



Antibacterial Activity of $\text{TiO}_2\text{-SnO}_2$ (2:1) Hollow Sphere Against Gram Positive and Gram Negative Bacteria

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Absrtract

In the present study, reported $\text{TiO}_2/\text{SnO}_2$ (2:1) Hollow spheres were successfully prepared by using the hydrothermally synthesized colloidal carbon spheres as sacrificial template. The morphology and structure of the spheres was characterized by SEM, EDS, XRD, UV-Vis, and FTIR. The Anti bacterial activity of $\text{TiO}_2/\text{SnO}_2$ (2:1). Hollow sphere tested against multi drug resistance bacteria viz both Gram Positive (*Staphylococcus aureus*) and Gram Negative (*Escherichia coli*). Further $\text{TiO}_2\text{-SnO}_2$ (2:1) hollow sphere show higher antibacterial activity against *Staphylococcus aureus* than *Escherichia coli*. The results will be presented.

Keywords: Hollow Spheres, Hydrothermal synthesis, Carbon sphere, Antibacterial Activity.

1. Introduction

Many researchers are trying to overcome the resistance of the drug resistant pathogenic strains by using nanotechnology¹. *Staphylococcus aureus* (G-positive) and *Escherichia coli*(G-negative) are the bacterial strains that have widely developed resistance against standard drugs an source of diversified spectrum diseases^{2,3}. In this regard, nanomaterials play an important role becuasae it can be used as an alternative to organic drugs⁴. Nanomatgerials can intgeract and kill bacteria cells by destruction of protein, destruction of DNA through production oxygen reactive species (ROS), the release of cation and by damaging peptidoglycan layer and cell membrane. When nanomaterials are exposed to radiation , they produce ROS that include $\text{O}^\cdot$, H_2O_2 and $\text{oH}^\cdot$. These peroxide and radicals play decisive role in the destruction of bacterial cell⁵.

Recently inorganic hollow spheres with nanometer-to-micrometer dimensions have attracted extensive attention due to their characteristics such as low density, high specific surface area, different optical properties superior thermal and mechanical stability. These properties have received great attention in the general synthesis of functional materials because of their various potential applications in drug delivery, catalysis, photonic crystals, coatings, chemical storage etc⁶⁻⁹.

For example TiO₂, ZnO, SnO₂ hollow spheres exhibit excellent photocatalytic performance, an anode material for lithium ion batteries with high efficiency and shows antibacterial activity on microorganism¹⁰⁻²⁰. The hollow nano-micro spheres synthesized by using carbonaceous polysaccharide microspheres as template prepared from saccharide solution²¹.

Herein, report a simple and general method to successfully fabricate TiO₂, SnO₂ hollow spheres and their composite hollow sphere TiO₂-SnO₂ in the ratio of 2:1. Studied the antibacterial activities of as synthesized hollow sphere against Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*

2. Experimental

2.1 Synthesizing Carbon Spheres: In 60 ml deionized water dissolve the 8 grams of the glucose and stirring for 60 min. Filled this solution in an autoclave about ¾ volume of it. Heat up this by using hot air oven at the temperature of 180°C for 12 hours. Sonicate this solution for 3 hours. The obtained sample was washed by centrifuge with methanol and deionized water for three times each and dried at hot air oven at 80°C for 12 hours, the resultant sample was characterized by SEM and confirms the Carbon sphere.

2.2 Preparing TiO₂-SnO₂ (2:1) Hollow Spheres: Using a carbon sphere template, TiO₂-SnO₂(2:1) hollow spheres were produced. After three hours of ultrasonication in 150 milliliters of methanol, 0.3 gm carbon spheres, 4.320 gm (90 mM) titanium tetra isopropoxide, 2.365 gm (45 mM), and tin chloride penta hydrate were dissolved. Subsequently, the suspension was allowed to come to room temperature for 12 hours while being stirred magnetically. After that, it was centrifuged three times using methanol and DI water until a clear result was achieved. Lastly, the goods were dried in a hot air oven for four hours at 80 °C, and they were calcined in a muffle furnace for four hours at 500 °C while heating at a rate of four degrees Celsius per minute. This is the method used to prepare TiO₂-SnO₂ hollow metal spheres (2:1).

