



Investigation of the Influence of pH on the Microstructure, Morphology, Optical Properties, and Environmental Remediation Efficacy of Molybdenum Trioxide (MoO₃) Nanoparticles

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ABSTRACT

Molybdenum trioxide (MoO₃) is a versatile transition metal oxide with promising applications in photocatalysis, optoelectronics, and antimicrobial systems due to its unique structural, optical, and electronic properties. In this study, undoped MoO₃ was synthesized using a facile wet-chemical approach under varying pH conditions (9, 10, and 11). The samples were characterized using X-ray diffraction (XRD), Field emission Scanning electron microscopy (FE-SEM), Photoluminescence (PL), Ultraviolet-visible (UV-Vis) spectroscopy, and antibacterial activity tests. The structural and optical properties, including crystallinity, defect states, and bandgap energy, were investigated in detail. The XRD analysis revealed a slight variation in crystallite size and strain with pH, while the FE-SEM analysis highlighted the morphological transformations from layered structures to dispersed nanoparticles. PL and UV-Vis analyses demonstrated pH-dependent bandgap tuning and defect-mediated optical transitions. Finally, the antibacterial activity of MoO₃ against *Escherichia coli* was evaluated, demonstrating enhanced bactericidal effects over time. These findings underscore the potential of MoO₃ as a multifunctional material for environmental and biomedical applications.

Keywords: Undoped MoO₃; pH variation; Structural analysis; Morphological studies; Antibacterial study.

1. INTRODUCTION

Molybdenum trioxide (MoO₃), a layered transition metal oxide, has garnered significant attention due to its exceptional optical, structural, and catalytic properties. Structurally, MoO₃ crystallizes in an orthorhombic lattice, consisting of distorted MoO₆ octahedra arranged in a layered configuration. This unique structural framework facilitates ion intercalation and anisotropic behavior, which are advantageous for numerous applications. The wide bandgap of MoO₃, ranging between 2.9 and 3.2 eV, facilitates its utility in optoelectronics, photocatalysis, and energy storage systems (Ragab *et al.* 2025; Gautam *et al.* 2024). Furthermore, MoO₃ exhibits redox-active properties, making it an efficient catalyst for oxidation reactions and a sensitive material for gas sensing applications (Liu *et al.* 2021).

One of the key attributes of MoO₃ is its tunability, which allows its structural, electronic, and optical properties to be optimized for specific

applications. The synthesis parameters, such as pH, precursor concentration, and calcination conditions, play a pivotal role in defining the material's morphology, defect density, and crystallinity (Hussain *et al.* 2022; Cheng *et al.* 2024). Among these parameters, pH is highly critical as it directly impacts the nucleation and growth mechanisms during synthesis. For instance, Mo precursors are more prone to aggregation under acidic conditions, leading to larger particle sizes and higher defect densities. In contrast, alkaline conditions promote deprotonation and efficient coordination of MoO₆ units, resulting in improved crystallinity and reduced defect states. Variations in synthesis pH can significantly influence the optical and catalytic properties of MoO₃ and its potential applications in antibacterial activity and photocatalysis (Ikram *et al.* 2024).

MoO₃ has versatility and extends across multiple domains, including energy storage, environmental remediation, and biomedicine. In energy storage, MoO₃ has been explored as an electrode material in lithium-ion batteries due to its high theoretical capacity

and ability to intercalate ions reversibly (Babu *et al.* 2025). Its photocatalytic capabilities make it suitable for degrading organic pollutants under UV or visible light, addressing environmental pollution challenges (Paul *et al.* 2017). MoO₃ exhibits antibacterial activity, especially against gram-negative bacteria like *Escherichia coli*, by generating reactive oxygen species (ROS) that disrupt cell membranes and inhibit microbial growth (Alghamdi, 2025).

The current study focuses on elucidating the effect of pH on the structural, optical, and antibacterial properties of MoO₃ synthesized via a wet-chemical approach. Characterization techniques, including XRD, FESEM, PL, and UV-Vis spectroscopy, are utilized to investigate the pH-dependent variations in morphology and crystallinity. These analyses aim to establish a direct correlation between the synthesis conditions and the functional performance of MoO₃, contributing to the development of optimized materials for diverse technological applications.

