Research Article

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Fabrication of CS-TiO_2-Ag Nanocomposites by Laser Ablation for Antimicrobial Applications and Water Purification

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Abstract

In this study, we fabricated a series of nanocomposites using a neodymium-doped yttrium aluminum garnet (Nd:YAG) laser that was operated with specific parameters, as follows: wavelength, 1 064 nm; energy output, 500 mJ; pulse number, 500; and frequency, 1 Hz. Four distinct types of nanocomposites were produced: chitosan (CS); chitosan-titanium oxide (CS-TiO_2); chitosan-silver (CS-Ag); and a composite of chitosan, titanium oxide, and silver (CS-TiO_2-Ag). A transmission electron microscopy (TEM) analysis was employed to characterize these nanocomposites, revealing particle sizes of 13, 15, 34, and 32 nm for CS, CS-TiO_2, CS-Ag, and CS-TiO_2-Ag, respectively. We further evaluated the antimicrobial efficacy of these nanocomposites against two prevalent bacterial strains, i.e., *Escherichia coli* and *Klebsiella*. Our observations indicated varying degrees of bactericidal effectiveness, as represented by the diameters of the killing zones. More specifically, for *E. coli*, the inhibition diameters were 20 mm (CS), 36 mm (CS-Ag), 38 mm (CS-TiO_2), and 40 mm (CS-TiO_2-Ag). Similar results were observed for *Klebsiella*, with inhibition diameters of 28 mm (CS), 35 mm (CS-Ag), 38 mm (CS-TiO_2), and 43 mm (CS-TiO_2-Ag). Moreover, the CS-TiO_2-Ag nanocomposite was further studied regarding its potential for use in environmental applications, especially water purification. An experiment combining 30 mL of the CS-TiO_2-Ag nanocomposite with 30 mL of contaminated water resulted in successful purification, as corroborated by a subsequent analysis. In conclusion, this study offered important insights into the fabrication of chitosan-based nanocomposites and their respective antimicrobial performances against *E. coli* and *Klebsiella*. Furthermore, it illustrated the promising potential of the CS-TiO_2-Ag nanocomposite for use in water purification applications, thus demonstrating its potential for broader environmental utility.

Keywords: chitosan-titanium-oxide-silver (CS-TiO_2-Ag); optical properties; transmission electron microscopy (TEM); *Escherichia coli*; *Klebsiella*; antibiotic

Introduction

Nanomaterials, which are defined by dimensions under 100 nm, are at the forefront of modern technological innovation. The unique attributes of these materials, such as their diminutive size coupled with a large surface area, have paved the way for new and exciting applications in various sectors, including water purification and antibacterial treatments. This advancement offers potential solutions for contemporary environmental challenges, including water pollution and bacterial resistance. A key area of focus in this context is the problem of antibiotic resistance associated with many types of bacteria. However, studies have shown that nanomaterials can effectively kill these resistant bacteria [1–3].

