



Original Research Article

Formulation and Characterization of Rifampicin-Loaded Nanoparticles for Enhanced Tuberculosis Therapy**Kojo Asante¹, Anna Johansson², Noah Brown³, Sunday Olajide Awofisayo⁴**¹. Department of Drug Development, Faculty of Pharmacy, Kwame Nkrumah University of Science and Technology, Ghana². Department of Clinical Pharmacology, Karolinska Institutet, Sweden³. Department of Pharmaceutical Sciences, University of Sydney, Australia⁴. Department of Clinical Pharmacy and Biopharmacy, Faculty of Pharmacy, University of Uyo, Nigeria**ABSTRACT**

Tuberculosis (TB) remains a major global public health challenge, complicated by prolonged treatment regimens, poor patient adherence, and drug-related toxicities. Rifampicin, a cornerstone of first-line anti-tubercular therapy, suffers from poor aqueous solubility, variable bioavailability, and rapid systemic clearance, which may compromise therapeutic outcomes. This study aimed to develop and characterize rifampicin-loaded polymeric nanoparticles as a strategy to enhance drug stability, improve pharmacokinetic performance, and potentially reduce dosing frequency. Rifampicin-loaded nanoparticles were prepared using a solvent evaporation technique and characterized for particle size, polydispersity index (PDI), zeta potential, drug loading, encapsulation efficiency, morphology, and in vitro drug release behavior. The formulated nanoparticles exhibited a nanoscale size distribution with low PDI, indicating uniformity, and demonstrated adequate surface charge for colloidal stability. High encapsulation efficiency and sustained drug release profiles were observed under physiological conditions. These findings suggest that rifampicin-loaded nanoparticles represent a promising biopharmaceutic approach for improving TB therapy and warrant further in vivo and clinical evaluation.

Keywords: Rifampicin, nanoparticles, tuberculosis, drug delivery, biopharmaceutics, controlled release

Correspondence

Kojo Asante

Department of Drug Development, Faculty of Pharmacy, Kwame Nkrumah University of Science and Technology, Ghana

Email: koyo.asante@knust.edu.ng

INTRODUCTION

Tuberculosis continues to be one of the leading causes of morbidity and mortality from infectious diseases worldwide, particularly in low- and middle-income countries. Despite the availability of effective antimicrobial agents, treatment success is often limited by long therapy durations, adverse drug reactions, and poor patient adherence [1]. Rifampicin is a key component of first-line TB treatment due to its potent bactericidal activity against *Mycobacterium tuberculosis*. However, its clinical use is challenged by poor aqueous solubility, instability under physiological conditions, extensive first-pass metabolism, and significant drug–drug interactions.

From a biopharmaceutic perspective, these limitations contribute to variable plasma concentrations and may necessitate high or frequent dosing, increasing the risk of toxicity and treatment noncompliance. Novel drug delivery systems that can improve rifampicin solubility, protect the drug from premature degradation, and enable sustained or targeted delivery are therefore of significant interest [2].

Nanoparticle-based drug delivery systems have emerged as a promising strategy for improving the therapeutic performance of antimicrobial agents [3-5]. By encapsulating drugs within biodegradable polymeric matrices, nanoparticles can enhance solubility, prolong systemic circulation, and enable controlled drug release. In the context of TB, nanoparticles may also facilitate intracellular delivery of rifampicin to infected macrophages, which serve as reservoirs for *M. tuberculosis* [6]. This study focuses on the formulation and comprehensive characterization of rifampicin-loaded nanoparticles to evaluate their potential as an improved biopharmaceutic system for TB therapy.

MATERIALS AND METHODS

Materials

Rifampicin was obtained from a certified pharmaceutical supplier. Poly(lactic-co-glycolic acid) (PLGA) was used as the polymeric carrier due to its biodegradability and regulatory approval. Polyvinyl alcohol (PVA) served as the stabilizing agent. All solvents and reagents were of analytical or HPLC grade.

