IJMPR 2023, 7(8), 20-26

International Journal of Modern Pharmaceutical Research

www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Review Article
SJIF Impact Factor: 5.273

FORMULATION AND EVALUATION OF METRONIDAZOLE LOADED SILVER NANOPARTICLES

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Received on: 21/06/2023
Revised on: 11/07/2023
Accepted on: 01/08/2023

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ABSTRACT

Nanotechnology, where nano signifies incredibly minute structures ranging from 1 to 100 nm, can be defined as the characterization, fabrication, investigation, and application of nanosized materials. Various nano particles offer certain unique advantages as pharmaceutical delivery systems, which is one of the emerging approaches for using nanotechnology successfully in medicine. A rapidly expanding topic, the creation of non-biodegradable nanoparticles is essential for both nanotechnology and nanomedicine applications. Our focus in this inquiry will be on creating and analyzing silver nanoparticles that have been loaded with metronidazole for antibacterial purposes. The metronidazole-loaded silver nanoparticles were created using conventional chemical reduction techniques. We investigated the metronidazole-loaded silver nanoparticles using zeta potential, TEM, *in vitro* release kinetics and photo correlation spectroscopy (PCS). The resulting elements were globular, with average particle sizes of 5 to 20 nm, and had zeta potentials ranging from -25.9 to -37.3 mV. The release kinetics were in accordance with zero order kinetics, and r2 was greater than 0.94. According to the dissolution data, the release of the Ag-nanoparticles that are loaded with metronidazole is inversely related to the dimension of the nanoparticles, or the release is enlarged with lesser elements. The consequences propose that the metronidazole-loaded nanoparticles (NPs) would be constant in p'ceutical formulations and with no trouble absorbed at the disease site. The antibacterial properties of the silver nanoparticles with metronidazole were shown to be particularly potent against a wide variety of microorganisms. Salmonellae, S. coccus, and E. coli were the bacteria that were investigated.

KEYWORDS: Silver nanoparticles, metronidazole, cytotoxicity, microplate assay.

INTRODUCTION

A rapidly expanding area of research in the realm of nanotechnology is the bio-synthesis of nanoparticles. Because of their unique properties, AgNPs have been the subject of extensive investigation and are a significant class of nanomaterials.[1]

It is simple to create nanoparticles using a variety of physical and chemical techniques. The manufacture of metronidazole-loaded silver nanoparticles involves the chemical reduction of metal salts utilizing a variety of reducing agents while a stabilizer is present. Silver-containing salts can be reduced to create Ag nanoparticles using plummeting agents such ($NaBH_4$), (N_2H_4), formaldehyde, etc.[2]

However, the majority of chemical techniques used to make nanoparticles contain toxic, dangerous compounds that pose a biological risk and are occasionally not environmentally friendly. As a result, there is a rising need to create methods that are low-cost, non-toxic, and environmentally benign for the manufacture of metronidazole-loaded silver nanoparticles. An innovative and environmentally benign method for creating nanoparticles is the utilization of plants and microbes.[2, 3]

Since they come into view to have a significant possible for addressing the issue of the growth of bacterial multidrug confrontation, NPs are at the present seen as feasible options to antibiotics.[5] The science community has paid a lot of attention to silver nanoparticles.[6] The antiseptic and antibacterial properties of silver make it effective next to both Gram+ and Gram- bacteria, and it has long been used to treat a variety of ailments.[7] The development of a novel class of antimicrobials using silver nanoparticles was thought to be particularly promising in recent years[8, 5, and 9], providing a completely novel approach to eradicating a wide variety of pathogenic bacteria.

