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FORMULATION AND EVALUATION OF SILVER NANOPARTICLE OF CURCUMIN

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ABSTRACT

Curcumin, a natural polyphenolic compound obtained from Curcuma longa, exhibits a wide range of pharmacological activities including antimicrobial, antioxidant, and anti-inflammatory effects. However, its therapeutic application is limited due to poor solubility, low bioavailability, and rapid degradation. The present study aimed to formulate and evaluate curcumin-loaded silver nanoparticles to enhance its antimicrobial efficacy and physicochemical properties. Curcumin was extracted from fresh turmeric using the Soxhlet extraction method and further purified by column chromatography. Silver nanoparticles were synthesized by chemical reduction using silver nitrate and sodium borohydride, followed by stabilization with polyethylene glycol (PEG 6000). Curcumin was then incorporated into the nanoparticle system to obtain curcumin-loaded silver nanoparticles. The prepared formulations were evaluated for various parameters including particle size, pH, homogeneity, entrapment efficiency, stability, and antibacterial activity against Escherichia coli using the disk diffusion method. Among the developed formulations, F2 was found to be optimized, showing uniform nanoparticle size distribution, high drug entrapment efficiency, acceptable pH (6.0–7.0), improved solubility, and good stability. The formulation demonstrated significant antibacterial activity, indicating a synergistic effect of curcumin and silver nanoparticles. The results suggest that curcumin-loaded silver nanoparticles can serve as a promising approach for enhancing the therapeutic efficacy of curcumin and may be used in antimicrobial and pharmaceutical applications.

KEYWORDS: Curcumin, Silver Nanoparticles, Nanotechnology, Antimicrobial Activity, Drug Delivery, PEG Stabilization, Soxhlet Extraction, Column Chromatography, Bioavailability Enhancement, Nanomedicine.

INTRODUCTION

Introduction to Silver Nanoparticles of Curcumin

Nanotechnology has emerged as one of the most advanced and rapidly developing fields in pharmaceutical and biomedical sciences. Nanoparticles are ultrafine particles having dimensions in the range of 1–100 nm and exhibit unique physicochemical and biological properties compared to bulk materials. Due to their small size, large surface area, enhanced reactivity, and improved drug delivery potential, nanoparticles are widely used in medicine, cosmetics, diagnostics, and therapeutics. Among various nanoparticles, silver nanoparticles (AgNPs) have gained significant attention because of their excellent antimicrobial, antioxidant, anti-inflammatory, antiviral, and anticancer properties.[1]

In recent years, the increasing prevalence of microbial resistance against conventional antibiotics has become a serious global health problem. Excessive and irrational use of antibiotics has led to the emergence of multidrug-resistant microorganisms, reducing the effectiveness of available antimicrobial therapies. Therefore, researchers are continuously exploring alternative approaches using natural compounds and nanotechnology-based formulations to develop safer and more effective antimicrobial systems.[2]

Curcumin is a natural polyphenolic compound isolated from the rhizomes of Curcuma longa commonly known as turmeric. Curcumin possesses a wide range of pharmacological activities including antimicrobial, antioxidant, anti-inflammatory, antiviral, wound healing, hepatoprotective, and anticancer effects. Due to these medicinal properties, curcumin has been extensively used in traditional medicine and pharmaceutical research.[3]

Despite its remarkable therapeutic potential, curcumin exhibits certain limitations such as poor water solubility, low absorption, rapid metabolism, chemical instability, and poor bioavailability, which restrict its clinical application. To overcome these drawbacks, nanoparticle-based drug delivery systems have been developed to improve the solubility, stability, and therapeutic efficacy of curcumin.[4]

Silver nanoparticles possess broad-spectrum antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, fungi, and viruses. AgNPs exert antimicrobial action by disrupting microbial cell membranes, generating reactive oxygen species, damaging intracellular proteins, and interfering with DNA replication. Combining curcumin with silver nanoparticles produces synergistic antimicrobial activity and improves the stability and effectiveness of curcumin.[5]

The present study focuses on the formulation and evaluation of curcumin-loaded silver nanoparticles using chemical reduction method. The formulation includes curcumin, silver nitrate, sodium borohydride, polyethylene glycol (PEG), and distilled water for the preparation of stable nanoparticles with enhanced antimicrobial activity.[6]