2. MATERIALS AND METHODS

The MoO₃ samples were synthesized using a facile wet-chemical method. A 0.2 mol quantity of hexa ammonium heptamolybdate tetrahydrate, used as the precursor, was dissolved in 100 ml of deionized water under continuous stirring for 30 minutes to ensure complete dissolution. Then, 25 ml of concentrated nitric acid (HNO₃) was added dropwise, and the solution was stirred for 30 minutes. To adjust the pH to 9, ammonia (NH₃) was added as the precipitating agent. The homogeneous mixture was stirred at 85 °C for 3 hours, followed by aging at room temperature for 4 hours to stabilize the precipitate.

The resulting precipitate was centrifuged at 3000 rpm for 10 minutes and thoroughly washed multiple times with acetone and ethanol to remove impurities. The washed material was dried at 120 °C for 24 hours, followed by calcination at 500 °C for 3 hours to enhance crystallinity and phase purity. This procedure was repeated for the samples prepared at pH 10 and pH 11 by adjusting the pH during the synthesis step.

The samples synthesized at different pH levels were stored and characterized to assess their structural, optical, and antibacterial properties. This methodology ensures reproducibility and highlights the impact of pH on the material's final properties.

3. RESULTS AND DISCUSSION

3.1 XRD Analysis

The XRD analysis of MoO₃ samples synthesized at pH levels 9, 10, and 11 reveals significant insights into their crystallinity and structural properties.

The XRD patterns display sharp and well-defined peaks, characteristic of orthorhombic MoO₃, as confirmed by matching with the JCPDS No. 05-0508 reference data. This confirms the successful synthesis of the desired phase for all samples, irrespective of pH.

Crystallographic parameters, such as crystallite size, strain, and dislocation density, were quantitatively analyzed using the Scherrer equation and Williamson-Hall plots. The average crystallite size for all pH levels was approximately 14.27 nm, indicating that the variation in pH did not significantly affect the crystalline domain size. However, subtle differences in strain (~ 0.1027) and dislocation density ($\sim 0.00491 \text{ nm}^{-2}$) were observed, suggesting a minor influence of pH on lattice distortions and defect densities. These results highlight the role of pH in altering the microstructure of MoO₃.

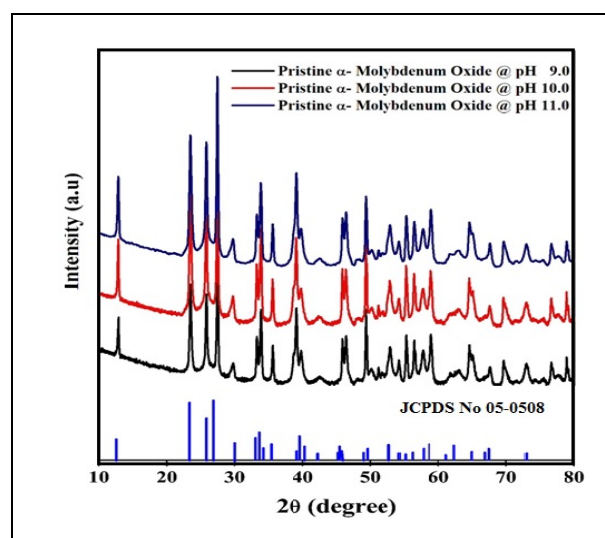


Fig. 1: XRD diffractograms of undoped MoO₃ at pH 9, 10, and 11

Notably, the XRD patterns (Fig. 1) show that higher pH levels (10 and 11) result in sharper and more intense diffraction peaks compared to pH 9. This indicates improved crystallinity and reduced structural defects at alkaline conditions. The enhanced crystallinity at higher pH can be attributed to the promotion of uniform nucleation and growth dynamics, driven by the increased availability of hydroxide ions, which facilitate better coordination of MoO₆ octahedra. Conversely, at pH 9, the relatively broader peaks and reduced intensity suggest slightly higher defect densities and lattice distortions, likely due to incomplete deprotonation and less efficient nucleation.

In conclusion, the XRD analysis demonstrates that the synthesis pH plays a pivotal role in modulating the structural integrity and crystallinity of MoO₃. These findings underscore the importance of pH optimization in tailoring the structural properties of MoO₃ for advanced applications.

