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Biosynthesis of mesoporous silica supported silver nanoparticles for antibacterial evaluation

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Abstract

Nanotechnology's game-changing applications have boosted the importance of synthesizing nanomaterials with carefully controlled dimensions. Biosynthesis of nanomaterials is a developing field that seeks to synthesize various nanoparticles. Leaf extract from *Azadirachta indica* will be used to bio generate silver nanoparticles on mesoporous silica spheres for this study. The leaf extract will be processed using ethanol. A modified version of Stober's method will be used to synthesize mesoporous silica balls. Spectroscopy, x-ray diffraction, scanning electron microscopy, and other cutting-edge tools will be employed to characterize the produced silica supported silver nanoparticles. The disc diffusion method or the Agar well diffusion method will be used to evaluate the antibacterial efficacy of the synthesized nanoparticles.

KEYWORD: (TEOS) Tetra ethyl orthosilicate, (TMB) Trimethyl benzene, (CTAB) Hexadecyl trimethylammonium bromide, (MSNs) mesoporous silica nanospheres, (NPs) nanoparticles, (FTIR) Fourier transform infrared spectroscopy, (UV VISI) Ultra violet, (XRD) X –Ray diffraction, (SEM) Scanning electron microscopy

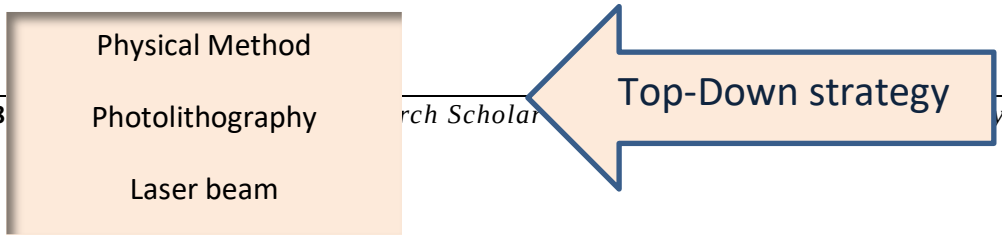
INTRODUCTION

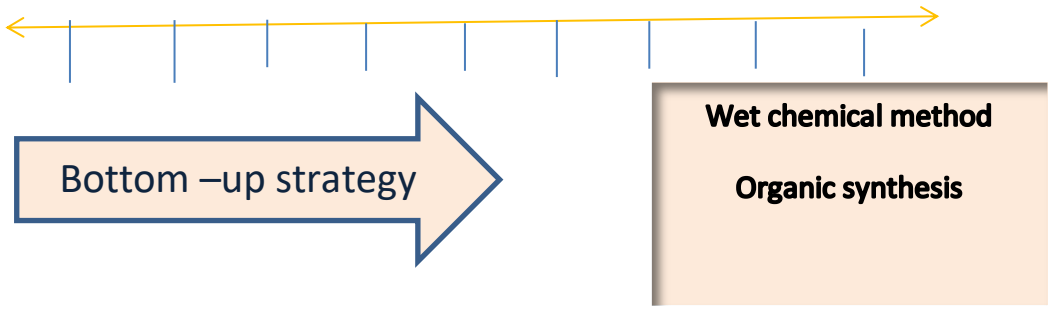
The fundamental principle of Nano science is that material properties are dimension-dependent. Although "nano science" and technology have been around for a while, they are rapidly developing subjects that are causing revolutions in all sciences on par with what genomics and proteomics did lately for the biological sciences. The size of a nanosac, or approximately 10^-9 m is the basis for the use of the prefix Nano (Hochella Jr., 2002).

The diminutive Latin term from which "Nano" is derived. One nanometer is one thousand millionth of a metre (1nm=10 to the ninth power) (Rangasamy, 2011).

One of Nano science's most important uses is in nanotechnology. Definition: Nanotechnology is the study and application of materials with dimensions of 100 nm or less (Mody et al., 2010). The medical sciences find significant use for nanotechnology. Exponential increase in recent years has attested to its significance in the fight against cancer. Nanotechnology-created nanoparticles provide significant untapped potential in the realm of therapeutic cancer development due to their adaptability in terms of design and tuning (Misra et al., 2010).

Both methods are used in the synthesis of nanomaterials in nanotechnology. The two most prevalent methods for creating nanoparticles are the top-down and bottom-up approaches. The **"top down"** method involves dismantling macroscale structures into smaller ones. Particles on the order of a few microns in size can be made using this method. If you want to create a certain type of nanomaterial, you can use a top-down method, which is a simpler tool that relies on either separating the bulk material or simplifying the creation process. The lack of surface structure is the main issue with top-down approaches. Nanowires fabricated via the top-down method, for instance, are rough and riddled with surface contaminations and structural faults on the nanoparticles' outer layers. High-energy wet ball milling, electron-beam lithography, gas phase condensation, aerosol, and spray are all examples of such processes. The term **"bottom up approach"** is used to describe the method by which complex Nano structures are built from their component atoms and molecules. Means to construct something from the ground up, either atomically, molecularly, or cluster-by-cluster. Bottom-up methods for the synthesis of luminescent nanoparticles have been widely reported, and include, among others, all organometallic chemical route, revere micelle route, sole gel synthesis, and colloidal precipitation, template, assisted sole gel electrodeposit ion, etc.





