Preparation and characterisation of silver nanoparticle coated on cellulose paper: evaluation of their potential as antibacterial water filter

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ABSTRACT
This study investigates the ability of cellulose filter paper coated with silver nanoparticles to remove Escherichia coli from drinking water. The cellulose filter paper was coated with silver nanoparticles by a chemical reduction method using two different ratios of sodium borohydride and silver nitrate. In consideration of drinking-water quality standards and non-carcinogenic health risks, the optimum sodium borohydride:silver nitrate ratio for coating the cellulose filter paper was determined by comparing the silver in the effluent after E. coli removal. For both ratios, 100% E. coli removal was realised. In terms of the silver in the effluent, only the first two lowest concentrations for both ratios of sodium borohydride and silver nitrate were compliant with the drinking-water quality standards, demonstrating hazard quotients (HQs) between 0.084 and 0.484. On the basis of the highest level of E. coli removal with the lowest HQ value, the optimum sodium borohydride:silver nitrate ratio for coating the cellulose filter paper as an antibacterial water filter was 2:1 molar ratio (0.002 M:0.001 M). Silver nanoparticle-coated cellulose filter paper was found to be an inexpensive and easy-to-use emergency antibacterial water filter to generate clean drinking water.

1. Introduction
The World Health Organization (WHO) and United Nations Children’s Emergency Fund [1] reported that 1.8 billion people consume faecally contaminated drinking water. The presence of specific faecal bacteria in drinking water is an indicator of faecal contamination. Insufficient treatment, cross-contamination, and poor maintenance of the distribution network are all common causes of faecal contamination of drinking water.[2,3] The assessment of faecal contamination of drinking water relies on indicator bacteria such as coliforms, Pseudomonas aeruginosa, and Escherichia coli. E. coli is a more specific
indicator of faecal contamination in drinking water than any other indicator.\cite{4,5} \textit{E. coli} can be detected in different matrices, including drinking water and sediment by improved testing methods. Additionally, \textit{E. coli} testing method recommended by the United States Environmental Protection Agency analyses enzyme that is selective for the \textit{E. coli} organism and separates \textit{E. coli} from non-faecal thermotolerant coliforms.\cite{5}

During emergency situations, clean drinking water is vital to save lives. Unsafe drinking water poses the greatest danger to public health because it can lead to outbreaks of diseases, like cholera and gastroenteritis.\cite{6,7} Thus, the main priority during emergency situations should be to provide clean potable drinking water. Two conventional modes of providing clean drinking water during emergencies are either water supplied from a packaged treatment plant or trucked water from such a plant and the use of point-of-use water treatment systems.\cite{8} Trucked water is expensive, difficult in terms of logistics, and ineffective, considering the poor facilities used to boil and store water. By contrast, a point-of-use water treatment system has been proved effective, economical, energy efficient, and easy to use.\cite{9,10} Although point-of-use water treatment has been shown to improve water quality and reduce diarrhoea incidents during emergencies, further investigation and adaptation is necessary to overcome its existing limitations, including insufficiency supply during emergencies in large geographical areas or countries.\cite{11} Thus, the search for an affordable and effective point-of-use water treatment system for simple intervention during various emergency situations is still ongoing.

