Investigating Silver Nanoparticles as Alternative Disinfectant

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Abstract

Eleven Disinfectant by-products (DBPs) have been regulated in the United States, but approximately 600-700 drinking water DBPs have been reported in the literature. It is speculated that more than 50% of the halogenated material formed in chlorinated drinking water is still unknown and could pose toxicological risk to human health. Further, some strains of bacteria have developed resistance against disinfectants like chlorine dioxide, and a quaternary ammonium compound due to prolonged and continual use of these disinfectants. The risks associated with conventional disinfectants have prompted the exploration of alternative disinfectants such as bromine and silver nanoparticles. This review aims to overview silver ions as a potential disinfectant. While the antibacterial properties of silver have been known since roman times, advancements in the nanotechnology and the fact that it does not forms DBP's have propelled the use of silver ion as a possible disinfectant. There have been umpteen number of studies inferring silver being an excellent disinfectant against bacteria, but there is little evidence showing similar statistics for the antiviral and antiprotozoal action of silver against these microorganisms. Interestingly, metal ions are shown to enhance the efficacy of the system well beyond that predicted by the individual ion. This synergistic effect between silver ions and other antimicrobials such as copper ions, potassium permanganate, hydrogen peroxide has been observed against a variety of micro-organisms including bacteria, viruses, and oocysts. Moreover, other applications of silver ions as nanoparticles (AgNP) coatings on matrix capped with different polymers like polyurethane, copolymer beads, polyvinylpyrrolidone (PVP) have also shown to enhance performance against disinfecting water from micro-organisms. However, in order to use AgNPs in water purification systems, there is a need to identify the limitations of silver nanoparticles as a disinfectant. This review will further summarize the impediments to the progression from chlorine to alternatives such as AgNPs as disinfectants.

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Introduction

Different ancient civilizations throughout the world, like the ancient Greeks, Phoenicians, and Egyptians had used silver compounds as food and water preservative. In 1884, the first scientifically documented medical use of silver by German obstetrician C.S.F. Crede was recorded. He introduced 1% silver nitrate solution as an eye solution for the prevention of Neonatal conjunctivitis. By 1940, in the United States, various forms of silver-based solution, ointment were available for the treatment of wounds. Further, topically used silver sulfadiazine cream was used as a standard antibacterial treatment for cutaneous wounds until quite recently. It was the discovery of penicillin and antibiotics that silver use was restricted to jewellery, utensils, monetary currency, etc.

Exploring Alternatives

Risks associated with the current disinfection techniques, including the formation of DBPs have prompted to use to explore alternatives to the current chlorine water disinfection methods. One such risk involved with chlorination is the formation of disinfection by-products (DBPs). When chlorine comes in contact with natural organic matter (NOM), carcinogenic compounds such as trihalomethanes (THMs) and haloacetic acids (HAAs) can be formed. Another problem associated with chlorination comes from multi-drug resistant bacterial species. As such, species such as Cryptosporidium cannot effectively be treated with chlorine. While newer techniques such as the use of UV-irradiation and ozonation have been to address this problem but the cost of operation is quite high and the power consumption of UV/ozone generating systems is high.

With the advent of nanotechnology, the efficiency of silver disinfection has improved and the toxicity concerns have been subsided to an extent. The oligodynamic effect of AgNP is strong for bacterial cells while being relatively non-toxic to mammalian cells and does not easily provoke microbial resistance. (*1*) (*2*) The germicidal effect of AgNP occurs at 40 to 200 µg/L for gram-positive and 250 µg/L for gram-negative bacteria (*3*). This is well below the drinking water guidelines of the EPA according to which the exposure limit should not exceed 0.1 mg/L for silver in water. The disinfectant value, however, is subject to change with different matrixes and the method employed to prepare AgNPs. One of the main engineering challenges is to incorporate AgNPs on composites that can greatly increase the efficiency of water disinfection.