Nanotechnology and Its Importance

Nanotechnology involves the manipulation and application of materials at the nanoscale level. Nanoparticles exhibit unique optical, chemical, magnetic, and biological properties due to their extremely small particle size and increased surface area. These properties make nanoparticles highly useful in pharmaceutical formulations, targeted drug delivery systems, tissue engineering, diagnostics, and antimicrobial therapy.[7]

Nanotechnology offers several advantages in drug delivery systems such as:

- Improved drug solubility

- Enhanced bioavailability
- Controlled drug release
- Increased therapeutic efficacy
- Reduced toxicity
- Site-specific drug targeting
- Improved stability of drugs

Nanoparticles can penetrate biological membranes more effectively and improve cellular uptake of drugs, thereby enhancing therapeutic action.[8]

Among metallic nanoparticles, silver nanoparticles are considered highly important due to their strong antimicrobial properties and biomedical applications.[9]

Silver Nanoparticles and Their Importance

Silver nanoparticles are Nano sized particles of silver having particle sizes generally below 100 nm. AgNPs possess remarkable antimicrobial, antioxidant, anti-inflammatory, and wound healing properties. They are widely used in pharmaceutical formulations, wound dressings, coatings, medical devices, cosmetics, textiles, and water purification systems.[10]

Silver nanoparticles exhibit broad-spectrum antimicrobial activity against various pathogenic microorganisms. Their antimicrobial mechanism includes:

- Disruption of microbial cell membrane
- Generation of reactive oxygen species
- Inhibition of protein synthesis
- Damage to microbial DNA
- Interference with cellular respiration

These mechanisms ultimately lead to microbial cell death.[11]

Due to their strong antimicrobial action, AgNPs are considered promising alternatives to conventional antibiotics against resistant microorganisms.[12]

Curcumin and Its Pharmaceutical Importance

Curcumin is the principal active constituent of turmeric obtained from Curcuma longa belonging to the family Zingiberaceae. Turmeric has been traditionally used in Ayurveda and herbal medicine for centuries due to its therapeutic properties.[13]

Curcumin possesses several pharmacological activities including:

- Antimicrobial activity
- Antioxidant activity
- Anti-inflammatory activity
- Anticancer activity
- Antiviral activity
- Wound healing activity

- Hepatoprotective activity

Curcumin acts as a potent antioxidant by scavenging free radicals and reducing oxidative stress. It also inhibits inflammatory mediators responsible for inflammation and tissue damage.[14]

Curcumin has shown effectiveness against various bacterial and fungal infections due to its ability to inhibit microbial growth and biofilm formation.[15]

Limitations of Curcumin

Although curcumin possesses numerous medicinal properties, its therapeutic application is limited because of:

- Poor water solubility
- Low absorption
- Rapid metabolism
- Poor bioavailability
- Chemical instability
- Rapid systemic elimination

These limitations reduce the effectiveness of curcumin when administered alone. Therefore, advanced drug delivery systems such as nanoparticles are required to improve its therapeutic performance.[16]

Nanoparticle formulations help protect curcumin from degradation, improve its solubility, enhance cellular uptake, and provide sustained drug release.[17]

Need for Curcumin-Loaded Silver Nanoparticles

The increasing incidence of antibiotic resistance has created an urgent need for alternative antimicrobial therapies. Combining natural bioactive compounds with nanotechnology provides a promising approach for developing effective antimicrobial formulations. [18]

Curcumin-loaded silver nanoparticles offer several advantages:

- Enhanced antimicrobial activity
- Improved stability of curcumin
- Better solubility and bioavailability
- Controlled drug release
- Reduced microbial resistance
- Synergistic therapeutic effect
- Improved penetration into microbial cells

Silver nanoparticles act as carriers as well as antimicrobial agents, thereby enhancing the overall therapeutic efficacy of curcumin.[19]

The present study therefore aims to formulate and evaluate silver nanoparticles of curcumin for improved antimicrobial activity and pharmaceutical applications.[20]

Concept of Curcumin-Loaded Silver Nanoparticles

Curcumin-loaded silver nanoparticles are nanosized delivery systems in which curcumin is incorporated or loaded into silver nanoparticles. These formulations combine the therapeutic properties of curcumin with the antimicrobial properties of silver nanoparticles.[21]

The formulation process generally involves:

1. Synthesis of silver nanoparticles
2. Stabilization using polymers such as PEG
3. Incorporation of curcumin into nanoparticles
4. Characterization and evaluation of nanoparticles

The resulting nanoparticles possess improved physicochemical properties and enhanced biological activity compared to pure curcumin.[22] Curcumin-loaded silver nanoparticles therefore provide better therapeutic effectiveness than conventional curcumin formulations.[30]

Ideal Characteristics of Curcumin Silver Nanoparticles should possess small particle size, good stability, uniform distribution, high drug loading capacity, controlled drug release, good antimicrobial activity, non-toxic nature, biocompatibility, high entrapment efficiency, suitable physicochemical properties.[31]

Figure 1: Curcuma Longa.

METHODOLOGY

MATERIALS

The following materials were used in the preparation and evaluation of Curcumin Loaded Silver Nanoparticles.

Herbal Ingredient

1. Turmeric

Biological Name: Curcuma longa

Fresh turmeric was used as the natural source of curcumin. Curcumin possesses antioxidant, antimicrobial, anti-inflammatory, and therapeutic properties. It acts as the major bioactive compound in the nanoparticle formulation.

Chemicals and Excipients Used: Silver Nitrate ($AgNO_3$), Sodium Borohydride ($NaBH_4$), Polyethylene Glycol (PEG 6000), Acetone, Chloroform, Methanol, Distilled Water.

Equipment used: soxhlet apparatus, round bottom flask, heating mantle, column chromatography, centrifuge, magnetic stirrer, oven, pH meter, weighing balance, measuring cylinder.

METHODS

Extraction of Curcumin by Soxhlet Extraction Method

Fresh turmeric was washed thoroughly and grated into small pieces. The wet grated turmeric was used directly without drying. The grated turmeric was placed inside filter paper/thimble and inserted into the Soxhlet extractor. Acetone was added into the round bottom flask attached to the Soxhlet apparatus. The flask was heated using a heating mantle.

Acetone evaporated and travelled upward into the condenser. Acetone vapour condensed into liquid form and dripped onto the turmeric sample.The solvent dissolved curcumin and other bioactive compounds from turmeric. After filling of extraction chamber, the solvent containing extracted compounds siphoned back into the flask. The extraction process was repeated several times to maximize extraction efficiency. The extract accumulated in the flask and acetone was evaporated to obtain concentrated turmeric extract containing curcumin.

Separation of Curcumin by Column Chromatography

A clean and dry glass column was fixed vertically using a stand. Silica gel slurry was prepared using chloroform and carefully poured into the column without formation of air bubbles. The crude curcumin extract was dissolved in minimum quantity of chloroform. The sample was mixed with silica gel to form free-flowing powder and loaded onto the packed silica column. Elution was performed using chloroform and methanol solvent system. Gradient system used: 100% chloroform, 95:5 (Chloroform: Methanol), 90:10 Increasing methanol concentration gradually, Different coloured bands appeared in the column, Yellow/Orange band → Curcumin. Other coloured bands → Impurities Separation occurred based on polarity differences. Eluted fractions were collected separately in test tubes. Solvent was evaporated from collected fractions to obtain purified curcumin.

Synthesis of Silver Nanoparticles (AgNPs)

A 0.01 M silver nitrate solution was prepared using distilled water (Solution A). 0.02 M sodium borohydride solution was added dropwise into Solution A under continuous stirring. The reaction mixture was stirred vigorously for 1 hour at: 25°C, 80°C, The prepared nanoparticle suspension was centrifuged at 10,000 rpm for 10 minutes. The precipitate was washed three times using distilled water to remove impurities. The purified nanoparticles were dried in an oven.

Preparation of PEG-Coated Silver Nanoparticles

PEG 6000 (2% w/w) was dissolved into the prepared AgNP solution. The mixture was stirred continuously for 1 hour at 25°C and 80°C. This produced PEG-stabilized silver nanoparticles with improved stability and reduced aggregation.

Preparation of Curcumin Loaded Silver Nanoparticles

Preparation of Curcumin Solution A 0.001 M curcumin solution was prepared. The curcumin solution was added into the AgNP or PEG–AgNP system. The mixture was stirred vigorously for 1 hour at 25°C and 80°C. This resulted in formation of curcumin-loaded silver nanoparticles.

