NEW TRENDS ON MICROBIOLOGICAL WATER TREATMENT

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Silver nanoparticle-decorated porous polypropylene filter is prepared by physical vapor deposition method using a modified Balzers 760 coating machine. Silver nanoparticles were generated by electron beam bombardment of the silver metal. A 45nm layer of the silver nanoparticles were subsequently deposited on the polypropylene filter homogenously. The nano silver-coated filters were characterized using scanning electron microscopy, transmission electron microscopy and atomic force microscopy. The antibacterial efficiency of the nano silver-coated filters was evaluated using a custom-made experimental set up and the membrane filter method. A zone of inhibition test was also performed to compare the bactericidal effect of coated versus non-coated filters. At a flow rate of 3L/hr, the output count of Escherichia coli was zero after 6.5 hours filtration when the input water had a bacterial load of 10^3 colony-forming units (cfu) per milliliter. The inductively coupled plasma/mass spectrometry (ICP/MS) results showed that the 45nm layer of the silver nanoparticles are stable on the water filter and are not washed away by water flow even after 6.5h filtration.

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1. Introduction

Clean water is essential to human life as well as the rest of the ecosystem. Nowadays, we are experiencing difficulties in responding to the increasing demand for reliable water sources. There are many approaches available for the disinfection of water. Each method has some practical difficulties and disadvantages. Nanotechnology is an emerging advanced technology with significant potential in various fields including solving water purification problems. Using nanotechnology methods it is possible to convert metal ions into nanoparticles for medical and non-medical [1] applications. The major property that makes nanoparticles attractive is that they are extremely small in size (1-100nm), which provides them with a higher surface area per unit mass. Nanomaterials often show unique and considerably enhanced physical, chemical and biological properties compared to their macro-scaled counterparts [2]. Studies have shown that the size, morphology, stability and other physicochemical properties of the metal nanoparticles are strongly influenced by the experimental conditions. These include kinetics of interaction of metal ions with reducing agents and adsorption processes of stabilizing agents with metal nanoparticles

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[2,3]. Hence, the design of a synthesis method in which the size, morphology, stability and other properties of nanoparticles are controlled has become a major field of interest [4]. Silver ions have been widely used as an effective water disinfectant or antimicrobial material for many centuries [5]. The antimicrobial action of silver ions has been widely reported against a broad spectrum of microorganisms [6-9] along with a lack of negative effects such as undesirable taste, odor and color [10-17]. Silver ions are being widely used in health care to control microorganisms, especially in water supply systems. The most widely known bactericidal mechanism of the silver ion is its interaction with the thiol groups of the L-cysteine residue of proteins and subsequent inactivation of their enzymatic functions [18-20]. Silver ion is a highly reactive species, readily binding to negatively charged proteins and chloride ions. While some other bactericidal mechanisms of silver ions, such as the release of potassium [20] and binding to DNA and RNA [21] have also been reported, generation of intracellular reactive oxygen species (ROS) by silver ions is strongly supported as an alternative mechanism. This relies on a number of evidences including: (*i*) silver ions were found to induce an increase in the respiration rate and the generation of intracellular ROS during this process [22,23] and (*ii*) the interaction between silver ions and the thiol groups could interrupt essential enzymes in the respiratory chain, such as NADH and succinate dehydrogenase, thereby obstructing adequate electron transfer to oxygen [24-26]. Although these are plausible reasons for ROS generation, very little experimental evidence of silver-induced intracellular ROS-generation and consequent bactericidal activity has been demonstrated. Regardless of the mechanism of action, the bactericidal effect of silver is now well established.

The WHO recommended that any water intended for drinking should contain fecal and total coli form counts of 0 in any 100ml sample [27,28]. The US Environmental Protection Agency (US EPA) proposed that the maximum contaminant level of silver ion in water must be less than 0.10mg/L [29,30]. Construction of water purification systems to comply with these regulations is the topic of research and study in several laboratories worldwide.

In this study, nano silver particles were deposited on the cylindrical polypropylene water filters using a modified Balzers machine [31,32]. Characterization of the filters was carried out using the scanning electron, transmission electron and atomic force microscopy techniques. The antibacterial property of the filters was evaluated using the membrane filter method as well as the zone of inhibition test. Furthermore, the quantity of silver released from the filters was determined using inductively coupled plasma mass spectrometry (ICP-MS).

