

# Nano-Scale Mo-MoO<sub>3</sub> Entrapped in Engineering Thermoplastic: Inorganic Pathway to Bactericidal and Fungicidal Action

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**Abstract**— In our contemporary endeavor, metallic molybdenum (Mo) and semiconducting molybdenum trioxide (MoO<sub>3</sub>) nanostructures have been simultaneously generated via solid state reaction between molybdenum (III) chloride (MoCl<sub>3</sub>) and polyphenylene sulfide (PPS) at 285°C in unimolar ratio for different time durations, namely, 6h, 24h and 48h. The resultant nanocomposites (NCs) revealed formation of predominantly metallic Mo for all the samples. However, MoO<sub>3</sub> gradually gained prominent position as secondary phase with rise in reaction time. The present study was intended to investigate the antibacterial potential of metal-metal oxide-polymer NCs i.e. Mo-MoO<sub>3</sub>-PPS against microorganisms viz., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Aspergillus fumigatus*. The antibacterial activity of the NCs was evaluated by agar well diffusion investigation. Maximum sensitivity concentrations of NCs were determined by finding out minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC). Moreover, the NCs prepared at reaction time of 48h exhibited best MBC values and were tested with time kill assay which revealed that the growth of *S. aureus* was substantially inhibited by Mo-MoO<sub>3</sub>-PPS NCs. This synchronized formation of Mo-MoO<sub>3</sub> nanostructures in an engineering thermoplastic may have potential antimicrobial applications in biomedical devices and components. *Prima-facie* results on antifungal activity are indicative of the fact that these materials can show anti-cancer behavior.

**Index Terms**— Nanocomposites, engineering thermoplastic, solid state reaction, antibacterial/ fungal activity.

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## I. INTRODUCTION

Among transition metals, molybdenum is cheaper and readily available as compared to the noble metals which can exhibit plasmonic behavior and demonstrate catalytic [1] as well as biosensing applications [2]. Curiously, research on molybdenum nanoparticles has not been adequately pursued among transition metals. There are sporadic reports pertaining to the synthesis of molybdenum nanostructures [3-7]. Mandal *et. al.* have prepared such nanostructures by introduction of  $\gamma$ -ray irradiation of the radioisotope <sup>99</sup>Mo into the solution of ammonium molybdate [8]. It is worthy to consider that molybdenum nanostructures have mostly been prepared by various plasma techniques such as RF plasma [3], DC magnetron sputtering [4, 5], electron cyclotron resonance plasma [6] etc. Another energy and resources intensive technique involved in such kind of synthesis is wire electrical explosion method [9]. Furthermore, molybdenum tri-oxide is an important n-type wide band gap layered semiconductor material. At nanoscale, MoO<sub>3</sub> has been prepared using different techniques like solvothermal [10], hydrothermal [11], sol-gel [12], etc and has been exploited for various applications such as catalysis [13], biosensor [14], gas sensor [15], energy storage and conversion [16],

electrochromic display [17] and antimicrobial activity [18]. Obtaining metal-metal oxide based nanocomposite can be important from the perspective of various technological applications. For example, incorporation of plasmonic noble metals such as gold and silver nanoparticles along with materials such as TiO<sub>2</sub>, ZnO etc for catalytic, environmental and sensor applications have been well-reported [19, 20, 21]. In this framework, generating Mo-MoO<sub>3</sub> based NCs can be advantageous from the standpoint of possible applications particularly in biosensing and catalysis. Additionally, compatibility of molybdenum may also be superior with its own oxide or sulfide. Like, many transition metals [22, 23] and metal oxides [24], it is anticipated that Mo-MoO<sub>3</sub> NCs can also exhibit antimicrobial behaviour.

