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Formulation and Characterization of Nifedipine loaded Nanostructured Lipid Carriers coated with Fenugreek Seed Polysaccharide

Dr. S. C. Arora[2], Anuradha Kush[1], Chahat Singla[1], Renu Rani[3]

Department of Pharmaceutics, R.K.S.D. College of Pharmacy, Ambala road, Kaithal, India

Abstract: ***Nanostructured lipid carriers (NLCs) are a recent approach for the delivery of poorly soluble drugs with low oral bioavailability. Nanostructured Lipid Carriers (NLCs) are mixture of solid lipids along with irreconcilable liquid lipids. The objective of the current study was to formulate and characterize Nifedipine loaded NLCs coated with Fenugreek Seed Polysaccharide for particle size, entrapment efficiency and in vitro drug release after 24h. NLCs of Nifedipine coated with Fenugreek Seed Polysaccharide were prepared by solvent injection method. A complete 2^3 factorial design was used for the evaluation of the prepared Nifedipine NLCs. The size and the morphology of the particles in suspension have been determined by electron microscopy studies and in vitro drug release studies by dialysis technique. Optimized formulation display 337nm average particle size and -52.4mV zeta potential that transmit good stability of NLCs dispersion. In vitro release study showed burst release for initial 0.5 h followed by sustained release up to 24h. SEM study confirmed smooth surface discrete spherical; nano-sized particles. These studies demonstrated that Nifedipine loaded NLCs coated with Fenugreek Seed Polysaccharide a promising method to improve the stability of NLCs.***

Keywords: Nanostructured lipid carriers (NLCs), Fenugreek Seed Polysaccharide, Physical stability, Particle size, In vitro release studies.

1. Introduction

The oral route is the most imperious route for administering varieties of drugs. It has been comprehensively used for both conventional and novel drug delivery systems. In recent decades, Lipid-based drug delivery systems are promising drug carriers due to their ability to improve solubility of poorly water-soluble drugs which eventually enhance the oral bioavailability [1].The first generation of lipid nanoparticle called solid lipid nanoparticles (SLNs), are composed of an aqueous dispersion nanoparticles with a solid lipid matrix that is stabilized by one or more of surfactant layer. However, SLNs has some limitations, such as drug loading insufficiency and drug expulsion during storage. Therefore, there is essentiality to create second generation of lipid nanoparticles, the nanostructured lipid carriers (NLCs). In contrast to SLNs, dispersions of NLCs are formed of a blend of solid lipid with liquid lipid, which provide a higher payload and prevent drug expulsion during storage [2]. Higher drug loading is replanted to the differences in the chemical structure between liquid and solid lipids result in distortion of a perfect crystal and accommodation of drug in molecular form or in amorphous clusters [3]. NLCs formulations offers advantages such as prolonged drug release, biocompatibility and easy of scaling-up its production [4]. Many natural polymers, such as Fenugreek Seed Polysaccharides, Black Gram Seed Polysaccharide and Tamarind Seed Polysaccharide have been applied to improve the performance of lipid nanoparticle [5,6]. In recent years, polymer-coated NLCs have been widely studied to optimize the performance of NLCs, including physical stability, entrapment efficiency and *in vitro* drug release of the drug [7]. For the preparation of polymer-coated NLC, polymers could be added in the system before the formation of NLCs through the coating process [8]. Fenugreek (*Trigonella foenum graecum*) is isolated from the ripe raw Fenugreek, used extensively as spice, herb, food and medicine [9]. It includes active ingredient like galactomannan [10]. Fenugreek galactomannan is present within the seed endosperm. It has moisture retention property which prevents seed from drying [11]. It is the important source of soluble dietary fiber (17–50%) of dry seed weight [12]. Due to the presence of high galactose content it shows more water solubility [13]. Fenugreek is already established as gelling agent, binder, disintegrate, suspending agent, matrix-former and release-retardant [14]. The aim of the present study was to develop Nifedipine loaded Nanostructured Lipid Carriers coated with Fenugreek Seed Polysaccharide to improve the physical stability of NLCs. Nifedipine, a lipophilic substance, was used as a model to investigate the preparation process and releasing mechanism. Nifedipine loaded NLCs were incorporated in Fenugreek Seed Polysaccharide was investigated through the study of entrapment efficiency, physical stability study and *in vitro* release study to evaluate the efficacy of this incorporation.