Nanotechnology provides a new mode for removing \textit{E. coli} through point-of-use water treatment during emergencies.\cite{10,12} Extensive research into silver nanoparticles has revealed their broad spectrum of antibacterial properties, high surface area-to-volume ratio, and strong and broad-spectrum antimicrobial activities.\cite{13,14} Praveena and Aris (2015) reviewed low-cost antibacterial filters, namely, ceramic, polyurethane, agricultural waste, and fibre coated with silver nanoparticles. These low-cost filters for point-of-use water treatment systems have removed between 92\% and 100\% of \textit{E. coli} during emergency applications.\cite{14–17} Although ceramic filters coated with silver nanoparticles can remove 97.8\%–100\% of \textit{E. coli}, the concentration of silver in the effluent exceeds the drinking-water quality standards.\cite{17} Moreover, incorporating silver into ceramics involve combustion, long preparation time with low flow rate, thus this technology is unsuitable during emergencies. Rice husk ash used as activated carbon, which contains nearly 95\% of silica mass, is a renewable resource for silver nanoparticle immobilisation. However, the preparation of rice husks requires combustion before being coated with silver nanoparticles.\cite{15} Using polyurethane foam is unsuitable for emergency applications because of the complex procedure of polyelectrolyte multilayer modification.\cite{18} Among these low-cost materials, cellulose substrates have attracted the most research interest for its successful track record as an antibacterial water filter. Cellulose makes a good filter because it is inexpensive, abundant, sustainable, and renewable; it also allows for rapidly absorption of silver nanoparticles during the coating process.\cite{19–21} The silver ions from the silver nanoparticles in the cellulose filter paper bind to the thiol group of the membrane protein and lead to membrane damage. Interference of silver ions in DNA replication can also result in cell death.\cite{22,23} The mechanisms of silver nanoparticles as antibacterial agents remain unclear. However, Larimer et al. \cite{24} suggested that the efficacy of silver nanoparticles can be ascribed to their direct contact with the cell wall of the contaminant organism. Bottino et al. \cite{25} and Heidarpour et al. \cite{12} demonstrated that
silver nanoparticles inhibit microbial growth by attaching to the cell membrane. Silver nanoparticles severely damage the cell’s major functions, such as enzyme-signal regulation, cell oxidation, and respiration. Brown and Su et al. explained that the bactericidal activity of silver nanoparticles depends on the electrostatic attraction between the positively charged silver nanoparticles and the negatively charged cell surfaces of E. coli. This finding has been used as a practical basis in using silver nanoparticles to remove pathogenic bacteria from drinking water. Dankovich and Gray, Heidarpour et al. and Nover et al. have successfully proved the antimicrobial activities of silver nanoparticles.

This paper presents the antibacterial properties and usefulness of cellulose filter paper coated with silver nanoparticles in removing E. coli from drinking water for different sodium borohydride:silver nitrate ratios. The optimum sodium borohydride:silver nitrate ratio for coating cellulose filter paper is determined using the following criteria: (1) bacteria removal, (2) silver in the effluent, and (3) health risk. The determination of the best sodium borohydride and silver nitrate ratio may help public health organisations employ an inexpensive, easy, and rapid preparation method to remove E. coli from drinking water at the point of use, which is often vulnerable during emergencies.

2. Materials and methods

The preparation of silver nanoparticle-coated cellulose filter paper can be divided into eight stages, namely, material preparation, silver nanoparticle-coated paper preparation, silver nanoparticle characterisation, bactericidal testing, filtration process, silver-in-effluent analysis, and E. coli removal effectiveness assessment, and morphology observation. All these stages are examined in this study.

In the material preparation, Whatman cellulose filter paper from high-quality cotton liner with 98% alpha cellulose content was used. The high percentage of alpha cellulose in the paper represents the amount of unmodified cellulose insoluble in sodium hydroxide solution and the pure form of cellulose. Among all cellulose types, alpha cellulose has the highest degree of polymerisation and forms provides most stable material.

