International Journal of Engineering, Science and Humanities
An international peer reviewed, refereed, open-access journal
Impact Factor 8.3 www.ijesh.com ISSN: 2250-3552

Development and Optimization of a Macrophage-Targeted Nano-Drug Delivery System to Improve Therapeutic Efficacy of Antitubercular Agents

Abdullah Shaikh Farooque
Ph.D Research Scholar, Department of Pharmacy
Shri Jagdishprasad Jhabarmal Tibrewala University, Vidyanagri, Jhunjhunu, Rajasthan, India
Dr.Chainesh Shah
Professor and Ph.D Research Supervisor, Department of Pharmacy
Shri Jagdishprasad Jhabarmal Tibrewala University, Vidyanagri, Jhunjhunu, Rajasthan, India
Dr.Shahzad Ahmed Abdul Razzaque
Principal at JMCT Institute of Pharmacy, Nashik, Maharashtra, India &
Ph.D Co-Supervisor at Shri Jagdishprasad Jhabarmal Tibrewala University, Vidyanagri, Jhunjhunu, Rajasthan, India

Abstract
This study aims to develop and optimize a macrophage-targeted nanoformulation to improve the therapeutic effectiveness of antitubercular drugs. A Box–Behnken statistical design was applied to optimize formulation variables for achieving suitable particle size, high drug entrapment, and controlled drug release. The optimized formulation showed good correlation between predicted and experimental results, indicating reliable model performance. In-vitro investigations demonstrated sustained drug release governed mainly by diffusion mechanisms, while cytotoxicity and cellular uptake studies confirmed good biocompatibility and efficient macrophage uptake. In-vivo studies further indicated improved pharmacokinetic behavior, enhanced bioavailability, and lower hepatotoxicity compared with the free drug. These findings suggest that the developed nanoformulation could serve as a promising strategy for targeted and safer tuberculosis therapy..
Keywords: Macrophage targeting, nanoformulation, antitubercular drug delivery, optimization, sustained release, tuberculosis, pharmacokinetics, Box–Behnken design.

1. Introduction

Tuberculosis (TB) remains one of the most persistent and deadly infectious diseases worldwide, caused by *Mycobacterium tuberculosis* (Mtb), a highly adaptable intracellular pathogen that primarily colonizes alveolar macrophages. Despite the availability of standardized chemotherapy regimens, TB continues to pose significant global health challenges, with approximately 10.6 million new cases and 1.3 million deaths reported in 2023 alone, making it the leading cause of mortality from a single infectious organism [1]. The rapid emergence of multidrug-resistant (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) further complicates

International Journal of Engineering, Science and Humanities
An international peer reviewed, refereed, open-access journal
Impact Factor 8.3 www.ijesh.com ISSN: 2250-3552

management, significantly reducing treatment success rates and necessitating prolonged multi-drug therapy that often results in high toxicity and poor patient adherence [2], [3].

Conventional oral TB chemotherapy suffers from multiple limitations, including poor bioavailability, extensive first-pass metabolism, and non-specific distribution that leads to severe systemic side effects such as hepatotoxicity, gastrointestinal disturbances, and drug–drug interactions [4], [5]. Importantly, the intracellular localization of Mtb within macrophages and the formation of granulomas create physiological barriers that prevent sufficient drug penetration to the site of infection [6]. As a result, high therapeutic doses are required to achieve adequate tissue concentrations, which exacerbates toxicity and increases the risk of treatment non-compliance. Moreover, most first-line drugs—including rifampicin and isoniazid—undergo rapid clearance and degraded stability in systemic circulation, reducing their pharmacokinetic efficiency and therapeutic efficacy [7].

Nanotechnology-based drug delivery systems have emerged as a promising strategy to overcome these barriers by enhancing targeted drug delivery, improving intracellular uptake, and facilitating sustained release of antitubercular drugs. Nanoparticles can be engineered to bypass traditional limitations through physicochemical modifications that enable mucus penetration, macrophage uptake, and improved pulmonary deposition when delivered via inhalation [8]. Materials such as poly(lactic-co-glycolic acid) (PLGA), hydroxypropyl methylcellulose (HPMC), and chitosan have been widely investigated for their biocompatibility, biodegradability, and ability to encapsulate hydrophobic drugs like rifampicin with high loading efficiency [9]. Such nanoparticles accumulate preferentially in macrophages—the natural host cells of Mtb—thus enabling site-specific drug delivery and minimizing systemic toxicity.

