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FORMULATION DESIGN AND *IN VITRO* CHARACTERIZATION OF FELODIPINE NANO-SUSPENSION

Suryakanta Nayak *[1], Dibyasundar Panda [2] and Ajaya Kumar Patnaik [3]

Department of Pharmaceutics [1], L.B. Rao Institute of Pharmaceutical Education and Research, Khambhat, Anand, Gujarat-388620, India
Department of Pharmaceutics Royal College of Pharmacy & Health Sciences [2], Andhapasara Road, Berhampur, Ganjam, Odisha – 760002, India
Department of Chemistry[3], Khallikote (Auto.) College, Berhampur, Odisha-760001, India

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Correspondence to Author:
Suryakanta Nayak

Lecturer
At/Po-Nuapatna, Cuttack, Odisha-754035, India.

E-mail: suryakantanayak.4u@rediffmail.com

ABSTRACT: Felodipine is a member of the dihydropyridine class of calcium channel antagonists (calcium channel blockers) and is insoluble in water. To counter act above side effects, to enhance bioavailability of felodipine in blood stream and to make drug to more targeting, felodipine drug is being designed as nanosuspension formulation using hydroxyl propyl methyl cellulose and hydroxyl propyl cellulose as surfectants as well as rate controlling polymer. Drug polymers interactions were studied by FT-IR spectroscopy. Precipitation method has been used to prepare nanosuspension particles of poorly soluble drug. The prepared nanosuspensions were characterized for droplet size, pH, viscosity, polydispersity index, refractive index, surface morphology study by Scanning Electron Microscopy (SEM), drug content, percentage transmittance, zeta potential, *in vitro* skin permeation and drug release kinetic studies. The droplet size was in the ranges of 61.2±0.58 (F3) to 91.4±0.79 nm (F5). The polydispersity index of various felodipine nanosuspension formulations was in the ranges of 0.271±0.14 to 0.651±0.21. Most nanosuspension possesses a very low viscosity and, therefore, their application may be convenient. The pH value of all the felodipine nanosuspension formulation was in the skin pH range, which was nearer to neutral pH range, demonstrating that all the felodipine nanosuspension will be non toxic, non irritating and non allergic. The felodipine nanosuspension formulation F3 containing 0.75% HPMC K4M, could be concluded as the best optimized formulation for safe management of hypertension.

INTRODUCTION: A pharmaceutical nano suspension is defined as very finely colloid, biphasic, dispersed, solid drug particles in an aqueous vehicle, size below 1µm, without any matrix material, stabilized by surfactants and polymers, prepared by suitable methods for Drug Delivery applications, through various routes of administration like oral, topical, parenteral, ocular and pulmonary routes [1].

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Depending on the production technique applied changes in crystalline structure of drug particles may also occur. An increasing amount of amorphous drug fraction could induce higher saturation solubility [2].

It was hypothesized that nanosuspensions will enhance drug flux resulting from higher trans-membranous concentration gradients. Nanosuspensions differ from nanoparticles. In nanosuspension technology, the drug is maintained in the required crystalline state with reduced particle size, leading to an increased dissolution rate and therefore improved bioavailability [3].

Felodipine is a member of the dihydropyridine class of calcium channel antagonists. The

bioavailability of Felodipine extended-release tablets are influenced by the presence of food. Felodipine also possesses unwanted side effects [4, 5]. The main objective of the present study to design nanosuspension formulation using hydroxyl propyl methyl cellulose and hydroxyl propyl cellulose as surfactants as well as rate controlling polymer with objective to make drug release more targeting.

MATERIALS AND METHODS:

Felodipine was obtained as gift sample from Ranbaxy Ltd., New Delhi. Hydroxyl propyl methyl cellulose and hydroxyl propyl cellulose were procured from S.D. Fine chemical, Kolkata. All other chemicals are of analytical grade, were procured from authorized dealer.

Methodology:

Drug polymers interaction study (FTIR):

Drug polymers interactions were studied by FT-IR spectroscopy (Shimadzu IR spectrophotometer, model 840, Japan). The spectra were recorded for drug, polymers (HPMC and HPC) and drug - polymer physical mixtures (1:5).

