Multifunctional nanocomposites of chitosan, silver nanoparticles, copper nanoparticles and carbon nanotubes for water treatment: Antimicrobial characteristics

Rania E. Morsi a, *, Ahmed M. Alsabagh a, Shima A. Nasr b, Manal M. Zaki b

a Egyptian Petroleum Research Institute, Nasr City, Cairo P.B. 11727, Egypt
b Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Egypt

1. Introduction

The United Nations and World Health Organization are currently pushing the water filter industry to develop sustainable solutions to empower many nations with the ability to filter their own water whether for use in the treatment of drinking water or industrial wastewater treatment before discharge into the open water. An effective purification active material is favorable to be able to remove several contaminants in the same treatment process to the standard limits. Numerous approaches have been studied for the development of cheaper and more effective materials containing natural polymers. Among these are polysaccharides such as chitin and chitosan [1], their derivatives [2] and their composites [3]. Chitosan is a polysaccharide biopolymer obtained from the deacetylation of chitin, which is present in the exoskeletons of some crustacean shells. Its chemical structure consists of β (1 → 4)-2-amino-2-deoxy-β-D-glucaminit[4]. Due to its unique biological characteristics; biocompatibility, non-toxicity and biodegradability in addition to being economically facile processed from chitin [5], chitosan has been widely used in many applications [6]. It is known to have good complexing ability through specific interactions of its amino and hydroxyl groups with heavy metals from various wastewaters [7]. In addition, the antimicrobial activity of chitosan [8], gives it an exceptional properties regarding the multi-tasks applications. Moreover, the modification of chitosan has been considered of significant interest due to the further more gained characteristics. Chitosan has been modified either chemically [9], as blends [10], as composites [11] and nanocomposites [12]. Among materials recently used for chitosan modification, Carbon nanotubes (CNTs), for example, have captured much attention worldwide; CNTs have unique size distributions, hollow-tube structures and high specific surface areas which allow their applications in many fields [13]. Many research studies have shown the superior efficiency of CNTs in the treatment of different pollutants [14]. In addition, silver NPs have also attracted intensive research interest because of their important applications in antimicrobial [15] and many other applications [16]. The copper nanoparticles have also been found to have an antimicrobial activity [17]. Accordingly, modifications of chitosan with different nanomaterials have been expected to further improve its antimicrobial activity.

Although disinfection methods currently used in water treatment can effectively control microbial pathogens, research in the past few decades have revealed a dilemma between effective disinfection and formation of harmful disinfection byproducts. More
recently, several natural and engineered nanomaterials, including chitosan, silver nanoparticles (AgNPs), copper nanoparticles (CuNPs) and Carbon nanotubes, have also been shown to have strong antimicrobial properties. Unlike conventional chemical disinfectants, these antimicrobial nanomaterials are not strong oxidants and are relatively inert in water. Therefore, they are not expected to produce harmful disinfection byproducts. If properly incorporated into treatment processes, they have the potential to replace or enhance conventional disinfection methods.

In our previous work [3], multifunctional bi- (chitosan/silver nanoparticles, chitosan/copper nanoparticles and chitosan/carbon nanotubes) nanocomposites and ter- (chitosan/silver nanoparticles/copper nanoparticles/carbon nanotubes) nanocomposites were successfully prepared and comprehensively characterized using different techniques to verify the structural modifications to be used as active material that can be used as purification systems. The prepared nanocomposites were evaluated for heavy metals removal; Cu, Cd and Pb were chosen as representative examples for heavy metal contaminants in waste water. It is well proved that the prepared multifunctional nanocomposites can be used effectively to clean water from heavy metal.

In this study, the prepared multifunctional nanocomposites are evaluated for water disinfection against microbial pollution. The antimicrobial activity was observed against Gram-positive, Gram-negative bacteria and fungal strains. Exceptional properties are expected due to the presence of a combination of different dimensional shapes; spherical NPs and nanotubes inside the chitosan matrix in its multifunctional nanocomposite.

2. Materials & methods

2.1. Materials

Chitosan was extracted from marine shrimp waste materials and was found to have a degree of deacetylation of 85% as determined with potentiometric titration and molecular weight of 2122 kDa as determined from the intrinsic viscous measurement. Multiwall carbon nanotubes (CNTs) with an average diameter 60 to 110 nm were kindly supplied from EPRI nanotechnology center. Silver nanoparticles (Ag NPs) and copper nanoparticles (Cu NPs) were of particle size 40–60 nm, respectively. All other reagents were of analytical grade and used as received without further purification.

