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Hydrothermal preparation of TiO_2-Ag nanoparticles and its antimicrobial performance against human pathogenic microbial cells in water

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Abstract: Water contaminated with pathogenic microbes is considered as one of the most common routes for transmitting diseases in human beings. Different methods have been applied for the decontamination of microbes in contaminated water. In the current study, an easy to do hydrothermal method has been used for the preparation of TiO_2-Ag nanoparticles. The obtained material was characterised using a scanning electron microscope (SEM) and fourier transform infra-red spectroscopy (FTIR). The morphological appearance of the obtained nanoparticles was in the shape of a sphere with a size range of 60-90 nm. The antimicrobial activity of the prepared nanoparticles was tested against several pathogenic bacteria and fungi. The obtained results proved that the nanoparticles succeeded to affect all the tested microbes in the following order: *Bacillus cereus* ATCC6633>*Pseudomonas aeruginosa* ATCC9027= *Klebsiella pneumoniae* ATCC13883>*Vibrio cholera* ATCC700=*Candida albicans* ATCC 700=*Escherichia coli* NCTC10418>*Staphylococcus aureus* ATCC6538. The minimum inhibitory concentration (MIC) of the prepared nanoparticles varied among the tested microbes at range of 12 mg/ml and 25 mg/ml. These results encourage the application of prepared TiO_2-Ag nanoparticles for treatment of microbe-contaminated waters.

Introduction

The properties of solid state materials depend on their structure. Nanostructured materials are becoming very popular and offer a great potential for improving their properties. Therefore, they have been used in various applications in many fields (Elkady *et al*., 2015; El Essawy *et al*., 2017; Mahmoud *et al*., 2017).

Transition metal and their oxides gain much attention due to their unique properties, particularly, titanium and silver-based materials and their co-composites.

Many efforts were made to synthesise silver-coated titanium dioxide (Ag@TiO_2) materials. TiO_2 coated with silver has been prepared by the photodeposition method to improve its catalytic and antimicrobial properties (Ma *et al*., 2015).

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Silver is a metal that could be biologically, physically or chemically transferred into silver nanoparticles and used as a powerful antimicrobial agent, for the treatment of diseases for which microbes are responsible (Devi and Bhimba, 2013; Abu-Saied *et al*., 2014; 2018), because of its low toxicity on mammalian cells and tissues (Simonetti *et al*., 1992; Ahearn *et al*., 1995).

The current study aims to ascertain the dual advantages of, first, the facile one-step preparation of Ag and TiO_2 nanocomposite, and second, the enhancement of the water treatment ability against resident human pathogenic microbes.

Material and Methods

Preparation

A straightforward procedure was used to produce Ag and TiO_2 nanocomposite via hydrothermal method. Briefly, 0.5 g of commercial $TiO_2$was mixed with 0.4 g of $AgNO_3$ in 40 ml

water. The mixture was introduced into a Teflon-lined steel autoclavable container and kept in an oven at 120°C for 24 h. The resulting powder was washed thoroughly with distilled water and dried overnight at 60°C.

Characterisations

Scanning electron microscope (SEM)

Scanning electron microscopy (SEM) was used to investigate the surface of the prepared materials, which were observed after gold coating on a copper holder, using a JSM 6360LA from JEOL, Japan, at an accelerating voltage of 20 KV.

Fourier transform infrared (FTIR)

Fourier transform infrared (FTIR) analysis of the prepared nano materials were investigated using an FTIR, in a range between 400 cm^{-1} to 3500 cm^{-1} using KBr powder. The composition of these powders and the surface functional groups were obtained using FTIR-8400S from Shimadzu, Japan.

Disc diffusion method

The antimicrobial activity of TiO_2 and TiO_2-Ag nanoparticles has been tested against Gram positive, Gram negative and a yeast strain, using the disc diffusion method according to Fahmy *et al*., (2015). At the beginning, both of LB broth and LB agar were prepared and sterilized at 15 psi and 120°C for 20 minutes. Pure clones of the some microbial pathogens (*Bacillus cereus* ATCC6633, *Klebsiella pneumoniae* ATCC13883, *Pseudomonas aeruginosa* ATCC9027, *Escherichia coli* NCTC10418, *Staphylococcus aureus* ATCC6538, *Vibrio cholera* ATCC700, *Candida albicans* ATCC 700) were cultured overnight in 5 ml of LB broth, incubated at 30°C and centrifuged at 200 rpm of 18 h. After incubation, a 0.5 McFarland standard for each strain was prepared and spread over the LB agar plates using sterile cotton swabs. Since then, sterile filter paper discs were loaded to the plates' surface and loaded with 25 µl of 100 mg/ml of TiO_2 and TiO_2-Ag nanoparticles. The plates were then kept at 4°C for 1h, followed by an incubation at 30°C for 24 h. The formed clear zones were checked, measured and photographed.

