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Research article

Synthesis and characterization of cefixime loaded silver nanoparticles for antibacterial activity against *staphylococcus aureus*

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ABSTRACT

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. Anyways 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 Polyvinylpyrrolidone.

The prepared AgNPs and Cefixime-loaded AgNPs were subjected to characterization like particle size, FT-IR, X-ray diffraction. In addition, the AgNPs draw much interest in the account of their powerful antibacterial movement. The antibacterial activity of AgNPs and Cefixime-loaded AgNPs was checked against the bacterial culture of *Staphylococcus Aureus*. The zone of inhibition AgNPs was checked against microorganisms. The results of this study demonstrate the antibacterial activity of AgNPs and Cefixime-loaded Silver Nanoparticles against the bacterial culture of *Staphylococcus Aureus* with synergistic activity.

Keywords: Silver Nanoparticle, Antibacterial activity, Multidrug Resistance, SEM, Antibiotics.

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INTRODUCTION

Nanotechnology is an emerging interdisciplinary revolution in several therapeutic areas over the last decade, including medicine, and the drug delivery system. The essence of this new technology features a significant impact within the field of diagnosis and drug delivery. The AgNPs and Cefixime-loaded AgNPs were set up by the chemical reduction method.

Nanotechnology is a rapidly expanding field, encompassing the development of man-made materials in the 1-100 nanometer size range [1]. Nanotechnology has its importance it eventually reduces dose and also has a superior bioavailability than larger particles. Currently, many methods and approaches have been reported for the synthesis of Silver Nanoparticles by using chemical, physical, photochemical, and biological routes. The nanotechnology revolution has begun and shows enormous promise in the field of drugs [2]. In the past, several Nanoparticles-based drug delivery systems were developed for the treatment of cancer, diabetes, pain, asthma, allergy, infections, and so on. The significant benefits of using a nanoscale agent are more effective and convenient routes of administration, lower therapeutic toxicity, extended drug bioavailability, as well as ultimately reduce healthcare costs [3].

Nowadays antibiotics have a lot of side effects as well as administration of high doses 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 a viable alternative to antibiotics and appear to possess a high potential for bacterial multidrug resistance. In particular, AgNPs have attracted much attention in the 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 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 [5].

The combination of NPs with antibiotics offers enhanced antimicrobial effects compared to single-molecule alone. This combination offers numerous advantages, such as no risk of developing resistance in the pathogen, lower cytotoxic effects, and no health risk. The present investigations aimed to chemically synthesize

silver nanoparticles and study their antimicrobial effects against common strains and multidrug-resistant strains of *S Aureus*. Moreover, the combinatorial effects of Cefixime with silver nanoparticles were evaluated against pathogens to eliminate the possible chances of developing antibiotics resistance and as a future strategy [6].

MATERIAL AND METHOD

Material

Silver nitrate, Cefixime Trihydrate, Sodium borohydrate, ascorbic acid and all chemical are purchased from Hi-media (AR Grade)

Preparation of Silver Nanoparticles

One of the most popular methods to synthesize silver nanoparticles is the use of ice-cold sodium borohydride to reduce silver nitrate. A large excess of sodium borohydride is needed to reduce the ionic silver and to stabilize the formed nanoparticles [7, 8]. In which add 0.002M sodium borohydride ($NaBH_4$) to an Erlenmeyer flask. An ice bath is used to slow down the reaction and give better control over the final particle size/shape. Stir and cool the liquid for about 20 minutes and drip 0.001M silver nitrate ($AgNO_3$) into the stirring $NaBH_4$ solution at approximately 1 drop per second. Stop stirring as soon as all of the $AgNO_3$ is added then color changes to light yellow which indicates the formation of silver nanoparticles [9,10].

