Synthesis, Characterization and Antimicrobial Activity of Copper-Metal Organic Framework (Cu-MOF) and Its Modification by Melamine

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Abstract
Copper-Metal organic framework (Cu-MOF) and melamine/Cu-MOF (MCu-MOF) samples were prepared by the hydrothermal process. The produced powder was dried and characterized by using the FTIR, X-ray techniques. The thermal-gravimetry (TG) analysis was performed to detect the thermal stability of the product. On other hand, the morphology and the surface area of this powder were carried. The bioactivity of the powder was carried by measuring the inhibition zone diameter around samples in (mm). The results of characterization showed that on the IR spectra a series of absorption peaks at 970, 1500 and 1640 cm\(^{-1}\) were appeared which characterized the formation of Cu-MOF, while an addition peaks were observed at 3121, 3324, 3415 and 3467 cm\(^{-1}\) and attributed to the incorporation of melamine into Cu-MOF. X-ray patterns of the prepared samples show sharp peaks at 7.4 and 8.5 specified to Cu-MOF. The intensity of these peaks increases by adding melamine which indicate the improving the crystallinity. Moreover two peaks at 26, 30 attributed to the incorporate melamine in the Cu-MOF. The surface area of Cu-MOF is equal to 1350 m\(^{2}\) g\(^{-1}\) while increase to 1410 m\(^{2}\) g\(^{-1}\) by incorporates melamine into the Cu-MOF. The thermal behavior (TG) of the Cu-MOF showed three sequence stages attributed to the moisture evaporation, degradation of the Cu-MOF and forming the Cu–O as end product respectively. For melamine incorporated to Cu-MOF (MCu-MOF), the TG profile split the main degradation into two parts resulting from the presence of melamine. To study the morphology of these samples by both FESEM and HRTEM were examined. The bioactivities of the both samples were tested against microbial strains. The results showed that the Cu-MOF and MCu-MOF have insignificant antimicrobial activity against gram positive of bacteria and Fungi. While for gram negative of bacteria it is observed a considerable effect.

Keywords Metal organic framework (MOF) · Antimicrobial · Characterization · Surface area and thermal behavior

1 Introduction

Bacterial contamination creates many problems in different areas such as food, medical, water, soil, etc. These contaminations cause different disease for the human, environment and sometimes cause a microbial corrosion for the building and the construction units [1–6]. These effects consumed lot of efforts and costs. For these reasons many trials were done to reduce the bacterial effects. Different chemicals, drugs and other developed materials were used. The literature survey shows that the antibacterial materials can be divided in three classes, the first and oldest one in the organic or inorganic materials, the second class represents the metal oxides and the more advanced one is the metal organic frameworks (MOFs) [7–9].

In general MOFs refer to a porous class of materials from metal ions and organic fragments. In the MOF built, the inorganic cations distribute on node and the organic ligands as bridge, forming a three dimensional structure with high porosity [10–12]. The MOFs have many advantages such as form a frameworks skeleton, controllable pore size, low density and high homogeneous surface area and have different active functional groups. It can be synthesized with commercially available reagents. Copper (Cu) was selected as a classical transition metal which is considered to be one of the most attractive elements for use in the preparation of MOFs due to its abundance of resources, low cost, non-toxic

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properties, and most importantly high complexation strength [13].

Different methods were used to prepare MOFs matrix such as solvo-thermal process, hydrothermal synthesis, electro chemical techniques and microwave [14–17]. MOFs can be used as metal ions source and liberate the inorganic cations such as Ag, Zr, Cu and Ni. These ions were continue release from the skeleton and forming an organic unit. Both the two fragments offer a durable antibacterial activity.

Ghafuri et al. [18] synthesized Cu-MOF by hydrothermal method with 1,3,5-tricarboxylic acid linkers and used for Catalyst for the Synthesis of 1,8-Dioxo-octa-hydro Xanthene. Mollabagher et al. [19] prepared Cu-MOF by one-pot reaction and used for catalysis for tacrine derivatives. Flores et al. prepared Cu-MOF -74 [20] and used for catalytic the generation of vanillin. Nivetha et al. [21] prepared and used Cu-MOF for electro-catalytic hydrogen evolution reaction. Recently (2017–2022), Sahar et al. [22–29] studied the synthesis and characterization a different nano-particles ceramic materials by different techniques. They found different applications to the produced nano-oxides such as energy storage, removal of some heavy materials.

Several efforts were done to improve the physical and chemical properties of MOFs [30]. These improvements mainly modified the structure of the molecules to increase the surface area. Melamine as a compound rich with nitrogen is prefer in this work to form melamine-MOF.

