The efficacy of silver as a bactericidal agent: advantages, limitations and considerations for future use

Michael A. Butkus, Lance Edling and Michael P. Labare

ABSTRACT

This work examined the efficacy of silver as a bactericidal agent against *Escherichia coli*. The minimum inhibitory concentration (MIC) for Ag(I) was observed to be between 0.1 mg Ag/l and 0.05 mg Ag/l following a 24-h incubation period at 25°C. Additional forms of silver that were effective included Ag_2O, a protein-based silver and $AgCl_{(s)}$. All of these forms of silver resulted in MICs that were comparable to Ag(I). Predictions from a chemical equilibrium modelling system indicated that a silver-histidine complex may have contributed to the observed bactericidal activity. A MIC for colloidal metallic silver (Ag^0) was not observed up to a total silver concentration of 82 mg Ag/l—the highest concentration evaluated. Moreover, aqueous silver was not detected at this total silver concentration. It was concluded from these findings that cationic Ag(I) or Ag(I)-complexes were responsible for the bactericidal activity of silver. In the batch systems evaluated, the MICs increased with time, over a 72-h incubation period, to values above the US Environmental Protection Agency Secondary Standard for silver, for all forms of silver tested. A desorption kinetics study indicated that less than 10% of silver was readily leachable from a granular activated carbon surface that was first saturated with silver.

Key words | disinfection, *E. coli*, minimum inhibitory concentration, point-of-use filters, silver

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INTRODUCTION

It has been known since at least 1000 BC that water kept in silver vessels could be made potable (Russell & Hugo, 1994). The bactericidal effects of silver have been known since the mid 1800s (Ravelin, 1869; Chambers *et al*., 1962). Given contact times on the order of hours, silver has been shown to be an effective disinfectant against coliforms (Chambers *et al*., 1962; Bell, 1991). The mechanisms of the bactericidal activity of silver have been attributed to a reaction between silver and thiol groups, silver and amino acids, and the binding of silver to key functional groups in enzymes (McDonnell & Russell, 1999). Silver has also been shown to inhibit the respiratory chain (Bragg & Rainnie, 1973) and inhibit phosphate uptake (Schreurs & Rosenberg, 1982) in *Escherichia coli*. Finally, silver has been reported to bind to DNA, however, the bactericidal activity of this observation remains unclear (McDonnell & Russell, 1999). Although silver is not typically used as a large-scale disinfectant in most developed nations, it is commonly used to prevent microbial growth in point-of-use filters (Tobin *et al*., 1981; Reasoner *et al*., 1987; Bell, 1991); as a co-disinfectant for swimming pool water, which allows for lower chlorine levels in pools (Yahya *et al*., 1990, 1992; Beer *et al*., 1999) and as a co-disinfectant in hospital hot water systems (Lin *et al*., 2002). The use of silver as a drinking-water disinfectant is also popular in Europe (Russell & Hugo, 1994). Moreover, silver is used in developing nations for treating drinking water (see Chaudhuri *et al*., 1994). In water, at concentrations sufficient for bactericidal activity, Ag(I) does not impart taste, colour or odour and has no apparent detrimental effects on mammalian cells (Yahya *et al*., 1990). The only known negative health effect is argyria, an irreversible darkening of the skin and mucous membranes, which has been

caused by prolonged silver therapy (Russell & Hugo, 1994).

For point-of-use applications, silver has been impregnated into activated carbon (Tobin *et al.*, 1981; Reasoner *et al.*, 1987; Bell, 1991) or ceramic filter candles (Chaudhuri *et al.*, 1994). By desorbing from these water purification devices, silver can automatically provide a residual for users who may not have access to other disinfectants or understand the concept of disinfection and how to determine/measure a safe quantity of disinfectant for potable water. In addition, silver may be useful in potable water storage systems that have long contact times such as cisterns (Owen & Gerba, 1987) and water storage tanks used in the field by the military.

Two of the principal drawbacks associated with the use of silver as a disinfectant are the need for long contact times (Chambers *et al.*, 1962; Bell, 1991) and the existence of silver-resistant organisms (Belly & Kydd, 1982; Richards *et al.*, 1984; Gupta *et al.*, 1998). Moreover, several reports on the use of silver-impregnated water treatment devices suggest that silver provides little benefit as a disinfectant over the life of a typical point-of-use granular activated carbon (GAC) filter or filter candle (Reasoner *et al.*, 1987; Chaudhuri *et al.*, 1994; Bell, 1991). The causes for the reported inability of silver to effectively disinfect water in point-of-use applications remains largely unreported although silver-resistant bacteria may have been implicated in some cases (Tobin *et al.*, 1981; Belly & Kydd, 1982).

