Synthesis titanium dioxide nanoparticles doped with silver and Novel antibacterial activity

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Abstract: Pure and Ag doped TiO$_2$ nanoparticles were synthesis via a hydrothermal process and have anti-bacterial activity were examined against Escherichia coli, Staphylococcus aureus. The effect of Ag doping on TiO$_2$ to rutile phase transformation was investigated by X-ray diffraction (XRD), Scanning electron microscopy (SEM),(EDX), Fourier transform infrared (FTIR) spectroscopy, and (UV). The average size of the TiO$_2$ was in the range of (12.6)nm, and the doped TiO$_2$+Ag had an average size of (12)nm. The antibacterial activity of the TiO$_2$ and TiO$_2$+Ag were evaluated against E. coli , S. aureus using the release of cellular materials method. The results showed that TiO$_2$+Ag nanoparticles as a novel DNA-mediated antibacterial agent. The TiO$_2$+Ag nanoparticles were observed to destroy the bacterial cells by permeating the bacterial nucleic acid and cytoplasmic membrane, resulting in the loss of cell wall integrity, nucleic acid damage, and increased cell-wall permeability.

Keywords: TiO$_2$; TiO$_2$+Ag; Hydrothermal synthesis; Antibacterial activity.

1. Introduction

The titanium dioxide (TiO$_2$) was received great attention in recent years, with regard to its unique photosynthetic activity. Since 1977, following Frank and Bard [1] to confirm that TiO$_2$ can be used in the hydrolysis of cyanide; there is great and growing interest in environmentally, medical and biology applications of titanium dioxide [1, 2]. Scientific researched has confirmed its good effectiveness in dissolving a wide range of pollutants, whether organic or inorganic [3,4]. Photo-oxidation was also demonstrated as a method for purifying some microbes for the first time. White titanium is also used, among other things, as a cosmetic, as a white pigment in cream and powder for the face, and as a sunblock [5]. TiO$_2$ is also present in three main crystalline forms in nature: anatase, rutile, and brookite. Where anatase has been recognized as having the strongest properties in photocatalysis as it is used in multiple applications, it is used as a carrier for particles that are biologically active, an example is silver.

Not much information exists on the biological activity of anatase other than ultraviolet irradiation, which is important for photocatalytic activity. Also, no information is available on the logical biological activity...
of amorphous titanium dioxide, which acts as a vector for silver ions or silver nanoparticles. We also prepared different forms of TiO$_2$ - amorphous and anatase (both doped with silver) and compared their anti-bacterial activity without ultraviolet irradiation. Biological activity has also been shown against positive and negative criminally responsible bacteria, aerobic and anaerobic bacteria, fungi and parasites. Where factors showed that chemotherapy is in contrast to antibiotics that have little effect dynamically [6]. The minerals contained by the bacteria from the medium can be cultured by a physical absorption of ions on the surface of the bacteria (Van der Waals force), through the exchange of ions or connections between the metal ion and a group of large and large molecules related to the elements, for example (oxygen, sulfur and nitrogen). The element silver is toxic to microorganisms and works to poison respiratory enzymes and their components in the work of microbial electron transfer. In addition, it is possible to perform the interaction of silver with structural proteins such as fimbriae - where the protein structures on the surface of the cell are in bacteria, and are responsible for attachment to the artificial surface or cells of microorganisms[7]. Where further research relates to the accumulation of silver element with bacterial cells and its interaction with cytosolic proteins and mitochondrial enzymes and the creation of nucleic acid or Ribonucleic acid. Microorganisms have very weak resistance to silver [8]. It is also possible for bacteria to resist metal ions by reducing the formation of proteins for the outer membrane, and that the system for flow cytometry (active or chemically growing and its delivery to cells in a way is complex inside cells. Bacteria can convert ions into their less toxic form. Silver's very excellent properties make sure it can be applied biologically and medicinally, for example a method synonymous with killing bacteria[9].

Basically, it is the work of a modern method in order to create very small particles of TiO$_2$ saturated with silver, which is a modern type of nanocomposite, as it has been shown to have very good efficacy in resisting microbes without a catalyst such as ultraviolet radiation[10]. In vitro strains (Staphylococcus aureus, gram-positive and gram-negative Escherichia coli) are responsible for many hospital infections, for example: urinary tract infections and wound infections. We also mentioned that the silver nanocomposites impregnated with titanium dioxide can be used in many biological, pharmacy and medical applications without the use of ultraviolet radiation.

2. Materials and Methods

2.1 Titanium Isopropoxide (TIP) [Ti(OCH(CH$_3$)$_2$)$_4$], Acetic acid[CH$_3$COOH], Ethanol [CH$_3$CH$_2$OH], Silver nitrate [AgNO$_3$].

