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Formulation And Characterization Of Copper Nanoparticle-Based cream To Enhance Wound Healing.

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Abstract:

The use of nanoparticles in medical applications has gained significant attention due to their unique properties, such as increased surface area, high reactivity, and the ability to interact at the cellular level. This study focuses on the formulation and characterization of a copper nanoparticle-based cream aimed at enhancing wound healing. Copper nanoparticles (CuNPs) are known for their antimicrobial, anti-inflammatory, and tissue regeneration properties, making them ideal candidates for wound healing applications. The copper nanoparticles were synthesized using a green synthesis method, ensuring biocompatibility and environmental safety. The formulated cream was characterized for its physicochemical properties, including viscosity, pH, and stability. Additionally, the size, shape, and surface morphology of the copper nanoparticles were analyzed using techniques such as transmission electron microscopy (TEM) and dynamic light scattering (DLS). The antimicrobial activity of the cream was evaluated through in vitro tests against common wound pathogens, while its cytotoxicity and biocompatibility were assessed using human fibroblast cell lines. The wound healing potential was investigated in an ex vivo model, demonstrating accelerated wound closure and improved tissue regeneration. The results indicate that copper nanoparticle-based creams can significantly enhance wound healing by promoting faster tissue repair, reducing infection risk, and minimizing inflammation. This formulation presents a promising approach for the development of advanced wound care therapies.

Keywords: Copper nanoparticles, wound healing, nanoparticle-based cream, green synthesis, antimicrobial activity.

1. INTRODUCTION

Wound healing is a complex and dynamic process that involves the coordinated interaction of various cellular, biochemical, and physiological mechanisms. However, delayed or impaired wound healing remains a significant clinical challenge, often leading to chronic wounds that are susceptible to infection and tissue damage. Despite advances in wound care products, there is a continuous need for innovative approaches to accelerate the healing process, reduce infection rates, and promote tissue regeneration. In recent years, nanotechnology has emerged as a promising field in medicine, offering novel solutions for wound care through the application of nanoparticles (NPs)[1-5].

Among the various types of nanoparticles, copper nanoparticles (CuNPs) have garnered considerable attention due to their unique properties. Copper is an essential trace element in the body, playing a critical role in collagen synthesis, angiogenesis, and the regulation of inflammation—all of which are fundamental processes in wound healing. CuNPs exhibit several beneficial characteristics, such as antimicrobial activity, anti-inflammatory effects, and the ability to enhance tissue regeneration, making them ideal candidates for developing wound healing agents [6-10].

Incorporating CuNPs into topical formulations, such as creams, offers a promising strategy to deliver these therapeutic effects directly to the site of injury. Moreover, CuNPs can be synthesized using environmentally friendly methods, ensuring biocompatibility and reducing potential side effects [11-16]. This study aims to formulate a copper nanoparticle-based cream and evaluate its potential to enhance wound healing. The formulation will be characterized for its physicochemical properties, and its antimicrobial activity, cytotoxicity, and efficacy in promoting wound closure will be assessed. By leveraging the unique properties of CuNPs, this study seeks to contribute to the development of advanced wound healing therapies that offer faster recovery, reduced infection, and improved tissue regeneration.

2. MATERIALS AND METHODS

2.1. Material used List of Chemicals:

Curcumin, Erythro Pharma Pvt Limited. Talab Katta, Hyderabad., Ethanol, Labogens, Ludhiana, Punjab, PEG4000, glycerin, Trietholamine Himedia, Plot No.C40, Road No.21Y, MIDC Wagle Industrial Estate, Thane, Maharashtra, Stearic acid, Qualikems Fine Chemicals Pvt. Ltd, Sadar Thana Road, Delhi-06,, Cetyl alcohol, Central Drug House, New Delhi, India, Liquid paraffin, Qualikems Fine Chemicals Pvt. Ltd, Sadar Thana Road, Delhi-06, Isopropyl myristate, Himedia, PlotNo.C40, RoadNo.21Y, MIDC Wagle Industrial Estate, Thane, Maharashtra, Methylparaben, Qualikems Fine Chemicals Pvt. Ltd, Sadar Thana Road, Delhi-06, Tween 80, Qualikems Fine Chemicals Pvt. Ltd, Sadar Thana Road, Delhi-06.

2.2 Methods of preparation of Cream

Preparation of topical Cream

1. **Preparation of Oil Phase:** In a beaker, combine the oil phase ingredients: 4 g of beeswax, 2 g of cetyl alcohol, 5 g of stearic acid, 10 g of mineral oil, and 5 g of isopropyl myristate. Heat the mixture in a water bath to 70-75°C until all the components are completely melted and mixed.

2. **Preparation of Water Phase:** In another beaker, combine the water phase ingredients: 5 g of glycerin, 60 g of distilled water, and 2 g of triethanolamine. Heat the mixture to 70-75°C.

3. **Emulsification:** Slowly add the heated water phase to the heated oil phase while continuously stirring. This can be done using a magnetic stirrer or a high-shear mixer. Add 5 g of Polysorbate 80 to the mixture and continue stirring to form a stable emulsion.

4. **Incorporation of Curcumin-Loaded Copper Nanoparticles:** Gradually add 1 g of the prepared curcumin-loaded copper nanoparticles to the cream base. Ensure uniform distribution by stirring continuously.

5. **Cooling and Homogenization:** Continue stirring the mixture while allowing it to cool to room temperature. This will help to achieve a smooth and uniform cream consistency. If using a preservative (e.g., 0.1 g of methylparaben), add it during the cooling phase and stir well.

6. **Addition of Fragrance (optional):** Add a few drops of fragrance if desired and mix thoroughly.

7. **Final Product:** Transfer the cream into suitable containers.

8. **Determining the Physicochemical Properties of Nanoparticles: -**

In this work, we aim to develop a smart drug delivery system consisting of Curcumin loaded with copper nanoparticles entrapped in and a Cream-based delivery system for enhancing the wound healing process.

Preparation of Copper Nanoparticle via Chemical Reduction

Procedure

1. Preparation of copper salt solution:

Dissolve an appropriate amount of copper (II) sulphate pentahydrate in deionized water

2. Addition of stabilizer: -

Add a small amount of stabilizer like PVA to copper salt solution to prevent nanoparticle aggregation. Stir the mixture until fully dissolved.

3. Addition of reducing agent: -

Prepare a fresh, dilute solution of Ascorbic acid. Slowly add the reducing agent to the copper solution under constant stirring Color change (often to brown or red) indicates the formation of copper nanoparticles.

4. Isolation of nanoparticles: -

After formation, centrifuge the solution to separate the nanoparticle from the liquid and wash the

nanoparticle with deionized water or ethanol to remove any impurities or by-products.

