Antibacterial properties of Ag and Ag/AgCl nanoparticles from radish and tea extracts for water treatment applications
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ABSTRACT
Silver nanoparticles (AgNPs) have antibacterial properties and are widely used for water disinfection. This technology is commercially applied in point-of-use water treatment as a post-treatment for filtrate water. However, the current process of synthesizing AgNPs has several disadvantages including the use of hazardous chemicals, consumption of a large amount of energy and the formation of hazardous byproducts. Here, we report an alternative and green synthesis using plant extracts. In this work, the plant extracts came from radish (R) and tea (T), and the AgNPs were derived from a microwave irradiation method. The AgNPs synthesized by chemical-based microwave irradiation (Ag-C) were also used as a control material. The novel method produced a smaller size of nanostructures with good dispersion ability and less agglomeration than those from chemical synthesis. The antibacterial properties of AgNPs on Gram-negative bacteria *Escherichia coli* (*E. coli*) and Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) were investigated. The results revealed that AgNPs from both green synthesis and chemical-based methods inactivated both types of bacteria. The green-synthesized AgNPs from radish juice provided a higher percentage of inhibition of *E. coli* than that of *S. aureus*. The inactivation rates of the AgNPs increased with increasing concentration of AgNPs. As the concentration of the Ag/AgCl-R and Ag-R increased from 150 μg/mL to 300 μg/mL, complete inactivation required a reduced time for the reaction from 300 minutes to only 30 minutes. Finally, the Ag/AgCl-R and Ag-R offered high antibacterial activity while the Ag-T provided the lowest antibacterial activity. This work provides an alternative method for the eco-synthesis of antibacterial nanomaterials for water treatment.

Key words | Ag, antibacterial, green nanomaterials, toxicity, water treatment

INTRODUCTION

The use of silver in water to remove bacteria and other microorganisms is widely known. This technology is commercially applied for point-of-use (POU) water treatment in households. It is also used as a post-treatment of filtrate water in water treatment plants. Silver nanoparticles (AgNPs) are an important development (Varma 2014) and have attracted the attention of many researchers and scientists due to their superb antibacterial properties. However, AgNPs are limited by their synthesis, which requires dangerous chemicals, large amounts of heat, and the formation of hazardous byproducts (Varma 2014). The conventional method of AgNP synthesis requires a reductive reaction. This reaction must be held at a constant temperature of approximately 60–80 °C for a long period (Wang et al. 2005; Hebbalalu et al. 2013). Recently, the green synthesis of AgNPs has been presented by using a microwave-based method (Hebbalalu et al. 2013). This method has several advantages over the conventional method especially in controlling the small size of nanostructures, the size distribution, and the degree of crystallization (Nadagouda et al. 2013).
Also, microwave heating requires shorter reaction times and less energy and achieves better product yields.

Using plant extracts rather than hazardous chemicals is another green chemistry approach that is widely used in synthesizing AgNPs. Many previous works have reported the synthesis of AgNPs via plant extracts. These include green tea (Camellia sinensis), Nelumbo nucifera Gaertn., Eucalyptus hybridra, Helianthus annuus, Rosa sinensis, Acalypha indica, and Tinospora cordifolia Miers (Leela & Vivekanandan 2008; Dubey et al. 2009; Krishnaraj et al. 2010; Philip 2010; Jayaseelan et al. 2011; Santhoshkumar et al. 2011). Here, we focused on green tea (Camellia sinensis) and radish (Raphanus sativus).

Indeed, green tea (Camellia sinensis) has received significant attention for the synthesis of green AgNPs (Vilchis-Nestor et al. 2008; Moulton et al. 2010; Virkutyte & Varma 2015). The epicatechin or tea extract is used as a reducing and capping agent. The nanosize of the particles is controlled by varying the concentration of tea extract. The extracts can act as reducing and stabilizing agents in the synthesis of AgNPs. The resulting particles exhibited highly efficient, single-photon induced luminescence that could be manipulated by changing the silver ion concentration (Vilchis-Nestor et al. 2008).

