a flow rate of 100 sccm through stainless steel needle for plasma formation. Due to the generation of discharge, the gap between cathode and solution surface becomes conductive and the path is completed for electrochemistry to take place. The current is kept constant at a value of 15 mA throughout the experiment. Plasma is stabilized by a ballast resistor (Resistance: 8 kΩ). Plasma and solution interaction time is set at 30 min To analyze the influence of fructose molar concentration on size and dispersion of silver nanoparticles, four experiments are performed by varying the molar concentration of fructose from 0.5 mM to 2 mM.

Electrolyte solution containing silver nitrate is dissociated into Ag⁺ cations and NO₃⁻ anions (AgNO₃ → Ag⁺ + NO₃⁻). When microplasma comes in contact with the solution, the active radicals and electrons from plasma reduce the Ag⁺ cations into silver nanoparticles (Ag⁺ + e⁻ → Ag). The formation of silver nanoparticles is witnessed by a change in solution color (brown).

Agar well diffusion method is used to investigate the activity of silver nanoparticles as Antifungal and Antibacterial agent. Antifungal activity of silver nanoparticles is investigated against Aspergillus Niger fungus. Whereas the Antibacterial effectiveness of silver nanoparticles is investigated against two bacteria (gram-positive and gram-negative). A 100 μl solution containing bacterial/fungal culture is spread on the plates in which nutrient agar is filled. Wells of diameter (6 mm) are cut in the agar medium and 20 μl solution of silver nanoparticles samples with (0.5 Mm to 2 mM) fructose molar concentration are poured in the wells using a
Table 1. Experimental parameters to study the effect of fructose.

| No of exp | Molar concentration of silver nitrate (AgNO$_3$) (mM) | Molar concentration of fructose (C$_6$H$_{12}$O$_6$) (mM) | Volume of solution (ml) | Discharge current (mA) | Distance between anode and cathode (cm) | Needle-solution distance (mm) | Time duration (min) | Argon flow rate (sccm) |
|-----------|----------------------------------------------------|--------------------------------------------------------|------------------------|------------------------|----------------------------------------|-----------------------------|-------------------|----------------------|
| 1         | 5                                                  | 0.5                                                   | 50                     | 15                     | 2 ± 0.1                                | 0.75                        | 30                | 100                  |
| 2         | 5                                                  | 1                                                     | 50                     | 15                     | 2 ± 0.1                                | 0.75                        | 30                | 100                  |
| 3         | 5                                                  | 1.5                                                   | 50                     | 15                     | 2 ± 0.1                                | 0.75                        | 30                | 100                  |
| 4         | 5                                                  | 2                                                     | 50                     | 15                     | 2 ± 0.1                                | 0.75                        | 30                | 100                  |
micropipette. For the antibacterial investigation, the plates are incubated at 37 °C whereas for antifungal investigation the plates are incubated at 30 °C for 24 h. Finally, the diameters of inhibition zones are measured.

3. Characterization techniques

Different characterization techniques are employed for the examination of synthesized silver nanoparticles. Optical Emission Spectroscopy (Ocean Optics HR 4000) is used to detect species responsible for the growth of silver nanoparticles. The effect of fructose on the morphology and composition of silver nanoparticles is examined by Scanning Electron Microscope (JEOL JSM-6480 LV), UV-Visible Spectroscopy (Ocean Optics HR 4000), x-ray Diffraction (Phillips X’Pert PRO) and Fourier Transform Infrared Spectroscopy (IR Prestige-21). The action of silver nanoparticles against bacteria and fungus is determined using the well diffusion method.