Engineering biocompatible, antibacterial, and functionalized compounds that can be employed is important due to bacteria's apparent resistance to antibiotics and metal ions. Numerous metallic nanoparticles have been thoroughly examined, including copper, gold, titanium, and silver. Thinly scattered

metallic silver exhibits unique qualities often associated with noble metals, including electrical conductivity, chemical stability, catalytic activity, as well as other specialized qualities like optical behavior, non-linearity, anti-bacteriostatic effects, etc. Metallic nanoparticles have different physical characteristics from ions and bulk materials. Due to its morphological form and enhanced active surface, this construct demonstrates additional qualities including strong catalytic activity.[3] Among the several noble metals, silver is a superior option for biological systems, bio-organisms, and pharmaceuticals. Since the poisonousness of silver ions and their combinations for microorganisms has been established, they are employed in wound dressings and used to create uncontaminated diet packaging material. The nanoparticles enter the cell and cause damage by interacting with molecules like DNA and protein that contain phosphorus and sulfur. By incorporating AgNPs, the specific bacterium is successfully "suffocated" and eliminated by the enzymatic metabolism of O2.[4]

MATERIALS AND METHODS

Requirements

Agarized Czapek Dox, AgNO3 Na3C6H5O7, Dulbecco's Modified Eagles Med (DMEM), glutamine, metronidazole, and MTT dye were all given by Sigma Aldrich (Germany). El Gomhoria CO. (Egypt) supplied HCL, HNO3, and deionized water. None of the compounds were further purified before usage.

Methods

The preparation of the Ag nanoparticles

The Frens method, which was also utilized to create gold nanoparticles, is used to create silver nanoparticles by citrate reduction of AgNO3.[10] Aqua regia (mixed 3 fractions HCl, 1 fraction HNO3) was used to clean a three-neck round bottom flask (100 mL), which was then cleaned with distilled water and covered with Al_2O_3 fuel to block light. AgNO3 solutions (1 mM, 100 mL) were boiled, refluxed, and continuously stirred. In situ additions of several tri-sodium citrate solutions with concentrations ranging from 38.8 to 40 mM (10 mL) were made. It had been noted that the hue of the solution had changed from colorless to golden. The solution has been abatement for a total of 15 min after the color change. The mixture had been swirled awaiting it had cooled to RT after the heat had been removed.

Ag Nanoparticles categorization

Particle size analyzes

Using photon correlation spectroscopy (Malvern Instrument, UK), the particles' imply diameter and PI were assessed in solution form immediately following synthesis. 2 millilitres of AG nanoparticles were introduced to the PCS's quartz cell. 3 measurements were made, each at a 90-degree angle to the source of the incident light.

Dimensions of Zeta Potential

We used a laser zeta metre (Malvern zeta seizer 2000, Malvern) to calculate the surface zeta potentials. The suspension electrolyte used was NaCl (2 x 10-2 M NaCl), which was diluted with double-distilled water (50 millilitre) made from liquid nanoparticle samples (5 millilitre). The pH was then set to the suitable level. 30 minutes of sample shaking. After shaking, the pH of equilibrium was calculated, and the metallic particles' zeta potential was assessed. The surface potential of the AG nanoparticles was calculated using a zeta potential. Every situation was given a 3-measure average assessment.

Transmission electron microscopy (TEM)

The dimension and shape of the Ag nanoparticles were examined using TEM (Jeol, Japan). The microscope was accelerated at an 80 kV voltage. The Ag samples were diluted in purified water (1:10) before a 20-L aliquot was applied on a grid that had been dusted with carbon. After the solution had been applied for 1 minute, the surplus was wiped off the grid with filter paper. The networks were allowed to dry in the grid box for 2 hours before imaging.

