History of Medicine, 2023, 9(1): 2183–2194
DOI: 10.17720/2409-5834.v9.1.2023.283

# Preparation and Evaluation of Felodipine Drug Nanoparticles

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**Received:** 20 January 2023 **Accepted:** 15 April 2023

**Citation:** Hamed HE (2023) Preparation and Evaluation of Felodipine Drug Nanoparticles. History of Medicine 9(1): 2183–2194. https://doi.org/10.17720/2409-5834.v9.1.2023.283

**Abstract**

The aim of this study is to formulate and evaluate felodipine nanoparticles using solvent anti solvent technology. Felodipine is a calcium-channel blocker with low aqueous solubility and bioavailability. Felodipine prepared as nano particles in order to improve its solubility and dissolution rate. Twelve formulas were prepared and different stabilizing agents were used with different concentrations such as poly vinyl alcohol (PVA), poloxamer 407 and HPMC k15. The ratios of drug to stabilizers used to prepare the nanoparticles were 1: 1 and 1:2.

The prepared nanoparticles were evaluated for particle size, entrapment efficiency, dissolution study, Fourier transform infrared spectroscopy, differential scanning calorimetry, and atomic force microscopy. The percentage of drug entrapment efficiency of F1-F12 was ranged from 72% ± 1 to 93.2 % ± 1. On the other hand dissolution rate increasing as the particle surface area is increase due to reduction of particle size to the nano range.

**Keywords**
Felodipine, Nanoparticles, Particle Size,

*The oral route is the most common and preferred and membrane permeability. Many poor water-soluble routes for drug administration due to its convenience and drugs come to the BCS classes of II and IV. When drug administered orally in process of drug absorption from oral route for such types solid dosage form like tablet, capsule; firstly it undergo of drugs is occurs via dissolution rate limiting step [(1)]. Several formulation techniques exist for the manufacturing of nanosuspension, precipitation has been applied to prepare submicron particles, especially for the poorly soluble drugs [(2)].*

*Felodipine (FLD) is a dihydropyridine calcium-channel blocker used in the treatment of elevated blood pressure and angina pectoris (m.w. 384.3 Dalton, m.p. 145C°, pKa 5.39, practically insoluble in aqueous medium, freely soluble in acetone, ethanol, methanol and in methylene chloride). FLD is more selective vasodilator and have fewer cardiac action than non-dihydropyridine calcium-antagonists. But this advantage is absent due to poor bioavailability of this medicament, which (although the drug is absorbed totally from the GIT) is simply 15% of the dose is available in blood circulation when it is administered orally. The low bioavailability of felodipine is attributed to its low aqueous solubility due to first pass metabolism [(3)]*

*The aim of this study is formulate and evaluate felodipine nanoparticles to enhance solubility and dissolution rate of drug.*

## Materials and Method

### Materials

*Felodipine powder was purchased from Baoji Guokang Bio- Technolog, PVA (JP&SB Converting Services, Spain), poloxamer 407(HIMEDIA (Mumbai, India), HPMC k15 from Avonchem, Engeland, ethanol (Scharlau Chemie, S.A. Spain). All other chemicals were of analytical reagent grade.*

### Method

### Preparation of Felodipine Nanosuspension

*Nanosuspensions of felodipine were prepared by the solvent evaporation technique, which is also termed as anti-solvent precipitation method. felodipine powder was dissolved in ethanol (5 ml) at room temperature. This was poured into 20 ml of water containing different types of stabilizer (alone and in combination) maintained at room temperature and subsequently stirred at agitation speed of 500 revolution per minute (rpm) on magnetic stirrer for 60 min.to allow the volatile solvent to evaporate[4]. The resultant organic solution of drug (organic phase) was added drop by drop by means of a plastic syringe positioned with the needle directly into aqueous solution of stabilizer. Felodipine insoluble in water, therefore, it will precipitate with stabilizer. The ratios of drug to stabilizer used to prepare the nanosuspension were 1:1 and 1:2, as shown in table (1) and (2).*

**Table (1): Composition of Felodipine Nanosuspension Using Different Stabilizers at Drug: Stabilizer Ratio 1:1.**

| *Formula* | *Felodipine (mg)* | *Poloxamer407 (mg)* | *HPMC k15 (mg)* | *PVA (mg)* | *Ethanol (ml)* | *Water (ml)* |
|---|---|---|---|---|---|---|
| *F1* | *5* | *5* | | | *5* | *20* |
| *F2* | *5* | | *5* | | *5* | *20* |
| *F3* | *5* | | | *5* | *5* | *20* |
| *F4* | *5* | *2.5* | *2.5* | | *5* | *20* |
| *F5* | *5* | *2.5* | | *2.5* | *5* | *20* |
| *F6* | *5* | | *2.5* | *2.5* | *5* | *20* |