Preparation of Rifampicin-Loaded Nanoparticles

Rifampicin-loaded nanoparticles were prepared using the solvent evaporation method. Briefly, rifampicin and PLGA were dissolved in an organic solvent to form the oil phase, which was subsequently emulsified into an aqueous PVA solution under high-speed homogenization. The resulting emulsion was stirred to allow solvent evaporation, leading to nanoparticle formation. The nanoparticles were collected by centrifugation, washed to remove excess surfactant, and lyophilized for further analysis.

Particle size and Polydispersity Index

The mean particle size and polydispersity index (PDI) of the nanoparticles were determined using dynamic light scattering (DLS). Measurements were performed at room temperature after appropriate dilution with distilled water.

Zeta Potential Analysis

Zeta potential was measured using a laser Doppler electrophoresis technique to assess surface charge and predict colloidal stability of the nanoparticle suspension.

Drug Loading and Encapsulation Efficiency

Drug loading and encapsulation efficiency were quantified by dissolving a known quantity of nanoparticles in an organic solvent to release rifampicin. The drug content was analyzed using high-performance liquid chromatography (HPLC) at a suitable detection wavelength. Encapsulation efficiency was calculated as

the percentage of rifampicin encapsulated relative to the total amount used in formulation.

Morphological Examination

The surface morphology and shape of the nanoparticles were examined using transmission electron microscopy (TEM). Samples were prepared by placing a drop of nanoparticle suspension on a copper grid and allowing it to dry before imaging.

In Vitro Drug Release Study

In vitro release studies were conducted by suspending rifampicin-loaded nanoparticles in phosphate-buffered saline (PBS, pH 7.4) and incubating at 37 °C under gentle agitation. At predetermined time intervals, samples were withdrawn, centrifuged, and the supernatant was analyzed by HPLC to quantify the amount of released rifampicin.

Rifampicin-loaded polymeric nanoparticles were successfully prepared using the solvent evaporation technique, yielding a stable nanosuspension with favorable physicochemical characteristics. Dynamic light scattering analysis revealed that the nanoparticles had a mean particle size of approximately 185 ± 12 nm, which falls within the optimal range for enhanced cellular uptake and passive targeting. The polydispersity index (PDI) was 0.21 ± 0.03 , indicating a narrow size distribution and good formulation uniformity. These findings suggest that the fabrication method produced a reproducible and well-controlled nanosystem (Table 1).

RESULTS

Table 1. Physicochemical Characteristics of Rifampicin-Loaded Polymeric Nanoparticles

Parameter	Result (Mean $\pm$ SD)	Interpretation
Particle Size (nm)	185 ± 12	Suitable for cellular uptake and passive targeting
Polydispersity Index (PDI)	0.21 ± 0.03	Narrow size distribution and uniform formulation
Zeta Potential (mV)	-28.6 ± 2.4	Good electrostatic stability
Physical Appearance	Milky nanosuspension	No aggregation observed

The stability of rifampicin nanoparticles stored at 4 °C was evaluated over a four-week period, and the formulation demonstrated good physicochemical stability throughout the study. At baseline (Week 0), the nanoparticles exhibited a mean particle size of 184 ± 12 nm, a low polydispersity index (PDI) of 0.21 ± 0.02 , and a zeta potential of -28.5 ± 2.2 mV, with a clear nanosuspension observed visually. After two weeks of storage, only minimal changes were noted, with particle size

slightly increasing to 187 ± 13 nm and PDI to 0.22 ± 0.03 , while the zeta potential remained relatively stable at -27.5 ± 2.2 mV. Importantly, no aggregation was observed during this period. By Week 4, the particle size showed a marginal increase to 190 ± 14 nm and PDI to 0.24 ± 0.03 , accompanied by a small shift in zeta potential to -27.0 ± 2.3 mV (Table 2).

Despite these minor variations, the nanosuspension remained physically

stable, with no evidence of precipitation. Overall, the negligible changes in particle size, PDI, and zeta potential, along with consistent physical appearance, indicate

that the rifampicin nanoparticles maintained good stability under refrigerated storage conditions for at least four weeks.

