

"Temperature-Dependent Synthesis of CuO-Based Nanocomposites with Glucose and GO: A Strategy to **Combat Antimicrobial Resistance**"

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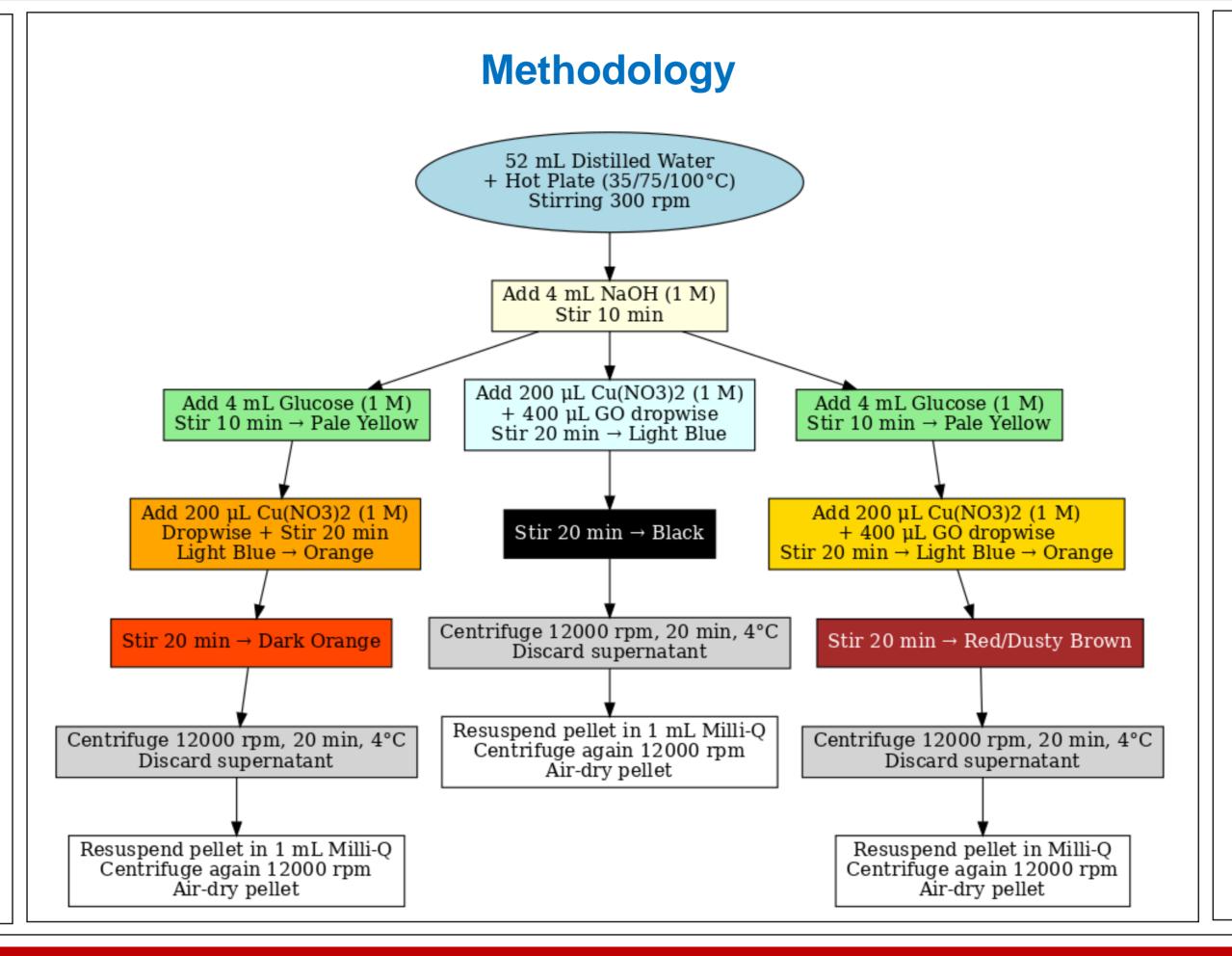
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Abstract

