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# Powerful colloidal silver nanoparticles for the prevention of gastrointestinal bacterial infections

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**Abstract**
In this work we have demonstrated a powerful disinfectant ability of colloidal silver nanoparticles (NPs) for the prevention of gastrointestinal bacterial infections. The silver NPs colloid was synthesized by a UV-enhanced chemical precipitation. Two gastrointestinal bacterial strains of *Escherichia coli (*ATCC 43888-O157:k-:H7*)* and *Vibrio cholerae* (O1) were used to verify the antibacterial activity of the as-prepared silver NPs colloid by means of surface disinfection assay in agar plates and turbidity assay in liquid media. Transmission electron microscopy was also employed to analyze the ultrastructural changes of bacterial cells caused by silver NPs. Noticeably, our silver NPs colloid displayed a highly effective bactericidal effect against two tested gastrointestinal bacterial strains at a silver concentration as low as $\sim 3\,\mathrm{mg\,l^{-1}}$. More importantly, the silver NPs colloid showed an enhancement of antibacterial activity and long-lasting disinfectant effect as compared to conventional chloramin B (5%) disinfection agent. These advantages of the as-prepared colloidal silver NPs make them very promising for environmental treatments contaminated with gastrointestinal bacteria and other infectious pathogens. Moreover, the powerful disinfectant activity of silver-containing materials can also help in controlling and preventing further outbreak of diseases.

**Keywords:** colloidal silver nanoparticles, gastrointestinal bacteria, infectious pathogens, disinfectant agent, environmental treatments

**Classification numbers:** 2.05, 4.02

## 1. Introduction

Diarrhea from gastrointestinal infections remains one of the leading causes of morbidity and mortality worldwide [1]. These diarrhea diseases not only occur in developing countries with low levels of hygiene and sanitation, but are also recognized in developed countries [2]. Food and waterborne pathogens are the main factors for the outbreak of these diseases, the transmission of these pathogens endangering public health [3]. In 2011, an outbreak of diarrhea caused by an unusual serotype of Shiga-toxin-producing *Escherichia coli* (O104:H4) began in Germany with a large number of cases of diarrhea in which there were 3167 cases without the hemolytic–uremic syndrome (16 deaths) and 908 cases

 

with the hemolytic-uremic syndrome (34 deaths) [4]. In recent years, a number of highly infectious diseases caused by gastrointestinal bacteria have tended to break out in Vietnam. Hien *et al* [5] reported that there were 8304 cases infected by *Vibrio cholerae* (*V. cholerae*) in the north of Vietnam from 2000 to 2009, with six times higher than the number of cases infected by this bacterial strain during the 10 years before. More importantly, authors also confirmed 8064 cases infected by *V. cholerae* in 22 provinces from the outbreak of cholera that occurred in 2009.

To prevent further spread of the gastrointestinal pathogens, disinfection methods should be carried out properly to eliminate these pathogens from infected environmental areas, and effective treatments should also be carried out for patients in hospitals and the community. There are several chemical disinfectants used to kill these pathogens such as alcohols, chlorines, chloramines, hydrogen peroxide, formaldehyde, etc [6]. Among them, chloramine B agent [7] was widely recommended for use in disinfecting water sources and on-site surfaces with a reasonable concentration. There are some advantages of using chloramine B to disinfect the water, it can be used for a long period of time, and the quality of the chemical does not decrease. It is very good for disinfecting the environment and easy to use in a short time. However, the disadvantages of using chloramine B are that users need to have some knowledge of chemistry in order to use the chemical efficiently. It can be hard for people who do not know much about this chemical since they need to measure the exact amount of chemical for it to react well in water or nothing will happen. If the amount of chloramine B is over the limit, then when people use it to drink, it can cause many dangerous symptoms, even cancer. Also, it is hard to keep and might be difficult to find in those places where people cannot get access to different chemicals [7]. Moreover, the activity of chloramine B will be decreased after short-term exposure to light. Due to that, other disinfectant agents for long-term effective and safe disinfection needed to be found to overcome the above-mentioned adverse effects.

In the recent decade, with the rapid development of nanotechnology, many nanoparticles and nanocomposites have been studied toward applications in treatment for endangering diseases or environments [8, 9]. Among them, silver and its compounds have been used extensively in many bactericidal applications [10, 11]. As a natural antibiotic, silver has been widely applied in water purification, aqueous paints, textiles and antibacterial coatings [12, 13]. Special attention has been focused on the production of nanoscale silver particles (silver NPs) because of their exhibited stronger antimicrobial activity and wider range of green applications [14, 15]. Silver NPs were found to exhibit very strong antimicrobial activity against a wide range of micro-organisms including bacteria, viruses, fungi as well as microbial strains that are resistant to multiple antibiotics. Due to the potent activities of silver-containing materials, they may help in reducing infections when employed either as dressing for a range of wounds including burns and ulcers to prevent bacterial infection and improve wound healing, or as hand gels to disinfect skin for clinical and personal hygiene purposes [16]. In addition, silver NPs have an important advantage over conventional antibiotics as they kill all pathogenic micro-organisms, and no organism has ever been reported to readily develop resistance to them, and hence silver NPs may be used for treating various bacterial infections. The previously published studies on bactericidal activity have proven that silver NPs could destroy bacteria at very low concentrations (units of $\mathrm{mg\,l^{-1}}$) [17, 18], which do not reveal acute toxic effects to human cells [19, 20]. With the above-mentioned advantages, silver NPs can be considered as a potential decontaminant agent for the prevention of bacterial infections.

