BACTERIAL MEDIATED COPPER NANOPARTICLE SYNTHESIS USING BACTERIA – *ESCHERICHIA COLI* AND ITS CHARACTERIZATION

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE

OF

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

Submitted by:

PRACHI CHOUDHARY

2K21/MSCBIO/32

Under supervision of

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Bawana Road, Delhi – 110042

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CANDIDATE'S DECLARATION

I, Prachi Choudhary, Roll No. 2k21/MSCBIO/32, student of M.Sc in Biotechnology, hereby declare that the Major project Dissertation titled "**Bacterial mediated copper nanoparticle synthesis using *E.coli* and its characterization**" which is submitted by me to the Department of Biotechnology, Delhi Technical University Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associate ship, Fellowship or other similar title or recognition.

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CERTIFICATE

I hereby certify that the Project Dissertation titled "**Bacterial mediated copper nanoparticle synthesis using *E.coli* and its characterization**" which is submitted by Prachi Choudhary, Roll No. 2K21/MSCBIO/32, Department of Biotechnology, Delhi Technological University in partial fulfillment of the requirement for the award of the degree of Master of Technology, is the record of the project work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to this university or elsewhere.

30/05/2023

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"BACTERIAL MEDIATED COPPER NANOPARTICLE SYNTHESIS USING BACTERIA – *ESCHERICHIA COLI* AND ITS CHARACTERIZATION"

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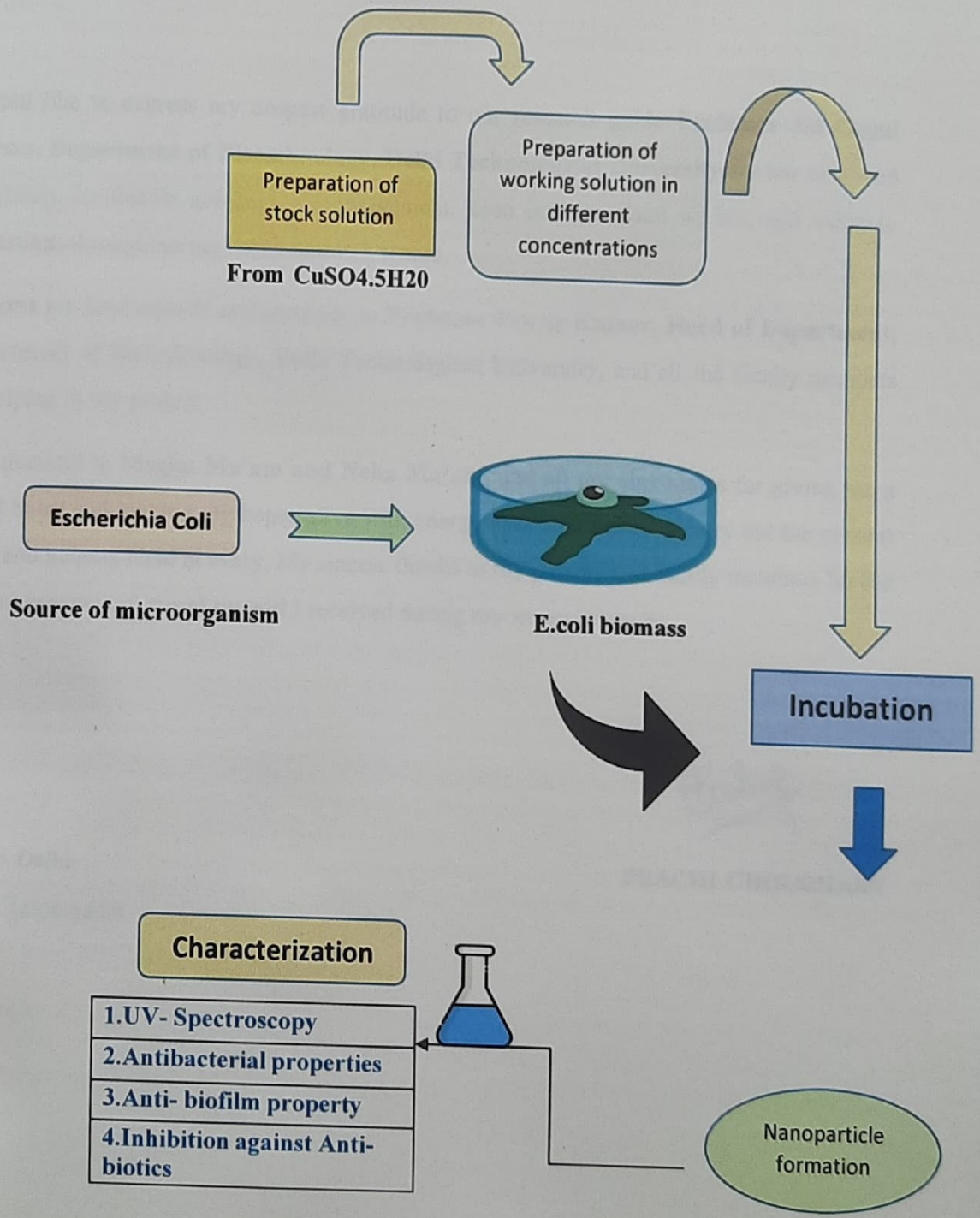
ABSTRACT

The bacterial-mediated synthesis of copper nanoparticles (CuNPs) has gained significant attention as a sustainable and eco-friendly approach for the production of nanomaterials. This method utilizes microorganisms, such as E. coli, to reduce copper ions into nanoparticles, eliminating the need for hazardous chemicals and energy-intensive processes. In this abstract, we summarize the key aspects and findings of bacterial-mediated synthesis of CuNPs. We discuss the advantages of this approach, including its green nature, cost-effectiveness, and scalability. In this article, we basically describe the methodology of synthesis of bacteria-mediated copper nanoparticles using the bacteria E.coli. Also, why we are using this particular bacteria only? Then, the characterization of the Cu NP's is done by 5 different methods–UV-Spectroscopy we will check the antibacterial property against S.aureus and bacteria obtained the zone of inhibition anti-biofilm property of nanoparticle is also studied, antibiotic property analyses is also done. Furthermore, the bacterial synthesis method offers control over the size, shape, and surface properties of the CuNPs. By manipulating the growth conditions and parameters, researchers can tune the characteristics of the nanoparticles to suit specific applications. CuNPs exhibit remarkable antimicrobial properties, making them effective against bacterial and fungal infections. They can disrupt the integrity of microbial cell membranes, inhibiting their growth and proliferation. Additionally, CuNPs demonstrate excellent catalytic activity, enabling them to facilitate various chemical reactions. In conclusion, bacterial-mediated synthesis of CuNPs offers a sustainable and efficient alternative for their production. The ability to control their properties, combined with their diverse range of applications, makes them highly valuable in different fields. Continued research and development in this area are crucial to unlocking the full potential of bacterial-mediated synthesis of CuNPs and advancing sustainable nanotechnology.

GRAPHICAL ABSTRACT



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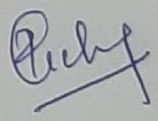

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CONTENTS

TOPICS NO.	PAGE
TOPIC NAME	i
CANDIDATE DECLARATION	ii
CERTIFICATE	iii
ABSTRACT	iv
ACKNOWLEDGMENT	v
CONTENTS	vi
LIST OF FIGURES	vii
LIST OF TABLES	viii
CHAPTER 1 INTRODUCTION	1-2
CHAPTER 2 REVIEW OF LITERATURE	3-6
CHAPTER 3 MATERIAL AND METHODS	6-8
CHAPTER 4 CHARACTERIZATION	9-16
CHAPTER 5 APPLICATIONS	17-18
CHAPTER 6 RESULT	19-23
CHAPTER 7 CONCLUSION	24
REFERENCES	25-27
PLAGIARISM REPORT	28 -32
LIST OF PUBLICATION	33

LIST OF FIGURES

FIGURE 1: Stock solution of copper sulfate solution.

FIGURE 2: *E.Coli* Biomass –prepared by primary culturing of bacteria.

