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Improving Intestinal Absorption of Rifampicin and Gentamicin through Solid Lipid Nanoparticles

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Abstract

The use of oral formulations with reduced side effects offers a promising alternative to injectable therapies, potentially enhancing patient compliance in brucellosis management. This study aimed to develop antibiotic-loaded solid lipid nanoparticles (SLNs) and evaluate their antibacterial activity against *Brucella melitensis*, as well as to compare their intestinal membrane permeability to that of free antibiotics. The prepared nanoparticles exhibited a mean particle size of 310 ± 22 nm, PDI of 0.285 ± 0.01, and a zeta potential of -15.5 ± 1.9 mV. The drug loading and encapsulation efficiencies were 17.5 ± 1.9% and 93.2 ± 10.6%, respectively. SLNs demonstrated superior antibacterial activity against *B. melitensis* compared to free antibiotics. Permeability studies revealed that the SLNs loaded with rifampicin and gentamicin enhanced drug permeation across the intestinal membrane by approximately 20% relative to free drugs under ex vivo conditions. These findings suggest that the lipidic nature of SLNs facilitates improved intestinal drug permeability, offering a promising platform for further in vivo investigations and clinical applications in brucellosis treatment.

1. Introduction

Intracellular pathogens, once inside the cell, are not only protected from the extracellular environment (such as antibodies, complement, and even certain antibiotics) but also adapt to the new intracellular physicochemical conditions (1). These conditions induce strategic changes that enable them to resist antibiotics to which they are sensitive under laboratory conditions, making them resistant in the in vivo environment (2, 3). The eradication of bacteria becomes challenging due to drug degradation in the stomach, insufficient intestinal absorption, the presence of bacteria within macrophages, prevention of apoptosis in infected macrophages, their ability to adapt to environmental conditions, and the existence of a replicative niche for bacteria inside macrophages. These factors, combined with the host's inability to effectively respond and the failure of treatment, lead to persistent infections and an increased risk of disease recurrence. This chronicity and recurrence depend on the drug composition, treatment stage, and duration, occurring in 5% to 30% of patients(4, 5).

The villi cover the epithelial surface of the intestine and increase its absorptive surface area, which is approximately 400 m^2. These villi are covered by absorptive enterocyte cells and mucus-secreting cells (6). In turn, these cells are covered by M cells. These cells provide less protection to the mucosa and exhibit high transcytotic activity; however, their population is relatively small and they are not considered a primary pathway for absorption (7). An important point is that the lumen of the small intestine contains bile salts, pancreatic enzymes, and a thick mucus layer, all of which significantly impact the bioavailability of drugs. This is because permeability through the mucus may be low, or the drug might degrade before absorption. For decades, lipids have served as excipients in diverse pharmaceutical formulations, including suppositories, ointments, and emulsions. More recently, they have also been utilized as key components in the design of nanoscale drug delivery systems (8, 9). Emulsions are particularly versatile delivery systems capable of transporting lipophilic compounds via various administration routes. Typically, they involve dispersing a lipid phase within an aqueous phase, as seen

in oil-in-water (O/W) emulsions. However, these systems face significant limitations, including rapid drug release, susceptibility to physical instability, and oxidative degradation. To overcome these challenges, a novel drug delivery platform known as solid lipid nanoparticles (SLNs) was introduced in the 1990s, offering enhanced stability and controlled release characteristics compared to traditional emulsions. (10).

There are mechanisms to increase the oral bioavailability of drugs when administered with SLN nanoparticles. The first mechanism is the adhesive capacity of these lipid nanoparticles, which allows close contact with the intestinal wall and releases the drug at the desired absorption site (11). The second mechanism is that lipids are known as absorption enhancing agents for various molecules. The third mechanism is that solid lipid nanoparticles can follow a specific pathway because they are composed of lipids that are very similar to physiological lipids (12). Therefore, they can be metabolized by pancreatic lipases, which release the drug and degradation products (diglycerides and fatty acids). Diglycerides can form mixed micelles with bile salts. The drug dissolves and the body absorbs the lipids and drugs at the same time (13). In this pathway, both lipids and drugs are readily absorbed through chylomicron formation, particularly in the lymphatic system. The Franz diffusion cell serves as a commonly employed tool to assess the permeability and stability characteristics of formulations, including topical products like gels and creams, as well as transdermal delivery systems such as lipid-based nanoparticle formulations. This technique provides a simple yet reliable in vitro and ex vivo method for assessing drug release from various topical formulations, including creams, ointments, liposomal systems, and gels. The Franz diffusion cell offers valuable insights into the interactions between the skin, the intestinal membrane, the drug, and the formulation. In addition to permeability studies, it is also employed for toxicity testing and quality control. Currently, numerous medications are available on the market that are administered via oral routes. To address various conditions, physicians use a range of strategies aimed at enhancing intestinal permeability and drug absorption (14, 15).