Recently, several attempts have been made to control infectious pathogens and contaminations, such as development of biosensing methods for rapid detection of infectious viruses. Electrochemical DNA biosensors and immunosensors were successfully used for detection of a variety of viral pathogens such as herpes virus, Japanese encephalitis virus, or influenza A-type virus [21–24]. In addition to virus contamination detection, bacterial/viral inactivation is an option to reduce infection risk and prevention of outbreak. In this respect, we focused on development of silver-based nanomaterials for antimicrobial applications. In previous works [25–28], we developed finely dispersed colloidal silver NPs by using a modified photochemical method (named as modified Tollens technique). It was found that with the use of simultaneous UV irradiation during the reduction process, the formation of colloidal silver NPs with controllable diameter and narrow size distribution could be achieved. Our studies revealed that the developed colloidal solutions of silver NPs could exist in the form of very stable aqueous dispersion up to several months and exhibited a noticeable antibacterial activity at laboratory scale tests [25]. We found that the concentration of silver leading to a complete inhibition of bacteria growth was revealed as low as $1.0\,\mathrm{mg\,l^{-1}}$, and that found in our work was noticeably lower than described in earlier reports $\sim$3–$10\,\mathrm{mg\,l^{-1}}$. Our findings indicated that an advanced synthetic technique and greater stability of fine silver NPs' dispersions resulted in the significant enhancement of their antibacterial activity [26]. In addition, we also developed some application tests using silver NPs for antibacterial purposes at laboratory scale. Potential applications of prepared silver NPs for antibacterial-masterbatches, acrylic emulsion paints as well as a coating layer on cotton textiles were demonstrated [27, 28].

In this study, colloidal silver NPs prepared by UV-enhanced chemical precipitation will be investigated to kill some gastrointestinal bacteria, and we will demonstrate them as a powerful decontaminant agent for controlling these infectious pathogens in a safer way, and as a longer-term protection over conventional chloramin B disinfection agent. Furthermore, the as-prepared silver NPs colloid could serve as an effective disinfectant agent used in treatment of environments containing infectious pathogens.

## 2. Materials and methods

### *2.1. Production of UV-enhanced chemically precipitated silver NPs*

All reagents were of analytical grade and used without further purification. In a typical experiment, 1.7 g

($1.0 \times 10^{-2}$ mol) of silver nitrate (Aldrich, 99.9%) was dissolved in 100 ml of deionized water. Then the solution of silver nitrate was precipitated with 0.62 g ($1.55 \times 10^{-2}$ mol) of sodium hydroxide (Aldrich, 99%). The obtained precipitate of silver oxide ($Ag_2O$) was filtered and dissolved in 100 ml of aqueous ammonia (0.4% w/w, $2.3 \times 10^{-2}$ mol) until a transparent solution of silver ammonium complex, $[Ag(NH_3)_2]^+_{(aq)}$ was formed. Next, 2.5 g ($8.9 \times 10^{-3}$ mol) of oleic acid (Sigma-Aldrich, 99%) was added dropwise into the obtained complex and the resulting solution was gently stirred for 2 h at room temperature until complete homogeneity of the reaction mixture was achieved. Finally, 2 g ($1.11 \times 10^{-2}$ mol) of glucose was added to the mixture at room temperature with gentle stirring. The reduction process of silver complex solution (in quartz glass) was initiated with UV irradiation. UV treatment was carried out for 8 h under vigorous stirring without additional heating. A UV lamp ($\lambda = 365$ nm, 35 W) was used as light source to stimulate the reduction process. Details of the synthesis process can be found elsewhere [25]. The process of silver NPs colloid preparation under UV treatment was controlled by tuning the irradiation time and the pH of the medium. Samples of aqueous dispersion of silver NPs colloid with different silver concentrations in the range from 1 to 10 mg $l^{-1}$ were prepared for the present study.

### 2.2. Physical and chemical measurements

The crystalline structure of nanosilver sample was analyzed by *x*-ray diffraction (XRD, Bruker D5005) using Cu*Ka* radiation ($\lambda = 0.154$ nm) at a step of 0.02° ($2\theta$) at room temperature. Transmission electron microscopy (TEM, JEOL-JEM 1010) was conducted to determine the morphology and size distribution of the nanosilver. The composition of the nanosilver particles was characterized by energy-dispersive x-ray (EDX-Emax Horiba, S4800 Hitachi). The silver concentration was determined using the method of atomic absorption spectroscopy (AAS, Shimadzu AA-6300).

A feature of absorption spectra of nanosilver particles was measured by UV–Vis absorbance spectra through the formation of an intense and broad band in the visible range which is called the surface plasmon resonance (SPR). Quartz cuvettes with a 10 mm path length were used for the measurement of dispersion spectra.

### 2.3. Microbiological assays

*2.3.1. Bacteria strains and media.* The growth of cell cultures was executed in a Luria–Bertani (LB) medium (1% tryptone, 0.5% yeast extract and 1% NaCl, pH 7). For that reason, the culture medium containing bacteria was kept in an incubator for 24 h at the temperature 37 °C, then the content of bacterial culture in it was $10^6$–$10^8$ CFU $ml^{-1}$, where the CFU is the colony forming unit.

The bacteria strains of *E. coli* (ATCC 43888-O157:k-:H7) and *V. cholerae* (O1) as the Gram-negative model were chosen as test cultures. These strains were obtained from the National Institute of Hygiene and Epidemiology (NIHE) in Hanoi.

*2.3.2. Surface disinfection assay on agar plates.* The surface disinfection assay was used for performing the antibacterial activity tests on agar plates. Aqueous dispersions of silver NPs of varying concentrations (1–10 mg $l^{-1}$) were prepared from the initial silver colloidal solution.

Next, 5 ml of each nanosilver solution was added onto Petri plates containing 15 ml of nutrient agar medium. The total volume on each Petri plate was kept to 20 ml, and the mixture was solidified with agar after 15 min. A 10 $\mu$l suspension of *E. coli* or *V. cholerae* bacteria was pipetted and spread onto the surface of the agar medium containing the silver NPs with 5 circulars marked at regular intervals. The Petri plates were incubated at 37 °C for 24 h in a shaking incubator (150 rpm) to encourage bacterial cell growth. The intensity of the bacterial growth on the agar plates with the silver NPs of variable concentration was monitored by the naked eye and stereo microscope (ZMS800, Nikon).

