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Synthesis, Characterization, and Antibacterial Activity of Metal Doped Copper Oxide MCM-41 Nanocomposites

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Abstract: The present study aimed to synthesize the CuO-MCM-41-Ag nanocomposites by hydrothermal method and evaluate their antibacterial activity by disc diffusion method. The synergistic effects of the transition metal-based nanocomposites are supposed to possess highly strong antimicrobial actions. SEM and TEM analyzed the morphological features of the synthesized Copper xide-MCM-41-silver nanocomposites. According to these morphological analyses, the biosynthesized CuO-MCM-41-silver nanocomposites are composed of regularly distributed hexagonal-shaped particles in aggregated form. The crystallinity of the prepared nanocomposites was confirmed through the XRD technique. From the FTIR results, the peaks obtained are closely Si-OH deformational vibrations, stretching vibrations of the surface Si-O_2 groups, and various other groups. The antibacterial activity of nanocomposites was evaluated on both Gram-negative and Gram-positive bacteria. It was monitored that the MIC of *Staphylococcus aureus, Bacillus aureus, Pseudomonas aeruginosa,* and *Escherichia coli* was 0.135 mL^{-1}. The nanocomposites synthesized by the hydrothermal study were good antibacterial against specific gram-positive and gram-negative bacterial strains.

Keywords: copper oxide, MCM41, nanocomposites, antibacterial activity.

金属掺杂氧化铜美孚物质41纳米复合材料的合成、表征和抗菌活性

摘要：本研究旨在通过水热法合成事情-41-银纳米复合材料的氧化铜-美孚成分，并通过圆盘扩散法评估其抗菌活性。过渡金属基纳米复合材料的协同作用被认为具有很强的抗菌作用。扫描电子显微镜和透射电子显微镜分析了合成的物质41银纳米复合材料的氧化铜-美孚组合物的形态特征。根据这些形态分析，事情-41-银纳米复合材料的生物合成的氧化铜-美孚组合物由规则分布的六角形颗粒聚集而成。所制备的纳米复合材料的结晶度通过X射线衍射分析技术得到证实。从傅里叶变换红外光谱结果来看，得到的峰很接近硅羟基变形振动、表面二氧化硅基团的伸缩振动和各种其他基团。纳米复合材料对革兰氏阴性和革兰氏阳性细菌的抗菌活性进行了评估。监测金黄色葡萄球菌、金黄色杆菌、铜绿假单胞菌和大肠杆菌的异氰酸甲酯为0.135毫升-1。通过水热研究合成的纳米复合材料对特定的革兰氏阳性和革兰氏阴性菌株具有良好的抗菌性。

关键词：氧化铜, 物质的流动组成41, 纳米复合材料, 抗菌活性

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1. Introduction

The idea of nanomedicine originated from the basic concept that nanomaterials and the apparatus and ingredients used for the synthesis of nanomaterials can be used and introduced into man's body to achieve cellular repairs at the subcellular level. Nanomedicine nowadays has spread out in so many areas, each of them embodying the central vision that the capability to structure materials and instruments at the molecular scale can carry vast fast benefits in the research and practice of drugs [1].

The word "nanotechnology" can be defined as using materials that have at least a minimum of 1 dimension less than 100 nm [2]. This field has a broad scope in chemistry, pharmacy, materials science, and biology to prepare new material properties that can be used to create synthetic protocols to synthesize many electrical appliances, biologicals, superior resources, client objects [3]. The area of nanotechnology is one of the chief extensively used areas for the latest work and evolution in primarily all applied fields. Nanocomposites are composed of many phase ingredients, i.e., metals, polymers inorganic porcelains. In such nanocomposites components, one element's minimum size has one dimension less than 100 nm [4].

Nanocomposites are materials of the modern age that have an annual growth rate of 25% because of their efficient properties, and they have unique pattern probabilities and properties. Nanocomposites have been employed to the asylum a wide diversity of arrangements, i.e., one-dimension, two-dimension, three-dimension more shapeless elements synthesized from diffidently different ingredients and synthesized at the nanoscale. The synthesis method of nanocomposites may be different from that of the chemical and mechanical properties of their respective components, mostly depending upon the type of polymers employed. Mainly it depends upon the corrosion-resistant property. Some nanocomposites are environment friendly because of the ecofriendly method of preparation of nanocomposites. Suitable selection of nanomaterial or polymer is important in attaining nanocomposites' desired properties, which have many useful applications [5].