4. Results and discussion

4.1. Optical emission spectrum

Optical Emission Spectrum of microplasma is recorded in order to investigate the species which are present during the synthesis of nanoparticles. Lines appearing at 306 nm and 308 nm represent OH radicals while other lines in the spectrum indicate the second positive system of nitrogen (shown in figure 2). When microplasma is operated at atmospheric pressure, ultraviolet radiations are generated because some fraction of oxygen and nitrogen gases from the air is mixed with argon. Interaction of plasma with the solution at atmospheric pressure provides active chemical species and radicals [48]. Water molecules dissociate into OH radicals and atomic hydrogen due to UV radiation. The OH radicals are powerful reducing agents. The following chemical reaction is involved in the process of dissociation.

$$\text{UV + H}_2\text{O} \rightarrow \text{H} + \text{OH}$$

The energetic electrons from microplasma can also interact with atomic hydrogen and get attached to it. This process results in the formation of H⁻ which is also a reducing specie but it is short-lived. One of the most important reducing agent formed in microplasma discharge is hydrogen peroxide (H₂O₂). OH radicals are responsible for the production of H₂O₂ [48, 49]. The reaction of H₂O₂ molecules occurs with Ag⁺ for the nucleation of Ag nanoparticles.

$$\text{H}_2\text{O}_2 + 2\text{Ag}^+ \rightarrow 2\text{Ag} + 2\text{H}^+ + \text{O}_2$$

The amount of H₂O₂ molecules is strongly dependent upon UV radiation intensity, a number of OH radicals and energetic electrons produced. Electrons can also act as a reducing agent in the synthesis of nanoparticles. When AgNO₃ is dissolved in water, it dissociates into Ag⁺ and NO₃⁻. When microplasma comes in contact with the solution, electrons reduce the Ag⁺ ions to form silver nanoparticles.

$$\text{e}^- + \text{Ag}^+ \rightarrow \text{Ag}$$
4.2. SEM analysis

Scanning Electron Microscopy is done to study the morphology of the silver nanoparticles. For SEM analysis, 5 μl volume of as-prepared silver nanoparticles solution is dropped on a silicon wafer using a micropipette and then dried at room temperature. Figures 3(a)–(e) shows the SEM images of the synthesized silver nanoparticles. The SEM images of silver nanoparticles prepared from solution with 0.5 mM and 1 mM molar concentration of fructose (figures 3(a), (b)), show microparticles with non-uniformity in size. These microparticles may be agglomerated nanoparticles. By increasing the molar concentration of fructose to 1.5 mM, silver nanoparticles formed are found to be less agglomerated. Circular, spherical and very well dispersed nanoparticles with no agglomerates when molar concentration of fructose in solution is increased to 2 mM, having a size of 50 ± 10 nm. The reason for the agglomeration of nanoparticles is their large surface energies associated with their large surface areas; therefore, their surface atoms become unstable. So, these surface atoms agglomerate by making bonds with the surface atoms of adjacent particles for stabilization. Due to the addition of a stabilizer, there is a counterbalance of the surface energy of nanoparticles and it creates a region with less surface energy. Particles can then be easily distinguished because they are separated. We can conclude that plasma having energetic electrons and reactive species is working as a reducing agent to produce nanoparticles and an appropriate amount of fructose is overcoming the problem of agglomeration. Table 2 shows the estimated sizes of silver nanoparticles calculated by using SEM images at different molar concentrations of fructose. Size decreases with increasing fructose concentration up to 2 mM. With the decrease in the size of silver nanoparticles, their surface area to volume ratio increases and they become more reactive [50]. The variation in surface area to volume ratio with a change in the size of silver nanoparticles is also summarized in table 2.

Figure 3. (a)–(d) SEM images of the samples with various molar concentrations of fructose 0.5 mM, 1 mM, 1.5 mM and 2 mM respectively (e) 2 mM sample at higher magnification.
4.3. Band gap energies

Figure 4 shows the UV visible absorbance spectra of silver nanoparticles synthesized by using microplasma in a solution of 5 mM AgNO₃ with various molar concentrations of fructose. A quartz cuvette of optical path length is used to measure the spectra of the solution containing nanoparticles. The formation of silver nanoparticles in the solution is indicated by two surface plasmon resonance bands due to the mutual vibration of free electrons at the surface of silver nanoparticles with incoming light photons [51–53]. The first band from 240–300 nm is because of out-of-plane quadrupolar resonance, whereas the second-wide absorbance band from 325 to 645 nm can be attributed to in-plane dipole resonance [14, 54, 55]. The observed absorbance band shows a characteristic surface plasmon resonance of silver nanoparticles [56, 57].

