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FABRICATION, OPTIMIZATION AND *EX-VIVO* CHARACTERIZATION OF FEBUXOSTAT LOADED NANOSTRUCTURED LIPID CARRIER BY 3^2 FULL FACTORIAL DESIGN

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ABSTRACT

Nano Structured Lipid Carriers (NLCs) are potential alternative of the tradition colloidal drug carrier. The main benefit is related to the well-known concept that lipids used in nanoparticle formulation promote drug oral absorption because they go through the same physiological mechanism as food lipid digestion. The objective of the present work is to use potential carrier NLCs for the oral delivery of poorly soluble drug Febuxostat (FEB). FEB-NLCs were formulated by High Speed Homogenization with Ultra sonication method using Capmul GMS 50 K as solid lipid and Transcutol HP as a liquid lipid. The 3^2 full factorial design utilized to study the effect of influence of solid lipid to liquid lipid ratio and concentration of surfactant, on the particle size and entrapment efficiency. Mean particle size and entrapment efficiency were found for all prepared formulation in the range of 196.66±2.08 nm to 368.33±2.51 nm and 74.80±0.52 % to 86.47±0.749 % respectively. Zeta potential was -15.2mv which is negative enough to give good stability. TEM study shows good spherical morphology of particle with diameter less than 200nm. *In-vitro* and *Ex-Vivo* study revel that NLCs gives biphasic release of the drug; first initial burst release and then sustained release up to 24 hour. The formulated NLCs dispersion was successfully converted in to the free flowing powder using trehalose as a cryoprotectant. In conclusion, FEB-NLCs potential carrier for the oral delivery and controlled release of drug which may reduce dosing frequency and improve patient compliance.

Keywords: Nano structured Lipid Carrier, High Speed Homogenization with Ultra sonication, 3^2 full factorial design, Lyophilization, *In-vitro*, *Ex-vivo*.

1. INTRODUCTION

Among all other routes such as parenteral, ocular, intranasal, dermal, transdermal and vaginal; oral route is most preferred route for the delivery of varieties of drug. Oral route used for both conventional and novel delivery of the drug. Oral route mostly preferred route for its vast qualities [1]. Gout is an ancient disorder associated with deposits of monosodium urate (uric acid), particularly in the joints and kidney. Gout is a most common and treatable type of inflammatory arthritis that affects almost 4% of adults in USA [2]. Gout is a rheumatic inflammatory condition that develops in some people who have high level of uric acid in their blood. The acid converted in to needle like crystal in some joints and bones and cause sudden and severe episode of pain, tenderness, redness warmth and swelling. It is usually associated with elevated serum uric levels (> 7mg/dL), and is often associated with the other metabolic disorders [3].

Febuxostat is Xanthine oxidase inhibitor, which is used in the treatment of gout. Febuxostat is classified under BCS class II in accordance with BCS classification. It has water solubility of only 0.0183 mg/mL, therefore it is necessary to improve solubility and dissolution characteristics of the drug, which results in improvement in oral bioavailability of the drug. Improvement in oral bioavailability can be achieved by reducing or by passing hepatic first pass metabolism of the drug [4-6].

Conventional dosage forms have limitation of hepatic metabolism of drug. This problem can be overcome by preparing novel dosage form such as polymeric nanoparticles, lipidic nanoparticles, micro emulsion, nano emulsion, self-micro emulsifying drug delivery system and self-nano emulsifying drug delivery system [7].

In the beginning of 1990, a substitute carrier system of liposomes, emulsion, and polymeric nanoparticle were

developed named solid lipid nanoparticles (SLNs) and is the first generation of lipid nanoparticle [8]. Polymeric nanoparticles and other lipidic formulation shows solvent related toxicity, rapid uptake by RES system, short shelf-life, interaction with the cell membrane that is overcome by preparing first generation lipidic nanoparticle. First generation lipid nanoparticles offers many advantages like safe to use, low toxicity, ease of commercialization and by passing of hepatic first pass metabolism but on the other side have drawbacks like low drug loading capacity, drug expulsion during manufacturing and storage period and perfect crystalline structure of SLNs may possess physical instability of system [8-9]. The aforementioned SLN drawbacks boosted the development of the 2nd generation lipid nanoparticle termed as Nanostructured lipid carriers (NLCs) in1999 [10]. In comparison to SLNs, NLCs are formed by mixing of spatially different lipid molecules, correspond to a blend of a solid and a liquid lipid. The presence of oil prevents perfect recrystallization of solid lipid and improvement of drug solubility in lipid matrix. The less-order inner-structured of NLCs allow, (1) High drug loading capacity (2) Reduce dug expulsion during storage (3) Modulation of drug release pattern by varying composition of lipids [11-14].

In the present work, FEB-NLCs were developed by High Speed Homogenization with Ultra Sonication method. The advantage of the method is the avoidance of usage of organic solvents, as organic solvent is not preferred because it generally leads toxicity and other problems [15-16].

2. MATERIAL AND METHOD

2.1. Material

Febuxostat was gifted from ZydusCadila Health care, Ahmedabad, Gujarat. Dynasan 114, 116, 118 and Miglyol 812 were gifted from Sasol, Germany. Precirol ATO 5, Compritol 888 Pellets, Transcutol HP were gifted from Gattefosse Pvt. Ltd., France. Capmul MCM, Capmul GDB, Capmul GMS 50K, Captex 355, Captex 200 P were gifted from Abitec Corporation Ltd., India. All other reagents and chemical used in research work were of pharmaceutical grade.

2.2. Methods

2.2.1. Selection of Solid Lipid

2.2.1.1. Solubility of drug in solid lipid

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. Solid Lipids used for this study were Dynasan 114, Dynasan 116, Dynasan 118, Precirol ATO 5, Compritol 888 pellets and Capmul GMS 50K. The experiment was conducted in triplicate [12, 17, 18].

