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Original Research Paper

PREPARATION AND EVALUATION LIPID NANOPARTICLES OF FENOFIBRATE OBTAINED BY SPRAY DRYING TECHNIQUE

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ABSTRACT

This work aimed to prepare a formulation for fenofibrate a poor water soluble drug characterized by incomplete oral bioavailability, bitter taste, and tendency to destabilize in aqueous media. Regarding to the good solubility of fenofibrate in lipid materials, lipid nanoparticles seemed to be an excellent way to overcome these issues. In an attempt to prevent eventual stability problems linked to lipid nanoparticles, we dried the prepared nanosuspensions into redispersible powder, in association with adapted drying carriers such as aerosol 200. The comparison took into account quality of the obtained powder and its redispersibility, palatability, drug stability during the process and the effect of the drying method on particle size, entrapment efficiency. The morphology of dried nanoparticles was found to be spherical in shape by scanning electron microscopy (SEM) observation. The X-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC) analysis indicated that there was no substantial crystalline change in the spray dried nanoparticles compared with pure drug. The *in vitro* dissolution rate of fenofibrate was significantly increased by reducing the particle size. The amorphous fenofibrate nanoparticles showed dramatic improvement in rate as well as extent of *in-vitro* drug dissolution.

Keywords: Fenofibrate, Lipids nanoparticles, Spray drying, Dissolution studies.

INTRODUCTION

Formulation of aqueous dispersions of lipid nanoparticles is an elegant way to enhance and control drug bioavailability, improve stability, and mask bitterness of some drugs.^{14,22} They are interesting carriers for oral delivery of lipophilic and, to a certain extent, hydrophilic substances. The maximum advantage from a lipid formulation could only be drawn if the drug remains in lipid solution throughout its residence in the gastrointestinal (GI) tract.¹¹ However, the performance of lipid formulations and the fate of the drug in the GI tract depend on 3Ds that occur simultaneously viz., dispersion, dilution, and

digestion of the formulation. Due to these 3Ds, lipid formulations often result in change in their composition, structure, and potential loss of their solvent capacity. Altogether, these processes may cause precipitation of drug to occur, and thus, the advantage of a lipid formulation is lost.^{11,13} For this reason, selection of oil phase that has high solubilization capacity for the drug is crucial in any lipid formulation. Besides, oil (consists of various types of triglycerides) is digested in the gastrointestinal tract and may play a major role in determining rate and extent of dissolution lipid nanoparticles in solid form one of the lipid-based

drug delivery systems prepared by incorporation of liquid excipients into powders by solidification, is a promising drug delivery system for poorly water-soluble compounds as it combines the advantages of lipid nanoparticles solubility and bioavailability enhancement with those of solid dosage forms high stability with various dosage form options. Solid form nanoparticles produces such as nanoemulsions of droplet size less than 200 nm upon mild agitation in aqueous media such as gastrointestinal fluids. These fine droplets of nanoemulsions have the advantage of presenting the drug in dissolved form with a large interfacial surface area for drug absorption, which results in enhanced more uniform and reproducible bioavailability.¹⁵ However, these lipid nanoparticle dispersions can present some instability phenomena (lipid crystallization, particle size increase, gelling, etc.). In order to prevent eventual issues, lipid nanoparticles are dried by spray-drying or freeze-drying techniques into redispersible powders. Spray drying (SD) is achieved by atomization of liquid through a nozzle; this liquid is instantaneously dried by a co-current air flow.⁶⁻⁸ Solution containing lipid nanoparticles can be sprayed and quickly be dried within air flow. In this work, a spray drying (SD) was performed. This comparison took into consideration: quality of the obtained powder; redispersibility of powder in water, palatability, nanoparticles size after redispersion; drug stability during the process; dissolution profile of fenofibrate from the dried powders.^{12,14,16} We prepared lipid nanoparticles containing lipids for the delivery of fenofibrate from the established composition of solid form lipids nanoparticles by spray drying with Aerosil 200 as the inert solid carrier. Colloidal silica (Aerosil® 200), a nonporous hydrophilic form of silica, is one of the most important carriers that enables fast drug dissolution by improving the wettability of the drug particles, and it was confirmed that drugs become molecularly dispersed within the matrix formed with silica particles and evaluated it the solid state characteristics of the prepared solid lipids nanoparticles were investigated using scanning electron microscopy (SEM), differential