Reaction Equation: -

$$2Cu^{2+}+4NaBH_4+6H_2 \rightarrow 2Cu\ (nanoparticles)+4NaBO_2+12H2$$

5. Characterization: -Characterization of the copper nanoparticle using techniques like UV-VIS spectroscopy, and FTIR (Fourier transform infrared spectroscopy) for size, structure, and composition analysis

6. Methods of incorporation of drugs into Cream bases: -

In addition to the active drug, ingredients in Cream preparations can include oleaginous components, aqueous components, emulsifying agents, stiffeners, penetration enhancers, preservatives, and antioxidants. Oleaginous Creams may be prepared by levigation and fusion.

7. Levigation

Levigation involves dispersing and/or grinding an insoluble drug into small particles while wet. Mixing a base and other components over an Cream slab using a spatula can carry it out. Components such as liquid petrolatum serve as levigating agents by promoting the wetting of powders for incorporation on into bases. Hydrophobic Creams w/o emulsions and suspensions are typically prepared by a levigation process to incorporate a powder and/or a small quantity of water or hydrophilic component into an oil base.

8. Fusion

The fusion process involves melting components (such as paraffin, stearyl alcohol, white wax, yellow wax, and high molecular weight PEGs) together to form a homogeneous solution. The fusion method is used when the base contains solids that have higher melting points (e.g., waxes, cetyl alcohol, or glyceryl monostearate). This process is employed only when the components are stable at fusion temperatures. Hydrophilic o/w emulsions (such as water-removable Creams and Creams) are typically prepared by the fusion process. The hydrophobic components are melted together and added to the aqueous phase/water-soluble components containing an emulsifying agent with constant mixing until the mixture congeals. Normally, drug substances are in fine-powered forms before being dispersed in the vehicle.

2.3 Characterization of Cream

The characterization of Cream involves evaluating its physico-chemical properties, stability, and performance to ensure quality, safety, and therapeutic efficacy. are semi-solid emulsions, typically either oil-in-water (O/W) or water-in-oil(W/O), used for topical application. Below are the key parameters assessed during the characterization of Cream

Appearance and Texture

Evaluation: The visual and tactile properties of the Cream are assessed, including color, consistency, smoothness, homogeneity, and the absence of visible particles or phase separation.

Importance: Aesthetic appeal and sensory attributes are critical for user acceptance and compliance.

pH Measurement

Evaluation: The pH of the Cream is measured using a pH meter to ensure it falls within a range suitable for skin application, typically between 4.5 and 6.5.

Importance: A pH close to the skin's natural pH helps prevent irritation, making the product more compatible with the skin barrier.

Spreadability

Evaluation: This measures how easily the Cream spreads on the skin surface, which affects ease of application and user experience.

Testing Method: Spreadability is often assessed by placing a specific amount of Cream between two glass slides and applying standardized pressure, then measuring the diameter of the spread area.

Viscosity and Rheology

Evaluation: The viscosity of the Cream is measured using a viscometer to assess its flow properties and consistency.

Rheological Behavior:

Creams should exhibit non-Newtonian, shear-thinning behavior, where the viscosity decreases with an increase in shear rate, making them easier to spread during application.

Drug Content Uniformity

Evaluation: The concentration of the active ingredient is assessed to ensure uniform distribution throughout the Cream.

Testing Method: Samples from different parts of the Cream batch are analyzed using techniques such as HPLC (High-Performance Liquid Chromatography).

In Vitro Release Study

Evaluation: This study simulates the release of the active ingredient from the Cream using synthetic membranes to predict drug permeation through the skin.

Testing Method: A Franz diffusion cell apparatus is commonly used to evaluate the release profile.

Stability Testing

Evaluation: Stability testing is conducted under different environmental conditions (e.g., temperature, humidity) to assess the Cream's physical, chemical, and microbiological stability over time.

Parameters Monitored: Changes in color, consistency, pH, drug content, and microbial load.

Microbial Limit Test

Evaluation: Ensures that the Cream is free from harmful microbial contamination.

Testing Method: The total aerobic microbial count and the presence of pathogens (e.g., Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans) are checked according to pharmacopoeial standards.

Emulsion Type and Stability

Evaluation: Creams are categorized into either oil-in-water (O/W) or water-in-oil (W/O) emulsions. The type of emulsion affects the Cream texture, spreadability, and moisture retention.

Testing Method: Techniques like dye solubility, conductivity measurements, and dilution tests help confirm the

emulsion type.

Importance: The emulsion type influences the Cream's moisturizing properties, absorption rate, and user preference.

Water Retention and Moisturizing Effect

Evaluation: The water-holding capacity of the Cream is assessed to determine its ability to retain moisture and prevent transepidermal water loss (TEWL).

Occlusivity: The ability of the Cream to form a barrier on the skin that locks in moisture, promoting hydration.

Sensory Evaluation and User Acceptability

Evaluation: Sensory characteristics such as greasiness, tackiness, cooling effect, and residual feeling on the skin are evaluated through user trials.

Potential Irritation and Sensitization Testing

Evaluation: Creams are tested for potential irritation or allergic reactions using in vitro skin models or clinical patch testing.

Characterization of formulation

Characterization of Copper Nanoparticle-Loaded curcumin-based Cream

The characterization of a copper nanoparticle-loaded curcumin-based Cream involves several analytical and physical tests to confirm its stability, efficacy, and safety for topical application. These tests are essential to ensure that the formulation meets quality standards and delivers therapeutic benefits effectively.

Particle Size and Distribution Analysis

Purpose: Determines the average particle size of the copper nanoparticles within the Cream and ensures their uniform distribution.

Method: Scanning Electron Microscopy (SEM) is used to measure the particle size and analyze the dispersion within the Cream matrix.

Surface Morphology

Purpose: Analyze the surface and structural morphology of the copper nanoparticles within the Cream.

Method: Scanning Electron Microscopy (SEM) reveals the shape, texture, and dispersion of the nanoparticles in the Cream matrix.

Drug Loading and Encapsulation Efficiency

Purpose: Determines the amount of curcumin loaded onto the copper nanoparticles and the efficiency of encapsulation.

Method: UV-Vis Spectrophotometry is used to quantify the curcumin concentration in the formulation. High-Performance Liquid Chromatography (HPLC) can also be used for accurate quantification.

Calculation:

$$\textbf{Encapsulation Efficiency}\ (\%) = \frac{\textbf{Amount of drug encapsulated}}{\textbf{Total amount of drug added}} \times \mathbf{100}$$

Zeta Potential Analysis

Purpose: Measures the surface charge of the nanoparticles within the Cream to assess stability.

Method: Zeta potential measurements are performed using a zeta potential analyzer.