Most studies have not evaluated all forms of silver for coliforms grown in a rich medium, a scenario that might provide for high numbers of actively metabolizing bacteria. An improved understanding of the bactericidal efficiency of silver might be constructive in improving applications, e.g. in developing nations, where silver may be the only barrier against pathogenic strains of coliforms and other silver-sensitive microorganisms, and in quantifying the effective life of silver-impregnated point-of-use devices. In addition, an understanding of the speciation of bactericidal forms of silver under these conditions could lead to more efficacious use of silver as a disinfectant. It was hypothesized that some of the reported shortcomings of silver in the literature may have been the result of the form of silver used, the speciation of silver, the presence of impurities in the water and the method of silver delivery. The objectives of the work reported herein were to: (1) evaluate the efficacy of silver for *E. coli*, an enteric bacterium and opportunistic pathogen against which silver has been proven somewhat effective; (2) identify bactericidal forms of silver and their minimum inhibitory concentrations (MIC) in a rich medium; (3) determine the speciation of bactericidal forms of silver in a rich medium; (4) investigate the influence of typical water impurities on the MIC; and (5) evaluate granular activated carbon as a substrate for delivering silver in point-of-use devices for continuous flow and batch systems. The findings reported herein may explain why silver has been reported to be an ineffective bactericidal agent and may lead to improved efficiency of silver disinfection in some applications.

METHODS AND MATERIALS

Solution analysis and cleaning procedures

The total silver concentration was quantified using atomic adsorption (AA) spectrophotometry (GBC 908AA, Arlington Heights, IL) following digestion in HNO_3 and H_2O_2 (for samples that contained organic constituents). Continued calibration verification (CCV) was conducted by using a midrange standard (2.5 mg Ag/l). The aqueous silver concentration was determined via a colorimetic procedure (Hatch, Loveland, CO). The method detection limit for AA and colorimetric methods were 0.03 mg Ag/l and 0.05 mg Ag/l. The total organic carbon content of the protein-based silver was determined with a total organic carbon analyser (Model 700, I/O Inst., College Station, Texas). All glassware was soaked in 10% HNO_3 for a minimum of 24 h and rinsed in deionized water to remove adsorbed silver (Chambers, 1956) and other contaminants prior to use.

Organisms and growth media

Escherichia coli (ATCC 33876) was grown in nutrient broth (Difco Laboratories, Detroit, MI) at 25°C and

150 rpm. Cultures were monitored by optical density at a wavelength of 600 nm (OD_{600}), grown overnight to log phase and used when $OD_{600} = 1.0$ (log phase).

Minimum inhibitory concentration (MIC)

The MIC was determined as described by Carson *et al.* (1995). Briefly, a series of 1:2 dilutions of silver were prepared in microtitre plates over the range of 100 to 9.5×10^{-5} mg Ag/l. The microtitre plates were inoculated with an overnight culture of *E. coli* (log phase) suspended in nutrient broth to an $OD_{600\,nm}$ of *ca.* 0.068. The plates were incubated at 25°C for up to 72 h. The OD was measured with an Anthos HT2 ELISA (microtitre) plate reader (Anthos Labtec Instruments Co., Austria) preset to a wavelength of 620 nm. Selected experiments were run in triplicate to validate the method. In all cases, the nutrient broth buffered the media at *ca.* 6.8 pH units. Because the influence of pH on the MIC has been documented (Chambers *et al.*, 1962) it was not evaluated as part of this work. In addition, because photoreduction of Ag(I) has been reported to have a negligible influence on the bactericidal efficiency of silver (Chambers, 1956; Chambers *et al.*, 1962), exposure to visible light during the short periods when the microtitre plates were set up or counted was considered negligible. The forms of silver studied as part of this work include: $AgNO_3$, Ag(I) (Alfa Aesar, Ward Hill, MA); protein-based silver, organic-Ag (Aqua Limpia, Mexico); colloidal Ag_2O (Alfa Aesar, Ward Hill, MA); and a 100 nm diameter colloidal metallic silver, Ag^0 (Aldrich, Milw. WI).

