

PREPARATION OF NOVEL CHITOSAN-GRAPHENE OXIDE/TIN OXIDE NANOCOMPOSITES FOR ANTI-BACTERIAL ACTIVITIES

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Herein, the novel chitosan-graphene oxide/tin oxide (CS-GO/SnO₂) hybrid composites were derived by one-pot reaction. The different SnO₂ and GO percentages were used to blend with chitosan to structuralize the CS-GO/SnO₂ nanocomposites. CS-GO and CS-GO/SnO₂ hybrid formation were structurally ascertained by Fourier-transform infrared spectral studies. X-ray diffraction outcomes evidently proved the formation of amorphous CS-GO and semicrystalline CS-GO/SnO₂ hybrids. Surface properties variations extensively conferred with scanning electron micrographs for CS-GO and CS-GO/SnO₂. The antibacterial performance of CS-GO and CS-GO/SnO₂ were established in terms of zone of inhibition against different microorganisms such as *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*.

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1. Introduction

The human society and medically developed countries are keenly taking strict summaries from the various severe acute respiratory syndrome based attacks and recent COVID-19 epidemic and necessities to reach the next level of clinical efforts and discoveries.[1] Undeniably, initiation of novel antibiotics with the efficacious remedial without toxicity is one of the ultimate accomplishment in the medicinal science.[2] Human society is being hungered to receive the effective antibiotics to protect from numeral viruses and infections.[3] The severe toxicities or damage can be extended to other tissues due to intake of inept antibiotic drugs which would be ensuing the expensive medicinal treatment and growth of medical death rate. Antibacterial are essential to kill the harmful pathogens or diminish its progress without habitually distressing adjacent tissues.[4] From the earlier findings, structurally fabricated antibiotics/antibacterial are having, rather than natural systems, fore stubborn capability against harmful germs.[5]

Nanomaterials are facilitated to use in the various industrial areas including transportation, food safety, information technology, energy, environmental, homeland security, and medicine.[6-8] The potential to shape the material arrangements at enormously trivial scales is core advantage for nanomaterials to realize their precise behaviors which significantly extending their use in the various materials science devices.[9, 10] The extension of nanotechnology towards medicine which called nanomedicine appeals to invent the accurate solutions or treatments for diagnosis and epidemic prevention.[11, 12] Nanomedicines can be offered the durable antimicrobial interactions with pathogens owing to their high surface area/low volume ratio and low dimensions than microorganism.[2, 13] The nanomaterials antibacterial activity is oriented with the surface reactive oxygen species and metals terminated metal ions.[14] The bactericidal behaviors are boosted by the attraction of bacteria's negative surface charge by nanomaterials' positive surface charge.[15] In addition, nanomaterials' structure and magnitudes have greatly being inclined with the antimicrobial performances.[16, 17]

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Dimension of the nanomaterials also lead the possibility to blend with other materials to form as a composites or hybrids with a vision to improve the material properties. Among the various candidates, graphene is a two-dimensional sp^2 bonded carbon and highly attracted by scientists because of their extensive thermal, mechanical and biological uses.[18] Also, chitosan is one of the natural polymers produced by the alkaline deacetylation of chitin, and non-noxious polymer with bio-adhesive behaviors along with the capability to rapidly bind the intestine barrier.[13, 19, 20] The various metal oxide hybrid systems were deeply studied as the auspicious material for antibacterial applications owing to their synergistic behavior.[2, 18] This work is focused to synthesis the chitosan-graphene oxide (CS-GO) and chitosan-graphene oxide/tin oxide (CS-GO/SnO₂) hybrid systems by sol-gel reaction. The antibacterial properties investigation were performed against *Escherichia coli* (*E. coli*), *Salmonella typhi* (*S. typhi*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Bacillus subtilis* (*B. subtilis*) microbes.

2. Materials and methods

2.1. Chitin preparation

The chitosan was extracted by the deacetylation process using chitin. Freshly collected crab shells were purified in seawater and then dried out in daylight. A 50 g milled dehydrated shells were liquefied in 5% hydrochloric acid (HCl) at room temperature with persistent stirring of 2 h. Then, the resultant were rinsed using di-ionized (DI) water to eradicate all the contaminations by demineralization process. A 5% sodium hydroxide (NaOH) was blended with resultant powder for 24 h for deproteinization. The collected final powder was purified with DI water to attain neutral pH and oven at 60°C at 1 h.