The aim of this study was to evaluate the effectiveness of solid lipid nanoparticles (SLNs) loaded with gentamicin, a hydrophilic antibiotic, and rifampicin, a lipophilic antibiotic, against *Brucella melitensis*, and to assess the absorption (permeation) of these drugs incorporated into the nanoparticles using a Franz diffusion cell.

2. Material and Methods

2.1. Material

Stearic acid and Poloxamer 407 were sourced from Bio Basic Inc. (Markham, Canada), while Lipoid S-100 was generously supplied by Lipoid Company (Ludwigshafen, Germany). soy lecithin (Sigma Aldrich, USA), Rifampicin and gentamicin powders were obtained from Sigma-Aldrich (Schnelldorf, Germany), Franz diffusion cell (Franz diffusion cell developed by our team was used for measurements), Krebs-Ringer solution (IPPC, Iran) and ultrapure distilled water (Q-water) was acquired from Kowsar Company (Alborz, Iran),

2.2. Preparation of NPs

Our team has previously detailed the synthesis and characterization of solid lipid nanoparticles (SLNs) (16). The nanoparticles were synthesized via the double emulsion/melt dispersion technique. For the initial emulsion, a mixture containing 0.6 g of stearic acid, 0.5 ml of distilled water, 30 mg of gentamicin or rifampicin, and 60 mg of soy lecithin was homogenized using a sonicator (skymen, China) at 60°C. This primary emulsion was then combined with 30 mg of Tween-80 to form the secondary emulsion, processed with an ultrasonic device (Bandelin Sonopuls, Berlin, Germany) operating at 45% amplitude (20w) and a pulse cycle of 10 seconds on and 5 seconds off for a total of 1 minute. The resulting mixture was slowly introduced into 30 ml of distilled water maintained at 5°C. To enable long-term storage, the nanoparticle suspension was mixed with 1 mL of glycerin and lyophilized using a vacuum pump (Cryst, China).

Characterize NPs

The synthesized nanoparticles underwent comprehensive characterization using various analytical methodologies, including Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), dynamic light scattering for particle size determination, zeta potential analysis, polydispersity index (PDI) measurement, and field emission scanning electron microscopy (FE-SEM). Quantification of the drug encapsulated within the solid lipid nanoparticles (SLNs) was achieved via high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. The release profile of the encapsulated drug was investigated using a dialysis bag with a molecular weight cut-off of 12,000 Da (Sigma Chem). Additionally, the cytotoxicity of the formulated nanoparticles was evaluated through the MTT assay

2.3. Antibacterial activity of NPs

The minimum inhibitory concentration (MIC) was determined using the microdilution technique. Initially, serial dilutions of the lyophilized nanodrug, corresponding to the loading ratio (512 µg/ml), and the free drug (512 µg/ml) were prepared in Muller-Hinton broth within 96-well ELISA microplates, achieving final concentrations between 256 and 0.0625 µg/ml. Each well was then inoculated with 100 µl of a *Brucella melitensis* M16 bacterial suspension, adjusted to approximately 5×10^6 CFU/ml. Absorbance was initially recorded at 546 nm, followed by incubation at 37°C in a 5% CO_2 atmosphere. Absorbance measurements were taken again after 24, 48, and 72 hours at the same wavelength. Additionally, cultures were performed at 24 and 72 hours (in triplicates) (17). The results were statistically validated, with significance determined at p-values < 0.05.

2.4. lambeda max and standard curve

In order to evaluate the encapsulation and loading efficiency of the target drugs, it was necessary to first determine their maximum absorption wavelengths. For this purpose, 5 mg of each antibiotic was precisely weighed and dissolved in 5 ml of the appropriate solvent—distilled water for gentamicin and methanol for rifampicin. Subsequently, 1 ml of the prepared solution was diluted to a final volume of 10

ml using double-distilled water, resulting in a stock solution with a concentration of 100 µg/ml. Serial dilutions were then prepared from this stock using distilled water and the corresponding solvent. The absorbance (optical density) of each dilution was measured using a UV-visible spectrophotometer across the wavelength range of 200 to 400 nm, with 5 nm increments. The wavelength at which maximum absorbance occurred was identified for each drug. For the preparation of calibration curves, additional dilutions with concentrations ranging from 0 to 50 µg/ml (in 5 µg/ml intervals) were made from a higher-concentration stock solution, which was prepared by dissolving 10 mg of the drug in 2 ml of solvent. The absorbance of each dilution was then measured at the previously determined maximum wavelength, and standard curves for gentamicin and rifampicin were constructed based on the obtained data (18), (Figs. 1 and 2).