Preparation of Cefixime loaded AgNPs

Cefixime-loaded silver Nanoparticles were prepared by using a 0.001 M aqueous solution of drug to 100 ml of synthesized AgNPs prepared by the continuous stirring method under the ultrasonication to improve the interaction between the antibiotic and AgNPs. In the process mix both the solutions (i.e.$NaBH_4$ and $AgNO_3$), Ag ions were reduced and clustered to form monodispersed Nanoparticles as a transparent sol in the aqueous medium [11, 12]. Add the polyvinylpyrrolidone to the solution of prepared silver nanoparticles. Add enough solid polyvinyl alcohol (PVA) to give a 4% solution. To get the PVA to dissolve, slowly add it to the stirred, hot, silver colloid solution. Then pour the mixture into a mold leaving air bubbles and undissolved PVA in the beaker. Evaporate in a toaster oven for about 30 minutes. The prepared silver nanoparticles show light yellow to brownish-yellow color. The required quantity of drug is dissolved in the solvent for the preparation of the drug solution. Then it is added simultaneously with silver nitrate solution into the reducing agent [13, 14].

Characterization of AgNPs and Cefixime Loaded AgNPs

UV-VIS spectroscopy

UV spectroscopy is a very useful and reliable technique for the primary characterization of synthesized nanoparticles which is also used to monitor the synthesis and stability of AgNPs. AgNPs have unique optical properties which make them strongly interact with specific wavelengths of light [15, 16]. In addition, UV-vis spectroscopy is fast, easy, simple, sensitive, and selective for different types of NPs. The absorption of AgNPs depends on the particle size, dielectric medium, and chemical surroundings. The synthesis of AgNPs was confirmed by using a UV-Vis spectrophotometer (Shimadzu 1800) in the range of 210 to 900 nm, by detecting sharp peaks at the expected wavelength [17, 18].

Fourier Transform Infra-Red spectroscopy (FTIR)

FTIR spectrum of drug-loaded silver nanoparticles was recorded using Bruker Fourier transform Infrared spectroscopy (FT-IR) (with spectrum 2000 analysis software) in the range of 400 to 4000 cm^{-1} to identify the possible molecules responsible for the reduction of metallic ions and to confirm capped AgNPs having drug encapsulated [19] .

Particle Size Determination

Particle size determination was carried out employing a 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) [18,22,23].

X-ray diffraction Analysis

The X-ray diffraction (XRD) measurement of AgNPs was carried out using Cu-Kα 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: [16,17]

$$D=(k\lambda/\beta \cos \theta)$$

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

λ is the wavelength of X-ray and θ is brags angle.

K is constant (geometric factor = 0.94).

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 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 [24,25].

Scanning electron microscopy

Scanning electron microscopy is a surface imaging technique in which an electron beam interacts with a sample generating different signals, which reflect the atomic composition and morphology of the surface. SEM uses backscattering electrons and secondary electrons emitted by the sample to construct the three-dimensional image of the sample analyzed. Once these electrons escape from the surface of the sample, they are detected by a photomultiplier [26,27].

However, many nanoparticles are invisible to the electron microscope, because they do not deviate the electron beam enough, therefore, the preparation of the sample requires a coating with a thin layer of metal that creates a conductive layer on the sample. This procedure inhibits surface wear, reduces thermal damage, and improves the secondary electron signal required in the SEM. The size, size distribution, and morphology of the nanoparticles can be obtained directly from SEM, the purity of the sample and its degree of aggregation can also be inferred from SEM images [28,29].

Antibacterial activity

This examination was pointed toward deciding the MIC of AgNPs and Cefixime-loaded AgNPs against *Staphylococcus Aureus.* 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, 30]. The main reasons for bactericidal properties of AgNPs interfere with the integrity of the bacterial cell by binding to essential cellular structures, 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, 31, 32]. AgNPs disturb the DNA replication and terminate the bacteria [23, 33].

