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DEVELOPMENT AND CHARACTERIZATION OF NANOCARRIER RIFABUTIN CONTAINING SOLID LIPID NANOPARTICLES FOR THE TREATMENT OF TUBERCULOSIS

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ABSTRACT

This research focuses on the development and characterization of Rifabutin-loaded solid lipid nanoparticles (SLNs) for improved tuberculosis treatment. Rifabutin, an effective anti-tubercular agent, was encapsulated in SLNs using a microemulsion technique. The study optimized various formulation parameters, including lipid composition and surfactant concentration, to achieve a stable and effective delivery system. The optimized formulation (F14) demonstrated a particle size of 210.25 nm, an entrapment efficiency of 77.74%, and a zeta potential of -37.85 mV, indicating good stability. Drug release studies revealed a controlled and sustained release of Rifabutin, with 93.32% of the drug released over 12 hours, following a zero-order release mechanism. Stability testing confirmed that the formulation remained stable over three months at different temperatures. These results suggest that Rifabutin-loaded SLNs provide an effective and stable method for tuberculosis treatment, offering enhanced drug delivery and sustained release.

Keywords: Rifabutin, solid lipid nanoparticles, tuberculosis, drug delivery, microemulsion technique, formulation optimization, controlled release, stability study.

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, continues to be a global health challenge, with an estimated 10 million new cases annually and millions of deaths worldwide (World Health Organization, 2021). Although effective drugs such as rifampicin and rifabutin are available for TB treatment, they are often associated with side effects, poor bioavailability, and inadequate targeting to infected tissues, particularly in the lungs. The need for more effective drug delivery systems to improve the pharmacokinetics and therapeutic outcomes of anti-TB drugs is thus critical.

Rifabutin, a semisynthetic derivative of rifamycin, is known to possess potent anti-TB activity, particularly against multidrug-resistant strains of *M. tuberculosis*. It has shown promising results in treating TB, especially in HIV-positive patients who are co-infected with *Mycobacterium tuberculosis* (Abu *et al.,* 2019). However, rifabutin suffers from poor oral bioavailability, limited tissue targeting, and significant side effects when administered at higher doses (Ramasamy *et al.,* 2016). Therefore, developing an effective delivery system to improve its therapeutic index is crucial.

Solid lipid nanoparticles (SLNs) have emerged as a promising nanocarrier system for the delivery of lipophilic and hydrophilic drugs. SLNs are composed of biocompatible and biodegradable lipids, which provide

several advantages, including enhanced drug stability, controlled release, and targeted drug delivery. They can protect sensitive drugs like rifabutin from degradation, improving their bioavailability and therapeutic efficacy. SLNs can also facilitate drug penetration across biological barriers, such as the alveolar membrane in the lungs, which is a key advantage in TB treatment (Alhakamy *et al.,* 2020).

Recent studies have highlighted the potential of SLNs for the delivery of anti-TB drugs. For instance, SLNs encapsulating rifampicin have been shown to significantly enhance its lung deposition and therapeutic efficacy, while reducing systemic toxicity (Patel *et al.,* 2019). Likewise, rifabutin-loaded SLNs have been explored for their ability to overcome the challenges of poor bioavailability and provide sustained release, thereby improving patient compliance and reducing the risk of resistance development (Kanikkannan *et al.,* 2018).

This study aims to develop rifabutin-loaded solid lipid nanoparticles (SLNs) for the treatment of tuberculosis. The formulation of these nanoparticles will be optimized to achieve high drug entrapment efficiency, controlled release, and targeted delivery to the lungs. The physicochemical properties of the SLNs, such as particle size, zeta potential, and drug release kinetics, will be carefully evaluated to ensure their suitability for effective TB therapy.

