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Formulation and Evaluation of Solid Lipid Nanoparticles of Water Soluble Drug: Isoniazid

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Abstract:
The Solid lipid nanoparticles (SLN) of hydrophilic dug isoniazid (INH), a first line antubericular drug are developed and the entrapment efficiency of drug in the SLN has been improved. The poor incorporation of water-soluble drugs is the problem associated with the incorporation of hydrophilic drugs in to SLN. The SLNs were prepared by ethanol injection method using tristearin and phospholipon 80 H using 3^2 factorial design. Different combinations of tween 80 concentrations and varied sonication time were used to prepare SLN. The two operating variables sonication time and tween 80 concentrations were found to have significant effect on particle size, entrapment efficiency of SLN but not on the drug release. The prepared SLNs were characterized for surface morphology by SEM analysis, entrapment efficiency, zeta potential, FTIR, DSC, invitro diffusion studies. The prepared SLN were spherical in shape and possess mean average size of 164.9nm.As the preparation of SLN suffer from the drawback of poor incorporation of water soluble drugs the present work is focused to assess the various formulation and process parameters to enhance the incorporation of isoniazid in to SLN. The drug solubility in the dispersion medium plays an important role in improving entrapment efficiency. The SLN could be alternate method for delivery for INH with prolonged drug release profiles and better therapeutic effect can be achieved for the treatment of tuberculosis.

Keywords: *Solid lipid Nanoparticles, hydrophilic drugs, isoniazid, entrapment, invitro diffusion.*

INTRODUCTION:

Polymeric and lipid based sub micron sized carrier systems such as SLNs have attained a great deal of interest during the past decades (1, 2) The 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 SLN as successful drug delivery systems. (3)

SLNs combine the advantages of both polymeric nanoparticles and liposome's such as possibility of controlled drug release and drug targeting, increased drug stability, incorporation of lipophilic and hydrophilic drugs etc. SLN production techniques include high shear homogenization and ultrasound, high pressure homogenization, hot homogenization, cold homogenization, solvent emulsification and evaporation, etc. Lipidic carriers used to prepare SLNs can be highly purified lipids such as tristearin or tripalmitin, hard fats such as stearic acid or behenic acid, waxes such as cetyl palmitate and acylglycerol mixtures such as compritol or glyceryl monostearate (4). For the lipophilic drugs SLNs serves as potential drug delivery but aqueous solubility of the drug serves as a limiting factor for its absorption. Although 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. The mechanism of action of INH is not clearly known but there is evidence that it inhibits the synthesis of mycolic acid, an essential component of the bacterial cell wall, and also combines with an enzyme that is uniquely found in INH-sensitive strains of mycobacterium. Resistance to INH can occur due to reduced intracellular penetration of the drug. Isoniazid is a biopharmaceutical classification system class III drug (high solubility and low permeability) having an aqueous solubility of approximately 125 mg ml^{-1}. The drug is characterized by a short half-life ranging from 1 h to 4 h, 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 because it

occurs in the protonated form at acidic pH (pKa = 2) (5). Therefore, it can be considered as a good candidate for the development of a site-specific release formulation. Mainly by acetylating and dehydrazination it is inactivated; the rate of acetylation depends on the individual natures and subject to individual variation. Hepatotoxicity and peripheral neuritis are the conditions associated with Long-term continuous therapy with INH (6). It is thus, important to have a drug formulation with controlled release of INH, which can be achieved by formulating the INH loaded SLNs.

The objective of the present study was to adopt a simple approach for the INH SLN, using 3^2 Factorial design. Two formulation variables Tween 80 concentration and Sonication time were studied to optimize the formulation for maximum entrapment efficiency (EE). In addition to EE, the particle size and Drug release were also considered as response using DESIGN EXPERT 8.0.5 software. .The other characterizations such as zeta potential, SEM, FTIR, DSC were carried out to determine the lipid nature size of prepared formulation etc.