Formulation design and preparation of felodipine nanosuspension:

Precipitation method has been used to prepare nanosuspension particles of poorly soluble drug. The drug, Felodipine was dissolved in ethanol. Then this solution was mixed with a miscible anti-solvent that is water in presence of surfactants that is hydroxyl propyl methyl cellulose (HPMC K4M) and hydroxyl propyl cellulose (HPC) at concentrations of 0.25, 0.5, 0.75 and 1.0 % of respectively. Rapid addition of a drug solution to the anti-solvent leads to the super saturation of drug in the mixed solution and generation of ultra fine or amorphous drug solids.

Optimized formulations of nanosuspension were prepared by dissolving 2 % w/w of Felodipine in a 10 % w/w stabilizer. Stabilizer, lecithin is used to wet the drug particles thoroughly; prevent Ostwald's ripening and agglomeration of nanosuspensions, providing steric or ionic barrier. Co-surfactant, bile salt is used to influence phase behavior when micro emulsions are used to formulate nanosuspensions. Ethanol is used as organic solvent. Then, 30 % w/w mixture (surfactant: co-surfactant at ratio of 1:1) was added slowly to the stabilizer, followed by the slow addition of distilled water to adjust the final preparation to 100 % w/w. Sorbitol was used as osmogen. Sodium chloride was used as pH adjustment agent. Methyl paraben was used as preservative. All components were mixed and stirred at 3000 rpm.

Characterization of felodipine nano-suspension.

Nanosuspension droplet size analysis:

Droplet size distribution is one of the important physicochemical characteristics of a nanosuspension, was measured by a diffusion method using a light-scattering particle size analyzer Coulter LS-230. It measures the size distribution using the diffusion of laser light by particles.

Polydispersity index:

The average diameters and polydispersity index of Felodipine nanosuspension formulations were measured by photon correlation spectroscopy. The measurements were performed at 25^{o}C using a He-Nelaser [6].

Viscosity determination:

The viscosity of the nanosuspension formulations was determined using a Brookfield Cup and Bob Viscometer (Brookfield Engi- neering Laboratories, Middleboro, MA) at 25 ± 0.3 °C and 100 rpm without diluting the nanosuspension formulations using spindle number 2.

Refractive Index:

The refractive index, n, of a medium is defined as the ration of the speed, c, of a wave such as light or sound in a reference medium to the phase speed, Vp, of the wave in the medium [7].

$$n=c/Vp \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots [1]$$

It was determined using an Abbes type refractrometer (Nirmal International) at 25 ± 0.5°C.

Scanning Electron Microscopy (SEM):

The size and shape of nanosuspension were also determined by SEM. The nanosuspensions were spread on a sample holder and dried using vacuum. They were subsequently coated with gold (JFC 1200 fine coater, JEOL, Japan) and examined using

scanning electron microscope (JSM 6301F, JEOL, Japan).

Drug content:
About 10 ml of each nanosuspension formulation was taken and dissolved in 10 ml isotonic solution and kept overnight. About 10 mg (similar as in formulation) of drug was taken and dilution was made to 10µg/ml. The dilutions were filtered and analyzed using UV-Visible spectrophotometer for their content uniformity. The absorbance of the nanosuspension formulations were read using one cm cell in a UV-Vis spectrophotometer. The instrument was set at 362 nm. The drug content in each nanosuspension formulation was calculated based on the absorbance values of known standard solutions. The drug entrapment efficacy of various felodipine nanosuspension was calculated by using following formula:

Entrapment efficiency (%) = [(Drug content)/ (Drug added in each formulation)] × 100.

Zeta potential:
Zeta potential is a technique which is used to measure the surface charge properties and further the long term physical stability of nanosuspensions, the instrument which is used to measure the surface charge is known as Zeta PALS .The measurements were carried out with diluted nanosuspension formulations and its values were determined from the electrophoretic mobility of the oil droplets. The minimum zeta potential of ±20 mv is desirable [8].

Percentage transmittance*:*
Percentage transmittance of the prepared nanosuspension formulations was determined spectrophotometrically using UV-VIS Spectrophotometer.

