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Asian Journal of Pharmaceutical Research and Development

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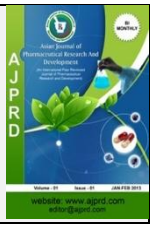

Open Access Research Article

# Synthesis of Silver Nanoparticles for Antibacterial Activity against Staphylococcus Aureus and Escherichia Coli

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## A B S T R A C T

In Recent year's medication obstruction is a quickly developing issue over the whole world in the treatment of infectious diseases. The widespread use of broad-spectrum antibiotics produced antibiotic resistance for many human bacterial pathogens. Anyway right now, nanotechnology research has been engaging more in restorative businesses with various advantages because of the way that surface area to volume proportion of Nanoparticles is quite large. In this exploration work, Silver Nanoparticles (AgNPs) are prepared by using a reducing agent like sodium borohydride and capping agent polyvinylpyrolidine.

The AgNPs were set up by the chemical reduction method. Nanotechnology has its own importance it eventually reduce dose and also have a superior bioavailability than larger particles. The prepared AgNPs were subjected to characterization like particle size, X-Ray diffraction. In addition, the AgNPs drawing much interest in account of their powerful antibacterial movement. The antibacterial activity of AgNPs checked against the bacterial culture of Staphylococcus Aureus and E, coli. The zone of inhibition AgNPs was checked against both microorganisms. The results of this study demonstrate antibacterial activity of AgNPs against the bacterial culture of Staphylococcus Aureus and E, coli.

**Key words:** AgNPs, Antibacterial activity, Multidrug Resistance, Antibiotics.

**A R T I C L E I N F O:** Received 15 August 2021; Review Complete; 25 Sept. 2021 Accepted; 10 Oct. 2021 Available online 15 Oct. 2021

**Cite this article as:**

Nitin P*, Avari J, Synthesis of Silver Nanoparticles for Antibacterial Activity against Staphylococcus Aureus and Escherichia Coli, Asian Journal of Pharmaceutical Research and Development. 2021; 9(5):67-73. **DOI:** http://dx.doi.org/10.22270/ajprd.v9i51029

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## INTRODUCTION

Nanotechnology is an emerging interdisciplinary revolution in several therapeutic areas over the last decade, including medicine, and drug delivery system. The essence of this new technology features a great and significant impact within the field of diagnosis and drug delivery. Nanotechnology is a rapidly expanding field, encompassing the development of man-made materials in the 1-100 nanometer size range [1]. Currently, many methods and approaches have been reported for the synthesis of AgNPs (AgNPs) by using chemical, physical, photochemical and biological routes. The nanotechnology revolution has begun and shows enormous promise in the field of drug [2]. In the past, number of Nanoparticles-based drug delivery system developed for the treatment of cancer, diabetes, pain, asthma, allergy, infections, and so on. The significant benefits using nanoscale agent are more effective and convenient routes of administration, lower therapeutic toxicity, extend the drug bioavailability, as well as ultimately reduce health-care costs [3].Now a day's antibiotics have lot of side effect as well as administration of high dose to the patient. The aim to synthesize the AgNPs is to procure the synergistic activity by the drug and to enhance synergistic activity by reducing the dose of antibiotics [4] .

Nanoparticles are viable alternative to antibiotics and appear to possess a high potential for bacterial multidrug resistance. In particular, AgNPs have attracted much attention towards field of nanotechnology. In the past years,

it was found that silver was very useful as an antiseptic and antimicrobial agent against Gram-positive and Gram-negative bacteria due to its low cytotoxicity [5] .From a structural point of view, AgNPs have at least one dimension in the range of 1 to 100 nm and more importantly, as particle size decreases, the Surface area-to-volume ratio greatly increases [6] .

### Mechanism of AgNPs

AgNPs show antibacterial effect by attaching to the cell membrane of bacteria and also penetrating inside the bacteria. The Nanoparticles preferably attack the respiratory chain and inhibit cell division. The release of silver ions in the bacterial cells enhances their bactericidal activity. Silver is used in different forms such as metals, nitrates, and sulfadiazine. Nanoparticles are effectively a bridge between bulk materials and atomic or molecular structures [7] .

In ancient days Romans treated their water with silver coins, a tradition still being continued in many societies and even in space programs for purifying water. The use of silver as an antimicrobial agent a German obstetrician used 1% silver nitrate solution to eliminate blindness caused by postpartum infections in newborns. US Food and Drug Administration approved colloidal silver for wound treatment. The 0.5% silver nitrate solution use in the burn area. 1% silver sulfadiazine (SSD) cream, which has become one of the leading topical antimicrobial agents used to treat burn wound infections over the last four decades [8,9] .