MATERIALS AND METHODS:

MATERIALS:

INH was purchased from Yarrow chemicals Ltd. (Mumbai, India). Phospholipon R 80 H was a gift sample from Lipoid (Ludwigshafen, Germany). Tristearin was procured from TCI Chemicals (India) Private Ltd. All other reagents used in this study were of analytical grade.

METHOD OF PREPARATION OF SLN DISPERSION:

Preparation of INH loaded SLNs and the process of optimization: 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 Tween 80 concentration (as stabilizer) and sonication time were studied to optimize the formulation for maximum entrapment efficiency (EE). In addition to EE, the particle size and % drug release were also considered as response using Design expert 8.0.5 trial software. Further, the optimized operating parameters were employed for the fabrication of INH SLN.

Nine different formulations were prepared by using different concentrations of tween 80 and sonication time to determine the effect of surfactant and sonication time on the potency of the SLNs. The variable parameters used during formulation development are given in Table No.1.

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Table.No.1 Variables used in the formulation development

S.No	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9
Tween80 Concentration	0.5ml	0.5ml	0.5ml	1ml	1ml	1ml	1.5ml	1.5ml	1.5ml
Sonication time	6min	12min	15min	6min	12min	15min	6min	12min	15min

Table.No.2 Selection of tween 80 concentrations and sonication time by using 3^2 factorial designs from this below table:

Ingredients	*%*	*Factor for CCD*
Model Drug	0.05	Constant
Tristearin	0.05	Constant
Tween 80 concentration	0.5-1.5	Vary
Sonication time	6min to 15min	Vary
Phospholipon	0.05	Constant

Table.No.3 Factor and their levels applied in the Design Experiment

Factor	*Name*	*Units*	*Type*	*Low Actual*	*High Actual*	*Low Coded*	*High Coded*
A	Tween 80 Concentration	ml	Numeric	0.5	1.5	-1.00	1.00
B	Sonication time	min	Numeric	6	15	-1.00	1.00

Generation of design matrix using design expert software 8.0.5 based on pre-selected formulation factors and chosen response.

Experimental Design

A 3^2 factorial design with 13 runs used to study the influence of chosen independent variables on the response selected and to see whether this design is capable of attainment of the true optimal tween composition and sonication with a better control release formulation having predetermined responses. Following Table shows the independent variables and their levels applied in the optimization

Characterization of prepared Slns:

Fourier Transform infrared (FTIR) spectroscopic analysis: The FTIR spectra of INH, tristearin, phospholipon 80 H, INH loaded SLNs and physical mixture of lipids and drug in 1:1 ratio were recorded using FTIR spectrophotometer in the range of 4000-650cm^{-1} (9).

Measurement of particle size, polydispersity index and zeta potential: Particle size distribution of INH loaded SLNs was determined by laser scanning technique using Malvern instrument after appropriate dilution with distilled water. The mean particle size, polydispersity index and zeta potential were calculated for each formulation maintained at 25^0 C and polydispersity index will measure the size distribution of nanoparticles population (10-12).

Scanning Electron Microscopy (SEM): The SEM analysis of prepared SLN was performed for morphological studies. The formulations are poured in to circular aluminum stubs using double adhesive tape, and coated with gold in HUS -5GB vaccum evaporator , and observed in Hitachi S-3000N SEM at an acceleration voltage of 10 Kv and a magnification of 5000X.(13)

Differential Scanning Calorimetry (DSC): DSC analysis was performed in order to investigate the melting and recrystallization behavior of crystalline materials like SLNs. The samples were sealed in aluminium pans and measurements were recorded using DSC instrument. The samples were heated from 25 to 200^0 C at a heating rate of 10^0 C /min under nitrogen atmosphere. (14-16)

Total drug content: From the prepared SLN formulation 1ml of suspension is dissolved in the 10 ml of 7.4 PBS buffer and ethanol mixture. The amount of isoniazid was determined using UV spectrophotometer at 266nm.The placebo formulation prepared similarly to drug loaded SLN is used as blank. The total drug content was calculated. (17)

Entrapment efficiency (EE): The prepared SLN dispersion was centrifuged at 15000 rpm for 30min at 0^0 C using REMI cooling centrifuge. Then the supernatant is analysed for the free drug content.