In addition, an agar plate on which no silver NPs were added was conducted for comparative purposes; this agar plate was positioned in the center and marked with (+). The test was carried out in triplicate to ensure reproducibility.

*2.3.3. Turbidity assay in liquid media.* Silver NPs colloids with varying silver concentrations in the studied range of 1–10 mg $l^{-1}$ were prepared. The nanosilver particles were added to the bacteria suspensions at different silver concentrations, and incubated at 37 °C with shaking at 200 rpm for 24 h. The turbidities of bacteria were observed and a clear tube demonstrated no bacteria growth. The minimal concentration of the nanosilver particles in the tube remaining clear after 24 h is minimum inhibitory concentration (MIC). A negative control tube contained only LB broth for comparative purpose.

### 2.4. Ultrathin sectioning sample preparation of bacteria cells

In order to obtain further understanding of the bactericidal and interaction mechanism of the silver NPs, ultrathin sectioning technique was carried out to observe the ultrastructural changes of bacterial cells destroyed by action of the silver NPs. After two strains of bacteria (*E. coli, V. cholerae*) were exposed to the colloidal silver NPs, the samples were collected and fixed by 2.5% glutaraldehyde in cacodylate buffer (0.1 M) for 30 min at room temperature. The fixed samples were washed by cacodylate buffer three times for 10 min each and transferred to 1% $OsO_4$/cacodylate buffer for 1 h. The samples were then dehydrated by using a series of alcohol with 50, 70, 80, 90 and 100% (two times × 5 min), and then propylene oxide (three times × 5 min). The samples were infiltrated and finally embedded in Epon 812 at 60 °C for 24 h. The polymerized samples were sectioned into ultrathin slices 60–90 nm in thickness, and placed on collodion-coated copper grids (300 meshes). The analyses of ultrastructural changes of interior of the bacteria cells were conducted by transmission electron microscopy (TEM, JEM 1010, JEOL).

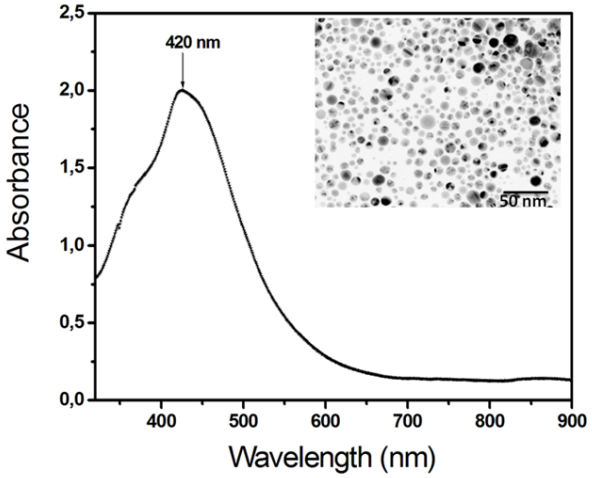


**Figure 1.** The UV-visible absorption spectrum of the colloidal solution of silver NPs at a silver concentration of 10 ppm. The inset displays the TEM image of the as-prepared silver NPs.

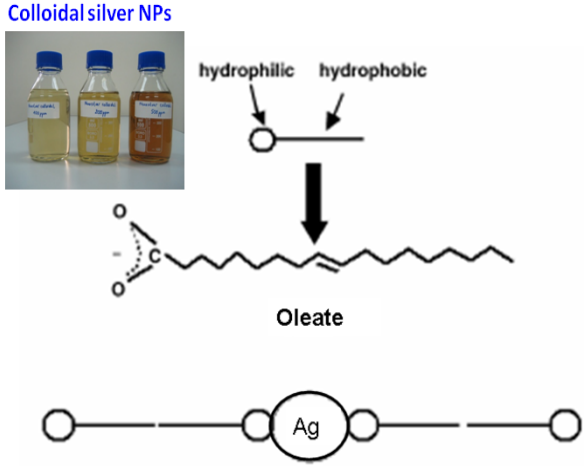


**Figure 2.** The formation of the stabilizing layer of oleate ions ($RCOO^-$) on silver particle surface. The inset displays the colloidal solutions of silver NPs prepared at different concentrations.

## 3. Results

### *3.1. Characterization of UV-enhanced chemically precipitated silver NPs*

In order to control the process of silver NPs formation we used UV irradiation at the stage of reducing silver ammonium complex. It was found that the UV treatment of the reaction medium containing silver complex, surfactant (oleic acid) and glucose resulted in formation of highly stable dispersion of small (9–10 nm in average size) silver NPs with narrow size distribution [25, 26]. A time period of 8 h was found to be optimal for the complete reduction of silver complex. The UV irradiation causes excitation of $[Ag(NH_3)_2]^+$ ions followed by electron transfer from the glucose molecule to $Ag^+$, thus producing $Ag^0$ atoms which then form clusters and seeds:

$$[Ag(NH_3)_2]^+ + RCHOH \xrightarrow{h\nu} Ag^0 + 2NH_3 + H^+ + R\dot{C}OH,$$

$$nAg^0 \rightarrow (Ag_n)^0,$$

where RCHOH represents glucose in its cyclic form. The UV treatment leads to the substantially simultaneous formation of a large amount of silver nuclei which then start to grow. This results in small dimensions and narrow size distribution of the finally obtained silver NPs [29, 30].