Minimum Inhibitory Concentration (MIC) determination

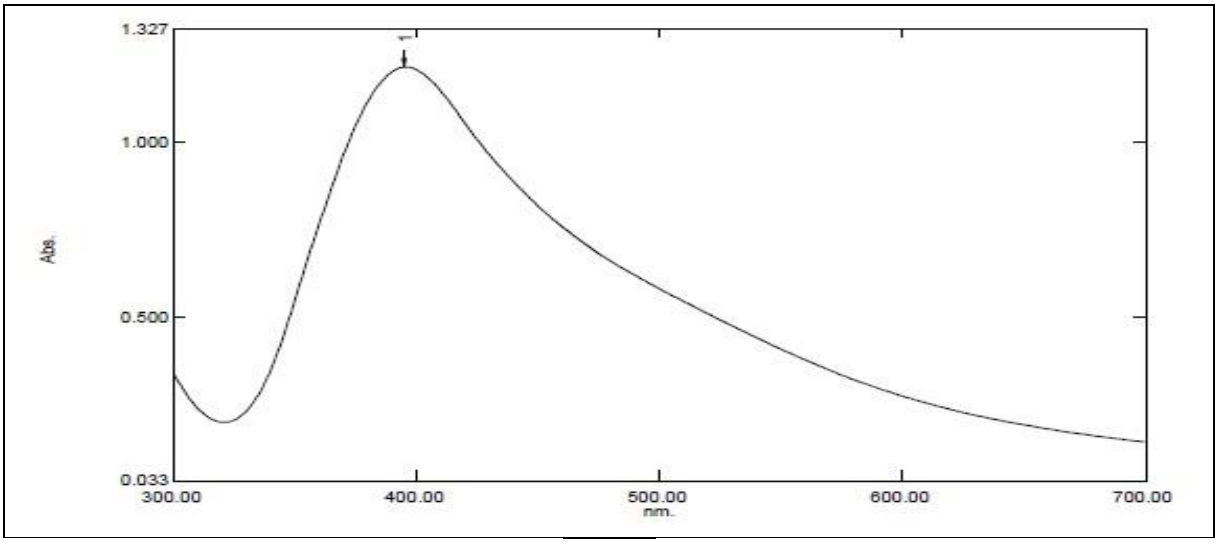
Antibacterial activity of the synthesized AgNPs and Cefixime-loaded AgNPs was studied by the standard disc diffusion method. The overnight grown bacterial culture of *Staphylococcus Aureus* was taken for study [24,34]. The preparation of nutrient media was done by taking 20 g of solidified nutrient media (Soyabean casein digest media) with 2 % of Agar added in 500 ml of distilled water and sterilized in an autoclave at 15 lb of pressure and 121^0c temperature [25]. This mixture was poured equally into Petri-plates. Keep this plate to solidify, after solidification bacterial cultures were spread on the surface of solidified agar with help of a spreader. The bore of 8 mm was made up of the lower surface of solidified media [26, 27]. Then organisms to be tested were inoculated in four bores (8 mm diameter) in different dilutions solutions [28,35]. The plates containing different concentrations of AgNPs were incubated at 37^0C 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. The dilutions of synthesized AgNPs and Cefixime loaded AgNPs varying from 0.030 mg/ml to 0.620 mg/ml were prepared.