In the silver nanoparticle-coated paper preparation, cellulose filter paper was coated with silver nanoparticles by a chemical reduction method, with sodium borohydride as the reducing reagent. Sodium borohydride was used as the reducing agent and stabiliser because it induces the most uniform and smallest of nanoparticles among all reducing agents. Sheets of Whatman cellulose filter paper (10 cm × 10 cm × 8 mm) were individually immersed in 40 mL of silver nitrate solutions at various concentrations ranging from 0 M to 0.1 M for 30 min. Then, each cellulose filter paper was rinsed with 95% ethanol for 1 min to flush unabsorbed silver nitrate from the cellulose filter paper. Next, each cellulose filter paper was placed in either a 2:1 or a 10:1 molar-ratio sodium borohydride: silver nitrate solution for 1 h. These ratios were compared to understand the role of sodium borohydride in terms of particle size and distribution. After this step, the cellulose filter paper was washed in 30 mL of ultrapure water for 30 min, and excess water absorbed by cellulose filter paper was removed by drying the sheet in a drying oven at 60 °C for 2.5 h. Untreated cellulose filter paper not immersed in a solution of silver nitrate and sodium borohydride served as control.
In the silver nanoparticle characterisation, the distribution of silver nanoparticles in the cellulose filter paper was examined with a Hitachi HT7700 transmission electron microscope to measure the sizes of the silver nanoparticles in individual specimens of cellulose filter paper. A Hitachi S-3700N scanning electron microscope attached to an energy-dispersive X-ray spectroscopy (EDX) detector was used to record the images of silver nanoparticles in cellulose filter paper.

Acid digestion was performed to quantify the silver concentrations in the cellulose filter paper. Cellulose filter paper was digested using 5 mL of nitric acid and 5 mL of 30% hydrogen peroxide.[31] Hydrogen peroxide was used to assist in the complete oxidation of the organic matter to release additional metals into the solution.[32] After heating the sample to 75 °C, the digestion was complete when a white residue remained, and the solution evaporated completely. After the cooling process, the samples were filtered using 0.45 μm filter paper. Untreated cellulose filter paper was also digested similarly as the control sample. Then, an inductively coupled plasma mass spectrometer (ICPMS; Agilent 7500a) was used to quantify the silver concentration in the cellulose filter paper.

In the bactericidal testing, the performance of the silver nanoparticles in the cellulose filter paper to deactivate E. coli was performed using a non-pathogenic strain of E. coli (ATCC 11229). This organism is ideal for our study because of its relatively small size and shape (approximately 2.5 μm); it is also a specific indicator of faecal contamination of drinking water.[33] Based on the method of Lechevallier et al.,[2] E. coli sample was cultured by pouring E. coli (ATCC 11229) to 1 L of autoclaved lauryl sulphate nutrient broth, and the suspension was agitated using a shaker water bath at 37 °C and 160 rpm overnight. During the midexponential growth phase, E. coli growth was measured with an absorbance of approximately 1.0 at 600 nm by ultraviolet-visible (UV-VIS) spectrophotometry. E. coli was separated from the lauryl sulphate nutrient broth by centrifuge method for 10 min at 3000 rpm, and the separated bacteria were resuspended in deionised water and diluted until an absorbance value of 0.1 (monitored with a spectrophotometer), which is equivalent to 10^8 colony-forming units (CFU)/mL.

In the filtration process, a water sample containing E. coli was used as a model for contaminated water. The water sample was passed through a sheet of silver nanoparticle-coated cellulose filter paper. The silver nanoparticle-coated cellulose filter paper was horizontally placed on a plastic grid, with 10 replicates done for each paper sample. The effluent was isolated for E. coli colony to check E. coli removal effectiveness. For E. coli isolation, 100 mL of effluent was filtered under vacuum through a 0.45 μm Whatman filter paper. E. coli retained on the surface of the filter paper was placed on lauryl sulphate broth and incubated at 42 °C—44 °C for 14 h. Yellow colonies were counted directly as E. coli present in the effluent.[34]

In the morphology observation, samples of E. coli were imaged using a field-emission scanning electron microscope (Hitachi S-3700N) with EDX detector before and after the filtration process. Scanning electron microscopes are known to provide high-resolution three-dimensional visualisation of microorganisms adherent to surfaces. An Agilent 7500a ICPMS was used to analyse the silver concentrations in the effluent after filtration.

