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# DEVELOPMENT, OPTIMIZATION AND CHARACTERIZATION OF FEBUXOSTAT LOADED SOLID LIPID NANO PARTICLES

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## ABSTRACT

Febuxostat (FEB) belongs to BCS class II, having low solubility and high permeability. Due to low solubility it is having low oral absorption that ultimately results in low oral bioavailability. Poor oral bioavailability leads to reduction in plasma drug concentration and overall reduction in therapeutic effects. The method use for the development of solid lipid nanoparticles (SLN) is high speed homogenization (HSH) with ultra-sonication; the advantage of the method is the avoidance of usage of organic solvents, as organic solvent is not most preferred because it generally leads toxicity and other problem. Optimized sonication times, rotation of HSH, speed of HSH were 2 minutes, 10,000 RPM and 10 minutes respectively. Using $3^2$ full factorial design, drug: lipid ratio (1:18) and surfactant concentration (0.7%) were optimized. Optimized batch of FEB SLN had mean particle size 193.4±4.23 nm, Polydispersibility index 82.93 ±1.6 % and surface charge -21.5 mv. From FTIR, and DSC studies of FEB, Lipids and optimized formulation it can be conclude that FEB was properly loaded in SLN. From TEM results, we can conclude that prepared SLN had Spherical shape & smooth surface. From in- vitro and Ex -vivo drug release profile, it can be concluded that FEB SLN gave sustained release as 100% CDR obtained after 24 hours.

**Key words: High speed homogenization with ultra-sonication, SLN, *Ex vivo*, TEM, Full factorial design**

## 1. INTRODUCTION

Designing and development of newer drug delivery system which gives maximum therapeutic effect and controlled release is a benchmark. Drug should be delivered safely within precise time at target site **[1]**. Recently, significant effort has been taken to develop nanotechnology for drug delivery, as it offers a suitable means of delivering low molecular weight drugs, as well as macromolecules such as peptides, proteins or genes to cells and tissue.

The reason behind formulating Solid Lipid Nano carriers is to improve solubility of drug and overall it may improve oral bioavailability. SLN will give initial burst release after that sustain release up to long time, which will maintain the plasma drug concentration throughout the period of treatment. SLN will also avoid the hepatic first pass metabolism of drug by direct lymphatic absorption of drug and it will directly go into systemic circulation **[2-4]**. Due to this dose reduction and dosing frequency may be reduce and ultimately toxicity is minimized. For the development of used lipids are physiologically acceptable, biodegradable and possess GRAS (generally recognized as safe) status approved by regulatory authorities for human use, which have generally low or no toxicity **[5]**. The method use for the development of SLN is high speed homogenization with ultra-sonication; the advantage of the method is the avoidance of usage of organic solvents, as organic solvent is not most preferred because it generally leads toxicity and other problem **[6-8]**.

Solid lipid nanoparticles (SLNs) were introduced in 1991; they represent an alternative carrier system in contrast to traditional colloidal carriers such as emulsions, liposomes and polymeric micro particles and nanoparticles **[9-11]**. Nanoparticles made from solid lipids are attracting major attention as novel drug carriers for intravenous applications as they have been proposed as an alternative particulate carrier system. The system comprises of spherical solid lipid particles in the nanometer ranges, which are dispersed in water or in aqueous surfactant solution. Generally, they are made of solid hydrophobic core having a monolayer of phospholipid coatings. The solid core contains the drug dissolved or dispersed in the hot melt fat matrix. The hydrophobic chains of phospholipids are embedded in the fat matrix. They have potential to carry hydrophilic or lipophilic drugs as well as diagnostics **[12-14]**.

## 2. MATERIALS AND METHODS

Febuxostat was gifted from Zydus Cadila Health care, Ahmedabad, Gujarat. Dynasan 114, 116, 118were gifted from Sasol, Germany. Precirol ATO 5, Compritol 888

Pellets were gifted from Gattefosse Pvt. Ltd., France. Capmul GDB, Capmul GMS 50K, were gifted from Abitec Corporation Ltd., India. All other reagents and chemical used in research work were of pharmaceutical grade.

**2.1 METHODS**

**2.1.1 Preformulation study**

**2.1.1.1 Solubility**

Solubility study was performed to check the tendency of different solvents to dissolve Febuxostat. Solubility of drug in different solvents was done by quantitative method. The solubility determination of febuxostat in various solvents was performed by adding febuxostat in increments of 1 mg until it failed to dissolve further in the fixed 1ml of solvent. Amount of drug dissolved in solvents was determined. The experiment was conducted in triplicate

**2.1.1.2 Compatibility study (FEB and excipients)**

Compatibility study of FEB and excipients were employed to determine compatibility between of FEB and excipients. It was carried out by DSC.