Nowadays, metal oxide nanoparticles attain huge attention because of their properties like catalysts, sensing appliances, biomedical applications, antibacterial and antifungal agents, fillers, opacifiers, semiconductors. Metal oxide nanoparticles are also used to prepare makeups and microelectronics [6, 7]. Copper oxide nanoparticles which are metal oxide nanoparticles are the material of interest nowadays because of their antifungal and antibacterial properties; that is why they are extensively used for treating the diseases caused by these organisms [8, 9]. CuO is a semiconductor metal with exceptional optical, electrical and magnetic characteristics, and it possesses different properties. That is why it is employed for different purposes like preparing supercapacitors, near-infrared filters, magnetic storage media, sensors, catalysis, semiconductors, etc. [10-12]. The MCM-41 and silver can further increase the biological activities of CuO.

2. Materials and Methods

2.1. Materials

Copper (II) sulfate (CuSO4 .5H2 O ≥ 99.0%), nutrient agar, and nutrient broth were procured from Sigma Aldrich Co., Ltd., Pakistan. Bacterial strains were obtained from the department of pharmacy, university of Sindh, Jamshoro, Pakistan. All the chemicals were analytical grade and directly used without any purification. The deionized water (>17 MΩ cm–1) from a Milli-Q water system carried out fundamental research.

2.2. Method

2.2.1. Preparation of Nanocomposites

The copper oxide-MCM-41-silver nanocomposites were prepared by the hydrothermal method [13]. Accurately weighed 2.4 grams, cetyl trimethyl ammonium bromide was dissolved in ten ml of deionized water and mixed for half an hour with gentle warming till the mixture became clear. Afterward, 10 ml NH4OH solution was added and mixed again for ten minutes, and pH was maintained between nine to twelve. Then ten ml tetraethyl orthosilicate was added dropwise and stirred again for three hours. During stirring, the pH was maintained at 10. After 0.5 grams, copper sulfate was dissolved in ten ml water and dropwise added to the same solution. Then it was kept to teflon autoclave and stored at 110 ^{0}C in the oven for thirty-six hours. Afterward, the aliquot was washed away with ethyl alcohol and D/water, filtered, and dried for twelve hours in the oven at 80 ^{0}C. Then aliquot was ground into a uniform powder with Mortar and Pestle. The powder was then heated at 400 ^{0}C for 4 hours.

2.2.2. Silver Coating

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The coating of synthesized nanocomposites was done with the solution dispersion method. Accurately weighed 0.5 g synthesized nanocomposites were dissolved in 50 ml water and sonicated for 10 minutes. Then it was kept on a magnetic stirrer for 30 minutes. Then silver nitrate solution was prepared by weighing 0.1-grams silver nitrate, dissolved in 15 ml of water, and sonicated for 5 minutes. Both the solutions were mixed dropwise and again stirred for 3 hours. Then sodium borohydride solution was prepared by dissolving 0.5 grams of $NaBH_4$ in 15 ml of distilled water.

Afterward, the borohydride solution was added to the previously prepared solution was stirred for one hour. Then the mixture was filtered, and the material above filter paper was dried in the oven at 70 ^{0}C for one hour. For four hours, the dried sample was heated in a muffle furnace at 400 ^{0}C.

3. Results

2.1. Antibacterial Activity Determination

The testing of synthesized nanocomposites against specific bacterial strains was evaluated by employing Disc Diffusion Method with slight modification [14]. The gram-positive and gram-negative strains of the organism were obtained from the department of pharmacy, university of Sindh, Jamshoro, Pakistan and were inoculated from stored slants (4°C) into the nutrient broth before determination of antibacterial activity. Then prepared inocula were incubated at 37°C for one day.

Then the Petri dishes were taken, which had freshly prepared nutrient agar, and 100 µl of bacterial inocula were added. Medium for bacterial strain is nutrient agar. Then nanocomposites sample (5 µl of 10 mg/ml DMSO) infused filter paper discs were kept over the swabbed surface.

The same amount of reference (ampicillin) was also taken and treated similarly to that of synthesized nanocomposites. To confirm the nontoxicity of DMSO, the DMSO impregnated discs were employed as a negative control.

Plates were then incubated for one day at 37°C. Inhibition zone diameter around drug and reference treated discs were measured to nearest mm by caliper and were documented.

For confirmation of the crystalline structure of synthesized nanocomposites, XRD analysis was performed. The figure shows the X-ray diffraction pattern of synthesized nanocomposites. The characteristic peaks of diffraction obtained at 2θ values of 22^o correspond to the plane orientation (300) of MCM-41, 37^o, 39^o, 48^o, 53^o, 58^o, 61^o, 68^o, 73^o respectively for the planes of (002), (111), (-202), (020), (202), (-113), (-311), (220) and (-222) which represent the monoclinic phase of CuO. In contrast, the peaks at 78 ^{o} correspond to Ag's plane orientation (311).