scanning calorimetry, (DSC) and X-ray powder diffraction (XRPD). Furthermore, the dissolution rate of fenofibrate were evaluated compared to a pure drug powder. Fenofibrate is a lipid-regulating agent that has chemical, pharmacological, and clinical similarities to the other fibrate drugs, such as clofibrate and gemfibrozil. Fenofibrate is a Biopharmaceutical Classification System (BCS) Class II drug with a high dose number.¹⁹⁻²² Thus, it can be assumed that the low oral bioavailability of fenofibrate is due to its solubility and dissolution limitations. Researchers have tried various methods (eg, cyclodextrin complexation, micronization, solid dispersion) to overcome these limitations. Furthermore, it is reported that absorption of fenofibrate is increased by ~35% when it is administered with food rather than in a fasting state. Thus, formulating a lipid-based system of fenofibrate can be viewed as an option for improving its oral bioavailability. Fenofibrate is available in various doses (54 mg, 67 mg, 100 mg, 160 mg, and 200 mg). The main objectives of the study were to develop and evaluate an optimal lipids nanoparticle in dry solid form formulation containing fenofibrate.

MATERIALS AND METHODS

Materials

Lipid nanoparticles were prepared using two lipid materials; other substances were added to optimize and stabilize nanosuspensions and to dry them. All substances are summarized in Table 1 Stearoyl macrogol-32 glycerides (Gelucire® 50/13) is a non-ionic, water dispersible surfactant composed of well-characterized PEG-esters, a small glycerol fraction and free PEG. Drop point is comprised between 46 and 51 °C (Supplier data). Fenofibrate was gifted from BASF Pvt Ltd Mumbai. Methanol and aerosol 200 was purchased from S.D. Research lab Fine Chemicals, India. Water was filtered through Millipore 0.22 µm filter before used.

Nanosuspension Preparation

The lipid materials; Gelucire®50/13 melted at respectively 60 °C, fenofibrate was then added and stirred until complete dissolution. Deionized water was heated at the same temperature and

vigorous stirring. This aqueous phase was added to lipid phase using a high shear stress homogenizer Ultra-Turrax® (IKA, Heidelberg, Germany) for 5 min at 10,000 rpm. Then the suspension was ultrasonicated for 5 min using an ultrasonicator at 25 KW. At the end, the nanosuspension was cooled at room temperature. Formulation table as given below

Characterization before Drying

Nanoparticle size measurement

Size of the obtained drug particles was measured by dynamic light scattering (DLS) using Zetasizer® (Malvern Instruments Ltd., UK) at room temperature. Before analysis, the drug suspension was diluted using deionized water and softly agitated for 10 min. The measurement was made in triplicate and mean diameter and polydispersity index (PDI) given by the cumulant method was reported.

Entrapment efficiency of feofibrate in lipid nanoparticles

Entrapment efficiency is defined as the percentage of drug incorporated into nanoparticles relative to the total drug added. It was determined by ultracentrifugation: SP content was measured in nanosuspension before centrifugation; the samples were centrifuged using ultracentrifuge (Remi Pvt Ltd) at 40,000 rpm for 1 h at 15^oC and the amount of SP in the supernatant was finally quantified.

Entrapment efficiency is calculated as follows

$EE (\%) = \frac{\text{total amount of API-free amount of}}{\text{API total amount of API}} \times 100$

Lipid Nanoparticles Drying

Solid state formulations are having long term stability compared to liquid state. So, stable batches of preliminary trial were tried to convert in a powder by spray drying technology. Thus, aerosol 200 at a concentration of 5%w/v of total formulation was dissolved in the resultant nanosuspension (NS). This suspension was spray dried to get dried powder using LSD-48 mini Spray Dryer [JISL, PVT, and Mumbai, India]. Operation parameters in spray dryer were: inlet temperature: 115 °C, outlet temperature: 55 °C, aspiration: 1200 rpm and feed pump: 21rpm.

