# Three-dimensional Nanoporous Cu-BTC/Graphene Oxide Nanocomposites with Engineered Antibacterial Properties Synthesized via a One-pot Solvosonication Process

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## Abstract

Cu-based nanostructures are a well-known class of antibacterial nanomaterials with broad antibacterial properties. In this study, a facile and one-pot solvosonication process is introduced to synthesize Cu-BTC/graphene oxide nanocomposites with controlled morphological and structural properties. The size range of synthesized Cu-BTC nanoparticles is controlled through the synthesis process by adjusting the content of graphene oxide nanosheets in the synthesis precursor solution. A wide range of sizes and morphologies are achieved via this strategy and the size range of Cu-BTC nanoparticles from 30-40 nm to 15-20 nm are obtained by increasing the content of graphene oxide in the precursor solution from 0.005 to 0.15 mg/ml. We believe an increase in the number of available sites on the basal plane of graphene oxide for the nucleation of Cu-BTC nanocrystals is the main reason for the smaller size range of Cu-BTC nanoparticles with higher concentrations of graphene oxide in the precursor solution.

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directly affected by the structure and morphology of Cu-BTC nanoparticles on the basal plane of graphene oxide nanosheets. The synthesized nanocomposite with the smallest size range of Cu-BTC nanoparticles presents the highest antibacterial activity. Consequently, the results presented here suggest that the antibacterial activity of Cu-BTC/graphene oxide nanocomposites can be engineered by controlling the structural and morphological properties of Cu-BTC nanoparticles through the synthesis process.

**Keywords:** Antibacterial nanomaterials; Cu-BTC nanoparticles; Nanocomposites; Graphene oxide; Solvosonication.

# **1. Introduction**

Bacterial infections are serious problems that can lead to different health issues such as the contamination of the drinking water, tissue damage, food spoilage, and implant failure [1, 2]. Moreover, despite great developments through past decades on the treatment of bacterial infections via antibiotics, the ability of microorganisms to develop resistance to antibiotics resulted in a global research interest on the introduction of antibacterial materials and nanomaterials with controlled structures and properties, as well as facile and fast synthesis processes [3]. Although a wide range of antibacterial materials and nanomaterials have been developed and reported in the literature through recent years, the promising antibacterial properties of metal-based nanoparticles and nanostructures have led to a growing interest in the introduction of novel and simple methods for the fabrication of antibacterial metal-based nanomaterials and nanostructures [4-6].

Among different antibacterial metal-based nanoparticles and nanostructures, copper-based particles and nanoparticles have attracted great interest due to their broad antibacterial properties and their high potential to damage bacteria's proteins and lipids [7]. Although Cu is

generally an inert metal, Cu ions (Cu<sup>+</sup> and Cu<sup>2+</sup>) that form through the release of Cu to an aqueous medium have a high potential to not only damage the bacteria membrane with binding with different organic substances but also provide bacteria toxicity [8, 9].

Besides the direct antibacterial applications of Cu nanoparticles, porous nanostructures such as Cu-based coordination polymers have also received great attention recently for antibacterial purposes [10, 11]. These porous nanostructures, as the third-generation antibacterial agents, can present advantages such as high specific surface areas, tunable pore and particle size ranges, and adjustable Cu ion release rates [10].

Cu-BTC is one of the main copper ion-containing coordination polymers that can present interesting antibacterial activity in nanoparticle and nanocomposite forms [10]. Recent studies on Cu/H<sub>3</sub>BTC nanoparticles have confirmed the concentration-depended antibacterial activity of these nanoparticles against *E. coli* and *S. aureus* [12]. Moreover, Cu-BTC nanoparticles (Cu-BTC NPs) can also be incorporated into different nanocomposites to introduce the antibacterial activity to resulting nanocomposites. The incorporation of Cu-BTC NPs in the structure of chitosan films can lead to the formation of antibacterial polymeric films with a slow release of copper ions and reduced cytotoxicity and, as a result, simultaneously kill bacteria and improve vessel regeneration in local infection therapy [1].

Different synthesis processes are available in the literature to fabricate Cu-BTC NPs with different structural and morphological properties [13, 14]. The synthesis process of Cu-BTC coordination polymers dictates the morphology and the crystal structure of these Cu-based nanoporous materials [15]. The most conventional synthesis process of Cu-BTC is the hydrothermal synthesis process, which mostly leads to the formation of Cu-BTC crystals with the octahedral morphology [13, 15]. However, different morphologies of Cu-based crystals can be synthesized by controlling the synthesis condition [16].

Cu-BTC microcrystals can also be synthesized on the basal plane of graphene oxide (GO) nanosheets to fabricate Cu-BTC/GO nanocomposites [17]. Generally, there is no data on the antibacterial properties of this new class of nanocomposite. However, a recent study on the antibacterial properties of CuO NPs/GO nanocomposites has revealed that CuO NPs synthesized on GO nanosheets' surface can significantly improve the inhibiting performance of GO nanosheets against *E. coli* and *S. typhimurium* bacteria [2].

In this work, Cu-BTC/GO nanocomposites with different morphologies are synthesized through a new solvothermal synthesis process with different contents of GO nanosheets in the precursor solution of the synthesis process. Structural and morphological studies are used to investigate the impacts of different contents of GO in the synthesis precursor solution on the structure and morphology of synthesized nanocomposites. Moreover, the antibacterial properties of synthesized nanocomposites are studied against *E. coli* and *S. aureus*. Our results revealed that the antibacterial activity of Cu-BTC/GO nanocomposites can be improved by adjusting the morphology and size of Cu-BTC NPs via controlling the content of GO nanosheets in the solvothermal synthesis precursor solution. The main advantage of the protocol presented in this work for the fabrication of Cu-BTC/GO nanocomposites that affects the antibacterial activity of final products.

## 2. Experimental

## 2.1. Materials

Graphite powder was from LECO Co. Moreover, potassium permanganate (KMnO<sub>4</sub>), sulfuric acid (98%, H<sub>2</sub>SO<sub>4</sub>), hydrogen peroxide (30%, H<sub>2</sub>O<sub>2</sub>), hydrogen chloride (37%, HCl), copper

acetate (Cu(CH<sub>3</sub>COO)<sub>2</sub>·xH<sub>2</sub>O), benzene tricarboxylic acid (BTC), dimethyl sulfoxide (DMSO), and N,N-dimethylformamide (DMF) were from Merck Chemicals Co.

