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Formulation and evaluation of silver nanoparticles as antibacterial and antifungal agents with a minimal cytotoxic effect

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Abstract

Preparation of non-biodegradable nonoparticles is a fast growing field, which is vital in both nanomedicine and nanotechnology applications. In this investigation, our attention will be focused on the preparation and evaluation of colloidal silver nanoparticles as antibacterial and antifungal agents. The colloidal silver nanoparticles have been prepared employing standard chemical reduction methods. The colloidal silver nanoparticles were characterized using transmission electron microscopy TEM, zeta potential, photo correlation spectroscopy PCS, and in vitro release kinetics. The particles thus obtained were spherical in shape and having an average particles size of 5-20 nm , zeta potentials of -25.5 to -38.3 mV, and the release kinetics was following zero order kinetics with r^2>0.96. The dissolution data indicates that the release of the silver nanoparticles is inversely correlated with the size of the nanoparticles i.e. the release increased with smaller particles. The results suggest that the Ag NPs would be stable in the pharmaceutical preparations and will be easily to the infection site. The colloidal silver nanoparticles were found to be very efficient antibacterial agents for different types of bacteria. The bacteria studied were namely: E. coli, S. coccus, Salmonellae, and P. aeruginosa. The associated antifungal effects were also investigated for Aspergillus and Pencillium. . Cytotoxicity of the nanoparticle was studied using human fibroblast cell line. It was concluded that cytotoxicity is concentrations dependant. The results provided strong evidence that could warrant the consideration of silver nanoparticles as antibacterial and antifungal agent that could circumvent the side and passive effects of the conventional antibiotics.

Keywords: Silver nanoparticles, antibacterial, antifungal, cytotoxicity, micro-plate assay, release kinetics.

Introduction

Noble metals nanoparticles synthesis attracts an increasing interest owing to their new and different characteristics as compared with those in the macroscopic phase. Nanoparticles have several attractive applications in various fields, such as medicine and biotechnology [1]. Upon reaching nanoscale, like other nanomaterials and primarily by virtue of extremely small size, silver nanoparticles exhibit remarkably unusual physicochemical properties and biological activities.

Silver nanoparticles have the properties of a high surface area, very small size (<20 nm) and high

dispersion. Moreover, colloidal silver solutions (CSSs) have an increased interest due to their antimicrobial properties, with direct applications in pharmacology, veterinary medicine and so on. The interaction of metal nanoparticles with microorganisms (from fungi to viruses, e.g. HIV) is an expanding field of research [2]. It is believed that the mechanism of the antibacterial effect of silver nanoparticles involve their absorption and accumulation by bacterial cells and accompanying shrinkage of the cytoplasm membrane or its detachment from the cell wall. Alternatevely, silver ions might interact with the S–H bounds of the proteins which is leading to the inactivation of them [3]. Upon binding of the nanoparticls to DNA, DNA molecules become condensed and loose their ability to replicate, which may be the main mechanism by which the nanoparticles inhibit the bacterial replications with [4]. Understanding the forces governing the colloidal stabilities [5] is an essential requirement for the preparation of colloidal nanoparticles. Particles can be stabilized either electrostatically or sterically. Sterical stabilization is accomplished by adsorbing either polymers or surfactants onto the surfaces of the particles to overcome nanoparticles agglomeration [6]. While, the electrostatic stabilization is attained by capping with charged molecules, which form chemical bonds with/or chemisorbs onto the particles [7].

Silver powder was believed to have a beneficial healing effect for ulcers [8]. Silver compounds were major weapons against wound infection in recent year's with the advent of antibiotics [9].

In this paper we focus on the preparation and characterization of silver nanoparticle and the study of their antimicrobial and antifungal effects on four types of bacteria; namely, E. coli, S. coccus, Salmonellae and P. aeruginosa and the fungal species Aspergillus and pencillium. Micro plate based assays will be utilized for monitoring bacterial growth and inhabitation. Manipulation of the best dose would provide safe formulas in future. Therefore, the aim of this article will include a brief study for the cytotoxicic effect of differ colloidal silver nanoparticle on human fibroblast cell line via long term of use.

Materials and methods

Materials

Silver nitrate $AgNO_3$, tri-sodium citrate $Na_3C_6H_5O_7$ maconci agar medium ,agarized Czapek Dox ,Dulbecco's Modification of Eagles Med (DMEM) ,glutamine, penicillin, streptomycin and MTT dye , all were provided by Sigma Aldrich (Germany). Hydrogen chloride HCL, Nitric acid HNO_3 and deionized water were provided by El Gomhoria CO. (Egypt). All chemicals were used without additional purification.

