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Hybrid Nanocomposites of Montmorillonite/Copper Oxide, Synthesis and Evaluation as Effective Growth Inhibitors in Different Biological Systems

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ABSTRACT

The external and interlamellar spaces of montmorilonite (MMT) were used as solid support for synthesis of CuO nanoparticles (NPs) at room temperature by the chemical reduction method. In this project, Copper Nitrate plus water ($Cu(NO_3)_2$. xH_2O) and Sodium Hydroxide (NaOH) were used as Copper precursor and reducing agent respectively. Then, MMT/Cu^{2+} nanocomposites were stabilized with different range of the weight percent of Polyethylene glycol (PEG). The solids were characterized by X-Ray diffractometry (XRD), Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Fourier transform infrared (FT-IR) and UV-Visible spectroscopy. The antibacterial activities of different size of CuO NPs in MMT were investigated against Gram-positive, Staphylococcus aureus, Gram-negative bacteria and Escherichia coli, by the disk diffusion method using Muller-Hinton agar (MHA). These results were showed that smaller CuO NPs were found to have higher antibacterial activities. By this method we were able to obtain CuO NPs with different sizes and making them applicable to medical applications and can be used as effective growth inhibitors in different biological system.

Keyword: Nanoparticles; Montmorillonite; CuO; Antibacterial activity; Biological systems.

1. INTRODUCTION

Recently attentions have been focused on the research of Nanoparticles. Nanoparticles are important materials for fundamental studies and diversified technical applications, because of their size dependent properties or highly active performance due to the large surface areas [1]. Nanoparticles including various types such as nano carbon, carbon nano tubes, nano clays and metal oxides [2]. Metal oxide nano particles (NPs) have been applied in host of uses, such as manufacture of both commercial and personal products [3]. The bactericidal effectiveness of metal nanoparticles has been one of the applications. Agglomeration between nanoparticles is one of the common problems when pure nanoparticles are used alone [4] to overcome agglomeration,

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(*) Corresponding Author - e-mail: sajjadsedaghat@yahoo.com preparation of nanoparticles based on clay compounds [5]. Clays are ubiquitous and cheap. Important characteristics of clays responsible for the varied applications are particle size, surface chemistry, particle shape, surface area, and other properties specific to a particular application [6]. A clay comprises silicate layers having 1 nm thick planar structure. It has been shown that the interlamellar spaces of clays silicate can be supports the nanoparticles. Montmorillonite as the major clay mineral with interlamellar spaces have adsorption ability, swelling, ion exchange properties that suitable way to supported particles as well as to control the particle size [7]. The copper oxide is the simplest member of the family of copper compounds and exhibits a range of physical properties such as high temperature superconductivity, electron correlation effects and spin dynamics [8]. Limited information on the possible antimicrobial activity of nano CuO is available. CuO is cheaper than silver, easily mixed with polymers and relatively stable in terms of both chemical and physical properties. Highly ionic metal oxides nano particulate, such as CuO, may be particularly valuable as antimicrobial agents [9-13]. In this study, the different-sized CuO NPs were synthesized in the lamellar space of the MMT in the aqueous solution. We used the MMT as the protective colloid preventing the CuO NPs from aggregation, and found that MMT also assisted in the chemical reducing process of copper. The antibacterial activities of CuO/MMT and CuO NPs formed in the lamellar space of MMT were investigated and compared with one another. The aims of this study were to obtain CuO NPs with different sizes and dissimilar antibacterial activities.

2. EXPERIMENTAL

2.1. Chemicals

All reagents in this work were of analytical grades and used as received without further purification. ($Cu(NO_3)_2 \cdot xH_2O$) (99.98%) was used as the copper precursor, which was obtained from Merck (Germany). MMT powder, used as a solid support for CuO NPs, was purchased from Merck (Germany). The NaOH (98.5%) was used as reducing and PEG was used as stabilizer. All the aqueous solutions were prepared indouble distilled water.

2.2. Synthesis of CuO/MM nanocomposites

For the synthesis of CuO/MMT NPs, the MMT contents of the samples were 1 g that mixt with 0.1 g $Cu(NO_3)_2.XH_2O$ and 50 mL double distil water and stirred for 24 hours at room temperature. after that added the different mount weight of PEG [0.05wt%(S1), 0.1wt%(S2), 0.15wt%(S3), 0.2wt%(S4), 0.5wt%(S5), 1.00wt%(S6)]. A freshly prepared NaOH (1 M) solution was then added to the suspensions under continuous stirring in the 60°C bath for one hour. The suspensions of CuO/MMT Nps obtained, and the precipitates washed 4 times using double distilled and dried for 24 hours in oven.

