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A Research Paper On Formulate And Evaluate Copper Nanoparticles As An Effective Antibacterial Agent In Topical Drug Delivery System

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Abstract

Copper has been utilized to make nanoparticles because it is readily available. However, aggregation and rapid oxidation are difficulties in the research sector. In the current work, pure copper nanoparticles were produced chemically in the presence of a chitosan stabilizer. The quality of the nanoparticles was confirmed using a variety of characterization techniques, including as ultraviolet visible spectroscopy, transmission electron microscopy, X-ray diffraction, Fourier transform infrared spectroscopy, and field emission scanning electron microscopy. The antibacterial and antifungal abilities of the nanoparticles were tested on a number of important pathogens, including Candida albicans, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella choleraesuis, and methicillin-resistant Staphylococcus aureus. The development of the microorganism is affected by the chitosan medium, according to studies on the issue. The size of the copper nanoparticles that were created varied from 2-350 nm depending on the quantity of the chitosan stabilizer.

Keywords: aqueous media, chemical production, antibacterial action, copper nanoparticles, and chitosan.

Introduction:

Antibacterial agents are compounds that, in most cases, do not harm the tissue around them but either kill bacteria or prevent their development. The term "antibiotics" was first used by Selman Waksman in 1941 to refer to the antibacterial compounds that many microorganisms produce. Antibacterial compounds have been employed in a wide range of sectors, including the textile industry, water treatment, food packaging, and medicine. As a result of antibiotic misuse, antibiotic resistance genes are becoming increasingly prevalent in several bacterial species. Many well-known antibiotics have shown resistance in at least one type of bacteria. As a result, this problem has been the subject of much research.

"Nanoparticles" (NPs) are solid colloidal particles having a size between one and 1000 nm (one micron), according to the Encyclopaedia of Pharmaceutical Technology. In fact, NPs demonstrate a range of potential therapeutic applications: They have the ability to focus medication on the intended target, reducing side effects and improving drug absorption. NPs can interact with mucosal surfaces and avoid endolysosomal compartments, among other things. The kinetic properties of drug release can also be changed by NPs. When particle size falls below one micrometre, the saturation solubility rises, according to the Ostwald-Freundlich equation.

The Noyes-Whitney equation predicts that NPs will have a higher saturation solubility and greater surface area, both of which will speed up the rate of dissolution. Comparatively, the solubility of particles larger than one micron (normal size) is a constant that solely varies on the temperature and solvent for each distinct component. The latest advancements in nanotechnology have generated a great lot of interest in figuring out the antibacterial activity of metals at the nanoscale. The use of metallic NPs results in decreased concentration and increased antibacterial and antifungal action. Even still, there are worries about how dispersing metallic NPs can damage the environment or put people in risk.

For instance, discharging silver into the environment might contaminate the ecosystem [3]. Numerous metallic NPs have been thoroughly investigated for their antibacterial capabilities, including alumina [10–12], silver [13–14], iron [15–19], gold [20–22], magnesium [23–25], titanium [26–27], and zinc oxide [28–29]. Despite the great efforts made in the use of metallic NPs with antibacterial characteristics, we are still a long way from perfect metallic NPs with efficient activity. As a novelty discussion paper, this review will give an outline of the major advancements made in the field of copper nanoparticles (Cu NPs), which are exploited as antibacterial agents.

Much interest was generated by the potential of the nanoscale particles in biological and pharmaceutical applications. Nanoscale particles can easily make contact with bacterial membranes. Studies using atomic force microscopy, transmission electron microscopy, and laser confocal imaging revealed that the use of nanoparticles significantly affected the integrity of the bacterial cell membranes, leading to bacterial cell death. The development in the synthesis of metallic NPs has led to a new class of antibacterial materials. Highly ionic metallic NPs are of tremendous interest because of their distinctive crystal morphologies, very large surface areas, and many reactive surface sites.

Topical drug delivery System:

Topical drug delivery is the application of a drug-containing formulation to the skin with the aim of limiting the drug's pharmacological or other effects to the skin's surface or deeper layers in order to treat cutaneous conditions like acne or the skin-related symptoms of a more widespread illness like psoriasis. The Benefits of Natural Products as Antibacterial Agents.

Through the topical formulation, the medication must be able to permeate the skin as efficiently as feasible.

Three primary purposes serve topical formulations:

• To help hydrate skin thanks to its emollient properties.

• To heal a portion of skin that is healthy or injured, or to shield it from the elements.

• To apply medicine straight to the skin.

Advantages of Topical Drug Delivery System:

• Easy to use and convenient.

• Prevents the first-pass action, potentially avoiding liver and digestive system enzyme inactivation.

• By removing the application from the skin's surface, drug treatment can be swiftly discontinued.

• Preventing digestive compatibility problems.

• Providing medications with a limited biological half-life with a constrained therapeutic window.

• A rise in patient adherence.

• Lower doses compared to forms of oral treatment.

• Allow users to self-medicate.

• Achieving effectiveness by continuously ingesting medicine while using a lower total daily dose.

• Prevents variations in patient and between-patient medication levels.

• A significantly wider region than the nasal or buccal cavities for application.

Disadvantages of Topical Drug Delivery System:

• Dermatitis or skin irritation may be brought on by the medicine or its excipients.

• Some medicines' low skin permeability.

• It is more challenging to absorb medications with larger particles via the skin.

• The potential for allergic reactions.

• Only drugs with very low plasma concentration requirements for action may be utilised.

Antibacterial Properties of Copper nanoparticles:

Copper is a widely accessible metal and one of the important trace metals that may be found in the majority of living things. Among the many industrial applications for nanoscale copper are gas sensors, high temperature superconductors, solar cells, and wood preservatives. This metal has long been researched as a possible antibacterial substance. CuSO4 and Cu(OH)2 are two examples of the copper-containing materials used in conventional inorganic antibacterial agents. Aqueous copper solutions, intricate copper species, or copper-containing polymers are also used as antifungal agents.

The control of legionella in hospital water distribution systems using the copper and silver ionisation technique is one of the most common applications for this metal in the modern healthcare setting. It has been demonstrated that copper ions exert antibacterial effects on a wide range of bacteria, including Staphylococcus aureus, Salmonella enterica, Campylobacter jejuni, Escherichia coli, and Listeria monocytogenes.

The only metal with antibacterial properties, according to the American Environmental Protection Agency (EPA), is copper. Within two hours of contact, this chemical kills 99% of most diseases. This metal occasionally possesses features that are better than those of other expensive metals with antibacterial properties, such silver and gold. For instance, the Cu NPs showed a stronger antibacterial activity than the silver NPs when tested against E. coli and Bacillus subtilis (B. subtilis). Bacteria, yeast, and viruses can be removed from copper surfaces using a procedure known as "contact killing" (contact-mediated killing). Contact mortality from copper was reported to begin occurring at a rate of no less than seven to eight logs per hour after the prolonged incubation period. There were no microorganisms on the copper surfaces. Therefore, it is advised to utilise copper as a self-sanitizing substance.

Copper Bacterial Resistance:

As was mentioned in preceding sections, copper has been employed as an antibacterial agent in medicine. The most widespread copper-resistant bacteria were isolated from microorganisms related to people, pets, and plants. The mechanisms that various microorganisms maintain copper homeostasis vary. For instance, two chromosomally encoded systems called cue and cus control how resistant E. coli is to copper. The periplasmic protein CueP is a distinct component of the cue system that is vital in copper resistance. Some bacteria, notably E. coli, have the PCO (plasmid-borne copper resistance) system, which allows them to live in situations with high copper concentrations. Other Gram-negative bacteria, such as Yersinia pestis, Yersinia pseudotuberculosis, Yersinia enterocolitica, Citrobacter koseri, and Erwinia carotovora, include CueP-like proteins.

The Toxicity Mechanisms of Copper Nanoparticles against Bacteria:

One of the most well-known toxicity mechanisms for NPs is the contact between the bacterial cell membrane and NPs, which leads to the loss of the bacterial membrane integrity and eventually culminates in the death of the bacterium. The toxicity mechanism of Cu NPs has been shown to be enhanced by a variety of factors, including temperature, pH, bacteria, NP concentration, and aeration.

Cu nanoparticles have been shown to have antibacterial effects on the functioning of bacterial cells in a number of ways, including adhesion to the Gram-negative bacterial cell wall due to electrostatic interaction (Figure 1), altering the structure of the intracellular proteins, and interacting with phosphorus- and sulfur-containing materials like DNA. Additionally, a detailed investigation was conducted to look into the processes behind the antibacterial action of Cu NPs using the biological tool E. coli.

The results showed that treatment of E. coli cells with Cu-NPs at the minimum bactericidal concentration (MBC) increased the formation of reactive oxygen species (ROSs) in the cells by 2.5 times. Furthermore, a considerable amount of protein oxidation, DNA deterioration, and lipid peroxidation were caused by the NP-mediated increase in ROS level, which eventually led to cell death.