**2.1.1.2.1 DSC Study**

The differential scanning Calorimetry (DSC, Metller Germany) was commonly used to investigate compatibility of drug and excipients used. In the formulation of SLN, heat was utilized so thermal behavior was necessary to examine. As a function of heat there may be chemical or physical changes occurs within the product during which heat may be gain or loss, this behavior can be explained by DSC. Thermogram of samples was recorded by Differential scanning calorimeter. Samples were weighed directly in aluminum pan and scanned at 50-300 ˚C temperature under dry nitrogen atmosphere at the heating rate of 10 ˚C / min. Differential Scanning Calorimetry (DSC) was employed to determine Interference Study. Standard aluminum DSC pans were used. Nitrogen gas was transported through the DSC at a rate of 50 ml/minute. The temperature was increased 10 °C/minute between 25 °C – 250°C. 10 mg physical mixture (30 mg FEB and 30 mg lipid) were added to the aluminum pans for the analysis.

**2.1.1.2 Selection of lipid**

**2.1.1.2.1 Solubility study**

Solubility study was employed to determine solubility of FEB in various lipids (Dynasan 114, Dynasan 118, Compritol 888 ATO, and Precirol 5 ATO, Capmul GMS 50K). The solubility determination of febuxostat in various solid lipids was performed by melting 1 gm of solid lipid (which was heated at 5˚C above their melting point) and solubility was checked by adding febuxostat in increments of 1mg until it failed to dissolve. The experiment was conducted in triplicate.

**2.1.1.2.2 Partition coefficient study**

1000 mg lipids were melted in beaker. Lipids were melted above 10°C of above its Melting Points. Dissolved 20 mg of FEB into melted lipids.10 ml distilled water, heated at the same temperature of melted lipids. Mixtures were stirred for 45 minutes and cooled at room temperature. Aqueous phase was separated and centrifuged. 1ml supernatant was taken and diluted with methanol up to 10ml and analyzed at 315 nm using UV visible spectrophotometer. The partition coefficient (PC) was calculated by equation given below **[15]**.

$$PC = (A_i - A_w)/A_w$$

**Where,**

**$A_i$ is initial amount of Febuxostat taken (10 mg)**

**$A_w$ is the amount of Febuxostat in the aqueous phase.**

**2.1.1.3 Selection of surfactant**

FEB loaded solid lipid nanoparticles were prepared using two different types of surfactants, Poloxamer 188 and Poloxamer 407 using 1% concentration of each. Surfactant was selected on the basis of mean particle size (MPS) and % drug entrapment (PDE).

***2*.1.2 Optimization of process parameter**

Process parameters were optimized by trial error batch. Process parameter includes sonication time, Speed of rotation of HPH, Time of rotation of HPH. Drug: lipid ratio (1:15) and surfactant concentration (1%) were kept constant in each batch. sonication time (2min,4min,6min), Speed of rotation of HPH (10,000 RPM,15000 RPM, 17,000 RPM) and Time of rotation of HPH (7 min,10 min,15 min) were changed respectively.

**2.1.3 Preparation method for SLN**

High speed homogenizer and ultra-sonication method was selected for preparation of SLN because compare to other methods they are easy, less time consuming and effective. The SLN were prepared using High speed homogenization and ultrasoniaction method. Lipid was melted at $10^0$c above its melting point and FEB was dissolve in melted lipid. Surfactant was dissolved into double distilled water and heated to same temperature as of melted lipid. Transfer hot aqueous surfactant solution into FEB lipid mixture. Mixture was homogenized using high speed homogenizer at Different RPM, Minutes and Different sonication cycles were given. Dispersion was centrifuged at 8000 RPM for 10 min to remove lipid and unentrapped FEB as they settled down into centrifuge tube. Dispersion was filtered using Whatman filter (pore size 46*57). Supernatant was collected containing FEB-SLN.

**2.1.4 Optimization of Formulation parameters**

Optimization of formulation parameter like Drug: lipid ratio and surfactant concentration were optimizing by Design Expert 10.0 trial version. In order to

optimize the formulation, the drug: lipid ratio (X1) and surfactant concentration (X2) were chosen as independent variables shown in **Table 1**. These two factors that might affect the nanoparticle formulation and three levels of each factor were selected and arranged according to a $3^2$ full factorial experimental Design. Mean particle size ($Y_1$) and % FEB entrapment ($Y_2$) were selected as dependent factors.

**Table 1: Independent Parameter with Levels**

| Independent Parameter | -1 | 0 | +1 |
|---|---|---|---|
| Drug: Lipid Ratio (X1) | 1:10 | 1:15 | 1:20 |
| Surfactant Concentration (X2) | 0.5 % | 1% | 1.5 % |

**2.1.4.1 Check point analysis**

A check point analysis was performed to confirm the role of derived polynomial equation and contour plots in predicting the responses in the preparation of solid lipid nanoparticles. Three check point values of independent variables (X1 and X2) were taken at any one point from each contour plot and theoretical values of dependent variables were calculated by substituting the values to respective polynomial equation. Solid lipid nanoparticles were prepared experimentally at 2 points and evaluated for the responses. Each batch was prepared three times and mean values were determined. Differences of the theoretically computed values of dependent variables and the mean values of experimentally obtained value of dependent variables were checked.

**2.1.4.2 Optimization of formulation with desirability function**

Optimization was performed to find out the level of independent variables (X1 and X2) that would yield a maximum value of % EE and minimum value of particle size. The desirability function was used for optimization of the formulation. The application of the desirability function combines all the responses in one experiment and gives the possibility of predicting optimum levels for the independent variables. By considering these facts, the optimization was performed through the software by setting the desired constraints for various variables to obtain the optimized batch which has maximum desirability that gives maximum % EE and minimum particle size.