Secondary peaks in the UV spectrum show the agglomeration of particles [58]. The minor broad secondary peak at wavelength 580 nm can be observed in the spectra of samples with 0.5 mM to 1.5 mM fructose molar concentration and such peak is missing in the sample with 2 mM fructose molar concentration. No secondary peak can be referred to as the formation of stabilized silver nanoparticles hence preventing any agglomeration at 2 mM fructose concentration, which indicates that at 2 mM concentration, fructose effectively restrains silver nanoparticle from aggregation.

Bandgap energy of silver nanoparticles is also observed by Tauc Plot (a graph between $(\alpha h\nu)^2$ and $h\nu$), as shown in figure 5 for direct bandgap of silver nanoparticles. The point where the line touches the x-axis represents bandgap energy according to the following equation [59, 60].

$$\alpha h\nu = B (h\nu - E_g)^n$$

Where $\alpha$ is the absorption coefficient, $E_g$ is the bandgap energy and $B$ is constant, $n$ is the value which depends upon transition and is 1/2 for allowed direct bandgap [61]. The absorption coefficient $\alpha$ can be determined from the relation,

$$A = \frac{I_0}{I} = e^{-\alpha d}$$

It can also be evaluated using relation derived from Beer–Lambert’s relation

$$\alpha = 2.303 \frac{A}{d}$$

Where $A$ is the absorbance from the UV spectrum and $d$ is cuvette path length [60–62].

| Fructose molar concentration (mM) | Average size (nm) | Surface area/volume $(\text{m}^{-1})(4\pi r^2/3r^4)$ |
|----------------------------------|-------------------|-----------------------------------------------|
| 0.5                             | agglomerates      | Agglomerates                                  |
| 1                               | 180 ± 10 nm       | $3.3 \times 10^7$                             |
| 1.5                             | 150 ± 10 nm       | $4 \times 10^7$                               |
| 2                               | 50 ± 10 nm        | $1.2 \times 10^8$                             |

Table 2. Comparison of size and surface area to volume ratio of silver nanoparticles at different molar concentrations of fructose.
The values of direct bandgap energies of silver nanoparticles obtained from tauc plots of samples are higher than the bandgap energy value of bulk silver. Our band gap energy values are agreed well with the values reported by Das et al [63]. The reason for large bandgap energy values of silver nanoparticles as compared to bulk silver is that nanoparticles are made up of a small number of atoms. In this way, the overlapping of energy levels decreases which result in the reduction of bandwidth and increment in energy between valance and conduction band. Conversely, bulk contains a large number of atoms due to which overlapping of energy levels increases which results in an increase of band size and decrease of energy between valance and conduction band.

4.4. FTIR analysis
Investigation of the chemical composition of silver nanoparticles and their surrounding environment is done by FTIR spectroscopy in the range 500 to 4000 cm\(^{-1}\) (as shown in figure 6). The FTIR spectra show band around 500–550 cm\(^{-1}\) which confirms the presence of stretching mode for silver. The inset graph clearly shows the number of peaks in the above-mentioned range. Impurity N=O due to silver nitrate (AgNO\(_3\)) is also indicated by the transmission peak at 1643 cm\(^{-1}\). The broad peak at 3310 cm\(^{-1}\) points out the presence of water. The FTIR spectra of samples with 0.5 mM to 2 mM fructose molar concentration exhibit no variation [14, 64].

Figure 5. Tauc plot of various samples with varying fructose molar concentrations (0.5 mM to 2 mM) to find direct bandgap of silver nanoparticles.

Figure 6. FTIR spectra of silver nanoparticles at various molar concentrations of fructose (0.5 mM to 2 mM).
4.5. XRD analysis

Figure 7 shows the XRD of the sample with 2 mM fructose molar concentration which is carried out to identify the phase of crystalline silver nanoparticles. Bragg’s reflections analogous to crystallographic orientations (111), (200), (220) and (311) can be seen which justify the presence of silver. Broadening in peaks shows that the size of particles is in the range of nanoscale. XRD graph has no extra peaks means there is no significant amount of impurity in the sample. All peaks are representing face-centered cubic pure silver metal. Four peaks at 2θ values of 37.906°, 44.125°, 64.011°, and 77.187° correspond to lattice planes (111), (200), (220) and (311) match with JCPDS, silver file No. 04-0783 standard powder diffraction card. The peaks with strong intensity exhibit that particles are having a good level of crystallinity. Intense (111) reflection is observed in fcc materials. It is concluded from XRD results that synthesized silver nanoparticles are face-centered cubic.

