Antibacterial, antioxidant and physicochemical investigations of tin dioxide nanoparticles synthesized via microemulsion method

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Abstract
The Tin dioxide nanoparticles (SnO2 NPs) were synthesized by modified microemulsion method and were screened for antibacterial and antioxidant applications. The crystalline nature was explored by using x-ray diffraction (XRD) analysis and the calculated crystallite size is 24.68 nm. The morphology was examined by scanning and transmission electron microscopies (SEM and TEM) and particles based on TEM image is 79.10 nm. The elemental analysis was performed by energy dispersive x-ray (EDX) and only desired elements was detected. The optical activity was investigated using diffuse reflectance spectroscopy (DRS) and band gap derived via Tauc plot is 2.82 eV. The surface functional moiety was detected by Fourier transform infrared spectroscopy (FTIR). The agar well diffusion technique was manipulated to screen the SnO2 NPs against selected bacteria whereas DPPH free radical scavenging activity was also performed. The activity of both samples was noticed to increase with increasing the amount of samples in the experiment. The SnO2 NPs with IC50 value of 1.50 g was found more active than the ascorbic acid with IC50 of 2.42 g.

1. Introduction
Humans and microbes have peculiar and beneficial association, some of them are advantageous while other are highly toxic concerning to the health of human. The disease causing microbes developed the drug resistant strain owing to the excess and inappropriate manipulation of broad spectrum antibiotics that can defy scientific treatment. The resistant bacteria against many antimicrobial agents was declared is global risk for human health. The quest for secure and alternate antimicrobial agents is on in order to subdue such resistant microorganisms. Thus, the development of nanotechnology and the production of nanoparticles have provide an effective way for treatment of infectious diseases to combat antibiotic resistance bacteria. Recently, the oxides of metal and metalloid are extensively studied to explore their biological potential and encounter drug resistant pathogens.

The SnO2 is n-type semiconductor attracting the attention of researcher due to their distant properties and tunable morphologies, which shows marvellous efficacy in photocatalysis, dye sensitized solar cells, humidity sensor and optoelectronic devices (Anusha et al 2018, Sagadevan et al 2018). SnO2 has got the entrancing properties like non-toxicity, high transmittance in visible region, high chemical stability and a wide band gap (3.6–4 eV) (El Radaf and Abdelhameed 2018, Zhang et al 2018, Chanu et al 2020). SnO2 NPs also exhibit properties like the antibacterial, antioxidant and biocompatibility and are reported for the antibacterial activity against T. viridea (Rehman et al 2019b). The inactivation of gram-negative and gram-positive Escherichia coli Staphylococcus aureus respectively in dark conditions under UV has been reported using SnO2 NPs(Mohan et al 2018).
Previously a number of methods have been utilized for the synthesis of SnO₂ NPs including sol-gel method, laser ablation, evaporative decomposition, wet chemical and green synthesis (Haq et al 2016, Singh et al 2019). However, the microemulsion process for the fabrication of SnO₂ NPs was yet reported, this method provide better control over size and morphology of the nanomaterials (Rehman et al 2019a).

This research work was planned to synthesize SnO₂ NPs via microemulsion methods with minor modification and characterized using XRD, EDX, TEM, SEM, UV-DR and FTIR spectroscopies. To the best of our knowledge based on the available literature, the SnO₂ NPs synthesized via microemulsion process were not being screened for antioxidant and antibacterial activity. The as-synthesized NPs were analysed for antibacterial activity against Escherichia coli (E. coli), Staphylococcus aureus (S. aureus) and Pseudomonas aeruginosa (P. aeruginosa) using agar well diffusion method. The DPPH free radical scavenging efficacy of SnO₂ NPs was also explored.

2. Material and methods

2.1. Materials

The laboratory grade chemicals including tin dioxide dehydrates, ethanol, ethanediol, sodium hydroxide, ascorbic acid, agar nutrient, DPPH were purchased for sigma Aldrich and used without further purification. The glassware were washed with 15 % (v:v) nitric acid solution followed by rinsing with deionized water. The deionized water was utilized throughout the research work.