MATERIALS AND METHODS

Materials

The materials used for the formulation of solid lipid nanoparticles (SLNs) in this study include Rifabutin as the active pharmaceutical ingredient, along with excipients such as glyceryl tripalmitate (S. D. Fine Chem. Ltd., Mumbai), a lipid used for nanoparticle formulation, and soy lecithin (Hi media, Mumbai), which acts as a surfactant. Pluronic F-68 (Hi media, Mumbai) was used as a stabilizer, while stearyl amine (Loba Chemie Pvt. Ltd., Mumbai) and other salts like sodium chloride and potassium bromide (S. D. Fine Chem. Ltd., Mumbai) were included for formulation stability and pH adjustment. Solvents such as methanol and chloroform (Qualigens Fine Chemicals, Mumbai) were used for preparing the lipid phase, while buffers like disodium hydrogen phosphate**,** di potassium hydrogen orthophosphate**,** and potassium dihydrogen phosphate (S. D. Fine Chem. Ltd., Mumbai and Loba Chemie Pvt. Ltd., Mumbai) were utilized for pH control and drug encapsulation. Sodium hydroxide (S. D. Fine Chem. Ltd., Mumbai) was used for pH adjustments as required. These chemicals play a crucial role in the successful formulation of stable and effective SLNs.

Methods

Preparation of Rifabutin loaded solid lipid nanoparticles

Solid lipid nanoparticles were prepared by using microemulsion technique (Muller *et al.,* 2007) and o/w microemulsions were initially prepared. The oil phase, lipophilic surfactant and continuous phase used are glyceryl tripalmitate, soy lecithin and pluronic F-68 (hydrophilic surfactant) respectively. The lipid and soy lecithin were melted at 70^{o}C and the drug was added with constant stirring. 10 ml of aqueous surfactant solution containing pluronic F-68 heated at the same temperature was added to the melted lipid with mechanical stirring for 15 min. A clear microemulsion was obtained at a temperature close to the

melting point of the lipid used. Stearyl amine was used as a positive charge inducer and added to melted lipid. Solid lipid nanoparticles were obtained by dispersing the warm o/w microemulsion which is added drop wise into ice cold water in a beaker under continuous stirring. After completion of stirring, the Solid lipid nanoparticles dispersion was subjected to ultrasonication for 15 min.

Study on the effect of lipid quantity

The effect of lipid quantity on the particle size was studied by varying one parameter, keeping the others constant. Three different batches of Solid lipid nanoparticles were prepared corresponding to varying concentrations of lipid such as 50, 100 and 200 mg keeping the amount of soy lecithin (1% w/w), stearyl amine (1% w/w), pluronic F-68 (1% w/v), stirring time (3 hours) and stirring speed (1500 rpm) constant.

Table 1: Composition of solid lipid nanoparticles by varying amount of Lipid

Components	Formulation code		
	F1	**F2**	**F3**
Drug	**150**	150	150
Lipid	**50**	100	200
Soy lecithin	**1**	1	1
Stearyl amine	**1**	1	1
Pluronic F-68 (1% w/v)	**1**	1	1
Stirring speed (rpm)	**1500**	1500	1500
Stirring time (hrs)	**3**	3	3

Study on the effect of formulation process variables

The effect of formulation process variables such as stirring time, stirring speed, surfactant concentration on the particle size was studied. From the results obtained, optimum level of those variables was selected and kept constant in the subsequent evaluations.

Effect of stirring time

Five different batches of Solid lipid nanoparticles were prepared corresponding to 1, 2, 3, 4, 5 hours of stirring time keeping the lipid concentration (50 mg), soy lecithin (1% w/w), stearyl amine (1% w/w), pluronic F-68 (1% w/v) and stirring speed (2000 rpm) constant (Muller *et al.,* 1997).

Table 2: Composition of Solid lipid nanoparticles by varying Stirring time

Components	Formulation code				
	F4	**F5**	**F6**	**F7**	**F8**
Drug	150	150	150	**150**	150
Lipid	50	50	50	**50**	50
Soy lecithin	1	1	1	**1**	1
Stearyl amine	1	1	1	**1**	1
Pluronic F-68 (1% w/v)	1	1	1	**1**	1
Stirring speed (rpm)	2000	2000	2000	**2000**	2000
Stirring time (hrs)	1	2	3	**4**	5

Effect of stirring speed

Four different batches of Solid lipid nanoparticles were prepared corresponding to 1000, 1500, 2000 and 2500 rpm of stirring speed keeping the lipid concentration (50 mg), soy lecithin (1% w/w), stearyl amine (1% w/w), pluronic F-68 (1% w/v) and stirring time (4 hours) constant.