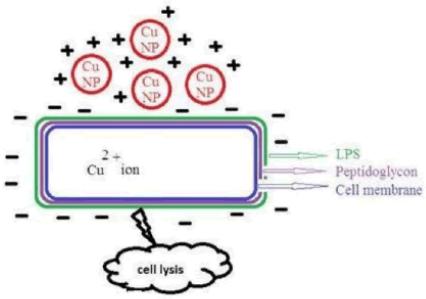


Figure 1: The antimicrobial activity of Cu NPs through the electrostatic interaction with the cell wall of gram-negative bacteria

Synthesis of Copper-based Nanoparticles:

Cu-based nanoparticles may be made using five different techniques: chemical processing, thermal processing, electrochemical synthesis, photochemical techniques, and sonochemical techniques. The "chemical treatment," which has been the most popular technique among them, has been utilised to create Cu-based NPs in some of the most innovative methods.Green synthesis has recently been used to produce eco-friendly NPs that don't produce any toxic waste. Green nanobiotechnology is a form of preparation technique that replaces conventional physical and chemical synthesis with secure biotechnological instruments. NPs are produced with this technique by using biological processes such as bacteria, fungus, plants, enzymes, or their byproducts like proteins.

The Copper Particles in Nano-scale as Antibacterial Agents:

In 2008, Ruparelia et al. investigated the antibacterial properties of copper and silver nanoparticles (NPs) on S. aureus, B. subtilis, and E. coli. The Cu NPs outperformed the silver particles in disc diffusion tests, minimum bactericidal concentrations, and minimum inhibitory concentrations (MICs) against B. subtilis. This is believed to be the result of the Cu NPs' higher affinity for the amines and carboxyl groups on the surface of B. subtilis.

Silver nanoparticles demonstrated a more potent antibacterial impact on S. aureus and E. coli than Cu nanoparticles did. Copper oxide (CuO) NPs have a novel applicability in antimicrobial applications, according to Ren et al. The metal oxide NPs were produced using the thermal plasma (Tesima TM) technology, which enables the continuous gas phase manufacture of bulk nano-powders. The produced CuO NPs in suspension were effective against a number of bacterial pathogens, including S. aureus, meticillin-resistant S. aureus, Staphylococcus epidermidis, E. coli, and Pseudomonas aeruginosa (P. aeruginosa), with MBCs ranging from 100 mg/ml to 5000 mg/ml.

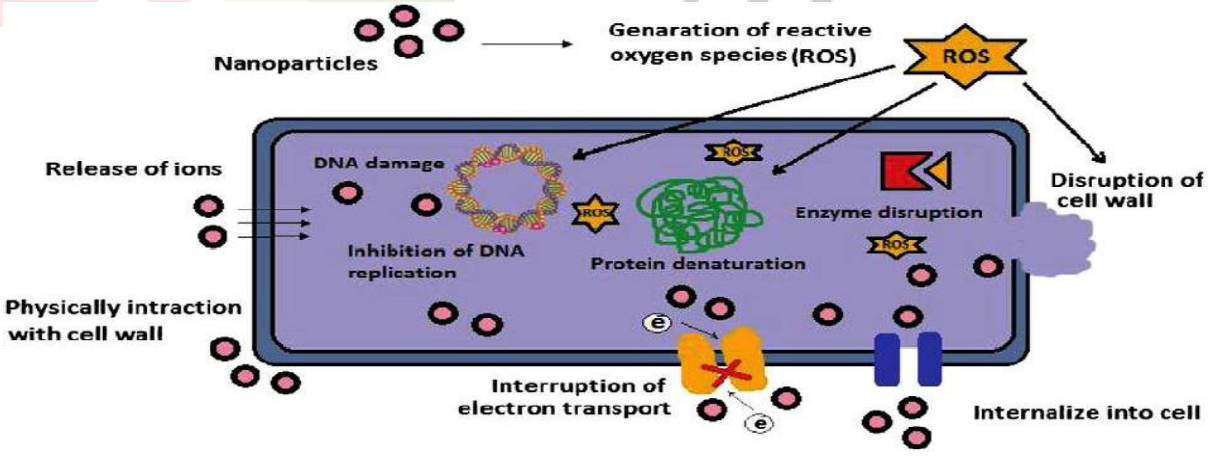
In 2010, Raffi and associates investigated the efficacy of employing Cu NPs as antibacterial agents against E. coli. Both liquid and solid growth media had their antibacterial activity assessed. Colony forming units (CFU) were utilised to determine the antibacterial potency of the generated NPs on solid medium. The behaviour of Cu NPs in liquid media was studied by measuring the optical density (OD) of various Cu NP concentrations at a predetermined wavelength. The results of both experiments on growth inhibition were very congruent. Scanning electron microscopy (SEM) pictures of Cu NPs-treated bacterial cells also demonstrated the

formation of pits and holes in their cell walls as well as a change in their typical rod-shaped appearance. The results also showed that the concentration of Cu NPs had an impact on how effective they were against bacteria; low concentrations only produced a delay in the lag phase, showing copper's role as a micronutrient for bacteria. On the other hand, at higher doses, they showed bacterial growth suppression.

The same year, a different study team tested the toxicity of aggregated zero valent Cu NPs (ZVCN) against E. coli using a centroid mixture type of experiment. Temperature, pH, aeration rate, NP concentration, and bacterial concentration were the five environmental parameters that were assessed since it was believed that they had a substantial influence on how damaging the NPs were to bacteria. According to their research, the examined factors' main and secondary effects both have a positive influence on lowering the toxicity of Cu NPs.

Overall, ZVCN will be most detrimental to nanoparticles in acidic conditions, higher temperatures, high aeration, and high concentrations of NPs and bacteria.

When one of the independent variables is changed, the toxicity of NPs is significantly influenced. Carbon nanotubes were employed by Mohan and colleagues to improve the antibacterial properties of Cu NPs. Synthesised Cu nanoparticles were grafted onto surfaces of multiwall carbon nanotubes (MWCNT). According to their research, the presence of carbon
nanotubes enhanced the surface area of Cu NPs, which led to a decrease in the number of E.
coli colonies in the Cu-MWCNT system as compared to the pure Cu-NPs and MWCNT. In
comparison to pure Cu NPs (52% 1.8), Cu-MWCNT was shown to have a higher antibacterial
effectiveness (% kill) against E. coli (75% 0.8). It is believed that Cu-MWCNT exerts its bactericidal activity



by the release of Cu ions, their entrance into the bacterial cells, and the subsequent disruption of metabolic processes. Cu-MWCNT can be used as a biocidal composite and antibacterial system coupled in biomedical devices.

In contrast to those that had undergone a chemical reduction procedure, Theivasanthi and Alagar discovered that Cu NPs created using the electrolysis approach exhibited a greater antibacterial activity against E. coli bacteria in a separate investigation. The use of electricity during the fabrication of Cu NPs enhanced their

antibacterial capabilities. Overall, the scientists argued that this material might be used for water purification, antimicrobial packaging, and air filtering. A novel polypropylene antibacterial agent with incorporated Cu metal or CuO NPs was evaluated by Delgado et al. Their study found that the type of Cu NPs utilised in composites had an impact on their ability to kill microbes. Because the CuO NPs did not need the creation of an oxide layer, leading to rapid ion release rates, it was shown that they had a stronger antibacterial impact than the Cu metallic NPs in killing E. coli. CuO NPs also included metal that was already oxidised, whereas Cu metal needed to first form an oxide layer. This resulted in the particle disintegration of the CuO NPs.

In 2012, Chatterjee and colleagues established a simple, dependable method for the generation of Cu NPs by reducing CuCl2 in the presence of the stabiliser gelatin. Instead of the usual cell size of roughly 2.5 m, NP treatment made E. coli cells filamentous, with an average filament size ranging from 7 to 20 m. The NPs were particularly powerful against E. coli even at considerably lower doses. Gram-positive B. subtilis and S. aureus, as well as an E. coli strain that was resistant to a number of drugs, were also demonstrated to be favourably impacted by the antibacterial capabilities of the generated NPs.

An aqueous solution of copper nanoparticles having bactericidal action against both gramme negative and gramme positive bacteria at nanomolar concentrations was made using starch as the green capping agent. In vitro studies on the 3T3L1 cells revealed that the capped NPs displayed cytotoxicity at far higher doses than the Cu ions. According to the results, the newly developed starch-capped water-soluble Cu NPs are a strong candidate for a number of applications, including photothermal treatment or cellular imaging.

In another noteworthy experiment, the antibacterial activity of CuO NP against Legionella pneumophila was investigated. A whole-genome microarray demonstrated that CuO NPs had a significant influence on the expression of genes involved in metabolism, transcription, translation, DNA replication and repair, pathogenicity, and unidentified/hypothetical proteins.

A separate study team showed that the antibacterial impact of CuO NPs was dependent on the particle size and that antibacterial activities were greatly boosted against both Gram-positive and -negative bacterial strains using the extremely stable minimum-sized monodispersed CuO NPs.

Thekkae Padil et al. used green technology to produce extremely stable CuO NPs by employing gum karaya, a naturally occurring polysaccharide component in plants. Lower particle size CuO NPs exhibited stronger antibacterial activity. The scientists noted that bed liner, active cotton bandages, and wound dressing are a few potential applications for CuO NPs, which may be produced utilising a simple, moderate, and environmentally friendly approach.

Thermal breakdown methods were used to produce CuO NPs, and their antioxidant and antibacterial properties were investigated. Their produced NPs demonstrated free radical scavenging activity up to 85% in 1 hour when compared to other metal oxide NPs. Additionally, P. aeruginosa and E. coli were successfully eradicated by

the CuO NPs' potent antibacterial activity. The amount of NPs significantly slowed bacterial development.

Usman and colleagues produced pure Cu NPs using chitosan polymer as a stabilising agent. They found that the chitosanstabilized NPs were effective against fungi like Candida albicans, as well as Gram-positive pathogens like methicillin-resistant S. aureus and B. subtilis and Gram-negative pathogens like Salmonella choleraesuis and P. aeruginosa and Gram-negative bacteria like B. subtilis and S. aureus. Additionally, the generated NPs demonstrated higher antibacterial activity against Gramnegative pathogens including P. aeruginosa compared to Grampositive microorganisms. Together, the researchers have developed a simple and affordable process for creating Cu nanoparticles that may one day be applied in medical and biological fields.