**Table (2): Composition of Felodipine Nanosuspension Using Different Stabilizers at Drug: Stabilizer Ratio 1:2**

| *Water (ml)* | *Methanol (ml)* | *PVA (mg)* | *HPMC k15 (mg)* | *Poloxamer407 (mg)* | *Felodipine (mg)* | *Formula* |
|---|---|---|---|---|---|---|
| *20* | *5* | | | *10* | *5* | *F7* |
| *20* | *5* | | *10* | | *5* | *F8* |
| *20* | *5* | *10* | | | *5* | *F9* |
| *20* | *5* | | *5* | *5* | *5* | *F10* |
| *20* | *5* | *5* | | *5* | *5* | *F11* |
| *20* | *5* | *5* | *5* | | *5* | *F12* |

### Evaluation of the prepared nanosuspension

### Particle size and size distribution

*Particle size determination was done by using Angstrom Advanced Inc. ABT-9000 USA particle size analyzer which is a dynamic light scattering works by measuring the intensity of light scattered by the molecules in the sample as a function of time, at scattering angle 90° and a constant temperature of 25 °C.*

*From the analysis, the average particle size which is also called volume moment mean, (mean diameter in nm) reflects the size of those particles which constitute the bulk of the sample volume and it was measured for all the prepared formulas. The polydispersity index (PDI) which is a measure of the width of the size distribution of each formula of felodipine nanosuspension also determined, it is a measure of the distribution of particle size of nanoparticles obtained from a particle analyzer, PDI*

*is an index of spread or variation or width within the particle size distribution. Also, the analyzer determines the specific surface area of each sample*[5] .

## Determination of drug entrapment efficiency (EE) of nanosuspension

*The freshly prepared nanosuspension was centrifuged at 6,000 rpm for 20 min. The supernatant solution was filtered and separated. 1 ml of this filtrate was diluted with water and the absorbance at maximum λ max was measured by UV spectrophotometer using water as blank. It was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken.*

*The experiment was performed in triplicate for each batch and the average was calculated. The entrapment efficiency (EE %) could be achieved by the following equation* [6]*: entrapment efficiency = (Weight initial drug- Weight free drug) / Weight initial drug.*

## In vitro dissolution profile of nanosuspension

*The dissolution was performed using dialysis membrane-60 (HIMEDIA) in 900 ml, it carried out by using USP type II apparatus.*

*Samples of 5ml were withdrawn at predetermined intervals, and the samples were 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 min respectively).*

*Replaced with fresh dissolution medium. The samples were filtered through a 0.45µm membrane filter and diluted if necessary. Absorbance of these solutions were measured at 237 nm using UV-visible spectrophotometer*[7].

## Freeze Drying of Nanosuspension

*The principle of freeze-drying method is that small amounts of a product will be frozen in and thereafter it will be placed under vacuum. Through vacuum the frozen liquid sublimates. The ice immediately changes into vapor, without to defrost first, also called sublimate. During process, the outside part will be the first part that dewaters.*

*After that, the water is removed closer and closer till the core of the product. Hereby the structure of the product stays intact. Due to the vacuum the ice will evaporate immediately without turning into water again. The freeze-dried products is usually of high quality, mainly because the temperature stays low during the whole process.*

*The lyophilization cycle includes overnight freezing (for about 20 hours) at -60 °C, 150 mTorr vacuum following by drying for about 24 hours at 120 mTorr vacuum conditions* [8].

## In vitro dissolution study

*An in vitro dissolution test was conducted in a dissolution apparatus according to the USP paddle method. The temperature was maintained at 37 ± 0.5°C, and the stirring rate was at 50 rpm.*

*The commercial felodipine nanosuspesion accurately weighed bulk drug and nanosuspensions were dispersed in 900 ml of dissolution medium (0.1 N HCL). 5 ml samples were drawn, and the same volume of fresh dissolution medium was added at 5, 10, 15, 20, 30, ....120 min, respectively. Then, the samples were filtered through a 0.1-µm syringe filter immediately before dilution, when necessary.*

*Drug content was determined with a UV spectrophotometer at 237 nm for 0.1 N HCL (pH 1.2)* [9]

## Fourier transform infrared spectroscopy

*The Fourier transform infrared spectroscopy (FT-IR) spectra were recorded for pure drug and optimized formulation using KBr pellet technique. The pellets were prepared using KBr hydraulic press under hydraulic pressure of 150 kg/cm2. The spectra were scanned over 3600-400 cm−1 at ambient temperature with a resolution of 4 cm−1, using FT-IR 2500 apparatus and spectra were recorded* [10]