Adsorption/desorption kinetics

A 50-ml continuous flow stirred tank reactor (CFSTR) was used to measure the rate of partitioning between Ag(I) ($AgNO_3$) and activated carbon (Pittsburgh Activated Carbon, Type F-400). An advantage of this approach is that diffusion limitations are significantly reduced (Yin *et al.*, 1997), and as desorbed solute is continuously removed from the reactor, desorption of solute is not inhibited by previously desorbed solute (Yin *et al.*, 1997). Sorbent media were maintained in the CFSTR via a 1 μm glass fibre filter. A 5 mg/l (as Ag) stock solution was formed by adding sufficient $AgNO_3$ to a 10 mM $NaNO_3$ solution. This silver stock solution was pumped through the reactor at a steady rate of 10 ml/min. Adsorption (desorption) of Ag(I) onto (from) the glass and other component surfaces was accounted for as described below (see equations (1) and (2)). Temperature was maintained at 25 ± 0.3°C via a water bath. The pH of the stock solution was maintained at 7 ± 0.05 units via NaOH or HNO_3. Exposure to the atmosphere was not a concern because bicarbonate and carbonate ions have very low stability constants for complexation with Ag(I) at these pH values (Herrin *et al.*, 2001*a*). The concentration of silver in the CFSTR influent and effluent was quantified every 5 min via atomic adsorption spectrophotometry. Blanks and CCV were analysed after approximately every five sample intervals. Adsorption kinetics of silver onto the sorbent surface, $q_a(t_i)$, was quantified via the following equation (Schnabel & Fitting, 1988; Yin *et al.*, 1997):

$$q_a(t_i) = \frac{\sum \left[\left(C_{i,eff}^{blank} - C_{i,eff}^{sorbent} \right) \frac{Q \Delta t_i}{\forall} \right] + \left[\overline{C}_{i,reactor}^{blank} - \overline{C}_{i,reactor}^{sorbent} \right]}{W} \quad (1)$$

where:

$C_{i,eff}^{blank}$ = CFSTR effluent silver concentration for the ith sampling period without a sorbent in the reactor, mg/l;

$C_{i,eff}^{sorbent}$ = CFSTR effluent silver concentration for the ith sampling period with a sorbent in the reactor, mg/l;

Q = volumetric flow rate, 0.01 l/min;

Δt_i = time interval between sampling periods, 5 min;

$\forall$ = reactor volume, 0.05 l;

$\overline{C}_{i,reactor}^{blank}$ = average CFSTR silver concentration during the ith sampling period without a sorbent in the reactor, mg/l;

$\overline{C}_{i,reactor}^{sorbent}$ = average CFSTR silver concentration during the ith sampling period with a sorbent in the reactor, mg/l; and

W = sorbent concentration, 100 mg/l.

Desorption of silver from the sorbent surface, $q_d(t_i)$, was quantified via the following equation (Schnabel & Fitting, 1988; Yin *et al.*, 1997):

$$q_d(t_i) = \frac{\sum\left[\left(C_{i,eff}^{sorbent} - C_{i,eff}^{blank}\right)\frac{Q\Delta t_i}{\forall}\right] + \left[\overline{C}_{i,reactor}^{sorbent} - \overline{C}_{i,reactor}^{blank}\right]}{W} \quad (2)$$

Adsorption and desorption onto the system components were below the detection limit after 10 min. Consequently, the influent silver concentration for each time step was substituted for the blank effluent concentration (*ca.* 5 mg Ag/l for adsorption and 0 mg Ag/l for desorption).

Equilibrium calculations

The predominant species of silver in the nutrient broth under various water quality conditions were determined via the MINEQL+ chemical equilibrium modelling system (version 4.5, Environmental Research Software, Hallowell, ME). This model employs a thermodynamic database to compute equilibrium speciation in complex aqueous systems. Model predictions used in this work were based on the composition of nutrient broth (Table 1), a specific silver concentration, and carbonate species (open system). All model calculations were corrected for ionic strength and conducted at 25°C. Because MINEQL+ allows for only 25 components, tryptophan was not considered (due to lack of thermodynamic data) and a sensitivity analysis was conducted to identify and discard the following negligible species (based on their inability to form significant complexes with silver): methionine, isoleucine, threonine and tyrosine.