Radish (Raphanus sativus) is another plant extract used in this work. It is in the Brassicaceae family. This plant is rich in ascorbic acid and folic acid and is a good source of vitamin B6. It has been reported that vitamins B1, B2, and C function both as reducing and capping agents for nanoparticles (Varma 2012). Thus, the radish extract possibly provides a smaller size of nanostructures with good dispersion ability and less agglomeration, which are the prerequisite characteristics of a good disinfection agent. Currently, most published works have focused on the characteristics and properties of AgNPs from plants. However, fewer works have reported the potential applications of those AgNPs in detail, especially their disinfection ability for water treatment.

In this study, we focused on the synthesis of AgNPs with radish (Raphanus sativus) and green tea (Camellia sinensis) extracts and investigated their antibacterial activities for use in water disinfection. To the best of our knowledge, this is the first work that has reported the disinfection ability of AgNPs obtained from radish and green tea extract. In this work, the radish extract was used to synthesize AgNPs (Ag-R) and Ag/AgCl (Ag/AgCl-R) using a microwave irradiation method. We also prepared AgNPs from tea extract (Ag-T) using a microwave irradiation method. AgNPs from a chemical-based AgNP (Ag-C) reduction process were also synthesized for comparison. Scanning electron microscopy (SEM) and X-ray diffraction (XRD) analyses were performed to confirm the formation of AgNPs. The antibacterial properties of those AgNPs on Gram-negative bacteria Escherichia coli (E. coli) and Gram-positive bacteria Staphylococcus aureus (S. aureus) were investigated, and we calculated the disinfection rates of E. coli using those AgNPs. The antibacterial activity was discussed on the basis of the AgNPs’ characteristics.

**METHODOLOGY**

**Chemicals**

All chemicals used in the experiment were analytic reagents (AR). The silver nitrate (AgNO₃) was provided by Aldrich; glucose and polyvinyl pyrrolidone (PVP) were obtained from Merck. The sodium hydroxide (NaOH) and sodium chloride (NaCl) were from Sigma-Aldrich.

**Synthesis of AgNPs**

For the preparation of radish juice, 50 g of radish root in 100 mL water was blended twice (10 minutes each). The mixture of juice and pulp was filtered (Whatman, No. 41) to obtain radish juice. AgNP synthesis followed the tropical method (Kou & Varma 2022a) in which 0.3 mmol of AgNO₃ and 6 mL radish juice were mixed at room temperature. The mixture was irradiated in the microwave at 100 °C for 15 minutes. The AgNPs precipitated and were separated from the mixture. The AgNPs from this process are termed Ag-R.

For Ag/AgCl-R, we followed the method of Kou & Varma (2022b). The 0.2 mmol AgNO₃ was dissolved in water (2 mL). Then, a NaCl solution (0.5 M) was added dropwise under vigorous magnetic stirring at room temperature. A white precipitate was formed, and 2 mL of radish juice was added to the mixture before being irradiated with microwave energy. After the reaction was complete,
the AgCl/Ag nanoparticles were harvested by centrifugation. For the tea extract, 1 g of green tea was boiled in 50 mL of water and filtered through a filter paper (Whatman, No. 41). Then, 2 mL of 0.1 N AgNO₃ was mixed with 10 mL of tea extract and shaken to ensure thorough mixing. The reaction mixture was allowed to settle at room temperature.

For chemical-based AgNPs, we used a technique from the literature (Wang et al. 2005). The AgNPs were made by reducing the AgNO₃ in PVP aqueous solution. The AgNO₃ solution was prepared by adding 3.4 g of AgNO₃ into 20 mL distilled water. The PVP solution was prepared by dissolving PVP, glucose and NaOH in 60 mL distilled water with heating to 60°C. The AgNO₃ solution was then added to the PVP solution. The particles from the mixture were separated after the reaction. All AgNPs were washed five times with de-ionized water and dried in a vacuum oven before use.

**Material characterization**

The surface physical morphology of the AgNPs was examined using a scanning electron microscope (SEM) (Leo1455VP). The crystal size of the AgNPs was examined using a transmission electron microscope (TEM) (Philips/Tecnai 12). All AgNP samples were mounted on a copper stub for SEM analysis and a copper grid for TEM analysis. Then, the samples were sputter coated with a thin layer of gold before examining by SEM or TEM. The samples were also analyzed with XRD (JDX-3530, Jeol, Japan) using CuKα radiation in the range of 20°–70°. The particle size and the hydrodynamic radii of the AgNPs were determined by dynamic light scattering (DLS) at 90°. Values for point of zero charge (pHₚzc) were determined with a Zetasizer Nano series instrument (Malvern, UK).