2.2.1.2. Partition-Coefficient

Weighed quantity of drug (20 mg) was added in to melted solid lipid of 1gm, mixed properly to form homogenous blend. 10ml of distilled water was added (Pre-heated at the temperature of melted solid) in to solid mass. Mixture was shaken on isothermal orbital shaker to reach equilibrium followed by separation of aqueous phase through centrifugation at 5000 rpm for 5 min using cooling centrifuge. 1ml of aqueous phase was taken and diluted upto10 ml with methanol. Drug content was analysed spectroscopically at 315 nm using UV visible spectrophotometer and calculated using below equation [18-20]:

% Partition = {(Amount of drug added-Amount of drug in aqueous phase)/Amount of drug added} x 100

2.2.2. Selection of Liquid Lipid

2.2.2.1. Solubility of drug in liquid lipid

The solubility determination of febuxostat in various liquid lipids was performed by taking 2ml of fixed liquid lipids and solubility was checked by adding febuxostat in increments of 1mg until it failed to dissolve further in the liquid lipids. The amount of drug solubilised in liquid lipid was determined. Liquid lipids used for the study were Capryol 90, Transcutol HP, Miglyol 812, Captex 355 EP, Captex 200 P. The experiment was conducted in triplicate [18, 21].

2.2.3. Formulation of FEB loaded NLCs

The Febuxostat loaded NLCs were formulated using high speed homogenization with ultrasonication method. The lipidic phase composed of calculated amount of liquid lipid, Transcutol HP and solid lipid, Capmul GMS 50K were taken in beaker and melted in hot plate at the temperature above 5°C of the melting point of solid lipid, after that calculated amount of drug was added in the molten lipid. The aqueous phase comprise of Poloxamer 188 dissolved in distilled water. Aqueous phase was heated at the same temperature as the temperature of molten lipid. At the same temperature, aqueous phase was mixed with lipid phase and homogenized using high speed homogenizer then hot coarse o/w emulsion obtained was sonicated using

frontline probe sonicator (Frontline Electronics and Machinery Pvt. Ltd). The prepared colloidal dispersion of NLCs was transferred into centrifuge tube equipped with compact cooling centrifuge (REMI Lab Word) and centrifugation was carried out for 8000 rpm 15 min and 10°C to separate precipitated NLC [22].

2.2.4. Optimization of Process Parameter

Optimization of process parameter was done by taking 3 level of 3 different process namely Time of High Speed Homogenization (HSH), Speed of the High Speed Homogenizer, Sonication Time. Total 9 batches were formulated with different process parameters and comparison was done by taking two dependent variables, %EE and Particle Size. The formulation was prepared by fixing 2 independent variable X1 (Level: 0) and X2 (Level: -1) as described in Table 1.

Table 1: Optimization factors with levels

Factors	Levels		
Independent Variables	Low (-1)	Medium (0)	High (+1)
X1 Liquid lipid (to solid lipid)	10	20	30
X2 Concentration of Surfactant	0.5	1	1.5
Dependent Variables	**Response**		
Y1	% EE		
Y2	Particle Size (nm)		

2.2.5. Optimization of Formulation Parameter

A complete 3^2 full factorial design was utilized to study the effect of independent variables on the dependent variables in the formulation of NLCs. Independent variables were selected for the study were solid lipid to liquid lipid ratio (X1) and concentration of surfactant (X2). Each independent factor had three levels, High, Medium and low. Dependent variables were % Entrapment Efficiency (%EE) (Y1) and Particle size (Y2).

2.2.5.1. Interaction between factors

The statistical evaluation of all the obtained data was carried out by analysis of variance (ANOVA) using DOE software. The results of ANOVA (P value) showed the effect of various independent variables on the particle size and %EE. After regression analysis of all the formulations, full polynomial model was obtained followed by omission of non-significant terms ($p > 0.05$) to obtain reduced model of analysis. This equation represents the effects of independent variables on the dependent variables.

2.2.5.2. Evaluation of model/Check point analysis

Evaluation of model was done by check point analysis to evaluate the dependability of the model and was performed to verify the effectiveness of the established contour plot and reduced polynomial equation in the development of NLCs. Two check point batch was prepared and evaluated through comparing experimental and predicted value of responses. Each formulation was fabricated thrice and average was calculated.

2.2.5.3. Preparation of Optimized formulation based on the desirability function

Optimization was carried out to ascertain the level independent variables (X1and X2) that would provide data of Y1 and Y2. At the time of developing the formulation, the response has been united to design the product of required attribute. The main function of the desirability was to join every response in single experiment and provide the probability of predicting highest level for independent variables.

2.2.6. Lyophilization of optimized batch of FEB-NLCs

Lyophilization or freeze drying is the generally used method in the pharmaceutical field to convert aqueous dispersion formulation into dried free flowing solid powder form, to prolong the physical and chemical stability of the formulated nanoparticle during storage time period. The freeze drying or lyophilization phase produces various stresses during the procedure, so it is essential to add cryoprotectant to protect the sample and avoid stress condition. For the lyophilization of the prepared FEB-NLCs; Trehalose was used as a cryoprotectant in the ratio of 1:1, i.e. 1 part of NLCs dispersion and 1 part of 1% Trehalose solution in distilled water. Trehalose had chosen among all other sugar, because trehalose offers many advantages including less hygroscopic, exhibit higher glass transit temperature which gives prolong stability to nanoparticles, low chemical reactivity.

2.2.7. Evaluation and Characterization of optimized batch of FEB-NLCs

2.2.7.1. Particle Size

The mean particle size of FEB-NLCs and particle size distribution expressed by the poly dispersibility index (PDI) of the prepared NLCs were determined using Dynamic Light Scattering (DLS) technique with particle size analyser (Malvern instruments). Particle size was

measured by diluting the dispersion with distilled water and then analysed by particle size analyser.

2.2.7.2. Entrapment efficiency (%EE)

%EE was calculated by measuring the amount of free non-entrapped febuxostat present in supernatant and sediment from the prepared FEB-NLC. 5ml of NLC dispersion was centrifuged using cooling centrifuge with the speed of 8000 rpm for 15 min. at 10°C. After centrifugation, supernatant was collected and lipid was precipitated by adding methanol, as the drug is soluble in methanol and lipids precipitated out. The solution was filtered with filter paper and by appropriate dilution to the solution, analysed in UV-VIS spectrophotometer at 315 nm. The concentration of free drug was found out and %EE was calculated by following equation.

%EE ={(Amount of febuxostat added-Amount of free febuxostat)/ Amount of febuxostat added} x100

2.2.7.3. Zeta potential

The measurement of the magnitude of the electric charge and repulsion/attraction between particles is one of the most promising tool for the stability of the system. The electrical charge on the surface of the particle shows the physical stability of the nanoparticle. The surface charge of prepared FEB-NLCs was measured by measuring zeta potential of the dispersion. For the measurement of zeta potential, 1ml of the dispersion was diluted with 10 ml distilled water and then analysed by (Zetasizer Nano ZS; Malvern Instrument, Malvern, UK).