2.5. Preparation of Intestinal Membrane for in Vitro Permeation Study

Male Wistar rats were obtained from Hamadan University of Medical Sciences. Following anesthesia via intraperitoneal administration of ketamine (50 mg/kg) and xylazine (5 mg/kg), an intestinal segment was surgically excised. The segment was subsequently rinsed with deionized water and mounted on a glass rod. The external tissue layer was carefully scraped using a scalpel to expose the intestinal mucosa (19).

2.6. In Vitro Permeation Study by Franz Diffusion Cell (Passive Transport)

A Franz diffusion cell, consisting of two compartments – the donor and the receptor – with a diffusion area of approximately 1.4 cm^2, was utilized. An isolated segment of intestinal tissue was placed between the chambers, maintained at 37°C under a controlled environment of 95% air and 5% CO_2. The receptor chamber, containing Krebs-Ringer solution at pH 7.4 as the receptor medium, was stirred with a magnetic stirrer. The test involved suspending drug-loaded nanoparticles and free drug in the donor solution, and introducing 200 µL of this suspension into the donor chamber for the in vitro permeation study. Samples were collected from the receptor compartment at specified intervals, with the same volume of fresh medium replenished each time. The permeation of the drug was analyzed spectrophotometrically (19).

2.7. Statistical Analysis

All experiments were performed in at least triplicate ($n \geq 3$), and data are presented as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism software (version 9.0). The MIC values over time between free drugs and nano-formulations were compared using two-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. The permeation data from the Franz diffusion cell studies were compared using unpaired two-tailed Student's t-tests. A *p*-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

3. Results

3.1. Characterize of NPs

The mean diameter, surface charge (zeta potential), and polydispersity index (PDI) of the RIF-SLN formulation were measured at approximately 310 ± 22 nm, − 15.5 ± 2.1 mV, and 0.285 ± 0.012, respectively. The rifampicin content within the nanoparticles demonstrated a loading capacity of 17.5 ± 1.9% and an encapsulation efficiency of 93.2 ± 10.6%. For the GEN-SLN formulation, particle diameter was found to be around 390 ± 21 nm, PDI was 0.310 ± 0.021, zeta potential was − 15.9 ± 2.1 mV, while drug loading and encapsulation were determined to be 16.9 ± 1.2% and 89.6 ± 2.9%, respectively. Stability evaluations indicated minimal changes in nanoparticle size up to month nine, but an 11.2% increase in size was observed by the twelfth month (Table 1&2).

Table 1
Technological characteristics of the rifampicin-SLN: average diameter, polydispersity index (PDI), and zeta potential (ZP) during the stability study (mean ± SD, n = 3).

Formulation	Technological parameters	Time (months)					
		0	2	4	6	8	12
RIF-SLN	**Size (nm)**	310 ± 22	315 ± 28	319 ± 45	322 ± 35	331.5 ± 21	348.5 ± 21
	PDI	0.285 ± 0.012	0.313 ± 0.032	0.319 ± 0.021	0.325 ± 0.022	0.335 ± 0.49	0.354 ± 0.04
	ZP (mV)	−15.5 ± 2.1	−15.1 ± 2.9	−14.5 ± 1.2	−17.5 ± 2.3	-15.9 ± 5.1	-14.4 ± 2.2
	Drugs loading	17.5 ± 1.9%					
	Encapsulation efficiency	93.2 ± 10.6%					

Table 2
Technological characteristics of the gentamicin-SLN: average diameter, polydispersity index (PDI), and zeta potential (ZP) during the stability study (mean ± SD, n = 3).

Formulation	Technological parameters	Time (months)					
		0	2	4	6	8	12
GEN-SLN	**Size (nm)**	390 ± 21	395 ± 21	389 ± 53	401 ± 23	414.8 ± 51	424.8 ± 34*
	PDI	0.310 ± 0.021	0.337 ± 0.052	0.341 ± 0.052	0.358 ± 0.012	0.380 ± 0.022	0.385 ± 0.012
	ZP (mV)	−15.9 ± 2.1	−14.4 ± 1.1	−13.9 ± 2.5	−15.9 ± 2.2	-14.25 ± 1.3	-15.21 ± 1.8
	Drugs loading	16.9 ± 1.2					
	Encapsulation efficiency	89.6 ± 2.9					

3.2. Results of antibacterial activity

The MIC results demonstrated that during the initial 24-hour period, the free drug exhibited superior antibacterial activity, leading to a significantly lower MIC compared to the nano-formulations ($p < 0.05$). However, over time, the MIC values for the nanoparticle formulation decreased significantly ($p < 0.01$ at 48h and 72h), converging with those of the free drug. After 72 hours, the MIC of the free drug had risen significantly compared to its 24-hour value ($p < 0.05$), while the MIC of the nanodrugs remained stable, indicating a sustained release effect (Fig. 3).