Viscosity and Spreadability

Purpose: Evaluate the Cream consistency, ease of application, and spreadability.

Method: A viscometer or rheometer is used to measure viscosity, while a spreadability test is conducted by placing a fixed amount of Cream between two glass slides and measuring the spread diameter under standardized pressure.

In Vitro Drug Release Studies

Purpose: Simulates the release of curcumin from the Cream to predict its delivery through the skin.

Method: A Franz Diffusion Cell apparatus is used with a synthetic membrane to evaluate the release profile of curcumin.

Stability Testing

Purpose: Evaluate the physical, chemical, and microbiological stability of the Cream under various storage conditions (temperature, humidity, and light exposure).

Method: Stability testing includes monitoring changes in appearance, viscosity, pH, drug content, and microbial load over a period of time.

Accelerated Stability Testing: Conducted at 40°C and 75% relative humidity to predict long-term stability.

2.4 Formulating Copper Nanoparticle-Loaded Curcumin-Based Cream

To prepare a copper nanoparticle-loaded Curcumin-based Cream, the formulation process focuses on incorporating copper nanoparticles as a drug carrier and stabilizer for enhanced topical drug delivery. Below is the step-by-step guide and rationale for the preparation:

Formulation Process

2.4.1 Preparation of Copper Nanoparticles

2.4.2 Synthesis of Copper Nanoparticles:

Dissolve 0.2 g of Copper Sulfate ($CuSO_4$) in 100 mL of distilled water.

Add a reducing agent (e.g., ascorbic acid or sodium borohydride) dropwise under constant stirring to facilitate the reduction of Cu^{2+} ions to Cu^0 nanoparticles.

Stabilize the nanoparticles using a surfactant or polymer such as polyvinyl alcohol (PVA) or polysorbate 80 to prevent aggregation.

2.4.3 Characterization of Copper Nanoparticles:

Determine the size and morphology of the nanoparticles using Scanning Electron Microscopy (SEM).

Conduct UV-visible spectrophotometry to confirm nanoparticle formation by observing characteristic surface plasmon resonance (SPR) peaks (~570-590 nm for copper).

2.4.4 Loading Curcumin into Copper Nanoparticles

2.4.4.1 Drug Dissolution:

Dissolve 0.1g of Curcumin in 50 mL of ethanol to form a clear solution. Ensure complete dissolution through continuous stirring.

2.4.4.2 Drug Loading

Mix the Curcumin solution with the prepared copper nanoparticles under mild stirring to allow the adsorption of the drug onto the nanoparticle surface.

Encapsulation efficiency can be improved by optimizing the pH (preferably ~6-7) and ensuring uniform distribution

Encapsulation Efficiency (EE%):

$$\textbf{EncapsulationEfficiency}(\%) = \frac{\textbf{Amountofdrugencapsulated}}{\textbf{Total amount ofdrug added}} X100$$

2.5 Formulation of Cream

Methods of preparation of Cream Preparation of topical Cream: -

1. Melting of beeswax and base: -

Melt 5 gram of beeswax in water bath at around 70- 75ºC and add 60gram of white petrolatum to melted beeswax and mix thoroughly until fully melted and homogenized.

2. Incorporation of emollients: -

add 10 g of lanolin to the melted mixture and continue stirring until completely incorporated add slowly 10g olive oil while continuously stirring to maintain homogeneity.

3. Addition of emulsifier: -

add 5 g of peg 4000 to the mixture, stirring constantly to ensure it well-dispersed.

4. **Incorporation of Humectants: -**

Add 5g of propylene glycol and mix thoroughly. propylene glycol helps retain moisture in the Cream.

5. **Preparation of aqueous phase: -**

warm 4g of distilled water in a separate container to around 40-45 ºC and slowly add the warm water to the oil phase stirring constantly to form an emulsion.

6. **Incorporation of curcumin–loaded copper nanoparticles: -**

Gradually add the prepared curcumin–loaded copper nanoparticle to the emulsified mixture ensuring uniform distribution by stirring continuously.

3 **Cooling and homogenization: -**

Allow the mixture to cool while stirring occasionally to prevent phase separation and ensure uniform consistency.

8. **Stabilization and Packaging: -**

Adjust the pH of the Cream to 5.5–6.5 using citric acid or triethanolamine, ensuring compatibility with the skin. Conduct stability tests under different temperature and humidity conditions. Package the Cream in airtight containers to prevent contamination and degradation.

2.6 Preparation of curcumin–copper nanoparticle suspension

Preparation of Organic and Aqueous Phases

Organic Phase (Curcumin Solution)

Dissolve 2g of Curcumin in 100 mL of ethanol at room temperature.

Stir the solution continuously to ensure complete dissolution, forming a clear organic phase.

Aqueous Phase (Copper Nanoparticles):

Prepare a 200mL aqueous solution by dissolving 0.1g of polyvinyl alcohol (PVA) or polysorbate 80 (as an emulsifier) under constant stirring.

Add pre-synthesized copper nanoparticles (prepared as described earlier) to the aqueous phase and mix thoroughly.

Formation of Curcumin–Copper Nanoparticle Complex

Dropwise Addition:

Add the organic phase (Curcumin solution) dropwise into the aqueous phase containing copper nanoparticles under continuous stirring.

Perform this step at room temperature to promote the adsorption of Curcumin onto the surface of the copper nanoparticles.

Stirring:

Stir the combined mixture for 2 hours to ensure complete interaction between Curcumin and the copper nanoparticles.

Ultrasonication:

Subject the resulting suspension to ultrasonication for 10–15 minutes to reduce particle size and ensure uniform distribution of Curcumin-loaded copper nanoparticles.

Filtration and drying

Filtration: Pass the suspension through a filtration apparatus to remove any large aggregates

Drying: Use one of the following methods to obtain dry powder of curcumin–loaded copper nanoparticle **water bath:** remove residual solvent using a rotary evaporator.**Lyophilization freeze:** dry the suspension to obtain stable, dry nanoparticle

Centrifugation:

Centrifugation of the suspension at 15,000 rpm for 20-30 minutes to separate curcumin–loaded copper nanoparticles from the supernatant.

Discard the supernatant and resuspend the nanoparticle in distilled water.

Repeat the centrifugation step 2–3 times to wash the nanoparticles and remove any unabsorbed drug.

Characterization of Curcumin-Loaded Copper Nanoparticles

Particle Size and Polydispersity Index (PDI)

Purpose: Determine the average size and uniformity of the nanoparticles.

Surface Morphology

Purpose: Examine the shape, texture, and dispersion of nanoparticles.

Technique: Scanning Electron Microscopy (SEM).

Zeta Potential

Purpose: Assess the stability of nanoparticles in the suspension.

Technique: Zeta potential analysis.

