e-ISSN: 2320-4230, p-ISSN: 2961-6085

# Journal of Drug Discovery and Therapeutics

Available Online at www.jddt.in

CODEN: - JDDTBP (Source: - American Chemical Society)

Volume 13, Issue 04; 2025, 138-154

## Nanocarrier-Mediated Delivery of Isoniazid: Development of SLN and PLGA Nanoparticle Systems

**Mahesh Kumar, Tarun Virmani, Jagadish C Pati**

**School of Pharmaceutical Sciences, MVN University, Palwal, Haryana 121105, India**

**Received: 14-04-2025 / Revised: 20-05-2025 / Accepted: 26-06-2025**

**Corresponding author: Mahesh Kumar**

**Conflict of interest: No conflict of interest.**

**Abstract:**

Tuberculosis remains a serious global health problem requiring effective drug delivery systems to improve therapeutic outcomes. Isoniazid, a first-line antitubercular drug, exhibits limitations related to drug stability, controlled release, and bioavailability. The present study aimed to develop and characterize isoniazid-loaded solid lipid nanoparticles (SLN) and poly (lactic-co-glycolic acid) (PLGA) nanoparticles to enhance drug delivery efficiency. PLGA nanoparticles were prepared using a modified multiple emulsion solvent evaporation technique, while SLN were formulated using a hot high-shear homogenization method with glyceryl dibehenate as the lipid matrix and Tween 80 as the surfactant. The formulations were optimized using response surface methodology employing Box–Behnken and Central Composite designs. The prepared nanoparticles were characterized for particle size, polydispersity index, zeta potential, encapsulation efficiency, drug loading, and morphological properties using techniques such as SEM, TEM, FTIR, UV spectroscopy, and X-ray diffraction. The optimized SLN showed particle sizes of 200–250 nm with encapsulation efficiency of 66.31%, while PLGA nanoparticles exhibited sizes of 170–190 nm with encapsulation efficiency of 50.62%. In-vitro drug release studies demonstrated sustained drug release, with SLN releasing 89.94% and PLGA nanoparticles releasing 67.5% of drug over 360 minutes. The results indicate that both SLN and PLGA nanoparticles provide improved drug delivery and controlled release behavior, suggesting their potential as promising nanocarrier systems for enhanced tuberculosis therapy.

**Keywords:** Isoniazid; Solid lipid nanoparticles; PLGA nanoparticles; Nanoparticle drug delivery.

## Introduction:

Tuberculosis (TB) remains one of the most prevalent infectious diseases worldwide and continues to pose a major public health challenge, particularly in developing countries. The disease is caused by Mycobacterium tuberculosis and primarily affects the lungs, although it may also involve other organs. Effective treatment of tuberculosis relies on the long-term administration of first-line antitubercular drugs such as isoniazid, rifampicin, pyrazinamide, and ethambutol. Among these, isoniazid is considered one of the most important drugs due to its strong bactericidal activity against actively dividing mycobacteria.

Despite its effectiveness, conventional delivery of isoniazid presents several limitations, including rapid metabolism, instability in biological environments, and the need for prolonged therapy. These factors may lead to poor patient compliance and the emergence of drug resistance. Therefore, the development of advanced drug delivery systems capable of improving the pharmacokinetic properties and therapeutic efficiency of antitubercular drugs is of considerable importance.

Nanotechnology-based drug delivery systems have emerged as promising approaches for improving the solubility, stability, and bioavailability of drugs. Nanoparticles provide several advantages, including increased surface area, improved drug dissolution, targeted delivery, and sustained drug release. Among various nanoparticle systems, solid lipid nanoparticles (SLN) and polymeric nanoparticles such as poly (lactic-co-glycolic acid) (PLGA) nanoparticles have gained significant attention due to their biocompatibility, biodegradability, and ability to encapsulate both hydrophilic and lipophilic drugs.

Solid lipid nanoparticles are lipid-based carriers that combine the advantages of polymeric nanoparticles, liposomes, and emulsions while minimizing their limitations. They provide improved stability and controlled drug release. PLGA nanoparticles, on the other hand, are biodegradable polymeric systems widely used in pharmaceutical formulations because of their safety and controlled drug release characteristics.

Therefore, the present study was undertaken to develop and evaluate isoniazid-loaded SLN and PLGA nanoparticles as potential nanocarrier systems for improved drug delivery. The study focuses on formulation optimization, physicochemical characterization, and evaluation of in-vitro drug release behavior in order to determine the suitability of these nanoparticle systems for enhancing the therapeutic performance of isoniazid.

**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 [222].Thelipidphase, 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.

## OPTIMIZATION OF PLG AND SLN USING CENTRAL COMPOSITE DESIGN AND BOX-BEHNKEN

"In-vivo and in-vitro estimation of formulations of anti-tubercular drugs was performed using factorial designs, specifically the Box-Behnken design and the Central Composite design. Both designs were employed to evaluate the impact of multiple variables and their interactions on the response variables".

- **Box-Behnken Design**

TheBox-Behnken design is a type of response surface methodology (RSM) that allows for the systematic investigation of the effects of multiple formulation parameters (independent variables) on the outcome or performance of the formulation (dependent variables). In this study, three independent variablessuch as lipid concentration, surfactant concentration, and homogenization speed (example variables, to be replaced with your actual parameters) were evaluated at three levels each. The dependent variables or response variables included characteristics such as particle size, entrapment efficiency, and drug release, which are critical for the performance of anti-tubercular drug formulations. The design, which consists of a specific set of experimental runs based on design principles, offers the advantage of requiring fewer experiments than a full factorial design, while still allowing for the estimation of main effects, interaction effects, and quadratic effects. In this study, the Box-Behnken design was employed to optimize the formulation of anti-tubercular drugs using polynomial regression models. The optimization process was conducted using the Design-Expert software (trial version 11.0.3, State-Ease Inc., Minneapolis, USA). A total of 14 experimental runs, including 3 center points, were performed to ensure a comprehensive estimation of prediction variance across the design space.