A multitude of studies have endorsed the effectiveness of distinct types of nanomaterials in this domain. Notably, nanoscale metal oxides, such as titanium oxide and zinc oxide, exert antimicrobial actions through the mechanism of photocatalytic oxidation [4–6]. Similarly, nanosilver and nanogold particles have displayed antibacterial effectiveness by forming reactive chemical combinations that can disrupt bacterial cells [7, 8]. Furthermore, nanocharcoal, akin to biochar, demonstrates a capacity for effective water purification by absorbing impurities, as well as chemical and biological pollutants [9]. In the realm of antibacterial solutions, silver, in its nano form, has been renowned since ancient times for its antibacterial attributes, offering an intense and effective antibacterial action [10]. In this context, the bacterial genus *Klebsiella*, which belongs to the family Enterobacteriaceae and includes species such as *K. pneumoniae* and *K. granulomatis*, stands out. These bacteria can cause a variety of diseases if they spread to other parts of the body, such as the lungs, blood, or urinary tract [11]. Some strains of *Klebsiella* exhibit resistance to many anti-inflammatory drugs, including potent antibiotics such as carbapenems; however, nanomaterials have displayed effectiveness in combating these resistant strains [12,13]. *Escherichia coli* is another important bacterium in this scenario. As the dominant normal intestinal bacteria in human and animal intestines, *E. coli* strains can cause a variety of infections, ranging from urinary tract infections to severe diarrhea. Nanomaterials, such as zinc oxide and nanometer silver, have been shown to eradicate these bacteria by affecting the integrity of the cell membrane and disrupting bacterial metabolism [14−16]. Further exploration of natural compounds in this context revealed that chitosan, which is derived from the exoskeleton of crustaceans (such as shrimp and crab), holds significant promise in water-purification and bacterial-elimination applications because of its unique adsorption capabilities [17]. Titanium oxide, which is a frequently used nanomaterial, possesses robust photocatalytic oxidation properties, rendering it effective in eliminating bacteria and other organic matter in water [18]. The preparation of nanomaterials entails various methods. The chemical methods include sol–gel processing for stability [19], chemical deposition [20], chemical vapor deposition [21], thermal decomposition [22], and microwave-assisted synthesis [23]. The physical methods encompass evaporation–condensation [24], thermal spray [25], laser melting [26], and pulsed laser ablation [27]. In our previous research, a compound comprising chitosan, titanium oxide, and silver (CS-TiO_2-Ag) was fabricated using the method of pulsed laser ablation in liquids.

The focus of this research was the application of chitosan, titanium oxide, and silver for the dual purpose of bacterial extermination and water purification, based on their proven efficacy in tackling water pollution and bacterial contamination.

Experimental

Mueller–Hinton agar (a culture medium for bacteria), the CS-TiO_2-Ag nanocomposite, a 5-cm-long sponge with a diameter of 3 cm, a 12-cm syringe, a beaker, contaminated water, and an intravenous set (IV set) were used in the present study.

The nanocomposite was synthesized using a neodymium-doped yttrium aluminum garnet (Nd:YAG) laser with a wavelength of 1 064 nm, an energy level of 500 mJ, 500 pulses, and a frequency of 1 Hz according to the method detailed in our previous research. As shown in Fig. 1, analyses were performed using ultraviolet (UV)–visible (Vis) spectroscopy and transmission electron microscopy (TEM) in the liquid medium.

To prepare the Mueller–Hinton agar, 38 g of agar powder (CM0337B) was suspended in 1 L of distilled water, mixed well until complete dissolution, and then steam sterilized at 121 °C for 15 min. The sterilized liquid was then poured into a Petri dish and left to solidify.

The microbial inoculum was spread evenly across the surface of the agar plate using the disc diffusion method. A hole with a diameter of 6–8 mm was then drilled aseptically using a drill or a sterile cork tip. A 0.5-mL volume of the nano-antibacterial solution was applied, and the agar plates were incubated at 37 °C. The antibiotic diffused into the agar medium, thus inhibiting the growth of the test microbe strain [28].

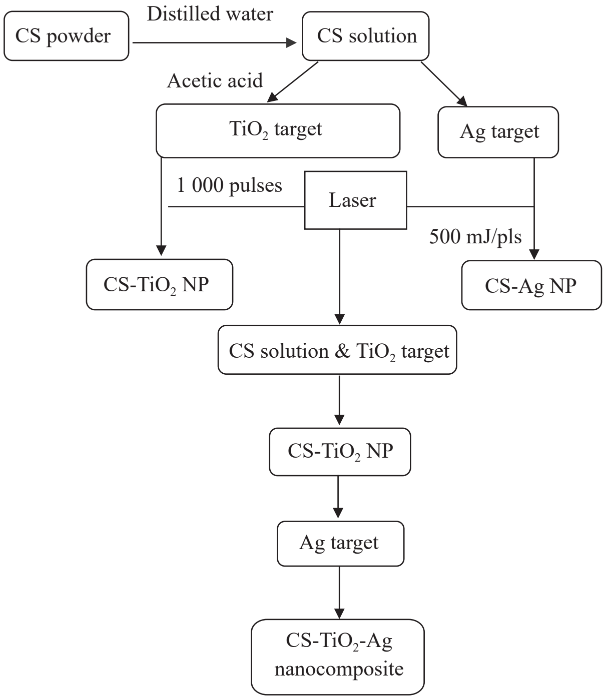

Fig. 1 Mechanism underlying the formation of the nanocomposite (CS-TiO_2-Ag).