In this work, nano-particle of Cu-MOF was prepared, characterized and their antibacterial effects were studied. Moreover the effects of melamine-Cu-MOF on the structure and the bioactivity were examined.

2 Experimental

2.1 Materials

Anhydrous N, N-dimethylformamide (DMF, 99%), terephthalic acid (H2BDC, 98%), melamine, ethanol and copper nitrate trihydrate (Cu(NO3)2, 99%) were obtained from Sigma Aldrich.

2.2 Synthesis of Cu-MOF

For the synthesis of Cu-MOF, 0.5 g H2BDC and 2 g Cu (NO3)2.3H2O were fully dispersed in 38 mL (DMF: ethanol, 25:13 mL) and then the obtained solution was transferred to a 50 mL polytetrafluoroethylene lined stainless steel autoclave and placed in an oven at 100 °C for 36 h. After that, it was taken out and cooling down at room temperature. Then, the product was separated and washed several times with DMF and water. Finally, Cu-MOF was obtained by drying the product at 80 °C oven for 12 h [30].

2.3 Synthesis of Melamine-Cu-MOF (MCu-MOF)

Cu-MOF (0.5 g) and melamine (M) (1.0 g) were dispersed in 50 mL and 100 mL absolute ethanol, respectively. The two dispersions were mixed and sonicated for 1 h then the mixture was transferred to a flask. When the condensation reflux reaction had lasted for 24 h at 100 °C, the product was centrifugally rinsed with absolute ethanol and vacuum desiccation at 50 °C overnight to obtain melamine-Cu-MOF (MCu-MOF).

3 The Characterization Techniques

To characterize the products, the dry samples exposed to different techniques:

3.1 Fourier Transforms Infrared (FTIR) Spectroscopy

FTIR spectra of the samples were obtained using a KBr disk technique and FTIR 6500 spectrometer (JASCO, Japan) in the range of 400–4000 cm⁻¹.

3.2 X-Ray Diffraction (XRD) Analysis

X-ray diffraction (XRD) patterns were performed with power D8 ADVANCE diffractometer (Germany) using CuKa radiation (1.542°A, 40 kV, 40 mA) in the 2θ range of 4–80. The acquisition parameters were as: a step size of 0.02 and a step time of 0.4 s.

3.3 The Thermal Analysis

The thermal analysis (TGA) was performed by USA Berkin–Elmer thermo-gravimeter. Samples of approximately 10 mg were heated from 50 °C to 800 °C with heating rate 10/m under a nitrogen atmosphere, and the flow of nitrogen was 50 mL/min.

3.4 Scanning Electron Microscope

The surface morphologies of the samples were carried out using a Quanta 200 FEG scanning electron microscope (SEM) (Quanta) at an accelerating voltage of 30 kV.
3.5 Transmission Electron Microscope (TEM)

The morphology and the particle size of the prepared samples were examined by using a transmission electron microscope (TEM) quanta FEG working at 100 keV.

3.6 BET Measurement

Nitrogen adsorption–desorption measurements (BET method) were performed at liquid nitrogen temperature (−196 °C) with an Auto-sorb BET apparatus from Quanta chrome Corporation. The BET analysis procedure is automated and operates with the static volumetric technique. Before each measurement, the samples were degassed firstly at 200 °C for 2 h, at 5 × 10⁻³ torr and then at room temperature for 2 h, at 0.75 × 10⁻⁶ torr. The isotherm methods were used to determine the specific surface areas using the BET equation.

4 Antimicrobial Assay

Antimicrobial activity of Cu-MOF and Melamine Cu-MOF (MCu-MOF) were evaluated against pathogenic Gram-positive bacteria (*Bacillus subtilis* ATCC6633, *Lactobacillus cereus* ATCC 14579 and *Staphylococcus aureus* ATCC29213), Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Salmonella enterica* ATCC 25566), and fungus (*Aspergillus niger* NRC53) by the agar diffusion technique. Bacteria were obtained from the American Type Culture Collection (ATCC) and Northern Regional Research laboratories (NRRL) while the fungal isolate was obtained from the culture collection of the Department of Chemistry of Natural and Microbial Products, National Research Centre, Cairo, Egypt. The microorganisms were passage at least twice to ensure purity and viability. The bacteria were maintained on nutrient agar medium and fungi were maintained on potato dextrose agar medium. About 50 mg of the prepared powder samples were applied on the inoculated agar plates and incubated for 24 h at 37 °C for bacteria and 72 h at 28 °C for fungi. The antimicrobial effect was evaluated by measuring the inhibition zone diameter around samples in (mm) [31].

