Preparation and Evaluation of Nanolipid Carriers of Bedaquiline- *In vitro* Evaluation and *in silico* Prediction

Nandhini Rajendhiran[1], Sayani Bhattacharyya[1*]

[1] Department of Pharmaceutics, Krupanidhi College of Pharmacy, Bengaluru, Karnataka, India.

ABSTRACT

Background: Bedaquiline, a potent antitubercular drug used in the treatment of multidrug-resistant strains, suffers from low oral bioavailability, a slow onset of therapeutic action, and side effects. This investigation proposes the development of nanocarriers for the drug to improve drug release and estimate its effect on oral absorption through an in-silico model. Initially, a custom design was investigated to estimate the effects of composition and process on the entrapment and particle size of the carriers. The nanocarriers were subjected to studies on surface characteristics, surface morphology, thermal properties, drug release, ex vivo permeation, and antimicrobial efficacy. In silico predictions of bioavailability and pharmacokinetic parameters of the optimized formulation were conducted using GastroPlus® software.

Results: The study revealed that bedaquiline entrapped in nano lipid carriers (65.5 nm) of glyceryl behenate and palm oil effectively increased the rate of drug release by more than 80% and led to a 3.5-fold increase in antimicrobial activity against Mycobacterium tuberculosis. Intestinal permeation was enhanced by 3.7 times. Predictions using GastroPlus® software indicated that the nano lipid carrier of bedaquiline could be a promising method for improving the drug's efficacy with better localization in the gastrointestinal compartments and improved pharmacokinetics, achieving 93% bioavailability.

Conclusion: It can be concluded that bedaquiline nanocarriers in a lipid matrix can serve as an effective tool for enhancing the efficacy of bedaquiline in the treatment of tuberculosis.

Keywords: Nano lipid carriers, Bedaquiline, Bioavailability, Permeation, *In Silico* prediction.

1. INTRODUCTION

Tuberculosis (TB), a contagious disease caused by *Mycobacterium tuberculosis*, is one of the leading causes of illness and death worldwide [1]. A major problem in the pharmacotherapy of TB is the occurrence of drug-resistant strains of bacteria [2]. Drug resistance is a man-made phenomenon and occurs due to non-compliance with the long dosage regimen [3]. Intrinsic resistance of the bacterial cell wall to drug penetration and bacterial mutations also contribute to resistance development [4]. The types of resistance include mono, poly, multi, and extensive. Resistance to first-line treatments, such as isoniazid and rifampicin, has become a significant therapeutic challenge in TB treatment, necessitating the development of novel strategies to overcome drug resistance and enhance drug potency against both drug-susceptible and resistant strains.

Bedaquiline is a diarylquinoline derivative indicated for multi-drug-resistant TB, typically used in combination therapy for the treatment of adult and pediatric patients [5]. The oral dose of the drug is 100 mg. It is categorized as a Biopharmaceutical Classification System (BCS) II drug, has a very slow

[*]***Corresponding author: Sayani Bhattacharyya***
sayanibh@gmail.com
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onset of action, and is extensively metabolized in the liver [6]. Side effects associated with oral bedaquiline delivery include cardiac arrhythmia, muscle stiffness, and joint pain [7]. According to available reports, the bioavailability of bedaquiline increases twofold in the presence of food [8]. The fatty component of food is a key factor in enhancing the absorption and bioavailability of lipophilic drugs [9].

Therefore, effective and safe development of nanotechnology methods would be beneficial in overcoming the challenges associated with oral drug administration. Nanocarriers can increase drug solubility, enabling lower doses and improving treatment safety and efficacy.

Nano lipid particles appear to be promising systems for the oral delivery of lipophilic drugs among the various types of nanoparticles examined, primarily due to their excellent surface-active characteristics, nontoxicity, stability, and good biocompatibility [8]. Additionally, their small particle size allows them to reach the cellular level, their high surface-to-volume ratio enhances interaction with target cells, and their improved water solubility and ability to penetrate thick bacterial cell walls make them effective carriers for overcoming therapeutic limitations [10].

Nano lipid carriers (NLCs), composed of physiological and biodegradable solid and liquid lipids, belong to second-generation nanoparticles. They are beneficial for drug targeting and improving the oral bioavailability of poorly aqueous soluble drugs [11]. An imperfect or less ordered structure is created in the lipid matrix, which plays a crucial role in enhancing drug entrapment and reducing drug expulsion, thereby providing a continuous release of the drug at a consistent rate. NLCs exhibit a bi-phasic drug release pattern, with an initial burst release from the outer layers followed by a slow release from the solid lipid core [12].

Therefore, the present study focuses on the preparation of nano lipid carriers of the anti-tuberculosis drug bedaquiline to enhance its solubility and bioavailability [6]. Oral drug absorption is a complex process, and predicting it in humans remains a challenge. Pharmaceutical research must translate in vitro data into in vivo data for the safe and effective development of pharmaceuticals. This study includes an in-silico prediction tool to estimate the viability of the proposal. The Advanced Compartmental Absorption Transit (ACAT) model in the GastroPlus® software (Simulations Plus, Lancaster, CA) considers physicochemical, physiological, and formulation factors for predicting oral absorption. It also detects the involvement of transporters and/or enzymes. Hence, the study includes the use of GastroPlus® software to correlate in vitro evaluations with the prediction of in vivo performance of NLCs of bedaquiline.

2. MATERIALS AND METHODS

Bedaquiline was provided as a gift sample from Viatris, Hyderabad, India. Stearic acid, palm oil, oleic acid, vitamin E, and olive oil were purchased from SD Fine Chemicals, Mumbai, India. Glyceryl behenate was gifted by Gattefosse Sas, Mumbai, India.

2.1 Screening of lipids for the preparation of bedaquiline loaded NLC

NLCs represent a binary system with an imperfect lipid core. The drug solubility in this less ordered structure determines its physicochemical properties and the final effectiveness of the formulation. The selection of solid and liquid lipids for preparing bedaquiline-loaded nano lipid carriers was performed using a solubility method [13]. For solid lipids, 10 mg of the drug was added to gradually increasing amounts of molten solid lipids (stearic acid, glyceryl monostearate, and glyceryl behenate). The mixture was heated in a controlled-temperature water bath to obtain a clear molten mass. The minimum amount of molten lipid required to solubilize the drug and form a clear molten state was estimated [14].

Various liquid lipids, including vitamin E, olive oil, palm oil, and oleic acid, were screened. A fixed volume (2 mL) of each liquid lipid was placed in an Eppendorf tube. Each tube, containing an excess of the drug, was shaken in a mechanical shaker for 24 hours at 37°C and 100 rpm. The tubes were then centrifuged at 5000 rpm for 15 minutes to separate the undissolved drug and collect the supernatant [15]. The supernatant was suitably diluted and analyzed chromatographically to estimate drug solubility. Chromatographic analysis was performed using a C18 column, with a mobile phase of acetonitrile and 0.1% trifluoroacetic acid in a 50:50 ratio, at a flow rate of 1 ml/min and ambient temperature. The sample was analyzed at 242 nm [16]. A calibration curve was generated in the dilution range of 50-200 µg/ml with a linearity equation of Y = 2.325X + 21664 (where Y represents peak area and X represents concentration (µg/ml)), and the correlation coefficient was calculated to be 0.9999.