In- vitro* skin permeation studies*:
In-vitro skin permeation studies were performed using porcine abdominal skin with a Franz diffusion cell having an effective diffusion area of 0.785 cm and 4 ml receiver chamber capacity. Full-thickness porcine skin was excised from the abdominal region and hair was removed with an electric clipper. The subcutaneous tissue was removed surgically, and the dermis side was wiped with isopropyl alcohol to remove adhering fat. The leaned skin was washed with distilled water and stored in the deep freezer at 0°C until further use. The skin was brought to room temperature and mounted between the donor and receiver compartments of the Franz diffusion cell, with the stratum corneum side facing the donor compartment and the dermal side facing the receiver compartment. The thickness of the skin was 50–70 lm as measured with a validated micrometer screw gauge.

The receiver chamber was filled with phosphate-buffered saline (PBS) solution pH 6.8, stirred with a magnetic rotor at a speed of 50 rpm, and maintained at a temperature of 37 ± 1 °C. A quantity of the nanosuspension diluted with 50 % water was used for the release studies. Since the nanosuspension had 2 % (20 mg/ml) of the drug, the final drug concentration in the suspension was 10 mg/ml which was equivalent to the amount in the marketed formulation. One ml of the optimized nanosuspension formulation was placed in the donor compartment and sealed with paraffin film to provide occlusive conditions. Samples were withdrawn at regular intervals (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 h) and sink conditions were maintained by replacement with fresh medium. Samples were filtered through a 0.45 to l m membrane filter and analyzed for drug content by a validated UV-Visible spectrophotometric method at 362 nm. Each experiment was conducted in triplicate [9].

***In - vitro* drug release kinetic study:**
To find out the mechanism of drug release from hydrophilic matrices, the dissolution data of tablets of each batch was treated with different kinetic equations, namely zero order kinetic study, first order kinetic study, Higuchi, Hixon-Crowell, Korsemeyer and Peppas [10].

RESULTS AND DISCUSSIONS: The physical mixture of 1:1 ratio of Felodipine, HPMC and HPC proves the compatibility of polymers with the Felodipine. In the FT-IR spectra of the physical mixtures of Felodipine studied, the bands in the range 3050–3250 cm^{-1}, assigned to the stretching vibrations of the N–H bonds in the dihydropyridine ring are not clearly distinguished. The differences also appear in the band corresponding to the vibrations of carbonyl groups in the ester bonds. In

the spectra of the felodipine the band occurs at 1690–1710 cm $^{-1}$ while in the spectra of the corresponding physical mixtures of polymers, complexes it is less broadened and % transmittance reduces. The differences also appear in the band corresponding to the amine group in the pyridine ring. In the spectra of the felodipine the band occurs at 3360-3380 cm^{-1} while in the spectra of the corresponding physical mixtures it is much broadened [11, 12].

All most all the nanosuspension formulations were slightly cloudy (Close to transparent) in appearance. The nanosuspension formulations were having characteristic taste and odor. The globule size analysis of the optimized formulations was done using a light-scattering particle size analyzer Coulter LS-230. The droplet size was in the ranges of 61.2±0.58 (F3) to 91.4±0.79 nm (F5). The deference in the droplet size between the formulations is not statistically significant ($p > 0.05$). There is only a marginal deference in the mean globule size of formulations. The minimum droplet size was obtained with nanosuspension formulation F3.

The polydispersity index of various felodipine nanosuspension formulations was in the ranges of 0.271±0.14 to 0.651±0.21. All the felodipine nanosuspension formulations were having low polydispersity index. The polydispersity index was maximum for nanosuspension formulation F1, suggesting non-uniformity in globule size. The polydispersity index was lowest for nanosuspension formulation F3, indicating uniformity in globule size. The viscosity of various felodipine nanosuspension formulations was in the ranges of 10.68±0.92 to 20.55±1.01 cp. Most nanosuspension possesses a very low viscosity and, therefore, their application may be convenient.

It was observed that the viscosity of all the formulations is less than 21 cP. The felodipine nanosuspension formulation F3, has the lowest viscosity (10.68±0.92 cP) which is highly significant ($p < 0.01$) as compared to the other formulations. Satisfactory refractive index was obtained with all the nanosuspension formulations. The refractive index was in the ranges of 0.48±0.44 to 0.82±0.21. The maximum refractive index was obtained with nanosuspension formulation F7; whereas minimum refractive index was obtained with nanosuspension formulation F5. Monitoring the pH value is important for determining the emulsions' stability because pH changes indicate the occurrence of chemical reactions that can compromise the quality of the final product. Forearm skin testing is standard in most clinical studies of skin and has pH values in the range of 4.2 to 5.9 for both sexes.