**Kirby-Bauer testing for antibacterial activity**

The Kirby-Bauer technique is a relatively quick and easy approach to semi-quantitative testing of antibacterial activities (Saravanan et al. 2011; Pant et al. 2013). In this work, antibacterial inactivation tests were carried out using *Escherichia coli* (*E. coli*, ATCC: 25922) and *Staphylococcus aureus* (*S. aureus*, ATCC: 25923) as test organisms. One colony of each bacteria was taken from a Petri dish. It was grown in nutrient broth medium (Müller-Hinton agar: beef infusion 300.0 mL, casein hydrolysate 17.5 g, starch 1.5 g, agar: 17.0 g). The pH was adjusted to neutrality at 25°C. The different types of AgNP were added as sterile disks to a final concentration of 100 μg/L. The disks were then gently placed in the agar and incubated. After incubation at 37°C for 24 hours, the zone of inhibition against the AgNPs was observed. The antibacterial activity of AgNPs was demonstrated by the diameter of the zone of inhibition versus the control Ag-C.

**Antibacterial activity test**

The antibacterial activities of the AgNPs were tested using two common bacterial strains: *E. coli* and *S. aureus*. In this test, the AgNPs and bacteria were stirred (250 rpm) together in a tryptic soy broth (TSB) and incubated at 37°C for 24 hours. The viable bacterial count was determined at different time intervals by plating the serial dilutions on agar plates, and we counted the number of colony forming units (CFU). The survival of the bacterial population was calculated by the equation:

\[
\text{Survival (\%)} = \left( \frac{P_r}{P_i} \right) \times 100
\]

where \(P_i\) and \(P_r\) are the CFU of the bacteria before and after contacting the AgNPs.

**RESULTS AND DISCUSSION**

**Morphology and characteristics of AgNPs**

The morphologies of Ag-R, Ag/AgCl-R, Ag-T, and Ag-C are shown in Figure 1(a)–(d). The surface morphology of all AgNPs from SEM analysis shows that the particles had a uniform crystalline phase. Surface morphologies of Ag/AgCl-R and Ag-R appeared as discrete particles while those of Ag-T and Ag-C were agglomerates. In addition, the TEM images of AgNPs are shown in Figure 2. The discrete pattern of nanomaterials is shown by the TEM image of Ag-R nanoparticles that were synthesized with radish
Figure 1  | SEM images of various types of AgNPs: (a) Ag/AgCl-R, (b) Ag-R, (c) Ag-T and (d) Ag-C.

Figure 2  | TEM images of (a) Ag-R, synthesized with radish extract, and (b) Ag-C, synthesized without extract.
extract (Figure 2(a)). The agglomerate pattern of nanomaterials is seen via TEM imaging of Ag-C – these were synthesized using a chemical-based method without organic extract (Figure 2(b)).

The difference in the accumulation pattern of these AgNPs may result from the presence of extracts used in material synthesis. The nanoparticles are capped with organic molecules from the extracts. This prevents particle agglomeration (Varma 2012). The SEM data suggest that vitamin B6 in the radish provides better dispersion characteristics for the AgNPs than the polyphenol from tea extract.

Table 1 shows crystal size, particle size, and pH\textsubscript{pzc} of four AgNPs. The crystal sizes of the nanoparticles were obtained from TEM analysis, and the particle sizes were measured using dynamic light scattering. The point of zero charge (pH\textsubscript{pzc}) was measured with a zetasizer. The crystal sizes of the Ag/AgCl-R, Ag-R, and Ag-T were slightly smaller than that of Ag-C. The particle sizes of those nanoparticles were also smaller than that of Ag-C. This information is evidence that the organic molecules from plant extracts tend to cap the AgNPs and can prevent the agglomeration of AgNPs.