### 2.2. Synthesis of graphene oxide (GO)

GO nanosheets were synthesized using graphite powder as the precursor based on the modified Hummers technique [1, 2]. In a typical synthesis process, 1 g of graphite powder was mixed with 100 mL of H<sub>2</sub>SO<sub>4</sub> using a magnetic stirrer at room temperature. Then, to oxidize graphite powder, 6 g of KMnO<sub>4</sub> was gradually added to the mixture and was kept at 75–80 °C for 4 h. Then, 300 mL of distilled water was added and the mixture was further agitated at room temperature using a magnetic stirrer. To terminate the oxidation reaction, 50 mL of H<sub>2</sub>O<sub>2</sub> was added to the mixture after 15 min of stirring. The product was washed with 10% HCl solution (5 times) and distilled water (5 times) via centrifugation to reach the neutral pH. The obtained graphite oxide powder was dispersed in the desired concentrations (0.005, 0.05, 0.1, and 0.15 mg/mL) in distilled water by sonication using a Hielscher UP400S homogenizer (20 kHz, 400 W, 80% power). The GO suspensions were then centrifuged to remove aggregates and unstable particles.

## 2.3. Synthesis of copper-based metal-organic nanoparticles and nanocomposites

A one-pot solvosonication process was employed for the fabrication of Cu-based metal-organic nanoparticles (Cu-BTC NPs) and Cu-BTC/GO nanocomposites. In a typical synthesis process of Cu-BTC NPs, 0.199 gr (1mmol) of copper acetate and 0.105 gr (0.5 mmol) of BTC were dissolved in a solution of 15 ml of DMF and 15 ml of distilled water. The mixture was sonicated for 45 min using an ultrasonic bath (Free 9 Tecno-Gaz, 300 W, 50 Hz). The synthesized

precipitates were filtered and washed three times with DMF. The obtained powders were then dried at room temperature.

Cu-BTC/GO nanocomposites were synthesized by adding 0.199 gr of copper acetate and 0.105 gr of BTC to a solution consisting of 15 ml of DMF and 15 ml of the GO suspension with a specific concentration of GO. The mixture was then sonicated for 45 min. The precipitates were filtrated, washed, and dried at room temperature. The concentration of GO nanosheets in the GO suspensions used for the synthesis of Cu-BTC/GO nanocomposites were 0.005 (NC-1), 0.05 (NC-2), 0.1 (NC-3), and 0.15 mg/mL (NC-4), as reported in **Table 1**. A schematic of the protocol followed in this study for the fabrication of samples is presented in **Figure 1**.

Table 1. GO content in the precursor solutions used for the synthesis of nanocomposites

Sample	Cu-BTC NPs	NC-1	NC-2	NC-3	NC-4
GO content (mg/mL)	0	0.005	0.05	0.1	0.15



Figure 1. Synthesis protocol: a schematic of the protocol followed in this study for the synthesis of samples

#### 2.5. Characterization

Atomic force microscopy (AFM) results were obtained via a Dualscope DS 95–200, DME atomic force microscope on a freshly cleaved mica substrate. A scan rate of 20  $\mu$ m/s on the noncontact mode was used and the microscope was equipped with a rectangular cantilever with aluminum coating. Transmission electron microscopy (TEM) images were provided using a Philips CM10 electron microscope (100 kV). Fourier transform infrared (FTIR) spectra were obtained using a Nicolet IR100 spectrometer according to the ASTM E 1252-98. X-ray diffraction (XRD) analyses were carried out using an Inel Equinox 3000 diffractometer equipped with a Cu ka radiation source ( $\lambda = 1.54056$  Å) at 40 kV and 30 mA. The field emission scanning electron microscopy (FESEM) images were carried out via a TESCAN Mira 3 scanning electron microscope (15 kV).

Nitrogen adsorption/desorption isotherms were provided using a Micromeritics TriStar II Plus instrument at 77 K. Brunauer, Emmett and Teller (BET), Barrett, Joyner, and Halenda (BJH) and t-plot theories were fitted on isotherms to calculate textural parameters. Moreover, single-point BET analyses were carried out using a ChemBET 3000 Chemisorption Analyzer at a detector current of 150 mA. Raman spectra were recorded on a LabRAM HR Evolution Raman spectrometer at 532 nm. In addition, dynamic light scattering (DLS) and zeta potential measurements were carried out on a Microtrac Nanotrac Wave II at 25 °C (solvent: water, the refractive index of the solvent: n=1.33).

The antibacterial behavior of fabricated samples was investigated using the micro-well diffusion method described in the literature [12]. Gram-negative *Escherichia coli* (*E. coli* - ATCC 10536) and Gram-positive *Staphylococcus aureus* (*S. aureus* - ATCC 29737) bacteria were used here to investigate the antibacterial behavior of the samples through the agar well diffusion assay [18]. First, each microorganism was grown in a 100 ml conical flask with 10

ml of tryptic soy broth (TSB, Merck) on a rotary shaker at 37 °C for 5 h at 200 rpm. The bacteria was then diluted to  $10^4$ – $10^5$  CFU/ml for further studies.

Micro-well dilution assay was used to calculate the minimum inhibitory concentration (MIC) of each sample for *E. coli* and *S. aureus* bacteria [12]. In a typical assay, 100 ml of a bacterial suspension  $(10^4-10^5 \text{ CFU/ml})$  was added to a micro-well (6 mm in diameter). Different concentrations of samples (3 mg, 2 mg, 1 mg, 0.5 mg, 0.25 mg, 0.125 mg, 62.5 µg, and 3.125 µg) were added to different wells containing different bacterial suspensions. Suspensions were then incubated at 37 °C for 24 h (Memmert GmbH, INB400) and then the concentration of samples in wells with no visible bacterial growth was considered as the MIC of samples.

