Abstract: Surface and groundwater sources, especially in urban areas, have been reported to be highly contaminated with faecal matter. Hence, removal of faecal-derived microorganisms in potable water is essential in protecting community health. Currently in many countries, chlorination is the mostly adopted disinfection technique in water treatment. However, disinfection by-products such as trihalomethanes that are produced due to post-chlorination bring about a risk of carcinogenic effects. Hence, finding alternative disinfection techniques are needed to avoid such health-related impacts due to disinfection by-products. In this study, the potential antimicrobial activity of silver oxide nanoparticles (SONPs) were studied. Synthesized-SONPs were characterized using ESEM-EDX, FT-IR, and XRD analytical methods. The antibacterial potential of SONPs was estimated using inhibition zone diameter, well-diffusion and broth dilution (optical density) methods. Synthesized-SONPs were found in the nano-range between 1 - 95 nm. SONPs had an irregular radial distribution pattern over the nutrient agar and the maximum distribution was identified within a dosage of 0.05 - 0.075 mg of SONPs. The inhibition zone around the SONPs in nutrient agar was increased with the increase of SONPs dosage and, the maximum inhibition zone was observed at 0.05 g of SONPs. The maximum growth of the inhibition zone in well-diffusion method was exhibited for 120 hours as 22.55 mm per 0.03 g and 20.63 mm per 0.04 g of SONPs. In broth dilution method, an excellent percentage of growth inhibition was exhibited, which was 85% and 81% for 0.02 and 0.03 g of SONPs. The results in this study provided an insight that SONPs strongly interacted with Escherichia coli and acted as a powerful antibacterial agent.

Keywords: Chlorination, disinfection, Escherichia coli, groundwater, growth inhibition, well diffusion.

1. INTRODUCTION

Providing safe potable water free of pathogenic microorganisms is a critical health concern all over the world. Surface and groundwater sources of potable water are in general, contaminated with faecal matter. The consumption of faecally polluted water has led to outbreak of water-borne diseases such as diarrhoea, gastrointestinal illnesses caused by various bacteria, viruses, and protozoa (Craun et al. 2006). World Health Organization (WHO) has declared that about 3.4 million of annual deaths are attributed to water-borne diseases, of which 99.8% of the deaths have been reported in developing countries, and most victims have been children (WHO, 2014). Hence, provision of safe drinking water is a high priority to protect the health of communities. At present, chlorine is often used as a disinfectant in water treatment and supply processes, especially in developing countries. The use of chlorine to disinfect potable water has been reported to be producing disinfection by-products such as trihalomethanes and halo-acetic acids (Bon et al. 2012). Such disinfection by-products, even in small amounts, have been reported to pose risk of cancer, act as mutagens, responsible for reproductive problem and miscarriage, and cause heart, kidney, lungs, liver and central nervous system problems (WHO, 2005). People in urban and peri-urban areas depend highly on pipe-borne water often rich in residual chlorine, which is provided as a buffer against contamination. Prolonged consumption of chlorinated water can bring about greater risks of adverse health effects. Hence, finding an alternative reliable disinfectant method is a vital need to replace conventional chemical disinfection methods such as chlorine, chloramines, and chlorine dioxide. Recent advances made with nanoparticles and potential use of such material for disinfection provide new pathways to look into in relation to providing safe potable water.

Silver oxide nanoparticles (SONPs) are popular due to their disinfection properties such as antimicrobial activity, good conductivity, catalytic activity and chemical stability (Frattini et al. 2005).
Several nanomaterials, including nano-silver/fiberglass (Nangmenyi et al. 2009), nano-silver/zeolite (Mahmood et al. 1993), nano-silver/sand (Matsumura et al. 2003), nano-silver/graphene oxide (Das et al. 2011), amino acid functionalized nano-silver/graphene oxide sheet (Chandraker et al. 2016), and nano-silver (Xie et al. 2014) have been developed to be used as a disinfectant in drinking water treatment. Even though, SNPs are commonly used as antibacterial agents, the antibacterial action of SNPs has not been reported to be remarkably high for potential commercialization. However, SONPs have been proved to be an effective alternative disinfection technique in the recent past. The detailed information about antibacterial attributes of SONPs to inhibit the growth of Escherichia coli has not been previously reported. Hence, this study investigates the antibacterial properties of SONPs in the removal of Escherichia coli bacteria in potable water under different environmental conditions and dosages of synthesized-SONPs by two different methods such as well-diffusion and broth dilution (Optical density) methods. Escherichia coli was selected to investigate antimicrobial properties of SONPs because such a group of bacteria can be commonly found in faecally polluted waters.

