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Research Article

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Synthesis of Iron Oxide–Tin Oxide Nanoparticles and Evaluation of their Activities against Different Bacterial Strains

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Abstract: Nanoparticles were found having applications in various fields of life but recently in diagnostic and therapeutic fields of medicine. Iron oxide-tin oxide (Fe_2O_3-SnO_2) nanoparticles were synthesized via hydrothermal method with various concentrations of sodium dodecyl sulfate (SDS) surfactant. Their characterization was done by Fourier Transform Infrared Spectroscopy (FT-IR), Thermogravimetric Analysis (TGA), Transmission Electron Microscope (TEM), Scanning Electron Microscope (SEM) and X-ray Diffraction (XRD) powder techniques. Antibacterial activities of these metal oxides nanoparticles were determined and they were found active against gram-positive *B. subtilis* and *B. lichniformis* and gram-negative *E. coli*, *P. aeruginosa* and *K. pneumonia*.

Keywords: Toxicology; Nanoparticles, Anti-Bacterial; Gram-Positive; Gram-Negative Bacteria.

1. INTRODUCTION

As the time passes, science has progressed very much. Initially penicillin was extracted from *Pencillium* a fungal class and used as antibiotic [1]. Development of science leads to the synthetic organic drugs which became in common practice to treat bacterial diseases [2, 3]. Increasing use of such drugs developed resistance in bacteria and these antibiotics became less efficient [4]. Decrease in efficiency of drugs motivate scientists to synthesize such drugs which are more efficient against bacteria and bacteria find difficulty in developing resistance against them, a drug which effects multisystem of bacteria. Organic antibiotics are found less stable (less shelf life) than inorganic antibiotics at elevated temperature or pressure [5].

Various nanoparticles of metal oxides have been synthesized and are found a good inhibitor of different bacterial strains [5, 6] Activity of nanoparticles is directly dependent on the bacterial strain i.e. gram positive and gram negative as they have differences in their cell wall. Electrostatic interactions are

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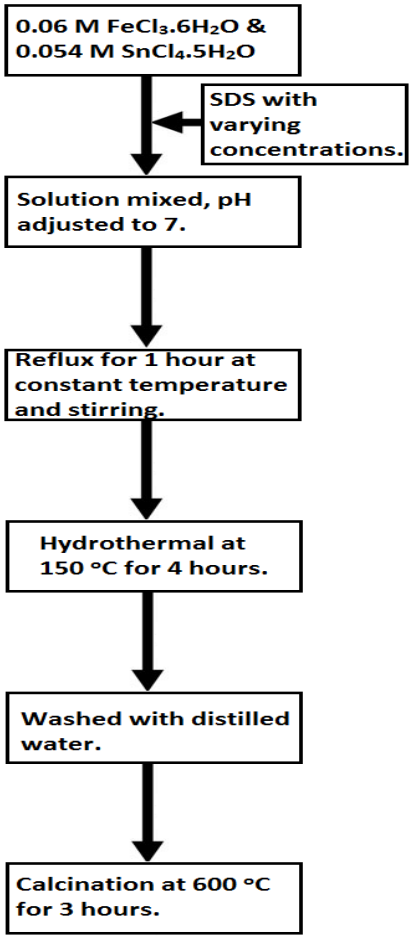

Figure 1. Schematic diagram of synthesis of iron oxide- tin oxide nanoparticles

directly responsible for the attachment of nanoparticles to bacteria. These interactions changes the integrity of cell membrane of bacteria and toxic free radicles are released which induce oxidative stress on bacteria [6].

Different methods have been adopted to synthesize nanoparticles like sol gel method [7, 8] hydrothermal method [9], surfactant assisted method [10], deposition-precipitation method [11], wet-oxidation method [12]. Metal oxide nanoparticles synthesized by any of these mentioned method is used against bacterial strains by adopting various protocols.

Zero-valent iron efficiently reduced the growth of *Escherichia coli* [13]. Silver nanoparticles prepared form silver nitrate is used against *Staphyloccocus aureus* and *Escherichia coli* and they are more efficient against *E. coli* than *S. aureus* [14]. CuO, NiO, Sb_2O_3, ZnO are used by dispersion method [15] and CuO and Ag are used by disc diffusion method [16] against various strains and are found effective.

Work is in progress to identify the mechanism of action of nanoparticles. Many hypothesis have been drawn among these bacterial cell disruption, damage of DNA, permeability of cell membrane changes and damage of cellular proteins are under consideration. All these processes occur because of heavy metal ion toxicity [5, 14-16].

