AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

# Formulation Development and Comparative Pharmacokinetic Evaluation of Felodipine Nanoemulsions in SD Rats

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## ABSTRACT

The present study involves the formulation and evaluation of o/w nanoemulsions with two simple edible oils in micro-liter quantities, avoiding large quantities of surfactants and co-surfactants. The nanoemulsions were prepared by high energy emulsification technique. The process optimization was based on the particle size, size distribution and entrapment efficiency in relation with the quantity of oil and concentration of surfactant. The percent drug content was determined by HPLC with UV detector. The particle size, polydispersity index (PDI), and zeta potential of nanoemulsions were determined by using particle sizer. Stability studies at 4°C for two months, centrifugation and freeze-thaw cycling were carried out. Pharmacokinetic studies of nanoemulsion and marketed dosage form were performed in male SD rats and blood plasma samples were analyzed by LC-MS/MS. The particle size, polydispersity index (PDI), and zeta potential of nanoemulsions were found to be in the range of 26.8±0.72 to 154.6±11.4 nm, 0.09±0.01 to 0.28±0.06 and 0.07±0.01 to -28±0.65 mv respectively. Transmission electron microscopy (TEM) and stability studies revealed the physical stability of the nanoemulsions. The percent drug content was found to be in the range of 73.74±3.79 to 101.16±1.35. The oral bio-availability was significantly increased in nanoemulsion compared with the marketed dosage form. These results showed a successful incorporation of felodipine into nanoemulsion with high drug loading efficiency and good stability.

**Key words**: Sesame oil, olive oil, felodipine, sonication, Oral bioavailability.

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Received 5 April 2012, Accepted 20 April 2012

Please cite this article in press as: Reddy P *et al.*, Formulation Development and Comparative Pharmacokinetic Evaluation of Felodipine Nanoemulsions in SD Rats. American Journal of PharmTech Research 2012.

# INTRODUCTION

Hypertension is one of the major causes of mortality in the world (causes 60,000 deaths/year and is directly related to more than 2, 50,000 deaths from stroke). Over one million people per year suffer adverse reactions from doctor prescribed drugs[1]. Felodipine is an oral calcium channel blocker and belongs to dihydro pyridine class. Chemically it is 3-ethyl 5-methyl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate. It is lipophilic in nature, highly photosensitive and exhibits poor aqueous solubility. It undergoes extensive first pass metabolism and exhibits poor bioavailability (10-15%) [2-3]. A few drug delivery systems containing felodipine such as chitosan micro particles[4], self emulsifying drug delivery systems[5], the hydrogel template method[6] and micro emulsions[7] were developed. However, these methods present difficulties related to high production costs, low stability and low drug loading. Lymphatic delivery is an alternate choice to avoid first pass metabolism in drug delivery and improves bio-availability, because intestinal lymph vessels drain directly into thoracic duct, further in to the venous blood, thus by passing the portal circulation. Lipid based formulations such as nanoemulsion may enhance oral drug absorption by lymphatic transport via transcellular path way, by increasing gastrointestinal membrane permeability or transit time or by modifying the metabolism of drug[8-9]. Nanoemulsions are thermodynamically stable, transparent or translucent dispersions of oil and water having a size range of 50-200 nm[10]. Nanoemulsions can be used as excellent vehicles in pharmaceutical field for the parenteral, oral and ocular or transdermal delivery of poorly permeable lipophilic drugs[11]. The objectives of the present investigation were to study the feasibility of using sesame oil or olive oil as sole lipid phase in the formulation and development of stable nanoemulsions with high drug loading efficiency, to evaluate the effect of oil phase, type and concentration of surfactants and the effect of sonication time on particle size, zeta potential, and drug loading efficiency, to study *in vitro* drug release characteristics and to carry out the comparative pharmacokinetic studies with commercial formulations in rats.

# MATERIALS AND METHODS

## Materials

Felodipine was a gratis sample from Dr. Reddy's Laboratories (Hyderabad, India), Brij-97, Pluronic-F-68, sodium cholate, Tween-80, Span-80 and dialysis tubing cellulose membrane (size: 43 mm × 27 mm) were purchased from Sigma Aldrich, USA. Sesame oil (pharmaceutical grade) was purchased from Thiagarajan Agro Products Pvt Ltd., Chennai, India. Olive oil

(pharmaceutical grade) was purchased from Figaro, Sri Roda Foods Pvt Ltd., New Delhi India. All other chemicals, water and solvents are of HPLC grade and purchased from S.D. Fine Chemicals, India.