2.2 Method of preparation of nanocarriers of Bedaquiline:

A custom design was applied to observe the effects of material and process attributes on the development of NLCs of bedaquiline using JMP software V16. Extensive preformulation work was carried out to identify the parameters and their levels for the design.

The independent variables included the solid-to-liquid lipid ratio, surfactant concentration, homogenization time, and speed, while the amount of drug and total lipid content of the formulations were kept constant. The drug content in each formulation was maintained at 10% w/w of the total lipid. The dependent variables measured were drug entrapment and particle size, as outlined in Table 1. Fourteen experimental trials were conducted according to the design.

Table 1 Variables with coded levels

Independent variables	**-1**	**0**	**+1**
Solid Lipid: Liquid Lipid	70:30	80:20	90:10
Conc of SAA (%)	5	7.5	10
Homogenization Time (min)	5	7.5	10
Homogenization Speed (rpm)	5000	7500	10,000
Dependent Variables			
Particle size (nm)	Minimize		
Entrapment efficiency (%)	Maximize		

*SAA- Surfactant

Bedaquiline-loaded NLCs were prepared using the high shear homogenization method. The drug, solid lipids, and liquid lipids were accurately weighed and melted in a water bath at 70°C with continuous stirring. A surfactant solution of Tween 80 in water (1.5% w/v) was maintained separately at 70°C. The surfactant solution was gradually added to the lipid dispersion at high speed for a sufficient time as per the design. The resulting emulsion was poured into a petri dish and allowed to solidify on ice with gentle stirring for 15 minutes [17].

2.3 Evaluation of nanocarriers of bedaquiline

2.3.1 Particle size analysis

Dynamic light scattering (DLS) was used to estimate the particle size of the nano lipid carriers, utilizing a Horiba SZ-100 (Germany). The samples were dispersed in Millipore water, and the analysis was conducted at 25°C. The particle size for each trial formulation was recorded as the average of three trials ± SD.

2.3.2 Estimation of entrapment efficiency

Entrapment efficiency measures the effectiveness of

the carriers in encapsulating the drug. Approximately 5 mg of bedaquiline-loaded NLCs were dispersed in 10 ml of methanol and vortexed. The sample was filtered using a 0.45 µm syringe filter and analyzed chromatographically to estimate the total drug (Td). A separate quantity of the sample was dispersed in distilled water, centrifuged at 10,000 rpm for 30 minutes, and the supernatant was collected, diluted, and analyzed chromatographically to estimate the free drug (Fd) [18]. Three trials were conducted for the estimation, and the % entrapment efficiency was calculated using the formula:

% Entrapment efficiency = {(Td -Fd) ÷ Td} × 100

The same procedure was repeated for blank formulations in all experimental trials to account for the effects of excipients during estimation. After completing all experimental trials, model validation and optimization were performed at a significant level of $p < 0.05$. The optimized formulation (Fopt) was prepared and subjected to further analysis.

2.3.3 Powder X ray diffraction study (PXRD)

To characterize the crystalline state of the drug in the formulation, powder X-ray diffraction (Rigaku SmartLab 3kW, Japan) was performed for the pure drug and formulation Fopt using Cu Kα radiation at 25°C. The scanning was conducted at a 2θ (diffraction angle) range from 2 to 70 degrees. The diffraction pattern was recorded with a vertical goniometer [19].

2.3.4 Differential Scanning calorimetry (DSC)

Thermal analysis of the pure drug bedaquiline and formulation Fopt was performed using a differential scanning calorimeter (SHIMADZU DSC-60). A precisely weighed sample (5 mg) enclosed in an aluminum pan was heated at a predetermined scanning rate (10°C/min) from 30°C to 400°C. Dry nitrogen gas (at a flow rate of 25 mL/min) was used as the carrier gas [20].

2.3.5 Scanning electron microscopy (SEM)

The surface characteristics of the pure drug and formulation Fopt were examined using scanning electron microscopy with a gold sputtering technique (Hitachi 3400S, Japan). The powder samples were fixed onto a stub, gold was sputtered onto the samples, and the sample slides were subjected to vacuum drying. The samples were investigated at 10 kV, and photographs were taken at different magnifications [21].

2.3.6 *In vitro* drug release study

The in vitro release of the drug from formulation Fopt was estimated using the dialysis bag method (molecular weight cut-off of dialysis membrane: 12-14 kDa; pore size: 2.4 nm). The dialysis bag was pre-soaked in biorelevant media (Fed State Simulated Intestinal Fluid [FESSIF], pH 6.8) for 12 hours. The formulation Fopt (10 mg equivalent bedaquiline) dispersed in 2 mL of water was placed into the dialysis bag and immersed in a beaker containing 100 mL of FESSIF. The beaker was magnetically stirred at 100 rpm, and the temperature was maintained at 37°C. Specific volumes of aliquots were withdrawn at regular intervals and replenished with equal volumes of media. Sampling was conducted for 24 hours. The samples were analyzed at 242 nm using the established chromatographic technique. The cumulative amount of drug release was estimated. The data were analyzed according to different kinetic models: zero-order, first-order, Higuchi, and Korsmeyer-Peppas models [12, 22].

2.3.7 *Ex vivo* permeation study

Ex vivo permeation studies were performed for Fopt and the pure drug. Freshly excised goat ileum from a local slaughterhouse was used for the study. The ileum was cut into 5.5 cm pieces and washed initially with saline solution, followed by phosphate buffer (pH 7.4). A weighed quantity of 10 mg equivalent of the sample, dispersed in 1 mL of water, was introduced into the ileum and then placed in a beaker containing 50 mL of FESSIF (pH 6.8) under magnetic stirring. The temperature was maintained at 37±0.5°C with continuous aeration. At each time point, 1 mL of the sample was withdrawn at specified intervals up to 24 hours and replaced with an equal volume of phosphate buffer. The samples were analyzed chromatographically at 242 nm. A plot of cumulative permeation of bedaquiline versus time was

created. Apparent permeability (Papp) was calculated using the following equation:

$$Papp = \frac{Q}{ACT}$$

Where Q=total amount of drug permeation, A= surface area of the intestine, C=initial concentration of drug, t=total time of permeation study.[23]

The same process was repeated for pure drug and apparent permeability was compared with the optimized formulation.