As evidence, figure 1 shows a UV–Vis spectrum of the colloidal solution of silver NPs at a silver concentration of 10 ppm. As observed from figure 1, an optical absorption band with a maximum at 420 nm was found, which is a typical feature of the absorption of metallic silver NPs due to the surface plasmon resonance (SPR), indicating the presence of silver NPs in the solution. It was found that the as-prepared aqueous dispersions of silver NPs were very stable against aggregation during several months (∼12 months). There is no aggregation behavior of silver NPs as confirmed by the TEM observation (see inset of figure 1). This can be explained by the formation of stabilizing oleate bilayer on the surface of silver particles. Taking into account the stability of the aqueous dispersions of the as-obtained silver NPs, oleic acid most likely forms a double layer between silver surface and liquid medium (figure 2). Since the UV treatment, a large amount of silver nuclei was initially formed. The remaining silver ions get adsorbed on the surface of already formed particles and attract oppositely charged oleate ions. These oleate ions form the first capping layer on the surface of the silver particles (see figure 2). Since the carboxyl group of the oleate ion is directed toward the silver surface, the first capping layer is highly hydrophobic from the outside. Therefore, the oleate ions from the solution start to attach to the first layer forming stable bilayer structure with carboxyl group directed toward liquid medium. Similarly charged bilayer structures protect silver particles from aggregation and, thus, act as particle stabilizers. As a result, this leads to excellent long-term stability of silver NPs colloid prepared, up to several months [25].

### *3.2. Antibacterial activity of colloidal silver NPs against gastrointestinal bacteria*

In order to verify highly effective antibacterial activity of as-prepared silver NPs colloid against some infectious gastrointestinal pathogens; two strains of *E. coli* (ATCC 43888-O157:k-:H7) and *V. cholera* (O1) were chosen for our present study and these strains were supplied from the National Institute of Hygiene and Epidemiology. Antibacterial activity of the silver NPs colloid against *E. coli* and *V. cholera* bacteria was evaluated by means of surface disinfection assay in agar plates, turbidity assay in liquid media [31, 32].

First, we used the surface disinfection method to evaluate the surface antibacterial effect of the silver NPs colloid. For qualitative assessment (by sight) of antibacterial activity agar plates containing silver NPs with different concentrations were inoculated with bacterial suspension $10^6$ CFU. Figures 3(a)–(d) display the observations of bacteria grown on agar plates after 24 h at different concentrations of the silver NPs ranging from 0 mg $l^{-1}$ (control sample), and from 2 to 6 mg $l^{-1}$ with counter clockwise direction.

For *E. coli* bacteria, it can be seen from figure 3(b) that, for a control sample (0 mg $l^{-1}$ reaction mixture without adding silver nitrate), the bacteria grown easily within

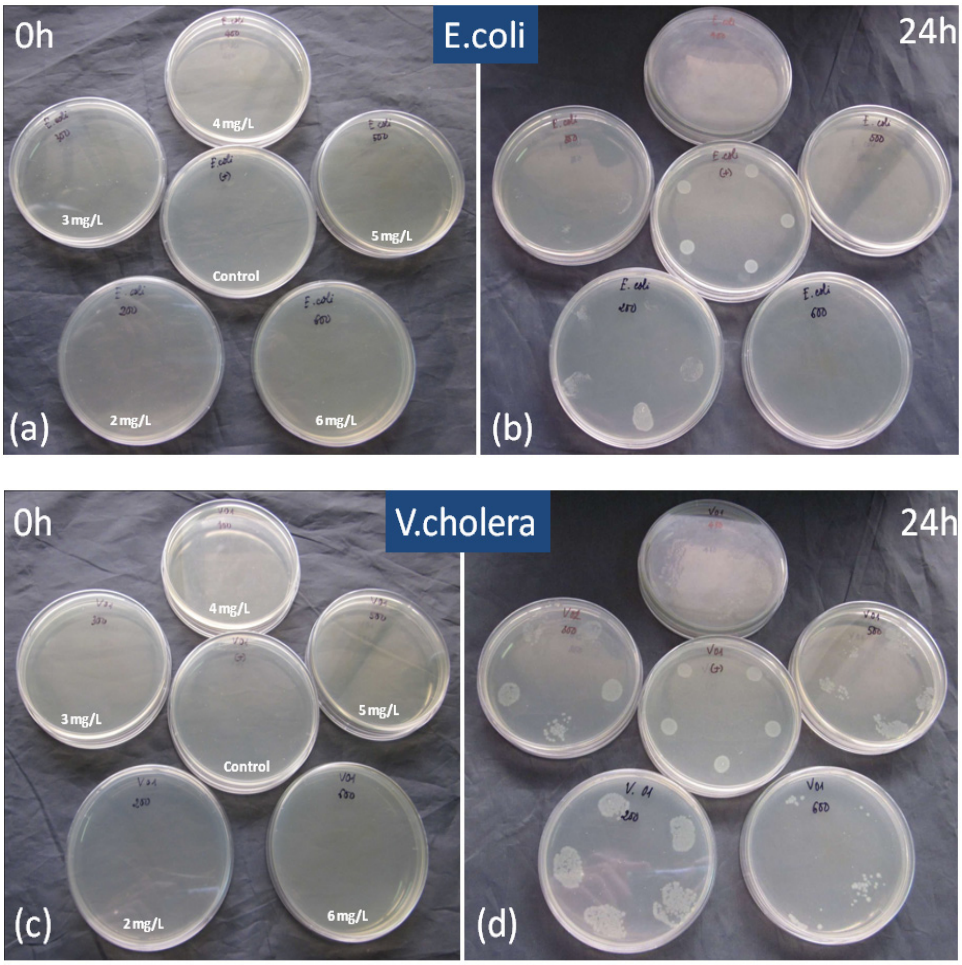


**Figure 3.** The observations of bacteria growth on agar plates from the beginning (0 h) and after incubation at 37 °C for 24 hours with different concentrations of the silver NPs ranging from 0 mg $l^{-1}$ (control sample), and from 2 to 6 mg $l^{-1}$ with counter clockwise direction for *E. coli* (a, b) and *V. cholera* (c, d) bacteria.

5 marked circulars on the surface of agar plates. As the silver amount increased, from 2 to 6 mg $l^{-1}$, the activity of suppression of bacteria growth was increasing as observed from figure 3(b). When the silver content reached 3 mg $l^{-1}$, a complete inhibition in bacteria growth was observed.