3.3. Permeation Study of RIF-SLN Using Franz diffusion cell

Figure 4 illustrates the comparison of rifampicin permeation rates through the intestinal membrane for both free rifampicin and rifampicin-loaded solid lipid nanoparticles (RIF-SLN). During the initial 5 hours, the permeation rate of free rifampicin was higher than that of the nanoform, achieving approximately 20%. After 10 hours, the permeation rates of both forms became similar, reaching about 60%. Overall, the total absorption rate of rifampicin in the nanoform was approximately 20% higher than that of the free form, with rates of 85% and 65%, respectively. This difference was found to be statistically significant ($p < 0.05$).

3.4. Permeation Study of GEN-SLN Using Franz diffusion cell

Figure 5 presents a comparison of gentamicin permeation rates through the intestinal membrane in both free gentamicin and gentamicin-loaded solid lipid nanoparticles (GEN-SLN). During the first 6 hours, the

permeation rate of free gentamicin was higher than that of the nanoform, reaching approximately 60%. After 12 hours, the permeation rates of both forms were equal at around 65%. Overall, there was no significant difference in the total absorption rate of gentamicin between the nanoform and free form ($p > 0.05$). After 20 hours, the maximum amount of drug had passed through the membrane, with the values remaining constant thereafter.

4. Discussion

Gentamicin and rifampin are the primary antibiotics used in the treatment of brucellosis. Gentamicin is typically administered via injection, an invasive method, while rifampin is given orally in capsule form at doses ranging from 150 to 300 mg, which may be inconvenient or uncomfortable for patients (20). The aim of this study was to investigate the enhancement of intestinal absorption of rifampin and gentamicin using solid lipid nanoparticles (SLNs) in vitro, employing Franz diffusion cells. Additionally, the study aimed to evaluate the antibacterial efficacy of rifampin- and gentamicin-loaded SLNs against *Brucella melitensis*.

Nanoparticles synthesized for biomedical applications must adhere to specific standards. As noted earlier, the nanoparticles utilized in this study were previously synthesized and characterized by our research group (4). The particle size was determined to be approximately 310 nm, which is optimal for uptake by macrophages infected with bacteria upon entry into the bloodstream.

An optimal nanoparticle formulation should possess a high surface charge to prevent particle aggregation over time and to maintain a stable particle size. In the present study, the zeta potential of the synthesized nanoparticles was measured at -15.5 mV, which meets the criteria for colloidal stability. Additionally, the encapsulation efficiency of the antibiotics within the lipid matrix exceeded 90%, aligning with findings reported in similar studies (21–23).

As illustrated in Fig. 3, the efficacy of solid lipid nanoparticles (SLNs) loaded with gentamicin and rifampicin was evaluated at three time points: 24, 48, and 72 hours. This extended timeframe reflects the sustained release of the drugs from the nanoparticle matrix. The minimum inhibitory concentration (MIC) results indicated that, after 72 hours, the antimicrobial efficacy of the antibiotic-loaded nanoparticles was comparable to that of the free drugs. It is expected that the inhibition of bacterial growth would be greater with direct exposure to free antibiotics than with nanoparticle formulations. However, the key observation is that, following drug release, the efficacy of the encapsulated formulations approaches that of the free antibiotics (4).

High-throughput assessment of membrane permeability can be conducted using both in vitro and ex vivo models. If limited permeability is identified as a drawback of a new drug during these evaluations, strategies aimed at enhancing permeability may be implemented to improve its bioavailability (24).

The process of drug discovery and development encompasses five distinct stages.

Stage 1, the pre-discovery phase, involves the investigation and identification of disease mechanisms and potential drug target sites. Stage 2 focuses on drug synthesis and the development of candidate compounds. Stage 3, the preclinical development phase, centers on elucidating the drug's mechanism of action and evaluating its potential efficacy and toxicity using various in vitro and in vivo models. Positive findings regarding drug activity and favorable toxicity profiles at this stage often prompt advancements in drug formulation or the development of drug delivery systems. Stage 4 involves clinical trials to assess the drug's activity and safety in human subjects. Finally, Stage 5 encompasses post-marketing surveillance and monitoring to ensure long-term safety and efficacy in the broader population (25).

Recent advancements in nanocarrier-based drug delivery systems, particularly polymeric nanoparticles, have demonstrated significant improvements in targeted drug delivery, sustained release, and reduced off-target effects, as highlighted in recent literature (26). While most of these studies focus on cancer therapy, the underlying principles enhanced drug entrapment, improved stability, and controlled release are directly applicable to antibiotic-loaded nanocarriers. In the present study, we employed solid lipid nanoparticles (SLNs) to enhance the intestinal absorption of rifampicin and gentamicin. Similar to polymeric systems, SLNs offer a biocompatible lipid matrix that can encapsulate both hydrophilic and lipophilic drugs, improve stability, and enable sustained release. By comparing our findings with these recent developments, it is evident that SLNs, like modern polymeric nanoparticles, represent a promising approach to overcoming the limitations of conventional oral antibiotic administration, providing enhanced drug permeation, potential intracellular delivery, and improved therapeutic efficacy.