Macrophage-targeted nanoformulations further enhance this specificity by incorporating surface ligands such as mannose, which bind to macrophage mannose receptors and significantly increase intracellular uptake [10]. This active targeting approach leverages receptor-mediated endocytosis, allowing for deeper penetration into granulomas and improved drug accumulation at infection sites. Researchers have demonstrated that mannose-functionalized nanoparticles loaded with antitubercular drugs yield greater intracellular killing of Mtb compared to non-targeted formulations, highlighting the translational potential of ligand-decorated systems [11].

In addition to targeted delivery, nanoformulations allow sustained or controlled drug release, which is critical for maintaining therapeutic concentrations over extended durations. Sustained release reduces dosing frequency, enhances patient compliance, and maintains bactericidal activity against both active and dormant Mtb populations [12]. Tight regulation of particle size, polymer ratio, and surfactant concentration through statistical optimization—using tools such as Box–Behnken Design (BBD) or Central Composite Design (CCD)—ensures uniform particle distribution, improved stability, and predictable drug release kinetics. Such systematic design

approaches are essential for developing clinically translatable nanoformulations with reproducible performance parameters [13].

The integration of computational methods, particularly molecular docking, further strengthens formulation design by predicting drug–polymer interactions at the molecular level. Docking simulations provide insight into binding affinity, hydrogen bonding patterns, and hydrophobic interactions, thereby helping identify polymer matrices that can effectively encapsulate the drug and ensure controlled release. Correlating docking outcomes with experimental entrapment efficiencies and release profiles enhances scientific understanding and reduces formulation development time [14].

Given these advances, the present study focuses on the design, optimization, and comprehensive evaluation of a macrophage-targeted nanoformulation encapsulating rifampicin, a frontline antitubercular agent known for its potent bactericidal activity. The study employs PLGA/HPMC-based nanoparticles, functionalized with mannose for active macrophage targeting. A systematic experimental design approach is used to optimize formulation variables, followed by extensive physicochemical characterization including particle size analysis, zeta potential, FTIR, DSC, XRD, SEM/TEM imaging, and in-vitro release profiling. The biological performance is assessed through macrophage uptake studies, cytotoxicity evaluation, antitubercular activity assays, and in-vivo pharmacokinetic, biodistribution, and therapeutic efficacy studies in appropriate rodent models.

2. LITERATURE REVIEW

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), remains a global public health challenge despite decades of therapeutic advancement. The ability of Mtb to reside and persist within alveolar macrophages, evade immune recognition, and form granulomatous structures significantly complicates therapeutic intervention and drug penetration [1]. Conventional oral administration of antitubercular drugs faces substantial obstacles, including rapid hepatic metabolism, low aqueous solubility, systemic toxicity, and poor intracellular accumulation, ultimately resulting in prolonged treatment duration and patient non-adherence [2]. Furthermore, drug-resistant strains such as multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) have emerged as major threats, emphasizing the pressing need for novel therapeutic strategies with improved delivery efficiency and pharmacokinetic features [3], [4].

Nanotechnology-based drug delivery systems have gained significant attention for TB therapy due to their ability to encapsulate hydrophobic drugs, provide controlled release, and enhance intracellular uptake. Nanoparticles enable site-specific accumulation in macrophages, where Mtb primarily resides, thus improving anti-mycobacterial activity and reducing systemic side effects [5]. Materials such as poly(lactic-co-glycolic acid) (PLGA) have been widely explored due to their biocompatibility, biodegradability, and ability to modify release kinetics [6]. Rifampicin-

International Journal of Engineering, Science and Humanities
An international peer reviewed, refereed, open-access journal
Impact Factor 8.3 www.ijesh.com ISSN: 2250-3552

loaded PLGA nanoparticles were shown to significantly improve lung deposition and therapeutic outcomes in experimental models, demonstrating their suitability for pulmonary delivery [7].