To the best of our knowledge, there are only two reports corresponding to the synthesis of Mo-MoO<sub>3</sub> nanostructures [25, 26]. The technological applications of metal-metal oxide nanostructured system can be *better* realized by providing a flexible yet robust platform/support, viz. preferably embedding it in polymer matrix. Herein, a facile solid-solid reaction technique for the concurrent *in-situ* formation of Mo-MoO<sub>3</sub> nanostructures within an engineering thermoplastic matrix of PPS is proposed with prospective antibacterial coating application in biomedical devices. Microbial infection is a major cause of concern in medical industry. Biomedical devices, components and implants are major carriers of such infections. Therefore, as a preventive measure to restrict/eliminate the bacterial colonization, it is imperative to fabricate/coat biomedical devices, components and implants using NCs with ingrained antimicrobial inorganic nanostructured materials. In the proposed assembly of Mo-MoO<sub>3</sub>-PPS NCs, PPS can impart the flexibility of extruding it into a desired shape of any biomedical component or implant while entrapped nano-scale Mo-MoO<sub>3</sub> can exert antibacterial action. As a preliminary effort to support our hypothesis, we have performed the study concerning antimicrobial activity of the prepared powders of Mo-MoO<sub>3</sub>-PPS NCs. We expect that this study opens up the door for NCs based inorganic pathways to develop bactericidal and fungicidal components and coatings for biomedical industry.

## II. MATERIALS AND METHODS

### A. Experimental work

All the reagents were of analytical grade and used without further purification. In the present work, Mo and MoO<sub>3</sub> nanostructures are simultaneously generated within polyphenylene sulfide (PPS) matrix via solid state reaction between MoCl<sub>3</sub> (Alfa Aesar) and PPS (Aldrich Chemicals). PPS acts as a stabilizing agent for the generated nanoparticles [27]. In a typical procedure, MoCl<sub>3</sub> and PPS are mixed homogeneously in a known molar ratio (1:1) in a pestle mortar using acetone. The resultant admixtures are kept in a furnace for 6h, 24h and 48h at melting temperature of PPS i.e. 285°C and finally allowed to cool naturally to room temperature. The as-prepared powder samples (denoted as N6, N24, N48) display different colors such as blue, grey and black for the reaction time of 6h, 24h and 48h, respectively. The powders thus obtained were in the form of bigger chunks which could be easily crushed to fine powder using pestle-mortar.

### Structure and morphology determination

The structural information on virgin powder samples was obtained using X-ray diffraction (Bruker D8 Advance) technique. The diffracting angle 2( $\theta$ ) was varied between 10 - 80° range and the observed XRD peaks were compared with standard JCPDS data. The surface morphological features were investigated by field emission scanning electron microscopy (FESEM) using HITACHI S-4800 instrument. The microstructural aspects of the samples were examined by high resolution transmission electron microscopy (HRTEM) with Tecnai G2-20U-Twin instrument (FEI, Netherlands) at an acceleration voltage of 200 KV. The samples for HRTEM were prepared by dispersing fine powder of the resultant product in isopropyl alcohol. A drop of dispersion was then transferred on carbon coated grid for further analysis.

### B. Antimicrobial activity study

Primary antimicrobial activity was ascertained by studying the zone of inhibition. It was followed by determining minimum inhibitory concentration (MIC) and minimum bactericidal / fungicidal concentration (MBC/MFC). MIC and MBC/MFC are the lowest concentrations (of NCs, in the present case) at which a tested compound inhibits growth or

kills more than 3 log (99.9%) of microorganisms. The lowest concentration of NCs showing visual growth inhibition was considered as the MIC. All MIC and MBC values reported were based on experimental repeats in triplicate. Finally, time kill assay was performed against the NCs sample showing the highest MBC value.

### C. Zone of inhibition

All the NCs samples were sonicated in 10% DMSO and used for further investigations. The antibacterial and antifungal activity of the resultant nanocomposites (NCs) was judged against selected microorganisms by determining the diameter of inhibition zone (DIZ) in agar well diffusion test. DIZ reflects magnitude of susceptibility of the microorganism. The concentrations used for antimicrobial assay were varied in the range of 25, 50, 75, 100 and 500 µg/well.

