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Optimization and characterization of isoniazid nanoparticles for tubercular management

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ABSTRACT

Pulmonary drug delivery has been attractive for both local and systemic drug delivery as a non-invasive route that provides a large surface area, thin epithelial barrier, high blood flow and the avoidance of first-pass metabolism. Tuberculosis (TB) is a disease caused by bacteria that are spread from person to person through the air. Biodegradable Nanoparticles were effective drug delivery systems. For this various polymers have been used in drug delivery as they effectively deliver the encapsulated drug to a target site and thus enhance the therapeutic benefit and minimizing the side effect. Nanoparticles are better than liposome's in tissue targeting in increasing stability of drugs and posses useful controlled release properties. And hence only in this study the main aim is to formulate and evaluate solid lipid nanoparticles containing Isoniazid by using solvent evaporation method. But among the two formulation it was concluded that Solvent evaporation method followed by ultrasonication was an optimized technique for the preparation of SLN nanoparticle, which lead to better results like high entrapment efficiency, good percentage yield, high drug content and Span 80 was a better choice of surfactant to reduce the particle size and leads to uniform distribution of SLN in its phase.

Keywords: SLN Nanoparticles, Isoniazid, Characterization and formulation methods

INTRODUCTION

Nanotechnology, as applied to medicine, brought significant advances in the diagnosis and treatment of disease. The desired applications in medicine include drug delivery, neutraceuticals, both in vitro and in vivo diagnostics and production of improved biocompatible materials. Nanoparticles are emerging as a class of therapeutics for cancer and can show improved efficacy, while simultaneously decreasing side effects, owing to properties such as more targeted localization in tumors and active cellular uptake. Oral route is the easiest and most convenient route for non invasive administration. Oral drug delivery system is the most cost- effective and leads the world wide drug delivery market. The oral route is the preferred route for chronic drug therapy. Numerous potent lipophilic drugs exhibit low

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bioavailability due to their poor aqueous solubility. Approximately 35-40% of new drug candidates have poor water solubility. When a drug is administered by oral route the first step for it to get solubilized and then absorbed [1-7].

MATERIALS AND METHODS

Isoniazid procured from Hetero Laboratories Pvt Ltd Hyderabad, Cholesterol from Himedia, Mumbai, Stearic acid from Loba Chemie and other ingredients used in the research obtained from Merck, Mumbai.

Solvent Evaporation followed by Ultrasonication

Isoniazid, Cholesterol and Span 60 are dissolved in ethanol and kept for some time in bath sonicator .The aqueous medium is prepared by dissolving tween 80 / Sodium lauryl sulfate (SLS) in distilled water and kept for stirring in magnetic stirrer for 15 mins. Upon evaporation of the solvent, the lipid phase is slowly added into the aqueous phase under continuous stirring. The nanoparticles dispersion is formed in the aqueous medium. The solution was kept in probe sonicator at different pulse rate. Now repeat the same experiment with same amount of solvent, span 60 by adding stearic acid [8-12].

Table 1: Composition of various formulation of Isoniazid SLN-Solvent evaporation method (Ultrasonication)

Trial Formulation	FS1	FS2	FS3	FS4	FS5	FS6
Isoniazid (mg)	20	20	20	20	20	20
Cholesterol (mg)	200	200	200	-	-	-
Stearic acid (mg)	-	-	-	200	200	200
Span 60(mg)	100	100	100	100	100	100
Tween 80(ml)	0.5	1	1.5	0.5	1	1.5
Distilled Water(ml)	50	50	50	50	50	50
Ethanol(ml)	10	10	10	10	10	10
Sonication time (Pulse rate)	5 min	10 min	15 min	5 min	10 min	15 min

Solvent Evaporation followed by Homogenization

Isoniazid, Cholesterol and Span 60 are dissolved in ethanol and kept for some time in bath sonicator .The aqueous medium is prepared by dissolving tween 80 in distilled water and kept for stirring in magnetic stirrer for 15 mins. Upon evaporation of the solvent, the lipid phase is slowly added into the aqueous phase under continuous stirring. The nanoparticles dispersion is formed in the aqueous medium. The solution was kept in Homogenization at different RPM speed .Now repeat the same experiment with same amount of solvent, span 60 by adding stearic acid [9].