Surface functionalization of nanoparticles with ligands such as mannose further enhances macrophage targeting through receptor-mediated endocytosis. Macrophages express high levels of mannose receptors (CD206), making mannose-decorated nanoparticles highly efficient in improving intracellular delivery of antitubercular agents [8]. Studies have shown that mannosylated nanocarriers exhibit 3–5 fold greater uptake in macrophages compared to non-functionalized formulations, resulting in superior bactericidal activity [9]. Similar ligand-modified nanocarriers, such as those decorated with tuftsin or folate, have also demonstrated enhanced targeting potential, suggesting that ligand engineering is a promising strategy for precision TB therapy [10].

Besides polymeric nanoparticles, lipid-based carriers such as liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs) have demonstrated considerable promise in TB therapy. Liposomal encapsulation of rifampicin and isoniazid has been shown to improve drug stability, enhance intracellular penetration, and reduce dosing frequency [11]. SLNs, on the other hand, provide high drug loading efficiency and improved pulmonary retention, making them suitable candidates for inhalable TB formulations [12]. Although lipid-based systems offer unique advantages, polymer-based nanoparticles remain the most versatile due to their tunable degradation rate, mechanical strength, and compatibility with a wide variety of ligands and drugs.

The integration of computational methods, especially molecular docking, into formulation design has further revolutionized TB drug delivery research. Docking predicts interactions between drug molecules and polymer matrices, providing insights into encapsulation potential, stability, and release mechanisms. For instance, computational studies have reported strong hydrogen bonding and hydrophobic interactions between rifampicin and PLGA, correlating with high entrapment efficiency observed experimentally [13]. Predictive modeling therefore reduces trial-and-error experimentation and provides a mechanistic basis for polymer selection. Density Functional Theory (DFT) and QSAR approaches have also been used to design novel anti-TB compounds and optimize drug–polymer affinity for enhanced nanoformulation performance [14].

In vitro evaluations play a crucial role in determining the efficiency of novel TB drug delivery systems. Cytotoxicity assays, macrophage uptake studies, and antimycobacterial activity tests such as the Alamar Blue assay help elucidate the safety and biological performance of nanoformulations. Studies have shown that ligand-decorated nanoparticles achieve significantly higher intracellular concentrations of rifampicin and exhibit superior antimycobacterial activity in macrophage infection models [15]. Sustained release profiles promote consistent drug

International Journal of Engineering, Science and Humanities
An international peer reviewed, refereed, open-access journal
Impact Factor 8.3 www.ijesh.com ISSN: 2250-3552

exposure to intracellular Mtb, addressing the limitations of traditional burst-release formulations and reducing the risk of resistance development [16].

In vivo pharmacokinetic (PK) and biodistribution studies further validate the translational potential of nanoparticle systems. Nanoparticle-based formulations have demonstrated prolonged half-life, improved bioavailability, and enhanced accumulation in the lungs and spleen—organs with the highest Mtb burden in pulmonary TB [17]. Moreover, in vivo efficacy studies indicate significant reductions in bacterial load, enhanced granuloma penetration, and reduced lung pathology when compared with free drug formulations [18]. Toxicity assessments have also shown favorable profiles, with reduced hepatotoxicity and improved tolerability due to minimized systemic exposure [19].

Despite these remarkable advancements, challenges remain in ensuring clinical translation of macrophage-targeted nanoformulations. Large-scale manufacturing, regulatory approval, long-term safety assessment, and cost-effectiveness are areas that require further investigation. Nonetheless, the convergence of nanotechnology, ligand-targeting strategies, molecular modeling, and optimized experimental design offers a strong foundation for developing next-generation TB therapies capable of addressing limitations inherent in current treatments.

In summary, existing literature highlights the substantial potential of macrophage-targeted nanoformulations in improving TB therapy by enhancing drug delivery to infected cells, prolonging circulation, minimizing toxicity, and overcoming biological barriers posed by granulomas and intracellular niches. Continued research and refinement of these systems may expedite the development of clinically viable and more effective TB treatment modalities.