RESULTS AND DISCUSSION

Preparation of AgNPs and Cefixime loaded AgNPs

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

Figure 1: UV Spectra of Silver Nanoparticles

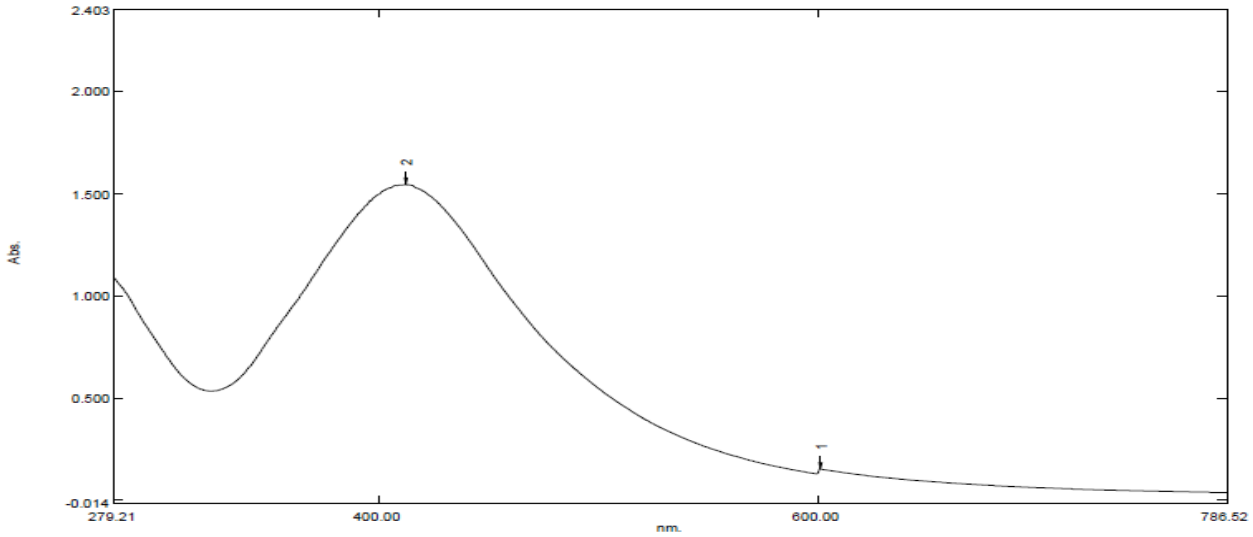


Spectra of silver colloids contain a strong Plasmon band close to 423 nm, which confirms that silver ions were reduced to Ag° in the aqueous phase. We found that as the concentration of silver increased the absorption band became sharper. The reaction solution

turned yellow, characteristic of the spherical particles when the entire silver precursor had been added.

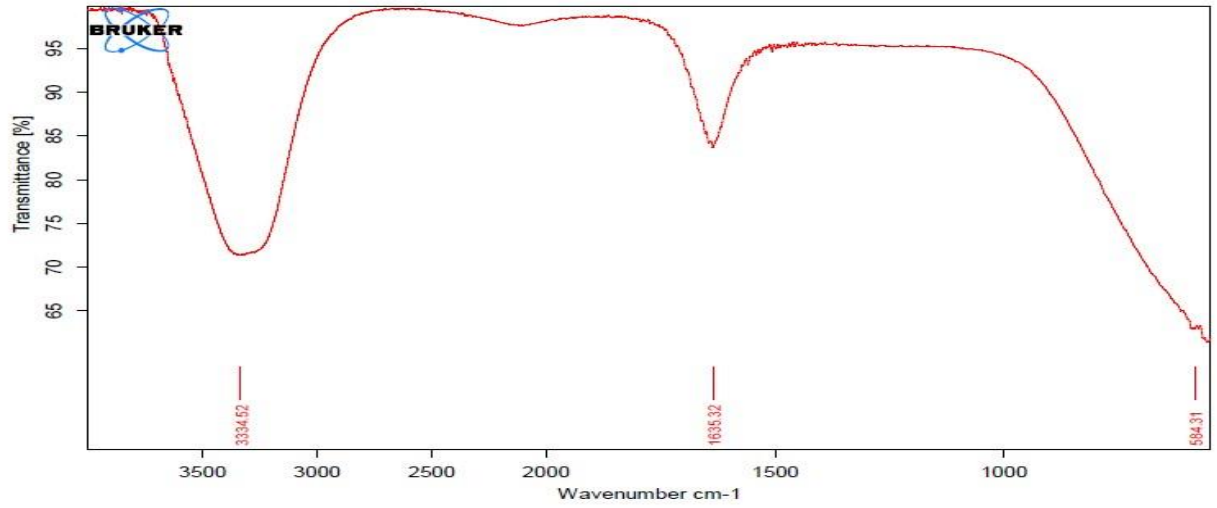
After the formulation of AgNPs, the confirmation was characterized by using a UV spectrophotometer (Shimadzu 1800). The formation of these can be confirmed employing spectrum for the colloids that Plasmon band is observed near 416 nm, which confirms that the silver ions were reduced to Ag° in watery phase. The prepared AgNPs can change the absorption spectra at different wavelengths according to the synthesized range of Nanoparticles in the solution. The spectrum mention in Figure 1 it is clear that there is the formation of nanoparticles in the range of 200-600 nm. The UV spectrum is the primary characterization to confirm the synthesis of AgNPs.