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

In vitro diffusion studies: This is performed by using a modified franz diffusion cell at 37^0 C which is fitted with a dialysis membrane having a molecular weight cut off 3500 Da.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 analysed using UV-Visible spectrophotometer at 266 nm (18).

RESULTS AND DISCUSSION:

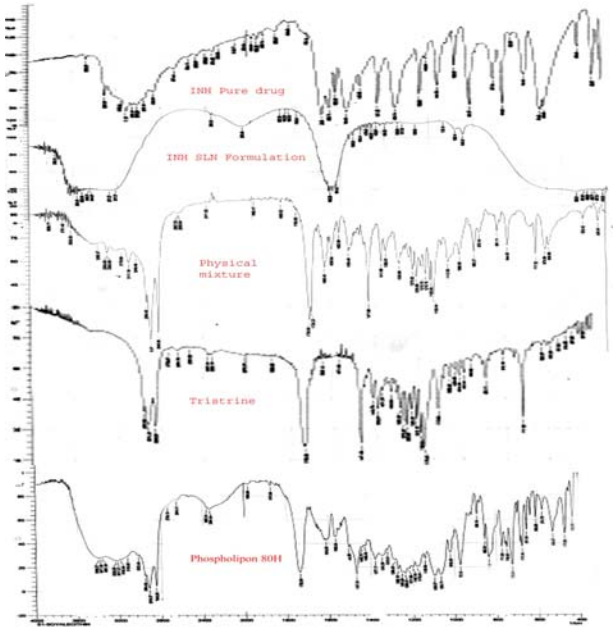
SLNs were prepared by solvent injection technique which relies on the rapid diffusion of solvent across the solvent –lipid interface with aqueous phase; hence the rate of diffusion of organic solvent through the interface seems to be critical parameter for particle size determination[19]. In the present work ethanol was selected as miscible solvent due to its solubilising potential for tristearin, and phospholipon .The smaller particle size is achieved due to addition of tween 80 and HSPC in the organic phase.

FTIR spectroscopy: FTIR spectroscopy was used to investigate the interactions between

lipid, drug and other excipients. From the FTIR graphs of pure drug, optimised formulation and physical mixture it is confirmed that there are no particular interactions between the lipids and drug .FTIR Spectra of pure drug, lipidic excipient, physical mixture of lipid and drug and drug loaded SLN are shown in the fig 1.The spectrum of physical mixture was equivalent to the spectrum of crystalline drug with sharp vibrational bands indicating crystallinity. This showed that there are no interactions with simple physical mixing of drug, lipid carrier and other excipents. The FTIR spectrum of INH showed a strong C=O stretch band (Amide I) around 1650 cm^{-1} and an Amide II due to N-H bend at 1620 cm^{-1}. These peaks were, however, completely masked in the FTIR spectrum of SLN.

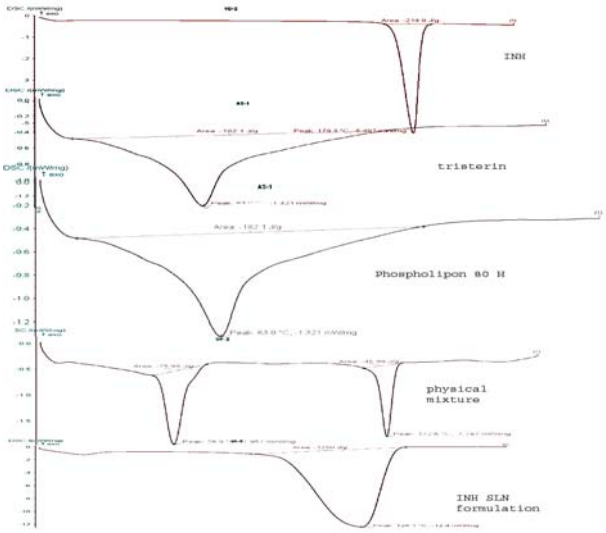
Table.No.4 Ftir interpretation of INH SLN comparative to pure drug and excipients:

Functional group	Pure drug	formulation	Physical mixture
C=o stretch vibrations	1668cm	1645	1668.43
C-N sym stretch	1556	1539	1558.48
C-c ring stretch	1412	1394, 1435	1411.89
N-H stretch	3303	3271.27	3304.06
C-C stretch	1060	1045	1060.85
N-X stretch	1141.08	1134.12	1112.93



DSC:

In the development of SLNs the confirmation of desired physical state of matrix lipid is of crucial importance which can be determined by the DSC. When the DSC thermograms of the bulk lipids and corresponding SLNs are compared the difference in the position and shape of the signals are usually observed. The DSC curve of the pure drug INH shows that it is in crystalline anhydrous state, exhibiting a sharp exothermic peak at 179.3 °C (ΔH-214.9J/ g), corresponding to its melting point 175 ◦C [20], and for the formulation peak is at 128.1°C (ΔH-1750j/g), for physical mixture two peaks are observed at 76.9 C (ΔH-75.98j/g) and 172.6°C (ΔH-45.99j/g).



SEM: The SEM photograph of optimised formulation reveals that particles are roughly spherical and somewhat uniformity is observed. Fig 3 shows the SEM photographs of INH loaded SLN.

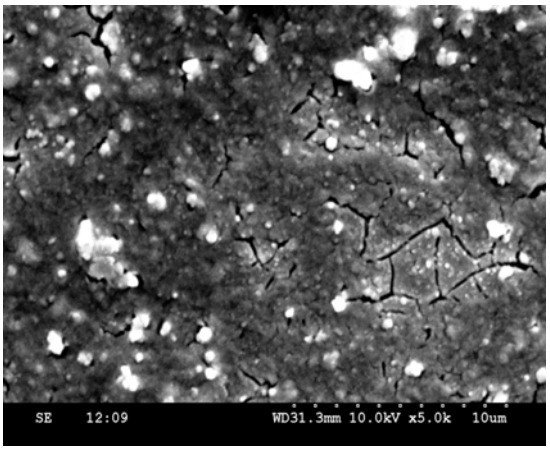


Fig.No.3 SEM Photographs of INH SLN

Table.No.5 INH formultion Zeta potential, particle size measurements

Formulation	*Variable parameter*		*size Average (d.nm):*	*Polydispersity index*	*Zeta potential*
	Tween 80	*Sonication time*			
F1	0.5ml	6min	194.2	0.212	-7.94
F2	0.5ml	12min	187.5	0.219	-7.60
F3	0.5ml	15min	185.7	0.228	-7.40
F4	1ml	6min	180.1	0.230	-7.23
F5	1ml	12min	179.4	0.236	-6.83
F6	1ml	15min	178.4	0.246	-6.12
F7	1.5ml	6min	169.4	0.264	-5.24
F8	1.5ml	12min	165.7	0.278	-4.98
F9	1.5ml	15min	164.8	0.293	-4.13

Particle size determination: The particle size of the formulations was determined by laser scattering technique using Malvern instruments. The mean particle size and width of distribution (polydispersity index) for INH loaded SLNs were measure by LD. Thus the SLNs showed narrow distribution width and considerable narrow particle size. By using solvent injection method SLNs are having good dispersion quality. The measurement of zeta potential allows for prediction about the storage stability of colloidal particles (21), as the particle aggregation will be less to the charged particles. For the prepared SLNs the Zeta Potential (mV): particle size and polydispersity index are tabulated below and size distribution charts of two formulations shown in fig 4and 5.