2.3 Characterization : TiO₂-SnO₂ hollow metal Spheres(2:1) sample crystal structure of the samples were determined by an X-ray diffraction (PAN ALYTICAL XPERT3 Powder), operated at 45 kV and 40 mA using Cu Kα radiation ($\lambda = 0.15406$ nm) at a scanning rate of 4° min⁻¹ ranging from 15° to 85°. Studied the percentage of composition samples with the EDS (ZEISS ULTRA 55 Field Emission Scanning Electron Microscope embeded with smart EDX)

Observe the morphologies and size of the samples were analyzed by Scanning Electron Microscopy (SEM, ZEISS ULTRA 55 Field Emission Scanning Electron Microscope) .

With the help of UV-VIS Spectrophotometer (Agilent Technologies Cary Services UV-Vis NIR Spectrometer), the prepared sample absorbance peaks studied.

Different IR frequencies absorbance of samples analyzed by FTIR prestige 21, Shimadzu with in wave number 400 cm^{-1} to 4500 cm^{-1}

2.4 Anti bacterial Activity

Antibacterial activities of the test compounds TiO_2 , SnO_2 , and $\text{TiO}_2\text{-SnO}_2$ (2:1)

On Gram-positive (*S.aureus*) and Gram-negative(*E.coli*) bacterial strains was carried out by the agar well diffusion method. Nutrient agar medium prepared was inoculated with 18hours old cultures of the test organism and poured into a Petri plate. The medium in the plates were allowed to solidify at room temperature with in a laminar air flow. After media gets solidified 4 cups of 5mm diameter were made in each plate at equal distance. The stock solutions of the test compound were prepared with a constant concentration of $50\text{ }\mu\text{g/mL}$. The concentration was placed in the cups by means of sterile micropipettes. In each plate one cup was used for standard drug (Ciprofloxain) also taken at $50\text{ }\mu\text{g/mL}$. The petri dishes thus prepared were incubated in incubator for 24hrs at 37°C and later examined by measuring the zones of inhibition.

3. Results and Discussion

3.1 XRD

The XRD pattern of studied sample carbon sphere, TiO_2 , SnO_2 and $\text{TiO}_2\text{-SnO}_2$ (2:1), hollow metal spheres are shown in Fig 1. From Figure 1(a) it is evident that the carbonsphere have the amorphous nature. The crystalline size of the particles was calculated from the width of XRD peak by using Scherrer's equation $D = 0.9\lambda/\beta\cos\theta$, where D = crystallite size, λ is wavelength(0.154nm), β is full width of peak at half maximum intensity and θ is Bragg's diffraction angle. The related data of D estimated for studied crystallites is given in Table 1.

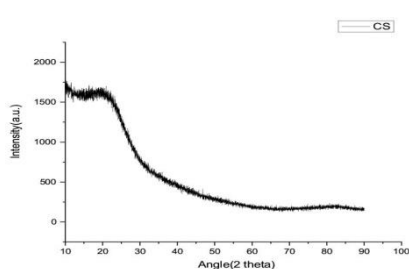


Fig.1(a)

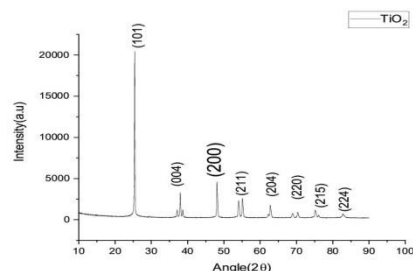


Fig.1(b)

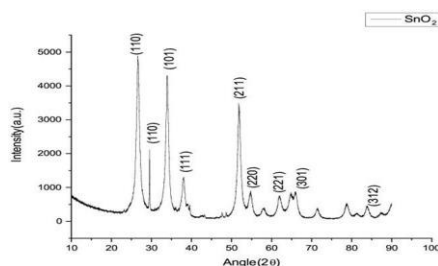


Fig.1(c)

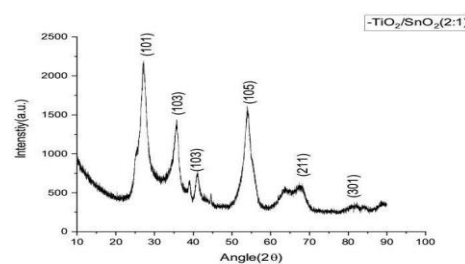


Fig.1(d)

Fig1: (a)Carbon Sphere (b) TiO_2 (c) SnO_2 (d) $\text{TiO}_2\text{-SnO}_2$ (2:1)

Table 1: Average diameter of the prepared hollow spheres

Name of the Sample	Average Diameter D(nm)
TiO ₂	30.65
SnO ₂	4.72
TiO ₂ -SnO ₂ (2:1)	1.11

