MoO₃ Nano-Bricks as Novel Antimicrobial Agents

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ABSTRACT

Multiple drug resistance in microorganisms has impersonated critical vulnerability to existing antibiotics; hence substitutes/or options to encounter resistant pathogenic microbes are desirable. Our focus in this study was the synthesis and characterization of Molybdenum oxide (MoO₃) nano-bricks and to explore them in terms of their antimicrobial potential. MoO₃ nano-bricks were successfully synthesized by hydrothermal method using (NH₄)₆Mo₇O₂₄·4H₂O precursor and calcination at 500 °C for 2 h. The synthesized MoO₃ nano-bricks determined antibacterial activity against four bacterial isolates and one fungal isolate. The MoO3 bricks were characterized using standard physicochemical characterization viz. XRD, SEM, FTIR, and EDX. In the present investigation, characteristic antibacterial properties of MoO3 nano-bricks against Gram +ve (S. aureus ATCC 29213 and S. epidermidis ATCC 12228) and Gram -ve (E. coli ATCC 35218 and K. pneumoniae ATCC 700603) bacteria is noted. The antifungal activity was tested using C. albicans ATCC 10231 as model organism. Molybdenum oxides generate acidic medium and demonstrated potent antimicrobial action for various pathogenic bacterial strains causing infections. The MoO3 nano-bricks depicted broad spectrum antimicrobial potential which strongly recommends their use as material of choice as potential antimicrobial material to be used in food industry, water purification, textile industry etc.

Keywords: MoO₃ nano-bricks, Antimicrobial, Broad spectrum antibiotics, Multiple drug resistance.

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I. INTRODUCTION

Nowadays antimicrobial resistance is comprehensible global health threat. Synthesis and establishment of novel antimicrobials is unable to follow fast appearance of bacterial resistance to nearly all drugs which are in use since discovery of Penicillin [1]. Therefore effective substitutes to encounter against multiple drug resistant (MR) pathogenic microbes are required [2]. Nanostructured materials deserve increasing attention owing to outstanding antimicrobial potential even at very small quantity. Recently, in addition photoelectrochemical properties, transition metal oxide (TMOs) nanotextured materials have also been scrutinize for antimicrobial action. These nanotextured particles are celebrated owing to superfluous stability and nontoxic character. Antimicrobial materials are incredibly essential in fabric industries, for water purification, drugs and food protective material. A lot of organic compounds have depicted antibacterial action, but the majority of these compounds show toxicity as well. Therefore there is emerging need for metal oxide based nanotextured material to be utilized as antibacterial material owed to nontoxic and high stability as aforementioned [3]. In this regard silver nanoparticles have broadly been investigated as well as utilized as they have exceptional antibiotic capabilities against a large group of microorganisms [4], [5]. Nonetheless; the silver is expensive and release of lethal silver ions have restricted its uses [6]. Molybdenum trioxide (MoO₃) has acknowledged substantial notice during past some years due to manifold uses in a variety of different branches [7], [8]. Various methods have been reported for the synthesis of MoO₃ and its analogues such as electrodeposited [9], thermal evaporation [10], chemical vapor deposition (CVD) [11], sol-gel process [12], electrochemical processes, cation-exchange resin [13] etc. Moreover; some of the nanostructured morphologies of MoO3 have also been reported. In the present study we made use of hydrothermal method for synthesis of MoO3 nano-bricks. Not many investigations have reported antimicrobial activities. The antibacterial effect of MoO₃ nanoparticles has previously been shown for some microorganisms [14]-[16]. Furthermore, Zollfrank et al. demonstrated antimicrobial action of MoO₃ and correlated it to its surface acidity which results due to formation of molybdic acid intermediate [14], [15]. Likewise, the toxic effect of MoO₃ against pathogenic bacteria has also been reported by K. Krishnamoorthy et al. [14]. In the present study we have synthesized MoO₃ nanobricks by facile hydrothermal method and evaluated these nano-bricks for broad spectrum antibacterial agents. Hitherto, there is not any report about antifungal activity of MoO₃. We evaluated these synthesized nano-bricks for antifungal activity taking in consideration pathogenic fungus C. albicans.

II. EXPERIMENTAL PROCEDURE

To synthesize MoO₃ nano-bricks, 1 g ammonium heptamolybdate tetrahydrate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O)$ was suspended in deionized water (40 ml) with constant shaking at normal temperature. 2 M HCl has been supplemented drop wise to suspension to get pH ~2 and forms transparent solution. The final infusion was transferred to autoclave reactor and heated at 180 °C for 6 h. Following chemical reaction, the obtained precipitates have been washed and filtered with water and dehydrated at 80 °C overnight. The attained residue has been heated at 500 °C for 2 h to get the final product. The synthesized powder samples were characterized for their physico-chemical properties. The crystalline phase was characterized by X-ray diffractometer (Rigaku Co., Japan) with Cu Kα (λ=1.54056 Å) radiation midst 2θ range of angles as of 20 to 60 degree. The shape of powder crust has been investigated by using Scanning electron microscope (JEOL JSM, Japan) operated with Energy-dispersive X-ray (EDX). Spectroscopic categorization was carried out by a Fourier-transform infrared (FT-IR) spectrophotometer having a range of 400–4000 cm⁻¹.

