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Silver Nanoparticle Formulation Development and Evaluation: *In-Vitro* Anti-Microbial Evidences

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ABSTRACT:

There is a rapidly expanding discipline dedicated to the creation of non-biodegradable nanoparticles for use in nanomedicine and nanotechnology. The purpose of this study is to create and test the efficacy of colloidal silver nanoparticles as an antibacterial and antifungal agent. Standard chemical reduction techniques have been used to prepare the colloidal silver nanoparticles. The nanoparticles of colloidal silver were studied using TEM, zeta potential, photo correlation spectroscopy, and in vitro release kinetics. The resulting particles were spherical, with an average particle size between 5 and 50 nm and zeta potentials between -10.0 and -30.0 mV; their release kinetics followed zero-order kinetics with a correlation coefficient of 0.96 or higher. According to the results of the dissolution tests, the release of silver nanoparticles is proportional to their size, i.e., the release is higher for smaller particles. These findings point to the stability of Ag NPs in pharmaceutical formulations and their potential for rapid delivery to the site of infection. There was a wide range of bacterial species that were killed by the colloidal silver nanoparticles. Bacteria such as Escherichia coli, Salmonella, and Pseudomonas aeruginosa were the focus of the research. Aspergillus and Penicillium's antifungal properties were also studied. Nanoparticle cytotoxicity was investigated in human fibroblast cells. The results indicated that cytotoxicity depends on concentration. In light of these findings, silver nanoparticles may be worth investigating further as a potential antibacterial and antifungal agent that would avoid the drawbacks of conventional antibiotics while yet being effective.

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INTRODUCTION

The production of nanoparticles composed of noble metals has garnered growing attention due to their unique and distinct properties when compared to their macroscopic counterparts. Nanoparticles possess numerous advantageous uses across diverse disciplines, including but not limited to medicine and biotechnology [1]. Upon reaching the nanoscale, silver nanoparticles demonstrate unique physicochemical properties and biological activity, similar to other nanomaterials. These distinctive characteristics can be attributed to their exceptionally small size [2, 3].

Silver nanoparticles possess several distinctive characteristics, including a significantly large surface area, minuscule dimensions, and exceptional dispersion capabilities. Furthermore, there has been a growing interest in colloidal silver solutions owing to its antibacterial capabilities, which have direct implications in fields such as pharmacology and veterinary medicine, among others. The investigation into the interaction between metal nanoparticles and microbes is a rapidly growing area of study [4]. The antibacterial activity of silver nanoparticles is thought to be mediated through their absorption and subsequent accumulation within bacterial cells, resulting in the shrinking of the cytoplasmic membrane or its dissociation from the cell wall. In an alternative scenario, it is plausible that silver ions might potentially engage in interactions with the S–H bonds present in proteins, hence resulting in their subsequent inactivation. The attachment of nanoparticles to DNA leads to the condensation of DNA molecules and subsequent loss of their replicative capacity. This phenomenon is likely the primary mechanism via which the nanoparticles impede bacterial replication [5, 6].

A fundamental prerequisite in the production of colloidal nanoparticles is the comprehension of the underlying dynamics that dictate their stability. Particles can achieve stabilization through either electrostatic or steric means. Steric stabilization is achieved through the adsorption of polymers or surfactants onto the surfaces of particles, effectively preventing their agglomeration. On the other hand, electrostatic stabilization is accomplished by capping the particles with charged molecules, which establish chemical bonds with or chemisorb onto the particles [7, 8].

It was widely believed that silver powder possessed therapeutic properties that might effectively aid in the healing of ulcers [9]. In recent years, silver compounds have emerged as significant agents in combating wound infections, particularly in conjunction with the introduction of antibiotics. This work centers on the synthesis and characterization of silver nanoparticles, as well as the investigation of their antibacterial and antifungal properties against four bacterial strains, namely Escherichia coli, Staphylococcus coccus, Salmonella, and Pseudomonas aeruginosa, along with the fungus species Aspergillus and Penicillium [10, 11]. The objective of this work is to conduct a concise investigation on the cytotoxic effects of several colloidal silver nanoparticles on a human fibroblast cell line through prolonged exposure.