### D. Minimum inhibitory concentration (MIC)

MIC was determined by exposing the micro-organisms to the predetermined concentrations of NCs. Micro-organisms were grown in Mueller Hinton (M-H) broth comprising various concentrations of NCs and 105 CFU/ml of bacterial cells at 37 °C in incubator shaker (REMI CIS – 24 Plus) with shaking speed of 150 rpm for 24 h. The final concentrations of NCs used in the experiment were 0.2, 0.4, 0.6, 0.8, 1.0 and 1.5 mg/ml.

### F. Minimum bactericidal / fungicidal concentration (MBC/MFC)

Possibility of misinterpretation of the results due to the turbidity of insoluble compounds, if any, in determining the MBC was avoided by sub-culturing the above MIC serial dilutions in freshly prepared nutrient agar plates and incubation at 37 °C for 48 h. MBC was regarded as the concentration of the NCs that did not show any growth on a new set of agar plates due to prevention of the bacterial colony growth on this solid media.

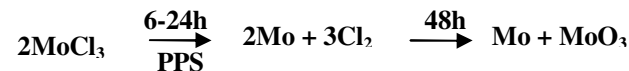
### E. Time Kill Assay

The time-kill curve was used to analyze post-treatment bacterial viability and to define the minimum time necessary to reach an inhibitory or bactericidal effect. Examination of the growth curves of bacterial cells was accomplished using *Staphylococcus aureus* through the time kill assay. The cells of *S. aureus* were exposed to NCs via M-H broth at different

concentrations (1/2 MIC, MIC and 2MIC) of NCs. Bacterial concentration was adjusted to 105 CFU/ml and each culture was incubated in a shaking incubator at 37 °C. Growth curves of bacterial cell cultures were calculated through repeated measure of the optical density (O. D.) using ELICO SL 210 double beam U-V visible spectrophotometer at 600 nm.

## III. RESULT AND DISCUSSION

MoCl<sub>3</sub> is known to melt/decompose at 410 °C, however, in the present reaction, the temperature was maintained at 285 °C, the melting point of PPS. Since MoCl<sub>3</sub> is homogeneously admixed in the PPS matrix, PPS may act as a catalyst reducing the decomposition temperature of MoCl<sub>3</sub> from 410 °C to 285 °C or below. PPS also acts as a capping agent preventing the oxidation of Mo. However as the reaction time increases, oxidation of Mo begins to take place due to partial degradation of PPS. The overall reaction is presented in the form of equation below:



### A. XRD

Figure 1 presents the typical XRD patterns of the as-prepared samples (N6, N24 and N48) which indicate the predominant formation of cubic phase of metallic molybdenum (JCPDS card no.42-1120) and minor phase of monoclinic MoO<sub>3</sub> (JCPDS card no.85-2405). As the reaction time increases, the resultant samples show little change in the relative intensity or sharpness for peaks corresponding to metallic molybdenum. However, the peak intensities were found to increase for molybdenum trioxide as reaction time increased from 6h to 48h. Especially, peak intensity around (011) plane (which is a minor intensity peak in monoclinic MoO<sub>3</sub>) was also appreciably increased with increase in the reaction time. It may be speculated that the molybdenum (100) surface provided suitable nucleation sites for the growth of (011) MoO<sub>3</sub> plane. From the diffractograms, it is evinced that the proposed solid-state reaction involving molybdenum precursor leads to formation of metallic molybdenum and semiconducting molybdenum oxide within PPS matrix. It is conjectured that as the reaction time increases, metallic

molybdenum gradually oxidizes to molybdenum oxide.