Silver nanoparticles are most commonly studied as anti-bacterial agent. They attack on the

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bacterial cell membrane and also attack on the phosphorous group of different components in bacteria like DNA and RNA [17-19]. Silver nanoparticles are also used in combination with other antibiotic drugs and the efficiency of drugs is found increased because of silver nanoparticles [20]. Graphene, graphene oxide, graphite and its oxide are also used against *E.coli* and graphene oxide is found more active than graphene and graphite oxide but it is less efficient than graphite [21].

Along the bacteriostatic or bactericidal properties, nanoparticles can also be used to detect bacteria at very low concentration. This is achieved by using bio-functional magnetic nanoparticles [22].

In the present work iron oxide-tin oxide nanoparticles were synthesized at various SDS (Sodium dodecyl sulfate) concentrations by hydrothermal method and their activities against gram-positive and gram-negative bacterial strains were determined. Bacillus strains were used as gram-positive test organisms and clinical isolates of gram-negative rods were used as gram-negative test organisms. The main objective of this study was to evaluate the anti-bacterial effects of nanoparticles against various strains. Iron oxide was chosen because it has various applications regarding biomedicine due to its different properties [23] while tin oxide was selected because it showed good catalytic activities in different reactions [24].

Table 1. Reactants concentrations used for the synthesis of iron oxide-tin oxide nanoparticles

Sr. No.	Sample Code	Concentration of SDS (M)	Concentration of $FeCl_3.6H_2O$ (M)	Concentration of $SnCl_4.5H_2O$ (M)
1	D1	0.012	0.060	0.054
2	D2	0.010	0.060	0.054
3	D3	0.008	0.060	0.054
4	D4	0.006	0.060	0.054
5	D5	0.004	0.060	0.054

2. EXPERIMENTAL

2.1 Chemicals

Tin (IV) chloride pentahydrated ($SnCl_4.5H_2O$) sigma Aldrich, Iron (III) Chloride hexahydrated ($FeCl_3.6H_2O$) Merck, Sodium dodecyl sulfate (SDS) Merck, Sodium Hydroxide (NaOH) Merck and distilled water.

2.2 Synthesis of Metal oxide Nanoparticles

0.06 M $FeCl_3.6H_2O$ and 0.054 M $SnCl_4.5H_2O$ were mixed well and clear solution of SDS in required concentration shown in Table 1 was added. The pH of whole reaction mixture was adjusted to 7 using NaOH. It was then stirred under reflux at constant temperature for one hour. 9 mL of solution was transferred to Teflon hydrothermal bomb and kept at 150 ^oC for 4 hours. After 4 hours, Teflon hydrothermal bomb was allowed to cool and brown viscous product was recovered. With the help of centrifuge, product was separated out and washed with 30 mL distilled water. Product was then oven dried and grinded well. Calcination was done at 600 ^oC using muffle furnace for 3 hours [9]. The schematic diagram of synthesis was shown in Fig. 1.

2.3. Characterization Techniques

Functional group identification and thermal stability were done by MIDAC 2000M Series FT-IR (Fourier Transform-Infra Red Spectroscopy) and TA series SDT Q600 TGA-DSC (Thermogravimetric analysis-Diffrential scanning calorimeter) respectively. Particle size was determined using Philips CM12, 80 kV Transmission Electron Microscope (TEM) and surface morphology along with chemical

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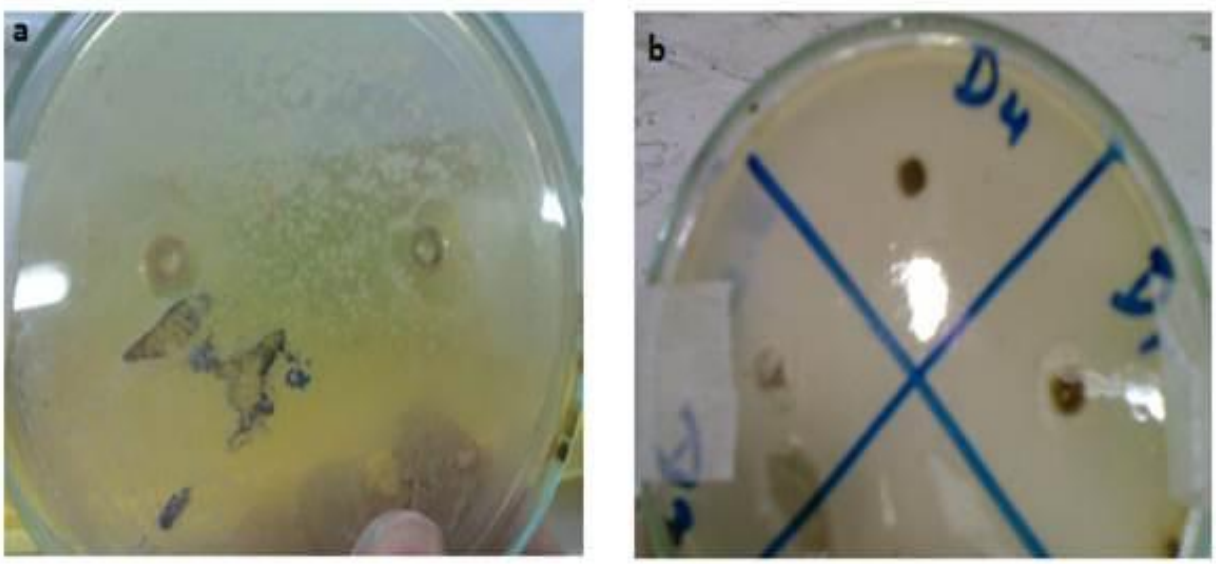

