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SYNTHESIS CHARACTERIZATION, OPTICAL AND ANTIBACTERIAL STUDIES OF Co-DOPED SnO_2 NANOPARTICLES

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This study was involved to synthesize, characterize and investigate the antimicrobial properties of Cobalt-doped tinoxide (Co-doped SnO_2) nanoparticles. Cobalt-doped tinoxide (Co-doped SnO_2) nanoparticles were prepared by using a simple and cheap co-precipitation method. The synthesized and Cobalt-doped tinoxide (Co-doped SnO_2) nanoparticles were characterized by using different characterization techniques i.e. Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD) analysis, and Ultraviolet-visible (UV-Vis) spectroscopy. The antimicrobial activity of the synthesized Cobalt-doped tinoxide (Co-doped SnO_2) nanoparticles was carried by agar well diffusion method against both Gram-negative (*Escherichia coli*) and Gram-positive bacteria (*Bacillus subtilis*). Results from XRD, SEM, EDX and UV-Vis analyses demonstrated successful synthesis of Co-doped SnO_2 nanoparticles as seen in their tetragonal structures. The average grain size of the synthesized nanoparticles was found to be 24.8 nm. The optical properties were studied by measuring the energy band gap and were found 1.50 eV for un-doped SnO_2 nanoparticles, and it decreases to 1.48 eV for Co-doped nanoparticles. Co-doped SnO_2 nanoparticles were exhibited the significant and maximum antibacterial activity against both bacterial strains with the zone of inhibition (ZOIs) of 16 ± 0.8 mm and 22 ± 1.6 mm for *Escherichia coli* (*E.coli*) and *Bacillus subtilis* (*B. subtilis*) respectively. Co-doped SnO_2 nanoparticles showed good activity against both Gram-negative and Gram-positive bacteria confirming these as future broad spectrum antibacterial. Thus, the preparation is a good candidate for further development into therapeutic formulations.

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1. Introduction

Nanotechnology is considered as new generation technology. This new field is greatly influencing the economy of the world by producing novel and great valued products, product usage and more efficient manufacturing methods [1]. Nanotechnology is leading to the production of many types of nanoparticles such as metal, metal oxide, doped and un-doped metal and metal oxide etc. Among these, doped metal oxide nanoparticles are of much important having many technological applications such as gas sensors, optical and electronic devices, catalysts, rechargeable batteries [2] and for environmental remediation [3,4]. Moreover nanoparticles show good antibacterial properties [5]. For obtaining desirable properties of the synthesized nanoparticles, controlled synthesis and synthesis method play an important role and solution phase method are very good in this regard [6]. SnO_2 is a very unique material with its important properties such as low operating properties, high thermal stability, and high degree of transparency in visible region of spectrum. It is also an important n-type semiconductor with wide band energy (3.6-3.8 eV) [7]. Because of its excellent optical, electrochemical, and catalytic properties it is being used in many practical applications such as solid-state sensors, solar cells, Li-batteries and optoelectronic devices [8-10]. SnO_2, similar to other wide band gap energy materials such as TiO_2, SiO_2 and ZnO_2, shows good photo catalytic activity. Metal doped SnO_2 nanoparticles have been successfully

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utilized for the photocatalytic decomposition of many organic compounds and dyes [11]. The antibacterial studies of the doped and un-doped SnO_2 nanoparticles are also being studied [12-14]. There are different methods like mechanochemical method [15], solvothermal [16] sol-gel method, chemical co-precipitation method [17], simple chemical precipitation method and other methods are available for the synthesis of metal doped SnO_2 nanoparticles [18]. In the present study Co-doped SnO_2 nanoparticles were synthesized by using simple co-precipitation method and their characterization, optical and biological properties were studied [19]. Human beings have been facing different type of diseases since ancient times caused by pathogenic microbes. Among these pathogenic microbes, bacteria have a large share in spreading different infections and fatal diseases. Scientists are trying to develop various chemical agents known as medicines for fighting against these pathogens. Among these agents nanoparticles are proving very efficient against various diseases. Nanoparticles have some special qualities which make them an efficient alternative to conventional medicines. These have very small size and easily permeable through cell membrane and transport in every part of the body in a very short period of time as compared to conventional medicines. Therefore it is of great importance to know and to understand antimicrobial effects of the synthesized nanoparticles. A large number of nanoparticles are being synthesized and their antimicrobial behavior also being studied. Behavior of SnO_2 nanoparticles against pathogenic microbes is studied through bioassay. Different studies have shown that the concentrations as well as size are the main factors that mainly control the bioactivity of nanoparticles. The studies on the antibacterial activity of SnO_2 nanoparticles show that the antibacterial effect increases with increasing the concentration of nanoparticles and doping enhances the antibacterial properties of the nanoparticles synthesized. The SnO_2 nanoparticles have higher activities against *E.coli* as compared to *S. aureus* and *T. viridea* [20]. Our study focuses on enhancing these properties by doping.