## Differential scanning calorimetry (DSC)

*DSC investigations were performed using DSC apparatus model DSC-6. Samples of about 5 mg of pure drug powder and selected formula are placed in an aluminum pan and the experiment was carried out under nitrogen atmosphere at a flow rate of 40 mL/min and scanning rate of 10°C/min in the range of 15-300°C* [11]

## High performance liquid chromatographic (HPLC)

*A simple high performance liquid chromatographic method has been developed and validated for the quantitative determination of mirtazapine in human plasma. A reversed-phase Cl8 column was used for the determination of mirtazapine with a mobile phase composed of 0.01M ammonium acetate solution (pH 4.2) and acetonitrile (75:25, v/v%) at a flow rate of 1.2 mL/min. Terazosin hydrochloride was used as an internal standard. The fluorescence detector was set at excitation and emission wavelengths of 290 and 350 nm, respectively. Intra- and inter-day precision and accuracy were acceptable for all quality control samples including the lower limit of quantification of 3 ng/Ml[12].*

## Atomic force microscopy (AFM)

*The AFM is capable of scanning the surfaces in controlled environmental conditions and is complementary to SEM imaging and also can measure the particle size of the nanoparticles accurately.*

*The size and surface morphology of mirtazapine nanoparticle were confirmed by atomic force microscopy after drying of the formula. Samples were determined in tapping mode, exerting pyramidal cantilevers with Pt probes. All results were recorded under ambient laboratory condition and scanning frequency of 2Hz.*

*Resonance frequency was 79.491 kHz and a constant force in the range 2.5-10$Nm^{-1}$, driving amplitude 334.6mv. silicon chip was newly operated by peeling off its upper layer to Form the sample. Particle size, 3D-dimension graph and histogram of particle size distribution were obtained[13].*

## Statistical Analysis

*The results of the experiments are given as a mean samples ± standard deviation (SD) and were analyzed according to t-test and one way analysis of variance (ANOVA) using Sigma Plot 11 software at which the results would be significant if $p<0.05$ , highly significant if $p<0.01$ and the results would be non - significant if $p>0.05$ .*

## Results and Discussion

## Evaluation of nanosuspension

## Particle size analysis

*The particle size of F1-F6 at drug : stabilizer ratio 1:1 was ranged from 129 - 429 nm measured by particle size analyzer (as shown in table 3) while for F7-F12 at drug : stabilizer ratio 1:2 the particle size ranged from 79 - 315 nm as in (table 4) using poloxamer 407 , HPMC k15 and PVA as primary stabilizers ; Although of this mechanism , HPMC gave larger particle size in both ratios 1:1 and 1:2 drug : stabilizer in F2 and F8.*

*HPMC k15, due to their hydrophobic nature tend to adsorb onto the hydrophobic part of felodipine nanoparticles and thus providing stabilization to the nanoparticles, preventing agglomeration and thus viscosity of it increases [14]*

*Polydispersity index is a parameter used to define the particle size distribution obtained from the particle size analyzer. Polydispersity index gives degree of particle size distribution at range from 0.021 to 0.380 depending on formulation variables. The formula F9 showed lowest PDI (0.029) at drug : stabilizer ratio 1:1 and 0.112 at drug : stabilizer ratio 1:2, as seen in (table 3) ; that indicate good uniformity of nanoparticle size. Uniformity of particle size is determined by polydispersity index values in which the low value means the best uniformity.*

*The range of PDI values (0-0.05) means (monodisperse system), 0.05-0.08 (nearly monodisperse), 0.08-0.7 (mid-range polydispersity), and >0.7 (very polydisperse) From the obtained results, one can conclude that the PVA and poloxamer 407 are not suitable as a primary stabilizer for nanoparticles because of poor adsorption and affinity of poloxamer to the drug molecules[15].*

## Effect of polymer concentration on the size of Felodipine nanoparticles

*The effect of the stabilizer concentration on the particle size was investigated by depending on two ratios 1:1 of drug : stabilizer in the preparation of F1-F6 and 1:2 of drug : stabilizer in the preparation of F7-F12. Not only the type of stabilizer affect on the particle size, but also the concentration of the stabilizers used.*

*Stabilizer concentration also influences on the adsorption affinity of non-ionic stabilizers to particle surface. In general as the concentration of stabilizer increase the particle size decrease at fixed drug concentration , which indicated that the drug particle surface was sufficiently enveloped by the stabilizer*

*molecules*

*The concentration of stabilizer may give negative effect (decrease particle size) or positive effect of on particle size (increase particle size). It can also influence on the adsorption affinity of non-ionic stabilizers to particle surface. In general, as the concentration of stabilizer increases the particle size decreases at fixed drug concentration, which indicated that the drug particle surface was sufficiently enveloped by the stabilizer molecules[16].*

