

Aggregation Study of Ag-TiO₂ Composites

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Received October 21st, 2011; revised November 30th, 2011; accepted December 7th, 2011.

ABSTRACT

Most of the toxicity data presented in the literature are obtained in relatively simple media, like distilled water. The literature reported that nanoparticles agglomerate immediately upon being added to cell culture media and if the agglomerates are used directly in antimicrobial studies, the interpretation of the toxicity results tends to be complicated. Six different molar ratios Ag-TiO₂ composites were synthesized by a reduction method using two different commercial TiO₂ particles as base materials and were used to find the aggregate size in distilled water and Mueller-Hinton Broth, and to obtain the minimum inhibitory concentrations (MIC) against *E. coli* and *E. faecalis*. To evaluate the evolution of the Ag-TiO₂ particle size (z -average) three dilutions of each of the synthesized composites 100 µg/ml, 250 µg/mL and 500 µg/ml were realized in deionized water and Mueller Hinton broth. It was found that Ag-TiO₂ composites increased in size after being diluted in Mueller-Hinton Broth, but once they grew in size, they remained constant for 24 minutes, and after this time, did not affect the MIC for the microorganisms involved.

Keywords: Ag-TiO₂, Composites, Nanoparticles, Particles, Aggregate Size, MIC

1. Introduction

The most common method of producing silver nanoparticles is a chemical reduction of silver salt dissolved in water with a reducing compound [1,2]. Silver nanomaterials exhibit broad-spectrum biocidal activity toward bacteria, fungi, viruses and algae [3].

Several factors have been reported to influence silver nanoparticle toxicity like particle size, shape, pH, ionic strength and the presence of divalent cations and macromolecules [4-8]. The stability of silver nanoparticles also influences toxicity since the formation of aggregates tends to decrease biocidal activity [9,10]. Most of the toxicity data presented in the literature are obtained in relatively simple media like distilled water.

It was found that TiO₂, when irradiated with UV radiation, acted as an antimicrobial [11]. It had been reported that photocatalytic and antimicrobial properties of TiO₂ can be improved by growing particles of a noble metal like Ag, Au or Cu over its surface or inside a matrix [12-14]. Composites of silver coatings over titanium dioxide nanoparticles are used in products to produce antibacterial activity [15].

The literature reported that nanoparticles agglomerate

immediately upon being added to cell culture media and if the agglomerates are used directly in antimicrobial studies, the interpretation of the toxicity results tends to be complicated. Agglomerates of nanoparticles have been shown to exert lower antibacterial effects as compared to well dispersed nanoparticles [16]. It was reported that the presence of proteins within the nanoparticle solution can stabilize the silver nanoparticles against aggregation [16-19].

It was the purpose of this study to evaluate the aggregate size of Ag-TiO₂ composites in deionized water and Mueller Hinton Broth, and analyze the bactericidal activity of the composites using two bacterial strains.

2. Materials and Methods

2.1. Materials

Two commercial TiO₂ particles were used as a base materials, Degussa P25 and DuPont™ Ti-Pure R-902, AgNO₃ (Sigma Aldrich, ACS reagent) was used as a precursor, NaBH₄ (Sigma Aldrich, ACS reagent) were used as a reducing agent and NH₄OH (30% w/w aqueous solution, Sigma Aldrich, ACS reagent), were used to adjust de pH.

2.2. Synthesis of the Composites

Silver nanoparticles were synthesized over the surface of two different commercial TiO₂ particles. The composites with three molar ratios were prepared following a method reported by Nino-Martinez *et al.* and they demonstrated that the nature of the nanoparticles is elemental silver [12].

All preparations started as follows: 0.2 g of TiO₂ particles were dispersed in 100 mL of deionized water by ultrasound for five minutes. Afterwards the 1:10, 1:25 and 1:50 molar ratios Ag-TiO₂ composites were obtained by addition of 0.0425 g, 0.0169 g, 0.00845 g of AgNO₃ respectively. The solution was magnetically stirred for 30 min at pH 7, then predetermined amount of NaBH₄, previously dissolved in deionized water, was added. The pH of the reaction was adjusted to 10 by adding NH₄OH, and magnetically stirred for another 30 min.

2.3. Characterization

The composites obtained were characterized by using Dynamic Light Scattering in a Malvern Zetasizer Nano Zs. Transmission electron microscopy (TEM) analysis were performed on a JEOL JEM-1230 at an accelerating voltage of 100 kV.

2.4. Bacterial Strains

Two bacteria were evaluated, *Escherichia coli* (ATCC 25922) Gram-negative and *Enterococcus faecalis*, (ATCC 29212) Gram-positive.