2. Experimental

The chemicals used were obtained from Merck (Germany) and were used without further purification. All the chemicals were of analytical grade. The chemicals used were $SnCl_4.2H_2O$ (Tin tetra chloride Dehydrate), Co $(NO_3)_2.6H_2O$ (Cobalt nitrate Hexahydrate), NH_4OH (Ammonia solution), NaOH, Nutrient agar, Nutrient broth and Methanol.

2.1 Bacterial strains

The human bacterial pathogens culture collections such as *Escherichia coli* which cause different infections like urinary tract infections, food poisoning, neonatal meningitis and *Bacillus subtilis*, produces infections like sepsis, pneumonia and meningitis [21,22] were obtained from PCSIR laboratories Lahore, Pakistan.

2.2 Synthesis of Co-doped SnO_2 nanoparticles

The synthesis of the Co-doped SnO_2 nanoparticles was carried out using co-precipitation method. For the synthesis of Co-doped SnO_2, 0.5 M solution of $SnCl_4.2H_2O$ and 0.01 M solution of cobalt nitrate hexahydrate were prepared in deionized water. Both the solutions were prepared by well shaking and sonication. Nano-crystalline Co-doped SnO_2 particles were prepared by mixing above two solutions in appropriate quantities. Then mixture was stirred for 30 min. After mixing, the precipitation of the dissolved chemicals was achieved by addition of a 5 M NH_4OH solution drop by drop with continuous stirring for about 30 min. In this method the process of precipitate formation was controlled by pH and temperature change. Precipitates were then filtered, washed away properly with de-ionized water in order to eliminate any chloride or other impurity. Then these particles were first dried at room temperature (32 $^{\circ}$C) and then in an oven at 110 $^{\circ}$C for 12 h. Finally the synthesized particles in fully dried form were ground to fine powder.

2.3 Characterization of Co-doped SnO_2 nanoparticles

Energy dispersive X-ray technique was employed for the determination of the composition and morphology of the synthesized un-doped and Mn-doped ZnO nanoparticles. In addition, characterization

of the crystalline structure of the nanoparticles was done using the XRD i.e. PANalytical X'Pert diffractometer instrument with Cu-Kα radiation (wavelength 0.154 nm) operating at 40 kV and 30 mA. Measurements were scanned for diffraction angles (2θ) ranging from 20 to 90^o with a step size of 0.02^o and a time per step of 1 s. The absorption spectrum and band gap energies of the synthesized nanoparticles were determined by UV-visible spectrophotometer (Spectra Flash SF 550, Data color Inc., USA), while their morphological features were determined using SEM (Jeol, 5910LV).