Minimum bactericide concentration (MBC) values were defined as the lowest concentration of each sample yielding colony counts <0.1% of the initial inoculum [19]. After MIC tests, 5  $\mu$ L of suspensions from wells with no visible bacterial growth was placed on an agar plate (Nutrient Agar, Merck). Plates were then incubated for an additional 24 h at 37 °C. The minimum concentration of each sample required for colony counts <0.1% of the initial inoculum after 24 h of the subculturing was reported as the MBC of the sample.

For the diameter of the inhabitation zone measurements, 100  $\mu$ L of the diluted bacterial suspension (10<sup>4</sup>–10<sup>5</sup> CFU/ml) was first evenly spread over an agar Luria Bertani (LB) plate. A sample suspension in DMSO (3, 0.5, and 0.25 mg/ml) was then placed in wells on LB agar plates via pipette [12]. Inoculated plates were then incubated at 37 °C for 24 h and the formation of inhibitory zones around each hole was assessed visually and diameters were measured using a millimeter scale. Measurements were repeated three times to calculate standard deviation values.

# 3. Results and discussion

# 3.1. Characterization of GO nanosheets

The thickness of synthesized GO nanosheets was investigated using AFM results (**Figure 2**). GO nanosheets had a mean thickness of around 1.5 nm (Figure 2a), which was in the thickness-range of GO reported in the literature (< 2 nm) [20, 21]. Moreover, the TEM results of synthesized GO nanosheets (Figure 2b) presented a folded structure with wrinkled edges, confirming the low thickness of synthesized nanosheets. FTIR results were used to investigate the functionality of synthesized GO nanosheets (Figure 2c). Bands observed at 3424 and 1717 cm<sup>-1</sup> were related to stretching vibrations of –OH (hydroxyl, COOH, and H<sub>2</sub>O, mostly carboxyl groups) and –C=O (COOH groups) bonds on the basal plane and edges of GO [21-23].



*Figure 2. Characterization of GO nanosheets:* (*a*) *AFM images, (b) TEM image, (c) FTIR spectrum, and (d) XRD pattern of synthesized GO nanosheets* 

The band at 1619 cm<sup>-1</sup> was assigned to the in-plane asymmetric stretching of sp2-hybridized C=C bonding in the backbone of GO nanosheets (overlapping with C=O stretching vibrations of ketones) [24]. Moreover, the band observed at 1415 cm<sup>-1</sup> was assigned to the stretching vibrations of C-O  $\gamma$ -region (mostly epoxides and ketones) [25, 26]. Furthermore, bands at 1041 and 1180 cm<sup>-1</sup> were related to C-O  $\alpha$ -region (ethers, hydroxyls, and carboxyls) and  $\beta$ -region (mostly ketones) stretching vibrations, respectively [21, 25]. Consequently, FTIR results confirmed the successful surface functionalization of GO nanosheets with different oxygen-containing functional groups. Presence of these groups resulted in the observed large interlayer spacing of GO, as observed in the XRD of nanosheets (Figure 2d). The characteristic

diffraction peak of GO nanosheets was observed at  $2\theta$  values around  $10^{\circ}$  [27], corresponding with an interlayer spacing of 0.88 nm.

## 3.2. Impacts of the GO content on the morphology of nanocomposites

Morphological characteristics of the synthesized Cu-based metal-organic nanoparticles (Cu-BTC NPs) and Cu-BTC/GO nanocomposites synthesized from solvosonication precursor solutions containing 0.005 (NC-1), 0.05 (NC-2), 0.1 (NC-3) and 0.15 mg/mL (NC-4) GO nanosheets were studied using FESEM images (**Figure 3**). Cu-BTC NPs synthesized via the solvosonication process presented a semi-spherical nanoparticle-form morphology, similar to the morphology of Cu-BTC metal-organic framework (MOF) synthesized by the hydrothermal process using DMF [28], and with ultrasound irradiation under solid-state conditions [29].



Figure 3. Morphology studies: FESEM images of Cu-BTC NPs and NC samples with different content of GO nanosheets in the precursor solution. NC-1 (0.005 mg/ml GO), NC-2 (0.05 mg/ml GO), NC-3 (0.1 mg/ml GO) and NC-4 (0.15 mg/ml GO).

With the incorporation of GO nanosheets, Cu-BTC crystals formed on the surface of GO nanosheets, and a shift in the geometry of Cu-BTC NPs was observed. Interestingly, the shape of the synthesized Cu-BTC NPs in the presence of 0.005 mg/mL GO (NC-1), was similar to the well-known octahedral geometry of HKUST-1 MOF crystals, synthesized via the hydrothermal process [30-32].

As the concentration of GO nanosheets in the precursor solution increased to 0.05 mg/mL (NC-2 in Figure 3), Cu-BTC NPs with both octahedral and semi-spherical morphologies were observed, suggesting a variation in the structure of Cu-BTC NPs with an increase in the concentration of GO in the precursor solution. Interestingly, Cu-BTC NPs with common morphologies (such as octahedral and cubic morphologies) were observed to form as a sublayer of Cu-BTC NPs with semi-spherical morphologies in the structure of both NC-1 and NC-2. This observation suggests that when the concentration of GO nanosheets in the precursor solution was low (0.005 mg/ml in NC-1 and 0.05 mg/ml in NC-2), first a layer of Cu-BTC NPs with the octahedral morphology formed on the basal plane of GO nanosheets and semi-spherical Cu-BTC NPs then nucleated on the surface of this layer (see FESEM images of NC-1 and NC-2 in Figure 3).

The morphology of Cu-BTC NPs synthesized in the presence of 0.1 (NC-3) and 0.15 mg/mL (NC-4) GO in the precursor solution was more semi-spherical (see FESEM images of NC-3 and NC-4 in Figure 3), close to the one observed for Cu-BTC NPs synthesized without GO (see FESEM images of Cu-BTC NPs in Figure 3). Moreover, not only the particle diameter of Cu-BTC NPs in nanocomposites decreased with an increase in the content of GO in the precursor solution, but also the diameter range distribution of Cu-BTC NPs in the structure of nanocomposites became narrower with an increase in the content of GO (see particle diameter size histograms in Figure 3).

