



Journal of Applied Sciences

ISSN 1812-5654

science
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Water Disinfection Using Novel Cyclodextrin Polyurethanes Containing Silver Nanoparticles Supported on Carbon Nanotubes

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Abstract: This study explores the use of nano-sized materials for the removal of bacteria in water. Silver nanoparticles immobilized onto carbon nanotube and cyclodextrin polymers were synthesized in our laboratories and the resultant polymers were characterized by various techniques such as BET, TEM, SEM, EDX and then tested for their efficacy at removing pathogenic bacteria from environmental water samples these environmental water samples were collected from surface and ground water sources where *E.coli* and *V. cholerae* were isolated from the environmental samples in relatively high numbers. These samples were subjected to synthesized polymers and a reduction in bacterial colony forming units was observed.

Key words: Bacteria, carbon nanotubes, cyclodextrins, nanoparticles

INTRODUCTION

Surface and groundwater are the main sources of potable water in South Africa. Groundwater contributes 9% to the country's water supply while the rest is sourced from surface water (77 from surface water, 14% from sewage and effluent purification and 9% from ground water) (van Vuuren, 2009). Groundwater is naturally of good microbiological and chemical quality when compared to surface water. However heterotrophic and coliform bacteria have been reported in groundwater. Sources of pollution of surface and ground water include municipal solid waste, sewage sludge and industrial waste and effluent (Momba *et al.*, 2003; Theron *et al.*, 2008). These pollution sources carry with them chemical pollutants and microorganisms including enteric pathogens such as *Escherichia coli*, *Shigella* sp., *Salmonella* sp. and *Vibrio* sp. which are causative agents of various diseases that can result in death (Alcamo, 2001). Consequently, the disinfection of surface and ground water is of public health importance.

Conventional drinking water disinfection methods employed in water treatment have their own disadvantages. Widely used disinfectants include chlorine, chloramine, ozone and UV light (Freese and Noziac, 2004). Each of these can have some problems, for example chlorine dissolves in water to form hypochlorous acid, which in turn reacts with natural organic matter

(NOM) (e.g., humic and fluvic acids) to form numerous disinfection by-products (DBPs), including trihalomethanes (THMs), chlorinated biphenyls and other halogenated hydrocarbons (Genthe and Kfir, 1995). These compounds are either endocrine disruptors (EDCs) or carcinogenic (cancer causing) in humans. Such disinfection by-products and their ill health effects challenge research into developing other methods of disinfecting water, which do not result in toxic by-products while ensuring the good quality of water is not compromised (Schutte and Focke, 2007).

Novel nanomaterials offer the potential for the treatment of surface water and groundwater contaminated with inorganic, organic and bacterial pollutants. In our laboratories, cyclodextrin polymers containing a small percentage of carbon nanotubes exhibited the ability to decrease the concentration priority organic pollutants in water at concentrations as low as parts per million (ppm) (Salipira *et al.*, 2007). Likewise bioactive silver nanoparticles are emerging to be good candidates for antibacterial use because of their large surface area (Morones *et al.*, 2005). Silver nanoparticles have been immobilized on carbon materials such as activated carbon and carbon aerogels and have been used as bactericidal materials (Ibarra *et al.*, 2007; Zhang *et al.*, 2004). The use of silver as an antibacterial agent dates back to ancient times when silver vessels were used to keep and purify water, wine and vinegar (Liau *et al.*, 1997).

From this point of view, this study was designed to prepare cyclodextrin polyurethanes containing a small amount of carbon nanotubes supporting silver nanoparticles. The synthesized polyurethanes were then used for the disinfection of model (laboratory) and environmental water samples.

MATERIALS AND METHODS

The experimental study was conducted in the months of May 2007 to July 2008, using both environmental waters and sterile distilled water seeded with pathogenic bacteria. Microbiological analyses were performed in aseptic conditions under a biohazard safety cabinet Class II (Vivid Air Filtration and Ventilation Suppliers, SA) in the laboratory. Tests for turbidity, temperature, pH and conductivity of environmental water samples were also considered as these factors play an important role in the disinfection process. All microbiological tests were performed in triplicates before and after disinfection while physicochemical tests were done only before disinfection.