Kinetics study for the release

AgNPs with a cutoff of Mw=12KDa were put in a dialysis bag. The container was submerged in a 30 mL, PBS solution (pH 7.4). Over the course of 10 hours, a steady discharge of Ag nanoparticles was observed. Calculations were made for the release rate and kinetic order. Using the Z-5000 from Hitachi, Japan, atomic absorption spectrophotometry was used to quantify the concentration of silver emitted. To summarize, the silver nanoparticle solution was routed at 120 oC in con'c HNO3 in an oil bath for 2 hours to achieve whole solvation, and the Ag conc's was then determined by Air/Acetylene Flame using a Ag flame, Fuel flow rate 0.8 to 1.2L/min, and Atomize temperature 1100 oC.[11,12]

Anti- Bacterial effect by Micro-plate assay

The microbiology division of Al-Azhar University in Cairo, Egypt, supplied the Escherichia coli XL-1 blue, Salmonella XL-1, Staphylococcus ATCC 2784, and Pseudomonas aeruginosa ATCC 2484 strains. Bacteria were grown for 24 hours at 37 °C on maconci agar medium plates. They were eventually re-suspended in maconci solution and brought to an optical density of 0.5 at 595 nm. To create cells with an about 10^6 counts/ml concentration, they were then diluted in Maconci broth medium. This was confirmed by the plate reader counts. Cells were exposed to doses of silver nanoparticles ranging from 10 M to 160 M. Growth kinetics were investigated using a 96-well plate and 96 microplate readers (Tecn Infinity M200, Switzerland). Turbidity may be measured at wave lengths between 400 and 700 nm in order to prevent any interference with silver absorption, which is known to occur at 450 nm. Optical density (OD) at 700 nm was used to monitor bacterial growth in the culture media. Replicate measurements of

the samples were taken at a temperature of 37 oC. The data were collected every 30 minutes, and shaking was done in between measurements. The survival was calculated from the last point of the growth curve in comparison to a control value.

The cytotoxic effect of the Ag nanoparticles and the MTT assay

Person fibroblast cells, designated as HBT68, were graciously given by Dr. Aly Fahmy of VACSERA. These cells were raised in Dulbecco's Modification of Eagles Medium (DMEM), which also contained fetal bovine serum (10%V/V), glutamine (2 mM), and metronidazole (1 g/mL). In order to obtain 75% confluency, a Person fibroblast cell line was multiplied in 6 well plates at a density of 5×10^4 cells/ml in DMEM and incubated for 24 hours at 37 °C with 5% CO2. Following that, the cells were exposed to growth media containing 200 mL of Ag nanoparticles at conc's ranging from 5 to 30 mg per 100 mL. The cells were stained with MTT dye after being washed three times with PBS solution. The MTT solution was injected into each well in an amount of 50 µl, and it was then incubated for a further four hours at 37 °C with 5% CO2. The insoluble formazan creation generated within living cells was rendered soluble by adding DMSO: glycine buffer (125 µl). The intensity of the solutions was assessed by a device (Tecn Infinity M200, Switzerland) and the relative cell viabilities were computed as a percentage of the control. The cells were then placed on the plate and kept there for four hours while being incubated at 37 °C and 5% CO2. The cell viability was evaluated using a microplate reader (Tecn Infinity M200, Switzerland).

Visualizing cell viability

After being taken away from the growing average, the cells underwent three PBS solution rinses before being stained with MTT dye. The MTT solution was injected into each well in an amount of 50 l, and it was then incubated for a further4hrs at 37 °C with 5% CO2. Using a stereoscope microscope (Nikon SMZ 800, Japan), photos of the samples were taken. The cell counts were calculated by straight counting.

Conclusion and dialogue

Particles' sizes are measured

Using photon correlation spectroscopy (PCS), the mean particle size diameter and polydispersity indices were all determined in solutions immediately following synthesis. The metronidazole-loaded silver nanoparticles' size, granulometric distribution, and number of particles as well as their occupied volume have all been recorded. The data showed that the particles' sizes ranged from 5 to 50 nm. Nanoparticles with low polydispersity indices were detected, according to particle size analyses. The outcomes are listed in table 1 and shown graphically in figure **Table1:** Using photon correlation spectroscopy, the metronidazole-loaded silver nanoparticles' particle size distribution and polydispersity indices;

