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Study the Effects of Pure Tin Oxide Nanoparticles Doped with Cu, Prepared by the Biosynthesis Method, on Bacterial Activity

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Abstract

In this study, pure SnO2 nanoparticles were doped with 2% at wt. of copper by biosynthesis method. As raw materials, SnCl2.2H2O, CuCl2.2H2O, and ESM biomaterial were used as eggshell membranes. Samples were annealed at 550 ° C for 3 h. The bacterial activity against E-coli Gram-negative and S. aureus Gram-positive strains and higher inhibition of concentrations in S.a-ureus was obtained from E-coli. The MIC method was used for the minimum inhibitory concentration. The results of XRD showed that the samples crystallized within the tetragonal rutile type and that the average crystal size is pure SnO2 and SnO2: Cu (24.2, 16.6) nm respectively .SEM and AFM tests were also carried out.The UV-Vis studies revealed reflection spectroscopy at the energy gap of SnO2, and SnO2: Cu 2% is (4.30, 4.35) eV respectively. The AFM results showed the roughness rate of the prepared samples (6.34, 9.13) nm respectively. An EDX test was also performed for the prepared samples. The aim of the work is to create pure SnO2 nanoparticles through active biosynthesis study the effect of doping it with Cu and study its effect on its structural and optical properties as well as how to use it as an antibacterial.

Keywords: ESM, E-coli& S. aureus, MIC method, SnO_2 biosynthesis, SnO_2:Cu.,Structure of SnO_2.

Introduction

SnO2 is the least expensive and least toxic metal oxide nanoparticle. It is an n-type semiconductor with a 3.6 eV direct bandgap [1] Because of its unique physical and chemical properties, it has a wide range of applications in the fields of sensors[2], optical electrons [3], medicine [4], and energy storage devices [5]. Because of two critical factors [6]: a) Increased surface area and b)Quantum effect, There are several methods for synthesizing and preparing tin oxide nanoparticles, including sol-gel [7], solvothermal [8], pulsed laser deposition [9], chemical precipitation [10], and green synthesis [11]. SnO2 NPS has been developed because it is less expensive and non-toxic. Tin oxide is an important material for the following reasons: It has a high degree of transparency in the visible spectrum, strong chemical and physical interaction with the absorbent species, Low operating temperature, strongly thermally stable in air up to 500 °C [12].

The most readily available vital waste whereas the eggshell membrane ESM within the eggshell contains collagen proteins containing various amino acids, glycine, and alanine, Uranic acid contains many amino acids, carboxylates, carbonyls, and aldehyde functional groups [13], where they act as reducing agents to form a nano-metal oxide, and the

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ESM model was used in this case because SnO_2 nanoparticles are assembled using natural biosynthesis rather than toxic chemicals. It appears that the use of ESM is appropriate for medical and antibacterial applications. Mataji Palaz discussed the morphology and functional groups of ESM, as well as the field applications of their systems [14]. Chang et al. [15]. Gold nanocomposites using ESM and studying their bio-properties. Wang et al[16]. obtained nano carbonate with ESM and studied its fluorescent application. Also, SnO_2 NPS was prepared and studied pure and doped with Cu, as copper has high electrical and thermal conductivity and is the most conductive mineral for electricity and heat after silver [17,18]. The study aims to form pure SnO_2 nanoparticles doped with Cu by active biosynthesis and use the prepared materials for medical applications

Materials and Methods

Materials:

$SnCl_2.2H_2O$ company Qualikems (India), $CuCl_2.2H_2O$ CDH, India), Deionized water (Local, Iraq), Eggshell membrane ESM Local by hand, Iraq) .For preparation of SnO_2 NPS pure and 2% Cu

Preparation of pure SnO_2 and doped with Cu nanoparticles by using ESM as bioactivity