Sampling of environmental test water: Surface and ground water were used as test waters for this study. The main purpose of our sampling was to obtain environmental samples to evaluate the antibacterial activity of the silver impregnated polymers in a realistic scenario. Surface water was collected from two treatment plants which were the Magalies Klipdrift treatment plant and the Magalies Temba treatment plant while ground water was collected from two randomly selected boreholes situated in Delmas. Surface water samples collected from two points along the flow path of the treatment plants. Samples were collected from raw water inlets and after filtration. Magalies Klipdrift treatment plant receives its raw water from the Pienaars River and the Rooderplot Dam. Magalies Temba treatment plant receives its raw water from the Leeukraal Dam. For each sampling points, three water samples were considered. Sampling collection was performed according to standard procedures for microbial testing (APHA, 1989) and analyses were done within 4 h of collection.

Physico-chemical analyses of environmental test water: Temperature and conductivity were measured using potable meter (Eutech, model Cyberscan PC 300), while pH was measured using a potable pH meter (WinLab Data Line). Also, turbidity was measured using an electronic turbidity meter (HACH, model 2100N). All physicochemical parameters were measured on site following the manufacturers' instructions.

Microbiological analyses of environmental waters:

Water samples were analyzed using standard methods (APHA, 1989). *Escherichia coli* and *Vibrio cholerae* and were detected after the membrane filtration technique using filters with 0.45 μm pore size and 47 mm diameter (Millipore). Different volumes were filtered depending on the type of water used. Saline water was used as a diluent for the 10 mL volumes to spread the bacteria evenly over the filter membrane. The filter membranes were placed onto Thiosulphate Citrate Bile Sucrose agar (TCBS) and chromocult media (Biolab) for the isolation of *V. cholerae* and *E. coli* respectively and incubated at 37°C for 24 h. Microbiological analyses were carried out in triplicates for each water samples.

Preparation of spiked water samples: Pathogenic *Escherichia coli* (ATCC 25925) were obtained from the American Type Culture Collection. The strain was then reconfirmed by cultural, morphological and biochemical tests. The manipulation of the bacterial strain was done under a biohazard safety cabinet Class II (Vivid Air Filtration and Ventilation Suppliers, SA).

Two loops of *E. coli* ATCC 25925 were grown in sterile nutrient broth (200 cm³) under constant aeration on a rotary shake incubator for 24 h at 37°C at a speed of 117 rpm. Cells from the nutrient broth were then centrifuged at 4000 rpm for 10 min and the supernatant was disposed. The cells were then washed using Phosphate Buffered Solution (PBS) for several times (Momba and Cloete, 1996). The washed cells were then re-suspended in tetra sodium pyrophosphate, a surfactant to prevent clumping of cells. The concentration of the cells harvested was determined by first serially diluting the initial biomass suspension in sterile saline solution. The enumeration of the initial concentration was done using the colony count method. The spiked water samples were prepared by adding the biomass suspension into appropriate volumes of sterile distilled water in separate 1 L sample bottles. Counts for viable *E. coli* in spiked water were confirmed by serially diluting the spiked water followed by culturing using the spread plate method (APHA, 1989).

Disinfection of test waters: Silver impregnated carbon nanotube co-cyclodextrin polymers (Ag MWNT/CD) were synthesized in our laboratories by initially impregnating carbon nanotubes with silver nanoparticles. Silver impregnation was done by the reduction of a silver salt using a weak reducing agent in a polyvinylpyrrolidone solution. The silver impregnated carbon nanotubes were then polymerized into cyclodextrin polyurethanes using a bifunctional linker. Characterization of these materials

was carried out by transmission electron microscopy, scanning electron microscopy, energy dispersive x-ray. Metal content of the polyurethanes was done by acid digestion of the polyurethanes and analyzing the metal using atomic absorption spectroscopy. These polyurethanes contained 0.019% (by weight) of silver. For comparative studies, multiwalled carbon nanotubes and cyclodextrin polymers (MWNT/CD), containing no silver, were also evaluated for their antibacterial character. The polymers all contain approximately 1% by weight of carbon nanotubes.

The multi-walled carbon nanotubes polymer cyclodextrin (MWNT-CD) (0.3 g) and silver impregnated carbon nanotubes cyclodextrin polymer (Ag-MWNT-CD) (0.3 g) were packed in separate empty solid phase extraction (SPE) cartridges (Salipira *et al.*, 2008). The cartridges were connected to a separation funnel which contained collected environmental samples (250 mL) and a collecting flask. The water samples were allowed to filter through by gravitational force at an average flow rate of 5 mL min⁻¹. Prior use, plastic ware were sterilized by soaking them in a 5% bleach solution for 24 h (Momba and Cloete, 2006). The bleach was washed off using sodium thiosulphate and rinsed with sterile distilled water. Glassware was sterilized by autoclaving (Momba *et al.*, 2006). The final viable count was again determined using the colony count method.