3.1.1 The Halder-Wagner (HW) Plot

The Halder-Wagner (HW) plot for the undoped MoO₃ (Fig. 2) illustrates the relationship between peak broadening and crystallite size and strain effects. The x-axis ($\frac{\beta}{\tan \theta \sin \theta}$) reflects the reciprocal dependence of peak broadening on crystallite size and strain, while the y-axis ($\frac{\beta}{\tan \theta}$)² indicates the square of peak broadening, proportional to strain effects. The linear fit equation, $y = a + bx$ provides insights, with the intercept, $a = 3.33792 \times 10^{-5}$ linked to the crystallite size and the slope, $b = 0.0028$ associated with the strain.

From the intercept (Fig. 2) the crystallite size is estimated to be approximately 49.51 nm, based on the Scherrer formula. The positive slope corresponds to a microstrain of 0.0265, indicating the presence of tensile strain in the material. The moderate fit reflects contributions from both crystallite size and strain to the peak broadening; in addition, it suggests that defects or anisotropy may play a role. This analysis highlights the nanoscale structure of undoped MoO₃ and its strain-related characteristics, potentially influencing its electrochemical and structural properties.

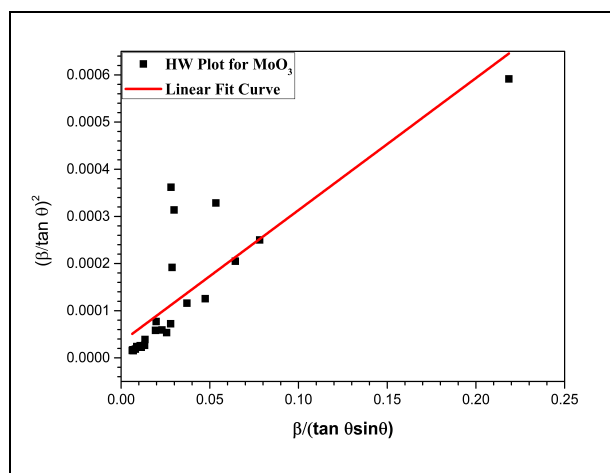


Fig. 2: HW plot of undoped MoO₃ at pH = 11

3.1.2 The Williamson-Hall (WH) plot

The WH plot for undoped MoO₃ demonstrates the relationship between peak broadening, crystallite size, and strain effects. The x-axis $4\sin \theta$ represents the strain contributions from lattice imperfections, while the y-axis $\beta \cos \theta$ reflects the size and strain-induced broadening (Fig. 3). The linear fit equation, $y = a + bx$ yields an intercept, $a = 0.00243$ associated with the crystallite size and slope, $b = 9.97557 \times 10^{-4}$ linked to the strain.

From the intercept (Fig. 3) the crystallite size is estimated to be approximately 52 nm. The analysis reveals a nanoscale crystallite size derived from the

intercept using the Scherrer equation. The positive slope corresponds to tensile strain within the lattice, but the scattered data points and poor fit suggest complex broadening mechanisms. While the WH plot provides insights into the structural properties of undoped MoO₃, further refinement using complementary models or additional data may enhance the understanding of its strain and size-related characteristics.

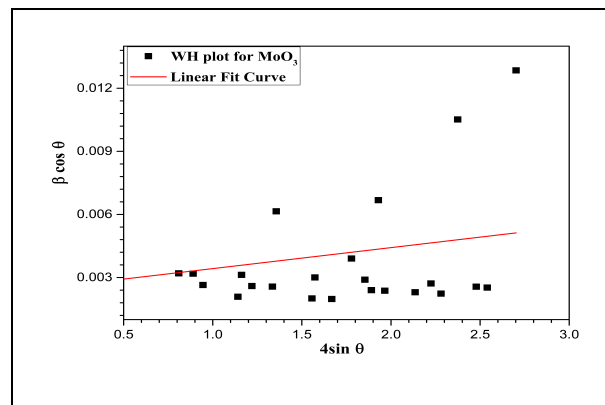


Fig. 3: WH plot of undoped MoO₃ at pH = 11

3.2 SEM Analysis

The SEM images in Fig. 4 provide a clear illustration of how the synthesis pH significantly affects the surface morphology of MoO₃ nanoparticles. At pH 9 (Fig. 4 a), the particles exhibit a compact, layered structure with substantial agglomeration. This morphology suggests that the acidic medium facilitates rapid nucleation and growth, which limits the time available for particle separation. Consequently, the resultant particles tend to cluster, forming bulk-like structures with reduced surface area. This characteristic morphology at lower pH levels can impact the functional properties of MoO₃, particularly in applications requiring high surface area, such as catalysis and adsorption.