A simple and cost-effective approach has been used to synthesize biocompatible nanocomposites of graphene oxide, glucose and copper oxide nanoparticles, GO stabilized copper oxide nanoparticles and GO stabilized glucosecapped copper oxide nanoparticles) are synthesized at 35, 75, and 100°C. All the nanocomposites are characterized for their physical properties and studied for their antibacterial properties. Antibacterial testing against laboratory strains of Escherichia coli and Bacillus anthracis shows that all the nanocomposites synthesized at 35°C possess better antibacterial activities compared to other nanocomposites and GO. In this study, the order of antibacterial activity for synthesized nanocomposites observed is Cu/Glu/GO > Cu/Glu > Cu be further explored to be an alternative antibacterial agent to overcome antimicrobial resistance in near future.

Graphical Abstract e transfer Penetration resulting in cellular leakage Wrapping/entrapment membrane Synthesis Oxidative stress at 35, 75 & 100°C Oxidation of essential Mitochondrial cellular components Dysfunction (for other microbes) Protein damage Lipid Lipid bilayer peroxidation DNA damage Copper ions Cu/Glu/GO Cu/Glu/GO nanocomposite



Color test

The net reaction between an aldehyde and the Cu II^{+ ions} is shown in equation 1.

 $RCHO + 2 Cu^{2+} + 5 OH^{-} \rightarrow RCOO^{-} + Cu_{2}O + 3 H_{2}O - (Eq 1)$

While in the case of Cu/GO nanocomposites, precipitation observed is of black color indicating the formation of cupric oxide (CuO) (12).

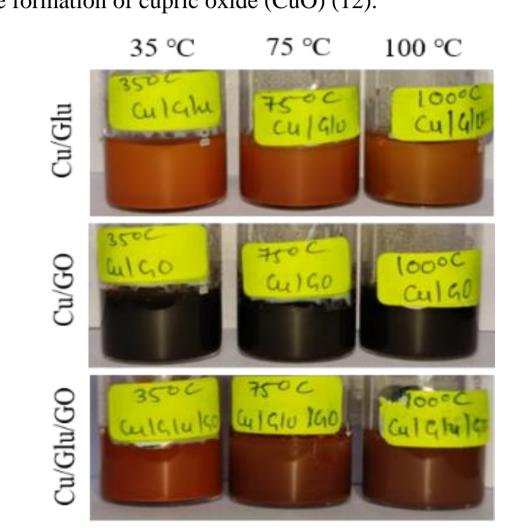


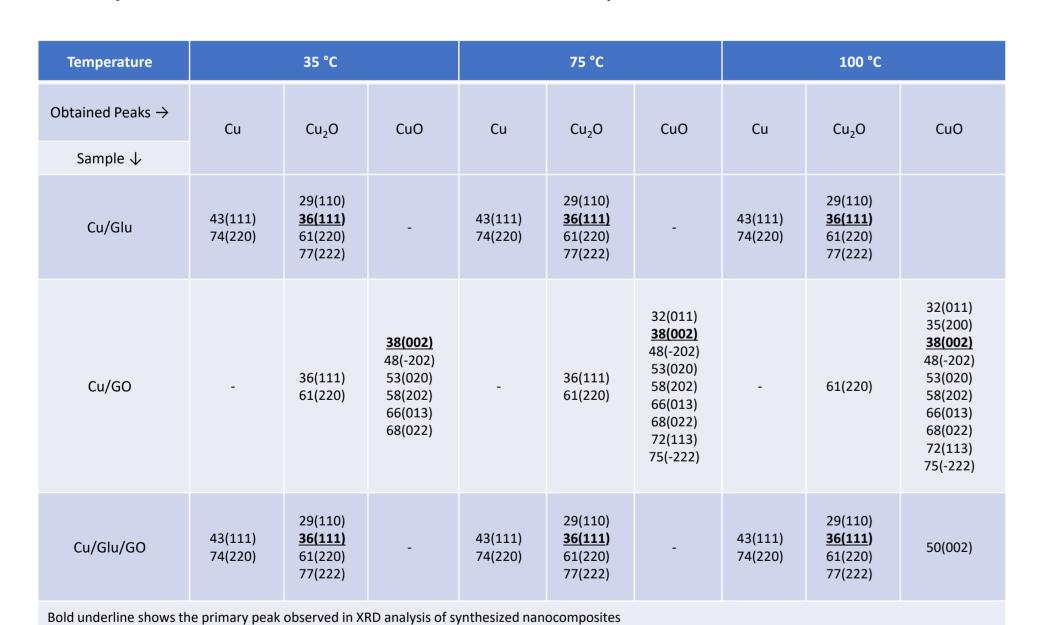
Figure 1 Color change reaction of Cu/Glu, Cu/GO, and Cu/Glu/GO nanocomposites synthesized at 35, 75, and 100°C.

