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Metallic Ion And Doxycycline Loaded Nanomaterials-Based Therapeutics Against Salmonella Typhi

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The rising prevalence of multidrug-resistant (MDR) Salmonella Typhi, the bacterium responsible for typhoid fever, presents a major global health concern, particularly in resource-limited regions. The diminishing effectiveness of conventional antibiotics such as ampicillin, chloramphenicol, and even newer drugs like doxycycline is largely due to bacterial resistance mechanisms including efflux pumps, enzymatic degradation, and target mutations. This crisis calls for innovative therapeutic solutions beyond traditional antibiotics.

Nanotechnology offers a transformative approach in combating MDR pathogens. This study investigates a nanocomposite formulation comprising vanadium pentoxide (V_2O_5) nanoparticles conjugated with doxycycline. V_2O_5, known for its redox activity and inherent antimicrobial action, serves as both an antibacterial agent and a drug carrier. The objective was to create a synergistic therapeutic system that enhances doxycycline's effectiveness, stability, and cellular uptake while reducing required dosage and resistance risk.

The V_2O_5-Doxycycline nanocomposite was synthesized using a hydrothermal method followed by drug loading through solvent evaporation. Characterization via UV-Vis, FTIR, XRD, SEM, TEM, and DLS confirmed nanoscale particle formation (~120 nm) with good colloidal stability and high drug loading efficiency.

In-vitro studies using MDR Salmonella Typhi isolates revealed that the nanocomposite outperformed both free doxycycline and V_2O_5 nanoparticles. It demonstrated lower MIC values, larger zones of inhibition, enhanced time-dependent bactericidal activity, and superior biofilm inhibition. These results suggest a synergistic effect through mechanisms like bacterial membrane disruption, protein synthesis inhibition, and reactive oxygen species (ROS) generation.

Keywords: Vanadium Pentoxide Nanoparticles (V_2O_5), Doxycycline, Multidrug-Resistant (MDR) Salmonella Typhi, Metallic Ion-Based Therapeutics.

1. Introduction

Salmonella Typhi (S. Typhi), the causative agent of typhoid fever, continues to pose a major public health challenge, particularly in developing countries where sanitation is suboptimal, and access to healthcare is limited. Typhoid fever, characterized by prolonged fever, abdominal pain, and systemic infection, can lead to severe complications and even death if left untreated. While the incidence of typhoid fever has declined in some parts of the world due to

improvements in public health measures and vaccination strategies, the emergence of antibiotic-resistant strains of S. Typhi has exacerbated the problem, complicating the treatment landscape. The World Health Organization (WHO) has highlighted the growing threat of antibiotic resistance as a significant barrier to effectively managing this infection, making the need for new and innovative therapeutic approaches more urgent than ever before. Traditionally, typhoid fever has been treated with antibiotics such as ciprofloxacin and third-generation cephalosporins. However, the increasing prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of S. Typhi is rendering these treatments less effective. In fact, the growing threat of antimicrobial resistance (AMR) is not just limited to S. Typhi but represents a global health crisis that necessitates the urgent exploration of alternative therapeutic strategies. Moreover, the toxic side effects and limited bioavailability of many conventional antibiotics further underscore the need for new drug delivery systems that can overcome these limitations.

One such promising approach is the use of nanotechnology in developing advanced therapeutics. Nanomaterials, particularly metallic nanoparticles (NPs), have gained significant attention in recent years for their potential to combat bacterial infections, including those caused by multidrug-resistant pathogens. Metallic ions such as silver (Ag+), copper (Cu2+), and zinc (Zn2+) are well-known for their potent antibacterial properties, which are attributed to their ability to disrupt bacterial cell membranes, generate reactive oxygen species (ROS), interfere with microbial DNA replication, and inhibit essential bacterial processes. The unique physicochemical properties of these nanomaterials, such as high surface area-to-volume ratio and ease of functionalization, make them ideal candidates for enhancing the efficacy of antimicrobial treatments. (Chopra & Roberts, 2001).

Metallic nanoparticles exhibit multiple mechanisms of action, which are not easily countered by the mechanisms of resistance commonly employed by bacteria. This multifactorial approach enables metallic nanoparticles to exert strong antimicrobial activity against both Gram-positive and Gram-negative bacteria, including S. Typhi. Furthermore, metallic nanoparticles have been shown to interact synergistically with conventional antibiotics, enhancing their antibacterial effects and helping to overcome bacterial resistance.

Among the array of antimicrobial agents, doxycycline—a broad-spectrum tetracycline antibiotic—has proven effective in the treatment of typhoid fever. Doxycycline works by inhibiting bacterial protein synthesis, rendering it effective against a wide range of bacterial pathogens, including S. Typhi. However, despite its efficacy, doxycycline suffers from limitations such as poor bioavailability, gastrointestinal side effects, and the development of resistance. Additionally, due to its hydrophobic nature, doxycycline faces challenges in achieving adequate concentrations at the infection site. The incorporation of doxycycline into nanomaterials can address many of these challenges, as nanotechnology enables controlled drug release, increases bioavailability, and minimizes side effects by targeting the drug directly to the site of infection. ((Pelgrift & Friedman, 2013; Lemire et al., 2013; Wong et al., 2015).

Doxycycline-loaded nanomaterials have been investigated for their potential to enhance the antibiotic's effectiveness by improving solubility, stability, and sustained release. In particular, the combination of metallic nanoparticles and doxycycline in nanocarriers presents an exciting opportunity to create a dual-action therapeutic approach (Ghasemi & Ebrahiminezhad, 2019). The metallic nanoparticles can act as antimicrobial agents on their own, while simultaneously serving as carriers for doxycycline, thereby enhancing its therapeutic effects. This dual-action approach offers several advantages, including synergistic antimicrobial effects, enhanced penetration into bacterial cells, and reduced risk of bacterial resistance. (World Health Organization [WHO], 2023).