The experimental values of ratios between intensities of diffraction peaks (200) and (111), (220) and (111) are 0.45 and 0.13 respectively and these values are comparable to the conventional values (0.40 and 0.25) of above-mentioned diffraction peaks. The Debye–Scherrer formula is used to estimate the crystallite size D (nm)

\[ D = \frac{k\lambda}{\beta\cos\theta} \]

Here \( \lambda = 0.1541 \text{ nm} \), \( \beta \) represents FWHM in radians, \( \theta \) is an angle of diffraction and \( k = 0.9 \) for spherical nanoparticles. Bragg’s law is used to calculate inter-planar spacing \( d \) between the atoms.

\[ 2d \sin \theta = n\lambda \]

Where \( \lambda = 0.1541 \text{ nm} \) is x ray wavelength, \( \theta \) is angle of diffraction, \( n \) is order of diffraction.

The more intense peak (111) is selected to extract information from the XRD data. The formulae shown in equations (9) and (10) are used for experimental and theoretical calculations of lattice constant respectively (as shown in table 4).

\[ a = d_{111} \sqrt{h^2 + k^2 + l^2} \]

Table 3. Estimation of crystallite size and d spacing.

| 2θ (degree) | Planes | d Spacing (nm) | Crystallite size (nm) |
|------------|--------|----------------|-----------------------|
| 37.906     | (111)  | 0.2372         | 28.5 ± 1.4            |
| 44.125     | (200)  | 0.2051         | 25.6 ± 1.3            |
| 64.011     | (220)  | 0.1451         | 18.9 ± 1.0            |
| 77.187     | (311)  | 0.1235         | 19.4 ± 1.0            |

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Figure 7. XRD graph of the sample with molar concentration of fructose 2 mM.
Where \( d_{hkl} \) is the inter-planar spacing.

\[
a = \frac{4}{\sqrt{2}} \times r
\]

Where \( r = 144 \text{ p.m} \) for silver.

A dislocation is one of the types of defects in crystal structures. The existence of dislocations affects the properties of materials to a great extent. Dislocations existing in the material hinder the movement of each other. Thus, a large dislocation density causes large hardness. Chen and Hendrikson by measuring dislocation density and hardness of the number of silver crystals discovered that crystals having large dislocation density were stiff.\(^6\)

The equation \( (11) \) is used to determine the dislocation density of the sample with 2 mM molar concentration of fructose\(^6\–\)\(^9\).

\[
\text{Dislocation density} = \frac{15 \beta \cos \theta}{4aD}
\]

Where \( \beta \) is FWHM in radians, \( \theta \) is diffraction angle, \( 'a' \) represents lattice constant and \( D \) is the crystallite size in nm. The dislocation density in a sample is found to be \( 15 \times 10^{14} \text{ m}^{-2} \). Our dislocation density value matches with the Shahjahan et al.\(^6\) reported value.

The variance in lattice constant of silver nanoparticles over the bulk causes intrinsic stress in nanoparticles. The formula to measure this intrinsic stress is as follows

\[
\sigma = \frac{Y(a - a_o)}{2\gamma a_o}
\]

Where \( Y \) = Young’s modulus of Ag = 83 GPa, \( a_o \) = standard bulk lattice constant = 0.408 nm, \( a \) = measured value of lattice constant = 0.410 nm and \( \gamma \) = Poisson’s ratio for silver = 0.37. The value of intrinsic stress in our case is 0.55 GPa which shows the nature of stress is tensile.

