Studies on preparation of TiO$_2$ Nanoparticles and its loaded groundnut shell activated carbon and their antibacterial activity

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Abstract

Preparation of titanium dioxide (TiO$_2$) nanoparticles and its loaded groundnut shell activated carbon (TiO$_2$/GNSAC) nanoparticles had been undertaken using sol-gel method and their application in antibacterial activity against human pathogens. The X-ray diffraction (XRD) pattern results reveal that the synthesized TiO$_2$ nanoparticles and TiO$_2$/GNSAC nanoparticles are in the tetragonal structure. The existence of functional groups was determined by Fourier transform infrared spectroscopy (FT-IR). The surface morphology of samples was studied by scanning electron microscope (SEM). We confirmed that the synthesized TiO$_2$/GNSAC have an effective antibacterial activity against V. cholerae bacteria than GNSAC and TiO$_2$ nanoparticles.

Keywords: TiO$_2$, TiO$_2$/GNSAC, Antibacterial activity

Introduction

Antibacterial activity agents are very important in the textile industry, water disinfection, medicine and food packaging. Organic compounds are used for disinfection but some disadvantages, including toxicity to the human body, therefore the interest in inorganic disinfectants such as metal oxide nanoparticles is increasing (Hajipour et al., 2012). Nanoparticles are classified majorly into two types viz., organic and inorganic nanoparticles. The nanoparticles of carbon are called the organic nanoparticles. Magnetic nanoparticles, noble metal nanoparticles (platinum, gold and silver) and semiconductor nanoparticles (titanium dioxide, zinc oxide and zinc sulfide) are classified as inorganic nanoparticles (Kathiresan and Asmathunisha, 2013). Many possibilities of applications in the areas of physics, chemistry, pharmacy, surface coating agents, textile sizing, agriculture, biochemistry and so on (Wu et al., 2003; Fortner et al., 2005; Li et al., 2005). Surface properties of titanium nanotubes have attracted more and more attention because of their prospects in photocatalysts, antibacterial and solar cells. Among various phases of titania reported, anatase shows a better photocatalytic activity and antibacterial performance (Yang et al., 2005; Hu et al., 2006, Sakatani et al., 2006). Furthermore, Anatase TiO$_2$ was also found to possess an effective antibacterial property (Carp et al., 2004). A wide range of materials such as, TiO$_2$ (Akhavan, 2009), SiO$_2$ (Oh et al., 2006), Al$_2$O$_3$ (Chang et al., 2007), Zeolites (Inoue et al., 2002) and activated carbon fibers [Chen et al. 2005] were studied against antibacterial activity. In the present work, in order to study the antibacterial activity performance against V. cholerae human pathogens, we synthesized TiO$_2$ and TiO$_2$/GNSAC nanoparticles using sol-gel method.
Materials and Methods
Preparation of TiO$_2$ and TiO$_2$ loaded GNSAC nanoparticles

The titanium dioxide (TiO$_2$) was synthesized by sol-gel method at room temperature directly from titanium tetra isopropoxide (C$_{12}$H$_{26}$O$_7$Ti) and 2-propanol (C$_3$H$_6$O) using a similar method by adding 2.8 g of titanium tetra isopropoxide (C$_{12}$H$_{26}$O$_7$Ti) to 90 mL of 2-propanol. After stirring for 5 min at a room temperature, 30 mL of water was added drop wise with vigorous stirring for 2 hrs to obtain a homogeneous gel which was then calcined at 500 °C for 2 hrs to obtain TiO$_2$. The TiO$_2$/GNSAC catalyst was prepared by a similar method by adding 2.8 g of GNSAC (50 wt% with regard to the TiO$_2$) to the titanium tetra isopropoxide 2-propanol solution. After stirring, the solution was filtered using whatman filter paper (11mm) and washed several times by ethanol and deionized water. The precipitate formed was dried at 100 °C for 5 hrs to evaporate water and organic residues and then ground using an agate mortar to avoid agglomeration. Finally, the powder was calcinated at 500 °C in muffle furnace for 2 hrs to obtain TiO$_2$/GNSAC.

