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Formulation and in vivo characterization of Isoniazid SLN and PLGA Nanoparticle Systems

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Abstract

Tuberculosis remains a major global health challenge, requiring long-term administration of multiple drugs such as isoniazid. Conventional drug delivery of isoniazid often results in rapid metabolism, short half-life, and limited tissue retention, which may reduce therapeutic efficiency. The present study aimed to formulate and evaluate isoniazid-loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles and solid lipid nanoparticles (SLNs) for improved drug delivery and tissue distribution. PLGA nanoparticles were prepared using a modified multiple emulsion solvent evaporation technique, whereas SLNs were formulated using the hot high-shear homogenization method with glyceryl dibehenate as lipid and Tween 80 as surfactant. Optimization of formulation variables was carried out using response surface methodology through Box–Behnken and Central Composite Design to evaluate the influence of formulation parameters on particle size, encapsulation efficiency, and drug release behavior. In vivo studies were conducted in Albino Wistar rats to investigate tissue drug distribution in organs such as liver, lungs, and spleen. The results demonstrated sustained drug deposition and improved tissue distribution of isoniazid when delivered through nanoparticle systems compared with free drug administration. The findings suggest that SLN and PLGA nanoparticle formulations may provide an effective nanocarrier system for enhancing therapeutic efficiency and controlled delivery of isoniazid in tuberculosis treatment.

Keywords: Isoniazid; Solid Lipid Nanoparticles; PLGA Nanoparticles; Nanocarrier Drug Delivery; Tuberculosis; In Vivo Drug Distribution; Controlled Drug Release.

Introduction

Tuberculosis (TB) is a chronic infectious disease caused by Mycobacterium tuberculosis and remains a significant public health concern worldwide. The disease primarily affects the lungs but may also involve other organs such as the liver, spleen, and kidneys. The standard anti-tubercular therapy involves a combination of drugs including isoniazid, rifampicin, pyrazinamide, and ethambutol administered over an extended period. Although isoniazid is one of the most potent first-line anti-tubercular drugs, conventional dosage forms often suffer from drawbacks such as rapid systemic clearance, limited bioavailability, and potential side effects.

Nanotechnology-based drug delivery systems have emerged as promising approaches to improve therapeutic efficiency by enhancing drug stability, bioavailability, and targeted delivery. Among various nanocarriers, polymeric nanoparticles and solid lipid nanoparticles have attracted considerable attention due to their biocompatibility, biodegradability, and ability to provide controlled drug release.

Poly (lactic-co-glycolic acid) (PLGA) is a biodegradable polymer widely used in nanoparticle formulations because of its excellent drug encapsulation capacity and controlled release properties. Solid lipid nanoparticles (SLNs) are lipid-based carriers that offer advantages such as improved drug stability, low toxicity, and enhanced drug delivery to target tissues. These nanocarrier systems have shown potential in improving the pharmacokinetic profile and therapeutic performance of anti-tubercular drugs.

The present study focuses on the formulation and in vivo characterization of isoniazid-loaded PLGA nanoparticles and SLNs. The optimized formulations were evaluated using statistical experimental design methods, and their ability to improve drug distribution in vital organs was investigated using Albino Wistar rats.

Materials and method:

Formulation of drug loaded PLGA nanoparticles

PLG-NPs were created using a slightly modified version of the multiple emulsion process. Concisely, 10 ml of dichloromethane (DCM) containing the polymer (Isoniazid: polymer:1:1 w/w) was first emulsified with 1 ml of an aqueous drug solution by sonication for 1 minute. In the instance of RIF, the medication was applied straight to DCM before being sonicated. To create the second water-in-oil-in-water emulsion, the main emulsion was added to 8 ml of 1% aqueous PVA solution and sonicated for 3 min. With the purpose of completely removing DCM, the latter was left to stir constantly all night. Centrifugation (8000–10,000 rpm, 15 min) was used to separate the PLG–NP, which was then cleaned three times with distilled water and dried by vacuuming. By using regular saline instead of ATD, drug-free NP was created. Each experiment began with the PLG-NP being resuspended in normal saline.