2.2. Synthesis of SnO₂ NPs

A stock solution was prepared by dissolving 0.225 g of SnCl₂.2H₂O in 1000 ml deionized water. For each reaction, an emulsion was prepared by mixing of 10 ml of deionized water, 10 ml ethanol and 20 ml ethanediol. To this emulsion, 60 ml of the prepared stock solution of SnCl₂.2H₂O was slowly added with constant heating and stirring of (50 °C) and (250 rpm) respectively. The pH of the reaction mixture was fixed at 10 by adding 0.5 M NaOH and reaction was allowed stir for 60 min under same condition. The white precipitate materialized was aged for 24 h and collected via filtration followed by thorough washing using deionized water and ethanol. The final product was dried in oven at 150 °C for 6 h and later stored in air tight veil.

2.3. Characterization

The XRD pattern of SnO₂ NPs was recorded in the range of 20 to 80 utilizing Panalytical X-pert pro, where Cu source was used. The FWHM values of the diffraction peaks was used to calculate the crystallite size. The morphological analysis was carried out through TEM model JEM 2010 with accelerating voltage of 100 keV and SEM model 5910 (Japan). The EDX model INCA 200 (UK) coupled with SEM was operated from elemental analysis. The DRS model lambda-950 was run in the between 400 to 1000 nm and band gap energy was determined through Tauc’s plot. The KBr pellet was analyzed by FTIR model Nicolet 6700 (USA) in the range of 4000 to 400 cm⁻¹ to identify the functional groups present on the surface of SnO₂ NPs.

2.4. Antimicrobial activity assay

The antibacterial activity of the SnO₂ NPs was investigated against the bacteria pathogens using agar well diffusion method. The Agar nutrient used to culture bacteria. The bacteria used were obtained from microbial biotechnology laboratory, department of Zoology, UAJ&K. The two GNB (E. coli and P. aeruginosa) and one GPB (S. aureus) bacteria were used during this study. The overnight bacterial culture was dissolved using agar medium, which was freshly prepared and then poured into the sterilized petri dishes and allowed for solidification. This process was performed in a laminar flow at room temperature and wells with diameter of 5 mm were made in each plate by using sterilized micropipette tip and to remove the agar plug sterilized needle was used. The stock suspension of each SnO₂ NPs were prepared in deionized water by ultrasonic dispersion of 50 mg in 500 ml and different volumes (20, 40, 60, 80 and 100 μl) of the suspension was transferred into each well and was incubated at 37 °C for overnight. After 24 h, the activity of SnO₂ NPs was checked by measuring the zone of inhibition in millimeter (mm) around each well (Haq et al 2018a). The bacteria used were obtained from microbial biotechnology laboratory, department of Zoology, UAJ&K. The two Gram-negative bacteria (GNB) i.e. Escherichia coli (E. coli) and Klebsiella pneumonia (K. pneumonia) and one Gram-positive bacteria (GPB) Staphylococcus aureus (S. aureus) bacteria were used during in this study.

2.5. DPPH radical scavenging assay

The DPPH scavenging activity of SnO₂ NPs was examined by the method reported previously with some modifications (Rehana et al 2017). The stock solution of SnO₂ NPs with various concentrations (10, 20, 30, 40 and 50 mg l⁻¹) was prepared. For the typical procedure, 1 uL of each stock solution was mixed 1 ml of
DPPH solution and the total volume of 3 ml was obtained by adding DMSO and was incubated for 30 min at room temperature (25 °C±2°C). Afterward, the reaction mixture was analyzed via UV-Visible spectrophotometer and the absorbance at 517 nm was recorded. The percentage scavenging activity at different concentration of SnO2 NPs was determined by equation (1), where \(A_i\) is the absorbance of sample and \(A_o\) is the absorbance of control.

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\%\text{Radical scavenging potential} = \frac{A_o - A_i}{A_o} \times 100
\]

3. Results and discussion

3.1. XRD analysis
The XRD pattern (Fig. 1) gives characteristic peaks for SnO2 NPs at 2\(\theta\) position 26.45, 33.79, 37.86, 51.99 and 65.34, belonging to the miller indices of (110), (101), (200), (211) and (301) respectively. The diffraction peaks detect in the x-ray diffractogram with hkl values are matched with the peaks listed in the reference card 01-077-0448, confirming the synthesis of SnO2 NPs with tetragonal geometry with space group of P42/mnm and space number 136. The reference card proposed that the length of a and b coordinates of the tetragonal structure are 4.739 Å whereas the length of c coordinate is 3.1869 Å. All the three angle \(\alpha\), \(\beta\) and \(\gamma\) of 90° suggesting the formation of a perfect tetragonal shaped crystal. The average crystallite size was found to be 24.68 nm with lattice strain of 0.462 %.