2.3.8 Antimicrobial Study

The test organism studied was Mycobacterium tuberculosis (H37RV Strain, ATCC No. 27294). The anti-mycobacterial activity of the pure drug and Fopt was assessed using the microplate Alamar Blue assay (MABA). The microdilution broth technique was used to determine the minimum inhibitory concentration (MIC). Sterile 96-well microplates were initially filled with 200 µL of sterile deionized water, followed by 100 µL of Middlebrook 7H9 broth. Serial dilutions of the compounds (100 to 0.2 µg/mL) were prepared on the plates. The plates were incubated for five days at 37°C. Freshly prepared Alamar Blue reagent and 10% Tween 80 (1:1 mixture) were added to the plates and incubated for an additional 24 hours. A blue color in the wells indicated no bacterial growth, while the formation of pink color was considered growth. The minimum inhibitory concentration (MIC) was estimated [24].

The measurement of colony-forming units (CFU) was carried out for the pure drug and Fopt using Middlebrook 7H9 broth supplemented with 10% OADC at the MIC concentration, infected with the Mycobacterium strain. The agar plates were incubated for 48 hours at 37°C. After the incubation period, the CFUs were quantified [25].

2.3.9 *In silico* prediction of oral absorption of bedaquiline NLCS

GastroPlus® 9.5 was used to predict the possible absorption model of the bedaquiline NLCs in a human model. [26] The pharmacokinetics were evaluated with ADMET Predictor v8.5.0.0. [27] An absorption simulation model was created for Fopt for a 100 mg oral dose. The physicochemical properties predicted by GastroPlus® version 9.5 (Simulations Plus) and ADMET Predictor version 8.5 (Simulations Plus) were used for the simulation and are listed in Table 2.

Table 2 Physicochemical properties of Bedaquiline predicted by GastroPlus

Property	Predicted
Molecular formula	C32H31BrN2O2
Molecular weight	555.52
Predicted log P	6.1
Dosage form (human)	IR tablet
Dose (mg)	100
Dosing interval (h)	56
Mean Precipitation time	900
Diffusion coeff (cm^2/S)	0.54 X 10^{-5}
Solubility (mg/ml)	2.89X 10^{-3} at pH 8.52
Drug Particle density(g/ml)	1.2
Particle size in radius(µm)	0.065
Dosage volume (ml) (human)	250

The human physiology was represented as a series of compartments, each defined with the default values for the drug absorption process, as listed in Table 3.

The outcome of the model was evaluated for oral bioavailability and pharmacokinetics and compared with data available in the literature.

3. RESULT

4. *Screening of lipids for the preparation of bedaquiline loaded NLC*

The solubility of bedaquiline was determined in various liquid and solid lipids. Among the tested lipids, bedaquiline demonstrated the highest solubility in glyceryl behenate and palm oil, as shown in Table 4.

4.1 Evaluation of nanocarriers

A total of 14 experimental runs were generated using a custom design with three center points, employing JMP version 16 software. The experiments varied the composition, homogenization speed, and time to assess their effects on entrapment efficiency and particle size. The entrapment efficiency of the drug ranged from 56% to 87%, while the particle size ranged from 60 to 70 nm. The recorded responses are detailed in Table 5.

Table 3 Default Gastrointestinal Physiology in GastroPlus

GI Compartment	ASF	pH	Transit time (min)	Volume (ml)	Bile
Stomach	0	1.30	0.25	46.56	0
Duodenum	2.741	6	0.26	41.56	2.8
Jejunum 1	2.719	6.2	0.93	154.2	2.33
Jejunum 2	2.719	6.4	0.74	122.3	2.03
Ileum 1	2.709	6.6	0.58	94.29	1.41
Ileum 2	2.688	6.9	0.42	70.53	1.16
Ileum 3	2.677	7.4	0.29	49.83	0.14
Caecum	7.221	6.4	4.19	47.49	0
Asc Colon	21.49	6.8	12.57	50.33	0

Table 4 Solubility of bedaquiline in different lipids

Lipids	Solubility (mg/gm)
Stearic acid (S)	0.51±0.37
Glyceryl monostearate (S)	1.09±0.24
Glyceryl behenate (S)	1.65±0.19
Vitamin -E (L)	0.63±0.21
Olive Oil (L)	0.33±0.05
Palm oil (L)	0.88±1.10
Oleic acid (L)	0.13±0.10

*S and L denotes solid and liquid lipid respectively

Table 5 Evaluation of NLCs as per custom design.

Formulation code	SL: LL	%Conc of SAA	Homogenization time (min)	Homogenization speed(rpm)	% EE	Particle size(nm)
F1	1	-1	1	1	70.52±0.06	66.1±11.4
F2	0	0	0	0	62.39±0.04	68.0±14.2
F3	1	1	1	-1	73.26±0.05	68.1±25.1
F4	-1	-1	1	1	65.13±0.05	66.2±20.2
F5	1	1	0	1	70.12±0.01	66.8±13.7
F6	-1	1	1	-1	58.39±0.02	70.8±19.2
F7	0	0	0	0	58.45±0.03	69.1±12.8
F8	-1	1	-1	1	56.25±0.04	65.6±24.1
F9	1	-1	-1	1	59.38±0.07	60.5±21.7
F10	1	-1	1	-1	68.26±0.08	60.1±17.4
F11	1	1	-1	-1	81.30±0.04	60.3±10.6
F12	1	-1	-1	-1	87.16±0.03	60.5±19.3
F13	0	0	0	0	59.59±0.01	68.2±22.5
F14	-1	-1	-1	-1	68.51±0.03	69.1±15.6

The data for both responses were analyzed statistically at a significance level of $p<0.05p < 0.05p<0.05$. Model validation was conducted using an actual vs. predicted plot, as shown in Fig. 1.

The main factors affecting the responses are illustrated in the response surface diagrams, which highlight the positive impact of homogenization conditions on particle size and formulation factors on the percentage of drug entrapment, as presented in Fig. 2.

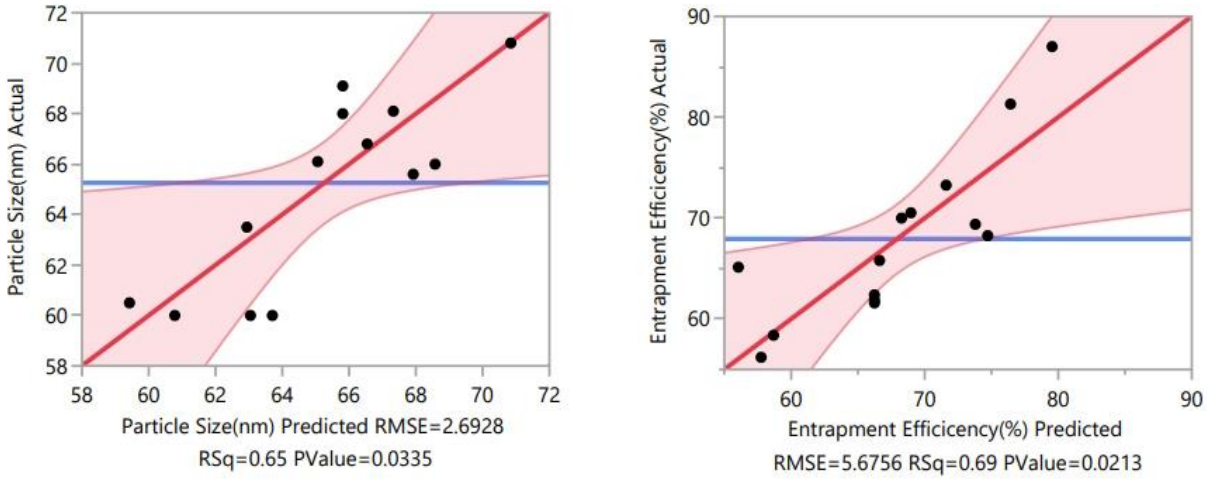

Fig.1 Predicted vs. actual plot for the responses.