*It was observed that with an increase in surfactant concentration in the nanosuspension from the particle size of the nanosuspension decreases. This was due to the decrease in relative viscosity, which led to decrease in particle size. It means that hydrodynamic diameter of particle decreased with increase in the concentration of the surfactant.The concentration of surfactant affected on particle size because too little concentration of stabilizer induces agglomeration or aggregation and too much concentration promotes Ostwald ripening[17].*

*As shown in table (3,4) the size range of particles is decrease , respectively, these results indicated that mean size of particles showed a regular decrease with increasing the concentration of poloxamer.*

*These effects may be due to a process of a primary covering of the newer surfaces competing with the aggregation of the uncovered surfaces. Hence, an elevation in ratio of surfactant in the primary dispersion results in rapid enclosing of the newly formed particle surfaces. There was an optimum concentration of surfactant, above which the increase in concentration did not result in a decrease in particle size due to saturation point; these results are in agreement when poloxamer was used as stabilizer at different ratios[18].*

*Poloxamer is a block co-polymer, can act as a surfactant, responsible for the hydrophobic association with the molecules of drug. The inhibition of the crystal growth is mainly related to the hydrophobic part (polypropylene oxide group PPO) in the pluronic polymer, while the second chain which is (the hydrophilic oxide) (PEO) can provide steric hindrance against particles aggregation[19].*

*These effects may be due to a They stabilized the system by steric stabilization where the surfactant adsorbed onto the drug particle surface leading to reduce in the surface tension and increasing the nucleation rate. On the other hand, the adsorption of surfactant makes the particles less hydrophobic and thereby reduces the hydrophobic forces of attractions (van der Waals interactions) and that reduced particle growth and aggregation[20].*

## Effect of combination of two polymers on the size of felodipine nanoparticles

*The particle size of ( F1-F6) of drug : stabilizer ratio 1:1 was ranged from 129-429 nm measured by particle size analyzer (table 3) , (F7- F12) of drug : stabilizer ratio 1:2 was ranged from 79-315 nm (table 4).*

*At ratio1:2 drug : stabilizer large particle size show in combination of poloxamer 407 and HPMC k15 gave higher size than alone that show in F10 (232nm). In F7that contain poloxamer 407 alone get particle size 157 nm, that mean HPMC has a higher affinity to adsorb felodipine than Poloxamer, these results due to that the combination lead to increase viscosity of the disperse media, so it is ineffective combination and cannot stabilize the nanoparticulate system [21].*

*Nanoparticles formulation generally requires addition of appropriate stabilizers to lower the free surface energy of the nanoparticles and prevent particle aggregation and/or particle growth.*

*The high surface free energy of nanoparticles is readily lowered by lowering the solid–liquid interfacial tension upon addition of surfactants[22].*

*Uniformity of particle size is determined by polydispersity index values in which the low value means the best uniformity when used PVA polymer.*

**Table (3): Particle Size, Polydispersity Index and EE% of Formulas at Drug: Stabilizer Ratio 1:1.**

| *Formula* | *Stabilizers* | *Particle size* | *PDI* | *EE%* |
|---|---|---|---|---|
| *F1* | *Poloxamer 407* | *219* | *0.131* | *83.9* |
| *F2* | *HPMC k15* | *429* | *0.380* | *72* |
| *F3* | *PVA* | *129* | *0.229* | *85.3* |
| *F4* | *Poloxamer407+ HPMC k15* | *371* | *0.234* | *89.1* |

| | | | | |
|---|---|---|---|---|
| *F5* | *Poloxamer407+PVA* | *211* | *0.321* | *78.9* |
| *F6* | *HPMC k15 +PVA* | *252* | *0.123* | *88* |

**Table (4): Particle Size, Polydispersity Index and EE% of Formulas at Drug: Stabilizer Ratio 1:2.**

| *EE%* | *PDI* | *Particles size* | *Stabilizers* | *Formula* |
|---|---|---|---|---|
| 88.2 | 0.079 | 157 | Poloxamer 407 | F7 |
| 93.1 | 0.192 | 315 | HPMC – k15 | F8 |
| 93.2 | 0.021 | 79 | PVA | F9 |
| 87.8 | 0.051 | 232 | Poloxamer 407 + HPMC | F10 |
| 90.9 | 0.211 | 197 | Poloxamer407 + PVA | F11 |
| 79.6 | 0.341 | 119 | HPMC + PVA | F12 |

## Determination of drug entrapment efficiency of nanosuspension

*The Percentage drug entrapment efficiency of the formulations from 72 % – 93.2 % ( table 3,4) The drug entrapment efficiency of F9 was high when compared to other formulations. Drug entrapment efficiency was significantly affected by ratio of drug: stabilizer(1:2) with higher EE% (93.2) in F9 which could be due to decreased partitioning of mirtazapine into the outer aqueous phase and better dispersion obtained by adding a hydrophilic stabilizers[23].*