The software optimization process was utilized to select the optimized formulation and based on the preceding modeling achieved by the software. To determine the most suitable mathematical model, various polynomial models were evaluated using statistical parameters. This statistical confirmation of the polynomial equation was carried out following the methodology described by Baruah et al. in 2018 [227].

- **Central Composite Design**

The "Central Composite design" (CCD) is another prominent reaction surface design. It is an extension of the factorial design that includes a set of center points and additional runs at different distances from the center to allow for the estimation of quadratic and interaction effects. This design is particularly useful for studying non-linear relationships between factors and responses.

In the present study, for evaluating formulations of anti-tubercular drugs, the “Central Composite design” was employed to examine the effects of factors liketemperature, pH, drug concentration, or the presence of co-solvents. In-vitrodissolution studies, microbial susceptibility testing, or in-vivopharmacodynamicand pharmacokinetic evaluations can be directed using this design

to assess the impact of these factors on drug performance.

Both the Box-Behnken and Central Composite designs offer efficient and statistically robust approaches for evaluating multiple factors simultaneously and optimizing formulations of anti-tubercular drugs. By systematically varying the levels of the factors and analyzing the resulting responses, researchers can gain insights into the effects of different formulation parameters and identify the optimal formulation conditions for improved drug efficacy and safety.

**Characterization of nanoparticles**

- **Particle size, physical stability, and surface charge**

A Zetasizer Nano S was used to conduct photon correlation spectroscopy (PCS) analysis of the average particle size (Malvern Instruments, UK). Specimen were kept in polystyrene cuvettes after the appropriate dilution in 0.45 m pore filtered clean water, and assessments were done at 25.0±0.1°C (1:100) Polydispersityindex and average particle size were used to represent the results (PI). A Zetasizer Nano Z was used to determine the zeta potentialby employing surface charge to estimate particle mobility in an electric field (Malvern Instruments, UK). Samples were used for this, and after being appropriately diluted in filtered, cleaned water, they were put in a special cuvette with a potential of around 150 mV. Threeduplicate samples were prepared for eachformulation measurement.

- **Drug Loading (DL) and Encapsulation efficiency (EE)**

Following preparation, non-incorporated isoniazid loading was removed from the PLGA and SLN dispersions by size exclusion chromatography on Sephadex G-25/PD-10 columns. Once acetonitrile was used to dissolve the nanoparticles, which aided in the lipid phase's precipitation, it was possible to evaluate the incorporation of the isoniazid/ / in PLGA loaded nanoparticles and in SLN. Centrifugation was used to separate the supernatant, which included the isoniazid/ / loaded SLN. Utilising a microplate spectrophotometer reader and UV-visible spectrophotometry, the concentration of the free drug in aqueous phase was determined.

The medications EE and DL were calculated using the following formula:

$$EE(\%) = \frac{W_{loaded\ drug}}{W_{initial\ drug}} \times 100$$

$$DL(\%) = \frac{W_{loaded\ drug}}{W_{lipid}} \times 100$$

where,

"$W_{initial}$drug = weight of the drug used,

$W_{loaded}$ drug = weight of encapsulated drug that was detected in the supernatant after nanoparticle purification, solubilization, and centrifugation.

$W_{lipid}$ = the weight of the lipid vehicle."

- **Morphological analysis**

The morphology of SLN and PLGA was examined using TEM. For observation, the specimens were mounted on copper racks with carbon membranes covering them. For staining, "phosphotungstic acid" at 2% (w/v) was applied for 2 minutes. Following that, they were examined at 120 kV using a Microscope, and photos were taken using a Gatan Orius camera.

- **Determination of Stability**

The SLN and PLGA suspension stability was evaluated under various circumstances, including its freeze-dried form at 37ºC in a desiccator and its aqueous suspension at 53ºC. After six and twelve months of storage at 53°C, the zeta potential, PI, and mean particle diameterof SLN and PLGA were assessed for suspension stability. Moreover, utilizing a Malvern Mastersizer 2000, laser diffractometry (LD) was used to identify bigger particles beyond the PCS's measurement range (Malvern Instruments, UK). Using this apparatus, each sample's size distribution was measured five times, and at least three duplicate samples were run (n=3).

Also, the impact of freeze-drying was evaluated. For this, prepared SLN and PLGA formulations were split into two equal-sized aliquots. For a comparison analysis of the physicochemical parameters, while the other (reference) was kept at 53°C, one aliquot was stored in the freezer for an entire day and then overnight frozen.

- **Thermal analysis**

DLS (dynamic light scattering) was utilisedtoestimate how temperature affects the physical stability of SLN and PLGA suspensions. Particle size measurement was carried out in a quartz cell with samples that had been adequately diluted with purified water while the sample was heated at the rate of 0.5°Celcius /min from 25 to 90°C. Every 0.5°C, measurements of the particle size were taken. Measurements were made in triplicate (n=3) for each sample. After these thermal experiments, the morphology of SLN and PLGA was evaluated by TEM in the manner previously reported.

- **Differential scanning calorimetry (DSC) studies**

A DSC Q200 calorimeter was utilized for the measurements (TA Instruments, DE, USA). Accurately weighed glyceryl dibehenate, Tween 80, and /isoniazid/ bulk materials, as well as SLN and PLGA dispersions (empty and loaded with /isoniazid/), were added to aluminum pans before being measured and sealed against an empty control pan. The aluminum pan underwent heating at a rate of 10°C/min to a temperature range of -20°C to 240°C, and thermograms were recorded at each temperature.

