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Original Article

PREPARATION AND *IN VITRO* CHARACTERIZATION OF FELODIPINE LOADED EUDRAGIT® RS100 NANOPARTICLES

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ABSTRACT

Objective: Felodipine, a calcium channel blocker, has been widely used for the treatment of hypertension and cardiovascular diseases. Being poorly soluble in nature felodipine shows poor and variable bioavailability. Poor bioavailability not only leads to the frequent dosing but also shows very poor patient adherence. Hence, in the present study an approach has been made to develop felodipine nanoparticle formulation using eudragit® RS100 polymer keeping in the view to the slow release of felodipine.

Methods: The felodipine nanoparticles were prepared using solvent evaporation technique (single emulsion technique). The particles sizes and zeta potential was measured using zeta-sizer. The particle morphology was also studied using scanning electron microscope (SEM) and atomic force microscope (AFM). The *in vitro* release of felodipine from the nanoparticles was carried out in phosphate buffer pH 6.8.

Results: Felodipine loaded eudragit® RS100 nanoparticles showed the particle size were in nano size range (492 to 576 nm) with positive zeta potential. The drug entrapment efficiency was varied with the drug polymer ratios. The scanning electron microscopy showed the spherical particle sizes. Atomic force microscopy study revealed the disc like shape of the prepared nanoparticles. The *in vitro* drug release study exhibited the sustained release of felodipine with reduced burst release as compared with pure drug powder.

Conclusion: The felodipine nanoparticles were prepared and characterized. The encouraging results suggest further *in vivo* studies to evaluate the bioavailability parameters as well as suitability of the formulation.

Keywords: Felodipine, Eudragit® RS100, Nanoparticles.

INTRODUCTION

The elevated blood pressure is a major risk factor in the development of cardiovascular diseases (CVD). It is common in both developed and low- and middle-income countries [1]. The recent study reveals that the achievement of desired blood pressure targets has become a challenge to reduce the risk of morbidity and mortality [2]. There are number of medicines used for the treatment of high blood pressure and patients might need to take their medicine for several years.

Calcium channel blockers (CCBs) are widely used for the treatment of systemic arterial hypertension. The antihypertensive effect of CCBs is due to the inhibition of voltage dependent L- type calcium channels in vascular smooth muscle and heart that causes arterial dilatation and smooth muscle relaxation. Felodipine, a dihydropyridine calcium channel blocker, has been widely used for the treatment of hypertension [3]. Felodipine is a lipophilic crystalline powder and practically insoluble (BCS Class-II) in water (solubility-0.5mg/l) [4].

Felodipine has poor and variable bioavailability, which leads to the multiple daily dosing. The multiple daily dosing sometimes exhibits fluctuation of plasma drug concentration and also lead to poor patients' adherence. The bioavailability of poorly water soluble drugs can be improved by various approaches like solubilisation, use of co-solvents, salt formation, micronization and complexation with cyclodextrins [5, 6, 7].

The bioavailability issue of the drugs' can be addressed by making the particle size within the nano-meter range. Nanoparticles have the diameter less than 1μm and have been used to improve the solubility and dissolution rate of poorly soluble drugs. Poorly soluble drugs can be formulated in the form of nanoparticles alone, or with the combination of pharmaceutical excipients. The polymeric nanoparticulate systems have been considered as promising carriers for the drug delivery [8, 9] and sustained oral drug delivery will be beneficial to the patients for the long term treatment. The widely used nanoparticulate engineering processes for the synthesis of nanoparticles are solvent evaporation, high pressure homogenization [10], nano-precipitation [11], emulsion diffusion etc.

Eudragit® RS100 is the co-polymer of poly (ethylacrylate, methyl-methacrylate and chlorotrimethyl-ammoniumethyl methacrylate) containing quaternary ammonium group. Eudragit® RS100 is commonly used for the formulation of controlled and sustained release dosage forms [12]. It is insoluble in physiological pH and capable of swelling, which represents the good material for the drug dispersion [13]. Eudragit® RS100 has been previously used for delivery of antihypertensive drugs [14].

In present study an attempt has been made to develop felodipine loaded eudragit® RS100 nanoparticles keeping in the view to get more effective delivery of felodipine. So, felodipine loaded nanoparticles were prepared and characterized with the aim to achieve the slow release of felodipine.

MATERIALS AND METHODS

Materials

Felodipine was a kind gift from Cadila Healthcare Limited (Ahmedabad, India). Eudragit® RS100 (Evonik Industries AG, Germany) was obtained from Sandoz Ltd. Mumbai. Lutrol® F-68 (Poloxamer 188) was obtained from Sigma Aldrich, Mumbai. Distilled- deionized water was prepared with Milli-Q plus System (Elix 10, Millipore corp. India). All other chemicals used were of the highest available grade.

