

Green Chemistry Derived Nanocomposite of Silver-modified Titania for Disinfectant

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ABSTRACT

A significant accomplishment of this study centers on developing nanocomposite materials via green synthesis techniques for inactivation of prokaryotes like *Escherichia coli* (*E. coli*). The motivations of this study are to: (1) implement fundamental research in nanochemistry for biological application; (2) understand the mechanism(s) of inactivation of bacteria; and (3) to integrate advanced instrumentation to aid understanding of antibacterial effects of novel nanoparticles. The originality of this study focuses on use of bottom-up synthesis to produce Ag-modified TiO₂ nanocomposite particles with decreased band gap energy in the visible light range. In addition, several characterization techniques were used to study the nanostructure of these nanocomposites.

Keywords: Nanocomposite of Ag-TiO₂, Colloidal Synthesis, Characterization, Antibacterial Performance

1 INTRODUCTION

Titania (TiO₂) is one common photocatalyst, which has been extensively studied for applications in the field of catalysis and disinfection [1, 2]. While the importance silver (Ag) as a bactericide has been well-documented [3,4], the mode of action remains to be explored. Other approaches include the use of Ag modified TiO₂ nanoparticles (Ag-TiO₂ NPs) as photocatalyst [5], however, its use as a disinfectant request ultraviolet light. This research suggests that Ag-TiO₂ NPs can be prepared in a cost-efficient and environment-friendly manner [6]. Modification of TiO₂ using Ag resulted in reduced band gap energy as measured by ultra-violet visible light (UV-VIS) spectroscopy. The decreased band gap will allow us to eliminate bacteria using visible light instead of UV radiation. Significance of this study was to develop environmental-friendly synthesis of nanomaterials, and characterization, including use of several state-of-the-art instrumentation to analyze nanostructure of nanocomposite for in-depth study of inactivation of bacteria.

2 EXPERIMENTAL

All chemicals unless otherwise specified were obtained from Sigma-Aldrich (St Louis, MO), and solvents from

VWR International (West Chester, PA). The reagents were reagent grade and were used without further purification. Doubly-distilled and 0.2 micron filtered (Milli-Q) water was used in the dissolution of our compounds. A number of formulations were attempted to optimize the final composition of Ag-TiO₂. Various reducing agents such as ascorbic acid, sodium citrate, sodium borohydride and dimethylamine borane (DMAB) were used for the complete chemical reduction of silver ion. In addition, the nanostructure of the Ag-TiO₂ was systematically studied using several analytical techniques, including transmission electron microscopy equipped with X-ray energy dispersive spectroscopy, UV-VIS spectroscopy, X-ray powder diffraction, and zeta-potential to determine the particle size and its distribution, crystalline phase structure and surface stability. Furthermore, antibacterial effect of Ag-TiO₂ colloid was conducted under visible light.

2.1 Materials

Starting material of titanium (IV) butoxide (Ti(OⁿBu)₄) was added into acetic acid (CH₃COOH) aqueous solution with continuous agitation. The acetic acid was used to control the hydrolysis rate of TiO₂ in our experiments. Arabic gum (AG, a dispersing agent) of 3.00 mass % solutions was prepared and stirred for min at 60 °C. Into this AG solution, the second starting material AgNO₃ (molar ratio of Ag: Ti was controlled at 1:20), which was dissolved in 30.00 ml of Milli-Q water, was added. The Ag⁺ cation was then reduced using the above reducing agents. The concentration of Ag was kept at 1.06×10⁻³ mol/L.