2.4 Evaluation of antibacterial activity

The antibacterial activity of Co-doped SnO_2 nanoparticles counter to pathogenic bacteria classes like *Escherichia coli* and *Bacillus subtilis* was carried out by following the method as described by [23,24]. Antibacterial activity of Co-doped SnO_2 nanoparticles on the growth of organisms was checked by agar well diffusion method. Standard antibiotics; cephradine and ampicillin were taken as positive control and distilled water served as negative control for all the species. Solutions of the Co-doped SnO_2 nanoparticles were prepared in distilled water by ultra-sonication. Different concentrations of nanoparticles were prepared; 1 M, 0.75 M, 0.5 M and 0.1 M. The concentration of the reference antibiotics was 1 mg/cm^3.To check the antimicrobial activity nutrient agar was used. The stock slants of bacterial culture were taken and transferred into nutrient broth to prepare inoculum. Then these cultures were placed in the incubator shaker for 24 hours at 37 ºC for growth. Then 25 ml nutrient agar was added in each petri dish and these were inoculated with 5 ml inoculum of the respective microorganism. After solidifying the nutrient agar a set of four holes of diameter 4 mm at four peripheral positions in each petri dish were made with the help of a sterilized borer. These holes were properly marked and filled with standard solutions and nanoparticles solutions. Then these plates were placed for 1 hour in flat positions. After 1 hour the incubation period was on the go for next twenty four hours at temperature of 37 ºC. When the incubation period was over the petri plates were taken out from incubator and the diameters of zones produced around the wells were examined and recorded. Each experiment was made in triplicate and the inhibition zones are given as the mean ± standard deviation.

2.4.1 Determination of minimum inhibitory concentration (MIC)

The MIC was determined by recommended methods in [25] with some modifications (NCCLS, 2000). Briefly, the sterile tubes were incubated aerobically at 37 °C for 24 h, which contained 5 mL Muller- Hinton (MH) broth (Difco, USA) with approximate 5×10^9 CFU bacterial cells, without nanoparticles (the control group) and various concentrations of nanoparticles. The concentration of tube without visible growth of the bacterial cells was the MIC.

2.4.2 Determination of minimum bactericidal concentration (MBC)

To evaluate the MBC, 100 μL of sample from each tube without visible growth was transferred into MH agar plate (Difco, USA), and then incubated aerobically for another 24 h [26]. The concentration of the tube without growth was the MBC (in this test, the population in agar plate less than 10 was regarded no growth). All the measures were triplicate.

2.5 Statistical analysis

Statistical analysis was done by one-way or two-way ANOVA and the Tukey post-test using Graph Pad Prism v6.04 software (Graph Pad Software, Inc., La Jolla, CA, USA). Differences were considered statistically significant at $p<0.05$. Group sizes are indicated in the figure legends. All values are expressed as mean ± SD.

3. Results

3.1 Characterization of synthesized nanoparticles X-ray Diffraction analysis

The XRD patterns for Co-doped SnO_2 nanoparticles are shown in Figure 1. The XRD pattern of Co-doped SnO_2 nanoparticles was uniform and no impurities were observed in the spectrum as shown in Figure 1. Moreover, the XRD pattern in the spectrum showed intense and sharp peaks which confirmed

the crystalline nature of the Co-doped SnO_2 nanoparticles. The average crystallite size of the doped nanoparticles was found to be 24.86 nm as calculated by XRD diffraction pattern of the particles given in Table 1. The XRD spectra indicate that all the peaks are in good agreement to the distinctive peaks of single phase SnO_2 with tetragonal structure (JCPDS file No.039178). Moreover, small broadening at the bottom of peaks indicated that the crystalline size was small and in nanosize range. Hence, results were shown that there is found an increase of crystallinity in the synthesized Co-doped SnO_2 nanoparticles when compared to the reported pure SnO_2 nanoparticles [16,18]. This physical process is due to the fact that a portion of the metal oxide ions formed stable solid solutions with SnO_2 and the metal oxide ions occupy the regular lattice site in SnO_2. Thus, it may lead to the introduction of point defects and change in stoichiometry owing to charge imbalance. This results a distortion in the crystal structure of the host compound. When the metal oxide ions occupy the regular lattice site in SnO_2, the interference takes place between Co-doped metal ions and those of SnO_2 lattice, and owing to this the crystallinity of the Co-doped metal oxides of SnO_2 nanoparticles tends to enhance than that of pure SnO_2 [12,18]. The grain size of the synthesized nanoparticles was calculated by using the sheerer formula.