Although acute toxicity of silver within the environment is dependent on the supply of free silver ions, investigations have shown that these concentrations of Ag+ ions are too low to cause toxicity. The dietary intake of silver is calculable at 70-90µg/day [9] .The synergistic effects of the antibiotics were attributed to complexes formed between the antibiotics and the AgNPs, resulting in the release of a high concentration of silver ion on the bacteria cell wall, thereby inhibiting the growth of bacteria [10] .

## MATERIAL AND METHODS

### Material

Silver nitrate, Sodium borohydrate and ascorbic acid all chemical are made by Hi-media (AR Grade)

### Methods

### Formulation of AgNPs

In formulation of AgNPs, Silver nitrate (AgNO3) was used as a precursor material while Sodium borohydrate and Ascorbic acid (C6H8O6) were used as the reducing agent and surfactant, respectively [11] .

The concentrations of Sodium borohydrate and ascorbic acid were varied in order to observe the effect of these parameters especially on the size and morphology of the AgNPs. In detail, 80 ml of AgNO3 was first heated to 60°C and was then added (with vigorous stirring) to 20 ml of a sodium borohydrate and ascorbic acid solution that was pre-heated to 60°C. The mixture was stirred for 20 minutes. Then heating was stopped and the solution cooled to room temperature with continuous stirring. The as synthesized AgNPs was characterized by XRD (using model D5000 Siemens Diffractometer). In plan of AgNPs silver nitrate (AgNO3) was utilized as an antecedent material while sodium borohydrate and ascorbic acid were utilized as the reducing agent and surfactant, individually [12-14] .

**Table 1:** Different Methods of Nanoparticles formulation

| Physical methods | Chemical methods | Biological method |
|---|---|---|
| Ion beam technique | Sol gel method | Using plant extracts |
| Electric arc deposition | Sol gel method | Using plant extracts |
| Mechanical methods | Co precipitation | Using microorganisms |
| Vapour deposition | Micro emulsions | Using enzymes |
| Sputter deposition | Hydrothermal synthesis | Using agricultural waste |
| Molecular beam epitaxy | Sonochemical synthesis | |

### X-ray diffraction Analysis

The X-ray diffraction (XRD) measurement of AgNPs was carried out using Cu-K$\alpha$ radiation source in scattering range $m(2\theta)$ of 20– 70 on the instrument operating at a voltage of 45 kV and a current of 40 mA. The presence, crystalline nature, phase variety, and grain size of synthesized AgNPs were determined by X-ray diffraction spectroscopy [15]. The particle size of the prepared samples was determined by using Scherer's equation as follows:

$$D = K\lambda\ \beta 1/2 \cos\theta\ ,$$

Where, $D$ is average crystallite size and $\beta$ is line broadening in radians (full width at half maximum of the peak in radians).

$\lambda$ is wavelength of X-ray and $\theta$ is brags angle.

*K* is constant (geometric factor = 0.94). [16]

At the point when the crystallite size diminishes from mass to nanoscale measurements, the XRD tops widen. The Scherer condition, = κλ (D β θ cos), quantitatively portrays the widening of a top at a specific diffraction point (θ), as it relates the translucent space size (D) to the width of the top at half of its tallness (β). The Scherer steady, κ, is commonly viewed as 0.91 however can differ with the morphology of the crystalline domains. The X-ray wavelength (λ) is a steady that relies upon the sort of X-beams utilized. Each peak can be assessed autonomously and should produce a consistent crystalline domain size as long as the sample can be roughly approximated as uniform round particles. Note that, in the Scherer condition, the diffraction point is in radians (not degrees) and relates to θ and not 2θ as is normally plotted in a XRD design. Likewise note that translucent space size doesn't really correspond to p article size, as particles can be polycrystalline, containing different crystalline domains [17]

**Particle size Determination by Laser particle size analyzer**

Particle size determination was carried out by means of laser Diffractometer, using an Omec instrument Co ltd. Model Omec LS (POP)9. Measurements were taken in the range between 0.1 and 1000 μm. The instrument was set on the following parameters, particle refractive index 0.54, particle absorption coefficient 4, water refractive index 1.33, and general calculation model for irregular particles. Three measurement cycles of each were taken, and the data obtained were averaged by software LS (POP) [18] .