2.3. Evaluation of antibacterial activity

The invitro antibacterial activity of the samples was evaluated according to the disc diffusion method using Mueller-Hinton agar (MHA) with determination of inhibition zones in millimetres, Escherichia coli (ATCC 25922), E. coli O157:H7 (ATCC 43895), Staphylococcus aureus (ATCC 25923), were used for the antibacterial effect assay. Briefly, the sterile paper discs (6 mm) impregnated with 20 μL of CuO/MMT NPs (A1), MMT/Cu^{2+} (A2), MMT (A3) were suspended in the sterile distilled water and left to dry for 24 hours at 37°C in a sterile condition. The bacterial suspension was prepared by making a saline suspension of isolated colonies selected from the 18 to 24 hours of tryptic soy agar plate. The surface of MHA was completely inoculated using a sterile swab, steeped in the prepared suspension of bacterium. Finally, the impregnated discs were placed on the inoculated agar and incubated at 37°C for 24 hours. After incubation, the diameter of the growth inhibition zones was measured.

3. RESULTS AND DISCUSSION

To prepare the stable CuO NPs via the chemical reducing method, the choice of a suitable stabilizer and reducing agent was important. In this research, MMT suspension was used as the appropriate support for reducing CuO/MMT.

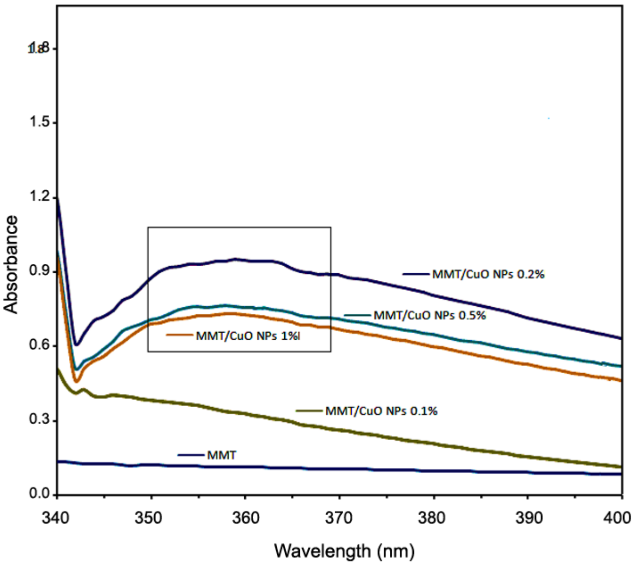

Figure 1: *UV-Visible absorption spectra of CuO/MMT suspension For different range of the weight percent of Polyethylene glycol (PEG [0.1wt%(S2), 0.2wt%(S4), 0.5wt%(S5), 1.00wt%(S6)] ,and MMT suspension.*

3.1. Ultra-Violete spectroscopy

The copper SPR bands were detected around 400 nm (Figure 1). These absorption bands were assumed to correspond to the CuO NPs smaller than 10 nm. While there was no UV absorption peak of MMT when use alone (Figure 1). The growth of the plasmon peak at 350-370 nm indicated the formation of CuO NPs in different range of the weight percent of Polyethylene glycol (PEG) [0.2wt%(S4), 0.5wt%(S5), 1.00wt%(S6)] .

3.2. Powder X-ray diffraction

As shown in Figure 2, the characteristic peak for original MMT at about 2θ= 3.3° these d-spacing values were direct proof that very small numbers of CuO NPs might be intercalated between adjacent MMT lamellar layers. Therefore, the CuO NPs formed at the latter location were the cause of the increase in basal spacing. In these samples, the intensities of the reflections were significantly lower, whereas their half-widths were larger than those of undoped clay minerals, whereby the highly ordered parallel lamellar structure of the mineral were disrupted by the metal nanoparticles formation. In addition, all the CuO/MMT NPs had a similar diffraction profile and the PXRD peaks at 2θ for 0.1wt% PEG at 5.47°,and for 0.5wt% PEG at 4.3°, 6.01°, 16.24 and for 1.00wt% PEG at 6.04°, 7.09° ,16° and 27° (Figure 2). In addition, there was a characteristic peak at about 2θ= 3.3° related to the MMT clay as a stable substrate. Due to the CuO NPs phase were also found to increase along with the increased CuO NPs in the solid support matrices.