Sample number	Z-average (nm)	Polydispersity index
S1	5 ± 0.4	0.05 ± 0.004
S2	15 ± 0.2	0.03 ± 0.003
S3	50 ± 0.1	0.08 ± 0.002

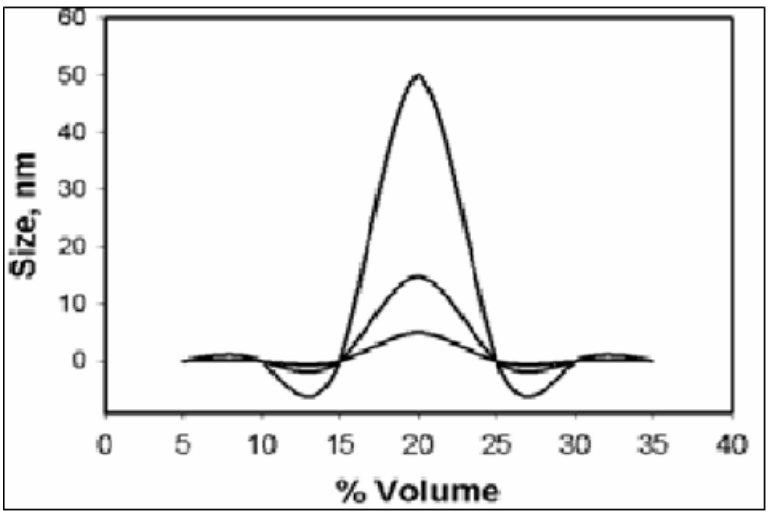


Figure 1. Particle number and occupied volume (% of volume) are expressed against the particle number and mean average particle size (nm).

Zeta potential

In Table 2 and Fig. 2, the dimensions of S1's zeta potential in solution are compiled. The observed zeta values for the generated nanoparticles were established to range among 25.9 and 37.3 mV. These worth's offer this thorough stabilization of the nanoparticles at varied pH levels, which may be the major cause of particle sizes with a limited size distribution index.

Table 2: Ag nanoparticle zeta potential tests at various pH ranges.

pH	Zeta potential (mV)
4	−25.9±0.36
7.4	-29.1±0.40
9	-37.5± 0.32

This work showed that silver nanoparticles with metronidazole attached were stable over a wide pH range and had no tendency to aggregate or form self-assembled aggregates.

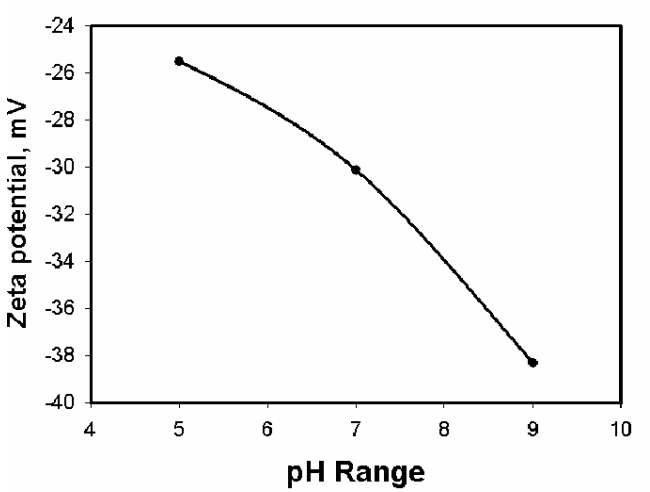


Figure 2. Spherical Ag nanoparticles' zeta potential.

Transmission electron microscopy

TEM was used to study them. Different fields were used to measure the particle sizes and shape. The electro-micrograph's silver particles were spherical and had well defined particle sizes. It is obvious that the Ag nanoparticles are round in form and have tightly regulated particle sizes. Additionally, and as would be predicted, preparation circumstances have a significant impact on particle size.