The order of crystalline size of as-synthesized hollow spheres was TiO₂>SnO₂> TiO₂-SnO₂(2:1). Lower size of TiO₂-SnO₂(2:1) than TiO₂ and SnO₂. It may be due to the composition of TiO₂ with SnO₂

3.2 SEM

SEM images of the obtained samples was shown in fig2(a) to 2(d)

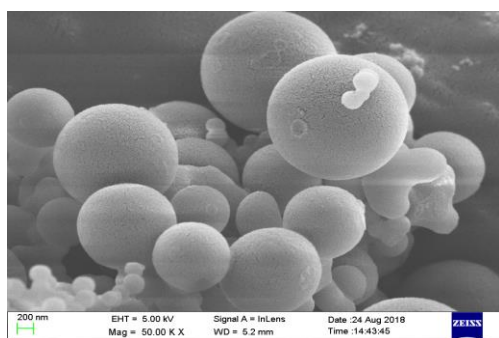


Fig. 2(a)

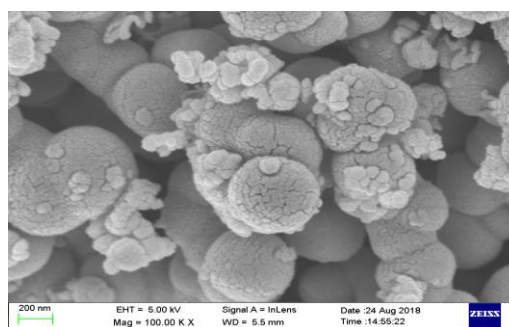


Fig. 2(b)

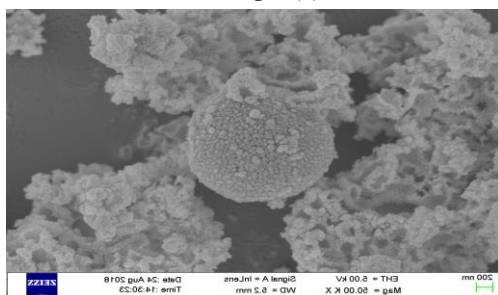


Fig. 2(c)

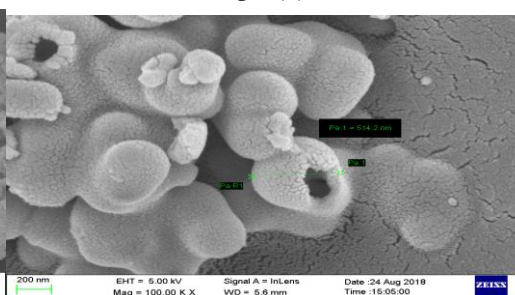


Fig. 2(d)

Fig2: SEM images of (a) Carbon Spheres, (b) TiO₂ metallic hollow spheres, and (c) SnO₂ metallic hollow spheres (d) TiO₂-SnO₂(2:1) hollow metallic spheres,

Figure 2a, 2b, 2c and 2d shows SEM images of respective samples prepared by hydrothermal treatment. Fig2(a) shows clearly the carbon spheres are of uniform spherical morphology. The average diameter of carbon spheres is found to be in the range of 600 to 1700 nm and an average of 1070 nm. Fig2(b) depicts TiO₂-SnO₂(2:1) hollow spheres are observed as the average diameter is about 419 nm. This value is very much less than the carbon hollow spheres. This shrinkage may be attributed to the loss of carbon spheres as a gas flow (decarbonization and dehydration of carbon spheres) during the process of calcinations.

EDS

The EDS spectrum of the Carbon Sphere , TiO_2 , SnO_2 , $\text{TiO}_2\text{--SnO}_2$ (2:1) hollow spheres are shown below figure 3.

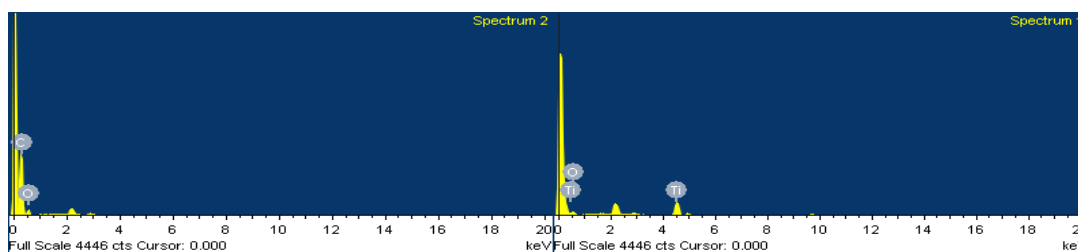


Fig. 3(a)