Methods

The Synthesis of the Silver nanoparticles

The Silver nanoparticles are synthesized by the citrate reduction of $AgNO_3$ via Frens method that was used for gold nanoparticles synthesis [10]. 3-neck round bottom flask (100 mL) was cleaned with aqua regia (3 parts HCl, 1 part HNO_3), rinsed with deionized water and covered with alumina fuel to prevent light. Ag NO_3 solutions (1mM, 100 mL) were heated to boiling and refluxed while being constantly stirred. Different concentrations of tri-sodium citrate solutions ranged from 38.8 to 40 mM (10 mL) were added in situ. The solution color had been noticed to change from colorless to golden yellow. After the color change, the solution has been refluxed for an additional 15 min. The heat was then turned off and the solution had been stirred until it cooled to room temperature.

Characterization of Silver Nanoparticles

Particle size analyzes.

Mean particle size diameter and polydispersity index were all measured in a solution form directly after synthesis, using photon correlation spectroscopy (Malvern instrument, UK). Silver nanoparticles (2 mL) were added to the quartz cell of the PCS. Measurements were taken in triplicates at 90° to the incident light source.

Zeta Potential Measurements

Surface zeta potentials were measured using the laser zeta meter (Malvern zeta seizer 2000,

Malvern). Liquid samples of the nanoparticles (5ml) were diluted with double distilled water (50 mL) using NaCl as suspending electrolyte solution (2×10^{-2} M NaCl). The pH was then adjusted to the required value. The samples were shaken for 30 minutes. After shaking, the equilibrium pH was recorded and the zeta potential of the metallic particles was measured. A zeta potential was used to determine the surface potential of the silver nanoparticles. In each case, an average of three separate measurements was reported.

Transmission electron microscopy (TEM)

The size and morphology of the silver was studied via transmission electron microscopy (TEM) (Jeol, Japan). The microscope was operating at an accelerating voltage of 80 kV. The silver samples were first diluted (1:10) in distilled water, and an aliquot (20 µL) was applied onto a carbon coated grid. The solution was then left for 1 minute, and the excess was removed from the grid by blotting with a filter paper .The grids were placed in the grid box for two hours to dry before imaging.

Kinetics of the release study

Silver nanoparticles were placed in a dialysis bag with a cut off of Mw=12KDa. The bag was suspended in a phosphate buffer saline PBS solution (30 mL, pH 7.4). A continuous release of silver nanoparticles was measured over a period of 10 h. The release rate and order of kinetics were calculated. The concentration of silver released was determined by atomic absorption spectrophotometry (Z-5000, Hitachi, Japan), Briefly the solution of silver nanoparticles was processed at 120 ºC in concentrated nitric acid in an oil bath for two hours for complete solvation and then the silver concentration was measured by Air/Acetylene Flame via using silver flame, Fuel flow rate 0.9 to 1.2L/min and Atomize temperature 1100°C [11, 12]. The percentage of silver released was calculated using equation (1)

$$\% \text{ cumulative silver released} = [W_t / W_c] \times 100.$$

Where W_c is the total silver content in the dialysis bag and W_t is the silver content in the PBS medium at a time.

Micro-plate assay

Anti- Bacterial effect

Escherichia coli XL-1 blue, Salmonella XL-1 and Staphylococcus ATCC 2784 and Pseudomons aeruginosa ATCC 2484 were purchased from the department of microbiology (Al-Azhar University, Cairo, Egypt). Bacterial cells were allowed to grow for 24 hours on a maconci agar medium plates (37 °C). They were then re-suspended in maconci medium to reach an optical density of 0.5 at 595 nm. This followed by their dilution with maconci broth medium to yield an approximate cells concentration of 10^6 counts/ml. This was confirmed by the plate reader counts. The cells were exposed to a range of silver nanoparticles concentrations ranging from 10 µM to 160µM. The growth kinetics were analyzed in 96 wells plate and monitored by a micro-plate reader (Tecn Infinity M200, Switzerland). The turbidity could be measured at wave length between 400-700 nm and to avoid any interference with silver absorbance that is known to occur at 450 nm, the bacterial growth in the culture medium was monitored by measuring the optical density OD at 700 nm. The samples were measured in replicates at a temperature of 37 °C. Shaking was carried out between measurements and the data were recorded every 30 minutes. Survival was calculated from the last point in the growth curve, as related to a control value.

Anti-fungal effect

Pencillium LB111 and Aspergillus AGS 530 were purchased from the department of microbiology (Al-Azhar University, Cairo, Egypt). The antibiogram method was used [13] for fungal cell growth on mycotoxins (aflatoxin). Petri vessels with agarized Czapek Dox medium were inoculated by spreading fungi medium in 96 well micro plates and were incubated for 24 hrs, at 37 ºC. The cells were exposed to solutions of silver nanoparticles with different concentrations ranging from 10 µM up to 140 µM. Growth kinetics' in the 96 well micro plate was optically monitored using a micro plate reader (Tecn Infinity M200, Switzerland). Again, the turbidity was measured at: 400- 700 nm: in order to avoid any interference with characteristic silver absorbance peak at 450

nm, the growth of fungi in the culture had been monitored by measuring the optical density "O.D" at 700 nm. Samples were measured in replicates, at 37 °C with a constant shaking between each successive measurement, the data were recorded every 30 minutes. Survival was calculated from the last point in the growth curve, related to a control value.