2.2.7.4. In-Vitro Drug Release Study

In-vitro drug release study was carried out using dialysis sac diffusion method [22]. The dialysis bag used had a molecular weight of 12,000-14,000 and was activated by first removing glycerol from the dialysis tube by washing it in running water for 3-4 hours. Sulphur compound was removed by treating with a 0.3% (W/V) sodium sulphide solution at 70°C for one minute, washed with hot water (60°C) for two minutes, followed by acidification with 0.2% sulphuric acid, and rinsed with hot water to remove the acid [23, 24]. The dialysis tube was then further used for the *in-vitro* study. Volume equivalent to 10 mg of FEB-NLCs dispersion was added into a thoroughly sealed (double-folding on both sides) dialysis bag which was immersed in 200ml of PBS (pH 7.4) maintained at 37±0.5°C. The agitation in the beaker was managed by magnetic bead stirrer and aluminium foil was utilized to cover the beaker to avoid loss of solvent during process. At predefined time intervals, 5ml of sample was withdrawn from the dissolution medium and the amount of FEB released into dissolution medium was analysed spectrophotometrically at 315nm. The volume of dissolution medium was kept constant by addition of replacement volume of PBS after each sampling.

2.2.7.5. Ex-Vivo Drug Release Study

To find out the drug permeation from the intestinal membrane, *Ex-Vivo* drug study was performed. The literature review gives conclusion that the absorption of nanoparticle occur through small intestine, which contains high amount of payer's patches and then goes into lymphatic system. *Ex-Vivo* study was carried out by using intestinal portion of chicken from the slaughter house. From the whole gastrointestinal tract of the chicken, ileum portion was identified, cut into appropriate segment and immediately placed into the aerated ringer PSS solution. The mesenteric and adhering tissues were removed with care and cleaned by washing with PSS solution. One end of the ileum was tied with thread and 1ml of NLCs dispersion was placed into it. Another end was also tied to avoid leakage. A beaker containing 200ml of PBS (pH 7.4) with continuous agitation and aeration constant temperature of 37±0.5°C was maintained. After preparing whole experimental set up, the tissue was placed into beaker. At predefined time intervals, 5ml of sample was withdrawn from the dissolution medium and the amount of FEB released into dissolution medium was analysed spectrophotometrically at 315nm. The volume of dissolution medium was kept constant by addition of replacement volume of PBS after each sampling.

2.2.7.6. TEM (Transmission Electron Microscopy)

The morphological characteristic of the optimized formulation of NLCs dispersion was investigated using TEM. NLC dispersion was diluted with distilled water and a drop of it was placed on copper grid slide and sample was allowed to air dry. The air dried sample was then analysed under TEM to capture image.

2.2.7.7. Differential scanning calorimetry (DSC)

DSC study was performed to characterize physical state of FEB in Lyophilized NLCs. 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.

2.2.7.8. Fourier transform infrared spectroscopy (FTIR)

It is necessary to identify the interaction that may occur during the manufacturing process of the NLCs. The IR spectrum of formulated freeze dried NLCs was measured by FT-IR spectrometer. In this process, sample was mixed with KBr and compressed to form thin pellet and then used for testing. The recording range for the measurement was 4000-400 cm^{-1}

2.2.8. Stability Studies

The main aim of the stability study is to examine the aspect of the characteristic of drug change with the various parameters such as temperature, humidity, light and with time. Further, it is helpful to form re-analysis period and shelf life for the formulation as well as prescribed storage condition. Stability test should cover to examine those aspects of the pharmaceutical formulation that changes at the time of storage and are likely to affect quality, potency and safety. Physical stability of the optimized formulation was done by keeping them in glass vial at room temperature (25±2°C) and in the refrigerator temperature (4-8±2°C) for 1 month. After the completion of time period, the samples were further analysed for the mean particle size and %EE [25].

3. RESULTS AND DISCUSSION

3.1. Solubility of Drug in solid lipid

The selection of lipid and other excipients have prime importance in the development of stable formulation. To maintain a drug that isn't very soluble, Febuxostat's solubility in solid lipids is determined by the drug's solubility. The solubility of drug in solid lipid was done by quantitative method. The drug shows highest solubility in Capmul GMS 50K (78.33±0.94 mg/gm) and in the Dyanasan 118 (6±0.816 mg/gm), Dynasan 116 (3.33±0.471 mg/gm), Dynasan 114 (1.933±0.249 mg/gm), Precirol ATO 5 (49.1±0.603 mg/gm), Compritol 888 pellets (18.33±1.247 mg/gm), Capmul GDB (10.33±0.471 mg/gm) shows lowest solubility or solution become cloudy.

3.2. Solubility of drug in Liquid Lipid

For the fabrication of NLCs; along with solid lipid, liquid lipid has important role in the solubilisation of drug and also for the entrapment efficiency of the drug. Selection of liquid lipid was done by solubility method. From the fig. 1 it can be concluded that Transcutol HP has higher capacity to dissolve Febuxostat (142.66 ± 2.05 mg/2ml). All other lipids shows lower solubility like Capryol 90 (17.9±0.535mg/2ml), Capmul MCM (15.7±0.294mg/2ml), Capmul MCM (15.7±0.294mg /2ml), Miglyol 812 (15.6±0.294mg/2ml), Captex 355EP (11.93±0.492mg/2ml), Captex 200 P (12.6± 0.535mg/2ml). Transcutol was selected as a liquid lipid for the development of the NLCs along with Capmul GMS 50K for the development of NLC.