Figure 2: UV spectra of drug-loaded Silver Nanoparticles



Spectra of silver nanoparticles colloids contain a strong Plasmon band close to 415nm, which confirms that silver ions were reduced to Ag^{o} in the aqueous phase. In fig 2 the spectra were also observed in the range of 200-600 nm and no separate peak for Cefixime was observed which means that the drug is completely encapsulated within the silver nanoparticles.

Figure 3: FTIR spectra of AgNPs

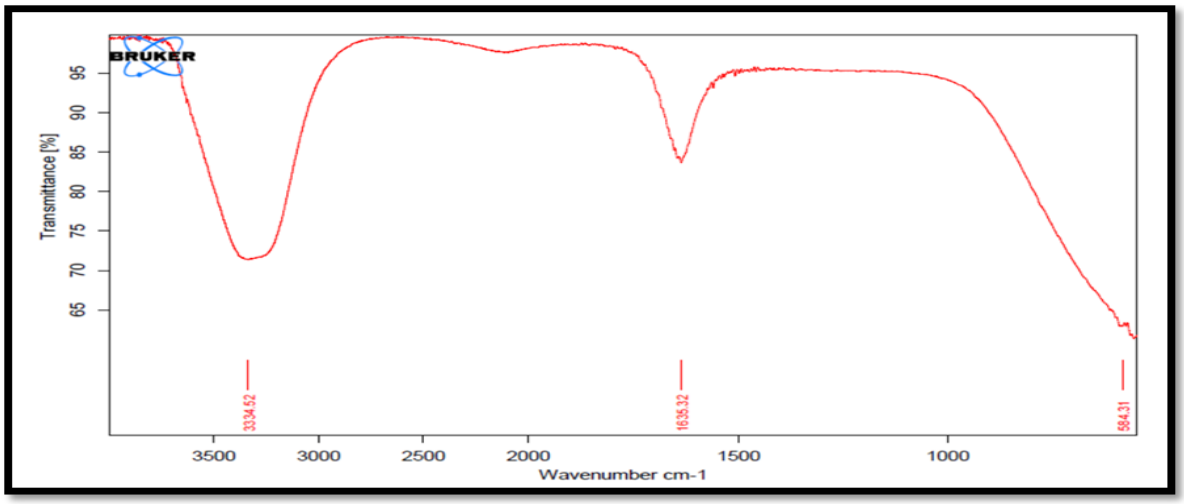


FT-IR Spectroscopy of silver nanoparticles were carried out to identify the presence of various functional groups in biomolecules responsible for the bio reduction of Ag and capping/stabilization of silver nanoparticles.

The observed intense bands were compared with standard values to identify the functional groups. FTIR spectrum shows absorption bands at 3509, 3597,3436, 2792,2579, 1785, 1597, 1451, 1306, 1240 and 863 cm^{1} indicating the presence of capping agent with the nanoparticles (Figure: 3). The bands at 3436 cm^{-1} in the spectra correspond to O=H stretching vibration indicating the presence of alcohol and phenol. Bands at 2792 and 2579 cm^{-1} regions arising from C-H stretching of the aromatic compound were observed. The band at 1785 cm^{-1} was assigned for C-C stretching (non-conjugated). These functional groups have a role in the stability/capping of AgNPs as reported in many studies. It may be concluded from the FTIR spectroscopic study that AgNPs are formed

and observed successfully with no interaction with another excipient.

Figure 4: FTIR spectra of Cefixime loaded AgNP



FT-IR Spectroscopy of Cefixime loaded silver nanoparticles

FTIR spectra of the optimized batch of Cefixime loaded AgNPs show the peaks of silver nanoparticles but do not show the frequencies (Figure: 4) of the encapsulated drug, hence it can be considered that the drug is completely incorporated inside the silver nanoparticles