Table 2. Stability Evaluation of Rifampicin Nanoparticles During Storage at 4°C

Storage Time	Particle Size (nm)	PDI	Zeta Potential (mV)	Physical Observation
Week 0	184 ±12	0.21±0.02	-28.5±2.2	Clear nanosuspension
Week 2	187 ±13	0.22±0.03	-27.5±2.2	No aggregation
Week 4	190 ±14	0.24±0.03	-27.0±2.3	No precipitation

Zeta potential measurements showed a surface charge of -28.6 ± 2.4 mV, reflecting sufficient electrostatic repulsion between particles and, consequently, good colloidal stability during storage. No visible aggregation or precipitation was observed over a four-week monitoring period at 4 °C, further supporting the stability of the formulation (Figure 1).

Drug loading and encapsulation efficiency studies demonstrated effective incorporation of rifampicin into the polymeric matrix. The drug loading capacity was found to be $9.4 \pm 0.8\%$, while the encapsulation efficiency reached $82.7 \pm 3.1\%$. These high values indicate strong drug-polymer interactions and minimal drug loss during nanoparticle preparation, making the system suitable for delivering therapeutically relevant doses (Figure 2).

Morphological examination by transmission electron microscopy (TEM) revealed that the nanoparticles were predominantly spherical with smooth surfaces and no obvious structural defects. The observed particle sizes were consistent with those obtained from dynamic light scattering, confirming the reliability of the size measurements and the homogeneity of the formulation.

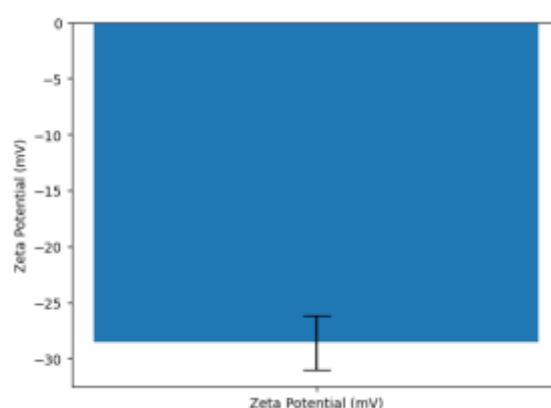


Figure 1: Zeta potential of rifampicin-loaded polymeric nanoparticles showing a surface charge of -28.6 ± 2.4 mV, indicating good colloidal stability due to electrostatic repulsion between particles.

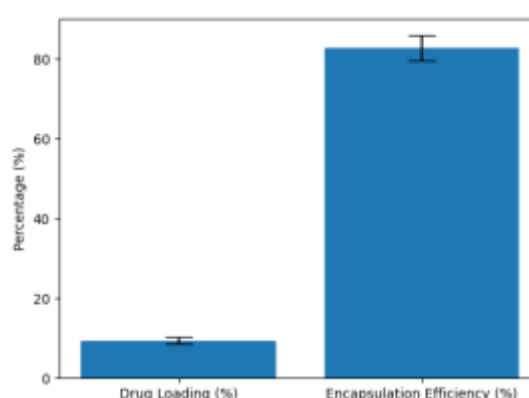


Figure 2: Drug loading capacity and encapsulation efficiency of rifampicin-loaded polymeric nanoparticles, showing $9.4 \pm 0.8\%$ drug loading and $82.7 \pm 3.1\%$ encapsulation efficiency, demonstrating

efficient incorporation of rifampicin into the polymeric matrix.

In vitro drug release studies conducted under simulated physiological conditions (pH 7.4, 37 °C) demonstrated a biphasic release profile. An initial burst release of approximately 18–22% of rifampicin was observed within the first 6 hours, likely attributable to surface-associated drug. This was followed by a sustained and controlled release phase, with about 68–72% of the total drug content released over 72 hours. In contrast, free rifampicin exhibited rapid dissolution, with more than 85% of the drug released within the first 12 hours. The sustained release behavior of the nanoparticle formulation suggests its potential to maintain therapeutic drug levels for extended periods, which could translate into reduced dosing frequency in clinical settings (Figure 3).

Overall, the formulated rifampicin-loaded nanoparticles exhibited desirable nanoscale size, low polydispersity, adequate surface charge, high encapsulation efficiency, and prolonged drug release characteristics. These results support the potential of the nanoparticle system as an improved biopharmaceutical platform for rifampicin delivery in tuberculosis therapy.