**Solubility determination**

The shake flask technique was used to test the solubility of, isoniazid, and in both acidic (pH - 1.2) and basic solutions (pH 6.8). During 12 hours at room temperature (28°C± 1°C) on a rotary flask shaker, excess isoniazid was added separately to 20ml of each fluid taken in a 25mL stoppered conical flask. Following shaking for 12 hours, 2 mL aliquots were taken out at 1-hour intervals and promptly filtered through a 0.45-disc filter. By measuring absorbance at 475, 263, and 268 nm while using the matching fluid as a blank, the filtered samples were appropriately diluted and tested for, isoniazid, and. Shaking persisted until the two estimates that followed were identical. To ascertain the impact of surfactant on drug solubility, the solubility of, isoniazid, and in 0.1 N HCl containing various doses of SLS was also evaluated at room temperature (28°±1°C). The solubility tests were conducted in three replicates.

**In-vitro dissolution rate**

Using a USP XXI 8-station dissolution rate test device with a paddle spinning at 50 rpm, the dissolution rate of the three anti-tubercular tablets under investigation was assessed at 37°C. During the two-hour dissolving process, 900 mL of 0.1 N hydrochloric acid (pH 1.2)/0.1 N hydrochloric acid with 0.1% SLS and 0.02% ascorbic acid were utilized as the dissolution medium. Samples of the dissolving medium were taken at intervals of 0, 5, 10, 20, 30,

45, 60, 90, and 120 minutes and filtered through a 0.45-inch nylon disc filter. By measuring absorbance at 475, 263, and 268 nm while using the matching fluid as a blank, samples were appropriately diluted with the appropriate dissolving media and then analyzed for, isoniazid, and. The dissolution tests were performed in triplicate.

## Results and discussion

### Organoleptic properties

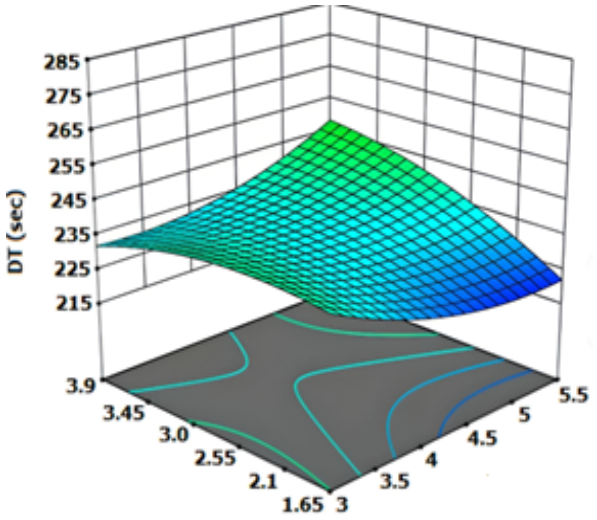
The drug isoniazid was a white, odourless substance that tasted initially pleasant but became bitter after being swallowed.

### Bulk characterization

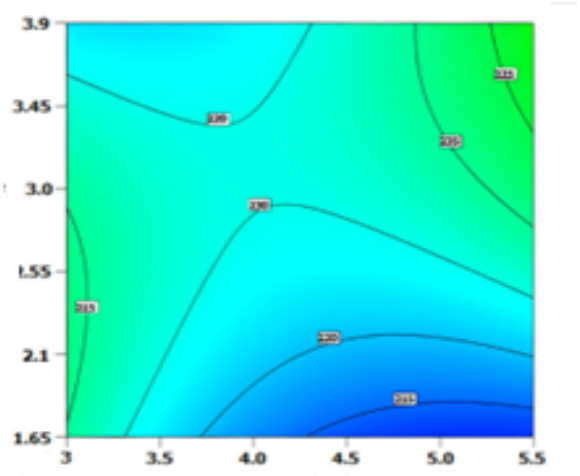
There were no polymorphs seen in the growth of rodlike isoniazid (INH) crystals in methanol, ethanol, or isopropanol.

### 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).

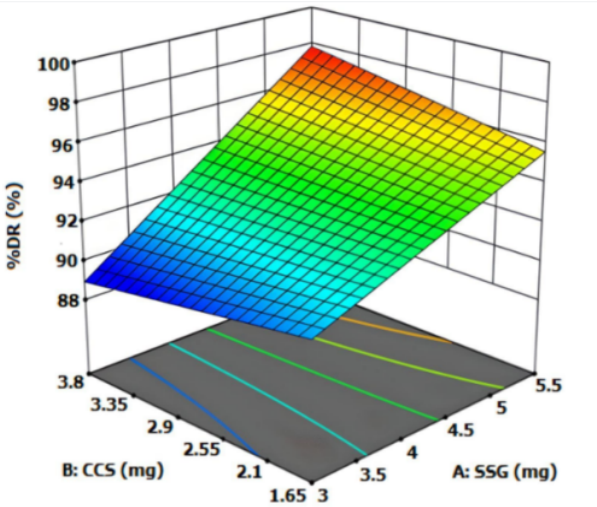



**Response surface plots Effect of INZ PLG NPs on response Y1 (disintegration time)**




**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)**

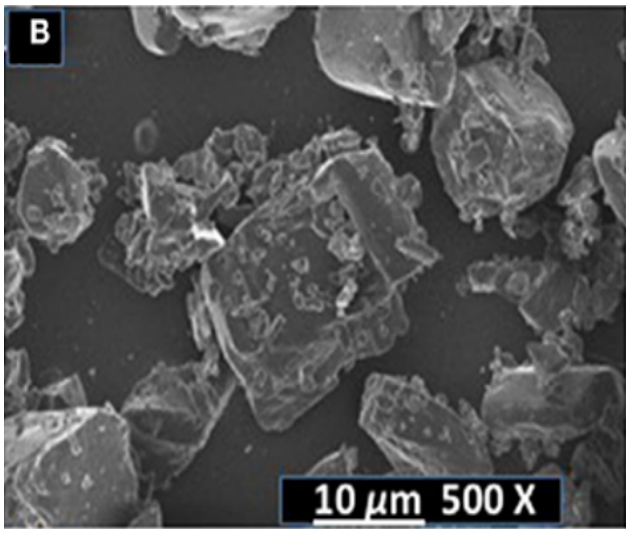
**Effect of Independent Variables on Disintegration Time (Y1) and Dissolution (%) (Y2) Based on Factorial Design**