Appropriate quality control and assurance measures were implemented to achieve higher data confidence against bias and variability. Ten replicate samples were run to estimate variability resulting from the analytical procedure.[34] Sample blanks were used to test for bias from the possible contamination of blank water, which consisted of distilled
Log reduction value (LRV) was determined by comparing the amount of colonies before and after the filtration process (Equation (1)). The silver residue in the effluent from the silver nanoparticle-coated cellulose filter was compared with the Malaysian Drinking-Water Quality Standards [35] and United States EPA standards and guidelines. [36] Silver-in-efluent analysis was conducted as part of the health-risk assessment on potential of non-carcinogenic risks. [19] According to the WHO, [37] silver is a non-carcinogenic element, but silver intoxication can result from chronic silver exposure. Hazard quotient (HQ) is the ratio of the average daily dose (ADD) to the reference dose (RfD), as expressed in Equation (2). Further details on health-risk assessment for silver can be obtained from previous studies, [38,39]

\[
HQ = \frac{ADD}{RfD},
\]

where

\[A = \text{number of } E. \text{ coli before filtration},\]
\[B = \text{number of } E. \text{ coli after filtration.}\]

\[
HQ = \frac{ADD}{RfD},
\]

where

\[ADD = \text{average daily dose (mg/kg/day)},\]
\[RfD = \text{Reference dose (mg/kg/day)}.
\]

3. Results and discussion

3.1. EDX images of silver nanoparticles in cellulose filter paper

Figure 1 shows the SEM images together with the EDX spectrum of the silver nanoparticle-coated cellulose filter paper. The peaks of silver in the EDX images proved proof of the attachment of silver nanoparticles to the cellulose filter paper. Figures 2 and 3 show the silver nanoparticle sizes obtained using a transmission electron microscope. The
transmission electron microscope images revealed that the particle size for a 2:1 sodium borohydride:silver nitrate molar ratio was between 5 and 31 nm, with 12 nm having the highest frequency (11%) of occurrence. For a 10:1 sodium borohydride:silver nitrate molar ratio, the particle sizes ranged between 5 and 24 nm, with 7 nm having the highest frequency (13%). These findings support the previous studies of Zhu et al. [40] and Dankovich and Gray,[10] who concluded that a higher molarity of sodium borohydride formed smaller and more uniform silver nanoparticles. Song et al. [35] showed that low sodium borohydride:silver nitrate ratios (lower than 2) produced low concentrations of silver nanoparticles because of an insufficient reduction reaction. A small amount of sodium borohydride likewise resulted in boron hydroxide production through hydrolysis, which reduces the electron density of surfaces and causes intense aggregation of silver nanoparticles. By contrast, a large amount of sodium borohydride prevented boron hydroxide ions from being absorbed into the silver nanoparticle surfaces, resulting in small and well-dispersed silver nanoparticles.

Figure 2. Particle size distribution of silver nanoparticles for 2:1 molar ratio NaBH4:AgNO3.

Figure 3. Particle size distribution of silver nanoparticles for 10:1 molar ratio NaBH4:AgNO3.
3.2. *E. coli* removal using silver nanoparticle-coated cellulose filter paper

Table 1 shows the *E. coli* measurement for the water sample with $10^8$ CFU/mL after filtration. For both ratios, the LRVs for *E. coli* removal were from 7 to 8. The morphological changes in *E. coli* were observed using a scanning electron microscope. Figure 4(a) shows the undamaged *E. coli* cell surface before passing through the silver nanoparticle-coated cellulose filter paper, whereas Figure 4(b) shows the *E. coli* cell surface after the filtration process using the silver nanoparticle-coated cellulose filter paper. After filtration, obvious pits can be observed on the *E. coli* cell surface. The formation of these pits can increase cell permeability and lead to cytoplasmic cell leakage.