Powder Characterization

Drying yield

The drying yield corresponds to the amount of obtained powder divided by the dry mass of all substances used in the formulation of nanosuspension added to drying carrier mass. The drying yield (DY) was determined with the following equation:

$DY = \frac{\text{mass of dried powder total}}{\text{mass of initial solid in formula}} \times 100$

Loss on drying

Approximately 1 g powder was analyzed using Sartorius MA 45 (Sartorius Göttingen, Germany), an infrared heating moisture analyzer. Weighed powder was exposed to a temperature of 110 °C for 20 min. The difference between initial and final mass reported to initial mass represents the percentage of loss on drying (LOD) that inform about the moisture content of powders.

$LOD (\%) = \frac{\text{mass before drying}}{\text{mass after drying}} \times 100$

Powders particle size measurement

Measurement was determined by LASER diffraction (LD) using Master Sizer 2000 (Malvern Instruments Ltd., Worcestshire, UK). Each analysis was done in triplicate. The size distributions were characterized using the diameters at the 10, 50 and 90 percentile (d10%, d50% and d90%, respectively). Sample obtained by FD was softly mixed using a spatula, and measured.

Scanning electron microscopy

Scanning electron microscopy (SEM) analyses were performed on dried nanoparticles. Gelucire samples was analyzed. A Hitachi 5800 (Japan) scanning electron microscope was used. Samples were metalized with palladium at 20 keV.

X-ray Diffraction [XRD]

XRD studies were performed to study effect of milling and spray drying on the crystallinity of lornoxicam. The XRD studies of pure drug, Poloxamer 407, aerosil and spray dried powder were carried out using X-ray diffractometer [XRD] [Brucker AXS, model D8 advanced, Germany] Standard runs were taken using 40kV voltage, 40mA current and scanning rate of 0.02°/min over a 2θ range of 5–50°.

Differential Scanning Calorimetry [DSC]

DSC studies were performed to investigate the effect of surfactants, milling process and drying process on the inner structure of lornoxicam and to confirm crystallinity result obtained by XRD. DSC studies were carried out using thermal analyzer (TA SDT-2790). The samples were hermetically sealed in an aluminum pans and heated at constant rate of 10 °C/min over a temperature range of 0-300 °C. Inert atmosphere was maintained by purging nitrogen gas at a flow of 50 mL/min.

Dissolution studies

Preliminary dissolution tests under gastric conditions, intended for selecting the spray dried nanosuspension system with superior dissolution properties were performed using the United States Pharmacopoeia (USP) dissolution apparatus II at 50 rpm. A sample equivalent to 8 mg of lornoxicam was placed in the dissolution vessel containing 900 mL of 0.1N HCL maintained at 37 ±0.5 °C and aliquots were withdrawn at suitable time intervals for 60 min, and filtered immediately through 0.1µm PTFE syringe filter [Whatman Inc Clifton, NJ, USA]. Subsequently, same volume of fresh medium was added to the dissolution vessel. Quantification of the samples was done by UV analysis at 378 nm. The experiment was performed three times and the mean values were plotted *versus* time.

Nanoparticles Characterization on Reconstituted Powders

- Nanoparticles size measurement and dissolution testing on dried powders was determined after redispersion of powder in purified water as previously mentioned for nanosuspensions.
- Redispersibility of powders was estimated by handshaking method; the reconstituted suspensions are shaken until complete dispersion was obtained, the redispersibility easiness was notated as follows: +++ For easy to disperse, +for difficult to disperse, ++ for inter-mediate.

RESULT AND DISCUSSION

Particle sizes of gelucire nanoparticles (table 2); this is due to the self microemulsifying property of gelucire. And entrapment efficiency of nanoparticles is up to 90%. Actually a part of SMG forms additional colloidal structures (micelles, super cooled melts, drug nanoparticles) with dynamic phenomena. The spray dried solid form nanoparticles of fenofibrate nanoparticles had good flowability. Properties due to the presence of aerosil200, which is regarded as a suitable excipients for the solid dosage forms. Any change in interfacial film influences the surface curvature of the drop let leading to differences in the droplet size.^{21,22} The emulsion droplet size and polydispersity index (PDI) of lipids nanoparticles are shown in table 2. The droplet size and PDI were 91.45 ± 3.56 nm and 0.257 ± 0.019 for lipids nanoparticles and it may be stated that or suggested the capability of the lipid components of lipid nanoparticles to retain its emulsification properties irrespective of physical form change. The results suggested that the oil phase used in this study positively influenced the formation of relatively nanosized droplets. Both labrasol and Gelucire are widely used in the pharmaceutical and food industries due to their excellent safety profile. These lipid-based components were expected to help the emulsion formed in the stomach be readily restructured into mixed micelles even in the absence of biliary phospholipids,