2.2 Characterization

A Tecnai F20-G² transmission electron microscopy (TEM) (FEI Company, Hillsboro, Oregon) was used to characterize the nanoparticles and its size distribution. X-ray energy dispersive spectroscopy (EDS) technique was also used to obtain elemental composition Ag-TiO₂ nanocomposite. An atomic-resolved lattice fringe of these nanocomposite materials was characterized by high resolution TEM (HRTEM). The samples were dispersed in absolute ethanol and deposited onto the carbon-coated copper (Cu) grid. An X-ray powder diffraction (XRD) was employed to determine the crystalline phase and average crystallite size of the nanocomposite. The XRD patterns

were collected using a Rigaku multiflex diffractometer (Rigaku Americas Incorporation, Houston, TX), equipped with a Cu target at 40 kV and 44 mA. The scanning range was varied between 30 and 80 degrees at a scanning rate of 2°/min. The phase structure was identified using the Rigaku Jade 7.0 software package. A ZetaPALS (Brookhaven Instruments Corporation, New York, USA) was utilized to measure the particle size distribution and surface energy to evaluate the stability of Ag-TiO₂ nanocomposite. The ZetaPALS utilizes dynamic light scattering to measure the electrophoretic mobility of negatively-charged colloid.

2.3 Inactivation of Bacteria

Pure cultures of *Escherichia coli* (*E. coli*, TG₁) were maintained as frozen glycerol stocks in the Department of Biochemistry at Vanderbilt University. *E. coli* cultures were grown on Bacto-Tryptone (2×YT) agar plates or in 20 ml of sterile 2×YT or Luria-Bertani (LB) broth. The cells were grown overnight, aerobically on a rotatory shaker at 37 °C. Cells were harvested from overnight culture by centrifugation for 10 min at 4 °C and 6000 rpm. The cells were re-suspended and diluted to a final concentration of 1×10⁷ cfu/ml in a 0.05M KH₂PO₄ and 0.05M K₂HPO₄ (pH 7.0) buffer solution.

3 RESULTS AND DISCUSSION

Synthesis of Ag-TiO₂ nanocomposite was developed in this study to prevent the aggregation of the particles during the synthesis process. The primary objectives of this work were to characterize the samples derived from colloidal chemistry and to attempt to prevent particles from agglomeration with a surfactant capable to improve their dispersion in the final product. This process has the advantage to improve the wetting and dispersion of the surface of the Ag-TiO₂ composite. A continuous external TiO₂ thin layer was then deposited onto the Ag core successively. The present work has successfully overcome the negative attributes of the existing products and procedure of Ag-TiO₂ fabrication (shown in *Figure 1*).

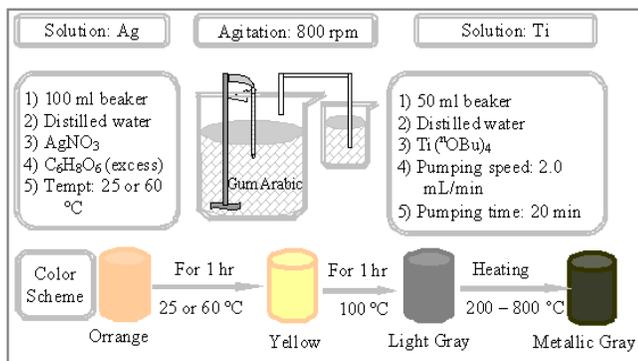


Figure 1: Fabrication of the Ag-TiO₂ via Bottom-up Green Chemical Approach.

3.2 Nanostructural Characterization

The X-ray powder patterns for Ag-TiO₂ nanocomposite are depicted in *Figure 1*. Results clearly indicate the presence of silver (Ag) in the form of highly crystalline face-centered cubic (*fcc*) structure (PDF 01-089-3722 with lattice constants of 4.0855 Å and 90 °). The diffraction from TiO₂ crystals indicates an anatase structure was obtained (PDF 03-065-5714 with lattice constants of 3.7855/9.514 Å and 90 °). *Figure 2* display four specimens with identical spectra, which indicates that the reproducibility was excellent. The insert was the Ag XRD pattern, which did not show in the spectra of Ag-TiO₂ composite due to its low concentration.