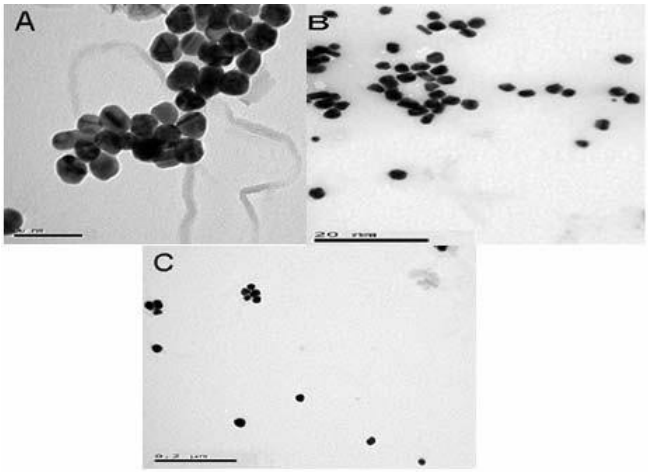


Figure 3. TEM pictures of a silver disc.

Kinetics of the Silver nanoparticles release

When a sample is rest in a dialysis bag and discharged into the discharge medium from side to side a dialysis casing. The early stages of the testing produced a low concentration of silver emissions, thus atomic absorption was used to assess each sample. The method was demonstrated to be efficient and extremely responsive in detecting the low proportion of Ag used, particularly at the start of the trial. Throughout the investigation's 10-hour duration, a steady flow of silver was observed. The silver concentration and dissolution rate followed zero order kinetics with an $r^2 > 0.94$. The cumulative drug discharged% for the Ag nanoparticles discharged into the media vs. time (h). The release profile demonstrated that sample 1 of the Ag nanoparticles released more quickly than samples 2 and 3. Approximately 83% of the silver nanoparticles S1, 73% of S2, and approximately 65% of S3 were released over the course of the experiment, or after 10 hours.

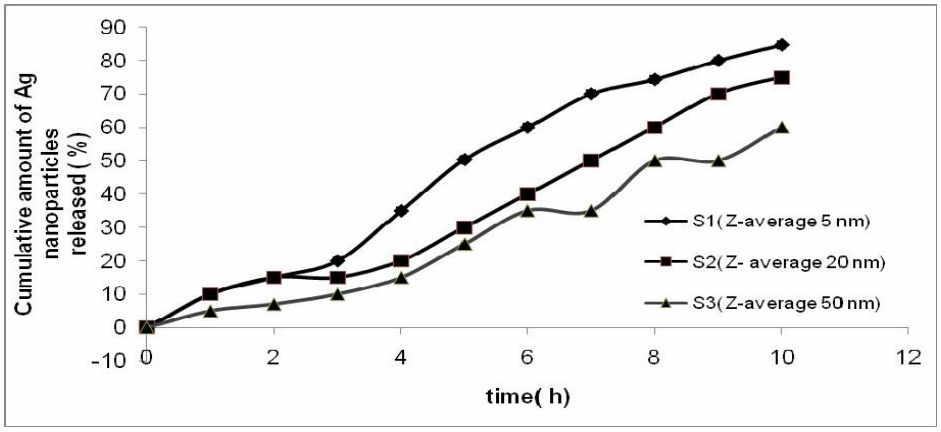


Figure 4. Silver released cumulatively measured using atomic absorption spectrophotometry as a function of time.

Anti Bacterial Effects

The four common bacterial species that were used to assess S1's antibacterial activity were P. aeruginosa, Salmonella, E. coli, P. aeruginosa, and S. coccus. The results of Ag nanoparticles on the suppression of antibacterial enlargement were monitored using OPD on a microplate booklover at con'c of the NPs ranging from 10 to 150 M. The consequences show how many bacterial species have unique and inborn morphological, physiological, and metabolic characteristics. It is significant to note that contrasted to the additional 3 Gram negative bacteria, Salmonella, E. coli, and P. aeruginosa, S.coccus is more susceptible to the antibacterial effects of Ag NPs. The degree of permeability determines the difference between Gram+ and Gram- bacteria's outer walls, with gram negative cells having an exclusion threshold for molecules with a MW greater than roughly 600Da.