For *V. cholera* bacteria, it can be seen from figure 3(d) that, at concentrations from 2 to 5 mg $l^{-1}$ there was evident bacteria growth. When the silver content reached 6 mg $l^{-1}$, a relatively complete inhibition in bacteria growth was observed. This indicates that the required silver concentration for effective inhibition in bacteria growth against *V. cholera* bacteria was two times higher than of that against *E. coli* bacteria. This is due to the difference in biological activity of individual bacterial species. Based on the surface disinfection analysis, it is revealed that the silver NPs colloid displayed noticeable antibacterial effect against *E. coli* and *V. cholera* bacteria. This silver NPs colloid prepared is promising for uses as a potential decontaminant agent for controlling bacterial infections.

Next, we further evaluated the antibacterial property of the silver NPs colloid for both *E. coli* and *V. cholera* bacteria by measuring the microbial viability of bacteria incubated with the nanosilver. When bacteria (either *E. coli* or *V. cholera*) were cultured in LB liquid media containing the nanosilver for 24 h, the mixture containing only LB broth became turbid (see figure 4), this suggests that bacteria in such a mixture medium rapidly proliferated. However, the medium containing the nanosilver remained pellucid (figure 4), indicating few bacteria proliferated. These results showed that the silver NPs colloid prepared could effectively prevent the bacterial growth. It can be seen that at the silver concentration of about 2 mg $l^{-1}$ the *E. coli* bacterial growth was inhibited, whereas at the silver concentration over 2 mg $l^{-1}$ the *V. cholera* bacterial growth could be prevented. Based on turbidity analysis, we found that the silver concentration of 3 mg $l^{-1}$ could completely inhibit the bacterial growth for both *E. coli* and *V. cholera* bacteria. Our obtained studies indicate that the silver NPs colloid exhibits antibacterial performance in LB liquid media better than that in agar plates media. This result from the increased biological interactions between silver NPs and bacteria in LB liquid media, hence the silver NPs kill tested bacteria more effectively [33].

### *3.3. Ultrastructural and morphological analyses*

In order to provide rudimentary insights into the interaction and bactericidal mechanism between the silver NPs and the *E. coli* and *V. cholera* bacteria, ultrastructural and morphological analyses of the electron microscopic technique

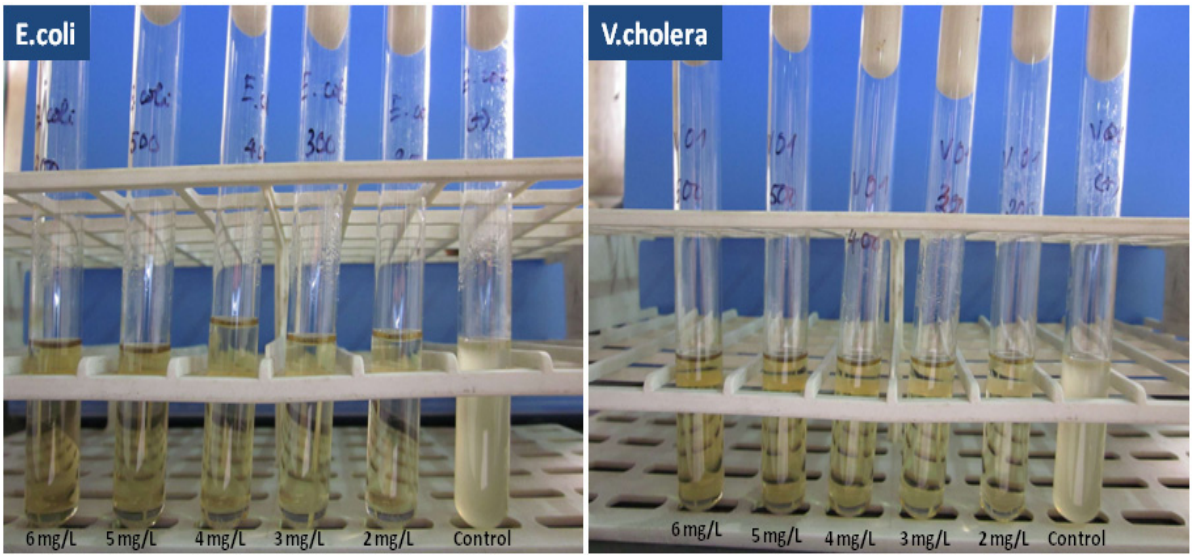


**Figure 4.** LB liquid medium turbidity assays were carried out to evaluate the antibacterial activity of silver NPs colloid toward *E. coli* (ATCC 43888-O157:k-:H7) and *V. cholera* (O1) bacteria. The photographs were taken after incubation for 24 h at 37 °C.