2. Experimental Materials

The Silver used in this study was of high grade quality (purity: 99.99%) and was supplied by Merck (Germany). The Balzers 760 coating machine, purchased from Balzers Company (Germany), has the following specifications. It can be used to evaporate a variety of metals in order to create a very thin layer of nanoparticles on the substratum. It has rotary and high vacuum diffusion pumps and a chamber area (888mm W, 923mm H, 950mm D) equipped with water cooled walls. The coating machine is also equipped with a thickness monitor system in order to measure the thickness of the created nanoparticle layers. The cylindrical polypropylene filters constructed using multi-layers of the polymer were purchased from Omran Mahab Co. (Tehran, Iran) with an average pore-size of 9.86μm. The dimensions of the water filter are 25cm (height) by 6.2cm (diameter). It possesses high filtering efficiency and able to remove dirt, rust, dust, silt, algae and other sediments but will not remove harmful bacteria and microorganisms such as Escherichia Coli. Therefore, it can not be used where the water quality is unknown or is unsafe. E. coli (ATCC strain 8739, USA) was selected as an indicator of fecal contamination of water. Eosin methylene blue agar, EMB, was purchased from Merck (Germany).

3. Methods and techniques

The Balzers 760 machine can be used to cover the surface of flat objects with thin metal layers but can not be employed for the coating of cylindrical objects *per se*. In order to be able to coat the cylindrical water filters with a homogenous layer of silver nanoparticles, a remotely controlled shaft was installed inside the chamber of the Balzers machine. The polypropylene water filter was placed on the shaft and the rotation of the shaft was adjusted to 20 rounds per minute [31] (Heidarpour et al. 2010). A molybdenum crucible was used for melting of silver metal. This is because silver has melting and boiling points of 961.78°C and 2162°C respectively, while the melting and boiling points of molybdenum are 2623°C and 4639°C. Therefore, molybdenum can be considered as a suitable crucible for silver. About 3 grams of silver was put in the pot crucible and placed in the chamber within a distance of 50mm from the electron beam. A 45nm layer of silver is coated on the polypropylene water filter at an evaporation rate of 2nm/s. By subtracting the weight of silver and crucible before and after coating, the weight of evaporated silver was obtained. The average amount of silver used for coating of each of the water filters was 2.15gr.

Transmission electron microscopy (TEM) was performed to measure and evaluate size and morphology of the nanosilver particles obtained by the Balzers 760 machine. TEM measurements of Ag nanoparticles were carried out by using a Philips EM 201 instrument (The Netherlands) with an electron beam emitted at 80 kV. For recording the TEM photograph, the samples were dispersed in distilled water using a bath sonicator for 20min and a few drops were spread on the grid of the TEM.

Bacterial attachment to the surface of the filters was visualised using a JEOL JSM-6400 scanning electron microscope (Japan). For this, square-shaped samples with the approximate size of 1cm by 1cm were cut from the filter surfaces and visualized by the SEM. Prior to SEM analysis of the filters containing bacteria, the samples were treated with 4% glutaraldehyde for 24h at 4ºC followed by two rounds of washing with 0.1M sodium cacodylate buffer and a post-fixation in 1% osmium tetroxide (2h, 4ºC). The samples were then dehydrated using a series of different concentrations of acetone (35- 100%) followed by drying and sputter-coating the samples with gold.

The pore sizes of the nano silver-coated polypropylene filters were analyzed by a Hitachi S-3400N scanning electron microscopy (SEM, Japan). For this, square-shaped samples with the approximate size of 1cm by 1cm were cut from the surface of the filters. The samples were dried at room temperature in a controlled environment. The images were captured in SEM mode at the desired magnification.

The thickness of nanosilver layer covering the surface of the water filters was measured by atomic force microscopy (AFM). For this, a square-shaped sample with the size of 1cm by 1cm was cut from the filter surface and analyzed by an atomic force microscope (AFM, Quesant Instrument Corporation, USA) in contact mode. In this technique, the feedback mechanism is the force measured between a tip and sample. In contact mode AFM, the tip and sample are brought into contact and a feedback circuit maintains a constant tip-sample force during scanning, usually by maintaining a constant cantilever deflection.