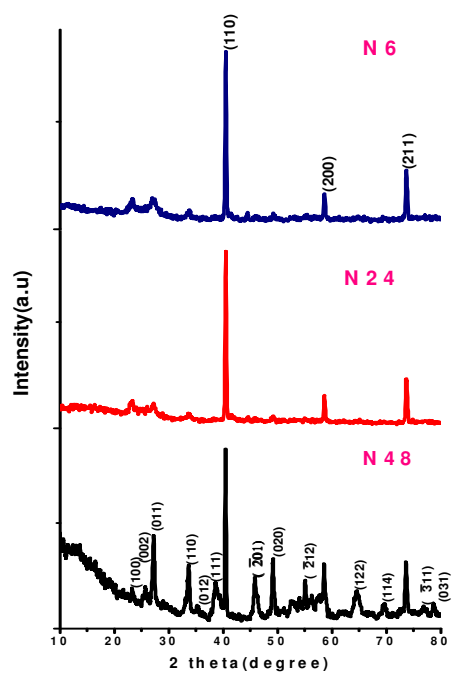


Fig. 1. XRD patterns of the resultant powdered samples.

### B. FESEM

FESEM images of the resultant composite samples are displayed in Figures 2(a)–(f). FESEM images for samples corresponding to 1:1 molar ratio of Mo salt:PPS prepared with different reaction intervals (6h–48h) elucidate nanoscale formation of molybdenum/molybdenum oxide embedded within the PPS matrix possessing mixed morphologies such as rods, sheets as well as spherical and irregular particles.

The information pertaining to the morphological features obtained from the FESEM images for each sample is summarized below: (i) For sample N6, formation of spherical and irregular sheet shaped nanostructures was noticed as displayed in figure 2a and b. The size of the spherical nanostructures was ~ 20-40 nm and irregular sheet nanostructures varied from ~ 100-400 nm in size. (ii) The FESEM images for the sample N24 reveals the generation of irregular sheet like structures (Figure 2c and d). A predominance of irregular shaped sheets was clearly visible. Size of the sheet like structure is found to be increased as compared to that of the sample N6. The irregular shaped nanostructured sheets displayed width varying from ~ 100-800

nm and were observed to be ~ 10-30 nm thick. (iii) Hierarchically entangled sheets like nanostructures were detected in case of sample N48 (Figure 2e and f). The width as well as thickness of sheets decreased as compared to other samples and it was observed that these sheets tend to self assemble forming complex hierarchical nanostructures. The width of these nano-sheets varies from 100 nm to 200 nm and thickness varies from 10–20 nm. Interestingly, as reaction time was increased from 6h–48h, more number of nanosheet like structures have been noticed implying that prolonged heat treatment favors 2-D growth.

### C. HRTEM

HRTEM images of the resultant samples are reproduced in figure 3. The gist of microstructural information for the NCs is as follows:

(A) The HRTEM image corresponding to sample N6 (figure

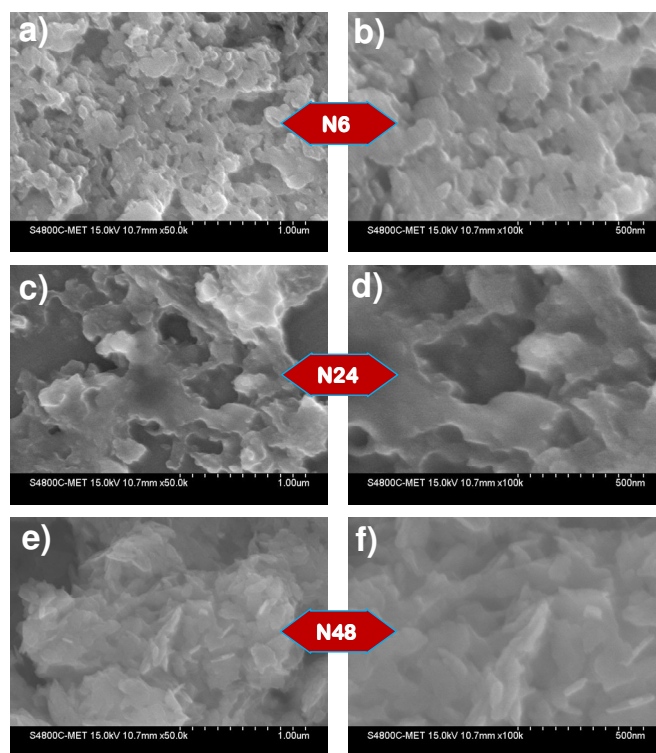


Fig. 2. FESEM images of the resultant powders corresponding to lower magnification (a, c, e) and, higher magnification. (b, d, f) for samples N6, N24 and N48, respectively

3a, at low magnification) showed formation of sheet like structures. The width of the sheets varied from 20 nm-50 nm, while the length varied from 50 nm-120 nm. Higher magnification image obtained on a representative sheet (figure

3b) revealed crystalline structure. The recorded lattice spacing of 0.232 nm matched well with *d*-spacing corresponding to (110) plane of cubic metallic molybdenum. The inset of the figure 3b represents the selected area electron diffraction (SAED) pattern of N6 sample revealing its polycrystalline nature. (B) At low magnification, for the sample N24 (figure 3c), growth of the sheets - most probably by coalescing of

be attributed to the presence of native MoO<sub>3</sub>. (C) For sample N48, low magnification image (figure 3e) illustrated formation of hierarchical chunk like structures which appeared to be formed due to agglomeration of smaller sheet like structures. Similar trend was also detected in the FESEM image (figure 2e) of the same sample. Polycrystalline structure was revealed at higher magnification (figure 3f). The lattice spacing ~ 0.201 nm could be indexed as (201) plane of monoclinic MoO<sub>3</sub>. The SAED pattern in the inset of the figure 3f confirmed the polycrystalline nature of the sample.

#### D. Antimicrobial activity

The anti-microbial activity of the resultant NCs was studied

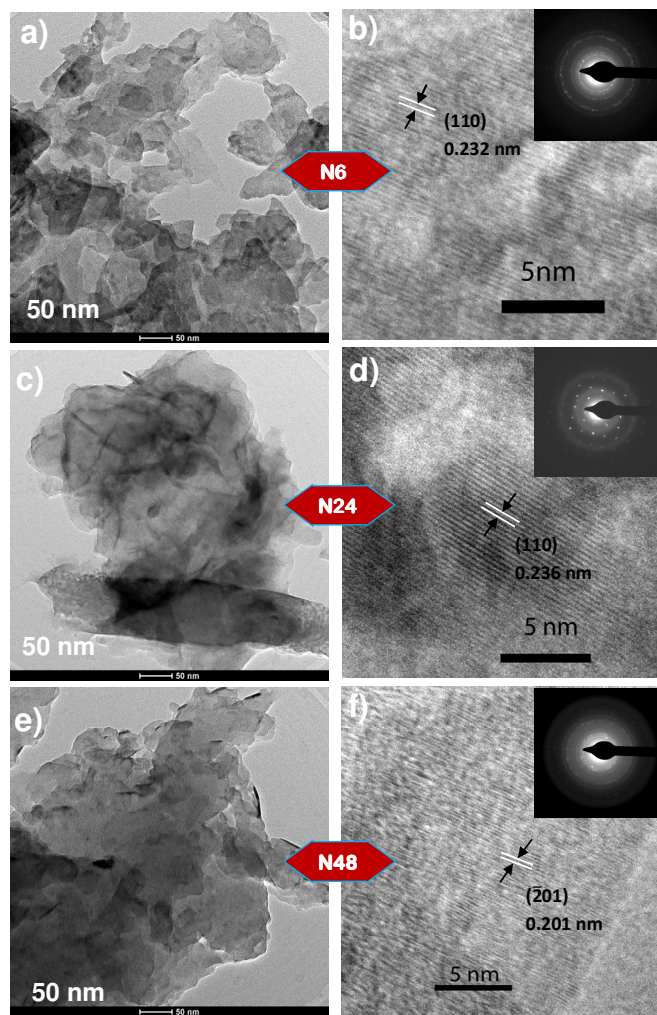


Fig. 3. The higher (b, d, f) and lower (a, c, e) magnification HRTEM images of the resultant NCs corresponding to N6, N24 and N48 samples, respectively.