2. Materials and Methods

2.1 Materials

Glyceryl monosterate (GMS) and Nifedipine were purchased by Balaji Chemicals (Surat, India). Poloxamer 188 was purchased from Crystal Chemicals (Gangtok, India). Oleic acid was purchased by Nice Laboratory Reagents, (Kochi, India). Fenugreek Seed Polysaccharide was purchased from Local market (Kaithal, India).

2.2 Design of the experiment

A complete 2^3 factorial design was utilized to study the effect of three independent variables namely solid lipid, liquid lipid and polysaccharide on entrapment efficiency of drug in prepared formulations. Variables and levels used for optimization of Nifedipine loaded NLCs are shown in **Table 1**. Based on pre-formulation studies Glyceryl monosterate

(GMS), Oleic acid and Fenugreek Seed Polysaccharide were selected as solid lipid, liquid lipid and Polysaccharide, respectively.

Table 1: 2^3 Factorial design for preparation of Nifedipine NLCs

Factor	Low level (-)	High level (+)
Amount of Oleic acid (Liquid lipid)	10mg	20mg
Amount of GMS (Solid lipid)	100mg	200mg
Amount of Fenugreek Seed Polysaccharide	40mg	50mg

2.3 Preparation of Nifedipine loaded NLCs including Fenugreek Seed Polysaccharide

Nifedipine loaded NLCs were prepared by the solvent injection technique. To prepare the inner phase Nifedipine, Glyceryl monosterate and Oleic acid were dissolved in 4ml of isopropyl alcohol and heated at 60°C. The resulting solution injected rapidly in 20ml of aqueous phase containing specified amount of poloxamer 188 and Fenugreek Seed Polysaccharide. Then, it was continuously stirred at 400 rpm for 30 min on a magnetic stirrer and then 0.1 N HCL (8ml) was added to the dispersion. Thereafter, centrifugation was done at 3000 rpm for 30 min and 4% poloxamer 188 (by weight) in 10ml double distilled water was used to re-suspend the aggregates with stirring at 1000 rpm for 10 min. The formed NLCs were filtered, washed with distilled water and dried at room temperature and stored [15,16]. An overview of the composition of the NLCs is outlined in **Table 2**.

Table 2: Composition of the prepared Nifedipine NLCs dispersion

Batch code	Nifedipine	Poloxamer 188	Glyceryl monosterate	Fenugreek Seed Polysaccharide	Oleic acid
N_1	30mg	40mg	100mg	40mg	10mg
N_2	30mg	40mg	100mg	40mg	20mg
N_3	30mg	40mg	200mg	40mg	10mg
N_4	30mg	40mg	200mg	40mg	20mg
N_5	30mg	40mg	100mg	50mg	10mg
N_6	30mg	40mg	100mg	50mg	20mg
N_7	30mg	40mg	200mg	50mg	10mg
N_8	30mg	40mg	200mg	50mg	20mg

3. Characterization of Prepared Nanostructured lipid carriers (NLCs)

3.1 Surface and shape analysis by Scanning Electron Microscopy

The shape and surface characteristics of NLCs were analyzed by scanning electron microscopy (Meta-litesizer) operating at 10 kV. The samples were mounted on an aluminum stub with adhesive tape and excess samples were removed and coated with gold for 20 seconds. Metal stub was located in E-1010 Ion sputter for 20 minutes under vacuum. After 20 minutes samples were analyzed under scanning electron microscope [17,18].

3.2 Particle size and Zeta potential

The particle size and zeta potential of optimize NLCs formulation N_7 were measured by (Malvern Instruments) after suitable dilution with distilled water. Zeta potential is estimate of surface charge of dispersed particles in relation to dispersion medium. The experiment was executed using clear disposable zeta cell, water as dispersant which having refractive index (RI) -1.330 and viscosity (cps) -0.88 and the temperature was kept constant at 25°C[19,20].

