Antibacterial Properties of Silver-Doped Titania**

J. Thiel, L. Pakstis, S. Buzby, M. Raffi, C. Ni, D. J. Pochan, and S. I. Shah*

Silver has been known to exhibit strong cytotoxicity towards a broad range of micro-organisms. Silver composites with a tailored slow silver-release rate are currently being investigated for various applications.[1] Silver has an oligodynamic effect, that is, silver ions are capable of causing a bacteriostatic (growth inhibition) or even a bactericidal (antibacterial) impact. Nanometer-sized inorganic particles and composites display unique physical and chemical properties and represent a unique class of materials in the development of novel devices, which can be used in numerous physical, biological, biomedical, and pharmaceutical applications.[2] Silver composites have applications in many industries, such as aerospace, surface coatings (e.g., in refrigerators, food processing, kitchen furniture), and for use in hospitals. Research indicates that silver is also effective in purification systems for disinfecting water or air.[3–6] However, in order to make the use of silver economical, there is a need to find cheaper ways of using silver in potential applications without jeopardizing its functionalities.

The bactericidal behavior of silver nanoparticles is attributed to the presence of electronic effects, which are a result of the changes in the local electronic structure of the surfaces of the smaller-sized particles. These effects are considered to be contributing towards an enhancement of the reactivity of silver-nanoparticle surfaces. It has been reported that ionic silver strongly interacts with thiol groups of vital enzymes and inactivates them. It has been suggested that DNA loses its replication ability once the bacteria have been treated with silver ions.[1] Two-dimensional (2D) electrophoresis and protein-identification analyses of the antibacterial action of silver nanoparticles have revealed an accumulation of envelope-protein precursors. Silver nanoparticles target the bacterial membrane, which leads to the destabilization of the plasma-membrane potential and the depletion of the levels of intracellular adenosine triphosphate (ATP), resulting in bacterial cell death.[7]

Bacteria cells grow by a process called binary fission in which one cell doubles in size then splits in half to produce two identical daughter cells.[8] If a bacterial population in an environment is without any growth restrictions by nutrient or metabolic products, the number of bacteria increases as an exponential function of time.[9] Bacterial growth can be depicted generally by the growth curve shown in Figure 1.

There is always a lag phase, where bacteria adapt themselves to the growth media and conditions. During this period, individual bacteria mature but they are not yet able to divide themselves. At the log or exponential phase, individual bacteria reproduce at their maximum rate; there is no restriction by nutrient or metabolic products. During the stationary phase, the growth rate declines and finally growth ceases due to the depletion of nutrients. This phase is reached as the bacteria begin to exhaust the resources that are available to them. At the death phase, bacteria run out of nutrients and begin to die.[9]

The bacterial cell colonies on agar plates are detected by viable cell counts, while in the solution the bacteria concentration affects the optical density of the solution, which can be monitored by optical techniques. Viable cell counts are the counted number of colonies that have developed after a sample has been diluted and spread over the surface of a nutrient medium solidified with agar and contained in a petri dish. Biomass in a nutrient culture medium is usually estimated from the turbidity. Liquid cultures are turbid because microbes have a higher refractive index than water and, thus, they scatter light. The turbidity can be measured by the attenuation of light as a result of scattering from a liquid-culture sample.[9]

Figure 1. Growth curve of E. coli bacteria.
This study is aimed at the synthesis and characterization of silver-doped titania nanoparticles by the sol–gel technique. Experimental results of synthesis and characterization of silver-doped titania particles and their antibacterial properties and kinetics of cytotoxicity are presented. The sol–gel synthesis technique is a promising low-temperature route that provides good homogeneity and control over the concentration and distribution of dopants in the matrix for the preparation of nanocomposites. Both the metallic dopant and the oxide matrix develop simultaneously in this process, which leads to a uniform dopant distribution throughout the nanoparticles. Nanoparticle agglomeration is inhibited in the network matrix, thus limiting the particle size. Annealing of metal oxide composites is an important step since the size, distribution, and number density of the nanoparticles are dependent on the annealing temperature, environment, and duration.[10]

The antibacterial effect of our nanoparticles was tested against Escherichia coli (E. coli) bacteria both in liquid-nutrient growth medium and on agar plates. The antibacterial efficacy was determined by using pure titania nanoparticles and silver-doped titania nanoparticles. The results were compared against those of pure silver nanoparticles. Silver atoms in silver-doped titania nanoparticles continuously interact with the bacterial cells and thus they exhibit an excellent toxicity against the E. coli bacteria.