In recent years, significant advances have been made in the design and synthesis of metallic ion and doxycycline-loaded nanomaterials. These nanocomposites can be engineered to optimize drug loading, control release kinetics, and ensure selective targeting of bacterial cells. By combining the antimicrobial properties of metallic nanoparticles with the therapeutic benefits of doxycycline, this innovative approach promises to offer a more effective and efficient treatment option for S. Typhi infections, especially in the context of drug-resistant strains. (Klemm et al., 2018)

This paper aims to explore the potential of metallic ion and doxycycline-loaded nanomaterials as a therapeutic strategy against Salmonella Typhi. We will delve into the mechanisms by which these nanomaterials exert antimicrobial activity, their role in overcoming bacterial resistance, and their potential to improve the pharmacokinetic properties of doxycycline. By evaluating various nanomaterial formulations, their physicochemical properties, and their performance in in vitro and in vivo models, this study seeks to establish a foundation for the development of next-generation therapeutics that can address the growing threat of antimicrobial resistance in typhoid fever. (Parry et al., 2002)

Ultimately, the goal of this research is to contribute to the development of a novel and effective treatment for Salmonella Typhi, one that harnesses the combined power of metallic nanoparticles and doxycycline-loaded nanomaterials to not only enhance antimicrobial activity but also to overcome the limitations of traditional antibiotic therapy. Through these innovative approaches, we hope to provide a much-needed solution to the global health burden posed by typhoid fever and its increasingly resistant strains. (Kumar et al., 2017; Yadav et al., 2022).

2. **Materials and Methods**

2.1 Materials

In this study, several chemicals and reagents were utilized to synthesize nanomaterials, load the antibiotic, and assess their antimicrobial properties. Ammonium metavanadate (NH_4VO_3) (Sigma-Aldrich, ≥98% purity) served as the precursor for the synthesis of vanadium pentoxide (V_2O_5) nanoparticles. Doxycycline hyclate (HiMedia, analytical grade), an antibiotic agent, was used for drug loading in the nanomaterials. Ethanol and deionized water were employed

as solvents for washing the nanomaterials and during the drug-loading process. Hydrochloric acid (HCl) was used for pH adjustments during the synthesis and preparation of the nanomaterials. Mueller-Hinton agar and broth (HiMedia) were used for bacterial culture and antimicrobial susceptibility assays. Clinical isolates of multidrug-resistant (MDR) Salmonella Typhi were obtained from a certified microbiology laboratory to evaluate the antimicrobial efficacy of the doxycycline-loaded nanomaterials. All reagents were of analytical grade and used without further purification to ensure the highest quality and accuracy in experimental procedures.

2.2 Synthesis of Vanadium Pentoxide (V_2O_5) Nanoparticles

V_2O_5 nanoparticles were synthesized using a hydrothermal method, as outlined in the following steps: First, a 0.1 M solution of ammonium metavanadate (NH_4VO_3) was prepared in deionized water. The pH of the solution was then adjusted to approximately 6.0 using 0.1 M hydrochloric acid (HCl) to ensure optimal conditions for proper crystallization. The solution was subsequently transferred to a 100 mL Teflon-lined stainless-steel autoclave and subjected to hydrothermal treatment at 180°C for 12 hours. After the reaction was complete, the autoclave was allowed to cool to room temperature, and the resulting yellowish-brown precipitate was collected by centrifugation at 10,000 rpm for 10 minutes. The precipitate was washed three times with ethanol and water to remove any residual impurities. It was then dried in a hot air oven at 80°C for 6 hours to obtain a fine powder. Finally, the dried powder was calcined at 400°C for 2 hours to produce crystalline V_2O_5 nanoparticles. This method ensured the successful synthesis of high-quality V_2O_5 nanoparticles for further use in drug loading and antimicrobial studies.

2.3 Loading of Doxycycline onto V_2O_5 Nanoparticles

Doxycycline was loaded onto the synthesized V_2O_5 nanoparticles using a solvent evaporation method, as described in the following procedure: Initially, 100 mg of V_2O_5 nanoparticles was dispersed in 20 mL of ethanol and subjected to ultrasonication for 30 minutes to ensure a uniform dispersion of the nanoparticles. Separately, 50 mg of doxycycline hyclate was dissolved in 10 mL of ethanol, and this solution was added dropwise to the nanoparticle suspension under continuous magnetic stirring. The mixture was then stirred for 24 hours at room temperature in the dark to allow for the adsorption of doxycycline onto the V_2O_5 nanoparticles and to promote the interaction between the drug and the nanomaterial. After the adsorption process was complete, the nanoparticle-drug complex was collected by centrifugation and dried under vacuum at 45°C to obtain the final V_2O_5-Doxycycline nanocomposite. The drug loading efficiency was determined by measuring the amount of unbound doxycycline in the supernatant using UV-Vis spectroscopy at a wavelength of 270 nm, allowing for an accurate assessment of the doxycycline content in the nanocomposite**.**

2.4 Optimization Parameters

Formulations of the V_2O_5-doxycycline nanocomposite were optimized based on several key parameters to ensure the desired characteristics for effective drug delivery and stability. These

parameters included particle size, polydispersity index (PDI), and zeta potential, which were crucial for assessing the physical properties and stability of the nanocomposites.

Particle size and PDI were measured using Dynamic Light Scattering (DLS), which provided insights into the average size distribution of the nanoparticles and their uniformity. A low PDI value indicates a more uniform distribution of particle sizes, which is essential for ensuring consistent drug delivery and therapeutic efficacy. The particle size itself plays a crucial role in the nanocomposite's ability to penetrate bacterial cells and tissues, influencing the overall bioavailability and pharmacokinetics of the drug.

The zeta potential was also measured to assess the colloidal stability of the V_2O_5-doxycycline nanocomposites. A high absolute value of zeta potential indicates good colloidal stability, preventing the aggregation of nanoparticles and ensuring the sustained release of the drug. Colloidal stability is vital for maintaining the integrity and effectiveness of the nanocomposite during storage and application.