## In vitro dissolution study

*The dissolution profile of the formulas that in nanosize were studied in 0.1N HCl of pH 1.2 to determine the best formula that gives the best release in first 20 min.*

*From the study, the results showed that the formula F9 that contain PVA stabilizers gave the best release in 20 min in comparison with other formulas and the formula shows a maximum cumulative percentage drug release of 100 % within 20 min. From the above result depending on the particle size and PDI will be the formula no. F9 which has the mean particle size (79 nm) and E E% (93.2).*

*F9 was considered as the selected formula because it had the highest dissolution rate, highest entrapment efficiency percentage and low particle size when compared with another formula. This formula is selected for zeta potential measurement study, lyophilization, and further study.*

*The release of F9 was compared with the pure drug in media of 0.1N HCl and in phosphate buffer pH6.8 ( figure 5) the maximum cumulative percentage drug release of F9 was 99.7 % within 20 minutes, whereas the pure drug having a release of 99.1 % in 50 minutes. In phosphate buffer solution (pH 6.8) F15having a release of 94.5 % in 40 minutes.*

*This may be attributed to the fact that the reduction of drug particle size caused an increase in the surface area and consequently enhanced the contact between nanoparticles and dissolution medium. The obtained results are in good accordance with Noyes–Whitney equation which states that the increase in saturation solubility and the decrease in particle size lead to an increased dissolution rate[24].*