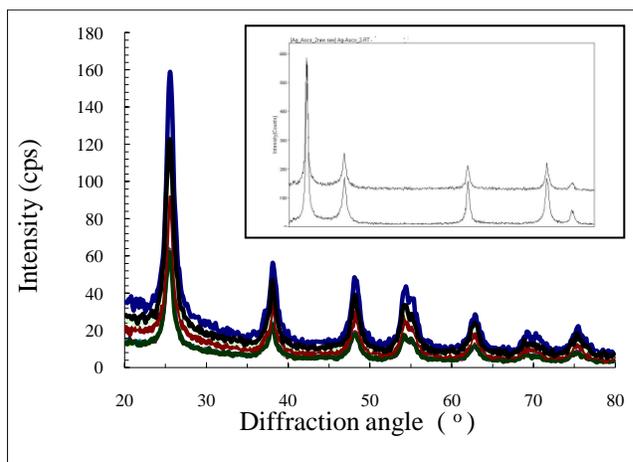


Figure 2: X-ray Powder Diffraction Spectra of Ag-TiO₂.

Results of TEM morphological and nanostructural studies are summarized in *Figure 3*. The results indicate that mono-dispersive and highly crystalline Ag-TiO₂ nanocomposites were obtained. The composite's appearance is near-spherical with the average size ranged from 20 to 50 nm in diameter. Particle agglomeration occurred and was presumably due to the high surface tension of the ultrafine nanocomposite particles. The fine particle size will result in a large surface area that will, in turn, enhance the nanocomposite catalytic activity. The Ag and TiO₂ distribution indicates that Ag was used as a seed to allow for TiO₂ deposition. TiO₂ formed continuous and successive membranes around the Ag core, which was consequently subjected to decreased band gap energy.

X-ray energy dispersive spectroscopic (EDS) analysis indicate that nanocomposite consisted mainly of Ag, Ti, and O (*Figures 4*). The spectra (*left insert*) of L_α for the Ag metal at 2.984 eV and L_n at 2.806 eV is well-indexed with the standard metallic Ag. Similarly, the spectra (*right insert*) Ti occurred at 4.510 eV and resulted from a K_α electron configuration and another one occurred at 0.452 eV and resulted from an L_α configuration. An insignificant peak at 4.930 eV caused by K_β was also detected.

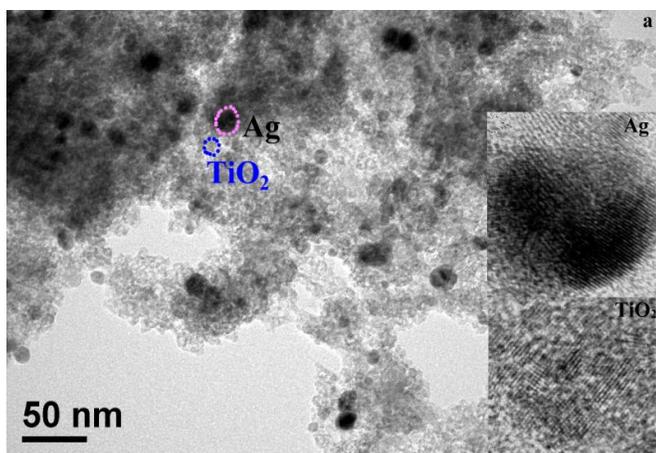


Figure 3: TEM image of Ag-TiO₂ nanocomposite (top insert: Ag fringe; bottom insert: TiO₂ fringe).

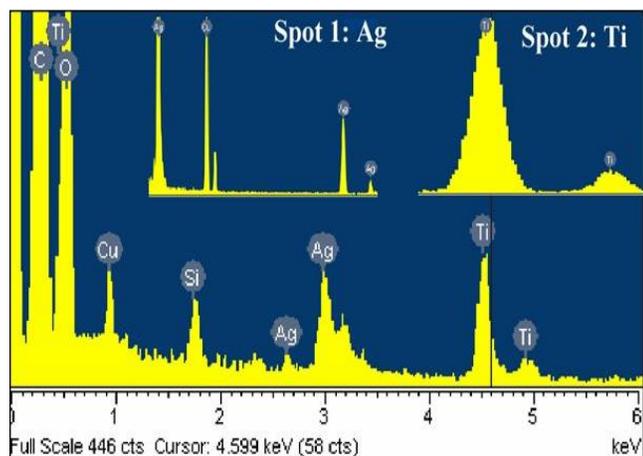


Figure 4: EDX spectra of Ag-TiO₂ nanocomposite (left insert: Ag spectrum; right insert: TiO₂ spectrum).