Nanoparticle leaching studies: Silver impregnated carbon nanotubes and cyclodextrin polymers were packed in SPE cartridges and were connected to the disinfection set up.

Distilled water (100 mL) was passed through the polymers at flow rates of 5, 30 and 85 mL min⁻¹. The eluted water was then analysed for the presence of leached out silver using atomic absorption spectroscopy (Ibarra *et al.*, 2007).

Statistical analysis: Statistical analysis was done using the SPSS computer statistical software (version 13.0). Test of significance was carried out using the Student's Independent T-Test at 95% confidence interval.

RESULTS

Physiochemical quality of samples: Table 1 illustrates the physiochemical characteristics of environmental samples. The temperatures ranged from 12.5-14.3°C, conductivities from 16.4-79.1 mS m⁻¹, pH from 7.13-8.23 and turbidity readings ranged from 1.1-7.4 NTU.

Characteristics of environmental waters before and after disinfection: The enteric pathogens frequently encountered in water sources include bacteria such as *Escherichia coli*, *Vibrio cholerae* and *Salmonella* sp. These are usually transmitted to humans by ingestion of contaminated water or food and cause various diseases. Table 2 shows the initial bacterial profile and CFU counts of the water samples. Bacterial species isolated from environmental samples were *E. coli* and *V. cholerae*. The highest *E. coli* counts was 1.5×10⁴ cfu 100⁻¹ and was detected in Temba raw water while the highest *V. cholerae* counts was 7.4×10³ cfu/100 mL detected in groundwater collected from from Delmas A3 borehole.

Table 1: Physio-chemical quality data (results obtained from a set of three experiments)

Parameter	Temba raw	Temba filtered	Klipdrift raw	Klipdrift filtered	Delmas A3	Delmas A7
Turbidity (NTU)	7.0-7.4	2.42-2.6	5.10-55.4	1.7-2.1	1.3-1.6	1.1-1.3
Conductivity (mS m ⁻¹)	54.2-57	56.0-57.1	78.0-79.1	52.45-53.1	18.8-19.2	16.4-16.9
pH	7.66	8.23	7.26	7.23	7.7	7.13
Temperature (°C)	13.0-13.2	13.5-13.9	14.0-14.6	11.8-12.3	13.7-14.2	12.5-12.9

Limits for no risk: Turbidity: 0 to 1 NTU; Conductivity: <70 mS m⁻¹; pH: 6.0 to 9.0; Temperature: 15 to 25°C (SANS 241, DWAF, 1996). A3 and A7 are borehole water samples

Table 2: Bacterial characteristics of environmental waters before and after treatment with cyclodextrin based polymers (average results after a set of three sets of results)

Bacteria species	Temba raw	Temba filtered	Klipdrift raw	Klipdrift filtered	Delmas A3	Delmas A7
Initial counts in water samples before treatment with polymers (cfu/100 mL)						
<i>Escherichia coli</i>	1.5×10 ⁴	3.9×10	1.4×10 ³	6.0×10	7.4×10 ³	2.2×10 ²
±SD	6.2×10 ³	9	6×10 ²	12	1.1×10 ³	40
<i>Vibrio cholerae</i>	2.3×10	0	2	0	5.3×10 ²	8.5×10
±SD	8	-	2	-	98	9
Counts after treatment with Ag MWNT/CD polymers (cfu/100 mL)						
<i>Escherichia coli</i>	1.3×10	0	9	0	2.0×10	7
±SD	3.2	-	3	-	3.9	1.7
<i>Vibrio cholerae</i>	0	0	0	0	7	1
±SD	-	-	-	-	1.1	1
Counts after treatment with MWNT/CD polymers (cfu/100 mL)						
<i>Escherichia coli</i>	8.9×10 ²	2.2×10	5.5×10 ²	1.0×10	3.1×10 ²	1.7×10 ²
±SD	92	5.2	53	1.7	26.5	4.4
<i>Vibrio cholerae</i>	5	0	0	0	1.9×10 ²	7.3×10
±SD	1	-	-	-	6	14

Table 3: Bacterial counts of spiked water samples before and after treatment with polymers (after averaging a set of three sets of results)