2.2 Preparation TiO$_2$ nanoparticles by hydrothermal method

We used 50 ml of ethanol and it was mixed for 5 minutes, then we used 5 ml of acetic acid with it added to ethanol and stirred with a magnetic stirrer for 5 minutes and using 6.3 ml of TIP we added it using a pipette to a beaker that contained a mixture of acetic acid. And ethanol, which was mixed for a period of (5 min). and after the stirrer (We then transfer it to the autoclave made of stainless steel teflon lined (100 ml) and seal it to heat at 200 °C after the heating process in a period of 10 hours, we cool the autoclave to ambient temperature and this is before the magnetic nanoparticles are taken out, and then we wash them Change with deionized water and ethanol several times, and then dry them over night in a vacuum at 60 °C.

2.3 Preparation TiO$_2$+Ag Nanoparticles by hydrothermal method

To synthesize the TiO$_2$+Ag nanoparticles, a hydrothermal method was also used. This is a similar process to preparing pure nanoparticles except (0.5 wt% Ag) was added to the above homogenized solution with magnetic stirring and then transferred to an autoclave for sterilization.)
2.4. Characterization of TiO$_2$+Ag NPs

The prepared TiO$_2$ nanoparticles (NPs) were distinguished using structural and optical techniques. XRD characterization of NPs in powder diffraction condition was performed using Shimadzu XRD 6000 with a Cu-Kα radiation source at an angle of 2θ = 10°–80°. We studied the molecular vibrations of the samples with an 8000 Series Shimadzu FTIR spectroscopy system, and a scanning electron microscope (SEM; Philips) was used to examine the morphological features of NPs. And also the optical properties are study using Shimadzu UV-Vis absorption spectrum.

2.5 Antibacterial activity of TiO$_2$+Ag NPs

2.5.1 The release of cellular substances

We conducted this investigation in order to release cellular materials present in living organisms that are treated with peptone water and be sterile. Where we sowed sterile peptone water into gram positive and gram negative bacterial strains in order to incubate them for a period of twenty-four hours. Hence, we added TiO$_2$ and TiO$_2$ + Ag NPs that were prepared at a concentration of 50 μg / mL in medium.

After a certain period of time (0, 30, 60, 120 minutes) was eluted from the treatment of the bacteria, the peptone medium was discarded at 3500 cycles per minute in order to obtain the buoyant material and be clear. Where we determined the absorbance of the clear supernatants that we obtained at 220 nm. Where we presented the results in order to investigate the percentage of the absorbed material in each period at 220 nm with a specified time [11].

3. Results and Discussions:

3.1 Structural properties of TiO$_2$ and TiO$_2$+Ag

The figure (1-a) show the XRD patterns of pure samples, and figure (1-b) shows the doped Ag. Whereas, it is possible to catalog the observed peaks according to the quadrangular structure of the anatase phase of TiO$_2$ (JCPDS, No. 861157). As all samples are in an excellent degree of crystallization, a slight increase in crystallization has been observed in silver activators without any change in their phase. Also, no clear peaks were seen showing the presence of silver in the sample, as this indicates that silver does not enter the crystal formation, while it is highly uniformly distributed over the TiO$_2$ surface [12].

It was also concluded that the expansion of the X-ray diffraction peaks of the anesthetic samples is due to the small grain size. As it was explained in Table (1), there was no clear effect of the percentage of silver weight on the crystal size of the particles. We also calculated the sizes of the nanocrystals of the samples using the Debye-Scherer equation. Table (1) shows that the crystalline size of nanotitania has been reduced with silver doping.

| Samples             | Crystallite size D$_{XRD}$ (nm) |
|---------------------|---------------------------------|
| Pure TiO$_2$        | 12.6 (nm)                       |
| 0.5wt% TiO$_2$+Ag   | 12 (nm)                         |
Table 1. The particle sizes of the samples

| Sample          | Particle Size (nm) |
|-----------------|--------------------|
| TiO$_2$         | 11–23              |
| TiO$_2$+Ag      | 10–17              |

Figure 1-a. Shows the XRD patterns of Pure TiO$_2$ nanoparticles

Figure 1-b. Shows the XRD patterns of the sample TiO$_2$+Ag nanoparticles

3.2 Morphological properties analysis

Scanning Electron Microscopy The size and morphological features of the prepared NPs were further analyzed using scanning electron microscopy. A scanning microscope image of the same batch of samples is demonstrated in Figure(3) and provides an indication of the achievement of a large quantity of uniform NPs (both TiO$_2$ and TiO$_2$+Ag), with a mean size of about 11–23 nm for TiO$_2$, and 10–17 nm for TiO$_2$+Ag. Figure (3) show SEM micrographs of the samples that are pure and of the anesthetized samples using Ag, the visibility of many particles that are spherical, and strong X-ray peaks that are associated with Ti Kα and Ag Kα were found in the EDX spectrum. Of Ag in the TiO$_2$ matrix according
to the silver atomic ratio of each sample. It showed the uniform nature of particles with no change in particle morphology due to Ag doping[13].