2. METHODOLOGY/MATERIALS AND METHODS

2.1 Synthesis of SONPs

In this study, SONPs were synthesized with a simple liquid-phase chemical reduction method proposed by Yong et al. (2013). 20 g of the Polyethylene glycol (PEG) was dissolved in 1 L of deionized water before being heated up to 50 °C. The solution was stirred for another 1 hour to ensure PEG was completely dissolved to form a homogeneous solution. The aqueous PEG solution was then filtered to remove impurities present, if any. Silver nitrate salt was dissolved in deionized water and added into PEG solution. Then pH of the PEG and silver nitrate mixture was set at pH 9.8 to 10 and stirred vigorously. pH of the mixture was maintained as 9.8 - 10 for 1 hour. For the pH adjustment, 0.1 M NaOH solution was added. The solution was continuously stirred for 1 hour to complete the chemical reaction. After the formation of nanoparticles, the solution was filtered to separate the particles from the solution. Particles obtained were rinsed with distilled water several times before it was rinsed again with ethanol. The particles were dried in an oven at 60 °C overnight to obtain the SONPs.

2.2 Characterization of SONPs

Morphological characteristics and elemental composition of the SONPs were identified using an Environmental Scanning Electron Microscopy (ESEM - Carl Zeiss EVO 18 Secondary Electron Microscope) coupled with Energy-Dispersive X-ray Spectroscopy (EDX analyser, ZI element). Phase identification of crystal structure in SONPs was performed using the X-ray Powder Diffraction (XRD - D8 Advance Bruker Diffractometer with filtered Co Kα radiation). Particle size distribution was calculated using the Scherrer formula;

\[ D = \frac{n\lambda}{\beta \cos \Theta} \]  

Where \( D \) is the crystalline size, \( n \) is a constant (if the particles are assumed to be spherical, \( n = 0.9 \)), \( \lambda \) is the wave length of the X-ray radiation, \( \beta \) is the line width and \( \Theta \) is the angle diffraction (Amir et al. 2015). Fourier Transform Infrared Spectroscopy (FTIR – ALPHA) was performed in the adsorption mode at ambient temperature in the spectral range of 500 – 4000 cm\(^{-1}\) to identify functional groups of the SONPs.

2.3 Morphological characteristics Escherichia coli bacteria and colony

The morphological characterization of bacteria was observed with an ESEM. A 1-mL of bacterial suspension was collected by centrifugation at 5000 rpm and was washed with sterile deionized water three times, followed by fixing with 3% glutaraldehyde solution for 4 hours. The samples were dehydrated with sequential treatment of 30, 50, 70, 90, and 100% ethanol for 10 minutes. Finally, the dried cells were gold sputter-coated, and imaged using ESEM. Bacterial colonies were examined using Electronic Optical Microscopy (EOM) (Axio Lab., A1 Carl Zeiss),
2.4. Antibacterial activity of SONPs

The antibacterial activity of SONPs was studied using gram-negative *Escherichia coli* bacteria. Firstly, the distribution pattern through nutrient agar (HiCrone clostridium selective agar) of SONPs was investigated in accordance with different SONPs dosages, such as 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.175, and 0.2 g. Then a range of suitable SONPs dosages that inhibit bacterial growth significantly were determined with different SONPs dosages (a range of 0.001 to 1.0 g). Petri dishes with nutrient agar inoculated by bacterial suspension were incubated in 37 °C for 24 hours. SONPs were placed on the agar medium using cotton pellets in three replications. The inhibition zone diameter was measured using a Vernier Calliper and mean diameters were analysed. The suitable SONPs dosages were determined to conduct the well-diffusion and broth dilution methods (See below).

2.4.1. Well-diffusion method

After identifying the range of suitable SONPs dosage, the bactericidal effects were studied in the nutrient agar medium using the well-diffusion method. 0.1-mL of *Escherichia coli* bacterial suspension was inoculated in semi-solidified 10 mL of nutrient agar in petri dishes and spread evenly. Small wells about 11.35 mm diameter in size were made in the middle of the petri dishes. Different dosages of SONPs such as 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08 and, 0.09 g/mL were added into each well with three replications and a control sample without SONPs. All samples were incubated at 37 °C for 24 hours. The inhibitory action of the SONPs was determined measuring the diameter of the inhibition zone in millimetres around the each well using a Vernier Calliper and mean values were analysed.