**2.1.5 Lyophilization**

Freeze drying technique was used to improve stability of SLN and to prevent the leakage of Entrapped FEB. Optimized Batch of FEB loaded SLNS was freeze dried using trehalose as cryoprotactant to preserve the size and shape. Further this SLNs dispersion was subjected to two stage of freeze drying.

1. The resultant dispersion was deep freezed at -70°C for 24 hr in Deep

Feezer, (Amancio Lab, Mumbai) form dry ice cake.

2. The formed dry ice cake containing vials transferred to the Heto Freeze-dry system (Heto dry, Denmark) and lyophilized at the -70°C and for 24 hours. After Lyophilization, effect of Lyophilization on size and % FEB content and drug release were studied.

**2.1.5.1 Optimization of cryoprotactant**

Cryoprotactant (trehalose): Total solid content of FEB SLN (1:3, 1:5) were taken optimized and selected on the basis of mean particle size (MPS) and % FEB content.

**2.1.6 Evaluation and Characterization of optimized FEB-SLN**

**2.1.6.1 Particle size analysis**

The particle size analysis of the formulations was performed using a Malvern zetasizer 2000MS device (Malvern Instruments, Worcestershire, UK) and laser diffraction with a beam length of 2.40mm.

**2.1.6.2 % Drug entrapment efficiency**

Entrapment efficiency of Febuxostat loaded SLN dispersion was carried out by considering free drug present in supernatant and sediment. After centrifuged of 10 ml of dispersion supernatant was collected and lipid was precipitated by adding Methanol, as drug is soluble in methanol but lipid was not. Precipitate was filtered and filtrate was collected as solution no 1. Sediment was further precipitated by adding methanol and it was also filtered and filtrate collected as solution no 2. Both the solution was mixed and analyze by UV Visible spectrophotometer at 315 nm to find out free drug concentration. % entrapment efficiency was calculated by using following equation:

$$(\%)\ EE = \frac{\text{Amount of Febuxostat added - Amount of free Febuxostat}}{\text{Amount of Febuxostat added}} \times 100$$

**2.1.6.3 Surface Charge**

Zeta potential is useful for assessment of the physical stability of colloidal dispersions. Zeta potential can be measured by determination of the movement velocity of the particles in an electric field (electrophoresis measurements). Practically all aqueous colloids are electronegative, with the general range of zeta potential being -14 to -30 millivolts.

**2.1.6.4 Transmission electron microscopy (TEM)**

Optimize formulation diluted with distilled water. Carbon coated copper grid used to hold that drop of dispersion followed by drying. To measure the Surface

morphology Transmission Electron Microscope used in which this grid mounted and pictures captured at a different resolution.

**2.1.6.5 Differential scanning calorimetry study (DSC)**

DSC study was performed to characterize physical state of FEB in SLN. DSC was performed by using differential scanning calorimeter (DSC-60, Shimadzu Corporation, Japan). Nitrogen gas was transported through the DSC chamber at a rate of 50 ml/minute.

**2.1.6.6 *In vitro* drug release:**

Dialysis bag method used to evaluate the *In vitro* release of FEB SLN. One end of the dialysis bag (Molecular weight cut off 10000-12000) was tied with the thread and examined for any leakage. It was filled with 2.0 ml of dispersion equivalent to 1 mg and remaining open end was tied up using thread. The packed sac acted as a donor compartment. The dialysis bag was then immersed in glass beaker containing 200 ml of phosphate buffer 7.4 as the receptor compartment at 37°C ± 2°C. The content of beaker was stirred using magnetic bead stirrer and the beaker was covered with an aluminum foil to prevent loss of solvent during the experiment. At selected time intervals, 5 ml samples were withdrawn from the dissolution medium and replaced with the same amount of freshly prepared dissolution medium maintained at the same condition. The sample was analyzed by UV spectrophotometer for FEB at 315 nm and the percent drug release was calculated. All of the formulations were studied for *In vitro* drug release in triplicate. Models, like zero order, first order, and Higuchi, Hixson Crowell and Peppas models were applied to drug release data to understand the mechanism of drug release from the SLN. Same procedure was followed for to obtain drug release data of freeze dried SLN.

**2.1.6.7 *Ex vivo* study**

Chicken duodenum was used for permeability study obtained from slaughter house. Small intestine was immediately excised and placed into ice-cold bubbled Ringer buffer. The tissue was rinsed with ice-cold standard Ringer buffer to remove luminal content and then cut into segments. The intestine was tied at one side and then dispersion was placed into duodenum. The other side of intestine was also tied. The tissue was placed into beaker with continue aeration and constant temperature 37 ± 0.5 $^{\circ}$C. The receiver compartment was filled with 100 mL phosphate buffer 7.4. Samples were taken from receptor compartment at predetermine time interval (30, 60, 120, 180, 240 min) and replaced with equal volume of buffer. Samples were analyzed for drug content using UV-Visible spectrophotometer at 315 nm.