Silver ions as a Potential Disinfectant

The chemical and physical of a matrix such as micro-porosity, insolubility, mechanical strength, and stability are some important parameter that needs to taken into consideration when fabricating silver particles on a matrix. Different matrixes such as methacrylic acid copolymer beads, polystyrene resin beads, polyurethane (PU) foams and AgNPs confined in nanoscale cages have been used as solid support due to their unique characteristics. One such method incorporates the combination of AgNPs and Ag^+ ions in the form of core-shell structure embedded in a matrix. Because of the shell material coating, the properties such as thermal stability of the core particle can be modified so as to make the overall particle stability and dispersibility of the core particle increase. Thus, the advantage is straightforward: coating the Ag^+ particle with silver compound having very less solubility in water will prevent the leaching of Ag or Ag^+ and in turn will be less toxic to the mammalian cell. The studies done on this regard, have been promising. Ghosh et. al. have reported brilliant results against both E. coli and S. aureus. Figure I. shows the percent survival versus exposure time in the dark and on exposure to light for films of all the samples. The authors used Ag-AgI/agarose film as the matrix for core-shell structure for the experiment. In-situ AgNPs of size 15-25 nm were synthesised in an agarose matrix. The method involved the reduction of Ag^+ ions to Ag^0 by dissolving chlorine free agarose in 100 mL of Millipore water under boiling conditions and 0.5mmol of $AgNO_3$ in 20mL of Millipore water. 5 mL of 0.25 mmol KI solution was added at 90 °C obtain Ag-AgI/agarose matrix. (*4*)

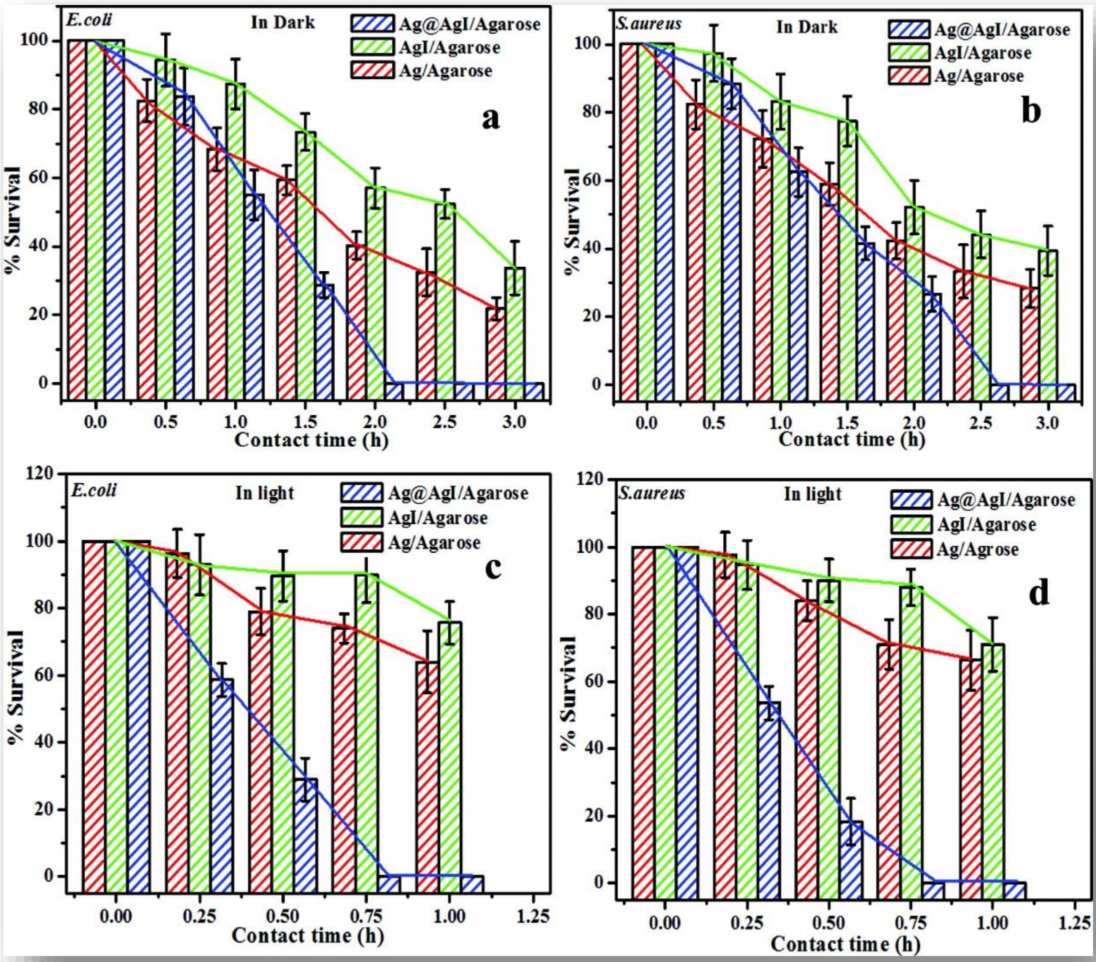

Figure 1: Percentage Survival: In dark, the percentage survival of E. coli remains above 80% at the end of the contact time of 30 min for all the samples. But after this contact time, there is a steep decrease in survival and zero is reached at the end of 2 h in the case of Ag-AgI/agarose film. In light, the rate of killing of the bacteria is still faster in the case of Ag-AgI/agarose, and it took only 45 min to kill both Gram-negative and Gram-positive bacteria in saline solution. (*4*)

