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Role of silver nanoparticles in imparting antimicrobial activity of titanium dioxide

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ABSTRACT

In the present work, facile syntheses for titanium dioxide (TiO_2), Silver (Ag), and Ag/TiO_2 nanoparticles were applied through sol-gel technique. Structural and morphological characterizations were followed by UV, TEM, and SAED. The cup agar diffusion method was followed to investigate the bactericidal activities against three common bacterial strains being *Escherichia coli* (Gram-negative), *Bacillus subtitles* and *Staphylococcus aureus* (Gram positive). The bactericidal activities were evidenced by the inhibition zones of the bacterial growth around the resultant substrates. The results prove promising bactericidal activity of Ag and Ag/TiO_2 nanophases compared to TiO_2 anatase phase nanoparticles possessing no bactericidal activity. The combined presence of silver nanoparticles along with the anatase phase induced a promising bactericidal activity.

1. Introduction

Despite the increased knowledge of microbial pathogenesis and application of modern therapeutics, the morbidity and mortality associated with the microbial infections remain high [1]. Recently, several investigators [2] have studied the effect of silver (Ag) on the antibacterial performance of titanium dioxide (TiO_2). The activities of silver-coated TiO_2 particles and bioactive films against *Escherichia coli* and *Staphylococcus aureus* were also verified. The achieved composite films reported for Cardio Vascular Disease (CVD) was exhibited both self-regeneration and bacterial destruction [3]. Titania matrices with uniformly distributed silver in low concentration were proved to be promising antibacterial agents [4]. Silver nanoparticles were known to increase the surface area which is essential property for bacterial growth inhibition. Such higher activity was attributed to the trapping of the conduction band electrons by Ag particles which prevent the electron-hole recombination. Consequently, the redox reaction on the TiO_2 surface is thus enhanced. However, the photoactivity of Ag-substituted TiO_2 has not been extensively investigated [5]. In addition, the photocatalytic behavior of Ag/TiO_2 nanoparticles was evaluated using the degradation of a methyl orange (MeO) aqueous solution under ultraviolet light irradiation and the results showed excellent performance in photocatalytic applications [6].

The present study aims to synthesis TiO_2, Ag and Ag/TiO_2 nanoparticles dispersed in silica sol-gel with bactericidal activities. Three different standard bacterial strains namely *Escherichia coli*, *Bacillus subtitles* (ATCC 6633) and *Staphylococcus aureus* (ATCC 29213) bacteria were studied through the cup agar diffusion method.

2. Materials and methods

All the chemicals used in the experimental work were prepared by pure and analytical grades. Titanium dioxide (TiO_2) nanoparticles were prepared by hydrolysis and condensation of titanium tetra-isopropoxide (TTIP). Silver nanoparticles were prepared by reducing silver nitrate ($AgNO_3$) in an aqueous solution, 0.294 g sodium citrate [7]. Detailed experimental procedures are available (Supplementary 1).

Physical and structural characterization was carried out by UV–vis spectroscopy, transmission electron microscopy (TEM) and selected area electron diffraction (SEAD) (Supplementary 2). The antibacterial activities were evidenced by the inhibition zones of bacterial growth on the resultant substrates. Studying the antibacterial activity of the prepared nanoparticles was performed by the cup agar diffusion method [8] (Supplementary 3).

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3. Results and discussion

The UV–vis spectrum shown in Fig. 1(a) reveals silver nanoparticles formation by exhibiting the typical surface plasmon absorption maxima at 454 nm which indicating the presence of spherical or roughly spherical Ag nanoparticles [9]. Fig. 1 (b) clarifies surface plasmon absorption at 263 nm corresponding to Ag/TiO_2 nanoparticles.

The TEM photographs (Fig. 1(c),(d)) indicate that the as-prepared nano-powders consist of well-dispersed grain agglomerates with a narrow size distribution whereas the radii of the individual particles are between 20 and 40 nm. The electron diffraction was carried out on a limited number of crystals (Fig. 1(e),(f)). The diffraction patterns are consistent with the planes (111), (200), (220), (222) and (311) of pure face-centered cubic (FCC) silver structure [10].

The bactericidal activity of the prepared TiO_2 nanoparticles dried in a microwave for 15 min and calcined at temperature 450 °C with 4 h soaking was negative as no detected effect was recorded. On the other hand, Fig. 2(a) demonstrates that Ag nanoparticles (Ag NPs) inhibited the *E. coli* bacterial growth. The inhibition zones diameters increase from 1.00 ± 0.05 to 1.20 ± 0.06 cm upon increasing the Ag NPs concentration in the agar plate media from 5% to 10% and then to 20%, respectively. These results could be expected because the driving force of silver ions diffusion from the bulk to the surface is larger the higher the silver content. The Ag NPs could also inhibit the *B. Subtilus* bacterial growth (Fig. 2(b)). The inhibition zone diameters in the agar plates increase from about 1.00 ± 0.05 to 1.10 ± 0.06 cm and then to about 1.30 ± 0.07 cm upon increasing the concentration of Ag NPs from 5% to 10% then to 20%, respectively. The Ag NPs could also inhibit the *S. aureus* bacterial growth similar to *E. coli* (Fig. 2 (c)). The inhibition zone diameter increases from about 1.00 ± 0.05 to 1.20 ± 0.06 cm upon increasing the Ag NPs concentration in the agar plate media from 5% to 20%, respectively. The synthesized Ag NPs proves a detectable increase in bactericidal activity against Gram positive and Gram negative bacteria in parallel with increasing Ag NPs concentration. A direct proportional relation between Ag NPs concentrations in the agar plate and the inhibition zone was achieved and hence the antibacterial activity. The inhibitory action of silver nanoparticles is higher against G^- bacteria compared to G+. This may be attributed to the difference in peptidoglycan layer thickness of the G^+ bacterial cell wall preventing, therefore, the silver ions actions [11]. A possible mechanism of bactericidal activity of silver nanoparticles is due to their interaction with nucleic acids and impairment of DNA replication. Consequently, formation of electron-dense granules containing silver ions in the cytoplasm of the bacterial cell is followed by cell viability loss and death [12].