| Run | X1: INZ PLG NPs | Y1: Disintegration Time (s) | Y2: % Dissolution |
|---|---|---|---|
| 1 | Low | 180 | 62.5 |
| 2 | High | 135 | 74.3 |
| 3 | Low | 200 | 58.7 |
| 4 | High | 145 | 69.2 |
| 5 | Low | 170 | 65.9 |

| | | | |
|---|---|---|---|
| 6 | High | 125 | 78.6 |
| 7 | Low | 190 | 61.4 |
| 8 | High | 130 | 76.1 |

**Physiochemical characterization**

Figure display the SEM images of isoniazid (INZ), and The SEM image of isoniazid co-crystals reveals sharp-edged, shattered hexagonal particles, suggesting crystalline fragility and fragmentation upon formation. In the case of, grinding produced dusty, fine particles, indicating a different morphology compared to its native crystalline form.

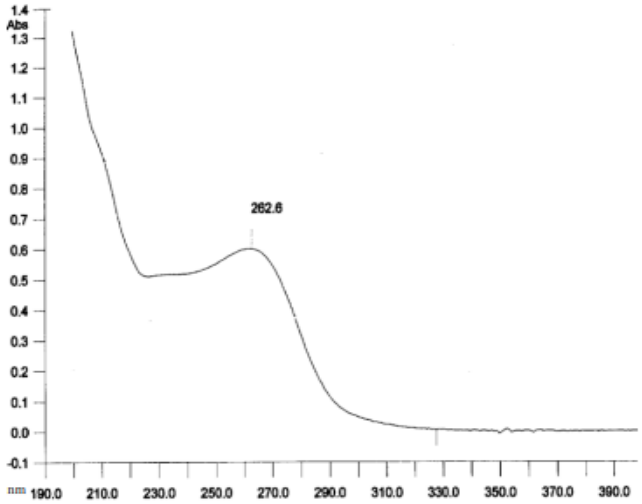



**SEM images of isoniazid**

**Ultravioletspectrum**

- **Isoniazid**

Figure shows an illustration of the UV-Vis spectrum. At 262.6 nm, the absorption maxima were recorded.




**UV-vis Spectrum of Isoniazid**

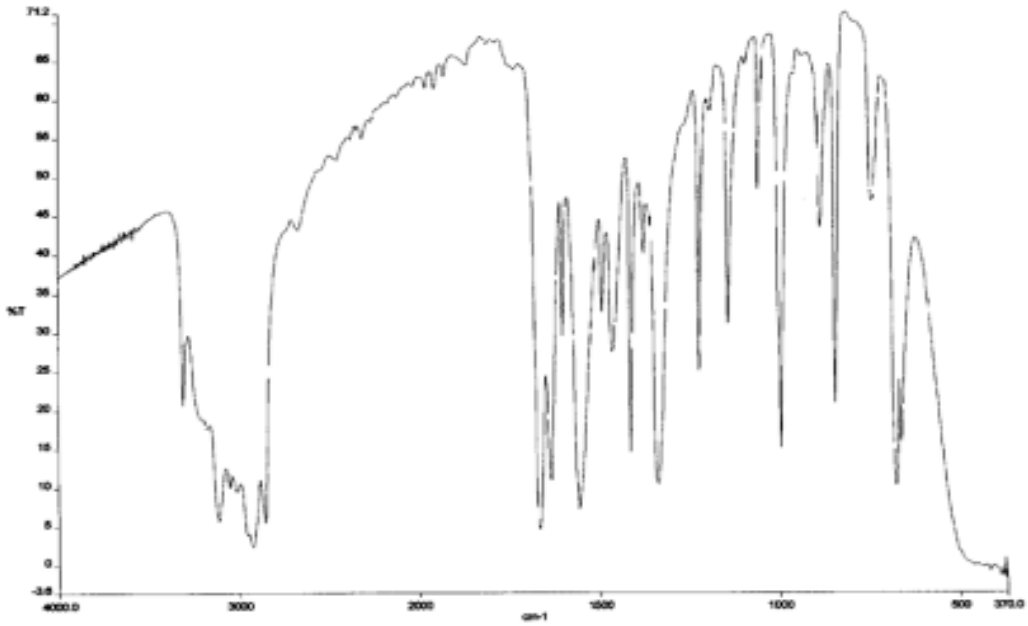
**Infraredspectrum**

**Isoniazid**

The spectrum in figure attests to the existence of the relevant functional groupings. Table 2.2 provides an analysis and tabulation of the findings

**IR absorption bands and corresponding functional groups in organic compounds**.

| IR Absorption band ($cm^{-1}$) | Functional Groups |
|---|---|
| 1661.1 and 1635 | -$NH_3$ asymmetric bending |
| 1603.3 | -C=N stretching |
| 1667.2 | -C=O stretching |
| 3174.3 | Aromatic C-H stretching |
| 3300.0-3000.0 | -NH, -NH bonded stretching |
| 1556.7,1492.3 and 1463.8 | Aromatic ring vibration |



