Design and optimization of febuxostat loaded nano lipid carriers using full factorial design

shailendra bhatt¹, ruchi kamboj¹, JAI BHARTI SHARMA¹, Manish Kumar¹, vipin saini², SHAIENDRA mandge³

¹MM college of pharmacy, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala, Haryana, India

²Maharishi Markandeshwar University, Solan, HP, India

³Pacific University, Udaipur, Rajasthan, India

INTRODUCTION: The main objective of this work was to develop nanostructured lipid carriers to improve the oral bioavailability of Febuxostat.

METHODS: The well known technique high shear homogenization followed by bath sonication with slight modifications was used to prepare Febuxostat loaded nanostructured lipid carriers using oleic acid and stearic acid as liquid lipid and solid lipid respectively. A total 3² full factorial design was employed to examine the effect of two independent variables namely X1 (liquid lipid to solid lipid ratio) and X2 (concentration of surfactant) on the Y1 (particle size) and Y2 (% entrapment efficiency) of drug. The prepared nanostructured lipid carriers were evaluated for particle size, polydispersity index, zeta potential and (%) entrapment efficiency.

RESULTS: The highest solubility of the drug was found in the stearic acid (solid lipid) and oleic acid (liquid lipid), which were further chosen for the preparation of nano lipid carriers. The result of present study showed increase and decrease in entrapment efficiency and particle size respectively with the increase in liquid lipid to solid lipid ratio. The small particle size observed with the increased concentration of surfactant. The particle size and (%) entrapment efficiency of optimized formulation was found to be 99 nm and 80% respectively. Zeta potential and polydispersity index of all the formulations were found within the range. The optimized formulation showed higher drug release compared to plain drug suspension, this may be due to the presence of the higher amount of liquid lipid. TEM study showed that the particles are round in shape and have smooth surface. Stability studies showed that the NLC formulation can be stored for a longer period of time under the room condition.

DISCUSSION AND CONCLUSION: Febuxostat loaded NLC were successfully prepared using full 3² factorial design and further can be used for oral delivery of Febuxostat for the treatment of gout.

Keywords: Febuxostat, Nanostructured Lipid Carriers, Oral Drug Delivery, Oleic Acid.

Shailendra Bhatt, MM college of pharmacy, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala, Haryana, India

Shailendra Bhatt

shailu.bhatt@gmail.com

0000-0001-9405-3311

8107663903

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INTRODUCTION

The oral route is the most convenient route for drug administration as it offers multiple selectivities of dosage forms and high patient compliance.¹ Not just the solubility but also the other inherent characteristics of the drugs, like physiological milieu in the gastrointestinal tract (GIT) which includes pH value, and bile salts, slow down the absolute absorption of the drug.^{2,3}

In the mid-'90s, Professor R.H. Müller (Germany) and Professor M. Gasco (Italy) started to research on the capability of lipid-based drug delivery system i.e. solid lipid nanoparticles (SLN).⁴ But the significant disadvantage of such dosage form was the drug loading. The incorporation of a liquid lipid in the preparation of nanoparticle helps more drug to incorporate in the nanoparticle and enhance the physical stability of the nanocarriers. This liquid lipid containing nanoparticles was further named nanostructured lipid carriers (NLC).^{5,6} NLC are made up of biocompatible solid lipids and liquid lipids.⁷ NLC are better than other colloidal carrier because the drug loading capacity of NLC is better than other carriers and thus, have been investigated to more extent in pharmaceutical modernization.⁸ Nanostructured lipid carriers are also helpful in other routes of administration like dermal, oral, ocular and pulmonary, in topical transporter, NLC shows high drug release, drug targeting, proper absorption of lipophilic and hydrophilic drug molecules.^{9,10}

Gout, one of the most common crystal-induced arthropathies and, is caused due to the inflammation of arthritis. The other problems that arise by gout are metabolic syndrome, renal disease, and cardiovascular disease.¹¹ Hyperuricemia is an abnormality, mostly found in children and adolescents. Even the pediatricians cannot measure the level of uric acid in children because of its low concentration in the serum. According to a recent study, hyperuricemia occurs due to obesity and non-communicable diseases like cardiovascular disorders. More attention has been given to hyperuricemia in children and adolescents.^{12,13}

Febuxostat (FEB) is another, powerful, non-purine particular inhibitor of xanthine oxidase, which is utilized to treat hyperuricemia in grown-ups with gout.¹⁴ FEB is BCS class II drug and in acidic (pKa~3.08) nature.¹⁵ According to the FDA, Febuxostat tablets show low oral bioavailability of 49.9% which is due to its low water solubility and low enzymatic degradation.¹⁶ The aim of this research was to develop the Febuxostat loaded NLC formulation using high shear homogenization technique followed by bath sonication method.