Preparation of felodipine nanoparticles

The felodipine nanoparticles were prepared with the different ratios of drug and eudragit® RS100 polymer using the solvent evaporation

(single emulsion) technique with slight modification [14, 15]. Felodipine (equivalent to 10% w/w dry weight of polymer) was dissolved in acetone containing 100 mg of the eudragit® RS100 polymer at room temperature. The resultant solution was added into 25 ml aqueous phase containing 0.5% (w/v) of poloxamer-188 with a constant flow rate (0.5 ml/min). The mixture was homogenized (VIRTIS, Cyclone IQ, USA), at constant agitation speeds of 15000 rpm in an ice bath. The formed emulsion was kept at room temperature for overnight under gentle stirring to evaporate the organic solvent. The nanosuspension was then freeze dried (-80 °C and <10 mm mercury pressure, Freezone 6lt, Labconco Corp., MO) to get powdered nanoparticles and kept at freeze for further use.

Determination of particle size and zeta potential

Particle size of the prepared nanoparticles was measured by Photon Correlation Spectroscopy (PCS) with Zetasizer 3000 (Malvern Instruments, Malvern, UK). The Refrigerator dried powder was suspended in Milli-Q water (1mg/ml) at 25 °C and sonicated for 25 sec in an ice bath (VC 505, Vibracell Sonics, USA) before measurement. The mean particle diameter and size distribution of the suspension were carried out for three times for each batch of sample under identical conditions and mean values were reported. The Zeta potential value was also measured using same suspension and same equipment.

Determination of drug entrapment efficiency by RP-HPLC method

The drug entrapment efficiency (EE) was estimated by reverse phase High Performance Liquid Chromatography (RP-HPLC) method [16]. The drug loaded nanoparticle solution (1 mg/ml) was prepared in methanol and 20 µL of the sample was injected manually to HPLC system equipped with Shimadzu LC-20AD PLC pump. The chromatographic separation was achieved by using Phenomenex C18 (150×4.6 mm, 5µ) analytical column.

The flow rate was maintained at 1.0 mL/ min and the measurements were made at 240 nm in ambient condition. The amount of the felodipine in the nanoparticles was determined from the peak area correlated with the standard curve. The standard curve was prepared under the same identical condition. The entrapment efficiency (EE) was calculated using the following equations-

$$EE\left(\%\frac{w}{w}\right)=\frac{\text{Weight of the drug in nanoparticles}}{\text{Weight of the drug added}}\ \text{x}\ 100$$

Scanning electron microscopy (SEM)

The scanning electron microscopy (SEM) (JEOL JSM-5610LV) was used to analyze the nanoparticle shape and surface morphology. Completely moisture free lyophilised powdered samples were consigned on aluminium stubs using adhesive tapes and coated with gold using sputter coater (JEOL auto fine coater, Japan) and observed for morphology at an acceleration voltage of 20 kV.

Atomic force microscopy (AFM)

Surface morphology of the prepared nanoparticles was studied using atomic force microscopy (AFM) (JPK NanoWizard II, JPK instrument, Berlin, Germany). The nanoparticle suspension was prepared with milliQ water and dried overnight in air on a clean glass surface. To avoid damage of the sample surface, all measurements were conducted in intermittent contact mode and the tip to sample distance was kept constant. The scan speed of 2 Hz and 312 kHz resonant frequency was used to obtain images [17].

***In vitro* drug release study**

The *in vitro* drug release study was carried out using rotating basket method [18, 19]. The drug loaded nanoparticles and pure felodipine (each containing 5 mg felodipine) were suspended in glass bottles containing 100 ml of phosphate buffer pH 6.8 [14]. Glass bottles were placed in beaker and kept in incubator shaker throughout the study (37° C, 50 rpm). At specified time interval 10 ml samples were collected and centrifuged at 13000 rpm for 30 min. The supernatant was collected and analysed by RP-HPLC at 240 nm. The precipitate was resuspended in 10 ml of fresh phosphate buffer and transferred to the glass bottle. All the measurements were carried out in triplicate.

Statistical Analysis

For statistical analysis the experimental data was tested by one-way analysis of variance (ANOVA). Data represented as mean values ± SD (standard deviation). The values of $p < 0.05$ (*) were indicative of significant difference.