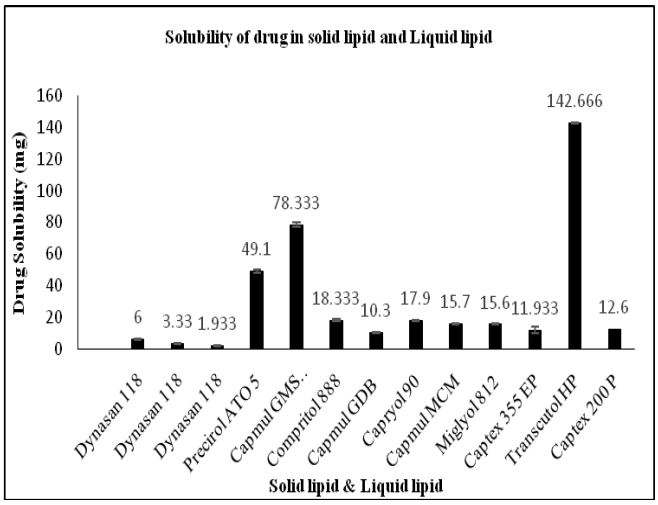


Fig. 1: Graphical representation of solubility of Febuxostat in Solid Lipid and liquid lipid

3.3. Partition-Coefficient

Determination of partition behaviour of drug is one of the important criteria for the selection of solid lipid in the development of nanoparticles of the poorly water soluble drug. Partition behaviour of the drug gives knowledge about the extent of the drug partitioned towards lipidic phase. Partition behaviour of the drug in the lipids is most important measures to controlling the parameters namely entrapment efficiency and drug release profile. From results, it was concluded that Febuxostat have higher partition behaviour towards Capmul GMS 50K, and from the solubility results, the drug has higher solubility in Capmul GMS discussed earlier. So according to both the results, Capmul GMS 50 k was selected for the solid lipid in the formulation of nanoparticles. The graphical representation of Partition is depicted in the Fig. 2.

3.4. Optimization of the process parameters

The effect of process parameters is much more important in the formulation of NLCs. These 3 parameter sonication time, HSH RPM, HSH time found importance in the formulation. Optimization was done by considering the results of %EE and particle size. Effect of different parameters are shown in table 2.

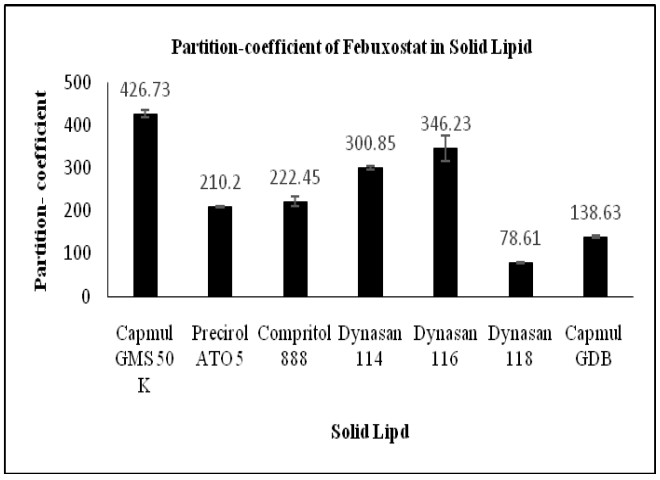


Fig. 2: Graphical Representation of partitioning behaviour of Febuxostat in Solid Lipid

3.4.1. Effect of Sonication Time

As the sonication time varies from 1-2 min, %EE increases and particle size decreases, which was desired too. 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.

3.4.2. Effect of Speed 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.

3.4.3. Effect of HSH Time

As the HSH 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 increases. From results, it can be concluded that 10 min HSH time is optimum for the effective formulation of Nanoparticle.

From the results of optimization of process parameters, 2min sonication time, 10,000 RPM and 10 min HSH Time was selected for further study.

Table 2: Optimization of Process Parameter

Batch Code	Sonication Time (Min)	Speed of HSH	HSH Time (Min)	% EE	Particle Size (nm)
NLC 1	1	15,000	10	74.43 ± 0.568	323.46 ± 2.32
NLC 2	2	15,000	10	78.46 ± 0.668	253.86 ± 1.44
NLC 3	3	15,000	10	73.22 ±1.01	325.43 ± 1.10
NLC 4	2	10,000	10	76.56 ± 0.545	163.833 ± 1.45
NLC 5	2	15,000	10	72.23 ± 0.405	332.7 ± 2.36
NLC 6	2	20,000	10	75.88 ± 0.575	793.66 ± 2.67
NLC 7	2	15,000	5	75.63 ± 0.442	453.066 ± 2.61
NLC 8	2	15,000	10	82.03 ± 0.485	381.7 ± 1.24
NLC 9	2	15,000	15	78.55 ± 0.460	427.033 ± 2.02

Mean ± SD, n = 3

3.5. Optimization of formulation parameters

Table 3 shows that %EE varies from 74.80±0.520 % to 86.47±0.749 % and particle size varies from 196.66± 2.08nm to 301.66±2.51nm. With the help of ANOVA and constructing polynomial equation, variation in the size of particle and %EE were evaluated.

Y1 (%EE) = +79.81+2.98* X1 +1.58 * X2

Y2 (Particle Size) = +208.56-22.00* X1 +60.67 X2 - 23.25* X1*X2 +7.67* $X1^2$+38.67* $X2^2$

For %EE, The Model F-value of 23.50 implies that the model is significant. There is only a 0.15% chance that a "Model F-Value" being large due to noise. In this case X1, X2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model. The effect of SL:LL ratio and conc of surfactant on %EE is shown in Fig. 3 (A) and (B) in 3D Surface plot and counter plot respectively. Solid to liquid lipid ratio shows positive influence on %EE. Increase in the amount of liquid lipid will significantly increase %EE. The strength of surfactant positively affects the drug entrapment; as the amount of surfactant increases, improvement in the drug entrapment was observed.

