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Synthesis, characterization of Ag/TiO_2 nanocomposite: Its anticancer and anti-bacterial activities

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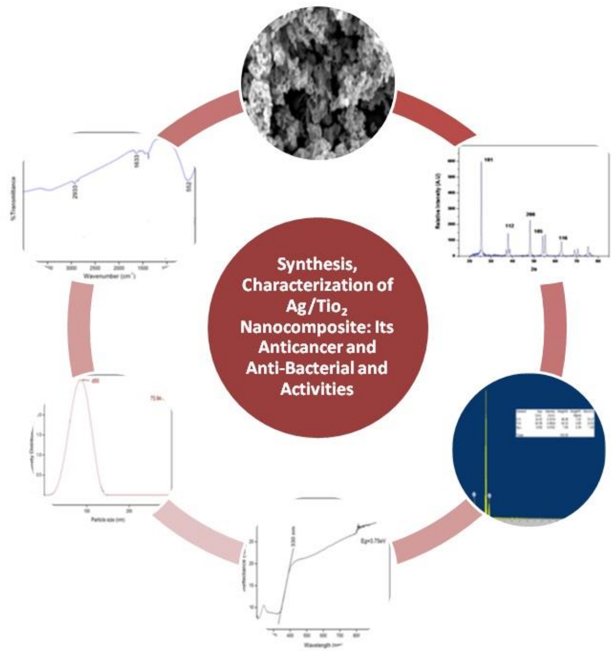
Graphical abstract



Abstract

TiO_2 nanomaterials have gained much interest due to their importance of wide area application. In this study, TiO_2 nanocomposites were modified with Ag NPs by the stober method. The synthesized nanocomposites were characterized by XRD, FTIR, scanning electron microscope (SEM) and energy dispersive X-ray spectroscope (EDAX), UV-DRS. Antibacterial activities were determined against the bacterial pathogens as well as *in vitro* cancer cell lines and significant of nanocomposite's anticancer activity were analyzed. Ag/TiO_2 nanocomposite possesses efficient cytotoxic activity towards the A549 cell lines and can be used further for *in vivo* trials. Ag/TiO_2 nanocomposites showed good antibacterial activity against different strains of bacteria *Staphylococcus aureus, Shigella flexneri* and *Bacillus sp*. The zone of inhibition was found to be higher in synthesized Ag/TiO_2 nanocomposites as compared to standard antibiotic streptomycin.

Keywords: Nanomaterials, TiO_2, bacteria, strains, antibacterial.

1. Introduction

In recent times, industrial wastewater treatment has become a critical issue due to its damaging and overwhelming effects on our environment and the environment.

For the past few decades, much research has been conducted to explore the nanomaterial which focuses on synthesis methods, characterization, components, and applications in various interdisciplinary fields. Nowadays, nanomaterials are widely used in medical fields such as drug delivery, implants, coating, antibacterial activity etc. Silver-based nanomaterials are attractive to the researchers due to their antibacterial activity, as they generally possess excellent mechanical properties and can release heavy metal ions to cause damage to bacterial DNA (Goudarzi *et al.*, 2016; Muflikhun *et al.*, 2017; Amiri *et al.*, 2014; Hajipour *et al.*, 2012). Silver and titanium dioxide nanomaterials have been explored by many researchers as they can be synthesized through various methods such as Sol-Gel, Micelle, and Inverse Micelle, Hydrothermal, Solvothermal, Electrode position, Chemical Vapor Deposition, Physical Vapor Deposition, HVPG, and Sonochemical (Zhang *et al.*, 2017; Zheng *et al.*, 2018; Besinis *et al.*, 2014; Muflikhun *et al.*, 2017).