FIGURE 3: Turbidity shown after biomass inoculation and incubation in the copper working 9solution.

FIGURE 4: Green color nanoparticle synthesized from *E.Coli* bacteria.

FIGURE 5: Graph of UV-spectrophotometer depicting Wavelength V/S OD.

FIGURE 6: Bar graph showing % of inhibition after anti-biofilm activity of copper nanoparticles.

FIGURE 7: A representative picture of an agar plate demonstrating areas where CuNPs-applied *S.Aureus* growth was inhibited.

FIGURE 8: figure showing -No zone of inhibition shown on the growth of *E.coli* against erythromycin drug .

LIST OF TABLE

TABLE NO.	NAME
TABLE NO. 1	Table 1: Spectroscopy reading at 500micro liter and 1000micro liter
TABLE NO. 2	Table 2: Biofilm Eradication by CuNPs. Here OD Control,% of inhibition is shown
TABLE NO. 3	Table 3: Zone of Inhibition against S. aureus (CuNPs)

CHAPTER 1

INTRODUCTION

In recent years, nanoparticle synthesis using biological systems has gained significant attention due to its eco-friendly and cost-effective nature. Among various biological systems, bacteria have emerged as promising candidates for nanoparticle synthesis due to their unique metabolic pathways and ability to produce nanoparticles with controlled size, shape, and composition. One such bacterium that has garnered considerable interest is Escherichia coli (E. coli).[1] E. coli-mediated synthesis of copper nanoparticles offers numerous advantages over conventional chemical methods, including scalability, reduced cost, and environmentally friendly production.

Gram-negative E. coli bacteria are typically found in warm-blooded animals' and humans' intestines. It is frequently employed and the subject of in-depth research in several molecular biology and biotechnology domains. Researchers have harnessed the potential of E. coli to produce nanoparticles by exploiting its cellular machinery and metabolic pathways. Copper nanoparticles synthesized by E. coli exhibit unique properties that make them attractive for diverse applications.[2]

The synthesis of copper nanoparticles by E. coli primarily involves two main steps: the reduction of copper ions and subsequent stabilization of the formed nanoparticles. Copper ions, usually in the form of copper salts, are introduced to the E. coli culture medium. The reduction of copper ions to metallic copper nanoparticles is facilitated by various enzymes and biomolecules present in the bacterial cells. These biomolecules act as reducing agents and play a crucial role in controlling the size and shape of the resulting nanoparticles.

The ability of E. coli to produce copper nanoparticles in a controlled and reproducible manner makes it an excellent biological system for nanoparticle synthesis. The process can be optimized by adjusting various parameters such as the concentration of copper ions, growth conditions of the bacteria, and duration of the synthesis process. Moreover, genetic engineering techniques can be employed to modify E. coli strains, enhancing their nanoparticle synthesis capabilities.

One of the significant advantages of E. coli-mediated synthesis of copper nanoparticles is its scalability. Bacterial cultures can be easily scaled up to produce large quantities of nanoparticles, making it suitable for industrial applications. Additionally, the cost of production is significantly lower compared to traditional chemical methods, as bacterial synthesis eliminates the need for expensive reagents and high-energy processes. The use of E. coli also offers environmental benefits by reducing the production of hazardous waste and minimizing the ecological impact.

The properties of copper nanoparticles synthesized by E. coli make them suitable for various applications across multiple fields. One of the notable applications is their antimicrobial activity. Copper nanoparticles have been shown to possess potent antimicrobial properties, inhibiting the growth of a wide range of bacteria, fungi, and viruses.[3] This makes them valuable for use in healthcare settings, where the prevention and control of infections are of utmost importance. Copper nanoparticles can be incorporated into coatings for medical devices, wound dressings, and textiles, providing a protective barrier against microbial colonization.

Another prominent application of E. coli-synthesized copper nanoparticles is in water purification. These nanoparticles exhibit excellent adsorption capabilities, allowing them to efficiently remove contaminants from water sources. They can effectively remove heavy metals, organic pollutants, and even bacteria, providing a cost-effective and environmentally friendly solution for clean water production. Copper nanoparticles can be incorporated into filtration systems or used as additives in water treatment processes to enhance their efficiency.

Copper nanoparticles synthesized by E. coli also find applications in catalysis. They function as efficient catalysts for a variety of chemical processes thanks to their special qualities, including large surface area and reactivity.[1] They can be used in organic synthesis, hydrogenation, and oxidation reactions, improving reaction rates and selectivity. The ability to tailor the shape and size of the nanoparticles allows for the design of specific catalysts with enhanced performance.

In the field of electronics and optoelectronics, E. coli-synthesized copper nanoparticles offer exciting opportunities. Copper nanoparticles exhibit excellent electrical and thermal conductivity, making them suitable for applications in conductive inks, printable electronics, and flexible circuitry.

Energy storage is another area where copper nanoparticles synthesized by E. coli hold promise. They can be used to improve the performance and stability of electrode materials in batteries

and supercapacitors. The high electrical conductivity and large surface area of the nanoparticles facilitate efficient charge transfer, leading to enhanced energy storage capacity and cycling stability.[4]

Nanomedicine is an emerging field where E. coli-mediated copper nanoparticles show significant potential. The unique properties of copper nanoparticles, such as their small size and ability to penetrate cells, make them valuable for various biomedical applications. They can be functionalized with specific ligands to target and deliver therapeutic agents to diseased cells or tissues, thereby enhancing the efficacy of treatments. Copper nanoparticles can also be used in diagnostic imaging techniques, improving the sensitivity and accuracy of medical diagnoses.

Lastly, E. coli-synthesized copper nanoparticles have implications in environmental remediation. They can be employed for the degradation of organic pollutants through advanced oxidation processes. In order to help clean up polluted surroundings, copper nanoparticles may also be used to remove heavy metals from contaminated soil and water sources.

In conclusion, the synthesis of copper nanoparticles using E. coli as a biological system offers numerous advantages and holds promise for various applications. The scalability, cost-effectiveness, and eco-friendliness of this approach make it attractive for industrial production. The unique properties of copper nanoparticles synthesized by E. coli make them suitable for applications in antimicrobial agents, water purification, catalysis, electronics, energy storage, nanomedicine, and environmental remediation.[5] Further research and optimization of the synthesis process are expected to unlock additional applications and enhance the performance of these nanoparticles, paving the way for their widespread utilization in various industries.

CHAPTER 2

LITERATURE OF REVIEW

Due to its cutting-edge materials and uses, nanotechnology is becoming more and more important as a science. Particles with a diameter of 1 to 100 nm are known as nanoparticles (NPs). Metal nanoparticles are widely employed in industrial catalysis and in chemical sensing devices, medicinal applications, cosmetics, etc.; they have unique chemical and physical characteristics due to their tiny size and high surface-to-volume ratio. Due to their widespread application in a variety of commodities, including consumer goods, healthcare products, and industrial materials, nanoparticles are becoming more common in our daily life. Although nanoparticles may have a number of benefits, there are concerns regarding their safety as well as potential harm to both human health and the environment.

Here are some examples of how nanoparticles are used in our daily lives:

a) **Sunscreens:** Many sunscreens contain nanoparticles of titanium dioxide and zinc oxide, which provide UV protection and reduce the risk of skin cancer. These nanoparticles are used because they are transparent and do not leave a white residue on the skin.

b) **Cosmetics:** Nanoparticles are also used in cosmetics such as lipsticks, eye shadows, and moisturizers. They can improve the texture, color, and absorption of these products.

c) **Food packaging:** Some food packaging materials use nanoparticles to improve their strength, flexibility, and resistance to moisture and oxygen.

e) **Electronics:** Since they may increase the performance and longevity of electronic equipment like computer chips and screens, nanoparticles are utilized in their manufacture.

f) **Medicine:** Medical uses for nanoparticles include medication delivery systems and diagnostic equipment. They can lessen unwanted effects while also enhancing the efficiency and specificity of medications.