Satisfactory pH was obtained with all the nanosuspension formulations. The pH was in the ranges of 6.42±0.34 (Nanosuspension formulation F3) to 6.91±0.35 (Nanosuspension formulation F8). The pH value of all the felodipine nanosuspension formulation was in the skin pH range, which was nearer to neutral pH range, demonstrating that all the felodipine nanosuspension will be non toxic, non irritating and non allergic. Morphology and structure of the nanosuspension were studied using Scanning electron microscopy. The nanosuspension appears dark and almost spherical in shape. All droplets are having almost uniform shape and size. All most all the nanosuspension formulation showed good drug content. The drug content of various felodipine nanosuspension formulations was in the ranges of 68.4±1.08 to 92.3±0.98 % (**Table 1**).

The nanosuspension formulation F8 has lowest drug content. The felodipine nanosuspension formulation F3, has the highest drug content (92.3±0.98 %) which is highly significant ($p < 0.01$) as compared to the other formulations. Zeta potential is a technique which is used to measure the surface charge properties and further the long term physical stability of nanosuspension.

The zeta potential of various felodipine nanosuspension formulations was in the ranges of 12.13±0.16 to 13.31±0.33 mV, as given in **Table 1**. All most all the nanosuspension formulation showed uniform Zeta potential, demonstrating that all nanosuspension formulation would be stable. The transmittance values of various felodipine nanosuspension formulations were in the ranges of 1.25±0.25 to 1.88±0.32 % (**Table 1**).

TABLE 1: REFRACTIVE INDEX, pH, DRUG CONTENT, ZETA POTENTIAL AND TRANSMITTANCE OF THE FELODIPINE NANOSUSPENSION FORMULATIONS. EACH VALUE ARE REPRESENTED AS MEAN ± STANDARD DEVIATION (n = 3). STANDARD ERROR OF MEAN (SEM) < 0.335.

Formulation	Refractive Index (X±S.D.)	pH value (X±S.D.)	Drug content (%) (X±S.D.)	Zeta potential (mV)(X±S.D.)	Transm-ittance. (%) (X±S.D.)
F1	0.79±0.22	6.65±0.31	69.3±1.08	12.63±0.19	1.75±0.71
F2	0.57±0.34	6.44±0.26	76.5±0.97	12.81±0.28	1.68±0.59
F3	0.62±0.25	6.42±0.34	[a]92.3±0.98	12.13±0.16	1.88±0.32
F4	0.53±0.48	6.84±0.54	82.4±0.88	12.41±0.34	1.25±0.25
F5	0.48±0.44	6.69±0.29	87.9±1.07	13.22±0.44	1.58±0.66
F6	0.66±0.31	6.51±0.43	85.7±1.04	12.52±0.51	1.84±0.44
F7	0.82±0.21	6.75±0.22	75.3±1.01	13.05±0.23	1.32±0.51
F8	0.58±0.19	6.91±0.35	68.4±0.87	13.31±0.33	1.76±0.28

All most all felodipine nanosuspension formulations were able to release drug in controlled manner over extended period of time. The *in vitro* drug dissolution study revealed that all nanosuspension formulations released the drug up to 9 h. The felodipine nanosuspension formulation F5 and F6 released 100 % of drug in 9 h only, where as nanosuspension formulation F1, F2, F4 and F7 released all drug in 10 h and nanosuspension formulations F3 and F8 released complete drug in 12 h (**Fig. 1** and **2).**