MTT assay and the cytotoxic effect of the silver nanoparticles

Human fibroblast cell HBT68 cells (generously gifted by Dr/Aly Fahmy, VACSERA) were cultured in Dulbecco's Modification of Eagles Medium (DMEM) containing fetal bovine serum (10%V/V) glutamine (2 mM); penicillin (100 U/mL) and streptomycin (1 µg/mL). Human fibroblast cell line was grown in 6 well plates at a density of 5×10^4 cells/ml in DMEM and incubated to 75% confluency after 24h in 5% CO_2 at 37 °C. The cells were then exposed to silver nanoparticles (200µL) at different concentrations ranging from (5 -30 mg per100 mL) of growth medium. The cells were then washed with PBS solution three times and stained with MTT dye. MTT solution (50 µl) was added to each well, followed by incubation at 37°C and 5% CO_2 for a further 4 hours. The insoluble formazan product formed within living cells were solubilized by adding DMSO:glycine buffer (125 µl). The intensity of the solutions was measured using (Tecn Infinity M200, Switzerland) with relative cell viabilities being determined as a proportion of the control. After this treatment, the cells were added to the plate and incubated for four hours in 5% CO_2, at 37 °C. The cell viability was evaluated using micro plate reader (Tecn Infinity M200, Switzerland).

Imaging of the cell viability

The cells were removed from culture medium, washed with PBS solution three times and stained with MTT dye. MTT solution (50 µl) was added to each well, followed by incubation at 37°C and 5% CO_2 for a further 4 hours. The samples were imaged via (Nikon SMZ 800, Japan) stereoscope microscope. The cells numbers were determined by direct counting method.

Result and discussion

Particle size measurements

Mean particle size diameter and polydispersity indices were all measured in solutions directly after synthesis, using photon correlation spectroscopy (PCS). The size of the colloidal silver nanoparticles, their granulometric distribution has been recorded, expressed against the particles number and their occupied volume. The data illustrated that the size of the particles was between 5 and 50 nm. Particle size analysis showed the presence of nanoparticles with low polydispersity indices (Table 1). The size distribution was in concurrence with other nanos-systems described in the literature [14-17]. By studying the dynamic light scattering, it is clear that the silver nanoparticles has a narrow size distribution for the nanoparticles that have the z-average ranged from 5 to 50 nm, and mono sized for the particles that have the average z-equivalent to 20 nm. The results are tabulated in table 1 and depicted in figure 1.

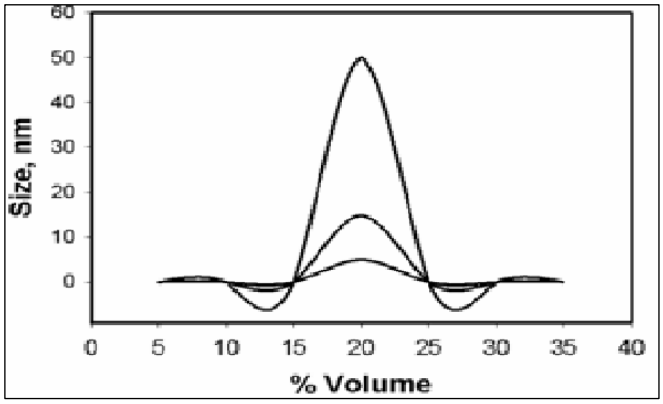


Figure 1. Particle size distribution, using photon correlation spectroscopy, the mean average particle size (nm) are expressed against the particles number and their occupied volume (percentage of volume).

Table1: The particle size distribution and the polydispersity indices of the colloidal silver nanoparticles using photon correlation spectroscopy.

Sample number	Z-average (nm)	Polydispersity index
S1	5±0.5	0.05±0.005
S2	15±0.3	0.03±0.002
S3	50±0.2	0.08±0.001

Zeta potential

Table 2 and Fig 2 are summarizing the zeta potential measurements of S1 in a solution form. For the obtained nanoparticles, zeta values were measured and found to fall between −25.5 and −38.3 mV. These values provide full stabilization of the nanoparticles at different pH, which may be the main reason in producing particle sizes with a narrow size distribution index. Values of the zeta potentials of the citrate treated silver nanoparticles in addition to their narrow size distributions provide satisfactory evidence about their little tendency towards aggregation. This behavior unambiguously suggests the presence of strong electric charges on the particles' surfaces to hinder agglomeration. These values were found to fall in the negative side which showed the efficiency of the capping materials in stabilizing the nanoparticles by providing intensive negative charges that keep all the particles away from Reddick threshold [15, 16, 18, and 19]. Charged particles are able to interact across long distances via electrostatic forces and the surface charge may be disrupted as a way to induce nanoparticles assembly by modifying the condition of the reaction.