Antibacterial studies were carried out on silver-doped titania samples that had been annealed in air at 600 °C for one hour. The X-ray diffraction (XRD) patterns of the samples annealed at 600 °C are shown in Figure 2. For the samples with silver-dopant concentrations of 0.0, 0.39, and 0.72 atom % silver, titania was found to be in the purely anatase phase. However, as can be seen in Figure 2, samples doped with 1.01 atom % silver show the beginning of the transformation from the anatase phase to the rutile phase, which is the most stable phase in bulk titania. The recorded XRD patterns were analyzed so as to determine the intensity, position, and width of the peaks. Full width at half-maximum (FWHM) data was used along with Scherrer’s formula to determine the average particle size. Scherrer’s formula is given by:

$$ t = \frac{0.9 \lambda}{\beta \cos \theta} \quad (1) $$

where $\lambda$ is the X-ray wavelength, $\beta$ is the FWHM and $\theta$ is the Bragg diffraction angle.[13] Figure 3 shows the average particle size for anatase titania, as determined by Scherrer’s formula, as a function of doping concentration. Transmission electron microscopy (TEM) images of each of the four samples (0.0, 0.39, 0.72, and 1.01 atom % silver) are shown in Figure 4. Although the size distribution of each of the samples was large, the average particle sizes match with those calculated from the XRD data.

The average particle size of the silver-doped titania nanoparticles decreased as the dopant concentration was increased from 0.0 to 0.72 atom % silver. However, as the dopant concentration increased further to 1.01 atom % silver, the average particle size started to increase. The amorphous-to-anatase-to-rutile phase transformations of titania are known to be nucleation and growth processes during which nuclei that are formed at the grain boundaries grow in size with increasing temperature. The addition of dopant ions causes grain-boundary pinning in which grain growth is limited by the symmetry-breaking effects of the dopant at the boundary, resulting in smaller particle sizes.[10] As the anatase-to-rutile transformation begins, as is observed in the case of 1.01 atom % silver dopant, adjacent anatase particles begin to coalesce by diffusion of atoms to form larger anatase particles before converting to the rutile phase.[12,13]

The optical density of the liquid-nutrient growth medium as a function of time is shown in Figure 5. The increase in the optical density reflects the amount of bacterial cell growth in the sample. Neither the pure titania nor the 0.39 atom % silver-doped titania showed any inhibition effect on the bacteria density at either of the test concentra-
1.5 g of these were added in the flask. However, as the amount of silver-doped titania particles added to the bacterial culture solution was increased to 10 g per 130 mL of solution in each flask, the inhibitory effect on the bacterial growth was appreciable. After an incubation period of 45 min, bacterial growth in the flasks containing 10 g of particles declined sharply. It was observed that in the flasks containing 10 g of 1.01 atom% silver-doped titania particles 30% of bacterial cells survived, whereas for the flasks incubated with 10 g of 0.72 atom% silver-doped titania, there was complete bacterial inhibition. No further changes in the growth patterns were noticed in the remaining observation period.

The effect of 0.39, 0.72, and 1.01 atom% silver-doped titania particles and that of pure silver on colony-forming units (CFUs) of *E. coli* observed on agar plates as a function of particle concentration is shown in Figure 6. As expected, the number of CFUs decreased as the silver-doped titania particle concentration was increased from 0.00 to 50.95 µg cm$^{-2}$. At concentrations above 25.46 µg cm$^{-2}$ there was no notable bacterial growth on the agar plates spread with 0.72 and 1.01 atom% silver-doped titania and pure silver on agar plates.
silver-doped titania and pure Ag samples. However, while the amount of observed CFUs on the 0.39 atom % silver-doped titania samples steadily decreased, even at sample concentrations of 50.95 μg cm⁻², at least 10 % of the bacteria still survived. Therefore, it may be concluded that 0.39 atom % silver-doped titania inhibited bacterial growth but did not have a complete toxic effect on the E. coli bacteria. It is remarkable that pure silver has a lower effect on the bacteria than the two titania samples with the higher doping concentrations. In all the investigated cases, the 0.72 atom % silver-doped samples were more effective than the 1.01 atom % silver-doped samples. The probable reason for samples with a lower concentration of silver being more effective is the difference in the particle sizes of the samples. As can be seen from Figure 3, the 0.72 atom % silver-doped titania sample has a much smaller average particle size (25.7 nm) than the 1.01 atom %-%doped sample (47.1 nm). This can be translated into a larger surface-to-volume ratio with less particle agglomeration (Figure 4c and d). This results in a more efficient bactericidal behavior as more silver atoms are available at the surface of the particle, thus giving a higher antibacterial efficacy against E. coli bacteria.