Titanium dioxide containing silver synthesized by different methods with excellent photo catalytic activities can efficiently be used to degrade R6G dyed, water purification, medical applications virus inactivation, self-cleaning and food safety/sensing (Seery *et al.*, 2007; Chin *et al.*, 2011; Xiong *et al.*, 2011; Samiei *et al.*, 2016; Liga *et al.*, 2011; Laohhasurayotin and Pookboonmee, 2013; Chau *et al.*, 2007). The studies reported that silver-titanium dioxide nanocomposites materials are widely known for their anti-pathogenic ability. As an antibacterial agent with efficacy against various strains of Gram-positive bacteria (B. subtilis, *S. aureus,* MRSA), Gram Negative bacteria (*E. coli, K. pneumoniae, P. aeruginosa*), and fungi (*C. albicans*) (Lungu, 2014)

Nanomaterials containing Ag are well-known as an effective antibacterial activity. The extract of Ag ion is the most biologically efficient viral study of bacterial, and fungi. Metallic Ag nanoparticles catch the attention of great interest in recent years on clarification of their omission of antimicrobial competence.

Some studies also indicate that antibacterial activity increased with increase concentration of TiO2-SiO2-Ag nanocomposites and the elevated concentrations were able to bring about complete inhibition of bacterial cells.

This study aims to explore and reveal the mechanical properties of silver and titanium dioxide nanocomposites materials with anti-bacterial capabilities. In this current study, we used the stober method to synthesize Ag/TiO_2 nanocomposites.

2. Materials and methods

2.1. Synthesis of Ag/TiO_2 nano composite

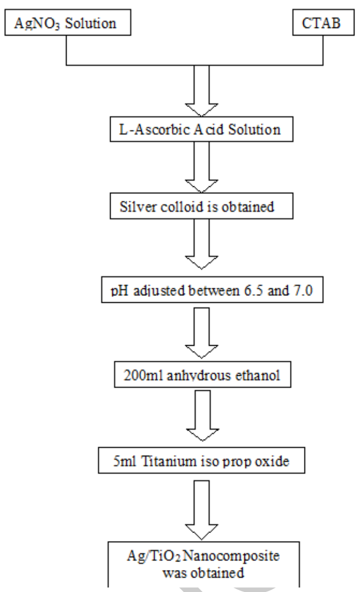


Figure 1. Synthesis of Ag/TiO_2 nanocomposite.

The silver colloids were synthesized by chemical reduction method in alkaline environment using L-Ascorbic Acid as reducing agent, N, N, N, N- Cetyl Trimethyl Ammonium Bromide (CTAB) as capping agent and Silver Nitrate solution as precursor. The CTAB is added to silver nitrate solution and stirred for half an hour and L-ascorbic acid solution is added to reduce the silver nitrate solution to silver nanoparticles. The pH of the prepared silver colloid is adjusted to 6.6-7.0 by adding ammonium hydroxide solution. Titania was grown onto the surface of silver nanoparticles to form Ag/TiO_2 nanocomposite by means of the Stober method. The silver colloid is mixed with 200ml of anhydrous ethanol and allowed to stir for an hour. After stirring for an hour, 5ml of titanium iso prop oxide was added. The solution containing silver colloid, ethanol and titanium iso prop oxide is continuously stirred for 24h. The solution after 24h of continuous stirring is dried in the oven at 60^{o}C to obtain the Ag/TiO_2 nanocomposite. If required the obtained nano powder can be sintered at 400^{o}C for 4h to make it further dry and to remove moisture content. The typical conditions used in this work are: [$AgNO_3$] = 0.1M, [$C_6H_8O_6$] = 0.1M, [$C_{19}H_{42}BrN$] = 0.1g, [NH_4 $(OH)_2$] = 0.5M. The growth condition used here is: [C_2H_5OH] = 200ml and [titanium isopropoxide] = 5ml. Figure 1 shows the schematic of the synthesis of Ag/TiO_2 nanocomposite (Yang *et al.*, 2012; Skorb *et al.*, 2008; Liu *et al.*, 2019; Sikora *et al.*, 2017).