Table 2: Composition of various formulation of Isoniazid SLN-Solvent evaporation method (Homogenization)

Trial Formulation	FH1	FH2	FH3	FH4	FH5	FH6
Isoniazid (mg)	20	20	20	20	20	20
Cholesterol (mg)	200	200	200	-	-	-
Stearic acid (mg)	-	-	-	200	200	200
Span 60 (mg)	100	100	100	100	100	100
SLS (%)	0.5	1	1.5	0.5	1	1.5
Distilled Water (ml)	50	50	50	50	50	50
Ethanol (ml)	10	10	10	10	10	10
Homogenization (RPM)	1000	2000	3000	1000	2000	3000

Optimization Modus operandi

Physical, chemical and biological properties all must be given due consideration in the selection of components and processing steps for the dosage form. The final product must be one that meets not only the requirements placed on it from a bioavailability standpoint, but also the practical mass production criteria of process and product reproducibility. While undergoing formulation it should be understand the theoretical formulation and target processing parameter, as well the ranges for each excipients and processing parameter.

Optimization technique provides both the depth of understanding and a ability to explore and defend ranges for the formulation and processing factors. With the rational approaches to the selection of the several excipients and manufacturing steps for a given product, one qualitatively selects a formulation. Optimization was an useful tool to quantitate a formulation that can be qualitatively determined. The word optimize is defined as follows i.e., to make as perfect, effective and functional as possible.

In developing a dosage form one must be undergo logical steps, carefully control the variables and changing one at a time until a satisfactory system is produced. No matter how the dosage form is designs, but the trial and error method will be improve the quality of the dosage form [10-15].

Characterization of SLN

The methods for the characterization should be perceptive to the key parameters of the performance of SLNs. Several parameters which have to be considered in characterization are as follows

Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) analysis was performed using Nietzsche DSC 200PC (Nietzsche, Selb, Germany). The instrument was calibrated with indium (calibration standard, >99.999%) for melting point and heat of fusion. A heating rate of 100C/min was employed in the range of 25–2000C. Analysis was performed under nitrogen purge (20 mL/min). The samples were weighted into standard aluminium pans and an empty pan was used as reference.

Scanning electron microscopy [26]

Scanning electron microscopy (SEM) was conducted to characterize the surface morphology of the SLNs. The samples were mounted on alumina stubs using double adhesive tape, coated with gold in HUS-5GB vacuum evaporator. Then the sample was observed in Hitachi S-3000N SEM at an acceleration voltage of 10KV and a magnification of 5000X.

Particle size determination

The average particle size, polydispersity index and zeta potential of the lipid particulate dispersions were determined using a Zetasizer (DTS Ver.4.10, Malvern Instruments, UK). The sample of dispersion was diluted to 1:9 v/v with double distilled water to ensure that the light scattering intensity was within the instrument's sensitivity range. Double distilled water was filtered through 0.45 μm membrane filters (Pall Life sciences, Mumbai, India) prior to particle size determination.

Zeta Potential

Zeta potential is the difference in the potential between the surface of tightly bound layer and the electro neutral region of the solution.

Total drug content

From the prepared SLN formulation 1ml of suspension is dissolved in the 10 ml of 6.8pH PBS buffer and ethanol mixture. The amount of Isoniazid was determined using UV spectrophotometer at 263nm.The placebo formulation prepared similarly to drug loaded SLN is used as blank. The total drug content was calculated.

Determination of Entrapment Efficiency (EE)

The EE was determined by analyzing the free drug content in the supernatant obtained after centrifuging the SLN suspension in high speed centrifuge at 16000 rpm for 30 min at 30°C using Remi cooling centrifuge (Mumbai, India).

The EE was calculated as follows:

EE= {total drug content-free drug content/ total drug content}*100

In vitro drug release

Drug release from the formulations was studied invitro using dialysis membrane . Membrane was soaked in double-distilled water for 12 h before mounting in a diffusion cell. This is performed by using a modified Franz diffusion cell at 370^0C which is fitted with a dialysis membrane having a molecular weight cut off 3500Da.The membrane was soaked in boiling distilled water for 12 hours before mounting in a Franz diffusion cell. SLN dispersion 2 ml is placed in to the donor compartment and the 20ml of PBS is used to fill receptor compartment. With one hour interval 1ml of sample is withdrawn and analyzed using UV Visible spectrophotometer at 263 nm [16-18].

Invitro drug release kinetics

Different kinetic models such as zero order (cumulative amount of drug released vs. time), first order (log cumulative percentage of drug remaining vs. time), Higuchi model (cumulative percentage of drug released vs. square root of time), korsmeyer-peppas model and Hixson crowell model were applied to interpret the drug release kinetics from the formulations. Based on the highest regression values for correlation coefficients for formulations, the best-fit model was decided.