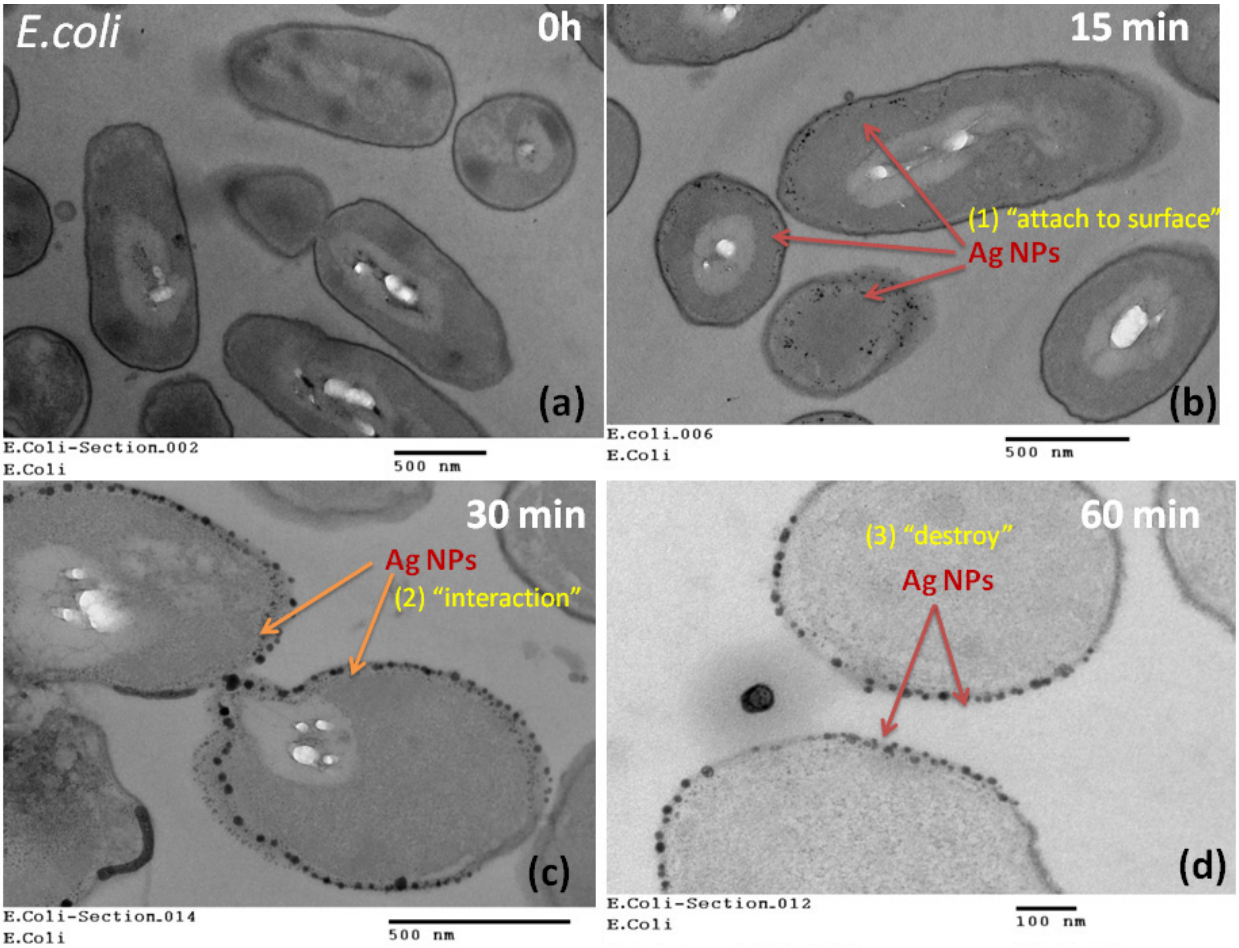


**Figure 5.** The TEM images showing different stages of interaction of silver NPs with *E. coli* bacterium after (a) 0 h, (b) 15 min, (c) 30 min and (d) 60 min.

were conducted. The colloidal solution of silver NPs was dropped onto the surface of *E. coli* and *V. cholera* grown on agar plates. After 15, 30 and 60 min, *E. coli* and *V. cholera* were taken out and underwent the sectioning method for TEM observation. At different magnifications and sections (figures 5 and 6), many silver NPs bindings around both the *E. coli* and *V. cholera* cell membranes as well as inside the cells were found. As observed, the silver NPs first attached to the surface of the cell membrane, penetrating further inside the bacteria. It should be noted that only silver NPs with sufficiently small diameters penetrated into the cells. The cytoplasm was destroyed as the silver NPs penetrated the