The observed variation in the morphology, size range, and size range distribution of Cu-BTC NPs in the structure of synthesized nanocomposites can be related to the difference in the nucleation of Cu-BTC NPs synthesized with different GO contents in the precursor solution [33]. An increase in the content of GO in the precursor solution may result in the additional sites for the nucleation of Cu-BTC NPs, which resulted in the homogeneous distribution of Cu-BTC NPs throughout the surface of GO nanosheets and narrow size distribution of Cu-BTC NPs [34]. Consequently, the morphology of synthesized nanocomposites was related to the content of GO nanosheets in the precursor solution.

## 3.3. Microstructural studies

N<sub>2</sub> adsorption/desorption results of Cu-BTC NPs, NC-2, and NC-4 (as model samples) were used to investigate the texture of synthesized samples (Figure 4). The isotherm of synthesized Cu-BTC NPs was a Type-IV adsorption isotherm with a small hysteresis, indicating the formation of a mostly mesoporous structure (inset in Figure 4 for BJH results). The BET surface area of synthesized Cu-BTC NPs (244 m<sup>2</sup>/g), was lower than the surface area of Cu-BTC NPs synthesized via hydrothermal and solid-state synthesis processes (> 1000 m<sup>2</sup>/g) [35, 36] and also lower than the surface area of Cu-BTC/GO nanocomposites (Table 2). However, an increase in the content of GO nanosheets in the precursor solution resulted in a decrease in the surface area of the samples (Table 2). Moreover, the average pore size of the Cu-BTC NPs sample was around 22.7 Å, which was close to the reported average pore size of microporous Cu-BTC NPs in the literature [37, 38]. Interestingly, the average pore size of the NC-2 sample was lower than the Cu-BTC NPs sample, and closer to the theoretical average pore size of Cu-BTC nanocrystals (9 Å [35, 36]). This behavior can be related to the discussed difference between the morphological behavior of Cu-BTC NPs synthesized without GO in the precursor solution (Cu-BTC NPs in Figure 3) and with a low concentration of GO in the precursor solution (NC-1 and NC-2 in Figure 3). As expected, an increase in the content of GO in the precursor solution from 0.05 (NC-2) to 0.15 mg/mL (NC-4) resulted in an increase in the average pore size of the sample to a value close to the Cu-BTC NPs sample (20.4 Å).



*Figure 4. Textural characteristics:* N<sub>2</sub> adsorption-desorption isotherm of the Cu-BTC NPs sample. The inset presents the pore diameter distribution in the structure of the Cu-BTC NPs sample synthesized without GO in the precursor solution.

Sample	BET area	Pore volume	Average BET	GO content	
	(m <sup>2</sup> /g)	(cm <sup>3</sup> /g)	pore size (Å)	(mg/mL)	
Cu-BTC NPs	244.5	0.13	22.75	0	
NC-2	775.3	0.31	15.99	0.05	
NC-4	312.9	0.16	20.45	0.15	

Table 2. Surface area and pore volume of Cu-C NPs, NC-2 and NC-4 nanocomposites

XRD results were used to further investigate the role of GO content in the precursor solution on the microstructural characteristics of Cu-BTC NPs in Cu-BTC/GO nanocomposites (**Figure 5**). The XRD pattern of Cu-BTC NPs synthesized via solvosonication without GO (Figure 5a) presented a polycrystalline microstructure. The position of main peaks observed in the XRD pattern of Cu-BTC NPs synthesized in this work via the solvosonication process was identical to the position of Cu-BTC MOFs reported in the literature ( $2\theta = 6.9^\circ$ ,  $9.5^\circ$ ,  $11.6^\circ$ ,  $13.4^\circ$ ,  $14.9^\circ$ ,  $16.6^\circ$ ,  $17.5^\circ$ ,  $19.1^\circ$ ,  $20.1^\circ$ ,  $23.2^\circ$ ,  $25.9^\circ$ ,  $29.4^\circ$ ,  $35.3^\circ$ , and  $39.1^\circ$ ) [39-41]. The relative intensity ratio of the peaks to the intensity of the 440 peak observed in the XRD pattern of Cu-BTC NPs was unlike the common reported XRD pattern for Cu-BTC MOFs. This observed XRD pattern was expected, considering that the morphology of Cu-BTC NPs synthesized in this work was semi-spherical and different from the common octahedral morphology of Cu-BTC MOFs prepared via the hydrothermal synthesis technique [30-32].



*Figure 5. The microstructure of samples:* XRD patterns of (a) Cu-BTC NPs and NC samples with different content of GO in the precursor solution. (b) NC-1 (0.005 mg/ml GO), (c) NC-2 (0.05 mg/ml GO), NC-3 (0.1 mg/ml GO) and NC-4 (0.15 mg/ml GO) nanocomposites.

Presence of GO in the synthesis precursor solution of all synthesized Cu-BTC/GO nanocomposites (Figure 5b and c) resulted in the formation of a new crystal lattice plane (i.e.,  $2\theta = 21.1^{\circ}$ , hkl = 600). A similar peak was previously reported in the XRD pattern of HKUST-1 crystals synthesized at room temperature using ethanol/water solutions [42]. The intensity of this peak at 21.1° and the peak observed at 23.2° (hkl = 444) increased noticeably with an increase in the content of GO in the precursor solution (Figure 5b and c). The position of the main XRD peak ( $2\theta = 23.2^{\circ}$ ) in Figure 5c was completely different from the characteristic peak of re-stacked GO nanosheets (expected at 2 $\theta$  values around 28° [43]). Consequently, two structural phenomena can be considered for the appearance of the peak observed at 23.2° in the

XRD patterns of Cu-BTC/GO nanocomposites: (i) the partial re-stacking of GO with intercalated Cu-BTC NPs after the synthesis process, and (ii) the formation of Cu-BTC NPs with different microstructural characteristics on the surface of GO nanosheets.