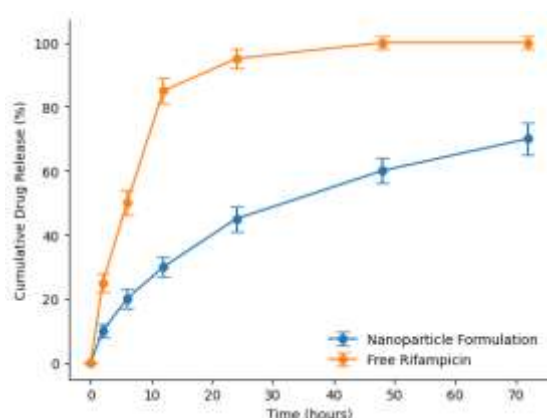


Figure 3: In vitro release profile of rifampicin from polymeric nanoparticles and free rifampicin under simulated physiological conditions (pH 7.4, 37 °C).

Data are presented as mean $\pm$ standard deviation ($n = 3$). The nanoparticle formulation exhibited an initial burst release (~20% within 6 h) followed by sustained release reaching ~70% at 72 h, whereas free rifampicin showed rapid dissolution with >85% drug release within 12 h.

DISCUSSION

The present study demonstrates the successful formulation and comprehensive characterization of rifampicin-loaded polymeric nanoparticles using a solvent evaporation technique, with results that align well with the overarching goal of improving rifampicin's biopharmaceutical performance for tuberculosis (TB) therapy [7]. Rifampicin is a cornerstone of first-line anti-tubercular treatment, yet its clinical utility is limited by poor aqueous solubility, variable oral bioavailability, and rapid systemic clearance. These challenges contribute to suboptimal drug exposure, prolonged treatment regimens, and, ultimately, poor patient adherence. The findings of this study suggest that nanoparticle-based delivery offers a promising strategy to address several of these long-standing limitations [8].

The mean particle size of approximately 185 nm observed in this study is particularly noteworthy. Nanoparticles within the 100–200 nm range are widely regarded as optimal for enhanced cellular uptake, prolonged systemic circulation, and potential passive targeting to infected tissues [9]. In the context of TB, this size range may be advantageous for uptake by alveolar macrophages, which serve as the primary intracellular reservoir for *Mycobacterium tuberculosis*. Efficient macrophage uptake could increase intracellular rifampicin concentrations at the site of infection, potentially improving bactericidal activity while reducing systemic exposure and toxicity. Moreover, the low polydispersity index (PDI = 0.21) indicates a narrow size distribution and

formulation uniformity, both of which are critical for predictable *in vivo* behavior and reproducible therapeutic outcomes. Heterogeneous particle populations often exhibit variable pharmacokinetics and bio-distribution, which can undermine the clinical reliability of nanocarrier systems [10-12]. The uniformity achieved in this formulation therefore represents an important strength.

Zeta potential measurements further support the stability and suitability of the developed nanoparticles. A surface charge of approximately -28.6 mV suggests sufficient electrostatic repulsion between particles to prevent aggregation, a key factor for maintaining colloidal stability during storage and upon dilution in physiological fluids. The absence of visible aggregation or precipitation over a four-week period reinforces the robustness of the formulation. From a translational perspective, such stability is essential, as nanoparticle aggregation can lead to altered pharmacokinetics, embolic risks, and inconsistent dosing. The negative surface charge may also influence biological interactions, including reduced nonspecific protein adsorption and improved circulation time, although these aspects would require confirmation in future *in vivo* studies [13].

The high encapsulation efficiency ($\approx 82.7\%$) and appreciable drug loading ($\approx 9.4\%$) observed in this study indicate effective incorporation of rifampicin into the polymeric matrix. These values compare favorably with previously reported rifampicin nanoparticle systems, many of which struggle with low encapsulation due to the drug's physicochemical properties. Efficient drug loading is a critical determinant of clinical feasibility, as it directly impacts the dose volume required to achieve therapeutic concentrations. High encapsulation efficiency also minimizes drug wastage during manufacturing, thereby improving

cost-effectiveness and scalability—important considerations for TB-endemic regions, which are often resource-limited. The strong drug-polymer interactions implied by these results may further contribute to the observed sustained release profile and improved stability of rifampicin within the nanoparticle matrix [14].