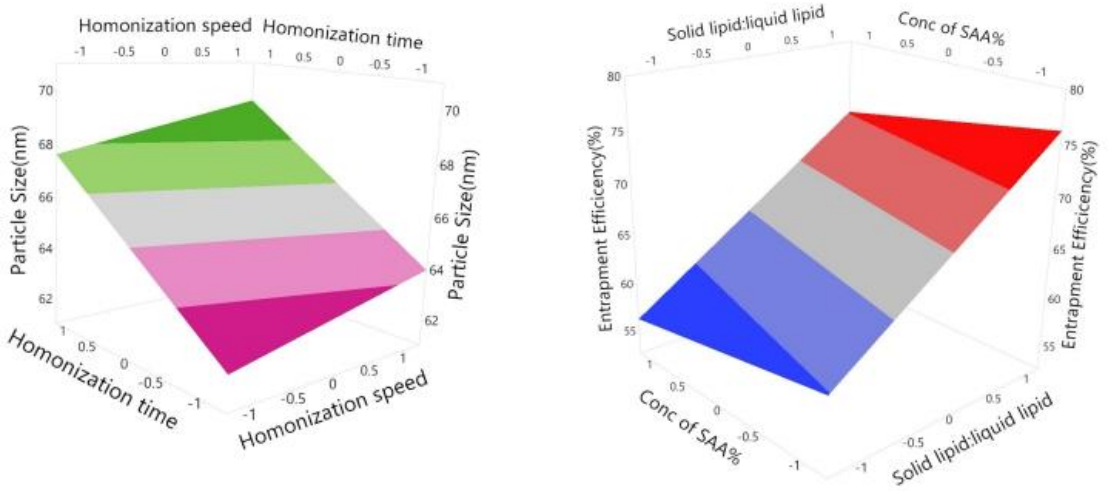

Fig.2 3D surface plots of the responses with the factors.

Mathematical optimization was carried out with a desirability factor set above 0.5. The optimization predicted a formulation with coded values for lipid ratio, surfactant concentration, homogenization time, and homogenization speed of 1:0:1:0, yielding a desirability of 0.568, as shown in Fig. 3. The prediction estimated a particle size of 66.19 nm and a drug entrapment percentage of 70.28%. The optimized NLCs were formulated according to these predicted conditions and analyzed for their responses. The formulation Fopt exhibited a particle size of 65.57 ± 11.3 nm and a drug entrapment percentage of 66.82 ± 0.89%. The percentage bias between the predicted and observed responses was less than 5%.

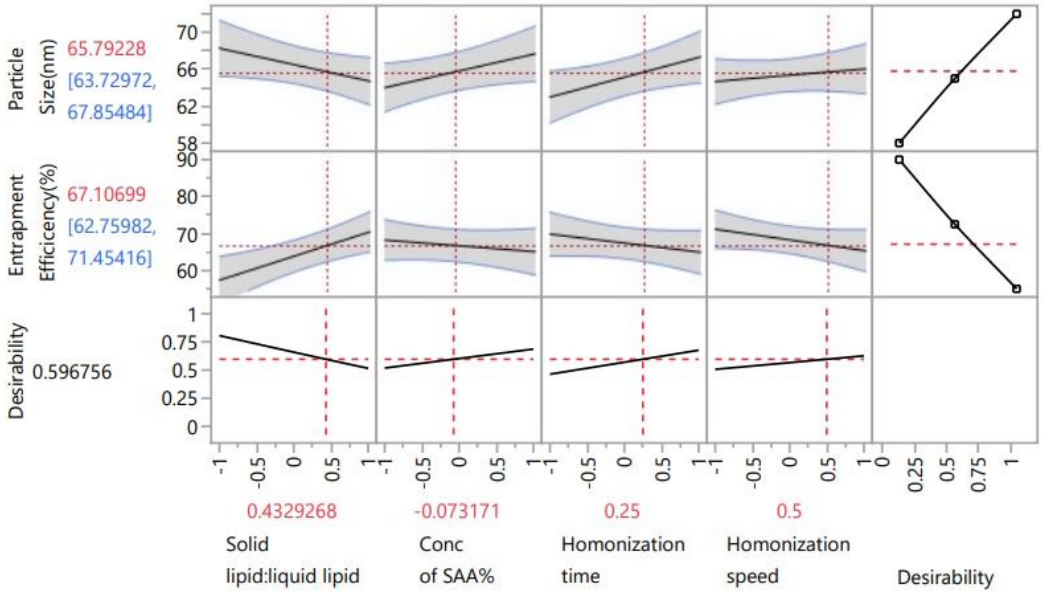

Fig.3 Prediction profiler for the custom design

4.2 PXRD

The PXRD patterns of the pure drug and Fopt are shown in Fig. 4. The pure drug exhibited high-intensity characteristic peaks at 2θ values of 6.11°, 19.36°, 21.53°, 23.18°, and 36.67°. In the formulation, the intensity of these peaks was reduced.

4.3 DSC

The thermogram of the pure drug bedaquiline showed a characteristic endothermic peak at 180°C. In contrast, the thermogram of the formulation Fopt did not exhibit a peak at the same temperature, as shown in Fig. 4.

4.4 SEM

The SEM images of the pure drug and Fopt are shown in Fig. 5. Surface characterization was performed at different magnifications, revealing the morphology of both the pure drug and the formulation.