Materials and methods

The chemicals used in this study were silver nitrate (AgNO3), tri-sodium citrate (Na3C6H5O7), maconci agar medium, agarized Czapek Dox, Dulbecco's Modification of Eagles Med (DMEM), glutamine, penicillin, streptomycin, and MTT dye. These chemicals were obtained from Sigma Aldrich. The chemical compounds hydrogen chloride (HCl), nitric acid (HNO3), and deionized water are being discussed. All compounds were utilized without any further purification.

Silver nanoparticles preparation

The manufacture of silver nanoparticles involves the reduction of AgNO3 using citrate, following the Frens process often employed for the synthesis of gold nanoparticles. The 3-neck round bottom flask was subjected to a cleaning process with aqua regain, followed by rinsing with deionized water. Subsequently, the flask was coated with alumina fuel in order to block the entry of light. Silver nitrate (AgNO3) solutions were subjected to boiling and reflux conditions with continuous stirring. In situ, several amounts of tri-sodium citrate solutions, ranging from 38.8 to 40 mM, were introduced. The observed phenomenon involved a transition in the solution's color, shifting from a state of being colorless to assuming a hue of golden yellow. Following the alteration in color, the solution underwent reflux for an extra duration of 15 minutes. Subsequently, the heat source was deactivated, and the solution was agitated

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until it reached ambient temperature [11, 12].

Silver Nanoparticles Characterization

Particle size analyses and Zeta Potential Measurements

The mean diameter of particles and the polydispersity index were determined by employing photon correlation spectroscopy immediately after the synthesis process, while the particles were in solution. The quartz cell of the PCS was supplemented with silver nanoparticles. The experiment involved the acquisition of duplicate measurements at a 90° angle relative to the source of incident light. The surface potential of the silver nanoparticles was determined by employing a zeta potential measurement. In each instance, a mean value derived from three distinct measurements was documented [13, 14].

Transmission electron microscopy

The dimensions and structure of the silver were investigated using transmission electron microscopy. The microscope was functioning with an accelerating voltage of 80 kilovolts. The silver samples were initially diluted using distilled water, and a portion of the diluted solution was subsequently deposited onto a grid that had been coated with carbon. Subsequently, the solution was allowed to stand undisturbed for a duration of one minute, following which any surplus material was eliminated from the grid using the process of blotting, utilizing a filter paper. The grids were allowed to dry in the grid box for a duration of two hours prior to imaging [15, 16].

Anti- Bacterial activity

The strains Escherichia coli XL-1 blue, Salmonella XL-1, Staphylococcus ATCC 2784, and Pseudomonas aeruginosa ATCC 2484 were acquired from the microbiology department. Bacterial cells were cultured for a duration of 24 hours on agar plates using maconci medium. Subsequently, the specimens were re-suspended in Maconci medium until they attained an optical density of 0.5 at a wavelength of 595 nm. The specimens were subjected to multiple measurements under controlled conditions at a temperature of 37 ºC. The process of shaking was performed intermittently in between measurements, and data collection occurred at regular intervals of 30 minutes. Survival was determined by evaluating the final data point on the growth curve in relation to a control value [17,18].

Anti-fungal activity

The antibiogram technique was employed to assess the proliferation of fungal cells in the presence of mycotoxins. Petri dishes with agarized Czapek Dox medium were inoculated by evenly distributing a fungal medium over 96 well microplates. The plates were then incubated at a temperature of 37ºC for a duration of 24 hours. The cellular specimens were subjected to varying doses of silver nanoparticles, spanning from 10 µM to 140 µM. The samples were subjected to many measurements, conducted at a temperature of 37 ºC, with consistent agitation between each subsequent measurement. Data collection occurred at 30-minute intervals. Survival was determined by evaluating the final data point on the growth curve in relation to a control value [19-21].

MTT assay and cytotoxic study

The HBT68 human fibroblast cells were cultivated in Dulbecco's Modification of Eagles Medium supplemented with fetal bovine serum and glutamine. Subsequently, the cells underwent a triple washing procedure using a PBS solution, followed by staining with MTT dye. The measurement of solution intensity was conducted by determining the relative cell viabilities as a proportion of the control. Following the completion of the treatment, the cells were introduced onto the plate and subjected to incubation for a duration of four hours under the conditions of 5% carbon dioxide concentration and a temperature of 37 °C. The assessment of cell viability was conducted with a microplate reader [22-24].