2.2. Preparation of the bi- and multifunctional-composite

The preparation of the bi- and ter- multifunctional nanocomposites was carried out using simple addition as in our previous work. Briefly, chitosan was dissolved in 2% acetic acid to prepare 1% polymer solution and filtered to remove any remaining impurities. Five weight percentages of CNTs, Ag NPs and Cu NPs with respect to chitosan weight, were dispersed individually in ethanol by sonication for 20 min and added dropwise to the previously prepared chitosan solution under stirring. The mixture was stirred for 4 h and the formed nanocomposite was further sonicated for 30 min for homogenization. The nanocomposite was then precipitated in a mixture of NaOH solution and ethanol, filtered, washed several times with deionized water till neutral and finally vacuum dried to constant weight at 60 °C and 850 mb. In the case of the multifunctional nanocomposite, the three nanomaterials (AgNPs, CuNPs and CNTs) were added sequentially to chitosan solution under the same conditions.

2.3. Characterization of the prepared nanocomposites

Transmittance Electron Microscopy (TEM) imaging was performed using a Jeol-JEM Japan 2100 operating at 200 kV, the samples were prepared for imaging by sonication of samples in ethyl alcohol and depositing onto a copper coated carbon grid and then let the solvent to evaporate.

2.4. Antimicrobial activity

2.4.1. Waste water sample collection

The 24 h flow-proportional wastewater samples (SL) were taken from the influent (INF) and effluent (EFF) as well as the mixed liquor from the aerobic chamber and return activated sludge (RAS) in three following days in Marriotta region.

2.4.2. Enumeration and isolation of fecal bacteria

The antibacterial activity of the prepared nanocomposites was tested against two bacterial strains; Staphylococcus aureus and E. coli as representative examples for gram positive and gram negative bacteria, respectively in addition to one fungal strain; Aspergillus flavus.

Detection and enumeration of E. coli and S. aureus were carried out by means of membrane filtration according to ISO 9308-1:2000 and ISO 7899-2:2000, respectively. Wastewater samples were diluted and filtered through 0.45 mm cellulose acetate filters in triplicate. Next, in order to detect fecal coliforms, filters were placed on mFC agar (Merck) and incubated at 44 °C for 24 h. Blue colonies, regarded as presumptive E. coli (n 14 153) were selected and subculture onto the nutrient agar, then kept in 4 °C for further investigation. Staphyloccoccal spp were determined using Staphylococcal selective agar (Merck) according to Slanetz-Bartley at 37 °C for 48 h. Representative colonies, dark red or maroon (n 14 199), were taken and kept on nutrient agar in 4 °C for further investigations. Isolation of A. flavus from waste water was obtained by direct spread of the sample on the plate of sabourands dextrose agar (SDA) supplemented with chloramphenicol, The agar plates were incubated at 28–30 °C and fungal growth was observed after 5–7 days, Positive cultures were sub cultured on SDA for the isolation of a pure, single colony for identification. The identification of filamentous fungus was based primarily on the macroscopic and microscopic morphology[slide culture using 0.05% Trypanblue in Lectophenol stain and the use of color atlas] [18].

2.4.3. In vitro antibacterial activity “Testing bactericidal or fungicidal activity”

1 mL of a bacterial test suspension adjusted to 1.5 × 10^6 to 5.0 × 10^8 cfu/mL using McFarland standard and was added to 1 mL interfering substance. (5% yeast extract). The mixture was maintained at 20 °C ± 1 °C for 2 min ± 10 s. Then 8 mL of the product test solution were added and the mixture was maintained at 20 °C ± 1 °C for 10, 15, and 30 min exposure time. At the end of the contact times an aliquot was taken adding 1 mL sample to a tube containing 8 mL of sterile saline and 1 mL water mixed by vortexing, and left at 20 °C. After mixing, duplicate 1.0 mL volumes were poured plated with tryptone soya agar for Staph. aureus and E.coli and Sabarada dextrose agar for A. flavus. All samples were incubated at 37 °C for 48 h prior to counting in case of bacteria and 25C for 3–5 days for A. flavus. The Log Reduction values are calculated by subtracting the reduction of the viable count from the initial inoculum.