Fig. 3(b)

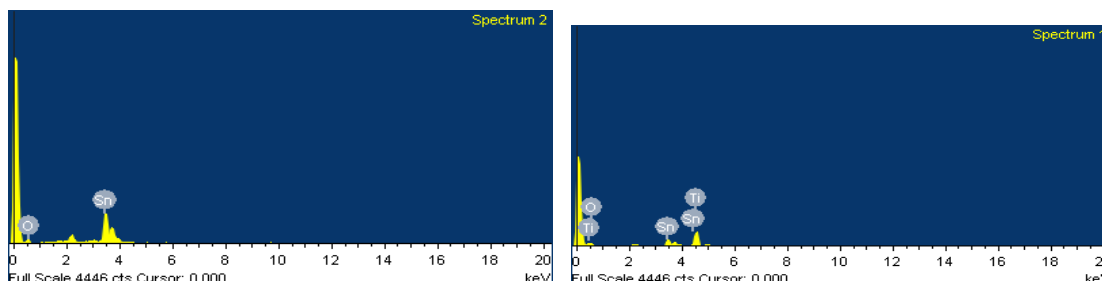


Fig. 3(c)

Fig. 3(d)

Fig3: EDS spectrum of (a) Carbon Spheres, (b) TiO_2 metallic hollow spheres, and (c) SnO_2 metallic hollow spheres (d) $\text{TiO}_2\text{--SnO}_2$ (2:1) hollow metallic spheres,

Table 2: The elemental chemical composition of prepared hollow spheres

Hallowspheres- →	CS		TiO_2		SnO_2		$\text{TiO}_2\text{--SnO}_2$ (2:1)		
Element---→	C(%)	O(%)	Ti(%)	O(%)	Sn(%)	O(%)	Ti(%)	Sn(%)	O(%)
Weight(%)	72.31	27.69	41.87	58.13	69.12	30.88	35.70	25.72	38.57
Atomic(%)	77.67	22.33	19.39	80.61	23.18	76.82	22.10	6.42	71.48

The elemental and oxygen composition of prepared samples are shown in Table-3 . The results indicate that Tin ions are more easily absorbed on the surface of carbon spheres than Titanium ions in the hydrothermal process. After calcinations carbon sphere was oxidized into gas flow out and Titanium ions and Tin ions oxidized into TiO_2 and SnO_2 respectively. Finally forming forming such $\text{TiO}_2\text{--SnO}_2$ (2:1) composite hollow spheres

3.3 UV-Vis

The UV-Vis spectrum of the Carbon Sphere , TiO_2 , SnO_2 , $\text{TiO}_2\text{--SnO}_2$ (2:1) hollow spheres are shown below figure 4.

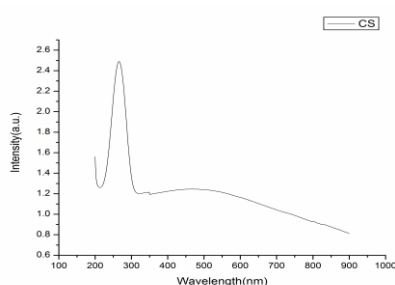


Fig. 4(a)

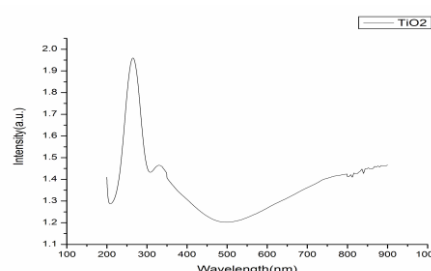


Fig. 4(b)

Fig. 4(c)

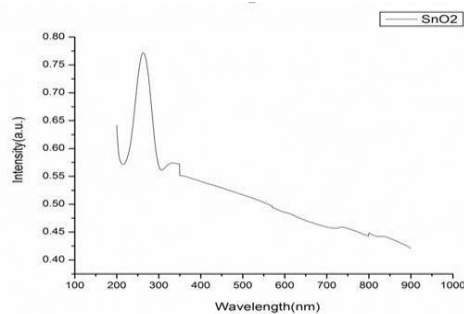


Fig. 4(d)