The obtained data follows the JCPDS File No. 04-0783, which confirmed the crystal structure of the development of copper oxide MCM-41 nanocomposites. The average particle size came out to be 15 nm as determined using Debye Scherrer equation 1.

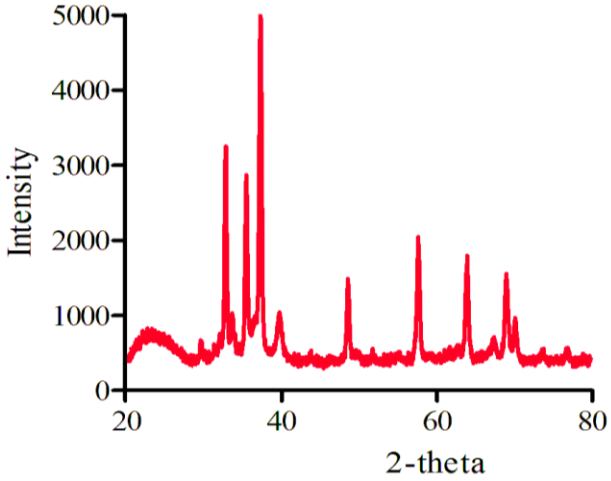

Fig. 1 XRD pattern of CuO-MCM-41 nanocomposites

To find the size, shape, and dispersity of nanocomposites, TEM was performed. Fig. 2 shows the TEM micrograph of synthesized copper oxide MCM-41 nanocomposite. Findings showed that synthesized copper oxide-MCM-41 nanocomposites are highly ordered mesoporous particles with long-range order and a regular two-dimensional hexagonal pore structure. The TEM image of copper oxide MCM-41 demonstrated that copper oxide is incorporated into the rough surface of MCM-41, as shown in the image. The results clearly illustrated that small size (3-100 nm) copper oxide particles are incorporated into MCM-41, indicating that MCM-41 nanoparticles are the best support and can produce widely distributed and small size nanoparticles.

For FTIR, nanocomposites were mixed with potassium bromide pressed into tablets, and the spectrum was obtained on a Bluker EQUINOX 55 Fourier Transform Infrared Spectrometer.

FTIR spectroscopic technique is widely employed for quantitative and qualitative analysis of every molecule. It is performed to identify the functional groups and bonds a molecule possesses. In quantitative analyses, FTIR is used to show the effect of nanofiller on characteristics of nanocomposites film by the change in band intensity of FTIR spectra. In the current study, FTIR was performed for evaluating possible bond formation between copper oxide nanomaterials and MCM-41. FTIR spectra of the synthesized sample were recorded to show that the synthesized sample exhibited absorption bands at approximately 2924, 1636, 961, 800 cm^{-1}, and 1478 cm^{-1}. The Si-OH deformational vibrations of adsorbed molecules produce the absorption band at approximately 1636 cm^{-}

[1]. The 800 cm^{-1} corresponds to the stretching vibrations of surface Si-O_2 groups' external asymmetric Si-O stretching modes. The absorption band at approximately 2924 $cm^{-1}$is possibly a consequence of the formation of copper complexes in silica micelle and template.

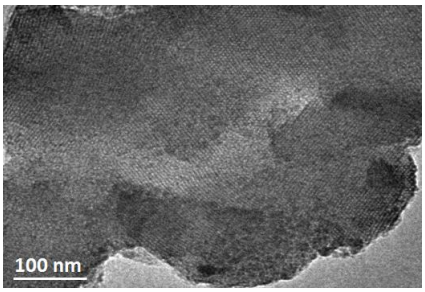

Fig. 2 Transmission Electron Microscopy of the CuO-MCM-41 nanocomposites

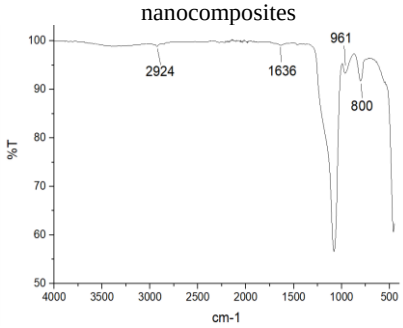

Fig. 3 FTIR of the CuO-MCM-41 nanocomposites

2.2. Antibacterial Activity of As-Synthesized CuO-MCM-41-Ag Nanocomposite

The disk diffusion approach also specifies that the tested pathogens are effectively regulated by nanoscale CuO-MCM-41-Ag and their results are illustrated in Table 1.