3. Results and discussion

3.1. X-Ray diffraction studies

The X-ray diffraction measurements were carried out to study the crystalline quality and crystallographic orientation of the Ag/TiO_2 nanocomposites. Figure 2 shows the XRD pattern of Ag/TiO_2 and exhibits characteristic peaks at 2θ=25.37°, 48.12°, 53.97° and 55.10°, corresponding to (101), (200), and (105) planes, respectively, which belong to typical anatase-TiO_2 materials (JCPDS no.89–4203). The sharp and narrow diffraction of the peaks demonstrated that all Ag/TiO_2 was of good crystalline in quality. This X-ray diffraction data is used to determine dimensions of unit cell, crystal structure, texture coefficient, standard deviation, crystalline and the calculated crystalline parameters are listed in Table 1. The grain size of the crystallite is calculated using Scherrer's formula and the average crystallite size is calculated from the broadening of the corresponding sharp peaks in Ag/TiO_2 and found to be 37.66 nm (Subash *et al.*, 2013).

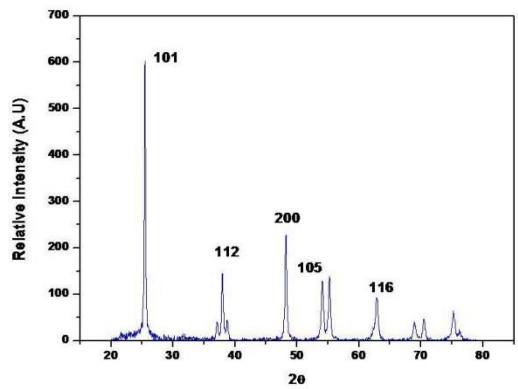


Figure 2. X-Ray Diffraction patterns of Ag/TiO_2 Nanocomposites.

3.2. FTIR analysis

Figure 3 shows the FT-IR spectrum of the synthesized samples. The peak 2933 cm^{-1} shows the CH_2 bending vibration. Wave number 1633 cm^{-1} indicates the presence of C=C stretching vibrations. The peak value 552 cm^{-1} in the Ag/TiO_2 sample indicates the Ti-O stretching vibrations (traversa *et al.*, 2001).

3.3. SEM and EDX analysis

The morphological purpose of Ag/TiO_2 , SEM analysis was made and it recognized that the samples are spherical in nature. Figure 4 showed that Ag/TiO$_2$ nanoparticles were with a number of aggregates respectively and it revealed that the nanoparticles are spherical in nature. The elemental dispersive analysis (EDX) completes the chemical profile of the Ag/TiO$_2$, the chemical profile was noted as 60.58 % of titanium and 10.58% of Ag.

Table 1. Structural Properties of Ag/TiO$_2$ Nanocomposites

Ag/TiO$_2$	2 θ		D		Crystal Size (nm)	Lattice constant Aº	
	(Std)	(Obs)	(Std)	(Obs)		JCPDS 89-4203	(Obs)
101	25.304	25.475	3.517	3.497	34.74	a=3.785	a = 3.810
200	48.037	48.256	1.892	1.886	40.61	c= 9.514	c= 9.112

Table 2. Antimicrobial activity of Ag/TiO$_2$ treated against bacterial pathogens

Test organisms	Zone of inhibition in millimeter (in diameter)	
	Ag/TiO$_2$	Standard Streptomycin 30µg
Staphylococcus aureus	24	34
Shigella flexneri	32	29
Bacillus sp	38	32

Table 3. Anticancer effect Ag/Ti O$_2$ on A549 cell line

S.No.	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.062	12.50
2	500	1:1	0.107	21.57
3	250	1:2	0.165	33.26
4	125	1:4	0.204	41.12
5	62.5	1:8	0.247	49.79
6	31.2	1:16	0.316	63.70
7	15.6	1:32	0.375	75.60
8	7.8	1:64	0.413	83.26
9	Cell control	-	0.496	100

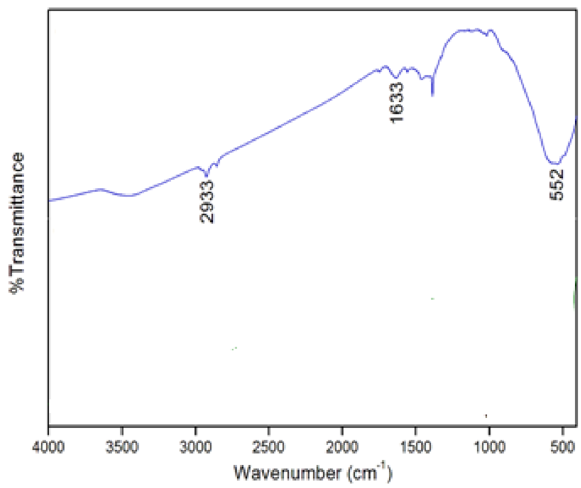


Figure 3. FTIR spectrum of Ag/TiO$_2$.