**Infrared spectrum of Isoniazid**

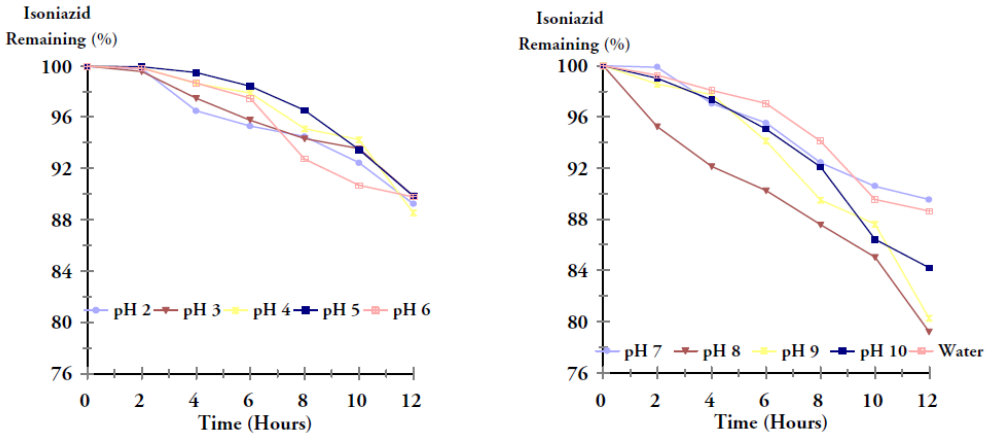
**Solubility analysis**

**Drug solubility tests at varying pH levels, with and without phosphate buffer pH solubility of Isoniazid, and**

| Buffer solvent | Isoniazid |
|---|---|
| 2.00±0.01 | 130.23±0.80 |
| 3.00±0.01 | 131.14±0.55 |
| 4.00±0.01 | 130.65±0.93 |
| 5.00±0.01 | 129.56±0.33 |
| 6.00±0.01 | 129.58±0.98 |
| 7.00±0.01 | 128.56±0.01 |

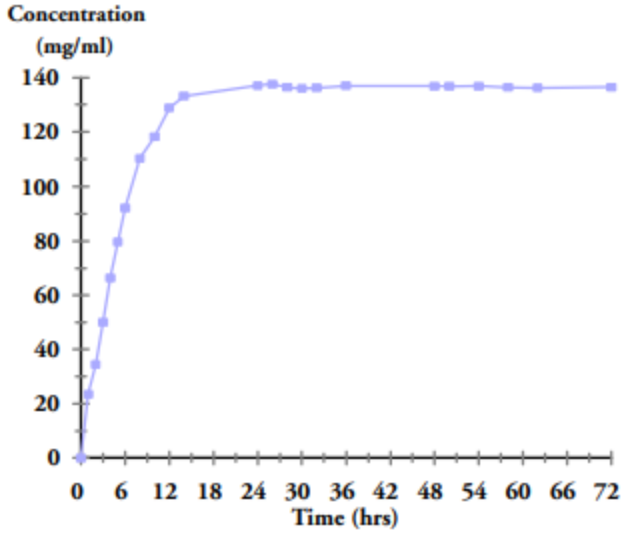
| | |
|---|---|
| 8.00±0.01 | 130.22±0.08 |
| 9.00±0.01 | 127.45±0.22 |
| 10.00±0.01 | 129.45±0.41 |
| **Unbuffered** | |
| 7.01±0.01 | 128.81±0.66 |

**a) Drug stability tests at varying pH levels, with and without phosphate buffer**




**pH stability profiles of Isoniazid**

**b) Dissolution study**




**Dissolution: Isoniazid solubility time curve**

- **Partition Coefficient (logP)**

**Partition coefficient of Isoniazid**

| Drug | logP |
|---|---|
| Isoniazid | -0.68 |

**Physical and chemical analysis of drug-loaded nanoparticles**

**Particle size, distribution and zeta potential**

**Physicochemical characterization of drug loaded SLN**

| Characterization | Isoniazid |
|---|---|
| Partical size | 200-250nm |
| Drug encapsulation efficiency (%) | 66.317±5.83 |
| Polydispersity index | 0.355±0.04 |
| Drug loading (mg/g polymer) | 663±45 |
| In vitro release (% of encapsulated drug) | 67-70% |
| Zeta potential | −22.20 ± 1.32 |

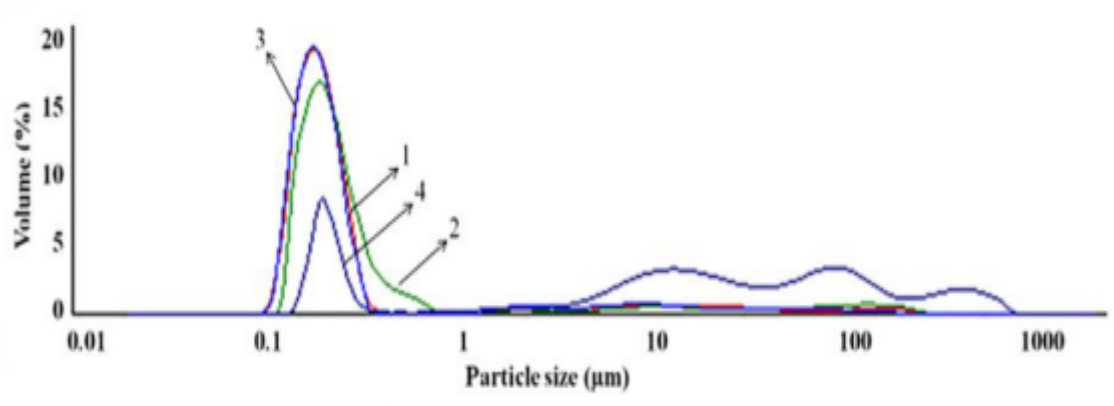
The solid-lipid nanoparticle-loaded versions of isoniazid physicochemical properties. Particle size, drug encapsulation effectiveness, zeta potential, drug loading capacity, polydispersity index, and invitro release of an encapsulated drug are a few examples of the physiochemical characteristics that were stated.