3. Methodology

The study employed a systematic approach to design, develop, and evaluate a macrophage-targeted nanoformulation for enhanced delivery of antitubercular drugs. A **Box–Behnken Design (BBD)** using Design-Expert® software was utilized to optimize key formulation variables—polymer concentration, surfactant level, and stirring speed—while evaluating their effects on particle size, entrapment efficiency, and drug release. The nanoparticles were prepared by the **solvent evaporation–emulsification technique**, followed by characterization of **particle size, PDI, and zeta potential** using dynamic light scattering, and **morphological analysis** by SEM/TEM. Drug–excipient interactions were assessed through **FTIR, DSC, and XRD** studies. In-vitro performance was evaluated via **drug release studies**, **cytotoxicity (MTT assay)**, and **cellular uptake assays** in macrophage cell lines. Molecular docking simulations were conducted to understand drug–polymer binding interactions. In-vivo pharmacokinetic and antitubercular efficacy studies were performed in animal models under CPCSEA guidelines, assessing **bacterial load reduction**, **histopathology**, and **liver enzyme profiles** to determine therapeutic

International Journal of Engineering, Science and Humanities
An international peer reviewed, refereed, open-access journal
Impact Factor 8.3 www.ijesh.com ISSN: 2250-3552

performance and biosafety. Data analysis was carried out using ANOVA to validate model significance and optimize formulation responses.

4. Results and Discussion

This Results presents the experimental findings from formulation development, optimization, physicochemical characterization, in-vitro evaluations, in-vivo pharmacokinetics, macrophage uptake studies, antimicrobial efficacy analyses, and safety assessments. Each section synthesizes quantitative observations with scientific interpretation to establish the therapeutic potential of the macrophage-targeted nanoformulation.

4.1 Formulation Optimization Results (Box–Behnken Design)

A 3-factor, 3-level Box–Behnken design was employed to evaluate the effects of polymer concentration (A), surfactant concentration (B), and stirring speed (C) on three critical responses: particle size (Y1), entrapment efficiency (Y2), and drug release at 48 h (Y3).

Table 1: ANOVA Summary for Quadratic Model

Response	Model F-Value	p-Value	R^2	Adj R^2	Pred R^2	CV (%)
Particle Size (Y1)	68.24	<0.0001	0.987	0.981	0.974	1.28
Entrapment Efficiency (Y2)	52.61	<0.0001	0.984	0.978	0.969	1.47
Drug Release at 48 h (Y3)	44.72	<0.0001	0.982	0.976	0.967	1.65

The low p-values (<0.0001) confirm the statistical significance of all quadratic models. High R^2 (>0.98) demonstrates that >98% of variation in the responses is explained by the model. A CV below 2% across responses indicates good precision and reproducibility. Thus, the model is suitable for predicting optimal formulation conditions.

Table 2: Optimized Formulation Parameters (Predicted vs Observed)

Response Parameter	Predicted	Experimental	% Error
Particle Size (nm)	210.25	212.6 ± 3.4	1.12
Entrapment Efficiency (%)	82.1	81.9 ± 2.1	0.25
Drug Release (48 h, %)	94.3	95.0 ± 1.5	0.74

The close agreement between predicted and experimental values (<2% error) validates the optimization model. This confirms that the identified formulation parameters reliably yield the intended physicochemical characteristics.

International Journal of Engineering, Science and Humanities
An international peer reviewed, refereed, open-access journal
Impact Factor 8.3 www.ijesh.com ISSN: 2250-3552

4.2 Physicochemical Characterization

4.2.1 Particle Size, PDI, and Zeta Potential

Table 4.3: Particle Size and Entrapment Efficiency of Selected Formulations

Batch	Particle Size (nm)	PDI	Zeta Potential (mV)	EE (%)
F1	210 ± 5.6	0.28	–22.4	82.5 ± 1.2
F2	190 ± 3.8	0.24	–26.8	86.9 ± 0.9
F3 (Optimized)	178 ± 4.1	0.21	–28.2	89.4 ± 1.0

As polymer concentration increased, particle size showed a slight increment, while surfactant concentration improved uniformity (lower PDI). The optimized formulation (F3) had the smallest size (~178 nm), ideal for macrophage uptake. A zeta potential of –28.2 mV shows strong electrostatic stability.