To create the filter, a sponge was placed inside a syringe, and an IV set was inserted into the bottom of the syringe, with the other end placed in a beaker (as shown in Fig. 2). Before the filtration process, the sponge was soaked in the nanocomposite for 10 min, and 30 mL of contaminated water was then added to the sponge, for filtration, with or without the nanocomposite. An absorbance analysis of the contaminated water was performed before and after filtration (after a 5-min waiting period).

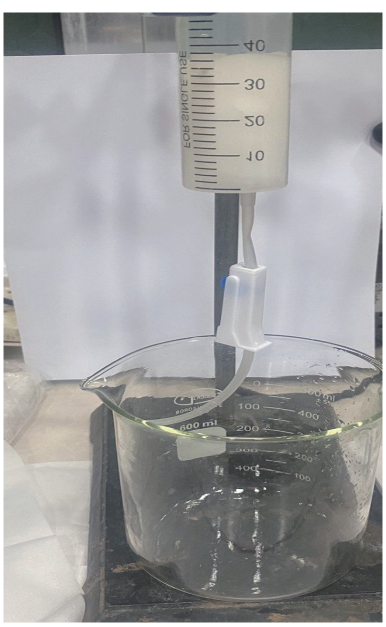

Fig. 2 Water-purifying filter.

Results and Discussion

UV–Vis spectroscopy revealed that the peak of CS-Ag was located at 400 nm, whereas that of CS-TiO_2 was detected at 224 nm. In turn, for the CS-TiO_2-Ag nanocomposite, there was no shift in the peak position. However, there was a notable change in the absorption intensity caused by the increased concentration of the nanoparticles within the solution [29]. Specifically, there was an increase in the intensity of CS-TiO_2 and a decrease in the intensity of CS-Ag within the CS-TiO_2-Ag composite. This change in intensity was attributable to the formation of a core–shell structure, suggesting that the silver nanoparticles are encapsulated by titanium [30]. This is further depicted in Fig. 3.

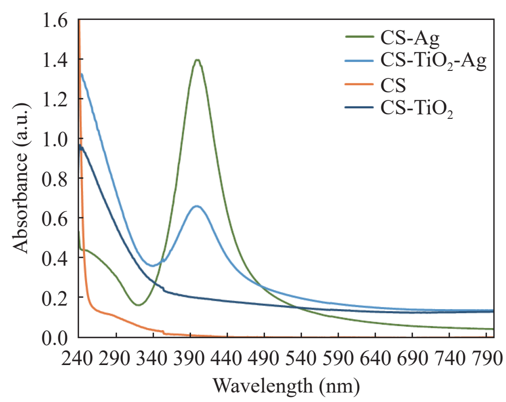

Fig. 3 UV–Vis spectra of CS-Ag, CS-TiO_2, CS-TiO_2-Ag, and CS.

TEM images revealed that chitosan particles exhibit heterogeneity in their shape while predominantly adopting a spherical form, as depicted in Fig. 4. Under usual circumstances, chitosan tends to display a fibrous or plate-like structure. However, in the observed sample, the chitosan polymer adopted a spherical morphology. The shape of the chitosan particles can be affected by various factors, such as the polymer's concentration, the preparation method used, the temperature, and the pH [31]. Our observations indicated that the average size of these particles was approximately 13 nm, as illustrated in Fig. 5.