2.2.1. Determination of MIC

MIC values are used to find the efficiency of nanoparticles. MIC is the lowest quantity of antibacterial agent used to inhibit the growth of bacteria. The bacterial inhibition efficacy of biosynthesized CuO/MCM-41/Ag nanomaterials may be quantitatively determined by mixing a dilute solution of CuO-MCM-41-Agwith *Bacillus aureus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Escherichia coli* in artificial media. After 24 hours of incubation, MIC was found to be 0.135 mL^{-1}.

Table 1 Growth inhibition of different bacterial strains by copper oxide-MCM-41 nanocomposites (10 mg/ml) by disc diffusion method

S.No	Bacterial strains	Diameter of growth inhibition zone (mm) (10mg/ml)	Positive control	Negative control (DMSO)
1	*Bacillus aureus*	23	15	-
2	*Staphylococcus aureus*	25	17	-
4	*Pseudomonas aeruginosa*	24	23	-
5	*Escherichia coli*	23	20	-

3. Discussion

Nanomedicine is a biomedical application of nanotechnology. Nanomedicine ranges from the biomedical application of nanomaterials and biological instruments to nanoelectronic biosensors and even likely future uses of molecular nanotechnologies, such as biological devices. Recent difficulties for nanomedicine include understanding the issues regarding the toxic nature and environmental hazards associated with using these nanomaterials. Nanomedicine is a promising tool in the research field to provide important biomedical devices and drug carriers in the upcoming days. The National Nanotechnology initiative expects novel biomedical uses in the drug manufacturing industry, including advanced drug delivery systems, novel treatments, and in vivo imaging. Nanomedicine research is getting funding from the US national health common fund program, supporting four nanomedicine development centers. Using nanotechnology drug delivery to specific cells may be possible by employing nanomaterials.

The present study is inconsistent with a study conducted by P.G Bhavyasree et al. [15]. The authors analyzed the green synthesis of CuO/Carbon Nanocomposites employing leaf extract of Adhatoda vasica Nees, characterization, and antibacterial activity. The reference study used almost similar characterization techniques, and the antimicrobial activities were determined according to NCCLS 1993 (National Committee for Clinical Laboratory Standards). In the reference study, the antimicrobial activities were determined using the agar well diffusion method. The resulting zone of inhibition against microorganisms was fairly circular since there was a convergent lawn of growth. Unlike this, the current study used the disc diffusion Method to determine the antimicrobial activities and used a chemical method to synthesize the Nanocomposites.

The present study is in line with the study made by Abdullah Alswat et al. [16]. The authors made a study on the synthesis and characterization of zeolite /Zinc oxide -copper oxide nanocomposites. In the reference study, the nanocomposites were synthesized using a chemical method like this research. Similar strains of bacteria like *Escherichia coli* and *Bacillus aurus* were taken in the reference research, and the similarly remarkable antibacterial activity of prepared nanocomposites was found. In the reference study, the

noticeably enhanced activity of NCs was due to the release of Cu^{+2}, which interacts with the cells being negatively charged. The NCs penetrate the cell membrane and adhere to the bacteria wall, thus will destroy the cell.

The present study is inconsistent with a study performed by Rajapaksha et al. [17]. The authors conducted a study on the antibacterial activity of graphene-oxide CuO nanocomposites in the reference study. A similar gram-negative *E.Coli* was taken, and the study concluded, like the current study, that the synthesized Nanocomposites possess excellent antibacterial activities.

4. Conclusion

It was confirmed that the nanocomposites of CuO-MCM-41-Ag were prepared successfully, having a porous structure. The synthesized nanocomposites showed efficient antibacterial action. According to results from XRD, hydrothermally prepared CuO-MCM-41-Ag nanocomposites were crystal structured in nature, and the purity was good enough. The crystal size of prepared CuO-MCM-41-Ag nanocomposites was too determined from the XRD pattern by Scherrer equation, and it was within range of 50-55 nm, so it confirmed that nanocomposites were prepared. Antibacterial activity of prepared CuO-MCM-41 nanocomposites against Gram-negative (*Pseudomonas aeruginosa* and *E. coli*) and gram-positive (*S. aureus* and *Bacillus aureus*) bacterial strains was assessed by measuring inhibition zone. It showed good antibacterial activity against selected bacterial strains. Therefore, the developed nanocomposites can be used as a novel antibacterial agent against specific gram-positive and gram-negative bacterial strains. Due to the available resources, the antibacterial activity of the CuO-MCM-41 nanocomposites was performed. In the future, the prepared nanocomposites can also be evaluated to diagnose and treat cancer.

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