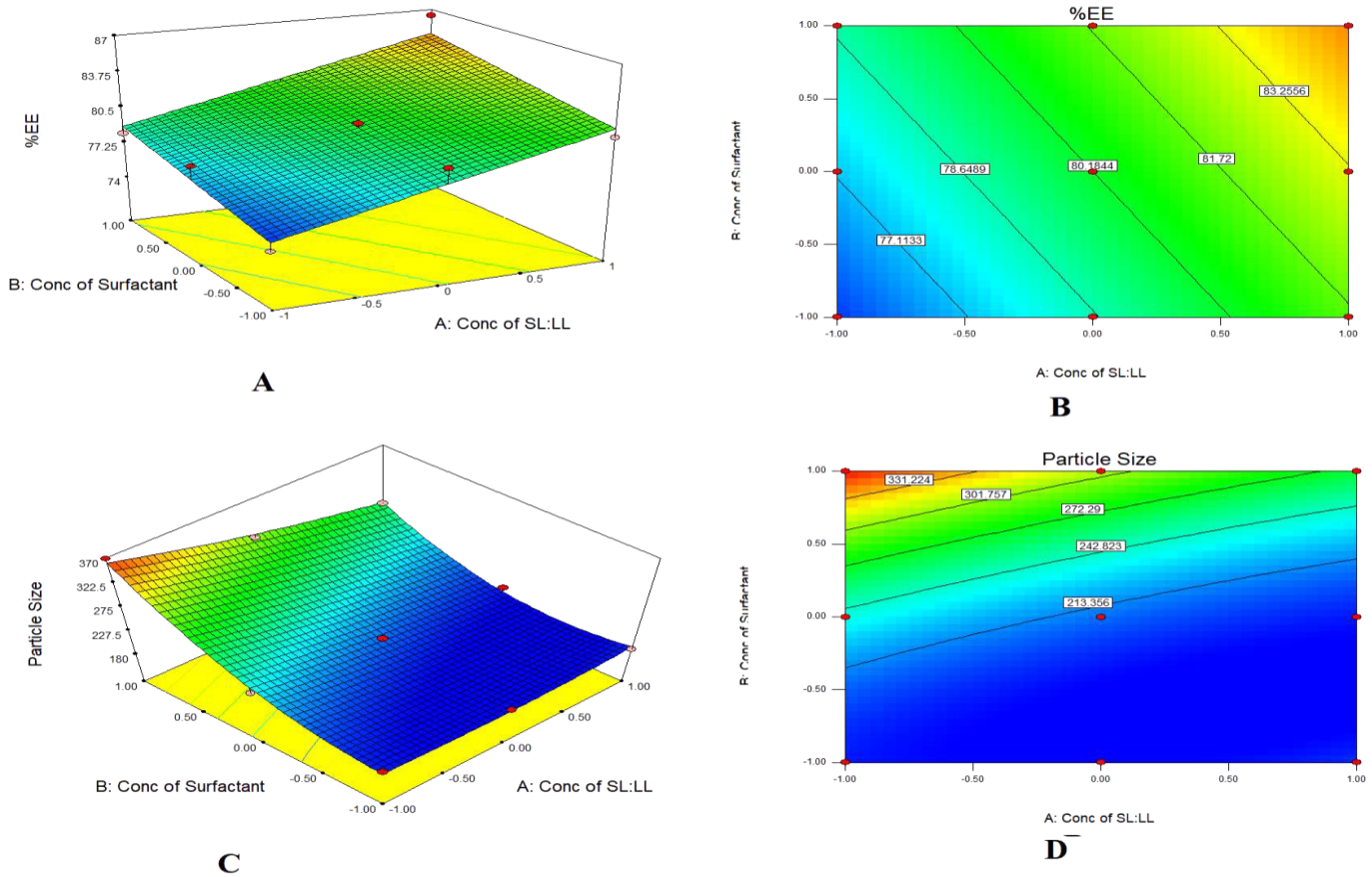
For particle Size, The Model F-value of 61.43 implies the model is significant. There is only a 0.32% chance that a "Model F-Value" being large due to noise. In this case X1, X2, X1X2, $X2^2$ are significant model

terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model. The effect of SL:LL ratio and conc of surfactant on particle size is shown in Fig. 3 (C) and (D) in 3D Surface plot and counter plot respectively. As per polynomial equation and graph, it can be concluded that SL: LL ratio has negative effect on the particle size i.e. increase in the amount of liquid lipid that reduces the size of nanoparticles. Second variable, concentration of surfactant shows positive effect on the size of nanoparticle i.e. increases in the conc. of surfactant that increases the size of nanoparticle. Both the variables conjointly give positive effect on particle size.

Table 3: Optimization of Formulation Parameter

Formulation Code	SL:LL Ratio (X1)	Concentration of Surfactant (X2)	Drug (mg)	% EE (Y1)	Particle size(nm) (Y2)
NLC 10	-1	-1	50	74.80 ± 0.520	196.66 ± 2.08
NLC 11	-1	0	50	78.39 ± 0.695	226.33 ± 1.52
NLC 12	-1	+1	50	78.12 ± 0.13	368.33 ± 2.51
NLC 13	0	-1	50	79.9 ± 0.137	188.66 ± 1.527
NLC 14	0	0	50	80.53 ± 0.330	212.33 ± 2.51
NLC 15	0	+1	50	80.63 ± 0.320	301.66 ± 2.51
NLC 16	+1	-1	50	80.86 ± 0.295	189.66 ± 1.52
NLC 17	+1	0	50	81.96 ± 0.189	200.66 ± 2.51
NLC 18	+1	+1	50	86.47 ± 0.749	265.66 ± 3.05

Mean ± SD, n = 3



(A) 3D Surface plot (B) Counter Plot and on Particle size (Y2) (C) 3D Surface plot (D) Counter Plot

Fig. 3: 3D surface plot and Counter plots showing effect of Solid lipid to liquid lipid ratio (X1) and Concentration of Surfactant (X2) on Effect on %EE (Y1)

3.5.1. Evaluation of Model/Check Point Batch Analysis

To confirm the polynomial equation which shows variables affect by factor, 2 check point batches were prepared. By comparing experimental value and predicted value, no major changes was observed as shown in table 4.

3.5.2. Preparation of Optimized Batch Based On Desirability Function

During the optimization of the formulation, all the responses were considered to find out the desirability characteristic of the formulation. The desirability function combines all the responses into one variable to predict the optimal levels for the independent variables. The desirability value of 0 represents unacceptable response and 1 represents the most significant and desired value for the response. The response of the factorial formulation is shown in table 4 that suggested SL: LL Ratio 70: 30 %w/w and concentration of surfactant 0.97% with 0.855 desirability.

3.6. Evaluation and Characterization of Optimized Batch of Formulation

3.6.1. %EE and Particle size

The drug entrapment efficiency and particle size of optimized batch was found to be 81.64% and 205.4nm respectively. It was observed that the given result is nearer to the software prediction i.e. 82.68% and 192.11 nm, drug entrapment efficiency and particle size respectively. Particle size distribution graph of optimized batch is shown in Fig. 4 (A).