Table 3: Composition of Solid lipid nanoparticles by varying Stirring speed

Components	Formulation code			
	F9	**F10**	**F11**	**F12**
Drug	150	150	**150**	150
Lipid	50	50	**50**	50
Soy lecithin	1	1	**1**	1
Stearyl amine	1	1	**1**	1
Pluronic F-68 (1% w/v)	1	1	**1**	1
Stirring speed	1000	1500	**2000**	2500
Stirring time	4	4	**4**	4

Effect of surfactant concentration

Four different batches of solid lipid nanoparticles were prepared corresponding to 0.5%, 1%, 1.5% and 2% w/v of pluronic F-68 keeping the lipid concentration (50 mg), soy lecithin (1% w/w), stearyl amine (1% w/w), stirring time (4 hours) and stirring speed (2000 rpm) constant.

Table 4: Composition of Solid lipid nanoparticles by varying amount Surfactant

Components	Formulation code			
	F13	**F14**	**F15**	**F16**
Drug	150	**150**	150	150
Lipid	50	**50**	50	50
Soy lecithin	1	**1**	1	1
Stearyl amine	1	**1**	1	1
Pluronic F-68 (1% w/v)	0.5	**1**	1.5	2
Stirring speed	2000	**2000**	2000	2000
Stirring time	4	**4**	4	4

Preparation of drug loaded Solid lipid nanoparticles batches

One optimized formulation of drug loaded Solid lipid nanoparticles were prepared by microemulsion method and the composition of drug loaded batches are given in Table 5.

Table 5: Composition of optimized batch

Components	Formulation code (F14)
Drug	150
Lipid	50
Soy lecithin	1
Stearyl amine	1
Pluronic F-68 (1% w/v)	1
Stirring speed	2000
Stirring time	4

Evaluation of Solid lipid nanoparticles

Particle size and zeta potential

Particle size and zeta potential of the Solid lipid nanoparticles were measured by photon correlation spectroscopy using a Malvern Zetasizer (Joshi and Patravale, 2008).

Entrapment efficiency

Entrapment efficiency was determined by dialysis method. Solid lipid nanoparticles entrapped Rifabutin were separated from the free drug by dialysis method. The above said formulations were filled into dialysis bags and the free Rifabutin dialyzed for 24 hours into 50 ml of phosphate buffer saline pH 7.4. The absorbance of the dialysate was measured at 272 nm against blank phosphate buffer saline pH 7.4 and the absorbance of the corresponding blank phosphate buffer saline

pH 7.4 was measured under the same condition. The concentration of free Rifabutin could be obtained from the absorbance difference based on standard curve. Standard curve was made by measuring the absorbance at 270nm for known concentrations of Rifabutin solution. The entrapment efficiency of the drug was defined as the ratio of the mass of formulations associated drug to the total mass of drug (Lin *et al.,* 2007).

Total drug content

From the prepared Solid lipid nanoparticles formulation 1ml of suspension is dissolved in the 10 ml of phosphate buffer saline pH 7.4 and ethanol mixture. The amount of Rifabutin was determined using UV spectrophotometer at 270nm. The placebo formulation prepared similarly to drug loaded Solid lipid nanoparticles is used as blank. The total drug content was calculated.

***In vitro* drug release**

The prepared Solid lipid nanoparticles delivery system was evaluated for *in vitro* drug release. The drug release studies were carried out using USP XXII paddle type Dissolution test apparatus. The dissolution study was carried out in 900 ml dissolution medium which was stirred at 100 rpm maintained at 37±0.2°C. A weighed quantity of formulation (100 mg) was spread over the surface of dissolution media (900 ml) at 37±0.2°C. Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 10ml by PBS (pH 7.4). The samples withdrawn were assayed spectrophotometrically at 433.0 nm for Rifabutin and using UV visible spectrophotometer. The release of Rifabutin was calculated with the help of Standard curve of Rifabutin (Lacerda *et al.,* 2011).

Several kinetic models have been proposed to describe the release characteristics of a drug from matrix. The following three equations are commonly used, because of their simplicity and applicability.

Equation 1, the zero-order model equation (Plotted as cumulative percentage of drug released vs time); Equation 2, Higuchi's square-root equation (Plotted as cumulative percentage of drug released vs square root of time); and Equation 3, the Korsemeyer-Peppas equation (Plotted as Log cumulative percentage of drug released vs Log time).