MATERIALS

Febuxostat was received as a gift sample from Arbindo pharmaceuticals, Dehradun, India. Oleic acid, Methanol AR and Sodium hydroxide pellets were purchased from Qualikems fine chemicals, Mumbai, India. Disodium hydrogen phosphate and potassium dihydrogen phosphate were purchased from S.D Fine chemicals, Ltd, Mumbai, India. Stearic acid and Tween 80 were purchased from Central drug house Ltd. Vardaan house, Daryaganj, New Delhi India. All the ingredients were of analytical grade.

METHODS

Selection of lipids

Solid and liquid lipid was selected on the basis of extreme solubility of the drug in various lipids.¹⁷ The solid lipid was selected on the basis of solubility studies of the drug in stearic acid, glyceryl monostearate, cetyl alcohol, palmitic acid and liquid lipid was examined in castor oil,

Isopropyl myristate and oleic acid. The solid lipid was accurately weighed and heated to 70°C in glass vials. To these vials, 10 mg of Febuxostat was added with continuous stirring. The mixture was cooled to room temperature and examined for precipitation microscopically. The solid lipids that did not show any precipitation were selected for further studies.¹⁸

Formulation of Febuxostat loaded NLC

Design of the experiment

A full 3² factorial design was utilized to study the effect of two independent variables X₁ (liquid lipid to solid lipid concentration) and X₂ (concentration of surfactant) on the particle size (Y₁) and entrapment efficiency (Y₂) of the drug. Table 1 represents the 3² factorial design along with factors and levels.

Preparation of Febuxostat loaded NLC

The NLC was prepared by high shear homogenization followed by bath sonication with slight modifications.⁸ The lipid phase was melted in a water bath at 85°C. Then the drug was added to melted lipid. Both the lipid phase and the aqueous phase were prepared separately. The surfactant solution or aqueous phase was prepared by adding Tween 80 in water. A preheated surfactant solution was added to the melted lipid to form a suspension. By high shear homogenizer (Remi motors Ltd, Mumbai), it was further homogenized for 15 min at 12000 rpm. For uniform size distribution, all the formulations were subjected to bath sonication (Hwashin technology SEOUL, Korea) for 5 min.¹⁹ Thereafter, the dispersion was centrifuged at 10,000 rpm at 10°C for 60 min in cooling centrifuge. The sedimented soft pellet was separated from supernatant and resuspended in 20 ml of distilled water containing 2-3% tween 80 as stabilizer with stirring for 10 minutes and again subjected to ultrasonication for 1 min to get the desired particle size.

Evaluation of Febuxostat loaded NLC

Particle size

The particle size and zeta potential were measured after proper dilution with distilled water. The scattering angle was fixed at 173°C and temperature was maintained at 25°C.

Entrapment efficiency

The formulation was centrifuged at 10,000 rpm and the supernatant was collected which is further used to calculate the entrapment efficiency (EE). Then dilution was made up to 10ml with PBS pH 7.4 and Febuxostat content was determined using a UV spectrophotometer at 315 nm. The entrapment efficiency of the drug was calculated as follows:⁸

$$(\%) \text{ Entrapment efficiency} = \frac{\text{Total amount of drug} - \text{drug in supernatant}}{\text{Total amount of drug}} \times 100$$

Optimization of formulation

The optimization was done using the design master. The optimized formulation was selected on the basis of the highest (%) entrapment efficiency and minimum particle size.

Interaction between the factors

The one way ANOVA was used to statistically evaluate all the results. The 'p' value gives the impact of different independent variables on dependent responses like entrapment efficiency and particle size. Further, by omitting non-significant terms (p > 0.05) from the full polynomial model, the reduced model was generated. The effect of independent variables on the responses was evaluated using the reduced polynomial model.