Encapsulation Efficiency

Purpose: Measure the amount of Curcumin successfully loaded onto copper nanoparticles. **Technique**: UV-Vis Spectrophotometry or High-Performance Liquid Chromatography (HPLC).

Determination of Melting Point

Purpose: Assess the purity of the drug-loaded nanoparticles and confirm structural integrity.

Method: Capillary tube method.

Load the powdered sample into a sealed capillary tube (2–3mm in height). Gradually increase the temperature in a melting point apparatus and record: Initial melting temperature: When the drug starts to melt. Final melting temperature: When the drug is completely melted.

Stability Testing

Purpose: Evaluate the stability of the nanoparticles under various conditions.

Method: Store the nanoparticles at different temperatures (e.g.,25°C,40°C) and monitor changes in size, zeta potential, and drug release over time.

Determination of Solubility

The greatest quantity of solute that may dissolve at equilibrium is the IUPAC definition of solubility, which is the capacity of a solute to dissolve in a solvent. Ten milliliters of each solvent—distilled water, ethanol, methanol, chloroform, acetone, phosphate buffer 7.4, and DMSO—were employed in different test tubes at room temperature in order to measure equilibrium solubility. Before precipitation occurred, around 10 mg of LU was added gradually while being shaken for five minutes at a time. Weighing the remaining medication was done when the precipitate continued. By deducting the residual quantity from the original amount, the amount of LU dissolved was determined (Adeyeye and Brittain, 2008).

Determination of partition coefficient

The partition coefficient is defined as the partition of a unionized drug distributed between the immiscible organic and aqueous phases at equilibrium.

$P_{o/w}=(C_{oil}/C_{water})$

The partition coefficient is a parameter for measuring the lipophilicity of a drug and its capability to pass the biological membrane. Determination of the partition coefficient for Curcumin was done by the shake flask method.10mg of Curcumin was added to 25ml of distilled water and 25ml of methanol; It was shaken separately for 30 minutes. Then, both phases were mixed in a separating funnel and again shaken for 4 hours in a mechanical shaker and then stood to separate the phases. The total quantity of drug present in both phases was determined by UV(Shimadzu UV-VIS-Spectrophotometer 1800)and compared with the quantity of the drug taken initially(Adeyeye and Brittain, 2008).

Scanning Electron Microscopy Analysis

Scanning electron microscope JEOL model JSM-6390LV, USA, has been utilized at STIC, Cochin University, Kerala, India, to perform the morphological study of CU-NPs. Nanoparticle Tracking and Analysis System (NanoSight Ltd., UK) for nanoparticle size measurement

The Nanoparticle Tracking and Analysis (NTA) system of Nano Sight Ltd. in the UK was employed to determine the size of the nanoparticles. The sample was diluted in ultrapure water to achieve a particle concentration of 10^7 to 10^9 particles/ml according to the manufacturer's guidelines. The calibrated sample was injected into the LM20 laser module through sterile syringes. The NTA program, which follows the movement of the particles under laser light to find their size, analyzed the moving particles in the liquid sample due to Brownian motion.

The NTA 2.3 software processed the laser light diffraction data to provide results in terms of the mean (average particle size) and mode (most frequently observed size). The modal value was taken as the size of the nanoparticle. The Stokes-Einstein equation underlies the particle size calculations in NTA.

Zeta-potential

The zeta–potential measurement for copper nanoparticles (CuNPs) loaded with curcumin can provide information on the surface charge, stability, and colloidal behavior of the nanoparticle. here is a general procedure you might follow to measure the zeta–potential of these loaded nanoparticles.

Sample Preparation

Copper nanoparticles (CuNPs)

Ensure the CuNPs are well synthesized and functionalized, and stored in an aqueous medium for zeta potential measurements.

Curcumin Loading: If not yet loaded, mix the CuNPs with a curcumin solution (e.g., dissolved in ethanol or DMSO) under gentle stirring or sonication to achieve adequate surface loading.

Dispersion Once loaded, disperse the curcumin-loaded CuNPs in deionized water or a suitable buffer (e.g., PBS, pH 7.4) as the zeta potential is pH-dependent. Dilute to a concentration suitable for measurement, typically around 0.1 mg/ml.

pH Adjustment: Measure the zeta potential at different pH levels to understand the influence of pH on stability. Adjust the pH of the sample using HCl or NaOH.

Zeta Potential Analysis

Measure the zeta potential by applying an electric field and analyzing particle movement. The result will indicate a positive or negative zeta potential value.

Repeat the measurement 2-3 times to confirm reproducibility and obtain an average value.

Low absolute zeta potential (<|30|mV) indicates potential aggregation or instability in the dispersion, which may require surface modification or stabilization steps.

Wound-Healing Properties of Creams Containing Copper Nano-particles

To prepare wound-healing Creams containing organic and inorganic nano-particles, copper nano-particles were synthesized and their surface was modified using various factors. The physicochemical characteristics of the particles were determined, technologies for preparation were developed, and Cream compositions were optimized as described.

Scanning electron microscopic images of sample 1 copper nano-particles are presented in Fig. 2 (A and B, respectively). The particles are microcrystalline structures covered with a semitransparent film of copper oxide. It should be noted that the copper oxide of Cu1-H2O sample 1 is represented by large angulated layered particles located on the surface; nano-particles of Cu1-Ox sample 2 show an almost ideal spherical surface that is covered with small islands of the oxide. The main physicochemical properties of modified copper nanoparticles are shown in Table 2. The average size of particles calculated using particle size distribution plots depends on the modification conditions and was equal to 86.0, 103, and 119 nm for samples 1, 2, and 3, respectively. The copper nanoparticles that were studied in this work varied in their phase composition. The content of crystalline copper, for example, was 84 and 96 volume1 % in samples 1 and 2, respectively, as detected by X-ray analysis. The content of crystalline copper in sample 3 was relatively low (0.5 volume1 %), so the sample was considered to consist of copper oxide nanoparticles.

3. Result And Discussion

3.1. UV-Visible Spectroscopy: Preparation of standard solution of Curcumin for UV Visible Spectroscopy

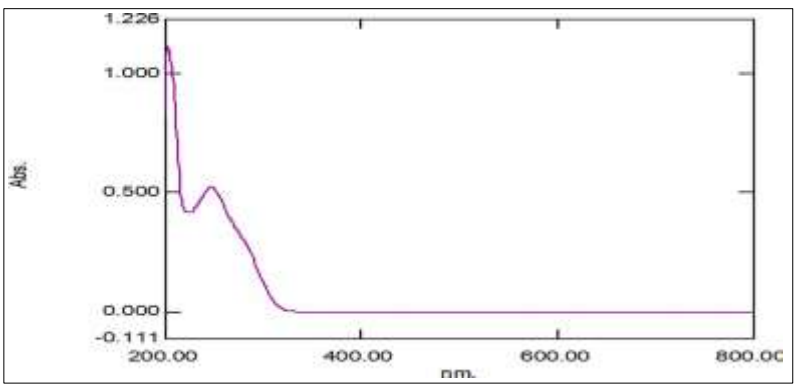


Figure 1 UV-visible spectra of curcumin.