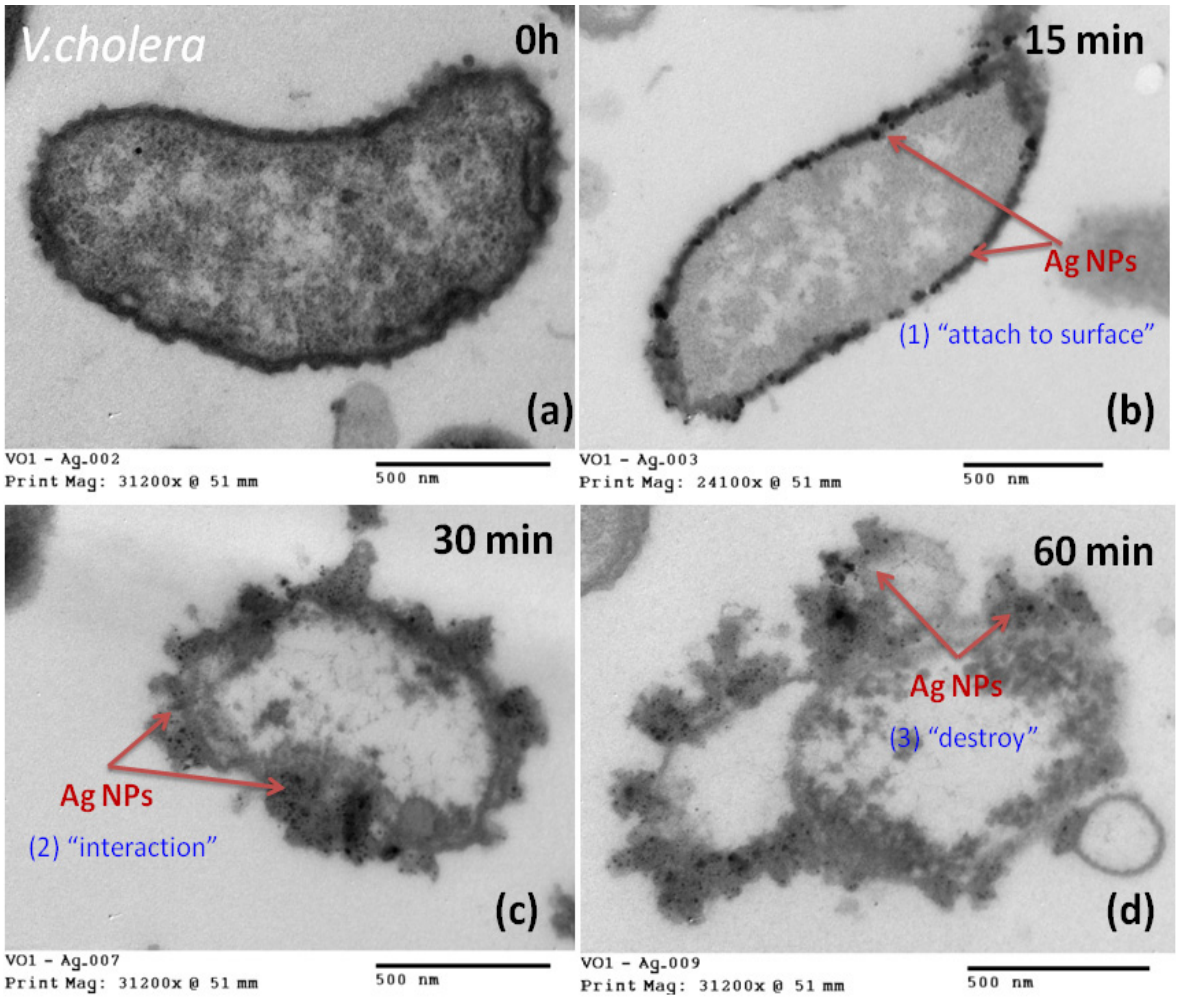


**Figure 6.** The TEM images showing different stages of interaction of silver NPs with *V. cholera* bacterium after (a) 0 h, (b) 15 min, (c) 30 min and (d) 60 min.

cell (figures 5(c) and 6(c)). This proves how *E. coli* and *V. cholera* cells can be eliminated by the silver NPs.

The changes took place in the cell membrane morphology, producing a significant increase in their permeability. This affects the proper transport through the plasma membrane, leaving the bacterial cells incapable of properly regulating transport through the plasma membrane, resulting eventually in cell death [34–36]. As shown in figures 5(b) and (d) and 6(b) and (d), in addition to being fixed to the cell membrane, the silver NPs are capable of penetrating through it to be distributed inside a bacterium. After interacting with the *E. coli* and *V. cholera* bacteria, the silver NPs adhered to the cell wall of the bacteria and penetrated the cell membrane, resulting in the inhibition of bacterial cell growth and multiplication.

Based on obtained observations (figures 5 and 6), the bactericidal action of our silver NPs was proposed to divide into three stages according to the morphological and structural changes found in the bacterial cells: (i) the silver NPs with sufficiently small diameters attach to the surface of the cell membrane and drastically disturb its proper functions such as permeability and respiration; (ii) then the silver NPs are able to penetrate inside the bacteria and cause further damage by possibly interacting with sulfur- and phosphorus-containing compounds and lose their activity; (iii) inside a bacterium, the silver NPs can interact with DNA, causing the latter to lose its ability to replicate, which may lead to the cell death; finally (iv) the silver NPs release silver ions $Ag^+$, which will have an additional contribution to the antibacterial activity of the nanosilver [37].

Here it should be noted that the bactericidal actions of silver NPs ($nAg^0$) and silver ion ($Ag^+$) are much different from the viewpoint of structural changes. In the case of the silver ions $Ag^+$, the antimicrobial action of silver ions is linked with interactions with the thiol group compounds found in the respiratory enzymes of bacterial cells. The silver ions enter into the bacterial cells by penetrating through the cell wall and consequently turn the DNA into condensed form which reacts with thiol group proteins and results in cell death. The silver ions also interface with the replication process. For the case of *E. coli*, the silver ions act by inhibiting the uptake of phosphate and releasing phosphate, mannitol, succinate, proline and glutamine from *E. coli* cells [38].

### *3.4. Evaluation of disinfection effect of colloidal silver NPs and chloramine B (5%)*

From the application point of view, it is important to check the uses of nanosilver as a potential decontaminant agent for controlling bacterial infections. We conducted