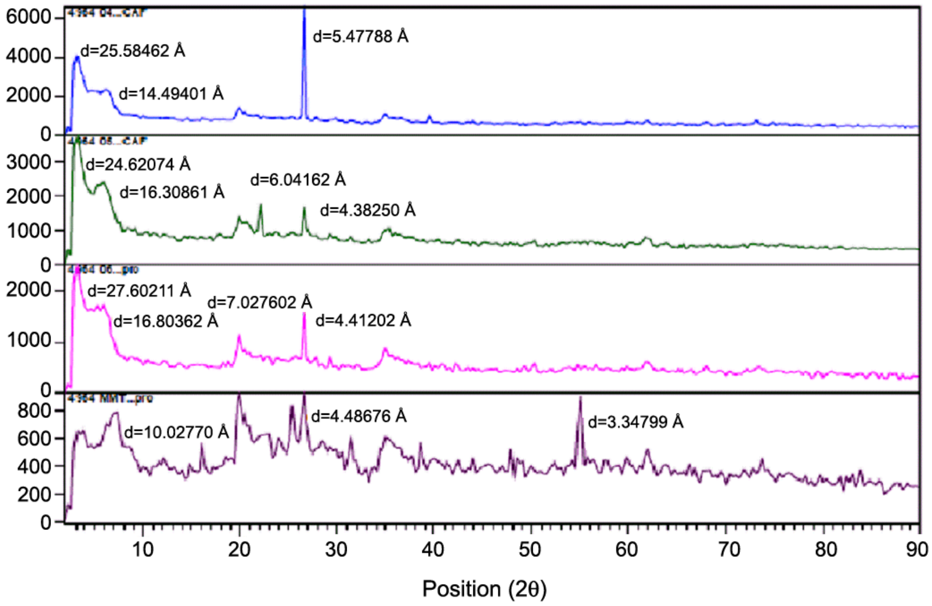

Figure 2: *PXRD patterns of MMT and CuO/MMT NPs for determination of d-spacing for different range of the weight percent of Polyethylene glycol.*