Although nanoparticles have numerous advantages, there are also worries about their possible negative effects on human health and the environment. Certain forms of nanoparticles may be

hazardous and may have negative consequences on both human health and the environment, according to certain research. Therefore, it is crucial to maintain research into the possible hazards and advantages of nanoparticles and to make sure they are handled properly and safely.

Bacterial-mediated synthesis of copper nanoparticles is a relatively new and emerging field of research. It involves the use of bacteria to produce copper nanoparticles through a process known as biosynthesis. By using biological agents like bacteria, fungus, and plants, copper ions are reduced to copper nanoparticles throughout the biosynthesis process. Because they can grow quickly and create a lot of nanoparticles, bacteria are a particularly appealing choice for this procedure. The process is mediated by enzymes and other biomolecule present in the bacterial cells.[6]

The synthesizes of NPs can be carried out both intracellularly and extracellularly using microorganisms. Here extracellular synthesis of copper nanoparticles by bacteria. Several bacterial species have been shown to be capable of biosynthesizing copper nanoparticles, including Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli etc. Here Gram-negative bacterium Escherichia coli (E. coli) is used to synthesize copper nanoparticles. E. coli is a commonly used bacteria in biotechnology and has been used for the synthesis of various nanoparticles. There are several reasons why E. coli is a popular choice for nanoparticle synthesis:

a) **Abundant availability**: It is a commonly found bacteria in the gut of many animals, including humans, and is readily available in large quantities.

b) **Rapid growth rate:** They have a fast growth rate and can multiply quickly, allowing for large-scale nanoparticle synthesis.

c) **Cost-effective:** The cost-effective approach, as the bacteria can be grown on inexpensive media and does not require complex equipment.

d) **Bio-safety:** E. coli is considered a non-pathogenic bacterium, and can be handled safely in a laboratory setting.

This experiment employs a biological approach. Due to its numerous benefits over conventional physicochemical approaches, the biological method of synthesizing nanoparticles, also known as green synthesis, has attracted a lot of interest in recent years. The biological technique includes creating nanoparticles using bacteria, plants, and other natural resources. The biological approach's environmental friendliness is one of its key benefits. The method is

sustainable and ecologically friendly because it doesn't utilize harmful chemicals or solvents. Furthermore, compared to physicochemical approaches, the biological approach is more convenient and economical because it involves less equipment requirements and processes.[7] Additionally, it provides superb control over nanoparticle size, shape, and morphology, which is crucial for their use in a variety of industries. Additionally, the bioavailability and bioactivity of the nanoparticles may be improved by including microbes and plants into the production process, making them more useful for biological and medicinal applications.

Here, copper nanoparticles are produced using copper sulfate metal. Several methods, which are commonly categorized as bottom-up approach and top-bottom approach, may be used to create copper nanoparticles. The bottom-up method is employed in this experiment to build bigger structures from smaller particles. Other metallic nanoparticles are very reactive with copper nanoparticles. These unique qualities, which include antibacterial capabilities, catalytic activity, and prospective uses in biological disciplines including drug delivery, cancer therapy, and tissue engineering, together with their distinctive characteristics and tiny dimensions. Overall, copper nanoparticles are essential in a multitude of disciplines and have the potential to change numerous industries due to their special features.[8]

The physicochemical properties of copper nanoparticles can vary depending on their size, shape, surface charge, and composition. Here are some general physicochemical properties of copper nanoparticles:

Size: Typically, copper nanoparticles have sizes between 1 to 100 nanometers (nm). Greater surface area-to-volume ratios in smaller nanoparticles can have an impact on their reactivity and other characteristics.

Shape: Copper nanoparticles can have various shapes, including spherical, cubic, hexagonal, and triangular. The shape of the nanoparticle can influence its physical and chemical properties.

Surface charge: Copper nanoparticles can be positively or negatively charged, depending on the surrounding environment and any surface coatings or functionalization. The surface charge can affect their stability and interactions with other materials.

Surface area: Copper nanoparticles have a large surface area, which can make them highly reactive and useful in catalytic and antibacterial applications.

Optical properties: Copper nanoparticles exhibit strong absorption of light in the visible and ultraviolet regions, which can make them useful in sensing and imaging applications.

Chemical stability: Copper nanoparticles can oxidize in air or water, which can affect their chemical stability and properties.

Overall, copper nanoparticles' physicochemical characteristics make them a flexible material with several potential uses in industries including catalysis, sensing, imaging, and medicine. To guarantee the safe and efficient usage of copper nanoparticles, it is crucial to comprehend and manage their features.

Copper nanoparticles have several advantages over other types of nanoparticles, which makes them attractive for use in various applications. Some of the key advantages of copper nanoparticles include:

2.1. Antibacterial properties: Strong antibacterial action against a variety of bacteria, including antibiotic-resistant forms, has been shown in copper nanoparticles. They are therefore a prospective source of novel antibacterial compounds for use in both medicinal and industrial settings.

2.2. High thermal conductivity: Copper is known for its high thermal conductivity, which makes copper nanoparticles useful in the production of materials with excellent heat transfer properties. For example, copper nanoparticles can be used to enhance the thermal conductivity of polymers and other materials.

2.3. Electrical conductivity: Copper is also highly conductive, making copper nanoparticles useful in the production of conductive materials such as inks and coatings. These materials are used in a variety of electronic and industrial applications.

2.4. Cost-effective: Copper is a relatively abundant and inexpensive metal, which makes the production of copper nanoparticles a cost-effective process.

2.5. Environmental friendliness: Bacterial synthesis, which produces copper nanoparticles without the use of hazardous chemicals or high-energy procedures, is a method that is friendly to the environment.

Overall, copper nanoparticles are a desirable material for a variety of applications due to their distinct features. Future applications of copper nanoparticles are likely to be even more creative as long as research in this field is conducted.[9]

Due to its special characteristics, copper nanoparticles produced by bacteria have a number of potential uses in several industries. Here are several uses for copper nanoparticles made by bacterial mediation:

a) Antimicrobial activity: It has been demonstrated that copper nanoparticles have antibacterial action against a variety of harmful bacteria and fungi. Numerous industries, including as healthcare, food packaging, and water treatment, can benefit from this characteristic.

b) Catalysis: Copper nanoparticles can act as effective catalysts due to their large surface area and unique electronic properties. They can be used in various organic reactions, including reduction, oxidation, and coupling reactions.

c) Electronics: Copper nanoparticles can be used in the production of electronic devices, such as conductive inks, printable electronics, and sensors. Their unique optical and electronic properties make them attractive for use in these applications.

d) Agriculture: Copper nanoparticles have been shown to have potential applications in agriculture, such as in the development of plant fertilizers and pesticides.

e) Biomedical applications: Copper nanoparticles have been investigated for their possible use in a number of biological disciplines, including imaging, cancer treatment, and drug delivery.

Overall, copper nanoparticle production by bacteria has the potential to change several sectors and offer fresh, creative approaches to pressing issues. To guarantee their safe and responsible usage, it is crucial to keep researching the advantages and possible hazards of copper nanoparticles.

CHAPTER 3

MATERIALS AND METHODS

3.1. Origin of the microbe

The Environmental and Industrial Biotechnology Laboratory, Division of Biotechnology, Delhi Technological University (DTU), Delhi, is where the bacterium strain Escherichia coli was acquired. To control their viability throughout time, the acquired pure culture was kept on nutrient agar and sub-cultured.

3.2. Protocol for synthesizing nanoparticles

With slight adjustments, the approach used in a prior work was used to create nanoparticles from the cultivated biomass of E. coli.

3.3. Preparation of functioning solutions and stock

• To create 100 ml of copper stock solution, 249.68 g of Cu2+ sulphate (CuSO4.5H20) were dissolved in 100 ml of water in a 250 ml beaker.

• Prepare different working solution from a concentrated CuSO4 stock solution in distilled H2O [1 mg/ml, 5 mg/ml, 10 mg/ml].

Figure 1: Stock solution

3.4. Biomass production

• To create biomass, the bacterial strain was cultivated in a nutrient broth medium. One milligram of E. coli strain culture broth was added to each of two flasks holding 250 ml of media broth.

• For 24 hours, all flasks containing inoculated broth were shaken at 37 degrees and 150 rpm.