5 Results and Discussion

5.1 Characterization the Prepared Samples

Both of the Cu-MOF and Melamine-MOF samples were characterized by using the following technique:-

5.2 Fourier Transforms Infrared (FTIR) Spectroscopy

The absorption bands of IR for both samples are illustrated in Fig. 1. For Cu-MOF powder, several bands were observed. For Fig. 1a, at 488 cm⁻¹ a specific peak characterized the Cu–O bonding was observed while another one at 721 cm⁻¹ attributed to the stretching modes of Cu–O. Different peaks characterized the organic ligand were appeared. A peak which between 663 and 766 cm⁻¹ was observed on the IR chart, this peak may attribute to the aromatic ring of H₂BDC (Terphthalic acid). Furthermore absorption band characterized the aromatic C=C was observed at 1390 cm⁻¹. Also, three absorption bands were observed at 970, 1500 and 1640 cm⁻¹ and attributed to the vibration of C–O, –C=C– and C=O groups respectively of the H₂BDC. The appearance of three bands on the IR charts proved the successful preparation of Cu-MOF. These findings are in agreement with the previous work [30]. Figure 1 represents the melamine-MOF (MCu-MOF) sample. On the charts a series of the absorption bands at 3121, 3324, 3415 and 3467 cm⁻¹ characterized the stretching of the NH₂ was observed which characterized the melamine molecule. It is noteworthy that the absorption bond at 1651 which attributed to C=O stretching of the aromatic ligand was reduce by adding the melamine to the Cu-MOF, while the strong stretching vibration of C≡N bond was formed at the triazine
These findings indicate the incorporation of the melamine into the Cu-MOF [33].

### 5.3 X-Ray Diffraction Spectroscopy (XRD)

To study the structure and the crystallinity of the MOF and melamine-MOF samples, the powder was exposed to X-ray measurements. Figure 2 represents the X-ray diffraction of the both samples. Firstly, it is observed that sharp definite peaks were observed and the sharpness and intensities of the peaks increase by introducing the melamine unite through the MOF network. On Fig. 2a for the Cu-MOF framework, a series of sharp peaks with definite intensities at 2-theta equal about 7, 13.8, 15.7, 19.6, 30.0, 37.0, 42.5, 62.5 and 74 were appeared. The presence of their peaks corresponding the crystal planes [2 2 2], [3 3 3], [4 2 0], [4 2 2], [7 7 3], [8 8 2], [4 4 0], and [5 3 3] crystal planes respectively in agreement with the previous work [34]. For Fig. 2b, similar patterns were observed with two remarks:

1. The appearances of some bands indicate that the structure of the network still formed in spite of introducing the melamine molecule.
2. The presence of the peaks at 26 and 30 on the X-ray chart of melamine-MOF and increase the intensity of the peaks indicate the improving of the crystallinity by adding the melamine.

Moreover two peaks on Fig. 2b chart were appeared at 2-theta equals to 26, 30 [35]. These remarks lead to conclude that there are an interaction between the Cu-MOF and melamine as conclude from the IR chart. The average particle size of the obtained products was calculated by using Scherer equation by applying the following equation: 

\[ t = \frac{1}{4k} \frac{b}{\cos(\theta)} \]

where, \( t \) is the crystallite size, \( b \) is the breadth of the observed diffraction line at its half intensity maximum, \( k \) is the so-called shape factor and \( l \) is the wavelength of X-ray source used in XRD. The average crystallite diameter of Cu-MOF equals to 86 nm and decreased to become equal 50 nm by adding melamine (MCu-MOF).

### 5.4 The Thermal Analysis

Figure 3a, b, represents the TG curves of Cu-MOF and MCu-MOF. For Cu-MOF, Fig. 4a, the thermal gravimetric

![Fig. 2 XRD of Cu-MOF and MCu-MOF](image)

![Fig. 3 TGA of Cu-MOF and MCu-MOF](image)

![Fig. 4 SEM of a Cu-MOF and b MCu-MOF](image)
curves it is showed that the curve passes through three definite regions. The first one lies between the room temperature and 300 °C, this stage represents the water loss however it is chemical or physical absorbed water. The second step accompanied by great weight loss between 300 and 420 °C, it represents the main degradation of Cu-MOF built. The third step lies above 420 and it represent the formation of Cu–O as residual of the degradation [35]. For MCu-MOF, fourth stages were appeared through the main degradation. This phenomenon may be attributed to splitting the organic fragment from the inorganic copper fragments. The results of the TG analysis showed that both the Cu-MOF and MCu-MOF are thermally stable until 400 °C and the stability increases by adding melamine to 700 °C as shown in Fig. 3b.