X-ray Powder Diffraction Analysis Cu/Glu (75 °C) Cu/Glu (35 °C) Cu/Glu (100 °C) Cu/GO (75 °C) Cu/GO (35 °C) Cu/GO (100 °C) -202 020 202 220 022 013 -222 -202 020 202 220 013 202 -202 020 202 220 022 022 013 Cu/Glu/GO (75 °C) Cu/Glu/GO (35 °C) Cu/Glu/GO (100 °C) 5 15 25 35 45 55 65 75 85 5 15 25 35 45 55 65 75 85 5 15 25 35 45 55 65 75 85 2Ө (degree)

Figure 2 XRD Spectrum of Cu/Glu, Cu/GO, Cu/Glu/GO synthesized at (a) 35°C, (b) 75°C, (c) 100°C

Effect of composition of nanocomposites and synthesis temperature

Table 1 XRD analysis for Cu/Glu, Cu/GO, and Cu/Glu/GO synthesized at 35 °C, 75 °C, and 100 °C



Raman Analysis

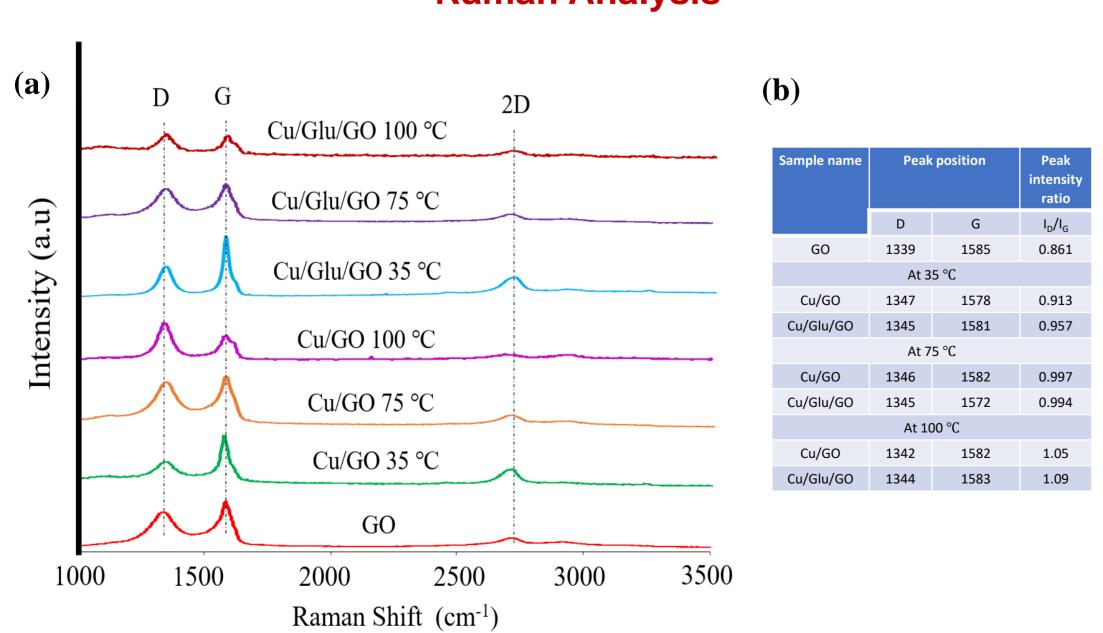
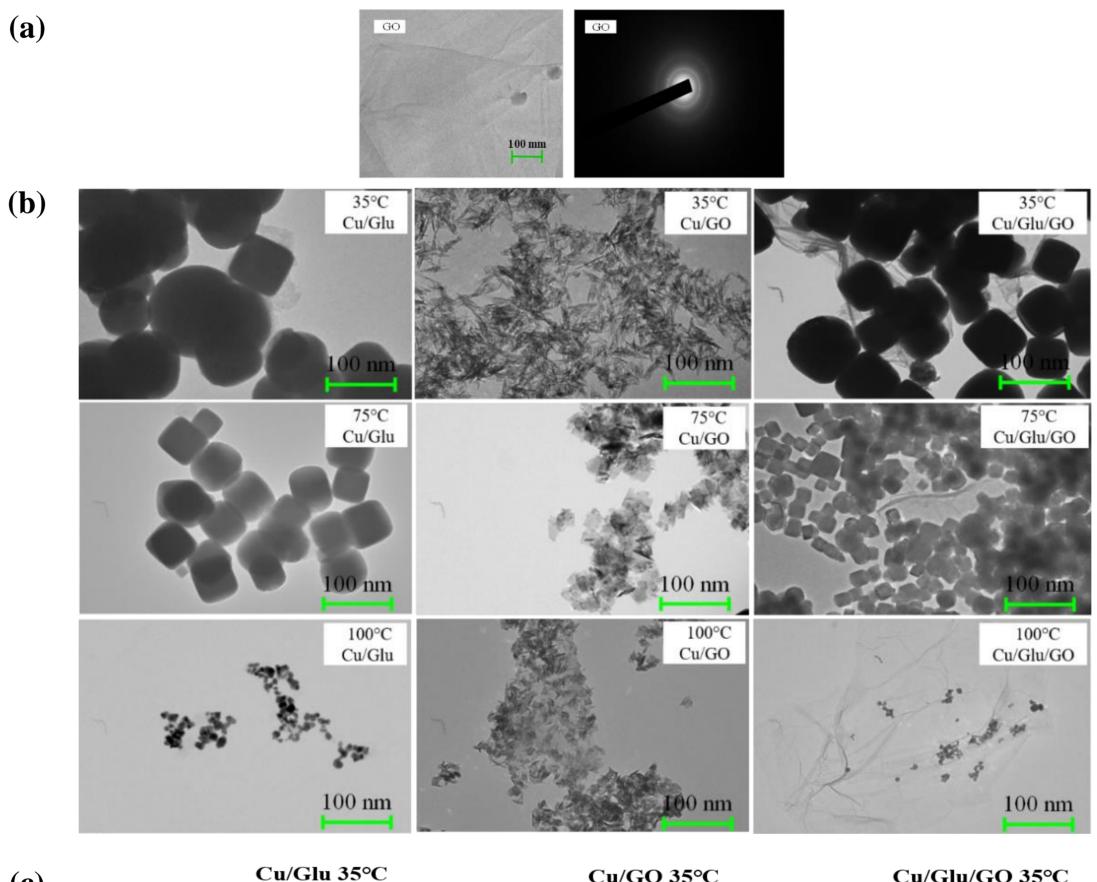