To examine the bactericidal effect of the nano silver-coated polypropylene water filters, a custom-made pilot plant was used as shown in Fig. 1. Initially 15L of distilled water was inoculated with 10^3cfu/mL E-coli bacteria. A Stainless steel centrifugal pump (1–phase, 1/2 suction/discharge, Max Head 20m), was used for feed and recirculation. The flow of water was measured by rotameter and the temperature was controlled by a cooling device inserted into the feed tank. Two gauges were used to measure the pressure at the entrance and exit to the housing of the silver-coated polypropylene water filter (Fig. 1). The flow rate was adjusted to 3L/hr and the pressure difference before and after the water filter was 0.1bar. The flux (hydraulic conductivity) of the coated and uncoated filters, defined as the flow volume/unit time per unit cross-section area of a porous medium, was calculated according to [33] Brassington and Australian National Committee on Irrigation and Drainage (ANCID 2000) [34].

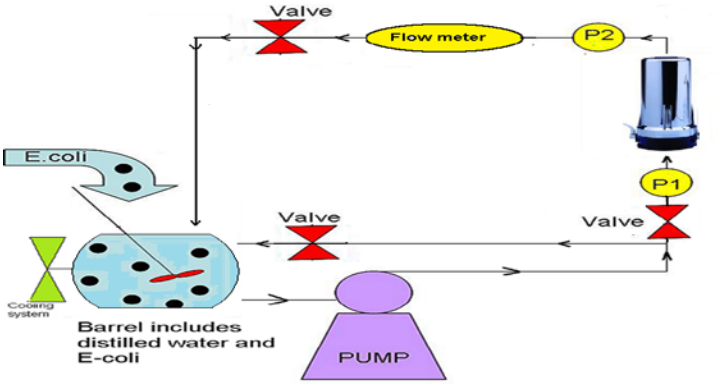

Fig. 1. Design of the experimental set-up for the evaluation of antibacterial efficiency of the nano silver-coated polypropylene filters. Distilled water includes E-coli and was circulated in the system by a centrifugal pump. P1 and P2 are manometers.

In order to further prove the antibacterial efficiency of the nano silver filters, the zone of inhibition test was also conducted. For this, Eosin methylene blue (EMB) agar was poured onto disposable sterilized Petri dishes and was allowed to solidify. An amount of 10mL of the bacteria-loaded water (10^3 cfu/mL) was streaked over the plate and was spread uniformly. Pieces from both of the nano silver-coated and uncoated water filters were gently placed over the solidified agar gel in different Petri dishes. Plates were incubated at 37ºC for a time period of 24h before evaluation.

In order to quantify the amount of silver particles released from the filters to the water, the silver concentration in the treated water sample was measured by using inductively coupled plasma mass spectrometer (Varian ICP/MS, Palo Alto, CA, USA). For this, a 100mL sample of the distilled water, filtered for 6.5 hours using the nano silver-coated filter, was analyzed by ICP/MS [35].

4. Results and discussion

Polypropylene water filters coated with a 45nm layer of nano silver were manufactured by physical vapor deposition (PVD) method using a modified Balzers 760 machine [32]. One advantage of the PVD method is that the thickness of the silver layer can be measured and controlled during the coating process. Consequently, employing the PVD method the accuracy of coating is much more than the other available methods such as the sputtering and chemical vapor deposition techniques [36,37]. The Balzers 760 machine does not allow coating all surfaces of objects in any single run. Therefore, it was subjected to appropriate modifications to enable coating all surfaces of the cylindrical filters homogenously.

Evaluation of the particle size and morphologies of silver nanoparticles was performed using the transmission electron microscopy (TEM). TEM studies revealed that the nano silver particle size distribution ranged from 9.27 to 18.42nm with average particle diameter of 13.85nm. The nanoparticles observed through TEM have polygonal (or circular) geometry (Fig. 2). Due to particle overlapping or aggregation, some of the nano silver particles seem to possess bigger particle sizes.