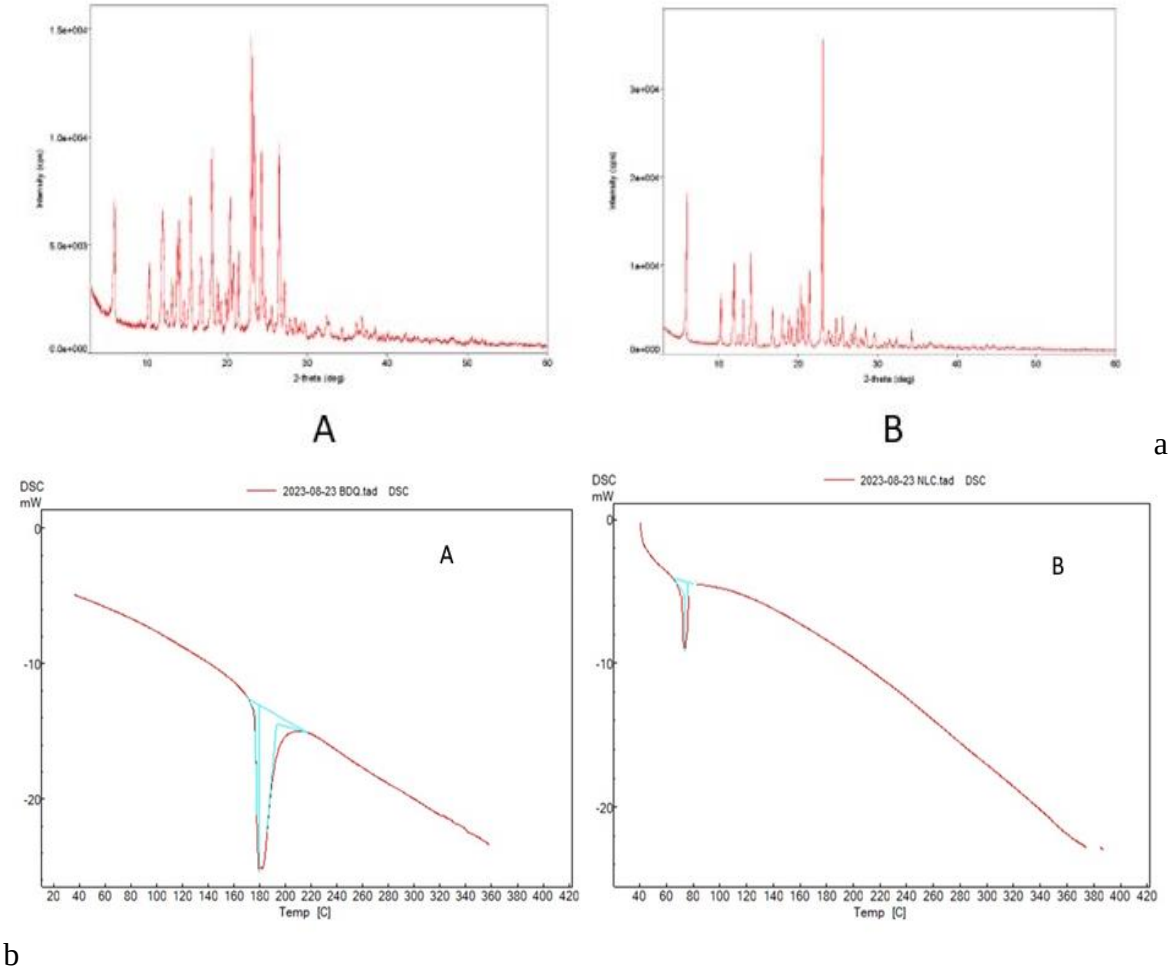

Fig.4 PXRD (a) and DSC thermogram (b) for pure drug: A Fopt: B

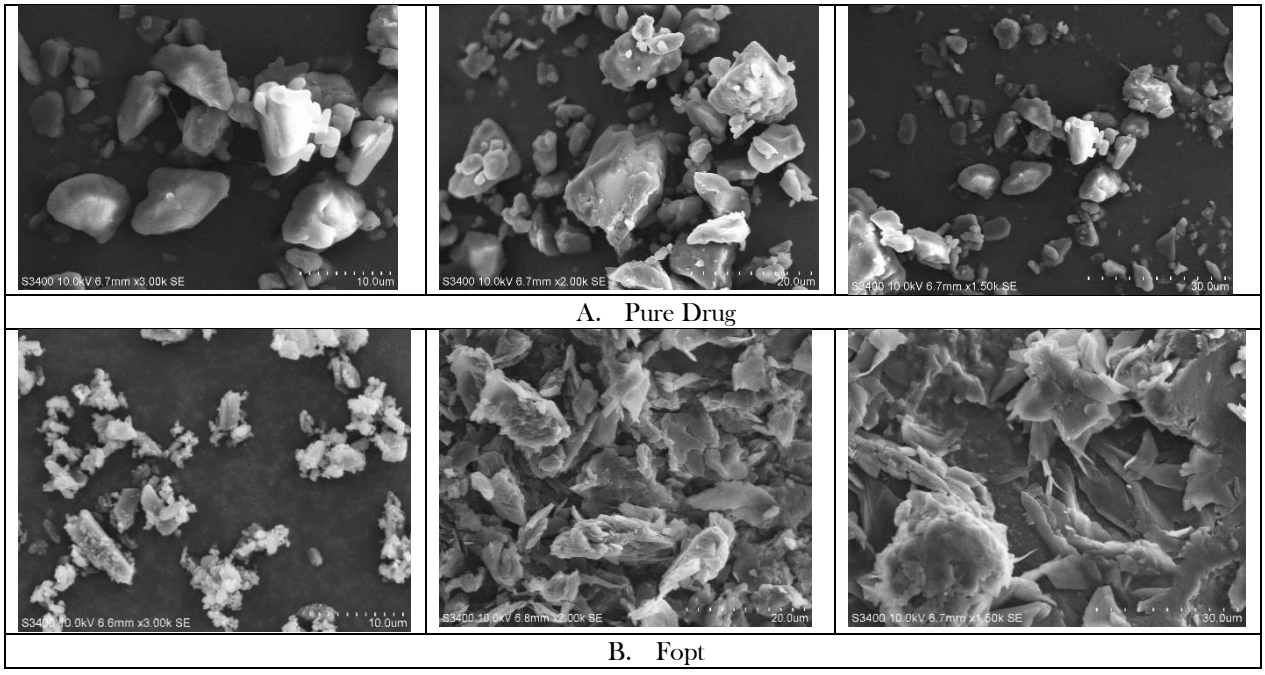

Fig.5 SEM image of pure drug (A) SEM image of Fopt (B)

4.5 In vitro drug release study

The in vitro release study was conducted over 24 hours, revealing a biphasic release profile of the drug from the nanocarriers, as shown in Fig. 6. Initially, a rapid release of the drug was observed, followed by a sustained release phase from the NLCs.