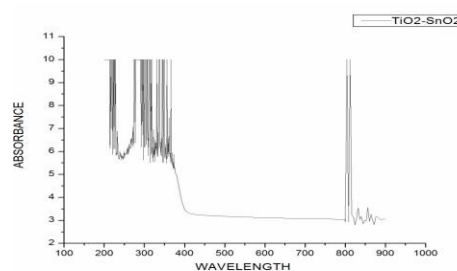
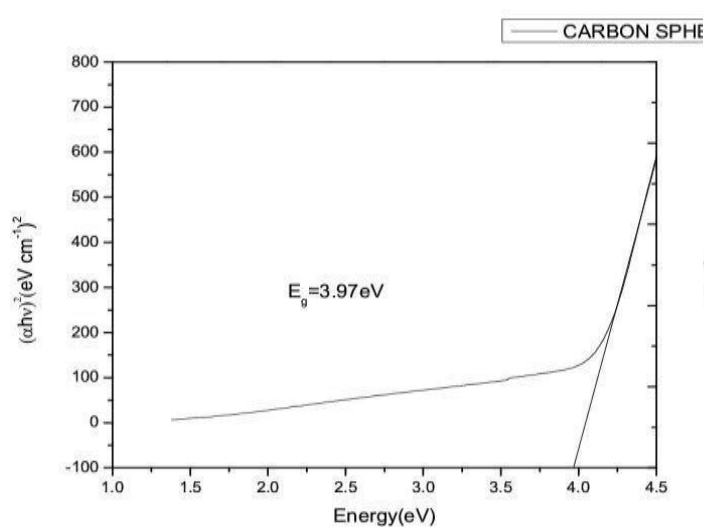
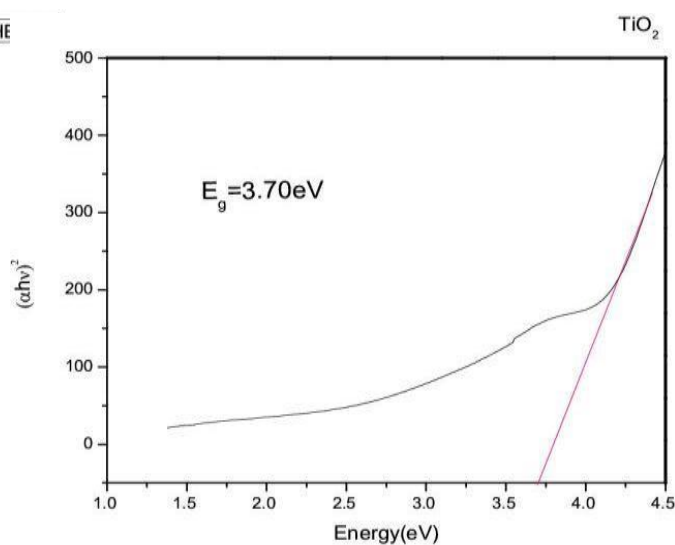


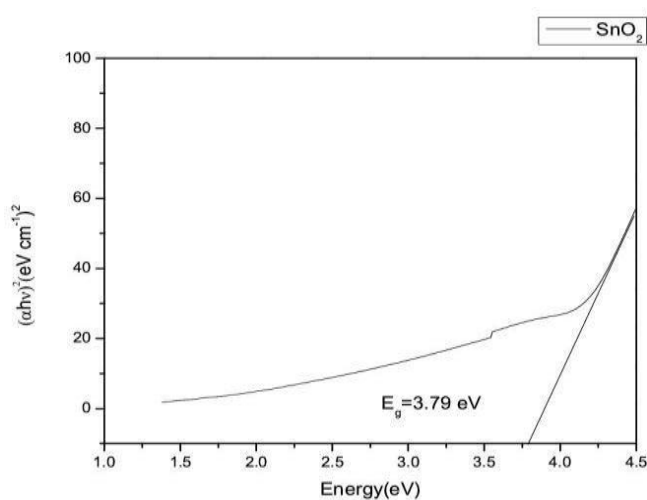
Fig4: UV-Vis spectrum of (a) Carbon Spheres, (b) TiO₂ metallic hollow spheres, and (c) SnO₂ metallic hollow spheres (d) TiO₂-SnO₂(2:1) hollow metallic spheres