When the pH is adjusted to 10 (Fig. 4 b), the morphology undergoes a noticeable transformation. The particles become smaller and exhibit a granular structure, accompanied by increased porosity. This intermediate morphology is indicative of slower growth kinetics compared to pH 9. The reduced rate of nucleation allows better separation of particles and prevents excessive aggregation. The increased porosity at this pH level is advantageous for enhancing the accessibility of active sites, particularly in catalytic and photocatalytic applications.

At pH 11 (Fig. 4 c), the particles are more uniformly distributed and have a size range of approximately 52.4 nm to 62.3 nm. The alkaline environment promotes enhanced deprotonation of hydroxyl groups in the precursor, resulting in improved coordination of MoO₆ octahedra during nucleation. This

leads to the formation of well-dispersed nanoparticles with minimal aggregation. The uniformity and smaller particle size achieved at pH 11 are highly desirable for applications requiring consistent performance, such as in optoelectronics and energy storage systems.

In summary, the SEM analysis highlights the critical role of pH in controlling the microstructure of MoO₃. The observed variations in particle size, porosity, and distribution directly correlate with the growth kinetics influenced by the synthesis pH, making it a vital parameter for tailoring the material's properties.

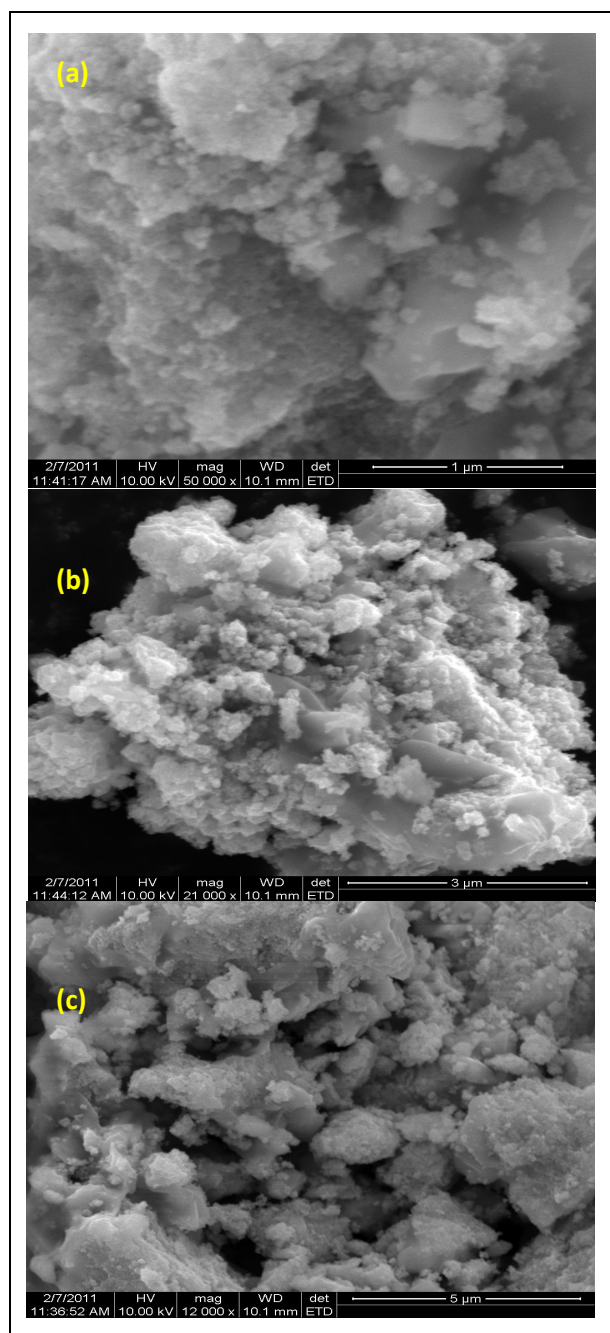


Fig. 4: SEM Images of undoped MoO₃ at (a) pH = 9 (b) pH = 10 (c) and (c) pH = 11

3.3 PL Analysis

The PL spectra of MoO₃ nanoparticles synthesized at different pH levels (Fig. 5) exhibit significant variations in their optical properties, reflecting the influence of synthesis conditions on defect density and electronic transitions. At pH 9 and 11, a dominant emission peak was observed at 470 nm, which corresponds to band-to-band recombination. This indicates a bulk-like emission characteristic of MoO₃ with minimal defect states, as these conditions promote higher crystallinity and reduced non-radiative recombination pathways.