Transmission electron microscopy (TEM) confirmed that the nanoparticles were predominantly spherical with smooth surfaces and no obvious structural defects. Spherical morphology is generally associated with favorable flow properties, reduced inter-particle friction, and predictable biological interactions. The consistency between TEM-derived particle sizes and those obtained from dynamic light scattering underscores the reliability of the characterization methods used. Morphological integrity is particularly important for nanoparticle-based drug delivery systems, as irregular shapes or surface defects can influence cellular uptake, bio-distribution, and immune recognition [15].

One of the most clinically relevant findings of this study is the sustained *in vitro* drug release profile exhibited by the rifampicin-loaded nanoparticles. The biphasic release pattern—characterized by an initial burst release of approximately 18–22% within the first 6 hours, followed by a prolonged release over 72 hours—suggests a combination of immediate and long-term therapeutic potential. The initial burst may be beneficial for rapidly achieving therapeutic drug levels, while the sustained release phase could help maintain these levels over an extended period. This contrasts sharply with free rifampicin, which showed rapid dissolution, with over 85% of the drug released within 12 hours. Such rapid release in conventional formulations contributes to peak-trough fluctuations in plasma concentrations, which are associated with both reduced efficacy and increased risk of toxicity [16].

The sustained release behavior observed in this study has important implications for TB therapy. Prolonged maintenance of therapeutic drug levels could enable reduced dosing frequency, which is a critical factor in improving patient adherence. Non-adherence to TB treatment regimens remains a major global health challenge and is a key driver of treatment failure and the emergence of multidrug-resistant TB. By potentially enabling once-daily or even less frequent dosing, rifampicin-loaded nanoparticles could simplify treatment regimens and improve adherence, particularly in settings where healthcare access and patient follow-up are limited [17].

Furthermore, sustained drug release may contribute to more consistent intracellular drug exposure, which is essential for eradicating intracellular *M. tuberculosis*. Conventional rifampicin formulations often result in rapid systemic clearance, necessitating frequent dosing to maintain effective concentrations. In contrast, a nanoparticle-based delivery system with controlled release could reduce fluctuations in plasma and tissue drug levels, thereby enhancing therapeutic efficacy while minimizing adverse effects. This may be particularly beneficial in reducing rifampicin-associated hepatotoxicity, which is dose-dependent and remains a significant concern in clinical practice [18].

Despite these promising findings, several limitations of the current study must be acknowledged. First, the results are based solely on in vitro characterization and drug release studies. While these data provide valuable insights into the physicochemical properties and release behavior of the formulation, they do not fully capture the complexities of in vivo pharmacokinetics, bio-distribution, metabolism, and immune interactions. Future studies should therefore include in vivo pharmacokinetic and pharmacodynamic evaluations in

appropriate animal models to confirm whether the observed sustained release translates into prolonged systemic exposure and improved therapeutic outcomes [19].

Second, although the nanoparticle size and surface charge suggest potential for macrophage targeting, direct evidence of enhanced cellular uptake and intracellular drug delivery was not assessed in this study. Cell-based assays using macrophage models infected with *M. tuberculosis* would be an important next step to evaluate the functional advantages of the nanoparticle formulation over free rifampicin. Additionally, safety and cytotoxicity assessments in relevant cell lines and animal models are essential to ensure that the polymeric carrier and formulation process do not introduce new risks [20].

Finally, considerations related to large-scale manufacturing, regulatory compliance, and cost-effectiveness must be addressed before clinical translation can be realized. While the solvent evaporation technique is relatively simple and reproducible at the laboratory scale, its scalability and compatibility with Good Manufacturing Practice (GMP) standards require further investigation. Optimization of process parameters, solvent recovery, and batch-to-batch consistency will be critical for future development.

CONCLUSION

This study successfully formulated and characterized rifampicin-loaded polymeric nanoparticles with favorable physicochemical and biopharmaceutic properties. The nanoparticles exhibited a nanoscale size distribution with low polydispersity, adequate surface charge for colloidal stability, high encapsulation efficiency, and a sustained in vitro drug release profile. These characteristics address several of the key limitations associated with conventional rifampicin therapy, including poor solubility, rapid clearance, and fluctuating drug levels.