The release rate and mechanism of release of drug from the prepared microcapsules were analyzed by fitting the release data into

Zero-order equation,

$Q = K_0 t$, Where, Q is the amount of drug release at time, t and K_0 is the release rate constant.

First order equation

Log $Q = K_1 t$, Where Q is the percent of drug release at time, t and K_1 is the release rate constant.

Higuchi's equation

$Q = K_2 t^{½}$, Where, Q is the percentage of drug release at time t and K_2 is the diffusion rate constant.

Peppa's equation

$Mt/M\infty = Ktn$, Where $Mt/M\infty$ is the fractional release of the drug, t is the release time, K is a constant incorporating structural and geometric characteristic of the release device, „n‟ is the release exponent indicative of mechanism of release[18-25]. For non-Fickian (anomalous/zero order) release, „n‟ value is between 0.5 to 1.0; for Fickian diffusion, $n < 0.5$; for zero order release, $n = 1$; „n‟ is estimated from linear regression of log $(Mt/M\infty)$ Vs log t.

RESULTS AND DISCUSSION

Evaluation of Isoniazid SLN

Scanning electron microscopy (SEM)

Shape and surface morphology of the SLNs prepared with optimized parameters was observed by research microscope and scanning electron microscopy. The study revealed that most of the SLNs were fairly spherical in shape, the surface of the particle showed a characteristic smoothness, and that the particle size was in the nanometric range, as depicted by SEM. Some of the particles were found to be in clusters as shown in the Figure 1.

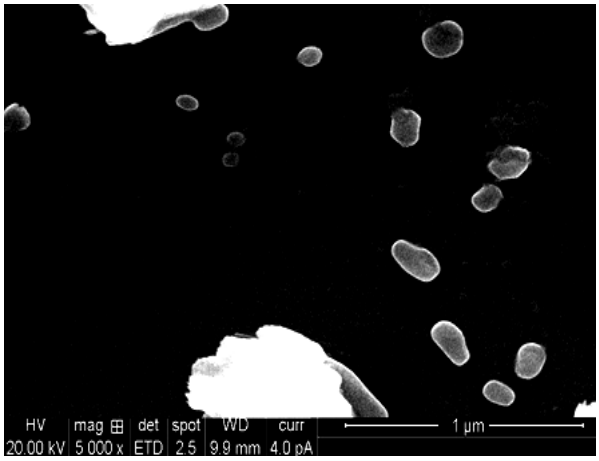

Figure 1: SEM – surface morphology of SLN

Differential Scanning Calorimetry (DSC)

Thermogram of pure drug shows a sharp endothermic peak at 177.74°C, which corresponds to its melting point, represented in figure. Formulation also showed endothermic peak at 177.00°C, which corresponds to the melting point of the drug. The DSC thermogram revealed that there was significant difference between the drug and the excipients. From the thermogram it was evident that melting point of Isoniazid was changed when it was formulated as solid lipid nanoparticles.