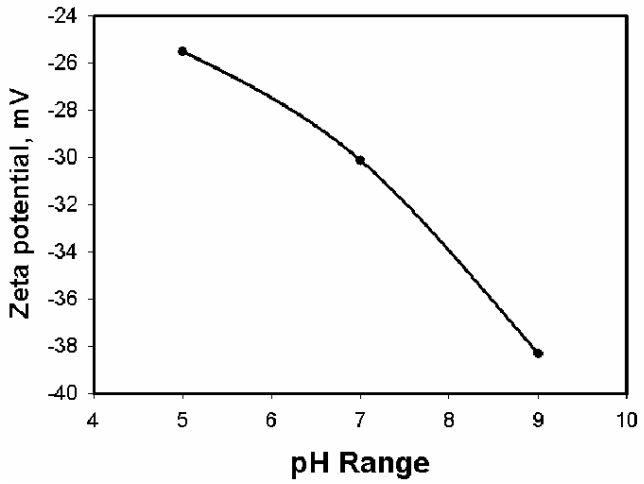


Figure 2. Zeta potential of spherical silver nanoparticles. Zeta potential (mV) values were measured at different pH ranges.

Table 2: Zeta potential measurements using silver nanoparticles at different pH ranges.

pH	Zeta potential (mV)
5	-25.5±0.38
7.4	-30.1±0.41
9	-38.3±0.33

In this work, colloidal silver nanoparticles were proven stable over a wide range of the pH with no tendency for aggregation or formation of self assembled aggregates. This observation is contrary to suggestions that surface modification is mandatory to the particles [15, 16 and 18]. It universally accepted that charged particles are capable of interacting across a long distance scale via electrostatic repulsive forces. Disruption of the surface charges plays a paramount role in the assembly of the nanoparticles [18]. Therefore, using these nanoparticles as a drug may provide a new horizon in avoiding bacterial and fungal resistance. In the studied series (S1) the electric charges were strong enough to hinder agglomeration and to provide stabilization of the nanoparticles. That suggests that the Ag NPs particles and thus their solution is stable, which is also in accordance with the result reported before for colloidal nanoparticles stability [15, 16 and 18].

Transmission electron microscopy

Figure 3 is showing the size and the morphology of the S1, S2 and S3. They were studied using transmission electron microscopy (TEM). The morphology and the diameters of the particles were measured using different fields. The silver particles in the electro-micrograph were in a spherical form with a well defined particle size. The size has been reported as the mean diameter as shown in figure 3. It is clearly apparent that the silver nanoparticles have spherical form with a well-controlled particle size. Also and as would be expected, the particle size strongly depends preparation conditions. The average particle size of the measured particles was as small as 5 to 50nm, which is in agreement with the results obtained from the PCS data.

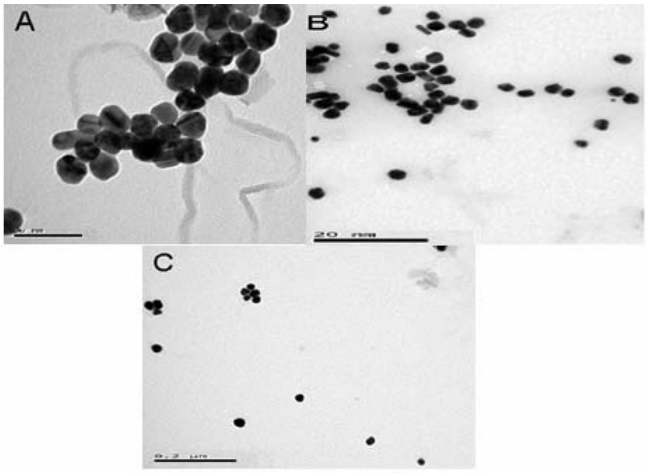


Figure 3. TEM images of spherical Silver, The scale bars were 50nm (A) and magnifications 80 kx, the scale bars were 20 nm (B) and magnifications100 kx and the scale bars were 200 nm (C) and magnifications 50 kx.

Table 3: the viability of the Cell using MTT assay as measured using micro-plate.