**High performance liquid chromatography analysis**

RP HPLC method developed in the laboratory using HPLC (Shimadzu-10ATVP) equipped with two pumps (Model-10ATVP) and Shimadzu –UV-Visible detector (SPD-10ATVP), Phenomenex C-8 column, Size: 250×4.6mm for the determination of felodipine content. The Analyte was eluted isocratically using mobile phase Acetonitrile: Water in the ratio of 31:69 at a flow rate of 1mL/min. Detection was done at a wavelength of 240nm.

**Selection of oil phase**

Oil phase was selected based on oil/water partition coefficient studies of felodipine. Different oils such as sesame oil, soya bean oil, olive oil were used for the investigation of the oil-water partition coefficient of Felodipine. A 5mg of the drug was dissolved in 2.5ml of oil and 2.5ml of HPLC water pH-6.8± 0.2 was added and shaken for 5 hours using a mechanical shaker and kept aside for overnight at room temperature (25° C) to achieve equilibrium. The aqueous layer was separated by a separating funnel, centrifuged to remove any entrapped oil globules or particles and the supernatant liquid was separated. From the supernatant a volume was taken and made up to 10ml with methanol, filtered, degassed and 20µL was injected into HPLC to determine the felodipine content[12]. Then oil/water partition coefficient was calculated using the formula $k = C_{water}/C_{oil}$ [13].

**Preparation of Nano Emulsion by high energy emulsification method**

Felodipine was incorporated into nanoemulsion by high energy emulsification method[14]. The aqueous phase composed of nonionic surfactant and HPLC water, whereas the oil phase contained dissolved drug. The oil phase and aqueous phase were prepared separately. A quantity of drug and oil were taken in a beaker and total weight was determined. To this mixture 200 µl of chloroform was added to dissolve the drug in oil. Then nitrogen gas was purged to evaporate the chloroform. Again the weight of the beaker was taken to confirm the complete removal of chloroform. At the beginning of the study various nanoemulsions were prepared using 100, 150 and 200 µl of sesame oil and Brij 97. Almost 100% drug loading efficiency was achieved with 10mg of the drug in 100 µl of oil. The particle size and polydispersity index were increased with increasing quantity of oil. Therefore the ratio of oil to water was fixed at 1:100 for all the nanoemulsions and further optimization was done using process variables like sonication time and formulation variables like oil phase, type and concentration of surfactants. Aqueous phase

was prepared by dissolving the surfactant in HPLC water pH 7.12±0.02 to produce surfactant solution. Initially 10 ml of surfactant solution was added to the oil phase and stirred by high shear stirrer (RQ-127A, Remi Motors, India) for 25 min at 6000 rpm to produce a coarse emulsion. Then this coarse emulsion was sonicated using probe sonicator (Bandelin Sonoplus, Heinrichstrab 3-4 D-12207, Berlin, Germany) at 37 to 40HZ in continuous mode to produce nanoemulsion. Ultra sonication was carried out at different time intervals of 5, 10 and 15 min using probe sonicator in continuous mode to optimize the sonication time. To investigate the effect of type of surfactants and their concentrations on droplet size and zeta potential of nanoemulsions, formulations without co-surfactants were prepared. Poloxamer-F68, Sodium Cholate, Tween-80 and Brij-97 were used as surfactants in different concentrations viz., 0.5%, 1% and 1.5%. In-order to investigate the effect of combination of surfactants on particle size, polydispersity and zeta potential nanoemulsions were also prepared using a combination of Lecithin and Poloxamer in 1:1 ratio. The compositions of nanoemulsions were given in Table 1.

**Table 1: Composition of felodipine loaded nanoemulsions**

| Code | Felodipine (mg) | Oil Phase volume (µl) | | Wt of surfactant (mg) | | | | Sonication Time (min) |
|---|---|---|---|---|---|---|---|---|
| | | Sesame oil | Olive oil | Brij 97 | Tween 80 | Lecithin | Poloxamer | |
| F1 | 10 | 100 | - | 50 | - | - | - | 15 |
| F2 | 10 | 100 | - | 100 | - | - | - | 15 |
| F3 | 10 | 100 | - | 150 | - | - | - | 15 |
| F4 | 10 | 100 | - | - | 50 | - | - | 15 |
| F5 | 10 | 100 | - | - | 100 | - | - | 15 |
| F6 | 10 | 100 | - | - | 150 | - | - | 15 |
| F7 | 10 | - | 100 | 50 | - | - | - | 15 |
| F8 | 10 | - | 100 | 100 | - | - | - | 15 |
| F9 | 10 | - | 100 | 150 | - | - | - | 15 |
| F10 | 10 | - | 100 | - | 50 | - | - | 15 |
| F11 | 10 | - | 100 | - | 100 | - | - | 15 |
| F12 | 10 | - | 100 | - | 150 | - | - | 15 |
| F13 | 10 | 100 | - | - | - | 75 | 75 | 15 |
| F14 | 10 | 100 | - | - | - | 75 | 75 | 15 |
| F15 | 10 | 100 | - | - | - | 75 | 75 | 15 |