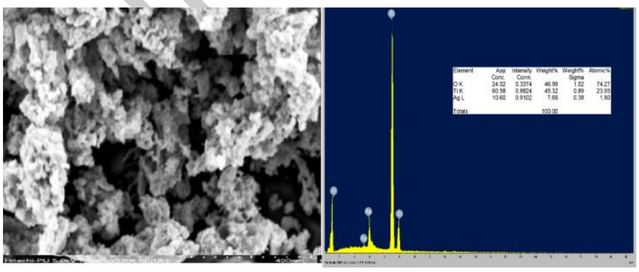


Figure 4. SEM and EDAX analysis of Ag/TiO$_2$.

3.4. Analysis of particle size by particle size analyzer: PSA

Figure 4 shows the particle size distribution of Ag/TiO$_2$. The dispersive medium for the sample is prepared by tri ethanolamine and deionized water in the ratio 1:3. 3g of prepared samples were dispersed in the dispersive medium by **sonication of the** mixture for duration of 10 min. The particle size measurement increases with increasing time period and temperature conditions. From the Figure 5, it is found that the particle size distribution of Ag/TiO$_2$ 75.94 nm.

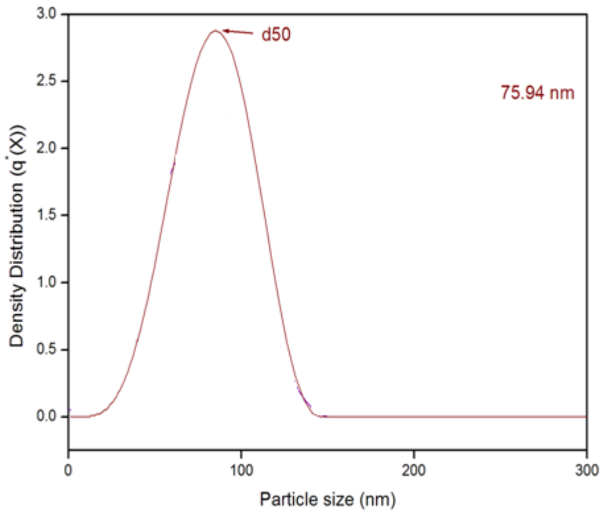


Figure 5. Particle size distribution of Ag/TiO$_2$.

3.5. Absorption spectrophotometer (UV-DRS)

Figure 5 and show the UV-DRS analysis of Ag/TiO$_2$. UV-DRS reflectance mode measurement is one of the powerful techniques in determining the optical properties of powder form of samples. It measures the wavelength of light when it reflects the UV light rays. The reflection band of Ag/TiO$_2$ is obtained as 330 nm from Figure 6 and the band gap energy was calculated to be 3.75 eV and also confirmed that the synthesized nanocomposites are wide band gap semiconducting materials.

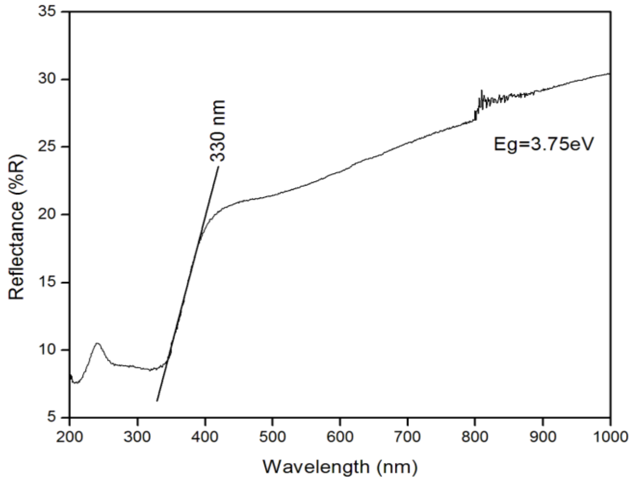


Figure 6. UV-DRS analysis of Ag/TiO_2.