**PLG NPS drug loaded nanoparticles**

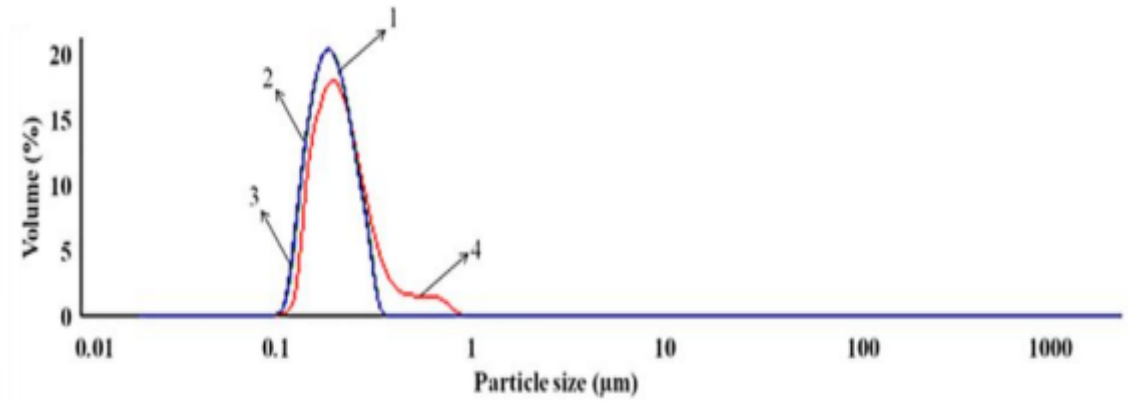
**Physicochemical characterization of drug loaded PLG-NP**

| | Isoniazid |
|---|---|
| Particle size | 170-190nm |
| Drug encapsulation efficiency (%) | 50.62 ± 2.33 |
| Polydispersity index | 0.345 ± 0.004 |
| Drug loading (mg/g polymer) | 610±40 |
| In vitro release (% of encapsulated drug) | 10-12% |
| Zeta potential | −25.12± 2.08 |

**Particles size**



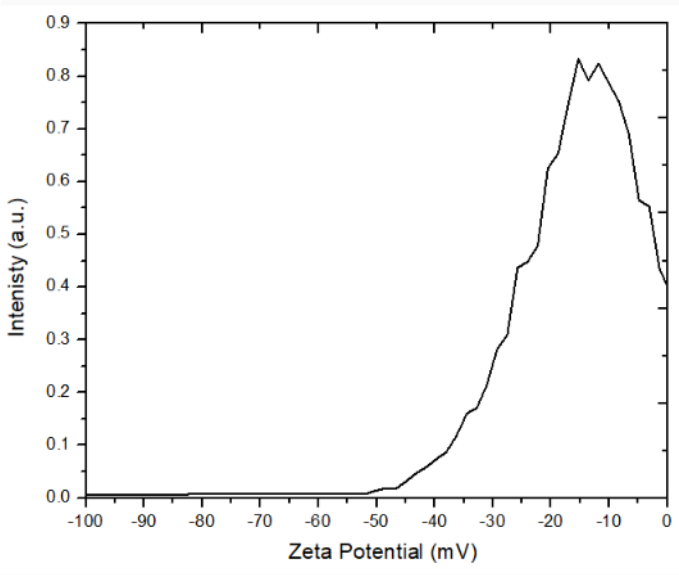

**Particle size distribution of drug loaded PLGA NPs after formulation Isoniazid**



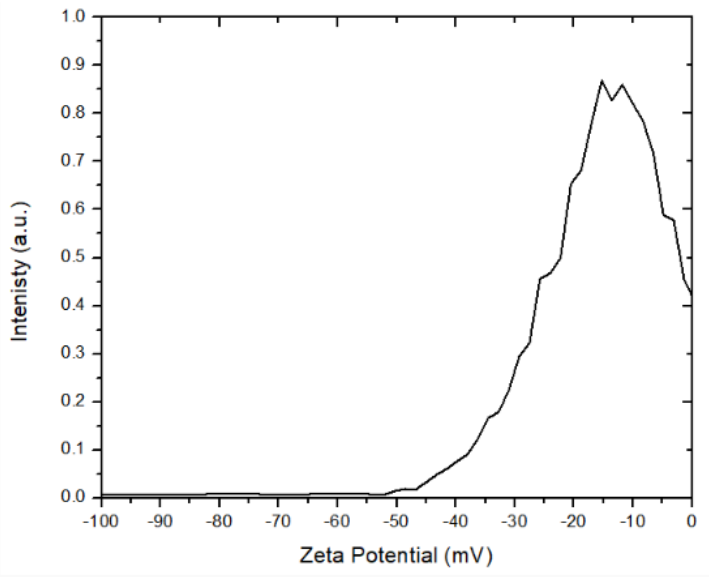

**Particle size distribution of drug loaded SLN after formulation Isoniazid free drug.**

- **Zeta potential**

The formulation of SLN (Isoniazid) and PLGA drug-loaded nanoparticles underwent a zeta potential analysis