Figure 2. Zone of inhibition developed by **a** sample D2 (Fe_2O_3.SnO_2, SDS 0.010M) and D3 (Fe_2O_3.SnO_2, SDS 0.008M) against *B. subtilis*. **b** sample D1 (Fe_2O_3.SnO_2, SDS 0.012M) and D4 (Fe_2O_3.SnO_2, SDS 0.006M) against *B. lichniformis*

composition was determined by Hitachi S3400 SEM (Scanning Electron Microsope) which has accelerating voltage of 15.0 kV. X-ray diffraction pattern was taken to determine the hkl value using PANalytical X' Pert PRO diffractometer.

2.4. Bactericidal Study

2.4.1. Bacterial Strains

Bacillus subtilis, *Bacillus licheniformis* were gram positive strains taken from the Biochemistry Lab, GCU, Lahore and *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were the clinical isolates from local hospital.

2.4.2. Anti-bacterial Assay

0.2 g nutrient broth was dissolved in 25 mL distilled water in erlenmeyer flask. Cotton plug the flask and sterilized it in autoclave at 121 ^oC for 15 min. Inoculums of all the strains were developed in this growth media. A loop full of bacterial strain was added in it and it was left over night at 37 ^oC in shaking incubator. This was diluted up to 10^{-2} before inoculating the plates.

2 g agar and 0.8 g nutrient broth were mixed to make 100 mL solution in an erlenmeyer flask. Cotton plug the flask and sterilized it. Poured about 15 to 20 ml of agar in petri plate, solidified it and then inoculate it with the bacterial strain. Wells having diameter of 0.4 cm were developed in which 10 µL of sample suspension was added. Suspensions of all samples were prepared by dissolving 20 mg of product in1 mL distilled water. These plates were kept for 24 hours at 37 ^oC and zones of inhibition were observed. Fig. 2 showed these observed zones.

3. RESULTS AND DISCUSSION

3.1. Characterization

The FT-IR of sample D3 (Fe_2O_3.SnO_2 with 0.008 M SDS synthesized) was taken before and after calcination at 600 ^oC for 3 hours. Fig. 3 showed the FT-IR spectra of sample D3 which clearly indicated

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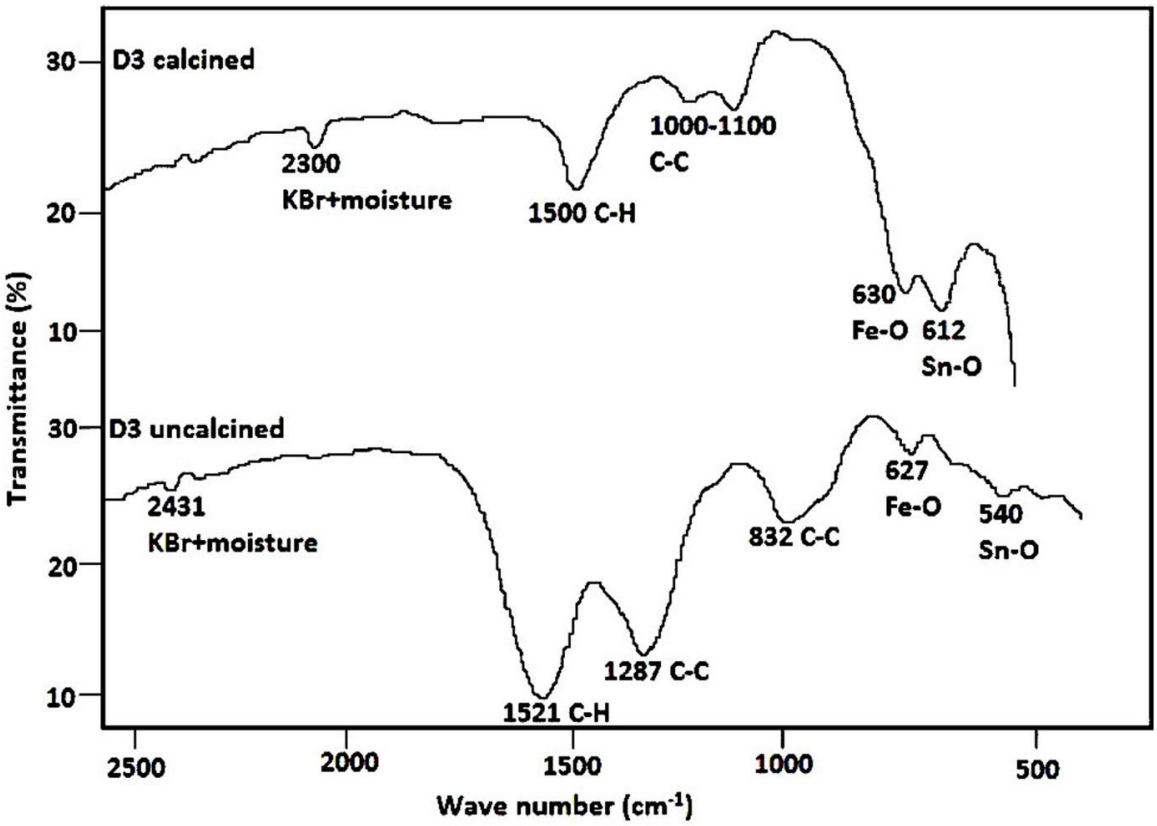