In-vitro drug release studies

The *in vitro* drug release study of a plain drug suspension and prepared NLC was carried out as per the previously published method with slight modification using the dialysis sac method.¹⁹ An

accurately measured amount of plain drug solution and prepared NLC equivalent to 5 mg of FEB were introduced into the sac and both the ends of the sac were properly tied with the help of thread. The sac was hanged in 200 ml of phosphate buffer pH 7.4 with 1% polysorbate 80 and kept on a magnetic stirrer. The receptor compartment temperature was maintained at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ at a predetermined time, aliquots of 5ml were withdrawn with the help of pipette and replace with fresh buffer at each time. The sample was filtered using a membrane filter ($0.45\mu\text{m}$) and analyzed spectrophotometrically at 315nm. The same procedure was repeated for blank solutions.

Characterization of optimized Febuxostat loaded NLC

Surface morphology study

Transmission Electron Microscopy (TEM) (Philip Tecnai – 20, USA) with an accelerating voltage of 120.0 kv was used to study the surface morphology of optimized formulations. The NLC dispersion was prepared in distilled water and a drop of dispersion was placed on the carbon-coated copper grid followed by drying.

Stability study

According to ICH regulations, Freeze-dried optimized formulation was used to carry out stability studies. In order to check the stability of these samples, they were stored in vials for three months and kept at atmospheric conditions of $25 \pm 2^{\circ}\text{C}/60 \pm 5\% \text{RH}$ and $40 \pm 2^{\circ}\text{C}/75 \pm 5\% \text{RH}$ in stability chamber (Macro scientific work Pvt Ltd, Delhi, India). Also, at specified intervals, like 0, 15, 30, 60, and 90 days, physical appearance and entrapment efficiency of these samples were determined.

RESULTS AND DISCUSSION

Selection of solid and liquid lipid

The drug solubility was found to highest in stearic acid and oleic acid, solid and liquid lipid respectively than compared to other lipids. As they did not show precipitation in the solubility studies of Febuxostat, thus selected for the preparation of NLC.

Evaluation of optimized NLC

Zeta potential and polydispersity index

The zeta potential evaluates the stability of colloidal dispersion. It measures the degree of repulsion between similarly charged particles to prevent the aggregation of particles. The polydispersity is ranging from 0 to 1 and its index is the measurement of width of the particle size distribution. The index near 0 has narrow size distribution. The mean polydispersity index value and zeta potential of drug-loaded NLC formulations F₁ to F₁₂ varied in the range of 0.135 to 0.452 and -12.3 to -28.3mv respectively (Table 1). The optimized formulation F₈ showed zeta potential of -17.9 mv (Figure 1). From previous studies it was found that only the electrostatic repulsion is not responsible for the stability of nanoparticles but to formulate stable nanoparticles, steric stabilizer also has a significant effect. The high concentration of surfactant compensates missing electrostatic repulsion and stabilizes the dispersion for a long duration.²⁰ The high concentration of tween 80 provides the steric stability to NLC.

Optimization of formulation

Entrapment efficiency

The entrapment efficiency of all the NLC formulations was remarkably increased from was 65.2 ± 1.25 to $80 \pm 2.27\%$ (Table 1). For optimized formulation, EE was found to be 80%. Incorporation of liquid lipids to solid lipids results in the more drug to entrap in the NLC.²¹ The polynomial equation of the full model for EE was obtained by regression analysis. The full model is as follows:

$$Y_1 = 74.23 + 5.70X_1 + 1.83X_2 - 0.9375X_1^2 + 0.2625X_2^2 - 0.7750X_1X_2$$

The Non-significant terms from the full model were removed ($P > 0.05$) to obtain a reduced model as follows:

$$Y_2 = 74.23 + 5.70X_1 + 1.83X_2 - 0.9375X_1^2 + 0.2625X_2^2$$

The higher positive coefficient in the equation indicates the raising variable increasing the response and effect of factor on the response. Based on the P-value X_1 , X_2 , X_1X_2 and X_1^2 factors were found to be significant. For the given model, the calculated F value was found very low than the tabular F value ($\alpha = 0.05$, 2), which clearly demonstrates that the omitted terms do not significantly affect the prediction of EE. From the study, it was found that by increasing the surfactant concentration and liquid lipid to solid lipid ratio, entrapment efficiency increases significantly. The high solubility of drug in the melted lipids and more space provided for the accommodation of the drug resulted in high entrapment efficiency. The high proportion of liquid lipid helps in increasing the drug solubility in the lipid matrix. Hence, high % EE was observed. The 3-D surface response plot for % entrapment efficiency is shown in figure 2.