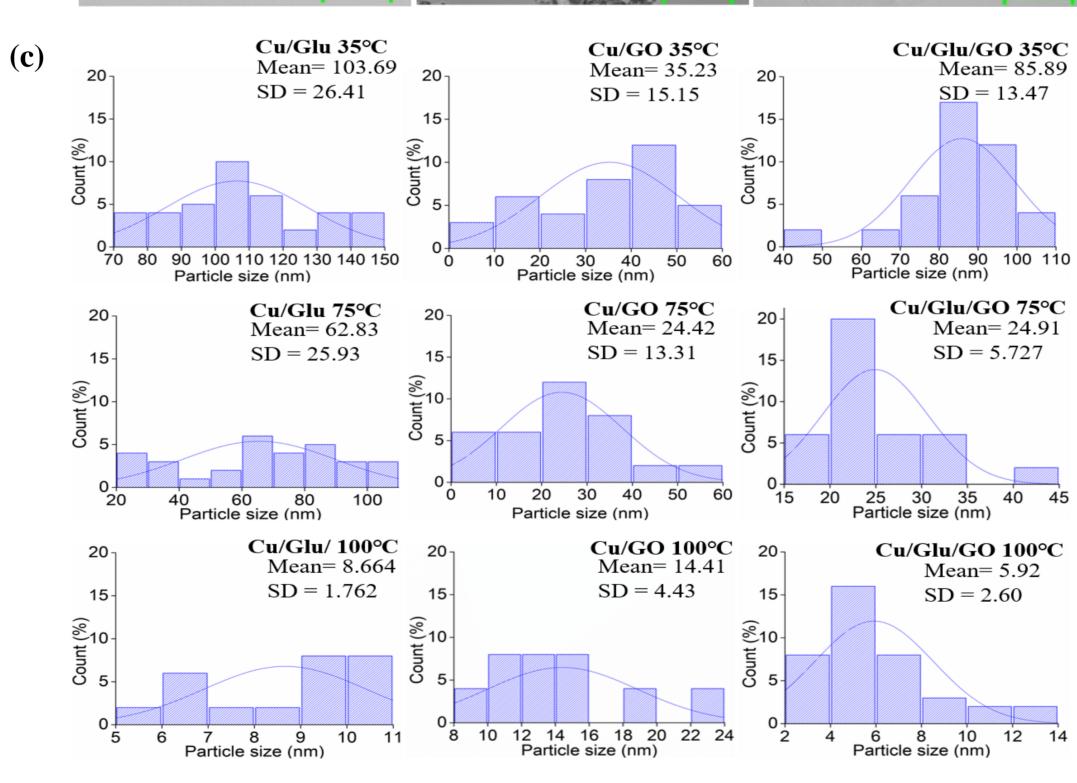
Figure 3 (a) Raman spectra of GO and Cu/GO, Cu/Glu/GO synthesized at 35, 75, and 100°C and (b) Raman shift and peak intensity ratio (ID/IG) of GO and Cu/GO, Cu/Glu/GO synthesized at 35, 75, and 100°C.

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Transmission Electron Microscopic Analysis