As can be seen from Figure 1, at the end of 2 hours, the Ag-AgI/agarose film show 0% survival of bacteria. This is further validated in Fig 2, where the TEM studies shows the E.coli and S.aureus before and after contact. (*4*)

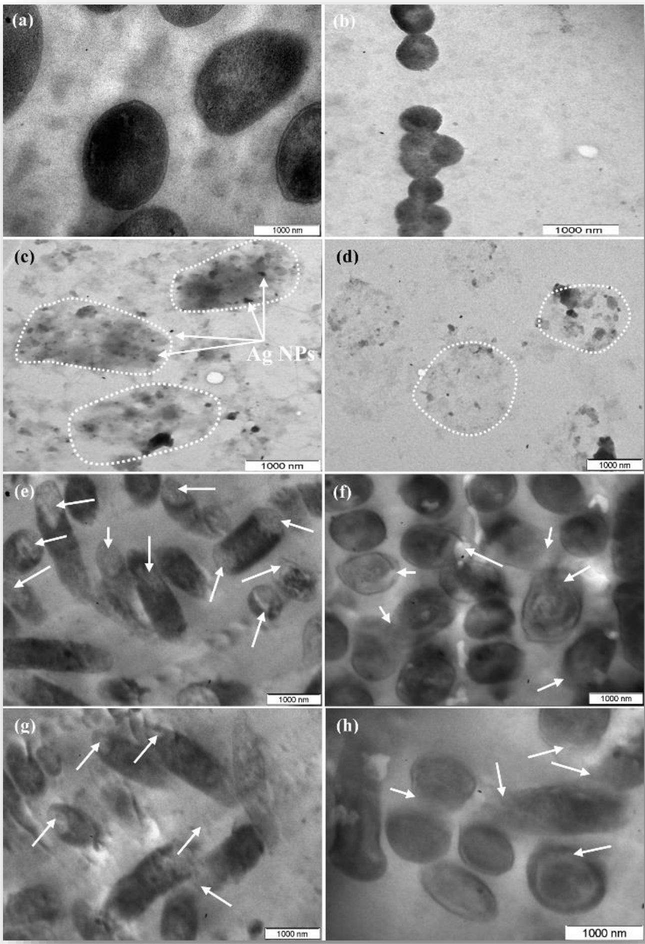

Figure 2: TEM images of bacteria: healthy (a) E. coli and (b) S. aureus In the case of Ag/agarose (c,d), AgNP particles can be seen inside cell after diffusing through the bacteria's outer membrane causing a loss of cellular integrity and cytoplasm material disintegrated throughout the solution. In the case of Ag-AgI/agarose films (e,f) the bacteria shows interrupted stretches, and electron-dense material has accumulated in the periplasmic space. AgI/agarose film (g,h) shows more damage than (e,f). (*4*)

The author also checked the cytotoxicity effect by treating HeLa cells with Ag-AgI/agarose coated glass with time. They observed that Ag-AgI/agarose did not exhibit toxicity even when it was held in it for more than 2 months. (*4*)

Another study by Jain et. al. showed similar results by coating AgNP articles on polyurethane (PU) foam. After a contact time of 5 and 10 min, two strains of E. Coli- MTCC 1302 and ATCC 25922 showed zero output count for all dilutions. They also checked the antibacterial action for a flow rate of 0.5 L/min and concluded no E. Coli detection suggesting the possibility of domestication of this technology. This was later confirmed by multiple studies. (*5*) (*6*) This technology can be used water purification in under-developed nation where E. coli is a problem.