RESULTS AND DISCUSSION

Particle size and Zeta potential (ζ) measurement

The particle size has direct impact on the stability, cellular uptake, drug release and biodistribution. The mean particle sizes of the prepared nanoparticles as measured by the Photon Correlation Spectroscopy (PCS) were in size range of 492 to 576 nm and the size distributions were monodispersed (0.214 to 0.842) in all the formulations (**Tab. 1)**. There were no noticeable differences between the sizes of nanoparticles obtained with different drug polymer ratio, as similar findings was reported earlier for the nanoparticles of anti hypertensive drugs with eudragit® RS100 [14] (**Tab. 1**). The formulation (FEN1) showed the smaller particle size than the formulation (FEN3) (**Fig. 1**), but exhibited less entrapment efficiency and low zeta potential. The zeta potential of the formulated nanoparticles measured in water, exhibited positive values of +8.9 to +19.8 mV (**Tab. 1)**. The positive zeta potential value is due to the quaternary ammonium group present in the eudragit® RS100 polymer and suggested that the drug was encapsulated with the polymer. The positive zeta potential values can facilitate an effective adhesion of the nanoparticles with the negatively charged mucus of the gastro-intestinal tract, prolonging the effective residence time of the formulations.

Surface morphological properties of nanoparticles

The surface morphology was determined using scanning electron microscopy. The SEM image of nanoparticles revealed spherical shape with smooth surface (**Fig. 2**). The AFM investigations showed disc like shape of the particles with slight aggregation. This is because of freeze drying of the product. It was also proven that the particles are surrounded by a soft layer (**Fig. 3**). The particle sizes obtained by SEM were relatively smaller than that of the particle sizes obtained by Zetasizer. The electron microscope exhibits only the nanoparticle surface; whereas the Zetasizer measured the particles sizes surrounded hydrodynamic layer.

Drug entrapment efficiency

The entrapment efficiency of nanoparticles is influenced by the characteristics of the polymer, drug, surfactant etc. The high entrapment efficiency results from the more affinity of the drug and polymer to the same solvents. The low entrapment is due to the high affinity of drug and polymer to the different solvents. In the present study the drug entrapment efficiency were affected by the polymer and drug ratio in the formulations. The increased entrapment efficiency was due to the greater proportion of polymer in the formulation [20]. It was also shown by Jana *et al*., [14], that the nebivolol- eudragit® RS100 nanoparticles have the encapsulation efficiency of 89%. Therefore, we prepared different formulation of the nanoparticles taking different ratio of drug- polymer. The formulation (FEN3) having drug- polymer ratio of 1:4, shows the entrapment efficiency of 75.87% (**Tab. 1**). Smaller particle size (FEN1) may be the reason of the decrease in the encapsulation efficiency. To improve the encapsulation efficiency of the nanoparticles the parameter drug- polymer ratio was taken into consideration. The result showed that, the increase in the drug-polymer ratio up to 1:4, the encapsulation efficiency was increased. This may be due to the higher proportion of polymer present in the formulation. As the amount of drug increased a more porous polymeric structure form, with large number of channels and hollow spaces, through which the drug can easily escape to the outer phase, thereby decrease the drug inside the polymeric matrix of the nanoparticles [21]. Though the nanoparticle formulation (FEN1) exhibited the smaller particle size, it showed the low entrapment

efficiency than the formulation (FEN3). So, the nanoparticles formulation (FEN3) having drug- polymer ratio of 1:4 with agitation speed of 15000 RPM was selected and used for the further studies.

***In vitro* drug release study**

The drug release rate from the prepared nanoparticles was influenced by the drug-polymer composition. It was a complex phenomenon which may occur between the drug and polymer, including entrapment of the drug in the polymer and the adsorption of drug on the surface of the polymer matrix as a result of electrostatic adhesion [22]. The *in vitro* drug release profile from the intact drug powder and prepared nanoparticles are shown in Fig. 4. The felodipine-eudragit® RS100 nanoparticles showed slower release of felodipine in comparison with intact drug powder. Within the first hour a burst release of about 36 % was observed for felodipine intact powder.

The initial burst release was reduced to 16 % and a slow release was observed for felodipine loaded eudragit® RS100 nanoparticles for at least 72 h. The slow release was due to the formation of polymeric wall around the drug. It signifies that the prepared nanoparticles possess sustained release properties. After 6 h the rapid drug release was identified and this could be as a result of polymer erosion in the surface of nanoparticles. It suggests that the drug release from the prepared nanoparticles is the combination of dissolution, diffusion and erosion mechanism.