![Figure 2](image1.png) ![Figure 3](image2.png)

**Figure 2.** SEM Micrographs of (a) Pure TiO$_2$, (b) TiO$_2$+Ag nanoparticles.

**Figure 3.** EDX images (a) Pure TiO$_2$, (b) TiO$_2$+Ag

### 3.3 Optical properties of TiO$_2$ and TiO$_2$+Ag

Figure (5) demonstrates optical absorption spectra of TiO$_2$ and TiO$_2$+Ag nanoparticles. The band gap will decreases due introducing silver into TiO$_2$ as a Nobel metal .The optical properties of TiO$_2$ and silver doped TiO$_2$ nanoparticles are determined from absorbance measurements in the range 200-400 nm (UV-VIS-NIR) by spectrophotometer. Compared to the pure rutile TiO$_2$, with the silver-doped sample exhibits a significantly enhanced absorption in the Ultraviolet range (265-305 nm) and shift of absorption band to lower wavelength (higher energy) owing to the Plasmon resonance effect of silver nanoparticles. The Absorbance spectra of calcined TiO$_2$ and silver doped TiO$_2$ which indicates that there is increase in the
absorbance and showed a shift in optical absorption in Ultra violet region as a result of incorporation of silver nanoparticles into TiO$_2$ matrix. In the composite of TiO$_2$+Ag nanoparticles it is observed that the peak at 280 nm decreases as the accumulated electrons in the TiO$_2$ are transferred to Ag nanoparticles[14]. This phenomenon is observed due to the reduction of average particle size from silver doped TiO$_2$ ratio TiO$_2$ pure which facilitates the transition of electrons.

![Figure 4. Show (a) pure TiO$_2$ and (b) TiO$_2$+Ag nanoparticles](image)

3.4 Chemical properties of TiO$_2$ and TiO$_2$+Ag

The infrared spectra of all the synthesized TiO$_2$ powder in the range 4000-400 cm$^{-1}$ wave number are shown in figure(6) the vibration bands at 3385 cm$^{-1}$ and 1628 cm$^{-1}$ are characteristic of the OH group adsorbed to the surface of TiO$_2$. In addition, the band in the region 650-500 cm$^{-1}$ which is the characteristic vibrational modes of TiO$_2$ (stretching mode of Ti–O bond) is also detected. Ag doping influenced on FTIR spectra that it reduced a concentration of photo-generated OH radicals on powder surfaces[15].
3.5 Antibacterial activity of TiO₂ and TiO₂+Ag

3.5.1 The release of cellular materials

The absorbed cellular materials, which were sorted by the living organisms that were treated by the nanoparticles at 220 nm, were shown in figure (8), and we measured the areas in which the inhibition occurred after the organisms were exposed to the nanoparticles (TiO₂ and TiO₂ + Ag). And show them in figure (7). Whereas, in this method, the OD of the culture medium is linked at 220 nm over time, and (TiO₂ + Ag) has a high potential to cause cell membrane damage to living organisms if compared with TiO₂. All the results also showed that (pure TiO₂ and TiO₂ + Ag) were the main causes of increasing the permeability in the cytoplasmic membrane of bacteria. In addition, the cytoplasmic membrane of bacteria acts as a barrier to prevent leakage of ions [16,17].
Figure 6. Antibacterial activity of TiO$_2$+Ag nanoparticles against *S. aureus* and *E. coli*: (1) Negative control; (2) Pure TiO$_2$; and (c) TiO$_2$+Ag.
Figure 7. Effects of Ag-TiO$_2$ nanoparticles in bacterial cellular materials release: (1) Negative control; (2) Pure TiO$_2$; and (3) TiO$_2$+Ag.

Conclusions

We prepared pure TiO$_2$ and Ag doped TiO$_2$ nanocrystals in anatase phase by hydrothermal method. The study by XRD and SEM analyzes demonstrated that activators of Ag reduced the crystal size of pure TiO$_2$ of (12.6 to 12 nm) due to increase in surface area without any phase change. Also the optical spectrum is measured by UV-Vis spectroscopy observes happening decreases in the band gap TiO$_2$ after doping by use Ag. And Ag doping on the FTIR spectra caused a reduction in the concentration of the hydroxide radicals that are both emergent and shown on the surfaces of the powder. It was also concluded, that TiO$_2$ + Ag NPs showed a greater inhibition activity on cultured organisms compared to TiO$_2$, as nanoparticles were more effective on Staphylococcus aureus than E. coli. Consequently, results were obtained
indicating that a successful preparation and characterization of TiO$_2$ and TiO$_2$ + Ag nanoparticles were obtained, which showed potential antibacterial activity against *S. aureus, E. coli*.

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