Pure tin oxide nanoparticles were prepared and doped with Cu by biosynthesis method using an eggshell membrane ESM.1g of $Sncl_2.2H_2O$ was taken and dissolved in 25 ml of deionized water and stirred for 1 hour for complete dissolution and homogeneity, then ESM was added at 0.25g for 24 hours. The eggshell membrane was filtered and washed to separate the unwanted components. At 550 ° C for 3 days, the ESM changed color from pale yellow to brown, and pure SnO_2 NPs were obtained. Preparation of pure nano-tin oxide doped with Cu.$CuCl_2.2H_2O$ was doped by 2% in addition to being doped by 1.12 g of $SnCl_2.2H_2O$ and 50 ml of water, according to equation [19]:

$$\% \text{ dopants } = \frac{M\ dopants(x) molarity}{M\ dopants + M\ origin} \quad \ldots..1$$

M dopants(x): The molar concentration doped $CuCl_2.2H_2O$.% dopants: doped concentrations. M origin : The molar concentration of the original material $SnCl_2.2H_2O$.

Where molarity is given by relation[20]:

$$M = \frac{W}{m.Wt} \times \frac{1000}{V} \quad \ldots..2$$

V: The volume of deionized water, W: the weight, $m.Wt$: Molecular weight.

It was placed on stirring for 1 hour, then weighted 0.5 g of ESM and put in the solution for 24 hours, then separated the unwanted materials and washed the substance in deionized water and ethanol, then annealed the precipitate at 550 °C for 3 hours.

Determination of the effect of bacterial activity of prepared nanoparticles

Determination of the effect of antibacterial activity of SnO_2 and SnO_2: Cu NPs by Well Diffusion assay.

The medium Mueller Hinton agar was prepared in accordance with the instructions. company's instructions prepared for the medium, dissolved in a specified amount of D.W., and entered into an autoclave at a temperature of 121°C and pressure at 15 pounds/inch2 for 15 minutes. It was poured into Petri dishes.

The bacteria to be measured were placed in dishes and then refrigerated at 4 °C. The effect of the prepared substance was spread on it, and the bacteria Four different types of bacteria were tested for activity. There are two types of gram-positive and gram –negative of SnO_2 Pure and SnO_2:Cu NPs are prepared by using ESM, The wells were made inside the Petri dish, and the material was placed in the wells. The Petri agar was incubated in an incubator at 37°C for 24 hours. The bacteria were local isolates that were activated by nutrients. agar and used in the assays. If bacterial growth extends to the nanoparticle-containing well, the bacterial strain is considered resistant to the nanoparticles. If there is this indicates that the nanoparticle has cleared the area around the antibiotic well. harmed the bacteria. to determine whether a bacterial strain is sensitive to an antibiotic, the size of the inhibition

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zone can be measured and compared. to control well [21].

Determine the Minimum inhibitor concentration MIC as the Antibacterial activity of SnO_2 pure and doped with Cu Nanoparticle.

The antibacterial activity of SnO_2 was measured, and SnO_2:Cu 2% of the nanoparticles were then evaluated against gram-positive bacteria. Staphylococcus aureus and gram-negative bacteria Escherichia coli by serial dilution by determining the minimum inhibitory concentration MIC in the cultural broth. 0.7 mL of the medium was taken in a test tube. 0.7ml of The test substance was added to the tube. 70 µl of the bacterial strains E-Coli, and S. aureus, and the serial dilution was performed at five concentrations. 50%, 25%, 12.5%, 6.25, and 3.125% of SnO_2 nanoparticles were obtained from the Eggshell membrane ESM at a stock concentration of 0.25 g/ml SnO_2 The control was SnO2:Cu NPs obtained from ESM 0.25, 0.5 g. tubes unexposed. All samples were incubated for 24 hours at 37 °C.