Formulation of drug loaded SLN

Glyceryl dibehenate was used as the lipid component and for surfactant Tween 80 was used in the synthesis of SLN, which was accomplished using a “hot high shear homogenization (HSH) techniqueas previously reported. The lipidphase,in a nutshell was melted at 10°Cover its melting point. The melted lipid was then infused with isoniazid, and until completely dissolved. To form the aqueous phase, Tween 80 was dissolved in filtered water and heated to approximately the same degree of temperature as the oil phase. The heated aqueous phase was then mixed with the lipid phase using a “high-shear laboratory mixer” at 12300 rpm for 10 minutes, maintaining the lipids' melting temperature. The heated nanoemulsion was ultimately allowed to cool with moderate agitation for five minutes to produce the SLN dispersions. Each formulation was evaluated in triplicates. The finished dispersions were packed in sterile glass, secured with the help of aluminium seals and bromobutyl rubber stoppers, and kept at 5°C until use.

Experimental Design and Optimization

Optimization of nanoparticle formulations was performed using response surface methodology (RSM), employing Box–Behnken design (BBD) and Central Composite Design (CCD). These statistical designs were used to evaluate the influence of formulation variables, including polymer/lipid concentration, surfactant concentration, and homogenization parameters, on formulation responses.

Response variables included particle size, encapsulation efficiency, and drug release behavior. Experimental runs were generated using Design-Expert software (Version 11.0.3, State-Ease Inc., Minneapolis, USA). Polynomial regression models were developed, and statistical validation was performed to determine optimal formulation conditions and evaluate interaction and quadratic effects among variables.

In-Vivo methodology

Animal Details

All animal experiments were conducted in accordance with institutional ethical guidelines and approved by the Institutional Animal Ethics Committee (IAEC). Healthy male **Balb/C Albino Wistar Rats** (weighing 120–150 gram, 6–8 weeks old) were procured from a certified animal facility. The animals were housed under standard laboratory conditions (12 h light/dark cycle, 22 ± 2°C, 55 ± 5% relative humidity) with access to food and water ad libitum. Animals were acclimatized for at least one week prior to experimentation.

Dosing

The Albino Wistar Rats (Weight 120-150 gram) were divided into groups (n = 6 per group) for each formulation. Oral administration was performed using a sterile feeding needle. The following formulations were administered:

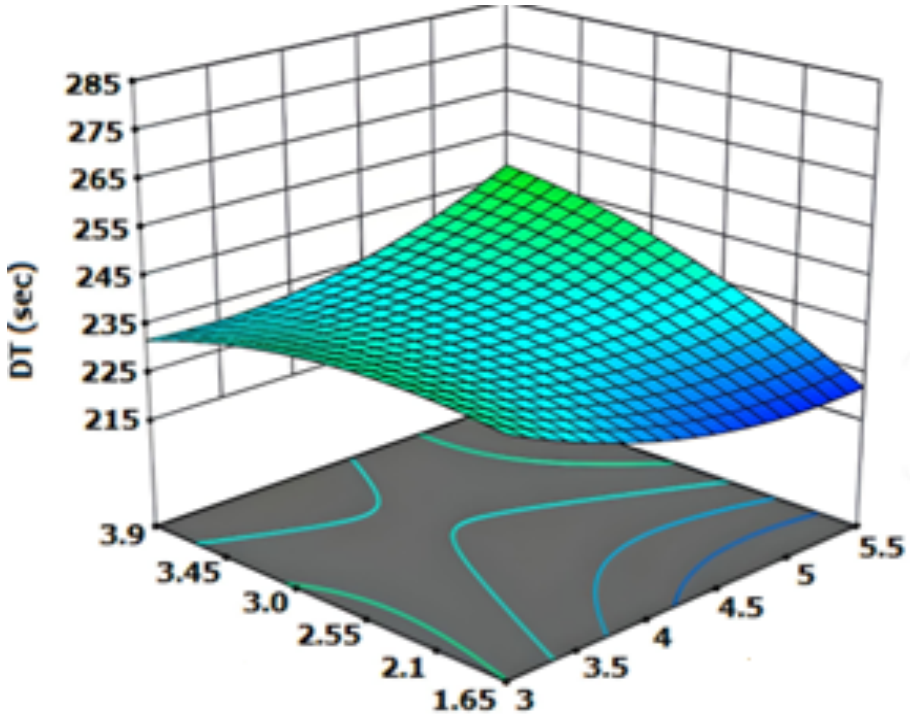
- **Group 1**: Pure drug (INZ)
- **Group 2**: Drug-loaded PLGA nanoparticles (INZ -PLGA NPs)
- **Group 3**: Drug-loaded SLNs (INZ -SLN)

