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RESEARCH ARTICLE

SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF Ag-DOPED TIO_2 NANOFIBERS VIA ELECTROSPINNING.

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Abstract

Pure TiO_2 and Ag-doped TiO_2 nanofibers were synthesized by electrospinning of a sol–gel consisting of titanium isopropoxide, silver nitrate and polyvinylpyrrolidone (PVP). Calcination of the electrospun mats at 500 ^oC led to produce well morphology Ag-doped TiO_2 nanofibers. The heat treated nanofibers are characterized by X-ray diffraction (XRD), Scanning electron microscopy (SEM) and Energy Dispersive X-Ray spectroscopy (EDX), etc. XRD analysis of calcined nanofibers confirms the anatase phase formation of pure and doped TiO_2 nanofibers. The antibacterial activity of TiO_2 and Ag-doped TiO_2 nanofibers was investigated against both gram positive (*Staphylococcus aureus*) and gram negative (*Pseudomonas aeruginosa*, *Escherichia coli*) bacteria. From the results, it was observed that TiO_2 shows the bactericidal property against the *S. aureus, E. coli, and P. Aeruginosa* bacteria which is enhanced by the presence of silver.

Introduction:-

Titanium dioxide (TiO_2) is a semiconducting oxide which is useful in many industrial applications due to its non-toxic nature, relative abundance, resistance to corrosion, photostability and low cost [1]. New physical and chemical properties arise when the size of TiO_2 becomes smaller down to the nano scale. Several methods have been employed for the preparation of TiO_2 nanostructures because of its wide-ranging potential applications. The main advantage of the nanostructural materials is the high surface to volume ratio, and possesses special characteristics due to the axial ratio aspect [2-4]. Among the one-dimensional nanomaterials, nanofibers have novel and improved features because of the long axial ratio; consequently good research has been done by the fabrication of various types of TiO_2 nanofibers/nanotubes [5-7]. Recently, metal-doping strategy has been introduced to enhance the physical and chemical properties of TiO_2 nanostructures [8-11].

The chemical modification of TiO_2, by doping the lattice with transition-metal ions, has proved efficient in the propagation of the absorption threshold towards the visible region [13]. Silver is one of the good doping materials among the transition-metals because silver particles are known to act as better electron traps, preventing photo-generated charge carrier recombination and thereby facilitating electron excitation by creating a local electrical field [12]. In addition, silver-doped titanium dioxide nanomaterials are known to their effects on the improvement of photocatalytic activity of TiO_2 and antibacterial activity [14]. These results indicated that Ag/TiO_2 composted

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materials may contain the advantages of both TiO_2 and silver: silver has a higher antibacterial activity, and TiO_2 can last longer, and able to be controlled by visible light [15].

In this study, pure and doped-TiO_2 (Ag-TiO_2) nanofibers were prepared via a combination of sol-gel and electrospinning methods. The prepared nanofibers were characterized by X-ray diffraction (XRD), Transmission electron microscopy (TEM), Energy Dispersive X-Ray Sprectroscopy (EDX). Furthermore, the antibacterial activities of the pure TiO_2 and Ag-doped TiO_2 nanofibers against Gram-positive *Staphylococcus aureus (S. aureus)*, and Gram-negative *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*) bacteria was investigated under visible light.

Materials and Methods:-

Titanium tetraisopropoxide (TIPP) (98% purity), poly(vinylpyrrolidone) (PVP) (M.Wt. = 3,60,000 and glacial acetic acid were purchased from Sigma-Aldrich and Merck. All the reagents used were of analytical grade without any further purification. Muller-Hinton agar media was obtained from Hi-Media, India. Silver nitrate (purity > 99 %) used as a dopant in the TiO_2 nanofibers and AHL (Acyl Homoserine Lactone) were obtained from Sigma. All the glassware's were washed twice with deionized water and stored in air-tight container until use. *S. aureus* MTCC 7443, *E.coli* MTCC 739 and *P. aeruginosa* MTCC 2297 were obtained from Microbial Culture Collection, Institute of Microbial Technology, Chandigarh, India.

Preparation of pure and Ag-doped nanofibers:-

In a typical procedure, solution-A was prepared by mixing 8 mL of titanium tetraisopropoxide with 16 mL of acetic acid and 16 mL of ethanol under stirring at room temperature. Solution-B was prepared by adding 10 wt% PVP and 0.5 wt % $AgNO_3$ to 40 mL of ethanol under stirring at room temperature. After 60 min., solution-A was added to solution-B drop wise and stirred for 24 h. This precursor mixture was loaded into a glass syringe equipped with a 21 G stainless steel needle which was connected to a high voltage supply (30 kV). A voltage of 20 kV was applied between the needle and the collector. The distance between the needle and the target was 12 cm. A flow rate of 50 μL/min was maintained using a syringe pump. Electrospinning process was carried out at the room temperature. The obtained nanofibers were left exposed to air for approximately 1 hour to allow complete hydrolysis of titanium tetraisopropoxide and then subjected to calcination at a temperature of 500 ^oC for 2 hours to remove residual organic chemicals and PVP.