3. Results and discussion

3.1. Chitosan/Ag NPs/Cu NPs/CNTs bi- and ter- multifunctional nanocomposites

Figs. 1 and 3 represent the TEM images of chitosan/Ag NPs bi-nanocomposite and chitosan/CNTs bi-nanocomposites and chitosan/Ag NPs/Cu NPs/CNTs multifunctional nanocomposite with schematic diagrams of the suggested structures and preparation
of bi- and multifunctional nanocomposites. The TEM image of the chitosan/Ag NPs and chitosan/CNTs bi-nanocomposites, as representative examples of the spherical nanoparticles and nanotubes structures; respectively, show that the nanomaterials are attached and embedded inside to the polymer chains. For the MFC, carbon nanotubes are attached to chitosan matrix and the spherical Ag and Cu NPs are embedded inside changing the overall morphology and form a characteristic polymer based matrix due to the presence and combination of different dimensional shapes; spherical nanoparticles and nanotubes. More detailed structure confirmation was in our previous work [3].

3.2. Antimicrobial activity of chitosan/Ag NPs/Cu NPs/CNTs bi- and ter- multifunctional nanocomposites

Water is considered as a suitable medium for the microbial growth specially when contaminated with some organic pollutants which could be suitable nutrients for the microorganisms. In this case, an immediate action for removal or inactivation of the microorganisms should be taken. Chitosan is a good model as a natural disinfectant against waterborne pathogens [19] and its based nanocomposites play an added vital role in the control of microbial growth specially when companied with nanoparticles such as silver, copper and carbon nanotubes (CNT) [16,17,20].

The antimicrobial activity of the prepared nanocomposites was studied using two different initial concentration of the tested material; 1% and 2% at three different contact times; 10, 15 and 30 min. The antimicrobial activities represented as “Log Reduction” values, are represented in Figs. 4–5 and the detailed results are listed in supplementary data Tables 1–3. The tested microorganisms showed varied response and the Log Reduction values were found to be directly related to the concentration of the active substance and the contact time.

For Staphylococcus aureus, chitosan showed Log Reduction value of 0.87 after 10 min contact time, 1.87 after 15 min and 3.87 after 30 min when using initial concentration of 1% (Fig. 4a) and when the concentration increased to 2% (Fig. 4b), the Log Reduction value increased to 4.9 after 30 min contact time. Modification of chitosan with CNTs showed no enhancement in its activity at 1% initial concentration but showed significant enhancement after relatively longer contact time, 30 min using 2% nanocomposite concentration as shown in Fig. 4. Upon modification of chitosan with Cu NPs, a great enhancement was achieved against Staphylococcus aureus which is more pronounced at 10 min contact time where Log Reduction value was increased from 0.87 for chitosan to 1.57 for chitosan/Cu NPs nanocomposite, from 1.87 to 2.13 after 15 min and a slight increase was obtained after that for higher concentrations and higher contact time. A similar behavior was observed for chitosan/Ag NPs. The maximum antibacterial activity against Staphylococcus aureus was achieved using the MFC which showed exceptional Log Reduction values reached up to 6.4.
Against *E. coli*, the same behavior was obtained by chitosan. Log Reduction value was 1, 1.09 and 2.39 for 1% initial chitosan concentration after contact time of 10, 15 and 30 min; respectively. Using 2% initial concentration, the Log Reduction value increased to 1.79, 2.19 and 2.89 after 10, 15 and 30 min respectively. On the other hand, modification of chitosan with CNTs showed a significant enhancement against *E. coli*. The Log Reduction value increased linearly to 1.59, 1.69 and 2.69 after 10, 15 and 30 min respectively using 1% initial concentration and 2.09, 3.39 and 3.62 using 2% at the similar contact times. Upon modification of chitosan with Cu NPs, the chitosan/Cu NPs nanocomposite showed enhanced results as compared with chitosan but still less that chitosan/CNTs. The antibacterial activity order against *E. Coli* was chitosan> chitosan/Cu NPs> chitosan/CNTs > chitosan/Ag NPs > MFC.