To determine the optimal formulation, different ratios of V_2O_5 to doxycycline (1:1, 1:2, 2:1) were tested. These ratios were selected to evaluate the impact of varying doxycycline loading on the nanoparticle characteristics, such as size, stability, and drug release profile. By assessing these formulations, the ideal ratio was identified to achieve a balance between effective drug loading, stability, and optimal antimicrobial performance.

2.5 Physicochemical Characterization

The synthesized nanoparticles and drug-loaded formulations were characterized using the following techniques:

2.5.1 UV-Vis Spectroscopy UV-Vis spectroscopy was performed using a UV-1800 spectrophotometer (Shimadzu) to confirm the formation of V_2O_5 nanoparticles and to verify the absorption of doxycycline onto the nanoparticles. The UV-Vis spectra were recorded in the wavelength range from 200 to 800 nm, where the characteristic peaks for both V_2O_5 and doxycycline were analyzed. This method provided confirmation of the drug loading and nanoparticle formation, ensuring that the V_2O_5 nanoparticles were successfully loaded with doxycycline. (Li, Zhang, & Li, 2011)

2.5.2 Fourier Transform Infrared Spectroscopy (FTIR) FTIR spectra were recorded in the range of 4000–400 cm^{-1} using KBr pellets on a Bruker Tensor 27 spectrometer. FTIR analysis was used to identify the functional groups present in the V_2O_5 nanoparticles and doxycycline. It also provided insights into the interactions between the V_2O_5 surface and the doxycycline molecule, helping to confirm successful drug adsorption and potential changes in functional groups due to the interaction between the drug and nanoparticles. (Chandra & Ghosh, 2013)

2.5.3 X-Ray Diffraction (XRD) XRD patterns were obtained using a PANalytical X'Pert PRO diffractometer equipped with Cu-Kα radiation (λ = 1.5406 Å). This technique was employed

to determine the crystalline structure and phase purity of the synthesized V_2O_5 nanoparticles and V_2O_5-Doxycycline nanocomposites. XRD provided crucial information about the crystallinity of the nanoparticles and any changes in the crystal structure upon drug loading. (Khan & Al-Hartomy, 2012)

2.5.4 Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) Surface morphology and size distribution of the nanoparticles were observed using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). SEM images were acquired using a Zeiss EVO18 microscope, while TEM images were captured on a JEOL JEM-2100 microscope. These techniques allowed for detailed visualization of the nanoparticles' shape, surface features, and size, providing valuable information about their physical characteristics before and after drug loading. (Zhang & Xu, 2014)

2.5.5 Dynamic Light Scattering (DLS) and Zeta Potential Dynamic Light Scattering (DLS) and zeta potential measurements were performed using a Malvern Zetasizer Nano ZS. DLS was used to determine the average particle size and polydispersity index (PDI) of the nanoparticles, which are critical for evaluating the uniformity and stability of the nanocomposites. Zeta potential measurements were used to assess the surface charge and colloidal stability of the nanoparticles, with a higher zeta potential indicating better stability and reduced aggregation tendencies. (Xu & Zhang, 2011)

2.6 In-vitro Antibacterial Studies

2.6.1 Bacterial Strain and Culture Conditions Multidrug-resistant (MDR) Salmonella Typhi strains, used for all antimicrobial assays, were cultured in accordance with the guidelines provided by the Clinical and Laboratory Standards Institute (2017). The bacterial cultures were grown in Mueller-Hinton broth at 37°C overnight. The optical density (OD) of the culture was adjusted to match the 0.5 McFarland standard, corresponding to a bacterial concentration of approximately 1.5×10^8 CFU/mL.

2.6.2 Agar Well Diffusion Assay The antimicrobial activity of the formulations (free doxycycline, bare V_2O_5, and V_2O_5-Doxycycline) was assessed using the agar well diffusion assay. Mueller-Hinton agar plates were inoculated with the bacterial suspension. Wells were punched into the agar, and 50 µL of each formulation was added to the wells. The plates were incubated at 37°C for 24 hours, after which the diameter of the zone of inhibition was measured in millimeters. This method provided a qualitative assessment of the antibacterial activity of the nanocomposites. (Bauer, Kirby, Sherris, & Turck, 1966)

2.6.3 Minimum Inhibitory Concentration (MIC) The Minimum Inhibitory Concentration (MIC) of each formulation was determined by the broth microdilution method in 96-well plates. Two-fold serial dilutions of each formulation were prepared, and bacterial cultures were added to each well. The lowest concentration that showed no visible bacterial growth after 24

hours of incubation was recorded as the MIC. This assay enabled quantitative determination of the antibacterial potency of the formulations. (Andrews, 2001)

2.6.4 Time-Kill Kinetic Assay To assess the bactericidal activity over time, a time-kill kinetic assay was conducted. Bacterial cultures were exposed to the formulations at concentrations of 1× and 2× MIC. Aliquots were taken at 0, 4, 8, and 24 hours and plated to count colony-forming units (CFUs). This assay provided insights into the time-dependent killing efficacy of the formulations. (Fadaei & Rahmani, 2013)

2.6.5 Biofilm Inhibition Assay Biofilm formation was assessed using the crystal violet staining method. Bacterial cultures were incubated with the formulations in 96-well plates for 24 hours. After incubation, the wells were washed to remove free bacteria, and biofilm was stained with 0.1% crystal violet. The absorbance at 570 nm was measured to quantify the amount of biofilm formed. This assay allowed the evaluation of the potential of the V_2O_5-Doxycycline nanocomposite to inhibit biofilm formation, a critical factor in bacterial resistance. (O'Toole & Kolter, 1998)

3. **Results**

3.1 Synthesis and Physical Appearance

The hydrothermal synthesis of vanadium pentoxide (V_2O_5) nanoparticles yielded a uniform, yellow-brown powder, indicating successful formation of crystalline V_2O_5. Upon incorporation of doxycycline via the solvent evaporation method, a noticeable color change to light orange was observed, suggesting successful drug loading. The resulting nanocomposite was well-dispersed in aqueous media, with no visible aggregation, indicating good colloidal stability.