The antibacterial activities of the prepared Ag/TiO_2 nanoparticles were tested against the three common bacterial strains. The results demonstrate that Ag/TiO_2 nanoparticles could inhibit the *E. coli* growth (Fig. 3(a)). The inhibition zone diameters increase from about 1.20 ± 0.06 to 1.60 ± 0.08 cm and up to 1.80 ± 0.09 cm. This is in parallel with the increasing loaded silver content through higher Ag/TiO_2 nanoparticles in the agar plate media from 5% to 10% and then to 20%, respectively. Increasing silver content enhanced the driving force of Ag^+ diffusion from the bulk to the surface. Also, the inhibition zone was more than that formed by applying Ag nanoparticles seldom.

Ag/TiO_2 nanoparticles exhibit bactericidal activity against *B. subtitles* (Fig. 3(b)). Increased diameters of the inhibition zones

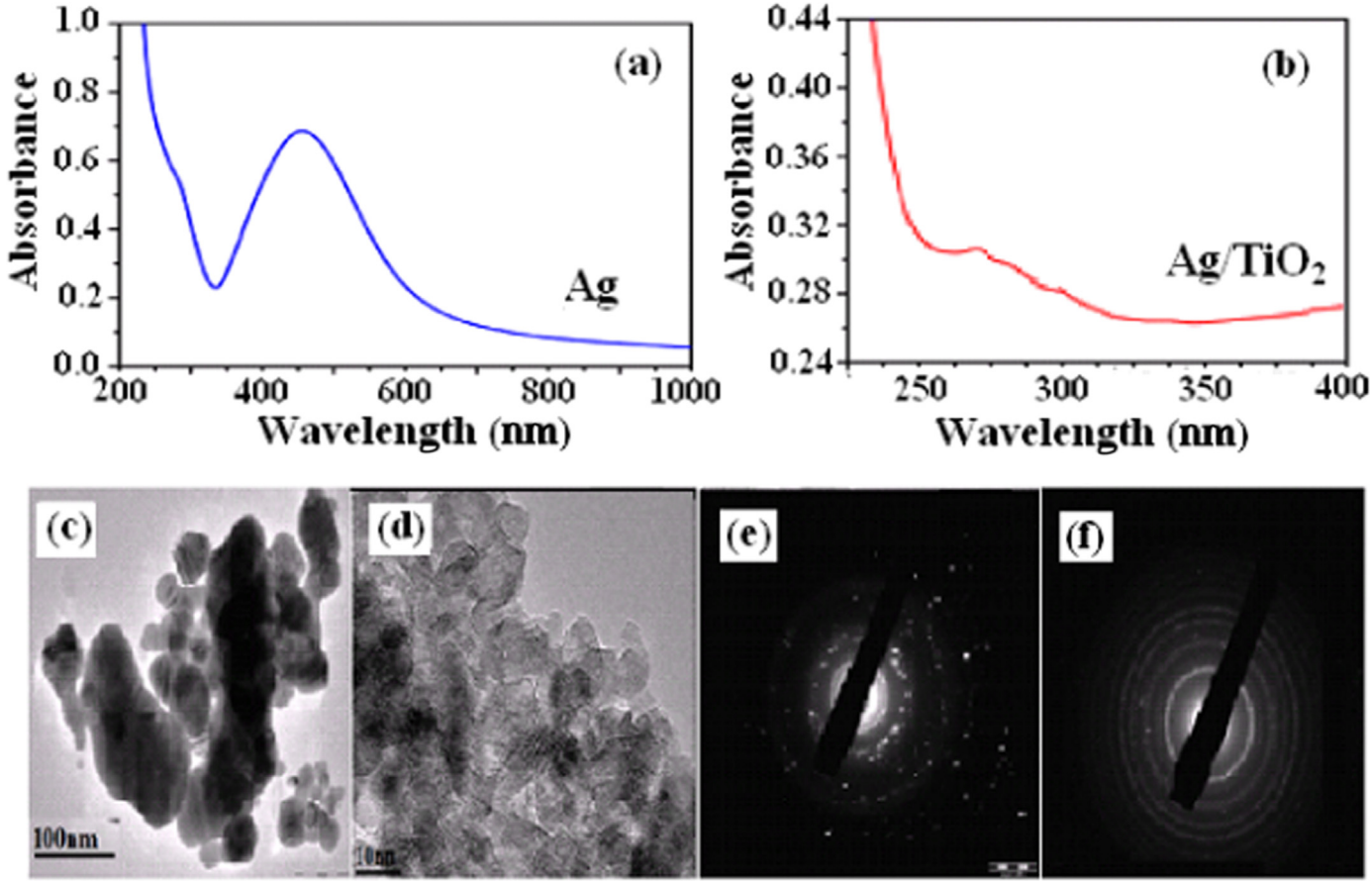

Fig. 1. Surface plasmon resonance of (a) Ag and (b) Ag/TiO_2 nanoparticles and their corresponding TEM micrographs (c) and (d) and SAED (e) and (f).