Use of Bottom Up and Top Down Approach

Because nanoparticles are so versatile, we'll go over each possible application in detail below. Because of their magnetic, optical, and chemical characteristics, ferrite nanoparticles are practically ubiquitous. The medical and manufacturing fields can both benefit from using nanoparticles. Their applications range from the biomedical to the technological, including water purification and catalysis. Most commonly, nanoparticles facilitate current flow in electrical chips used in circuses (Kolahalam et al., 2019).

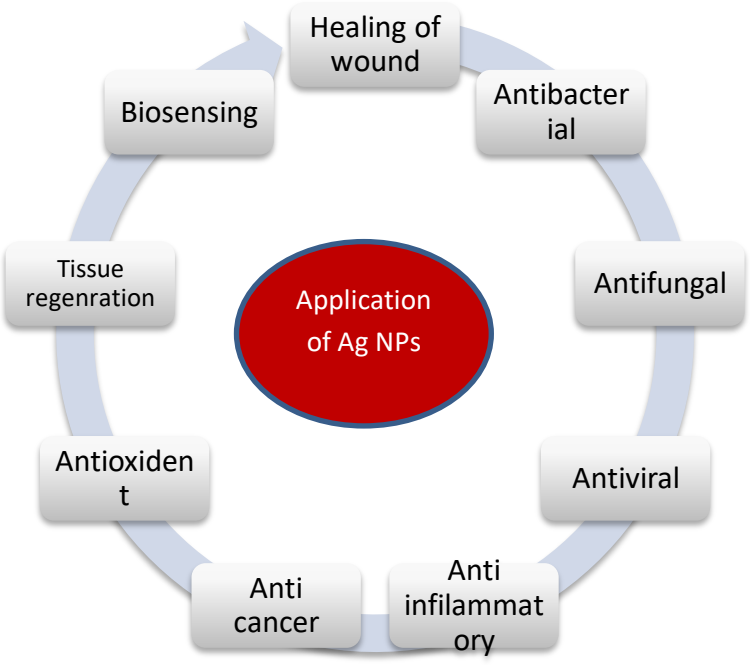


Fig 1.2: Application of Ag NPs

A "Green synthesis" refers to the production of nanoparticles from renewable resources like flavonoids and terpenoids. When present in the extract, these terpenoids and flavonoids serve

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as a capping and reducing agent. Although conventional techniques have been in use for quite some time, recent research has demonstrated that green techniques are superior for producing NPs due to lower failure risks, lower costs, and easier characterization. Physical and chemical ways of producing NPs have imposed numerous stresses on the environment as a result of their toxic consequences. The synthesis of NPs from plants is undeniably straightforward; in only a few minutes to a few hours, a metal salt can be prepared at room temperature using plant extract. Modern society has come to value plant extract for its vitamin and amino acid content (Gour & Jain, 2019).