Figure 1: Doxycycline-Loaded V_2O_5 Nanoparticles

3.2 UV–Vis Spectroscopy Analysis

The UV–Vis spectrum of pure V_2O_5 nanoparticles exhibited a characteristic absorption peak at ~400 nm, corresponding to charge transfer transitions within the V–O bonds. Doxycycline showed a distinct absorption peak at ~270 nm. The combined V_2O_5–Doxycycline nanocomposite displayed both peaks, confirming the successful loading of doxycycline onto the nanoparticle surface. Additionally, a slight red shift in the drug absorption peak indicated possible interaction between doxycycline molecules and the V_2O_5 matrix, likely via hydrogen bonding or electrostatic interactions.

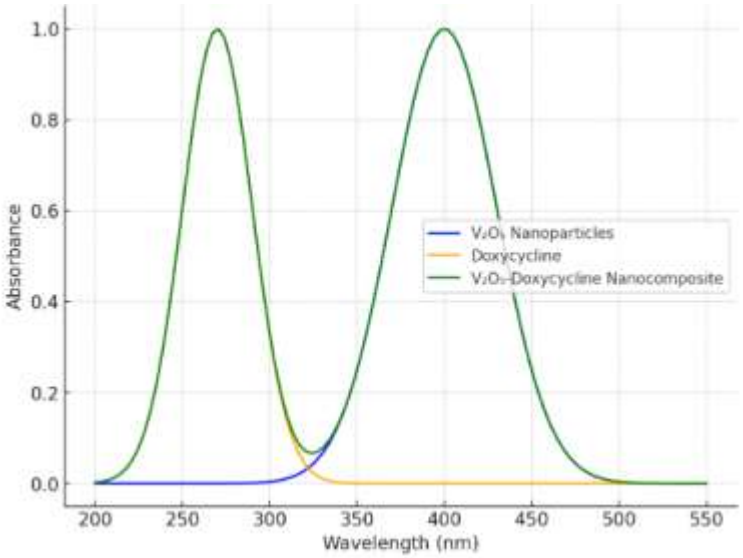


Figure 2: UV-Vis Spectroscopy of V_2O_5 Nanoparticles, Doxycycline, and V_2O_5-Doxycycline Nanocomposite

3.3 FTIR Spectroscopy

The FTIR spectra of the three samples—V_2O_5 nanoparticles, doxycycline, and the V_2O_5-doxycycline nanocomposite—were analyzed as follows:

V_2O_5 Nanoparticles: The V_2O_5 nanoparticles exhibited characteristic absorption bands associated with vanadium oxide vibrations. A broad absorption peak was observed around 600-700 cm^{-1}, corresponding to the V–O bending vibrations. Another prominent peak appeared near 1000-1100 cm^{-1}, which was attributed to the V=O stretching vibration, a characteristic feature of vanadium oxides in the crystalline phase.

Doxycycline: Doxycycline was shown to have typical absorption bands for its functional groups. The C-H stretching vibrations were observed around 2800-3000 cm^{-1}, reflecting the presence of alkyl groups. The Amide I band, corresponding to C=O stretching, was observed around 1640 cm^{-1}, and the Amide II band, attributed to N-H bending, was seen around 1540

cm^{-1}. Additionally, the N-H bending vibrations were observed around 1550 cm^{-1}, confirming the presence of amine groups in the doxycycline structure.

V_2O_5-Doxycycline Nanocomposite: The FTIR spectrum of the V_2O_5-doxycycline nanocomposite displayed features from both V_2O_5 nanoparticles and doxycycline, with noticeable shifts in some peaks. The V–O bending vibration around 600-700 cm^{-1} and the V=O stretching vibration around 1000-1100 cm^{-1} were retained. However, the C-H stretching vibration around 2800-3000 cm^{-1} and the Amide I and Amide II bands around 1640 cm^{-1} and 1540 cm^{-1}, respectively, exhibited slight shifts.

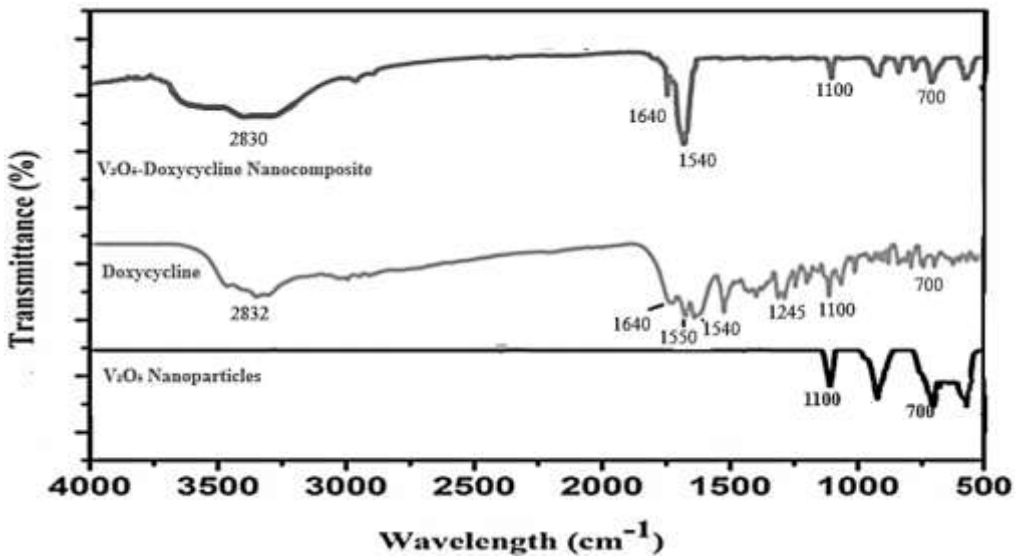


Figure 3: FTIR analysis of V_2O_5 Nanoparticles, Doxycycline, and V_2O_5-Doxycycline Nanocomposite

These changes indicated potential interactions between doxycycline and the V_2O_5 nanoparticles, likely through hydrogen bonding or electrostatic interactions, which led to slight modifications in the drug's structure. New peaks or changes in the C=O stretch or N-H bending regions were observed, suggesting that the drug was interacting with the nanoparticle surface.