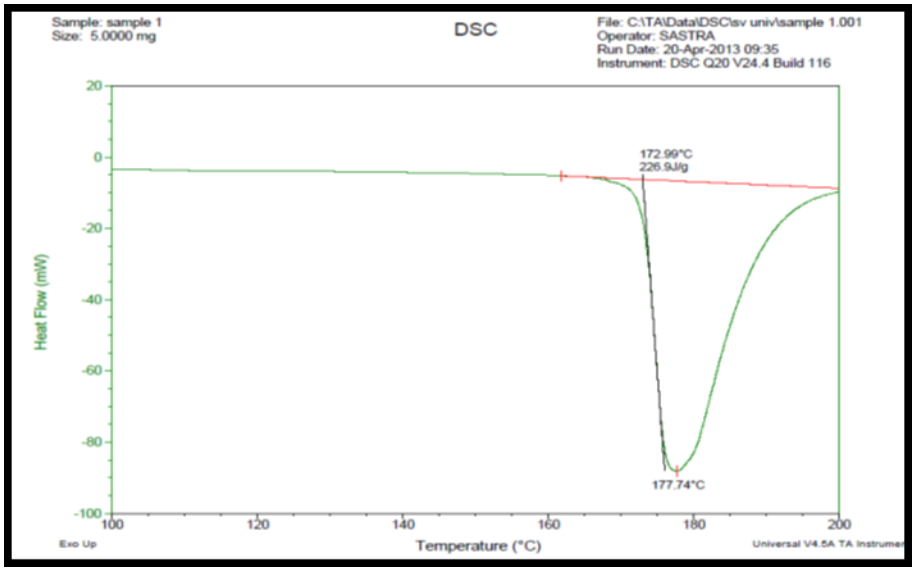

Figure 2: DSC Thermogram of pure Isoniazid

The Isoniazid and solid lipid nanoparticle of formulation were subjected for DSC studies. DSC thermograph showed that difference in the melting point of the formulation when compared with drug. This was due to thermal transition behavior. Decrease in the melting point of the drug was due to decrease in the crystallinity of the compound. it was observed that the heat of fusion for pure drug was 41.86 J/g, where ΔH for Isoniazid and prepared solid lipid nanoparticle formulation was 7.719 J/g and 18.23 J/g respectively.

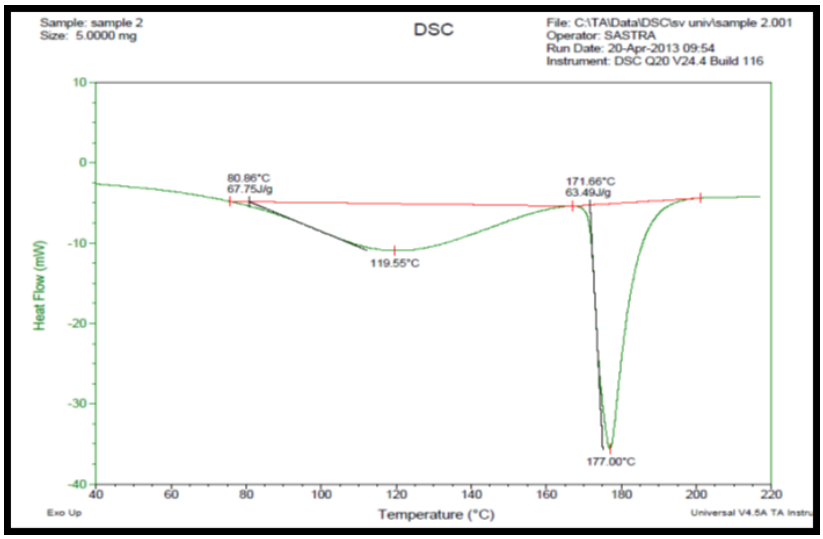

Figure 3: DSC Thermogram of SLN formulation

Evaluation of isoniazid SLN –prepared by solvent evaporation method folllowed by ultrasonication

The particle size analysis revealed that, the SLNs were in the nanometer range. The size of the nanoparticles was affected by the sonication time and the concentration of tween 80. The size of the Isoniazid loaded SLNs were found to be between 273.3 nm to 368.6 nm.The stability of the formulated SLNs was evaluated by measuring the zeta potential of the SLNs by the Malvern particle size analyzer.

Zeta potential of isoniazid loaded formulations was in the range of --23.38 ± 2.40 to -35.26 ± 2.28 mV and Polydispersity index was found to be between 0.234±0.028 to 0.326±0.012. From the results it shows that as sonication time and surfactant concentration increases with decrease in particle size to nanometric range. And if concentration of surfactant i.e.tween 80 increases with decrease in Poly Dispersibility index which shows good dispersibility particles and stability by increasing the concentration of tween 80.

Table 3: Effect of Sonication time &Tween 80 on Particle size, PDI and Zeta Potential

Formulation	Sonication time (min)	Tween 80 concentration %	Mean Particle size (nm)	Poly Dispersibility Index	Zeta Potential (mV)
FS1	5	0.5	368.6±3.9	0.326±0.012	-28.70 ± 2.28
FS2	10	1.0	346.4±1.8	0.312±0.018	-23.38 ± 2.40
FS3	15	1.5	273.3±4.1	0.263±0.024	-35.26 ± 1.84
FS4	5	0.5	340.5±3.1	0.358±0.020	-26.84 ± 1.82
FS5	10	1	288.6±1.4	0.244±0.018	-27.44 ± 2.80
FS6	15	1.5	278.00±1.8	0.234±0.028	-26.85± 1.80