Antibacterial activity was evaluated using the disk diffusion method against Escherichia coli. Nutrient agar plates were prepared and inoculated with Escherichia coli culture. Sterile discs containing nanoparticle formulation were placed on agar surface. Plates were incubated at suitable temperature for 24 hours. Zone of inhibition around discs was measured.

Larger zone of inhibition indicated stronger antibacterial activity of the formulation.

Evaluation Parameters

The prepared formulation was evaluated for:

1. Particle size
2. Morphology
3. Stability
4. Antibacterial activity
5. Drug entrapment efficiency
6. pH determination
7. Homogeneity
8. Appearance
9. Solubility
10. Physicochemical stability

RESULT

EVALUATION OF SILVER NANOPARTICLE OF CURCUMIN

Different formulations of Curcumin Loaded Silver Nanoparticles were prepared by varying the concentration of curcumin, PEG 6000, and silver nitrate to obtain optimized nanoparticle formulation with suitable particle size, stability, drug entrapment efficiency, antimicrobial activity, and physicochemical properties.

Table 1: Formulation Development Table.

Ingredient	F1	F2	F3
Curcumin	0.001 M	0.002 M	0.003 M
Silver Nitrate ($AgNO_3$)	0.01 M	0.01 M	0.01 M
Sodium Borohydride ($NaBH_4$)	0.02 M	0.02 M	0.02 M
PEG 6000	1%	2%	3%
Distilled Water	q.s.	q.s.	q.s.

OBSERVATION

Among all formulations, F2 showed the best results in terms of nanoparticle stability, particle size distribution, drug entrapment efficiency, homogeneity, and antimicrobial activity. Therefore, F2 was selected as the optimized formulation for further evaluation studies.

Optimized Formula Composition (F2)

Sr. No.	Ingredient	Quantity
1	Curcumin	0.002 M
2	Silver Nitrate ($AgNO_3$)	0.01 M
3	Sodium Borohydride ($NaBH_4$)	0.02 M
4	PEG 6000	2%
5	Distilled Water	q.s.

Evaluation of Silver Nanoparticle of Curcumin

Evaluation and characterization are essential steps in nanoparticle formulation development. In the present study, Curcumin Loaded Silver Nanoparticles were evaluated to ensure suitable physicochemical properties, stability, antimicrobial activity, and formulation quality. The evaluation process helps determine whether the nanoparticles possess acceptable appearance, particle size, stability, homogeneity, entrapment efficiency, and therapeutic performance.

1. Organoleptic Evaluation

Organoleptic evaluation includes colour, appearance, texture, and homogeneity of the nanoparticle formulation.

- **Colour:** Yellowish-brown colour was observed due to curcumin and silver nanoparticle formation.
- **Appearance:** The formulation appeared smooth and uniformly dispersed.
- **Texture:** Fine nanoparticle suspension without coarse particles was obtained.
- **Homogeneity:** Uniform dispersion without aggregation was observed.

This indicated successful nanoparticle preparation and acceptable physical appearance.

2. Determination of pH

The pH of nanoparticle formulation should remain within acceptable limits to ensure stability and compatibility. One ml of formulation was diluted with distilled water. The solution was stirred properly. pH was measured using a calibrated digital pH meter.

Observation

The prepared formulation showed pH within acceptable range (approximately 6.0–7.0), indicating formulation stability and suitability for pharmaceutical application.

3. Particle Size Analysis

Particle size plays an important role in nanoparticle stability and therapeutic efficacy. Particle size was determined using suitable particle size analyzer.

The nanoparticles showed nano-sized particle distribution with uniform size range indicating successful nanoparticle synthesis.

4. Entrapment Efficiency

Entrapment efficiency determines the amount of curcumin successfully incorporated into nanoparticles. Nanoparticle suspension was centrifuged. Free drug present in supernatant was measured. Entrapment efficiency was calculated using standard formula.

Observation

The optimized formulation showed high drug entrapment efficiency indicating effective incorporation of curcumin into silver nanoparticles.

5. Stability Study

Stability studies were performed to evaluate physical and chemical stability of nanoparticles. The formulation was

stored under: room temperature, refrigerated condition, and elevated temperature Observations were made periodically for: colour, aggregation, pH, homogeneity, sedimentation. Observation

No significant changes in colour, pH, or aggregation were observed during storage indicating good nanoparticle stability.