Solid State Characterization of Lipid Nanoparticles SEM studies

The SEM pictures of fenofibrate powder (figure1A) appeared as smooth-surfaced, irregularly shaped, flat crystals in shape. However, the converted solid nanoparticles (figure1B) appeared as smooth-surfaced particles without any crystalline shape, indicating complete adsorption of lipids containing amorphous drug inside the pores of Aerosil 200.

DSC studies

The DSC thermograms were shown in figure 2 Pure crystalline fenofibrate showed two small endothermic peaks at about 85 °C. This endothermic peak of fenofibrate is in compliance

with previous report.¹⁸ No obvious peak for fenofibrate was found for the spray dried nanoparticle indicating that the drug must be present in amorphous or molecularly dissolved state in lipids.^{12,13,17}

XRD studies

The internal physical state of fenofibrate in the solid form nanoparticles was further verified using XRPD diffractograms (figure 3). Pure fenofibrate powder, showed prominent diffraction peaks in the range of 5-50 How theta ever, no obvious peaks representing crystals of fenofibrate were seen for the spray dried nanoparticles, indicating the absence of crystalline structure of fenofibrtae in the formulation. Spray dried nanoparticle formulation should disperse quickly and completely when subjected to aqueous environment under mild agitation. The efficiency of self-emulsification can be estimated by measuring the rate of emulsification and the droplet size distribution. The rate of emulsification of spray dried nanoparticle formulations is measured by visual observation as reported previously. It was observed that emulsification time of spray dried nanoparticles was 19 ± 2 s for spray dried nanoparticle, (table 2). The efficiency of self-emulsification of surfactant and co-surfactant is much related to their hydrophilic-lipophilic balance (HLB) value. Generally, surfactants with HLB 12-15 are regarded as being of good efficiency for self-emulsification. Thus, labrasol with HLB value of 13 (known to possess good self-emulsification ability) was selected as primary surfactant in SNEDDS for proper self emulsification. The results from zeta potential analyses of both formulations were shown in table 2. The zeta potential of liquid lipids and solid lipids were $+1.40 \pm 0.22$ and $+1.16 \pm 0.35$, respectively. There was no significant difference between the charges of the two formulations. It is reported that

in addition to particle size, zeta potential also plays an important role in the interactions with mucus of the gastrointestinal tract. It was reported earlier that the positively charged droplets could have better interaction with the mucus of the gastrointestinal tract as the intestinal cells carry negative charges with the presence of mucosal fluid .Because of this reason, it is likely that the solid form lipids nanoparticles would enhance the intestinal absorption of fenofibrate.

In Vitro Release Study

Fenofibrate release profile spray dried nanoparticles was presented in figure 4 The percentage dissolution of pure drug at 30 min was $39.72 \pm 1.05\%$ and $89.45 \pm 1.03\%$ from pure drug, and solid form lipids nanoparticles respectively. The solid carrier used (Aerosil 200) in the present study did not interfere the dissolution of pure drug from the solid nanoparticles. At the end of the study, the dissolution was around 94% for formulation. The results of the release profile suggested that the spray dried nanoparticles preserved the enhancement of in vitro dissolution of fenofibrate.

CONCLUSION

Lipid nanoparticles were an efficient way to enhance dissolution and stability properties of fenofibrate. Spray drying process produced dry nanosuspension with high stability compared to liquid formulation. Batch was analyzed for particle size and % drug content. Stable fenofibrate dry nanosuspension batch was evaluated by SEM, XRD, DSC, and *in-vitro* dissolution. The amorphous fenofibrate nanoparticles showed dramatic improvement in rate as well as extent of *in-vitro* drug dissolution. The improvement can be attributed to amorphization and surface area, reduced particle size.