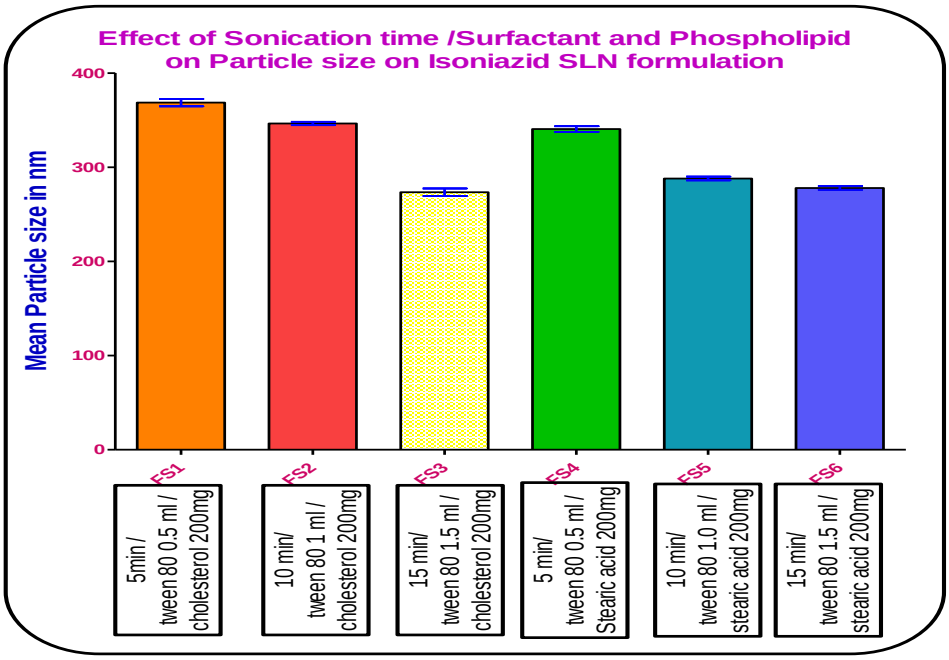

Figure 4: Effect of Surfactant & Phospholipid, Sonication time on particle size on Isoniazid SLN

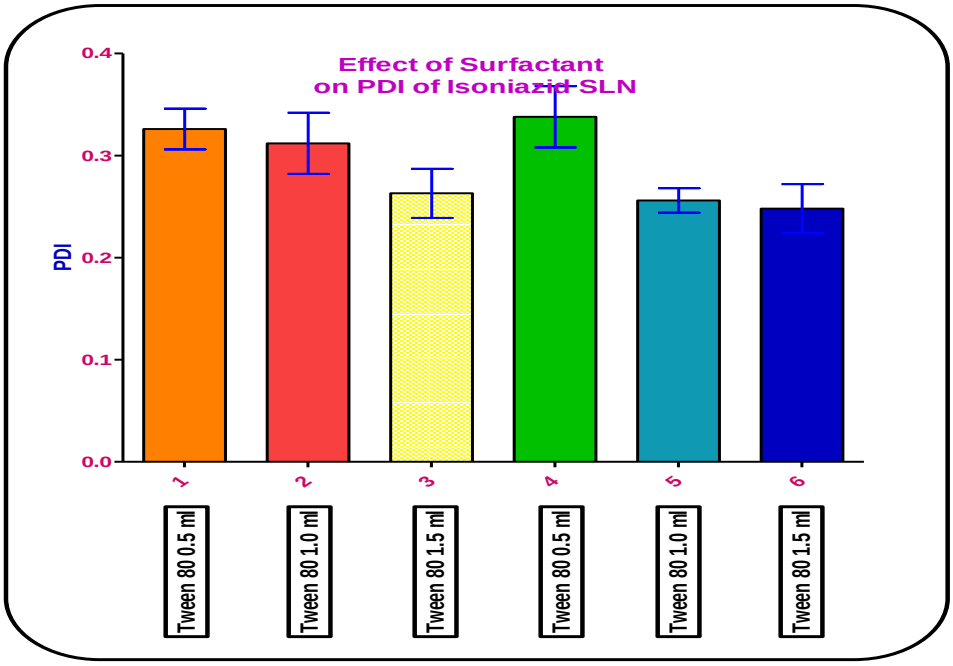

Figure 5: Effect of tween 80 on PDI of Isoniazid SLN

Drug content

The prepared formulations were analyzed for drug content. It was observed that the drug content in the prepared solid lipid nanoparticles was satisfactory and the drug was uniformly distributed in all the formulations.

The percentage drug content is highest for FS3 formulation was about 98.91 ± 2.050 and lowest for FS4 was about 88.29 ± 2.045.This may be due do the concentration of Phospholipid concentration in the formulation.