**Measurement of particle size and zeta potential**

The average particle size, polydispersity index (PDI), and Zeta potential were measured by photon correlation spectroscopy (PCI) using a Malvern particle sizer (Nano ZS Malvern Instruments Ltd., UK). The PDI represents the uniformity of the particle size and size distribution of the nanoemulsion. The prepared formulations were diluted with HPLC water pH-7.12±0.02. The diluted nanoemulsions were kept in the cuvette with an attached dip cell. The cuvette was placed inside the instrument and the observations were recorded at 90° light

scattering angle and temperature was maintained at 25 °C. During the measurement, average particle count rate was maintained between 50 to 500kcps. The zeta potential was also measured by using the same instrument with inbuilt software based on the electrophoretic mobility of globules and the Helmholtz-Smoluchowski equation

$$\text{Zeta potential (Zp)} = 6\pi\upsilon\eta/\varepsilon\chi$$

Where Zp is in volts, υ = migration velocity cm/sec, η = viscosity of the medium in poise, ε = dielectric constant of the external medium, and χ = potential gradient in volts[15-17].

**Determination of felodipine content in the formulations**

A volume of felodipine nanoemulsion was taken from the bulk and dissolved in 5ml of methanol. From this 1ml was taken and appropriate dilutions were made with phosphate buffer saline (PBS) pH 7.4 to get a concentration of 4µg/ml. Then filtered, degassed and 20 µl was injected in to HPLC. The drug content was determined by the above mentioned method.

***In vitro* drug release studies**

The *in vitro* drug release studies of felodipine were carried out using a dialysis bag diffusion technique (43mm x 27mm size, mol. wt. cutoff 12000 or greater, Sigma-Aldrich, USA). The bag containing 1ml of nanoemulsion was placed and immersed in a 50 ml beaker containing 25ml of PBS or PBS containing 1% tween 80. The entire system was kept at 37°C±0.5°C with continuous magnetic stirring. The samples were withdrawn at periodical time intervals (0min, 15min, 30min, 1, 2, 4, 6, 8 and 24 hrs), and replaced with equal volume of fresh medium to maintain sink conditions. The samples were filtered through 0.22 µ membrane filter, degassed in a bath sonicator (Spincotech, India) and injected in to HPLC column. All experiments were performed in triplicate[18].

**Stability of the optimized formulations**

**Effect of storage at 4°C on particle size and zeta potential of nanoemulsions**:

he formulations F3, F6, F8, F9 and F12 were selected based on particle size, zeta potential, drug loading efficiency and reproducibility and kept for stability studies for 2 months at 4°C. Particle size and zeta potential were measured as described above at periodical time intervals.

**Heating and cooling cycling:**

Heating and cooling cycling was conducted by storing the formulations at 4°C for 48 hrs followed by 45°C for 48hrs for each cycle and the stability was tested after four cycles. The stable preparations were subjected to centrifugation test.

**Centrifugation test:**

The selected formulations were centrifuged at 5000 rpm for 5 hrs and at 10000 rpm for 30 min and were observed for creaming or phase separation. The formulations that are showing no phase separation in heating and cooling cycling and centrifugation were selected and subjected to freeze thaw cycling.

**Freezing and thawing:**

Three freeze thaw cycles between -20°C and +25°C with storage at each temperature for not less than 48 hrs was done for the optimized formulations[19].

**Transmission electron microscopy**

The particle size and morphology were observed with transmission electron microscopy (TEM) analysis. The samples were placed on form var-coated copper grid. Then the samples were negatively stained with 50µl of 2% phospho tungustic acid for one minute and air dried. Excess liquid was blotted with whatman filter paper. Then the samples were observed under Philips (TECNAI – FE12) Transmission Electron Microscope (120 kV) [20].

