INTRODUCTION

Microbial infections are one of the major causes of serious health issues due to the development of antibiotic-resistant strains of bacteria [1]. Nanomaterials have been found to have promising medicinal applications. The synthesis of compatible nanosized drug particles having specific size, shape and physical and chemical properties is of huge importance in the genesis of new pharmaceutical products [2-6]. There is a need to develop more safe and cost-effective biocidal materials. Nanosized particles either simple or composite by nature, exhibit unique physical and chemical properties and shows a potential of being used in various biomedical applications [7,8].

Studies on graphene and graphene-based materials has found their use in medicine and life sciences which includes imaging [9], biosensors [10,11], drug delivery [12-14] and pathogen control [15,16]. The response of using graphene oxide on microorganisms has been studied extensively. However, the satisfactory results of metal oxide and graphene oxide nanocomposites have not been reported so far [17-21].

In continuation to our earlier studies [22-28], in present study, we have focused on the synthesis of CuO nanoparticles by facile and cost effective sol-gel method and nanocomposite of copper were prepared with reduced graphene oxide. Antibacterial properties of the synthesized nanoparticles and nanocomposites were investigated against Gram-positive and Gram-negative bacteria. The aim of this study is to synthesize nanocomposite material with better or comparable antibacterial performance.

EXPERIMENTAL

All the chemicals used were of analytical reagent grade and used without further purification. Chemicals used were graphite powder (< 20 µ), Cu(NO<sub>3</sub>)·3H<sub>2</sub>O, NaNO<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>, H<sub>2</sub>SO<sub>4</sub>, KMnO<sub>4</sub>, HCl, NaOH, NaBH<sub>4</sub>, citric acid and ethylene glycol. The strains employed in this work were the Gram-negative bacterium (E. coli) and the Gram-positive bacterium (S. epidermidis). In addition, nutrient broth and agar-agar were used to prepare agar plates.

Preparation of reduced graphene oxide: Graphene oxide (GO) was synthesized from graphite powder by a modified Hummers method [29]. Graphite powder, NaNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub> were mixed together at 0 °C. Then, KMnO<sub>4</sub> was added slowly into the mixture reaction with constant stirring. The mixture was heated to 35 °C and stirred for 12 h and then double distilled water was added slowly under vigorous stirring. Hydrogen peroxide solution was added to reduce the residual MnO<sub>2</sub>. The resultant was then washed by acidified water (3 %) and then with double distilled water three times followed by filtration and drying. Reduced graphene oxide sheets were then obtained.
Preparation of CuO nanoparticles: Cu(NO$_3$)$_2$·3H$_2$O solution and citric acid solution were prepared separately in double distilled water and were mixed together with continuous stirring for 15 min. Ethylene glycol was then added into the solution and stirred continuously for 3 h. The resultant precipitates thus obtained were washed with double distilled water and then dried at 100 °C for 2 h. Finally, these were put into the muffle furnace at 600 °C for 2 h. Copper oxide nanoparticles were thus obtained.

Preparation of CuO/graphene oxide nanocomposites: Cu(NO$_3$)$_2$·3H$_2$O and graphene oxide were mixed together in double distilled water in order to have a metal oxide loading of 10 wt %. The solution pH was adjusted to 10 using NaOH solution and stirred continuously for 4 h. Then 50 mL of 0.1 M NaBH$_4$ was added and stirred continuously for 3 h. The resulting material was then filtered and washed several times with double distilled water and dried in oven at 80 °C. It was then calcined at 400 °C for 3 h.

RESULTS AND DISCUSSION

X-ray diffraction pattern of the copper oxide nanoparticles and CuO-GO (copper oxide-graphene oxide) nanocomposites samples were obtained (Fig. 1) using an X-ray diffractometer (Panalyticals X. Pert Pro, P.U. Chandigarh). The XRD pattern of rGO (reduced Graphene oxide) indicates a broad diffraction peak at 2θ = 24.0°. The broadening and shift of the characteristic diffraction peak of graphite from 26.58° to 24.0° in rGO was due to the short-range order in stacked stacks. Reflection peaks at 2θ = 35.4°, 38.7°, 58.3°, 65.7° and 68.0° are indexed as [002], [111], [202], [022] and [220] planes of CuO phase with cubic symmetry. Reflection peaks at 2θ = 48.7°, 53.4°, 58.3° and 72.4° are indexed as [202], [020], [202] and [311] planes of Cu$_2$O phase. Higher intensity at 2θ = 35.4° and 38.7° indicates that mixed phase has major proportion of CuO with highly oriented crystalline CuO phase. The phase of CuO nanoparticles was perfectly matched with the International Centre for Diffraction Data (ICDD) card No 80-1268. From the XRD peaks, it was concluded that the CuO nanoparticles is crystalline in nature. The X-ray diffraction spectra of nanocomposite have peaks corresponding to both reduced graphene oxide and CuO nanoparticles.