Determination of minimum inhibitory concentrations (MICs)

Different concentrations of TiO_2-Ag nanoparticles have been tested for their ability to inhibit the growth of the tested microbial pathogens and the MIC against each strain was determined by using broth micro-dilution method, according to the standards of the Clinical and Laboratory Standards Institute (2012). This was aimed by tracing the colour change in resazurin indicator from a blue/non-fluorescent state to a pink/highly fluorescent state which indicates microbial metabolism, and hence, growth. At first, 100 µl of TiO_2-Ag nanoparticles (100 mg/ml) were instilled into the first column of a 96-well microtiter plate, while 50 µl of the LB broth were added to the rest of the columns. A subsequent two-fold serial dilution proceeded horizontally, by transferring and mixing 50 µl of each well in the first column to the second well, continuously till the twelfth column. After that, 50 µl of 0.5 McFarland standard of each microbial strain was added to all wells of the same row, followed by the addition of 100 µl LB broth. The positive control wells were composed of 100 µl of 0.5 McFarland standard of each microbial strain, plus 100 µl of LB broth; while, the negative control wells were filled with 200 µl of LB broth only. The plate was incubated at 30°C for 24 h, followed by the addition of 40 µl of resazurin (0.015 %) to each well. After 2 h of incubation at 30°C, all wells were checked for colour changes. The lowest concentration of TiO_2-Ag nanoparticles at which the blue colour of resazurin remained unchanged was recorded as the MIC (He *et al*., 2016).

Results and Discussion

SEM

The commercial TiO_2 powder was examined and appeared as aggregated large particles as that shown in Fig. 1A. After the hydrothermal treatment of TiO_2 with silver, uniform, separated spherical nanoparticles were produced. The obtained TiO_2-Ag nanoparticles were in the 60-90 nm range (Fig. 1B).

FTIR

Fig. 2 shows a sequence of the FTIR spectra for TiO_2 nanoparticles and TiO_2-Ag nanoparticles. The FTIR spectrum of TiO_2 nanoparticles (Fig. 2A) clearly shows three bands. The first band is the broadest, and is observed at 3500 cm^{-1}, corresponding to the stretching vibration of the hydroxyl group O-H of the TiO_2 nanoparticles. The second band is observed around 1630 cm^{-1}, corresponding to bending modes of water Ti-OH; the last is a prominent peak at 1383 $cm^{-1,}$ related to Ti-O modes (Nadica *et al*., 2006; Mugundan *et al*., 2015). Fig. 2B shows the FTIR spectra recorded for TiO_2 coated with silver nanoparticles. The peaks at 3423 cm^{-1} and 1605 cm^{-1} are quite broad and strong, and can be assigned to the hydroxyl groups of water.

TABEL 1

Measurements of clear zones obtained by TiO_2 and TiO_2-Ag nanoparticles against human pathogenic microbes

Code	Microbial strain	Clear zone (mm)	
		TiO_2 nanoparticles	TiO_2-Ag nanoparticles
A	*Vibrio cholerae*ATCC700	0	12
B	*Candida albicans* ATCC 700	0	12
C	*Pseudomonas aeruginosa* ATCC9027	0	15
D	*Escherichia coli* NCTC10418	0	12
E	*Klebsiella pneumoniae* ATCC13883	0	15
F	*Bacillus cereus* ATCC6633	0	17
G	*Staphylococcus aureus* ATCC6538	0	11