Subhankari and Nayak presented another fascinating Cu NP synthesis using a novel biological approach and ginger (Zingiber officinale) extract. It was shown that pure ginger extract was less effective against E. coli when compared to Cu NPs created using a green synthesis technique. The researchers made note of the fact that this method is safe for the environment, employs cheap, non-toxic materials, and may be useful for water purification, regulating air quality, and antimicrobial packaging.

Utilising aqueous Acalypha indica leaf extract, very stable CuO NPs were created utilising green chemistry. It was found that the particles formed were efficient against Candida albicans, Pseudomonas fluorescens, and E. coli. The MTT experiment revealed that they were also effective cytotoxicants against MCF-7 breast cancer cell lines.

Agarwala et al.'s studies assessed the antibiofilm activity of CuO and iron oxide NPs against multidrug resistant uropathogens that generate biofilms. CuO nanoparticles were shown to have dose-dependent antibiofilm properties and to be more toxic than iron oxide nanoparticles.
Hydrothermal approach was used by Giannousi et al. to manufacture Cu, Cu2O, and Cu/Cu2O NPs in a cost- and environmentally friendly way. Cu-based NPs caused substantial ds CT-DNA degradation as well as dose-dependent pDNA degradation. Additionally, Cu2O NPs showed a stronger antibacterial impact when used against Gram-positive pathogens. Thus, the potential reaction pathway was examined. The findings demonstrated lipid peroxidation and ROS generation.

In order to produce Cu NPs, Parikh and colleagues have also examined the use of green nanotechnology. Because Datura Meta leaf extract may reduce metal ions in NPs, it was used for biosynthesis. The following advantages of the recommended course of action: It is economical, quick, straightforward, simple, and eco-friendly. In tests against E. coli, Bacillus megaterium, and B. subtilis, it was shown that Cu NPs displayed better antibacterial activity than the extract did.

Tomasz and associates produced and investigated further nanostructured Cu in 2015. Copper nanoparticles evaluated against clinical methicillin-resistant S. aureus strains and other Gramme positive bacteria showed a potent antibacterial activity. It was shown that Cu NPs have substantially stronger antibacterial qualities than

Ag NPs. Additionally, the developed NPs demonstrated antifungal activity against species of candida. In order to avoid biofilms and reduce bacterial or fungal adherence at a lower cost than silver, the generated copper NPs can be used.

Cu NPs' ability to prevent P. aeruginosa from forming biofilms was investigated by Lewis Oscar et al. The authors proposed employing their recommended NPs as coating agents to prevent biofilm growth on surgical tools and medical devices since Cu NP treatments at 100 ng/ml caused a 94% drop in biofilm development.

In a separate study, the cytotoxicity of the generated CuO NPs in colon cancer cells was examined. CuO NPs were shown to inhibit the development of HT-29 human colon cancer cells by downregulating the apoptosis-controlling proteins Bcl-2 and Bcl-xL.

Cu NPs have drawn a lot of attention from researchers recently due to their antibacterial effect against diverse microorganisms. The ability of copper nanoparticles to act as strong antibacterial agents in biomedical and industrial applications is demonstrated by this.

Materials & Methods:

The list of materials utilized is shown below. The chemicals were made for laboratories.

Resources used in study

S. No.	MATERIAL	VENDOR
1.	Mupirocin	Anclaima Health Sciences Pvt. Ltd., India.
2.	Copper sulphate	Thermo Fisher Scientific Pvt. Ltd., Mumbai, India.
3.	Tri sodium citrate	Nice chemicals Pvt. Ltd., Mumbai, India.
4.	Propylene glycol	S.D. Fine Chem. Ltd., Mumbai, India.
5.	Methanol	LOBA CHEMIE Pvt. Ltd., Mumbai, India.
6.	Ethanol	LOBA CHEMIE Pvt. Ltd., Mumbai, India.
7.	Carbopol 940	Lubrizol advanced material Europe BVBA, Belgium.
8.	Methyl paraben	Lubrizol advanced material Europe BVBA, Belgium.
9.	Propyl paraben	Lubrizol advanced material Europe BVBA, Belgium.
10.	Triethanolamine	Nice chemicals Pvt. Ltd., Mumbai, India.
11.	Chloroform	LOBA CHEMIE Pvt. Ltd., Mumbai, India.
12.	Octanol	LOBA CHEMIE Pvt. Ltd., Mumbai, India.

13.	Acetone	LOBA CHEMIE Pvt. Ltd., Mumbai, India.
14.	Disodium hydrogen phosphate	S.D. Fine Chem. Ltd., Mumbai, India.
15.	Ether	LOBA CHEMIE Pvt. Ltd., Mumbai, India.
16.	Sodium hydroxide	S.D. Fine Chem. Ltd., Mumbai, India.
17.	Hexane	LOBA CHEMIE Pvt. Ltd., Mumbai, India.
18.	n-Propanol	S.D. Fine Chem. Ltd., Mumbai, India.
19.	Hydrochloric acid	RANKEM laboratory reagent, NEW DELHI.

INSTRUMENTS:

List of apparatus and equipment's used in research work

S. NO.	EQUIPMENTS	VENDOR
1.	Electronic single pan balance	Danwer scale
2.	Digital pH meter	Eutech instruments
3.	Hot air oven	Shivaki equipment
4.	UV-Visible spectrophotometer	Shimadzu (UV-1700)
5.	Micro centrifuge	Hi-con Pvt. Ltd.
6.	Homogenizer	Elektrocraft India Pvt. Ltd.
7.	Magnetic hot plate	Scope Enterprises
8.	Magnetic stirrers	Scope Enterprises
9.	FT-IR spectrometer	Bruker
10.	Refrigerated centrifuge	Eltak
11.	Franz diffusion cell	MGI Borosilicate Glass
12.	Brookfield viscometer	Fungilab expert
13.	Separating funnel	Borosil Glass Works Ltd.
14.	Vials	Borosil Glass Works Ltd.
15.	Stability chamber	Scope Enterprises
16.	Optical microscope	Medico

17.	Other glassware	Borosil Glass Works Ltd.
18.	Melting point apparatus	Scope Enterprises

MUPIROCIN:

Pharmacokinetic

Absorption: When mupirocin was applied to the lower arm of healthy male volunteers and then their arm was occluded for 24 hours, there was no detectable systemic absorption (less than 1.1 nanograms of mupirocin per millilitre of whole blood). These participants' stratum corneum still contained detectable radioactivity 72 hours following the treatment.

Metabolism: Mupirocin is rapidly broken down after intravenous or oral administration. Monic acid, the main metabolite, has no antibacterial properties.

Excretion: Renal excretion is the main method of mononic acid elimination.

Elimination: The elimination half-life of mupirocin after intravenous treatment was 20 to 40 minutes for mupirocin and 30 to 80 minutes for monic acid in an experiment with 7 healthy adult male volunteers.

Microbiology: Mupirocin is an antibacterial RNA synthetase inhibitor made via fermentation with the Pseudomonas fluorescens bacterium. When used topically, mupirocine can reach quantities that are bactericidal. The influence of wound secretions on the minimum inhibitory concentrations (MICs) of mupirocin has not been established, despite the fact that mupirocin is heavily protein bound (more than 97%).

Mechanism of Action:

Mupirocin suppresses the production of bacterial proteins, RNA, and cell walls in S. aureus by binding to the isoleucyl-transfer RNA synthetase (Ile-tRNAsynthetase) enzyme in a reversible and specific manner. Mupirocin was shown to have little to no inhibitory effects on other tRNA synthetases while being a powerful inhibitor of Ile-tRNA synthetase. By forming an enzyme isoleucyladenylate complex, ile-tRNA synthetase catalyses the synthesis of isoleucyl-tRNA in two distinct steps. Mupirocin does not affect the transfer of isoleucine from the enzyme-Ile-AMP complex to tRNA but competes with isoleucine to impede the development of the enzyme-Ile-AMP complex. The affinity of mupirocine for mammalian Ile-tRNA synthetase is significantly diminished.

Mechanism of Resistance

The creation of a modified isoleucyl-tRNA synthetase or the genetic transfer of a plasmid mediating a new isoleucyl-tRNA synthetase are the two causes of mupirocin resistance. There have been more reports of high-level plasmid-mediated resistance (MIC 512 mcg/mL) in S. aureus isolates, and this resistance is more common in coagulase-negative staphylococci. Methicillin-resistant staphylococci are more likely than methicillin-susceptible ones to exhibit mupirocine resistance.

Cross Resistance

Mupirocin does not exhibit cross resistance with other kinds of antimicrobial drugs because of the way it works.

Antimicrobial Activity

Mupirocin has been demonstrated to be effective against S. aureus and S. pyogenes isolates that are sensitive. The following in vitro data are available, however it's unclear what they mean clinically. The majority of Staphylococcus epidermidis isolates are susceptible to mupirocine.

Dosage and Administration

The location of the superficial skin infection should be treated topically with 2% mupirozin ointment. In most patients, the dosing schedule should be 2 or 3 times daily for 5 to 14 days, while chronic or more severe conditions may require prolonged use.

Clinical use

Ecthyma, impetigo, folliculitis, and furunculosis. Mupirocin can be used to avoid bacterial contamination of tiny wounds, incisions, and other clean lesions as well as to stop infection of abrasions, cuts, and other minor wounds.