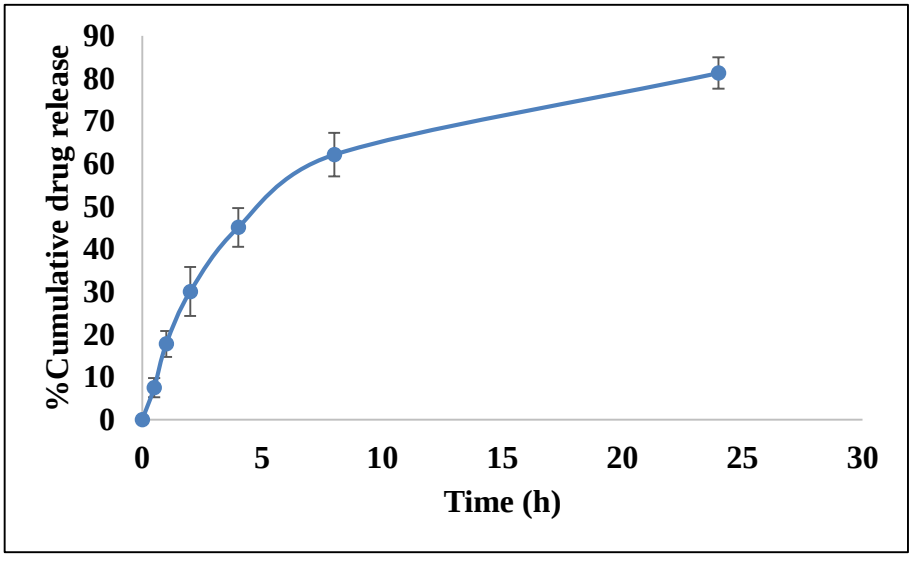

Fig.6 In vitro drug release study of Fopt (mean(n=3) ± SD)

Various kinetic models were explored for the in vitro release pattern. It was found that the regression coefficient (R^2) was highest for the Higuchi release model (R^2 = 0.999), and the release also followed non-Fickian diffusion according to the Korsmeyer-Peppas model (release exponent n = 0.744).

4.6 Ex vivo permeation study

The ex vivo permeation study was performed using the non-inverted intestinal sac method. The optimized formulation (Fopt) demonstrated 3.7 times greater potential for diffusion and permeation compared to the pure drug, as shown in Fig. 7.

4.7 Antimicrobial Study

The formulation was evaluated for antibacterial activity. The formulations proved to be effective, with significant antibacterial efficacy. The minimum inhibitory concentration (MIC) for Mycobacterium tuberculosis (Vaccine strain, H37RV strain) was found to be 1.6 µg/mL for formulation Fopt and 0.08 µg/mL for bedaquiline. After 48 hours of incubation, the colony-forming units (CFUs) for the pure drug and Fopt were found to be 35 ± 4.5 and 10 ± 2.5, respectively. A significant reduction in CFUs was observed at the MIC concentrations of the formulation compared to the pure drug.

4.8 In silico prediction of oral absorption of bedaquiline NLCs

The ADMET predictor revealed low penetration of the formulation through the blood-brain barrier (BBB), 97% inhibition of P-glycoprotein (Pgp) and breast cancer resistance protein (BCRP) transporters, and the likelihood of drug absorption in various gastrointestinal compartments, as shown in Fig. 8. The predicted outcome supports enhanced absorption of the drug through the intestine. The absorption rate in the Advanced Compartmental Absorption and Transit (ACAT) model was calculated using Log D. The predicted bioavailability (F) and pharmacokinetic parameters are presented in Table 6. The predicted plasma concentration-time graph, shown in Fig. 9, indicates very low hepatic and biliary clearance.

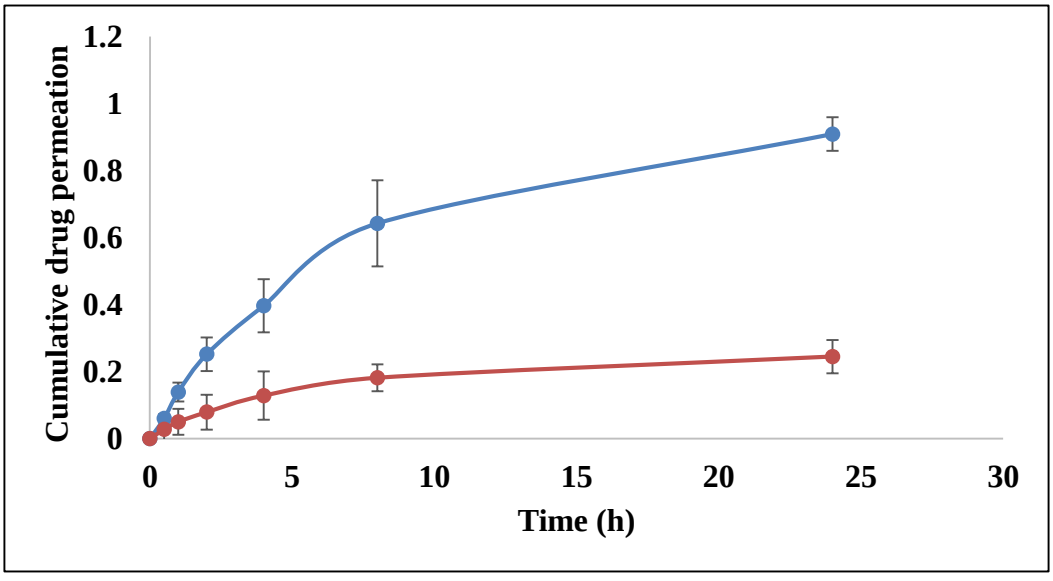

Fig.7 Ex vivo permeation study (mean(n=3) ± SD)

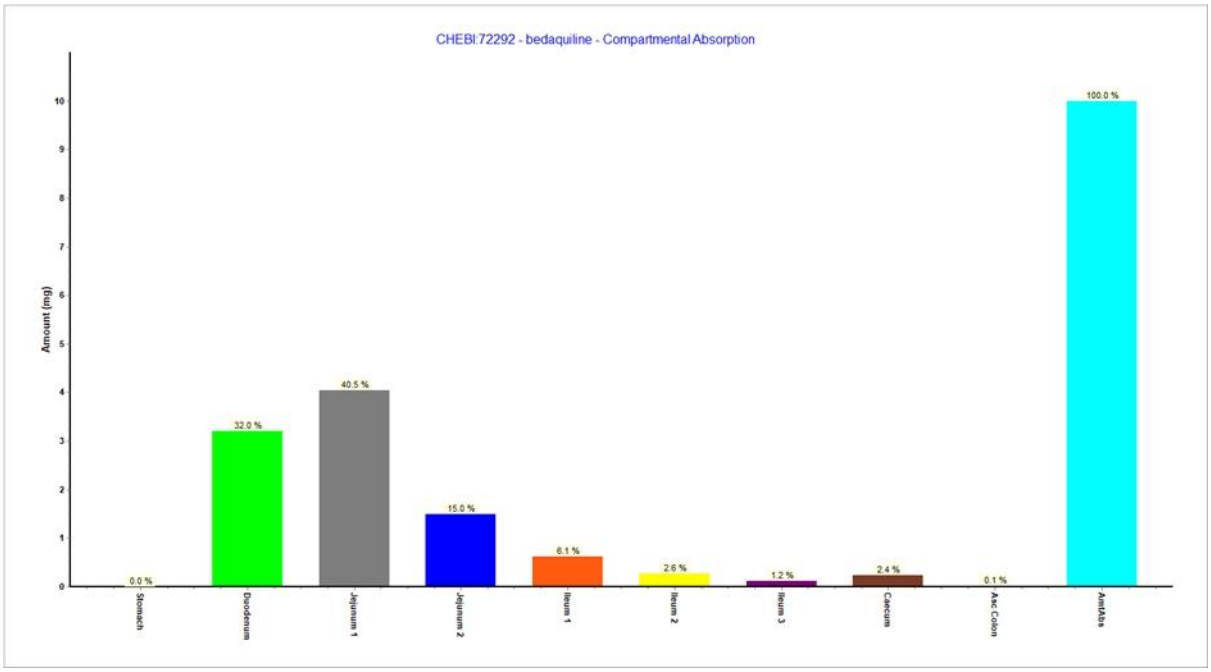

Fig.8 GastroPlus® prediction of drug absorption from Fopt in different GI compartments.