Results





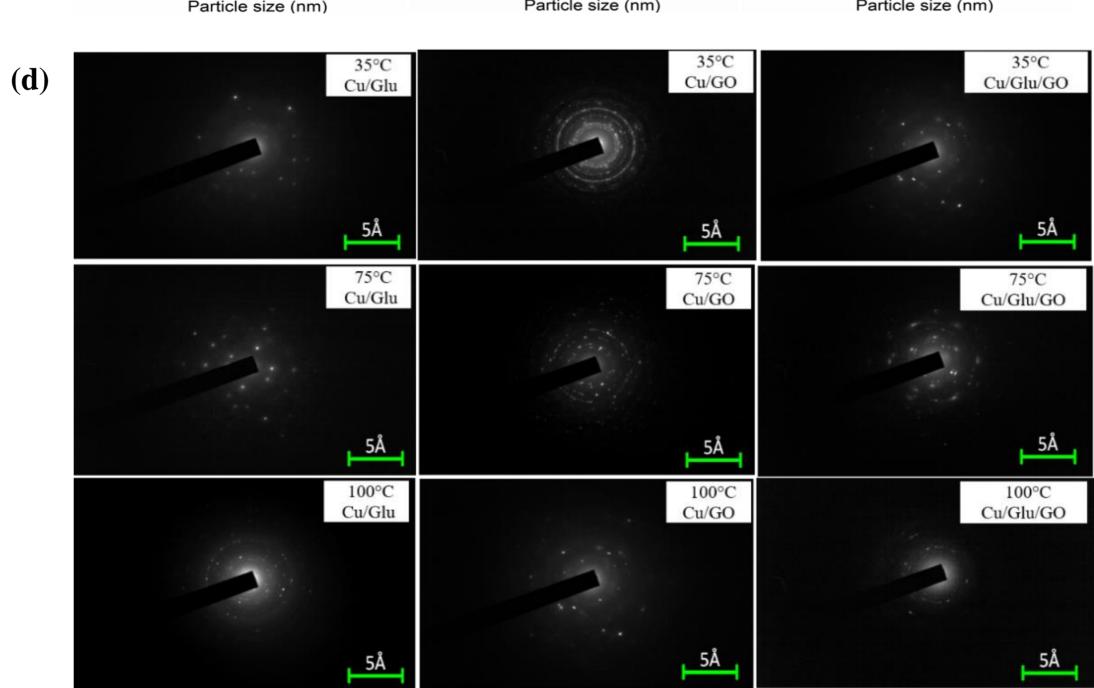
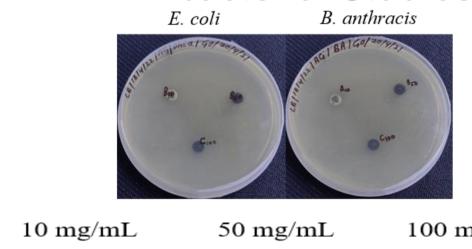


Figure 4 (a) TEM images and inset: SAED pattern of GO, (b) TEM images of Cu/Glu, Cu/GO, and Cu/Glu/GO synthesized at 35, 75, and 100°C, and (c) Histograms showing the average size range of copper nanoparticles in Cu/Glu, Cu/GO, and Cu/Glu/GO synthesized at varied temperatures (d) SAED pattern of synthesized nanocomposites.

Antibacterial Studies



(a)