**2.1.7 Stability studies for Freeze dried FEB SLN**

Stability study of freeze dried FEB SLN was performed over a period of 45 days accelerated condition ($40^0 \pm 2^0$C and 75% ± 5% RH) and stability study was performed by the parameters like Man particle size and % drug content. Test for all parameters were performed for the optimized batch at the initial time, after 15 days, 30 days and 45 days.

## 3. RESULT AND DISCUSSION

### 3.1 Preformulation study

#### 3.1.2 Solubility study

The amount of drug dissolved in particular solvent described in **Table 2**. Febuxostat having highest solubility in acetone as compared with methanol, although for the calibration curve of Febuxostat methanol used as a solvent. Because acetone having cut off wavelength at 330 nm. Cut off wavelength may be defined as a region where the solvent absorbs the UV or Visible light. At this wavelength measurement must be avoided because it is difficult to determine the absorbance is come from solvent or your analyte. The λ max of Febuxostat is 315 nm so it is better to use methanol instead of using acetone for a calibration curve.

#### 3.1.3 Compatibility study (FEB and excipients)

##### 3.1.3.1 DSC study

DSC study was carried out to check compatibility between FEB and excipients. Endothermic peak of FEB, and Capmul GMS 50 K were observed at $212.31^0$c, $64.54^0$c respectively. In Physical Mixture of drug and different lipids (1:1) endothermic peak of lipid observed, but no endothermic peak of FEB observed due to solubilisation of drug into lipids. DSC graph of FEB and physical mixture are shown in **Figure 1**.

#### 3.1.4 Selection of lipid

##### 3.1.4.1 Solubility study:

Solubility study was carried out for selection of lipid. Various lipids were melted and FEB was dissolved in melted lipids. Solubility of FEB in various lipids is shown in **Table 4**. From the data it can be concluded that solubility of Febuxostat is 78.33 ± 6.944 mg in 1 gm of Capmul GMS 50 K. This is highest among all the solid lipids, so in the formulation of SLN as a solid lipid Capmul GMS50 K was taken.

##### 3.1.4.2 Partition coefficient study

Partition coefficient study was carried out for selection of lipid. After Solubility study in lipids, partition study of drug between two phase like lipid and aqueous phase is also important since the drug although high solubility in lipid may precipitate out in aqueous media *In vivo* if its partition coefficient is low. Therefore, partition study was conduct in several lipids. Results are shown in **Table 3**. Capmul GMS 50 K having highest partition co efficient and majority of the drug goes into lipidic phase as compared with the aqueous phase. For

this reason, Capmul GMS 50 K is use as a solid lipid in the preparation of SLN.

**3.1.5 Surfactant selection**

Surfactant selection was carried out on bases of mean particle size (MPS) and% drug entrapment (PDE). With 1% Poloxamer 188 and 1% Poloxamer 407 obtained particle size were 229.45±0.25, 322.40±0.29 respectively and % drug entrapment 78.85±2.58, 57.52±0.22 respectively **(Table 4)**. For SLN preparation, Minimum Particle Size and High Drug Entrapment Require. Results showed that low particle size & high % drug entrapment observed in Poloxamer 188 compare to Poloxamer 407. Particle size require for lymphatic absorption is 100- 400nm that was obtained in Poloxamer 188. Results obtained here met both the desirable criteria (MPS and PDE). So, Poloxamer 188 is selected for formulation of solid lipid nanoparticles.

**3.2 Optimization of Process parameter**

Before optimization of formulation parameter, process parameters were optimized to check their effect on formulation. Results of optimization of process parameters are shown in **Table 5**.

**A) Effect of Sonication**

As the sonication time varies from 1-2 min, %EE increases and particle size decreases which are desired. At 3 min of sonication time %EE decreases and Particle size increases as compared to **2 min of sonication time**. Result shows that 2 min. sonication time is optimum and therefore it was selected.

**B) Effect of rotation of HSH**

At the 10,000 RPM maximum %EE and minimum particle size was obtained as compared with 15,000 and 20,000 RPM. So **10,000 RPM** was selected as an optimum HSH RPM.

**C) Effect of time of HSH**

As the sonication time varies from 5-10 min. increase in %EE and reduction in particle size was observed. But by further increase in the sonication time i.e. 15 min it shows that further reduction in %EE and particle size was increases. From results it can be concluded that **10 min HSH time** is optimum for the effective formulation of Nanoparticle

**3.3 Optimization of Formulation parameter**

For particle size response Y1, The Model F-value of 48.19 implies the model is significant. There is only a 0.46% chance that a "Model F-Value" this large could occur due to noise. In this case A, B, AB, B2 are significant model terms. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Pred R-Squared" of 0.8562 is in reasonable agreement with the "Adj R-Squared" of 0.9672."Adeq

Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 19.083 indicates an adequate signal. This model can be used to navigate the design space. Drug: lipid ratio and surfactant concentration had shown positive effect on particle size means high concentration of Lipid and surfactant will cause rise in particle size. Combined effect of drug: lipid ratio and surfactant concentration had shown positive effect on MPS.