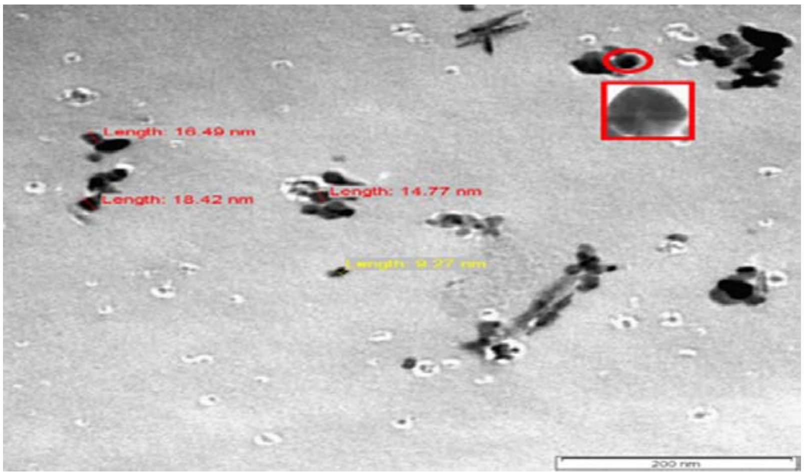

Fig. 2. Transmission electron microscopy (TEM) image of the silver nano particles. The nano silver particles are circular or polygonal shaped (inset).

The atomic force microscopy technique was employed to measure the thickness of the coated nano silver layer on the polypropylene water filters. The average thickness of the nano silver layer on the surface of the water filters obtained by the AFM technique is 44.70nm (Fig. 3). This is very close to the thickness of 45nm reported by the microprocessor of the coating machine. It appears that the silver nanoparticle binding is on the surface of the polypropylene filters.

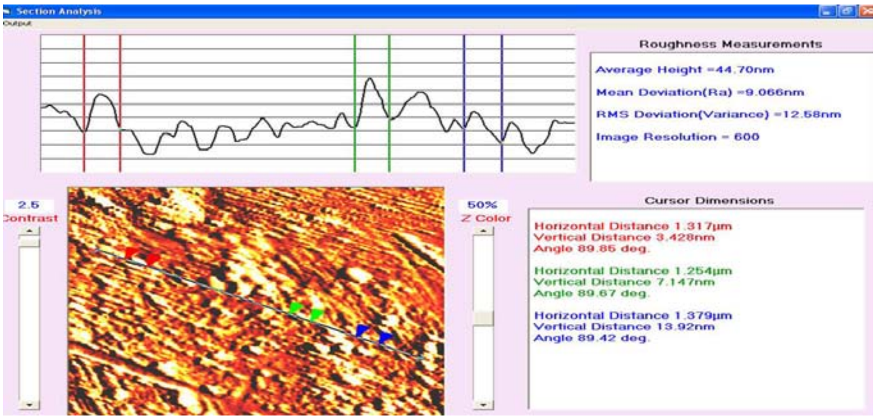

Fig. 3. A representative atomic force microscopy (AFM) image of 45nm nano silver water filter. The average thickness of the nano silver layer is 44.70nm.

Scanning electron microscopy (SEM) was conducted to measure the pore size of the coated filters. The pore size of 45nm layer of the nano silver, coated the polypropylene filter, depicted in Fig. 4 ranged from 0.612µm to 1.35µm in diameter, as measured by SEM. After analyzing 10 SEM micrographs, the average pore size of the filters covered with nano silvers was calculated to be 950nm. The average pore size of the uncoated water filters were 9860nm. Therefore, coating with a 45nm layer of nano silvers has caused an approximate ten-fold reduction in the pore-size of the polypropylene filters.