Regarding the CS-Ag nanocomposite, its particles exhibited an almost spherical form and resembled a core–shell structure, as illustrated in Fig. 6. The size of these nanoparticles was approximately 34 nm, as depicted in Fig. 7. This could be attributed to the encapsulation of the silver surface by chitosan. By

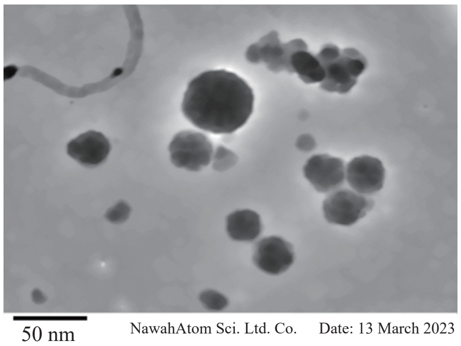

Fig. 4 TEM image of CS nanoparticles.

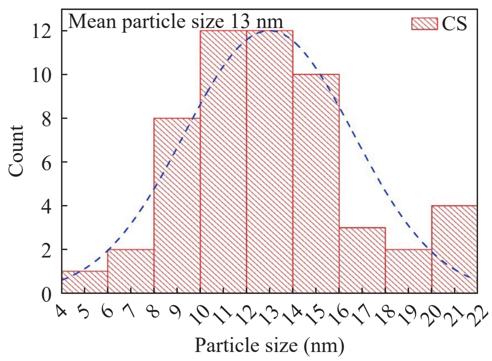

Fig. 5 Distribution of the size of a CS nanoparticle.

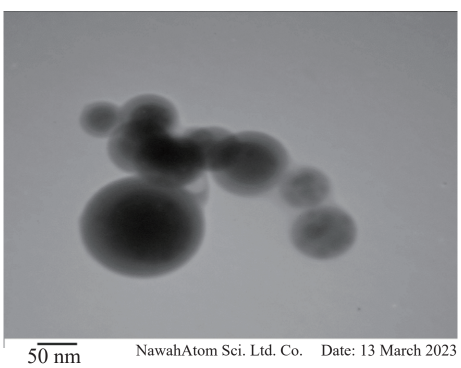

Fig. 6 TEM image of CS-Ag nanoparticles.

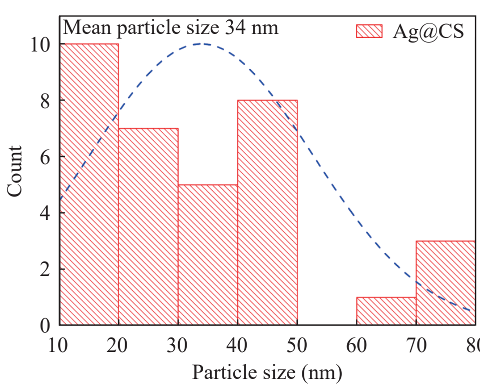

Fig. 7 Distribution of the size of a CS-Ag nanoparticle.

serving as an encapsulating agent, chitosan can deposit itself on the surface of silver particles, which results in the creation of a core–shell structure. In this structure, the core is constituted by silver, whereas the shell is composed of chitosan [32].

The CS-TiO_2 nanocomposite exhibited a hexagonal structure enveloped by spherical chitosan particles, as depicted in Fig. 8. This could be attributed to the introduction of acetic acid during the preparation of chitosan, which affects the growth and crystallization of titanium oxide particles, thus giving rise to the hexagonal shape [33]. The average size of the CS-TiO_2 nanocomposite was around 15 nm, as shown in Fig. 9.

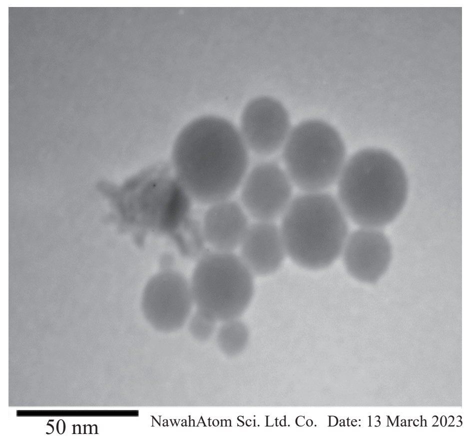

Fig. 8 TEM image of CS-TiO_2 nanoparticles.