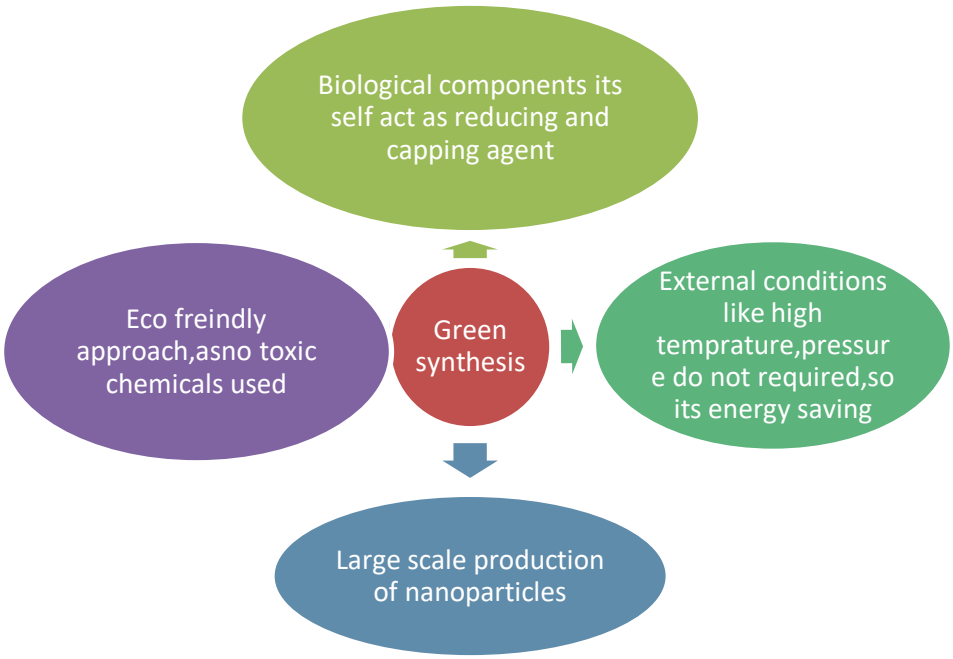

Figure 1.3: Green Synthesis of nanoparticles

The meliaceae (mahogany) family is where neem calls home. Azadirachta indica is a plant with a long history of use as medicine in India and neighbouring countries. It has been recognised for over 200 years as one of the most adaptable medicinal plants available. Likewise, there is a broad spectrum of biological details. Each and every part of the tree can be used to create effective home medicines for a wide range of common human ailments and historical ills. Silver and gold nanoparticles have been synthesised using Azadirachta indica leaf extract. The antibacterial activity of biosynthesized silver nanoparticles may be increased when capped with the neem leaf extract, which is the plant's most notable property (Lalitha et al., 2013).

To stabilise the nanoparticles, neem contains phytochemicals such terpenoids and flavanones, which act as reducing and capping agents. Neem leaf extract converts silver salt into

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nanoparticles (AgNPs). The neem extract coating gives the produced nanoparticles superior antibacterial action.According to (Verma & Mehata, 2016).

The anti-inflammatory, anti-pyretic, anti-fungal, anti-gastric ulcer, hypoglycemic, diuretic, anti-bacterial, anti-malarial, anti-pandemic, etc. properties of Neem plant leaves have led to its widespread use. The Ayurvedic term for "curing ailment" is "SarvaRogha-Nirvarini," and neem (Nimba) has been used for centuries to treat a.The US National Academy of Sciences has dubbed this worldwide system of problem-solving "The Tree of Problem Solving" (Senthilkumar et al., 2018).

1.9 Metallic nanoparticles

Nanoparticles of metal, often called microparticles, are a type of substance with special qualities that makes it useful in many fields, including business, agriculture, and the home. New MNPs have been rapidly created and mass-produced to fulfil the expanding demand for nanotechnology-based products and gadgets. Reference: (Peralta-Videa et al., 2016).

Green chemistry on mesoporous silica spheres for the creation of silver nanoparticles is the most dependable technology currently available. For the synthesis of silver nanoparticles mesoporous silica nano spheres are used due to their large surface area and higher porosity, and bio functionality of pores.

The stober technique creates mesoporous silica spheres, which can be used to make both silica and non-silica particles. Stobber process actually called the sole gel process in which the hydrolysis and condensation of salinol group in a different solutions are used.MSNs synthesized by this process use tetraortooxysilane ,tetraethyl orthosilicates(TEOS),surfactant and solvent.The antibacterial activity of silver NPs generated through biosynthesis is highest. Synthesized silver NPs lose some of their useful properties due to their tendency to aggregate. Therefore, we prepare the nanoparticles on MSN to prevent the aggregation. Nanoparticles' functional qualities are improved thanks to the mesoporous silica sphere's ability to prevent aggregation (Mehmood et al., 2017).

RESEARCH METHODOLOGY

3.1. Materials

Ethanol (>98%), Tetraethyl orthosilicate (>98%), ammonia solution in water (28-30%), (CTAB), trimethyl benzene (TMB), silver nitrate, neem plant extract, NaOH solution to

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maintain basic pH, and HCl solution to maintain acidic pH will be used to synthesise silica-supported silver nanoparticles.

3.2. Plant collection

The university of Sialkot's Botany department confirmed in March 2023 that samples of *Azadirachta indica* leaves collected from several locations in Narrowal District, Narrowal, Pakistan were indeed authentic.

3.3. Extraction of plant

The surface impurities were washed away from the collected *Azdiracta indica* leaves by first washing them in regular tap water and then in deionized water.After being cleaned and dried, the leaves were ground into a powder.The quantity of fine powder was measured, and it came in at 95 grammes.One half of the fine powder weighed 50 grams, while the other half weighed 45 grams.To a container holding 300 milimeter of ethanol, 50 grammes of leaf extract was added.Then, for 30 minutes, the mixture was sonicated at room temperature to ensure uniform blending.The liquid was sonicated, then transferred to a stirring machine and heated to 60 degrees Celsius for thorough mixing.Wattsman No. 1 filter paper was used to filter the mixture.The rotary evaporator was then used to remove the solvent from the final product.A jelly-like result was formed after the solvent was removed; this was then dried in a hot plate to produce powder at the appropriate temperature.The process resulted in a solid extract form.The extracted material was kept cold (4 degrees Celsius) for further use