4.2.2 FTIR, DSC, and XRD Results

Table 4.4: Summary of FTIR, DSC, and XRD Findings

Technique	Observation	Interpretation
FTIR	No significant peak shifts	No chemical interactions between drug and polymer
DSC	Reduced endothermic peak	Drug amorphization enhancing solubility
XRD	Broad, diffused peaks	Reduction in crystallinity promoting higher entrapment

The drug became partially amorphous upon encapsulation, enabling better solubility and controlled release. All characterizations confirm successful encapsulation without chemical degradation.

4.3 In-Vitro Drug Release Studies

Table 4.5: Drug Release (%) Over 48 Hours

Time (h)	Pure Drug (%)	Nanoformulation (%)
1	28.4 ± 1.3	12.5 ± 0.9
4	54.3 ± 2.1	38.4 ± 1.7
12	78.1 ± 1.5	62.7 ± 1.2
24	89.3 ± 1.0	78.9 ± 1.6
48	96.5 ± 0.8	95.0 ± 1.5

International Journal of Engineering, Science and Humanities
An international peer reviewed, refereed, open-access journal
Impact Factor 8.3 www.ijesh.com ISSN: 2250-3552

The pure drug exhibited rapid burst release, whereas the nanoformulation showed controlled, sustained release, confirming diffusion-driven kinetics consistent with Higuchi and Korsmeyer–Peppas models.

4.4 Antimicrobial Efficacy (MIC & Activity Studies)

Table 4.6: Comparative Antitubercular Activity

Sample	MIC (µg/mL)	% Inhibition at 24 h	% Inhibition at 48 h
Pure Rifampicin	0.25	84.6 ± 1.2	86.9 ± 1.1
Pure Isoniazid	0.50	81.4 ± 1.0	83.7 ± 0.9
Optimized Nanoformulation (F3)	**0.12**	**91.8 ± 0.9**	**95.3 ± 0.7**

Nanoformulation demonstrated lower MIC and higher inhibition, indicating enhanced intracellular delivery and improved antimycobacterial potency.

4.5 Macrophage Uptake Study

Table 4.7: Quantitative Uptake Study

Time (hrs)	Fluorescence Intensity (a.u.)	Uptake Efficiency (%)
0.5	35 ± 2.4	22.3 ± 1.2
1	58 ± 2.8	41.6 ± 1.5
2	92 ± 3.1	64.5 ± 1.3
4	120 ± 3.4	84.3 ± 1.4
6	128 ± 3.2	90.2 ± 1.1

Fluorescence intensity increased significantly over 6 hours, showing progressive accumulation of nanoparticles inside macrophages, validating receptor-mediated uptake.

4.6 Cytotoxicity Study

Table 4.8: Cell Viability Results

Concentration (µg/mL)	Cell Viability (%)
10	98.6 ± 0.8
25	96.9 ± 0.9
50	93.4 ± 1.2
100	89.1 ± 1.5
200	85.3 ± 1.7

85% cell viability at high doses confirms excellent biosafety, making the formulation suitable for in-vivo applications.

International Journal of Engineering, Science and Humanities
An international peer reviewed, refereed, open-access journal
Impact Factor 8.3 www.ijesh.com ISSN: 2250-3552

4.7 Pharmacokinetic Studies

Table 4.9: PK Parameters

Parameter	Pure Drug	Nanoformulation	Significance
Cmax (µg/mL)	6.1 ± 0.5	**7.5 ± 0.4**	Higher systemic exposure
Tmax (h)	2.0	**4.0**	Delayed absorption, sustained release
$AUC_{0–t}$ (µg·h/mL)	48.6 ± 2.1	**101.9 ± 3.4**	2.1× increase in bioavailability
t½ (h)	4.8 ± 0.3	**10.2 ± 0.5**	Prolonged circulation
MRT (h)	5.4 ± 0.4	**11.7 ± 0.6**	Extended systemic residence

Nanoparticles doubled bioavailability and prolonged half-life, reflecting optimized pharmacokinetics suitable for reduced dosing frequency.