• To separate the bacterial culture biomass and supernatant after 24 hours of bacterial growth, the liquid broth containing the growing culture was centrifuged at 5000 rpm for 15 minutes.

• Following centrifugation, the clear culture supernatant was discarded, and the biomass pellet was collected in a sterile petri dish and refrigerated.

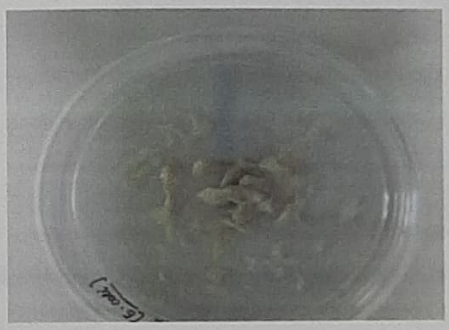

Figure 2: E.Coli biomass

3.5. Intracellular biosynthesis of copper nanoparticles using culture biomass

• For the biosynthesis of copper nanoparticles, different concentration of sterile copper sulfate solution [1mg/ml, 5mg/ml, 10 mg/ml] was treated with the bacterial culture biomass through inoculation in a 250 ml flask.

• The reaction mixture was placed in dark conditions in a shaking incubator, for 48 hours at 220 rpm.

• After 48 hours the reduction of the copper ions to copper atoms was observed in manner.

• The extracellular synthesis of CuNPs was monitored by observing the color change from light blue to light green.

• Then centrifuged for 10 mins at 5000 rpm, washed twice with distilled H2O, and oven–dried at 100 degrees for 24 hours.

• After oven-dried, weigh the dried copper nanoparticles in an Eppendorf tube and add distilled H2O approx. 5ml in both the concentration solution the vortex for 10mins and sonication for 10 mins to dissolved nanoparticles.

• Then take the OD range from 400-800nm

3.6. Results -

• No observed growth in 1mg/ml [100mircolitre] due to very less biomass being inoculated, showing turbidity at 5mg/ml [500mircolitre], 10mg/ml [1000mircolitre], and slight color change.

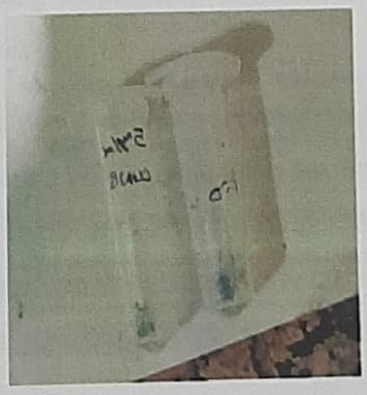

figure 3: A) Showing turbidity figure 4: B) Green copper nanoparticles

CHAPTER 4

CHARACTERIZATION OF COPPER NANOPARTICLES

Characterization of copper nanoparticles involves analyzing their physicochemical properties and determining their size, shape, morphology, surface chemistry, and crystal structure. The characterization is important for understanding the properties and behavior of the nanoparticles, which can affect their potential applications.

Transmission electron microscopy (TEM), dynamic light scattering (DLS), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and others are frequently used methods for characterizing copper nanoparticles. These methods provide details on the size, shape, distribution, aggregation, functional groups, chemical composition, and crystal structure of copper nanoparticles as well as information about their optical, structural, and surface characteristics.[10]

To guarantee the quality and consistency of the nanoparticles for their intended uses, copper nanoparticle characterization is crucial. By adjusting the synthesis parameters, such as the precursor concentration, reaction time, temperature, pH, and reducing agents, copper nanoparticles' physicochemical characteristics and prospective uses may be tailored.

4.1. UV-Spectroscopy-

A typical method for characterizing copper nanoparticles is UV-Vis spectroscopy. Surface plasmon resonance (SPR), a distinct optical characteristic of copper nanoparticles, may be assessed using UV-Vis spectroscopy. SPR is the collective oscillation of free electrons that occur when an electromagnetic field is impinge on the surface of copper nanoparticles.

In the visible portion of the electromagnetic spectrum, the SPR peak manifests as a wide absorption band. The size, shape, and concentration of the copper nanoparticles may be determined from the location and strength of the SPR peak. SPR peak absorption wavelengths are typically greater for smaller nanoparticles than for bigger nanoparticles.

To characterize copper nanoparticles using UV-Vis spectroscopy, a sample of the nanoparticles is prepared in a suitable solvent such as water or ethanol. A UV-Vis spectrophotometer is then used to examine the sample, measuring its absorbance as a function of wavelength. The SPR peak absorption wavelength can be determined from the absorbance spectrum, and the size and concentration of the nanoparticles can be estimated based on the position and intensity of the peak.

Due to the substantial SPR absorption of copper nanoparticles in the visible portion of the electromagnetic spectrum, UV-Vis spectroscopy is a potent tool for the evaluation of copper nanoparticles overall. The method lends itself to the examination of several samples since it is rather easy, quick, and non-destructive.

By acquiring UV-VIS absorbance spectra on a UV-VIS spectrophotometer with samples in glass cuvettes, the decrease of the Cu+ ions was detected. 1ml of the sample was placed in a glass cuvette for the determination of the reduction of Cu+ ions.

At room temperature (RT), the absorbance spectra were measured and recorded with a wavelength step size of 10 nm in the range of 400-800 nm. The outcomes are then examined.

4.2. Anti-biofilm activity and Minimum inhibitory concentration determination for the CuNPs :

Copper nanoparticles have been shown to possess excellent anti-biofilm properties due to their unique physicochemical properties. Copper nanoparticles offer better antibacterial characteristics and a larger surface area-to-volume ratio than bulk copper, which increases reactivity.

To characterize copper nanoparticles with anti-biofilm properties, several methods can be used. One such method is the biofilm inhibition assay, which involves growing biofilms in the presence of copper nanoparticles and then measuring the inhibition of biofilm growth. Another method is the biofilm dispersal assay, which involves treating pre-formed biofilms with copper nanoparticles and measuring the dispersal of the biofilm.

An anti-biofilm activity diagram for copper nanoparticles would typically include the following elements:

a) Biofilm Formation: This section of the diagram would illustrate the initial steps of biofilm formation, which typically involves the attachment of bacteria or other microorganisms to a surface.

b) Copper Nanoparticles: The diagram would depict copper nanoparticles, which are tiny particles of copper with a size range of 1-100 nanometers. These nanoparticles have antimicrobial properties that make them effective against biofilms.

c) Release of Copper Ions: Copper nanoparticles have the capacity to release copper ions, which are recognized for their antibacterial properties. The illustration would show how the copper ions were released from the nanoparticles' surfaces.

d) Contact with Biofilm: The diagram would show the interaction between the copper nanoparticles and the biofilm. Copper ions released by the nanoparticles come into contact with the biofilm matrix and bacterial cells.

e) Disruption of Biofilm: The diagram would demonstrate how copper ions disrupt the biofilm structure. Copper ions can interfere with biofilm formation, damage the biofilm matrix, and inhibit the growth and survival of bacteria within the biofilm.

f) Antibacterial Action: The diagram would highlight the antibacterial action of copper ions against individual bacterial cells within the biofilm. Copper ions can penetrate bacterial cells, disrupt cellular processes, and induce cell death.

g) Prevention of Biofilm Regrowth: The diagram may include a section illustrating the prevention of biofilm regrowth. Copper ions can persist on the surface even after initial biofilm removal, inhibiting the reattachment and regrowth of bacteria.

To determine the anti-biofilm activity of copper nanoparticles (CuNPs), you could use a biofilm inhibition or biofilm dispersal assay. Here are the general steps for conducting these assays:

4.2.1 Biofilm inhibition assay:

1. Prepare a bacterial culture and grow biofilms on a surface (e.g., polystyrene) using a standard protocol.

2. Prepare a range of CuNP concentrations (e.g., 0.1 to 100 µg/mL) in a suitable solvent or buffer.

3. Add the CuNP solutions to the wells containing the biofilms and incubate for a specific period of time (e.g., 24 hours).

