PHARMATUTOR
PHARMACY INFOPEDIA
ISSN: 2347-7881

Research Article

Formulation and Evaluation of Solid lipid nanoparticles: Isoniazid

Umatiya Imran*, Chintan Aundhia, A.K.Seth, Sachin Chauhan, Nirmal Shah
Department of pharmacy, Sumandeep Vidhyapeeth University,
Piparia, Waghodia, Vadodara, Gujarat, India
imran_umatiya@yahoo.com

ABSTRACT

Tuberculosis is an infectious disease caused by Mycobacterium Tuberculosis which attacks lungs and other parts of the body. Isoniazid is a hydrophilic drug that is having a first line antituberculosis drug. Entrapment Efficiency has been improved by preparing Solid Lipid Nanoparticles (SLNs). Two different variable Stearic acid and Tween 80 were used by using w/o/w double emulsion-solvent evaporation method by using 3^2 factorial designs. Particle size, Surface Morphology, Entrapment efficiency and In-vitro diffusion studies were evaluated for prepared SLNs. Different combination of Surfactant and Lipid were found to have significant effect on entrapment efficiency but not on drug release. The prepared SLNs were found in spherical shape and 678 nm particle size was found. % Entrapment efficiency were found from 31.90±0.41 to 74.89±0.67. Present Work is focused on increase incorporation of drug into SLN. Work indicates that formulation of SLNs loaded INH can give reliable therapeutic effect for the treatment of tuberculosis by prolonged action.

Keywords: Solid lipid nanoparticles, Isoniazid, 3^2 factorial designs

INTRODUCTION

Tuberculosis, or TB (tubercle bacillus) is a common, and in many cases lethal, infectious disease caused by various strains of mycobacterium usually Mycobacterium tuberculosis. Tuberculosis typically attacks the lungs, but can also affect other parts of the body. It is spread through the air when people who have an active TB infection cough, sneeze, or otherwise transmit their saliva through the air.[1]

Isoniazid also known as isonicotinyl hydrazine (INH) is an organic compound that is the first-line medication in prevention and treatment of tuberculosis. INH is an antimycobacterial agent, which is bactericidal for both extracellular and intracellular organisms. It is the primary drug for the treatment of tuberculosis when the disease is caused by isoniazid-sensitive strains of the M. tuberculosis. [2]

The solid lipid nanoparticles (SLNs) possess a lipid core matrix in the nanometer range stabilized by a layer of surfactants. They have been used as ideally suited drug delivery systems for the proteins vaccines and other drugs for controlled release compared to other colloidal drug delivery systems. Their ability to penetrate through several anatomical barriers, sustained release of their contents, and their nanometer size range makes the implementation of SLNs as successful drug delivery systems. SLNs combine the advantages of both polymeric nanoparticles and liposome is such as possibility of controlled drug release and drug targeting, increased drug stability,

How to cite this article: : I Umatiya, C Aundhia, AK Seth, S Chauhan, N Shah; Formulation and Evaluation of Solid lipid nanoparticles: Isoniazid; PharmaTutor; 2014; 2(10); 129-135

PHARMATUTOR
PHARMACY INFOPEDIA
ISSN: 2347-7881

incorporation of lipophilic and hydrophilic drugs.[1]

SLN's can be prepared by various technique high shear homogenization and ultrasound, high pressure homogenization, hot homogenization, cold homogenization, solvent emulsification and evaporation methods. Both hydrophilic and lipophilic drugs can be incorporated in to SLNs loading of hydrophilic drugs is a great challenge as the drug has maximum tendency to partition in the water during the preparation process. INH is a hydrophilic drug, which is effective drug for the treatment of tuberculosis. Isoniazid is a biopharmaceutical classification system class III drug (high solubility and low permeability) having an aqueous solubility of approximately 125 mg/ ml. The drug is characterized by a short half-life ranging from 1h to 4h, depending on the rate of metabolism. INH has a pronounced absorption from all the three sections of the small intestine and from intramuscular injection sites. INH is less permeated through the stomach and is mainly absorbed through the intestine.[3]

MATERIALS AND METHODS

Materials: INH & PVA was purchased from Loba chemie pvt. Ltd (Mumbai, India) Tween 80, Methanol, & DCM purchased from Sulab Laboratories (Baroda, India) Stearic Acid Molychem (Mumbai, India

Methods: Isoniazid (INH), a hydrophilic drug has been used in the present investigation to determine the process variables effecting the incorporation of hydrophilic drugs in to SLN. In the present study, a simple approach for the fabrication of SLN of the basic molecule INH was adopted, using 3^2 factorial designs. Two formulation variables Stearic Acid and Tween 80 conc. were studied to optimize the formulation for maximum entrapment efficiency (EE). Nine different formulations were prepared by using different concentrations of tween 80 and stearic acid. The variable parameters used during formulation development are given in Table No.1.