Adverse effect

Headache, redness, nausea, stomach discomfort, and burning at the application site are adverse reactions.

RESULTS & DISCUSSION

The primary goal of the current research was to use chemical reduction to create a gel of copper nanoparticles. Under transmission electron microscopy (TEM), spherical nanoparticles with smooth surfaces were discovered during characterisation. CuNPs' zeta potential, whose negative charge shows the presence of strong electric charges on the particle surfaces that prevent agglomeration, gives adequate proof of the particles' low inclination to aggregate. When compared to the typical copper powder diffraction peaks, the X-ray diffraction pattern of the synthesised nanoparticles revealed diffraction peaks at 2, indicating the purity of the copper. The particle size characterization studies supported the viability of the synthesis of copper nanoparticles in the growth media and gave us an idea of the size and shape of the particles. Zone of inhibition formulation shown increased action for S. aureus, per the findings of antibiotic tests.

The tests conducted in the previous part serve as the foundation for the results and discussion.

Preformulation Studies:

Organoleptic Properties:

The following drug qualities were assessed, and the findings are as follows:

Organoleptic Properties of Mupirocin

Test	Specification	Observation
Colour	White	White
Odour	Odourless	Odourless
Appearance	Crystalline powder	Crystalline powder

To authenticate the identification of the medicine, the observed observations were compared to the pharmacopoeia's standards, and it was discovered that the observations met the requirements.

Solubility analysis-

To ascertain a drug's solubility in various solvents, solubility experiments are carried out. The ratio of the solute to the solvent is used to express solubility.

Mupirocin was discovered to be soluble in chloroform, methanol, acetone, ethanol, distilled water, and PBS with pH values of 5.5, 6.8, and 7.4.

Solubility profile of mupirocin

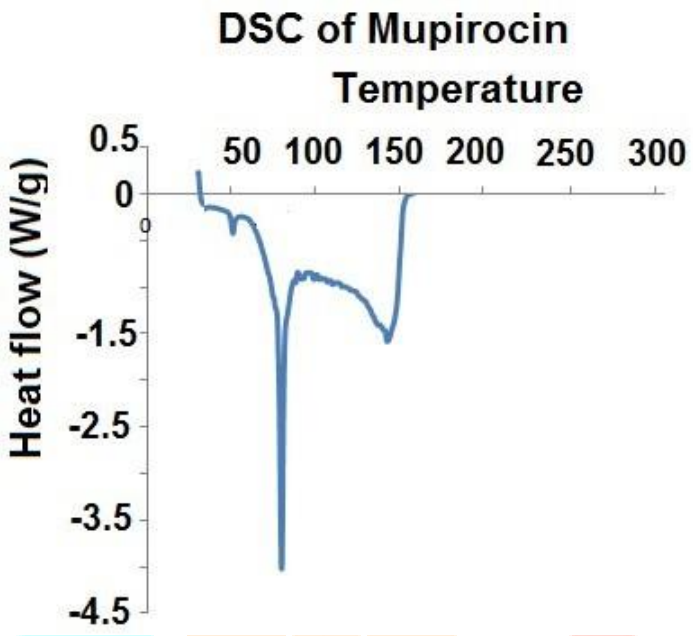
Qty. of drug	Solvent	Qty. of solvent	Inference
500 mg	Methanol	5 ml	Freely soluble
500 mg	Acetone	5 ml	Freely soluble
500 mg	Chloroform	5 ml	Freely soluble
100 mg	Ethanol	10 ml	Sparingly soluble
100 mg	0.1 N HCl	10 ml	Sparingly soluble
10 mg	Distilled water	10 ml	Slightly soluble
10 mg	PBS pH 6.8	10 ml	Slightly soluble
10 mg	PBS pH 7.4	10 ml	Slightly soluble

Melting point determination-

Mupirocin's melting point was discovered to be 78 0C. Three melting points were tested, and the average was recorded. At 780C, a quick change from solid to liquid occurred, demonstrating the sample's purity and lack of contaminants.

Differential scanning calorimetry-

The DSC thermograms displayed a prominent endothermic peak that was in line with the mupirocin melting point of 77.31°C. The mupirocin DSC thermograms show



DSC thermograms of mupirocin

Determination of Partition coefficient-

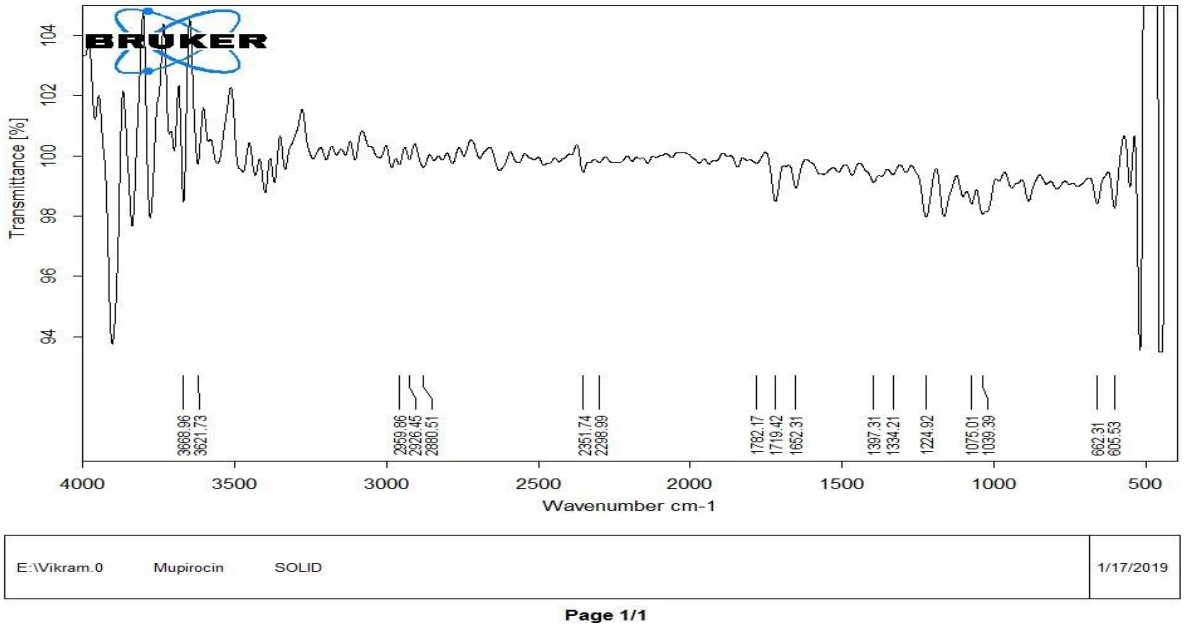
The partition co-efficient was calculated from three separate measurements. Therefore, the partition coefficient of 2.45±0.03 indicates that the medication is lipophilic.

Determination of pH of drug-

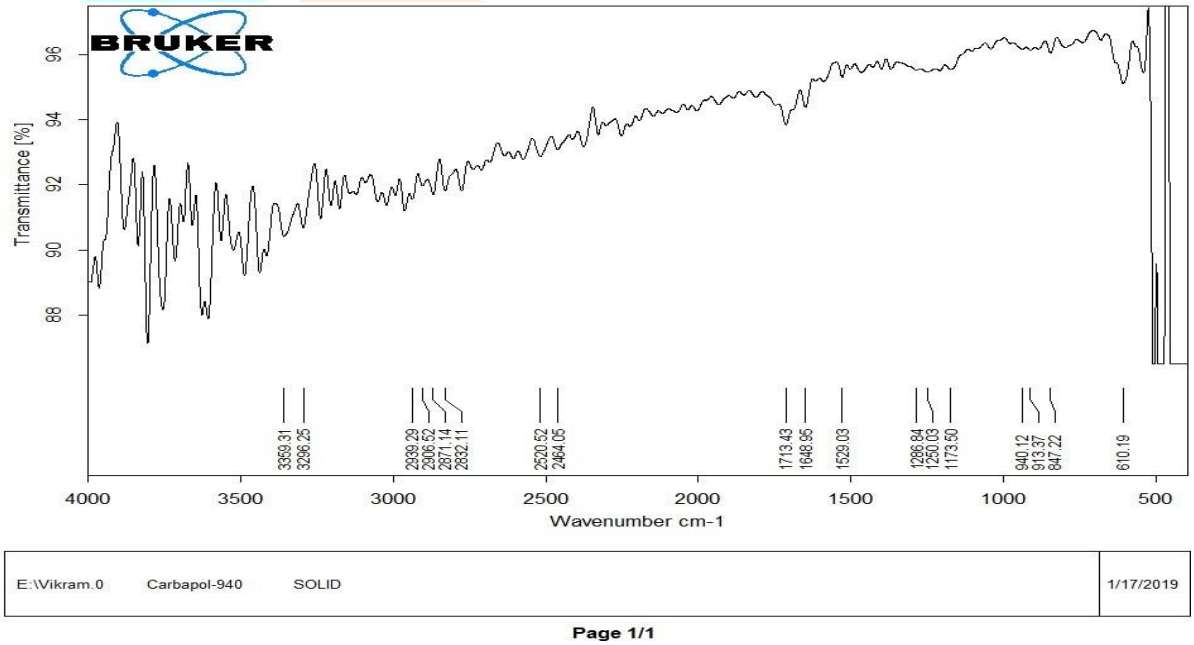
Three pH readings were taken, and the average was recorded. Mupirocin's pH was thus determined to be 4.1.