Bacteria have different membrane structures on which their Gram-positive or Gram-negative classifications are based. The membrane’s structural differences lie in the organization of a key component known as peptidoglycan. Gram-negative bacteria including E. coli exhibit only a thin peptidoglycan layer (ca. 2–3 nm) between the cytoplasmic membrane and the outer membrane. We believe that when the silver-doped titania particles were dispersed in the growth media the silver atoms present in these particles interacted with the bacterial cells and adhered to the bacterial cell walls. The overall charge on the bacterial cell surface at biological pH values is negative, which is due to the excess number of carboxylic and other groups that upon dissociation make the cell surface negative.[14] The bacteria and the silver atoms in the nanoparticles have opposite charges, and these electrostatic forces may be the reason for their adhesion and bioactivity.

Silver-doped titania nanoparticles have been demonstrated to be useful and effective in bactericidal applications and present a reasonable alternative for the development of new bactericides. Silver-doped titania nanoparticles with silver-doping concentrations from 0.0 to 1.01 atom % silver and grain sizes ranging between 26 nm and 56 nm were synthesized via a sol–gel technique and studied against grain sizes ranging between 26 nm and 56 nm. To synthesize silver-doped titania nanoparticles, silver acetylacetonate was used as the silver precursor and Ti(IV) chloride as the titanium precursor. The silver precursor was first dissolved in ethanol then Ti(IV) chloride was added dropwise to this solution. During the addition of the titanium precursor the temperature of the resultant solution increased and gas evolution began. When the addition was completed the resulting yellow-colored solution was allowed to rest in a fume hood until the solution returned to room temperature and all gas evolution stopped. This solution was then placed into a furnace and dried in air at 80 °C for 24 h. The concentration of the silver dopant in various samples was controlled stoichiometrically by varying the amount of the silver precursor dissolved in ethanol. Composite samples with target compositions of 0.0, 0.5, 1.0, and 1.5 atom % silver were prepared. After drying, the samples were annealed in air for one hour at 600 °C. The silver concentrations of the doped titania samples were determined by X-ray photoelectron spectroscopy (XPS) analysis employing Al Kα (hv = 1486.6 eV) excitation radiation. The peak positions were internally referenced to the C 1s peak at 284.6 eV.

Figure 7 shows the XPS survey spectrum of the 0.72 atom % silver-doped titania nanoparticles. As expected, only peaks associated with Ti (2p, 3s, 2p, 2s), O (2p, 2s, 1s), Ag (3d), and C (1s) were observed. The silver concentrations were determined from the Ag 3p peak area from the survey scans and were found to be 0.0, 0.39, 0.72, and 1.01 atom % silver. The inset of Figure 7 shows the high-resolution XPS spectrum of the Ag 3d region of the 0.72 atom % silver-doped titania sample. Both the positions (367.81 and 373.82 eV) and the separation (6.01 eV) of the peaks indicate that silver is present in metallic form (Ag⁰). [15]

The antibacterial efficacy of silver-doped titania nanoparticles, both in liquid-nutrient growth medium and on agar plates was...
studied. *E. coli* bacteria (BL 21), frozen in glycerol at –80 °C and 2xYT media (5 g NaCl, 2.5 g yeast extract, and 5 g tryptone, dissolved in 500 mL distilled water), were used for both studies. Frozen bacteria cells were grown overnight in a nutrient medium to prepare cultures for the solution studies. The prepared cell culture (125 mL) was then added to a nutrient solution (5 mL) containing ampicillin and chloramphenicol (5 μl). These bacteria cultures were then incubated in the dark in the presence of silver-doped titania nanoparticles of each of the dopant concentrations and using two different quantities (1.5 g and 10 g), independently. The bacteria cultures were then incubated in a shaker incubator at 37 °C and 200 rpm for 5.5 h. Shaking provided the bacteria aeration and homogeneity. The bacteria were healthiest at mid log phase (Figure 1). The optical density of solution was measured every 30 min by use of a UV/Vis spectrophotometer at a wavelength of 600 nm. Control flasks were used containing growth medium, bacterial culture, and titania nanoparticles only. Agar was added to the nutrient solution to make plates. After autoclaving the solution, it was poured and solidified in petri dishes. The silver-doped titania samples at concentrations of 0.00, 1.27, 3.18, 6.37, 25.46, and 50.95 μg cm⁻² were mixed with the bacterial solution and spread on the plates. Samples of 0.0, 0.39, 0.72, and 1.01 atom% silver-doped titania, as well as pure silver nanoparticles with an average particle size of 15 nm were used. After completion of spreading, all petri dishes were incubated in the dark at 37 °C for 24 h and the number of CFUs was counted. Experiments were performed in triplicate and sterile conditions were maintained.

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