Figure 3. FT-IR spectra of sample D3 (Fe_2O_3.SnO_2 synthesized with 0.008M SDS concentration) before and after calcination at 600 ^oC for 3 hours

the difference in sample before and after calcination. After calcination, Sn-O peak changed its position from 540 cm^{-1} to 612 cm^{-1} [9] which showed the formation of metal oxides from metal hydroxides. The peak intensities of Fe-O and Sn-O became observable after calcination. Peak around 630 cm^{-1} was because of Fe-O stretching vibration which became a little more observable in calcined sample [25]. C-C and C-H peaks intensities reduced sharply and they were observed in the range of 800 cm^{-1} to 1400 cm^{-1} and 1500 cm^{-1} to 1600 cm^{-1} respectively [26] which may be due to the ash or residue of SDS burning after calcination. The number and intensity of C-C and C-H peaks also decreased after calcination and these may be due to the combustion/ash of SDS residues.

TGA-Thermogram of sample D3 (Fe_2O_3.SnO_2 with 0.008 M SDS synthesized) shown in Fig. 4 indicated that Fe_2O_3.SnO_2 was quite stable and only 11% weight loss was observed above 60 ^oC. The set temperature range was from 0-800 ^oC. TGA-thermogram showed two stages of weight loss. First stage showed a little weight loss of 5% which starts at 60 ^oC due to the moisture absorbed by the sample. Second stage began from 200 ^oC and completed at 800 ^oC with almost 6% weight loss because of decomposition of Fe_2O_3 to Fe_3O_4 in nitrogen at about 400 ^oC [27] and weight loss by SnO_2[28].

TEM images showed that the particles had sizes in nano-range and their size varies as the concentration of SDS varied. Fig. 5 showed the TEM image of D3 (Fe_2O_3.SnO_2 with 0.008 M SDS synthesized).

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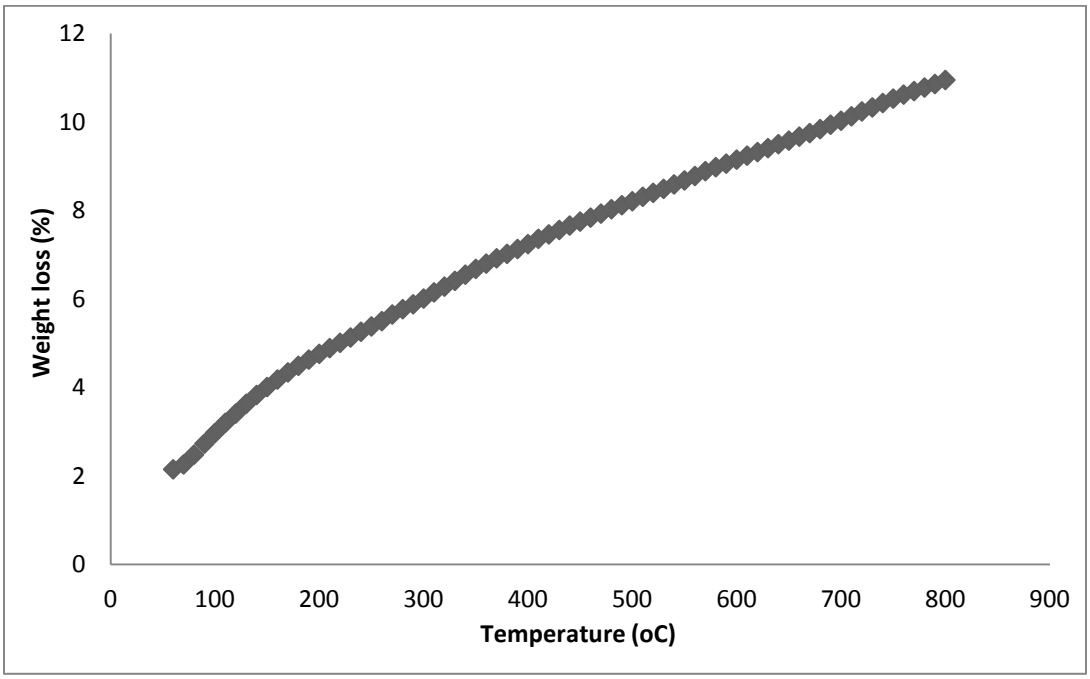