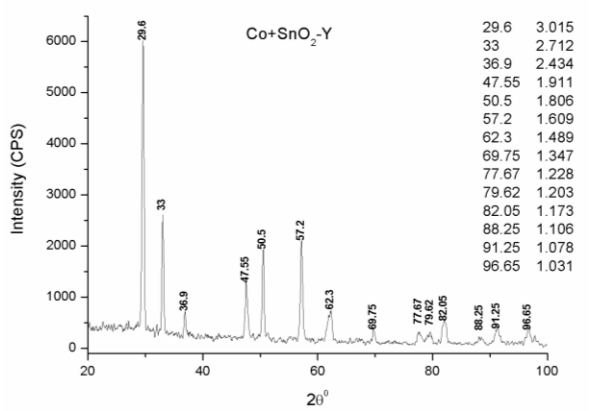


Fig. 1. X-ray diffraction pattern of the Co-doped SnO_2 nanoparticles

The crystallite sizes of all the samples were estimated by using Scherrer's formula and the values are listed in the Table 1.

Table 1. Crystallite size of Co doped SnO_2 nanoparticles

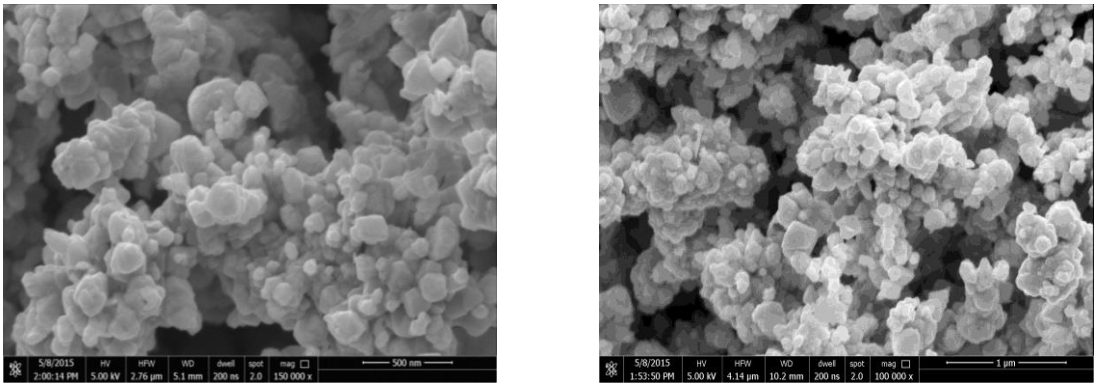
2 θ (deg) of the Intense peak	FWMH Intense peak (θ_2-θ_1)	FWMH Intense peak (β) radians	Grain Size (D) nm	Average Grain Size Nm
29.7	0.31	0.0058	24.9	
29.6	0.32	0.0057	24.4	24.8 nm
33.0	0.34	0.0059	25.2	

Values are mean ± SD (n = 3)

3.2 SEM analysis

The morphological and structural properties of Co-doped SnO_2 nanoparticles were observed using field scanning electron microscopy (SEM). From the Figure 2 (a) and (b) it is simply experiential that the synthesized nanoparticles were in nanometer range. SEM images were indicated that synthesized nanoparticles are in tetragonal shape. Some nanoparticles are well separated from each other while most were present in agglomerated form. The SEM investigations of synthesized nanoparticles reveal their crystallites nature. The same structural behavior of Co-doped SnO_2 nanoparticles was observed in XRD

results in terms of particles size. Moreover, Figure 2 also indicates that the synthesized nanoparticles had good crystalline nature. A close relationship was observed between the results of XRD and SEM.



(a) (b)

Fig. 2. SEM image of Co-doped SnO_2 Nanoparticles. a) at 500 nm b) at 1µm

3.3 EDX analysis

EDX studies were carried out in order to examine the chemical formation and composition of the synthesized nanoparticles and also to confirm the presence of Co-doped SnO_2 nanoparticles. Figure 3 shows the EDX spectrum for the synthesized Co-doped SnO_2 nanoparticles. EDX spectra confirmed the presence of the constituents i.e. Cobalt (Co), oxygen, (O) and tin (Sn) in the synthesized nanoparticles along with the trace amounts of impurities like Aluminum (Al) and Chlorine (Cl). It is clear from the Figure 3 that Co ions are successfully incorporated in the host SnO_2 material. Hence, EDX spectra affirmed the presence of Co-doped SnO_2 nanoparticles in the synthesized sample. It was clear from the EDX pattern that the nanoparticles were successfully synthesized. The consistent and sharp peaks with tin oxide and cobalt-tin oxide demonstrated that both synthesized nanoparticles were crystalline in nature [9]. Hence, synthesized nanoparticles were obtained in their pure forms.

