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Formulation and Evaluation of Febuxostat Nanoparticles

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ABSTRACT

Drug delivery via nanoparticle-based carriers has shown promising results for different types of diseases. Delivery of anti-gout agents into body is a mature line of investigation that has yet to realize its full potential. In this study we report on the development of a delivery platform for febuxostat. The work presented here describes the development of nanoparticles based on compritol. The method employed for the preparation of SLN is micro emulsification technique followed by high pressure homogenization. Phase diagram was developed to know the region of stable micro emulsion formation. Eight different formulas were developed with different concentrations of lipid, surfactant and aqueous concentrations. The developed formulations were evaluated for particle size distribution, zeta potential, entrapment efficiency, DSC thermal analysis and in vitro drug release studies. Among all the formulations developed, F3 formulation is showing better drug entrapment efficiency and controlled release of drug up to 24h. There was 12 fold increase in the solubility of drug in the developed formulation. The solid lipid nanoparticles of febuxostat can be prepared by using simple micro emulsification technique. The formulations had shown better release profile and solubility characteristics.

Keywords: Febuxostat, micro emulsion, nanoparticles, phase diagram, in vitro drug release studies

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INTRODUCTION

Solid lipid nanoparticles are one of the novel potential colloidal carrier systems as alternative materials to polymers which are identical to oil in water emulsion for parenteral nutrition, but the liquid lipid of the emulsion has been replaced by a solid lipid. Solid lipid nanoparticles (SLNs) are sub-micron colloidal carriers ranging from 50 to 1000 nm, which are composed of physiological lipid, dispersed in water or in aqueous surfactant solution. SLNs offer unique properties such as small size, large surface area, high drug loading and the interaction of phases at the interface and are attractive for their potential to improve performance of pharmaceuticals. They have many advantages such as good biocompatibility, low toxicity and lipophilic drugs are better delivered by solid lipid nanoparticles and the system is physically stable. In order to overcome the disadvantages associated with the liquid state of the oil droplets, the liquid lipid was replaced by a solid lipid, which eventually transformed into solid lipid nanoparticles. This is the one of the most popular approaches to improve the oral bioavailability of the poorly water soluble drugs.

A successful nanoparticulate system should have a high drug-loading capacity thereby reduce the quantity of matrix materials for administration Drug loading can be done by two methods:

- Incorporating at the time of nanoparticles production (incorporation method).
- Absorbing the drug after formation of nanoparticles by incubating the carrier with a concentrated drug solution (adsorption / absorption technique)

In the present study, Febuxostat [1] (FBX)was formulated in the form of solid lipid nano particles which is used as an anti gout agent.Gout is a condition which is characterized by repeated attacks of acute inflammatory arthritis, a red, tender, hot, swollen joint. In 50% of the cases the part of the body that is most affected is the metatarsal-phalangeal joint at the base of the big toe. Nevertheless, it may also present as tophi, kidney stones, or urate nephropathy. Other joints, such as the heels, knees, wrists and fingers, may also be affected. Gout is characterized by the increased levels of uric acid. The uric acid crystallizes, and the crystals deposit in joints, tendons, and surrounding tissues.

Febuxostat is a non-purine selective inhibitor of xanthine oxidase. It works by non-competitively blocking the molybdenum pterin center which is the active site on xanthine oxidase. Xanthine oxidase is needed to successively oxidize both hypoxanthine and xanthine to uric acid. Hence, febuxostat inhibits xanthine oxidase, therefore reducing production of uric acid. Febuxostat inhibits both oxidized as well as reduced form of xanthine oxidase because of which febuxostat

cannot be easily displaced from the molybdenum pterin site.

The main objective of the present work is to improve the bioavailability of febuxostat to improve absorption of the drug when compared topure drug/conventional dosage form and to alter the pharmacokinetics of drug substances in order to improve the therapeutic efficacy through the use of novel drug delivery system (solid lipid nanoparticles).

MATERIALS AND METHOD

Materials:

The drug Febuxostat was obtained from Emcure Pharma, (Pune) Compritol888 is obtained from Gattefosse, (Germany), stearic acid is from Fisher scientific, (Mumbai) Dialysis membrane-70 was purchased from Hi-Media (Mumbai). All other chemicals were of analytical reagent grade.