Figure 4. TGA- Thermogram of sample D3 after calcination (Fe_2O_3.SnO_2 synthesized with 0.008M SDS concentration)

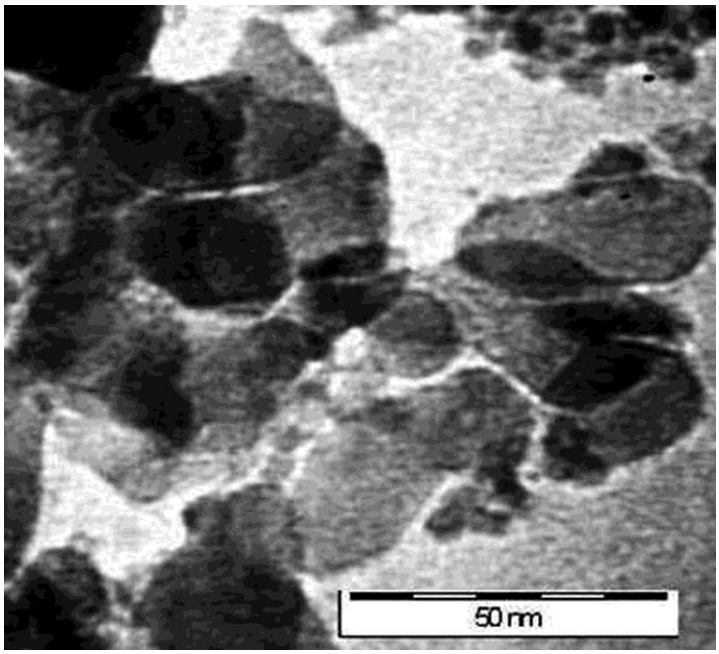

Figure 5. TEM image of sample D3 (Fe_2O_3.SnO_2 synthesized with 0.008 M SDS)

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The results of TEM indicated that the particle size of iron oxide-tin oxide nanoparticles ranges between 8 to 11 nm.

SEM-EDX image of sample D3 (Fe_2O_3.SnO_2 with 0.008 M SDS synthesized) was shown in Fig. 6. It was observed that the sample D3 (Fe_2O_3.SnO_2 synthesized with 0.008 M SDS) had pyramidal shape and porous morphology. EDX spectra in Fig. 7 confirmed the presence of iron and tin in the sample. Iron was present 11.38 % and tin percentage in the sample was 6.88 while percentage of oxygen and carbon was observed as 72.53 and 9.21 % respectively. The XRD pattern of D3 (Fe_2O_3.SnO_2 with 0.008 M SDS synthesized) presented in the Fig. 7 showed the hkl value of SnO_2 and Fe_2O_3. The hkl values 101 and 002 were for SnO_2 at 2θ value 33.68 and 57.79. These values correspond to the tetragonal SnO_2 (ICSD 39173 (PDF 77-447) with crystal parameters a=b= 4.7382 A^o and c=3.1871 A^o. The XRD lines having hkl values 110, 116, 300, 119 and223 at 2θ values 35.43, 54.021, 63.672, 72.09 and 78.750 were for hematite rhombohedral Fe_2O_3 with crystal parameters a=b=5.0356 A^o and c=13.7489 A^o ((ICSD 64599 (PDF 79-7). The characterization of rest of the samples was similar and confirmed the results.