2.4.2. Broth-dilution (optical density) method

The bacterial growth kinetics were studied using 10 mL of MacConkey Broth Purple nutrient medium, which was poured in each nine test tubes plugged with sterile cotton. The experiment was conducted with the three replications and the control. MacConkey Broth Purple nutrient medium in three sets of nine test tubes were inoculated by 0.1 mL of *Escherichia coli* suspension. Different dosages of SONPs such as 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, and, 0.09 g/mL were added into test tubes. The growth kinetics of the *Escherichia coli* was observed by determining the optical density in 600 nm using UV/Vis spectrophotometer. The control samples were prepared, excluding the SONPs in the bacterial inoculated nutrient broth. The percentage of growth inhibition was calculated using:

\[
\text{% of growth inhibition} = \frac{(OD_c - OD_t)}{OD_c} \times 100
\]  

Where \(OD_c\) and \(OD_t\) represent the optical density of the control and test samples of SONPs, respectively.

3. RESULTS AND DISCUSSION

3.1. Characterization of SONPs

The preliminary investigation of the formation of SONPs and their morphological characteristics were identified using the X-ray diffraction spectrophotometer (Figure 1). The prominent peak at 38.2°, 44.4°, 64.5° and 77.4° according to 2θ values could be attributed to the crystallographic planes of SNPs in (111), (200), (220) and (311). The prominent peak at 38.2° confirmed the formation of crystalline silver, which has also been observed by Das et al. (2011) and Jalali et al. (2015). The crystalline size of the SONPs calculated using Scherrer formula represented a range between 1-95 nm. The ESEM images of SONPs were appeared to be in the form of small spherical shape with smooth edges and confirmed the presence of agglomeration, which may have been due to chemical and magnetic interactions among the particles (Figure 2). An agglomeration of nanoparticles due to high surface reactivity increased the size of SONPs. Previous studies also reported agglomeration of the SONPs (Devaraj et al. 2013) and the agglomeration process increased the stability of colloidal SONPs due to the influence of van der Waals and electrostatic forces (Chen et al. 2007). The material was composed only with silver (89.97%) and oxygen (10.03%) elements as evident from EDX analysis (Figure 3).
Figure 1 XRD Analysis of the Synthesized Crystalline SONPS, Peaks At The 38.2°, 44.4°, 64.5° And 77.4° Represented the Formation of Crystalline Phases of SONPs

Figure 2 ESEM Analysis of the Synthesized SONPs, Particles Were Agglomerated and Spherical in Shape

Figure 3 EDX Analysis of the Synthesized SONPs, Peaks Illustrated Available High Content of 89.97% of Silver Elements And 10.03% of Oxygen Elements in the Sample).

FTIR analysis peaks (Figure 4) were observed at the ~3394 cm⁻¹, ~1649 cm⁻¹, ~1370 cm⁻¹, and ~883 cm⁻¹. Peaks at ~3394 cm⁻¹, ~1649 cm⁻¹ were assigned to stretching and bending vibration of hydroxyl groups (Jyoti et al. 2016). The O-H stretching and bending vibration manifested surface adsorbed water, resulting in the formation of the hydroxide, broad peak at ~1387 cm⁻¹ assigned for stretching of the N-O due to residual silver nitrate used for synthesizing SONPs (Mohiti-Asli et al. 2014).

3.2. Morphological characteristics Escherichia coli bacteria and colony

ESEM analysis of the Escherichia coli bacteria showed that they grew in culture media as rod-shaped bacterial cells and circular-shaped bacterial colonies and showed terracing and continued outward expansion with sectorial subpopulations (Figure 5). Same observation has been reported in the study conducted by Shapiro (1987).
3.3. Antibacterial activity of SONPS