6. Homogeneity Test

Homogeneity determines uniform distribution of nanoparticles throughout the formulation. The formulation was visually examined for aggregation, lumps, or sedimentation. The nanoparticle formulation showed excellent homogeneity without phase separation or aggregation.

7. Antibacterial Activity

Antibacterial activity was evaluated using disk diffusion method against Escherichia coli. Nutrient agar plates were inoculated with bacterial culture. Sterile discs containing nanoparticle formulation were placed on agar surface. Plates were incubated for 24 hours. Zone of inhibition was measured.

The prepared Curcumin Loaded Silver Nanoparticles showed significant antibacterial activity against E. coli with clear zone of inhibition. This confirmed enhanced antimicrobial effectiveness of the nanoparticle formulation.

8. Solubility Study

Curcumin possesses poor water solubility which limits its therapeutic effectiveness. Nanoparticle incorporation improved the aqueous solubility and dispersion of curcumin compared to pure curcumin.

9. Sedimentation Study

Sedimentation study determines physical stability of nanoparticle suspension. The formulation was stored undisturbed and observed for sediment formation. Minimal sedimentation was observed indicating good suspension stability and nanoparticle dispersion.

10. Centrifugation Test

Centrifugation study helps evaluate nanoparticle stability under stress conditions. The formulation was centrifuged at high speed and observed for phase separation. No significant phase separation or aggregation was observed after centrifugation.

Table 2: Evaluation Parameters of Optimized Formulation (F2).

Sr. No.	Evaluation Parameter	Observation
1	Colour	Yellowish-brown
2	Appearance	Smooth suspension
3	pH	6.0 – 7.0
4	Particle Size	Nano range
5	Homogeneity	Uniform
6	Entrapment Efficiency	High
7	Stability	Stable
8	Solubility	Improved
9	Sedimentation	Minimal
10	Antibacterial Activity	Significant inhibition
11	Aggregation	Absent
12	Centrifugation Test	Stable

RESULT AND DISCUSSION

The evaluation parameters included organoleptic properties, pH determination, particle size analysis, entrapment efficiency, homogeneity, antibacterial activity, stability studies, sedimentation study, centrifugation test, and solubility analysis.

1. Organoleptic Properties

The prepared Curcumin Loaded Silver Nanoparticles showed yellowish-brown colour due to the presence of curcumin and formation of silver nanoparticles. The formulation exhibited smooth appearance with uniform nanoparticle dispersion and absence of visible aggregates or coarse particles.

The nanoparticle suspension remained homogeneous and stable throughout the observation period. The appearance and texture indicated successful nanoparticle synthesis and proper formulation development.

2. PH Determination

The pH of the prepared nanoparticle formulation was found to be within the range of 6.0–7.0. This pH range is considered suitable for pharmaceutical and biomedical applications.

Maintenance of acceptable pH helps improve nanoparticle stability and reduces the possibility of irritation or degradation of the formulation.

PEG coating also contributed to stabilization of the nanoparticle system.

3. Particle Size Analysis

Particle size analysis confirmed the formation of nanoparticles within nano range. Uniform particle size distribution was observed in the optimized formulation.

Smaller particle size increases surface area and improves drug solubility, absorption, antimicrobial activity, and therapeutic effectiveness.

The reduction process using sodium borohydride successfully converted silver ions into nano- sized silver particles.

4. Entrapment Efficiency

The optimized formulation showed high entrapment efficiency indicating successful incorporation of curcumin into silver nanoparticles. Efficient entrapment improves drug stability, sustained release, and therapeutic activity of curcumin. PEG coating also helped improve drug loading and nanoparticle stability.

5. Homogeneity Test

The prepared nanoparticle formulation showed excellent homogeneity without aggregation, phase separation, or sedimentation. Uniform distribution of nanoparticles indicates proper mixing and stabilization during formulation preparation. PEG acted as an effective stabilizing agent and prevented nanoparticle aggregation.

6. Solubility Study

Curcumin possesses poor water solubility in its pure form. After nanoparticle incorporation, improved aqueous solubility and dispersion were observed. Improved solubility enhances bioavailability and therapeutic effectiveness of curcumin. Nanoformulation significantly improved dispersion characteristics of curcumin in aqueous medium.