A. Antimicrobial Activity

Antibacterial activity of MoO3 nano-bricks has been tested in liquid broth following Amna et al (2011) method [17]. The bacterial strains were cultured (106 CFU) with different concentration of MoO₃ nano-bricks. Double dilution method has been applied to test out the minimum inhibitory concentration (MIC) of the MoO₃ nano-bricks. The concentrations used in this study were 400, 200, 100 and 50 μg/ml respectively. The growth kinetics was monitored at every 4 h by checking the OD with spectrophotometer in order to verify the MIC. A constant incubation temperature of 37 °C has been kept and rpm of 150 was maintained in a rotary shaker. The entire incubation period to monitor growth inhibition in presence of MoO₃ nano-bricks was 16 h. The change in absorbance was calculated at 600 nm by Ultraviolet spectrophotometer. The bacterial and fungal strains had obtained from ATCC. The representative Gram +ve isolates used are Staphylococcus aureus ATCC 29213 and Staphylococcus epidermidis ATCC 12228. Likewise, the MoO₃ nano-bricks were also tested against representative Gram -ve isolates such as Escherichia coli ATCC 35218 and Klebsiella pneumoniae ATCC 700603. To test antifungal activity of MoO₃ nano-bricks, Candida albicans ATCC 10231 was used in the present research. The Candida albicans ATCC 10231 fungus was cultured and maintained in the Sabouraud's dextrose agar. Briefly, after spreading the plates with representative fungus, the inoculated plates were kept in incubator for 30 minutes for incubation. For the fungal culture the growth was monitored after 72 h incubation period. The experiments were executed in triplicates.

III. RESULTS AND DISCUSSION

XRD is a trustworthy method for examining crystallinity. XRD patterns of MoO₃ nano-bricks synthesized by a hydrothermal method at 180 °C.

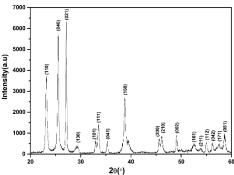


Fig. 1. XRD spectra of synthesized MoO₃ nano-bricks calcined at 500 °C.

All diffraction crests have been assigned to orthorhombic (α) MoO₃ (JCPDS no. 05-0508) [18] without contamination. Existence of strong crystalline peaks at 2θ values of 23.1° , 25.5°, 27.08°, 29.33°, 32.91°, 33.59°, 35.31°, 38.75, 45.63, 46.16, 49.0, 52.59, 54.95 and 58.65° correspond to the crystal planes of (110), (040), (021), (130), (101), (111), (041), (150), (200), (210), (002), (161), (112), and (001) confirms the formation of MoO₃ nano-bricks. There is no impurity peak present in the spectrum.

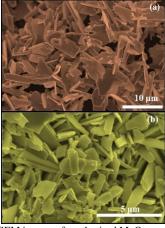


Fig. 2. SEM images of synthesized MoO₃ nano-bricks.

Fig. 2 (a, b) shows the morphology of the MoO₃ nanobricks from SEM at low and high magnifications. The sample shows homogenously spread nano-bricks having a width of 700-800 nm and length of about 1 to 2 micrometers.

The EDX spectrum was taken to quantify chemical composition of the MoO₃ nano-bricks (Fig. 3). The existence of Mo and O was identified. It was worth pointing out that atomic ratio of Mo: O was around 1:3 indicating the chemical formula of MoO₃ nano-bricks.

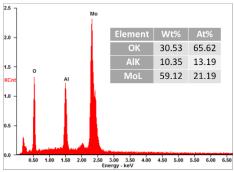


Fig. 3. EDX spectra of synthesized MoO₃ nano-bricks.

The functional groups of MoO₃ nano-bricks have been assigned by infrared in the range from 400–4000 cm⁻¹ (Fig. 4). The band from 1000-400 cm⁻¹ assigned to stretching and bending vibrations of metal-oxygen representative bonds. The peaks at 980 cm⁻¹ and 914 cm⁻¹ represents Mo=O stretching vibrations. The broad band at 580 cm⁻¹ corresponds to vibration of Mo-O bonds [19], [20]. The bands at 3490 cm⁻¹ and 1618 cm⁻¹ assigned to stretching and bending vibrations of O-H bonds of water molecules, respectively.