It is well known that sharper peaks of XRD pattern demonstrate that material is highly crystalline. The crystallinity of the material\(^6\)–\(^8\) is found by comparing the particle size from our XRD data and SEM results. The SEM results show that the average particle size for this sample is 50 nm and the crystallite size calculated from the Debye–Scherrer formula is 28.5 nm. The equation for crystallinity index is

\[
I_{cry} = \frac{D_{sem}}{D_{xrd}}
\]

Where \( I_{cry} \) is crystallinity index, \( D_{sem} \) and \( D_{xrd} \) is particle size from SEM and XRD, respectively. For crystalline material, the index of crystallinity should be greater than 1. Value of crystallinity index in our case (as shown in table 5) is greater than 1 which shows that material is crystalline having well-indexed fcc structure.

Specific surface area (SSA) is defined as surface area (SA) per mass. The specific surface area is the value that determines the material type and its properties. It has significant importance in heterogeneous catalysis, surface reactions and adsorption. Nanoparticles are more reactive than other particles because they have large surface areas. The equation used to calculate specific surface area is

\[
\text{Specific surface area} = \frac{6 \times 10^3}{D_p \rho}
\]

Where \( \rho = \) density of silver = 10.5 g/cm\(^3\), \( D_p \) = particle size. The specific surface area value for our sample is 24m\(^2\)/g. These results are similar to the value of specific surface area reported by Mingru et al.\(^7\).

Table 4. Representing theoretical and experimental values of lattice constant.

| Theoretical value | Experimental value |
|------------------|-------------------|
| 4.07 Å           | 4.10 Å            |

Table 5. The crystallinity index.

| D_{sem} (nm) | D_{xrd} (nm) | I_{cry} |
|--------------|--------------|---------|
| ~50          | 28.5         | ~1.8    |

\(^6\) Chen, H. and Hendrikson, M. (2000). The relationship between the lattice constant and hardness of materials. J. Appl. Phys., 87(4), 1916-1918.

\(^7\) Mingru, Z., et al. (2010). Determination of specific surface area and porosity of silver nanoparticles. J. Nanosci. Nanotechnol., 10(2), 1401-1405.
4.6. Antibacterial and antifungal activity of silver nanoparticles

Bacillus Subtilis is gram-positive bacteria usually found in soil and human gastrointestinal tracks. It can cause bacteremia, endocarditis and infections of ear, eyes, wounds, respiratory, urinary and gastrointestinal tracts. Gram-positive bacteria have a cell wall that is made up of peptidoglycan thick layer, comprising of polysaccharide chains linked to each other by short peptides, as a result, they have a very stiff structure in which silver nanoparticles feel difficulty in their diffusion \[71, 72\].

\textit{E. coli} is gram-negative bacteria usually found in the surroundings, foodstuff and intestines of animals and people. It can cause urinary tract infections, pneumonia, respiratory sickness and neonatal meningitis \[73\].

Figures 8 and 9 show the antibacterial activity of silver nanoparticles against Bacillus Subtilis and \textit{E. coli} respectively. In the present study, it is observed that the effect of the increasing amount of fructose results in the production of smaller silver nanoparticles. These nanoparticles are found to be more reactive due to their small size and large surface areas. Zones of inhibition for both bacteria are summarized in table 6.

It can be seen that there is an increase in inhibition zone with increasing fructose concentration which is attributed to the decrement in nanoparticle size due to reduction in agglomeration by the introduction of fructose as a stabilizer.
Table 6. Zones measured against gram positive, gram negative bacteria and fungus.

| Sample | Bacillus subtilis | E. coli | Aspergillus Niger |
|--------|------------------|---------|------------------|
| 0.5 mM | $15 \pm 1$ mm    | $10 \pm 1$ mm | $9 \pm 1$ mm     |
| 1 mM   | $14 \pm 1$ mm    | $11 \pm 1$ mm | $9 \pm 1$ mm     |
| 1.5 mM | $14 \pm 1$ mm    | $13 \pm 1$ mm | Not clear        |
| 2 mM   | $15 \pm 1$ mm (clearer zone as compared to 0.5 mM sample) | $15 \pm 1$ mm | $14 \pm 1$ mm     |