Particle size

The particle size measurement was required to confirm the nano-range of the produced particles and presented as z-average diameter. The particle size of all the formulations was in the range of 99-229nm (Table 1). The formulation F₈ showed considerably smaller mean particle size of 99nm. From the data obtained from the experimental design, it was confirmed that the model is significant. The polynomial equation of the full model for the particle size was obtained by regression analysis. The full model is as follows:

$$Y_2 = 179.58 - 53.17X_1 - 15.17X_2 - 13.25X_1^2 - 8.25X_2^2 + 3.75X_1X_2$$

The Non-significant terms from the full model were removed ($P > 0.05$) to obtain a reduced model as follows:

$$Y_2 = 179.58 - 53.17X_1 - 15.17X_2 - 13.25X_1^2 - 8.25X_2^2$$

Based on the P-value X_1 , X_2 , X_1^2 and X_2^2 factors were found to be significant. For the given model, the calculated F value was found very low than the tabular F value ($\alpha = 0.05$, 2), which clearly demonstrates that the omitted terms do not significantly affect the prediction of particle size. From the above study it was found that when the liquid lipid to solid lipid ratio increases, particle size decreases.^{22,23} The concentration of surfactant (X_2) is also a critical factor that significantly affects the particle size. The increase in the surfactant concentration in NLC formulation reduces the interfacial tension between the lipid and dispersion. Thus NLC with smaller particle size formed. The particle size of optimized formulation was found to be 99nm. The 3D surface response plot for particle size is shown in Figure 3.

Characterization of optimized Febuxostat loaded NLC

Surface morphology study

From TEM study it was found that NLC particles are spherical in shape and have a smooth surface (Figure 4). The shape of NLC is in correlation with the previous findings. TEM study also proved that the particle size of NLC is less than 100nm which is reliably equal to the size of particles calculated by Zetasizer.

In-vitro drug release

The percent cumulative drug release over a period of 12 hours was studied (Figure 5). The NLC showed a high burst release in the second hour. As compared to a plain drug suspension, optimized formulation (F₈) showed enhancement in drug release and it is due to the presence of a higher amount of liquid lipid. More liquid lipid stays at the outer shell of nanoparticles outside shell of particles become delicate and shown essentially greater solubility for hydrophobic drugs

which gives burst release in drug profile. The drug release was then fitted into the zero-order, first-order and Higuchi kinetic model as shown in figure 6. The drug release characteristics in NLC were best fitted to the Higuchi model. The correlation was determined for kinetics models. The value of R^2 as shown in table 2 indicates the drug release characteristics were best fitted to Higuchi kinetic model.

Stability study

This table provides data for the stability of the optimized formulation of (F₈). No change in physical appearance was observed at given stability conditions. It was also observed that there is a slight decrease in the entrapment efficiency in accelerated conditions. The results show that storage of lipid-based formulation is not suitable under the accelerated temperature, as under this condition or temperature the drug starts degrading. Thus, with the help of this table, it can be concluded that the NLC formulated can be stored for a longer period of time under the room condition rather than the accelerated condition. The data for the stability study are shown in table 3.