Characterization

The X-ray diffraction XRD pattern of tin oxide nanoparticles was recorded by Card No.96-900-9083 using CuKα,λ=0.154 nm. The particle morphology and size were examined by scanning electron microscopy SEM ZEISS model Sigma VP with a magnification of 50.00KX and EDX Oxford instruments in the UK. Optical properties were analyzed using ultraviolet diffusion. Reflection Spectroscopy with UV-VIS Shimadzu within the wavelength range of 280 nm-900nm. The AFM revealed the shape of SnO2 NPs in the DME Denmark model, and the cumulative distribution plot revealed the average grain

Results and Discussion

XRD Analysis

The XRD pattern of pure SnO2 doped with 2% at wt. of Cu nanoparticles and annealed at 550 °C for 3 hours is shown in Fig 1. All samples were identified as tetragonal rutile, and the results are in agreement with JCPDS standard card No. (96-900-9083) where the diffraction peaks coincide with the standard data from (110), (101), and (211), and the most obvious peak is (110). There are no identical peaks for Cu The results match those of the researchers Sagadevan et al., [22], and this leads to the conclusion that there are two types of doped SnO2 with Cu: substitutive and interstitial. In this case, some ions are replaced by Sn + with a Cu + ion based on similar radius Sn, Cu (0.69,0.73) Å, respectively. The average grain size of the nanoparticles was calculated based on Scherrer's equation [23].

$$d = \frac{\kappa\lambda}{\beta\cos\theta} \quad \text{.....3.}$$

d: is the mean crystalline size, K is the constant = 0.89, λ is the wavelength of the incident beam λ=1.5418Å Cukα, β is the full-width for a half maximum is the Bragg diffraction angle in degrees. It was found that the average crystal size as shown in Tab. 1, for pure SnO2 is 24.2nm and for the doped with 2%at wt of Cu16.60 nm. The presence of doping reduces the crystal size, which means that the presence of copper ions in SnO2 hinders the growth of crystal grains. From the figure, we notice that with the presence of copper ions, the intensity of the XRD peaks increases, which indicates that the doping increases crystallinity.

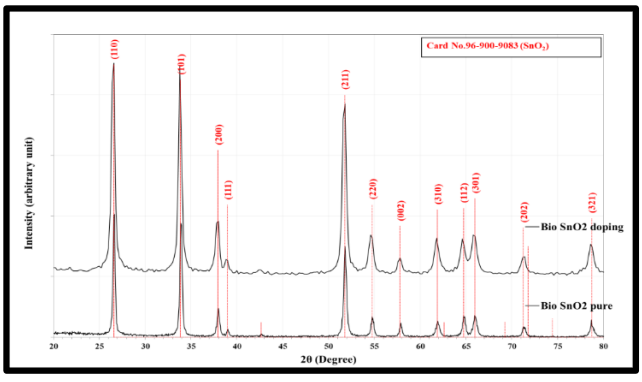

Figure 1. X-ray diffraction pattern of a) pure Sno_2 nanoparticles .b) SnO2 NPS doped with Cu 2%

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Table 1. the grain size of the pure and doped nanoparticles