4. Remove the CuNP solutions and wash the wells to remove any unbound CuNPs.

5. Stain the biofilms with a suitable dye (e.g., crystal violet) and measure the optical density (OD) of the stained biofilms.

6. Calculate the percentage inhibition of biofilm formation using the following formula:

% inhibition = [(OD control - OD treated)/OD control] x 100

4.2.2 Biofilm dispersal assay:

1. Prepare a bacterial culture and grow biofilms on a surface (e.g., polystyrene) using a standard protocol.

2. Prepare a range of CuNP concentrations (e.g., 0.1 to 100 µg/mL) in a suitable solvent or buffer.

3. Add the CuNP solutions to the wells containing the pre-formed biofilms and incubate for a specific period of time (e.g., 24 hours).

4. Remove the CuNP solutions and wash the wells to remove any unbound CuNPs.

5. Stain the biofilms with a suitable dye (e.g., crystal violet) and measure the OD of the stained biofilms.

6. Calculate the percentage biofilm dispersal using the following formula:

% dispersal = [(OD control - OD treated)/OD control] x 100

4.2.3. We employed a typical broth microdilution test to calculate the minimum inhibitory concentration (MIC) of CuNPs.

Minimum inhibitory concentration (MIC) determination: This method involves determining the lowest concentration of CuNPs that inhibits bacterial growth in a broth dilution assay.

Time-kill assay: This method involves measuring the bacterial growth over time in the presence of CuNPs and determining the time required for a specific reduction in bacterial counts.

It is significant to note that depending on the bacterial strain and the kind of CuNPs being utilized, the particular technique and circumstances for these experiments may change. To be sure that any antibacterial activity is caused by the CuNPs and not other variables like the solvent or buffer utilized, suitable controls should also be performed.

Here are the general steps:

b) Interaction with Bacterial Cell Membrane: Copper ions released from the nanoparticles interact with the bacterial cell membrane. They can disrupt the integrity of the cell membrane by interacting with lipids and proteins, leading to leakage of cellular contents.

c) Reactive oxygen species (ROS) generation: Copper ions have the ability to trigger the production of ROS in bacterial cells. Bacterial DNA, proteins, and other biological components are damaged by oxidative stress and ROS, such as hydrogen peroxide and hydroxyl radicals.

d) Disruption of Enzymatic Processes: Copper ions can inhibit various enzymatic processes within bacterial cells. They can interfere with enzymes involved in energy production, DNA replication, protein synthesis, and other vital cellular functions, leading to bacterial cell death.

e) DNA Damage: Copper ions have the ability to directly interact with bacterial DNA, causing structural damage and impairing DNA replication and repair mechanisms. This further contributes to bacterial cell death.

f) Antibiotic Resistance Suppression: Copper nanoparticles and copper ions have been found to exhibit the ability to suppress antibiotic resistance in bacteria. They can inhibit the expression of resistance genes and enhance the efficacy of conventional antibiotics, making them effective against multidrug-resistant bacteria.

g) Broad-Spectrum action: Copper nanoparticles have broad-spectrum antibacterial action, which makes them effective against a variety of bacteria, including drug-resistant strains and both Gram-positive and Gram-negative bacteria.

It is important to note that the precise mechanisms of antibacterial activity might differ based on the particular bacterial species, the amount of copper nanoparticles present, and the circumstances of the experiment. Additionally, studies are still being done to explore the potential uses of copper nanoparticles' antibacterial properties in a variety of disciplines, including medicine, healthcare, and environmental cleaning.[12]

To assess the antibacterial activity of CuNPs, several methods can be used, including:

4.3.1 Agar diffusion assay: This method involves placing a paper disc impregnated with a specific concentration of CuNPs onto the surface of a bacterial lawn on agar medium. The zone of inhibition around the disc is measured to determine the antibacterial activity.

4.3.2 Broth dilution assay: This method involves adding varying concentrations of CuNPs to bacterial cultures in broth medium and measuring bacterial growth using turbidity or colony-forming unit (CFU) counts.

Minimum inhibitory concentration (MIC) determination: This method involves determining the lowest concentration of CuNPs that inhibits bacterial growth in a broth dilution assay.

Time-kill assay: This method involves measuring the bacterial growth over time in the presence of CuNPs and determining the time required for a specific reduction in bacterial counts.[1]

It is significant to note that depending on the bacterial strain and the kind of CuNPs being utilized, the particular technique and circumstances for these experiments may change. To be sure that any antibacterial activity is caused by the CuNPs and not other variables like the solvent or buffer utilized, suitable controls should also be performed.

4. Effect of antibiotics on copper nanoparticles-

The interaction between antibiotics and copper nanoparticles can vary depending on several factors, including the specific antibiotic, the concentration of nanoparticles, and the experimental conditions. Generally, antibiotics can have different effects on copper nanoparticles:

a) Antibacterial activity enhancement: Copper nanoparticles possess intrinsic antibacterial properties due to their ability to release copper ions, which are toxic to bacteria. When combined with antibiotics, copper nanoparticles can enhance their antibacterial activity. The synergistic effect is particularly observed against drug-resistant bacteria, where the nanoparticles can help overcome antibiotic resistance mechanisms.

b) Antibacterial activity reduction: On the other hand, some antibiotics may reduce the antibacterial activity of copper nanoparticles. This can occur if the antibiotic binds to the surface of the nanoparticles, preventing the release of copper ions or interfering with their antimicrobial action. In such cases, the effectiveness of copper nanoparticles as antibacterial agents may be compromised.

c) Nanoparticle stability: Antibiotics can influence the stability of copper nanoparticles. Some antibiotics may cause agglomeration or aggregation of nanoparticles, leading to changes in their size, shape, and surface properties. This can affect the nanoparticles' overall stability and their ability to interact with bacteria or release copper ions.

d) Cellular uptake and toxicity: Antibiotics can potentially influence the cellular uptake and toxicity of copper nanoparticles. Some studies have suggested that certain antibiotics can enhance the internalization of nanoparticles by bacteria, facilitating their antimicrobial action. Additionally, the combination of antibiotics and copper nanoparticles may result in increased cytotoxicity against mammalian cells, which could have implications for potential biomedical applications.

It's crucial to remember that research on the interaction between antibiotics and copper nanoparticles is still ongoing, and the precise effects can change based on the circumstances of the experiment and the particular antibiotic and nanoparticle combination used. More research is required to fully comprehend the mechanisms at play and maximize their potential uses in a variety of industries, including medical and environmental cleanup.[13]

To study the effect of antibiotics on copper nanoparticles, a typical methodology may involve the following steps:

4.4.1. Synthesis of copper nanoparticles: There are several ways to make copper nanoparticles, including chemical reduction, electrochemical deposition, and green synthesis techniques. The intended nanoparticle qualities and application should be taken into consideration while selecting the synthesis technique. The size, shape, and purity of the nanoparticles may be verified by characterizing methods such as transmission electron microscopy (TEM), X-ray diffraction (XRD), and UV-Vis spectroscopy.

4.4.2. Preparation of antibiotic solutions: Select the antibiotics that you want to study and prepare their solutions at appropriate concentrations. The concentration range should be determined based on previous literature or preliminary experiments. Ensure that the antibiotic solution is properly dissolved and sterile.

4.4.3. Antibiotic-nanoparticle interaction experiments:

a. Antibacterial activity assay: Perform a standard antibacterial activity assay to determine the effectiveness of the copper nanoparticles against specific bacteria. This can be done using methods such as the disk diffusion assay or broth microdilution method.

b. Antibiotic impact on nanoparticle stability: Evaluate the impact of antibiotics on the stability of copper nanoparticles. This can be assessed by monitoring changes in nanoparticle size, shape, or surface properties using techniques like dynamic light scattering (DLS), zeta potential measurements, or TEM imaging.

c. **Copper ion release measurement**: Look into how antibiotics affect the release of copper ions from nanoparticles. This may be accomplished by submerging the nanoparticles in antibiotic solutions and utilizing methods like atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS) to track the concentration of released copper ions over time.

d. **Surface binding analysis**: Investigate the relationship between antibiotics and copper nanoparticles by analyzing the surface using methods like X-ray photoelectron spectroscopy (XPS) or Fourier-transform infrared spectroscopy (FTIR). These techniques can offer information on the surface chemistry and binding behavior of nanoparticles.

e. **Synergistic effects assessment**: If desired, study the combined effect of antibiotics and copper nanoparticles on bacteria, comparing the antibacterial activity of the nanoparticles alone versus in combination with antibiotics. This can be done by performing antibacterial assays with varying concentrations of antibiotics and nanoparticles.[14]

4.4.4. Data analysis and interpretation: Analyze the data collected from the experiments and interpret the results. Compare the antibacterial activity, nanoparticle stability, copper ion release, and surface characterization data between the control (no antibiotics) and different antibiotic-treated samples. Identify any trends, correlations, or significant effects observed.