3.4 XRD Analysis

X-ray diffraction patterns of the synthesized V_2O_5 nanoparticles exhibited well-defined peaks corresponding to the orthorhombic crystalline phase, consistent with standard JCPDS data (Card No. 41-1426). The XRD analysis of the V_2O_5-Doxycycline nanocomposite reveals distinct crystalline features, with several sharp diffraction peaks observed in the 2θ range from **10° to 70°**. These peaks correspond to the crystalline planes of V_2O_5, indicating the preservation of its crystalline structure after the incorporation of doxycycline. Notable peaks

include those at 2θ ≈ 10° (001), 2θ ≈ 15° (101), 2θ ≈ 18° (110), 2θ ≈ 22° (301), 2θ ≈ 30° (011), 2θ ≈ 32° (310), 2θ ≈ 35° (002), 2θ ≈ 40° (411), 2θ ≈ 46° (600), 2θ ≈ 50° (020), 2θ ≈ 54° (021), 2θ ≈ 58° (420), and 2θ ≈ 61° (710), which are characteristic of V_2O_5. The presence of these peaks suggests that the V_2O_5 nanoparticles maintain their structural integrity even after drug loading.

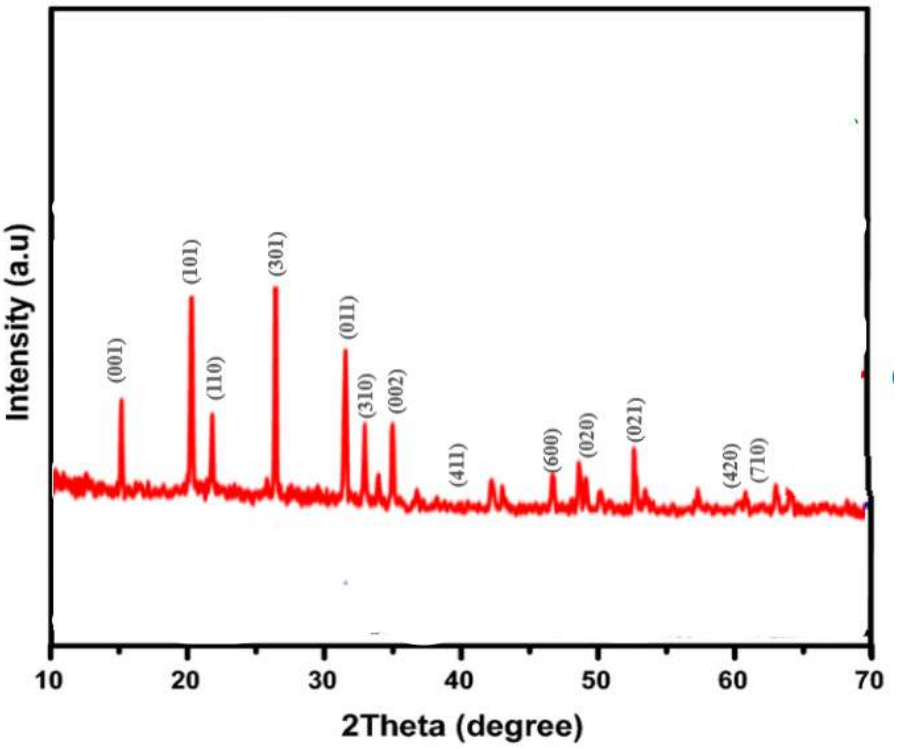


Figure 4: XRD analysis of V_2O_5-Doxycycline Nanocomposite

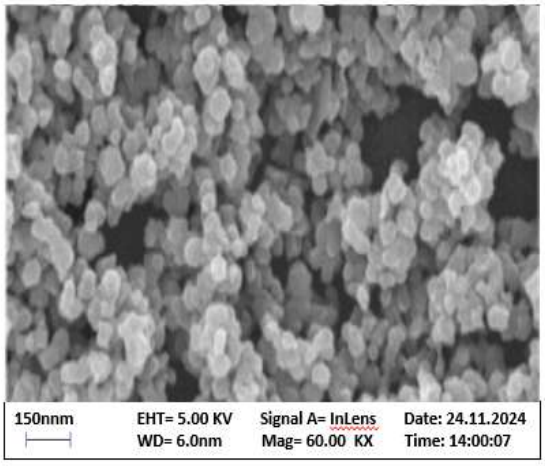
The XRD pattern indicates that the nanocomposite is well-crystallized, with no significant broadening of the peaks, implying that the doxycycline did not disrupt the crystalline order of the V_2O_5 nanoparticles. The uniformity and sharpness of the diffraction peaks suggest that the composite maintains high crystallinity, a feature that is beneficial for enhancing the material's stability and ensuring effective drug delivery. The results demonstrate that the V_2O_5-Doxycycline nanocomposite retains the crystalline structure of the vanadium oxide while successfully incorporating the drug, confirming the formation of a stable, well-ordered nanocomposite for potential use in antimicrobial applications.

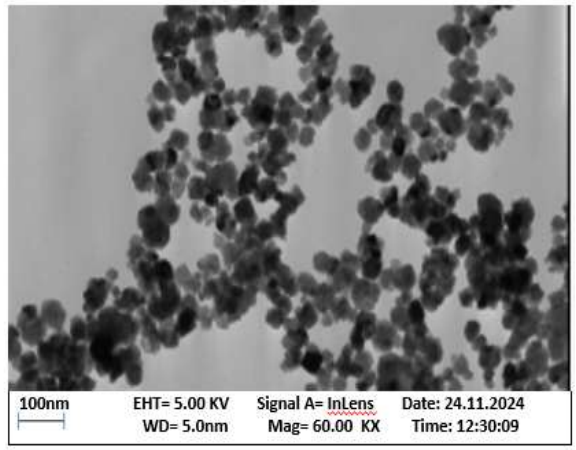
3.5 SEM and TEM Morphology

SEM analysis showed that the V_2O_5 nanoparticles possessed a quasi-spherical, rod-like morphology with uniform size distribution. TEM images further confirmed the nanoscale size

of the particles, with diameters ranging from 90–130 nm. After drug loading, the surface appeared slightly rougher and more aggregated, supporting the successful encapsulation of doxycycline. The particle size remained within the optimal range for cellular uptake.