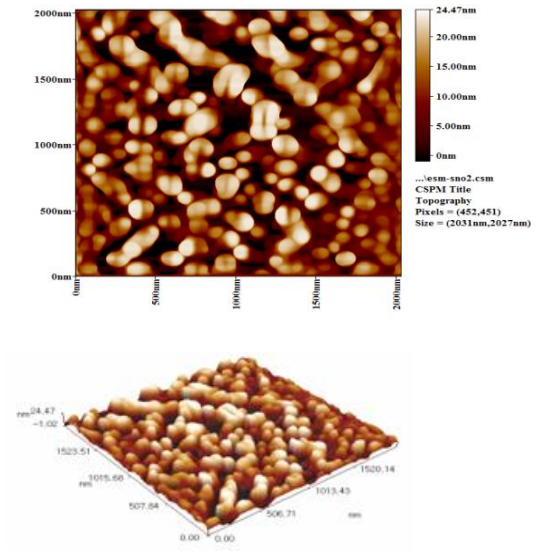
Cu%	G.S (nm)
0	**24.2**
2	**16.60**

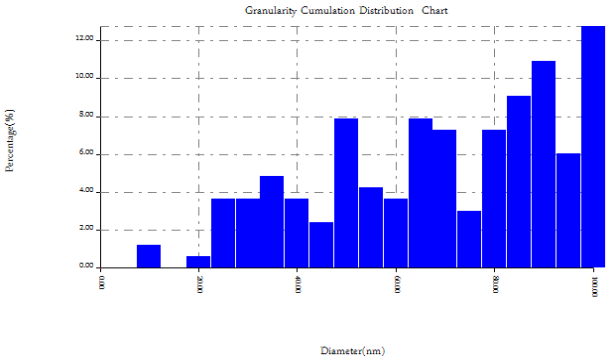
AFM Examination

Fig 2 shows an atomic force microscope image of the pure SnO2 sample surface and SnO2 doped with 2% at wt. of Cu and annealed at 550 ° C prepared in a bio-based method. The 2D and 3D images show that the SnO2 topography for all samples has spherical shapes as shown in Fig 2. Moreover, in the area that has been investigated by AFM, it is found that the roughness average of pure SnO2 NPS and SnO2:Cu at 550 °C annealing are 6.34 nm and 9.13 nm, while the grain size is 66.9 nm and 67.4 nm, respectively. The grain size increased in doped samples because crystallinity defects occurred, which leads to an increase in the size due to the addition of Cu, as well as increasing roughness, as shown in Table 2

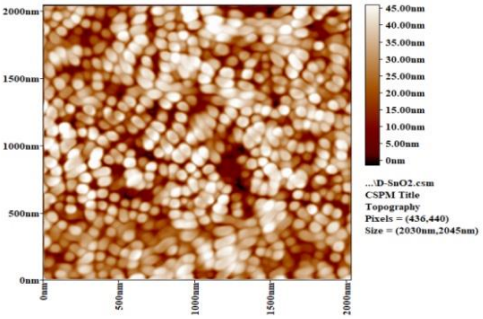
Table 2. The grain size and roughness of pure SnO2 nanoparticles doped with Cu, annealing at 550°C

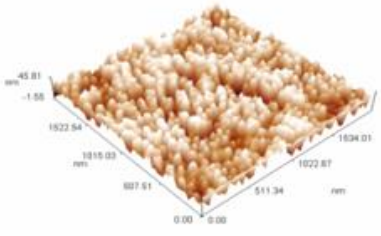
Cu doping concentration W%	average size (nm)	Roughness (nm)	RMS
0	**66.9**	**6.34**	**7.31**
2	**67.4**	**9.13**	**11**

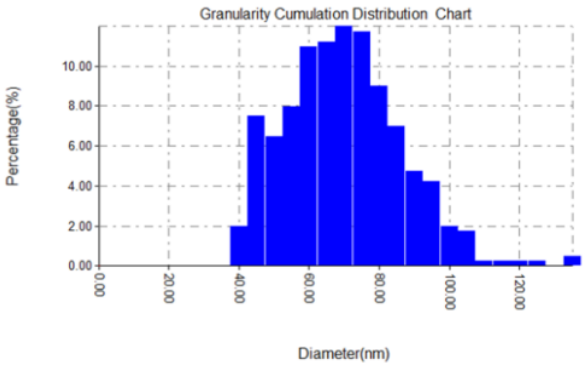




(A)







(B)

Figure 2. The AFM of (A): pure SnO2 Nano nanoparticles in 2D and 3D and Granularity Cumulation Distribution Report, and (B) SnO2: Cu in 2D and 3D and Granularity Cumulation Distribution Report, annealing at 550°C.

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SEM Scan Results

By examining SEM images of pure SnO2 and SnO2:Cu samples at 2% wt concentration and annealing at 550°C prepared by biosynthesis using eggshell membrane as shown in Fig 3, the form and size of the grains can be determined. Since the SEM images give clarity about the surface morphology and the average particle size shown in this figure for the pure SnO2 is 68.02nm and for SnO2:Cu is 100 nm which proves that the prepared samples are within the nanoscale domain [24].