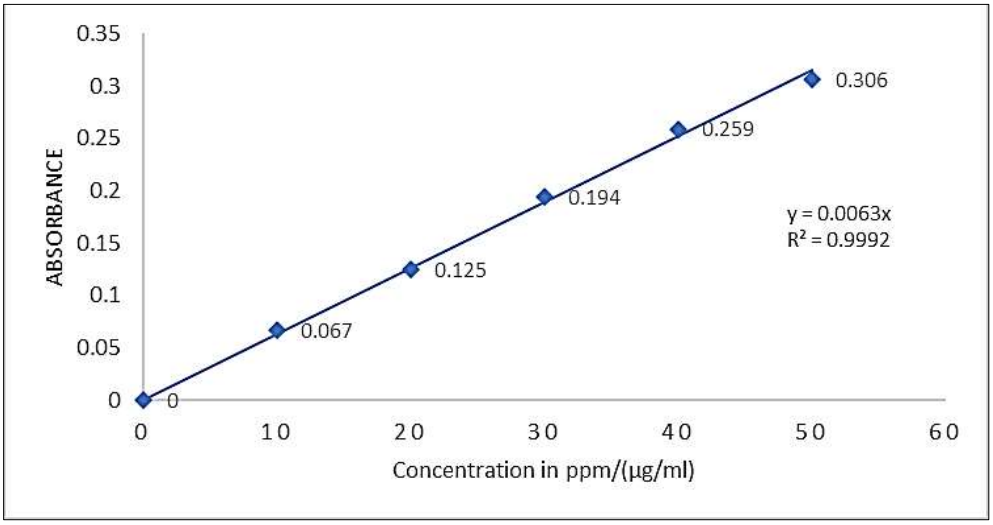


Figure 2 UV calibration curve of curcumin

Table 1: Calibration curve of curcumin

Concentration in PPM/(µg/ml)	Absorbance
0	0
10	0.067
20	0.125
30	0.194
40	0.259
50	0.306

3.2 Fourier Transforms Infrared Analysis: -

Following the encapsulation of Cu-NPs by CU, the C=O peak at 1629 cm−1 shifts toward a higher frequency and appears as a less intense band.

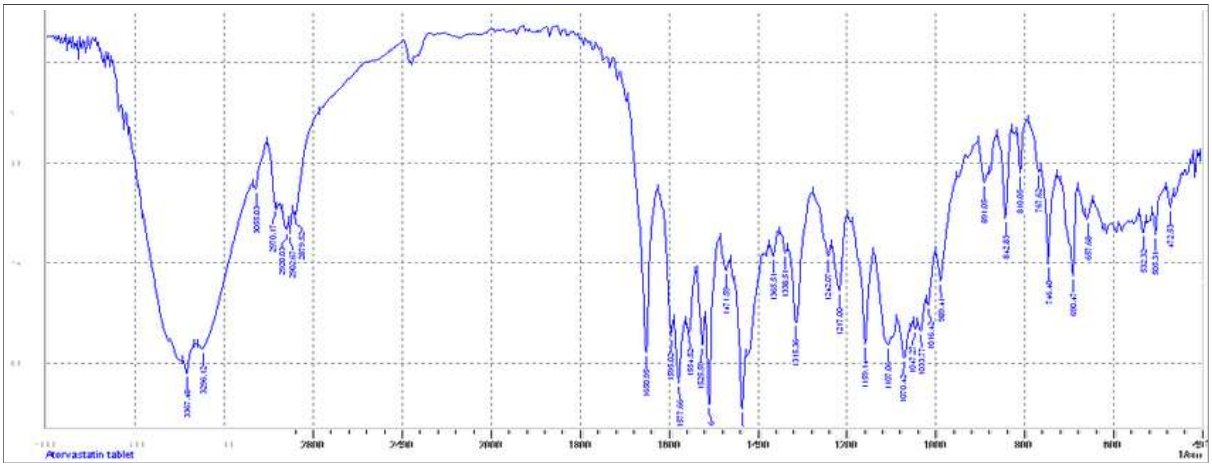


Figure: 3 FTIR Spectra of a curcumin and b Cu-NPs

Fourier Transforms Infrared Analysis: -

Titurate that curcumin in with, powdered potassium halide (KBr). Insert a portion of the mixture in a special die and press it under a vacuum under high pressure mount the resultant disc in a suitable holder, identification was done by comparing the obtained spectrum to the reference spectrum.

Characteristic FTIR Peaks for Curcumin

Wavenumber (cm^{-1})	**Functional Group/Mode of Vibration**	**Description**
3300–3500	–OH stretching (Hydroxyl group)	Indicates the presence of hydroxyl groups in the structure. Broad peak due to hydrogen bonding.
2920–2850	–CH stretching (Aliphatic C–H bonds)	Represents symmetric and asymmetric stretching of methyl and methylene groups.
1710–1750	C=O stretching (Carbonyl group)	Corresponds to the lactone ring carbonyl stretching vibration.
1600–1650	C=C stretching (Aromatic rings)	Indicates the aromatic framework in the Curcumin structure.
1510–1560	C=C stretching in aromatic rings	Represents the conjugated system within the phenyl rings.
1200–1250	C–O stretching (Ester functional group)	Indicates the presence of ester groups in the structure.
1000–1100	C–F stretching (Fluorine substitution)	A characteristic peak for the fluorophenyl group present in Curcumin.
650–800	Aromatic ring bending vibrations	Represents out-of-plane bending of aromatic C–H bonds.