CONCLUSION

The NLC was successfully prepared and optimized using high shear homogenization followed by bath sonication technique. A full 3^2 factorial design was utilized to study the effect of liquid lipid to solid lipid ratio and surfactant concentration on the particle size and entrapment efficiency of the drug. Based on the solubility studies, stearic acid and oleic acid were selected as the solid and liquid lipid respectively and tween 80 was used as the surfactant. From the above study, it was found that both liquid lipid to solid lipid ratio and surfactant concentration significantly affect the particle size and (%) entrapment efficiency. The entrapment efficiency was found to be increased by increasing the surfactant concentration and liquid lipid to solid lipid ratio. The high % EE resulted from the high proportion of liquid lipid, which helps in increasing the drug solubility in the lipid matrix. The increase in surfactant reduces the interfacial tension between the lipid and dispersion resulted in a decrease in Particle size. The zeta potential and polydispersity index of all the formulation were within range. The high concentration of tween 80 compensates missing electrostatic repulsion and provides the steric stability to NLC. The TEM images showed the spherical globules nano-sized particles with a smooth surface area and particle size of NLC is obtained less than 100nm which is in good agreement with zetasizer results. *In-vitro* drug release study showed the sustained release of drug up-to 12 hours. Stability study showed that the formulation possesses good stability at room temperature where the accelerated temperature is not an appropriate storage condition for lipid-based formulation. Febuxostat loaded NLC were successfully prepared and can be used for oral delivery of FEB in the treatment of gout.

CONFLICT OF INTEREST

No conflict of interest declared by authors

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Table 1. Experimental design for Febuxostat loaded NLC

Formulation code	X ₁	X ₂	Zeta potential	Polydispersity Index	Particle size	Entrapment efficiency
F ₁	0	1	-15.1	0.144	145	76.8±2.13
F ₂	-1	1	-18.7	0.152	198	70.3±2.42
F ₃	0	-1	-25.9	0.412	189	72.9±2.76
F ₄	0	0	-12.3	0.136	182	74.2±1.89
F ₅	1	0	-14.8	0.131	109	79.5±2.57
F ₆	-1	0	-20.7	0.346	215	67.8±1.77
F ₇	0	0	-21.2	0.128	182	74±1.94
F ₈	1	1	-17.9	0.187	99	80±1.58
F ₉	0	0	-27.3	0.254	182	73.9±2.63
F ₁₀	1	-1	-12.9	0.437	115	78±2.54
F ₁₁	0	0	-25.5	0.135	181	74.1±1.87
F ₁₂	-1	-1	-28.3	0.452	229	65.2±2.11
X ₁ = liquid lipid to solid lipid ratio (-1= 1:9, 0 = 5:5, +1= 9:1) X ₂ = concentration of surfactant (-1= 3%, 0 = 4%, +1= 5%)						

Table 2. Slope and R² values of drug release kinetics by various models

S. No.	Model	Slope	R ²
1.	Zero Order	6.570	0.973
2.	First Order	-0.069	0.95

3.	Higuchi equation	0.038	0.978
4.	Korsemeyer Peppas equation	1.089	0.638

Table 3. Stability study data for optimized formulation (F₈)

		25 °C ± 2 °C/60% ± 5% RH		40 °C ± 2 °C/75% ± 5% RH	
S.N.O	Time(Days)	Physical Appearance	Entrapment efficiency ± SD (%)	Physical appearance	Entrapment efficiency ± SD (%)
1	0	White colored suspension throughout the study	80±2.79	White suspension throughout the study	80±2.79
2	15		79.87±2.68		79.97±2.63
3	30		79.80±2.57		78.52±2.52
4	60		79.74±3.52		77.44±1.15
5	90		78.85±2.32		75.93±2.12

fig 1

System

Temperature (°C): 25.0

Count Rate (kcps): 190.6

Cell Description: Clear disposable zeta cell

Zeta Runs: 12

Measurement Position (mm): 2.00

Attenuator: 6

Results

	Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -17.9	Peak 1: -17.9	100.0	4.91
Zeta Deviation (mV): 4.91	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 0.0262	Peak 3: 0.00	0.0	0.00
Result quality : Good

fig 2

Design-Expert® Software
Trial Version
Factor Coding: Actual

EE (%)

● Design points above predicted value

○ Design points below predicted value

65.2 80

X1 = A: liquid to solid ratio

X2 = B: surfactant

Fig 3

Design-Expert® Software
Trial Version
Factor Coding: Actual

partical size (nm)

● Design points above predicted value

○ Design points below predicted value

99 229

X1 = A: liquid to solid ratio

X2 = B: surfactant

Fig 4

Fig 5

Fig 6