smaller sheets - was observed. The crystalline nature was noticed when higher magnification image was obtained on the representative sheet like structure (figure 3d). The lattice spacing of 0.236 nm corresponded to (110) plane of cubic metallic molybdenum. The SAED pattern of N24 sample (inset of figure 3d) disclosed the single-crystalline nature. The slight increase in the *d* value corresponding to (110) plane of cubic metallic molybdenum for both N6 and N24 samples can

Table I  
ZONE OF INHIBITION OF NCS AT DIFFERENT CONCENTRATIONS AGAINST THE MICROORGANISMS.

Concentration of Nanoparticles (µg)	Micro organisms				
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>A. fumigatus</i>
<b>Inhibition halo diameter (mm)</b>					
<b>N6</b>					
25	00	00	00	00	00
50	00	00	00	00	00
75	00	00	00	00	00
100	00	00	00	05	00
500	00	06	12	07	09
<b>N24</b>					
25	00	00	00	00	00
50	00	00	00	00	00
75	00	00	00	00	00
100	00	00	00	05	00
500	05	06	15	07	09
<b>N48</b>					
25	00	00	00	00	00
50	00	00	00	00	00
75	00	00	00	00	00
100	00	00	00	09	00
500	07	09	17	10	11

The results revealed that all the NCs acted as excellent antimicrobial agents against both bacteria and fungi. All the NCs exhibited the highest zone of inhibition against *S. aureus*. The inhibition halo diameter was found to be 12, 15 and 17 mm for N6, N24 and N48 NCs, respectively. The zone of inhibition increased with the gradual rise in concentrations of NCs. The N48 sample exhibited highest antibacterial activity and highest antifungal activity as compared to other

nanostructured composites used in the present study. Significant zone of inhibition was obtained for concentration of 500 µg. The higher concentration required for such activity may be attributed to the entrapment of the synthesized nanoparticles within the polymer matrix. An additional aspect of this study was that for concentration of 100 µg, considerable (5 mm-9 mm) inhibition halo diameter was noted for *K. pneumoniae* colony treated with the NCs. *A. fumigatus* when treated with the NCs samples displayed zone of inhibition from 9 to 11 mm. Interestingly, to the best of our knowledge, this is the first report on antifungal activity against *A. fumigatus* using any kind of NCs system.

#### E. MIC/MBC/MFC

The MIC/MBC/MFC values for bacterial as well as fungal colonies treated with NCs samples were provided in Table 2. Since, MIC (data not shown) and MBC/MFC values (Table 2) for all the samples and concentrations matched with each other, the formulations of NCs were considered to be bactericidal /fungicidal. The maximum sensitivity towards MBC values (0.8 mg/ml) was recorded for sample N48 in all the bacterial cultures. However, sample N24 has also induced similar sensitivity in *P. aeruginosa*, *S. aureus* and *K. pneumoniae*. Curiously, MFC value for *A. fumigates* is noted to be 1.5 mg/ml.