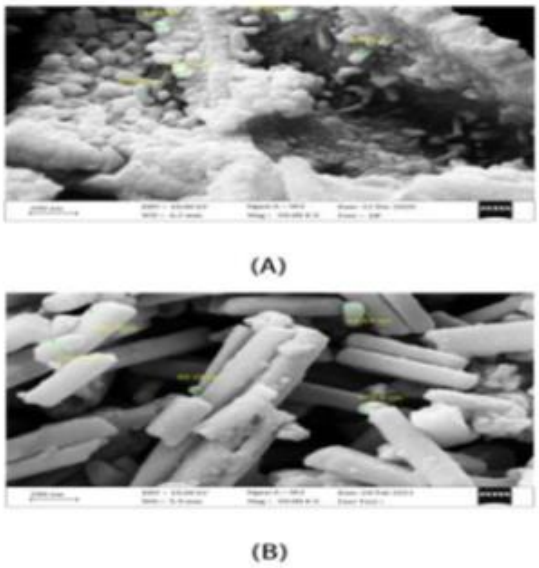

Figure 3. SEM images of (A): pure SnO2 at 550°C annealing temperature, (b): SnO2: Cu at 550°C annealing temperature.

EDX Results

EDX describes the elemental composition in the as-prepared samples (SnO2 and SnO2:Cu) and that Cu ions were successfully incorporated into the SnO_2 lattice, These nanoparticles have spherical forms and are less than 100 nm in size. The EDX results are shown in Fig 4, which shows the rising peaks representing the quantity of Sn ,O, Cu, and C. The C element is present in the eggshell membrane because it contains carbonyl and carboxyl which contain element C. The actual composition of the rated EDX- prepared materials is scheduled in Table 3 below:

Table 3. The actual composition of the rated EDX- prepared materials is scheduled

EDXComposition	*Sn (wt%)*	*O(wt%)*	*Cu(wt%)*	*C(wt%)*
pure	89.4	8.9	0	1.7
2% Cu	0.1	2.5	44.8	9.8

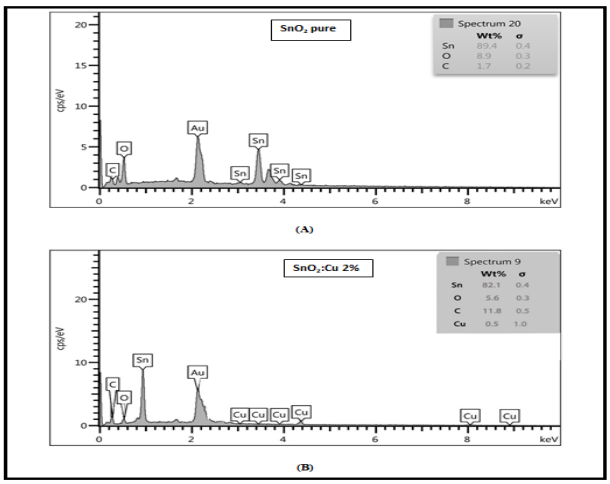

Figure 4. EDX image of. A) Pure SnO_2. B) SnO_2: 2% Cu

Optical Studies

In order to better understand the effect of copper doping on the optical properties of SnO_2 nanoparticles, This UV-VIS spectroscopy analysis was performed. Fig 5, shows the variation in absorbance with wavelength within the range of 280nm-900nm for the pure SnO_2 samples and those doped with Cu ions as shown in Tab.4, where the band gap is calculated using the Planck equation [25].

$$E= \frac{1240}{\lambda} \text{ ev}\ldots\ldots 4.$$

The absorbance decreases as the wavelength increases. This means that the incident photon is unable to excite the electron and transfer it from the valence to the conduction band, because its energy is less than the energy gap's value [26]. Table 4 shows the values of the energy gap for the permissible direct transmission of the pure and 2%wt doped SnO2 nanoparticles. The values of the energy gap increase with doping and the reason for that is due to the fact that the doping leads to reducing the dangling bonds, filling voids, and reducing defects that are formed during the preparation process.