2.5. Antibacterial Test

The applied antibacterial test was the standard microdilution method (NCCLS-CLSI N7 A7 Vol. 26 No. 2, 1996), which determines the minimum inhibitory concentration

(MIC, as the minimum concentration of tested substance that inhibited the growth of the bacterial strain). The MIC was determined on 96-well microdilution plates. Microorganisms (10⁵ CFU/mL) were exposed to serial dilutions of Ag-TiO₂ particles with Mueller-Hinton Broth (Fluka), and the endpoints were determined when no turbidity in the well was observed after 24 hours of incubation at 37°C. All assays were carried out in triplicate and the Ag-TiO₂ composites were used in the form in which they had been prepared.

3. Results and Discussion

3.1. Characterization

Six different samples were synthesized with Ag-TiO₂ molar ratios 1:10, 1:25 and 1:50, three with TiO₂ P25, and three with TiO₂ R-902 as base materials.

TEM images shows that TiO₂ P-25 had a size between 10 - 70 nm, TiO₂ DuPont R902 had a size 200 - 300 nm. The Ag nanoparticles deposited on TiO₂ P25 surface had a size between 5 to 40 nm, and Ag nanoparticles deposited in TiO₂ R902 surface had a size between 5 to 50 nm. **Figures 1(a)** and **(b)** shows the TEM images for 1:10 Ag-TiO₂ R902 and 1:50 Ag-TiO₂ R902 composites. **Figures 1(c)** and **(d)** shows the TEM images for 1:10 Ag-TiO₂ P25 and 1:50 Ag-TiO₂ P25 composites.

In DLS analyses, Ag-TiO₂ composites present an overall particle diameter (z-average) 377.5, 288, 282, 254.6, 252.4 and 251.5 nm, for samples Ag-TiO₂ R902 1:10, Ag-TiO₂ R902 1:25, Ag-TiO₂ R902 1:50, Ag-TiO₂ P25 1:10, Ag-TiO₂ P25 1:25, Ag-TiO₂ P25 1:50 respectively. The polydispersity index (PDI) was below 0.3 in all cases.

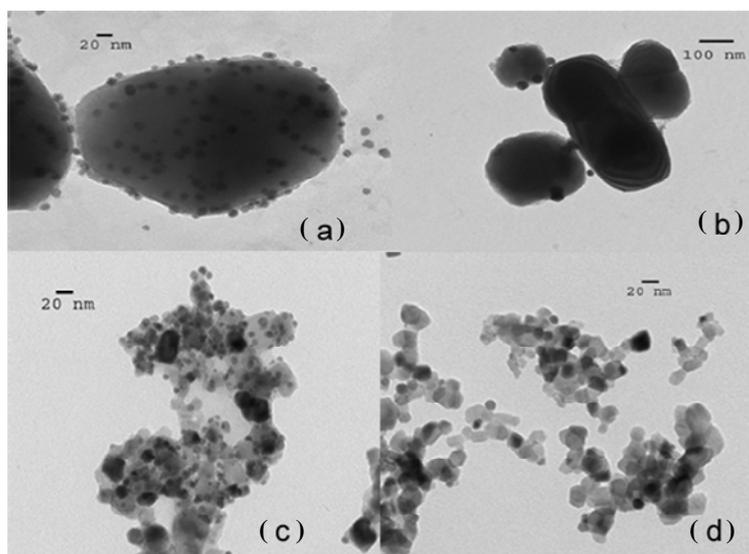


Figure 1. TEM images of Ag-TiO₂ composites (a) 1:10 Ag-TiO₂ R902; (b) 1:50 Ag-TiO₂ R902; (c) 1:10 Ag-TiO₂ P25; (d) 1:50 Ag-TiO₂ P25.

The zeta potential of the same samples were -58.8 ± 6.92 , -47.8 ± 6.03 , -46.78 ± 5.9 , -37.78 ± 4.8 , -49.8 ± 5.89 and -58.8 ± 6.92 . **Figures 2(a)** and **(b)** show the DLS results for 1:10 Ag-TiO₂ R902 and 1:50 Ag-TiO₂ R902 composites. **Figures 2(c)** and **(d)** show the DLS results for 1:10 Ag-TiO₂ P25 and 1:50 Ag-TiO₂ P25 composites.

According to the DVLO Theory, the stability of particle dispersions depends on the balance between attractive and repulsive forces between the particles. With electrostatic stabilization, the zeta potential of the particles provides a repulsive force. In practice if the zeta potential of the particles is higher than 30 mV or lower than -30 mV the dispersion is stable [19].