**Particle size Determination by XRD**

The particle size 'D' was calculated for the samples using Scherer's equation

$$\frac{dy}{dx} = \frac{0.9\lambda}{(W\cos\theta)}$$

Where '$\lambda$ is the wavelength of x-ray, 'W' is FWHM (Full width at half maximum), 'ɵ' is the diffraction angle and 'D' is particle diameter (Size). When the crystalline domain size calculated by the Scherer equation matches the average diameter of particles determined Particle size analyzer. This observation suggests that the particles are single crystals rather than polycrystalline X-ray diffraction [18, 19] .

**In-Vitro Antibacterial Activity**

This examination was pointed toward deciding the MIC and MBC of AgNPs against Staphylococcus Aureus and Escherichia coli. The antibacterial effects of silver are mostly attributed to silver ions [20] . AgNPs continuously release silver ions in an aqueous microenvironment. Because of the bigger surface area of AgNPs, they show a stronger and better bactericidal effect [21] . The main reasons for bactericidal properties of AgNPs interfere with the integrity of the bacterial cell by binding to essential cellular structural, particularly to their SH-groups. AgNPs also generate reactive oxygen species (ROS) and free radicals which damage the bacterial cell wall and inhibit the respiratory enzymes [22]. AgNPs disturb the DNA replication and terminate the bacteria [23] .

**Minimum Inhibitory Concentration (MIC) determination**

Antibacterial activity of the synthesized AgNPs was studied by the standard disc diffusion method. The overnight grown bacterial culture of Staphylococcus Aureus and Escherichia, coli. Were taken for study [24] .The dilutions of synthesized AgNPs varying from 0.030 mg/ml, 0.070 mg/ml, 0.150mg/ml, 0.310 mg/ml, and 0.620 mg/ml were prepared. The preparation of nutrient media was done by taking 20 g of solidified nutrient media (Soyabean casein digest media) with 2 % of Agar added in 500 ml of distilled water and sterilized in autoclave at 15 lb of pressure and 1210c temperature [25] . This mixture was poured equally into Petri-plates. Keep this plate to solidify, after solidification bacterial cultures were spread on surface of solidified agar with help of spreader. The bore of 8 mm was made up to the lower surface of solidified media [26, 27] . Then organisms to be tested were inoculated in four bores (8 mm diameter) in different dilutions of AgNPs (0.030 mg/ml, 0.070 mg/ml, 0.150mg/ml, 0.310 mg/ml, and 0.620mg/ml) solutions [28, 29,30] . The plates containing different concentration of AgNPs was incubated at 370C and then examined for confirmation, the appearance of a clear area around the bore was observed [31]. The diameter of such zones of inhibition was measured using a meter ruler, and the mean value for each organism was recorded and expressed in millimeters [32,33] .

## RESULT & DISCUSSION

The AgNPs were successfully prepared by chemical reduction method with low cost. The process utilizes, in the aqueous solution, the mixing of Silver Nitrate as organic precursor. The sodium borohydrate and ascorbic acid were use as reducing agents for preparation of AgNPs. The concentrations of Sodium borohydrate and ascorbic acid were varied in order to observe the effect of these parameters especially on the size and morphology of the Ag NPs.