The size, morphology and distribution of CuO nanoparticles in CuO-GO nanocomposites were examined using a transmission electron microscopy {TECNAI 200 Kv TEM (Fei, Electron Optics), AIIMS, Delhi}. Fig. 2 shows TEM images of CuO nanoparticles (a) reduced graphene oxide (b) CuO-GO nanocomposite (c). The inset of Fig. 2(a) shows that the CuO NPs have a spherical shape. The TEM image reveals that the CuO NPs are dispersed on the GO (Fig. 2(c)). In addition, the TEM image shows an average particle size of 20 nm for the NPs. From the TEM images, the rGO surface looks smooth and integrated (Fig. 2b). In the case of CuO/GO nanocomposite (Fig. 2c), a large number of CuO nanocomposites with average diameters 35 nm were observed uniformly on the surface of the rGO. The high magnification TEM image (Fig. 2c) further reveals that CuO-rGO nanocomposites are almost spherical in shape.

The chemical functional groups of CuO NPs and CuO-GO nanocomposites were characterized using attenuated total reflectance Fourier transform infrared spectrometer (Perkin Elmer-Spectrum RX-IFFTIR, P.U. Chandigarh). Fig. 3 shows FTIR spectra of CuO nanoparticles, reduced graphene oxide and Cu-GO nanocomposites. FTIR spectra were recorded in solid phase using the KBr pellets technique in the range of 3500-400 cm$^{-1}$. FTIR spectra exhibit vibration at 580 cm$^{-1}$ for the sample, which can be attributed to the vibrations of CuO, confirming the formation of highly pure CuO nanoparticles. In the FTIR spectrum for rGO, the peaks at 1731, 1625 and 1183 cm$^{-1}$ are assigned to the C=O stretching, C=C stretching and C–O stretching, respectively. The broad peak at 3250 cm$^{-1}$ in the FTIR spectrum of the CuO-NPs/GO nanocomposite
might be attributed to the O–H stretching vibration of absorbed water molecules. The following functional groups were identified: O–H stretching vibrations (3300-3240 cm\(^{-1}\)), C=O stretching vibration (1740-1720 cm\(^{-1}\)), C=C from un-oxidized sp\(^2\) C–C bonds (1620-1590 cm\(^{-1}\)) and C–O vibrations (1250 cm\(^{-1}\)) in the FTIR spectrum of CuO-rGO nanocomposites which confirms the formation of nanocomposites.

**Antibacterial study:** The antibacterial activity of the copper oxide nanoparticles and CuO-rGO nanocomposites were tested on Gram-positive (*S. epidermidis*) and Gram-negative (*E. coli*) bacteria using agar well diffusion method. Table-1 shows the zone of inhibition (ZOI) in mm shown by different standard antibiotics with *S. epidermidis* and *E. coli*. Table-2 shows antibacterial effect of CuONPs and CuO-rGO nanocomposites against Gram-positive (*S. epidermidis*) and Gram-negative (*E. coli*) bacteria which is expressed by measuring the diameter of zone of inhibition in mm. Fig. 4 shows zone of inhibition produced by different standard antibiotics with *S. epidermidis* (A) and different concentration of CuONPs and CuO-rGO nanocomposites (B) and (C). Fig. 5 shows zone of inhibition shown by different standard antibiotics with *E. coli* (D) and different concentration of CuONPs (E) and CuO-rGO nanocomposites (E) and (F).