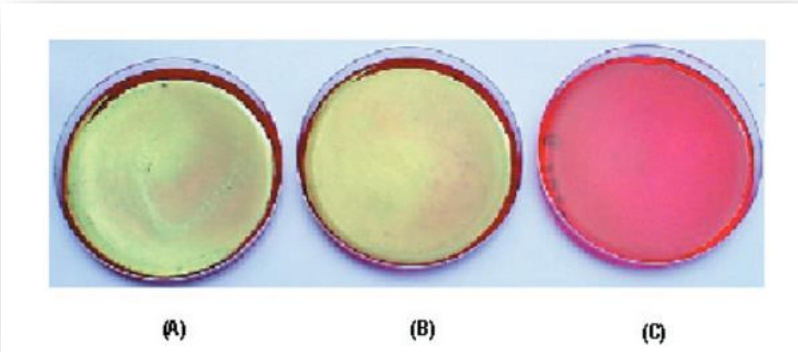

Figure 3: Test tube result for E. Coli MTCC 1302 for 10^{-2} dilution after 5-min exposure. A: Initial count. B: After exposure to pure polyurethane (PU). C: After exposure to nanoparticle-coated PU. The bacterium count was zero in C. (*7*)

Antimicrobial Property of AgNPs for Different Micro-Organisms

This section aims at comparing the effective removal of different micro-organisms from the data found in literature. Broadly, the amount of research done on finding the effectiveness of AgNPs against bacteria is much more than that of viruses and oocysts. Therefore, finding a common focal point for comparison for these micro-organisms requires careful examination. Multiple mechanisms have been proposed for the mechanism of silver disinfection. According to one theory, Ag atoms are believed to attach to the thiol, sulphydryl, amino, imidazole, phosphate and carboxyl groups of membrane or enzyme proteins to cause enzyme deactivation. Other theory postulates that Ag^+ ions denature DNA molecule by interacting with the purine and pyrimidine base pairs, thus disrupting the hydrogen bonding. (*1*) (*2*) (*8*)

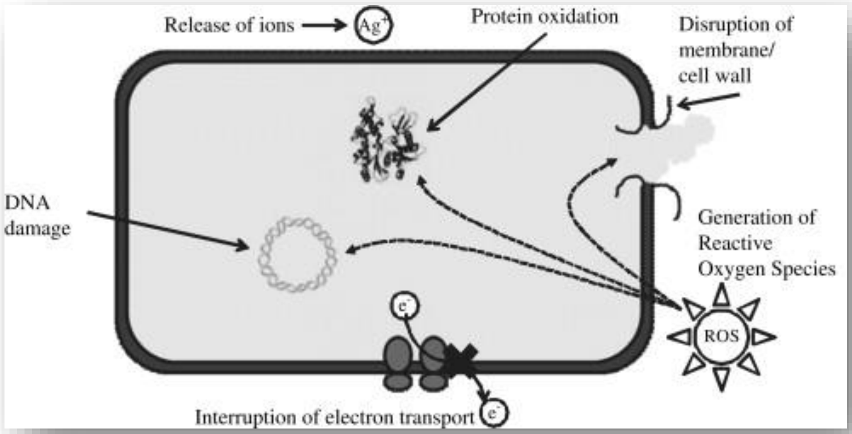

Figure 4: Various mechanisms of antimicrobial activities by AgNP (*9*)

Table 1, herein, summarizes the pathogen removal performance of silver against Cryptosporidium P. Fundamentally, oocyst response to AgNPs differs from the bacterial response. While the bacterial study found silver ions to be more cytotoxic than AgNPs (*10*), these results indicate that AgNPs appear to be slightly more toxic than silver ions to oocysts. This implies that the impact of AgNP action is an intrinsic property of the NP itself.

References	Pathogen types	Pathogen reduction performance	Types of silver
	Cryptosporidium P.	**% excystation**	
Cameron et al. (*11*)		33.33%	AgNPs (at 500 µg/ml)
		62.00%	Ag ions (at 500 µg/ml)
Su et. al. (*12*)		42.70%	AgNPs (at 500 µg/ml)
		71.40%	Ag ions (at 500 µg/ml)
Abebe et. al. (*13*)		0% (majority)	Proteinate capped AgNP
		80%	PVP-capped AgNP
Quilez et. al. (*14*)		0.00%	5% of 48% hydrogen peroxide + 0.05% silver nitrate

Table 1: Pathogen reduction performance for Silver against Cryptosporidium Parvum

Table 2, herein, compares the efficacy of silver against different family of viruses. There is a paucity of research to determine the interactions of metal nanoparticles with viruses and only until recently some studies have come up with results showing that metal nanoparticles can be effective antiviral agents against virus. The general trend observed for these studies indicates that the antiviral effect of silver nanoparticles was due to the nanoparticles, rather than just to the silver ions present in the solution.