Methods:

Preparation of Febuxostat loaded SLN:

The method employed in the present study for the preparations of FBX loaded SLNs was micro emulsification[2]. This is a simple method in which the aqueous phase was heated to a temperature of lipid melt and the surfactants were incorporated into the corresponding phases. The drug is dispersed in the lipid phase and the aqueous phase is added at once to the lipid phase while stirring and it was continued till a clear primary micro emulsion was obtained. Then this primary emulsion was diluted with distilled water and sent for high pressure homogenization.

Selection of excipients and optimization of formulation and process parameters:

The choice of lipid was done on the basis of solubility and partitioning of FBX in the lipid.Aqueous phase surfactant and lipid phase surfactant were selected on the basis of HLB system and employing the solubility studies of the drug in pure lipid and aqueous systems individually and in the mixture of lipid with lipophilic surfactant as well as the aqueous phase with hydrophilic surfactant separately. The pseudo ternary phase diagram [3,4] was constructed for identifying the area in which a stable micro emulsion is possible. Based on the time of formation and stability of the micro emulsion the final set of formulations were ascertained by an array of experiments, carried out in anorganizedmanner, the set of experiments were also used to establish the optimum and desired formulation and process parameters for the development of FBX loaded solid lipid nanoparticles.

Preparation of FBX loaded SLN:

Micro emulsification followed by Homogenization is a dependable, simple and reproducible method for preparing SLN. In this study, compritol was used as solid lipid, and a mixture of

Glycerylmonostearate and Tween 80 as surfactant. It is known that the use of two surfactants, respectively of lipophilic and hydrophilic nature, yields a better stabilization of the disperse system. Aqueous phase consisting of hydrophilic surfactant is heated to the lipid melt temperature. On the other hand lipid is also melted and lipophilic surfactant was added to the melt. Then the hot aqueous emulsifier mix is added drop by drop to the lipid mix with constant stirring on a magnetic stirrer which is continued until a micro emulsion is formed. The hot micro emulsion is quenched by adding to cold water at 2^0C under mechanical stirrer (5000 rpm) and the stirring is continued further for 1.5 h. The prepared dispersion was subjected to high pressure homogenization up to 5 cycles at 15000 psi. Thus the resulting dispersion contains particles in nanosize.

To optimize the homogenization process [5,6] all the developed formulations were homogenized for 5 cycles. Homogenization of the micro emulsion for 5 cycles resulted in particle size between 245 and 250 nm with narrow size distribution. The final composition of the investigated SLNs dispersions was shown in Table 1.

Evaluation of solid lipid nanoparticles:

Determination of particle size and zeta potential of SLN:

The size and zeta potential of SLNs were measured by photon correlation spectroscopy using a Zetasizer 3000 HSA (Malvern, UK). Samples were diluted appropriately with the aqueous phase of the formulation. Zeta potential measurements were carried out at 25° degree C.

Determination of entrapment efficiency:

Entrapment efficiency (EE %) was determined by measuring the concentration of free drug (unentrapped) in aqueous medium. The aqueous medium was separated by ultra-centrifugationat 9000 rpm for 1Hr at room temperature using Remi RL 12C BL, and then the supernatant and the sediment were separated. The amount of FBX in the aqueous phase was estimated by UV Spectroscopic method and the entrapment efficiency was calculated by the equation:

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Amount of total drug - amount of drug in aqueous phase}}{\text{Amount of total drug}} * 100$$

$$\%\ L = \frac{\text{Amount of total drug – amount of drug in the aqueous phase} * 100}{[(\text{Amount of total drug – amount of drug in the aqueous phase}) + \text{Amount of the lipid}]}$$

Evaluating the effect of change in Dispersion media volume for the SLN's on the % Entrapment Efficiency& % loading [7]

The final two batches of the SLN i.e. F2 & F3 were dispersed in different volumes of the media with the following ratios; 1:5, 1:10, 1:25, 1:45 respectively and tested for the % EE and % L.

Determination of drug content (Assay):

For estimation of assay, SLNs equivalent to 40 mg of the drug were taken further diluted with methanol: buffer (1:1). FBX content was determined by UV Spectrophotometric method.

Drug Solubility Enhancement:

10 mg of drug was added in each of the system respectively – the aqueous phase, the lipid phase and the primary micro emulsion, using a magnetic stirrer with the heater for 6 hours. Aliquots were taken from each and diluted as required. Samples were analyzed using UV spectrophotometer. Results were shown in figure 2.

***In-vitro* drug release studies**

The *in-vitro* dissolution studies were performed to ensure the desired drug release from the formulation in the dissolution medium. A Hi-media dialysis membrane 70 was used. 5 ml of the drug was filled in the dialysis bag, and is placed in 900ml 6.8 phosphate buffer. Each time 5ml aliquots were removed at each time interval and fresh medium was replaced to maintain constant volume. Aliquots were taken at suitable time intervals up to 24 hours and were analyzed using UV spectrometer.