The zeta potential measurements showed that the pH\textsubscript{pzc} value of Ag-C was 3.1 while the pH\textsubscript{pzc} values of Ag/AgCl-R, Ag-R, and Ag-T were 2.0, 2.7, and 2.8, respectively. This indicates that the plant extracts also shifted the pH\textsubscript{pzc} of the AgNPs towards a low acidic pH. Thus, AgNPs from radish (or Ag-R) are capped by organic molecules better than AgNPs from tea extract – this results in less agglomeration. Polyphenol from tea extract has been shown to reduce or cap nanoparticles (Varma 2012). However, in this work, vitamin B6 from radish provides better dispersion of AgNPs than the polyphenol from tea extract.

The crystalline nature of the AgNPs was demonstrated by X-ray diffraction (Figure 3). It reveals two major compositions of nanoparticles: Ag and AgCl. For all AgNPs, the peaks at 20 values of 38.0, 44.0, 64.5, 77.5, and 81.5 correspond to the spectrum of Ag. This result indicates that the particles were indeed silver. AgCl composition only appeared in the Ag/AgCl-R. The AgCl peaks were at 20 values of 27.9, 32, 32.5, 46, and 55. AgCl dominated the composition of Ag/AgCl-R – there is no evidence of Ag\textsubscript{2}O in any AgNPs.

**Antibacterial activity**

The antibacterial testing data using the Kirby-Bauer approach with *E. coli* and *S. aureus* are shown in Figure 4 and Figure 5, respectively. The AgNP samples formed inhibition zones in this test. The control experiment without AgNPs was also conducted for comparison.

The diameters of the inhibition zones are summarized and presented in Table 2. The diameters of the inhibition zones for the control (without AgNPs), Ag-C, Ag-T, Ag-R, and Ag/AgCl are 0.01 ± 0.02, 6.50 ± 0.50, 8.75 ± 0.25, and 9.50 ± 0.10 mm against *S. aureus*, respectively. The Ag-C had the lowest antibacterial activity for both bacteria species. The Ag-R and Ag/AgCl-R showed high antibacterial activity versus *E. coli* and *S. aureus*.

A comparison of the antibacterial activity of AgNPs between *E. coli* and *S. aureus* at 5 and 120 minutes is shown in Figure 6. The inactivation of Gram-negative species (*E. coli*) using the AgNPs was greater than that of Gram-positive species (*S. aureus*) for all AgNPs. The number of *E. coli* drastically decreased upon application of Ag/AgCl-R and Ag-R at 120 minutes. The different inactivations between *E. coli* and *S. aureus* might be due to the fact that the pH\textsubscript{pzc}
values of all AgNPs are in an acidic region. Thus, the Gram-negative species of *E. coli* are more susceptible than the Gram-positive species of *S. aureus*.

The effect of different concentrations of Ag/AgCl-R, Ag-R, and Ag-T on the inactivation of *E. coli* is shown in Figure 7. The AgNPs were prepared by incubating the bacteria with various concentrations of AgNPs. For Ag/AgCl-R and Ag-R, the doses of nanoparticles were 35, 70, 150, and 300 µg/mL. For Ag-T, the doses of nanoparticles were 150, 300, 450, and 600 µg/mL.

The inactivation rate and antibacterial efficacy of AgNPs in each condition are shown in Table 3. The inactivation rates of the AgNPs increased with increasing concentration of AgNPs. Clearly, the Ag/AgCl-R and Ag-R provided high antibacterial activity; Ag-T provided the lowest antibacterial activity. As the concentration of Ag/AgCl-R and Ag-R increased from 150 µg/mL to 300 µg/mL, complete
Figure 6 | Antibacterial activity of AgNPs (100 μg/mL) on E.coli and S. aureus at 5 and 120 minutes.

Figure 7 | Antibacterial activity (N/N0) of AgNPs on E. coli using different concentrations as a function of time.
inactivation time dropped from 300 minutes to 30 minutes. However, 50 μg/mL of either type of AgNP is too low to inactivate bacteria. From Table 3, we see that the inactivation rate at 300 μg/mL of Ag/AgCl-R was much higher than at 150 and 75 μg/mL. The inactivation rate accelerated as a function of AgNP concentration. For the Ag-T, the inactivation rate and antibacterial ability were not as good as those using Ag/AgCl-R and Ag-R. Increasing the dose of Ag-T from 150 to 600 μg/mL can increase the inactivation percentage from 12.2% to 40.5%. The complete inactivation was not seen at the doses of Ag-T used here. This might be because of the agglomeration of Ag-T.