In contrast, at pH 10, the PL spectrum reveals an emission peak at a shorter wavelength of 369 nm. This shift suggests the presence of shallow defect states, such as oxygen vacancies, which act as intermediate electronic states within the bandgap (Mamatha *et al.* 2022). These defects trap carriers, lead to radiative recombination at lower energy levels. The reduced PL intensity at pH 10 further confirms the prevalence of non-radiative recombination centers, which quench the overall emission. The intermediate pH likely induces structural distortions and incomplete nucleation, increasing the defect density.

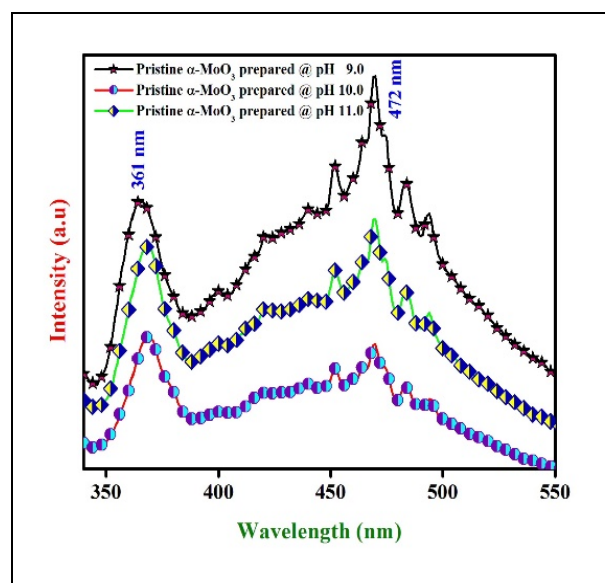


Fig. 5: Photoluminescence spectra of undoped MoO₃ at pH 9, 10, and 11

The pH-dependent shifts in emission properties suggest that the alkaline synthesis environment (pH 11) is favorable for reducing defect states and enhancing structural order. Enhanced deprotonation and efficient MoO₆ coordination under alkaline conditions contribute to improved crystallinity, as indicated by the intense and stable emission peak at 470 nm. Conversely, the acidic environment at pH 9 also supports good crystallinity but with slightly more aggregation, leading to comparable emission characteristics.

This analysis highlights the critical role of synthesis pH in tuning the defect density and electronic properties of MoO_3 . The ability to control the optical properties through simple adjustments in synthesis conditions, positions MoO_3 as a promising material for optoelectronic and photocatalytic applications (Joya *et al.* 2019). Future studies could explore further defect engineering strategies to optimize its photoluminescence behavior.

3.4 UV Analysis

The UV-Vis absorption spectra of MoO_3 nanoparticles synthesized at different pH levels (Fig. 6) reveal notable variations in optical properties. Strong absorption in the ultraviolet region is observed for all samples, with the absorption edge shifting depending on the pH. Using the Tauc plot method, the optical bandgap energies were calculated to be in the range of 3.0 to 3.2 eV. These values align with the intrinsic bandgap of orthorhombic MoO_3 , indicating its semiconducting nature and suitability for UV-driven photocatalysis (Koriche *et al.* 2025).

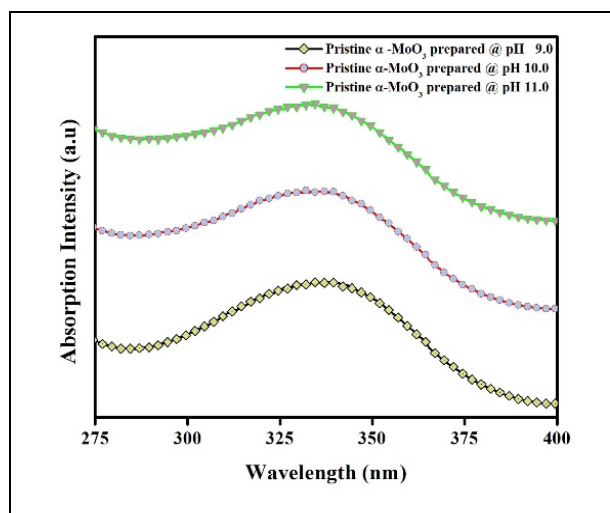


Fig. 6: UV-Vis spectra of undoped MoO_3 at pH 9, 10, and 11

At pH 11, the sample exhibited a narrower bandgap and higher absorption intensity, indicative of improved crystallinity and reduced defect density. This enhanced optical behavior can be attributed to the alkaline synthesis environment, which promotes deprotonation and efficient coordination of MoO_6 octahedra, leading to well-ordered crystal structures. The reduced defect density at this pH minimizes mid-gap states that typically act as non-radiative recombination centers, allowing for stronger and more efficient absorption.