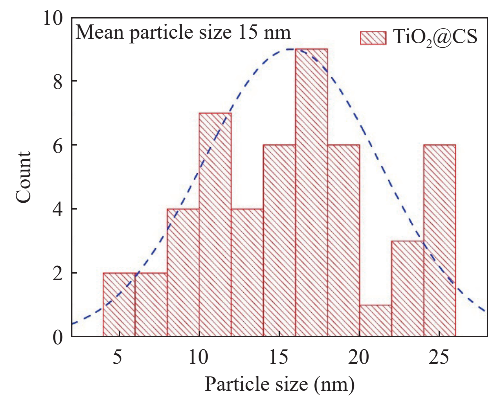

Fig. 9 Distribution of the size of a CS-TiO_2 nanoparticle.

In turn, the CS-TiO_2-Ag nanocomposite presented a spherical form, as depicted in Fig. 10. The effect of chitosan on silver nanoparticles and titanium oxide nanoparticles can vary according to the conditions and concentrations utilized during particle preparation. Moreover, chitosan possesses active groups that have the capacity to interact with the surface of silver and titanium oxide particles. This contributes to the stabilization of the particles, thereby reducing their propensity to aggregate [34]. The mean size of the CS-TiO_2-Ag nanocomposite was approximately 32 nm, as illustrated in Fig. 11.

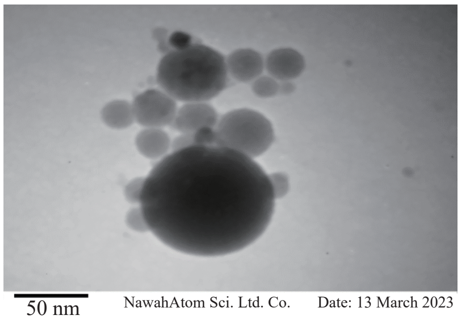

Fig. 10 TEM image of CS-TiO_2-Ag nanoparticles.

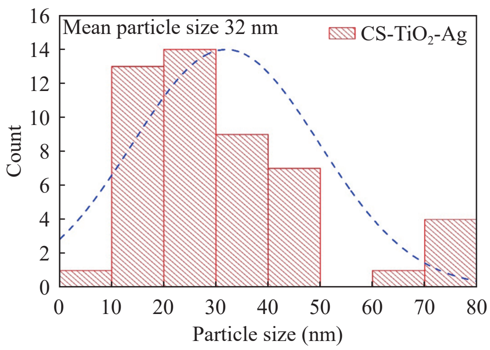

Fig. 11 Distribution of the size of a CS-TiO_2-Ag nanoparticle.

Nanocomposite Applications

Antimicrobial applications

E. coli bacteria were exposed to several compounds, including CS, CS-TiO_2-Ag, CS-Ag, and CS-TiO_2, each at a volume of 200 μL. After a 24-h incubation period at 37 °C, the highest bacterial inhibition was observed for the CS-TiO_2-Ag nanocomposite, which yielded an inhibition zone of 40 mm. This was followed by the CS-TiO_2 nanocomposite, with an inhibition zone of 38 mm. In turn, the CS-Ag composite displayed an inhibition zone of 36 mm, whereas the CS polymer had the smallest effect, inhibiting bacterial growth over a zone of only 20 mm (Fig. 12). Furthermore, even after an incubation period of 72 h, these inhibition zones remained consistent, suggesting that the compounds eradicated the bacteria effectively.

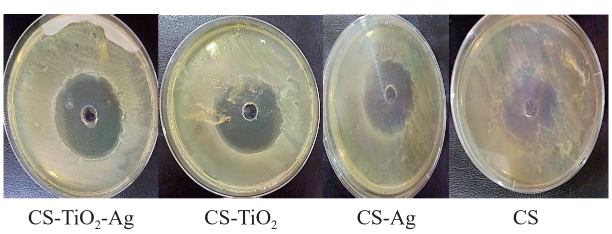

Fig. 12 Bacterial cell (*E. coli*) death.