Result and discussion

Particle size study

The measurements of mean particle size diameter and polydispersity indices were conducted on solutions immediately following synthesis, employing photon correlation spectroscopy. The dimensions of the colloidal silver nanoparticles, together with their granulometric distribution, have been documented and quantified in terms of both particle count and occupied volume. The data presented in the study indicated that the particles had sizes ranging from 5 to 50 nm. The

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results of the particle size study indicated the existence of nanoparticles characterized by low polydispersity indices. The size distribution observed in this study aligns with the size distributions reported in previous studies on nanosystems as documented in the literature [25, 26]. Through an examination of dynamic light scattering, it becomes evident that silver nanoparticles exhibit a limited range of sizes, with an average z-average ranging from 5 to 50 nm. Furthermore, it is shown that the particles with an average z-equivalent of 20 nm had a uniform size distribution. The findings have been compiled and presented in Table 1 and visually shown in Figure 1.

Table1: The particle size and particle size distribution

Sr. No.	Sample Number	Particle size (nm)	PDI
1.	F1	10.01	0.014
2.	F2	15.10	0.147
3.	F3	35.47	0.214
4.	F4	55.00	0.019

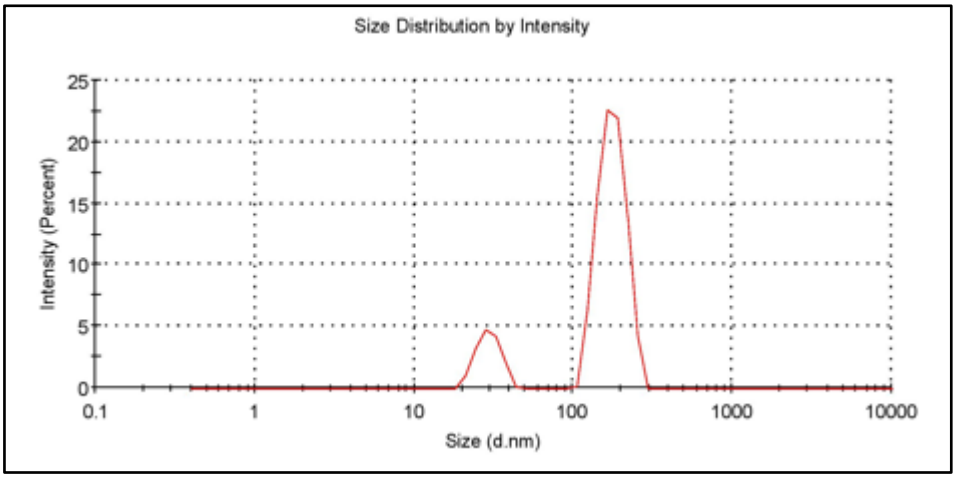

Figure 1: The particle size and particle size distribution

Zeta potential

Table 2 and Figure 2 provide a summary of the zeta potential readings for all batches in a solution format. The nanoparticles that were acquired underwent zeta value measurements, which revealed a range of -20.00 to -30.00 mV. The observed values offer comprehensive stabilization of the nanoparticles across various pH levels, potentially serving as the primary factor contributing to the production of nanoparticles with a limited size distribution index. The zeta potentials of the silver nanoparticles treated with citrate, along with their narrow size distributions, offer compelling evidence of their minimal propensity for aggregation. The observed behaviour strongly indicates the existence of potent electric charges on the surfaces of the particles, which serve to impede their tendency to aggregate [27, 28].