***In vivo* Pharmacokinetic analysis**

Comparative pharmacokinetic studies were carried out for the formulation F8 with the commercial tablet suspension. Felodipine tablet was taken and crushed into powder in a mortar and pestle. To this 10 ml of 1 % tween-80 solution was added and triturated to prepare a fine suspension with strength of about 1mg/ml. The experimental protocol was approved by institutional animal ethics committee of vimta labs limited, Hyderabad (study number: VLL/0510/NG/D016). Male Sprague Dawly rats of approximately 6-8 weeks of age weighing between 200 and 240gms were used. The animals were acclimatized for a period of 5 days. All the rats had free access to reverse osmosis generated potable water and standard animal diet. Throughout the study period, room temperature and relative humidity were maintained at 20° C±2° C and 30% to70% RH respectively. Illumination was controlled to give 12 hrs dark cycles during the 24hrs period.

Overnight fasted rats were used for the study. Prior to the initiation of the study rats were weighed for the body weights. Twelve rats were randomized based on their body weights and distributed equally into 2 groups. One group of rats was received Felodipine tablet suspension and another group of rats received felodipine nanoemulsion. Both the formulations were administered by oral gavage at the dose of 1mg/Kg. The dose volume administered was 1ml/kg body weight. Following oral administration approximately 0.3ml of blood samples were collected after anaesthetizing with isofurane from a group of 3 animals per time point from respective group at respective time intervals that is pre-dose (0), 5min, 0.25,0.5, 1, 2, 4, 6, 8 and

24hrs post dosing from retro-orbital plexus in pre-labeled eppendorf tubes containing 20µl of 10% K2EDTA. The blood samples collected as pre-dose at (0) time were taken as control.

**Analysis of blood samples by liquid chromatography/mass spectroscopy/mass spectroscopy**

A volume of study sample, calibration curve samples and quality control samples were transferred to the pre-labeled ria vials, 10µl of internal standard (4µg/ml amlodipine) was added and vortexed, 2.5 ml of Tertiary Butyl Methyl Ether (TBME) was added to all the samples. These vials were placed on a shaker for 10 minutes and centrifuged for 10 minutes at 4000 rpm at 20°C and supernatant was transferred in to pre-labeled ria vial and evaporated under a stream of nitrogen at 35°C until dryness, reconstituted the dried residue with 300µl of mobile phase and vortexed. Samples were loaded in to pre-labeled auto-injector vials and 10 µl of samples were injected onto LC-MS/MS system containing HPLC (AGILENT 1200 series (VLS-UTL/HPLC/02) and Mass spectrophotometer (AB MDS Sciex 4000,VLS-UTL/MASS/01) with a Column of Hypurity Advance, 100 X 4.6mm, 5µ.The column oven temperature was maintained at 40°C, peltier temperature at 10°C and the mobile phase was 20mM Ammonium acetate: Methanol (20:80 v/v) with a flow rate of 1ml/min and an injection volume of 10µl. The separation was conducted under isocratic conditions and the total run time was within 3.2 minutes. The ion source was heated nebulizer with positive polarization. The detection ions were 384.10 amu (parent) to 338.10 amu (product) and 409.30 amu (parent) to 238.10 amu (product) for felodipine and the internal standard amlodipine respectively. Then the pharmacokinetic parameters were calculated by non-compartmental analysis by winN online $^{(R)}$ 5.2 soft ware.

**Statistical analysis:**

The pharmacokinetic parameters of the olive oil nanoemulsion and marketed tablet suspension were compared by the student t-test. A p-value of less than 0.05 was considered as statistically significant.

## RESULTS AND DISCUSSION

Emulsions are composed of oil phase, aqueous phase and surfactant (emulsifying agent). An essential component of the emulsion is the internal lipid core which constitutes the drug dissolved in oil and is surrounded by a thin layer of non-ionic surfactant. Therefore at the beginning of the study vegetable oils such as sesame oil, soybean oil and olive oil were selected, because theses oils are of vegetable origin, bio-compatible and used as edible oils from ancient times[21-22]. Moreover they are easily available and literature survey revealed that felodipine loaded nanoemulsions were not reported with these oils. The oil water partition coefficients

($K_{o/w}$) of the drug in theses oils were carried out and given in table 2. Based on these $K_{o/w}$ values, olive oil and sesame oil were used for the present study, as the rate of drug release depends on the nanoemulsion composition, solubilization properties of the oil and also related to the partition coefficient of the drug in oil/ water system which in turn depends on the solubility of drug in oil[23]. More specifically for efficient transport of the drug from the formulation in to the systemic circulation, the drug must first pass from the lipid phase to the aqueous phase and then in to the GIT lumen. In addition to this, these oils were also stable at room temperature and they do not become rancid like other oils due to the presence of natural antioxidants. Therefore nanoemulsions loaded with felodipine were prepared using these oils by high energy emulsification technique and evaluated for particle size, polydispersity, zeta potential, drug content, *in vitro* drug release studies, stability and *in vivo* pharmacokinetic studies.