Particle size distribution

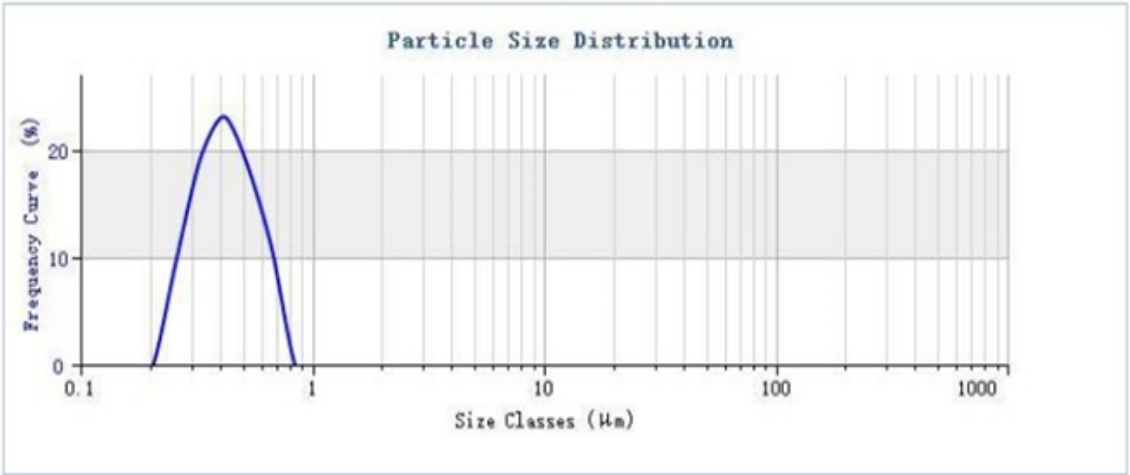
The size distributions of the AgNPs were determined by the Malvern instrument Particle size distribution curve reveals that AgNPs obtained are poly dispersed in nature (Figure:5),with an average diameter of 1-100 nm.

Table 1: 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 (%)**	**Vol (%)**	**Size (µm)**	**Vol (%)**	**Vol (%)**	**Size (µm)**	**Vol (%)**	**Vol (%)**
0.059	0.36	0.36	2.781	0.00	100.0	29.9	0.00	100.00	321.62	0.00	100.0
0.072	12.4	12.76	3.527	0.00	100.0	48.0	0.00	100.00	517.20	0.00	100.0
0.137	37.6	87.04	5.671	0.00	100.0	60.9	0.00	100.00	655.86	0.00	100.0
0.147	12.9	100.00	7.192	0.00	100.0	77.3	0.00	100.00	750.00	0.00	100.0
0.189	0.00	100.00	9.120	0.00	100.0	98.0	0.00	100.00	0.00	0.00	0.00

From the above data, particle size distribution was observed which is shown in Figure 5

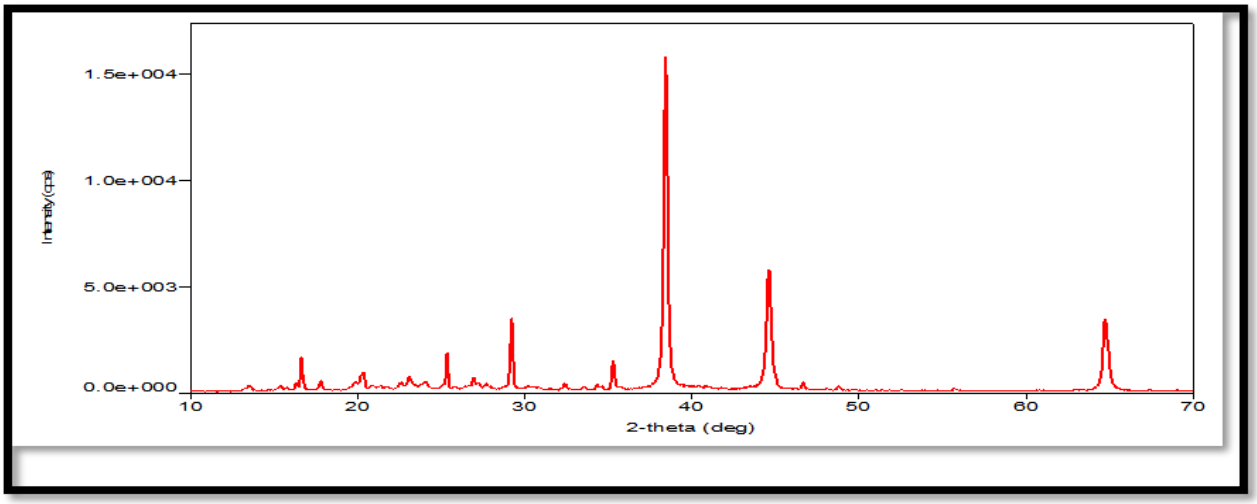
Figure 5: Particle size distribution of Cefixime Loaded AgNPs