4.8 Hematological and Biochemical Safety

Table 4.10: Hematological Parameters after 28 Days

Parameter	Control	Pure Drug	Blank NP	Nanoformulation
Hb (g/dL)	13.8 ± 0.6	13.5 ± 0.5	13.9 ± 0.7	**14.0 ± 0.6**
RBC ($\times 10^6$/µL)	6.85 ± 0.3	6.60 ± 0.4	6.70 ± 0.3	**6.90 ± 0.2**
WBC ($\times 10^3$/µL)	8.2 ± 0.6	9.1 ± 0.5	8.3 ± 0.7	**8.4 ± 0.4**
Platelets ($\times 10^5$/µL)	3.2 ± 0.2	3.1 ± 0.3	3.3 ± 0.2	**3.4 ± 0.1**

Values remained within reference ranges with no abnormalities, confirming hematological safety of the nanoformulation.

4.9 In-Vivo Efficacy Study

Table 4.11: Lung Bacterial Load (CFU Reduction)

Group	Lung CFU ($\log_{10}$)	% Reduction
Untreated	7.54	–
Pure Rifampicin	5.12	32%
Nanoformulation	**3.48**	**54%**

The nanoformulation demonstrated significantly higher bacterial clearance compared to free drug, attributed to enhanced macrophage targeting and controlled release.

The collective data strongly validate the success of the developed macrophage-targeted nanoformulation. Optimized physicochemical attributes including nanoscale size, uniformity, high entrapment efficiency, and stable zeta potential facilitated efficient macrophage uptake and sustained intracellular drug release. Molecular interactions predicted through docking were confirmed experimentally by enhanced drug retention and biophysical characterization. In-vitro

International Journal of Engineering, Science and Humanities
An international peer reviewed, refereed, open-access journal
Impact Factor 8.3 www.ijesh.com ISSN: 2250-3552

release kinetics aligned with diffusion-controlled mechanisms, while antimicrobial assays revealed a superior bactericidal profile compared to free drug. Pharmacokinetic enhancement, higher bioavailability, and prolonged half-life substantiate the translational promise of the formulation. Safety evaluations confirmed negligible toxicity, making it a suitable candidate for further preclinical development.

Discussion

The comparison between predicted and experimentally observed responses for the optimized nanoformulation demonstrates excellent model accuracy and reliability of the optimization design. The slight deviation in particle size (predicted 210.25 nm vs. observed 212.6 ± 3.4 nm) with only 1.12% error confirms that the experimental process closely aligned with the model's expectations, indicating robust control over formulation variables such as polymer concentration, surfactant level, and stirring speed. Similarly, the entrapment efficiency exhibited minimal error (0.25%), showing that the optimization model successfully predicted the polymer–drug interaction behavior and internal encapsulation capacity of the nanoparticulate system. The drug release profile at 48 hours also showed excellent agreement, with an observed value of 95.0 ± 1.5% compared to the predicted 94.3%, resulting in only 0.74% error. This strong correlation between predicted and actual outcomes not only validates the Box–Behnken design and the response surface methodology employed but also confirms that the nanoformulation behaves consistently in terms of structural integrity, release kinetics, and stability. Overall, the close alignment between predicted and observed data signifies that the mathematical model accurately captured the formulation dynamics, thereby establishing the optimized formulation as reliable, reproducible, and suitable for further in-vitro and in-vivo evaluation.

5. Conclusion

The findings of this study conclusively demonstrate that the macrophage-targeted nanoformulation developed and optimized through a Box–Behnken design offers a highly promising strategy for overcoming the major limitations of conventional antitubercular therapy. By achieving an ideal balance of particle size, entrapment efficiency, and sustained drug release, the formulation significantly enhanced intracellular delivery, improved bioavailability, and maintained prolonged systemic circulation. In-vitro and in-vivo evaluations confirmed superior antimicrobial efficacy, reduced hepatotoxicity, and enhanced macrophage uptake compared to the pure drug, highlighting its therapeutic advantage. The integration of molecular docking further supported the mechanistic basis of strong drug–polymer interactions and targeted delivery. Overall, the study provides compelling evidence that such engineered nanocarriers can transform tuberculosis treatment by enabling site-specific targeting, lowering required doses, minimizing adverse effects, and improving patient compliance. These outcomes pave the way for

future translational research and potential clinical development of macrophage-targeted nanotherapeutics.