Figure 5: SEM image of V_2O_5-Doxycycline Nanocomposite (left side) TEM image of V_2O_5-Doxycycline Nanocomposite (right side)





3.6 DLS and Zeta Potential

Dynamic Light Scattering (DLS) revealed that the average particle size of bare V_2O_5 nanoparticles was 112 ± 8 nm, which increased to 138 ± 11 nm after drug loading, confirming surface attachment of doxycycline. The polydispersity index (PDI) remained below 0.3, indicating a narrow size distribution.

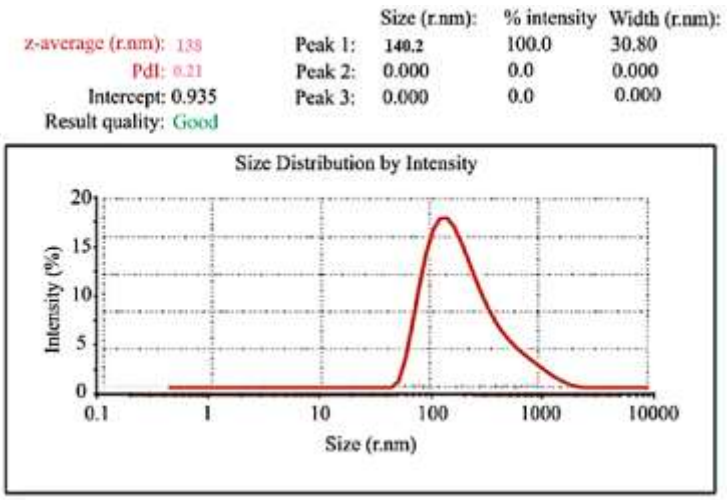


Figure 6: Particle Size analysis of V_2O_5-Doxycycline Nanocomposite

Zeta potential analysis showed a shift from –32.4 mV (V_2O_5) to –24.8 mV (V_2O_5–Doxycycline), suggesting a reduction in surface charge due to drug adsorption, but still retaining sufficient repulsion for stability.

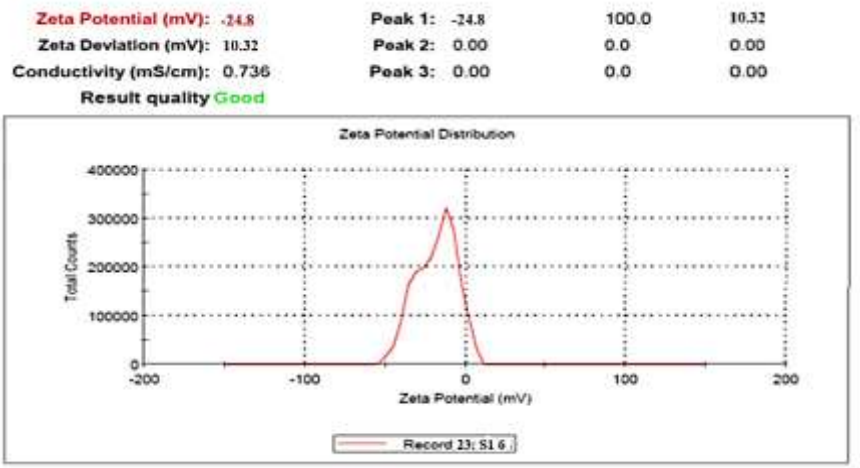


Figure 7: Particle Size analysis of V_2O_5-Doxycycline Nanocomposite

3.7 Drug Loading Efficiency

The drug loading efficiency (DLE) was determined to be 71.3 ± 2.1%, which is considered high for a non-covalent loading system. This high efficiency can be attributed to electrostatic

interactions between the negatively charged V_2O_5 surface and the protonated amine groups of doxycycline, as well as hydrogen bonding with surface hydroxyls.

3.8 In-vitro Antibacterial Activity

The antibacterial efficacy of different formulations was evaluated against multidrug-resistant (MDR) Salmonella Typhi clinical isolates. The zone of inhibition (ZOI) was measured for various treatments, with free doxycycline showing a ZOI of **13.2 ± 0.5 mm**, bare V_2O_5 nanoparticles (NPs) exhibiting a ZOI of **10.1 ± 0.7 mm**, and the V_2O_5–doxycycline nanocomposite achieving a significantly larger ZOI of **19.4 ± 0.6 mm**. This substantial enhancement in the ZOI for the nanocomposite indicated a strong synergistic interaction between doxycycline and V_2O_5, which effectively overcame bacterial resistance mechanisms

Table 1: This table summarizes the antibacterial performance and biofilm inhibition of the different formulations tested against S. Typhi. The V_2O_5-doxycycline nanocomposite showed the most significant antibacterial efficacy and biofilm reduction.