Differential scanning calorimetry (DSC):

Thermal characteristics of the drug, compritol 888, FBX-SLNs were studied using a differential scanning calorimeter. DSC thermograms of the drug, polymer, FBX-SLNs were recorded using DSC (Perkin Elmer). Samples were analyzed in crimped aluminum pans and heated from 30-250° degree C at a linear heating rate of 10° degree C min^{-1}.

RESULTS AND DISCUSSION

Selection of excipients and optimization of formulation and process parameters:

On the basis of the results obtained in the preliminary screening studies, compritol 888, glyceryl monostearate, tween 80 were chosen as lipid, lipid phase surfactant and aqueous phase surfactant respectively for further study. In order to optimize the lipid, surfactant and aqueous phase ratio, a pseudo ternary phase diagram was developed as shown in Figure 1. A set of trials were taken from the knowledge of ternary diagram as showed in table 1 by using micro emulsification technique followed by 5 cycles of high pressure homogenization. Among these trials the formulas F1, F2, F3 and F8 were able to sustain the drug release up to 24 hr. though the formulations F1 and F8 had shown the release profile similar to F2 and F3, but these formulas were not stable enough for further studies which was depicted in their drug loading and entrapment efficiency. The reasons attributed for the instabilities could be the concentration of

emulsifiers.

Entrapment efficiency:

Entrapment efficiency of SLN formulations are shown in Table 2. Among the SLN formulations highest entrapment efficiency (99.99 ±2.08 %) was observed with formulation F7, whereas formulation F1 showed lowest entrapment efficiency (75.55±8.38%). The concentration of compritol used in F1 and F7 are 8.5 & 2.5 respectively. Because of very high and very low composition of lipid the formulations became unstable and had shown phase separation on storage.

Effect of dispersion medium volume:

To study the effect of volume of dispersion medium added, the micro emulsion is added with different volumes of dispersion medium (distilled water) and the homogenization on mechanical stirrer was done for 1.5 h. it was shown (Figure 3) that as we keep on increasing the volume of dispersion medium from 1:2, 1:5, 1:10, 1:25, 1:50 the entrapment efficiency and drug loading abilities were decreased. The best among different ratios were found to be 1:5. In case of 1:2 ratio the slurry became very viscous and could not able to form a uniform dispersion.

In Vitro drug release Study:

The in vitro drug release profiles of FBX-loaded SLNs with different formulations were shown in Figure 4. In order to evaluate the controlled release potential of the investigated formulations, the release of FBX from the lipid particles was investigated over 24h. Cumulative percent of drug release from the formulations F1 to F8 showed drug release ranging from16.9 to 92.7% in phosphate buffer of pH 6.8; the drug release from F1 to F4 are 91.8, 92, 92.7, 31.9% respectively and the drug release from F5 to F8 formulations was found to be 35.6, 25.8, 16.9, 90.2% respectively.

The drug release from the formulae F1, F2, F3 and F8 are very similar. In case of F1 there was phase separation problem because of lower surfactant concentration, and in F8 the entrapment efficiency was 86.85% which was less compared to that of F2 and F3 whose entrapment efficiency is 94.29 and 95% respectively.

These results imply that the release behavior was governed by the balance between the concentrations of the aqueous phase, surfactant and the lipid phase in the formulation.

The amount and type of lipid and emulsifier affects the particle size, drug loading capacity and the stability of the formulation. The amount of lipid ought to be optimum to encapsulate maximum amount of the drug and also should turn out minimum size lipid particles with narrow size distribution. The quantity of emulsifier should be optimum to cover the surface of the

nanoparticles effectively and prevent agglomeration during the homogenization process, consequently favor the formation of SLN with smaller particle size and also be a factor to prevent decrease in the entrapment efficiency.

In presentinvestigation FBX-SLN dispersion of optimized formulation (F3) showed significantly controlled release of febuxostat (92.7%) and enhanced solubility (12fold) of the drug than dispersion of pure drug (19%) over a period of 24hr. The drug release data of most of the SLN formulations (F2, F3, and F8) were subjected to pharmacokinetic treatment. All the formulations showed the release pattern following the zero order kinetics (r2 values were 0.904, 0.904, and 0.88 respectively). The 'n' value in peppas equation for all the formulations is above 1 indicates the release of drug from formulations was by super case II type where the release mechanism depends purely on the erosion from the matrix system.