Preparation of mesoporous silica spheres by stobber process

"Modifications to the stobber method were used to create mesoporous silica spheres. One hundred millilitres of the beaker were removed, rinsed in distilled water, ethanol, and air dried. Then, 16 ml of water and 4 ml of ethanol were added to the beaker, bringing the total volume to 20 ml.0.6g of CTAB, a white powder surfactant, were included into the mixture. After covering the mixture with aluminum foil, it was sonicated for 15 minutes at room temperature to ensure even distribution of ingredients. A clear solvent was obtained using sonication. The PH of the clear solvent was kept between 12 and 13 with the addition of 0.5 milliliters of a 30% ammonia solution. After making sure the PH was stable, 0.9 ml of trimethyl benzene and 2 ml of tetraethyl orthosilicates were added all at once. Two hours were spent stirring the solution. After 2 hours of constant stirring, the solution thickened noticeably. For 15 minutes, the mixture was spun at 8000 RPM in a centrifuge. White precipitates settled to the bottom of the centrifuge tubes after the process. Precipitates were collected and cleaned with ethanol after

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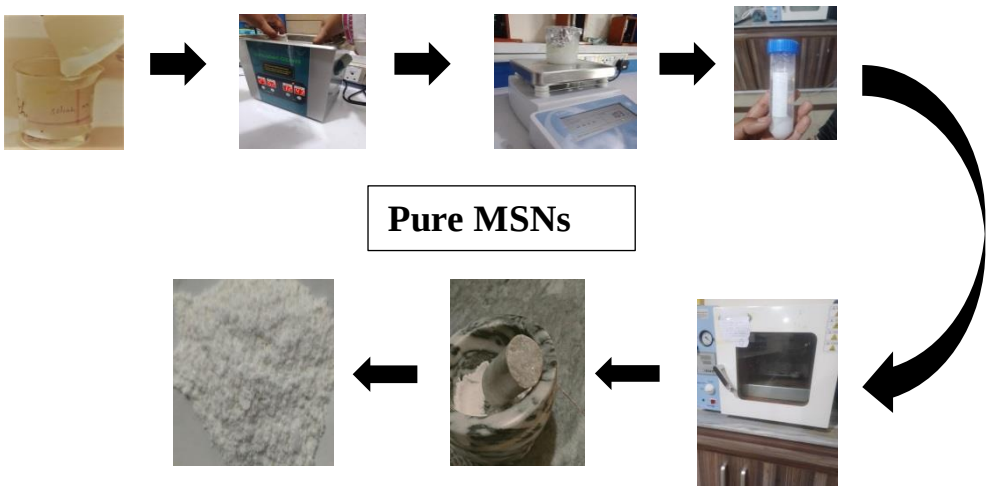
the solvent in the tube was removed. After centrifuging the solution for 5 minutes, it was shook vigorously to remove any sediment from the bottom of the tube. This cycle of washing was done three times. The resulting precipitates were washed for a third time, then dried in an oven at 120 °C for 12-16 hours. The MSN hardened into a solid state after being exposed to air, and was further powdered using a mortar and pestle. Once the fine powder was collected, it was placed in the tube for later use.



Synthesis of pure MSNs using stobber process

Biosynthesis of silver Nanoparticles

A solution of 0.2 gram of mesoporous silica nanospheres (MSN) in 200 milliliters of distilled water was used to synthesize silver nanoparticles. The 0.2g of MSN was added, and after 15 minutes of stirring, the mixture was transferred to a sonicator to be thoroughly mixed at room temperature. The acidity of the solution was kept in the 2-to-3 range to provide uniform dispersion. The required pH was kept at with an HCL solution. The solution became transparent after adding 2 cc of HCL, showing that the MSN was evenly distributed throughout the liquid. The pH was kept constant at 2.8 through constant stirring, and the mixture was thoroughly mixed at all times. A precursor of 1mm silver nitrate (0.034) was added after MSN dispersion. The plant extract was then added, which was diluted in 25 ml of ethanol, and the mixture was kept on the stove and constantly stirred. After that, the mixture was boiled for 5 minutes. Silver nanoparticles formed after the color of the solution changed from dull yellow to dark brown during the boiling process. The initial pH of the solution was low, but a more basic pH range was required for the formation of silver nanoparticles. NaOH solution was made by dissolving