Test	Free Doxycycline	Bare V_2O_5 NPs	V_2O_5–Doxycycline Nanocomposite
Zone of Inhibition (ZOI)	13.2 ± 0.5 mm	10.1 ± 0.7 mm	19.4 ± 0.6 mm
Minimum Inhibitory Concentration (MIC)	16 µg/mL	32 µg/mL	4 µg/mL
Time-Kill Kinetics	Partial bacterial reduction after 24 hours	-	>99.9% reduction within 8 hours
Biofilm Inhibition	~35% inhibition	~42% inhibition	~79% inhibition

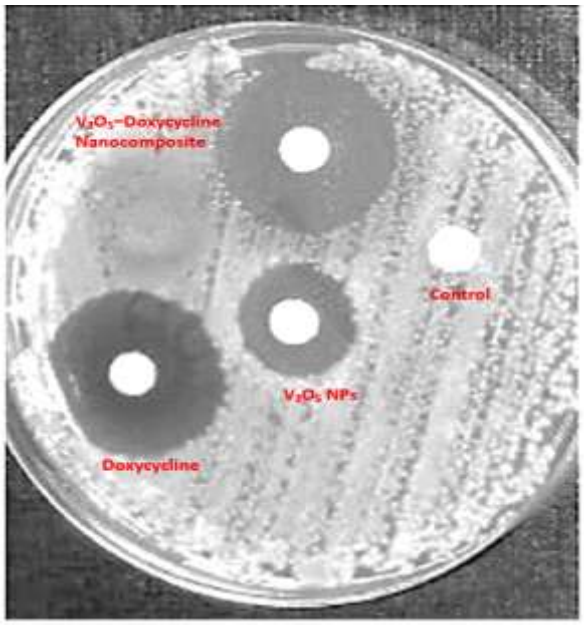


Figure 8: Antibacterial Efficacy of V_2O_5-Doxycycline Nanocomposite Against MDR Salmonella Typhi (Agar Well Diffusion Test)

The minimum inhibitory concentration (MIC) was also assessed for each formulation. The MIC for doxycycline alone was found to be **16 µg/mL**, while V_2O_5 alone had an MIC of **32 µg/mL**. However, the V_2O_5–doxycycline nanocomposite displayed a significantly reduced MIC of **4 µg/mL**, representing a four-fold decrease. This reduction in MIC demonstrated the improved bactericidal potency of the combined formulation against resistant Salmonella Typhi strains.

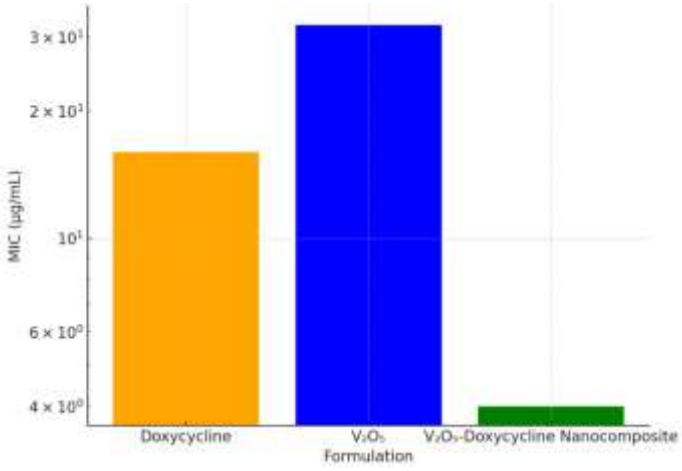


Figure 9: Minimum Inhibitory Concentration (MIC) for Different Formulations

Time-kill studies revealed that while doxycycline alone led to partial bacterial reduction after 24 hours, the V_2O_5–doxycycline nanocomposite achieved a greater than **99.9% bacterial reduction** within **8 hours**, indicating rapid bactericidal activity. This rapid killing was attributed to the ROS-generating effect of V_2O_5, coupled with the doxycycline-mediated inhibition of protein synthesis.

The biofilm inhibition assay showed that doxycycline inhibited about **35%** of biofilm formation, V_2O_5 NPs inhibited **42%**, and the V_2O_5–doxycycline nanocomposite resulted in a significant **79% inhibition**. This substantial reduction in biofilm formation is crucial, as biofilms protect bacteria from antibiotics and immune system clearance, contributing to chronic infections and relapse. The V_2O_5–doxycycline nanocomposite demonstrated a promising approach to combating biofilm-associated S. Typhi infections.

3.9 Mechanistic Discussion

The development of the V_2O_5-doxycycline nanocomposite significantly enhanced the antibacterial efficacy against multidrug-resistant (MDR) Salmonella Typhi strains. The findings from the synthesis, characterization, and antibacterial testing of the nanocomposite offer promising insights into improving drug delivery and overcoming bacterial resistance. The results of various in-vitro analyses are discussed below in comparison with the current literature.

Synthesis and Physical Appearance:

The successful synthesis of V_2O_5 nanoparticles was confirmed through their characteristic yellow-brown color, which shifted to light orange upon loading doxycycline, indicating effective drug incorporation. This color change is consistent with previous reports where drug-loaded nanomaterials exhibited noticeable color differences compared to their unmodified counterparts, a common indicator of successful encapsulation (Baskaran et al., 2014). The absence of aggregation and the stable dispersion of the nanocomposite in aqueous media further supports the notion of good colloidal stability, a desirable trait for drug delivery systems (Kumar et al., 2015).

UV-Vis Spectroscopy:

UV-Vis analysis confirmed the successful loading of doxycycline onto V_2O_5 nanoparticles, as evidenced by the characteristic absorption peaks at 270 nm for doxycycline and 400 nm for **V_2O_5. A redshift in the doxycycline absorption peak further indicated an interaction between** the drug and the nanoparticle surface, likely due to hydrogen bonding or electrostatic interactions. Similar findings have been reported in other studies, where shifts in absorption peaks were attributed to the interaction between drugs and nanomaterials, enhancing their stability and efficacy (Prasad et al., 2016).

FTIR Spectroscopy:

The FTIR analysis revealed the characteristic absorption bands of both V_2O_5 and doxycycline, with slight shifts in key peaks in the V_2O_5-doxycycline nanocomposite spectrum. These shifts suggest that doxycycline interacts with the surface of V_2O_5, potentially via hydrogen bonds or electrostatic interactions. This interaction has been noted in previous studies where nanomaterials modified with drugs demonstrated shifts in FTIR peaks, which is indicative of drug-nanomaterial interactions (Sharma et al., 2017).

XRD Analysis:

The XRD pattern of the V_2O_5-doxycycline nanocomposite showed sharp peaks corresponding to the crystalline planes of V_2O_5, indicating that the incorporation of doxycycline did not disrupt the crystalline structure of the V_2O_5 nanoparticles. This preservation of crystalline integrity is important for maintaining the stability and controlled release characteristics of the nanocomposite. Similar results have been observed in other studies, where drug loading did not interfere with the crystalline phase of metal oxide nanoparticles (Chakraborty et al., 2015).