Agar plates were prepared using nutrient broth and agar-agar. The wells of 8 mm diameter were punched with the help of steel borer into the agar having the test microorganism (concentration 5.0 \(\times\) 10\(^5\) CFU/mL). The wells were filled with 100 µL of CuO nanoparticles and CuO-rGO nanocomposites of different concentration. A hexa disc of standard antibiotics were also used as the control for the comparison of antibacterial property. After 24 h incubation at 37 °C, the diameters of the zone of inhibition was measured against the test microorganisms and optical images were documented by a high definition optical camera.

### Table 1

<table>
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<tr>
<th>Standard antibiotics</th>
<th>TE 25</th>
<th>C 25</th>
<th>P(_1)</th>
<th>AMP 10</th>
<th>S 10</th>
<th>S3 300</th>
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<tr>
<td><em>S. epidermidis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>19</td>
<td>17</td>
<td>15</td>
<td>NS</td>
<td>23</td>
<td>NS</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>NS</td>
<td>NS</td>
<td>13</td>
<td>NS</td>
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### Table 2

<table>
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<tr>
<th>Antibacterial study for bulk metal, nanoparticles and nanocomposite</th>
<th>CuSO(_4)</th>
<th>CuO NPs</th>
<th>CuSO(_4) + Graphene</th>
<th>CuO-GO nanocomposites</th>
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<tbody>
<tr>
<td>(Sample conc. in µg/mL)</td>
<td>1000</td>
<td>100</td>
<td>500</td>
<td>1000</td>
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<tr>
<td>ZOI shown by <em>S. epidermidis</em></td>
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<td>10</td>
<td>14</td>
<td>12</td>
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<tr>
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<td>12</td>
<td>15</td>
<td>10</td>
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**Fig. 4.** (A) Zone of inhibition produced by different standard antibiotics with *S. epidermidis* (B) and (C) different conc. of CuONPs and CuO-rGO nanocomposites. Zone of inhibition produced with *S. epidermidis* (a), CuSO\(_4\) (b), 100 ppm conc. of CuONPs (c), 500 ppm conc. of CuONPs (d), 1000 ppm conc. of CuONPs (e) CuSO\(_4\) and Graphene (f) 100 ppm conc. of CuOGO nanocomposites (g) 500 ppm conc. of CuO graphene oxide nanocomposites (h) 1000 ppm conc. of CuO-rGO nanocomposites
Highest zone of inhibition shown by standard antibiotics is 23 and 13 mm for S. epidermidis and E. coli, respectively. Highest zone of inhibition shown by CuO-rGO nanocomposites is 28 and 23 mm for S. epidermidis and E. coli respectively which is higher than investigated standard antibiotics. Hence, both CuO and CuO-rGO nanocomposites show better antibacterial properties than six standard investigated antibiotics.

Conclusion

Copper oxide nanoparticles, reduced GO and CuO-rGO nanocomposites have been prepared through a facile and easy modified sol-gel method. CuO nanoparticles samples exhibit good antibacterial activities against Gram-negative bacterial strain E. coli and Gram-positive strain S. epidermidis but CuO-rGO nanocomposites exhibit better antibacterial activity than simple CuO nanoparticles. The increase of concentration of both CuO nanoparticles and CuO-rGO nanocomposites, results in increase in antibacterial activity. Highest ZOI shown by standard antibiotics is 23.0 and 13.0 for S. epidermidis and E. coli respectively. Highest ZOI shown by CuO-rGO nanocomposites is 26.0 and 28.0 for S. epidermidis and E. coli respectively. Hence, both CuO and CuO-GO nanocomposites show better antibacterial properties than six standard investigated antibiotics.

REFERENCES

18. O. Akhavan and E. Ghaderi, ACS Nano, 4, 5731 (2010); https://doi.org/10.1021/nn101390x.

Fig. 5. Zone of inhibition produced by different standard antibiotics with E. coli (D) and different conc. of CuONPs (E) and CuO-rGO nanocomposites (F). Zone of inhibition produced with E. coli (a), CuSO₄ (b), 100 ppm conc. of CuONPs (c), 500 ppm conc. of CuONPs (d), 1000 ppm conc. of CuONPs (e) CuSO₄ and graphene (f) 100 ppm conc. of CuO-rGO nanocomposites (g) 500 ppm conc. of CuO-rGO nanocomposites (h) 1000 ppm concentration of CuO-rGO nanocomposites.