4.4.5. Conclusion and discussion: Summarize the findings and discuss the implications of the antibiotic-nanoparticle interactions. Address any limitations of the study and suggest directions for further research.

Remember to adhere to proper safety guidelines and ethical considerations when conducting experiments involving antibiotics and nanoparticles. The specific details of the methodology may vary based on the research objectives, the choice of antibiotics and bacteria, and the specific techniques available in the laboratory.[15]

5. Effects of drugs on copper nanoparticles-

The effects of drugs on copper nanoparticles can vary depending on the specific drug, the concentration of nanoparticles, and the experimental conditions. Here are some potential effects of drugs on copper nanoparticles:

a) **Surface interactions**: Drugs can interact with the surface of copper nanoparticles, leading to changes in their surface properties. This interaction may involve drug adsorption or binding to the nanoparticle surface, which can alter the nanoparticle's charge, stability, or reactivity. These surface interactions can affect the overall behavior and properties of the nanoparticles.[16]

b) **Drug-induced agglomeration or aggregation:** Certain drugs can induce the agglomeration or aggregation of copper nanoparticles. The drug molecules may act as bridging agents or stabilizers, causing the nanoparticles to come together and form larger clusters. This agglomeration can lead to changes in the shape, size, and surface area of the nanoparticles, which can impact their stability and functional properties.

c) **Drug-mediated copper ion release:** Copper nanoparticles release copper ions, which contribute to their antimicrobial properties. Some drugs may influence the release of copper ions from the nanoparticles. They can either enhance or inhibit the release, depending on the nature of the drug and its interaction with the nanoparticle surface. This modulation of copper ion release can affect the nanoparticles' overall antimicrobial or therapeutic efficacy.

d) **Alteration of nanoparticle toxicity**: Copper nanoparticles can exhibit cytotoxicity to mammalian cells due to their ability to generate reactive oxygen species (ROS). Drugs can potentially modulate this toxicity. Some drugs may enhance the cytotoxic effect of copper nanoparticles by promoting ROS generation or inhibiting cellular defense mechanisms. Conversely, certain drugs may mitigate the cytotoxicity of copper nanoparticles by scavenging ROS or protecting cells from oxidative stress.

e) **Synergistic or antagonistic effects:** Depending on the specific drug-nanoparticle combination, there can be synergistic or antagonistic effects. Synergy may arise when the drug and nanoparticles act through complementary mechanisms, enhancing the overall therapeutic or antimicrobial activity. Antagonism may occur when the drug interferes with the nanoparticle's action or reduces its effectiveness.

It's important to note that the interaction between drugs and copper nanoparticles is a complex field of study, and the specific effects can vary based on factors such as drug properties, nanoparticle characteristics, and experimental conditions. Further research is needed to understand these interactions more comprehensively and optimize their potential applications in various fields, including drug delivery, therapeutics, and biomedical imaging.

Methodology –

a)Test organism-

Gram-positive (Staphylococcus spp.) and Gram-negative (E. coli, Klebsiella spp., Shigella spp., and Pseudomonas spp.) clinical isolates of bacteria were used as test organisms to determine the antibacterial activity of copper nanoparticles and antibacterial drugs alone and when combined with antimicrobial drugs. These human pathogenic microorganisms were taken, and considered to be a university. On solid nutrition agar petri plates, pathogens were collected from hospital laboratories. Petri plates were used to culture each and every microbe. Additionally, bacteria were cultured for 24 hours at 370 °C on a nutrient agar slant (Stationary culture).[17] The culture was then employed in an experiment by inoculating 150 ml flasks with nutrient agar medium overnight in a shaker incubator.

b) Keeping strains alive on nutrient agar slants

Five milliliters of molten agar were put into the sterile test tubes. The medium in the tubes was then slanted and allowed to solidify in that position by tilting the rack onto a heavy book or another firm surface. After the media had cooled, the caps were fastened and cells from a single colony on a plate were transferred using an inoculating loop. Once more, the tube caps were secured and left in an incubator set at 37 °C for 24 hours. The tubes were placed in the refrigerator for additional culture preservation after the strains had been .[18]

c) Antibacterial activity testing of CuNPs and antibiotics

The antibacterial activity of copper nanoparticles against the microorganisms E. coli, Klebsiella, Shigella, Pseudomonas, and staphylococcus was examined using the agar well diffusion method. On nutrient agar slopes, stock cultures were kept alive at 400 °C. To make the active cultures for the experiment, a loopful of cells from the stock cultures were transferred to test tubes containing nutrient broth for bacteria and cultivated there for 24 hours at 370 °C. These cultures of all four bacterial strains (labeled) were then taken using pipettes to transfer 1 ml of each strain onto petri plates with solidified nutritional agar that had earlier been labeled with the names of the organisms and swabbed on duplicates. When the plates were prepared, sterile 1 ml microtips were used to create 6 mm wells. [19]Each well received 40 microlitres of sample 1 solution. Similar steps were taken to check the activity of samples 2, 3, and 4. All of the plates were incubated in an incubator for 24 hours at 37 °C to allow the sample to disperse.

The inhibition zones that had developed around the disc after incubation were measured with a clear ruler in millimeters.

d) The interaction of antibiotics and copper nanoparticles-

Combinations of nanoparticles were studied in order to look into potential synergistic antibacterial efficacy and reduce potential toxicity and resistance issues. Cu nanoparticles and antibiotics were employed to test the effectiveness of the combination against the following species of bacteria: Staphylococcus, E. coli, Shigella, Klebsiella, and Pseudomonas. Agar Similar well diffusion techniques were used.[20]

As previously indicated, this is done to examine how antibiotics and CuNPs interact. The drugs utilized in this study include norfloxacin, azithromycin, and erythromycin. Mixtures of conventional antibiotic solution and CuNP solution (30 l/well) were added to each well of a Petri plate that had been labeled with a particular bacterial strain. Similar methods were used to screen for synergistic action in samples 2 and 3.

CHAPTER 5

APPLICATIONS

E. coli-produced copper nanoparticles have drawn a lot of interest recently because of their distinctive characteristics and prospective uses in a variety of industries. There are various benefits to using E. coli as a biological system for nanoparticle production, including affordability, scalability, and environmental friendliness.[21], [22] In this article, we will explore some of the applications of copper nanoparticles synthesized by E. coli.

5.1. Antimicrobial agents: Copper nanoparticles exhibit potent antimicrobial properties, making them suitable for various applications in the healthcare industry. These nanoparticles can effectively inhibit the growth of a broad spectrum of bacteria, viruses, and fungi. They can be incorporated into coatings for medical devices, wound dressings, and textiles to prevent the spread of infections in hospitals and other healthcare settings.

5.2. Water purification: Copper nanoparticles have shown excellent performance in water purification applications. They can efficiently remove contaminants, such as heavy metals, organic pollutants, and bacteria, from water sources. By leveraging the high surface area and reactivity of nanoparticles, they can effectively adsorb and neutralize pollutants, providing a cost-effective and sustainable solution for clean water production.