For % drug entrapment response Y2, The Model F-value of 17.42 implies the model is significant. There is only a 2.00% chance that a "Model F-Value" this large could occur due to noise. In this case A, B, $B^2$ are significant model terms. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.The "Pred R-Squared" of 0.6084 is not as close to the "Adj R-Squared" of 0.9112 as one might normally expect. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response tranformation, outliers, etc."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 12.255 indicates an adequate signal. This model can be used to navigate the design space. Drug: lipid ratio and surfactant concentration had shown positive & negative effect on Drug Entrapment respectively means high concentration of Lipid will increase drug entrapment due to availability of lipid content for drug solubility. High concentration of surfactant will affect size of particle which will cause reduction in drug entrapment. Combined effect of drug: lipid ratio and surfactant concentration had shown negative effect on MPS

***Effect of Drug: lipid ratio and Surfactant concentration on particle size and DE***

Response surface plot for particle size is shown in **Figure 2**. From the graph it can be observed that drug: lipid ratio had a positive effect particle size. As increase in drug to lipid ratio and surfactant concentration due to high content of drug and lipid, high Particle size was observed. Contour plots for particle size is shown in **Figure 3**.

***Effect of Drug: lipid ratio and Surfactant concentration on Drug Entrapment***

Response surface plot for %drug entrapment is shown in **Figure 2**. It can be observed that % Drug to lipid ratio ha positive effect on PDE because accumulation of higher content of drug and lipid which will increase solubilization of drug into lipid which will increase PDE. As increasing Concertation of Surfactant which will decrease PDE because reduction of particle size affect drug entrapment.

**Figure 3** shows Contour plot for % drug entrapment

**3.3.1Check point Analysis**

A check point analysis was performed to confirm the prediction in order to validate the equation that describes the influence of the factors on the dependent variables. Two check point batches were prepared (CP1 and CP2). **Table 7** shows the actual and predicted value of independent parameters.

**3.3.2 Optimization using desirability function**

Desirability function was utilized to optimize the best batch .after studying the effect of the independent variables on the responses, the levels of the variables that gives the optimum responses were determined. The optimized batch with level of different factors, results and desirability is shown in **Table 7**.

**3.3Lyophilization**

Freeze drying technique was used to improve stability of SLN and to prevent the leakage of Entrapped FEB. Optimized Batch of FEB loaded SLNS was freeze dried using trehalose as cryoprotectant to preserve the size and shape. Trehalose was used as cryoprotectant because Trehalose seems to be a preferable cryoprotectant for biomolecules. It has many advantages in comparison with the other sugars as: less hygroscopicity, an absence of internal hydrogen bounds which allows more flexible formation of hydrogen bonds with nanoparticles during freeze-drying, very low chemical reactivity and finally, higher glass transition temperature.

Effect of varying ratio of total solid content FEB SLN: Trehalose (1:3 and 1:5) is shown in **Table 8**.

From the obtained result, 1: 5 was selected because it showed optimum particle size and high drug content compared to 1:3.

**3.4 *In vitro* drug release**

*In vitro* drug release study of SLN and suspension of pure drug performed by dialysis bag method and data of %CDR shown in **Figure 4**. From the results it can concluded that FEB-SLN gives first initial burst release of the drug 17.75 ± 1.14 %, initial burst release of the drug might be due to the presence free drug on the surface of nanoparticle. This mechanism is responsible for the initial burst release from the nanoparticle. After that SLN gives sustained release of drug up to 26 hr., the extended release of the drug might be due to the presence of solid lipid. The lipophilic nature of the solid lipid is responsible for the extended release of the drug. It is clearly visible from the data and figure that SLN formulation gives sustained release up to several hours when it compared with the pure suspension of drug. After 5 hours almost 100% drug was released and drug loaded in SLN gives release up to 26 hours.

**3.5 Ex- vivo drug release study**

*Ex vivo* permeability study was relevant approach to evaluate the absorption enhancing effects of a colloidal drug carrier system on intestinal tissue. The increase in permeability of SLN may be due to enterocyte of villi, M cells of Peyer's patches, paracellular and transcellular pathway. **Figure 5** shows permeability of SLN up to one day.SLN was absorbed through lymphatic system and met to systemic circulation. %CDR after 4 hours was observed 32.98 ± 1.04 SLN are permeable from small intestine. *Ex vivo* drug release curve is shown in figure 5. So from the results it can be conclude that SLN were permeable from intestine.

**3.6 Stability studies for freeze dried of FEB SLN**

As described in method, stability studies of freeze dried of FEB SLN were performed and results for all parameters at each time point reported and shown in **Table 9**.

As shown in **Table 9**, there was not major change shown in mean particle size and % drug content. After 45 days, mean particle size and % content 203.12 ± 1.20 nm and 78.12 ± 1.12 respectively. From result it can be concluded that freeze dried FEB SLN stable up to 45 days.