**Table-2: Determination of oil water partition coefficient of felodipine**

| **Oil** | **$K_{o/w}$** |
|---|---|
| Sesame oil | 0.34±0.1 |
| Oliver oil | 0.67±0.05 |
| soybean oil | 0.074±0.02 |

Data represents Mean±SD, n=3

**Measurement of particle size and zeta potential**

The physical properties such as particle size, PDI and zeta potential are essential parameters in predicting the physical stability of nanoemulsions. The mean particle sizes of nanoemulsions were in the range of 26.8±0.72 to 154.6±11.4 nm and the PDI of nanoemulsions were in the range of 0.09±0.01 to 0.28±0.06 which shows a narrow particle size range and size distribution in all formulations. With increasing sonication time the globule size was reduced. This is because various processes like breakup of droplets, adsorption of surfactants and droplet collision occur during emulsification. This shows that the emulsification process is a dynamic process and events that occur in a micro second range could be very important[24-25].

Surfactants play a major role in the formation and stabilization of nanoemulsions. They prevent the coalescence of drops by reducing the interfacial tension and by forming a stearic barrier against droplet coalescence[26]. Nonionic surfactants such as Pluronic-F-68, sodium cholate, Tween-80, and Brij-97 were employed for the present study as these materials have highest biocompatibility and most effective surface properties[27-29]. However nanoemulsions were not formed with Pluronic-F-68 and sodium cholate, further study was discontinued with these surfactants. Nanoemulsions prepared with combination of surfactants possess large particle size compared to nanoemulsions prepared with single surfactant. This increased droplet size was due

to the expansion of the interfacial film by the second surfactant which acts as co-surfactant[30]. The quantity of surfactant also affects the particle size, drug loading efficiency and *in vitro* drug release studies of nanoemulsions. At low concentrations the amount of emulsifier was not sufficient to cover the surface of the droplets formed during the sonication leading to particle aggregation. However use of excess amount of emulsifier can cause decrease in entrapment efficiency, burst release, formation of other colloidal species like liposomes and micelles and may even cause toxic effects[31]. Hence to ensure the formation of small and uniform particle size and a short emulsification time as well as to avoid the use of co-surfactant and to keep the surfactant concentration at a minimum, the surfactant concentration was used up to 1.5%. Among all these nanoemulsions prepared with sesame oil as oil phase, non-ionic surfactants brij 97 and tween 80 possesses narrow particle size and size distribution. The mean particle size and PDI of the nanoemulsions were very low in case of formulations using Brij-97 and this may be due to its high surface effective properties. All the successful felodipine loaded nanoemulsion formulations had zeta potentials between -0.07±0.01 to -24.8±1.69 mv. The presence of free fatty acid esters as contaminants in the oil phases and/or surfactants may be responsible for this negative charge[32]. The pH of all nanoemulsions, which was found to be in the range of 6.8±0.03 to 7.2±0.02, may also contribute to this negative zeta potential. All the characterization parameters were shown in the Table 3.