2.2. Preparation of graphene oxide

An amended Hummers and Offeman procedure was employed to produce graphene oxide (GO). About 3 g of graphite powder was blended with 12 ml of intense sulfuric acid (H₂SO₄), subjected to hot plate for 5 h at 80°C. Then, it was sonicated at room temperature for 15 min and filtrated with 200 nm porous filter to recover the pre-oxidized graphite. Further, 15 g of potassium permanganate and 2 g of pre-oxidized graphite powder were liquefied into 120 ml of H₂SO₄, stirred for 2 h in an ice-water bath to warrant the below 10°C temperature. The 30 wt.% hydrogen peroxide (120 ml) aqueous solution was mixed with the above solution, placed for room temperature vigorous stirring for 2 h. Final product of GO was extracted after the filtration and successive cleansing with HCl and DI water.

Table 1. Composition of the CS-GO and CS-GO/SnO₂ composites.

Material code	Sample	Sources		
		GO (w/v) %	SnCl ₂ (w/v) %	Chitin (g)
NC1	CS-SnO ₂	0.5	0	0.25
NC2	CS-GO/SnO ₂	0.5	0.5	0.25
NC3	CS-GO/SnO ₂	1.0	1.0	0.25
NC4	CS-GO/SnO ₂	1.5	1.5	0.25
NC5	CS-GO/SnO ₂	2.0	2.0	0.25

2.3. Nanocomposites synthesis

A sol-gel reaction was successfully followed to prepare the hybrid composites as reported earlier. The mixture of derived chitin (0.25 g) mixed acetic acid (2%) and 0.5% (w/v) GO (30 ml) were subjected to constant bath stirring. Bath temperature was gradually elevated to 70°C and fixed for 2 h. NaOH 45% (w/v) aqueous (30 ml) was blended gradually until a black gluey precipitate formation. After 24h, the supernatant liquid was extracted, and the precipitates was

cleaned and filtered by suction pump. The final CS-GO residue was dried at 110°C for 2 h in a hot oven, which labeled as NC1.

The mixture of derived chitin (0.25 g) mixed acetic acid (2%), 0.5% (w/v) SnCl_2 (30 ml) and 0.5% (w/v) graphene oxide (30 ml) were kept in the constant bath stirring. Bath temperature was gradually elevated to 70°C and fixed for 2 h. NaOH 45% (w/v) aqueous (30 ml) was blended gradually until a dark black precipitate formation. Then, after the above mentioned cleaning procedure to remove impurities, and then final product of CS-GO/ SnO_2 was stored, named as NC2, for analyses. Similarly, earlier procedure was tailed to synthesize the NC3, NC4 and NC5 composites with 1.0, 1.5 and 2.0% (w/v) metal chloride and graphene oxide concentrations, respectively, and their details are given in Table 1.