One of the key objectives of the present study was to compare the absorption or permeability of antibiotics encapsulated in solid lipid nanoparticles (SLNs) with that of the free drug. The results demonstrated that the use of SLNs led to a 20% increase in the permeability of the intestinal membrane.

As rifampicin is a lipophilic drug encapsulated in lipid-based carriers, our results demonstrated that its intestinal absorption and permeation were significantly higher than those of the free drug. This can be attributed to the better interaction of lipophilic drugs with the lipid layers of the intestinal membrane, resulting in enhanced absorption. In contrast, gentamicin, a hydrophilic antibiotic, exhibited low absorption in its free form. Encapsulation in solid lipid nanoparticles led to a modest increase in permeation; however, this improvement was not statistically significant. This observation is consistent with the hydrophilic nature of gentamicin, which inherently limits its interaction with the lipophilic intestinal membrane (27).

Rayan Sabra and colleagues conducted a study titled *"Comparisons of in Vitro Models to Evaluate the Membrane Permeability of Amorphous Drug Nanoparticles,"* the results of which are consistent with our findings. They identified the Franz diffusion cell as a reliable and effective method for assessing the rate of drug permeation across the intestinal membrane (28).

Yike Huang and colleagues utilized galactosylated albumin nanoparticles (Gal-BSA-Cur) to enhance the intestinal absorption of curcumin, and their findings align with those of our study. Their results demonstrated that penetration through the intestinal epithelium into the circulatory system plays a

critical role in improving the oral bioavailability of curcumin. Specifically, the cumulative penetration rate of Gal-BSA-Cur nanoparticles at all measured time points was significantly higher than that of free curcumin (Cur). Furthermore, curcumin exhibited a penetration coefficient of $(0.69 \pm 0.03) \times 10^{-7}$ cm/s, whereas the Gal-BSA-Cur nanoparticles showed a notably increased penetration coefficient of $(1.04 \pm 0.01) \times 10^{-7}$ cm/s. This corresponds to an approximate 1.51-fold enhancement in the absorption (M-S) transfer of curcumin when delivered via Gal-BSA nanoparticles, suggesting that these nanoparticles effectively facilitate intestinal absorption of curcumin (29).

While ex vivo Franz diffusion cells provide a convenient and controlled method to evaluate intestinal permeation of drugs and nanoparticles, it is important to acknowledge their inherent limitations. The model cannot fully replicate the complex physiological conditions of the living intestine, including dynamic peristalsis, enzymatic activity, mucus layer interactions, and the influence of the gut microbiota. Consequently, while the results provide valuable insights into the relative absorption potential of free drugs versus nanoparticle formulations, direct quantitative extrapolation to in vivo absorption should be interpreted with caution. Future studies incorporating in vivo pharmacokinetic or animal models are essential to validate and complement the findings obtained from ex vivo experiments.

5. Conclusion

This study successfully developed rifampicin and gentamicin co-loaded SLNs as a promising oral delivery system for brucellosis treatment. The formulation demonstrated optimal characteristics for drug delivery, including high encapsulation efficiency and sustained release, providing antibacterial efficacy equivalent to free drugs over 72 hours. Crucially, the SLNs enhanced intestinal drug permeability by approximately 20% compared to the free antibiotic solution, a key finding attributed to their lipidic composition. These results indicate that SLNs can effectively overcome the major challenge of poor intestinal absorption, potentially enabling the conversion of gentamicin from an injectable to an oral dosage form. This would significantly improve patient compliance and treatment outcomes for long-term brucellosis management. Future work should focus on validating these promising results through *in vivo* pharmacokinetic and efficacy studies in animal models of brucellosis, which is the essential next step toward clinical translation.

Declarations

Ethics approval and consent to participate

All experimental procedures involving animals were conducted in accordance with the relevant guidelines and regulations of the Ethics Committee of Hamadan University of Medical Sciences. The study protocol was reviewed and approved by the Ethics Committee of Hamadan University of Medical Sciences (Approval No.: IR.UMSHA.REC.1402.168).

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analyzed during this study can be obtained from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Study conception and design were carried out by SM.H and M.A. Data collection, analysis, and interpretation were performed by N.M., S.K., F.K., and M.A. The initial draft of the manuscript was prepared by SM.H. Critical revision of the manuscript for substantial intellectual input was conducted by S.K., M.A., SM.H., M.A., P.K., and F.K. The final version of the manuscript was approved for submission by M.A. and SM.H.