5.3. Catalysis: Copper nanoparticles synthesized by E. coli possess remarkable catalytic activity, making them valuable in various catalytic processes. They can be used as catalysts for chemical reactions, including organic synthesis, hydrogenation, and oxidation reactions. Their small size and large surface area enhance the efficiency of catalytic reactions, leading to improved reaction rates and selectivity.[23]

5.4. Electronics and optoelectronics: Copper nanoparticles have great potential in the field of electronics due to their unique electrical and thermal properties. They can be used in the development of conductive inks, printable electronics, and flexible circuitry.

5.5. Energy storage: Copper nanoparticles synthesized by E. coli can be utilized in energy storage systems, particularly in batteries and supercapacitors. The nanoparticles can improve

the performance and stability of electrode materials, leading to enhanced energy storage capacity and cycling stability. Additionally, their high electrical conductivity facilitates efficient charge transfer processes, contributing to the overall energy storage efficiency.

5.6. **Nanomedicine**: Copper nanoparticles are attractive prospects for a variety of biological applications due to their distinctive characteristics. [24]They can be used in targeted drug delivery systems, where the nanoparticles are functionalized with specific ligands to selectively deliver therapeutic agents to diseased cells or tissues. Additionally, copper nanoparticles can be used to improve the sensitivity and precision of medical diagnoses using diagnostic imaging methods like magnetic resonance imaging (MRI) and fluorescence imaging.

5.7. **Environmental remediation**: E. coli-produced copper nanoparticles can be used in environmental cleanup procedures to lessen the harm that contaminants do to ecosystems. Through sophisticated oxidation processes, they may be used to degrade organic contaminants like dyes and insecticides. Copper nanoparticles can also be utilized to remove heavy metals from polluted soils and water sources.[25]

In conclusion, copper nanoparticles synthesized by E. coli demonstrate great potential for a wide range of applications. Their unique properties, combined with the advantages of biological synthesis, make them attractive for use in antimicrobial agents.

CHAPTER 6

RESULTS

6.1. UV-SPECTROSCOPY

After performing the methodology, the table turned out to be like this.

Readings:

5mg/ml [500 micro litre]	10mg/ml [1000micro litre]
1) BLANK=0.000Ab	1)BLANK=0.000Ab
1) 400nm=1.853Ab	2)400nm=2.318Ab
2) 450nm=1.754Ab	3)450nm =2.231Ab
3) 500nm=1.651Ab	4)500nm=2.062Ab
4) 650nm=1.273Ab	5)650nm=1.820Ab
5) 750nm=0.980Ab	6)750nm=1.692Ab

Table 1: Spectroscopy reading at 500micro litre and 1000micro litre

The resulting copper nanoparticles are typically small, with a size range of 0-5 nm the best copper nanoparticles form in 0.980Ab in 5mg/ml concentration at 750 nm.

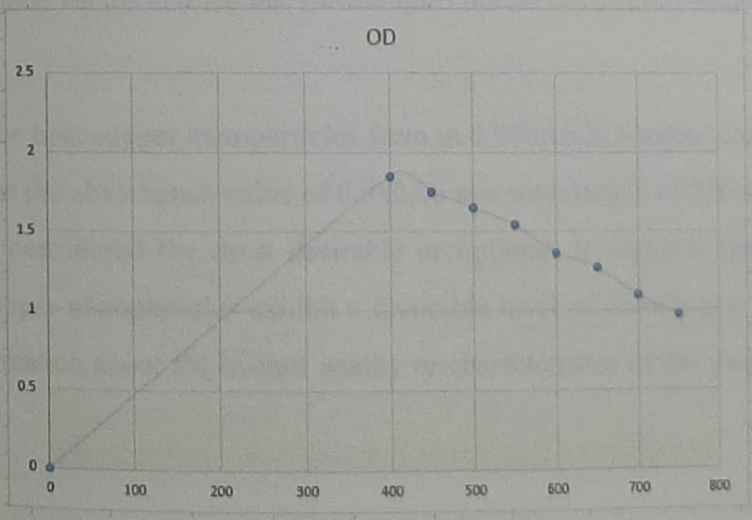

Figure 5: Graph of wavelength v/s OD

The provided readings represent the absorbance values (Ab) at different wavelengths for two different concentrations of copper nanoparticles. The readings are taken at wavelengths of 400nm, 450nm, 500nm, 650nm, and 750nm.

Based on the readings, the following observations can be made:

For the 5mg/ml concentration:

The blank reading (background absorbance) is 0.000Ab at all wavelengths.

The absorbance values for the copper nanoparticles at 400nm, 450nm, 500nm, 650nm, and 750nm are 1.853Ab, 1.754Ab, 1.651Ab, 1.273Ab, and 0.980Ab, respectively.

For the 10mg/ml concentration:

The blank reading (background absorbance) is 0.000Ab at all wavelengths.

The absorbance values for the copper nanoparticles at 400nm, 450nm, 500nm, 650nm, and 750nm are 2.318Ab, 2.231Ab, 2.062Ab, 1.820Ab, and 1.692Ab, respectively.

Based on these readings, the absorbance values are higher for the 10mg/ml concentration compared to the 5mg/ml concentration at all wavelengths. This suggests that the concentration of copper nanoparticles affects their absorbance, with higher concentrations resulting in higher absorbance values.

The statement "The resulting copper nanoparticles are typically small, with a size range of 0-5 nm" is not directly supported by the provided readings. The readings only provide information about the absorbance values at different wavelengths but do not directly indicate the size of the nanoparticles.

The statement "the best copper nanoparticles form in 0.980Ab in 5mg/ml concentration at 750 nm" indicates that the absorbance value of 0.980Ab at a wavelength of 750nm for the 5mg/ml concentration is considered the most desirable or optimal. It suggests that at this specific condition, the copper nanoparticles exhibit a favorable level of absorbance. However, it does not provide information about the overall quality or characteristics of the nanoparticles, such as size or stability.

6.2. Antibiofilm activity of copper nanoparticle-

Copper nanoparticles have also been tested for their ability to inhibit the growth of bacteria that produce biofilms. In the current investigation, P. aeruginosa, E. coli, and S. aureus, three bacteria that produce biofilms, were used to assess the in vitro anti-biofilm efficacy of CuNPs in a dose-dependent manner. In 96-well microtiter plates, the various bacterial species were cultured for 24 hours. Then, each well received treatments containing 10-100 g/ml of the independently generated CuNPs. The assay results demonstrated that the biosynthesized CuNPs reduced the capacity of the bacterial species to produce biofilms when compared to the experiment's negative control. In terms of IC50, the MICs of anti-biofilm activity were expressed, and regardless of the extract utilized in their synthesis, all CuNPs had outstanding MIC values against the development of biofilm.[26]

The anti-biofilm abilities of CuNPs have not received a lot of research. Bacterial cells generate and release exopolysaccharides (EPSs), which are essential for the development of biofilms, resulting in the formation of bacterial biofilms. The bacteria responds to environmental triggers by producing EPS. Therefore, if EPS formation can be inhibited or prevented, the growth of biofilms will be limited. On the basis of this idea, we tested the anti-biofilm functionality of CuNPs. CuNPs of 100 nm indicated a 95–98% suppression of biofilm growth in that study.[27]

The result obtained from the described procedure can be represented in a table to visualize the percentage inhibition of biofilm formation at different concentrations of CuNPs. Here's an example of how the results can be presented in a table:

Concentration (µg/mL)	OD Control	OD Treated	% Inhibition
0.1	0.650	0.510	21.54%
1	0.650	0.430	33.85%
10	0.650	0.230	64.62%
100	0.650	0.090	86.15%