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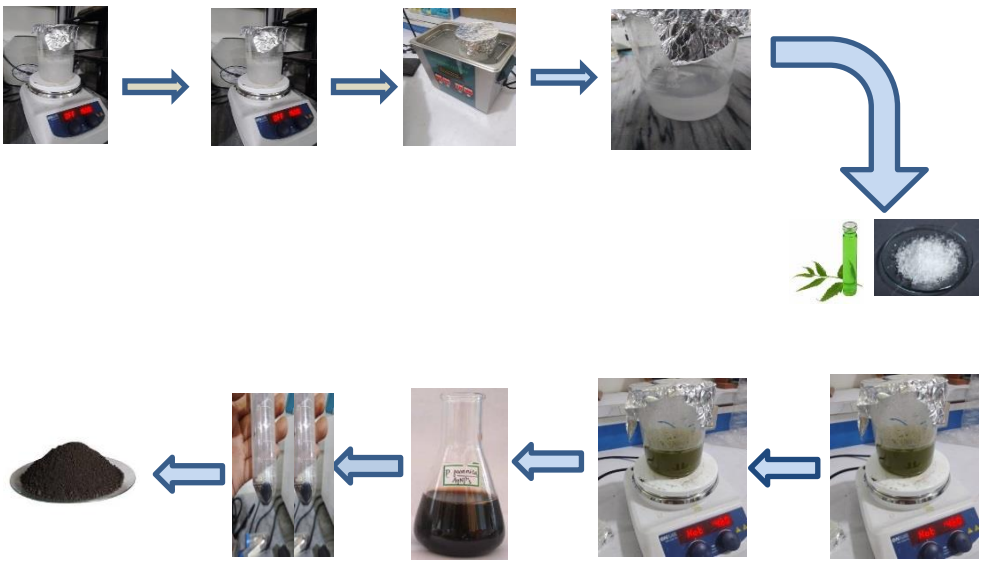
0.4g of NaOH into 10 ml of distilled water and mixing until the salt was completely dissolved. The pH was adjusted by adding 2 ml of NaOH solution to keep the range of the solution between 8 and 9. The pH was tested and reported as 8 after the addition of NaOH solution. All the while, the solution was being stirred constantly and then allowed to cool to room temperature. The solution was then transferred to the centrifuge tube and spun for 15 minutes. To eliminate any residual chemicals and biomolecules, the resultant product was washed three times with ethanol before being isolated. The product was then dried in an oven at 120 degrees Celsius for a whole day. Once the silver nanoparticles were obtained,



Biosynthesis of silver nanoparticles using plant extract

RESULTS AND DATA ANALYSIS

UV-Vis spectroscopy

Analysing the UV-visible absorption spectrum provides insight into the electrical nature of the optical band gap in the material. Absorption in the near UV spectrum occurs when electronic changes inside the sample are in sync with those outside. Energy in the band gap and UV absorption in SNPs are connected. The UV absorption edge provides a reliable approximation of the band gap for any system.

For the first characterization of the synthesised nanomaterials, UV-visible spectroscopy is a very beneficial instrument . In order to study the synthesis and permanancy.Many assistances are related with its use, such as being time effective, simple, and definite to various kinds of

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NPs. It is also significant to particle characterization of colloidal suspensin of AgNPs, UV-vis has been used. In AgNPs, the conduction and valence bands seem to be quite near to one another, allowing electrons to transfer about easily and produce an SPR absorption band. The size of the particles, the dielectric medium, and the chemical environment all have an effect on how much AgNP absorbs. Surface plasmon peaks are used to capacity the quantity of metal NPs, which range in size from 2 to 100 nm. SPR peaks in the visible region provide as indication of the occurrence of NPs.

According to a research by reseacher , the normal peak for AgNPs was seen about 430 nm.Furthermore, dynamic light scattering (DLS) was used to evaluate the nanoparticles' average particle size, which was appeared to be 58 0.05 nm.

Uv visiblr spectra for silver nanoparticles was plotted using origin software. Graph was plotted between wavelenth and obsorbance, ony one peak obtaines at 430nm at this place peaks fokss for silver nanoparticles appered and 430 place give the presence of SNPs.

In a work by researcher very unchanging spherical AgNPs were shaped using a green method. A discrete band between 420 and 430 nm was originate in this study's use of UV-visible spectroscopy to look at the stability of nanoparticles over three months. In a succeeding green examination, researcher stated the synthesis of non-spherically shaped AgNPs produced two distinct peaks at 363 and 426 nm.

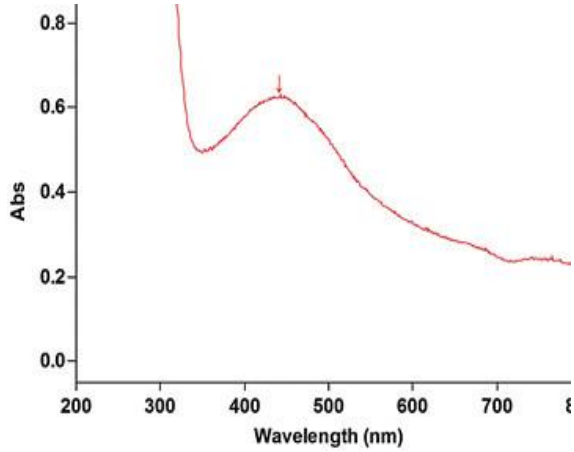

Figure 4.1: Uv visible spectra of synthesized silver nanoparticles

XRD analysis of samples.