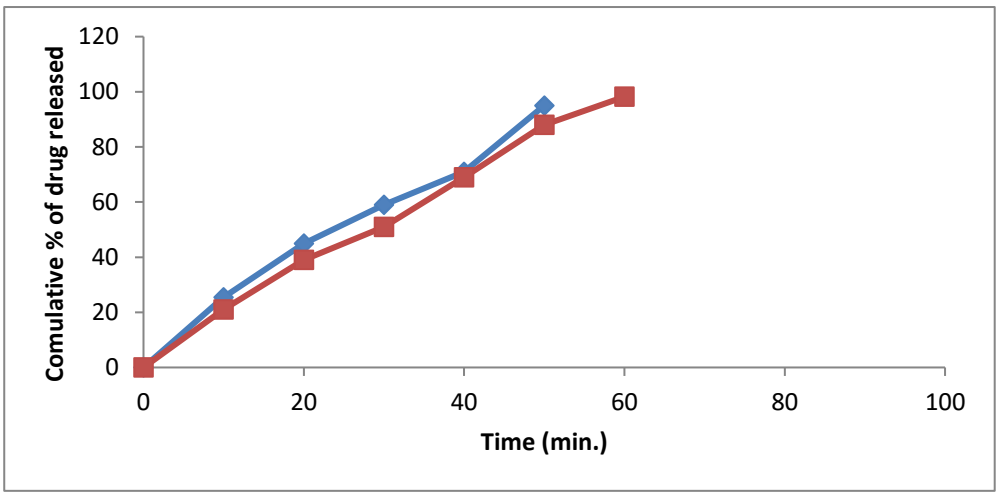


**Figure (1): In vitro drug release profile of felodipine formulation nanosuspension in 0.1N HCl at 37°C (F1,F2)**

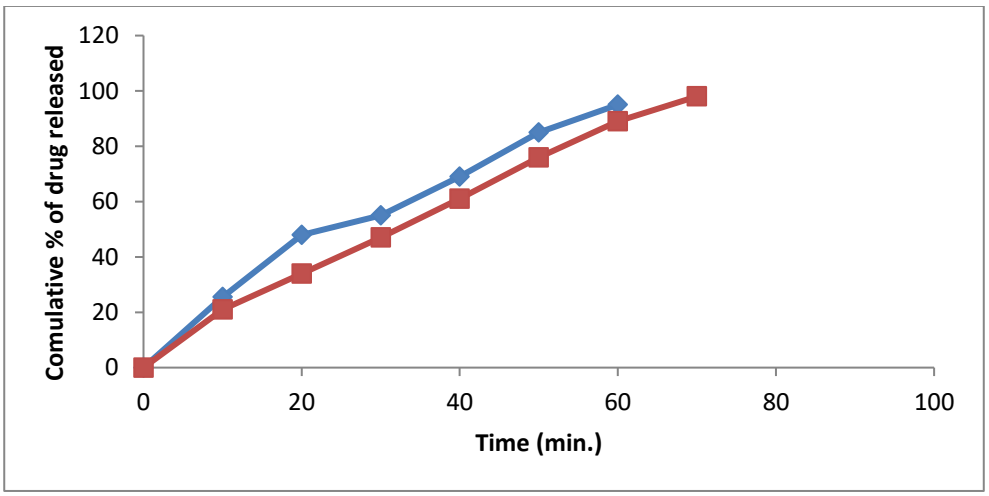


**Figure (2): In vitro drug release profile of felodipine formulation nanosuspension in 0.1N HCl at 37°C (F3,F4)**

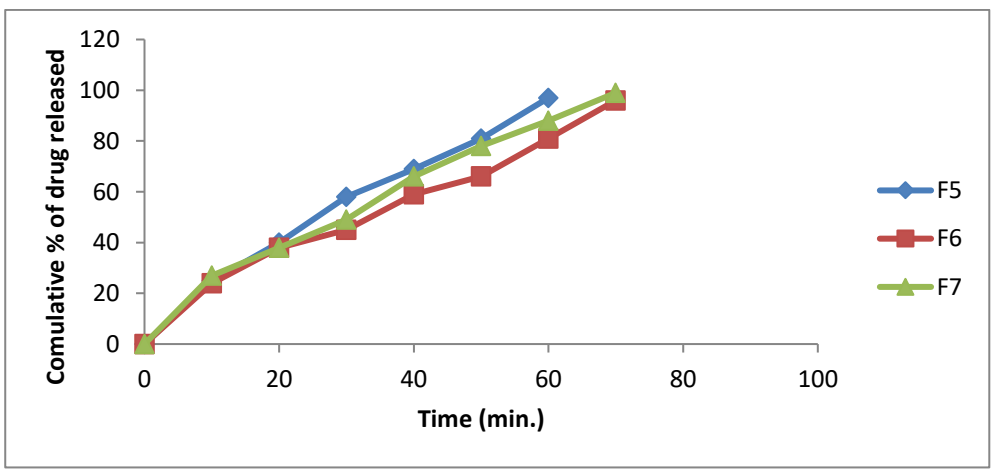


**Figure (3): In vitro drug release profile of felodipine formulation nanosuspension in 0.1N HCl at 37°C (F5,F6,F7)**

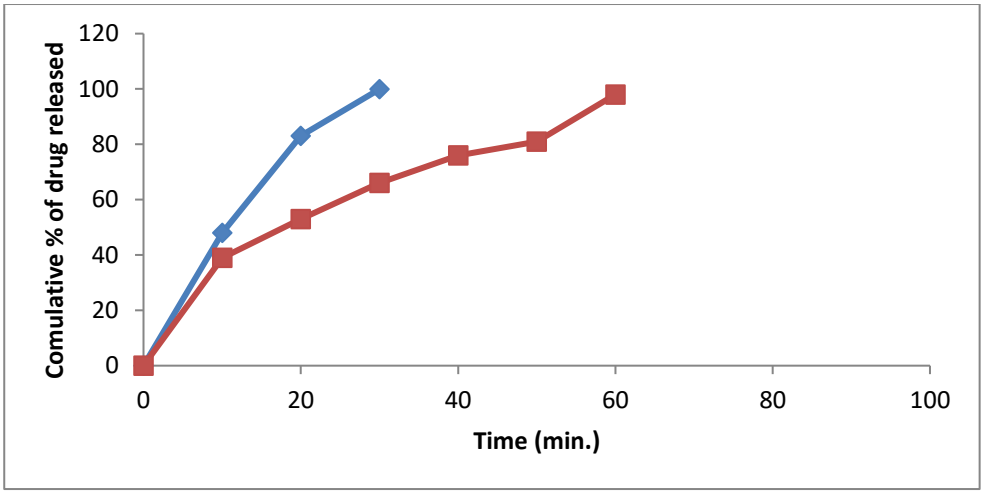


**Figure (4): Dissolution profile of felodipine (F9) nanosuspension in 0.1N HCl (pH 1.2) and in phosphate buffer (pH6.8) at 37C°**

## Drug Content in Lyophilized Powder

*Percentage drug content of lyophilized powder found to be 96.43% of felodipine when determined by UV-visible spectrophotometer at λ max 237 nm.*

## Fourier Transform Infrared Spectroscopy

*FTIR spectra of felodipine showed characteristic peaks at 3371.57 (N-H Str., Secondary), 2989.66 (C-H Str., -CH3), 3068.75 (C-H Str., Aromatic), 1689.64 (C=O Str.), 769.60 (C-Cl Str.) cm-1. There were no considerable changes in the IR peaks of the spherical agglomerates when compared to pure drug.As showen in figure (5).*