Characterization

The functional groups were determined by Fourier transform infrared spectroscopy (FT-IR). Spectra of the samples were recorded using NICOLET AVATAR 330. The samples were scanned 16 times per minute and spectra were recorded in the region 4000-400 cm$^{-1}$ with wave number accuracy of 0.01 cm$^{-1}$. The crystal structures and particle size of GNSAC, TiO$_2$ and TiO$_2$/GNSAC were analyzed by X-ray diffraction (XRD) measurement which was carried out by using XPERT-PRO diffractometer system using Cu-Kα radiation ($\lambda = 1.5406\text{Å}$), as in the 2θ range from 10 to 80°, operating at 30 mA and 40 kV. The morphology of the products was explored using scanning electron microscope JEOL: JSM-5610LV equipped with energy dispersive spectrometer (Bruker) at an accelerating voltage of 20 kV.

Antibacterial activity test

The antibacterial activity of GNSAC, TiO$_2$ and TiO$_2$/GNSAC nanoparticles was tested against the bacterial pathogens using disc diffusion assay. The pathogens namely Vibrio cholerae was obtained from Raja Sir Muthaiya Medical College, Annamalai University. Whatman filter paper (No.1) disc of 6mm diameter were impregnated with 10µl of the solution containing GNSAC, TiO$_2$ and TiO$_2$/GNSAC obtained from a concentration of 100 mg/mL and these disc were evaporated at 37 °C for 24 hrs. References standard disc were prepared with Ampicillin (50 µg/mL) to compare the antibacterial activity results of GNSAC, TiO$_2$ and TiO$_2$/GNSAC. After drying, the disc with GNSAC, TiO$_2$ and TiO$_2$/GNSAC and Ampicillin were placed on Muller Hinton Agar (MHA) plates were the bacterial culture was swabbed on the surface of the agar and incubated for 24 hrs at 37 °C. After incubation, plates were examined for clear zone around the disc with GNSAC, TiO$_2$ and TiO$_2$/GNSAC and Ampicillin. A clear zone with diameter more than 2 mm was taken as an antibacterial activity and the results were expressed in mm. The plates were incubated at 37 °C for 24 hrs to measure zone of inhibition. The mean was calculated by performing the experiments in triplicates.

Results and Discussion

Fourier transform infrared spectroscopy (FT-IR)

Fig.1a-c shows the FT-IR spectra of GNSAC and TiO$_2$, TiO$_2$/GNSAC nanoparticles after calcination at 500 °C for 2 hrs. In Fig.1a for the groundnut shell activated carbon, of the sharp peak located at 2931 cm$^{-1}$ corresponds to aliphatic C-H stretching vibrations in methyl and methylene groups, while the band at 1599 cm$^{-1}$ to C=C stretching of aromatic group (Mahmoodi et al., 2011). The peak at 1188 cm$^{-1}$ is assigned to the stretching vibration P=O-C (aromatic) linkage and P=OOH (Hadoun et al., 2013). The broad band at 1300-900 cm$^{-1}$ in GNSAC has a maximum at 1078 cm$^{-1}$. The adsorption in this region is the characteristics for phosphorous. These bands arise as a result of phosphoric acid impregnation during GNSAC synthesis. Spectra of both TiO$_2$ and TiO$_2$/GNSAC are found to have significant hydroxyl groups on their surface.

A broad band is observed in between 3600 and 3000 cm$^{-1}$ is related to the O-H stretching mode of hydroxyl group, indicating the presence of moisture in the samples. The characteristic peak at 1633 cm$^{-1}$ is associated with the O-H bending vibrations of the absorbed water molecules. The two observed peaks at 3412 and 1633 cm$^{-1}$ correspond to the surface adsorbed water and hydroxyl groups (Praveen el al., 2014).
Fig. 1. FT-IR spectra of (a) GNSAC (b) TiO\(_2\) and (c) TiO\(_2\)/GNSAC.

A new peak at 1064 cm\(^{-1}\) can be assigned to C-O stretching vibration. For the pure TiO\(_2\), the broad peak at range from 800-400 cm\(^{-1}\) is the contributions from the anatase titania. A broad absorption band between 800 and 450 cm\(^{-1}\) region is ascribed to the vibration absorption of the Ti-O-Ti linkages in TiO\(_2\)/GNSAC (Lu et al., 2008). The FT-IR results, strongly confirm the presence of hydroxyl groups in the structure of the TiO\(_2\)/GNSAC.