X-ray diffraction (XRD) allows for the exact measurement of the size distribution of nanomaterials and the measurement of millions of crystals. X-ray diffraction (XRD) is a common technique for characterising NPs. X-ray diffraction (XRD) is commonly used to

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determine the lattice parameters, phase, crystalline grain size, and crystalline structure. The XRD graph plotted using the following settings : The crystal structure of samples was evaluated using X-Ray diffraction technique. The XRD data was

Collected using a portable table top XRD machine.

Data was collected using following settings:

X-Ray source: Copper (Cu) tube with 1.54184(A),Scanning rate : 5 degree per min.

The XRD spectra was plotted using origin software .The crystal structure of synthesized nanoparticles and their crystallite size were calculated using origin software.

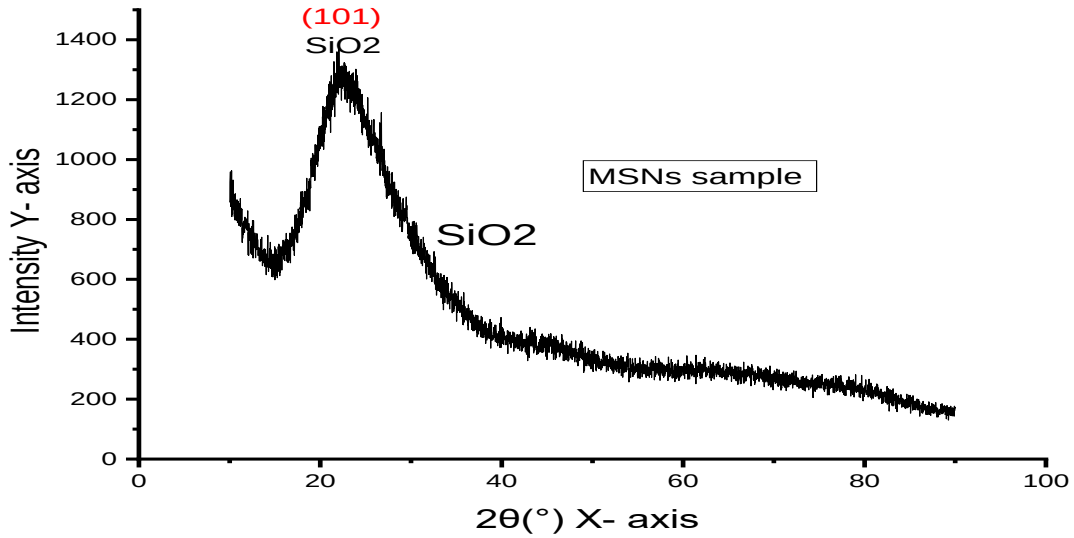

Figure : 4.2: **Determine the pure MSNs presence of silica in the sample.**

The graph was plotted 2 theeta on X – axis against intensity on y- axis a curve is obtained which contain the only one peak at 21 theeta value and crystal lattice value (101) deermine the presence of silica SiO2. This determine the presence of silcon dioxide.

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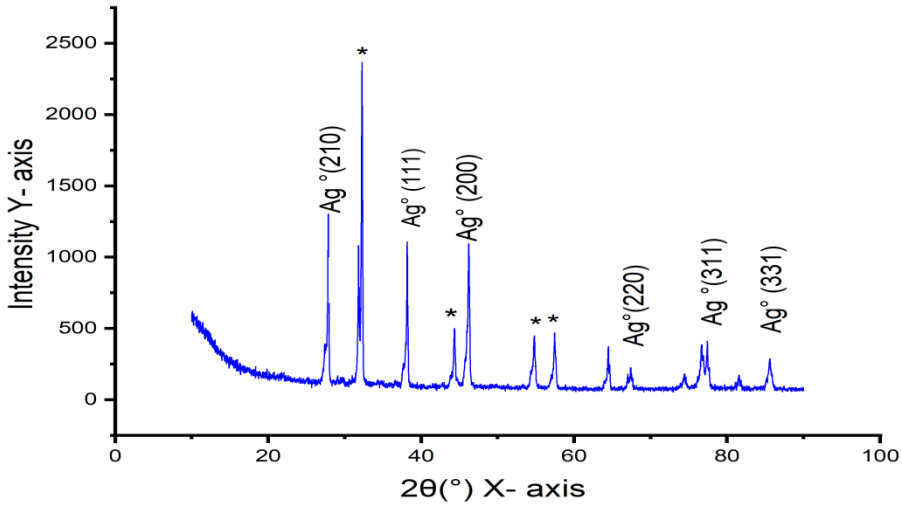

Figure 4.4 : XRD pattren of sample 1 determine the crystal structure and peaks obtained at (210).(110),(200).(220),(311),(331

We have plotted the XRD graph using the origin software.In the graph we have plotted 2 theeta value on X-axis and inthensity(au) in Y-axis. We obtained the peaks for silver nanoparticle. 6 peaks obtained for the pure silver and the peaks that are represensted by star indicate the impurities in the sample.The impirity belongs to some silver chloride atom present in the sample. 1st peak was obtained at 27° with hkl value (210) ,2nd peak was appeared at 38°with hkl value (110), 3rd peak obtained at (200) at 46°.Other peaks was appeared at 67°, 77°,85 ° with hkl value (220),(311),(331). Other peaks that are presented with star belong to impurities present in the sample.