3.3 Drug Entrapment Efficiency

Entrapment efficiency of the formulation was determined by diluting the formulation with 0.1 N HCl until pH of the formulation reached to 1.5 to 2. At this pH, formulation undergoes aggregation and separation. Formulation was centrifuged at 3000 rpm for 30 min. Supernatant was taken out, diluted with Phosphate Buffer 6.8 and quantified via UV Spectrophotometer [21, 22].

$$\% \text{ EE} = (W_{total} - W_{free}) / W_{total} \times 100$$

Where W_{total} is the total amount of drug in used in preparing formulation, W_{free} is the amount of the drug in supernatant.

3.4 *In vitro* drug release Studies

In vitro drug release from the Nanostructured lipid carriers was tested with dialysis technique. Dialysis bag of cellulose dialysis membrane was soaked in the distilled water overnight. 1ml of the preparations was placed in dialysis bag and sealed both ends with threads. *In vitro* drug release studies were carried out in Phosphate-buffered saline (PBS) pH 6.8 at 37°C on magnetic stirring moving at a speed of 50 rpm for 24hrs. Samples were drawn back at predetermined time intervals and replaced with fresh media. Samples were filtered and then analyzed using UV spectroscopy at λ_{max} of 223 nm [23,24].

3.5 Stability study

Freeze-dried optimized NLCs formulation N_7 was subjected to stability studies as per ICH guidelines. The samples were placed in vials and kept at 25±2 °C/60 ±5% RH and 4±2 °C C/75 ± 5% RH atmospheric conditions using stability chamber over period of three months. The samples were analyzed physical appearance at specified time intervals (0, 15, 30, 60, 90 days of storage). Cumulative drug release study was also switched out at the end of stability study for both storage conditions [25, 26].

4. Result and Discussion

4.1 Surface and shape analysis by Scanning Electron Microscopy

Surface and shape analysis of the optimized NLCs formulation N_7 captured by SEM was shown in **Figure 1**. The SEM image revealed that NLCs were of uniform, distinct, and spherical or oval shapes with little or no agglomeration indicating that prepared NLCs were homogeneously dispersed. The sizes of NLCs obtained by

SEM were in close agreement with the results obtained by a litesizer.

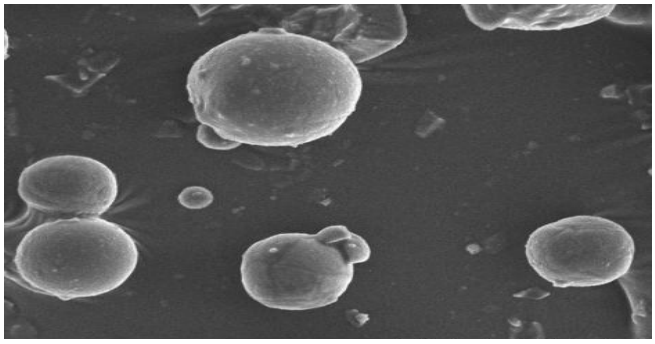

Figure 1: SEM photograph of Nifedipine loaded NLCs coated with Fenugreek Seed Polysaccharide

4.2 Particle size and zeta potential

The particle size of the optimized NLCs formulation N_7 showed considerably mean size of 337 nm as shown in **Figure 2**. Zeta potential is imperative for analyzing stability of colloidal dispersion during storage. The zeta potential of optimized formulation was found to be -52.4 mV as shown in **Figure 3**, which imparts good stability of NLCs dispersion.