Table 1: Nanoparticle composition (% w/w)

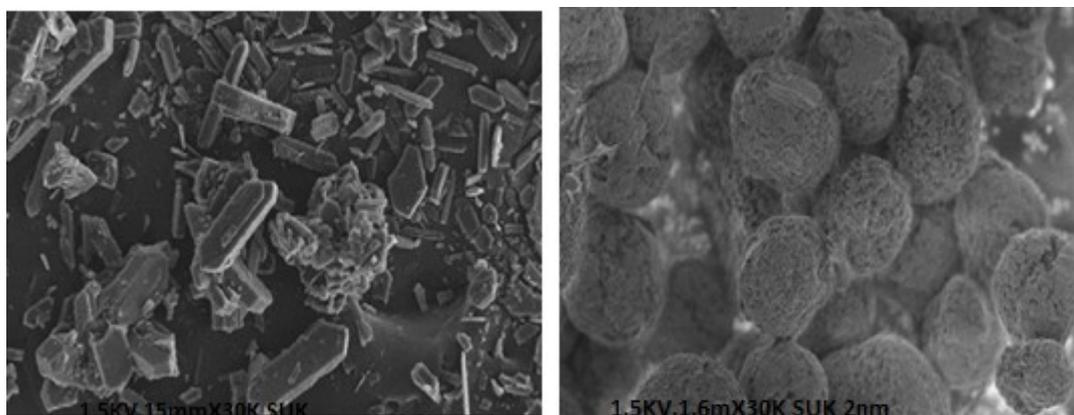
Name of chemical	Weights in gram	Function
Fenofibrate	0.6	Active Pharmaceutical Ingredients
Gelucire®50/13	10	Lipid Material
Labrasol®	2	Surface Active Agent
Aerosil® 200	3	Drying Carrier
Deionzied Water	100 ml	Vehicle

Table 2: Nanoparticle properties

Lipid nanosuspension	Gelucire nanoparticles
Mean particle size (nm)	91.45
Polydispersity index (%)	0.256
Fenofibrate content in nanosuspension (mg/g of nanosuspension)	5.69
Entrapment efficiency	89.02

Table 3: Properties of powders obtained by spray drying process

Drying yield (%)	82.4
Redispersibility	++
Loss on drying (%)	1.90
Entrapment efficiency (%)	94.56
Particle size distribution (µm)	3.9(D10),21.3(D50),132.7(D90)