3.2. SEM analysis
The morphological study of the SnO2 NPs was conducted through SEM analysis. The micrograph (Fig. 2) reveals that the majority of the particles are highly agglomerated and seen to connect with each other, however few individual particles with clear boundaries were also observed varied in shape and size. The different size cavities were also observed in the SEM image. The particles size estimated are ranging from 92.78 to 98.42 nm with average size of 95.60 nm.

3.3. TEM analysis
TEM analysis (figure 3) provide further realization into the morphology and particle size distribution profile of the SnO2 NPs. The images revealed that the irregular shaped particles are unevenly distributed. The small size particles get agglomerated during the dehydration process forming rigid solid structure with various morphologies. It is been reported that the agglomerated particles are more stable than individual particles due to
**Figure 2.** SEM Micrograph of SnO$_2$ NPs synthesized via microemulsion method.

**Figure 3.** High and low magnification TEM Micrograph of SnO$_2$ NPs synthesized via microemulsion method.
the dissipation of their energies (Sirelkhatim et al 2015). The particles size ranging from 54.82 to 92.56 nm with an average size of 79.10 nm.

3.4. EDX analysis

The EDX spectrum of SnO$_2$ NPs is shown in figure 4 exhibit a sharp peak at 0.3 keV that is ascribed to O while a group of sharp bands from 3.5 to 4 keV along with a very small signal at 2.6 keV attributed to the Sn and Cl, respectively. The EDX analysis disclosed that the synthesized SnO$_2$ NPs exhibits stoichiometric composition of O and Sn whereas the small amount Cl also found as an impurity. The weight percentage estimated are 78.7, 20.2 and 1.1% corresponding to Sn, O and Cl, respectively. The presence of Cl is might be due to the use of SnCl$_2$ salt during synthesis, whoever the weight % of Cl almost negligible as compared to Sn and O.

3.5. DRS analysis

The electronic state of the SnO$_2$ NPs were determined from the transmittance spectra as given as inset in figure 5, shows that the sample is translucent in a wide range of wavelength. The transmittance edge wavelength was determined by coinciding the sharp raising portion of the UV-vis curve with x-axis of the DRS spectrum according to previous finding [1]. The transmittance edge is located at 376.78 nm suggest a significant absorbance of light in the visible region. The band gap energy for SnO$_2$ NPs was enumerated from Tauc’s plot by joining sharp rising portion with horizontal axis of the $(hv/nT)^2$ against $hv$ is 2.82 eV. The band gap energy found lower than that reported previously, which might be to the larger particle size of SnO$_2$ NPs.
3.6. FTIR analysis
The FTIR spectrum of the SnO\textsubscript{2} NPs is presented in figure 6, possess a broad band ranging from 3421–3240 cm\textsuperscript{-1} and a less intense small peak at 1621 cm\textsuperscript{-1} assigned the stretch and bend vibrational mode of O-H moiety (Haq et al 2019\textsuperscript{a}). The peak in the range of at 1244–921 cm\textsuperscript{-1} are because of the Sn-O vibration in the lattice structures (Haq et al 2019\textsuperscript{b}). The wide range bands at 771–503 cm\textsuperscript{-1} exists owing to the homogenization of two peaks at 686 and 538 cm\textsuperscript{-1} are assigned vibrational of Sn-O-Sn and Sn-O, respectively (Haq et al 2020).