7. Antibacterial Activity

The antibacterial evaluation using disk diffusion method showed significant inhibition against
Escherichia coli. Clear zone of inhibition was observed around the discs containing Curcumin Loaded Silver Nanoparticles. The enhanced antibacterial activity is due to membrane disruption caused by silver nanoparticles, antioxidant and antimicrobial properties of curcumin, increased penetration of nanoparticles into microbial cells. The combined effect of curcumin and silver nanoparticles produced synergistic antimicrobial action.

8. Sedimentation Study

Minimal sedimentation was observed during storage studies. The formulation remained uniformly dispersed without significant settling of particles. This indicates acceptable physical stability of the nanoparticle suspension. PEG coating contributed to stabilization and reduced sediment formation.

9. Centrifugation Test

The formulation remained stable after centrifugation and no phase separation or aggregation was observed. This result confirmed good physical stability and compatibility of formulation components.

10. Stability Study

The prepared nanoparticle formulation was stored under different temperature conditions for stability analysis. No significant changes were observed in colour, pH, homogeneity, particle aggregation, appearance. The formulation remained physically and chemically stable throughout the study period. PEG stabilization improved shelf stability and prevented nanoparticle aggregation.

DISCUSSION

The results obtained from the present study confirmed successful formulation and evaluation of Curcumin Loaded Silver Nanoparticles with acceptable physicochemical and antimicrobial properties. Curcumin is well known for its antioxidant, anti-inflammatory, and antimicrobial activities, but its poor aqueous solubility and low bioavailability limit its therapeutic effectiveness. Incorporation of curcumin into silver nanoparticles significantly improved its solubility, stability, and antimicrobial performance.

Silver nanoparticles demonstrated excellent antimicrobial properties due to their ability to disrupt microbial cell membranes, generate reactive oxygen species, and interfere with bacterial cellular functions. The combination of curcumin and silver nanoparticles produced synergistic antimicrobial activity against Escherichia coli. The chemical reduction method using sodium borohydride proved effective for synthesis of stable silver nanoparticles. PEG 6000 acted as an efficient stabilizing agent by preventing nanoparticle aggregation and improving homogeneity and shelf stability.

The optimized formulation exhibited uniform nanoparticle distribution high drug entrapment efficiency, improved aqueous solubility, acceptable pH, good physical stability, significant antibacterial activity. Stability studies confirmed that the prepared nanoparticles remained stable without major changes in colour, pH, or aggregation during storage.

Compared to conventional curcumin formulations, Curcumin Loaded Silver Nanoparticles provide several advantages enhanced antimicrobial activity, improved drug stability, better solubility and dispersion, increased bioavailability, sustained therapeutic action, reduced particle aggregation, potential biomedical applications. The nanoparticle system developed in the present study may therefore serve as a promising pharmaceutical and antimicrobial formulation for future therapeutic applications. Overall, the results support that Curcumin Loaded Silver Nanoparticles are stable, effective, safe, and suitable for pharmaceutical and biomedical applications.

CONCLUSION

The present study entitled **"Formulation and Evaluation of Silver Nanoparticle of Curcumin"** was successfully carried out with the objective of developing a stable, effective, and pharmaceutically acceptable nanoparticle formulation possessing enhanced antimicrobial and therapeutic activity. Curcumin, the principal bioactive constituent obtained from Curcuma longa (turmeric), possesses excellent antioxidant, antimicrobial, anti-inflammatory, and medicinal properties. However, its pharmaceutical application is limited because of poor aqueous solubility, low bioavailability, rapid degradation, and poor absorption. To overcome these limitations, silver nanoparticle-based drug delivery system was selected for the present work. In this study, curcumin was successfully extracted from fresh turmeric using Soxhlet extraction method with acetone as solvent. Further purification and separation of curcumin were performed by column chromatography using chloroform and methanol solvent system. The extraction and purification procedures produced satisfactory yield of curcumin suitable for nanoparticle formulation.

Silver nanoparticles were synthesized successfully using silver nitrate and sodium borohydride through chemical reduction method. PEG 6000 was incorporated as stabilizing agent to improve nanoparticle stability and prevent aggregation. Curcumin was then loaded into the prepared silver nanoparticle system to obtain Curcumin Loaded Silver Nanoparticles.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research work.

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