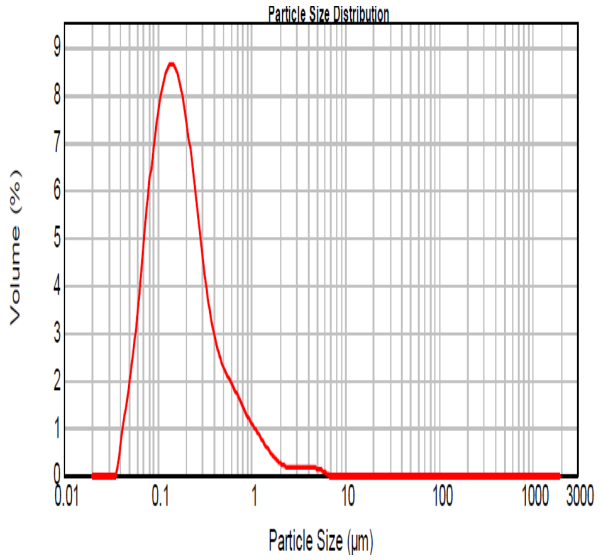

Fig. 1: Mean particle size distributions of felodipine – eudragit® RS100 nanoparticles (FEN3) prepared with the drug polymer ratio (1:4)

Table 1: Physico-chemical characterisation of felodipine loaded eudragit®RS100 nanoparticles (Data represents mean ± SD)

Batch	Drug-Polymer ratio	Particle size (nm) ± SD (n = 3)	Polydispersity index	Zeta potential (mV) ± SD (n = 3)	Entrapment Efficiency (% w/w)
FEN1	1:2	492 ± 1.80	0.483 ± 0.053	+14.1 ± 0.47	57.78 ± 0.480
FEN2	1:3	517 ± 3.26	0.356 ± 0.078	+17.6 ± 0.56	69.89 ± 0.861
FEN3	1:4	526 ± 1.41	0.214 ± 0.007	+19.8 ± 0.81	75.87 ± 0.242
FEN4	2:1	576 ± 2.21	0.842 ± 0.088	+8.9 ± 0.79	46.47 ± 0.645

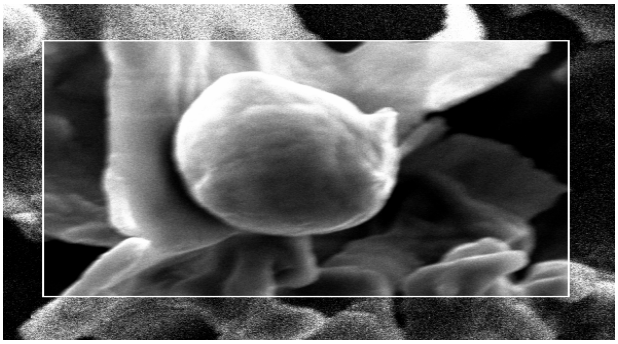

Fig. 2: Scanning electron microscopy image of felodipine – eudragit® RS100 nanoparticles (FEN3).

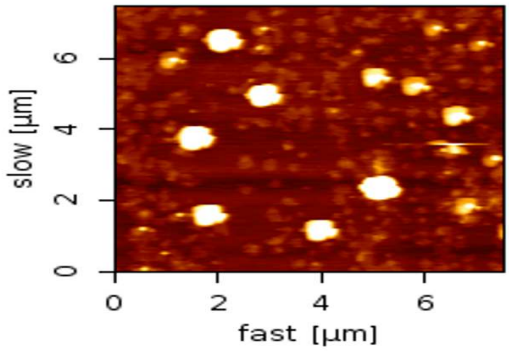

Fig. 3: AFM image of felodipine – eudragit® RS100 nanoparticles (FEN3).

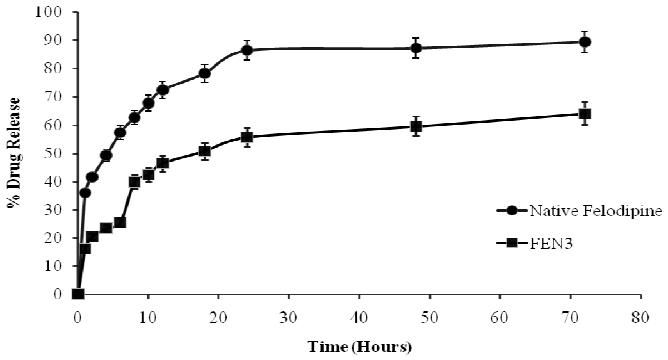

Fig. 4: *In vitro* drug release profile of native felodipine and felodipine – eudragit® RS100 nanoparticles (FEN3). Data as mean ± standard error of mean (n=3)

CONCLUSIONS

Felodipine loaded eudragit® RS100 nanoparticles were successfully formulated using emulsion-solvent evaporation technique. The formulation was able to improve the physicochemical characteristics of the drug. The variation on particle size of the nanoparticles was observed with changing the drug polymer ratio. The noticeable changes were also observed in drug entrapment with increasing the polymer amount. The prepared drug loaded nanoparticles showed slow release of the felodipine with reduced burst release in comparison with intact drug powder. Thus, the felodipine-eudragit® RS100 nanoparticles may provide an effective platform for nanotech drug delivery systems and the prepared formulation may further be used for *in vivo* study.

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