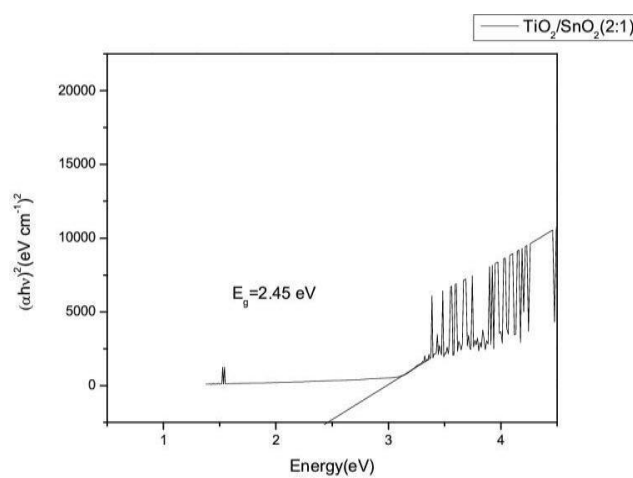
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Fig5: Tauc plot of (a) Carbon Spheres, (b) TiO₂ metallic hollow spheres, and (c) SnO₂ metallic hollow spheres (d) TiO₂-SnO₂(2:1) hollow metallic spheres

Fig.4 a,b,c depicts, the wavelength of the absorption edge for carbon spheres, TiO_2 and SnO_2 hollow spheres are located around 266nm, 265 nm, and 263 nm respectively. This can be attributed to the intrinsic absorption, due to the band gap transition. Whereas fig 4d reveals that $\text{TiO}_2\text{-SnO}_2(2:1)$ hollow spheres shows much stronger absorbance intensity nearly 361 nm with larger absorbance region when compared to TiO_2 and SnO_2 individual hollow spheres. This stronger absorbance may be due to heterogeneous structural formation of composite oxide.

Figure 5 a, b,c, shows the optical band gap energies of the prepared samples by using Tauc plot. It is evident that the band gap energies of carbon sphere, TiO_2 , SnO_2 , and $\text{TiO}_2\text{-SnO}_2(2:1)$ hollow spheres are 3.97 eV, 3.70 eV, 3.79 eV, and 2.45 eV respectively. Among all the band gap energy of prepared composite hollow sphere is less than individuals.

3.4 FTIR

The FTIR spectrum of the Carbon Sphere, TiO_2 , SnO_2 , $\text{TiO}_2\text{-SnO}_2(2:1)$ hollow spheres are shown below figure 6.

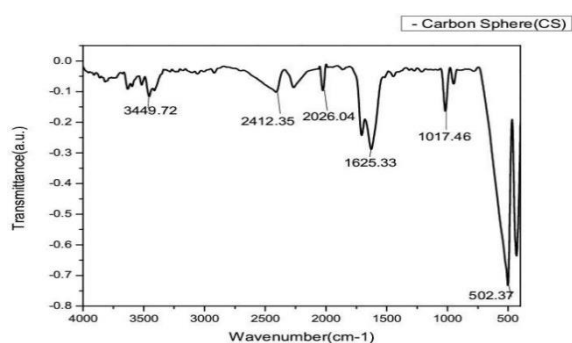


Fig 6(a)

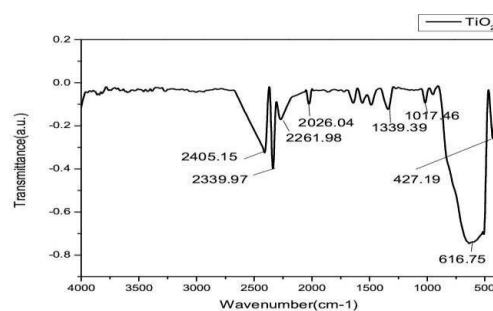


Fig 6(b)

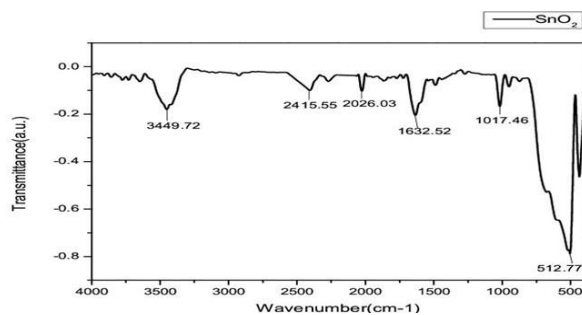


Fig 6(c)

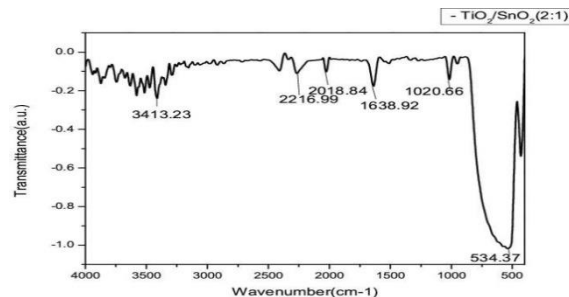


Fig 6(d)