Antibacterial activity of Nanofibers:-

In vitro antibacterial activity assay was carried out by using Kirby-Bauer method. The anti bacterial activity of both pure TiO_2 and Ag-doped TiO_2 nanofibers was screened by disc diffusion method by using Muller Hinton Agar (MHA). 20 mL of MHA were prepared and poured into sterile petri dishes and then allowed to solidify for 10 min. The three different bacterial pathogenic microorganisms such as *S. aureus, E. coli, and P. aeruginosa* were uniformly spreaded on the surface of MHA plate at a concentration of 10^5 to 10^6 CFU/mL. The different concentrations of pure and Ag-doped TiO_2 nanofibers (5, 10, 15, 20 μL/disc) were loaded on 6mm sterile disc and incubated at 37^oC for 24 hours in the visible light. After the period of incubation, the formation of inhibition zones around the disc was measured using high antibiotic zone scale.

Characterization of TiO_2 nanofibers:-

Surface morphology of nanofibers was studied by field-emission scanning electron microscope (FESEM, JEOL JSM-6701F, Japan). The phase and crystallinity were characterized by using Rigaku X-ray diffractometer (Rigaku Co., Japan) with Cu Kα (λ = 1.54056 A^o) radiation over a range of 2θ angles from 20^o to 80^o and elemental analysis of the nanofibers were studied by energy dispersive X-ray spectroscopy (EDX) (EMAX Energy EX-200, HORIBA Ltd., Japan).

Results and Discussion:-

The XRD patterns of TiO_2 and Ag-TiO_2 nanofibers which were calcined at 500°C in air for 2 hours are shown in Figure 1. As shown in both spectra, the results confirm the formation of pure anatase form of titanium dioxide. The strong diffraction peaks at about 25.6(101), 38.1(004), 48.5(200), 54.7(105), 55.3(211), 63.0(204), 68.9(116), 70.8(220) and 76.1(215), respectively indicate the formation of the anatase phase (JCPDS 71- 1166). In the case of the nanofibers obtained from calcination of Ag-doped nanofibers, additional peaks at 2θ values of 38.11^o, 44.29^o, 64.43^o and 77.48^o corresponding to the crystal planes (111), (200), (220) and (311) respectively confirm presence of silver metal in addition to TiO_2 peaks [16]. From the XRD profile, it appears that Ag doping does not influence the crystalline structure of TiO_2. Figure 2 shows the FE-SEM images of the TiO_2 nanofibers before calcination (Figure 2 (a)) and after calcination at 500 ^{o}C (Figure 2 (b)). Figure 3(a) shows the SEM image of Ag-doped TiO_2 nanofibers after calcination. A difference between the surface morphology of pure TiO_2 and Ag-doped TiO_2 was observed. It was presumed that the doping with Ag brought the surface roughness. Figure 3(b) shows the EDX spectrum of Ag-doped TiO_2 nanofibers after calcination indicates the presence of Ti and Ag elements. There are no impurities present in the sample, which indicate the absence of carbon element originating from PVP after heat treatment.

Antibacterial activity of TiO_2 (doped and undoped) nanofibers:-

The bactericidal activity of the crude and annealed samples of TiO_2 and Ag-doped TiO_2 were investigated against Gram (+ve) and Gram (−ve) bacteria, as presented in the Table 1 and 2. The *in vitro* antibacterial activity of the nanofibers was tested against human pathogenic bacteria such as *S. aureus, P. aeruginosa,* and *E. coli* using disk diffusion method. The 18 mm clear inhibitory zone appeared around 20 μL Ag-doped TiO_2 nanofibers against *E. coli* after incubation for 24 hours followed by *P. aeruginosa* (17 mm), and *S. aureus* (14 mm) suggesting that both the synthesized pure TiO_2 and Ag-doped TiO_2 nanofibers showed phenomenon bactericidal effect. Based on the zone of inhibition analysis, shown in Table 2, it was observed that when the media was subjected to Ag-doped TiO_2 nanofibers, inhibition zone of *S. aureus, P. aeruginosa,* and *E. coli* was increased dramatically.