Sr.No	1	2	3	4	5	6	7	8	9
Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9
Stearic Acid	100mg	200mg	300mg	100mg	200mg	300mg	100mg	200mg	300mg
Tween Acid	0.5ml	1.0ml	1.5ml	0.5ml	1.0ml	1.5ml	0.5ml	1.0ml	1.5ml

Table.No.1 Detail of code for experimental design

Methods of preparation of SLN: Constant weight of Drug & 5% PVA were used. Stearic acid, Tween 80 were taken according to the 3^2 factorial design.

Solvent injection technique: The solid lipid (stearic acid) was dissolved in organic solvent [e.g. methanol and DCM (3:2)]. Then this organic solvent mixture was slowly mixed into the surfactant, which contained drug. These mixture stirred by sonicater for 2-3 minutes. Primary emulsion mixtures through an injection needle stirred in to PVA solution at 1500 rpm for 2 hrs. The presence of surfactant within the aqueous phase helps to produce lipid droplets at the site of injection and stabilize the formed SLNs until solvent diffusion was complete by reducing the surface tension. Drug polymer ratio were taken 1:5, 1:10 and 1:15.[4-5]

Evaluation of SLN:

1) Drug entrapment efficiency:

Separation of free drug: Analysis of INH from SLN was done by separating free drug from the nanoparticles dispersion. The separation was done by centrifugation of nanoparticles. Then, the nanoparticles pellets and supernatant were separated out.

PHARMATUTOR
PHARMACY INFOPEDIA
ISSN: 2347-7881

Direct method: In this method, analysis of drug from SLN was done by dissolving in phosphate buffer pH 7.4. The dispersion was then allowed to stand for overnight for complete dissolution of drug. Then, absorbance was taken against phosphate buffer as a blank on UV-Visible Spectrophotometer. The percentage entrapment was calculated by using following equation.

%Drug Entrapment =Drug entrapped in SLN's/Total drug taken *100

Indirect method: In this method, analysis of drug from SLN was done by appropriately diluting supernatant in phosphate buffer and absorbance was taken against phosphate buffer as a blank on UV-Visible Spectrophotometer. To find out percentage entrapment following equation was used. [8-9]

%Drug Entrapment = Total Drug taken-drug in supernatant/Total Drug taken *100

2) Scanning Electron Microscopy

The SEM analysis of prepared SLN was performed for morphological studies. SEM is an instrument that produces largely magnified image by using electrons instead of light to form an image. Electron gun produces a beam of electrons, which follow the vertical path through the microscope between electromagnetic fields and lenses towards the sample due to which electrons and x-rays are ejected from sample [10-11]

3) Measurement of particle size and zeta potential:

Zeta potential of a nanoparticle reflects the electric potential of particles and is used to characterize the surface charge properties and to determine whether the charged particle is encapsulated within the centre or adsorbed on to the surface of nanocapsule. The Particle size and Zeta potential of nanoparticles were recorded using Zetasizer. The samples that gave good In-vitro release was subjected to zeta potential analysis.[12]

4) In vitro diffusion studies:

In-vitro drug diffusion of nanoparticles in present research work is carried out by Dialysis Bag diffusion method. A 4–5 cm long portion of the dialysis tubing was made into a dialysis sac by folding and tying up one end of the tubing with thread. It was then filled up with phosphate-buffered and examined for the leaks. The sac was then emptied and 1 ml of the nanosuspension, liquid nanosuspension was accurately transferred into sacs, which served as the donor compartments. The sacs were once again examined for leak and then suspended in the glass beakers containing 50 ml phosphate-buffered, which become the receptor compartment. At predetermined time intervals, 3 ml samples were withdrawn from the receptor compartment and analyzed spectrophotometrically. Fresh buffer was used to replenish the receptor compartment at each time point. [13]

5) Stability Study:

The stability study was carried out for optimized formulation as per ICH guidelines. The nanoparticle of the best formulation were placed in screw capped glass container and stored at various ICH storage condition for a period of 60 days. The samples were analyzed for physical appearance and for the drug content at regular interval of 15 days.[14]

Results and Discussion: Solid lipid nanoparticles were prepared by w/o/w double emulsion solvent evaporation method. Drug can be effectively loaded in lipid which resulting less toxic and that can exhibit enhanced efficiency.