No sign of GO characteristic peaks was observed in the XRD patterns of the synthesized Cu-BTC/GO nanocomposites (around 10° in Figure 2d), suggesting the complete exfoliation of GO nanosheets through the synthesis process of Cu-BTC/GO nanocomposites. In addition, the higher specific surface areas of synthesized NC-2 and NC-4 nanocomposites (775 and 312 m<sup>2</sup>/g, respectively), compared to the neat synthesized Cu-BTC NPs (244 m<sup>2</sup>/g), also confirm the complete exfoliation of GO through the synthesis process. Therefore, the partial re-stacking of GO nanosheets observed in the FESEM images of the NC-4 sample in Figure 3 can only be related to the partial aggregation of Cu-BTC/GO nanocomposites through the drying process.

Interestingly, the microstructure of synthesized NC-4 was close to the Cu-BTC NPs synthesized without GO nanosheets in the precursor (Figure 5a). Most of XRD peaks related to the structure of Cu-BTC NPs were observed in the XRD pattern of NC-4 (Figure 5c). Moreover, the intensity of peaks related to the 440 and 511 lattice planes (19.1° and 17.5°, respectively) increased noticeably in the XRD pattern of NC-4, compared to the peak intensity of 444 and 600 planes (23.2° and 21.1°, respectively). This structural behavior change suggests the formation of Cu-BTC NPs in the structure of NC-4 with microstructural characteristics close to the Cu-BTC NPs synthesized without any GO nanosheets in the precursor solution. Therefore, the microstructure and morphology of Cu-BTC NPs in the structure of Cu-BTC/GO nanocomposites can be engineered by controlling the conditions of the synthesis process.

FTIR results were used to further study the structure of synthesized Cu-BTC NPs and Cu-BTC/GO nanocomposites (**Figure 6**). Main characteristic bands of Cu-BTC NPs were observed at around 1374, 1443, 1581, and 1644 cm<sup>-1</sup> in the FTIR spectrum of Cu-BTC NPs

(Figure 6a). Bands observed at 1443 and 1644 cm<sup>-1</sup> were assigned to -O-C-O- bonding (symmetric and asymmetric stretching vibrations, respectively), and the bands at 1374 and 1581 cm<sup>-1</sup> were related to C=C stretching vibrations in the backbone of BTC in Cu-BTC NPs [44-46]. Moreover, bands related to the Cu-O bonding in the structure of Cu-BTC NPs were observed in the range of 670-770 cm<sup>-1</sup>, indicating the successful formation of Cu-BTC NPs [44]. The band at 1105 cm<sup>-1</sup> is also known to be the characteristic band of C–O–Cu bonding in the structure of Cu-BTC NPs [47, 48].



*Figure 6. Surface chemistry of samples: FTIR spectra of (a) Cu-BTC NPs, (b) NC-1 (0.005 mg/ml GO), (c)* NC-2 (0.05 mg/ml GO), NC-3 (0.1 mg/ml GO) and NC-4 (0.15 mg/ml GO) nanocomposites.

No significant difference was observed in the FTIR spectra of the neat Cu-BTC NPs (Figure 6a) and Cu-BTC/GO nanocomposites (Figure 6b and c). The only difference was in the

intensity of bands assigned to the C-H stretching vibrations (around 2916 and 2865 cm<sup>-1</sup>), compared to the intensity of other structural bands. The main reason for this behavior was the complete coverage of GO surface with Cu-BTC NPs in the structure of synthesized Cu-BTC/GO nanocomposites (see Figure 3). Moreover, no band was observed in the range of 1680–1750 cm<sup>-1</sup> in all of the FTIR spectra, indicating the absence of free H<sub>3</sub>BTC ligands in the structure of Cu-BTC NPs and synthesized Cu-BTC/GO nanocomposites [40].

## 3.4. Raman studies

Raman spectroscopy results were used to investigate the structural characteristics of both Cu-BTC NPs and Cu-BTC/GO nanocomposites (**Figure 7**). Raman results can provide a deep understanding of the structural characteristics of Cu-BTC NPs synthesized with and without GO nanosheets in the precursor of the solvosonication process. Moreover, as Cu-BTC NPs covered the entire surface of Cu-BTC/GO nanocomposites (see FESEM and FTIR discussions), Raman spectroscopy can be a versatile and effective tool to study the structure, defects status, and functionalities of GO nanosheets in synthesized Cu-BTC/GO nanocomposites [49].



*Figure 7. Structural properties:* Raman spectra of (a) Cu-BTC NPs (labeled as 1), (b) NC-1 (labeled as 2), (c) NC-2 (labeled as 3), NC-3 (labeled as 4) and NC-4 (labeled as 5) nanocomposites (insets: Raman spectra of samples in 100-1100 cm<sup>-1</sup>, 1100-1800 cm<sup>-1</sup> and 2400-3100 cm<sup>-1</sup> ranges).

Main Raman bands of synthesized Cu-BTC NPs at 177 (\*), 273 (\*), 499 (\*\*), 664 (·:), 741 (·:), 826 (·:), 1003 (#), 1459, 1548 and 1611 cm<sup>-1</sup> completely confirm the successful formation of Cu-BTC with the mesoporous structure [50, 51]. Raman bands at 177 and 273 cm<sup>-1</sup> can be assigned to the Cu–Cu dimer moieties [51]. In addition, the bands at 500 and 664 cm<sup>-1</sup> were related to the stretching modes of Cu–O coordination bond, and Cu<sub>2</sub>O bonding, respectively [46, 50, 51]. Two bands observed at 741 and 826 cm<sup>-1</sup> were assigned to out-of-plane ring C–H bending [19, 46]. Moreover, bands at 1003 and 1611 cm<sup>-1</sup> were related to stretching modes of Cu–BTC NPs [46, 52]. Furthermore, two bands observed at 1459 and 1611 cm<sup>-1</sup> were assigned to symmetric and asymmetric vibration of C–O<sub>2</sub> bonding, respectively [46, 52].

Almost no significant change was observed in the position and the relative intensity ratio of main Cu-BTC NPs bands (the intensity ratio of bands to the intensity of the band labeled as ::) in the Raman spectra of Cu-BTC/GO nanocomposites (see the inset in Figure 7a). Moreover, D and G bands of GO nanosheets in the first-order Raman spectra of all synthesized Cu-BTC/GO nanocomposites were observed at around 1350 and 1580 cm<sup>-1</sup>, respectively [53]. In addition, broad 2D bands of GO nanosheets were observed in the second-order Raman spectra of all nanocomposites at around 2700 cm<sup>-1</sup> (Figure 7b and c). The intensity ratio and the position of these main bands provide information on the structural defects of GO in the structure of Cu-BTC/GO nanocomposites [54]. Moreover, fitting the shoulders of these bands can provide further information on the structural characteristics of GO in the structure of Cu-BTC/GO nanocomposites [54].