**Table 2: Febuxostat solubility in various solvents**

| Solvent (1 ml) | Soluble drug (mg) |
|---|---|
| **Methanol** | **22.33 ± 0.471** |
| **Chloroform** | **18.33 ±1.247** |
| **Ether** | **5.33 ± 1.247** |
| **Acetone** | **62.33 ± 2.054** |
| **Toluene** | **6 ± 0.816** |
| **Dichloromethane** | **10.66 ± 0.942** |

**Mean ± SD, n=3**

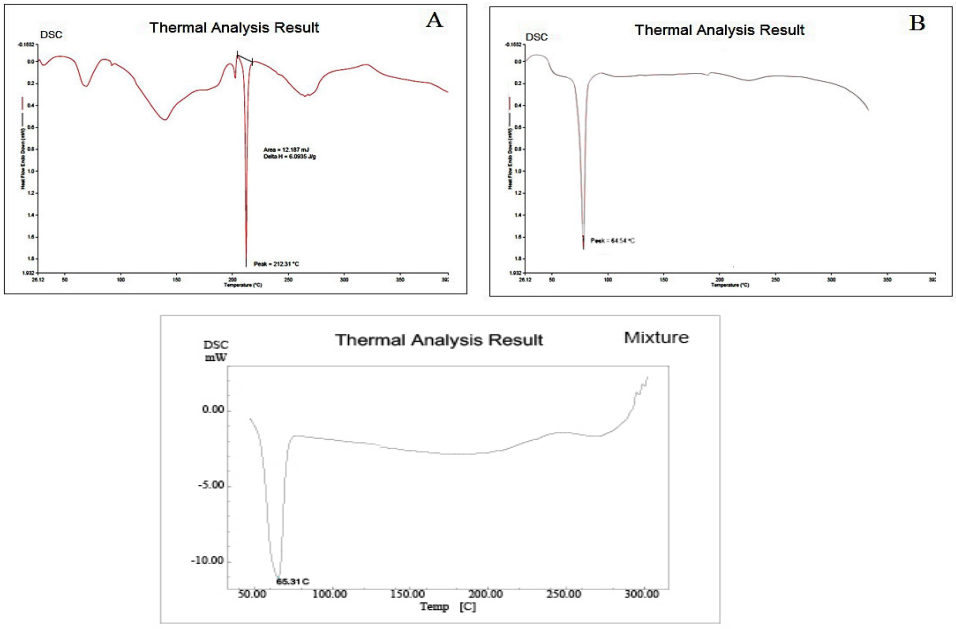


**Figure 1: DSC Thermogram of A: FEB B: Lipids C: mixture of drug and lipid**

**Table 3: solubility and Partition coefficient study**

| Solid Lipid | Drug solubility (mg) | Drug in lipidic phase | Drug in aqueous phase |
|---|---|---|---|
| Capmul GMS 50K | 78.33 ± 0.944 | 19.95 ± 0.00152 | 0.045 ± 0.00129 |
| Precirol ATO 5 | 49.1 ± 0.603 | 19.90 ± 0.001 | 0.09 ± 0.0008 |
| Compritol 888 | 18.33 ± 1.247 | 19.91 ± 0.0051 | 0.36 ± 0.481 |
| Dynasan 114 | 1.933 ± 0.249 | 19.93 ± 0.001 | 0.06 ± 0.0010 |
| Dynasan 116 | 3.33 ± 0.471 | 19.94 ± 0.0064 | 0.05 ± 0.0063 |
| Dynasan 118 | 6 ± 0.816 | 19.75 ± 0.0045 | 0.24 ± 0.0004 |

Mean ± SD, n=3

**Table 4: Selection of surfactant**

| Drug: lipid ratio | Surfactant concentration | Mean Particle Size(nm) | %Drug Entrapment |
|---|---|---|---|
| 1:15 | 1% (Poloxamer 188) | 229.45±0.25 | 78.85±2.58 |
| 1:15 | 1% (Poloxamer 407) | 322.40±0.29 | 57.52±0.22 |

Mean ± SD, n=3

**Table 5: Optimization of Process parameter**

| Parameter | | Particle Size* | % Drug Entrapment* |
|---|---|---|---|
| Sonication Time(min) | 1min | 323.46±2.32 | 74.43±0.568 |
| | 2min | 253.86±1.44 | 78.46±0.668 |
| | 3min | 325.43±1.10 | 73.22±1.01 |
| For optimization of Sonication Time, HSH speed 15,000 RPM and HSH Time 10 min was kept constant | | | |
| HSH Speed (RPM) | 10,000 | 163.83±1.45 | 76.56±0.545 |
| | 15,000 | 332.7±2.36 | 72.23±0.405 |
| | 17,000 | 693.66±2.67 | 75.88±0.575 |
| For optimization of HSH speed, Sonication time 2min and HSH Time 10 min was kept constant | | | |
| HSH Time (MIN) | 7min | 453.066±2.61 | 75.63±0.442 |
| | 10min | 323.7±1.24 | 82.03±0.485 |
| | 15min | 427.033±2.02 | 78.55±0.460 |
| For optimization of HSH time, Sonication time 2min and HSH speed 15,000 RPM was kept constant | | | |