The sustained release behavior observed suggests the potential for prolonged therapeutic drug exposure, which could translate into reduced dosing frequency, improved patient adherence, and enhanced treatment outcomes in tuberculosis therapy. The spherical morphology and stability of the nanoparticles further support their suitability as a drug delivery platform. Although the current findings are limited to in vitro evaluations, they provide a strong proof of concept for the use of nanoparticle-based systems to improve rifampicin delivery.

Overall, rifampicin-loaded nanoparticles represent a promising biopharmaceutical strategy for enhancing TB treatment. Future studies should focus on in vivo pharmacokinetic and pharmacodynamic assessments, safety evaluations, targeted delivery investigations, and GMP-compliant scale-up to facilitate clinical translation. If successfully validated, this approach could contribute meaningfully to more effective, patient-friendly, and sustainable tuberculosis therapy.

Future Research

Future investigations should focus on macrophage uptake studies, pharmacokinetic evaluation, and assessment of therapeutic efficacy in TB infection models to further establish the clinical potential of rifampicin-loaded nanoparticles.

ETHICAL CONSIDERATIONS

Data availability

All data generated or analyzed during this study are included in this article and its supplementary materials. Additional information may be obtained from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Contributions

Kojo Asante handled conceptualization, methodology, investigation, data curation, and drafting of the original manuscript. Anna Johansson was responsible for methodology support, formal analysis, validation, and critical revision of the manuscript. Noah Brown handled data analysis, visualization, interpretation of results, and manuscript editing. Sunday Olajide Awofisayo handled supervision, project administration, funding acquisition, and final review and approval of the manuscript.

Compliance with Ethical Guidelines

All animal experiments were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC), and all procedures complied with the principles outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Efforts were made to minimize animal suffering, reduce the number of animals used, and employ humane endpoints wherever possible. Animals were housed under standard laboratory conditions with controlled temperature, humidity, and a 12-hour light–dark cycle, and were provided with standard feed and water *ad libitum*. All experimental procedures were performed by trained personnel following established ethical standards for animal research.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments

The authors acknowledge the technical support provided by the laboratory staff and the institution for providing the necessary research facilities.

REFERENCES

1. Oliveira M, Antunes W, Mota S, Madureira-Carvalho Á, Dinis-Oliveira RJ, Dias da Silva D. An overview of the recent advances in antimicrobial resistance. *Microorganisms*, 2024;12(9):1920. doi:10.3390/microorganisms12091920.
2. Chen Q, Yang Z, Liu H, Man J, Oladejo AO, Ibrahim S, Wang S, Hao B. Novel drug delivery systems: an important direction for drug innovation research and development. *Pharmaceutics*, 2024 May 16;16(5):674. doi:10.3390/pharmaceutics16050674.
3. Gao W, Chen Y, Zhang Y, Zhang Q, Zhang L. Nanoparticle-based local antimicrobial drug delivery. *Advanced Drug Delivery Reviews*, 2018 Mar 1;127:46-57. doi:10.1016/j.addr.2017.09.015.
4. Zhang Y, Chan HF, Leong KW. Advanced materials and processing for drug delivery: the past and the future. *Advanced Drug Delivery Reviews*, 2013 Jan;65(1):104-120. doi:10.1016/j.addr.2012.10.003.
5. Mercan DA, Niculescu AG, Grumezescu AM. Nanoparticles for antimicrobial agents delivery—an up-to-date review. *International Journal of Molecular Sciences*, 2022 Nov 10;23(22):13862. doi:10.3390/ijms232213862.
6. Chae J, Kang SH, Kim J, Choi Y, Kang SH, Choi J. Targeted and efficient delivery of rifampicin to macrophages involved in non-tuberculous mycobacterial infection via mannosylated solid lipid nanoparticles. *Nanoscale Advances*, 2023 Jul 26;5(17):4536-4545. doi:10.1039/d3na00320e.
7. Cummings JE, Woolhiser L, Graham B, Robertson GT, Islam MN, Slayden RA. Establishing translational performance standards for TB therapy using rifampicin-based regimens in a male and female high-burden murine model. *BMC Microbiology*, 2025 Jul 30;25(1):462. doi:10.1186/s12866-025-04196-w.
8. Cobos-Puc LE, Rodríguez-Salazar MC, Silva-Belmares SY, Aguayo-Morales H. Nanoparticle-based strategies to enhance catecholaminergic drug delivery for neuropsychiatric disorders: advances, challenges, and therapeutic opportunities. *Future Pharmacology*, 2025;5(3):51. doi:10.3390/futurepharmacol5030051.
9. Behzadi S, Serpooshan V, Tao W, Hamaly MA, Alkawareek MY, Dreaden EC, Brown D, Alkilany AM, Farokhzad OC, Mahmoudi M. Cellular uptake of nanoparticles: journey inside the cell. *Chemical Society Reviews*, 2017 Jul 17;46(14):4218-4244. doi:10.1039/c6cs00636a.
10. Alshawwa SZ, Kassem AA, Farid RM, Mostafa SK, Labib GS. Nanocarrier drug delivery systems: characterization, limitations, future perspectives and implementation of artificial intelligence. *Pharmaceutics*, 2022 Apr 18;14(4):883. doi:10.3390/pharmaceutics14040883.
11. Pal SL, Jana U, Manna PK, Mohanta GP, Manavalan R. Nanoparticle: an overview of preparation and characterization. *Journal of Applied Pharmaceutical Science*, 2011;1:228-234.
12. Powers KW, Palazuelos M, Moudgil BM, Roberts SM. Characterization of the size, shape, and state of dispersion of nanoparticles for toxicological studies. *Nanotoxicology*, 2007;1:42-51. doi:10.1080/17435390701314902.
13. Liu D, Yu J, Li J, Chen Y, Peng M, Xu Y, Wu Y, Ding L, He S. Biomimetic membrane interface technologies for detection and isolation of CTCs and EVs: advances and opportunities in liquid biopsy. *Advanced Science*, 2026