ENTRAPMENT EFFICIENCY

Entrapment efficiency was performed for all nine batches (F1 – F9). The result obtained for a different batch varies from **31.90±0.45** to

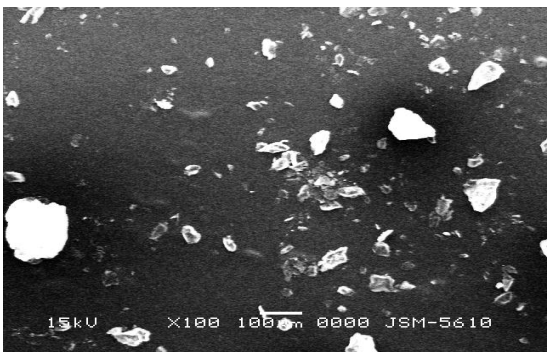
PHARMATUTOR
PHARMACY INFOPEDIA
ISSN: 2347-7881

74.89±0.67. It has been observed that with increase in lipid concentration entrapment efficiency also increases. The maximum entrapment efficiency was found **74.89±0.67** obtained in Batch F8.

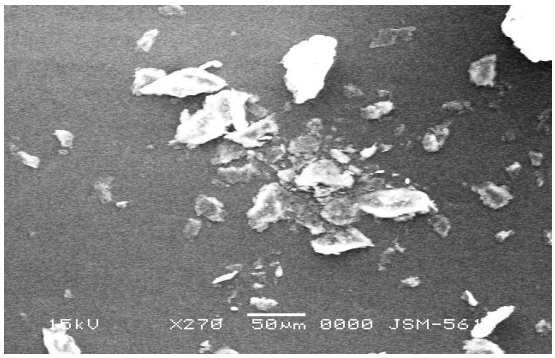
BATCH	D:P RATIO	%EE
F1	1:5	31.90±0.41
F2	1:5	45.33±0.87
F3	1:5	44.67±0.37
F4	1:10	48.44±0.34
F5	1:10	55.23±0.78
F6	1:10	57.86±1.23
F7	1:15	61.37±0.81
F8	1:15	74.89±0.67
F9	1:15	68.37±0.97

Table no 2: % Entrapment efficiency

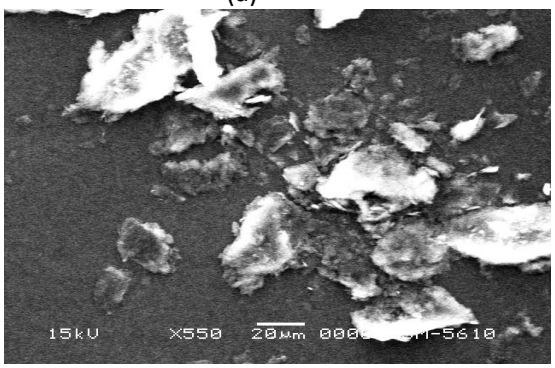
SCANNING ELECTRON MICROSCOPY:



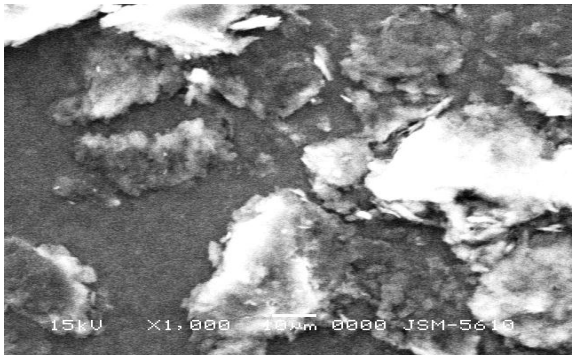
(a)



(b)



(c)



(d)