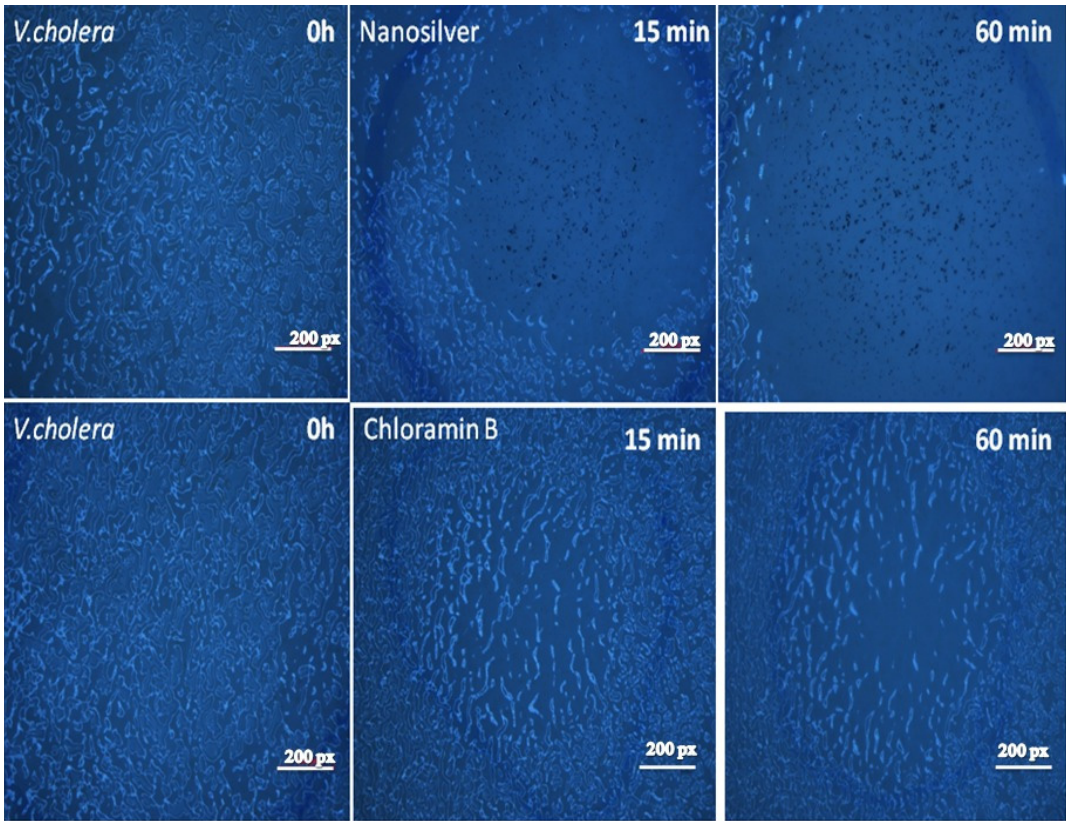


**Figure 7.** The stereomicroscopic images of *V. cholera* bacteria controlled by our silver NPs and chloramin B (5%) at different treatment time.

a comparative evaluation of disinfection effect of silver NPs colloid and chloramin B (5%) conventionally used in controlling bacterial infections. Figure 7 shows fluorescent images of *V. cholera* bacteria controlled by our silver NPs and chloramin B (5%) at different treatment time. The obtained results indicate that the silver NPs colloid displayed stronger disinfection effect as compared to the chlolarmin B. At the time of 15 min, the silver NPs colloid showed the highly effective disinfection ability against *V. cholera* (O1) bacteria. The *V. cholera* bacteria was mostly destroyed after 60 min when treated by the silver NPs colloid, whereas this bacteria largely remained when treated by chloramin B solution (5%). This reveals that as-prepared silver NPs colloid displayed enhanced antibacterial performance and longer lasting bacterial inactivation efficiency comparative to and better than that of the chloramin B disinfection agent conventionally used.

With this advantage, the silver NPs colloid is very promising for use in treating environments contaminated with infectious pathogens (bacteria or virus); this will help to effectively prevent and control the outbreak of diseases occurring in the future.

## 4. Discussion

From results on disinfection evaluation, it has been demonstrated that the silver NPs colloid are a powerful decontaminant agent for controlling gastrointestinal bacterial infections. However, there are some emerging questions on potential risks [39] involved in using these silver NPs for clinical and disinfectant purposes, as it is very difficult to remove colloidal silver NPs by virtue of their chemical and physical properties. Silver is a recognized cause of argyrosis and argyria [40] and potential clinical toxicity of silver to human cells has also been mentioned [41, 42]. Therefore, maximum contamination levels for silver in drinking water (100 ppb) [43] and the occupational exposure limit to various forms of silver ($0.01\,\mathrm{mg\,m^{-3}}$) [44] have been established in order to avoid the accumulation of silver in the human body. Recent reports concluded that silver NPs have the potential to give rise to health and ecotoxicity issues in a concentration- and size-dependent manner. In order to overcome this problem, new approaches for development of silver-containing nanocomposites were proposed. The first approach is to use oxide nanomagnetic system as a core and silver nanoparticles as the shell, and utilize the resulting core-shell nanostructures for antimicrobial activity in their colloidal state and subsequently remove them from the medium by means of an external magnetic field. These core-shell nanostructures can be successfully removed from the medium by a static magnetic field which provides a solution to prevent uncontrolled exposure of the potentially hazardous silver nanoparticles to contamination of the environment. An alternative approach is to use a nanohybrid structure by decorating the silver nanoparticles on the nanocarbon materials including silver-carbon nanotubes (Ag-CNTs) [45] or silver-graphene (Ag-Grp) [46] in order to reduce toxicity of silver NPs, thus helping in preventing potential contamination to the environment. It is noteworthy

that these silver-decorated nanohybrids are very different from isolated and dispersed colloidal silver NPs, and it appears that the strong interactions between the silver nanoparticles and the CNTs or Grp surfaces make the silver NPs less toxic due to the fact that they are not able to release themselves into the environment. Noticeably, Chudasama *et al* [47] and Prucek *et al* [48] indicated that magnetic nanocomposites of iron oxide and silver nanoparticles Ag@$Fe_3O_4$ and $g$-$Fe_2O_3$@Ag are exploited to destroy bacterial and fungal infections. Both kinds of silver-containing nanocomposites are particularly applicable for a targeted magnetic delivery of silver nanoparticles in medical and disinfection applications. In addition, the biocompatibility and antimicrobial activity of Ag/CNT and Ag/Grp hybrid nanocomposites was also reported [49, 50], the results revealed that these Ag/CNT and Ag/Grp hybrid nanocomposites may be useful for applications in biomedical devices and antibacterial control systems. With the above-mentioned advantages, the silver-containing core-shell and/or hybrid nanocomposites will open new opportunities for future environmental treatments and biomedical devices applications. Further works in synthesis and application development of these novel silver-containing nanocomposites are in working progress.

## 5. Conclusion

In this work, we investigated the antibacterial and disinfectant activity of colloidal silver NPs which were prepared by a UV-enhanced photochemical method. The experiments were carried out with two gastrointestinal bacterial strains of *E. coli* (ATCC 43888-O157:H7) and *V. cholerae* (O1) by means of surface disinfection assay on agar plates and turbidity assay in liquid media. It was found that our silver NPs colloid exhibited strongly effective antibacterial effect against tested gastrointestinal bacteria at a silver concentration as low as $\sim 3\,\mathrm{mg\,l^{-1}}$. Interestingly, the silver NPs colloid displayed enhanced antimicrobial performance and longer lasting disinfectant effect as compared to the conventional chloramin B disinfection agent. With these exhibited advantages, the as-prepared colloidal silver NPs are very promising for use in treatment of environments containing gastrointestinal bacteria or other infectious pathogens.

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