**Table 3: Characterization of felodipine loaded nano emulsions**

| Code | Particle size (nm) | Polydispersity | Zeta potential (mv) | Drug content (percent) |
|---|---|---|---|---|
| F1 | 127.50±3.8 | 0.23±0.05 | -0.07±0.01 | 96.67±6.5 |
| F2 | 71.60±1.7 | 0.12±0.01 | -8.69±0.83 | 97.04±4.3 |
| F3 | 26.80±0.72 | 0.13±0.01 | -18.20±1.05 | 101.2±1.4 |
| F4 | 120.90±4.2 | 0.17±0.01 | -28±0.65 | 78.6±5.8 |
| F5 | 64.29±1.9 | 0.27±0.04 | -24.83±1.69 | 73.89±4.7 |
| F6 | 48.42±0.8 | 0.20±0.01 | -20.37±0.77 | 73.74±3.9 |
| F7 | 86.2±2.7 | 0.09±0.01 | -18.76±0.7 | 98.4±5.1 |
| F8 | 69.30±1.62 | 0.11±0.01 | -14.38±1.06 | 100.67±3.0 |
| F9 | 47.31±1.73 | 0.278±0.04 | -13.57±0.92 | 99.68±7.2 |
| F10 | 154.6±11.4 | 0.28±0.06 | -18.88±0.24 | 81.3±2.68 |
| F11 | 61.80±1.3 | 0.26±0.02 | -15.89±0.36 | 86.42±4.7 |
| F12 | 45.96±2.18 | 0.233±0.01 | -18.5±1.36 | 85.92±3.5 |
| F13 | 115.4±2.9 | 0.194±0.01 | -6.5±0.9 | 75.5±3.6 |
| F14 | 84.9±3.4 | 0.15±0.06 | -5.6±0.86 | 76.9±6.2 |
| F15 | 84.19±1.9 | 0.178±0.01 | -4.4±0.94 | 78.94±5.9 |

Data represents Mean±SD, n=3

**Determination of drug content and *in vitro* drug release studies**

Drug content was determined in all the nanoemulsions and given in table 3. Among all these prepared nanoemulsions, five compositions (F3, F6, F8, F9 and F12) that showed narrow particle size and polydispersity and high drug content were selected, *in vitro* drug release studies were carried out in phosphate buffer saline pH 7.4 (PBS). The cumulative percent release of felodipine from nanoemulsions was in the range of 7.76±0.56% to 9.8±0.29% and was increased to 25.9±1.55% for the formulations F3, F6, F9 and F12 when the receptor medium PBS was replaced by PBS containing 1%Tween-80, where as in composition F8 it was increased to 65.3±3.04. The correlation coefficient was in the range of 0.6 to 0.976. The quantity of surfactant affects not only particle size but also drug loading efficiency and *in vitro* drug release studies of nanoemulsions and it was revealed in composition F8. The cumulative percent drug released vs time plots were shown in the Figure 1. The log percent drug release Vs time plots revealed that the drug release from the nanoemulsions was found to have followed first order kinetics.

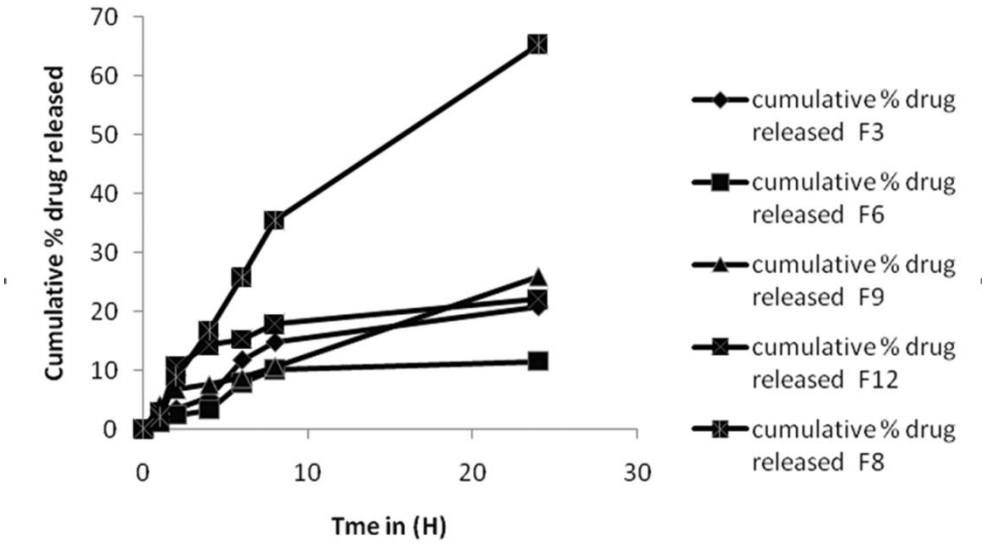


**Figure 1: Cumulative % felodipine released from nano emulsions in PBS with tween 80.**

**Stability studies**

Stability studies were conducted for all nanoemulsion compositions (F3, F6, F8, F9 and F12) that posses narrow globule size, high drug content and best drug release characteristics by storing them at 4°C for 60 days. The particle size and zeta potential were determined after 30 days and 60 days and the results were reported in Table 4 and 5. After storage at 4°C no significant change in the zeta size and zeta potential were observed in optimized formulations. After centrifugation at 5000 rpm for 5 hrs and at 10000 rpm for 30 min, no phase separation or creaming was found upon visual observation. Nanoemulsions that are stable in centrifugation, heating and cooling

cycles were subjected to freezing and thawing. At the end of 3 cycles, the particle size was slightly increased, however no significant difference in zeta potential was observed.