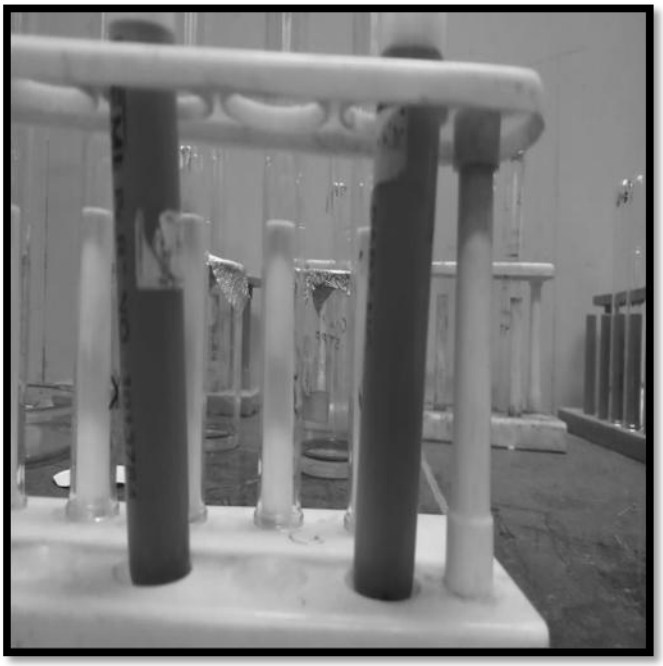


**Fig 1: Prepared AgNPs**

After formulation of AgNPs the confirmation of synthesized AgNPs was characterized by using UV spectrophotometer (Shimadzu 1800). The formation of these can be confirmed by means of spectrum for the colloids that Plasmon band is observed near 416 nm, which confirms that the silver ions were reduced to Ag° in watery phase. The prepared AgNPs can change the absorption spectra at different wavelength according the synthesized range of Nanoparticles in the solution

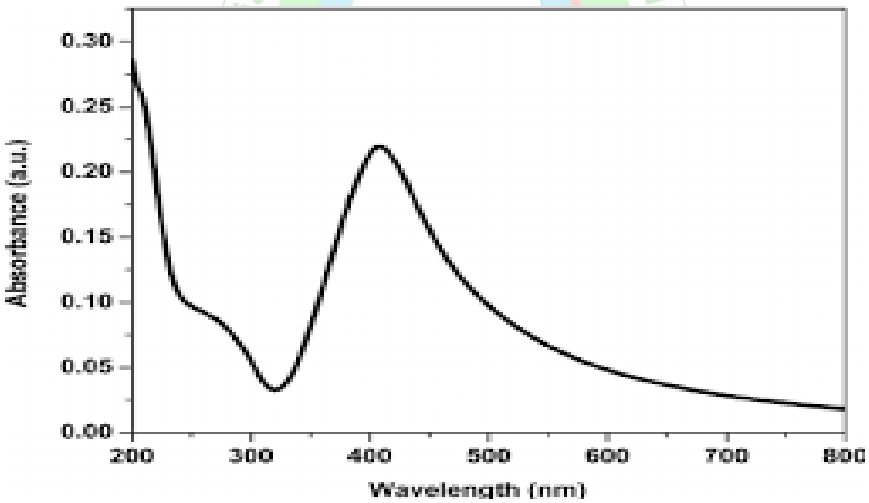



**Fig 2: UV spectra of prepared Silver Nanoparticles**

As per the absorption spectrum mention above it is clear that there is formation of AgNPs in the range of 1-200 nm. UV spectrum is primary characterization to confirm the synthesis of AgNPs.

***Particle size***

Particle size determination was carried out by means of laser Diffractometer, using an Omec instrument Co ltd. Model Omec LS (POP) 9. In which parameters of the instruments has been set. Material Refractive Index was 1.52, Dispersant RI 1.333 , Obscuration 0.45% was set then result was taken the average particle size of AgNPs was found in the range of 1- 200 nm.

**Table 2: Particle size distribution with volume %**

| Material | Material RI: | Dispersant Name: | Dispersant RI: | Obscuration % | Result Range(µm): | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Dx(10) | Dx(25) | Dx(50) | Dx(75) | Dx(90) | Dx(97) |
| AgNP | 1.52 | Water | 1.333 | 0.45 | 0.311 | 0.355 | 0.417 | 0.489 | 0.557 | 0.633 |
| **Size (µm)** | **Vol (%)** | **Vol (%)** | **Size (µm)** | **Vol (%)** | **Size (µm)** | **Vol (%)** | **Vol (%)** | **Size (µm)** | **Vol (%)** | **Vol (%)** |
| **0.059** | 0.36 | 0.36 | 2.781 | 0.00 | 29.9 | 0.00 | 100.00 | 321.62 | 0.00 | 100.0 |
| **0.072** | 12.4 | 12.76 | 3.527 | 0.00 | 48.0 | 0.00 | 100.00 | 517.20 | 0.00 | 100.0 |
| **0.137** | 37.6 | 87.04 | 5.671 | 0.00 | 60.9 | 0.00 | 100.00 | 655.86 | 0.00 | 100.0 |
| **0.147** | 12.9 | 100.00 | 7.192 | 0.00 | 77.3 | 0.00 | 100.00 | 750.00 | 0.00 | 100.0 |
| **0.189** | 0.00 | 100.00 | 9.120 | 0.00 | 98.0 | 0.00 | 100.00 | 0.00 | 0.00 | 0.00 |

From the above data particle size distribution was observed which shown in Fig: 3