FTIR analysis-

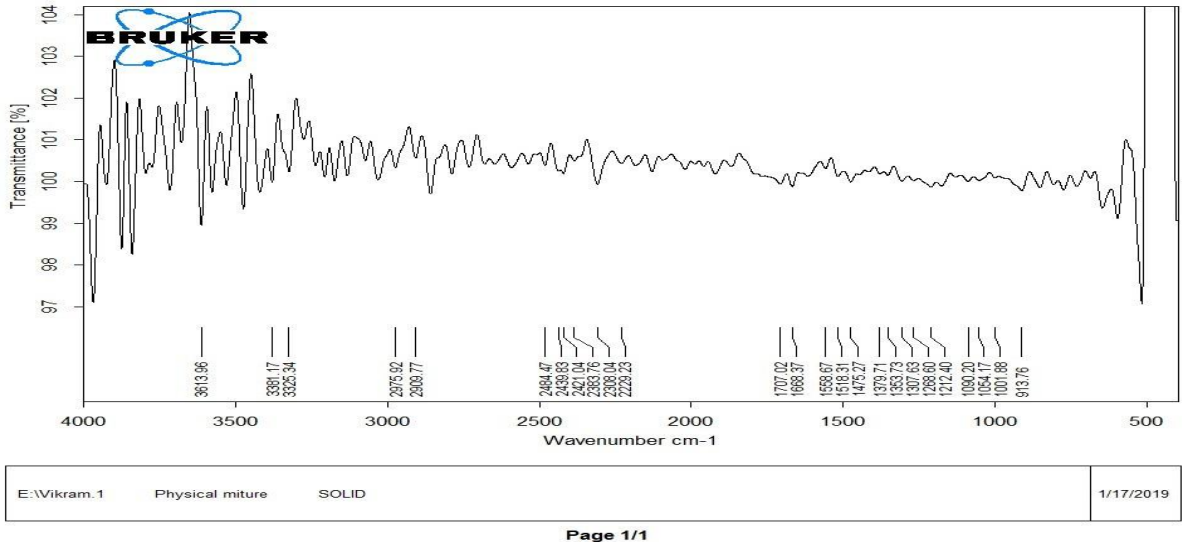
Drug characterization was done using FTIR spectroscopic analysis. The resulting FTIR spectra were compared to the mupirocin pharmacopoeia-recommended spectrum. Fingerprint areas and diagnostic peaks were discovered to be similar. These characteristic peaks are helpful in drug detection. For medication compatibility investigations, FTIR was performed on carbopol 940 and a combination that included mupirocin. The findings demonstrated that, when considered collectively, there are no interactions between the elements.



FTIR of Mupirocin



FTIR of Carbopol 940



FTIR of Physical mixture

The drug sample was genuine and devoid of any significant types of contaminants because all the groups were present at the same value.

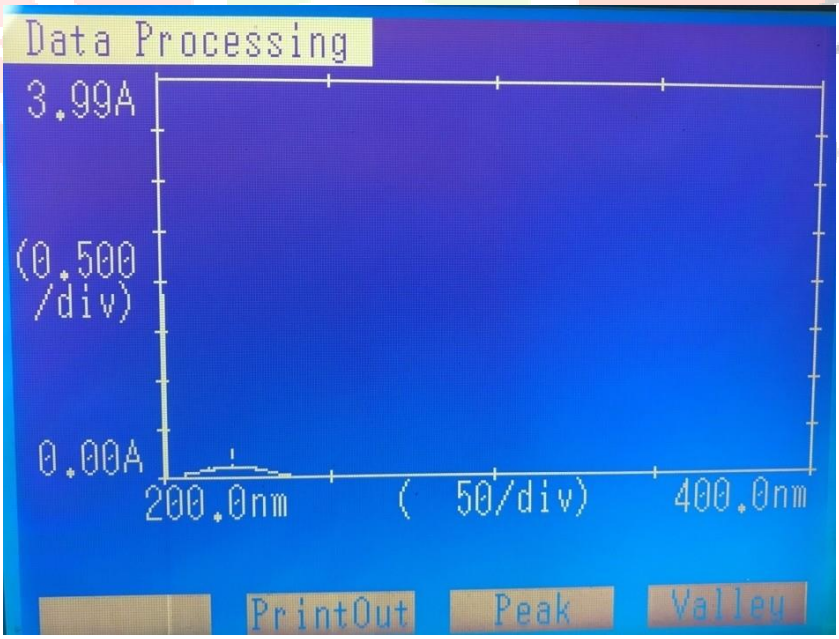
Comparison between peaks obtained in drug and in mixture

Peak obtained in drug (frequency cm^{-1})	Description	Peak obtained in mixture (frequency cm^{-1})
3621.73	OH group	3613.96
2959.86	C-H stretch of CH_3	2975.92
2351.74	C=O stretch of carboxylic acid	2383.76
1397.31, 1334.21	C-H bending of CH_3, CH_2	1379.71, 1307.63
1075.01	C=O stretch of ether	1090.20

Analysis by UV-Visible spectrophotometry

Preparation of standard graph

Stock solution of Mupirocin: By combining 100 mg of mupirocin with 100 ml of methanol, a stock solution containing 100 g/ml was created. Through the use of a UV spectrophotometer, dilutions between 10 and 100 g/ml were scanned to determine the maximum between 200 and 400 nm. The maximum was discovered to be at 220 nm for mupirocin.

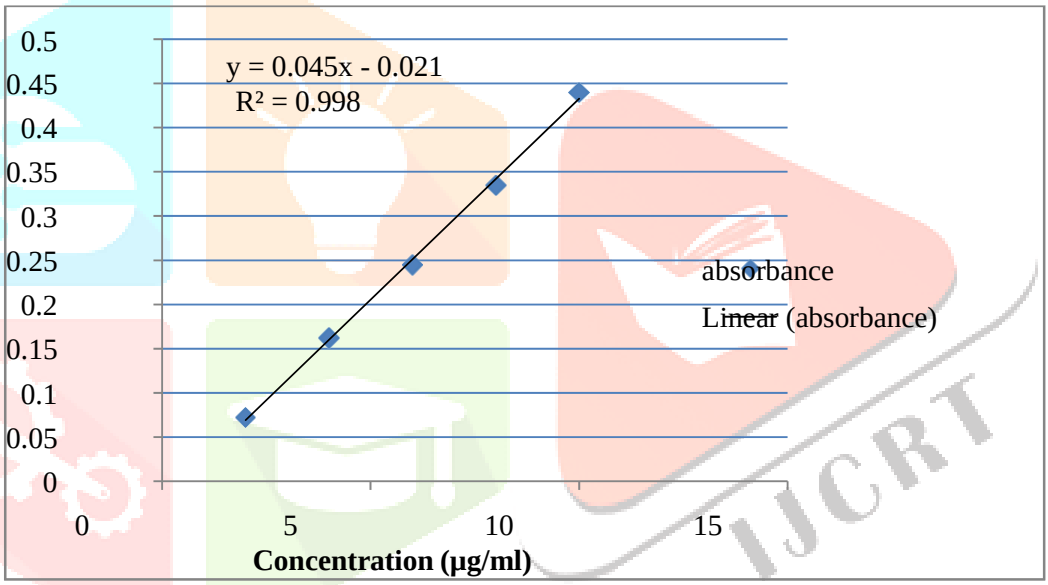


Standard Curve of Mupirocin

Preparation of calibration curve in methanol: From this solution of conc. 2 µg/ml, 4 µg/ml, 6µg/ml, 8 µg/ml, 10 µg/ml were prepared.

Absorbance different dilutions of drug at 220 nm in methanol

S.NO.	Conc. (µg/ml)	Abs.
1	2	0.072
2	4	0.162
3	6	0.245
4	8	0.335
5	10	0.440



Standard calibration curve of mupirocin at 220 nm.

EVALUATION OF NANOPARTICLES

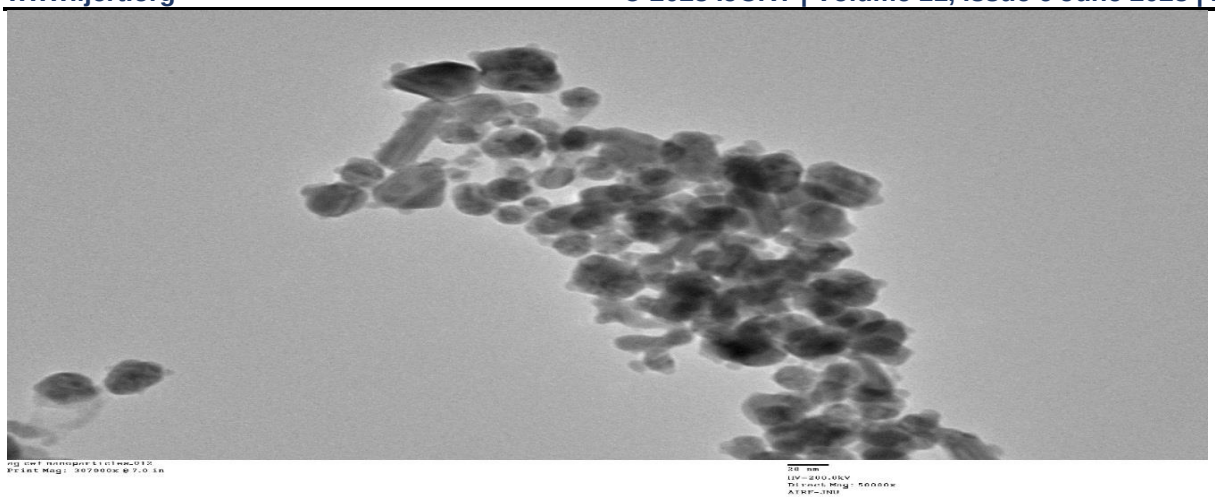
Drug entrapment efficiency

Drug entrapment efficiency was calculated as by formula:

$$\% \text{ Entrapment efficiency} = \frac{\text{Amount of entrapped drug recovered}}{\text{Total amount of drug}} \times 100$$