**Zeta potential of PLGA nanoparticles loaded with Isoniazid.**



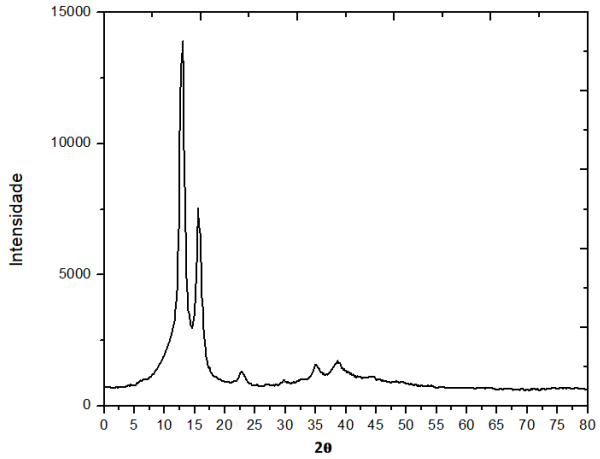

**Zeta potential of SLN nanoparticles loaded with Isoniazid**

After analyzing the data, it is clear that drug loaded SLN exhibits greater nanoparticle stability than PLGA NPs.

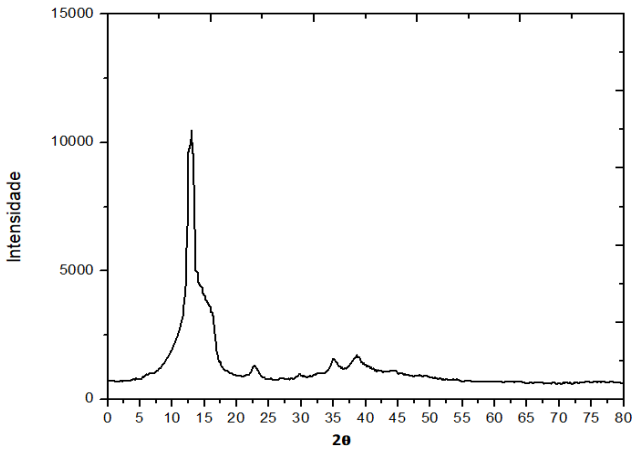
**Morphological analysis**

**X-RAY Diffraction (Xrd)**

Pure INZ's diffraction spectrum revealed that the substance was crystalline in form with several distinct peaks. According to the XRD analysis, SLNs lacked all of the key INZ characteristic peaks, suggesting that when they were incorporated into the lipid matrix, INZ became very amorphous.



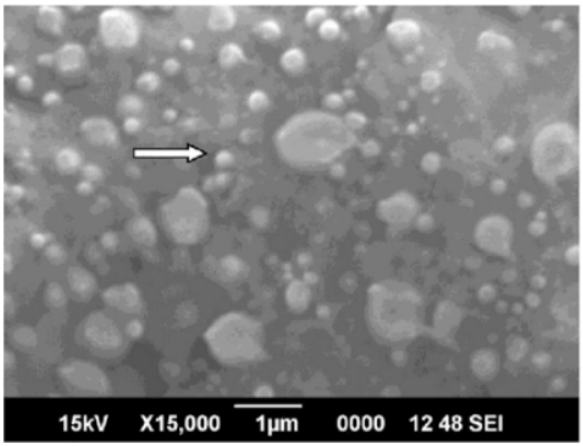

**XRD patterns individual of drug loaded SLN isoniazid**



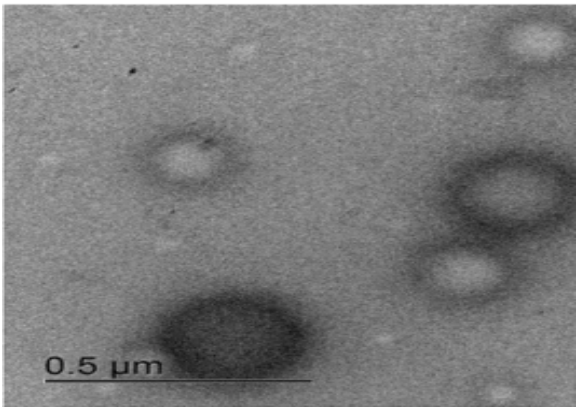

**XRD patterns of PLGA NPs isoniazid**

## TEM

TEM scans indicated that the SLNs were spherical in form and nanometric in size. Both the TEM and the dynamic light scattering methods produced results that were in excellent agreement with the particle size of the SLNs




**TEM analysis of drug loaded SLNPs Isoniazid**




**TEM analysis of drug loaded PLGA NPs Isoniazid**

## IN-VITRO DRUG RELEASE STUDIES

### In-vitrorelease rate (SLN NPs)

- **Isoniazid:** At the beginning (0 minutes), no isoniazid has released. However, the release rate increases progressively, with 36.6% releases at 60 minutes, 62.4% at 180 minutes and 89.94% at 360 minutes.
- The release of the drug sustained on encapsulating within the SLNs and maximum release is obtained for isoniazid SLNs

**In-vitro release rate (SLN NPs)**

| Drugloaded nanoparticles | % Drug Releases | | | | | |
|---|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 120min | 180 min | 360 min |
| Isoniazid | 0 | 22.8 | 36.6 | 45.3 | 62.4 | 89.94 |

### In-vitrorelease studies- (PLGA NP's)

**Isoniazid:** The release of isoniazid also begins with 0% at 0 minutes, indicating no initial releases. As time passes, the release rate gradually rises. At 30 minutes, 21.2% of isoniazid has released, which further increases to 56.3% at 180 minutes and 67.5% at 360 minutes.

- The PLGA nanoparticles showed sustained release of the drug.