PARTICLE SIZE ANALYSIS

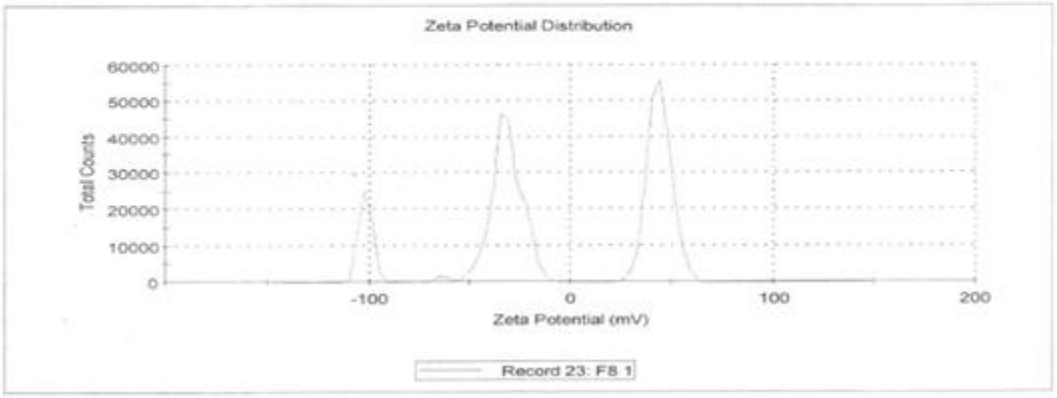
Particle size determination was done from a Malvern Mastersizer instrument, which gave particle size range of 678 nm with maximum intensity and volume.

PHARMATUTOR
PHARMACY INFOPEDIA
ISSN: 2347-7881

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-7.46	Peak 1:	44.0	44.6	5.99
Zeta Deviation (mV):	52.2	Peak 2:	-31.6	43.1	7.70
Conductivity (mS/cm):	3.99	Peak 3:	-102	11.6	3.17

Result quality : See result quality report



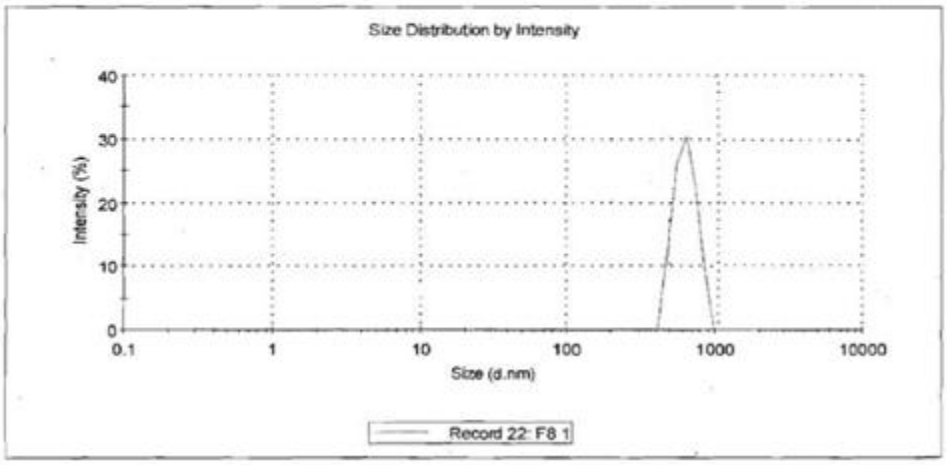
ZETA POTENTIAL

Zeta potential was determined and it was found to be -**7.46 mv**. Zeta potential determines the physical stability of solid lipid nanoparticle. Zeta potential is an indirect measurement of the thickness of the diffusion layer, i.e. can be used to predict long-term stability

Results

		Size (d.nm):	% Intensity	Width (d.n...
Z-Average (d.nm):	723	Peak 1:	678	100.0	218.2
PdI:	0.427	Peak 2:	0.000	0.0	0.000
Intercept:	0.597	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report



In-Vitro STUDIES

The In-Vitro drug release of drug INH from the various SLNs was carried out by using dialysis method in phosphate buffer solution 7.4pH for 24 hours. The cumulative percentage release of Isoniazid from the

PHARMATUTOR
PHARMACY INFOPEDIA
ISSN: 2347-7881

prepared SLNs varied from **82.91±0.38** to **95.09±0.27.** Maximum release found from **F8** Batch and Minimum release found from **F5** batch,

Sr.no	Time(Hrs)	F5	F8
1	00	0	0
2	01	35.88±0.41	45.45±0.29
3	02	47.99±0.28	60.32±0.12
4	04	72.71±0.31	81.32±0.15
5	08	78.76±0.48	86.50±0.19
6	12	79.15±0.17	91.09±0.52
7	24	82.91±0.38	95.09±0.27

Table No 3: % CDR of formulation F5 & F8

STABILITY STUDIES

Stability testing was done for an optimized batch, %Drug Remaining was calculated for 2 months at an interval of 15 days for 25 ºC ,4ºC & 40ºC

Formulation	Tested after time(days)	Physical Appearance	%of Drug Remaining ±SD
Batch F8	15	No change	100
	30	No change	100
	45	No change	99.56±0.1
	60	No change	98.01±0.1