3.7. Antimicrobial activity
The antibacterial property of SnO\textsubscript{2} NPs against GNB i.e. E. coli, P. aeruginosa and GPB i.e. S. aureus was deduced by agar well diffusion method. As a result of activity, clear zone of inhibitions were formed around the wells (figure 7), which were measured in millimeters (nm) in listed table 1. The result demonstrates that by raising the concentration of suspension in wells, antibacterial activity of SnO\textsubscript{2} NPs was enhanced. The exact mechanism of the antibacterial activity of SnO\textsubscript{2} NPs is still not known, however it believed that the Sn cation release in aqueous solution have the capacity to inhibit the growth bacterial species. The SnO\textsubscript{2} NPs showed greater activity against GNB instead of GPB. This is ascribed to the differentiation in chemical structure of the cell wall of both type bacteria. The thick peptidoglycan layer present in the cell wall of GPB provide more strengthen and shows greater resistance toward the infiltration of SnO\textsubscript{2} NPs (Haq et al 2018\textsuperscript{b}). Whereas the cell wall of GNB is made up of soft layer of peptidoglycan, which facilitate the entry of penetrating agent. The enhanced activity of SnO\textsubscript{2} NPs against GNB was may also be due to the strong negative surface due to the presence of phospholipid and lipopolysaccharide, which provide binding site for Sn cation to attached in the surface of bacteria and rapture the outer membrane (Shah et al 2019). The partial negative surface of GPB is due to the presence of teichoic acid act as poor binding site for the metal cation, which led less growth inhibition (Haq et al 2020). In aqueous suspension of SnO\textsubscript{2} NPs reactive species are generated which are Sn cation, super oxide radical anions and hydroxyl radicals. The SnO\textsubscript{2} NPs discharge Sn ions, which interact with thiol group of vital bacterial enzyme resulting in the inactivation and ultimate death of microorganisms. The light contact with SnO\textsubscript{2} NPs surface result in the excitation of electron, which produce oxygen ion on reaction with absorbed oxygen, which lead to the formation of H\textsubscript{2}O\textsubscript{2} on combining with H\textsubscript{2}O molecule. Thus H\textsubscript{2}O\textsubscript{2} disturb the cytoplasmic functions by penetrating in the bacterial cell and prove itself fatal for the microorganisms (Shah et al 2019).

3.8. Antioxidant property
The free radicals belongs to different chemical origin containing one or more and are highly unstable, extract an electrons from other molecules to get stability led to the denaturation of the target molecule (Gültekin et al 2016). These highly reactive species are continuously formed inside the human body and have potential to
damage the short lived molecules and cellular components like protein, lipids and DNA (Fouda et al 2020). DPPH is stable free radical and become stable upon accepting hydrogen or an electron to form stable diamagnetic molecule. The damaging effect of free radical species including cancer, heart diseases and neurodegenerative disorder may be reduced by scavenging free radicals (Rehana et al 2017). The alcoholic solution DPPH radicle has deep violet color and shows strong absorbance at 517 nm. The DPPH radical get reduced by accept hydrogen or electron from the antioxidant and turn to light yellow color with a significant decrease in the absorbance maxima. The decrease in the absorbance maxima are may due to the normalization/reduction of DPPH ions which are responsible for absorbance. The synthesized SnO2 NPs was subjected to the antioxidant activity in dose-dependent manner whereas and the ascorbic acid was used as standard. The obtained results were presented in figure 8, shows that the antioxidant activity increases with increasing concentration of SnO2 NPs and ascorbic acid. This enhanced antioxidant activity is due to the larger number of SnO2 NPs at higher concentration to combat more reactive oxidant species (DPPH radical cation) and thus, the percentage radical scavenging potential of SnO2 also increased. The sample with smaller IC_{50} value have greater

Table 1. The zones of inhibition in millimeter (mm) showing the antibacterial activity of SnO2 NPs at different concentration.

| Volume of SnO2 NPs | S. aureus | E. coli | P. aeruginosa |
|--------------------|-----------|---------|---------------|
| 20                 | 5         | 4       | 6             |
| 40                 | 7         | 6       | 8             |
| 60                 | 8         | 8       | 9             |
| 80                 | 9         | 9       | 10            |
| 100                | 10        | 11      | 12            |
| Solvent            | —         | —       | —             |

Figure 7. Experimental photographs showing antibacterial activity of SnO2 NPs (a) = E. coli, (b) = P. aeruginosa and (c) = S. aureus.
hydrogen donating ability to stabilize the unstable free radicals. This reveals the higher free radical scavenging efficacy of SnO₂ NPs. The small IC₅₀ value suggest that the antioxidant potential of SnO₂ NPs (IC₅₀ = 1.50) was higher the ascorbic acid (IC₅₀ = 2.42).

4. Conclusions

The SnO₂ NPs was successfully synthesized by microemulsion method using ethanol, ethanediole and deionized water. The highly crystalline nature with nano-sized crystallite of SnO₂ NPs was confirmed by XRD analysis. The smooth surface and agglomerated particles of SnO₂ NPs were identiﬁed via SEM and TEM analysis. The EDX analysis conﬁrm the elemental composition of SnO₂ NPs whereas the smaller band gap determined through Tauc plot was due to the larger particles size. The antibacterial activity of SnO₂ NPs was found higher against the GNB as compared to GPB and this difference in activity was attributed to difference in the cell wall composition and surface negative charge. The DPPH free radical scavenging activity of SnO₂ NPs was found to be increased with the increase in concentration of sample solution.

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