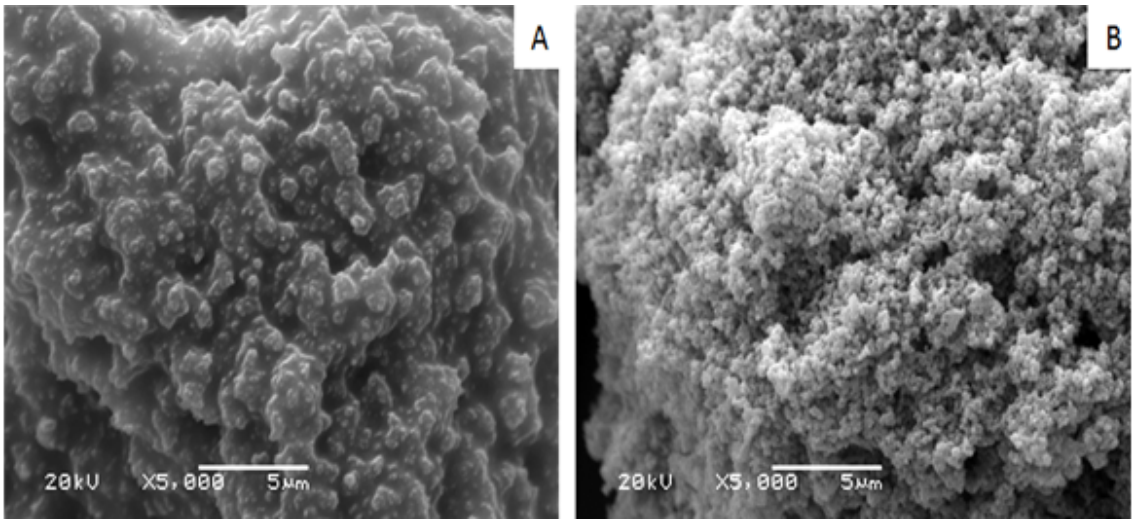

FIGURE 1. SEM micrograph of (A) as-received TiO_2, (B) hydrothermal prepared TiO_2-Ag nanoparticles.

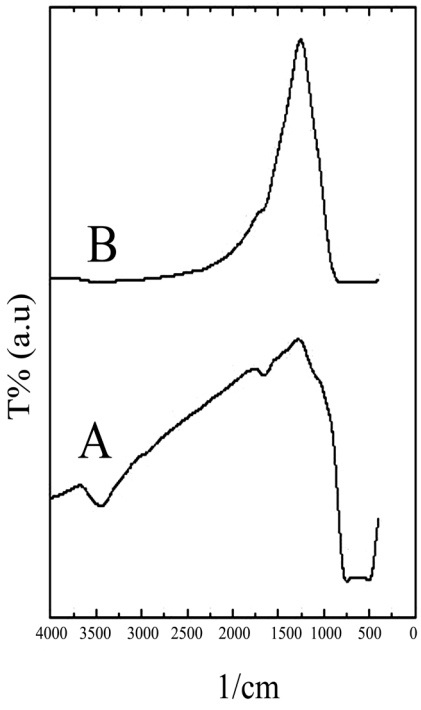

FIGURE 2. FTIR spectra of (A) as-received TiO2, (B) hydrothermal prepared TiO2-Ag nanoparticles.

Disc diffusion method

The ability of TiO_2 and TiO_2-Ag nanoparticles to cease the growth of different microbial pathogens has been investigated. As shown in Fig. 3 and Tab. 1, the effect of TiO_2-Ag nanoparticles on seven tested microbes was slightly varied. The most affected bacterial pathogen was the *Bacillus cereus* ATCC6633 strain, which was highly-affected, compared to the *Staphylococcus aureus* ATCC6538 strain, which was the least affected. The TiO_2-Ag nanoparticles were able to form a clear zone with 17 mm and 11 mm diameters for the *Bacillus cereus* ATCC6633 strain and the *Staphylococcus aureus* ATCC6538 strain, respectively. However, it showed the same antimicrobial pattern towards the *Pseudomonas aeruginosa* ATCC9027 and the *Klebsiella pneumoniae* ATCC13883 group or the *Vibrio cholera* ATCC700, *Candida albicans* ATCC 700, and *Escherichia coli* NCTC10418 group. Their effect on *Pseudomonas aeruginosa* ATCC9027 and *Klebsiella pneumonia* ATCC13883 was somehow higher than that recorded for *Vibrio cholera* ATCC700, *Candida albicans* ATCC 700 and *Escherichia coli* NCTC10418. It almost showed a 15 mm clear zone against the first group and a 12 mm clear zone against the second. It is worth mentioning that the pattern of TiO_2-Ag nanoparticles against the tested pathogens was as follows: *Bacillus cereus* ATCC6633 >*Pseudomonas aeruginosa* ATCC9027=*Klebsiella pneumoniae* ATCC13883>*Vibrio cholerae*ATCC700=*Candida albicans* ATCC 700=*Escherichia coli* NCTC10418>*Staphylococcus aureus* ATCC6538. On the other hand, the TiO_2 nanoparticles failed to represent any detectable antimicrobial activity against all the tested microbial pathogens. It could be attributed that the investigated antimicrobial activity of the TiO_2-Ag nanoparticles is related to the presence of silver nanoparticles and the TiO_2 alone is not probably considered as an antimicrobial agent.