Table 4: Check point batch analysis and optimized batch with desirability function

Check Point Batch	Experimental Value		Predicted Value	
	%EE*	Particle Size*	%EE	Particle Size
NLC 19	81.46±0.189	240±0.221	82	233
NLC 20	78.14±0.51	172.8±0.35	77.5	194.9
Optimized Batch with Desirability Function				
Formulation Code	**Drug (mg)**	**SL: LL Ratio (%)**	**Concentration of Surfactant (%)**	**Desirability**
NLC 21	50	70:30	0.97%	0.855

** Mean ± SD, n = 3*

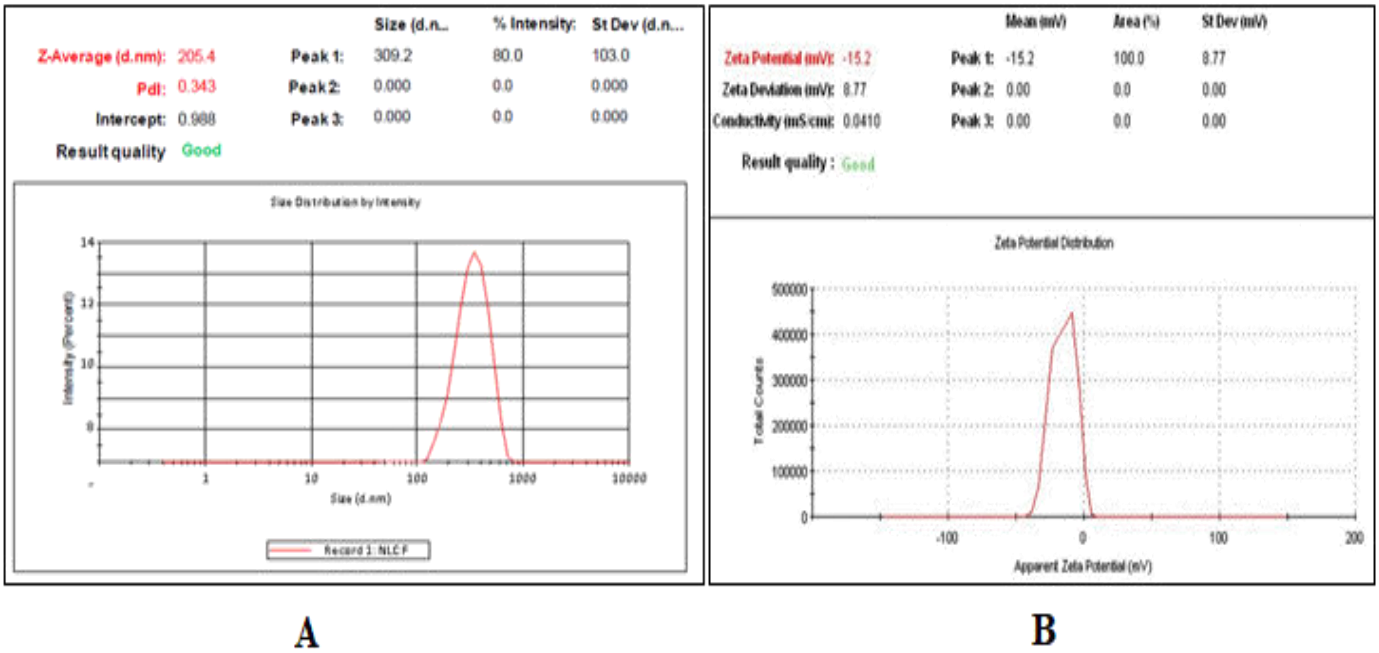


Fig. 4: (A) Particle size distribution graph of optimized batch of FEB- NLCs (B) Zeta Potential curve of optimized batch of FEB-NLCs

3.6.2. Zeta Potential

The storage stability of the colloidal system can be evaluated by measuring zeta potential of the system. Nanoparticles with the zeta potential range between -10 to + 10 mv considered as a neutral, Zeta potential, greater than + 10mv and less than -10 mv are considered as a strong cationic and anionic in nature. As most of the cell membranes possess negative charge, the nanoparticles with positively charged particles generally displays cell wall disruption toxicity. Positive charge possesses a membrane destabilizing and destructive effect on cell membrane resulting from an interaction of positive and negative charged of particles. Therefore, positively charged particles or lipids are not approved by FDA for clinical use. FEB-NLCs show zeta value -15.2mv which is negative and gives higher stability to the system. The zeta potential curve of optimized batch is shown in Fig. 4 (B).

3.6.3. In-Vitro Drug release study from FEB-NLC

In-Vitro drug release study of NLC and suspension of pure drug was performed by dialysis bag method. From the results, it can concluded that FEB-NLC gives first initial burst release of the drug 17.75±1.14 %, initial burst release of the drug might be due to the presence of free drug on the surface of nanoparticle, another reason might be due to presence of liquid lipid located on the outer shell of nanoparticle, the Transcutol enriched outer layer of nanoparticle possess a soft and considerably higher solubility for the febuxostat. Which, in turn, improves drug solubility and results in a drug-enriched shell that can be easily released via matrix or erosion diffusion mechanisms. This mechanism is responsible for the initial burst release from the nanoparticle [23-25]. The other possible justification for burst release is the larger surface area of FEB-NLC as well short diffusion distance for Febuxostat from the particle matrix into the dissolution medium, it was observed that liquid lipid adheres to the lipidic matrix which reduces the diffusion path length of lipid matrix [23]. After that NLC 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 [24]. It is clearly visible from the data and figure that NLC formulation gives sustained release up to several hours when it is compared with the pure suspension of drug. After 5 hours almost 100% drug was released and drug loaded in NLC gives release up to 26 hour. The graphical representation is shown in Fig. 5.

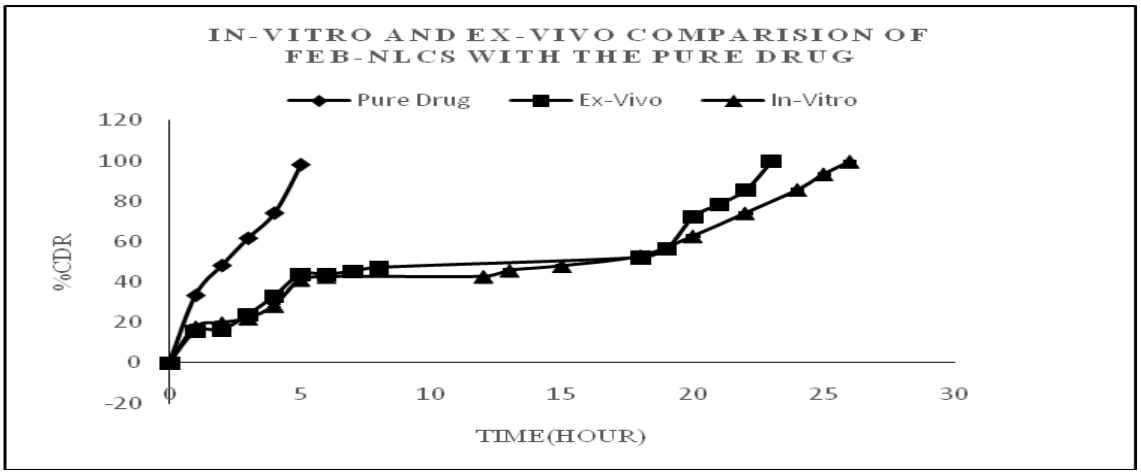


Fig. 5: In-Vitro and Ex-Vivo Drug Release study of FEB-NLC and compared with pure drug dispersion of Febuxostat