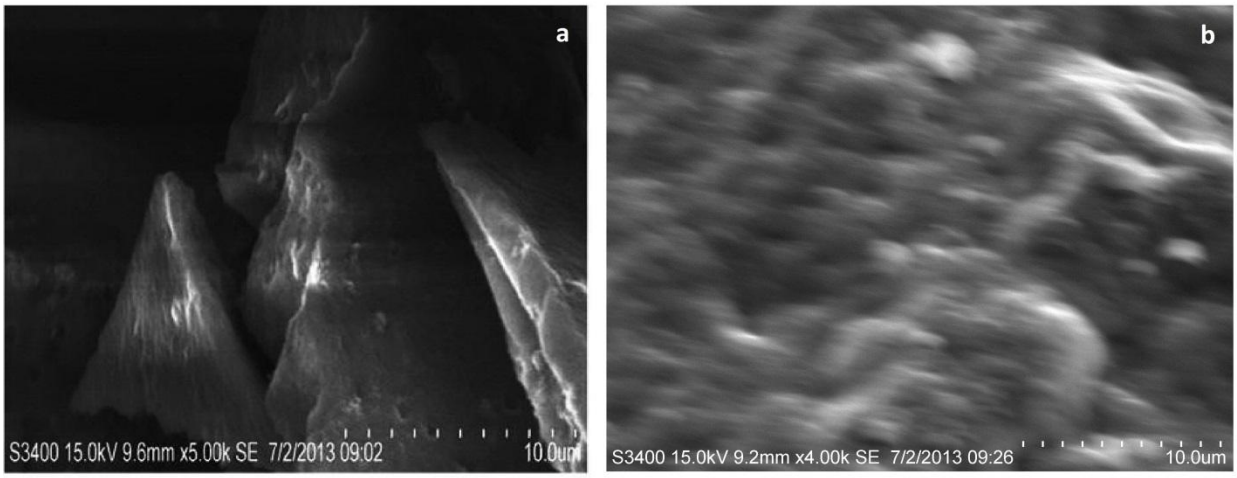

Figure 6. SEM image of sample D3 (Fe_2O_3.SnO_2 synthesized with 0.008 M SDS), **a** showed shape **b** showed porous morphology

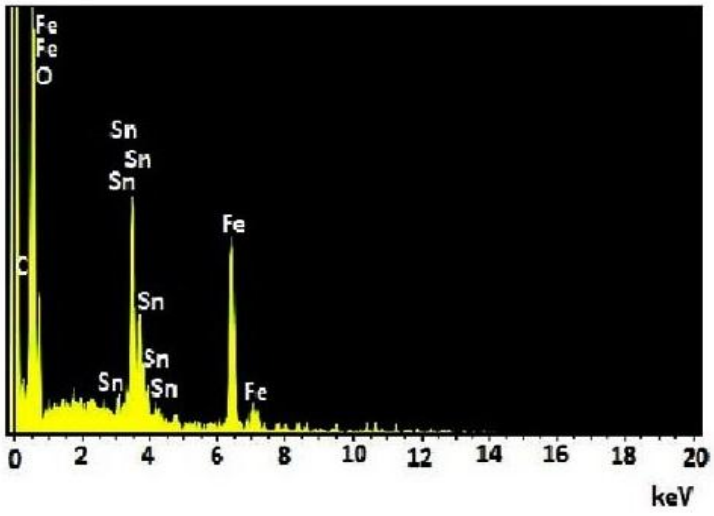

Figure 7. EDX spectra of sample D3 (Fe_2O_3.SnO_2 synthesized with 0.008 M SDS)

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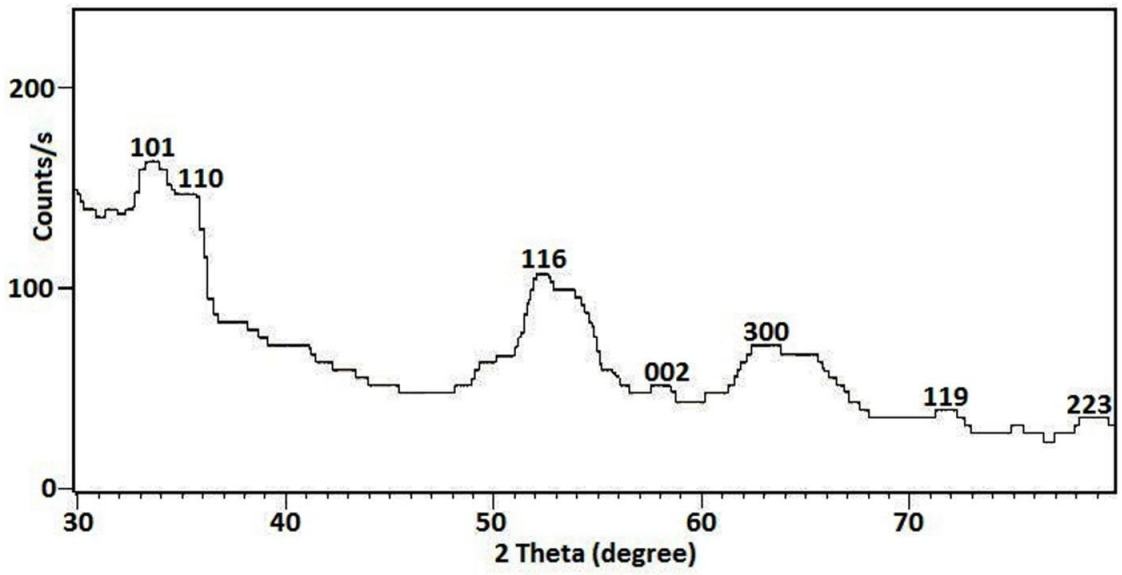