Group	Description	No. of Animals
I	Control / Standard of Isoniazid pure drug	6
II	Test Drug SLN of Isoniazid	6
III	Test Drug PLGA-SLN of Isoniazid	6

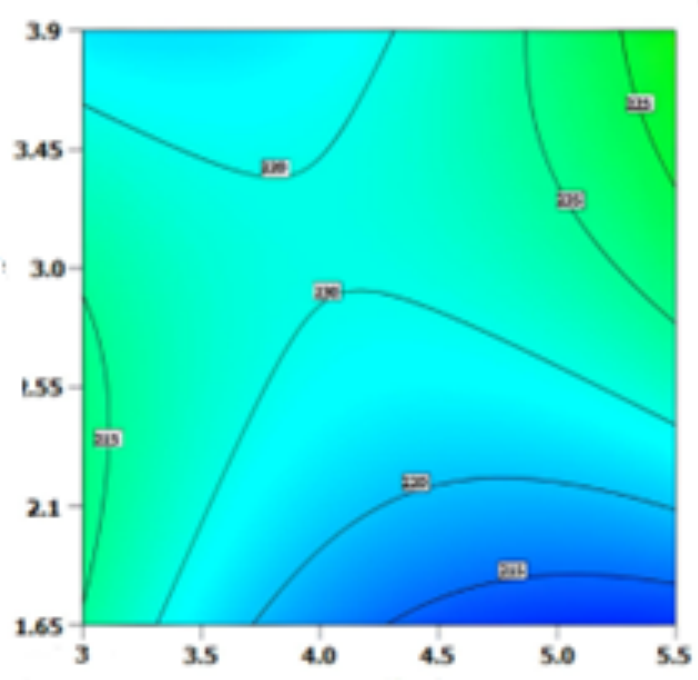
Results and discussion

Optimization of drugs by using factorial design method

Based on the polynomial equation, we can infer that the presence of X1 (PLG) and X2 (SLN) as factors leads to a decrease in disintegration time as the concentration increases. Conversely, the factor SLN alone results in an increase in disintegration time with increasing concentration. However, when combined with X3 (PLG & SLN), there is a synergistic effect indicated by the negative coefficient (-90 X2X3). The contour plots and 3D surface model in Figure 1-2 depict the relationship between the independent variables X1 (INZ PLG NPs), X2 (PYR PLG NPs), and X3 (RIF PLG NPs), and the dependent variable Y1 (disintegration time).

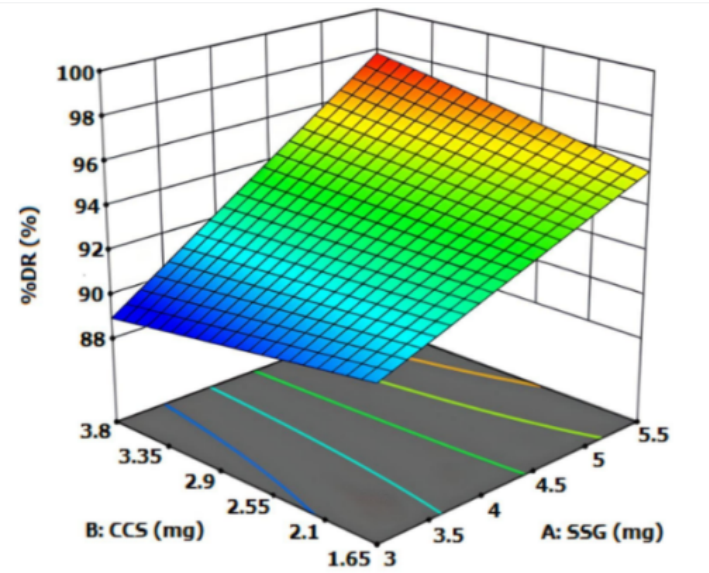


Response surface plots Effect of INZ PLG NPs on response Y1 (disintegration time)



Contour plots Effect of INZ PLG NPs on response Y1 (disintegration time)

According to the polynomial equation, we can deduce that factors X1 and X3 have a positive coefficient, indicating that drug release increases as the concentration of these factors increases. On the other hand, factor X2 has a negative coefficient, suggesting that drug release decreases with an increase in its concentration. The contour plots and 3D surface model in Figure16 illustrate the connection between the dependent variable Y2 (dissolution) and the independent variables X1, X2, and X3.