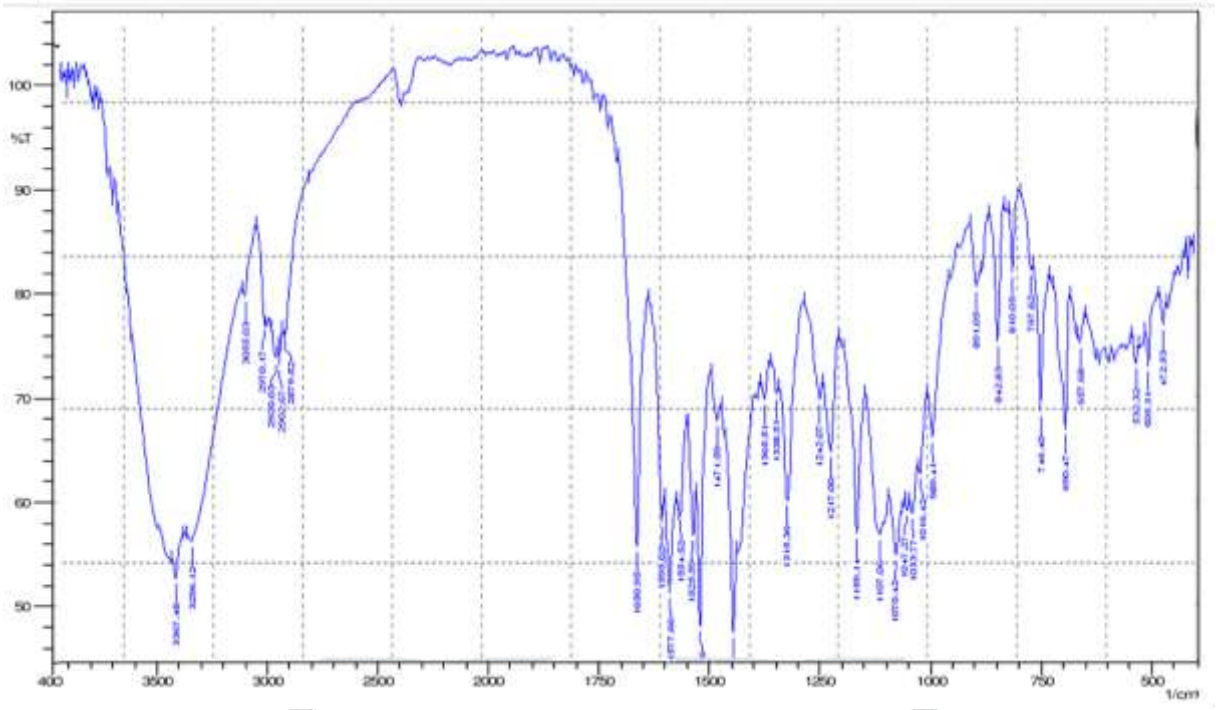


Figure 4: FTIR of Formulation Curcumin loaded Copper Nanoparticles

Zeta Potential Analysis

Zeta potential measures the surface charge of copper nano-particles in suspension, which indicates colloidal stability and aggregation potential electric field is applied to cordial suspension and the velocity of particle movement is measured to determine the zeta potential. zeta potential value negative charge indicating colloidal stability generally value greater than ±30mV suggest good stability.

Wound-Healing Properties of Creams Containing Copper Nano-particles

Cu NPs have a high antibacterial response capacity since they are prone to interacting with the bacteria membrane. In addition, they can penetrate the bacteria bio-film and release ions inside it, compromising its integrity, where the rigidity of the membrane is an important determinant of antibacterial efficiency.

Our results have shown that Cu NPs are structurally designed to have a rough surface to facilitate adhesion to the bacterial membrane, helping to reduce or prevent the formation of bacteria.

This bactericidal task occurs within a few minutes of encountering the bacteria; therefore, it is fast-acting, efficient, and long-term since it lasts over time without losing its bactericidal activity. However, it has been shown that Cu NDs demonstrate a much higher antibacterial effect than Cu NPs using laser radiation and can almost completely lyse bacterial membranes.

Stability Table for Copper nano-particle Loaded with Curcumin– based Cream formulation

Parameter	Initial (Day)	1Month	2 Month	3 Months	Evaluation Method
Appearance	Smooth yellow	No change	No change	Slight darkening	Visual Inspection
pH	6.5±0.2	6.4±0.2	6.3±0.3	6.2±0.3	pH meter
Viscosity (cPs)	5000±100	4950±120	4900±150	4800±150	Viscometer
Particle size (nm)	50±5	52±6	54±6	55±7	Scanning electron microscopy
Zeta potential(mV)	-30±2	-29±3	-28±3	-27±4	Zeta potential analyzer
Curcumin content (%)	100%	98%	97%	96%	UV-Vis spectroscopy
Antimicrobial activity (Zone of inhibition in mm)	12mm	12mm	11mm	10mm	Agar diffusion test