Fig6: FTIR spectrum of (a) Carbon Spheres, (b) TiO_2 metallic hollow spheres, and (c) SnO_2 metallic hollow spheres (d) $\text{TiO}_2\text{-SnO}_2(2:1)$ hollow metallic spheres

The FTIR spectrophotometer operated at a resolution of 4cm^{-1} records a spectrum of wavenumber range in between 4000 and 400 cm^{-1} . From the above fig 6 it is observed that the frequency bands occurs from 4000 to 1500 cm^{-1} is functional group region which confirms O-H, C-H stretch and C triple bond C. And less than 1500cm^{-1} gives finger print region which confirms the aromatic ring formation.

4. Antibacterial Activity

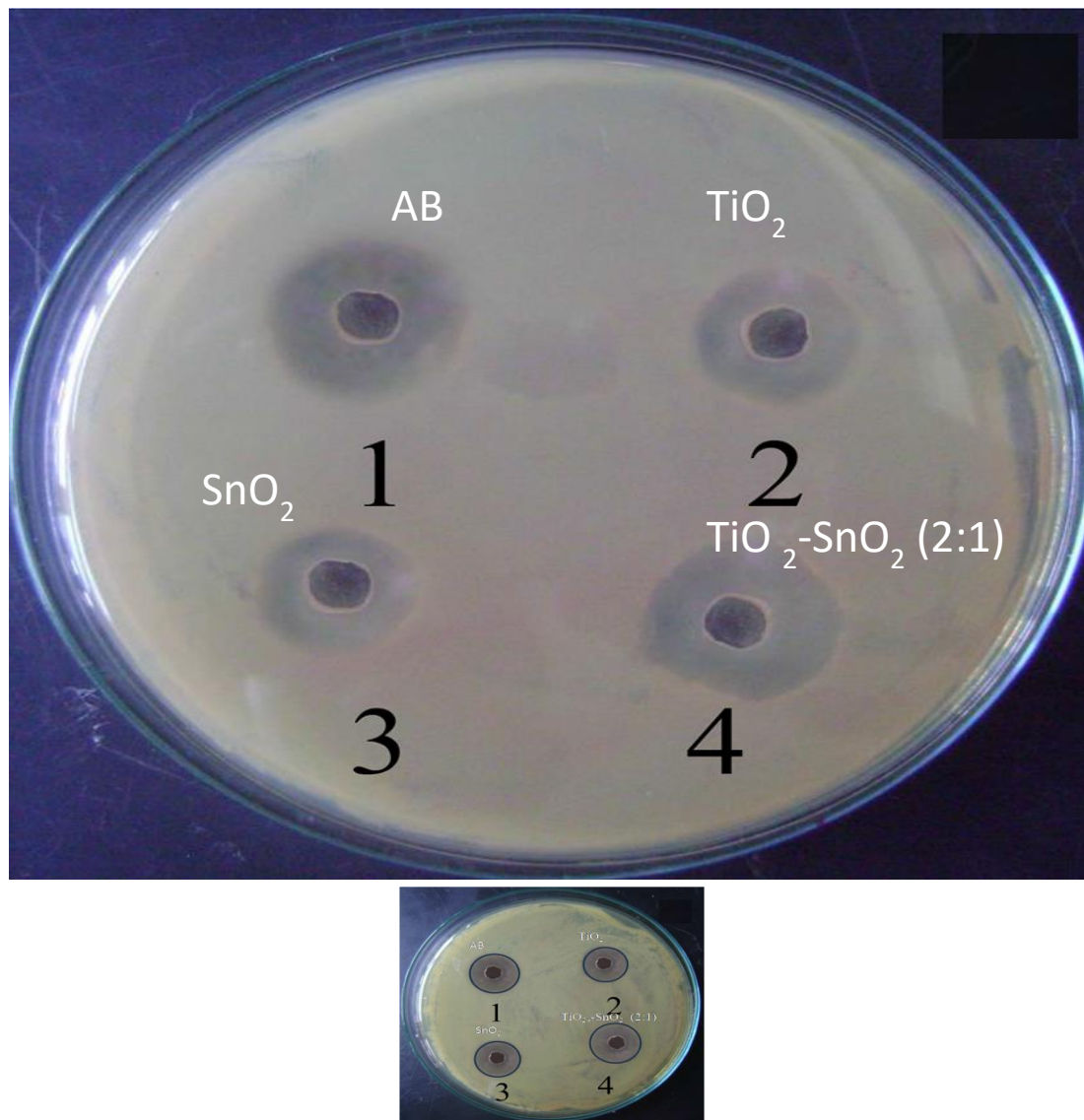


Fig.7 : Antibacterial activity study of Metal oxide/composite metal oxide hollow spheres on Gram-positive (*S. aureus*) and Gram-negative(*E. coli*)