In order to evaluate the stability and the electrokinetic behavior of the Ag-TiO₂ colloid, the zeta-potential (known as effective surface potential, denoted as ζ) was measured by imposing an electrical field (2 V/cm) across the colloid particles. The measured ζ allowed an estimation of the degree of aggregation. It was found that zetapotential essentially ranged from (minus) -20 to -85 mV according to the various fabrication parameters of the TSNC. With increasing pH, the absolute ζ values decreased and were essentially constant at basic solution. The large zetapotential of negative charges minimized particle aggregation due to electrostatic repulsion. In the finer particle region (10 - 20 nm), the zetapotential was from (minus) -55 to -45 mV. The less negative zetapotential ranging from -25 to -15 mV can be attributed to the crystal growth to form larger particle with size of 50 to 70 nm.

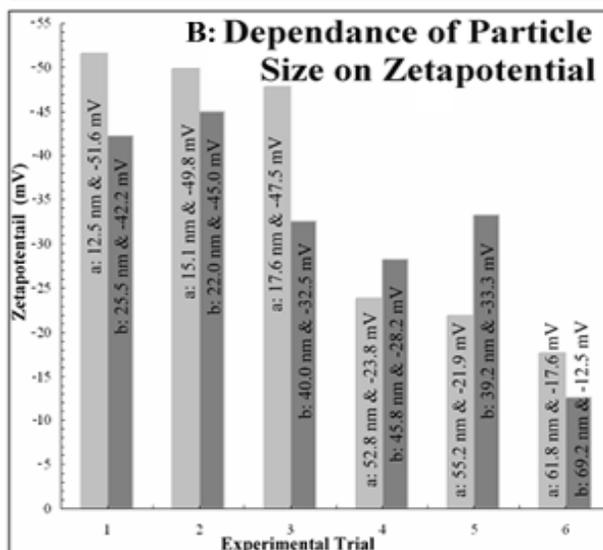
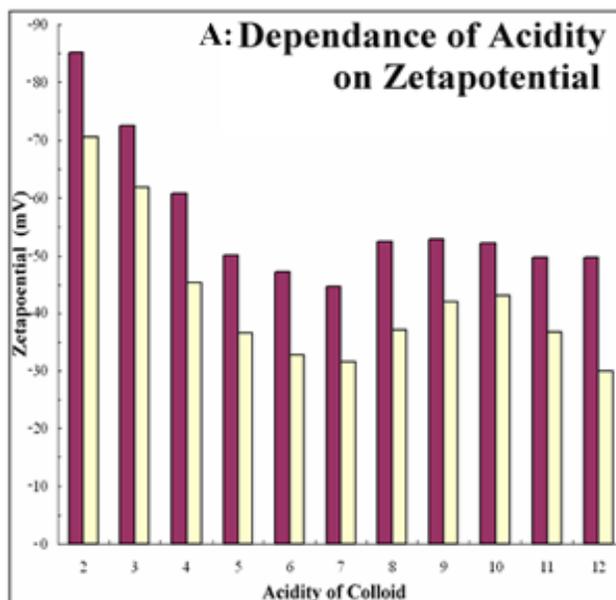


Figure 5: The electrokinetics analysis of Ag-TiO₂ nanocomposite to evaluate the surface energy (A); the particle size distribution (B) of Ag-TiO₂ nanocomposite corresponding to the zeta potential.

3.3 Inactivation of Bacteria

The nanocomposite optical absorption properties were consistent with noble metal nanoparticles. The GS derived Ag-TiO₂ were successful in the removal of biological impurities from drinking and underground water supplies. *E. coli* were inactivated using Ag-TiO₂ under visible light at ambient temperature and pressure. The results of the study indicated that nanocomposites could be specifically designed to prevent growth of bacteria in water (Figure 6 A to C). Significance of work is development of Ag-TiO₂ for inactivation of bacteria.