Sample	Initial (cfu/100 mL)	After treatment with Ag MWNT/CD (cfu/100 mL)	After treatment with MWNT/CD (cfu mL ⁻¹)
Sample A	8.5×10 ⁷	8.8×10 ³	6.0×10 ⁶
±SD	1.5×10 ⁶	2.3×10 ²	1.1×10 ⁶
Sample B	2.0×10 ⁶	0	2.9×10 ⁴
±SD	3.6×10 ⁵	-	3.0×10 ³

DISCUSSION

Physicochemical properties of water samples: Some of the parameters were within the target water quality ranges while others were above the target ranges (SANS 241, DWAF, 1996). For example, the conductivities and pH values for all environmental water samples were within the target ranges. All water samples collected during the study period had turbidity values ranging between 1.1 and 7.4 NTU. Raw water collected from Temba and Klipdrift treatment plants had the highest turbidity values (7.0-7.4 and 5.10-5.4 NTU, respectively) which exceeded 5 NTU which possesses a possible health risk (DWAF 1996). Turbidity values higher than 1 NTU is a matter of concern in terms of the performance of the disinfection process. Studies by previous investigators have reported that the effectiveness of the disinfection process is linked to the turbidity of the water (Momba *et al.*, 2006). These investigators pointed out the lower the turbidity (<1 NTU), the higher the efficiency of the disinfection process. It is important to note that turbidity in water is caused by the presence of suspended matter, which usually consists of a mixture of inorganic matter such as clay and soil particles and organic matter. The latter can be both living matter such micro-organisms and non-living matter such death algae cells (DWAF, 1996). Measuring the turbidity of water is therefore a good indication of the concentration of the suspended matter in water. The temperatures were quite low because the samples were collected in winter. The highest sample temperature was 14.6°C obtained from Klipdrift raw water samples whilst the lowest temperature 11.8°C. The average temperature was 13.1°C (Table 1).

Characteristic of raw surface water before and after disinfection: From results shown in Table 2, it can be noted that Temba treatment plant had 1.5×10⁴ cfu/100 mL of *E. coli* and 23 cfu/100 mL of *Virio cholerae* while Klipdrift treatment plant had 1.4×10³ cfu/100 mL and only 2 cfu/100 mL of *Vibrio cholerae*. Upon disinfection with the silver polymer, a 3 log reduction of *E. coli* from both Temba and Klipdrift raw water was observed. The silver free polymer reduced bacterial counts by 2 logs in Temba

water and a 1 log reduction from Klipdrift raw water at the end of the 90 min study period. There was complete removal of *V. cholerae* by the silver impregnated polymers whilst the multiwalled carbon nanotubes and cyclodextrin polymer recorded 5 cfu/100 mL after disinfection.

Characteristic of filtered surface water before and after disinfection: Filtered surface water collected from the two treatment plants had low bacterial counts when compared to the raw water. Temba plant had 39 cfu/100 mL of *E. coli* while Klipdrift had 60 cfu/100 mL. *V. cholerae* was not observed from both plants after filtration. The low bacterial counts or the absence of *V. cholerae* can be attributed to the treatment processes employed in the water works. These include pre-chlorination in the case of Klipdrift and filtration for both plants. After disinfection with the silver polymer, no bacteria were isolated from both Temba and Klipdrift filtered water samples. The MWNT/CD polymer reduced the counts from 39 to 22 cfu/100 mL for Temba plant and 60 to 10 cfu mL⁻¹ for Klipdrift. These counts exceeded by far the limits allowed by the South African Water Quality standards (SANS 241, 2006). Consequently this study suggests that the MWNT/CD cannot be considered for the disinfection of drinking water in terms of the removal of pathogenic bacteria from surface water.

Characteristic of raw ground water before and after disinfection: Both *Vibrio cholerae* and *Escherichia coli* were isolated from groundwater samples. From borehole A3, 7.4×10³ cfu/100 mL of *E. coli* was found while the borehole A7 had 2.2×10² cfu/100 mL of *E. coli*. Counts for *Vibrio cholerae* in both borholes A3 and A7 were 5.3×10² and 8.5×10 cfu/100 mL, respectively. Worth noting is that ground water samples recorded the highest *V. cholerae* counts compared with surface water. Naturally, groundwater is of excellent microbiological quality (Foster, 1995). However, the presence of high numbers of pathogenic bacteria, especially *V. cholerae* in ground water suggests contamination of the water bed in this area by faecal contaminants. Poor sanitation could be the cause of the recent diarrhoea outbreaks which were suspected to be directly linked to poor water quality in the Delmas area (Lang, 2007). Many rural municipalities that rely on ground water as their main water sources have adapted some measure of disinfection. These municipalities have small water treatment units which are classified under small water treatment plants. It has been reported that South African small water treatment plants face challenges such as poor administration, lack of

human resources and insufficient financial capacity (Obi *et al.*, 2007). Hence the Delmas cases could have stemmed from improper handling of problems associated with the water quality management.