To evaluate the evolution of the Ag-TiO₂ particle size (z-average) there were realized three dilutions of each of the six synthesized composites 100 $\mu\text{g/ml}$, 250 $\mu\text{g/mL}$ and 500 $\mu\text{g/ml}$ in deionized water and Mueller Hinton broth. It is interesting to note that the particles size remained stable in deionized water for about 24 minutes, 235 - 290 nm for Ag-TiO₂ R902 1:10 (**Figure 3(a)**), 240 - 290 for Ag-TiO₂ R902 1:25, 257 - 285 nm for Ag-TiO₂ R902 1:50, 170 - 190 nm for Ag-TiO₂ P25 1:10, 160 - 200 nm for Ag-TiO₂ P25 1:25 and 190 - 210 nm for Ag-TiO₂ P25 1:50. In contrast the particles became larger in Mueller Hinton Broth but once they grew in size, they remained constant for about 24 minutes, 280 - 360 nm for Ag-TiO₂ R 9021:10 (**Figure 3(b)**), 260 - 360 nm for Ag-TiO₂ R 902 1:25, 260 - 350 nm for Ag-TiO₂ R 902 1:50, 190 - 215 nm for Ag-TiO₂ P25 1:10, 180 - 220 nm for Ag-TiO₂ P25 1:25 and 210 - 240 nm for TiO₂ P25 1:50. The size of de Ag-TiO₂ composites was influenced by the TiO₂ base materials in both media.

Steric stabilization is used for nanoparticle dispersion stabilization, where a stabilizer is added to the dispersion

and it is adsorbed onto the particle surface, preventing them from coming close to one another [16-19]. It is believed that nanoparticles are covered by proteins immediately upon contact with a cell culture media and physiological environment, resulting in a protein arrangement also referred to as protein corona on the particle surface [16] and this protein corona is exchanging with other nearby proteins [20]. Mueller-Hinton Broth is a complex system containing a lot of different proteins. Therefore, it is possible that Ag-TiO₂ composite adsorb proteins from Mueller-Hinton Broth, as indicated by an increase in the particle size (z-average), which could make them become more stable. The identification of the protein corona composition was not the focus of this work.

3.2. Antibacterial Results

Minimum inhibitory concentration values were obtained (**Table 1**). The six different samples have antibacterial activity. The TiO₂ particles present no antibacterial activity. The test was performed on dark and it is reported that TiO₂ particles in dark condition present no antibacterial activity [12,15] which is consistent with our results. The six composites show antibacterial activity without light. We evaluated the antibacterial activity after 24 minutes of exposure of the Ag-TiO₂ composites in Mueller-Hinton Broth, and this did not affect the MIC for the microorganisms involved. According to Lynch and coworkers [20] if the protein corona exchanges with other proteins in the medium faster than the time it takes for the particle to attach to the bacteria surface, then the particle-bacteria interactions will not be greatly affected by the presence of the corona. Therefore the MIC of the composite would not be affected. We found the best results in Ag-TiO₂ 1:10 composite.

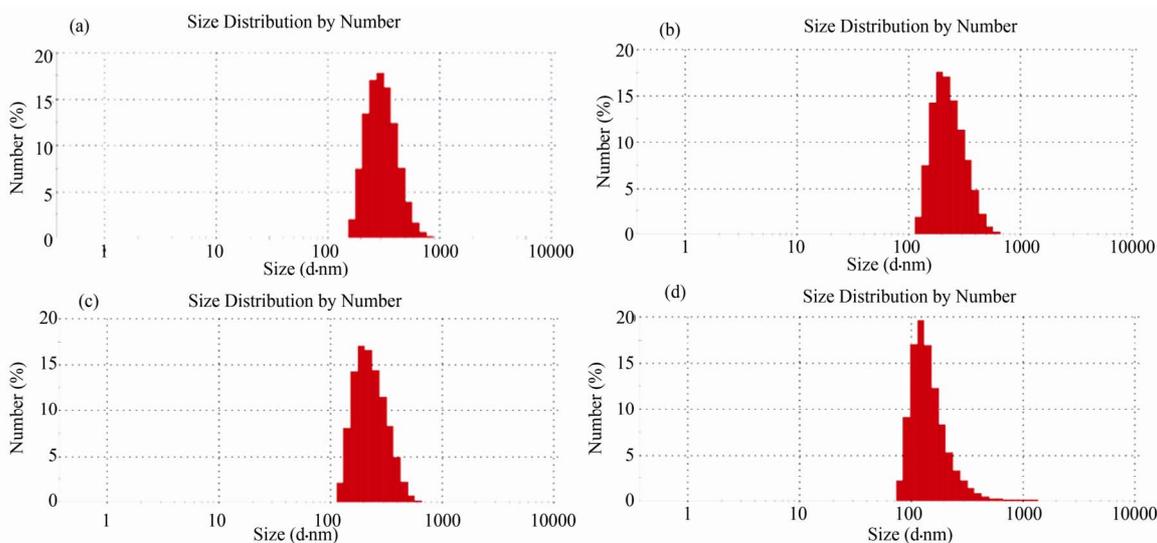


Figure 2. DLS results (a) 1:10 Ag-TiO₂ R902; (b) 1:50 Ag-TiO₂ R902; (c) 1:10 Ag-TiO₂ P25; (d) 1:50 Ag-TiO₂ P25.