X-ray diffraction (XRD)

The XRD patterns of the prepared TiO\(_2\) and TiO\(_2\)/GNSAC nanoparticles calcination at 500 °C for 2 hrs and GNSAC are shown in Fig. 2a-c. Figure 2a the diffraction profile of GNSAC exhibits two broad peaks at 2θ = 26 and 43° which are similar to the peaks of crystalline carbonaceous structure and the absence of sharp peak reveals a predominantly amorphous structure.

Fig. 2. XRD patterns of (a) GNSAC (b) TiO\(_2\) and TiO\(_2\)/GNSAC.
Fig. 2b-c show sharp peaks exhibiting the crystalline nature of TiO\textsubscript{2} and TiO\textsubscript{2}/GNSAC nanoparticles. X-ray diffraction studies indicate that the materials synthesized are pure anatase TiO\textsubscript{2} phase with the tetragonal structure. The obtained results are well agreed with the corresponding reported JCPDS value (Card No. 71-1166). Average particle size of TiO\textsubscript{2} and TiO\textsubscript{2}/GNSAC nanoparticles are calculated using the Scherrer’s formula given by,

\[ D = \frac{\lambda}{\beta \cos \theta} \]  

Where D is the average crystallite diameter (nm), K is the Scherrer’s constant (0.9), \(\lambda\) is the wavelength (1.5406 Å), \(\beta\) is the Full width at half maximum, and \(\theta\) is the Bragg angle. The average crystallite size of TiO\textsubscript{2} and TiO\textsubscript{2}/GNSAC are calculated to be 18.79 and 8.7 nm, respectively. The XRD peaks exactly match with the anatase phase of TiO\textsubscript{2} showing that GNSAC modification does not change the phase. The broadening of peaks implies the decrease in crystalline size of TiO\textsubscript{2} (Jamil et al., 2012).

**Scanning electron microscopy (SEM)**

The surface morphology of GNSAC, TiO\textsubscript{2} and TiO\textsubscript{2}/GNSAC nanoparticles are shown in Fig. 3a-c. Pure TiO\textsubscript{2} is observed to exhibit surface particle agglomeration. GNSAC showed that the cavities on the surfaces of the carbon sample resulted from the evaporation of the H\textsubscript{3}PO\textsubscript{4} during carbonization, leaving the space open that was previously occupied by H\textsubscript{3}PO\textsubscript{4}. During impregnation, the molecules of the chemical impregnating agent diffused into the texture of the lignocellulosic material. On carbonization at the desired temperature, the chemical impregnating agent evaporates and makes the remaining carbon texture porous (Alothman, 2013). TiO\textsubscript{2}/GNSAC exhibits a rough porous surface resulting from the growth of TiO\textsubscript{2} nanoparticles on GNSAC surface.

![Fig. 3](image.png)

**Antibacterial activity**

The results of the antibacterial activity of GNSAC, TiO\textsubscript{2}, and TiO\textsubscript{2}/GNSAC confirmed an antibacterial effect on the diameters of the inhibition zones. All samples were tested against Gram-negative (V. cholerae) bacteria. The GNSAC, TiO\textsubscript{2} and TiO\textsubscript{2}/GNSAC were tested for its antibacterial activity using Muller Hinton Agar (MHA) plates, prepared by growing bacterial lawn of V. cholerae. Each plate referred to an individual samples as shown in Fig. 4.

Each plate also had positive standard (S) and negative control (C). The positive standard (S) was ampicillin (50µg/ml) which is an antibiotics and the negative control (C) was 50 µl of DMF. The zone of sensitivity of conjugate was compared with the positive standard. Each disk was prepared containing 50 µl of samples and was placed on agar plate along with both positive standard and negative control incubated at 37 °C for 24 h. During the incubation period, the zones of inhibition were measured. It is apparent that the samples showed inhibition zone against almost the test organisms (Paskalis Sahaya Murphin Kumar et al., 2014) are shown in Table 1.
In TiO$_2$/GNSAC (Fig. 4) showed that the maximum zone of inhibition was found against gram positive *V. cholerae* (7 mm) and it was higher than that of the positive standard ampicillin (13 mm).

**Conclusion**

The antibacterial activity of TiO$_2$ and TiO$_2$/GNSAC nanoparticles were tested against gram positive bacteria *V. cholerae* using disc diffusion method. The TiO$_2$/GNSAC nanoparticles shows maximum zone of inhibition than GNSAC and TiO$_2$ nanoparticles. The higher antibacterial activity of TiO$_2$/GNSAC nanoparticles can be attributed due to reduced crystallite size and increased surface area.

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**References**