In this work, based on the literature recommendations [49, 55], shoulders in the first-order Raman spectra of nanocomposites were fitted using five functions: D, G, D', D", and D\* bands. As the intensity of bands related to Cu-BTC NPs in the Raman spectra of Cu-BTC/GO nanocomposites (see the intensity of bands in Figure 7b and c) were much lower than that of GO-related bands, the overlapping impacts of Cu-BTC NPs bands in the 1200-1800 cm<sup>-1</sup> region of Raman spectra in Figure 7b and c were neglected. Moreover, the second-order Raman spectra of nanocomposites were fitted via four functions: 2D, 2D', D+D', and G\* bands [56]. Fitting parameters of first-order and second-order Raman spectra of synthesized Cu-BTC/GO nanocomposites are summarized in **Figure 8**.



Figure 8. Fitting parameters of GO-related Raman bands: (a) position of fitted D and G bands, (b)  $A_D/A_G$  and  $A_D/A_G$  area ratios of fitted first-order Raman bands, (c) position of fitted D\* and D'' bands, and (d) position of fitted 2D and D+D' second-order Raman bands of nanocomposites

The positions of G and D (also known as D1 [57]) bands and their relative intensities in the five-functions fitted Raman spectra of carbon-based materials can be considered as representatives of the graphitic structural perfectness and the disorder degree of carbon-based materials [56-58]. The G mode of graphite, which can lie in the range  $1500-1630 \text{ cm}^{-1}$ , has  $\text{E}_{2g}$  symmetry and represents the in-plane bond-stretching motion of pairs of C sp<sup>2</sup> atoms [55]. Moreover, the D band is a breathing mode of  $A_{1g}$  symmetry (graphene layer edges) and only becomes active in the presence of structural disorder [55]. Therefore, the intensity ratio or the area ratio of D to G bands is expected to increase with increasing the disorder degree in the graphitic structure of carbon-based materials [57, 58].  $A_D/A_G$  ratios lower than 3.5 are known to represent low disorder degrees in the structure of carbon-based materials (graphene edge

disorders in particular) [57]. As a result, the  $A_D/A_G$  ratios of synthesized nanocomposites in Figure 8b suggest the low degrees of edge disorders in the structure of nanocomposites.

The D' band is another important first-order Raman characteristic of GO, which represents the structural disorder of graphitic lattice due to surface disorders ( $E_{2g}$ -symmetry). Features of this band can provide information on the status of surface disorders in the structure of GO nanosheets. Moreover, the  $A_{D'}/A_{G}$  ratio in low-defect GO can be considered as a measure for the type of surface disorders, with values around 0.14 for vacancies, values around 0.08 for sp<sup>3</sup> defects (most probably surface functionalities), and values around 0.29 for grain boundaries [58]. Consequently,  $A_{D'}/A_{G}$  ratios calculated for nanocomposites in this work (Figure 8b) suggest that the dominant disorder type in the structure of GO nanosheets in synthesized nanocomposites was a combination of sp<sup>3</sup> defects and grain boundaries. The observed shifts in the type of dominant surface disorder of GO nanosheets in the structure of nanocomposites can be related to the content of surface functionalities occupied by Cu-BTC NPs through the synthesis process and as a result, the observed change in the morphology of Cu-BTC NPs in synthesized nanocomposites (see insets in Figure 3 and XRD discussions).

The D\* band is widely considered as a representative of functionalities and ionic impurities in the structure of GO nanosheets [57, 58]. The position of this peak is expected to shift to lower frequencies when the oxygen content in the structure increases [58]. As shown in Figure 8c, the position of this band shifted to higher wavenumbers as the content of GO nanosheets increased in the precursor solution. Since an increase in the concentration of GO nanosheets results in the presence of more oxygen-containing functional groups in the structure, this behavior should be considered as proof for interactions between surface oxygen groups of GO nanosheets and Cu-BTC NPs in the structure of nanocomposites. Moreover, no significant and systematic change in the position of 2D and D + D' bands in Figure 8d with increasing GO concentration in the precursor solution was observed. Therefore, it can be concluded that the percentage of C sp<sup>2</sup> atoms remained unchanged, as the position of these bands can be considered as a measure of C sp<sup>2</sup> percentage in the structure of GO nanosheets [56].

The  $A_{D}*/A_{G}$  ratio is a widely used measure for the content of oxygen in the structure of GObased nanostructures. Consequently, this parameter can be employed to investigate the content of functional groups on basal plane and edges of GO. The  $A_{D}*/A_{G}$  ratio of around  $0.15 \pm 0.02$ is known to represent an oxygen percentage of 20-22% in the structure of GO nanosheets. The  $A_{D}*/A_{G}$  ratio of NC-4 was 0.16, which was close to the  $A_{D}*/A_{G}$  ratio of NC-1 (0.15). This indicates that, despite an increase in the content of GO in Cu-BTC/GO nanocomposites from sample NC-1 to NC-4, oxygen containing functional groups on the basal plane and edges of GO nanosheets were probably involved in interactions with Cu-BTC NPs.

To further investigate the possible interaction mechanism between Cu-BTC NPs and surface functional groups on the basal plane and edges of GO, FTIR spectra of samples presented in Figure 8 were fitted via Gaussian and Lorentz functions, according to the method described in the literature (**Figure S1**). Fitting results were normalized to the **A** peak to compare the relative intensity of bands assigned to the functional groups of GO (**Figure S1**). Intensity ratios of FTIR fitted bands (**Table S2**) and the intensity ratios of Raman bands (Figure 8) were then used to investigate the role of possible interactions between GO and Cu-BTC NPs on the growth mechanism of Cu-BTC NPs on the surface of GO nanosheets.