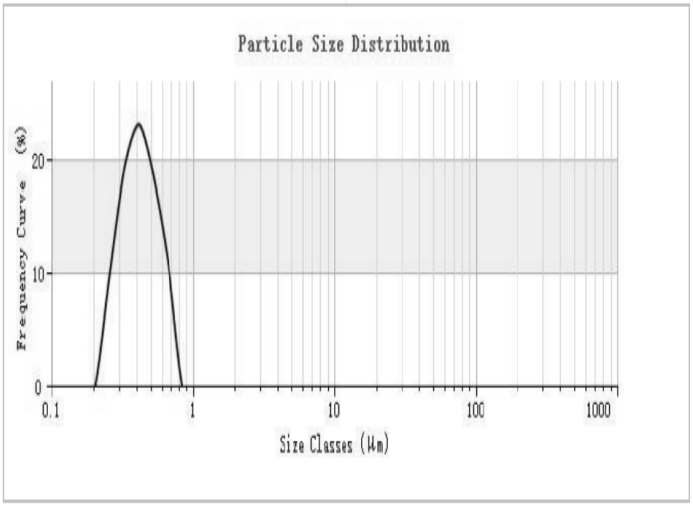



**Fig 3: Particle size distribution**

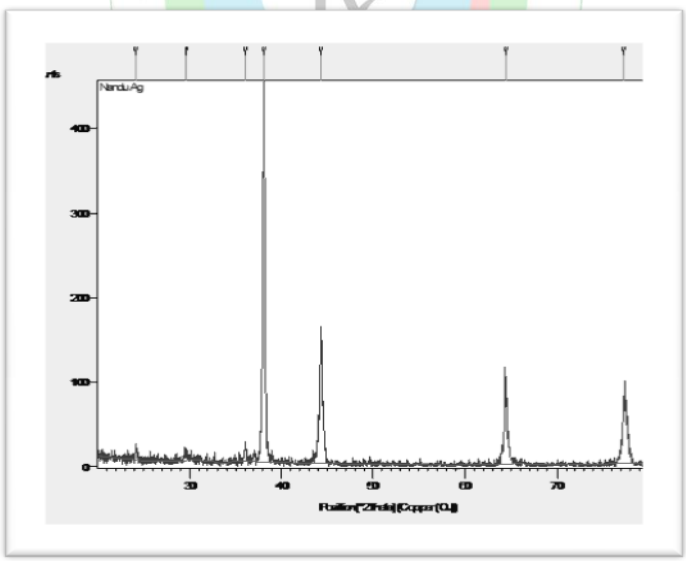



**Fig 4: XRD spectrum of AgNPs**

The Nanoparticles synthesized in this method were characterized using powder form. The evaluations are shown in Table 3. Diffracting angle in degree, FWHM(radians) , d spacing(nm), Rel. Int. [%] .By considering the values given in table the particle size 'D' was calculated for the samples using Scherer's equation (Cullity & Stock, 2001). From the calculation the particle size of synthesized Nanoparticles was observed in the range of 1-200 nm. Which was again compared with the particle size obtain by the Particle size analyzer. The result obtain by both the methods was matched with each other.

**Table 3:** XRD Data of Prepared AgNPs

| Diffracting angle in degree (expt.) | Diffracting angle in degree (JCPDS) | FWHM(radians) | d spacing(nm) | d spacing(nm) JCPDS data. | Rel. Int. [%] |
|---|---|---|---|---|---|
| 24.2131 | 24.2125 | 0.5904 | 3.67585 | 3.6729 | 2.39 |
| 29.6854 | 29.6875 | 0.5904 | 3.00952 | 3.0068 | 1.70 |
| 36.0417 | 36.0375 | 0.2952 | 2.49201 | 2.49023 | 3.60 |
| 38.0910 | 38.0875 | 0.2460 | 2.36253 | 2.36078 | 100.00 |
| 44.2875 | 44.2875 | 0.2460 | 2.04529 | 2.0436 | 34.23 |
| 64.4234 | 64.4125 | 0.2460 | 1.44628 | 1.4453 | 22.76 |
| 77.3330 | 77.3375 | 0.3000 | 1.23290 | 1.23284 | 16.10 |