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Figures

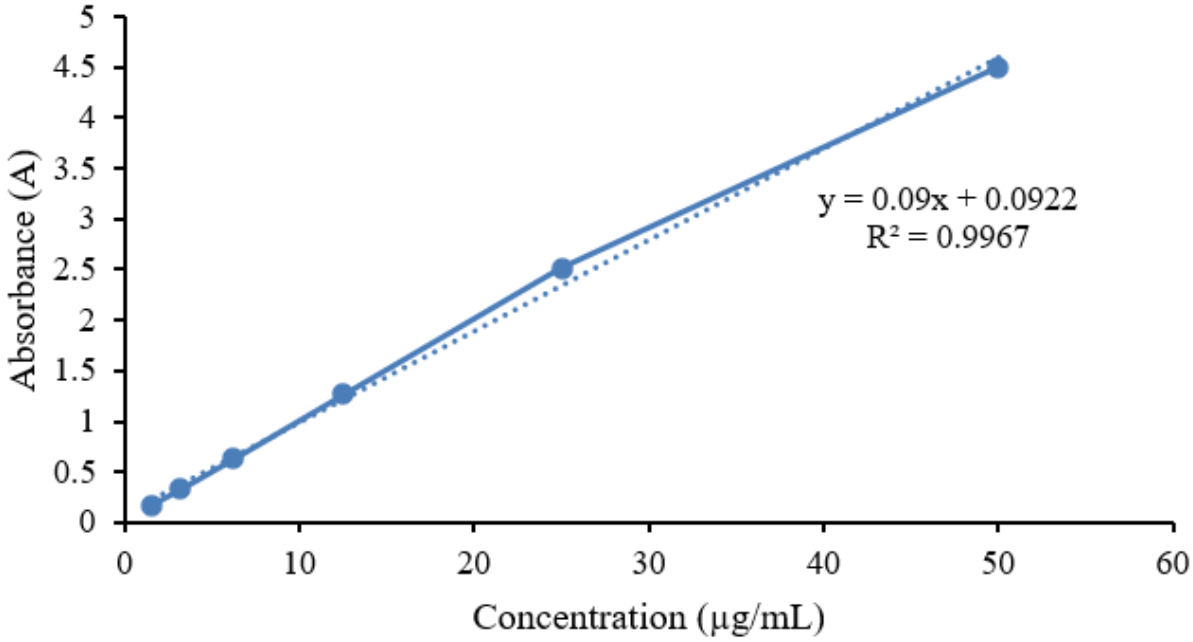


Figure 1

Standard calibration curve of gentamicin constructed by plotting concentration versus absorbance for quantitative analysis. The curve was used as an internal standard reference for subsequent measurements.

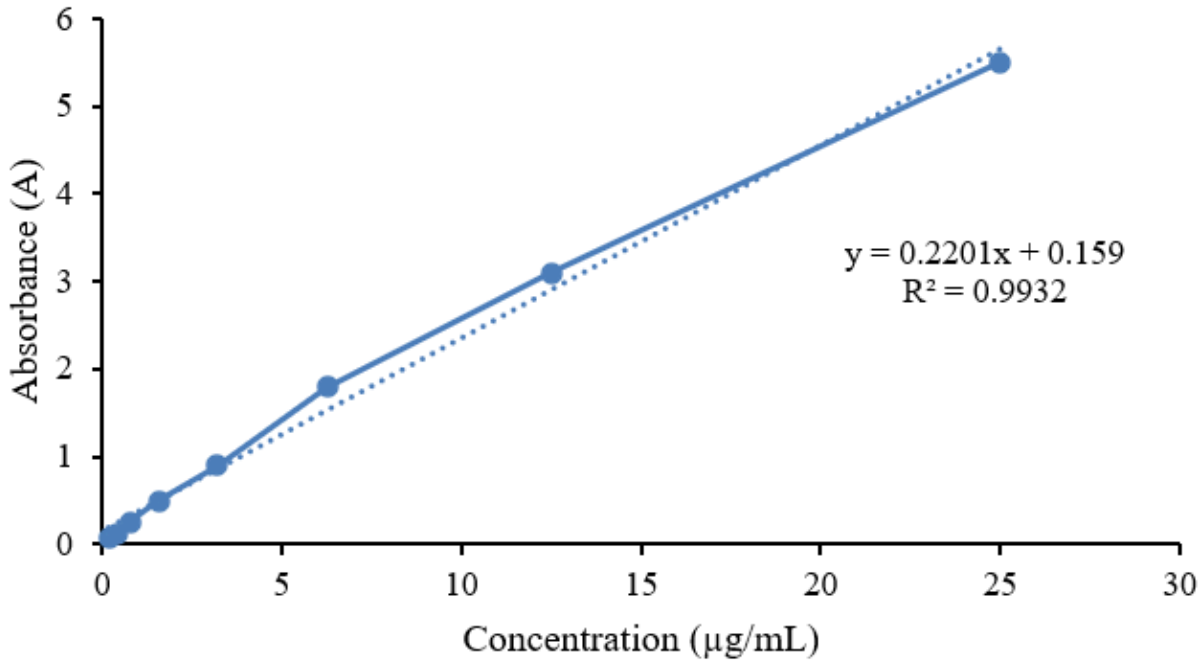


Figure 2

Standard calibration curve of rifampicin constructed by plotting concentration versus absorbance for quantitative analysis. The curve was used as an internal standard reference for subsequent measurements.