The nucleation of Cu-BTC nanoparticles in the presence of GO nanosheets is reported to be a fast nucleation process that mainly initiates on structural defects of GO nanosheets [59]. These defects can be either molecular imperfections in the backbone of GO nanosheets (nanopores) or oxygen-containing functional groups on the basal plane of GO [59]. Moreover, the surface fluctuation of GO nanosheets, which is known to be related to the presence of functional

groups, can further facilitate the nucleation of Cu-BTC NPs on the surface of GO nanosheets [59]. Consequently, not only the presence of oxygen-containing functional groups on the basal plane of GO nanosheets can provide nucleation sites for the growth of Cu-BTC NPs, these functional groups can also control the surface fluctuation of GO nanosheets [60] and as a result, the growth rate of Cu-BTC NPs.

The suggested growth mechanism for the crystallization of Cu-BTC NPs on the surface of GO nanosheets suggest that epoxy groups on the basal plane of GO act as the seed sites for the crystallization of Cu-BTC NPs [36]. A comparison between the intensity ration of fitted bands related to the structure of Cu-BTC/GO nanocomposites prepared in this work (Table S2) suggests that an increase in the relative intensity of bands assigned to hydroxyl groups of GO nanosheets was observed in the FTIR spectra of nanocomposites with increasing the content of GO in the precursor solutions. However, no sign of bands related to epoxides and carboxyls groups of GO (expected at around 1041, 1180, and 1415 cm<sup>-1</sup>, see Figure 2) was observed in the FTIR spectra of Cu-BTC/GO nanocomposites. Since the formation of interactions between materials with carboxyls groups on the surface and Cu-BTC NPs also reported in the literature [61], it can be concluded that molecular imperfections and the epoxides and carboxyls groups of GO acted in this work as the seed sites for the crystallization process. The growth process of Cu-BTC NPs continued until in the Cu-BTC nanocrystals reached a defect or other growing nanocrystals in the structure [36].

## 3.5. Antibacterial properties and the role of microstructure

Impacts of the structural characteristics of prepared samples on their antibacterial activity were investigated using the diameter of the inhabitation zone, minimum inhibitory concentration (MIC), and minimum bactericide concentration (MBC) results against *S. aureus* and *E. coli* 

(**Figure 9**), as two model bacteria that are known to be responsible for a large number of the bacterial infections among common species [1]. Cu-BTC NPs presented clear inhibition zones for both Gram-positive and Gram-negative bacteria (Figure 9a and b). The diameters of the inhabitation zone for Cu-BTC NPs were 14 mm and 10 mm against *S. aureus* and *E. coli* bacteria, respectively (Figure 9d). This is a medium antibacterial activity compared to Cubased porous nanostructures with antibacterial activities in the literature [10, 12].



Figure 9. Antibacterial properties: formation of the inhibition zones for Cu-BTC NPs and NC-4 samples against (a) Gram-positive S. aureus (ATCC 29737) and (b) Gram-negative E. coli (ATCC 10536), inhibition zones of NC-1, NC-2, and NC-3 samples against S. aureus and E. coli, (d) measured diameters of the inhabitation zones of Cu-BTC NPs, NC-1, NC-2, NC-3, and NC-4 samples against S. aureus and E. coli at a sample concentration of 3 mg/ml, (e) minimum inhibitory concentration (M.I.C.) and minimum bactericide concentration (M.B.C.) of Cu-BTC NPs, NC-1, NC-2, NC-3, and NC-4 samples against S. aureus and E. coli

The diameter of the inhabitation zone of NC-1, NC-2, and NC-3 samples against *S. aureus* was all lower than that of the Cu-BTC NPs sample (Figure 9 c and d). In addition, the NC-1 sample presented no antibacterial activity against *E. coli*, even at concentrations as high as 3 mg/ml. As discussed through structural studies, the main difference between NC-1 and Cu-BTC NPs samples was in the morphology of NPs. The morphology of Cu-BTC NPs synthesized in the

presence of 0.005 mg/mL GO (NC-1) was similar to the reported morphology of Cu-BTC MOFs. Same morphology-related antibacterial behavior against *E. coli* has been reported in the literature for Cu2O crystals with different particle geometries [33].

Although the antibacterial activity of the NC-1 nanocomposite against *E. coli* was lower than the Cu-BTC NPs, the antibacterial activity of NC-2 and NC-3 nanocomposites against *E. coli* increased with the content of GO in the precursor solution (Figure 9d). The highest antibacterial activity against both Gram-positive and Gram-negative bacteria in this work was obtained with the NC-4 sample. The diameter of the inhabitation zone of the NC-4 nanocomposite was 21 mm and 12 mm against *S. aureus* and *E. coli* bacteria, respectively. The lowest MIC and MBC values were also observed for the NC-4 sample (Figure 9e). Considering the discussed structural differences between fabricated samples, it can be postulated that the antibacterial activities of prepared samples were significantly affected by the morphology and the crystalline nature of Cu-based NPs.

Three main antibacterial mechanisms have been proposed in the literature for Cu-based nanoparticles: (1) cellular uptake, (2) ion dissolution, and (3) reaction oxide species (ROS) generation [2]. In these mechanisms, the release of copper ions ( $Cu^{2+}$  and  $Cu^{+}$ ) in water plays an important and controlling role. It has been suggested that the release of copper ions increases in the presence of carbon nanotubes [8]. The same phenomena can be considered as the main reason behind the observed improved antibacterial activity of NC-4.

As discussed through FESEM, XRD, and Raman studies, the structure and the morphology of Cu-BTC NPs synthesized in the structure of NC-4 (the highest content of GO nanosheets in the synthesis precursor solution) was similar to the structure of Cu-BTC NPs synthesized without GO in the precursor solution. Moreover, Cu-BTC NPs synthesized without GO in the precursor solution and antibacterial activity. Furthermore, the weight

percentage of Cu in the structure of the NC-4 sample was even less than the Cu-BTC NPs sample due to the presence of GO nanosheets in the structure of the NC-4 nanocomposite. Therefore, the observed improved antibacterial activity of NC-4 can be related to the synergistic effects of both Cu-BTC NPs and GO nanosheets.