The antibacterial activity of annealed TiO_2 nanofibers is slightly more than crude TiO_2 nanofibers, because annealing at 500 °C converts the amorphous phase of the nanofibers to pure anatase phase, and shows an indirect band gap of 3.2 eV [11]. Due to the indirect band gap of anatase phases present in the annealed TiO_2 nanofibers, they showed enhanced antibacterial activity than crude TiO_2 nanofibers. However, the pure TiO_2 (crude and annealed) nanofibers showed less photocatalytic activity, while doping with silver improves the efficiency under visible-light irradiation. When compared with the pure TiO_2 nanofibers, the antibacterial activity of Ag-doped TiO_2 nanofibers is high and also they showed enhanced antibacterial activity on Gram-negative bacteria. Gram-positive bacteria have more negatively charged peptidoglycan than Gram-negative bacteria in the cell wall, and due to this interaction, gram-positive bacteria may allow less Ag^{+} to reach the plasma membrane than gram-negative species [17]. This is attributed to the high antibacterial activity of Ag-doped TiO_2 on Gram-negative bacteria.

The generation of reactive oxygen species (ROS) such as hydroxyl and peroxide radicals could be the cause of the antibacterial activity which involves in the degradation of the cell wall and cytoplasmic membrane. This leads to leakage of cellular contents then cell lysis and may be followed by complete mineralisation of the bacterial organism.

The experimental results presented in this work show that the antibacterial activity of Ag-doped nanofibers can be used for the effective growth inhibition of Gram-positive and Gram-negative bacteria, making them applicable to diverse antimicrobial control systems.

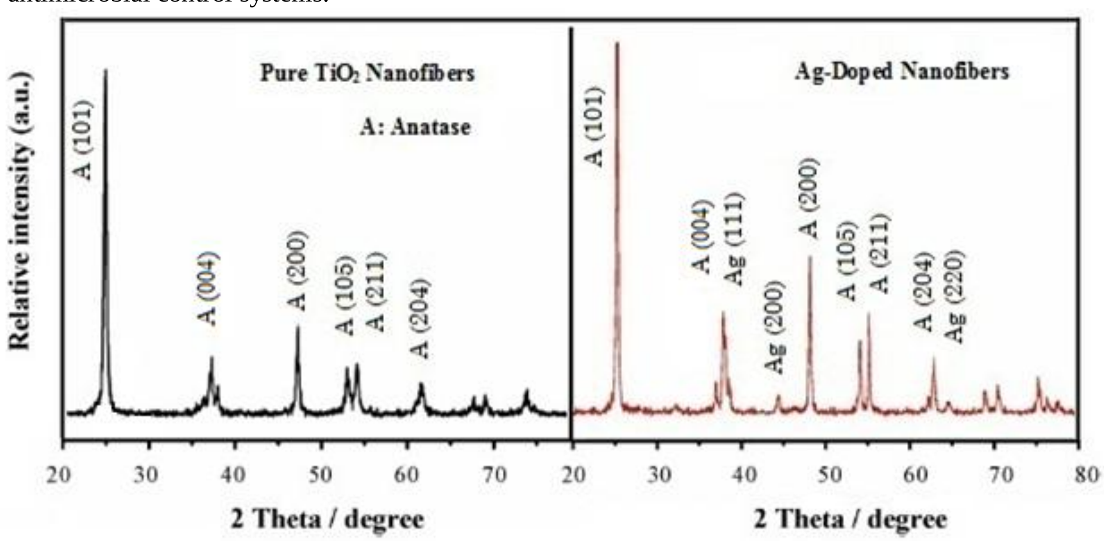

Figure 1:- XRD profile of pure TiO_2 and Ag-doped TiO_2 nanofibers.

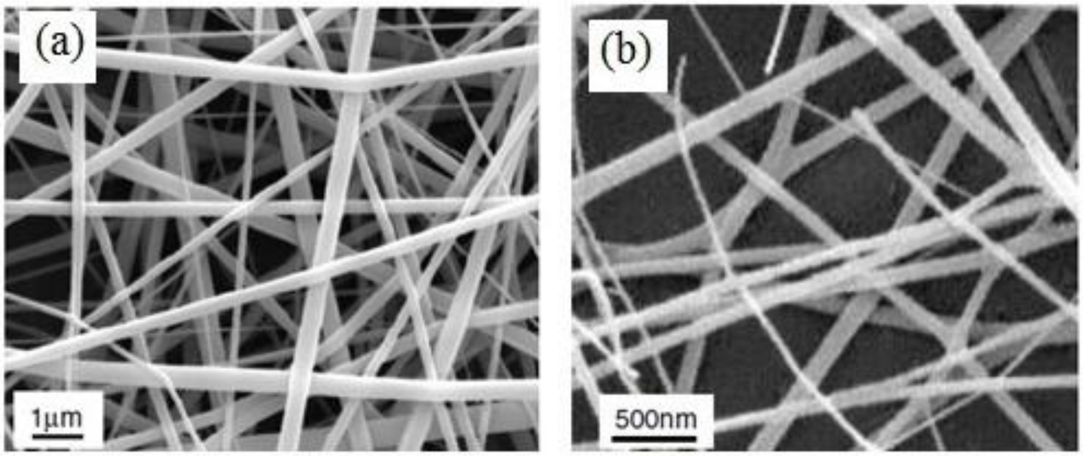

Figure 2:- FE-SEM images of a) pure TiO_2 nanofibers before calcination b) pure TiO_2 nanofibers after calcination at 500 ^oC.