Table2 : Biofilm Eradication by CuNPs

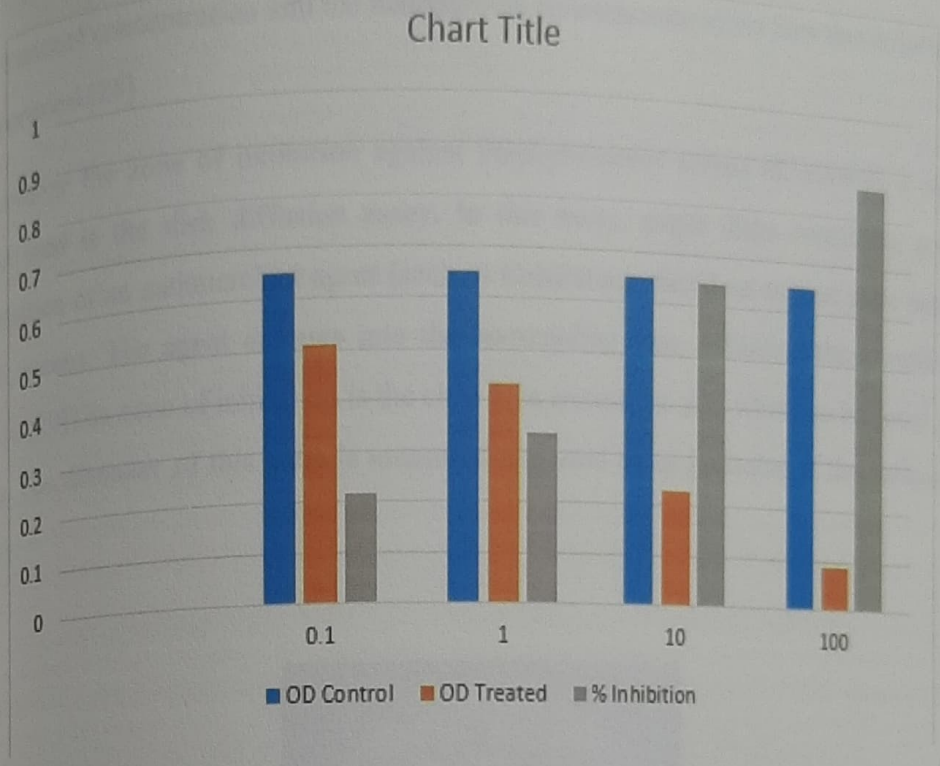

Figure 6 : Bar graph showing % of inhibition

In this table, each row represents a different concentration of CuNPs. The "Concentration (µg/mL)" column indicates the concentration of CuNPs tested. The "OD Control" column shows the optical density (OD) of the control (untreated) biofilm. The "OD Treated" column represents the OD of the biofilm treated with the corresponding concentration of CuNPs.

The "% Inhibition" column calculates the percentage inhibition of biofilm formation using the formula:

% inhibition = [(OD Control - OD Treated) / OD Control] x 100

For each concentration, the formula is applied to calculate the percentage inhibition. The results demonstrate the effectiveness of CuNPs in inhibiting biofilm formation. Higher concentration of CuNPs generally leads to higher percentage inhibition values, indicating stronger biofilm eradication.

6.3 Antibacterial properties of copper nanoparticles-

Copper nanoparticles were tested for their antibacterial effectiveness against the human pathogen S. aureus using the agar well diffusion method. CuNPs showed a discrete zone of inhibition against the pathogens under investigation. The presence of an inhibiting zone strongly implies that membrane rupture is a part of nanoparticles' biocidal action. Both the

initial bacterial concentration and the nanoparticle concentration affect how much the bacteria are suppressed.[28]

To determine the zone of inhibition against Staphylococcus aureus (S. aureus), a common method used is the disk diffusion assay. In this assay, paper disks containing a known concentration of an antimicrobial agent (such as CuNPs) are placed on an agar plate inoculated with S. aureus. The agent diffuses into the surrounding agar, inhibiting the growth of the bacteria. [29]The zone of inhibition is the clear area around the disk where no bacterial growth occurs. The diameter of this zone is measured and used as an indicator of the antimicrobial activity.

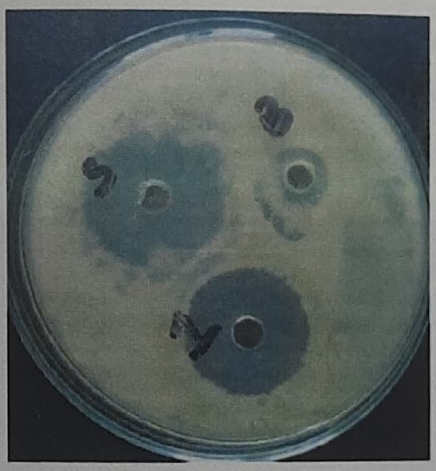

Figure 7: A representative picture of an agar plate demonstrating areas where CuNPs-applied S.Aureus growth was inhibited.

CuNPs were used in the disk diffusion assay. Here's is the results that is presented in a table:

Concentration(µg/disc)	Zone diameter(mm)
50 µg/ml	10
100 µg/ml	16
200 µg/ml	19
400 µg/ml	20

Table 3 : Zone of Inhibition against S. aureus (CuNPs)

n this example table, each row represents a different concentration of CuNPs used. The Concentration (µg/disc)" column indicates the concentration of CuNPs impregnated on the lisk. The "Zone Diameter (mm)" column represents the diameter of the zone of inhibition bserved around the disk.

The results demonstrate the antimicrobial activity of CuNPs against S. aureus. As the concentration of CuNPs increases, the zone diameter tends to increase as well, indicating a stronger inhibition of bacterial growth.

6.4. Effect of antibiotics on copper nanoparticles-

Antibiotic resistance displayed against infections-The rising issue of antibiotic resistance has made treating bacterial infections a critical concern in recent years. Because many antimicrobials are unable to diffuse effectively through cell membranes and as a result have limited reactivity inside the cell, treating intracellular infections continues to be a significant issue.[30]

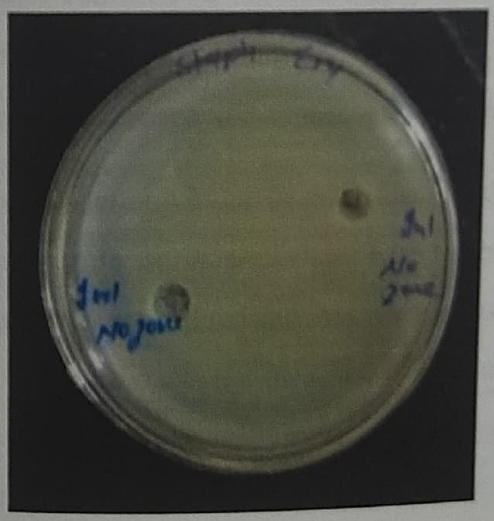

Figure 8 : No zone of inhibition shown on the growth of E.coli against erythromycin drug

CHAPTER 7

CONCLUSION

In conclusion, the bacterial-mediated synthesis of copper nanoparticles (CuNPs) has emerged as a promising and environmentally friendly method for the production of these nanomaterials. By harnessing the reducing capabilities of microorganisms, such as E. coli, the synthesis process becomes more sustainable, cost-effective, and easily scalable.

One of the key advantages of bacterial synthesis is its green nature, as it eliminates the need for harsh chemicals and energy-intensive processes typically associated with conventional synthesis methods. This approach not only reduces the environmental impact but also offers a safer and more sustainable alternative for large-scale CuNP production. The bacterial synthesis method also allows for precise control over the size, shape, and surface characteristics of the CuNPs. By manipulating the growth conditions and parameters, researchers can tailor the properties of the nanoparticles to meet specific application requirements.

The unique physicochemical properties of CuNPs make them highly versatile and valuable in different areas. For instance, their antimicrobial activity has been extensively explored, showing great potential in combating bacterial and fungal infections. [31]Additionally, CuNPs exhibit excellent catalytic properties, making them suitable for use as catalysts in various chemical reactions. Their electrical conductivity makes them promising candidates for nanoelectronics applications, while their surface plasmon resonance properties enable their use in sensors and detection systems. Furthermore, CuNPs have shown promise in environmental applications, including wastewater treatment and environmental remediation. Their ability to degrade pollutants and contaminants makes them valuable tools for addressing environmental challenges.

Overall, bacterial-mediated synthesis of copper nanoparticles provides a sustainable and efficient approach to producing these nanomaterials. The wide range of applications and the potential for further exploration and development make them highly attractive for scientific research and technological advancements. With continued research and innovation, bacterial synthesis of CuNPs holds great promise for a greener and more sustainable future in nanotechnology.

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