Figure 8. XRD pattern of sample D3 ($Fe_2O_3.SnO_2$ synthesized with SDS concentration 0.008M)

3.2. Bactericidal Activity

Bactericidal activities of $Fe_2O_3.SnO_2$ synthesized with different SDS concentrations were determined against five different strains. *Bacillus subtilis*, *Bacillus licheniformis* were gram positive strains and *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were gram negative strains used for this purpose. Zone of inhibitions were measured and the results were shown below in Table 2.Observations showed that the products are more active against gram positive strains.

Sample D1 $Fe_2O_3.SnO_2$, which was synthesized with 0.012 M SDS found less active against the selected strains. The zone of inhibition observed by it against *B. subtilis* was 0.7 cm and 0.65 cm against *B. lichniformis* which were the smallest zones shown by these samples. It also did not showed any activity against gram-negative strains. The reason of such behavior was that highest amount of SDS was used in the synthesis of this sample so more number of pores were developed in it on calcination [9-10]. This number of pores reduces the activity of sample against bacterial strains.

Sample D2 for which 0.010 M SDS was used to synthesized the sample of $Fe_2O_3.SnO_2$ showed good activity in comparison with sample D1($Fe_2O_3.SnO_2$, which was synthesized with 0.004 M SDS) against gram-positive bacterial strains. But it also did not show any activity against gram negative strains. 0.875 cm and 1.6 cm were the zones of inhibition observed against *B. subtilis* and *B. lichniformis* respectively. As slightly less amount of surfactant i.e. SDS was used for its synthesis so a little less number of pores were developed which increases its activity against bacterial strains.

D3 sample of $Fe_2O_3.SnO_2$, was synthesized with 0.008 M SDS showed activity against gram-positive as well as one strain of gram-negative bacteria. It was found active against *B. subtilis*, *B. lichniformis* and *E. coli* with 0.75 cm, 0.8 cm and 0.725 cm zone of inhibitions respectively. In this sample less amount of SDS was used for synthesis as compare to other two samples discussed above so slightly less amount of pores were developed which increases its anti-bacterial range to gram-negative strain.

Sample D4 $Fe_2O_3.SnO_2$, which was synthesized with 0.006 M SDS showed activity against just only two strains. Among these one was gram-positive and one was gram-negative. 3.25 cm zone of

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Table 2. Zone of inhibition developed against bacterial strains by $Fe_2O_3.SnO_2$ (with various SDS concentrations)

Sr. No.	Sample ($Fe_2O_3.SnO_2$) (0.06 M $FeCl_3.6H_2O$, 0.05 M $SnCl_4.5H_2O$) SDS	Zone of inhibition (cm)				
		B. subtilis	*B. lichniformis*	*E. coli*	*P. aeruginosa*	*K. pneumonia*
1	D1 0.012 M	0.7	0.65	0*	0*	0*
2	D2 0.010 M	0.875	1.6	0*	0*	0*
3	D3 0.008 M	0.75	0.8	0.725	0*	0*
4	D4 0.006 M	3.25	0*	0*	0.5	0*
5	D5 0.004 M	1.075	2.35	0*	2.05	0*

* No activity observed against these strains by the samples in current experimental conditions.

inhibition was developed by it against *B. subtilis* which was the biggest zone developed against this strain by all these samples but it did not show any activity against the other gram-positive strain i.e. *B. lichniformis*. In the discussed above samples it was the first which showed activity against *P. aeruginosa* with 0.5 cm zone of inhibition. The improved activity was because of less number of pores as less amount of SDS was used in synthesis.

Sample D5 of $Fe_2O_3.SnO_2$ was the sample in this set which was synthesized with the smallest amount of SDS i.e. 0.004 M. It showed activity against both gram-positive strains and *P. aeruginosa* of gram-negative strains. 1.075 cm inhibition zone was developed against *B. subtilis* but bigger zones of inhibition were developed against *B. lichniformis* and *P.aeruginosa* which were 2.35 cm and 2.05 cm respectively. As lesser amount of SDS was used for D5 sample synthesis so lesser the number of pores were developed and in comparison to other synthesized samples good activity against the selected bacterial strains were observed.

In general, these nanoparticles were found good against gram-positive bacteria rather than gram-negative. This was due to the differences in the cell wall composition of these two bacteria. But on average sample D5 $Fe_2O_3.SnO_2$, which was synthesized with 0.004 M SDS was found most active against bacterial strains.