Table 1 | Composition of nutrient broth (BD Diagnostic Systems, Sparks, MD)

Species	Concentration (M)	Species	Concentration (M)
Ca	1.6E-05	Phenylalanine	7.5E-04
K	1.5E-03	Serine	1.5E-03
Cl	1.3E-03	Tryptophan	4.7E-05
PO_4	3.1E-04	Valine	1.4E-03
Mg	2.5E-03	Arginine	2.3E-03
Na	7.6E-03	Cysteine	4.2E-05
SO_4	4.3E-04	Isoleucine	7.7E-04
Alanine	5.9E-03	Lysine	1.5E-03
Aspartic acid	2.2E-03	Glycine	1.7E-02
Glutamic acid	4.5E-03	Proline	5.5E-03
Histidine	4.8E-04	Threonine	8.5E-04
Leucine	1.6E-03	Tyrosine	3.6E-04
Methionine	4.7E-04		

Table 2 | Minimum inhibitory concentration (MIC) for *E. coli* as a function of silver form following a 24-h incubation period at 25°C

Form of silver	MIC, total silver (mg Ag/l)
Ag(I)	$0.10 \geq MIC \geq 0.05$
Organic-Ag	$0.10 \geq MIC \geq 0.05$
Ag_2O	$0.14 \geq MIC \geq 0.07$
Ag^o	$MIC^* > 82$
$AgCl_{(s)}$	$0.40 \geq MIC \geq 0.20$

*This value represents the total silver measured via atomic absorption spectrophotometry following digestion. Aqueous silver was less than the detection limit (0.1 mg/l).

RESULTS AND DISCUSSION

Forms of silver that have been reported to be effective disinfectants include metallic silver (Ag^0), Ag(I), Ag_2O and protein-based forms of silver (organic-Ag) (Chambers *et al.*, 1962; McDonnell & Russell, 1999). The bactericidal activity of each of these forms of silver has been tested in order to assess their efficacy under the rich growth conditions encountered in nutrient broth for *E. coli*. Table 2 illustrates the minimum inhibitory concentration (MIC) for several forms of silver following a 24-h incubation period. The silver concentration (for dissolved forms) was measured colorimetrically and/or estimated based on the quantity added and number of serial dilutions. The total

silver concentration was measured with atomic absorption spectrophotometry following sample digestion and/or estimated based on the quantity of silver added and number of serial dilutions. The MIC for Ag(I) (formed by dissolving $AgNO_3$ in deionized water) was observed to be between 0.1 mg Ag/l and 0.05 mg Ag/l. The range for the MIC for Ag(I) is just below the US Environmental Protection Agency (USEPA) Secondary Drinking Water Standard for silver (0.1 mg Ag/l). While this strain of *E. coli* (ATCC 33876) has been genetically engineered to serve as a transformation host for the plasmid, pMOB48, the MIC for Ag(I) is comparable with the literature MIC values for *E. coli* (Ghandour *et al.*, 1988) and *Legionella pneumophila* (Lin *et al.*, 2002). In addition, Chambers *et al.* (1962) reported that Ag(I) was bactericidal at concentrations as low as 0.04 mg/l for *E. coli* following 2 h of contact in distilled water at 20°C. Although this observation was not the MIC (as defined herein), it does indicate that the constituents of the nutrient broth used in the work reported here did not have a profound influence on the bactericidal efficiency of silver.

The predominant species of silver in the nutrient broth were determined via the MINEQL+ chemical equilibrium modelling system. Figure 1(a) presents the speciation of silver in nutrient broth for a total silver concentration of 0.10 mg Ag/l at 25°C. Interestingly, the model predicted that almost 100% of the total silver was complexed with histidine ($AgHIS_2$) and that cationic Ag(I) was *c.* 6.8×10^{-7} mg Ag/l at $6.5 < pH < 7$. Silver has been reported to complex with many of the amino acids and constituents commonly found in growth media (Belly & Kydd, 1982; Liau *et al.*, 1997; McDonnell & Russell, 1999; Herrin *et al.*, 2001*b*). Indeed, MINEQL+'s thermodynamic database contains complexation constants between Ag(I) and all but one of the reported nutrient broth organic constituents identified in Table 1. The mechanism associated with the biostatic activity of $AgHIS_2$ remains unclear. However, the bioaccumulation and toxicity of silver complexes have been reported elsewhere (Gupta *et al.*, 1998; Reinfelder & Chang, 1999; Bury & Hogstrand, 2002). At 0.10 mg Ag/l, silver chloride species were only predominant at pH values less than 6. Illustrated in Figure 1(b), at the highest total silver concentration examined (100 mg Ag/l), $AgCl_{(s)}$ was predominant at all circumneutral pH values. However, silver was observed to be biostatic at all concentrations above the MIC suggesting that the presence of chloride did not significantly influence the results. This topic is addressed in more detail below.