Fig no: 4 INH F3 Size distribution chart

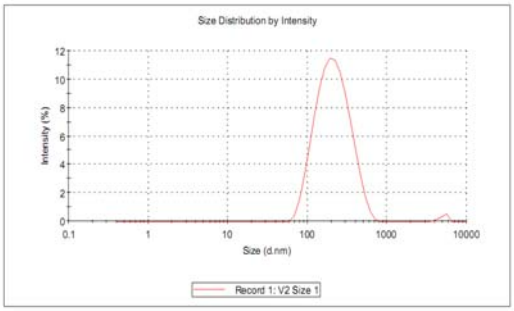
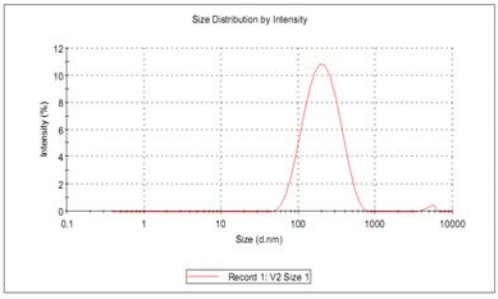


Fig no: 5 INH F9 Size distribution chart



n-vitro diffusion studies: Modified Franz diffusion cells with dialysis membrane were used in our study. This dialysis membrane allowed the transfer of drug immediately in to receiver compartment .The % drug release of isoniazid from 9 different formulation of SLNs is shown in the figure (6, 7). The % of isoniazid released from SLNs up to 24 hours is in the table no 6.

Fig no : 6 Comparative release profile of INH SLN in 7.4 PBS for F1-F6

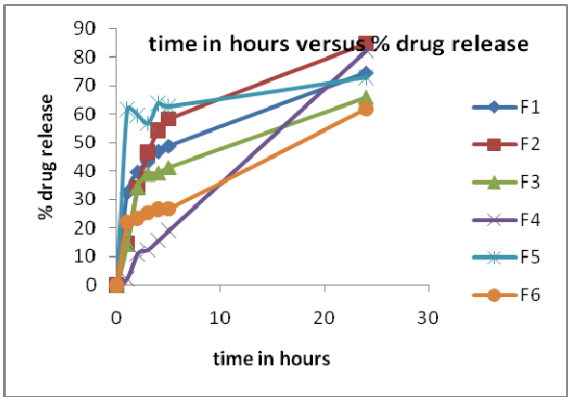


Fig No: 7 Comparative release profile of INH SLN in 7.4 PBS for F7-F9

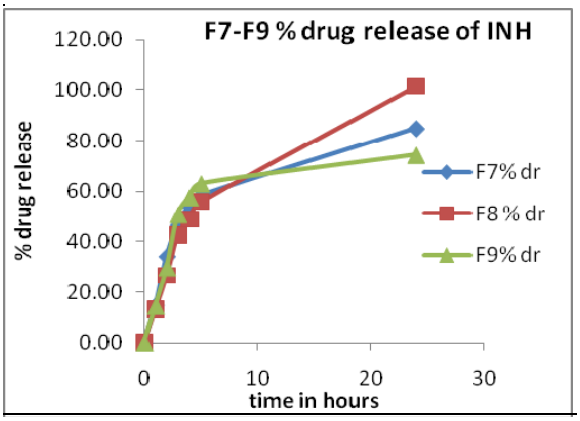


Table.No.6 Percentage drug Release of INH SLN

S.No	Formulation	% Drug release
1	F1	74.45
2	F2	84.85
3	F3	65.96
4	F4	82.32
5	F5	72.76
6	F6	61.83
7	F7	72.56
8	F8	76.3
9	F9	65.4

Table.No.7 Table showing total, free drug content and EE of INH

Formulation	Free drug Content (mg)	Total drug content (mg)	%EE
F1	37	42.8	13.5
F2	40	41.6	3.846
F3	36.25	42	13.69
F4	24.5	34.5	28.9
F5	32.4	41.33	21.57
F6	31.16	39.5	21.1
F7	30.2	43.4	32.1%
F8	28.5	42.6	35.2%
F9	31.3	43.8	30.1%

EE: The EE of the fabricated batches was in the range of 3.99 to 35.2%.The total drug content and free dug content of the 9 batches are shown in the table No.7

Generation of design matrix using design expert software 8.0.5 based on preselected formulation factors and chosen response.