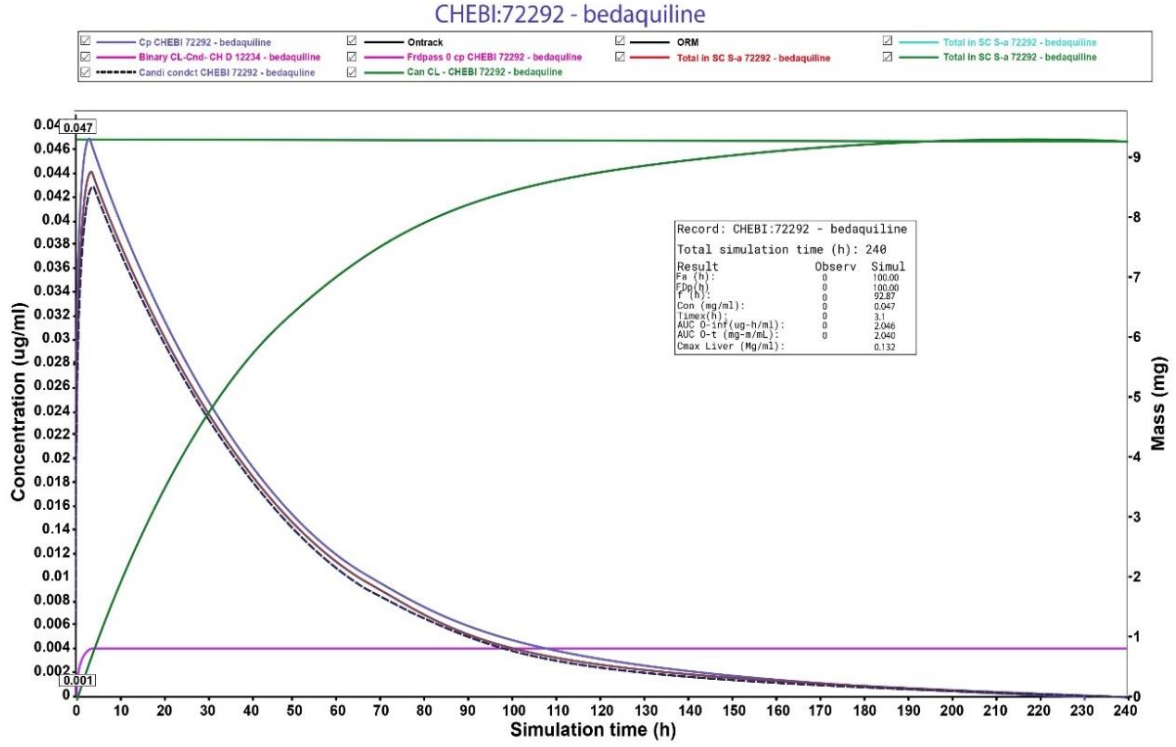

Fig.9 GastroPlus® prediction of plasma concentration of Fopt following oral administration

Table 6 Predicted bioavailability (F) and pharmacokinetics parameters

Parameters	**Predicted**	**CV%**	**Reported data**[28]
Bioavailability (F%)	92.869	1.57	*15.1*
Cmax (µg/ml)	0.468	16.079	0.24
Tmax (h)	3.1	27.202	4.3
AUC $_{0\text{-inf}}$ (µg-h/ml)	20.456	32.	43.5

5. DISCUSSION

The solubility of the drug in solid and liquid lipids is a crucial determinant for the entrapment and successful formulation of nano lipid carriers (NLCs). Lipids were screened to identify those with good affinity for the drug, ensuring high entrapment efficiency in the lipid carriers. Based on the solubility studies, glyceryl behenate and palm oil were selected as the solid and liquid lipids, respectively, for the preparation of bedaquiline-loaded nano carriers.

Bedaquiline belongs to Biopharmaceutical Classification System (BCS) class II, characterized by low solubility, a slow onset of action, and improved oral bioavailability in the presence of fatty food. Thus, the primary focus of this study was to develop a delivery system to enhance the solubility and dissolution of the drug using lipid components. Bedaquiline-loaded NLCs were proposed for this purpose. The quality objectives were to achieve high entrapment of the drug in a nanosized lipid carrier.

A custom design approach was chosen to closely monitor the effect of process and material attributes with the most optimal set of design points to meet the experimental needs for the preparation of NLCs of bedaquiline. This design generated a minimum number of trial runs, and three center points were included to avoid bias. Custom design diagnostics, such as D, G, and A efficiency, were found to be less than 100 and were 75.9, 59.2, and 72.93, respectively. These values satisfied the evaluation of the design for the diagnostics of the factors and the 14 experimental runs. The factor sensitivity analysis and response surface diagrams indicated that homogenization speed and time had a significant effect on particle size, whereas the formulation components, such as lipid ratio and surfactant concentration, had a substantial impact on drug entrapment. The optimized product exhibited less than 5% bias from the design prediction for both drug entrapment and particle size. Therefore, it can be concluded that the custom design approach can be successfully employed for the formulation of lipid nano carriers of bedaquiline and can yield nanosized formulations with high entrapment efficiency.

The PXRD pattern of the pure drug revealed its crystalline nature, with the peak intensity drastically reduced in the formulation Fopt [24]. This reduction in peak intensity might be due to the molecular dispersion of the drug in the solid and liquid lipids.

The broad endothermic peak at 180 ℃ in the DSC thermogram represents the melting point of the pure drug. The optimized formulation did not show the peak at the same temperature, indicating that the drug was completely dispersed in the lipids. The additional peak at 70 ℃ in the optimized formulation might indicate the presence of free lipids in the sample [30].

The surface morphology of the pure drug revealed its crystalline structure with angular-shaped flakes. The nanoparticles were discrete, with minimal conglomeration, and were in the nanometric range. The surfaces of the NLCs appeared irregular and rough.

The in vitro drug release study exhibited a biphasic release pattern. In the initial hours, drug release was rapid due to the displacement of the drug from the irregular outer surface of the NLCs, followed by a consistent release from the core of the lipid matrix [12]. The lipophilic nature of bedaquiline and its tight binding to the core of the NLCs

might be responsible for the consistent release of the drug. The rate kinetic of the release study indicated a case II transport mechanism, supporting the preliminary burst release from the outer crust, accompanied by diffusion through the inner core. Hence, it can be concluded that the initial accelerated and subsequent passive release of the drug from the carriers may offer promising results for better efficacy and improved oral bioavailability.