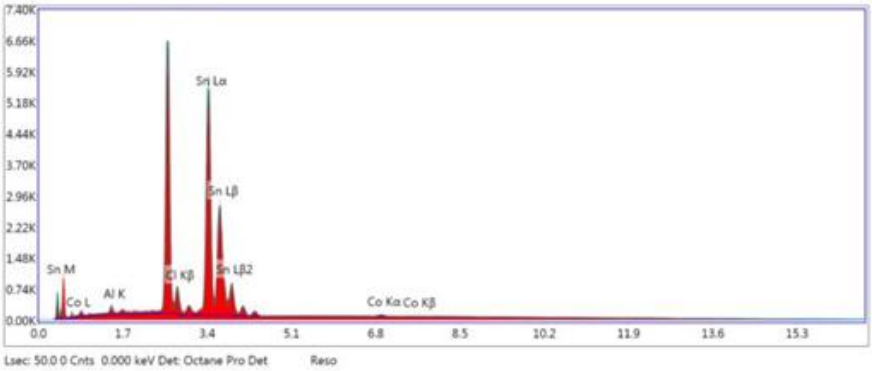


Fig. 3. EDX spectra of Co-doped SnO_2 nanoparticles

3.4 Optical measurements

Optical measurements have been performed in order to confirm the substitution of Co^{2+} for Sn^{4+} at room-temperature by UV-vis spectroscopy. A specific minimum amount of energy is required for this transition. Measurement of band gap plays a vital role in semiconductors. The band gap energy of an insulator is large (> 4 eV), but is lower for a semiconductor (< 3 eV) [16]. The value of *E*g is achieved by the intersection between the photon energy axes and linear fit [17]. Being semiconductors, the band gap energy of Co-doped SnO_2 nanoparticles should be less than 3 eV. So that UV-VIS-spectrophotometer measurement found band gap 3.36 eV for un-doped SnO_2 nanoparticles, and it decreases to 1.48 eV for

the synthesized Co-doped SnO_2 nanoparticles as shown in Figure 4. This decrease in the energy band gap was already observed in Co and Mn-doped SnO_2, [16,18] as well as in other transition metal doped oxides. This observation can be explained on the basis of the sp-d exchange interactions between the band electrons and the localized d electrons of the Co^{2+} ions substituting Sn^{4+} ions. The s-d and p-d exchange interactions give rise to negative and positive corrections to the conduction-band and valence band edges, respectively, leading to a band gap narrowing. This is in agreement with the band gap energy value for Co-doped SnO_2 nanoparticles in this study, which was less than 3 eV (Fig, 4).