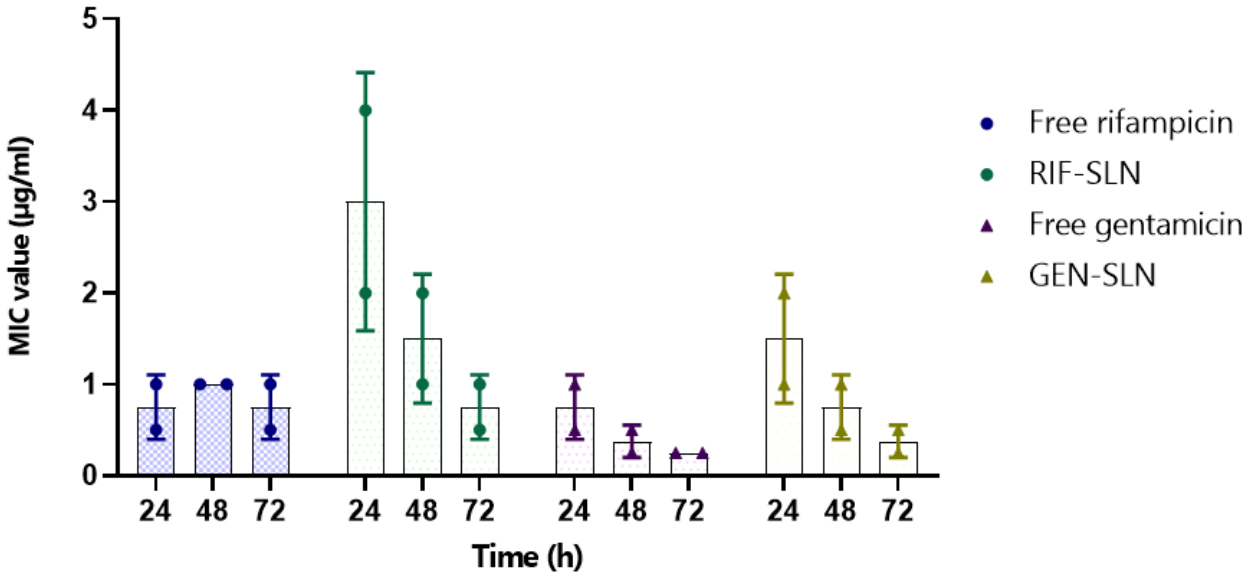


Figure 3

Minimum inhibitory concentration (MIC) values obtained after treatment with free drugs and corresponding nanoformulated drugs. Data are presented as mean ± standard deviation (SD) from [n = 3] independent experiments.