XRD study

The crystalline nature of nanoparticles was confirmed by X-ray crystallography. The XRD pattern of the Synthesized AgNPs and Cefixime loaded AgNPs is shown in Figure: 6. The Nanoparticles synthesized in this method were characterized using powder form. The evaluations are shown in Table: 2. Diffracting angle in degree, FWHM (radians), d spacing (nm), Rel. Int. [%].

Figure 6: XRD spectrum of Cefixime loaded AgNPs



By considering the values given in the table the particle size 'D' was calculated for the samples using Scherer's equation). From the calculation, the particle size of synthesized nanoparticles was observed in the range of 1-100 nm. Which was again compared with the particle size obtained by the Particle size analyzer. The result obtained by both the methods was matched with each other

Table 2: 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

Scanning Electron Microscopy

From the results, it is noticed that the examined particles consist of several nanoparticles in the range of 1-100 nm to a few micrometers in size. The surface morphology of AgNPs and Cefixime loaded AgNPs shown in Fig: 7 and Fig: 8 simultaneously. From the studies it revealed that spherical nature of particles synthesized from silver metal.

Figure 7: SEM images of AgNPs

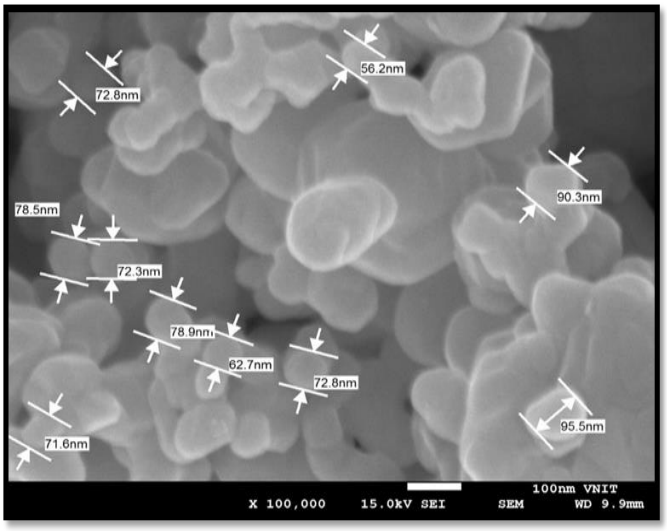
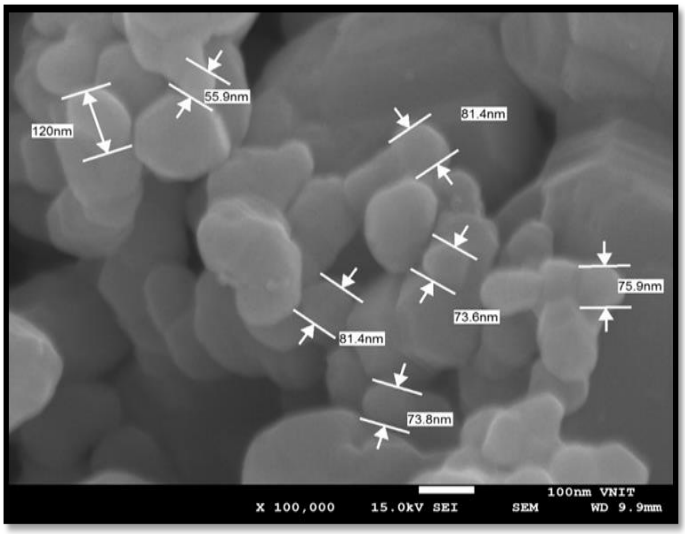