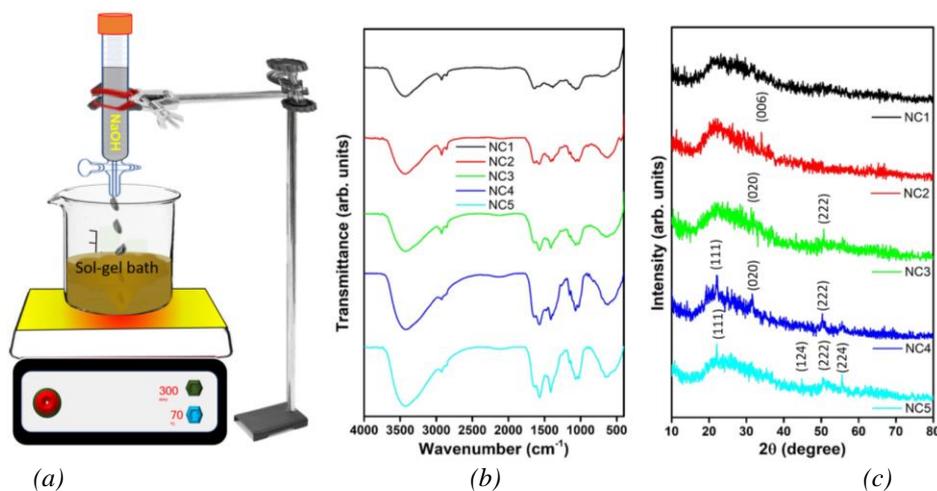


Fig. 1. (a) Illustration for synthesis of CS-GO/ SnO_2 by sol-gel reaction; (b) XRD and (c) FTIR profiles of CS-GO and CS-GO/ SnO_2 nanocomposites.

2.4. Antibacterial activity and characterization

Well diffusion procedure by sterilized Mueller Hinton agar (MHA) was engaged to assess the antibacterial properties of hybrid composites. The different organism of *E. coli*, *S. Typhi*, *P. aeruginosa* and *B. subtilis* were brushed out the MHA broth surface, then cultured. The CS-GO and CS-GO- SnO_x nanocomposites were exposed on the different well of the agar. Further, MHA cultured plates were developed at 37°C for 24 h, and the area of inhibition were appraised in mm. All the experiments were triplicated.

The following analytical instruments were used to characterize the prepared nanocomposite samples such as Hitachi-S3000 H scanning electron microscopy (SEM), Thermo-Nicolet-380 Fourier transform infrared (FTIR) spectroscopy and X'Pert PRO PANalytical X-ray diffractometer with Cu K_α radiation wavelength of 0.15406 nm (XRD).