Mean ± SD, n=3

**Table 6: Values of Particle size and % drug entrapment of Febuxostat loaded solid lipid nanoparticle as per $3^2$ full factorial designs**

| Batch No. | A | B | $Y_1$ (nm)* | $Y_2$ (%)* |
|---|---|---|---|---|
| 1 | -1 | -1 | 187.55±5.23 | 76.82±2.12 |
| 2 | -1 | 0 | 198.66±6.56 | 80.39±3.12 |
| 3 | -1 | +1 | 255.66±1.13 | 75.12±2.56 |
| 4 | 0 | -1 | 186.60±5.12 | 81.98±4.23 |
| 5 | 0 | 0 | 210.33±8.23 | 82.53±5.12 |
| 6 | 0 | +1 | 278.56±10.12 | 77.23±5.12 |
| 7 | +1 | -1 | 194.66±7.23 | 82.86±3.12 |
| 8 | +1 | 0 | 224.33±3.81 | 83.96±6.16 |
| 9 | +1 | +1 | 366.54 ±1.34 | 79.32±4.82 |

Mean ± SD, n=3

Y1 = +206.17+22.61 * A+59.99 * B+23.94* A * B+38.49* B2

Y2 = +82.85+2.30* A-1.67 * B -0.46* A * B -0.83 * A2 -3.40 * B2

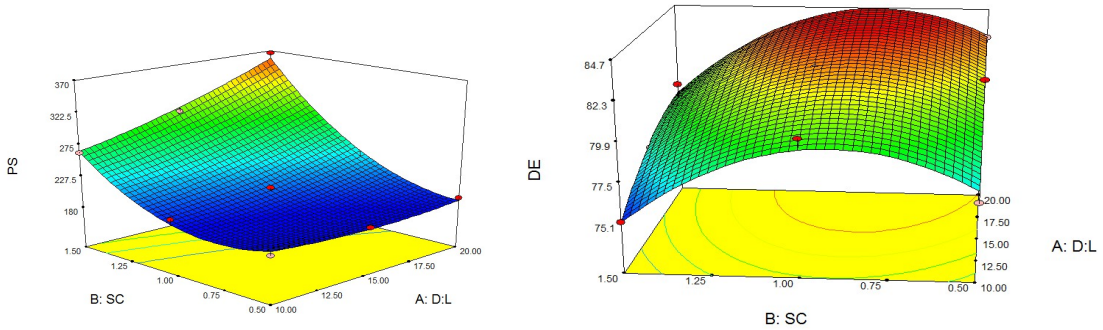


**Figure 2: Three-dimensional response surface plots for particle size and Drug entrapment**

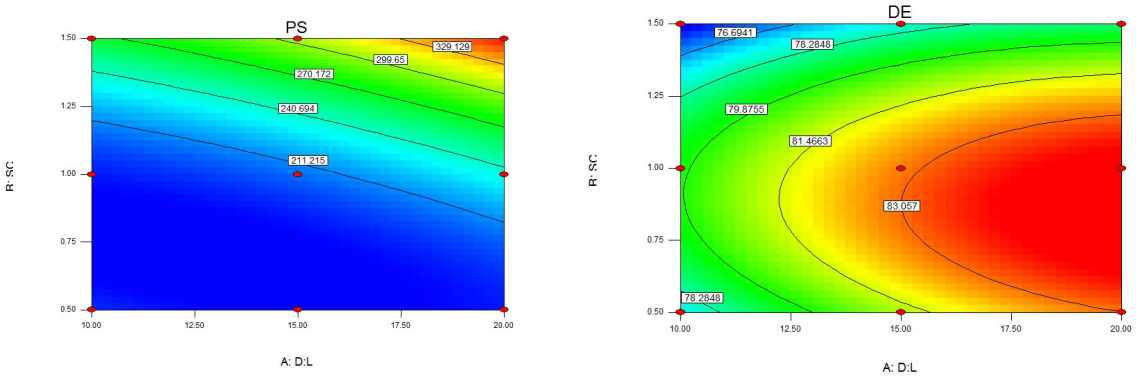


**Figure 3: Contour plots for % drug entrapment and particle size**

**Table 7: Check point batches and optimized batch**

| Check Point Batch | Measured Value | | Predicted Value | |
|---|---|---|---|---|
| | Particle Size | % drug entrapment | Particle Size | % drug entrapment |
| CP1 | 199.12±2.7 | 85.56±1.21 | 192.64 | 83.96 |
| CP2 | 210.21±1.2 | 82.12±1.45 | 202.84 | 80.67 |
| Optimized batch using desirability function | | | | |
| Drug :lipid ratio | Surfactant concentration | Particle Size | %drug entrapment | Desirability |
| 1:18.50 | 0.70 % | 192.64 | 83.96 | 0.983 |

**Table 8: Optimization of lyoprotectant**

| For trehalose concentration 1:3 | | | |
|---|---|---|---|
| Before | | After | |
| Particle Size | %drug Entrapment | Particle Size | % Drug Content |
| 193.4 Nm | 83.96 % | 184.52 nm | 76.98 % |
| **For trehalose concentration 1:5** | | | |
| Before | | After | |
| Particle Size | %drug Entrapment | Particle Size | %drug Content |
| 193.4 nm | 83.96 % | 189.62 Nm | 81.42 % |