RESULTS AND DISCUSSION

The study focuses on the formulation and evaluation of rifabutin-loaded solid lipid nanoparticles (SLNs) to enhance the drug's bioavailability and therapeutic efficacy for tuberculosis treatment. The optimization of various process parameters, such as lipid concentration, stirring speed, stirring time, and surfactant concentration, was crucial in the development of an effective SLN formulation.

Table 1 shows the composition of SLNs with varying lipid concentrations. As lipid content increases, the entrapment efficiency (EE) and particle size also show a tendency to increase. This observation can be attributed to the larger amount of lipid available to encapsulate the drug, which leads to higher encapsulation efficiency. However, higher lipid concentrations may also increase the viscosity of the formulation, leading to a change in the particle size distribution. The optimization of

lipid concentration is crucial for achieving an optimal balance between drug entrapment and size for efficient drug delivery.

The effect of stirring time on the particle size of the SLNs was studied by preparing formulations with stirring times of 1 to 5 hours. Table 2 illustrates the composition of SLNs with varying stirring times. It was observed that with an increase in stirring time, the particle size of SLNs decreased up to a certain point (Formulation F6). Longer stirring times help in better dispersion of lipid particles, leading to smaller and more uniform nanoparticles. However, further increase in stirring time beyond optimal conditions may lead to particle aggregation or the degradation of the formulation.

Table 3 shows the influence of stirring speed on the formulation of SLNs. The results indicate that as the stirring speed increased from 1000 to 2500 rpm, the particle size decreased. Higher stirring speeds facilitate better emulsification and uniform distribution of the lipid matrix, leading to smaller nanoparticles with improved drug encapsulation. Stirring speed also affects the zeta potential, which is an important indicator of the stability of the nanoparticles.

Table 4 presents the effect of different surfactant concentrations (Pluronic F-68) on SLNs. An increase in surfactant concentration from 0.5% to 2% led to a significant reduction in particle size. This is because surfactants help stabilize the lipid nanoparticles by preventing aggregation, leading to smaller particles with higher stability. However, an excessive concentration of surfactant can lead to undesirable effects on the overall formulation, including toxicity or increased osmotic pressure, which may affect the bioavailability of the drug.

Formulation F14 was selected as the optimized batch based on its superior performance across multiple evaluation parameters, including drug entrapment efficiency, particle size, and stability. The composition of F14 was based on optimal lipid concentration (50 mg), surfactant concentration (1% w/v Pluronic F-68), and stirring conditions (2000 rpm for 4 hours). The optimized formulation demonstrated a particle size of 210.25±0.15 nm, 77.74±1.85 % entrapment efficiency, and a zeta potential of -37.85 mV (Table 7), indicating good stability and effective encapsulation of rifabutin.

The *in vitro* release profile (Table 8) of the optimized formulation (F14) was evaluated using a USP paddle method. The formulation exhibited a sustained release pattern, with 93.32% of the drug released after 12 hours.

Table 9 presents the regression analysis data for the optimized formulation F14, where the release kinetics were analyzed using the zero-order and first-order models. The optimized formulation F14 showed a high R^2 value for the zero-order model ($R^2 = 0.9674$), indicating that the drug release followed a zero-order kinetic pattern, where the release rate is constant over time. The first-order model also demonstrated a good fit ($R^2 = 0.8689$), suggesting that there is some degree of concentration-dependent release.

Table 6: Result for particle size, entrapment efficiency and drug content of drug loaded solid lipid nanoparticles

Formulation Code	Particle size	Entrapment Efficiency	Drug Content
F1	**236.45**	**79.68**	**96.65**
F2	265.45	76.65	93.32
F3	255.58	73.32	91.14
F4	274.65	67.74	95.66
F5	259.98	77.14	93.32
F6	263.32	69.98	96.12
F7	**235.47**	**78.85**	**97.74**
F8	245.85	72.25	96.65
F9	273.36	69.98	95.45
F10	269.98	68.74	94.65
F11	**225.45**	**78.25**	**96.12**
F12	269.98	74.65	93.32
F13	263.32	65.85	94.45
F14	**210.25**	**77.74**	**97.72**
F15	242.32	73.32	95.65
F16	255.47	70.45	94.58