Conversely, the sample synthesized at pH 10 showed a slight bandgap widening and lower absorption intensity. This behavior is attributed to the presence of defect states, such as oxygen vacancies, which introduce

shallow trap levels within the bandgap. These defect-mediated transitions alter the absorption properties and reduce the material's ability to absorb UV light efficiently. The results for pH 9 also align with the intrinsic behavior of MoO_3 , although the absorption intensity is slightly lower due to possible aggregation and higher defect density at lower pH levels.

These findings are consistent with the photoluminescence (PL) results, further highlighting the influence of pH on the optical properties of MoO_3 . The higher absorption at pH 11 emphasizes its potential for photocatalytic applications, where efficient light absorption and minimal defects are critical for performance (Koriche *et al.* 2025).

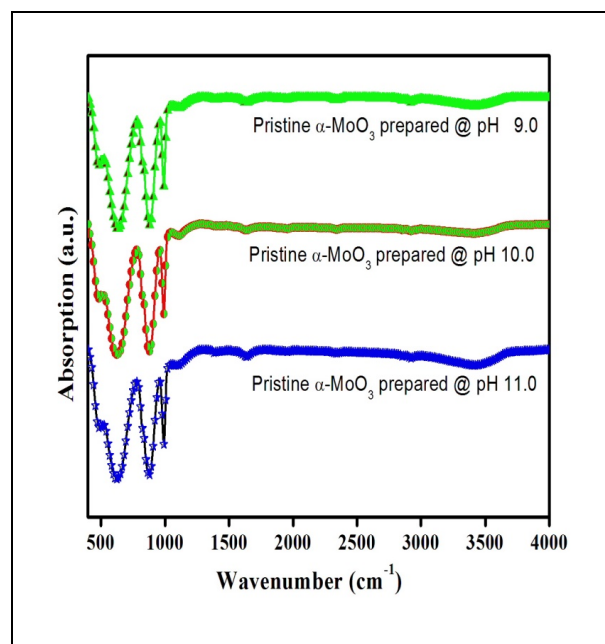


Fig. 7: FT-IR spectra of pristine MoO_3 prepared at pH 9, 10, and 11

3.5 FT-IR Analysis

The FTIR spectra (Fig. 7) of undoped MoO_3 synthesized at different pH levels reveal substantial variations in the material's surface chemistry and vibrational characteristics, underscoring the impact of pH on its structural dynamics. At lower pH levels, particularly under acidic conditions, the spectra prominently feature the stretching vibrations of terminal $\text{Mo}=\text{O}$ bonds, typically observed within the range of 960–1000 cm^{-1} . These bands are characteristic of isolated $\text{Mo}=\text{O}$ bonds located at the surface of MoO_3 , indicating minimal interaction with surrounding species. This vibrational mode reflects the inherent structural integrity of MoO_3 synthesized in acidic environments (Bile *et al.* 2025).

As the synthesis pH transitions toward alkaline conditions, significant changes occur in the FTIR spectra,

primarily due to the interaction of hydroxyl ions (OH^-) with $\text{Mo}=\text{O}$ bonds. This interaction results in the formation of surface $\text{Mo}-\text{OH}$ groups, leading to a reduction in the intensity and broadening of the $\text{Mo}=\text{O}$ stretching bands. This effect highlights the hydroxylation of the material's surface, which alters its vibrational properties and introduces new features associated with $\text{Mo}-\text{OH}$ bonds. These additional vibrational modes appear at lower wavenumbers, indicating structural modifications at the molecular level (Yakubu *et al.* 2024). Furthermore, the bridging $\text{Mo}-\text{O}-\text{Mo}$ modes, typically observed between $820\text{--}870\text{ cm}^{-1}$, also exhibit sensitivity to pH variations. The increasing alkalinity induces shifts in these bands' position and intensity, attributed to modifications in the bond length and angle of the $\text{Mo}-\text{O}-\text{Mo}$ linkages. These changes are consistent with the structural alterations caused by the incorporation of hydroxyl groups and the deprotonation of surface species under alkaline conditions (Taha *et al.* 2023).