The antibacterial activities of both Cu-BTC NPs and NC-4 samples were higher against *S. aureus*, compared to *E. coli*. This behavior can be related to the presence of the bacterial outer membrane in gram-negative bacteria that improves the resistance of *E. coli* against bacteria toxicity mechanisms (**Figure 10**). The main proposed mechanism of the antibacterial activity of Cu NPs is based on the formation of Cu ions (Cu<sup>2+</sup> and Cu<sup>+</sup>) with the release of Cu to the water [8, 12]. The possible conversion of Cu<sup>+</sup> to Cu<sup>2+</sup> in water can lead to the formation of H<sub>2</sub>O<sub>2</sub>, which can further facilitate the dissolution of Cu ions.



*Figure 10. Antibacterial activity mechanisms:* mechanisms involved in the antibacterial behavior of Cu-BTC NPs and NC-4 samples against S. aureus and E. coli. The NC-4 sample presented a larger diameter of the inhabitation zone against S. aureus due to the smaller diameter range of Cu-BTC NPs in the structure of the NC-4 sample. The dissolution of Cu<sup>2+</sup> and Cu<sup>+</sup> ions, the formation of ROS, and the intracellular uptake were the main mechanisms involved in the bacteria toxicity of Cu-BTC NPs and NC-4 samples against both bacteria studied in this work. The bacterial outer membrane of E. coli was the main reason for the observed higher resistance of E. coli against synthesized samples.

The release of Cu ions and the antibacterial act of such ions in water is known to be a complex reaction with a series of intermediate reactions. In proposed interaction mechanisms for the cell toxicity of cooper-based nanoparticles, the formation of  $Cu^+$  is widely considered in the literature as the first possible step in the formation of copper ions [8, 62]:

Cu-based NPs + 
$$O_2 \rightarrow Cu^+ + O_2^{\bullet}$$
 (1)

$$Cu^{+} + O_2 \rightarrow Cu^{2+} + O_2^{\bullet}$$
<sup>(2)</sup>

Moreover, the proposed interaction mechanism between copper ions and bacterial sulfhydryl groups (RSH) can lead to the formation of  $H_2O_2$  and ROS [8, 62]:

$$2Cu^{2+} + 2RSH \rightarrow 2Cu^{+} + RSSR + 2H^{+}$$
(3)

$$2Cu^{+} + 2H^{+} + O_{2} \rightarrow 2Cu^{2+} + H_{2}O_{2}$$
(4)

$$O_2^{\bullet} + H^+ \to HO_2^{\bullet} \tag{5}$$

$$HO_2 \cdot + H^+ + e^- \to H_2O_2 \tag{6}$$

The hydrogen peroxide formed through equations 3-6 not only can facilitate the release of copper ions, it also assists the formation of reactive oxide species including hydroxyl radicals, which are known to have not only strong oxidizing potentials but also able to cause DNA strand breaks and affect gene expression [8, 62]:

$$H_2O_2 + O_2^{\bullet} \rightarrow OH^{\bullet} + OH^{-} + O_2$$
(7)

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^{\bullet} + OH^{\bullet}$$
(8)

Studies on the concentration of copper ions in the presence of bacteria suggest that the bacteria can also uptake the dissolved Cu ions directly [8]. Cu ions can damage the bacteria cell membrane and also affect the metabolic processes such as respiration osmotic stress [8]. Moreover, reactions of Cu ions with thiol and amino groups in proteins can lead to the

formation of ROS [8, 63]. Oxidizing ROS radicals can also cause different damages to the bacteria, leading to the bacteria cell toxicity [8].

In addition to the important aforementioned impacts of the released coper ions on main cellular toxicity mechanisms of Cu-BTC NPs, i.e. direct cellular uptake of copper ions, formation of reaction oxide species, and damages to the bacterial membrane, the role of GO nanosheets on the high antibacterial performance of Cu-BTC/GO nanocomposites should also be addressed. GO is known to have the highest antibacterial activity among different graphene-based materials (i.e., graphite, graphite oxide, GO and reduced-GO) [64]. Sharpe edges of GO nanosheets can cause irreversible damages to the bacterial cell [64]. Moreover, oxygen-containing functional groups on the basal plane and edges of GO can not only improve the formation of ROS but also affect microbial processes by disturbing the cellular structure [64]. Consequently, the observed improved antibacterial performance of the NC-4 sample, compared to the Cu-BTC sample, can be related to the additional cellular toxicity of the NC-4 sample caused by the presence of GO nanosheets in the structure.

## 4. Conclusions

In this work, Cu-BTC/GO nanocomposites with different morphologies and structures were synthesized through the solvothermal synthesis of Cu-BTC NPs in the presence of different contents of GO nanosheets. The nucleation and growth of Cu-BTC NPs took place on the basal plane of GO nanosheets and therefore, the size and morphology of the synthesized Cu-BTC NPs in the structure of Cu-BTC/GO nanocomposites were directly related to the content of GO nanosheets in the precursor solution.

The size range of Cu-BTC NPs changed from 30-40 nm to 15-20 nm when the content of GO nanosheets in the precursor solution increased from 0.005 to 0.15 mg/ml. Moreover, Raman

and XRD results revealed that the structural characteristics of Cu-BTC NPs were also related to the GO content in the precursor solution. The microstructure of the Cu-BTC/GO nanocomposite synthesized with 0.15 mg/ml of GO in the precursor solution (NC-4) became similar to the Cu-BTC NPs sample synthesized without GO. We suggested that the content of available sites for the nucleation of the Cu-BTC crystals increased with the increase of the GO content in the precursor solution, and as a result, Cu-BTC NPs with smaller nanoparticle size ranges were obtained in the structure of the NC-4 sample.

Interestingly, antibacterial studies against *S. aureus* and *E. coli* suggested that the antibacterial activity of Cu-BTC/GO nanocomposites increased with the decrease of Cu-BTC nanoparticle size, and the largest diameter of the inhabitation zone for both studied bacteria was observed for the NC-4 sample. Our results confirmed that the antibacterial activity of Cu-BTC/GO nanocomposites can be controlled by tuning the structural and morphological properties of Cu-BTC NPs through the synthesis process.

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