3.6. Antibacterial activity of Ag/TiO nanoparticles

The antibacterial activity of Ag/TiO nanoparticles was analyzed in vitro using a well diffusion method. The bactericidal effect was confirmed in contradiction of gram negative *bacteria S. flexner*i and gram positive pathogens like *S. aureus* and *Bacillus sp.* The antibacterial activity of Ag/TiO_2 nanocomposites was given in Table 2. Previous studies reported that TiO_2 nanotubes own antimicrobial accomplishment, and the device involves a prompt and reversible substantial first phase, and a cellular second phase (Kaviyarasu *et al.*, 2017; Angel Ezhilarasi *et al.*, 2016; Kaviyarasu *et al.*, 2015; Kaviyarasu and Devarajan, 2013; Magdalane *et al.*, 2017; Kaviyarasu *et al.*, 2016). Another study compared the antibacterial activity of Ti substrate with Ti coated with EG, PEG 400, PEG 600 and PEG 1000 against *E. coli*. Among these, Ti substrate showed little activity (Kaviyarasu *et al.*, 2017). This reveals that Ti substrate has not much antibacterial activity. Studies about the bactericidal activity of ZrO_2 nanoparticles disclosed that they exhibit greater activity against *S. aureus* and *B. subtilis* (Fuku *et al.*, 2016). These studies reveal that the Ag/TiO_2 nanocomposites activity towards bacteria leads to an inhibition zone even greater than standard antibiotics (Chanderiya *et al.*, 2021; Pandey *et al.*, 2020; Pandey *et al.*, 2020).

Solvent used : DMSO (Dimethyl Sulphoxide) **Standard used** : Streptomycin 30 µg.

3.7. Cytotoxicity behavior

The significant candidate of anticancer drugs is the ability to induce tumor cell apoptosis. The basic cytotoxic assay to assess anticancer activity is the MTT assay. In the present study, various Ag/TiO_2 nanocomposites were treated against A549 cell lines. The percentage of cell viability was gradually decreased against increasing concentration of nanoparticles Table 3 Ag/TiO_2 nanoparticles showed obvious results against A549 cell lines. The nanoparticles showed good activity against A549 cells even at lower concentration like 7.8 µg/ml. The IC_{50} value of Ag/TiO_2 nanoparticles is 49.79. This indicated that nanoparticles showed intense activity against the A549 cell line. A recent study (Chanderiya *et al.*, 2021) showed the anticancer activity of TiO_2 nanoparticles against A549 cells, which confirmed that cell viability decreased only after 72 hrs of treatment. But in the present study, the results illustrated that Ag/TiO_2 nanoparticles impart cytotoxic effects towards the A549 cell line with 24 hrs treatment. It is evident that the Ag/TiO_2 nanocomposite possesses efficient cytotoxic activity towards the A549 cell lines. This indicated that Ag/TiO_2 nanocomposite can be further tested for *in vivo* trials.

4. Conclusions

Ag/TiO_2 nanocomposites have been successfully synthesized by the Stober method. The XRD analysis confirmed that synthesized nanocomposites are crystalline in nature and the average calculated crystallite size was found to be 37.66 nm. UV-DRS characterization of the prepared nanocomposites shows that the band gap energy of Ag/TiO_2 is 3.75 eV and confirms that the prepared nanocomposites is a wide band gap semiconducting material. The synthesized NPs showed great activity against the bacterial pathogens as well as *In Vitro* cancer cell lines. An Ag/TiO_2 nanocomposite possesses efficient cytotoxic activity towards the A549 cell line and can be used further for *in vivo* trials. Ag/TiO_2 nanocomposite showed good antibacterial activity against different strains of bacteria *Staphylococcus aureus, Shigella flexneri* and *Bacillus sp*. The zone of inhibition was found to be higher in synthesized Ag/TiO_2 nanocomposite as compared to standard antibiotic streptomycin. Foregone discussion showed that these NPs can be considered further for *in vivo* studies

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