Conversely, the application of the nanomaterials to *Klebsiella* bacteria under the same conditions for 24 h yielded inhibition zones of 43, 38, 35, and 28 mm for CS-TiO_2-Ag, CS-TiO_2, CS-Ag, and CS, respectively, as depicted in Fig. 13(a). However, after a 72-h incubation period, the bacteria re-emerged, as shown in Fig. 13(b). This evidence suggests that, although the nanocomposites successfully inhibited *Klebsiella* bacteria, they only fully eradicated the *E. coli* bacteria. This can be explained by the fact that *E. coli* bacteria carry a positive charge, whereas *Klebsiella* bacteria possess a negative charge.

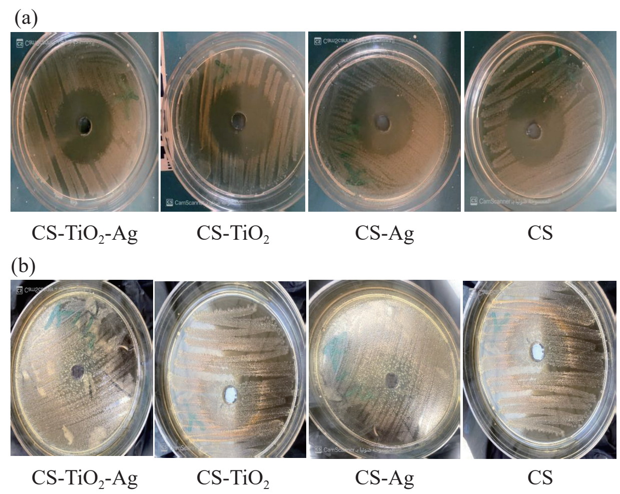

Fig. 13 (a) Bacterial cell (*Klebsiella*) death. **(b)** Bacterial cell (*Klebsiella*) inhibition.

Water purification

The efficacy of the filter depicted in Fig. 14 was evaluated regarding water purification by measuring the adsorption of the contaminated water. Initially, the absorption intensity was measured as 2.54. After passing the water through the filter, the absorbance increased to 2.77, suggesting the successful capture and removal of pollutants by the filter. The same process was then repeated with the application of nanoparticles after a time interval of 10 min, at which

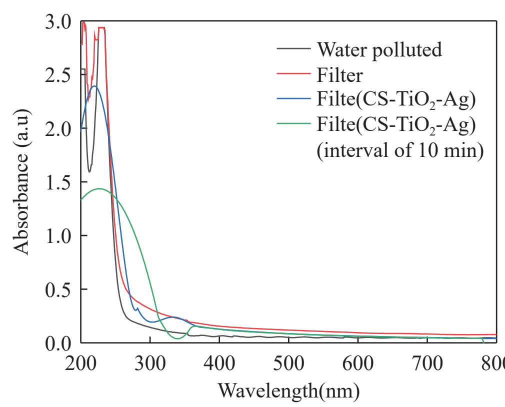

Fig. 14 UV–Vis spectra of the polluted water, sponge filter only, and sponge filter with the CS-TiO_2-Ag nanocomposite.

point the absorbance was recorded as 1.33, which signifies the sustained efficiency of the filter in pollutant elimination .From these observations, it can be deduced that the filter has an effective performance in water purification, achieving an efficacy rate of 75%.

Conclusion

In summary, the findings of this research led to the assertion that the nanomaterials, i.e., CS, CS-TiO_2, CS-Ag, and CS-TiO_2-Ag, exhibited a significant efficacy in eliminating *E. coli* bacteria and inhibiting the growth of *Klebsiella* bacteria. Furthermore, the CS-TiO_2-Ag compound proved to be effective in purifying water, by achieving an efficacy rate of up to 75%. The investigations carried out using TEM demonstrated that these compounds have sizes in the nano range, although their dimensions varied. These insights could pave the way for the development of new applications in water treatment and highly efficient bacterial control strategies.

CRediT Author Statement

Tebark Abd Zaid Hassoun: Main researcher and author of the initial manuscript. **Amer Al-Nafiey**: Corresponding author responsible for editing and arranging the manuscript. **Jinan A. Abd**: Project manager overseeing the research.

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Conflict of Interest

The authors declare no conflicts of interest in relation to this research manuscript.

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