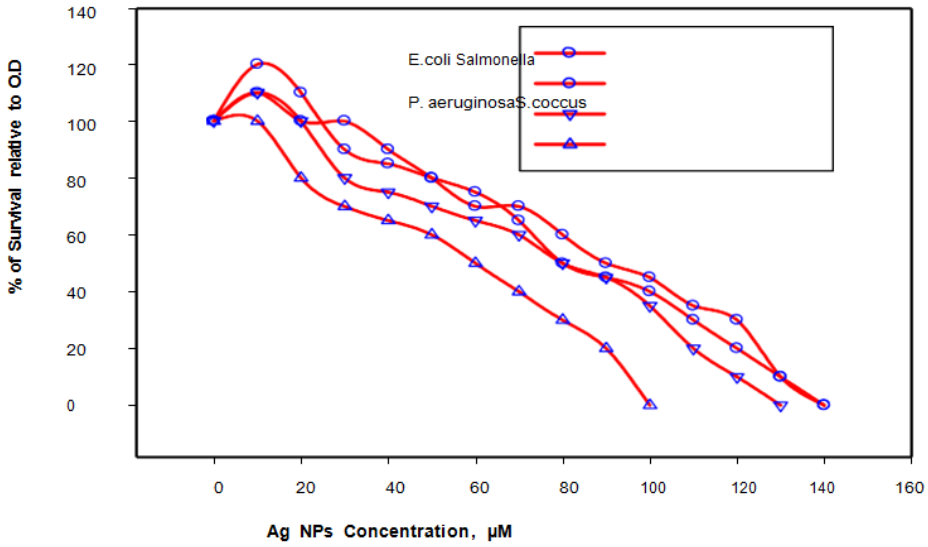


Figure 5. The curve showing bacterial strain survival under various silver nanoparticle concentrations.

Assessment of Cytotoxicity

Studying the cell viability qualitatively

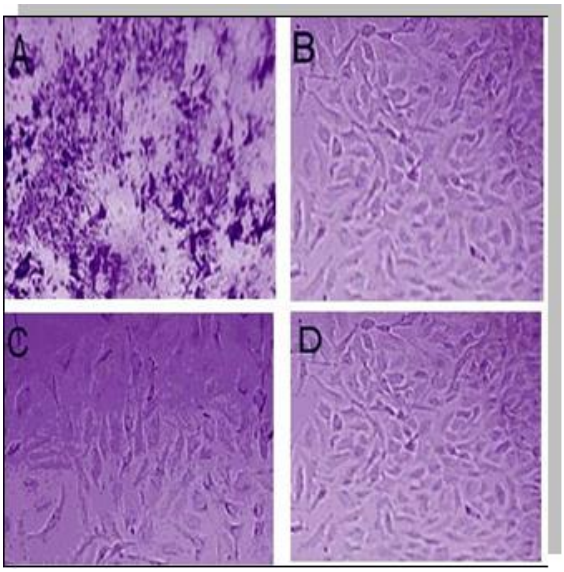
The MTT dye has been used to conduct qualitative study on the cellular cytotoxicity when utilized as a stain for person fibroplast cells. According to the concentration of AgNPs used and the span of the treatment period, silver nanoparticles (5 nm) are shown in Figure 7 to exhibit a variety of hazardous behaviors (data not reported). The MTT assay was employed to quantitatively evaluate the in vitro cytotoxicity of the particles after they had been administered to human fibroblast cells at various dosages of silver nanoparticles. For eight days, NPs in the concentration range of 0.05 to 0.33 mg/mL were used. Merely cells that are still alive after 8 days of contact to Ag can successfully metabolize a dye to produce a purple precipitate that can be counted and viewed using a microplate reader in this test. After 8 days of post-treatment with Ag nanoparticles (0.05 mg/mL), the cells demonstrated good cell viability. It was establish that each well contained roughly 2.91 106 live cells. This shows that exposure to silver nanoparticles at low concentrations (0.05 mg/mL) has no impact on the cell's ability to develop. Cell development was impacted by silver nanoparticle concentrations as low as (0.05 mg/mL). However, action of the cells with 0.2 mg/mL of nanoparticles showed low cell viability while treatment