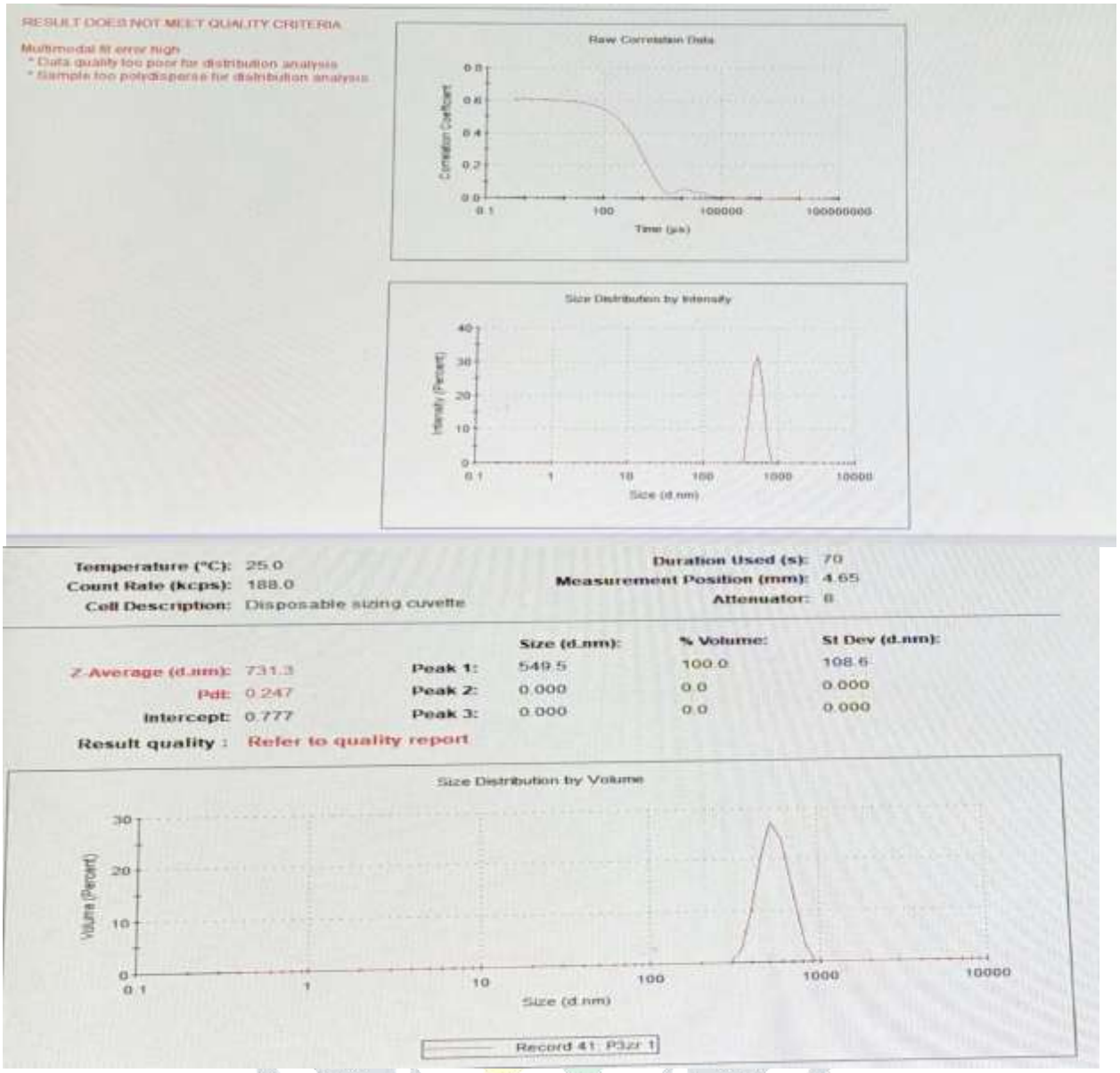


Figure 5: zeta potential

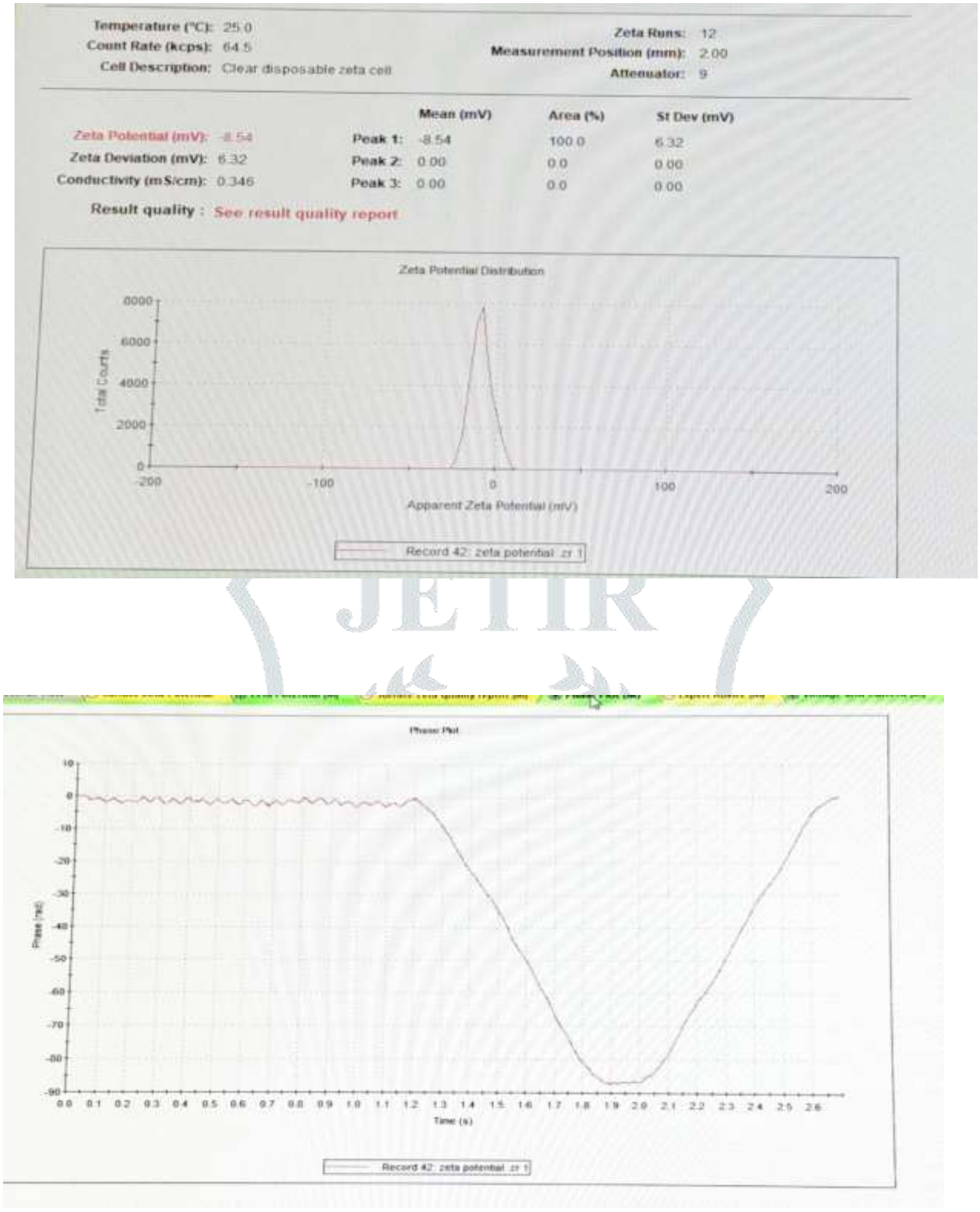


Figure 6: zeta potential

Characterization of copper nano-particle

Characterizing copper nano- particle (CuNPs) requires a comprehensive suite of technique to evaluate their physical, chemical, structural and functional properties.

Morphology and size analysis

By giving a three-dimensional impression of particle structure and agglomeration, scanning electron microscopy (SEM) aids in the observation of surface topography. An electron beam scans the sample's surface after it has been put on a substrate coated and placed in a SEM chamber. the surface morphology, roughness, and particle

agglomeration information of the data output.