3.6.4. Ex-Vivo Drug Release Study

The intestinal permeability of the nanoparticle was examined through *Ex-vivo* permeation study. From the data it can be concluded that formulation was easily permeable through the intestinal membrane via M cells of the payer's patches, transcellular, paracellular pathway and enterocytes of villi. NLC gives sustained release up to 23 hour, %CDR detected was 99.86±0.09. *Ex-Vivo* permeation study curve of FEB-NLC and pure drug is shown in Fig. 5. From the data, it is clearly concluded that NLC is permeable from intestine.

3.6.5. TEM (Transmission Electron Microscopy)

The morphological shape of nanoparticle is shown in Fig. 6. TEM image describe the discrete particle size with smooth surface and spherical shape. It also shows particle size <200 nm, confirming the nanosize of particle.

3.6.6. DSC (Differential Scanning Calorimetry)

Fig. 7 shows the sharp endothermic peak of Febuxostat at 212.31°C which represent the melting point of the drug. Fig. 7B shows the endothermic peak of Capmul GMS 50K at 65.27°C. DSC Thermogram of physical mixture depicted in Fig. 7C shows two different peaks at 215.65°C and 64.54°C of FEB and Capmul GMS 50 K respectively. Fig. 9D shows DSC thermogram of optimized batch of FEB-NLCs. Thermogram shows one endothermic peak at 69.37°C representing melting point of Capmul GMS 50K but absence of the peak within the melting range of FEB which gives indication of either solubilisation or conversion of drug from crystalline to amorphous form in the lipidic matrix.

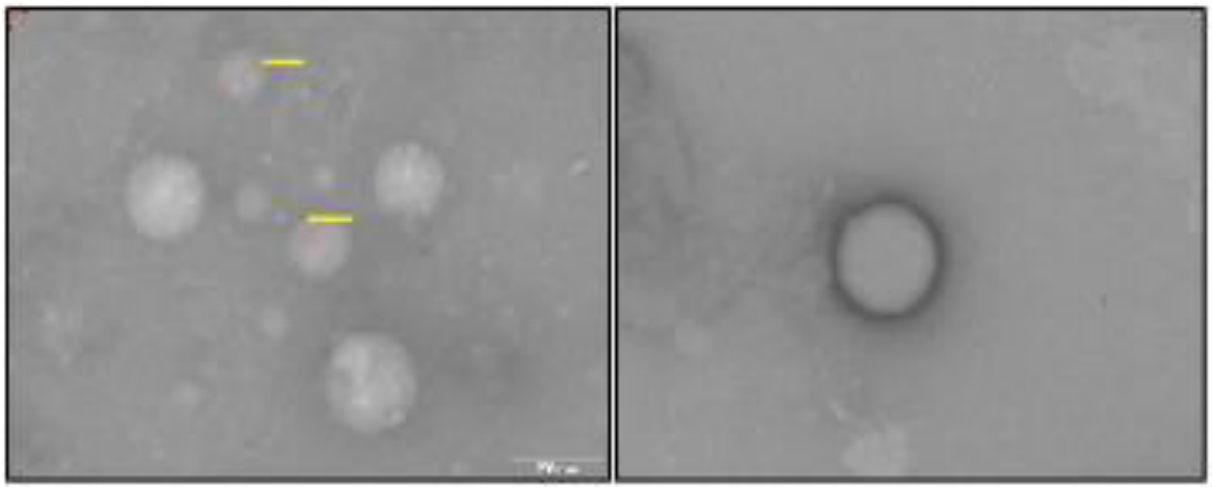
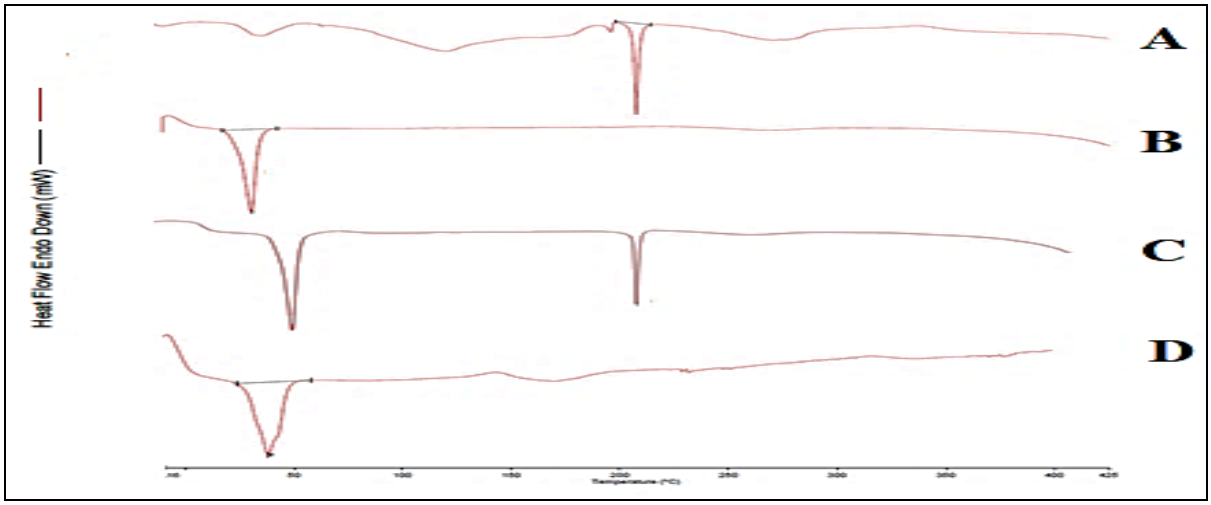