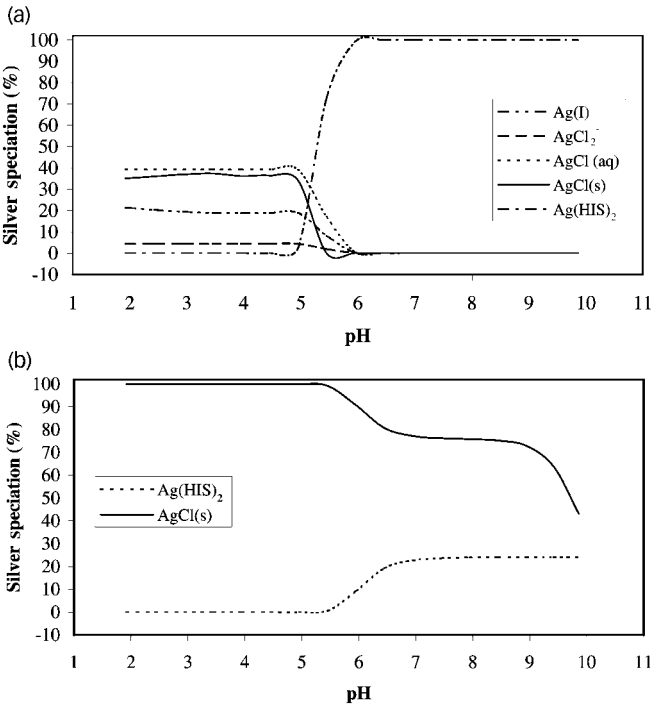

Figure 1 | (a) Speciation of silver in nutrient broth as a function of pH for a total silver concentration of 0.1 mg/l at 25°C. (b) Speciation of silver in nutrient broth as a function of pH for a total silver concentration of 100 mg/l at 25°C.

The biostatic capability of a protein-based silver disinfectant, so called 'colloidal silver' (referred to as organic-Ag in this work), which has been impregnated into some filter candles for use in developing nations (personal communication, Mr Reid Harvey, Arsenic Research Group, Dhaka), was also evaluated under rich growth conditions. The total organic carbon content of the stock organic-Ag solution was found to be *c.* 2500 mg/l. Chambers *et al.* (1962) reported that Ag(I), which was released from organic-Ag compounds, was responsible for bactericidal effects in these systems and not the protein-Ag compound itself. The MIC for the organic-Ag evaluated herein was observed to be in the same range as that for Ag(I), which supports the findings of Chambers

et al. (1962). In light of the congruence between the MIC for organic-Ag and Ag(I), it appears that the constituents of nutrient broth did not influence the release of Ag(I) from the organic-Ag compound and reduce the bactericidal activity of this form of silver.

Because both Ag_2O and Ag^0 have been reported to be bactericidal (McDonnell & Russell, 1999), they were evaluated under the rich growth conditions considered in this work. Table 2 illustrates that the MIC for the Ag_2O was comparable to that for Ag(I). At the MIC, the range of total silver concentrations was equivalent to the dissolved silver concentrations. It is presumed that dissolution of Ag_2O resulted in the formation of Ag(I), which inhibited growth at concentrations greater than the MIC. Bactericidal effects, if any, of colloidal Ag_2O could not be confirmed due to the dissolution of the Ag_2O colloids. Recently, Bellantone *et al*. (2002) reported that the bactericidal activity of a Ag_2O-doped bioactive glass against *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* was the result (exclusively) of leaching of Ag(I) from the glass matrix.