3.3. Silver concentration in effluent after filtration

The amount of silver in the effluent after filtration was considered to evaluate the impact of the cellulose filter paper on drinking-water quality. Figures 5 and 6 show the concentrations of silver in the effluent for both ratios. The results show that only the first two lowest concentrations for both ratios of sodium borohydride:silver nitrate comply with the Malaysian Drinking-Water Quality Standards [36] and the guidelines and regulations of

![Figure 4. SEM images of *E. coli* (a) before filtration without silver contact (b) after filtration with contact of silver nanoparticle (surface cell of *E. coli*).](image-url)
the United States Environmental Protection Agency,[41] which respectively state that the maximum contaminant level of silver ions in drinking water must be less than 0.05 and 0.10 mg/L. The retention of silver nanoparticles in the cellulose filter paper depends on the incorporation method.[42] Nanostructures retention in paper matrices is a challenge due to weak interactions (H-bonding and van der Waals forces) between cellulose surfaces and nanostructures. Thus, various additives have been used to improve retention of the nanostructures in paper matrices.[43] Moreover, the excess silver may also be due to the reduced stability of silver nanoparticles in the cellulose filter paper.[44] The chemical reduction method using sodium borohydride is preferred because of the desirable properties of borohydride, including high reactivity (compared with citrate), ease of handling, and non-toxicity.[45] Borohydride acts not only as a reducing agent but also as an ion stabiliser, which prevents silver ions from aggregating. Moreover, hydroxyl and ether groups in the cellulose fibre play an important role in the stabilisation of metal nanoparticles.[15]

### 3.4. Health risk assessment involving silver effluent after filtration

According to Borm et al. [46], human health risk assessment should be included as an important dimension in understanding the impacts of nanoparticles on human populations. Thus, for a conservative prediction of the risk of a solution to human health, a more detailed human health risk assessment is necessary. Our health risk assessment shows non-carcinogenic risks for the highest concentrations for both ratios. Figures 5 and 6 show that only the two lowest concentrations for both ratios of sodium borohydride and silver nitrate in the cellulose filter paper comply with the Malaysian Drinking-Water Quality Standards. Moreover, the two lowest concentrations for both ratios of sodium borohydride and silver nitrate in the cellulose filter paper also have HQ values between 0.084 and 0.484 indicated that there is no any health risk through ingestion. The optimum molar ratio of sodium borohydride: silver nitrate was determined by comparing the E. coli removal percentages against the drinking-water quality standards and by the lowest HQ values. Thus, 0.002 M:0.001 M sodium borohydridesilver nitrate ratio is selected to be coated on the cellulose filter paper as an
antibacterial water filter, taking into account the need for less silver with low potential of leaching from the cellulose filter paper coated with silver nanoparticles and low health risks.

3.5. Comparison of different materials used as antibacterial water filters

Table 2 shows a comparison of different materials used as antibacterial water filters. Given that reports on silver nanoparticle-coated cellulose filter paper are unavailable, we are unable to compare our results with those of a similar work. The closest study was conducted by Dankovich and Gray [10] and it involved blotting paper. Blotting paper contains natural fibre with nitrocellulose, which is a popular binding matrix for blotting paper with an excellent affinity to proteins and high blocking capacity. By contrast, cellulose filter paper is made of a high-quality cotton liner of 98% alpha cellulose.

According to He et al. [15], strong interactions between Ag ions, hydroxyl, and ether groups of nanoporous structures of cellulose fibre result in the strong attachment of Ag to cellulose fibre. This strong attachment plays an important role in the stabilisation of metal nanoparticles and results in the low mobility of silver nanoparticles and the formation of

Table 2. Comparison between E. coli removal efficiency and silver concentration in effluent with other materials.