Table 2: Zeta potential

Sr. No.	pH	Zeta Potential
1.	6.0	-10.00
2.	7.3	-15.10
3.	8.1	-20.47
4.	9.0	-30.00

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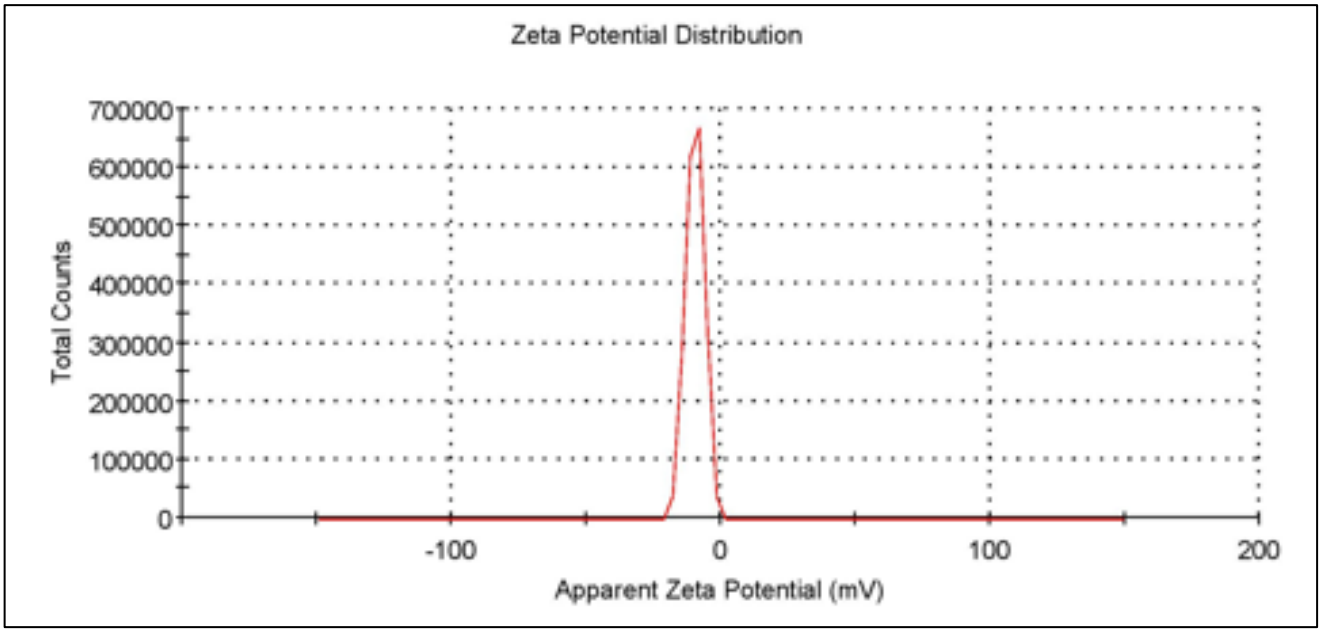

Figure 2: Zeta potential

This study demonstrates the stability of colloidal silver nanoparticles throughout a broad pH spectrum, exhibiting no propensity for aggregation or the creation of self-assembled aggregates. This observation contradicts the notion that surface modification of the particles is obligatory. The phenomenon of charged particles interacting through electrostatic repulsive forces at significant distances is widely acknowledged in the scientific community.

TEM study

Figure 3 depicts the dimensions and structural characteristics of the S1, S2, S3, and S4. The specimens were examined via transmission electron microscopy. The measurement of particle shape and diameter was conducted across several fields. The silver particles observed in the electro-micrograph had a spherical morphology and have a distinct and consistent particle size. The dimensions have been documented as the average diameter, as depicted in Figure 3. The silver nanoparticles have a distinct spherical morphology, characterized by a precisely regulated particle size. Furthermore, as anticipated, the particle size is heavily influenced by the preparation circumstances. The observed particles had an average size ranging from 5 to 50nm, a finding that aligns with the results derived from the Particle Size Distribution data.