From the ex vivo study, it was found that the apparent permeability of the optimized formulation was 3.7 times greater than that of the pure drug. The nanosized formulation facilitated drug dissolution and permeation. The enhancement in permeation might be due to the availability of the solubilized drug at the site of absorption. The pure drug exhibited lower permeation due to its poor solubility.

The antimicrobial study demonstrated the efficacy of the nano lipid carrier of bedaquiline in controlling bacterial growth compared to the pure drug. The presence of lipids might have favored drug penetration through the bacterial cell wall [31], resulting in a reduction in the number of Mycobacterium colonies.

Bedaquiline exhibits poor oral bioavailability, a slow onset of action, and extensive hepatic metabolism, as reported in the literature [6]. The absorption of the drug is dissolution-rate limited. The absorption of the drug increases in the presence of lipids [6]. Therefore, the present study explored the in silico evaluation of NLCs of bedaquiline to overcome these limitations.

The ACAT absorption model was used to simulate the oral absorption of bedaquiline NLCs from the intestinal tract. The effect of bile salts on the dissolution of the drug was considered for each region of the gastrointestinal tract [32]. This effect was compared with the in vitro and ex vivo solubility and diffusion, respectively, in biorelevant media. It was predicted that the NLCs of bedaquiline might improve oral bioavailability by approximately 92% and overcome P-gp efflux transport. The time to reach maximum plasma concentration (tmax) was predicted to be significantly shorter compared to the pure drug. The pharmacokinetics (Cmax, AUC0-inf) of the drug were also found to be improved in the ACAT model for oral absorption in humans. The enhancement in pharmacokinetics and bioavailability might provide a better and safer pharmacotherapy for the treatment of TB.

Therefore, the overall study established that a nano lipid carrier of bedaquiline might ensure a safer and more effective regimen for the treatment of TB.

6. CONCLUSION

The present study aims to address the drawbacks associated with the oral administration of bedaquiline. A biocompatible platform for the drug was created using solid and liquid lipids. A statistical design was applied to develop nano-sized lipid carriers of bedaquiline using glyceryl behenate and palm oil. The optimized product demonstrated improved drug release and permeation through in vitro and ex vivo studies compared to the pure drug. The antimicrobial efficacy was significantly enhanced. The in-silico simulation for oral absorption showed promising results. The prediction indicated that both bioavailability and tmax could be improved significantly, while hepatic metabolism could be controlled to enhance the overall pharmacokinetics of the drug. Hence, the proposed approach can be explored in animal models for further confirmation of the outcomes and subsequent research on TB. It can be concluded that nano lipid carriers of bedaquiline offer a novel platform to overcome the challenges associated with its oral administration.

Abbreviations

TB	Tuberculosis
BCS	Biopharmaceutical classification system
NLCs	Nano lipid carriers
ACAT	Advanced Compartmental Absorption Transit
PEG	Polyethylene glycol

Vit E	Vitamin E
DLS	Dynamic light Scattering
TD	Total Drug
FD	Free Drug
SD	Standard deviation
Fopt	Optimized formulation
PXRD	Powder X ray diffraction study
DSC	Differential Scanning Calorimetry
SEM	Scanning electron microscopy
MABA	Microplate Alamar Blue assay
MIC	Minimum inhibitory concentration
CFU	Colony form units
R^2	Regression coefficient
GI	Gastrointestinal
ADMET	Absorption, distribution, metabolism, excretion and toxicity
ACAT	Advanced compartmental absorption and transport model

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Author contributions

SB was involved in the conceptualization, design, supervision, and analysis of the research outcomes. NR was involved in data generation and analysis. Both authors contributed to the preparation of the manuscript.

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Availability of data and materials

All data are presented in the manuscript. Queries regarding the data can be addressed to the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data supporting the conclusions are included within the article.

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تحضير وتقييم حاملات الدهون النانوية للبيداكويلين – التقييم في المختبر والتنبؤ بالسيليكو

ناندیني راجيندیران[1] و سایاني بهاتاشاریا[1]*

[1] قسم الصيدلة، كلية كروبانيدهي للصيدلة، بنغالورو، كارناتاكا، الهند.

ملخص

الخلفية: يعاني البيداكيلين، وهو عقار قوي مضاد للسل، يستخدم في علاج السلالات المقاومة للأدوية المتعددة، من انخفاض التوافر البيولوجي عن طريق الفم، وبطء بداية التأثير العلاجي، وآثار جانبية. يقترح البحث تطوير ناقلات نانوية للدواء من خلال تحسين إطلاق الدواء وتقدير تأثيره في الامتصاص عن طريق الفم من خلال نموذج السيليكو. في البداية، تم دراسة تصميم مخصص لتقدير التركيب وتأثيرات العملية على الانحباس وحجم الجسيمات للناقلات. خضعت الناقلات النانوية لدراسة خصائص السطح، وتشكل السطح، والحرارة، وإطلاق الدواء، والنفاذ خارج الجسم الحي، ودراسة فعالية مضادات الميكروبات. في التنبؤ السيليكو بشأن التوافر البيولوجي ومعلمات الحرائك الدوائية للتركيبة المُحسّنة، تم تنفيذها من خلال برنامج GastroPlus®.

النتائج: كشفت الدراسة أن البيداكويلين المحصور في ناقلات الدهون النانوية (65.5 نانومتر) من غليسيريل بيهينات وزيت النخيل زاد بشكل فعال من معدل إطلاق الدواء بأكثر من 80٪، وأدى إلى تكثيف النشاط المضاد للميكروبات ضد المتفطرة السلية بمقدار 3.5 مرة. تم تعزيز تخلل الأمعاء بمقدار 3.7 مرة. تم توسيع النتائج التي تم الحصول عليها للتنبؤ بالتوافر الحيوي عن طريق الفم من خلال برنامج GastroPlus® وكشفت عن حقيقة أن حامل الدهون النانوية للبيداكويلين يمكن أن يكون طريقة واعدة لتحسين فعالية الدواء مع مزيد من التوطين في الأجزاء المعدية المعوية، وتحسين الحرائك الدوائية بنسبة 93٪. التوافر البيولوجي. الاستنتاج ومن ثم يمكن أن نستنتج أن الناقلات النانوية للبيداكويلين في المصفوفة الدهنية يمكن أن تكون بمثابة أداة فعالة لتعزيز فعالية البيداكويلين في علاج مرض السل.

الكلمات الدالة: حاملات الدهون النانوية، بيداكيلين، التوافر الحيوي، التخلل، في تنبؤ السيليكو.

* ***المؤلف المراسل: سایاني بهاتاشاري***

sayanibh@gmail.com

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