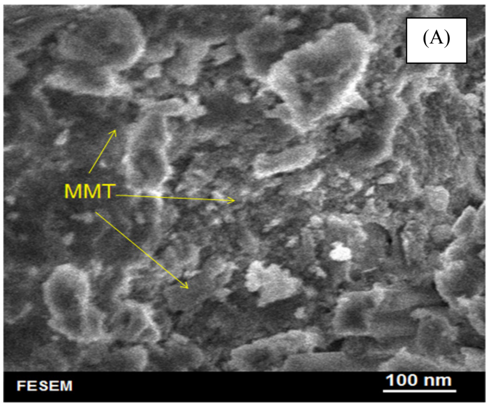

Figure 3: *SEM micrographs for pure MMT.*

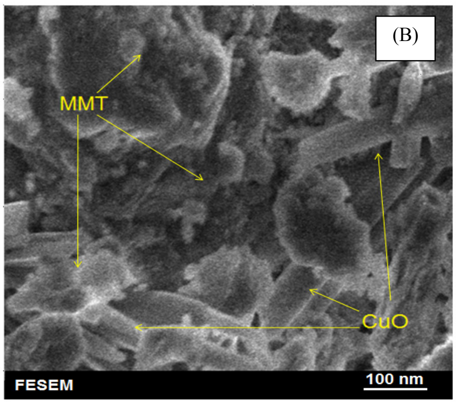

Figure 4: *SEM micrographs for CuO/MMT NPs.*

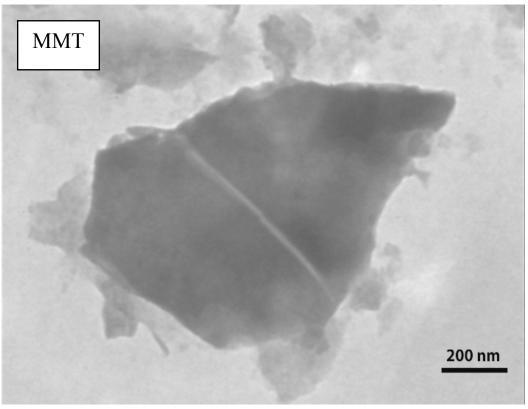

Figure 5: *TEM images of MMT.*

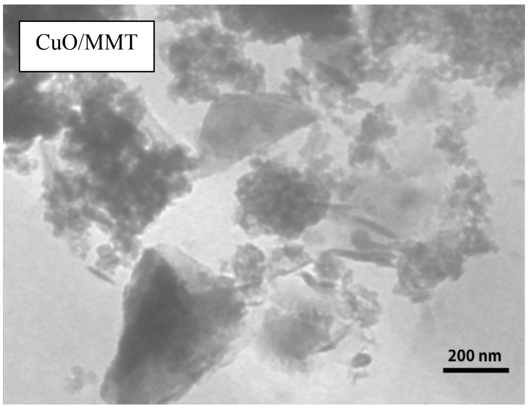

Figure 6: *TEM images of CuO/MMT NPs.*

3.3. Morphology

SEM images of the MMT and CuO/MMT NPs are presented in Figures 3 and 4. The surface morphology of MMT demonstrates a layered surface with some large flakes, which is a typical structure for MMT. The exterior morphology for CuO/MMT NPs, show layered surfaces with large flakes and without significant morphological differences between them. TEM images of CuO/MMT NPs and MMT are shown in Figures 5 and 6. In This figures the influence of nanoparticles between the MMT interlayers and the formation of CuO NPs can be seen.

3.4. FT-IR Chemical analysis

The FT-IR spectrum of MMT (Figure 7) showed the vibration bands at 3633 cm^{-1} for O-H stretching, 3423 cm^{-1} due to the inter-layered O-H stretching, at 1652 and 1531 cm^{-1} for H-O-H bending, 1126, 1011, and 910 cm^{-1} for Si-O stretching. The FT-IR spectrum of PEG (Figure 8) showed the vibration bands at 3600-3200 cm^{-1} for O-H was stretching, 2960-9850 cm^{-1} for C-H stretching, 1450-1300 cm^{-1} for C-H bending, 1260-1000 cm^{-1} for C-O stretching and 1050-1150 cm^{-1} for C-O-C stretching. The FT-IR spectrum of CuO/MMT NPs (Figure 9) showed the vibration bands at 1384 cm^{-1} for Cu^{2+}-O^{2-} Stretching. The FT-IR spectra demonstrated the inflexibility of silicate layers and non-bond chemical interface between the silicate layers and CuO NPs in CuO/MMT NPs. Peak areas shifted to low wave numbers and the intensity of

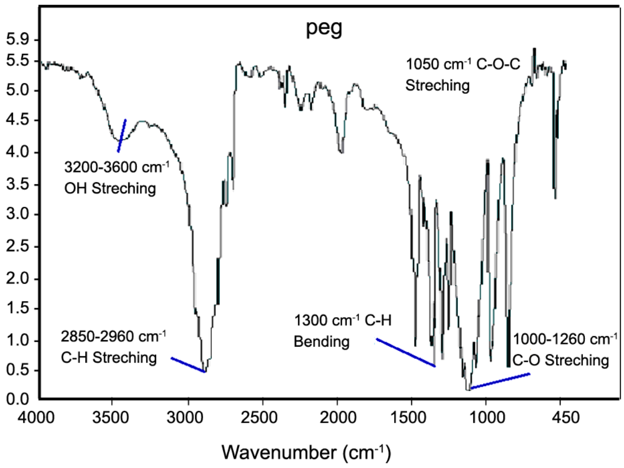

Figure 7: *FT-IR spectra for PEG.*

peaks appearing in the range of 1384 cm^{-1} increased.