**Table 4: Effect of storage at 4°C on particle size**

| Code/day | F3 | F6 | F8 | F9 | F12 |
|---|---|---|---|---|---|
| $^{t}$day | 26.80±0.72 | 48.42±0.8 | 69.30±1.62 | 47.31±1.73 | 45.96±2.18 |
| 30$^{th}$ day | 28.125±0.05 | 49.24±0.97 | 70.12±0.61 | 49.84±1.45 | 47.82±1.5 |
| 60$^{th}$ day | 28.7±0.86 | 49.65±0.28 | 70.48±0.64 | 49.92±2.36 | 48.73±0.85 |

Data represents Mean±SD, n=3

**Table 5: Effect of storage at 4°C on zeta potential**

| Code/day | F3 | F6 | F8 | F9 | F12 |
|---|---|---|---|---|---|
| 1$^{st}$ day | -18.20±1.05 | -20.37±0.77 | -14.38±1.06 | -13.57±0.92 | -18.5±1.36 |
| 30$^{th}$ day | -18.85±0.8 | -16.65±0.14 | -14.32±0.59 | -14.87±1.7 | -18.48±1.4 |
| 60$^{th}$ day | -18.68±0.69 | -16.44±0.17 | -14.52±0.17 | --14.64±0.8 | -18.29±1.6 |

Data represents Mean±SD n=3

**Table 6: Pharmacokinetic parameters after oral administration of Felodipine nanoemulsion and felodipine commercial tablet suspension**

| Parameter | Nanoemulsion | Oral tablet suspension |
|---|---|---|
| Cmax* | 72.42±4.25 | 52.58±4.25 |
| Tmax** | 3.33±1.15 | 3.33±1.15 |
| Kel** | 0.07±0.01 | 0.06±0 |
| T1/2** | 10.37±1.75 | 12.59±0.78 |
| AUC0-t (ng*hr/ml)* | 745.3±50.28 | 533.21±19.71 |
| AUC0-inf (ng*hr/ml)* | 952.05±106.77 | 703.69±14.21 |
| AUMC* | 6063.36±566.59 | 4265.24±146.19 |
| MRT** | 8.13±0.52 | 8.00±0.13 |

***p*<0.01when compared with tablet formulation using student's t test.

***p*>0.05when compared with tablet formulation.

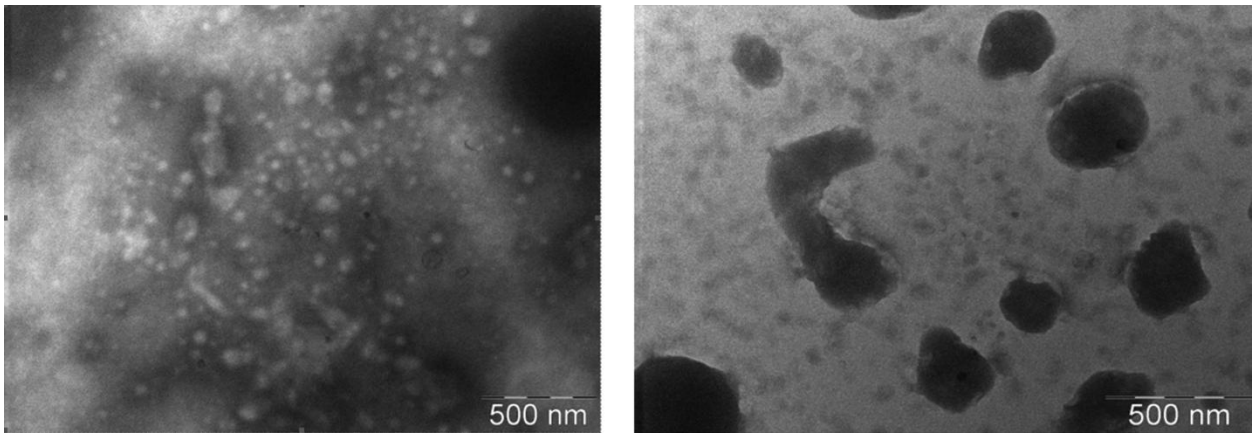


**Figure 2: Transmission electron micrographs of the blank (A) and Felodipine loaded nano emulsions (B). Both the nanoemulsions prepared with olive oil. The scale bar represents a distance of 500nm in Pictures (A) and (B).**

**Transmission electron microscopy**

Transmission electron microscopic studies were carried out for the formulation F8 to observe the physical properties of nanoemulsion droplets. Transmission electron micrographs revealed that the droplets were spherical, homogeneous and no signs of precipitation. The droplet size was correlated with the results from particle size analysis. All these results were presented in figure 2.