### *Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)*

Determination of MIC was done by taking 0.030 mg/ml, 0.070 mg/ml, 0.150 mg/ml, 0.310 mg/ml, 0.620 mg/ml of conc. of AgNPs and inoculated to well and incubated for 24 hrs. zone of inhibition was measured against the same bacterial culture result shows that 0.030 mg/ml concentration of silver does not show zone of inhibition whereas 0.070 mg/ml concentration of AgNPs showed zone of inhibition against *Staphylococcus Aureus and Escherichia Coli.* From this it confirms that 0.070 mg/ml was the minimum inhibitory concentration of Ag NPs.
Then MBC of AgNPs was determined by taking 0.070 mg/ml 0.150mg/ml, 0.310 mg/ml, 0.620 mg/ml and inoculated to well and incubate for 24 hours. 0.070 mg/ml conc. shows the zone of inhibition after that it was kept under observation no visible bacterial growth reappear on area of zone of inhibition that menace 0.070 mg/ml conc. consider as Minimum bactericidal concentration. This was done by observing pre and post-incubated agar plates. In this study, The MIC and MBC of AgNPs against gram positive and gram negative bacteria were determined and were found to be effective at 0.070 mg/ml. (Tables 4). The AgNPs showed MIC at concentration of 0.070 mg/ml against the culture of both gram positive and gram negative bacteria and same concentration act as Minimum Bactericidal Concentration. (Shown in Table: 4 & 5).

**Table 4:** MIC and MBC Determination of Ag NPs against S, Aureus

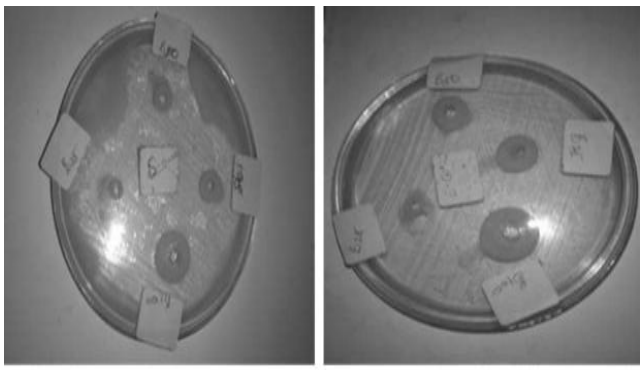
| | MIC observations | | | | | MBC observations | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Staphylococcus Aureus | | | | | Staphylococcus Aureus | | | |
| Conc. of silver NP | 0.030 mg/ml | 0.070 mg/ml | 0.150mg/ml | 0.310 mg/ml | 0.620mg/ml | 0.070 mg/ml | 0.150mg/ml | 0.310 mg/ml | 0.620 mg/ml |
| Zone of Inhibition | - | + | + | + | + | + | + | + | + |

Positive (+): Indicating Zone of Inhibition; Negative (–): Indicating No Zone of Inhibition

**Table 5:** MIC and MBC Determination of Ag NPs against E,coli

| MIC observations | | | | | | MBC observations | | | |
|---|---|---|---|---|---|---|---|---|---|
| E, Coli | | | | | | E, Coli | | | |
| Conc.of silver NP | 0.030 mg/ml | 0.070 mg/ml | 0.150mg/ml | 0.310 mg/ml | 0.620mg/ml | 0.070 mg/ml | 0.150mg/ml | 0.310 mg/ml | 0.620 mg/ml |
| Zone of Inhibition | - | + | + | + | + | + | + | + | + |

Positive (+): Indicating Zone of Inhibition; Negative (–): Indicating No Zone of Inhibition



**Staphylococcus Aureus (5a) Escherichia Coli (5b)**
**Figure: 5 Determination of MIC by AgNPs**

## CONCLUSION

The AgNPs were synthesized by chemical reduction method. In this method silver nitrate ($AgNO_3$) work as precursor which further interact with reducing agent and stabilizing agent. UV -VIS absorption spectrum and XRD result give the confirmation about synthesis of AgNPs. The size of prepared Nanoparticles was confirmed by Scherer equation and particles size analyzer. The resulted sizes compared with each other which showed the average particles size in the range of 1-200 nm. In further study the MIC and MBC of AgNPs against *S. Aureus* and *E. coli* was determined and found to be effective at 0.070 mg/ml. The AgNPs show MIC and MBC at concentration of 0.070 mg/ml against the culture of *Staphylococcus Aureus and Escherichia Coli.*

**Conflict of Interest:** None.

## ACKNOWLEDGEMENT

Author is thankful to HOD, Department of Pharmaceutical Sciences, R.T.M. Nagpur University, Nagpur to carried out experimental work in research laboratory and for fruitful discussion.

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