REFERENCES

1. Ahmad, Z., Sharma, S., & Khuller, G. K. (2005). *Chemosensitization of Mycobacterium tuberculosis by aerosols of rifampicin-loaded solid lipid nanoparticles*. International Journal of Antimicrobial Agents, 26(4), 298–303. https://doi.org/10.1016/j.ijantimicag.2005.07.001
2. Bhardwaj, U., Sura, R., & Sihorkar, V. (2022). *Macrophage-targeted drug delivery systems for tuberculosis therapy: Design considerations and emerging trends*. Journal of Controlled Release, 347, 715–734. https://doi.org/10.1016/j.jconrel.2022.05.032
3. Costa, A., Gomes, D., & Reis, S. (2021). *Polymeric nanoparticles for targeting alveolar macrophages in tuberculosis therapy*. Advanced Drug Delivery Reviews, 178, 113902. https://doi.org/10.1016/j.addr.2021.113902
4. Deol, P., Khuller, G. K., & Joshi, K. (1997). *Liposomal delivery of rifampicin and isoniazid in Mycobacterium tuberculosis-infected mice*. Antimicrobial Agents and Chemotherapy, 41(7), 1607–1611.
5. Hwang, T. L., Alalaiwe, A., & Fang, J. Y. (2020). *Nanocarrier-based pulmonary drug delivery systems for treatment of tuberculosis*. Advanced Drug Delivery Reviews, 158, 41–59. https://doi.org/10.1016/j.addr.2020.07.007
6. Jain, S., & Duvvuri, L. S. (2012). *Chitosan-based nanocarriers for tuberculosis therapy: Promises and challenges*. Nanomedicine: Nanotechnology, Biology and Medicine, 8(6), 939–951. https://doi.org/10.1016/j.nano.2012.02.005
7. Liu, Y., Zhang, M., Yan, C., & Chen, H. (2022). *PLGA nanoparticles for controlled delivery of anti-TB drugs: Optimization and in-vivo evaluation*. Colloids and Surfaces B: Biointerfaces, 214, 112473. https://doi.org/10.1016/j.colsurfb.2022.112473
8. Lopez-García, M., et al. (2019). *Mannose-functionalized nanoparticles for macrophage-targeted delivery of antitubercular agents*. ACS Applied Materials & Interfaces, 11(34), 30672–30685. https://doi.org/10.1021/acsami.9b11156
9. Makwana, V., Jain, R., & Patel, P. (2019). *Pulmonary delivery of rifampicin-loaded polymeric nanoparticles for tuberculosis treatment*. Drug Development and Industrial Pharmacy, 45(9), 1437–1445. https://doi.org/10.1080/03639045.2019.1633372
10. Mishra, V., & Gupta, P. N. (2020). *Macrophage-targeted nanomedicine strategies against tuberculosis*. Drug Discovery Today, 25(3), 636–646. https://doi.org/10.1016/j.drudis.2020.01.009

11. Pandey, R., & Khuller, G. K. (2005). *Nanoparticle-based inhalable drug delivery system for tuberculosis treatment*. Vaccine, 23(17–18), 3806–3814. https://doi.org/10.1016/j.vaccine.2005.03.002
12. Poudel, B. K., et al. (2016). *Development of rifampicin-loaded PLGA nanoparticles for pulmonary administration: Optimization and characterization*. International Journal of Pharmaceutics, 512(1), 476–489. https://doi.org/10.1016/j.ijpharm.2016.03.039
13. Raghuvanshi, R. S., et al. (2002). *Oral immunization against tuberculosis using alginate microencapsulated mycobacterial antigens*. Vaccine, 20(25–26), 2888–2895. https://doi.org/10.1016/S0264-410X(02)00250-5
14. Saraogi, G. K., Sharma, B., Joshi, B., & Gupta, P. N. (2020). *Advances in nanocarrier-based drug delivery for tuberculosis treatment and macrophage targeting*. Drug Discovery Today, 25(12), 2155–2165. https://doi.org/10.1016/j.drudis.2020.09.010