Figure: 8: SEM images of Cefixime loaded AgNPs



Antibacterial assay

MIC of AgNPs and Cefixime loaded AgNPs

Determination of Minimum Inhibitory Concentration (MIC) was done by taking 0.030 mg/ml to 0.620 mg/ml of the concentration of AgNPs inoculated to well and incubated for 24 hrs. The zone of inhibition was measured against the same bacterial culture result shows that 0.030 mg/ml concentration of AgNPs does not show zone of inhibition whereas 0.070 mg/ml concentration of AgNPs showed zone of inhibition against *Staphylococcus Aureus.*

Table 3: MIC of AgNPs & Cefixime loaded AgNPs against *S. Aureus*

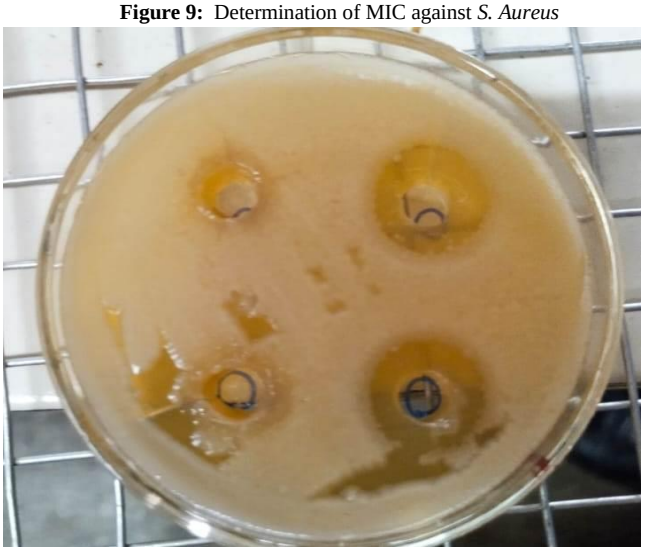
	MIC observations						***MIC observations***				
	Staphylococcus Aureus						***Staphylococcus Aureus***				
Conc. of AgNPs	0.030 mg/ml	0.070 mg/ml	0.150m g/ml	0.310 mg/ml	0.620m g/ml	Conc. of Cef. loaded AgNPs	0.030 mg/ml	0.070 mg/ml	0.150m g/ml	0.310 mg/ml	0.620 mg/ml
Zone of Inhibition	-	+	+	+	+	Zone of Inhibition	+	+	+	+	+

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

It confirms that 0.070 mg/ml was the minimum inhibitory concentration of AgNPs. The zone of inhibition of AgNPs against *S. Aureus* at different concentrations is shown in Table: 2 and Figure: 9 Determination of MIC for Cefixime loaded AgNPs was done by taking 0.030 mg/ml to 0.620 mg/ml of conc. of Cefixime loaded AgNPs inoculated to well and incubated for 24 hrs. The zone of inhibition was measured against the same bacterial culture result shows that 0.030 mg/ml concentration of Cefixime loaded AgNPs showed a zone of inhibition against *Staphylococcus Aureus.* From this, it confirms that 0.030 mg/ml was the minimum inhibitory concentration of Cefixime-loaded AgNPs.

From the above results, AgNPs show the Zone of inhibition at conc. 0.070 mg/ml whereas Cefixime loaded AgNPs shows the zone of inhibition at conc. of 0.030 mg/ml. from these results, it concludes Cefixime loaded AgNPs require less conc. to produce the same antibacterial effect because of synergistic activity.

Figure 9: Determination of MIC against *S. Aureus*



CONCLUSION

The AgNPs were synthesized by the chemical reduction method. In this method silver nitrate ($AgNO_3$) works as a precursor which further interacts with a reducing agent and stabilizing agent. UV-VIS absorption spectrum and XRD result confirming the synthesis of AgNPs. The size of prepared Nanoparticles was measured by laser Diffractometer confirmed by Scherer equation and a particle size analyzer.

The morphology of nanoparticles was evaluated by using SEM. The resulting sizes compared with each other showed the average particles size in the range of 1-200 nm. In a further study, the MIC of AgNPs and Cefixime-loaded AgNPs against *S. Aureus* was determined and found to be effective at 0.070 mg/ml for AgNPs and 0.030 mg/ml for Cefixime loaded AgNPs. The Cefixime loaded AgNPs shows the same effect at low concentration due to synergistic activity.

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Conflict of Interest: None

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