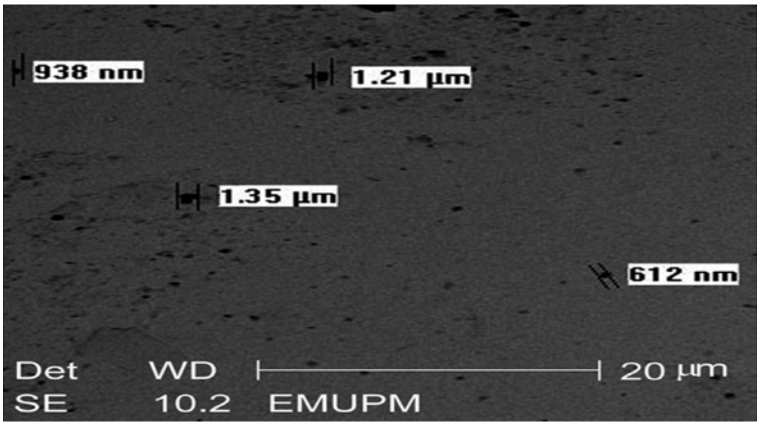

Fig. 4. Scanning electron microscopy (SEM) image of 45nm Ag-coated water filter.

The flux (hydraulic conductivity) of the filters, defined as the rate of movement of water through a porous medium [33,34] was calculated to be 43×10^{-3}cm/s for the uncoated filters and 4.6×10^{-3}cm/s for the nano silver-coated filters. This indicate that the flux of uncoated filters was almost ten times more than the flux of the coated filters and is in accordance with the SEM analysis that revealed a ten-fold difference between the pore sizes of coated and uncoated filters. The flux measurement data clearly support the fact that the hydraulic conductivity of water that passes through a filter depends on the size and connectivity of the pores [38].

The antibacterial property of the filters was evaluated using the membrane filter method [32]. After circulating the distilled water inoculated with 10^3cfu/mL E-coli through the pilot plant for 15min, a 100ml sample was assessed for the presence of the bacteria. Figure 5 depicts presence of approximately 1000cfu/mL of E-coli in the water sample before filtration (control). Figure 6 shows bacterial count for the water sample after 2h filtration with the nano silver-coated filter. As shown in Figure 6, after 2h filtration the number of E-coli colonies reduced to 486cfu/mL. This is while the number of E-coli colonies in the treated water using uncoated filters (control) was about 1000cfu/mL. The number of E-coli in the water treated with the coated filter for 2h is significantly reduced when compared to the number of the bacteria in the control (untreated and treated with uncoated filter) water sample.

Fig. 5. Membrane filter assay for detection of E-coli in the water sample before filtration. The approximate number of bacteria is 1000cfu/mL.

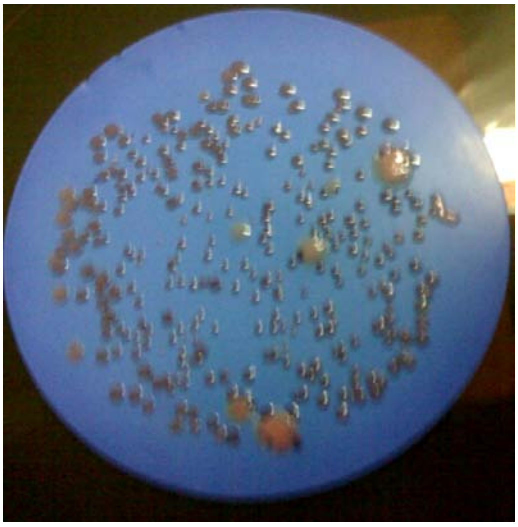

Fig. 6. Membrane filter assay of 45nm nano silver-coated filter for detection of E-coli in the treated water sample after 2 hours filtration. The number of bacteria is equal to 486cfu/mL.

When filtration was continued for another 2 hours using the nano silver coated filter, after a total of 4 hours filtration, only 40 colonies of E-coli remained in the water sample (Fig. 7). This is while the number of E-coli colonies in the treated water using uncoated filters (control) was still about 1000cfu/mL even after 6 hours filtration. However, after 6 hours filtration, 12 colonies of E-coli remained in the water sample filtered with the coated filters (Fig. 8).