The distribution pattern of SONPs through the nutrient agar was investigated using different dosages (0.025 to 0.2 g) incorporated on cotton pellets. The irregular radial distribution pattern was manifested by the SONPs as shown in Figure 6. The maximum and well-distributed zone diameter was observed in a range of 0.05 to 0.075 mg of SONPs. To identify appropriate SONPs dosages which inhibit maximum bacterial growth, a range of 0.001 to 1.0 g of SONPs dosages was therefore tested as the suitable SONPs dosage. The mean inhibition zone diameter was analysed to select the SONPs dosage giving rise to maximum bacterial inhibition. The results showed that the inhibition zone around SONPs rich in nutrient agar was increased with the increase of SONPs dosage and with the mass of 0.05 g of SONPs, the maximum inhibition zone was observed (Figure 7). Even after 1 hour, it was observed that the formation of inhibition zone had started around the trial composed of 0.05 g of SONPs dosage.

Antibacterial activity of SONPs was then determined using the well-diffusion method for *Escherichia coli* using different dosages such as 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, and 0.09 g/ml (Figure 8 (a)). These values were decided based on the range of dosages that gave the maximum inhibition zone. *Escherichia coli* were comparatively more sensitive to the SONPs dosages selected. The maximum growth of inhibition zone was measured after 24, 48, 96, and 120 hours. After 24 hours, formation of the inhibition zone began to appear. The growth of the inhibition zone of SONPs was exhibited clearly in 48 hours (Figure 9). However, no change was observed for the trials with 0.01, 0.02, 0.08 and 0.09 g of SONPs in 48 hours. The inhibition zone observed for other trials in 48 hours were 21.45 mm for 0.03 g of SONPs, 19.35 mm for 0.04 g of SONPs, 14.1 mm for 0.05 g of SONPs, 12.5 mm for 0.06 g of SONPs, and 12.1 mm for 0.07 g of SONPs, respectively; the maximum growth of the inhibition zone was observed for 120 hours as 17.35 mm for 0.02 g of SONPs, 22.60 mm for 0.03 g of SONPs, 21.43 mm for 0.04 g of SONPs, 15.80 mm for 0.05 g of SONPs, 16.20 mm for 0.06 g of SONPs, 15.30 mm for 0.07 g of SONPs and no change was observed for 0.08 and 0.09 g of SONPs. 0.03 and 0.04 g of SONPs dosage in well-diffusion method showed the mean maximum growth inhibition zone as 22.2 mm and 20.3 mm, respectively.
In the broth dilution method, test tubes were prepared with 10 mL of culture media, including SONPs and *Escherichia coli*; a suspension was taken to determine the antibacterial effects of the SONPs at the same dosages mentioned above. The colour variation of the suspension medium was determined using the UV/vis spectrophotometer in 600 nm. The results indicated that different dosages of SONPs showed different affinities for the antimicrobial activity. With higher dosages such as 0.08 and 0.09 g of SONPs antibacterial activity was observed to be marginal. Maximum growth inhibition percentage of 85 and 81% were observed at 0.02 and 0.03 g of SONPs dosages, respectively (Figure 8 (b)). SONPs dosages within the range 0.01 to 0.07 g showed a substantial growth inhibition of *Escherichia coli* bacteria. The growth inhibition decreases considerably when the carbon source in the media is lowered (Xie et al. 2014). With the limited carbon source in the media with time, it has been observed that the production of lipopolysaccharide decreases making the bacteria more vulnerable for the actions of SONPs for inhibition of growth (Xie et al. 2014) and hence death. When the cell membrane of *Escherichia coli* interacted with SONPs, the process has been found to alter the cell response-potential of fatty acid composition, surface charge, protein content, cell surface hydrophobicity, lipid composition and bacterial cellulose, which lead to the inhibition of the bacterial growth (Kaczorek et al. 2013). The results of the present study provided an insight that SONPs considerably interact with *Escherichia coli* acting as a powerful antibacterial agent to improve the microbiological quality of potable water.
4. CONCLUSIONS

SONPs were synthesized successfully and different bacterial growth inhibition methods, such as inhibition zone, well-diffusion and broth dilution (Optical density) methods were used to identify the faecal decontamination potential of different dosages of SONPs as an alternative disinfection technique. SONPs dosages of 0.03 and 0.04 g showed effective bacterial inhibition in the well-diffusion method and 0.02 and 0.03 g SONPs exhibited excellent percentage of growth inhibition in the broth dilution method. The present study indicated that SONPs could be effectively and efficiently used as an alternative disinfection product for faecally contaminated potable water.

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6. REFERENCES