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Table 4. of the energy band gap for pure and doped nanoparticle concentrations with Cu

Concentration Cu ratios%	Band gap (Eg)	wave length (λ) nm
0	4.30	288
2	4.35	285

It is known that semiconducting nanoparticles have a distinct absorption edge and that shifting the material to the nanoscale leads to its deviation towards short wavelengths. The pure SnO2 nanoparticles should have a wide energy gap due to the quantum confinement of the electron gap pair formed as a result of photon absorption of energy. The slight increase in the energy gap after doping with Cu is the result of a slight decrease in the crystal grain size, this was given by the interpretation of the XRD results, where a slight blueshift was observed for these peaks, indicating a decrease in the energy gap. Also, this slight improvement in optical range difference with an increase of Cu concentration by 2% may be due to the presence of grid defects or stacking errors.

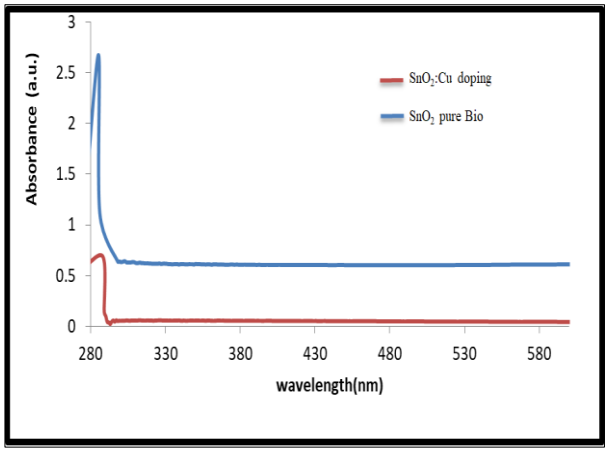

Figure 5. Shows the absorbance spectrum of pure Sno$_2$ NPs& doped with Cu.

Fourier Transform Infra-Red spectroscopy (FTIR)

This procedure has been carried out in order to pinpoint the biomolecules and reduction compounds in charge of producing the SnO2 NPs produced by the eggshell membrane. Fig 6 shows the FTIR spectrum for SnO_2 NPs. The bands at 34093cm-1_3568cm-1 are given to the O-H and N-H stretching vibration of phenols and proteins from the eggshell membrane M.Buck[27],Other peaks at 1364 cm-1 represent to C-H stretch of alkanes ElżbietaDrzymała[28]. Peaks at 1631_1633cm-1 were identified as belonging to the O-H bending mode in the protein's characteristic amine I group. The Peak at 1223cm^{-1} represents C-O Phenol, Peak at 1714cm-1 represents C=O Fan Liu*, Yu Liu, Jia Chuan Chen, Zhen Wang[29], while the peak at 623 – 619 cm^{-1} region corresponds to the stretching modes of the Antisymmetric Sn-O-Sn Yang et. Al[30]. These bands are characteristic of Sn- OH terminal bonds of the SnO $_2$ crystalline phase at 1110_1114cm^{-1} [31].

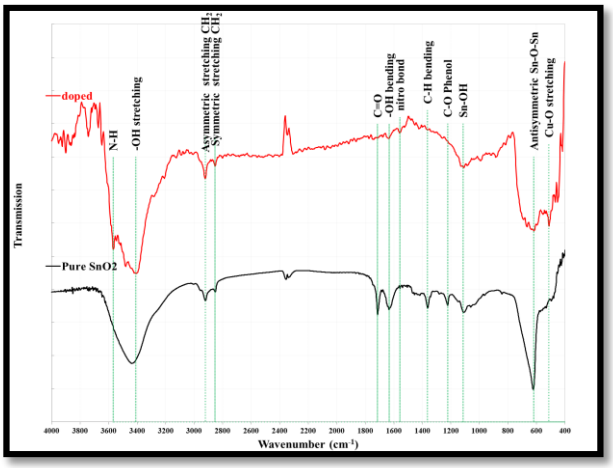

Figure 6. FTIR spectra of pure tin oxide nanoparticles synthesized by active biosynthesis using eggshell membrane