Pores were developed after calcination due to removal of SDS (anionic surfactant) which is a templating agent as studied in our previous work[9-10]. Morphology and surface area were effected by different calcination temperatures at 600 ^oC and 800 ^oC. The number of pores formed after calcination varies as the amount of SDS varied in the synthesis of product. Thus, these pores greatly influenced their activity against bacteria and it was found that as the SDS concentration decreased activity against bacterial strains increased.

Many hypotheses have been given regarding the action of nanoparticles against bacteria. Study is in process to develop complete mechanism. It was concluded from the conducted studies that the nanoparticles interact with cell membrane and rupture it caused the cell to undergo lysis or they developed bond with DNA nucleotides and broke it down into pieces. Fig. 13 showed these processes.

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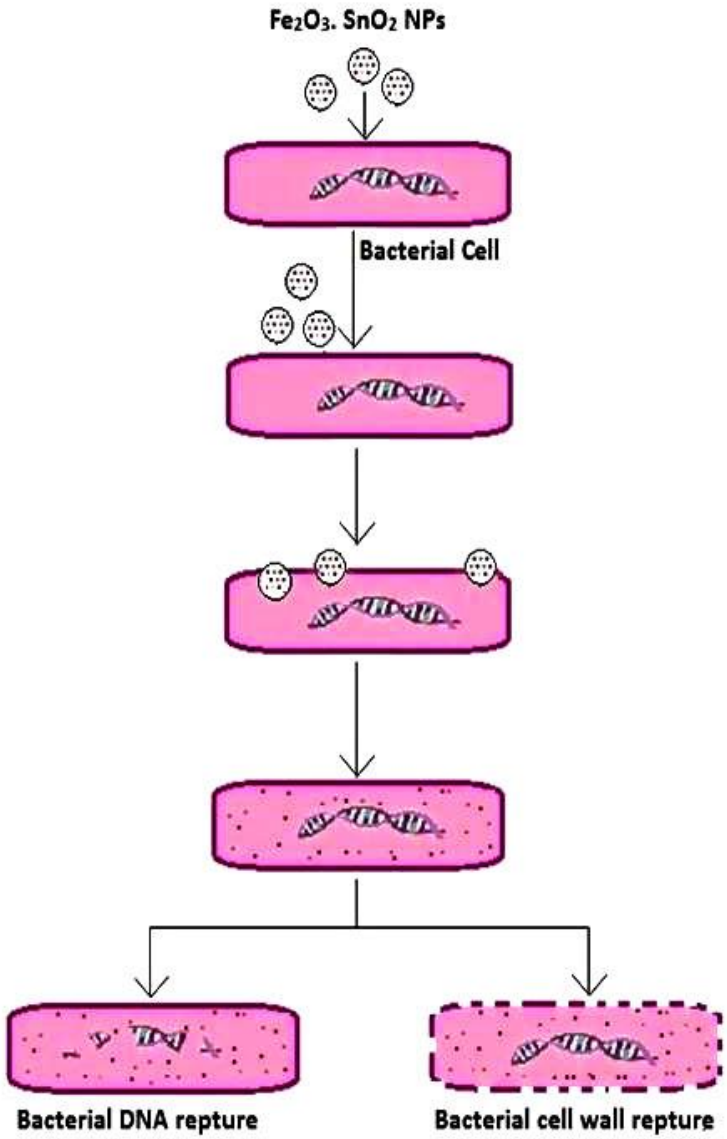

Figure 9. Bactericidal mechanism of action shown by nanoparticles.

4. CONCLUSION

$Fe_2O_3.SnO_2$ nanoparticles of size ranges from 8-11 nm were synthesized using hydrothermal method with various concentrations of SDS. Their activities against different bacterial strains were determined. SDS (surfactant) which is a templating agent creates pores in the products on calcination. These pores played an important role in determining the activities of samples against bacteria and their number is directly related to the concentration of surfactant (SDS) used. More the surfactant more will be the pores. Sample D5 synthesized with the minimum SDS concentration i.e. 0.004 M showed maximum activity on average against bacterial strains. The zone of inhibition developed by it against *B. subtilis* was 1.075 cm which was less than D4 sample but zones of inhibition against *B. lichniformis* was 2.35 cm and *P. aeruginosa* was 2.05 cm were greater than all other samples. Sample D1 which is synthesized with 0.012 M SDS (maximum SDS concentration) showed activity against only gram-positive bacteria i.e. *B.* subtilis was 0.7 cm and *B. lichniformis* was 0.65 cm. It was found that lesser the number of pores in the

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sample more active it will be against the bacterial strains. This is probably because pores are filled by the plasmic fluid and reduces the activity of particles against DNA or cell membrane.

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The authors declare no conflict of interest

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