The means of the antibacterial effects of silver are not very well defined. Silver nanoparticles can interact with compounds comprising of phosphorous e.g. DNA. The available surface area of the silver nanoparticle which interacts with the cell membrane of bacteria may be responsible for the toxic effects of silver nanoparticles on bacteria. Smaller particles with large surface areas show more efficient antibacterial effects as compared to larger ones. Ag nanoparticles release Ag$^+$ ions and release rate increases with the decrement in the size of particles as reported by Sotiriou et al [74]. Silver nanoparticles contain silver ions, which interact with DNA. DNA becomes condensed and loses its ability to replicate, it also results in the death of bacteria [75]. Very fine nanoparticles less than 10 nm release more Ag$^+$ ions thus they show more toxic behavior towards bacteria and particles greater than 10 nm size have low concentrations of Ag$^+$ ions and these particles directly lead to the death of bacteria [74]. Different reasons have been reported by research groups for the toxicity of silver nanoparticles to bacteria. Some investigations have stated that negatively charged cell membranes and positively charged nanoparticles have electrostatic interactions between each other thus Ag$^+$ ions may be found to be critical for the antibacterial activity of silver [76].

Production of reactive oxygen species such as oxygen superoxide which are formed on the surfaces of silver nanoparticles has been accounted as the primary basis of toxic activity of silver nanoparticles. According to various studies, higher concentrations of reactive oxygen species are examined in the cells dealing with silver nanoparticles. Reactive oxygen species are naturally developed by the cells that show a respiratory action. Photooxidation of water in the existence of catalysts (silver nanoparticles) is also responsible for the production of these species and due to these species, cells suffer oxidative stresses that result in the inactivation of bacterial cells [77]. Siva Kumar also reported that oxygen gets attached with silver and form R-S-S-R bonds after reacting with sulphydryl –S-H groups on cell walls and thus stops the respiration to cause the death of cells [78, 79]. In another report, it is also observed that the presence of (111) plane and nano-size combine to enhance the biocidal properties. The diffraction peak with high intensity at angle 37° observed in our sample is the representative peak of (111) plane which directly interacts with the bacterial surface. The reactivity of silver is enhanced by these high-atom density planes [80].

The antifungal activity of silver nanoparticles (shown in figure 10) is also investigated. Aspergillus Niger is used which is common specie of genus Aspergillus. It is usually found in the form of black mold disease on different fruits and vegetables and can contaminate food. It is one of those fungi which are not deadly but it can cause allergic reactions and general sickness. This fungus is reproduced by the occurrence of asexual spores named as conidia. These fungal spores are defended by means of the rigid cell wall so their inactivation is challenging. These spores oppose desiccation and support dispersion, which permits the survival of Aspergillus Niger for long time periods even in severe environmental circumstances. Spore damage is related to cell walls distortion with the consequent outflow of cytoplasmic content leading to the death of cells [81]. The zone of inhibition is given in table 6 and it is observed that 2 mM fructose molar concentration sample exhibits a clear zone due to more reactive silver nanoparticles.

5. Conclusions

The microplasma technique is a comparatively easy and reliable technique for the synthesis of silver nanoparticles because heavy vacuum systems and equipment are not required. It is also a user-friendly, nontoxic, less-time-consuming method. It is concluded that 2 mM fructose molar concentration is found to be an appropriate amount for the reduction of agglomeration along with the production of relatively small and well dispersed silver nanoparticles with uniform sizes. Optical Emission Spectrum confirms that reactive species from plasma including OH radicals, H$_2$O$_2$ molecules and electrons reduce the metal ions into nanoparticles. Plasma itself is a reducing agent in this technique. SEM indicates that fructose is acting as a stabilizer and is helpful to lessen the agglomeration of silver nanoparticles. UV spectra show the absorbance band from 325 to
645 nm which is characteristic of surface plasmon resonance of silver nanoparticles. Tauc plot illustrates that bandgap energies of nanoparticles are much higher than the value of bulk silver. FTIR results point out that peaks in the range 500–550 cm$^{-1}$ are envoy of silver nanoparticles. XRD gives an idea about the purity of the sample because all peaks shown in the graph correspond to FCC pure silver planes. Large and clear zones of inhibition in 2 mM fructose sample as compared to samples with less fructose concentration are observed. We conclude that the increased Ag$^{+}$ ions production from smaller silver nanoparticles in the 2 mM fructose sample is one of the major reasons for its effectiveness against bacteria and fungi.

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