of the cells with a quantity of silver equal to 0.10 mg/mL showed good cell vitality with 2.1106 cells per well. The presence of silver nanoparticles led to the discovery of 1.8 106 cells per well. Finally, treatment with 0.30 mg/mL of colloidal Ag nanoparticles demonstrated extremely limited cell viability. It was establish to be as low as 1.05 106 cells per well, which were all remained alive after the adding of Ag nanoparticles, as shown in table 3 and figures 7 and 8.

Because nanoparticles frequently cover the perinuclear region, the hypothesis that they can be harmful to cells has been documented in the literature. It is assumed that this happens during the endocytotic pathway. Molecular motor proteins and functionalized silver nanoparticles are said by Suh et al. to be the driving forces behind this controlled intracellular movement of nanocarriers. Therefore, utilizing a sufficient concentration of nanoparticles is crucial to maintaining their safety. Furthermore, it was evident that cytotoxicity depends on particle size and concentration (data not shown). Silver nanoparticle use in pharmaceutical preparations can be safely started by determining the safe dose for human cells.

Table 3: the viability of the Cell using MTT assay as.

Conc. (mg/mL)	Number of cell (million)	Control
0.04	2.88	2.0
0.08	2.20	2.0
0.12	1.98	2.0
0.16	1.01	2.0
0.24	0.98	2.0



Figiure 7. The cytotoxicity activity study of silver nanoparticles on human fibroblast cell HS68 cells after eight days of addition of various silver nanoparticle concentrations using (A) 0.24mg/mL of silver, (B) 0.16mg/mL of silver, (c) 0.8mg/mL of silver, and (D) 0.04mg/mL of silver

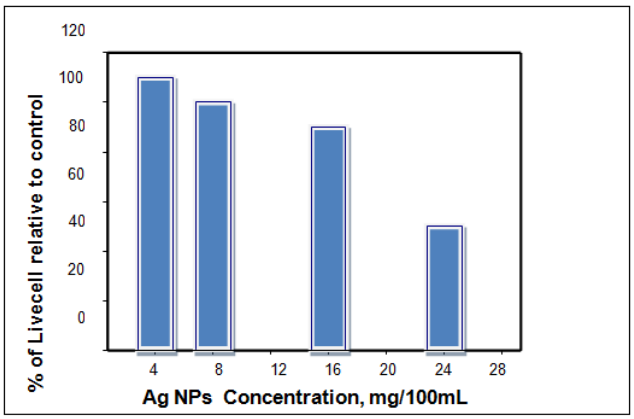


Figure 8. A quantitative evaluation of the cytotoxicity of silver nanoparticles on the human fibroblast cell HBT68 was performed using a microplate reader.

CONCLUSION

This study shows that solutions containing Ag NPs with a size range of 5–50 nm and their stability over a broad pH range. The size distribution of the silver particles became more homogenous and narrow when they were coated with citrate. These results are essential for comprehending how various cell types react to cytotoxicity in various ways. Ag NPs are safe to use in food chemistry, therapy, molecular imaging, and additional medical sectors due to their lack of obvious toxicity when utilized for an extended length of time at this concentration. The findings offer convincing proof that silver nanoparticles may be used as antibiotics to eradicate different types of bacteria while avoiding the unfavorable side effects and inactive effects of traditional antibiotics. It will eventually be important to ensure that the design of nanomaterials results in solutions that are both effective and secure, which calls for such stimulating environmental and toxicological research.

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