Conc. (mg/mL)	Number of cell (million)	Control
0.05	2.91	2.1
0.10	2.1	2.1
0.20	1.8	2.1
0.30	1.05	2.1

Kinetics of the Silver nanoparticles release

Figure 4 is showing the release kinetic of Sample 1, Sample 2 and Sample 3when placed in a dialysis bag and released into the release medium through dialysis membrane [19-21]. The measurement of each sample was conducted using atomic absorption due to the low concentration of silver released in the early stages of the experiments. The method was proven valid and very sensitive in recognizing the low percent of the silver used especially at early stage of the experiment. Continuous release of silver has been observed over the period of the study (10 hr). The rate of dissolution and the concentration of silver was obeying the zero order kinetics with r^2>0.96. The silver nanoparticles that released to medium are expressed as cumulative drug released % versus time (h). This is similar to the patterns observed with other drugs [22-24]. The release profile showed that the rapid release of the silver nanoparticles sample 1 in comparison to the release of sample 2 and Sample 3. Throughout the duration of the experiment i.e after 10 h, about 85% of the silver nanoparticles S1, about 75% of the S2 and about of 60% of S3 were released consequently.

This rapid release was correlated with the particle size and increasing surface area of the silver nanoparticles. Therefore, small nanoparticles underwent more rapid release kinetics than the bigger nanoparticles due to higher diffusability of the small particles. There was a difference in the final cumulative drug released throughout the study. Therefore, samples might be rapidly released when incorporated in a dosage form, only when they have a small particle size down to 5 nm. This consequently may enhance their absorbtion when applied in vivo. The above results suggest that the silver nanoparticles would be stable and rapidly released when applied at the infection sites.

Anti Bacterial Effects

The antibacterial activity of S1 was assayed against four common bacterial species namely: E.coli, Salmonella, P. aeruginosa and S. coccus. The effects of silver nanoparticles on the anti bacterial growth inhibition was monitored by optical density measurements using a micro-plate reader, for different concentrations of the NPs.

varying from 10 to 150 μM. The results reflect the innate and diverse morphological, physiological and metabolic characteristics diversity of different kinds of bacteria. It is noteworthy here that in the case of S.coccus Gram positive, the Ag NPs are more effective as anti bacterial agents as compared to the other three bacteria; namely: E. coli, Salmonella and P. aeruginosa Gram negative. Due to the difference between outer wall if Gram positive and Gram negative bacteria is given by the degree of permeability, with exclusion limit for substances with a molecular weight of more than approximately 600Da for gram negative cells.

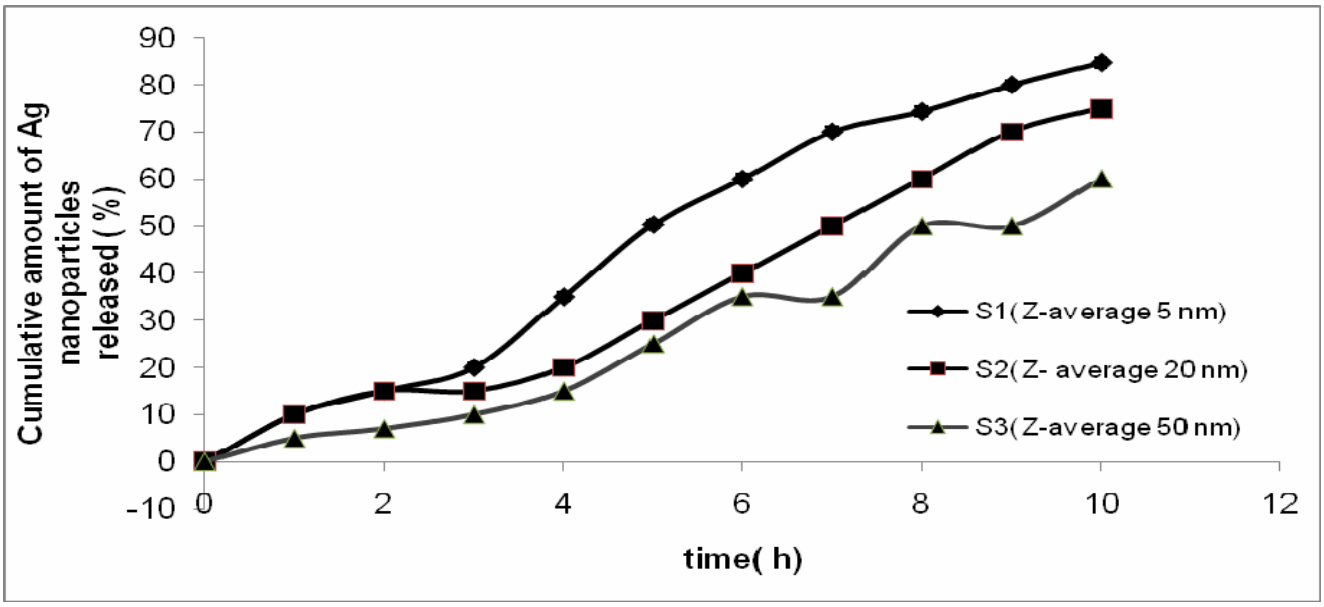


Figure 4. Cumulative amount of silver released **%** versus time using atomic absorption spectrophotometry.