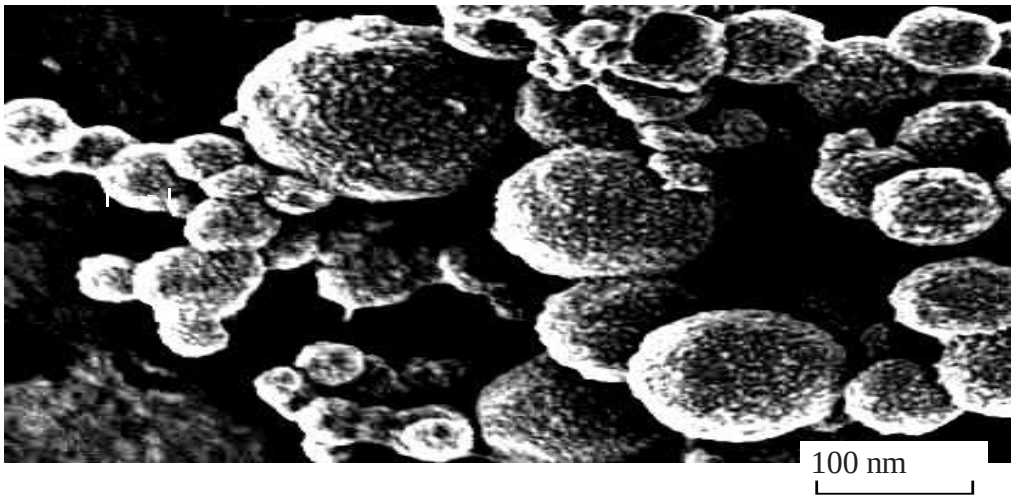
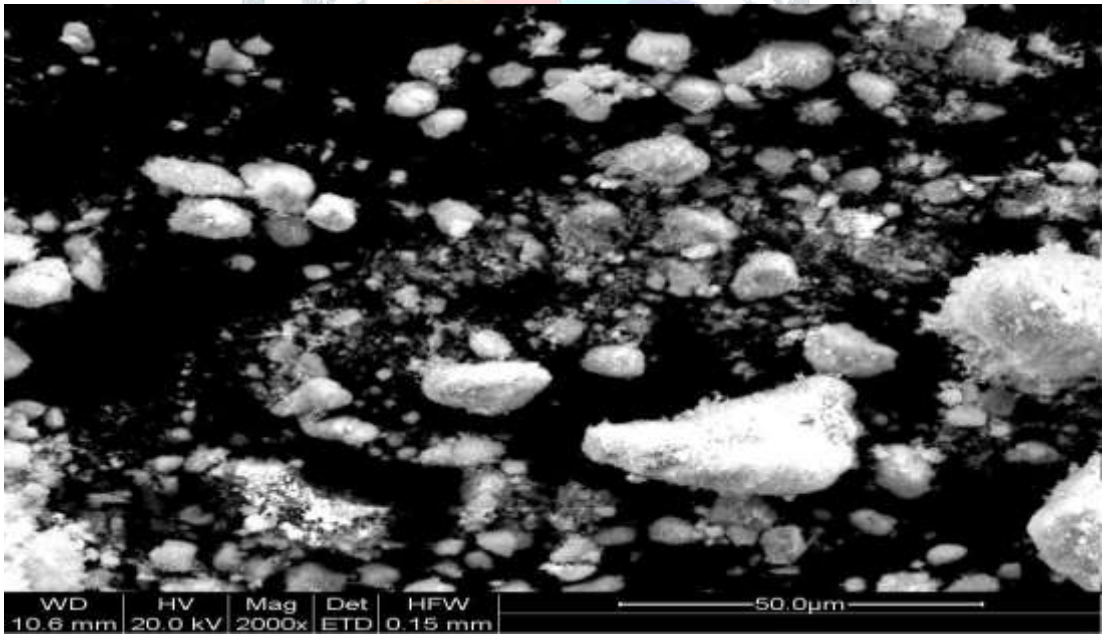


Figure: 7 SEM image of CuNPs

Scanning Electron Microscopy Analysis: -

The synthesis of nanoparticles is further corroborated by the characterization of CuNPs by scanning electron microscopy (SEM). As can be observed in Fig. 4, the morphology of the nanoparticles was a very small rod shape with numerous patches on its surface. It is found that the diameter of the nanoparticles is 178 nm. Also, as evident from (Fig. 4) of Sample 1 Cu-H2O powder, the mean CU-NP size measured by the nanoparticle tracking and analysis system using LM 20 is 173 nm, which is the mode value (most common particle size).



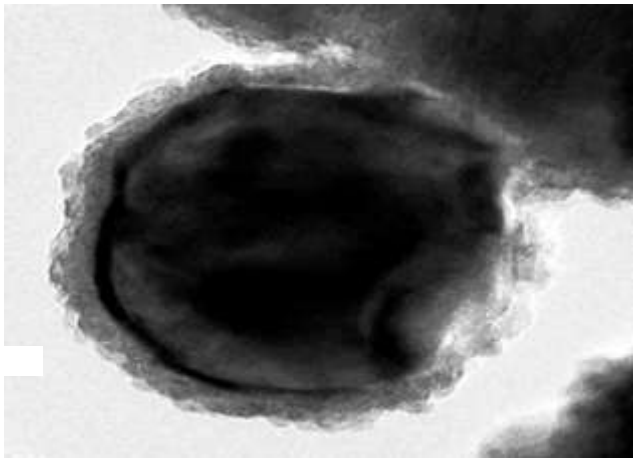


Figure 8: SEM image of CU-NPs

4. Summary and Conclusion

The work concentrated on characterizing and assessing curcumin-loaded copper nanoparticles (Cu-NPs) for possible usage in a range of applications, such as antibacterial and wound healing properties.

UV- Visible Spectroscopy : Curcumin's absorption properties were assessed using UV-Visible Spectroscopy, and it was discovered that its greatest absorbance occurred at 256 nm. A calibration curve was created that demonstrated a strong correlation value (r = 0.999) and a linear connection between concentration and absorbance. Curcumin's highest absorption wavelength, according to additional research on the compound in methanol, was 424 nm.

Fourier Transform Infrared Spectroscopy (FTIR)

Curcumin and Cu-NPs' functional groups were examined using Fourier Transform Infrared Spectroscopy (FTIR). The presence of curcumin is confirmed by the FTIR spectra, which showed numerous important peaks, including those for hydroxyl (–OH), carbonyl (C=O), and aromatic (C=C) groups. Peak changes were seen following curcumin encapsulation in Cu-NPs, suggesting interactions between the curcumin and Cu-NPs. One such interaction was a decrease in the carbonyl peak, which supports the encapsulation process.

Cu-NPs had a potent antibacterial impact because of their interaction with bacterial membranes, according to tests on the wound-healing capabilities of the ointments that contained them. After three months, a little decline in antibacterial activity was noted, but the cream formulation continued to be effective over time.

Lastly, the shape and size of Cu-NPs were examined using **Scanning Electron Microscopy (SEM),** which revealed rod-like structures that were around 173 nm in size. This further validated the synthesis and stability of Cu-NPs.

The effective production and encapsulation of curcumin into copper nanoparticles (Cu-NPs) is confirmed by the findings of the UV-visible spectroscopy, FTIR, Zeta Potential, and SEM analyses. The encapsulation technique did not impair curcumin's functional characteristics, and the curcumin-loaded Cu-NPs showed notable stability.

With strong antibacterial activity that held up over time, the compound demonstrated encouraging wound-healing qualities. After three months, the stability tests showed that the Cu-NPs were still stable and effective, which qualified them for long-term usage in ointments and other formulations. Because of their antibacterial and restorative qualities, curcumin and copper nanoparticles together have a lot of promise for use in medicinal applications.

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