% E.E. was found to be 65.2%

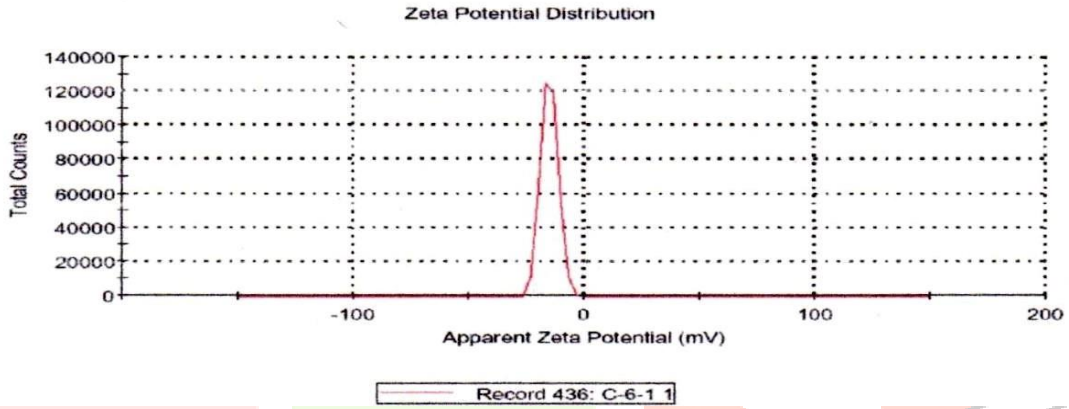
Transmission electron microscopy (TEM): Formulation was determined to be the optimal formulation, thus it was put through a TEM to produce the image of nanoparticles below at a magnification of 13.0 x 4000 with a scale bar of 200 nm. Under transmission electron microscopy (TEM), spherical, unilamellar vesicles with a smooth surface were discovered.



TEM of Copper nanoparticles

Zeta potential measurement:

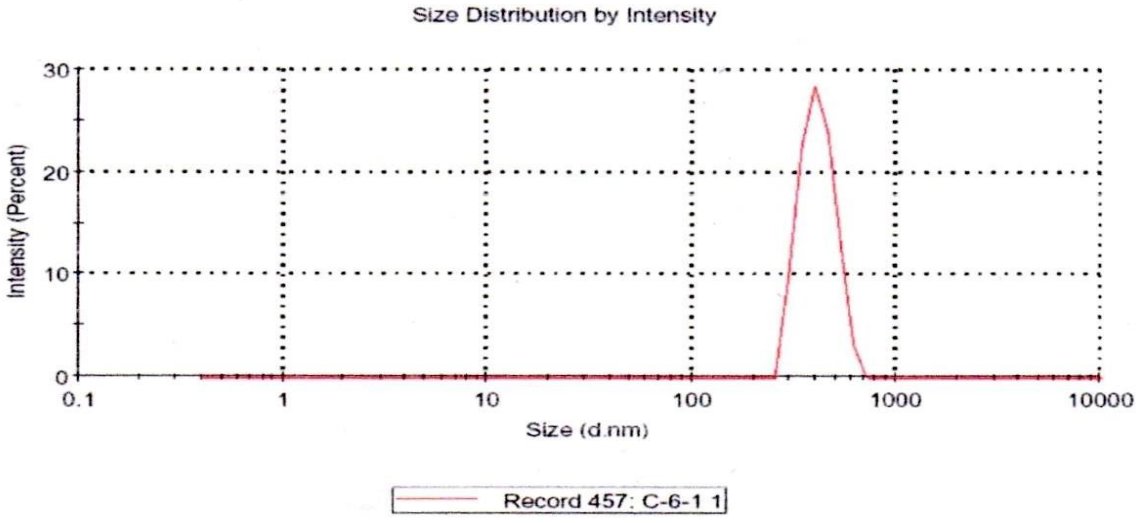
Zeta potential (mV): - 15.1 ± 3.69 mV



Zeta potential Copper nanoparticles

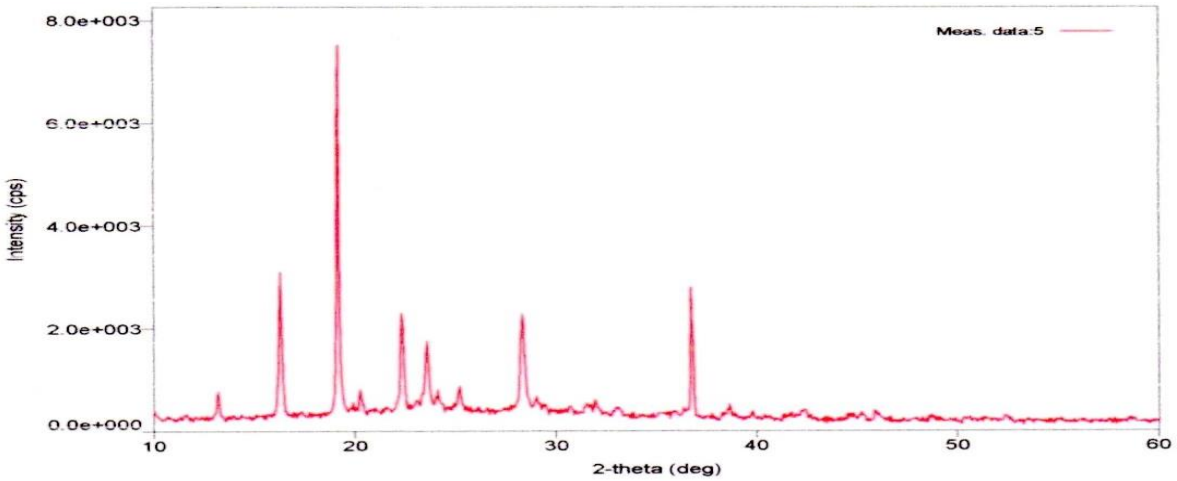
Particle size measurement:

Z- Average (d.nm): 413.0± 30.2nm



Particle size Copper nanoparticles

X- ray diffraction (XRD)



X-ray diffraction of Copper nanoparticles

X-ray diffraction pattern reveals that Cu nanoparticles exhibit crystalline structure.

PHYSICAL EVALUATIONS OF NANOPARTICLE GEL

The nanoparticle gel formulation of mupirocin was evaluated for the following:

Organoleptic characteristics

Colour = light pale

Odour = characteristic

Appearance = translucent

Phase separation = no

Occlusiveness = yes

Washability = washable

Determination of pH of gel base and nanoparticle gel-

The freshly made nanoparticle gel's pH was discovered to be 7.1.

Viscosity

By using a Brookfield viscometer, it was discovered that the viscosities of the carbopol 940 gel base and nanoparticle gel were 73,200 and 72,300 cP, respectively.

Spreadability

Nanoparticle gel's spreadability was determined to be 13.29 g.cm2/sec. The spreadability data demonstrated that nanoparticle gel was more efficient, i.e., it performed best.

Extrudability study

Positive results were observed for the nanoparticle gel's extrudability.

Percentage yield

It was discovered that the nanoparticle gel had a 95.78% yield.

Homogeneity and grittiness

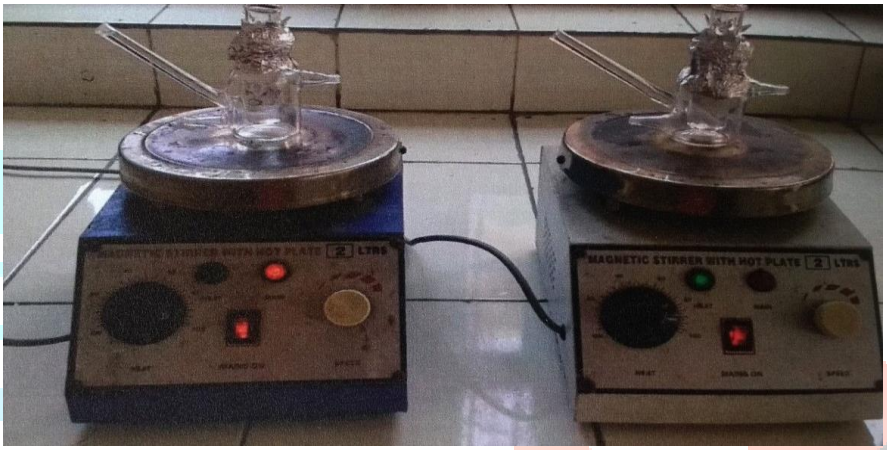
The nanoparticle gel was discovered to be homogenous and without roughness.

Copper content determination

5.9 micrograms of copper were found in every milligram of nanoparticles.