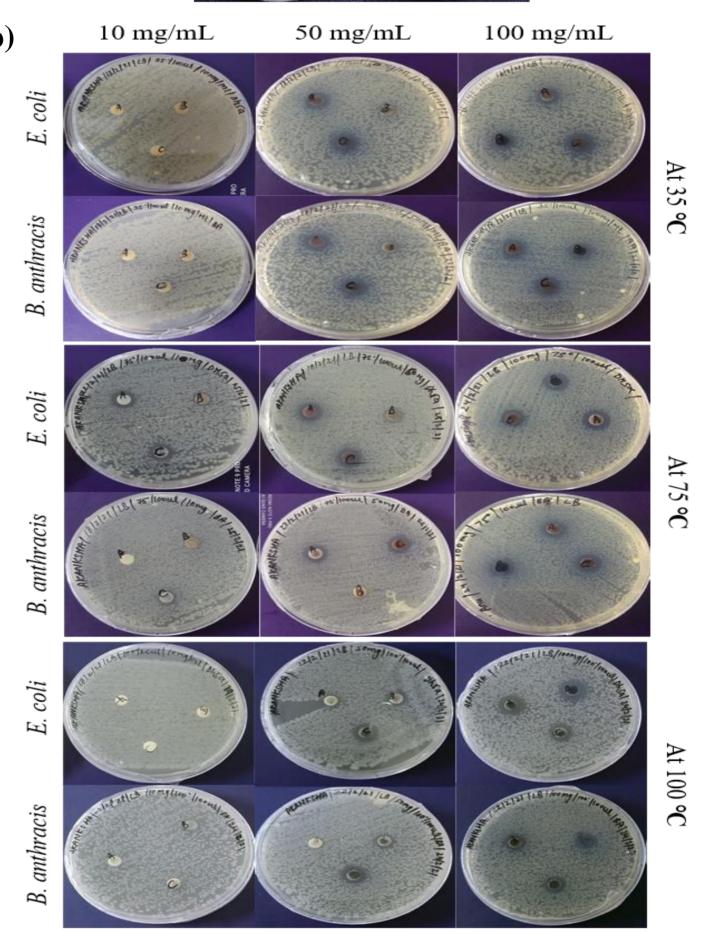


Figure 5 (a) Antibacterial activity studies of GO at different concentrations (10, 50, and 100 mg/mL) against B. anthracis and E. coli and (b) Antibacterial activity studies of (A) Cu/Glu, (B) Cu/GO (C) Cu/Glu/GO synthesized at 35, 75, and 100°C against E. coli and B. anthracis at different concentrations (10, 50, and 100 mg/mL) of nanocomposite.

Table 2 showing ZOI (mm) obtained against B. anthracis and E. coli with different concentrations of nanocomposites. (± Standard Deviation)

Sample Name	Sample Concentrati on	ZOI (mm) for samples synthesized at 35 °C		ZOI (mm) for samples synthesized at 75 °C		ZOI (mm) for samples synthesized at 100 °C	
		E. Coli	B. anthracis	E. Coli	B. anthracis	E. Coli	B. anthracis
Cu/Glu	10 mg/mL	6	6	6	6	6	6
Cu/GO		6	6	6	6	6	6
Cu/Glu/GO		6	6	7	6	6	6
Cu/Glu	50 mg/mL	10.33	10	9	8	6.67	6.63
Cu/GO		6.34	6.34	6.33	6.33	6	6
Cu/Glu/GO		12.33	10.67	10	9.33	8.33	8
Cu/Glu	100 mg/mL	11.67	10.67	11	8	9.33	7.8
Cu/GO		8.33	7.33	8.33	8.33	8	8
Cu/Glu/GO		12.33	12	11.67	10.67	10	10

- **Conclusions** In this study, Glucose-capped copper oxide nanoparticles (Cu/Glu) were synthesized as
- a control, and copper-GO nanocomposites were synthesized with and without glucose (i.e., Cu/Glu/GO and Cu/GO, respectively) and studied for their antibacterial activity. Structural studies revealed that copper nanoparticles possess oxide forms, and the size of nanoparticles is reduced with increasing temperature, while chemical structure analysis of GO shows that it is also reduced with increasing temperature.
- · The shape of copper oxide nanoparticles is successfully controlled by glucose.
- · All the synthesized nanocomposites were found to have better antibacterial activity
- against laboratory strains of E. coli and B. anthracis when compared to GO.
- Nanocomposites synthesized at 35°C show better antibacterial activity.
- In this study, the order of antibacterial activity observed is Cu/Glu/GO > Cu/Glu > Cu/GO, suggesting Cu/Glu/GO to be a potential antibacterial agent · However, the mechanism of action of the synthesized nanocomposite includes the production of reactive oxygen species, cation release, biomolecule damage, ATP

depletion, and membrane interaction caused by metal nanoparticles and membrane

stress against bacteria while contacting with the sharp corner basal planes of GO, which results in disruption and damage to the cell membranes.

• Fine edges of GO sheets act as nano knives, cutting bacterial cell membrane, leads to exposure of intracellular components, followed by cell death. • It can be stated that a 100 mg/mL concentration of Cu/Glu/GO synthesized at 35°C can

be used as an efficient alternative antibacterial agent; however, cytotoxicity analysis can

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Acknowledgments

help to explore its clinical usage.

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