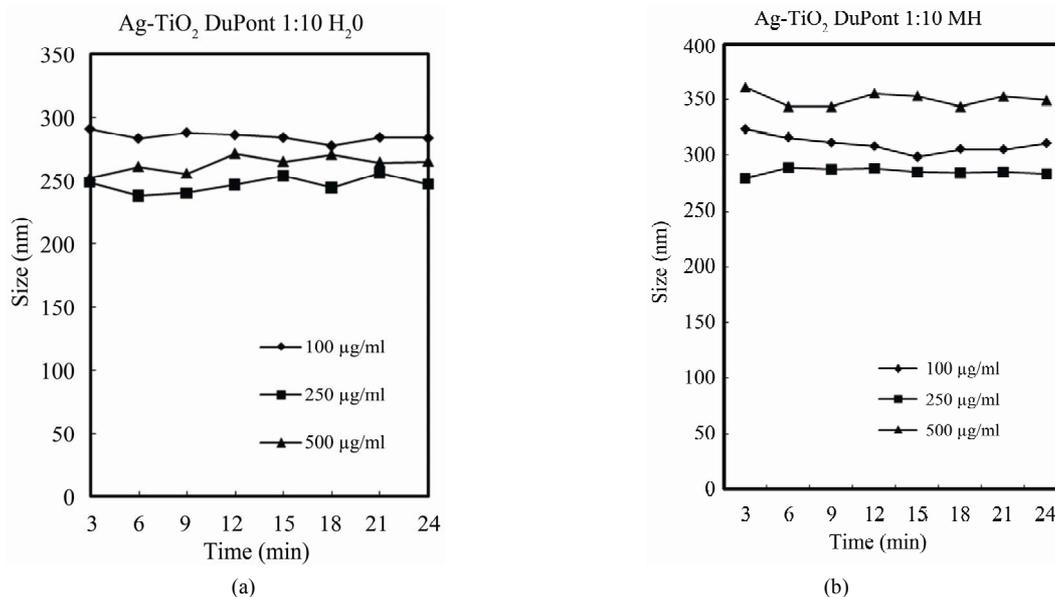


Figure 3. Temporal evolution of the Ag-TiO₂ DuPont 1:10 composite size (z-average): (a) deionized water; (b) Mueller Hinton Broth.

Table 1. Minimum inhibitory concentration of Ag-TiO₂ nanoparticles against *E. coli* and *E. faecalis*.

Material	MIC (µg/mL)	
	<i>E. coli</i> (Gram-negative)	<i>E. faecalis</i> (Gram-positive)
Ag-TiO ₂ 1:10 (P25)	500 ± 0 500 ± 0 ^a	500 ± 0 500 ± 0 ^a
Ag-TiO ₂ 1:10 (R902)	500 ± 0 500 ± 0 ^a	375 ± 144.3 375 ± 144.3 ^a
Ag-TiO ₂ 1:25 (P25)	500 ± 0 500 ± 0 ^a	1000 ± 0 1000 ± 0 ^a
Ag-TiO ₂ 1:25 (R902)	500 ± 0 500 ± 0 ^a	1000 ± 0 1000 ± 0 ^a
Ag-TiO ₂ 1:50 (P25)	1000 ± 0 1000 ± 0 ^a	1000 ± 0 1000 ± 0 ^a
Ag-TiO ₂ 1:50 (R902)	1000 ± 0 1000 ± 0 ^a	1000 ± 0 1000 ± 0 ^a
TiO ₂ (P25)	>2000 ^b	>2000 ^b
TiO ₂ (R902)	>2000 ^b	>2000 ^b

4. Conclusions

Six different molar ratios Ag-TiO₂ composites were prepared and characterized. We found that Ag-TiO₂ composites increased in size with respect to deionized water when be diluted in Muller-Hinton Broth, but once they grew in size, they remained constant for 24 minutes, and did not affect the MIC for the microorganisms involved. The Ag-TiO₂ 1:10 composites showed promising results as an antibacterial agent against *E. faecalis* Gram-positive and *E. coli*. Gram-negative bacteria.

5. Acknowledgements

M. E. Noriega-Treviño, J. E. Morales-Sánchez and J. M. Guajardo-Pacheco would like to thank CONACYT for grant of scholarships.

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