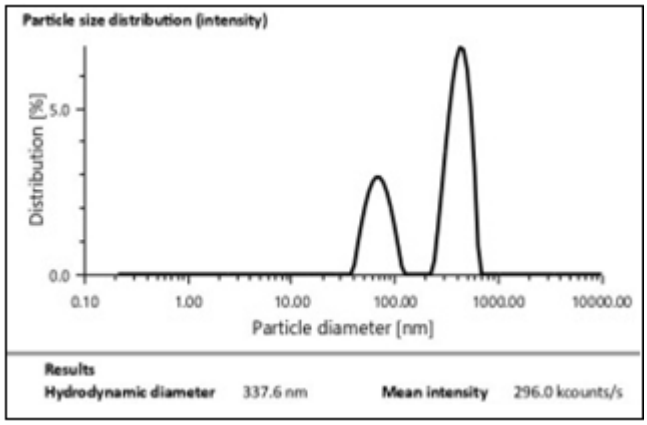

Figure 2: Particle Size Analysis of Nifedipine loaded NLCs coated with Fenugreek seed polysaccharide

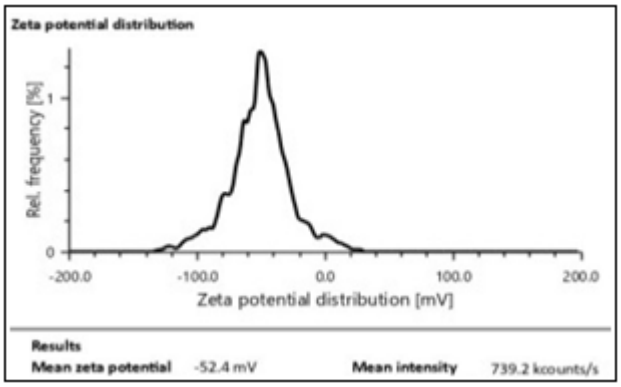

Figure 3: Particle Size Analysis of Nifedipine loaded NLCs coated with Fenugreek seed polysaccharide

4.3 Drug Entrapment Efficiency

The Nifedipine loaded NLCs using Fenugreek Seed Polysaccharide were formulated using different concentrations of solid lipid, liquid lipid and Fenugreek Seed Polysaccharide by the Solvent injection technique as shown in **Table 1**. Out of eight formulations (N_7) seems to exhibit good physical stability indicated by high entrapment efficiency value as shown in the **Table3**

Table 3: Entrapment Efficiency of NLCs

Batch Code	*Entrapment Efficiency (%)*
N_1	75.32
N_2	77.17
N_3	79.08
N_4	79.88
N_5	82.32
N_6	80.13
N_7	**86.88**
N_8	80.46

4.4 *In vitro* drug release Studies:

The *in vitro* drug release study was performed using dialysis technique in Phosphate Buffer pH 6.8. *In vitro* release profile of Nifedipine loaded NLCs formulations portrayed in **Figure 4** and showed burst drug release for initial 0.5h followed by slow and sustained release up to 24 h. However from the data, it was found that drug release profile of RLX was improving from formulations N_1 to N_8. The NLCs formulation containing 200mg of GMS, 10mg of Oleic acid and 50mg of Fenugreek Seed Polysaccharide (N_7) showed considerable improvement in release profile (92.95±0.31) compared to other NLCs formulations.