| Type of materials                                      | E. coli removal efficiency (%) | Silver concentration in filtered water (mg/L) | Reference                        |
|--------------------------------------------------------|--------------------------------|-----------------------------------------------|----------------------------------|
| Cellulose filter paper                                  | 99—100                         | 0.02—0.03                                     | Present study                    |
| Blotting paper                                          | 99—100                         | 0.0475                                        | Dankovich and Gray [10]          |
| Ceramic materials (clay from Indonesia)                 | NA                             | 10                                            | Rayner et al. [17]               |
| Ceramic materials (clay mixed with sawdust)             | 92                             | 0.02                                          | Kallman et al. [47]              |
| Ceramic filters (manufactured by combining 40% soil, 10% flour, and 50% grog) | 97.8-100% | <0.1                                         | Oyanedel-Craver and Smith [48]  |
| Polyethersulphones                                      | 100                            | 0.005                                         | Diagne et al. [16]               |
| Polyurethane foams                                      | 100                            | 0                                             | Jain and Pradeep [14]            |
| Rice husk ash                                           | NA                             | <0.1                                          | He et al. [49]                   |
| Activated carbon fibres with activated with phosphoric acid | 100                            | 10                                            | Chen et al. [50]                 |
monodispersed silver nanoparticles. This phenomenon holds true at low silver-ion concentrations. However, at high silver concentrations, a large amount of silver ions are attached to cellulose fibres, leading to large and widely distributed particles after reduction. A stabiliser is needed to control the growth of larger silver nanoparticles. This explanation likewise rationalises why only low concentrations of silver for both ratios in this study meet the drinking-water quality standards and have low HQ values. Polyurethane foam showed better *E. coli* removal and lower silver amount in the effluent because this material uses an electrochemical method for the synthesis of nanoparticles. Electrochemical synthesis is more effective as this method releases minimal chemical by-products and low levels of excess silver cations.[51] However, according to Jain and Pradeep,[14] the preparation of polyurethane foams require repeated washing and air drying. These requirements make the preparation process lengthy and more costly. Similarly, preparations of antibacterial materials, such as ceramics and rice husks, involve combustion as the main preparation process and lose effectiveness over time due to release of silver from the filters which depend amount of silver applied, filter pore structure and water chemistry, making these materials unsuitable for use during emergency situations.[12,19]

3.6. **Capability of silver nanoparticle-coated cellulose filter paper**

In this study, the measured flow rates are approximately 330 mL/s using cellulose filter paper with an area of 80 cm², which is sufficient to supply approximately 7 L of water to meet the drinking-water requirements of a person during emergency.[52] However, the real capacity of this cellulose filter paper needs to be tested using field samples (low and high turbidity) need to be focused in next study. The high flow rate and low cost (approximately 1–2 USD/sheet) of the filter paper can address the issues of maintenance and replacement especially during emergency situations. However, long-term research and development of a better coating mechanism for silver nanoparticles can help obtain further information on the efficacy of cellulose filter paper as an antibacterial water filter. Importantly, this study provides a better understanding of materials that can be prepared in a few hours and easily used for point-of-use water purification by fitted it into a funnel for use in emergencies as an antibacterial water filter.

4. **Conclusions**

Cellulose filter paper was coated with silver nanoparticles using a chemical reduction method, with sodium borohydride:silver nitrate ratios of 2:1 and 10:1. The efficiency this cellulose filter in *E. coli* removal ranged from 99% to 100%. Moreover, the performance of the silver nanoparticle-coated cellulose filter paper was evaluated in terms of silver in the effluent and compliance with the drinking-water quality standards. The optimum 2:1 molar ratio (0.002 M:0.001 M) of sodium borohydride and silver nitrate to be coated on cellulose filter paper was determined using the *E. coli* removal percentages as basis, comparing the percentages with drinking-water quality standards, and obtaining the lowest HQ value. Further research on the development of a better coating mechanism for silver nanoparticles is necessary to quantify *E. coli* removal and silver in the effluent as a function of material reusability and filter life span. Although further research is still necessary, the performance
of silver nanoparticle-coated cellulose filter paper and its short and easy preparation method suggests its suitability as an antibacterial water filter during emergency situations.

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