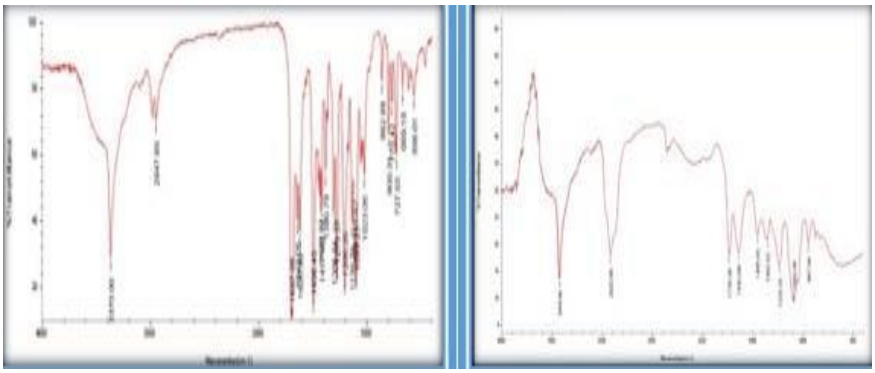

**Figure (5) : FTIR spectrum of pure drug and selected formula (F9)**

## Differential Scanning Calorimetry

*The DSC thermograms of pure felodipine and its spherical agglomerates with F9 are presented in Fig. 6. A single endotherm at 146.28 °C was ascribed to drug melting. There was a negligible change in the melting endotherms of prepared spherical agglomerates compared to pure drug (FI-2 = 145.46 °C), indication that the drug lost the crystallinity state and converted to an amorphous form[25] .*

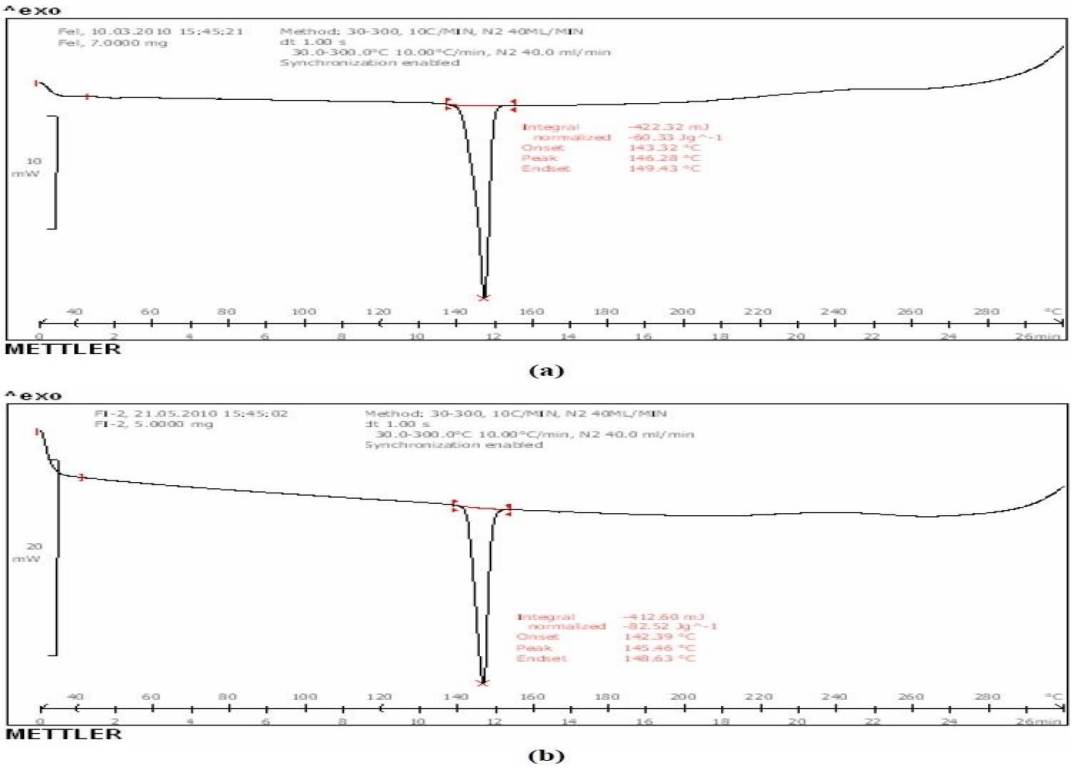


**Figure (6): DSC Thermogram of pure Powder and selected formula (F9)**

## Assay Amount of Felodipine by HPLC

*Assay for felodipine was determined using HPLC technology to be compared with the UV spectroscopy. Figure (7) shows the HPLC chromatogragram of felodipine as pure powder in the mobile phase. [The retention time of felodipine in the HPLC chromatogram was 7.13 minutes , for lyophilized powder of felodipine nanosuspension for best formula (F9) the retention time in the HPLC chromatogragram was 7.11 minutes, as shown in figure (8)].*