References	Virus	Family	Types of Silver	Pathogen reduction performance
				% inhibition of viral replication
Lara et. al. (*15*)	Human immunodeficiency virus type 1 (HIV-1)	Retroviridae	PVP-coated silver nanoparticles (1–10 nm)	~90%
Sun et. al. (*16*)			Silver nanoparticles fabricated in Hepes buffer	98%
Baram-Pinto et. al. (*17*)	Herpes simplex virus type 1 (HSV-1)	Herpesviridae	MES-coated silver and gold nanoparticles (4 nm)	At low virus loads (25 and 250 pfu), , viral infection was completely blocked by the Ag-MES nanoparticles. At higher virus loads (2500 pfu), an average of 67 plaques/well was observed, which is nearly a 97% decrease in the number of plaques
Lu et. al. (*18*)	Hepatitis B virus (HBV)	Hepadnaviridae	Silver nanoparticles fabricated in HEPES buffer solution; (10–50 nm)	10nm: 80% at 50 µM concentration, 50 nm: 92% at 50 µM concentration

Table 2: Pathogen reduction performance for silver against Virus

Table 3, investigates the pathogen removal performance of silver against bacteria. These micro-organisms have been studied well and numerous studies have been published. Silver does an excellent job against bacteria. Again, with the exception of MS2 bacteriophage, AgNP provides good reduction performance. Synergistic effect of metal ions can be utilised with a development of silver and copper loaded TiO_2 nanowire membrane (Cu-Ag-TiO2) to successfully inactivate bacteriophage MS2 and E. Coli. (*19*)

References	Pathogen types	Pathogen reduction performance		Types of silver
		% removal	LRV	
Lantagne et. al. (*20*)	E. coli		>2	AgNP
	Cryptosporidium parvum		4.3	
	MS2		<1	
Fahlin et. al. (*21*)	E. coli		2.9	AgNP
Campbell et. al. (*22*)	E. coli		>3	AgNP
Halem et. al. (*23*)	E. coli		7	AgNP
	Clostridium spores		3.3-4.9	
	MS2 bacteriophage		<1	
Brown et. al. (*24*)	E. coli	99		Ag+
	MS2 bacteriophage	90-99		

Table 3: Pathogen reduction performance for silver against Bacteria

Limitations of Silver Nanoparticles for Water Treatment

The rapid growth of nanotechnology has propelled the use of nanomaterials in the environmental applications of nanomaterials. Their use in decentralized or point-of-use water treatment and reuse systems have attracted attention from scientists due to aging distribution systems and cost to transport water. These small-scaled particles tend to be excellent adsorbents, sensors due to their large surface area.

But it is this small scale of these particles that raises eyebrows. Consequently, factors like hydrodynamic behaviour, bioavailability, toxicity and pathogenesis of nanoparticles in aquatic organisms have to be kept in mind before switching to alternative disinfectants. Another factor that needs to be evaluated is the cost of silver/silver solutions from which NPs are made from. One kg of $AgNO_3$ costs $1200-$1400 which makes it an expensive disinfectant to work with. Retention of AgNPs and the sustainability of antimicrobial activity are also some factors over time are some challenges that needs to be studied in more detail. There have been a few studies regarding AgNP retention in the rat model but nothing conclusive can be said if as the sample size is very small. Argyrosis is a condition in which excessive exposure to the elemental silver turns the skin blue. A few cases including one of Libertarian candidate for the US Senate in 2002 and 2006 caught media attention when he ingested colloidal silver causing a blue coloration of his skin. A few more cases have been reported but no known death has occurred due to excessive amount of silver ingestion.

Conclusion

Before adopting any technology, massive amount of research has to conducted in order to know the implications of it, in the long run, on the environment. Although AgNP coated surfaces could be used as a replacement for the current disinfectant, chlorine; problems particularly of metal leaching and toxicity to the aquatic organisms are of particular concern. Thus, future studies need to explore leaching and toxicity of silver nanoparticles to ensure that AgNPs are not disturbing the ecosystem. Furthermore, substantial contributions are needed in order to make the use AgNPs more scalable and economical for water purification systems.

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