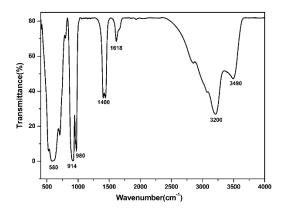


Fig. 4. FT-IR spectra of MoO₃ nano-bricks.

The in vitro antimicrobial action of MoO₃ nano-bricks against four bacterial strains (Gram -ve and two Gram +ve) was examined by means of MIC technique as shown in the figure 5. In order to screen the antibacterial activity, four varying concentrations (200, 100, 50 and 25 mg/tube) of the MoO₃ nano-bricks have been used. The observed MIC of MoO₃ nano-bricks was found to be 25 mg for Gram positive strains (S. epidermidis and S. aureus). Whilst for Gram negative strains (E. coli and K. pneumonia) it was found to be 50 mg. The MoO₃ nano-bricks were found more effective against S. epidermidis and S. aureus strains. Furthermore, regarding all selected pathogenic strains, it has been observed that with the amplification in MoO₃ nano-bricks; the bacteriostatic effect was also improved. Perceptible changes in growth kinetics have been observed in case of all the strains between 4-8 h of incubation time. The uppermost value of MoO₃ nano-bricks particles (200 mg) has established excellent reduction in bacterial cells of selected pathogens. The logarithmic stages were established between 4 to 8 h time-points (Fig. 5).

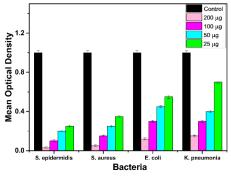


Fig. 5. A bar graph showing optical density (600 nm) of spent medium from S. epidermidis, S. aureus, E. coli and K. pneumonia after exposure with different concentrations of MoO3 nano-bricks.

MoO₃ nano-bricks have shown inhibition of all tested isolates. The outcome of this research signifies that growth inhibition is reliant to strength of MoO₃ nano-bricks. The sensitivity of the Gram positive strains towards MoO₃ nanobricks might be due to the difference in the structure of cell membrane [15]. Few studies have documented the antimicrobial activity of MoO₃ nano-bricks [14]-[16]. Nevertheless; hitherto there is no study which account for antifungal activity of MoO₃. Our research investigation for the first time reports antifungal potential of MoO₃ nanobricks. The antifungal activity of MoO₃ nano-bricks unwrap a suitable substitute to accessible antimicrobial drugs. The representative fungal isolate utilized was C. albicans. Herein; it was found that high concentration (200 µg) of MoO₃ nanobricks possesses commendable antifungal potential (Fig. 6).

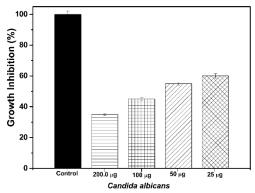


Fig. 6. In vitro antifungal activity of nano-bricks against C. albicans. Absorbance for control without MoO3 nano-bricks and with different concentrations of MoO₃ nano-bricks was determined. Fungal growth was expressed as % growth of control (i.e. growth in Sabouraud dextrose without MoO₃ nano-bricks).

The outstanding antifungal and antibacterial of MoO₃ nano-bricks has been suggested due to the release of hydroxonium ions (H₃O⁺) creating an acidic environment [15]. In present study, an acidic pH could be created by dissolution of MoO3 nano-bricks into molybdic acid (H₂MoO₄) due to molybdate [HMoO₄] and subsequent release of H₃O⁺ ions, which disperse inside the membranes of microbial cells; thereby restricting enzyme action, protein immovability as well as disorganizing nucleic acids organization [15], [21]. This is a non-specific mechanism, which could explain efficacy of MoO3 nano-bricks for microbial isolates. In contrast to a previous study that evaluated biocidal activity of MoO3 coated surfaces exclusively against multiple resistant bacteria isolates [22]. Herein, the MoO₃ nano-bricks have been in parallel tested for both fungal as well as bacteria isolates. MoO₃ nano-bricks are evidently effective against tested isolates including C. albicans fungus. Molybdenum is a non-toxic element turning MoO₃ nano-bricks into a very promising biocidal material in order to trim down microbial contamination.

IV. CONCLUSION

Conclusively, the main endeavor of current investigation was synthesis and characterization of MoO₃ nano-bricks and to explore them in terms of their antimicrobial potential, an alternative to combat multidrug resistant pathogens. MoO₃ nano-bricks were successfully synthesized by hydrothermal method. The MoO₃ nano-bricks have fully been differentiated by XRD, SEM, EDX and FTIR. The outcomes of our study verify earlier studies which have specified that MoO₃ nanomaterial is very effective against multiple drug resistant bacteria. MoO₃ nano-bricks possess antibacterial activity against bacterial and fungal isolate. Our data is in admirable harmony with the earlier research. The MoO₃ nano-bricks depicted broad spectrum antimicrobial potential which strongly recommends their use as material of choice for potential antimicrobial agents.

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