The Minimum Inhibitory Concentration(MIC) value of synthesized TiO_2 , SnO_2 , and $\text{TiO}_2\text{-SnO}_2(2:1)$ hollow spheres to inhibit the growth of bacteria was recorded by testing zone formation against gram positive(*S. aureus*) and gram negative (*E. coli*) against Ciprofloxacin antibiotic is shown in figure?. The zone inhibition values shown in Table? given below

Table 3: Antibacterial activity study of Metal oxide/composite metal oxide hollow spheres on Gram-positive (*S. aureus*) and Gram-negative(*E. coli*)

Bacterial Pathogens	Zone Inhibition of Hollow Spheres with constant concentration 50 $\mu\text{g/mL}$ in mm			
	TiO_2	SnO_2	$\text{TiO}_2\text{-SnO}_2(2:1)$	Ciprofoxacin(AB)
Gram positive (<i>S. aureus</i>)	14	16	17.50	19
Gram negative (<i>E. coli</i>)	13	14	15	17

From Table-3 it is observed that the antibacterial activity by observing zone inhibition, which is more when used the Composite $\text{TiO}_2\text{-SnO}_2(2:1)$ hollow spheres than individual hollow spheres TiO_2 and SnO_2 in both Gram positive and Gram negative bacteria. And more over these are close with the values against antibiotic Ciprofloxacin.

Conclusion

After preparing carbon spheres used as sacrificial template and synthesized the hollow spherical structures of TiO_2SnO_2 and $\text{TiO}_2\text{-SnO}_2 (2:1)$ successfully. On observing the XRD pattern, it is evident that the composite hollow sphere have lower crystalline size when compare with individuals which gives the structure of composite hollow sphere is heterogeneous. The compositions of the samples is obtained by Energy Dispersive X-ray Spectroscopy and also the absorbance of the wavelengths by the hollow spheres observed through UV-Vis spectrometer. Tauc-Plot is used to observed the optical energy band gap. FTIR studies observed the molecular bonds in the sample. The study of antibacterial activity shows that the $\text{TiO}_2\text{-SnO}_2(2:1)$ composite metal oxide hollow sphere having more antibacterial activity than the individuals.

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References

1. Zhuoran Wu a 1, Brian Chan b 1, Jessalyn Low b, JustinJang Hann Chu c d e f, Hwee Weng Dennis Hey g, Andy Tay a b h Microbial resistance to nanotechnologies: An important but understudied consideration using antimicrobial nanotechnologies in orthopaedic implants October 2022 16, 249-270 J. Biactive Materials
2. Timothy J. Foster* Antibiotic resistance in *Staphylococcus aureus*. Current status and future prospects FEMS microbiology review, fux007,41, 2017,430-449
3. Poirel L.Madec J.Lupo A.Schink A.Kieffer N.Nordmann P.Schwarz S. 2018. Antimicrobial Resistance in *Escherichia coli*. Microbiol Spectr 6:10.1128/microbiolspec.arba-0026-2017. <https://doi.org/10.1128/microbiolspec.arba-0026-2017>
4. Patra, J.K., Das, G., Fraceto, L.F. *et al.* Nano based drug delivery systems: recent developments and future prospects. *J Nanobiotechnol* **16**, 71 (2018). <https://doi.org/10.1186/s12951-018-0392-8>
5. Fatemeh Aflakian, Farshad Mirzavi, Hammed Tanimowo Aiyelabegan, Anvar Soleimani, Jamshid Gholizadeh Navashenaq, Iman Karimi-Sani, Abolfazl Rafati Zomorodi, Roghayyeh Vakili-Ghartavol, Nanoparticles-based therapeutics for the management of bacterial infections: A special emphasis on FDA approved products and clinical trials, European Journal of Pharmaceutical Sciences, Volume 188, 2023, 106515, ISSN 0928-0987, <https://doi.org/10.1016/j.ejps.2023.106515>.
6. Merkel TC, Freeman BD, Spontak RJ, He Z, Pinnau I, Meakin P, et al. Science 2002;296:519–22.
7. Yang J, Ying JY. Nature Mater 2009;8