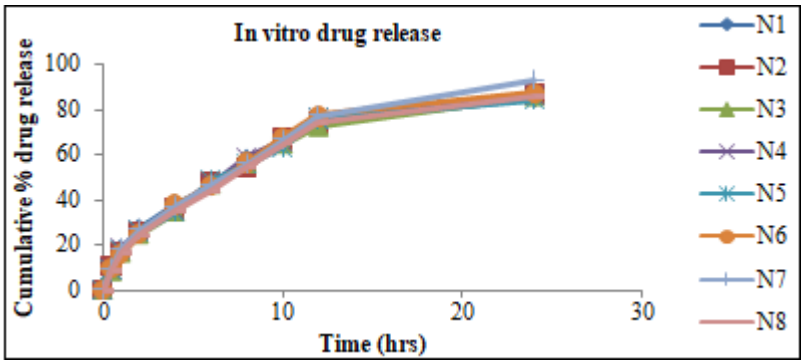

Figure 4: *In-vitro* Drug release profiles of NLCs

4.5 Stability study

The physical stability of Nifedipine loaded NLCs formulation (N_7) was intimated by visible phase separation and all dispersion remained in homogenous state upon storage at 4±1°C and room temperature (25± 2°C) for 90 days. The mean particle size of optimize NLCs formulation (N_7) after storage at both temperatures is shown in **Table 4**. Non-significant increase was observed in the particle size for the sample stored at 4±1°C over the monitored period while those stored at room temperature (25± 2°C) showed increase in the mean particle size. **Figure 5** shows the cumulative % drug release of the optimize NLCs formulation (N_7) after storage at 4±1°C and room temperature (25± 2°C). At the end of study, it was observed that cumulative % drug release for the formulation kept at 4±1°C was satisfactory but showed significant reduction at room temperature (25± 2°C). The result shown for 4±1°C and room temperature (25± 2°C) supports that at room temperature (25± 2°C) there was small degradation of drug and room temperature (25± 2°C) is not a satisfactory storage condition for lipid based formulation.

Table 4: Effect of aging on particle size on NLCs (N_7) at 4 ± 1°C and 25± 2°C temperature

Sr. No.	Days	Physical Change	Particle size(nm) (at 4 ± 1°C)	Particle size(nm) (at 25± 2°C)
1	0	No Change	337±0.72	337±0.72
2	15	No Change	337.3±0.43	337.9±0.53
3	30	No Change	337.8±0.56	341.8±0.66
4	45	No Change	338.1±0.34	349.1±0.34
5	60	No Change	338.9±0.67	359.9±0.57
6	75	No Change	339.5±0.57	371.5±0.87
7	90	No change	340.2±0.41	393.2±0.91

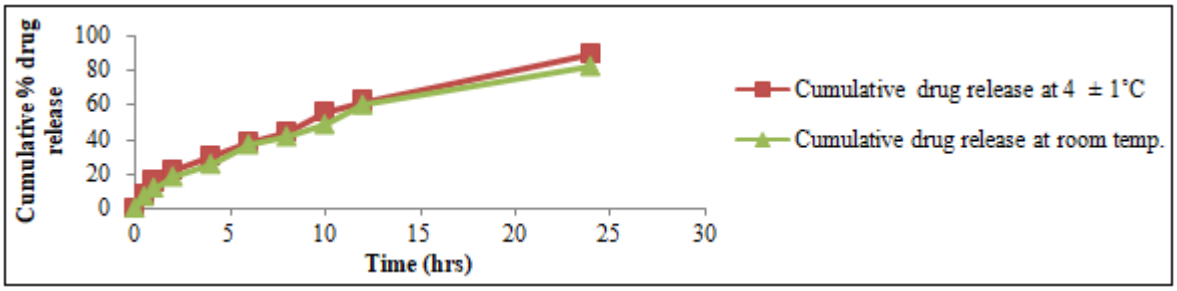

Figure 5: Cumulative % drug release at 4 ± 1˚C and at room temperature

5. Conclusion

In the present study, an attempt was made to improve bioavailability of poorly soluble Nifedipine by preparing nanostructured lipid carrier. NLCs were prepared by solvent injection method and optimized batch was formulated by using Glyceryl monosterate, oleic acid, and Fenugreek Seed Polysaccharide as solid lipid, liquid lipid and polysaccharide respectively. Which exhibit high entrapment efficiency and sustained release of drug up to the period of 24 h. Particle size and SEM study confirms nano sized discrete spherical globules with smooth surface area. Stability study of optimized formulation at cold condition (4±1°C) and room condition (25± 2°C) shows extremely stable formulation for the period of three months at 4±1°C and support the fact that dried lyophilized nano-carriers may remain stable for longer period of time at cold condition (4±1°C). It can be concluded from the result obtained that the NLCs developed for oral delivery of Nifedipine with Fenugreek Seed Polysaccharide showed better stability and higher entrapment efficiency, easy to scale up. The results of the present study showed that the problems associated with the oral bioavailability of Nifedipine can be overcome by using Nanostructured lipid carriers' preparation including Fenugreek Seed Polysaccharide.

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