A MIC for colloidal Ag^0 was not observed up to a total silver concentration of 82 mg Ag/l—the highest concentration evaluated. Moreover, aqueous Ag(I) or Ag-complexes were not detected at this silver concentration. Thus, oxidation of Ag^0 to Ag(I) was not significant under the conditions tested. Contrary to what has been previously reported (McDonnell & Russell, 1999), and in parallel with conclusions drawn by Russell & Hugo (1994) based on the observations of Gibbard (1937), the results of this study clearly demonstrate that Ag^0 was not effective at inhibiting growth at concentrations two orders of magnitude above the USEPA Secondary Drinking Water Standard. This finding may help elucidate some of the problems reported in the literature concerning microbial growth in point-of-use silver-impregnated carbon water treatment devices (Tobin *et al*., 1981; Reasoner *et al*., 1987; Geldreich & Reasoner, 1990; Bell, 1991). Using scanning electron microcopy and energy-dispersive X-ray analysis, Hoskins *et al*. (2002; see also Biniak *et al*., 1999) recently reported that silver-impregnated filters are composed mainly of Ag^0. Thus, Ag^0 bound to a GAC surface would not be expected to provide significant bactericidal activity and could lead to microbial growth in point-of-use GAC filters. Hoskins *et al*. (2002) postulated that oxidation of Ag^0 occurs in the presence of oxygen, with the carbon surface acting as a catalyst, and results in the desorption of Ag(I). The rate of this reaction and pore diffusion probably influence the release of Ag(I) from GAC filters.

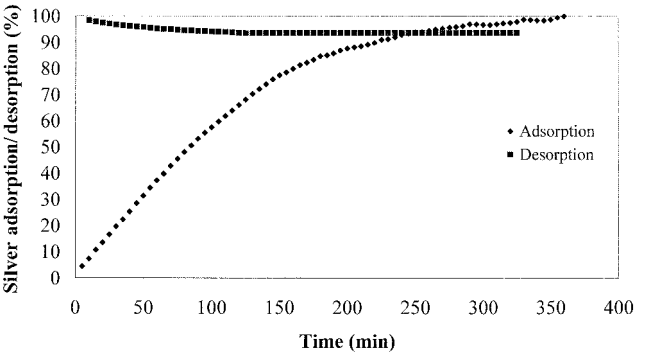

Figure 2 | Adsorption and desorption rates for Ag(I) onto GAC at 25°C. The ordinate values for adsorption and desorption were calculated via equations (1) and (2). Adsorption and desorption rates were quantified at 25°C in a 10 mM $NaNO_3$ solution with pH maintained at 7±0.05 units via NaOH or HNO_3.

A silver saturated GAC filter was tested to determine the quantity of leachable Ag(I) that could be delivered under conservative flow conditions. Silver nitrate was used for these experiments because it was presumed that this salt would not form significant precipitates on the GAC surface (Biniak *et al*., 1999). Figure 2 illustrates adsorption and desorption kinetics for Ag(I), sorbate, and granular activated carbon (GAC), sorbent. The ordinate values were derived from equation (1) (adsorption) and equation (2) (desorption) for each time step. The adsorption experiment was run until the GAC was saturated with Ag(I) (reactor influent equalled reactor effluent). The desorption experiment was run until the reactor effluent concentration was below the method detection limit for three consecutive samples. The data indicate that only *ca.* 9% of the adsorbed Ag(I) is capable of readily desorbing from the GAC surface. Additional Ag(I) desorption might have gone undetected because of limitations caused by pore diffusion (Kang *et al*., 1998) and/or the kinetics of silver oxidation (Hoskins *et al*., 2002). Regardless, it is likely that in some cases, the rate of Ag(I) desorption may not be sufficient to maintain Ag(I)>MIC in the carbon pore spaces of GAC point-of-use water treatment devices.