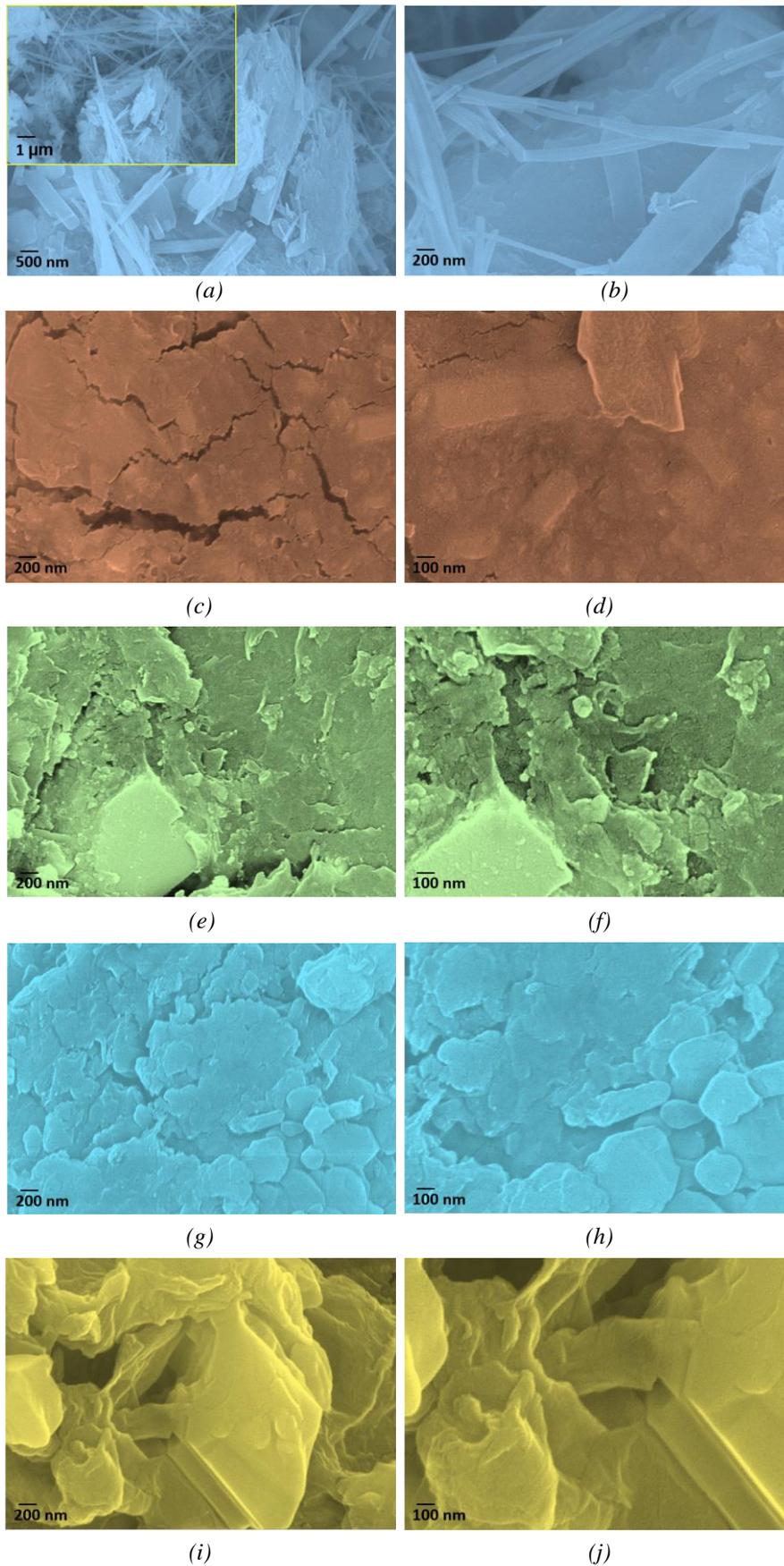


Fig. 2. SEM micrographs of (a-b) NC1, (c-d) NC2, (e-f) NC3, (g-h) NC4 and (i-j) NC5 nanocomposites.

3. Results and discussion

CS-GO and CS-GO/SnO₂ nanocomposites were successfully driven by sol-gel reaction and their schematic preparation is given in the Fig. 1a. Fig. 1b displays the FTIR spectra of NC1, NC2, NC3, NC4 and NC5 nanocomposites. FTIR spectrum reveals the C-N in-plane banding, OH in plane and -CH₃ out of plane bands at 1152, 1384, and 1237 cm⁻¹, respectively for NC1 composite. FTIR spectra exposes the O-H stretching band around ~3400 cm⁻¹ for CS-GO and CS-GO/SnO₂ (NC1-NC5).[21] The C-H symmetric and asymmetric vibrations observe at ~2925 and ~2850 cm⁻¹ for NC1-NC5 composites.[22] A C=C vibration exhibits for NC1-NC5 nanocomposites at 1640 cm⁻¹ due to GO presence.[23] The characteristic vibration for metal from SnO_x interaction with C=O group perceives at ~1568 cm⁻¹. [24] For CS-GO/SnO₂ (NC2-NC5) nanocomposites, C-H bending and CH₃ group symmetry peaks acquire at ~1468 and ~1414 cm⁻¹, respectively. Bending vibration of C-H band emerges at ~1067 and ~1026 cm⁻¹. [25] Out of plane O-H bending exposes at 900 cm⁻¹, whereas amido group transmittance obtains at 708 cm⁻¹ for NC1 composites. The C-O ring and polysaccharide related bands perceive at 893 and 820 cm⁻¹, respectively. For CS-GO/SnO₂ (NC2-NC5) nanocomposites, the band at ~660-620 cm⁻¹ implies the antisymmetric and symmetric vibrations of Sn-O-Sn recognized from SnO₂ on CS-GO surface.[26]

Fig. 1c shows the XRD patterns of NC1, NC2, NC3, NC4 and NC5 nanocomposites. Amorphous natured XRD pattern unveils for CS-GO (NC1) nanocomposites. The semicrystalline pattern with low intense (006) orthorhombic SnO₂ peak emerges for NC2 CS-GO/SnO₂ nanocomposites (JCPDS: 78-1063). For NC3 nanocomposites, (020) and (222) lattice orientations obviously exhibit with amorphous background due to equal amount of GO inclusion. Similarly, NC4 and NC5 CS-GO/SnO₂ nanocomposites produces the semicrystalline SnO₂ peaks as given in the Fig. 1c. FTIR and XRD outcomes demonstrate the improvement of CS-GO/SnO₂ nanocomposites.