Fig. 6: SEM image of FEB-NLC



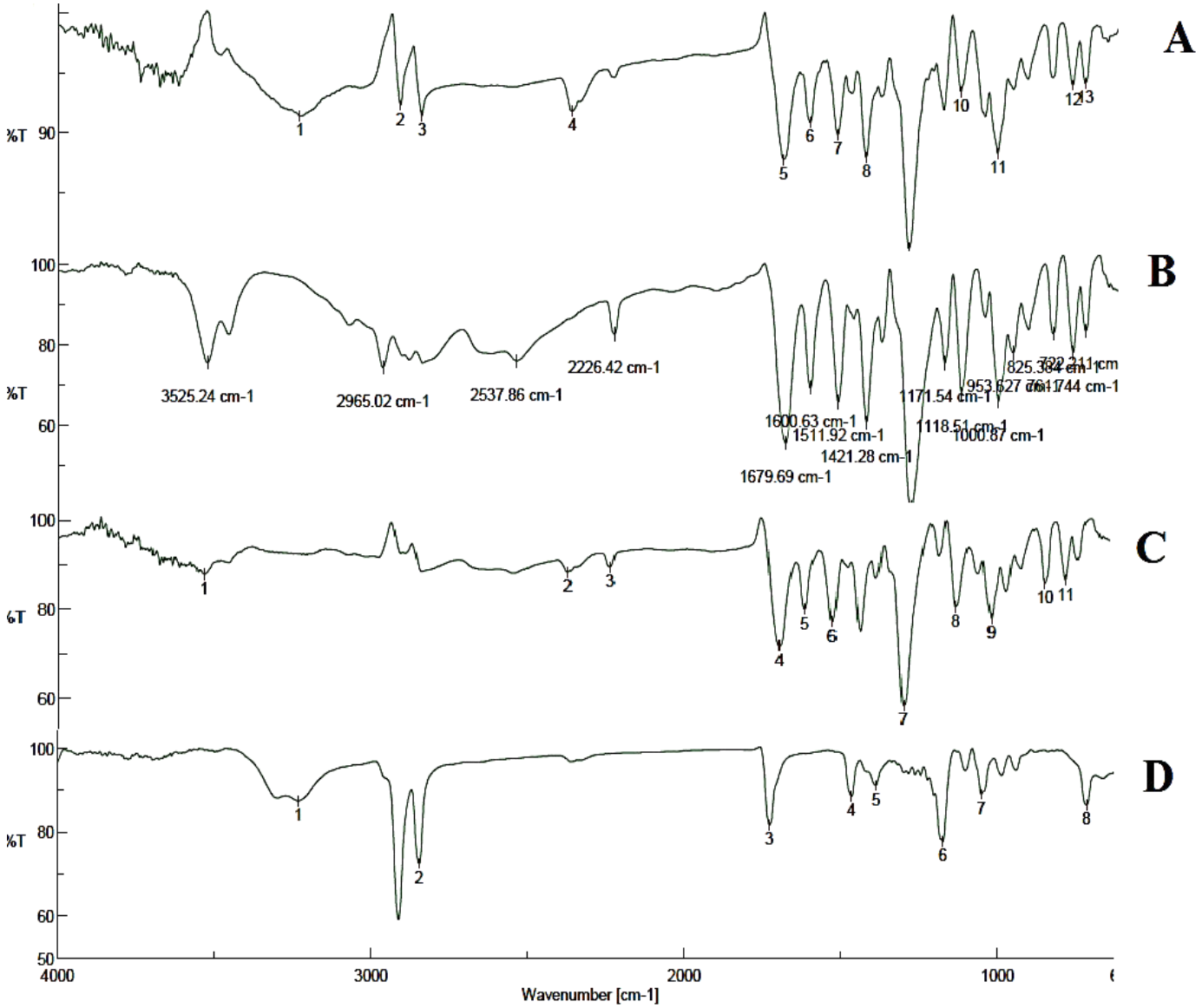
(A) Febuxostat (B) Capmul GMS 50K (C) Physical Mixture of Febuxostat and Capmul GMS 50K (D) lyophilized Optimized Batch

Fig. 7: DSC Thermogram

3.6.7. FT-IR

FT-IR spectrum of Febuxostat, Febuxostat + Capmul GMS 50 K, Febuxostat + Poloxamer 188 are shown in fig. 8 A, B and C respectively. From the spectrum, it can be concluded that there was no major changes observed in the peak of drug and drug+excipient mixture when compared with the standard peak. Ultimately, it can be assumed that there was no

interaction between drugs and excipients used in the formulation of NLCs. The FI-TR spectrum of optimized lyophilized batch shown in fig. 8D represents retention of all the important functional group peaks of drug (O-H Stretching at 3232.11 cm^{-1} , C=N Stretching at 2846.42 cm^{-1} , COOH Group 1728.87 cm^{-1}). Thus the drug was supposed to remain unchanged after the lyophilization.



(A) FEB Pure Drug (B) FEB + Capmul GMS 50 K (C) FEB + Poloxamer 188 (D) lyophilized Optimized Batch

Fig. 8: FT-IR Spectrum

3.7. Stability Studies

Stability study was performed to provide conclusion that the formulation remains stable for the specific period of time. Stability study shows measured %EE and particle size of the dispersion to ensure that the product remain unchanged. At the time of preparation, % EE was 82.68±0.49 % and particle size 192.11±1.49 nm was observed. At the room temperature, the formulation remains stable for 7 days but after that aggregation was observed. Particle size was increased (230.54±1.74nm) and %EE (79.12±1.46 %) was slightly reduced. At the refrigerating condition, the product is physically stable; %EE and particle size were found 80.83±0.62 % and 198.44±0.88 nm respectively. At the refrigerated condition, % EE and particle size shows minor changes and remains stable. From the stability we can conclude that the refrigerator condition is more favourable in case of stability of Nanoparticle colloidal system.

4. CONCLUSION

In the current study, FEB-NLCs were successfully formulated by high speed homogenization with ultra-sonication method. Ratio of solid to liquid lipid and concentration of surfactant plays crucial role on the particle size and drug entrapment. The formulated NLCs dispersion was successfully converted into free flowing powder by lyophilization of dispersion. The result of *In-vitro* and *Ex-vivo* study shows improved rate of the drug release from FEB-NLCs as compared with pure drug. This is because of change in the physical structure of drug, reduction in particle size, higher surface area so that better wettability and improvement in solubility. In future, from the I*n-vivo* study on can prove the increased in bioavailability, which ultimately leads to reduce dose frequency and patient compliance.

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Conflict of interest

Authors have no conflict of interest regarding this research work.

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