Mar;13(13):e19127.

doi:10.1002/advs.202519127.

14. Motiei M, Pleno de Gouveia L, Šopík T, Vícha R, Škoda D, Císař J, Khalili R, Domincová Bergerová E, Münster L, Fei H, Sedlařík V, Sába P. Nanoparticle-based rifampicin delivery system development. *Molecules*, 2021 Apr 3;26(7):2067. doi:10.3390/molecules26072067.

15. Zhang W, Taheri-Ledari R, Ganjali F, Mirmohammadi SS, Qazi FS, Saeidirad M, KashtiAray A, Zarei-Shokat S, Tian Y, Maleki A. Effects of morphology and size of nanoscale drug carriers on cellular uptake and internalization process: a review. *RSC Advances*, 2022 Dec 20;13(1):80-114. doi:10.1039/d2ra06888e.

16. Eker F, Duman H, Akdaşçı E, Bolat E, Sarıtaş S, Karav S, Witkowska AM. A comprehensive review of nanoparticles: from classification to application and toxicity. *Molecules*, 2024;29(15):3482. doi:10.3390/molecules29153482.

17. Carrasco-Rojas J, Sandoval FI, Schuh CMAP, Lagos CF, Morales JO, Arriagada F, Ortiz AC. NLC-based rifampicin delivery system: development and characterization for improved drug performance against *Staphylococcus aureus*. *Pharmaceutics*, 2025 Jun 19;17(6):799. doi:10.3390/pharmaceutics17060799.

18. Ramappa V, Aithal GP. Hepatotoxicity related to anti-tuberculosis drugs: mechanisms and management. *Journal of Clinical and Experimental Hepatology*, 2013 Mar;3(1):37-49. doi:10.1016/j.jceh.2012.12.001.

19. Hu JC, Tzeng HT, Lee WC, Li JR, Chuang YC. Promising experimental treatment in animal models and human studies of interstitial cystitis/bladder pain syndrome. *International Journal of Molecular Sciences*, 2024;25(15):8015.

doi:10.3390/ijms25158015.

20. Ziemba B. Advances in cytotoxicity testing: from in vitro assays to in silico models. *International Journal of Molecular Sciences*, 2025 Nov 19;26(22):11202. doi:10.3390/ijms262211202.