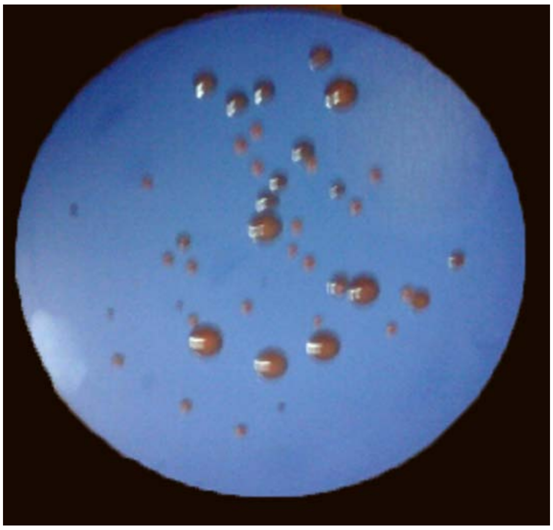

Fig. 7. Membrane filter assay of 45nm nano silver-coated filter for detection of E-coli in the treated water sample after 4 hours filtration.

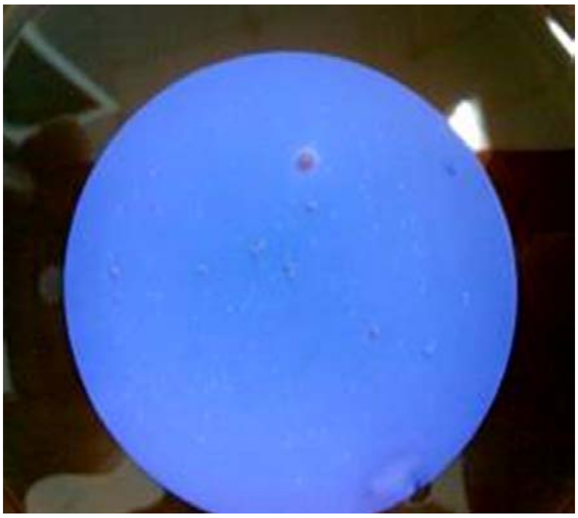

Fig. 8. Membrane filter assay of 45nm nano silver-coated filter for detection of E-coli in the treated water sample after 6 hours filtration.

When filtration was continued for another 30min (i.e. after a total of 6.5hours filtration), using the nano silver-coated filters, there was no bacterium detected in the treated water (Fig. 9). The number of E-coli colonies in the treated water using uncoated filters (control), however, was still about 1000cfu/mL at the end 6.5 hours filtration. These results show that the antibacterial efficiency of the nano silver-coated filters is in line with the WHO requirements for drinking water, which state that any water intended for drinking should contain fecal and total coli form counts of 0 in any 100mL sample [28].

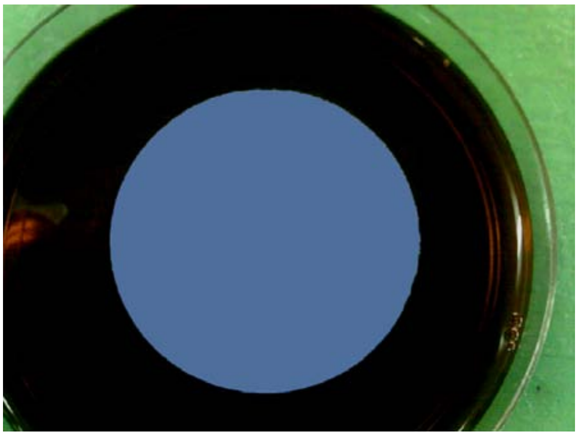

Fig. 9. Membrane filter assay for detection of E-coli in the treated water sample after 6.5h filtration. There is no bacterium in this image.

For further proof that the manufactured filters are efficient in removing E-coli from drinking water, the scanning electron microscopy analysis was performed. Figure 10 depicts the SEM of a section cut from the surface of the nano silver-coated filter, after 6.5hr filtration process. The SEM image depicts bacterial attachment to the surface of the water filter (Fig. 10).