In vitro release study

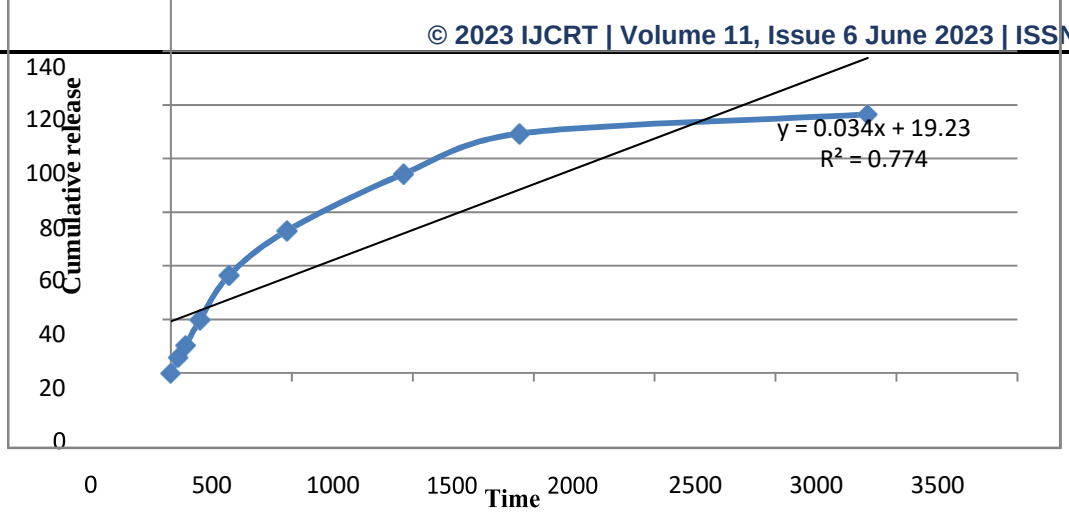
To measure the amount of medicine released at various intervals of time, an in vitro release research was carried out.



Franz diffusion cell for drug release study

Release of drug from formulation

Time (min.)	% cumulative release of mupirocin gel in 7.4 pH PBS
0	0
30	5.7
60	10.1
120	19.8
240	36.3
480	53.1
960	74.2
1440	89.3
2880	96.5



Release of drug from formulation in PBS at pH 7.4

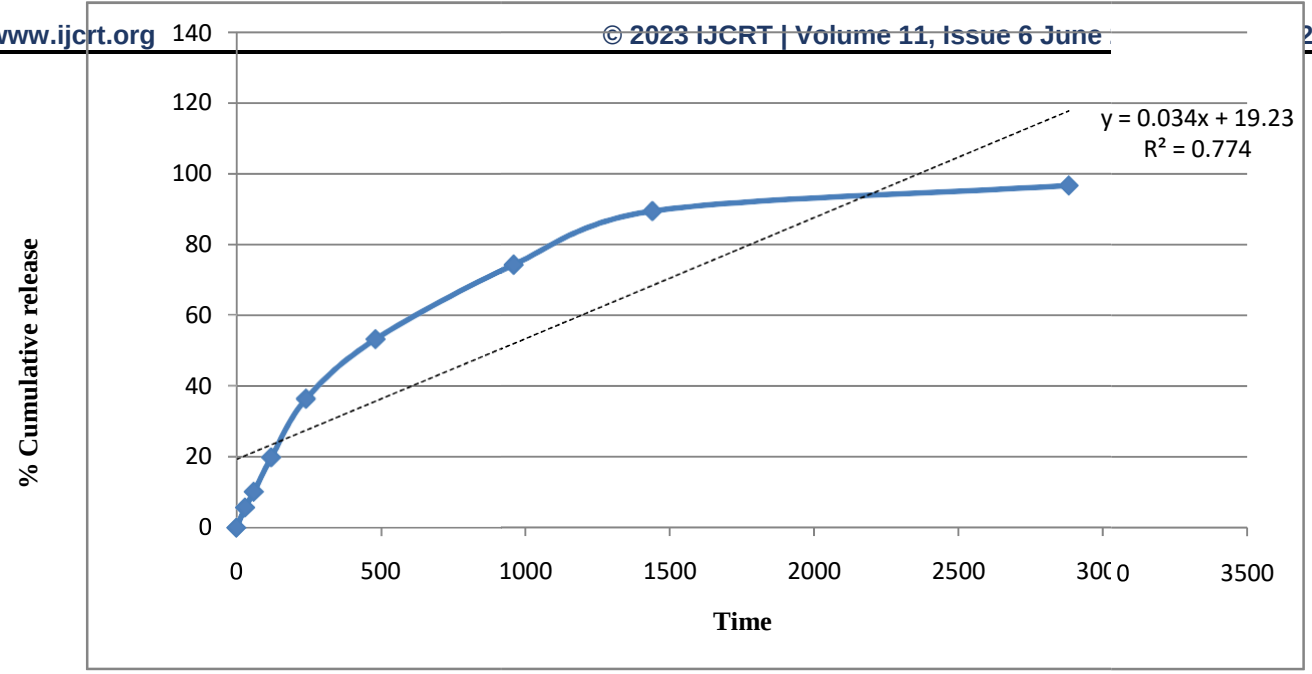
Kinetics of drug release

Different kinetic models were used to analyse the release kinetics of the pH 7.4 formulation in PBS. The information below was gathered.

Drug release data of formulation in PBS at pH 7.4

Time (min.)	Log time	Square root of time	% cumulative release of formulation	Log% cumulative release of formulation	% cumulative remaining	Log% cumulative remaining
0	0	0	0	0	100	2
30	1.48	5.48	5.7	0.76	94.3	1.97
60	1.75	7.75	10.1	1.00	89.9	1.94
120	2.08	10.95	19.8	1.29	80.2	1.90
240	2.38	15.49	36.3	1.56	63.7	1.80
480	2.68	21.91	53.1	1.73	46.9	1.66
960	2.98	30.98	74.2	1.86	25.8	1.40
1440	3.15	37.95	89.3	1.94	10.7	1.03
2880	3.46	53.67	96.5	1.97	3.5	0.58

ZERO ORDER PLOT

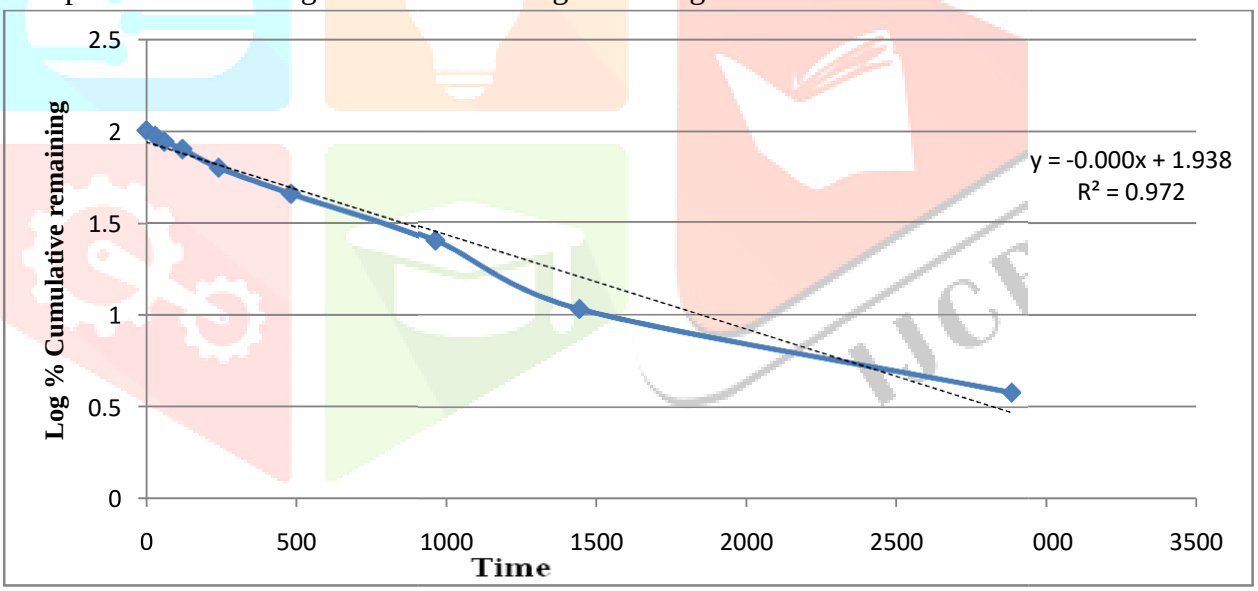


Graph was plotted between % cumulative drug release Vs time

Zero order plot for drug release kinetics of formulation in PBS at pH 7.4

FIRST ORDER PLOT

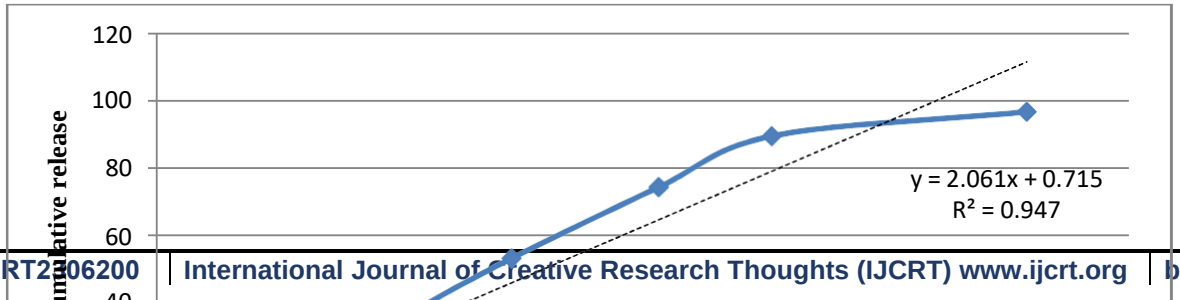
Graph was plotted between log % cumulative drug remaining Vs time