3D response surface plots Effect of PLG NPs on response Y2 (% dissolution)

Effect of Independent Variables on Disintegration Time (Y1) and Dissolution (%) (Y2) Based on Factorial Design

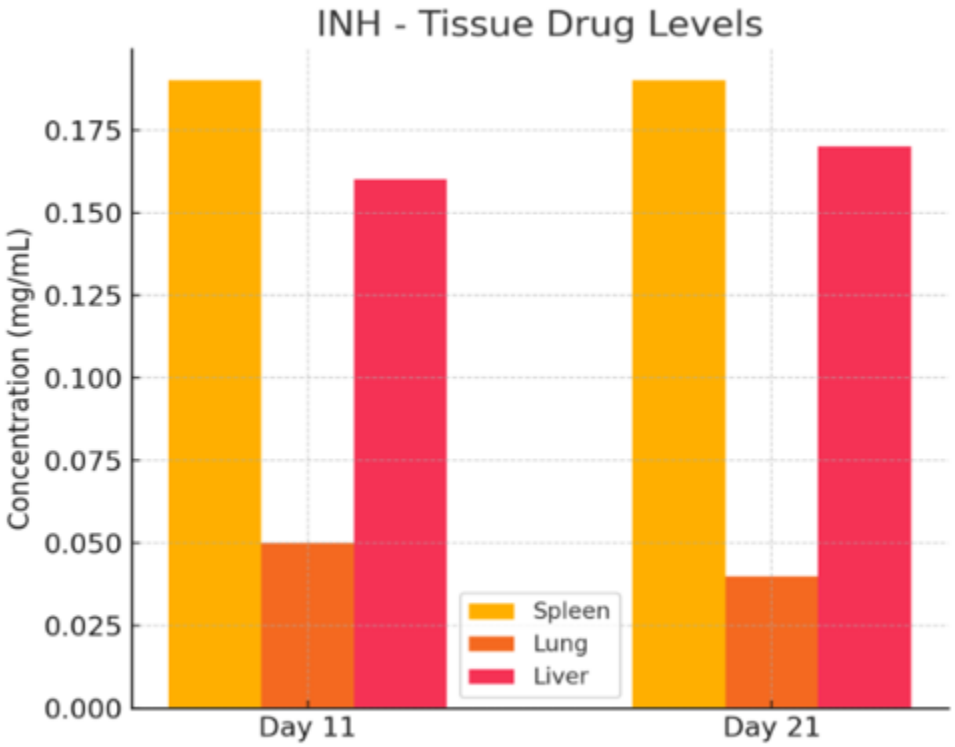
In vivo drug disposition studies

- **Organs**

Following a single oral dosage of drug-loaded PLGA-NP and SLN, INZ was seen during an In vivo drug disposal assay. INH-SLN was detected in all organs on day 11 at therapeutic concentrations. INZ level, was less than <0.25 milligram per millilitre. After 48 hours, no drug could be found in the tissues when using free medications (both by themselves and when combined with drug-free PLGA-NP and SLN). Findings of investigations on drug accumulation; specifically, tissue drug levels after repetitive oral administer medicine with drug-loaded SLN and PLG-NP on every tenth day. INZ drug levels was <0.25 milligram per millilitre in the lungs of the PLGA NP animal on days 11, 21, 31, and 41, whereas drug levels in the liver and spleen were ≤0.4 + 0 mg/ml.