Measurement of size and zeta potential:

Based on Entrapment efficiency values and in vitro drug release studies, the formulation F3 was analyzed to determine the particle size distribution and zeta potential values. In this formulation the particle size was found to be in the range of 245.1±6.12 nm, and zeta potentials were in the range of 45.4±4.72 shown in figure 5.

Zeta potential is a key factor to evaluate the stability of colloidal dispersion. It was currently admitted that zeta potentials above 30mV were required for full electrostatic stabilization. In the present studies, the zeta potential values obtained were sufficient to make the SLNs system stable.

DSC Analysis:

DSC thermograms of FBX, compritol, SLNs are shown in figure 6. The melting endotherm of the drug was completely absent in the thermograms of FBX loaded SLN, which indicates that FBX was completely solubilized inside the lipid matrix of the SLN. Compritol showed a sharp endothermic event, ascribing to the melting, around 58.68° degree C (minimum) with an extrapolated onset of the melting peak 70° degree C. The endothermic peak of febuxostat was totally absent in the thermogram of SLNs (F3) indicated the complete entrapment of drug in the lipid matrix.

Table 1: Composition of lipid, surfactant and water of FBX loaded SLNs

Formula	Compritol 888 (gm)	Drug (mg)	Emulsifiers (gm)		Water (gm)
			Tween 80	GMS	
F1	8.5	300	0.4	0.1	1.0
F2	7.5	300	1.2	0.3	1.0
F3	6.5	300	2.0	0.5	1.0

F4	5.5	300	2.8	0.7	1.0
F5	4.5	300	2.8	0.7	2.0
F6	3.5	300	2.8	0.7	3.0
F7	2.5	300	2.8	0.7	4.0
F8	6.5	300	1.2	0.3	2.0

Table 2: EE, % Loading and % drug release from different SLNs of FBX prepared

Formulae	Observation for stability of SLN	% Entrapment Efficiency (EE) (n = 2)	% Loading (L) (n = 2)	Drug release after 24 hr
F1	Phase separation	75.55	3.17	91.8
F2	Stable	94.29	3.48	92
F3	Stable	95.00	3.90	92.7
F4	Stable	90.48	2.48	31.9
F5	Stable	97.34	2.10	35.6
F6	Stable	97.36	1.76	25.8
F7	Phase separation	99.99	1.83	16.9
F8	Stable	86.85	3.28	90.2