Anti-bacterial activity of SnO_2 NPs pure and doped with Cu

Biosynthesis of SnO_2 NPs showed excellent antibacterial activity against multiple bacterial pathogens by Petri-agar methods, such as Gram-positive bacteria Staphylococcus aureus, Streptococcus pyogenes, and Gram-negative bacteria Escherichia coli, Pseudomonas aeruginosa, as obtained from the eggshell membrane ESM, as shown in Fig 7 The diameter of the inhibitory zone varies. The results showed that Pseudomonas aeruginosa occupied greater inhibition areas than Staphylococcus aureus compared to Escherichia coli and Streptococcus pyogenes, which could be due to differences in cell wall composition. The

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diameter of the inhibition zone was 37 mm, the highest inhibition area for all bacterial pathogens The reason is that it has an effect on the metabolism of bacteria as an enzyme inhibitor, and that SnO_2 Pure has no inhibition zone, unlike S. aureus bacteria. where the outer membrane of the bacteria provides a strong interaction with the prepared samples and the presence of Sn enhances the substance and the association of NPs with the bacteria and thus the bactericidal activity [32] and the doping area of the material is higher than pure because the particle size is smaller in the doped than in the pure [33]. The experiment was conducted under aerobic conditions at a temp of 37°C.

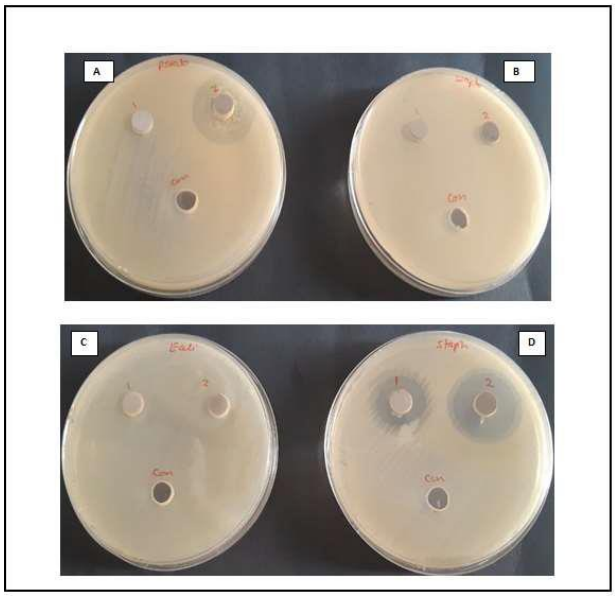

Figure 7. Antibacterial activity of 1) SnO2 NPs, 2) SnO2:Cu 2% from ESM for bacterial strains.

A) *Pseudomonas aeruginosa*, B) *Streptococcus pyogenes*, C) *Ecoli*, D) *S. aureus*.

this study the Escherichia coli strain as well as Staphylococcus aureus were used for the ESM biomaterial. It can be seen that Staphylococcus aureus disrupted the formation of peptide cross-links to the cell wall more than E-coli for different concentrations of the substance 50%, 25%, 12.5%, 6.25%, 3.125% respectively of the pure substance and doped with Cu. Whereas, in the E-coli strain, complete inhibition was obtained in pure SnO_2 at a concentration of only 50%, while SnO_2:Cu was inhibited at concentrations 12.5%, 25%, and 50% only. As for the Staphylococcus aureus strain, pure SnO_2 was inhibited at a concentration of only 50%, and SnO_2: Cu was inhibited for all concentrations of 3.125%, 6.25%, 12.5%, 25%, and 50%, respectively. The reason for this is that the peptides in the S. aureus strain interact with Sn + 2 ions and direct the growth of SnO_2 nanoparticles [34] as shown in Fig 8, so these peptides can achieve an easy interaction environment as they ensure biocompatibility. The major part of S. aureus is built of interconnected glycan-oligopeptide chains, and glycan strings are interconnected by parallel, shell-like layers [35]. The cells of Escherichia coli are surrounded by a complex cell wall consisting of two concentric lipid bilayers, the outer membrane, and the cytoplasmic membrane with a circumferential space between them[36]. The outer membrane is filled with lipopolysaccharides connected with complex lipids with fatty acid tails, and the space between the membranes is filled with peptidoglycan and a group of small soluble proteins. E-coli cell wall thickness and Gram-negative bacteria are 4nm [37].