Fig. 2 shows SEM micrographs of CS-GO and CS-GO/SnO₂ hybrid composites. Nanorod and course like structured grains exhibits for NC1 CS-GO as shown in the Fig. 2a and their inset. The higher magnification image obviously visualizes the nanorod type of grains with a micrometer length and ~50 nm diameter for NC1 (Fig. 2b). The patterned array with agglomerated nano-sized grains observes for SnO₂ incorporated NC2 CS-GO/SnO₂ hybrid (Fig. 2c-d). The voids and pinholes exhibit between the grains bunches. Finely dispersed nanograins agglomeration with different sizes (Fig. 2e-f) appear when the increase to 1.0% (w/v) of GO and SnO₂ in the NC3 hybrid. The higher magnification image clearly proves the constituents of smaller size spherical grains to produce the larger size grains. Fig. 2g shows different sizes of nanoarray structured grains for NC4 hybrid. The voids and hillocks natured grains visibly expose in the SEM micrograph of NC4 as shown in a Fig. 2h. For NC5 hybrid composites with the source concentration of 2.0% (w/v) for GO and SnO₂, the leaves like grains agglomerates on the CS-GO/SnO₂ nanocomposites surface (Fig. 2i-j). The perceived surface properties outputs for CS-GO and CS-GO/SnO₂ also prove the enormous variations due to inclusion of metal oxide matrix.

Fig. 3 shows the detailed antibacterial activities of NC1-NC5 nanocomposites against *S. Typhi*, *P. aeruginosa*, *E. coli*, and *B. subtilis* germs in terms of zone of inhibition. For evaluation, earlier outputs using streptomycin (SMN) as a standard antibiotic, and pure chitosan (CS) are used in the zone of inhibition performance.[14] The triplicated zone of inhibition results mean values are presented here for NC1-NC5 nanocomposites. A agar plate photograph demonstrates the antibacterial performances of NC1, NC2, NC3, NC4 and NC5 against *S. Typhi* and their zone of inhibition variations provide in the Fig. 3a-b, respectively. The observed performance reveals the better inhibition in NC4. Similarly, NC1, NC2, NC3, NC4 and NC5 nanocomposites produces the zone of inhibition of 21, 22, 22, 25, and 25 mm, respectively against *E. coli* germs (Fig. 3c-d). The antibacterial properties of NC1, NC2, NC3, NC4 and NC5 nanocomposites versus against *P. aeruginosa* derive by zone of inhibition as shown in the Fig. 3e-f. Figure 3g-h show the preventing rate of NC1, NC2, NC3, NC4 and NC5 nanocomposites for *B. subtilis* microorganism. The antibacterial bactericidal process significantly improve for nanocomposites structure due to ease of

dispersion the pathogen cell wall and improved lipophilic behavior of chelation to make inactive the pathogens by their complex behavior.[2, 27] From the antibacterial results, CS-GO/SnO₂ geometry is supposed to distressed the microorganism cell diffusion hindrance and inhibit the microorganism progression.