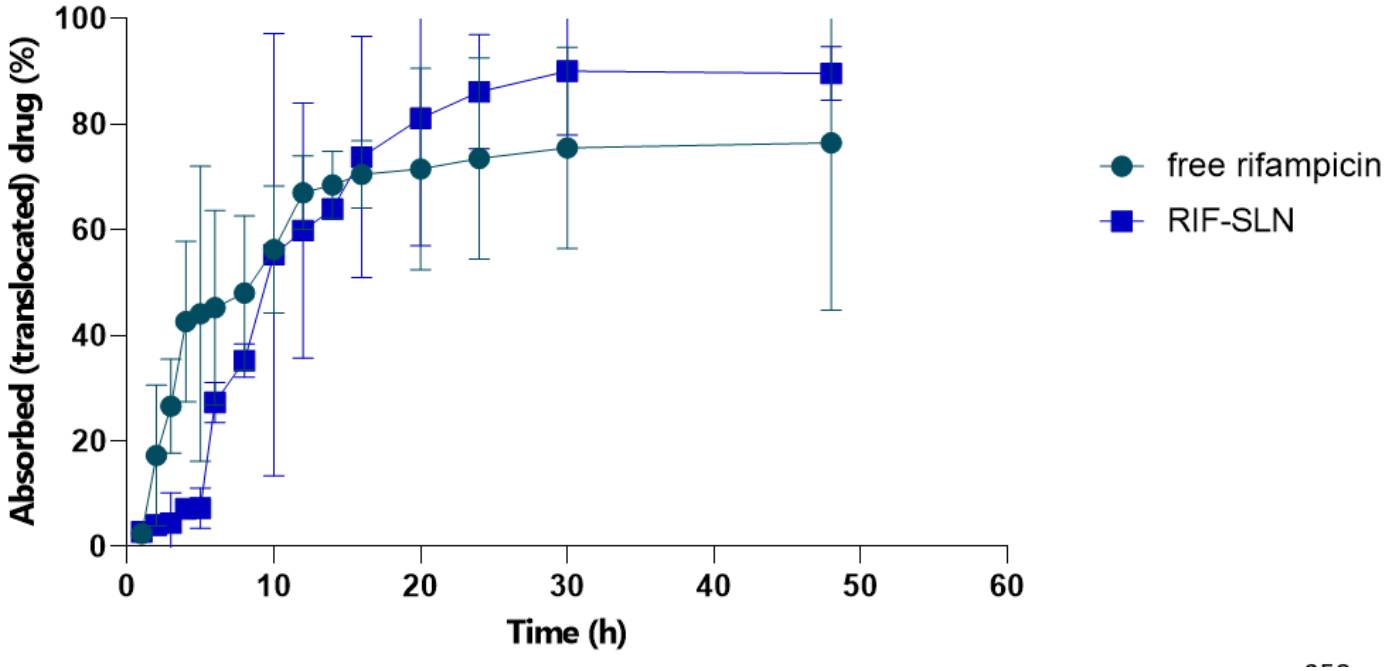


Figure 4

In vitro rifampicin penetration flux measured using Franz diffusion cells. Data are expressed as mean ± standard deviation (SD) from [n = 3] independent experiments.

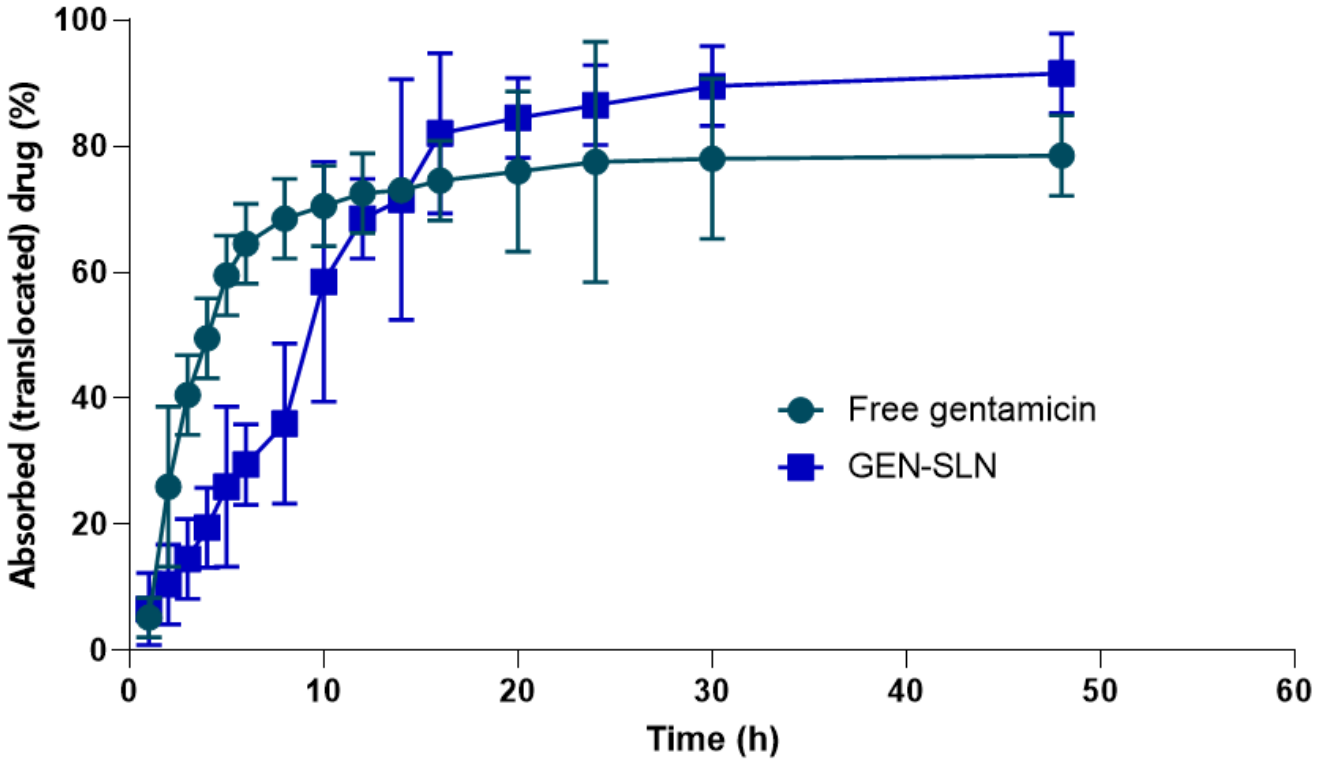


Figure 5

In vitro gentamicin penetration flux measured using Franz diffusion cells. Data are expressed as mean ± standard deviation (SD) from [n = 3] independent experiments.