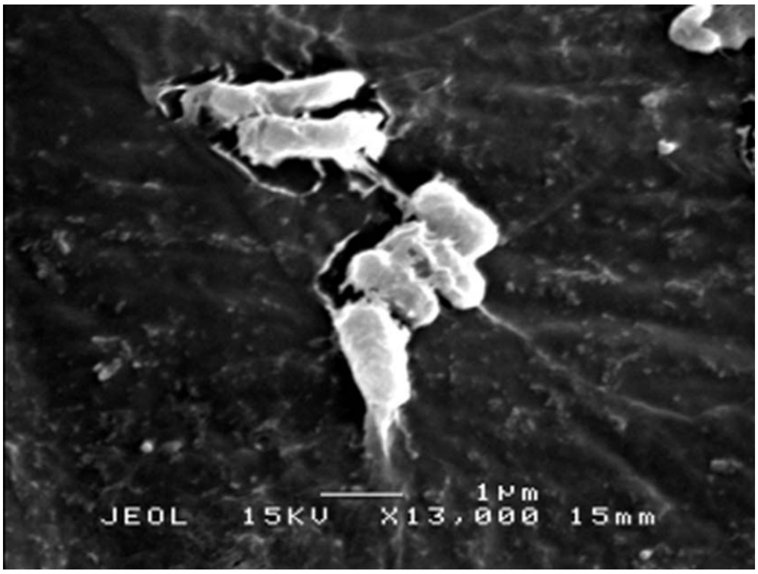

Fig. 10. Representative scanning electron micrograph of E-coli cells attached to the surface of the nano silver-coated polypropylene water filter.

In order to obtain more evidence of the bactericidal efficiency of the nano silver-coated water filters, the zone of inhibition test was also conducted. The zone of inhibition observed for a sample of the nano silver-coated water filter confirms the antibacterial action of the nano silver particles (Fig. 11). This is while there was no zone of inhibition for the uncoated polypropylene water filter, as shown in Fig. 12. The green color around the uncoated polypropylene water filter shows the bacterial growth zone (Fig. 12).

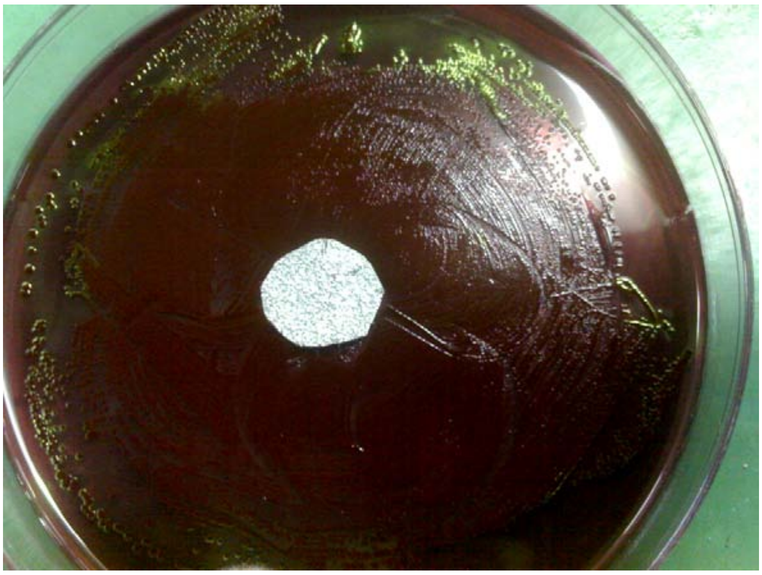

Fig. 11. Zone of inhibition test result of the nano silver-coated polypropylene water filter for E-coli with nutrient agar plating.

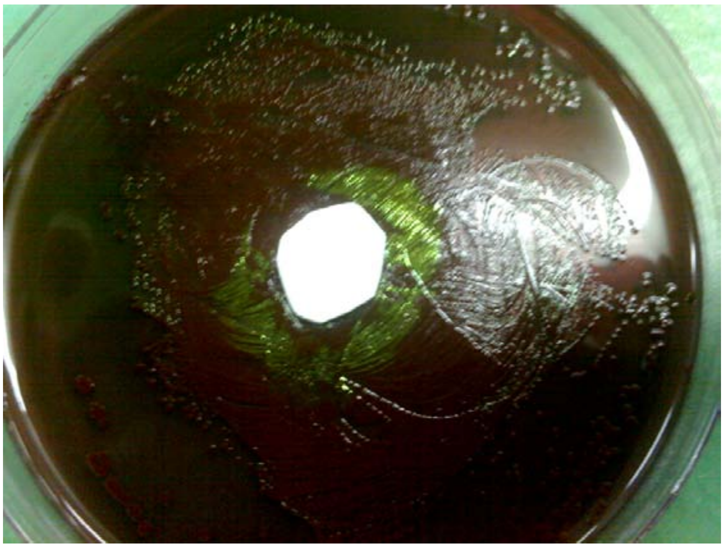

Fig. 12. Zone of inhibition test result of the uncoated polypropylene water filter for E-coli with nutrient agar plating.