A

Figure1: SEM

B

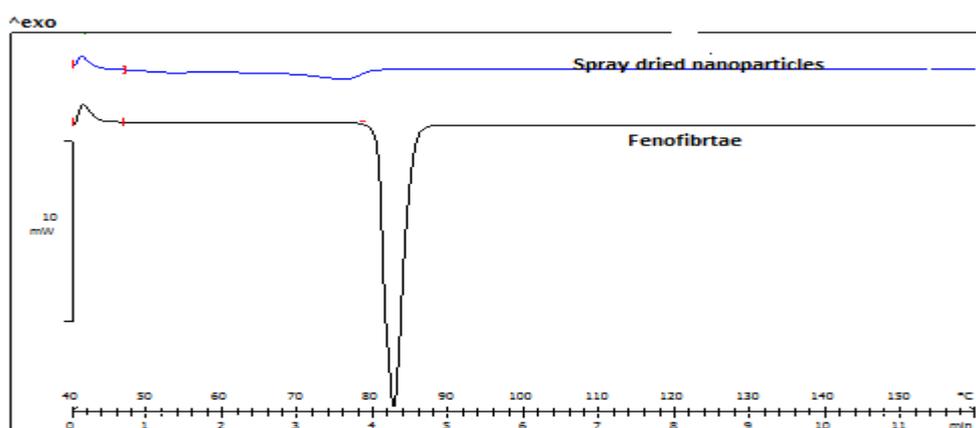


Figure2: DSC

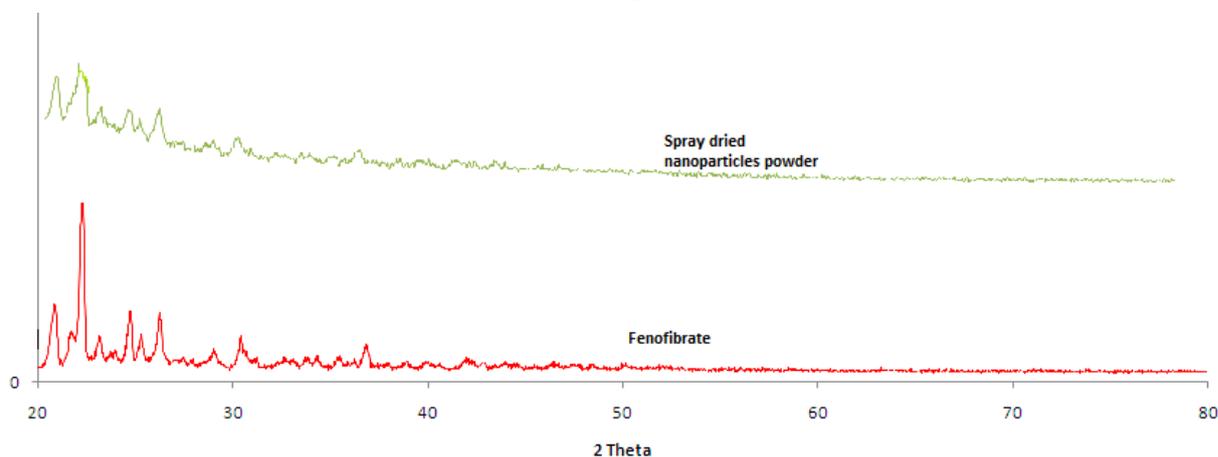


Figure3: XRD

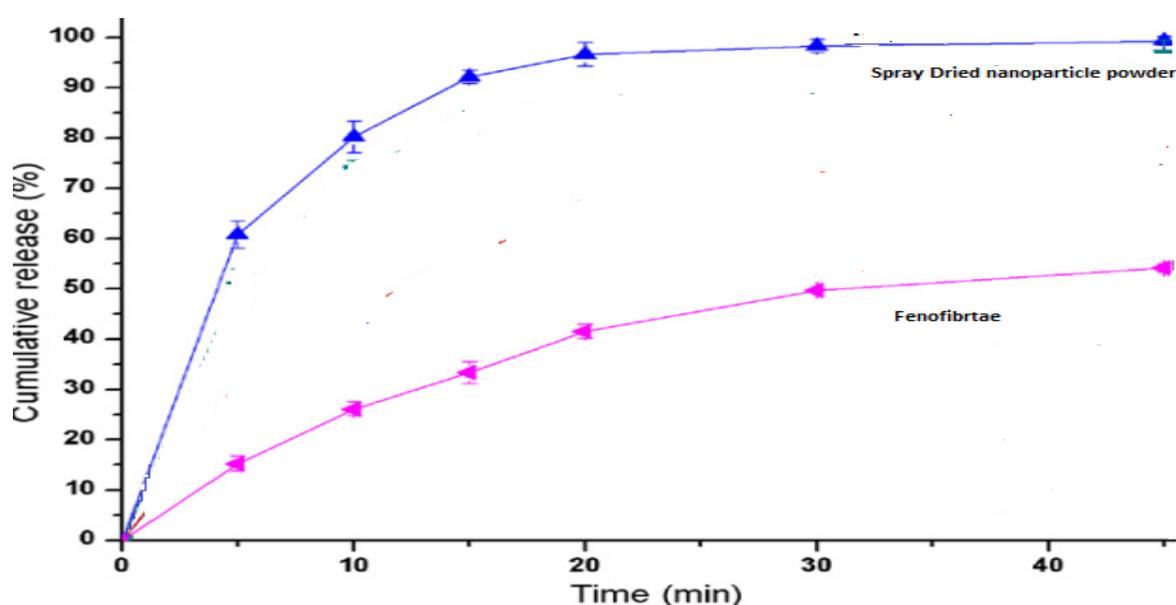


Figure4: Dissolution Studies

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