Figure 8. Bacterial activity by MIC for different concentrations of pure SnO_2 &SnO_2:Cu

Conclusion

A simple, economical, and environmentally friendly route has been developed for the synthesis of SnO2 nanoparticles using an active biosynthesis method using an eggshell membrane. Crystal size was calculated by XRD data of nanoparticles using, and nanoparticle size was measured from SEM data obtained from nanoparticle size. Shows the XRD pattern. The tin oxide nanoparticles formed from

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this structure are tetragonal crystals, and the crystal size is 24.2nm, and 16.60nm for the pure samples and doped with 2 wt% Cu respectively. AFM results showed that the size of the granules inside the nanoscale is spherical in different sizes ranging from 66.9nm to 67.4nm. The highest roughness is 9.13 nm for the sample doped with 2% Cu. The roughness of the pure samples is 6.34 nm.

This method by which SnO_2 nanoparticles are obtained is environment-friendly for commercial production as it does not involve the use of hazardous and toxic sealing agents. Moreover, the concentration of the capping agent has a significant effect and can be seen in the crystal size, and a shift was seen in λ max.

Also, from the compositional results, energy gap values, absorbance increase, roughness, and other results obtained, it is clear that the prepared samples can be used in different applications such as solar cells, gas sensors, and different medical applications such as cancer and as an antibacterial activity to inhibit the action of bacteria.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in Al-Karkh University of Science, Baghdad, Iraq.

Authors' Contribution Statement

N. K. A. proposed the idea of research and guidance in how to prepare, as well as preparing the workplace. D. S. Sh. worked on preparing samples and conducting tests for them and verified the analytical methods. N. K. A. investigated and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript

Journal Declaration:

N. K. A. is an Editor for the journal but did not participate in the peer review process other than as an author. The authors declare no other conflict of interest.

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دراسة تأثير الجسيمات النانوية لأكسيد القصدير النقي والمخدر بالنحاس المحضر بطريقة التخليق الحيوي على النشاط البكتيري

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الخلاصة

في هذه الدراسة ، تم تشويب جسيمات SnO2 النانوية بنسبة 2٪ بالوزن من النحاس بطريقة التخليق الحيوي. تم استخدام المواد الحيوية SnCl2.2H2O و CuCl2.2H2O و ESM كأغشية قشر البيض كمواد خام. تم تلدين العينات عند 550 درجة مئوية لمدة 3 ساعات. تم الحصول على الفعالية البكتيرية ضد سلالات المكورات العنقودية الذهبية سالبة الجرام و S. aureus إيجابية الجرام وتثبيط أعلى لتركيزات المكورات العنقودية الذهبية من E- coli. تم استخدام طريقة MIC لأدنى تركيز مثبط. أظهرت نتائج XRD أن العينات تبلورت ضمن النوع رباعي الزوايا الروتيل وأن متوسط حجم البلورة ل SnO2 النقي و SnO2: Cu كان (24.2 ، 16.6)نانومتر على التوالي ، كذلك تم اجراء اختبارات SEM و.AFM كشفت دراسات UV-vis التحليل الطيفي للانعكاس عند فجوة الطاقة لـ SnO2 ، و SnO2: Cu 2٪ هو (4.35،4.30) الكترون فولت على التوالي ، وأظهرت نتائج AFM معدل الخشونة للعينات المحضرة حيث كان(9.13،6.34) نانومتر على التوالي. كما تم أجراء فحص ال EDX للعينات المعدة. الهدف من العمل هو إنشاء جزيئات نانوية SnO2 نقية من خلال التخليق الحيوي النشط ودراسة تأثير تعاطي المنشطات ب Cu ودراسة تاثيرها على خواصها التركيبية والبصرية وايضا في كيفية استخدامها كمضاد بكتيري.

الكلمات المفتاحية:ESM،E-coli& S. aureus، طريقةMIC، التخليق الحيويSnO2 ، تركيب SnO2، SnO2:Cu.