Table 4: Drug content for the prepared formulations

Sl. No.	Formulation code	% Drug content
1	F S1	94.68±2.047
2	F S2	97.87±2.040
3	F S3	98.91±2.050
4	F S4	88.29±2.045
5	F S5	91.45±2.025
6	F S6	92.55±2.060

*Standard deviation (n=3)

Percentage yield, encapsulation efficiency

During the formulation of solid lipid nanoparticles, the percentage yield obtained after the whole process was not equal to 100 %. So, there was deviation in the percentage yield of the compound. Especially in the freeze drying process, the percentage yield of the product was calculated after freeze drying.

The percentage yields of the formulations lies between 90.62 ±2.85 to 97.18±3.24 .Encapsulation efficiency gives the amount of drug entrapped in the solid lipid.

This may be due do the concentration of Phospholipid concentration in the formulation. Concentration of phospholipid and homogenization time increases with increase in Percentage yields and Entrapment efficiency of Isoniazid in SLN.

Table 5: Percentage yield and EE for prepared formulations

S.No	Formulation code	Percentage yield	% Encapsulation efficiency
1	FS1	97.18± 3.24	86.51±2.72
2	FS2	94.06± 3.44	90.24±2.24
3	FS3	90.62±2.85	88.16±2.06
4	FS4	92.18±2.44	80.73±3.84
5	FS5	95.62±2.80	83.76±3.08
6	FS6	93.12±3.84	85.08±2.22

*Standard deviation (n=3)

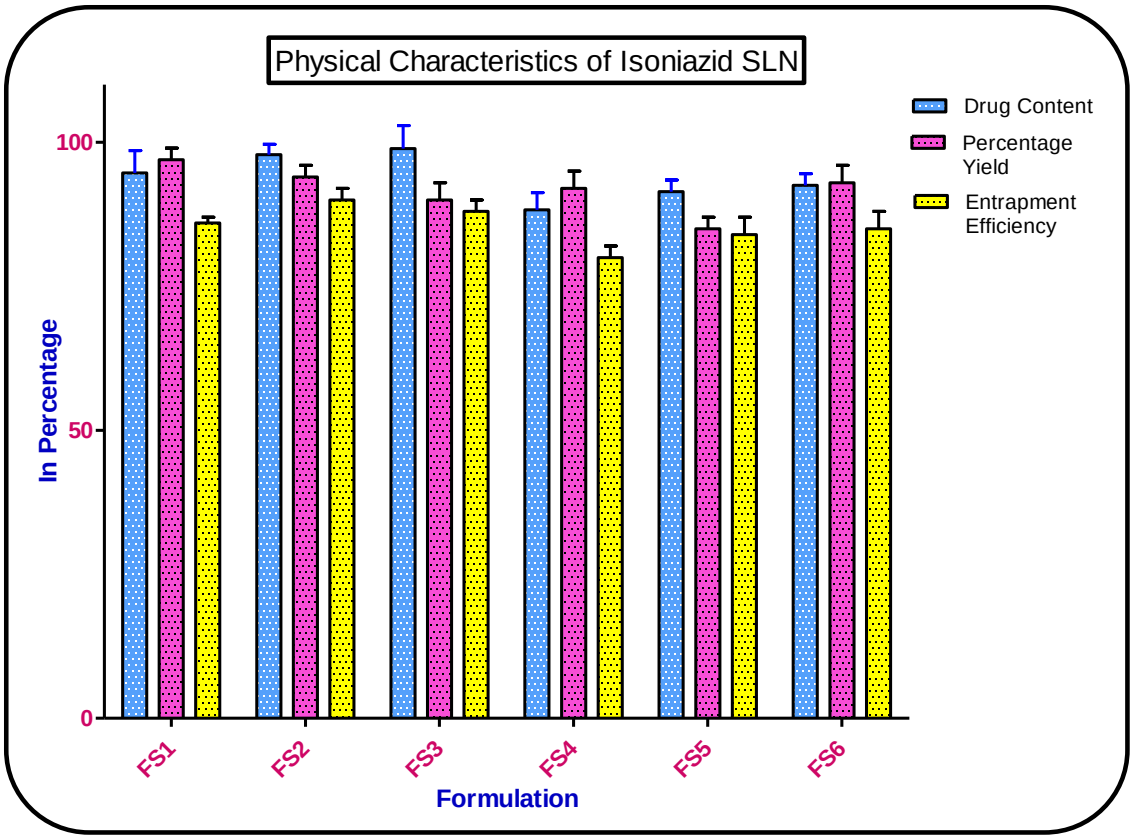

Figure 6: Physical Characteristics of Isoniazid SLN

Invitro drug release

The *invitro* drug release studies for all six formulations of Isoniazid loaded SLNs were carried out in pH 6.8 phosphate buffer using dialysis membrane and Franz diffusion apparatus.