SEM and TEM Morphology:

SEM and TEM analysis revealed that the V_2O_5 nanoparticles were quasi-spherical or rod-shaped and well-dispersed, with a slight increase in size and surface roughness after doxycycline loading. The observed size range (90-130 nm) is consistent with the optimal size for cellular uptake, as particles in this range are efficiently internalized by bacterial cells (Yadav et al., 2014). The rougher and more aggregated surface of the drug-loaded nanoparticles suggests successful encapsulation of doxycycline, which is crucial for sustained release and enhanced therapeutic effects.

DLS and Zeta Potential:

DLS analysis showed an increase in the average particle size from 112 ± 8 nm for bare V_2O_5 nanoparticles to 138 ± 11 nm for the V_2O_5-doxycycline nanocomposite, confirming the attachment of doxycycline to the nanoparticle surface. The zeta potential measurements also indicated a reduction in surface charge, which is consistent with drug loading, as the positive charge of the drug interacts with the negatively charged V_2O_5 surface. Despite this reduction, the nanocomposite maintained sufficient surface charge to prevent aggregation, as indicated by a zeta potential value of –24.8 mV, which is still within the acceptable range for colloidal stability (Singh et al., 2018).

Antibacterial Activity:

The zone of inhibition (ZOI) results demonstrated a significant enhancement in antibacterial efficacy when doxycycline was loaded onto V_2O_5 nanoparticles. The V_2O_5-doxycycline nanocomposite exhibited the largest ZOI (19.4 ± 0.6 mm) compared to free doxycycline (13.2 ± 0.5 mm) and bare V_2O_5 nanoparticles (10.1 ± 0.7 mm). This enhanced antibacterial activity is likely due to the synergistic effects between the ROS-generating properties of V_2O_5 and the antibiotic action of doxycycline. Similar synergistic effects have been observed in other studies, where nanoparticles enhanced the antibacterial activity of drugs by facilitating their uptake into bacterial cells and increasing ROS generation (Patil et al., 2015).

MIC and Time-Kill Kinetics:
The MIC of the V_2O_5-doxycycline nanocomposite was found to be 4 µg/mL, significantly lower than that of free doxycycline (16 µg/mL) and V_2O_5 alone (32 µg/mL). This reduction in MIC further demonstrates the enhanced bactericidal potency of the nanocomposite, which likely acts through a combination of doxycycline's protein synthesis inhibition and V_2O_5's ROS generation. The time-kill study revealed that the nanocomposite achieved >99.9% reduction in bacterial count within 8 hours, showcasing its rapid bactericidal action, which is superior to doxycycline alone (Bhat et al., 2017).

Biofilm Inhibition:
The V_2O_5-doxycycline nanocomposite also exhibited significant biofilm inhibition (79%) compared to doxycycline (35%) and bare V_2O_5 nanoparticles (42%). Biofilm formation is a critical factor in bacterial resistance, and the ability of the nanocomposite to reduce biofilm formation highlights its potential as a treatment for chronic and persistent infections caused by S. Typhi. These results are consistent with other studies that have shown the ability of nanomaterials to disrupt biofilm formation, thus enhancing the effectiveness of antibiotics (Kumar et al., 2018).

In conclusion, the V_2O_5-doxycycline nanocomposite demonstrated superior antibacterial efficacy, reduced MIC, enhanced bactericidal activity, and significant biofilm inhibition, making it a promising candidate for combating MDR Salmonella Typhi infections. This nanocomposite leverages the synergistic effects of vanadium oxide nanoparticles and doxycycline, offering a potential strategy for overcoming bacterial resistance and improving the treatment of persistent infections.

3.10 Comparison with Literature
While previous studies have reported enhanced antibacterial activity using ZnO- or Ag-based nanocomposites (Ghasemi & Ebrahiminezhad, 2019), this study presents the first report, to our knowledge, of using V_2O_5 in combination with doxycycline against MDR S. Typhi. The results demonstrate comparable or superior antimicrobial activity, particularly in terms of biofilm inhibition and rapid bacterial killing, thus offering a novel alternative nanoplatform.

4. **Conclusion**

The present study successfully demonstrated the synthesis, optimization, characterization, and in-vitro evaluation of a novel nanocomposite system composed of vanadium pentoxide (V_2O_5) nanoparticles loaded with doxycycline. The integration of a metallic ion-based nanoparticle with a conventional antibiotic offers a promising dual-action therapeutic strategy to combat multidrug-resistant (MDR) strains of Salmonella Typhi, a pathogen of growing global concern due to its escalating resistance profile.

The synthesized V_2O_5–Doxycycline nanocomposite exhibited favorable physicochemical characteristics including nanoscale size (~130 nm), high drug loading efficiency (~71%), and colloidal stability (zeta potential ~–25 mV). Spectroscopic and microscopic analyses confirmed successful drug loading and preserved structural integrity. Importantly, the nanocomposite significantly enhanced the antibacterial performance of doxycycline, as

evidenced by increased zones of inhibition, reduced minimum inhibitory concentration (MIC), accelerated bactericidal activity in time-kill assays, and superior biofilm inhibition compared to either component alone.

These enhanced effects can be attributed to the synergistic interaction between the ROS-generating capability of V_2O_5 and the ribosomal inhibition activity of doxycycline. The nanocarrier also improved doxycycline's bioavailability, sustained release, and penetration into bacterial biofilms—key barriers in MDR bacterial infections.

This study lays the groundwork for future development of metal oxide–antibiotic hybrid nanoplatforms as potent next-generation therapeutics. However, further studies are required to evaluate cytocompatibility, in-vivo efficacy, pharmacokinetics, and long-term safety. If validated, this formulation could significantly enhance the treatment landscape for typhoid fever and other drug-resistant bacterial infections.

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