Outer wall of the Gram negative act as permeability barrier due to the presence of a lipopolysacharide layer that is able to exclude macromolecules and hydrophilic substance, therapy, being responsible for the intrinsic resistance of gram negative bacteria [25, 26]. E. coli, completely died with conc. 150µM, Salmonella completely died with conc. 140µM, P. aeruginosa completely died with conc. 130µM and S. coccus completely died with conc. 100µM as figured in Figure 5. Overall the differences in four types of bacteria are attributed to genome islands encoding a lot of toxins [27].

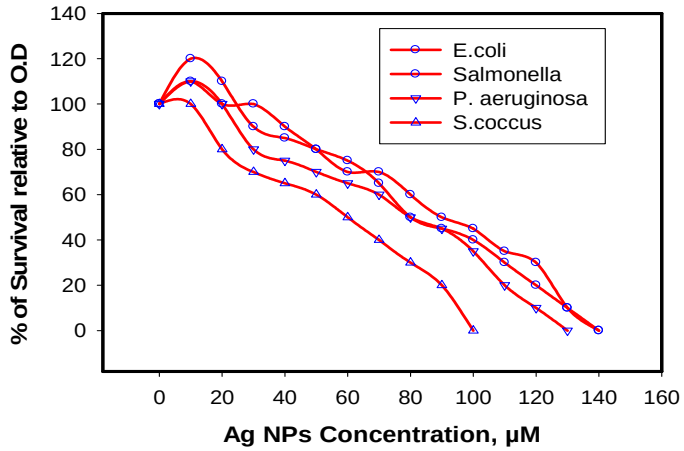


Figure 5. The survival Curve of Bacterial Strains at different concentrations of Silver nanoparticles.

It has been generally believed that the mechanism of the antibacterial effects of silver ions Ag^+ involves their absorption and accumulation by the bacterial cells that would lead to shrinkage of the cytoplasm membrane or its detachment from the cell wall [3, 28]. At lower concentration, silver nanoparticles directly damage the cell envelope by penetrating the cell and then silver binds to the DNA, this complex prevents the DNA replication by displacement of hydrogen bonds between adjacent nitrogen of purines and pyrimidines [4, 29]. As a result, DNA molecules become condensed and lose their ability to replicate upon the infiltration of Ag^+ ions.

The silver ions also interact with the S–H bonds of the proteins, thus blocking and inactivating them [2-5, 28].Therefore bacterial species that namely "E.coli, Salmonella and P. aeruginosa" and "S. coccus" were susceptible to the lethal effects of our prepared unmodified silver NPs. This outcome is agreement with the finding of Kim and pal et al., who's investigated observe signification killing of micro organism using silver NPs [30]. Also this outcome is agreement with the finding of pal et al., who mentioned that the silver nanoparticles undergo shape dependent interaction with gram negative bacteria [31], But our synthetic silver nanoparticles were more effective on both gram

negative and gram positive bacterium due to our belief that the inhibition of the growth of microorganisms is not related to penetration of outer cell wall but is also dependent on the size of silver and depend on the capping material as well. Additional factors, albeit not completely understood, could come into play; e.g. the activity of the silver NPs and the kinetics and mechanism of permeability. The result thus obtained clearly indicates that the unmodified nanoparticles with their smaller size and unique surface properties are more potent [1, 8, 9].

In addition, the nature of the surface treatment plays a paramount role in the ability of the nanoparticles to inhibit the growth of microorganisms. In this work, Ag NPs were small in size due to using of citrate as a capping material instead of using polymers. Using citrates as capping materials may provide higher particles stability than that provided by the polymer. Therefore, a higher penetration efficiency of the bacterial cell membrane was recognized.

The result thus obtained clearly indicates that the unmodified nanoparticles with their smaller size and unique surface properties are more potent [1,8 ,9]. In addition, the nature of the surface treatment plays a paramount role in the ability of the nanoparticles to inhibit the growth of microorganisms.

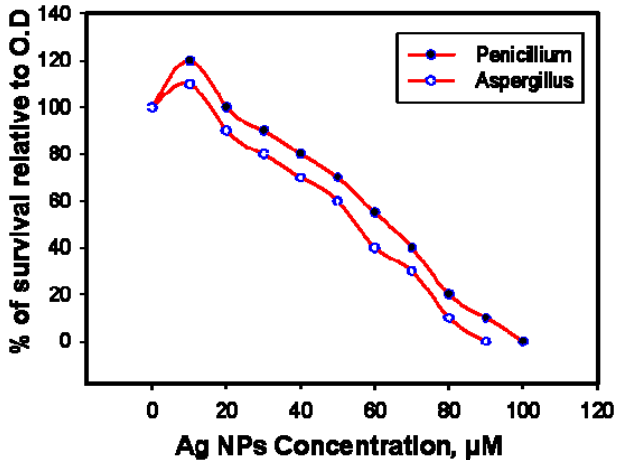


Figure 6. Survival curve of fungal strains using silver nanoparticles.