5.5 Scanning Electron Microscope (SEM)

To study the morphologies of the prepared samples, the prepared powder exposed to SEM examination. Figure 4a and b represents the images of the SEM. The images showed that for the Cu-MOF and MCu-MOF have rods and long–bar structure. These rods are distributed randomly while more clearance of the MCu-MOF is observed. This emphasis the IR and the X-ray measurements. The surface area of Cu-MOF and MCu-MOF were calculated from the BET measurements, for the prepared Cu-MOF, the specific surface area equals to 1350 m² g⁻¹ and pore volume 0.78 cm³ g⁻¹ while the MCu-MOF has specific surface area 1410 m² g⁻¹ and pore volume 0.90 cm³ g⁻¹ as shown in Table 1. The results indicate that both the surface area and pore volume improves by introducing the melamine molecules into the Cu-MOF. In all cases, they have high surface area which candidate to use in different applications such a removal of some heavy metals and their antibacterial and anti-fungi effects.

5.6 Transmission Electron Microscope (TEM)

To clarify the morphology of the prepared samples, the HRTEM was examined. The TEM image reveals the sheets structure with uniformly distributed nano-particles (Cu-nano-particles) which observed previously at the last stage of the thermal degradation. Figure 5a and b show that the produced samples are formed in nano-particles scale, for Cu-MOF equal 50 nm and still unchanged by adding melamine. This results are in agreement with SEM measurement.

5.7 Bioactivity Test

The antibacterial activity of both Cu-MOF and MCu-MOF were tested for five bacterial species, (Bacillus subtilis ATCC6633, Lactobacillus cereus ATCC 14579 and Staphylococcus aureus ATCC29213), Gram-negative bacteria (Escherichia coli ATCC 25922 and Salmonella enterica ATCC 25566), and fungus (Aspergillus niger NRC53). It is found that Cu-MOF and MCu-MOF compounds have insignificale activities when compared with terphthalic acid, while for Escherichia coli ATCC 25922, MCu-MOF have a considerable effects, where the inhabitation zone increase from 10 to 17 mm. For Fungus, Aspergillus niger

| Materials  | BET surface area (m² g⁻¹) | Pore volume (cm³ g⁻¹) |
|------------|--------------------------|----------------------|
| Cu-MOF     | 1350                     | 0.78                 |
| MCU-MOF    | 1410                     | 0.9                  |

Fig. 5 TEM a Cu-MOF and b MCu-MOF
NRC53, both the Cu-MOF and MCu-MOF have a potent effects where the inhibition zone increases from zero for terphthalic acid to record 11 mm as shown in Table 2 and Figs. 6, 7, 8, 9, 10 and 11.

6 Conclusion

From the analysis of the obtained data it may conclude that:

(i) Both Cu-MOF and MCu-MOF can be formed by applying the hydrothermal route.

(ii) By using the same procedure, melamine can be incorporated with melamine without distortion the frame work.

Table 2 Antimicrobial activity of prepared samples

| Microorganism       | Inhibition zone (mm) | Cu-MOF | MCu-MOF | Terphthalic acid |
|----------------------|----------------------|--------|---------|-----------------|
| Gram positive bacteria |                      |        |         |                 |
| Bacillus subtilis     | 11                   | 10     | 10      |                 |
| Lactobacillus cereus  | 10                   | 10     | 11      |                 |
| Staphylococcus aureus | 10                   | 10     | 9       |                 |
| Gram negative bacteria |                    |        |         |                 |
| Escherichia coli      | 11                   | 17     | 10      |                 |
| Salmonella enterica   | 9                    | 9      | 9       |                 |
| Fungi                 | Aspergillus niger    | 11     | 11      | 0               |

Fig. 6 Antibacterial activity of Cu-MOF, MCu-MOF and Terphthalic acid on Bacillus subtilis

Fig. 7 Antibacterial activity of Cu-MOF, MCu-MOF and Terphthalic acid on Lactobacillus cereus

Fig. 8 Antibacterial activity of Cu-MOF, MCu-MOF and Terphthalic acid on Staphylococcus aureus

Fig. 9 Antibacterial activity of Cu-MOF, MCu-MOF and Terphthalic acid on Escherichia coli
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