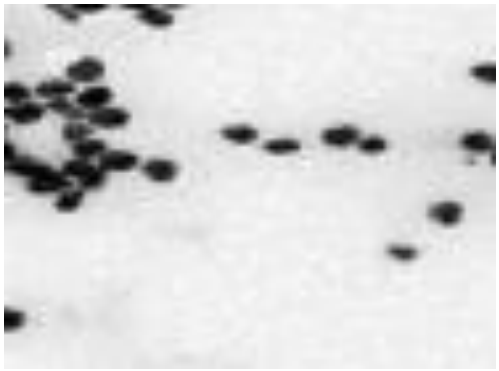

Figure 3. TEM images of spherical Silver

Anti-Bacterial Study

The antimicrobial efficacy of S1 was evaluated against four prevalent bacterial strains, specifically: Escherichia coli, Salmonella, Pseudomonas aeruginosa, and Staphylococcus coccus. Optical density measurements were employed utilizing a micro-plate reader to evaluate the impact of silver nanoparticles on the prevention of bacterial growth. This was done across various concentrations of the nanoparticles. The findings demonstrate the inherent and varied diversity in the morphological, physiological, and metabolic properties of many bacterial species.

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Antifungal study

The antifungal effects of colloidal silver nanoparticles were examined through experimental testing. The measurements of growth inhibitory concentration were obtained by utilizing fractional concentration values. The potential antifungal properties of silver ion solutions can be attributed to their ability to interact with proteins, resulting in protein inactivation, as well as their direct interaction with DNA. This interaction can induce mutations and hinder the replication capacity of DNA. This solution is capable of traversing the cell wall with ease due to the minute size of its constituent elements. The buildup of substances on the cell membrane has the potential to cause cell lysis.

Cytotoxicity

The qualitative assessment of cellular cytotoxicity in human fibroblast cells was conducted using MTT as a dye. In Figure 4, the observed cytotoxic behaviour of silver nanoparticles varies based on the concentration of the silver nanoparticles utilized and the duration of the treatment period. The quantitative assessment of the cytotoxicity of silver nanoparticles was conducted in vitro using the MTT test. Human fibroblast cells were exposed to different doses of Ag NPs for evaluation.

Table 3: Cell viability using MTT assay

Sr. No.	pH	Zeta Potential
1.	6.0	-10.00
2.	7.3	-15.10
3.	8.1	-35.47
4.	9.0	-25.00

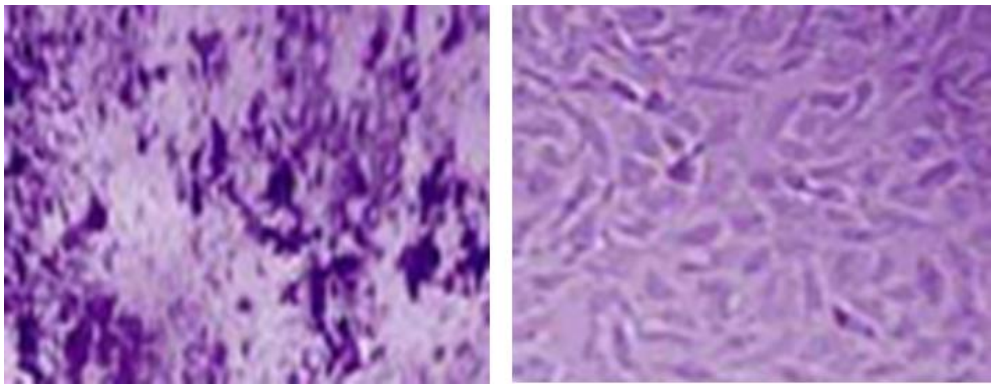

Figure 4. The cytotoxicity activity analysis of silver nanoparticles

CONCLUSION

This study proposes that silver nanoparticles with a particle size ranging from 5-50 nm exhibit stability in their fluids across a broad pH range. Upon capping silver particles with citrate, the distribution of particle size became narrower and more monodisperse. The dissolution data demonstrates an inverse relationship between the size of the silver nanoparticles and their release, specifically indicating that the release is enhanced with decreasing particle size. The findings indicate that the silver nanoparticles are likely to exhibit stability within medicinal formulations and possess a high likelihood of reaching the site of infection. The nanoparticles exhibit a significant antimicrobial activity against both bacteria and fungi. The findings provide robust data that supports the potential use of silver nanoparticles as an alternative antibiotic treatment for eradicating various bacterial and fungal strains. This approach may offer advantages by mitigating the adverse and unintended consequences associated with conventional antibiotic therapies.

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Conflict of Interest
None

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