Antifungal effect

The colloidal silver nanoparticles were tested to evidence their antifungal properties. The antifungal properties of silver nanoparticles against two fungal species "Aspergillus and pencillium", are shown in figure 6. The growth inhibitory concentration measurements were measured using fractional concentration from (10 to 100 µm). The antifungal activity of silver ions solution may be due to the interaction with protein, which leads to the inactivation of protein and direct interaction with DNA, this interaction creates the mutation and stops the replication ability of DNA [3,4,28]. This solution can also go through the cell wall easily, since those materials are very small in size. This accumulation in the call memebrane can induce the cell lysis [2,35]. Also the antifungal activity of silver nanoparticles may be attributed to the disruption of trans-membrane energy metabolism. In addition to the disruption of the membrane electron transport chain may be disrupted by formation of insoluble compounds in the cell wall.

The formation of insoluble compound may be due to the inactivation of cell wall sulphydryl group. On the other hand, silver ions can create mutation in fungal DNA by displacing the hydrogen bonds [2,3,5,28] They also can dissociate the enzyme complexes, which are essential for respiratory chain and membrane permeability disruption of membrane bounded enzymes and lipids could cause the cell lysis [2,35]. Due to the difference in outer cell wall genome of Pencillium and Aspergillus. Therefore Pencillium was more resisting to silver NPs than the Aspergillusp hence Pencillium needed a higher concentration of silver nanoparticles (100µM), while the Aspergillus were completely died upon treated with 90µM of silver. Therefore Aspergillus and pencillium are susceptible to the lethal effects of the prepared silver due to their smaller size and lack of polymers on their surfaces.

The Pencillium is needed higher concentration of the silver nanoparticles than Aspergillusp due to the difference in outer cell wall genome of the two

fungi species, which it have been reported in litrature [36, 37].

It is our belief that the inhibition of the growth of microorganisms is not related only to the penetration of outer cell wall but is also dependent on the size of silver. In this work, citrate was used as as a capping material for silver nanoparticles instead of using polymers due to the fact that some of the fungal species are highly resistant towards some polymer such as chitosan since those species contain chitosan biopolymer in their cell wall.

Aspergillus is the best example for chitosan resistant fungus [32]. The interaction of the surface modified nanoparticles with the peptide glycol layer of the cells has a remarkable effect on the inhibition of growth of microorganisms [33]. This outcome is in agreement with the finding of Berger TJ, et al., who's investigated and observed antifungal properties of metallic nanoparticles[34]. This out come also is in agreement with the finding of of other group who investigated the antifungal effects of silver NPs [29]. The latter group used modified silver chitosan nanoparticles for investigation of the antifungal effects nanoparticles. The results obtained clearly indicate that the unmodified nanoparticles with their smaller size and unique surface properties are more potent than the modified nanoparticles with their polymeric shield [1,8,9]. In addition, the nature of the surface treatment plays a paramount role in the ability of the nanoparticles to inhibit the growth of microorganisms.

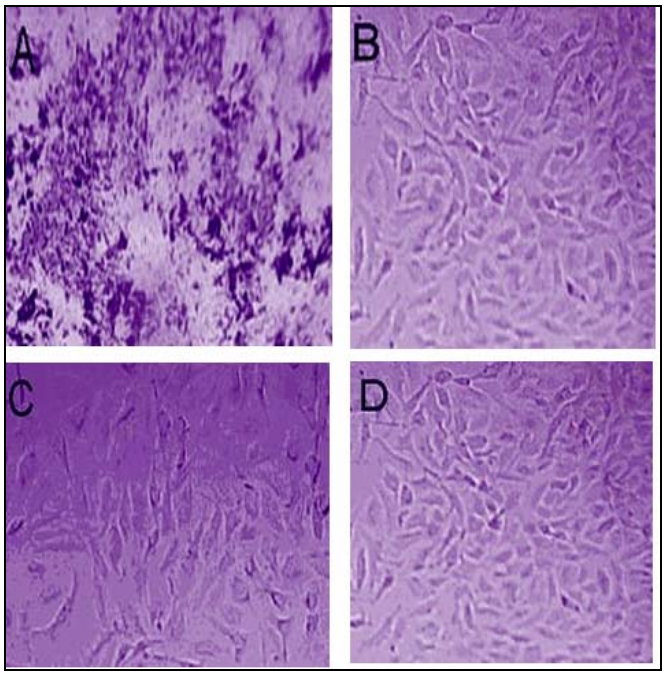
Cytotoxicity evaluation

Qualitative studying of the cell viability

The cellular cytotoxicity has been studies qualitatively using the MTT as a dye when used a stain for the human fibroplast cells. Figure 7, shows silver nanoparticles (5nm) , which exhibit different cytotoxic behavior according to the concentration of the used silver NPs(Figure 7) and due the length of the treatment period (data not shown).