***In vivo* Pharmacokinetic analysis**

The *in vivo* bio-availability studies were performed for the formulation F8 which showed maximum drug release in *in vitro* release studies. The mean plasma concentration vs time profiles of the felodipine nanoemulsion and tablet suspension after administration of a single dose of 1mg/kg body weight were shown in Figure 3. The pharmacokinetic parameters were computed by non compartmental analysis using Winn online software and the results were summarized in the Table 6. Pharmacokinetic parameters revealed that the extent of oral absorption and hence oral bioavailability of felodipine was enhanced with nanoemulsion compared with that of marketed tablet suspension.

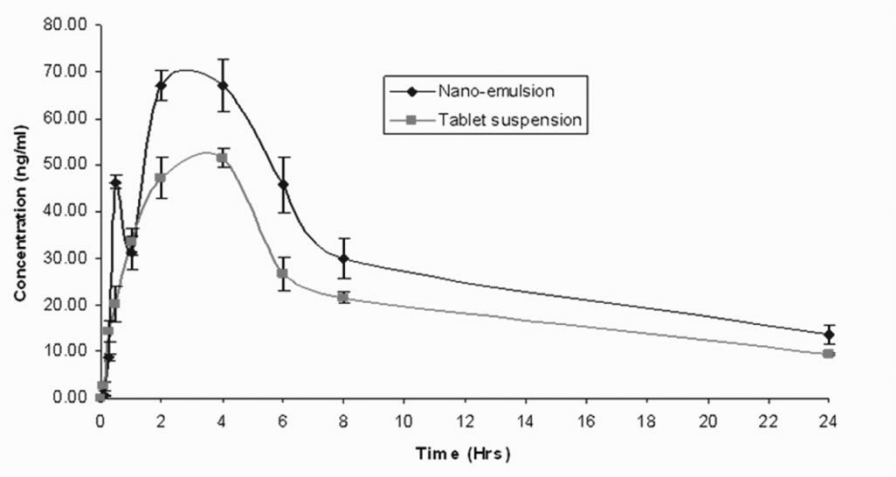


**Figure 3: Plasma concentration (ng/ml) - time profile of Felodipine following nanoemulsion and tablet suspension administration at dose of 1 mg/kg to Sprague Dawley rats.**

The nanoemulsion was more effective in enhancing oral absorption and availability of felodipine in plasma. The average plasma concentrations were 66.94±3.36 and 47.29±4.28 after 2 hours following felodipine with nanoemulsion and oral tablet suspension respectively. Plasma concentration of felodipine when administered with the nanoemulsion remained higher than with the tablet suspension for up to 8 hours. Felodipine administration in olive oil nanoemulsion showed a significantly higher ($p<0.01$) $C_{max}$ and AUC in plasma and no significant difference in $t_{max}$, $t_{1/2}$ and MRT compared to that of tablet suspension. AUC is expected as an indicator of the extent of absorption, where as $C_{max}$ and $T_{max}$ are considered as estimates of the absorption rate[33].

This may be explained by the fact that the presence of omega-6 and omega-3 PUFA (poly unsaturated fatty acids) in olive oil which are essential fatty acids and are not produced by the human body may enhance the rate of oral absorption[34]. The small droplet size, and hence the large surface area, lymphatic transport through the transcellular pathway [35] may also contribute to the increased bio availability.

## CONCLUSION

Felodipine loaded sesame oil and olive oil nanoemulsions were successfully prepared by high energy emulsification technique. The resultant nanoemulsions possessed good physicochemical properties and high percentage yield of the drug. The *in vitro* drug release studies followed first order kinetics. The *in vivo* bioavailability of felodipine was enhanced with nanoemulsion compared to tablet suspension.

## ACKNOWLEDGEMENTS

The authors acknowledge Dr. Reddy's Laboratories, Hyderabad for providing gift sample of Felodipine, S.N Vanita Pharmacy Maha Vidyalaya, Hyderabad, V. Vinay Kumar and M. Sailaja of IICT, Hyderabad and vimta labs, Hyderabad.

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