This pH-dependent behavior demonstrates that FTIR is a powerful tool for studying the structural and chemical changes in MoO_3 . The ability to tune surface hydroxylation and vibrational properties through pH control enhances its potential for applications in catalytic and environmental remediation.

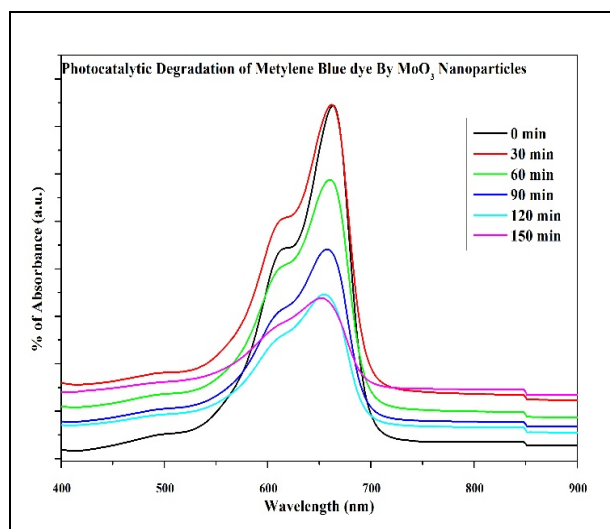


Fig. 8: Photocatalytic degradation of Methylene blue dye by MoO_3 nanoparticles

3.6 Photocatalytic Degradation of MoO_3 on Methylene Blue Dye

The photocatalytic degradation of methylene blue (MB) dye using MoO_3 nanoparticles (Fig. 8), shows a progressive reduction in absorbance with time. Initially, at 0 minutes, the absorption spectrum exhibits a strong peak at approximately 660 nm, which is characteristic of methylene blue. Over time, this peak diminishes significantly, indicating the breakdown of the dye molecules due to photocatalytic activity.

At 30 minutes, a noticeable reduction in the peak intensity is observed, suggesting that the photocatalytic process has started. As the reaction proceeds, the absorbance continues to decrease, with pronounced reductions at 60 and 90 minutes, highlighting the efficiency of MoO_3 nanoparticles in accelerating the degradation process under UV or visible light. By 120 minutes, the peak becomes much smaller, and at 150 minutes, it nearly disappears, demonstrating the near-complete degradation of the dye.

This behavior can be attributed to the generation of reactive oxygen species (ROS), such as hydroxyl radicals ($\bullet\text{OH}$) and superoxide anions ($\text{O}_2^{\bullet-}$), by MoO_3 nanoparticles under light irradiation (Priyadharsini *et al.* 2024). These ROS are highly reactive and attack the dye molecules, breaking them into smaller, non-toxic fragments or mineralizing them completely into CO_2 and H_2O . The high photocatalytic activity of MoO_3 can be linked to its wide bandgap ($\sim 3.0\text{--}3.2\text{ eV}$), which allows efficient electron-hole pair generation and minimizes recombination under irradiation.

The results confirm the effectiveness of MoO_3 nanoparticles as a photocatalyst for the degradation of organic dyes. This highlights their potential for environmental remediation, particularly in wastewater treatment, where the removal of hazardous dyes is critical. Future work could explore the reusability and scalability of MoO_3 for industrial-scale applications.

3.7 Antibacterial Activity Zone Inhibition Method

The zone of inhibition method is a widely used technique for evaluating the antibacterial efficacy of materials against various microbial strains. In this study, nutrient agar was prepared by dissolving 1.5 g of beef extract and 2.5 g of peptone in 500 ml of water, followed by pH adjustment to 7.2 using 10 N NaOH. Subsequently, 10 g of agar was added to the solution, and the medium was sterilized in an autoclave. To prepare agar plates, approximately 20–25 ml of the sterilized agar medium was poured into sterilized Petri dishes under aseptic conditions to prevent contamination. After cooling and solidification, these plates were used as a growth medium for *E. coli*.