**Percent Release Rate**

| Drugloaded nanoparticles | % Release Rate | | | | | |
|---|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 120 min | 180 min | 360 min |
| Isoniazid | 0 | 21.2 | 31.6 | 45.1 | 56.3 | 67.5 |

## Conclusion

The present study successfully developed and characterized isoniazid-loaded solid lipid nanoparticles and PLGA nanoparticles as potential nanocarrier systems for improved drug delivery. The prepared formulations exhibited nanoscale particle sizes with acceptable polydispersity index and zeta potential values, indicating good stability of the nanoparticles. Encapsulation efficiency and drug loading studies demonstrated effective incorporation of isoniazid into both SLN and PLGA systems.

Morphological analysis using SEM and TEM confirmed the formation of spherical nanoparticles with uniform size distribution. X-ray diffraction studies indicated a reduction in the crystallinity of the drug after incorporation into the nanoparticle matrix, suggesting successful encapsulation. In-vitro drug release studies revealed sustained and controlled drug release profiles, with SLN formulations showing relatively higher drug release compared to PLGA nanoparticles.

Overall, the developed nanoparticle formulations demonstrated improved physicochemical characteristics and enhanced drug release behavior compared with conventional drug forms. These findings suggest that SLN and PLGA nanoparticles may serve as promising drug delivery systems for improving the therapeutic efficiency of isoniazid in tuberculosis treatment. Further in-vivo studies and clinical investigations are required to confirm their potential application in advanced tuberculosis therapy.

## References

1. R. Sankar, N. Sharda, and S. Singh, "Behavior of decomposition of  in the presence of isoniazid in the pH range 1–3," Drug Development and Industrial Pharmacy, vol. 29, no. 7, pp. 733–738, 2003. doi: 10.1081/DDC-120022009.
2. T. T. Mariappan and S. Singh, "Regional gastrointestinal permeability of  and isoniazid (alone and their combination) in the rat," Int. J. Tuberc. Lung Dis., vol. 7, no. 8, pp. 797-803, Aug. 2003.
3. F. Dalenconetal., "Atovaquone and rifabutine-loaded nanocapsules: formulation studies," Int. J. Pharm., vol. 153, no. 1, pp. 127–130, Jul. 1997.
4. Valverde-Fraga L, Haddad R, Alrabadi N, Sánchez S, Remuñán-López C, Csaba N. Design and in vitro assessment of chitosan nanocapsules for the pulmonary delivery of rifabutin. European Journal of Pharmaceutical Sciences. 2023 Aug 1;187:106484.
5. Silveira N, Longuinho MM, Leitão SG, Silva RS, Lourenço MC, Silva PE, Pinto MD, Abraçado LG, Finotelli PV. Synthesis and characterization of the anti-tubercular phenazine lapazine and development of PLGA and PCL nanoparticles for its entrapment. Materials Science and Engineering: C. 2016 Jan 1;58:458-66.
6. zurMühlen A, Schwarz C, Mehnert W. Solid lipid nanoparticles (SLN) for controlled drug delivery–drug release and release mechanism. European journal of pharmaceutics and biopharmaceutics. 1998 Mar 1;45(2):149-55.
7. Dixit N, Maurya SD, Sagar BP. Sustained release drug delivery system. Indian Journal of Research in Pharmacy and Biotechnology. 2013 May 1;1(3):305.
8. C. Allen, D. Maysinger, and A. Eisenberg, "Nano-engineering block copolymer aggregates for drug delivery," Colloids Surf. B Biointerfaces, vol. 16, no. 1–4, pp. 3–27, Nov. 1999.
9. Patel R. Fatty acid-based solid lipid nanoparticles and their effects on metabolism and permeability of drugs (Doctoral dissertation, Kingston University).
10. Zeng L, An L, Wu X. Modeling drug-carrier interaction in the drug release from nanocarriers. Journal of Drug Delivery. 2011;2011(1):370308.
11. Kamaly N, Yameen B, Wu J, Farokhzad OC. Degradable controlled-release polymers and polymeric nanoparticles: mechanisms of controlling drug release. Chemical reviews. 2016 Feb 24;116(4):2602-63.
12. M. Beck-Broichsitteretal., "Development of a biodegradable nanoparticle platform for sildenafil: formulation optimization by factorial design analysis combined with application of charge-modified branched polyesters," J. Control. Release, vol. 157, no. 3, pp. 469–477, Feb. 2012.
13. O. Kayser, C. Olbrich, S. L. Croft, and A. F. Kiderlen, "Formulation and biopharmaceutical issues in the development of drug delivery systems for antiparasitic drugs," Parasitology Research, vol. 90, pp. S63-S70, Jun. 2003.
14. T. Feczkó, J. Tóth, G. Dósa, and J. Gyenis, “Optimization of protein encapsulation in PLGA nanoparticles,” Chem. Eng. Process., vol. 50, no. 8, pp. 757–765, Aug. 2011.
15. A. K. Behera, B. B. Barik, S. Pandya, and S. Joshi, "Formulation and evaluation of Isoniazid loaded-∑-polycaprolactone nanoparticles," J. Pharm. Res., vol. 5, no. 2, pp. 798–802, Feb. 2012.
16. Montoto SS, Sbaraglini ML, Talevi A, Couyoupetrou M, Di Ianni M, Pesce GO, Alvarez VA, Bruno-Blanch LE,

Castro GR, Ruiz ME, Islan GA. Carbamazepine-loaded solid lipid nanoparticles and nanostructured lipid carriers: physicochemical characterization and in vitro/in vivo evaluation. Colloids and Surfaces B: Biointerfaces. 2018 Jul 1;167:73-81.

17. K. Dillen et al., "Factorial design, physicochemical characterisation and activity of ciprofloxacin-PLGA nanoparticles," Int. J. Pharm., vol. 275, no. 1–2, pp. 171–187, May 2004.