The process of optimization by 3^2 full factorial design needs that experimentation should be completed so that mathematical model can be generated. The number of experiment required for the studies depend upon number of independent variables selected by formulator. The responses were measured for each trial and then linear, interactive or quadratic model if fitted by carrying out multiple linear regression analysis and F-statistics to identify significant terms. A statistical model incorporating interactive terms and polynomials is eq (1)

$$Y=b_0+b_1 X_1 + b_2 X_2 +b_{12} X_1 X_2 +b_{11} X_1^2 +b_{22} X_2^2 (1)$$

Where Y is dependent variable , b_0 is arithmetic mean response of nine runs and b_i (b_1, b_2, b_{12} . b_{11} b_{22}),is estimated for corresponding factor X_i (X_1, X_2 , X_{12}, X_{11}) which represents the average results of changing one factor at a time from its low to high value. The interaction term(X_1 X_2) depicts the changes in response when two factors are simultaneously changed. To determine the nonlinearity polynomial terms (X_1^2 , X_2^2) are included. The effect of tween 80 concentration and sonication time on particle size , entrapment efficiency and drug release can be understood by using response surface plots and cotour plots (fig no 8-13) generated using design expert software..

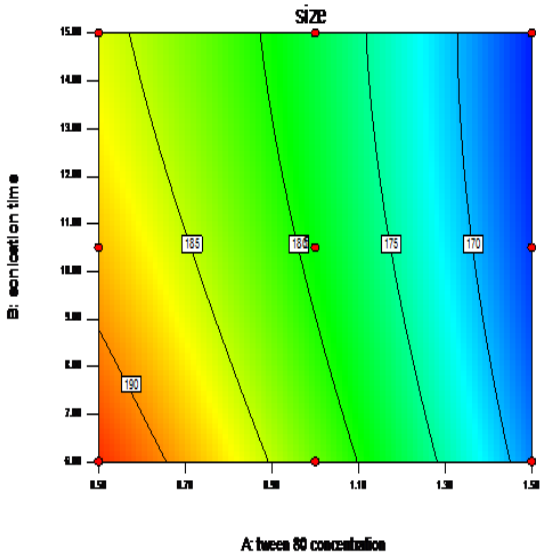


Fig no :8 Countour plot showing the effect of tween 80 concentration (X1) and sonication (X2) on the response particle size (Y1).

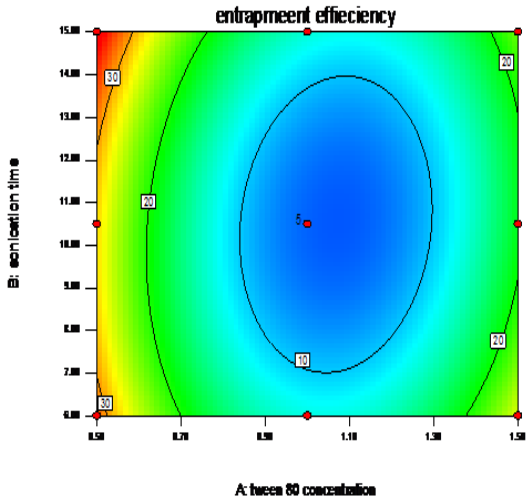


Fig.No: 9 Contour plot showing the effect of tween 80 Concentration (X1) and sonication (X2) on the response entrapment efficiency (Y2).

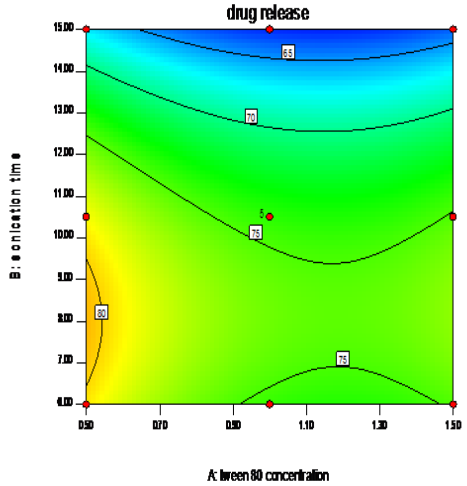


Fig .No.10 Contour plot showing the effect of tween 80 concentration (X1) and sonication (X2) on the % drug release at the end of 24th hour (Y3).