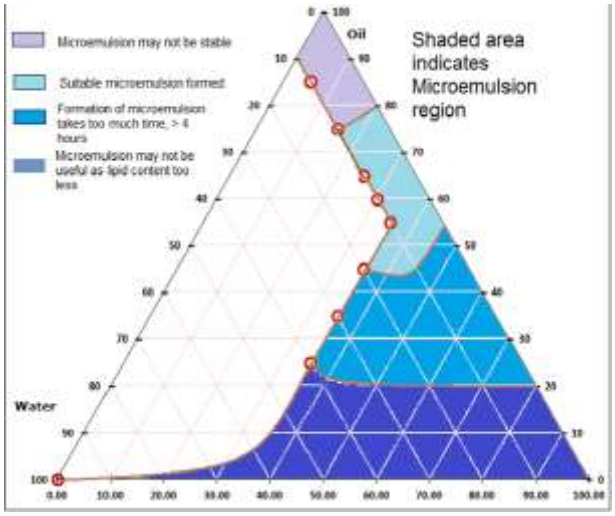

Figure 1: Phase diagram of water surfactant and compritol system

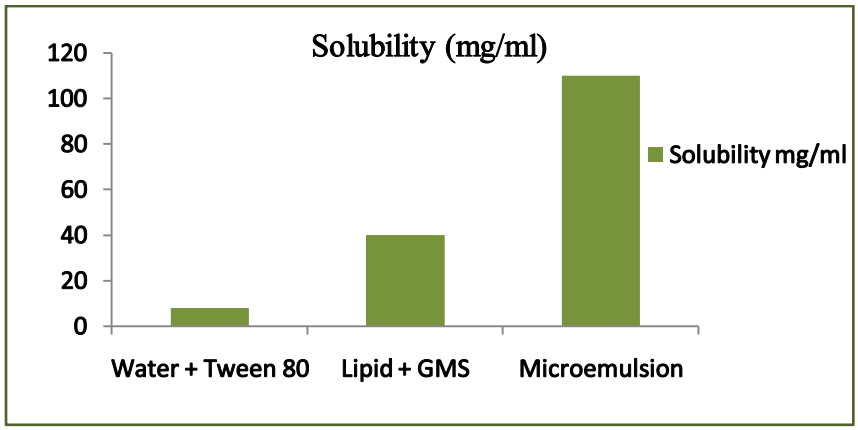

Figure 2: Solubility of Febuxostat in different media

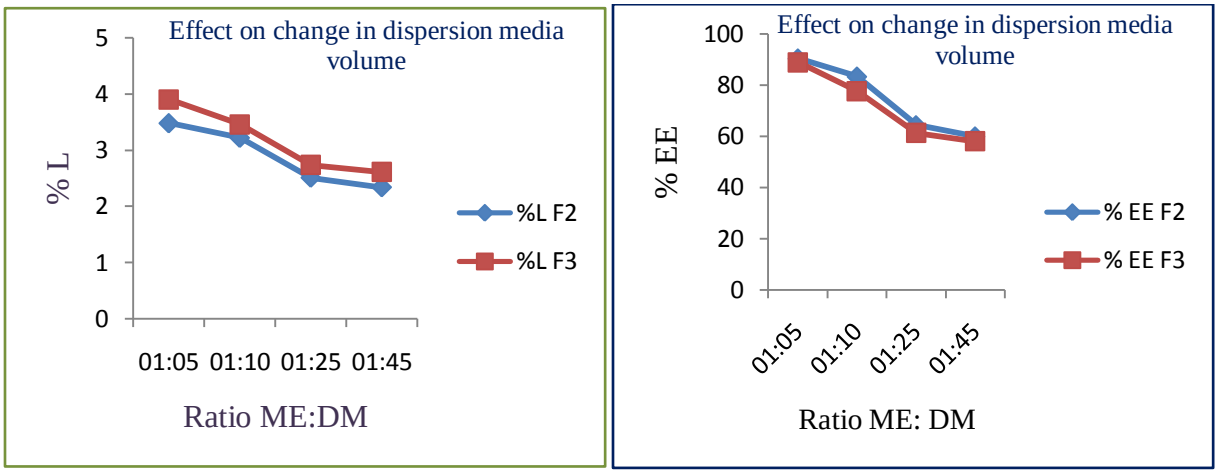

Figure 3: Effect of volume of dispersion media

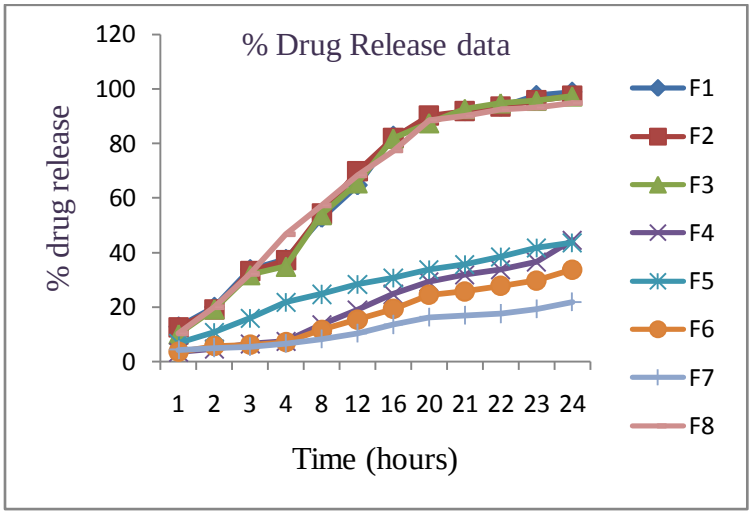

Figure 4: Percent Febuxostat release from various formulations

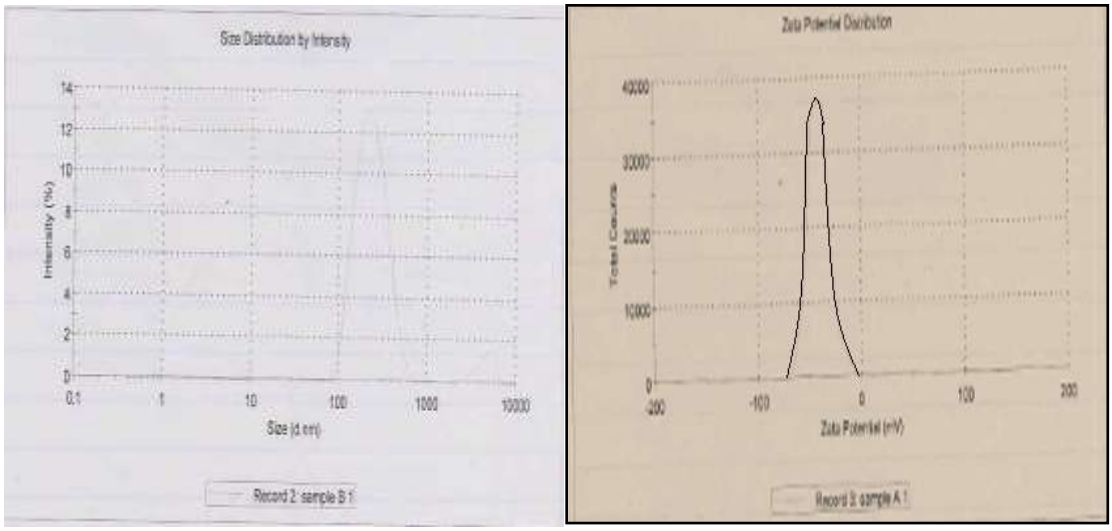

Figure 5: Particle size and Zeta potential for F3 formulation

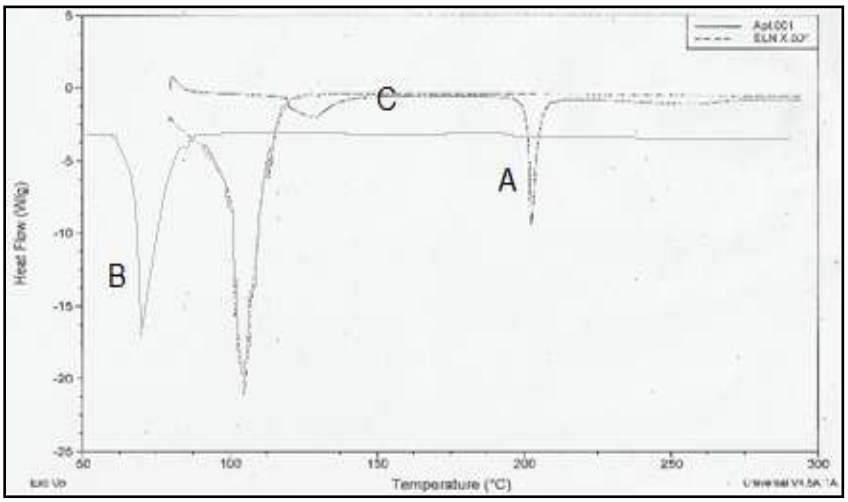

Figure 6: DSC thermograms of Pure Febuxostat, Compritol and F3 formulation

Entrapment efficiency:

Entrapment efficiency was calculated for all the formulations prepared. By comparing the observations of entrapment efficiency and % drug loading F2 and F3 formulations had shown good entrapment efficiencies in comparison with other formulations. This could be a reason for uniform drug release from these formulations.

In Vitro drug release Study:

Based on the in vitro drug release studies conducted in phosphate buffer of pH 6.8 the formulas F2 and F3 were found to sustain the release of drug up to 24 hr with uniform release at unit time intervals. without any phase separation and entrapment efficiency issues like in F1 and F8.

Effect of dispersion medium volume:

The effect of dispersion medium was done by using 1:2, 1:5, 1:10, 1:25, 1:50 ratio of micro emulsion and dispersion medium. It was clearly showed by the entrapment efficiency that the best ratio was 1:5.

Measurement of size and zeta potential:

The particle size distribution (245±6.12nm) and zeta potential (45.4±4.72) studies shown that the formulation F3 had the SLNs in acceptable range.

CONCLUSION:

Micro emulsification followed by high pressure homogenization method is suitable to produce SLN of around 250 nm size. Lipophilic drugs like FBX can be successfully loaded with compritol, and nonionic surfactants like tween 80. The entrapment efficiency and the drug release profile depend on the concentration of lipid and surfactant mixture employed. The drug release rate decreases for SLN with a higher aqueous phase concentration. DSC analysis showed

the complete entrapment of febuxostat in the prepared formulations. The results of the in-vitro drug release studies demonstrated significantly controlled release of FBX (92%) from FBX-SLN. All the formulations showed the release pattern following the zero order kinetics (r2 values were 0.904, 0.904, and 0.88 respectively). The 'n' value in peppas equation (1.198) indicates the erosion type of drug release from the developed formulations.

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