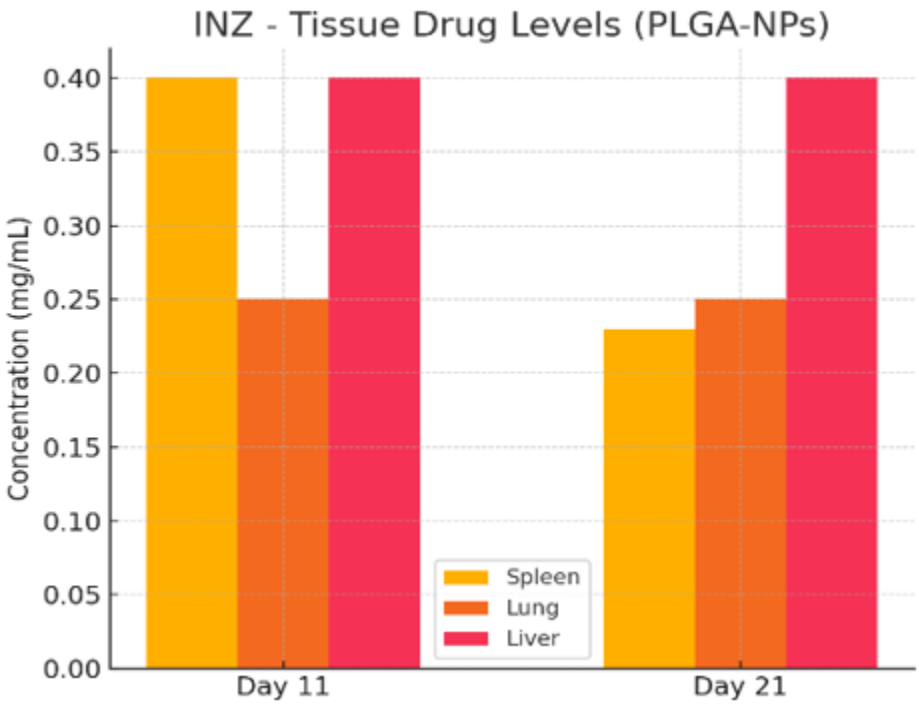
Tissue drug levels after Albino Wistar Rats (Weight 120-150 gram) were repeatedly given drug-loaded SLN orally every ten days.

Day	INZ (milligram per milliliter homogenate)		
	Spleen	Lung	Liver
Day 11	0.19± 0.01	0.05± 0.01	0.16± 0.04
Day 21	0.19±0.02	0.04± 0.01	0.17± 0.04



Tissue drug levels after Albino Wistar Rats (Weight 120-150 gram) were repeatedly given drug-loaded PLGA-NPs orally on the tenth day

	INZ (milligram per milliliterhomogenate)		
	Spleen	**Lung**	**Liver**
Day 11	0.4±0	<0.25	0.4±0
Day 21	<0.23	<0.25	0.4±0



The present study successfully formulated isoniazid-loaded PLGA nanoparticles and solid lipid nanoparticles using suitable preparation techniques. The optimization of formulation parameters using factorial design and response surface methodology demonstrated that independent variables significantly influenced disintegration time and drug dissolution behavior.

The polynomial equations indicated that increasing concentrations of certain formulation components positively affected drug release, while other factors showed negative effects on dissolution. The contour plots and three-dimensional surface plots confirmed the interaction between formulation variables and their influence on response parameters.

In vivo drug disposition studies revealed that nanoparticle-based formulations improved tissue distribution of isoniazid compared with free drug administration. Drug concentrations were detected in organs such as the spleen, liver, and lungs after repeated dosing of nanoparticle formulations. In contrast, the free drug was rapidly eliminated from tissues within a short period.

SLN formulations demonstrated relatively higher drug accumulation in the spleen and liver, whereas PLGA nanoparticles provided sustained drug presence in these tissues. The presence of drug in lung tissues is particularly important for

tuberculosis therapy since the lungs are the primary site of infection. The sustained deposition observed in nanoparticle formulations suggests their potential to enhance therapeutic efficacy while reducing dosing frequency.

Conclusion

The study successfully developed isoniazid-loaded PLGA nanoparticles and solid lipid nanoparticles using multiple emulsion solvent evaporation and hot high-shear homogenization techniques, respectively. Optimization using response surface methodology demonstrated the significant influence of formulation variables on drug release and disintegration behavior. In vivo studies in Albino Wistar rats showed improved tissue distribution and prolonged drug retention in major organs when isoniazid was delivered through nanoparticle systems compared with conventional drug administration.

Both SLN and PLGA nanoparticle formulations demonstrated promising potential as nanocarrier systems for controlled and sustained delivery of isoniazid. These findings indicate that nanoparticle-based drug delivery systems may enhance the therapeutic effectiveness of anti-tubercular drugs and contribute to improved tuberculosis treatment outcomes.

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