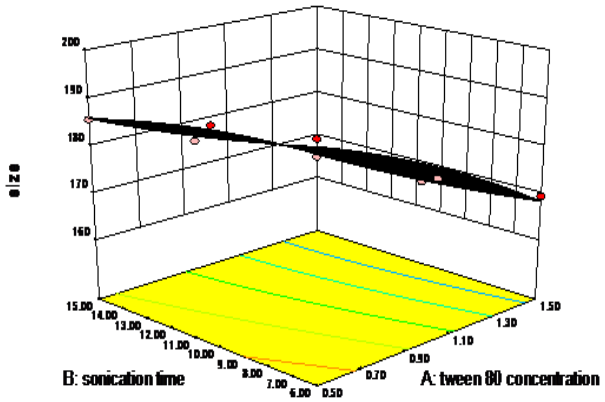


Fig.No.12 Response surface plots showing the effect of tween80 concentration (X1) and sonication time (X2) on the response particle size (Y1).

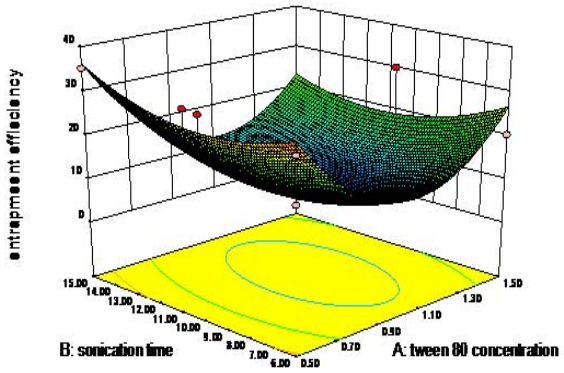


Fig.No.11 Response surface plots showing the effect of tween80 concentration (X1) and sonication time (X2) on the response EE (Y2).

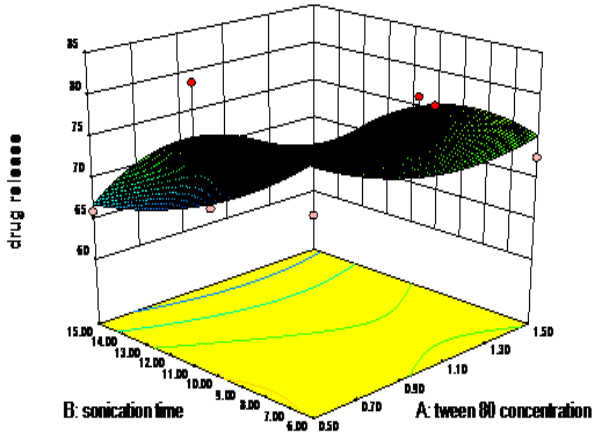


Fig.No.13 Response surface plots showing the effect of tween80 concentration (X1) and sonication time (X2) on the response % drug release (Y3).

Table. No. 8 ANOVA for Response Surface Quadratic Model of EE:

Source	*Sum of squares*	*df*	*Mean square*	*F values*	*P values*	*Significant/ Not significant*
Model	1403.90	5	280.78	5.31	0.0247	Significant
A-tween 80 concentration	123.31	1	123.31	2.33	0.1707	Not significant
B-sonication time	0.51	1	0.51	9.64E-0.03	0.9245	Not significant
AB	16.32	1	16.32	0.31	0.5959	Not significant
A^2	777.98	1	777.98	14.71	0.0064	Significant
B^2	95.12	1	95.12	1.80	0.2218	Not significant

ANOVA for Response Surface Quadratic Model of particle size:

Source	*Sum of squares*	*df*	*Mean square*	*F values*	*P values*	*Significant/ Not significant*
Model	808.71	5	161.74	101.29	□0.0001	Significant
A-tween 80 concentration	759.38	1	759.38	475.33	□0.0001	Significant
B-sonication time	36.51	1	36.51	22.86	0.0020	Significant
AB	3.80	1	3.80	2.38	0.1667	Not significant
A^2	8.93	1	8.93	5.59	0.05	Significant
B^2	2.00	1	2.00	1.25	0.29996	Not significant

ANOVA for Response Surface Quadratic Model of drug release

Source	*Sum of squares*	*df*	*Mean square*	*F values*	*P values*	*Significant/ Not significant*
Model	328.19	5	65.64	3.18	0.0817	Not significant
A-tween 80 concentration	20.17	1	20.17	0.98	0.3559	Not significant
B-sonication time	217.68	1	217.68	10.55	0.0141	Significant
AB	0.44	1	0.44	0.021	0.8878	Not significant
A^2	22.34	1	22.34	1.08	0.3328	Not significant
B^2	88.36	1	88.36	4.28	0.0773	Not significant

From the ANOVA table of Particle size Model F-value of 101.29 implies the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. From the ANOVA table of EE Model F-value of 5.31 implies the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

The fitted equations' relating the responses is clearly shown in the Following equation

Final equations in terms of coded factor

$EE=6.84 -4.53\ X_1 + 0.29X_2 + 2.02X_1\ X_2 + 16.78\ X_1^2 + 5.87\ X_2^2$(3)

Particle Size$=179.11 -11.25\ X_1 -2.47\ X_2 + 0.98X_1\ X_2 - 1.80\ X_1^2 + 0.85\ X_2^2$(4)

%drug release$=74.18 -1.83\ X_1 -6.02X_2 + 0.33X_1\ X_2 + 2.81\ X_1^2 -5.66\ X_2^2$(5)

Final equations in terms of Actual factors

EE=101.88 -133.9Tween80 concentration – 5.123Sonication time – 0.8977 Tween80 concentration*Sonication time+67.13Tween80 $conc^2$+0.28Sonication $time^2$ (6)

Size=209.633-12.663Tween80 concentration– 1.864Sonication time+0.433 Tween80 concentration* Sonication time–7.193Tween80 $concentration^2$+0.04Sonication $time^2$(7)

Percent drug release=209.633-12.663Tween80 concentration 1.864Sonication time+0.433Tween80 concentration* Sonication time-7.193Tween80 $concentration^2$+0.04Sonication $time^2$(8)

Summary of ANOVA results for response surface quadratic models of % drug release, %EE, and particle size.

Response	*Model*	*Sum of squares*	*F value*	*Prob>F*
EE	Quadratic	1403.90	5.31	0.024<0.05
Drug release	Quadratic	328.19	3.18	0.081>0.05
Particle size	Quadratic	808.71	101.29	>0.0001

CONCLUSION:

The present work objectives was to assess the various formulation and process parameters to enhance the incorporation of water soluble drug (INH) in to SLN prepared by solvent injection method by using central composite design and to study the influence of choosen independent variables on the responses selected. The hydrophilic drug INH had been successfully incorporated in to SLNs and the purpose of controlled release has been achieved. Results show that on increasing the concentration of tween 80 from 0.5-1.5% w/v a decrease in particle size was observed. This may be due to the decrease of surface tension between organic and aqueous phase that possibly allows the formation of initially smaller solvent droplets at the site of solvent injection and causes decreased particle size. Two operating variables, concentration of Tween 80, and sonication time were found to have a significant effect on particle size and entrapment efficiency (EE) ($p>0.005$). However effect of these variables on drug release was found to be not significant but the release profiles of INH loaded SLNs are amenable to slow delivery of the drug to afford at least once in two days for administration. The major outcome of this work was the successful entrapment of a hydrophilic drug with in a liquid core. Despite of the low zeta potential the prepared SLN were stable. It can be concluded that using tween 80 concentrations in optimum concentration i.e. 1ml and sonication for more time during the process of formulation better narrow size is achieved and by this SLN approach and preparation by solvent injection method the drug release can be sustained and may lead to the avoidance of frequent drug administration.

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