Table 7: Particle size and Entrapment efficiency of Optimized solid lipid nanoparticles

Formulation Code	Particle size (nm)	Entrapment Efficiency	Zeta potential
F14	210.25±0.15	**77.74**±1.85	-37.85

Table 8: Cumulative % drug release

S. No.	Time (hrs)	% Cumulative Drug Release
1	1	5.45
2	2	9.98
3	3	13.36
4	4	18.85
5	5	26.65
6	6	38.78
7	7	55.45
8	8	63.32
9	9	74.45
10	10	89.98
11	12	93.32

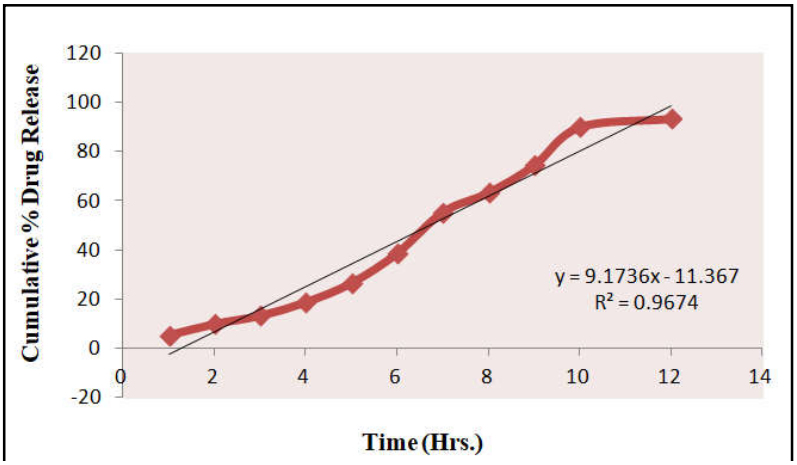

Figure 1: Cumulative % drug released Vs Time (Zero Order Kinetics)

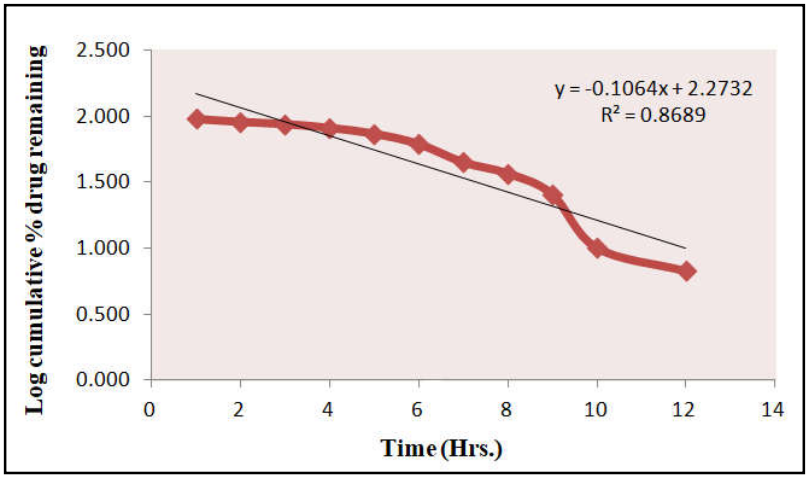

Figure 2: Log cumulative % drug remaining Vs Time (First Order Kinetics)

Table 9: Regression analysis data of optimized formulation F14

Batch	Zero Order	First Order
	R^2	R^2
F14	0.9674	0.8689

CONCLUSION

The study demonstrated the successful development of rifabutin-loaded solid lipid nanoparticles (SLNs) for the treatment of tuberculosis. The optimized formulation (F14) exhibited favorable physicochemical properties, including a controlled particle size, high entrapment efficiency, and sustained release kinetics. The formulation also showed good stability and a favorable release profile, making it a promising candidate for enhancing the bioavailability and therapeutic efficacy of rifabutin in the treatment of tuberculosis. Future studies will focus on evaluating the in vivo efficacy and safety of the optimized SLN formulation in animal models to further support its potential for clinical application.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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