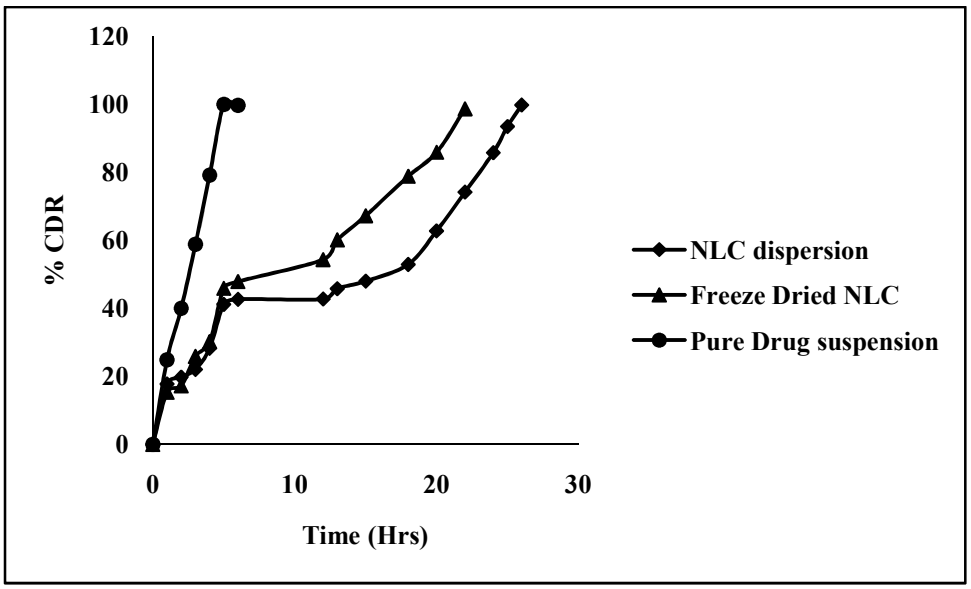


**Figure 4: *In vitro* drug release profile**

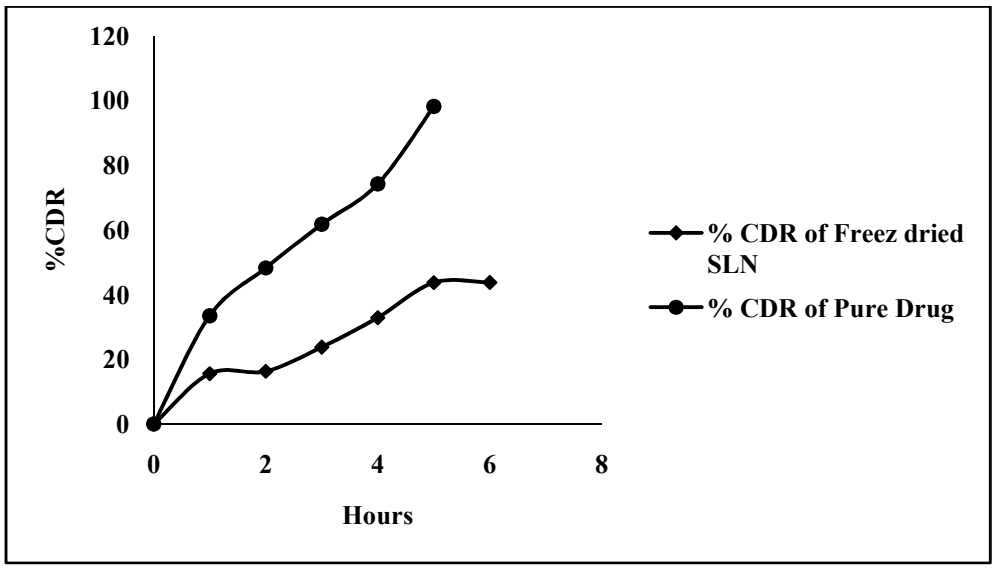


**Figure 5: *Ex vivo* drug release study**

**Table 9: Stability study of freeze dried FEB SLN**

| Days | Particle Size | % Drug content |
|---|---|---|
| 0 | 193.4 ± 1.98 | 81.42 ± 0.12 |
| 30 | 196.56 ± 2.11 | 79.67 ± 1.65 |
| 45 | 203.12 ± 1.20 | 78.12 ± 1.12 |

Mean ± SD, n=3

## 4. CONCLUSION

In the present investigation, an attempt was made to prepare and characterize FEB loaded solid lipid nanoparticles by hot homogenization–ultrasoniaction method. Optimized sonication times, rotation of HSH, speed of HSH were 2 minutes, 10,000 RPM and 10 minutes respectively. Using $3^2$ full factorial design, drug: lipid ratio (1:18) and surfactant concentration

(0.7%) were optimized. Optimized batch of FEB SLN had MPS 193.4±4.23 nm, PDE 82.93 ±1.6 % and surface charge -21.5 mv. From FTIR, and DSC studies of FEB, Lipids and optimized formulation it can be conclude that FEB was properly loaded in SLN. From TEM results, we can conclude that prepared SLN had Spherical shape & smooth surface. From *in- vitro* and *Ex -vivo* drug release profile, it can be concluded that FEB SLN gave sustained release as 100% CDR obtained after 24 hours.

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## 6. CONFLICT OF INTEREST

The authors have no conflict of interest.

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