The exact mechanism of antibacterial action of silver is not completely understood. However, there are some literature on the effect of silver ions on the bacterial structure and function [39]. Three main mechanisms have been proposed for bacterial inactivation with silver [20]. These mechanisms are: (*i*) interaction of silver with thiol (sulphydryl, SH) groups of the bacterial proteins; (*ii*) structural changes in bacterial cell membranes; and (*iii*) interaction of silver with the bacterial nucleic acids. In case of E. coli, silver acts by inhibiting the uptake of phosphate and releasing phosphate, mannitol, succinate, proline and glutamine from the E. coli cells [9,40].

After proving the antibacterial efficiency of the nano silver-coated filters, the next step was to evaluate the amount of silver particles released from the filters to the water. The inductively coupled plasma/mass spectrometry (ICP/MS) was used according to US EPA Method 6020 [35] to determine any amount of silver nano particles in the water sample after 6.5h filtration. The output count of nano silver particles in the filtered water sample was nil, indicating the stability of the manufactured filters and their ability to retain the silver nanoparticles on their surface. This result complies with the US Environmental Protection Agency (US EPA), which states that the maximum contaminant level of silver ion in the drinking water must be less than 0.10 mg/L [29,30].

Since there is no report on the coating of polypropylene water filters with silver nanoparticles in the literature, results of the present study cannot be compared directly with those of a similar work. Nano silver coating has been carried out on other types of water filters, using techniques different than the physical vapour deposition method with respect of process time and simplicity. In a recent study, silver nanoparticles were coated onto polyurethane foams by overnight exposure of the foams to the silver nanoparticle solutions [27]. Although the filters were able to completely eliminate pathogenic bacteria, they were not adequately characterised and the manufacture procedure required repeated washing and air-drying, which make it lengthy and practically very difficult to scale-up [27]. More recently, Bielefeldt [35] reported antibacterial efficiency of silver-coated ceramic water filters. In general, the ceramic filters were found to be effective, but showed loss of effectiveness with time and indicated a release of microbes into subsequent volumes of water passed through the system. Furthermore, the bactericidal efficiency of the silver-coated ceramic filters for *ca*. 8L water after 4 hours filtration was not 100% [35] while the silver-coated polypropylene filters reported here were able to kill 100% of the bacteria in 15L water. Other studies on silver-coated ceramic water filters have generally reported high pathogen disinfection efficiency for the filters, which are typically new filters with fresh silver coating. For example, Oyanedel-Craver [41] reported 99–100% disinfection of E. coli pulse spiked onto ceramic filter disks. Brown [42] loaded 600L of water with 10^4–10^7 cfu/mL of E-coli to three ceramic filters and observed 99–99.9999% (2–6 log) disinfection. Over time as batches of water were treated, the silver leaches out into the water. This decreases the amount of silver left on the ceramic filters and may impact on pathogen inactivation. One major advantage of the nano silver-coated polypropylene filters manufactured in this study is that even after 6.5 hours continuous filtration there was no silver leakage to the effluent water.

5. Conclusions

Cylindrical polypropylene water filters were coated with a 45.0nm layer of nano silver particles using a modified Balzers machine. The antibacterial efficiency of the filters was evaluated in a custom-made pilot plant. After 6.5h filtration, the nano silver-coated filters were able to remove 100% of the E-coli contamination when the input water had a bacterial load of 10^3cfu/mL and a flow rate of 3L/hr. The standard "zone of inhibition" test was also performed to confirm the antibacterial properties of the filter. The inductively coupled plasma/mass spectrometry examination revealed that there was no nano silver particle in the filtered water sample. These results are in agreement with the WHO requirements for drinking water and suggest the possibility of the use of the nano silver-coated filter in drinking water purification.

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