Upon disinfection of ground water with silver impregnated polymer, there was a 2 log reduction of *E. coli* from both borehole A3 and A7 where the resulting counts were 20 and 7 cfu/100 mL, respectively. For the removal of *V. cholerae*, silver impregnated polymers reduced *V. cholerae* counts by 2 logs where the silver polymer recorded 7 cfu/100 mL for A3 and 1 cfu/100 mL for A7. However, the silver free polymers had a significantly lower removal of both bacterial species ($p \leq 0.05$) from both ground water sources with the exception of *E. coli* from A3 where a 1 log reduction was observed after treatment. The poor performance of the silver free polymer was also observed during the treatment of groundwater.

Comparative analysis of disinfection techniques: Some of the techniques used in the disinfection of water include chlorination, ozonation and UV radiation (Freese and Noziac, 2004). Chlorine is a widely used disinfectant because of its effectiveness, low capital and running costs and it is relatively easy to handle (White, 1999). All the above-mentioned techniques at correct dosages can give 2 to 3 log reduction of bacterial counts in water (Freese *et al.*, 2003). This is similar to the reduction obtained by the silver impregnated carbon nanotubes and cyclodextrin polymers. The removal of bacteria by the silver impregnated polymers was observed to be significantly higher than the carbon nanotubes and cyclodextrin polymers ($p \leq 0.05$). Hence silver impregnated polymers synthesized in our laboratories performed competitively with conventional disinfection techniques. However some optimization studies on the polymers still need to be carried out to achieve maximum bacterial reductions.

Silver leaching out studies: Some concerns have been raised regarding the toxicity of engineered nanomaterials towards the environment and humans. In this study, it was necessary to assess the leachability of silver nanoparticles into the water treated with the silver polymers. Treated water was found to contain less than 0.1 mg L^{-1} of silver because silver levels in treated water were below the instrument's detection limit. According to the World Health Organizations *Water Quality Guidelines* for drinking water, silver levels of 0.1 mg L^{-1} could be tolerated without any health risk. This concentration gives a total dose over 70 years of half the human NOAEL (no- observed- adverse- effect- level) of 10 g (WHO,

2006). Hence possible leaching of silver into the water when using silver impregnated polymers dose not pose any significant health risk since levels are below 0.1 mg L^{-1} .

Effect of the nature of sample on the disinfection properties of silver: Comparing the performance of silver impregnated polymer with the other polymer at removing bacteria in environmental samples and model samples synthesized in the laboratory, there is a great difference at the removal of bacteria from both environmental and spiked water samples spiked with *E. coli* (ATCC 25925). From spiked water samples, a higher bacterial removal capacity was observed compared to the bacterial removal capacity in environmental samples. The silver polymer exhibited approximately a 3 logs removal of *E. coli* when using raw water from Temba. Also, approximately 4 logs and 6 logs of *E. coli* were removed from 'Samples A and B', respectively. In this study, the nature of samples was observed to affect the efficacy of the silver polymer. The possible factor that contributed to this could be the presence of other constituents in environmental water samples such as turbidity which is indicative of the concentration of dissolved suspended solids. Turbidity in general has negatively impacted the performance of silver polymer.

CONCLUSIONS

A low bacterial removal from raw unfiltered water samples implies that the polymer is not an ideal disinfectant for such waters. However high bacterial removal by the silver impregnated polymers from filtered water samples suggests that the polymers can be positioned after filtration is performed to get maximal removal of bacteria. Silver impregnated polymers promise to be good candidates for complementing the currently employed disinfection methods

ACKNOWLEDGMENTS

Support from the National Research Foundation (NRF), Mintek's Nanotechnology Innovation Centre (NIC) and University of Johannesburg (UJ) and the Tshwane University of Technology's Water Care Department is gratefully acknowledged.

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