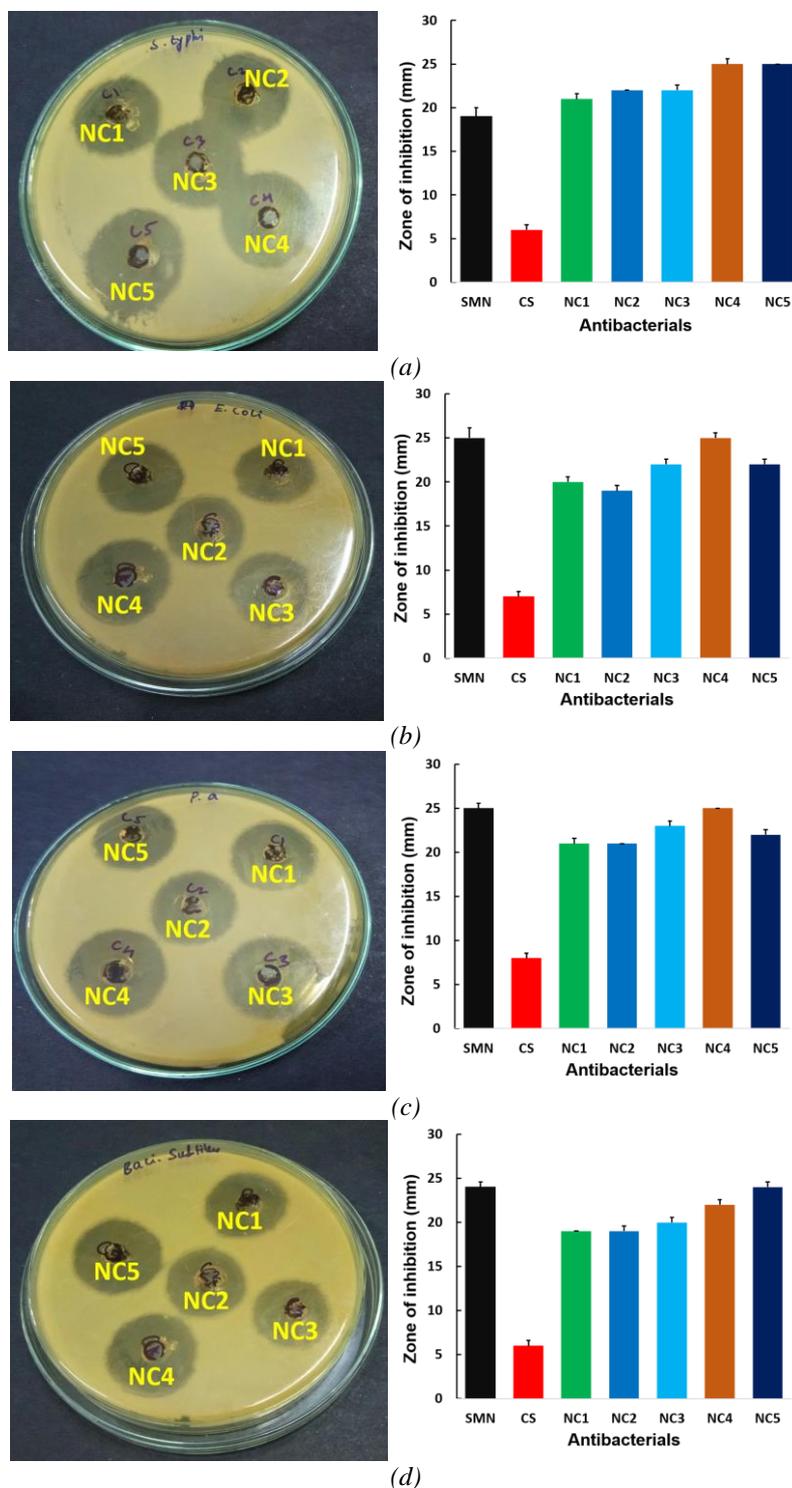


Fig. 3. Antibacterial activity of CS-GO and CS-GO/SnO₂ nanocomposites: Performance against (a) *S. typhi* (b) *E. coli*, (c) *P. aeruginosa* and (d) *B. subtilis* organisms and their zone of inhibition with mean \pm standard deviation from the triplicated tests.

4. Conclusions

One-pot reaction was successfully used to derive the CS-GO and CS-GO/SnO₂ nanocomposites. FTIR and XRD studies were plausibly proved the coexistence of SnO₂ with CS and GO in the CS-GO/SnO₂ nanocomposites. Morphological variations were evidently picturized by SEM images for different source concentration using prepared CS-GO/SnO₂ nanocomposites. The antibacterial activity of CS-GO and CS-GO/SnO₂ were seasoned against *E. coli*, *S. Typhi*, *P. aeruginosa* and *B. subtilis* germs. The superior immune activity was exhibited in CS-GO/SnO₂ nanocomposites against all the microorganisms. Among, the better activity was produced by NC4 nanocomposites versus *P. aeruginosa*, *E. coli*, and *S. typhi* organism whereas NC5 nanocomposites have showed the considerably improved activity against *B. subtilis* organism. The acquired results derived the importance of CS-GO/SnO₂ nanocomposites against various hazardous microorganism for the human society.

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