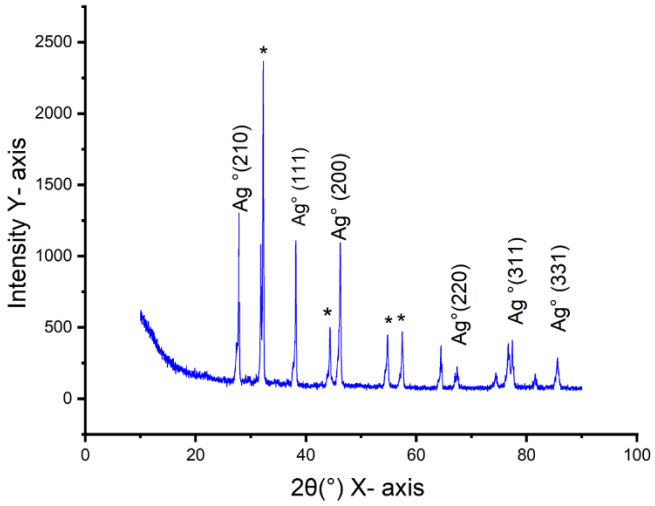

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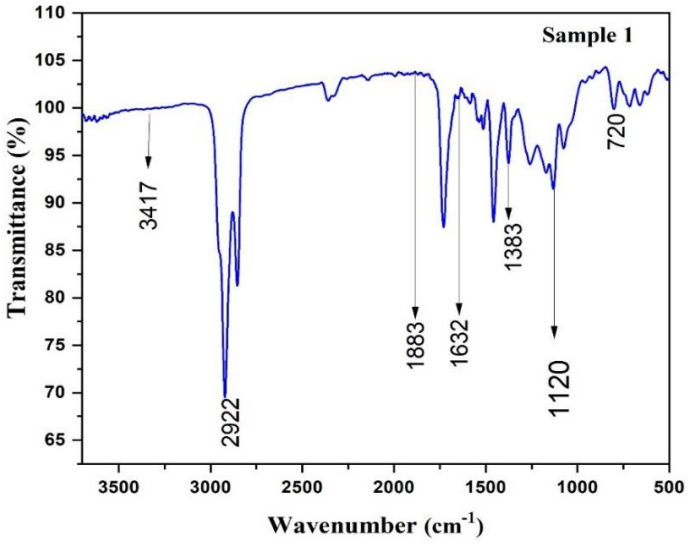
Figure 4.3: Determine the crystal structure of silver nanoparticles (210).(110),(200).(220),(311),(331).

We have plotted the XRD graph using the origin software.In the graph we have plotted 2 theeta value on X-axis and inthensity(au) in Y-axis. We obtained the peaks for silver nanoparticle. 6 peaks obtained for the pure silver and the peaks that are represensted by star indicate the impurities in the sample.The impirity belongs to some silver chloride atom present in the sample. 1st peak was obtained at 27° with hkl value (210) ,2nd peak was appeared at 38°with hkl value (110), 3rd peak obtained at (200) at 46°.Other peaks was appeared at 67°, 77°,85 ° with hkl value (220),(311),(331). Other peaks that are presented with star belong to impurities present in the sample.

FTIR analysis of samples.

This study's results demonstrated that the synthesized AgNPs controlled functional groups, and that distinguished peaks could be found at 1120, 1383, 1883, 720, 1632, and 3416 cm–1, corresponding to primary alcohols and NO3, as well as the C–N stretching vibrations of aromatic and aliphatic amines, respectively. The scientists in this study manmade green nanoparticles from silver nanoparticles and analyzed those using FTIR after preparing green nanoparticles from *Trigonella foenum-graecum*. The findings exposed distinguished bands of absorption for both AgNPs and seed extracts of Trigonella foenum. The study's peaks were set at about 3264, 1636, and 1961 c1, which stand for −OH, NH, and ═C–H, correspondingly.



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Experimental conclusions from FTIR spectroscopy recommended that these results might be matching to those that have been studied and debated away in the context of the green path synthesis of silver nanoparticles, mainly in rights related to microbiology.FTIR spectra of silver nanoparticles was plotted by using origin software.The plotted graph contain the peaks at 3417, 2922, 1632, 1883,1383, 1120, 720.