For microbial inoculation, *E. coli* cultures were prepared, and a gram-staining procedure was performed to confirm the gram-negative nature of the bacterium. The smear was stained with crystal violet, followed by Gram's iodine, and decolorized with alcohol. Safranin was then used as a counterstain, revealing the characteristic red coloration of gram-negative bacteria due to their thin peptidoglycan layer. The bacterial inoculum was prepared by suspending the culture in sterile water, and 1 ml of the suspension was evenly spread onto the agar plates using a sterile pipette and an

'L' rod. Plates were incubated at 30 °C for 48 hours to allow bacterial growth.

To evaluate the antibacterial activity of MoO₃, circular samples of the material were placed on the inoculated agar plates, creating localized zones of inhibition where bacterial growth was suppressed. These zones, visible as clear areas around the material, indicate the diffusion of antimicrobial agents or ROS generated by MoO₃ into the surrounding medium, leading to bacterial cell death. Control plates without MoO₃ served to verify the absence of contamination and natural bacterial growth. After incubation, the diameter of the inhibition zones was measured to quantify the antibacterial effectiveness of MoO₃. This method is robust, reproducible, and provides direct evidence of the bactericidal properties of the tested material.

The antibacterial activity of MoO₃ was evaluated against *E. coli* using a zone of inhibition test. At 12 hours (Fig. 9 a), the inhibition zones were visible but less distinct, indicating the initial phase of bacterial cell death. By 24 hours (Fig. 9 b), the zones were more pronounced, confirming enhanced bactericidal efficacy over time.

The antibacterial activity is attributed to the generation of ROS and the oxidative stress induced by MoO₃. These ROS disrupt bacterial cell membranes and induce cell death. The findings highlight the potential of MoO₃ as an antimicrobial agent for environmental and biomedical applications.

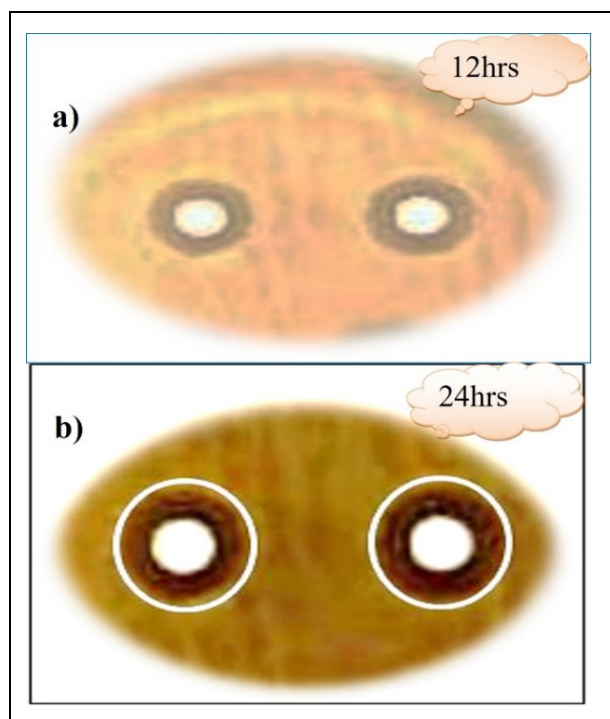


Fig. 9: Antibacterial activity of undoped MoO₃ after: (a) 12 hours, and (b) 24 hours

4. CONCLUSION

The study highlights the significant influence of pH on the structural, optical, and functional properties of undoped MoO₃ nanoparticles. Microstructural properties are evaluated through Standard analytical formulae. pH mediated strain induced particle size variations are unveiled through Williamson Hall plot and Halder Wagner plot method. Upon increasing the pH values of the growth medium size of the crystallites gets minimized which infers the chemical potential rendered by the pH varying agent create more strain in the crystal lattices. Higher pH levels enhance crystallinity, reduce defects, and improve surface morphology, as shown by XRD and SEM analyses. Optical studies confirm pH-dependent bandgap tuning and emission characteristics, with better absorption and reduced defect states at pH 11. It is also identified that pH 11.0 grown particles exhibit minimum shift in the band edge absorption value compared to their pH 9.0 and 10.0 grown particles, which reveal that pH 11.0 of the medium prevents the aggregation of nucleates. Such state limits the further growth of individual particles during synthesis. FTIR reveals surface hydroxylation effects, while photocatalytic tests demonstrate efficient dye degradation and strong antibacterial activity against *E. coli*. These findings showcase the adaptability of MoO₃ for applications in environmental remediation, optoelectronics, and biomedicine, emphasizing the importance of synthesis conditions.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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