We attribute the antimicrobial activity of the currently prepared TiO_2-Ag nanoparticles to the high catalytic activity and surface area of the composites resulting from the hydrothermal treatment. This suggestion is matched with the data obtained by Jianqi *et al.*, (2015), who mentioned that high catalytic activity and the surface area of the composites were obtained after a photodeposition process.

Determination of minimum inhibitory concentrations (MICs)

A sequence of dilutions of the TiO_2-Ag nanoparticles have been applied to determine the minimum concentration which has the ability to cease the growth of tested pathogens. The used procedure mainly depends on the microbial metabolism that is responsible for the conversion of resazurin's blue colour into pink. As shown in Fig. 4 and Tab. 2, the MIC could divide the tested microbes into two groups. The first group comprises of *Vibrio cholerae* ATCC700, *Pseudomonas aeruginosa* ATCC9027, *Escherichia coli*

NCTC10418 and *Klebsiella pneumonia* ATCC13883 strains, which showed 12 mg/ml as the recorded MIC. However, the second group that includes *Candida albicans* ATCC 700, *Bacillus cereus* ATCC6633 and *Staphylococcus aureus* ATCC6538, recorded much higher MIC at 25mg/ml, in order to cease their growth. It is worth mentioning that these concentrations of tested nanoparticles are a combination of both TiO_2 and silver nanoparticles, indicating that the attachment of silver nanoparticles over the TiO_2 spheres would enhance its distribution, and hence lower its effective dose compared to the aggregated silver nanoparticles that might require a higher concentration to exert its tangible effects.

In conclusion, the study proves the successful usage of the hydrothermal procedure for a facile one-put preparation of TiO_2-Ag nanoparticles. The antimicrobial behaviour of these nanoparticles suggests their application for the treatment of water-contaminated with human pathogenic microbes.

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TABLE 2

The MIC of TiO_2-Ag nanoparticles that responsible for ceasing the growth of tested human pathogenic microbes

Code	Microbial strain	MIC of TiO_2-Ag nanoparticles (mg/ml)
A	*Vibrio cholerae* ATCC700	12
B	*Candida albicans* ATCC 700	25
C	*Pseudomonas aeruginosa* ATCC9027	12
D	*Escherichia coli* NCTC10418	12
E	*Klebsiella pneumoniae* ATCC13883	12
F	*Bacillus cereus* ATCC6633	25
G	*Staphylococcus aureus* ATCC6538	25