In fact, the Ag(I) concentration for desorption from the saturated GAC studied here, quickly fell below 0.1 mg Ag/l before 35 min (based on raw data for Figure 2) at the conservative flow to mass (of GAC) ratio of 6 l/(h · g) investigated. Such conditions could lead to microbial growth in a GAC point-of-use water treatment device as previously reported (Tobin *et al.,* 1981; Reasoner *et al*., 1987; Geldreich & Reasoner, 1990; Bell, 1991). Point-of-use carbon treatment devices are commonly operated at a slightly lower flow to mass (of GAC) ratio of *ca.* 0.2 l/(h · g) to 1.3 l/(h · g) (Reasoner *et al*., 1987), which implies that they might deliver Ag(I) above the MIC for a longer period of time in some cases. However, even at lower flow to mass ratio in this range, Tobin *et al*. (1981) reported that Ag(I) did not exceed 0.05 mg Ag/l—the lower limit of the MIC reported herein. These observations may help explain the reported failure of silver as a disinfectant in point-of-use filters. Because only a small fraction of silver bound to GAC surfaces may be readily leachable as Ag(I), the useful life of silver-impregnated activated carbon filters should be based on the time (number of pore volumes for a particular flow rate) over which the desorbing silver concentration remains above the MIC (Ag(I)>0.05 mg/l) at the rated flow rate for the device. In addition, other forms of silver, e.g. organic-Ag, which might allow for a greater quantity of Ag(I) to desorb from silver-impregnated point-of-use devices should be identified and evaluated. On the other hand, the concentration of the Ag(I) in the filter effluent should not exceed the USEPA Secondary Drinking Water Standard for silver.

In a manner analogous to GAC, Ag(I) has also been reported to be reduced to Ag^0 on the surface of montmorillonite; resulting in reduced bactericidal activity (Ohashi & Oya, 1992; Besrest *et al*., 1995). We also observed the formation of metallic silver colloids while attempting to quantify the adsorption rate between Ag(I) (sorbate) and clay from a filter candle device (data not shown). It is likely that reduced silver on silver-impregnated clay filter candles resulted in the decreased bactericidal efficiency reported in the literature (Ohashi & Oya, 1992; Chaudhuri *et al*., 1994).

Chloride has been reported to decrease the bactericidal activity of silver (Russell & Hugo, 1994; Lin *et al*., 2002). As discussed above and illustrated in Figures 1(a) & (b), the presence of chloride influenced the speciation of Ag because the nutrient broth used in this work contained *ca.* 45 mg Cl/l (see Table 1). Perhaps surprisingly, the MIC observed for Ag(I) after 24 h of incubation under the conditions examined in this work is comparable to the MIC of 0.27 mg/l reported by Gandhour *et al*. (1988) for *E. coli* that were grown in a chloride-free medium at circumneutral pH values and 37°C (Table 3). The congruence between the results reported herein and the work by Gandhour *et al*. (1988) may be the result of an increase in 'accessible' anionic complexes in the presence of higher chloride concentrations in the nutrient broth as reported by Gupta *et al*. (1998), and/or the result of the higher incubation temperature. The efficacy of using a $AgCl_{(s)}$ precipitate as a form of silver was also evaluated. In this case $AgCl_{(s)}$ was formed by combining equimolar concentrations of $AgNO_3$ and NaCl to form a 1000 ppm Ag stock solution, which was then thoroughly mixed, added to the culture in the microtitre plates and serially diluted according to the method discussed above. As illustrated in Table 2, the MIC range increased only slightly. This increase may have been the result of dilution error caused by transferring the colloidal $AgCl_{(s)}$ solution or limitations in the rate of dissolution of $AgCl_{(s)}$. In either case, using $AgCl_{(s)}$ as a form of silver did not have a substantial influence on the MIC under these growth conditions although the upper value of the range is greater than the USEPA Secondary Drinking Water Standard for silver.

Hardness has been reported to negatively influence the bactericidal efficiency of silver (Russell & Hugo, 1994). MINEQL + predicted that the speciation of silver would not change (data not shown) in the presence of 300 mg/l

Table 3 | Influence of selected water quality parameters on the MIC for *E. coli* following a 24-h incubation period at 25°C

Parameter	MIC (mg Ag/l)
Cl free medium*	0.27≥MIC≥0.1
Hardness (300 mg/l as $CaCO_3$)	0.10≥MIC≥0.05
Turbidity (25 NTU)	0.10≥MIC≥0.05

*Adapted from Ghandour *et al.* (1988).