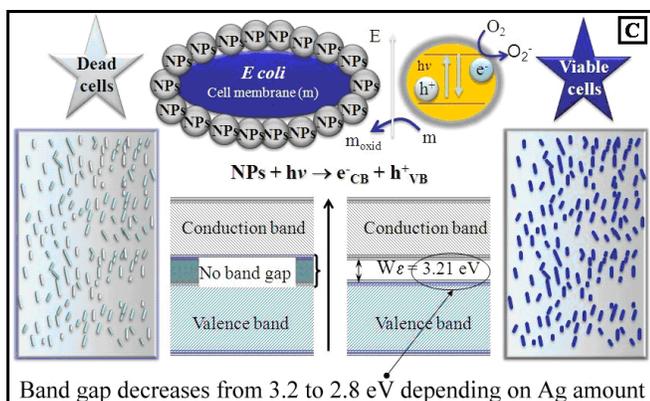
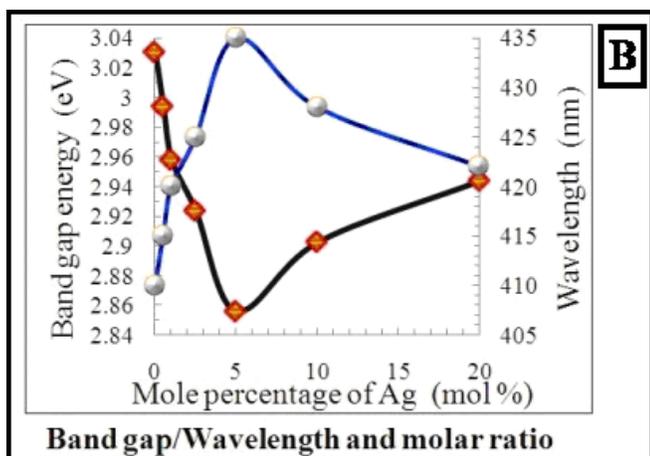
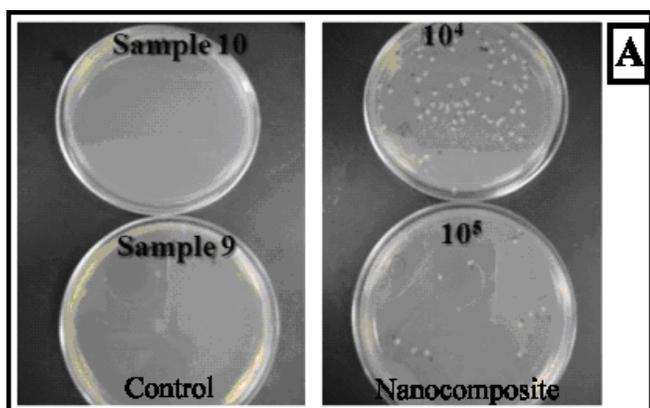


Figure 6: The inactivation of *E. coli* using Ag-TiO₂ nanocomposite (A); UV-VIS spectroscopic study of the band gap decrease of TiO₂ modified by Ag (B); and hypothesis of inactivation mechanism of *E. coli* (C).

4 CONCLUSION

The colloidal suspensions of the nanocomposites are composed of metal and ceramic (Ag-TiO₂). They were found to be extremely stable over a prolonged time period, and were identified using zetapotential data at various pH with negative surface charges. Morphologically, nanocomposites were found to be composed of near-

spherical particles that were highly crystalline. The nanocomposites were mono-dispersed with particles varying in size from 15.8 to 22.5 nm, depending upon nanocomposite solution pH. Nanocomposite elemental composition studies indicated that the molar ratio of Ag and Ti was approximately 1 to 20. The binding energies and energy differences of Ag and Ti were well indexed with their associated standard spectra. Nanocomposite optical absorption properties were consistent with noble metal nanoparticles. The ‘green chemistry’ derived Ag-TiO₂ composites are applicable for the removal of biological impurities from drinking and underground water supplies. *E. coli*, a gram-negative model microorganism was effectively inactivated using the nanocomposite under visible light at ambient temperature and pressure. The results of the study indicated that nanocomposite could be specifically designed to prevent growth of bacteria in water.

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