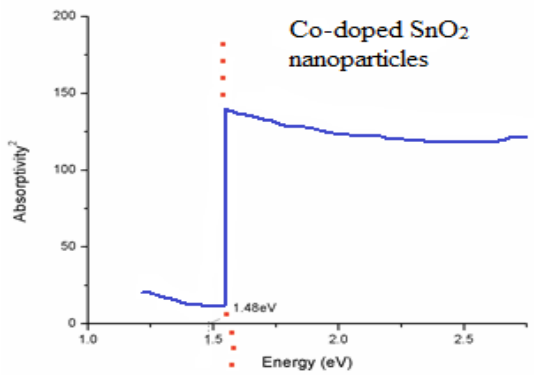


Fig. 4. Band gap determination of Co-doped SnO_2 nanoparticles

3.5 Antibacterial activity

Antibacterial activity of Co-doped SnO_2 nanoparticles on the growth of organisms was checked by agar well diffusion method, MIC and MBC measurements. The activity was a function of cobalt doping and crystallite size. The representative of Gram positive bacteria was *Bacillus subtilis* and of Gram negative bacteria were *Escherichia coli*. Co-doped SnO_2 nanoparticles were dispersed in autoclaved water by ultra-sonication and by dispersing these nanoparticles at different desired concentrations. Antibacterial activity of Co-doped SnO_2 nanoparticles against the chosen bacteria is given on the basis of inhibition zone (mm) in Figure 5 (a) and (b). Co-doped SnO_2 nanoparticles with the concentration of 500 µL were exhibited the significant and maximum antibacterial activity against both bacterial strains i.e. *Escherichia coli* and *Bacillus subtilis* with zone of inhibition of (16 ± 0.8 mm) and (22 ± 1.6 mm) respectively. It is clear that the synthesized nanoparticles show good activity against bacteria as compared to standard antibiotic Cephradine. The antibacterial action of the prepared nanoparticles increases as the concentration increases. The antibacterial activity of the prepared nanoparticles is also comparable with Ampicillin Trihydrate which was also used as standard antibiotic.

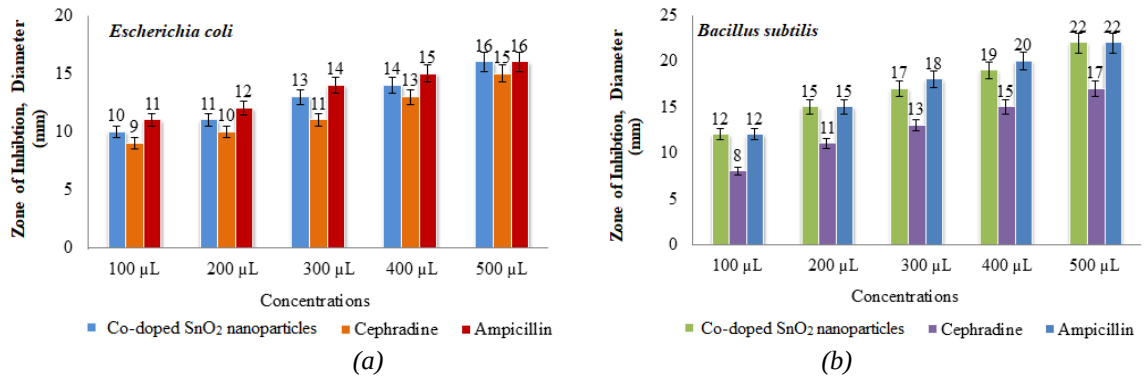


Fig. 5. Zones of inhibitions of Co-doped SnO_2 nanoparticles against E. coli (a) and B. subtilis strains (b)

A standard testing protocol was employed that is applicable to inorganic metal oxides and composite materials such as Co-doped SnO_2 nanoparticles. The MIC of the agent is the concentration at which the solution becomes turbid. A lower MIC corresponds to higher antibacterial effectiveness. The MBC is the lowest concentration (μg ml^{-1}) at which a compound will kill more than 99% of the added bacteria. In terms of both MIC and MBC, we found an inverse relationship between the particle size and activity. The findings are summarized in Figure 6. The MIC was generally observed to be in 9 mM and 11 mM and MBC was 27 mM and 31 mM (depending on the particular bacterial strain). Gram negative bacteria *E. coli* (MIC 11 mM and MBC 31 mM) was more resistant than Gram positive bacterial strains *B. subtilis* (MIC 9 mM and MBC 27 mM).

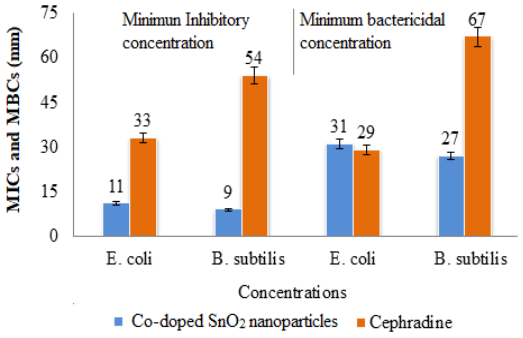


Fig. 6. Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations of Co-doped SnO_2 nanoparticles against different bacterial strains

The difference in activity against these two types of bacteria can be explained by the different structures and chemical composition of the cell surfaces. The cell wall of Gram-negative bacteria is different from the Gram-positive bacteria by having an outer membrane that covers the peptidoglycan layer. The positive surface charge of the metal nanoparticles facilitates their binding to the negatively charged surface of the bacteria which may result in an enhancement of the bactericidal effect. The activity of nanoparticles enhances because of increased surface to volume ratio. Cobalt doping further enhances the activity.