Microorganism	Concentration mg/ml		
	N6	N24	N48
<i>P. aeruginosa</i>	1.0	0.8	0.8
<i>S. aureus</i>	1.0	0.8	0.8
<i>E. coli</i>	1.0	1.0	0.8
<i>K. pneumoniae</i>	1.0	0.8	0.8
<i>A. fumigatus</i>	1.5	1.5	1.5

The antimicrobial activity of NCs was striking, especially, because there are no reports associated with metal/metal oxide nanostructures embedded within the polymer matrix and still

exhibiting antimicrobial activity. However, Ana M. Díez-Pascual *et. al.* studied the antibacterial activity on ZnO-PPS NCs [28]. It may be noted that bare nanoparticles always exhibit higher MBC/MFC values at lower concentration but their application can be limited owing to their potential cytotoxicity, mixing with body fluids and lack of platform. Coating of such nanoparticles on biomedical devices and components is also tedious, expensive and rigid. However, such drawbacks can be overcome in case of polymer NCs but at the cost of lower MBC/MFC values at lower concentration.

#### F. Time kill assay

Since, NCs sample N48 has revealed maximum antibacterial activity, it was allotted for time kill assay against *Staphylococcus aureus*. The cells of *S. aureus* exhibited different growth curve patterns when exposed to the different concentrations of N48 NCs as illustrated in the figure 4. In this investigation, for control as well as sub-inhibitory concentration (½ MIC), lag phase ended at 6h and log phase lasted up to 16h. Subsequent to this, steady state growth was noted. However, the degree of growth was found to be smaller for sub-inhibitory concentration. Nonetheless, at inhibitory concentrations i.e. MIC and 2 × MIC, least to no growth was observed till 10h implying that significant delay occurred before the initiation of log phase. During the log phase (10-14h), very small scale growth was observed. Thus, it can be concluded that inhibitory concentrations caused drastic growth inhibitions and consequently can be used as antibacterial biomedical components. It is speculated that release of Mo<sup>2+</sup> ions from Mo as well as MoO<sub>3</sub> lead to the killing of bacterial

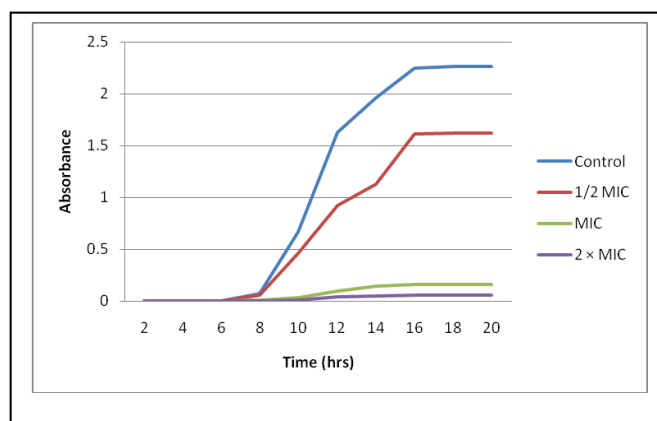


Fig. 4. Kill-Growth (time) curves of *Staphylococcus aureus* cells exposed to different concentrations of N48 NCs

cells presumably by disruption of the cell wall.

## V CONCLUSION

Solid-solid reaction protocol involving  $\text{MoCl}_3$  and PPS for the synthesis of Mo-MoO<sub>3</sub>-PPS NCs was developed. Metallic Mo was noted to be the dominant phase. As the reaction proceeds from 6h to 48h on time-scale, the tendency to generate MoO<sub>3</sub> was found to be enhanced. The synthesized NCs were subjected to antimicrobial activity by determining MBC/MFC and time kill curves. The higher concentration of the NCs required for attaining the antimicrobial activity may be attributed to the entrapment of the synthesized nanostructures within the polymer matrix which can hamper the direct exposure of active surface area of nanoparticles to microbial cells. However, this work is significant as it unlocks the prospect of fabricating the biomedical devices and components (or coating on them) using such antimicrobial NCs. The preliminary results of antifungal activity are indicative of the fact that these materials can potentially demonstrate anti-cancer properties as it is a thumb rule that antifungal materials are also anti-carcinogenic in nature. More investigations in this direction are underway.

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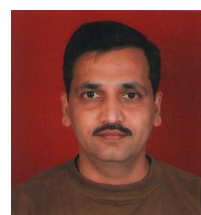
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