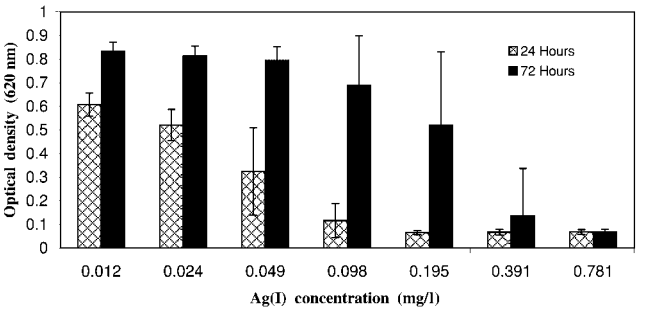

Figure 3 | Influence of incubation time on the Ag(I) MIC for *E. coli* incubated at 25°C. Error bars represent one standard deviation.

Table 4 | Influence of time on upper range of the MIC for *E. coli* for several of the silver species examined

Form of silver	MIC, 72 h (mg Ag/l)	MIC (72 h)/ MIC (24 h)
Ag(I)	$0.78 \geq MIC \geq 0.39$	7.8
Organic-Ag	$0.78 \geq MIC \geq 0.39$	7.8
Ag_2O	$1.10 \geq MIC \geq 0.55$	7.9
$AgCl_{(s)}$	$1.56 \geq MIC \geq 0.78$	3.9

of Ca hardness as $CaCO_3$. Congruently, as illustrated in Table 3, Ca hardness at this concentration did not influence the MIC for Ag(I) under these growth conditions. The data suggest that Ca hardness does not influence the bactericidal activity of silver even when the water is very hard. Lin *et al*. (2002) also reported that moderate hardness (100 mg/l of Ca hardness as $CaCO_3$) did not negatively influence the bactericidal activity of Ag(I) against *L. pneumophila*.

Because silver is known to have a high affinity for particulate matter in natural systems (Wen *et al*., 1997), the effect of high turbidity was also examined by dissolving kaolin clay in the nutrient broth, which resulted in a final turbidity of 25 NTU. (The background turbidity of the media was 2.4 NTU.) The MIC was unchanged under these conditions (Table 3), a finding that was unexpected in light of the reported reduction of silver into Ag^0 on the surface of montmorillonite (Ohashi & Oya, 1992). It is presumed that the reactions between silver species and nutrient broth, and silver species and *E. coli* may be more thermodynamically favourable than between silver species and kaolin.

Silver is known to accumulate in bacteria including *E. coli* (Belly & Kydd, 1982; Ghandour *et al*., 1988; Efrima & Bronk, 1998). Accumulation of silver in batch systems may lead to an increase in the MIC because inactive bacteria may bind silver thereby reducing the effective aqueous silver concentration. The MIC for all incubation experiments was monitored up to 72 h following the start of incubation. Figure 3 illustrates (see also Table 4) that the MIC increased significantly with time for Ag(I) to a value that is greater than the USEPA Secondary Drinking Water Standard following 72 h of incubation. Table 4 illustrates the MIC for 72 h and the change in MIC from 24 h to 72 h for all of the forms of silver tested. In all cases the MIC increased by a factor of 4 to 8 between 24 and 72 h. Adsorption of silver from deionized water and nutrient broth to the microtitre plates, under aseptic conditions, was not detected over a 72-h incubation period. These results suggest that the influence of biological accumulation of silver should be considered in batch systems and in systems that have point-of-use silver-impregnated water treatment devices because it could lead to a decrease in aqueous silver concentration. Indeed, this problem could certainly be exacerbated if silver resistant bacteria were able to grow in these filters, as reported in the literature (Tobin *et al*., 1981; Reasoner *et al*., 1987; Geldreich & Reasoner, 1990; Bell, 1991), and accumulate silver.

CONCLUSIONS

The MIC for Ag(I) was observed to be between 0.1 mg Ag/l and 0.05 mg Ag/l following a 24-h incubation period at 25°C for *E. coli*. The results suggest that Ag(I) or Ag(I)-complexes were the only forms of silver with significant bactericidal activity. The effectiveness of silver as a bactericidal agent did not change significantly in the presence of chloride, increased hardness, or high turbidity. Because only a small fraction of silver bound to

GAC surfaces may be readily leachable as Ag(I), the useful life of silver-impregnated point-of-use filters should be based on the time in which the desorbing silver concentration remains above the MIC (Ag(I)>0.05 mg/l) at the rated flowrate for the device. The accumulation of silver by microorganisms should also be considered in batch systems because the effective silver concentration could fall below the MIC. Finally, future studies with silver and other target bacterial strains are warranted.

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