4. Discussion

In the present study Co-doped SnO_2 nanoparticles were synthesized successfully by using co-precipitation method. Tin Chloride dehydrates and Cobalt Nitrate hexahydrate solutions were mixed and then nanoparticles were precipitated by using Ammonia solution. The characterization and structural formation of the prepared nanoparticles was carried out by using usual sensitive instruments like UV-VIS spectrophotometer, powder X-ray Diffraction and scanning electron microscope. The XRD diffraction patterns indicate that the synthesized nanoparticles are single phase with tetragonal structure. The images obtained from SEM analysis shows that only few particles with spherical shape have been synthesized and most are with irregular shapes. Some nanoparticles are well separated from each other while most are present in agglomerated form because no capping agent has been used. The elemental composition was confirmed and estimated with EDX analysis. The EDX spectra and results confirmed the doping of cobalt in SnO_2 as well as the elemental composition of the synthesized nanoparticles. The band gap observed and calculated for SnO_2 nanoparticles was found smaller than the reported value of bulk SnO_2 .This decrease can be due to the strong quantum confinement effect of the nanoparticles. When these SnO_2 are doped with Co, the band gap energy is further decreases. Results of SEM, XRD, EDX and bad gap energy data were found supportive each other. A close agreement of our results was observed with the previously reported data [17,18].

The doped SnO_2 nanoparticles exhibited a sufficient antimicrobial activity against *Gram*-negative as well as *Gram*-positive bacteria. However the antibacterial effect was more prominent against *Bacillus subtilis* than that of *Escherichia coli.* The antibacterial effect increases with increasing the concentration of SnO_2 nanoparticles. This study confirms the use of metal nanoparticles as future antibacterial [27]. Proposed mechanism for the action of Co-doped SnO_2 nanoparticles to destroy the bacterial cell is shown in Figure 7 [28].

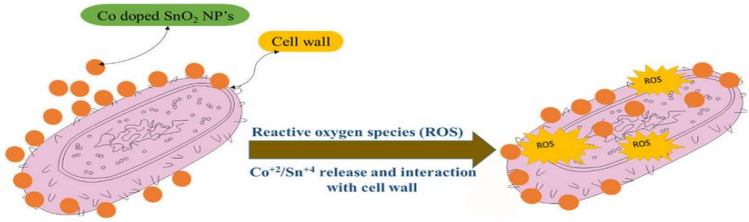


Fig. 7. Proposed mechanism for the action of Co-doped SnO_2 nanoparticles to destroy the bacterial cell

While the root cause of such differential antibacterial activity is still to be completely explained, the plausible mechanisms as shown in Figure 7 could be involved in the interaction between Co-doped SnO_2 NPs and bacterial species. Accumulation of Co-doped SnO_2 NPs on the surface of bacterial strains due to adsorption-desorption chemical-physical or either in the cytoplasmic lipid membrane or cell membrane disruption due to inhibition of NPs and leading to cell death [27]. It is assumed that the prepared Co-doped SnO_2 NPs penetrate in to the cell wall of bacteria in the form of their respective ions i.e. Co^{+2} and Sn^{4+} and bind with the surface of bacterial strains through electrostatic interaction mechanism as bacterial surface rich with electron and have negative charge. As a result of electrostatic interaction mechanism between Co-doped SnO_2 NPs and surface of bacterial strains produced reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion $O_2^{\cdot -}$, hydroxyl radicals (OH_3), and organic hydro peroxides (OHPs) on the surface of cell wall, which leads to the cell damage due to the oxidative stress on the cell wall.

5. Conclusion

The results of this study indicate that the synthesized Co-doped SnO_2 nanoparticles possess potent and desirable biological properties such as antibacterial activities as Co-doped SnO_2 nanoparticles showed better and effective results against all employed bacterial strains in contrast to employed standard drugs. This was due to the fact of physical characteristics of the Co-doped SnO_2 nanoparticles (large surface with increase grain size and smaller particle size). Thus these synthesized nanoparticles hold enormous potential for use in the cosmetic, nutraceutical and pharmaceutical industries.

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