The in vitro release profile obtained for all six Isoniazid loaded SLN formulations FS1 to FS6. The cumulative percent drug release of Isoniazid loaded SLNs after 24 hrs was found to be 90.13 %, 89.23 %, 92.23 %, 80.1 %,82.16% and 84.46% for F-1 to F-6 respectively.

From the results it was observed that, all the formulation shows better control release of drug from the SLN. But smaller particles leading to faster drug release due to larger surface area. In general the drug release from all formulation followed a steady pattern. It was observed that the drug release from the formulations decreased as in the tween 80 concentration.

In the formulations the cholesterol having tween 80 concentration with 1.5 ml shows the good release. The drug release may be mainly controlled by drug diffusion through the lipid matrix respectively.

Table 6: *Invitro* drug release of Isoniazid SLN (FS1-FS6)

Time (hr)	FS1	FS2	FS3	FS4	FS5	FS6
0.5	0	0	0	0	0	0
1	3.83± 2.05	3.73±2.021	4.59±0.05	4.21±0.038	4.02±0.020	4.78±0.025
2	8.49±2.04	7.21±2.020	8.71±2.02	7.28±1.06	6.21±1.05	6.72±2.02
3	12.94±2.03	11.87±2.02	13.4±2.06	10.52±2.03	8.6±2.02	9.60 ±2.05
4	17.26±2.04	15.45±2.09	20.13±2.04	17.16±2.04	13.71±2.03	15.35±1.05
5	24.01±2.04	22.92±2.08	27.9±2.03	23.19±1.04	20.3±2.04	24.05±1.04
6	29.32±2.03	26.32±2.03	32.69±2.04	27.78±2.02	26.59±1.04	28.16±2.03
7	33.95±2.03	31.95±2.06	36.88±2.04	32.69±2.05	29.96±1.06	30.09±2.04
8	39.7±2.04	38.64±2.06	45.49±2.05	37.86±1.03	37.52±1.06	36.79±1.04
9	46.62±2.05	44.88±2.05	50.53±2.04	41.9±1.08	40.7±2.04	43.72±1.03
10	55.23±2.03	52.35±2.03	56.28±2.03	49.6±1.03	47.34±2.04	50.42±2.03
12	61.23±2.05	59.34±2.01	67.08±2.03	56.87±2.09	55.57±1.03	57.43±2.04
24	90.13±2.02	89.23±2.07	92.23±2.02	80.1±2.03	82.16±2.06	84.46±2.04