Table No 4: Stability study of formulation batch F8 stored at 25 ºC ±2º(60%±5% RH)

Formulation	Tested after time(days)	Physical Appearance	%of Drug Remaining ±SD
Batch F8	15	No change	100
	30	No change	99.24±0.01
	45	No change	98.07±0.05
	60	No change	97.98±0.35

Table No 5: Stability study of formulation batch F8 stored at 4 ºC ±2ºC

Formulation	Tested after time(days)	Physical Appearance	%of Drug Remaining ±SD
Batch F8	15	No change	100
	30	No change	98.99±0.03
	45	No change	98.03±0.01
	60	No change	96.16±0.01

Table No 6: Stability study of formulation batch F8 stored at 40 ºC ±2ºC (75%±5% RH)

CONCLUSION

The objective of the present work was to formulate SLN of antituberculer drug Isoniazid.SLN was prepared by solvent evaporation method by using 3^2 full factorial design. Results show that on

PHARMATUTOR
PHARMACY INFOPEDIA
ISSN: 2347-7881

increasing the ratio of drug: Stearic acid from 1:1 to 1:15 and Tween 80 conc. 0.5 -1.5 ml increase in entrapment efficiency was observed. The may be due to decrease of surface tension between organic phase and aqueous phase that possibly allows the formation of initially smaller solvent droplets at the site of solvent injection and causes decrease particle size as well as increase entrapment efficiency.

Two independent variable conc. of Stearic acid and Tween 80 were found to have significant effect on dependent variable entrapment efficiency but not on drug release profile. The major outcome of this work was the successfully entrapment of a hydrophilic drug within lipid core.

It can be concluded that using drug: Stearic acid ratio 1:15 concentrations in optimum concentration and Tween 80 1.0 ml during the process of formulation better entrapment and drug release profile achieved and by this SLN approach and preparation by solvent evaporation method the drug release sustained and may lead to avoidance of frequent drug administration.

↓ REFERENCES

1. Sarathchandiran I, "A Review on nanotechnology in solid lipid nanoparticles", IJPDT,2012,2(1):45-61
2. Ekambaram P, "Solid lipid nanoparticles: A Review", Sci.Revs.Chem.Commun.2010, 2(1):80-102
3. Nair R, "Formulation and Evaluation of Solid Lipid Nanoparticles of Water Soluble Drug: Isoniazid", J. Pharm. Sci. & Res,2011,3(5):1256-1264
4. Gulbake A, "Solid lipid nanoparticles bearing oxybenzone:In-vitro and In-vivo Evaluation" Journal of microencapsulation,2010, 2(3): 223-226
5. Sanjay S, "Formulation and Evaluation of Solid Lipid Nanoparticles of Water Soluble Drug: Zidovudine". Chem. Pharm. Bull 2010; 58(5):650-655
6. Kausik M, "Formulation development and evaluation of solid lipid nanoparticles of aceclofenac using solvent injection method, Journal of Drug Delivery & Therapeutics 2012,2(4):97-100
7. Kheradmandnia S, "Preparation and characterization of ketoprofen-loaded solid lipid nanoparticles made from beeswax and carnauba wax. ", NBM, 2010,6:753-759
8. Zhiwen Z, "Solid lipid nanoparticles loading candesartan cilexetil enhance oral bioavailability: in vitro characteristics and absorption mechanism in rats" Nanomedicine,2012,8:740-747
9. Vishvajit A. "Solid lipid nanoparticles as drug delivery system", Int. J. Pharm,2010;1(3):1-9
10. Umaretiya G, "Formulation and characterization of solid lipid nanoparticles dry powder inhaler containing Triamcinolone acetonide", IJRPC ,2011;1(3):662-673
11. Schwarz C, "Freeze-drying of drug-free and drug-loaded solid lipid nanoparticles (SLN)", Int.J. Pharma,1997,157: 171–179
12. Chakrapani M, Rahul N, "Formulation and Evaluation of Solid Lipid Nanoparticles using Lipids of Natural Origin", JITPS 2012,.3(3):80-87
13. Soo-Jeong L, "Formulation parameters determining the physicochemical characteristics of solid lipid nanoparticles loaded with all-trans retinoic acid", Int.J.Pharm2002,243(67): 135–146
14. Jameel AM, "Formulation, Characterization, and in vitro Evaluation of Methotrexate Solid Lipid Nanoparticles", Research J. Pharm. and Tech, 2009, 2(4): 611-16.