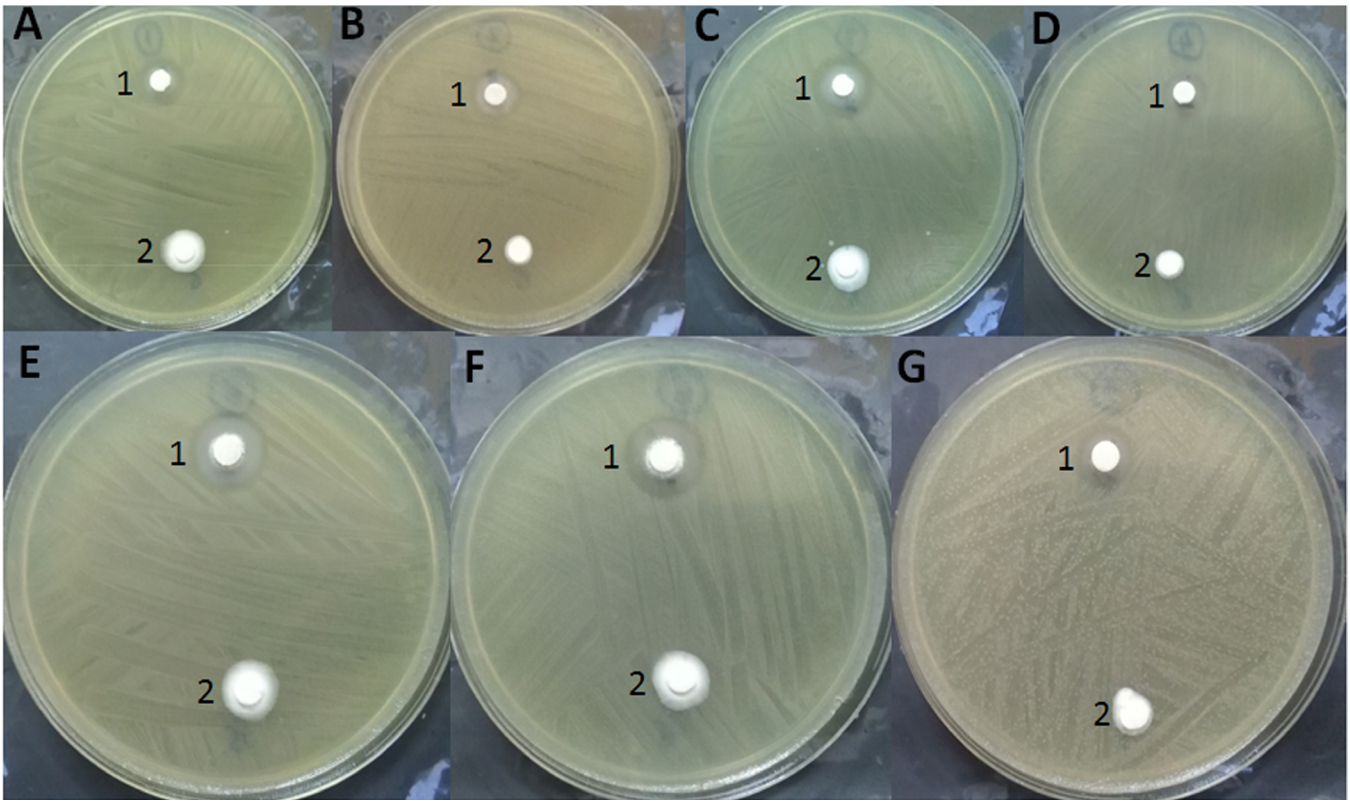

FIGURE 3. Antimicrobial activity of plain TiO_2 and TiO_2-Ag nanoparticles against human pathogenic microbes. A:*Vibrio cholerae* ATCC700, B: *Candida albicans* ATCC 700, C: *Pseudomonas aeruginosa* ATCC9027, D: *Escherichia coli* NCTC10418, E: *Klebsiella pneumoniae* ATCC13883, F: *Bacillus cereus* ATCC6633, G: *Staphylococcus aureus* ATCC6538.

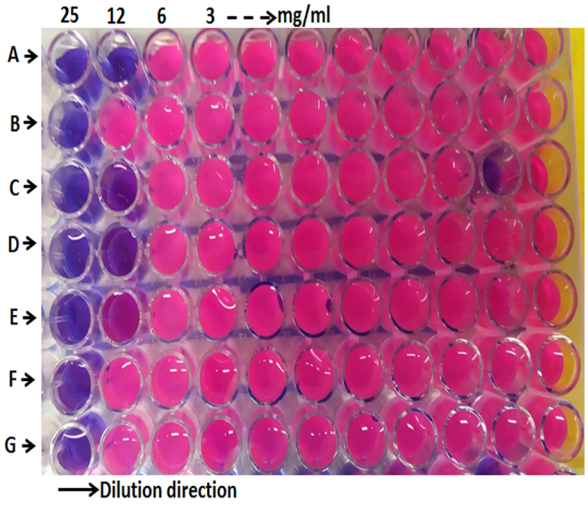

FIGURE 4. The MIC of tested TiO_2-Ag nanoparticles against the tested human pathogenic microbes. A: *Vibrio cholerae* ATCC700, B: *Candida albicans* ATCC 700, C: *Pseudomonas aeruginosa* ATCC9027, D: *Escherichia coli* NCTC10418, E: *Klebsiella pneumoniae* ATCC13883, F: *Bacillus cereus* ATCC6633, G: *Staphylococcus aureus* ATCC6538.

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