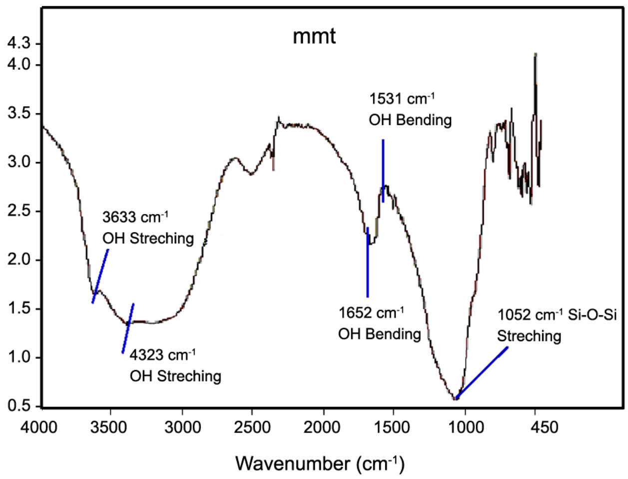

Figure 8: *FT-IR spectra for the MMT.*

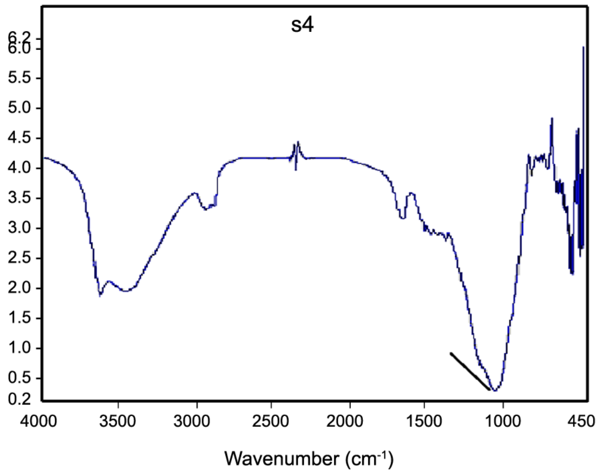

Figure 9: *FT-IR spectra for CuO/MMT NPs.*

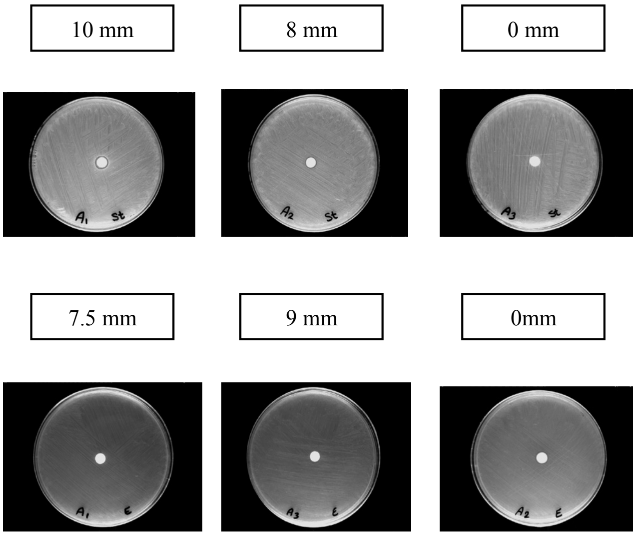

Figure 10: *Comparison of the inhibition zone test between Gram-negative and Gram-positive bacteria (Escherichia coli, Staphylococcus aureus) form CuO/MMT (A1), MMT/Cu^{2+} (A2), MMT (A3) respectively.*

3.5. Antibacterial activity

The results and images of inhibition zones are presented as the average values in Table 1 and Figure 10, respectively. Table 1 shows that the MMT/Cu^{2+} and CuO NPs in MMT suspension gave high and similar antibacterial activity against Gram negative and Gram-positive bacteria. The diameters of inhibition zone in the agar plate are given in mm. The results are presented in Table 1. The suspension of MMT showed no antibacterial activity. The MMT/Cu^{2+} and CuO/MMT NPs suspension for all tested bacteria showed high antibacterial activity.

4. CONCLUSIONS

The CuO NPs were successfully prepared from the $Cu(NO_3)_2$/MMT suspension with different range of the weight percent of Polyethylene glycol (PEG). By using NaOH at room temperature, the surface of MMT fostered the nucleation of CuO NPs during the reduction process.

A small number of CuO NPs intercalated between bordering MMT lamellar layers, but the majority of the CuO NPs indicated by the PXRD signal, and that appeared in the TEM, form simply at the outer surface of MMT layers. The antibacterial activities of CuO/MMT NPs at the different particle size of CuO NPs showed antibacterial activity against the Gram-negative and Gram-positive bacteria.

These results show that the antibacterial resistance of CuO NPs in MMT can be modified with the size of CuO NPs, and decreases with the increase in particle size. Needless to say, further studies are required to investigate the biological effects of CuO/MMT NPs on the types of bacteria, in order to extend this subject area.

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Table 1: *Comparison of the inhibition zone test between Gram negative and Gram-positive bacteria (Escherichia coli, Staphvlococcus avreus) form (A1), (A2) and (A3) respectively.*

Bacterial	Inhibition zone (mm)		
	A1	A2	A3
E coli	7.5	9	0
S. avreus	10	8	0

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