The antibacterial activity of Ag is dependent on Ag⁺, which binds strongly to electron donor groups on biological molecules like sulfur, oxygen, or nitrogen (Ghaffari-Moghaddam & Eslahi 2014). The silver ions act by displacing other essential metal ions such as Ca²⁺ or Zn²⁺ (Boomi et al. 2015). The aggregation of the particles plays a significant role in this testing. As the aggregation of particles increases, the effective surface-to-volume ratio of the particles decreases, and the interaction between particles and the cell wall of the bacteria also decreases.

The SEM data and size analysis showed that the Ag/AgCl-R and Ag-R were capped with radish extracts that can prevent particle aggregation. Thus, the Ag/AgCl-R and Ag-R can effectively inhibit the growth of bacteria. There are various mechanisms for the action of silver nanoparticles on the bacterial cell (Prabhu & Poulose 2012). These mechanisms include: (i) the ability of AgNPs to anchor to and penetrate the bacterial cell wall (Sondi & Salopek-Sondi 2004); (ii) the formation of free radicals by the AgNPs that can damage the cell membrane and make it porous (Danilcauk et al. 2006; Kim et al. 2007); (iii) release of Ag⁺ that can interact with the thiol groups of many vital enzymes and inactivate them (Matsumura et al. 2003; Feng et al. 2008); and (iv) the AgNPs can modulate signal transduction in bacteria to stop bacterial growth (Shrivastava et al. 2007). Here, we show that the Ag/AgCl-R and Ag-R provided high antibacterial activity while the Ag-T provided the lowest antibacterial activity. This work describes an alternative, eco-friendly synthesis that results in a product with strong antibacterial properties that can be used in disinfection.

**Table 3** | Comparison of the inactivation rate and antibacterial efficiency of AgNPs on *E. coli* using different concentrations of AgNPs

<table>
<thead>
<tr>
<th>AgNPs</th>
<th>Concentration of AgNPs (mg/mL)</th>
<th>Inactivation rate (mg/mL-min)</th>
<th>Antibacterial efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag/AgCl-R</td>
<td>35</td>
<td>0.0072</td>
<td>22.3</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>0.0015</td>
<td>95.6</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.0047</td>
<td>99.2</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.0333</td>
<td>100.0</td>
</tr>
<tr>
<td>Ag-R</td>
<td>35</td>
<td>0.0024</td>
<td>15.3</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>0.0025</td>
<td>80.5</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.0054</td>
<td>91.4</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.0250</td>
<td>100.0</td>
</tr>
<tr>
<td>Ag-T</td>
<td>150</td>
<td>0.0045</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.0075</td>
<td>24.5</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>0.0038</td>
<td>32.7</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>0.0076</td>
<td>40.5</td>
</tr>
</tbody>
</table>

**CONCLUSION**

In this work, radish juice and tea extract were used to synthesize AgNPs using a microwave irradiation method. AgNPs synthesized by chemical-based microwave irradiation were also used as control materials. The resulting AgNPs from plant extracts had a smaller size as nanostructures with good dispersion ability and less agglomeration of nanoparticles than AgNPs via traditional synthesis. The antibacterial effects of Ag-R and Ag/AgCl-R can inactivate both *Escherichia coli* (*E. coli*) and Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) effectively. The green-synthesized AgNPs from radish juice provided the highest percentage of AgNP inhibition in *E. coli* and *S. aureus*. The inactivation of Gram-negative species (*E. coli*) with AgNs was greater than that of Gram-positive species (*S. aureus*) for all AgNPs. The inactivation rates of the AgNPs increased with increasing concentrations of AgNPs. As the concentration of Ag/AgCl-R and Ag-R increased from 150 μg/mL to 300 μg/mL, complete inactivation required a shorter time (300 minutes versus only 30 minutes). Finally, the Ag/AgCl-R and Ag-R provided high antibacterial activity while the Ag-T provided the lowest antibacterial activity. Green chemistry for AgNP synthesis is an alternative method that is eco-friendly and effective against bacteria. These nanomaterials can be used to disinfect water.
REFERENCES


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First received 5 February 2015; accepted in revised form 29 July 2015. Available online 13 August 2015