Antibacterial activity of silver nanoparticles

In the University of Sialkot's biotechnology lab, the antibacterial activity of produced silver nanoparticles was evaluated using the agar disk diffusion method. S. aureus and Escherichia coli bacteria's antibacterial activity against each compound**.** In test tubes, pure cultures of bacteria were introduced to L.B. broth (PH=7) and left to develop for a full day at 37°C. The bacterium suspensions were made with a sterile, isotonic solution. The next step involved creating a 9:1 dilution of bacteria in a test tube by mixing 1 mL of turbid bacterial culture with 9 mL of distilled water.

Preparation of Nutrient Agar Medium

Agar solution was prepared by dissolving 6.25g of nutritional agar powder in 250mL of distilled water. To dissolve them, fully combine them. It was sterilised in an autoclave set at 120 °C for 15 minutes with the pH maintained at 7. After autoclaving, all of the experiments were carried out in a laminar flow. Pour the liquid medium into sanitized Petri plates after allowing it to settle. A clean environment should be used to prepare agar in order to prevent contamination. After it has set, the agar is ready to be used.

4.3.3 Determination of Antibacterial Activity

The antibacterial activity of silver nanoparticles was evaluated using the standard procedure, the agar disk diffusion technique. After melting and adding 20 ml of the by turning it. This method is referred to as "carpet culture" or "lawn culture." Five minutes of drying the plates is necessary to ensure that the medium fully absorbs the inoculum.

Before handling the antibiotic discs, sterilize the forceps with alcohol. Ideally, discs should be spaced 24 mm apart. To establish proper contact and prevent misplacing, gently touch each disk with the forceps. Plates were covered with paraffin tape and incubated in an incubator at 37°C for around 24 hours. The antibacterial activity was ascertained by measuring the zone of inhibition's millimeter diameter and comparing it to reference values.

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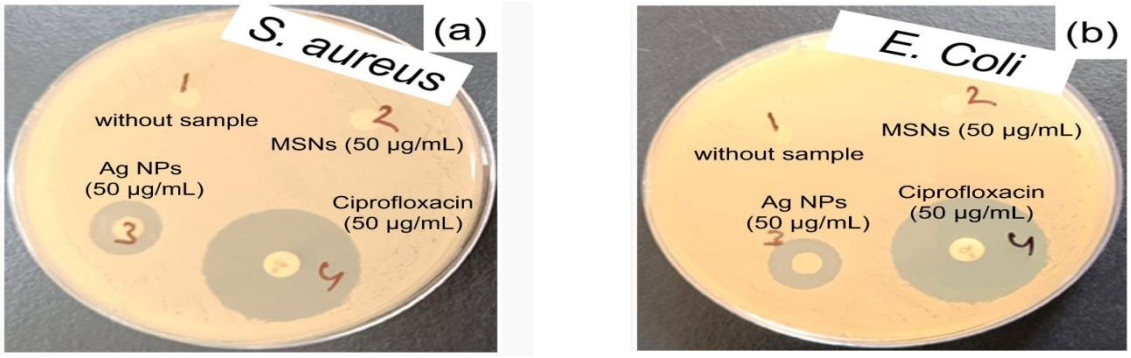
sterile nutritional agar medium to petri plates, it was allowed to cool and harden for half an hour. The inoculum is dipped into a sterile cotton swab, which is then pressed against the tube wall to remove any surplus media. Swipe the whole surface area of the plate.



Antibactarial activity of silver nanoparticles usin *S.aureus* and *E. coli*

S. aureus			*E. Coli*		
Sample	**Concentration**	**Zone of inhibition**	**Sample**	**Concentration**	**Zone of inhibition**
MSNs	---	---	MSNs	---	---
Ag@MSNs	(50 µg/mL)	~12 mm	Ag@MSNs	(50 µg/mL)	~11 mm
Control	(50 µg/mL)	~20 mm	Control	(50 µg/mL)	~20 mm

Table4.1 shows the zone of inhibition for silver nanoparticles and control ciprofloxacin.

CONCLUSION

Biosynthesis of silver nanoparticles using neem plant extract have been found from this method to produce NPs process high reducing and capping properties, Which are exhibited in the formation of aqueous silver nanoparticle dispersion, through with little variations. They are formed after 5 minutes of mixing silver nitrate. A simple rapid production of silver NPs have been achieved by using neem plant extract. The green production rout is done at room temperature using water and leaves using biological material, therefore neem plant leaf extract was good for the synthesis of silver nanoparticles. X-Ray diffraction spectroscopy was used to

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measure the particle size of synthesized silver nanoparticles. XRD confirm the particle size of NPs which is 18.31nm. Uv visible spectroscopy give the absorption of light at 430nm. The most effective antibacterial action was revealed by synthesized AgNPs against human pathogens as *E.coli* and Staphylococcus aureus. Upcoming industrial and medicinal difficulties might be readily met by scaling up the above-mentioned environmentally friendly synthesis process of AgNPs.

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