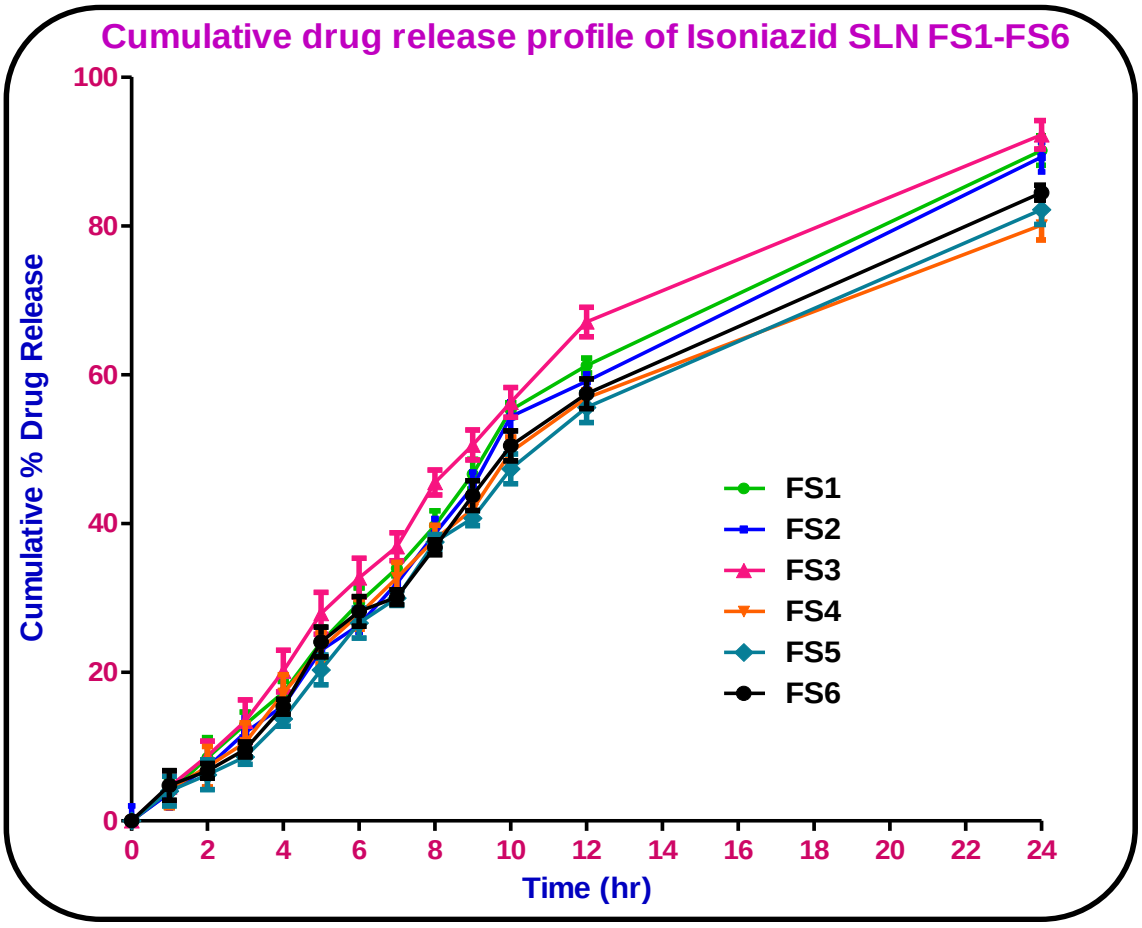

Figure 7: Cumulative *Invitro* Drug Release Studies for Isoniazid SLN FS1-FS6

In-vitro drug release kinetics

The release kinetics of Isoniazid loaded SLNs are evaluated by fitting the data into various kinetic models like first order, zero order, Higuchi, Peppas, and Hixson–Crowell equations. The drug release kinetic data of Isoniazid loaded SLNs respectively. It was proved that Zero order model R^2 values of all SLN formulation were 0.9903 to 0.9974 respectively which are higher than other models. So it was concluded that all the formulations follow Zero order kinetics, which release the same amount of drug at unit time and it is the ideal method of drug release to achieve pharmacological prolong action.

The values of release exponent (n) of all the formulations lies within of n = 0.5-1 have been observed, which are regarded as Non-fickian diffusion mechanism. Based on the results, the release of Isoniazid from SLNs best-fitted in Peppas fitting kinetics and the possible mechanisms for the drug release might be diffusion of the drug from the matrix and matrix erosion resulting from degradation of lipids.

Table 7: *In vitro* release kinetics for the prepared solid lipid nanoparticle formulations

Release Model		Formulation Code					
		FS1	**FS2**	**FS3**	**FS4**	**FS5**	**FS6**
Zero order	R^2	0.9919	0.9923	0.9974	0.9944	0.9903	0.9918
First order	R^2	0.8782	0.8775	0.8902	0.934	0.9077	0.9097
Hixson Crowell	R^2	0.9951	0.9951	0.9911	0.9926	0.9962	0.9953
Higuchi	R^2	0.9318	0.9327	0.9536	0.9437	0.9268	0.9308
Peppas	R^2	0.9852	0.9881	0.9879	0.9841	0.9757	0.9738
	n	0.891	0.887	0.863	0.8668	0.914	0.877
Best Fit Model		Peppas	Peppas	Peppas	Peppas	Peppas	Peppas
Model		Non-Fickian diffusion mechanism	Non-Fickian diffusion mechanism	Non-Fickian diffusion mechanism	Non-Fickian diffusion mechanism	Non-Fickian diffusion mechanism	Non-Fickian diffusion mechanism

*Standard deviation (n=3)

CONCLUSION

In this current research, SLN are formulated using with various excipients especially surfactant and process especially ultrasonication and homogenization time. Which are evaluated for Particle size, Zeta potential and Poly dispersibility index (PDI). It shows size of SLN decreases with increase in concentration of surfactant along with increased sonication and homogenization time. Concentration of surfactant (SLS/Tween 80) increases means, the size of SLN decreases and Zeta potential (surface charge) increases. Finally it was concluded that desired SLN was formed when the concentration of the surfactant (tween 80 and sodium lauryl sulfate) was maintained at 1.5% and Ultrasonication at 15 pulse/min, Homogenization (3000 RPM) under solvent evaporation method. Hence it has been concluded the method adopted for the formulation has been very conducive to prepare Isoniazid SLN nanoparticles.

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