The in vitro cytotoxicity of silver nanoparticles was evaluated quntitatively using MTT assay using Human fibroblast cells that were treated with various concentrations of Ag NPs. The concentration of the NPs used was in a range from 0.05 to 0.33 mg/mL for eight days. In this assay, only cells that are viable after eight days of exposure to the silver are capable of metabolizing a dye efficiently and produce a purple colored precipitate which is analyzed and counting using a micro plate reader. After eight days of post-treatment of the cells with silver nanoparticles (0.05 mg/mL), the cells showed excellent cell viability. They were found to be approximately 2.91×10^6 alive cells per well. This means that exposure of the cell to a low concentration as (0.05mg/mL) of silver nanoparticles did not affect and cell growth. However, treatment of the cells with a concentration of silver equivalent to 0.10mg/mL showed a good cell viability with 2.1×10^6cells per well while the treatment with 0.2 mg/mL of concentrations of nanoparticles human fibroblast cell cells showed limitation of cell viability. It was found 1.8×10^6

cells /well after addition silver nanoparticles. Finally the treatment with a concentration of a 0.30mg/mL of colloidal silver nanoparticles showed very low cell viability. It was found to be as low as 1.05×10^6cells/well which was still alive after addition silver nanoparticles as tabulated in table 3 and figure 7 and 8. The idea of the nanoparticles cytotoxicity to the cells was reported in literature and presumed to happen during the endocytotic pathway, because the nanoparticles tend to cover the perinuclear area. Suh et al attributed this directed intracellular movement of nanocarriers as an active transport process driven by molecular motor proteins and functionalized silver nanoparticles [38]. Therefore, using adequate concentration of nanoparticlces is essential to keep the safe use of them. Also it was clear that cytotoxicity is a concentration dependant and particle size dependant (data not shown). Determination of the safe concentration for the human cells providea a safe start for using silver nanoparticles in the pharmaceutical preparation.



Figiure 7. The cytotoxicity activity analysis of silver nanoparticles on human fibroblast cell HS68 cells after eight day's of addition of different concentrations of silver nanoparticles using (A) 0.30mg/mL of silver, (B) 0.20mg/mL of silver, (c) 0.10mg/mL of silver and (D) 0.05mg/mL.

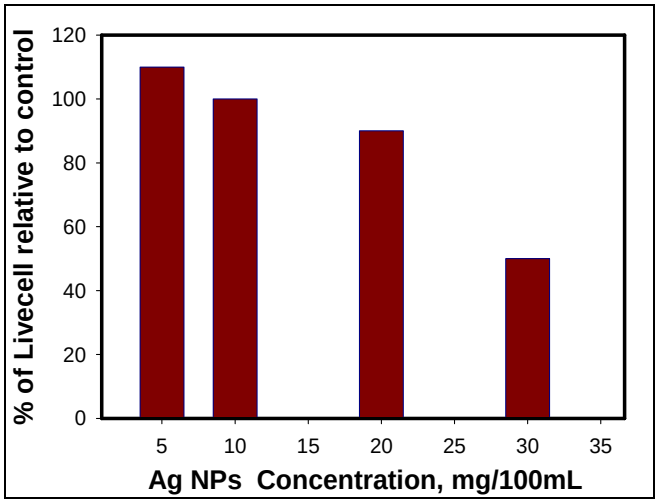


Figure 8. Quantitative measurement of cell viability and the cytotoxicity of silver nanoparticles on human fibroblast cell HBT68 when measured by micro plate reader.

Conclusion

This work suggests the silver nanoparticles particle of the size ranging from 5-50 nm and their solutions are stable over a wide range of the pH. The distribution of particles size of silver became narrower and mono distributed upon capping them with citrate. The dissolution data indicates that the release of the silver nanoparticles is inversely correlated with the size of the nanoparticles i.e. the release increased with smaller particles. The results suggest that the Ag NPs would be stable in the pharmaceutical preparations and will be easily to the infection site. The nanoparticles show high anti bacterial and antifungal effect.

These results are clearly important to recognize the varying degrees of cytotoxicity to a variety of cells. The lack of any noticeable toxicity of Ag NPs via long term use with this concentration provides new opportunities for the safe application in molecular imaging, therapy, food chemistry and other medical application. The results provided strong evidence that could warrant the consideration of silver nanoparticles as antibiotic for killing different type of bacteria and fungi and could circumvent the side and passive effects of other antibiotics .Ultimately, such positive environmental and toxicological studies will be imperative to ensure the nanomaterials design process yields both effective and safe technologies.

Acknowledgments

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