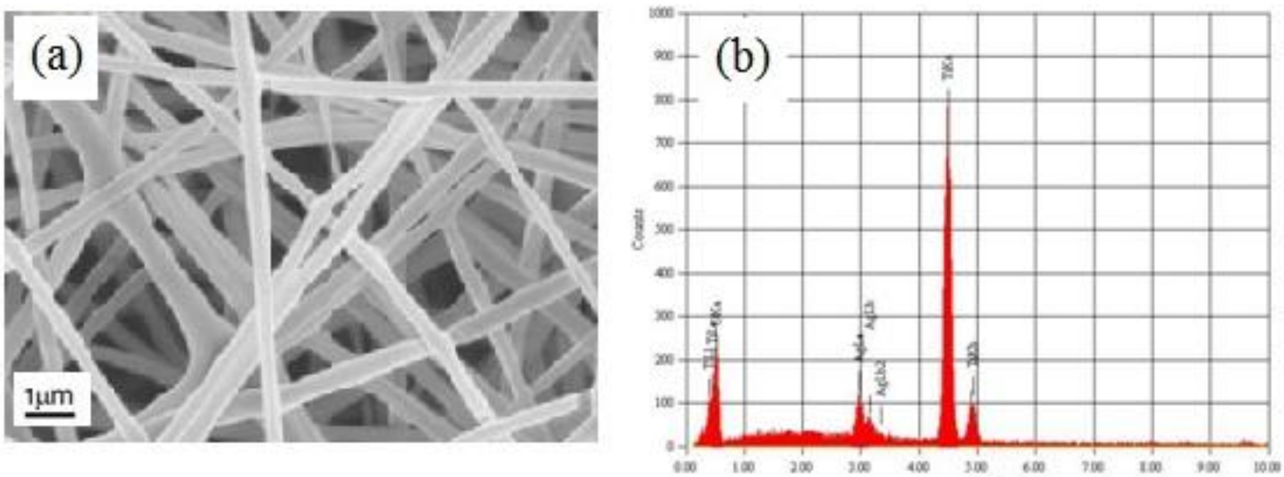

Figure 3:- (a) FE-SEM image of Ag-doped TiO_2 nanofibers after calcination at 500 ^oC b) EDX spectrum of Ag-doped TiO_2 nanofibers.

Table 1:- Antibacterial activity of crude pure TiO_2 and Ag-doped TiO_2 nanofibers

S.No.	Pathogenic bacteria	Zone of inhibitions (mm)							
		Concentration of pure TiO_2 nanofibers				Concentration of Ag-doped TiO_2 nanofibers			
		5 µL	10 µL	15 µL	20 µL	5 µL	10 µL	15 µL	20 µL
1	*S. aureus*	-	1	3	6	-	2	4	5
2	*P. aeruginosa*	-	3	4	7	2	5	6	8
3	*E. coli*	-	4	6	7	2	5	6	8

Table 2:- Antibacterial activity of annealed pure TiO_2 and Ag-doped TiO_2 nanofibers

S.No.	Pathogenic bacteria	Zone of inhibitions (mm)							
		Concentration of pure TiO_2 nanofibers				Concentration of Ag-doped TiO_2 nanofibers			
		5 µL	10 µL	15 µL	20 µL	5 µL	10 µL	15 µL	20 µL
1	*S. aureus*	4	6	9	13	6	8	12	14
2	*P. aeruginosa*	6	7	11	14	6	10	12	17
3	*E. coli*	7	9	12	17	9	12	17	18

Conclusion:-

The pure TiO_2 and Ag-doped TiO_2 nanofibers were successfully prepared by electrospinning method. The heat treated nanofibers were characterized by X-ray diffraction (XRD), Scanning electron microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX) etc. XRD analysis of calcined nanofibers confirms the anatase phase formation of pure and Ag-doped TiO_2 nanofibers. The antibacterial activity was tested using bacteria *S. aureus, E. coli, and P. Aeruginosa*. The obtained experimental results showed that bioactivity of the nanofibers differed depending on microbial strain, Ag content and Ag-doped nanofibers have the advantages of both TiO_2 and silver metal.

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