As antifungal agents, all tested materials showed linear response with contact time against *A. flavus* using 1% initial concentration.
while when using 2% most materials show steady state after contact time of 15 min but chitosan/AgNPs and the multifunctional nanocomposite still show linear response with the increase of contact time.

Numerous mechanisms are suggested related to the antimicrobial activity of chitosan. One of the most accepted mechanisms is that the positive charge of chitosan probably interacts with negatively charged microbial cell membranes and form electrolyte complexes with the microbial surface compounds forming an impermeable layer around the cell which prevents the transport of the essential solutes into the cell [19]. On the other hand, chitosan can chelate with the essential nutrients inhibiting thus the growth of bacteria.

The modification of chitosan with different nanoparticles was found to greatly modify the surface morphological structure as well as the charge density. The antimicrobial characteristics of chitosan were greatly enhanced by the added nanomaterials. Chitosan bi-nanocomposites (chitosan/Ag NPs, chitosan/Cu NPs and chitosan/CNTs) exhibited further enhanced inhibition efficiency against the tested microbes. This could be attributed, beside the effect of chitosan, to the great affinity of metal nanoparticles towards the amino and carboxylic groups. Nanoparticles release inside the bacterial cells and may interact and intercalate with the nucleic acid strands of DNA and may also release inside the microbial cells and disrupt biochemical processes. It is believed that Ag NPs after penetration into the bacterial cells acts to deactivate their enzymes generating hydrogen peroxide which in turn affects the cell viability and causing bacterial death [21]. The high affinity of silver towards sulfur and phosphorus is thus the key element of its antimicrobial effects. It is demonstrated that a similar mechanism of action of copper nanoparticles is as silver ones [17]. Chitosan/Cu NPs may bind with DNA molecules and lead to disorders by cross-linking within and between the nucleic acid strands and may also disrupt biochemical processes. The potency of chitosan/CNTs against microbes could be explained by an efficient contact with the surface of bacterial cell by hydrophobic interactions resulting in perturbation of bacterial cell integrity and then cell membrane damage. The mechanism may be associated with length-wrapping on the bacterial walls and membranes and allowing a loss of bacterial membrane potential, demonstrating complete destruction of microorganism [20].

The multifunctional nanocomposite was found to have superior antimicrobial characteristics most probably due to the combination of different dimensional nanoparticles in the chitosan matrix and the effect is more pronounced at relatively short contact time as shown in Fig. 7 where the materials are highly differentiable at low concentration; 1% and short contact time; 10 min.

![Comparison between the antimicrobial characteristics of the prepared nanocomposites against the tested microbes at the lowest used contact time (10 min) and concentration (1%).](image-url)
The multifunctional nanocomposites of chitosan could be applied broadly as antimicrobial agent against different microbial strains; Gram positive and Gram negative bacteria as well as fungal strains. These finding along with the previously obtained results in our previous work (which concluded that the prepared MFC showed also superior heavy metals adsorption characteristics) allow this material to act as a promising multi-tasking agent in wastewater treatment.

4. Conclusion

Nanocomposites of chitosan and silver nanoparticles, copper nanoparticles and carbon nanotubes have been successfully prepared as bi- and Multifunctional nanocomposites. Modifications of chitosan with metal nanoparticles and carbon nanotubes were found to greatly modify the structural morphology of the produced nanocomposites. Chitosan have antimicrobial properties against Gram positive, Gram negative and fungal strains and the antimicrobial activity was significantly enhanced by copper, silver nanoparticles and CNTs. The prepared materials are highly differentiable at low concentration; 1% and short contact time; 10 min. The multifunctional (chitosan/Ag NPs/Cu NPs/CNTs) nanocomposite has a combination of different dimensional nanoparticles and was found to have superior antimicrobial characteristics more pronounced at relatively short contact time. The multifunctional nanocomposites of chitosan could be applied broadly as antimicrobial agent against different microbial strains; Gram positive and Gram negative bacteria as well as fungal strains in addition to the heavy metals uptake characteristics reported in our previous work. These findings suggested that the prepared multifunctional nanocomposite can act as a promising integral single material for multi-contaminant water treatment; heavy metals removing and water disinfection against different microbes.

Acknowledgements

This work was supported by internal funding program of Egyptian Petroleum Research Institute (EPRI). The authors would like to express their acknowledgement to EPRI nanotechnology center.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijbiomac.2017.01.032.

References