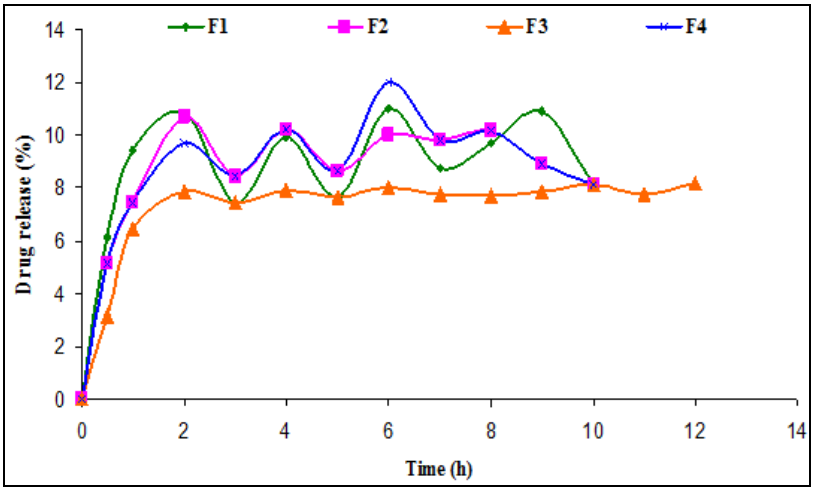

FIG. 1: *IN VITRO* DRUG RELEASE COMPARATIVE PROFILE OF FELODIPINE NANOSUSPENSION FORMULATION F1 TO F4. ALL POINTS (PERCENTAGE DRUG RELEASE) REPRESENT MEAN VALUE (n = 3).

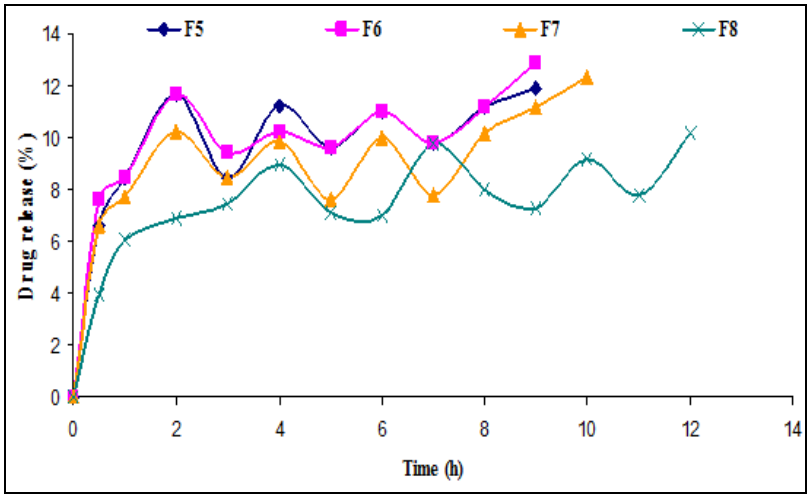

FIG. 2: *IN VITRO* DRUG RELEASE COMPARATIVE PROFILE OF FELODIPINE NANOSUSPENSION FORMULATION F5 TO F8. ALL POINTS (PERCENTAGE DRUG RELEASE) REPRESENT MEAN VALUE (n = 3).

The more controlled and constant manner drug release was observed from felodipine controlled release nanosuspension formulation F3 (Containing HPMC K4M 80 mg and ethyl cellulose 75 mg) as it released it 100 % of drug up to 12 h with minimum fluctuation of drug (Felodipine) concentration in blood stream. From the release kinetics data **Table 2**, it was confirmed that, the control release formulations F1 to F9 obeyed zero order kinetic model, independent of time and concentration.

TABLE 2: *IN VITRO* DRUG RELEASE KINETIC STUDIES OF DIFFERENT FELODIPINE NANOSUSPENSION FORMULATIONS

Formulation	Zero order kinetics	First order kinetics	Higuchi equation	Korsemeyer-Peppas	Release Exponent (n)
	Regression co-efficient (r^2)				
F1	0.924	0.7261	0.8349	0.972	1.28
F2	0.861	0.7967	0.7665	0.906	1.66
F3	0.909	0.786	0.8159	0.933	1.12
F4	0.927	0.750	0.856	0.937	1.015
F5	0.898	0.589	0.809	0.873	1.035
F6	0.887	0.714	0.797	0.925	1.235
F7	0.899	0.658	0.810	0.927	1.176
F8	0.913	0.740	0.816	0.942	1.164

All the tablet formulations obeyed Korsemeyer and Peppas kinetic model which confirms the diffusion controlled release. The diffusion co-efficient data indicates that the tablet formulations F3 to F8 released the drug by diffusion following Fickian transport mechanism, where as tablet formulations F1, F2 and F9 released the drug by diffusion following non-Fickian transport mechanism.

CONCLUSION: The felodipine nanosuspension formulation F3 also released drug in a constant manner irrespective of time with minimum fluctuation in drug concentration in blood stream explaining exhibition of less side effects. Thus the felodipine nanosuspension formulation F3 containing 0.75% HPMC K4M, could be concluded as the best optimized formulation for safe management of hypertension.

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