First order plot for drug release kinetics of formulation in PBS at pH 7.4

HIGUCHI'S MODEL

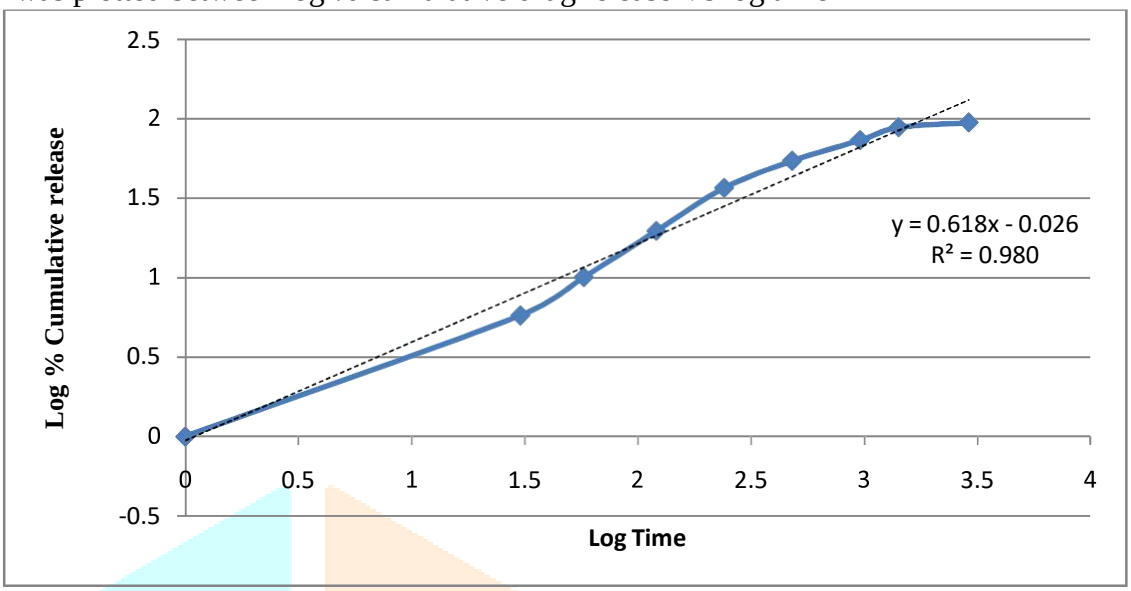
Graph was plotted between % cumulative drug release Vs square root of time



Higuchi plot for drug release kinetics of formulation in PBS at pH 7.4

KORSMEYER-PEPPAS MODEL

Graph was plotted between log % cumulative drug release Vs log time



Peppas plot for drug release kinetics of formulation in PBS at pH 7.4

Kinetics of drug release of formulation in PBS at pH 7.4

Plot	K_0	R^2
Zero order	0.077	0.774
First order	0.000	0.972
Higuchi	4.75	0.947
Peppas	1.41	0.980

Equations for the zero order, first order, Higuchi, and Korsmeyer Peppas release models were fitted with the data from the in vitro release experiments. The significance of the derived regression coefficients served as the foundation for data interpretation.

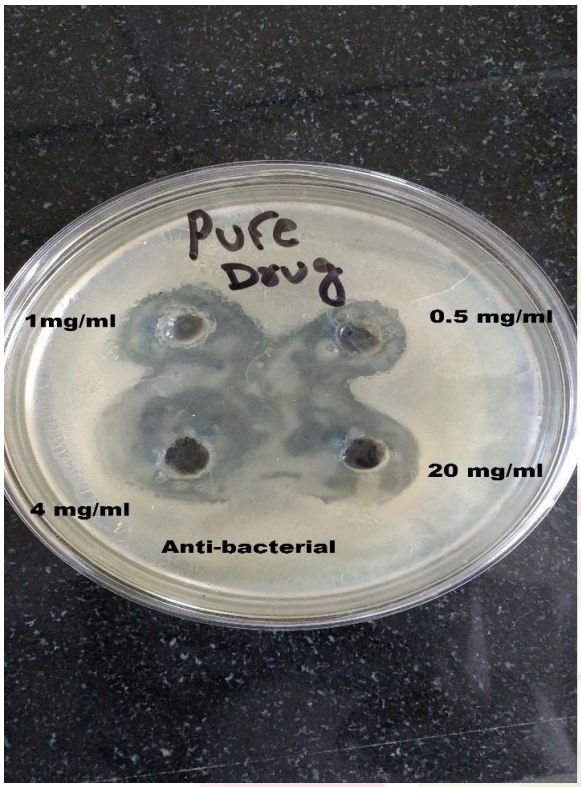
With an R2 value of 0.980, it was determined from these results that the Peppas model was the most appropriate.

Antimicrobial activity

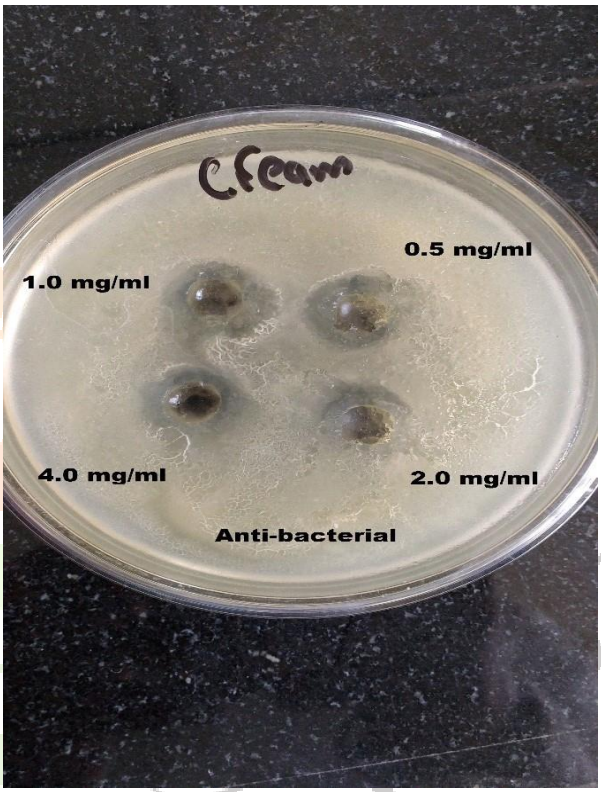
On S. aureus, antimicrobial activity was assessed using the cup-and-plate technique.

Antimicrobial activity by cup-plate method

Concentration (µg/ml)	Pure Mupirocin	Formulation
0.5	9 ± 1 mm	6± 2 mm
1	12 ± 2 mm	9± 2 mm
4	17 ± 1 mm	13 ± 3 mm
20	18± 3 mm	17 ± 1 mm
30	18± 2 mm	18 ± 2mm
40	19± 2 mm	18 ± 1 mm
MIC	20 µg	30 µg



Antibacterial activity of pure drug mupirocin



Antibacterial activity of copper nanoparticle gel containing mupirocin

Future Perspectives:

Antibiotics are effective in treating the majority of bacterial infections; nevertheless, the development of microbial resistance reduces the benefits of antibacterial medications in the treatment of infectious diseases. Metallic nanoparticles have proven to be effective antibacterial agents against a range of bacterial species, including fungi and Gram-positive and -negative bacteria. For use in treating microbiological infections, copper nanoparticles have lately been the topic of substantial investigation. "Nanoparticles" (NPs) are solid colloidal particles having a size between one and 1000 nm (one micron), according to the Encyclopaedia of Pharmaceutical Technology. Among the many industrial applications for nanoscale copper are gas sensors, high temperature superconductors, solar cells, and wood preservatives. Additionally, the generated NPs demonstrated higher antibacterial activity against Gramnegative pathogens including P. aeruginosa compared to Grampositive microorganisms. Together, the researchers have developed a simple and affordable process for creating Cu nanoparticles that may one day be applied in medical and biological fields.

Discussion:

The creation of a gel of copper nanoparticles by chemical reduction was the main objective of the current study. Spherical nanoparticles with smooth surfaces were observed during characterization under transmission electron microscopy (TEM). The lack of agglomeration is sufficiently demonstrated by the zeta potential of CuNPs, whose negative charge indicates the presence of significant electric charges on the particle surfaces. The X-ray diffraction pattern of the synthesised nanoparticles displayed diffraction peaks at 2, demonstrating the purity of the copper, when compared to the conventional copper powder diffraction peaks. The copper nanoparticle production in the growth media was supported by the particle size characterization assays, which also provided information on the size and shape of the particles.

Conclusion:

The present work aims to develop a topical copper nanoparticle gel containing mupirocin and use the potential of Cu NPs as a carrier to enhance the action of the medicine. We synthesized and assessed the Cu NPs containing mupirocin in order to develop an optimal formulation with enhanced antibacterial activity that is suitable for topical application. Gramme (+ve) bacteria like Staphylococcus and others cause infections, which are treated with mupirocin. But one of the biggest problems with mupirocin is the development of drug resistance. The Cu NPs formulation changes the chemical structure of mupirocin, making it effective against resistant bacteria. A chemical reduction process was used to create the mupirocin-containing Cu NPs, and they were then evaluated. The formulation is based on Peppa's R2 value notion. The production and evaluation of a copper nanoparticle gel containing mupirocin using a chemical reduction process. developed an optimised formulation, estimated drug release, and investigated kinetic models that complied with the formulation. Mupirocin-containing Cu NPs-based gel beat pure medicine in its capacity to treat S. aureus because to its prolonged release.

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