*From the results it was found that no significant difference between the two methods for the assay of felodipine pure powder and felodipine lyophilized powder.*

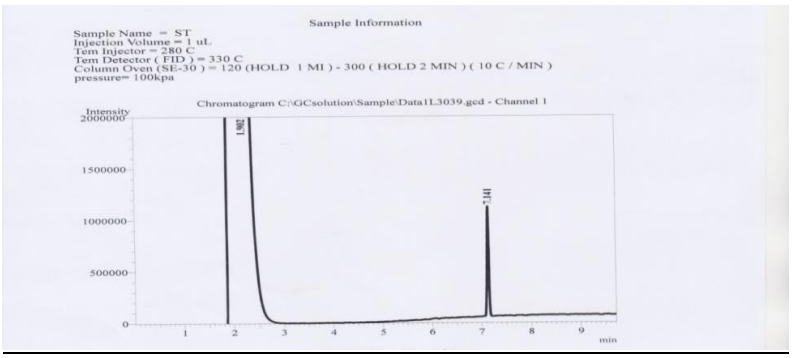


**Figure (7): HPLC Chromatogragram of Felodipine Pure Powder**

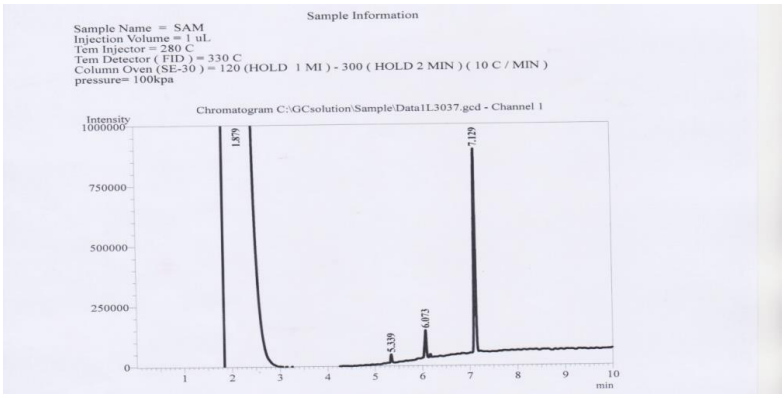


**Figure (8): HPLC Chromatogragram of Lyophilized Powder for Felodipine in the Selected formula, F9**

## Evaluation of surface morphology

***Atomic force microscopy study***

*It is an instrument that measure the properties of surfaces. AFM is capable of scanning the surfaces in controlled envi- ronmental conditions and is complementary to SEM imaging. With the high precision of the AFM, in principle it is possible to determine the dimensions of nanoparticles with high accuracy. AFM allows the visualization of samples with resolution in three dimensions x-, y- and z-directions in atmospheric or submerged conditions[26] The morphological analysis of felodipine pure powder performed by AFM showing spherical shaped nanoparticles (figure 9) . It was found to be stable, and no aggregation of particles could be observed. For a precise determination of single particle dimensions, size and distribution, microscopic techniques are required.*

*The particle size of F9 obtained by AFM was comparable to or equal to that measured by ABT-9000 nano laser and this agreement in particle size measurements provide the good size distribution and the stability of felodipine nanparticles[27].*

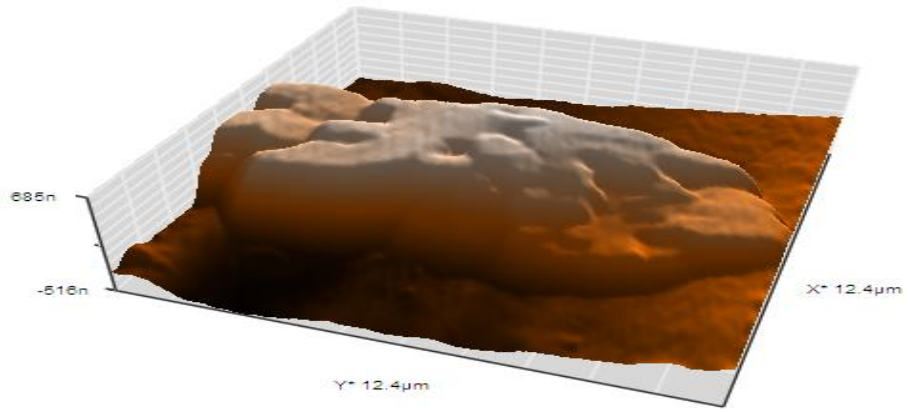


**Figure (9): 3D-Morphology of F9**

## Conclusion

*Nano particulate systems have great potentials, being able to convert poorly soluble, poorly absorbed and labile biologically active substance into promising deliverable drugs.*

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